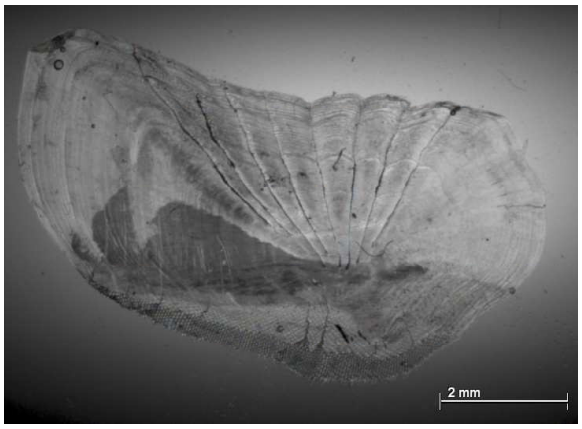
Red mullet (*Mullus surmuletus*) and striped red mullet (*M. barbatus*) otolith and scale exchange 2011



Red mullet (*Mullus surmuletus*) and striped red mullet (*M. barbatus*) otolith and scale exchange 2011

Mahé, K., Elleboode, R., Charilaou, C., Ligas, A., Carbonara, P. & Intini, S., 2012. Red mullet (*Mullus surmuletus*) and striped red mullet (*M. barbatus*) otolith and scale exchange 2011, 30pp.

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1. Introduction

The Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) meeting in 2011 recommended a small exchange :

"7.2.1.1.3 Red mullet (Mullus surmuletus) and striped red mullet (M. barbatus) An exchange for a new set of M. barbatus otoliths from the Mediterranean should be examined and new sets of M. surmuletus otoliths from the Mediterranean, the Gulf of Biscay and the English Channel should be organised, in order to detect differences between areas. PGCCDBS recommends a small exchange in 2011 in order to clarify the ageing in these species and to compare age reading from otoliths and scales (PGMED)."

5 countries took part to this exchange :

- Spain
- Greece
- Cyprus
- Italy
- France

The objectives of this exchange were:

- to investigate the levels of agreement on age readings
- to analyse the relative differences between age readers and calcified pieces (otoliths and scales)

2. Participants

5 readers participated to this exchange.

Table 1 : List of the readers.

Name	Country	Institute
Romain Elleboode	France	IFREMER
Charis Charilaou	Cyprus	DFMR
Alessandro Ligas	Italy	CIBM
Carbonara Pierluigi/ Intini Simona	Italy	COISPA

3. Material

175 *Mullus surmuletus* and 202 *Mullus barbatus* specimens have been examined by all Institutes :

- Balearic Islands, *Mullus surmuletus*, burned and unburned otoliths, 100 read in its entirely (IEO Institute)
- Balearic Islands, *Mullus surmuletus*, scales, 95 (IEO Institute)
- Bay of Biscay, *Mullus surmuletus*, burned and unburned otoliths, 75 read in its entirely (IFREMER Institute)

- Southern Spain, *Mullus barbatus*, burned and unburned otoliths, 100 read in its entirely (IEO institute)
- Southern Spain, *Mullus barbatus*, scales, 68 (IEO Institute)
- Southern Adriatic sea, *Mullus barbatus*, unburned whole otoliths, 102 (COISPA Institute)

540 images of 377 otoliths and 163 scales were used (Fig. 2 and 3; Tab. 2). 2 images sets have been realised for *Mullus surmuletus* in Balearic Islands and for *Mullus barbatus* in the Southern Spain to compare the age estimation between both calcified pieces.

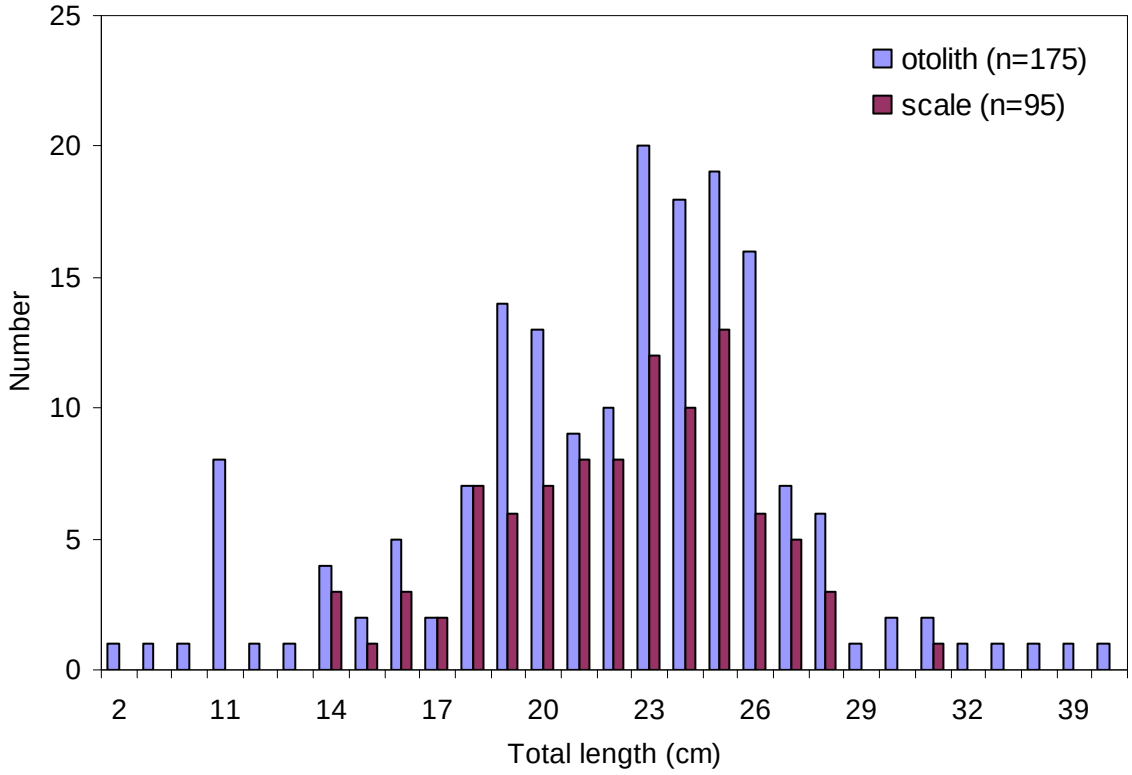


Figure 2 : Histograms of *Mullus surmuletus* samples by calcified pieces.

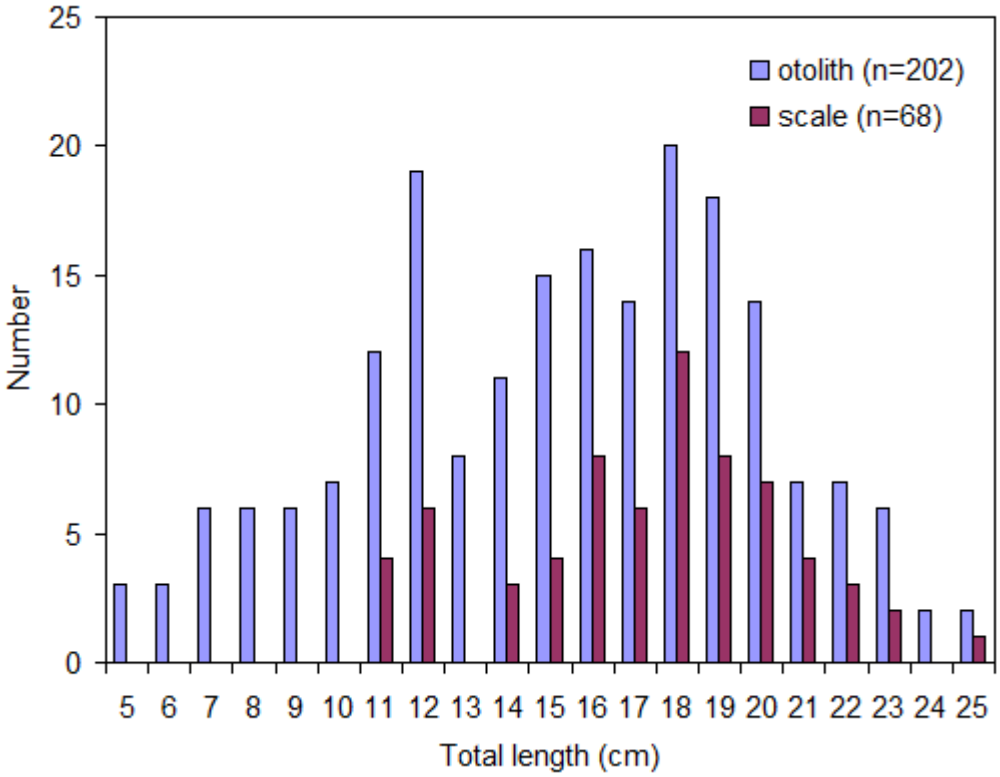


Figure 3 : Histograms of *Mullus barbatus* samples by calcified pieces.

Table 2 : Mean total length (range) of fish (cm) by species, calcified piece and sex.

Species	Calcified piece	Female	Immature	Male	Total
Mullus barbatus	otolith	17.6 (9-25.2)	7.1 (5-8.5)	14.6 (9-20.5)	15.7 (5-25.2)
	scale	18.3 (11.4-25.2)		15.5 (11.2-18.6)	17.7 (11.2-25.2)
Mullus surmuletus	otolith	23.2 (4-44)	17.6 (11-28)	22.3 (2-35)	22.3 (2-44)
	scale	23.9 (17.6-31.7)		19.5 (14-25.5)	22.7 (14-31.7)

4. Reading procedure

A video per scale has been carried out while varying lighting to contribute to the interpretation.

The interpretation should be done based on the following guidelines established at the last Workshop (Report on WKACM 2009 (ICES, 2009)) :

i. As a first step, a blind reading has to be performed at the beginning without any information given except the date of capture.

ii. Selection of a suitable measurement axis (Fig. 3); it is proposed the axis joining the *sulcus* and the *nucleus* of the otolith.

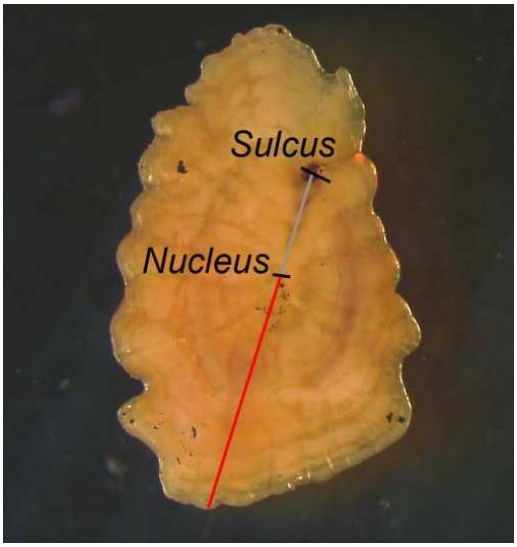


Figure 3 : Surface of the whole otolith presenting the Axis joining the *sulcus* and the *nucleus* as a suitable measurement axis.

iii. Translucent true rings should be visible all around the whole otolith in order to be considered as annual rings.
iv. 1st of January is considered as the date of birth. As a result, if a translucent ring is observed at the edge of the otolith at the first part of the year, it should be counted as *annulus*. In contrary, if a translucent ring is observed at the edge of the otolith at the second part of the year, it should not be counted as annulus.

Each reader must interpret the calcified structure as individual and must not be influenced by other readings of the calcified structure on the same fish. This exchange will be used to compare the readings between otoliths and scales.

Each reader must complete the column of age reading quality such as :

AQ1: Easy to age with high precision.

If a scale of 1-100 is applied, where 100 represents the highest readers confidence in age reading and 1 indicates no confidence in the age reading. Age quality 1 (AQ1), will apply to approximately the top 25 % of the possible quality ratings. AQ1 is an indication that the age data is considered reliable for stock assessment.

AQ2: Normal quality.

Age quality 2 (AQ2), will apply approximately to age readings comprised between 25 and 75 percentiles of possible quality ratings. AQ2 is an indication that the age data is sufficiently reliable to be used for stock assessment purposes but an improvement is required.

AQ3: Difficult to age with acceptable precision.

Age quality 3 (AQ3), will apply to approximately the lowest 25 % of the possible quality ratings. AQ3 is an indication that there are serious

concerns about the reliability of the age data and/or its value to stock assessment WGs.

5. Results

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

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

The 6 samples were not read by all readers (Tab. 3).

Table 3 : Number of readers by species and calcified piece.

	Mullus surmuletus		*Mullus barbatus*	
	whole otolith	scale	whole otolith	scale
Bay of Biscay	4			
Balearic Islands	4	2		
Southern Spain			4	2
Southern Adriatic			3	

5.1. Precision[1]

Results among sets of calcified pieces showed a large difference (Tab. 4 and 5). A set of *Mullus surmuletus* otoliths from the Bay of Biscay presented better percentage of agreement (82% ; Tab. 5). On 75 otoliths, 34 were read with 100% agreement (45%) and thus a CV of 0%. The modal age of these fishes was comprised between 0 and 3 years. The second set with good percent agreement was composed by otoliths from *Mullus barbatus* from the Southern Adriatic (68.6% ; Tab. 5). On 102 otoliths, 20 were read with 100% percentage agreement (19.6%) and thus a CV of 0%.
The other sets of this exchange, for both species, showed a very lower precision under 50% percentage agreement. The reason of these results was not species while it occurs in both species. In Balearic Islands and southern Spain, we compared readings from otoliths and scales but the difference observed between calcified pieces did not explain these results. In Balearic Islands and southern Spain, the results only from otoliths presented respectively coefficient of 37.6% and 48.5% and percentage agreement of 47.3% and 52.8%.
Difference of precision could be largely due to the sampling area (it is easier to estimate age in the Atlantic than in the Mediterranean sea) and the composition of the samples (all *Mullus barbatus* from the Southern Adriatic presenting 100% percentage agreement, had the modal age at 0 year. Inversely, modal age of the fish from the Balearic Islands and the southern Spain began to 1 year).

Table 4 : Coefficient of Variation (range) for each set of images by species, calcified pieces and area.

	Mullus surmuletus		*Mullus barbatus*	
	whole otolith	scale	whole otolith	scale
Bay of Biscay	42.4% (0-200%)			
Balearic Islands	32.3% (11-31%)			
Southern Spain			47.8% (0-115%)	
Southern Adriatic			59.6% (0-173%)	

Table 5 : Percent agreement (range) for each set of images by species, calcified pieces and area.

	Mullus surmuletus		*Mullus barbatus*	
	whole otolith	scale	whole otolith	scale
Bay of Biscay	82% (50-100%)			
Balearic Islands	45.9% (0-80%)			
Southern Spain			48.7% (25-100%)	
Southern Adriatic			68.6% (33-100%)	

[1] Precision is defined as the variability in the age readings. The precision's errors in age readings are better described by the coefficient of variation (CV) by age group. This measure of precision is independent of the closeness to the true age (ICES, 2007).

5.2. Relative bias (Accuracy) [2]

Minimal requirement for age reading's consistency is the absence of bias among readers and through time. The hypothesis of an absence of bias between two readers or between a reader and the modal age estimated can be tested non-parametrically with a one-sample Wilcoxon signed rank test. This table shows inter-reader bias test and reader against modal age bias test (-: no sign of bias ($p>0.05$); *: possibility of bias ($0.01<p<0.05$); **: certainty of bias ($p<0.01$)).

The tables 6. 7. 8 and 9 showed these analyses for the set coming from different areas and from both species of *Mullus*.

Table 6 : Inter-reader bias test and reader against modal age bias test for the set of *Mullus surmuletus* coming from the bay of Biscay.

	France RE	Italy AL	Italy CI	Cyprus CC
	Reader 1	Reader 2	Reader 3	Reader 4
Reader 1				
Reader 2	*			
Reader 3	–	*		
Reader 4	*	* *	* *	
MODAL age	–	* *	–	*

Table 7 : Inter-reader bias test and reader against modal age bias test for the set of *Mullus surmuletus* coming from the Balearic island.

	France RE	Italy AL	Italy CI	Cyprus CC	France RE	Italy AL
	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6
Reader 1						
Reader 2	* *					
Reader 3	* *	* *				
Reader 4	* *	* *	* *			
Reader 5	* *	* *	* *	* *		
Reader 6	* *	* *	–	* *	* *	
MODAL age	* *	* *	–	* *	* *	–

Table 8 : Inter-reader bias test and reader against modal age bias test for the set of *Mullus barbatus* coming from the southern Spain.

[2] In absence of calcified structures of known age, the age readings can be compared to modal age, which is defined as the age determined for an individual structure whose most of the readers have a preference. Relative bias can be defined as a systematic over- or underestimation of age compared to the modal age. The age reading comparisons to modal age provide a low estimate of relative bias compared to absolute bias, when most readers have a similar serious bias in age reading (ICES, 2007).

	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	Cyprus CC Reader 4	France RE Reader 5	Italy AL Reader 6
Reader 1						
Reader 2	* *					
Reader 3	* *	* *				
Reader 4	* *	* *	* *			
Reader 5	* *	* *	* *	* *		
Reader 6	* *	*	* *	* *	* *	
MODAL age	* *	* *	* *	* *	* *	* *

Table 9 : Inter-reader bias test and reader against modal age bias test for the set of *Mullus barbatus* coming from the southern Adriatic sea.

	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3
Reader 1			
Reader 2	*		
Reader 3	* *	* *	
MODAL age	* *	* *	* *

It should be noted that there were certainty of bias among readings (and between 2 readings of the same reader) from otoliths and from scales and modal age.

5.3. Age reading quality

Age reading quality was estimated by all readers (Tab. 10).

Table 10 : Level of Age reading quality by readers and all readers of the otoliths (readers 1, 2, 3, 4) and scales (reader 6) by species and by areas.

Species	Area	Level of Quality	reader 1	reader 2	reader 3	reader 4	reader 6	Total
M. surmuletus	Bay of Biscay	AQ1		3	20			23 (7.6%)
		AQ2	75	70	55	75		275 (91.6%)
		AQ3		2				2 (0.6%)
	Balearic Islands	AQ1			11		5	16 (4.1%)
		AQ2		36	78	100	40	254 (64.8%)
		AQ3		64	8		50	122 (31.1%)
M. barbatus	Southern Spain	AQ1		1	29			31 (15.0%)
		AQ2		78	54			134 (65.0%)
		AQ3		21	17			41 (19.9%)
	Southern Adriatic	AQ1		30	6			36 (12.1%)
		AQ2		52	64			116 (39.0%)
		AQ3	99	20	26			145 (48.8%)

The images of *Mullus surmuletus* in the Bay of Biscay were almost all classified into AQ2 (91.6%). For the others sets, Quality level 2 decreased for Quality level 3 (Difficult to age with acceptable precision).

6. Executive Summary

There were an exchange and a workshop in 2009 of Red mullet *Mullus barbatus* and Striped red mullet *Mullus surmuletus* (WKACM). The Otolith Exchange Scheme 2011 was the second exercise for the Red mullet *Mullus barbatus* and Striped red mullet *Mullus surmuletus*. 5 readers participated in this exchange. The images collection (n=540) came from the Bay of Biscay, the Balearic Islands, the Southern Spain and the Southern Adriatic sea. 175 *Mullus surmuletus* and 202 *Mullus barbatus* have been examined to all Institutes. Among 540 images, there were 377 otoliths and 163 scales. 2 images sets have been realised for *Mullus surmuletus* in Balearic Islands and for *Mullus barbatus* in the Southern Spain to compare the age estimation between both calcified pieces.

Results among sets of calcified pieces showed a large difference (Tab. 4 and 5). A set of *Mullus surmuletus* otoliths from the Bay of Biscay presented better percentage of agreement (82% ; Tab. 5). On 75 otoliths, 34 were read with 100% agreement (45%) and thus a CV of 0%. The modal age of these fishes was comprised between 0 and 3 years. The second set with good percent agreement was composed by otoliths from *Mullus barbatus* from the Southern Adriatic (68.6% ; Tab. 5). On 102 otoliths, 20 were read with 100% percentage agreement (19.6%) and thus a CV of 0%.

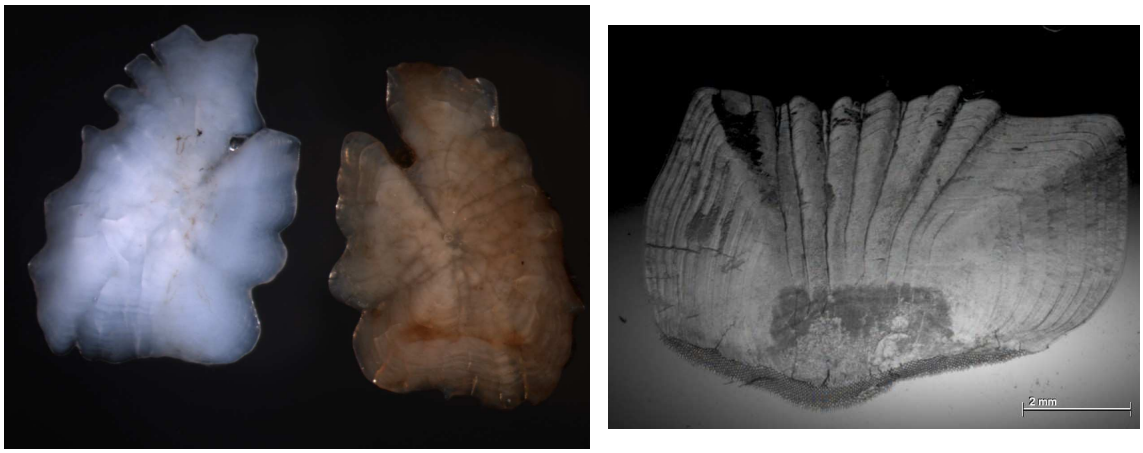
The other sets of this exchange, for both species, showed a very lower precision under 50% percentage agreement. The reason of these results was not species while it occurs in both species. In Balearic Islands and southern Spain, we compared readings from otoliths and scales but the difference observed between calcified pieces did not explain these results. In Balearic Islands and southern Spain, the results only from otoliths presented respectively coefficient of 37.6% and 48.5% and percentage agreement of 47.3% and 52.8%.

Difference of precision could be largely due to the sampling area, indeed (it is easier to estimate age in the Atlantic than in the Mediterranean sea). This is due to the environmental characteristics (e.g. higher seasonal differences in the temperature) generally in the otoliths of Atlantic stocks results in the formation of more clear and distinguishable annuli than their Mediterranean counterparts. Moreover difference of precision could be due also to the and the composition of the samples (all *Mullus barbatus* from the Southern Adriatic presenting 100% percentage agreement, had the modal age at 0 year. Inversely, modal age of the fish from the Balearic Islands and the southern Spain began to 1 year). Indeed one of the critical point for the ageing in *Mullus barbatus* is to distinguish the first hyaline ring from(ICES, 2009).

It should be noted that there were certainty of bias among readings from otoliths and from scales and modal age. Moreover, there is certainty of bias between the readings from different calcified pieces of the same fish.

The images of *Mullus surmuletus* in the Bay of Biscay were almost all classified into AQ2 (91.6%). For the others sets, Quality level 2 decreased for Quality level 3 (Difficult to age with acceptable precision).

In this context where the lack of validated age data is a major source of uncertainty for the application of analytical stock assessments models, as well as to the reliability of the assessment results, the age validation and calcified structures formation studies should be of high priority.



Age estimated from 3 to 9 years from the scales or from the otoliths. *Mullus surmuletus* was sampled March 2011 in the Balearic Island. This is a female of 28 cm TL.

Age estimated from 2 to 6 years from the otoliths. *Mullus barbatus* was sampled March 2011 in the Southern Spain. This is a female of 23.2 cm TL.

7. References

Eltink, A. T. G. W., Newton, A. W., Morgado, C., Santamaria, M. T. G., Modin, J., 2000. Guidelines and Tools for Age Reading. **(**PDF document version 1.0 October 2000) Internet : http://www.efan.no

Eltink, A. T. G. W., 2000. Age reading comparisons. (MS Excel workbook version 1.0 October 2000) Internet : http://www.efan.no

ICES. 2007. Report of the Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS), 5–9 March 2007, Valetta, Malta. ACFM:09. 115p.

ICES. 2009. Report of the Workshop on Age Reading of Red mullet *Mullus barbatus* and Striped mullet *Mullus surmuletus* (WKACM), 30 March - 3 April 2009, Boulogne sur Mer, France. ICES CM 2009\ACOM:44. 42 pp.

8. Appendix 1 : Details results of *Mullus surmuletus* Otolith Exchange (VIIIab)

The number of age readings, the coefficient of variation (CV), the percentage of agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups combined indicate the precision in age reading by reader and for all age readers combined.

NUMBER OF AGE READINGS

	MODAL age	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	Cyprus CC Reader 4	TOTAL
	0	29	29	29	29	116
	1	28	28	28	28	112
	2	5	5	5	5	20
	3	4	4	4	4	16
	4	5	5	5	5	20
	5	3	3	3	3	12
	6	-	-	-	-	-
	7	1	1	1	1	4
Total	0-7	75	75	75	75	300

COEFFICIENT OF VARIATION (CV)

	MODAL age	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	Cyprus CC Reader 4	ALL Readers
	0	0%	199%	199%	0%	63.1%
	1	34%	46%	56%	47%	39.8%
	2	34%	20%	0%	0%	16.9%
	3	0%	15%	15%	0%	4.1%
	4	12%	12%	11%	25%	16.3%
	5	0%	25%	20%	13%	14.1%
	6	-	-	-	-	-
	7	-	-	-	-	-
Weighted mean	0-7	16.0%	98.3%	100.2%	19.9%	42.4%
	RANKING	1	3	4	2	

PERCENTAGE AGREEMENT

	MODAL age	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	Cyprus CC Reader 4	ALL
	0	100%	79%	79%	100%	90%
	1	89%	75%	75%	82%	80%
	2	60%	80%	100%	100%	85%
	3	100%	75%	75%	100%	88%
	4	60%	40%	80%	80%	65%
	5	100%	0%	67%	33%	50%
	6	-	-	-	-	-
	7	0%	0%	100%	100%	50%
Weighted mean	0-7	89.33%	70.67%	78.67%	89.33%	82.00%
	RANKING	1	4	3	1	

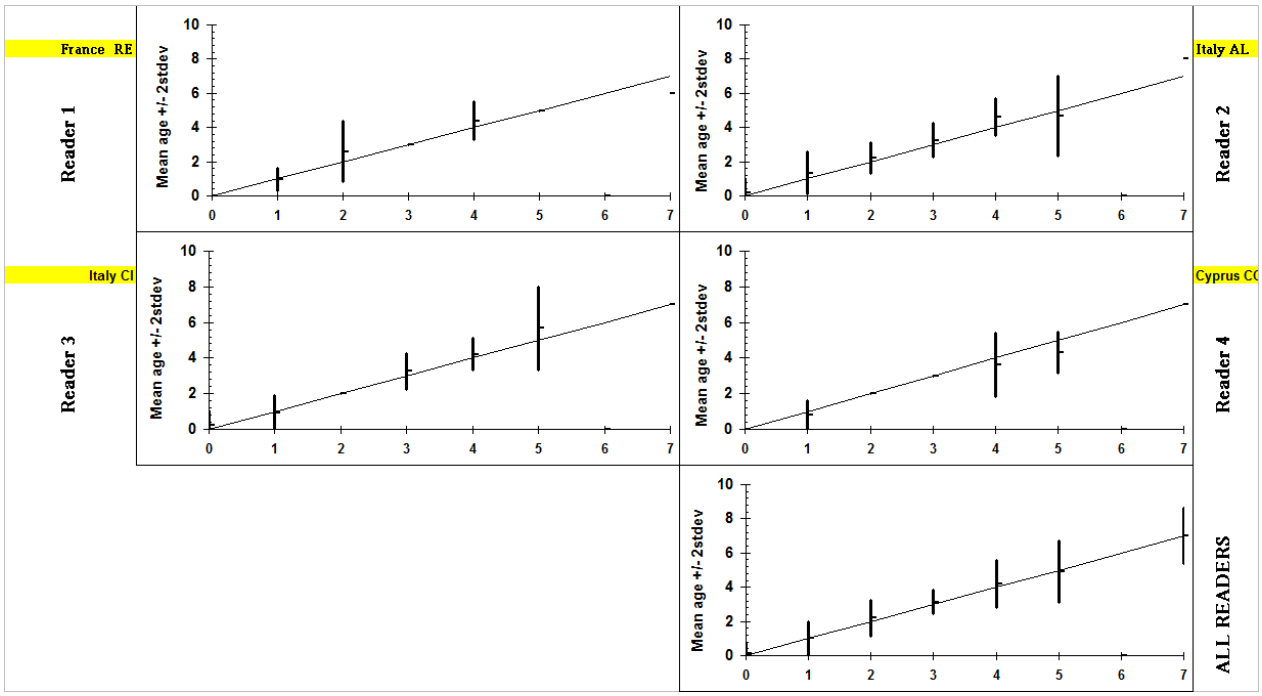
RELATIVE BIAS

	MODAL age	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	Cyprus CC Reader 4	ALL
	0	0	0	0	0	0
	1	0	0	0	0	0
	2	1	0	0	0	0
	3	0	0	0	0	0
	4	0	1	0	0	0
	5	0	0	1	-1	0
	6	-	-	-	-	-
	7	-1	1	0	0	0
Weighted mean	0-7	0.04	0.27	0.09	-0.12	0.10
	RANKING	1	4	3	1	

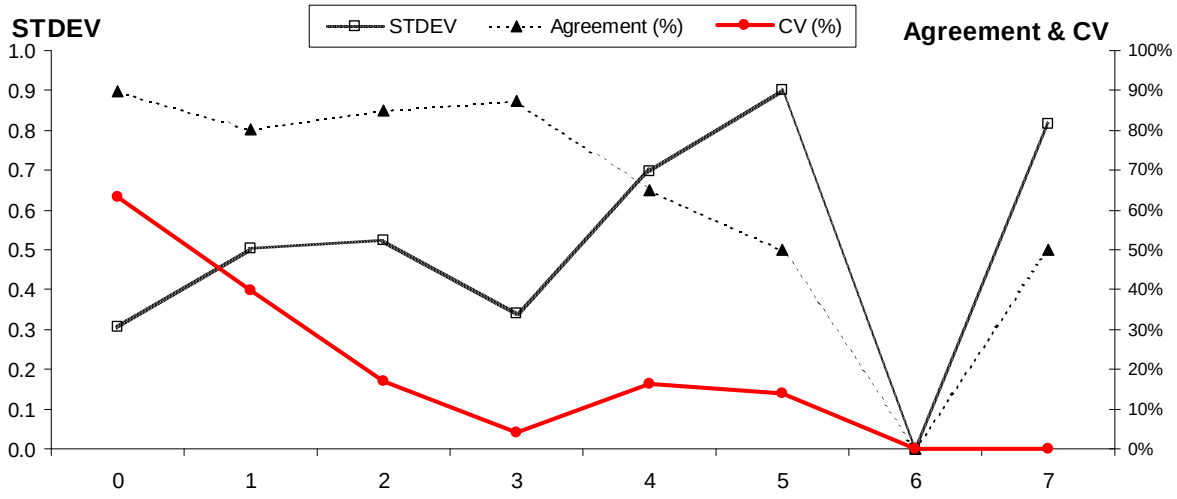
Overall ranking

	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	Cyprus CC Reader 4
Ranking Coefficient of Variation	1	3	4	2
Ranking Percentage Agreement	1	4	3	1
Ranking Relative bias	1	4	3	1
OVERALL RANKING	1	4	3	2

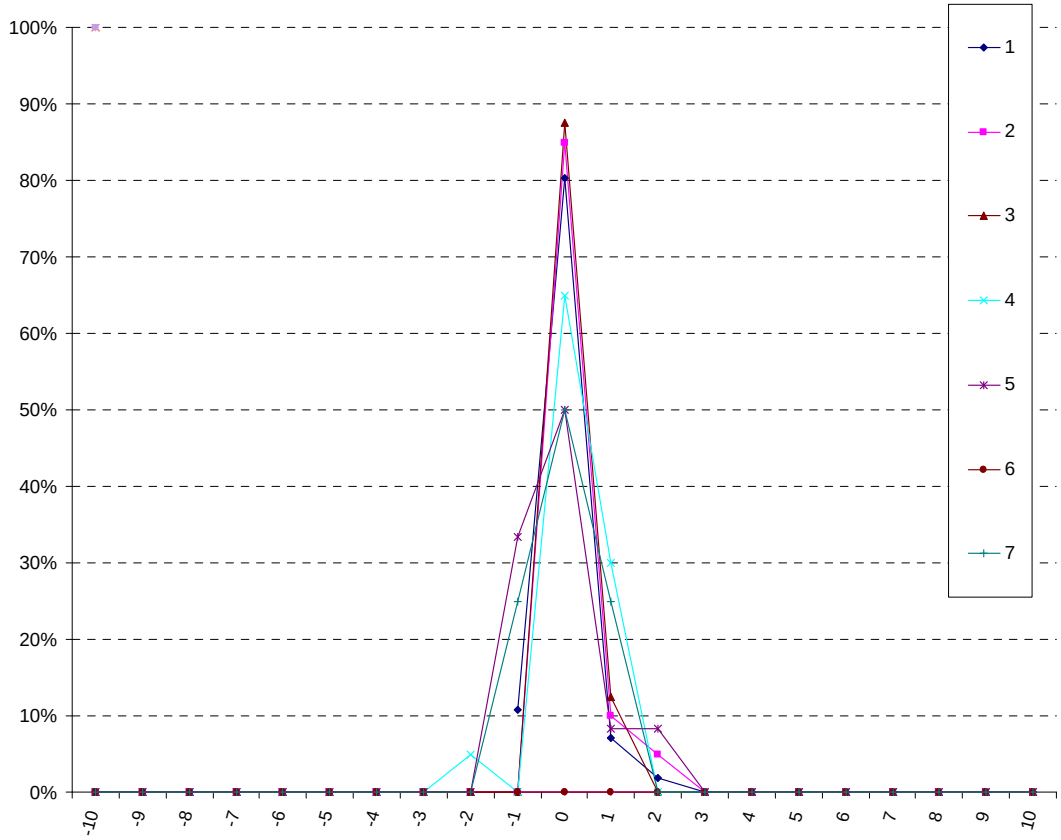
In the age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.



The coefficient of variation (CV%), percentage of agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percentage of agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.



The distribution of the age reading errors in percentage by MODAL age as observed from the whole group of age readers in an age reading comparison to MODAL age. The achieved precision in age reading by MODAL age group is shown by the spread of the age readings errors. There appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.



9. Appendix 2 : Details results of *Mullus surmuletus* Otolith and Scale Exchange (Balearic Islands)

The number of age readings, the coefficient of variation (CV), the percentage of agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups combined indicate the precision in age reading by reader and for all age readers combined.

NUMBER OF AGE READINGS

	MODAL age	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	Cyprus CC Reader 4	France RE Reader 5	Italy AL Reader 6	TOTAL
	0	-	-	-	-	-	-	-
	1	3	3	3	3	-	3	15
	2	14	14	14	14	1	13	70
	3	32	32	32	32	4	30	162
	4	30	30	30	30	4	29	153
	5	16	16	14	16	3	15	80
	6	5	5	4	5	3	5	27
Total	0-6	100	100	97	100	15	95	507

COEFFICIENT OF VARIATION (CV)

	MODAL age	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	Cyprus CC Reader 4	France RE Reader 5	Italy AL Reader 6	ALL Readers
	0	-	-	-	-	-	-	-
	1	17%	0%	0%	0%	-	78%	53.8%
	2	34%	24%	17%	78%	-	37%	44.5%
	3	23%	19%	17%	37%	29%	24%	30.5%
	4	22%	21%	18%	32%	12%	26%	29.1%
	5	18%	23%	18%	36%	0%	22%	28.1%
	6	7%	12%	14%	14%	9%	45%	28.6%
Weighted mean	0-6	22.6%	20.3%	17.0%	38.9%	12.6%	28.8%	32.3%
RANKING		4	3	2	6	1	5	

PERCENTAGE AGREEMENT

	MODAL age	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	Cyprus CC Reader 4	France RE Reader 5	Italy AL Reader 6	ALL
	0	-	-	-	-	-	-	-
	1	0%	100%	0%	100%	-	33%	47%
	2	29%	79%	86%	29%	0%	46%	53%
	3	22%	78%	72%	31%	25%	43%	49%
	4	60%	37%	63%	3%	75%	62%	46%
	5	44%	56%	43%	0%	100%	47%	40%
	6	80%	0%	50%	0%	67%	0%	30%
Weighted mean	0-6	40.00%	59.00%	63.92%	18.00%	60.00%	47.37%	45.96%
RANKING		5	3	1	6	2	4	

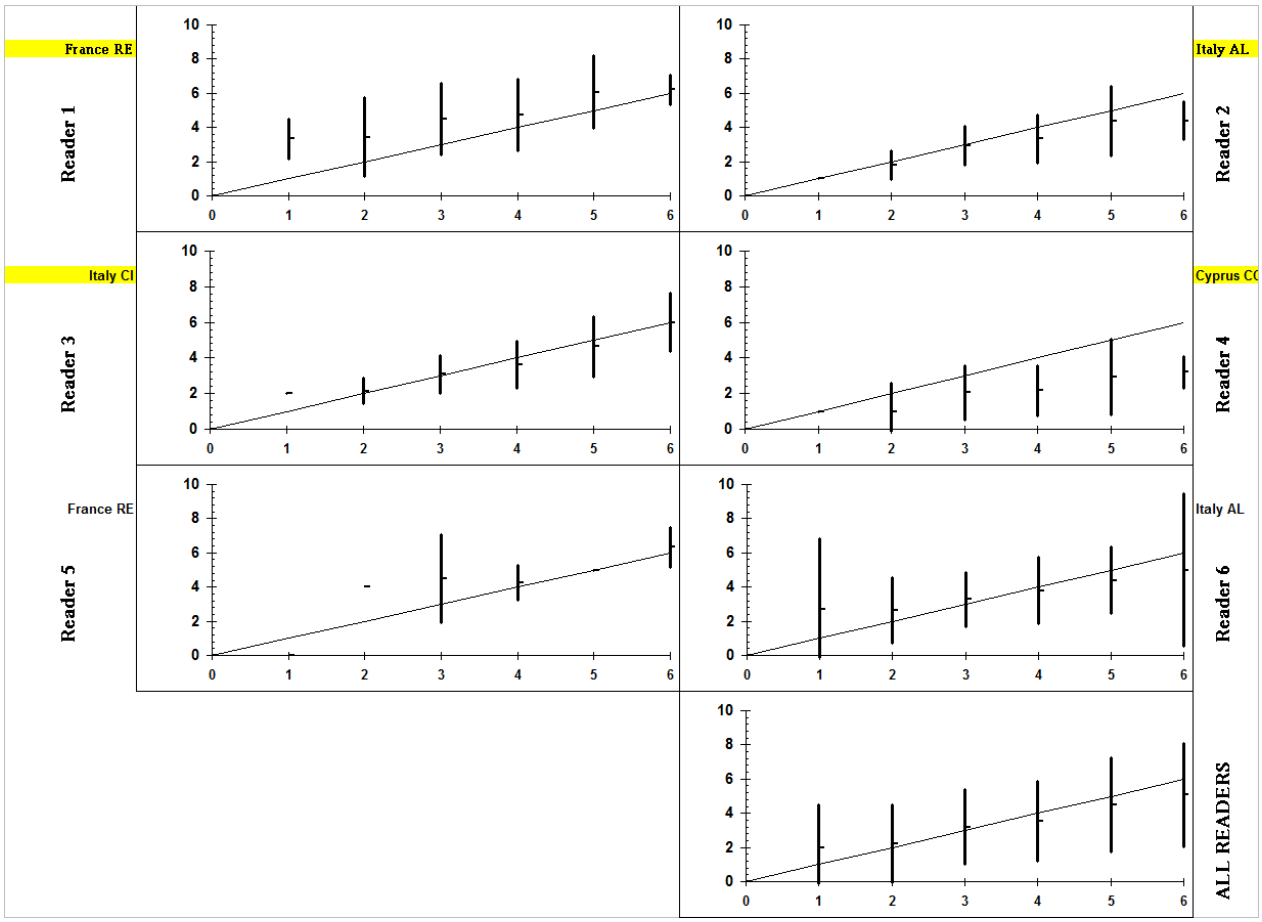
RELATIVE BIAS

	MODAL age	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	Cyprus CC Reader 4	France RE Reader 5	Italy AL Reader 6	ALL
	0	-	-	-	-	-	-	-
	1	2	0	1	0	-	2	1
	2	1	0	0	-1	2	1	0
	3	1	0	0	-1	2	0	0
	4	1	-1	0	-2	0	0	0
	5	1	-1	0	-2	0	-1	-1
	6	0	-2	0	-3	0	-1	-1
Weighted mean	0-6	1.14	-0.43	-0.08	-1.46	0.67	0.01	-0.24
RANKING		5	3	1	6	2	4	

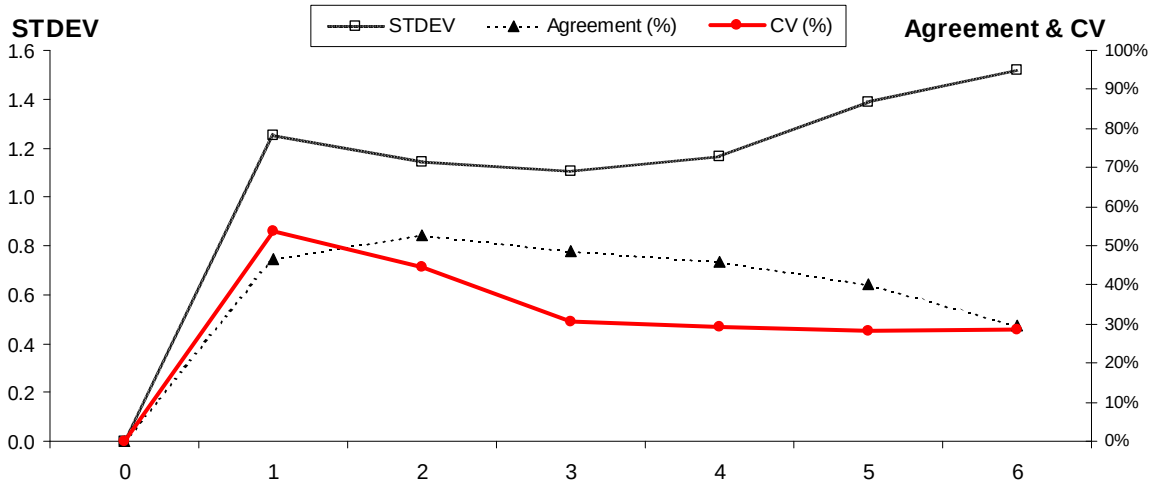
Overall ranking

	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	Cyprus CC Reader 4	France RE Reader 5	Italy AL Reader 6
Ranking Coefficient of Variation	4	3	2	6	1	5
Ranking Percentage Agreement	5	3	1	6	2	4
Ranking Relative bias	5	3	1	6	2	4
OVERALL RANKING	5	3	1	6	2	4

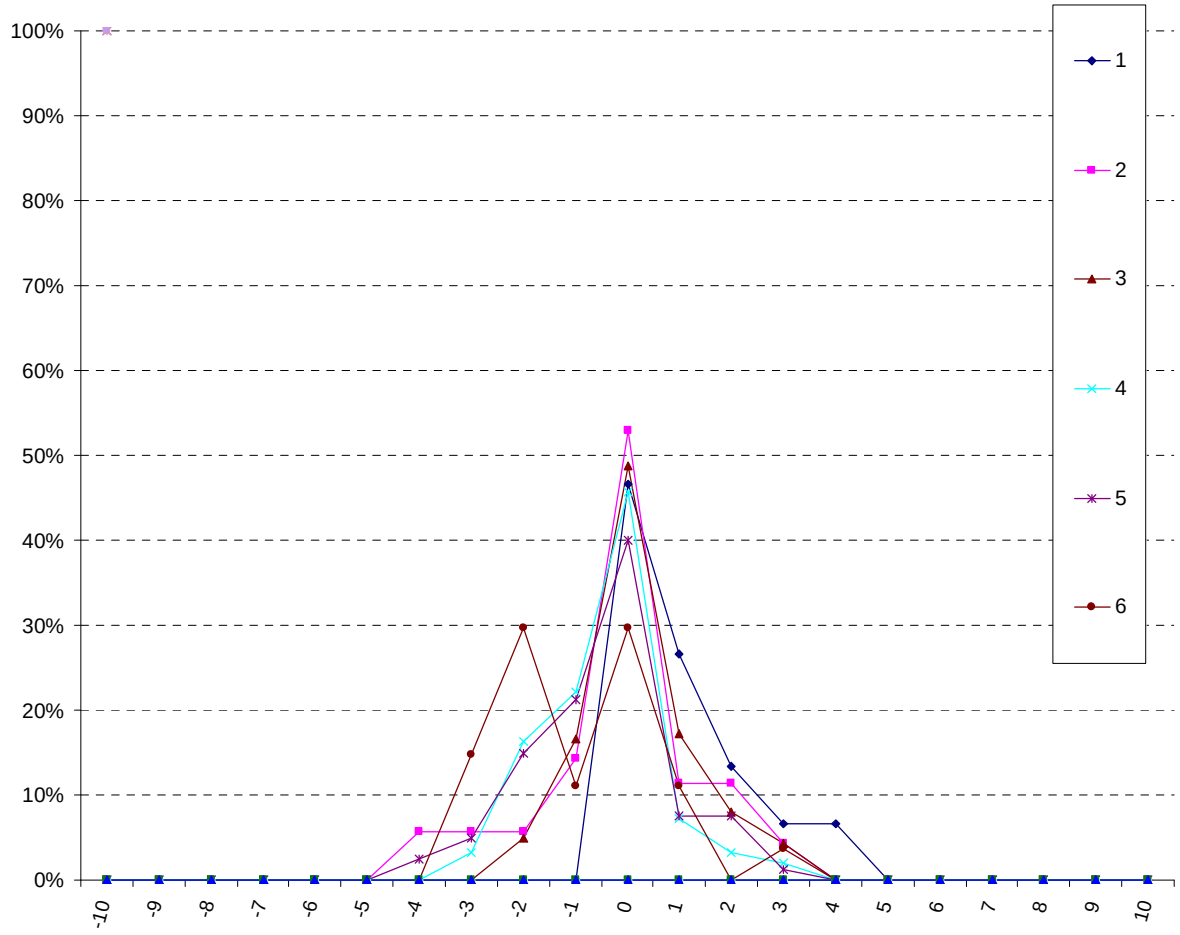
In the age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.



The coefficient of variation (CV%), percentage of agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percentage of agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.



The distribution of the age reading errors in percentage by MODAL age as observed from the whole group of age readers in an age reading comparison to MODAL age. The achieved precision in age reading by MODAL age group is shown by the spread of the age readings errors. There appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.



10. Appendix 3 : Details results of *Mullus barbatus* Otolith and Scale Exchange (Southern Spain)

The number of age readings, the coefficient of variation (CV), the percentage of agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups combined indicate the precision in age reading by reader and for all age readers combined.

NUMBER OF AGE READINGS

	MODAL	France RE	Italy AL	Italy CI	Cyprus CC	France RE	Italy AL	
	age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	TOTAL
	0	-	-	-	-	-	-	-
	1	16	16	16	16	5	5	74
	2	41	41	41	41	29	28	221
	3	34	34	34	34	27	27	190
	4	9	9	9	9	6	6	48
Total	0-4	100	100	100	100	67	66	533

COEFFICIENT OF VARIATION (CV)

	MODAL	France RE	Italy AL	Italy CI	Cyprus CC	France RE	Italy AL	ALL
	age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Readers
	0	-	-	-	-	-	-	-
	1	37%	27%	33%	50%	41%	25%	56.6%
	2	30%	53%	20%	99%	32%	40%	57.2%
	3	17%	30%	19%	49%	23%	27%	34.9%
	4	8%	27%	24%	43%	0%	22%	38.1%
Weighted mean	0-4	24.8%	38.5%	22.1%	69.3%	26.0%	31.7%	47.8%
	RANKING	2	5	1	6	3	4	

PERCENTAGE AGREEMENT

	MODAL	France RE	Italy AL	Italy CI	Cyprus CC	France RE	Italy AL	
	age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	ALL
	0	-	-	-	-	-	-	-
	1	25%	94%	25%	81%	20%	20%	51%
	2	63%	39%	73%	32%	14%	68%	49%
	3	79%	24%	79%	18%	56%	37%	49%
	4	89%	11%	56%	0%	100%	0%	42%
Weighted mean	0-4	65.00%	40.00%	66.00%	32.00%	38.81%	45.45%	48.59%
	RANKING	2	4	1	6	5	3	

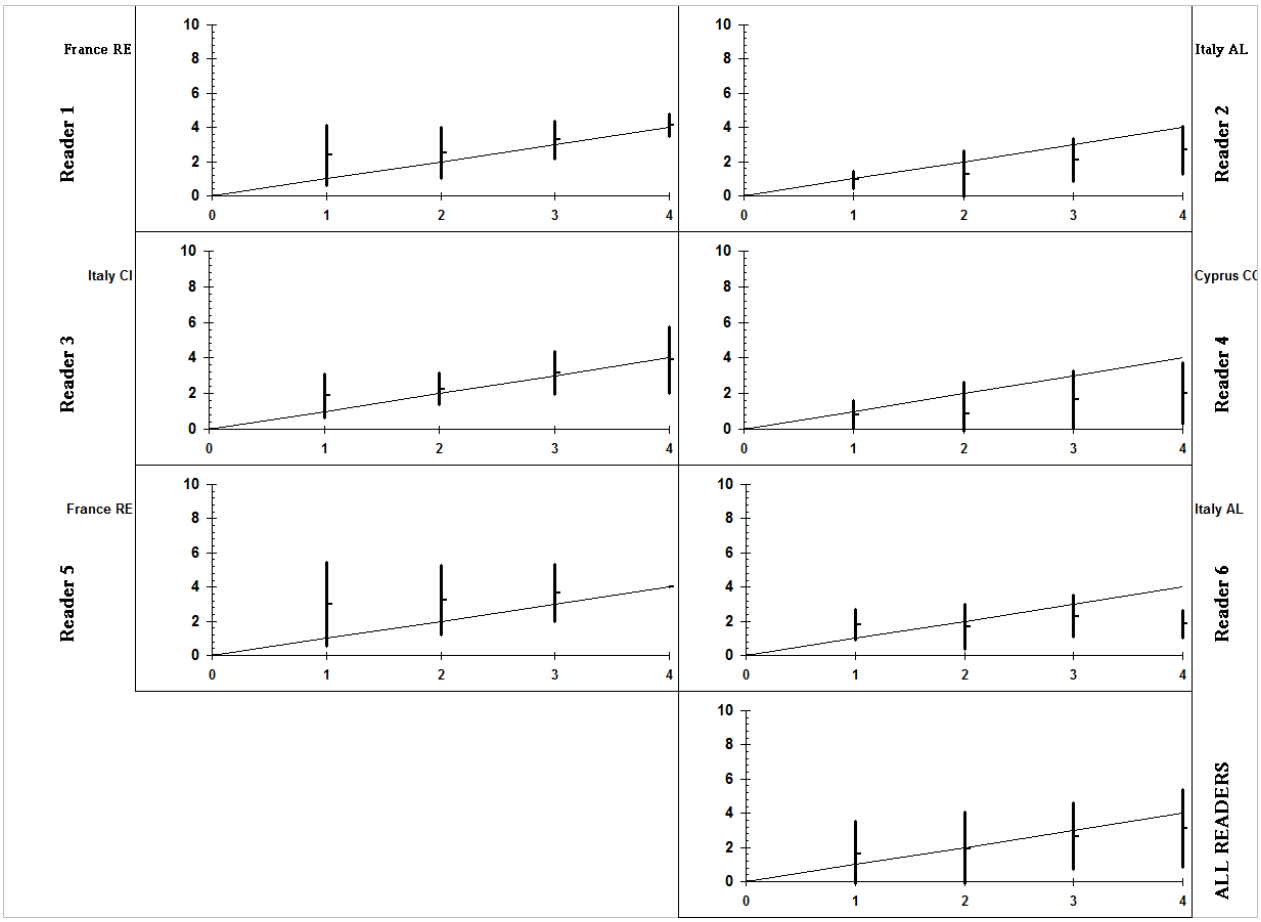
RELATIVE BIAS

	MODAL	France RE	Italy AL	Italy CI	Cyprus CC	France RE	Italy AL	
	age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	ALL
	0	-	-	-	-	-	-	-
	1	1	0	1	0	2	1	1
	2	1	-1	0	-1	1	0	0
	3	0	-1	0	-1	1	-1	0
	4	0	-1	0	-2	0	-2	-1
Weighted mean	0-4	0.53	-0.74	0.29	-1.13	0.94	-0.56	-0.41
	RANKING	2	4	1	6	5	3	

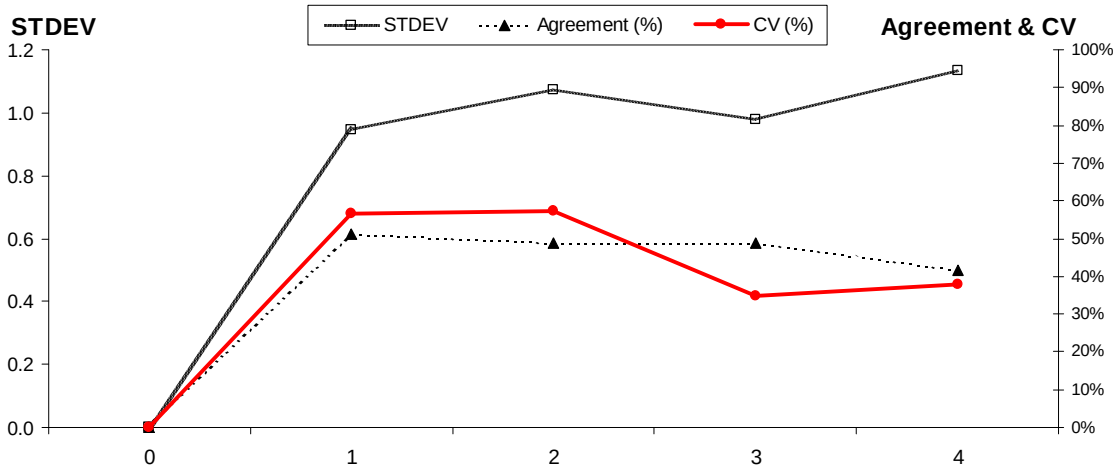
Overall ranking

	France RE	Italy AL	Italy CI	Cyprus CC	France RE	Italy AL
	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6
Ranking Coefficient of Variation	2	5	1	6	3	4
Ranking Percentage Agreement	2	4	1	6	5	3
Ranking Relative bias	2	4	1	6	5	3
OVERALL RANKING	2	4	1	6	4	3

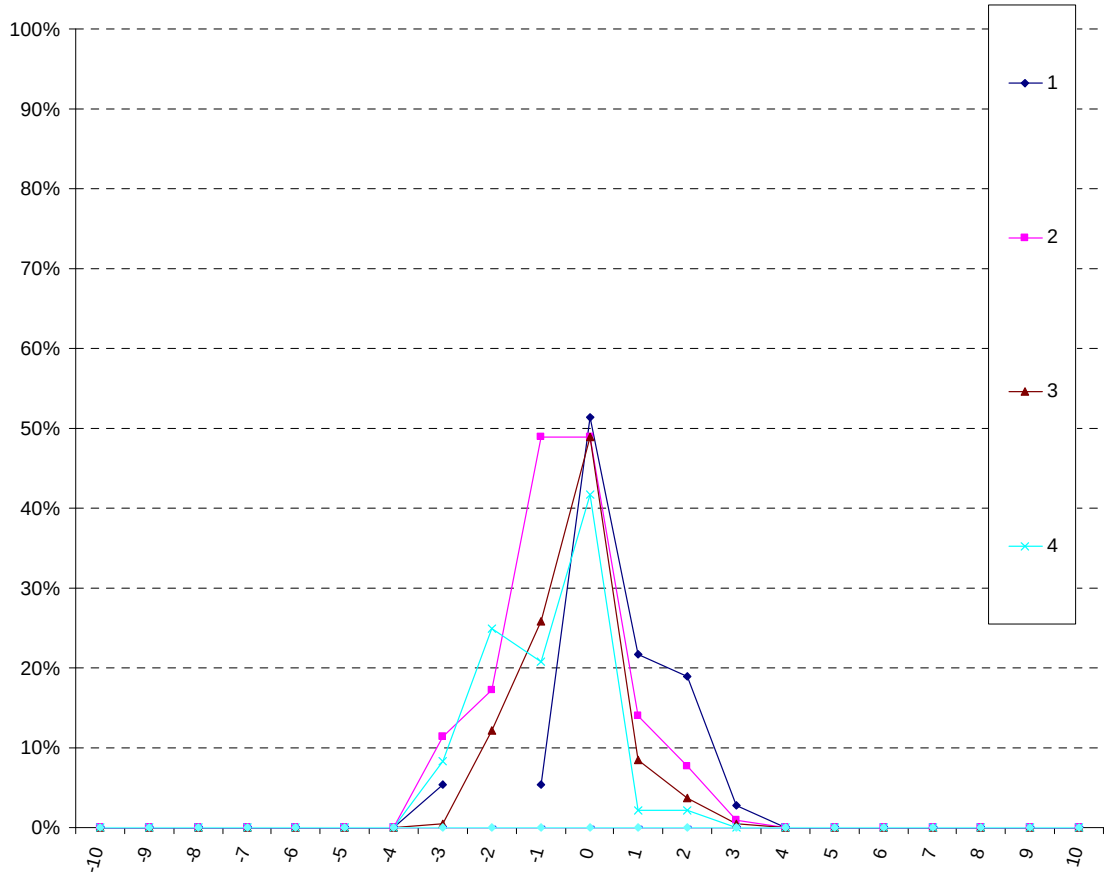
In the age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.



The coefficient of variation (CV%), percentage of agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percentage of agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.



The distribution of the age reading errors in percentage by MODAL age as observed from the whole group of age readers in an age reading comparison to MODAL age. The achieved precision in age reading by MODAL age group is shown by the spread of the age readings errors. There appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.



11. Appendix 4 : Details results of *Mullus barbatus* Otolith Exchange (Southern Adriatic)

The number of age readings, the coefficient of variation (CV), the percentage of agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups combined indicate the precision in age reading by reader and for all age readers combined.

NUMBER OF AGE READINGS

	MODAL age	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	TOTAL
	0	31	31	31	93
	1	25	25	22	72
	2	19	18	18	55
	3	19	19	19	57
	4	6	6	6	18
	5	2	2	1	5
Total	0-5	102	101	97	300

COEFFICIENT OF VARIATION (CV)

	MODAL age	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	ALL Readers
	0	188%	0%	159%	95.0%
	1	37%	115%	41%	74.6%
	2	15%	41%	21%	29.9%
	3	21%	32%	17%	28.8%
	4	0%	21%	10%	18.5%
	5	16%	35%	-	21.2%
Weighted mean	0-5	73.2%	43.9%	68.0%	59.6%
RANKING		3	1	2	

PERCENTAGE AGREEMENT

	MODAL age	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	ALL
	0	77%	100%	68%	82%
	1	88%	44%	59%	64%
	2	89%	39%	61%	64%
	3	79%	47%	53%	60%
	4	100%	17%	83%	67%
	5	50%	50%	100%	60%
Weighted mean	0-5	83.33%	59.41%	62.89%	68.67%
RANKING		1	3	2	

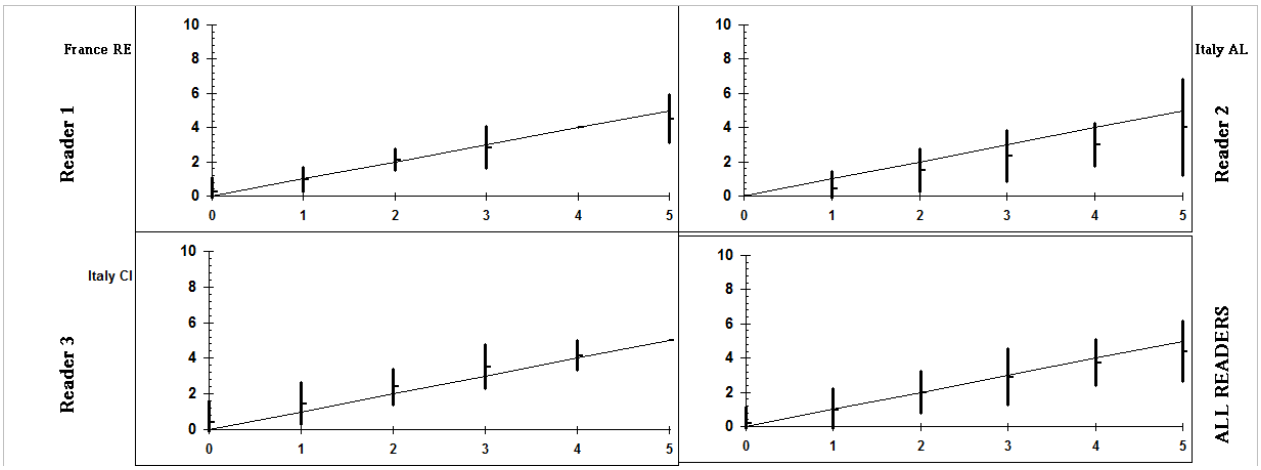
RELATIVE BIAS

	MODAL age	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3	ALL
	0	0	0	0	0
	1	0	-1	0	0
	2	0	-1	0	0
	3	0	-1	1	0
	4	0	-1	0	0
	5	-1	-1	0	-1
Weighted mean	0-5	0.04	-0.44	0.41	0.08
RANKING		1	3	2	

Overall ranking

	France RE Reader 1	Italy AL Reader 2	Italy CI Reader 3
Ranking Coefficient of Variation	3	1	2
Ranking Percentage Agreement	1	3	2
Ranking Relative bias	1	3	2
OVERALL RANKING	1	3	1

In the age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.



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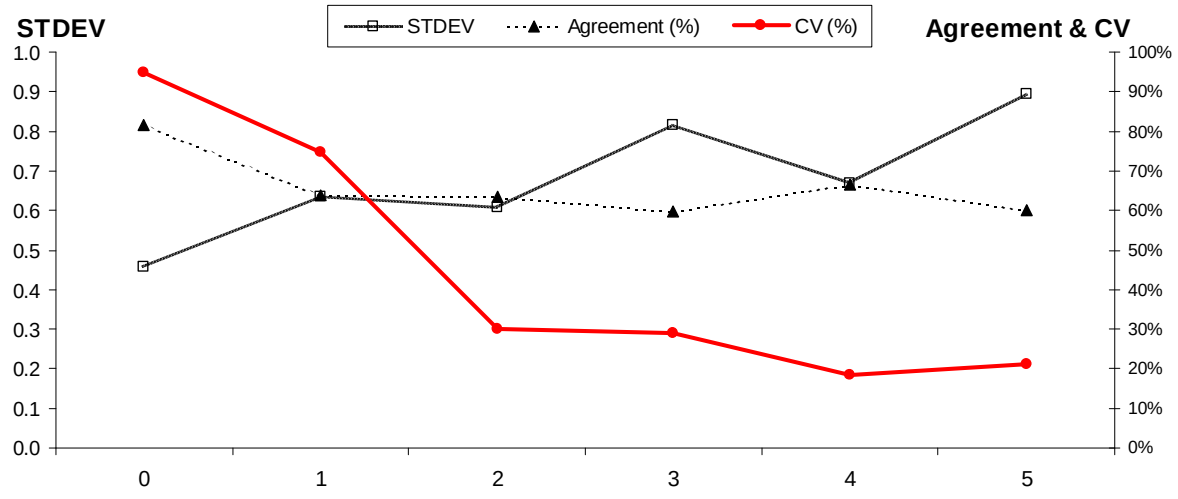
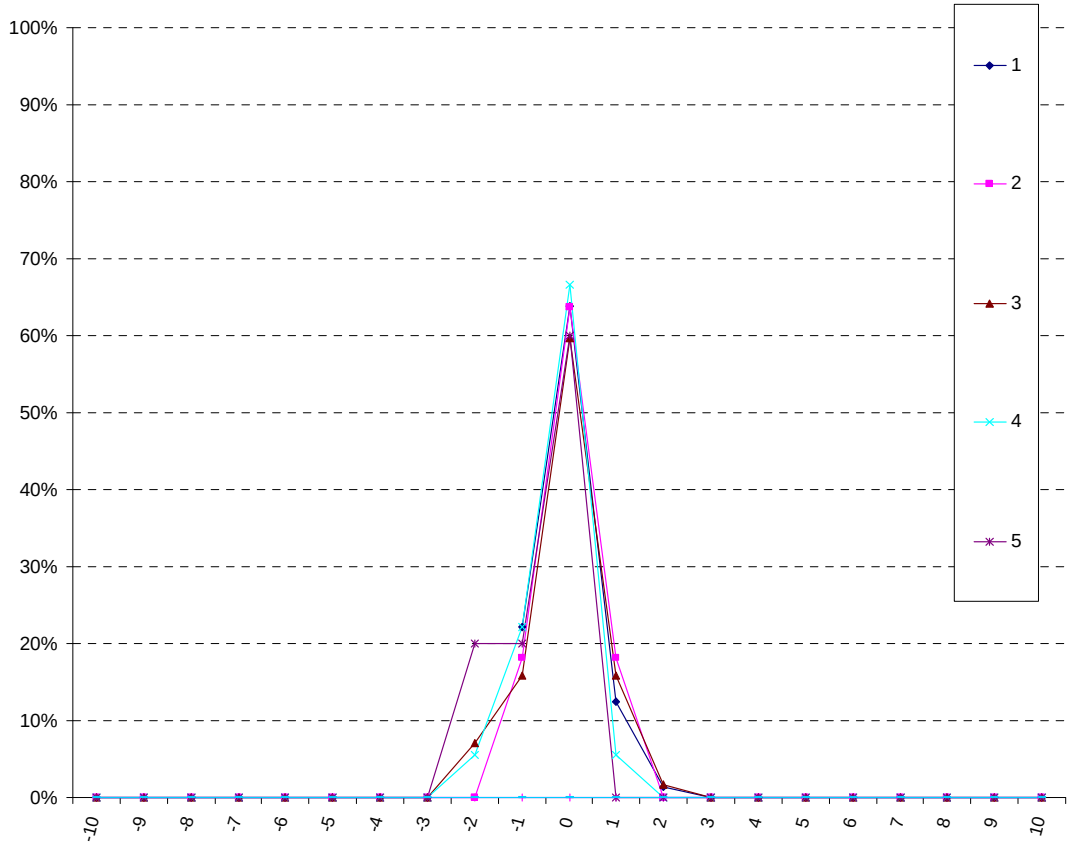
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