SWG-10-JM-01

International Consultations on the Establishment of the
South Pacific Regional Fisheries Management Organisation

Report of the Chilean Jack Mackerel Otolith Interpretation and Ageing Work Shop.

4 – 13 July, 2011.

Lima, Peru.

Report of the Chilean Jack Mackerel Otolith Interpretation and Ageing Workshop

1. WELCOME

The Executive Director of IMARPE, Mr. Godofredo Cañote, welcomed participants to the Chilean jack mackerel Otolith Interpretation and Ageing Workshop.

2. INTRODUCTION

The 9th Meeting of the SPRFMO Scientific Working Group-Jack Mackerel Sub-Group (SWG–JMSG) concluded that differences between the results of sensitivity analyses conducted during the 2010 jack mackerel assessment process emphasized the importance of obtaining correct age and growth information for the different fleets participating in the jack mackerel fishery. To improve future assessments, progress needs to be made with developing standardised and agreed otolith interpretation protocols, and developing correct growth curves and age-length keys, for jack mackerel caught in various regions. An otolith interpretation and ageing workshop was considered to be the best way to progress this work.

3. WORKSHOP ARRANGEMENTS

The workshop was hosted by the Instituto del Mar del Peru (IMARPE) in Lima, Peru, from 4 – 13 July 2011. The workshop was chaired by Rodolfo Serra and all attendees participated in preparation of the report. The draft agenda was adopted without modification and is provided in **Annex 1**; a list of participants is given in **Annex 2**.

4. TERMS OF REFERENCE

The following terms of reference for the WS were defined during the 9th Meeting of the SWG-JMSG held in Viña del Mar in October 2010:

- To read a selected set of otoliths and compare otolith readings to determine differences in ageing protocols and resulting ages obtained by different participants.
- To review reasons for differences in age-estimates and agree on a standardised otolith interpretation protocol for *Trachurus murphyi*.

5. NOMINATION OF RAPPORTEURS

Mr. Rodolfo Serra was nominated rapporteur of the WS.

6. MATERIALS AND METHODS

In preparation for this WS, and for the methodological aspects of the age reading comparison at the WS, the recommendations contained in the “Guidelines and tools for age Reading” by Eltink et al. (2000) were followed.

The WS started with presentations by participants on their current otolith reading techniques and results. A presentation was also given summarizing the biological aspects of jack mackerel relevant to age determination. The list of documents and presentations is attached in **Annex 3**. The techniques to be adopted by the WS for age readings and comparisons were then discussed and agreed.

Otolith samples were provided by the Instituto de Fomento Pesquero. A sample of 88 pairs of whole otoliths of fish of different sizes was used for the age readings (**Annex 4**). For fish larger than 45 cm FL, the left otolith was read as a whole otolith and the right otolith was cross-sectioned for reading. In all, 30 otolith pairs were examined in this way, with two being lost during preparation. Sectioned otoliths were used for the larger fish because ring formation in older (larger) fish becomes compressed as a result of slow otolith growth, so that rings are under-counted and ages are under-estimated if whole otoliths are read. The left otolith is preferred for whole otolith readings, so the right otolith was selected for cross-sections.

To speed up the circulation of the otolith samples between readers, 6 randomly selected otoliths were put on cards. For the whole otolith readings, otoliths were immersed in transparent oil in a Petri dish with a black base, and read at a magnification of 10x under incident light. Cross-sections were covered with oil and read at a magnification of 20x.

For comparison of age readings, it is necessary to establish the level of expertise and experience of the individual readers in reading jack mackerel otoliths. The otolith readers that participated in the WS show a large range in experience (T**able 1)** with reading jack mackerel otoliths. However, most of these readers have substantial experience reading otoliths of other species.

Table 1.

Reader Nº	**Experience**
1	Recent
2	Recent
3	Only 2007
4	Very recent
5	30 years
6	Not continuous
7	Recent
8	6 years

The different level of experience with jack mackerel otoliths complicates the analysis of the otolith reading results. The true ages of the fish are unknown, due to the fact that no validated otolith collection exists. Under such circumstances, Eltink et al. (2000) proposed that the modal age of readings by all readers be used as the best estimate of the true age of each fish. However, because the majority of the readers in the WS were relatively inexperienced with jack mackerel otoliths, the modal age of readings can be misleading, and perhaps biased away from the true value. Where, for example, inexperience results in under-ageing by the majority of otolith readers, the model age will be biased downwards from the true age. It was therefore decided to use the readings of the most experienced reader in the working group as a reference age.

For the reading of whole otoliths, a simple rule for the interpretation of whether a hyaline otolith edge indicated the appearance of another growth ring was agreed, as is shown in **Table 2**.

Table 2.

# Rings	Type of border	Age
1	Opaque	1
1	Hyaline	2
3	Opaque	3
3	Hyaline	4

For simplicity, only two types of otolith border were defined in this rule: opaque and hyaline. However, these could be narrow or broad and some difficulty remained with actually identifying the type of border for some otoliths. These difficulties contributed to differences in age readings, particularly for less experienced readers.

For the analysis of age readings, the Excel file provided by Eltink et al. (2000) was used. This provides criteria and analysis tools to measure agreement between readers, and to generate plots of the readings against the modal age and the age given by the reference reader. The modal age is calculated automatically from the age readings for each otolith and is the most frequently occurred age.

The statistical comparison between readings of 29 whole otoliths and their corresponding cross- sections, were also analyzed using the plugin tools included in two packages developed in R: FSA and NCStats libraries (Ogle 2010).

<u>Statistical tests of symmetry and bias</u>

The bias between readings was estimated with the method described by Hoenig et al. (1995) using a test based on the age-agreement table. The 1:1 diagonal represents fish for which the same age was obtained in all cases, whether considering two different readers, the same reader ageing different structures or different otolith preparation methodologies.

The test of symmetry uses a chi-square statistical test to determine if the age-agreement table is symmetrical around the diagonal (this being the null hypotheses) or not. If the age-agreement table is determined to be asymmetrical, then it can be concluded that there is a systematic difference in ages observed between different readers or between different otolith preparation methods.

Age precision

To evaluate age precision, which is the reproducibility of estimated ages either between or within readers, regardless of whether the estimated ages are accurate or not (Campana 2001), the percent of all paired readings that were in agreement were computed and other statistical measures were considered: The average percent error (APE) and the Coefficient of Variation (CV), following Beamish and Fournier (1981), were calculated as follows:

$$APE = \frac{\sum_{j=1}^{n} APE_j}{n}$$

$$APE_j = 100 * \frac{\sum_{i=1}^{R} \frac{|x_{ij} - \bar{x}_j|}{\bar{x}_j}}{R}$$

where APE_j is the average percent error for the jth fish, x_{ij} is the ith age estimate on the jth fish, x_j is the mean age estimate for the jth fish; R is the number of times that each fish was aged (assumed to be the same for all fish), and n is the number of aged fish in the sample.

To avoid assumptions that the standard deviation of the age estimates is proportional to the mean of the age estimates (Chang 1982), precision was also measured by using the coefficient of variation (CV):

$$CV = \frac{\sum_{j=1}^{n} CV_j}{n}$$

$$CV_j = 100 * \frac{s_j}{\bar{x}_j} = 100 * \frac{\sqrt{\sum_{i=1}^{R} \frac{(x_{ij} - \bar{x}_j)^2}{R-1}}}{\bar{x}_j}$$

where CV_j is the coefficient of variation for the jth fish and s_j is the standard deviation of the age estimates for the jth fish.

The CV measure is preferred because the coefficient of variation has known statistical properties. Campana (2001) showed that the CV was approximately 40% greater than the APE in practice. For relative comparisons, both the APE and CV were used. Campana (2001) suggests that 5% is an acceptable value of precision, based on studies for a number of different species. Values of CV greater than 5% would be considered to be relatively imprecise.

Image analysis

A further step in the analysis was the examination by all participants of the images of a number of whole otoliths and cross-sections projected on a screen. However, the cross-section images were not discussed in any detail, due to substantial difficulties with interpretation of cross-section images. Image analysis software was used for the examination of the whole otolith images, to improve the images and to take measurements of otolith and annulus radii.

7. RESULTS

7.1 From presentations and documents provided

The documents presented to the WS and the presentations made contain background information in regard to validations conducted in different countries, and criteria developed to check the quality of age determination. China validated ages using otolith weights (Gang Li et al., 2011). In Chile the first ring was validated with daily rings and the formation of annual rings was validated using analysis of the border in two strong year class from 1987 to 1994 (**Figure 1**), following strong year classes and with the bomb radio carbon method.

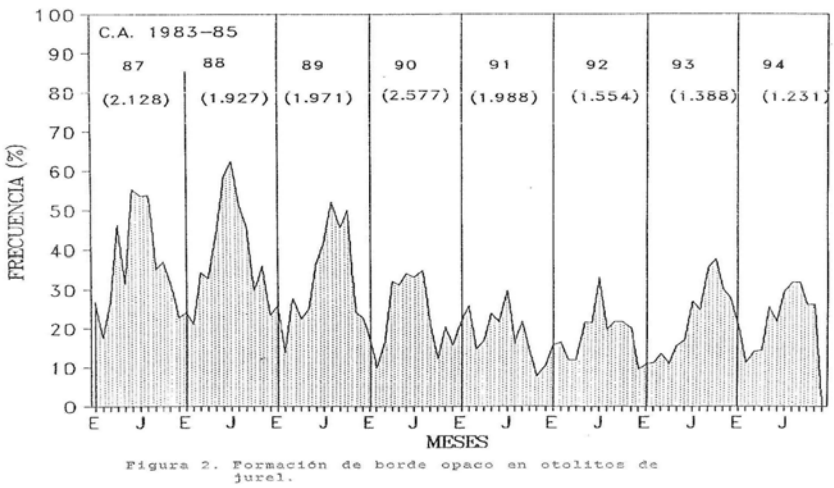


Figure 1. Seasonality of opaque ring formation in the jack mackerel. Source: Serra and Gili (1995).

Several methods for quality control and validation of age estimations were presented by Russia. The monthly progression of modal lengths in commercial catches was analyzed by AtlantNIRO for 1980-91. The progression of length distributions is consistent with results of Chilean carbon bomb analyses and the tracking of strong year classes. Daily rings count in some fish caught in 2009 confirms that jack mackerel currently grow at the same rate as in earlier years, attaining 15 cm FL in the first year.

A number of general approaches to investigating the validity of growth curves derived from otolith readings were summarized. Back-calculated lengths from annual rings in the otolith can be compared to other growth curves. Another approach using the length and age at first maturity (Ionas and Blinov 1976) provides a single growth curve that fits many species when lengths are plotted as the ratio of fish length and the length at first maturity, and ages are plotted as the ratio of the age to the age at first maturity. Otolith radius and annulus measurements used in some of these methods must be measured on the caudal part of jack mackerel otolith only. This part of the otolith undergoes little distortion with growth, whereas other parts of the otolith change substantially with age. The grooves on the otolith surface have also been found to correspond to annual growth zones and can be used for age determination.

7.2 Otolith readings

7.2.1 Whole otolith readings

87 otoliths were included in the final set of age readings. Otolith number 59 was not included because of uncertainty regarding the length of the fish. The final sample of otoliths included fish that ranged in age reading from 0 to 16 years of age.

Individual age readings were compared for each otolith with the modal age resulting from all readers and with the reference age reading by the most experienced reader. It is a measure of the number of readings for each otolith that coincide with the criteria (modal age or reference reader) and for that age. The result shows that the level of agreement against both was poor. **Table 3** shows the level of agreement using the criterion of 80% proposed by Eltink et al. (2000). It represent in percentage the number of coincidence with the modal age for each otolith and **Table 3** shows the number of agreement grouped for each age in accordance with the 80% criteria or more. The low levels of agreement result from the wide range in level of experience of the group in reading Chilean jack mackerel otoliths.

Table 3.
Level of agreement between readers for each age (80% or more).

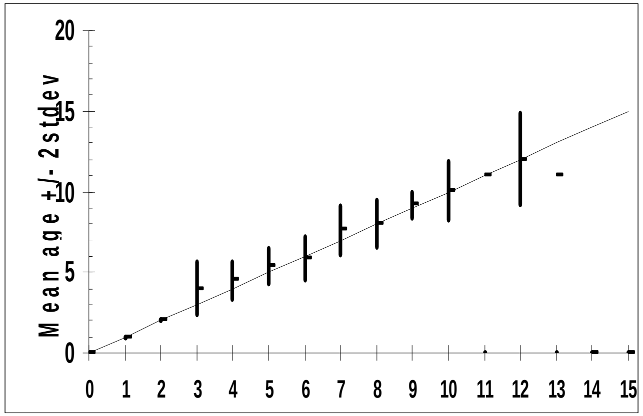
Age	Modal age	Reference reader
1	5	5
2	0	0
3	0	0
4	1	1
5 and more	0	0

The results of the readings compared against the modal age, i.e. the most frequently age occurred among all readers for each otolith, are shown for each reader in **Figure 2**, which show for each reader the mean age estimated from readings that coincide with a particular modal age and for all ages plus two standard deviation. The plots show fairly good agreement between readers up to about age 5, with reasonable agreement up to age 7. Above this age the trend is to systematically under-estimate age, as can be seen contrasting with the solid line that represent 1:1 ages (bisector). The last plot in **Figure 2** show better agreement of the overall mean age of all readers up to age 9 or 10; but the systematic underestimation above age 10 is clear.

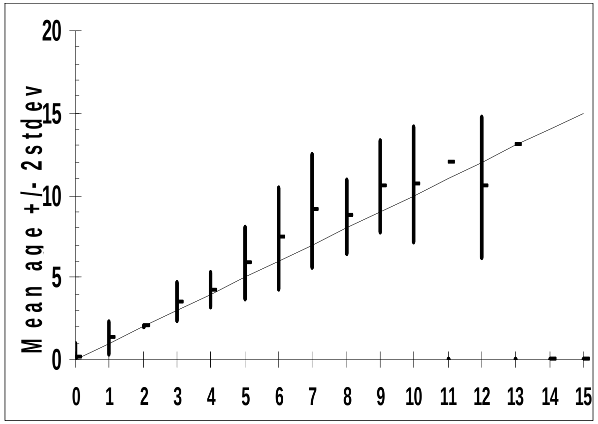
Similarly, the plots of readings from each reader against the reference reader (Nº 5) show good agreement up to about age 6 (**Figure 3**). Beyond this age, a systematic underestimation of age, compared to that assigned by the reference reader, is evident for all other readers. The same conclusion can be seen in the combined plot for all readers, where the overall trend is to systematically underestimate older ages compared with ages assigned by the reference reader.

Figure 2. In the age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age. The solid line represents 1:1 agreement (bisector). The X axis represents the modal age and Y axis the mean age estimated.

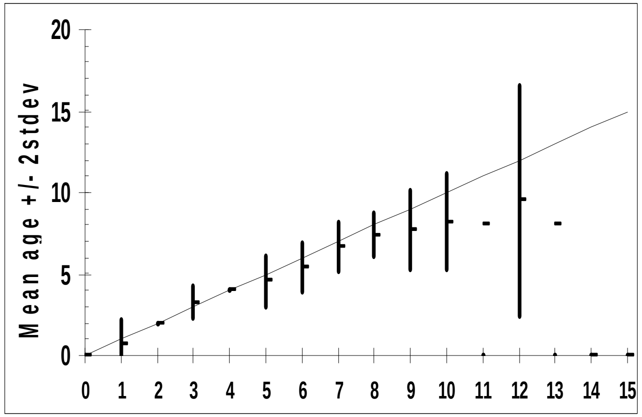
Reader 1



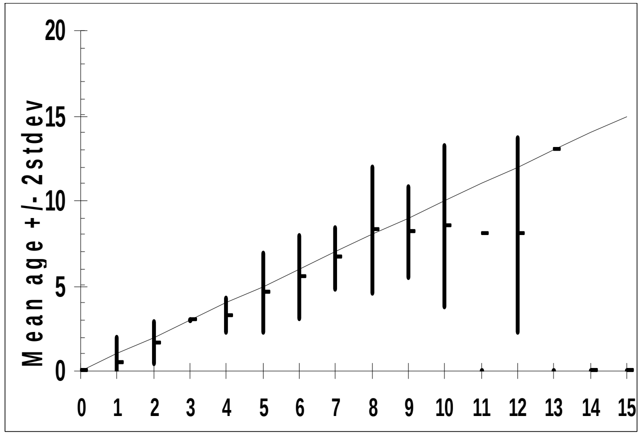
Reader 2



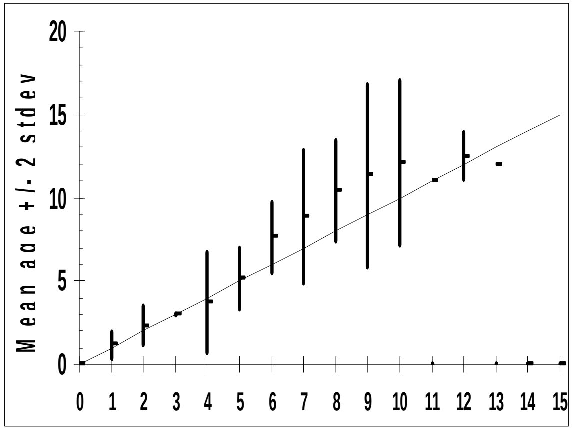
Reader 3



Reader 4



Reader 5



Reader 6

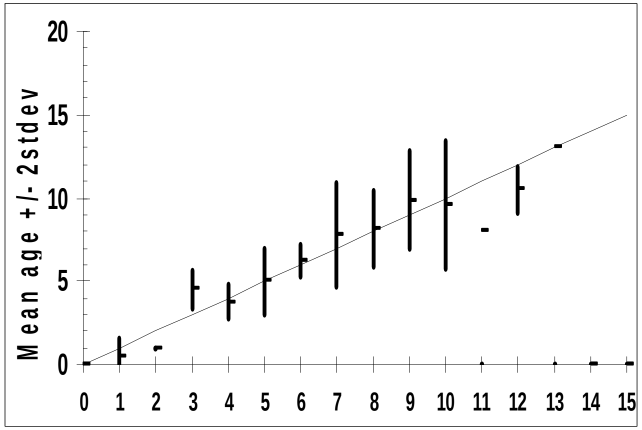
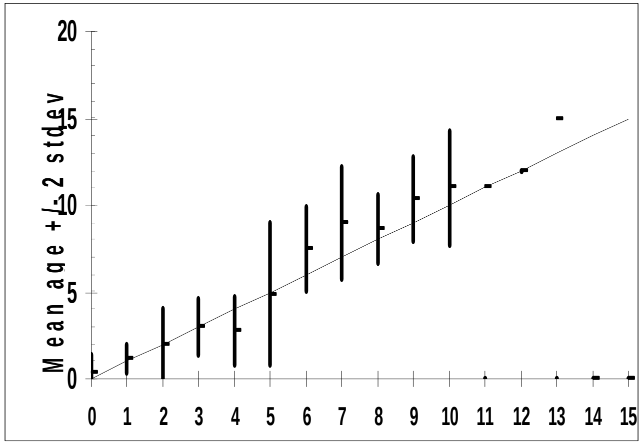
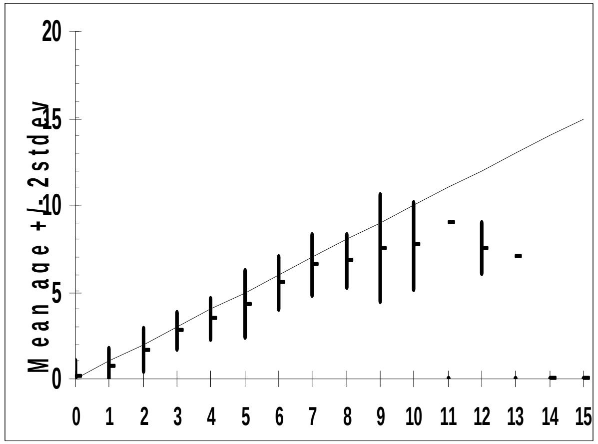


Figure 2. Continued

Reader 7



Reader 8



All readers

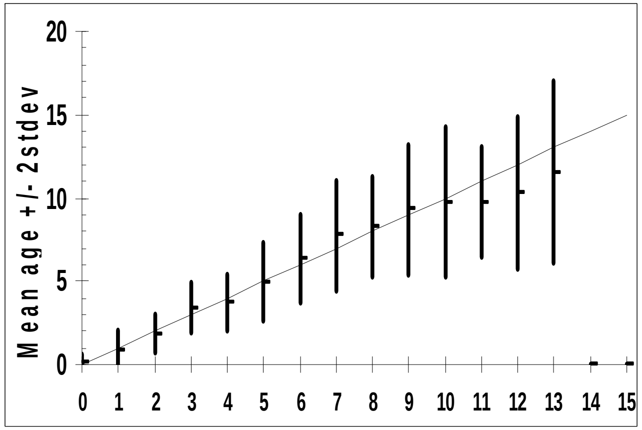
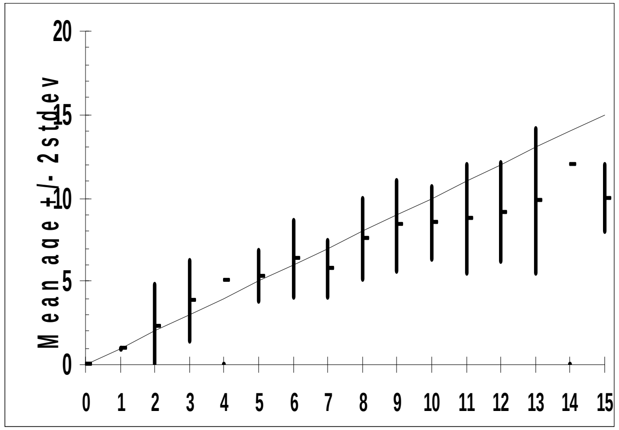
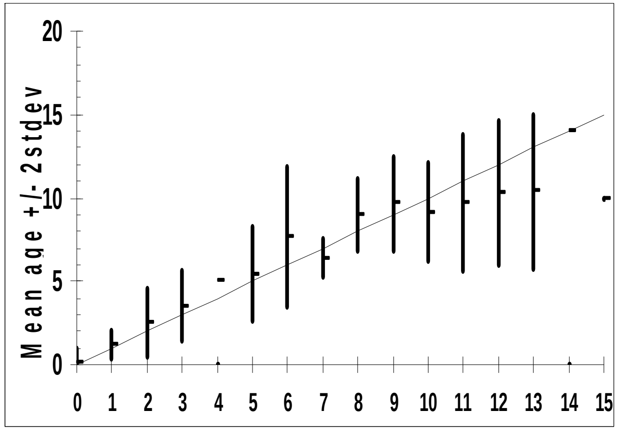


Figure 3. In the age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the REFERENCE READER age for whole otoliths readings. The solid line represents 1:1 agreement (bisector). The X axis represents the reference reader age and Y axis the mean age estimated.

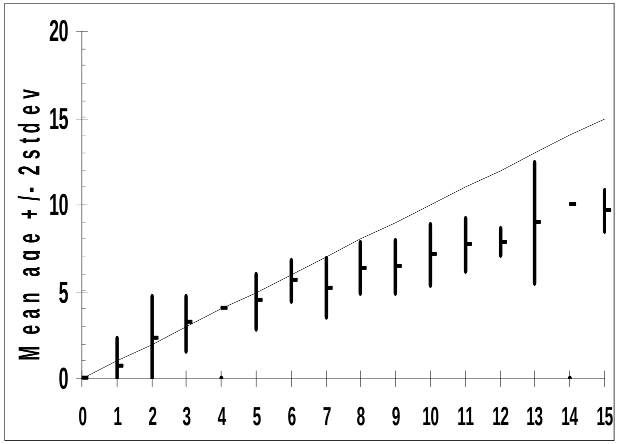
Reader 1.



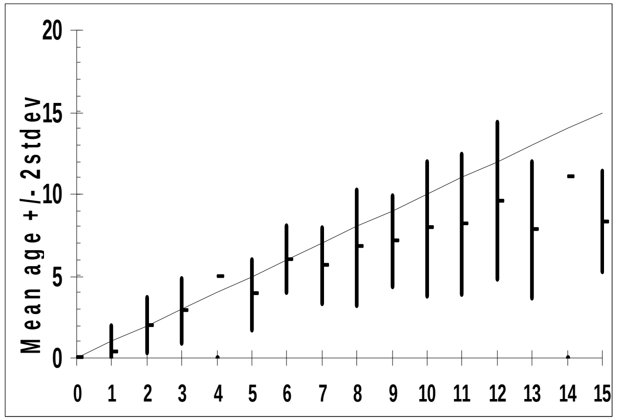
Reader 2.



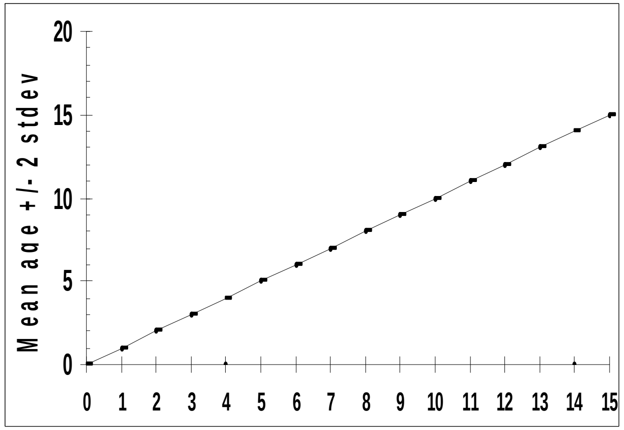
Reader 3.



Reader 4.



Reader 5.



Reader 6.

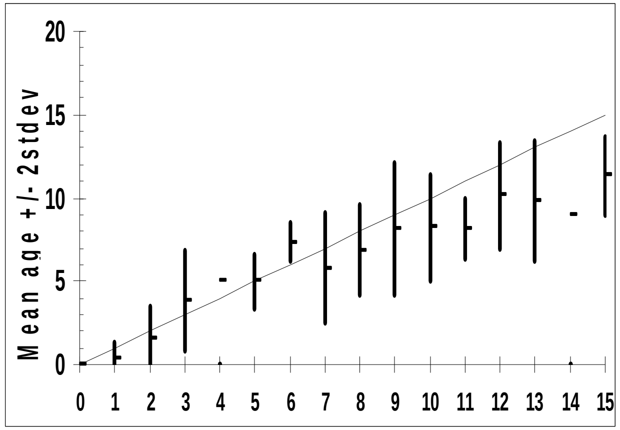
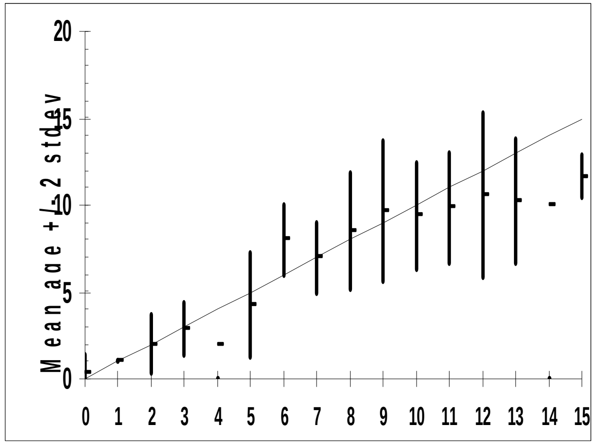
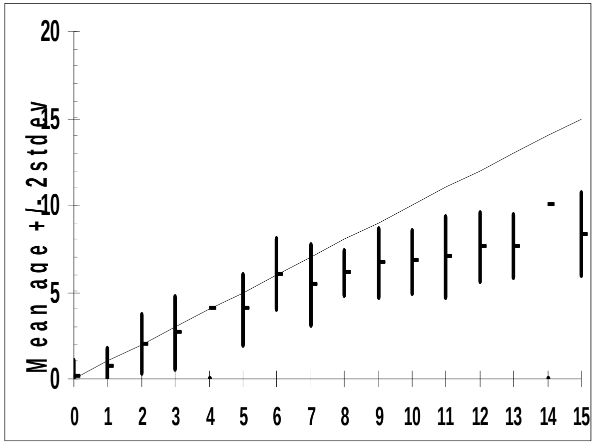


Figure 3. Continued

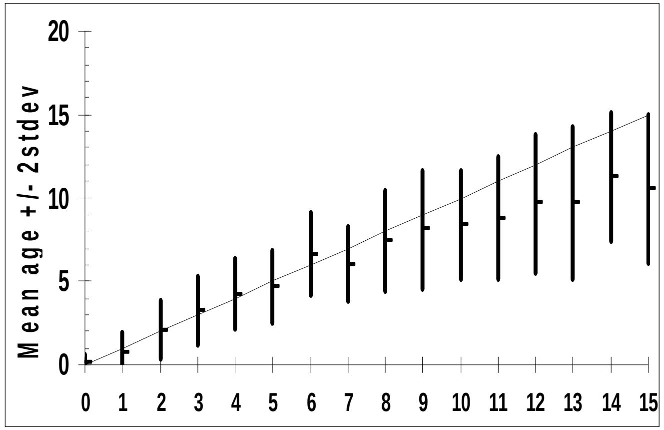
Reader 7.



Reader 8.



All readers.


Mean age +/- 2stdev
20
15
10
5
0
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

7.2.2. Otolith cross-section readings

Otolith cross-sections were prepared and read for fish over 45 cm FL. The chosen agreement criterion of 80% (Eltink et al. 2000) was not met for any age, and neither for the results comparing individual readers with the modal age or the reference reader.

Figures 4 and **5** show the plots of the cross-sectioned otolith readings compared with modal age and the age readings of the reference reader. In the plots against the modal age, the first 5 readers tend to over-estimate the younger ages and under-estimate the older ages, compared with the modal age. The others show a systematic under-estimation of the ages against the modal age. This is also shown in the all-readers plot for the last three ages. A similar pattern can be seen in the plots against the reference reader (**Figure 5**).

These results show that ages are likely to be under-estimated when reading whole otoliths for large (older) jack mackerel.

7.2.3. Comparison of whole otoliths against cross-section for each reader

Comparison of age readings obtained from whole otoliths and from cross-sections show that most readers under-estimated the age when reading whole otoliths, compared to cross-sections, although two readers over-estimated age from whole otoliths compared to cross-sections (**Figure 6**). The reference reader showed a high level of precision in age readings, with close correlation between readings done on whole otoliths and cross-sections.

Symmetry and bias between otolith preparation methods: whole otoliths and cross-sections

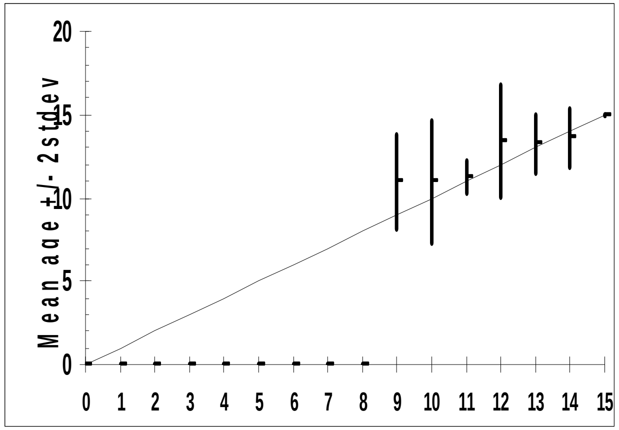
Comparisons of age readings obtained from whole otoliths and cross-sections for each reader are presented in **Figure 7**. **Table 4** summarizes Chi-square values for age readings of the 29 fish for which both whole otoliths and cross-sections were available. Two readers, numbers 3 and 8, showed significantly higher annulus counts using otolith cross-sections ($p < 5\%$). The reference reader (N^{o} 5) showed no significant differences between reading whole otoliths and cross-sections, likely due to his experience in reading otoliths of older jack mackerel, and in reading otolith sections.

Table 4. Summary of the chi-squared Test of Symmetry, comparing age readings obtained using whole otoliths and cross-sections. Readers who obtained significantly higher age readings ($p < 5\%$) using cross-sections are shaded.

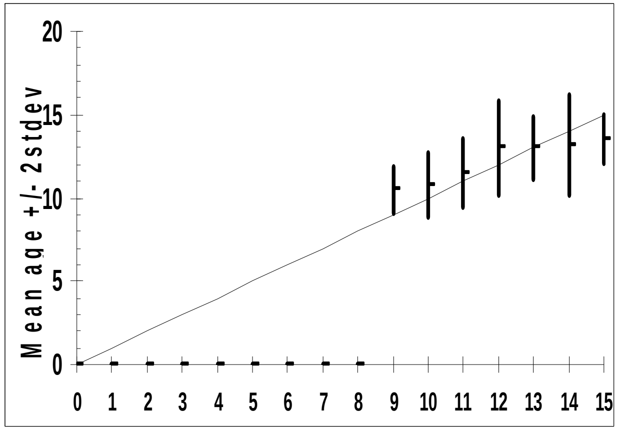
Reader	Df	Chi square	p-value
1	18	27	0.079
2	17	23	0.149
3	17	29	0.034
4	22	28	0.176
5	7	7	0.429
6	13	15.3	0.287
7	13	11.7	0.555
8	17	28	0.045

Figure 4. In the age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the MODAL age for cross-section of otoliths. The solid line represents 1:1 agreement (bisector). The X axis represents the modal age and Y axis the mean age estimated.

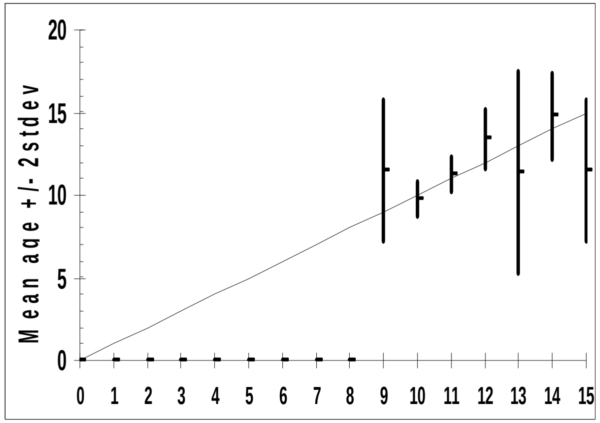
Reader 1



Reader 2



Reader 3



Reader 4

Reader 5

Reader 6

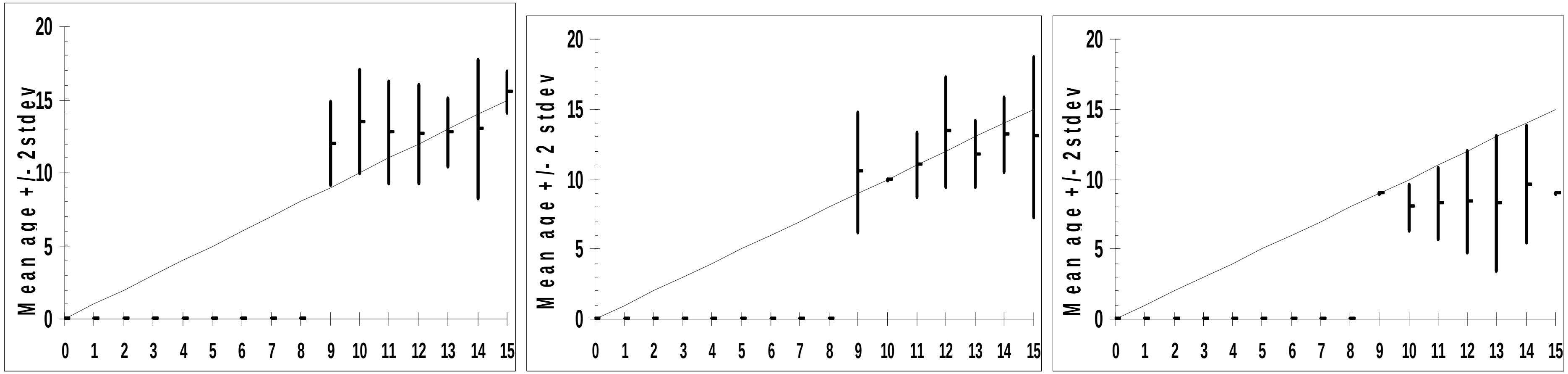
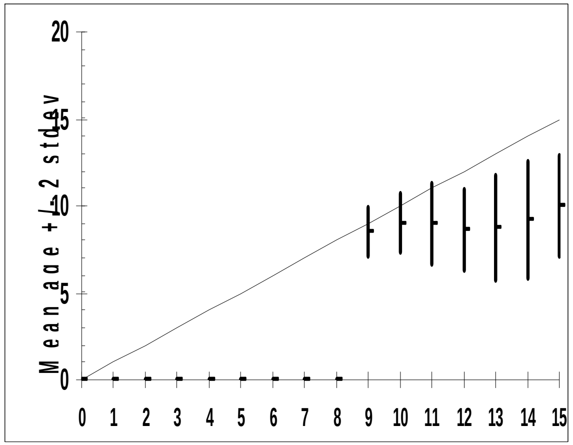
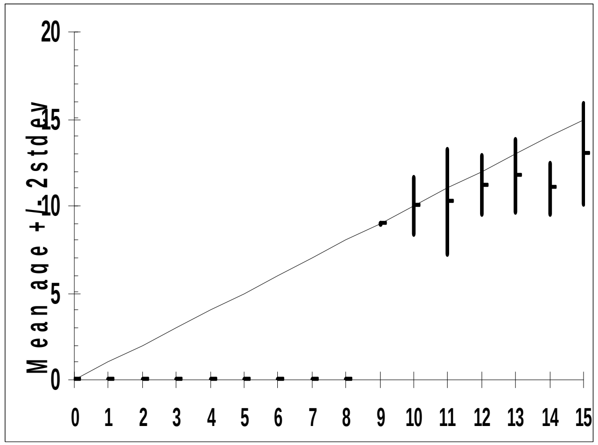


Figure 4. Continued

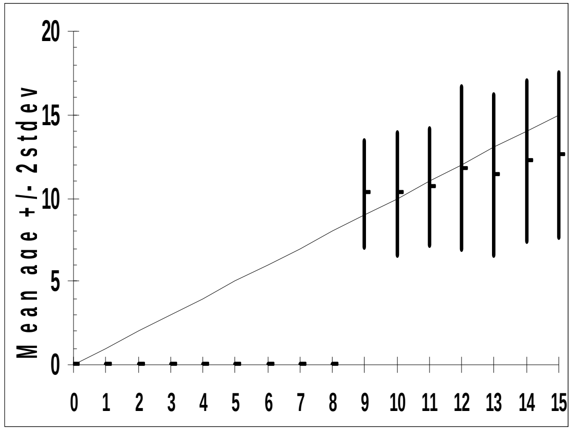
Reader 7.



Reader 8.



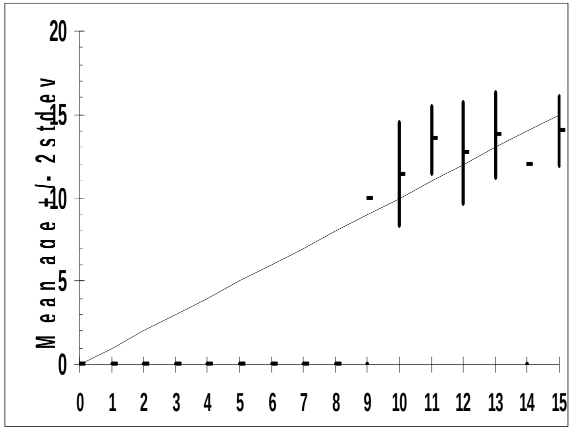
All readers.



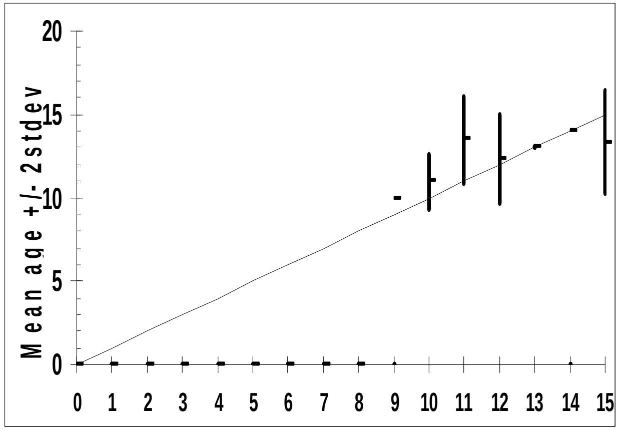
Mean age +/- 2stdev
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Figure 5. In the age bias plots below the mean age recorded +/- 2stdev of each age reader and all readers combined are plotted against the REFERENCE READER age for cross-section of otoliths. The solid line represents 1:1 agreement (bisector). The X axis represents the reference reader age and Y axis the mean age estimated.

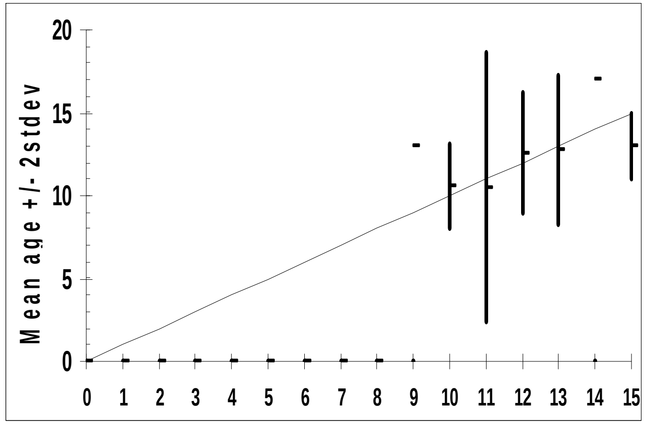
Reader 1



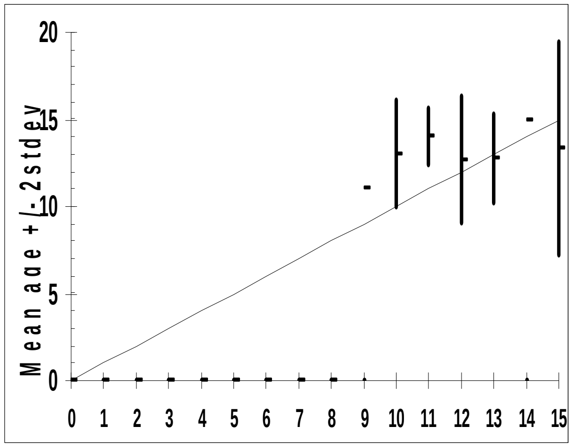
Reader 2



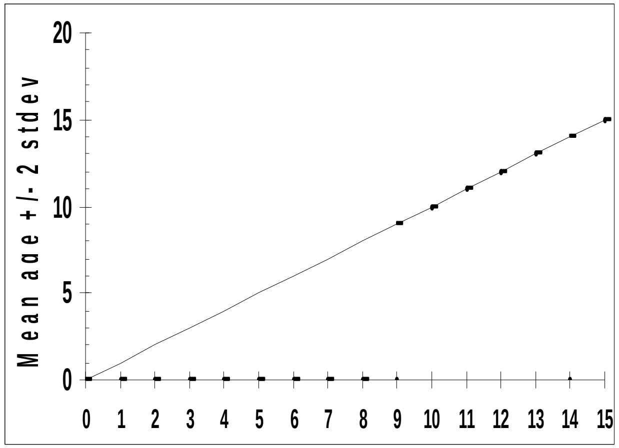
Reader 3



Reader 4



Reader 5



Reader 6

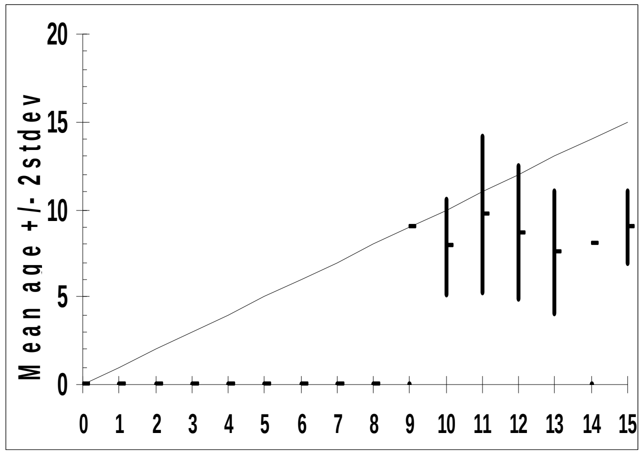
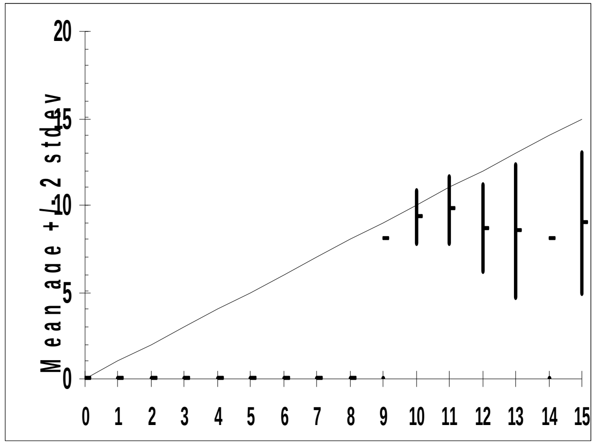
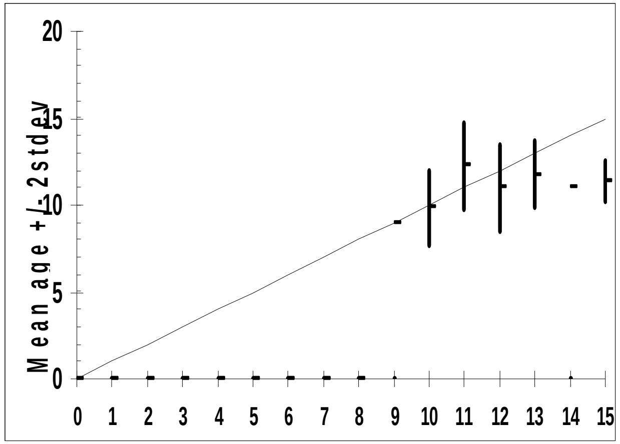


Figure 5. Continued

Reader 7.



Reader 8.



All readers.

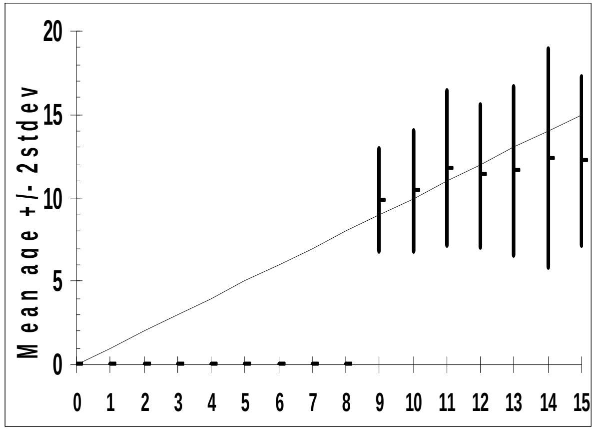
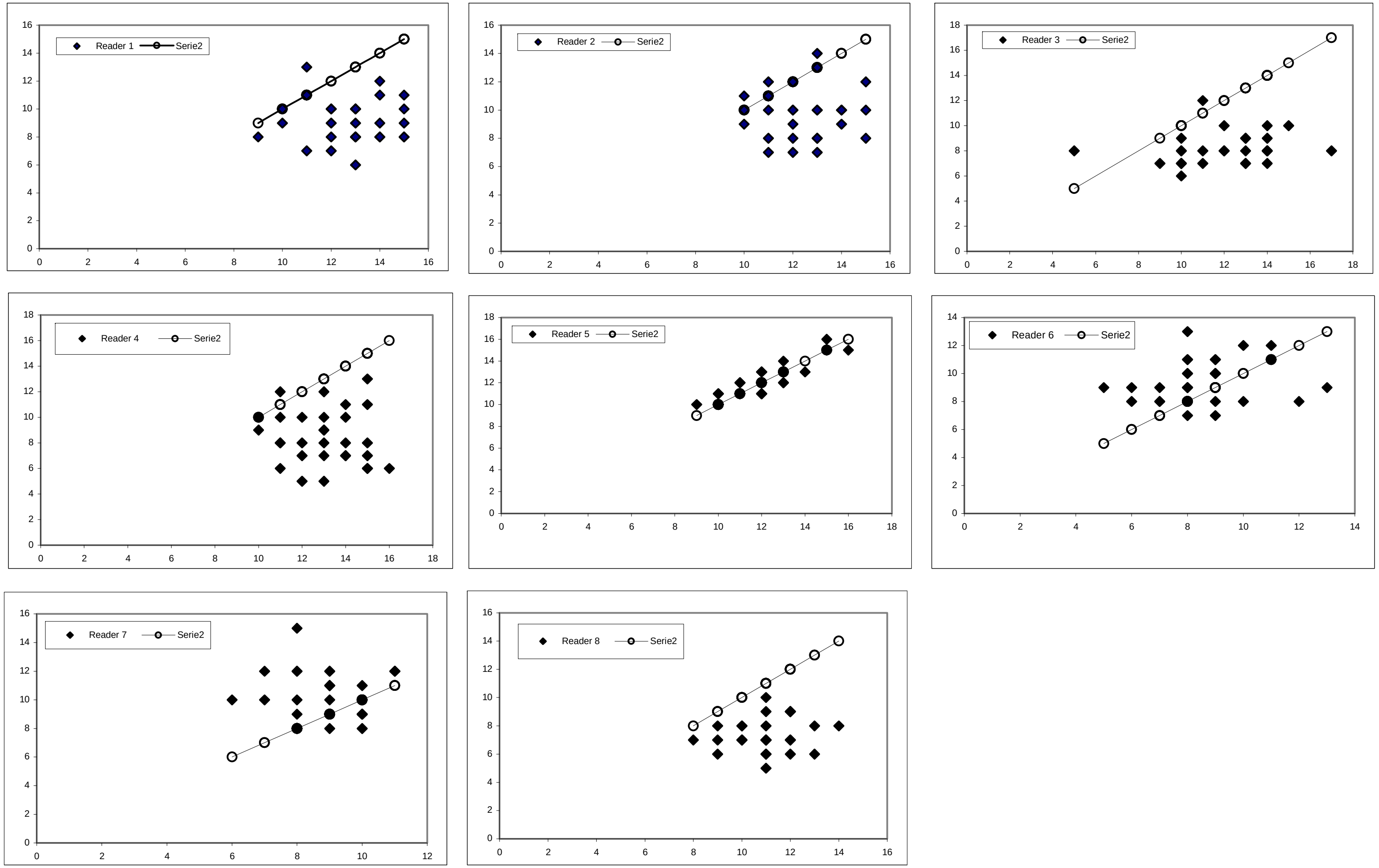
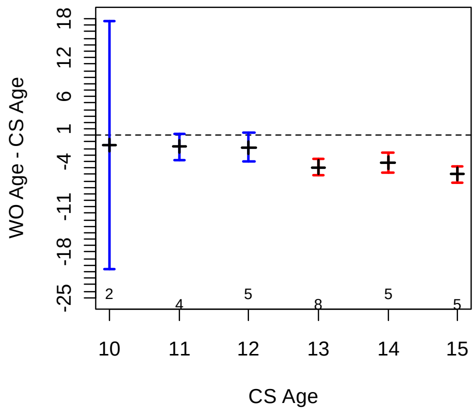


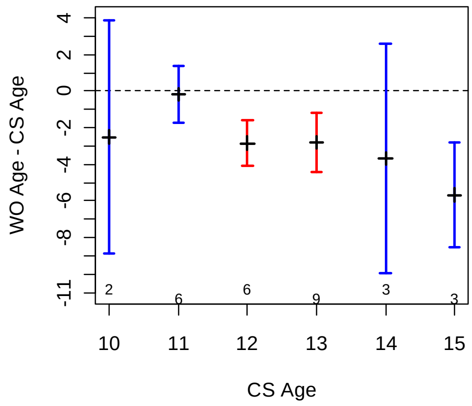
Figure 6. Results of reading same otolith: whole and cross section by each reader, comparing age readings obtained from whole otoliths against those from cross-sectioned otoliths. The line indicates the 1:1 line of agreement between whole and sectioned otolith age readings. The X axis represents the ages from sections otolith readings and Y axis from whole otolith readings.



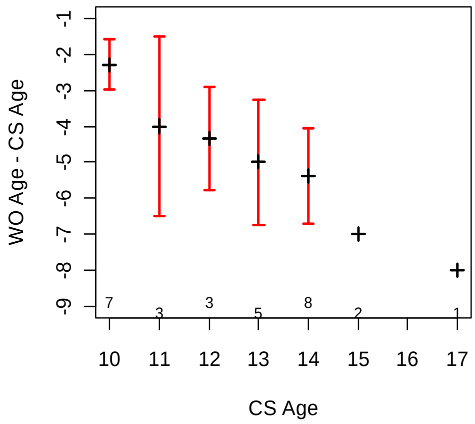
Reader 1



Reader 2



Reader 3



Reader 4

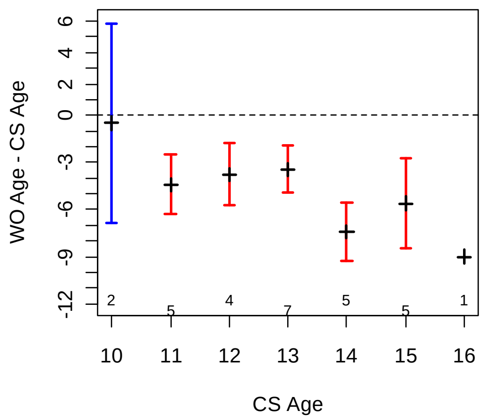
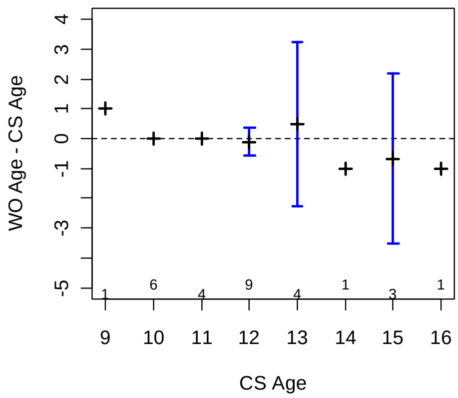
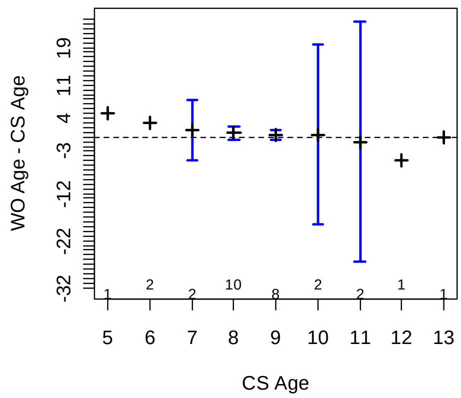


Figure 7. Differences between Cross Section (CS) ageing versus Whole Otolith (WO) ageing for readers 1 to 4. The bars represents the 95% confidence interval and the numbers below the number of observations.

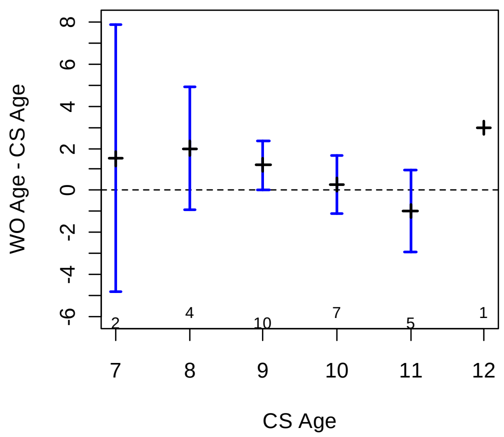
Reader 5



Reader 6



Reader 7



Reader 8

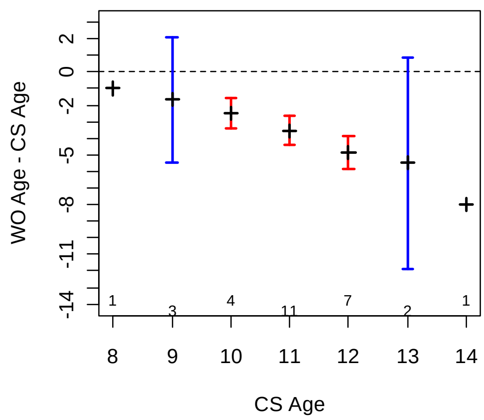


Figure 7 (continuation). Differences between Cross Section (CS) ageing versus Whole otolith (WO) ageing for readers 5 to 8. The bars represents the 95% confidence interval and the numbers below the number of observations.

7.2.5. Otolith image analysis

Participants then analyzed images of whole otoliths and cross-sections. However, images of otolith cross-sections were considered too difficult to analyze within the limited time of the workshop, so efforts focused on analyzing whole otolith images. 16 whole otolith images were examined, starting with smaller fish, but later concentrating on larger fish due to greater difficulty in reaching agreement on ages of older fish. Participants reached full agreement on ageing of some fish using these images, with some disagreement on others. However, the levels of disagreement were not as large as occurred with the actual otolith readings. One important aspect that emerged from image interpretation was the method, or rules, by which different participants examined each otolith. The key factors that influenced how readers interpreted otoliths related to:

- The relative position of the first ring (distance range from the nucleus).

- The incremental distance between annulus rings, which should decrease as age increases and growth rate slows.
- The ability to follow the ring around the otolith.

It is frequently difficult to identify the first ring, particularly in older fish, due to the fact that otolith growth and thickening obscures the early rings with newly deposited material. For similar reasons, it becomes difficult to follow rings around the otolith, particularly near the edge, due to the concave shape of the otolith and compression of rings in older fish. These problems can usually be resolved by using otolith cross-sections for larger fish. When a ring can still not be followed around the otolith, then it may be identified as being a false ring, or part of a split ring.

There are fewer such problems for younger fish, which have thinner otoliths with wider ring spacing. Reading of whole otoliths is quite effective for smaller fish, as is shown by the good agreement on young ages in the first reading exercise, and particularly in the results of the image analysis.

A few examples of detailed image analysis are given in **Annex 5**.

7.2.6. Results of a second reading

The results of a second reading, conducted after review of the results of the first reading, and practice with image analysis, showed closer agreement between most readers (**Figure 8**). All of them, except the reference reader (Nº 5), obtained higher ages for large fish compared with the first reading exercise. This is shown in **Figure 8**, which shows that almost all the second readings for most readers (other than readers 5 and 6) were higher than the first readings. The lines on these plots indicate the 1:1 line of agreement between the two readings. Reader 5 obtained virtually the same age readings in both reading exercises, and again showed high precision in age readings.

However the agreement between all readers for the same otolith is still poor, usually below the 80% agreement quality criterion. Agreement on second readings was about 33% for all the otoliths, which was a substantial improvement on the 19% agreement obtained for the same otoliths in the first reading exercise. In most cases, remaining age reading difficulties related to identifying the first one or two age rings. This has a propagating effect on counts of the subsequent rings, which might be clearly identified, but the overall age counts will differ from those of other readers as a result of missing the first one or two rings.

Although the level of absolute agreement (33%) was still quite low, **Figure 9** shows much closer age readings for older fish between most of the readers and the reference reader (reader 5). Reader 6 (and to some extent reader 7) still underestimated ages compared with the reference reader, whereas reader 8 overestimated ages in the second reading.

Precision of reading sessions

In **Table 5** and **Table 6**, statistical measures of the level of agreement between readers and the reference reader are summarized. Comparing the reduction in CV's between the first and the second trial of readings, an improvement in agreement was obtained, with substantial reduction in CVs of all readers compared to the reference reader. The CV's of 3 of the readers were reduced to less than 8% in the second reading.

Table 5. Precision indices and summary statistics: whole otolith first readings.

Comparison	N	Agree	APE	CV
Reader 1/Reference	29	13.8	13.54	19.15
Reader 2/ Reference	29	6.9	11.55	16.33
Reader 3/ Reference	29	0	19.68	27.83
Reader 4/ Reference	29	6.9	19.44	27.50
Reader 5/ Reference	29	100	0	0
Reader 6/ Reference	29	3.4	12.78	18.07
Reader 7/ Reference	29	13.8	9.69	13.70
Reader 8/ Reference	29	0	23.93	33.84

Table 6. Precision indices and summary statistics: whole otolith second readings. Readers that achieved a CV of < 8% are shaded.

Comparison	N	Agree	APE	CV
Reader 1/ Reference	30	30.0	4.04	5.71
Reader 2/ Reference	30	26.7	5.55	7.84
Reader 3/ Reference	30	26.7	8.16	11.54
Reader 4/ Reference	30	13.3	7.36	10.41
Reader 5/ Reference	30	100	0	0
Reader 6/ Reference	30	16.7	10.50	14.85
Reader 7/ Reference	30	36.7	5.11	7.22
Reader 8/ Reference	30	13.3	7.88	11.14

Figure 8. Results from the second readings of whole otoliths, comparing first and second readings for each reader. The line indicates the 1:1 line of agreement between first and second readings.

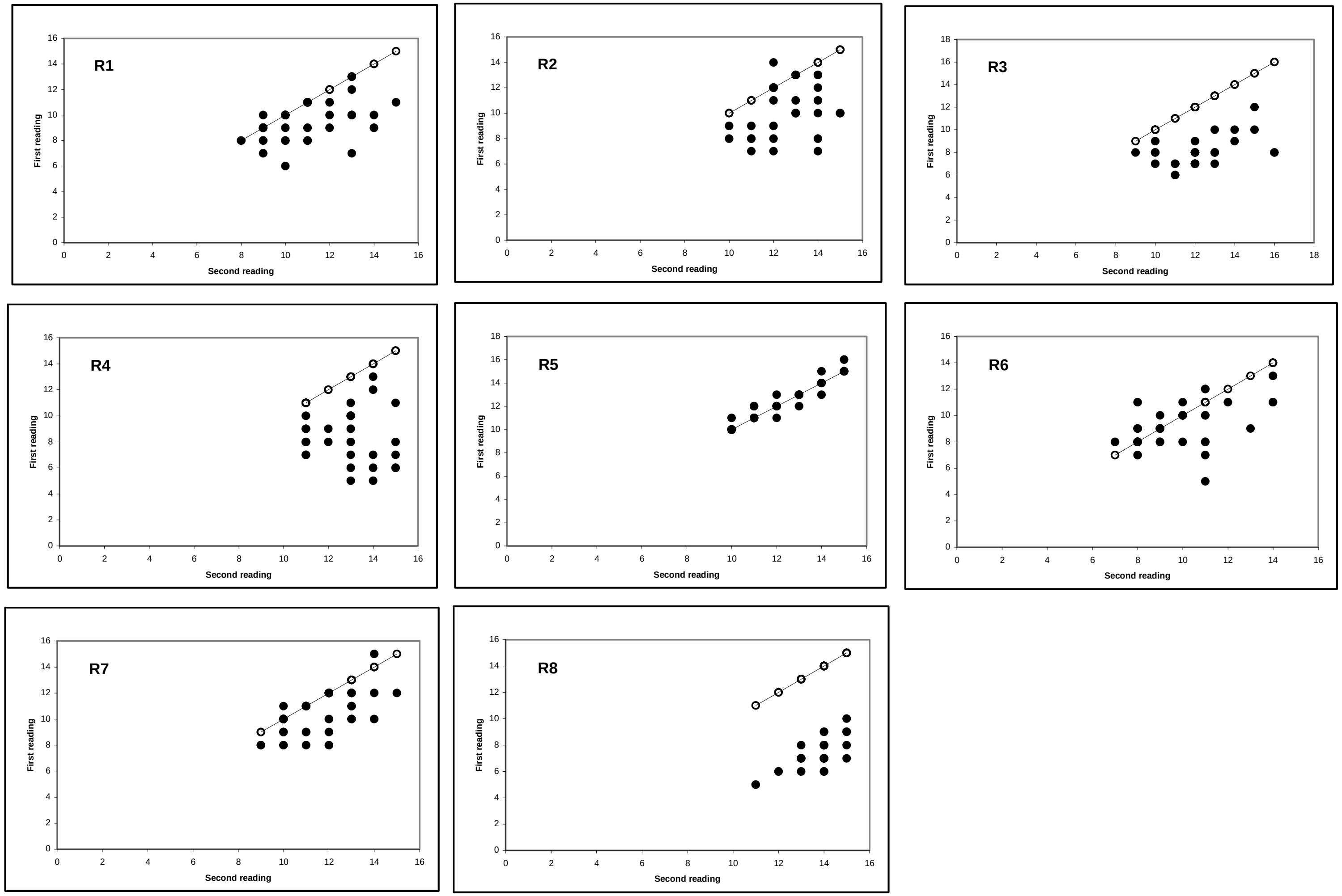
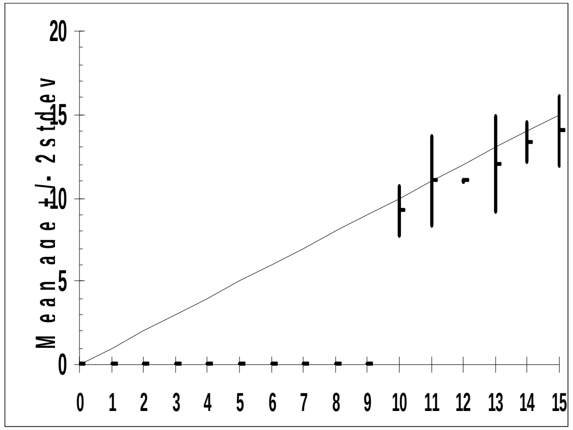
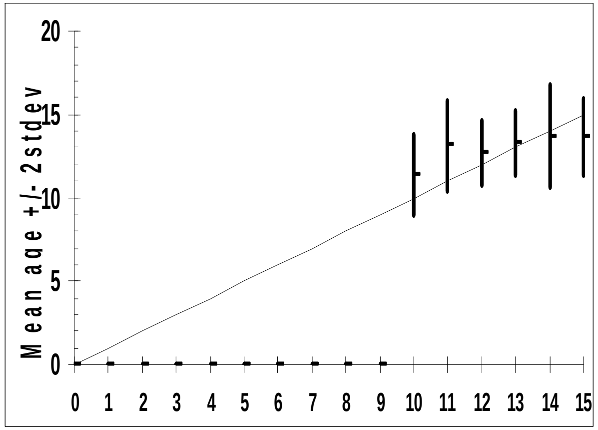


Figure 9. In the age bias plots below the mean age recorded +/- 2stdev of each age reader, and all readers combined, plotted against the REFERENCE READER. The solid line represents 1:1 ages (bisector). The X axis represents the reference reader age and Y axis the mean age estimated.

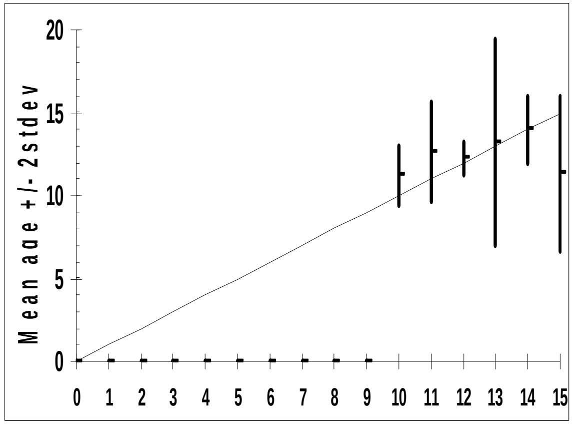
Reader 1



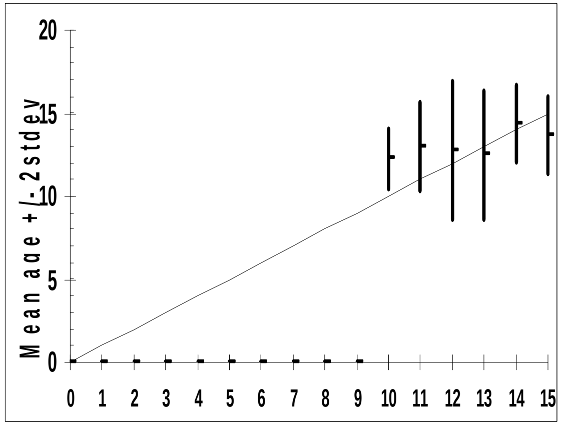
Reader 2



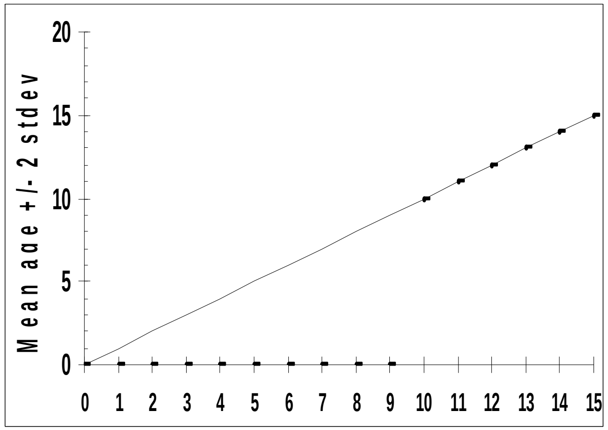
Reader 3



Reader 4



Reader 5



Reader 6

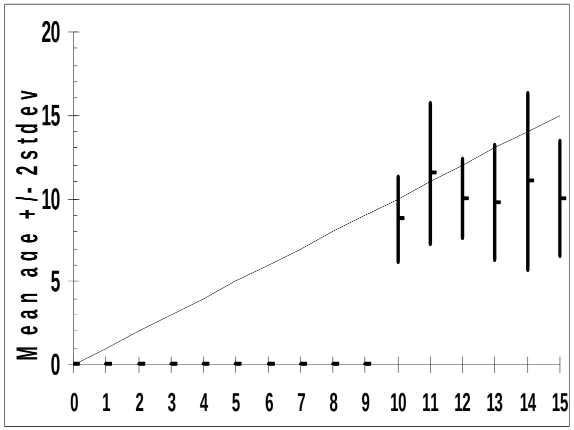
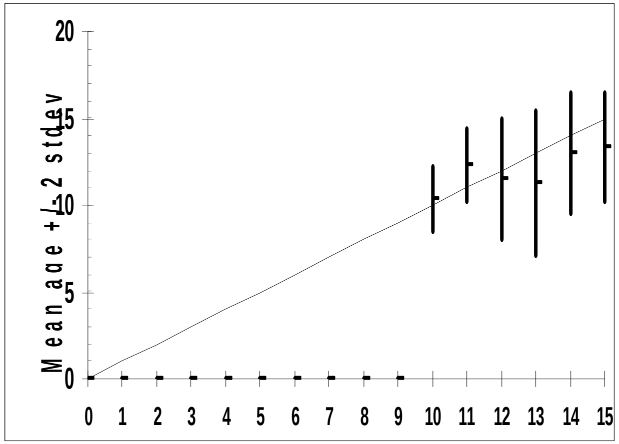
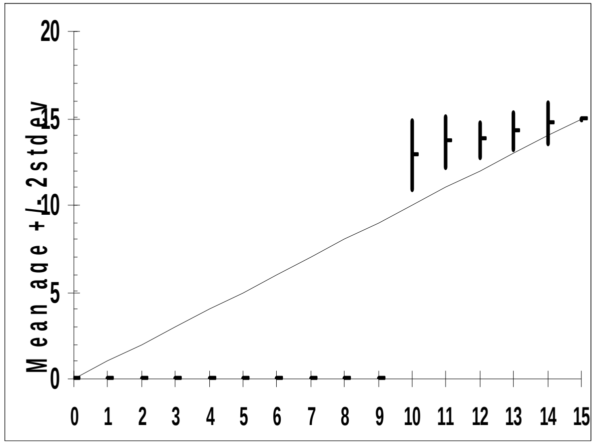


Figure 8. Continued

Reader 7.



Reader 8.



7.2.7 Main conclusions of the Workshop

- The results of the age reading exercises show particularly good agreement when ageing otoliths of juveniles.
- In juveniles it is far easier to identify the 1st and 2nd ring, although it is not always possible to do so.
- For fish up to about age 11 there are reasonably high levels of agreement between readers. However, there are still high CVs on age readings, and statistically significant differences between readers.
- In older fish, and particularly using whole otoliths, it is frequently difficult to identify the first one or two rings, and this then affects age readings for subsequent rings.
- Use of otolith sections results in better ageing of larger fish (> 40cm FL) than using whole otoliths.
- Cooperative training, exchange of otoliths and joint interpretation of otolith images results in substantial improvements in agreement between readers, and better ageing of older fish.

Differences in the level of experience of the readers, associated differences in otolith interpretation methods and rules used by different readers contributed substantially to differences between age-readings. It was very useful to have participants with different levels of experience at the workshop, to identify reasons for the differences in interpretation, and to allow less-experienced readers to benefit from the experience of others.

For some readers, the lack of information on the size of fish made otolith interpretation more difficult. Ensuring that otolith readers are unaware of fish lengths is usually considered to be important for maintaining the objectivity of readers, preventing them from making assumptions on fish age from the fish lengths. However, good consistency between otolith readings is rarely achieved without length information, even for a fish with a short spawning season. Extended spawning creates additional difficulties for interpreting otolith rings. For jack mackerel, extended spawning means that the first ring can represent a period of growth ranging from a few months to a full year, depending on the difference between the times of hatching and ring formation. This can mean that fish from different generations might be incorrectly merged into a single year-class.

It was also noted that otolith readers at different research centres may not have enough of a spread of otoliths over the year, or over different age classes, to be able to develop reliable otolith interpretation protocols relating to edge type and timing of annulus formation. This is because many laboratories do not collect samples across the full year, or across the full range of habitats for juvenile and adult fish.

8. RECOMMENDED STANDARDISED OTOLITH INTERPRETATION PROTOCOL FOR *TRACHURUS MURPHYI.*

During the discussions of results and the review of otolith images, a number of criteria and rules were applied by different readers that helped in the identification of annual rings. These criteria and rules should form the basis of an interpretation protocol for the proper age determination of jack mackerel.

The criteria and rules identified by workshop participants are recommended as a starting point for a standardised jack mackerel otolith interpretation protocol that can be improved later. The main purpose of this initial recommended protocol is to reduce bias in future age readings by participants in the jack mackerel fishery. It should be noted, however, that there will always be uncertainty in otolith readings, and some level of disagreement will always remain. Collaborative work should continue to improve the otolith interpretation protocol, and to improve agreement between otolith readers.

Recommended Otolith Interpretation Rules:

- From previous investigations of daily growth, the radius of the first annulus may be between 1.5 and 2.5 mm. This criterion should be used to identify the first annual ring. Large serrations in the shape of rings are an indication that they may be false rings.
- Consistency and a regular decrease in the width of subsequent rings is a second important criterion for identifying annual rings. Split rings were often observed in the first three years. The steady decrease in spacing between annual rings can be used to recognize split rings.
- Many additional false rings (minor growth checks) may be visible and make it difficult to identify true annual rings in the central part of otolith when magnification is more than 20x. Higher magnification may be needed to distinguish closely spaced rings near the edge of otoliths for larger fish, so it is recommended that different magnifications be used for the central and marginal zones of larger otoliths.
- Annual rings should be well defined and possible to follow around the otolith. This is not always possible, particularly near the edge due to the concave shape of the otolith, and the thickening of otoliths in older (larger) fish. The best approach for large fish is to compare readings of whole and cross-sectioned otoliths. When it is not possible to follow a ring around the otolith, then it may be a false ring or split ring.
- The entire otolith or otolith section should be examined when doing age reading, including the caudal zone and the rostrum. This is particularly important when the caudal zone is difficult to read, in which case it is necessary to examine the rostrum. Identification of false and split rings should also be checked on the rostrum.
- For larger fish (40 cm FL and larger), age readings should be confirmed using otolith cross-sections to avoid under-estimation of age. Ring deposition in larger fish occurs across more by thickening of the otolith, and older rings are particularly difficult to read at the otolith edge, particularly using whole otoliths.

9. OTHER RECOMMENDATIONS

- Participants in the workshop agreed that collaborative discussions on otolith interpretation should continue. Improvements in agreement between otolith readers will benefit from the regular exchange of images of otoliths between the research institutes involved in jack mackerel ageing.
- Inter-sessional work should continue to improve otolith interpretation by the workshop participants, and to increase the level of experience in reading Chilean jack mackerel otoliths. Photographic images are particularly suitable for this purpose, eliminating the practical difficulties with circulating otolith collections between countries. Images can also be examined simultaneously by all participants.
- Otolith images for exchange should be export in a format and resolution that ensure adequate quality for image interpretation, while still allowing images to be easily exchanged. There may need to be some standardization of image analysis software.
- Participants should continue to work inter-sessionally on validation of jack mackerel ageing and growth.

10. REFERENCES

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

Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59: 197-242.

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

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Hoenig, J. M., M. J. Morgan, and C. A. Brown. 1995. Analysing differences between two age determination methods by tests of symmetry. Canadian Journal of Fisheries and Aquatic Systems 52: 364-368.

Ionas, V., and V. Blinov. 1976. Summarized relationship between linear growth of the commercial fish and their age. Rybnoe Khoziaistvo Nº 9, pp 29-51.

Ogle, D. H. 2010. Age-comparisons – Precision and Accuracy Vignette. 8 p.

Serra, R. and R. Gili. 1995. Identificación de anillos anuales en otolitos de jurel. Informe de Taller, IFOP. Junio, 1995.

Walford, I. 1946. A new graphic method of describing the growth of animals. Biol. Bull. 90(2):141-142.

ANNEX 1. Adopted Agenda

Chilean jack mackerel otolith interpretation and ageing WS.
Lima, Peru.
July, 2011.

July 4th
09:00 – 17:30 hrs

Welcome to WS participants by Admiral Jorge Brousset Barrios, President of IMARPE Directive Board.

1. Opening of the WS by Rodolfo Serra (Coordinator).
2. Adoption of the Agenda
3. Nomination of Rapporteurs
4. Methodological approach for the analysis of readings results.
    - Terms of reference.
    - Presentations from Chile, China, EU (Poland), Peru and Russia.
        i. Results of otolith reading (method, validation, mean length at age).
    - Some biology relevant to age determination (R. Serra).
    - Discussion on the methodology to be adopted for age readings comparison.

July 5th
09:00 – 17:30 hrs

1. Reading session of by all participants (whole otoliths).
   (40 otolith)
   The otolith will be assembled into groups of 5 to speed the otolith rotation among readers.

July 6th
09:00 – 17:30 hrs

2. Reading session of by all participants (whole otoliths).
   (40 otolith)

July 7th
09:00 – 17:30 hrs

3. Reading session of by all participants (cross sections).
   (32 otolith; group of 4)

July 8th

1. Discussion of the results.
2. Level of agreement
3. Comparison of the readings among participants.

July 9th
09:00 – 17:30 hrs

4. Comparison of the readings among participants.
5. Review of images of whole otoliths and sections.
6. Discussion of criteria for the interpretation of rings.

July 10th

Free

July 11th
09:00 – 17:30 hrs

7. Discussion of criteria for the interpretation of rings.
8. Agreement on the criteria for the identification of annual rings.

June 12th
09:00 – 17:30 hrs

9. Agreement on the criteria for the identification of annual rings.
10. Report writing.

June 13th
09:00 – 17:30 hrs

11. Adoption of report.
12. WS closure.

ANNEX 2. List of Participants.

Name	Afiliation	email
Rodolfo Serra (Chair)	Instituto Fomento Pesquero, Chile.	rodolfo.serra@ifop.cl
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Marcia Neira	Fishery Research Institute, Chile	mneira@inpesca.cl

ANNEX 3. List of Documents and Presentations

List of documents

Cerna, F. and V. Bocic. Age validation and growth function of Chilean jack mackerel (*Trachurus murphyi*) off Chile**.** Fishing Research Division. Instituto de Fomento Pesquero (IFOP). Chile.

Gang Li, Li-jin Zou, Xiao-ron Zou, Ying-qi Zhou, Min Zhang. Age validation, relationship between age and otolith weight in Chilean jack mackerel (Trachurus murphyi) off Chile watrers. College of Marine Science, Shanghai Ocean University, Shanghai, China; Key Laboratory of Sustainable Exploitation of Oceanic Fisheries Resources. Ministry of Education, Shanghai Ocean University. Shanghai, China.

Ojeda, V., V. Bocic and L. Muñoz. Methodology used to determine the Jack Mackerel *Trachurus Murphyi*) age in Chile. CHILEAN JACK MACKEREL WORKSHOP. Santiago, Chile. 2008. CHJMWS pap #8.

Presentations

Age and growth of jack mackerel from Peruvian waters. T. Dioses. IMARPE.

Age validation and growth function of Chilean jack mackerel (*Trachurus murphyi*) off Chile**.** F. Cerna. IFOP.

Results of Polish research of Chilean jack mackerel. K. Trella. National Marine Fisheries Research Institute.

Some biology and life cycle of *Trachurus murphyi*. R. Serra. IFOP.

Annex 5. Jack Mackerel Otolith Image Interpretation Examples

Annulus identification criteria for jack mackerel otoliths

First Ring

There is general agreement on the identification of the first ring on juvenile fish aged 1 to 3. At these ages, the first annual growth ring is characterized as a strong band that can be followed around the otolith. In some cases this annulus is made up of multiple thin split rings (double or triple). Readers 2, 5 and 8 identify the first annual ring as occurring at a radius from 1.5 to 2.5 mm (**Figure 1**). It should be noted that some otoliths show a false juvenile growth check near to the nucleus, well within the first annual ring (**Figure 1**).

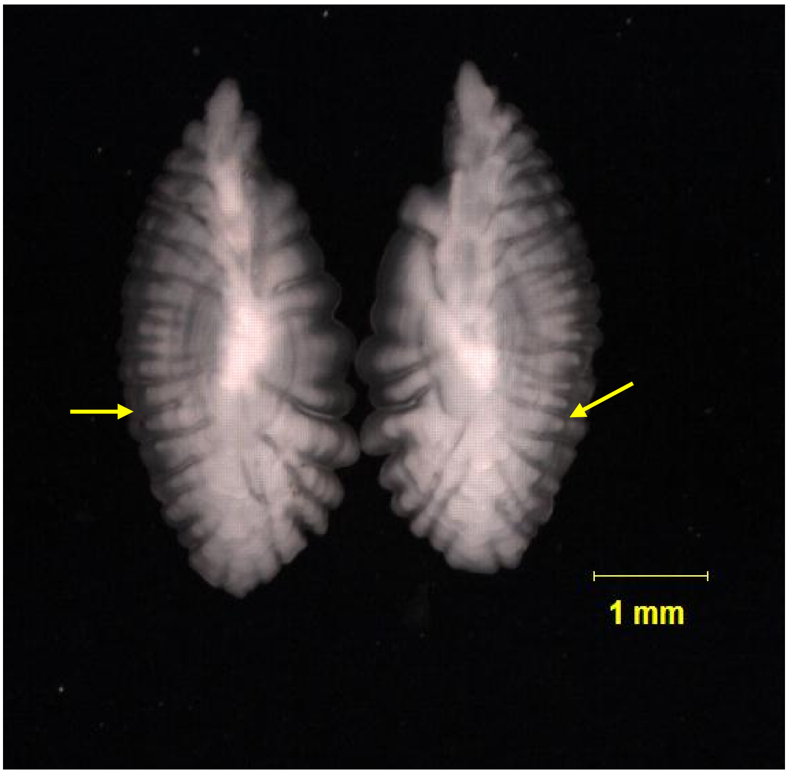

Figure 1. Otolith of a fish of 15 cm FL with one annulus and an opaque border. All readers agreed on the identification of this first annulus (marked with the arrow) (image WOJM-10)

For older fish, different readers applied different criteria to identify the first annulus. Readers 2, 5, 6, 7 and 8 considered, in most cases, the first annulus to be the first ring with a radius inside the range 1.5 to 2.5 mm. Reader 1 identified the first annulus as the ring that readers 2, 5 and 8 identify as the second annulus (**Figure 2**).

Second Ring

In some cases, where readers 2, 5 and 8 identified the second annulus, readers 6 and 7 identified a false ring, identifying the 2nd ring where readers 2, 5 and 8 identified the 3rd annulus.

These differences arise in otoliths with well defined hyaline rings, but where the distance between these rings does not always follow the expected pattern of decreasing distance between annuli (**Figure 2**). Readers 2, 5 and 8 considered that growth can vary, and attached more importance to the presence of clearly defined rings that can be followed around the otolith, but not necessarily have a regular pattern of decreasing distance between annuli, particularly for fish < 7 years of age.

Figure 2. Otolith of a fish of 58 cm FL with alternative age readings from 11 – 13 years. The green points mark rings identified by reader 1, red points by reader 2, black points by reader 7 and large red point to the left by reader 6. The yellow lines indicate radius measurements for the 3 first rings, being 2.1 mm, 2.4 mm and 2.8 mm respectively (image WOJM-88).

Where annuli in otoliths are well defined, and clearly show a pattern of decreasing distance between annuli, absolute agreement was reached between readers (**Figure 3**).

Third and more rings

For otoliths for which there is agreement on identification of the first and second annuli, it is likely that there will also be agreement on the older ages (**Figure 3**).

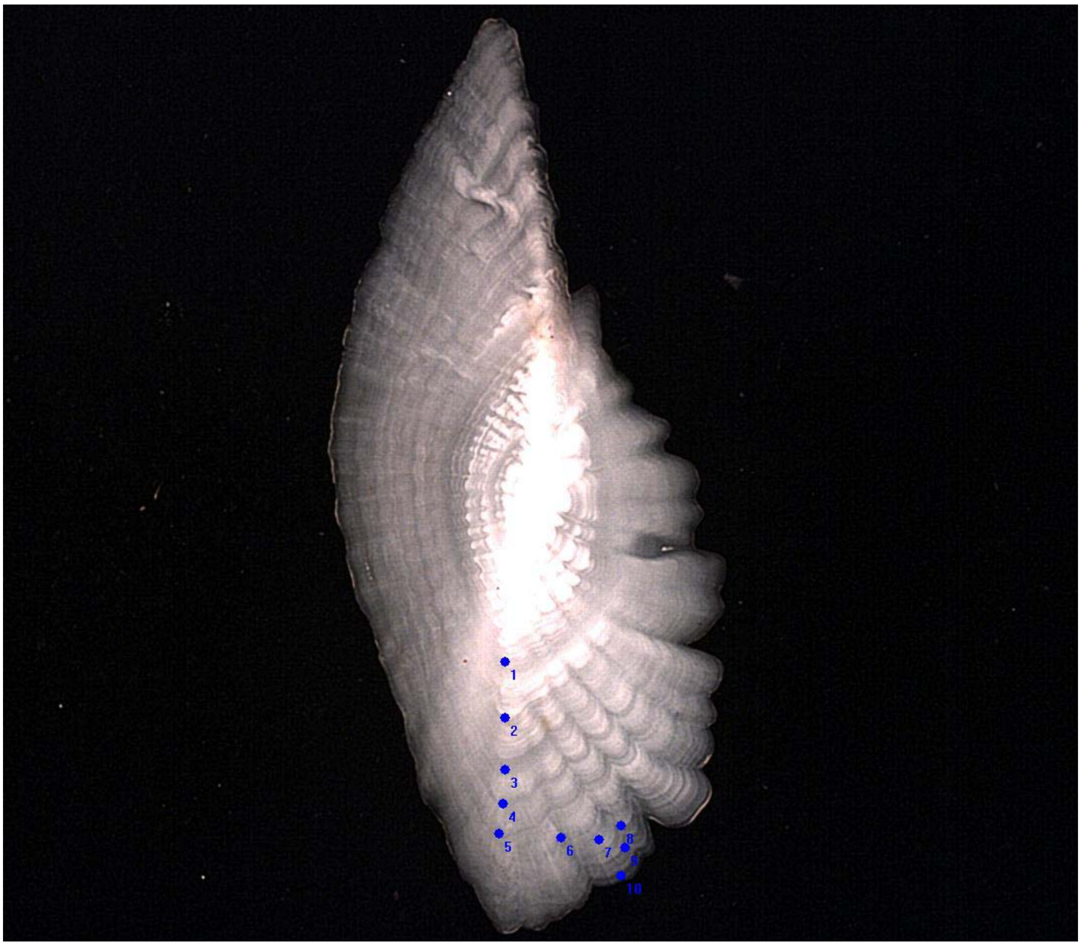

Figure 3. Otolith of a fish of 47 cm FL with 10 clear annuli. Full agreement was reached on reading this otolith image (image WOJM62).

Older fish age > 9 years

The otoliths of jack mackerel often show frequent multiple or split rings between annuli, which correspond to repeated, short, slow growing periods within a year. This appearance of short-term growth checks can be confused with the closely spaced annuli that occur in larger fish, complicating age readings for older fish and generating differences between readers.

For older fish, readers 2, 5 and 8 conducted counts around the whole caudal area, following annuli around the otolith. When rings are difficult to follow continuously around the caudal area, these readers prefer to read them on the rostrum. In this case, to determine final counts, the total number of rings on the rostrum should be compared with the counts from the caudal zone (**Figure 4**). In contrast, readers 1, 6 and 7 only read the caudal area, considering the main criterion to be the progressive decrease in spacing between annuli. These readers do not read the rostrum, and consider that the identification of true rings is more difficult in this part of the otolith.

For large fish, readings of the whole otolith should be complemented by reading a transverse cross-section of the otolith.

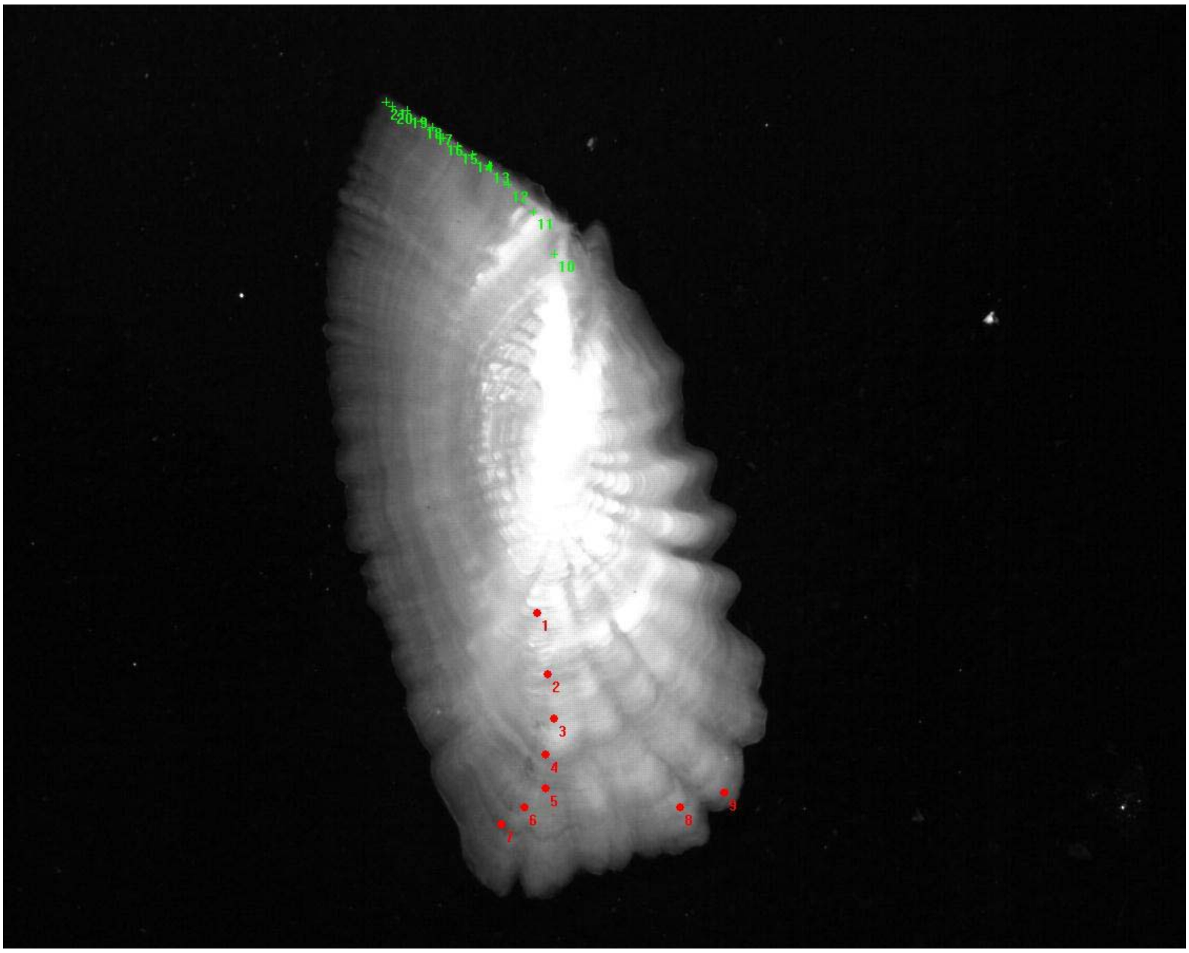

Figure 4. Otolith of a fish of 54 cm FL. The image shows the difference between caudal and rostral readings. At the caudal end it is possible to count 9 rings at the magnification used (10x,) while at the rostrum, 13 rings can be counted (image WOJM85).