Report of the 4th International Ageing Workshop on European Anglerfish

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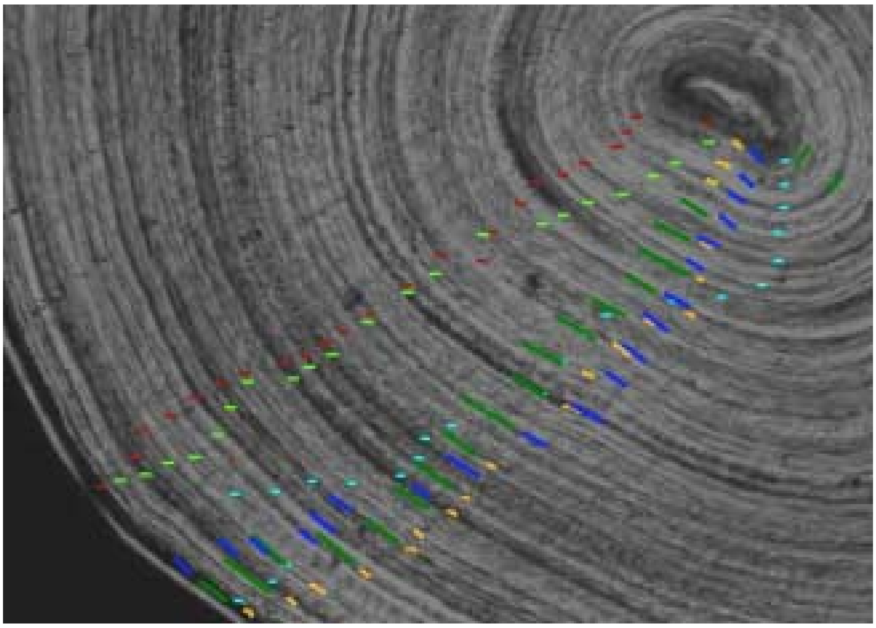
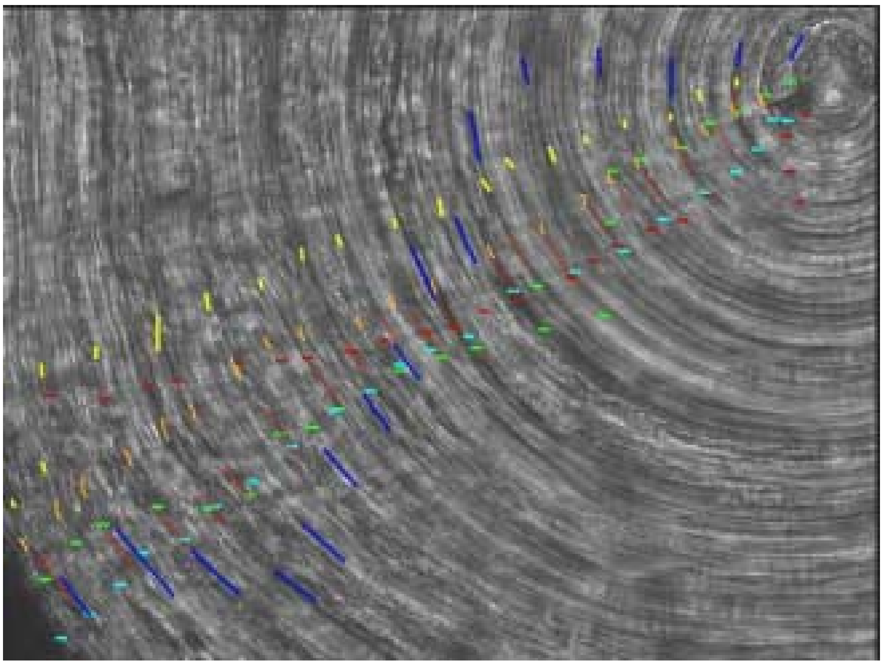
by

Landa, J., Duarte, R., Quincoces, I., Dupouy, H., Bilbao, E., Dimeet, J., Lucio, P., Marçal, A., McCormick, H. and Ni Chonchuir, G.





Contents

1. INTRODUCTION

White (*Lophius piscatorius*) and black anglerfish (*Lophius budegassa*) are two important species of groundfish fisheries in the North East Atlantic.

Both black and white anglerfish are typical bottom living species, the former having a depth range between 70 m and 800 m and the latter extending to depths >1000 m (Dardignac, 1988; Azevedo and Pereda, 1994). Black anglerfish have a more southern distribution (Mediterranean and Eastern North Atlantic from British Isles to Senegal) compared to white anglerfish (Mediterranean, Black Sea and Eastern North Atlantic from Barents Sea to the Straits of Gibraltar), but there is considerable overlap.

In the last two decades several studies on reproduction, age reading and growth have been carried out. The spawning season of *L. piscatorius* is poorly described in the literature, but is generally from late winter to summer (Afonso-Dias and Hislop, 1996; Quincoces et al., 1998a; Duarte et al., 2001). For *L. budegassa* the spawning period is described from October to March for the Iberian coast (Azevedo, 1996 and Duarte et al. 2001) and between May and July in the Cantabrian Sea (Quincoces et al., 1998b). The growth of *L. piscatorius* and *L. budegassa* has been studied based on sagitae otoliths (Crozier, 1989 and Tsimenidis, 1984) and *illicia* (Dupouy *et al*., 1986; Duarte *et al*., 1997; Landa and Pereda, 1997 and Landa *et al*., 2001). Studies comparing the two structures showed that age estimates based on *illicia* readings resulted in higher agreement between readers. (Dupouy pers. com.; IFREMER, 1991; Staalasen, 1995).

Age readings based on *illicia* have been applied for stock assessment of both species. In spite of recent ageing studies, there are still some doubts concerning age validation and ageing precision between readers. The three previous workshops were carried out with different objectives and in different situations concerning biological knowledge of the growth of both species. The first ageing workshop for anglerfish was carried out in 1991 (IFREMER, 1991) involving readers from France and Spain. The main objective was to define clearly an ageing structure to perform ageing for stock assessment purposes. Best results were obtained with the *illicium*, which was adopted. The second workshop held in 1997 (IFREMER, 1997) was conducted with additional participation by Portugal and the main objective was to increase ageing precision between readers. The third workshop was carried out in 1999 (Anon., 1999) with additional participation by Ireland and the main objective was to establish and describe ageing criteria in order to increase the ageing precision between experienced readers.

The present and fourth workshop is undertaken as part of the EU Study Contract No.99/013 (“Genetic characterization and stock structure of the two species of anglerfish (*Lophius piscatorius* and *L. budegassa*) of the Northeast Atlantic” - GESSAN).

Prior to the workshop an *Illicia* Exchange was performed with the main objectives as follows:

- To analyse and to compare the age readings mainly by means of precision (CV), accuracy (bias), agreement and mean length at age.
- To analyse the measurement of the first ring.
- To analyse the new age readings from samples of younger and older fish.

This exchange program included digitised images where each reader identified the annual rings.

The main objectives of the fourth Ageing Workshop were:

- To analyse and discuss the results of the *illicia* exchange, in order to clarify the main problems of anglerfish age reading:
    - To discuss disagreements in age readings, based on statistical analysis of exchange results,
    - To analyse and discuss the digitised images that were obtained from the *illicia* exchange.
- To review work developed for Age Validation, namely, the analysis of length distributions from surveys and the analysis of results, obtained so far, from the mark-recapture study.
- To elaborate an ageing guide including images with highest agreement between readers.

The present report was structured in order to cover the objectives shown above. Therefore, section 2 contains a list of the participants of the workshop, section 3 contains the statistical analysis of the *illicia* exchange results, section 4 contains a summary of the *illicia* image analysis and discussions, with a list of the *illicia* with the highest ring identification agreement, and section 5 contains a review of the developed work on age validation and the main results are presented.

2. PARTICIPANTS

Eli Bilbao	AZTI	Spain
Joel Dimeet	IFREMER	France
Rafael Duarte (coordinator)	IPIMAR	Portugal
Hervé Dupouy	IFREMER	France
Jorge Landa (coordinator)	IEO	Spain
Paulino Lucio	AZTI	Spain
António Marçal	IPIMAR	Portugal
Helen McCormick	Marine Institute	Ireland
Grainne Ni Chonchuir	Marine Institute	Ireland
Iñaki Quincoces (coordinator)	AZTI	Spain

Addresses of participants are in Annex 1.

3. *ILLICIA* EXCHANGE PROGRAMME IN 2001

3.1. OBJECTIVES OF THE EXCHANGE

This is the 2nd *Illicia* Exchange that has taken place. The 1st Exchange took place in 1998, prior to the 3rd Anglerfish Ageing Workshop carried out in 1999 (Anon., 1999).

The recommendations of the 3rd Workshop were taken into account in this exchange, and the objectives of this International Ageing Exchange on European Anglerfish were:

- To continue with regular *illicia* reading exchanges among all readers involved in age determination of anglerfish.
- To involve new readers in age determination of anglerfish for stock assessment purposes.
- To analyse and to compare the age readings by different methods, such as: precision (CV), accuracy (bias), agreement, mean length at age, etc.
- To analyse the measurement of the first ring (horizontal diameter) in both species.
- To analyse the new age readings from samples of younger and older fish.

- To test the usefulness of circulating digital images as a new way of comparing the *illicia* readings. Section 4 of this report contains the discussion of these images.

3.2. EXCHANGE PARTICIPANTS

The reader codes, names and expertise of the readers who participated in the *illicia* exchange are presented in the following table:

Code	Reader	Institution	Country	*L. piscatorius*	*L. budegassa*
R1	Hervé Dupouy	IFREMER	France	**	**
R2	Joël Dimeet	IFREMER	France	*	*
R3	Rafael Duarte	IPIMAR	Portugal	**	**
R4	António Marçal	IPIMAR	Portugal	*	*
R5	Iñaki Quincoces	AZTI	Spain	**	**
R6	Jorge Landa	IEO	Spain	**	**
R7	HelenMcCormick	MIFRC	Ireland	*	*
R8	Grainne NiConchuir	MIFRC	Ireland	*	*

* No experienced reader
** Experienced reader

3.3. MATERIAL AND METHODS

3.3.1. Samples

Two *illicia* collections from each of the two species were prepared for the exchange. These collections were accompanied by digital images of each *illicium*.

The collection of *L. piscatorius* for the exchange program consists of 86 *illicia* in total: 44 *illicia* from the Northern Stock (Divisions VIIIa,b,d) prepared by AZTI from commercial sampling; and 42 *illicia* from the Southern Stock (Divisions VIIIc and IXa) prepared by IEO from commercial sampling and research surveys. The *illicia* from the Northern Stock covered a length range between 21 and 143 cm. Those of the Southern Stock, covered a length range between 10 and 144 cm, thus completing the extremes of length ranges observed for this species (Graph 1).

The collection of *L. budegassa* for the exchange program consists on 76 *illicia*: 36 *illicia* from the Northern Stock (Divisions VIIIa,b,d) prepared by AZTI from commercial sampling; and 40 *illicia* from the Southern Stock (Divisions VIIIc and IXa) prepared by IPIMAR from commercial sampling and research surveys. The *illicia* from the Northern Stock covered a range between 14 and 74 cm. The *illicia* from the Southern Stock ranged between 10 and 97 cm, thus completing the extremes of length ranges observed for this species (Graph 2).

3.3.2. Age reading

In this Exchange, 8 readers belonging to 5 institutes from 4 countries participated. This reflects an increase in the number of new readers of this species. Of the eight readers who participated in the exchange, all of them read the collection of *L. piscatorius*, and only one of them (R1) was not able to read that of *L. budegassa*, due his late involvement in the workshop. Also, of the eight readers, six were able to annotate the annual rings on the digitised images.

A protocol (Annex 2) was prepared in advance of the exchange and circulated amongst readers. This gave information on the collection preparation, ageing and measurement methodology and a time schedule for ageing amongst readers.

The observation of the *illicia* was carried out using an optical microscope under transmitted light. The recommendation of the protocol (based on the recommendations of the previous workshops) was to use the same magnification (100 x) for all readers. Most of the readers used 100x magnifications, but R1 and R2 read part of the exchange *illicia* using 50x magnification.

Information on the total length and the time (quarter) of capture of each specimen was given to the readers. Wherever possible, each reader attributed an age to each *illicium* and gave information about the ageing credibility and the first ring measurements.

The age credibility was classified according to one of four levels of readability: u - unreadable; b - low; m - medium; h - high.

The first ring diameter was measured in micrometers (um) and measured in the direction of the longest axis.

When the readers attributed more than one age to the same *illicium*, the first age was considered. Ages that had a low credibility were also included in the analysis (i.e. 7? was considered as 7).

3.3.3. Data analysis

Several methods have been used, up to the present time to analyse the results of these exchanges or to examine between-reader bias, such as the paired t-test, the Bowker´s test of symmetry and the Wilcoxon rank sum test (ICES, 1994). Other methods were used to compare levels of agreement between groups of readers (in order to test the reproducibility of the results from ageing structures, stocks or preparation methods), the average percent error (APE) from Beamish and Fournier (1981), the index of precision (D) from Chang (1982) and the chance-corrected measure from O'Connell and Dobson (1984). Some of these methods have been considered inappropriate in performing multiple paired comparisons when more than two readers are involved in ageing of the same collection, or because they did not provide an appropriate measure of ageing agreement between readers.

The concerted action group EFAN (European Fish Ageing Network) proposed that a certain degree of standardisation was required if exchanges of age determination material were to be made efficiently. A guideline with the tools for age reading comparisons was made by Eltink *et al.* (2000), and an Excel workbook "AGE COMPARISONS.XLS" (Version 1.0) was produced by Eltink (2000). This workbook was developed for an easy and fast analysis of age reading results and it ensures that age reading comparisons are carried out in a more standardised way, which will enable stock assessment experts to estimate the effect of reading errors on stock assessments. The readings of both species of anglerfish from this Exchange were analysed for the first time, using this workbook. Eltink's workbook mainly estimates the age reading errors that affect precision and accuracy.

- Precision is defined as the variability in the age readings, and it is estimated by the coefficient of variation (CV).

- Accuracy is defined as the closeness of a measured value to its true value. In the absence of calcified structures of known age, the accuracy is estimated by the relative bias. This bias is a systematically over- or underestimation of age compared to the modal age.
- Another statistic estimated by the workbook and related to the accuracy, is the weighted mean percentage agreement, which indicates agreement with respect to the modal age.
- Also the mean length at age is estimated in this workbook.

It provided the following detailed results:

- Number of age readings, CV (%), agreement (%), relative bias and the ranking of age readers according CV, agreement and bias, by age reader and by modal age.
- Age composition and mean length at age by reader, inter-reader and reader against modal age bias tests.
- Number of age readings, CV's, percentage agreements and relative bias, by quarter and by modal age
- Tables for plotting the age bias plots: 2stdv., mean age, 2stdv.+mean age and 2stdv.-mean age by modal age;
- Number of *illicia* by modal age for which 80% or more agreement is reached.
- Age bias plot figures.
- CV (%), agreement (%) and standard deviation plotted against modal age.
- Distribution of the age reading errors in percentage by modal age.
- Relative bias by modal age.
- Estimated mean length at age by age reader.

Other additional analyses, not offered by Eltink's workbook, were performed using SPSS software (SPSS Inc., 10.1.3) and Excel (Microsoft 98) spreadsheets.

- Box-Whisker Plot of mean age by reader.
- Euclidean distances between readers (Cluster analysis)
- Box-Whisker Plot for the average 1st ring diameter.
- Average 1st ring diameter by age, by reader.
- Ageing credibility percentages by reader

3.4. RESULTS AND DISCUSSION

3.4.1. *Lophius piscatorius*

Results are presented for both the Northern and Southern collections together as no differences were observed in an exploratory analysis.

3.4.1.1. Ageing data

Table 1 presents the readings by reader and basic information about the *illicia*. Following the methodology described by Eltink (2000), the modal age was determined by a selected group of experienced readers and not by the whole group. These experienced readers were: R1 (Hervé Dupouy), R3 (Rafael Duarte), R5 (Iñaki Quincoces) and R6 (Jorge Landa). Their readings are coloured in yellow. The readers whos' age readings are used for anglerfish stock assessment, are in red.

Eltink (2000) warns about a problem that occurs with the estimation of the modal age when all age readings are different and no modal age is possible. This happens when the number of age readers that participate for the modal age is low (4 readers). This problem was solved by inserting the mean age (also calculated from the experienced readers).

The percentage of agreement between all readers and the precision (obtained by the coefficient of variation) are also presented.

3.4.1.2. Number of age readings, coefficient of variation (CV), agreement and relative bias by reader.

3.4.1.2.1. Number of age readings

In Table 2.1 we can observe that all the readers aged practically the whole sample (between 83 and 86 *illicia*) and some readers rejected only 2 or 3 illicia.

3.4.1.2.2. Coefficient of variation (CV)

In Table 2.2, the CV (= stdv./ mean age) indicates the precision (variability) in age reading by modal age. The CV is independent of the closeness to the true age. As Figure

2 shows, stdv. increases with ages, while CV remains far more stable since it is much less age dependent. Therefore, CV is a better index for the precision in age reading. Its value was 25%.

The weighted mean of CV, highlights the high precision of the experienced R1 and R3 and the low precision of inexperienced R7, but the values of the weighted mean seem very influenced by low precision in the first ages.

Figure 2 shows that, for all readers, the highest CV was estimated for ages 0 and 1 (216% and 39%, respectively). It could indicate specific problems in the readings of these ages. However, stdv. is at its' lowest for these ages. For ages 0 and 1, high values of CV are more related to the very low value of the mean age (0 and 1) because to calculate those CV values, it is necessary to divide the very low value of the stdv. by 0 or by 1. With the exception of very young fish, no problems in age reading are indicated because low CV's values appear. For ages older than 7 years, CV decreases to values around 10%.

3.4.1.2.3. Weighted mean percent agreement

The mean percent agreement indicates the agreement with respect to the modal age. Its value was just 47 % (Table 2.3).

Figure 2 shows that the agreement is very dependent of the age, decreasing as the ages increased. A high agreement of around 70% is observed between 0 and 3 years, an agreement of around 50% between 4 and 14 years, and a low agreement of around 30% for ages older than 15 years.

In Table 2.3, it highlights the low agreement of the inexperienced R2 and R7, but the high agreement of the other inexperienced R4 and R8. Also it highlights the relative lower agreement (40%) of the experienced R1 in relation to the rest of experienced readers (60%).

3.4.1.2.4. Relative bias

The Relative bias is a systematically over- or underestimation of age compared to the modal age (considered as the true age). Table 2.4 shows mainly an underestimation of age of R1 and R2, and an overestimation of R5.

In Figure 4, the relative bias by modal age estimated by all readers combined, shows important bias for age 13 and for the ages older than 19. The low number of readings at these ages explains the extreme values at these ages.

3.4.1.2.5. Overall ranking

Based on the results from the CVs, percentages of agreement and relative bias for each reader, an overall ranking was obtained (Table 2.5). Readers R3, R4, R6 and R8 were located at the top of this ranking, and only two of them (R3 and R6) are experienced readers. The lowest positions were obtained by two inexperienced readers (R2 and R7).

3.4.1.2.6. Age bias plots, the mean age recorded (+/- 2stdv.) of each age reader and all readers

In Figure 1, the age bias plots, the mean age recorded (+/- 2stdv.) by each age reader and all readers combined are plotted against the modal age.

In relation to the precision, the experienced R1, R5 and R6, and the inexperienced R8 present low stdv. values along most of the age range. The experienced R3 and R4 show higher stdv. in older ages. R7 shows high stdv. across the entire age range.

In relation to the accuracy, the mean ages of R3, R5, R6 and R8 are very close to the modal age. The mean ages of R4 and R7 deviate from the modal age in older ages. The experienced R1 and the R2 show smaller mean ages than the modal age across the whole range of ages.

For all readers, the pattern of the stdv. is observed to increase with age. Readings were very close to the modal ages.

3.4.1.2.7. Age reading errors in percentage by modal age

Figure 3 shows the distribution of the age reading errors in percentage by modal age as observed for the whole group of readers. No relative biases from the normal distribution were observed except for age 13.

3.4.1.3. Age composition, mean length at age and Bias tests.

3.4.1.3.1. Age compositions estimated by each age reader and all age readers combined.

Table 3.1 shows that ages from 0 to 30 years were read. The box-whisker plots of the mean ages (Figure 6) shows the age composition by each age reader. It is observed that the medians of all the readers are close to 10 years, except for the R1 and R2 where they were around 8 years. The mean age also presents differences between readers, with slightly lower values than R1 and R2. Readers 3 and R4 showed narrower intervals because they did not estimate ages older than 26.

3.4.1.3.2. Estimated mean length at age by age reader and by all age readers combined.

In Table 3.2 the values of the mean length at age for each reader are observed. Figure 7 shows that the most significant differences in mean length at age appear in R1 and R2, in comparison with the other readers, in particular between ages 3 and 13.

Figure 5 also shows the mean lengths at age estimated for each reader. Some differences are observed in the mean lengths of some ages between readers, this is due to the small number of samples for those ages. However the most obvious is the higher mean length observed by experienced R1 and the inexperienced R2, at ages 2 to 13.

In the Figure 8 the differences in mean length at age are compared amongst the experienced readers. We can see that the readings of R5 and R6 are practically the same and that R3 differs only in some higher ages. The major difference is observed in R1 with respect to the other expert readers, between the ages of 4 and 13. This difference is around 1 and 2 age classes (around 7.5 cm).

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

The results of the comparison of readings between pairs of readers (Table 3.3) show that R1, R2 and R5 disagree more frequently with the rest of the readers. There is only one case without significant difference between this group and the rest of the readers. The readings of R1 are only similar to R2 and vice versa. The rest of the readers have three and four comparisons with non significant bias.

3.4.1.4. Criterion 80% of agreement

Table 4 shows that more than 80% of agreement between all readers was obtained for just ten *illicia,* all of them lower than 4 years old.

3.4.1.5. Dendrogram

In Figure 9 the dendrogram obtained from cluster analysis, points out a great closeness between the experienced R6 and the inexperienced R8. R5 and R3 were observed to be in close proximity as were the other inexperienced readers R2 and R4. The experienced R1 and the inexperienced R7 were observed to be very far from the rest.

3.4.1.6. First ring

In Figure 10, the box-whisker plots of the first ring diameter by reader show three different groups of readers. The median and the modal diameter of most readers were very close to each other and located between 260 and 280 µm. The mean values of R1 and R8 were respectively slightly higher and lower than these. R2 shows much higher mean values.

Figure 11 shows the graphical representation of the average fist ring diameter by age for each reader. R2 shows very high values. R1 also presents higher values of the first ring in *illicia* younger than 7 years. R8 shows slightly lower values than the other readers. Excluding R2, we observe that the diameter ranges between 200 and 325 µm.

Also a continuous increase of the diameter is observed with age. Up to 4 years of age the diameter ranges from 200 and 250µm. It increases between the ages of 4 to 7. From 7 onwards, a slight increase is observed. This increasing diameter with age is probably due to the distance from the base of the *illicia* to which the cut of the section is made, as opposed to natural causes.

3.4.1.7. Magnifications

All the readers read with 100X, except R1 and R2. R1 read half of the collection (42 *illicia*) with 50X and the other half with 100X. R2 read a third of the collection (26 *illicia*, all those of lengths higher than 72 cm of the Northern Stock) with 50X and the rest with 100X. Maybe this had an influence in the differences of these readings.

3.4.1.8. Ageing credibility percentages by reader

Table 5 shows that "Medium" credibility level is the most frequent for most readers. R1 and R6 consider a high proportion of "High" credibility levels, and for R8 the "High" credibility level is the most frequent.

3.4.2. *Lophius budegassa*

Results are presented for Northern and Southern collections together as no differences were observed in an exploratory analysis.

3.4.2.1. Ageing data

Table 1 presents the readings by reader and basic information about the *illicia*. Following the methodology described by Eltink (2000), the modal age was determined by a selected group of experienced readers and not by the whole group. These experienced readers were: R1 (Hervé Dupouy), R3 (Rafael Duarte), R5 (Iñaki Quincoces) and R6 (Jorge Landa). Their readings are coloured in yellow. The readers who's their readings are used for anglerfish stock assessment, are in red.

Eltink (2000) warns about a problem that occurs with the estimation of the modal age when all age readings are different and no modal age is possible. This happens when the number of age readers that participate for the modal age is low (4 readers). This problem was solved by inserting the mean age (also calculated from the experienced readers).

The percentage of agreement between all readers and the precision (obtained by the coefficient of variation) are also presented.

3.4.2.2. Number of age readings, coefficient of variation (CV), agreement and relative bias by reader.

3.4.2.2.1. Number of age readings

In Table 2.1 we can observe that all the readers aged practically the entire sample (between74 and 76) and only 2 readers read 72 *illicia*.

3.4.2.2.2. Coefficient of variation (CV)

In Table 2.2, the CV (= stdv./ mean age recorded) indicates the precision in age reading by modal age. The CV is independent of the closeness to the true age. As Figure 2 shows, stdv. increases with ages, while CV remains far more stable since it is much less age dependent. Therefore, CV is a better index for the precision in age reading. Its value was 16.6%.

In the weighted mean of CV, highlights the high precision of the expert readers R5 and R6 and the low precision of inexperienced reader R7 and R8. But these values of the weighted mean seem very influenced by the low precision in the first ages.

Figure 2 shows that, for all readers, the highest CV was estimated for ages 1 and 2 (63% and 38%, respectively). It could indicate specific problems in the readings of these ages. However, their stdv. have the lowest values. In these ages 1 and 2, these high values of CV are more related to the very low value of the mean age (1 and 2) because to calculate those CV values, it is necessary to divide the very low value of the stdv. by 1 or by 2. Except in the first ages, no problems in age reading are indicated because low CV's values appear.

3.4.2.2.3. Weighted mean percent agreement

The mean percent agreement indicates the agreement with respect to the modal age. Its value was just 44 % (Table 2.3).

Figure 2 shows that the agreement is very dependent of the age, decreasing with age. A high agreement of around 70% is observed between 1 and 4 years, an agreement of around 50% between 5 and 10 years; and a low agreement of around 30% for ages older than 10 years.

Table 2.3, highlights the lower agreement of the inexperienced R2 than the rest of inexperienced readers. It also highlights the lower agreement (29%) of the experienced R3 in relation to that of the rest of experienced readers (around 75%).

3.4.2.2.4. Relative bias

The Relative bias is systematically over- or underestimation of age compared to the modal age (considered as the true age). The Table 2.4 shows mainly an underestimation of age of R2 and R3, and an overestimation of R5.

In Figure 4, the relative bias by modal age estimated by all readers combined, shows important bias for the age 9, 13, 14, 18, 22 and 23. The extreme values at ages 14, 18 and older than 20 are explained by the low number of readings at these ages.

3.4.2.2.5. Overall ranking

Based on the results from the CVs, percentages of agreement and relative bias for each reader an overall ranking was obtained (Table 2.5). The experienced R6 and R5 and the inexperienced R4 were located at the top of this ranking. The inexperienced R2 and the experienced R3 obtained the lowest position.

3.4.2.2.6. Age bias plots, the mean age recorded (+/- 2stdv.) of each age reader and all readers

In Figure 1, the age bias plots, the mean age recorded (+/- 2^{nd} stdv.) of each age reader and all readers combined are plotted against the modal age.

In relation to the precision, the expert R3 and inexperienced R4, R7 and R8 show a higher stdv. for ages older than 10 years.

Related to the accuracy, the mean ages of the experienced R5 and R6 are very close to the modal age. The experienced R3 and the inexperienced R2 present smaller mean age than the modal age for practically the whole range of ages. The inexperienced R4, R7 and R8 present mean ages deviating from the modal age in ages older than 10 years.

For all readers, the pattern of the stdv. is observed as it increases with age. Readings were very close to the modal ages.

3.4.2.2.7. Age reading errors in percentage by modal age

The distribution of the age reading errors in percentage, by modal age as observed from the whole group of age readers in an age reading comparison to the modal age (Figure 3) shows no relative bias from the normal distribution except for age 13 and 14.

3.4.2.3. Age composition, mean length at age and Bias tests.

3.4.2.3.1. Age compositions estimated by each age reader and all age readers combined.

Table 3.1 shows that ages from 0 to 26 years were read. The box-whisker plots of the mean ages (Figure 6) shows the age composition by each age reader. It is observed that all medians are placed between 6 and 8 years old so there is no marked differences between readers. R2 and R3 presented a mean age value lower than the rest of the readers that gave very close mean age values. Also, R2, R3 and R4 gave the narrowest reading intervals.

3.4.2.3.2. Estimated mean length at age by age reader and by all age readers combined.

In Table 3.2 the values of the mean length at age for each reader are observed. Figure 7 shows that the most significant differences in mean length at age appear in R2 and R3, with respect to the other readers, between ages 3 and 15.

Figure 5 also shows the mean lengths at age estimated for each reader. Some differences are observed in the mean lengths of some ages of some readers with respect to the rest because of the scarce number of samples for those ages. However the most obvious is the higher mean length at ages 3 to 15 observed by experienced R3 and inexperienced R2.

Figure 8 compares the differences in mean length at age amongst the experienced readers. We can see that the readings of R5 and R6 are practically the same and that R3 has a slightly higher mean length at age.

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

The results of the comparison of the readings between pairs of readers (Table 3.3) show that R2 and R3 are the readers that coincide less with the rest of the readers, since they present no significant bias cell between them. The rest of readers have 3 and 4 comparisons with non significant bias.

3.4.2.4. Criterion 80% of agreement

The Table 4 shows that more than 80% of agreement between all readers was obtained for just 6 *illicia,* most of them between 2 and 4 years old.

3.4.2.5. Dendrogram

Figure 9, the dendrogram obtained from cluster analysis, points out very clearly 3 different groups of readers. The first cluster consists of R2 and R3. R4, R5 and R6 belong to the second one and finally, a third cluster is formed by R7 and R8. The closest readers calculated by the squared Euclidean distance are R5 and R6 (expert readers). However, R3, being another expert reader, is located far apart from the other experts.

3.4.2.6. First ring

In Figure 10, the box-whisker plots of the first ring diameter by reader show that all readers except for R7 presented low variation in the diameter measurements. R4, R5, R6, R7 and R8 appeared to locate the first ring very similarly (between 75 and 90 μm) but R2 and R3 placed the first ring closer to the *illicia* centre.

Figure 11 shows the graphical representation of the average fist ring diameter by age for each reader. R2 shows slightly lower values and R8 shows slightly higher values than the other readers.

Also it is observed a continuous yet slight increment of diameter with age was observed, with 70-80 μm for lower ages and around 90 μm for the older ones. This slight increase with age is probably due to the distance from the base of the *illicia* to which the cut of the section is made, rather natural causes.

3.4.2.7. Magnifications

All the readers read with 100X except R6 who read the most of the *illicia* with 200X magnification but no differences regarding this different reading method has been found in R6’s readings.

3.4.2.8. Ageing credibility percentages by reader

Table 5 shows that “Medium” credibility level is the most frequent for most readers, except for R8, who considers “High” credibility level for most of the *illicia*.

3.5. CONCLUSIONS

3.5.1. *Lophius piscatorius*

- Except for the inexperienced R7, no other problems in precision of age reading by reader are indicated. This is because of low CV's values.

- There was low agreement between inexperienced R2 and R7, and of the experienced R1.

- Related to the accuracy, an underestimation of age of R1 and R2, and an overestimation of R5 are observed.

- The most significant differences in mean length at age appear in R1 and R2 with respect to the other readers, with higher mean lengths between ages 3 and 13. This difference is around 1 or 2 age classes.

- In the previous 3rd Workshop, the mean values of the diameter of the 1st ring had a high variation (between 200 and 450 μm), even for the expert readers (among 200-270 μm). However, for all the readers participating in the present workshop (experienced and inexperienced, except for R2), the 1st ring see diameter seems fairly standardized, lying between 260 and 280 μm.

3.5.2. ***Lophius budegassa***

- No problems in precision of age reading by reader are indicated because low CV's values appear.

- There is low agreement between the inexperienced readers and the experienced R3.

- Related to the accuracy, an underestimation of age of R2 and R3, and an overestimation of R5 are observed.

- The most significant differences in mean length at age appear in R2 and R3 with respect to the other readers, with slightly higher mean lengths between ages 3 and 15.

- In the previous 3rd workshop, the mean values of the diameter of the 1st ring had a high variation (between 50 and 350 μm), even amongst the expert readers (among 50-150 μm). However, for all the readers of the present workshop (experienced and inexperienced), the 1st ring seems standardized, located between 60 and 100 μm.

4. DISCUSSION OF *ILLICIA* IMAGES

During the Exchange, *illicia* sections and their respective digitised images were circulated. Each reader was asked to mark the annual rings on the images, using an image analysis software program. Images from the following readers were obtained:

Code	Reader	Institution	Country	*L. piscatorius*	*L. budegassa*
R1	Hervé Dupouy	IFREMER	France	1/2 Images	
R2	Joel Dimeet	IFREMER	France		1/2 Images
R3	Rafael Duarte	IPIMAR	Portugal	Images	Images

R4	António Marçal	IPIMAR	Portugal	Images	Images
R5	Iñaki Quincoces	AZTI	Spain	Images	Images
R6	Jorge Landa	IEO	Spain	Images	Images
R7	Helen McCormick	MIFRC	Ireland	Images	Images
R8	Grainne NiConchuir	MIFRC	Ireland	Images	Images

At the Workshop, the position of the rings annotated by each reader on these images was compared. A certain *illicia* number with low agreement was examined and discussed, and an agreed age was established for each one of them. Also the location of the first ring was agreed. This activity was especially useful to unify the approach amongst the expert in identifying the location of the 1st ring in more difficult *illicia*. Likewise it was very useful for the inexperienced readers, where the differences were higher (especially in the location of the first ring). For the majority of the images, it was observed that disagreements in ring location occurred especially in the first 5 rings. A high agreement in ring location was obtained after ring 5, in the middle part of the *illicia* section.

The following *illicia* were considered to have a high agreement:

Lophius piscatorius

Agreed age	File name	Collection area	Comments
1	14 3b-1 1999	South	Total Agreement by experienced readers
2	289-97-5b-4	North	Total Agreement by experienced readers
3	67-99-5b-3	North	Total Agreement by experienced readers
3	2-1b-4 2000	South	Total Agreement by experienced readers
4	60-99-3a-3	North	Total Agreement by experienced readers
5	73-99-1b-4	North	Total Agreement, better image
5	13-4a-21999	South	Total Agreement by experienced readers
6	2b-5	North	Modal Age agreed, but different rings selected
8	66-99-4a-7	North	Disagreement on 3rd and 4th rings
9	11-00-1a-1	North	Good agreement by R5 &R6, R3= N-1
10	84-99-2A-3	North	Total Agreement by experienced readers
11	15-4b-5 1999	South	R5&3=11, R6=12, however chose very similar rings
12	114-99-2a-1	North	Disagreement on one ring

12	8-4b-3-2000	South	R3 differs on ring 1 and 11
13	238-97-1a-4	North	R1, 5 &6 =13, similar rings, R3=11
14	100-99-3b-3	North	R5&6 in total agreement, R3=11
16	77-99-5b-8	North	Total Agreement by experienced readers
24	9-1b-3 1999	South	Age 24-29, image looks good, different rings chosen
25	9-1b-2 1999	South	Total Agreement by experienced readers

Lophius budegassa

Agreed age	File name	Collection area	Comments
1	A-2	South	Total Agreement by experienced readers
2	A-7	South	Total Agreement by experienced readers
3	B-3	South	Total Agreement by experienced readers
4	19-1998-5b-8	North	Total Agreement by experienced readers
5	C-7	South	Total Agreement by experienced readers
6	3-1999-3b-2	North	Total Agreement by experienced readers
7	39-1998-3a-3	North	Total Agreement by experienced readers
8	G-7	South	R3 missed 3rd ring
9	6-1999-3b-4	North	R5&R6=9, similar rings, R3=6
10	237-1997-5a-5	North	Total Agreement by experienced readers
11	92-1999-5b-10	North	Agreement on all rings except 1st by R3
12	K-6	South	R5&6 = 12, R3=8, Good illustration of applying different ageing criteria.
15	N-1	South	R5&6=15, R3=13
19	N-5	South	R3=17, R6=19 and R5=20, R5&6 chose similar rings
21	O-3	South	R3=16, R5&R6=21, but chose some different rings

An Anglerfish Ageing Guide (Annex 3) with these images, the ageing criteria and the methods of mounting and sectioning was elaborated.

5. TRIALS OF GROWTH VALIDATION

5.1. INTRODUCTION

Fish growth studies must underlay on an accurate and reliable individual age determination. The accuracy of an age estimation method needs to be finally based on the age validation. Validation could be understood either as the confirmation of the temporal meaning of the different changes or marks observed in the fish or in their calcareous structures or also as the accuracy of the age determination. Validation process could imply different steps or degrees in the goal achievement (Francis, 1995).

Age validation could be achieved by indirect or direct methods: if they inform on population growth rates estimated by consistent evidences or they straight assess the temporal increment of the individual fish length or the fish calcified structures marks, respectively.

The validation of the growth of both species of anglerfish has been required since the studies of growth based on skeletal pieces began but that is difficult to get. As expected, validation of the anglerfish ageing was not possible within the short period of years, since it is a part of the growth study that needs several years of investigation.

Until now, 2 projects have offered the first results about the validation of the anglerfish growth in the Southern shelf of the Atlantic European waters: DEMASSESS project and GESSAN project. During DEMASSESS project (2000), three different ways were used to try of validating the growth in anglerfish: the study of the length frequency distributions of the catches, the back-calculation, both in the Northern Stock, and the study of the results of the mark-recapture experiments. During GESSAN project, two different ways were used: the study of the length frequency distributions of specimens caught in the research trawl surveys in the Southern Stock and the study of the results of the mark-recapture experiments took place until now days.

5.1.1. Indirect validation methods

<u>5.1.1.1. Length frequencies based methods</u>

Fish populations that reproduce seasonally are characterized by regular influxes of new recruits. Adequately sampled, they usually reveal a size structure featuring a train of wave-like modes (length frequency polygons), especially in the first few years of life (Weatherley, 1972). The length frequency analysis methods are carried out in two different ways: following a single cohort along time or analysing the length composition of different age classes.

Fish recruitment can be variable. Sometimes a combination of favourable conditions results in an unusually large recruitment (i.e. a new year-class of exceptional strength is added to the population). The members of such a year-class may progress, like other ones, but persisting as a notable strong size mode. On the other hand, also unfavourable conditions at spawning or during egg-larval development can sometimes produce a very small subsequent year-class easily detectable, by comparison with other strong or medium year classes, in a regular monitoring sampling (Weatherley, 1972).

Different mathematical methods have been deployed during the last decades to analyse more precisely the modal components of the length distributions, one of them is MULTIFAN.

5.1.1.2. Back-calculation methods

They consist on the assumed relation between the fish length at the moment of its capture and some measurable marks present in determinate calcareous structures. It is assumed also that marks formation follows a regular pattern linked to a temporal period.

5.1.2. Direct validation methods

They are based on experiments on external and internal tagging and recapture of anglerfish.

For the first time an anglerfish tagging program with a uniformized methodology was initiated along the Northern and Southern stocks in DEMASSESS project (2000) and followed during GESSAN project.

The aims of the tagging and recapture experiments consist basically on:

5.1.2.1. Knowledge of the fish length increment within a certain time period

Fish of known size can be captured, marked and later, when recaptured, its size increment in the period elapsed can be determined with accuracy. Thus both the increase in size and the time passed result well know and measured.

5.1.2.2. Marking of calcareous structures

When fish are marked by special substances, determinate marks in their hard structures (otoliths, *illicia*, fins) can be produced and detected, and the periodicity of the ageing pieces increments could be validated.

Markers are those substances (i.e. tetracycline) that applied to the fish by means of either injecting or dissolving it in seawater by ambient exposure, are deposed in the calcareous structures and can be easily detected.

5.2. RESULTS

The results obtained by the different ways used indicates that the growth estimated from the *illicia* reading during last years seems not to be so far from the possible true growth of these species.

5.2.1. Indirect validation methods

5.2.1.1. Length frequencies based methods

The anglerfish lengths frequency data from commercial catches of the Northern Stock resulted consistently incomplete and lacked of some lengths classes to carry out satisfactorily the MULTIFAN study to both anglerfish species during DEMASSESS project (2000).

The length frequency distributions of *L. piscatorius* caught in the research trawl surveys carried out in September-October from 1994 to 2001 and in April 1997, in the Northern Spanish continental shelf (Southern Stock: ICES Div. VIIIc and IXa) were studied and some modal classes of the first years was identified on them (Annex 4; Landa, 2002a). The clearest recognizable of them and visible in most years was that around 18 cm (probably age class 1). Another less clear modal age class was observed around 10 cm and probably associated with the age class 0. Other age classes were identified around 29 cm (supposed age 2) and around 35 cm (age 3). An adjustment along the months between these modal lengths and the mean length at first ages estimated in the last International Ageing Exchange Programme on European Anglerfish was observed. The modal class of 26 cm observed in the survey in April 1997 corroborated the growth between the ages 1 and 2, when presenting an intermediate value between these modal ages from September surveys. Likewise this value of 26 cm of modal class coincided with the value of mean length for age 2 estimated for the same season of the year in the International Ageing Exchange Programme.

5.2.1.2. Back-calculation methods

A study on back-calculation by image analysis of *illicia* to calculate the mean length by age class was satisfactorily applied to both anglerfish species of the Northern Stock during DEMASSESS project (2000). An isometric relationship between body length and *illicia* radius was found for both species of anglerfish. Annual mean length of black and white anglerfish (ages 2-14) estimated by back-calculation did not differ significantly from mean length values obtained by *illicia* readings carried out by ageing methods.

5.2.2. Direct validation methods

It was not possible to mark as many anglerfish as other species (i.e. small pelagic fish) due to the scarce number that are captured compared to other species and to that not all the anglerfish present the appropriate conditions to survive after tagging. Although the recapture percentage was similar to that of other species where this technique has been successful, the scarce recaptures number make very difficult that there are, in a short term, the enough ones to offer significant results on growth validation.

Most recaptured anglerfish were less than one year in the sea and they did not allow making definitive conclusions about the growth rates and the periodicity of the ring deposition. However, four anglerfish were recaptured with useful information to growth validation.

A definitive conclusion can only be made with more recaptures of individuals that were during a long time (more than one year) in the sea, to consider the seasonal growth variations along the year.

5.2.2.1. *L. piscatorius*

5.2.2.1.1. Knowledge of the fish length increment within a certain time period

2 specimens tagged and recovered by IEO validated the growth estimated by *illicia* reading (Annex 5; Landa, 2002b).

They spent 9 and 11 months at sea before recapture and it is the first records with such a long time between tagging and recapture. The growth of these specimens recaptured after tagging was very close to that estimated by *illicia* reading in the International Ageing Exchange Programme on European Anglerfish and also to that estimated from the routinely annual age length keys.

5.2.2.1.2. Marking of calcareous structures

Only an increasing fixing of this substance at the outer edge of the *illicium* of specimens recovered between a few days and 2 months is observed (Landa, 2002b).

5.2.2.2. *L. budegassa*

5.2.2.2.1. Knowledge of the fish length increment within a certain time period

1 specimen tagged and recovered by IFREMER validated the growth estimated by *illicia* reading (Annex 6; Dupouy et al., 2002).

It spent 14 months at sea before recapture and it is the first record with such a long time between tagging and recapture. The growth of this specimen recaptured after tagging was very close to that estimated by *illicia* reading in the International Ageing Exchange Programme on European Anglerfish and also to that estimated from the routinely annual age length keys.

5.2.2.2.2. Marking of calcareous structures

2 specimens tagged and recovered (one by IFREMER and the other one by IEO) validated the growth estimated by *illicia* reading (Annex 6; Annex 7; Dupouy et al., 2002; Landa, 2002c).

The internally mark of the *illicium* of the IFREMER specimen which spent 14 months at sea before recapture, suggested an age ranging from 4 to 5 years but the tetracycline mark was very large with outward and possibly inward diffusion which obscures the interpretation task (Annex 6; Dupouy et al., 2002). Alternatively, age reading from vertebrae provides an of 6 years for this 40.5 cm fish which is consistent with the age obtained using the growth rate estimated from the tetracycline mark. Moreover this is in good agreement with the age provided by Dupouy *et al.*, (1986) and Duarte *et al.*, 1997.

The *illicium* section of the IEO specimen recovered after 7 months showed the tetracycline deposited at its edge of over half the width of the last supposedly annual growth area of the *illicium* (between the supposed annual ring 8 and 9) (Annex 7; Landa, 2002). Therefore, the area with the tetracycline deposited and the part without tetracycline was considered an annual growth area. This help in the validation of growth pattern estimates by means of *illicia* reading for the first time.

6. RECOMMENDATIONS

- The annexed ageing guide to this document is recommended for use as reference guide for ageing *illicia* for expert readers and as training guide for inexpert readers. This document will be a live document and will be open to updating as and when more research is carried out and advances are made on ageing criteria.

- Anglerfish *illicia* exchanges should be conducted regularly for the purpose of checking the precision, agreement and accuracy of all readers involved in age determination.

- In order to improve ageing agreement, it is recommended that workshops are held regularly amongst the countries involved in stock assessment, and especially new readers from the North-Eastern Atlantic European Anglerfish Stock will be welcome. The collection of *Illicia* should include samples from all North Eastern Atlantic European Anglerfish Stocks.

- Both *illicia* sections and their respective digitised images should be circulated in future exchanges, as this has proven to be a very valuable exercise in this workshop.

The position of the rings should be annotated on the images and these will be compared at the following workshop.

- The measurement of the first ring (horizontal diameter) of each species is important to help locate its' position and its measurement should be requested in future exchanges.

- It is recommended that in future *illicia* exchanges, specimens of all the length range (specially younger fish) should be included in the samples.

- Digitising and multimedia systems are recommended to aid communal readings and discussions during next workshops.

- More studies based on life history events of Anglerfish are strongly recommended in order to identify the occurrences of some characteristic rings, which are very prominent in the *illicium*.

- Validation of age reading is necessary and this should be carried out on more than one structure. Methods of tagging and chemical marking should be used in addition to modal length distribution analysis.

7. SUMMARY AND CONCLUSIONS

This fourth workshop and a previous *illicia* exchange were performed to deal some problems in age reading. The objectives of the exchange were to compare the age readings (by means of precision, accuracy, agreement and mean length at age), to analyse the measurement of the first ring, to analyse the new age readings from samples of younger and older fish, to continue with the regular *illicia* reading exchanges and to involve new readers and to test the useful of circulating digitised images as a new way of comparing the *illicia* readings. In this exchange, 8 readers belonging to 4 countries (Ireland, France, Portugal and Spain) participated. Two *illicia* collections from each of the two species were studied: 86 *illicia* of *L. piscatorius* and 76 *illicia* of *L. budegassa*. Also, digitised images where each reader identified the annual rings were included. The main objectives of the fourth ageing workshop were to analyse and discuss the results of the *illicia* exchange, to review of the work developed for age validation and to elaborate an ageing guide including images with highest agreement between readers.

The results of the *illicia* exchange of *L. piscatorius* did not show problems in precision of age reading, except for one inexperienced reader. Acceptable overall agreement and only very low agreement of two inexperienced readers were observed. It showed good values for overall accuracy and similar mean lengths at age for most readers. An underestimation of age with higher mean lengths of two readers (around 1 and 2 age classes) and a slight overestimation of one of them was observed. The 1st ring was located for all the readers and its diameter was standardized between 260 and 280 µm.

In *L. budegassa* the analysis did not showed any problem special precision of age reading. Low agreement of the inexperienced readers and of one experienced reader was observed. It showed good values for overall accuracy and similar mean lengths at age for most readers. Slight under and overestimations of age and some slight differences in mean lengths at age in few readers were observed. The 1st ring was located for all the readers and its diameter was standardized between 60 and 100 µm.

Related to the images, the position of the rings annotated by each reader on them were compared to unify the approach among the expert readers in some concrete ring located in the central part of the *illicium* and that they were not easily identifiable. Likewise it was very useful for the inexpert readers, where the differences were higher (especially in the first ring). For the majority of the images, it was observed that disagreements in the ring location occurred especially in the first 5 rings. A high agreement in the ring location was obtained after ring 5, in the middle part of the *illicia* section.

An ageing *illicia* guide was elaborated. This includes elements from the protocol of *illicia* age determination, age reading criteria and reference collection of images of agreed ages.

The results obtained by the different ways of validation used indicated that the growth estimated from the *illicia* reading during last years did not seem to be so far from the possible true growth of these species.

In the length frequency distributions of *L. piscatorius* from the Southern Stock, some modal lengths of the first years (ages 0-2) were identified and an adjustment was observed between these modal lengths and the mean length at first ages estimated by *illicia* reading in the exchange.

The study on back-calculation by image analysis of *illicia* to calculate the mean length by age class was satisfactorily applied to both anglerfish species of the Northern Stock. The annual mean length of both anglerfish (ages 2-14) estimated by back-calculation did not differ significantly from mean length values obtained by *illicia* readings.

The growth of 2 specimens of *L. piscatorius* recaptured 9 and 11 months after tagging and that of 1 specimen of *L. budegassa* recovered 14 months after tagging, was very close to that estimated by *illicia* reading in the exchange and also to that estimated from the routinely annual age length keys.

Observing the marks in calcareous structure, 2 specimens of *L. budegassa* recovered 7 and 14 months after tagging, also validated the growth estimated by *illicia* reading.

8. WORKING DOCUMENT PRESENTED

8.1. *ILLICIA* EXCHANGE

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8.2. TRIALS OF GROWTH VALIDATION

- Landa, J. 2002. Validation trials of growth of white anglerfish (*Lophius piscatorius*) in the North-eastern Atlantic based on mark-recapture experiments. Working Document of the 4th International Ageing Workshop on European Anglerfish, IPIMAR, Lisbon, 14-18 January 2002.

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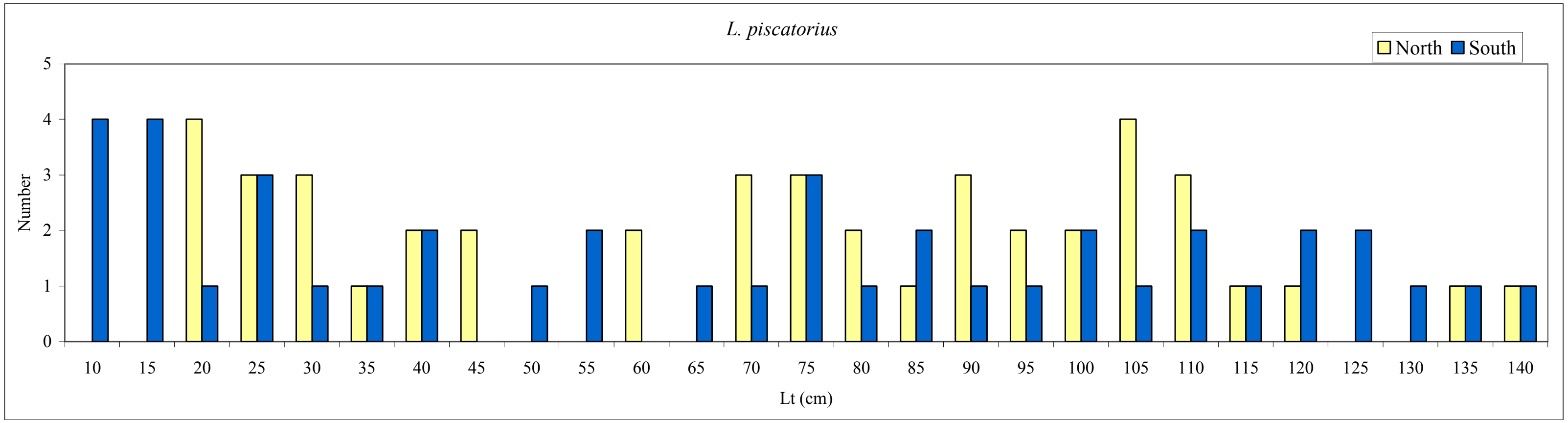
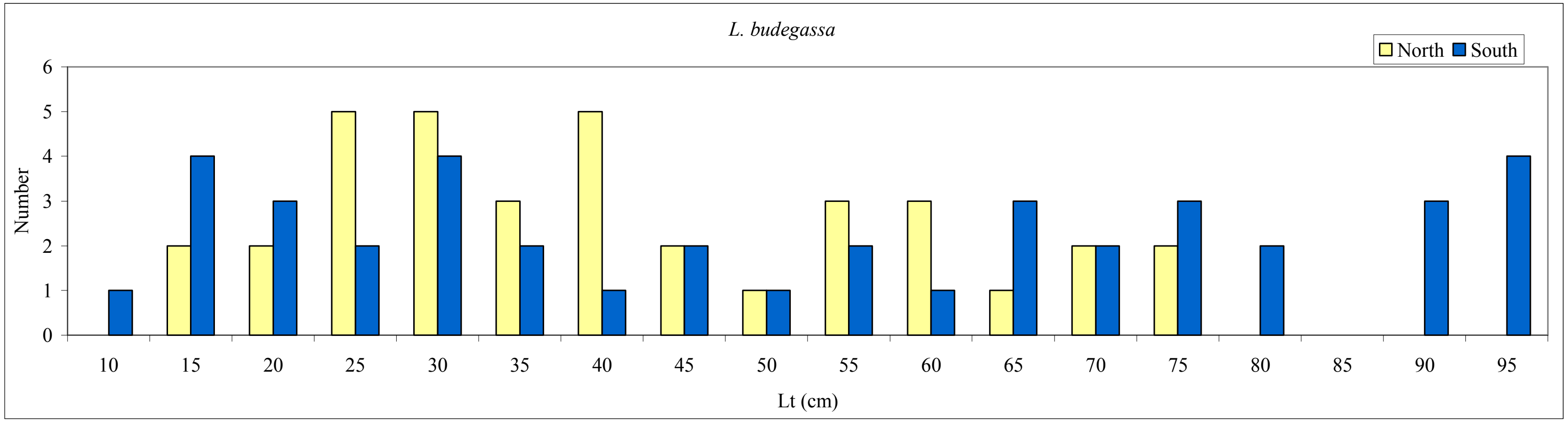


Graph 1. Length frequency distribution of *L. piscatorius* specimens studied from the northern and southern stocks.



Graph 2. Length frequency distribution of *L. budegassa* specimens studied from the northern and southern stocks.

Table 1

RANGE r. 1,3,5,6

Stratum	Sample year	Sample Section N	Fish Slide N	Fish length	Sex	Landing quarter	[illegible] Reader 1	Joël Reader 2	Rafael Reader 3	Antonio Reader 4	[illegible] Reader 5	[illegible] Reader 6	Helen Reader 7	Graeme Reader 8	MODAL age	Percent agreement	Precision CV
-	1999	1a-8	103	21.0	-	4	1	1	1	1	1	1	1	1	1	100%	0%
-	1998	1a-1	1	22.0	-	1	2	1	2	2	2	2	2	2	2	88%	19%
-	1997	5b-4	289	24.0	-	2	2	2	2	2	2	2	3	2	2	88%	17%
-	1997	2a-6	207	25.0	-	1	2	2	3	3	2	2	3	2	2	63%	22%
-	1999	1b-1	133	27.0	-	3	2	2	3	3	3	2	3	2	2	50%	21%
-	1999	6a-4	67	29.0	-	2	3	2	3	3	3	2	2	2	3	50%	21%
-	1999	5b-3	67	30.0	-	2	3	2	3	3	3	3	3	3	3	88%	12%
-	2000	5a-8	20	31.0	-	1	-	2	4	4	4	3	3	3	4	43%	23%
-	1999	3a-3	63	34.0	-	2	2	3	4	4	4	4	3	4	4	63%	22%
-	1999	1b-5	143	35.0	-	4	2	3	5	4	5	4	4	4	5	25%	26%
-	1999	1b-4	73	39.0	-	2	3	4	5	5	5	5	4	5	5	63%	17%
-	1997	2b-5	207	42.0	-	1	4	4	7	5	6	5	7	6	6	25%	22%
-	2000	5b-5	5	43.0	-	1	5	5	6	6	6	6	5	6	6	63%	9%
-	1997	3b-5	208	48.0	-	1	5	6	7	7	7	6	7	7	7	63%	12%
-	2000	2b-5	2	49.0	-	1	5	6	6	7	6	6	7	7	6	50%	11%
-	1999	2b-6	69	62.0	-	2	7	7	9	10	9	8	8	8	9	25%	13%
-	1999	4a-7	68	63.0	-	2	7	6	8	8	8	7	7	7	7	50%	10%
-	2000	1a-1	11	71.0	-	1	8	8	8	8	9	9	8	8	8	75%	6%
-	1999	4b-2	141	72.0	-	3	8	8	9	9	10	9	10	9	9	50%	8%
-	1999	3a-4	73	74.0	-	2	8	8	9	9	10	-	9	10	9	43%	9%
-	1999	2a-3	84	76.0	-	2	9	9	10	11	10	10	10	9	10	50%	7%
-	1999	4b-2	161	78.0	-	4	8	9	9	10	10	10	9	9	10	38%	8%
-	2000	1b-4	46	80.0	-	4	9	10	11	10	13	11	13	11	11	38%	13%
-	1997	5a-3	210	81.0	-	1	10	9	12	10	12	11	12	11	12	38%	10%
-	1997	1a-4	238	82.0	-	1	13	10	11	11	13	13	15	11	13	38%	14%
-	1999	1a-5	58	90.0	-	1	12	10	14	12	14	13	14	13	14	38%	11%
-	1997	4a-7	219	92.0	-	1	12	11	15	13	15	14	14	13	15	25%	11%
-	1999	2a-1	114	94.0	-	4	11	11	12	12	13	12	11	12	12	50%	6%
-	1999	5a-3	152	95.0	-	4	12	12	12	12	15	14	12	13	12	63%	9%
-	1999	3a-3	85	98.0	-	2	14	13	16	14	15	14	15	14	14	50%	6%
-	1999	3b-3	100	100.0	-	4	14	13	11	14	14	14	13	14	14	63%	8%
-	2000	5a-2	20	102.0	-	1	15	14	14	15	15	16	15	16	15	50%	5%
-	1999	1b-4	118	104.0	-	4	14	14	13	14	15	15	13	14	15	25%	5%
-	1999	5a-1	72	107.0	-	2	16	15	17	16	16	16	17	16	16	63%	4%
-	1999	5b-8	77	108.0	-	2	16	14	16	17	16	16	15	15	16	50%	6%
-	1997	2b-3	207	109.0	-	1	17	16	17	17	18	19	20	18	17	38%	7%
-	1999	2b-7	79	110.0	-	2	16	16	15	18	16	16	16	16	16	75%	5%
-	1998	1b-5	1	112.0	-	4	17	15	12	19	-	-	14	-	15	20%	18%
-	1998	2b-4	2	113.0	-	4	18	17	15	17	15	16	16	16	15	25%	6%
-	1999	4a-3	101	115.0	-	4	15	18	23	18	22	19	16	19	20	0%	14%
-	1997	1a-3	238	118.0	-	1	16	19	15	19	19	18	15	17	17	13%	10%
-	1999	4a-1	116	122.0	-	4	21	20	22	21	23	22	20	22	22	38%	5%
-	1997	2b-2	288	136.0	-	2	22	28	24	25	24	25	25	25	24	25%	5%
-	1999	4a-4	116	143.0	-	4	24	27	22	22	23	-	25	21	23	14%	9%
-	1999	10_1a	1	10.0	-	3	0	1	0	0	0	0	0	-	0	86%	265%
-	1999	10_1a	2	12.0	-	3	0	1	0	0	0	0	0	-	0	86%	265%
-	1999	14_2a	3	14.0	-	4	0	0	0	0	0	0	0	1	0	88%	283%
-	1999	14_3b	1	15.0	-	4	1	0	0	1	0	1	0	1	1	50%	107%
-	1999	14_2a	5	16.0	-	4	0	0	0	1	1	0	0	0	0	75%	185%
-	1999	14_3b	3	18.0	-	4	0	0	0	1	1	1	1	1	0	38%	83%
-	1999	13_3b	1	19.0	-	4	1	1	1	1	1	1	1	1	1	100%	0%
-	2000	01_1a	1	20.0	-	1	1	1	1	1	-	1	2	1	1	86%	33%
-	1999	14_2b	5	21.0	-	4	1	1	0	2	1	1	1	2	1	63%	57%
-	2000	01_1a	4	27.0	-	1	2	2	3	2	3	2	3	2	2	63%	22%
-	1999	13_5a	5	29.0	-	4	2	2	3	1	3	2	2	3	2	50%	31%
-	2000	02_1b	4	30.0	-	1	3	2	3	3	3	3	3	3	3	88%	12%
-	2000	06_2a	2	34.0	-	2	3	2	4	3	4	3	3	3	3	63%	21%
-	1999	13_3b	4	37.0	-	4	3	3	4	4	5	4	4	4	4	63%	17%
-	2000	02_2a	2	42.0	-	1	5	4	7	6	7	5	6	6	5	25%	18%
-	2000	06_5b	4	46.0	-	2	4	4	6	6	5	5	6	5	5	38%	16%
-	1999	13_4a	2	51.0	-	4	4	4	5	6	5	5	6	5	5	50%	15%
-	2000	07_3b	3	57.0	-	2	6	6	7	7	8	8	9	8	8	38%	14%
-	2000	07_4a	2	60.0	-	2	6	6	8	8	8	8	11	9	8	50%	20%
-	1999	15_1b	2	67.0	-	4	7	6	8	8	8	8	10	8	8	63%	14%
-	2000	03_1a	4	71.0	-	1	9	7	10	9	10	10	9	9	10	38%	11%
-	1999	11_3a	5	76.0	-	3	8	8	10	9	10	9	10	10	10	50%	10%
-	1999	15_4b	5	79.0	-	4	8	8	11	10	11	12	11	11	11	50%	15%
-	2000	03_3b	1	80.0	-	1	8	9	10	11	11	12	13	12	10	13%	16%
-	2000	06_4b	3	83.0	-	2	9	9	12	12	12	12	13	11	12	50%	13%
-	1999	12_1a	3	86.0	-	3	9	9	10	10	11	10	11	10	10	50%	8%
-	2000	04_1a	1	90.0	-	1	11	10	12	12	12	12	14	12	12	63%	9%
-	1999	07_4b	2	93.0	-	2	10	10	12	12	13	12	13	11	12	38%	10%
-	1999	16_5b	1	99.0	-	4	11	11	13	13	14	12	13	12	13	0%	9%
-	2000	04_3b	4	101.0	-	1	12	12	14	14	16	15	15	14	14	0%	10%
-	1999	08_2b	2	103.0	-	2	14	14	15	16	17	16	17	16	16	0%	8%
-	1999	12_5b	3	106.0	-	3	13	15	14	15	17	16	16	17	15	25%	9%
-	2000	05_1a	4	111.0	-	1	13	16	14	14	16	15	17	15	15	0%	9%
-	1999	08_5b	3	114.0	-	2	16	18	17	16	18	17	19	17	17	38%	6%
-	1999	17_5b	1	119.0	-	4	18	19	16	17	19	19	14	19	19	50%	10%
-	2000	05_4a	1	121.0	-	1	17	20	19	20	20	19	19	19	19	50%	5%
-	1999	09_1a	4	125.0	-	2	20	23	21	23	22	23	19	23	22	13%	7%
-	1999	09_1b	2	128.0	-	2	21	24	19	20	21	24	21	24	21	38%	9%
-	2000	05_5a	3	130.0	-	1	19	24	21	18	22	23	24	23	21	13%	10%
-	1999	09_1b	3	135.0	-	2	24	25	24	24	29	30	28	30	24	38%	10%
-	1999	18_1a	2	140.0	-	4	29	26	26	24	25	26	27	27	26	38%	6%
-	1999	09_1b	5	144.0	-	2	25	28	25	22	26	25	28	25	23	50%	8%
						Total read	85	88	88	88	84	83	88	83		47.8%	25.4%
						Total NOT read	1	0	0	0	2	3	0	3			

Table 2 The number of age readings, 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. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups combined indicate the precision in age reading by reader and for all age readers combined.

NUMBER OF AGE READINGS

MODAL age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	TOTAL
0	5	5	5	5	5	5	5	3	38
1	5	5	5	5	5	5	5	5	39
2	6	6	6	6	6	6	6	6	48
3	4	4	4	4	4	4	4	4	32
4	2	3	3	3	3	3	3	3	23
5	5	5	5	5	5	5	5	5	40
6	3	3	3	3	3	3	3	3	24
7	2	2	2	2	2	2	2	2	16
8	4	4	4	4	4	4	4	4	32
9	3	3	3	3	3	2	2	3	23
10	6	6	6	6	6	6	6	6	48
11	2	2	2	2	2	2	2	2	16
12	5	5	5	5	5	5	5	5	40
13	1	1	1	1	1	1	1	1	8
14	3	3	3	3	3	3	3	3	24
15	6	6	6	6	5	5	6	5	45
16	3	3	3	3	3	3	3	3	24
17	3	3	3	3	3	3	3	3	24
18	-	-	-	-	-	-	-	-	-
19	2	2	2	2	2	2	2	2	16
20	1	1	1	1	1	1	1	1	8
21	2	2	2	2	2	2	2	2	16
22	2	2	2	2	2	2	2	2	16
23	1	1	1	1	1	-	1	1	7
24	2	2	2	2	2	2	2	2	16
25	1	1	1	1	1	1	1	1	8
26	1	1	1	1	1	1	1	1	8
Total 0-26	85	84	86	86	84	83	84	83	675

COEFFICIENT OF VARIATION (CV)

MODAL age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	ALL Readers
0–26	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Weighted mean 0-26	7.8%	20.1%	11.8%	19.4%	16.3%	15.2%	27.2%	12.8%	29.4%
RANKING	1	7	2	5	4	6	8	3	

PERCENTAGE AGREEMENT

MODAL age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	ALL
0–26	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Weighted mean 0-26	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	47.4%
RANKING	6	8	2	3	5	1	7	4	

RELATIVE BIAS

MODAL age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	ALL
0–26	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Weighted mean 0-26	[illegible]	[illegible]	[illegible]	[illegible]	0.43	0.18	0.23	0.12	[illegible]
RANKING	8	7	2	1	6	4	5	3	

Overall ranking

	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8
Ranking Coefficient of Variation	1	7	2	5	4	6	8	3
Ranking Percentage Agreement	6	8	2	3	5	1	7	4
Ranking Relative bias	8	7	2	1	6	4	5	3
OVERALL RANKING	6	8	1	3	5	2	7	2

Table 3 <u>Upper table</u>: The age compositions estimated by each age reader and all age readers combined.
<u>Midle table</u>: The estimated mean length at age by age reader and by all age readers combined.
<u>Lower table</u>: 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.

AGE COMPOSITION

	Age	Hervé Reader 1	Joël Reader 2	Rafael Reader 3	Antonio Reader 4	Iñaki Reader 5	Jorge Reader 6	Helen Reader 7	Grainne Reader 8	TOTAL
	0	5	4	7	3	4	4	5	1	33
	1	5	7	3	7	5	6	4	6	43
	2	8	10	2	4	3	7	4	7	45
	3	6	3	7	6	6	4	9	5	46
	4	3	5	4	4	3	3	3	3	28
	5	4	1	3	2	5	5	1	3	24
	6	2	6	3	4	3	3	3	3	27
	7	3	2	4	3	2	1	4	3	22
	8	7	5	4	4	4	4	2	4	34
	9	5	6	4	4	2	3	4	5	33
	10	2	5	5	6	6	4	4	3	35
	11	3	3	4	3	3	2	4	6	28
	12	4	2	7	6	3	7	2	4	35
	13	3	2	2	2	4	2	7	3	25
	14	4	4	5	5	3	4	5	4	34
	15	2	3	5	2	6	3	6	2	29
	16	5	3	3	3	5	7	4	5	35
	17	3	1	3	4	2	1	3	3	20
	18	2	2	-	3	2	1	-	1	11
	19	1	2	2	2	2	4	3	3	19
	20	1	2	-	2	1	-	2	-	8
	21	2	-	2	1	1	-	1	1	8
	22	1	-	2	2	3	1	-	1	10
	23	-	1	1	1	2	2	-	2	9
	24	2	2	2	2	1	1	1	1	12
	25	1	1	1	1	1	2	2	2	11
	26	-	2	1	-	1	1	-	-	5
	27	-	1	-	-	-	-	1	1	3
	28	-	1	-	-	-	-	2	-	3
	29	1	-	-	-	1	-	-	-	2
	30	-	-	-	-	-	1	-	1	2
Total	0-15	74	72	75	72	69	70	74	70	679

MEAN LENGTH AT AGE

	Age	Hervé Reader 1	Joël Reader 2	Rafael Reader 3	Antonio Reader 4	Iñaki Reader 5	Jorge Reader 6	Helen Reader 7	Grainne Reader 8	ALL
	0	14.0	15.8	15.1	12.0	12.8	13.0	13.4	16.0	14.0
	1	19.2	17.9	20.0	19.7	19.0	19.0	19.8	17.8	18.9
	2	27.9	28.6	23.0	23.5	23.7	26.1	25.0	25.0	26.2
	3	33.2	35.3	28.1	29.2	28.7	31.3	29.1	30.8	30.2
	4	46.0	43.8	34.0	34.3	33.0	35.3	37.0	35.3	37.6
	5	45.5	43.0	41.7	40.5	41.4	43.8	43.0	45.0	43.1
	6	58.5	57.3	45.7	45.3	44.7	46.7	46.0	42.3	48.8
	7	64.0	66.5	47.3	51.3	45.0	63.0	50.5	53.3	53.8
	8	75.7	74.4	65.3	65.3	61.8	61.5	66.5	64.3	67.9
	9	79.2	80.7	71.5	73.3	66.5	73.0	70.0	71.4	74.2
	10	87.0	87.0	77.8	77.7	74.5	77.8	72.8	78.7	78.5
	11	94.3	96.0	85.3	79.3	81.7	80.5	79.8	83.0	84.6
	12	94.5	98.0	92.6	90.8	84.7	88.3	88.0	90.8	90.8
	13	99.7	99.0	101.5	95.5	87.3	86.0	91.3	92.3	93.1
	14	101.3	104.3	102.0	102.8	96.3	96.3	100.6	100.8	100.8
	15	108.5	108.3	107.2	104.0	100.7	105.3	101.5	109.5	104.6
	16	111.4	110.0	108.3	108.0	107.4	107.0	111.0	107.0	108.6
	17	114.0	113.0	110.0	112.3	104.5	114.0	107.0	112.7	110.8
	18	116.0	114.5	-	118.3	111.5	118.0	-	109.0	115.1
	19	130.0	118.5	124.5	115.0	118.5	116.0	120.0	118.3	119.1
	20	125.0	121.5	-	124.5	121.0	-	115.5	-	121.1
	21	125.0	-	127.5	122.0	128.0	-	128.0	143.0	128.3
	22	136.0	-	132.5	143.5	123.3	122.0	-	122.0	130.2
	23	-	125.0	115.0	125.0	132.5	127.5	-	127.5	126.7
	24	139.0	129.0	135.5	137.5	136.0	128.0	130.0	128.0	133.7
	25	144.0	135.0	144.0	136.0	140.0	140.0	139.5	140.0	139.8
	26	-	138.0	140.0	-	144.0	140.0	-	-	140.0
	27	-	143.0	-	-	-	-	140.0	140.0	141.0
	28	-	144.0	-	-	-	-	139.5	-	141.0
	29	140.0	-	-	-	135.0	-	-	-	137.5
	30	-	-	-	-	-	135.0	-	135.0	135.0
Weighted mean	0-15	84.2	87.0	83.5	87.0	88.9	84.8	84.6	87.6	72.9

Inter-reader bias test and reader against MODAL age bias test

	Hervé Reader 1	Joël Reader 2	Rafael Reader 3	Antonio Reader 4	Iñaki Reader 5	Jorge Reader 6	Helen Reader 7	Grainne 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)

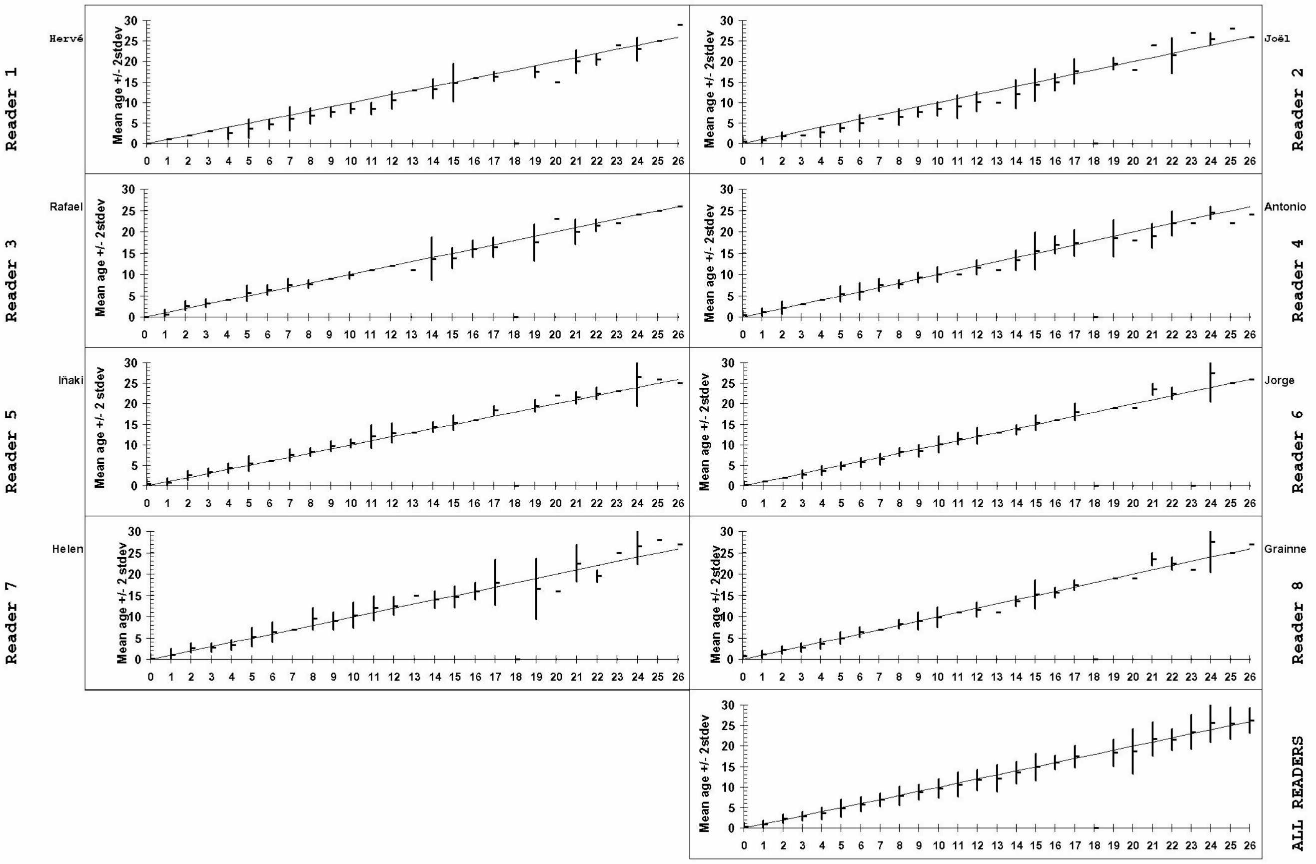
Table 4 Agreed collection
Criterion 80% agreement

MODAL AGE	n
0	3
1	3
2	2
3	2
4	0
5	0
6	0
7	0
8	0
9	0
10	0
11	0
12	0
13	0
14	0
15	0
16	0
17	0
18	0
19	0
20	0
21	0
22	0
23	0
24	0
25	0
26	0
	10

Table 5. Ageing credibility percentages by reader

Reader	Credibility			
	unreadable	low	medium	high
R1	1	12	43	44
R2	27	27	43	3
R3	0	13	63	24
R4	1	9	90	0
R5	1	36	53	8
R6	1	12	44	41
R7	0	19	56	26
R8	0	1	13	83
mean	6	16	51	33

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



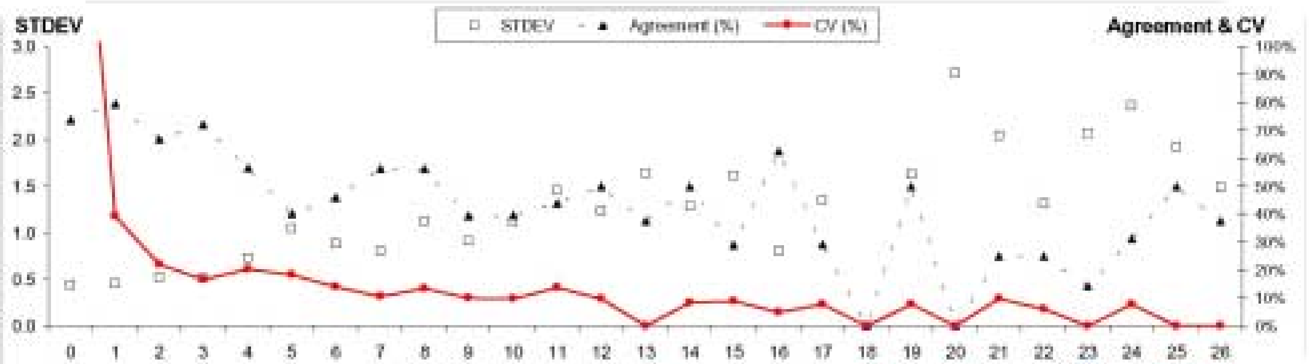


Figure 2 The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age.
CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

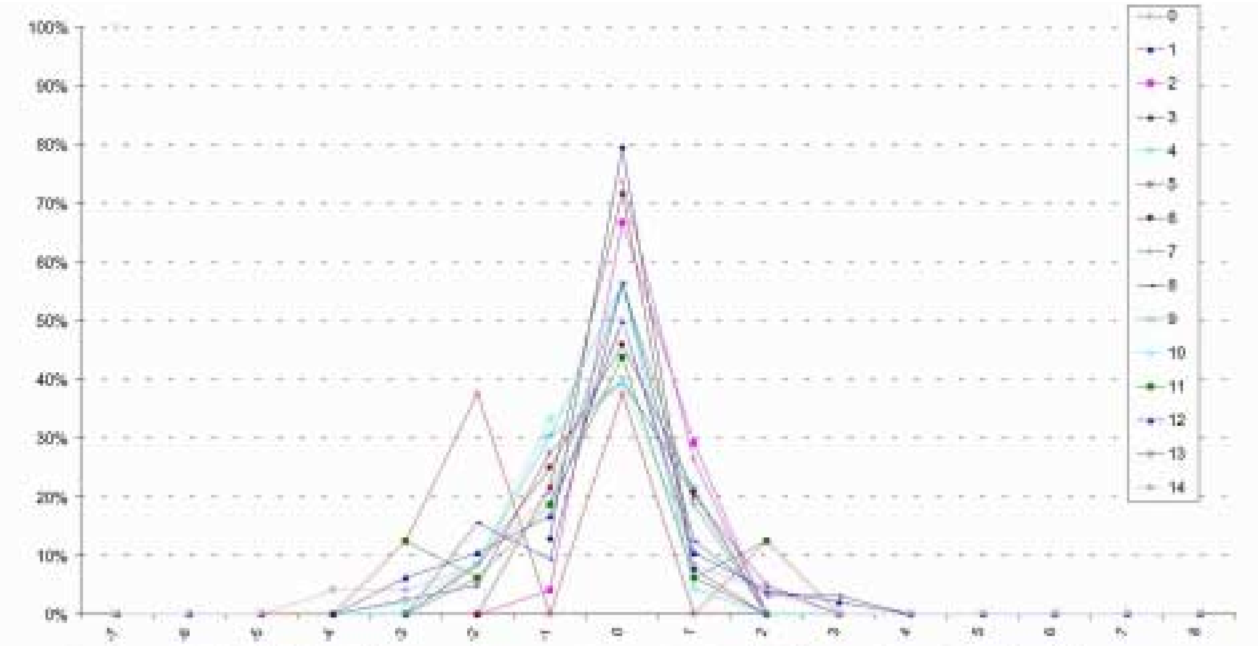


Figure 3 The distribution of the age reading errors in percentage by MODAL age as observed from the whole group of age readers in an age reading comparison to MODAL age. The achieved precision in age reading by MODAL age group is shown by the spread of the age readings errors. There appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias o ure

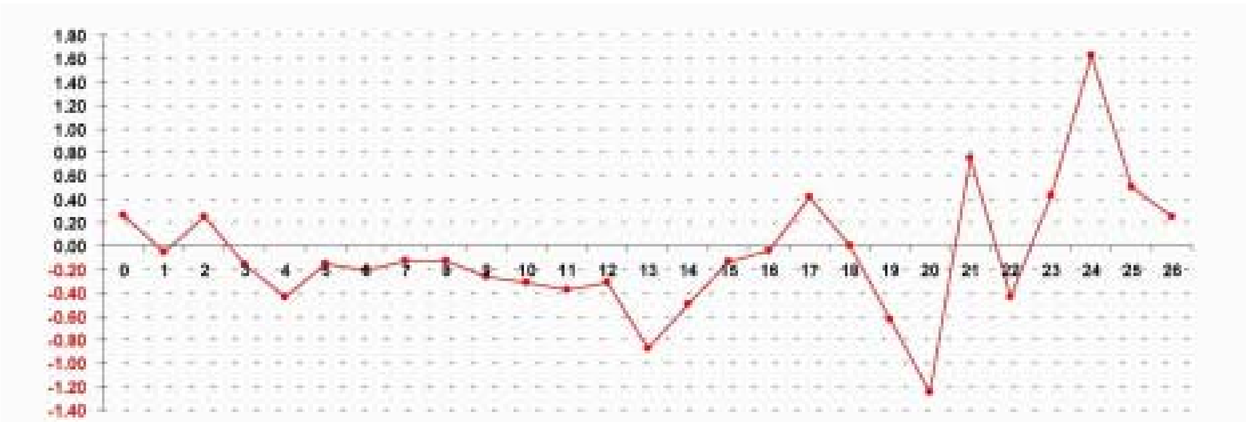


Figure 4 The RELATIVE bias by MODAL age as estimated by all age readers combined.

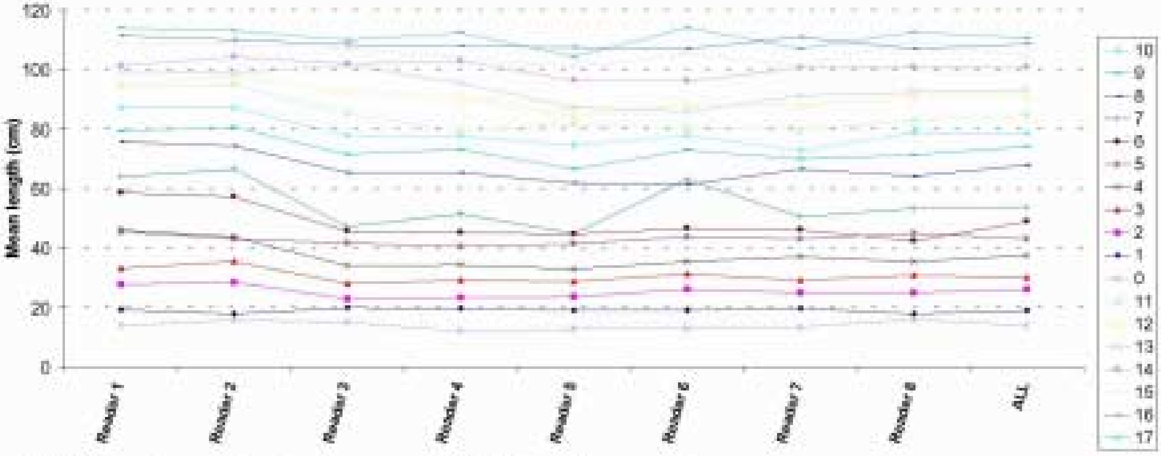


Figure 5 The mean length at age as estimated by each age reader.

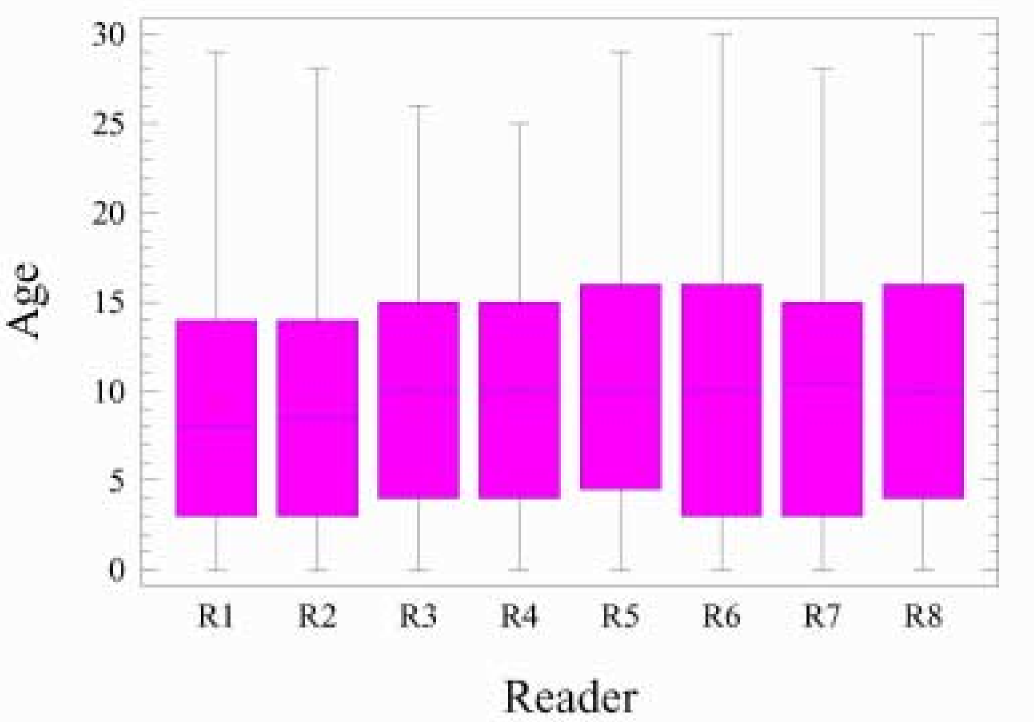


Figure 6 Box and Whisker plot of the ages asigned by reader

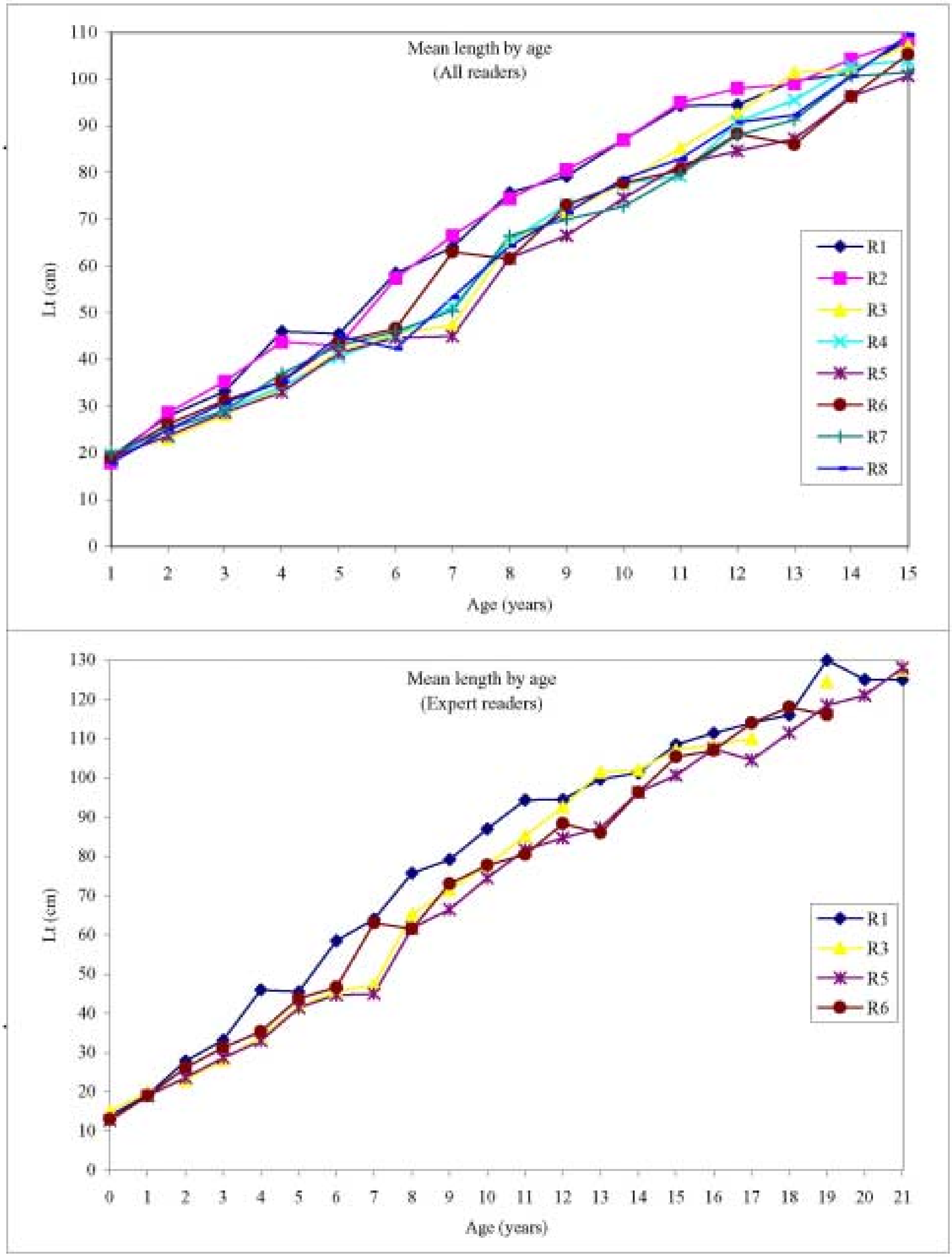


Figure 7 (above) and 8 (below). Mean lengths at age by age reader.

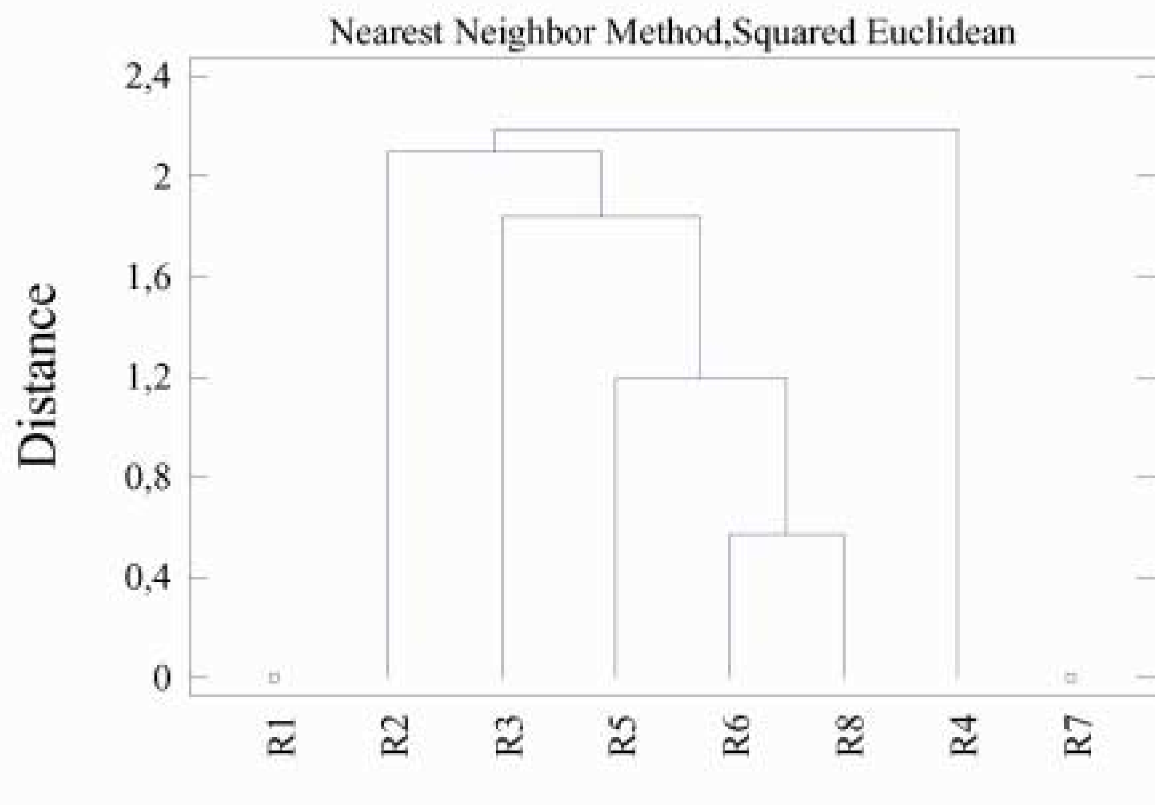


Figure 9 Cluster analysis by each reader age assignation

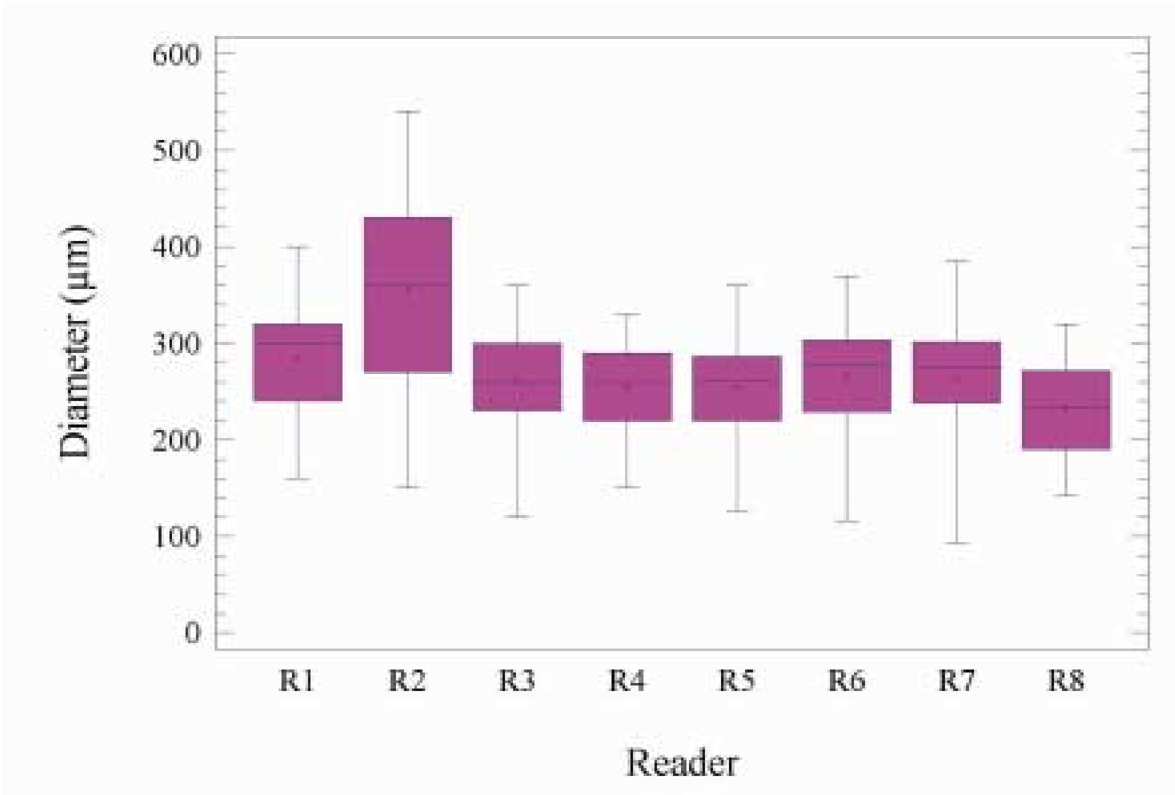


Figure 10 Box and whisker plot of the first ring diameters measured by each reader

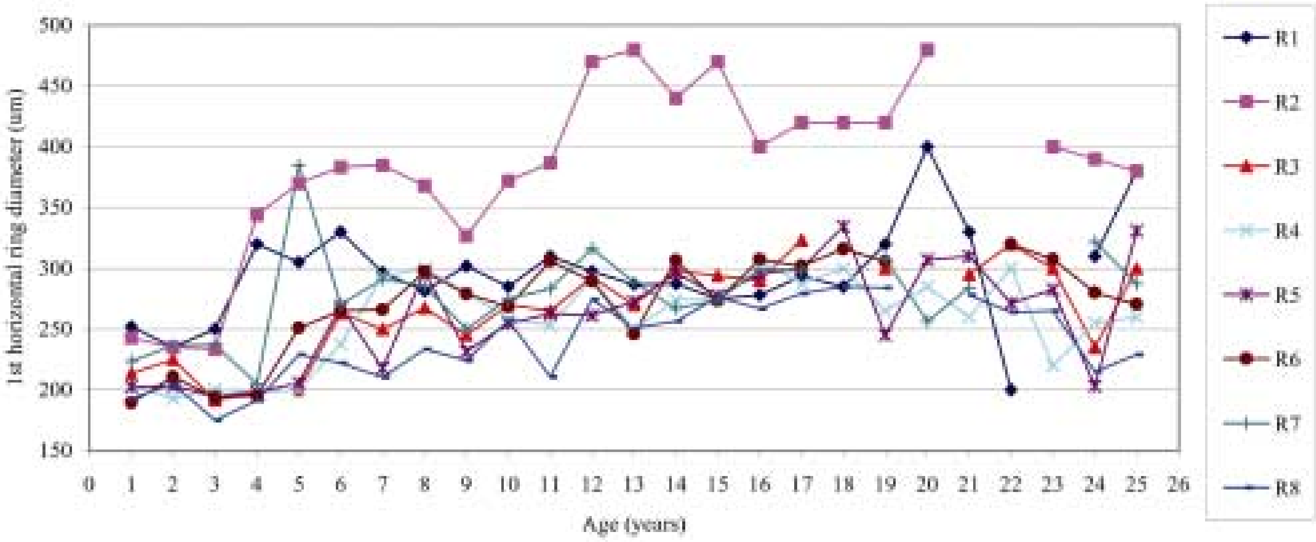


Figure 11. Mean fist ring diameter by age for each reader.

Table 1

Stratum	year	Sample Section N	Fish Slide N	Fish length	Sex	Landing quarter	Joël Reader 2	[illegible] Reader 3	Antonio Reader 4	[illegible] Reader 5	Jorge Reader 6	Helen Reader 7	Grainne Reader 8	RANGE [illegible] MODAL age	Percent agreement	Precision CV
-	1995	3a-1	50	14.0	-	2	2	3	3	3	3	3	3	3	86%	13%
-	2000	0a-4	20	15.0	-	4	1	1	-	-	-	-	-	-	0%	0%
-	1997	4b-1	253	16.0	-	2	3	3	5	4	3	3	3	3	71%	23%
-	1997	4b-7	323	17.0	-	1	3	5	5	5	4	5	5	5	71%	17%
-	1998	4a-6	18	23.0	-	1	3	3	4	4	4	3	4	4	57%	15%
-	1998	5b-2	18	24.0	-	2	4	3	3	4	3	5	3	3	57%	22%
-	1998	5b-6	18	26.0	-	2	4	3	4	4	4	4	3	4	71%	13%
-	1998	5b-7	18	25.0	-	2	4	3	4	3	3	4	3	3	57%	16%
-	1998	6b-8	18	25.0	-	2	5	4	4	4	4	4	3	4	71%	13%
-	2000	2b-5	41	26.0	-	3	-	3	4	-	-	3	2	3	50%	27%
-	1998	2a-5	23	27.0	-	2	4	3	4	5	5	5	3	5	43%	22%
-	1998	2a-3	23	28.0	-	2	4	4	4	5	5	5	5	5	57%	12%
-	1998	2b-7	23	30.0	-	2	5	6	5	5	6	5	5	6	29%	8%
-	2000	1a-7	30	30.0	-	4	5	4	5	6	6	6	6	6	57%	14%
-	1998	1a-7	1	33.0	-	1	5	5	6	6	6	6	6	6	57%	10%
-	1997	1b-2	228	34.0	-	4	5	5	5	6	6	6	6	6	57%	10%
-	1998	3b-2	3	35.0	-	2	6	6	6	6	6	7	6	6	71%	10%
-	1998	3b-5	3	36.0	-	4	5	5	5	7	6	7	6	6	29%	15%
-	1998	3a-5	16	38.0	-	2	7	7	7	7	7	8	6	7	71%	11%
-	1998	2a'-8	32	39.0	-	2	6	8	7	7	7	8	8	7	43%	10%
-	1998	2b'-8	32	39.0	-	2	6	7	7	7	6	7	7	7	71%	7%
-	1997	1a-5	228	40.0	-	1	6	6	7	-	-	7	-	6	60%	8%
-	2000	2a-4	17	41.0	-	4	7	5	7	8	7	7	7	7	71%	13%
-	2000	1a-1	35	43.0	-	3	7	8	6	7	7	8	8	7	43%	10%
-	1998	0a-1	39	50.0	-	2	8	8	7	10	9	8	9	8	29%	12%
-	1997	5a-4	231	51.0	-	4	8	13	9	11	9	13	12	11	14%	19%
-	1998	3b-6	8	52.0	-	4	8	8	8	9	9	9	8	8	43%	13%
-	1997	3a-4	245	54.0	-	1	9	15	11	11	11	14	10	11	43%	18%
-	1997	5a-5	237	59.0	-	1	9	10	10	10	10	8	10	10	71%	5%
-	1998	1a-4	37	60.0	-	2	10	11	10	10	10	10	10	10	86%	4%
-	1998	5b-10	32	60.0	-	4	10	12	10	11	11	12	11	11	43%	7%
-	1998	2b-2	31	62.0	-	2	11	12	10	11	11	13	11	11	57%	8%
-	1998	5b-1	187	66.0	-	4	11	9	12	11	11	12	12	11	43%	10%
-	1998	0a-1	11	67.0	-	4	12	18	12	15	13	14	14	15	14%	11%
-	2000	2b-4	12	73.0	-	4	13	15	13	16	15	16	16	16	29%	8%
-	1998	2a-11	31	74.0	-	2	14	14	14	16	16	13	15	16	29%	8%
-	-	A-1	-	10.0	-	-	1	1	1	1	1	0	0	1	71%	68%
-	-	A-2	-	11.0	-	-	1	1	1	1	1	0	2	1	71%	58%
-	-	A-3	-	13.0	-	-	2	1	1	2	2	0	1	2	43%	59%
-	-	A-4	-	14.0	-	-	1	1	2	2	2	1	2	2	57%	34%
-	-	A-6	-	14.0	-	-	3	1	3	2	2	1	2	2	43%	41%
-	-	A-7	-	16.0	-	-	3	2	3	2	2	2	2	2	86%	18%
-	-	B-1	-	18.0	-	-	4	6	-	-	-	2	3	-	0%	48%
-	-	B-2	-	20.0	-	-	4	3	3	3	4	2	3	3	57%	22%
-	-	B-3	-	24.0	-	-	4	3	3	3	3	2	3	3	86%	12%
-	-	C-1	-	25.0	-	-	4	3	4	4	4	4	4	4	86%	10%
-	-	C-2	-	26.0	-	-	5	4	4	4	4	4	4	4	86%	8%
-	-	C-3	-	26.0	-	-	5	3	4	4	4	4	3	4	57%	16%
-	-	C-6	-	27.0	-	-	5	5	4	4	5	4	4	5	43%	12%
-	-	C-7	-	28.0	-	-	6	5	5	5	5	6	4	5	57%	13%
-	-	D-1	-	32.0	-	-	6	4	5	6	5	5	5	5	14%	23%
-	-	D-7	-	35.0	-	-	6	5	6	7	7	6	6	7	29%	16%
-	-	E-1	-	37.0	-	-	7	6	6	7	7	6	6	7	43%	8%
-	-	F-2	-	42.0	-	-	7	6	7	8	8	7	9	8	29%	13%
-	-	G-1	-	46.0	-	-	7	6	7	9	9	7	8	9	29%	15%
-	-	G-7	-	47.0	-	-	8	7	8	9	8	7	10	8	57%	13%
-	-	H-1	-	52.0	-	-	8	9	10	11	10	9	12	10	29%	12%
-	-	H-7	-	54.0	-	-	9	9	10	10	10	9	12	10	43%	12%
-	-	I-6	-	57.0	-	-	10	9	12	11	12	9	14	11	14%	17%
-	-	J-1	-	61.0	-	-	10	10	12	12	11	11	15	11	29%	15%
-	-	J-8	-	63.0	-	-	11	11	15	13	12	9	13	12	14%	16%
-	-	K-6	-	66.0	-	-	11	9	10	12	12	11	11	12	29%	16%
-	-	L-1	-	67.0	-	-	12	9	15	14	15	13	18	13	14%	16%
-	-	L-7	-	69.0	-	-	11	11	17	16	15	14	19	14	14%	25%
-	-	M-4	-	72.0	-	-	13	13	20	16	17	16	20	16	14%	18%
-	-	N-1	-	75.0	-	-	12	15	17	15	15	15	17	15	43%	13%
-	-	N-2	-	75.0	-	-	12	13	18	18	17	17	17	18	0%	18%
-	-	N-3	-	76.0	-	-	12	13	17	16	15	16	20	15	29%	17%
-	-	N-4	-	77.0	-	-	11	11	18	15	14	18	19	13	0%	25%
-	-	N-5	-	87.0	-	-	16	17	20	20	19	19	24	19	29%	15%
-	-	O-1	-	87.0	-	-	18	17	22	19	19	25	21	19	14%	16%
-	-	O-2	-	87.0	-	-	17	18	22	22	19	19	19	20	0%	10%
-	-	O-3	-	91.0	-	-	18	16	21	21	21	23	24	21	43%	13%
-	-	O-4	-	95.0	-	-	20	19	22	25	24	25	18	22	0%	14%
-	-	O-5	-	97.0	-	-	20	21	22	24	22	19	17	22	29%	11%
-	-	P-3	-	92.0	-	-	17	17	19	21	20	19	14	19	29%	12%
						Total read	75	76	74	72	72	75	74		43.4%	16.6%
						Total NOT read	1	0	2	4	4	1	2			

Table 2 The number of age readings, 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. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups combined indicate the precision in age reading by reader and for all age readers combined.

NUMBER OF AGE READINGS

MODAL age	Joël Reader 2	Rafael Reader 3	Antonio Reader 4	Pati Reader 5	Jorge Reader 6	Helen Reader 7	Graham Reader 8	TOTAL
0	-	-	-	-	-	-	-	-
1	2	2	2	2	2	2	2	14
2	4	4	4	4	4	4	4	28
3	6	7	7	6	6	7	7	46
4	6	6	6	6	6	6	6	42
5	5	5	5	5	5	5	5	35
6	8	8	8	7	7	8	7	53
7	7	7	7	7	7	7	7	49
8	2	2	2	2	2	2	2	14
9	3	3	3	3	3	3	3	21
10	4	4	4	4	4	4	4	28
11	7	7	7	7	7	7	7	49
12	2	2	2	2	2	2	2	14
13	2	2	2	2	2	2	2	14
14	1	1	1	1	1	1	1	7
15	3	3	3	3	3	3	3	21
16	4	4	4	4	4	4	4	28
17	-	-	-	-	-	-	-	-
18	1	1	1	1	1	1	1	7
19	2	2	2	2	2	2	2	14
20	1	1	1	1	1	1	1	7
21	1	1	1	1	1	1	1	7
22	1	1	1	1	1	1	1	7
23	1	1	1	1	1	1	1	7
24	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-
Total 0-15	75	75	74	73	73	73	74	519

COEFFICIENT OF VARIATION (CV)

MODAL age	Joël Reader 2	Rafael Reader 3	Antonio Reader 4	Pati Reader 5	Jorge Reader 6	Helen Reader 7	Graham Reader 8	ALL Readers
0	-	-	-	-	-	-	-	-
1	0%	0%	0%	0%	0%	0%	141%	53.0%
2	43%	40%	41%	0%	0%	60%	29%	37.8%
3	24%	0%	22%	15%	13%	29%	13%	18.5%
4	22%	14%	0%	0%	0%	11%	14%	12.0%
5	26%	20%	12%	9%	9%	14%	20%	15.2%
6	10%	16%	13%	15%	8%	14%	8%	12.3%
7	12%	19%	12%	5%	8%	15%	13%	10.8%
8	8%	11%	0%	0%	0%	0%	7%	12.3%
9	8%	17%	8%	8%	0%	15%	7%	13.2%
10	8%	10%	0%	0%	0%	8%	15%	8.3%
11	11%	19%	11%	0%	8%	14%	15%	15.0%
12	0%	22%	0%	8%	0%	14%	12%	17.8%
13	9%	14%	17%	9%	9%	33%	17%	20.3%
14	-	-	-	-	-	-	-	-
15	9%	10%	18%	8%	8%	7%	10%	16.8%
16	8%	4%	19%	7%	0%	11%	14%	14.7%
17	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-
19	0%	0%	0%	3%	0%	3%	37%	13.7%
20	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-
Weighted mean 0-15	12.7%	13.8%	11.8%	5.7%	4.8%	16.0%	16.4%	10.6%
RANKING	4	5	3	2	1	6	7	

PERCENTAGE AGREEMENT

MODAL age	Joël Reader 2	Rafael Reader 3	Antonio Reader 4	Pati Reader 5	Jorge Reader 6	Helen Reader 7	Graham Reader 8	ALL
0	-	-	-	-	-	-	-	-
1	100%	100%	100%	100%	100%	0%	0%	71%
2	25%	25%	50%	100%	100%	25%	75%	57%
3	17%	100%	57%	67%	83%	57%	86%	67%
4	33%	33%	100%	100%	100%	60%	60%	71%
5	20%	60%	40%	80%	80%	60%	40%	54%
6	38%	38%	0%	67%	86%	38%	71%	48%
7	57%	29%	57%	86%	86%	29%	29%	55%
8	50%	0%	50%	100%	100%	0%	0%	43%
9	0%	0%	0%	67%	100%	33%	33%	33%
10	25%	25%	100%	75%	100%	25%	50%	57%
11	29%	0%	14%	86%	71%	14%	29%	35%
12	0%	0%	0%	50%	100%	0%	0%	21%
13	0%	0%	0%	0%	0%	50%	0%	7%
14	0%	0%	0%	0%	0%	100%	0%	14%
15	0%	33%	0%	67%	67%	33%	0%	29%
16	0%	0%	0%	50%	50%	25%	0%	18%
17	-	-	-	-	-	-	-	-
18	0%	0%	0%	0%	100%	0%	0%	14%
19	0%	0%	50%	0%	50%	100%	0%	29%
20	0%	0%	0%	0%	0%	0%	0%	0%
21	0%	0%	100%	100%	100%	0%	0%	43%
22	0%	0%	100%	0%	100%	0%	0%	29%
23	0%	0%	0%	0%	0%	0%	0%	0%
24	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-
Weighted mean 0-15	24.0%	29.3%	38.2%	68.1%	79.2%	26.0%	35.1%	44.0%
RANKING	7	6	3	2	1	4	5	

RELATIVE BIAS

MODAL age	Joël Reader 2	Rafael Reader 3	Antonio Reader 4	Pati Reader 5	Jorge Reader 6	Helen Reader 7	Graham Reader 8	ALL
0	-	-	-	-	-	-	-	-
1	0.00	0.00	0.00	0.00	0.00	-1.00	0.00	-0.14
2	0.25	-0.75	0.00	0.00	0.00	-1.00	-0.25	-0.25
3	0.00	0.00	0.57	0.33	0.17	0.86	-0.14	0.25
4	0.00	-0.67	0.00	0.00	0.00	-0.17	-0.50	-0.19
5	-0.80	-0.60	-0.80	-0.20	-0.20	0.00	-0.60	-0.43
6	-0.63	-0.88	-0.75	0.29	-0.14	0.13	-0.29	-0.34
7	-0.57	-0.43	-0.57	0.14	-0.14	0.29	0.14	-0.16
8	-0.50	-1.50	-0.50	0.00	0.00	-1.00	1.50	-0.29
9	-1.33	-2.33	-1.33	0.33	0.00	-1.00	-0.67	-0.90
10	-0.75	-0.50	0.00	0.25	0.00	-0.50	1.00	-0.11
11	-1.14	0.43	-0.14	0.14	-0.14	1.00	1.14	0.18
12	-1.50	-2.00	3.00	0.50	0.00	-2.00	0.00	-0.29
13	-1.50	-3.00	4.00	1.00	1.00	2.00	4.00	1.36
14	-2.00	-3.00	3.00	2.00	1.00	0.00	5.00	0.71
15	-0.67	-2.33	-1.33	0.33	-0.67	0.00	0.67	-0.52
16	-2.25	-2.75	1.00	1.00	0.50	0.00	2.00	-0.14
17	-	-	-	-	-	-	-	-
18	-2.00	-1.00	4.00	1.00	0.00	1.00	3.00	1.71
19	-2.50	-3.00	0.50	1.00	0.50	0.00	0.00	-0.50
20	-3.00	-3.00	2.00	2.00	-1.00	-1.00	-2.00	-0.71
21	-3.00	-4.00	0.00	0.00	0.00	2.00	0.00	-0.43
22	-3.00	-4.00	2.00	2.00	0.00	-3.00	-3.00	-1.29
23	-3.00	-4.00	-1.00	2.00	1.00	0.00	-3.00	-0.86
24	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-
Weighted mean 0-15	-0.90	-0.83	0.14	0.29	0.03	0.07	0.25	-0.11
RANKING	7	6	3	5	1	2	4	

Overall ranking

	Joël Reader 2	Rafael Reader 3	Antonio Reader 4	Pati Reader 5	Jorge Reader 6	Helen Reader 7	Graham Reader 8
Ranking Coefficient of Variation	4	5	3	2	1	6	7
Ranking Percentage Agreement	7	6	3	2	1	4	5
Ranking Relative bias	7	6	3	5	1	2	4
OVERALL RANKING	7	6	3	2	1	4	5

Table 3

Upper table: The age compositions estimated by each age reader and all age readers combined.
Midle table: The estimated mean length at age by age reader and by all age readers combined.
Lower table: 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.

AGE COMPOSITION

Age	Joël Reader 2	Rafael Reader 3	Antonio Reader 4	Iñaki Reader 5	Jorge Reader 6	Helen Reader 7	Grainne Reader 8	TOTAL
0	-	-	-	-	-	3	1	4
1	4	6	3	2	2	2	1	20
2	2	1	2	4	4	3	5	21
3	6	12	5	4	5	5	11	48
4	9	5	11	9	8	7	5	54
5	9	8	11	5	5	6	4	48
6	6	9	2	4	7	6	7	40
7	6	3	8	7	6	8	2	40
8	4	4	2	4	2	4	5	25
9	4	5	1	2	4	6	2	24
10	4	2	6	4	4	1	4	25
11	6	4	1	7	5	2	3	28
12	5	2	4	2	3	2	4	22
13	2	5	1	1	1	4	1	15
14	1	1	1	1	1	3	3	11
15	1	2	3	3	4	1	2	16
16	1	2	-	4	2	2	2	13
17	2	3	3	-	2	1	3	14
18	1	1	-	2	1	2	2	9
19	-	1	3	1	2	4	2	13
20	2	-	2	1	1	-	2	8
21	-	1	1	2	1	-	1	6
22	-	-	4	1	1	-	-	6
23	-	-	-	-	-	1	-	1
24	-	-	-	1	1	-	2	4
25	-	-	-	1	-	1	-	2
26	-	-	-	-	-	1	-	1
27	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-
Total 0-15	72	73	64	63	65	66	65	518

MEAN LENGTH AT AGE

Age	Joël Reader 2	Rafael Reader 3	Antonio Reader 4	Iñaki Reader 5	Jorge Reader 6	Helen Reader 7	Grainne Reader 8	ALL
0	-	-	-	-	-	11.0	10.0	10.8
1	12.5	12.7	11.0	10.5	10.5	14.0	12.0	12.1
2	13.0	18.0	16.0	14.5	14.5	18.7	16.6	15.8
3	18.8	22.9	19.2	20.8	20.6	20.6	22.2	21.2
4	24.0	26.2	25.7	24.1	23.4	25.6	25.6	25.0
5	30.7	31.3	29.5	26.0	28.4	26.3	26.8	28.8
6	36.5	37.5	40.0	32.6	33.7	32.7	34.1	34.9
7	41.0	41.3	41.8	38.1	38.8	40.6	40.0	40.2
8	50.3	49.3	50.0	40.5	44.5	48.5	43.8	46.1
9	54.8	59.2	51.0	49.0	49.6	53.7	46.0	53.2
10	59.5	60.0	57.8	55.8	58.3	60.0	55.0	57.3
11	67.0	67.3	54.0	57.4	60.6	63.0	62.3	62.3
12	72.0	61.0	62.8	63.0	61.7	63.0	55.8	63.3
13	72.0	69.8	72.0	63.0	67.0	63.5	63.0	67.5
14	74.0	74.0	74.0	67.0	77.0	63.3	72.0	70.2
15	87.0	63.0	85.0	73.0	70.8	75.0	67.5	70.0
16	87.0	79.0	-	72.8	75.0	72.0	69.5	74.8
17	89.5	88.7	73.3	-	73.6	75.0	82.3	81.8
18	91.0	87.0	-	73.5	87.0	78.5	91.0	83.0
19	-	95.0	81.3	87.0	87.0	90.8	73.0	85.3
20	96.0	-	79.5	87.0	82.0	-	74.0	84.8
21	-	87.0	91.0	91.5	91.0	-	87.0	91.5
22	-	-	91.5	87.0	97.0	-	-	91.7
23	-	-	-	-	-	91.0	-	91.0
24	-	-	-	97.0	95.0	-	89.0	92.5
25	-	-	-	95.0	-	87.0	-	91.0
26	-	-	-	-	-	95.0	-	95.0
27	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-
Weighted mean 0-15	47.4	47.1	53.2	53.0	51.4	51.8	52.1	45.9

Inter-reader bias test and reader against MODAL age bias test

#####	Joël Reader 2	Rafael Reader 3	Antonio Reader 4	Iñaki Reader 5	Jorge Reader 6	Helen Reader 7	Grainne Reader 8
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 4 Agreed collection

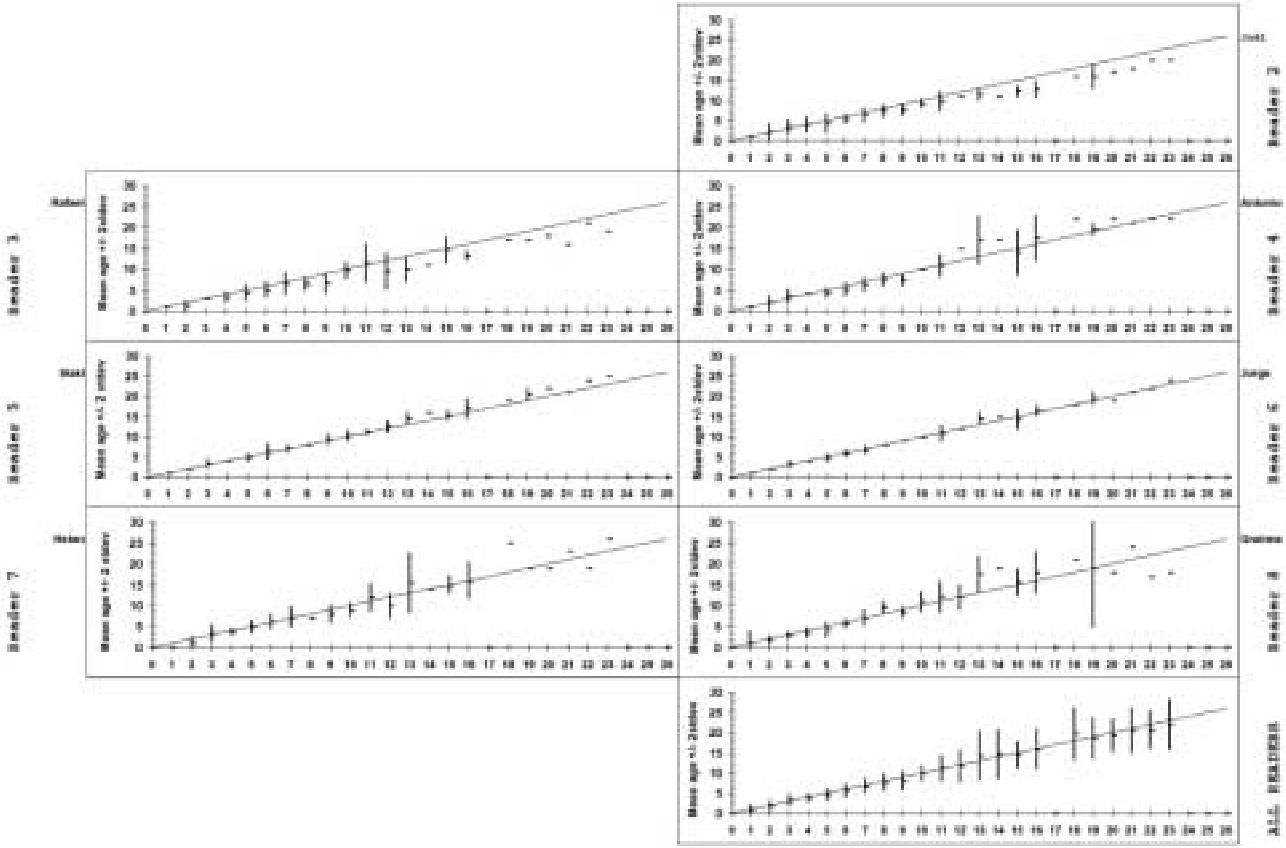
Criterion 80 % agreement

MODAL AGE	n
0	0
1	0
2	1
3	2
4	2
5	0
6	0
7	0
8	0
9	0
10	1
11	0
12	0
13	0
14	0
15	0
16	0
17	0
18	0
19	0
20	0
21	0
22	0
23	0
24	0
25	0
26	0
	6

Table 5. Ageing credibility percentages by reader

Reader	Credibility			
	unreadable	low	medium	high
R1	-	-	-	-
R2	-	-	-	-
R3	0	7	89	4
R4	1	21	76	1
R5	1	25	67	7
R6	0	8	62	30
R7	0	20	59	21
R8	1	5	36	57
mean	1	14	65	20

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



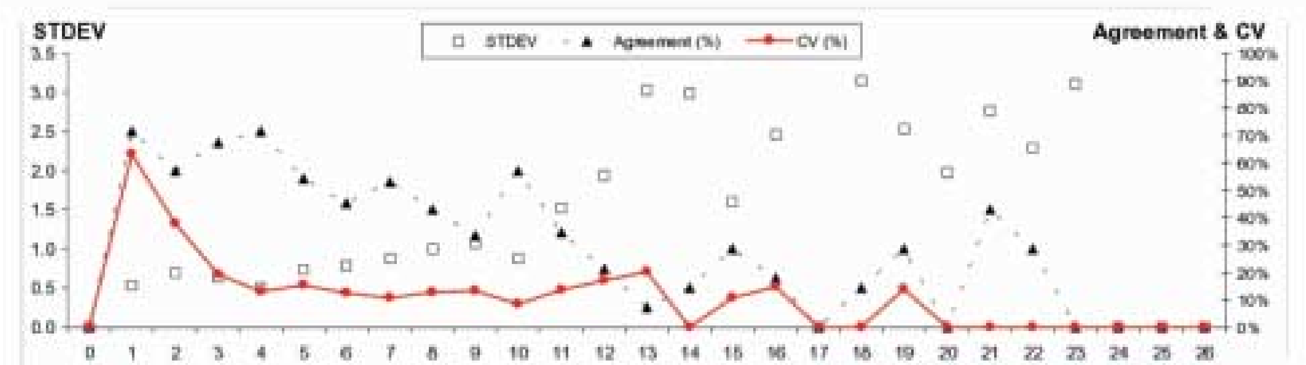


Figure 2 The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age.
CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

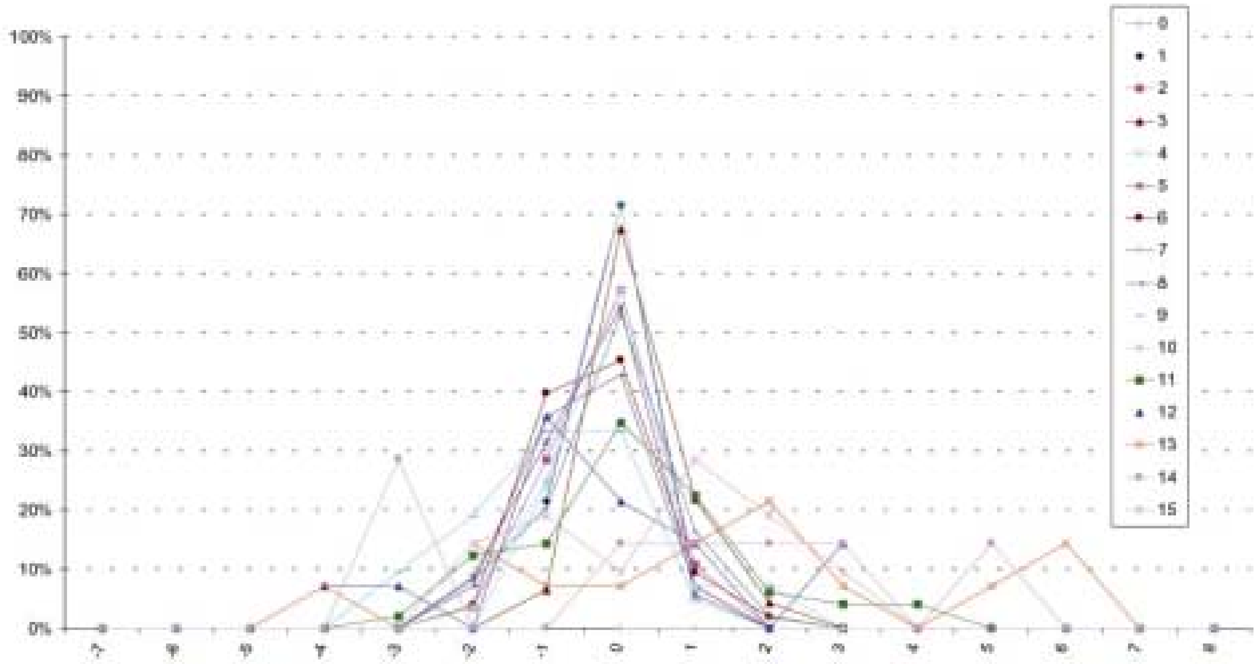


Figure 3 The distribution of the age reading errors in percentage by MODAL age as observed from the whole group of age readers in an age reading comparison to MODAL age. The achieved precision in age reading by MODAL age group is shown by the spread of the age readings errors. There appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.

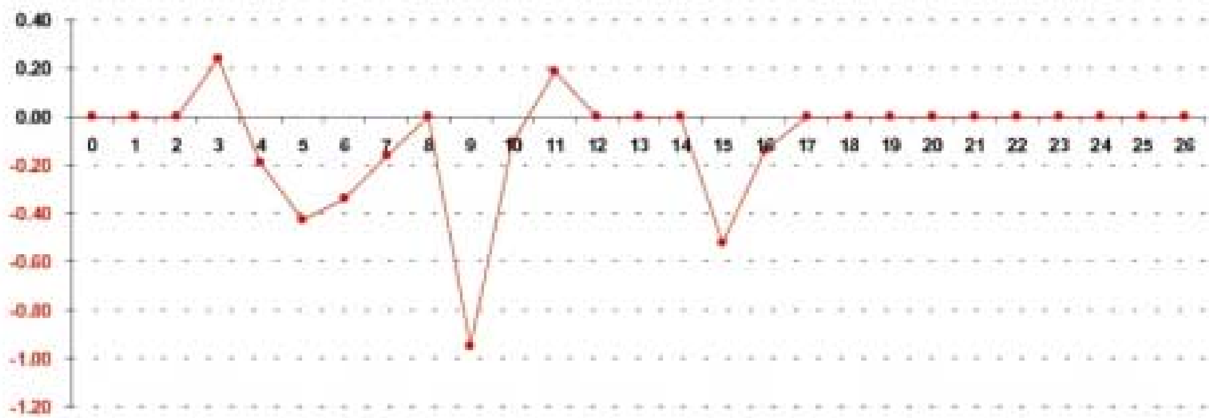


Figure 4 The RELATIVE bias by MODAL age as estimated by all age readers combined.

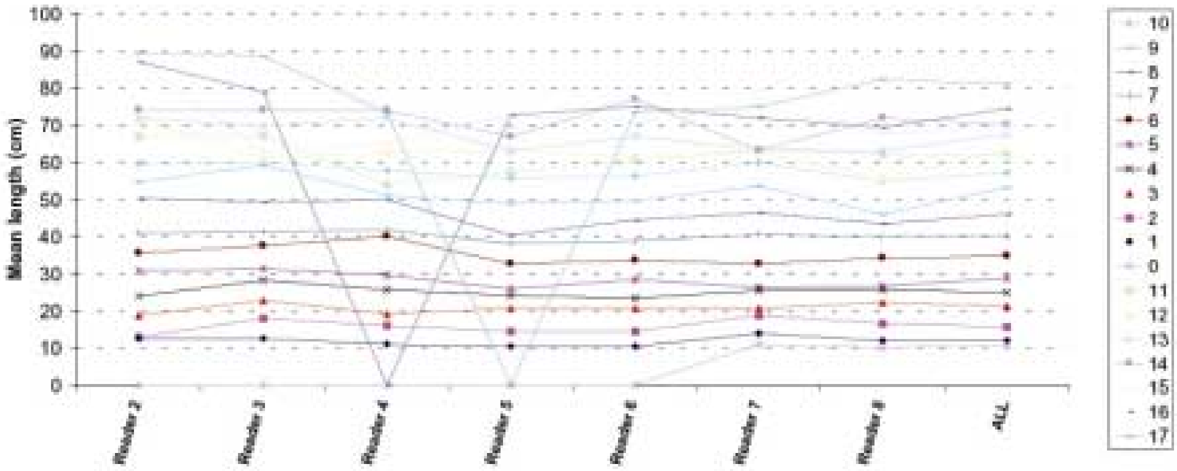


Figure 5 The mean length at age as estimated by each age reader.

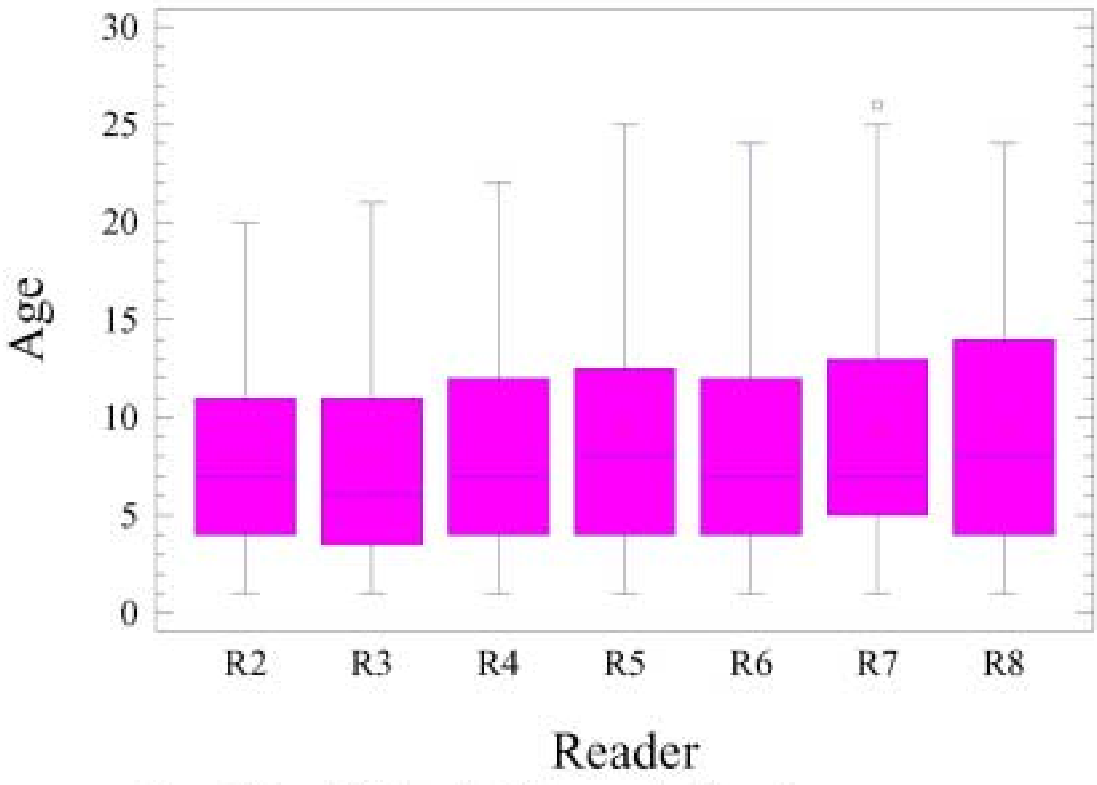
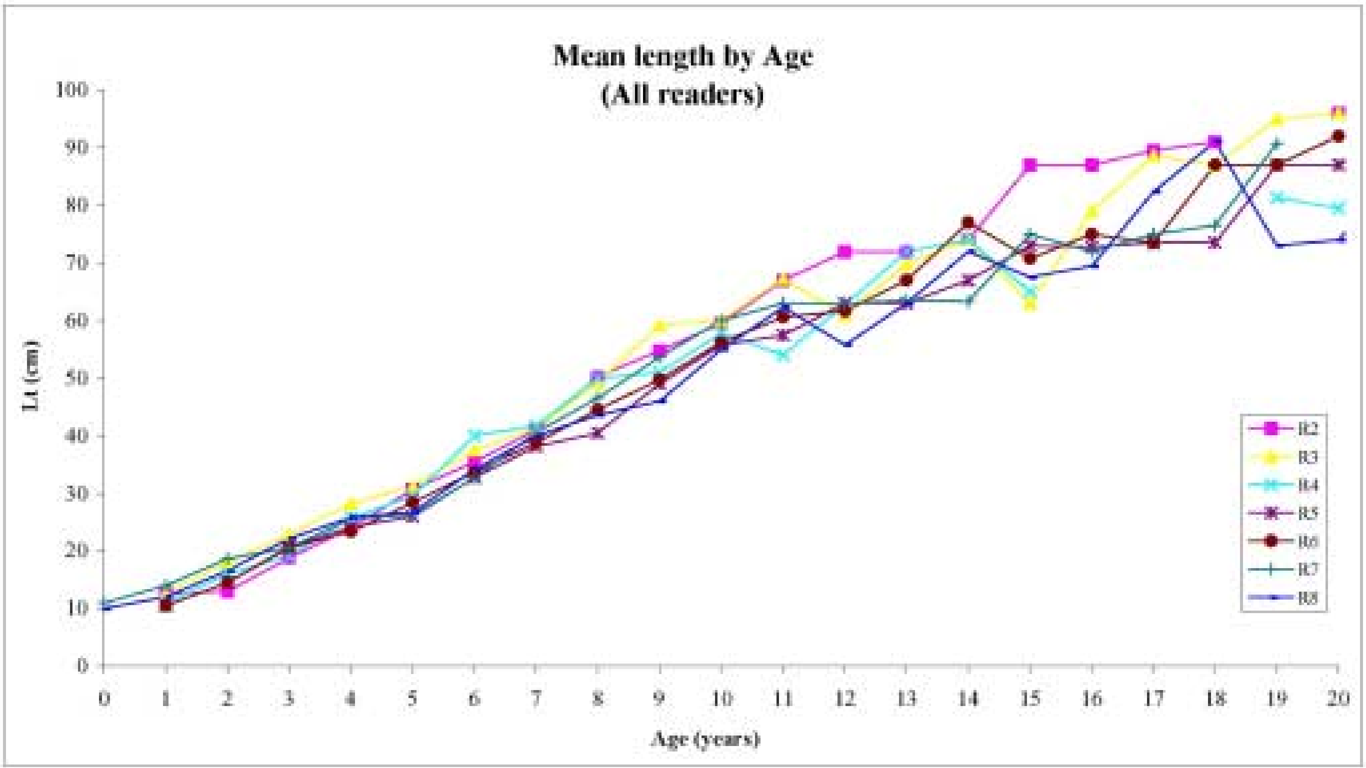


Figure 6 Box and Whisker plot of the ages asigned by reader



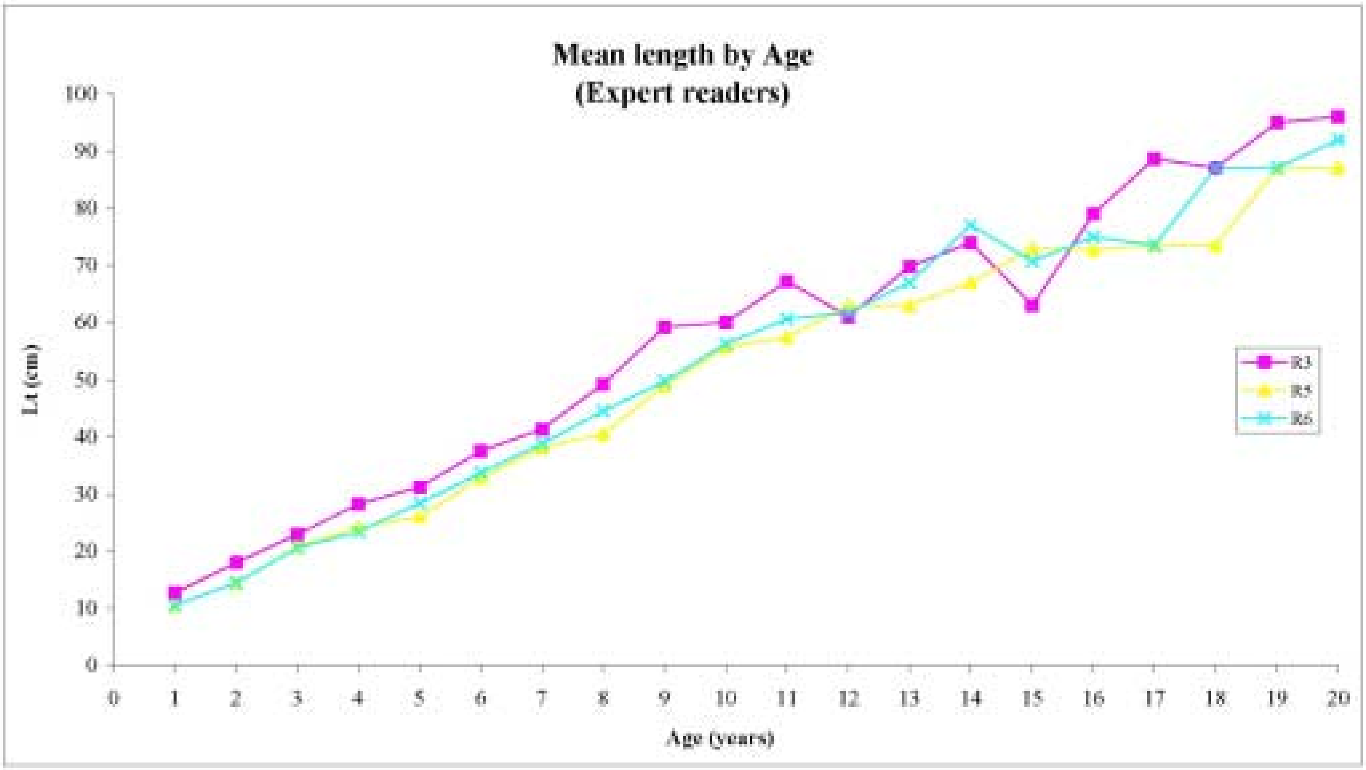


Figure 7 (above) and 8 (bellow). Mean lengths at age by age reader.

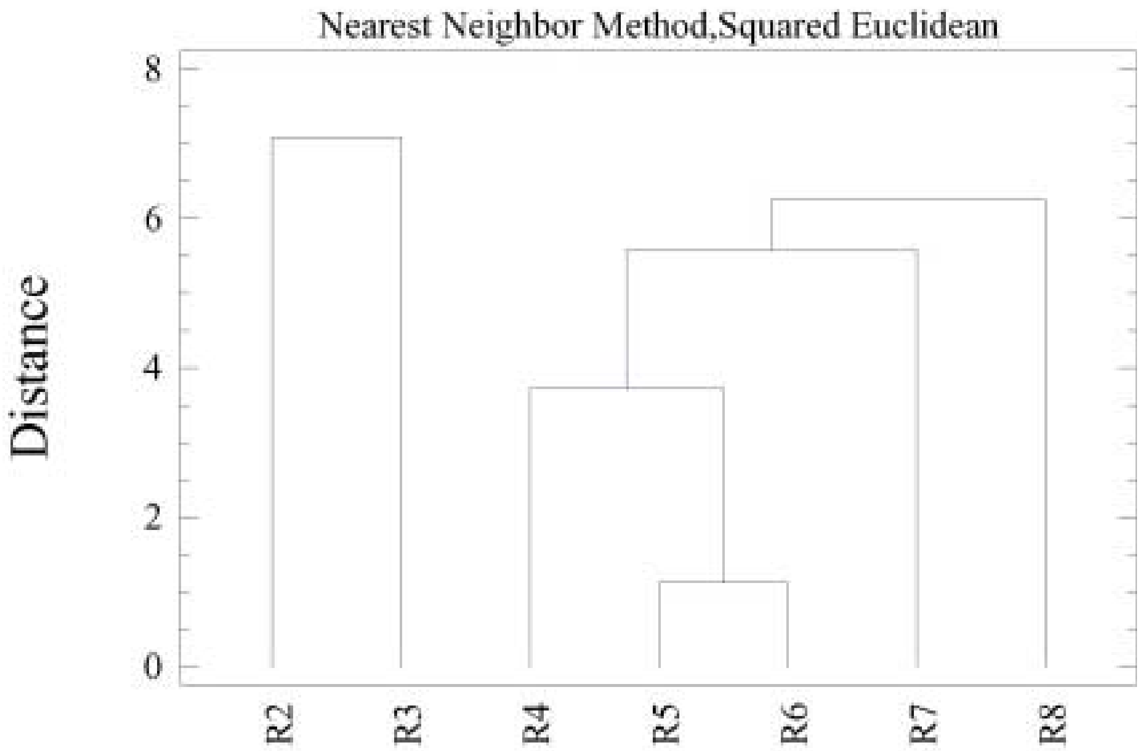


Figure 9 Cluster analysis by each reader age assignation

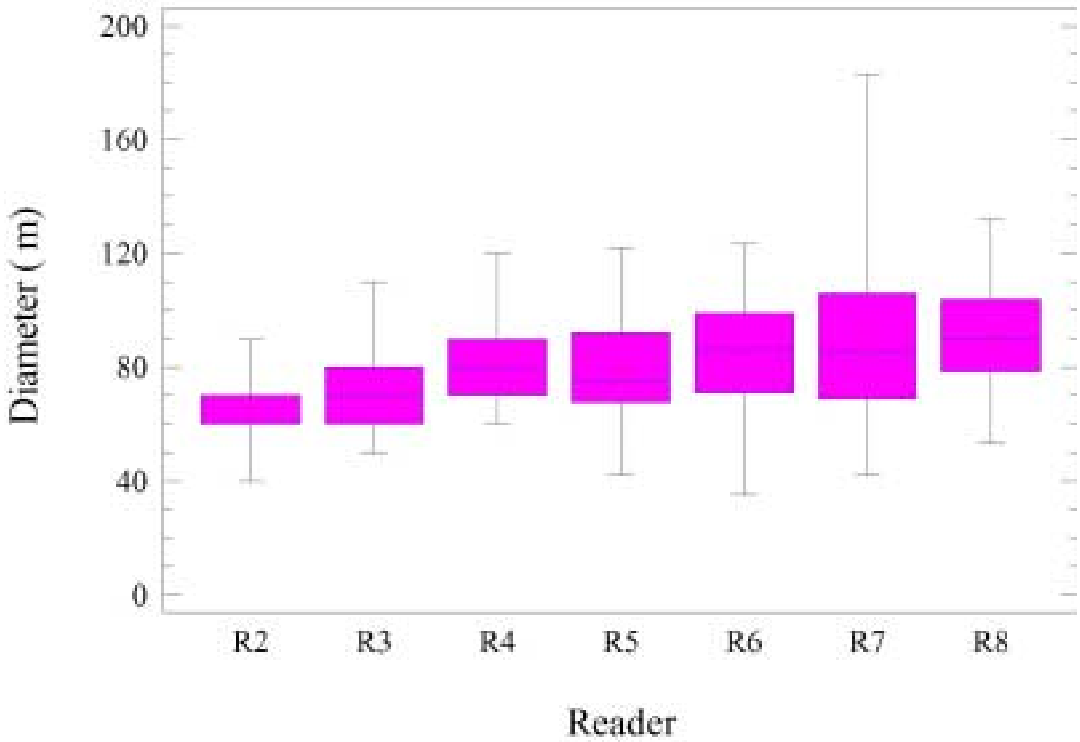


Figure 10 Box and whisker plot of the first ring diameters measured by each reader

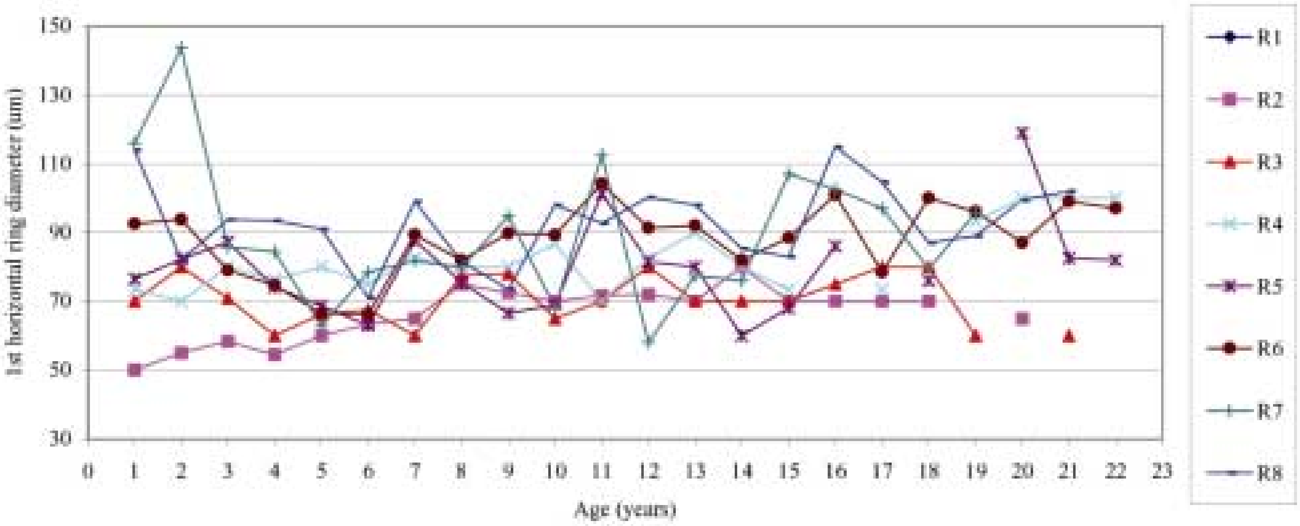


Figure 11. Mean fist ring diameter by age for each reader.

Annex 1

Participants in the 4th Ageing Workshop on European Anglerfish

PARTICIPANTS IN THE 4TH AGEING WORKSHOP ON EUROPEAN ANGLERFISH

Participants	Institution	Address	Telephone	Fax	e-mail	Illicia Exchange	
						Readings	Images
Eli Bilbao	AZTI	Txatxarramendi ugartea, z/g 48395 Sukarrieta Bizkaia Spain	+34 94 602 94 00	+34 94 687 00 06	ebilbao@suk.azti.es		
Joel Dimeet	IFREMER	8, Rue François Toullec 56100 Lorient France	+33 2 97873800	+33 2 97873801	joel.dimeet@ifremer.fr	X	
Rafael Duarte	IPIMAR	Av. Brasília 1449-006 Lisbon Portugal	+351 21 439 61 38	+351 21 301 59 48	rduarte@ipimar.pt	X	X
Hervé Dupouy	IFREMER	8, Rue François Toullec 56100 Lorient France	+33 2 97873800	+33 2 97873801	herve.dupouy@ifremer.fr	X	
Jorge Landa	IEO	Promontorio San Martín, s/n Apdo. 240 39080 Santander. Spain	+34.942.29.10.60	+34.942.27.50.72	jorge.landa@st.ieo.es	X	X
Paulino Lucio	AZTI	Txatxarramendi ugartea, z/g 48395 Sukarrieta Bizkaia Spain	+34 94 602 94 00	+34 94 687 00 06	plucio@suk.azti.es		
António Marçal	IPIMAR	Av. Brasília 1449-006 Lisbon Portugal	+351 21 439 61 38	+351 21 301 59 48	amarcal@ipimar.pt	X	X
Helen McCormick	Marine Institute	Abbotstown Laboratory Complex,Snugboro road, Abbotstown, Dublin 15 Ireland	+353 1 8228200	+353 1 8205078	helen.mccormick@marine.ie	X	X
Grainne Ni Chonchuir	Marine Institute	Abbotstown Laboratory Complex,Snugboro road, Abbotstown, Dublin 15 Ireland	+353 1 8228200	+353 1 8205078	grainnen@marine.ie	X	X
Iñaki Quincoces	AZTI	Txatxarramendi ugartea, z/g 48395 Sukarrieta Bizkaia Spain	+34 94 602 94 00	+34 94 687 00 06	iquincoces@suk.azti.es	X	X

Annex 2

Protocol for the *Illicia* Exchange Program in 2001

PROTOCOL FOR THE *ILLICIA* EXCHANGE PROGRAM IN 2001

Contents

1. INTRODUCTION

One of the objectives of Study Project UE DGXIV 99/013 “Genetic characterisation and stock structure of the two species of anglerfish (*Lophius piscatorius* and *L. budegassa*) of the Northeast Atlantic”, is to perform an exchange program of *illicia* between readers and conduct a new workshop.

IEO, AZTI and IPIMAR have prepared two collections: one of white Anglerfish (*Lophius piscatorius*) and another of black Anglerfish (*Lophius budegassa*), which include *illicia* from both Northern Stocks (Divisions VIIIa,b,d) and Southern Stocks (Divisions VIIIc and IXa).

The participants involved and the reading schedule is shown in Figure 1.

2. ORGANISATION OF THE COLLECTIONS

The methodology used to obtain the *illicia* sections is familiar to every reader: *Illicia* were mounted in a black resin and cut transversally using a double bladed sectioning machine.

The collections for this exchange program only contain one section from each *illicium.* Each set of *illicia* was fixed over a microscope slide using “Eukitt”.® resin.

To improve the readability of the *illicia* they have been finally covered also with "Eukitt" ® resin. Thus it is not necessary –according to our experience- to cover the sections again with a glycerine / alcohol mixture, except the IPIMAR collection.

The collection of white Anglerfish for the exchange program consists of 86 *illicia* in total: 44 *illicia* from the Northern Stock (Divisions VIIIa,b,d) and 42 *illicia* from the Southern Stock (Divisions VIIIc and IXa). These *illicia* are described by length in the files **PISC-AZTI_ILLICIA_COLLECTION.xls** and **IEO_ILLICIA_COLLECTION .xls** (Excel 97).

The collection of black Anglerfish for the exchange program consists on 76 *illicia*: 36 *illicia* from the Northern Stock (Divisions VIIIa,b,d) and 40 *illicia* from the Southern Stock (Divisions VIIIc and IXa). These *illicia* are described by length in the files **AZTI_ILLICIA_COLLECTION.xls** and **IPIMAR_ILLICIA_COLLECTION.xls** (Excel 97).

The *illicia* are mounted on numbered plates or microscope slides corresponding to the numbering order in the AZTI, IEO and IPIMAR's routine collections of *Lophius spp.* A complete description of all the plates used is presented in the sheets "Plates"/"Readings". This description describes the *illicia* that should be read because not all *illicia* contained in the plates are considered in the present exchange program.

It has to be pointed out that:

- Only *illicia* were mounted and no second dorsal fin rays.
- Each microscope slide contains only one section from each *illicium*.

All *illicia* have been cut usually at the same position -i.e. at about 5 mm from the *illicium* base, or more accurately between 4.5 mm (lower position) and 5.5 mm (upper position of the cut)- and all of them have a thickness of about 500 μm. (The total loss caused by the abrasion of the two saw cuts are estimated to be also around 500 μm).

3. GENERAL INSTRUCTIONS

3.1. M. Excel books

There are 3 CDs and each CD contains one of these 3 books:

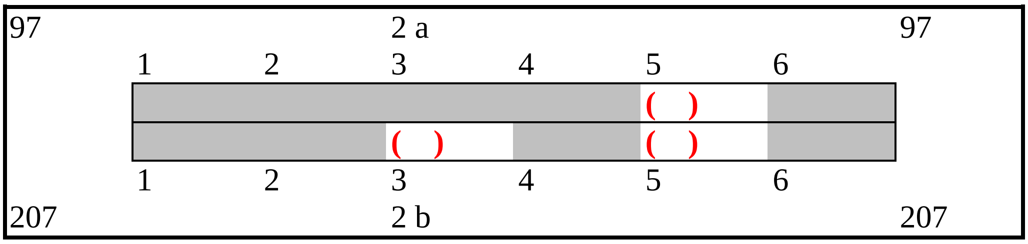
- **IEO_ILLICIA_COLLECTION.xls**
- **AZTI_ILLICIA_COLLECTION.xls**
- **IPIMAR_ILLICIA_COLLECTION.xls**

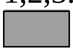
Each book contains some of the two parts (north or south) of the two collections (white and black Anglerfish). They have two sheets: plates and readings.

<u>3.1.1. Plates</u>

3.1.1.1. Explanation of the AZTI plates legends

A general overview of all the *illicia* collection is indicated graphically in the sheets “Plates” that is contained in the file “**AZTI_ILLICIA_COLLECTION.xls**”. The common scheme used in each microscope plate/slide is shown in the next diagram:



97: Number of the sampling year (i.e. 1997)
207: Number of plate/slide (in order of the routine AZTI collection)
2a/2b: Number and position of the rows. (a: upper row; b: lower row).
(The numbers (2,...) must not be taken into account: they correspond only to the AZTI’s routine sampling codes and indicate the order of files obtained in the processing of the black resin plates).
1,2,3... Number of order of each *illicium* in the row.
 The shaded boxes mark the *illicia* that must not be read.
( ) The numbers in closed brackets indicate the *illicia* to be read. These *illicia* have been highlighted on the slides with a circle

3.1.1.2. Explanation of the IEO plates legends

A general overview of all the *illicia* collection is indicated graphically in the sheets “Plates” that is contained in the file “**IEO_ILLICIA_COLLECTION.xls**”. The common scheme used in each microscope plate/slide is shown in the next diagram:

13						97
	1	2	3	4	5	
1a	(coloured)	(coloured)	( )	( )	(coloured)	
1b	( )	( )	(coloured)	( )	( )	
	1	2	3	4	5	

97: Number of the sampling year (i.e. 1997)
13/1a or 13/1b: Number of section (in the order of the routine IEO's collection).
1,2,3... Number of order of each *illicium* in the row.
(coloured box) The coloured boxes mark the *illicia* that must not be read.
( ) The numbers in closed brackets indicate the *illicia* to be read. These *illicia* have been highlighted on the slides with a circle

3.1.1.3. Explanation of the IPIMAR plates legends

A general overview of all the *illicia* collection is indicated graphically in the sheets "Plates" that is contained in the file "**IPIMAR_ILLICIA_COLLECTION.xls**". The common scheme used in each microscope plate/slide is exemplified in the next diagram:

							Plate A
1999 – 3rd Trim							
(IPIMAR code: 99.016 - 1)							
Order	1	2	3	4	5	6	7
Length (cm)	10	11	12	14	14	14	18
Illicium	X	X	X	X	X	X	X
2nd ray		X	X	X		X	

The IPIMAR collection is composed of 16 plates (A to P). Each plate contains 2 slices of the same *illicia* and reading can be performed on any slice. Each slice contains *illicia* followed in some cases by the respective 2nd dorsal fin ray (from left to right). An **X** specifies the presence of the 2nd ray. Read only the 40 *illicia* in yellow columns.

Very Important: For the IPIMAR collection it is necessary to <u>cover the slices with glycerine</u> (due to the ridges that appeared in the 1st Etalan cover). The glycerine is provided with the *Illicia* Collection.

<u>3.1.2. Readings</u>

All data will be entered in the sheets "Readings" of each Excel book. The sheets will be sent by E-mail (electronic copy) and mail (hard copy) to Jorge Landa (jorge.landa@st.ieo.es)

- Fill the name of reader and Institute, the date of reading and the magnification used in the reading. The same magnification (100 x) is recommended for all readings in order to avoid the possibility of reading more rings than those that are really significant. (Recommendation of "International ageing workshop on European monkfish". Lorient, 25-28 June 1991 and 9-11 July 1997. IFREMER, 1997).
- Attribute an age to each section.
- Attribute the respective ageing credibility using the following codes:

    u – unreadable

    b – low credibility

    m – medium credibility

    h - high credibility

    Example: 2m ⇒ age 2 and medium credibility

    3/4b ⇒ age 3 or 4 with bad credibility

    When two possible ages are indicated (e.g. 5/4), the first one is considered to be the more reliable (i.e. the age 5 in the 5/4 case).
- Assign a type of edge (if possible) using the following codes:

    h – hyaline

    o – opaque

    ? – doubtful
- Measure the first annual ring by means of the horizontal diameter. The measurements will be done comprising the more outer part of the zone of the first annual ring (estimated by the reader). The values should be expressed in micra (micrometers) units. You can measure the first annual ring on the image (with a program: eg. VISILOG) and for this reason we send you .bmp files; or measure directly on the section. For each *illicium* you have its square pixels / micra relationship that will help if you measure it on the image.
- All the *illicia* samples should be sent to the next reader, following the proposed schedule (Fig. 1).

3.2 Images

Each CD contains the images of the *illicia* to read from each collection. To annotate the annual rings on the images you need the program Paint Shop Pro 7.02 that is included on the CD. This program is based in layers and each layer will correspond to a reader.

- Open each image.
- Create a layer with your name.
- Annotate the annual rings on your layer (not on the original image using the paint brush, sign shape: horizontal, size: 10, hardness: 100. The colours used by each laboratory/reader could be: Rafael Duarte (IPIMAR): dark green; António Marçal (IPIMAR): dark blue; Jorge Landa (IEO): light blue; Iñaki Quincoces (AZTI): yellow; Helen McCormick (MI): light green; Grainne NiConchuir (MI): red; and the other possible readers: pink, orange,......etc, but ensure that a different colour is used for each reader

Warning: When you finish of counting rings on all the images, please create a new CD recording all the .psp files and send to the next reader. After the reading of each reader,

each image should have a new layer else with the new annotated rings.

3.3 Collections

4 boxes with their respective collections will circulate among the readers.

4. SUPPORT

If you have any doubts or need any kind of help about the northern samples please contact Iñaki Quincoces (iquincoces@suk.azti.es) at AZTI (tel: 34 94 602 94 00; fax: 34 94 602 94 01). Regarding southern *L. piscatorius* samples please contact Jorge Landa (jorge.landa@st.ieo.es) at IEO (tel: 34 942 29 10 60; fax: 34 942 27 50 72). Regarding southern *L. budegassa* samples please contact Rafael Duarte (rduarte@ipimar.pt) at IPIMAR (tel: 35 1 13 01 08 14; fax: 35 1 13 01 59 48).

5. PARTICIPANTS

Hervé Dupouy. IFREMER Lorient. France

Joël Dimeet. IFREMER Lorient. France

Rafael Duarte. IPIMAR Lisbon. Portugal

António Marçal. IPIMAR Lisbon. Portugal

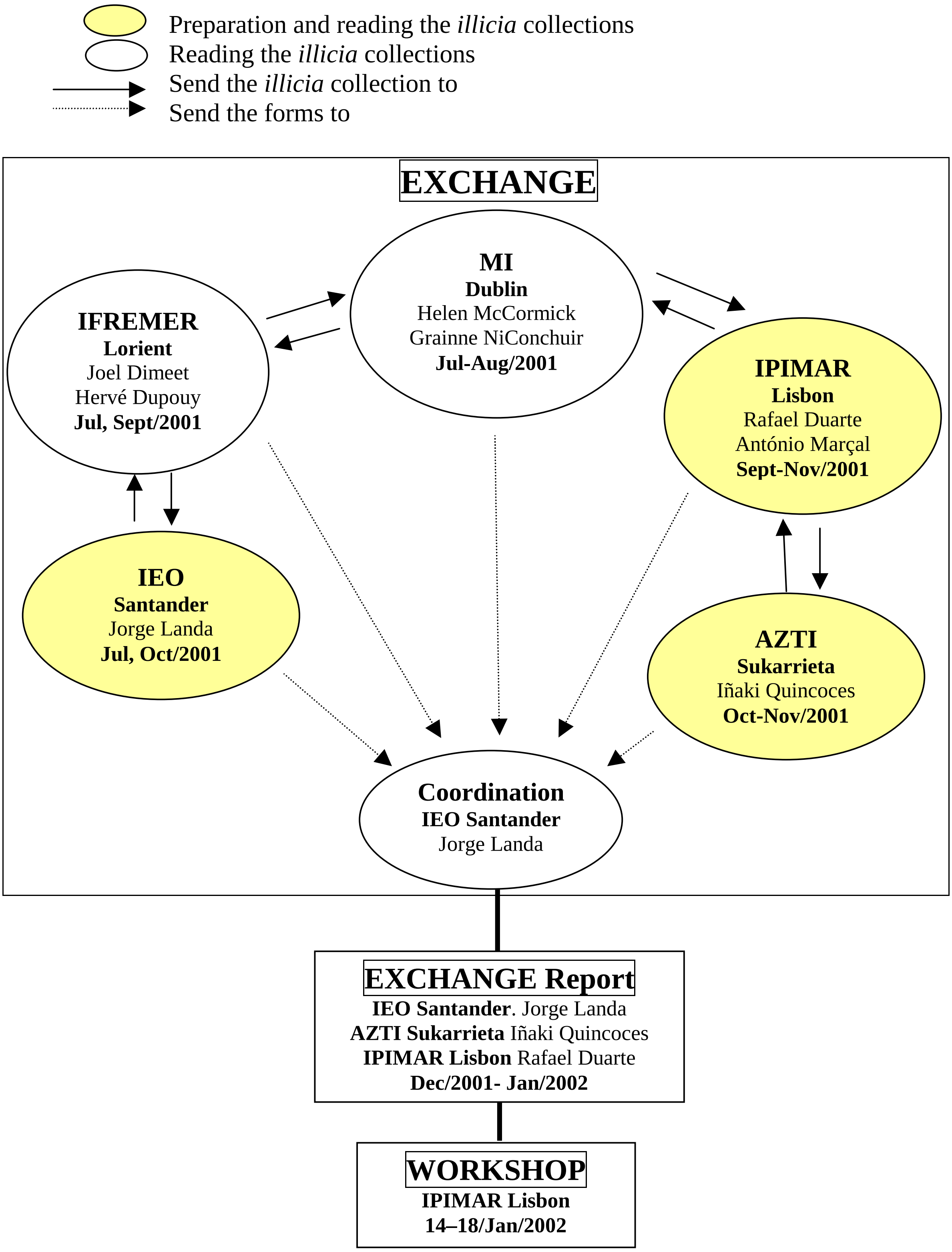
Iñaki Quincoces. AZTI. Sukarrieta. Spain

Jorge Landa. IEO Santander. Spain

Helen McCormick. MI Dublin. Ireland

Grainne NiConchuir. MI Dublin. Ireland

6. READING SCHEDULE



7. REFERENCES

A very useful reference for this exchange is:

ANON., 1999. 3rd International Ageing Workshop on European Anglerfish. (Lisbon 8-12 March 1999). 106 pp. (Please, pay special attention to the conclusions and recommendations).

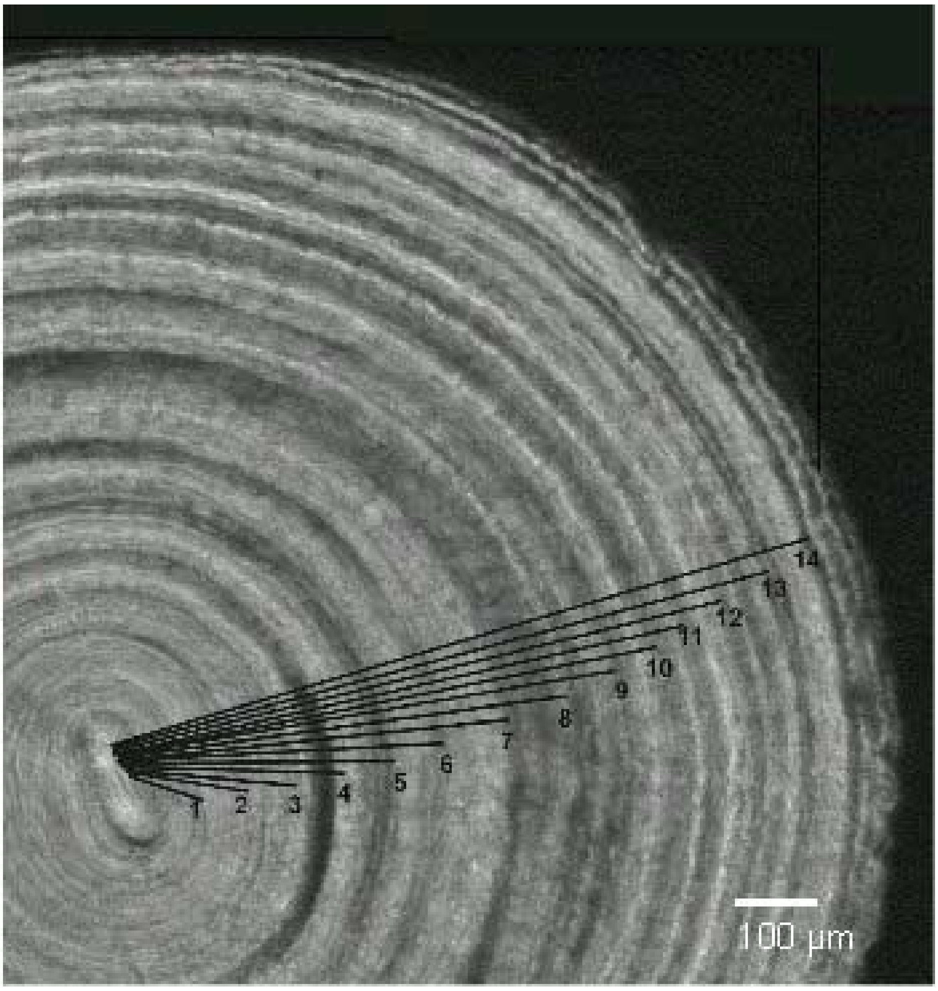
Annex 3

Anglerfish Ageing Guide

ANGLERFISH AGEING GUIDE

by

Duarte, R., Landa, J., Quincoces, I., Dupouy, H., Bilbao, E., Dimeet, J., Marçal, A., McCormick, H. and Ni Chonchuir, G.



Summary

The objective of the present ageing guide is to make a compilation of all the information necessary to age the two species of European anglerfish (*Lophius piscatorius* and *L. budegassa*). The used calcified structure is the first dorsal fin ray (*illicium*). Anglerfish ageing is generally recognised as a difficult task. Hervé Dupouy, from IFREMER, Lorient (France), started to implement in the eighties a routine ageing procedure, based on *illicia* transversal sections. After recognising the benefits of this procedure and the clearer annual ring identification, compared to otoliths, researchers from Spain and Portugal followed Hervé's work, in order to provide annual data for stock assessment. Since the beginning of the nineties, four *illicia* ageing workshops were held in order to improve methodologies and uniformity in the ageing criteria. This ageing guide results from the work developed during these workshops and the objective is to present all the necessary information to age anglerfish. This way, the introduction in section 1 makes a summary of the main biological particularities of these species, section 2 contains all the methodology to obtain *illicia* transversal sections, section 3 describes the ageing criteria and in section 4 are *illicia* images with marked annual rings.

Participants

(By alphabetical order of last name)

Eli Bilbao	AZTI	Spain
Joel Dimeet	IFREMER	France
Rafael Duarte (coordinator)	IPIMAR	Portugal
Hervé Dupouy	IFREMER	France
Jorge Landa (coordinator)	IEO	Spain
António Marçal	IPIMAR	Portugal
Helen McCormick	Marine Institute	Ireland
Grainne Ni Chonchuir	Marine Institute	Ireland
Iñaki Quincoces (coordinator)	AZTI	Spain

Contents

1. INTRODUCTION

Black anglerfish (*Lophius budegassa* Spinola, 1807) and white anglerfish (*L. piscatorius* Linnaeus, 1758) are important species in European fisheries. They are very similar, being distinguished by the colour of the peritoneum (*L. budegassa* black and *L. piscatorius* white) or by the number of rays in the second dorsal fin (*L. budegassa* 9-10 and *L. piscatorius* 11-12) (Caruso, 1986). Both black and white anglerfish are typical bottom living species, the former having a depth range between 70 m and 800 m and the latter extending to depths >1000 m (Dardignac, 1988; Azevedo and Pereda, 1994). Black anglerfish has a more southern distribution (Mediterranean and Eastern North Atlantic from British Isles to Senegal) compared to white anglerfish (Mediterranean, Black Sea and Eastern North Atlantic from Barents Sea to the Straits of Gibraltar), but there is considerable overlap (Fig. 1).

The spawning season of *L. piscatorius* is poorly described in the literature, but is generally from late winter to summer. Afonso-Dias and Hislop (1996) observed spawning females from November to May in Scottish waters, Quincoces *et al*. (1998a) described the spawning period between May and August in the Cantabrian Sea. In Norwegian waters it has been observed between June and August (Staalasen, 1995). The poor description of the spawning period is due to the difficulty in obtaining mature females from the fishery and research surveys. The spawning period is described better for *L. budegassa*. Azevedo (1996) observed spawning during October to March off the Iberian coast. Quincoces et al. (1998b) observed it between May and July in the Cantabrian Sea and Duarte et al. (2001) between November and February off the Iberian coast.

The maturation process of male and female gonads is well described in the literature (Afonso-Dias and Hislop, 1996 and Quincoces *et al*., 1998a,b). The length at first maturity seems to be quite long. Females of *L. piscatorius* mature at around 70-75 cm while males reach maturity at a shorter length (50 cm). For *L. budegassa*, females reach first maturity from 50 to 65 cm and males reach it between 35 and 40 cm.

The reproduction of the *Lophius* genera is very unique compared to other teleost species, the ovaries are ribbon like and the eggs are released in floating gelatinous matrixes (Armstrong et al., 1992; Afonso-Dias and Hislop, 1996 and Quincoces *et al*., 1998a,b). This unique characteristic makes it difficult to observe the species during routine larval-egg surveys.

The growth of *L. piscatorius* and *L. budegassa* has been studied based on Sagitae Otoliths (Crozier, 1989 and Tsimenidis, 1984) and *Illicia* (Dupouy *et al*., 1986; Duarte *et al*., 1997; Landa and Pereda, 1997 and Landa *et al*., 2001). Studies comparing the two structures showed that with *illicia* higher agreement between readers were achieved (Dupouy pers. com.; IFREMER, 1991, Staalasen, 1995). Observations have shown that *L. piscatorius* attains higher lengths than *L. budegassa*. In both species, females achieve greater lengths. For *L. piscatorius* females reach an L_{∞} between 160 and 170 cm compared to males with an L_{∞} between 110 and 130 cm (Dupouy *et al*., 1986 and Landa *et al*., 2001) and *L. budegassa* females reach an L_{∞} between 90 and 110 cm compared to males with an L_{∞} between 70 and 85 cm (Dupouy *et al*., 1986; Duarte *et al*., 1997 and Landa *et al*., 2001).

For age determination it is important to understand the early life cycle of the species. After spawning of *L. piscatorius*, there are indications of yolk sac duration of between 15 and 20 days and that the pelagic phase is around 3 to 4 months duration. Studies based on otolith daily increments shows that pelagic specimens between 7 and 11 cm are between 80 and 120 days (Hislop *et al*., 2001). Settlement probably occurs when specimens reach around 6 cm or greater (Russel, 1976 and Hislop *et al*., 2001). There is no data in the literature for the early life cycle of *L. budegassa* but it is assumed to be similar.

The early life cycle of the two species has a marked influence on the microstructure of the *illicia*. Observing the *illicium* under sweeping electronic microscopy (SEM), the area of its nucleus is distinguished (Fig. 2). This area, when being attacked by EDTA used for the preparation of the samples for its vision under SEM, presented multiple holes, possibly due to the heterogeneous crystallization of this area of the *illicium* (Quincoces, 2002). It shows an amorphous crystallization and in an axis different to that given in the rest of the *illicium*. This nucleus also is similar in length and in shape to the nucleus observed under photonic microscopy. It is considered to be a consequence of a change in the life cycle (changing from planktonic to benthic living), and is therefore designated as the benthic ring.

Age readings based on *illicia* have been applied for stock assessment of both species. In spite of recent ageing studies, there are still some doubts concerning age validation and ageing precision between readers. In order to improve agreement between readers and this way provide more precise data for stock assessment, four workshops were carried out with different objectives and in different situations concerning biological knowledge of the growth of both species. The first ageing Workshop for Anglerfish was carried out in 1991 (IFREMER, 1991) involving readers from France and Spain. The main objective was to define clearly an ageing structure to perform ageing for stock assessment purposes. Best results were obtained with the *illicium*, which was adopted. The second workshop held in 1997 (IFREMER, 1997) was conducted with additional participation by Portugal and the main objective was to increase ageing precision between readers. The third workshop was carried out in 1999 (Anon., 1999) with additional participation by Ireland and the main objective was to establish and describe ageing criteria in order to increase the ageing precision between experienced readers. The fourth workshop was held in 2002, after an *illicia* exchange that included digital images. The main objective was to discuss the exchange results and to analyse the digital images, where each reader marked the annual rings. The images were considered a very important tool to discuss ageing criteria.

2. MATERIALS AND METHODS

The interpretation of growth structures in *illicia* is widely accepted as one of the most reliable methods for ageing anglerfish. For this method it is necessary to prepare a section 0.5 cm from the base of the *illicia* with a thickness of 0.5 mm or less in which the age readings are performed using a microscope.

2.1. *Illicia* mounting and sectioning

The *illicia* mounting has the objective of including the *illicia* in resin that serves as a support for the sectioning. The material necessary is a mould, a set of chemical compounds and a cutting machine. The practical procedure for the *illicia* mounting and sectioning is also described.

2.1.1. The mould

The mould is basically a base plate with two longitudinal strips and three lateral strips. These five strips are screwed to the base as shown in Fig. 3. The base plate and the strips of the mould are composed of aluminium and the screws are made of stainless steel.

2.1.2. Materials

To prepare the blocks of black resin the following material is necessary (Fig. 4):

- Honey Wax (Mold Release Compound)
- Resin (SP106 / Multi-purpose Epoxy System)
- Slow Hardener (SP106 / Multi-purpose Epoxy System)
- Epoxi Pigment Black (The proportions used are 81% resin for 16% slow hardener for 3% epoxi pigment black)
- glass microscope slides
- glue (entellan)

2.1.3. Slicing machine

The slicing machine (Fig. 5) can be of different types. Nevertheless it is essential to have the following characteristics:

- Cutting speed of 2000 rpm or higher,
- Diamond sectioning blade,
- Cooling system

2.1.4. Preparation of the base and mounting the *illicia*

The inner surfaces of the mould are lightly smeared with a releasing agent (Honey Wax) and a first layer of resin is poured in the mould. The mould is then placed on a level surface (Fig. 6) and when the resin becomes viscous / sticky the mounting of the *illicia* can start.

A strip of "spaghetti" is placed at the extreme left-hand side of the mould. The *illicia* are placed in the mould in straight and parallel lines. The rows start at the top left-hand corner and work from left to right (Fig. 7). The "spaghetti" marks the left side of the slices and this way it is possible to identify each *illicium*. All this process can be made manually or using a specific hardware (Fig. 8).

When all the rows have been filled with *illicia*, and the resin is less viscous (to avoid the *illicia* moving), a further layer of liquid black resin is poured on top, filling the mould, which is placed again on a level surface.

2.1.5. Cutting the *illicia* sections

Each polyester block is set up in the machine and cut separately. A simple jig, permanently fixed to the table of the machine is used to hold the block under the cutting disc. The row of *illicia* is positioned under the cutting disc (Fig. 9) and the cutting process begins (following the specification of the different types of cutting-machine) resulting in sections of 0.5 mm or less in thickness.

2.1.6. Mounting the slices

Finally the sections are fixed permanently in glass microscope slides. This is done using glue, which fix them to labelled microscope slides (Fig. 10).

2.2. Observation

Microscopes / Software

An image analysis system is used, composed of a microscope (Zeiss) with a video camera (Sony Model DXC-930P / 3CCP Colour Video Camera CCD-IRIS). The magnification is 100X and transmitted light is used. This system allows the treatment of the images (mark the rings and measure distances) (Fig. 11).

3. AGEING CRITERIA

Anglerfish ageing using *illicia* consists of identifying dark and light rings (Fig. 12, Fig. 13). For age determination only the dark rings are counted. For this we assume that one dark ring represents one year growth. At times these rings are well defined and clearly visible, but most of the time, rings appear doubled and are not well defined, which makes ring identification very difficult. From the open discussion and communal interpretation of *illicia* sections during the 4th International Ageing Workshop on European Anglerfish, some peculiarities inherent to *illicia* ageing were defined:

- It is important to play with and adjust the light and focus of the microscope, to identify the pairs of dark and light rings, and to try to find a general pattern of growth. Unlike otoliths, where ring widths tend to decrease as you approach the edge, in *illicia,* rings remain a similar width apart throughout the section. Rings close to the edge may even be wider apart than those closer to the nucleus (Fig. 12, Fig. 13).

- Rings in *illicia* differ in composition. As a result, the surface appears rippled, alternating between high and low ridges. The differences in these levels relate directly to the dark and light rings. This characteristic is very apparent from research carried out using scanning electron microscopy (Fig. 14).

- Rings may not be visible in all the axes of the section. Defined rings, which are clearly visible in one part of a section may be less defined or even appear to double in another part of the section. The counting should be based upon the area where good contrast between rings exists.

- The next step is to identify the position of the first annual ring, and to confirm this by measuring its diameter. For this reason it is necessary to know the following.

  - The first well-marked ring usually observed is considered to be a consequence of a change in the life cycle (changing from planktonic to benthic living), and is therefore designated as the benthic ring (Fig 15, Fig. 16). The next ring is considered to be the first annual ring. When identifying the first annual ring, the diameter of the benthic ring can be of assistance. The distance of the first annual ring from the benthic ring is usually not greater than half the distance of the diameter of the benthic ring.

  - In *L. piscatorius*, the first ring tends to be oblong in shape and the mean horizontal diameter of the first ring tends to be between 200 and 300 μm (Fig. 15). For *L. budegassa*, the first ring tends to be circular in shape and the mean diameter of the first ring tends to be at 80 μm (between 60 and 100 μm) (Fig. 16).

- To identify the outer ring it is very important to look at the edge of the *illicium*. For this it is essential to know the quarter (or month) in which the sample was taken. This will determine whether or not the ring at the edge is to be counted in the age reading. At times the outer ring(s) are not visible in the whole *illicium*, this may be because the section has not been cut perpendicularly (Fig. 17). When a dark ring appears at the edge in Q1, it should be counted and included in the age reading. If a similar ring appears in Q4 it should not be counted or included in the age reading.

- It is recommended to read *illicia* of similar length group fish together, and also to begin with the clearest *illicia* sections. This is a good exercise to help train the eye in identifying the typical pattern of *illicia*. Because the first rings in younger fish are often difficult to define, it is easier to begin reading the *illicia* from the middle of the fish length range to establish the growth pattern of these first rings. For example in *L. piscatorius* this corresponds with lengths in the range of 60 to 90cm, and in *L. budegassa* it is between 50 to 70cm.

- The <u>length</u> of the fish can be a useful piece of information in ageing *illicia*. It is recommended to do a first reading and afterwards to check that the age reading lies within the possible mean fish length range at that age. For example for *L. piscatorius* a fish of 20cm will be aged between 1 - 3 years. For *L. budegassa* the same fish would be expected to be between 2 - 3 years.

- The ageing of this species is not easy. As an example we can see that within this *illicia* exchange, even with samples that were originally considered to be clear, the expert readers assigned the following credibility percentages. 55% were considered to be of medium credibility, and only 30 % were considered to be high, a further 15 % were found to be bad. It was also found that 2% of the *illicia* were unreadable.

- <u>Confusion after some ages (age 6)</u> may be related to first maturation or any other unidentified life – history event, which causes changes in the growth pattern.

4. REFERENCE COLLECTION OF *ILLICIA* IMAGES OF AGREED AGES

The following reference collections of illicia images of agreed ages were prepared.

4.1. *L. piscatorius*

Image number	Agreed age	File name	Collection area	Comments
1	1	14 3b-1 1999	South	Total Agreement by experienced readers
2	2	289-97-5b-4	North	Total Agreement by experienced readers
3	3	67-99-5b-3	North	Total Agreement by experienced readers
4	3	2-1b-4 2000	South	Total Agreement by experienced readers
5	4	60-99-3a-3	North	Total Agreement by experienced readers
6	5	73-99-1b-4	North	Total Agreement, better image
7	5	13-4a-21999	South	Total Agreement by experienced readers
8	6	2b-5	North	Modal Age agreed, but different rings selected
9	8	66-99-4a-7	North	Disagreement on 3rd and 4th rings
10	9	11-00-1a-1	North	Good agreement by R5 &R6, R3= N-1
11	10	84-99-2A-3	North	Total Agreement by experienced readers
12	11	15-4b-5 1999	South	R5&3=11, R6=12, however chose very similar rings
13	12	114-99-2a-1	North	Disagreement on one ring

14	12	8-4b-3-2000	South	R3 differs on ring 1 and 11
15	13	238-97-1a-4	North	R1,5 &6 =13, similar rings, R3=11
16	14	100-99-3b-3	North	R5&6 in total agreement, R3=11
17	16	77-99-5b-8	North	Total Agreement by experienced readers
18	24	9-1b-3 1999	South	Age 24-29, image looks good, different rings chosen
19	25	9-1b-2 1999	South	Total Agreement by experienced readers

4.2. *L. budegassa*

Image number	Agreed age	File name	Collection area	Comments
20	1	A-2	South	Total Agreement by experienced readers
21	2	A-7	South	Total Agreement by experienced readers
22	3	B-3	South	Total Agreement by experienced readers
23	4	19-1998-5b-8	North	Total Agreement by experienced readers
24	5	C-7	South	Total Agreement by experienced readers
25	6	3-1999-3b-2	North	Total Agreement by experienced readers
26	7	39-1998-3a-3	North	Total Agreement by experienced readers
27	8	G-7	South	R3 missed 3rd ring
28	9	6-1999-3b-4	North	R5&R6=9, similar rings, R3=6
29	10	237-1997-5a-5	North	Total Agreement by experienced readers
30	11	92-1999-5b-10	North	Agreement on all rings except 1st by R3
31	12	K-6	South	R5&6 = 12, R3=8, Good illustration of applying different ageing criteria.
32	15	N-1	South	R5&6=15, R3=13
33	19	N-5	South	R3=17, R6=19 and R5=20, R5&6 chose similar rings
34	21	O-3	South	R3=16, R5&R6=21, but chose some different rings

Figure 1. Distribution area of *L. piscatorius* and *L. budegassa*

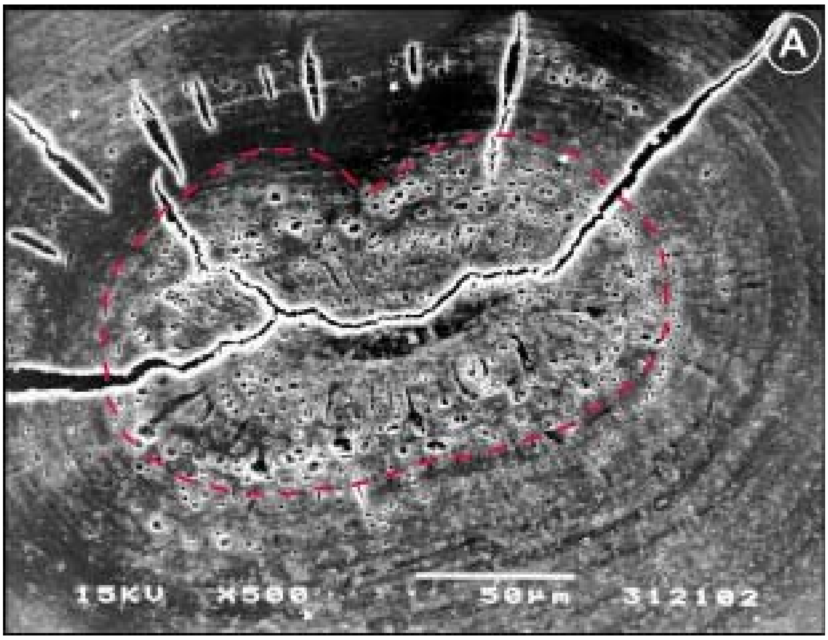


Figure 2. Microphotograph with sweeping electronic microscopy at 500X of the central area of an *illicium* of *L. piscatorius* of 63 cm and 8 years of age. The nucleus of the *illicium* is marked with a line discontinuous.

Figure 3. The mould.

Figure 4. Materials: Resin, pigment, wax and entellan.

Figure 5. Slicing machine.

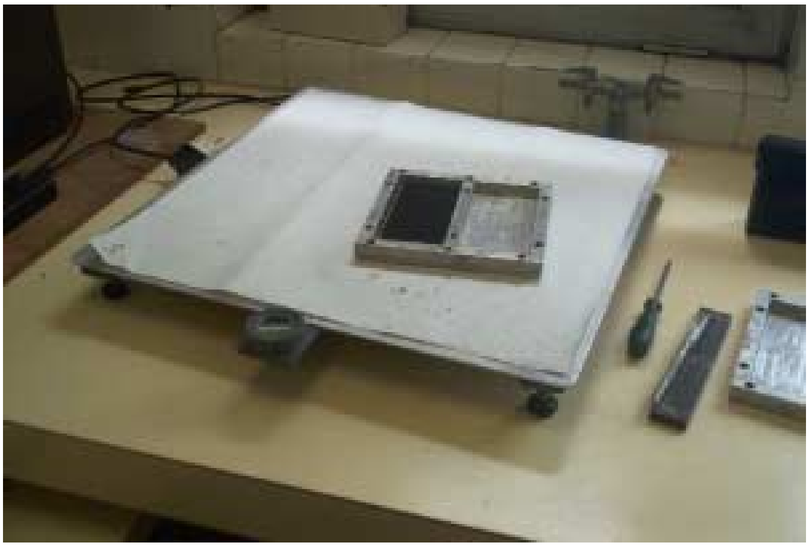


Figure 6. Level surface with mould.

Figure 7. Mould with a “spaghetti” on the left side. *Illicia* are placed from left to right.

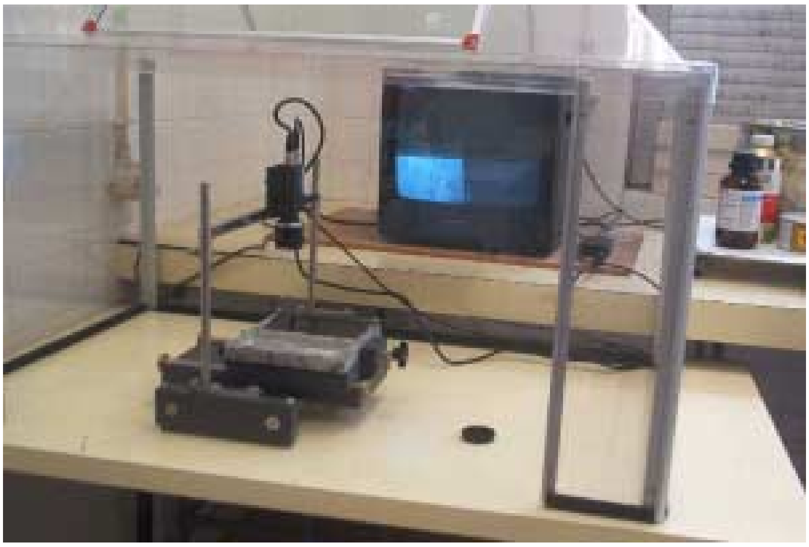


Figure 8. Mounting system.

Figure 9. Resin block positioned in the slicing machine, ready to begin the slicing process.

Figure 10. Microscope slide with two *illicia* sections.

Figure 11. Image analysis system.

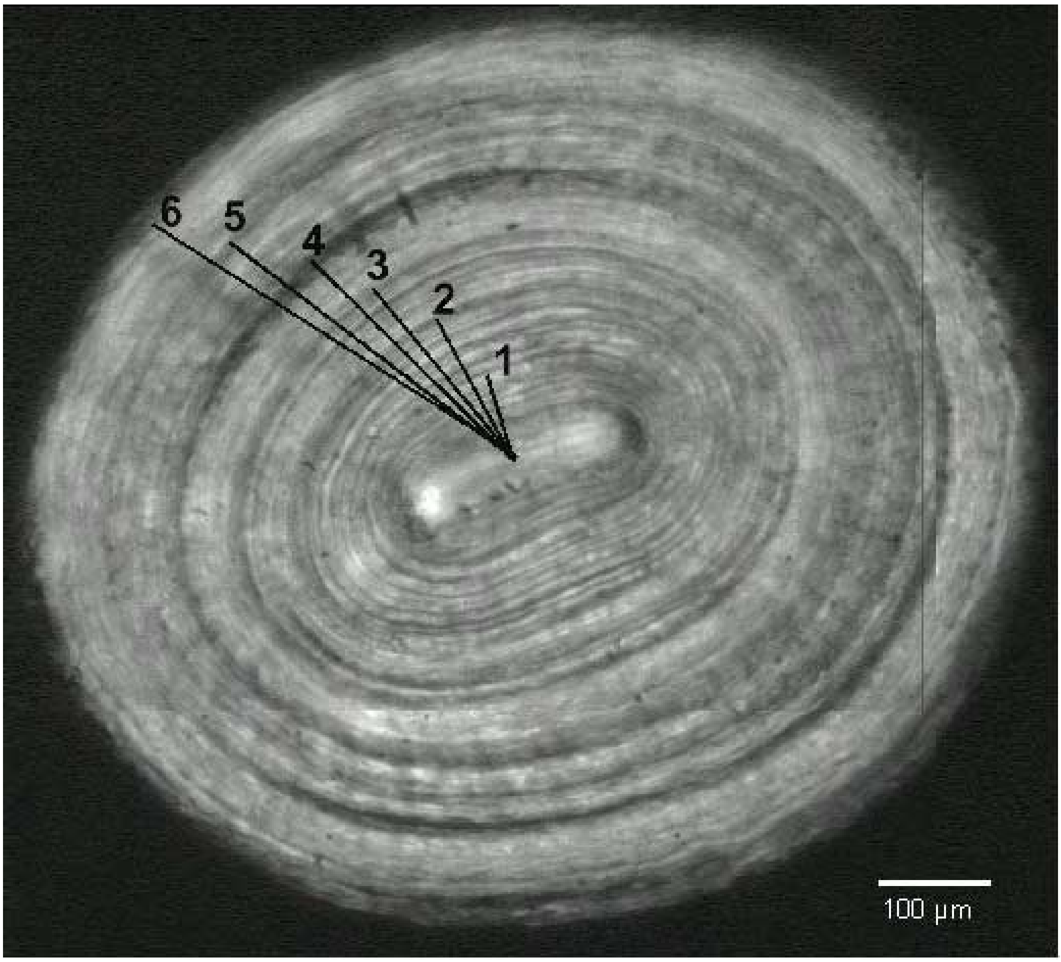


Figure 12. *Lophius piscatorius* with 51 cm in length. There are 6 annual rings visible. Distances between the majority of the rings are equivalent but rings 5 and 6 are more separated compared to 3 and 4 or 2 and 3.

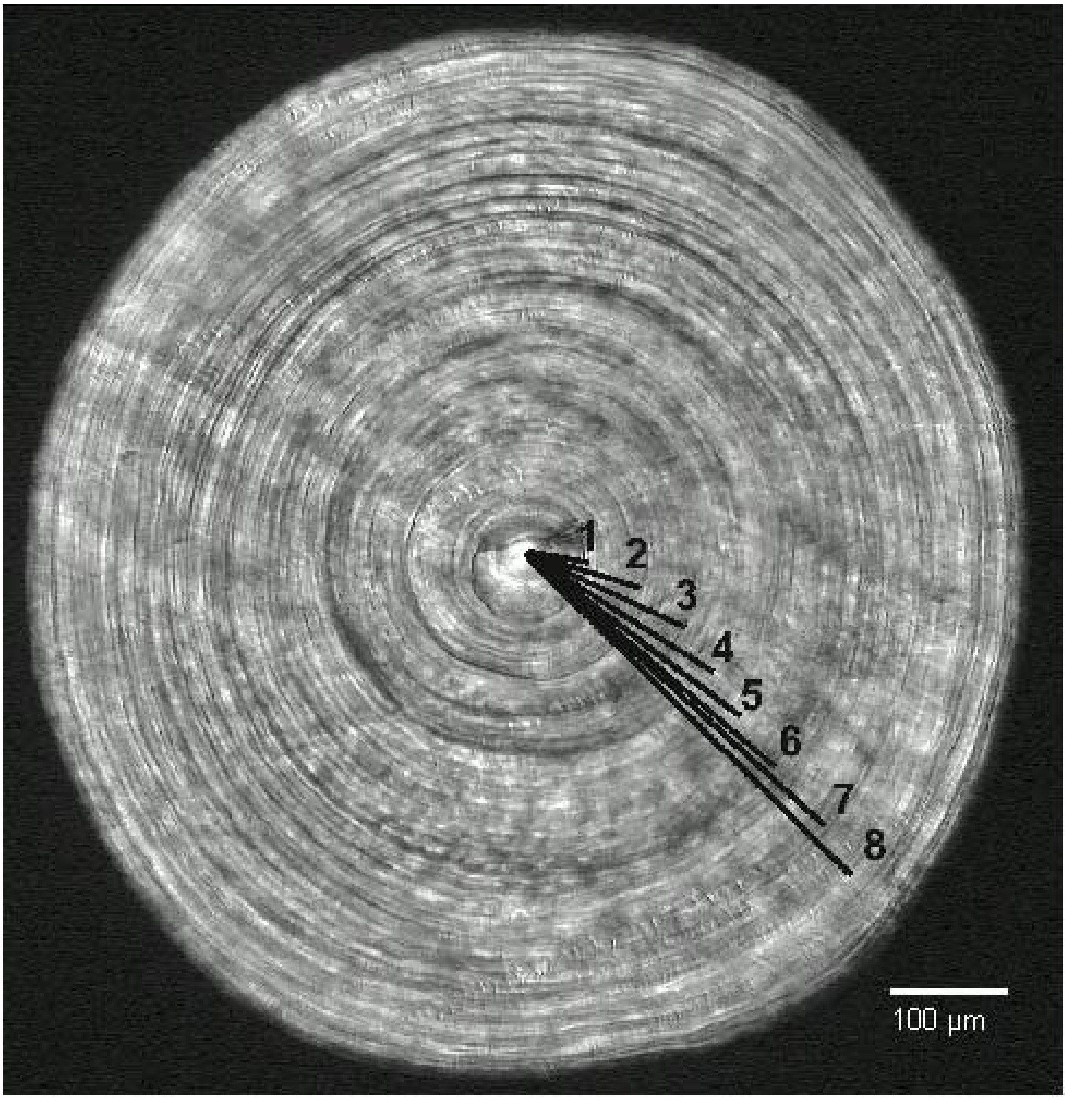


Figure 13. *Lophius budegassa* with 52.7 cm in length. There are 8 annual rings visible. Distance between rings 7 and 8 is greater compared to distance between rings 6 and 7 or 4 and 5.

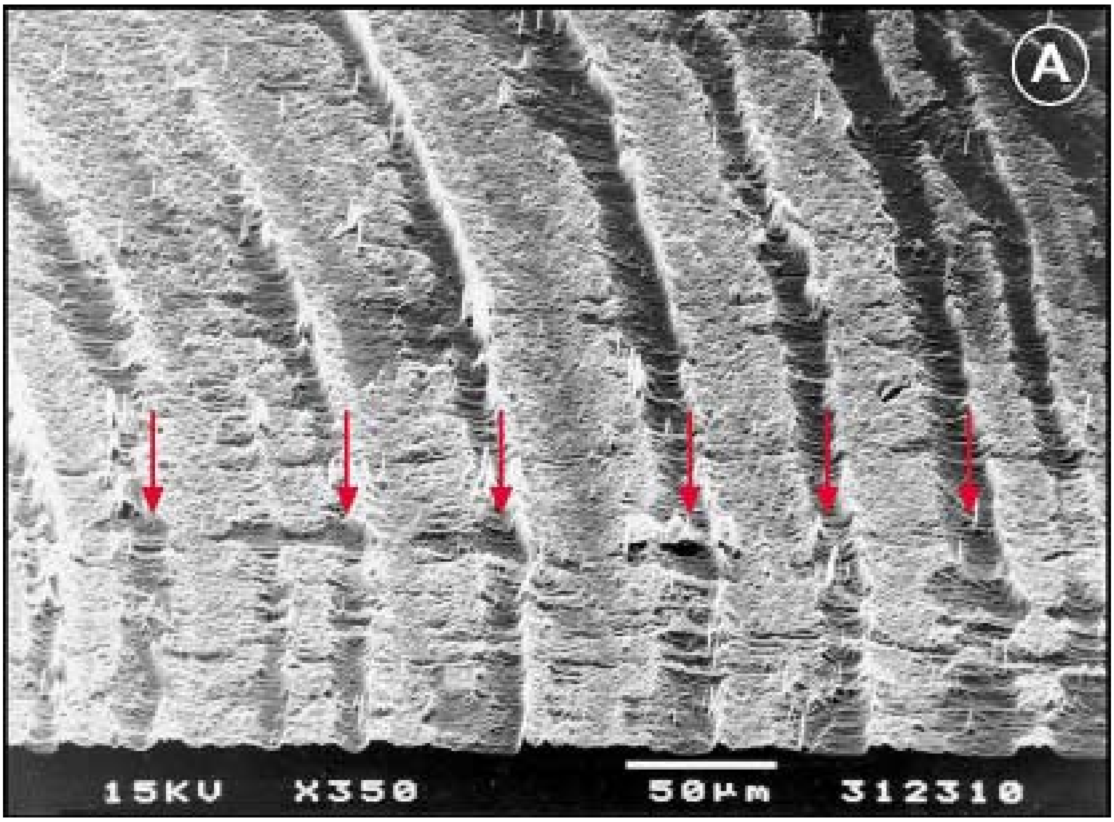


Figure 14. *Illicium* image obtained using scanning electron microscopy. The surface appears rippled, alternating between high and low ridges (dark and light rings).

A

B

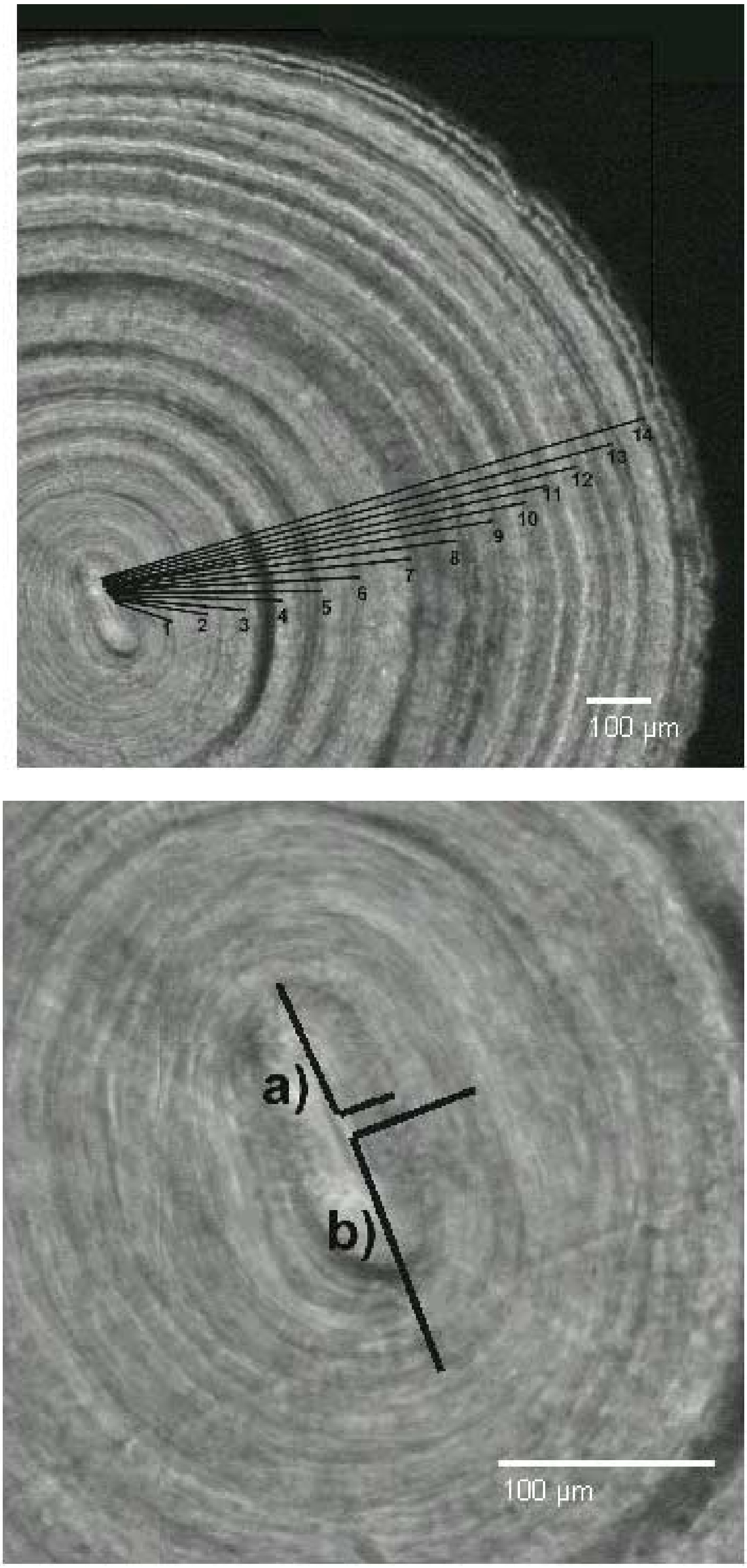


Figure15. *Lophius piscatorius* with 91 cm in length. In Image **A** are the identified annual rings (14 annual rings) and Image **B** contains for the same *illicium*, only the central part. Image **B** shows the oval shape of the first rings characteristic of this species and the benthic ring is marked as a) and the first annual ring is marked as b).

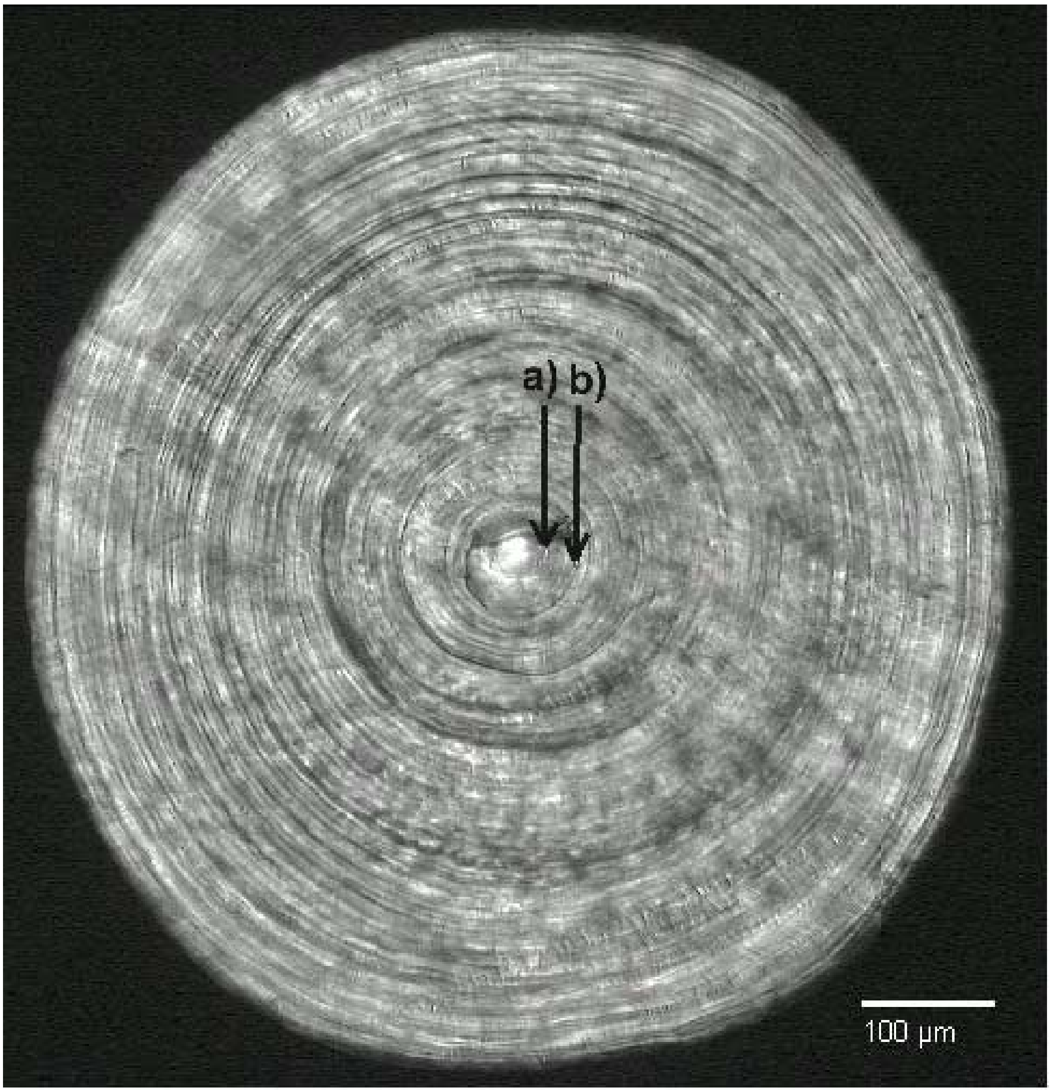


Figure 16. *Lophius budegassa* with 47.9 cm in length. Benthic ring is marked as a) and first annual ring is marked as b). Annual rings and nucleus tend to be circular in shape, what is a characteristic of this species.

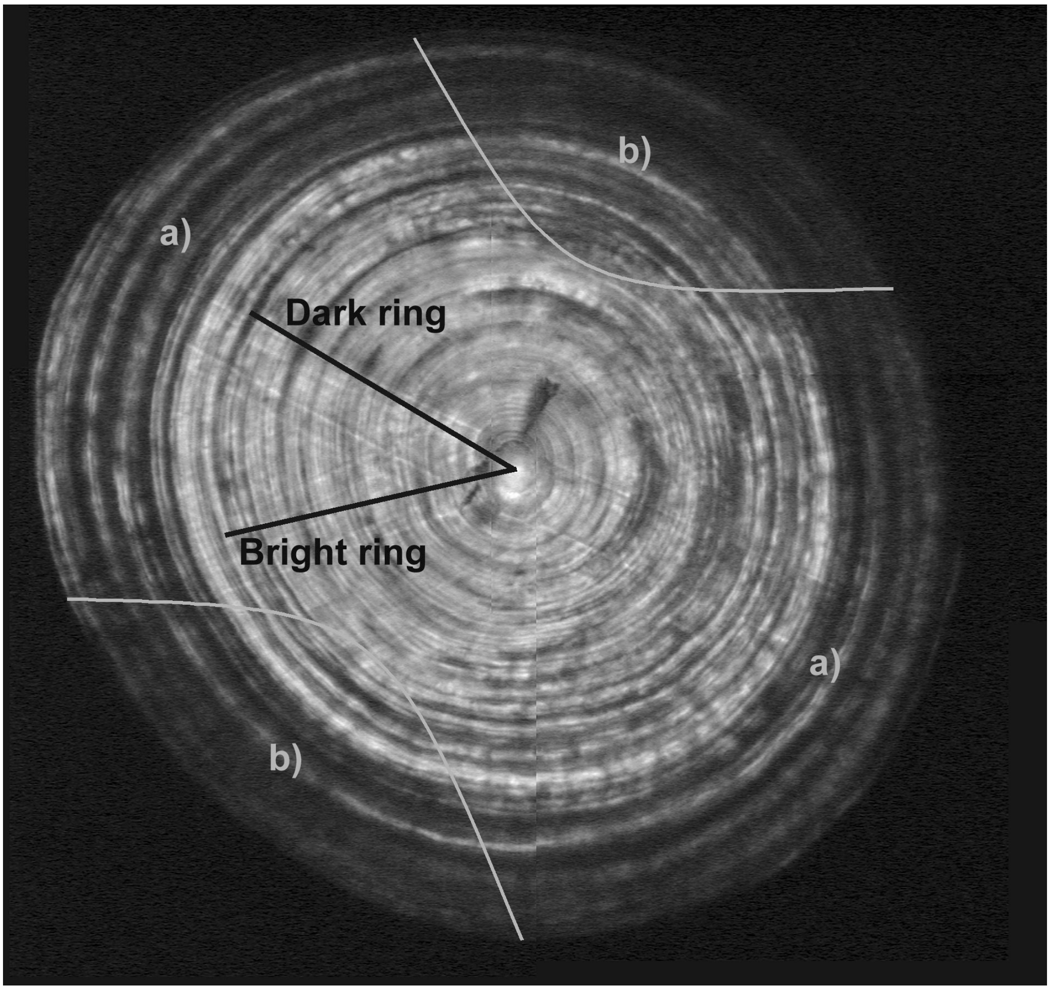


Figure 17. *Lophius budegassa* with 64 cm in length. Region a) of the *illicium* with good contrast between rings and region b) with low contrast. Some rings visible in region a) are not distinguishable in region b). Relative to the ring coloration, there are two well marked dark rings in a certain region of the *illicium* and in another region of the cut the dark part disappears and a bright part is very visible and easy to count.

Image 1. *L. piscatorius.* Agreed Age: 1. File name: 14 3b-1 1999. Collection South. Total Agreement by experienced readers.

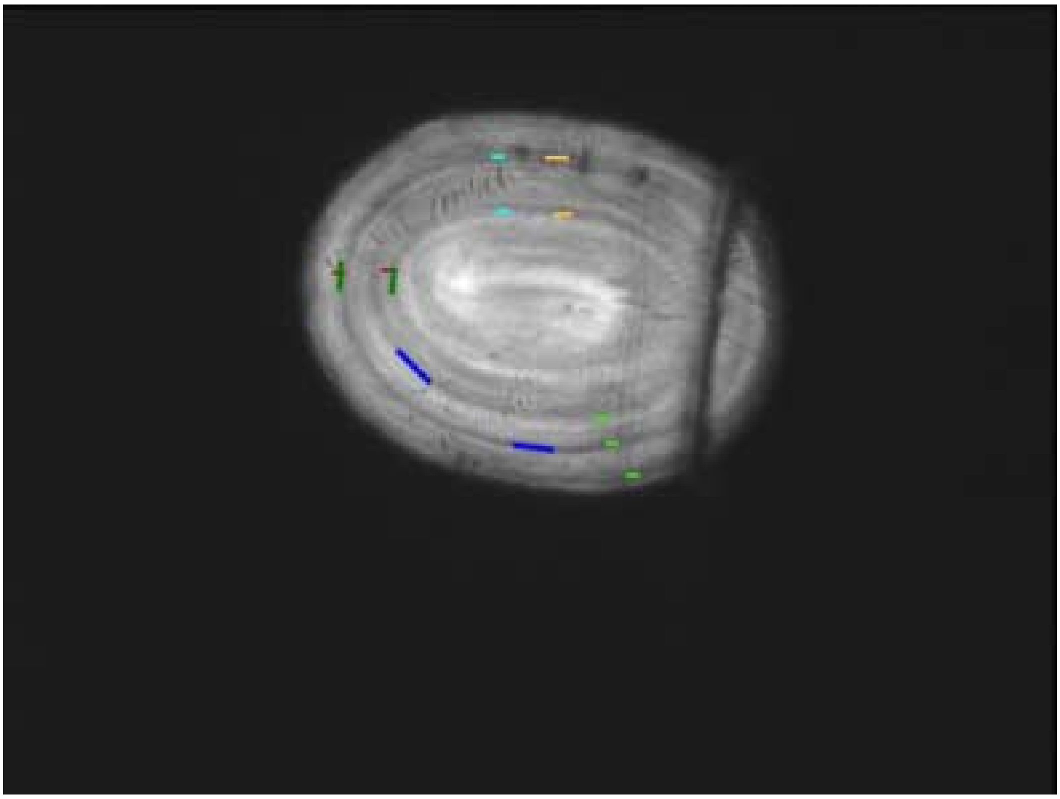


Image 2. *L. piscatorius.* Agreed Age: 2. File name: 289-97-5b-4. Collection North. Total Agreement by experienced readers.

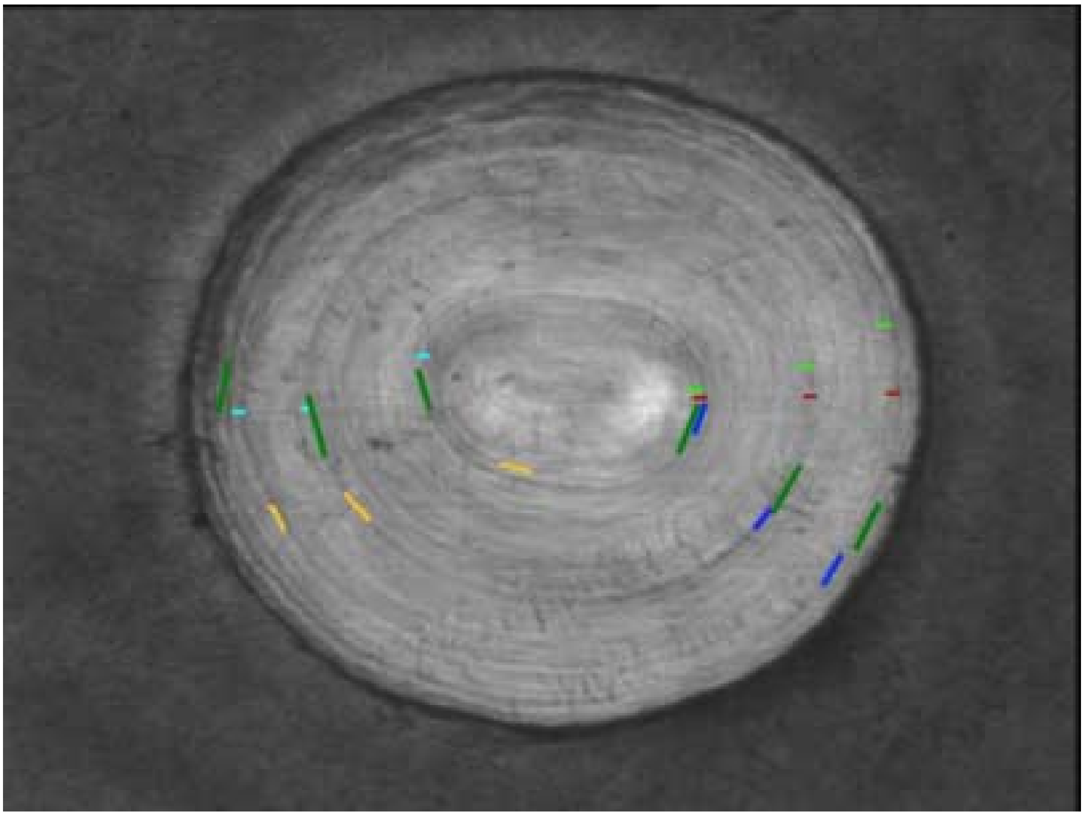


Image 3. *L. piscatorius.* Agreed Age 3. File name: 67-99-5b-3. Collection North. Total Agreement by experienced readers.

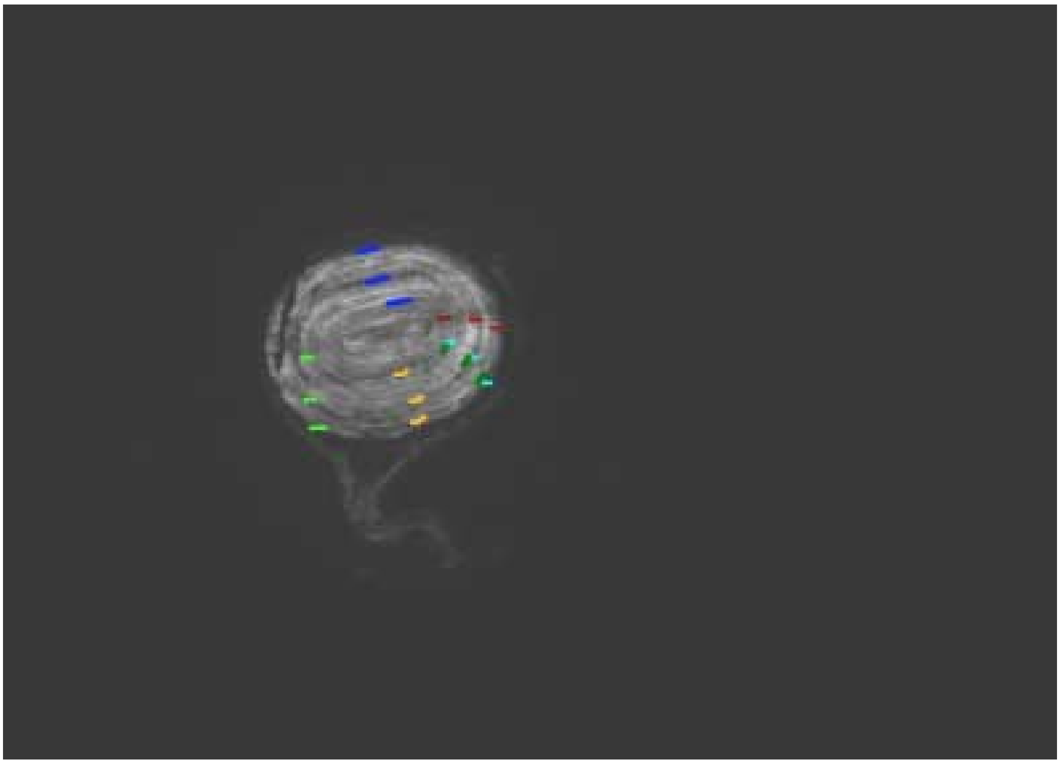


Image 4. *L. piscatorius.* Agreed Age 3. File name: 2-1b-4 2000. Collection South. Total Agreement by experienced readers.

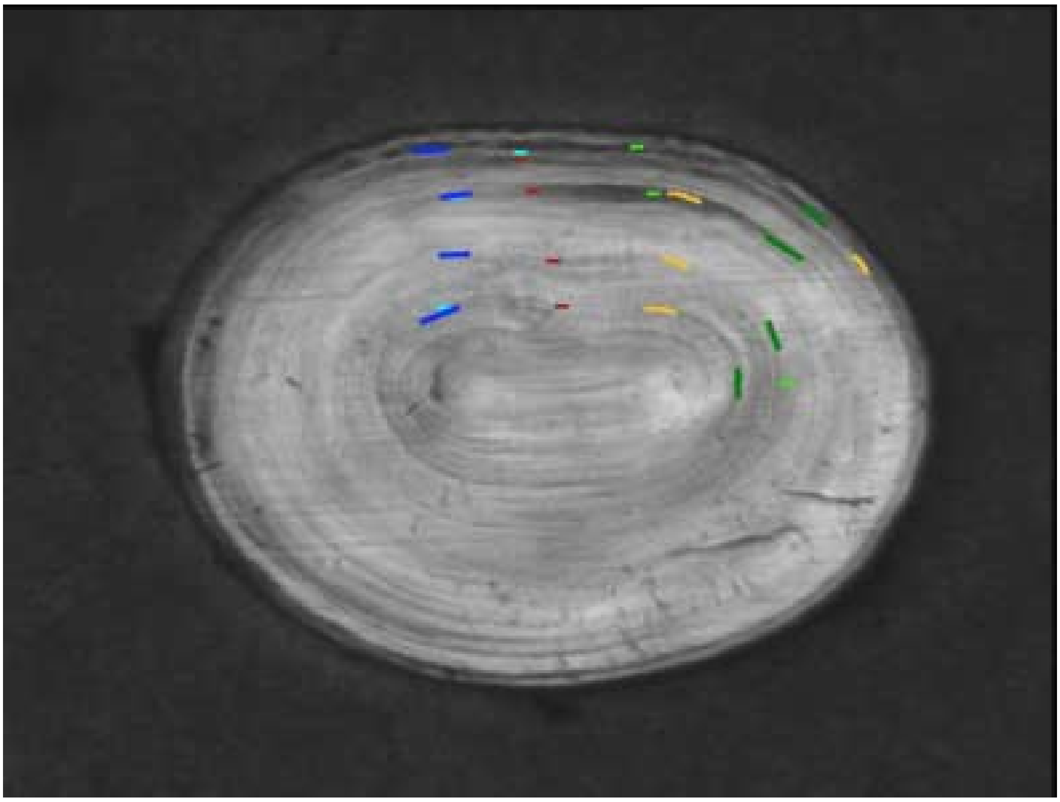


Image 5. *L. piscatorius.* Agreed Age 4. File name: 60-99-3a-3. Collection North. Total Agreement by experienced readers.

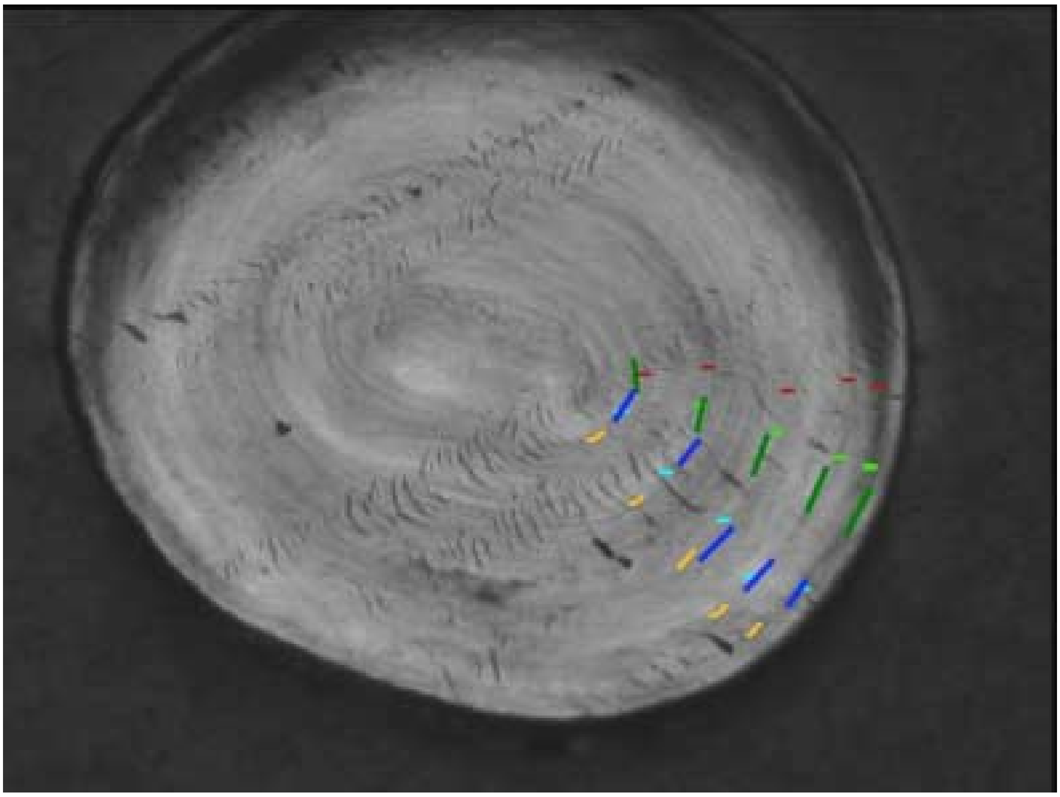


Image 6. *L. piscatorius.* Agreed Age 5. File name: 73-99-1b-4. Collection North. Total Agreement, better image.

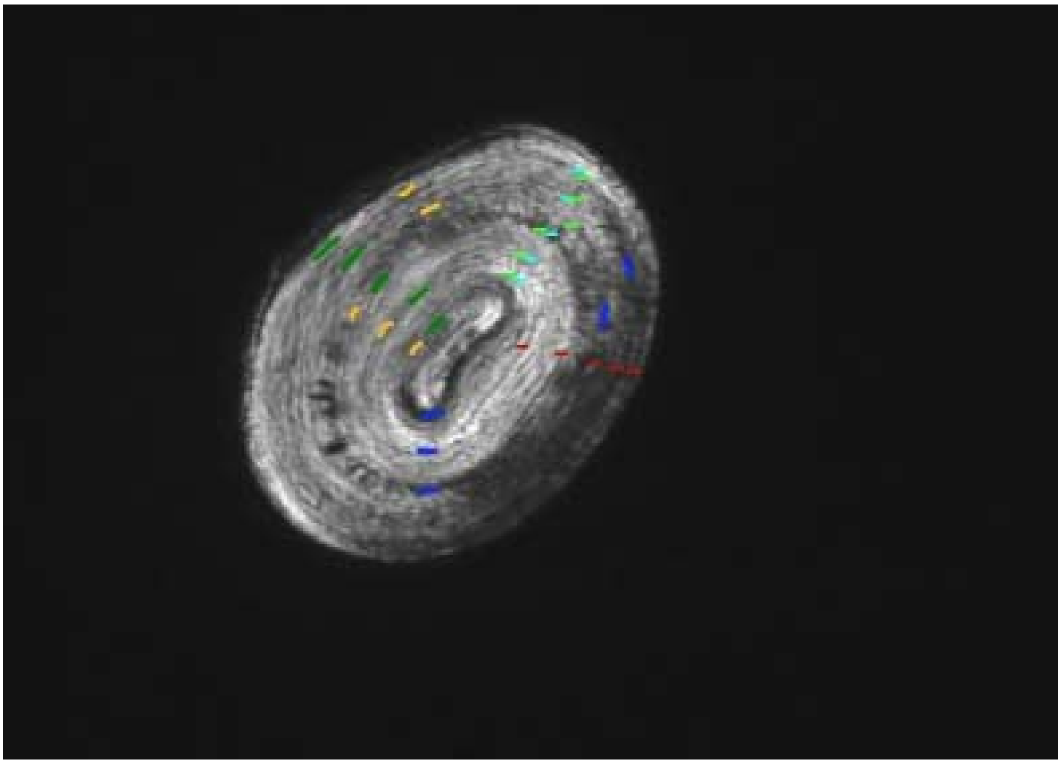


Image 7. *L. piscatorius.* Agreed Age 5. File name: 13-4a-21999. Collection South. Total Agreement by experienced readers.

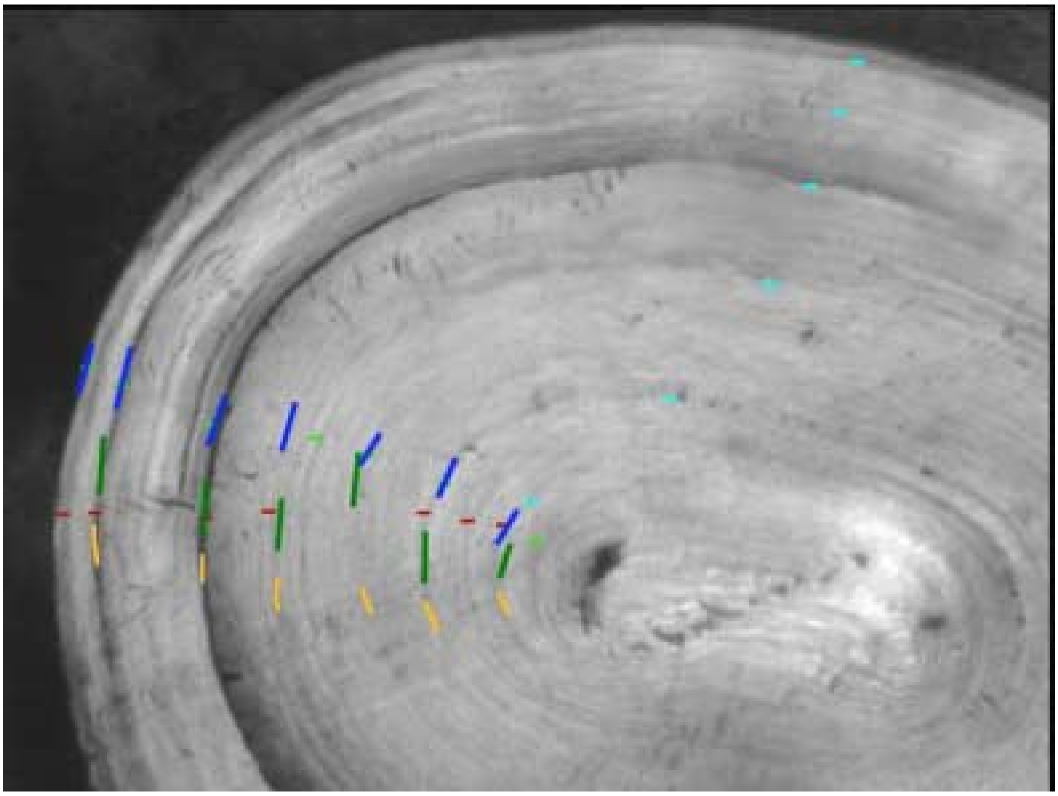


Image 8. *L. piscatorius.* Agreed Age 6. File name: 2b-5. Collection North. Modal Age agreed, but different rings selected.

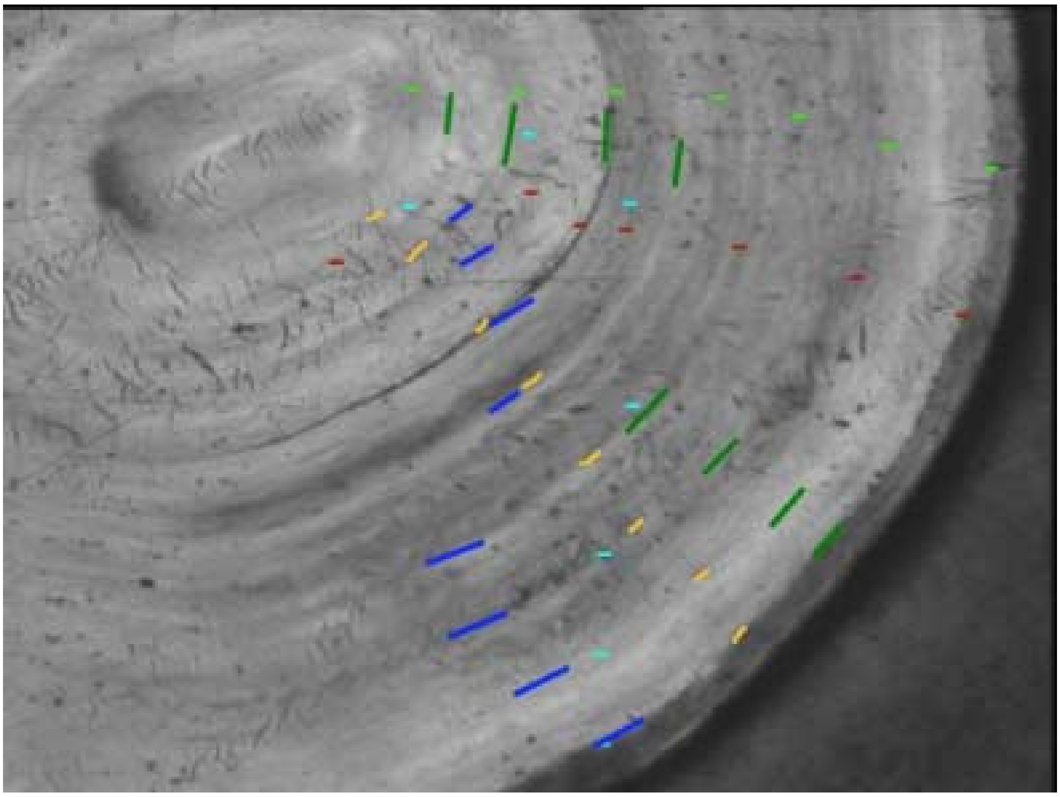


Image 9. *L. piscatorius.* Agreed Age 8. File name: 66-99-4a-7. Collection North. Disagreement on 3rd and 4th rings.

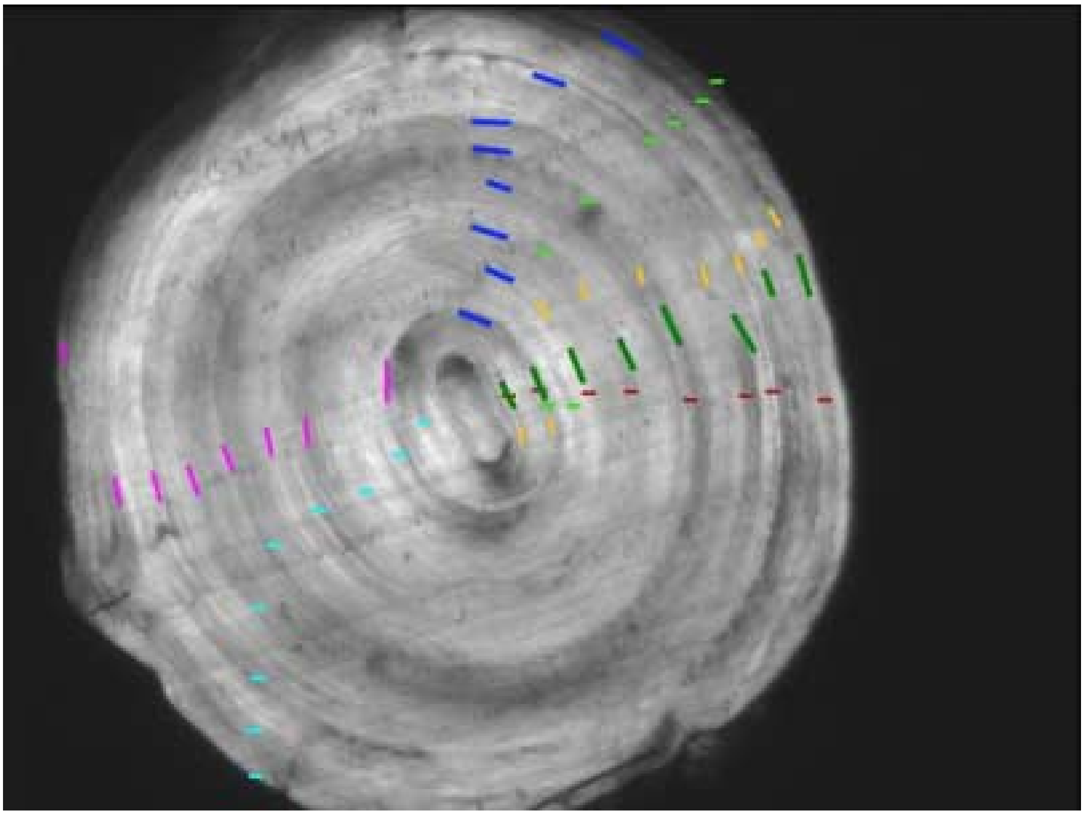


Image 10. *L. piscatorius.* Agreed Age 9. File name: 11-00-1a-1. Collection North. Good agreement by R5 &R6, R3= N-1.

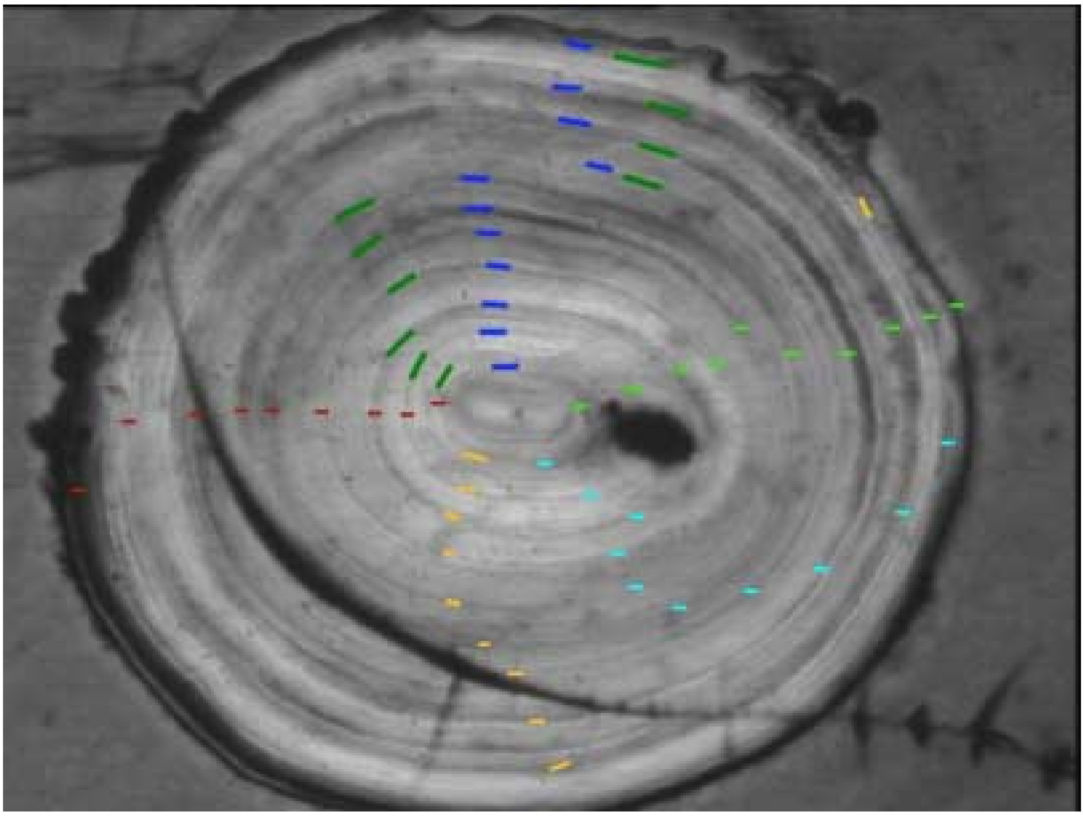


Image 11. *L. piscatorius.* Agreed Age 10. File name: 84-99-2A-3. Collection North. Total Agreement by experienced readers.

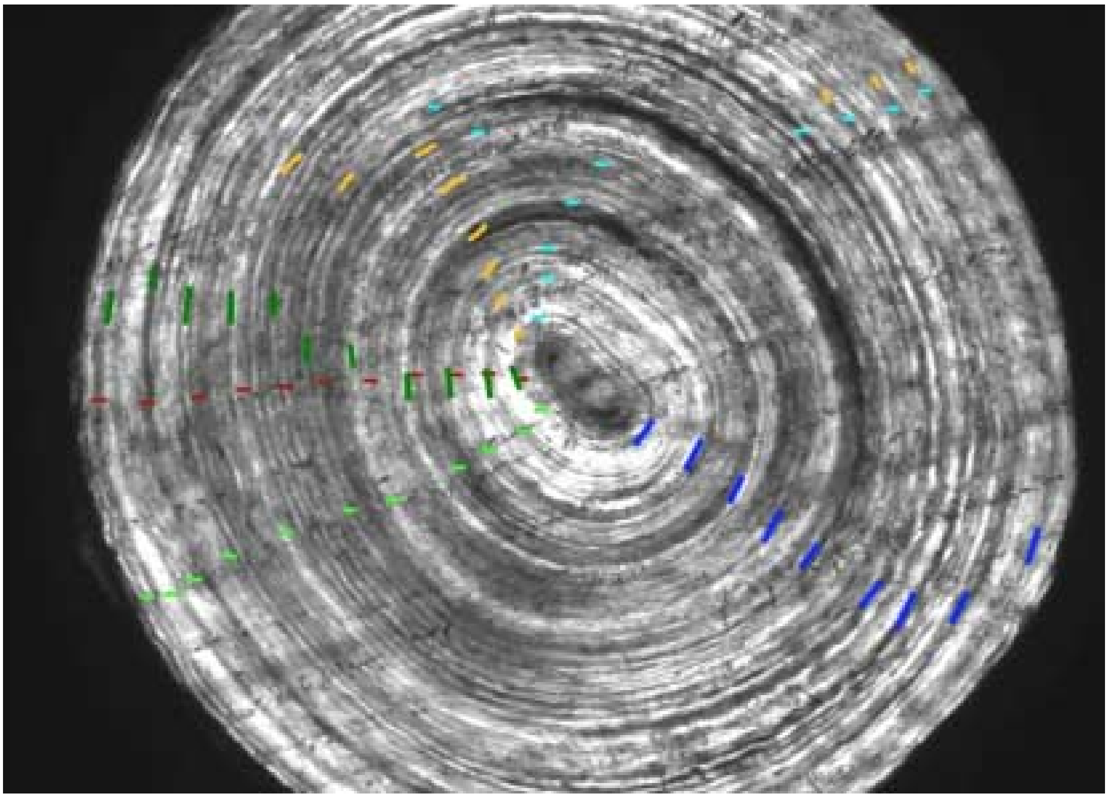


Image 12. *L. piscatorius.* Agreed Age 11. File name: 15-4b-5 1999. Collection South. R5&3=11, R6=12, however chose very similar rings.

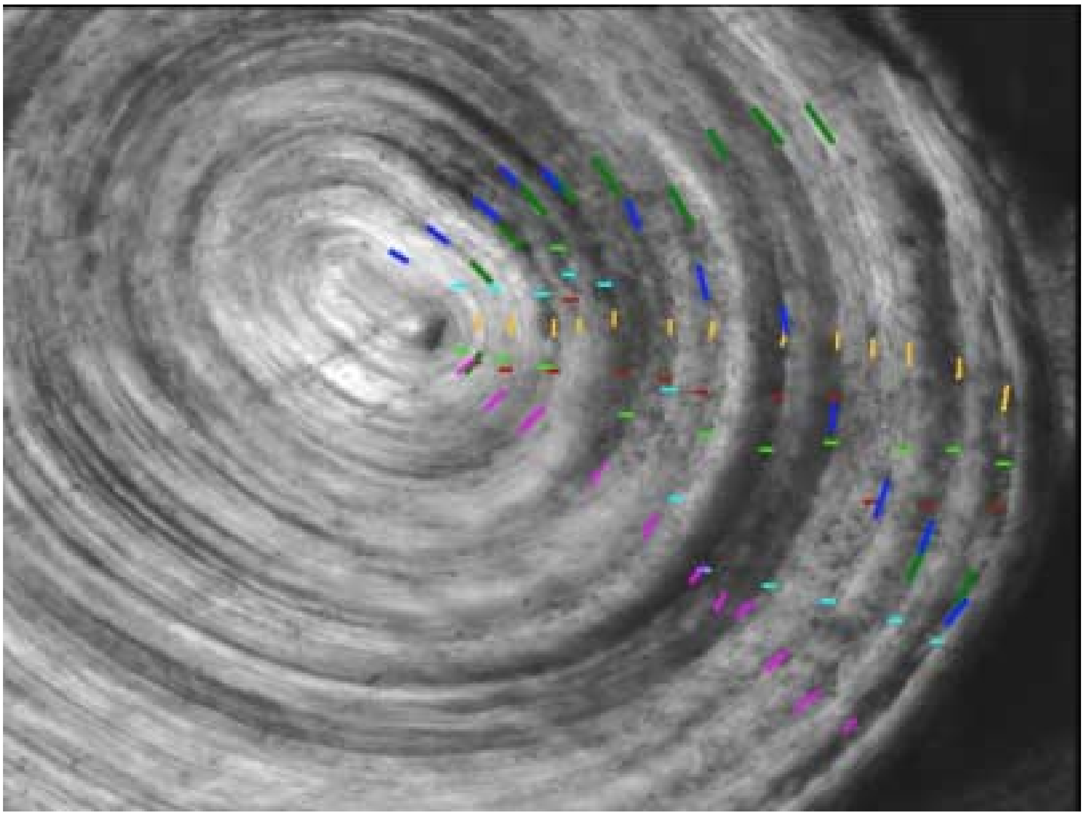


Image 13. *L. piscatorius.* Agreed Age 12. File name: 114-99-2a-1. Collection North. Disagreement on one ring.

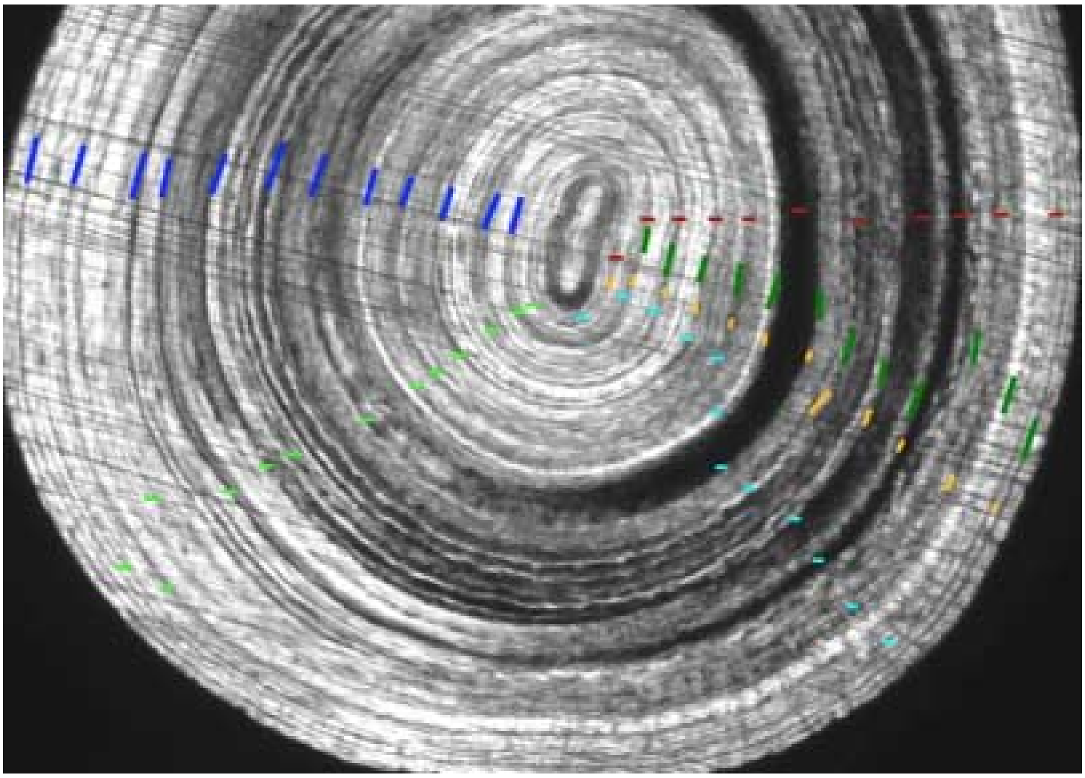


Image 14. *L. piscatorius.* Agreed Age 12. File name: 8-4b-3-2000. Collection South. R3 differs on ring 1 and 11.

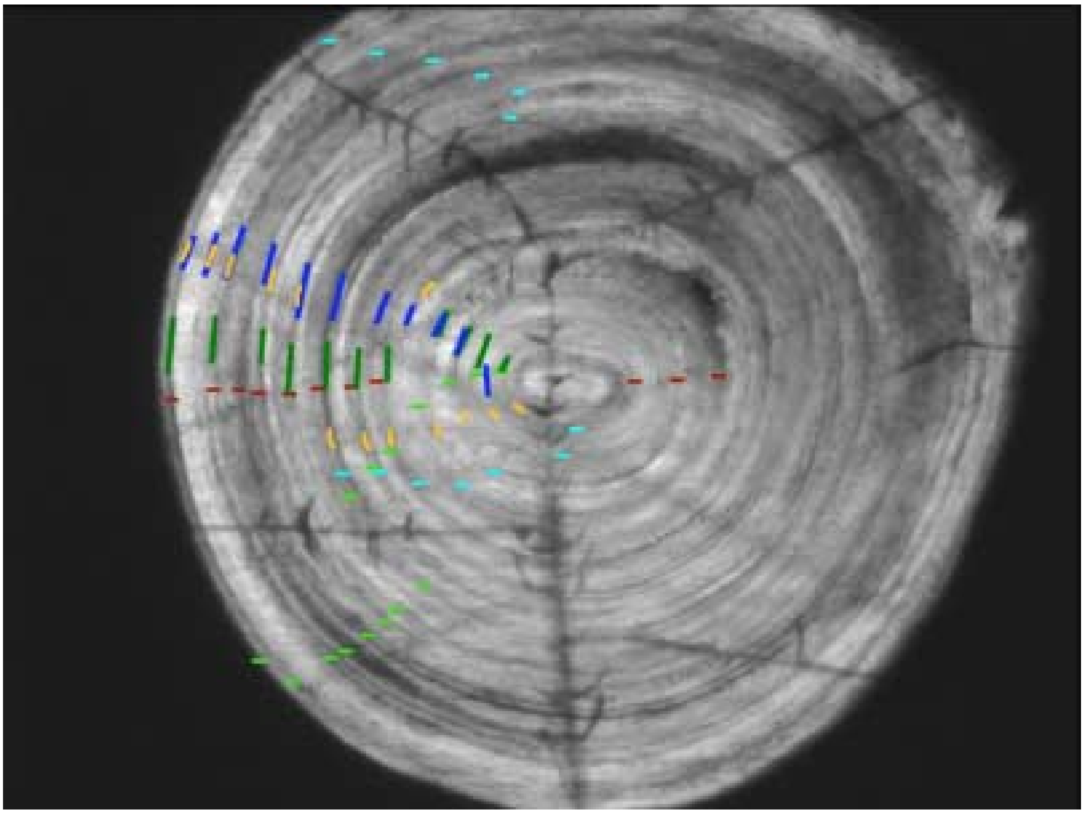


Image 15. *L. piscatorius.* Agreed Age 13. File name: 238-97-1a-4. Collection North. R1,5 &6 =13, similar rings, R3=11.

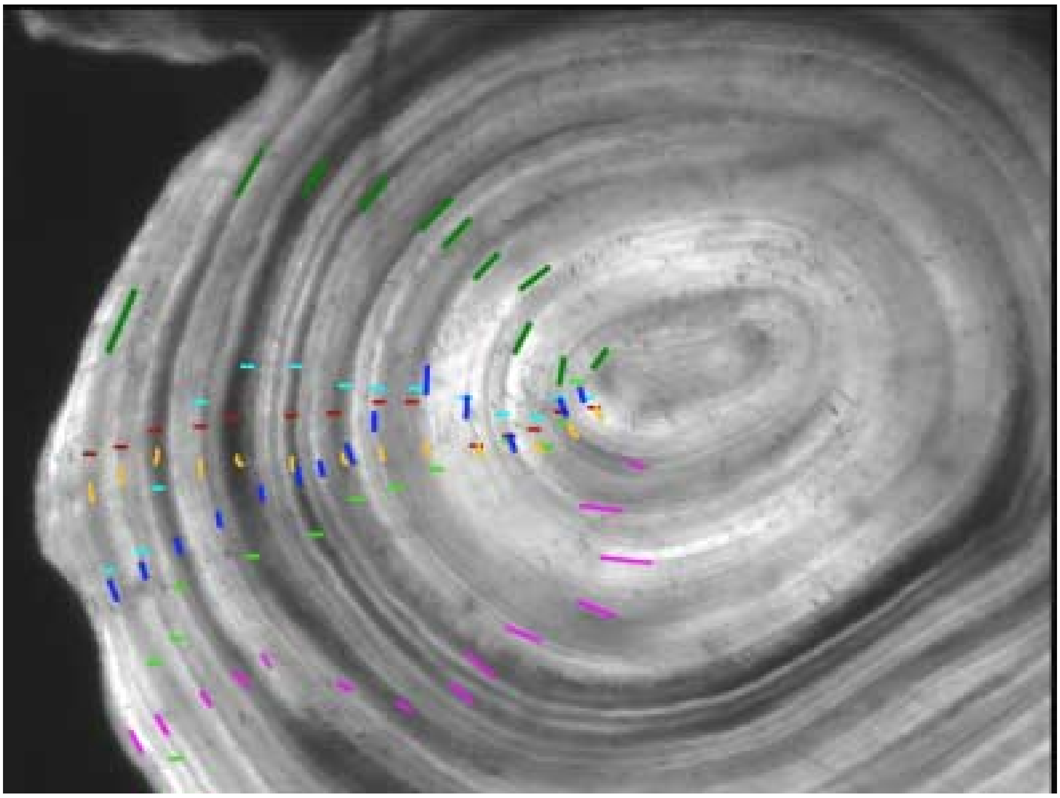


Image 16. *L. piscatorius.* Agreed Age 14. File name: 100-99-3b-3. Collection North. R5&6 in total agreement, R3=11.

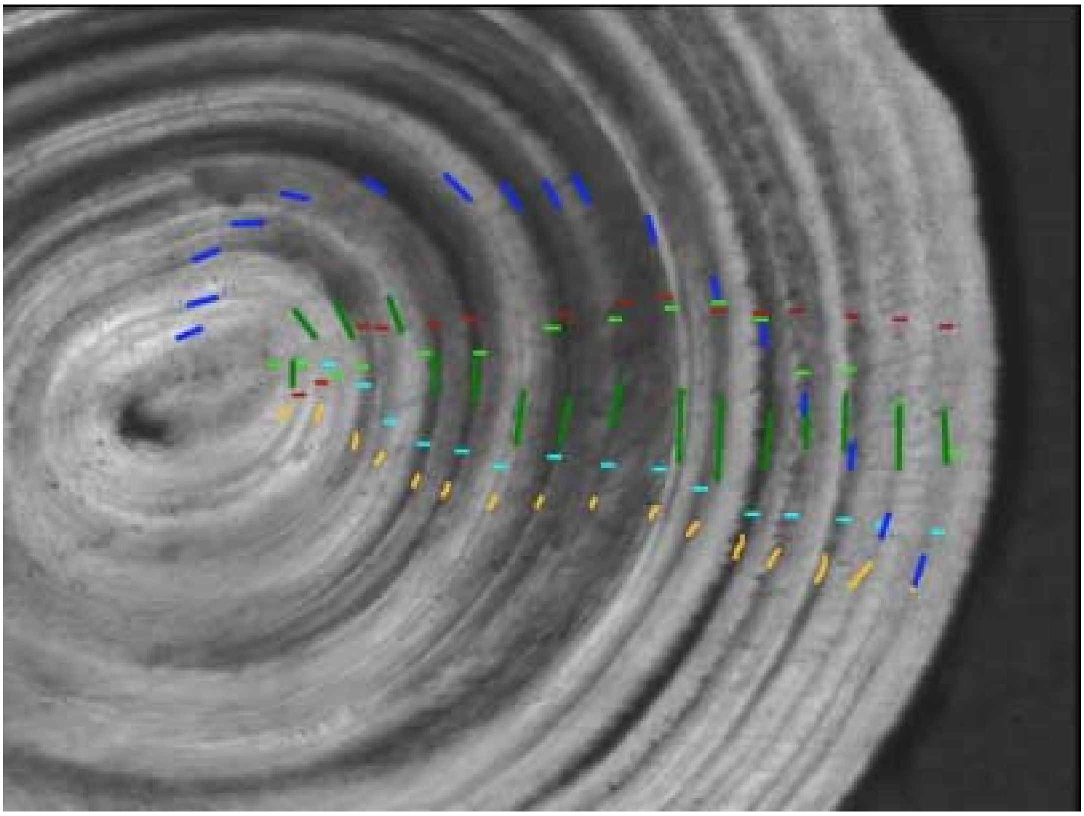


Image 17. *L. piscatorius.* Agreed Age 16. File name: 77-99-5b-8. Collection North. Total Agreement by experienced readers.

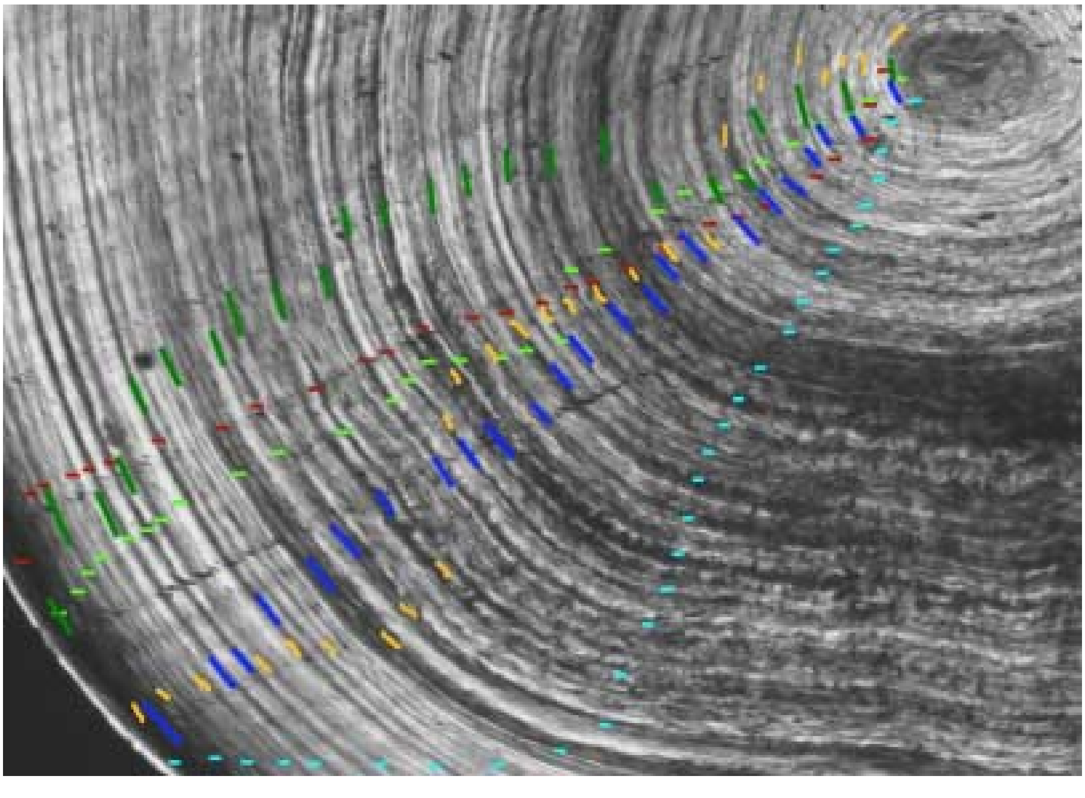


Image 18. *L. piscatorius.* Agreed Age 24. File name: 9-1b-3 1999. Collection South. Age 24-29, image looks good, different rings chosen.

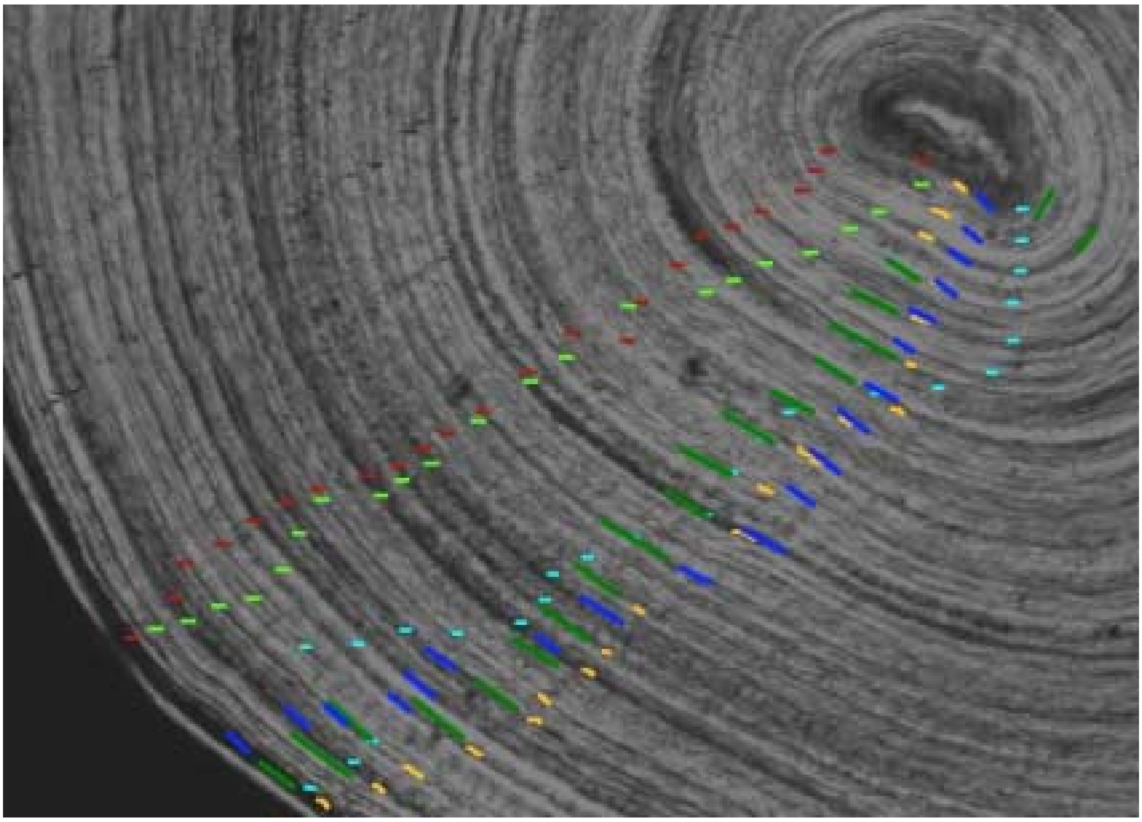


Image 19. *L. piscatorius.* Agreed Age 25. File name: 9-1b-2 1999. Collection South. Total Agreement by experienced readers.

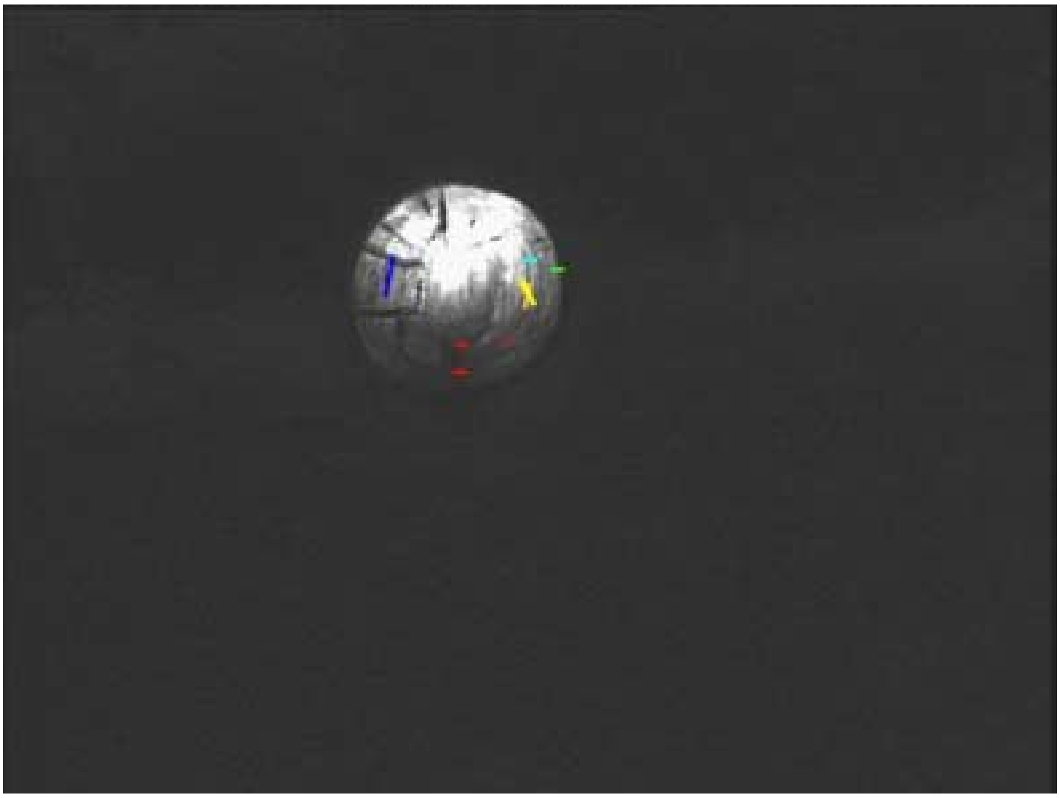


Image 20. *L. budegassa.* Agreed Age 1. File name: A-2. Collection South. Total Agreement by experienced readers.

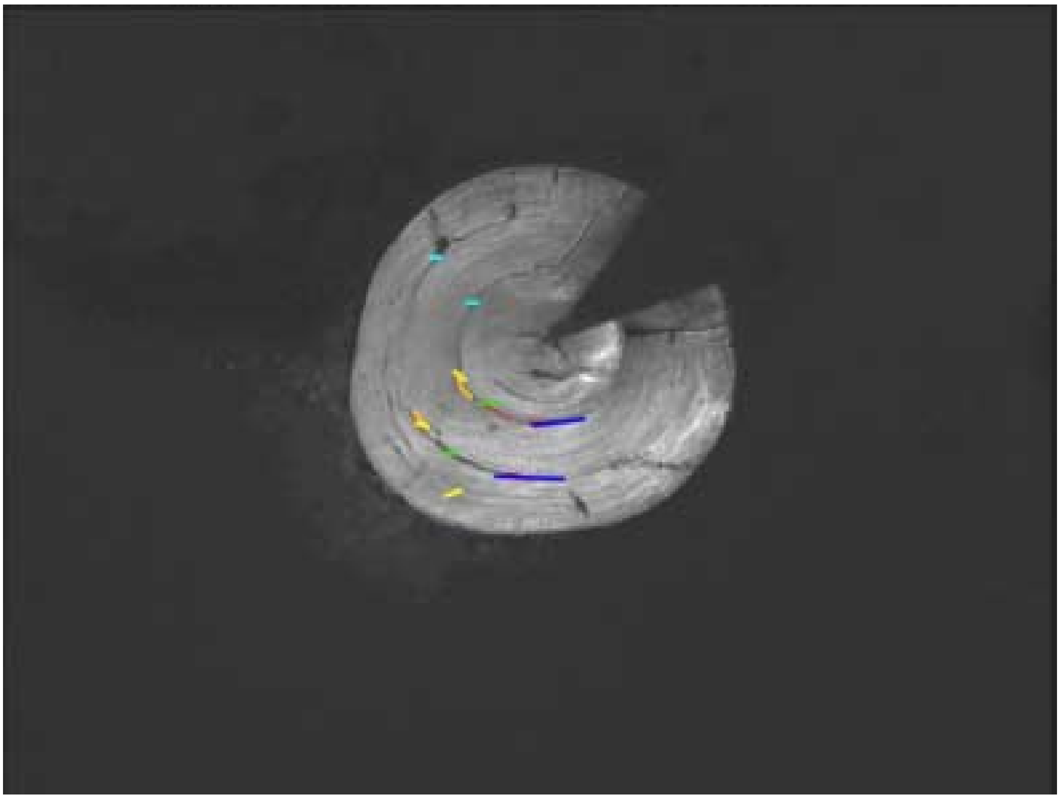


Image 21. *L. budegassa.* Agreed Age 2. File name: A-7. Collection South. Total Agreement by experienced readers.

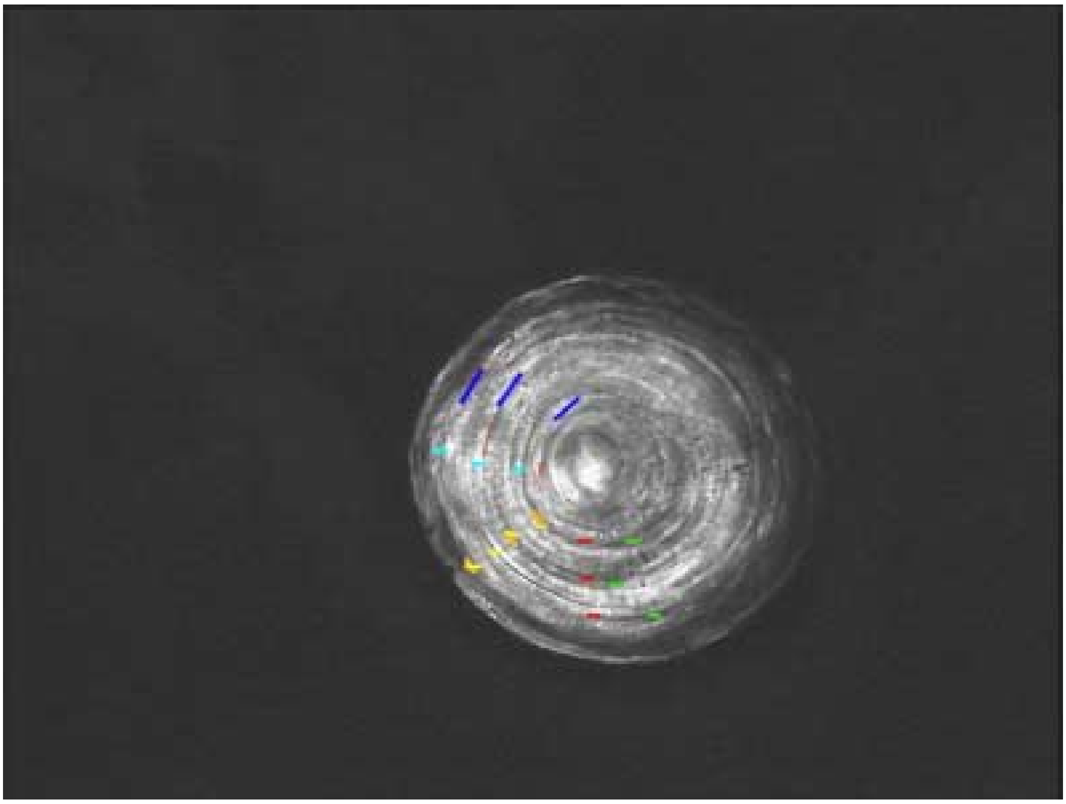


Image 22. *L. budegassa.* Agreed Age 3. File name: B-3. Collection South. Total Agreement by experienced readers.

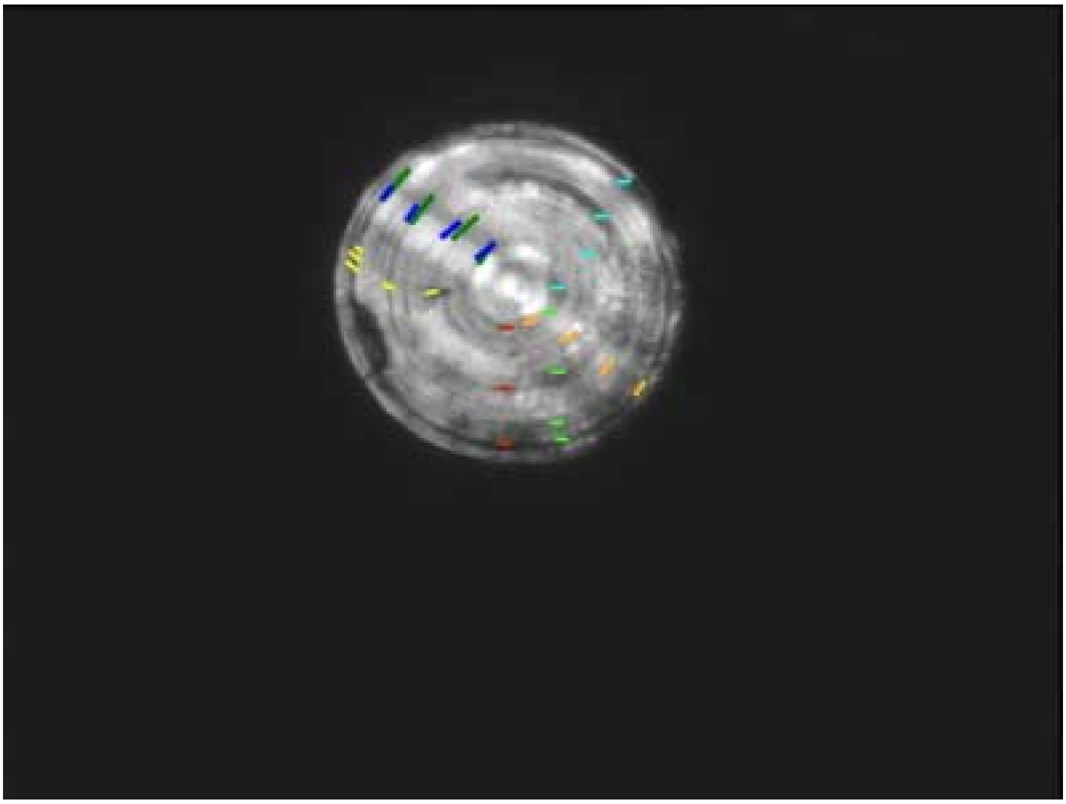


Image 23. *L. budegassa.* Agreed Age 4. File name: 19-1999-5b-8. Collection North. Total Agreement by experienced readers.

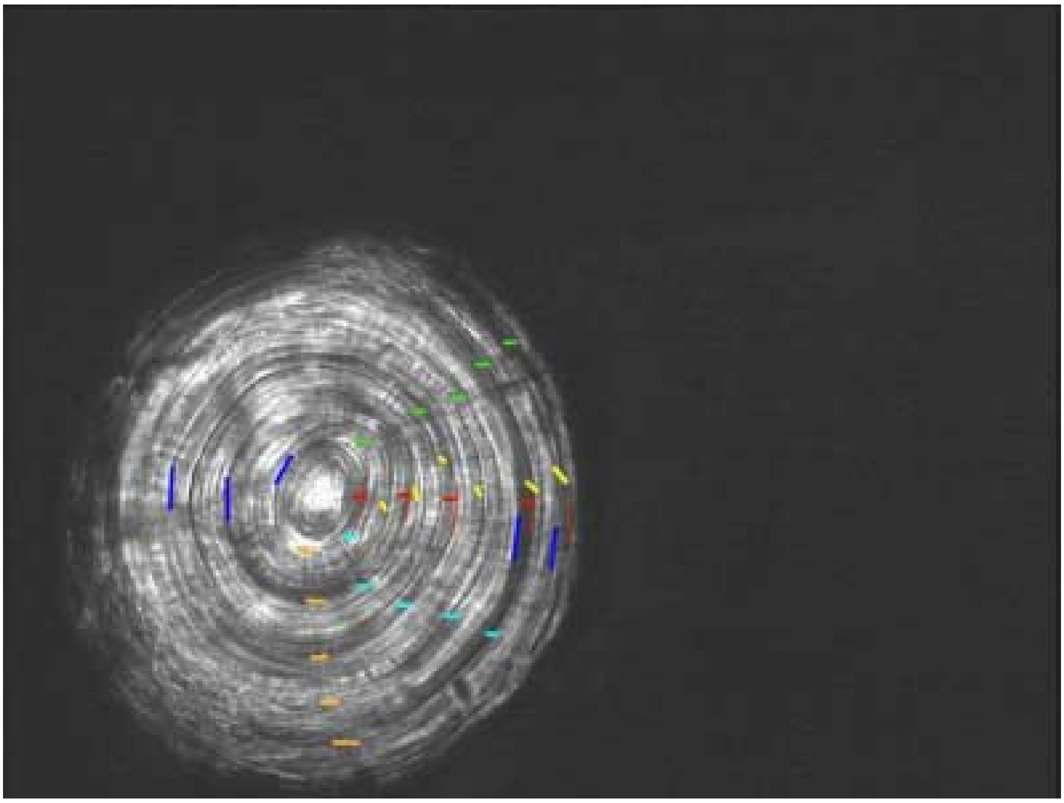


Image 24. *L. budegassa.* Agreed Age 5. File name: C-7. Collection South. Total Agreement by experienced readers.

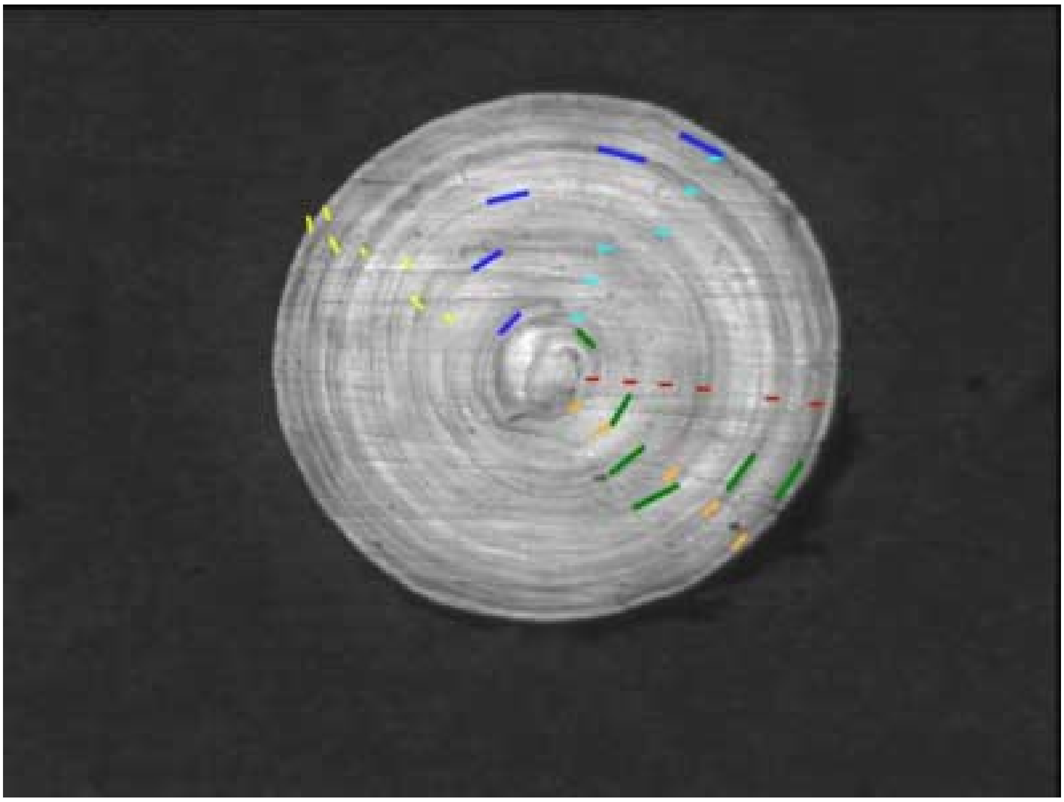


Image 25. *L. budegassa.* Agreed Age 6. File name: 3-1999-3b-2. Collection North. Total Agreement by experienced readers.

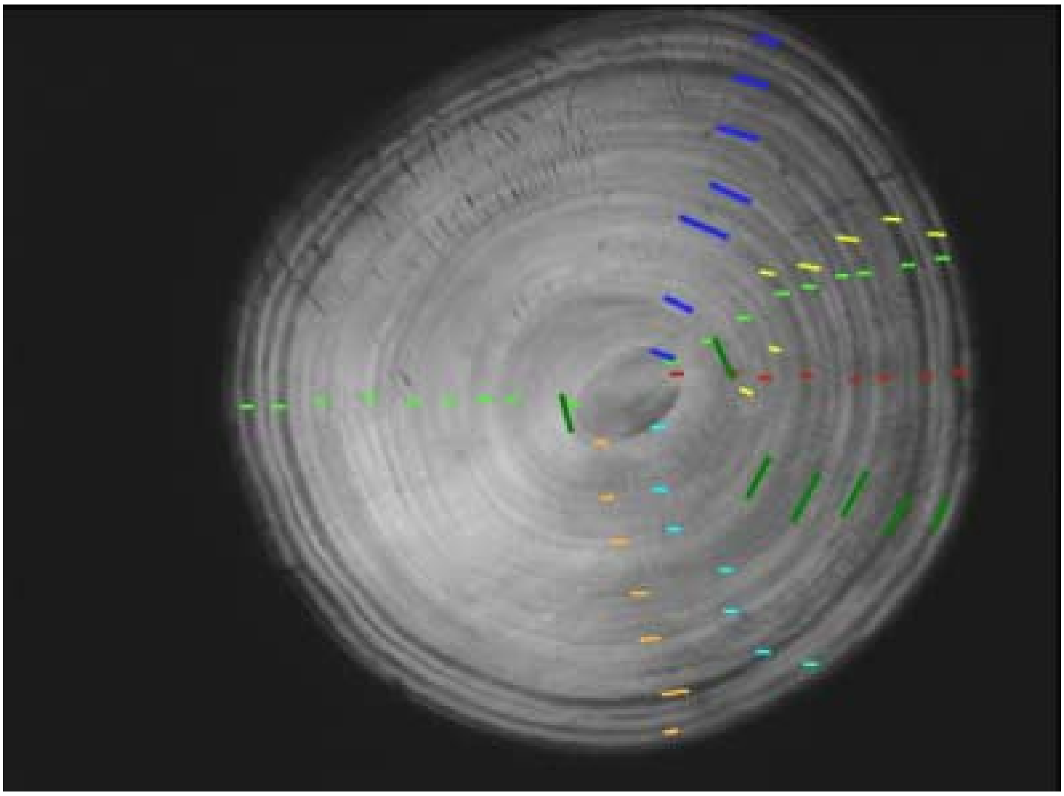


Image 26. *L. budegassa.* Agreed Age 7. File name: 39-1998-3a-3. Collection North. Total Agreement by experienced readers.

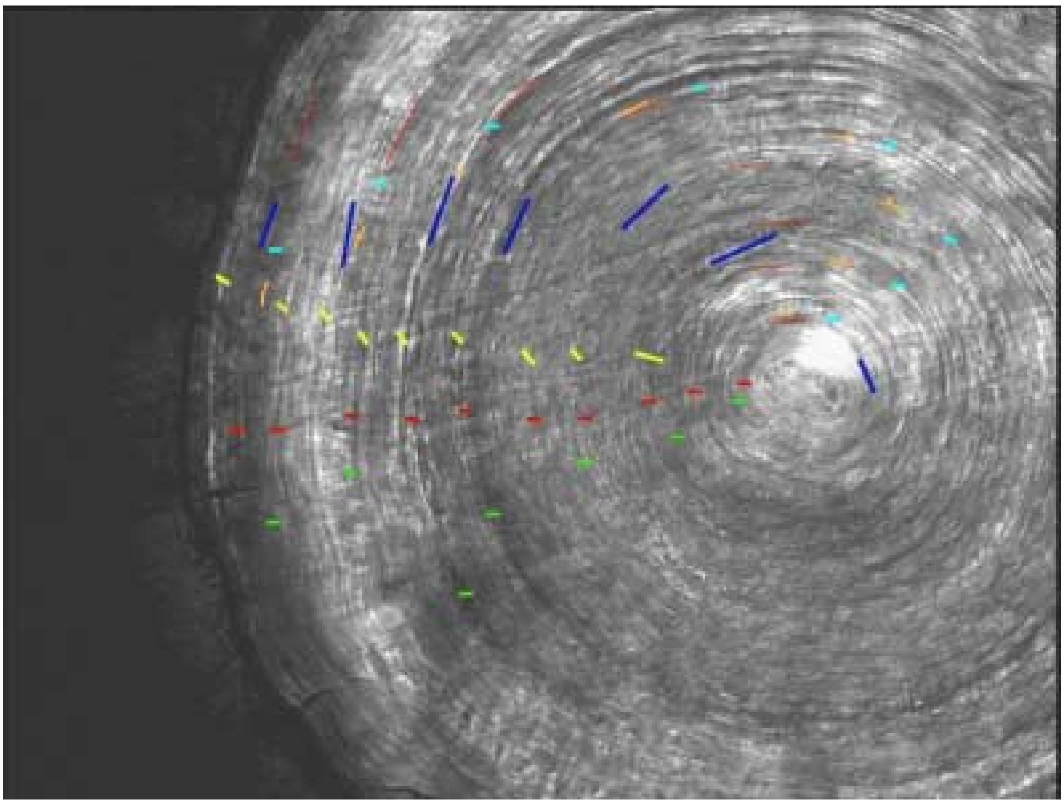


Image 27. *L. budegassa.* Agreed Age 8. File name: G-7. Collection South. R3 missed 3rd ring.

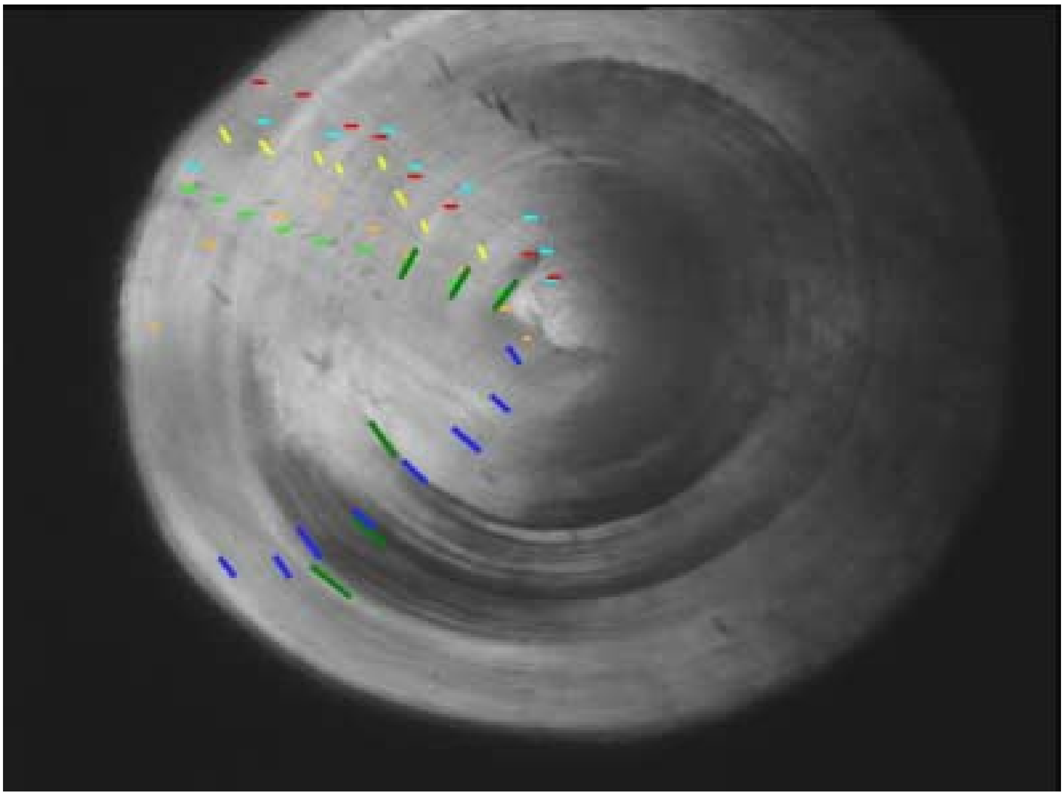


Image 28. *L. budegassa.* Agreed Age 9. File name: 6-1999-3b-4. Collection North. R5&R6=9, similar rings, R3=6.

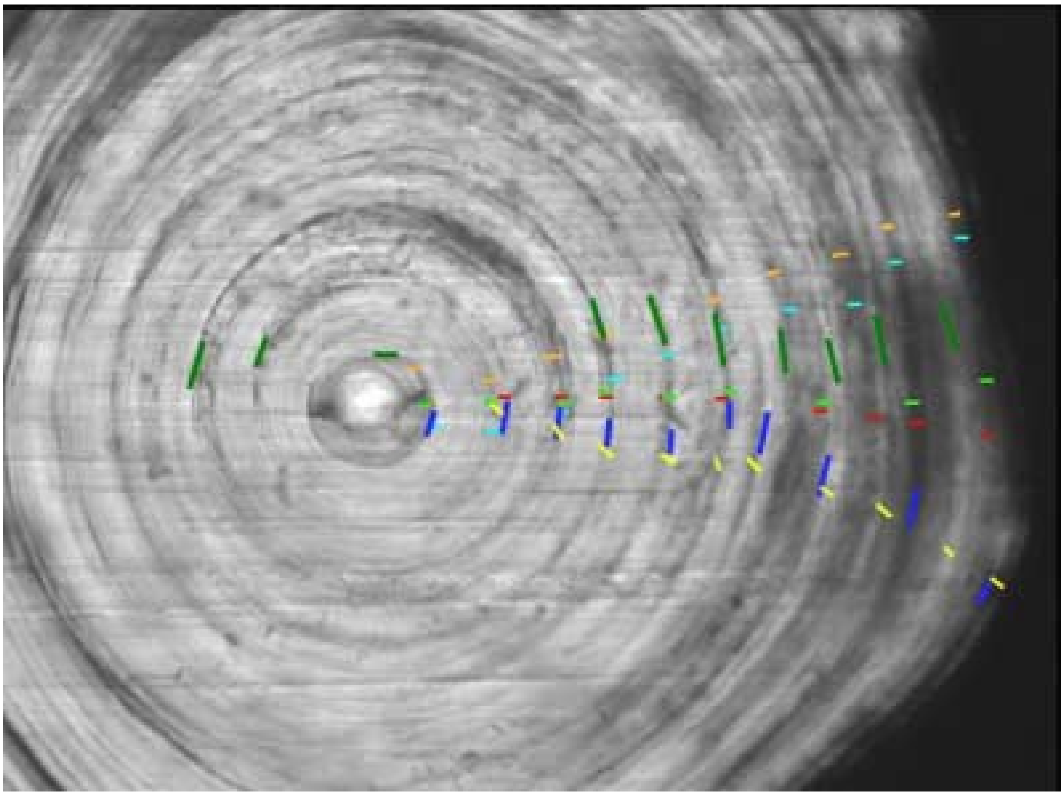


Image 29. *L. budegassa.* Agreed Age 10. File name: 237-1997-5a-5. Collection North. Total Agreement by experienced readers.

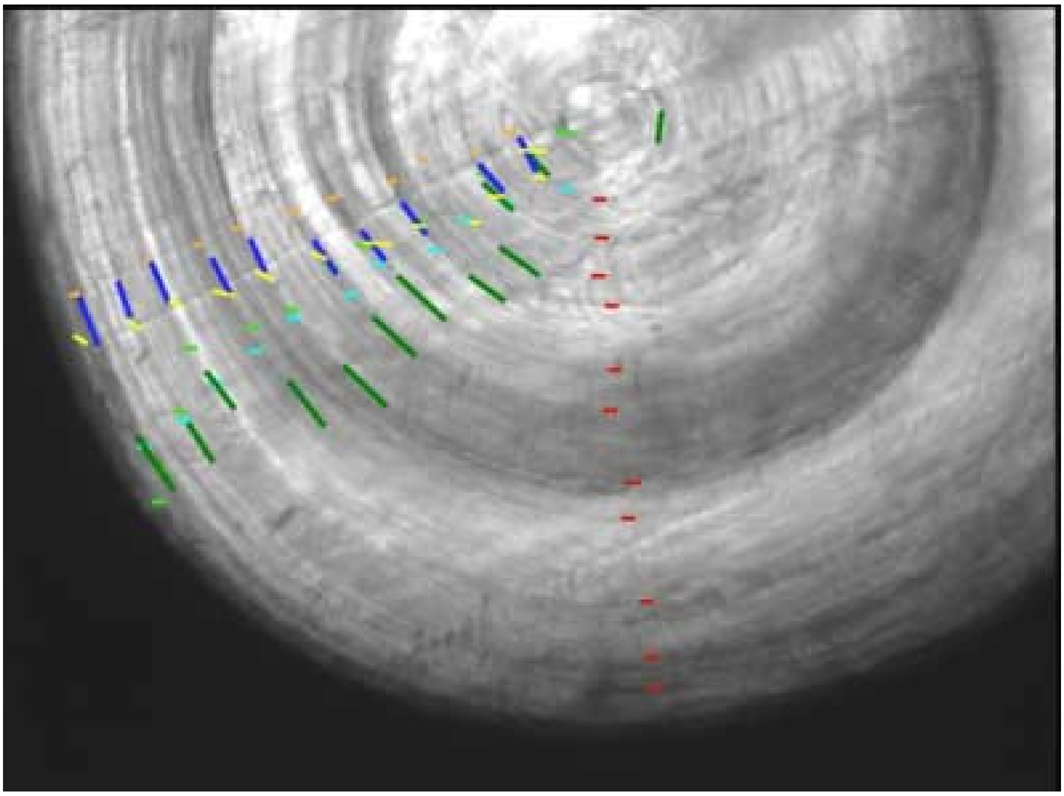


Image 30. *L. budegassa.* Agreed Age 11. File name: 92-1999-5b-10. Collection North. Agreement on all rings except 1st by R3.

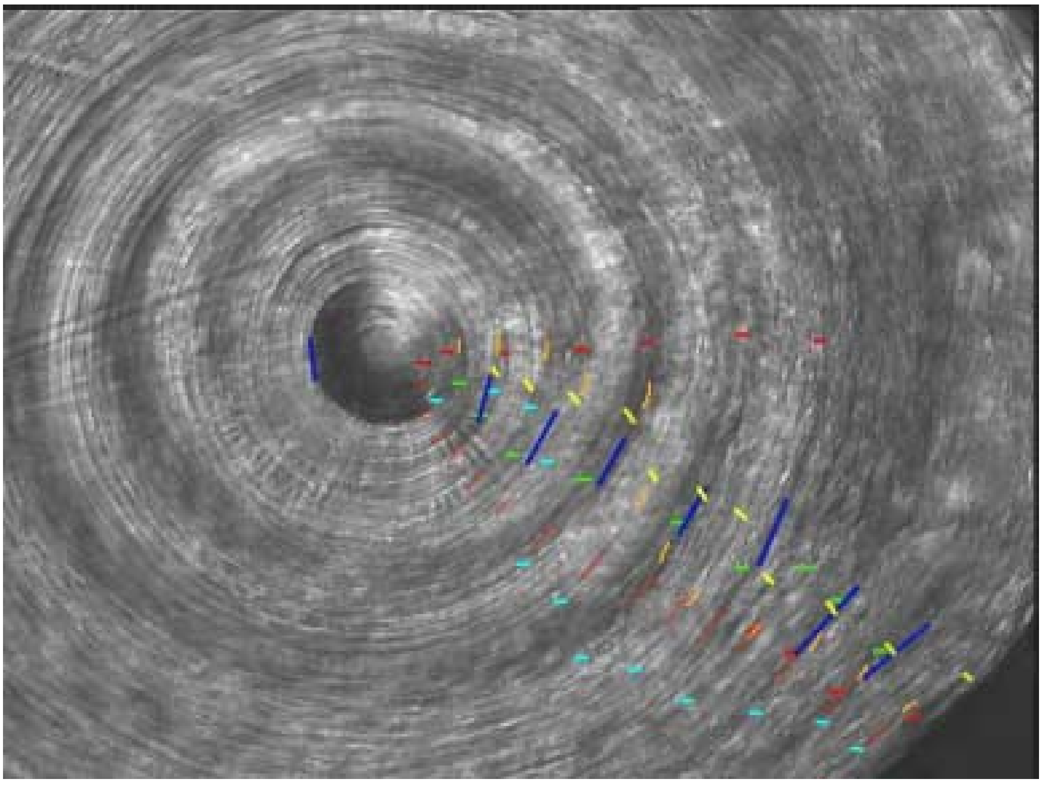


Image 31. *L. budegassa.* Agreed Age 12. File name: K-6 . Collection South. R5&6 = 12, R3=8, Good illustration of applying different ageing criteria.

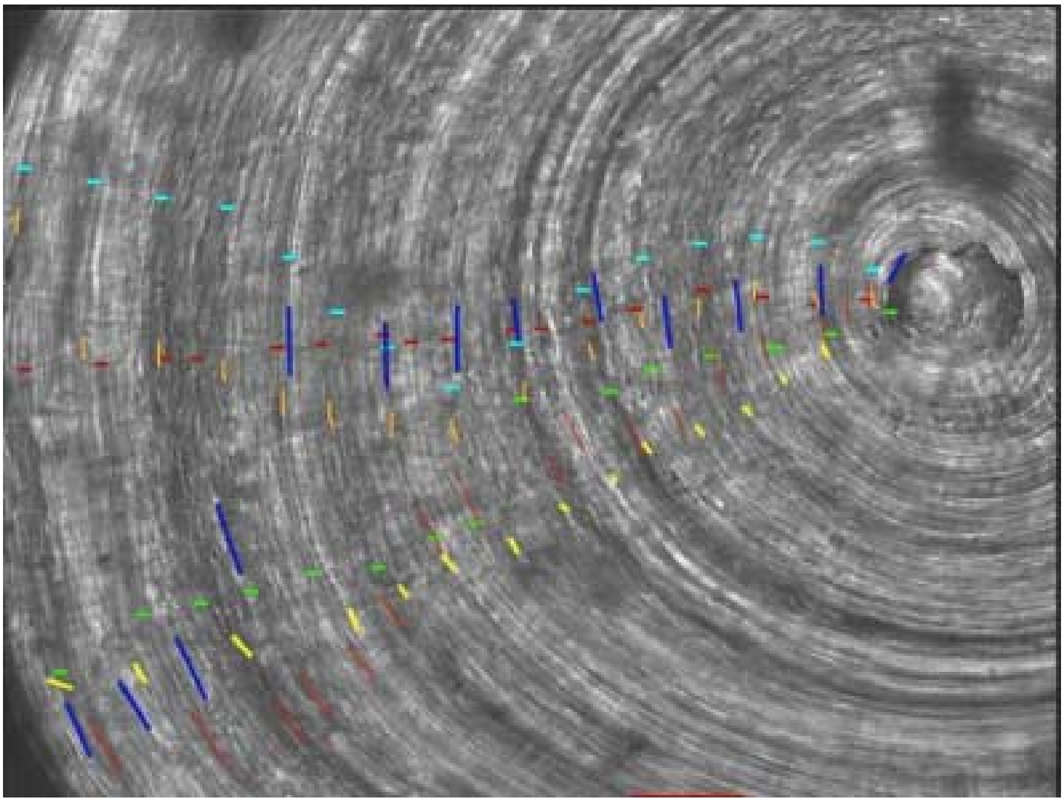


Image 32. *L. budegassa.* Agreed Age 15. File name: N-1. Collection South. R5&6=15, R3=13.

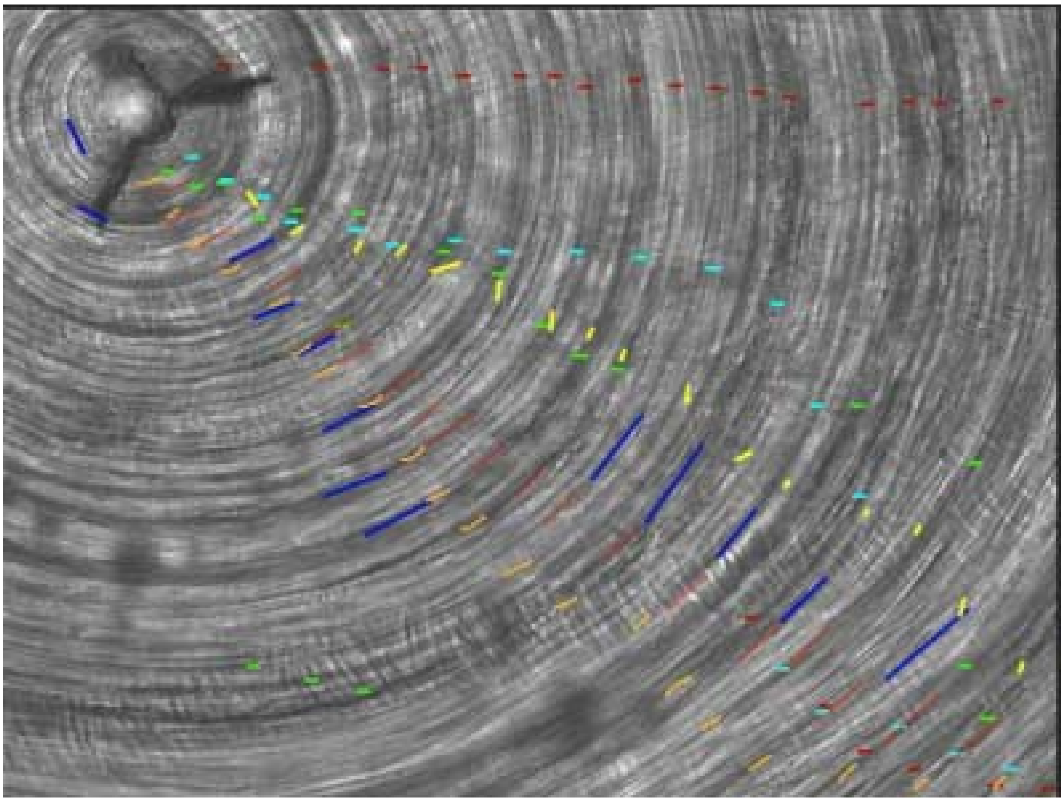


Image 33. *L. budegassa.* Agreed Age 19. File name: N-5. Collection South. R3=17, R6=19 and R5=20, R5&6 chose similar rings.

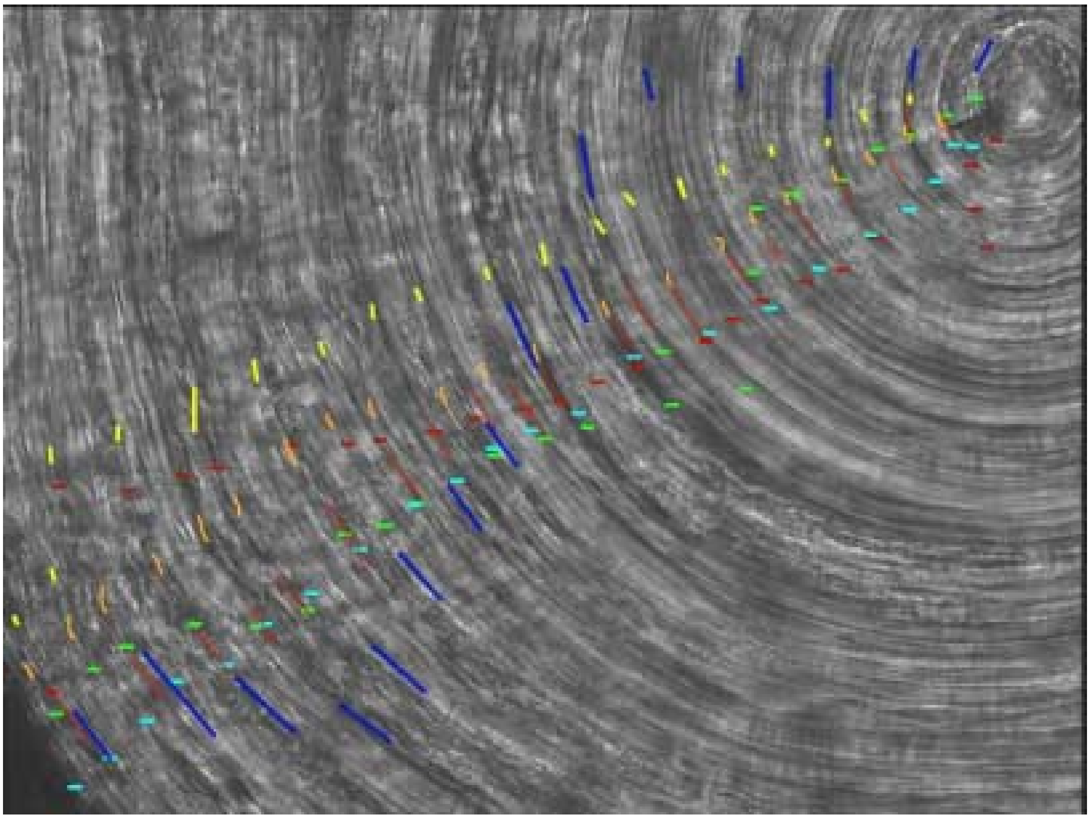


Image 34. *L. budegassa.* Agreed Age 21. File name: O-3. Collection South. R3=16, R5&R6=21, but chose some different rings.

Annex 4

Modal ages in white anglerfish (*Lophius piscatorius*) from demersal trawl surveys in the Northern Spanish continental shelf.

Working Document. 4th International Ageing Workshop on European Anglerfish.
IPIMAR, Lisbon, 14-18 January 2001.

MODAL AGES IN WHITE ANGLERFISH (*Lophius piscatorius*) FROM DEMERSAL TRAWL SURVEYS IN THE NORTHERN SPANISH CONTINENTAL SHELF.

J. Landa

Instituto Español de Oceanografía. Apdo 240. 39080 Santander. Spain.
e-mail: jorge.landa@st.ieo.es.

ABSTRACT

The length frequency distributions of specimens caught in the research trawl surveys carried out in September-October from 1994 to 2001 and in April 1997, in the northern Spanish continental shelf (ICES Div. VIIIc and IXa) were studied and modal lengths of the first year classes were identified. A length group about 18 cm, possibly corresponding to age class 1, was clearly identifiable most years. Another less clear modal length group was observed around 10 cm, likely associated with age class 0. Other length groups were identified around 29 cm (supposed age 2) and around 35 cm (possibly age 3). An adjustment along the months was observed between these modal lengths and the mean length at first age groups estimated in the last International Ageing Exchange Programme on European Anglerfish. The modal length 26 cm observed in the survey in April 1997 corroborated the growth between ages 1 and 2, since it is an intermediate value between these modal ages from September surveys. Likewise, the 26 cm modal length coincided with the value of the mean length for age 2 estimated for the same season of the year in the Exchange.

Keywords: anglerfish, growth, *Lophius piscatorius*, monkfish, Northern Spanish continental shelf

INTRODUCTION

The fishing for white anglerfish (*L. piscatorius*) represents a significant part of the yields obtained by the different fleets (France, Spain, Portugal, United Kingdom and Ireland) exploiting the demersal fisheries in western and southern European waters. White monkfish is an important component of the by-catches of both bottom trawl and artisanal fisheries in ICES Divisions VIIIc and IXa. The exploitation assessment of the northern stock (ICES Divisions VIIIa,b and Subarea VII) and southern stock (ICES Divisions VIIIc and IXa) of this species is made annually within the ICES Working Group on the Assessment of Southern Shelf Demersal Stocks.

Growth studies of anglerfish have been made by different methods: the study of modal length groups and the analysis of otoliths, *illicium* sections or vertebrae. The only studies on growth of the southern stock of this species by means of analysis of length distributions are those of Azevedo (1992), Duarte *et al.* (1997) and Landa and Pereda (1997). Azevedo (1992) estimated, for the first time, growth parameters for the southern stocks of *L. piscatorius* for both sexes combined using Shepherd's Robust Length Composition Analysis, SRLCA (Shepherd, 1987). Growth parameters for this species were estimated by the 1993 Working Group based on the mean lengths at age observed in direct reading of *illicia* and identification of modes in the length frequency distribution found in surveys. From these parameters an attempt was made to estimate age compositions of the catches by slicing length compositions (Anon., 1994). Duarte *et al.* (1997) and Landa and Pereda (1997) studied growth based on mean lengths at age derived from direct reading of transversal sections of *illicia* and identification of modes in the length composition from research surveys.

However, anglerfish is a difficult species to age, and more growth studies, such as the analysis of length frequency distributions, are necessary to obtain additional growth results for the validation of ageing.

MATERIAL AND METHODS

The length frequency distributions of specimens caught in the research trawl surveys conducted by the IEO (Instituto Español de Oceanografia) were studied. These surveys were carried out in September-October from 1994 to 2001 and one from April 1997, in the Cantabrian Sea and the Galician continental shelf (ICES Div. VIIIc and IXa).

RESULTS

Figure 1 shows the length frequency distributions of the specimens caught in the research surveys during September and October from 1994 to 2001, and those caught in a research survey in April 1997.

The modal ages considered as best defined in the length distributions on Fig. 1 were selected (Table 1). Table 2 shows the modal lengths estimated for these selected ages and the mean length at first ages estimated in the International Ageing Exchange Programme on European Anglerfish (Landa, 2002).

Table 3 presents the modal lengths at supposed age class 1, estimated from the length distributions of the IEO bottom trawl survey in September-October 1996, and at the same age class 6 months later, during the survey in April 1997.

DISCUSSION

The availability of samples collected in the same season of the year permitted to compare the modal length classes in the frequency distributions. Thus, in the length frequency distributions, some modal age classes of the first years were identified throughout the historical series of length distributions of the demersal trawl surveys carried out in September and October. The best defined was that of 17-20 cm modal length in the years 1994, 1995, 1996, 2000 and 2001 (Fig. 1). The mean length of this possible modal age during all these years was about 18 cm. Another less clear modal age class could be observed between 9 and 11 cm during the years 1994 and 1995. In spite of the fact that anglerfish was not fully recruited to the gear for these lengths, this could be considered as the immediately previous modal age to that of 18 cm. In 1996 a modal class could be identified around 29 cm, and it could be the following modal class to that of 18 cm. In 1996 another modal class was also observed around 35 cm.

Comparing the lengths of these modal classes with the mean length at first ages estimated in the International Ageing Exchange Programme on European Anglerfish (Landa, 2002), an adjustment among the length values along the months was observed (Table 2). Thus, age class 1 could be that of 18 cm in September and October. These values are similar to those about 17 cm obtained by Duarte *et al.* (1997), for the same age during the period from 1982 to 1993. Age class 2, that of 29 cm, is also very similar to that of 30 cm (Duarte *et al.,* 1997) for the same age during the former period. So is age class 3, that of 35 cm. Length group close to 10 cm is probably associated with age class 0, but as it is not fully recruited to the gear, its modal length of 10 cm could have a lower value. In *L. budegassa*, a modal class around 6 cm (age class 0) was observed in these same surveys. The spawning seasons of both species do not seem to be so different. Supposing a not so different growth of both species during the first months, we could think that the modal length 18 cm in *L. piscatorius* would be very hardly age class 0, and that the modal length 10 cm (or lower) could be age 0.

The research surveys carried out annually by the IEO in September and October include, among their aims, the estimation of the strength of recruitment of demersal species, mainly hake. In 1994 there was a significant recruitment of specimens smaller than 12 cm, considered as age 0, in the fishing area studied. In the same year, the strength of hake recruitment (age class 0) was outstanding, benefited by the hydrographic conditions in that year (Sanchez and Gil, 1997). The high capture of anglerfish specimens of age class 1 during the following year is also noteworthy, presumably from the good year class of 1994. The presence of anglerfish juveniles of age 0 was comparatively low in 1995 and practically null in 1996 and following years.

If we observe the abundant modal length 17 cm in the length distribution of September-October of 1996 (Fig. 1), and the modal length 26 cm in the survey in April of the following year 1997, we could hope that they belong to the same year class of 1996. The modal length 26 cm observed in the survey in April 1997 corroborated the growth between the ages 1 and 2, when presenting an intermediate value between these modal lengths from September surveys (18 cm and 29 cm, respectively) (Table 3). Likewise this value of 26 cm of modal class coincided with the value of mean length for age 2 estimated in the International Ageing Exchange Programme on European Anglerfish (Landa, 2002).

ACKNOWLEDGEMENTS

Thanks are extended to Francisco Sánchez, Orestes Cendrero and the people which participated in the IEO fishing surveys “DEMERSALES” from 1994 to 2001 and in April 1997.

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Table 1. Selection of the years of survey where the length distributions showed some definite modal ages.

age			
0	1	2	3
1994	1994	1996	1996
1995	1995		
	1996		
	2000		
	2001		

Table 2. Modal length (cm) at supposed age class 0, 1, 2 and 3 (in blond). Mean length at age from the International Ageing Exchange Programme on European Anglerfish (in italics).

	age			
month	0	1	2	3
1				
2				
3				
4				*30*
5			*26*	
6				
7				
8				
9				
10	**<10**	**18**	**29**	**35**
11		*19*		
12				

Table 3. Modal length (cm) at supposed age class 1, from the length distributions of the IEO bottom trawl surveys in Sept-Oct. 1996 and April 1997.

	year	
month	1996	1997
1		
2		
3		
4		**26**
5		
6		
7		
8		
9		
10	**17**	
11		
12		

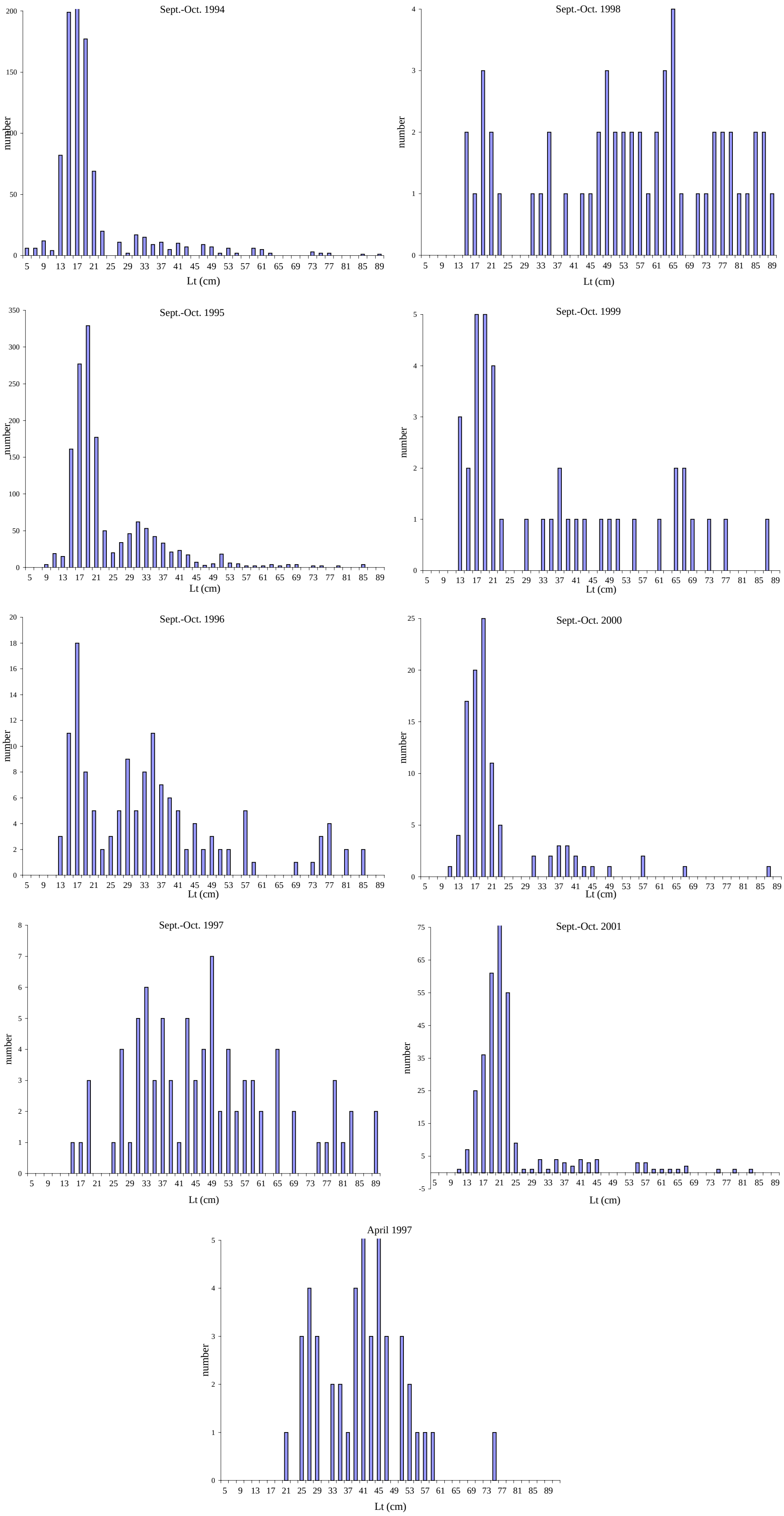

Figure 1. Length frequency distributions of specimens caught in the research surveys during September and October from 1994 to 2001, and those caught in a research survey in April 1997.

Annex 5

Validation trials of growth of white anglerfish (*Lophius piscatorius*) in the North-eastern Atlantic based on mark-recapture experiments.

Working Document. 4th International Ageing Workshop on European Anglerfish.
IPIMAR, Lisbon, 14-18 January 2001.

Validation trials of growth of white anglerfish (*Lophius piscatorius*) in the North-eastern Atlantic based on mark-recapture experiments.

J. Landa

Instituto Español de Oceanografía. Apdo 240. 39080 Santander. Spain.
e-mail: jorge.landa@st.ieo.es.

ABSTRACT

Of 481 white anglerfish tagged by IEO in South-western Ireland and North Iberian waters since 1995, 24 were recovered. Most were recovered within a month after tagging and no significant increase in their lengths was observed. The growth of two specimens recaptured 9 and 11 months after tagging was very close to that estimated by *illicia* reading in the International Ageing Exchange Programme on European Anglerfish and also to that estimated from the routinely annual age length keys. The recaptures of internally marked specimens were obtained after intervals between a few days and 2 months. In the *illicia* sections of these latter specimens, increasing fixing of this substance at the outer edge is observed.

Key words: Anglerfish, growth, *illicium*, *Lophius piscatorius*, mark, North-eastern Atlantic, recapture, recovery, tagging.

INTRODUCTION

The fishing for white anglerfish (*L. piscatorius*) (Fig. 1) represents a significant part of yields obtaining by the different fleets (France, Spain, Portugal, United Kingdom and Ireland) exploiting the bottom fisheries in western and southern European waters (ICES, 2001).

Growth studies of anglerfish has been made using different ways: following of modal length groups and analysed otoliths, *illicium* sections or vertebrae. However, anglerfish is a difficult species to ageing, and growth studies have to be continued and its validation is needed. The first anglerfish tagging experiences in the Northeast Atlantic, began in 1995 (Dupouy, pers. comm.; Pereda and Landa, 1997), were aimed as the basis to growth validation. Since then, a tagging program was undertaken by several European research institutions: IFREMER (Biseau and al., 1999), IEO (Pereda and Landa, 1997; Landa et al., 2001), AZTI (Lucio, pers. com.) and IPIMAR (Duarte, pers. com.).

This papers presents the first steps on growth validation based on the tagged and recovered specimens by IEO.

MATERIAL AND METHODS

The tagging experiments were carried out on trawl and gillnet commercial fleets, and in research surveys. Spaghetti T-bar tags were used for external tagging, and in addition, a dose of oxy-tetracycline hydrochloride (50 mg/kg of fish) was injected in most cases to form a deposit in the hard parts of the fish (bones, otoliths, fins) (Rijnsdorp and Visser, 1987).

RESULTS

IEO have tagged 481 white anglerfish in North Iberian waters (ICES Division VIIIc) and South-western Ireland (Division VIIjk) since 1995 (Tab. 1), with lengths ranging from 20 to 89 cm and a mean of 71 cm. Locations of white anglerfish tagging areas are shown in Fig. 2.

Twenty-four specimens were recaptured (ranging from 23 and 89 cm in length with a mean length of 75 cm). Juveniles and adult fish were tagged and recovered in similar numbers.

Growth in length

Twenty-one anglerfish were recaptured in the 3 months following the date of tagging, and their increase in length was not significant. The two white anglerfish recaptured 9 and 11 months after tagging were studied and it was observed increments in total length of 7 and 5 cm respectively in the cited periods (Tab. 3). This corresponds to an annual growth of 7.8 and 6.5 cm respectively assuming a constant growth along the whole year, necessary to be able to extrapolate the increments in months to the annual period.

Marks in hard parts

Knowledge of the process of fixing of tetracycline is basic to growth validation. Once the tetracycline has been injected, it is not immediately deposited in the bone tissue and takes around a month to disappear from the blood and deposit in the hard parts. Fish recaptured after a longer period of time had not been marked internally with tetracycline. The recaptures of internally marked specimens were obtained after intervals between a few days and 2 months. In the *illicia* sections of these latter specimens, increasing fixing of this substance at the outer edge is observed (Fig. 3).

DISCUSSION

Although a great part of recoveries took place shortly after tagging, fresh recoveries can be expected to occur in the future, since 252 white anglerfish were tagged in 2001.

The overall recovery index (5%) is higher to that estimated for other species in which tagging results are considered successful (Anon., 2000) (Tab. 2). This figure varied depending on the tagging area, probably in a direct relationship with the fishing gear used and the interest of the fishermen for communicating the recoveries.

Growth in length

The annual increments in length of the specimens recaptured were compared with the annual increments of the running mean length at age from the experienced readers of the International Ageing Exchange Programme on European Anglerfish (Landa, 2002) and with those obtained from the running mean length at age from the routinely annual age length keys of the southern and northern stocks in the period 1997-1999. In these cases the ages were obtained by *illicia* reading. The comparison with the growth from the International Ageing Exchange Programme offered an agreed and global ageing of several readers, although the mean lengths at age were estimated with a small number of samples. Likewise, in the comparison with the growth from the annual age length keys, although the number of readers that ageing these keys was small, the mean lengths at age were estimated with a high number of samples.

It was observed that the annual increments in length estimated in the specimens recaptured were very close to those obtained by *illicia* ageing so much compared to that from the Exchange as that estimated from the routinely annual age length keys (Tab. 3). It is difficult to obtain any conclusion with only two specimens recovered, but it seems that the growth estimated from the *illicia* during last years is not so far from the possible true growth evidenced by the recoveries.

Marks in hard parts

Recoveries of fish marked internally after long intervals of time would be definitive in the validation of growth of this species.

ACKNOWLEDGEMENTS

The study was partially supported by EU DG-XIV (Study contracts 95/038 “BIOSDEF”, 97/015 “DEMASSESS”, and 99/013 “GESSAN”). We are very grateful to I. Bruno, A.C. Fariña, J. Castro, J.C. Arronte, T. Patrocinio, J.A. Pereira, I. Preciado, P. Pereda, C. Pérez, A. Fernández, J. Barrado, H. Dupouy, P. Lucio, I. Quincoces, J.M. Salinas and C. Lordan.

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TABLES

Table 1. Number of white anglerfish tagged and recaptured by year.

Year	1995-1996	1997-1998	1999-2000	2001	Total
tagged	114	23	92	**252**	481
recaptured	0	5	10	9	24

Table 2. White anglerfish tagged and recaptured by area.

ICES Division	VIIjk	VIIIab	VIIIcEast	VIIIcWest	**Total**
tagged (n)	130	0	269	82	**481**
recaptured (n)	2	2	14	6	**24**
recaptured (%)	2%	-	5%	7%	**5%**

Table 3. Increase in length after tagging.

RECAPTURES					*ILLICIA* READING		
					Exchange	Southern stock	Northern stock
time(days)	time(month)	tagging Lt(cm)	variation Lt(cm)	variation Lt /t (cm/year)	variation Lt /t (cm/year)	variation Lt /t (cm/year)	variation Lt /t (cm/year)
440	14.4	22	no length data		-	-	-
327	10.8	60	7	**7.8**	**7.9**	**6.5**	**7.0**
282	9.2	78	5	**6.5**	**6.5**	**6.1**	**6.3**

FIGURES

Figure 1. White anglerfish (*Lophius piscatorius*).

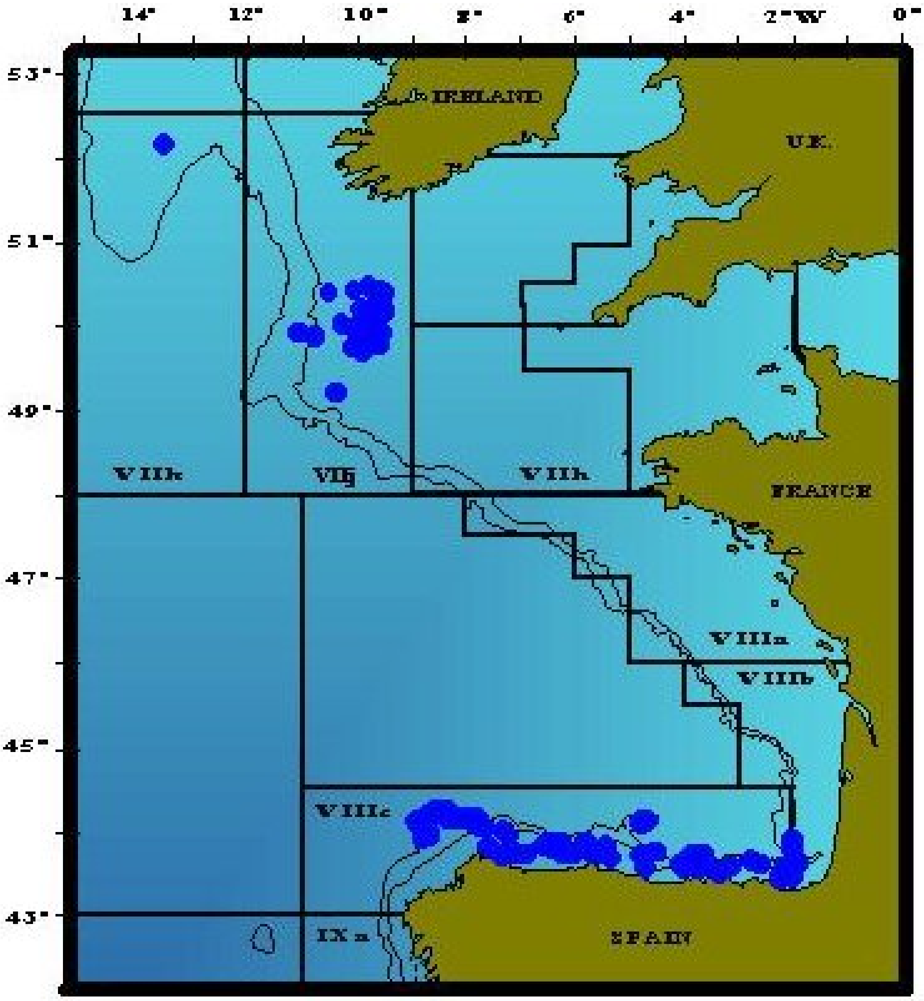

Figure 2. Locations of white anglerfish tagging areas.

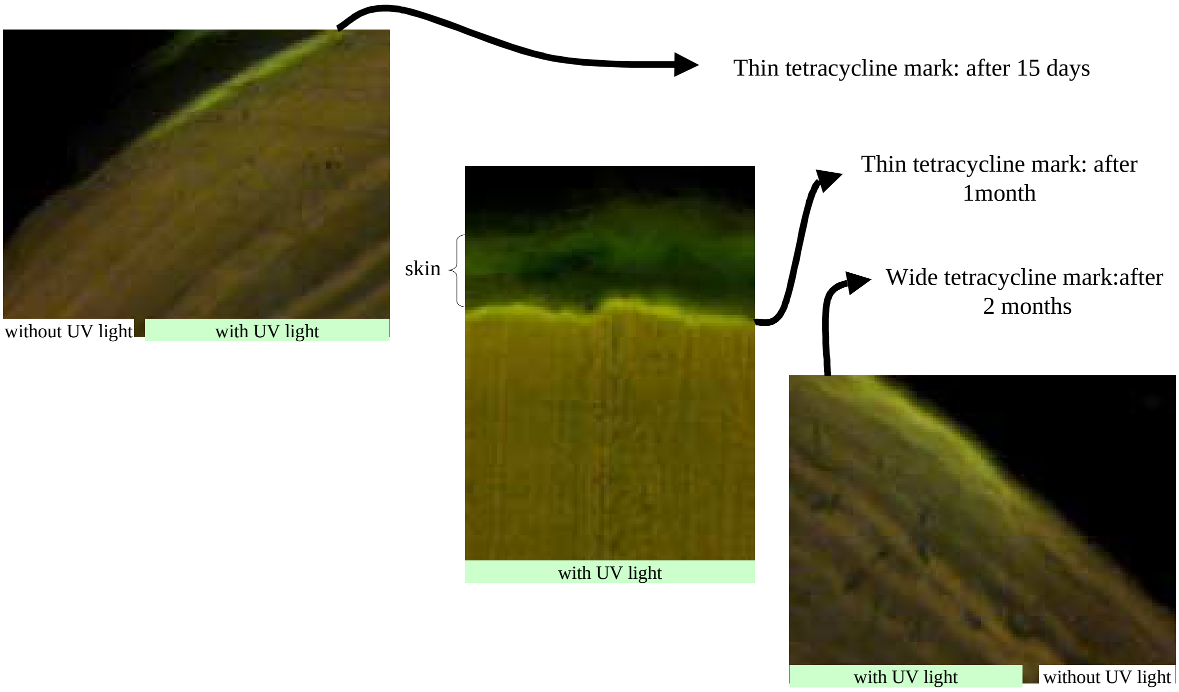

Figure 3. *Illicia* sections with the increasing fixing of tetracycline at the outer edge.

Annex 6

An attempt to validate the age of the black anglerfish (*Lophius budegassa*)
by marking calcified structures

Working Document. 4th International Ageing Workshop on European Anglerfish.
IPIMAR, Lisbon, 14-18 January 2001.

An attempt to validate the age of the black anglerfish (*Lophius budegassa*) by marking calcified structures

Hervé DUPOUY[1], Hélène de PONTUAL[2], Hervé TROADEC[2] (†),
Bernard KERGOAT[1] and André OGOR[2]

[1] IFREMER,
8, rue François TOULLEC
56100 LORIENT (France)

[2] LAboratoire de Sclérochronologie des Animaux Aquatiques
IFREMER DRV/RH/LASAA
BP 70
29280 PLOUZANE (France)

ABSTRACT

IFREMER laboratories of Lorient and LASAA were the first in Europe to tag anglerfish by external spaghetti tags and to mark calcified structures with tetracycline injections. The aims of the two tagging experiments (1995 and 1998), carried out on R/V " GWEN-DREZ ", were (i) to investigate the age and growth of fish, and (ii) to study the possible migrations of the two anglerfish species, *Lophius piscatorius* (white anglerfish) and *Lophius budegassa* (black anglerfish).

A total of 175 fish for both species were tagged and 6 fish have been recaptured until now. Most of them were recaptured within a few months after tagging except one black anglerfish which spent 14 months at sea before recapture. This is the first record with such a long time between tagging and recapture and it provides new data for age validation of black anglerfish.

INTRODUCTION

The age of anglerfish has been investigated by different means : Fulton (1902), Dupouy and Kergoat (1985), Gardner (1987), Discazeaux (1995), Landa and al., (1998), tried to follow increments of modal length groups; Guillou and Knock (1978), Tsimenidis and Ondrias (1980), Crozier (1989), analysed otoliths; Conolly (1920) analysed vertebrae ; a new method based on illicium sections has also been used (Dupouy *et al.* 1986 ; Peronnet and al., 1992 ; Duarte *et al*., 1997 ; Quincoces and al., 1998 ; Anon., 1999).

Large discrepancies have been noted between methods and authors. They are partly due to the different areas studied but also to the method used. To reduce these discrepancies, several International Ageing Workshop on European Anglerfish have been undertaken since 1991 (Anon., 1991, 1997,1999). The two first workshops recommended to use illicium sections for which statistical tests indicated that a best fit was obtained compared to that of otoliths. The third workshop (1999) defined the position of the first ring and noted good agreement in interpretation of illicia in both species of anglerfish.

But ageing by illicia or other methods remains difficult and validation is needed. Mainly for that reason, a tagging program was undertaken by IFREMER (Biseau and al., 1999), IEO (Pereda and Landa, 1998) and AZTI (Lucio, pers. com.).

IFREMER laboratories from Lorient and Brest conducted two research cruises, in 1995 and 1998 to mark anglerfish. One of the marked fish (*Lophius budegassa*) was recaptured 14 months after releasing. An attempt for age validation is ongoing. Some preliminary results are presented here.

MATERIAL AND METHODS

Two surveys, carried out on R/V "Gwen-Drez ", were devoted to tagging experiments (12-23 April 1995 and 17-29 April 1998). The boat is 25 m long and is powered by a 500 kw engine. It towed a bottom trawl specially designed to catch anglerfish and flatfish. (Figure 1).

Tows were of 30 minutes duration and were realised on the central stock of anglerfish (Subareas VII b-k and VIII abd). Fishing operations took place in the Bay of Biscay and in the Celtic Sea (Figure 2), mainly by 47°40'N and 005°30' W (area called " Le Poulailler ") and by 48°20' N and 009°15' W (area called " La Petite Sole ") at 100-200 m depth.

Once the trawl on board, fish was sorted, anglerfish species was determined by external observation (pigmentation of peritoneum by the gill hole under the pectoral fin) and fish were measured to the centimetre below. A yellow spaghetti tag of 15 cm long was fixed on the animal back (just behind the first dorsal fin). It had a reference number and specified that a 500 FF (76.2 euros) reward would be obtained for a fish sent back to IFREMER Lorient. Intra-peritoneal injection of Terramycine ®, a stable solution of tetracycline (TC) for veterinary use, was used at the recommended concentration of 50-75 $mg.kg^{-1}$ to mark calcified structures. After tagging and injection, fish was put under observation for about 30 minutes in a large tank with running seawater. During the first cruise, fish were released at the sea-bottom using a perforated plastic tank closed with a cover. When the tank reaches the sea-bottom, a messenger opens the cover which set the fishes free. This procedure was fixed because anglerfish is fragile but it is particularly time consuming and may be dangerous depending on conditions at sea. It was not used during the second cruise where tagged fish were directly released at sea.

RESULTS

A cumulative total of 175 tagged fish was obtained for the two surveys. Released fish consisted of 92 *Lophius piscatorius* and 83 *Lophius budegassa*. Fish length ranged from 25 to 103 cm and from 20 to 81 cm for white anglerfish and black anglerfish respectively.

Six fish were recaptured and one more signalled which tag was lost. Recaptured fish consisted of 3 *Lophius piscatorius*, 3 *Lophius budegassa* which characteristics are the following :

Species	Tag number	Release date	Release poisition	Total length at release (cm)	Recapture date.	Recapture position	Total length at recapture (cm)
Piscat.	02298	22/04/95	47°41 N 005°22 W	30	24/04/96	47°40 N 003°50 W	?
Piscat.	02269	20/04/95	47°40 N 005°31 W	45	01/05/96	49°59 N 004°44 W	?
Piscat.	06038	26/04/98	47°50 N 006°51 W	103	01/08/98	47°45 N 006°54 W	103
Budegas.	02211	13/04/95	47°45 N 005°32 W	40	02/08/95	47°40 N 004°23 W	40
Budegas.	06024	24/04/98	48°14 N 005°06 W	59	22/05/98	48°47 N 005°13 W	59
Budegas.	06062	28/04/98	47°41 N 005°07 W	35	10/06/99	47°40 N 004°10 W	40.5

Most recaptures occurred within a few months after fish releasing and thus somatic growth was not measurable except for the *Lophius budegassa* n°06062 which spent 14 months at sea before recapture. During this time, the fish growth was 5.5 cm.

Fish illicium, otoliths and vertebrae were extracted and preserved before subsequent observation.

Sections of illicium were obtained following a standard preparation method and examined using a compound microscope under transmitted and ultraviolet (UV) light to reveal fluorescent mark induced by TC (Figure 3). Illicium of fish n° 06062 presented a large TC mark. Three to four rings were observed between the centre and the TC mark and one ring between the TC mark and the edge suggesting an age of 4 to 5 years. Absolute growth between the inner TC mark and edge was estimated from measurements on 3 different radii and gave a growth rate of 57.7 ± 2.7 $\mu m.year^{-1}$ whereas the total radius was 230.9 ± 4.6 µm (mean ± 1 SE). Assuming a constant growth rate, the corresponding age at recapture would be 4.67 years.

Vertebrae were dried and observed with a binocular with variable (10-50x) magnification (Figure 4). The TC mark is clearly visible and corresponds to a narrow brown band, at 1.5 mm from the edge, whereas the total size of vertebra is of 8 mm. Five to six rings are visible from the centre to the TC mark and one other appears between the TC mark and the edge, suggesting an age of 5 to 5 years for a 35 cm black anglerfish and 6 years for a 40.5 cm fish.

The absolute growth rate of this vertebra estimated from the TC mark to the edge is 1.29 $mm.year^{-1}$. Assuming a constant growth rate, age at recapture would be 6.2 years. This is in good agreement with the vertebra ageing from ring count which suggests an age of 6 to 7 years.

Otoliths have not been examined yet but information will be obtained by the end of the year including data regarding other available specimens.

DISCUSSION

Tagging experiments on anglerfish carried out by IFREMER, provide useful information on growth and constitute an attempt to validate ageing methods. On a total of 175 tagged fish, 7 were recaptured. The percentage of recapture is about 4 % the same that obtained by Landa *et al.* (2001).

Most fish were recaptured within a few months after release except one *Lophius budegassa*. This fish spent 14 months at sea between tagging and recapture and showed an increase in total length of 5.5 cm.

The illicium of this fish suggests an age ranging from 4 to 5 years but the TC mark was very large with outward and possibly inward diffusion which obscures the interpretation task. Alternatively, age reading from vertebrae provides an of 6 years for this 40.5 cm fish which is consistent with the age obtained using the growth rate estimated from the TC mark. Moreover this is in good agreement with the age provided by Dupouy *et al.*, (1986) and Duarte *et al.*,1997.

Further work including otolith analysis has to be undertaken to understand the source of the discrepancy between interpretation of illicium and vertebrae. If the age estimated from the illicium analysis is the correct one, the turnover of the stock would be shorter than accepted at present and the yield could be upper on this species and stocks. Alternatively, the age estimated from the vertebrae analysis seems to be more reliable and is in good agreement with ages obtained from the age-length keys of *Lophius budegassa* which have been presented at the Southern shelf demersal fish W.G. since 1987 and no correction has to be made. This hypothesis is also the most careful for the stock.

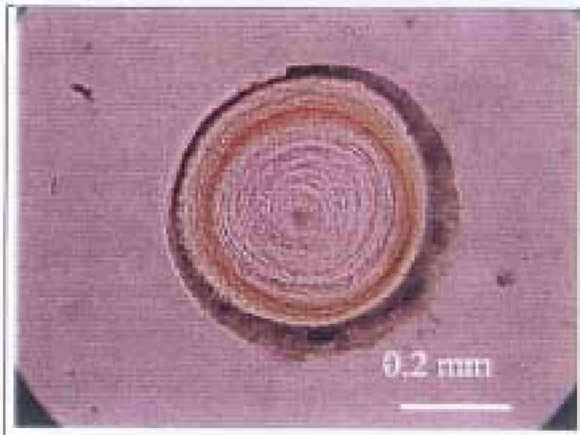

Figure 1: image in transmitted light of a section of illicium of *Lophius budegassa* (N° 6062) recaptured 14 months after its release

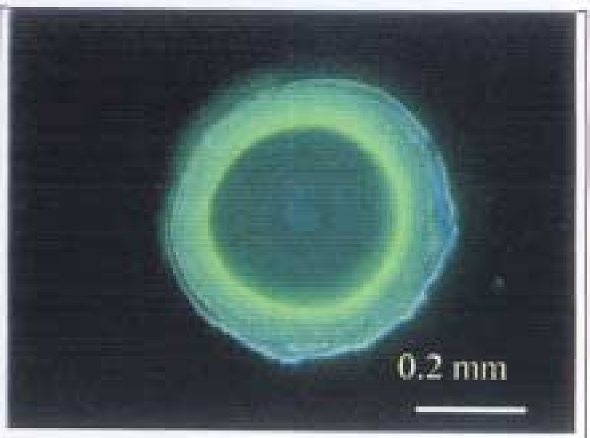

Figure 2 : the same section in fluorescent light.

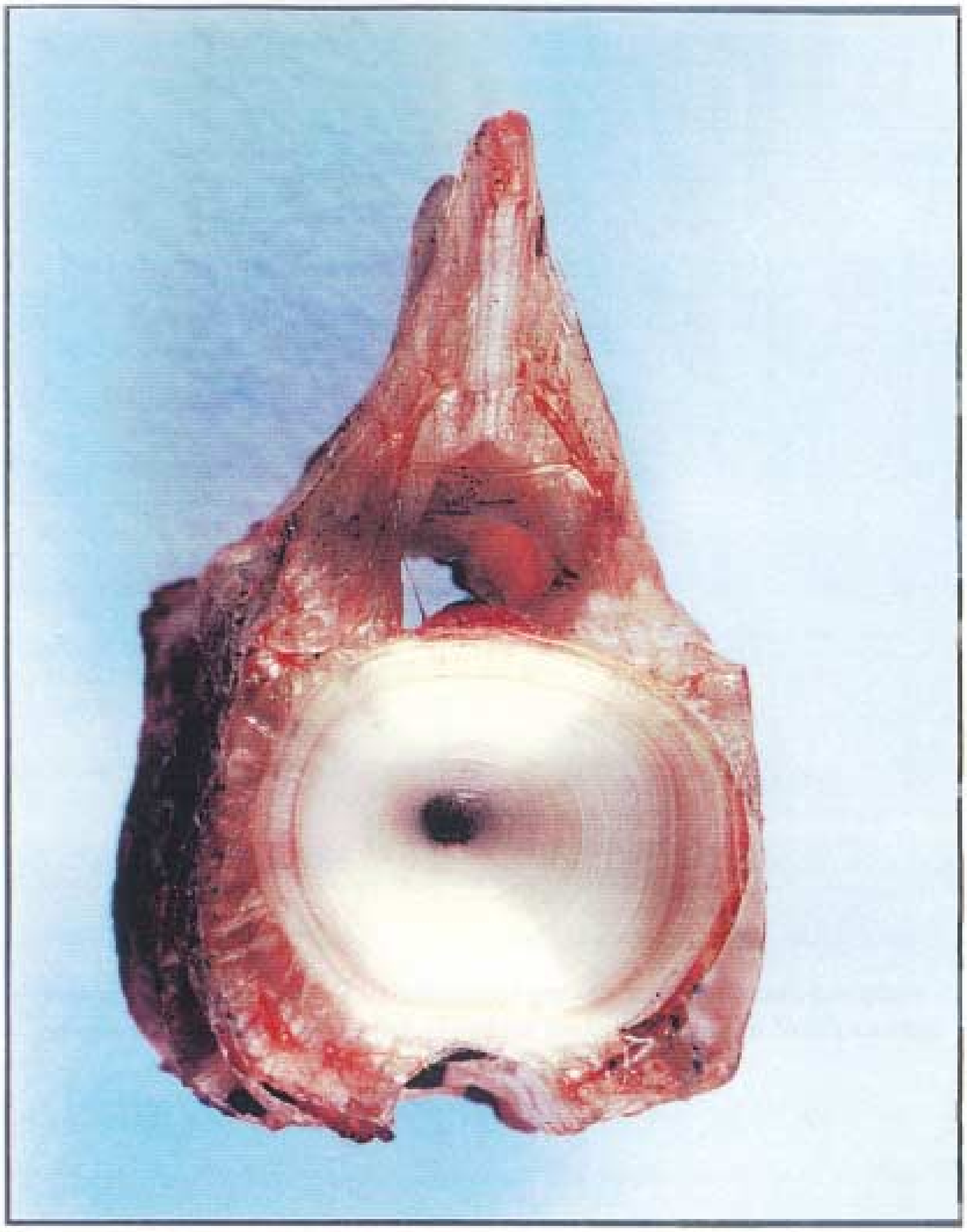

FIGURE 3 : VIEW OF THE VERTEBRA OF LOPHIUS BUDEGASSA WITH BROWN RING DEPOSITED BY TETRACYCLIN MARKER

Annex 7

Preliminary results about validation of growth based on mark-recapture experiences of black anglerfish (*Lophius budegassa*) in the North-eastern Atlantic.

Working Document. 4th International Ageing Workshop on European Anglerfish.
IPIMAR, Lisbon, 14-18 January 2001.

Preliminary results about validation of growth based on mark-recapture experiments of black anglerfish (*Lophius budegassa*) in the North-eastern Atlantic.

J. Landa

Instituto Español de Oceanografía. Apdo 240. 39080 Santander. Spain.
e-mail: jorge.landa@st.ieo.es.

ABSTRACT

Since 1995, IEO tagged 351 black anglerfish in North-eastern Atlantic waters: ICES Divisions VIIj (Northern anglerfish stock) and VIIIc and IXa North (Southern anglerfish stock). Six fish were recovered, giving a recovery index of 3.4%, the greater part of which was recaptured in less than 10 days. No appreciable increase in length was observed in these specimens. The *illicium* (1st dorsal fin ray) section of a specimen recovered after 7 months, showed the tetracycline deposited at its edge of over half the width of the last supposedly annual growth area of the *illicium* (between the supposed annual ring 8 and 9). Therefore, the area with the tetracycline deposited and the part without tetracycline was considered an annual growth area. This help in the validation of growth pattern estimates by means of *illicia* reading for the first time.

Key words: Anglerfish, growth, *illicium*, *Lophius budegassa*, mark, monkfish, North-eastern Atlantic, recapture, recovery, tagging.

INTRODUCTION

The fishing for black anglerfish (*L. budegassa*) represents a significant part of yields obtaining by the different fleets (France, Spain, Portugal, United Kingdom and Ireland) exploiting the bottom fisheries in western and southern European waters (ICES, 2001). While black anglerfish is a by-catch of Spanish bottom trawl fisheries, it is a target species of gillnet (“rasco”) together with the white anglerfish *Lophius piscatorius*. Smaller catches and lower numbers of live specimens caught limit the number of specimens that can be tagged annually and it will only be over a long time scale that relevant information on growth can be obtained.

The first anglerfish tagging experiments in the Northeast Atlantic, began in 1995 (Dupouy, pers. comm.; Pereda and Landa, 1997), were aimed as the basis to growth validation. Since then, a tagging program was undertaken by several European research institutions: IFREMER (Biseau and al., 1999), IEO (Pereda and Landa, 1997; Landa et al., 2001), AZTI (Lucio, pers. com.) and IPIMAR (Duarte, pers. com.).

Growth studies of anglerfish has been made using different ways: following of modal length groups and analysed otoliths, *illicium* sections or vertebrae. However, anglerfish is a difficult species to ageing, and growth studies have to be continued and its validation is needed.

This papers presents the first steps on growth validation based on the tagged and recovered specimens by IEO.

MATERIAL AND METHODS

External tags used were spaghetti T-bar type (model "FD-94 T-Bar Anchor"), inserted in the dorsal area of fish using a tagging gun (model "Mark II Regular Pistol Grip") supplied with 2 cm needles.

A dose of oxy-tetracycline hydrochloride (the brand used was "Terramicina 100") of 50 mg/kg of fish was injected (Weber and Ridgway, 1962; Rijnsdorp and Visser, 1987) using plastic syringes in the ventral area of the fish, just forward of the anus. This substance is deposited in the bone structure and leaves marks that are visible under UV light.

RESULTS

Since 1995, from 351 black anglerfish tagged by IEO, six have been recovered (Tab. 1). These recoveries corroborate the usefulness of tagging by demonstrating the survival of specimens.

Most of the specimens tagged and recaptured were adults. Total lengths of black anglerfish tagged and recaptured are shown (Tab. 2).

Division VIIIc East was the area where the highest number of specimens was tagged Fig. 1). All the recaptures came from this area, in which the estimated recovery index was 3.4%, a figure similar to that estimated for other species for which tagging results are considered successful (Anon., 2000).

Length increment

No appreciable increase in length was observed in any of the recaptured specimens, not even in the one recovered 7 months after tagging.

Age increment

The *illicium* (1st dorsal fin ray) section of the black anglerfish of 52 cm length recovered after 7 months, shows the tetracycline deposited at its edge (Fig. 2).

DISCUSSION

The tetracycline was deposited in a little over half the width of the last supposedly annual growth area of the *illicium* (between the supposed annual ring 8 and 9) and the time elapsed since tagging was a little over half a year (Fig. 2). Therefore, the area with the tetracycline deposited (situated closer to the edge) and the part without tetracycline (more internally) may be considered an annual growth area. These considerations, although preliminary, help in the validation of growth pattern estimates by means of *illicia* reading for the first time.

ACKNOWLEDGEMENTS

The study was partially supported by EU DG-XIV (Study contracts 95/038 “BIOSDEF”, 97/015 “DEMASSESS”, and 99/013 “GESSAN”). We are very grateful to I. Bruno, A.C. Fariña, J. Castro, P. Pereda, R. Duarte, J.C. Arronte, T. Patrocinio, J.A. Pereira, I. Preciado, C. Pérez, A. Fernández, J. Barrado, H. Dupouy, P. Lucio, I. Quincoces, J.M. Salinas and C. Lordan.

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TABLES

Table 1. Number of black anglerfish tagged and recaptured by year.

	1995-1998	1999	2000	2001	Total
tagged	14	20	67	250	351
recaptured	0	0	4	2	6

Table 2. Total length of black anglerfish tagged and recaptured.

	mean	min	max
tagged	53	14	85
recaptured	55	50	62

FIGURES

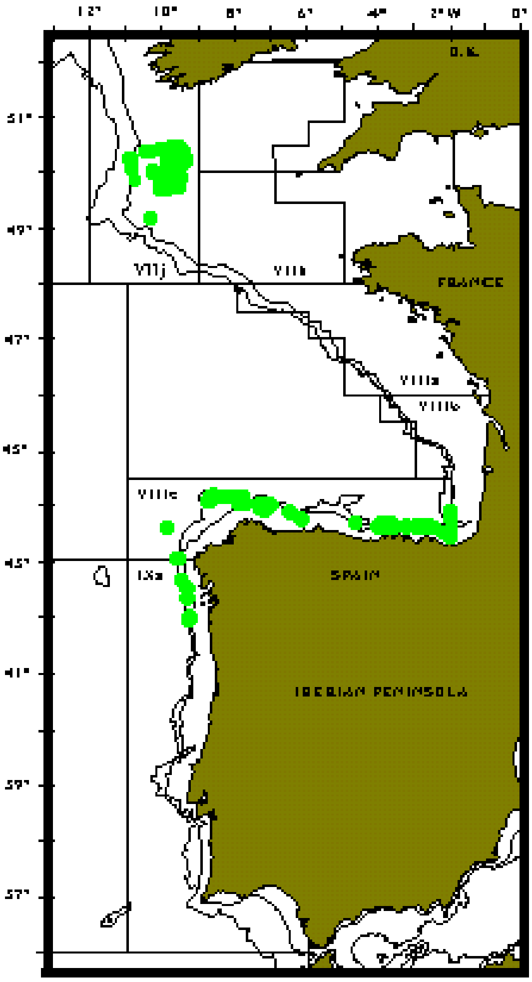

Figure 1. Locations of black anglerfish tagging areas.

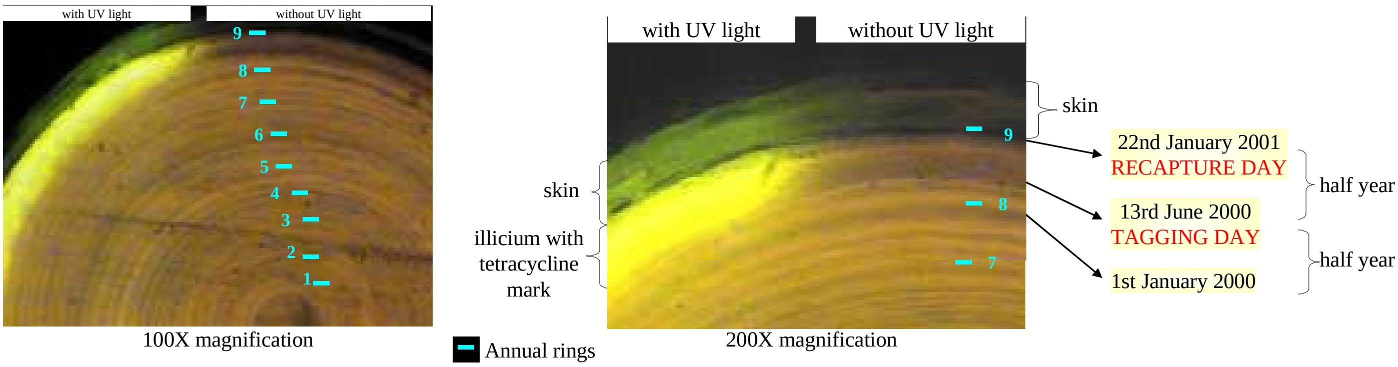

Figure 2. *Illicium* (1st dorsal fin ray) section of the black anglerfish of 52 cm length recovered after 7 months, showing the tetracycline deposited at its edge.