REPORT OF 3rd EXCHANGE on EUROPEAN HAKE
AGE READINGS
(SAMFISH)

by
Carmen Piñeiro and María Sainza
Instituto Español de Oceanografía
IEO, Vigo, Spain

carmen.pineiro@vi.ieo.es
maria.sainza@vi.ieo.es
http://www.ieo.es

1. Introduction

According to the Workplan of EUContract No. 99/009 (Improving Sampling of Western and Southern European Atlantic Fisheries – SAMFISH) an otolith exchange scheme should be undertaken on hake (Merluccius merluccius) for the Northern and Southern Stocks in order to calibrate the age readings.

The exchange was carried out following the recommendations of the EFAN Report 3-2000 on Guidelines and Tools for Age Reading Comparations, which is available in the EFAN home page. In order to check the precision in age reading and bias of the age readers of this species, the background for ageing hake was based on two reports of Hake Otolith Age Reading Workshops conducted previously (1997 and 1999). They are available in the EFAN home page (http://www.efan.no; Report 6-2000 and Report 7-2000).

2. Objectives:

- Analysis of the results of the hake otolith exchange in order to check the precision in age reading and bias of the age readers referred to at the last workshop.
- Also new readers become involved in hake age determination and need to get used to the otolith interpretation criteria.

3. Participants:

<table>
<thead>
<tr>
<th>Reader</th>
<th>Name</th>
<th>Institution</th>
<th>City</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reader 1</td>
<td>M. Saínza</td>
<td>IEO</td>
<td>Vigo</td>
<td>Spain</td>
</tr>
<tr>
<td>Reader 2</td>
<td>M. H. Afonso</td>
<td>IPIMAR</td>
<td>Lisbon</td>
<td>Portugal</td>
</tr>
<tr>
<td>Reader 3</td>
<td>C. Piñeiro</td>
<td>IEO</td>
<td>Vigo</td>
<td>Spain</td>
</tr>
<tr>
<td>Reader 4</td>
<td>J. Labastie</td>
<td>IFREMER</td>
<td>La Rochelle</td>
<td>France</td>
</tr>
<tr>
<td>Reader 5</td>
<td>S. Arego</td>
<td>AZTI</td>
<td>Sukarrieta</td>
<td>Spain</td>
</tr>
<tr>
<td>Reader 6</td>
<td>S. Warnes</td>
<td>CEFAS</td>
<td>Lowestoft</td>
<td>England</td>
</tr>
<tr>
<td>Reader 7</td>
<td>A. Payne</td>
<td>CEFAS</td>
<td>Lowestoft</td>
<td>England</td>
</tr>
<tr>
<td>Reader 8</td>
<td>T. Watson</td>
<td>CEFAS</td>
<td>Lowestoft</td>
<td>England</td>
</tr>
</tbody>
</table>
4. Material and Methods

A collection of 199 Hake otolith sections from two ICES areas (99 from Sub-area VII and 100 from Divisions VIIIc + IXa) was circulated among interested Institutes during 2001. The exchange scheme started on the 20th of January 2001 and finished in 31st of November (Figure 1). Digitised images from otoliths sections were stored in a CD Rom, which accompanied the exchange collection. Unfortunately the otolith collection was lost when the last institute sent it to the co-ordinator in Vigo but the digitised images still remain in the CD Rom.

The length range of the fish sampled was between 11cm and 83 cm. Information on catch date, sex and total length were available for the readers. The otolith sections were prepared in Vigo (IEO) with the same technique used in the previous exchanges. The length and age distribution sampled is shown in Figure 2.

Before circulating the otolith collection an ageing protocol was provide to all readers (Annex 1). In order to know whether the readers count the same rings, the first three rings and the check ring considered by reader for age estimation, were measured. For Reader 8, only distances of the first annual ring were considered for the analysis since the following rings measurement presented problems with units.

Regarding to the level of experience of the readers involved in the exchange there was differences. On one hand, there were hake readers traditionally involved in hake stock assessment, more over, there were readers who participated in the three hake otolith exchanges and on the other hand, there were new readers in this exchange. So initially, it was decided to split them into 4 groups:

- Readers involved in hake stock assessment: R1, R2, R3, R4, R5
- Most experienced readers: R3, R4
- Readers with some experience: R9, R10, R11, R12
- New readers: R6, R7, R8

The general criteria adopted for ageing is described in the report of the previous exchange (Hake Otolith Age Reading Workshop, 1999; EFAN 7-2000).

The analysis of the age reading results was done by mean the Excel ad-hoc Workbook “AGE COMPARATIONS. XLS” from A.T.G.W. Eltink from RIVO.

The basic requirement for age reading consistency is the absence of bias among readers and through time. One of the ways to test this absence of bias between two readers or between a reader and the modal age estimated is with a one-sample Wilcoxon signed rank test, which was, chosen in this exchange in order to compare with the previous test applied in the first exchange.

To study the variability in the precision of age determinations between readers an
extensive analysis was conducted to provide more details concerning individual’s performances:

1. **Exploratory data analysis (EDA)**

   - Determination of the modal age and of the difference between each readers’ age and the modal age. The modal age was calculated based on the results of readers involved in stock assessment: R1, R2, R3, R4 and R5. In case of bi-modality the modal age was estimated from the most experienced readers (R3 and R4), and when the analysis is performed for only these two readers, the bi-modality is solved with the modal age from the readers R1-R5 involved in stock assessment.

   - Graphical representation by reader for the sample using box-whisker plots (median and interquartil range by reader). The box-whisker plots were used to summarise the observations and are useful in observing and comparing the distribution of the otolith readings by reader.

2. **In terms of reproducibility measures:**

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

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

   \( n = \) number of otoliths  
   \( r = \) number of readings for each otolith  
   \( x_{ij} = \) the \( j \) value of age estimation for the \( i \) otolith  
   \( \bar{x}_i = \) average age calculated for the \( i \) otolith

   2.2) **The Mean Coefficient of Variation (CV)**

   
   \[
   CV = \frac{100}{n} \left[ \sum_{i=1}^{n} \left( \frac{sd}{x_i} \right) \right]
   \]

   \( sd = \) the standard deviation for the \( i \) otolith

   2.3) **The index of precision (D)** (Chang, 1982):

   
   \[
   D = \frac{100}{n} \left[ \sum_{i=1}^{n} \frac{CV}{r^{0.5}} \right]
   \]

   CV is described in 2.2)
These measurements are more appropriate than the conventional percent of agreement when comparing ages, since those take into account the average year class of fish.

3. In terms of grouping readers with different agreement between them:

- Hierarchical cluster analysis using average linkage (between groups) based on squared Euclidean measure for readers without transforming the data.

- Multiscaling dimension (MSD) to show the multidimensional space based on squared Euclidean measure for readers without the transformation of input data using an ordinal measure scale.

To know if there were differences in the ages assigned for Southern and Northern stock samples, a Kruskal-Wallis non-parametric analyses was carried out based on the readings from readers involved in hake stock assessment: R1, R2, R3, R4, R5

5. Results and discussion

The results of the readings and the basic information about otolith collection are summarised in Table 1

In general, the box-whisker plot for all readers shows that the ages attributed are very similar (average = 2.5 years) except readers R7, R8, and R9. The first results pointed out two main groups which it can be related to experience and inexperience readers (Figure 3a):

1/ R1, R2, R3, R4, R5, R6, R10, R11 and R12
2/ R7, R8, and R9

The box-whisker plot for first ring distance measured for all readers shows two main groups, the first group include R1, R2, R3, R4, R5, R9, R10 and R12 consisting of readers with some experience and the other group include mainly readers without experience: R6, R7, R8, and R11 (Figure 3b). The graphical representation illustrates that, readers with experience present similar average distances (1.3 mm).

The box-whisker plot for check ring distance measured for all readers pointed out that, with exception of readers R7 and R11, all readers can distinguish clearly this check (Figure 3c). Its average distance to the nucleus seems to be around 1.5 mm., this coincide with the distance reported by Piñeiro and Hunt, 1989. This is a very hopeful result since this check is the reference ring to start the counting.

The box-whisker plot for second and third ring distance measured indicates again that the majority of readers are in agreement and present similar average measurements with exception of R2, R7 and R11 readers. (Figure 4a,b). It should be noted that Reader R2 presents a very consistent pattern in the identification of the rings.

In relation with the location of the first three rings, the graphical indicates that in this
exchange there is the highest agreement among readers obtained until now. Only the lack of experience in hake ageing and the misinterpretation of the ageing criteria are likely causes for any discrepancies observed. Taken into account that this aspect is one of the major difficulties in the age interpretation of this species, the result is an encouraging sign.

Considering the bias plots for all readers together it can be observed that the mean age recorded is very close to the modal age and the deviations increase relatively with age. High bias was observed in readers R7 and R9 which are overestimated, and R8 which is underestimated relative to the modal age. Readers R5, R6 and R12 underestimate ages older than 6. However the results for all readers present less bias than the previous exchanges (Figure 5). A high precision is achieved by readers: R1, R2, R3, R4, R5, R11 and R12

The coefficient of variation (CV%) and percent agreement are plotted against modal age in Figure 6. This figure shows these results from all readers, experienced readers involved in hake ALK’s (R1-R5) and from the most experienced readers (R3, R4) to see how these measures change with experience. Firstly, from all readers the average of CV was 40 %. This value is so high mainly due to an important effect of age magnitudes in the calculation of CV’s. Especially in this exchange, where there was a big amount of younger ages in the sample (0,1,2). In fact, the CV’s are much higher for age 0 and afterwards the CV’s decreased very much, keeping around 20 % for ages older than three. Besides that there are difficulties in the discrimination of the first annual ring among the false rings. The percent agreement decreased as the age increased and the mean value obtained was 58%.

Secondly, from readers involved in hake ALK’s, the average of CV was 17% and this value was much higher for age 0, mainly due to the important effect of age magnitudes in the calculation of CV’s, already mentioned. For older ages, the CV’s decreased considerably to values around 10 %. The average percent agreement was 75 %. Finally from most experience readers, the average of CV was 9 % and the average percent agreement was 84%. As can be seen the level of experience affect considerably to the ageing precision and the level of agreement.

Wilcoxon’s test between readers is shown in Table 2. The results clearly emphasize the absence of bias for the first six readers (R1, R2, R3, R4, R5, R6). However bias still remained for the rest of the readers.

A plot of mean length at age (Figure 7) can serve to diagnose individual reader tendencies. Except for readers R7, R8, R9, the mean length at age estimated by reader is similar for the first 6 ages above that age the difference increases.

The results of APE, CV, and D indices in (%) for all readers are: 28, 40 and 12 respectively (Table 3). The fact of having a large number of young fish in the sample affects the results of APE and CV since these indices are very sensitive to differences in younger ages, especially 0 and 1. When the analysis is carried out using only the most experienced readers (R3 and R4) these indices decreased considerably to 6, 9 and 6. These results are much lower than those obtained in the previous exchanges (Figure 8) and indicate that there has been a clear improvement in ageing hake.
The dendogram obtained from the hierarchical cluster analysis point out also the presence of two main groups (Figures 9).

Group 1: R1, R2, R3, R4, R5, R11, and R12
Group 2: R6, R7, R8, R9 and R10

In order to clarify the groups, a plot of coordinates from MSD analysis was carried out (Figures 10). It can be seen in the plot that the first dimension splits the readers into two groups, supporting the results of the hierarchical cluster analysis.

The results of the Kruskal-Wallis analyses shown that no significant differences (p>0.05) exist between the ages assigned for the same length in both stocks (North and South).

In summary the exploratory and statistical analysis showed two main groups of readers: experienced and inexperienced. Therefore again it is recognised the importance of experience in hake ageing. The lack of experience in ageing is the most likely reasons for the observed discrepancies.

The significant achievement of this exchange is that for the first time, the ageing method for the first 6 ages has provided high level of agreement and very consistent age estimations, including readers partially experienced. For ages older than 6, the agreement amongst readers diminish.

Despite the improvement of all the readers, the results highlight the difficulties in ageing hake otoliths, mainly older fish, and justify the need for periodical exchange exercises amongst the countries involved in stock assessment, particularly focussed on older ages.

6. Conclusions:

From these results a clear improvement in ageing hake is observed comparing the results with those from previous exchanges it can be seen:

- The highest level of agreement in the location of the first three annual rings. This is the result of the adoption of the ageing criteria established.
- The exploratory and statistical analyses showed that ageing is very consistent and the check ring which is the reference ring to start the counting is very well distinguished for practically all readers.
- The results of APE, CV, and D indices in (%) for all readers are the best in the whole series of exchanges.
- The ageing method for the first 6 ages has provided the highest level of agreement and very consistent age estimations, including the readers partially
experienced. For ages older than 6, it is necessary to undertake studies on life history events such as reproduction process in relation to the pattern ring formation in the otoliths.

- Readers involved in stock assessment had a high level of agreement and precision reaching the mean values of 75% and 17% respectively. Furthermore, new readers showed an adequate interpretation of the ageing criteria.

- No significant differences exist (p>0.05) between otoliths from both stocks (North and South).

7. Recommendations

- Exchanges should be carried out among Institutes on a regular basis, especially for those who supply ALK’s to the assessment Working Groups. Furthermore, a workshop should be convened in the future since some ageing difficulties still remain, mainly for the older ages.

- Statistical tests on age data of each Institute should be carried out before they are combined for ALK’s.

- It is desirable that at least two readers in each Institute should be involved in Hake age determination.

- A training guide with digital images of otolith sections from ages 0 to 6 will be helpful to introduce the readers in the ageing criteria established. Likewise a reference collection to calibrate the experienced readers is recommended.

- It is necessary to validate the age estimation of this species by conducting more studies on the life history events of the fish.

- A secure transportation method is essential for the successful completion of the exchange otolith collection and this is responsibility of all participants.

8. Acknowledgements

This otolith exchange was possible to carried out with the participation of all readers from the six institutes involved and the success of the exchange is due to their efforts. Thank is extended to Valentin Trujillo for his suggestions. Thanks also to Guus Eltink form RIVO for permission to use his analytical software AGE COMPARISONS.XLS.
Table 1.- Results of age estimations by reader

<table>
<thead>
<tr>
<th>Nº</th>
<th>N°</th>
<th>length</th>
<th>Sex</th>
<th>month</th>
<th>R 1</th>
<th>R 2</th>
<th>R 3</th>
<th>R 4</th>
<th>R 5</th>
<th>R 6</th>
<th>R 7</th>
<th>R 8</th>
<th>R 9</th>
<th>R 10</th>
<th>R 11</th>
<th>R 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1006</td>
<td>23</td>
<td>3</td>
<td>18</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1007</td>
<td>23</td>
<td>3</td>
<td>17</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1008</td>
<td>22</td>
<td>3</td>
<td>16</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1009</td>
<td>23</td>
<td>3</td>
<td>15</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1010</td>
<td>22</td>
<td>3</td>
<td>14</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1011</td>
<td>21</td>
<td>3</td>
<td>13</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>1012</td>
<td>20</td>
<td>3</td>
<td>12</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>1013</td>
<td>19</td>
<td>3</td>
<td>11</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>1014</td>
<td>18</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>1015</td>
<td>17</td>
<td>3</td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>1016</td>
<td>16</td>
<td>3</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>1017</td>
<td>15</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>1018</td>
<td>14</td>
<td>3</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>1019</td>
<td>13</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
### Table 1. Results of age estimations by reader (continuation)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hake Otolith age reading: 3rd exchange 2001</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fish</td>
</tr>
<tr>
<td>N°</td>
<td>N°</td>
</tr>
<tr>
<td>14</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

*Note: The table continues with similar structures.*
Table 1.- Results of age estimations by reader (continuation).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hake Otolith age reading: 3rd exchange 2001:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total read</td>
</tr>
<tr>
<td>N°.</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>64</td>
</tr>
<tr>
<td>29</td>
<td>64</td>
</tr>
<tr>
<td>30</td>
<td>64</td>
</tr>
<tr>
<td>31</td>
<td>64</td>
</tr>
<tr>
<td>32</td>
<td>64</td>
</tr>
<tr>
<td>33</td>
<td>64</td>
</tr>
<tr>
<td>34</td>
<td>64</td>
</tr>
<tr>
<td>35</td>
<td>64</td>
</tr>
<tr>
<td>36</td>
<td>64</td>
</tr>
<tr>
<td>37</td>
<td>64</td>
</tr>
<tr>
<td>38</td>
<td>64</td>
</tr>
<tr>
<td>39</td>
<td>64</td>
</tr>
<tr>
<td>40</td>
<td>64</td>
</tr>
</tbody>
</table>
Table 2.- Wilcoxon signed rank test for the readings of otolith collection.

<table>
<thead>
<tr>
<th></th>
<th>IEO Reader 1</th>
<th>IPIMAR Reader 2</th>
<th>IEO Reader 3</th>
<th>TRENIE Reader 4</th>
<th>REME Reader 5</th>
<th>CEFAS Reader 6</th>
<th>CEFAS Reader 7</th>
<th>CEFAS Reader 8</th>
<th>MI Reader 9</th>
<th>MI Reader 10</th>
<th>IPIMAR Reader 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reader 1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Reader 2</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>*</td>
<td>--</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Reader 3</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Reader 4</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>*</td>
<td>--</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Reader 5</td>
<td>--</td>
<td>*</td>
<td>--</td>
<td>*</td>
<td>--</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Reader 6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>*</td>
<td>--</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Reader 7</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Reader 8</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Reader 9</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Reader 10</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Reader 11</td>
<td>**</td>
<td>--</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Reader 12</td>
<td>**</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>MODAL age</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

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

Table 3.- Average percent age error (APE), Mean coefficient of Variation (CV), and Index of precis obtained in the reading of otolith collection; n: number of otoliths read.

<table>
<thead>
<tr>
<th></th>
<th>APE</th>
<th>mean CV</th>
<th>mean D</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL READERS</td>
<td>28.2</td>
<td>40.1</td>
<td>11.6</td>
<td>199</td>
</tr>
<tr>
<td>READERS 3 AND 4</td>
<td>6.4</td>
<td>9.0</td>
<td>6.4</td>
<td>199</td>
</tr>
<tr>
<td>READERS 1,2,3,4 and 5</td>
<td>13.1</td>
<td>16.9</td>
<td>7.6</td>
<td>199</td>
</tr>
</tbody>
</table>
Figure 1.- Flow chart of activities during the Third hake otolith exchange.

- **IEO 1**
  - Vigo
  - C. Piñeiro
  - M. Sainza
  - M. Marín

- **IFREMER 2**
  - La Rochelle
  - J. Labastie

- **CEFAS 4**
  - Lowestoft
  - S. Warnes
  - A. Payne
  - T. Watson

- **MI 5**
  - Dublin
  - F. Woods
  - H. McCormick

- **AZTI 3**
  - Sukarrieta
  - S. Arego

- **IPIMAR 6**
  - Lisboa
  - C. Morgado
  - H. Afonso

**Dates:**
- 09/07/01
- 13/08/01
- 23/05/01
- 25/04/01

**Sent to IPIMAR:**
- 20/02/01
- 10/03/01

**Sent from IPIMAR:**
- 09/07/01
- 23/05/01
- 25/04/01
- 13/08/01

**Activities:**
- Sent the readings
- Sent otolith collection

*Page 12*
Figure 2.-Length frequency and age reading distributions of the collection sampled.
Figure 3.- Box-whisker plot of all readings (a), for first (b) and check ring (c) distances.
Figure 3 (cont.).- Box-whisker plot of check ring (c)

(c)
Figure 4.- Box–whisker plot for second (a) and third (b) ring distances measured for all readers.

(a)

(b)
Figure 5. Mean age recorded (±2*std) of each age reader and all readers combined against the modal age. The estimated mean age corresponds to MODAL age, if the estimated mean age is on the 1:1 equilibrium line (solid line). RELATIVE bias is the age difference between estimated mean age and MODAL age.
Figure 6.- The coefficient of variation (CV%) and percent of agreement (%) from ALL readers; experienced readers involved in ALK’s (R1, R2, R3, R4, R5,) and most experienced readers (R3, R4) are plotted against MODAL age.
Figure 7.- Mean length at age estimated by reader.
Figure 8.- Indices of Beamish and Fournier (APE), Coefficient of variation and the Index of Precision (D) observed for all readers combined, experienced readers (Involved in stock assessment: R1, R2, R3, R4, R5) and most experienced readers (R3 and R4) through all series of exchanges.

![Graph showing indices of Beamish and Fournier (APE), Coefficient of variation and the Index of Precision (D) for all readers and most experienced readers through all exchanges.](image-url)
Figure 9- Dendogram obtained from the hierarchical cluster analysis.

![Tree Diagram for 12 Variables](image)

Figure 10.- MSD plots analysis.

![Scatterplot 2D](image)
Dear Colleague,

According to the work plan of EU contract 99/009 (Improving Sampling of Western and Southern European Atlantic Fisheries – SAMFISH) several otolith exchange schemes will be undertaken. This protocol is concerned with European Hake exchange.

In order to continue the work initiated in 1997 and to check the precision in age reading and bias of the age readers a new hake otolith exchange will be carried out. At the start of the exchange it is very important the background of ageing this species and have some idea of the problems found so far, then please read two reports of Hake Otolith Age Reading Workshops conducted previously (1997 and 1999). They are available in the EFAN home page (http://www.efan.no: Report 6-2000 and Report 7-2000).

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

A collection of 200 Hake otolith sections from two ICES areas, (100 from Sub-area VII and 100 from Division VIIIc + IXa) will be exchanged among interested institutes during 2001. The otoliths sections were prepared in Vigo (IEO) with the same technique used in the previous exchanges.

PARTICIPANTS:

- AZTI, Sukarrieta (Spain)
- CEFAS, Lowestoff (U.K.)
- IPIMAR, Lisbon (Portugal)
- IFREMER, La Rochelle (France)
- IEO, Vigo, (Spain)
- MI, Dublin, (Ireland)

Before circulating the otolith collection the following information should be taken into account for the successful completion of the exchange:

1- Otolith interpretation should be done following the standard ageing criteria already established (consult Hake Workshops Reports 1997, 1999).

2- Annual growth cycle consist in one opaque and one translucent or hyaline zone. The first of January is conventionally adopted as hake birthday.
3- Is very important to determine the position of the First annual ring (or growth ring) because this will be probably one of the main sources of discrepancies among readers.

4- In the samples some rings considered for age estimations should be measured in order to know whether the readers count the same rings or not. Due to time consuming, we suggest to measure only the first three rings, check (Ch) and Rt. The measurements are not taken from the nucleus to the outer edges of the translucent rings, because the exact point of the nucleus is often not well defined so they should be taken as follows: (see otolith drawing).

   **R1 (Radius of the first year’s growth zone):** distance between the Centrum of the otolith section and the outer edge of first translucent ring.

   **Dr2 (Radius distance of the second year’s growth zone):** distance between outer edge of R1 and outer edge of the second translucent ring.

   **Dch (Check distance):** distance between outer edge of R1 and outer edge of the false annual ring (Quite frequent).

   **Dr3 (Radius distance of the third year’s growth zone):** distance between outer edge of R1 and outer edge of the third translucent ring.

   .....  

   **Drt (Radius of the total section):** distance between the edge of R1 and outer edge of section along the axes anterior-posterior

**Edge Type:**

   Hyaline: 1  
   Opaque: 2  
   Unknown: 3

**Reliability: (1,2,3)**  
1: minimum ... 3: maximum

*There will be an observation cell for comments*

5- An input form (Exhake_input.xls) provides all the information on date of capture, area, etc. The readers should record the age determinations and the ring measurements as numerical format on the spreadsheet. This should be identified with name of the reader so that individual problems can be resolved at the end of the exchange.

6- The otolith sections should be viewed with a binocular microscope on a black background under reflected light. The magnification used to measure rings will be X20 and the units should be recorded in the spreadsheet (Exhake_input.xls) in mm.

7- Digitised images (*.tif) for all the otoliths will be available in a CD Rom in order to help the reader interested in recording how he/she arrives at their estimated age. The CD Rom Images should accompany to the otolith collection in the running of the exchange.
The order of circulation is in the flowchart activity. Anyway, to speed up the exchange IEO institute (3 readers) will be the first one and for the reading we need whole January and part of February. The list below is ordered following the dates proposed:

Carmen Piñeiro  
carmen.pineiro@vi.ieo.es  
IEO  (January- February)  
Apartado 1553  
Vigo, Spain

Cristina Morgado  
cmorgado@ipimar.pt  
IPIMAR  (February-March)  
Avenida de Brasilia  
1400 Lisboa, Portugal

Jaques Labastie  
jlabas@ifremer.fr  
IFREMER  (March-April)  
BP7 17137 L’Houmeau  
La Rochelle, France

Paulino Lucio  
paulino@rp.azti.es  
AZTI  (Apr.-May)  
Txatxarramendi ugartea, s/n  
Sukarrieta (Bizkaia) , Spain

Steve Warnes  
S.Warnes@cefas.co.uk  
CEFAS  (May- Jun)  
Pakfield Road  
Lowestoft, Suffolk  
England, NR33 0HT

Fiona Woods  
fwoods@marine.ie  
MI  (Jun.-Jul)  
Abbotstwon  
Dublin 15, Ireland

Three further items:

1. Please contact the next institution or reader on the list before dispatch in order to reduce the possibility of losing time and inform me when the otoliths have been sent.

2. Once your institute has completed the readings, please return, as soon as possible, the spreadsheet to me via e-mail with all the age reading results following the recommendations here mentioned in order to carry out successfully the age reading comparisons.

3. Is recommended to send the otolith collection with the CD Rom images by special courier in order to speed up the exchange.
The Guus’s spreadsheet will be used for the analysis of the exchange results (if anyone wishes to look at this spreadsheet and the accompanying guidelines they should go to www.efan.no, click on the radio button ‘Guidelines’ and then select ‘Guidelines and Tools for Age Reading Comparisons’).

If you have any queries please contact me in Vigo.
Thank you in advance for your co-operation

Carmen Piñeiro
Instituto Español de Oceanografía
C. O. de Vigo
Apartado 1552, 36280 Vigo, Spain
carmen.pineiro@vigo.ieo.es
fax: +34(986) 49 23 51
telf: +34(986) 49 21 11

Hake otolith section drawing

Protocol of 3rd Hake Otolith Exchange 2001  Pag. 4