

# ICES WKAEH REPORT 2009

ICES ADVISORY COMMITTEE

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## Report of the Workshop on Age estimation of European hake (WKAEH)

9-13 November 2009

Vigo, Spain



**ICES**

International Council for  
the Exploration of the Sea

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l'Exploration de la Mer

## **International Council for the Exploration of the Sea Conseil International pour l'Exploration de la Mer**

H. C. Andersens Boulevard 44–46  
DK-1553 Copenhagen V  
Denmark  
Telephone (+45) 33 38 67 00  
Telefax (+45) 33 93 42 15  
[www.ices.dk](http://www.ices.dk)  
[info@ices.dk](mailto:info@ices.dk)

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## Executive summary

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In 2008, ICES PGCCDBS recommended conducting a Workshop on Age Estimation of European Hake (WKAEH). The WKAEH took place in Vigo, (IEO) Spain, from 9 to 13th November, 2009; (Chair: C. Piñeiro (IEO, Spain), Co-chair: H. de Pontual (IFREMER, France). The workshop was preceded by an otolith exchange between laboratories involved in the assessment of the hake stocks, in order to identify the current ageing problems arising between readers and to evaluate the state of art of age estimation according to the validation studies conducted so far. Readers from 8 research institutes (AZTI, IPIMAR, CEFAS, IM, IFREMER, IEO, AFBI NI, and VTI-DF) participated in the exchange, for which the aims were:

1. To evaluate the age estimation errors (accuracy and precision) based on a reference collection (otoliths marked with oxytetracycline).
2. To subsequently evaluate the relevance of the ageing method traditionally used to provide ALKs for stock assessment purposes.
3. To inter-calibrate readers, specifying the interpretation differences (ring positions).
4. To progress in the implementation of quality control and quality assurance (QC/QA) in the labs.

The exchange collection consisted of digital images of otolith sections from 104 tagged fish recovered during all seasons and for which size at recapture ranged between 25 cm and 67 cm. The exchange program and workshop were carried out following the recommendations of the EFAN (European Fish Ageing Network) Report 3-2000 on Guidelines and Tools for Age Reading Comparisons (Eltink *et al.*, 2000).

Given the relevance of the WKAEH for the assessment of hake stocks, and its terms of reference (TORs), a presentation session was organized during the workshop to promote a wide discussion on ageing and growth issues that are presently compromising the quality of Northern and Southern hake assessment (2010 Benchmark). The workshop was divided into four parts: (i) Reporting on the results of the exchange programme 2009; (ii) A presentation session of recent studies related with growth and complementary information on the species conducted so far; (iii) Working in two subgroups based on the expertise: Age Readers (G1) and Growth, Modelling /Assessment experts (G2); (iv) Plenary sessions for presenting and discussing the results of both subgroups

A number of six calibration exercises were undertaken for and during the workshop where a group of 15 readers participated. The overall results of the workshop confirm that the previous internationally agreed ageing criteria are neither accurate nor precise and provide overestimation of age. Interpretation of tagged material resulted in a general shift towards younger ages (from 0-10 to 1-5 years) for the same otolith/fish collection. This raises concern about the use, for previous stock assessments, of ALKs that were inaccurate and demonstrates the need to develop approaches allowing the integration of a "validated" growth model or age reading errors into the stock assessment model.

In general the comparison among the results of the exercises shows that irrespective of experience in reading hake otoliths, the age interpretation of hake otoliths was made easier when tagging information was available, improving the precision and agreement among readers in contrast with the age interpretation without tagging information. The complexity of age estimation of European hake remains and vali-

dated material is essential to further develop a new method of otolith interpretation for the age estimation of the species. A preliminary set of guidelines have been established to help the interpretation of otoliths but it will require further refinement using younger and older marked fish to study the structural growth pattern of the otolith.

Results on different approaches considered to incorporate estimated ageing errors in Age-Length keys (ALK) due to the wrong criteria of otolith interpretation for the species showed some promise but further work on growth models will be necessary and it was suggested to continue this work until the 2010 ICES benchmark. It was recommended to continue working on the analysis of tagging data, 'daily' ring counting and age readings to: (i) estimate a growth model or, (ii) develop an error transition matrix between ages identified with previous protocol and ages identified with tagged otoliths or daily ring counts. Both approaches would allow the integration of a growth model or age reading errors into the stock assessment model.

The workshop achieved quite a lot in terms of demonstrating that hake is a much faster growing species than was previously believed and recognised the necessity of working together towards a solution to improve the accuracy and precision of ageing for the assessment. The calibration exercises and general discussions proved positive, by bringing stock assessors, otolith readers and research scientists together, in order to identify the issues and associated consequences of age estimation of hake and to propose some clues to settle this matter.

Another workshop was recommended in three years to continue promoting standardization of methodologies and practices for age estimation of hake built on the work undertaken during the WKAEH 2009. It should be noticed that the availability of otoliths from a wider size/age range of tagged material is very necessary for this work.

## 1 Terms of reference

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2008/2/ACOM42. The Workshop on Age estimation of European hake [WKAEH] (Chair: Carmen Piñeiro\*, Spain) will take place in C.O. in Vigo, (IEO) Spain, by 9 to 13 November 2009 to:

- a. Review information on age estimations, otolith exchanges, workshops and validation work done so far.
- b. Analysis of the results of exchange programme between ageing labs, using a set of otoliths (images) collection partially from tagging material and from previous WK collection with the purpose of inter-calibration age readers involved in Stock assessment.
- c. Report on progress of the compilation of biometrics data of hake otoliths from Southern stock.
- d. To revise the age estimation procedures and explore the possibilities to use supplementary information for validating estimated age structures, this include:
  - i. Otoliths weight distributions
  - ii. Length distribution in surveys and catches.
- e. To develop mathematical methods for estimating hake catches age composition to be used by ICES WG.
- f. To join international experts on growth, age estimation and scientists involved in assessment in order to progress towards a solution.

Supporting information:

An otolith exchange exercise for European hake (*Merluccius merluccius*) will be undertaken during 2008 aimed at dealing with problems of hake age estimation. Following the outcome of this exercise, IEO is going to organize a workshop at the end of 2009.

Up to now France, Portugal, Spain, Ireland and England were participating in the otolith exchanges and workshops. However the age estimation of hake is a complex task that requires a concerted effort to join international experts on growth, age estimation and also scientists involved in assessment in order to progress towards a solution.

<b>PRIORITY:</b>	Age determination is an essential feature in fish stock assessment to estimate the rates of mortalities and growth. Assessment of hake stocks using age structured models has proved useful in establishing a diagnosis on stock status. However, the approach has several limitations and shortcomings such as stock structure, natural mortality and growth. Age data is provided by different countries and are estimated using international ageing criteria which have not been validated. Therefore, an otolith exchange programme and WK should be carried out in order to know the current situation of age estimation of hake which has been subject of concern of ICES WG HMM and make progress towards a solution.
<b>SCIENTIFIC JUSTIFICATION AND RELATION TO ACTION PLAN:</b>	<p>Hake age estimation method has not been validated although progress has been made regarding precision on age data along the last decade: international reading otoliths exchanges and workshops (1997, 1999, 2001, 2003, 2004).</p> <p>The results obtained indicated that the confident age range dropped from 5 to 3 years old, since WK2001 and this loss of precision is associated with applying ageing criteria, which are not validated.</p> <p>This problem has a potential effect on the drift of individual readers through time that is not evaluated since 2004.</p> <p>For the purpose of inter-calibration between ageing labs an appropriate exchange programme with a set of otoliths (images) collection partially from tagging material and from previous WKS collection will be carried out for next year.</p> <p>The aim of the workshop is to identify the current ageing problems between readers from both stocks through a reference collection. To identify the state of art of age estimation after validation studies conducted so far.</p>
<b>RESSOURCE REQUIREMENTS :</b>	Before starting the exchange programme, the scientific institutions should make a concerted effort to compile the existing tagging material (digital otolith images) that can be used as a reference collection.
<b>PARTICIPANTS:</b>	In view of its relevance to the DCR, and ICES WG, the Workshop try to join international experts on growth, age estimation and scientists involved in assessment in order to progress towards a solution.
<b>SECRETARIAT FACILITIES:</b>	
<b>FINANCIAL:</b>	Additional funding will be required for facilitate the attendance of the scientists. The workshop will be eligible under the EU - DCR.
<b>LINKAGES TO ADVISORY COMMITTEES:</b>	ACOM
<b>LINKAGES TO OTHER COMMITTEES OR GROUPS:</b>	
<b>LINKAGES TO OTHER ORGANIZATIONS COST:</b>	There is a direct link with the EU DCR and outcomes from this Workshop will be of interest to ICES WGHMM.

On IEO request and in agreement with the Professional Secretary for Advisory Services of the ICES, the original official statement of the WKAEH was modified on 24/03/09 as follow: WKAEH Chairs : Carmen Piñeiro IEO, Spain and H el ene de Pontual, Ifremer, France



## 2 Agenda and participation

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The agenda is given in Annex 1 and list of participants and age readers experience in Annex 2.

## 3 Introduction: (Tor a)

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Northern and southern hake (*Merluccius merluccius*) stock assessments have used age data based on otolith analysis since 1992. Age data for stock assessment are provided by different institutes and should follow quality control and quality assurance procedures regarding accuracy and precision issues. However, the age estimation method for hake had not been validated, although progress had been made regarding precision on age data throughout the last decade (international otolith exchanges and workshops held in 1997, 1999, 2001, 2003 and 2004; Piñeiro *et al.*, 2009). Preliminary results of a tag-recapture pilot experiment indicated that hake growth would be underestimated by a factor of 2 (de Pontual *et al.* 2003). The subsequent otolith exchange and workshop held in 2004 resulted in a loss of precision associated with applying ageing criteria that were likely to be incorrect (Piñeiro *et al.*, 2004). Consequently this workshop recommended firstly to suspend providing ALKs for the WGHMM until validated/accurate criteria were available (WGHMM Report, 2004, ICES CM 2005/ACFM and secondly, to allocate the effort to other tasks (tagging, otolith microstructure analysis, etc). However, ALKs are currently being provided by some institutions on the request of the WG.

The analyses of marked otoliths clearly showed that age overestimation is the reason for growth underestimation, a result that invalidated the age estimation method that provided estimates that were neither accurate nor precise (de Pontual *et al.*, 2006). Tagging experiments from both stocks (de Pontual *et al.*, 2003, 2006; Piñeiro *et al.*, 2007) have provided direct evidence supporting the fast-growth hypothesis. Moreover, daily growth studies also support the fast growth hypothesis during the first year of life (Kacher and Amara, 2005; Piñeiro *et al.*, 2008).

Considering the impact of bias in age estimation on stock assessment (Bertignac and de Pontual 2007), the Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) in March 2008 recommended a Workshop on Age Estimation of European Hake (WKAEH), with a preceding otolith exchange among laboratories involved in the assessment of the hake stocks. It was anticipated that the WKAEH results would be of major interest for the ICES roundfish workshop planned in 2010, where the two hake stocks will be benchmarked.

The WKAEH took place in Vigo, (IEO) Spain, from the 9th to 13th of November, 2009; (Chair: C. Piñeiro (IEO, Spain), Co-chair: H. de Pontual (IFREMER, France).

#### 4 Otolith exchange 2009 (Tor b)

An otolith exchange was conducted prior to the workshop (16 March -10 June 2009), using an otolith reference collection supplied by IFREMER. This collection consisted of two sample sets of digital images of marked otoliths (Fig. 1) recovered from tagging experiments carried out by IFREMER in the Atlantic waters (Bay of Biscay), from 2002 to 2007.

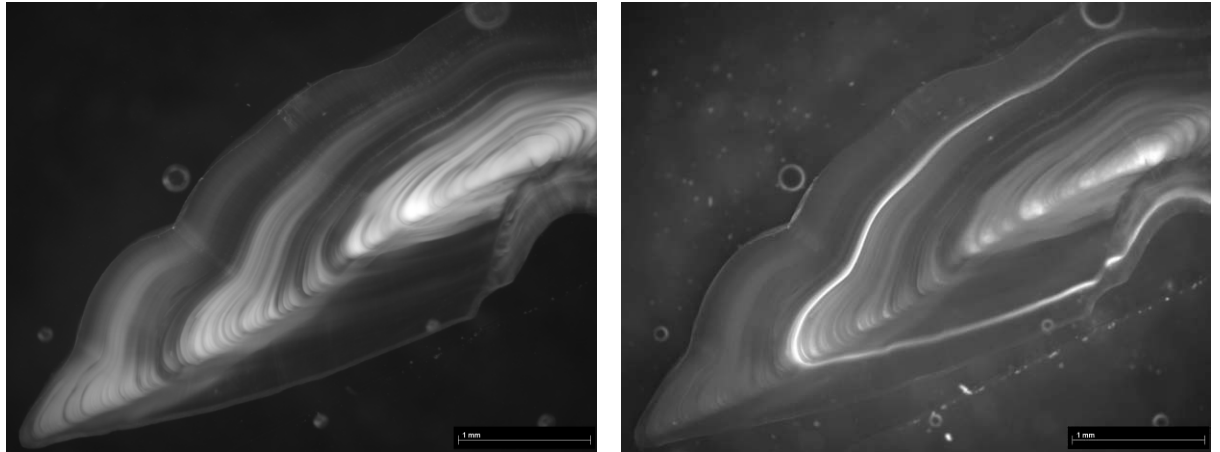


Figure 1: Example of a transverse section of a tagged hake otolith observed under a) reflected light –translucent zones (TZ) appear as dark areas and b) UV light revealing the oxytetracycline (OTC) mark deposited at tagging.

##### *Objectives and organization*

Readers from 8 research institutes (AZTI, IPIMAR, CEFAS, IM, IFREMER, IEO, AFBI NI, and VTI-DF) participated in the exchange, for which the aims were:

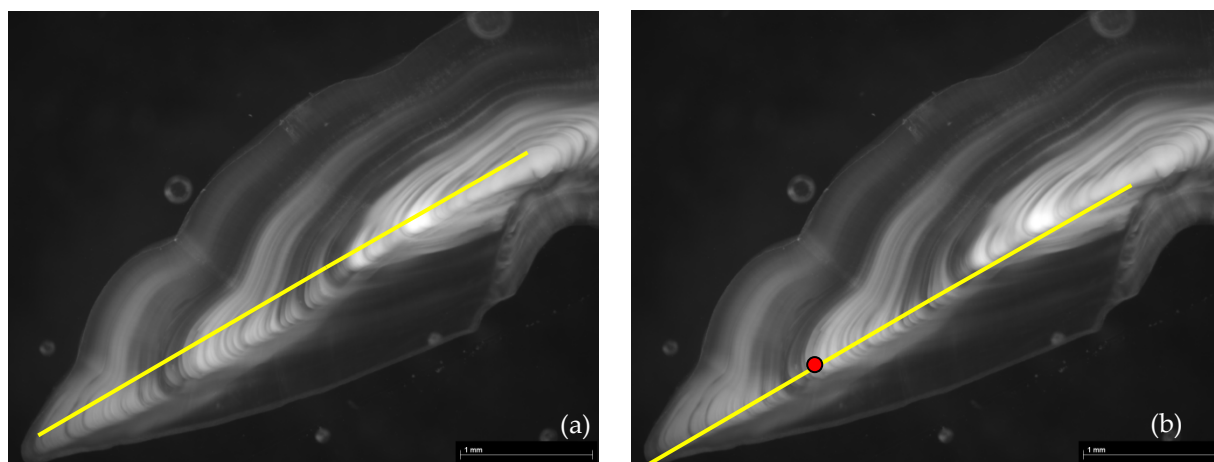
- 1) To evaluate the age estimation errors (accuracy and precision) based on a reference collection.
- 2) To subsequently evaluate the relevance of the ageing method traditionally used to provide ALKs for stock assessment purposes.
- 3) To inter-calibrate readers, specifying the interpretation differences (ring positions).
- 4) To progress in the implementation of quality control and quality assurance (QC/QA) in the labs.

Objectives 1 and 2 were achieved by comparing the individual age estimates obtained without tagging-provided information (hereafter referred to as blind interpretation), to those obtained with tagging-provided information (hereafter referred to as supervised interpretation).

More precisely, blind interpretation consisted of estimating individual ages from images under reflected light (scale bar provided, Fig. 2c), with the recapture date and sex as additional information. Experts were asked to follow the internationally agreed interpretation criteria described in Piñeiro, 2000, Piñeiro *et al.*, 2000, Piñeiro and Sainza, 2002, and Piñeiro *et al.*, 2009, although these criteria had been recently invalidated (de Pontual *et al.*, 2006). This request was specifically aimed at providing estimates of ageing errors in ALKs that were provided in recent decades for stock assessment purposes.

Supervised interpretation consisted of estimating individual ages from the same image set with the OTC mark position specified on the standardized transect (Fig. 2 b), as well as additional information, namely fish lengths (total length) at tagging and recapture and time at liberty (i.e. time elapsed between tagging and recapture).

Objectives 3 and 4 were addressed by comparing not only the age estimates, but also the positions of the rings interpreted as annuli (annual rings) by the readers. This was done using the recorded positions of the rings interpreted as annuli for each otolith and reader (see Fig. 2).



**Figure 2:** Example of the images provided for blind interpretation (a). A standardized transect (in yellow) is linked to the image and experts were asked to record their interpretation by marking the positions of the rings they interpreted as *annuli* on the transect. Example of the images provided for supervised interpretation (b). The position of the OTC mark (in red) is specified on the transect and experts are provided with additional information (fish lengths at tagging and recapture and time at liberty). Experts were asked to record their interpretation by marking the positions of the rings they interpreted as *annuli* on the transect.

A total of 4 exercises (Table 1.) were carried out during this exchange. Exercises 1 and 2 concerned experts involved in routine age estimation of hake. Exercise 3 concerned experts involved in routine age estimation of other species. Exercise 0 was carried out later by additional readers interested in attending the WKAEH. The expertise level in routine ageing (Annex 2) was heterogeneous for the latter group.

EXERCISE	TYPE	READER EXPERTISE	ANALYZED MATERIAL	ADDITIONAL INFORMATION
1	Blind interpretation	Hake (R1 to R5)	104 Images Type 1 (fig 2-a).	Catching date, Sex
2	Supervised interpretation	Hake (R1 to R5)	104 Images Type 2 (fig 2-b)	Catching date Time at liberty TL <sub>tag</sub> TL <sub>rec</sub> Sex
3	Supervised interpretation	Other species (R6 to R10)	104 Images Type 2 (fig 1-b)	Catching date Time at liberty TL <sub>tag</sub> TL <sub>rec</sub> Sex
0	Blind interpretation	Late participants (R11 to R14)	104 Images Type 1 (fig 1-a)	Catching date, Sex

Table 1: Synopsis of the exercises carried out during the 2009 hake otolith exchange with the information provided to the readers.

#### 4.1 Sample collection

The exchange collection consisted of digital images of otolith sections from 104 tagged fish recovered during all seasons and for which size at recapture ranged between 25 cm and 67 cm. The frequency distribution of the total length at recapture is given in the Figure 3 and time at liberty (from 155 days to 1066 days) *versus* length at recapture is given in Figure 4

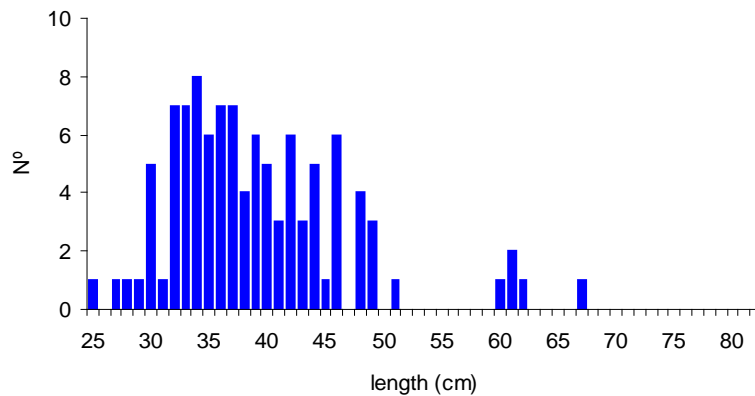


Figure 3: Length frequency distribution of samples analyzed during the exchange program conducted in the 2009.

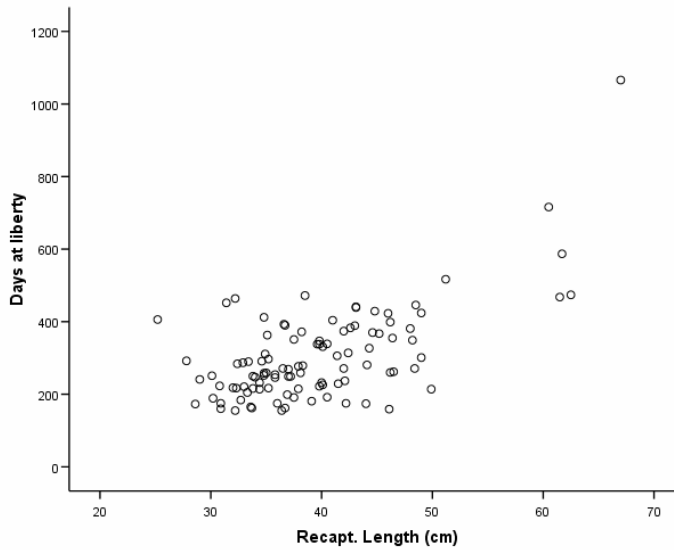


Figure 4: Length at recapture versus time at liberty of the analyzed samples.

**4.2 Methods**

The exchange program was carried out following the recommendations of the EFAN (European Fish Ageing Network) Report 3-2000 on Guidelines and Tools for Age Reading Comparisons (Eltink *et al.*, 2000).

Details regarding age reading protocols are provided in Annex 3. Image annotations by experts were performed using the GIMP2.6 software (freeware) whereas measurements (nucleus to rings distances) were subsequently taken by IEO using the dedicated software TNPC V4.1

(<http://www.ifremer.fr/lasaa/TNPC/Tnpc4.2English.htm>) developed under the Visilog software platform (NOESIS, France).

Statistical indexes and tests for estimating the degree of agreement between readings (Campana, 2001; Morales Nin and Panfili, 2002) were used for the analysis, as described below:

The Percentage of readings agreement (PA) is the ratio between the number of coincident readings and the total number of readings (in percentage). However, PA depends on the lifespan of the species. Therefore, Beamish and Fournier (1981) recommended the use of average percent error (APE), which is an index of reading precision useful for comparing series of observations defined as:

$$APE = 100\% \times \frac{1}{R} \sum_{i=1}^R \frac{|X_{ij} - \bar{X}_j|}{\bar{X}_j}$$

Where  $X_{ij}$  is the  $i$ th reader age estimation of the  $j$ th fish,  $\bar{X}_j$  is the mean age of the  $j$ th fish, and  $R$  is the number of times each fish is aged (reader). When averaged across many fish, it becomes an index of mean APE.

Chang (1982) suggested incorporating the standard deviation in the previous equation rather than the absolute deviation from the mean age. The resulting equation produces an estimate of the Coefficient of variation (CV), and does not assume that the standard deviation is proportional to the mean:

$$CV = 100\% \times \frac{\sqrt{\frac{\sum_{i=1}^R (X_{ij} - \bar{X}_j)^2}{R-1}}}{\bar{X}_j}$$

where CV can be averaged across a number of fish to produce a mean CV that is statistically more robust than APE, and more flexible. It should be remembered that the CV is very sensitive to low age values.

The analyses of the age reading results were performed using the Excel ad-hoc Workbook, "AGE COMPARATIONS.XLS" (Eltink *et al.*, 2000). This methodology assumed a reference age for comparison. Since there are no validated ages available, the reference age assumed is the modal from all readers. In case of bi-modality, the mean age was used. Intra-calibration of hake readers using exercise 1 and 2 by using the mean age was undertaken.

Box-whisker plots (median and interquartile range) were used to summarize and to compare the distribution of estimates between readers. They were built both for age estimate distribution analysis and ring-to-nucleus distances distribution analysis. It is worth noting that such representations do not take into account the repeated nature of the observations (data –either age estimates or distances measurements- are not independent). As a consequence such representation should be interpreted cautiously.

### 4.3 Results

The results of the age estimations by reader and the basic information about otolith collection by exercise conducted are summarized in Annex 4. The results of the analysis of the exchange are summarized by exercise in Table 2.

Exchange 2009				
Exercise	0 (blind)	1 (blind)	2 (Supervised)	3 (Supervised)
Readers	Late readers. R10 to R14	Hake readers. R1 to R5	Hake readers R1 to R 5	Other Expert readers R5 to R10
N	104	104	104	104
FL Range (cm)	25-67	25-67	25-67	25-67
APE (%)	49.7	30.7	16.8	10.9
CV (%)	66.8	41.2	21.8	14.4
PA (%)	3.6	46.3	73.9	83.1
Age Range (years)	0-10	0-10	1-5	1-5

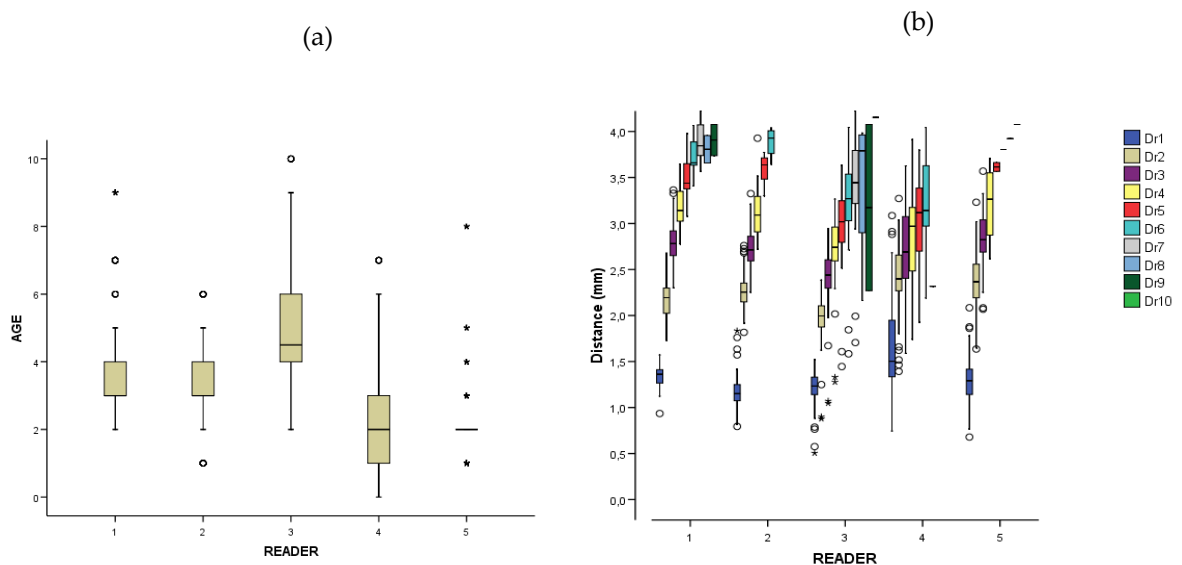
Table 2: Summary of the exchange results by exercise.

**Results and discussion regarding blind interpretation (precision of estimation)**

The results of exercise 1 and 0 (blind interpretation) clearly indicated a considerable imprecision (APE, CV) between readers with respective values of 30.7 and 49.7 % for APE, 41.2 and 66.8 % for CV and 46.3 and 3.6 % for PA (Table 2)

Considering the results for expert hake readers (Exercise 1), two of them (R4, R5) did not obviously used the agreed ageing criteria which partly explains the very low precision reflected high APE and mean CV values (Table 2). This is also confirmed by the box-whisker plot which shows the wide range of ages attributed by all readers with a mean value of 3.2 years (Figures 5 a).

The distribution of ring measurements by readers (Figure 5 b) highlights the between reader differences in the interpretation process of the otolith structures although readers 1 and 2 had roughly similar measurements distributions as they strictly applied the traditional ageing criteria.



**Figure 5: Box whisker plots of otolith interpretations from exercise 1 (a) ages and (b) distances from nucleus to rings considered by reader.**

The Wilcoxon signed rank tests on age estimates between hake readers are shown in table 3. The results indicate that age distributions are statistically identical between readers 1 and 2 on one hand and between 4 and 5 on the other hand.

	IEO	IPIMAR	MI	CEFAS	IFREMER
	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5
Reader 1	□	–	**	**	**
Reader 2	–	□	**	**	**
Reader 3	**	**	□	**	**
Reader 4	**	**	**	□	–
Reader 5	**	**	**	–	□

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

**Table 3: Wilcoxon signed rank tests on age distributions from exercise 1.**

***Comparison between blind and supervised interpretation process (are ALK accurate?)***

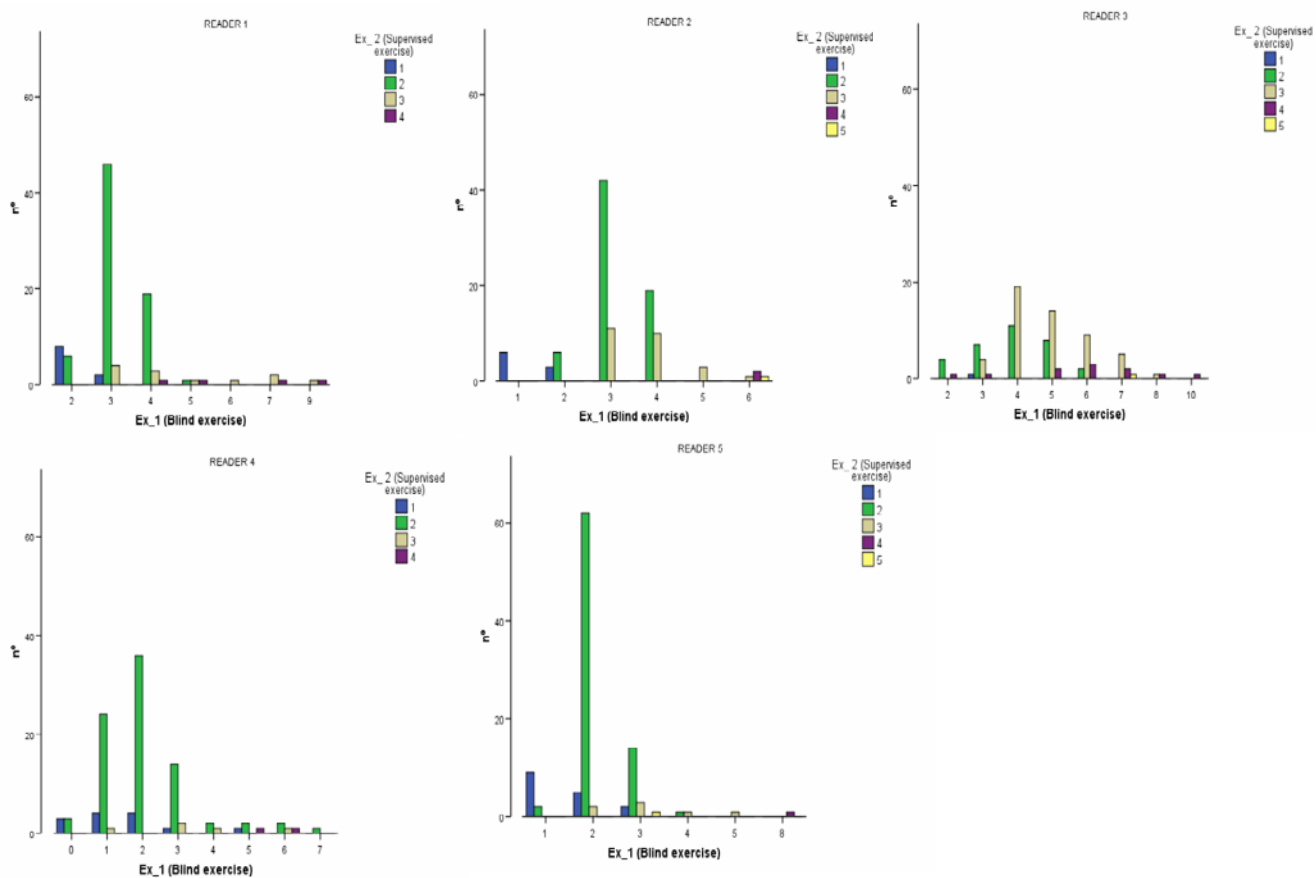
The comparison of exercises 1 versus 2 aimed at evaluating the differences between blind and supervised interpretation for each of the readers, both for reader calibration purposes and to specifically address the issue of accuracy of ALKs that were provided in the last decades.

The results of the cross comparison of the ages given by the hake readers and ages subsequently assigned using the OTC mark position and extra data are shown in Figure 6.

This graph shows that for most of the readers, age distributions are deeply modified when the estimation is supervised and ages that ranged from 0 to 10 in exercise 1 were assigned from 1 to 5 in exercise 2. Figure 6 also show that age distributions differ between readers at both blind and supervised interpretation.

The Wilcoxon signed rank tests indicated significant differences for all readers between the two exercises except for R4. This reader seems to have not changed the perception of the otolith structures once tagging information was provided and used their own ageing method in both exercises.





**Fig 6: Cross comparison of blind versus supervised interpretation (exercises 1 vs 2) of the marked otolith collection by reader. For each reader, blind age distribution is given by the sum of occurrences at each age of X axis. Corresponding supervised age distribution is given by the sum of occurrences of each color. For instance, otoliths that were assigned age 3 by R1 when interpreted blind were then assigned 1, 2 and 3 when interpretation was supervised and the 2 otoliths that were first assigned 9 by R1 were then assigned 3 and 4.**

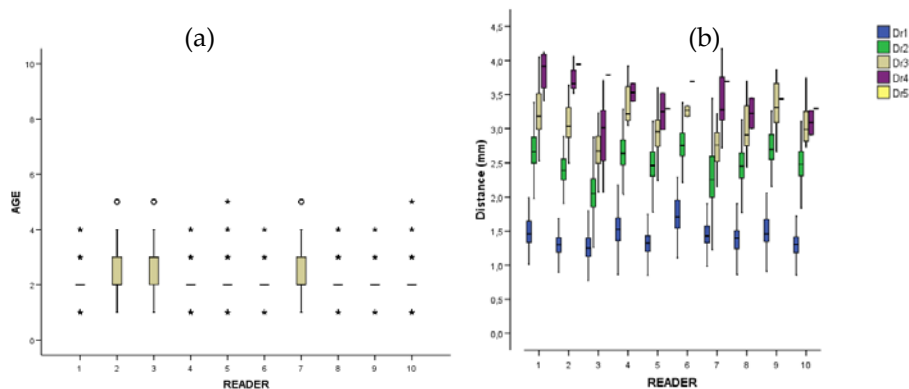
The main outcome of this analysis is the evidence that the agreed age estimation method leads to inaccurate estimates of ages (see R1 and R2) which confirm the conclusions of de Pontual *et al.* (2006). This constitutes a clear indication that ALKs previously provided for stock assessment were inaccurate. As a consequence, including aging errors into stock assessment is a critical issue which is addressed in section 4.4.

***Results and comments regarding supervised exercises 2 and 3 (towards a new age estimation method?).***

The results of the two supervised interpretation exercises (exercise 2 for hake experts and exercise 3 for other expert readers) show a considerable increase of precision (see table 2) with values of 16.8, 21.8, 73.9 for APE, CV and PA respectively for hake readers and values of 10.9, 14.4, 83.1 for APE, CV and PA respectively for other expert readers. Knowledge of the somatic growth in combination with the otolith growth increment between tagging and recapture date, has improved the agreement in the estimated ages by providing a reference for the growth rate of otolith. As previously mentioned the range of ages attributed was narrower than that of blind estimation

whatever the reader with a mean value of 2,2 years. However discrepancies still exist as shown by Figure 7a and Table 4.

Interpretational differences between readers were even more evident when analyzing the distributions of the positions of the rings considered by readers as annuli (Figure 7 b). Discrepancies between readers appeared from the first ring position and are subsequently propagated to the other rings. It can be noticed, however, that the distributions are roughly similar for R1 and R2 although this should be confirmed because such representation does not take into account the fact that observations are actually repeated ones.



**Figure 7: Distributions of age estimates (a) given by readers of exercises 2 and 3 (supervised interpretations) and corresponding distributions of ring distances to the nucleus (b).**

Pairwised comparisons of age distributions between readers (Wilcoxon signed rank tests) are provided in table 4. The results clearly show that, despite the use of a reference collection (tagged otoliths) together with additional information, significant differences exist between readers. Interestingly, two groups show consistent estimates: R1 and R2 on one side and R 4, R5, R9 and R10 on the other side. The differences between those two groups of readers may be partially related to the image magnification that was slightly higher than usually used by hake readers. However the overall results provide clear evidence that hake otolith interpretation is far from being a fixed issue.

Table 4: Wilcoxon signed rank tests for all readers of supervised age estimation exercises 2 and 3.

	IEO	IPIMAR	MI	CEFAS	IFREMER	AFVI	VTI-DF	AZTI	IEO	IFREMER
	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10
Reader 1	□	–	**	*	*	**	**	–	*	–
Reader 2	–	□	**	**	**	**	**	–	**	**
Reader 3	**	**	□	**	**	**	**	**	**	**
Reader 4	*	**	**	□	–	–	**	**	–	–
Reader 5	*	**	**	–	□	–	**	**	–	–
Reader 6	**	**	**	–	–	□	**	**	–	–
Reader 7	**	**	**	**	**	**	□	**	**	**
Reader 8	–	–	**	**	**	**	**	□	**	**
Reader 9	*	**	**	–	–	–	**	**	□	–
Reader 10	–	**	**	–	–	–	**	**	–	□

#### 4.4 Including ageing errors into stock assessment

One possible way of including age reading errors into stock assessment is through the computation of a transition matrix that allocates an otolith classified in a specific age without tagging information, into the expected age if it was classified with tagging information. Such statistic would allow an operational procedure to rebuild the time series of hake information, without requiring a growth model, and, more importantly, keeping the information about growth over time.

The data used for this exercise were the age readings of exercises 1 and 2 of the 2009 otolith exchange.

Note that for the rest of this section the term “tagged ages” will be used to refer to ages classification attributed with tagging information (referred to as "supervised estimation" in the rest of the document) and the term “untagged ages” for age attributed without tagging information (referred to as "blind interpretation").

Table 5: Number of readings by age and exercise

Age	Exercise			
	0	1	2	3
0	0	6	0	0
1	7	46	49	37
2	71	138	334	387
3	22	156	101	63
4	1	95	20	11
5	1	35	3	2
6	1	24	0	0
7	0	13	0	0
8	1	3	0	0
9	0	3	0	0
10	0	1	0	0

Figure 8 shows the readings of each reader by exercise. A regression line was included to help visualizing the analysis. It is clear that readers 1-5 and 10 have a distinct perspective of growth with and without tagging information. It's also clear that when the tag information is available all readers are more look-alike than when this information is not available.

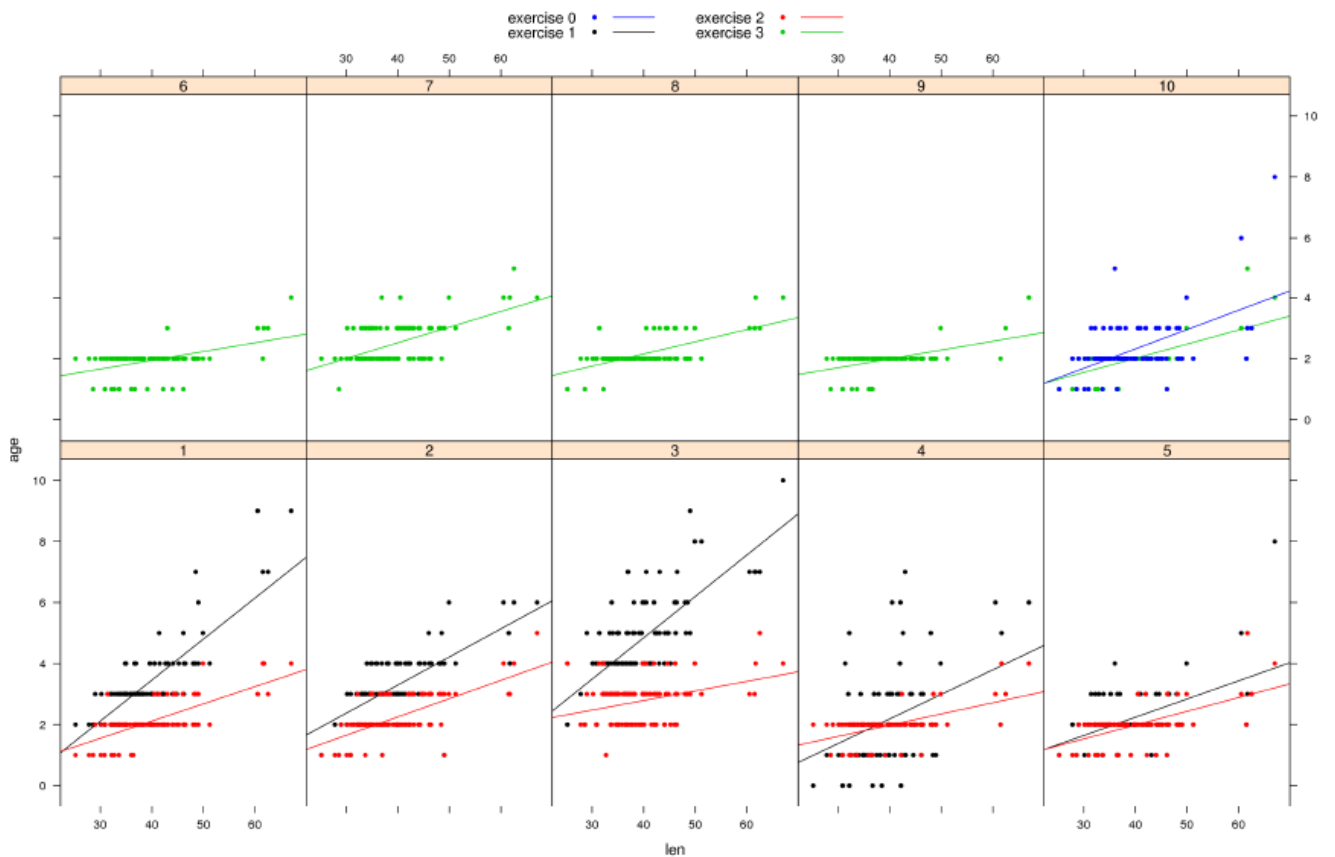
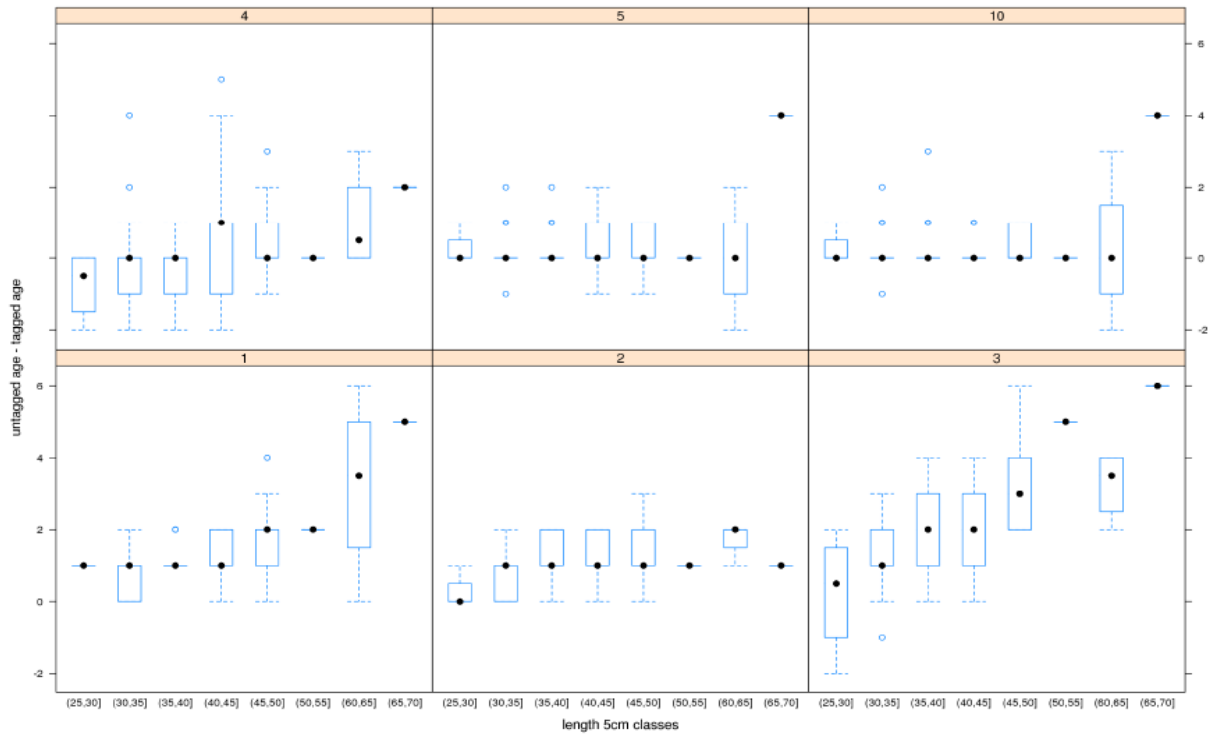


Figure 8: Age readings by length and readers for different exercises

Looking at differences between untagged ages and tagged ages (Figure 9) it can be seen that, with the exception of reader 3, all readers have a constant value over lengths although a high variability is present. Readers 1 and 2 have mostly a gap of 1 year, while readers 4, 5 and 10 have zero year gap. Reader 3 shows what could be considered a multiplicative error, which increases with the size of the fish.



**Figure 9: Age difference by length and reader**

Looking where the errors maybe coming from, Figure 10 plots the ratio untagged/tagged of the first ring distance. Most of these ratios are between 0.9 and 1, which means that readers in the presence of tagging information do not change their evaluation of ring 1. Some readers have a tendency to reduce the length of the first ring in small individuals, when in the presence of tagging information, while others do it in larger individuals.

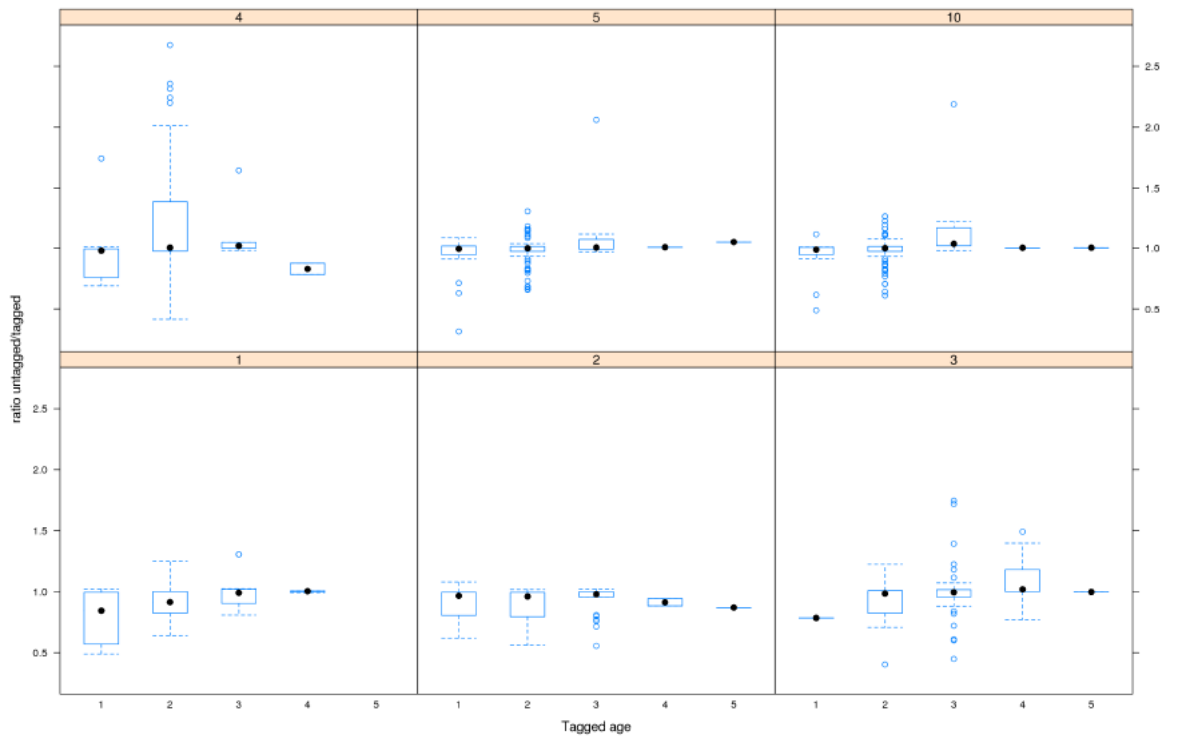


Figure 10: Ratio untagged/tagged of the 1<sup>st</sup> ring distances by tagged age and reader.

Figure 11 shows the same information for the second ring distance and the underestimation of the length of this ring becomes clearer. However the reduced number of observations makes it difficult to draw conclusions.

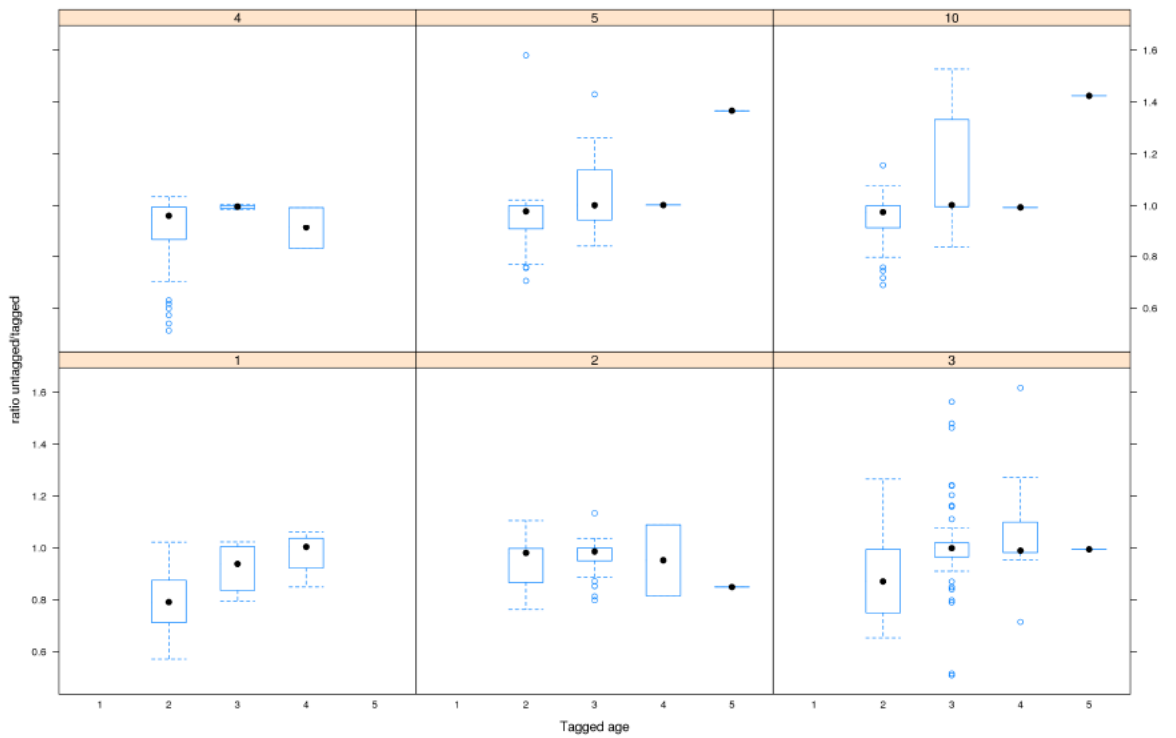


Figure 11: Ratio untagged/tagged of the 2<sup>nd</sup> ring distances by tagged age and reader.

From this exploratory analysis it is obvious that there is an underestimation of the growth rate, in face of the tagged otoliths presented to the group. The size of this bias is not clear due to the high variability of the readings and the distinct behaviour of the distinct readers. There is an obvious impact of the tagging information on the readings, which is higher in readers 1, 2 and 3. It is interesting to note that reader 10, not experienced in hake readings, also changed his evaluation when interpreting the tagged otoliths, although to a less extent.

As discussed before the idea is to have a matrix that reallocates the readings taking into account the information untagged/tagged information.

Figure 12 shows the probability of the allocation of an untagged age into tagged ages. For example, reader 4 classified some otoliths as age 0, without tagging information, and after looking at the tagging information, allocated 50% of these to age 1 and 50% to age 2. The expectations could have been to have something similar to age 3, where most of the readers, after analyzing the tagging information, allocated a high percentage of otoliths to age 2, a minor percentage stayed in age 3 and residual percentages were allocated to ages 0, 4 and 5. However for age 1 and 2 most of the otoliths are kept on the same age which is actually due to the data set (homogeneous size range of fish at tagging and time at liberty less than one year for a number of recoveries see figure 4 section 4.1).

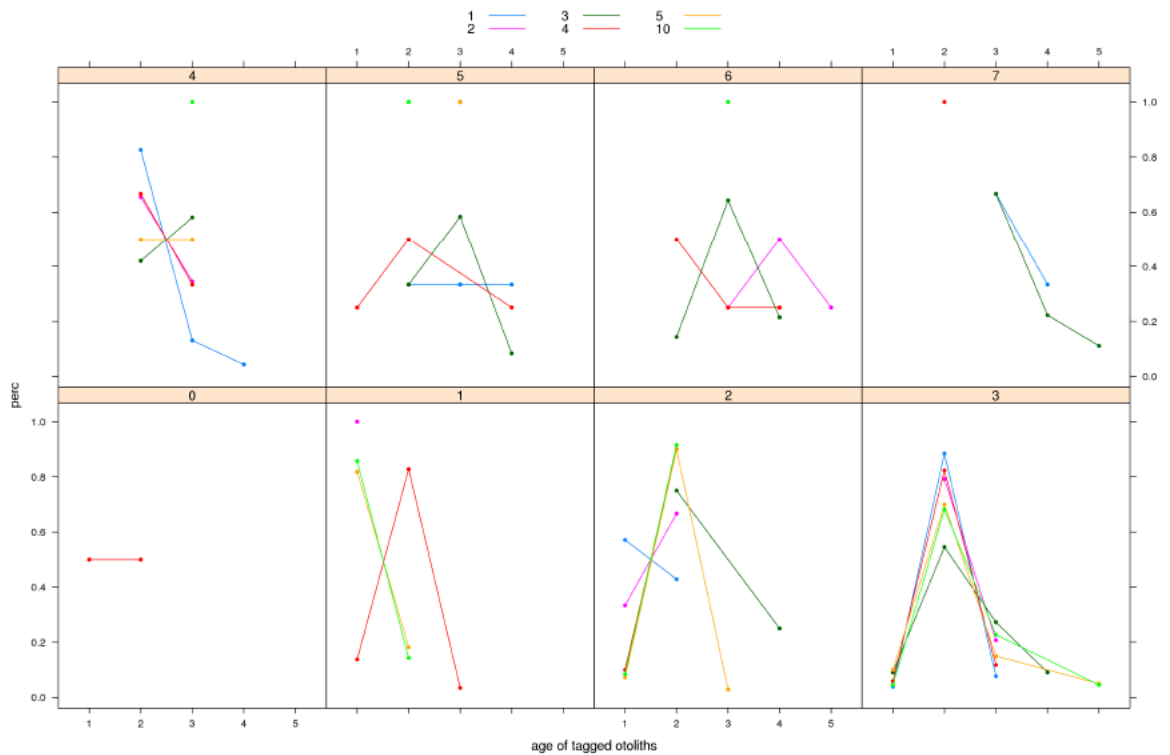


Figure 12: Probability of allocation of untagged ages to tagged ages by reader.

Figure 13 has the same information as figure 12 but collapses the readers so it computes overall allocation percentages.

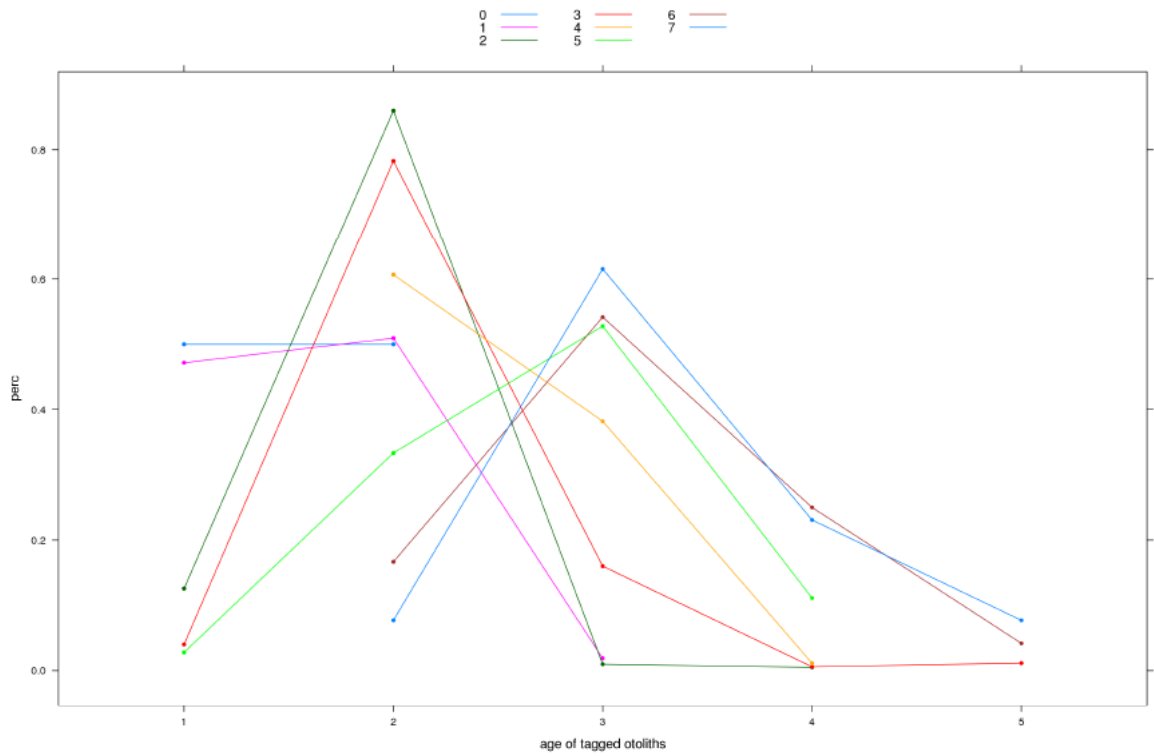


Figure 13: Probability of allocation of untagged ages to tagged ages.

Table 6 presents the transition matrix computed from this exercise.

Age difference	Untagged age										
	0	1	2	3	4	5	6	7	8	9	10
-6	0	0	0	0	0	0	0	0	0	0.67	1
-5	0	0	0	0	0	0	0	0.08	0.25	0.33	0
-4	0	0	0	0	0	0.03	0.17	0.62	0.75	0	0
-3	0	0	0	0	0	0.33	0.54	0.23	0	0	0
-2	0	0	0	0.04	0.61	0.53	0.25	0.08	0	0	0
-1	0	0	0.13	0.78	0.38	0.11	0.04	0	0	0	0
0	0	0.47	0.86	0.16	0.01	0	0	0	0	0	0
1	0.5	0.51	0.01	0.01	0	0	0	0	0	0	0
2	0.5	0.02	0	0.01	0	0	0	0	0	0	0

Table 6: Transition matrix - probability of allocation of otoliths classified to tagged ages when classified.



## 5 **Compilation of biometric data of hake otolith (Tor c)**

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In recent years the IEO has been created a database (2004-2007) of otolith weight from ICES Subareas VIIIa, b, VIIIc and IXa that includes surveys as well as otoliths from commercial sampling (N:17.000). It contains the typical fish information (TL, capture date, sex, etc) obtained in biological sampling and also the traditional age reading results (invalid method) with the associated otolith weight. The problems found with hake age estimation do not permit the use of otolith weight as a good descriptor of the relationship somatic growth and age in hake. However this database has been partially used for comparison between geographical areas and preliminary results were presented during this workshop. This work is being undertaken by a Ph.D. student within the IEO (for more information, see Annex 5).

Information on biometric parameters of hake otoliths from individuals collected in three important areas of their Northeast Atlantic distribution (Gulf of Cádiz, Galician Waters and Porcupine Bank), were investigated as a tool to detect differences between distant locations. There were no consistent differences between left and right otoliths and between sexes within the same length classes. The preliminary results indicated that Porcupine Bank increment of otolith weight by length class is significantly lower than in the rest of areas.

## 6 **Workshop WKAEH 2009 (Tor d,e,f)**

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Given the relevance of the WKAEH for the assessment of hake stocks, and its terms of reference (TORs), a presentation session was organized during the workshop to promote a wide discussion on ageing and growth issues that are presently compromising the quality of Northern and Southern hake assessment (2010 Benchmark).

Participants are listed in Annex 2 with detailed information regarding exercises where they participated.

The workshop was divided into four parts:

- 1) Reporting on the results of the exchange programme 2009,
- 2) A presentation session of recent studies related with growth and complementary information on the species conducted so far.
- 3) Working in two subgroups based on the expertise: Age Readers (G1) and Growth, Modelling /Assessment experts (G2).
- 4) Plenary sessions for presenting and discussing the results of both subgroups

The presentation session consisted in the following contributions which abstracts are reported in Annex 5:

- Hake Age Estimation: State of the Art (W. McCurdy *et al.*)
- Exploring WKAEH Exchange data towards a transition matrix (E. Jardim, see section 4.4 )
- Tagging and Recapture Results from Southern Stock (C. Piñeiro *et al.*)
- How fast does hake grow? Recent advances from mark recapture and rearing experiments, (H. de Pontual *et al.*)
- Fisheries connectivity within the northern hake stock: the pivotal role of Porcupine bank (P. Presa *et al.*)

- Daily growth study of Hake otoliths: Preliminar Results (L. Rodriguez, Phd Student Vigo)
- Study on biometric data of hake otoliths (L. Rodriguez, Phd Student Vigo).
- Analysis of length frequency distribution data for *Merluccius merluccius* from the ICES Divisions VIIIc and IXa, (M. P. Sampedro and N. Perez)
- Would be possible to approach to Model of Growth of Hake based on Daily Growth and Tagging Material?, (C. Pineiro *et al.*)
- Can we Model Otolith Growth and Opacity Patterns as a Response to Environmental Factors and Fish Metabolism?, (H. de Pontual *et al.*)
- Effects of growth in Southern Hake Assessment, (S.Cerviño)
- Growth issues related with Anglerfish by J. Landa.
- WebGR - Web Services for support of Growth and Reproductions Studies. (E. Jardin).

### 6.1 Revising age estimation method of hake (Tor c , subgroup G1)

The reader group first discussed thoroughly the interpretation of a sub sample of the tagged otoliths collection with the aims of reaching a consensual interpretation and deriving preliminary guidelines for hake otolith interpretation. This sub-sample was selected from the collection of tagged otoliths, based on the time at liberty (6, 12, 16, 24, 36 moths), in order to check for the timing of deposition of the translucent zone of this "time calibrated" part of the otolith (B area in Figure 14). Twelve otoliths were discussed in detail.

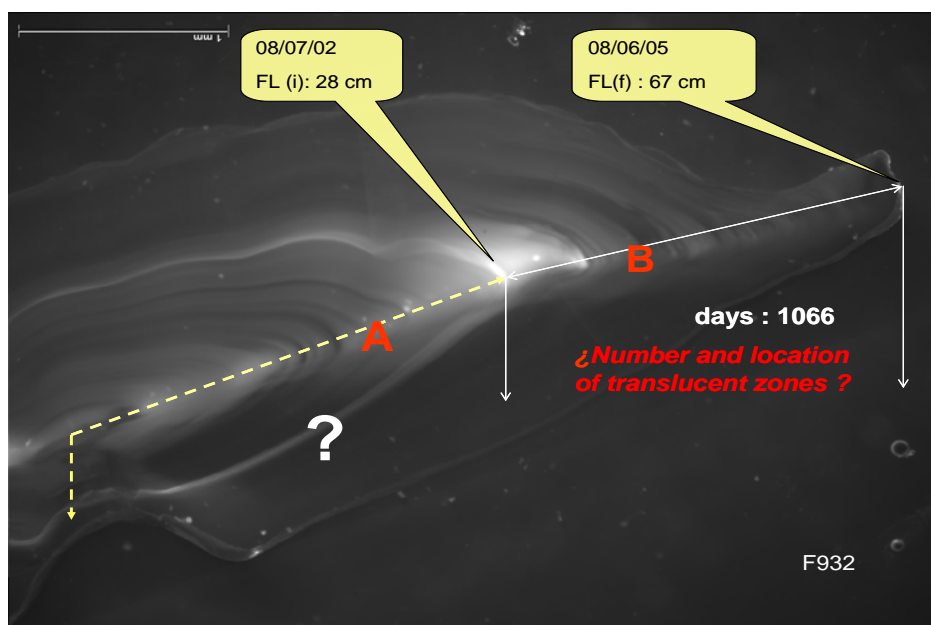
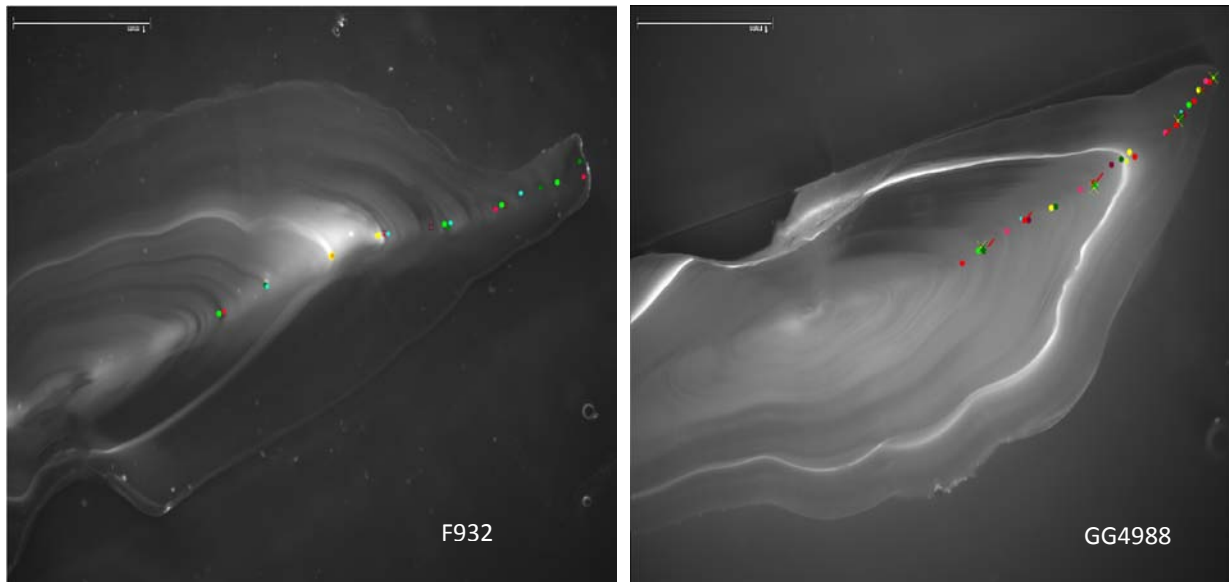


Figure 14: Example of tagged otolith (TS) showing the otolith growth before (A) and after tagging (B) which is very useful information to interpret the growth pattern for estimating the age and to locate the translucent zones.

The main conclusions of the discussion were that the appearance of the time-known part of the otolith varies considerably with no evident seasonal opacity variations. The interpretation of successive opaque and translucent zones proved to be difficult

even in the "known" part of the otolith. The use of the OTC reference mark position in the otolith structure is an important aid to assigning *annuli* around the time of tagging, however it is only of limited use in explaining the overall structure of the *annuli* within the otolith. The location of the rings presented a high variability as was clearly demonstrated by the discrepancies found (see for instance the Figure 15). The group found that the problems of interpretation are not restricted to only older ages (larger fish) but common across all age classes analyzed.



**Figure 15: Comparison of assignments of annuli by different readers (readers are represented by different colours).**

However the group agreed on the following consensual guidelines aimed at improving the precision of the age estimation:

- The growth pattern should be considered based on growth bands around the whole transversal section (TS), including both ventral and dorsal axes.
- Each annulus (annual translucent zone) consists of bands of several thinner translucent rings. Interpreting these individual components of the *annuli* as complete annuli, is a potential cause of over estimation of age when reading hake otoliths.
- Using different magnification helps to reveal the pattern of translucent and opaque bands. Changing the source of light from transmitted to reflect light can also help in the interpretation of the otolith.
- Marking the location of the *annuli* (annual translucent zones): For inter-calibration exercises, the coloured dots should be placed on the image at the point where each annulus ends and the following season's opaque growth begins. This is difficult to interpret in hake, but it is the outer edge of the last translucent band/ring in each group of these zones or rings, that the reader considers to represent an annulus.
- The growth pattern should be considered based on growth bands around the whole of the transversal section (TS), including both axes, ventral and dorsal.

Based on these guidelines, readers agreed to conduct two more inter calibration exercises (exercise 4 and 5). Exercise 4 consisted in individual reinterpretation of a subset of tagged otolith using related information in order to check for a potential increase of precision resulting from the discussion of preliminary guidelines.

Exercise 5 consisted in individual reinterpretation of a subset of untagged otoliths previously analyzed during the 2003-2004 exchange and workshop. The aim was to assess the impact of moving interpretation criteria on precision.

### 6.1.1 Sample collection

For exercise 4, a subset of 48 images of tagged otoliths was selected including samples which presented either poor or high agreement in exercise 2 and 3. This subset was individually interpreted using new guidelines related to modified growth estimation. Additional information was sex, tagging date and time at liberty (Annex 4). Fish length was not provided.

For exercise 5, a subset of 49 otolith images from the 2003 otolith exchange was selected according to their high/low agreement (corresponding to young/old fish) obtained in 2003 (detailed information in Annex 4). This subset was individually interpreted using new guidelines related to modified growth estimation. Fish length was not provided.

The fish length distributions of samples used in both exercises are shown in Figure 16.

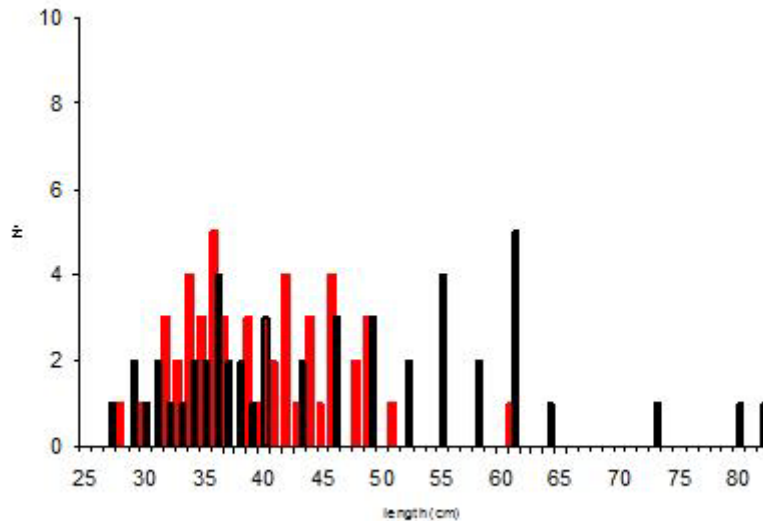


Figure 16: Length Frequency distributions of the sample sets used during the workshop for exercise 4 (in red) and exercise 5 (in black).

### 6.1.2 Methods

The data were analyzed using the same methodology as for the 2009 otolith exchange (see section 4.2).

### 6.1.3 Results

The precision indexes obtained for exercises 4 and 5 are summarized in table 7.

	WKAEH 2009			(WK 2004) <i>Exchange 2003</i>	
	Exer. 4 (OTC)	Exer. 5 (blind)	Exer. 5 (blind)	Based on ageing criteria	Based on ageing criteria
Number of exercise conducted					
Type of readers	All Readers	All Readers	Hake readers	All Readers	Hake readers
N	48	49	49	49	49
Fish TL (cm)	29-62	27-82	27-83	27-83	27-83
APE (%)	10.6	39.7	28.3	22.8	18.7
CV (%)	14.6	50.6	64.5	29.7	24.4
PA (%)	86.7	52	35.2	38.8	42.4
Age range (years)	0-5	0-7	0-7	0-14	0-14

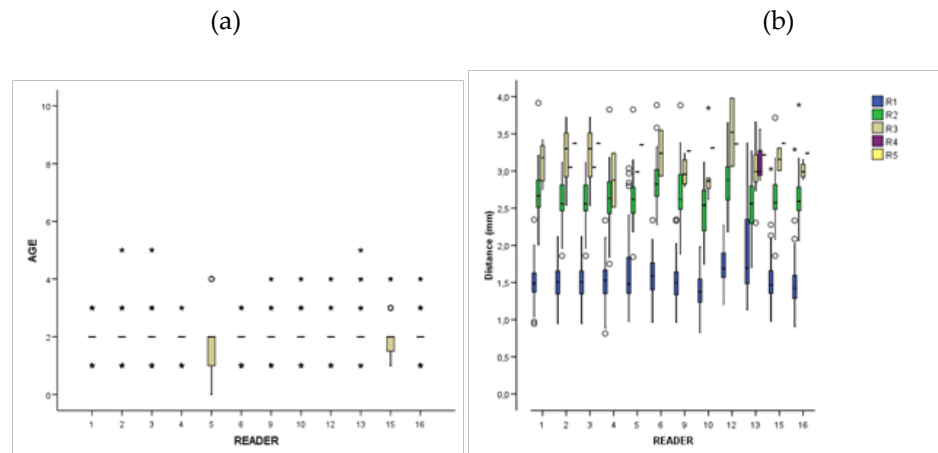
**Table 7: Summary of the workshop results by exercise conducted in comparison with the results of the exchange (2003) previously conducted to the WK 2004.**

Regarding exercise 4 (tagged otoliths), table 7 shows a good level of agreement (86.7%) and a low CV (14.6%), slightly better than the results obtained in exercises 2 and 3 (see table 2). The available tagging data, mainly consisted of fish tagged at ~20-30 cm with a limited number of individuals that had time at liberty greater than 2 calendar years. This is the reason why most of the individuals were allocated ages of 1 or 2 years (94%) with very few older fish present. About 70% of the sampled individuals were allocated a modal age of 2, by the readers of exercise 4. The differences in estimation of age ranged from full agreement to 4 years of difference. There was full agreement on 42% of the samples. There were 63% of samples with agreement higher than 90% which is the accepted value used in most age based stock assessments.

For readers that currently provide ALKs for assessment purposes, agreement was higher with no more than 1 year difference in ages assigned to the sample set. Full agreement was made on 40 of the 48 otoliths (83%). The influence of implied knowledge of fish size and image pattern information acquired (consciously or subconsciously), during previous examination of these images must be considered in this comparison. The readers were familiar with this sub-sample due to a short age range of recaptured tagged fish. It was previously read during the exchange program (two times) and the otoliths came from a tagging experiment where the average length of fish for tagging was about 30 cm. Therefore it was easily deducible that before the OTC ring there should be one or no more than two *annuli*. Consequently despite not having information on fish length at tagging date, there was an idea of the size of fish.

The box-whisker plot for all readers showed that the range of ages attributed was very narrow with a mean value of 1,9 years (Figure 17 a). Figure 17 b illustrates that

almost all readers with exception of Readers 12 and 13 showed the same median (1.5 mm) for the first annual ring considered.



**Figure 17: Box whisker plot of age readings conducted by all readers participating in the exercise 4, ages values (a) and ring distances (b).**

The results of exercise 5 regarding APE and CV in (%) undertaken during the workshop are presented together with the results of the previous exercise conducted in 2003 (WK2004) in order to see the differences after 6 years (Table 7). The values of APE and CV obtained now for all readers were higher (39.7 and 50.6% respectively) than in 2004 (22.8 and 29.7% respectively). The level of agreement obtained improved, with a value of 52% in contrast to the previous value of 38.8%. Regarding the hake readers, the results clearly show a considerable imprecision in 2009 compared to 2004, with values of 28.3 and 18.7% respectively for APE, and 64.5 to 24.4% for CV and PA of 35.2 in contrast 42.4% in 2004. These high levels of APE and CV demonstrate that the age estimation of hake otoliths by ALK readers is still not sufficiently precise and highlight the continuing difficulty for the hake readers to interpret the complex growth pattern structure when there is no additional information on tagging.

The results of the cross comparison of the ages given by hake readers that participated in both workshops 2009 and 2004 (exchange 2003) on the same subsample (untagged fish) and without information on fish size indicated a high variation of the ages assigned and differences in the mean value of age estimated (Figure 18) as expected using new interpretation guidelines.

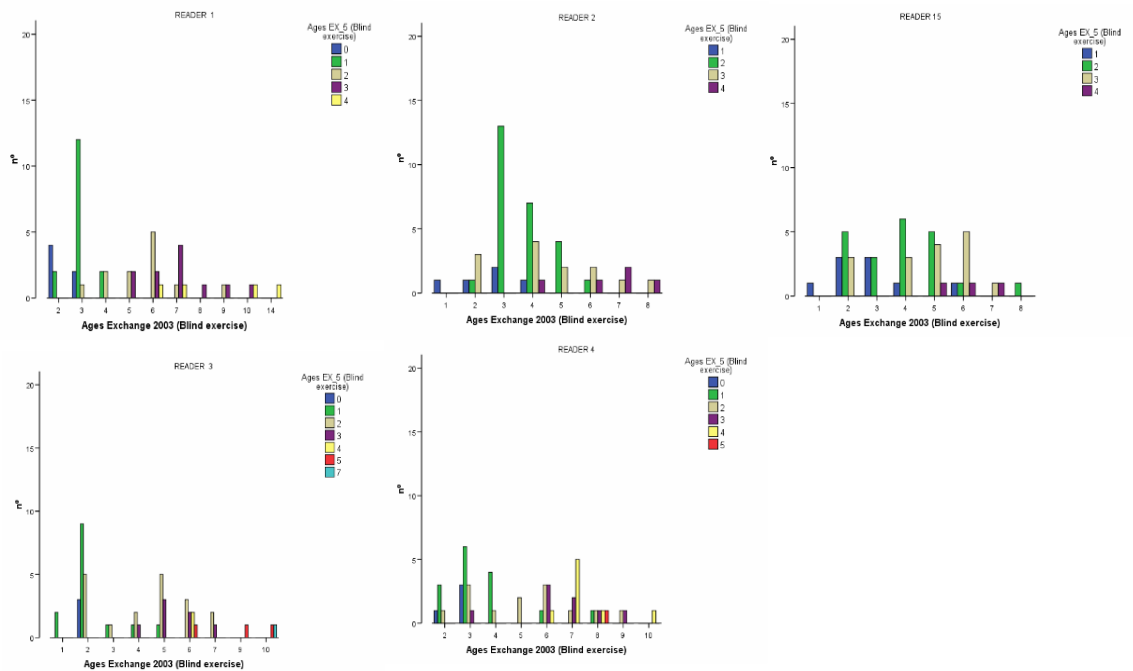


Figure 18: Cross comparison analysis of age estimated in the exercise 5 by hake readers.

The readers showed distinct perception of the otolith growth pattern before and after having been confronted to tagged otoliths and the species "new" growth pattern. The discrepancies between age estimates before and after tagging information are clear and although the variation observed was comparably high, there was a reduction in age range from 1 to 14 years, to 1 to 7 years.

The age distributions obtained by hake readers in both, the 2004 and 2009 workshops (Figure 19 a and b respectively) show differences in the median value of age estimated on the same sample taking into account the both growth models, slow and fast, going from 4 to 2 years.

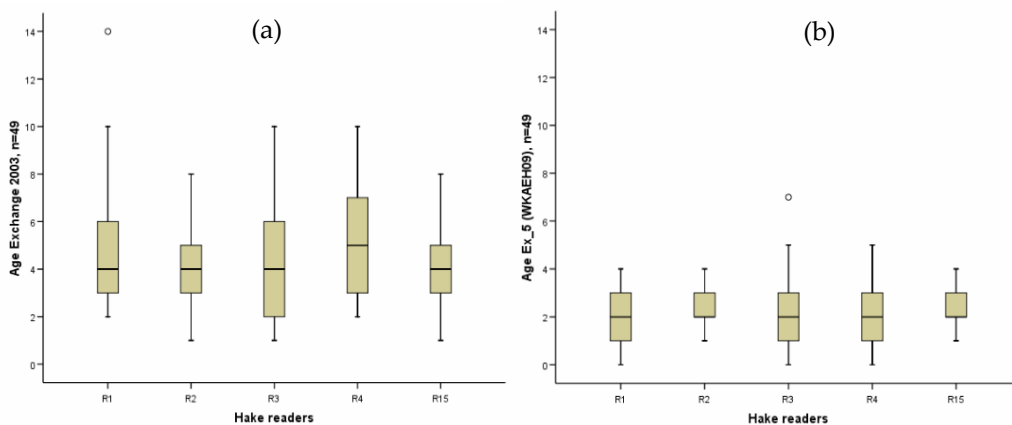


Figure 19: Box whisker plot of age readings conducted by hake readers participating in both workshops 2009 (a) and 2004 (b), showing the comparison of age estimates values.

General results can be summarized as follows:

- An overall comparison among the results of the all exercises showed that the precision and agreement for blind exercises (exercises 0,1 and 5) were low (APE: 28.3-49.7%, CV:41.2-66.8% and PA:3.6-52%). These results contrasted with the supervised exercises (exercises 2, 3 and 4) where the precision and agreement increased considerably (APE: 10.9-16.8%, CV:14.4-21.8%, PA:73.9-86.7%) with low differences among them. This demonstrated that being hake expert reader or not, the tagging information make easier the age interpretation of hake otoliths, improving the precision and agreement among readers.
- The analysis performed from tagging material confirms that the previous internationally agreed ageing criteria are neither accurate nor precise and provide overestimation of age. This raises concern about the use, for stock assessment, of ALKs that were inaccurate.
- Information on tagging and recapture resulted in a general shift towards younger ages (from 0-10 to 1-5 years) for the same size range of fish.
- Although extremely valuable for assigning annuli between time at tagging and time at recapture, the OTC mark is of less value for explaining the otolith structures between birth and the date of tagging. The appearance of the otolith region between tagging and recapture seems to vary independently of the season which complicates the interpretation of opaque and translucent zones (there are high discrepancies between readers regarding the positions of the assigned annuli).
- Ageing criteria based on tagged material cannot be provided, considering the difficulties found on tagging material with the otolith growth structures and their interpretation. However preliminary guidelines have been established to help the interpretation of otoliths.
- The tagged otolith collection is still missing oldest fish (i.e. fish more than 70 cm and with significant time intervals between tagging and recapture). More reference material is needed.

## **6.2 Methods for estimating hake catches age composition to be used by ICES (Tor d, *Subgroup 2*)**

The results of the 2009 hake otolith exchange (see section 4.) clearly confirm previous evidence (de Pontual *et al.* 2006) of ageing errors in Age-Length keys (ALK) due to wrong criteria of otolith interpretation for the species. As a consequence, the next ICES assessments will have to correct for this source of bias.

Two different approaches were considered:

- 1) Incorporate, in the Bayesian age-structured model currently used in ICES, a component dealing with probabilities of ageing errors when constructing an ALK. The priors can be estimated using IEO and IPIMAR data of the 2009 otolith exchange (tagged otoliths) as those labs kept the same reading criteria whereas other labs did not.
- 2) Use a new growth model estimated from tag-recapture data alone or including daily increment data (age 0).

It was decided to work about advantages/disadvantages of the two considered options:



### Approach 1

This approach consists in trying to incorporate estimated ageing errors in Age-Length keys (ALK) within a stock assessment model. The starting point was the Bayesian age-structured model currently used in ICES for the assessment of the southern hake stock, as detailed in the ICES WGHMM 2009 report (expanded to include discards information). An additional component was now incorporated in the model, capturing the probability that a fish whose true age is  $atrue$  is assigned age  $aread$  when constructing an ALK, with assigned ages constrained to be larger than or equal to true ages. Denoting the above mentioned probability by  $P(atrue \rightarrow aread)$ , it must then hold that the sum of  $P(atrue \rightarrow aread)$  for  $aread$  going from  $atrue$  to the oldest age considered in the assessment (8+) is equal to 1, for each true age  $atrue$ .

The stock assessment model considered contains a population dynamics process, which is age-structured and corresponds to true ages. Estimates of landed and discarded numbers-at-age, for the true ages, are obtained from the model by application of the Baranov catch equation. Relative indices of abundance-at-age can also be derived from the model, for the true ages, through catchability-at-age parameters for the different tuning fleets.

To acknowledge the fact that observations (landings, discards and relative abundance indices) relate to assigned ages ( $aread$ ) rather than to true ages ( $atrue$ ), all model derived quantities are converted from true to assigned ages using the probabilities  $P(atrue \rightarrow aread)$ . Stochastic observation equations link the observed data to these converted quantities.

Two possibilities could be considered:

- 1) To estimate  $P(atrue \rightarrow aread)$  externally and plug the estimated values into the assessment model
- 2) To estimate  $P(atrue \rightarrow aread)$  as part of the assessment, that is, at the same time than all other unknown parameters in the assessment model. The data on landings, discards and relative abundance indices, in combination with model assumptions, provide information about stock and fisheries parameters and the probabilities  $P(atrue \rightarrow aread)$ .

The second possibility was explored during the workshop, using the Bayesian approach as in the southern hake stock assessment. A prior distribution was constructed for the probabilities  $P(atrue \rightarrow aread)$  on the basis of the information provided by the ages read from the exchanged otoliths. For a same otolith, the ages read by the most expert hake readers were considered as “true” ages when the experts had tagging information available and as “assigned” ages when the experts lacked this information. For each of the “true” ages present in the exchanged otoliths, the proportion of times that each possible “assigned” age appeared in the exchanged otoliths was calculated and is displayed in the following matrix (rows represent “true” and columns “assigned” ages):

	0	1	2	3	4	5	6	7	8+
1	0	0.32	0.58	0.11	0	0	0	0	0
2	0	0	0.09	0.63	0.27	0.01	0	0	0
3	0	0	0	0.41	0.35	0.11	0.05	0.05	0.03
4	0	0	0	0	0.17	0.17	0.33	0.17	0.17
5	0	0	0	0	0	0	1	0	0

These proportions, as well as additional expert opinion from workshop participants, were used to guide the prior choices for the probabilities  $P(atriue \rightarrow aread)$ . To be more specific, these probabilities were defined as:

$$P(atriue \rightarrow aread) = \exp[x(atriue, aread)] / \sum_{a=atriue}^{8+} \exp[x(atriue, a)] ,$$

where  $x(atriue, aread)$  are independent normally distributed random variables with some suitable chosen mean values and variance equal to 0.25. The mean values were chosen so as to obtain prior medians of  $P(atriue \rightarrow aread)$  in (loose) agreement with the observed proportions shown in the preceding matrix and the additional expert opinion. This procedure resulted in the following prior medians and 95% central probability intervals for the matrix of  $P(atriue \rightarrow aread)$  values (rows represent true ages and columns read ages):

	0	1	2	3	4	5	6	7	8+
0	0.39 (0.15,0.69)	0.45 (0.19,0.74)	0.09 (0.03,0.26)	0.03 (0.01,0.08)	0	0	0	0	0
1	0	0.17 (0.06,0.39)	0.54 (0.27,0.79)	0.19 (0.07,0.44)	0.07 (0.02,0.19)	0	0	0	0
2	0	0	0.11 (0.04,0.27)	0.21 (0.08,0.46)	0.42 (0.19,0.70)	0.21 (0.07,0.46)	0	0	0
3	0	0	0	0.04 (0.01,0.12)	0.09 (0.03,0.23)	0.18 (0.06,0.4)	0.36 (0.16,0.63)	0.18 (0.06,0.4)	0.09 (0.03,0.22)
4	0	0	0	0	0.03 (0.01,0.09)	0.06 (0.02,0.17)	0.12 (0.04,0.31)	0.25 (0.09,0.52)	0.5 (0.24,0.75)
5	0	0	0	0	0	0.02 (0.01,0.08)	0.05 (0.01,0.16)	0.1 (0.03,0.3)	0.81 (0.57,0.93)
6	0	0	0	0	0	0	0.01 (0,0.05)	0.03 (0.01,0.11)	0.95 (0.85,0.99)
7	0	0	0	0	0	0	0	0.01 (0,0.03)	0.99 (0.97,1)
8+	0	0	0	0	0	0	0	0	1

The entire stock assessment model, incorporating these prior distributions on the probabilities of age reading errors, was coded in the free Bayesian software WinBUGS. Unfortunately, errors detected in the new part of the code as well as the large amount of time that takes running the program in WinBUGS, made it impossible to obtain results during the workshop. Further exploratory work along these lines will be continued during the coming weeks and months.

Results from a model run that finished after the workshop indicate that the approach shows some promise, but it is also highly complex. The model has a very large number of parameters, as it attempts to estimate the matrix of  $P(atrue \rightarrow aread)$  together with all other assessment model parameters. It also takes a very long time to run (about 4.5 days for the run performed). Being able to use an approach like this would still require a substantial amount of additional work, in order to fine tune model settings and choose appropriate prior distributions, so as to make sure model fits are good and results sensible (this is not the case for some of the outputs from the run conducted). Additionally, a faster platform than WinBUGS would be necessary. The use of AD Model Builder might potentially be a way forward in this respect.

**Approach 2**

The objective is to estimate a new and reliable growth model to be incorporated to the assessment model.

Two sets of data are available:

- Ifremer tagged data (ie observed incremented lengths at incremented ages)
- IEO estimated ages of 0 group juveniles at given lengths based on otolith "daily" increment counting.

Different options were considered:

- a) Non linear estimation of VB model using tag-recapture data

- b) Non linear estimation of VB model using daily increment data
- c) Two branch model using both sets of data
- d) A model with two likelihood components for both data sets.

Some preliminary results for the 3 options are reported below:

**Model a:** tagged data

$$\Delta L = (L^\infty - L1) (1 - e^{-(k\Delta T)})$$

with  $\Delta L = L2 - L1$  (length increment) where L1: length-at-release and L2: length-at-recapture

- $L^\infty$ : asymptotic length corresponding to null growth
- k: growth rate constant at which  $L^\infty$  is approached
- $\Delta T$ : elapsed time between release and recapture

data being weighted by the number of "days at liberty"

This model corresponds to that described by de Pontual *et al.* (Annex 5)

**Model b:** age data from daily increment counting (0 group)

$$\text{age} = t0 - \text{Log}((L^\infty - L) / L^\infty) / k$$

with - L: the fish length

- t0 the theoretical "age" of the fish at 0 size

This model provided unrealistic estimates of K and  $L^\infty$  ( $L^\infty$  too low)

**Model c:** two branch model exploiting tag data and daily data

$$y = b * ((L^\infty - L1) * (1 - e^{-(k * \Delta T)}) + (1 - b) * (t0 - \text{Log}((L^\infty - L) / L^\infty) / k)$$

(b being a dummy variable used for the two branch model)

This model provided unrealistic estimates of K and  $L^\infty$  ( $L^\infty$  too high)

**Model c bis:** same approach than the latter one but with "similar" variances

$$y70 = b * ((L^\infty - L1) * (1 - e^{-(k * \Delta T)}) / 70 + (1 - b) * (t0 - \text{Log}((L^\infty - L) / L^\infty) / k)$$

This model provided unrealistic estimates of K and  $L^\infty$  ( $L^\infty$  too low)

**Model d:** model with two likelihood components exploiting tag data and daily data

$$y = b * Y1 + (1 - b) * Y2$$

(as in previous models, b is a dummy variable)

with:

$$Y1 \text{ follows a normal distribution with mean } = ((L^\infty - L1) * (1 - e^{-(k * \Delta T)})$$

$$Y2 \text{ follows a normal distribution with mean } = (t0 - \text{Log}((L^\infty - L) / L^\infty) / k)$$

The variances of Y1 and Y2, which determine the scaling between the two likelihood components, are estimated and are not assumed to be equal.

This model provided the best adjustments and estimates of K,  $L^\infty$  and t0 within the range of what is to be expected for hake. It has the advantage of estimating all VB parameters using the best information available.

In conclusion, it was not possible to finish the work regarding the estimation of new growth parameters. The model with two likelihood components showed the best results but it must be further explored.

The idea of a more flexible VBGM with a different values of  $k$  based on the size/age has been proposed.

It has also been indicated that daily information is not fully reliable because it is based on otolith interpretation and because of the sampling period (spring survey). Temperature and food are two parameters that drive growth which is seasonal-dependant. It will be necessary to have data through the year.

Another approach to obtain a new growth model was proposed. Using the known otolith distances (nucleus-tetracycline; tetracycline-otolith edge) a back-calculation method could be applied. This idea was discussed because hake might likely be an opportunistic species which could stop the somatic growth at a certain moment while the otolith keeps growing. Besides, hake is a species with a highly variable individual growth. This phenomenon tends to make the length-otolith relationship non-linear, but linear within a given age. The advantage of this new approach would be the use of homogeneous data for the model which would permit avoiding the use of a two branch model that it is necessary using traditional tag model + daily growth model.

It has been suggested going on working on growth models by the 2010 ICES benchmark

## **7 Conclusions from exchange and WKAEH**

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1. The comparison among the results of the exercises shows that being hake expert reader or not, tagging information make easier the age interpretation of hake otoliths, improving the precision and agreement among readers in contrast with the age interpretation without tagging information.
2. Tagged material can only validate the period of otolith growth between tagging and recapture. Most tagged fish are in the size range 35 to 45 cm and more recoveries are required from fish with a longer time between release and recapture, to validate older ages. Daily growth studies may provide a better interpretation of otolith growth patterns in smaller fish.
3. The complexity of age estimation of European hake remains and knowledge of validated ages is essential to further develop the new interpretation for the age estimation of the species.
4. A preliminary set of guidelines have been established to help the interpretation of otoliths but it will required further refinement using tagging recaptures of younger and older fish in order to study the growth structure of the otolith in relationship with time and session to help identify true growth rates. It will take time for otolith readers to adjust to the new fast growth model for hake and the data generated will be used to correct the errors in the current growth model.
5. Different approaches were considered to incorporate estimated ageing errors in Age-Length keys (ALK) due to the wrong criteria of otolith interpretation for the species by incorporating the errors within a stock assessment model or using an new growth model estimated from tag-recapture data alone or including daily increment data (age 0). Results indicated that these ap-

proaches showed some promise but further work on growth models will be necessary and it was suggested going on working by the 2010 ICES benchmark.

6. It was very positive bringing stock assessors, otolith readers and research scientists together, to understand that hake are a much faster growing species that it was believed and it was recognised the necessity of working together towards a solution to handle age data for the assessment.

## 8 Recommendations

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1. It is recommended to replace the previous criteria for hake estimation with the current evolving guidelines for hake age estimation developed at this workshop. Further research is needed to develop these guidelines to increase the accuracy and precision of ALKs.
2. More validated data is required to increase the understanding of the hake otolith growth pattern. This could be achieved by tagging experiments in different areas, including the Mediterranean Sea, and experiments in controlled conditions. Research on the effects of environmental factors on otolith formation should be supported.
3. It is recommended that assessment readers re-read a common collection of circa 250 otoliths from previous years, using the new age estimation guidelines described in Section 6.1. This data could be used to investigate possibilities of providing a transition matrix from the old to the new ALKs. Involved participants will include MI, IPIMAR, IEO, IFREMER, AZTI, COISPA, HCMR
4. There should be an annual intercalibration exchange (circa 100 otoliths) in order to check future stability of agreement between age readers. For the next exchange, otoliths from previous workshops should be included in the sample set. These exchanges should be conducted using the new WebGR program which will be available in Jan 2010.
5. Another workshop should be performed in three years to continue promoting standardization of methodologies and practices for age estimation of hake based on the current work done. It is recommended readers continue working and discuss by correspondence.
6. Continue work on the analysis of tagging, 'daily' ring counting and age readings to: (i) estimate a growth model or, (ii) develop an error transition matrix between ages identified with previous protocol and ages identified with tagged otoliths or daily ring counts. Both approaches would allow the integration of a growth model or age reading errors into the stock assessment model.
7. The work undertaken during this WK could be published as a monographic article or in a publication in the ICES CRR series.

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## **Annex 1: Agenda**

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### Monday, 9 November 2009: Opening of meeting

15: 00-16:00: Welcome and Introduction to the Workshop (organising, logistics, time schedule) and ToRs

- Presentation of Hake Age Estimation: State of the Art (W.McCurdy)
- Reporting on the Results of the Exchange Programme 2009 (C. Piñeiro, and H. de Pontual)

16:00-16:20: Coffee Break

16:20-18:00: Discussion in a Plenary Meeting

- Agenda Revision : Discussion for Planning the Work for the Next Days and Assigning Responsibilities

### Tuesday, 10 November 2009:

09:00 – 10:30: Plenary Meeting: Presentation Session of Recent Studies related with growth conducted so far (aprox. 15 m each)

- i. Tagging and Recapture Results from Southern Stock (C. Piñeiro)
- ii. Recent advances on European Hake Growth: A synthesis on growth from tag-recapture experiments and experiment in controlled condition (H. de Pontual)

10:30– 11:00: Coffee break

11:00– 13:00: Continue the presentations on recent studies related with growth and complementary information on the species

- iii. Recent results of stock separation based on Genetic studies ( P. Presa; U.Vigo)
- iv. Daily growth study on hake (L. Rodriguez, Phd student Vigo)

13:00 – 14:30: Lunch Break (A catering service is organised at the lab)

14:30– 16:00: Contributions: Cont.

- v. Can we Model Otolith Growth and Opacity Patterns as a Response to Environmental Factors and Fish Metabolism? (H. de Pontual)

16:00-16:30: Coffee Break

16:30-18:00: Contributions: Cont.

- vi. Study on biometric data of hake otoliths (L. Rodriguez, Phd student Vigo).
- vii. Effects of growth in Southern Hake Assessment (S.Cerviño)
- viii. Growth issues related with Anglerfish by J. Landa.
- ix. Discussion and Revision of the plans for working the next days and assigning responsibilities.

Wednesday, 11 November 2009

09:00 – 10:30: Plenary Meeting: Contributions:

- Results of the Exchange otolith by E. Jardim
- Length distribution from catches (discards+ landings) :Progression analysis (P. Sampedro)
  - i. An approach to a Model of Growth based on DG and tagging Recapture.
- Planning of the age readings for the next days/ Discussion

Working into 2 Sub-Groups:

**Tasks by** Sub-Groups:

**G1.- Age readers participants: Tasks**

1. Results of otolith exchange 2009: Revise the Sources of problems found in the otolith interpretation: Identification of Sources of discrepancies, Interpretational Differences.
2. Examine the interpretation of those otolith (6 months, 1 year... at liberty)
  - a. Examining Ageing Criteria of Hake Otoliths Based on Tagging-Recapture Material.
  - b. Read again a subsample of the otolith exchange.
  - c. Evaluate the Relevance of Traditional Ageing Method (ALK for stock assessment) in Relationship to Tagging-Recapture Material.
3. To Progress in the Implementation of Quality Control and Quality Assurance (QC/QA) in the Laboratories.
4. Discussion and Evaluation of the Exchange: Report the Results, Responsibilities, make recommendation for further cooperation or Workshops

**G2.- Assessment people and growth experts: Tasks**

- a. Discussion and Evaluation of the results. (Impact on the Benchmark and assessment WG)
- b. Examining the potential of tagged material to provide new model of growth
- c. Growth models analysis: based on two sources of data tagging and daily growth

10:30– 11:00: Coffee break

11.00 – 13:00: Working in subgroups

13:00 – 14:30: Lunch

16.00-16.30: Coffee Break

16:30-18.00: Working in subgroups

20:00-20:30 Supper for all participants of Workshop

Thursday, 12 November 2009

09:00 – 10:30: Plenary (update) discussion on progress

Draft report Workshop: Structure

11.00 – 13:00: Working in subgroups

13:00 – 14:30: Lunch

14:30– 16:00: Working in subgroups

16:30-18.00: Each group presents its findings to a plenary discussion. Compilation of the report by group. Draft of the WKAEH Report.

Friday, 13 November 2009

09:00 – 10:30: Working in subgroups

10:30– 11.00: Coffee break

11.00 – 13:00: Draft report Workshop (revision)

13.00 – 14:30: Lunch

14:30 – 18:00: Plenary meeting: Recommendations and conclusion

Final report (revision)/end of workshop

## Annex 2: List of participants and Reader experience

Table 1: Participants in the workshop showing their participation group exercises and the institution and country they come from.

Participants	Groups according to task	Reader	Exercises participants	Institution	<i>email</i>	Country	
C. Piñeiro	Co-Chair			IEO	<a href="mailto:carmen.pineiro@vi.ieo.es">carmen.pineiro@vi.ieo.es</a>	Spain (Atl)	
H. de Pontual	Co-Chair			IFREMER	<a href="mailto:helene.De.Pontual@ifremer.fr">helene.De.Pontual@ifremer.fr</a>	France (Atl)	
E. Jardim	Assessment experts			IPIMAR	<a href="mailto:ernesto@ipimar.pt">ernesto@ipimar.pt</a>	Portugal (Atl)	
S. Cerviño				IEO	<a href="mailto:santiago.cervino@vi.ieo.es">santiago.cervino@vi.ieo.es</a>	Spain (Atl)	
P. Sampedro				IEO	<a href="mailto:paz.sampedro@co.ieo.es">paz.sampedro@co.ieo.es</a>	Spain (Atl)	
C. Fernandez				IEO	<a href="mailto:carmen.fernandez@vi.ieo.es">carmen.fernandez@vi.ieo.es</a>	Spain (Atl)	
M. Sainza	Hake Readers	R1	1,2,4,5	IEO	<a href="mailto:maria.sainza@vi.ieo.es">maria.sainza@vi.ieo.es</a>	Spain (Atl)	
S. Dores		R2	1,2,4,5	IPIMAR	<a href="mailto:sdores@ipimar.pt">sdores@ipimar.pt</a>	Portugal (Atl)	
S. Beattie		R3	1,2,4,5	MI	<a href="mailto:susan.beattie@marine.ie">susan.beattie@marine.ie</a>	Ireland (Atl)	
S. Warnes		R4	1,2,4,5	CEFAS	<a href="mailto:steve.warnes@cefasc.co.uk">steve.warnes@cefasc.co.uk</a>	UK England (Atl)	
J. L. Dufour		R5	1,2,4,5	IFREMER	<a href="mailto:jean.louis.dufour@ifremer.fr">jean.louis.dufour@ifremer.fr</a>	France (Med)	
W. McCurdy	Experts Readers in other species	R6	3,4,5	AFBI	<a href="mailto:willie.mccurdy@afbini.gov.uk">willie.mccurdy@afbini.gov.uk</a>	UK N. Ireland (Atl)	
D. Schroeder*		R7	3	VTI-DF	<a href="mailto:dorit.schroeder@vti.bund.de">dorit.schroeder@vti.bund.de</a>	Germany (Atl)	
I. Quincoces**		R8	3	AZTI	<a href="mailto:iquincoces@azti.es">iquincoces@azti.es</a>	Spain (Atl)	
J. Landa		R9	3,4,5	IEO	<a href="mailto:jorge.landa@st.ieo.es">jorge.landa@st.ieo.es</a>	Spain (Atla)	
J. Felix		R10	0,3,4,5	IFREMER	<a href="mailto:jerome.felix@ifremer.fr">jerome.felix@ifremer.fr</a>	France (Med)	
J. Rey		R11	0,		IEO	<a href="mailto:javier.rey@ma.ieo.es">javier.rey@ma.ieo.es</a>	Spain (Med)
E. Lefkaditou	Other participants Readers	R12	0,4,5	HCMR	<a href="mailto:teuthis@ncmr.gr">teuthis@ncmr.gr</a>	Greece (Med)	
J. L. Perez Gil		R13	0,4,5	IEO	<a href="mailto:joseluis.perez@ma.ieo.es">joseluis.perez@ma.ieo.es</a>	Spain (Med)	
I. Loureiro*		R14	0		IEO	<a href="mailto:i.loureiro@vi.ieo.es">i.loureiro@vi.ieo.es</a>	Spain (Atl)
S. Hoey		R15	4,5		MI	<a href="mailto:selene.hoey@marine.ie">selene.hoey@marine.ie</a>	Ireland (Atl)
P. Carbonara		R16	4,5		COISPA	<a href="mailto:carbonara@coispa.it">carbonara@coispa.it</a>	Italy (Med)

L. Rodriguez -Fdez		Daily G.	<a href="mailto:lorena.rodriguez@vi.ieo.es">lorena.rodriguez@vi.ieo.es</a>	Spain (Atl)
P. Presa	Other Experts	Genetist	<a href="mailto:pressa@uvigo.es">pressa@uvigo.es</a>	Univ. Vigo Spain

\* Readers who participated only in the Exchange but not in the workshop./ \*\* Participated during the Workshop as assessment expert.

Table 2: Experience of readers who participated in the exchange and the workshop, showing their the institution and country they come from.

Surame	Name	Institution	Country	Hake ( <i>M. merluccius</i> )	Demersal Species	Pelagic Species
Beattie	Susan	Marine Institute	Ireland	2003-2004	2002-2009	2007-2009
				2 300	22 400	3 300
Carbonara	Pietro	COISPA	Italy	2002-2009	2002-2009	2002-2007
				4 000	3 850	1 600
Dores	Sandra	IPIMAR	Portugal	2007-2009		
				9 000		
Dufour	Jean Louis	IFREMER	France	2002-2009	1982-2009	
				7 500	203 600	
Felix	Jerome	IFREMER	France		2008-2009	2008-2009
					9 300	1 000
Hoey	Selene	Marine Institute	Ireland	2002-2004	2006-2007	
				2 300	1 000	
Landa	Jorge	IEO	Spain		1991-2006	2002-2002
					28 100	400
Lefkaditou	Eugenia	HCMR	Greece	2009-2009		1997-2008

Surame	Name	Institution	Country	Hake ( <i>M. merluccius</i> )	Demersal Species	Pelagic Species
				800		680
Loureiro	Isabel	IEO	Spain	1998-2000	1998-2002	2003-2009
				3 000	9 000	10 000
McCurdy	Willie	AFBI NI	Ireland	2009-2009	1982-2009	1980-2009
				200	127 900	6 600
Pérez Gil	José Luis	IEO	Spain	2004-2009		
				6 600		
Piñeiro	Carmen Gloria	IEO	Spain	1986-2009	1990-2006	
				48 000	9 200	
Quincoces	Iñaqui	AZTI	Spain		1999-2004	
					12 000	
Rey	Javier	IEO	Spain	2000-2004	2004-2004	
				500	20	
Sáinza	María	IEO	Spain	1994-2008	2004-2008	1993-1993
				82 500	3 500	1 000
Schröder	Dorit	VTI SF	Germany			
				1 500	10 700	
Warnes	Steve	CEFAS	England	1986-2009	1978-1986	1976-2009
				48 000	54 000	297 000

### Annex 3: Protocol for reading and Instructions for Gimp

#### Protocol of Hake Otolith Exchange 2009

Dear all,

As you know the ICES PGCCDBS 2008 have recommended an age reading Workshop on Age Estimation of European Hake (WKAEH) with a previous otolith exchange among laboratories involved in the assessment of the hake stocks. IEO is in charge of organizing this exchange. This coming age reading workshop on hake will be held in Vigo, (IEO) Spain, from 9 to 13 November, 2009. *Chair: C. Piñeiro (IEO, Spain), Co-chair: H. De Pontual (IFREMER, France)*. Further information about TORs is available on the report of ICES PGCCDBS 2008.

The exchange will consist in three age reading exercises using digital images of otolith sections from Atlantic waters kindly supplied by IFREMER. The first two exercises will be done by hake age readers whereas the third one will concern expert readers involved in other species.

For analysing the age interpretation process, readers will have to annotate every annulus on each digitised image using the free program GIMP2.6 (see Appendix).

Considering the hold-up in the process, it would be ideal if all readers could read and annotate their interpretation in a maximum of one month, since the sample set is received. If there is any problem, please indicate me at which date you will be able to send your results.

The exchange will be conducted as follows:

#### 1. Exchange exercises:

1.1 Exercice 1, involving hake readers (see § 3. participants). This exercise consists in blind interpretation of otolith images (N:104) taken under reflected light (Tif-format) excel workbook with capture date and sex as extra information.

1.2 Exercice 2, involving hake readers. Interpretation of marked otolith images (N:104): Images with an OTC mark position with, capture date, sex, time at liberty and fish length at capture and recapture date as extra information.

1.3 Exercice 3, involving expert readers on other species. Interpretation of marked otolith images (N:104): Images with an OTC mark position with, capture date, sex, time at liberty and fish length at capture and recapture date as extra information.

Proposed schedule (up dated):

EXERCISE 1		EXERCISE 2		EXERCISE 3	
Start	End	Start	End	Start	End
March, 16	April, 16	May, 5	June, 10	May, 5	June, 10

#### 2. Data analysis.

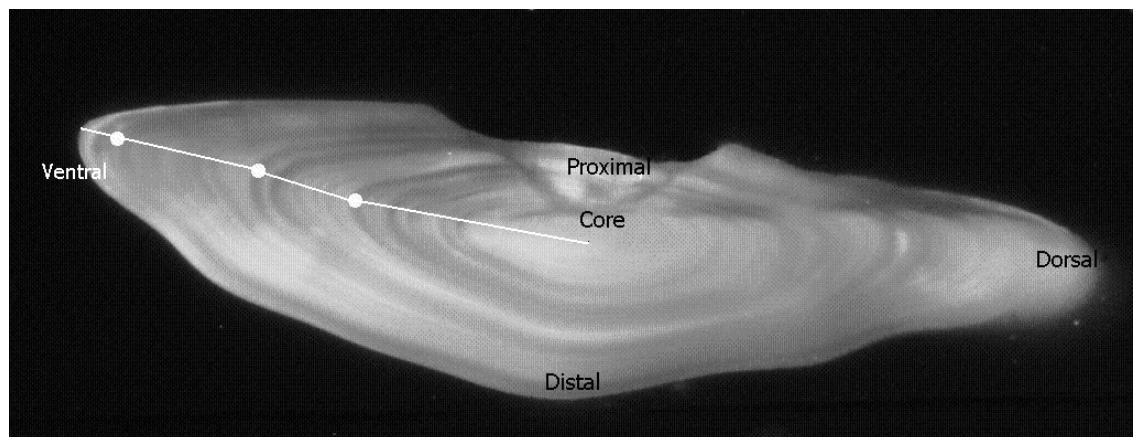
The analysis of the traditional age determination calibration will be performed using an Excel adhoc Workbook "AGE COMPARATIONS.XLS" from A.T.G.W. Eltink from RIVO following the recommendations of EFAN (Eltink et al., 2000)

Additionally, measurements using annual ring considered by each reader will be carried out by IEO by processing pictures (only in Tif-format) with a specialized software as TNPC.

### 3. Reading Procedure:

- 3.1. For exercise 1, readers should read the sample set in exactly the same way as they normally read for stock assessment (see the age interpretation method described in the Report of Hake Workshop 2004).
- 3.2. For exercise 2, and 3, readers should take into account the OTC mark position on the image and the corresponding growth zone between tagging mark and the otolith edge (between capture and recapture date)
- 3.3. Age estimation should be done by counting the translucent zones, preferably along the dorso-ventral axis from the nucleus to ventral edge as illustrated in the figure 1. Please only provide one age estimation per otolith image.
- 3.4. All readers are asked to annotate their own interpretation on the digitised images using the program GIMP2.6 Annotating images will consist in marking the positions of the annual translucent zones on a specific layer of the image (see instructions in Appendix)
- 3.5. The annual rings considered by the reader will be annotated on the transect visible on the digitised image (as in figure 1).
- 3.6. The images must be stored using the name of the reader (see Appendix 1)
- 3.7. Complete the excel workbook with the age estimated and save the file with your name "*Exercise \_ N# \_ Name of the reader*".
- 3.8. Notify the coordinator when the exercise is completed and send the excel file with readings by email.
- 3.9. The coordinator will provide you with a [ftp](ftp://vi.ieo.es) server at [ftp.vi.ieo.es](ftp://vi.ieo.es). to upload your digital images with annotations.
- 3.10. Additionally the reader should save a copy of the annotated images in a DVD with the name of the reader and country: i.e. **CG\_IEO\_HKEXH 2009**.

**Figure 1:** Picture with orientations on a hake otolith section showing the reading axis (dorso-ventral axis) to take into account for age reading: from the nucleus to ventral edge.





**4.-Age readers participants:**

The colours to be used by each participant are below. If there is a problem with the colour assigned you will contact me in order to solve it. The layer with the annotations will be saved with the reader name initials only (i.e. H9363x30\_CG.xcf for Carmen G.) as follows:

Participants	Reader	Layer name	Exercise N	Colour
M. Sainza, (IEO, Spain)	R1	MS	EX 2	red
S. Dores, (IPIMAR, Portugal)	R2	SD	EX 2	bright green
S. Beattie, (IM; Ireland)	R3	SB	EX 2	dark green
S. Warnes,(CEFAS; UK)	R4	SW	EX 2	blue
J. L. Dufour (IFREMER, France )	R5	JLD	EX 2	brown
Javier Rey	R11	JR	Ex 0	diagonal star (11) 13*13)
Eugenia Lefkaditou	R12	EL	Ex0	diagonal star (11) 13*13)
I. Qincoces (AZTI, Spain )	R8	IQ	EX 3	Orange
J. Landa (IEO, Spain)	R9	JL	EX 3	purple
J.F.Xerome (IFREMER, France )	R10	JFX	EX 3	Square pink
W. MacCurdy (AFVI, N. Ireland)	R6	WM	EX 3	yellow
D. Schroeder (VTI-DF), Germany	R7	DS	EX 3	Black

**5.-Digitised Images**

The software used for recording the interpretation by the age readers will be GIMP 2.6. A few points should be very clear to proceed with the annotation on the images (see appendix 1):

- 5.1 Do not change the magnification of the images. Any modification would compromise subsequent analysis and particularly examining different reader interpretations simultaneously.
- 5.2 Use the assigned colour (above) to annotate your images.
- 5.3 Create a new layer for annotations with your name initials.
- 5.4 DO NOT forget to save the file with the extension *\*xcf* for posterior analysis. This will allow simultaneous analysis of different interpretation layers for a given image. Save the file with your Name i.e. **H9363\_TAG\_cg.xcf** (see appendix 1).
- 5.5 Save as, this file again, with your annotations but with extension *\*Tif* (see appendix 1).
- 5.6 Please do not change the file names in any way to keep them standard.
- 5.7 The reader should annotate in the excel workbook in which exercise is participating ( the Exercise 1 or 2 )

5.8 The two files with annotated images ( **xcf** and **tif**) together with the completed excel file must be uploaded to a private **ftp** provided by the coordinator ( <ftp.vi.ieo.es>)

**Contact person:** If there is any issue, feel free to contact us.

Carmen Piñeiro: [carmen.pineiro@vi.ieo.es](mailto:carmen.pineiro@vi.ieo.es)

Maria Sainza: [maria.sainza@vi.ieo.es](mailto:maria.sainza@vi.ieo.es)

**Experience of the reader:** A brief statement describing the experience of the reader with this and other species and a summary of the quality management procedure used for this species at their Institute (no more than 100 letters). A datasheets called *experience reader* will be available in the same Excel adhoc Workbook.

Since IEO (Vigo) will be tracking the hake exchange, I would really appreciate if the participants inform me about the reading progress and if there is any concern with the time schedule or any other issue.

Due to the delayed in getting this exchange started, I would encourage all participants to read the otoliths and annotate the images as soon as they are received and then send them promptly back to me.

Good luck with your readings!

Carmen Piñeiro

C.O. de Vigo (IEO)

Carmen Piñeiro Alvarez

Instituto Español de Oceanografía

C.O. de Vigo

Apartado 1552, 36200 Vigo, Spain

[carmen.pineiro@vi.ieo.es](mailto:carmen.pineiro@vi.ieo.es)

Fax: +34(986) 492351

Telf: +34(986) 492111

#### **Instructions for annotating otolith images using GIMP 2.6**

All readers are asked to annotate each of the digitised images with their own interpretation (positions of the annual rings on a given transect) using the programme GIMP2.6. This program permits creating layers without any problem and is free downloaded at <http://www.gimp.org/downloads/>. Additionally there are tutorials available in several languages at <http://www.gimp.org/>

GIMP2.6. is an image manipulation programme, freely distributing piece of software for such task as photo retouching, image composition etc. It works on many operating systems, in many languages. The official GIMP web site contains information about downloading, installing, and using it.



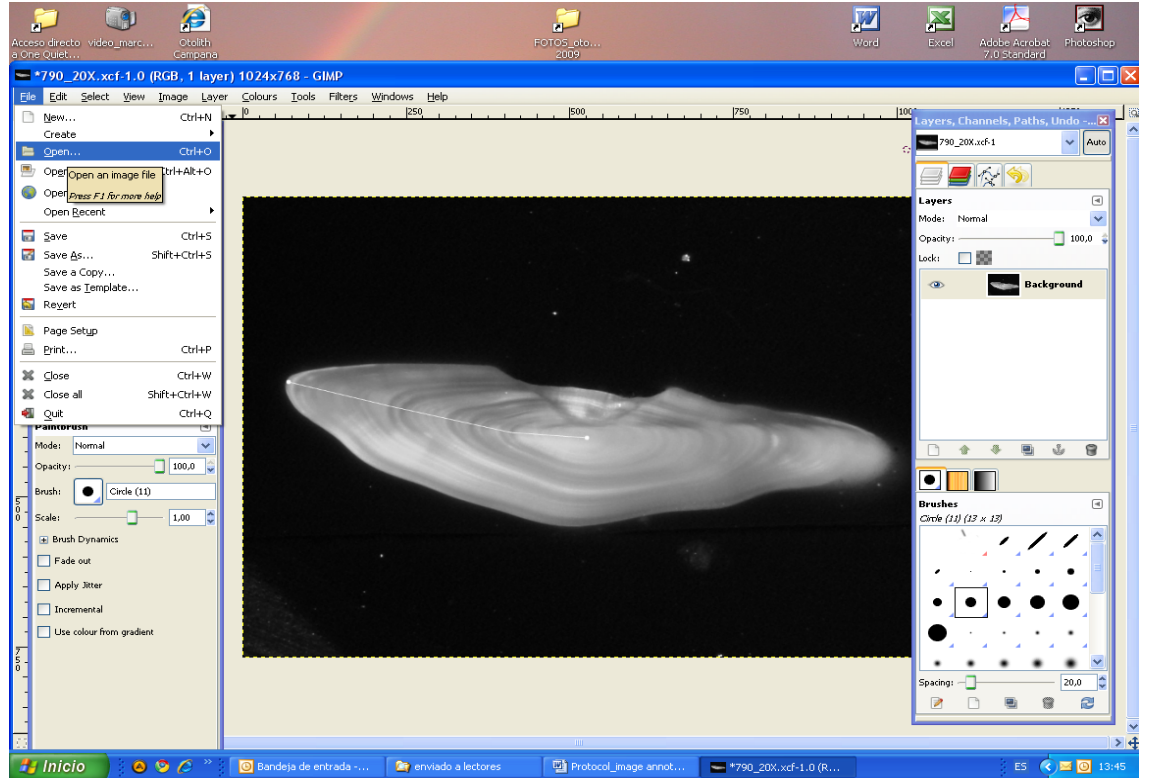
**A.- To annotate the annual rings on a layer using GIMP\_2.6, please follow the instructions below:**

1. Open the file (eg 790\_transect.tif) to work with, with the GIMP 2.6 programme
2. Add a new layer: click layer, Create a new new layer, (it has to be a transparent layer which is the default option)
3. Type your initials as layer name (for instance: cg for carmen gloria)
4. Block the background layer
5. From the toolbox
  - a. Select the colour you have been assigned (see section 3 of the protocol document ), left click on
  - b. Select the pencil with the circle (09) to indicate the positions of the rings that you are considering to be annual rings
6. Mark these positions on your layer on the transect line.
7. Save this file (button save as) with your initials in the GIMP format (eg "790\_transect\_cg.xcf") for subsequent analysis. Do not forget to change the extension of the file to \*.xcf
8. Save (save as) this file again, with the same name but with extension \*.Tif
  - a. (eg "790\_transect\_cg.tif")
  - b. Flatten image
  - c. Export
  - d. Save Tif (compression default: None)
9. At the end of the process you should have two files for the current otolith image:
  - a. One file such as 790\_transect\_cg.xcf with two layers: the background and your layer named with your initials, and
  - b. The corresponding flattened image in tiff format such as 790\_transect\_cg.tif

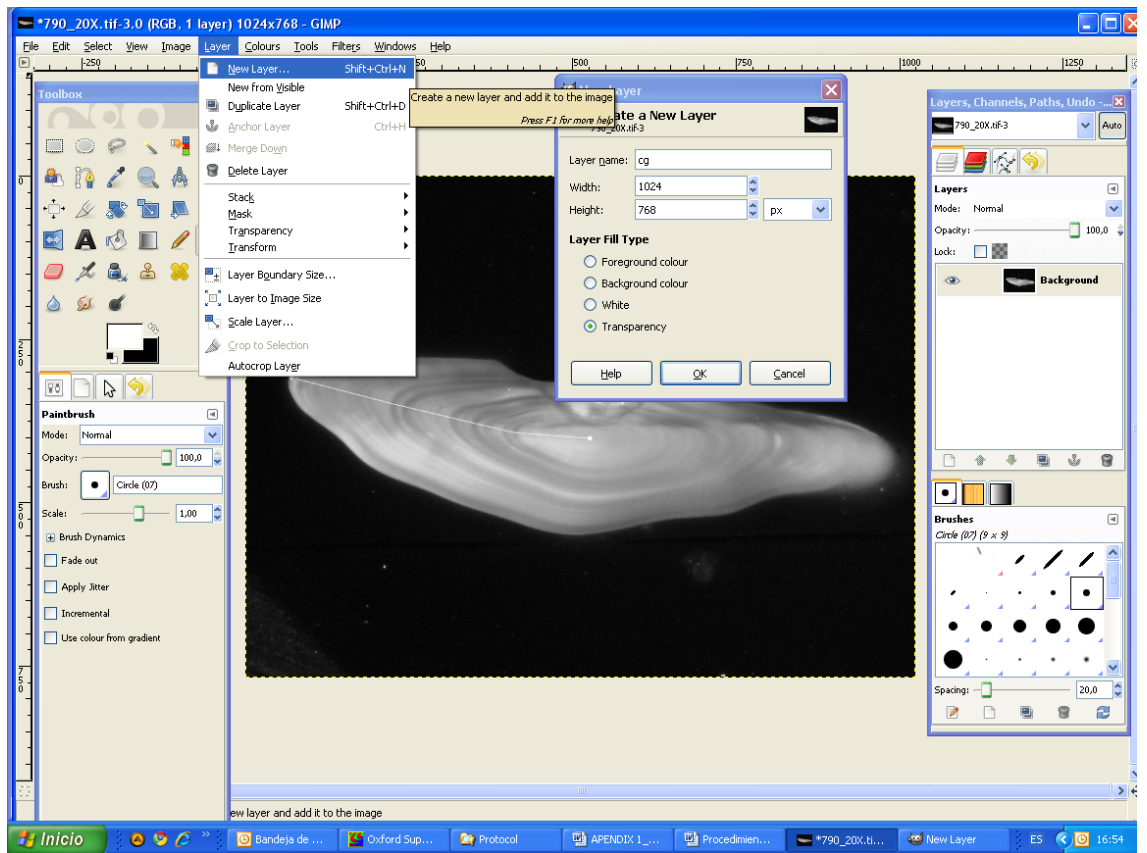
10. Proceed with the next otolith image.

### Instructions by mean of images:

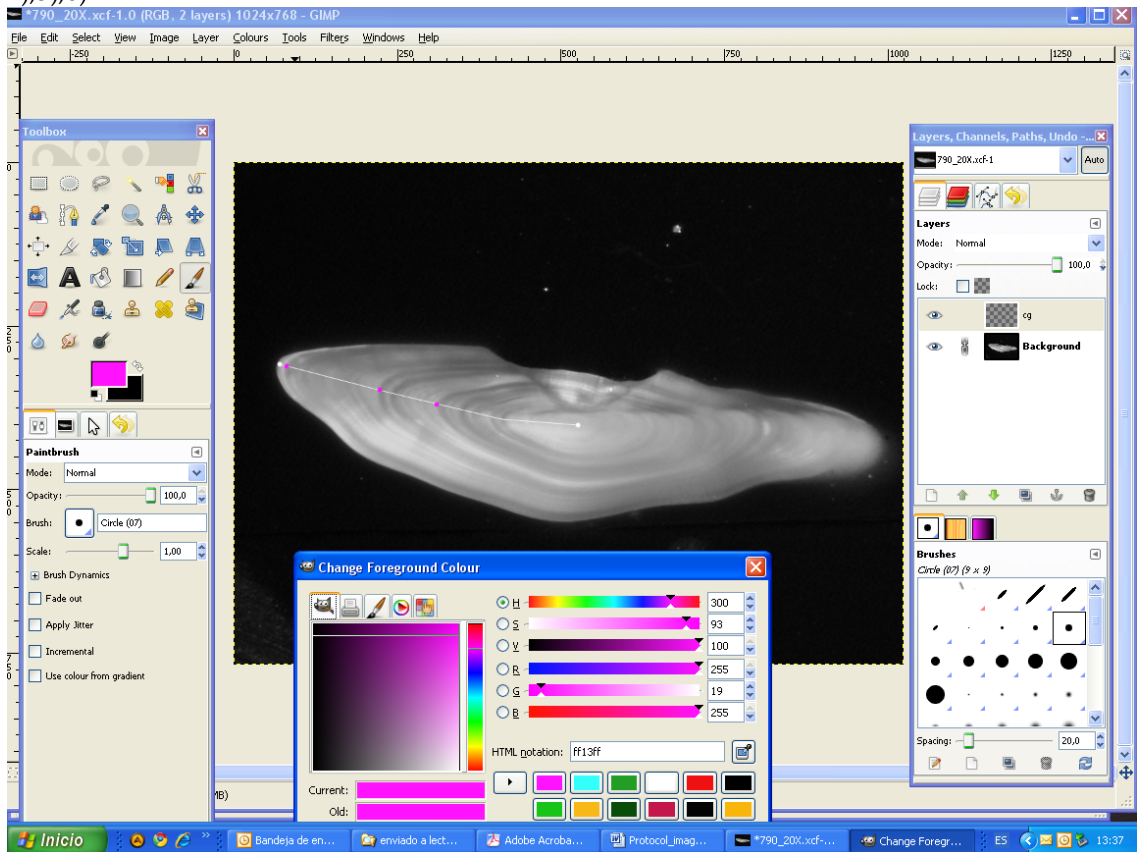
1)



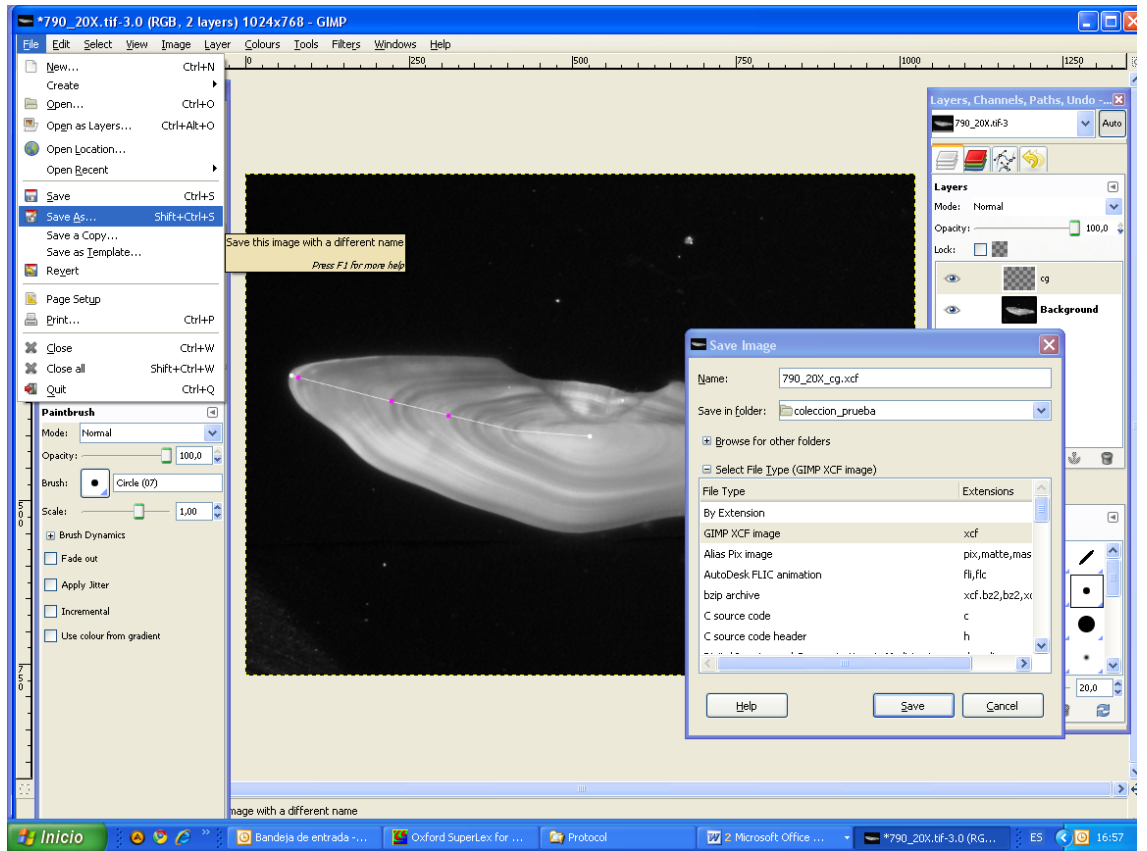
2),3)



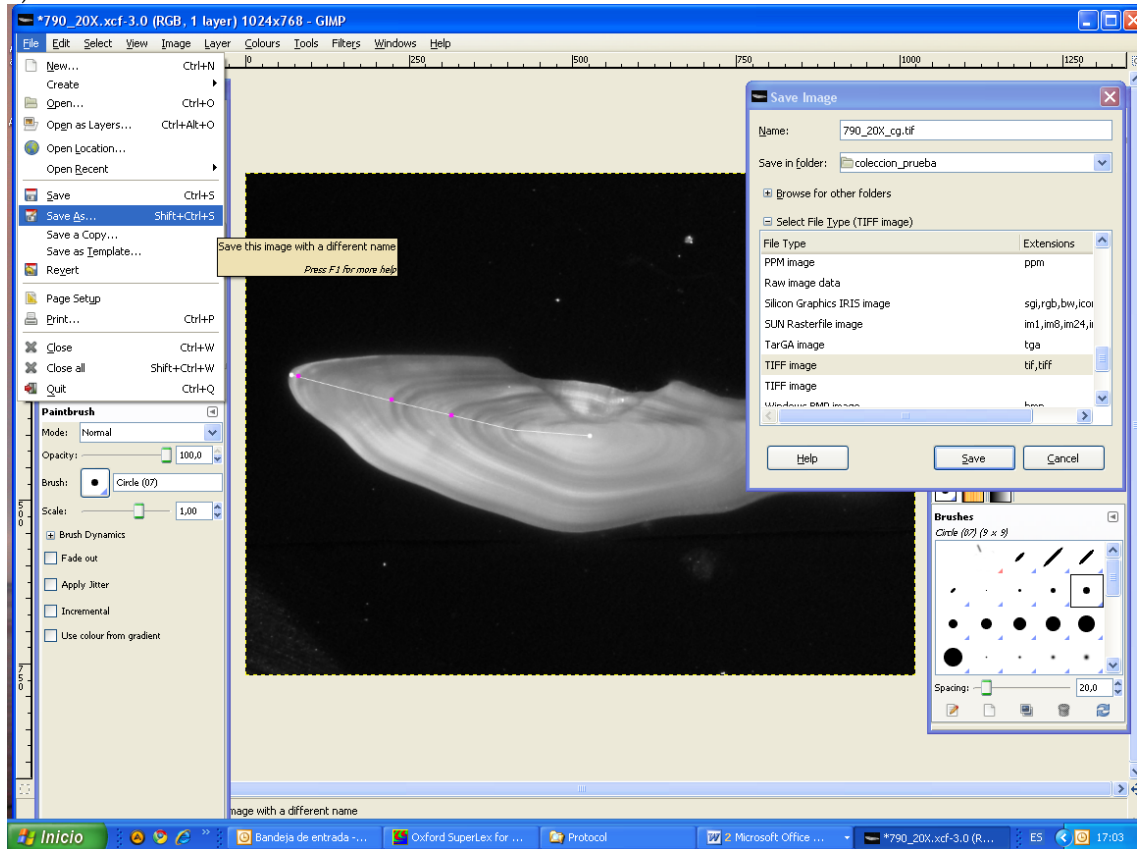
4),5),6)



7)



8)



#### **Annex 4: Results of the age estimation by reader and exercise with the basic information on otolith collection**

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See next page



Exercise 0: Exchange Program 2009												
Fish no	Fish length	Fish Sex	Capture month	IFREMER	IEO	HCMR	IEO	IEO	Mean age	Percent agreement	Precision CV	
				Reader 10	Reader 11	Reader 12	Reader 13	Reader 14				
290	42	2	3	3	1	2	3	6	3	40%	62%	
F932	67	1	6	8	2	5	9	9	7	0%	46%	
F01701	32	2	12	3	1	1	4	6	3	20%	71%	
F01997	37	2	8	2	1	2	4	5	3	0%	59%	
F02892	35	2	6	2	1	2	3	6	3	0%	69%	
F03363	49	1	9	3	1	4	4	3	3	40%	41%	
F03527	29	2	12	1	1	1	2	7	2	0%	109%	
F03654	61	3	6	6	2	4	5	10	5	0%	55%	
F03852	43	2	7	2	1	2	6	7	4	0%	75%	
F04561	38	1	3	2	2	3	4	7	4	0%	58%	
F04642	42	2	2	2	2	3	5	6	4	0%	50%	
F04661	46	2	12	1	1	2	5	4	3	0%	70%	
F04683	63	3	10	3	1	5	6	5	4	0%	50%	
F04953	35	1	4	2	1	2	5	4	3	0%	59%	
F06549	48	2	6	3	1	3	6	7	4	0%	61%	
F06638	34	1	11	2	1	1	4	6	3	0%	77%	
F06809	42	2	1	2	2	2	6	9	4	0%	76%	
F07809	43	2	7	2	2	2	5	7	4	0%	64%	
F08563	33	1	12	2	0	1	3	6	2	0%	96%	
F08588	36	1	11	1	1	1	5	3	2	0%	81%	
F09718	40	2	5	2	2	2	4	4	3	0%	39%	
F09759	40	1	1	2	2	2	4	3	3	0%	34%	
F11118	40	2	5	2	1	2	5	5	3	0%	62%	
GG00017	44	2	4	2	1	2	4	5	3	0%	59%	
GG01142	31	1	8	3	1	2	6	4	3	0%	60%	
GG01233	46	2	8	2	1	3	6	6	4	0%	64%	
GG02347	62	2	9	2	1	5	6	6	4	0%	59%	
GG02969	39	2	12	2	1	1	6	6	3	0%	81%	
GG03926	31	2	12	2	1	1	2	4	2	40%	61%	
GG03938	41	2	7	3	1	2	7	6	4	0%	68%	
GG03984	44	2	12	3	1	2	5	5	3	0%	56%	
GG04102	25	2	8	1	1	1	2	4	2	0%	72%	
GG04696	48	2	3	2	1	5	7	9	5	0%	70%	
GG04722	42	3	12	2	1	1	5	4	3	0%	70%	
GG05056	44	1	4	2	0	3	6	3	3	0%	77%	
GG05571	36	2	12	5	2	2	6	6	4	0%	49%	
GG05737	28	2	4	2	1	2	4	7	3	0%	75%	
GG05739	41	2	5	2	1	3	6	10	4	0%	83%	
GG05886	35	1	4	2	1	2	4	7	3	0%	75%	
GG06093	38	2	1	2	1	2	4	10	4	0%	96%	
h1185	37	2	11	2	2	2	5	7	4	0%	64%	
F11957	39	2	10	2	0	2	4	4	2	0%	70%	
h1400	49	2	7	2	1	2	6	6	3	0%	71%	
h1806	35	2	1	3	1	2	6	9	4	0%	78%	
F1847	32	2	10	2	1	1	4	5	3	0%	70%	
h2048	45	2	6	2	1	2	4	4	3	0%	52%	
h2049	31	1	1	2	1	3	2	4	2	0%	48%	
h2189	37	1	3	3	1	3	3	5	3	60%	47%	
h2949	38	1	6	2	1	2	5	7	3	0%	74%	
h3234	35	2	3	2	1	3	5	6	3	0%	61%	
h4259	31	3	11	1	1	2	3	6	3	0%	80%	
h43	45	1	7	2	0	2	5	4	3	0%	75%	
h4440	42	2	5	2	1	2	5	8	4	0%	80%	
GG4464	46	2	3	3	1	2	4	7	3	0%	68%	
h4660	32	1	1	2	1	1	4	4	2	0%	63%	
GG4816	47		3	3	2	2	5	6	4	0%	50%	
GG4988	62		2	3	2	3	5	7	4	0%	50%	
h5023	35	3	3	2	1	3	5	7	4	0%	67%	
h5038	46	2	7	3	1	2	7	7	4	0%	71%	
h5307	34	2	3	3	1	2	5	5	3	0%	56%	
h5667	33	1	1	2	1	1	5	10	4	0%	101%	
h5873	35	2	5	2	1	2	4	5	3	0%	59%	
F5906	35	1	7	2	1	2	4	4	3	0%	52%	
h6135	34	2	2	2	1	2	3	5	3	0%	58%	
h6146	34	2	2	2	1	2	4	3	2	0%	48%	
h6398	33	1	4	2	1	2	3	5	3	0%	58%	
F6493	38	2	1	2	1	2	3	9	3	0%	94%	
h6661	45	3	7	3	1	2	5	3	3	0%	53%	
h6693	40	1	2	2	1	2	7	9	4	0%	85%	
h6866	48	2	6	2	1	3	5	9	4	0%	79%	
h6970	33	1	2	2	1	2	3	3	2	0%	38%	
F6974	30	2	2	1	0	2	2	4	2	0%	82%	
h707	34	2	11	1	2	1	3	5	2	0%	70%	
h7235	50	2	2	4	1	4	7	9	5	0%	62%	
F7241	46	2	5	3	1	2	5	6	3	0%	61%	
F7330	51	1	11	2	1	3	6	7	4	0%	68%	
h748	33	2	3	2	1	2	3	10	4	0%	101%	
h7501	37	2	1	2	1	3	6	6	4	0%	64%	
h7588	30	1	1	2	1	2	5	4	3	0%	59%	
h7593	36	3	3	2	1	2	4	7	3	0%	75%	
h7743	38	2	3	3	1	3	4	4	3	40%	41%	
F7929	40	3	5	2	1	2	4	8	3	0%	82%	
h7958	38	1	6	2	1	2	3	3	2	0%	38%	
F8044	34	1	1	2	1	2	4	4	3	0%	52%	
F8067	43	1	8	2	1	2	5	3	3	0%	58%	
h8126	37	1	3	2	1	2	4	6	3	0%	67%	
h812	37	1	6	2	1	2	3	4	2	0%	48%	
h8247	40	1	6	2	1	2	4	4	3	0%	52%	
h8328	32	1	4	2	1	1	2	4	2	40%	61%	
h8410	35	1	3	2	1	2	4	7	3	0%	75%	
F8450	36	2	2	2	1	2	4	8	3	0%	82%	
h8672	38	2	4	2	1	2	4	3	2	0%	48%	
h8966	37	1	4	3	1	2	3	4	3	0%	44%	
F9051	29	2	2	2	2	2	4	5	3	0%	47%	
h9054	40	2	2	2	1	2	3	4	2	0%	48%	
h9105	40	1	2	2	1	2	4	6	3	0%	67%	
h913	34	1	2	2	1	2	5	7	3	0%	74%	
h9146	42	2	7	3	1	2	4	4	3	0%	47%	
h9158	41	2	6	3	1	3	5	6	4	0%	54%	
h9327	41	2	1	3	2	5	5	10	5	40%	62%	
h9363	32	1	2	2	1	2	3	5	3	0%	58%	
F9724	43	2	9	2	1	2	5	7	3	0%	74%	
ifr_408	37	1	3	2	2	2	2	3	2	0%	20%	
ifr_1356	49	2	5	2	1	-	2	3	2	50%	41%	
Total read				104	104	103	104	104				
Total NOT read				0	0	1	0	0		3.6%	63.8%	

**Exercise 1: Exchange Program 2009**

Fish no	Fish length	Fish Sex	Capture month	IEO	IPIMAR	MI	CEFAS	IFREMER	Modal age	Percent agreement	Precision CV	
				Reader 1	Reader 2	Reader 3	Reader 4	Reader 5				
290	42	2	3	3	4	6	3	3	3	60%	34%	
F932	67	1	6	9	6	10	6	8	6	40%	23%	
F01701	32	2	12	2	2	3	5	3	2	40%	41%	
F01997	37	2	8	4	3	4	1	1	4	40%	58%	
F02892	35	2	6	3	3	3	2	2	3	60%	21%	
F03363	49	1	9	7	5	6	3	2	5	20%	45%	
F03527	29	2	12	2	1	2	1	1	1	60%	39%	
F03654	61	3	6	9	6	7	6	5	6	40%	23%	
F03852	43	2	7	4	3	5	5	2	5	40%	34%	
F04561	38	1	3	3	3	3	3	2	3	80%	16%	
F04642	42	2	2	4	4	4	3	2	4	60%	26%	
F04661	46	2	12	5	4	6	3	1	4	20%	51%	
F04683	63	3	10	7	6	7	3	3	7	40%	39%	
F04953	35	1	4	3	3	3	2	2	3	60%	21%	
F06549	48	2	6	4	4	6	5	3	4	40%	26%	
F06638	34	1	11	2	2	2	2	1	2	80%	25%	
F06809	42	2	1	3	3	3	6	2	3	60%	45%	
F07809	43	2	7	4	3	4	7	2	4	40%	47%	
F08563	33	1	12	2	2	3	2	1	2	60%	35%	
F08588	36	1	11	3	3	3	2	1	3	60%	37%	
F09718	40	2	5	3	3	4	3	2	3	60%	24%	
F09759	40	1	1	4	4	5	3	2	4	40%	32%	
F11118	40	2	5	4	3	5	2	2	2	40%	41%	
GG00017	44	2	4	3	4	3	3	2	3	60%	24%	
GG01142	31	1	8	3	2	5	4	3	3	40%	34%	
GG01233	46	2	8	3	5	6	2	2	2	40%	50%	
GG02347	62	2	9	7	5	7	2	2	7	40%	55%	
GG02969	39	2	12	3	3	5	1	2	3	40%	53%	
GG03926	31	2	12	2	1	2	0	2	2	60%	64%	
GG03938	41	2	7	3	3	4	1	3	3	60%	39%	
GG03984	44	2	12	3	3	4	3	3	3	80%	14%	
GG04102	25	2	8	2	1	2	0	1	2	40%	70%	
GG04696	48	2	3	4	4	6	1	2	4	40%	57%	
GG04722	42	3	12	3	3	5	0	2	3	40%	70%	
GG05056	44	1	4	4	4	5	2	2	4	40%	39%	
GG05571	36	2	12	3	4	4	2	4	4	60%	26%	
GG05737	28	2	4	2	2	3	1	2	2	60%	35%	
GG05739	41	2	5	5	3	4	2	2	2	40%	41%	
GG05886	35	1	4	3	3	4	1	2	3	40%	44%	
GG06093	38	2	1	3	3	4	2	2	3	40%	30%	
h1185	37	2	11	3	3	4	0	2	3	40%	63%	
F11957	39	2	10	3	3	4	0	2	3	40%	63%	
h1400	49	2	7	4	4	5	1	2	4	40%	51%	
h1806	35	2	1	3	3	5	2	3	3	60%	34%	
F1847	32	2	10	2	3	3	0	2	2	40%	61%	
h2048	45	2	6	4	4	4	2	2	4	60%	34%	
h2049	31	1	1	2	1	2	1	2	2	60%	34%	
h2189	37	1	3	3	3	4	1	3	3	60%	39%	
h2949	38	1	6	3	4	5	1	2	3	20%	53%	
h3234	35	2	3	4	4	4	1	2	4	60%	47%	
h4259	31	3	11	2	3	4	1	1	1	40%	59%	
h43	45	1	7	3	3	5	2	2	3	40%	41%	
h4440	42	2	5	3	4	5	1	2	3	20%	53%	
GG4464	46	2	3	4	3	5	2	3	3	40%	34%	
h4660	32	1	1	3	2	4	2	2	2	60%	34%	
GG4816	47	3	3	4	4	7	3	3	4	40%	39%	
GG4988	62	2	2	4	4	7	5	3	4	40%	33%	
h5023	35	3	3	4	4	5	1	2	4	40%	51%	
h5038	46	2	7	3	4	6	1	3	3	40%	53%	
h5307	34	2	3	3	3	6	2	3	3	60%	45%	
h5667	33	1	1	2	3	4	2	2	2	60%	34%	
h5873	35	2	5	3	3	5	1	2	3	40%	53%	
F5906	35	1	7	3	3	4	2	2	3	40%	30%	
h6135	34	2	2	3	3	4	2	2	3	40%	30%	
h6146	34	2	2	2	3	4	2	2	2	60%	34%	
h6398	33	1	4	3	3	4	2	3	3	60%	24%	
F6493	38	2	1	3	4	5	2	2	2	40%	41%	
h6661	45	3	7	3	3	5	1	3	3	60%	47%	
h6693	40	1	2	3	4	6	1	2	3	20%	60%	
h6866	48	2	6	4	4	5	2	2	4	40%	39%	
h6970	33	1	2	2	3	4	2	2	2	60%	34%	
F6974	30	2	2	2	2	4	1	1	2	40%	61%	
h707	34	2	11	2	3	3	1	1	3	40%	50%	
h7235	50	2	2	5	6	8	4	4	4	40%	31%	
F7241	46	2	5	4	3	6	2	3	3	40%	42%	
F7330	51	1	11	4	4	8	2	2	4	40%	61%	
h748	33	2	3	3	3	5	1	2	3	40%	53%	
h7501	37	2	1	4	4	7	2	2	4	40%	54%	
h7588	30	1	1	3	3	4	2	2	3	40%	30%	
h7593	36	3	3	3	3	4	2	2	3	40%	30%	
h7743	38	2	3	3	4	6	2	2	2	40%	49%	
F7929	40	3	5	3	3	4	2	2	3	40%	30%	
h7958	38	1	6	3	3	4	1	2	3	40%	44%	
F8044	34	1	1	3	3	4	3	2	3	60%	24%	
F8067	43	1	8	4	3	5	1	2	3	20%	53%	
h8126	37	1	3	3	3	4	2	2	3	40%	30%	
h812	37	1	6	3	3	5	2	2	3	40%	41%	
h8247	40	1	6	3	3	3	2	2	3	60%	21%	
h8328	32	1	4	2	2	4	1	2	2	60%	50%	
h8410	35	1	3	3	3	4	2	2	3	40%	30%	
F8450	36	2	2	3	4	4	1	2	4	40%	47%	
h8672	38	2	4	3	3	4	2	2	3	40%	30%	
h8966	37	1	4	3	3	5	3	3	3	80%	26%	
F9051	29	2	2	3	2	5	2	2	2	60%	47%	
h9054	40	2	2	3	4	6	1	2	3	20%	60%	
h9105	40	1	2	3	3	6	3	2	3	60%	45%	
h913	34	1	2	3	4	5	1	2	3	20%	53%	
h9146	42	2	7	3	4	6	4	2	4	40%	39%	
h9158	41	2	6	4	4	6	3	3	4	40%	31%	
h9327	41	2	1	4	3	7	6	3	3	40%	39%	
h9363	32	1	2	3	3	4	3	2	3	60%	24%	
F9724	43	2	9	4	3	7	1	1	1	40%	78%	
ifr_408	37	1	3	3	1	3	3	2	3	60%	37%	
ifr_1356	49	2	5	6	1	4	2	2	2	40%	67%	
Total read				104	104	104	104	104		46.3%	41.2%	
Total NOT read				0	0	0	0	0				

**Exercise 2: Exchange Program 2009**

Fish no	Fish length	Fish Sex	Capture month	IEO Reader 1	IPIMAR Reader 2	MI Reader 3	CEFAS Reader 4	IFREMER Reader 5	MODAL age	Percent agreement	Precision CV
290	42	2	3	2	2	4	2	2	2	80%	37%
F932	67	1	6	4	5	4	4	4	4	80%	11%
F01701	32	2	12	1	2	3	1	1	1	60%	56%
F01997	37	2	8	-	3	-	2	1	2	33%	50%
F02892	35	2	6	2	3	2	2	2	2	80%	20%
F03363	49	1	9	3	3	3	3	2	3	80%	16%
F03527	29	2	12	1	1	2	1	1	1	80%	37%
F03654	61	3	6	3	4	3	3	3	3	80%	14%
F03852	43	2	7	2	3	3	2	2	2	60%	23%
F04561	38	1	3	2	2	2	2	2	2	100%	0%
F04642	42	2	2	2	2	2	2	2	2	100%	0%
F04661	46	2	12	2	2	4	1	1	2	40%	61%
F04683	63	3	10	3	4	5	3	3	3	60%	25%
F04953	35	1	4	2	2	2	2	2	2	100%	0%
F06549	48	2	6	2	3	3	2	2	2	60%	23%
F06638	34	1	11	2	1	2	1	1	1	60%	39%
F06809	42	2	1	2	2	2	2	2	2	100%	0%
F07809	43	2	7	3	3	3	2	2	3	60%	21%
F08563	33	1	12	1	2	1	1	1	1	80%	37%
F08588	36	1	11	1	2	2	1	1	1	60%	39%
F09718	40	2	5	2	2	2	2	2	2	100%	0%
F09759	40	1	1	2	2	2	2	2	2	100%	0%
F11118	40	2	5	2	2	2	2	2	2	100%	0%
GG00017	44	2	4	3	2	3	2	2	2	60%	23%
GG01142	31	1	8	3	2	4	2	2	2	60%	34%
GG01233	46	2	8	3	3	2	2	2	2	60%	23%
GG02347	62	2	9	4	3	3	2	2	3	40%	30%
GG02969	39	2	12	2	2	2	1	1	2	60%	34%
GG03926	31	2	12	2	1	2	1	1	1	60%	39%
GG03938	41	2	7	2	2	3	2	2	2	80%	20%
GG03984	44	2	12	2	2	2	2	1	2	80%	25%
GG04102	25	2	8	1	1	4	2	1	1	60%	72%
GG04696	48	2	3	2	2	3	2	2	2	80%	20%
GG04722	42	3	12	2	2	3	1	1	2	40%	46%
GG05056	44	1	4	2	2	2	2	2	2	100%	0%
GG05571	36	2	12	1	2	3	1	2	1	40%	46%
GG05737	28	2	4	1	1	2	2	1	1	60%	39%
GG05739	41	2	5	3	2	3	2	2	2	60%	23%
GG05886	35	1	4	2	2	2	2	2	2	100%	0%
GG06093	38	2	1	2	2	3	2	2	2	80%	20%
h1185	37	2	11	2	2	3	1	1	2	40%	46%
F11957	39	2	10	2	3	2	2	2	2	80%	20%
h1400	49	2	7	2	3	3	2	2	2	60%	23%
h1806	35	2	1	2	2	3	2	2	2	80%	20%
F1847	32	2	10	2	3	4	2	2	2	60%	34%
h2048	45	2	6	2	3	2	2	2	2	80%	20%
h2049	31	1	1	1	1	2	2	2	2	60%	34%
h2189	37	1	3	2	2	2	2	2	2	100%	0%
h2949	38	1	6	2	3	3	2	2	2	60%	23%
h3234	35	2	3	2	2	3	2	2	2	80%	20%
h4259	31	3	11	2	2	2	1	1	2	60%	34%
h43	45	1	7	2	3	3	2	2	2	60%	23%
h4440	42	2	5	2	2	2	3	2	2	80%	20%
GG4464	46	2	3	2	2	3	2	3	2	60%	23%
h4660	32	1	1	2	2	3	2	2	2	80%	20%
GG4816	47	3	3	2	3	3	2	2	2	60%	23%
GG4988	62	2	2	4	3	4	4	5	4	60%	18%
h5023	35	3	3	2	2	2	2	2	2	100%	0%
h5038	46	2	7	2	3	3	2	2	2	60%	23%
h5307	34	2	3	2	2	3	2	2	2	80%	20%
h5667	33	1	1	-	2	-	2	2	2	100%	0%
h5873	35	2	5	2	2	3	2	2	2	80%	20%
F5906	35	1	7	2	3	3	2	2	2	60%	23%
h6135	34	2	2	2	2	3	2	2	2	80%	20%
h6146	34	2	2	2	2	3	2	2	2	80%	20%
h6398	33	1	4	-	2	-	2	2	2	100%	0%
F6493	38	2	1	2	2	3	2	2	2	80%	20%
h6661	45	3	7	3	3	4	2	2	3	40%	30%
h6693	40	1	2	2	2	3	2	2	2	80%	20%
h6866	48	2	6	3	3	3	2	3	3	80%	16%
h6970	33	1	2	2	2	3	2	2	2	80%	20%
F6974	30	2	2	1	1	-	2	2	1	50%	38%
h707	34	2	11	1	2	2	1	1	1	60%	39%
h7235	50	2	2	4	3	4	3	3	3	60%	16%
F7241	46	2	5	2	2	2	2	2	2	100%	0%
F7330	51	1	11	2	3	3	2	2	2	60%	23%
h748	33	2	3	2	2	3	2	2	2	80%	20%
h7501	37	2	1	2	2	3	2	2	2	80%	20%
h7588	30	1	1	2	2	3	2	2	2	80%	20%
h7593	36	3	3	-	2	-	2	2	2	100%	0%
h7743	38	2	3	2	2	3	2	2	2	80%	20%
F7929	40	3	5	2	2	3	2	2	2	80%	20%
h7958	38	1	6	2	3	2	2	2	2	80%	20%
F8044	34	1	1	2	2	3	2	2	2	80%	20%
F8067	43	1	8	2	3	3	2	2	2	60%	23%
h8126	37	1	3	2	2	3	2	2	2	80%	20%
h812	37	1	6	2	2	2	2	2	2	100%	0%
h8247	40	1	6	2	2	3	2	2	2	80%	20%
h8328	32	1	4	-	2	-	2	2	2	100%	0%
h8410	35	1	3	2	2	2	2	2	2	100%	0%
F8450	36	2	2	2	2	2	2	2	2	100%	0%
h8672	38	2	4	2	2	3	2	2	2	80%	20%
h8966	37	1	4	2	2	2	2	2	2	100%	0%
F9051	29	2	2	2	2	3	2	2	2	80%	20%
h9054	40	2	2	2	2	4	2	2	2	80%	37%
h9105	40	1	2	2	2	3	2	2	2	80%	20%
h913	34	1	2	2	2	3	2	2	2	80%	20%
h9146	42	2	7	-	3	-	2	3	3	67%	22%
h9159	41	2	6	2	2	3	2	2	2	80%	20%
h9327	41	2	1	3	2	4	2	3	3	40%	30%
h9363	32	1	2	2	2	3	2	2	2	80%	20%
F9724	43	2	9	2	2	3	2	2	2	80%	20%
ifr_408	37	1	3	2	1	3	2	2	2	60%	35%
ifr_1356	49	2	5	3	1	3	2	2	3	40%	38%
Total read				98	104	97	104	104		73.9%	21.8%
Total NOT read				6	0	7	0	0			

**Exercise 3: Exchange Program 2009**

Fish no	Fish length	Fish Sex	Capture month	AFVI Reader 6	VTI-DF Reader 7	AZTI Reader 8	IEO Reader 9	IFREMER Reader 10	MODAL age	Percent agreement	Precision CV
290	42	2	3	2	3	2	2	2	2	75%	22%
F932	67	1	6	4	4	4	4	4	4	100%	0%
F01701	32	2	12	1	2	1	-	1	1	75%	40%
F01997	37	2	8	2	3	2	2	1	2	50%	41%
F02892	35	2	6	2	2	2	2	2	2	100%	0%
F03363	49	1	9	2	2	2	2	3	2	80%	20%
F03527	29	2	12	1	1	1	1	1	1	100%	0%
F03654	61	3	6	3	4	3	3	3	3	80%	14%
F03852	43	2	7	2	3	2	2	2	2	80%	20%
F04561	38	1	3	2	3	2	2	2	2	80%	20%
F04642	42	2	2	2	3	2	2	2	2	80%	20%
F04661	46	2	12	1	3	2	2	1	1	40%	46%
F04683	63	3	10	3	5	3	3	3	3	80%	26%
F04953	35	1	4	2	3	2	2	2	2	80%	20%
F06549	48	2	6	2	3	2	2	2	2	80%	20%
F06638	34	1	11	1	2	2	1	1	1	60%	39%
F06809	42	2	1	2	2	2	2	2	2	100%	0%
F07809	43	2	7	3	3	2	3	2	3	60%	21%
F08563	33	1	12	1	2	2	1	1	1	60%	39%
F08588	36	1	11	2	2	2	1	1	2	60%	34%
F09718	40	2	5	2	2	2	2	2	2	100%	0%
F09759	40	1	1	2	2	2	2	2	2	100%	0%
F11118	40	2	5	2	2	2	2	2	2	100%	0%
GG00017	44	2	4	2	3	3	2	2	2	60%	23%
GG01142	31	1	8	2	3	3	2	2	2	60%	23%
GG01233	46	2	8	2	2	3	2	2	2	80%	20%
GG02347	62	2	9	2	3	3	2	2	2	60%	23%
GG02969	39	2	12	1	2	2	2	2	2	80%	25%
GG03926	31	2	12	1	2	2	1	1	1	60%	39%
GG03938	41	2	7	2	3	2	2	2	2	80%	20%
GG03984	44	2	12	1	3	2	2	2	2	60%	35%
GG04102	25	2	8	2	2	1	-	1	2	50%	38%
GG04696	48	2	3	2	3	2	2	2	2	80%	20%
GG04722	42	3	12	1	3	2	-	2	2	50%	41%
GG05056	44	1	4	2	2	2	2	2	2	100%	0%
GG05571	36	2	12	1	2	2	1	2	2	60%	34%
GG05737	28	2	4	2	2	2	2	1	2	80%	25%
GG05739	41	2	5	2	3	2	2	2	2	80%	20%
GG05886	35	1	4	2	2	2	2	2	2	100%	0%
GG06093	38	2	1	2	2	2	2	2	2	100%	0%
h1185	37	2	11	1	2	2	1	1	1	60%	39%
F11957	39	2	10	2	2	2	2	2	2	100%	0%
h1400	49	2	7	2	3	2	2	2	2	80%	20%
h1806	35	2	1	2	3	2	2	2	2	80%	20%
F1847	32	2	10	2	2	2	2	2	2	100%	0%
h2048	45	2	6	2	2	2	2	2	2	100%	0%
h2049	31	1	1	2	2	2	2	2	2	100%	0%
h2189	37	1	3	2	2	2	2	2	2	100%	0%
h2949	38	1	6	2	2	2	2	2	2	100%	0%
h3234	35	2	3	2	3	2	2	2	2	80%	20%
h4259	31	3	11	1	2	2	1	1	1	60%	39%
h43	45	1	7	2	2	2	-	2	2	100%	0%
h4440	42	2	5	2	2	2	2	2	2	100%	0%
GG4464	46	2	3	2	3	3	-	3	3	75%	18%
h4660	32	1	1	2	2	2	2	2	2	100%	0%
GG4816	47	3	3	2	3	2	2	2	2	80%	20%
GG4988	62	2	2	3	4	4	3	5	3	40%	22%
h5023	35	3	3	2	2	2	2	2	2	100%	0%
h5038	46	2	7	2	2	2	2	2	2	100%	0%
h5307	34	2	3	2	2	2	2	2	2	100%	0%
h5667	33	1	1	2	2	2	2	2	2	100%	0%
h5873	35	2	5	2	2	2	2	2	2	100%	0%
F5906	35	1	7	2	2	2	2	2	2	100%	0%
h6135	34	2	2	2	3	2	2	2	2	80%	20%
h6146	34	2	2	2	2	2	2	2	2	100%	0%
h6398	33	1	4	2	3	2	-	2	2	75%	22%
F6493	38	2	1	2	2	2	2	2	2	100%	0%
h6661	45	3	7	2	2	3	2	2	2	80%	20%
h6693	40	1	2	2	3	2	-	2	2	75%	22%
h6866	48	2	6	2	3	3	-	2	2	50%	23%
h6970	33	1	2	2	2	2	2	2	2	100%	0%
F6974	30	2	2	2	2	2	2	2	2	100%	0%
h707	34	2	11	1	2	2	-	1	1	50%	38%
h7235	50	2	2	2	4	3	3	-	3	50%	27%
F7241	46	2	5	2	2	2	2	2	2	100%	0%
F7330	51	1	11	2	3	2	2	2	2	80%	20%
h748	33	2	3	2	3	2	2	2	2	80%	20%
h7501	37	2	1	2	4	2	2	2	2	80%	37%
h7588	30	1	1	2	3	2	2	2	2	80%	20%
h7593	36	3	3	2	3	2	2	2	2	80%	20%
h7743	38	2	3	2	2	2	2	2	2	100%	0%
F7929	40	3	5	2	2	2	2	2	2	100%	0%
h7958	38	1	6	2	-	2	2	2	2	100%	0%
F8044	34	1	1	2	3	2	2	2	2	80%	20%
F8067	43	1	8	2	2	2	2	2	2	100%	0%
h8126	37	1	3	2	2	2	2	2	2	100%	0%
h812	37	1	6	2	2	2	2	2	2	100%	0%
h8247	40	1	6	2	2	2	2	2	2	100%	0%
h8328	32	1	4	2	2	2	-	2	2	100%	0%
h8410	35	1	3	2	2	2	2	2	2	100%	0%
F8450	36	2	2	2	2	2	2	2	2	100%	0%
h8672	38	2	4	2	2	2	2	2	2	100%	0%
h8966	37	1	4	2	2	2	2	2	2	100%	0%
F9051	29	2	2	2	2	2	2	2	2	100%	0%
h9054	40	2	2	2	3	2	-	2	2	75%	22%
h9105	40	1	2	2	3	2	2	2	2	80%	20%
h913	34	1	2	2	2	2	2	2	2	100%	0%
h9146	42	2	7	2	3	3	-	3	3	75%	18%
h9158	41	2	6	2	3	2	2	2	2	80%	20%
h9327	41	2	1	2	4	3	-	3	3	50%	27%
h9363	32	1	2	2	2	2	2	2	2	100%	0%
F9724	43	2	9	2	3	3	-	2	2	50%	23%
ifr_408	37	1	3	2	2	2	2	2	2	100%	0%
ifr_1356	49	2	5	2	3	2	2	2	2	80%	20%
Total read				104	103	104	88	103		83.1%	14.4%
Total NOT read				0	1	0	16	1			



## Annex 5: Abstracts of presentations

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**Hake age estimation: State of the art**, by Willie McCurdy\*, C. Piñeiro, C. Morgado, M. Sainza.

*Institution: Agri-Food and Biosciences Institute (AFBI)(\*), Belfast, U. K.*

ICES CRR No. 296 is the first of a series of Cooperative Research Reports on age calibration, promoted by ICES PGCCDBS. This report reviews hake age estimation exchanges and workshops and presents a summary of the current status of the knowledge. Northern and southern hake stock assessments have used age data based on otolith analysis since 1992. During this period, considerable effort has been made to improve the precision of age data through successive age reading calibration exercises in 1997, 1999, 2001, and 2004. This goal was partially achieved and experts recently agreed on standard criteria that allowed reaching acceptable precision for ages up to 3 years. However, these criteria have never been validated. Hake are a difficult species to age and the complexity of the otolith macrostructure and growth variability is related to the long spawning season. Direct validation studies on age estimation for Hake in the NE Atlantic have not accomplished until very recently due to fragility of the species. Results from tagging experiments conducted for both stocks, have concluded that hake grows two-fold faster than considered previously. Daily growth studies have corroborated the fast growth hypothesis of this species while bioenergetics studies and an analysis of hake otolith macrostructure typology also support this hypothesis.

**Exploring WKAEH Exchange data towards a transition matrix**, by E. Jardim\*

*Institution: National Laboratory of Marine Research(\*), Unit of Marine Resources. Av. Brasília, 1449-006 Lisboa, Portugal (IPIMAR)*

The objective of this work is to compute a transition matrix that allocates an otolith classified in a specific age without tagging information, into the expected age if it was classified with tagging information. Such statistic would allow an operational procedure to rebuild the time series of hake information, without requiring a growth model, and, more importantly, keeping the information about growth over time.

**Tagging and Recapture Results from Southern Stock**, by C. Piñeiro\*, L. Rodríguez-Fernández, A. Leal, A. Gómez.

*Institución: Instituto Español de Oceanografía, Centro Oceanográfico de Vigo(\*), Subida a Radio Faro, 50. C.P.: 36390, Vigo, Pontevedra, España.*

*Correo electrónico: carmen.pineiro@vi.ieo.es*

Tagging experiments were conducted off the NW Iberian Peninsula during the period 2004-2009 to study both growth of hake (*Merluccius merluccius* L.) in the wild and movement, following the methodology described in Pontual *et al.* (2003). A total of 268 hauls were carried out between 30 and 130 m of depth, and 2715 alive tagged individuals within the size range 11-61 cm (TL) were released. The mean survival rate after capture and tagging was 70%. The recaptures obtained so far belong to two surveys and the recapture rate indicate an increase of recapture rate from 0.3% in 2004 (7 individuals) to 2% in 2009 (15 individuals). This increase is probably related with the 50 euros reward offered for each tagged fish returned to the laboratory. The time at

liberty varied from 14 to 466 days and the maximum distance observed between location of release and recapture was of 15 nautical miles. The mean somatic growth rate of all recaptured hake was  $0.029 \pm 0.013$  cm day<sup>-1</sup> (sexes combined), while the mean growth rate of the two hakes with over 340 days at liberty was  $0.052 \pm 0.003$  cm day<sup>-1</sup> (sexes unknown). These results indicate that conventional otolith age reading methods overestimate age and underestimate growth.

**How fast does Hake Grow? Recent advances from mark recapture and rearing experiments**, by H. de Pontual\*, A. Jolivet, F. Garren, R. Fablet and M. Bertignac

*Institution: IFREMER Centre de Brest(\*). Laboratoire de Biologie Halieutique. Z.I. Pointe du Diable. B.P. 70. 29280 Plouzané.*

Following the pilot experiment on hake tagging carried out in 2002, using a methodology specifically developed to catch and handle fish in good condition, we conducted four more tagging experiments in the Bay of Biscay in 2004, 2005, 2006 and 2007. A total of 27949 fish had been released among which 1200 tagged fish have been recovered until now. This corresponds to a mean recapture rate of 4.9% and times-at-liberty range from 1 to 1066 days. Somatic growth of the recoveries as well as somatic growth of fish reared in controlled conditions (aquaculture facilities) both confirms that European hake is a fast growing species with a growth potential similar to that of cod. We show that growth varies with size and sex and we propose new von Bertalanffy growth models, derived from the recovered tagged fish data. This data also reveal an inter annual growth variability.

Complementary experiments on hake successfully maintained in aquaculture facilities demonstrated that archival tagging should not drastically affect survival and growth compared to conventional tagging. Hence, two pilot experiments with DSTs (data storage tag) were carried out in the Bay of Biscay in 2006 and 2007. Nine fish with electronic tag surgically implanted in the peritoneal cavity have been recovered until now. The corresponding high resolution environmental records (pressure and temperature data at 30' interval) provide evidence of a general vertical migration pattern characterised by extended periods spent near the bottom during the day (tidal signal clearly visible on some sequences) and vertical movements (VM) at night probably to forage. During VM, fish could go across the thermocline and experienced large and abrupt temperature changes (eg from 12°C to 20°C). A signal processing method allowed to statistically analysing the characteristic features of VMs. Individual behaviour differed both in VM patterns and in horizontal movements. The available information remains too scarce to state on medium and long term migrations. However, the observed behavioural differences provide new keys for the understanding of both somatic growth variability and the complex structural patterns of hake otoliths.

These studies highlight the need to improve biological and behavioural knowledge of the specie. Such information is critical to improve assessment and management advice and hence for exploiting the resource in a sustainable way.

**Fisheries connectivity within the Northern Hake Stock: the pivotal role of Porcupine bank**, by P. Presa\*, A. Pita and M. Pérez.

*Institution: ECIMAT Marine Station - Faculty of Marine Sciences(\*), University of Vigo, 36310 Vigo, Spain;*

*E-mail: [pressa@uvigo.es](mailto:pressa@uvigo.es)*

Population genetics of exploited marine fishes allows testing the inter-annual stability of genetic structures as well as a better understanding of the migration patterns between fisheries. Herein we report spatio-temporal distribution patterns of microsatellite variation scored on 22 populations from the northern European hake stock. The architecture of this stock is characterised by a reduced gene flow between fisheries from opposite edges of its range and a high genetic admixture among central and southern fisheries. In this panmictic scenario that violates the Isolation-by-Distance model a southern-wards biomass radiation seems to occur consistently between the Celtic Sea and the Bay of Biscay. Porcupine bank and Grand Sole might play a crucial role at ensuring sustainability not only of other most northern hake fisheries but probably so of the southern hake stock. An integrative effort to interpret together the ecological approach and the genetic approach should be a priority to be considered in long-term management plan for this species.

**Daily Growth study of Hake otoliths: Preliminary Results** by L. Rodríguez-Fernández\*, F. Velasco, A. García, C. Piñeiro.

*Institución: Instituto Español de Oceanografía, Centro Oceanográfico de Vigo(\*). Subida a Radio Faro, 50.*

*C.P.: 36390, Vigo, Pontevedra, España.*

Daily growth of juvenile hake from three important areas in their Northeast Atlantic distribution (Gulf of Cádiz, Galician Waters and Porcupine Bank) were studied along two consecutive years in order to estimate and compare the growth rates of juvenile hake from distant locations in two consecutive years, 2004 and 2005, and analyse the existence of a seasonal growth pattern. A total of 146 right otoliths (sagittae) from individuals between 102 and 179 mm total length collected during the autumn bottom trawl surveys were analyzed following the methodology described by Piñeiro et al., 2008. The overall mean growth rate by area was very close, ranging from  $0.626 \pm 0.06$  mm/day ( $n=24$ ) in Porcupine Bank and  $0.674 \pm 0.07$  mm/day ( $n=36$ ) in Gulf of Cádiz. The estimated age-length relationship was explained by a power fit. The growth model indicates that one year hake may attain 24 cm long. By other hand, our results support that increments width change with time, so exist a seasonal growth pattern where growth slows down in samples collected in autumn while it increases in samples collected in spring. Finally in a next future work, growth rates and increments width in these areas will be studied considering geographical and environmental factors.



**Study on Biometric data of Hake otoliths** by L. Rodríguez Fernández\*, J. Santos, C. Piñeiro.

*Institution: Instituto Español de Oceanografía, Centro Oceanográfico de Vigo(\*), Subida a Radio Faro, 50. C.P.: 36390, Vigo, Pontevedra, España*

The variability in otolith biometric parameters was investigated as a tool to detect differences between distant locations. A total number 2122 otoliths (sagittae) from individuals between 5 and 88 cm total length collected in three important areas of their Northeast Atlantic distribution (Gulf of Cádiz, Galician Waters and Porcupine Bank), were weighed to the nearest 0.1 mg, digitised and analyzed. The images were analyzed using Leica image analysis software for the export of morphometric characteristics (length, width, perimeter and area). In this study was investigated the differences on biometric data of hake otoliths between distant locations. Two different approaches for pattern recognition on otolith morphometrics were done: (1) modelling the otolith weight and (2) multivariate analyses of morphometric otolith data. There were no consistent differences between left and right otoliths and between sexes within the same length classes. The sampling area determines otolith weight, thus in Porcupine Bank increment of otolith weight by length class is significantly lower than in the rest of areas.

**Analysis of Length frequency distribution data for *Merluccius merluccius* from the ICES Divisions VIIIc and IXa**, by M. P. Sampedro\*, N. Pérez\*\*.

*Institution: Instituto Español de Oceanografía*

*Centro Oceanográfico de A Coruña(\*), Paseo Marítimo Alcalde Francisco Vázquez 10, 15001 A Coruña.*

*Centro Oceanográfico de Vigo (\*\*), Subida a Radio Faro 50, 36390, Vigo, Pontevedra, España.*

The aim of the present working document was to report the results of an analysis of total catch length frequency distributions of *Merluccius merluccius*. From June 1999 to July 2000 monthly length frequency distributions of total catches (landings plus discards), both sexes combined, were obtained from samples collected by observers on-board. The peaks in length-frequency data in successive time periods were followed to estimate Von Bertalanffy growth parameters using the ELEFAN I algorithm. Although a main increase of smaller individual was observed in July 1999, and was repeated in June 2000, the recruits could be found through the year. It was not possible to follow the length components through the time period so the obtained growth parameter estimates were considered not realistic. A second length frequency analysis was performed using the Bhattacharya method. The modal length groups were not clearly followed in successive month samples. It was suggested the application of Bhattacharya method to quarterly length compositions that could improve the results of this analysis.

**Would be possible to approach to Model of Growth of Hake based on Daily Growth and Tagging Material?**, by C. Piñeiro\*, A. Garcia (IEO), H. De Pontual (IFREMER)

*Institution: Instituto Español de Oceanografía. Centro Oceanográfico de Vigo(\*). Subida a Radio Faro, 50.*

*C.P.: 36390, Apdo. 1552, Vigo, Pontevedra, España.*

We presented an idea to explore a new approach to obtain a new growth model for hake based on the results of two different studies. For young fish with survival problems in the tagging experiments (TL<25 cm) we proposed using daily growth results obtained by IEO and for larger fish, using the growth rates obtained from tagging and recapture results of IFREMER and IEO. The idea was opening a discussion to progress towards a solution to provide more feasible growth parameters for hake to be used in the assessment.

**Can we Model Otolith Growth and Opacity Patterns as a Response to Environmental Factors and Fish Metabolism?**, by Helene de Pontual\*, R. Fablet, L. Pecquerie, H. Hoie, A. Jolivet, R. Millner, H. Mosegaard, Sebastiaan A.L.M. Kooijman.

*Institution: IFREMER Centre de Brest(\*). Laboratoire de Biologie Halieutique. Z.I. Pointe du Diable. B.P. 70. 29280 Plouzané.*

Fish otoliths, calcified structures located in the inner ear are actual biological and environmental archives. Their accretionary growth results from a strict physiological control of the organism, but is influenced by the environmental conditions in which the fish lives. For instance, environmental variables such as temperature and salinity, as well as season-based or age-based metabolic variations are known to influence the deposit rate and the incorporation of chemical elements. This accretionary process often leads to the formation of a sequence of structures (rings), whose periodicity goes from the day to the season. Otolith analysis then offers a unique potential to reconstruct, at a daily and/or yearly scale, environmental parameters as well as individual life traits (age, growth and migration patterns) and population features (age structure, spatio-temporal distribution). Although otolith analysis is now recognized as an invaluable source of information, the extraction of the metabolic and environmental information archived in the otolith remains critical in numerous cases due to a lack of understanding of the mechanisms driving the biomineralization of the otoliths.

The DEB theory opens new avenues to address these issues. A DEB-based model of otolith formation, supported by experimental evidences, is presented. Stating otolith formation as a DEB product, otolith growth and opacity result of elementary metabolic fluxes (i.e., somatic growth and maintenance fluxes). In addition to this direct metabolic modulation, a biomineralization-specific temperature effect is considered as calcium carbonate precipitation is known to be temperature-dependent. A two-dimensional extension of the model is also proposed. From these two elementary components, the model conforms to the relationships observed empirically in different experiments regarding the effects of metabolism and environmental conditions (i.e., food availability and temperature) on global otolith characteristics (growth and opacity) and provide a clear interpretation of the interactions between these two (opposite in some cases) effects.

In addition to model calibration and evaluation for otolith patterns issued from rearing experiments, the potential of this DEB-based otolith model for understanding differences in otolith patterns is illustrated for two cod stocks, namely Southern North Sea and Barents Sea cod otoliths. We will further stress the interest of this modelling approach for analyzing individual life traits using model inversion.

**Effects of growth in Southern Hake Assessment**, by *Santiago Cervoño\**.

*Institution: Instituto Español de Oceanografía, Centro Oceanográfico de Vigo(\*), Subida a Radio Faro, 50*

*36390, Vigo, Pontevedra, España.*

It is well known that hake ageing is underestimated and preliminary results show that hake growths two times faster than expected. During last year assessment of Southern hake, a problem of accuracy on assessment results was detected through a retrospective assessment. An analysis of the effects of growth overestimation on Southern hake assessment is presented. The analysis is based on an age-length model implemented with GADGET. Two assumptions about growth were implemented: one with classical slow growth and another with fast growth ( $K_{fast} = 2 * K_{slow\ growth}$ ). Results for fast growth model show that: (1) improves the results accuracy detected by retrospective pattern; (2) recruitment trends fit best the surveys; (3) biological reference points are quite different ( $F_{max\ fast} = 2 * F_{max\ slow}$ ) and (4) projections under current Southern hake recovery plan drive the stock towards a faster recovery. The model diagnosis support a faster growth hypothesis and the analysis performed show that differences in growth have important consequences on assessment results and eventually on the advice.

**Growth issues related with Anglerfish**, by *J. Landa\**.

*Institution: Instituto Español de Oceanografía, Centro Oceanográfico de Santander(\*), Promontorio San Martín, s/n, 39004 Santander. SPAIN*

Studies on growth of both European anglerfish have focused on age estimation using illicia and otoliths. Age estimation in anglerfish is difficult, as it is in European hake.

Five age estimation workshops, involving age readers from several European countries, took place from 1991 to 2004. A standardized reading criteria in illicia was obtained and age estimations from illicia and otoliths were compared. Accuracy and precision reached a good level for illicia but remained low for whole otoliths. The illicium has become the standard structure for anglerfish age estimation in several European countries (France, Ireland, Portugal, Spain), and the one used as a basis for stock assessment (VPA) in the northern stock of both anglerfish.

Historical series (1996-2006) of captures at age (CaA) of both southern anglerfish stocks were obtained from age estimations based on the standardized reading criterion in illicia. Although those CaA series were ready to be used in a new age-based assessment (VPA), inconsistencies in the follow-up of cohorts using the CaA matrix were found, which could be due to a biased ageing criterion using illicia, lack of discard data and problems in catch estimates for some years. The same ageing criterion was being used for the northern anglerfish stocks; therefore a bias in the catch proportions at age was also detected there. Since 2007, age-based assessment has not been performed for both stocks of both anglerfish.

The growth patterns of white anglerfish in European Atlantic waters obtained with techniques alternative to the common age estimates from hard parts (i.e. tag-recapture, length frequency of the catches, and microstructural analysis of hard parts) were analysed. An overall faster growth rate of white anglerfish was estimated based on the results of those studies. After a comparison, estimates of growth based on illicia, which had been used in assessing the northern stock of white anglerfish, were underestimated.

A recent work has showed that the faster growth hypothesis fit better the data found for Porcupine Bank white anglerfish, providing reasonable tracking of large or small cohorts during the first age groups. However, more validated growth information in the different areas of the anglerfish distribution is necessary.

It is unproductive to continue with the age estimation in anglerfish using the biased ageing criterion and without new progress being made in age validation. A better approach to perform a new age-based assessment may be to use the new faster growth parameters, or to continue assessing with stock production models, as that used (ASPIC) in the southern anglerfish stock.

#### **Web Services for support of Growth and Reproductions Studies, by *E. Jardim\****

*Institution: National Laboratory of Marine Research (\*), Unit of Marine Resources. Av. Brasília, 1449-006 Lisboa, Portugal (IPIMAR)*

WebGR is a European project that aims to develop Open Source software for supporting studies of fish growth and reproduction. In particular it promotes the usage of online services to organize calibration workshops.

Calibration workshops are carried out for a long time between scientists "reading" otoliths to identify individual age, so that all scientists "tune" their interpretation of the ageing protocols. It has recently being extended to also cover identification of maturity stages with gonads. In general it can be applied to all situations where distinct scientists have to discuss the interpretation of a protocol to identify status of biological material.

The consortium is constituted by: Laboratório Nacional de Recursos Biológicos – IPIMAR (Portugal) – Consortium leader, The Agri-Food & Biosciences Institute (UK), AZTI Tecnalia Foundation (Spain), Federal Agency for Agriculture and Food (Germany), Johann Heinrich von Thünen Institute (Germany), Hellenic Centre for Marine Research (Greece), Instituto Español de Oceanografía (Spain), Institut français de recherche pour l'exploitation de la mer (France), Institute for Marine Resources & Ecosystem Studies (The Netherlands), Institute of Marine Research (Norway), Swedish Board of Fisheries (Sweden), Italian Society for Marine Biology (Italy). (For more information please visit <http://webgr.berlios.de>.)