ICES Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS)

# Report of the Blue Whiting Otolith Ageing Workshop

DIFRES Hirtshals, Denmark 13-16 June 2005

Co-sponsored by the Commission Regulation (EC) No 1639/2001

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The chapter 6 – Method and Manual – was written by the entire group during the workshop.

### **1. Introduction**

Blue whiting is a meso-pelagic gadoid widely distributed in the eastern North Atlantic and perform extensive migrations throughout their distribution area (Bailey 1982, Monstad 1990; see figure 1). From the wide distribution area follows a very diverse fishery on the blue whiting as many as 13 nations exploit the blue whiting population. The fishery is mainly carried out by combined purse-seiners/trawlers, though a few large industrial freezer trawlers participate along with some bottom trawlers and artisanal fishery. Figure 2 shows the distribution of the fishery by quarter for 2003 as reported by the Northern Pelagic and Blue Whiting Fisheries Working Group (WGNPBW) (ICES 2004).



Figure 1. Migration routes for the blue whiting in the Northern Atlantic. Tangen and Sveinbjörnsson.

Blue whiting is managed as being one stock, however morphological, physiological and genetic research has suggested that there may be several components of the stock (ICES 2000, Heino et al. 2003, Brophy and King 2004).



Figure 2. Total catches of blue whiting in 2003 by ICES rectangle. Grading of the symbols: small dots 10-100 t, white squares 100-1 000 t, grey squares 1 000-10 000 t, and black squares > 10 000 t. (ICES 2004)

During the recent WGNPBW comparisons of age distributions from surveys and landings originating from different age reading laboratories indicated differences in the interpretation of the structure in the otoliths resulting in different age estimates. As a result the WGNPBW recommended an otolith exchange programme to investigate the magnitude of the differences in age structure perception. Such an exchange was carried out between Norway and Ireland, results indicating a limited inconsistency among international blue whiting age readers (Power et al. 2004). ICES expert groups PGCCDBS and the Coastal States Scientific Working Group on Blue Whiting have recommended a further investigation of these discrepancies in age estimation and the present report gives the results from a workshop hosted by DIFRES aiming at securing consistent age readings at different laboratories.

The objectives of the workshop were manifold; apart from the overall goal of securing consistency in age estimation of blue whiting; updating and assembling age readers from all national laboratories handling blue whiting from the North Atlantic to exchanged views on methods and experiences was among the objectives. This had not been done for more than a decade among the participating laboratories. The aim of these exchanges was to create the basis for a manual for age determination of blue whiting for future reference.

The workshop consisted of 3 calibration exercises:

- Two separate traditional age calibrations
- An Image analysis calibration

In the present report the three calibrations will be treated individually and conclusions will be assembled in the final discussion.

## 2. Participants

Name	Institute	Country
Tommy Henriksen	DIFRES	Denmark
Helle Rasmussen	DIFRES	Denmark
Lotte Worsøe Clausen	DIFRES	Denmark
Sveinn Sveinbjőrnsson	Marine Reaserach Institute	Iceland
Susan Beattie	Marine Institute	Ireland
Eugene Mullins	Marine Institute	Ireland
Gavin Power	Galway/Mayo Institute of Technology	Ireland
Ronald Bol	RIVO	Netherlands
Jan de Lange	Havforskningsinstituttet	Norway
Øyvind Tangen	Havforskningsinstituttet	Norway
Kirsti Børve Eriksen	Havforskningsinstituttet	Norway
Tatiana Prokhorova	PINRO	Russia
Nikolay Timoshenko	Atlantniro	Russia
Jane Mills	FRS Marine Lab	Scotland
Manuel Meixide	Instituto Espanol De Oceanografia	Spain
Rosendo Otero	Instituto Espanol De Oceanografia	Spain

For participant contact information please refer to Annex 1.

### 3. Traditional age calibration part 1

The first traditional age calibration set was made up of 100 otoliths from blue whiting caught in ICES subdivision IVa during August 2003. The length distribution ranged from ~40 sc to ~65 sc, with highest representation of the smaller individuals. All otoliths had been stored in water for at least 24 hours prior to the workshop. The otoliths were read submerged in water under dissection microscopes by all participants.

The analysis of the results was performed using an Excel ad-hoc Workbook "AGE COMPARATIONS.XLS" from A.T.G.W. Eltink from RIVO following the recommendations of EFAN (Eltink et al., 2000). This analysis is based on a reference age when there are no validated ages available, which is the case for blue whiting. As reference age the modal age was chosen as the experience level and 'reading school' of the participants varied to some extent and choosing one reader or another as reference age was not an obvious task. However, the most experienced readers were weighed higher in the modal age and were used as 'true age' when no modal age could be reached.

The results from the traditional age calibration exercise displayed that the differences in perception of otolith structures between the participating age readers was not that alarming. The overall agreement was 86.5 % with a precision of 12.2% CV and in 57% of the otoliths the agreement was larger then 90%. Figure 3 shows the overall pattern of the readings, showing that the divergences of the interpretations of the otolith structures increased with modal age.



Figure 3. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

The relatively high Coefficient of Variation for ages above 5 indicates problems in reaching agreement on these higher ages. Figure 4 shows the relative bias by modal age indicating any trends in over-or under estimation of ages by all readers combined. Age 1 seem to be overestimated whereas the remaining ages more often are underestimated compared to the modal age.



Figure 4. Relative bias by modal age

Thus the relative bias appears to be skewed for the older ages. The older ages were consistently underestimated though some readers did overestimate the older ages compared to the modal age.

The inter-reader bias test is presented in Table 1. Generally, the bias level is low, though readers 10 and 13 have significant bias.

	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14	Reader 15
Reader 1		*	-	-	-	-	-	* *	-	* *	-	-	* *	-	-
Reader 2	*	Ī	* *	-	-	-	-	-	-	*	-	-	*	-	* *
Reader 3	-	**		-	-	-	-	* *	-	* *	-	-	**	-	-
Reader 4	-	- 1	- 1		-	-	-	*		* *			**	-	*
Reader 5	-	Γ-	-	-		-	-	* *	-	* *	-	-	* *	-	*
Reader 6	-	-	-	-	-		-	* *	-	* *	-	-	* *	-	-
Reader 7	-		- 1	-	-	-	]	*	-	* *	-	-	**	-	*
Reader 8	* *	-	* *	*	* *	* *	*	]	-	-	* *	* *	-	-	* *
Reader 9	-			-	-	-	-	-		* *	-	-	**	-	*
Reader 10	* *	*	* *	* *	* *	* *	* *		**		* *	* *	-	-	* *
Reader 11	-	-	-	-	-	-	-	* *	-	* *		-	**	*	-
Reader 12	-		- 1	-	-	-	-	* *	-	* *			**	*	-
Reader 13	* *	*	* *	* *	* *	* *	* *	- 1	**	-	* *	* *		-	* *
Reader 14	-	-	-	-	-	-	-	-	-	-	*	*	-		* *
Reader 15	-	* *	-	*	*	-	*	* *	*	* *	-	-	* *	* *	
MODAL	-	-	*	-	-	-	-	-	-	* *	-	-	* *	-	-

= no sign of bias (p>0.05)

= possibility of bias (0.01<p<0.05)

\* = certainty of bias (p<0.01)

Table 1. Inter reader bias

Although the length of the individual is not taken into account when estimating the age from the otolith, it is worth noting that with the widespread perception of age for each individual, the length at modal age 3 is contained within the range for modal age 2 (figure 5).



Figure 5. Modal age at length.

When dividing the material into two length groups; small being < 25 cm and large being > 25 cm, comparisons of percentage agreement for modal ages containing both groups, the larger length group tended to have higher percent agreement (figure 6).



Figure 6. Comparison of the percent agreement between two length categories. Though not statistically different, the larger length group at modal age 3 tended to have a higher percent agreement

Though the bias and disagreement in general in the present calibration may be considered as low, a difference in perception of age structures is clearly present. Not surprisingly was the disagreement highest on the older individuals and small old individuals. Though the calibration set was made up of younger fish originating from the same month and thus could be considered an 'easy' set, the discrepancies in interpretation of age structures in the otolith were still apparent. This fact was further explored in the image analysis calibration described below.

### 4. Image analysis age calibration

The image analysis age calibration was performed using both 'live' otoliths under the stereomicroscope and digitized images of the corresponding otoliths. The readers had the otolith exposed under the stereomicroscope while pointing at the age structures on the picture using the image analysis system tool and could consult the 'live' otolith if the pictures did not reveal all the desired otolith structures clearly. The image analysis system tool makes use of XY-coordinates corresponding to the points, the reader marks as age structures on the digitised image of the otoliths. Prior to the exercise the readers agreed on one axis from the centre and towards the edge along the rostrum along which all points should be placed. All reading on the digitised images were done by marking the first age structure as the first point and then marking all identified age structures along the agreed axis. All points logged on each individual otolith were then transferred into an Excel spreadsheet with the correct ID (otolith number, picture number and reader ID). The readers agreed to mark the outer edge of each translucent ring identified as an annual structure.

From the XY coordinates recorded by the age readers in the image analysis programme the first ring was calculated as the mean X and mean Y for each otolith and each reader. This starting point was then used to compare individual reader interpretations of translucent rings. Distances between each ring was calculated and compared among otoliths and readers. The data coordinates were further subjected to statistical analyses for the variance in different interpretations of the age structures and the span of different positions of the actual structures.

The spreadsheet program, which combined image analysis and plots, made it possible to demonstrate where the individual age readers interpret the rings directly on the digitised images of the otoliths. Some otoliths showed to be very difficult to reach a common interpretation of the age and the points counted as age structures were scattered along the otolith, however, most otoliths were agreed upon by the readers.

The most variation in interpretation of defining rings was observed for the  $2^{nd}$  and  $3^{rd}$  ring. An example of this is illustrated by figure 7.



Figure 7. Agreed age 3: Fourth ring very close to edge, ring measurments 55,66,71 and 73 total length. Fish Length: 28cm Catch date: 6/8/03 Area : IVa-44F5

The variation between otoliths in the median distance to successive rings is shown in figure 8 as cumulated frequency distributions of the position of each ring. This variation increased with ring number.



Figure 8. Cumulated frequency distribution of ring positions.

Though the definition of the first ring appeared to be the least problematic some variation arose around this structure as some readers interpreted a zone closer to the centre of the otolith as the first ring (figure 9). During discussions in the group this structure was defined as the 'Baileys zone'.



Figure 9. Example of 'Baileys zone' where the position of the first ring varies between readers.

The problem in defining the older age structures arose from the difficulty in defining split rings. From inspection of the position of points on otolith images it was seen that frequently some readers did not mark out rings that other readers interpreted as true annual structures. This occurred most frequently around the 3<sup>rd</sup> ring.

### 5. Traditional age calibration part 2

The objective of this exercise was to examine if the precision, in the age determination of blue whiting, increased between participant age-readers after calibration of ageing criteria. Furthermore, as volunteer age-readers represented a wide spectrum of age-reading experience, this study aimed at tentatively investigating the effects of age-reader experience on age determination precision.

### Materials & Methods

For the purpose of this study, several sets of otoliths were drawn from a blue whiting otolith database compiled by the Commercial Fisheries Research Group at the Galway - Mayo Institute of Technology (GMIT), Galway, Ireland. Otoliths were removed from fish of commercial origin, sampled from catches taken annually during February to April in ICES areas VIa, VIb, VIIc and VIIk, from years 2002 to 2005. For the construction of experimental otolith sets, otoliths were drawn randomly but were stratified by age to ensure each set of 100 otoliths had the same age structure as estimated by the GMIT age-reader. Furthermore each set of 100 otoliths contained two sets of 50 otoliths of identical GMIT estimated age structure. Within each set of 50 otoliths, both sagittal otoliths from selected fish were included to enable replicate age readings of otoliths from the same fish. These otoliths were scattered randomly through the sample. The composition of the experimental otolith sets was unknown to the participant age-readers and contained otoliths from several age classes of blue whiting. Ageing procedure (magnification, sample preparation etc.) followed the workshop calibration methodology. A schematic diagram of the otolith set composition can be seen in Fig. 10. Lastly, unknown to participant age-readers, samples were swapped between pre and post calibration age determinations to remove the possibility of the sample affect on results.



Fig. 10. Schematic diagram of otolith sample composition used in the present study.

### Data Analysis

During this investigation, experimental data was analysed through the European Fish Ageing Network's 'Age Reading Comparison Programme' (Eltink et al., 2000). This programme is useful for the identification of between reader bias and for the analysis of both between reader and overall ageing precision.

Age-readers and their respective ages were entered in the EFAN worksheet. Experience was assessed for each age-reader on the basis of the number of years involved in ageing blue whiting otoliths and the number of otoliths aged each year. This experience gradient was conducted to aid interpretation of results and also for the calculation of modal age, for which the age estimates of the two least experienced age-readers were excluded. Therefore, the modal age of the otolith sample was calculated on experienced age-reader estimates only (Age-readers 1-5).

### Results

### Pre Calibration Analysis

Overall, all age-readers were in agreement (over 80% agreement) on the age of 41% of studied otoliths (Fig. 11) in the pre-calibration exercise, the mean CV for all readers was 14.6%, with % agreement at 62.4% (Fig. 12).

Pre Calibration Agreed Collection										
Criterion 80% agreement										
MODAL AGE	n									
0	0									
1	0									
2	0									
3	7									
4	6									
5	6									
2	0									
3	7									
4	6									
5	6									
6	3									
7	0									
8	0									
9	0									
	41									

Fig. 11. Number of otoliths, by modal age, that achieved over 80% agreement between age-readers pre- calibration.

For all age-readers combined, relative bias was found to be minimal ( $\pm 0.03$ ) but for individual agereaders relative bias varied from  $\pm 0.81$  to -0.60 (Fig. 13). This indicates significant under and overageing of otoliths by age-readers, with the largest relative bias observed in less experienced age readers (Fig. 13). However, it must be noted that age-readers, from both experienced and less experienced backgrounds, were found to be significantly biased from the modal age. Results of the Wilcoxon inter-reader bias test are presented in Fig. 14, and indicate a large degree of significant relative bias between age-readers. Significant relative bias against modal age was observed for both experienced and less experienced age-readers alike. The nature of observed bias can best be described from the age bias plots in Fig. 15. In these plots, under-ageing of older age groups by experienced age readers is evident. Some over-ageing of younger age groups is also visible. Linear over-ageing by some less experienced age-readers signifies systematic miss-interpretation of growth structures within the otolith.

The coefficient of variation was observed to be highest for two year-old fish (modal age) but this was subsequently found to be a factor of a low number of observations of this age group combined with high variation within observations. CV for older age groups was observed to be quite similar (Fig. 16). Overall, CV was observed to be quite poor between age-readers (14.6%) considering that otoliths of relatively few age classes were involved in this study (age classes 2 - 8, Fig. 16). The distribution of age reading errors for each age-reader by modal age is presented in Fig. 17. The normal distribution of the errors indicated no large-scale bias present; however a limited skew is visible representing the under-ageing evident in the estimates of some age-readers. Fig. 18 best describes the general ageing trend and graphically represents the over-ageing of younger age groups and over-ageing of older age groups by all age-readers combined.

	COEF	FICIEN	T OF V/	ARIATIC	DN (CV)				
	MODAL	0	0	0	0	0	0	0	ALL
	age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Readers
	0	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-
	2	0%	28%	0%	0%	47%	28%	28%	26.9%
	3	14%	11%	21%	18%	19%	24%	21%	14.5%
	4	17%	10%	18%	14%	16%	13%	18%	14.6%
	5	7%	8%	24%	18%	15%	16%	17%	13.8%
	6	10%	11%	15%	10%	10%	16%	16%	13.6%
	7	7%	7%	25%	27%	8%	12%	6%	17.3%
	8	0%	0%	33%	24%	14%	17%	13%	17.4%
	9	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-
	11	-	-	-	-	-	-	-	-
	12	-	-	-	-	-	-	-	-
	13	-	-	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-
Weighted mean	0-15	11.0%	9.8%	19.9%	15.5%	15.1%	16.6%	17.4%	44.00/
R	ANKING	2	1	7	4	3	5	6	14.6%
	PERC	ENTAG	E AGR	EEMEN	T				
	PERC MODAL	ENTAG	<b>E AGR</b>	EEMEN	<b>T</b>	0	0	0	
	PERC MODAL age	ENTAG 0 Reader 1	E AGR	EEMEN 0 Reader 3	0 Reader 4	0 Reader 5	0 Reader 6	0 Reader 7	ALL
	PERC MODAL age 0	0 Reader 1	0 Reader 2	EEMEN 0 Reader 3	0 Reader 4 -	0 Reader 5 -	0 Reader 6 -	0 Reader 7 -	ALL -
	PERC MODAL age 0	0 Reader 1 -	0 Reader 2	0 Reader 3 -	0 Reader 4 -	0 Reader 5 - -	0 Reader 6 -	0 Reader 7 -	ALL - -
	PERC MODAL age 0 1 2	ENTAG 0 Reader 1 - 100%	0 Reader 2	EEMEN 0 Reader 3 - - 100%	0 Reader 4 - 100%	0 Reader 5 - - 50%	0 Reader 6 - - 50%	0 Reader 7 - 50%	ALL - - 71%
	PERC MODAL age 0 1 2 3	ENTAG 0 Reader 1 - - 100% 81%	E AGR	0 Reader 3 - 100% 69%	T 0 Reader 4 - - 100% 73%	0 Reader 5 - - 50% 87%	0 Reader 6 - - 50% 69%	0 Reader 7 - 50% 38%	ALL - - 71% 72%
	PERC MODAL age 0 1 2 3 4	ENTAG 0 Reader 1 - - 100% 81% 75%	E AGR 0 Reader 2 - - 50% 88% 76%	EEMEN 0 Reader 3 - - 100% 69% 64%	T 0 Reader 4 - - 100% 73% 79%	0 Reader 5 - 50% 87% 75%	0 Reader 6 - 50% 69% 68%	0 Reader 7 - 50% 38% 32%	ALL - - 71% 72% 67%
	PERC MODAL age 0 1 2 3 4 5	ENTAG 0 Reader 1 - 100% 81% 75% 86%	E AGR 0 Reader 2 - 50% 88% 76% 82%	EEMEN 0 Reader 3 - - 100% 69% 64% 29%	T 0 Reader 4 - - 100% 73% 79% 57%	0 Reader 5 - 50% 87% 75% 71%	0 Reader 6 - 50% 69% 68% 61%	0 Reader 7 - 50% 38% 32% 50%	ALL - 71% 72% 67% 62%
	PERC MODAL age 0 1 2 3 4 5 6	ENTAG 0 Reader 1 - 100% 81% 75% 86% 71%	E AGR 0 Reader 2 50% 88% 76% 82% 86%	EEMEN 0 Reader 3 - - 100% 69% 64% 29% 38%	T 0 Reader 4 - - 100% 73% 79% 57% 67%	0 Reader 5 - 50% 87% 75% 71% 67%	0 Reader 6 - - 50% 69% 68% 61% 38%	0 Reader 7 - 50% 38% 32% 50% 14%	ALL - 71% 72% 67% 62% 54%
	PERC MODAL age 0 1 2 3 4 5 6 7	ENTAG 0 Reader 1 - 100% 81% 75% 86% 71% 80%	E AGR 0 <u>Reader 2</u> 50% 88% 76% 82% 86% 80%	EEMEN 0 Reader 3 - 100% 69% 64% 29% 38% 0%	T 0 Reader 4 - 100% 73% 79% 57% 67% 40%	0 Reader 5 - 50% 87% 75% 71% 67% 60%	0 Reader 6 - - 50% 69% 68% 61% 38% 20%	0 Reader 7 - 50% 38% 32% 50% 14% 80%	ALL - 71% 72% 67% 62% 54% 51%
	PERC MODAL age 0 1 2 3 4 5 6 7 8	ENTAG 0 Reader 1 - 100% 81% 75% 86% 71% 80% 100%	E AGR 0 <u>Reader 2</u> 50% 88% 76% 82% 86% 80% 100%	0 Reader 3 - 100% 69% 64% 29% 38% 0% 33%	0 Reader 4 - 100% 73% 79% 57% 67% 40% 33%	0 Reader 5 - 50% 87% 75% 71% 67% 60% 33%	0 Reader 6 - - 50% 69% 68% 61% 38% 20% 0%	0 Reader 7 - 50% 38% 32% 50% 14% 80% 33%	ALL - 71% 72% 67% 62% 54% 51% 45%
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9	ENTAG 0 Reader 1 - - 100% 81% 75% 86% 71% 86% 100% -	E AGR 0 Reader 2 50% 88% 76% 82% 86% 86% 80% 100%	EEMEN 0 Reader 3 - - 100% 69% 64% 29% 38% 0% 33%	T 0 Reader 4 - - 100% 73% 79% 57% 67% 40% 33%	0 Reader 5 50% 87% 75% 71% 67% 60% 33%	0 Reader 6 - 50% 69% 68% 61% 38% 20% 0% -	0 Reader 7 - 50% 38% 32% 50% 14% 80% 33% -	ALL 71% 72% 67% 62% 54% 51% 45%
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10	ENTAG 0 Reader 1 100% 81% 75% 86% 71% 80% 100% -	E AGR 0 Reader 2 50% 88% 76% 82% 86% 80% 100% -	EEMEN 0 Reader 3 - 100% 69% 64% 29% 38% 0% 38% 0% 33% - -	T 0 Reader 4 - - 100% 73% 79% 57% 67% 40% 33% - -	0 Reader 5 50% 87% 75% 71% 67% 60% 33% -	0 Reader 6 - 50% 69% 68% 61% 38% 20% 0% - -	0 Reader 7 - 50% 38% 32% 50% 14% 80% 33% - -	ALL - 71% 72% 67% 62% 54% 51% 45% - -
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10 11	ENTAG 0 Reader 1 100% 81% 75% 86% 71% 86% 71% 80% 100% - -	E AGR 0 Reader 2 50% 88% 76% 82% 86% 80% 100% - -	EEMEN 0 Reader 3 - 100% 69% 64% 29% 38% 0% 38% 0% 33% - - -	T 0 Reader 4 - - 100% 73% 79% 57% 67% 40% 33% - - -	0 Reader 5 50% 87% 75% 71% 67% 60% 33% - -	0 Reader 6 - 50% 69% 68% 61% 38% 20% 0% - - -	0 Reader 7 50% 38% 32% 50% 14% 80% 33% - - -	ALL - 71% 72% 67% 62% 54% 51% 45% - - -
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10 11 12	ENTAG 0 Reader 1 100% 81% 75% 86% 71% 80% 100% - - -	E AGR 0 Reader 2 50% 88% 76% 82% 86% 80% 100% - - -	0 Reader 3 - - 100% 69% 64% 29% 38% 0% 33% - - - - -	0 Reader 4 - - 100% 73% 79% 57% 67% 40% 33% - - - - -	0 Reader 5 50% 87% 75% 71% 67% 60% 33% - - -	0 Reader 6 - - - - - - - - - - - - - - - -	0 Reader 7 - - - - - - - - - - - - - - -	ALL - 71% 72% 67% 62% 54% 51% 45% - - -
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10 11 12 13	ENTAG 0 Reader 1 - - - - - - - - - - - - - - - - - - -	E AGR 0 Reader 2 50% 88% 76% 82% 86% 80% 100% - - - - - -	0 Reader 3 - - - - - - - - - - - - - - - - - - -	0 Reader 4 - 100% 73% 79% 57% 67% 40% 33% - - - - - - -	0 Reader 5 50% 87% 75% 71% 67% 60% 33% - - - - - -	0 Reader 6 - - 50% 69% 68% 61% 38% 20% 0% - - - - - - - - -	0 Reader 7 - - - - 38% 32% 50% 14% 80% 33% - - - - - - - - - -	ALL - - 71% 72% 67% 62% 54% 51% 45% - - - - - -
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14	ENTAG 0 Reader 1 - - - - - - - - - - - - - - - - - - -	E AGR 0 Reader 2 50% 88% 76% 82% 86% 80% 100% - - - - - - - - - - -	0 Reader 3 - - 100% 69% 64% 29% 38% 0% 33% - - - - - - - - - -	0 Reader 4 - - 100% 73% 79% 57% 67% 40% 33% - - - - - - - - -	0 Reader 5 50% 87% 75% 71% 60% 33% - - - - - - -	0 Reader 6 - 50% 69% 68% 61% 38% 20% 0% - - - - - - - - - -	0 Reader 7 - 50% 38% 32% 50% 50% 14% 80% 33% - - - - - - - - - - - -	ALL - 71% 72% 67% 62% 54% 51% 45% - - - - - - - -
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	ENTAG 0 Reader 1 100% 81% 75% 86% 71% 86% 71% 80% - - - - - - - - - - - - -	E AGR 0 Reader 2 50% 88% 76% 82% 86% 80% 100% - - - - - - - - - - -	EEMEN 0 Reader 3 - - 100% 69% 64% 29% 38% 0% 38% 0% 33% - - - - - - - - - - - -	T 0 Reader 4 - - - - - - - - - - - - - - - - - - -	0 Reader 5 50% 87% 75% 71% 67% 60% 33% - - - - - - - - - - - - - -	0 Reader 6 - 50% 69% 68% 61% 38% 20% 0% - - - - - - - - - - - -	0 Reader 7 - 50% 38% 32% 50% 14% 80% 33% - - - - - - - - - - - - - -	ALL - 71% 72% 67% 62% 54% 51% 45% - - - - - - - - - - - - - - - - -
Weighted mean	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 0-15	ENTAG 0 Reader 1 100% 81% 75% 86% 71% 80% 100% - - - - - - - - - - - - - - - - - -	E AGR 0 Reader 2 50% 88% 76% 82% 86% 80% 100% - - - - - - - - - - - - - - - - - -	EEMEN 0 Reader 3 - 100% 69% 64% 29% 38% 0% 38% 0% 33% - - - - - - - - - - - - - - - - -	T 0 Reader 4 - - 100% 73% 79% 57% 67% 40% 33% - - - - - - - - - - - - - - - - -	0 Reader 5 50% 87% 75% 71% 67% 60% 33% - - - - - - - - - - - - - - - - -	0 Reader 6 50% 69% 68% 61% 38% 20% 0% - - - - - - - - - - - - - - - - -	0 Reader 7 50% 38% 32% 50% 14% 80% 33% - - - - - - - - - - - - - - - - -	ALL - 71% 72% 67% 62% 54% 51% 45% - - - - - - - - - - - - - - - - - - -

Fig. 12. Pre Calibration CV and percentage agreement against modal age for participant age-readers.

	RELA	TIVE BI	AS						
	MODAL	0	0	0	0	0	0	0	
	age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	ALL
	0	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-
	2	0.00	0.50	0.00	0.00	1.00	0.50	0.50	0.36
	3	0.06	0.13	0.25	0.00	0.07	0.31	0.75	0.23
	4	0.17	0.24	-0.24	0.08	-0.04	0.16	1.00	0.20
	5	0.14	0.11	-0.79	-0.04	0.07	-0.04	0.71	0.03
	6	0.38	-0.14	-0.86	0.05	-0.05	-0.43	1.05	0.00
	7	-0.20	-0.20	-2.40	-1.40	-0.40	-1.00	0.20	-0.77
	8	0.00	0.00	-2.00	-1.67	-1.00	-2.00	0.00	-1.00
	9	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	-	-	-
	11	-	-	-	-	-	-	-	-
	12	-	-	-	-	-	-	-	-
	13	-	-	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-
ean	0-15	0.16	0.08	-0.60	-0.10	-0.02	-0.11	0.81	0.03
R	ANKING	5	2	6	3	1	4	7	

Fig. 13. Pre Calibration relative Bias at modal age.

Inter-	reader l	oias tes	t and re	eader ac	ainst N	IODAL	age bia:
	0	0	0	0	0	0	0
	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7
Reader 1		*	I	* *	I	I	* *
Reader 2	*		* *	* *	I	* *	* *
Reader 3	_	* *		* *	1	I	* *
Reader 4	* *	* *	* *		*	*	* *
Reader 5	_	-	١	* *		*	* *
Reader 6	_	* *	١	* *	*		* *
Reader 7	* *	* *	* *	* *	* *	* *	
MODAL age	* *	_	* *	_	-	-	* *
		_	= no sign	of bias (p	>0.05)		
		*	= possibi	lity of bias	; (0.01 <p<0< th=""><th>.05)</th><th></th></p<0<>	.05)	
		* *	= certaint	y of bias (	p<0.01)		

Fig. 14. Results of pre-calibration inter-reader bias tests.

#### Blue whiting otolith set X HIRTSHALS, DENMARK JUNE 2005

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



Fig. 15. Age bias plots for individual age-readers and all age-readers combined pre calibration.



Fig. 16. Precision for all age-readers combined for modal age groups pre calibration



Fig. 17. The pre-calibration distribution of the age reading errors in percentage by MODAL age as observed from the whole group of age readers in an age reading comparison to MODAL age. The achieved precision in age reading by MODAL age group is shown by the spread of the age readings errors. There appears to be no RELATIVE bias, if the age reading errors are normally distributed.



Fig. 18. The pre-calibration RELATIVE bias by MODAL age as estimated by all age readers combined (Modal age is on the X axis, bias on the Y axis).

### Post Calibration Analysis

For the analysis of post calibration precision, the modal age was again calculated from age determinations of age-readers 1-5. Overall, age-readers achieved over 80% agreement on only 18% of otoliths aged (Fig. 19), compared to 41% in pre-calibration exercise. This represented a substantial decrease in age estimation consensus between age-readers.

CV increased from 14.6% in the pre calibration exercise to 17.5% post calibration (Fig. 20) signifying a decrease in precision between age-readers. The percentage agreement remained unchanged at 62.3% indicating that although there appeared to no change in the number of differing age estimates, there was an increase in the intensity of these differences. After calibration, the overall relative bias for all age-readers combined increased (from 0.03 pre calibration to -0.23 post calibration), with under-ageing evident (Fig. 21). For individual age-readers, relative bias ranged from -0.80 to +.02 in comparison to the modal age. Five age-readers were found to be significantly biased, to the modal age, post calibration (Fig. 22), with four of these age-readers systematically under-ageing otoliths (Fig 21). Furthermore, less experienced age-readers were again found to display the greatest relative bias (-0.80). Ironically this was in contrast to the over-ageing trend observed in the results of the pre calibration exercise.

Age bias plots once more best described the bias observed between age-readers, demonstrating under-ageing evident in the estimates of two age-readers (Fig. 23). This indicates a calibration related systematic bias in the interpretation of otolith age structures for these age-readers, as pre calibration biases of this nature were not obvious. Furthermore, some of the more experienced age-readers did not appear to alter ageing procedures post calibration. However, one experienced age-reader was found to be significantly negatively biased post calibration indicating no change in growth zone interpretation. Less experienced age-readers appeared to be more prone to alterations in growth zone interpretation due to the process of calibration.

CV was observed to be quite consistent for older age groups (Fig. 24), with two year-old fish once more displaying the highest CV between age readers (for reasons discussed earlier). The distribution of ageing errors from the modal age was observed to be normally distributed (post calibration); nevertheless, some relative negative bias was evident (Fig. 25). This bias (for all agereaders combined) was most evident in older fish i.e. age groups 5,6 and 7 (Fig. 26).

Post calibration Agreed Collection									
Criterion 80% agreement									
MODAL AGE	n								
0	0								
1	0								
2	2								
3	10								
4	4								
5	2								
6	0								
7	0								
8	0								
9	0								
	18								

Fig. 19. Number of otoliths, by modal age, that achieved over 80% agreement between age-readers post calibration.

	COEF	FICIEN	T OF V/	ARIATIC	DN (CV)				
	MODAL	0	0	0	0	0	0	0	ALL
	age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Readers
	0	-	-	-	-	-	-	-	-
	1	-	-	-	-	-	-	-	-
	2	20%	23%	0%	52%	37%	25%	25%	31.8%
	3	14%	13%	11%	9%	24%	17%	24%	17.6%
	4	21%	12%	19%	15%	18%	19%	19%	16.7%
	5	10%	5%	18%	14%	19%	14%	19%	15.8%
	6	6%	6%	24%	13%	20%	16%	21%	18.0%
	7	0%	8%	21%	11%	12%	8%	14%	15.0%
	8	-	_	-	_	_	_	-	-
	9	-	-	-	-	-	-	-	-
	10	-	-	-	-	-	_	-	-
	11	-	-	-	-	-	_	-	-
	12	-	-	-	-	-	-	-	-
	13	-	-	-	-	-	-	-	-
	14	-	-	-	-	-	-	-	-
	15	-	_	-	_	_	_	-	-
Weighted mean	0-15	13.4%	10.0%	16.0%	14.6%	20.8%	16.6%	20.5%	
	RANKING	2	1	4	3	7	5	6	17.5%
			-	-	-		•	•	
	PERC	ENTAG		EEMEN	т				
				EEMEN	<b>T</b>	0	0	0	
		ENTAG	E AGR	EEMEN 0 Reader 3	T 0 Reader 4	0 Reader 5	0 Reader 6	0 Reader 7	A1 1
	PERC MODAL age	0 Reader 1	0 Reader 2	0 Reader 3	0 Reader 4	0 Reader 5	0 Reader 6	0 Reader 7	ALL
	PERC MODAL age 0	0 Reader 1	0 Reader 2	0 Reader 3	0 Reader 4	0 Reader 5 -	0 Reader 6 -	0 Reader 7 -	ALL -
	PERC MODAL age 0 1 2	0 Reader 1 - 80%	0 Reader 2 - - 60%	EEMEN 0 Reader 3 - - 100%	T 0 Reader 4 - - 80%	0 Reader 5 - - 20%	0 Reader 6 - - 80%	0 Reader 7 - - 80%	ALL - - 71%
	PERC MODAL age 0 1 2 3	0 Reader 1 - 80% 81%	0 Reader 2 - - 60% 85%	EEMEN 0 Reader 3 - 100% 80%	0 Reader 4 - - 80% 93%	0 Reader 5 - - 20% 37%	0 Reader 6 - - 80% 85%	0 Reader 7 - - 80%	ALL - - 71% 74%
	PERC MODAL age 0 1 2 3 4	0 Reader 1 - 80% 81% 52%	E AGR 0 Reader 2 - 60% 85% 84%	EEMEN 0 Reader 3 - 100% 89% 60%	T 0 Reader 4 - - 80% 93% 72%	0 Reader 5 - 20% 37% 52%	0 Reader 6 - - 80% 85% 68%	0 Reader 7 - - 80% 44%	ALL - - 71% 62%
	PERC MODAL age 0 1 2 3 4 5	0 Reader 1 - 80% 81% 52% 70%	E AGR 0 Reader 2 - - 60% 85% 84% 03%	0 Reader 3 - 100% 89% 60% 48%	T 0 Reader 4 - - 80% 93% 72% 70%	0 Reader 5 - 20% 37% 52% 31%	0 Reader 6 - - 80% 85% 68% 59%	0 Reader 7 - - 80% 44% 44% 10%	ALL - 71% 74% 62% 57%
	PERC MODAL age 0 1 2 3 4 5 6	0 Reader 1 - 80% 81% 52% 79% 88%	E AGR 0 Reader 2 - - 60% 85% 84% 93% 88%	0 Reader 3 - 100% 89% 60% 48% 13%	T 0 Reader 4 - - 80% 93% 72% 79% 75%	0 Reader 5 - 20% 37% 52% 31% 25%	0 Reader 6 - - 80% 85% 68% 59% 38%	0 Reader 7 - - 80% 44% 44% 10% 13%	ALL - - 71% 74% 62% 57% 48%
	PERC MODAL age 0 1 2 3 4 5 6 7	0 Reader 1 - 80% 81% 52% 79% 88% 100%	E AGR 0 Reader 2 - 60% 85% 84% 93% 88% 50%	EEMEN 0 Reader 3 - 100% 89% 60% 48% 13% 50%	T 0 Reader 4 - - 80% 93% 72% 79% 75% 50%	0 Reader 5 - 20% 37% 52% 31% 25% 33%	0 Reader 6 - - 80% 85% 68% 59% 38% 67%	0 Reader 7 - - 80% 44% 44% 10% 13% 0%	ALL - 71% 74% 62% 57% 48% 51%
	PERC MODAL age 0 1 2 3 4 5 6 6 7 8	ENTAG 0 Reader 1 - - 80% 81% 52% 79% 88% 100%	E AGR 0 Reader 2 - - 60% 85% 84% 93% 88% 50%	EEMEN 0 Reader 3 - 100% 89% 60% 48% 13% 50%	T 0 Reader 4 - - 80% 93% 72% 79% 75% 50%	0 Reader 5 - 20% 37% 52% 31% 25% 33%	0 Reader 6 - - 80% 85% 68% 59% 38% 67%	0 Reader 7 - 80% 44% 44% 10% 13% 0%	ALL 71% 74% 62% 57% 48% 51%
	PERC MODAL age 0 1 2 3 4 5 6 7 8 8	ENTAG 0 Reader 1 - - 80% 81% 52% 79% 88% 100% -	E AGR 0 Reader 2 60% 85% 84% 93% 88% 50%	EEMEN 0 Reader 3 - 100% 89% 60% 48% 13% 50% -	T 0 Reader 4 - - 80% 93% 72% 79% 75% 50% -	0 Reader 5 - 20% 37% 52% 31% 25% 33% -	0 Reader 6 - - 80% 85% 68% 59% 38% 67% -	0 Reader 7 - - 80% 44% 44% 10% 13% 0% -	ALL - 71% 62% 57% 48% 51% -
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 9	ENTAG 0 Reader 1 - - 80% 81% 52% 79% 88% 100% - -	E AGR 0 Reader 2 - - 60% 85% 84% 93% 88% 50% - -	EEMEN 0 Reader 3 - - 100% 89% 60% 48% 13% 50% - -	T Reader 4 - - 80% 93% 72% 79% 75% 50% - -	0 Reader 5 - 20% 37% 52% 31% 25% 33% - -	0 Reader 6 - - - 80% 85% 68% 59% 38% 67% - -	0 Reader 7 - - 80% 44% 44% 10% 13% 0% - -	ALL - 71% 74% 62% 57% 48% 51% - -
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10	ENTAG 0 Reader 1 - 80% 81% 52% 79% 88% 100% - - - -	E AGRI 0 Reader 2 60% 85% 85% 84% 93% 88% 50% - -	EEMEN 0 Reader 3 - - 100% 89% 60% 48% 13% 50% - - - -	T Reader 4 - - 80% 93% 72% 72% 75% 50% - - - -	0 Reader 5 - 20% 37% 52% 31% 25% 33% - - -	0 Reader 6 - - 80% 85% 68% 59% 38% 67% - - - -	0 Reader 7 - - 80% 44% 44% 10% 13% 0% - - -	ALL - 71% 74% 62% 57% 48% 51% - - -
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10 11 12	ENTAG 0 Reader 1 - - 80% 81% 52% 79% 88% 100% - - - - - -	E AGRI 0 Reader 2 60% 85% 84% 93% 88% 50% - - -	EEMEN 0 Reader 3 - - 100% 89% 60% 48% 13% 50% - - - - - - -	T Reader 4 - - 80% 93% 72% 79% 75% 50% - - - - -	0 Reader 5 - 20% 37% 52% 31% 25% 33% - - -	0 Reader 6 - - 80% 85% 68% 59% 38% 67% - - - - - - -	0 Reader 7 - - 80% 44% 44% 10% 13% 0% - - - - - -	ALL - 71% 74% 62% 57% 48% 51% - - - -
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10 11 12 2	ENTAG 0 Reader 1 - 80% 81% 52% 79% 88% 100% - - - - - - - -	E AGRI 0 Reader 2 - 60% 85% 84% 93% 88% 50% - - - - - - - -	EEMEN 0 Reader 3 - 100% 89% 60% 48% 13% 50% - - - - - - - - - -	T Reader 4 - - 80% 93% 72% 79% 75% 50% - - - - - - - -	0 Reader 5 - 20% 37% 52% 31% 25% 33% - - - - - - -	0 Reader 6 - - 80% 85% 68% 59% 38% 67% - - - - - - - - - - - -	0 Reader 7 - 80% 44% 44% 10% 13% 0% - - - - - - - -	ALL - 71% 74% 62% 57% 48% 51% - - - - - - - - -
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14	ENTAG 0 Reader 1 - - 80% 81% 52% 79% 88% 100% - - - - - - - - - - -	E AGR 0 Reader 2 - - - - - - - - - - - - - - - - - - -	EEMEN 0 Reader 3 - - 100% 89% 60% 48% 13% 50% - - - - - - - - - - - - - -	T Reader 4 - - 80% 93% 72% 79% 75% 50% - - - - - - - - - -	0 Reader 5 - 20% 37% 52% 31% 25% 33% - - - - - - - - - -	0 Reader 6 - - - - - - - - - - - - - - - - - - -	0 Reader 7 - - 80% 44% 44% 10% 13% 0% - - - - - - - - - - - -	ALL - 71% 62% 57% 48% 51% - - - - - - - - - - - - -
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 5	ENTAG 0 Reader 1 - - 80% 81% 52% 79% 88% 100% - - - - - - - - - - - - - -	E AGR 0 Reader 2 - - - - - - - - - - - - - - - - - - -	EEMEN 0 Reader 3 - - 100% 89% 60% 48% 13% 50% - - - - - - - - - - - - - - - - -	T Reader 4 - - 80% 93% 72% 79% 75% 50% - - - - - - - - - - - - - - -	0 Reader 5 - 20% 37% 52% 31% 25% 33% - - - - - - - - - - - - - - - - -	0 Reader 6 - - - 80% 85% 68% 59% 38% 67% - - - - - - - - - - - - - - - - - - -	0 Reader 7 - - 80% 44% 44% 10% 13% 0% - - - - - - - - - - - - - - - -	ALL - 71% 74% 62% 57% 48% 51% - - - - - - - - - - - - - - - - - - -
	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 2 45	ENTAG 0 Reader 1 - 80% 81% 52% 79% 88% 100% - - - - - - - - - - - - -	E AGRI 0 Reader 2 60% 85% 85% 84% 93% 50% - - - - - - - - - - - - - - - - - - -	EEMEN 0 Reader 3 - - 100% 89% 60% 48% 13% 50% - - - - - - - - - - - - - - - -	T Reader 4 - 80% 93% 72% 75% 50% - - - - - - - - - - - - -	0 Reader 5 20% 37% 52% 31% 25% 33% - - - - - - - - - - - - - - - - -	0 Reader 6 - - - - - - - - - - - - - - - - - - -	0 Reader 7 - - 80% 44% 44% 10% 13% 0% - - - - - - - - - - - - - - - - - -	ALL - 71% 74% 62% 57% 48% 51% - - - - - - - - - - - - - - - - - - -
Weighted mean	PERC MODAL age 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 0-15	ENTAG 0 Reader 1 - 80% 81% 52% 79% 88% 100% - - - - - - - - - - - - -	E AGRI 0 Reader 2 60% 85% 84% 93% 88% 50% - - - - - - - - - - - - - - - - - - -	EEMEN 0 Reader 3 - 100% 89% 60% 48% 13% 50% - - - - - - - - - - - - -	T Reader 4 - 80% 93% 72% 79% 75% 50% - - - - - - - - - - - - -	0 Reader 5 - - 20% 37% 52% 37% 52% 35% 25% 33% - - - - - - - - - - - - - - - - -	0 Reader 6 - - - 80% 85% 68% 59% 38% 67% - - - - - - - - - - - - - - - - - - -	0 Reader 7 - - 80% 44% 44% 10% 13% 0% - - - - - - - - - - - - - - - - - -	ALL - 71% 74% 62% 57% 48% 51% - - - - - - - - - - - - - - - - - - -

Fig. 20. Post Calibration CV and percentage agreement against modal age for participant age-readers.

RELA	TIVE B	AS						
MODAL	0	0	0	0	0	0	0	
age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	ALL
0	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-
2	0.20	0.40	0.00	0.60	-0.80	-0.20	-0.20	0.00
3	0.11	0.07	-0.04	0.00	-0.67	-0.04	-0.59	-0.16
4	0.36	0.20	-0.04	0.36	-0.44	0.00	-0.52	-0.01
5	0.17	0.00	-0.69	0.25	-0.48	-0.31	-1.21	-0.33
6	-0.13	-0.13	-1.50	-0.38	-1.13	-0.88	-1.50	-0.80
7	0.00	-0.50	-1.00	0.67	-0.33	-0.33	-2.00	-0.46
8	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-
n 0-15	0.17	0.05	-0.40	0.20	-0.58	-0.20	-0.88	-0.23
RANKING	2	1	5	4	6	3	7	

Fig. 21. Post Calibration relative Bias at modal age.

Inter-	Inter-reader bias test and reader against MODAL age bia												
	0	0	0	0	0	0	0						
	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7						
Reader 1		* *	* *	-	* *	* *	*						
Reader 2	* *		_	* *	-	* *	* *						
Reader 3	* *	-		* *	I	* *	* *						
Reader 4	_	* *	* *		* *	*	* *						
Reader 5	* *	-	-	* *		* *	* *						
Reader 6	* *	* *	* *	*	* *		* *						
Reader 7	*	* *	* *	* *	* *	* *							
MODAL age	*	—	* *	* *	* *	* *	**						
		_	= no sign	of bias (p	>0.05)								
		*	= possibi	lity of bias	; (0.01 <p<0< td=""><td>.05)</td><td></td></p<0<>	.05)							
		* *	= certaint	ty of bias (	p<0.01)								

Fig. 22. Results of post calibration inter-reader bias tests.

### Blue whiting set Y Hirtshals, Denmark, June 2005

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



Fig. 23. Age bias plots for individual age-readers and all age-readers combined post calibration.





Fig. 25. The post calibration distribution of the age reading errors in percentage by MODAL age as observed from the whole group of age readers in an age reading comparison to MODAL age. The achieved precision in age reading by MODAL age group is shown by the spread of the age readings errors. There appears to be no RELATIVE bias, if the age reading errors are normally distributed.



Fig. 26. The post calibration RELATIVE bias by MODAL age as estimated by all age readers combined (Modal age is on the X axis, bias on the Y axis).

### Discussion

The results of the post calibration analysis indicate that, of the several age-readers tested, more experienced age-readers did not appear to dramatically alter ageing procedures. Less experienced age-readers were more prone to alterations in ageing criteria and subsequently post calibration bias became evident in several age-readers. The calibration exercise itself may have resulted in a conservative approach to age estimation by many age-readers, which manifested itself in a change from pre-calibration over-ageing to post-calibration under-ageing. The CV was consequently observed to increase from 14.6% to 17.5% across all age-readers. This is in contrast to what would have been expected from a calibration exercise. However, the nature of the age-reader calibration must be discussed here as it directly influences the results of this study. 'Split' rings, and their interpretation, were identified during the workshop as a source of ageing error for blue whiting otoliths. They are most prevalent in the otolith structure of older blue whiting. Such otoliths made up a large proportion of the otolith sample used in this experiment. This workshop calibration exercise was designed to (a) facilitate the identification of 'false' rings or 'split rings' in the blue whiting otolith structure, and (b) allow for the disregarding of these problematic rings when estimating the age of the fish. Therefore, it is not surprising that pre and post calibration estimates differed; the latter would have been expected to have lower values of CV. One would expect a significant increase in precision between age readers post calibration and less divergence from the modal age. This was not the case in this study and may have been due to the complexity of age structures chosen for this analysis, or a factor of varying levels of age-reader experience.

### 6. Method and manual

During discussions of the results from the age calibrations and the image analysis, the group agreed on the need for a set of 'rules of thumb' regarding various aspects of the age estimation of blue whiting. The guidelines for ageing of blue whiting are intended to guide new readers of blue whiting and keep experienced readers on track. The guidelines are to be developed into a larger manual comprising both the guidelines and the methods described in section 7 in the present report.

Otoliths are read whole immersed in water. Reading is considered easiest when removed from fish and read immediately. If otoliths are stored dry, soak for 24 hours beforehand. Make sure that the otoliths are proper cleaned before storage. This will enhance the winter rings. Under reflected light the winter rings appear translucent.

If the otolith is stored longer than 7 days in water the shape/composition of the otolith seem to change (due to unstable pH of the water), so the storage is recommended to be done dry.

Use reflected light and magnification X 6/6.4 against a black background where 12 e.p.u (eyepiece units) are equal to 2 mm. The magnification and the light intensity can be adjusted by the individual reader. When ageing an otolith that displays split rings, it may be useful to both 'zoom out' and 'focus out'. This will result in only the darkest translucent rings becoming visible. In older individuals the first annulus may be difficult to define due to overlaying otolith growth. In cases where it is difficult to define the e.p.u's for this age (see below) it may be an advantage to grind and polish to produce a section through the saggital plane where the first annulus then can be defined. Usually the characteristics of the 1<sup>st</sup> ring in the older individuals are a more undefined wavy appearance.

The otolith is interpreted by reading up the rostrum area and using the whole otolith pattern as a guide. Usually the clearest pattern is seen when the convex side of the otolith is facing down (sulcus side facing down). However, handling the otolith turning it in various directions may be a way of assuring the estimated age. With difficult otoliths, it is advised to read both the concave and convex sides of the otoliths to gain a better interpretation of the annuli.

Otoliths with translucent edge, sampled from the first half of the year, are aged by counting all translucent annuli, including the edge, if translucent. Fish sampled from the second half of the year, are aged by ignoring a translucent edge if present. This 'translucent edge' is the onset of the winter ring. This guide is particular useful for fish of ages 1-3. This onset varies with time by geographic location.

When using the measurement scale of the eyepiece unit the following reference guide may be applied as general guidelines (figure 27) (please refer to chapter 7.2 for background material for these distances):

Age	length (cm)	mean e.p.u
1	18-23	50+
2	23-26	60+
3	25-28	70+
4	27-30	(76)
5	29-33	(79)



Figure 27. Measurements of year rings.

For the older ages (4+) the distances between the rings may vary with sex. The mean e.p.u for these ages should be regarded as rough guidelines. When measuring the distances between rings point to the outer edge of the translucent zone. If the translucent zone is not finished on the edge, mark the edge of the otolith. As an additional measurement take the total otolith length.

A 'false' ring known as the 'Baileys zone' may appear inside the first winter ring, confusion can be eliminated by referring to the measurement scale below. This false zone has been linked to a change in behaviour from pelagic to demersal feeding and distribution in the first year of life. The split rings can be differentiated from annual rings as they cannot be followed around the whole otolith. If a ring is less than 48 e.p.u it probably is a Bailey's zone. As the otolith grows older the Baileys zone appear less visible. Even the first ring may disappear in large otoliths, thus having the measurement guide of the probable size of the first ring is very useful. During the first 3 years of growth the winter rings appear far apart and appear closer as the fish grow older.

Ancillary information such as fish length should not be over relied upon when estimating age. Other information such as, sex of fish and geographic sample location may be considered. For example, male and female fish of similar length may not necessarily be the same age. Sexual dimorphism is present in blue whiting, females grow faster than males, thus are younger in general at similar length, to male fish. Therefore knowledge of the sex of the fish is important for the ageing. There are morphometric differences between the sexes. In terms of spawning; the spawning is gradually later in the season moving from the South to the North. The spawning starts in the southern parts of the area in February and ends sometime during April in the northern parts of the area. Therefore the formation of the first winter ring can occur from October to January.

### 7. Accuracy and precision of age readings

During the workshop several presentations on methods for quality control and validation of age estimations were given. The following is a sum up of the most important discussions concerning these presentations.

### 7.1 Length based revision of age and cluster analysis

The interpretation of age structures appear to be easier in older individuals where the consecutive ring structure with the gradual growth each season becomes increasingly evident. The detection of possible false rings is also easier in these individuals as the interruption of the regularity of the ring formation is more evident. When such irregular rings/checks are accounted a way of validating the structure can be done by recalculation of fish length and assess if the result is in accordance with what could be expected.

A way to evaluate the back-tracked growth from the annual structures in the otolith is to compare the growth to growth curves in the literature. According to the empirical data (Walford 1946), the growth when plotted as fish length at age t and t+1 forms a straight line.

Another approach is to consider the length and age at first maturity (Ionas and Blinov 1976). For many fish species it has been found that their growth is described by a single curve when plotting the ratio of the length and the length of first maturation and, respectively, the ratio of the age to the age of the first maturation instead of fish length and age. To check age estimates on the basis of those criterions the diameter of the age structures ("ring") and the corresponding estimated fish lengths are needed.

Though fish length should not be heavily relied upon when estimating age these data combined with otolith measurements may facilitate age estimation. If two individuals of similar sex and length have otoliths of considerably different size it may be an indication of different age. The relation between otolith size and fish size is influenced by growth rate in many fish species (Lagardère 1989, Krivibok and Shatunovsky 1976, Secor and Dean 1989) and in blue whiting as well (Timoshenko 1982). Measuring the age structures of individuals with similar sex and length sampled within a short time period a simple graphical analysis indicates that e.g. blue whiting between 22.0-22.9 cm in length are subdivided into 2 groups on the basis of the relative otolith size and growth rate. This should be born in mind when back-tracking growth patterns from otolith structures.

Often it is necessary to subdivide a rather large sample of otoliths similar in size into different age groups. This may be done sufficiently accurate without assessment of the individual otoliths. This procedure is based on a simple assessment of numbers of growth zones in relation to otolith size and fish size. If the relative otolith size differs in two individuals of similar length and the smallest otolith (relative size) displays the highest number of growth zones, it must be assumed that not all the observed growth zones are true annual zones. The text table below shows an example of such an analysis (Timoshenko 2002).

	A	В	С	D	Е	F	G	Н		J	K	L	M
1	Длина	Длина	Ширина	Наличие	Темп	Границы	ЕСЛИ С >	F3;D=1;B/A	<СРЗНАЧ	(В/А) - ТО з	кспорт в г	юле М25	
2	рыбы	отолита	2-й зоны	3-й зоны	роста	облаков	ECNH C *	§F3;D=1;ΒΛ	А≻СРЗНАЧ	1(B/A) - TO	экспорт в г	поле МЗ2	695
3	24	11.5	653	1	0.479	675	ЕСЛИС>	F3;D=0;B/A	<СРЗНАЧ	(B/A) - TO 3	кспорт в г	юле МЗб	
4	24	11.1	646	1	0.463	655	еспис>	F3;D=0;B/A	>СРЗНАЧ	(B/A) - TO 3	кспорт в г	юле М40	_ 690 реф и_
5	24	10.4	647	1	0.433		ЕСЛИ F3>	C>F4;D=1;E	3/А<СРЗНА	\Ч(В/А) - Т(	Э экспорт	в поле	685 n
6	24	10.3	670	0	0.429	Ср.темп	ЕСЛИ F3>	C>F4;D=1;E	3/A>CP3H/	\Ч(В/А) - Т(	Э экспорт	в поле	
7	24	10.5	661	1	0.438	роста	ЕСЛИ F3>	C>F4;D=0;E	3/A <cp3h <="" td=""><td>\Ч(В/А) - Т(</td><td>Э экспорт</td><td>в поле</td><td>680 🕂 🏅 🔶 🦷</td></cp3h>	\Ч(В/А) - Т(	Э экспорт	в поле	680 🕂 🏅 🔶 🦷
8	24.1	11.1	648	0	0.461	0.463	ЕСЛИ F3>	C>F4;D=0;E	8/A>CP3H/	<b>\Ч(В/А) - Т(</b>	О экспорт	в поле	675 H
9	24.1	11.5	650	1	0.477		ЕСЛИ С <	F4;D=1;B/A	<cp3ha4< td=""><td>(В/А) - ТО з</td><td>кспорт в г</td><td>юле</td><td></td></cp3ha4<>	(В/А) - ТО з	кспорт в г	юле	
10	24.1	12.5	660	0	0.519		ЕСЛИ С <	F4;D=1;B/A	>СРЗНАЧ	(В/А) - ТО з	кспорт в г	юле	670 🔶 🔶
11	24.1	10.4	661	1	0.432		ЕСЛИ С <	F4;D=0;B/A	<cp3ha4< td=""><td>(В/А) - ТО з</td><td>кспорт в г</td><td>юле</td><td>ees 2</td></cp3ha4<>	(В/А) - ТО з	кспорт в г	юле	ees 2
12	24.1		670	0	0.456		ЕСЛИС <	F4;D=0;B/A	>СРЗНАЧ	(В/А) - ТО з	кспорт в г	юле	
13	24.1	10,5	690	1	0.436	Критерий				Критерий			_ 660 🗰 👬 📈 🗸
14	24.2	10.8	658	1	0.446	Ширина	Наличие	Темп		Ширина	Наличие	Темп	
15	24.2	11.3	662	1	0.467	2-й зоны	3-й зоны	роста		2-й зоны	3-й зоны	роста	
16	24.2	11.2	663	0	0.463	>=675	1	<=.463		>=675	1	>.463	_ 650 <b>[                                   </b>
17	24.2	11.3	663	0	0.467								
18	24.3	12.5	660	0	0.514	Ширина	Наличие	Темп		Ширина	Наличие	Темп	_ 646 H
19	24.3	11	661	0	0.453	2-й зоны	3-й зоны	роста		2-й зоны	3-й зоны	роста	L 640 🛏 ы
20	24.3	11.1	690	U	0.457	>=675	0	<=.463		>=675	0	>.463	24 25
21	24.4	12.1	663	1	0.496								24 25
22	24.4	11.9	664	0	0.488	Расши	ренный	і фильт	D			? X	
23	24 4	////XX	()))(()()()()()()()()()()()()()()()()(		0.467				r				Возраст 2
24	24.4	31.3	681	9	0:463	060260	TV D						Длина
25	24.4	11.4	679	0	0,467	Copaco	in d						24.1
26	24.5	11.3	660	1	0.461	Сфи	льтроват	гь список	на несте				24,7
27	24.5	11.4	661	1	0.465	6							24.9
28	24.5	11.3	663	1	0.461	(• GKG	пироват	ь результ	ат в друг	гое место			24.9
29	24.5	11	664	0	0.449	_							
30	24.5	11.1	663	1	0.453	Maxin Bla	ui auana	ROLL!		****	140	<b>1</b>	Возраст 3
31	24.6	11.6	660	0	0.472	Pickodha	ын длана	SUH.		\$4\$1:\$E\$	943		Длина
32	24.6	11.0	666	0	0.447	A		×.		1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	100		
33	24.7	10.5	650	1	0.425	диапази	н услови	141;		\$J\$18:\$L	\$20		-
34	24.7	12.0	661		0.486	-						-	Возраст 1 или 2
35	24.7	요 변환	680		0.449	Помести	ть резул	ьтат в ди	ianason:	M25		<u></u>	Длина
36	24.8	11.5	662	0	0.464								24.3
37	24.8	11.2	670	1	0.452								
38	24.9	12.2	662	0	0.490								Возраст 2
39	<u>24.9</u>	⊢ 12 N	664	L 0	0.482								Лпина 🗌

In the table column A contains fish length, column B contains otolith length and the width of the  $2^{nd}$  hyaline zone is listed in column C. In column D a '1' means that the  $3^{rd}$  is visible in the otolith and a '0' means that there are no hyaline zones visible between the  $2^{nd}$  zone and the edge of the otolith. The growth rate index is estimated as the ratio of the otolith length to the fish length, thus the growth rate is calculated in column E by dividing otolith length (column B) with fish length (column A).

Using the data on the width of 2<sup>nd</sup> hyaline zone, it is possible to divide the whole dataset into two or sometimes to three groups. The cells F3 and F4 define the separation criterion between the groups and are used by the excel-setup to place an individual otolith within a group.

The majority of otoliths within a group are displaying 'typical' growth within the defined limits, though there are also otoliths assigned to one age-group that may display atypical growth and thus needs further inspection (visual check of the hyaline zones). As an example could be that the central graph shown in column M displays the otoliths in which the  $2^{nd}$  hyaline zone is within the defined limits. The majority of these otoliths are classified as 2 year olds. However, some of these otoliths may display a  $3^{rd}$  hyaline zone and in this case the excel-setup will examine the growth rate of these individuals (from column E) to define whether it is within the high- or low-ends of the mean growth rate displayed by the whole dataset which is defined in cell F8. If the individual is showing a slow growth rate, i.e. the value in column E>F8 the growth is defined as slow and the individual is assigned the age 3. If the opposite is the case and the growth rate is high, the age assigned is 2. The excel-setup has12 possible variants of 'decisions' to assign age to an individual otolith based on growth rate comparisons.

Alternatively the dataset can be divided into growth rates prior to the above analysis or one can cluster by all data simultaneously. In addition some successful attempts to subdivide samples into age-groups by means of otolith weight have been made, though this approach requires rather large sample sizes (Timoshenko 1989).

7.2 Distances between annual structures following an entire year-class

In the general guidelines given in section 6 (Method and manual) some distances between annual growth structures are listed. These are mainly based on an extensive dataset provided by Norway on measurements of distances between annual growth structures for an entire year-class. The figures below represent measurements of 12542 individuals from samples from 1982 to 2000.



Figure 28. Age-length distribution



Figure 29. Length measurements of otoliths for each year ring. Mean length for each year ring is given in the figure legend.



Figure 30. Measurements of year rings for male and female. Mean length for each year ring. A 4 year female has come up to the same mean length as a 5 year old mail (see also fig.10).



Figure 31. Otolith length compared with the fish length. Calibration 12 marks; 2 mm.

### 7.3 Microstructure validation of age structures

Problems with correct age determination may arise from two that more than one translucent zone may be formed in a specific year thereby adding false winter zones to the total count as suggested for other species like sandeel.

Validation of annual ring formation from primary increment formation in otoliths has to either rely on a daily periodicity of the primary increments all year round or an annual cycle in the pattern of the otolith microstructure (Panella 1971). The following validation method of annual structures has been suggested by Arneri et al. 1998 for sprat. It is suggested by the Workshop-group that work similar to this is performed for blue whiting.

Studies of microstructures in sprat otoliths have demonstrated structural differences between what are defined as true and false translucent (winter) rings (Arneri et al., 1998). Sprat sagitta otoliths were taken from samples of age 0 and 1 in ICES subdivisions 22 and 25 (the Danish Belts, and the Bornholm basin of the Eastern Baltic respectively). Samples were taken from both commercial landings and scientific cruises to cover the major part of the year for age 0 and 1 sprat. Otolith pairs were individually placed in black trays immersed in alcohol for ageing and stored for later mounting on microscope glass slides. Mounting was performed with the sulcus side up in thermoplastic resin at 150°C allowing for repeated relocation of the otolith for grinding and polishing on both sides.

An objective lens with 40 times magnification and further ocular enhancing gave the necessary resolution of 7 to 10 pixels per micrometer to count and measure daily structures between 1 and 2  $\mu$ m wide. Pixel values, grey level and calibrated distances were used for calculation of daily increment widths formed during the larval period. Individual increment positions were identified by their relative changes in grey level values using running average filters.

During winter when the annual structure is deposited the width of the daily increments gradually reduces in width (figure 32). This pattern can be found in true winter rings in the otolith in sprat aged 0 - 2 years old (figure 33).



Figure 32. Development of daily increment width on the edge of sprat otoliths from indviduals caught during winter (Arneri et al., 1998)



Figure 33. Example of otolith microstructure on the edge of a sprat otolith caught during winter (Arneri et al., 1998)

In a false winter ring have a very different structure of the daily increment surrounding it. No gradual reduction of the daily increments is found either before or after the false translucent zone (figure 34). Thus, in otoliths where the age reader is in doubt whether a translucent zone is true or false when ageing the individual the validity of the ring can be examined using the otolith microstructure.



Figure 34. Example of otolith microstructure in relation to a false winter ring (Arneri et al., 1998)

### 8. Conclusions and recommendations

The overall result of the workshop exercises is that there is a general high agreement between readers. The image analysis exercise clarified that the lack of agreement can be referred to two reasons, the first being the position of the first ring where the Bower zone is clear. This is often seen in the younger individuals as the otolith is thinner and thus the structures more clear. The second reason to disagreement arose where some readers choose to leave out specific rings identified by other readers as true annual rings where the rings successive to the 2<sup>nd</sup> ring were split rings. The third exercise showed that, although precision decreased between age-readers post calibration, this was in part due to deviations in growth zone interpretation in less experienced participant agereaders. It may be argued that because of the difficulty in split ring identification and subsequent discount, a defined protocol may have to be introduced to reduce the potential of age-readers, particularly those less experienced, to diverge from an agreed ageing criteria. Previous studies have identified experience as an important contributing factor to precision, one report stating that, "new age-readers start at a high CV and work their way down" (Walsh & Burnett, 2002). It is most probable that this 'experience effect' contributed to the findings of the present study. There was a difference in age structures between the two first calibration sets and the third calibration sets, as the latter was a more heterogeneous set including a higher frequency of older individuals. The results showed clearly that otoliths of younger fish achieved better precision that older fish illustrating the continuing problem of age determination of older fish. This is a universal problem however this workshop recommends the use of a more heterogeneous age structured otolith collection for the exchange in 2007.

The workshop achieved quite a lot in terms of ironing out, through discussion and calibration, some of the major problems in ageing otoliths of blue whiting. The group reached agreement on a definition of an ageing protocol/guidelines mentioned in the present report and the aim is to employ these guidelines to eliminate some of the problems with e.g. split rings in the otolith structures. The group strongly recommends that all ageing laboratories processing blue whiting should include the guidelines developed during the workshop in their ageing manuals. If possible the ICES system should facilitate the distribution of these guidelines to all relevant laboratories. All participants in the workshop agreed to follow the defined guidelines in the present report. All labs are recommended to use measurement scales (e.p.u) and note down distances between age-structures in 'typical' individuals specific for their stock. It is the intention to compile a dataset consisting of measurements on distances between age-structures from all stocks and areas from which the groups get samples of blue whiting. This will be the basis of an international reference collection and is intended to be used in future workshops.

The present workshop was the first one to be held in decade and the difficulties in reaching agreement were not different from the ones encountered 10 years ago (Mexide 1990, 1995). This does call for a far more regular intercalibration of the age readers to prevent drift and keep track of interpretation of age structures so that the agreements and conclusions from the present workshop will continue to guide the age readers. The group therefore recommend an exchange to be established during 2006 followed by a workshop in 2007 to finalize the manual commenced in the present report. The set of agreed age otoliths which is a product of the present report should be included in an upcoming calibration.

Through the discussions at the workshop it became apparent that the knowledge of the various life history traits for the different stocks in different areas may differ and that these are not very well described in the literature. Therefore the group highly recommend an update of the work by Bailey 1970 concerning general blue whiting behaviour in major blue whiting areas.

In addition, all age readers would benefit from more information on the formation of otolith structures in blue whiting, especially the formation of split rings. The group recommends the inclusion of studies on otolith formation in general and the blue whiting physiology/growth/behaviour in relation to this.

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Annex: Participant addresses