Report of the

WORKSHOP ON SARDINE OTOLITH AGE READING AND BIOLOGY

IPIMAR
Lisbon, Portugal
27 June – 1 July, 2005

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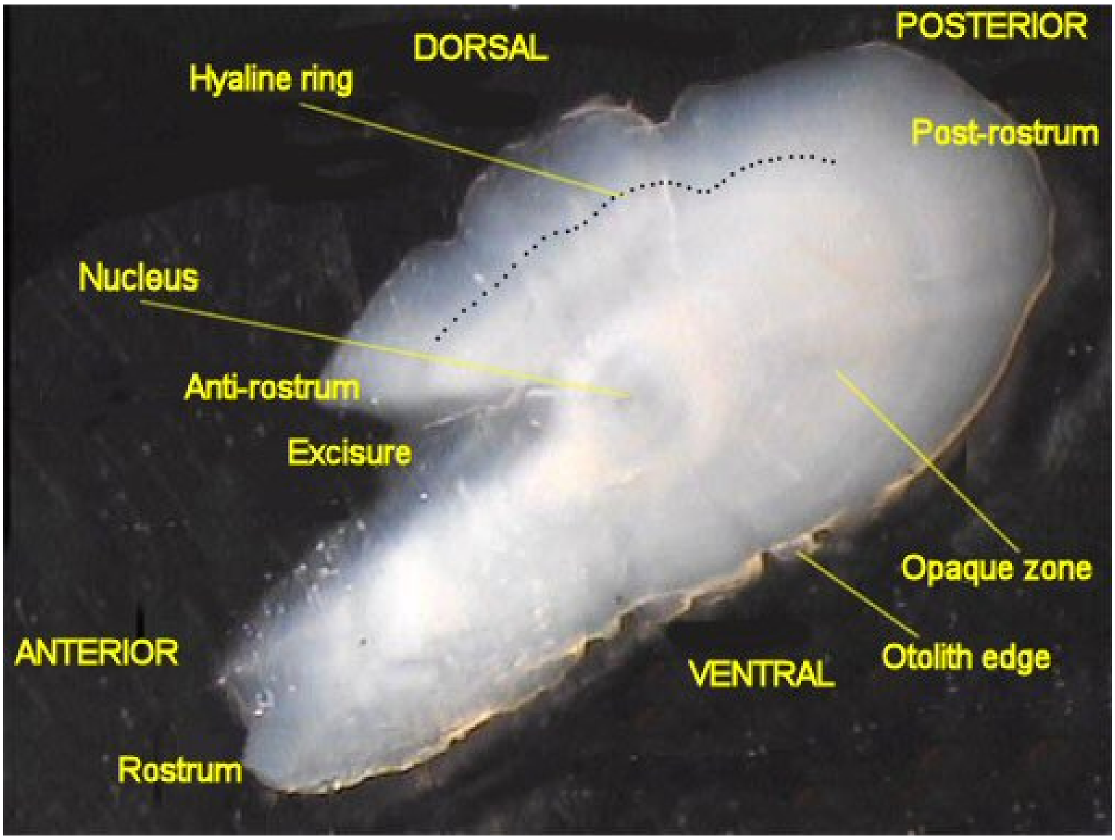
Workshop held within the framework of the EU Data Collection Regulation (DCR)

The Workshop participants

Some aspects of the activities carried out during the Workshop



View of sardine otolith: identification of main structural areas.

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ABSTRACT

The current exchange and workshop aimed to evaluate readers agreement and aging precision, to assess the extent of aging difficulties previously identified (identification of the first annual ring and aging of older individuals) and to propose guidelines for their minimization. The consistency of age readings in time (comparison of the 1980's, 1990's and the present time) and in space (comparison with Mediterranean and northwest African areas) was also explored and the consequences of the assumed birthdate for the estimation of growth were discussed. In addition, profiting from the experience of the workshop attendants, biological sampling methodologies (assignment of sexual maturity stages, visceral fat and stomach condition) were listed and discussed and standard protocols are recommended.

A total of 555 otolith pairs, grouped into 10 sets according to the different objectives and areas, were read by thirteen readers (from seven Institutes across five countries) following a common age reading protocol. For each otolith, the number of hyaline rings, the type of edge (hyaline/opaque), the age group (years) and the readability level (1-good, 2-medium, 3-difficult) were recorded. The modal age of each otolith, based on readings of five experienced readers, was assumed as the true age.

Otolith readability declined from the northern to the southern areas in the Atlantic and was intermediate in the northwestern Mediterranean samples. The exclusion of difficult otoliths did not affect the estimates of the mean length-at-age but improved considerably their precision. Within the Atlantic Iberian area, both the agreement among experienced readers and the CV by age group declined in comparison to the last Workshop. Two possible explanations are the shorter experience of some current readers and the fact that most samples were collected when the edge type classification is more uncertain (transition between winter/summer). Difficulties in the identification of the first annual ring and aging of older fish still persist while the identification of the otolith edge and whether to decide to account it for age assignment are additional problems. To minimize these problems, the workshop recommends that readers use either the anterior or posterior margin of the otolith to identify the edge type and follow its seasonal evolution in each area.

Overall, agreement with age readings from the 1980s and the 1990s was lower than current levels of between-reader agreement in samples from similar areas. The small sample sizes prevent firm conclusions about bias but the observed systematic differences in some ages/periods advise a more thorough evaluation of this issue.

Otoliths from the Mediterranean area showed generally low agreement levels (comparable to otoliths from southern Portugal) mainly due to the identification of the first annual ring. The workshop recommended the use of the diameter of the opaque core measured in juvenile fish otoliths as a gauge to help aging older individuals. Agreement between readers from the Atlantic Iberian and the NW African areas was considerably low. Iberian readers assign older ages to otoliths from the NW African areas while Moroccan readers assign younger ages to the otoliths from the Iberian areas, indicating different age reading criteria. The high opacity of otoliths from the NW African areas raises serious difficulties to aging. The use of alternative preparation techniques, such as soaking in water/alcohol, was recommended to enhance ring visibility in these otoliths.

The age reading protocol for sardine was updated and a standard sheet for the recording of age reading results was prepared. The organization of reference collections of otoliths (>80% agreement) within each area is recommended.

1.INTRODUCTION

Fish resources assessment requires an actual knowledge of fish populations' age structure in order to achieve appropriate fisheries management. This implies an accurate determination of fish age which requires for each species the set up of suitable methodologies and age reading criteria to be used in common by those engaged in this task.

Similarly to what happens in a significant number of fish species, age determination of Atlantic Iberian sardine is based on the analysis of otolith structure. The effort for the standardization of sardine otolith age reading methodology and for the improvement of the readings precision in the Northeast Atlantic began to be more prominent in the late 70's (FAO, 1978, 1979; Anon, 1981). This was the basis for the work subsequently developed for the Atlantic Iberian sardine, which involved otoliths exchanges and workshops on sardine age reading in this area (Anon, 1994a, 1994b; Carrera, 1996; ICES, 1997; Soares *et al*, 2002).

Pursuing this work, IPIMAR coordinated during 2004 a sardine otolith samples exchange for age reading comparisons, which involved researchers and technicians working in several fisheries research Institutions from Portugal, Spain, France, Germany and Morocco.

The objectives of this exchange were to:

1. Evaluate the agreement among otolith readers and the precision of age determination;
2. Assess whether the main problems identified in the previous workshop still persist and to what extent. Propose ways of minimizing the difficulties still existing;
3. Evaluate the consistency of age readings between the early 1980's , the early 1990's and the present time;
4. Evaluate the consistency of age determinations with sardines from the northwest African area;
5. Discuss the consequences of the assumed birthdate for the estimation of growth. Suggest alternatives;
6. Produce a "summer" reference collection (otoliths with more then 80% agreement between experienced readers) of sardine otoliths from different areas of the species distribution.

Following this exchange, a Workshop on Sardine Otolith Age Reading and Biology was held at IPIMAR, Lisbon, from 27 June to 1 July, 2005.

The main objectives of this Workshop were to discuss the results of the 2004 otolith exchange focusing on the main problems detected, review the sardine age reading criteria and propose measures in order to improve the precision of otolith age readings.

Profiting from the experience of most of the attendants in sardine biological sampling in different areas, this Workshop also discussed practical problems related with the application of scales for macroscopic assignment of sexual maturity stages, visceral fat and stomach condition (colour and fullness), in order to improve the quality and the accuracy of the biological information collected from sampling used in sardine stock assessment.

2.AGENDA

In order to achieve its objectives the Workshop followed the next agenda:

Date	Time	Item
June, 27	09:30-10:30	– Opening; – Introduction: antecedents and objectives of the Workshop; – Discussion and approval of the Agenda; – Information on current criteria for sardine otolith age group assignment: • in the Atlantic Iberian sardine distribution area; • in other areas.
	10:45-13:00	Presentation of results from the main collection
	13:00-14:30	Lunch
	14:30-17:30	– Discussion of results from the main collection
June, 28	09:30-13:00	– Presentation and discussion of results from collections of the 1980s and the 1990s.
	13:00-14:30	Lunch
	14:30-17:30	– Presentation and discussion of results from Northwest African collections. – Presentation of information on sardine biology and age reading on the Moroccan coast.
June, 29	09:30-13:00	– Discussion of other issues: • Alternative methodologies to improve the accuracy and precision of age readings; • Consequences of the assumed birthdate: which alternatives? – Presentation of information on sardine biology and age reading off the Greek coast. – Presentation of the analysis on the birthdate convention, readability and seasonality of the otolith edge and on interpretation of the first ring.
	13:00-14:30	Lunch
	14:30-17:30	– Conclusions: • Identification of the major difficulties in age readings and improvements since last workshop; • Conclusions about the consistency of age readings along time and among areas; • Review of the age reading criteria: differences among areas; • The otolith reference collection and future work; • Age reading protocol and recommendations.
June, 30	09:30-13:00	– Sardine biological sampling: • Methodology and criteria; • Discussion on the use of: ✓ Scales for macroscopic assignment of sexual maturity stages; ✓ Scale for visceral fat condition stages; ✓ Scale for stomach condition (colour and fullness).
	13:00-14:30	Lunch
	14:30-17:30	– Sardine biological sampling (continued): • A practical observation of sardine samples in laboratory: ✓ Discussion on the biological information collected and the application of scales (maturity, fat condition and stomach).
July, 1	09:30-13:00	– Conclusions and recommendations for sardine biological sampling; – Discussion on the Workshop report: structure, contributions, conclusions and recommendations.

3.PARTICIPANTS

Seventeen researchers and technicians from different research institutions usually involved in otolith age reading have attended this Workshop (Table 3.1).

Table 3.1 – List of participants

Name	Reader	Observer	Institute	e-mail
Eduardo Soares (Chairman)	✓	–	IPIMAR – Lisbon (Portugal)	esoares@ipimar.pt
Alexandra Silva	✓	–	IPIMAR – Lisbon (Portugal)	asilva@ipimar.pt
Alexandre Morais	✓	–	IPIMAR – Lisbon (Portugal)	amorais@ipimar.pt
Delfina Morais	✓	–	IPIMAR – Lisbon (Portugal)	–
Jorge Barra	✓	–	IPIMAR– CRIPSUL (Portugal)	–
Ana Canas	✓	–	IPIMAR– CRIPSUL (Portugal)	–
Quena Peleteiro	✓	–	IEO – Vigo (Galicia - Spain)	quena.peleteiro@vi.ieo.es
Isabel Loureiro	✓	–	IEO – Vigo (Galicia - Spain)	–
Begoña Santos	–	✓	IEO – Vigo (Galicia - Spain)	m.b.santos@vi.ieo.es
Iñaki Rico	✓	–	AZTI – Santander (Gipuzkoa - Spain)	irico@pas.azti.es
Luís Quintanilla	–	✓	IEO – Malaga (Spain)	luis.quintanilla@ma.ieo.es
Véronique Laurent	✓	–	Univ. Perpignan - Perpignan (France)	vlaurent@univ-perp.fr
Erwan Duhamel	✓	–	IFREMER - Lorient (France)	erwan.duhamel@ifremer.fr
Abdel Hakim Mesfioui	–	✓	INRH – Casablanca (Morocco)	mesfioui@inrh.org.ma
Ahmed El Youssoufi	✓	–	INRH – Casablanca (Morocco)	assidima@yahoo.fr
Eleutherios Pinakis	–	✓	HCMR - Greece	pinakis@her.hcmr.gr
Konstantinos Skarvelis	–	✓	HCMR - Greece	k_skarvelis@yahoo.com

4.OTOLITH SAMPLING, PREPARATION AND AGE READING CRITERIA

Profiting from the experience of participants, the methodology used for otolith sampling preparation and reading in different areas of sardine distribution was discussed during the workshop and is summarized in the table below.

Table 4.1 – Summary of the otolith sampling, preparation procedures and age reading criteria in the different areas.

Area	Samples	Otolith sampling	Otolith preparation and age reading
Atlantic Iberian Coast (Portugal+Spain)	Commercial catches; research surveys.	Commercial catches: 10 otolith pairs by length class (0.5 cm) and sample. Surveys: *Portugal* – 5-20 otolith pairs by length class and area (N,C,S); *Spain* – 40-50 otolith pairs from fish at random in all hauls.	Black plastic plaques and polyester resin (Potugal: Entellan; Spain: Eukitt). 2002 Workshop's age reading protocol (Soares *et al*, 2002).
Spanish Western Mediterranean coast (Northern Alboran Sea and NW Mediterranean)	Commercial catches; research surveys.	In each sample, 10 otolith pairs by length class (0.5 cm).	Black plastic plaques and polyester resin (Eukitt). 2002 Workshop's age reading protocol adapted: – 30X magnification; Post-rostrum for hyaline rings counting; – Special care for the interpretation of the 1st hyaline ring, mainly in young fish (ages 0 and 1) due to the high occurrence of false rings. In older specimens 1st ring interpretation referenced to the ulterior true rings.
French Atlantic Coast (Bay of Biscay)	Commercial catches; research surveys.	In each sample, 5 otolith pairs by length class (0.5 cm).	Black plastic plaques and polyester resin. 2002 Workshop's age reading protocol (Soares *et al*, 2002) adapted: – Otolith posterior area of for age reading; – Hyaline otolith edge in 1st semester considered as a sign that growth has not begun.
Moroccan coast	Commercial catches; research surveys.	In each sample, 10 otolith pairs by length class (0.5 cm).	Black plastic plaques and polyester resin. Protocol approved in Kaliningrad 2001 (FAO, 2002)
Greek coast	Commercial catches; research surveys.	In each sample, 25 otolith pairs by fish length class (1 cm).	Deep frozen (-90ºC) in Eppendorf tubes. Unfrozen and immerged in alcohol for observation. Age reading is applied in the photographs of the collected otoliths which are stored afterwards.

5.THE 2004 SARDINE OTOLITH EXCHANGE

5.1. Material and Methods

Thirteen researchers and technicians from 7 different research Institutions from Portugal, Spain, France, Germany and Morocco were involved in the 2004 Sardine Otolith Exchange (Tables 5.1.1 and 5.1.2). 555 otolith pairs distributed by 10 sets belonging to three sample collections from different sardine distribution areas were analysed for age assignment (Figure 5.1.1).

Five readers (**in Table 5.1.2) were considered experienced for age readings comparison purposes, as they are regularly involved in this activity and some of them are responsible for the preparation of sardine age-length keys (ALK) used in stock assessment in their respective countries.

The collection and preparation of the otolith samples followed the procedures described in a guide document which circulated among all the participants by e-mail preceding the beginning of the exchange (see Annex). The age reading protocol approved during the last sardine otolith Workshop held in Lisbon in 2002 (Soares *et al.*, 2002), included in that document, was used by all participants as their age reading common criteria.

For each of the otoliths, readers registered the number of hyaline rings, the type of edge (hyaline/opaque), the age group (years) and the readability (1-good, 2-medium, 3-difficult). The modal age of each individual was based on readings of experienced readers. Analyses of readability and edge type were also based on modal values considering only experienced readers.

The samples from the Atlantic Iberian Area (North Portugal and Galicia/Cantabria, Sets 5 to 8) for age reading consistency analysis were read only by the readers of their respective countries, i.e. Sets 5 and 6 from Portugal were only read by the Portuguese readers and Sets 7 and 8 from Spain only by the Spanish. Modal ages correspond to the original readings, i.e. ages attributed by the respective readers in the 1980's and 1990's.

The sardine otolith sample collection off Moroccan coast (Sets 9 and 10), were read by readers from the other areas in order to assess the consistency of their age readings on otoliths from a region distinct from the ones they are used to observe. The readings of Moroccan readers were used as the modal age.

Table 5.1.1 – Distribution of the otolith sample collections

Sample	Set	Number of otolith pairs	Length range (cm)	Area	Institute
Main collection (June-July 2003)	1	83	14.5 – 24.0	North Galicia/Cantabria	IEO
	2	92	16.5 – 23.0	South Portugal	IPIMAR
	3	40	17.0 – 22.6	Gulf of Biscay	CNRS
	4	42	13.0 – 18.4	W. Mediterranean (Lyon's Gulf)	CNRS
Collection for consistency in time (Spring)	5	50	11.0 – 21.0	North Portugal (80's)	IPIMAR
	6	49	14.3 – 22.9	North Portugal (90's)	IPIMAR
	7	50	15.5 – 25.5	North Galicia/Cantabria (80's)	IEO
	8	49	18.0 – 25.0	North Galicia/Cantabria (90's)	IEO
Collection for consistency in time Northwest Africa (Spring)	9	50	17.3 – 23.5	Morocco (Larache/Casablanca)	INRH
	10	50	12.5 – 12.5	Southern Morocco/Mauritania	INRH
TOTAL		555			

Table 5.1.2 – List of age readers and samples analysed.

		SET									
		Main collection (June-July 2003)				Consistency: N. Portugal and N. Galicia/Cantabria (Spring 80's and 90's)				Consistency NW Africa (Spring)	
Reader		1	2	3	4	5	6	7	8	9	10
		IEO	IPIMAR	CNRS	CNRS	IPIMAR	IPIMAR	IEO	IEO	INRH	INRH
Eduardo Soares (1) **	ES	✓	✓	✓	✓	✓	✓	-	-	✓	✓
Alexandra Silva (1)	AS	✓	✓	✓	-	-	-	-	-	✓	✓
Alexandre Morais (1)	AM	✓	✓	✓	✓	-	-	-	-	-	-
Delfina Morais (1) **	DM	✓	✓	✓	✓	✓	✓	-	-	✓	✓
Afonso Jorge (1) **	AJ	✓	✓	✓	✓	✓	✓	-	-	✓	✓
Quena Peleteiro (2) **	QP	✓	✓	✓	✓	-	-	✓	✓	✓	✓
Isabel Loureiro (2)	IL	✓	✓	✓	✓	-	-	✓	✓	✓	✓
Iñaki Rico (3) **	IR	✓	✓	✓	✓	-	-	-	-	✓	✓
Véronique Laurent (4)	VL	✓	✓	✓	✓	-	-	-	-	✓	✓
Erwan Duhamel (5)	ED	✓	✓	✓	✓	-	-	-	-	✓	✓
Ahmed El Youssufi (6)	AY	✓	✓	✓	✓	-	-	-	-	✓*	✓*
Said Semmoumy (6)	SS	✓	✓	✓	✓	-	-	-	-	✓*	✓*
Gudrun Gentschow (7)	GG	✓	✓	✓	✓	-	-	-	-	✓	✓

*Otolith sets jointly read by both Moroccan readers; ** Experienced readers.
(1): IPIMAR – Lisbon (Portugal); (2): IEO – Vigo (Galicia - Spain); (3): AZTI – Santander (Gipuzkoa - Spain); (4): Univ. Perpignan – Perpignan (France); (5): IFREMER – Lorient (France); (6): INRH – Casablanca (Morocco); (7): Germany??.

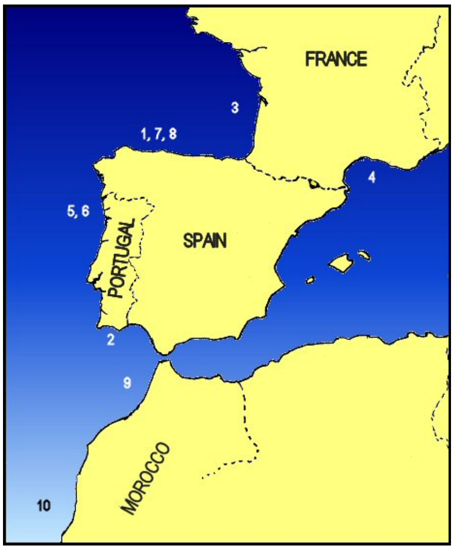

Figure 5.1.1 – Collection areas of 2004 exchange otolith sample sets.

Data were analysed using the workbook "Age reading comparison" of Eltink (2000) and following the recommendations of the "Guidelines and tools for age reading comparisons" (Eltink *et al.*, 2000).

5.2. Results and Discussion

5.2.1. Readability of the otoliths

Overall, 47% of the otoliths from Sets 1-4, 9 and 10, covering the Atlantic area between the Gulf of Biscay and Mauritania and the north western Mediterranean (see Table 5.1.1) have a medium readability while 23% and 29% were considered good and difficult, respectively (Table 5.2.1.1). Samples from the northern Atlantic areas, Gulf of Biscay (Set 3) and Cantabrian Sea (Set 1) and from the Gulf of Lyon (Set 4) have the clearest structure with low percentages of difficult otoliths (3-7%). Good otoliths are however scarcer in the Mediterranean sample. The structure of otoliths is more complex in the south of Portugal (Set 2) where 36% of the otoliths were considered difficult and the complexity increases towards the southern areas in the Atlantic. In Mauritania no good otoliths were observed and 78% of them were considered difficult to read.

Older otoliths present considerably less clear structure in all areas (Figure 5.2.1.1).

Table 5.2.1.1 - Otolith readability

Readability	Set 1	Set 2	Set 3	Set 4	Set 9	Set 10	Total
Good	41	12	65	26	2	0	23
Medium	51	52	33	69	50	22	47
Difficult	7	36	3	5	48	78	29

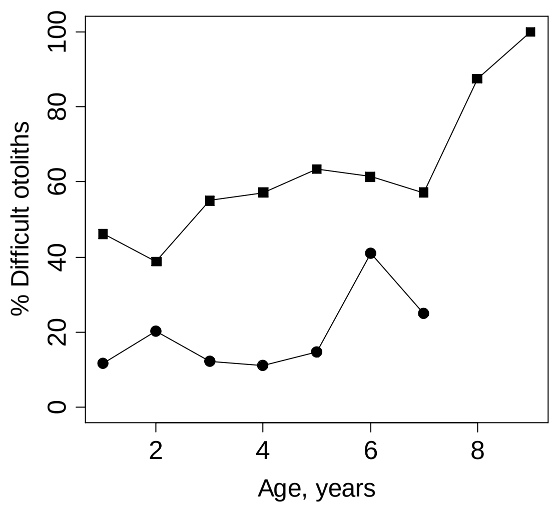

Figure 5.2.1.1 – Percentage of difficult otoliths by age group in data pooled from sets 1, 3, 4 (squares) and sets 2, 9, 10 (circles).

When difficult otoliths from Set 2 are excluded from the analysis, overall agreement of experienced readers with the modal age increased 7%, CV decreased 2% and relative bias became negligible (0.03). Within age groups 1 to 4, agreement remained above 70% showing a considerable improvement compared to 54-72% when all otoliths were included in the analysis (see also Table 5.2.2.3). Point estimates of mean length-at-age were comparable when all otoliths or only good+medium otoliths were included in the analysis (Figure 5.2.1.2). On the other hand, when difficult otoliths were excluded, the precision of the mean length-at-age increased considerably beyond age 2.

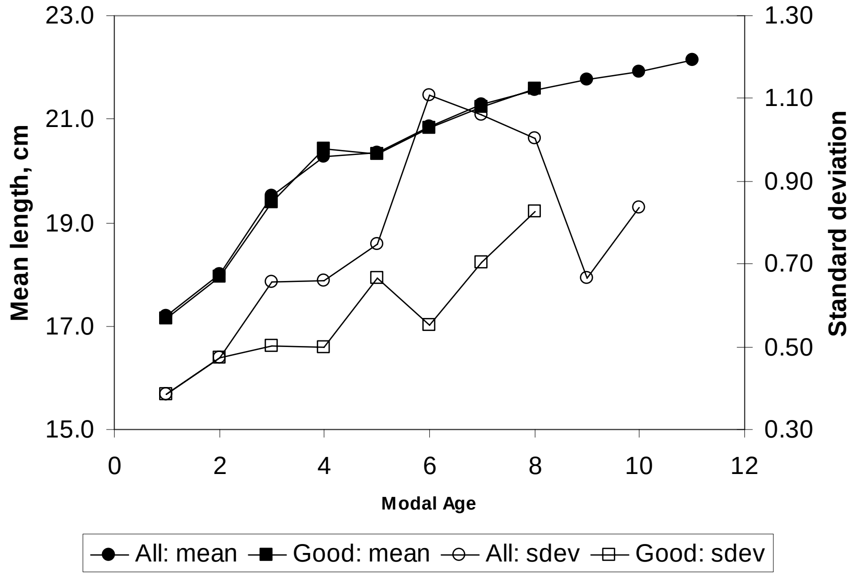

Figure 5.2.1.2 – Mean length at age comparison and standard deviation

5.2.2. Comparison of age readings

<u>Main collection (June-July 2003)</u>

SET 1 - *North Galicia/Cantabria*

The average percentage of agreement with the modal age across all ages and experienced readers in this set was 72% and the average coefficient of variation (CV) was 23% with higher values on the youngest age groups (45% at age 0 and 40% at age 1) (Table 5.2.2.1). Although the pattern of precision with age was variable among readers, in general they all tended to be less precise in younger ages. Mean agreement with the modal age decreased from 94% at age 0 to 40% at age 9 (Table 5.2.2.2).

Table 5.2.2.1 – SET 1 North Galicia/Cantabria: percentage of agreement with the modal age across all ages and readers, CV and reading bias.

Modal Age	Otolith N	CV	% Agreement	Bias
0	13	45	94	0.06
1	9	40	80	0.02
2	7	29	70	0.36
3	12	18	72	0.09
4	9	15	64	0.07
5	13	11	66	0.23
6	5	10	76	-0.20
7	13	12	63	-0.11
8	1		80	-0.20
9	1		40	-1.00
TOTAL	83	23	72	0.06

Table 5.2.2.2 – SET 1 North Galicia/Cantabria: Mean agreement (all readers) with the modal age and inter reader bias test.

	AJ	IR	DM	QP	ES	AM	AS	IL	ED	GG	SS	AY	VL
AJ	89	**	*	–	*	–	–	–	–	**	**	**	–
IR	67	76	**	–	**	–	**	–	**	*	**	**	*
DM	65	55	70	*	–	*	–	**	–	**	**	**	–
QP	60	64	42	43	*	–	*	–	**	**	**	**	–
ES	61	52	58	41	61	*	–	**	–	**	**	**	–
AM	65	51	57	55	55	61	*	–	*	**	**	**	–
AS	71	50	61	56	71	62	63	**	–	**	**	**	–
IL	64	57	47	59	47	55	61	59	**	**	**	**	*
ED	38	49	25	52	18	38	28	42	38	**	**	**	–
GG	53	55	37	64	47	46	49	63	31	54	–	*	**
SS	40	35	36	31	30	31	35	37	17	33	40	–	**
AY	41	38	37	32	32	31	32	38	21	36	67	39	**
VL	36	40	29	36	19	27	24	35	51	35	17	20	37
Modal age	–	**	*	–	*	–	*	–	*	**	**	**	–

– = no sign of bias (p>0.05)
* = possibility of bias (0.01<p<0.05)
** = certainty of bias (p<0.01)
(yellow box) = percentage of reading agreement between each reader and the modal age

From age bias plots (Figure 5.2.2.1) it was observed that among all readers AJ was the one who showed less biased readings. In general, all readers showed a trend to overestimate the younger fish ages and underestimate the older ones, although IR showed a general trend to underestimate all ages. The Moroccan readers (SS and AY) showed the less precise readings and a trend to considerably underestimate the older ages (age 3 onwards). Among experienced readers, agreement varied from 41% (QP-ES) to 67% (AJ - IR) (Table 5.2.2.2). Among these readers, QP showed no signs of bias in two of the four cases of inter-reader bias test (versus AJ and IR). Readers against modal age showed percentage values of agreement ranging from 43% (QP) to 89%

(AJ) (Table 5.2.2.2). These readers also were the only ones that showed no sign of bias against modal age.

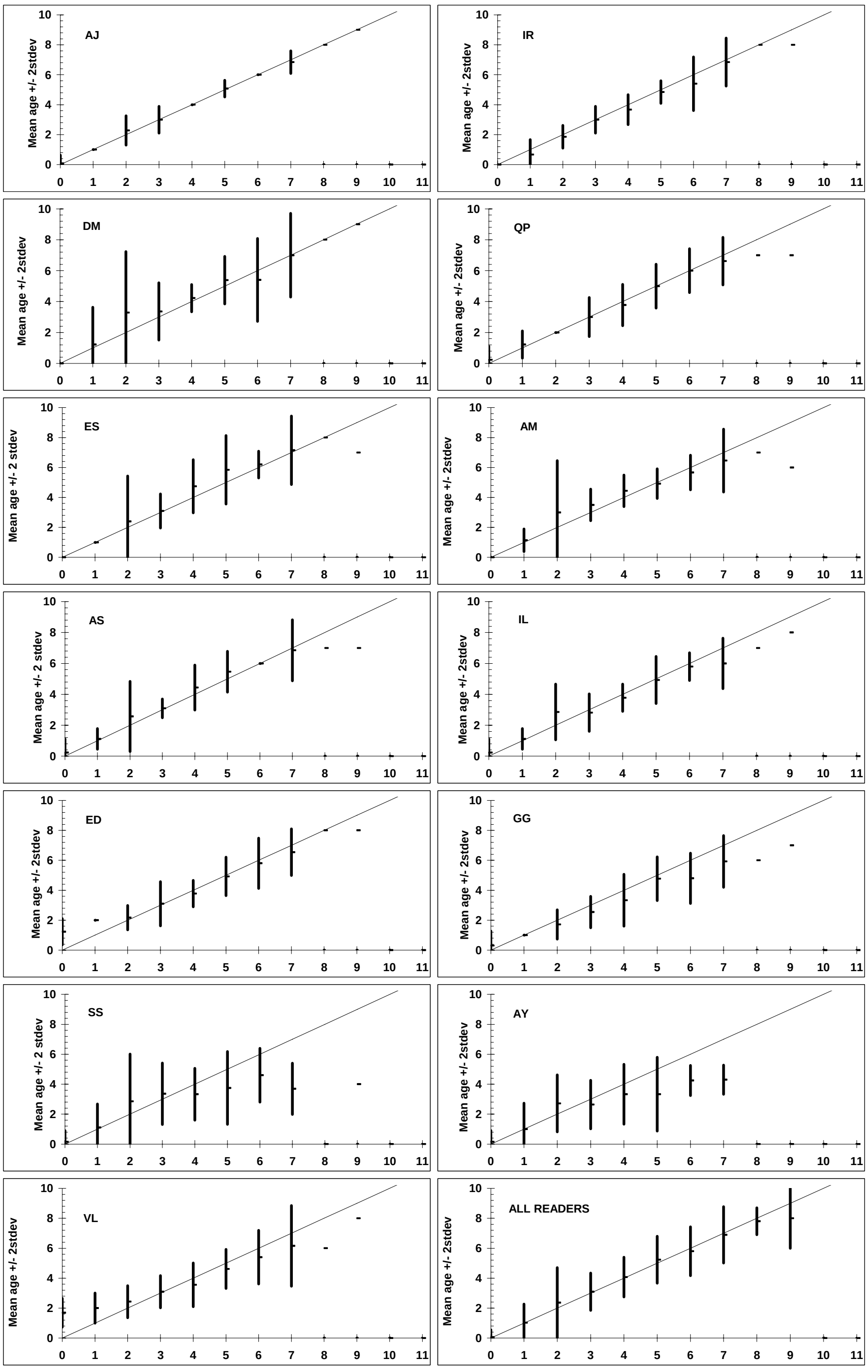

Figure 5.2.2.1 – SET 1 North Galicia/Cantabria: Age bias plots.

Among the inexperienced readers, percentages of agreement with modal age ranged from 37% (VL) to 63% (AS). Readers AM, IL and VL showed no signs of bias against modal age.

SET 2 – *South Portugal*

The average percentage of agreement with the modal age across all ages and readers in this set was 67% and the average CV was 14% with higher values on the youngest age groups (20% at age 1 and 17% at age 2) (Table 5.2.2.3). The general trend of readers to be less precise in younger ages was also observed. Mean agreement (all readers) with the modal age decreased from 72% at age 2 to 17% at age 10.

Table 5.2.2.3 – SET 2 South Portugal: percentage of agreement with the modal age across all ages and readers, CV and reading bias.

Modal Age	Otolith N	CV	% Agreement	Bias
1	6	20	64	0.17
2	16	17	72	0.08
3	12	14	57	0.19
4	7	15	54	0.09
5	18	13	46	-0.02
6	10	14	32	0.42
7	13	12	34	0.31
8	7	12	33	-0.23
9	2	7	26	0.00
10	1		17	-0.20
TOTAL	92	14	67	0.12

Table 5.2.2.4 – SET 2 South Portugal: average CV across all ages and readers.

Modal Age	AJ	QP	ES	DM	IR	AM	AS	IL	ED	GG	SS	AY	VL	All Readers
1	39	35	0	35	35	0	110	35	32	100	37	35	22	20
2	0	23	33	16	26	27	34	33	21	16	36	33	25	17
3	17	14	26	18	26	19	42	9	17	40	28	43	33	14
4	10	14	22	9	8	14	34	14	12	23	33	28	53	15
5	8	18	17	15	14	15	13	13	17	25	26	19	34	13
6	17	8	21	19	12	21	24	16	14	22	12	15	54	14
7	6	11	10	18	13	13	19	16	20	16	15	19	31	12
8	15	16	11	6	6	11	13	20	7	13	16	13	40	12
9	0	8	8	7	7	-	-	13	16	16	0	13	35	7
0-9	11	17	19	16	17	17	31	19	18	27	24	25	35	14

Table 5.2.2.5 – SET 2 South Portugal: percentage of agreement with modal age.

Modal Age	AJ	QP	ES	DM	IR	AM	AS	IL	ED	GG	SS	AY	VL	All Readers
1	67	83	100	83	83	100	50	83	17	33	50	83	0	64
2	100	81	60	88	75	75	50	75	63	88	56	56	69	72
3	75	75	73	92	58	73	55	92	75	25	25	17	8	57
4	86	71	71	86	14	71	57	71	67	57	29	29	0	54
5	83	61	50	56	56	39	61	33	44	44	28	39	6	46
6	90	80	50	30	40	20	30	0	44	30	0	0	0	32
7	85	62	54	31	46	45	45	8	23	33	0	8	0	34
8	71	43	57	57	71	29	29	0	33	29	0	0	14	33
9	100	50	50	50	50	-	0	0	0	0	0	0	0	26
10	100	0	0	100	0	-	0	0	0	0	0	0	0	17
0-10	85	68	60	64	55	55	48	43	46	44	24	28	15	67

Age bias plots show that there was a general trend among all readers to underestimate the age of older fish (>4 years) (Figure 5.2.2.6). Among the experienced readers, this trend was apparent for AJ and QP, but not for the three other readers.

Among experienced readers, percentage of agreement varied from 35% (ES- IR) to 63% (AJ - QP) (Table 5.2.2.6). All these readers showed bias between them. Reader against modal age showed percentage values of agreement ranging from 55% (IR) to 85% (AJ). Reader AJ was the only one that showed no sign of bias against modal age.

Among the inexperienced readers the percentage of agreement with modal age showed values ranging from 15% (VL) to 55% (AM), all readers showing bias against modal age.

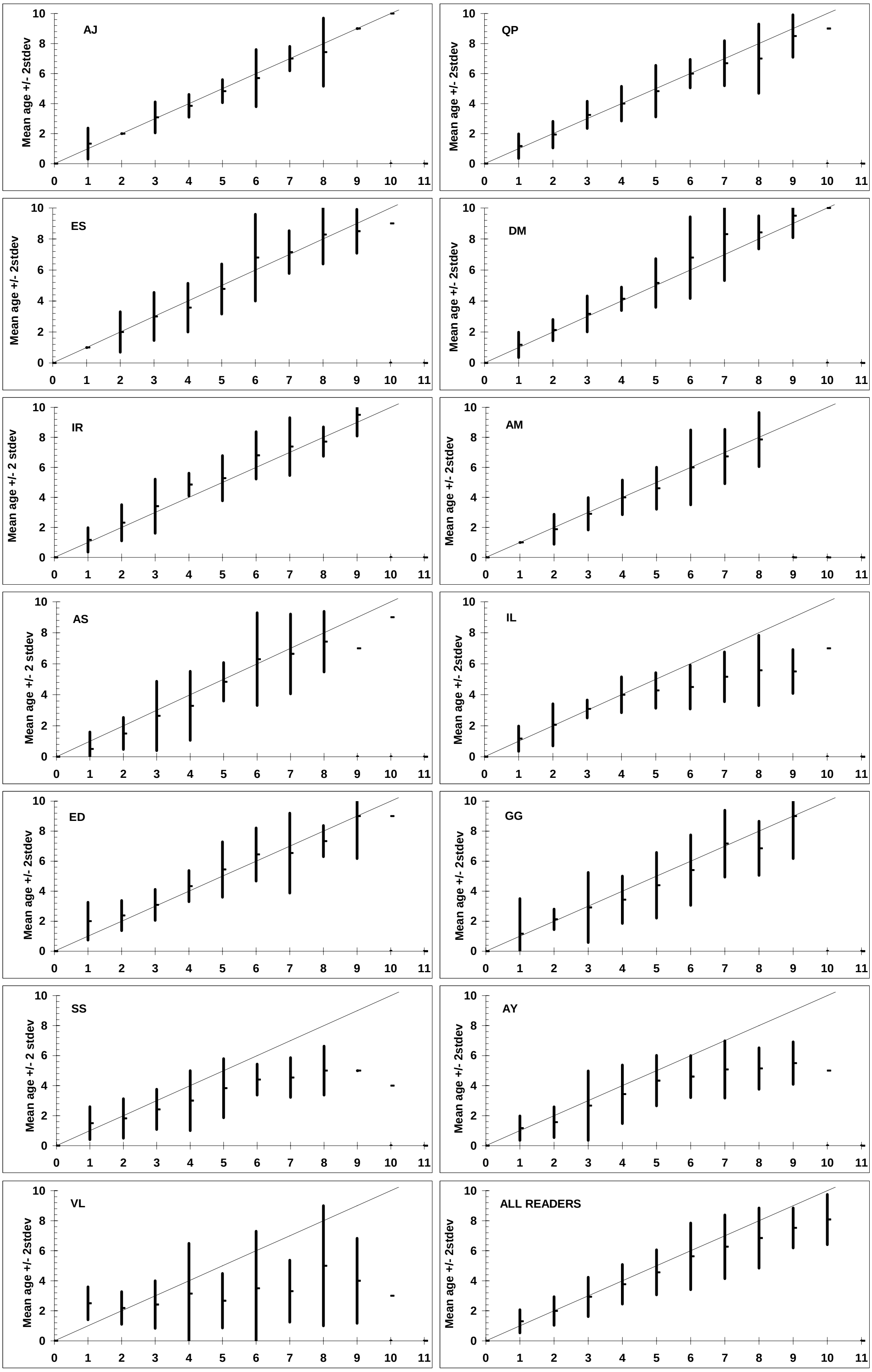

Figure 5.2.2.6 – SET 2 South Portugal: Age bias plots.

Table 5.2.2.6 – SET 2 South Portugal: Mean agreement (all readers) with the modal age and inter reader bias test.

	AJ	QP	ES	DM	IR	AM	AS	IL	ED	GG	SS	AY	VL
AJ	85	*	**	**	**	–	**	**	**	*	**	**	**
QP	63	68	**	**	**	–	*	**	**	–	**	**	**
ES	46	47	60	**	**	**	**	**	*	**	**	**	**
DM	53	47	54	64	**	**	**	**	**	**	**	**	**
IR	47	45	35	48	55	**	**	**	**	**	**	**	**
AM	49	43	52	55	41	55	**	**	**	*	**	**	**
AS	47	49	42	40	30	52	48	**	**	–	**	**	**
IL	47	36	39	39	28	49	31	48	**	**	**	**	**
ED	46	48	30	44	46	38	34	30	46	**	**	**	**
GG	43	40	34	40	38	34	30	31	35	44	**	**	**
SS	29	25	18	22	17	19	23	37	24	26	24	**	**
AY	29	33	28	24	21	36	27	43	17	36	42	28	**
VL	18	24	8	12	18	13	14	22	17	22	32	25	15
Modal Age	-	**	*	**	**	*	**	**	*	**	**	**	**

– = no sign of bias ($p>0.05$)
* = possibility of bias ($0.01<p<0.05$)
** = certainty of bias ($p<0.01$)
= percentage of reading agreement between each reader and the modal age

***SET 3** – Gulf of Biscay*

The average percentage of agreement with the modal age across all ages and readers in this set was 78% and the average CV was 16% with the highest value on the age group 2 (27%) (Table 5.2.2.7). Readers showed again a general trend to be less precise in younger ages. Mean agreement (all readers) with the modal age decreased from 98% at age 1 to 60% at age 6.

Table 5.2.2.7 – SET 3 Gulf of Biscay: percentage of agreement with the modal age across all ages and readers, CV and reading bias.

Modal Age	Otolith N	CV	% Agreement	Bias
1	10	4	98	0.02
2	11	27	72	0.11
3	10	16	80	-0.16
4	4	18	70	-0.40
5	4	14	55	-0.15
6	1		60	0.40
TOTAL	40	16	78	-0.05

Among experienced readers the percentage of agreement varied from 26% (ES – DM) to 90% (AJ – IR and AJ - QP) (Table 5.2.2.8) and reader against modal age showed agreements ranging from 56% (ES) to 93% (AJ and QP).

The age bias plot for all readers indicate underestimation of ages 3-5, and overestimation of age 6 (Figure 5.2.2.7). AJ, IR and AM generally showed low bias in readings, although AJ underestimated age 4 and IR and AM overestimated ages 5 and 6. Two of the experienced readers which showed average performance in Sets 1 and 2, DM and ES, had unexpectedly poor results in this Set. DM showed a trend to overestimate ages 2, 5 and 6 and ES underestimated from age 2 onwards.

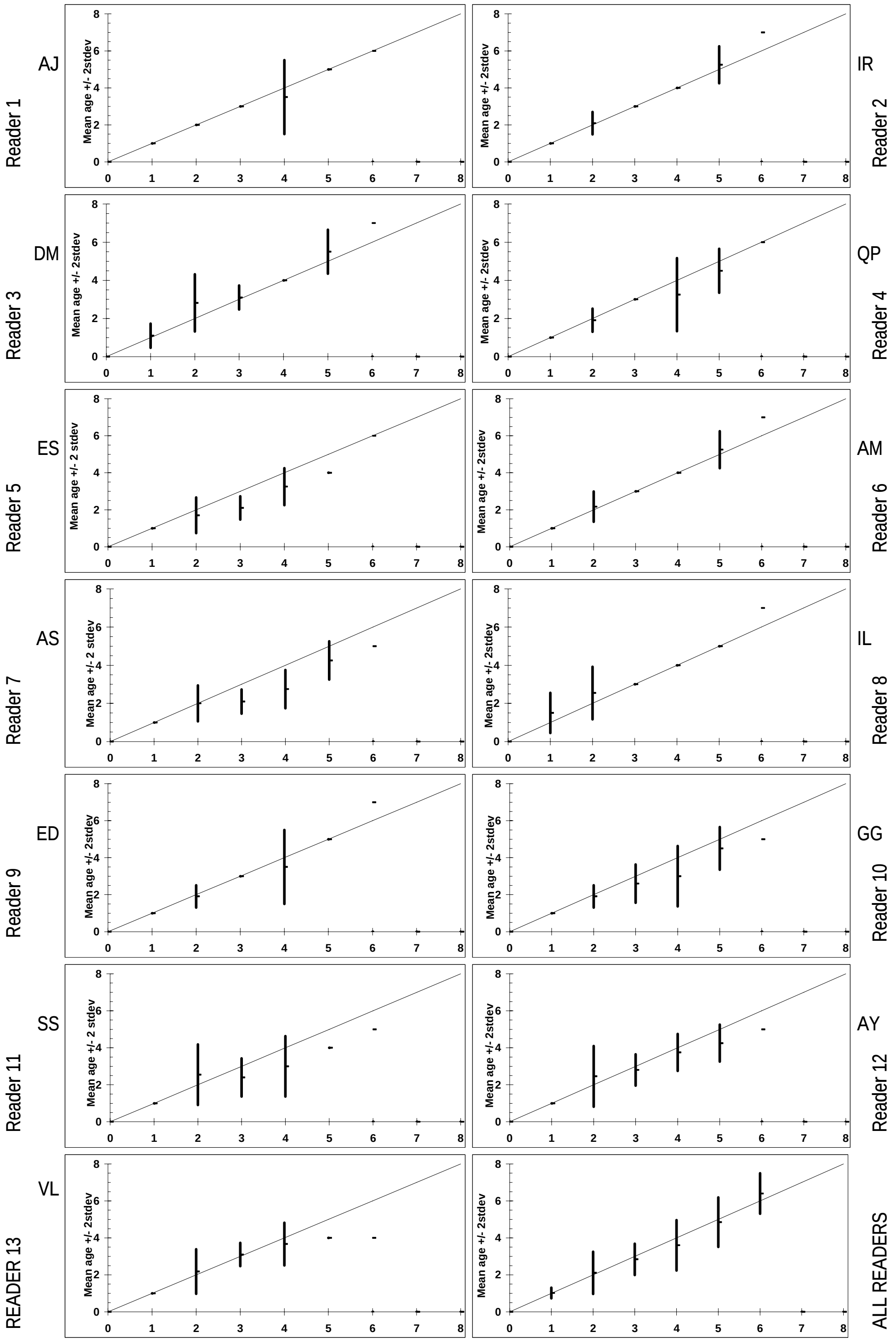

Figure 5.2.2.7 – SET 3 Gulf of Biscay: Age bias plots.

Table 5.2.2.8 – SET 3 Gulf of Biscay: Mean agreement (all readers) with the modal age and inter reader bias test.

	AJ	IR	DM	QP	ES	AM	AS	IL	ED	GG	SS	AY	VL
AJ	93	–	**	–	**	–	**	**	–	**	–	–	–
IR	90	88	**	*	**	–	**	*	–	**	*	–	–
DM	60	70	58	**	**	**	**	*	**	**	**	**	**
QP	90	80	55	93	*	*	*	**	–	*	–	–	–
ES	49	49	26	54	56	**	–	**	**	–	–	**	**
AM	91	97	79	79	42	85	**	–	–	**	**	*	–
AS	51	51	26	56	85	45	56	**	**	–	–	**	*
IL	70	75	65	60	31	76	31	68	**	**	–	–	–
ED	95	90	65	90	44	91	49	70	88	**	–	–	–
GG	75	68	43	85	64	67	72	48	78	78	–	–	–
SS	50	48	48	55	67	55	67	33	53	60	53	–	–
AY	75	73	53	75	56	73	59	55	73	70	68	80	–
VL	82	82	58	82	55	76	53	58	79	68	61	82	87
Modal Age	–	*	**	–	**	*	**	**	–	**	–	–	–

– = no sign of bias (p>0.05)
* = possibility of bias (0.01<p<0.05)
** = certainty of bias (p<0.01)
= percentage of reading agreement between each reader and the modal age

SET 4 – *Lyon's Gulf*

The average percentage of agreement with the modal age across all ages and readers was 65% and the average CV was 48% with higher values on the youngest age groups (Table 5.2.2.9). A general trend to be less precise in younger ages was again shown by all readers. Agreement with the modal age was lower in younger (ages 0-3) than in older individuals (ages 4 and 5) suggesting problems with the identification of the first ring.

Table 5.2.2.9 – SET 4 Lyon's Gulf: percentage of agreement with the modal age across all ages and readers, CV and reading bias.

Modal Age	Otolith N	CV	% Agreement	Bias
0	3	137	60	0.40
1	13	71	67	0.02
2	10	47	48	0.12
3	6	22	70	-0.10
4	6	8	83	-0.03
5	1	-	100	0.00
6	2	15	50	-0.20
7	1		80	-0.40
TOTAL	42	48	65	0.06

A general trend among all readers to underestimate older fish was not clearly apparent from age bias plots (Figure 5.2.2.8). Among experienced readers the percentage of agreement varied from 27% (ES - QP) to 57% (AJ - IR and AJ - QP) (Table 5.2.2.10). Most of these readers showed bias between them, except ES versus IR and QP versus DM. Reader against modal age bias test showed agreements ranging from 51% (ES) to 95% (AJ). Reader AJ was the only one that showed no sign of bias against modal age.

Among the inexperienced readers the percentage of agreement with modal age ranged from 12% (AM) to 64% (AY). Readers AM, ED and VL showed bias against modal age.

Table 5.2.2.10 – SET 4 Lyon's Gulf: Mean agreement (all readers) with the modal age and inter reader bias test.

	AJ	IR	QP	ES	DM	AM	IL	AY	SS	GG	ED	VL
AJ	95	*	*	**	*	**	–	–	–	–	**	**
IR	57	62	**	–	**	**	*	*	–	*	**	*
QP	57	36	60	**	–	**	–	–	*	–	**	**
ES	46	51	27	51	**	**	**	**	*	**	**	–
DM	55	43	48	37	57	**	–	*	*	–	**	**
AM	12	7	24	5	29	12	**	**	**	**	**	**
IL	50	45	38	46	48	10	55	–	*	–	**	**
AY	69	55	45	46	38	5	57	64	–	–	**	**
SS	40	43	31	56	31	5	50	55	43	*	**	**
GG	43	38	26	46	31	5	43	57	67	38	**	**
ED	17	37	17	24	10	0	15	15	20	15	20	*
VL	36	45	21	49	29	5	36	38	43	26	48	40
Modal Age	–	*	*	**	**	**	–	–	–	–	**	**

– = no sign of bias (p>0.05)
* = possibility of bias (0.01<p<0.05)
** = certainty of bias (p<0.01)
[yellow box] = percentage of reading agreement between each reader and the modal age

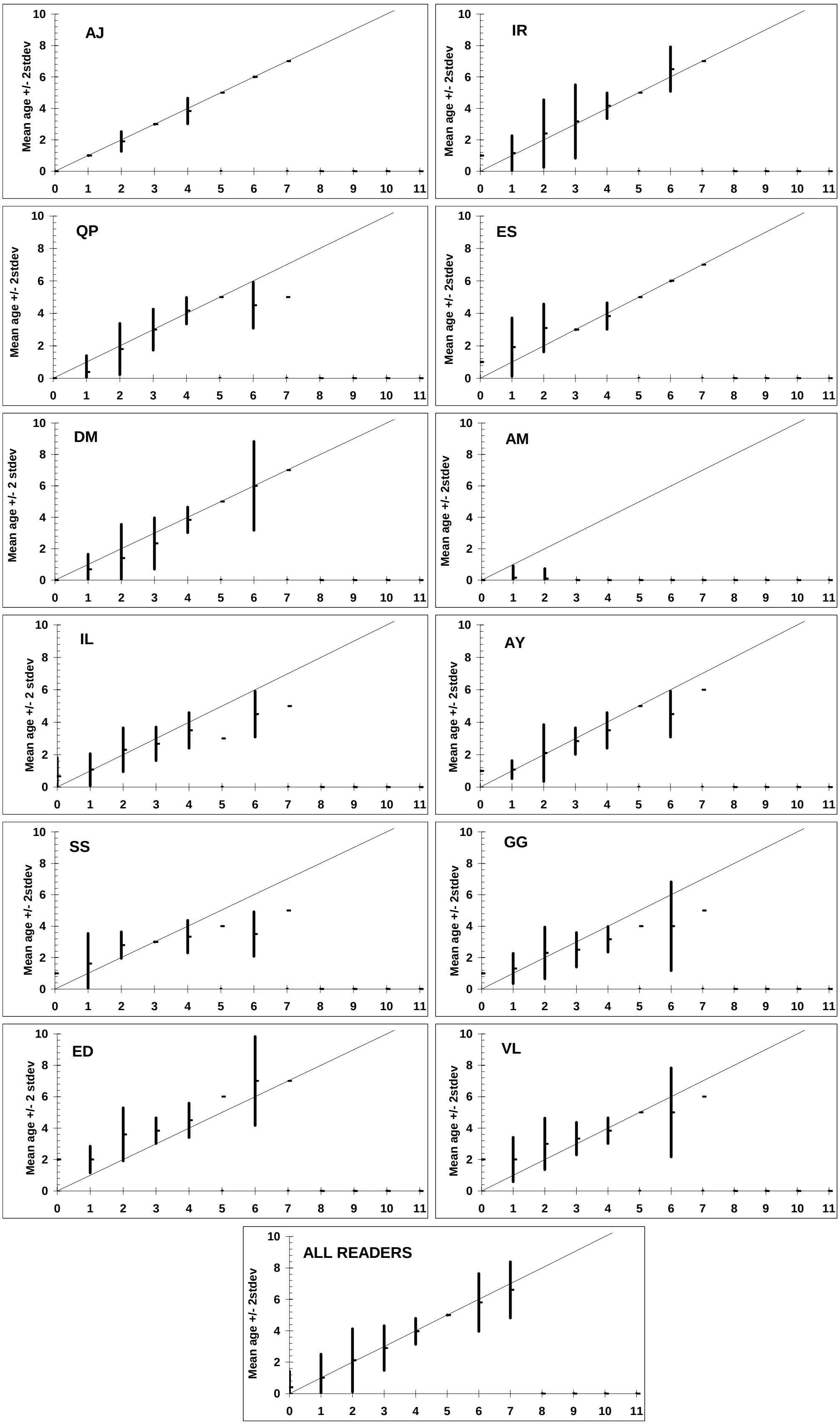

Figure 5.2.2.9 – SET 4 Lyon's Gulf: Age bias plots.

Collection for consistency in time (Spring)

***SET 5 –** North Portugal (80's)*

The average percentage of agreement with the modal age across all ages and readers was 62% and the average CV was 17% with the highest value (36%) at age 1 (Table 5.2.2.11). A trend about the variation of readings precision with modal age was not apparent, with the lowest value at age 1 (CV=36%). Nevertheless, a decreasing trend of mean agreement (all readers) with the modal age was observed (from 93% at age 0 to 41% at age 4).

Table 5.2.2.11 – SET 5 North Portugal (80's): percentage of agreement with the modal age across all ages and readers, CV and reading bias.

Modal Age	Otolith N	CV	Agreement	Bias
0	9	10	93	0.07
1	10	36	73	-0.20
2	10	9	63	-0.17
3	10	17	43	0.23
4	10	12	41	-0.38
TOTAL	49	17	62	-0.09

From age bias plots it was detected that in general readers tended to underestimate all ages, except ages 0 and 3, this one overestimated (Figure 5.2.2.10). AJ showed the most consistent readings in relation to the 80's. The other two Portuguese readers (DM and ES) showed both a similar trend to underestimate all ages except ages 0 and 3, this one overestimated.

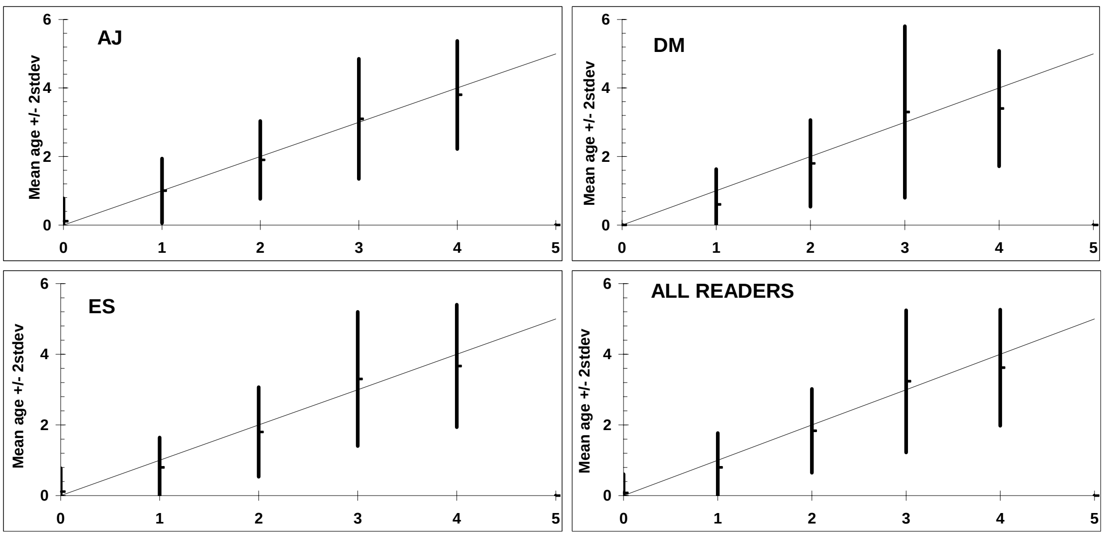

Figure 5.2.2.10 – SET 5 North Portugal (80's): Age bias plots.

***SET 6 –** North Portugal (90's)*

The average agreement with the modal age across all ages and readers was 46% and the average CV was 17% with the highest value (37%) at age 2 (Table 5.2.2.12). Precision of readings generally increased from younger to older ages. Readers were less precise at ages 1 and 2 (respectively with CV=35% and 37%). In general, there was a decreasing trend of mean agreement (all readers) with the modal age (from 67% at age 1 to 33% at age 8), except for age 7 (57%).

Table 5.2.2.12 – SET 6 North Portugal (90's): percentage of agreement with the modal age across all ages and readers, CV and reading bias.

Modal Age	Otolith N	CV	Agreement	Bias
1	6	35	67	0.33
2	6	37	61	-0.28
3	6	17	56	0.00
4	7	9	24	0.81
5	6	15	33	1.39
6	7	11	33	0.43
7	7	7	57	0.05
8	4	8	33	0.17
TOTAL	49	17	46	0.37

Based on age bias plots, it was observed that there was an underestimation of age 2 and an overestimation of ages 4, 5 and 6 (Figure 5.2.2.11). AJ was the reader who showed less biased readings, showing better consistency with the ones of the 90's. The other two Portuguese readers (DM and ES) were not so consistent showing both a similar trend to overestimate ages 4, 5, 6 and 8.

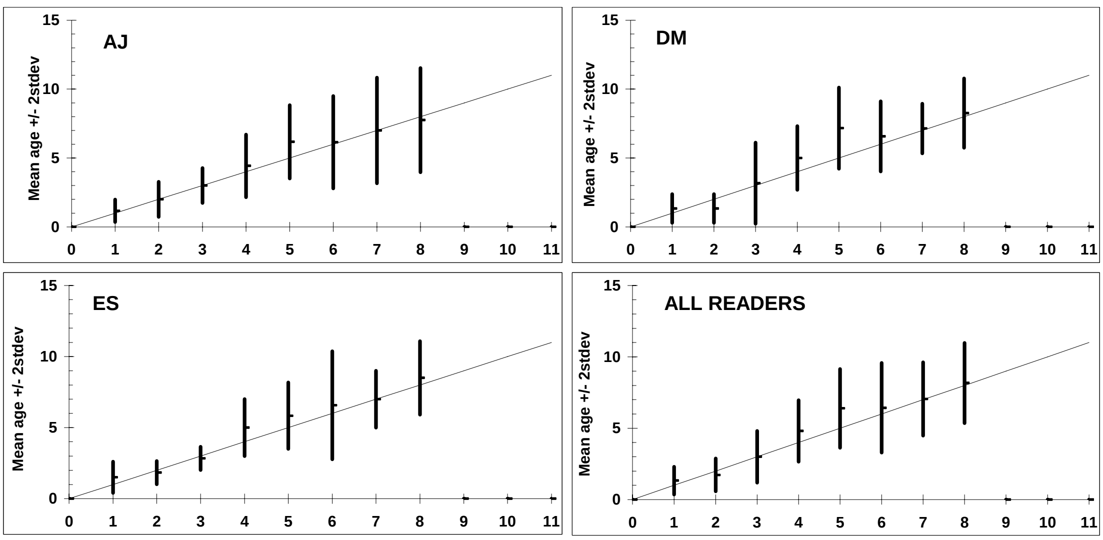

Figure 5.2.2.11 – SET 6 North Portugal (90's): Age bias plots.

SET 7 – *North Galicia/Cantabria (80's)*

The average agreement with the modal age across all ages and readers was 41% and the average CV was 13% with the highest value (15%) at age 9 (Table 5.2.2.13). Precision of readings did not show any trend in relation to modal age. Readers were less precise at ages 5 and 9 (respectively with CV=14% and 15%). In general, there was a decreasing trend of mean agreement (all readers) with the modal age (from 78% at age 1 to 0% at age 9).

Table 5.2.2.13 – SET 7 North Galicia (80's): percentage of agreement with the modal age across all ages and readers, CV and reading bias.

Modal Age	Otolith N	CV	Agreement	Bias
1	9	10	78	0.22
2	1		50	1.00
3				
4				
5	8	14	50	-0.56
6	8	10	44	-0.25
7	8	9	38	-0.75
8	9	11	28	-1.00
9	4	15	0	-2.38
10	2			
11	1			
TOTAL	50	13	41	-0.56

Based on age bias plots, it was observed that there was a general trend of both Spanish readers to underestimate older ages (>3). Present readings were not very consistent with the ones of the 80's (Figure 5.2.2.12).

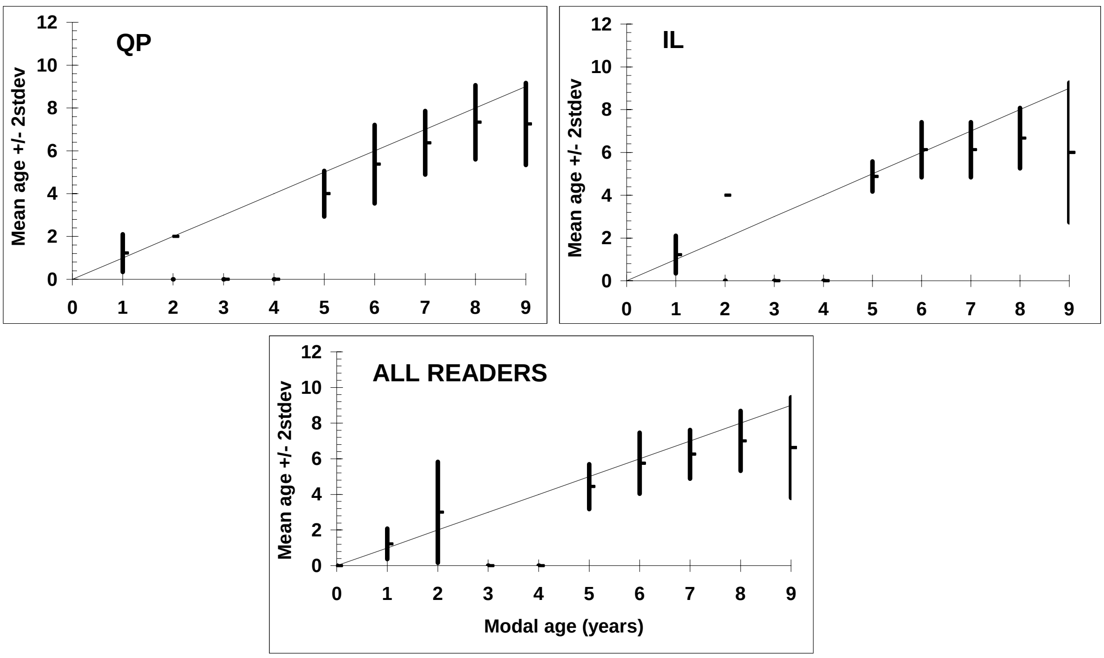

Figure 5.2.2.12 – SET 7 North Galicia (80's): Age bias plots.

SET 8 – *North Galicia/Cantabria (90's)*

The average agreement with the modal age across all ages and readers was very low (27%) and the average CV was 15% with the highest value (26%) at age 2 (Table 5.2.2.14). Precision of readings did not show any trend in relation to modal age. Readers were less precise at age 2 (CV=26%).

Table 5.2.2.14 – SET 8 North Galicia/Cantabria (90's): percentage of agreement with the modal age across all ages and readers, CV and reading bias.

Modal Age	Otolith N	CV	Agreement	Bias
1	3	16	83	0.17
2	4	26	63	0.50
3	4	16	75	0.13
4	4	9	75	0.00
5	4	7	0	-0.17
6	4	11	38	-0.88
7	4	9	13	-1.13
8	4	8		-1.63
9	4	8		-2.63
TOTAL	50	15	27	-0.46

Based on age bias plots, there was a general trend of both Spanish readers to underestimate older ages (>5) in relation to the 90's readings (Figure 5.2.2.13). QP showed some consistency at ages 1 and 2, but underestimated older ages (>5). IL showed a very low consistency against the 90's readings, overestimating younger ages and overestimating older ones. In general, the present readings only showed some consistency at younger ages (0 to 5).

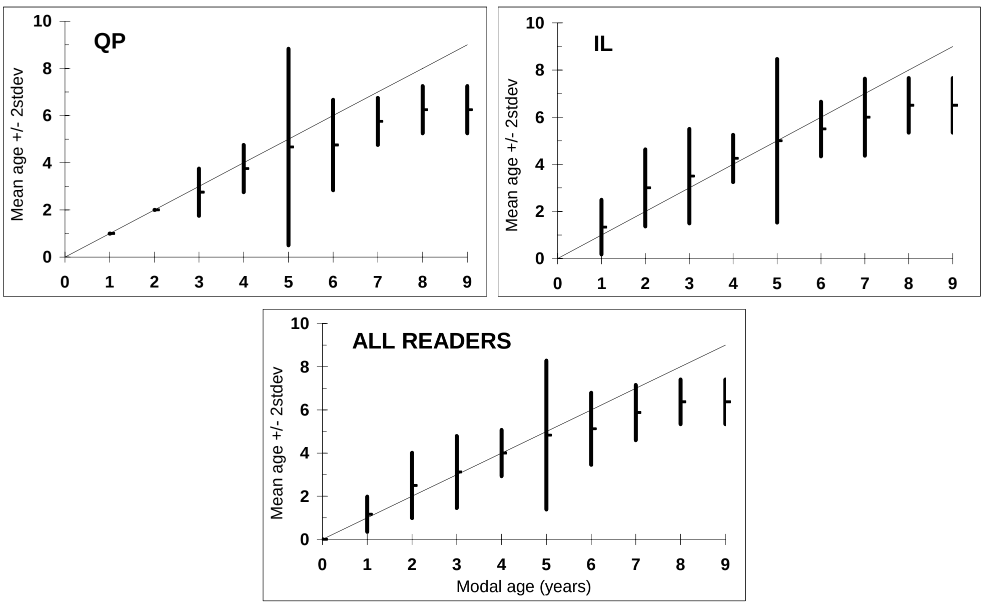

Figure 5.2.2.13 – SET 8 North Galicia/Cantabria (90's): Age bias plots.

Collection for consistency NW Africa (Spring)

SET 9 – *Morocco (Larache/Casablanca)*

The average agreement with the modal age across all ages and readers was very low (25%) and the average CV was 30%. Precision of readings decreased in relation to modal age and readers were less precise at age 2 (CV=30%) (Table 5.2.2.15). Ages only ranged from 2 to 4.

Table 5.2.2.15 – SET 9 Morocco (Larache/Casablanca): percentage of agreement with the modal age across all ages and readers, CV and reading bias.

Modal Age	Otolith N	CV	Agreement	Bias
2	3	11	87	0.20
3	23	30	31	0.35
4	24	31	21	0.49
TOTAL	50	30	25	0.41

Age bias plots showed a trend of all readers to overestimate all ages, except IR who showed an opposite trend (Figure 5.2.2.14).

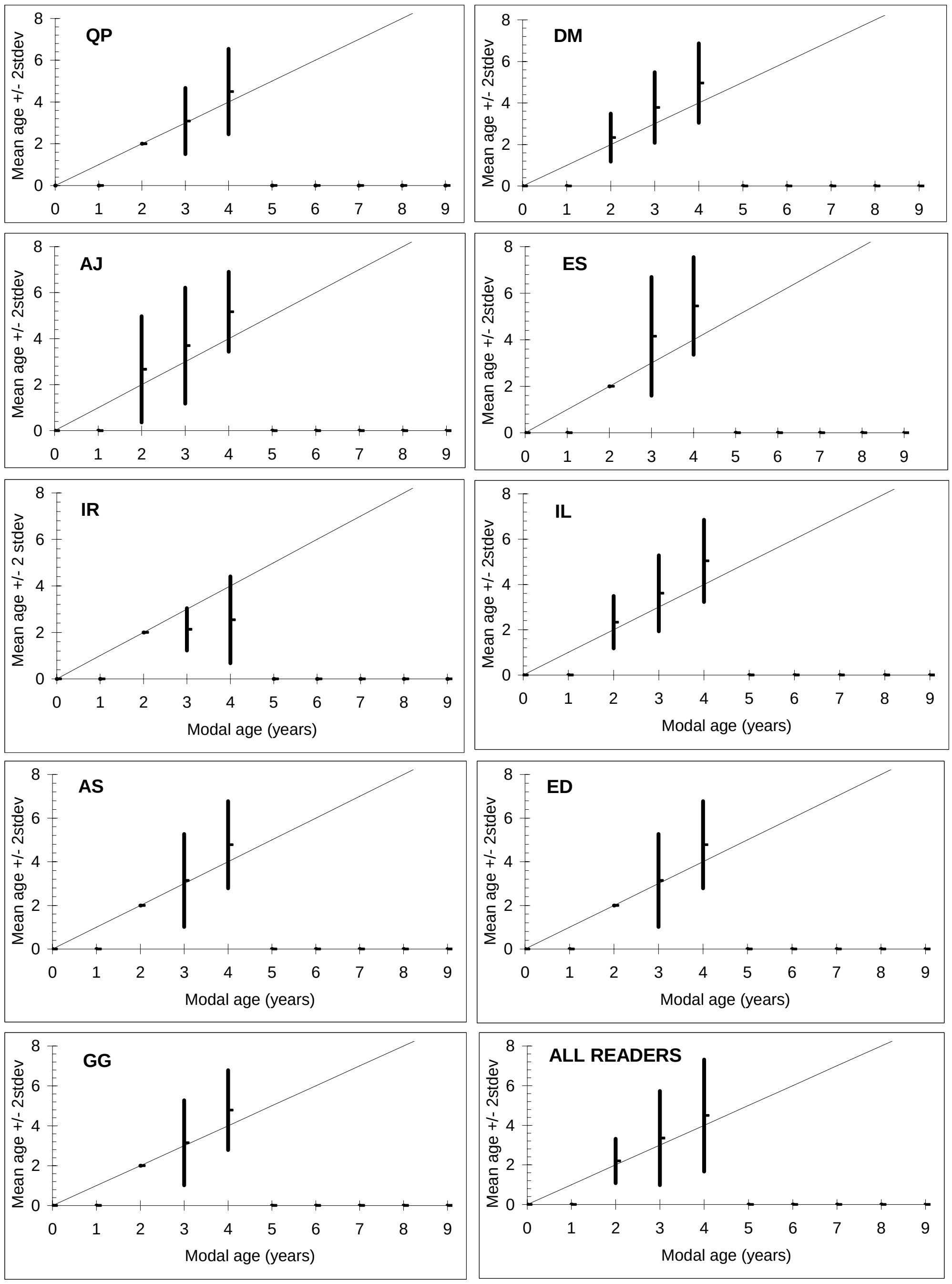

Figure 5.2.2.14 – SET 9 Morocco (Larache/Casablanca): Age bias plots.

SET 10 – *Southern Morocco/Mauritania*

Ages ranged from 1 to 5. The average agreement with the modal age across all ages and readers was also very low (32%) and the average CV was 33% (Table 5.2.2.16). Precision of readings decreased in relation to modal age and readers were less precise at ages 2 (CV=41%) and 3 (CV=42%).

Table 5.2.2.16 – SET 10 Southern Morocco/Mauritania: percentage of agreement with the modal age across all ages and readers, CV and reading bias.

Modal Age	Otolith N	CV	Agreement	Bias
1	2		75	0.35
2	7	41	45	0.45
3	14	42	21	0.25
4	17	28	26	0.28
5	5	16	45	0.15
TOTAL	45	33	32	0.26

Despite the inconsistency shown by some readers, age bias plots in general showed that these readings were more consistent with Moroccan ones than those in the precedent Set (Figure 5.2.2.15).

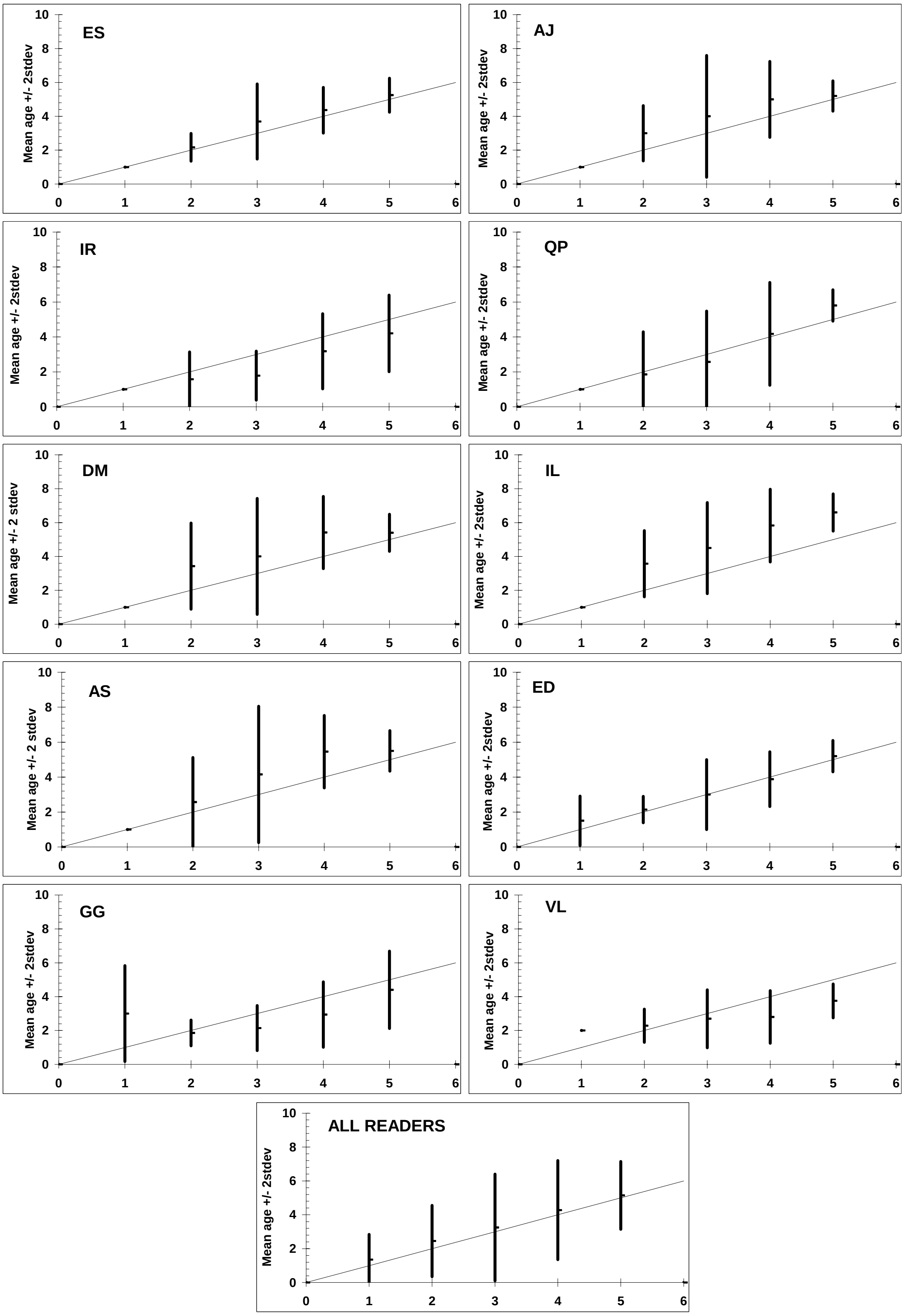

Figure 5.2.2.15 – SET 10 Southern Morocco/Mauritania: Age bias plots.

5.2.3. Classification of the otolith edge: seasonal pattern and agreement between readers

Data on the otolith edge type in commercial catch samples collected monthly off the North (Matosinhos/Póvoa), Central (Peniche) and South (Portimão) Portuguese coast since 1987 were explored to describe growth seasonality. The percentage of opaque and hyaline edges along the year was comparable among areas with the peak of hyaline edges in winter (January/February) and the peak of opaque edges in summer (June/August). On the other hand, the seasonality of the edge was dependent on the age of fish (Figure 5.2.3.1). The peak of opaque edges in younger individuals (ages 0 and 1) occurred in late spring (May-June) and moved gradually to mid summer (August) in fish of ages 6 and 7.

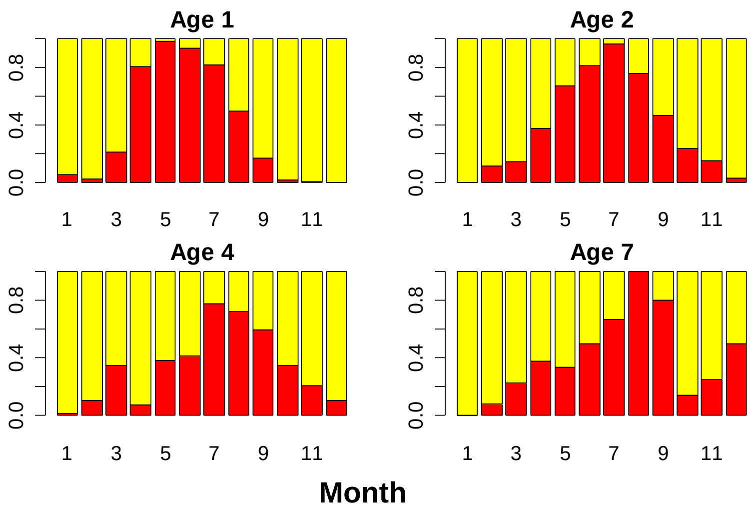

Figure 5.2.3.1 - Proportion of otoliths with opaque (red) and hyaline (yellow) edge by month off the northern Portuguese waters.

The classification of the otolith edge was compared among experienced readers for otolith Sets 1 and 2 (Table 5.2.3.1). The percentage of agreement between readers ranged from 39 to 96% being on average higher and more similar for the different readers for Set 2. Readers from the IPIMAR (AJ, DM and ES) showed better agreement possibly because they use the same side of the otolith for edge classification. QP shows systematic differences with IPIMAR readers attributing generally a lower number of hyaline edges. Disagreement of IR with the other readers occurs mainly on the first age groups.

The above differences on the classification of the otolith edge have possibly contributed to ageing discrepancies in the otolith collections from June-July (Sets 1-4) depending on how each reader interpreted the hyaline margin of each otolith. If the hyaline margin was considered to represent the end of the last slow growing season, it was included as an age ring. On the other hand if it was interpreted as the start of the next slow growing season it was not included in the age determination.

	AJ	IR	DM	QP	ES
AJ		64	92	46	81
IR	68		58	39	64
DM	73	59		40	84
QP	64	67	46		41
ES	72	58	96	45	

Table 5.2.3.1 - Percentage of agreement on edge type for otolith Sets 1 (above diagonal) and 2 (below diagonal).

5.2.4. Exchange main conclusions

A joint discussion on the structure of otoliths which raised more difficulties on age reading followed the presentation of the exchange results for each area. These otoliths were selected among those which showed lower average agreement with the modal age across all ages and readers. For this purpose it was used an image analysis system (Figure 5.2.4.1) consisting of a magnifying dissection microscope equipped with a high resolution video camera linked to a PC running the image analysis software NOESIS TNPC 4.1, VISILOG version 6.300, which was connected to a multimedia projector allowing all participants to watch the otoliths images on a large screen.

Figure 5.2.4.1 – Image analysis system used for the joint discussions on sardine otoliths structure.

These discussions helped to identify the structural differences among the otoliths from the different areas, to detect the main difficulties found by the readers during their analysis and contributed to the improvement of their reading accuracy in future work.

From the exchange results analysis it can be concluded that:

- For the Atlantic Iberian area the agreement among experienced readers was lower than that found during the last Workshop in 2002. The coefficient of variation (CV) which measures the difference between each age assigned by each reader to the same otolith has increased at ages 0-2.

- In relation to the last Workshop, the problems of first annual ring identification and the age assignment to older fish still persist. On the southern coast of Portugal these problems grow worse due to the weak contrast between otoliths opaque and hyaline zones. The identification of otolith edge and whether to decide to account it for age assignment are other recognized problems.

- Concerning the consistency of age readings currently carried out on otolith samples from the 80's and 90's when compared to the ones undertaken in those epochs it was found that there was a low reading agreement among current readers. Nevertheless, considering that only a trimester was analysed and that a very small number of otoliths was observed, this subject should be further investigated.

    - For the otoliths of the Portuguese coast, there was a general good age reading agreement with the 80's but relatively to the 90's, ages presently assigned are in general higher at ages 4-6 (although this is not apparent at ages 7-8).

    - Regarding the otoliths from the Spanish coast there was a trend to currently assign lower ages to fish older than 4 years than in the 80's and 90's.

- The analysis of consistency of age readings in the otoliths of the Mediterranean showed a level of agreement (65%) similar to that found for otoliths of the South of Portugal (Algarve). There was an increasing trend on the readings agreement with the age. The main problem detected in this area was the identification of the first annual ring.

- From the analysis of consistency of age readings on otoliths of NW Africa (Morocco (Larache/Casablanca) and Southern Morocco/Mauritania), a low age reading agreement was found between the Iberian sardine stock readers and the Moroccan ones. The former usually tend to assign older ages than the latter. On the other hand Moroccan readers tend to assign younger ages than the Iberian readers to the otoliths from the Iberian area. Besides, the greater opacity of Moroccan otoliths raises more difficulties in the identification of the annual rings.

– From this exchange it was observed that sardine from the different areas show different growth patterns and otoliths structure raising specific age reading problems in each area which need to be individually further investigated.

6. ALTERNATIVE METHODOLOGIES TO IMPROVE THE READABILITY OF SARDINE OTOLITHS

The otoliths structural differences among the different areas raise the need for the use of specific preparation techniques. In those areas where the otolith structure is less clear, it is advisable to try alternative otolith preparation techniques more suitable to clarify those structures and thus allowing more precise age readings, than the one currently used in the Atlantic Iberian area. In those areas where otoliths show a low contrast between hyaline and opaque zones, burning or polishing techniques are a choice to consider. In those areas where otoliths have a higher degree of opacity, immersion in water or alcohol for observation (as used by age readers from Greece), could improve readability as this procedure shows to be effective in the hyalinization of the otoliths.

7. CONSEQUENCES OF THE ASSUMED BIRTHDATE: WHICH ALTERNATIVES?

The birthdate criterion and the associated interpretation of the otolith margin came up as important issues during the workshop. For age determination purposes, it is assumed that sardine is born on the 1st of January and age is counted as civil years. Opaque zones are formed mainly during summer (fast growing season). Thus, a hyaline margin observed within the first half of the year is assumed to represent the last winter (slow growing season) and counted as an annual growth ring. A hyaline margin observed within the second half of the year corresponds to the following winter and it is not counted as an annual growth ring.

Off the West and South Iberian Peninsula, sardine has an extended spawning season (October-March). Individuals born in the start of the season may be classified in two different year-classes during their first year due to the aging criteria (see diagram in Figure 7.1). This may confound year-class strength and bias the initial growth trajectory of successive cohorts. Figure 7.2 shows the initial growth of the 1997-2000 cohorts in the Northern Portuguese coast, based on data collected during spring and autumn surveys. Some cohorts (1997 and 1999) have apparently little or no growth during the first year of life as a consequence of the age reading conventions; in fact, small juveniles observed in March are aged 1 year old due to the birthdate convention. These fish were possibly born in the previous civil year, within the first half of the spawning season, and present a totally opaque otolith. However, fish born in the same period will show a hyaline otolith margin in the following autumn and will be aged as 0-group according to the otolith margin convention.

The Workshop participants acknowledged the importance of these issues and agreed that changing the otolith margin convention for juveniles during the first semester of the year, would apparently solve the inconsistency of year-class classification. However, a more detailed analysis of otoliths of juvenile fish and a broader discussion on this subject in other Working Groups is needed for a clear perception of all the problems involved and of the consequences for stock assessment of adopting any alternative birthdate or margin convention.

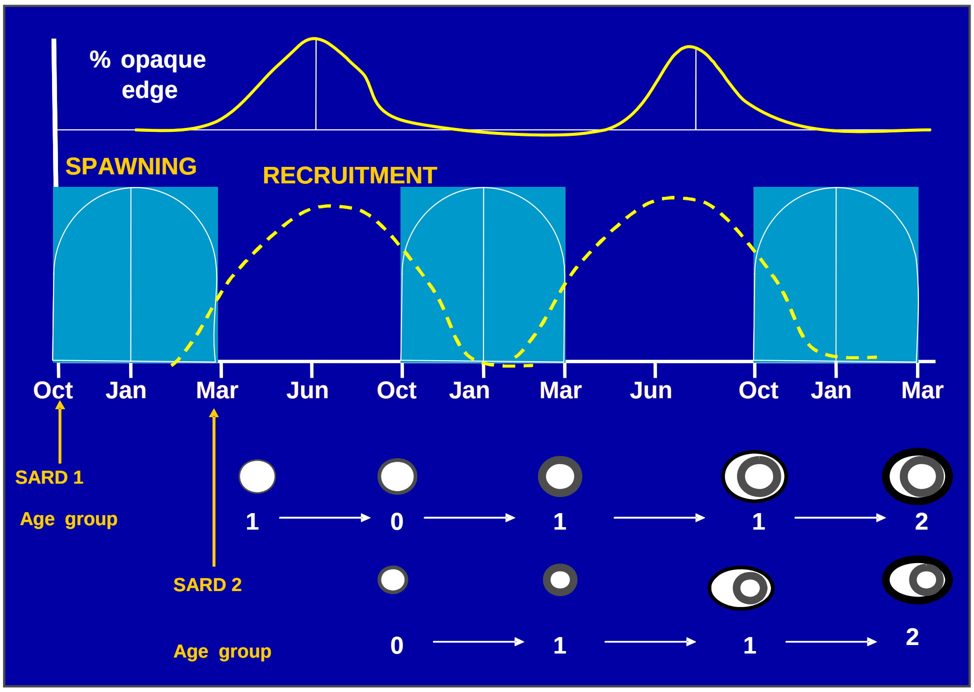

Figure 7.1 - Diagram of the birthdate and otolith margin conventions for sardine age determination.

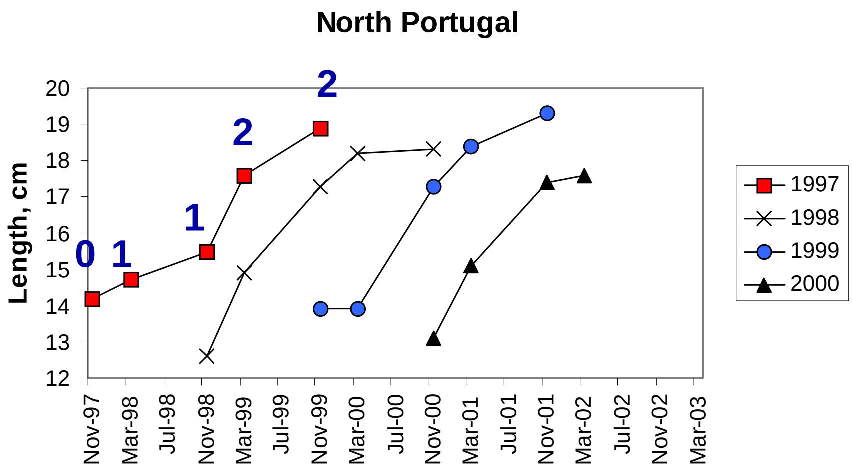

Figure 7.2 - Growth of 1997-2000 sardine cohorts based on data collected in November and March acoustic surveys. Text numbers correspond to fish age.

8. THE OTOLITH REFERENCE COLLECTIONS AND FUTURE WORK

The great importance of the existence of otolith reference collections for aiding age readers to improve their readings was also pointed out during this Workshop. These reference collections made with otoliths which age readings have reached high agreement (>80%) among different readers allow readers to calibrate their readings and help them to better interpret the otoliths structure in order to get more reliable readings.

IPIMAR showed an example of a draft of a "collection of agreed ages" of otoliths from different regions mainly of the Atlantic Iberian area. This was illustrated by digitalized images of otoliths which achieved at least 80% of age reading agreement among readers of both Iberian countries in previous otolith exchanges (see Annex).

Considering that each area has otoliths with specific structures raising different age reading problems, it was considered more adequate and useful the existence of specific sardine otolith reference collections in each area. The production of such regional reference collections, based on otoliths with at least 80% age reading agreement among readers, should be faced as a priority task within the improvement of the age reading data quality.

9. AGE READING PROTOCOL AND RECOMMENDATIONS

This Workshop agreed that readers should apply the following procedures and age reading criteria:

1. For each otolith, the number of (true) hyaline rings (excluding the edge), edge type, age assigned and readability (1 - good, 2 - medium, 3 - difficult), as well as false rings and other relevant characteristics of the otolith must be recorded (see Table 9.1). The percentage of opaque/hyaline edges for each sample must be also summarised;
2. In order to help in the identification of the 1st annual ring, the otolith opaque zone in juvenile sardines (less than 1 year old) in each area must be measured and used as a gauge for ageing older individuals;
3. The posterior or anterior edge of the otolith, depending on which side is more suitable for the age reading, must be preferably used for the identification of the edge type. In order to identify the growth period, the type of edge should be followed across the year in different ages;
4. Only clearly defined rings must be considered for the age determination. Nevertheless, if a faint ring occurs at a distance where a true ring should be expected (based on the diameter of the 1st annual ring) it must be also considered as a true ring for age assignment;
5. Different preparation techniques should be tried in those areas where otoliths show a more complex structure thus becoming more difficult to read. For example, burning and polishing the otoliths when there is little contrast between rings or soaking them in water/alcohol when they are very opaque, are techniques that could be applied in these areas;

6. A reference collection of otoliths with a high reading agreement (>80%) among readers must be prepared in each area. Each reader in each area should regularly calibrate his age readings with this reference collection;
7. Sardine growth patterns and otolith structures in the Atlantic Iberian coast, Mediterranean and Northwest Africa are different from each other raising specific age reading problems in each area. Therefore, this Workshop recommends that local workshops should be promoted and that a workshop joining readers from the 3 areas must periodically take place;
8. This Workshop agreed that any decision concerning the use of the birthdate criterion in sardine age assignment and its consequences in the stock assessment must be preceded by a more detailed analysis of juvenile fish otoliths and a broader discussion in other Working Groups.

Table 9.1 - Otolith observation record sheet

Area:
Date:

Sample ID	Otolith number	Fish total length	Number hyaline rings (true age rings)	Edge type O – opaque H – hyaline	Age group	Readability 1 – Good 2 – Medium 3 – Difficult	Diameter of opaque area (0 group fish)*	Obs. (false rings, etc.)

...

Summary of edge deposition (% of opaque edge in sample)
Age 1:
Age 3:
Age 5:

**To be filled only during the recruitment season*

10. SARDINE BIOLOGICAL SAMPLING

Profiting from the experience of some of the participants on sardine biological sampling this Workshop also discussed the problems rising from the application of scales of sexual maturation, visceral fat content and stomach condition (fullness and colour). The application of such scales in biological sampling frequently raises some difficulties, which may reflect on the stock assessment accuracy.

During this second part of the workshop, sardine samples collected off the Portuguese coast in different periods of the spawning season were analysed by some of the participants. Each participant independently assigned sexual maturation stages and in some cases visceral fat content and stomach condition to individual sardines. At the conclusion of each sample analysis, a general discussion on the differences in results among the participants took place in order to identify the main difficulties they have found and to clear out the criteria used by each one of them.

Results from this exercise were difficult to compare due to the differences in the scales used by each participant.

From the exercise and general discussion on this topic it was possible to compile the sampling methodologies (Section 10.1) and to prepare tables showing the different scales of sexual maturity visceral fat and stomach condition used by each laboratory (Table 10.1) and the correspondence between them (Table 10.2).

Table 10.1 – Scales used in sardine biological sampling analysis by the different Institutes: sexual maturity, visceral fat and stomach condition (fullness and colour).

Area			Lab	Sexual Maturity	Visceral Fat	Stomach Condition	
						Colour	Fullness
Atlantic Iberian	Portuguese coast		IPIMAR	1. Virgin/resting 2. Early development 3. Pre-spawning 4. Spawning 5. Post-spawning 6. Partial post-spawning	1. No fat 2. Thin thread of fat 3. Fat around all the gut 4. Body cavity full of fat	1. White 2. Orange 3. Brown 4. Dark green 5. Light green	1.Almost empty 2.Half filled 3.Filled 4.Bursting
	Spanish coast	Bay of Biscay Galicia Cadiz Bay	IEO	1. Virgin 2. Early development 3. Pre-spawning 4. Spawning 5. Partial post-spawning 6. Post-Spawning/resting	1. No fat; 2. Thin thread of fat; 3. Fat around all the gut; 4. Body cavity full of fat.	1. White 2. Orange 3. Brown 4. Dark green 5. Light green	0.Empty 1.Almost empty 2.Half filled 3.Filled 4.Bursting
		Bay of Biscay	AZTI	1. Virgin 2. Resting 3. Developing 4. Hydration 5. Spawning (no hydration, stage between 2 hydration processes) 6. Gonads degeneration	1. Absence or small amount of fat; 2. Fat around all the gut; 3. Guts totally covered with fat.	No data recorded	No data recorded
Western Mediterranean	Spanish coast	Northern Alboran Sea NW Mediterranean	IEO	1. Virgin 2. Early development 3. Pre-spawning 4. Spawning 5. Partial post-spawning 6. Resting	No data recorded	No data recorded	No data recorded
Atlantic	French Coast	Bay of Biscay	IFREMER	1. Virgin 2. Resting 3. Developing 4. Spawning 5. Partial post-spawning 6. Post-spawning	1. Little or no fat 2. Fat around all the gut 3. Body cavity full of fat	No data recorded	No data recorded
Mediterranean		Lyon's Gulf	IFREMER	1. Virgin 2. Resting 3. Developing 4. Spawning 5. Partial post-spawning 6. Post-spawning	1. Little or no fat 2. Fat around all the gut 3. Body cavity full of fat	No data recorded	No data recorded
Atlantic And Mediterranean	Moroccan coast		INRH	1.Virgin 2.Resting 3.Pre-spawning 4.Spawning 5.Post-spawning	1. Thin 2. Medium fat 3. Fat	1. Orange/ Brown 2. Green	No data recorded
Mediterranean	Greek coast		HCMR	1. Virgin 2. Early development 3. Pre-spawning 4. Spawning 5. Post spawning/resting (both partial and final)	No data recorded	No data recorded	No data recorded

Table 10.2 - Correspondence between the different scales of sexual maturity (A), visceral fat (B) and stomach condition – colour - (C) used by each laboratory.

A

IPIMAR	IEO	AZTI	IFREMER	INRH	GREECE	IEO (MEDIT)
1.Virgin/resting 2.Early development 3.Pre-spawning 4.Spawning 5.Post-spawning 6.Partial post-spawning	1.Virgin 2.Early development 3.Pre-spawning 4.Spawning 5.Partial post-spawning 6.Post-spawning/ resting	1.Virgin 2.Resting 3.Developing 4.Hydration 5.Spawning (no hydration stage between 2 hydration processes) 6.Gonads degeneration	1.Virgin 2.Resting 3.Developing 4.Spawning 5.Partial post-spawning 6.Post-spawning	1.Virgin 2.Resting 3.Pre-spawning 4.Spawning 5.Post-spawning	1.Virgin 2.Early development 3.Pre-spawning 4.Spawning 5.Post spawning /resting (both partial and final)	1.Virgin 2.Early development 3.Pre-spawning 4.Spawning 5.Partial post-spawning 6.Resting

B

IPIMAR	IEO	AZTI	IFREMER	INRH
1.No fat 2.Thin thread of fat 3.Fat around all the gut 4.Body cavity full of fat	1.No fat 2.Thin thread of fat 3.Fat around all the gut 4.Body cavity full of fat	1.Absence or small amount of fat; 2.Fat around all the gut; 3.Guts totally covered with fat.	1.Little or no fat 2.Fat around all the gut 3.Body cavity full of fat	1.Thin 2.Medium fat 3.Fat

C

IPIMAR	IEO	INRH
1.White 2.Orange 3.Brown 4.Dark green 5.Light green	1.White 2.Orange 3.Brown 4.Dark green 5.Light green	1.Orange/brown 2.Green

10.1. Biological Sampling Procedures

<u>Atlantic Iberian Area</u>

Portuguese coast

<u>IPIMAR</u>

The guidelines for biological sampling of sardine off Portuguese coast are reported in Annex. Shortly, in the Portuguese coast sardine samples of around 200 fish each are collected from commercial catches at fishing harbours twice a month and also at sea during research surveys.

Total individual length of the sampled fish is measured at the harbour in order to get the sample length distribution. For all the main Portuguese sardine sampling harbours (Póvoa de Varzim, Matosinhos, Peniche and Portimão), biological samples are taken from the same day/vessel as of the samples for length distribution for the National Sampling Program (PNAB).

Each biological sample is composed of a collection of about 10 fish per length class (0.5 cm). The extreme length classes are completed with fish taken from the sample fish container.

The following data are recorded from biological sampling:

- Total length (mm);
- Total weight (0.0 g);
- Gutted weight (0.0 g);
- Sexual maturity stage (see scale in Annex);
- Visceral fat stage (see scale in Annex);
- Gonad weight (0.00 g);
- Stomach colour and fullness (see scale in Annex);
- Liver weight (0.00 g).

The assignment of sexual maturation stages follows the criteria adopted for this stock (ICES, 1981) and is based on maturation classification scales by gonads macroscopic analysis adapted from the research work previously developed by Pinto and Andreu (1957), Pinto (1957) and Pinto and Barraca (1958) (see Annex).

Spanish Atlantic coast

<u>IEO</u>

In the Spanish coast IEO obtains biological samples from both research cruises and commercial catches.

During the Northern acoustic Spring surveys biological sampling is at random, 40-50 fish being sampled per haul (additional fish are sampled to try to cover the tails of the length distribution per area i.e. Portugal, Southern Galicia, Northern Galicia, Asturias-Cantabria, Bask Country, France). In the Gulf of Cadiz area and during the research surveys, 40 fish per haul are sampled at random. The following information is recorded for each fish:

- Total length (mm);
- Total weight (0.0 g);
- Sexual maturity stage (6 stages see Table 10.1);
- Otoliths are removed from each fish.

Since 2003 (following the SARDYN project recommendations) during the Northern acoustic surveys the following additional information is also recorded:

- Visceral fat stage (4 stages see Table 10.1);
- Stomach colour and fullness (4 and 5 stage scales respectively with, in the case of stomach fullness, an extra stage (0) to denote an empty stomach, see Table 10.1).

Samples from commercial catches obtained in their respective areas are processed at Vigo (IXaN), Coruña (VIIIcW), Santander (VIIIcE) and Cadiz (IXaS) labs. Sampling is carried out with the ultimate objective of covering as much as possible all length classes. Vigo, Santander and La Coruña[1] carry out length stratified sampling (2-3 samples per quarter) where the following information is collected:

- Total length (mm);
- Total weight (0.0 g);
- Sexual maturity stage (6 stages see Table 10.1);
- Visceral fat stage (4 stages see Table 10.1);
- Otoliths are removed from each fish.

Since 2004 (following the SARDYN project recommendations) the following additional information has also been collected in Vigo:

- Visceral fat stage (4 stages see Table 10.1);
- Stomach colour and fullness (4 and 5 scales respectively with in the case of stomach fullness an extra stage (0) to denote an empty stomach, see Table 10.1 and Annex);

Following the workshop recommendations since August 2005 gutted weight (0.0 g) is also being recorded in Vigo.

In Cadiz (IXaS) 50 fish are monthly selected at random to record the following biological characteristics:

- Total length (mm);
- Total weight (0.0 g);
- Sexual maturity stage (6 stages see Table 10.1);
- 5 otoliths per length class are removed from the sample.

<u>AZTI</u>
In the Bay of Biscay sardine samples of about 50 fish each (approximately 5 kg of fish) are collected from commercial catches and research surveys (generally undertaken in May). Five fish are taken from each length class (0.5 cm) in order to take biological data. The following information is recorded from commercial samples:

- Individual total weight;
- Individual total length;
- Sex determination;
- Stage of sexual maturation (see Table 10.1);
- Gonad weight;
- Otolith collection.

And from research surveys samples:

- Individual total weight;

[1] Random sampling started in 2005

- Individual total length;
- Sex determination;
- Stage of sexual maturation (see Table 10.1);
- Parasite occurrence on the liver (*Anisakis*);
- Stage of visceral fat content (see Table 10.1).

Spanish Mediterranean coast (North Alboran Sea and NW Mediterranean)

<u>IEO</u>
In this area sardines are regularly collected for biological sampling. In each sample the following individual data are recorded:

Total length;
Total weight;
Sex;
Stage of sexual maturation (6 stages, see Table 10.1);
Gonad Weight;
Otolith collection.

Greek coast

<u>HCMR</u>
Sardine is randomly sampled from commercial landings from all the main Greek fishing harbours. All samples come from the purse-seine fishery which is the main fishery for this species in Greek waters. The number of length samples taken is selected according to the regulation EC 1639/2001. Each sample for biological sampling is consisted of at least 50 specimens and length measurements are taken to the nearest 1mm. Otoliths for age composition, are sampled according to the regulation EC 1639/2001. Maturity, sex and weight are recorded from all the length samples.

The biological data recorded in each sample are the following:

- Total individual length (mm);
- Total individual weight (g);
- Individual eviscerated weight (g);
- Sex;
- Sexual maturity stage (a Nikolsky macroscopic scale for sexual maturity assignment to gonads, see Table 10.1);
- Gonad weight (g);
- Otoliths extraction (25 per 1 cm length class). Storage in Eppendorf tubes and preservation in deep freeze (-95ºC);
- Sex ratio, weight/length relationship and gonadosomatic relationship are also estimated and recorded.

French Atlantic coast (Bay of Biscay)

IFREMER

In Bay of Biscay samples of around 5 kg of sardine are collected from commercial catches all along the year in the different categories of length (4 categories determined by the number of fish per kg).

During the first semester, the following data are recorded each month from 80 individuals (two samples):

- Total individual length (0.5 cm);
- Total individual weight (g);
- Sex;
- Sexual maturity stage (6 stages, see Table 10.1).

The totality of otoliths of the first semester are collected during the French acoustic survey PELGAS, which is generally carried out in May. The sardine age/length key for the first semester is based on the survey data. During this survey, for each sampling trawl containing sardine a length distribution is obtained from a sample of 5 to 10 kg of sardines. 5 fish per length class are collected after and used for biological sampling:

- Total individual length (0.5 cm);
- Total individual weight (g);
- Sex;
- Sexual maturity stage (6 stages, see Table 10.1);
- Parasite occurrence (*Anisakis*);
- Visceral fat content (3 stages, see Table 10.1);
- Otolith collection.

In the second semester, all data are collected from samples taken from commercial catches. Each sample of about 5 kg of fish is used for length distribution and biological data are collected from 5 to 6 individuals by length class:

- Total individual length (0.5 cm);
- Total individual weight (g);
- Sex;
- Sexual maturity stage (6 stages, see Table 10.1);
- Otolith collection.

All the otoliths of this period are sampled from commercial catches. For that purpose 2 samples of about 40 fish each are collected each month.

CNRS – EPHE

Sardine biological research has been undertaken by CNRS. Samples (2571 individuals) were collected in the Bay of Biscay during three research surveys: PEL01 (April 28 to June 4, 2001), PELGAS02 (May 6 to June 10, 2002), and PELGAS03 (May 29 to June 24, 2003) on the research vessel *Thalassa*. In each trawl containing *Sardina pilchardus*, 50 individuals were randomly collected. Samples were stored on board at –30°C until dissection in the laboratory. The following biological data were recorded:

- Total individual length (mm);
- Standard individual length (mm)
- Total individual weight (g);

Otoliths were collected for age determination.

French Mediterranean coast (Lyon's Gulf)

<u>IFREMER</u>
Sardine samples are collected from commercial catches each quarter and during a research survey (PELMED) usually undertaken in July. All these samples have been frozen and stored awaiting an opportunity to be analyzed.

<u>CNRS – EPHE</u>
Samples of *Sardina pilchardus* were obtained during the research survey PELMED03 (July 13-28, 2003). In each trawl containing *Sardina pilchardus*, 50 individuals were randomly collected. Samples were stored onboard at –30 °C until dissection in the laboratory. The following biological data were recorded:

- Total individual length (mm);
- Standard individual length (mm)
- Total individual weight (g);

Otoliths were collected for age determination.

Moroccan coast (Atlantic and Mediterranean)

<u>INRH</u>
Samples are collected from commercial catches at the fishing harbours. Each sample is around 3-4 kg of fish. For biological sampling 10 fish per length class (0.5 cm) are used.

The following biological data are recorded:
- Total individual length (mm);
- Total individual weight (g);
- Total individual gutted weight (g);
- Gonads weight (g);
- Liver weight (g);
- Sex;
- Sexual maturity stage (5 stage scale, see Table 10.1);
- Collection of otoliths (10 per length class).
- Sex ratio, condition factor, weight/length relationship, gonadosomatic and hepatosomatic relationships are also estimated.

10.2. Recommendations

From the practical work and the discussions held during this Workshop some differences were found among the participating Institutes on the biological sampling methodology and on the scales used in the assignment of sexual maturity, visceral fat and stomach condition (see Table 10.1). Therefore the Workshop agreed to advise the continuity of the exchange of knowledge and experience in this area and to recommend the following standardisation of the biological sampling procedures:

1. Random/length stratified sampling;

2. Size classes of 0.5 cm;

3. Minimum set of characteristics:
    - total length
    - total weight
    - sex
    - maturity stage
    - fat stage

4. Desirable set of characteristics:
    - eviscerated weight
    - gonad weight
    - stomach fullness/colour;

5. Complete set of characteristics:
    - liver weight
    - parasites

6. Sexual maturity scale:
    - Calibration of macroscopic-microscopic sexual maturity stages;
    - The simplification of the macroscopic sexual maturation scale with the reduction from 6 to 5 stages was discussed in order to overcome the difficulties to discriminate between the pre-spawning stage 3 and the post-spawning stages. Nevertheless, it was considered as advisable to wait for the imminent publication of the results of the calibration between microscopic and macroscopic maturation stages to support this simplification.

7. Scale of visceral fat stages

8. Scale of stomach colour – The 3 stages scale below is recommended, since it has been validated (Cunha *et al.*, 2005):

    1 – White/cream coloured (corresponding to empty or almost empty stomachs);
    2 – Orange/Brown;

3 – Green.

9. Scale of stomach fullness – A scale with four stages (1-almost empty, 2-half full, 3-full and 4-bursting) as validated by Cunha *et al.*, 2005 is recommended. However, since empty stomachs (stage 0) have been often identified in samples collected in the Spanish coast, the addition of this stage should be explored and validated.

10. Otolith sampling.

11. FINAL CONCLUSIONS

This Workshop pointed out that much work is still to be done in the future in the otoliths age reading:

- The first annual ring identification and the age assignment to older fish are problems that still persist since the last Workshop held in 2002. These difficulties become worse in the southern areas where contrast between hyaline and opaque zones of otoliths is lower. Further research on alternative techniques to enhance the otolith structure and improve contrast between growth zones is needed to overcome this problem. Also, the measurement of opaque zone in otoliths of juvenile fish (<1 year old) in each area, will help to identify the first ring and its use as a template will help to age older fish;
- Specific problems arise in the different areas due to the different growth patterns and otolith structures, needing different solutions in what concerns otoliths preparation and age reading criteria and demanding specific workshops for their implementation;
- Alternative birthdate criteria and their consequences for the stock assessment must be further investigated;
- Regular otolith exchanges must also be promoted in order to detect difficulties, standardize methodologies and continuously improve the readings precision;
- Reference collections with otoliths which achieved at least 80% of age reading agreement among readers must be produced for each area in order to help improve readings agreement and precision.

On the other hand, incorporating the discussion of the problems of the application of scales for sexual maturity, visceral fat and stomach condition, this Workshop also allowed the mutual knowledge of the sardine biological sampling methodologies applied in the different areas and gave a contribution for their standardization.

Finally, this Workshop contributed for the improvement of future work within these matters and widened the opportunities of cooperation among the participants working in the different areas. Due to its importance for the knowledge of sardine biology and for the resource assessment, this kind of initiative should continue in the future.

12. CONTRIBUTIONS TO THE WORKSHOP

As a contribution to the Workshop the Moroccan participants presented the research work that is being carried out off the Moroccan Atlantic coast making an approach to the different aspects of sardine resource dynamics and biology, as age determination based on otolith analysis, growth, condition factor and populations' characterisation (Amenzoui *et al.*, 2005). Also Greek participants made an approach to the research work that has been carried out on the biology of sardine off Greek coast, focusing on the otoliths preparation and ageing methodology used in that region.

13. ACKNOWLEDGEMENTS

The 2004 otolith exchange and this workshop were held within the framework of the EU Data Collection Regulation (DCR).

Special thanks are due to all participants whose deep engagement enabled the accomplishment of the Exchange and Workshop's goals allowing their success. Their contributions also allowed the making of this report.

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Report of the

WORKSHOP ON SARDINE OTOLITH AGE READING AND BIOLOGY

IPIMAR, Lisbon, 27 June – 1 July, 2005

ANNEX

2004 SARDINE OTOLITH EXCHANGE

GUIDE DOCUMENT

1. Objectives:

The objectives proposed for the present exchange of sardine otoliths are to:

1. Evaluate the agreement among otolith readers and the precision of age determination;
2. Assess whether the main problems identified in the previous workshop still persist and to what extent. Propose ways of minimising the difficulties still existing;
3. Evaluate the consistency of age readings between the early 1980's , the early 1990's and the present time;
4. Evaluate the consistency of age determinations with sardines from the northwest African area;
5. Discuss the consequences of the assumed birthdate for the estimation of growth. Suggest alternatives;
6. Produce a "summer" reference collection (otoliths with more then 80% agreement between experienced readers) of sardine otoliths from different areas of the species distribution.

2. Sample sets of otoliths for the exchange:

Sample	Set	Number of otolith pairs (aprox.)		Area	Institute
		By Age Group	Sample Total		
Main collection (June-July 2003)	**1**	10	100	North Galicia/Cantabria	IEO
	2	10	100	South Portugal	INIAP/IPIMAR
	3	5	50	Gulf of Biscay	CNRS
	4	10	50	Western Mediterranean (Lyon's Gulf)	CNRS
Collection for consistency in time (Spring) (*)	**5**	5	50	North Portugal (80's)	INIAP/IPIMAR
	6	5	50	North Portugal (90's)	INIAP/IPIMAR
	7	5	50	North Galicia/Cantabria (80's)	IEO
	8	5	50	North Galicia/Cantabria (90's)	IEO
Collection for consistency with northwest Africa (Spring)	**9**	5	50	Morocco (Larache/Casablanca)	FAO/INRH
	10	5	50	Southern Morocco/Mauritania	FAO/INRH
TOTAL (aprox.)			600		

(*) The Portuguese collections for consistency in time should be read by INIAP/IPIMAR only and the Spanish collections by the IEO only.

The selection of otoliths should be based in previous age readings carried out in each Institute responsible for its preparation.

3. Circulation of the otolith sample sets:

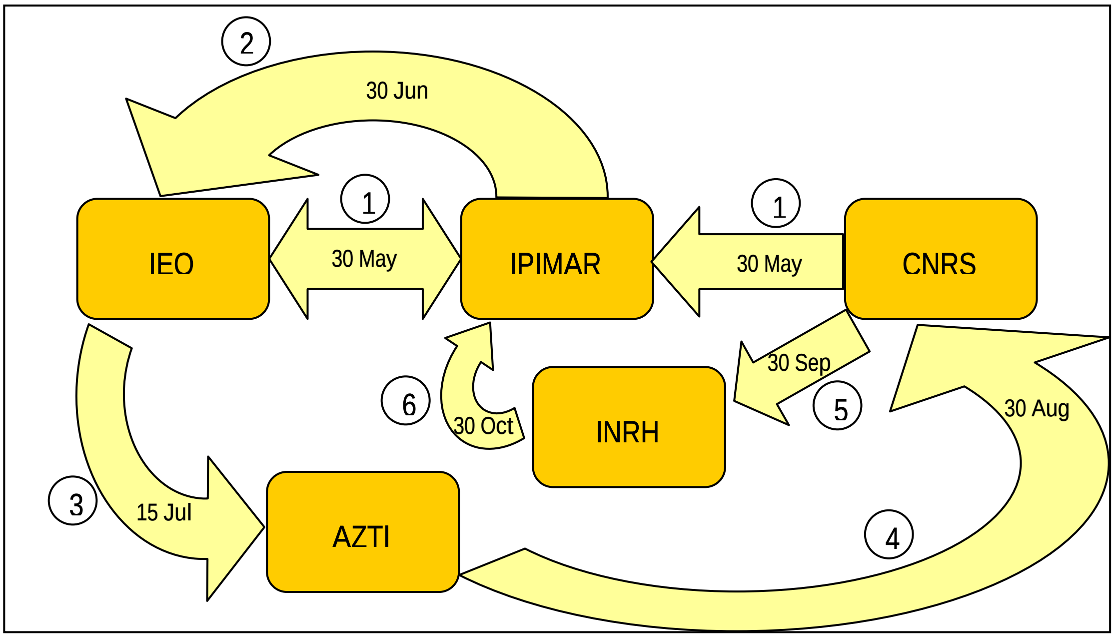
Each Institute must prepare standardised MS Excel worksheets of individual sample sets (see example below), with the area, date of sampling, observation and otolith numbers, individual fish total length and otolith location (plaque number and position). Each reader must use these worksheets to record their observations, i.e. the number of hyaline (age) rings (counted as winter rings) excluding the edge, the type of edge (opaque, hyaline), the age group and the otolith readability (1-good, 2-medium, 3-difficult). The number of false/double rings as well as other particular features of the otolith should be recorded in the OBS field. In order to properly identify each file, each reader must record his name in the respective cell and also include it in the files names, for example: PortNorth90s_Ed.xls.

All the files will be centralized at INIAP/IPIMAR and sent by e-mail to Eduardo Soares, *esoares@ipimar.pt* (see list below).

<u>Example of MS Excel worksheet heading for individual sample sets</u>

SARDINE OTOLITH EXCHANGE 2004								**READABILITY:1=GOOD; 2= MEDIUM; 3= DIFFICULT**							
READER:															
DATE:															
INSTITUTE	**DATE**	**QUARTER**	**AREA**	**PLAQUE_N**	**LINE**	**COLUMN**	**OBS_N**	**LENGTH**	**SEX**	**OTOL_N**	**EDGE_TYPE**	**HYAL_RINGS**	**AGE**	**READABILITY**	**OBS**
IPIMAR	29-03-1990	1	IXaN				17	19,1	M	241					

The collections should circulate among participants between May and October 2004 following the next scheme:



1) 30 May: IEO and IPIMAR exchange their otolith sets. CNRS sends the French collections to IPIMAR.
2) 30 June: IPIMAR sends IEO and CNRS collections to IEO.
3) 15 July: IEO sends all the collections (IEO, IPIMAR and CNRS) to AZTI.
4) 30 August: AZTI sends all the collections to CNRS**.**
5) 30 September: CNRS sends part of the collections to INRH.
6) 30 October: INRH sends the collections back to IPIMAR.

Readers must also follow the age reading protocol approved during the last sardine otolith workshop held in Lisbon in 2002 (see Annex).

IMPORTANT: In order to avoid any damage during mail transportation, special care must be put in the way the samples are packed and also specialised mailing services must be used.

Institutes participating: IPIMAR, IEO, AZTI, CNRS, INRH. The persons responsible for the otolith sets in each Institute are:

Name	Institute	Phone number	e-mail
Andrés Uriarte	Unidad de Investigación Marina Fundación AZTI (Instituto Tecnológico Pesquero y Alimentario) Herrera kaia Portualde z/g 20110 PASAIA Gipuzkoa, SPAIN	34 943-004800	auriarte@pas.azti.es
Eduardo Soares	IPIMAR, Av. Brasília 1449-006 Lisboa, Portugal	(351) 21-3027000	esoares@ipimar.pt
Véronique Laurent	CNRS Univ. Perpignan 66860 Perpignan cedex – France	33 4 68 66 20 55	vlaurent@univ-perp.fr
Quena Peleteiro	IEO- Centro Ocean. de Vigo,Cabo Estay – Canido 36200 Vigo, Spain	986-492111	quena.peleteiro@vi.ieo.es
Hakim Mesfioui	INRH, 2, Rue de Tiznit Casablanca, Maroc	(212) 022 22-20-90	mesfioui@inrh.org.ma

Workshop: The exchange will be followed by a Workshop. Proposed venue dates are February 2005, INIAP/IPIMAR, Lisboa

References:

Anon. 1994. Report of the Spanish-Portuguese workshop on otolith age readings of sardine in ICES Divisions VIIIc and IXa. Working document to the meeting of the Working Group on the Assessment of Mackerel, Horse mackerel, Sardine and Anchovy (ICES CM 1995/Assess:2), 19pp.

FAO, 1978. Rapport du groupe de travail sur l' unification de la determination de l' âge de la sardine (*Sardina pilchardus*, Walb.). Rome, FAO, 8pp., (COPACE/TECH. REP. No. 78/8).

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ICES, 1981. Rapport du groupe de travail pour l' evaluation des stocks de sardines dans les Divisions VIIIc and IXa. ICES CM 1981/H:72, Annex I, 10 pp.

ICES, 1982. Working Rapport du groupe de travail pour l' evaluation des stocks de sardines dans les Divisions VIIIc and IXa. ICES CM 1982/H:72, Annex I, 10 pp.

ICES, 1997. Report of the Workshop on sardine otolith age reading. ICES CM 1997/H:7, 47pp.

Soares, E., Morais, A., Silva, A., Carrera, P., Jorge, A., Rico, I., Peleteiro, Q., Evano, H. (*in press*). Report of the Workshop on sardine age reading (Lisbon, 28 January-1 February 2002). Relatórios Científicos e Técnicos do Instituto de Investigação das Pescas e do Mar, Série digital, http://ipimar-iniap.ipimar.pt/, ISSn 1645-863x.

Workshop on Sardine Otolith Age Reading (Lisbon, 28 January – 1 February, 2002)

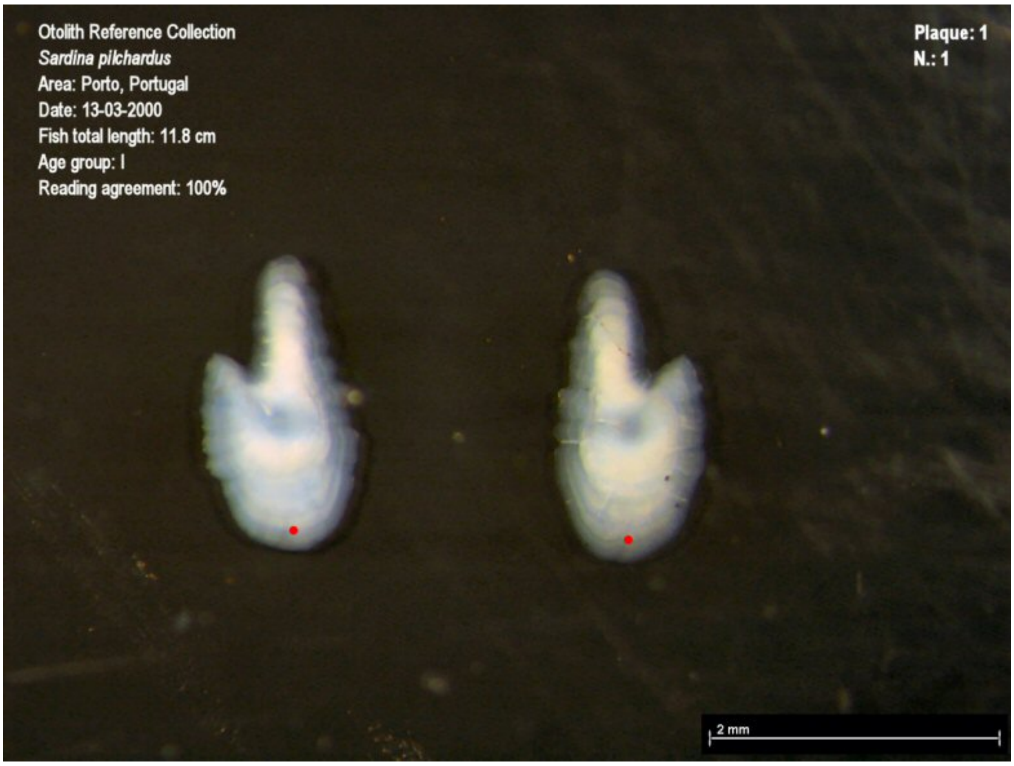
PROTOCOL FOR SARDINE AGE DETERMINATION

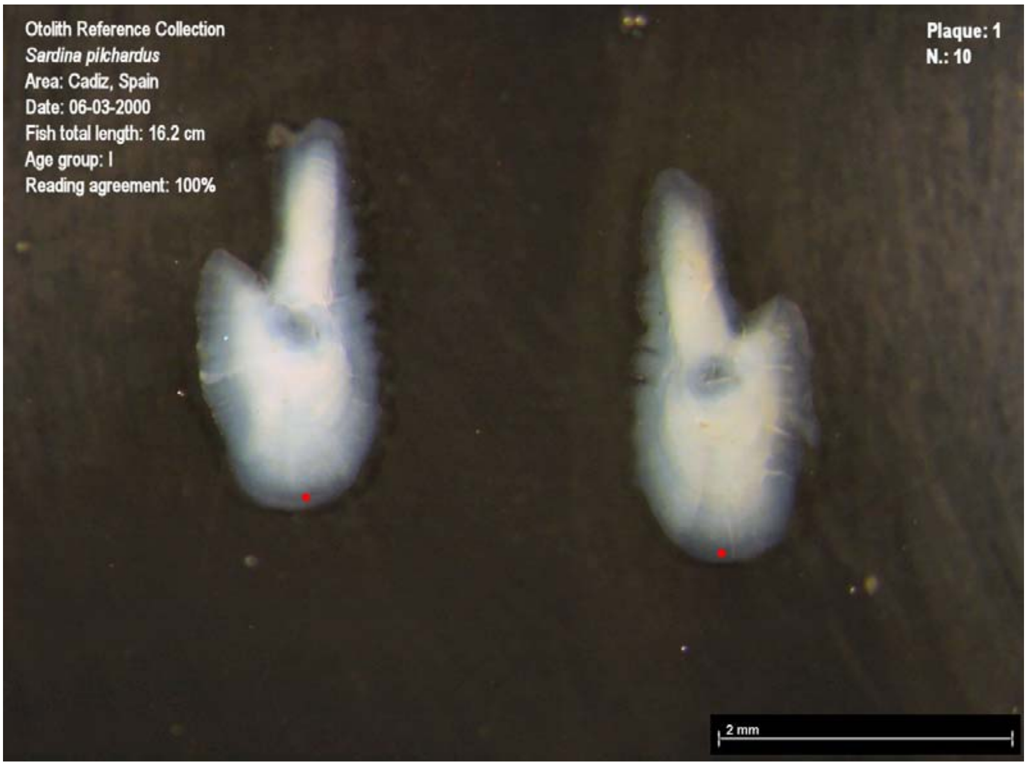
In order to standardise the sardine age assignments and to improve the age estimates, the Workshop held in Lisbon-Portugal, 2002 adopted the following protocol:

1. The first of January is adopted as the birthdate reference for age assignment purposes. Consequently, if an otolith is collected from a fish caught in the first semester of the year the age group assignment will correspond to the number of hyaline zones present. If the otolith is extracted from a fish caught in the second semester of the year the age group assigned will correspond to the hyaline zones completely formed, i.e. if the edge of the otolith is hyaline it will be not considered.
2. After extraction otoliths are washed thoroughly, dried, mounted and preserved in xylol resistant plastic plaques in a synthetic resin ("Eukitt" or "Entellan").
3. The observations of entire otoliths are made under reflected light against a black background using magnifying dissection microscopes with 20X magnification. Magnification should be increased near the otolith edge to improve the discrimination of narrow hyaline rings in older individuals.
4. It is always advisable to have pairs of whole otoliths available from individual sardine specimens when trying to interpret the ring structure.
5. A set of an opaque and a hyaline zone corresponds to one annual growth zone (*annulus*).
6. It is recommended to use the *anti-rostrum* as the most adequate zone to count hyaline rings for age assignment. However, since false rings are more evident in this region, the *rostrum* should be used in otoliths from the southern area.
7. Sometimes it may happen that other areas of the otolith, i.e. the dorsal part, are easier to read. In this case the age reading based on the analysis of these areas can be considered appropriate if the readings prove to be consistent.
8. In order to adopt a ring as an *annulus* it is recommended that the ring can be followed throughout the whole otolith contour. This rule must be applied specially for the first three *annuli*, since in the older specimens growth often slows down to such an extent that hyaline rings are very close together. In that case opaque and hyaline zones become more difficult to be identified.

OTOLITH REFERENCE COLLECTION

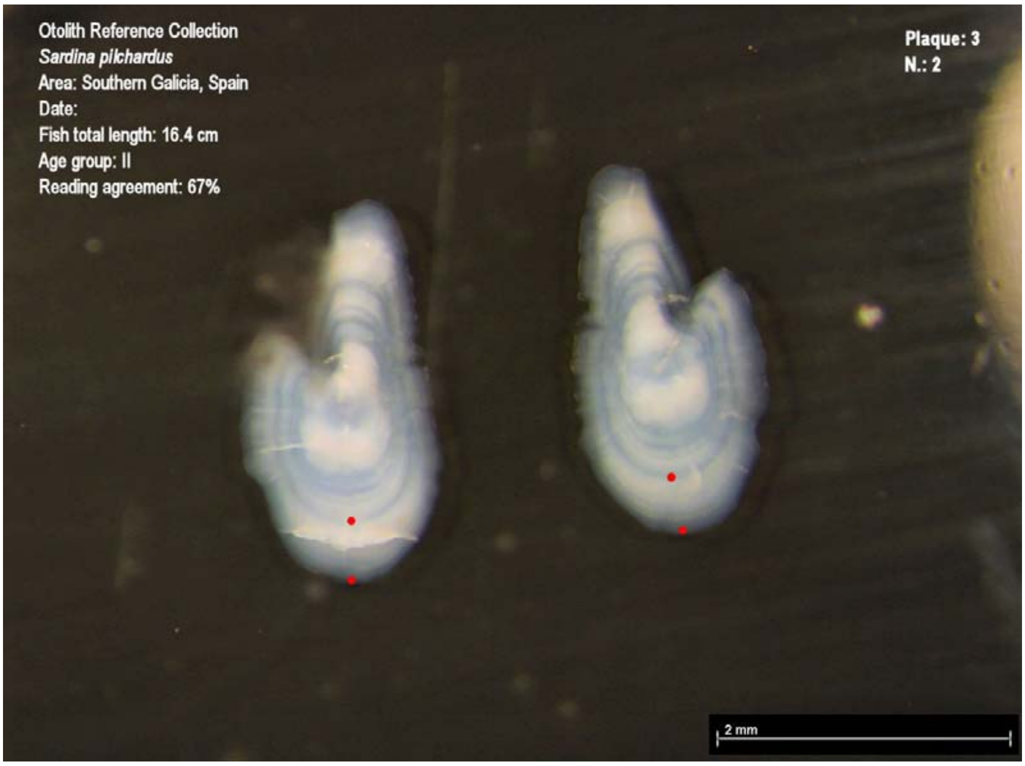
The following photos make part of a larger compilation which is the first attempt of IPIMAR sardine research team to build up a reference collection of sardine otoliths. These otoliths were collected from sardines caught in different regions off the Atlantic Iberian area and also off French and English coasts. They achieved at least 80% of age reading agreement among readers of both Iberian countries in previous otolith exchanges. This work is still in progress and a final version of a reference collection for Atlantic Iberian area is in its scope.


Otolith Reference Collection
Sardina pilchardus
Area: Porto, Portugal
Date: 13-03-2000
Fish total length: 11.8 cm
Age group: I
Reading agreement: 100%
Plaque: 1
N.: 1
2 mm

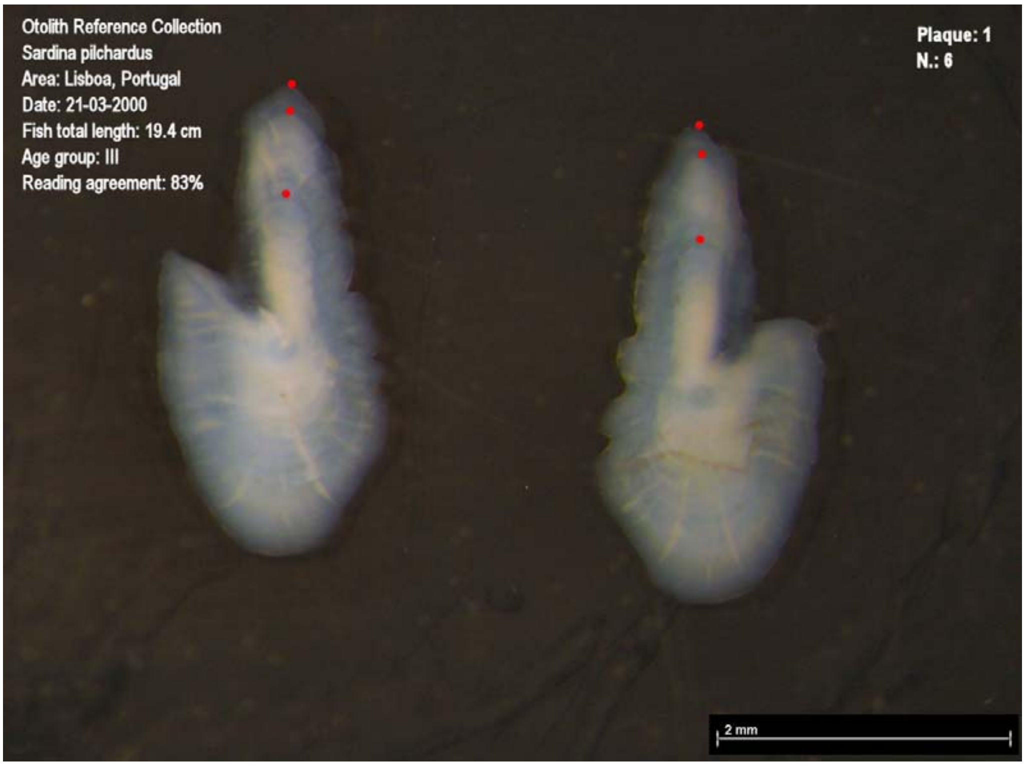

Otolith Reference Collection
Sardina pilchardus
Area: Cadiz, Spain
Date: 06-03-2000
Fish total length: 16.2 cm
Age group: I
Reading agreement: 100%
Plaque: 1
N.: 10
2 mm

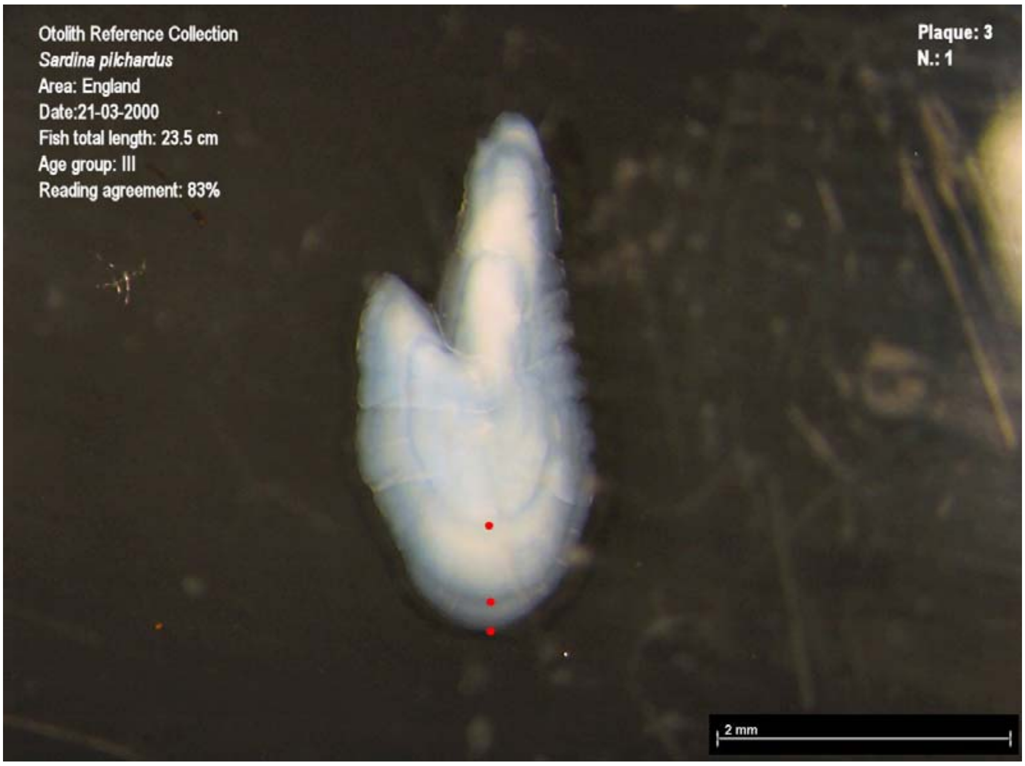
AGE GROUP I


Otolith Reference Collection
Sardina pilchardus
Area: Britany, France
Date: 20-03-2000
Fish total length: 19.4cm
Age group: II
Reading agreement: 100%
Plaque: 2
N.: 4
2 mm


Otolith Reference Collection
Sardina pilchardus
Area: Southern Galicia, Spain
Date:
Fish total length: 16.4 cm
Age group: II
Reading agreement: 67%
Plaque: 3
N.: 2
2 mm

AGE GROUP II



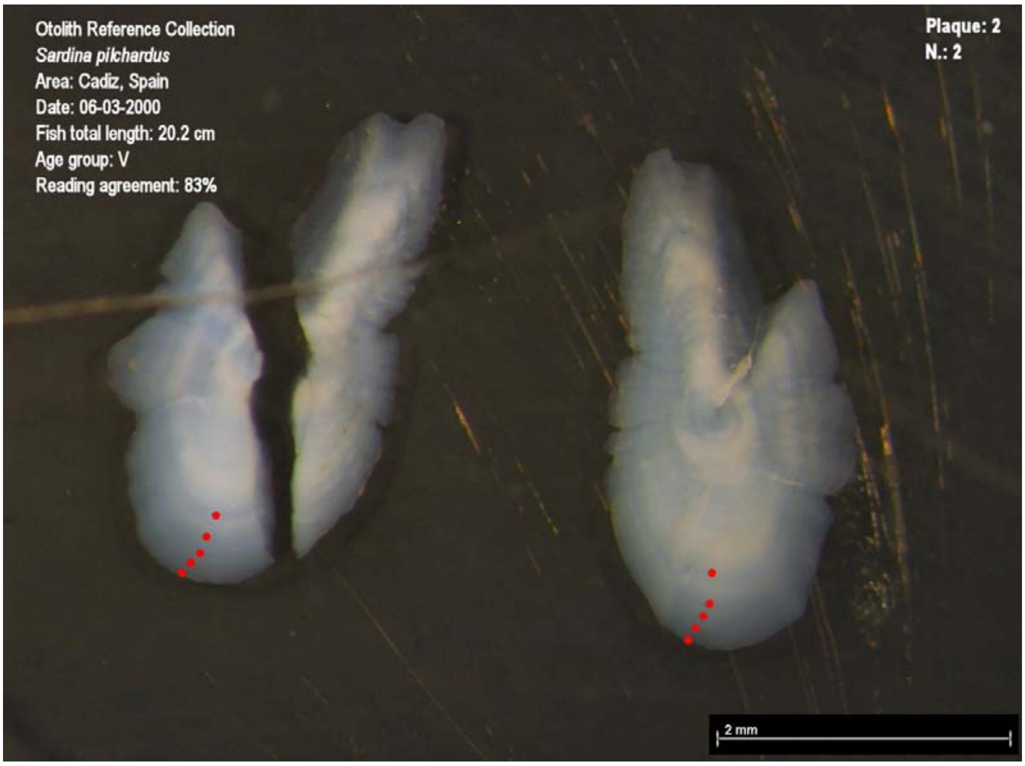


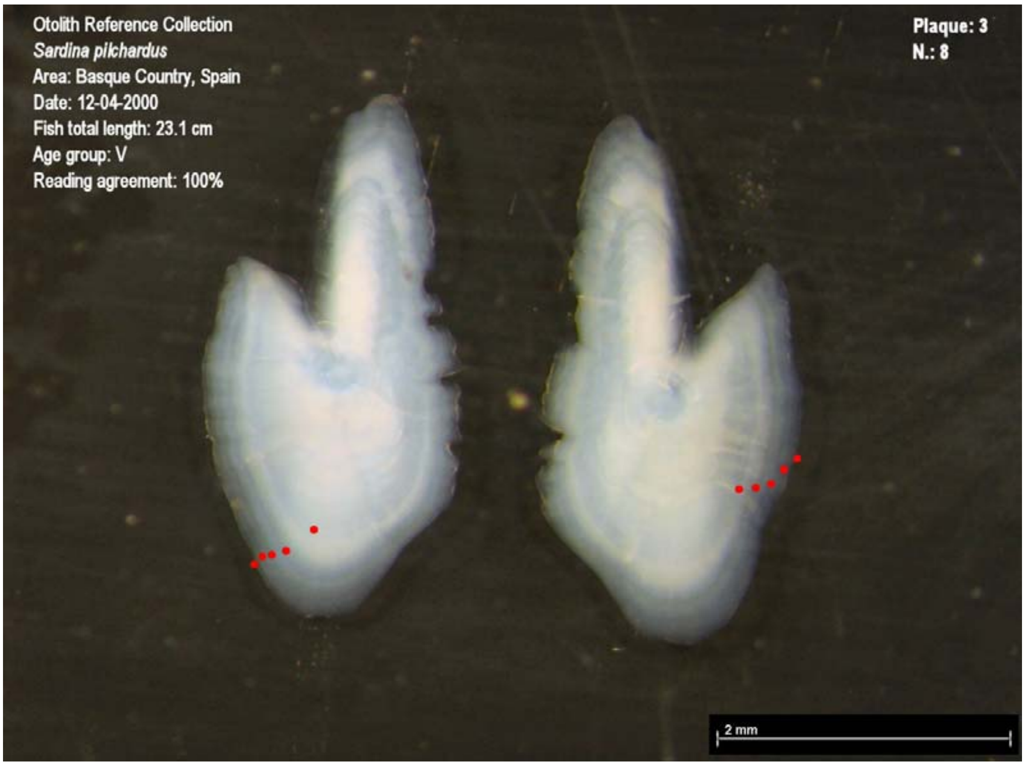
AGE GROUP III


Otolith Reference Collection
Sardina pilchardus
Area: Britany, France
Date: 19-03-2000
Fish total length: 22.5 cm
Age group: IV
Reading agreement: 83%
Plaque: 2
N.: 7
2 mm

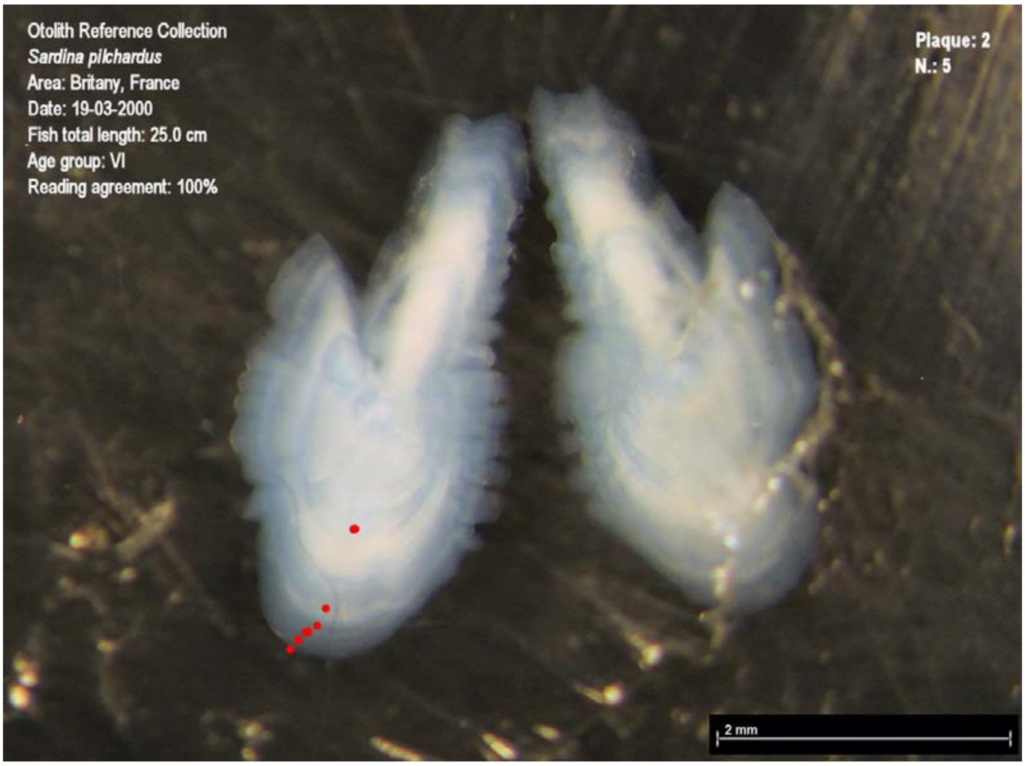

Otolith Reference Collection
Sardina pilchardus
Area: Northern Galicia, Spain
Date: 03-04-2000
Fish total length: 23.0 cm
Age group: IV
Reading agreement: 100%
Plaque: 3
N.: 4
2 mm

AGE GROUP IV


Otolith Reference Collection
Sardina pilchardus
Area: Cadiz, Spain
Date: 06-03-2000
Fish total length: 20.2 cm
Age group: V
Reading agreement: 83%
Plaque: 2
N.: 2
2 mm


Otolith Reference Collection
Sardina pilchardus
Area: Basque Country, Spain
Date: 12-04-2000
Fish total length: 23.1 cm
Age group: V
Reading agreement: 100%
Plaque: 3
N.: 8
2 mm

AGE GROUP V


Otolith Reference Collection
Sardina pilchardus
Area: Britany, France
Date: 19-03-2000
Fish total length: 25.0 cm
Age group: VI
Reading agreement: 100%
Plaque: 2
N.: 5
2 mm

AGE GROUP VI

GUIDELINES FOR BIOLOGICAL SAMPLING OF SARDINE OFF PORTUGUESE COAST

Sardine samples are periodically collected for biological data collection at the main Portuguese sardine landing harbours (Póvoa de Varzim, Matosinhos, Peniche, Sines and Portimão). The main guidelines for the samples preparation and data collection are described in the following lines as a reference for sardine biological sampling.

1. Sampling rules

1. Each sample contains about 200 sardines. All individual total lengths must be measured in order to get the sample length composition. At the main Portuguese sardine landing harbours (Matosinhos, Póvoa de Varzim, Peniche, Sines and Portimão), biological samples are collected from the same day/vessel of the samples for length distribution within the National Sampling Program (PNAB). Although the length distribution data are not saved in the computer database they must be recorded at the laboratory.

2. Biological sample:
   - 10 fish are collected by each length class (0,5 cm);
   - The extreme length classes are completed with fish taken from the fish container.

3. Biological sampling data:
   - Total individual length (mm);
   - Total individual weight (0,0 g);
   - Individual gutted weight (0,0 g);
   - Sex identification;
   - Sexual maturity stage (see scale in Annex);
   - Visceral fat stage (see scale in Annex);
   - Gonad weight (0,00 g);
   - Stomac colour (see scale in Annex);
   - Stomac filling state (see scale in Annex);
   - Liver weight (0,00 g)

4. Otolith extraction and collection:

Fish up to 13 cm total length (inclusive)	5 pairs by length class
Fish between 13.5 e 17.5 cm total length	8 pairs by length class
Fish longer than 18 cm (inclusive)	10 pairs by length class

   - In each sample, an observation number beginning in 1 must be attributed to each otolith pair;
   - The otolith plaques identification labels must include: the species identification (PIL), sampling harbour abbreviation (MAT, PEN, SIN, POR), date, fish gear type and observation number;
   - Mainly because of the extreme length classes, the number of the otoliths collected by length class, by area and month, must be controlled in the same way it is done during the sea research surveys (see control sheet in Annex);
   - During age reading on otoliths the number of annual hyaline rings, the type of edge and the number of false rings must always be recorded.

5. Data Base Rules

- Sex much be identified as M (Male), F (Female) or I (Indeterminate);

- Sexual maturation stages are numbered from 1 to 6 (both for males and females). When sex is indeterminate, the maturation stage record is kept as blank;
- The otolith margin is recorded as O – Opaque and H – Hyaline;
- Length class must always be recorded;
- A data revision must take place whenever sampling data recording is finished (directly from the PC monitor or from a print record).

PS: Specific database rules must be followed (in the case of PNAB database, capital letters must be used, the port names must be abbreviated, FAO code for species - PIL - must be used, etc.).

2. Biological sampling data record sheet

Sardine Biological Sampling Data Record

Sample N:____________ **Date**: ___/___/___ **Sampling site:**____________ **Sample weight (kg):**________	**Vessel:**____________ **Fishing site:**____________ **Depth (m):**________ **Catch weight (kg):**____________ **Fishing duration:** ____________	**Fishing gear type:**____________ **Sample state: Frozen** ☐; **Fresh:** ☐ **Observations.:**____________

Nº	Length (cm)		Total weight (g)	Gutted Weight (g)	Sex	Sexual Maturation Stage	Visceral fat stage	Gonad weight (g)	Stomach		Liver weight	N. of rings	Otolith margin type	Age
	Length Class	Total Length							Colour	Filling				
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														
16														
17														
18														

…

100														

Length class (cm)																			**Total**
Nº sampled sardines																			
Total weight (g)																			
Average weight (g)																			

3. Age reading record sheet

Sardine Biological Sampling
Age Reading Record

Sample N.:_______________ ; **Date**: ____/____/____ ; **Harbour:**_________________ ; **Reader:**___________________________

Nº	Length (cm)		N. of rings	Margin type	Age
	Length Class	Total Length			
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
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26					
27					
28					
29					
30					
31					
32					
33					
34					
35					
36					
37					
38					
39					
40					

Nº	Length (cm)		N. of rings	Margin type	Age
	Length Class	Total Length			
51					
52					
53					
54					
55					
56					
57					
58					
59					
60					
61					
62					
63					
64					
65					
66					
67					
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83					
84					
85					
86					
87					
88					
89					
90					

4. Otolith collection control sheet

Species: ______________________________ Harbour: ____________________________

Year: __________ Quarter: ____________

Length Class	Month: ________________	Month: ________________	Month: ________________
10.0			
10.5			
11.0			
11.5			
12.0			
12.5			
13.0			
14.0			
14.5			
15.0			
15.5			
16.0			
16.5			
17.0			
17.5			
18.0			
18.5			
19.0			
19.5			
20.0			
20.5			
21.0			
21.5			
22.0			
22.5			
23.0			
23.5			
24.0			
24.5			
25.0			

5. Scales for macroscopic assignment of sexual maturation stages to sardines

The assignment of sexual maturation stages to sardines from the macroscopic observation of gonads is based on scales adapted from Pinto and Andreu (1957), Pinto (1957) and Pinto and Barraca (1958).

Sardine undertakes a long spawning period off the Portuguese coast, which generally extends from October/November to March/April, with the highest peak in winter months and the resting period during summer. The knowledge of the spawning season is useful for the macroscopic assignment of sexual maturation stages, particularly when doubtful cases occur as it happens between stages 3 and 6 and 2 and 5. The first two have a higher probability of occurrence in the beginning of spawning season and the last two ones at the end of this season.

Table 1 – Scale of sardine sexual maturation (females)

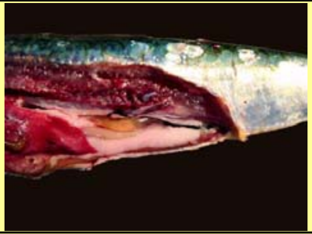
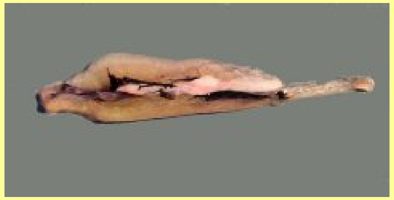
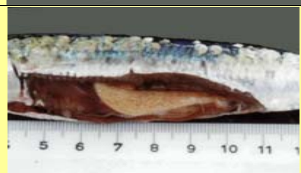
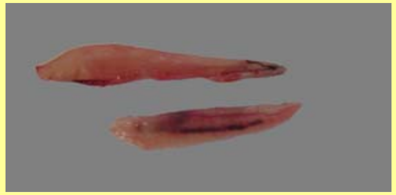
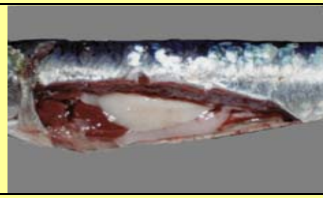
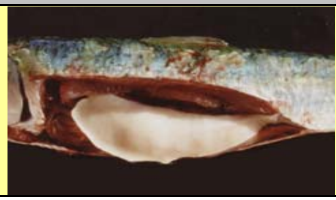
STAGE		MACROSCOPIC ASPECT OF GONADS		
1	*Virgin or resting stage*	Ovaries of small dimensions, translucent, almost uncoloured.		
2	*Developing stage*	Increasing size and beginning of opacification of ovaries. Pink or yellow colouration.		
3	*Pre-spawning stage*	Completely opaque ovocytes. Absence of hyaline zones in ovaries which are yellow-orange coloured. Ovocytes organized in well arranged rows.		
4	*Spawning stage*	Swollen and jelly like ovaries where some opaque ovocytes of the next batch can already be perceived.		
5	*Post-spawning stage*	Very flaccid ovaries with hemorrhagic zones and sometimes with necrotized ovules. Blood-coloured.		
6	*Post-spawning Recovering stage*	Similar to stage 3 but with a more hemorrhagic appearance. Ovocytes more irregularly arranged showing dispersed hyaline zones among them.		

Table 2 – Scale of sardine sexual maturation (males)

	STAGE	MACROSCOPIC ASPECT OF GONADS		
1	*Virgin or resting stage*	Double thin plated testicles with a cutting inferior margin. Variable size in adults and very small in virgin individuals. Virgin testicles are homogenously greyish coloured sometimes slightly pink while in adults they have a similar colour but not homogeneous. Testicles almost transparent, easily allowing an observation of the gonad internal vascularisation. Very consistent, any products susceptible of microscopic observation are not obtained by compression.		
2	*Developing stage*	Macroscopically testicles keep a firm consistency. Homogenous white ivory coloration sometimes greyish. Blood vessels or any other structure are not apparent at the surface. White and thick fluid is released from the gonad by compression.		
3	*Pre-spawning stage*	Swollen testicles showing the maximum size at this stage. Heterogeneous superficial colouration, showing numerous star like marks which correspond to terminal ramifications of the internal vascularisation of the gonad. An irregular mosaic spread all over the gonad surface is observed due to the fullness of the seminiferous channels with spermatozoids agglomerations.		
4	*Spawning stage*	Swollen and much vascularised testicles with a smooth and sparkling surface, not showing the star like marks of the previous stage. Superficial presence of mosaics corresponding to the seminiferous channels. White marble colouration, similar to the one of stages 2 and 3. Flaccid consistence which allows the formation of superficial depressions by compression. At this stage sperm is freed through the urogenital aperture when fish is compressed on the abdominal area.		
5	*Post- spawning stage*	Flaccid, slightly wrinkled and very thin testicles, which due to their transparency allow the observation of the internal vascularisation. After the total sperm expelling colouration changes from pinkish white to greyish white. Compression frees a fluid which is a mixture of cellular remains and a small number of spermatozoids.		
6	*Post- spawning /recovering stage*	Testicles similar to stage 2 but with bigger size and with similar colour to the previous stage with red blood shades.		

2. Scale for macroscopic assignment of visceral fat stages

The amount of visceral fat is subjected to a great variability and depends on the seasons, age and sex of the fish. The seasonal variations of the fat amount in fishes are directly related with feeding, spawning periods and the physiological state of individuals.

The fat stage in sardine seems to vary in accordance with fish length. Thus, for example in the case of sardines in the Mediterranean (Krvaric e Muzinic, 1950), smaller sized fish generally show lower fat stages than bigger ones. This may be related with the fact that younger individuals show a higher growth rate.

Sometimes it can happen that some adult fish show a lower fat stage than young ones. Usually this occurs when these fish are in spawning stage.

Planktivorous fish of temperate waters such as sardines and herrings have more water and less fat in their body tissues during spring, when plankton blooms occur, than during the post spawning seasons. When the amount of fat increases in tissues the amount of water increases only very slightly. Thus, considering that the amount of water keeps relatively constant, the weight increase of individuals fundamentally is a consequence of the fat accumulation in the tissues in visceral cavity (Wagner e Ramalho (1936) *in* Krvaric e Muzinic, 1950).

Several authors have proposed scales for macroscopic assignment of visceral fat stages to various species. For sardine off Portuguese coast a scale proposed by Furnestin (1939, 1943) (*in* Krvaric e Muzinic, 1950) is presently used (see Table 3). Fat accumulated in muscles was chemically analysed by this author who compared the results obtained with those based on an empirical scale. As the results obtained by both ways were very similar, Furnestin considered as valid the use of the empirical scale for the assignment of fat stages in sardines.

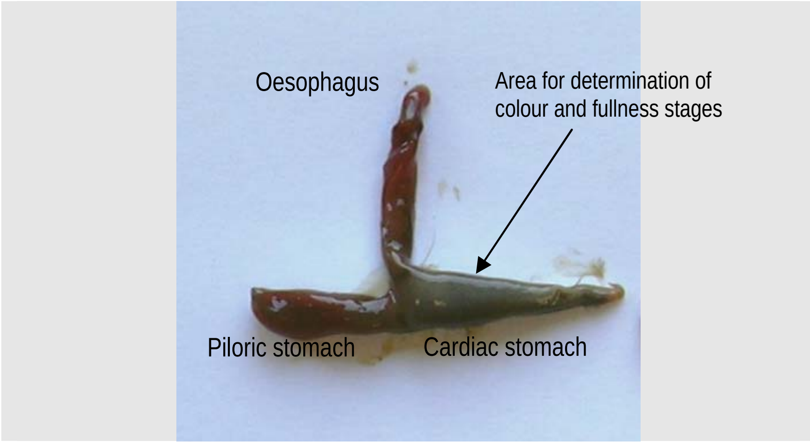
Table 3 – Scale for macroscopic assignment of visceral fat stages

Stage		Aspect
I	*Thin stage*	Absence of fat on the digestive tract.
II	*Low fat stage*	A thin string of fat along the intestines is visible.
III	*Medium Fat stage*	The string of fat becomes larger and can even wrap all the digestive tract.
IV	*High fat stage*	All the digestive tract is involved by fat as well as the abdominal cavity.

3. Stomach condition (colour and fullness)

Table 4 – Scale for assignment of stomach condition (colour and fullness) stages

Colour scale	Fullness scale
	**1 – Almost empty** **2 – Half full** **3 – Full** **4 – Bursting**



Colour	1	2	2
Filling	1	2	3

Colour	4	4	4
Filling	2	3	4

4. Occurrence of parasites

The following pictures show an ectoparasitic isopod which is found in the buccal cavity of sardine attached to the tongue apparently feeding on host's blood. Most probably it belongs to the family *Cymothoidea* that parasitize numerous families and species of fish, some of commercial importance, especially in warmer water regions (tropical and sub-tropical). Preliminary identification indicates that this species is *Ceratothoa oestroides*, although additional taxonomic verification is still in progress.

Although it was recently also detected in the Cantabrian Sea, routine biological sampling indicates that this parasite is more frequently found in sardines off the Portuguese coast in the Algarve region. Observations on live sardine show that usually a pair is found parasitizing healthy hosts (with the male considerably smaller) and that these parasites rapidly abandon moribund fish. The presence of females carrying eggs and the release of larvae in aquaculture tanks suggest that reproduction occurs within the buccal cavity of the host.

References

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Krvaric, J. and Muzinic, R., 1950. Investigation into the fat content in the sardine tissues. *Acta Adriatica*, 4(8) 1950: 291-313.

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Pinto, J.S., 1957. Essai d'une échelle de maturation sexuelle, pour les males de la sardine (*Sardina pilchardus* Walb.) basée sur l'histologie des testicules. *Notas e Estudos Inst. Biol. Mar.*, **15**.