

## **Workshop on Megrin Otolith Age Readings**

By

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### **Abstract**

Since 1997, there has not been an exchange and workshop on Megrin Ageing. The last one was carried out under the auspicious of EC Study Project Contract No. 95/038 "*Biological Studies of Demersal Fish* (BIOSDEF)" deployed from 1996 to 1998. To address possible problems that could have raised, an otolith exchange was recommended between readers involved in the assessment and a posterior workshop was carried out. The results of the Megrin otolith exchange conducted in 2004 indicate that the age estimation criteria adopted seem to be appropriate (see Annex 3). A second reading of a subset of the exchange otolith collection (n: 39 otoliths with their corresponding images) was undertaken during this workshop. In general the results indicate that the percentage of agreement increased. The results of this workshop indicate that the precision of age readings (CV) decreased, probably due to the smaller sample size of the collection although the average percent of error also decreases. Thus, the values of APE and CV in (%) for all readers were 16 and 21.5, and 18 and 12.6 % respectively for the real and image otolith collection. The analysis of these results shows that there are no serious deviations in the otolith readings. The comparison of these results with those from the previous workshop conducted in 1997 shows that the criteria is firmly established due to the consistency of the results.

**Key words:** Ageing, Megrin, Otolith, Digital images

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Annex 1: exchange 2003 a protocol circulated.

Annex 2: Agenda of workshop

Annex 3: Presentation of the analysis of the results of the exchange 2003

Annex 4: Report on Megrin Age Reading Second Workshop (Vigo, May 1997).

Annex 5. Collection of marked images

## **7.1 Introduction**

In March 2003 the Planning Group on Sampling (PGCCDBS) in Rome, recommended that otolith exchanges for a number of fish species should be carried out in 2004, 2005 and 2006. Megrim was one of this species. Although no specific problems have been detected in the readings provided to the Assessment Working Groups, the need of organising a Workshop on Megrim Readings was defined by the long period that has passed from the last Workshop.

The advantage of these workshops is that the age reading methods of all experts of this species are discussed and compared "in situ".

## **7.2 Terms of References**

- To analyze and present the results of the 3<sup>rd</sup> exchange.
- To check the precision and bias of age readers involved in stock assessment.
- Discuss on age reading problems found for young and/or old fish.
- Check the possibility of start using images instead of real otoliths in the exchanges.
- To incorporate new readers in Megrim age estimation.

The main lines of the work at the workshop are described in the Agenda (Annex 2).

In order to check the precision in age reading and bias of the age readers of this species, the background for ageing megrim was based on the Reports of Megrim Otolith and Fin Rays Age Reading Workshops conducted previously (1997).

The financial support for the meeting was obtained partially from the EC No 1543/2000 within the framework programme "National Data Collection and Management Programme".

## **7.3 Participants**

The workshop met in Sukarrieta from 29 November -1<sup>st</sup> December 2004 with the following participants:

Name	Institution	Country	email	Participating		Degree of Experience
				Exchange (Reader #)	Workshop (Reader #)	
Mark Etherton	CEFAS	United Kingdom	<a href="mailto:M.W.Etherton@cefas.co.uk">M.W.Etherton@cefas.co.uk</a>	R1	R1	Most expert ALK for WGHMM
Jorge Fontela	IEO	Spain	<a href="mailto:jorge.fontenla@vi.ieo.es">jorge.fontenla@vi.ieo.es</a>	R2	-	Most expert ALK for WGHMM
Amaia Gomez de Segura	AZTI	Basque Country (Spain)	<a href="mailto:amgomez@suk.azti.es">amgomez@suk.azti.es</a>	R3	R3	Expert ALK for WGHMM
Gordon Henderson	FRS	Scotland	<a href="mailto:G.I.Henderson@marlab.ac.uk">G.I.Henderson@marlab.ac.uk</a>	R4	-	Expert ALK for WGNSSDS
Peter Vingaard Larsen	DIFRES	Denmark	<a href="mailto:pvl@dfu.min.dk">pvl@dfu.min.dk</a>		R4	New reader
Afra Egan	Marine Institute	Ireland	<a href="mailto:afra.egan@marine.ie">afra.egan@marine.ie</a>	R5	R2	Expert ALK for WGNSSDS
Marina Santurtún	AZTI	Basque Country (Spain)	<a href="mailto:msanturtun@suk.azti.es">msanturtun@suk.azti.es</a>		R5	Expert ALK for WGHMM
Ayesha Power	Marine Institute	Ireland	<a href="mailto:ayesha.power@marine.ie">ayesha.power@marine.ie</a>	R6	R6	New reader
Sally Warne	CEFAS	United Kingdom	<a href="mailto:S.A.Warne@cefas.co.uk">S.A.Warne@cefas.co.uk</a>	R7	R7	Expert ALK for WGHMM
Antonio Marçal	IPIMAR	Portugal	<a href="mailto:amarcal@ipimar.pt">amarcal@ipimar.pt</a>	R8	R8	New reader
<b>Other participants:</b>						
Ane Iriondo	AZTI	Basque Country (Spain)	<a href="mailto:airiondo@suk.azti.es">airiondo@suk.azti.es</a>			
Iñaki Quincoces	AZTI	Basque Country (Spain)	<a href="mailto:iquincoces@suk.azti.es">iquincoces@suk.azti.es</a>			

*All the above otolith readers participating in the 2004 Megrim Otolith Exchange, participate in the Workshop except for R2 and R4 who*

*were unable to attend the Workshop. Also, a new reader R4 was incorporated to the Work*

## 7.4 Material and Methods

The workshop was carried out following the recommendations of the EFAN Report 3-2000 on Guidelines and Tools for Age Reading Comparisons.

The general criteria adopted for ageing the exchange collection is described in the report of the previous exchanges (Report of the Megrin Otolith Age Reading Workshop, 1997).

Firstly, the results of the analysis of the exchange were presented (Annex 3).

A discussion about the problems found followed the presentation of the results of the exchange. The major concern appears to be the no identification of the ring located at the edge of the otolith in the new readers. After all discussions on ageing results, this was followed by another discussion/review of the marked images done during the exchange. After reaching agreement, especially in relation to the edge identification, a second reading was carried out. Thus, a subset of 39 otoliths was selected according to their high/low agreement (corresponding to young/old fish) in the 2004 exchange collection with a length range of: 17-41 cm.

The second reading was performed also on the real otolith subsample and in the images obtained from them. In that sense, readers were required to mark where they consider the rings to be located. In this occasion the fish length corresponding to the otolith was not provided to the readers.

Readers who did not participated in this workshop were removed from the analysis. As readers changed between the exchange and the workshop it was also decided to choose other expert readers for calculating the modal ages on which analysis is based. In this occasion readers providing ALK to the working Groups (R1: Mark Etherton; R2: Afra Egan and R3. Amaia Gomez de Segura) were chosen.

Whilst the analysis of the second reading was undertaken, the entire group discussed with images of these otoliths the individual interpretation of every reader and age and the classification of the otolith edge type (opaque or translucent).

After this, a very qualitative comparison of the otolith readings from the exchange and the second reading in the workshop was carried out as the readers were not the same and also one of the experts chosen for the Modal Age calculation also changed.

Not enough readers, just eight, were present at the workshop to do a further analysis in relation to their level of experience, for instance, most expert readers (Modal readers) compared to expert readers and/or new readers.

As in the 2004 exchange, the analyses of the age reading results were performed using an Excel ad-hoc Workbook "AGE COMPARATIONS. XLS" from A.T.G.W. Eltink from RIVO.

The basic requirement for age reading consistency is the absence of bias among readers over time. To study the variability in the precision of age determinations among readers, an extensive analysis was conducted to provide more details concerning individual performances:

### 1. Exploratory data analysis (EDA)

Determination of the modal age and the difference between each reader's age and modal age was performed. The modal age was calculated on basis of the results of the readers from the readers providing ALK for stock assessment. In the case of bi-modality, the modal age was estimated by the mean age.

Box-whisker plots were used for the graphical representation of the sample by each reader (median and interquartile range by each reader). They are useful to observe and compare the distribution of the otolith readings by reader<sup>1</sup>.

Age bias plots show both types of age reading errors (affecting precision and accuracy) whenever otoliths of a known age are available. In this case the bias in age reading can only assess the precision.

### 2. In terms of reproducibility measures:

2.1) Average percent age error (APE) (Beamish and Fournier, 1981) is an index of reading precision used to compare a series of observations. The formula is as follows:

$$APE = \frac{100}{n} \sum_{i=1}^n \left( \frac{1}{r} \sum_{j=1}^r \frac{|x_{ij} - \bar{x}_i|}{\bar{x}_i} \right) \quad (1)$$

n = number of otoliths

r = number of readings for each otolith (readers)

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<sup>1</sup> The center line within the box gives the median of the distribution of averaged data. The upper and lower sides of the box give the 25 and 75 percentiles, respectively. The ends of the whisker give the 5 and 95 percentiles, respectively. There are data that fall well outside to the range which are called outliers

$x_{ij}$  = the  $j$  value of age estimation for the  $i$  otolith

$\bar{x}_i$  = average age calculated for the  $i$  otolith

2.2) Mean Coefficient of Variation (CV). The precision errors in age reading are best described by this coefficient by age group.

$$CV = \frac{100}{n} \left[ \sum_{i=1}^n \left( \frac{sd}{\bar{x}_i} \right) \right] \quad (2)$$

$sd$  = the standard deviation for the  $i$  otolith

This measurement is more appropriate than the conventional percent of agreement when comparing ages, since it take into account the average year class of fish. It should be remembered that the CV is very sensitive to low age values.

## 7.5 Results and Discussion

The results of the readings of the subset of 39 otoliths by reader, modal age and percentage of agreement are shown in the Table 1 and Table 1i.

The box-whisker plots for all readers and both formats of the collection are presented in Figure 2 & 2i. The results for the first and second readings show that the mean age was similar being 5 and 4 years old, respectively. In general, readers tend to give younger ages in the second reading of the image collection. For the real otolith collection no apparent differences in the readings are found.

The image analysis for the situation of the rings indicate that mostly all readers can allocate clearly these rings, except for R4, who just start reading this species at the workshop. Some discrepancies were found in otoliths of fish older than 5 or 6 six years old and olders. However, discrepancies were discussed and consensus was reached in most of the cases. This agreement pointed out the consistent ageing criteria for otoliths.

The age bias plots by each reader, and all readers combined for the second reading show that the majority of readers reached a high agreement for younger ages. Fish older than 6 years showed a higher level of variability in the ages assigned by R4, R5 and R6. This is



more evident in the age bias plot for each readers (Figure 3).

In the second reading and for the image collection, the amplitude of confidence intervals was decreased, in general, for all readers and the ages assigned were lower. For the real otolith collection, R5 tend to overestimate ages slightly. In the case of the image collection, R4 and R5 overestimate the ages. For this collection, Readers 3, 6, 7 and 8 tend to underestimate ages more than for the real otolith collection, However, in all cases the deviance from the modal age appeared to be very light, specially for the real otolith collection and, in the image collection, all readers combined tend to underestimates the ages above 7 (Figure 3 & 3i).

Considering the incorporation of new readers at the workshop and the no high variability trends, the ageing criteria established appeared to be well assimilated.

When the results of the second reading (both collections) were compared with the first age readings (both collections) using the percentage of agreement, the Average Percentage Error (APE) and the mean Coefficient of Variation (CV) (Table 2, 2i & 3) for the same subset of otoliths, it can be seen that the percentage of agreement increased, and both APE and mean CV remains very similar.

The inter-reader bias test (Wilcoxon's test) results for the second reading (both collections) are given in Table 4 & 4i. In general, the comparison indicates that there is a significant bias just for two readers, in the case of the real otolith collection and for the image collection just one readers differs from the modal age significantly.

As the second reading was based on otoliths selected according to their fish length, the number of otoliths for all ages were poorly represented and so, this lack of otoliths can affect the calculation of all indicators of precision.

Again in this workshop, it is important to note that the comparative analysis between the first and second readings cannot be completely deployed as the readers changed. However, comparisons between collections showed that very similar results are obtained.

In general, the decrease of APE and the increase of the percentage of agreement obtained from 1997 to 2004 exchanges and workshops show the consistency of the age readers.

## **7.6 Other contributions**

The Portuguese participant presented a small collection of four-spot megrim otolith (*Lepidorhombus bosci*) as this is the predominant species in the Portuguese landings. These otoliths were checked/read by the most experienced reader. The conclusion was that although

the otoliths differed in shape and growth rate, the ageing criteria used for Megrin could be applied.

## 7.7 Age Determination Criteria

- For a better interpretation of the age it was agreed that both otoliths should be kept free to enable manipulation of them to provide the best indication of the growth pattern.
- It was commented that for a better reading otoliths should not be soaked in water for more than 1 hour.
- Using ethanol for soaking the otoliths instead of water during readings was suggested.
- Reading the otoliths in fish length order is important to get a clearer picture of the pattern of ring development.
- It was agreed that both otoliths should be aged wherever possible and the rings counted in several places if there is a clear image of ring formation in more than one place. The preferred areas for ring interpretation are indicated in Figure 7.
- If the otoliths have been counted in more than one place and different numbers of rings have been identified, the true age should be taken as the age derived from what is considered to be the clearest section of the otolith for interpretation.
- If both otoliths have been counted and they are both queried e.g.  $n \neq n+1$ ,  $n+1 \neq n+2$ , then the modal age should be used, e.g.  $n+1$ .
- The opaque zone is deposited on the edge of the otoliths between March and October for Megrin from ICES Sub-areas VII and VIII and can be observed forming first on the edges of the otoliths from the more Southerly areas. (Anon., 1997)
- It is recognised that fish deposit their opaque and hyaline zones on some parts of the otolith edge before others. When this is observed, the edge type on the fastest growing area of the otolith should be accepted.
- When digital images are used for ageing fish, the identification of the edge should be carried out by checking the whole area of the otolith. The use of a higher magnification could help in this identification.
- The older the fish is, the later in the summer months the opaque zone is deposited (Anon., 1997).
- When the opaque edge starts to form, its deposition is very rapid (Dawson, 1991).
- Megrin usually matures at about age 2 in males and age 3 in females. (Anon., 1997). When the fish reach maturity the growth of it, as recorded on the otolith, is observed to slow down. Therefore, there are large opaque zones formed between the first, second and sometimes third hyaline zone and these are usually followed by more narrow opaque and hyaline zones. Sometimes in these large

opaque zones a hyaline ring appears that cannot be considered as a “true” annual ring. It is assumed that growth slows down when energy is diverted into spawning. However, it should be noted that this is not always the case, and it is not uncommon to find large, old immature fish.

- It is possible that fish mature earlier in the more Southerly areas where the temperature is higher. Therefore, it should be recognised that this is likely to affect the growth pattern of the otolith. (Anon., 1997).

## 7.8 Conclusions

- Age reading criteria for interpretation of the otoliths was established for new readers. The percentage of agreement was improved.
- In general the results of the otolith readings indicated that readers do not have problems ageing fish younger than 6. After that, the variability increased but agreement was still good for bigger otoliths.
- The Indices of Beamish and Fournier (Average percentage of error) decreased after the first reading, indicating that ageing agreement has improved.
- The new people involved in ageing megrim appeared to miss the last ring in the edge of the otolith during the exchange exercise. However, this problem has been solved during the workshop.
- It appears that readers from more Northern areas tend to underestimate age for medium to large sized fish (35 cm onwards) in comparison to readers from more southern areas. This situation may be caused by the possible different growth pattern between fish in different sea areas (Sub area VI and Bay of Biscay (Div. VIIIa,b,d)).
- The use of digital images of the otolith proved to be a good method for checking the ageing criteria followed by each readers and so the location of the rings.
- In general, the agreement between readers on the location of the rings was high.
- The workshop was very useful for experienced readers because it was a long time since readers of Megrim had gathered together to discuss the ageing criteria and it was proved that agreement was high.
- For new readers, it was a good chance to learn the ageing criteria.
- Good results were obtained from the digital images and their use is recommended in future exchanges. The need for another workshop is dependant on the results of these exchanges.

## 7.9 Recommendations

- Every three years or so., regular exchanges, of ageing structures for the purpose of checking the precision of all readers involved in age determination.
- Include a more diverse otolith collection with a higher number of otoliths and from different sea areas.
- Use of the images instead of the real otoliths.
- To carry out an experiment trying different soaking times for the otoliths before reading.
- An age determination guide is recommended to assist the individual reader in the correct interpretation of the otoliths, this should include marked images.
- A direct method of validation is needed to confirm age determination (e.g., otolith microchemical analysis, tagging programs...).

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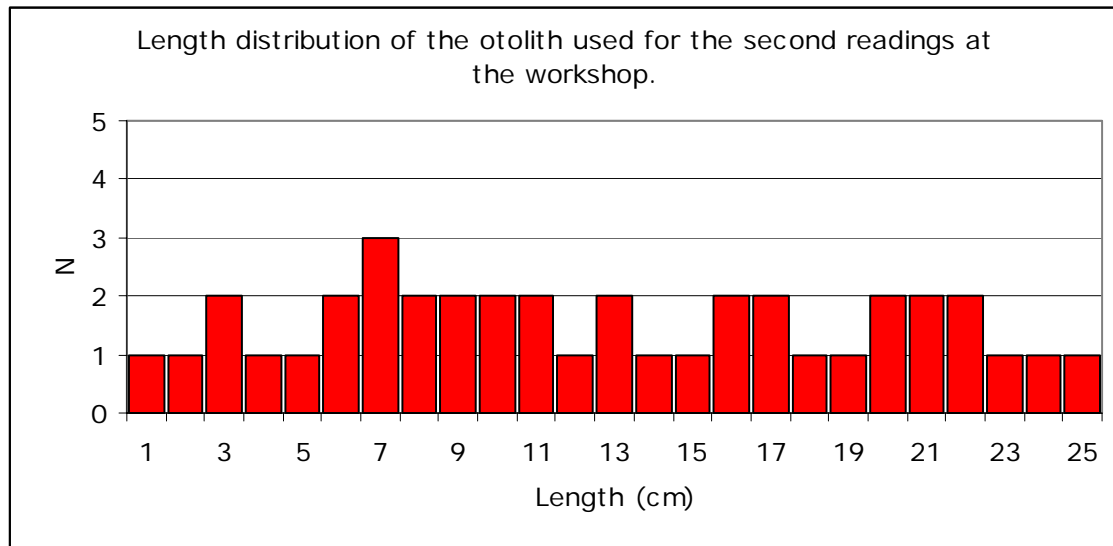
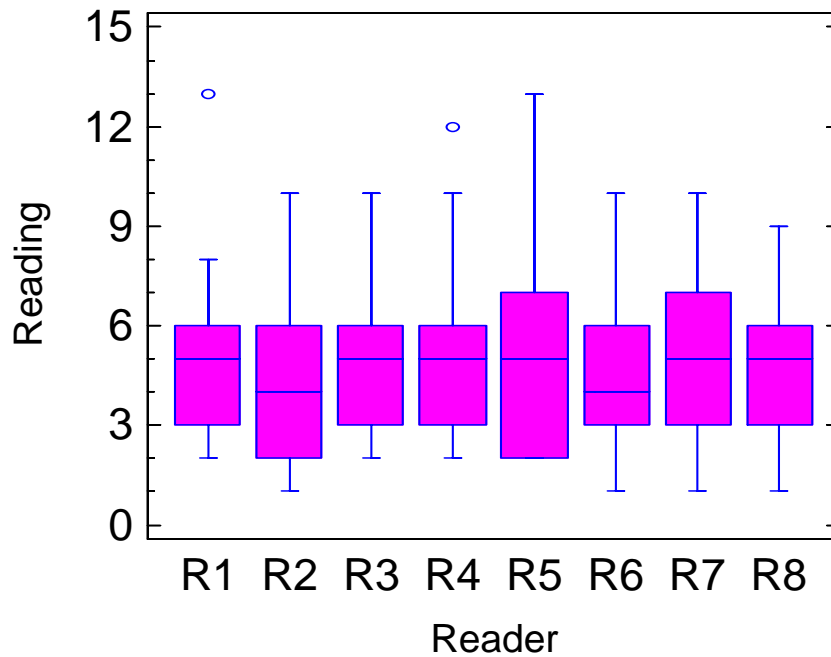


Figure 1. Length frequency distribution of samples from Megrin otolith used in the second reading during the workshop.

## Box-and-Whisker Plot for Real Otolith Collectic



## Box-and-Whisker Plot Image Otolith Collectio

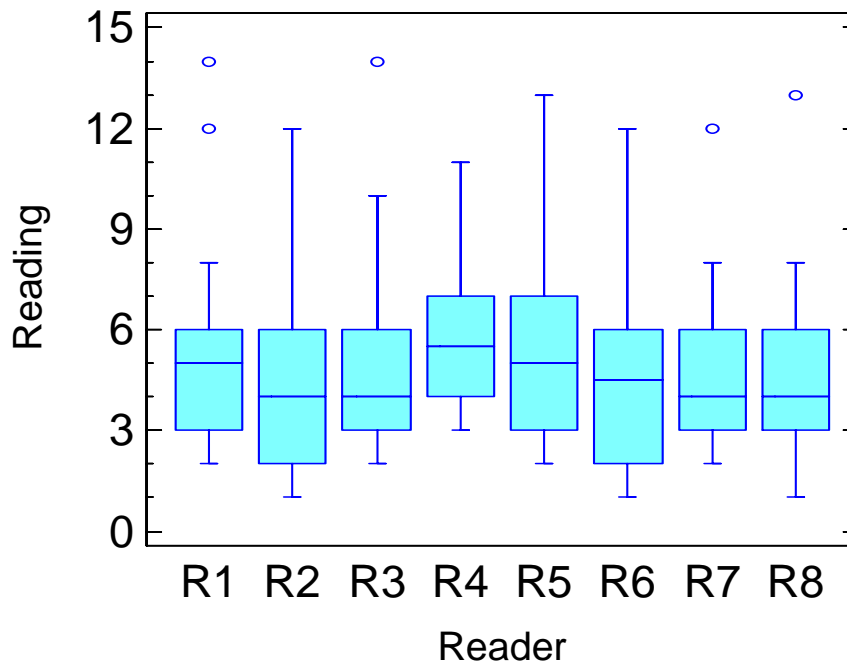


Figure 2 & 2i. Box-Whisker plots for each reader in relation to the whole set of otolith for each "collection". Above: Real Otolith Collection. Below: Image Otolith Collection



# REAL OTOLITH COLLECTION

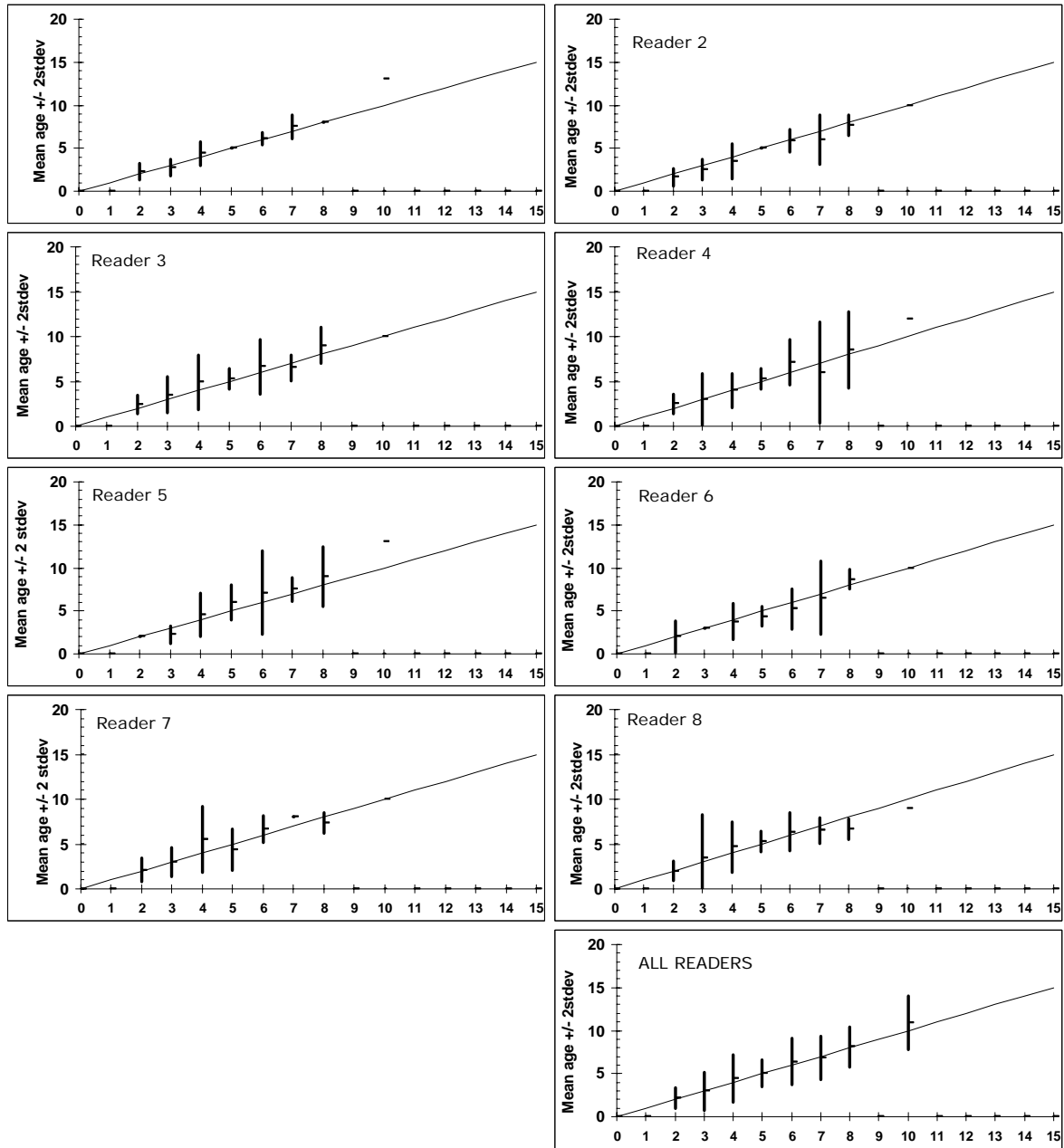


Figure 3. In the age bias plots below the mean age recorded  $\pm 2$ stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

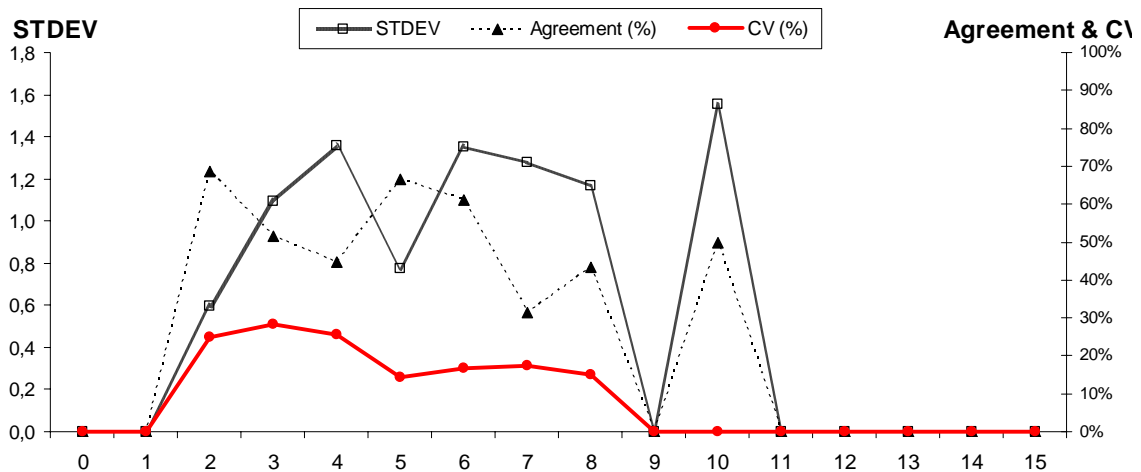


Figure 4. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL (and in some cases ABSOLUTE MEAN) age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

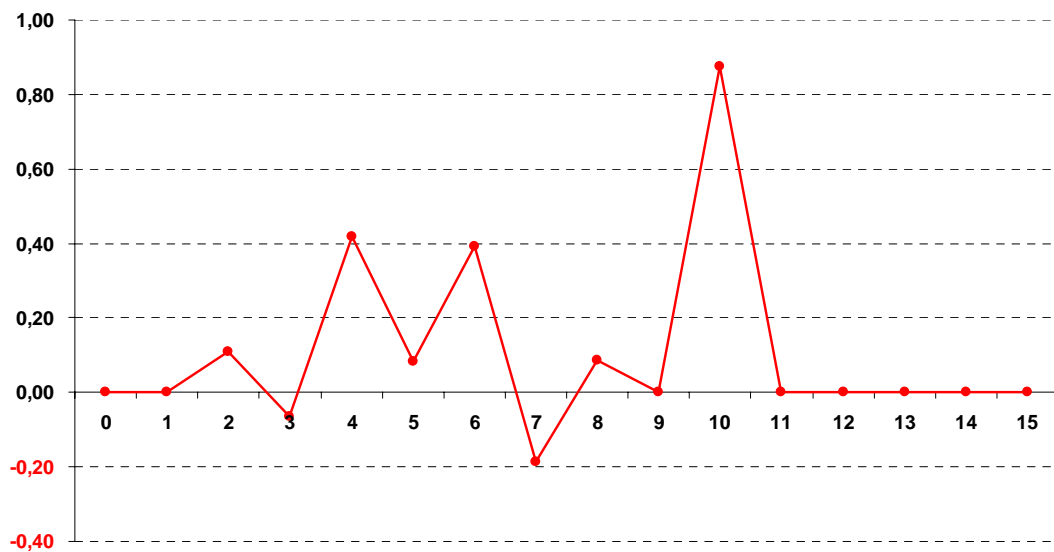


Figure 5. The RELATIVE bias by MODAL (in some cases ABSOLUTE MEAN) age as estimated by all age readers combined

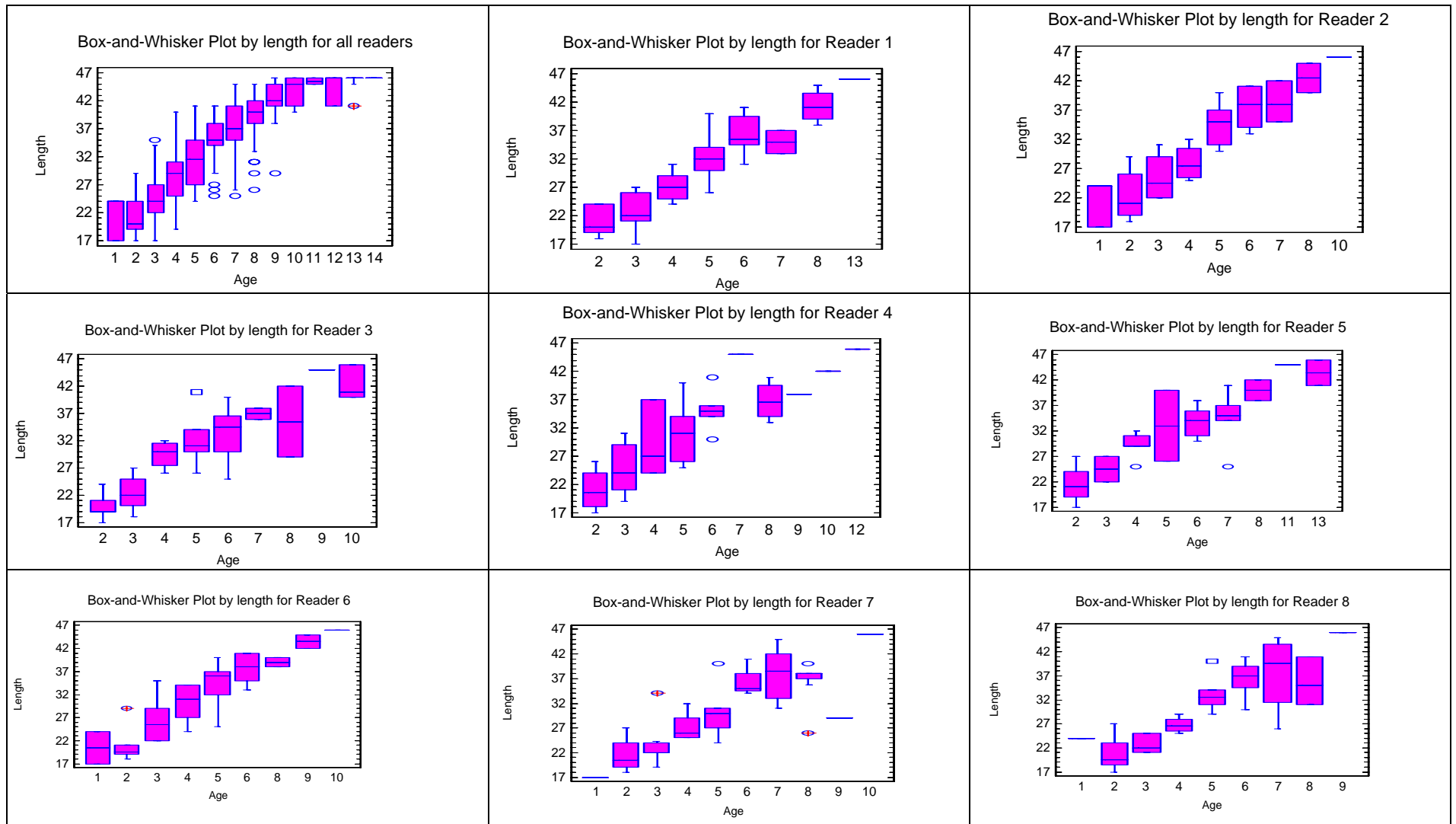


Figure 6. Box-Whisker plots of the length distribution (cm) by age obtained for all readers reading the real otolith collection

## IMAGE OTOLITH COLLECTION

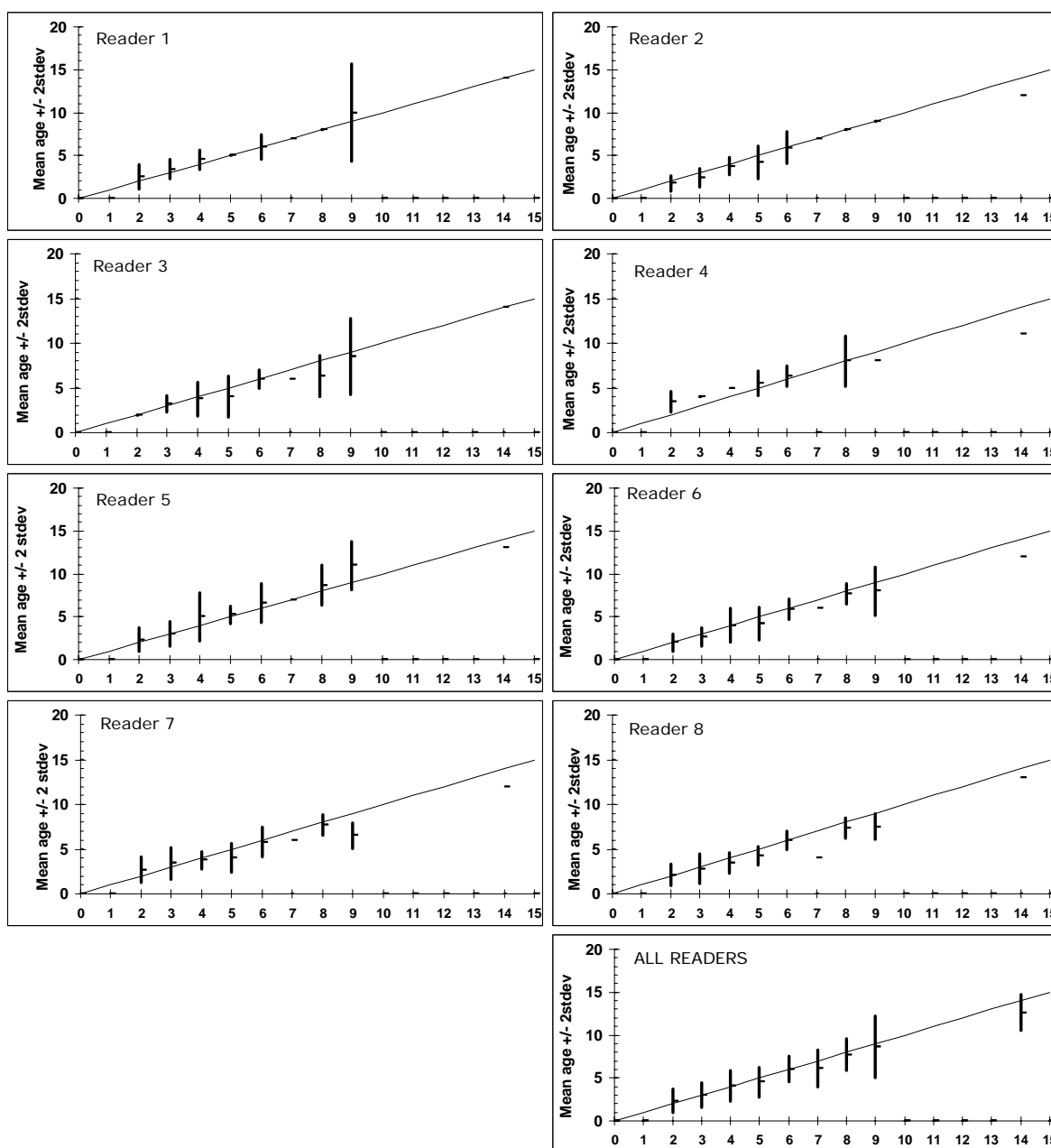


Figure 3i. In the age bias plots below the mean age recorded  $\pm$  2stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

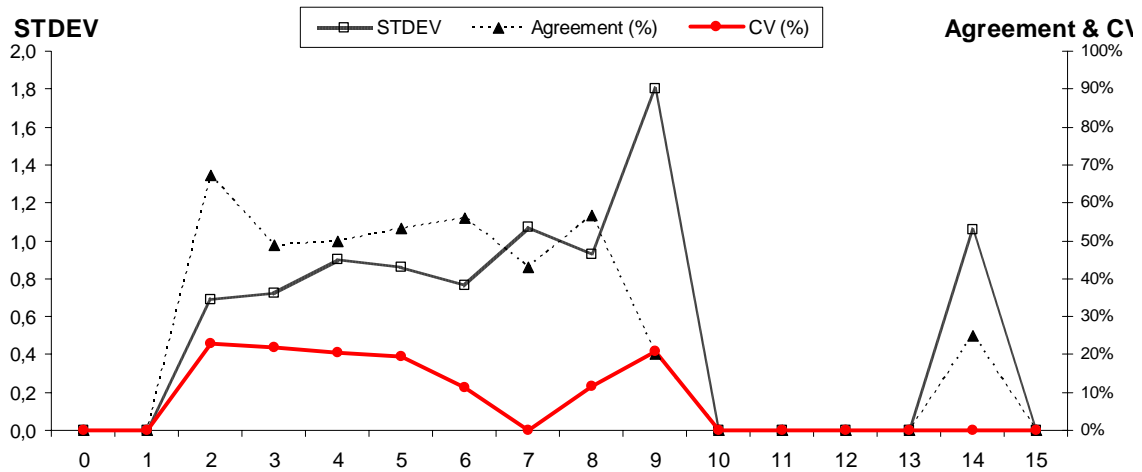


Figure 4i. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age (in some cases ABSOLUTE MEAN). CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

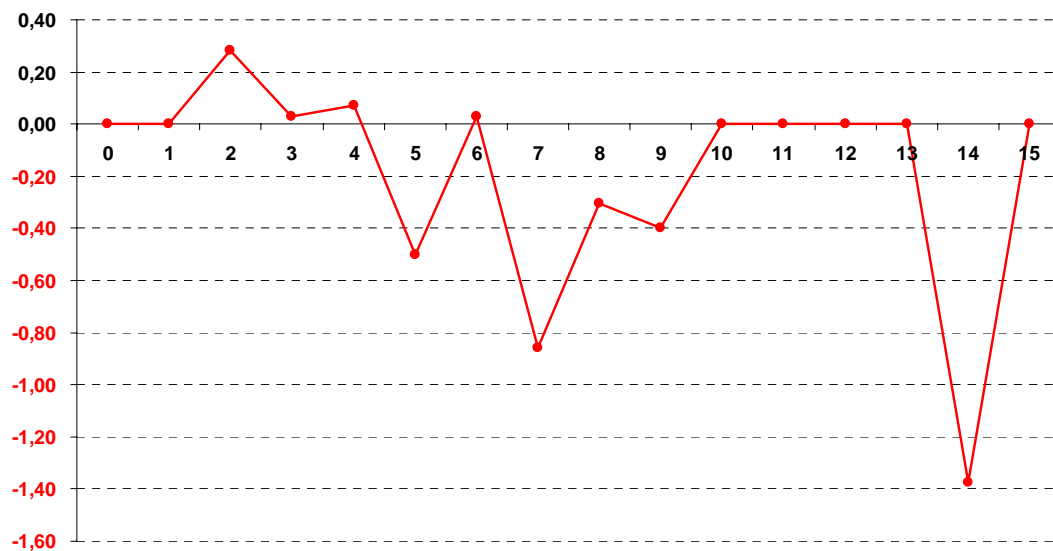


Figure 5i. The RELATIVE bias by MODAL(in some cases ABSOLUTE MEAN) age as estimated by all age readers combined

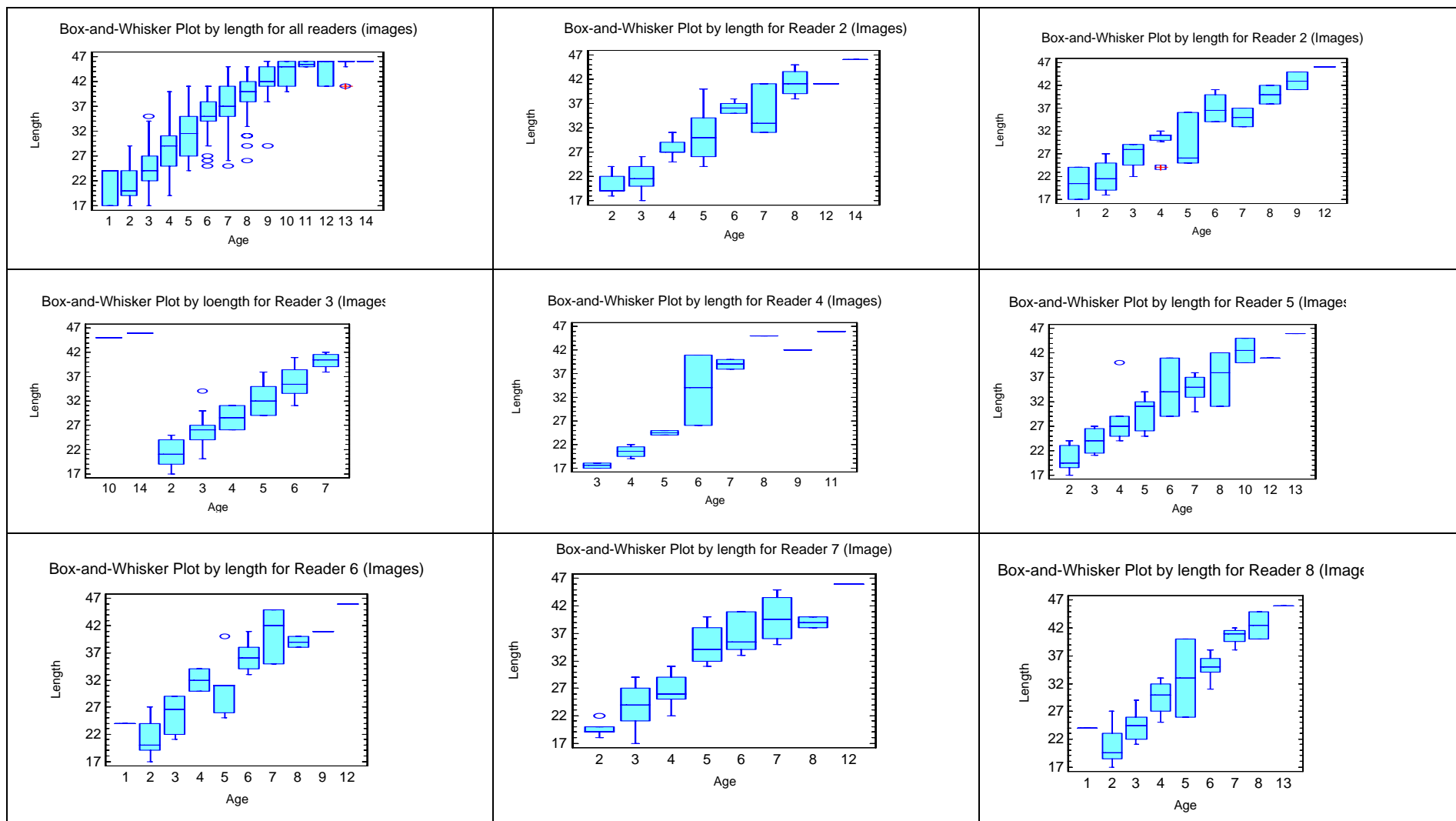


Figure 6i. Box-Whisker plots of the length distribution (cm) by age obtained for all readers reading the image otolith collection

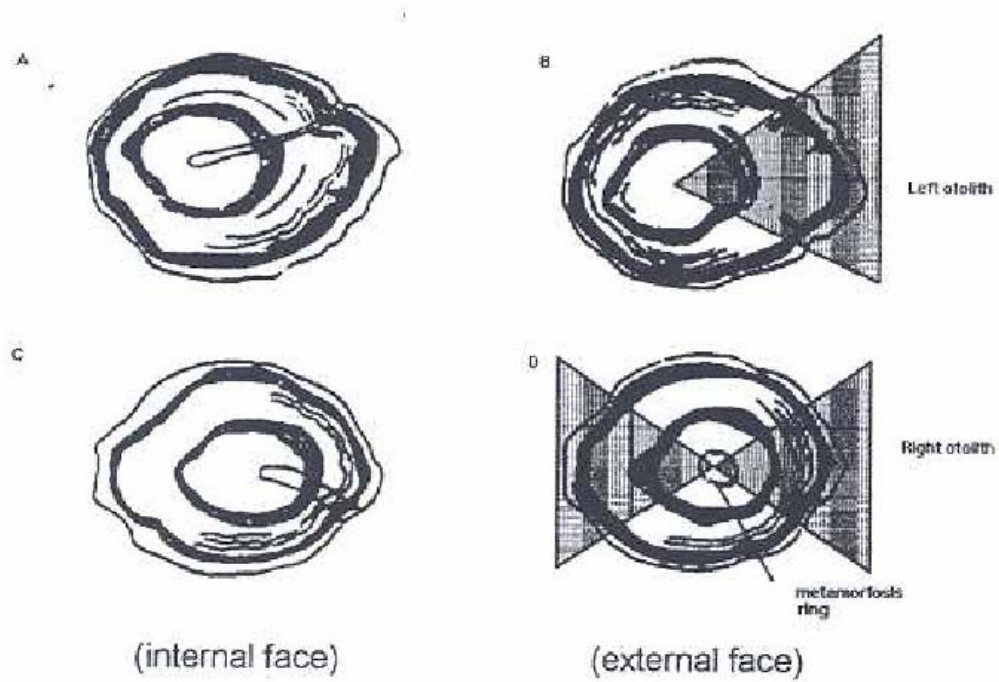


Figure 7. Shaded areas are the preferred areas for the readings.

Table 1. Readings of the Megrim Otolith Workshop (Real collection)

Sample		Fish	Fish	Landing	Mark	Afra	Amaia	Peter	Marina	Ayesha	Sally	Antonio	MODAL	Percent	Precision	
Stratum	year no	no	length	Sex												month
1		24/03/99.2(M)1	17.0		3	3	1	2	2	2	1	1	2	2	50%	40%
2		31/03/98.5(H)7	18.0		3	2	2	3	2	2	2	2	2	2	88%	17%
3		31/03/98.5(H)12	19.0		3	2	2	2	2	2	2	2	2	2	100%	0%
4		31/03/98.5(H)15	20.0		3	2	2	3	3	2	2	2	2	2	75%	21%
5		31/03/98.5(H)25	21.0		3	3	2	2	3	2	2	2	3	2	63%	22%
6		31/03/98.5(H)32	22.0		3	3	3	3	3	3	3	3	3	3	100%	0%
7		24/03/99.2(M)54	24.0		3	4	-	-	4	-	-	5	-	4	67%	13%
8		22/02/99.1(M)2	25.0		2	4	4	3	3	7	5	4	4	4	50%	30%
9		22/02/99.1(H)6	26.0		2	5	4	4	5	5	-	8	4	4	43%	28%
10		24/03/99.1(M)38	34.0		3	5	5	5	5	7	4	3	5	5	63%	23%
11		05/03/01.1(H)6	38.0		2	8	7	6	8	8	8	8	6	7	13%	12%
12		31/03/98.1(H)5	40.0		3	5	5	6	5	5	5	5	5	5	88%	7%
13		11/03/99.1(H)1	41.0		3	6	6	5	6	7	6	7	6	6	63%	10%
14		31/03/98.1(H)8	42.0		3	8	7	8	10	8	9	7	7	8	38%	13%
15		31/03/98.1(H)16	45.0		3	8	8	9	7	11	9	7	7	8	25%	17%
16		11/03/99.1(H)11	46.0		3	13	10	10	12	13	10	10	9	10	50%	14%
17		05/08/98.1(M)14	19.0		3	2	2	2	3	2	2	3	2	2	75%	21%
18		05/08/98.1(H)35	22.0		3	2	3	3	2	2	3	3	2	3	50%	21%
19		05/08/98.1(M)47	24.0		3	2	1	3	2	2	4	3	2	2	50%	39%
20		05/08/98.2(M)6	27.0		3	3	2	3	5	2	-	2	2	3	29%	41%
21		05/08/98.2(M)16	29.0		3	4	4	4	3	4	3	4	4	4	75%	12%
22		05/08/98.2(M)21	30.0		3	5	5	5	6	6	4	5	6	5	50%	13%
23		05/08/98.2(H)27	31.0		10	4	5	4	5	4	4	5	5	4	50%	12%
24		05/08/98.2(M)44	33.0		10	7	6	6	8	6	6	7	5	6	50%	14%
25		30/09/99.2(H)12	34.0		10	6	6	6	6	6	4	6	6	6	88%	12%
26		30/09/99.2(H)18	35.0		10	6	7	6	6	7	6	6	8	6	63%	12%
27		30/09/99.2(H)34	38.0		10	6	6	7	9	6	6	8	6	6	63%	17%
28		30/10/02.1(M)37	24.0		10	2	1	2	3	2	1	2	1	2	50%	40%
29		30/10/02.1(M)46	25.0		2	4	2	6	5	4	3	4	3	4	38%	32%
30		21/10/99.3(M)3	26.0		2	3	2	5	2	2	3	4	7	3	25%	51%
31		21/10/99.3(M)8	27.0		2	4	3	6	4	3	4	5	4	4	50%	24%
32		21/10/99.3(M)21	29.0		2	4	2	8	3	4	2	9	5	4	25%	57%
33		21/10/99.3(M)28	31.0		1	6	3	5	3	6	4	7	8	4	13%	35%
34		21/10/99.3(H)30	32.0		3	5	4	4	5	4	5	4	5	4	50%	12%
35		21/10/99.2(H)52	35.0		4	6	5	6	8	6	3	6	6	6	63%	24%
36		29/10/98.2(H)19	36.0		4	6	5	7	6	6	5	7	6	6	50%	13%
37		19/10/98.2(H)21	37.0		4	7	5	7	4	7	5	8	7	7	50%	22%
38		21/10/99.1(H)3	40.0		5	8	8	10	-	8	8	8	6	8	71%	14%
39		19/10/98.3(H)5	41.0		11	6	6	10	8	13	6	6	8	6	50%	32%
Total read					39	38	38	38	38	36	39	38				
Total NOT read					0	1	1	1	1	3	0	1			55,1%	21,5%



Table 2. The coefficient of variation (CV), the percent agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined for the REAL OTOLITH collection. 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.

COEFFICIENT OF VARIATION (CV)									
MODAL age	Mark Reader 1	Afra Reader 2	Amaia Reader 3	Peter Reader 4	Marina Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8	ALL Readers
0	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-
2	21%	32%	22%	21%	0%	46%	30%	27%	24,9%
3	18%	23%	29%	47%	22%	0%	27%	68%	28,3%
4	16%	29%	31%	24%	27%	28%	33%	30%	25,6%
5	0%	0%	11%	11%	17%	13%	27%	11%	14,5%
6	6%	11%	23%	17%	34%	22%	11%	17%	16,9%
7	9%	24%	11%	47%	9%	33%	0%	11%	17,3%
8	0%	8%	11%	25%	19%	7%	8%	9%	14,9%
9	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-
Weighted mean	0-15	11,8%	20,2%	22,1%	24,0%	19,2%	24,8%	22,5%	25,6%
RANKING		1	3	4	6	2	7	5	8
									21,5%

PERCENTAGE AGREEMENT									
MODAL age	Mark Reader 1	Afra Reader 2	Amaia Reader 3	Peter Reader 4	Marina Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8	ALL
0	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-
2	75%	63%	63%	50%	100%	63%	63%	75%	69%
3	75%	50%	75%	25%	25%	100%	50%	25%	52%
4	70%	44%	44%	20%	56%	38%	40%	44%	45%
5	100%	100%	67%	67%	33%	33%	67%	67%	67%
6	88%	63%	50%	50%	63%	63%	50%	63%	61%
7	50%	50%	50%	0%	50%	0%	0%	50%	31%
8	100%	67%	33%	0%	67%	33%	33%	0%	43%
9	-	-	-	-	-	-	-	-	-
10	0%	100%	100%	0%	0%	100%	100%	0%	50%
11	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-
Weighted mean	0-15	76,9%	60,5%	55,3%	34,2%	60,5%	52,8%	48,7%	50,0%
RANKING		1	2	4	8	2	5	7	6
									54,9%

RELATIVE BIAS									
MODAL age	Mark Reader 1	Afra Reader 2	Amaia Reader 3	Peter Reader 4	Marina Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8	ALL
0	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-
2	0,25	-0,38	0,38	0,50	0,00	0,00	0,13	0,00	0,11
3	-0,25	-0,50	0,50	0,00	-0,75	0,00	0,00	0,50	-0,06
4	0,40	-0,56	0,89	0,00	0,56	-0,25	1,50	0,67	0,42
5	0,00	0,00	0,33	0,33	1,00	-0,67	-0,67	0,33	0,08
6	0,13	-0,13	0,63	1,13	1,13	-0,75	0,63	0,38	0,39
7	0,50	-1,00	-0,50	-1,00	0,50	-0,50	1,00	-0,50	-0,19
8	0,00	-0,33	1,00	0,50	1,00	0,67	-0,67	-1,33	0,09
9	-	-	-	-	-	-	-	-	-
10	3,00	0,00	0,00	2,00	3,00	0,00	0,00	-1,00	0,88
11	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-
Weighted mean	0-15	0,26	-0,37	0,55	0,39	0,55	-0,25	0,49	0,16
RANKING		3	4	7	5	7	2	6	1

Overall ranking								
	Mark Reader 1	Afra Reader 2	Amaia Reader 3	Peter Reader 4	Marina Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8
Ranking Coefficient of Variation	1	3	4	6	2	7	5	8
Ranking Percentage Agreement	1	2	4	8	2	5	7	6
Ranking Relative bias	3	4	7	5	7	2	6	1
OVERALL RANKING	1	2	5	8	3	4	7	5

Table 3 & 3i.- Agreement (%), CV and APE values of otoliths reading from both collections and readers.

	Real Otolith Collection	Image Otolith Collection
N	39	39
% Agreement	54.9	52.4
CV	21.5	18.0
APE	16.0	12.6
Readers	ALL READERS	

Table 4. Bias tests: non-parametrically with a one-sample Wilcoxon rank sum test. The inter-reader bias test and the reader against MODAL age bias test.

<b>Inter-reader bias test and reader against MODAL age bias test</b>								
	Mark Reader 1	Afra Reader 2	Amaia Reader 3	Peter Reader 4	Marina Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8
Reader 1	—	* *	—	—	—	*	—	—
Reader 2	* *	—	* *	* *	* *	—	* *	*
Reader 3	—	* *	—	—	—	* *	—	—
Reader 4	—	* *	—	—	—	*	—	—
Reader 5	—	* *	—	—	—	* *	—	—
Reader 6	*	—	* *	*	* *	—	*	—
Reader 7	—	* *	—	—	—	*	—	—
Reader 8	—	*	—	—	—	—	—	—
MODAL age	*	* *	* *	*	*	—	*	—

—	= no sign of bias ( $p > 0.05$ )
*	= possibility of bias ( $0.01 < p < 0.05$ )
* *	= certainty of bias ( $p < 0.01$ )

Table 1i. Readings of the Megrin Otolith workshop (Image collection)

Sample	Fish	Fish	Landing	Mark	Afra	Amaia	Peter	Marina	Ayesha	Sally	Antonio	MODAL	Percent	Precision		
Stratum	year	no	length	Sex	month	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	age	agreement	CV
1	24/03/99.2	17,0	3	3	1	2	3	2	2	3	2	2	2	2	50%	31%
2	31/03/98.5	18,0	3	2	2	2	3	2	2	2	2	2	2	2	88%	17%
3	31/03/98.5	19,0	3	2	2	2	4	2	2	2	2	2	2	2	88%	31%
4	31/03/98.5	20,0	3	3	2	3	4	2	2	2	2	2	2	3	25%	30%
5	31/03/98.5	21,0	3	3	2	2	4	3	3	3	3	3	3	2	25%	22%
6	31/03/98.5	22,0	3	3	3	3	4	3	3	3	4	3	3	3	75%	14%
7	24/03/99.2	24,0	3	5	4	3	5	4	-	4	3	4	3	4	43%	20%
8	22/02/99.1	25,0	2	5	5	3	5	5	5	3	4	4	5	5	63%	21%
9	22/02/99.1	26,0	2	5	5	3	6	5	5	4	5	5	5	5	63%	19%
10	24/03/99.1	34,0	3	5	6	6	6	6	6	5	6	6	6	6	75%	8%
11	05/03/01.1	38,0	2	8	8	5	7	8	8	8	7	8	7	8	63%	14%
12	31/03/98.1	40,0	3	5	6	6	7	4	5	5	5	5	5	6	25%	17%
13	11/03/99.1	41,0	3	7	6	6	6	6	6	6	7	6	7	6	75%	7%
14	31/03/98.1	42,0	3	8	8	7	9	8	7	7	7	7	7	8	38%	10%
15	31/03/98.1	45,0	3	8	9	10	8	10	7	7	8	8	8	9	13%	14%
16	11/03/99.1	46,0	3	14	12	14	11	13	12	12	13	13	13	14	25%	8%
17	05/08/98.1	19,0	3	2	2	2	-	2	2	2	2	2	2	2	100%	0%
18	05/08/98.1	22,0	3	2	2	2	-	2	2	2	2	2	2	2	100%	0%
19	05/08/98.1	24,0	3	3	1	2	-	2	2	3	2	2	2	2	57%	32%
20	05/08/98.2	27,0	3	4	2	3	-	3	2	3	2	2	2	3	43%	28%
21	05/08/98.2	29,0	3	4	3	5	-	4	3	3	3	3	3	4	29%	22%
22	05/08/98.2	30,0	3	5	4	3	-	7	4	4	4	4	4	4	57%	29%
23	05/08/98.2	31,0	10	4	4	4	-	5	5	4	4	4	4	4	71%	11%
24	05/08/98.2	33,0	10	7	7	6	-	7	6	6	4	4	4	7	43%	17%
25	30/09/99.2	34,0	10	5	6	3	-	5	4	6	6	6	6	5	0%	23%
26	30/09/99.2	35,0	10	6	6	5	-	7	6	6	6	6	6	6	71%	10%
27	30/09/99.2	38,0	10	6	6	7	-	7	6	5	6	6	6	6	57%	11%
28	30/10/02.1	24,0	10	2	2	2	-	2	1	3	1	1	1	2	57%	37%
29	30/10/02.1	25,0	2	4	2	2	-	4	2	4	3	3	3	2	43%	33%
30	21/10/99.3	26,0	2	3	2	4	-	3	3	4	3	3	3	3	57%	22%
31	21/10/99.3	27,0	2	4	3	3	-	4	3	4	4	4	4	3	43%	15%
32	21/10/99.3	29,0	2	5	3	5	-	6	3	4	4	4	4	5	29%	26%
33	21/10/99.3	31,0	1	7	4	6	-	8	5	5	6	6	6	6	29%	23%
34	21/10/99.3	32,0	3	5	4	5	-	5	4	5	4	4	4	5	57%	12%
35	21/10/99.2	35,0	4	6	7	6	-	7	7	7	6	6	6	6	43%	8%
36	29/10/98.2	36,0	4	6	5	6	-	7	6	6	6	6	6	6	71%	10%
37	19/10/98.2	37,0	4	6	7	6	-	7	6	7	6	6	6	6	57%	8%
38	21/10/99.1	40,0	5	8	8	7	-	10	8	8	8	8	8	8	71%	11%
39	19/10/98.3	41,0	11	12	9	7	-	12	9	6	7	7	7	9	29%	27%
Total read					39	39	39	16	39	38	39	39	39			
Total NOT read					0	0	0	23	0	1	0	0	0		52,4%	18,0%

Table 2i. The coefficient of variation (CV), the percent agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined for the IMAGE OTOLITH collection. 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.

COEFFICIENT OF VARIATION (CV)										
MODAL age	Mark Reader 1	Afra Reader 2	Amaia Reader 3	Peter Reader 4	Marina Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8	ALL Readers	
0	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-
2	28%	25%	0%	16%	30%	25%	27%	28%	22,7%	
3	16%	23%	14%	0%	24%	21%	26%	30%	21,9%	
4	13%	13%	26%	-	28%	25%	13%	16%	20,6%	
5	0%	23%	29%	13%	10%	23%	20%	12%	19,3%	
6	12%	16%	8%	9%	17%	10%	14%	8%	11,4%	
7	-	-	-	-	-	-	-	-	-	-
8	0%	0%	18%	18%	13%	8%	8%	8%	11,7%	
9	28%	0%	25%	-	13%	18%	11%	9%	20,7%	
10	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-15	14,1%	16,0%	12,0%	9,6%	19,6%	17,0%	17,4%	16,3%	18,0%
RANKING		3	4	2	1	8	6	7	5	

PERCENTAGE AGREEMENT										
MODAL age	Mark Reader 1	Afra Reader 2	Amaia Reader 3	Peter Reader 4	Marina Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8	ALL Readers	
0	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-
2	56%	78%	100%	0%	78%	78%	44%	67%	67%	
3	60%	40%	80%	0%	60%	60%	20%	40%	49%	
4	50%	75%	25%	0%	50%	33%	75%	50%	50%	
5	100%	50%	50%	50%	75%	50%	25%	25%	53%	
6	56%	56%	78%	67%	22%	67%	33%	78%	56%	
7	100%	100%	0%	-	100%	0%	0%	0%	43%	
8	100%	100%	0%	0%	67%	67%	67%	33%	57%	
9	0%	100%	0%	0%	0%	50%	0%	0%	20%	
10	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-
14	100%	0%	100%	0%	0%	0%	0%	0%	25%	
15	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-15	61,5%	64,1%	61,5%	18,8%	51,3%	57,9%	35,9%	48,7%	52,4%
RANKING		2	1	2	8	5	4	7	6	

RELATIVE BIAS										
MODAL age	Mark Reader 1	Afra Reader 2	Amaia Reader 3	Peter Reader 4	Marina Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8	ALL Readers	
0	-	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-	-
2	0,56	-0,22	0,00	1,50	0,33	0,00	0,67	0,11	0,28	
3	0,40	-0,60	0,20	1,00	0,00	-0,40	0,40	-0,20	0,03	
4	0,50	-0,25	-0,25	1,00	1,00	0,00	-0,25	-0,50	0,07	
5	0,00	-0,75	-1,00	0,50	0,25	-0,75	-1,00	-0,75	-0,50	
6	0,00	-0,11	0,00	0,33	0,56	-0,11	-0,22	0,00	0,03	
7	0,00	0,00	-1,00	-	0,00	-1,00	-1,00	-3,00	-0,86	
8	0,00	0,00	-1,67	0,00	0,67	-0,33	-0,33	-0,67	-0,30	
9	1,00	0,00	-0,50	-1,00	2,00	-1,00	-2,50	-1,50	-0,40	
10	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-
14	0,00	-2,00	0,00	-3,00	-1,00	-2,00	-2,00	-1,00	-1,38	
15	-	-	-	-	-	-	-	-	-	-
Weighted mean	0-15	0,28	-0,31	-0,28	0,44	0,46	-0,32	-0,21	-0,36	-0,07
RANKING		2	4	3	7	8	5	1	6	

Overall ranking									
	Mark Reader 1	Afra Reader 2	Amaia Reader 3	Peter Reader 4	Marina Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8	
Ranking Coefficient of Variation	3	4	2	1	8	6	7	5	
Ranking Percentage Agreement	2	1	2	8	5	4	7	6	
Ranking Relative bias	2	4	3	7	8	5	1	6	
OVERALL RANKING	1	3	1	6	8	4	4	7	

Table 4i. Bias tests: non-parametrically with a one-sample Wilcoxon rank sum test. The inter-reader bias test and the reader against MODAL age bias test.

<b>Inter-reader bias test and reader against MODAL age bias test</b>								
	Mark Reader 1	Afra Reader 2	Amaia Reader 3	Peter Reader 4	Marina Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8
Reader 1		**	**	—	—	**	*	**
Reader 2	**		—	*	**	—	—	—
Reader 3	**	—		—	**	—	—	—
Reader 4	—	*	—		—	*	**	*
Reader 5	—	**	**	—		**	**	**
Reader 6	**	—	—	*	**		—	—
Reader 7	*	—	—	**	**	—		—
Reader 8	**	—	—	*	**	—	—	
MODAL age	*	**	*	—	**	*	—	*

—	= no sign of bias ( $p > 0.05$ )
*	= possibility of bias ( $0.01 < p < 0.05$ )
**	= certainty of bias ( $p < 0.01$ )

# **Megrim (*Lepidorhombus whiffiagonis*) Otolith exchange 2004**

## *Protocol for the megrim otolith exchange in 2004*

### **1. Introduction**

The Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) held in Rome in March 2003 considered megrim as one of the species demanding confirmation of the ages being assigned by the Fisheries Institutes as since the last ageing workshop for this species was carried out in 1997 in Vigo (Spain), no other workshop or exchange has been carried out.

The planning group indicated AZTI (Basque Country, Spain) as responsible for the organization of a megrim exchange and workshop in 2004, to focus on these improvements.

In order to compare age readings agreement, and also discrepancies between readers, an otolith exchange program was planned before the workshop. In this exchange, a collection of otoliths will be circulated among experienced and new readers.

The objectives of the exchange are:

- Estimate age reading agreement between readers.
- Analyze relative differences between reader agreements.

**2. Participants** (Please feel free to update this list with any other person in your lab participating in the exchange)

Ireland Mar. Inst. Gráinne Ní Chonchúir [Grainne.NiChonchuir@marine.ie](mailto:Grainne.NiChonchuir@marine.ie)

Portugal IPIMAR António Marçal [amarcal@ipimar.pt](mailto:amarcal@ipimar.pt)

Scotland (UK) FRS Marine Laboratory Maria Mathewson [M.Mathewson@marlab.ac.uk](mailto:M.Mathewson@marlab.ac.uk)

Scotland (UK) FRS Marine Laboratory Gordon Henderson [G.I.Henderson@marlab.ac.uk](mailto:G.I.Henderson@marlab.ac.uk)

Spain AZTI Amaia Gomez de Segura [amgomez@suk.azti.es](mailto:amgomez@suk.azti.es)

Spain AZTI Marina Santurtun [msanturtun@suk.azti.es](mailto:msanturtun@suk.azti.es)

Spain IEO Jorge Fontela [jorge.fontenla@vi.ieo.es](mailto:jorge.fontenla@vi.ieo.es)

UK CEFAS Sally Warne [S.A.Warne@cefas.co.uk](mailto:S.A.Warne@cefas.co.uk)

UK CEFAS Mark Etherton [M.W.Etherton@cefas.co.uk](mailto:M.W.Etherton@cefas.co.uk)

### **3. Otolith collection**

The otoliths chosen for the exchange came from 93 fish originated from market samples. Otoliths were chosen by fish length, sex and quarter trying to cover all quarters in a year and both sexes along the year for the marketable lengths found in the samples (17 - 51 cm). All otoliths are from ICES Div. VIIIa,b,d collected during different years. Whole otoliths (right and left) were imbibed in water during, at least 24 h. before the pictures were taken.

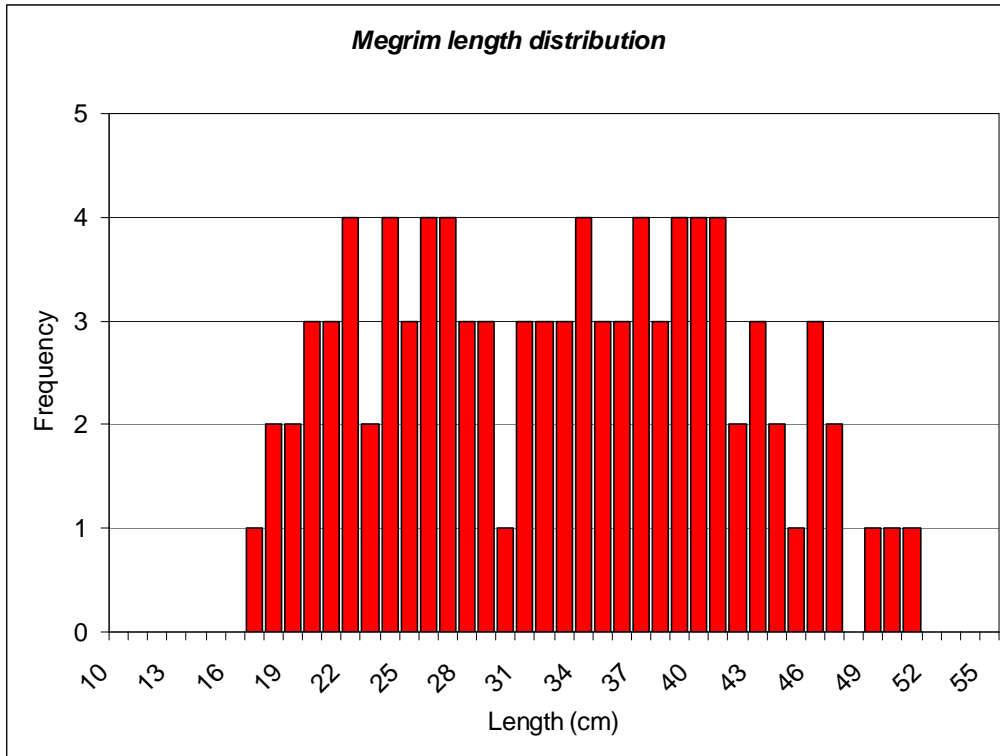


Figure 1. Length frequency for megrim otolith collection.

One collection is available but in two different formats:

- 1) "Image" or "Virtual" megrim otolith collection.
- 2) "Real" megrim otolith collection.

#### 4. Reading procedure

Digitize images of all otoliths (right and left, in the same image) are sent to all participants in a CD which contains these images, an Excel file (NorMegrim\_Otol\_Read.xls) with the image code and the length, sex and quarter belonging to the otolith of that image and an instruction document (INSTRUCTIONS.doc)

When the two otoliths from one fish were too big to be taken both in one image, pictures were taken individually for each otolith. Images were named using the same file code with an "a" or "b" for the left and right otolith, respectively.

The OTOLITH LIST (\_VIRT or \_REAL) in the Excel file (NorMegrim\_Otol\_Read.xls) provides all essential information for age reading (quarter (Q o M), fish length and sex). The participants are requested to input the age readings, the identification of the edge and the reliability of the reading in the otolith list.

In the Excel file of the respective collection (shaded areas), each reader should indicate the following information:

- Reader,
- Date,
- Institute,
- Age,
- Edge (if possible)
- Reliability (the credibility of the reading, from 1 (very bad) to 3 (good), this should reflect the confidence on the age given),
- Observations (if any comment about the otolith has to be included).

When readings have been completed, the Excel file should be renamed (for example, NorMegram\_Otol\_Read.xls should be renamed to NorMegram\_Otol\_Read\_Marina.xls, after containing Marina's readings). After this, the Excel file should be send by e-mail to Marina Santurtún (AZTI).

Otolith readings should be performed straight from the images. If more than one reader from an institute participates in the exchange, it is essential that these readers determine the age of the fish independently.

Please always try to enter an age even if the otolith or the image is complicated to read. When in doubt, please enter two ages (e.g. 7-8) being the first age the most probable age to be considered for the analysis.

The advantage of using images instead of real otoliths is the time saving for the otolith exchange; however we are very conscious of the reading difficulties when using images, especially if images are not good enough, large otoliths etc...

That is why we encourage the age readers to mark the images (using any painting tool) of the otoliths to facilitate tracking down where differences in age interpretation occur to be discuss later during the Workshop. When the amount of otoliths that cannot be read is "considerable" (to be decided by each reader...) the corresponding otolith will be read. In that case, it will be indicated (OBSERVATIONS) that the reading come from the "real" otolith.

In general, we encourage the readers to use just the "image" collection although it is also recommended to read the "real" collection as a way of checking (both templates area included). However, the idea is to start using digital otolith/illicia collections, at least for some species, to safe time and avoid quality lost of the real collections.

## **5. Time schedule for the exchange and workshop**

15 July 2004 ➔ All participants will have received the CD's with images and data lists.

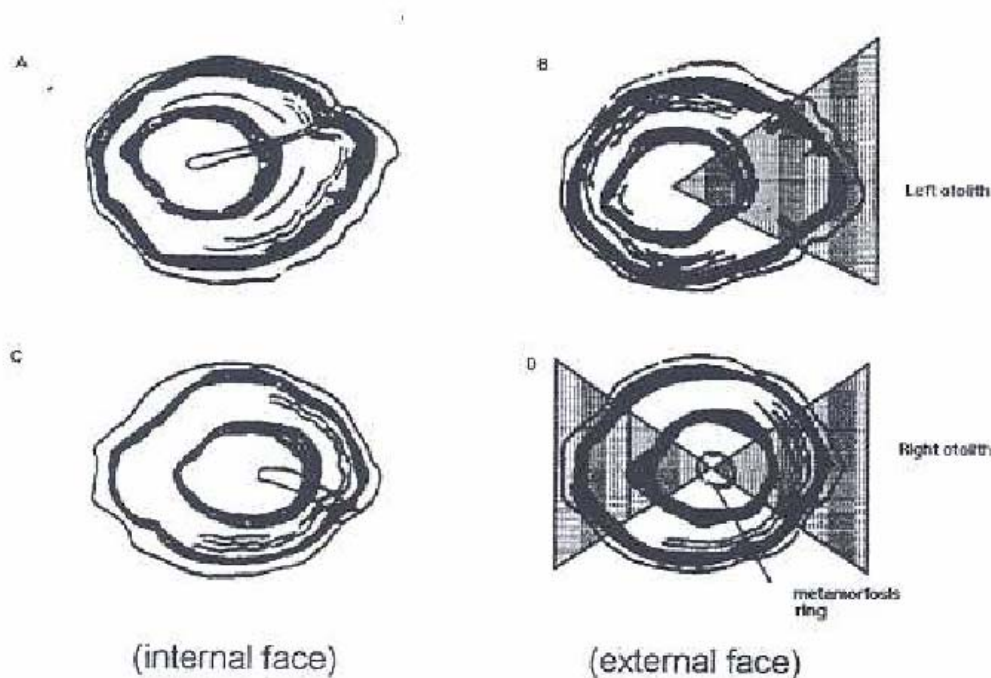
30 October 2004 ➔ All age readings to be returned to AZTI.

15- 22 November 2004 ➔ Draft report with the results of the exchange circulated among participants.



29 November-3 December 2004 →MEGRIM AGE READING WORKSHOP in AZTI.  
 March 2005→Final report submitted to PGCCDBS.

**6. Brief megrim otolith protocol** (from the Workshop on Megrim Otolith and Fin Rays Age Reading held in Vigo, May 1997)



The general criteria adopted for ageing megrim otoliths is based on the number of annual hyaline rings, using the following rules:

	Quarter 1	Quarter 2	Quarter 3	Quarter 4
<b>N rings Hyaline Edge</b>	Age = N	Age = N	Age = N-1 Early winter	Age = N-1 Early winter
<b>N rings Opaque edge</b>	Age = N+ 1 late winter	Age = N	Age = N	Age = N

**7. Comments**

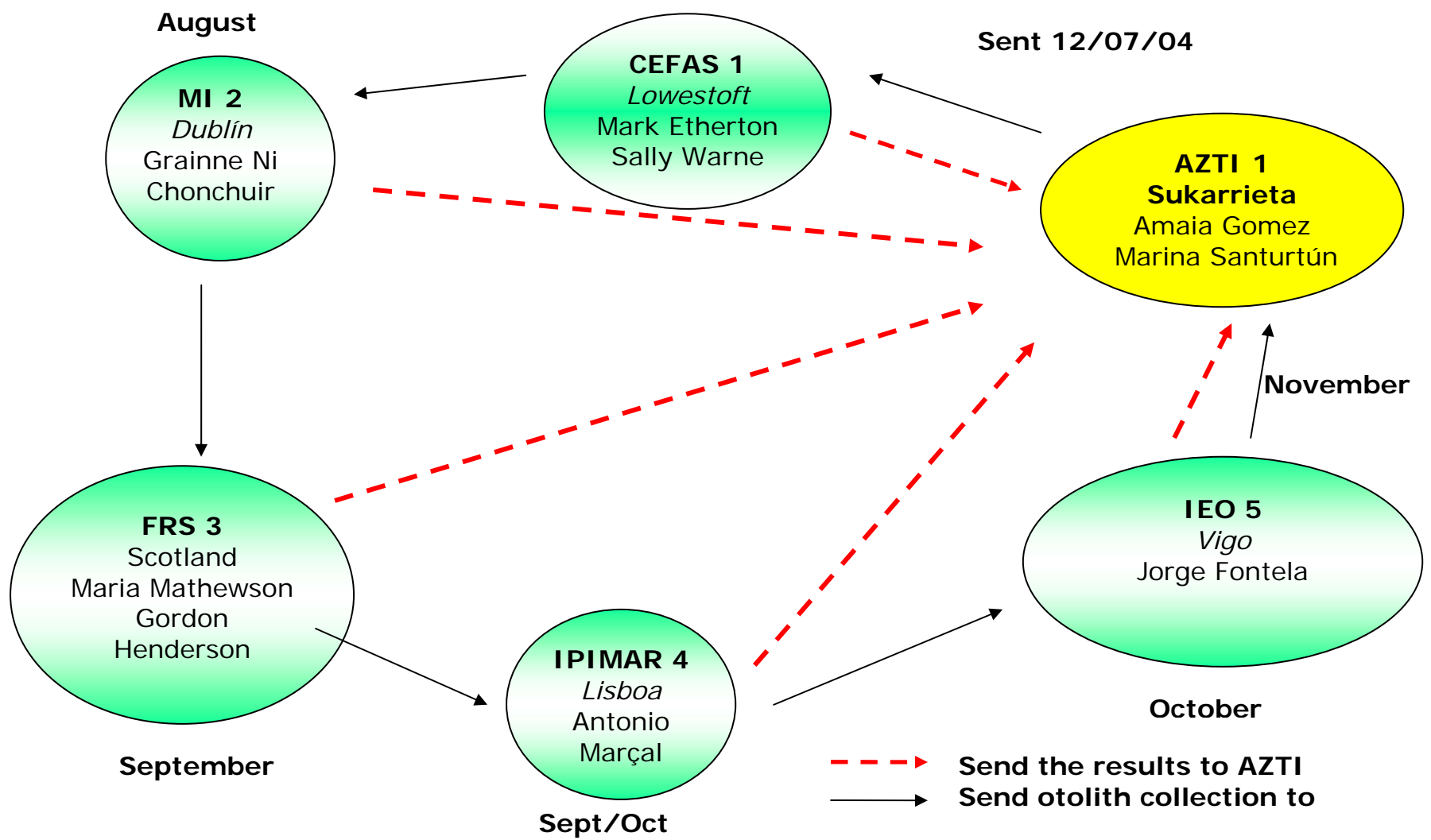
If you have any comments on the above described methods and time schedule, please inform us.

## 8. Participants Address

Name	Institute	Address	e-mail
*Gráinne Ní Chonchúir	Mar. Inst.	Marine Institute. Galway Technology Park. Galway (Republic of Ireland)	<a href="mailto:Grainne.NiChonchuir@marine.ie">Grainne.NiChonchuir@marine.ie</a>
*António Marçal	IPIMAR	Av. Brasília, 1449-006 Lisbon (PORTUGAL)	<a href="mailto:amarcal@ipimar.pt">amarcal@ipimar.pt</a>
*Maria Mathewson	FRS Marine Laboratory	PO Box 101 375 Victoria Road Aberdeen AB11 9DB (SCOTLAND)	<a href="mailto:M.Mathewson@marlab.ac.uk">M.Mathewson@marlab.ac.uk</a>
Gordon Henderson	FRS Marine Laboratory	Same as Maria	<a href="mailto:G.I.Henderson@marlab.ac.uk">G.I.Henderson@marlab.ac.uk</a>
*Amaia Gómez de Segura	AZTI	Unidad de Investigación Marina - Marine Research Division Txatxarramendi Ugarte a z/g 48395 Sukarrieta (Bizkaia) (SPAIN)	<a href="mailto:amgomez@suk.azti.es">amgomez@suk.azti.es</a>
Marina Santurtún	AZTI	Same as Amaia	<a href="mailto:msanturtun@suk.azti.es">msanturtun@suk.azti.es</a>
*Jorge Fontela	IEO	Apartado 1552, 36280 Vigo, (SPAIN)	<a href="mailto:jorge.fontenla@vi.ieo.es">jorge.fontenla@vi.ieo.es</a>
Sally Warne	CEFAS		<a href="mailto:S.A.Warne@cefas.co.uk">S.A.Warne@cefas.co.uk</a>
*Mark Etherton	CEFAS	Lowestoft Laboratory Pakefield Road Lowestoft Suffolk NR33 0HT (UNITED KINGDOM)	<a href="mailto:M.W.Etherton@cefas.co.uk">M.W.Etherton@cefas.co.uk</a>
<b>*Contact person to be sent the “real” otolith collection</b>			

## 9. Megrim "Real" otolith Exchange 2004 (Time Schedule)

Second half of July



# Megrim Otolith Ageing Workshop

## AZTI

### SUKARRIETA, 29 November-1 December 2004

#### Objectives:

1. Analyse and discuss the otolith exchange results for megrim (ages and images).
2. Perform a 2nd reading on both real otoliths and images in order to corroborate results from the exchange.
3. Discuss possible discrepancies between readers.
4. Give the chance to other scientist to get involved in the megrim otolith ageing procedure.

#### Participants:

Name	Institution	Country	email	Participating	
				Exchange	Workshop
Mark Etherton	CEFAS	United Kingdom	<a href="mailto:M.W.Etherton@cefas.co.uk">M.W.Etherton@cefas.co.uk</a>	X	X
Sally Warne	CEFAS	United Kingdom	<a href="mailto:S.A.Warne@cefas.co.uk">S.A.Warne@cefas.co.uk</a>	X	X
Jorge Fontela	IEO	Spain	<a href="mailto:jorge.fontenla@vi.ieo.es">jorge.fontenla@vi.ieo.es</a>	X	X
Amaia Gomez de Segura	AZTI	Spain	<a href="mailto:amgomez@suk.azti.es">amgomez@suk.azti.es</a>	X	X
Marina Santurtún	AZTI	Spain	<a href="mailto:msanturtun@suk.azti.es">msanturtun@suk.azti.es</a>		X
Ane Iriondo	AZTI	Spain	<a href="mailto:airiondo@suk.azti.es">airiondo@suk.azti.es</a>		X
Iñaki Quincoces	AZTI	Spain	<a href="mailto:iquincoces@suk.azti.es">iquincoces@suk.azti.es</a>		X
Gordon Henderson	FRS	Scotland	<a href="mailto:G.I.Henderson@marlab.ac.uk">G.I.Henderson@marlab.ac.uk</a>	X	
Afra Egan	Mar. Inst.	Ireland	<a href="mailto:afra.egan@marine.ie">afra.egan@marine.ie</a>	X	X
Ayesha Power	Mar. Inst.	Ireland	<a href="mailto:ayesha.power@marine.ie">ayesha.power@marine.ie</a>	X	X

Antonio Marçal	IPIMAR	Portugal	<a href="mailto:amarcal@ipimar.pt">amarcal@ipimar.pt</a>	X	X
Peter Vingaard Larsen	DIFRES	Denmark	<a href="mailto:pvl@dfu.min.dk">pvl@dfu.min.dk</a>		X

## Agenda:

Date	Time	Item
Monday, 29 <sup>th</sup> November 2004	9:30-10:45	Opening: Some logistics Objectives of the megrim otolith ageing workshop Revision of Agenda/Time table <ul style="list-style-type: none"> <li>1. 1.1 Megrim ageing based on Otolith results from the previous otolith workshop.</li> <li>2. 1.2. Results from projects developed by Spain, France and Portugal</li> </ul> Participants' explanations in relation to their work ageing with megrim and /or other species.
	10:45-11:00	Coffee break
	11:00-11:45	Results from the otolith exchange (Marina Santurtún) General discussion of the ageing results-

	11:45-13:30	Presentation of the new otolith collection for the readings (real otoliths and images). Organization of the work. 2 <sup>nd</sup> reading of the otoliths (images and real otoliths) previously selected.
	13:30-14:30	Lunch
	14:30-17:30	2 <sup>nd</sup> reading (continues)
Tuesday, 30 <sup>th</sup> November 2004	9:00-10:45	2 <sup>nd</sup> reading (continues)
	10:45-11:00	Coffee break
	11:00-12:30	2 <sup>nd</sup> reading (continues)
	12:30-13:30	Discussion of the age reading criteria
	13:30-14:30	Lunch
	14:30-17:00	Preliminary results from the otolith 2 <sup>nd</sup> reading(real and images)
	17:30 →	Social events: Visit to the Fishermen Museum Beer in the Bermeo Port Dinner
Wednesday, 1 <sup>st</sup> December 2004	9:30-10:45	Further result discussion?.../ Start writing the Workshop Report
	10:45-11:00	Coffee break
	11:00-13:30	Writing the report
	13:30-14:30	Lunch
	14:30-16:30	Finalizing the report and End of the meeting

### ***Results of the 3<sup>rd</sup> Exchange of Megrim Otoliths for Age determination***

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*Marine Research Division. AZTI Foundation. Txatxarramendi ugarte z/g.*  
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#### **Abstract**

The Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) recommended otolith exchange for different species that are assessed in the ICES framework. Thus, during the 2<sup>nd</sup> semester of 2004, a megrim otolith exchange was carried out. The preliminary results of the third megrim otoliths exchange indicate that the age estimation criteria has been maintained as the one adopted previously. The results indicate that the precision of age readings is around 21 % and slightly higher bias has been found in age readings of older fishes. Thus, the values of APE and CV in (%) for all readers are 16 and 14, and 21-19 % respectively for the real and image otolith collection. The preliminary analysis of these results shows that there are no serious deviations in the otolith readings. Nevertheless, and considering that seven years have already passed since the last exchange and workshop, it was necessary to held a workshop to get together megrim otolith readers to exchange suggestions and relevant improvement or information.

#### **Introduction**

The ICES fisheries advice depends on the quality of data from the commercial fisheries that in all cases has not been satisfactory. As part of the biological data, reliable age determination is an essential feature of fish stock assessment, as it provides information required to estimate growth and mortality rates.

Otolith processing methods and age reading methods might differ considerably among countries. Therefore, it is advised that otolith exchanges should be carried out on a regular basis. Workshops should be organised to address age reading observed problems.

The PG recommends that otolith exchanges for a number of fish species should be carried out in 2004, 2005 and 2006. Megrim was one of this species. Although no specific problems have been detected in the readings provided to the Assessment Working Groups, the need of

organising a Workshop on Megrim Readings was defined by the long period that has passed from the last Workshop, 7 years. Also, the advantage of these workshops is that the age reading methods of all experts of this species are discussed and compared "in situ".

The megrim otolith exchange was deployed along the 2<sup>nd</sup> semester of 2004 while the specific international workshop will be celebrated in November-December 2004, within the framework of the National Plan, 2004.

The objectives of the exchange are:

- Estimate age reading agreement between readers
- Analyze relative differences between reader agreements
- Incorporate new readers in the megrim age estimation.

This document presents the preliminary results obtained so far for this megrim otolith exchange

## **Material and Methods**

The exchange was carried out following the recommendations of the EFAN Report 3-2000 on Guidelines and Tools for Age Reading Comparisons, which is available on the EFAN home page.

No comparison between this exchange and the previous one was done just two readers participating in the exchange were also involved in the last exchange (1997).

The otoliths chosen for the exchange came from 93 fish originated from market samples. Due to some labelling problems with the images the collection was reduced to 87 otolith. Otoliths were chosen by fish length, sex and quarter trying to cover all quarters in a year and both sexes along the year for the marketable lengths found in the samples (17 - 51 cm) (Figure 1.)

All otoliths are from ICES Div. VIIIabd collected during different years. Whole otoliths (right and left) were imbibed in water during, at least 24 h. before the pictures were taken.

Just one collection was available but in two different formats:

- 1) "Image" or "Virtual" megrim otolith collection
- 2) "Real" megrim otolith collection



Digitize images of all otoliths (right and left, in the same image) were sent to all participants in a CD.

When the two otoliths from one fish were too big to be taken both in one image, pictures were taken individually for each otolith. Images were named using the same file code with an "a" or "b" for the left and right otolith, respectively.

The instructions for filling up the Data Bases for the readings provide all essential information for age reading (quarter (Q or M), fish length and sex). The participants were requested to input the age readings, the identification of the edge and the reliability of the reading in the otolith list.

Otolith readings should have been performed straight from the images. However, in some cases due to the bad quality of the image, the readings on some images could not be deployed and so the real otolith were used. In most of the cases readings were performed on the images and on the real otoliths separately, except for two readers.

The advantage of using images instead of real otoliths was the time saving for the otolith exchange, and also to avoid quality lost of the real collections. Besides the reading criteria used could be easily checked out when using digital support. In that sense, some of the age readers marked the otolith images to facilitate tracking down possible differences in age interpretation.

The general criteria adopted for ageing is presented in the report of the previous workshop (Megrim Otolith and Spines Age Reading Workshop, 1997).

A spreadsheet for a standardised analysis of the age reading comparisons ("AGE COMPARISONS. XLS") can also be found on the EFAN website ([www.efan.no](http://www.efan.no) under "Guidelines"). The standard analysis has been prepared by A.T.G.W. Eltink from RIVO.

Results from the otolith exchanges and age reading workshops will be reported to the PG and to the relevant ICES assessment working groups.

## 1. Exploratory data analysis (EDA)

The first approximation was the determination of the modal age and the difference between each reader's age and modal age. The modal age was calculated based on the results of the readers involved in the stock assessment: R1, R2, R3. Although the most experienced readers were

R1 & R2. If the modal age could not be estimated, then the rounded mean age was inserted instead. Box-whisker plots were used for the graphical representation of the sample (real otolith and images) by each reader (median and interquartile range by each reader). They were also used to summarise the observations and are useful in observing and comparing the distribution of the otolith readings by reader.

Also, Age bias plots are presented as they are considered as being a good way of showing the aging errors by reader (precision and accuracy). In this case the bias in age reading can only be shown as a relative bias (as no real age of the otolith is known).

2. In terms of reproducibility measures:

2.1) Average percent age error (APE), Beamish and Fournier (1981) is an index of reading precision to compare a series of observations. The formula is as follows:

$$APE = \frac{100}{n} \sum_{i=1}^n \left( \frac{1}{r} \sum_{j=1}^r \frac{|x_{ij} - \bar{x}_i|}{\bar{x}_i} \right) \quad (1)$$

n = number of otoliths

r = number of readings for each otolith (readers)

x<sub>ij</sub> = the j value of age estimation for the i otolith

$\bar{x}_i$  = average age calculated for the i otolith

2.2) The Mean Coefficient of Variation (CV). The precision errors in age reading are best described by this coefficient by age group

$$CV = \frac{100}{n} \left[ \sum_{i=1}^n \left( \frac{sd}{\bar{x}_i} \right) \right] \quad (2)$$

sd = the standard deviation for the i otolith

This measurement is considered to be more appropriate than the conventional percent of agreement when comparing ages, since it take into account the average year class of fish.

## Results

The results of the age estimations by reader and the basic information in relation to each of the otolith collection in both formats are summarised in Table 1 and Table 1i.

The Box-whisker plot for all readers and both formats of the collection shows that the range of ages attributed was wide with a mean value of 5 years (Figure 2 & 2i).

Considering the bias plots for all the readers combined it could be observed that the mean age recorded is very close to the modal age and that the deviations slightly increased from age 5 and over (Figure 3 & 3i). The main bias detected in the real otolith collection is for age 4 onwards. Readers R3 and R7 overestimated the ages meanwhile, for the image otolith collection, the overestimation is less detectable. R5 and R6, in general slightly underestimated the ages regarding the modal age. However, the bias detected is really small, as expected.

The precision errors by age reader are best described by the coefficient of variation (CV%) by age group because the CV might often differ by age group. The coefficient of variation (CV%) and percent agreement are plotted against modal age in Figure 4 & 4i. These figures shows the mean results for all the readers and indicate how the CV and Agreement in general decreases accordingly with the modal age.

Firstly for the real otolith collection, from all readers the average of CV was 20.6 %. This value is not very high. In fact, the CV's are higher for age 2 and afterwards the CV's decreased until 12 %. Mean percent of agreement for this collection is around 48 %, and is maintained above 50 % until age 8 and decreases again for ages 9 and 10 (Table 2.).

Secondly, for the image collection, from all readers the average of CV was 18.5 %. This value is not very high. In fact, the CV's are higher for age 3 and afterwards the CV's decreased, keeping around 13 % for ages older than 4. Mean percent of agreement for this collection is around 45.4 %, and is maintained around 53 % from age 2 to 8 and decreases again for ages 9 and 10 (Table 2i.).

The relative mean bias in relation to the modal age are presented in Figure 5 & 5i.

The results of the %Agreement, APE and CV in (%) are presented together in the Table 3. The values of APE and CV obtained for all readers were 16 and 14 and 21 and 19 respectively for each of the "collections". These high levels of precision and the low APE is a good sign of the assimilation of the ageing criteria by all readers.

## **Discussion**

In summary, the exploratory and statistical analysis showed that the age precision (CV) is quite low (20%) while the bias is quite reasonable.

In particular, the ageing method up to age 8 seems to be the same for almost all the readers. However, from the eight age onwards the results indicates the difficulty for the majority of the readers to interpret the ring pattern of older otoliths.

A plot of mean length at age by reader can be used to diagnose individual reader tendencies (Figure 6 & 6i). The figures show that, for the same age, some readers obtained larger range of length. Kruskal-wallis test between readers for ageing samples of the two formats showed significant bias for all readings obtained by R3 and R7 just for the real otolith collection. This significant bias are also quite conspicuous fro R5 (Table 4 & 4i).

## **Conclusions:**

- a. The level of agreement in the readings was very high for the majority of readers. This is the result of the good adoption of the ageing criteria established. Furthermore, new readers showed an adequate interpretation of the ageing criteria (R8).
- b. The most experienced readers involved in stock assessment had a very high mean level of agreement (from 66 to 77 %) and high mean levels of precision from 7 to 9%.
- c. The megrim apparently well established ageing method for mostly all ages provide a good quality to the age estimations used in assessment.

## **Recommendations**

Taking into account the good results obtained, the work in the future will be deployed towards international intercalibration exercises by means of images to ensure consistency and precision between readers over time.

## **References**

- Beamish, R. J., and Fournier, D.A. 1981. A method for comparing the precision of a set of age determinations. *Can. J. Fish. Aquat. Sci.*, 38: 982-983.

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

Guidelines and tools for age reading. Eltink, A.T.G.W., A.W. Newton, C. Morgado, M.T.G. Santamaria and J. Modin, 2000. Guidelines and tools for age Reading. Internet: <http://www.efan.no>

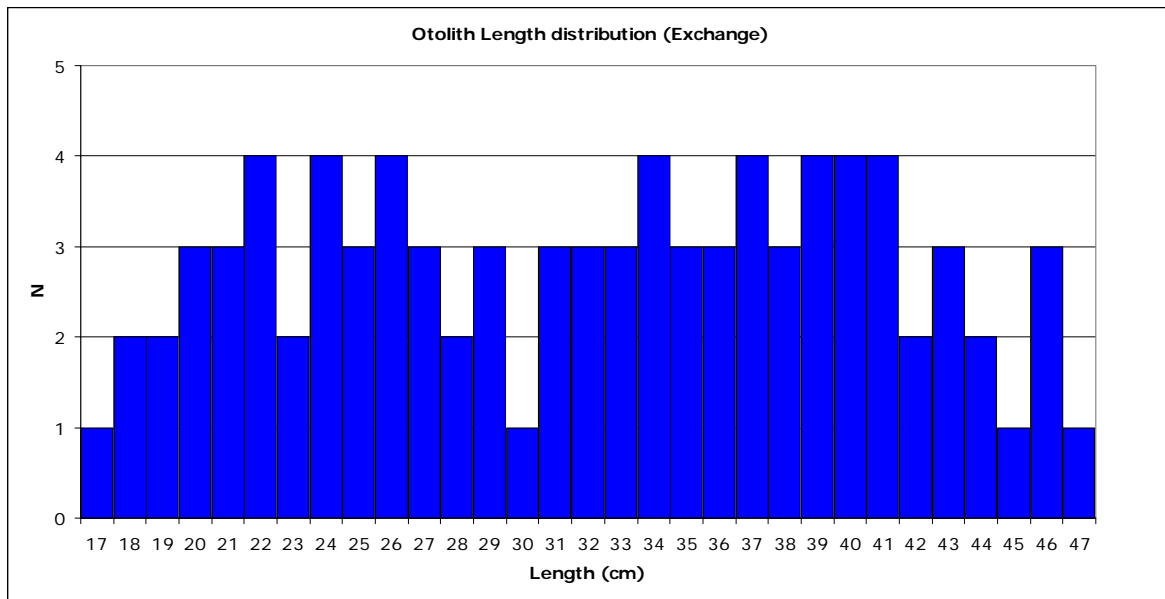
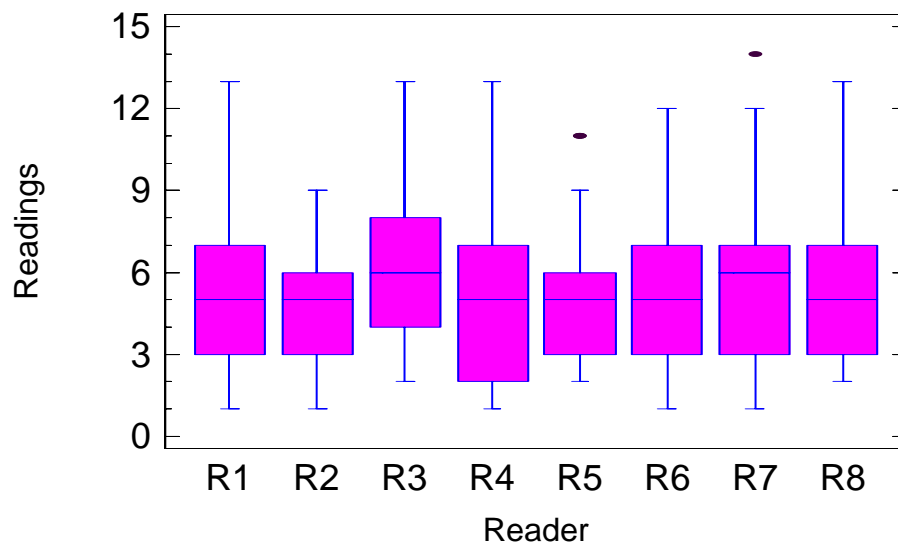


Figure 1. Length frequency distribution of samples from Megrim otolith exchange.

Box-and-Whisker Plot for Real Otolith Collection



Box-and-Whisker Plot for Image Otolith Collection

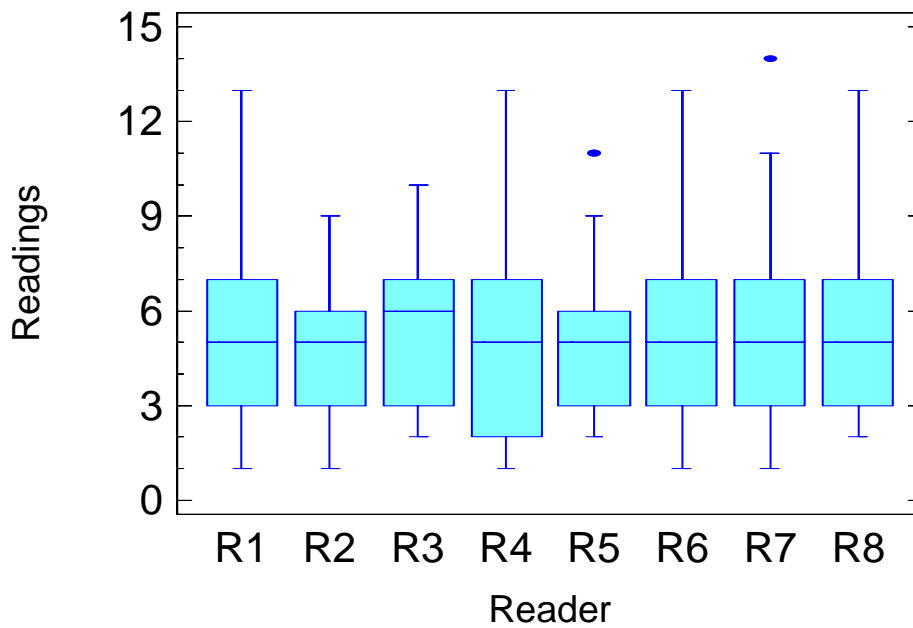


Figure 2. Box-Whisker plots for each reader in relation to the whole set of otolith for each "collection".

# REAL OTOLITH COLLECTION

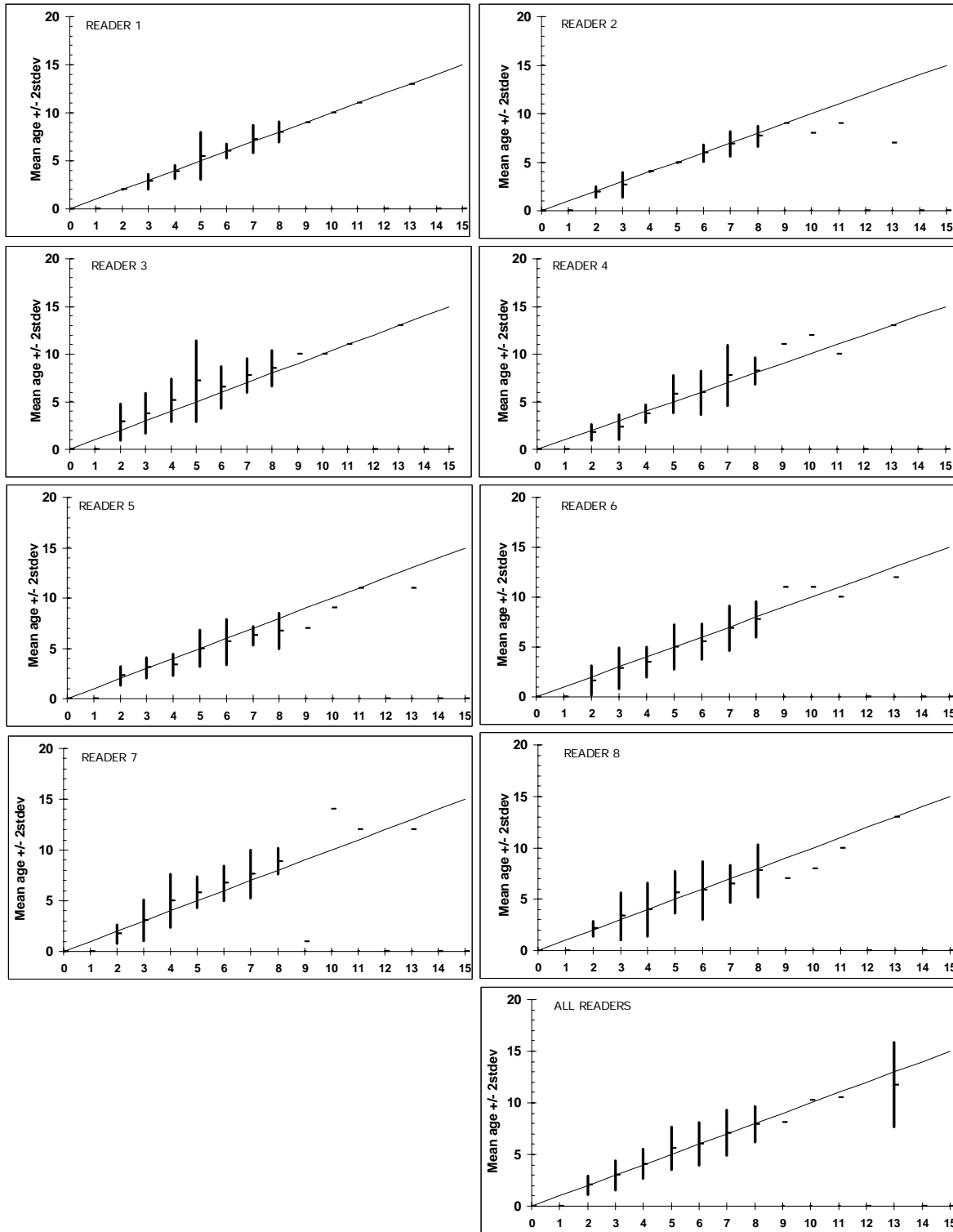


Figure 3. In the age bias plots below the mean age recorded  $\pm 2$ stddev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.



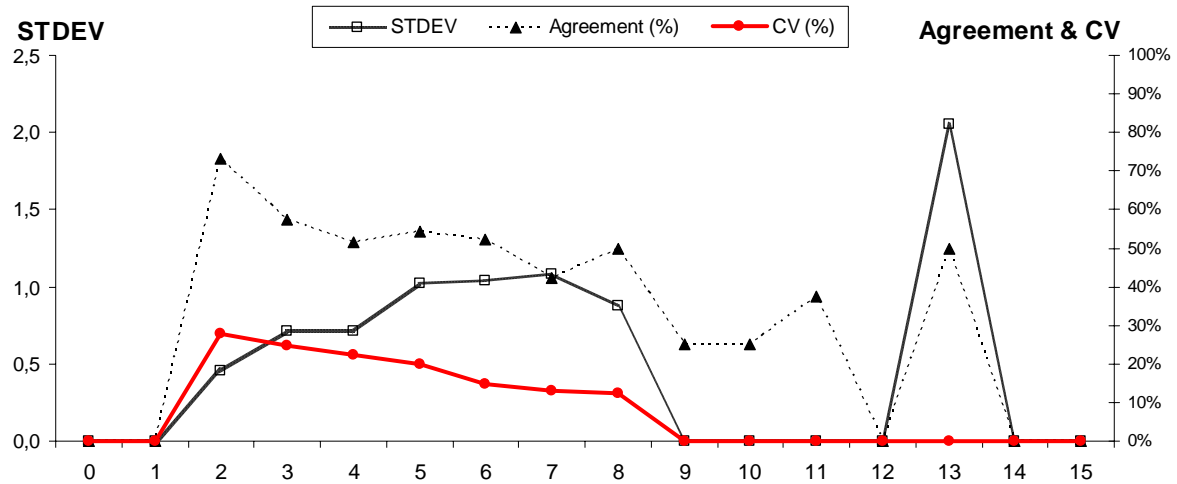


Figure 4. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL (and in some cases ABSOLUTE MEAN) age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

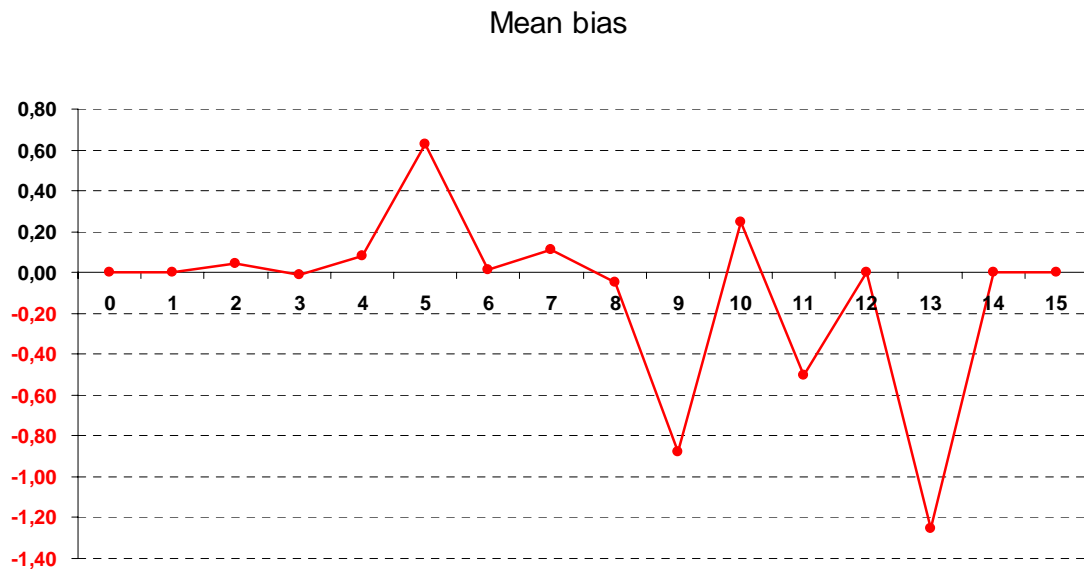


Figure 5. The RELATIVE bias by MODAL (in some cases ABSOLUTE MEAN) age as estimated by all age readers combined

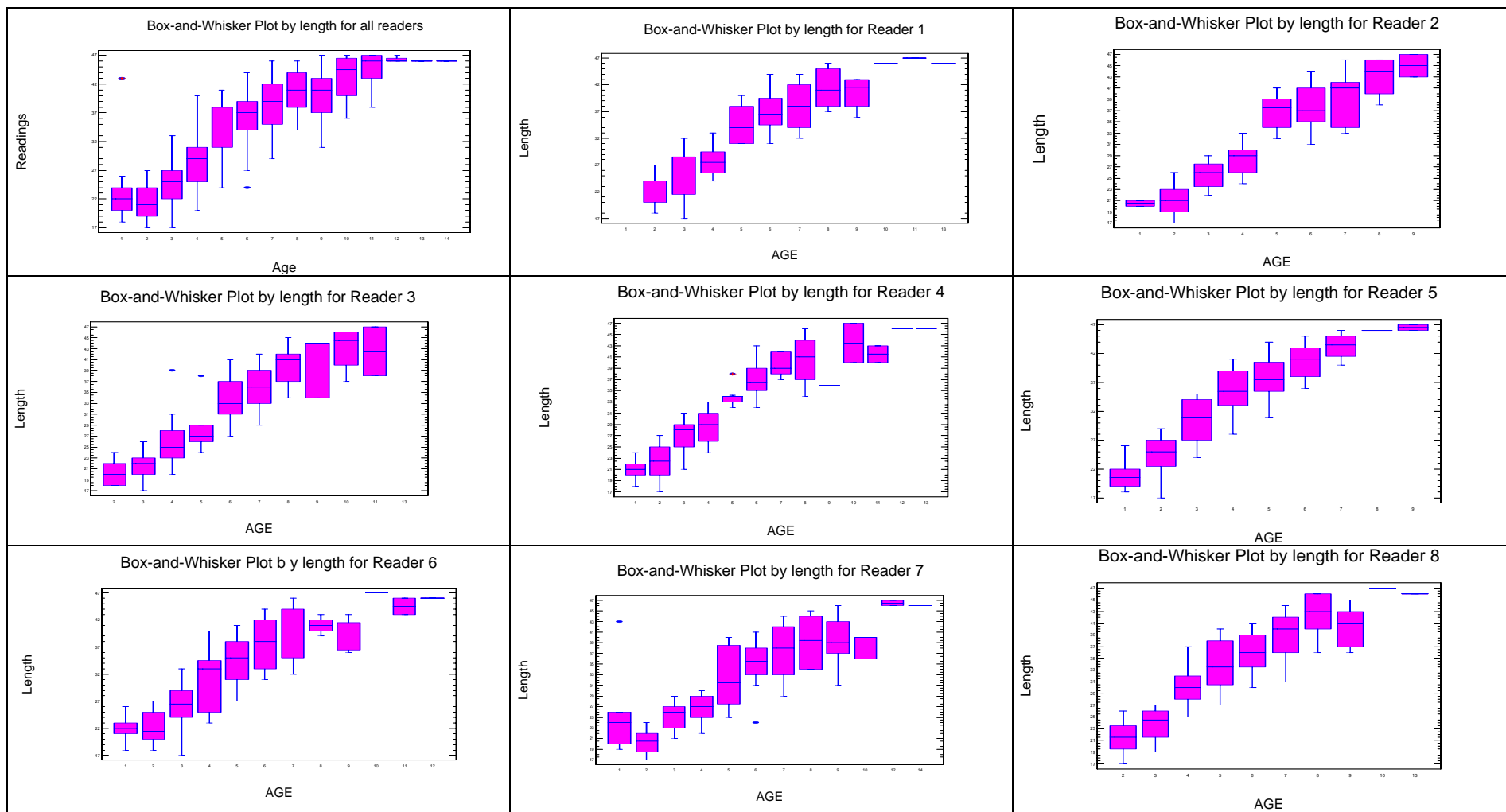


Figure 6. Box-Whisker plots of the length distribution (cm) by age obtained for all readers reading the real otolith collection

## IMAGE OTOLITH COLLECTION

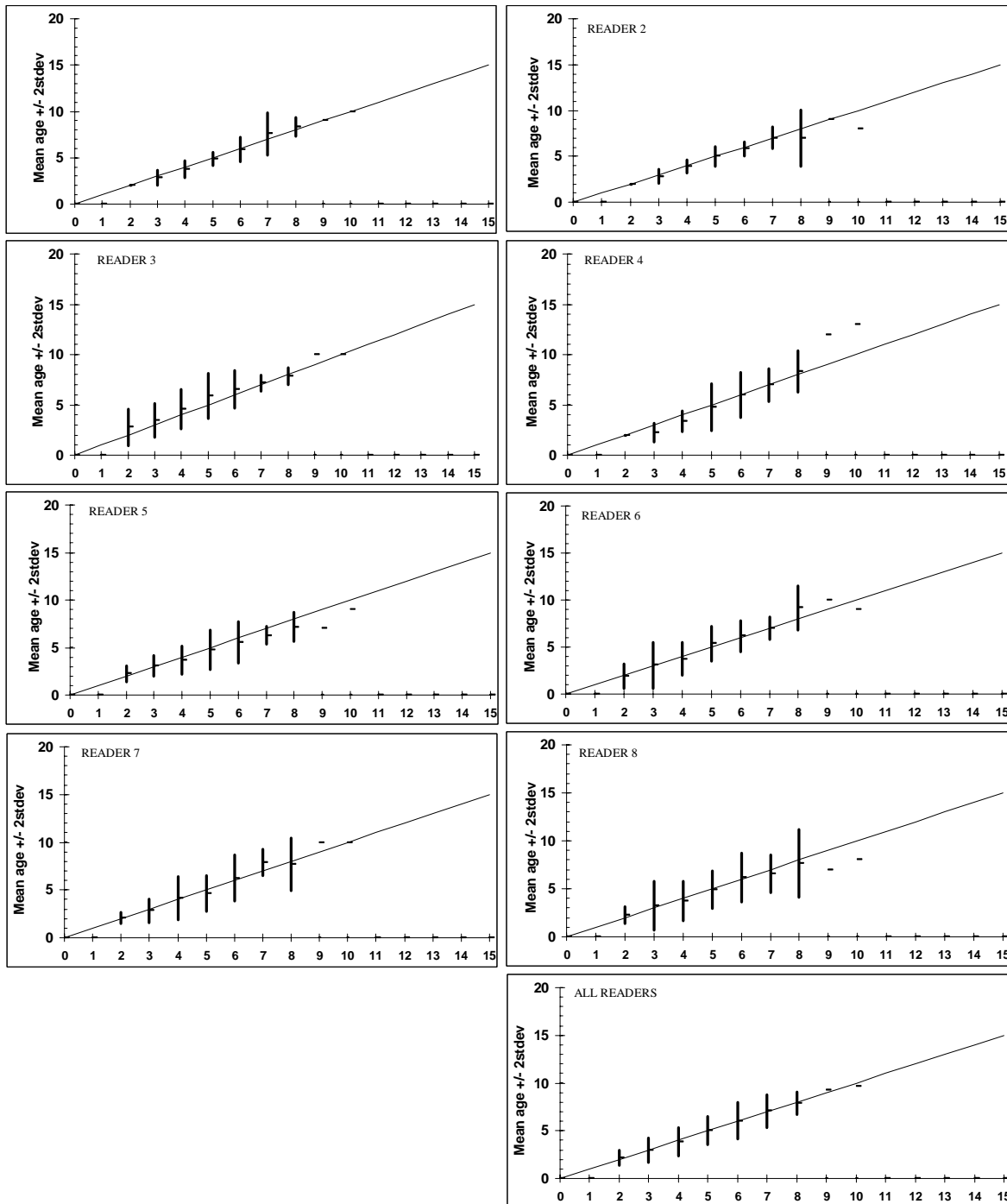


Figure 3i. In the age bias plots below the mean age recorded  $\pm 2$ stdev of each age reader and all readers combined are plotted against the MODAL age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.

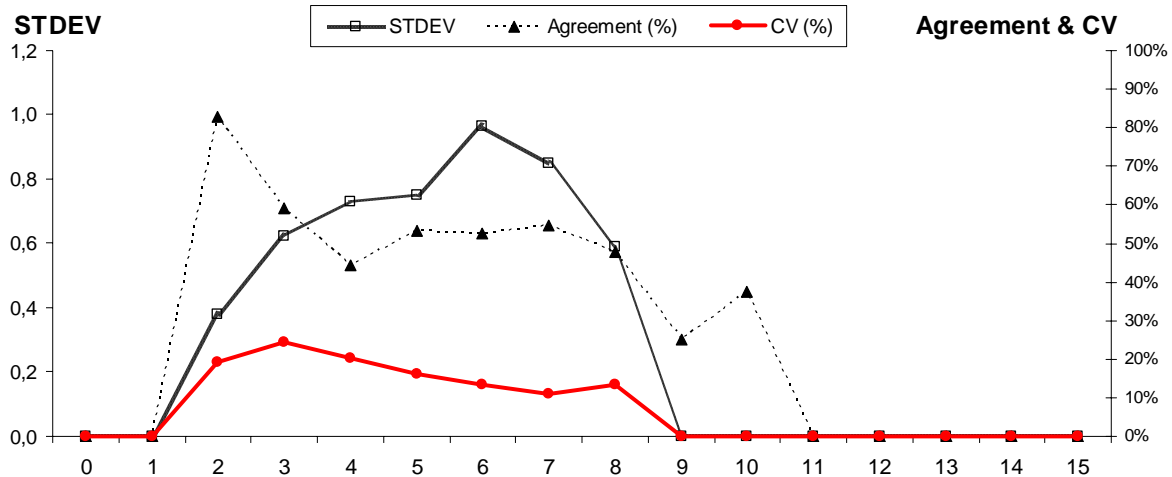


Figure 4i. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age (in some cases ABSOLUTE MEAN). CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

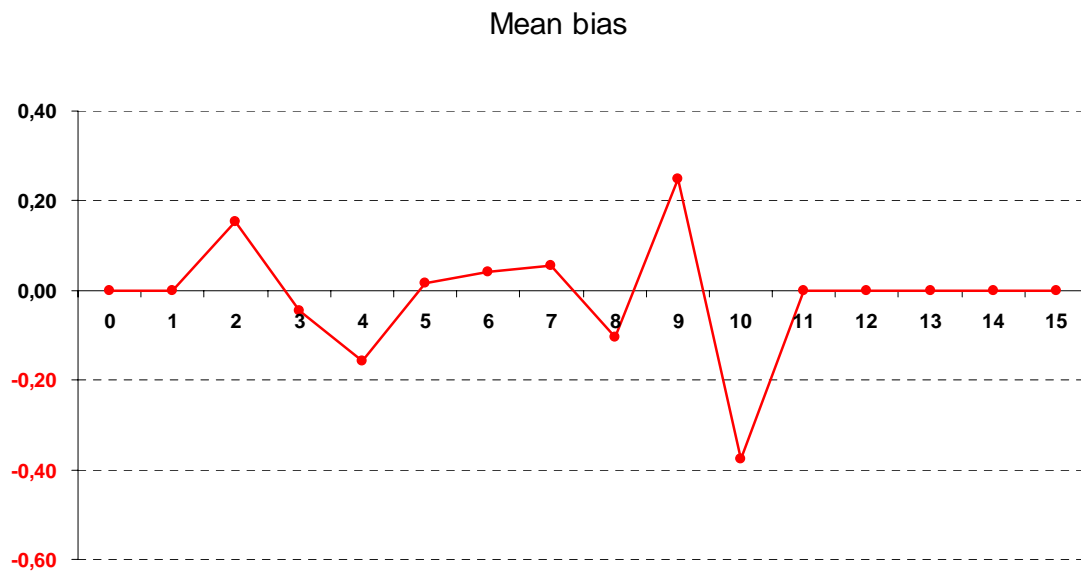


Figure 5i. The RELATIVE bias by MODAL(in some cases ABSOLUTE MEAN) age as estimated by all age readers combined.

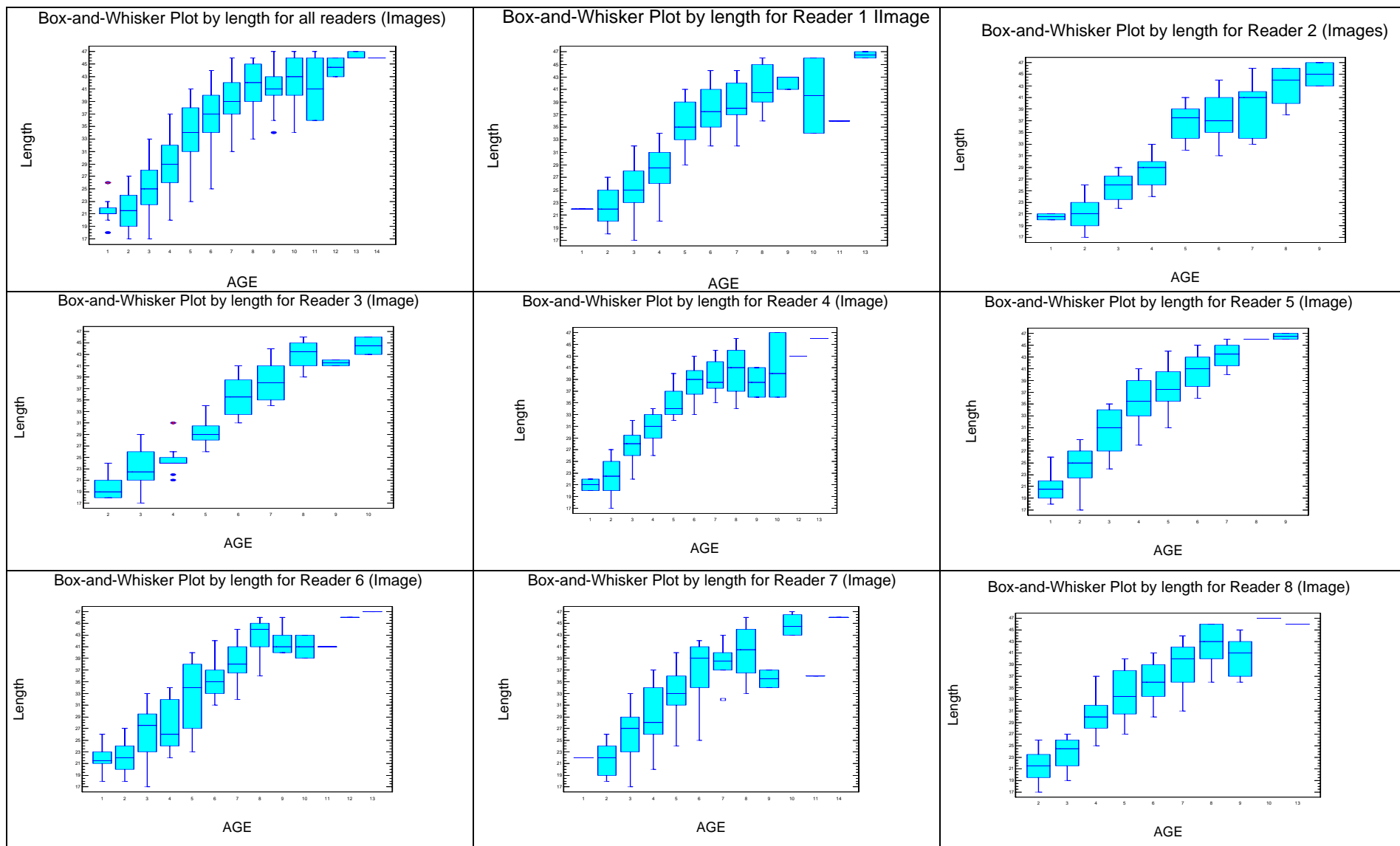


Figure 6i. Box-Whisker plots of the length distribution (cm) by age obtained for all readers reading the image otolith collection

Table 1. Readings of the Megrim Otolith exchange (Real collection)

Stratum	Sample year	no	Fish no	Fish length	Sex	Landing month	Mark Reader 1	Jorge Reader 2	Amaia Reader 3	Gordon Reader 4	Afra Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8	MODAL age	Percent agreement	Precision CV
Males	1999	Q1_17_1	1	17,0	1,0	3	3	2	3	2	3	3	2	2	3	50%	21%
Females	1998	Q1_18_2	2	18,0	2,0	3	2	2	2	2	2	2	2	2	2	100%	0%
Females	1998	Q1_19_2	3	19,0	2,0	3	2	2	2	2	2	3	2	3	2	75%	21%
Females	1998	Q1_20_2	4	20,0	2,0	3	2	2	2	2	2	2	2	2	2	100%	0%
Females	1998	Q1_21_2	5	21,0	2,0	3	3	2	3	3	2	2	3	3	3	63%	20%
Females	1998	Q1_22_2	6	22,0	2,0	3	4	3	3	3	3	3	4	3	3	88%	11%
Males	1999	Q1_24_1	7	24,0	1,0	3	4	4	5	4	4	4	6	3	4	63%	21%
Females	1999	Q1_25_1	8	25,0	2,0	2	4	4	4	4	3	3	5	3	4	50%	19%
Females	1999	Q1_26_2	9	26,0	2,0	2	4	4	5	4	3	4	4	3	4	63%	17%
Males	1998	Q1_27_1	10	27,0	1,0	3	4	4	4	4	3	3	5	3	4	50%	19%
Males	1998	Q1_32_1a/1b	11	32,0	1,0	2	6	6	7	6	5	5	6	4	6	50%	16%
Females	1999	Q1_34_1a/1b	12	34,0	2,0	3	6	5	6	6	5	4	8	4	6	38%	24%
Females	1998	Q1_37_2a/2b	13	37,0	2,0	3	6	6	6	6	6	6	6	4	6	88%	12%
Females	2001	Q1_38_2a/2b	14	38,0	2,0	3	7	8	7	8	7	6	8	5	7	38%	15%
Females	2001	Q1_39_2a/2b	15	39,0	2,0	3	6	6	6	6	6	5	6	6	6	88%	6%
Females	1998	Q1_40_2a/2b	16	40,0	2,0	3	5	5	6	6	5	4	5	5	5	63%	13%
Females	1999	Q1_41_2a/2b	17	41,0	2,0	3	6	6	6	6	5	5	6	6	6	75%	8%
Females	1998	Q1_42_2a/2b	18	42,0	2,0	3	7	7	8	7	7	6	7	7	7	75%	8%
Females	1999	Q1_43_2a/2b	19	43,0	2,0	3	9	8	8	8	8	9	9	9	8	50%	6%
Females	1998	Q1_44_2a/2b	20	44,0	2,0	3	7	7	9	8	6	6	8	7	7	38%	14%
Females	1998	Q1_45_2a/2b	21	45,0	2,0	3	8	8	8	8	7	7	8	9	8	63%	8%
Females	1999	Q1_46_2a/2b	22	46,0	2,0	3	13	7	13	13	11	12	12	13	13	50%	17%
Females	2002	M4_20_2	23	20,0	2,0	10	3	1	3	1	3	2	1	3	3	50%	47%
Females	2002	M4_21_2	24	21,0	2,0	10	2	1	2	1	3	1	2	2	2	50%	40%
Males	2002	M4_22_1	25	22,0	1,0	10	2	2	2	2	2	1	2	2	2	88%	19%
Females	2002	M4_23_2	26	23,0	2,0	10	2	3	3	2	3	1	2	2	3	38%	31%
Females	2002	M4_24_2	27	24,0	2,0	10	3	3	4	2	3	2	3	2	3	50%	26%
Females	2002	M4_26_2	28	26,0	2,0	10	2	2	3	2	2	1	1	2	2	63%	34%
Females	2002	M1_29_2a/2b	29	29,0	2,0	2	3	4	4	4	3	3	3	4	4	50%	15%
Females	2002	M1_31_2a/2b	30	31,0	2,0	2	6	6	4	4	6	6	6	4	6	63%	20%
Males	2002	M1_32_1a/1b	31	32,0	1,0	1	7	5	6	5	6	7	7	5	5	25%	15%
Males	1999	M1_33_1a/1b	32	33,0	1,0	3	7	7	7	6	6	6	7	6	7	50%	8%
Males	1999	M2_34_1a/1b	33	34,0	1,0	4	7	7	9	8	6	7	8	6	7	38%	14%
Males	1999	M2_35_1a/1b	34	35,0	1,0	4	7	6	7	8	6	7	6	6	7	38%	11%
Males	1999	M2_36_1a/1b	35	36,0	1,0	4	6	-	7	6	7	6	6	8	7	0%	12%
Males	1998	M2_37_1a/1b	36	37,0	1,0	5	8	-	8	8	6	9	9	6	8	43%	16%
Females	2002	M4_38_2a/2b	37	38,0	2,0	11	8	5	5	7	6	5	6	7	5	38%	18%
Females	2002	M4_39_2a/2a	38	39,0	2,0	11	7	5	4	7	5	8	7	6	6	0%	22%
Males	2002	M2_40_1a/1b	39	40,0	1,0	3	9	7	7	11	6	9	10	7	7	38%	21%
Females	1998	M2_41_2a/2b	40	41,0	2,0	5	8	7	8	8	6	8	8	7	8	63%	10%
Females	2002	M1_43_2a/2b	41	43,0	2,0	3	7	6	8	6	6	8	7	8	7	25%	13%
Females	2002	M1_44_2a/2b	42	44,0	2,0	3	6	6	9	8	8	7	7	8	6	25%	14%
Females	2002	M1_46_2a/2b	43	46,0	2,0	3	8	8	10	8	8	7	9	8	8	63%	11%
Females	1998	Q3_18_2	44	18,0	2,0	8	2	2	2	1	2	1	2	2	2	75%	26%
Males	1998	Q3_19_1	45	19,0	1,0	8	2	2	3	2	2	1	1	2	2	63%	34%
Males	1998	Q3_20_1	46	20,0	1,0	8	2	2	4	2	2	2	2	2	2	88%	31%
Females	1999	Q3_21_2	47	21,0	2,0	8	2	2	4	2	3	1	2	2	2	63%	39%
Females	1998	Q3_22_2	48	22,0	2,0	8	2	2	4	2	2	2	2	2	2	88%	31%
Males	1998	Q3_24_1	49	24,0	1,0	8	2	2	4	2	2	1	1	2	2	63%	46%
Males	1998	Q3_25_1	50	25,0	1,0	8	2	2	4	2	3	2	1	3	2	50%	39%
Males	1998	Q3_26_1	51	26,0	1,0	8	2	3	5	2	3	2	3	3	3	0%	34%
Males	1998	Q3_27_1	52	27,0	1,0	8	2	3	6	3	3	2	3	4	3	0%	39%
Males	1998	Q3_28_1a/1b	53	28,0	1,0	8	3	3	6	3	3	3	4	5	3	63%	31%
Males	1998	Q3_29_1a/1b	54	29,0	1,0	8	3	3	5	3	3	3	4	5	3	63%	25%
Males	1998	Q3_30_1a/1b	55	30,0	1,0	8	4	4	6	3	4	4	3	4	4	50%	27%
Females	1998	Q3_31_2a/2b	56	31,0	2,0	8	5	4	7	3	4	3	5	4	4	0%	30%
Males	1998	Q3_33_1a/1b	57	33,0	1,0	8	6	7	6	5	5	6	7	5	6	38%	14%
Females	1999	Q3_34_2a/2b	58	34,0	2,0	9	5	5	8	5	4	4	6	5	5	50%	24%
Females	1999	Q3_35_2a/2b	59	35,0	2,0	9	6	6	8	6	5	5	7	6	6	50%	16%
Females	1999	Q3_36_2a/2b	60	36,0	2,0	9	9	-	8	9	6	9	10	9	9	0%	15%
Males	1999	Q3_37_1a/1b	61	37,0	1,0	9	7	-	10	8	6	7	9	9	8	14%	18%
Females	1998	Q3_38_2a/2b	62	38,0	2,0	9	5	5	11	5	5	5	5	5	5	88%	37%
Females	1998	Q3_39_2a/2b	63	39,0	2,0	9	6	5	8	6	6	5	5	5	5	0%	18%
Females	1998	Q3_40_2a/2b	64	40,0	2,0	5	6	6	7	8	6	6	6	8	6	50%	14%
Females	1999	Q3_41_2a/2b	65	41,0	2,0	9	8	7	8	8	6	8	9	8	8	63%	11%
Females	1998	Q3_42_2a/2b	66	42,0	2,0	7	6	6	7	7	6	6	7	7	6	50%	8%
Females	1998	Q3_43_2a/2b	67	43,0	2,0	7	9	9	10	11	7	11	1	7	9	25%	40%
Females	1999	Q3_46_2a/2b	68	46,0	2,0	9	10	8	10	12	9	11	14	8	10	25%	20%
Females	2002	Q4_22_2	69	22,0	2,0	10	1	2	3	1	2	1	1	2	2	0%	46%
Males	2002	Q4_23_1	70	23,0	1,0	10	2	3	3	2	3	4	3	3	3	63%	22%
Males	2002	Q4_24_1	71	24,0	1,0	10	2	2	2	1	3	3	2	2	2	63%	30%
Males	2002	Q4_25_1	72	25,0	1,0	10	3	3	3	3	3	3	4	4	3	75%	14%
Males	1999	Q4_26_1a/1b	73	26,0	1,0	10	3	3	4	2	4	3	3	3	3	63%	21%
Males	1999	Q4_27_1a/1b	74	27,0	1,0	10	3	3	5	2	4	5	4	5	3	25%	29%
Females	1999	Q4_28_2a/2b	75	28,0	2,0	10	4	3	5	3	5	5	4	5	4	0%	21%
Males	1999	Q4_29_1a/1b	76	29,0	1,0	10	4	4	7	3	3	5	7	4	4	38%	35%
Males	1999	Q4_31_1a/1b	77	31,0	1,0	10	5	6	6	4	4	5	9	7	6	25%	29%
Females	1999	Q4_32_2a/2b	78	32,0	2,0	10	3	5	6	4	4	4	5	5	3	0%	21%
Males	1999	Q4_33_1a/1b	79	33,0	1,0	10	4	4	6	4	4	3	6	6	4	50%	26%
Females	1999	Q4_34_2a/2b	80	34,0	2,0	10	5	5	6	5	4	5	6	5	5	63%	13%
Females	1999	Q4_35_2a/2b	81	35,0	2,0	10	6	6	6	6	4	4	7	7	6	50%	20%
Females	1998	Q4_36_2a/2b	82	36,0	2,0	10	6	6	7	6	5	5	6	7	6	50%	13%
Females	1998	Q4_37_2a/2b	83	37,0	2,0	10	5	5	7	7	6	7	7	7	5	25%	14%
Females	1998	Q4_39_2a/2b	84	39,0	2,0	12	6	6	7	6	6	6	7	6	6	75%	7%
Females	1999	Q4_40_2a/2b	85	40,0	2,0	10	8	8	8	10	7	7	10	6	8	38%	18%
Females	1998	Q4_41_2a/2b	86	41,0	2,0	10	7	5	8	8	7	7	7	9	7	0%	16%
Females	1999	Q4_47_2a/2b	87	47,0	2,0	10	11	9	11	10	11	10	12	10	11	38%	9%
max		46,0					86	80	86	86	86	86	86	86			
min		17,0					0	6	0	0	0	0	0	0		47,8%	20,6%

Table 2. The coefficient of variation (CV), the percent agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined for the REAL OTOLITH collection. 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.

COEFFICIENT OF VARIATION (CV)									
MODAL age	Mark Reader 1	Jorge Reader 2	Amaia Reader 3	Gordon Reader 4	Afra Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8	ALL Readers
0	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-
2	0%	14%	33%	24%	21%	45%	27%	17%	28,0%
3	14%	24%	28%	28%	17%	36%	32%	35%	24,9%
4	9%	0%	22%	12%	15%	22%	26%	33%	22,3%
5	22%	0%	30%	17%	18%	22%	13%	18%	19,8%
6	6%	7%	17%	19%	20%	16%	13%	24%	14,9%
7	10%	9%	11%	20%	7%	16%	16%	14%	13,2%
8	7%	7%	11%	9%	13%	11%	7%	17%	12,3%
9	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-
Weighted mean	0-15	6,9%	8,7%	18,5%	16,1%	13,9%	21,4%	16,6%	19,0%
RANKING		1	2	6	4	3	8	5	7
									20,6%

PERCENTAGE AGREEMENT									
MODAL age	Mark Reader 1	Jorge Reader 2	Amaia Reader 3	Gordon Reader 4	Afra Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8	ALL
0	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-
2	100%	93%	50%	79%	71%	36%	71%	86%	73%
3	83%	75%	58%	42%	75%	50%	33%	42%	57%
4	88%	100%	38%	75%	38%	25%	25%	25%	52%
5	83%	100%	17%	50%	33%	50%	33%	67%	54%
6	88%	81%	50%	56%	38%	38%	44%	25%	52%
7	88%	63%	50%	13%	25%	25%	38%	38%	42%
8	75%	67%	75%	88%	25%	25%	25%	25%	50%
9	100%	100%	0%	0%	0%	0%	0%	0%	25%
10	100%	0%	100%	0%	0%	0%	0%	0%	25%
11	100%	0%	100%	0%	100%	0%	0%	0%	38%
12	-	-	-	-	-	-	-	-	-
13	100%	0%	100%	100%	0%	0%	0%	100%	50%
14	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-
Weighted mean	0-15	77,0%	71,1%	44,8%	49,4%	40,2%	29,9%	34,5%	37,9%
RANKING		1	2	4	3	5	8	7	6
									48,0%

RELATIVE BIAS									
MODAL age	Mark Reader 1	Jorge Reader 2	Amaia Reader 3	Gordon Reader 4	Afra Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8	ALL
0	-	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-	-
2	0,00	-0,07	0,86	-0,21	0,29	-0,36	-0,29	0,14	0,04
3	-0,17	-0,33	0,75	-0,67	0,08	-0,17	0,08	0,33	-0,01
4	-0,13	0,00	1,13	-0,25	-0,63	-0,50	1,00	0,00	0,08
5	0,50	0,00	2,17	0,83	0,00	0,00	0,83	0,67	0,63
6	0,00	-0,06	0,50	-0,06	-0,38	-0,50	0,75	-0,13	0,02
7	0,25	-0,13	0,75	0,75	-0,75	-0,13	0,63	-0,50	0,11
8	0,00	-0,33	0,50	0,25	-1,25	-0,25	0,88	-0,25	-0,05
9	0,00	0,00	1,00	2,00	-2,00	2,00	-8,00	-2,00	-0,88
10	0,00	-2,00	0,00	2,00	-1,00	1,00	4,00	-2,00	0,25
11	0,00	-2,00	0,00	-1,00	0,00	-1,00	1,00	-1,00	-0,50
12	-	-	-	-	-	-	-	-	-
13	0,00	-6,00	0,00	0,00	-2,00	-1,00	-1,00	0,00	-1,25
14	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-
Weighted mean	0-15	0,02	-0,23	0,71	0,02	-0,31	-0,24	0,34	-0,03
RANKING		1	4	8	1	6	5	7	3

Overall ranking								
	Mark Reader 1	Jorge Reader 2	Amaia Reader 3	Gordon Reader 4	Afra Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8
Ranking Coefficient of Variation	1	2	6	4	3	8	5	7
Ranking Percentage Agreement	1	2	4	3	5	8	7	6
Ranking Relative bias	1	4	8	1	6	5	7	3
OVERALL RANKING	1	2	6	2	4	8	7	5

Table 3.- Agreement (%), CV and APE values of otoliths reading from both collections and readers.

	Real Otolith Collection	Image Otolith Collection
N	87	87
% Agreement	48.0	45.4
CV	20.6	18.5
APE	15.5	13.8
Readers	ALL READERS	

Table 4. Bias tests: non-parametrically with a one-sample Wilcoxon rank sum test. The inter-reader bias test and the reader against MODAL age bias test.

Inter-reader bias test and reader against MODAL age bias test								
	Mark Reader 1	Jorge Reader 2	Amaia Reader 3	Gordon Reader 4	Afra Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8
Reader 1		*	**	—	**	*	**	—
Reader 2	*		**	—	—	—	**	—
Reader 3	**	**		**	**	**	*	**
Reader 4	—	—	**		*	—	**	—
Reader 5	**	—	**	*		—	**	**
Reader 6	*	**	**	—	—		**	*
Reader 7	**	—	*	**	**	**		*
Reader 8	—	—	**	—	**	*	*	
#####								
#####								
#####								
#####								
#####								
#####								
#####								
#####								
MODAL age	—	*	**	—	**	*	**	—

—	= no sign of bias ( $p > 0.05$ )
*	= possibility of bias ( $0.01 < p < 0.05$ )
**	= certainty of bias ( $p < 0.01$ )



Table 1i. Readings of the Megrim Otolith exchange (Image collection)

Stratum	year	Sample	Fish	Fish	Landing	Mark	Jorge	Amaia	Gordon	Afra	Ayesha	Sally	Antonio	MODAL	Percent	Precision	
		no	no	length	Sex												month
Males	1999	Q1_17_1	1	17,0	1,0	3	2	3	2	4	3	3	2	3	3	63%	20%
Females	1998	Q1_18_2	2	18,0	2,0	3	2	2	2	2	2	2	2	2	2	100%	0%
Females	1998	Q1_19_2	3	19,0	2,0	3	2	2	2	2	2	3	2	3	2	75%	21%
Females	1998	Q1_20_2	4	20,0	2,0	3	2	2	2	2	2	2	2	2	2	100%	0%
Females	1998	Q1_21_2	5	21,0	2,0	3	2	2	2	2	2	3	2	3	2	75%	21%
Females	1998	Q1_22_2	6	22,0	2,0	3	3	3	3	3	3	4	3	3	3	88%	11%
Males	1999	Q1_24_1	7	24,0	1,0	3	3	4	4	3	4	4	5	3	4	50%	19%
Females	1999	Q1_25_1	8	25,0	2,0	2	3	4	4	3	3	5	6	3	4	25%	29%
Females	1999	Q1_26_2	9	26,0	2,0	2	4	4	5	4	3	4	4	3	4	63%	17%
Males	1998	Q1_27_1	10	27,0	1,0	3	4	4	5	4	3	3	3	3	3	29%	15%
Males	1998	Q1_32_1a/1b	11	32,0	1,0	2	6	6	6	5	5	6	5	4	6	50%	14%
Females	1999	Q1_34_1a/1b	12	34,0	2,0	3	4	5	6	4	5	6	6	4	5	25%	19%
Females	1998	Q1_37_2a/2b	13	37,0	2,0	3	6	6	7	6	6	8	4	6	6	63%	18%
Females	2001	Q1_38_2a/2b	14	38,0	2,0	3	7	8	7	7	7	7	5	7	7	75%	12%
Females	2001	Q1_39_2a/2b	15	39,0	2,0	3	6	6	-	6	6	6	6	6	6	100%	0%
Females	1998	Q1_40_2a/2b	16	40,0	2,0	3	5	5	6	5	5	5	5	5	5	88%	7%
Females	1999	Q1_41_2a/2b	17	41,0	2,0	3	6	6	6	6	5	6	6	6	6	88%	6%
Females	1998	Q1_42_2a/2b	18	42,0	2,0	3	7	7	7	7	7	7	8	7	7	88%	5%
Females	1999	Q1_43_2a/2b	19	43,0	2,0	3	9	8	8	8	8	9	10	9	8	50%	9%
Females	1998	Q1_44_2a/2b	20	44,0	2,0	3	7	7	8	7	6	7	8	7	7	63%	9%
Females	1998	Q1_45_2a/2b	21	45,0	2,0	3	8	8	8	8	7	8	8	9	8	75%	7%
Females	1999	Q1_46_2a/2b	22	46,0	2,0	3	13	7	-	13	11	12	14	13	12	0%	20%
Females	2002	M4_20_2	23	20,0	2,0	10	4	1	3	1	3	2	4	3	3	0%	45%
Females	2002	M4_21_2	24	21,0	2,0	10	4	1	3	1	3	1	3	2	3	0%	52%
Males	2002	M4_22_1	25	22,0	1,0	10	3	2	3	2	2	2	2	2	3	25%	21%
Females	2002	M4_23_2	26	23,0	2,0	10	3	3	3	2	3	1	3	3	3	63%	30%
Females	2002	M4_24_2	27	24,0	2,0	10	3	3	4	2	3	2	3	2	3	50%	26%
Females	2002	M4_26_2	28	26,0	2,0	10	2	2	3	2	2	1	2	2	2	75%	27%
Females	2002	M1_29_2a/2b	29	29,0	2,0	2	5	4	3	4	3	3	4	4	4	63%	14%
Females	2002	M1_31_2a/2b	30	31,0	2,0	2	5	6	4	4	6	6	5	4	5	25%	19%
Males	2002	M1_32_1a/1b	31	32,0	1,0	1	7	5	6	5	6	7	7	5	6	25%	15%
Males	1999	M1_33_1a/1b	32	33,0	1,0	3	7	7	8	6	7	7	8	6	7	29%	12%
Males	1999	M2_34_1a/1b	33	34,0	1,0	4	10	7	7	8	6	7	9	6	7	38%	19%
Males	1999	M2_35_1a/1b	34	35,0	1,0	4	5	6	7	8	6	6	8	6	7	0%	16%
Males	1999	M2_36_1a/1b	35	36,0	1,0	4	11	-	6	10	7	7	8	8	8	0%	22%
Males	1998	M2_37_1a/1b	36	37,0	1,0	5	7	-	6	5	6	6	7	6	6	0%	11%
Females	2002	M4_38_2a/2b	37	38,0	2,0	11	6	5	6	7	6	7	8	7	7	38%	14%
Females	2002	M4_39_2a/2a	38	39,0	2,0	11	-	5	6	6	5	5	-	6	5	0%	10%
Males	2002	M2_40_1a/1b	39	40,0	1,0	3	-	7	6	6	6	7	-	7	7	0%	8%
Females	1998	M2_41_2a/2b	40	41,0	2,0	5	8	7	7	8	6	8	7	7	7	38%	10%
Females	2002	M1_43_2a/2b	41	43,0	2,0	3	7	6	7	6	6	7	7	8	7	50%	10%
Females	2002	M1_44_2a/2b	42	44,0	2,0	3	6	6	7	8	8	8	8	8	6	25%	12%
Females	2002	M1_46_2a/2b	43	46,0	2,0	3	8	8	8	8	8	8	8	8	8	100%	0%
Females	1998	Q3_18_2	44	18,0	2,0	8	2	2	2	2	2	1	2	2	2	88%	19%
Males	1998	Q3_19_1	45	19,0	1,0	8	2	2	2	2	2	2	2	2	2	100%	0%
Males	1998	Q3_20_1	46	20,0	1,0	8	2	2	3	2	2	2	2	2	2	88%	17%
Females	1999	Q3_21_2	47	21,0	2,0	8	2	2	4	2	3	1	3	2	2	50%	39%
Females	1998	Q3_22_2	48	22,0	2,0	8	2	2	4	2	2	2	2	2	2	88%	31%
Males	1998	Q3_24_1	49	24,0	1,0	8	2	2	4	2	2	2	2	2	2	88%	31%
Males	1998	Q3_25_1	50	25,0	1,0	8	2	2	4	2	3	2	2	3	2	63%	30%
Males	1998	Q3_26_1	51	26,0	1,0	8	2	3	4	2	3	2	3	3	3	0%	26%
Males	1998	Q3_27_1	52	27,0	1,0	8	2	3	-	3	3	2	3	4	3	0%	24%
Males	1998	Q3_28_1a/1b	53	28,0	1,0	8	3	3	5	3	3	3	3	5	3	75%	26%
Males	1998	Q3_29_1a/1b	54	29,0	1,0	8	3	3	5	3	3	3	3	5	3	75%	26%
Males	1998	Q3_30_1a/1b	55	30,0	1,0	8	3	4	5	3	4	3	3	6	4	0%	29%
Females	1998	Q3_31_2a/2b	56	31,0	2,0	8	4	4	5	3	4	3	5	4	4	50%	19%
Males	1998	Q3_33_1a/1b	57	33,0	1,0	8	5	7	6	5	5	6	6	5	5	0%	13%
Females	1999	Q3_34_2a/2b	58	34,0	2,0	9	5	5	7	5	4	4	4	5	5	50%	20%
Females	1999	Q3_35_2a/2b	59	35,0	2,0	9	6	6	7	5	5	5	5	6	6	38%	13%
Females	1999	Q3_36_2a/2b	60	36,0	2,0	9	8	-	-	9	6	8	11	9	9	0%	19%
Males	1999	Q3_37_1a/1b	61	37,0	1,0	9	7	-	-	8	6	7	9	9	8	0%	16%
Females	1999	Q3_38_2a/2b	62	38,0	2,0	9	5	5	7	5	5	5	5	5	5	88%	13%
Females	1998	Q3_39_2a/2b	63	39,0	2,0	9	8	5	8	7	6	10	7	5	8	25%	24%
Females	1998	Q3_40_2a/2b	64	40,0	2,0	5	7	6	-	8	8	9	8	8	8	0%	12%
Females	1999	Q3_41_2a/2b	65	41,0	2,0	9	5	7	9	8	6	9	6	8	7	0%	21%
Females	1998	Q3_42_2a/2b	66	42,0	2,0	7	6	6	9	7	6	6	7	6	6	63%	16%
Females	1998	Q3_43_2a/2b	67	43,0	2,0	7	9	9	10	12	7	10	10	7	9	25%	18%
Females	1999	Q3_46_2a/2b	68	46,0	2,0	9	10	8	10	13	9	9	10	8	10	38%	17%
Females	2002	Q4_22_2	69	22,0	2,0	10	1	2	3	1	2	1	1	2	2	0%	46%
Males	2002	Q4_23_1	70	23,0	1,0	10	2	3	3	2	3	5	2	3	3	50%	34%
Males	2002	Q4_24_1	71	24,0	1,0	10	2	2	2	2	3	2	2	2	2	88%	17%
Males	2002	Q4_25_1	72	25,0	1,0	10	3	3	3	2	3	3	3	4	3	75%	18%
Males	1999	Q4_26_1a/1b	73	26,0	1,0	10	2	3	3	2	4	3	2	3	3	50%	26%
Males	1999	Q4_27_1a/1b	74	27,0	1,0	10	3	3	3	2	4	5	4	5	3	38%	29%
Females	1999	Q4_28_2a/2b	75	28,0	2,0	10	4	3	5	3	5	5	3	4	4	25%	23%
Males	1999	Q4_29_1a/1b	76	29,0	1,0	10	5	4	5	3	5	3	4	4	5	38%	23%
Males	1999	Q4_31_1a/1b	77	31,0	1,0	10	4	6	6	4	4	6	5	7	6	38%	22%
Females	1999	Q4_32_2a/2b	78	32,0	2,0	10	3	5	6	3	4	4	3	5	5	0%	27%
Males	1999	Q4_33_1a/1b	79	33,0	1,0	10	4	4	6	4	4	3	3	6	4	50%	27%
Females	1999	Q4_34_2a/2b	80	34,0	2,0	10	5	5	5	5	4	5	5	5	5	88%	

Table 2i. The coefficient of variation (CV), the percent agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined for the IMAGE OTOLITH collection. 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.

COEFFICIENT OF VARIATION (CV)										
MODAL	Mark	Jorge	Amaia	Gordon	Afra	Ayesha	Sally	Antonio	ALL	
age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Readers	
0	-	-	-	-	-	-	-	-	-	
1	-	-	-	-	-	-	-	-	-	
2	0%	0%	33%	0%	20%	33%	13%	20%	19,4%	
3	14%	14%	24%	21%	17%	40%	21%	39%	24,3%	
4	12%	9%	21%	15%	21%	24%	27%	28%	20,4%	
5	7%	11%	19%	25%	22%	17%	20%	20%	16,0%	
6	11%	7%	14%	19%	20%	14%	19%	21%	13,3%	
7	15%	8%	6%	12%	8%	8%	9%	15%	11,1%	
8	6%	22%	5%	12%	11%	13%	18%	23%	13,3%	
9	-	-	-	-	-	-	-	-	-	
10	-	-	-	-	-	-	-	-	-	
11	-	-	-	-	-	-	-	-	-	
12	-	-	-	-	-	-	-	-	-	
13	-	-	-	-	-	-	-	-	-	
14	-	-	-	-	-	-	-	-	-	
15	-	-	-	-	-	-	-	-	-	
Weighted mean	0-15	7,0%	7,1%	15,7%	10,7%	13,1%	17,1%	14,0%	17,9%	18,5%
	RANKING	1	2	6	3	4	7	5	8	

PERCENTAGE AGREEMENT										
MODAL	Mark	Jorge	Amaia	Gordon	Afra	Ayesha	Sally	Antonio		
age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	ALL	
0	-	-	-	-	-	-	-	-	-	
1	-	-	-	-	-	-	-	-	-	
2	100%	100%	54%	100%	77%	62%	92%	77%	83%	
3	82%	82%	73%	27%	73%	45%	64%	27%	59%	
4	75%	88%	29%	38%	38%	25%	25%	38%	44%	
5	88%	75%	25%	50%	38%	50%	50%	50%	53%	
6	83%	83%	64%	33%	42%	58%	33%	25%	53%	
7	71%	71%	83%	43%	29%	71%	29%	43%	55%	
8	67%	67%	83%	50%	33%	33%	33%	17%	48%	
9	100%	100%	0%	0%	0%	0%	0%	0%	25%	
10	100%	0%	100%	0%	0%	0%	100%	0%	38%	
11	-	-	-	-	-	-	-	-	-	
12	-	-	-	-	-	-	-	-	-	
13	-	-	-	-	-	-	-	-	-	
14	-	-	-	-	-	-	-	-	-	
15	-	-	-	-	-	-	-	-	-	
Weighted mean	0-15	65,9%	66,3%	47,4%	37,9%	37,9%	37,9%	40,0%	31,0%	45,4%
	RANKING	2	1	3	5	5	5	4	8	

RELATIVE BIAS										
MODAL	Mark	Jorge	Amaia	Gordon	Afra	Ayesha	Sally	Antonio		
age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	ALL	
0	-	-	-	-	-	-	-	-	-	
1	-	-	-	-	-	-	-	-	-	
2	0,00	0,00	0,77	0,00	0,23	-0,08	0,08	0,23	0,15	
3	-0,18	-0,18	0,45	-0,73	0,09	0,09	-0,18	0,27	-0,05	
4	-0,25	-0,13	0,57	-0,63	-0,38	-0,25	0,13	-0,25	-0,16	
5	-0,13	0,00	0,88	-0,25	-0,25	0,38	-0,38	-0,13	0,02	
6	-0,08	-0,17	0,55	0,00	-0,50	0,17	0,25	0,17	0,04	
7	0,57	0,00	0,17	0,00	-0,71	0,00	0,86	-0,43	0,05	
8	0,33	-1,00	-0,17	0,33	-0,83	1,17	-0,33	-0,33	-0,10	
9	0,00	0,00	1,00	3,00	-2,00	1,00	1,00	-2,00	0,25	
10	0,00	-2,00	0,00	3,00	-1,00	-1,00	0,00	-2,00	-0,38	
11	-	-	-	-	-	-	-	-	-	
12	-	-	-	-	-	-	-	-	-	
13	-	-	-	-	-	-	-	-	-	
14	-	-	-	-	-	-	-	-	-	
15	-	-	-	-	-	-	-	-	-	
Weighted mean	0-15	0,00	-0,16	0,42	-0,08	-0,23	0,11	0,06	-0,05	0,01
	RANKING	1	6	8	4	7	5	3	2	

Overall ranking								
	Mark Reader 1	Jorge Reader 2	Amaia Reader 3	Gordon Reader 4	Afra Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8
Ranking Coefficient of Variation	1	2	6	3	4	7	5	8
Ranking Percentage Agreement	2	1	3	5	5	5	4	8
Ranking Relative bias	1	6	8	4	7	5	3	2
OVERALL RANKING	1	2	6	3	5	6	3	8

Table 4i. Bias tests: non-parametrically with a one-sample Wilcoxon rank sum test. The inter-reader bias test and the reader against MODAL age bias test.

<b>Inter-reader bias test and reader against MODAL age bias test</b>								
	Mark Reader 1	Jorge Reader 2	Amaia Reader 3	Gordon Reader 4	Afra Reader 5	Ayesha Reader 6	Sally Reader 7	Antonio Reader 8
Reader 1	—	—	**	—	*	—	—	—
Reader 2	—	—	**	—	—	*	*	—
Reader 3	**	**	—	**	**	**	**	**
Reader 4	—	—	**	—	—	—	—	—
Reader 5	*	—	**	—	—	**	**	**
Reader 6	—	*	**	—	**	—	—	—
Reader 7	—	*	**	—	**	—	—	—
Reader 8	—	—	**	—	**	—	—	—
#####								
#####								
#####								
#####								
#####								
#####								
#####								
#####								
MODAL age	**	**	**	**	*	**	**	**

—	= no sign of bias ( $p > 0.05$ )
*	= possibility of bias ( $0.01 < p < 0.05$ )
* *	= certainty of bias ( $p < 0.01$ )

# REPORT OF WORKSHOP ON MEGRIM OTOLITH AND FIN RAYS AGE READING

## 1. INTRODUCTION

Megrim is an important target species of groundfish fisheries in the North East Atlantic, which is annually evaluated at the ICES Working Group on the Assessment of Southern Shelf Demersal Stocks. England, France and Spain presently provide the ICES Working Group (Anon. 1997) with age length keys for Megrim from ICES Sub-areas VII and VIII.

One of the objectives of the EC Study Project Contract No. 95/038 "*Biological Studies of Demersal Fish*", was to conduct an age reading exchange for Megrim from ICES Sub-areas VII and VIII. The purpose of the otolith and fin ray exchange was to determine the ageing agreement between readers and identify any differences in the interpretation of the ages.

The age determination exchange highlighted differences in the interpretation of the age, it was therefore considered necessary to convene an age determination workshop to evaluate the results and resolve the differences. The following points were addressed :-

- Several of the otolith readers involved in the age determination of commercial samples, used in the ICES assessment Working Groups, have changed over the last five years.
- A previous otolith exchange, conducted in 1990, demonstrated a very poor level of agreement between all readers (Dawson, 1991).
- At an age determination Workshop, conducted in 1991, between French and Spanish readers quite a good agreement was obtained. However only France and Spain were represented and not all the different countries involved in the exploitation of Megrim.
- The recommendations of the Workshop on "*Sampling strategies for age and maturity*" (CM, 1994) considered it necessary to convene an age determination workshop when the readers change, or when new or inexperienced staff become involved with age determination in order to develop appropriate ageing criteria for a species to maintain adequate levels of precision between readers.

The results and discussions are presented in this report.

## 2. OBJECTIVES OF THE WORKSHOP

- To analyse and discuss the results of the age determination exchange in order to identify the age interpretation problem.
- To agree ageing criteria based on the discussions and interpretations between readers.
- To establish a protocol for Megrim age determination.

## 3. PARTICIPANTS

The workshop was held in Vigo (Spain), from 19 to 23 May 1997 with the following participants :

Bellail R., IFREMER, Lorient, France

Dawson W., CEFAS , Lowestoft, UK

Etherton M., CEFAS, Lowestoft, UK  
 Fontenla J., IEO, Vigo, Spain  
 Landa J., IEO, Santander, Spain  
 Loureiro I., IEO, Vigo, Spain  
 Mahe J.C., IFREMER, Lorient, France  
 Martinez J., AZTI, Txatxarramendi, Spain  
 (3 days)  
 Mc Cormick H., MIFRC, Ireland  
 Meixide M., IEO, Vigo, Spain

Pereda P., IEO, Santander, Spain (2 days)  
 Perez N., IEO, Vigo, Spain  
 Piñeiro, C., IEO, Vigo, Spain  
 (Chairperson)  
 Sainza M., IEO, Vigo, Spain (2 days)  
 Santurtún M., AZTI, Txatxarramendi,  
 Spain  
 Trujillo V., IEO, Vigo, Spain  
 Woods F., MIFRC, Ireland

#### 4. REVIEW OF MEGRIM BIOLOGY

##### 4.1 Distribution

Megrim (*Lepidorhombus whiffiagonis*) is a benthic species which is distributed along the coastal area of the Northern Atlantic. It is found from Faroes as far as Mauritania, but is mainly located in the West of the British Islands and the Bay of Biscay (64° N - 26° N) Figure 1. The species also occurs, although less frequently, in the Mediterranean Sea. (Charuau et al. 1992).

The bathymetric distribution of this species is from 50m to 800m of depth but is mainly found between 100 and 400m. of depth. In the Celtic Sea and the Bay of Biscay, Megrim is found between 50m to 500m, with the highest abundance at around 250m. (Poulard J.C. et al , 1993).

Three stocks are recognised for assessment purposes in the North Atlantic :-

- Two Northern stocks in the ICES Sub-areas VI and VII +Div. VIIIab
- One Southern stock in ICES Divisions VIIIc and IXa.

##### 4.2 Reproduction

Spawning mainly takes place in deep water along the continental shelf edge from February until April, with a peak in March/April (Table 1). Spawning starts in the South and gradually extends Northwards as the water temperature increases. After a few days the egg hatch (Warnes and Nichols, 1997) and the larvae are planktonic until they metamorphose. Little is known about juvenile Megrim after metamorphoses. It is thought that they settle on the sea bed along the shelf edge (Figure 2).

The Megrim's mean length at maturity stage tends to increase with the advancement of maturity and is also higher for the fish in the more Northern areas. As shown in Table 2 the maturity takes place at around age 3 for females and age 2 for males (Dawson, 1989).

##### 4.3 Migration

There is a migration of the adult fish from the more shallow water in the Western approaches to the deeper water along the continental shelf edge in December and January. Spawning commences in February and probably continues until May. After spawning there is a return migration back to the shallow areas where they remain until the next spawning season. Most fish do not migrate far; however, as the females get larger they tend to migrate into the more

shallow waters along the Celtic Sea and Bay of Biscay plateau. The larger older males follow a similar pattern, although their distribution does not extend so far to the East (Dawson; Mahe, pers comm).

#### 4.4 Growth

Megrim growth has been studied using different bony parts : otoliths and fin-rays. Age reading becomes more difficult as the fish become older, especially when 8 years plus. This increases the uncertainty of age determination for these fishes. The sectioned and stained otoliths technique described later in this report (Section 7) could help to solve this problem.

Growth parameters have been estimated by several authors since 1981 for different ICES areas. The growth pattern varies depending on the area the sample was taken. These variations are summarised in Table 3.

##### 4.4.1 Formation of the Opaque and Hyaline Zone

Deposition of the formation of the opaque and hyaline zones has been observed for each month of the year by several authors (Rodriguez and Iglesias, 1985; Dawson, 1991 ; Landa and Piñeiro, in prep.), therefore one opaque zone and one hyaline zone constitutes one year of growth (*annulus*). The opaque zone can be observed forming from March through to November and the hyaline zone from November through to February for most fish (Figure 3). No description of a direct method of validation for Megrim age determination is available in the literature. However, an indirect validation technique, for Megrim from ICES Divisions VIIIc, Ixa, has been described. This technique indicates that the interpretation criteria applied for Spanish age reading used in the assessment from this area is correct. The weak 1993 year class and the strong year class of 1991 can be followed in successive years up until the year class of 1996. (ICES C.M. 1998/Assess :4)

## 5. MATERIAL AND METHODS

A sample of 90 pairs of otoliths and fin rays from ICES Subareas VII and VIII caught from January to December, 1995 covering a length range from 18 to 55 cm were examined. The collection was provided by two laboratories IEO (Vigo) and IFREMER (Lorient). During the present workshop a second reading of the same exchange collection was conducted. Similarly, an independent second reading sample of 59 otoliths and fin rays from the same Subareas and the same length range was taken.

Only the collection of otoliths and fin rays and the corresponding dates of capture, as well as the sex, were sent to the readers of each country during the exchange. Fish length information was not given as this may have had some influence on the age determination.

In order to avoid any mix up of otoliths during the exchange period, the otoliths of the sample collection were not stored in envelopes as is the normal practice. Both whole otoliths, from the sample, were mounted on black slides and covered with Eukitt resin to view under a microscope by reflected light.

With the exception of one reader, most readers had some experience in age determination of Megrim. Not all readers that participated in the exchange provide age readings to the ICES

Working Group on the Assessment of Southern Shelf Demersal Stocks. Presently only three of the readers (R2, R3, R5) provide age data, therefore they are considered together in the analysis due to the importance of their interpretation for assessment purposes.

The observation of the otoliths was done mainly with a magnification of X15, using a stereo microscope under reflected light. Fin rays were read under a microscope with a magnification of 100X. For the discussion of the otolith interpretation criteria, a video camera and monitor was used.

During the otoliths exchange, the labels used for readers in the analysis were as follows:-

READERS	ID_OTO	READERS	ID_OTO
W.Dawson	R1	I.Loureiro	R6
M.Etherton	R2	J.Landa	R7
J.Fontenla	R3	F.Woods	R8
M.Santurtún	R4	R. Bellail	R9
J.C.Mahe	R5	H. Mc Cormick	R10

Wherever possible, each reader gave an absolute value to each otolith and fin rays. Doubtful ages were indicated by the mark "?" after the age. The identification code used for otoliths was 1 and 2 for the fins rays.

After the otoliths and fin ray exchange was completed and the data had been inputted, prior to the analysis, the imprecise ages were treated as follows:-

Age : ?	=> is excluded from the analysis
Age : 6-7	=> the age is excluded from the analysis
Age : 6 ?	=> 6
Age : 6 ? 7	=> 7, 7 is more sure than 6 ?, so is 7 ;

### 5.1 The Main Lines of the Work at the Workshop:-

The first set of otoliths and fin rays to be read were the exchange collection for the readers who had not aged the sample prior to the workshop. Results of the exchange and individual differences were thoroughly discussed to determine where there were discrepancies, in order to reach a consensus agreement.

The general criteria adopted for ageing each otolith was based on the number of annual hyaline rings, using the following rules:

Period	Quarter 1	Quarter 2	Quarter 3	Quarter 4
N rings Hyaline edge	age = N	age = N	age = N-1 early winter	age = N-1 early winter
N rings Opaque edge	age = N+1 late winter	age = N	age = N	age = N

A second reading was carried out using the ageing criteria agreed upon by all participants,

after discussions about the ageing differences. However, the surface of the resin where the otoliths were embedded had been damaged and consequently it was thought that the condition of the collection could affect age determination. The group, therefore, agreed to read a new sample of 59 otoliths and fin rays.

The reading of the new set was also carried out to avoid the bias of familiarity with the original sample. After the second sample had been aged the analysis and the evaluation of the results was completed.

The comparison of readings were made using an Excel spreadsheet, described by Eltink, (1994). The data analysis was carried out by participants, V. Trujillo and M. Meixide. From each otolith mean age, mode, range and standard deviation were estimated. The percentage of agreement between readers was also calculated. The box-whisker plot was used as an exploratory data analysis that summarised the observations and compared the distribution of the ages in the samples by each reader.

Following the recommendation given by the "Workshop on Sampling Strategies for Age and Maturity C:M., 1994", the statistical analysis applied was as follows:-

- The Wilcoxon's Rank Sum Test, to find out the bias between pairs of readers.
- A measures of agreement: The average percent error (APE), (Beamish and Fournier, 1981). The APE is an index of reading accuracy to compare series of observations, the formula is as follows:

$$I\% = \frac{100}{n} \sum_{i=1}^n \left( \frac{1}{r} \sum_{j=1}^r \frac{|x_{ij} - \bar{x}_i|}{\bar{x}_i} \right)$$

$n$  = number of otoliths

$r$  = number of readings for each otolith.

$x_{ij}$  = the  $j$  value of age estimation for the otolith/fin-ray  $i$ .

$\bar{x}_i$  = average age calculated for the otolith/fin-ray  $i$ .

The average percent error (APE), is considered more appropriate to compare ages than the conventional percent of agreement technique, because it incorporates the average year class of fish.

- Average age bias plots,  $\pm 2$  standard deviation, of each age reader and all age readers combined were plotted against modal age, which was considered to be the reference age.

## 6. RESULTS

The readings of the 149 samples exchanged by reader, combined with the basic information concerning each otolith and fin-ray (sample number, month of the capture, fish length, sex) are summarised in Tables 4-6. The first readings of the first sample was done with personal



criteria by reader.

To interpret the analysis of readings it was necessary to take into account the fact that the majority of readers were not familiar with fin rays. Only one reader was familiar enough with them to use them for stock assessment purposes. In addition, during the workshop, a new otolith reader (R10) participated in the age determination of all the samples.

The box-whisker plot, summaries the differences amongst the readers. The results of the three readings of otoliths and fin rays are shown in Figures 4, 5, 6. As indicated, the figures show a great deal of variability not only for otoliths but also for fin rays.

The percentage of agreement between readers was also calculated for otoliths and fin rays, the results are presented in Table 7. In general the agreement was not very high for the three readings. In the first reading the agreement for otoliths varied between 56 % and 21%, but the majority did not reach 50 % agreement. For the fin rays reading, the percent of agreement is lower, only one pair of readers reach 33 %. The consensus was better after discussion raising the agreement levels to 67 % for otoliths and 43 % for fin rays.

Tables 8 and 9, present the results of the Wilcoxon test for the first and the second sample. There is a clear bias between most readers. For the first and second otolith readings of sample one (Table 8), there were only 12 combinations of readers where there was no significant bias, for the same number of readers. In the fin rays sample, which was smaller with only with 7 readers, there were also few numbers of combinations with no significant bias. For the otolith's readings, readers R1 and R4 held the biggest difference with respect to the other readers. In fin rays readings the greatest difference were between readers R2 and R4.

A comparison between both, otolith and fin rays readings of the same fish was carried out in order to compare the difference in interpretation between the two methods used, for each reader. The results (Tables 8, 9), show that after discussion the majority of readers were consistent in their interpretations. It was also considered necessary to assess the interpretations of those readers involved in stock assessment. Therefore a comparison of otolith readers R2 and R3 and fin rays reader R5 was carried out. In the first sample, after discussion there was no bias. In the second sample (Table 9), there was one reader who was thought to show a difference (R2).

The average percent age error (APE) was calculated using the readings of all readers and also using the readings of those readers who were involved in age determination for stock assessment (Table 10 ). The results in general indicated an improvement in accuracy as more discussion and readings were carried out. The APE decrease. Thus, 21 - 11 for otolith, 26 - 10 for otolith vs fin rays, 26 - 23 for fin rays and 23 - 9 for readers involved in stock assessment..

Tables 11-16 show, by modal age group, the mean age recorded,  $2 (\pm)$  standard deviation, and number of age readings by reader as well as by all readers combined. The modal age was obtained taking into account the experienced readers and also all readers. The results indicated there were no differences between both cases, so the modal age obtained from all readers was used in the analysis. In general, the standard deviations for fin rays reading was higher than for otolith reading.

The age bias plots of each reader with respect to the modal age and all readers combined are

shown in the Figures 7-12. These plots enable the identification of readers that demonstrated a bias for each age group. These plots clearly show great variability; mainly for older age groups.

## 7. DISCUSSION

### 7.1 ANALYSIS OF AGE READING

All readers expressed concern that the otoliths had been mounted in resin instead of being free. Readers could not hand and manipulate the samples to view the most suitable surface for interpretation. The handling throughout the exchange damaged the resin and both these factors affected the readings and consequently the results of the exchange.

#### 7.1.1 First sample.

Initially, the results obtained in the analysis of the first sample indicated great variability for both otoliths and fin-rays age interpretations. The box-whisker plot (Figure. 4), shows the results of the first reading. The interquartile range of all otolith readings is between 3-9 and there are three groups, one of them includes the majority of readers, with a median value of 4; the other two are quite close and have the median values 5 and 3.

For fin rays sample there were fewer readers with a smaller sample. These factors could have affected the results. They indicate there were four groups of readers, the biggest has a median value of 6 and the interquartile range is between 3-11. As mentioned earlier, almost all of the readers had no experience of fin rays, thereby introducing great variability. After the discussion, there was no improvement in agreement, as shown in Figure 5 and 6. The differences in the ages assigned are more or less the same for otoliths, however with respect to the fin rays, Figure 5 indicates that after discussion a better understanding to fin rays interpretation was achieved.

The percentage agreement between readers (Table 7), indicates that the reading of otoliths and fin rays improved after discussion. In the first reading, the majority of agreement for otoliths are under 50 %, in the second reading agreements were over 50 %. The highest agreements were found between experienced readers. This improvement difference also occurs with fin rays, but the percentage agreements were smaller.

The Wilcoxon test (Table 8), shows a clear difference between most readers. The otolith readings did not improve with discussion. This is because this test takes into account the range of the age. After discussion, the combinations of readers where there was no difference was 12. The readers with the highest differences were R1, R4, R9 and R7 respectively.

In the fin rays sample, the improvement between the first to the second reading was higher (from 9-15). Most of the readers did not have any experience of fin rays interpretation. This gave the impression that the fin rays reading agreement was worst than in otoliths reading. After discussion, the number of combinations in fin rays reading with no significant difference increased from 9 to 11.

In the comparison of both methods of interpretation, otoliths and fin rays (Table 8), the results indicate the consistency of interpretation for each reader increased after discussion. The

majority of readers had no significant difference between themselves. For stock assessment purposes R2 and R3 read otoliths and R5 read fin rays, these readers were compared and the results demonstrated agreement in age determination between the two methods.

The discussion of the criteria of interpretation proved useful. It was demonstrated in the average percentage error analysis (Table 10), that the APE values of otolith are always lower than fin rays. This might be due to most readers being more familiar with otoliths. The results obtained for readers involved in stock assessment were consistently lower than for all readers, probably because of their experience.

Plots of the otolith first reading by reader (Figures 7-9), demonstrates great variability, showing that readers 1 and 4 tend to overestimate ages. The plots of all readers combined, shows greater bias from age 6 onwards. Plots of the fin rays by reader (Figure 8-10), show greater variability from ages older than 6.

There appears to be an improvement between the first and second reading in the precision as indicated from the APE.

### 7.1.2. Second Sample.

The time available at the workshop, only permitted a new small sample to be examined. Therefore it was necessary to take into account the small number of otoliths and fin rays included in the analysis.

The results of the box-whisker plot for otolith readings (Figure 6), indicates that there was no clear agreement after discussion. The variability for otoliths and fin rays is slightly lower than for sample 1, however the percentage of agreement decreased.

A similar trend was observed in the box-whisker plot for fin rays (Table 9). Both, otoliths and fin rays demonstrated small numbers with no significant difference and the problems seemed to remain. The comparison of otolith readings from R2, R3 and fin rays readings from R5, show no improvements. This was probably a result of insufficient numbers within the samples, with respect to the first sample results.

The new sample otolith plot (Figure 11a,b), indicated that there are no problems for ages younger than 4. Readers 1 and 4 continued to assign higher ages. The greatest difference observed was in ages older than 5. In general, all readers showed problems with ageing older fish. The readings of fin rays presented problems for most readers. (Figure 12 a, b).

The considerable differences, especially for older fish, suggests that the level of agreement in age estimation could be higher for younger fish. The group decided to restrict the analysis to the age range 10<sup>+</sup> as it was not included in the Working Group. The Wilcoxon test was repeated for this age range, however, the improvement was less important than it was expected and therefore the results are not presented in this Report.

## 7.2. AGE DETERMINATION

After the results of the otolith and fin ray exchange had been discussed, and a difference between most readers recognised, it was agreed that a sample of otoliths and the corresponding fin rays should be put under two different monitors and the age interpretation

using both methods discussed simultaneously.

After a discussion had taken place on 4 fish (aged 1,3,3,4), agreement had only been achieved between all readers for the one year old fish, it was felt that some readers were being influenced in their interpretation by others who had aged the fish before them and verbally given their ages. Therefore, it was suggested that each reader write down the age they had assigned the fish for both otoliths before the age of the fish was discussed. Ten readers aged the otoliths and three readers aged the fin rays. After all readers ages had been recorded, all the ages were read out and the ages discussed.

This approach was much more successful. Eight otoliths with the corresponding fin rays were discussed and most of the readers agreed on the ages. The fin ray ages agreed with the modal ages given for the otoliths for six out of the eight otoliths discussed. Where the fin rays ages differed they were both one year younger than the modal ages from the otoliths.

After the discussion of these otoliths and fins rays, all the readers agreed that they felt more confident with their age interpretation after discussing their ages with other readers. Occasionally, there were differences in the interpretation of the otolith ring structure. These differences were discussed fully and, with one exception, the differences were resolved.

The unresolved interpretation was where there were two rings close together for the first ring in one otolith. Three out of the ten otolith readers counted both rings, the remaining seven readers counted the second of the rings only and disregarded the first ring as a split ring. All readers agreed, however, that this type of ring structure within the first year was not common and readers felt that this was unlikely to be a serious problem in age determination from the otolith.

The three readers who interpreted the corresponding fin rays were usually in agreement. One reader was more experienced than the other two and gave an absolute age for all fin rays discussed (R5). The other two readers queried some of their ages e.g.  $n?n+1$ , one of the ages they assigned always agreed with the age given by the more experienced reader.

All readers felt that it was useful to see the corresponding fin ray displayed with the otoliths and generally gave confirmation of the otolith interpretation. Where the otolith readers did not all assign the same age to the otolith, the fin ray age tended to agree with the upper end of the range of ages given for the otolith which also tended to be the modal age.

It was felt that the experienced otolith readers were also able to achieve very good agreement in age determination from the fin ray. This demonstrated that age determination experience from otolith is fairly easily transferable to age determination using the fin ray method.

## 8. AGE DETERMINATION CRITERIA

After the general discussion had taken place between all readers on individual otoliths (Section 5.3), there followed a general discussion about which ageing criteria should be applied to help standardise age determination, both using otoliths and fin rays. The agreed criteria are summarised below:

### 8.1. Ageing criteria for otoliths

- For a better interpretation of the age it was agreed that both otoliths should be kept free to enable manipulation of them to provide the best indication of the growth pattern.
- It was agreed that both otoliths should be aged wherever possible and the rings counted in several places if there is a clear image of ring formation in more than one place. The preferred areas for ring interpretation are indicated in Figure 13.
- If the otoliths have been counted in more than one place and different numbers of rings have been identified, the true age should be taken as the age derived from what is considered to be the clearest section of the otolith for interpretation.
- If both otoliths have been counted and they are both queried e.g.  $n \neq n+1$ ,  $n+1 \neq n+2$ , then the modal age should be used, e.g.  $n+1$ .
- The opaque zone is deposited on the edge of the otoliths between March and October for Megrim from ICES Sub-areas VII and VIII.
- The opaque zone can be observed forming first on the edges of the otoliths from the more Southerly areas.
- The opaque can first be observed forming on the edges of the youngest fish.
- It is recognised that fish deposit their opaque and hyaline zones on some parts of the otolith edge before others. When this is observed, the edge type on the fastest growing area of the otolith should be taken as the edge type.
- The older the fish the later in the summer months the opaque zone is deposited.
- When the opaque edge starts to form, deposition of the opaque zone is very rapid.
- Megrim usually mature at about age 2 in males and age 3 in females. When the fish reach maturity the growth of the fish, as recorded on the otolith, is observed to slow down. Therefore, there are large opaque zones formed between the first, second and sometimes third hyaline zone and these are usually followed by more narrow opaque and hyaline zones. It is assumed therefore, that growth slows down when energy is diverted into spawning. It should be noted however, that this is not always the case, and it is not uncommon to find large, old immature fish.
- It is possible that fish mature earlier in the more Southerly areas where the temperature is higher. Therefore, it should be recognised that this is likely to affect the growth pattern on the otolith.

### 8.2 Ageing criteria for fin rays

- Transverse sections of fin rays are made by placing the caudal fin ray in black resin and cutting it in thin slices (0.4 mm), using the method described by Peronet et al., 1992
- The two best slices are then selected in order to make the readings on cuts showing the shape as in Figure 13, which generally gives the best image.
- Readings are made under a microscope at magnification 100 x. Special care has to be given to the lighting to give the maximum contrast. In most cases the condenser should be moved to its lower position. The use of phase contrast is to be avoided. The use of a video monitor is not recommended unless for teaching purpose as maximum definition is needed to identify the sharply defined rest lines.
- The basis of fin rays interpretation is generally the same as for otoliths. The winter deposits or rings are the structures counted. In fin rays they are called "*rest lines*" and are deposits of lamellar tissues in the winter season corresponding to the hyaline zone in otoliths. They are thin and well marked concentric lines that surround the medullar canal after the first deposit of opaque bone tissue in the first summer. Some changes in the

density of the opaque zone can be mistaken for rest lines.

- Generally the readings can be made anywhere on the cut as the rings are relatively evenly spaced. However, in older individuals the rest lines are better marked in the area shown in Figure 13. In cases of uncertainties the true age should be derived from the reading of the outer rings made in this area.
- It might be difficult in some case to distinguish between the crescent shaped medullar canal and the first ring. The use of a micrometer is then useful to compare the size of the uncertain structure with another one well differentiated on the same slide.
- The interpretation of the edge is very hard, almost impossible in the first and second quarters as the rest of the lines are so thin that they can only be seen after the deposit of enough opaque material (mostly end of second quarter and third quarter). So, for the first and second quarter where  $n$  rings are counted with a wide opaque edge, the age given should be  $n+1$ .

## 9. CONTRIBUTIONS TO THE WORKSHOP

A new method of otolith preparation was presented, by M. Etherton from CEFAS, UK, which may improve age determination of older fish:

### 9.1 Sectioned and stained otolith collection from CEFAS

The participants from CEFAS introduced a sample of sectioned and stained otoliths to the workshop as an alternative method of age determination of Megrim to compliment age determination from whole otoliths that are difficult. It has been decided to try this method because readers of other species, particularly sole, have found it benefits the ageing of older fish. The technique used to present the otoliths is the same as the one established to assist in the age determination of some sole at the CEFAS laboratory and has since been tried for Megrim.

#### 9.1.1 The process:

The otoliths are initially stored in paper envelopes without any cleaning. They are then set in moulds in a resin which comprises of:-

- Polyester fillable casting (styrene)
- Dense black colour paste
- Tack free additive (wax in styrene)
- Catalyst (Methyl ethyl ketone peroxide)

They are then left to cure. The resin is scored to mark the position to be sliced to ensure a cut that goes through the nucleus of the otoliths. The moulds are dismantled and the mounted otoliths are sectioned with a cutting disc.

The sectioning is carried out with an old blade as a new blade tends to rip the surface of the otolith and makes them unreadable. The blade is not cleaned because this would involve partly dismantling the machine and it is thought the advantages of cleaning the blade do not outweigh the disadvantages (time, health & safety, ease etc.). The rate of rotation of the blade is kept constant although the speed at which the block of resin is fed through is controlled by the operator.

After cutting, excess cutting fluid is wiped off and the sections are left to dry. Details of the species, area, date of sample, sample number and row number are recorded on the strip in pencil (which remains unaffected by the staining process). The strips are then put into plastic wallets until the whole sample is ready to be stained (this process is called the "dip").

The staining solution is made up as follows:-

- 1.0g Neutral red (weighed to 4 decimal places)
- 99.5ml distilled water (meniscus level with 99.5ml mark in a measuring cylinder)
- 0.5ml Acetic acid (measured using a pipette)

The dye solution is poured into a plastic bottle after each use. The bottle is shaken before each use as sediment has been noticed. Selected otoliths are placed horizontally in a built-in rack along the bottom of a plastic container with the thin edge facing upwards. The staining solution is poured into the container so that the otoliths are covered and left to soak for 15 minutes. The strips are then removed from the stain and rinsed in a bowl of tap water. The strips are then placed in a glass rack and left to dry overnight. The otoliths are put into plastic packets and the details recorded on the packet.

Points of note:-

- The sectioning and staining takes place at room temperature and this will vary.
- The calibration of the instruments is checked.

## 9.2 Description of Removing Otoliths From Under the Gill Cover

Some delegates mentioned that obtaining Megrim otoliths from commercial landings was difficult due to the damage that otolith extraction inflicts on the fish and the reluctance of fishermen to allow this on fish at the market. This was the case particularly for the French delegation and was cited as one reason why the use of fin rays has been introduced there. A solution was offered by the CEFAS representatives, who routinely use a method of otolith extraction in market samples that does not damage the fish. However, there are countries (e.g. Spain) which can not introduce this method because the fish owners consider this method of extraction damages the fish.

The method involves making a small cut on the underside (white side) of the fish along the edge of the operculum (gill cover) where it joins the flesh of the fish at the top of the operculum. The gill is then lifted to expose the area of the otolith cavity. The left-hand otolith (the one with the off-centre nucleus) can then be clearly seen beneath a thin layer of bone (opercular bone). This layer is cut away and the otolith removed with forceps. When the operculum is released, the fish is undamaged externally except for the small incision made to assist entry into the cavity and this is barely noticeable. It is worthy of note that on large individuals, the cut may not be necessary as the size of the fish means that merely folding the operculum back reveals enough of the cavity to allow otolith extraction. This technique is also used at CEFAS for other flat fish species including sole, plaice and lemon sole. An adapted version is also used for roundfish such as cod, whiting and haddock.

## 10. CONCLUSIONS

The workshop participants agreed that the co-operation between the countries concerned

needs to be intensified to solve the ageing problems encountered in this workshop. It was considered that this workshop was only one step further in trying to achieve a unified ageing technique.

- Age reading criteria to interpret the otoliths and fin rays was established. After that the percentage of agreement was improved.
- In general, results of otolith readings indicated that readers seems do not have problems for ages younger than 5. After that, the variability increased.
- The Indices of Beamish and Fournier (Average percentage of error) decreased after the first reading, indicating that ageing agreement has improved.
- The results demonstrated that the differences in age readings between whole otoliths and fin rays were mainly not significant for those readers who provide the ALK to the ICES Working Group for assessment.
- The level of agreement in age determination among readers involved in stock assessment was high mainly for those ages less than the 10 plus year class.
- Despite the problems encountered in age reading of Megrim it was pointed out that this workshop was very useful because it was the first time that so many readers of Megrim were together to discuss on its problems. It was recommended that another workshop be conducted in three years time.

## 11. RECOMMENDATIONS

- Regular exchange of ageing structures for the purpose of checking the precision of all readers involved in age determination.
- The experience of the readers and the good quality of the collection sample improve the ageing agreement.
- It is recommended that future exchanges involving whole otoliths, should include one embedded in resin (e.g. right) and the other one (left) in the packet. And an age given for both the left and the right otolith.
- It is very important to be accurate in the age estimations for the first 10 years of the fishes lives for stock assessment purposes, because ages >10 are aggregated into a 10 + group.
- It is recommended that another workshop is convened in 3 years time to review agreement.
- An age determination guide is recommended to assist the individual reader in the correct interpretation.
- Recommended that adequate training of readers supplying ages to the ICES W.G. on assessment is given.

Although this workshop improved the ageing agreement between readers, especially for the readers who supplied the ages to the working group, a direct method of validation is needed to confirm age determination (e.g., otolith microchemical analysis). Therefore it is strongly recommended that a direct method of validation is identified. This recommendation requires a dedicated project.



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Name	LAB.	E-mail	EXPERIENCE	
			Otoliths:read	Fin-rays: read
Wendy Dawson	Lowestoft	W.a.dawson@cefas.co.uk	24 years age determinate experience > 200.000 mackerel/ 30.000 pilchard/ 10.000 megrim/ 2.000 ling/ 1.000 red mullet/ 2.000 haddock/ 1.000 hake/ 2.000 angler fish also age determination of tropical pelagic and demersal species from Ecuador approx. 10.000 Scomber japonicus, thread herring, round herring, sardine and anchovy and demersal species.	
Mark Etherton	Lowestoft	M.W.etherton@cefas.co.uk	10.000 + / Mackerel=1000 Anglers=100 vertebrae	> 100
Jorge Landa	IEO, Santander	jorge.landa@st.ieu.es	megrim + four spot megrim approx. 10.000	angler fish approx. Approx. 300
Robert Bellail	Ifremer	rbellail@ifremer.fr	approx. 300. Others (Gadvid fish) more than 50.000 otoliths.	approx. 150
Jean-claude Mahé	Ifremer	jc@ifremer.fr	others approx. 6.000	3000
Marina Santurtún	Azti	marina@rp.azti.es	approx. 2.000 megrim otoliths. < 500 snappers.	
Jorge Fontenla	IEO, Vigo	jorge.fontenla@vi.ieu.es	4.000 cod. 7.000 megrim + four spot megrim.	
Isabel Loureiro	IEO, Vigo	isabel.loureiro@vi.ieu.es	2.000 megrim + four spot megrim. 1500 hake	
Fiona Woods	Marine Institute Fisheries Research Centre (Dublin).	FWOODS@FRC.IE	1 Year 10 months:14.000 otoliths cod, haddock, plaice, black sole, lohitling.Megrim: 1 year 3 months, 2.200 otoliths.	
Helen McCormick	Marine Institute Fisheries Research Centre (Dublin).	H.M.CORMICK@FRC.IE	5 1/2 years (approx. 12.000/year) of cod, haddock, whitina, plaice, blacsole and megrim.Megrim : 4 years, approx. 1.500/year.	
Carmen Piñeiro	G <sup>a</sup> IEO, Vigo	carmen.pineiro@vi.ieu.es	Hake sectioned 15.000/ Hake whole 10.000/ Megrim whole 3.000/ Four spot megrim (whole) 3.000/ D. cuneata 1.000/ Granadiers (sectioned) 500/ Blue whiting (s. and w.) 1.000/ P. maxima (whole) 300/G. argenteus (whole) 1.000.	

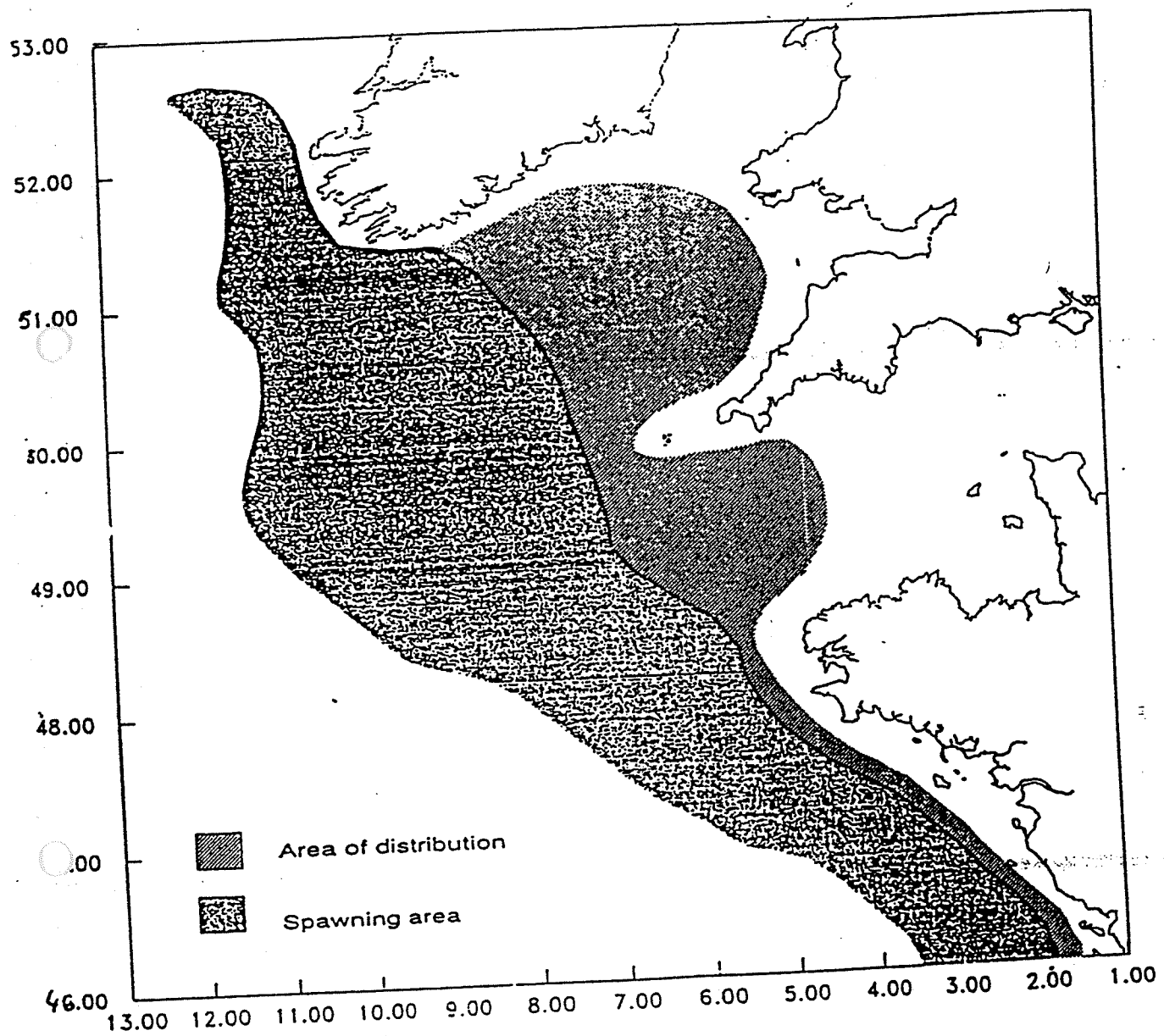


Figure 1.- Megrim distribution in the Celtic Sea and Biscay in March/April 1984-1993.

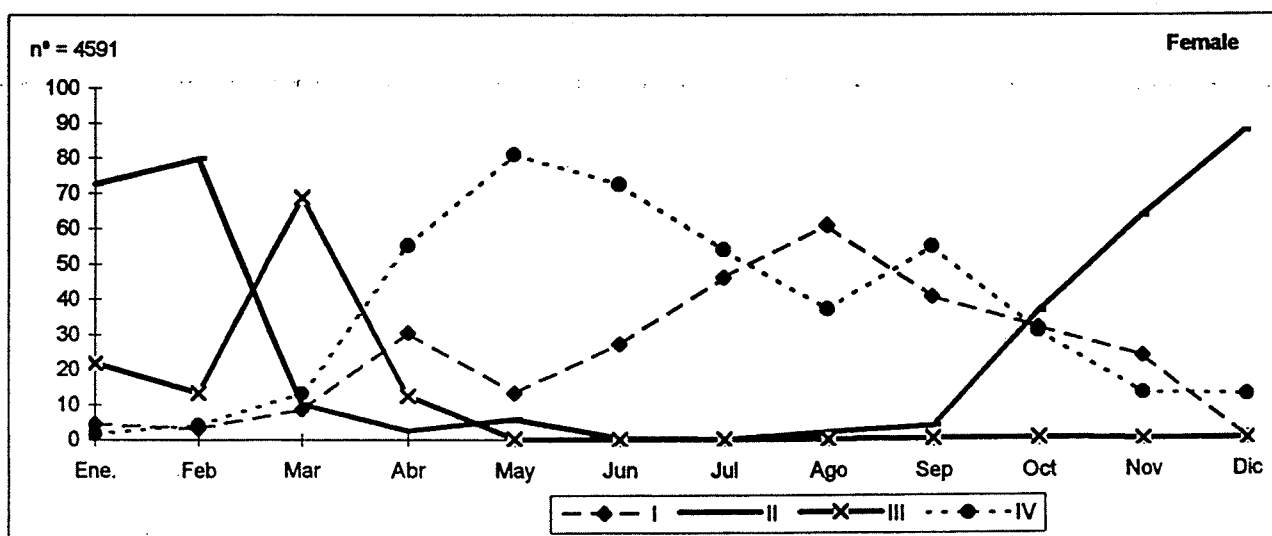
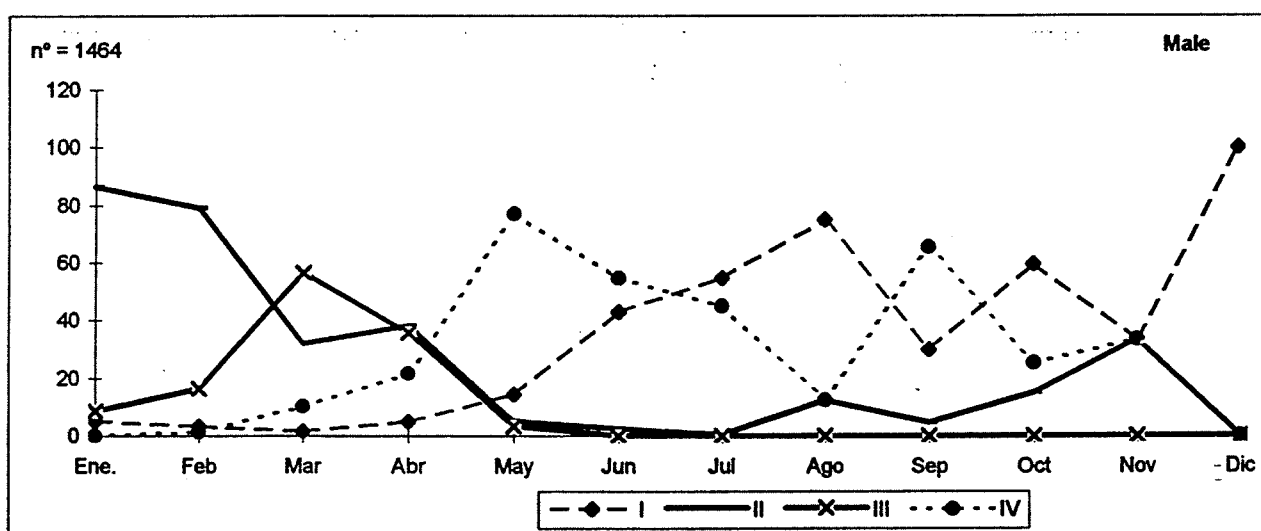
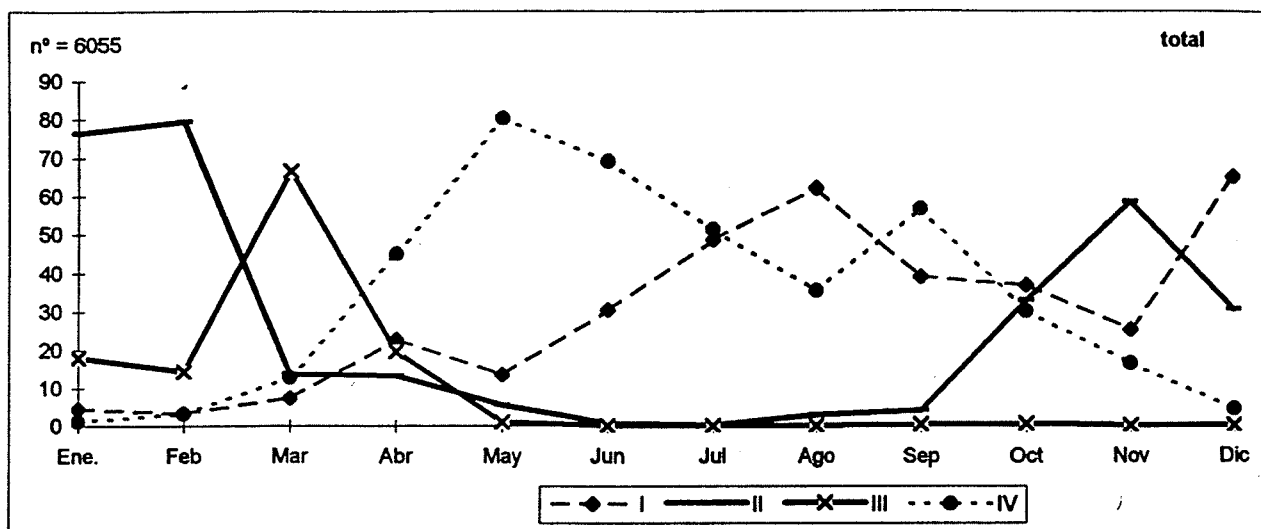


Figure 2.- Spawning period and maturity stage of Megrim along the year in Subarea VII (ICES). Fontenla and Pérez (unpublish data)

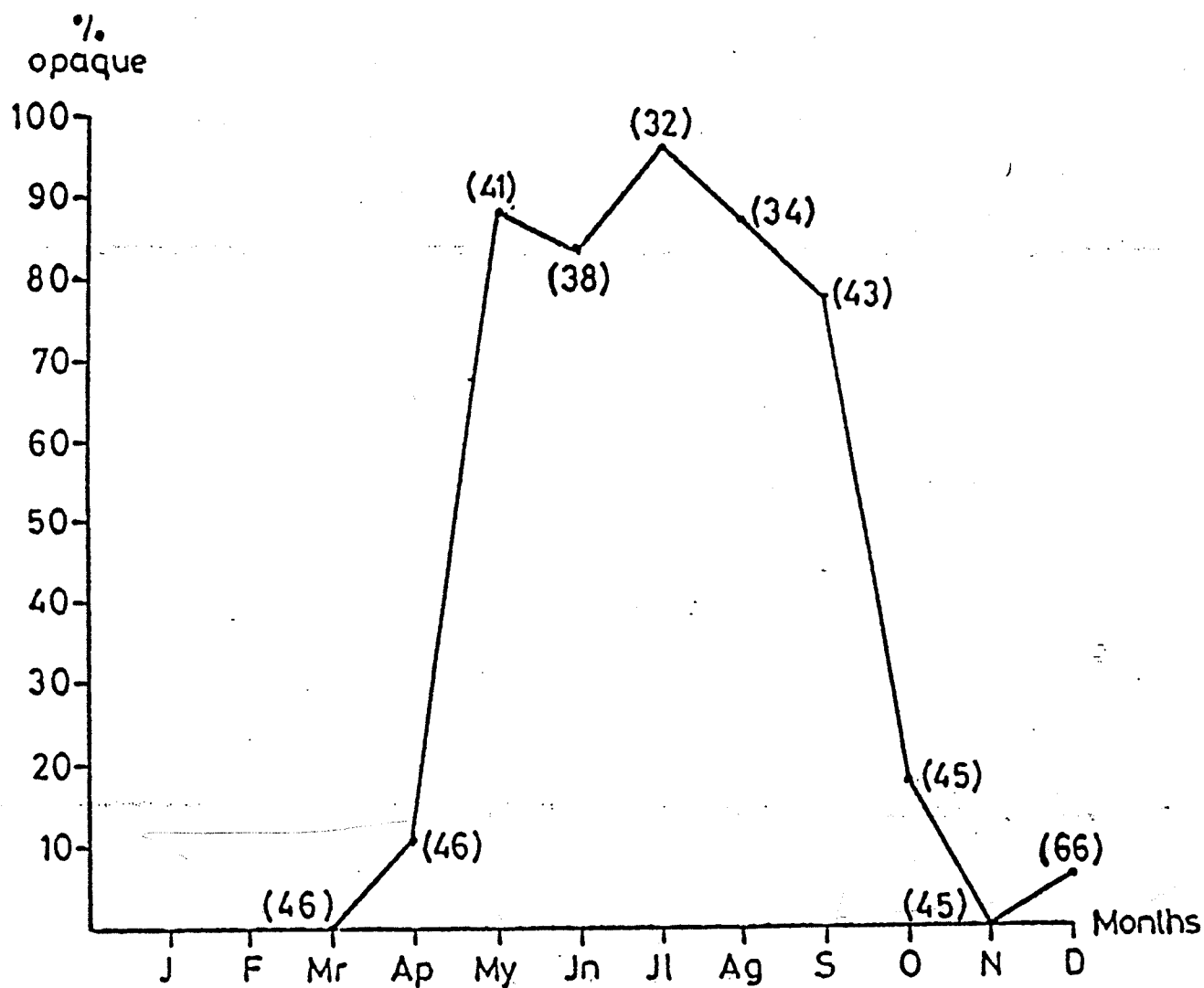


Figure 3.- Percentage of opaque edge otoliths along the year. The number brackets is the total otoliths examined each month. (Rodriguez & Iglesias, 1985).

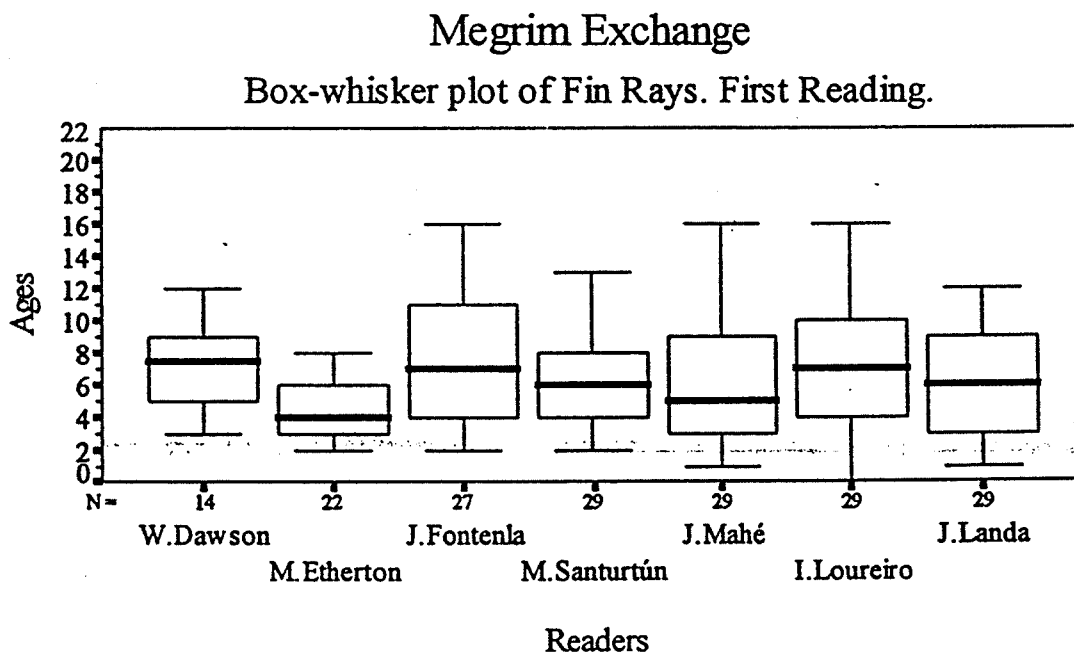
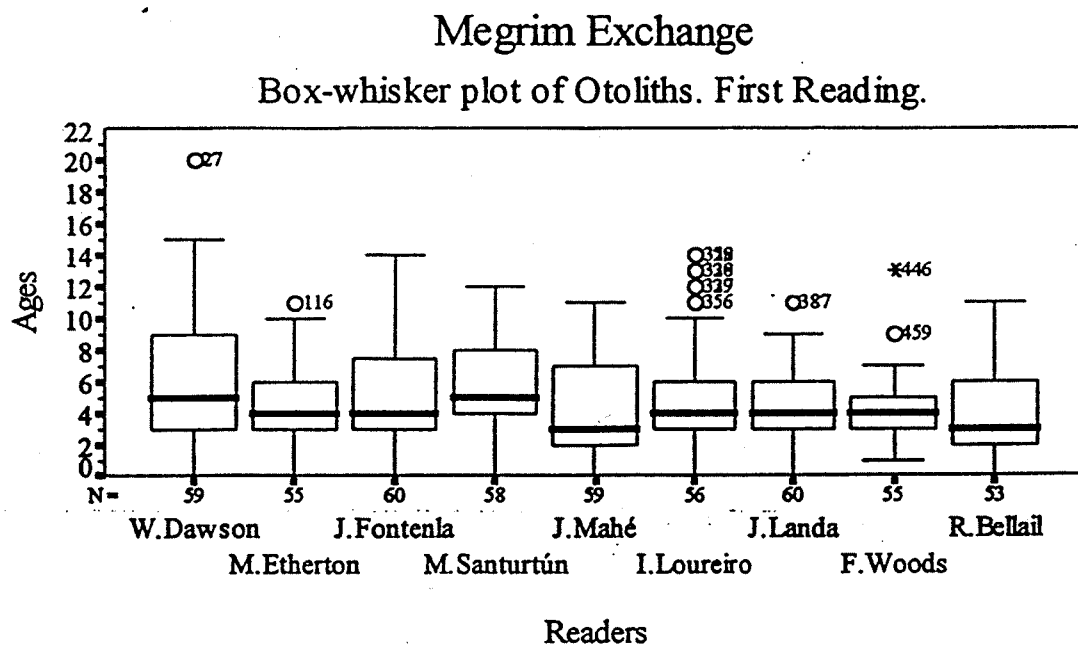


Figure 4.- Box and Whisker plot for the First reading of all readers.

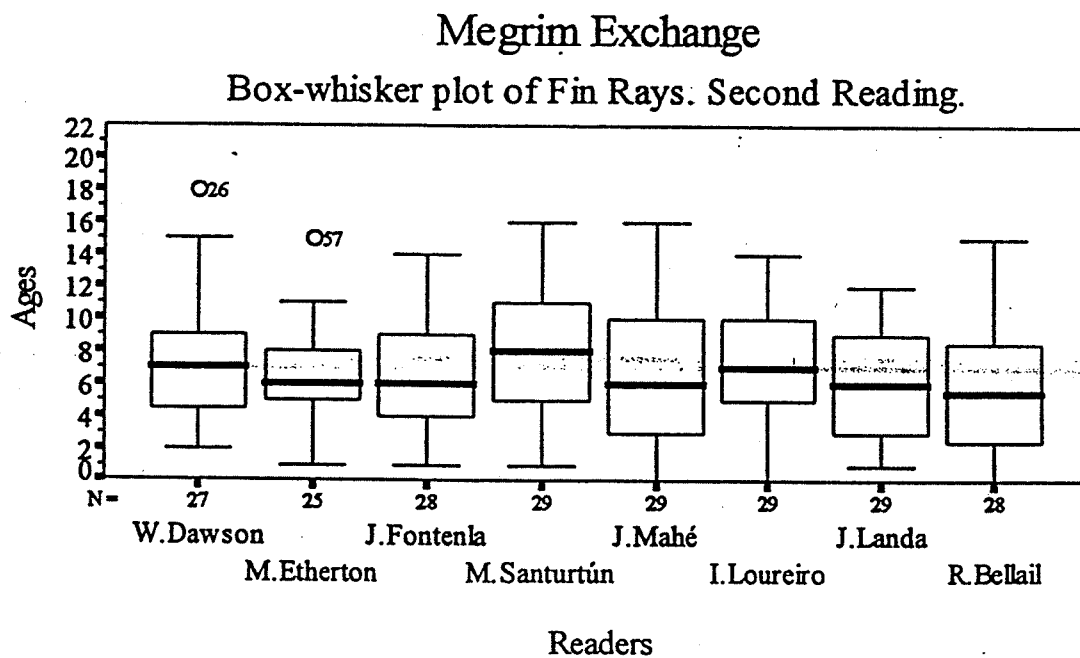
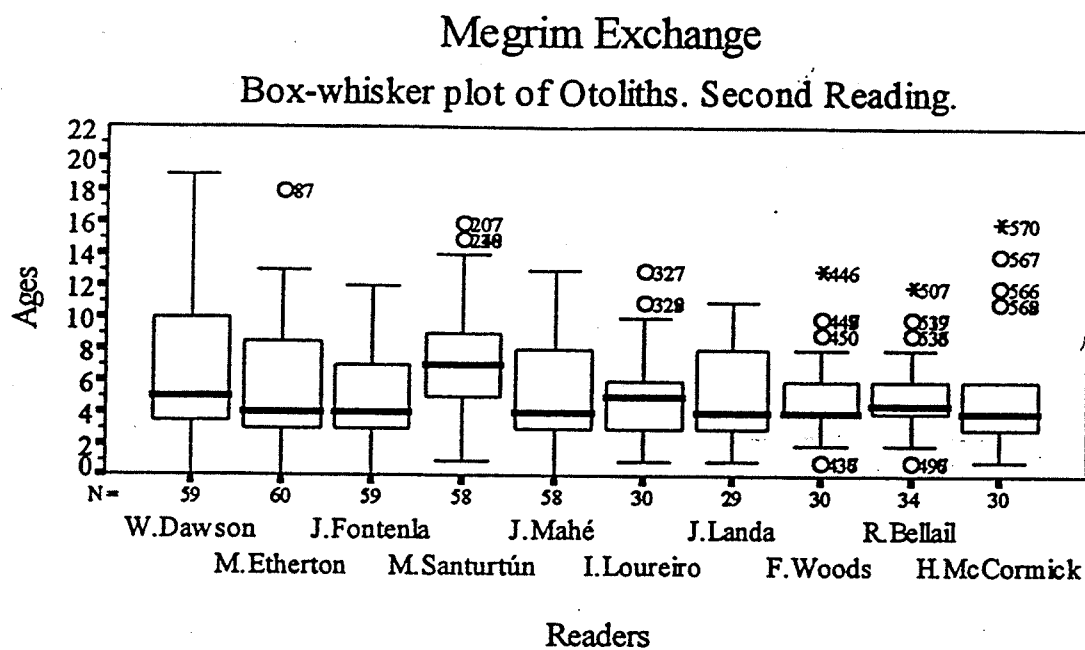


Figure 5.- Box and Whisker plot for the Second reading of all readers.



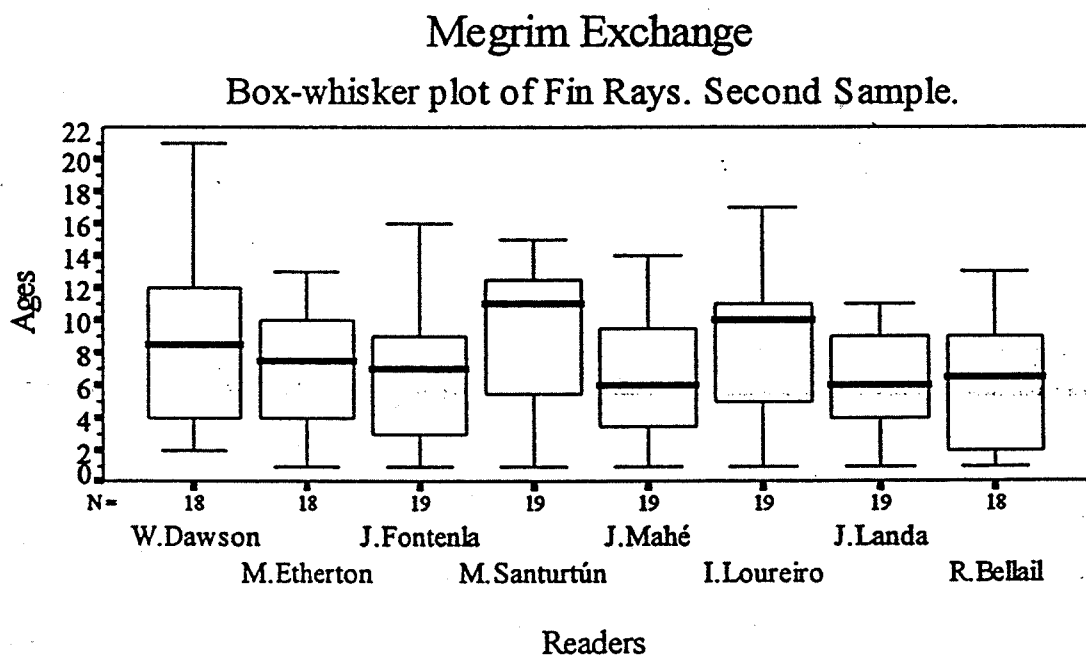
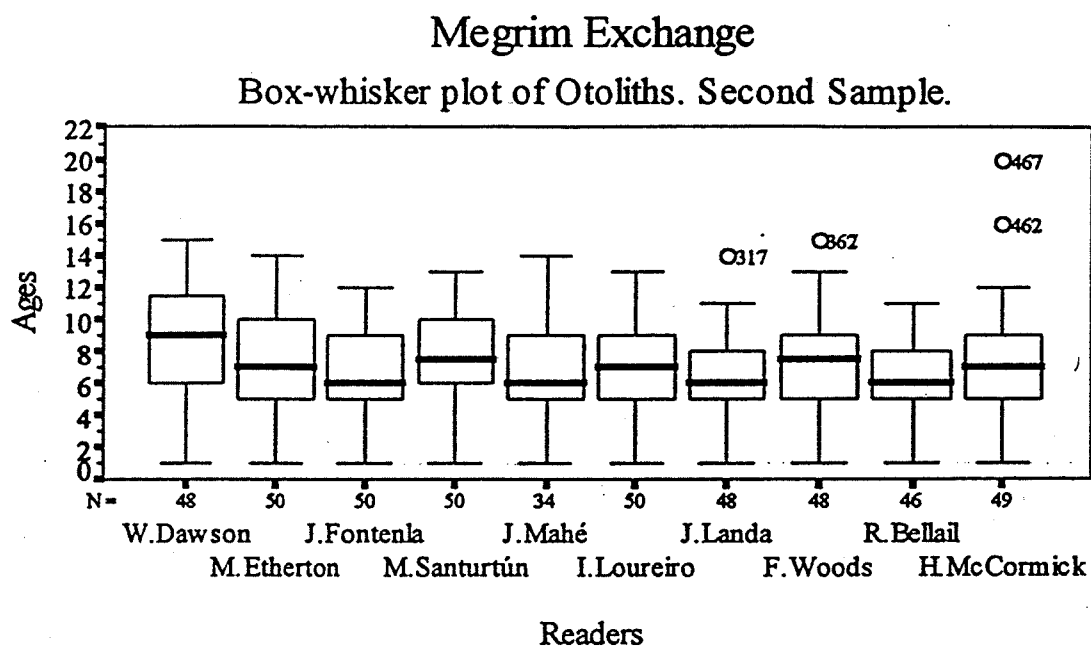


Figure 6.- Box and Whisker plot for the reading of Second Sample of all readers.

# MEGRIM EXCHANGE SAMPLE FIRST READINGS (OTOLITHS)

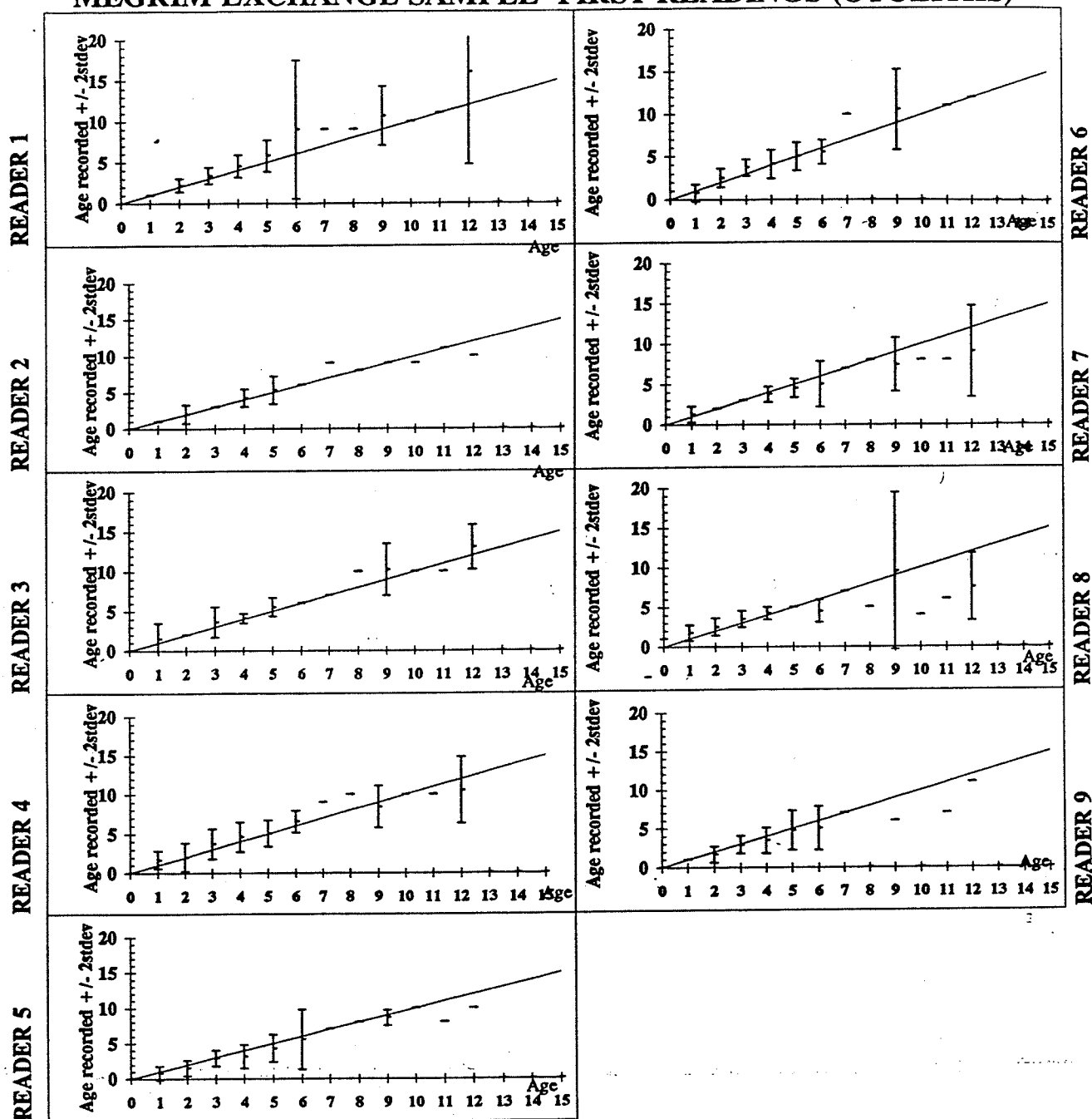


Figure 7a.- In above age bias plots average age  $\pm$  2stdev of each age reader is plotted against modal age.

Modal age	0	1	2	3	4	5	6	8	9	10	11	12	13	14	15
Age recorded	0.11	1.17	2.04	3.29	3.98	5.00	5.89	8.29	8.97	8.71	9.11	11.06	-	-	-
2*stdev	0.67	1.14	1.16	1.32	1.59	1.72	3.62	3.41	4.12	4.43	3.80	6.26	-	-	-

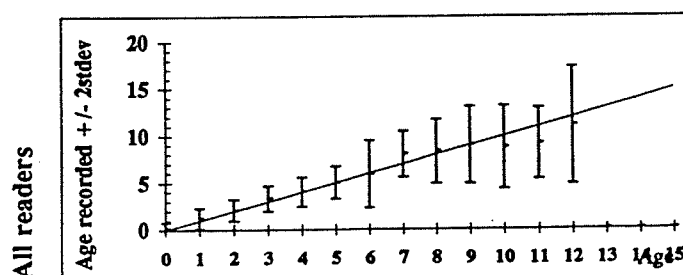
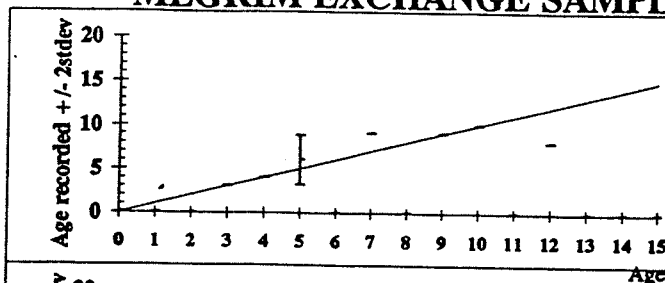


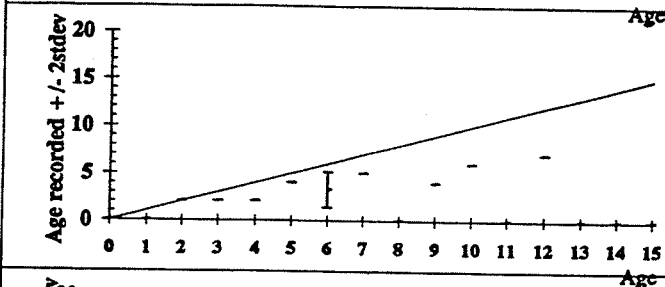
Figure 7b.- In above age bias plot average age  $\pm$  2stdev of all age readers is plotted against modal age.

# MEGRIM EXCHANGE SAMPLE FIRST READINGS (RAYS)

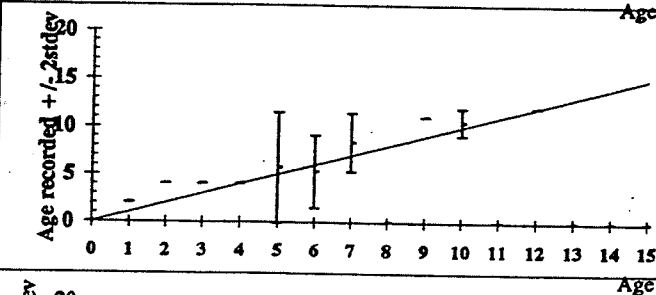
READER 1



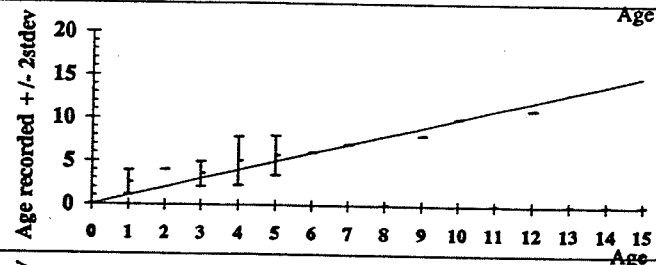
READER 2



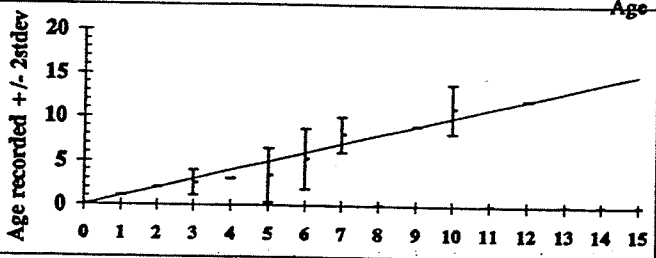
READER 3



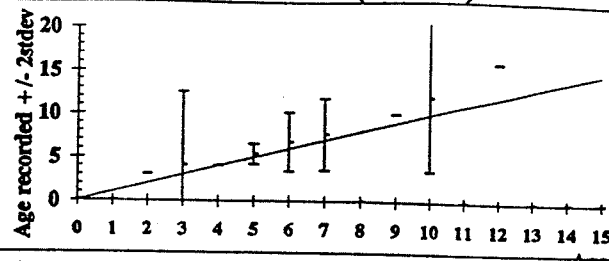
READER 4



READER 5



READER 6



READER 7

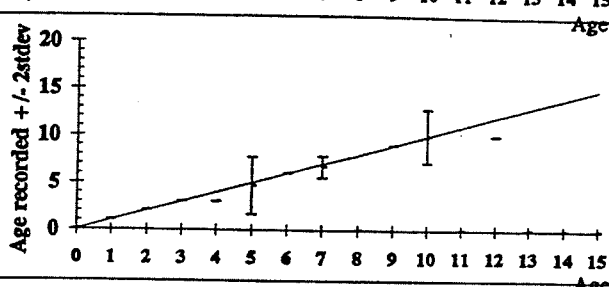


Figure 8a.- In above age bias plots average age +/- 2stddev of each age reader is plotted against modal age.

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,22	2,83	3,18	3,57	5,00	5,42	7,33	-	8,87	9,92	-	10,86	-	-	-
2*stddev	-	1,94	1,97	3,07	2,03	3,29	3,23	2,91	-	4,45	4,65	-	5,94	-	-	-

All readers

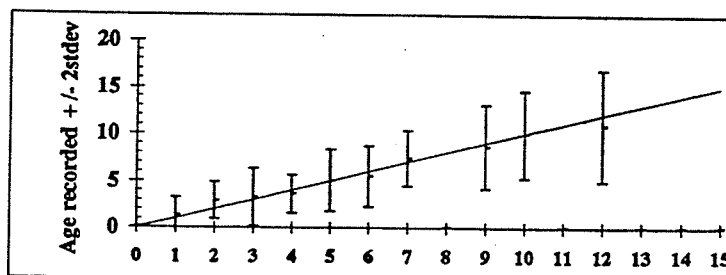


Figure 8b.- In above age bias plot average age +/- 2stddev of all age readers is plotted against modal age.

# MEGRIM EXCHANGE SAMPLE SECOND READING (OTOLITHS)

READER 1

READER 2

READER 3

READER 4

READER 5

READER 6

READER 7

READER 8

READER 9

READER 10

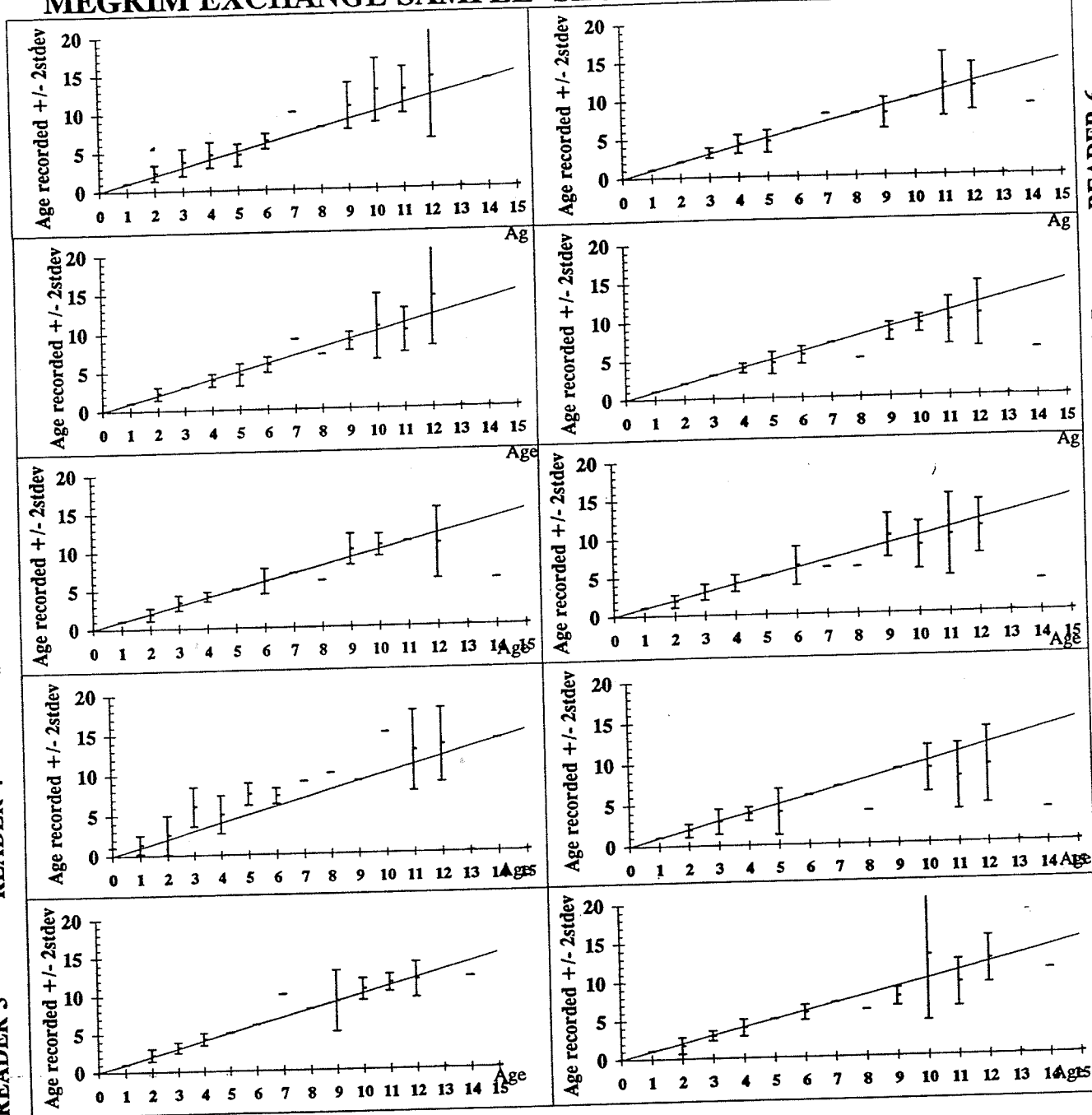


Figure 9a.- In above age bias plots average age  $\pm$  2stddev of each age reader is plotted against modal age.

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,10	1,03	2,03	3,40	4,16	4,95	6,08	8,00	6,80	9,00	10,79	10,68	11,93	-	8,89	-
2*stddev	0,63	0,32	1,11	2,15	1,37	2,10	1,39	2,83	3,50	2,60	4,64	4,15	5,10	-	8,09	-

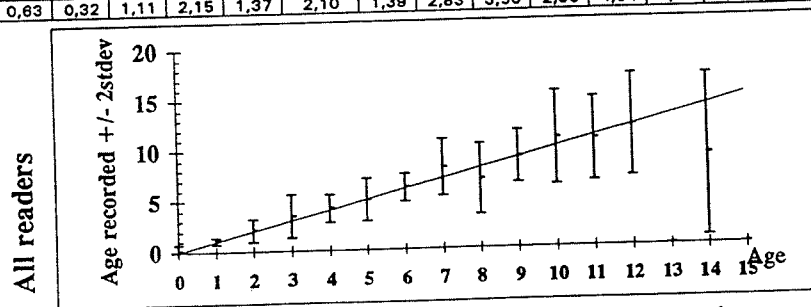


Figure 9b.- In above age bias plot average age  $\pm$  2stddev of all age readers is plotted against modal age.

# MEGRIM EXCHANGE SAMPLE SECOND READINGS (RAYS)

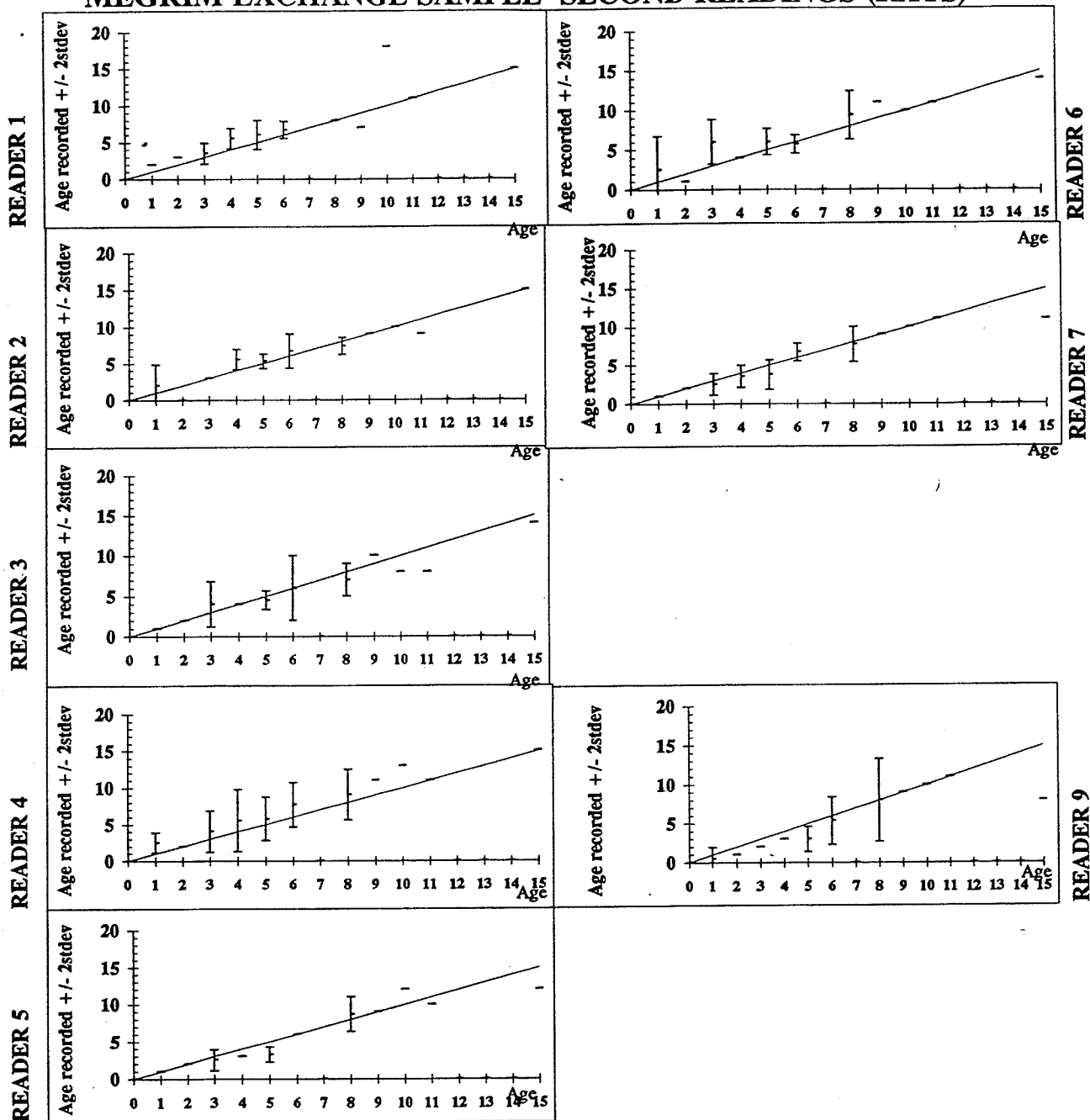


Figure 10 a.- In above age bias plots average age  $\pm 2$ stdev of each age reader is plotted against modal age.

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,53	1,86	3,47	4,25	4,66	6,33	-	8,13	9,38	11,38	10,25	-	-	-	13,00
2*stdev	-	2,12	1,38	2,91	2,48	2,81	2,41	-	2,86	2,60	6,14	2,33	-	-	-	5,01

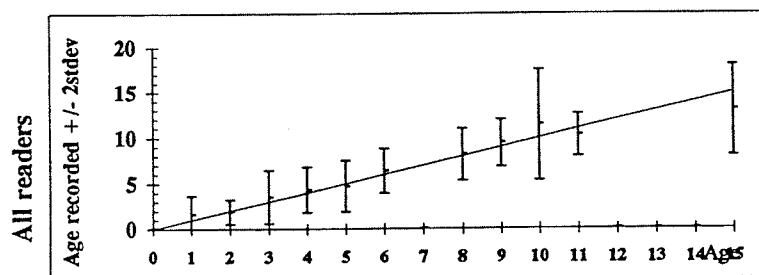
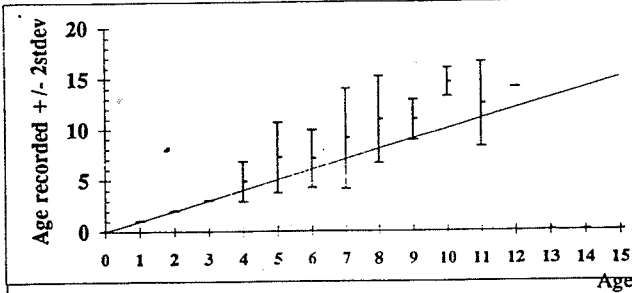


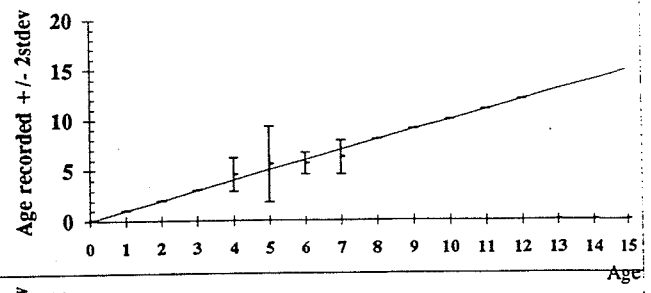
Figure 10 b.- In above age bias plot average age  $\pm 2$ stdev of all age readers is plotted against modal age.

# MEGRIM SECOND OTOLITH SAMPLE

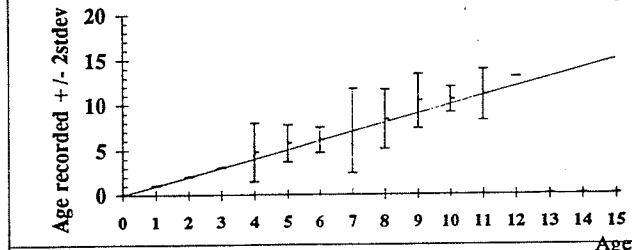
READER 1



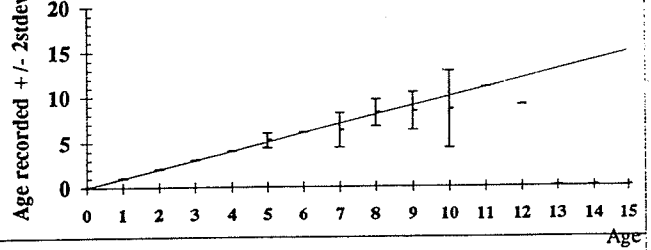
READER 6



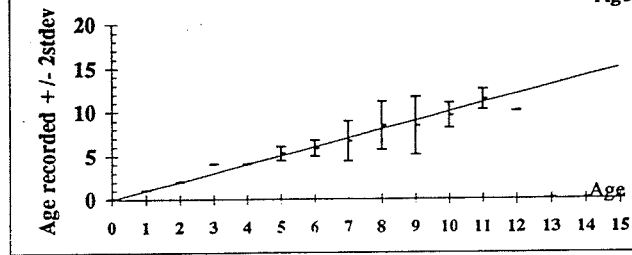
READER 2



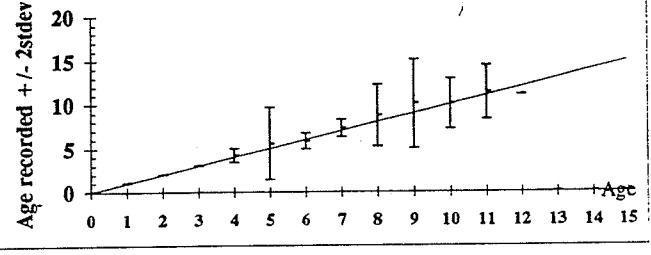
READER 7



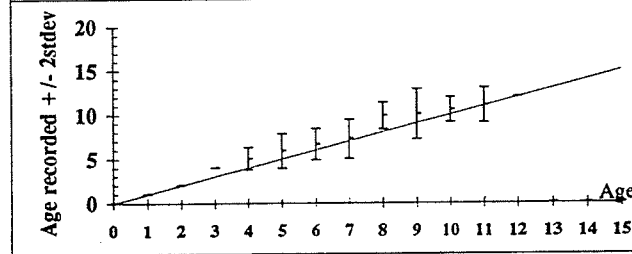
READER 3



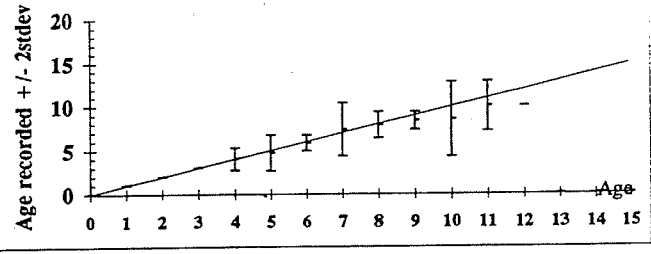
READER 8



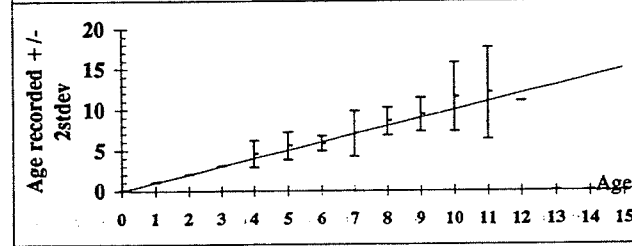
READER 4



READER 9



READER 5



READER 10

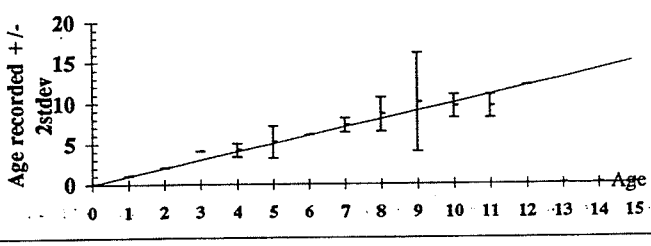


Figure 11a.- In above age bias plots average age +/- 2stdev of each age reader is plotted against actual age.

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	3,30	4,38	5,53	6,04	7,07	8,70	9,45	10,30	11,12	11,40	-	-	-
2*stdev	-	0,00	0,00	0,97	1,61	2,64	1,45	2,91	2,91	3,44	3,90	2,67	3,01	-	-	-

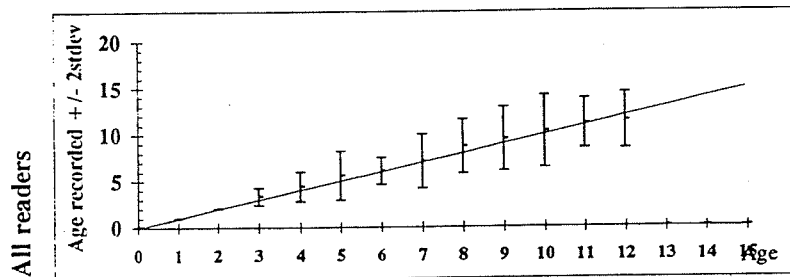


Figure 11b.- In above age bias plot average age +/- 2stdev of all age readers is plotted against actual age.

# MEGRIM SECOND RAY SAMPLE

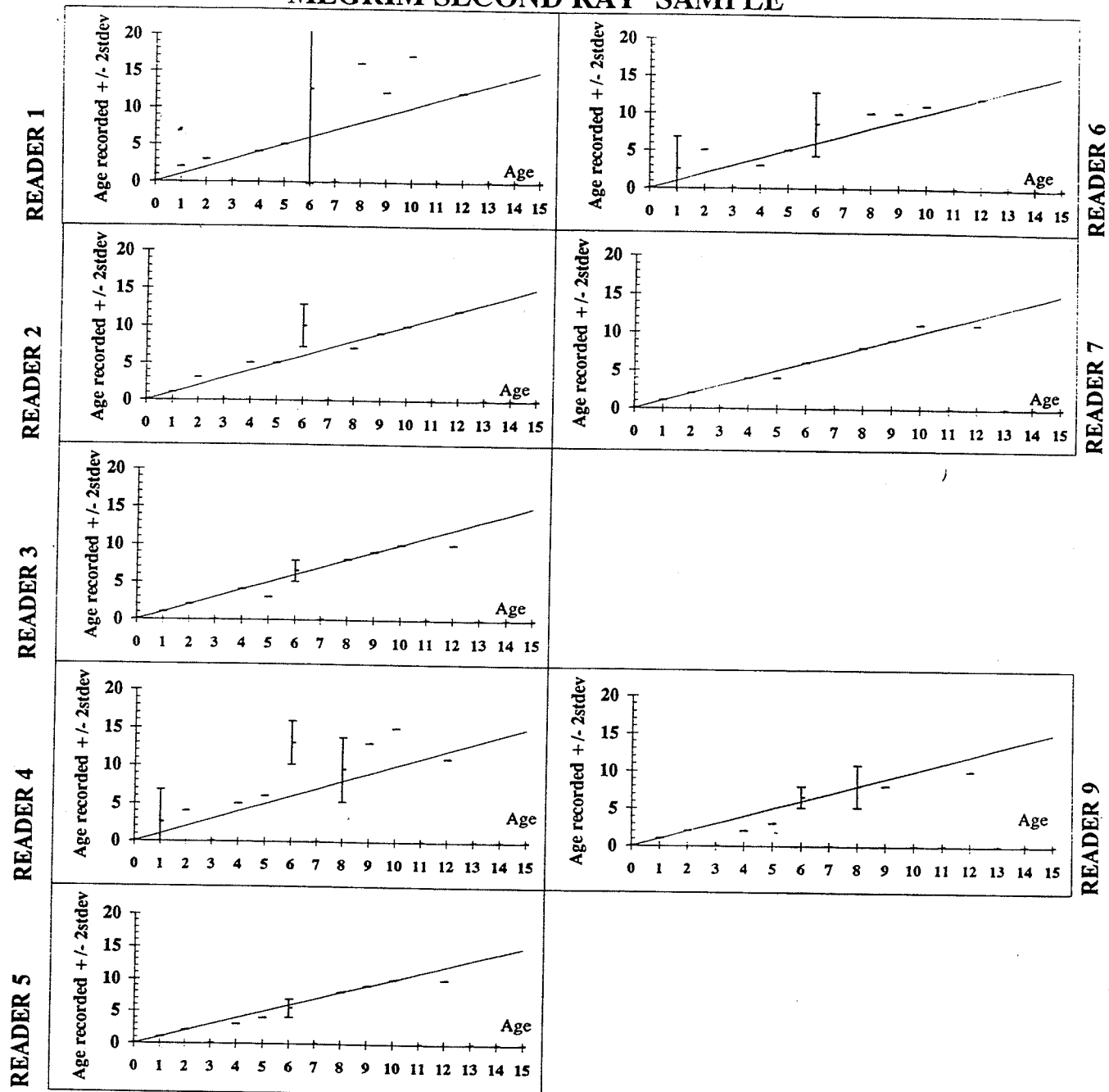


Figure 12 a.- In above age bias plots average age  $\pm$  2stddev of each age reader is plotted against actual age.

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,50	2,88	-	3,75	4,38	8,56	-	9,00	9,88	10,50	-	11,00	-	-	-
2*stddev	-	2,07	2,25	-	2,07	2,12	6,81	-	4,64	3,45	9,97	-	1,85	-	-	-

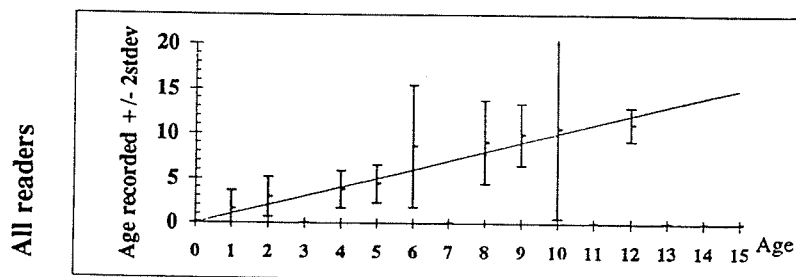


Figure 12 b.- In above age bias plot average age  $\pm$  2stddev of all age readers is plotted against actual age.

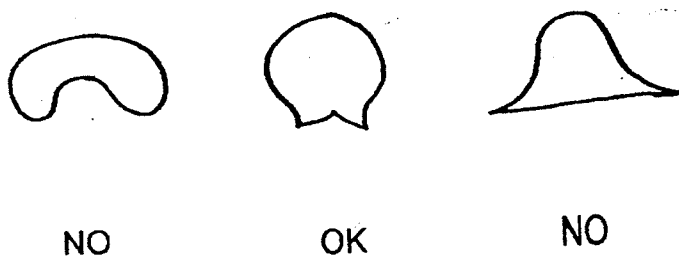
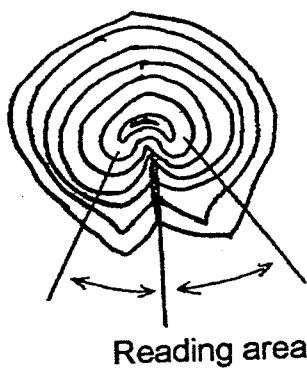
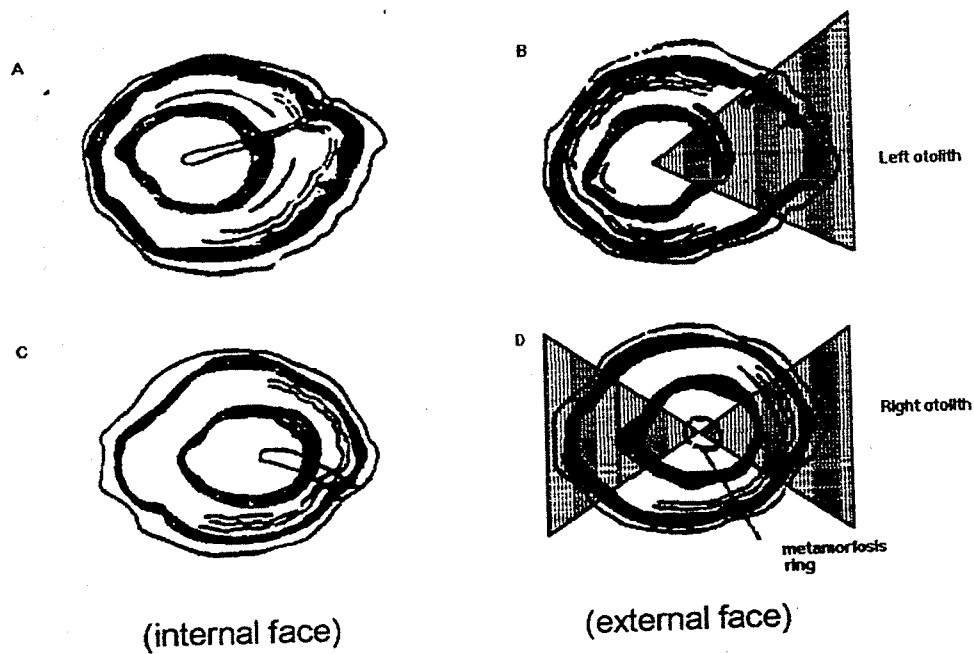


Figure 13.- Otolith and Fin-ray showing recommended areas for ring interpretation. Central cut of finray indicating the right position for reading (below)



Table 1.- Spawning Season of *L. whiffiagonis* in Subareas VI, VII, VIII and IX by different authors

Year	Area	Reproduction Period			
		Initial	Spawning Final	Pick	Recruitment to bottom
Dwivedi	IV-V	March	April-June	May-June	
Furneston	IV-VIIj,h		April-July	May	
Holt	VIIb,j		April-May		
Fontenla and Patiño	VIIj		February-April		
Pérez and Fontenla	VIIj	January	February-April	March	
Aubin-Ottenheimer	VIIj	January	February-April	March	
Aubin-Ottenheimer	VIIj	January	February-April	March	
Dawson	VIIj	January	February-April	March	
Dawson	VIIj	January	February-April	March	
Dwivedi	VIIj	January	February-April	March	
Fontenla and Patiño	VIIj	January	February-April	March	

Table 2.- Mean length at first maturity of *L. whiffiagonis* by different authors

Year	Area	Male			Female			Combined		
		Min.	Length	Age	Min.	Length	Age	Min.	Length	Age
1896	VIIb,j	27 cm			0 cm					
1990	VIIj									
1997	VIIj	14 cm	18 cm	2	8 c	20 cm	3	18 cm	2	2
1986	VIIj		25 cm			34 cm		19 cm	2-3	
1996	VIIj									
1990	VIIj	15 cm	15 cm	2	1 c	22 cm	3			
1965	VIIa,b	24 cm	26 cm		7 cm	29 cm				
1990	VIIc-Ixa		17 cm			18 cm		18 cm	1-2	

Table 3.- Summary of *L. whiffiagonis* growth parameters by ICES Division ( from Landa et al. 1996)

Div.	Author	Year	Period	Males				Females					
				$L_{\infty}$	K	to	n	$r^2$	$L_{\infty}$	K	to	n	$r^2$
VII	Conan et al.	1981	Mar.-Aug.	29,74	0,25	-1,59	6		67,65	0,12	-0,51	210	
	Rodriguez et al.	1984	Annual	39,36	0,29	0,14	181		63,13	0,11	-0,07	444	
	Aubin-Ottenheimer	1985	Annual	38,40	0,34	-0,06	72		60,20	0,14	-0,05	190	
	Moguedet al al.	1987	Annual	43,67	0,14	-1,76	184		65,20	0,09	-1,87	342	
	Peronnet et al.	1987	Annual	51,31	0,10	-1,88	204		66,80	0,11	-0,33	726	
	Peronnet et al.	1988	Annual	44,80	0,14	-1,76	230		66,80	0,11	-0,33	888	
	Peronnet et al.	1989	Annual	53,92	0,09	-2,12	387		69,92	0,10	-1,08	1287	
	Dawson	1990	March	34,40	0,16	-1,65	74	0,82	64,90	0,08	-2,40	192	0,99
	Present work	1991	Annual						66,00	0,11	-0,31	463	0,98
	Present work	1992	Annual	46,00	0,14	-1,25	171	0,99	66,00	0,13	0,38	256	0,98
VIIIa	Present work	1991	Annual	45,00	0,14	-1,85	54	0,99	59,00	0,12	-1,34	54	0,96
	Present work	1992	Annual	45,00	0,23	0,29	180	0,99	59,00	0,19	0,92	128	0,99
VIIIc2	Alperi	1983	Nov.-Dec.	32,37	0,38	-0,77	81		52,25	0,17	-1,58	124	
	Alperi	1984	June	34,29	0,26	-0,72	112		49,59	0,17	-0,82	156	
	Alperi	1990	Sept.	32,44	0,24	-2,20	148		51,52	0,12	-2,68	224	
VIIIc1	Present work	1991	Oct.	38,00	0,21	-0,91	122	0,93	53,00	0,17	-0,59	181	0,98
	Present work	1992	Oct.	38,00	0,20	-1,19	181	0,98	53,00	0,20	0,23	338	0,95
	Present work	1991	Sept.-Oct.	38,00	0,27	-0,22	24	0,98	53,00	0,26	0,17	37	0,99

Cod	Bony part*	Month	Sex	Length	Age R1	Age R2	Age R3	Age R4	Age R5	AgeR6	AgeR7	Age R8	Age R9
7192	1	1	2	30	7	5	7	6	2	6	5	5	6
1086	1	9	2	31	8	6	4	5	4	5	5	6	6
7334	1	1	1	31	7	6	6	5	5	5	5	5	6
136	1	1	2	32	6	6	6	6	5	5	5	5	5
956	1	8	2	32	8	7	6	8	7	6	5	5	4
7389	1	1	2	21	4	4	4	4	2	3	4	4	2
48	1	1	1	22	5	4	4	4	2	4	4	4	2
7391	1	1	2	22	4	3	4	4	3	4	3	4	4
1226	1	10	1	23	5	5	4	4	3	4	4	5	3
66	1	1	2	24	5	5	5	6	3	4	4	4	3
1138	1	9	2	18	2	2	2	1	1	3	2	2	2
36	1	1	1	19	4	3	4	4	2	3	3	2	2
7379	1	1	2	19	3	3	3	3	3	3	3	3	3
791	1	6	2	20	5	4	4	5	3	3	4	4	4
1119	1	9	1	20	4	4	4	5	3	5	3	4	3
1175	1	9	1	13	1	1	1	1	0	1	1	1	1
1170	1	9	2	15	2	1	2	1	1	2	2	2	1
1154	1	9	2	16	2	2	2	2	1	2	2	2	1
1142	1	9	1	17	3	3	2	2	2	3	2	3	2
1247	1	10	1	18	4	3	4	3	2	3	3	4	2
1233	1	10	1	24	6	4	4	4	2	5	4	4	4
772	1	6	2	25	4	5	4	4	4	3	4	4	4
7196	1	1	1	25	6	6	6	6	4	5	4	4	6
845	1	7	2	26	5	5	5	4	3	4	4	5	5
1329	1	10	1	26	4	5	5	4	4	4	4	5	4
697	1	6	2	53	12	9	8	9	8	9	7	13	6
147	1	2	2	54	20		14	12	10	12	11	6	
692	1	6	2	54	15	10	11	10	11	13	8	7	6
155	1	2	2	55	13		12		9	14	9	6	6
177	1	2	2	55	12		10	10	8	13	9	6	6
1	1	11	1	14	1	1	1	2	1	1	2	2	1
2	1	11	1	17	1	1	3	2	1	1	1	2	1
3	1	11	1	18	2	2	2	3	2	3	2	3	2
4	1	11	1	22	3	3	4	5	3	4	3	4	3
5	1	11	1	23	4	3	4	3	3	3	3	3	3
6	1	11	1	26	3	3	3	4	4	4	3	4	3
7	1	11	1	28	9	9	9	7	9	9	5		
8	1	11	1	31	9	9	11	7	9	10	7		6
9	1	11	1	35	12	10	12	9	10	12	7	9	11
10	1	11	1	40	12	6	6	7	7	6	6	5	4
11	1	11	2	12	0	0	0	0	0	0	0	1	0
12	1	11	2	15	1	1	1		1	0	1	2	1
13	1	11	2	18	2	2	2	3	2	2	2	3	2
14	1	11	2	19	4	4	4	5	4	5	3	4	4
15	1	11	2	23	4	3	3	2	2	4	3	3	4
16	1	11	2	24	3	3	3	5	3	4	3	3	3
17	1	11	2	27	3	3	3	4	3	4	3	4	3
18	1	11	2	29	3	3	3	4	3	4	3	4	3
19	1	11	2	31	5	4	5	5	4	6	4	5	3
20	1	11	2	32		3	6	4	3	4	3	3	2
21	1	11	2	34	4	4	4	7	4	5	4	4	4
22	1	11	2	38	8	8	7	8	7	6	6		7
23	1	11	2	40	11	9	11	9	6	7	6		
24	1	11	2	41	9	9	7	9	7	10	7	7	7
25	1	11	2	43	9	8	10	10	8		8	5	
26	1	11	2	46	11	11	10	10	8	11	8	6	7
27	1	11	2	49	13		11	9	8		9		
28	1	11	2	52	10	9	12	10	9	14	9	6	6
29	1	11	2	55	10	9	10	10	10		8	4	
30	1	11	2	56	10		12	11			8	6	
1	2	11	1	14				2	1	0	1		
2	2	11	1	17									
3	2	11	1	18				3	2	1	3		
4	2	11	1	22		2	4	6	3	6	6		
5	2	11	1	23			4	7	3	5	5		
6	2	11	1	26		3	4	6	5	7	6		
7	2	11	1	28			8	7	7	10	6		

Table 4.- First by reader of the m&amp;g rim otolith exchange.

\*Bony part: (1) Otolith (2) Fin-ray.

Cod	Bony part*	Month	Sex	Length	Age R1	Age R2	Age R3	Age R4	Age R5	AgeR6	AgeR7	Age R8	Age R9
8	2	11	1	31		4	5	6	7	9	6		
9	2	11	1	35	9	4	11	8	9	10	9		
10	2	11	1	40	10	6	11	10	10	9	9		
11	2	11	2	12			2	3	1	0	1		
12	2	11	2	15			4	4	1	1	2		
13	2	11	2	18		2	4	4	2	3	2		
14	2	11	2	19		3	4	4	1	3	2		
15	2	11	2	23	5		4	5	2	6	3		
16	2	11	2	24	4	2	4	6	3	4	3		
17	2	11	2	27	3	2	4	4	3	7	3		
18	2	11	2	29	4	2	4	4	3	4	3		
19	2	11	2	31	7	4	7	6	5	10	5		
20	2	11	2	32	7	4	9	5	5	5	6		
21	2	11	2	34		4	8	6	6	5	6		
22	2	11	2	38	9	5	10	7	8	7	7		
23	2	11	2	40	11	4	12	9	9	11	10		
24	2	11	2	41		5	7	7	9	6	7		
25	2	11	2	43		6	15	12	9	10	8		
26	2	11	2	46		6	10	10	12	15	11		
27	2	11	2	49	9	8	15	8	12	15	9		
28	2	11	2	52	12	7	16	10	16	14	9		
29	2	11	2	55	8	7	12	11	12	16	10		
30	2	11	2	56	7	6	14	13	14	12	12		

Table 4.- First by reader of the megrim otolith exchange.

\*Bony part: (1) Otolith (2) Fin-ray.

Cod	Bony part*	Month	Sex	Length	Age R1	Age R2	Age R3	Age R4	Age R5	Age R6	Age R7	Age R8	Age R9	Age R10
7192	1	1	2	30	6	6	7	7	6	6	6	6	6	5
1086	1	9	2	31	7	6	5	7	6	6	5	5	6	6
7334	1	1	1	31	7	7	6	9	10	7	5	6	6	6
136	1	1	2	32	6	6	6	8	6	6	6	6	6	6
956	1	8	2	32	8	7	6	10	8	8	5	6	4	6
7389	1	1	2	21	4	4	4	5	4	4	4	4	4	4
48	1	1	1	22	6	4	4	5	4	5	4	5		4
7391	1	1	2	22	5	3	4	5	3	3	3	4	4	3
1226	1	10	1	23	5	4	4	6	4	5	4	4	3	5
66	1	1	2	24	6	4	5	7	5	5	4	4	4	4
1138	1	9	2	18	3	2	2	4	2	2	2	2	2	2
36	1	1	1	19	4	3	4	3	4	4		4	4	3
7379	1	1	2	19	3	3	3	6	3	3	3	3	3	3
791	1	6	2	20	5	4	4	5	4	4	4	4	4	4
1119	1	9	1	20	5	4	4	5	4	5	3	4	5	3
1175	1	9	1	13	1	1	1	1	1	1	1	1	1	1
1170	1	9	2	15	2	2	1	1	2	2	2	1	1	1
1154	1	9	2	16	2	2	2	2	2	2	2	2	2	1
1142	1	9	1	17	3	3	2	2	3	2		2	2	2
1247	1	10	1	18	4	4	4	3	4	3	3	5	4	3
1233	1	10	1	24	4	3	4	5	5	4	4	3	3	4
772	1	6	2	25	4	4	4	6	4	4	4	4	4	4
7196	1	1	1	25	6	5	6	7	6	6	5	8	6	6
845	1	7	2	26	5	5	5	7	5	5	4	5	5	5
1329	1	10	1	26	5	4	5	6	5	5	4	4	4	4
697	1	6	2	53	12	12	8	12	11	10	9	13	8	12
147	1	2	2	54	9	18	12	16	13	13	12	10	12	14
692	1	6	2	54	12	11	10	13	8	11		10	6	11
155	1	2	2	55	12	13	12	12	11	11		10	8	11
177	1	2	2	55	12	12	10	15	10	10	9	9	8	16
1	1	11	1	14	1	1	1	1	1	1	1	1	1	1
2	1	11	1	17	1	1	1	2	1	1	1	1	1	1
3	1	11	1	18	2	2	2	2	2	2	2	2	2	2
4	1	11	1	22	4	3	4	6	4	3	3	3	3	3
5	1	11	1	23	3	3	4	4	3	3	3	2	1	3
6	1	11	1	26	3	3	3	5	3	3	3	3	3	3
7	1	11	1	28	9	8	9	9	9	7	8	11		8
8	1	11	1	31	11	9	11	10	11	10	8	11	8	8
9	1	11	1	35	14	11	11	13	12	11	11	12	10	9
10	1	11	1	40	14		6	14	12	9	6	4	4	11
11	1	11	2	12	0	0	0	1	0	0	0	0	0	0
12	1	11	2	15	1	1	1		1	1	1	1	1	1
13	1	11	2	18	2	2	2	4	2	2	2	2	2	2
14	1	11	2	19	4	4	4	5	4	4	4	4	4	4
15	1	11	2	23	5	3	3	7	3	4		3	3	2
16	1	11	2	24	3	3	3	6	3	3	3	3	3	3
17	1	11	2	27	4	3	3	7	3	3	3	3	3	3
18	1	11	2	29	3	3	3	8	3	3	3	3	3	3
19	1	11	2	31	4	4	5	8	5	4	5	5	3	5
20	1	11	2	32		3	6	8	4	4		2	2	2
21	1	11	2	34	4	4	4	5	4	4	4	4	4	4
22	1	11	2	38	8	7	7	9	8	6	7	9	8	7
23	1	11	2	40	11	9	11	9	7	8	9	9	9	7
24	1	11	2	41	10	9	7	9	10	8	7	6	7	7
25	1	11	2	43	12	9	10	9	11	9	8			8
26	1	11	2	46	13	10	10	13	12	11	9	11	9	11
27	1	11	2	49	15		11		11	10	9	10	10	10
28	1	11	2	52	13		11	15	11	14	10	7	6	11
29	1	11	2	55	10	9	9	12		10	8	11		12
30	1	11	2	56	11	9		15		10	10	7		0
1	2	11	1	14	4		2	1	0	0	1		1	
2	2	11	1	17										
3	2	11	1	18	3		2	2	2	1	2		1	
4	2	11	1	22	6	6	4	4	3	4	3		3	
5	2	11	1	23	5	5	4	7	3	4	4		3	
6	2	11	1	26	6	5	5	8	4	7	5		4	
7	2	11	1	28	7	5	6	9	8	8	6		11	

Table 5.- Second readings by reader of the megrim otolith exchange.

\*Bony part: (1) Otolith (2) Fin-ray.

Cod	Bony part*	Month	Sex	Length	Age R1	Age R2	Age R3	Age R4	Age R5	AgeR6	AgeR7	Age R8	Age R9	Age R10
8	2	11	1	31	8	7	6	8	8	9	7		6	
9	2	11	1	35	8	8	8	11	10	11	9		11	
10	2	11	1	40	7	9	10	11	9	11	9		9	
11	2	11	1	12	2	1	1	2	1	1	1		0	
12	2	11	2	15		3	1	3	1	4	1		1	
13	2	11	2	18	3			4	2	4	2			
14	2	11	2	19	3		3	3	2	5	2		2	
15	2	11	2	23	7	6	4	5	3	5	3		3	
16	2	11	2	24		5	5	5	3	6	3		3	
17	2	11	2	27	4	3	5	5	3	7	3		2	
18	2	11	2	29	5	5	4	5	3	6	4		2	
19	2	11	2	31	7	6	4	6	6	6	6		4	
20	2	11	2	32	6	6	8	8	6	5	7		7	
21	2	11	2	34	7	8	6	9	6	6	7		5	
22	2	11	2	38	8	7	7	8	8	8	7		7	
23	2	11	2	40	11	9	8	11	10	11	11		11	
24	2	11	2	41	13	8	11	12	9	9	8		8	
25	2	11	2	43	14	9	12	15	11	9	11		6	
26	2	11	2	46	18	10	8	13	12	10	10		10	
27	2	11	2	49	15	15	14	15	12	14	11		8	
28	2	11	2	52	13	11	10	16	16	10	12		15	
29	2	11	2	55	7	7	11	13	11	12	10		7	
30	2	11	2	56	10	6	12	15	13	10	12		13	

Table 5.- Second readings by reader of the megrim otolith exchange.

\*Bony part: (1) Otolith (2) Fin-ray.

Cod	Bony part*	Month	Sex	Length	AgeR1	AgeR2	AgeR3	AgeR4	AgeR5	AgeR6	AgeR7	AgeR8	AgeR9	AgeR10
107	1	1	2	33	8	5	5	7	5	5	5	8	5	5
1369	1	9	2	33	6	5	5	6	5	6	6	5	6	6
733	1	1	1	34	9	6	6	8	6	5	6	6	6	6
7371	1	1	2	34	7	5	7	7		5	7	7	6	7
205	1	8	2	35		6	7	7		6				7
993	1	1	2	28	10	7	6	9	6	7	6	7	9	7
7198		1	1	28	6	5	5	7	6	5	5	5	5	5
7381	1	1	2	29	9	6	5	9	7	7	6	5	5	7
7112	1	10	1	29	6	6	6	6	6	5	6	6	5	6
1387	1	1	2	30		6	5	8		8	7		5	7
206	1	9	2	41	7	7	6	5	5	6	6	5	5	7
952	1	1	1	41	12	11	9	12	9	9	8	15	8	16
161	1	1	2	42	12	11	8	6		7	7	7		8
1412	1	6	2	42	11	9	9	10	8	9	9	10	8	9
717	1	9	1	43	12	9	7	9		9	7	8	7	8
696	1	9	1	51	11	10	10	10	9	8	8	8	7	8
831	1	9	2	51	15	14	11	11	10	13	14	15		20
694	1	9	2	52	11	11	10	13	11	10	10	9	8	9
699	1	9	1	52	14	10	9	10	8	8	9	8	8	9
158	1	10	1	53	14	11	10	11	13	10	10	9	10	9
1	1	11	1	17	1	1	1	1	1	1	1	1	1	1
2	1	11	1	20	3	3	4	4	3	3	3	3	3	4
4	1	11	1	26	7	5	5	5	5	4	5	3	3	5
5	1	11	1	27	6	4	4	5	6	4	4	4	4	5
12	1	11	2	17	1	1	1	1	1	1	1	1	1	1
13	1	11	2	20	2	2	2	2	2	2	2	2	2	2
14	1	11	2	25	10	7	5	6	7	9	5	8	6	5
15	1	11	2	31	6	8	4	5	4	5	4	4	4	4
16	1	11	2	32	8	7	6	7	6	6	6	6	6	6
17	1	11	2	34	15	10	9	10	10	10	7	11	7	10
21	1	11	2	39	14	13	10	12	11	12	9	11	10	12
22	1	11	2	40	11	7	8	9	8	8	9	8	9	8
23	1	11	2	41	10	10	9	10	9	9	8	8	9	8
24	1	11	2		10	9	9	9	10	9	8	9	9	8
25	1	11	2	45	12	9	9	11	10	8	8	12	8	10
26	1	11	2	47	13	10	11	10	10	11	11	13	11	9
27	1	11	2	48	8	8	8	9	8	8	8	7	7	9
28	1	11	2	51	12	13	9	11	11	9	10	9	8	10
29	1	11	2	52	10		12	11		11		11		
30	1	11	2	55	14	12	11	12	14	11	11	10	9	10
1402	1	2	2	22	4	4	4	5	4	4	4	4	4	4
1411	1	2	2	20	4	4	4	4	4	4	4	4	4	4
1442	1	2	2	25	6	6	6	6	6	6	6	6	6	6
1357	1	10	2	27	9	6	6	10	8	8	7	9	8	7
1214	1	10	1	24	7	5	5	6	5	6	5	8	6	6
1215	1	10	1	21	5	5	5	5	5	4	5	4	4	4
1216	1	10	1	20	5	4	4	5	5	4	4	4	3	4
1245	1	10	1	18	4	4	4	6	4	6	4	5	5	4
1246	1	10	2	24	7	6	5	7	8	6	5	8	7	7
1249	1	10	1	26	10	10	5	8	9	9	7	9	8	9
1	2	11	1	17	2	1	1	1	1	1	1		1	
2	2	11	1	20	3	2	3	3	3	2	2		1	
4	2	11	1	26	4	5	4	5	3	3	4		2	
5	2	11	1	27	5	5	3	6	4	5	4		3	
12	2	11	2	17	2	1	1	4	1	4	1		1	
13	2	11	2	20	3	3	2	4	2	5	2		2	
14	2	11	2	25	7	4	3	6	6	7	4		3	
15	2	11	2	31	8	9	6	12	5	10	6		7	
16	2	11	2	32	8	6	5	8	5	8	6		5	
17	2	11	2	34	17	11	7	14	6	7	6		6	
21	2	11	2	39	9	8	10	12	10	13	7		9	
22	2	11	2	40	10	8	9	13	8	12	9		7	
23	2	11	2	41	16	7	8	8	8	10	8		7	
25	2	11	2	45	21	12	9	13	10	11	9		10	
26	2	11	2	47	12	9	9	13	9	10	9		8	
27	2	11	2	48			8	11	8	10	8		9	
28	2	11	2	51	12	12	10	11	10	12	11		10	
29	2	11	2	52	11	13	16	12	14	17	11		13	
30	2	11	2	55	17	10	10	15	10	11	11			

Table 6 .- Second sample. Readings by reader of the megrim otolith.

\* Bony part: (1) Otolith (2) Fin-ray.

Table 7 .- Percentage of Agreement between readers in the first and second sample (otoliths and fin-rays).

First reading, first sample (otoliths)									
* * *	R1	R2	R3	R4	R5	R6	R7	R8	R9
R1	56	56	32	31	27	32	32	42	
R2		53	30	44	30	55	41	47	
R3			33	32	27	40	40	45	
R4				23	41	33	36	24	
R5					21	48	24	55	
R6						36	31	23	
R7							40	43	
R8								37	
R9									

Second reading, first sample (otoliths)									
* * *	R1	R2	R3	R4	R5	R6	R7	R8	R9
R1	50	48	23	56	53	39	36	44	
R2		54	16	57	56	67	53	58	
R3			18	64	49	56	59	58	
R4				11	17	11	16	15	
R5					60	51	51	32	
R6						54	48	51	
R7							55	53	
R8								64	
R9									

Second reading, first sample (fin-rays)									
* * *	R1	R2	R3	R4	R5	R6	R7	R8	R9
R1	27	10	27	13	13	10	10		
R2		23	20	23	20	33	23		
R3			23	20	23	33	20		
R4				20	30	17	13		
R5					27	43	33		
R6						20	17		
R7							43		
R9									

First reading, first sample (fin-rays)									
* * *	R1	R2	R3	R4	R5	R6	R7		
R1	23	20	13	13	20	13			
R2		10	7	7	13	7			
R3			27	13	17	7			
R4				20	17	27			
R5					10	33			
R6						20			
R7									

Second sample (otoliths)									
* * *	R1	R2	R3	R4	R5	R6	R7	R8	R9
R1	30								
R2		46							
R3			28						
R4				26					
R5					36				
R6						46			
R7							38		
R8								42	
R9									

Second sample (fin-rays)									
* * *	R1	R2	R3	R4	R5	R6	R7	R8	R9
R1	21								
R2		21							
R3			16						
R4				21					
R5					11				
R6						16			
R7							21		
R9									

\* Stock Assessment readers

\* Stock Assessment readers



Table 8.- Wilcoxon Match-Pairs Signed-Ranks Test for the two readings of first sample.

	Otoliths									
	First reading									
	R1	R2	R3	R4	R5	R6	R7	R8	R9	
R1	■	**	*	*	**	*	**	**	**	**
R2	**	■	*	ns	**	ns	**	ns	**	**
R3	*	*	■	ns	**	ns	**	**	**	**
R4	*	ns	ns	■	**	ns	**	**	**	**
R5	**	**	**	**	■	**	ns	ns	ns	ns
R6	*	ns	ns	ns	**	■	**	ns	**	**
R7	**	**	**	**	ns	**	■	ns	ns	ns
R8	**	ns	**	**	ns	ns	ns	■	**	**
R9	**	**	**	**	ns	**	ns	ns	**	■

Otoliths	Fin Rays
First reading	First reading

	R1	R2	R3	R4	R5	R6	R7
R1		**	**	ns	ns	ns	ns
R2	**		**	**	**	**	**
R3	**	**		ns	**	ns	**
R4	ns	**	ns		ns	ns	**
R5	ns	**	**	ns		*	ns
R6	ns	**	ns	ns	*		**
R7	ns	**	**	**	ns	**	

## Consistency table

First reading	Fin Rays
R1	ns
R2	**
R3	**
R4	*
R5	*
R6	ns
R7	**

	First reading			
	ot R2	ot R3	fin R5	
ot R2		*	ns	
ot R3			ns	
fin R5		ns	ns	

Otoliths  
Second reading

	R1	R2	R3	R4	R5	R6	R7	R8	R9
R1		**	**	**	**	**	**	**	**
R2	**		**	**	ns	ns	**	ns	**
R3	**	**		**	ns	ns	**	ns	**
R4	**	**	**		**	**	**	**	**
R5	**	ns	ns	**		ns	**	ns	**
R6	**	ns	ns	**	**		**	ns	**
R7	**	**	**	**	**	**		ns	ns
R8	**	ns	ns	**	ns	ns	ns		**
R9	**	**	**	**	**	**	ns	**	

**Fin Rays**  
**Second reading**

	R1	R2	R3	R4	R5	R6	R7
R1		**	*	ns	ns	ns	**
R2	**		ns	**	ns	*	ns
R3	*			**	ns	ns	ns
R4	ns	**	**		**	*	**
R5	ns	ns	ns	**		ns	ns
R6	ns	*	ns	*	ns		*
R7	**	ns	ns	**	ns	*	

## Consistency table

Second reading	Fin Rays
R1	ns
R2	ns
R3	ns
R4	ns
R5	ns
R6	**
R7	*
R9	ns

	Second reading		
	ot R2	ot R3	fin R5
ot R2		ns	*
ot R3	ns		ns
fin R5	*	ns	

Table 9.- Wilcoxon Match-Pairs Signed-Ranks Test for the reading of Second sample.

# Otoliths

	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
R1	**	**	**	**	**	**	**	**	**	**
R2	**	**	**	**	**	**	**	**	**	**
R3	**	**	**	**	**	**	**	**	**	**
R4	**	**	**	**	**	**	**	**	**	**
R5	**	**	**	**	**	**	**	**	**	**
R6	**	**	**	**	**	**	**	**	**	**
R7	**	**	**	**	**	**	**	**	**	**
R8	**	**	**	**	**	**	**	**	**	**
R9	**	**	**	**	**	**	**	**	**	**
R10	**	**	**	**	**	**	**	**	**	**

# Consistency table

Second sample	Fin Rays
R1	ns
R2	ns
R3	ns
R4	**
R5	**
R6	*
R7	ns
R9	ns

Second sample	ot R2	ot R3	fin R5
ot R2	**	**	**
ot R3	**	**	ns
fin R5	**	ns	ns

# Fin Rays

	R1	R2	R3	R4	R5	R6	R7	R9
R1	**	**	**	**	**	**	**	**
R2	**	**	**	**	**	**	**	**
R3	**	**	**	**	**	**	**	**
R4	**	**	**	**	**	**	**	**
R5	**	**	**	**	**	**	**	**
R6	**	**	**	**	**	**	**	**
R7	**	**	**	**	**	**	**	**
R9	**	**	**	**	**	**	**	**

ns: no sign of bias ( $p > 0.05$ )  
 \*: possibility of bias ( $0.01 < p < 0.05$ )  
 \*\*: certainty of bias ( $p < 0.01$ )

Table 10.- Index of Beamish and Fournier (APE) for the three readings of otoliths and fin-rays, . Including the Index for the readers who are involved in Stock Assessment (\*)

Readings	Otoliths	Otoliths vs fin rays	Fin Rays	R2 (oto.) & R3 (oto.) & R5 (fin- ray) (*)
Sample 1, First reading	21	26	26	23
Sample 1, Second reading	17	21	21	15
Sample 2, First reading	11	10	23	9

# MEGRIM EXCHANGE SAMPLE FIRST READINGS (OTOLITHS)

Table 11.- By modal age are presented the average age recorded, 2\*stdev and number of age readings by reader and of all readers.

## READER 1

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,00	2,17	3,33	4,45	5,75	9,00	9,00	9,00	10,60	10,00	11,00	16,00	-	-	-
2*stdev	####	0,00	0,82	1,00	1,38	1,91	8,49	####	####	3,63	####	####	11,31	-	-	-
n	1	4	6	9	11	4	2	1	1	5	1	1	2	0	0	0

## READER 2

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,00	2,00	3,00	4,18	5,25	6,00	9,00	8,00	9,00	9,00	11,00	10,00	-	-	-
2*stdev	####	0,00	1,26	0,00	1,21	1,91	0,00	####	####	0,00	####	####	####	-	-	-
n	1	4	6	10	11	4	2	1	1	4	1	1	1	0	0	0

## READER 3

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,50	2,00	3,60	4,09	5,50	6,00	7,00	10,00	10,20	10,00	10,00	13,00	-	-	-
2*stdev	####	2,00	0,00	1,93	0,60	1,15	0,00	####	####	3,29	####	####	2,83	-	-	-
n	1	4	6	10	11	4	2	1	1	5	1	1	2	0	0	0

## READER 4

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,67	2,00	3,70	4,55	5,00	6,50	9,00	10,00	8,40	10,00	10,00	10,50	-	-	-
2*stdev	####	1,15	1,79	1,90	1,87	1,63	1,41	####	####	2,68	####	####	4,24	-	-	-
n	1	3	6	10	11	4	2	1	1	5	1	1	2	0	0	0

## READER 5

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	0,75	1,50	2,90	3,09	4,25	5,50	7,00	8,00	8,60	10,00	8,00	10,00	-	-	-
2*stdev	####	1,00	1,10	1,14	1,66	1,91	4,24	####	####	1,10	####	####	0,00	-	-	-
n	1	4	6	10	11	4	2	1	1	5	1	1	2	0	0	0

## READER 6

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	0,75	2,50	3,70	4,09	5,00	5,50	10,00	-	10,50	-	11,00	12,00	-	-	-
2*stdev	####	1,00	1,10	0,97	1,66	1,63	1,41	####	-	4,76	-	####	0,00	-	-	-
n	1	4	6	10	11	4	2	1	0	4	0	1	2	0	0	0

## READER 7

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,25	2,00	3,00	3,73	4,50	5,00	7,00	8,00	7,40	8,00	8,00	9,00	-	-	-
2*stdev	####	1,00	0,00	0,00	0,93	1,15	2,83	####	####	3,35	####	####	5,66	-	-	-
n	1	4	6	10	11	4	2	1	1	5	1	1	2	0	0	0

## READER 8

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	1,00	1,75	2,50	3,50	4,18	5,00	4,50	7,00	5,00	9,50	4,00	6,00	7,50	-	-	-
2*stdev	####	1,00	1,10	1,05	0,81	0,00	1,41	####	####	9,90	####	####	4,24	-	-	-
n	1	4	6	10	11	4	2	1	1	2	1	1	2	0	0	0

## READER 9

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,00	1,67	2,90	3,45	4,75	5,00	7,00	-	6,00	-	7,00	11,00	-	-	-
2*stdev	####	0,00	1,03	1,14	1,64	2,52	2,83	####	-	0,00	-	####	####	-	-	-
n	1	4	6	10	11	4	2	1	0	3	0	1	1	0	0	0

## ALL READERS

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,11	1,17	2,04	3,29	3,98	5,00	5,89	8,00	8,29	8,97	8,71	9,11	11,06	-	-	-
2*stdev	0,67	1,14	1,16	1,32	1,59	1,72	3,62	2,45	3,41	4,12	4,43	3,80	6,26	-	-	-
n	9	35	54	89	99	36	18	9	7	38	7	9	16	0	0	0

# MEGRIM EXCHANGE SAMPLE FIRST READINGS (RAYS)

Table.- 12 By modal age are presented the average age recorded, 2\*stdev and number of age readings by reader and of all readers.

## READER 1

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	-	-	3,00	4,00	6,00	-	9,00	-	9,00	10,00	-	8,00	-	-	-
2*stdev	-	-	-	####	0,00	2,83	-	####	-	####	####	-	####	-	-	-
n	0	0	0	1	2	2	0	1	0	1	1	0	1	0	0	0

## READER 2

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	-	2,00	2,00	2,00	4,00	3,25	5,00	-	4,00	6,00	-	7,00	-	-	-
2*stdev	-	-	####	####	0,00	####	1,91	0,00	-	####	0,00	-	####	-	-	-
n	0	0	1	1	2	1	4	2	0	1	2	0	1	0	0	0

## READER 3

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	2,00	4,00	4,00	4,00	5,67	5,25	8,33	-	11,00	10,50	-	12,00	-	-	-
2*stdev	-	####	####	####	0,00	5,77	3,79	3,06	-	####	1,41	-	####	-	-	-
n	0	1	1	1	2	3	4	3	0	1	2	0	1	0	0	0

## READER 4

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	2,50	4,00	3,50	5,00	5,67	6,00	7,00	-	8,00	10,00	-	11,00	-	-	-
2*stdev	-	1,41	####	1,41	2,83	2,31	0,00	0,00	-	####	0,00	-	####	-	-	-
n	0	2	1	2	2	3	4	3	0	1	2	0	1	0	0	0

## READER 5

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	2,50	3,00	3,33	5,25	8,00	-	9,00	11,00	-	12,00	-	-	-
2*stdev	-	0,00	####	1,41	0,00	3,06	3,42	2,00	-	####	2,83	-	####	-	-	-
n	0	2	1	2	2	3	4	3	0	1	2	0	1	0	0	0

## READER 6

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	0,00	3,00	4,00	4,00	5,33	6,75	7,67	-	10,00	12,00	-	16,00	-	-	-
2*stdev	-	0,00	####	8,49	0,00	1,15	3,42	4,16	-	####	8,49	-	####	-	-	-
n	0	2	1	2	2	3	4	3	0	1	2	0	1	0	0	0

## READER 7

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	3,00	3,00	4,67	6,00	6,67	-	9,00	10,00	-	10,00	-	-	-
2*stdev	-	0,00	####	0,00	0,00	3,06	0,00	1,15	-	####	2,83	-	####	-	-	-
n	0	2	1	2	2	3	4	3	0	1	2	0	1	0	0	0

## ALL READERS

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,22	2,83	3,18	3,57	5,00	5,42	7,33	-	8,57	9,92	-	10,86	-	-	-
2*stdev	-	1,94	1,97	3,07	2,03	3,29	3,23	2,91	-	4,45	4,65	-	5,94	-	-	-
n	0	9	6	11	14	18	24	18	0	7	13	0	7	0	0	0

# MEGRIM EXCHANGE SAMPLE SECOND READING (OTOLITHS)

Table 13.- By modal age are presented the average age recorded, 2\*stdev and number of age readings by reader and of all readers.

## READER 1

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,00	2,33	3,67	4,55	4,50	6,25	10,00	8,00	10,67	12,67	12,67	14,33	-	14,00	-
2*stdev	####	0,00	1,03	1,73	1,64	1,41	1,00	####	####	3,06	4,16	3,06	8,08	-	####	-
n	1	4	6	9	11	2	4	1	1	3	3	3	3	0	1	0

## READER 2

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,00	2,17	3,00	3,82	4,50	5,75	9,00	7,00	8,67	10,50	10,00	14,33	-	-	-
2*stdev	####	0,00	0,82	0,00	0,81	1,41	1,00	####	####	1,15	4,24	2,83	6,43	-	-	-
n	1	4	6	9	11	2	4	1	1	3	2	2	3	0	0	0

## READER 3

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,00	1,83	3,33	4,09	5,00	6,00	7,00	6,00	10,00	10,50	11,00	10,67	-	6,00	-
2*stdev	####	0,00	0,82	1,00	0,60	0,00	1,63	####	####	2,00	1,41	0,00	4,62	-	####	-
n	1	4	6	9	11	2	4	1	1	3	2	3	3	0	1	0

## READER 4

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	1,00	1,33	2,50	6,00	5,00	7,50	7,25	9,00	10,00	9,00	15,00	12,67	13,33	-	14,00	-
2*stdev	####	1,15	2,45	2,45	2,37	1,41	1,00	####	####	0,00	0,00	5,03	4,62	-	####	-
n	1	3	6	9	11	2	4	1	1	3	2	3	3	0	1	0

## READER 5

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,00	2,17	3,11	4,18	5,00	6,00	10,00	8,00	9,00	10,50	11,33	11,67	-	12,00	-
2*stdev	####	0,00	0,82	0,67	0,81	0,00	0,00	####	####	4,00	1,41	1,15	2,31	-	####	-
n	1	4	6	9	11	2	4	1	1	3	2	3	3	0	1	0

## READER 6

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,00	2,00	3,11	4,18	4,50	6,00	8,00	8,00	8,00	10,00	11,67	11,33	-	9,00	-
2*stdev	####	0,00	0,00	0,67	1,21	1,41	0,00	####	####	2,00	0,00	4,16	3,06	-	####	-
n	1	4	6	9	11	2	4	1	1	3	3	3	3	0	1	0

## READER 7

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,00	2,00	3,00	3,90	4,50	5,50	7,00	5,00	8,33	9,33	9,67	10,50	-	6,00	-
2*stdev	####	0,00	0,00	0,00	0,63	1,41	1,15	####	####	1,15	1,15	3,06	4,24	-	####	-
n	1	4	5	8	10	2	4	1	1	3	3	3	2	0	1	0

## READER 8

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,00	1,83	3,00	4,09	5,00	6,25	6,00	6,00	10,00	8,67	10,00	11,00	-	4,00	-
2*stdev	####	0,00	0,82	1,00	1,08	0,00	2,52	####	####	2,83	3,06	5,29	3,46	-	####	-
n	1	4	6	9	11	2	4	1	1	2	3	3	3	0	1	0

## READER 9

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,00	1,83	2,89	3,80	4,00	6,00	7,00	4,00	9,00	9,00	8,00	9,33	-	4,00	-
2*stdev	####	0,00	0,82	1,56	0,84	2,83	0,00	####	####	####	2,83	4,00	4,62	-	####	-
n	1	4	6	9	10	2	4	1	1	1	2	3	3	0	1	0

## READER 10

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,00	1,00	1,67	2,89	3,91	5,00	5,75	7,00	6,00	7,67	13,00	9,33	12,33	-	11,00	-
2*stdev	####	0,00	1,03	0,67	1,08	0,00	1,00	####	####	1,15	8,49	3,06	3,06	-	####	-
n	1	4	6	9	11	2	4	1	1	3	2	3	3	0	1	0

## ALL READERS

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	0,10	1,03	2,03	3,40	4,16	4,95	6,08	8,00	6,80	9,00	10,79	10,66	11,93	-	8,89	-
2*stdev	0,63	0,32	1,11	2,15	1,37	2,10	1,39	2,83	3,50	2,60	4,64	4,15	5,10	-	8,09	-
n	10	39	59	89	108	20	40	10	10	27	24	29	29	0	9	0

# MEGRIM EXCHANGE SAMPLE SECOND READINGS (RAYS)

Table 14.- By modal age are presented the average age recorded, 2\*stdev and number of age readings by reader and of all readers.

## READER 1

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	2,00	3,00	3,50	5,50	6,00	6,67	-	8,00	7,00	18,00	11,00	-	-	-	15,00
2*stdev	-	####	####	1,41	1,41	2,00	1,15	-	0,00	####	####	####	-	-	-	####
n	0	1	1	2	2	3	3	0	3	1	1	1	0	0	0	1

## READER 2

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	2,00	-	3,00	5,50	5,25	6,67	-	7,33	9,00	10,00	9,00	-	-	-	15,00
2*stdev	-	2,83	-	####	1,41	1,00	2,31	-	1,15	####	####	####	-	-	-	####
n	0	2	0	1	2	4	3	0	3	1	1	1	0	0	0	1

## READER 3

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	4,00	4,00	4,50	6,00	-	7,00	10,00	8,00	8,00	-	-	-	14,00
2*stdev	-	0,00	####	2,83	0,00	1,15	4,00	-	2,00	####	####	####	-	-	-	####
n	0	2	1	2	2	4	3	0	3	1	1	1	0	0	0	1

## READER 4

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	2,50	2,00	4,00	5,50	5,75	7,67	-	9,00	11,00	13,00	11,00	-	-	-	15,00
2*stdev	-	1,41	####	2,83	4,24	3,00	3,06	-	3,46	####	####	####	-	-	-	####
n	0	2	1	2	2	4	3	0	3	1	1	1	0	0	0	1

## READER 5

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	2,50	3,00	3,25	6,00	-	8,67	9,00	12,00	10,00	-	-	-	12,00
2*stdev	-	0,00	####	1,41	0,00	1,00	0,00	-	2,31	####	####	####	-	-	-	####
n	0	2	1	2	2	4	3	0	3	1	1	1	0	0	0	1

## READER 6

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	2,50	1,00	6,00	4,00	6,00	5,67	-	9,33	11,00	10,00	11,00	-	-	-	14,00
2*stdev	-	4,24	####	2,83	0,00	1,63	1,15	-	3,06	####	####	####	-	-	-	####
n	0	2	1	2	2	4	3	0	3	1	1	1	0	0	0	1

## READER 7

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	2,50	3,50	3,75	6,67	-	7,67	9,00	10,00	11,00	-	-	-	11,00
2*stdev	-	0,00	####	1,41	1,41	1,91	1,15	-	2,31	####	####	####	-	-	-	####
n	0	2	1	2	2	4	3	0	3	1	1	1	0	0	0	1

## READER 9

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	0,50	1,00	2,00	3,00	3,00	5,33	-	8,00	9,00	10,00	11,00	-	-	-	8,00
2*stdev	-	1,41	####	0,00	0,00	1,63	3,06	-	5,29	####	####	####	-	-	-	####
n	0	2	1	2	2	4	3	0	3	1	1	1	0	0	0	1

## ALL READERS

Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,53	1,86	3,47	4,25	4,65	6,33	-	8,13	9,38	11,38	10,25	-	-	-	13,00
2*stdev	-	2,12	1,38	2,91	2,48	2,81	2,41	-	2,85	2,60	6,14	2,33	-	-	-	5,01
n	0	15	7	15	16	31	24	0	24	8	8	8	0	0	0	8

# MEGRIM SECOND OTOLITH SAMPLE

Table 15 .- By modal age are presented the average age recorded, 2\*stdev and number of age readings by reader and of all readers.

<b>READER 1</b>																
Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	3,00	4,83	7,17	7,00	9,00	10,83	10,83	14,50	12,33	14,00	-	-	-
2*stdev	-	0,00	####	####	1,97	3,44	2,83	4,90	4,27	1,97	1,41	4,16	####	-	-	-
n	0	2	1	1	6	6	5	4	6	6	2	3	1	0	0	0
<b>READER 2</b>																
Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	3,00	4,67	5,67	6,00	7,00	8,33	10,33	10,50	11,00	13,00	-	-	-
2*stdev	-	0,00	####	####	3,27	2,07	1,41	4,69	3,27	3,01	1,41	2,83	####	-	-	-
n	0	2	1	1	6	6	5	5	6	6	2	2	1	0	0	0
<b>READER 3</b>																
Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	4,00	4,00	5,17	5,80	6,60	8,33	8,33	9,50	11,33	10,00	-	-	-
2*stdev	-	0,00	####	####	0,00	0,82	0,89	2,28	2,73	3,27	1,41	1,15	####	-	-	-
n	0	2	1	1	6	6	5	5	6	6	2	3	1	0	0	0
<b>READER 4</b>																
Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	4,00	5,00	5,83	6,60	7,20	9,83	10,00	10,50	11,00	12,00	-	-	-
2*stdev	-	0,00	####	####	1,26	1,97	1,79	2,19	1,51	2,83	1,41	2,00	####	-	-	-
n	0	2	1	1	6	6	5	5	6	6	2	3	1	0	0	0
<b>READER 5</b>																
Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	3,00	4,50	5,50	5,80	7,00	8,50	9,33	11,50	12,00	11,00	-	-	-
2*stdev	-	0,00	####	####	1,67	1,67	0,89	2,83	1,67	2,07	4,24	5,66	####	-	-	-
n	0	2	1	1	6	6	5	2	6	6	2	2	1	0	0	0
<b>READER 6</b>																
Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	3,00	4,50	5,50	5,60	6,20	8,00	9,00	10,00	11,00	12,00	-	-	-
2*stdev	-	0,00	####	####	1,67	3,74	1,10	1,67	0,00	0,00	0,00	0,00	####	-	-	-
n	0	2	1	1	6	6	5	5	6	6	2	3	1	0	0	0
<b>READER 7</b>																
Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	3,00	4,00	5,17	6,00	6,25	8,17	8,33	8,50	11,00	9,00	-	-	-
2*stdev	-	0,00	####	####	0,00	0,82	0,00	1,91	1,51	2,07	4,24	0,00	####	-	-	-
n	0	2	1	1	6	6	5	4	6	6	2	2	1	0	0	0
<b>READER 8</b>																
Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	3,00	4,17	5,50	5,80	7,25	8,67	10,00	10,00	11,33	11,00	-	-	-
2*stdev	-	0,00	####	####	0,82	4,15	0,89	1,00	3,50	5,06	2,83	3,06	####	-	-	-
n	0	2	1	1	6	6	5	4	6	6	2	3	1	0	0	0
<b>READER 9</b>																
Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	3,00	4,00	4,67	5,80	7,33	7,83	8,33	8,50	10,00	10,00	-	-	-
2*stdev	-	0,00	####	####	1,26	2,07	0,89	3,06	1,51	1,03	4,24	2,83	####	-	-	-
n	0	2	1	1	6	6	5	3	6	6	2	2	1	0	0	0
<b>READER 10</b>																
Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	4,00	4,17	5,17	6,00	7,20	8,50	10,00	9,50	9,50	12,00	-	-	-
2*stdev	-	0,00	####	####	0,82	1,97	0,00	0,89	2,10	6,07	1,41	1,41	####	-	-	-
n	0	2	1	1	6	6	5	5	6	6	2	2	1	0	0	0
<b>ALL READERS</b>																
Modal age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age recorded	-	1,00	2,00	3,30	4,38	5,53	6,04	7,07	8,70	9,45	10,30	11,12	11,40	-	-	-
2*stdev	-	0,00	0,00	0,97	1,61	2,64	1,45	2,91	2,91	3,44	3,90	2,67	3,01	-	-	-
n	0	20	10	10	60	60	50	42	60	60	20	25	10	0	0	0



**MEGRIM OTOLITH CATALOGUE USED DURING THE  
WORKSHOP FOR ESTABLISHING THE READING  
CRITERIA.**

Name	Institution	Country	Workshop Reader #	Color Code
Mark Etherton	CEFAS	United Kingdom	R1	
Afra Egan	Marine Institute	Ireland	R2	
Amaia Gomez de Segura	AZTI	Basque Country (Spain)	R3	
Peter Vingaard Larsen	DIFRES	Denmark	R4	
Marina Santurtún	AZTI	Basque Country (Spain)	R5	
Ayesha Power	Marine Institute	Ireland	R6	
Sally Warne	CEFAS	United Kingdom	R7	
Antonio Marçal	IPIMAR	Portugal	R8	

Image	Quarter	Otolith Code	Month	Length (cm)	Sex
1	1	24/03/99.2(M)1	3	17	1
2	1	31/03/98.5(H)7	3	18	2
3	1	31/03/98.5(H)12	3	19	2
4	1	31/03/98.5(H)15	3	20	2
5	1	31/03/98.5(H)25	3	21	2
6	1	31/03/98.5(H)32	3	22	2
7	1	24/03/99.2(M)54	3	24	1
8	1	22/02/99.1(M)2	2	25	2
9	1	22/02/99.1(H)6	2	26	2
10	a b	24/03/99.1(M)38	3	34	2
11	a b	05/03/01.1(H)6	2	38	2
12	1	31/03/98.1(H)5	3	40	2
13	1	11/03/99.1(H)1	3	41	2
14	a b	31/03/98.1(H)8	3	42	2
15	a b	31/03/98.1(H)16	3	45	2
16	a b	11/03/99.1(H)11	3	46	2
17	3	05/08/98.1(M)14	3	19	1
18	3	05/08/98.1(H)35	3	22	2
19	3	05/08/98.1(M)47	3	24	1
20	3	05/08/98.2(M)6	3	27	1
21	a b	05/08/98.2(M)16	3	29	1
22	3	05/08/98.2(M)21	3	30	1
23	a b	05/08/98.2(H)27	10	31	2
24	3	05/08/98.2(M)44	10	33	1
25	a b	30/09/99.2(H)12	10	34	2
26	a b	30/09/99.2(H)18	10	35	2
27	3	30/09/99.2(H)34	10	38	2
28	4	30/10/02.1(M)37	10	24	1
29	4	30/10/02.1(M)46	2	25	1
30	a b	21/10/99.3(M)3	2	26	1
31	a b	21/10/99.3(M)8	2	27	1
32	4	21/10/99.3(M)21	2	29	1
33	4	21/10/99.3(M)28	1	31	1
34	4	21/10/99.3(H)30	3	32	2
35	a b	21/10/99.2(H)52	4	39	2
36	4	29/10/98.2(H)19	4	36	2
37	a b	19/10/98.2(H)21	4	37	2
38	4	21/10/99.1(H)3	5	40	2
39	4	19/10/98.3(H)5	11	41	2

a left otolith

right otolith

Just shaded images are included in the catalogue as marked during the workshop.

1<sup>st</sup> QUARTER Image 2



Image 3



Image 4

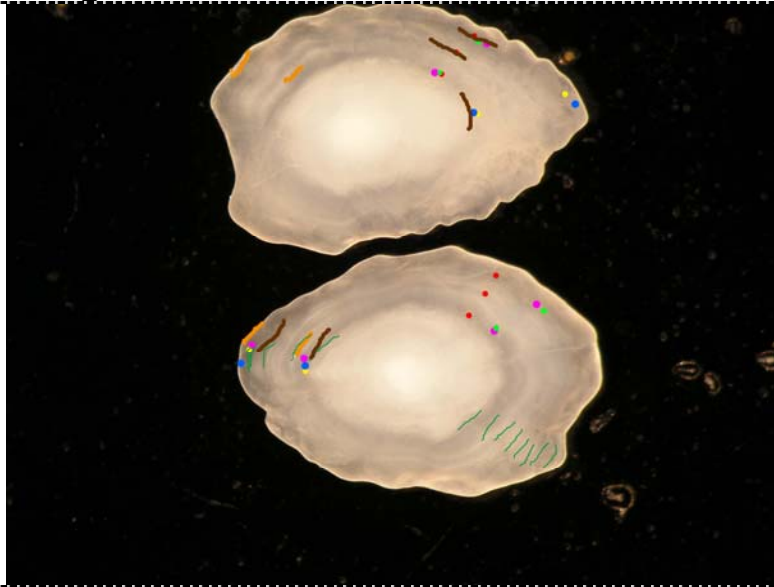


Image 6

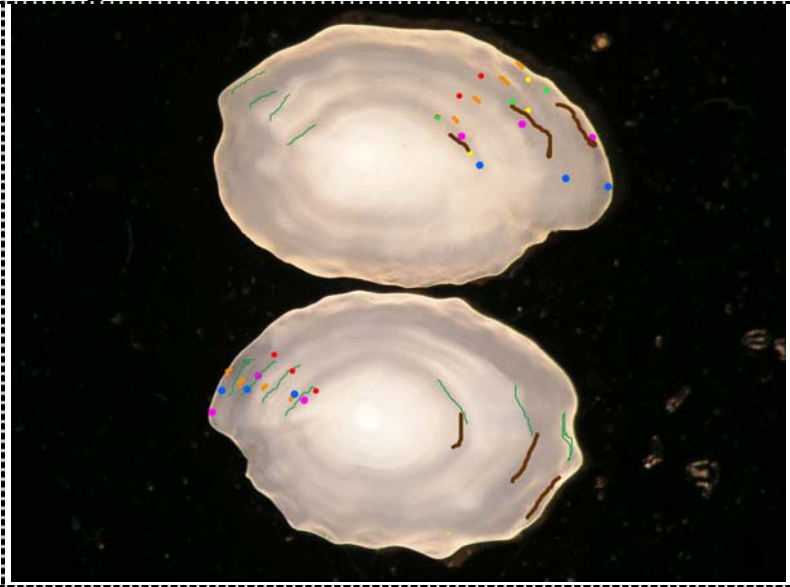


Image 10 a

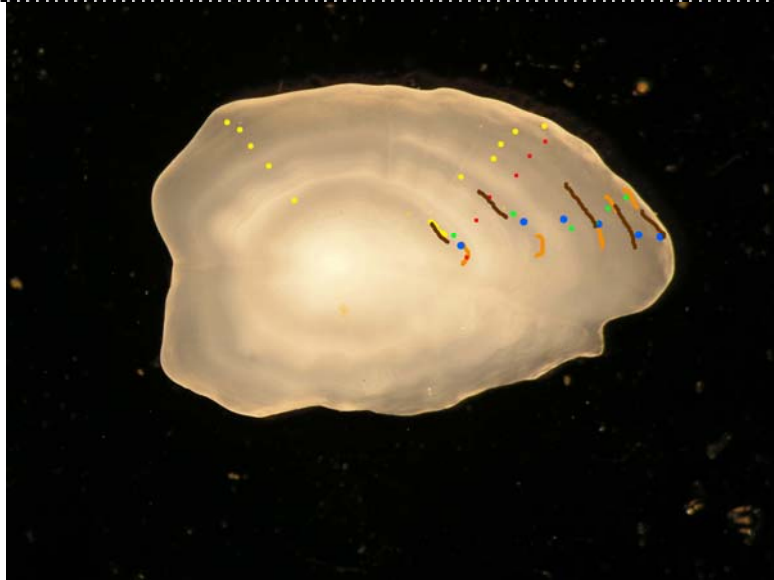


Image 10 b

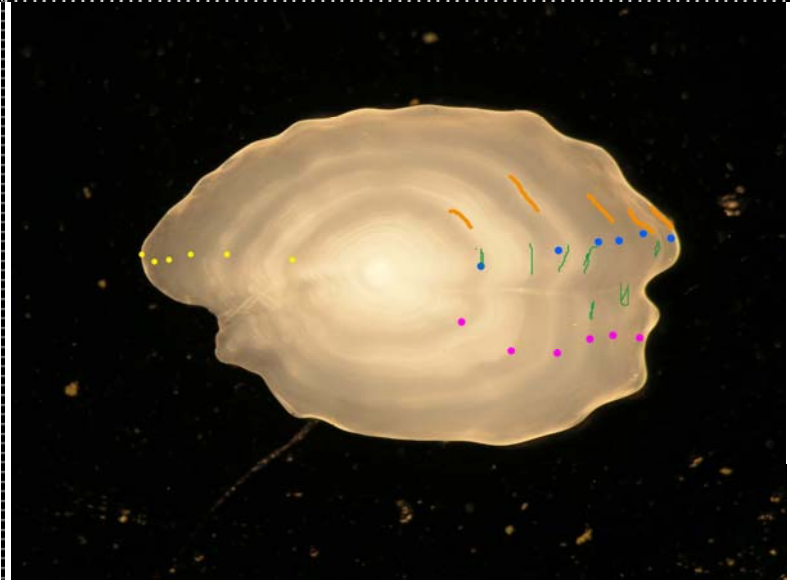


Image 11 a

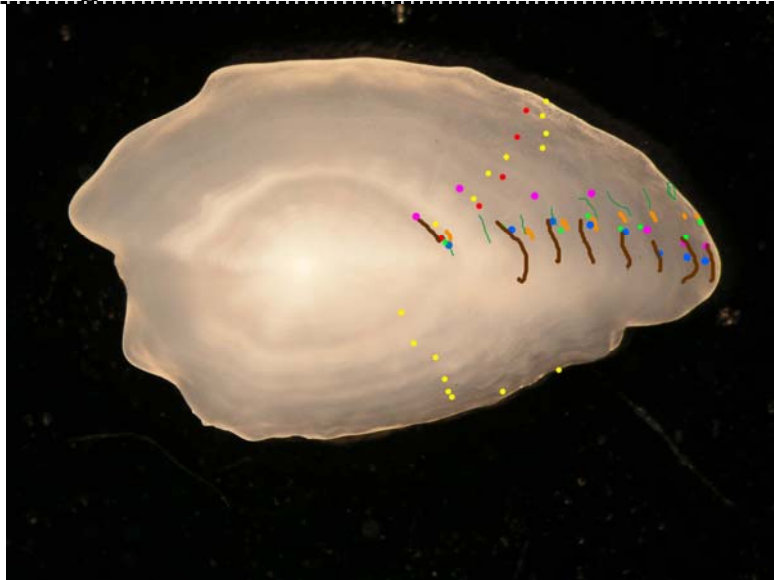


Image 11 b

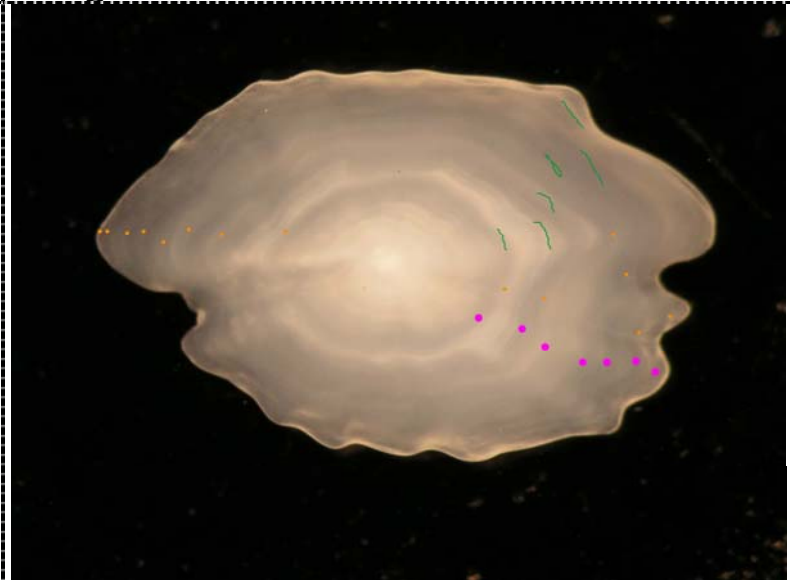


Image 14 a

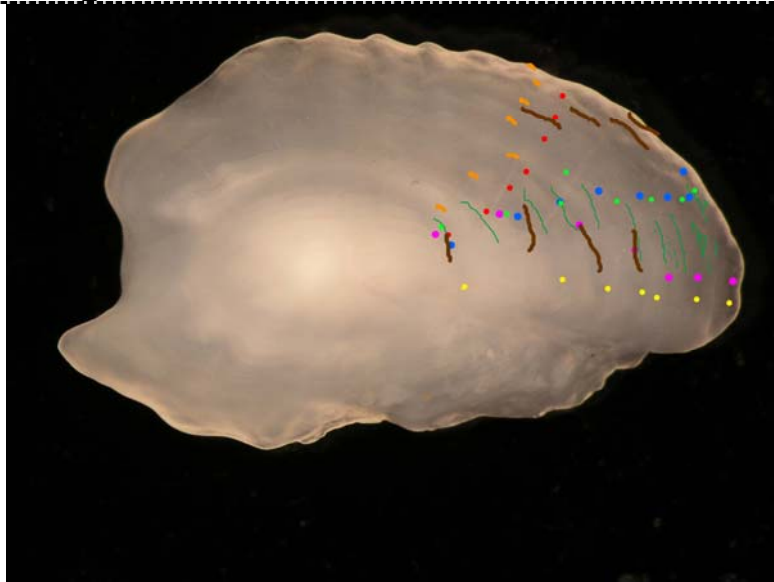


Image 14 b

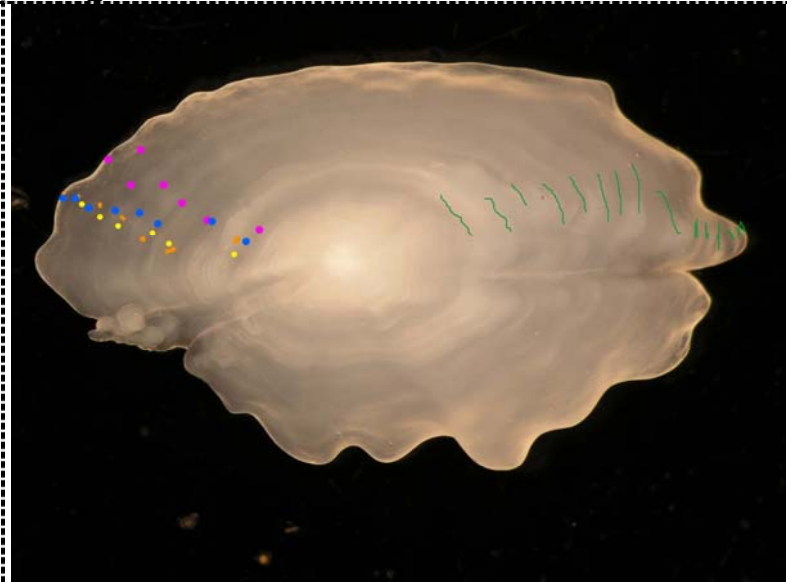


Image 15a

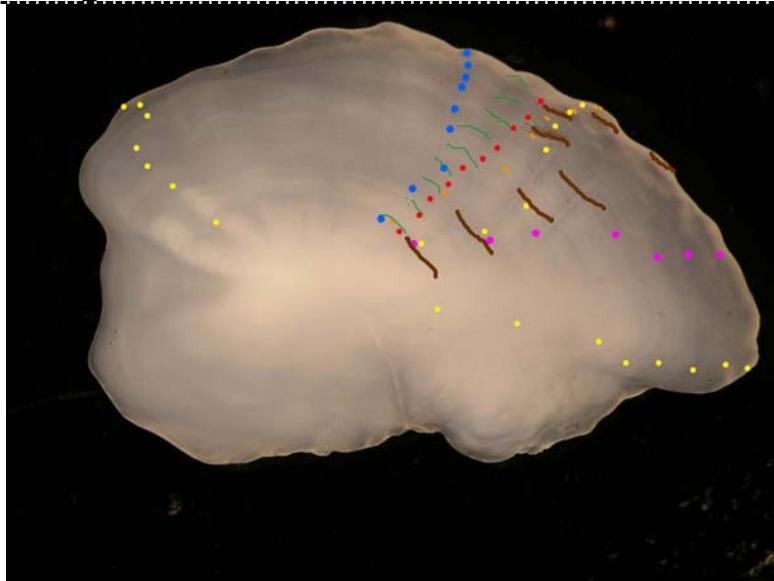


Image 15b

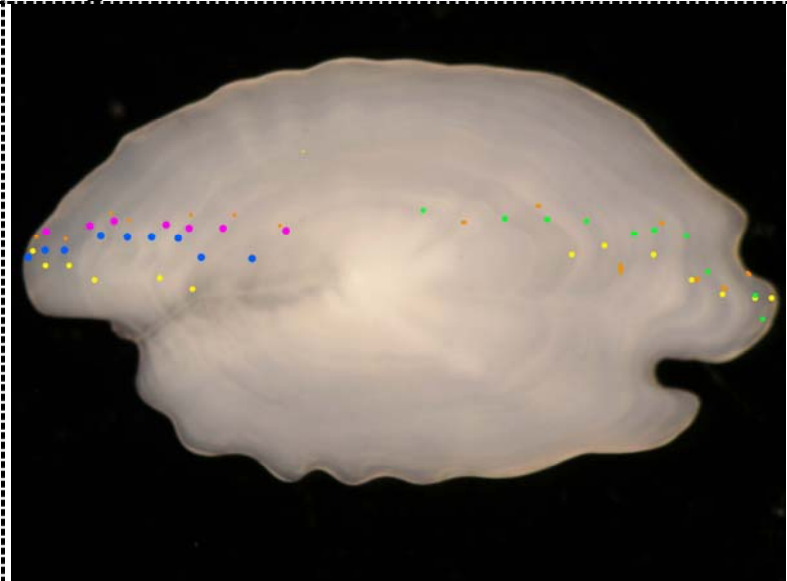




Image 16 a

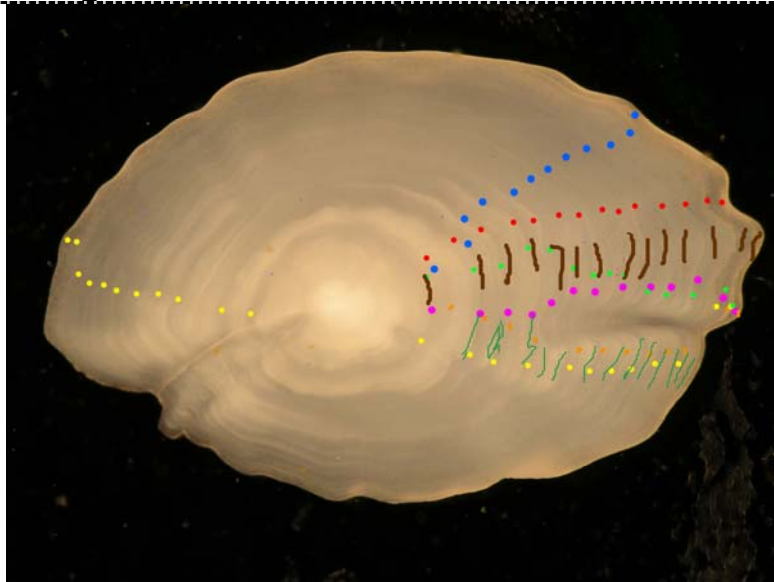
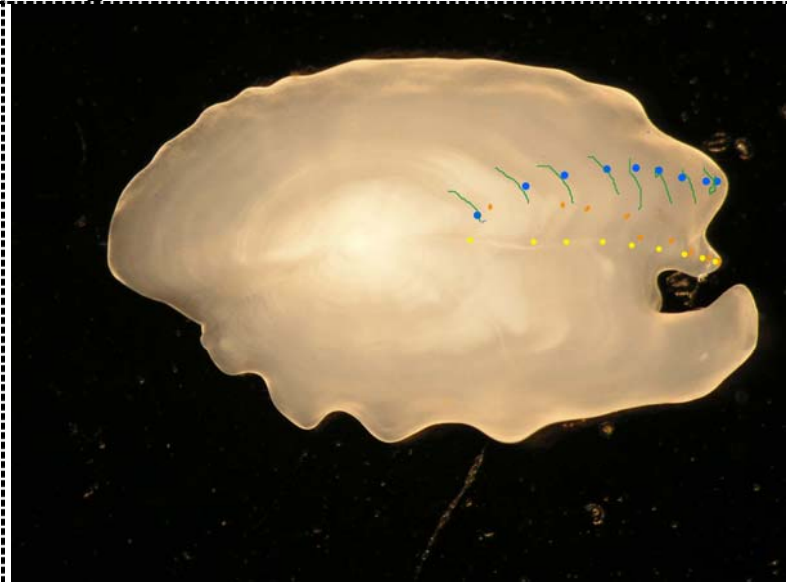


Image 16 b



3<sup>rd</sup> QUARTER Image 21 a

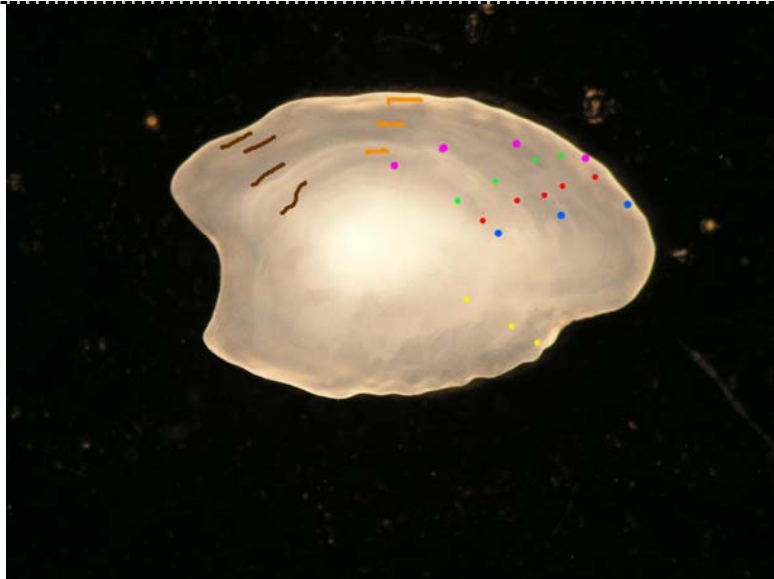


Image 21 b

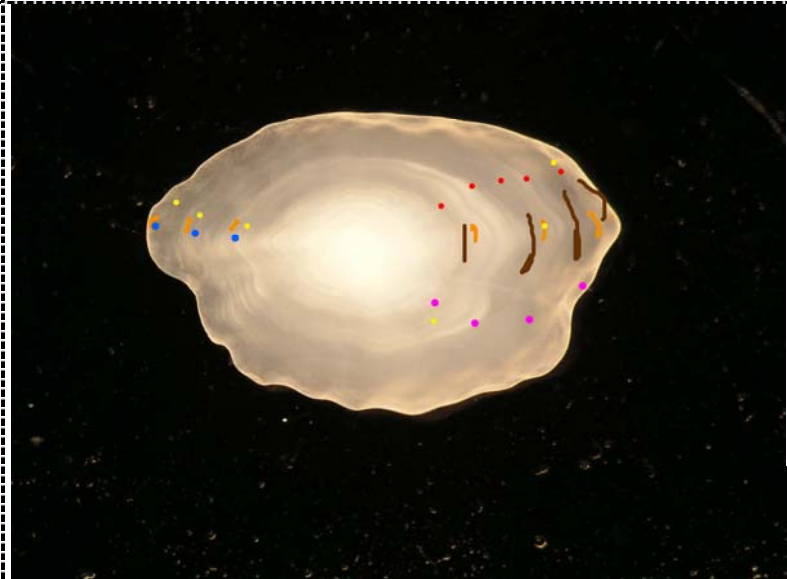


Image 23 a

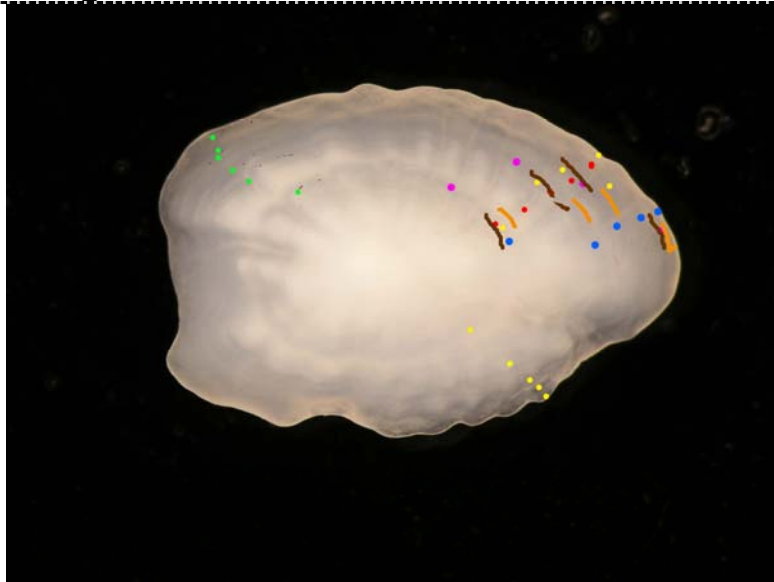


Image 23 b



Image 25 a

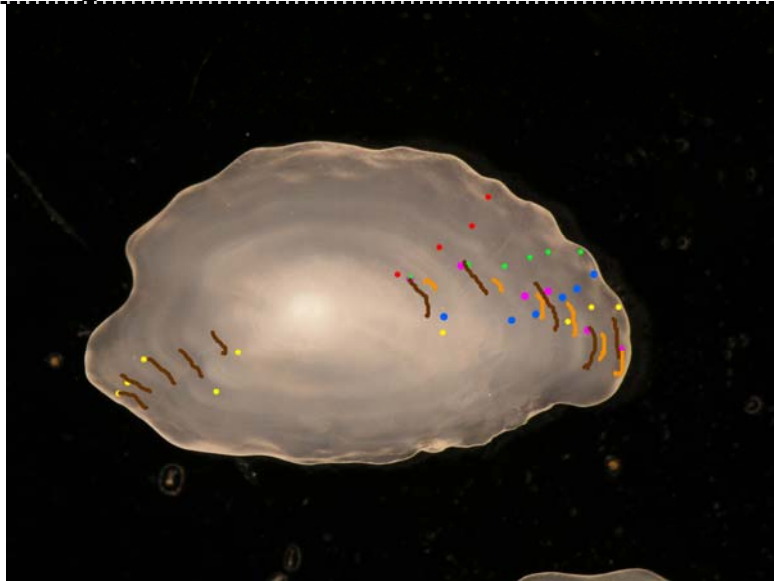


Image 25 b

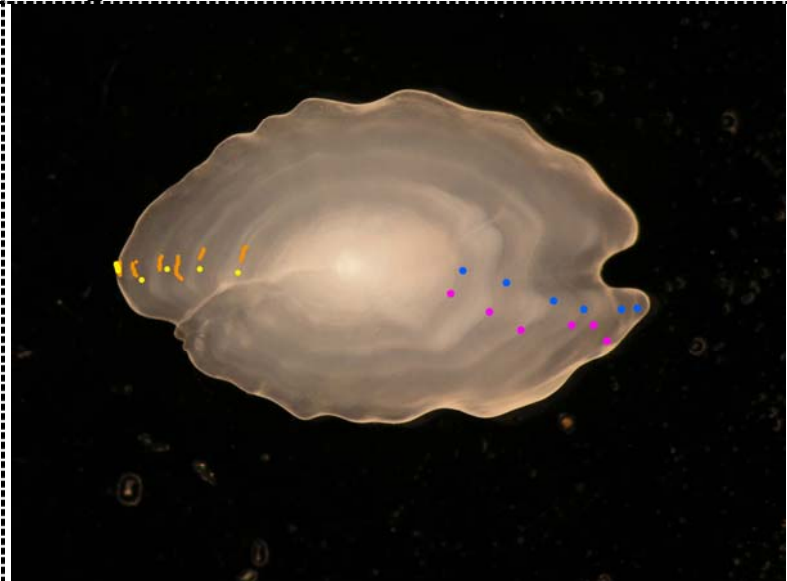






Image 26 a

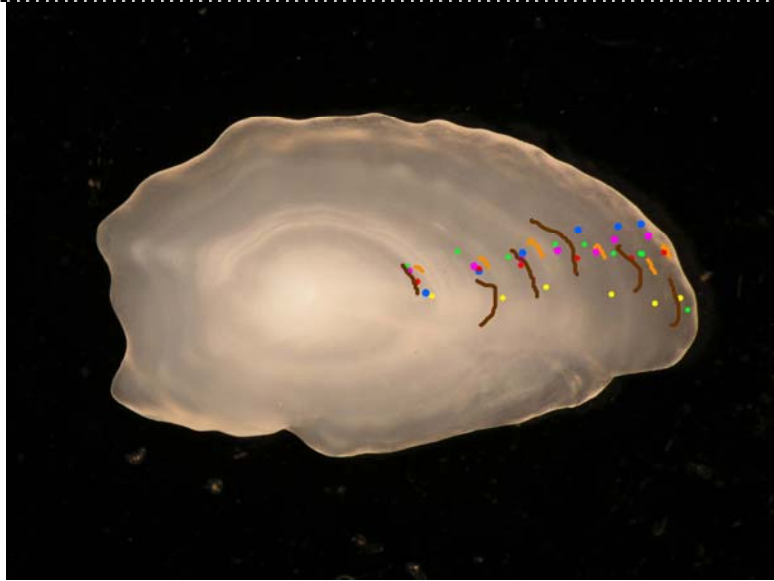
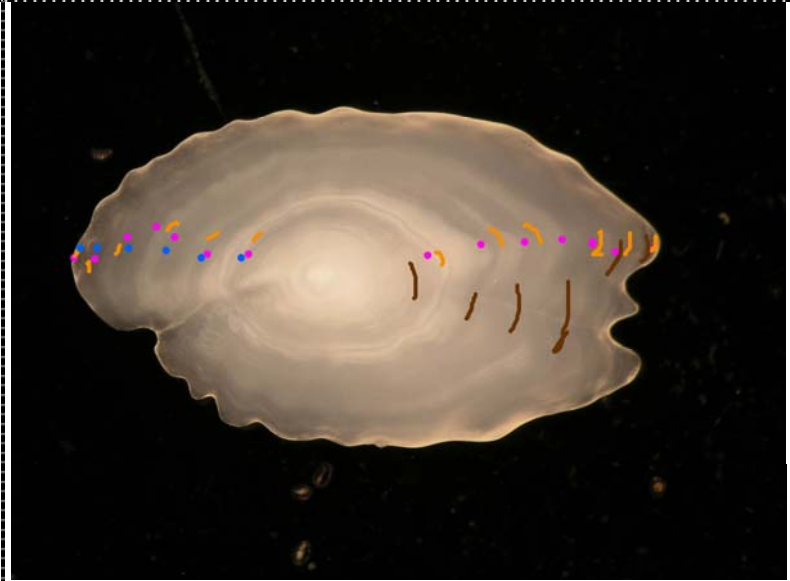


Image 26 b



4<sup>th</sup> QUARTER Image 28



Image 29



Image 30 a



Image 30 b



Image 31 a



Image 31 b

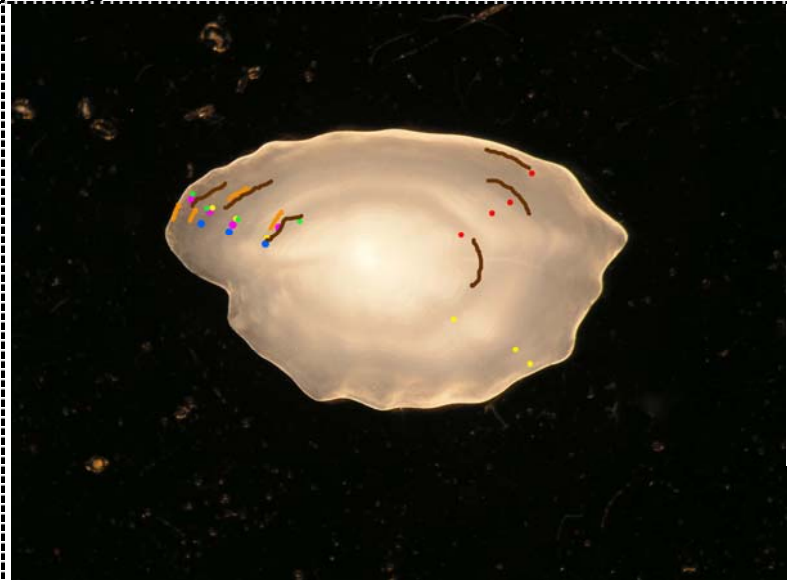


Image 35 a

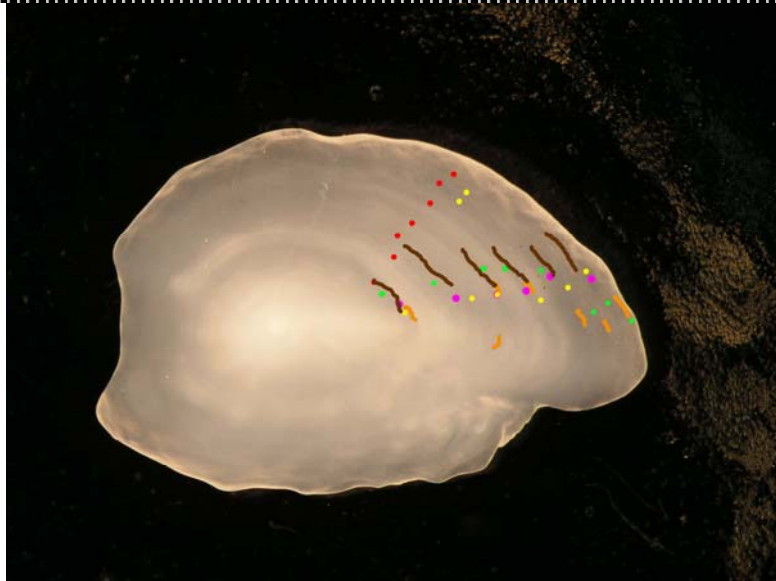


Image 35 b

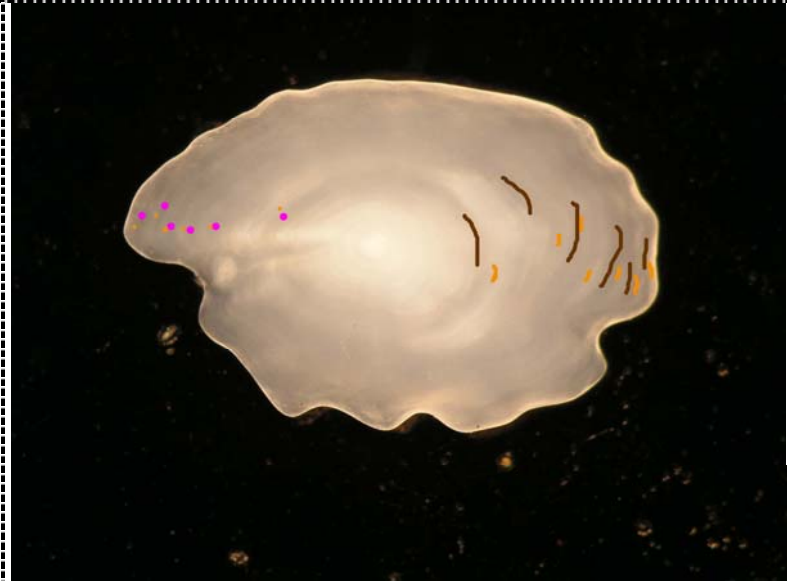


Image 37 a

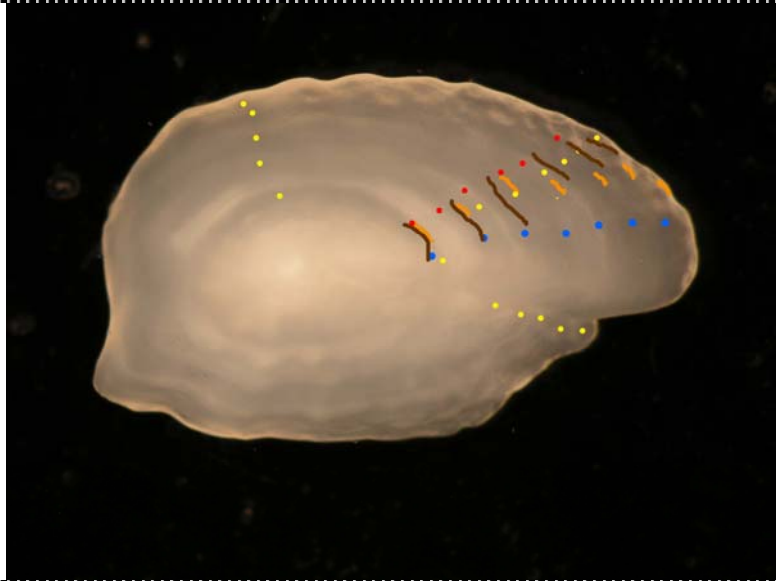


Image 37 b

