Working Document to ICES Workshop on Micro increment daily growth in European Anchovy and Sardine (WKMIAS). Mazara del Vallo, Italy, 21-25 October 2013

## ANCHOVY AND SARDINE MICROINCREMENT DAILY GROWTH EXCHANGE RESULTS

Begoña Villamor<sup>1</sup>, Gualtiero Basilone<sup>2</sup> and Mario La Mesa<sup>3</sup>

<sup>1</sup>Instituto Español de Oceanografía (IEO). CO de Santander, Spain <sup>2</sup> CNR-IAMC. Mazara del Vallo, Sicily, Italy <sup>3</sup>CNR-ISMAR. Ancona, Italy

## 1. Introduction

Based on main results produced in previous ICES workshops on ageing adult anchovy and sardine (WKARA 2009, WKARAS 2011), a focal point was to correctly identify the right position of the first ring (annulus) on sagittal otoliths of these species, being one of the main sources of error affecting ageing precision. One of the most common method to validate the timing and position of the first ring consists of counting of otolith microincrements (daily rings) in juveniles (young-of-the-year). Daily growth studies of anchovy and sardine are currently carried out in different European laboratories, principally to analyze the effects of environmental parameters on growth and survival, and thus to understand the factors affecting recruitment processes of these species. However, given the wide span of methodologies already existing within laboratories, ageing data are often difficult to compare, actually masking the contribute of environmental conditions of different growth rate patterns observed among areas.

Exchanges, workshops and checks of the procedures for annual age determination of anchovy and sardine otoliths have been made in the past (i.e., ICES WKARA 2009; ICES WKARAS 2011). However, very little has been done with respect to daily age

1

determination of these species, having been only performed during the SARP project (in 1992) (only larvae) and during SARDONE project (in 2008).

The Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) meeting in February 2012, identified anchovy and sardine as the species requiring confirmation of the micro increments daily growth interpretation and counting carried out by Fisheries Institutes. The planning group indicated that a workshop on anchovy and sardine should be organized in 2013.

Before the foreseen workshop on daily age reading (see below), it has been considered useful to plan an exchange programme of anchovy and sardines otolith images in order to ascertain the current level of precision among Institutes and the difficulties that the daily age reading of anchovy and sardine otoliths present.

To that purpose, an exchange programme of anchovy and sardine otoliths has been organized between June and September 2013 before the workshop on anchovy and sardine daily age determination (ICES WKMIAS) that will be held in Mazara del Vallo (Italy) on 21-25 October 2013.

## 2. Objectives

The exchange will have the following objectives:

- Evaluate the current precision in otolith daily age reading of anchovy and sardine among readers from fishery and surveys samples throughout the year.
- 2- Identify major difficulties in anchovy and sardine otolith microstructure interpretation for daily age determinations resulting in observed disagreements.
- 3- Report results to the Workshop on microincrement daily growth in European Anchovy and Sardine (ICES WKMIAS) that will take place in October to facilitate the discussions and progress of work.

## 3. Material and Methods

## 3.1 Participants in the exchange

A total of 11 readers with different levels of experience of anchovy and sardine otolith daily reading participated in the otolith exchange, from different research institutions from France, Spain, Portugal, Italy and Greece and from the different areas concerned (Tables 3.1.1 and 3.1.2). Nine of them participated in the exchange of otoliths of anchovy and another nine in the otoliths of sardine. Six of them participated in both exchanges. Anchovy readers had more experience (only two with low level) than sardine readers (five readers with low level).

**Table 3.1.1.** Participants in the ANCHOVY exchange programme, with reader's identification (ID), their associated institution/laboratory, country and level of experience of anchovy ageing.

Country/Laboratory	Participants in exchange	Readers or Not ?	Daily Age reading expertise level Anchovy	ID	Email
France/IFREMER	Erwan Duhamel				erw an.duhamel@ifremer.fr
	Patrick Grellier	reader	high	R1	Patrick.Grellier@ifremer.fr
Spain-Basque Country/AZTI	Unai Cotano				ucotano@azti.es
	Naroa Aldanondo	reader	higth	R2	Naroa.aldanondo@kaust.edu.sa
Curring Atlantic (JEO (Country dow)	D <sup>2</sup> - \/;11				h
Spain-Atlantic/ IEO (Santander)	Begona Villamor	age coordinator	11:-h		begona.villamor@st.leo.es
	Carmen Hernadez	reader	High	R3	carmen.nernandez@st.leo.es
Spain-Mediterranean/IEO (Malaga)	Alberto Garcia				alberto.garcia@ma.ieo.es
	Jose Mª Quintanilla	reader	higth	R4	jose.quintanilla@ma.ieo.es
Italy-Sicily/CNR-IAMC	Gualtiero Basilone	age coordinator			gualtiero.basilone@iamc.cnr.iit
	Salvatore Mangano	reader	low	R5	salvo_mangano@hotmail.com
Italy-Adriatic/CNR-ISMAR	Mario La Mesa	age coordinator			m.lamesa@ismar.cnr.it
	Fortunata Donato	reader	Hlgh	R6	f.donato@ismar.cnr.it
	Monica Panfili	reader	Hlgh	R7	m.panfili@an.ismar.cnr.it
	Elisa Domenella	reader	Low	R8	e.domenella@an.ismar.cnr.it
Greece/HCMR	Stylianos Somarakis				somarak@hcmr.gr
	Eudoxia Schismenou	reader	high	R9	schismenou@hcmr.gr

#### **Anchovy Exchange**

**Table 3.1.2.** Participants in the SARDINE exchange programme, with reader's identification (ID), their associated institution/laboratory, country and level of experience of sardine ageing.

Country/Laboratory	Participants in exchange	Readers or Not ?	Daily Age reading expertise level Sardine	ID	Email
France/IFREMER	Erwan Duhamel				erwan.duhamel@ifremer.fr
	Patrick Grellier	reader	high in Iarvae Low in juveniles	R1	Patrick.Grellier@ifremer.fr
Spain-Atlantic/ IEO (Vigo)	Isabel Riveiro				isabel.riveiro@vi.ieo.es
	Eduardo Lopez	reader	low	R2	eduardo.lopez@vi.ieo.es
Portugal/IPMA	Alexandra Silva				asilva@ipma.pt
	Andreia Silva	reader	low	R3	<u>avsilva@ipma.pt</u>
Spain-Mediterranean/IEO (Malaga)	Alberto Garcia				alberto.garcia@ma.ieo.es
	Francisco Alemany				francisco.alemany@ba.ieo.es
	Jose Mª Quintanilla	reader	high	R4	jose.quintanilla@ma.ieo.es
Italy-Sicily/CNR-IAMC	Gualtiero Basilone	age coordinator			gualtiero.basilone@iamc.cnr.iit
	Salvatore Mangano	reader	low	R5	salvo_mangano@hotmail.com
Italy-Adriatic/CNR-ISMAR	Mario La Mesa	age coordinator			m.lamesa@ismar.cnr.it
	Fortunata Donato	reader	High	R6	f.donato@ismar.cnr.it
	Monica Panfili	reader	High	R7	m.panfili@an.ismar.cnr.it
	Elisa Domenella	reader	Low	R8	e.domenella@an.ismar.cnr.it
Greece/HCMR	Stylianos Somarakis				somarak@hcmr.gr
	Eudoxia Schismenou	reader	Low	R9	schismenou@hcmr.gr

#### Sardine Exchange

## 3.2. Images of anchovy and sardine collection

For this exchange we have selected images by species and sampling area, with a total of 81 images distributed as follows:

**Anchovy:** 41 images of otoliths were analyzed for daily age assignment, distributed in 5 sets from different anchovy distribution areas (Figure 3.2.1 and Table 3.2.1):

- <u>Bay of Biscay</u> (ICES Subarea VIII), 10 images: 5 larvae-postlarvae from AZTI and 5 juveniles from IEO Santander.
- Western Mediterranean: 5 images (larvae-postlarvae) from IEO Málaga
- <u>Strait of Sicily</u>: 5 images (juveniles) from IAMC-CNR, Sicily
- <u>Adriatic Sea</u>: 11 images (6 larvae-postlarvae and 5 juveniles) from ISMAR-CNR, UOS Ancona
- North Aegean Sea: 10 images (5 larvae-postlarvae and 5 juveniles) from HCMR

		Anche	ovy samples			
SET- Area	Zone	Number of images	Length range SL (mm)	Year	Institute	Remarks
SET A- Bay of Biscay	ICES Division VIIIcb	10	20.4-106	2005-2007-2009	IEO Santander/AZTI	Larvae & juveniles
SET B- Western Mediterranean	Almeria Bay	5	14.7-20.7	2011-2012	IEO Malaga	Larvae
SET C- Strait of Sicily	GS16	5	56-69	2005	IAMC-CNR	Juveniles
SET D- Adriatic Sea	Manfredonia/ Ortona	11	26-63	1996-1997-2013	ISMAR-CNR	Larvae & juveniles
SET E- North Aegean Sea	North Aegean Sea	10	12.01-75	2007	HCMR	Larvae & juveniles
Total		41	12.01-106			

Table 3.2.1.	. Otolith	image ex	change	sample	data of	ANCHOVY
--------------	-----------	----------	--------	--------	---------	---------

**Sardine:** 40 images of otoliths were analyzed for daily age assignment, distributed in 5 sets from different sardine distribution areas (Figure 3.2.1 and Table 3.2.2).

- <u>Bay of Biscay</u> (ICES Divisions VIIIab): 5 images (larvae-postlarvae) from IFREMER.
- <u>Atlantic Iberian</u> (ICES Division IXa): 5 images (culture juveniles) from IEO Vigo and
   5 images (juveniles) from IPMA.
- Western Mediterranean: 5 images (larvae-postlarvae) from IEO Málaga
- <u>Adriatic Sea</u>: 10 images (5 larvae-postlarvae and 5 juveniles) from CNR-ISMAR, UOS Ancona
- North Aegean Sea: 10 images (5 larvae-postalarvae and 5 juveniles) from HCMR

Table 3.2.2. Otolith image exchange sample data of SARDINE

		Sardin	e samples			
SET- Area	Zone	Number of images	Length range SL (mm)	Year	Institute	Remarks
SET A- Bay of Biscay	ICES Division VIIIab	5	17.1-21.6	2012	IFREMER	Larvae
SET B- Atlantic Iberian	ICES Division IXa	10	54.2-136	04-05-08-09-10	IEO Vigo/IPMA	Juveniles (5 from culture)
SET C- Western Mediterranean	Almeria Bay	5	15-22.9	2010	IEO Malaga	Larvae
SET D- Adriatic Sea	Manfredonia/ Ortona	10	14-80	1997 - 2013	ISMAR-CNR	Larvae & juveniles
SET E- North Aegean Sea	North Aegean Sea	10	20.3-80	2007-2009	HCMR	Larvae & juveniles
Total		40	15-136			



**Figure 3.2.1**: Collection areas of 2013 otolith image exchange sample sets of anchovy (in red) and sardine (in green)

Each laboratory uploads their images in the European Age Readers Forum (EARF) (https://groupnet.ices.dk/AgeForum/default.aspx), in the appropriate folder of WKMIAS. The set of images have to be accompanied by the images objective micrometer calibrated to x100 and x1000 for anchovy and x200 and x1000 for sardine, following the recommendations of the SARDONE project and Morales-Nin et al. (2010). It was recommended to choose images that are better processed, as this greatly influences the interpretation of the micro increments.

## 3.3. Reading procedure

All readers were asked to annotate their interpretation based on the shared ageing criteria on each digitised image using the images analyzer of their lab.

We recommended that the interpretation should be done based on the following guidelines established at the SARDONE project Workshop and Morales-Nin et al., 2010:

- Sagittal otoliths have to be employed.
- Indistinctly right or left otoliths can be used.

- Readings have to be done along the post-rostrum axis.
- The length of the total radius in the post-rostrum axis has to be measured.
- The method used for both species, named Group Band Reading (GBR), consists on counting as one every repetitive cyclic set of growth bands or apparently groups of microincrements (usually 2 but occasionally more), assuming that they are subdaily marks in post-rostrum zones corresponding to the early juvenile period.
- The otolith nucleus should be read at 1000 magnification, whilst the rest of the otolith at x100 for anchovy and X200 for sardine. Whenever necessary to assess the growth pattern, higher magnification can be used.
- The grouping of sub increments forming growth bands (GB) is common in all individuals and starts very early in the life history of the fish. The width of the GB is conservative, no sudden changes occur. In some cases the sub increments are very clear and difficult the identification of the GB. However, once the GB is initiated, this pattern continues. Therefore the group banding interpretation has to be maintained.
- Counting of otolith growth increments commenced from the hatch check and the last ring was omitted. The last ring is considered incomplete since it does not represent a full day.
- <u>Anchovy</u>: The first increment corresponds to the hatch check with a radius between 3.5 and 5 μm (Aldanondo et al., 2008). The GB starts very early in life, with double bands, once formed this pattern has to be kept in all the otolith.
- <u>Sardine</u>: The hatching check appears at 5-7 μm (Alemany and Alvarez, 1994). The GB appears at 40-80 increments after the hatching check, once formed this pattern has to be kept in all the otolith.

Increment measures had to be reported in the readings results files. All readers were asked to annotate if any otolith image has some area where the increments have to be indirectly estimated because they were not well marked (especially the final margin of the otolith), then these increments had to be marked in red in the reading result file to allow identification.

## 3.4. Data Analysis

We have standard statistical analysis to investigate the daily age interpretation among readers. Precision was estimated using coefficient of variation (CV) of increment counting between the different readers (Chang, 1982) and also was evaluated using the average percent error ( $APE_{BF}$ ) of Beamish & Fournier (1981). Both APE and CV have been widely used as statistically measures of ageing precision in fishes (Campana, 2001).

As reference age, we used the mean age rather than the modal age, due to the large number of ages obtained in the daily age determination. Although the mean age estimate is not an indicator for the reliability of ageing structure, it may provide useful information regarding over- or underestimation of age by a structure irrespective of fish size class. In addition, we analyzed separately the sample of culture juveniles of sardine from the Atlantic Iberian set, because we knew the actual age of the specimens.

We compared the increment width reported by reader, in order to know the readings interpretation from each reader in larvae and juveniles. For this, we selected image otolith readings with high level of precision in the age determination.

## 4. Results

The preparation of otoliths images sets and uploads to EARF and submission to the coordinator was completed during July 2013 and the exchange programme was completed by the end of September. This report presents the results of all readers.

## 4.1 ANCHOVY results

#### 4.1.1 By areas

Table 4.1.1 details length and month of landing of the sets of otoliths images selected for the anchovy exchange programme by areas along with the ageing produced by each reader. The last four columns give mean daily age, standard deviation (SD) and precision of reading as the CV in relation to the average age and the  $APE_{BF}$ . This exercise showed differences among readers and among areas. The lowest differences were found in the set of the North Aegean Sea (CV = 9.0% and APE = 7.8%) and the highest in the set of the Strait of Sicily (CV = 34.9% and APE = 23.0%). (Figure 4.1.1)

Table 4 1 1		Otolith SET	(MKMIAS 2013	Otolith	Exchange)
Table 4.1.1	ANCHUVI	Otolith SET	(WKINIAS 2013		Exchange

Bay of Biscay

Western Mediterranean

North Across Sea

	Sample	Fish	ΠL	SL	Landing	IFREMER	AZTI	IEO-ST	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAF	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	APEBF
2005	m41_2	1	-	20.4	8	35	28	29	36	41	36	38	41	25	34	5.7	17%	13.5%
2005	m83_10	2	-	25.2	8	29	37	38	31	44	32	35	34	29	34	4.9	14%	10.9%
2005	m81_5	3	-	28.3	8	36	42	45	46	67	43	45	47	43	46	8.5	18%	10.6%
2005	m83_18	4	-	30.6	8	36	46	43	44	44	40	41	44	33	41	4.3	10%	8.0%
2005	m81_2	5	-	34.7	8	46	49	48	54	63	48	53	50	40	50	6.3	13%	8.7%
2007	P07_15_18	6	121	106.0	9	104	116	117	111	139	109	123	127	109	117	10.9	9%	7.0%
2009	P09_09_43	7	57	50.0	9	56	62	61	60	89	57	62	61	57	63	10.1	16%	9.3%
2009	P09_17_68	8	74	62.0	9	74	75	82	76	112	72	89	78	66	80	13.5	17%	11.5%
2009	D09_94_05	9	100	86.0	10	71	77	82	76	114	73	88	87	70	82	13.6	17%	11.6%
2009	D09_94_31	10	91.3	78.0	10	68	80	83	78	128	77	79	74	71	82	17.9	22%	12.7%
					Total read	10	10	10	10	10	10	10	10	10			45 39/	40.49/
				Total	NOT read	0	0	0	0	0	0	0	0	0			10.3%	10.4%

			_															
	Sample	Fish	TL	SL	Landing	IFREMER	AZTI	IEO-ST	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	APEBF
2011	OTO 362-TF0811	1	· •	19.9	8	29	30	26	28	37	35	31	34	23	30	4.5	15%	11.5%
2012	OTO 118a-TF0712	2	-	13.3	7	18	20	21	22	27	22	20	26	14	21	3.9	19%	13.2%
2012	OTO 118b-TF0712	3	-	13.3	7	20	20	19	21	25	21	20	24	16	21	2.6	12%	8.7%
2011	OTO 226 - TF0712	4	-	14.7	7	23	19	18	25	36	28	21	31	18	24	6.3	26%	20.7%
2012	OTO 96 - TF0712	5	-	20.7	7	38	32	32	31	45	32	30	44	22	34	7.2	21%	16.3%
					Total read	5	5	5	5	5	5	5	5	5			18.6%	1/1 1%
				Total	NOT read	0	0	0	0	0	0	0	0	0			10.078	14.178

Strait	of Sicily																	
	Sample	Fish	TL	SL	Landing	IFREMER	AZTI	IEO-ST	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAF	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	
2005	5-22	1	68	58.0	10	72		-	61	128	66	66	74	89	79	23.2	29%	20.9%
2005	5-30	2	81	69.0	10	108	-	-	92	164	95	95	92	83	104	27.4	26%	17.5%
2005	5-32	3	67	56.0	10	71	-	-	63	176	77	77	69	73	87	39.8	46%	29.6%
2005	5-47	4	69	58.0	10	66	-	-	76	147	-	58	82	71	83	32.3	39%	25.5%
2005	5-50	5	70	59.0	10	64		-	75	145	80	67	80	68	83	28.2	34%	21.6%
					Total read	5	0	0	5	5	4	5	5	5			24.0%	22.0%
				Total	NOT read	0	5	5	0	0	1	0	0	0			34.9%	23.0%

Adriat																		
	Sample	Fish	TL	SL	Landing	IFREMER	AZTI	IEO-ST	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	
1996	E149	1	55		3	65	-	-	69	-	86	85	86	60	75	11.9	16%	14.0%
1996	E481	2	26		9	36	30	22	37	39	41	41	44	18	34	9.0	26%	21.2%
1996	E666	3	46	39.0	12	65	64	69	70	74	83	78	74	48	69	10.1	14%	10.2%
1996	E685	4	35		12	54	48	53	53	55	58	59	68	48	55	6.1	11%	7.9%
1997	E696	5	75	63.0	1	83	-	-	80	-	118	128	111	81	100	21.3	21%	18.8%
1997	E807	6	66		2	85	-	-	89	-	98	104	103	67	91	14.0	15%	11.7%
2013	Ma 5	7	29	26.0	3	53	-	-	-	94	66	53	75	37	63	19.9	32%	24.3%
2013	Ma 17	8	32	28.0	3	-	36	-	-	67	73	77	73	33	60	19.9	33%	28.2%
2013	Ma 28	9	35	30.0	3	64	36	-	50	122	75	62	79	38	66	27.6	42%	29.9%
2013	Ma 57	10	40	34.0	3	89	-	-	72	125	117	80	119	48	93	28.7	31%	25.4%
2013	Ma 129	11	58	50.0	3	117	-	-	92	135	113	115	127	66	109	23.3	21%	15.8%
					Total read	10	5	3	9	8	11	11	11	11			04.0%	40.00/
				Total	NOT road	1	6	0	2	2	0	0	0	0			∠4.0%	18.9%

HOILI	Acgcunocu																	
	Sample	Fish	TL	SL	Landing	IFREMER	AZTI	IEO-ST	IEO-MA	CNR-IAMC	CNR-ISMAF	R CNR-ISMAR	RCNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	APEBF
2007	Ee_J_1-3	1	71	-	12	78	93	90	80	91	87	92	88	91	88	5.3	6%	4.6%
2007	Ee_J_1-23	2	53	-	12	70	81	81	76	77	78	76	77	72	76	3.6	5%	3.4%
2007	Ee_J_1-30	3	67	-	12	77	85	88	78	92	84	92	87	79	85	5.7	7%	5.4%
2007	Ee_J_1-32	4	59	-	12	71	81	79	73	82	75	83	82	76	78	4.3	6%	4.8%
2007	Ee_J_2-21	5	75	-	12	-	95	100	90	105	112	101	115	92	101	8.9	9%	6.9%
2007	Ee_L_11-1	6	-	30.5	7	32	33	33	33	39	33	35	34	37	34	2.3	7%	5.3%
2007	Ee_L_15-12	7	-	22.3	7	35	31	-	27	33	30	31	32	29	31	2.4	8%	5.5%
2007	Ee_L_S3-18	8	-	12.6	7	21	17	17	18	25	24	17	20	16	19	3.3	17%	13.7%
2007	Ee_L_S3-19	9	-	12.0	7	19	17	14	16	21	20	17	18	16	18	2.2	12%	9.8%
2007	Ee_L_S4-5	10	-	17.5	7	19	21	20	21	30	22	22	24	22	22	3.2	14%	8.9%
	Total r			Total read	9	10	9	10	10	10	10	10	10					
				Total	NOT read	1	0	1	0	0	0	0	0	0			9.0%	7.8%











**Figure 4.1.1.** ANCHOVY: Coefficient of variation (CV%), Average percent error (APE%) and standard deviation (SD) plotted against MEAN age, by sampling area.

All images of the Bay of Biscay, Western Mediterranean and North Aegean Sea were read by all readers. However, many images of Strait of Sicily and the Adriatic Sea sets remained unread (table 4.1.1). Some reasons for not reading these images were recorded by some of the readers, and were due to images calibration problems and the difficulty in identifying a growth pattern (unclear images). In these sets, the differences in the ages assigned by each reader are very large respect to the mean age (much greater than 10%). (Table 4.1.2 and Figure 4.1.2)

We look only at the clearer images sets (Bay of Biscay, Western Mediterranean and North Aegean Sea) to compare the results of each reader. In the Bay of Biscay set (larvae & juveniles) we found that 6 of the 9 readers have a significant agreement in the readings (the mean counts differ <10% from the mean age); R1 and R9 showed a small underestimation in their readings (between -12 and -15% relative to the mean) and R5 showed clear differences in the age interpretation criteria, over all in the older ages. Overestimation in fish age was observed in R5. In the Western Mediterranean Sea set (only larvae), the variability is higher among all readers. The differences are great in readers R5, R8 and R9, showing an overestimation in the readings of R5 and R8, and an underestimate in the reader R9. In North Aegean Sea set (larvae & juveniles), the differences are very small in the readings of all readers, showing a difference of less than 7% in 8 readers, resulting in a very high agreement among all readers in this area. The R5 has a difference of 13%, showing overestimation of younger ages (larvae). (Table 4.1.2 and Figure 4.1.2)

**Table 4.1.2.** ANCHOVY: Age differences (number of days and percentages) from the mean age bysampling area and reader.

Day UI D	iscay																	
Mean	IFRE	EMER	A	ZTI	IE	D-ST	IEC	D-MA	CNR	R-IAMC	CNR	ISMAR	CNR-	ISMAR	CNR-	ISMAR	HC	CMR
age	I	R1		R2		R3		R4		R5		R6		R7		R8	-	<b>R</b> 9
34	1	2%	-6	-18%	-5	-15%	2	5%	7	20%	2	5%	4	11%	6	18%	-9	-27%
34	-5	-15%	3	8%	4	11%	-3	-10%	10	28%	-2	-7%	1	2%	-1	-2%	-5	-15%
41	-5	-13%	5	12%	2	4%	3	7%	3	7%	-1	-3%	0	-1%	3	7%	-8	-20%
46	-10	-22%	-4	-9%	-1	-2%	0	0%	21	46%	-3	-7%	-1	-2%	1	2%	-3	-7%
50	-4	-8%	-1	-2%	-2	-4%	4	8%	13	26%	-2	-4%	3	6%	0	0%	-10	-20%
63	-7	-11%	-1	-1%	-2	-3%	-3	-4%	26	42%	-6	-9%	-1	-1%	-2	-3%	-6	-9%
80	-6	-8%	-5	-7%	2	2%	-4	-6%	32	39%	-8	-10%	9	11%	-2	-3%	-14	-18%
82	-11	-13%	-5	-6%	0	0%	-6	-7%	32	39%	-9	-11%	6	7%	5	6%	-12	-15%
82	-14	-17%	-2	-2%	1	1%	-4	-5%	46	56%	-5	-6%	-3	-4%	-8	-10%	-11	-13%
117	-13	-11%	-1	-1%	0	0%	-6	-5%	22	19%	-8	-7%	6	5%	9	8%	-8	-7%
Mean																		
days	-8	-12%	-2	-3%	0	-1%	-2	-2%	21	32%	-4	-6%	2	3%	1	2%	-9	-15%

#### Western Mediterranean

Mean	IFRE	EMER	A	ZTI	IEC	D-ST	IEC	D-MA	CNR	-IAMC	CNR-	ISMAR	CNR-	ISMAR	CNR-	ISMAR	HC	MR
age	1	R1	ŀ	R2	F	२३	F	R4	1	R5	-	R6		R7	F	र8	F	२9
21	-1	-3%	-1	-3%	-2	-8%	0	2%	4	21%	0	2%	-1	-3%	3	14%	-5	-22%
21	-3	-15%	-1	-5%	0	-1%	1	4%	6	28%	1	4%	-1	-5%	5	23%	-7	-34%
24	-1	-5%	-5	-22%	-6	-26%	1	3%	12	48%	4	15%	-3	-14%	7	27%	-6	-26%
30	-1	-4%	0	-1%	-4	-14%	-2	-8%	7	22%	5	15%	1	2%	4	12%	-7	-24%
34	4	12%	-2	-6%	-2	-6%	-3	-9%	11	32%	-2	-6%	-4	-12%	10	29%	-12	-35%
Mean																		
days	0	-3%	-2	-7%	-3	-11%	-1	-2%	8	<b>30%</b>	2	6%	-2	-6%	6	21%	-7	<b>-28%</b>

#### Strait of Sicily

Mean	IFRE	EMER	A	ZTI	IEC	)-ST	IEC	D-MA	CNF	R-IAMC	CNR-	ISMAR	CNR-	ISMAR	CNR-	ISMAR	HC	MR
age	F	R1	F	R2	F	3		R4		R5	-	R6		R7	F	R8	F	R9
79	-7	-9%	-	-	-	-	-18	-23%	49	61%	-13	-17%	-13	-17%	-5	-7%	10	12%
83	-19	-23%	-	-	-	-	-8	-9%	62	75%	-3	-3%	-16	-19%	-3	-4%	-15	-18%
83	-17	-21%	-	-	-	-	-7	-9%	64	77%	-	-	-25	-30%	-2	-2%	-12	-15%
87	-16	-18%	-	-	-	-	-24	-27%	90	103%	-10	-11%	-10	-11%	-18	-21%	-14	-16%
104	4	4%	-	-	-	-	-12	-12%	60	57%	-9	-9%	-9	-9%	-12	-12%	-21	-20%
Mean																		
days	-11	-13%	-	-	- 1	-	-14	-16%	65	75%	-9	-10%	-15	-17%	-8	-9%	-10	-11%

#### Adriatic Sea

Mean	IFRE	EMER	A	ZTI	IEC	D-ST	IEC	-MA	CNR	-IAMC	CNR-	ISMAR	CNR-	ISMAR	CNR-	SMAR	HC	MR
age	F	٦1	F	R2	F	२३	F	R4	ŀ	R5	F	R6	F	R7	F	88	F	R9
34	2	5%	-4	-12%	-12	-36%	3	8%	5	14%	7	20%	7	20%	10	29%	-16	-47%
55	-1	-2%	-7	-13%	-2	-4%	-2	-4%	0	0%	3	5%	4	7%	13	23%	-7	-13%
60	-	-	-24	-	-	-	-	-	7	-	13	22%	17	29%	13	22%	-27	-45%
63	-10	-16%	-	-	-	-	-	-	31	-	3	5%	-10	-16%	12	19%	-26	-41%
66	-2	-3%	-30	-45%	-	-	-16	-24%	56	86%	9	14%	-4	-6%	13	20%	-28	-42%
69	-4	-6%	-5	-8%	0	-1%	1	1%	5	7%	14	20%	9	12%	5	7%	-21	-31%
75	-10	-14%	-	-	-	-	-6	-8%	-	-	11	14%	10	13%	11	14%	-15	-20%
91	-6	-7%	-	-	-	-	-2	-2%	-	-	7	8%	13	14%	12	13%	-24	-26%
93	-4	-4%	-	-	-	-	-21	-22%	32	35%	24	26%	-13	-14%	26	28%	-45	-48%
100	-17	-17%	-	-	-	-	-20	-20%	-	-	18	18%	28	28%	11	11%	-19	-19%
109	8	7%	-	-	-	-	-17	-16%	26	24%	4	3%	6	5%	18	16%	-43	-40%
Mean																		
days	-5	-6%	-14	<b>-20%</b>	-5	-13%	-9	-10%	20	27%	10	14%	6	8%	13	18%	-25	-34%

#### North Aegean Sea CNR-ISMAR CNR-ISMAR CNR-ISMAR IFREMER AZTI IEO-ST IEO-MA CNR-IAMC HCMR Mean R3 age R1 R2 R4 R5 R6 R7 R8 R9 8% -3% -20% -9% 20% 14% -3% 3% -9% 18 -4 -2 2 0 -2 1 -1 3 -1 19 2 8% -12% -2 -12% -7% 29% 5 24% -2 -12% 0 1% -3 -17% -2 -1 6 22 31 -3 -15% -1 -6% -2 -10% -1 -6% 8 35% 0 -1% 0 -1% 5% 0 -1% 1 -2 3 4 13% 0 0% --4 -13% 2 7% -1 -3% 0 0% 2% -6% 1 34 76 78 -1 0 -1 -4% -2 -7% -1 -4% -4% 5 14% -1 -4% 1 2% -1 -2% 8% -6 -7 -8% 5 6% 5 6% -1% 1 1% 2 2% 0 -1% 1 1% -4 -2 -6% -3 -2% -9% 3 4% 1 1% -5 -6% 5% -4% 5 6% 4 5% 4 -6 3 85 2 -8 -9% 0 0% 3 4% -7 7 9% -1% 9% -8% -1 7 2% -7% 88 -10 -11% 5 6% 2 3% -8 -9% 3 4% -1 -1% 4 5% 0 0% 4% 101 -6 -6% -1 -1% -11 -11% 4 4% 11 11% 0 0% 13 13% -9 -9% -Mean -4 1 2 -2 -3 -3% 0 -1% 0 -4% -7% 4 13% 1 4% 0% 3% -5% days



**Figure 4.1.2.** ANCHOVY: Age differences in number of days (left) and percentage (right) from the mean age by sampling area and reader.

We compared the increment width reported by anchovy reader R2, R3, R4, R5, R7 and R9 (the other readers not recorded the increments width), in order to know the readings interpretation from each reader in larvae and juveniles. For this, we selected image otolith readings with high level of precision in the age determination in the Bay of Biscay (larvae and juvenile), Western Mediterranean (larvae) and North West Aegean (larvae and juvenile) sets. (Figures 4.1.3 to 4.1.4) In the Bay of Biscay, all readers appeared to apply the same reading criteria, except the reader R9 in larvae and the reader R5 in juveniles. R9 overestimated the increments width (thus

underestimating the age) in the sample of larvae (code m83-18; SL= 30.6 mm; Ageing precision= 10%CV and 8%APE); R5 perhaps have applied the IMR criteria (individual increments) up to 66 days, and from there applied the GBR criteria (group bands) in the sample of juveniles (codePO7\_15\_18; SL= 106.0 mm; Ageing precision= 9%CV and 7%APE), overestimating the age (Figure 4.1.3).



**Figure 4.1.3**. ANCHOVY. Comparison of the increments width results (by reader) from larvae (top panel) and from juveniles (bottom panel) of Bay of Biscay. The sample code, fish length (mm) and ageing precision (CV&APE) are indicated.

In the sample of the Western Mediterranean (Code: OTO118b-TF0712; SL= 13.3 mm; Ageing precision= 12% CV and 8.7%APE), there was greater variability in the increments width of larvae among readers. Again, the R9 overestimated the increments width underestimating the age of larvae. (Figure 4.1.4).



**Figure 4.1.4** ANCHOVY. Comparison of the increments width results (by reader) from larvae of the Western Mediterranean. The sample code, fish length (mm) and ageing precision (CV&APE) are indicated.

In the samples of the North Aegean Sea, high increment width variability was observed in larvae (Code sample: Ee\_L\_11-1; SL= 30.5 mm; Ageing precision= 7% CV and 5.3%APE) among all readers, although they appeared to use the same ageing criteria. The same results and argumentations can be made for juvenile samples (Code sample: Ee\_J\_1-3; SL= 71 mm; Ageing precision= 6% CV and 4%APE). The largest width increments reported by the reader R5 probably could be explained more by a calibration problem than to the real assignment of increment (Figure 4.1.5).



**Figure 4.1.5.** ANCHOVY. Comparison of the increments width results (by reader) from larvae (top panel) and juveniles (bottom panel) of the North Aegean Sea. The sample code, fish length (mm) and ageing precision (CV&APE) are indicated.

#### 4.1.2 Total areas

When we consider all areas together, the exchange showed differences among reader, with a CV of 18% and a APE of 13.8 % (Table 4.1.3). Showed a great variability among ages, not being any trend. The CV was smaller in the readings of the larvae (up to 55 days), although there are also large fluctuations. (Figure 4.1.6)

	Sample	Fish	TL	SL	Landing	IFREMER	AZTI	IEO-ST	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	
2005	m41_2	1	-	20.4	8	35	28	29	36	41	36	38	41	25	34	5.7	17%	13.5%
2005	m83_10	2	-	25.2	8	29	37	38	31	44	32	35	34	29	34	4.9	14%	10.9%
2005	m81_5	3	-	28.3	8	36	42	45	46	67	43	45	47	43	46	8.5	18%	10.6%
2005	m83_18	4	-	30.6	8	36	46	43	44	44	40	41	44	33	41	4.3	10%	8.0%
2005	DOT 15 19	0	- 121	34.7	8	40	49	48	04 111	120	48	23	5U 127	40	3U 117	0.3	13%	8.7%
2007	P09_09_43	7	57	50.0	9	56	62	61	60	89	57	62	61	57	63	10.3	16%	9.3%
2009	P09 17 68	8	74	62.0	9	74	75	82	76	112	72	89	78	66	80	13.5	17%	11.5%
2009	D09_94_05	9	100	86.0	10	71	77	82	76	114	73	88	87	70	82	13.6	17%	11.6%
2009	D09_94_31	10	91.3	78.0	10	68	80	83	78	128	77	79	74	71	82	17.9	22%	12.7%
2011	OTO 362-TF0811	1	-	19.9	8	29	30	26	28	37	35	31	34	23	30	4.5	15%	11.5%
2012	OTO 118a-TF0712	2	-	13.3	7	18	20	21	22	27	22	20	26	14	21	3.9	19%	13.2%
2012	OTO 226 - TE0712	3	-	14.7	7	20	19	19	25	20	21	20	24	18	21	6.3	26%	20.7%
2012	OTO 96 - TF0712	5	-	20.7	7	38	32	32	31	45	32	30	44	22	34	7.2	21%	16.3%
2005	5-22	1	68	58.0	10	72	-	-	61	128	66	66	74	89	79	23.2	29%	20.9%
2005	5-30	2	81	69.0	10	108	-	-	92	164	95	95	92	83	104	27.4	26%	17.5%
2005	5-32	3	67	56.0	10	71	-	-	63	176	77	77	69	73	87	39.8	46%	29.6%
2005	5-47	4	69	58.0	10	66	-	-	76	147	-	58	82	71	83	32.3	39%	25.5%
2005	5-50 E140	5	70	59.0	10	65	-	-	75	145	80	6/	80	68	83	28.2	34%	21.6%
1996	E 143	2	26		9	36	30	22	37	39	41	41	44	18	34	9.0	26%	21.2%
1996	E666	3	46	39.0	12	65	64	69	70	74	83	78	74	48	69	10.1	14%	10.2%
1996	E685	4	35		12	54	48	53	53	55	58	59	68	48	55	6.1	11%	7.9%
1997	E696	5	75	63.0	1	83	-	-	80	-	118	128	111	81	100	21.3	21%	18.8%
1997	E807	6	66		2	85	-	-	89	-	98	104	103	67	91	14.0	15%	11.7%
2013	Ma 5	7	29	26.0	3	53	-	-	-	94	66	53	75	37	63	19.9	32%	24.3%
2013	Ma 17 Ma 28	8	32	28.0	3	- 64	36	-	- 50	122	73	62	73	33	66	19.9 27.6	33%	28.2%
2013	Ma 57	10	40	34.0	3	89	-	-	72	125	117	80	119	48	93	28.7	31%	25.4%
2013	Ma 129	11	58	50.0	3	117	-	-	92	135	113	115	127	66	109	23.3	21%	15.8%
2007	Ee_J_1-3	1	71	-	12	78	93	90	80	91	87	92	88	91	88	5.3	6%	4.6%
2007	Ee_J_1-23	2	53	-	12	70	81	81	76	77	78	76	77	72	76	3.6	5%	3.4%
2007	Ee_J_1-30	3	67	-	12	77	85	88	78	92	84	92	87	79	85	5.7	7%	5.4%
2007	Ee_J_1-32	4	59	-	12	71	81	79	73	82	/5	83	82	76	78	4.3	6% 0%	4.8%
2007	Ee_J_2-21 Fe I_11-1	5 6	/5	30.5	12	- 32	33	33	33	39	33	35	34	92 37	34	2.3	9%	5.3%
2007	Ee L 15-12	7	-	22.3	7	35	31	-	27	33	30	31	32	29	31	2.4	8%	5.5%
2007	Ee_L_S3-18	8	-	12.6	7	21	17	17	18	25	24	17	20	16	19	3.3	17%	13.7%
2007	Ee_L_S3-19	9	-	12.0	7	19	17	14	16	21	20	17	18	16	18	2.2	12%	9.8%
2007	Ee_L_S4-5	10	-	17.5	7	19	21	20	21	30	22	22	24	22	22	3.2	14%	8.9%
					Total read	39	30	27	39	38	40	41	41	41			18.9%	13.8%
			ļ	Total	NOT read	2	11	14	2	3	1	0	0	0	ļ		10.070	10.070
			<b>SD</b> 60.0	)				An —	chovy sd	<b>/-All ard</b>	PEBF				CV & AI	Æ		
			50.0 40.0	)   )						Λ					- 50% - 40%			
			30.0		^						$\land$			$\wedge$	- 30%			
			10.0	, 🖍	$\swarrow$	$\bigvee$	Z	$\wedge$	<b>y</b> ♥	4	JY	<u> /                                   </u>	V	$\square$	10%			
			0.0	18 3	21 22	30 34	34 34	46	55 63 Mear	66 75 n Age (days)	78 80 8	2 83 87	91 100	104 11	⊥ 0% L7			

Table 4.1.3 ANCHOVY Otolith SET (WKMIAS 2013\_Otolith Exchange)



For all areas, we found that 7 of the 9 readers have a significant agreement in the readings (the mean counts differ <8% from the mean age); R9 showed a underestimation in their readings (-19% relative to the mean) and R5 showed clear differences in the age interpretation criteria in all ages.(Table 4.1.4 and Figure 4.1.7)

**Table 4.1.4.** ANCHOVY: Age differences (number of days and percentages) from the mean age by reader, for all areas.

Mean	IFRI	EMER	A	ZTI	IE	O-ST	IEC	D-MA	CNF	R-IAMC	CNR-	ISMAR	CNR-	ISMAR	CNR-	ISMAR	H	CMR
age	I	R1		R2		R3		R4		R5	1	R6		R7	1	R8		<b>R</b> 9
18	1	8%	-1	-3%	-4	-20%	-2	-9%	3	20%	-	-	-1	-3%	0	3%	-2	-9%
19	2	8%	-2	-12%	-2	-12%	-1	-7%	6	29%	5	24%	-2	-12%	0	1%	-3	-17%
21	-1	-3%	-1	-3%	-2	-8%	0	2%	4	21%	0	2%	-1	-3%	3	14%	-5	-22%
21	-3	-15%	-1	-5%	0	-1%	1	4%	6	28%	1	4%	-1	-5%	5	23%	-7	-34%
22	-3	-15%	-1	-6%	-2	-10%	-1	-6%	8	35%	0	-1%	0	-1%	1	5%	0	-1%
24	-1	-5%	-5	-22%	-6	-26%	1	3%	12	48%	4	15%	-3	-14%	7	27%	-6	-26%
30	-1	-4%	0	-1%	-4	-14%	-2	-8%	7	22%	5	15%	1	2%	4	12%	-7	-24%
31	4	13%	0	0%		-	-4	-13%	2	7%	-1	-3%	0	0%	1	2%	-2	-6%
34	4	12%	-2	-6%	-2	-6%	-3	-9%	11	32%	-2	-6%	-4	-12%	10	29%	-12	-35%
34	2	5%	-4	-12%	-12	-36%	3	8%	5	14%	7	20%	7	20%	10	29%	-16	-47%
34	1	2%	-6	-18%	-5	-15%	2	5%	7	20%	2	5%	4	11%	6	18%	-9	-27%
34	-5	-15%	3	8%	4	11%	-3	-10%	10	28%	-2	-7%	1	2%	-1	-2%	-5	-15%
34	-2	-7%	-1	-4%	-1	-4%	-1	-4%	5	14%	-1	-4%	1	2%	-1	-2%	3	8%
41	-5	-13%	5	12%	2	4%	3	7%	3	7%	-1	-3%	0	-1%	3	7%	-8	-20%
46	-10	-22%	-4	-9%	-1	-2%	0	0%	21	46%	-3	-1%	-1	-2%		2%	-3	-7%
50	-4	-8%	-1	-2%	-2	-4%	4	8%	13	26%	-2	-4%	3	6%	0	0%	-10	-20%
55	-1	-2%	-/	-13%	-2	-4%	-2	-4%	0	0%	3	5%	4	7%	13	23%	-/	-13%
60	- 7	-	-24	-40%	-	-	-	-		12%	13	22%		29%	13	22%	-21	-45%
63	-7	-11%	-1	-1%	-2	-3%	-3	-4%	20	42%	-0	-9%	-1	-1%	-2	-3%	-0	-9%
63	-10	-10%	- 20	-	-	-	16	-	50	49%	- 0	0% 1.40/	-10	-10%	12	19%	-20	-41%
60	-2	-3 %	-30	-43%	-	-19/-	-10	-24%	50	70/	9	20%	-4	-0%	5	20%	-20	-42 %
75	-4	-078	-5	-076	0	-1 /0	-6	-8%	5	1 /0	14	1/1%	10	12 /0	11	1/%	-15	-20%
76	-6	-8%	5	6%	5	6%	0	-1%	1	1%	2	2%		-1%		1%	-10	-20%
78	-7	-9%	3	4%	1	1%	-5	-6%	4	5%	-3	-4%	5	6%	4	5%	-2	-2%
79	-7	-9%	-	-	1	-	-18	-23%	49	61%	-13	-17%	-13	-17%	-5	-7%	10	12%
80	-6	-8%	-5	-7%	2	2%	-4	-6%	32	39%	-8	-10%	9	11%	-2	-3%	-14	-18%
82	-11	-13%	-5	-6%	0	0%	-6	-7%	32	39%	-9	-11%	6	7%	5	6%	-12	-15%
82	-14	-17%	-2	-2%	1	1%	-4	-5%	46	56%	-5	-6%	-3	-4%	-8	-10%	-11	-13%
83	-19	-23%	-	-	-	-	-8	-9%	62	75%	-3	-3%	-16	-19%	-3	-4%	-15	-18%
83	-17	-21%	-	-	-	-	-7	-9%	64	77%	-	-	-25	-30%	-2	-2%	-12	-15%
85	-8	-9%	0	0%	3	4%	-7	-8%	7	9%	-1	-1%	7	9%	2	2%	-6	-7%
87	-16	-18%	-	-	-	-	-24	-27%	90	103%	-10	-11%	-10	-11%	-18	-21%	-14	-16%
88	-10	-11%	5	6%	2	3%	-8	-9%	3	4%	-1	-1%	4	5%	0	0%	3	4%
91	-6	-7%	-	-	-	-	-2	-2%	-	-	7	8%	13	14%	12	13%	-24	-26%
93	-4	-4%	-	-	-	-	-21	-22%	32	35%	24	26%	-13	-14%	26	28%	-45	-48%
100	-17	-17%	-	-	-	-	-20	-20%	-	-	18	18%	28	28%	11	11%	-19	-19%
101	-	-	-6	-6%	-1	-1%	-11	-11%	4	4%	11	11%	0	0%	13	13%	-9	-9%
104	4	4%	-	-	-	-	-12	-12%	60	57%	-9	-9%	-9	-9%	-12	-12%	-21	-20%
109	8	7%		-	- 1	-	-17	-16%	26	24%	4	3%	6	5%	18	16%	-43	-40%
117	-13	-11%	-1	-1%	0	0%	-6	-5%	22	19%	-8	-7%	6	5%	9	8%	-8	-7%
Mean days	-5	-7%	-3	-7%	-1	-5%	-5	-7%	20	32%	1	3%	1	0%	4	8%	-11	-19%



**Figure 4.1.7.** ANCHOVY: Age differences in number of days (left) and percentage (right) from the mean age by reader, for all areas.

#### **4.2 SARDINE results**

## 4.2.1. By areas

Table 4.2.1 details length and month of landing of the sets of otoliths images selected for the sardine exchange programme by areas along with the ageing produced by each reader. The last four columns give mean daily age, standard deviation (SD) and precision of reading as the CV in relation to the average age and the  $\ensuremath{\mathsf{APE}_{\mathsf{BF}}}$  .

#### Table 4.2.1 SARDINE Otolith SET (WKMIAS 2013\_Otolith Exchange)

Bay	~ 1	Р		
Бау	oı	D	150	a

	Sample	FISH	1L	3L	Landing	IFREIVIER	IEO-VI	IP IVIA	IEO-IVIA	CINK-IMIVIC	CINK-ISIVIAR	CINK-ISIMAR	CINK-ISIVIAR	HUIWIR	wean		Frecision	Frecision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	
2012	St 483 - 07	1	20.45	19.5	4	26	23	25	19	18	23	27	22	26	23	3.2	14%	10.6%
2012	St 483 - 17	2	19.85	18.2	4	27	20	27	17	20	22	20	19	23	22	3.5	16%	12.6%
2012	St 533 - 11	3	21.06	19.7	5	25	22	21	18	23	20	22	22	22	22	1.9	9%	6.2%
2012	St 533 - 14	4	23.18	21.7	5	31	27	28	24	25	17	25	16	28	25	5.0	20%	15.1%
2012	St 551 - 14	5	18.79	17.1	5	23	23	21	16	27	23	22	20	23	22	3.0	13%	9.1%
				Т	fotal read	5	5	5	5	5	5	5	5	5			14 5%	10.7%
				Total I	NOT read	0	0	0	0	0	0	0	0	0			14.37	10.7 %

Atlantic	c Iberian																	
	Sample	Fish	TL	SL	Landing	IFREMER	IEO-VI	IPMA	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	
2010	sc_040810_6_1	1	67	54.2	8	69	68	113	93	76	-	71	77	67	79	16.0	20%	15.0%
2010	sc_180810_6_1	2	85	72.0	8	78	81	91	120	92	86	85	80	84	89	12.7	14%	9.4%
2010	sc_060910_2_1	3	82	67.0	9	88	89	123	119	108	95	90	96	90	100	13.5	14%	11.3%
2010	sc_210910_4_1	4	90	77.0	9	100	104	116	117	106	128	113	128	109	113	9.8	9%	6.8%
2010	sc_041110_3_2	5	109	92.0	11	116	147	130	150	130	124	119	147	111	130	14.5	11%	8.9%
2008	21_10_08_33_200x	6	160	136.0	10	185	278	257	214	162	252	251	293	123	224	56.9	25%	21.0%
2009	16_04_09_01_200x	7	144	123.0	4	217	222	267	255	151	237	175	265	150	215	46.4	22%	17.6%
2004	20_07_04_71_200x	8	138	117.0	7	136	214	189	175	160	290	177	184	121	183	48.9	27%	17.7%
2004	27_05_04_07_200x	9	84	71.0	5	108	168	116	122	116	124	120	119	116	123	17.4	14%	8.2%
2005	10_01_05_02_200x	10	99	84.0	1	141	154	185	138	144	213	135	221	104	159	38.7	24%	19.6%
-				1	Fotal read	10	10	10	10	10	9	10	10	10			40.00/	40.5%
				Total	NOT read	0	0	0	0	0	1	0	0	0			18.0%	13.5%

Wester	n Mediterranean																	
	Sample	Fish	TL	SL	Landing	IFREMER	IEO-VI	IPMA	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	
2010	OTO 18-TF1110	1	-	21.4	10	31	28	29	30	35	28	30	30	30	30	2.1	7%	4.3%
2010	OTO 563-TF1110	2	-	15.0	11	17	17	22	16	20	19	18	24	16	19	2.7	14%	11.4%
2010	OTO 497-TF1110	3	-	16.6	11	16	17	17	18	25	15	17	19	17	18	2.9	16%	10.4%
2010	OTO 492-TF1110	4	-	19.7	11	19	18	20	26	27	20	19	22	17	21	3.5	17%	13.1%
2010	OTO 385-TF1110	5	-	22.9	10	33	36	34	32	37	35	34	36	33	34	1.6	5%	3.8%
	· · · · · ·			1	Fotal read	5	5	5	5	5	5	5	5	5			11 7%	9.6%
				Total	NOT read	0	0	0	0	0	0	0	0	0			11.7 /0	0.070

	Sample	Fish	TL	SL	Landing	IFREMER	IEO-VI	IPMA	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	
2013	Ms 72	1	36	32.0	3	51	-	59	64	63	63	72	68	46	61	8.6	14%	10.8%
2013	Ms 82	2	46	39.0	3	84	97	85	96	98	96	92	101	86	93	6.3	7%	5.8%
2013	Ms 121	3	39	34.0	3	62	64	69	65	68	69	68	67	58	66	3.7	6%	4.5%
2013	Ms 151	4	25	22.0	3	36	41	44	50	54	41	40	42	34	42	6.3	15%	10.8%
2013	Ms 174	5	15	14.0	3	18	20	19	21	19	18	19	21	19	19	1.1	6%	4.6%
2013	Ms 201	6	30	26.0	3	58	-	45	63	61	53	62	59	42	55	8.0	14%	11.8%
2013	Ms 231	7	43	37.0	3	75	-	96	106	96	107	109	111	80	98	13.6	14%	11.0%
1997	Sa 75	8	56		1	69	-	84	74	73	99	93	95	75	83	11.6	14%	12.1%
1997	Sa 287	9	80		5	121	-	130	167	80	211	203	194	154	157	45.4	29%	23.0%
1997	Sa 298	10	65		5	97	153	-	147	100	162	153	164	85	133	32.7	25%	21.8%
					Total read	10	5	9	10	10	10	10	10	10			44.00/	44.00/
				Total	NOT read	0	5	1	0	0	0	0	0	0			14.3%	11.6%

	Sample	Fish	TL	SL	Landing	IFREMER	IEO-VI	IPMA	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	
2007	Sp_J_5_7	1	80	-	7	100	98	124	107	107	118	128	126	134	116	13.1	11%	9.8%
2007	Sp_J_10_8	2	76	-	7	127	128	140	119	134	160	155	128	163	139	16.1	12%	9.7%
2007	Sp_J_10_9	3	59	-	7	126	158	133	117	125	134	151	129	141	135	13.1	10%	7.5%
2007	Sp_J_11_7	4	64	-	7	101	149	163	107	149	140	165	141	151	141	22.5	16%	11.7%
2007	Sp_J_12_22	5	60	-	7	108	137	136	111	134	135	148	129	147	132	14.0	11%	7.9%
2009	Sp_L_2-3	6	-	34.4	2	59	65	65	66	63	62	66	66	66	64	2.4	4%	3.0%
2009	Sp_L_2-18	7	-	35.4	2	64	62	68	61	61	55	63	61	66	62	3.7	6%	4.2%
2009	Sp_L_3-15	8	-	21.2	2	28	29	32	31	33	24	28	30	31	30	2.7	9%	6.9%
2009	Sp_L_3-17	9	-	20.3	2	28	29	25	30	28	25	29	32	30	28	2.3	8%	6.1%
2009	Sp_L_19-16	10	-	25.5	2	34	36	30	37	32	34	39	38	37	35	2.9	8%	6.7%
				1	Total read	10	10	10	10	10	10	10	10	10			0.49/	7 40/
				Total	NOT read	0	0	0	0	0	0	0	0	0			9.4%	1.4%

This exchange showed differences among readers, particularly in older ages. The lowest differences were found in the set of the North Aegean Sea (CV = 9.4% and APE = 7.4%) and the highest in the set of Atlantic Iberian (CV = 18% and APE = 13.5%) (Figure 4.2.1). The differences were lower in the sardine compared to anchovy in all areas, except in the North Aegean Sea. In this area, the differences are similar to those of anchovy, being the area with the best ageing precision in the two species.

All images of all areas were read by all readers, except a reader who did not read half of the images of the Adriatic Sea set (Table 4.2.1). In general, in larvae the age differences respect to the mean age is less than 10% for almost all readers and in all areas, except in the sample from the Bay of Biscay, where the ageing precision is lower. In this area, the reader R1 has a difference of 17%, overestimating at young ages (larvae) and readers R4 & R8 underestimating them (-17% and -12% respectively). In the Western Mediterranean set, all readers have a difference of less than 9% from the mean age of larvae, but the reader R5 overestimating the age of the larvae (an average difference of 20%). In the case of juveniles the differences are higher (greater than 30% in some cases) among readers and areas, and especially in the set of Atlantic Iberian (Table 4.2.2 and Figure 4.2.2).





0%

10.0



**Figure 4.2.1.** SARDINE: Coefficient of variation (CV%), Average percent error (APE%) and standard deviation (SD) plotted against MEAN age, by sampling areas.

**Table 4.2.2.** SARDINE: Age differences (number of days and percentages) from the mean age by sampling area and reader.

Bay of Bi	iscay																	
Mean	IFR	EMER	IE	O-VI	IF	PMA	IEC	D-MA	CNR	-IAMC	CNR-	ISMAR	CNR-	ISMAR	CNR-	ISMAR	HC	CMR
age		R1		R2		R3		R4		R5		R6		R7		R8		R9
22	5	25%	-2	-8%	5	25%	-5	-22%	-2	-8%	0	2%	-2	-8%	-3	-12%	1	6%
22	3	15%	0	2%	-1	-3%	-4	-17%	1	6%	-2	-8%	0	2%	0	2%	0	2%
22	1	5%	1	5%	-1	-5%	-6	-27%	5	23%	1	5%	0	0%	-2	-9%	1	5%
23	3	12%	0	-1%	2	8%	-4	-18%	-5	-22%	0	-1%	4	16%	-1	-5%	3	12%
25	6	26%	2	10%	3	14%	-1	-2%	0	2%	-8	-31%	0	2%	-9	-35%	3	14%
Mean																		
days	4	17%	0	1%	2	8%	-4	-17%	0	0%	-2	-7%	1	2%	-3	-12%	2	8%

Atlantic Iberian

Mean	IFRE	EMER	IE	O-VI	IP	MA	IEC	-MA	CNR	-IAMC	CNR-I	SMAR	CNR-	ISMAR	CNR-	ISMAR	HC	MR
age	F	R1		R2	F	R3	F	R4	F	R5	F	86	F	R7	F	R8	F	R9
79	-10	-13%	-11	-14%	34	43%	14	17%	-3	-4%	-	-	-8	-10%	-3	-3%	-12	-15%
89	-11	-12%	-8	-8%	3	3%	32	36%	4	4%	-3	-3%	-4	-4%	-9	-10%	-5	-5%
100	-12	-12%	-11	-11%	23	23%	19	19%	8	8%	-5	-5%	-10	-10%	-4	-4%	-10	-10%
113	-13	-12%	-9	-8%	3	2%	4	3%	-7	-7%	15	13%	0	0%	14	12%	-4	-4%
123	-15	-12%	45	36%	-7	-6%	-1	-1%	-7	-6%	1	1%	-3	-3%	-4	-3%	-7	-6%
130	-14	-11%	17	13%	0	0%	20	15%	0	0%	-6	-5%	-11	-9%	16	12%	-19	-15%
159	-18	-12%	-5	-3%	26	16%	-21	-13%	-15	-10%	54	34%	-24	-15%	61	38%	-55	-35%
183	-47	-26%	31	17%	6	3%	-8	-4%	-23	-13%	107	59%	-6	-3%	1	1%	-62	-34%
215	2	1%	7	3%	52	24%	40	18%	-64	-30%	22	10%	-40	-19%	50	23%	-65	-30%
224	-39	-17%	54	24%	33	15%	-10	-4%	-62	-28%	28	13%	27	12%	69	31%	-101	-45%
Mean																		
days	-18	-13%	11	5%	17	12%	9	9%	-17	-8%	24	13%	-8	-6%	19	10%	-34	-20%

#### Western Mediterranean

Mean	IFRE	EMER	IE	O-VI	IP	PMA	IEC	D-MA	CNF	R-IAMC	CNR-	ISMAR	CNR-	ISMAR	CNR	ISMAR	H	CMR
age	I	R1		R2	1	R3		R4		R5	1	R6	F	R7		R8		R9
18	-2	-11%	-1	-5%	-1	-5%	0	1%	7	40%	-3	-16%	-1	-5%	1	6%	-1	-5%
19	-2	-9%	-2	-9%	3	18%	-3	-15%	1	7%	0	1%	-1	-4%	5	26%	-3	-15%
21	-2	-9%	-3	-14%	-1	-4%	5	24%	6	29%	-1	-4%	-2	-9%	1	5%	-4	-19%
30	1	3%	-2	-7%	-1	-4%	0	0%	5	16%	-2	-7%	0	0%	0	0%	0	0%
34	-1	-4%	2	5%	0	-1%	-2	-7%	3	8%	1	2%	0	-1%	1	3%	-1	-4%
Mean																		
days	-1	-6%	-1	-6%	0	1%	0	1%	4	<b>20%</b>	-1	-5%	-1	-4%	2	8%	-2	<b>-9%</b>

Mean	IFRE	EMER	IE	O-VI	IP	MA	IEC	D-MA	CNR	-IAMC	CNR-	ISMAR	CNR-	ISMAR	CNR-	ISMAR	HC	CMR
age	I	R1	F	R2	F	3	I	R4	F	R5	F	R6	1	R7	F	78	1	39
19	-1	-7%	1	3%	0	-2%	2	9%	0	-2%	-1	-7%	0	-2%	2	9%	0	-2%
42	-6	-15%	-1	-3%	2	4%	8	18%	12	27%	-1	-3%	-2	-6%	0	-1%	-8	-20%
55	3	5%	-	-	-10	-19%	8	14%	6	10%	-2	-4%	7	12%	4	7%	-13	-24%
61	-10	-16%	-	-	-2	-3%	3	5%	2	4%	2	4%	11	19%	7	12%	-15	-24%
66	-4	-5%	-2	-2%	3	5%	-1	-1%	2	4%	3	5%	2	4%	1	2%	-8	-12%
83	-14	-17%	-	-	1	2%	-9	-11%	-10	-12%	16	20%	10	12%	12	15%	-8	-9%
93	-9	-9%	4	5%	-8	-8%	3	3%	5	6%	3	3%	-1	-1%	8	9%	-7	-7%
98	-23	-23%	-	-	-2	-2%	9	9%	-2	-2%	10	10%	12	12%	14	14%	-18	-18%
133	-36	-27%	20	15%	-	-	14	11%	-33	-25%	29	22%	20	15%	31	24%	-48	-36%
157	-36	-23%	-	-	-27	-17%	10	6%	-77	-49%	54	34%	46	29%	36	23%	-3	-2%
Mean																		
days	-14	-14%	4	4%	-5	-4%	5	6%	-9	-4%	11	8%	10	9%	11	11%	-13	-15%

North A	egean	Sea																
Mean	IFRE	EMER	IE	O-VI	IF	PMA	IEC	D-MA	CNR	-IAMC	CNR	ISMAR	CNR-	ISMAR	CNR-	ISMAR	HC	MR
age		R1	F	R2		R3	1	R4	F	R5		R6	ŀ	R7	F	78	ŀ	२9
28	0	-2%	1	2%	-3	-12%	2	5%	0	-2%	-3	-12%	1	2%	4	13%	2	5%
30	-2	-5%	-1	-2%	2	8%	1	5%	3	12%	-6	-19%	-2	-5%	0	2%	1	5%
35	-1	-3%	1	2%	-5	-15%	2	5%	-3	-9%	-1	-3%	4	11%	2	7%	2	5%
62	2	3%	0	-1%	6	9%	-1	-2%	-1	-2%	-7	-12%	1	1%	-1	-2%	4	6%
64	-5	-8%	1	1%	1	1%	2	3%	-1	-2%	-2	-3%	2	3%	2	3%	2	3%
116	-16	-14%	-18	-15%	8	7%	-9	-8%	-9	-8%	2	2%	12	11%	10	9%	18	16%
132	-24	-18%	5	4%	4	3%	-21	-16%	2	2%	3	3%	16	12%	-3	-2%	15	12%
135	-9	-7%	23	17%	-2	-1%	-18	-13%	-10	-7%	-1	-1%	16	12%	-6	-4%	6	5%
139	-12	-9%	-11	-8%	1	0%	-20	-15%	-5	-4%	21	15%	16	11%	-11	-8%	24	17%
141	-40	-28%	8	6%	22	16%	-34	-24%	8	6%	-1	0%	24	17%	0	0%	10	7%
Mean																		
days	-11	-9%	1	1%	3	2%	-10	-6%	-2	-1%	0	-3%	9	7%	0	2%	8	8%



**Figure 4.2.2.** SARDINE: Age differences in number of days (left) and percentage (right) from the mean age by sampling areas and reader.

We compared the increment width reported by sardine readers R2, R3, R4, R5, R7 and R9 (the other readers not recorded the increments width), in order to know the readings interpretation from each reader in larvae and juveniles. For this, we choose the image otolith readings with high level of precision in the age determination of the all sets (Figures 4.2.3 to 4.2.6). In the larvae samples of the Bay of Biscay (Code sample: St 533 – 11; SL= 19.69 mm; Ageing precision: 9%CV and 6.2%APE) and Mediterranean Sea (Code sample: OTO 385-TF1110; SL= 22.9 mm; Ageing precision: 5%CV and 3.8%APE), all readers seem to apply the same reading criteria in the larvae, although it is noted great variability in the increments width among readers, especially in the first

increments after hatching. The reader R2 also shows great variability in the remaining increments width (Figure 4.2.3)



**Figure 4.2.3**. SARDINE. Comparison of the increments width results (by reader) from larvae of Bay of Biscay (top panel) and Western Mediterranean Sea (bottom panel). The sample code, fish length (mm) and ageing precision (CV&APE) are indicated.

In the juveniles sample of the Atlantic Iberian, high variability is observed in the increments width assigned by reader R2, R3 & R5 (Code sample: 27\_05\_04\_07\_200x SL= 71 mm; Ageing precision= 9%CV and 6.8%APE), and the other readers (R4, R7 & R9) appear to follow the same reading criteria (Figure 4.2.4).



**Figure 4.2.4**. SARDINE. Comparison of the increments width results (by reader) from juveniles of the Atlantic Iberian (ICES Division IXa). The sample code, fish length (mm) and ageing precision (CV&APE) are indicated.

In the samples of the Adriatic Sea, also variability is observed in the width of the first increments in the larvae (Code sample: Ms 174; SL= 14 mm; Ageing precision= 6% CV and 4.6%APE) among all readers, although seem to have the same reading criteria. The reader R7 seems to have had calibration problems in reading image. In juveniles (Code sample: MS82; SL=39 mm; Ageing precision= 7%CV and 5.8%APE), high variability is observed, though without any evident pattern of growth among readers (Figure 4.2.5). Some readers have noted that there were problems with the calibration of some images of this set, so they were not sure about radius and increment measurements from nucleus.



**Figure 4.2.5**. SARDINE. Comparison of the increments width results (by reader) from larvae (top panel) and juveniles (bottom panel) of the Adriatic Sea. The sample code, fish length (mm) and ageing precision (CV&APE) are indicated.

In the samples of the North Aegean Sea, high similarity is observed in the width increments in the larvae (Code sample: Sp\_L\_2-3; SL= 34.4 mm; Ageing precision= 4% CV and 3%APE) among all readers. Generally readers adopted similr ageing criteria in the sample of juveniles (Code sample: Sp\_J\_10\_9 ; SL= 59 mm; Ageing precision= 10% CV and 7.5%APE), although some variability is observed in the increments width of readers R2 & R5 (Figure 4.2.6)



**Figure 4.2.6**. SARDINE. Comparison of the increments width results (by reader) from larvae (top panel) and juveniles (bottom panel) of the North Aegean Sea. The sample code, fish length (mm) and ageing precision (CV&APE) are indicated.

#### 4.2.2 Total areas

When we consider all areas together, the sardine exchange showed differences among reader, with a CV of 13.7% and a APE of 10.6 % (Table 4.2.3). It showed a great variability in older ages (Figure 4.2.7).

	Sample	Fish	TL	SL	Landing	IFREMER	IEO-VI	IPMA	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Mean		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	
2012	St 483 - 07	1	20.5	19.5	4	26	23	25	19	18	23	27	22	26	23	3.2	14%	10.6%
2012	St 483 - 17	2	19.9	18.2	4	27	20	27	17	20	22	20	19	23	22	3.5	16%	12.6%
2012	St 533 - 11	3	21.1	19.7	5	25	22	21	18	23	20	22	22	22	22	1.9	9%	6.2%
2012	St 533 - 14	4	23.2	21.7	5	31	27	28	24	25	17	25	16	28	25	5.0	20%	15.1%
2012	St 551 - 14	5	18.8	17.1	5	23	23	21	16	27	23	22	20	23	22	3.0	13%	9.1%
2010	sc_040810_6_1	1	67	54.2	8	69	68	113	93	76	-	71	77	67	79	16.0	20%	15.0%
2010	sc 180810 6 1	2	85	72.0	8	78	81	91	120	92	86	85	80	84	89	12.7	14%	9.4%
2010	sc_060910_2_1	3	82	67.0	9	88	89	123	119	108	95	90	96	90	100	13.5	14%	11.3%
2010	sc_210910_4_1	4	90	77.0	9	100	104	116	117	106	128	113	128	109	113	9.8	9%	6.8%
2010	sc_041110_3_2	5	109	92.0	11	116	147	130	150	130	124	119	147	111	130	14.5	11%	8.9%
2008	21_10_08_33_200x	6	160	136.0	10	185	278	257	214	162	252	251	293	123	224	56.9	25%	21.0%
2009	16_04_09_01_200x	7	144	123.0	4	217	222	267	255	151	237	175	265	150	215	46.4	22%	17.6%
2004	20_07_04_71_200x	8	138	117.0	7	136	214	189	175	160	290	177	184	121	183	48.9	27%	17.7%
2004	27 05 04 07 200x	9	84	71.0	5	108	168	116	122	116	124	120	119	116	123	17.4	14%	8.2%
2005	10_01_05_02_200x	10	99	84.0	1	141	154	185	138	144	213	135	221	104	159	38.7	24%	19.6%
2010	OTO 18-TF1110	1	-	21.4	10	31	28	29	30	35	28	30	30	30	30	2.1	7%	4.3%
2010	OTO 563-TF1110	2	-	15.0	11	17	17	22	16	20	19	18	24	16	19	2.7	14%	11.4%
2010	OTO 497-TF1110	3	-	16.6	11	16	17	17	18	25	15	17	19	17	18	2.9	16%	10.4%
2010	OTO 492-TF1110	4	-	19.7	11	19	18	20	26	27	20	19	22	17	21	3.5	17%	13.1%
2010	OTO 385-TF1110	5	-	22.9	10	33	36	34	32	37	35	34	36	33	34	1.6	5%	3.8%
2013	Ms 72	1	36	32.0	3	51	-	59	64	63	63	72	68	46	61	8.6	14%	10.8%
2013	Ms 82	2	46	39.0	3	84	97	85	96	98	96	92	101	86	93	6.3	7%	5.8%
2013	Ms 121	3	39	34.0	3	62	64	69	65	68	69	68	67	58	66	3.7	6%	4.5%
2013	Ms 151	4	25	22.0	3	36	41	44	50	54	41	40	42	34	42	6.3	15%	10.8%
2013	Ms 174	5	15	14.0	3	18	20	19	21	19	18	19	21	19	19	1.1	6%	4.6%
2013	Ms 201	6	30	26.0	3	58	-	45	63	61	53	62	59	42	55	8.0	14%	11.8%
2013	Ms 231	7	43	37.0	3	75	-	96	106	96	107	109	111	80	98	13.6	14%	11.0%
1997	Sa 75	8	56		1	69	-	84	74	73	99	93	95	75	83	11.6	14%	12.1%
1997	Sa 287	9	80		5	121	-	130	167	80	211	203	194	154	157	45.4	29%	23.0%
1997	Sa 298	10	65		5	97	153	-	147	100	162	153	164	85	133	32.7	25%	21.8%
2007	Sp_J_5_7	1	80	-	7	100	98	124	107	107	118	128	126	134	116	13.1	11%	9.8%
2007	Sp_J_10_8	2	76	-	7	127	128	140	119	134	160	155	128	163	139	16.1	12%	9.7%
2007	Sp_J_10_9	3	59	-	7	126	158	133	117	125	134	151	129	141	135	13.1	10%	7.5%
2007	Sp_J_11_7	4	64	-	7	101	149	163	107	149	140	165	141	151	141	22.5	16%	11.7%
2007	Sp_J_12_22	5	60	-	7	108	137	136	111	134	135	148	129	147	132	14.0	11%	7.9%
2009	Sp_L_2-3	6	-	34.4	2	59	65	65	66	63	62	66	66	66	64	2.4	4%	3.0%
2009	Sp_L_2-18	7	-	35.4	2	64	62	68	61	61	55	63	61	66	62	3.7	6%	4.2%
2009	Sp_L_3-15	8	-	21.2	2	28	29	32	31	33	24	28	30	31	30	2.7	9%	6.9%
2009	Sp_L_3-17	9	-	20.3	2	28	29	25	30	28	25	29	32	30	28	2.3	8%	6.1%
2009	Sp_L_19-16	10	-	25.5	2	34	36	30	37	32	34	39	38	37	35	2.9	8%	6.7%
				1	Fotal read	40	35	39	40	40	39	40	40	40			13 7%	10.6%
				Total I	NOT read	1	6	2	1	1	2	1	1	1			13.1%	10.0%

Table 4.2.3 SARDINE Otolith SET (WKMIAS 2013\_Otolith Exchange)



Figure 4.2.7. SARDINE: Coefficient of variation (CV%), Average percent error (APE%) and standard deviation (SD) plotted against MEAN age, for all areas.

For all areas, we found that all readers have a significant agreement in the readings (the mean counts differ <8% from the mean age). (Table 4.2.4 and Figure 4.2.8)

Mean	IFRE	MER	IE	O-VI	IP	MA	IEC	D-MA	CNR	-IAMC	CNR-	ISMAR	CNR-	ISMAR	CNR-	ISMAR	HC	MR
age	F	R1	1	R2		R3		R4		R5	F	R6	F	R7	F	R8	F	R9
18	-2	-11%	-1	-5%	-1	-5%	0	1%	7	40%	-	-	-1	-5%	1	6%	-1	-5%
19	-2	-9%	-2	-9%	3	18%	-3	-15%	1	7%	0	1%	-1	-4%	5	26%	-3	-15%
19	-1	-7%	1	3%	0	-2%	2	9%	0	-2%	-1	-7%	0	-2%	2	9%	0	-2%
21	-2	-9%	-3	-14%	-1	-4%	5	24%	6	29%	-1	-4%	-2	-9%	1	5%	-4	-19%
22	5	25%	-2	-8%	5	25%	-5	-22%	-2	-8%	0	2%	-2	-8%	-3	-12%	1	6%
22	3	15%	0	2%	-1	-3%	-4	-17%	1	6%	-2	-8%	0	2%	0	2%	0	2%
22	1	5%	1	5%	-1	-5%	-6	-27%	5	23%	1	5%	0	0%	-2	-9%	1	5%
23	3	12%	0	-1%	2	8%	-4	-18%	-5	-22%	0	-1%	4	16%	-1	-5%	3	12%
25	6	26%	2	10%	3	14%	-1	-2%	0	2%	-8	-31%	0	2%	-9	-35%	3	14%
28	0	-2%	1	2%	-3	-12%	2	5%	0	-2%	-3	-12%	1	2%	4	13%	2	5%
30	-2	-5%	-1	-2%	2	8%	1	5%	3	12%	-6	-19%	-2	-5%	0	2%	1	5%
30	1	3%	-2	-7%	-1	-4%	0	0%	5	16%	-2	-7%	0	0%	0	0%	0	0%
34	-1	-4%	2	5%	0	-1%	-2	-7%	3	8%	1	2%	0	-1%	1	3%	-1	-4%
35	-1	-3%	1	2%	-5	-15%	2	5%	-3	-9%	-1	-3%	4	11%	2	7%	2	5%
42	-6	-15%	-1	-3%	2	4%	8	18%	12	27%	-1	-3%	-2	-6%	0	-1%	-8	-20%
55	3	5%	-	-	-10	-19%	8	14%	6	10%	-2	-4%	7	12%	4	7%	-13	-24%
61	-10	-16%		-	-2	-3%	3	5%	2	4%	2	4%	11	19%	7	12%	-15	-24%
62	2	3%	0	-1%	6	9%	-	-	-1	-2%	-7	-12%	1	1%	-1	-2%	4	6%
64	-5	-8%	1	1%	1	1%	2	3%	-1	-2%	-2	-3%	2	3%	2	3%	2	3%
66	-4	-5%	-2	-2%	3	5%	- T.	-	2	4%	3	5%	2	4%	1	2%	-8	-12%
/9	-10	-13%	-11	-14%	34	43%	14	17%	-3	-4%	-	-	-8	-10%	-3	-3%	-12	-15%
83	-14	-17%	-	-	1	2%	-9	-11%	-10	-12%	16	20%	10	12%	12	15%	-8	-9%
89	-11	-12%	-8	-8%	3	3%	32	36%		-	-3	-3%	-4	-4%	-9	-10%	-5	-5%
93	-9	-9%	4	5%	-8	-8%	3	3%	5	6% 20/	3	3%	-1	-1%	8	9%	-/	-1%
90	-23	-23%	- 11	-	-2	-2%	9	9%	-2	-2%	10	10%	12	12%	14	14%	-18	-18%
112	-12	-12%	-11	-11%	23	23%	19	19%	8	8%	-5	-5%	-10	-10%	-4	-4%	-10	-10%
115	-13	-12%	-9	-0%	- 3 - 0	2% 70/	4	3%	-/	-7 %	15	13%	12	0%	14	12%	-4	-4%
123	-15	-1-470	45	36%	-7	-6%	-1	-0 /0	-3	-6%	1	2 /0	-3	-3%	-4	-3%	-7	-6%
130	-14	-12/0	43	13%	-7	-078	20	15%	- 1	-078	-6	-5%	-3	-0%	16	-370	-10	-0 /0
132	-14	-112%	5	10/		3%	-21	-16%	2	2%	-0	-378	16	-370	-3	-2%	15	12%
133	-36	-27%	20	15%	-	570	14	11%	-33	-25%	20	22%	20	15%	31	24%	-48	-36%
135	-00	-7%	23	17%	-2	-1%	-18	-13%	-10	-7%	-1	-1%	16	12%	-6	-4%	6	5%
139	-12	-9%	-11	-8%	1	0%	-20	-15%	-10	-1%	21	15%	16	11%	-11	-8%	24	17%
141	-40	-28%	8	6%	22	16%	-34	-24%	-	- 470	-1	0%	24	17%	0	0%	10	7%
157	-36	-23%	-	-	-27	-17%	10	6%	-77	-49%	54	34%	46	29%	36	23%	-3	-2%
159	-18	-12%	-5	-3%	26	16%	-21	-1.3%	-		54	34%	-24	-15%	61	38%	-55	-35%
183	-47	-26%	31	17%	6	3%	-8	-4%	-23	-13%	107	59%	-6	-3%	1	1%	-62	-34%
215	2	1%	7	3%	52	24%	40	18%	-64	-30%	22	10%	-40	-19%	50	23%	-65	-30%
224	-39	-17%	54	24%	33	15%	-10	-4%	-62	-28%	28	13%	27	12%	69	31%	-101	-45%
	10	00/		40/		40/		00/	7	40/		20/	2	20/	7	E0/	10	70/
days	-10	-8%	4	1%	4	4%		U%	-1	-1%	Э	3%	3	3%	1	5%	-10	-1%

**Table 4.2.4.** SARDINE: Age differences (number of days and percentages) from the mean age by reader, for all areas.



**Figure 4.2.8.** SARDINE: Age differences in number of days (left) and percentage (right) from the mean age by reader, for all areas.

## 4.3. Culture juveniles of sardine from Atlantic Iberian set

Table 4.3.1 details length and month of landing of culture juveniles set (Atlantic Iberian) of otoliths images selected for the sardine exchange programme with the ageing produced by each reader. The last four columns in the top table show actual age, standard deviation (SD) and precision of reading as the CV in relation to the actual age and the APE<sub>BF</sub>; the bottom table shows mean age, standard deviation (SD) and precision of reading as the CV in relation to the actual age and the APE<sub>BF</sub>; the bottom table shows mean age, standard deviation (SD) and precision of reading as the CV in relation to the actual age and the APE<sub>BF</sub>.

	Sample	Fish	TL	SL	Landing	IFREMER	IEO-VI	<b>IPMA</b>	IEO-MA	CNR-IAMC	CNR-ISMAR	CNR-ISMAR	CNR-ISMAR	HCMR	Actual		Precision	Precision
year	no	no	(mm)	(mm)	month	R1	R2	R3	R4	R5	R6	R7	R8	R9	age	SD	CV	
2010	sc_040810_6_1	1	67	54.2	8	69	68	113	93	76	-	71	77	67	70	27.8	40%	15.3%
2010	sc_180810_6_1	2	85	72.0	8	78	81	91	120	92	86	85	80	84	85	11.1	13%	8.7%
2010	sc_060910_2_1	3	82	67.0	9	88	89	123	119	108	95	90	96	90	101	4.1	4%	11.6%
2010	sc_210910_4_1	4	90	77.0	9	100	104	116	117	106	128	113	128	109	116	8.3	7%	6.9%
2010	sc_041110_3_2	5	109	92.0	11	116	147	130	150	130	124	119	147	111	160	94.2	59%	18.5%
	•				otal read	5	5	5	5	5	4	5	5	5				
														-			<b>34 C0/</b>	40.00/
				Total I	NOT read	0	0	0	0	0	1	0	0	0			24.6%	12.2%
Mean A	Age Sample	Fish	π	Total I	NOT read	0	0 IEQ-VI	0 IPMA	0 IEQ-MA	0 CNR-IAMC	1 CNR-ISMAR	0 CNR-ISMAR	0	0 HCMR	Mean		24.6% Precision	12.2%
Mean A	Age Sample	Fish	TL (mm)	Total I SL (mm)	NOT read	0 IFREMER R1	0 IEO-VI R2	0 IPMA R3	0 IEO-MA R4	0 CNR-IAMC R5	1 CNR-ISMAR R6	0 CNR-ISMAR R7	0 CNR-ISMAR R8	0 HCMR R9	Mean age	SD	24.6% Precision CV	12.2% Precision APE <sub>BF</sub>
Mean A year 2010	Sample no sc_040810_6_1	Fish no 1	TL (mm) 67	SL (mm) 54.2	Landing month	0 IFREMER R1 69	0 IEO-VI R2 68	0 IPMA R3 113	0 IEO-MA R4 93	0 CNR-IAMC R5 76	1 CNR-ISMAR R6	0 CNR-ISMAR R7 71	0 CNR-ISMAR R8 77	0 HCMR R9 67	Mean age 79	<b>SD</b> 16.0	Precision CV 20%	Precision APE <sub>BF</sub> 15.0%
<b>Mean A</b> year 2010 2010	Age Sample no sc_040810_6_1 sc_180810_6_1	Fish no 1 2	TL (mm) 67 85	Total I SL (mm) 54.2 72.0	Landing month 8 8	0 IFREMER R1 69 78	0 IEO-VI R2 68 81	0 IPMA R3 113 91	0 IEO-MA R4 93 120	0 CNR-IAMC R5 76 92	1 CNR-ISMAR R6 - 86	0 CNR-ISMAR R7 71 85	0 CNR-ISMAR R8 77 80	0 HCMR R9 67 84	Mean age 79 89	<b>SD</b> 16.0 12.7	24.6% Precision CV 20% 14%	12.2% Precision APE <sub>BF</sub> 15.0% 9.4%
<b>Mean A</b> year 2010 2010 2010	Age Sample no sc_040810_6_1 sc_180810_6_1 sc_060910_2_1	Fish no 1 2 3	TL (mm) 67 85 82	Total I SL (mm) 54.2 72.0 67.0	Landing month 8 8 9	0 IFREMER R1 69 78 88	0 IEO-VI R2 68 81 89	0 IPMA R3 113 91 123	0 IEO-MA R4 93 120 119	0 CNR-IAMC R5 76 92 108	1 CNR-ISMAR R6 - 86 95	0 CNR-ISMAR R7 71 85 90	0 CNR-ISMAR R8 77 80 96	0 HCMR R9 67 84 90	Mean age 79 89 100	<b>SD</b> 16.0 12.7 13.5	24.6% Precision CV 20% 14% 14%	<b>Precision</b> <b>APE</b> <sub>BF</sub> 15.0% 9.4% 11.3%
<b>Mean A</b> year 2010 2010 2010 2010	Age Sample sc_040810_6_1 sc_180810_6_1 sc_060910_2_1 sc_210910_4_1	Fish no 1 2 3 4	TL (mm) 67 85 82 90	Total I SL (mm) 54.2 72.0 67.0 77.0	Landing month 8 9 9	0 IFREMER R1 69 78 88 100	0 IEO-VI R2 68 81 89 104	0 IPMA R3 113 91 123 116	0 IEO-MA R4 93 120 119 117	0 CNR-IAMC R5 76 92 108 106	1 CNR-ISMAR R6 - 86 95 128	0 CNR-ISMAR R7 71 85 90 113	0 CNR-ISMAR R8 77 80 96 128	0 HCMR R9 67 84 90 109	Mean age 79 89 100 113	<b>SD</b> 16.0 12.7 13.5 9.8	24.6% Precision CV 20% 14% 14% 9%	<b>Precision</b> <b>APE</b> <sub>BF</sub> 15.0% 9.4% 11.3% 6.8%
<b>Mean A</b> year 2010 2010 2010 2010 2010 2010	Sample no sc_040810_6_1 sc_180810_6_1 sc_210910_2_1 sc_210910_4_1 sc_041110_3_2	Fish no 1 2 3 4 5	TL (mm) 67 85 82 90 109	Total I SL (mm) 54.2 72.0 67.0 77.0 92.0	Landing month 8 9 9 11	0 IFREMER R1 69 78 88 100 116	0 IEO-VI R2 68 81 89 104 147	0 IPMA R3 113 91 123 116 130	0 IEO-MA R4 93 120 119 117 150	0 CNR-IAMC R5 76 92 108 106 130	1 CNR-ISMAR R6 - - 86 95 128 124	0 CNR-ISMAR R7 71 85 90 113 119	0 CNR-ISMAR R8 77 80 96 128 147	0 HCMR R9 67 84 90 109 111	Mean age 79 89 100 113 130	<b>SD</b> 16.0 12.7 13.5 9.8 14.5	<b>Precision</b> <b>CV</b> 20% 14% 14% 9% 11%	<b>Precision</b> <b>APE</b> <sub>BF</sub> 15.0% 9.4% 11.3% 6.8% 8.9%
<b>Mean A</b> 2010 2010 2010 2010 2010 2010	Sample no sc_040810_6_1 sc_180810_6_1 sc_060910_2_1 sc_210910_4_1 sc_041110_3_2	Fish no 1 2 3 4 5	TL (mm) 67 85 82 90 109	Total I SL (mm) 54.2 72.0 67.0 77.0 92.0	Landing month 8 9 9 11 Total read	0 IFREMER R1 69 78 88 100 116 5	0 IEO-VI R2 68 81 89 104 147 5	0 IPMA R3 113 91 123 116 130 5	0 IEO-MA R4 93 120 119 117 150 5	0 CNR-IAMC R5 76 92 108 106 130 5	1 CNR-ISMAR R6 95 128 124 4	0 CNR-ISMAR R7 71 85 90 113 119 5	0 CNR-ISMAR R8 77 80 96 128 147 5	0 HCMR R9 67 84 90 109 111 5	Mean age 79 89 100 113 130	<b>SD</b> 16.0 12.7 13.5 9.8 14.5	<b>Precision</b> <b>CV</b> 20% 14% 14% 9% 11%	<b>Precision</b> <b>APE</b> <sub>BF</sub> 15.0% 9.4% 11.3% 6.8% 8.9%

In general, the exchange shows greater differences between readers when we relate it to
the actual age (CV = 24.6%) than the mean age (CV = 13.6). The mean age is very similar to
the actual age in three otoliths image, and therefore the difference between readers is
very low with an ageing precision very high (CV between 4 and 13%). (Figure 4.3.1).

# Table 4.3.1 SARDINE Otolith SET (WKMIAS 2013\_Otolith Exchange): Cultures juveniles from Atlantic Iberian



**Figure 4.3.1.** Culture juveniles of SARDINE: Coefficient of variation (CV%), Average percent error (APE%) and standard deviation (SD) plotted against ACTUAL age (top panel) and MEAN age (bottom panel).

In general, readers R1 & R9 underestimate the ages respect to actual age (difference of 13% and 11% from the actual age, respectively), and readers R3 & R4 overestimate them (14% and 17% respectively. (Table 4.3.2 and Figure 4.3.2). All readers underestimate the older fish (160 days), with differences between 8 and 49 days (between 6 and 31%) with the actual age.

**Table 4.3.2.** Culture juveniles of SARDINE: Age differences (number of days and percentages) from the actual age.

		1000																
Actual	IFR	EMER	IE	O-VI	IP	MA	IEC	D-MA	CNR	-IAMC	CNR	ISMAR	CNR-	ISMAR	CNR-	ISMAR	HC	MR
age	I	R1	1	R2		R3		R4		R5		R6		R7	F	88	F	۲9
70	-1	-1%	-2	-3%	43	61%	23	33%	6	9%	-	-	1	1%	7	9%	-3	-4%
85	-7	-8%	-4	-5%	6	7%	35	41%	7	8%	1	1%	0	0%	-6	-6%	-1	-1%
101	-13	-13%	-12	-12%	22	22%	18	18%	7	7%	-6	-6%	-11	-11%	-6	-5%	-11	-11%
116	-16	-14%	-12	-10%	0	0%	1	1%	-10	-9%	12	10%	-3	-3%	12	10%	-7	-6%
160	-44	-28%	-13	-8%	-30	-19%	-10	-6%	-30	-19%	-36	-23%	-41	-26%	-14	-8%	-49	-31%
Mean	-16	-13%	-9	-8%	8	14%	13	17%	-4	-1%	-7	-4%	-11	-8%	-1	0%	-14	-11%
davs																		

Atlantic Iberian	(culture	iuveniles)



**Figure 4.3.2.** Culture juveniles of SARDINE: Age differences in number of days (left) and percentage (right) from the mean age by sampling areas and reader.

Readers generally adopted similar ageing criteria in the culture juveniles sample (Code sample: sc\_060910\_2\_1; SL= 67 mm; Actual Age= 101 days; Ageing precision= 4% CV) , although some variability is observed in the increments width of reader R5 (Figure 4.3.3)



**Figure 4.3.3**. Culture juveniles of SARDINE. Comparison of the increments width results (by reader). The sample code, fish length (mm) and ageing precision (CV&APE) are indicated.

## 5. Conclusions

• The Exchange exercise showed differences among readers and areas for both species.

	cv	′ %	API	Ξ%
Area	Anchovy	Sardine	Anchovy	Sardine
Bay of Biscay	15.3	14.5	10.4	10.7
Atlantic Iberian	-	18	-	13.5
Western Mediterranean	18.6	11.7	14.1	8.6
Strait of Sicily	34.9	-	23	-
Adriatic Sea	24	14.3	18.9	11.6
North Aegean Sea	9	9.4	7.8	7.4
Total	18.9	13.7	13.3	10.55

- Differences in the age determination were generally lower for sardine. However, in sardine, a greater variability was observed in the allocation and width of increments, suggesting that not all readers followed the same ageing criteria for this species, especially readers R2, R3 and R5 (and also probably R1). This could be because sardine readers have a low level of daily age expertise.
- The comparison with the actual age of sardine showed that sardine readers are generally in good agreement, with a greater deviation of readers R3 & R4. Nevertheless, all readers underestimated the older fish (160 days).
- In general, anchovy reader R5 tended to overestimate daily ages considerably in the anchovy images, showing clear differences in the age interpretation criteria, much more in the older ages (juveniles). Conversely, R9 tended to underestimate daily ages in the anchovy larvae.
- However, bearing in mind the inherent difficulties to interpret the daily micro increments in anchovy, generally most readers apply the same reading criteria in all areas.
- Good quality images generally provided high ageing precision for both species, for example those from the North Aegean Sea (Figures 5.1 & 5.2). Therefore, it should

be stressed the importance of obtaining clear images to properly interpret daily micro increments in this species.

- The reasons that might explain the agreement and discrepancies in the anchovy and sardine exchange can be summarized as follows: a) unclear images, in which was difficult to interpret well the pattern of daily growth due to under-or overpolishing, poor image acquisition or calibration problems; b) Difficulties in interpretation of subdaily increments, double structures or band zones (see Cermeño et al.., 2006; Cermeño 2008),
- Further reasons for discrepancies have not yet been examined in individual image cases of disagreement, as their will be thoroughly discussed in the upcoming workshop.
- It will be a good occasion to study and discuss the growth pattern of the sardine whose actual age is known from marine culture (Figure 5.3)



**Figure 5.1.** Example of an ANCHOVY juvenile image from the North Aegean Sea. Sample Code: Ee\_J\_1-3; SL= 71 mm; Mean age: 88 days; CV= 6%; APE= 4.6%.



**Figure 5.4.** Example of a SARDINE post larvae image from the North Aegean Sea. Sample Code: Sp\_L\_2\_3; SL= 34.4 mm; Mean age: 64 days; CV= 4%; APE= 3.0%.



**Figure 5.4.** Example of a SARDINE juvenile image from the marine culture (Atlantic Iberian). Code sample: sc\_060910\_2\_1; SL= 67 mm; Actual Age= 101 days; Ageing precision= 4% CV

## REFERENCES

Aldanondo, N., Cotano, U., Etxebeste, E., Irigoien, X., Álvarez, P., Martínez de Murguía, A. and Herrero, D.L. (2008). Validation of daily increments deposition in the otoliths of European anchovy larvae (*Engraulis encrasicolus* L.) reared under different temperature conditions. Fish. Res. 93: 257–264.

Alemany, F., Álvarez, F. 1994. Formation of initial daily increments in sagital otoliths of reared and wild *Sardina pilchardus* yolk-sac larvae. Marine Biology, 121: 35-39.

Anon. 1993. Report of the clupeidos otolith microstructure Workshop. Informe Técnico. Instituto Español de Oceanografía. No 145.

Beamish, R.J. and D.A. Fournier. 1981. A method for comparing the precision of a set of age determinations. Canadian Journal of Fisheries and Aquatic Sciences 38, 982-983.

Campana, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59, 197-241

Cermeño, P. Morales-Nin, B.and Uriarte, A., 2006. Juvenile European anchovy otolith microstructure. Scientia Marina, 70(3):553-557.

Cermeño, P., A. Uriarte, B. Morales-Nin, U. Cotano, P. Alvarez 2008. Setting up interpretation criteria for ageing juvenile european anchovy otoliths. Sci. Mar. 72(4)

Chang, W.Y.B. 1982. A statistical method for evaluating the reproducibility of age determination. Canadian Journal of Fisheries and Aquatic Sciences 39, 1208-1210.

ICES WKARA. 2009. Report of the Workshop on Age reading of European anchovy (WKARA). ICES CM 2009/ACOM:43

ICES WKARAS. 2011 Report of the Workshop on Age Reading of European Atlantic Sardine (WKARAS). ICES CM 2011/ACOM:42

Morales-Nin, B., N. Aldanondo and I. Alvarez. 2010. Age determination of larval and juvenile small pelagics: the importance of a common protocol. Rapp. Comm. int. Mer Médit., 39, 2010

SARDONE Project. Improving assessement and management of small pelagic species in the Mediterranean. Final Report of the SARDONE project. UE-FP6- 44294