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Report of the Seabass (*Dicentrarchus labrax*) Otolith and Scale Exchange Scheme 2013



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1. Introduction

The Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) meeting in 2012 recommended a large exchange (ICES, 2012) :

"3.2.1.10 Seabass scale and otolith exchange"

In 2011, only 2 countries (France, UK England) participated to the first exchange. A second exchange is carried out in 2013 to confirm the first observed difficulties to interpret the age of seabass with otoliths and/or scales.

The objectives of the exchange were:

- ❖ to investigate the levels of agreement on age readings
- ❖ to analyse the relative differences between age readers and techniques

2. Participants

All countries were informed. 12 countries answered and 3 positively. 3 readers participated in this exchange (Tab. 1).

Table 1 : List of the readers.

Country	Institute	Reader	Name
France	IFREMER	1	Karine Sevin
Belgium	ILVO	2	Martine Moerman
UK England	CEFAS	3	Alison Holmes

3. Sampling collection

A total of 223 fish was sampled onboard French research vessels (Gwen-Drez and Thalassa) during 3 international surveys (Fig. 1 & 2) :

- ❖ 29 fish from 2010 and 2011 from Evaluation des ressources Halieutiques de l'Ouest de l'Europe (EVHOE) in the bay of Biscay
- ❖ 149 fish from 2012 from Channel Ground Fish Survey (CGFS) in the Eastern English Channel
- ❖ 45 fish from 2012 and 2013 from International Bottom Trawl Survey (IBTS)

The length range of the fish was between 26 and 71 cm, with mean 42.3 cm (Fig. 1).



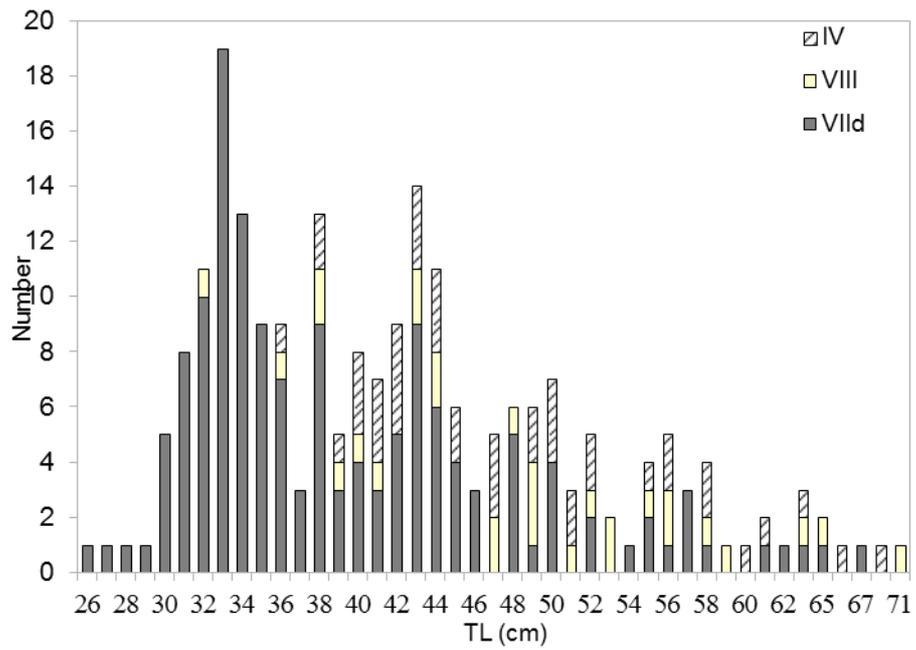


Figure 1 : Histograms of the samples.

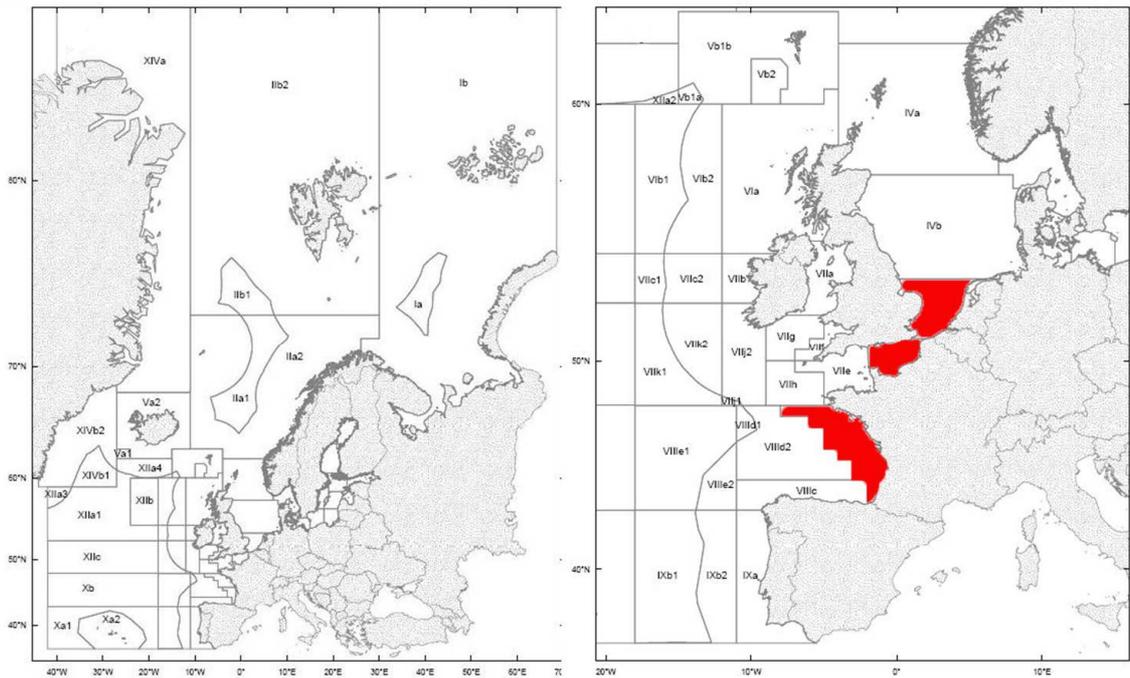


Figure 2 : ICES Areas of sampling 5 (red areas).

For each fish, the *Sagittae* otoliths and few scales were used to compare the age estimation between the both calcified pieces.

The scales are most probably the easiest structures to remove. However, it is very important to choose carefully the removal area in order to avoid regenerated scales. For seabass, the scales are removed under the pectoral fin, an area where regeneration is less frequent and where few visible traces are left for the future commercialisation of the fish (Fig. 3; Mahé *et al.*, 2009).



Figure 3 : Removal of scales under the pectoral fin (removal area in red) for seabass (images : Pierre Porché, IFREMER ; In Mahé *et al.*, 2009).

Among the sampled scales, the regenerated ones were sorted in order to keep only the readable scales which are the ones where there is a succession of rings starting from the *nucleus* (Fig. 4).

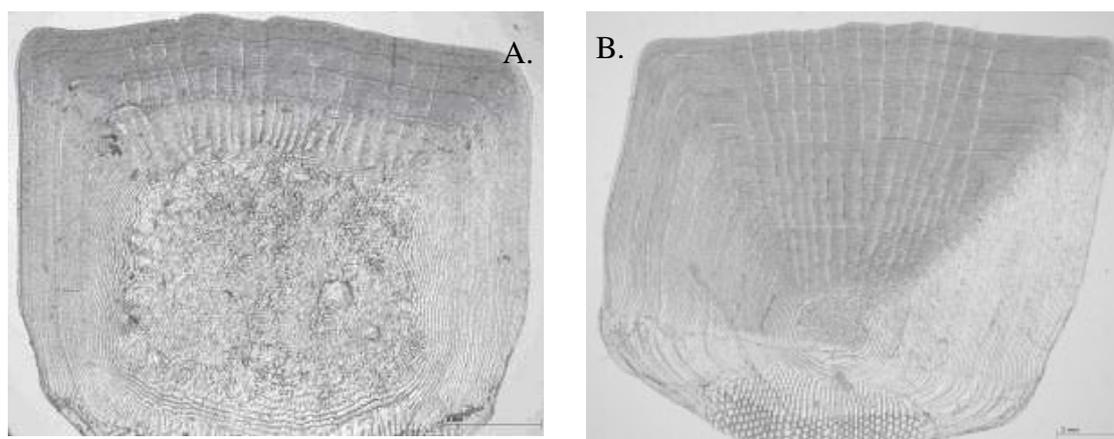


Figure 4 : Difference between a regenerated scale (A.) which does not enable all the growth rings to be seen and a non regenerated scale (B.). The scales are from the same individual (images : Jérôme Huet, IFREMER ; In Mahé *et al.*, 2009).

For each otolith, 2 images of otolith section were used in this exchange with reflected and transmitted light.

4. Reading procedure

Date of birth is set to the 1st of January as convention. One *annulus* consists of one opaque and one translucent zone. For the age estimation, we count the translucent zones.

All otoliths and scales were digitalised by TNPC software. All participants received all informations to participate to this exercise in the WebGR tool.

The WebGR tool was used to this exchange. The use of WebGR tool for the exchange has some advantages: (i) it can facilitate and accelerate the whole exchange process, (ii) annotated images are obtained for every otolith which enables to compare age readings directly and to identify possible sources of bias (iii) it is very easy for the chairman to compile the results.

However, the use of WebGR tool for the exchange present some limits: (i) the WebGR tool is not very intuitive tool (ii) it is not very easy to the scale exchange (some images or video for 1 fish) (iii) the WebGR could be jam (as during the half of the 2013 year) (iiii) it is not possible to upload always a large batch of images (problem with the format of the csv file with Windows 7).

5. Results

The spreadsheet (Eltink, 2000) was completed according to the instructions contained in Guidelines and Tools for Age Reading Comparisons by Eltink *et al.* (2000). Modal ages were calculated for each otolith read, with percentage agreement, mean age and precision coefficient of variation as a definition (for each otolith):

- ❖ percentage agreement = $100 \times (\text{no. of readers agreeing with modal age} / \text{total no. of readers})$.
- ❖ precision c. v. = $100 \times (\text{standard deviation of age readings} / \text{mean of age readings})$.

Age estimation of 223 fish was realised from otoliths and scales by 3 readers.

5.1. Precision¹

The analyse presented the results with 3 readers (each reader interpreted both otoliths and scales). Mean precision of age estimate for individual fish were Coefficient of Variation (CV) of 9.4% (13.1% during the 2011 exchange) and percent agreement to modal age of 68.6% (54.1% during the 2011 exchange) (Tab. 2). Among 223 fish, 84 were read with 100% agreement (37%) and thus a CV of 0%. There were variations in precision of age estimate between individual fish, with CV ranging from 0 to 33% (from 0 to 42.36% during the 2011 exchange) and percent agreement range from 0 to 100% (from 25 to 100% during the 2011 exchange) (Tab. 2). Appendix 1 examined the readings of individuals at each modal age and summarised the number of otoliths or scales read, the precision CV, percentage agreement for both calcified pieces.

¹ Precision is defined as the variability in the age readings. The precision's errors in age readings are better described by the coefficient of variation (CV) by age group. This measure of precision is independent of the closeness to the true age (ICES, 2007).

Table 2 : Precision of readings from otoliths, from scales and from both calcified pieces.

Used calcified pieces	Number	Percentage of Agreement (range)	CV (range)	Number of fish with 100% of agreement
Otolith	149	55.7%	13.4	22
Scale	74	78.4%	1.4	66
Both	223	68.6%	9.4	84

Precision of Age estimation from the scales was better than that's from the otoliths. However, the size of sampling for the otolith exercise (N=149) is twice as much as that's of the scale exercise (N=74, Tab. 2)

5.2. Relative bias (Accuracy)²

The minimal requirement for age reading's consistency is the absence of bias among readers and through time. The hypothesis of an absence of bias between two readers or between a reader and the modal age estimated can be tested non-parametrically with a one-sample Wilcoxon signed rank test (Tab. 3).

Table 3 : Inter-reader bias test and reader against modal age bias test (-: no sign of bias ($p>0.05$); *: possibility of bias ($0.01<p<0.05$); **: certainty of bias ($p<0.01$)) (A : both otoliths and scales; B : otoliths; C : scales).

A : both otoliths and scales (N=223)

	France Reader 1	Belgium Reader 2	UK England Reader 3
Reader 1			
Reader 2	**		
Reader 3	**	**	
MODAL age	**	**	**

² In absence of calcified structures of known age, the age readings can be compared to modal age, which is defined as the age determined for an individual structure whose most of the readers have a preference. Relative bias can be defined as a systematic over- or underestimation of age compared to the modal age. The age reading comparisons to modal age provide a low estimate of relative bias compared to absolute bias, when most readers have a similar serious bias in age reading (ICES, 2007).

B : otoliths (N=149)

	France Reader 1	Belgium Reader 2	UK England Reader 3
Reader 1			
Reader 2	**		
Reader 3	**	**	

MODAL age	**	**	**
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C : scales (N=74)

	France Reader 1	Belgium Reader 2	UK England Reader 3
Reader 1			
Reader 2	**		
Reader 3	**	**	

MODAL age	**	**	**
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It should be noted that there were certainty of bias among readings from otoliths, and from the scales and modal age.

6. Abstract

The ICES Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) identified the need of a seabass *Dicentrarchus labrax*) otolith exchange to take place in 2013. It was the second exchange after that's of 2011.

The IFREMER institute coordinated this exchange. A total of 223 fish from the bay of Biscay (ICES area : VIII, N=29), the Eastern English Channel (ICES area : VIId, N=149) and the North Sea (ICES area : IV, N=45) was sampled onboard French research vessels (Gwen-Drez and Thalassa) during 3 international surveys (EVHOE, CGFS and IBTS). The length range of the fish was between 26 and 71 cm, with mean 42.3 cm. For each fish, the *Sagittae* otoliths and few scales were used to compare the age estimation between the both calcified pieces.

Only 3 readers were participated from France (1 reader), Belgium (1 reader) and UK England (1 reader). During the first exchange in 2011, there were only 2 countries (France and UK England). Only images were used during this exchange. The analyses did not show a high mean precision of age estimate for individual fish with Coefficient of Variation (CV) of 9.4% and percent agreement to modal age of 68.6%. Among 223 fish, 84 were read with 100% agreement (37%) and thus a CV of 0%.

During this exchange, 2 different calcified pieces (otolith and scale) from the same sampling were analysed. The results showed precision of age estimation from the scales (Agreement = 78.4%; CV=1.4) was better than that's from the otoliths (Agreement = 55.7%; CV=13.4). However, the size of sampling for the otolith exercise (N=149) is twice as much as that's of the scale exercise (N=74, Tab. 2). Moreover, 2 readers (France and Belgium) preferred to analyse otoliths and only 1 reader the otoliths of seabass.

7. References

Eltink, A. T. G. W., Newton, A. W., Morgado, C., Santamaria, M. T. G., Modin, J., 2000. Guidelines and Tools for Age Reading. (PDF document version 1.0 October 2000) Internet : <http://www.efan.no>

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8. Appendix 1 : Details results of Seabass exchange

The number of age readings, the coefficient of variation (CV), the percentage of agreement and the RELATIVE bias are presented by MODAL age for each age reader and for all readers combined. A weighted mean CV and a weighted mean percent agreement are given by reader and all readers combined. The CV's by MODAL age for each individual age reader and all readers combined indicate the precision in age reading by MODAL age. The weighted mean CV's over all MODAL age groups combined indicate the precision in age reading by reader and for all age readers combined.

NUMBER OF AGE READINGS					
MODAL age	France Reader 1	Belgium Reader 2	JK England Reader 3	TOTAL	
0	-	-	-	-	
1	-	-	-	-	
2	1	1	1	3	
3	28	28	28	84	
4	39	39	39	117	
5	28	28	28	84	
6	34	34	34	102	
7	15	15	15	45	
8	14	14	14	42	
9	18	18	18	54	
10	11	11	11	33	
11	7	7	7	21	
12	4	4	4	12	
13	2	2	2	6	
14	1	1	1	3	
15	-	-	-	-	
Total	0-15	223	223	223	669

COEFFICIENT OF VARIATION (CV)					
MODAL age	France Reader 1	Belgium Reader 2	JK England Reader 3	ALL Readers	
0	-	-	-	-	
1	-	-	-	-	
2	-	-	-	-	
3	9%	9%	0%	16,0%	
4	11%	15%	4%	9,7%	
5	11%	8%	0%	6,6%	
6	11%	10%	5%	7,7%	
7	5%	9%	5%	5,3%	
8	5%	13%	3%	5,8%	
9	6%	11%	3%	5,6%	
10	11%	8%	0%	5,1%	
11	0%	10%	3%	4,3%	
12	0%	0%	0%	0,0%	
13	12%	10%	10%	11,9%	
14	-	-	-	-	
15	-	-	-	-	
Weighted mean	0-15	7,9%	9,2%	2,4%	9,4%
RANKING		2	3	1	

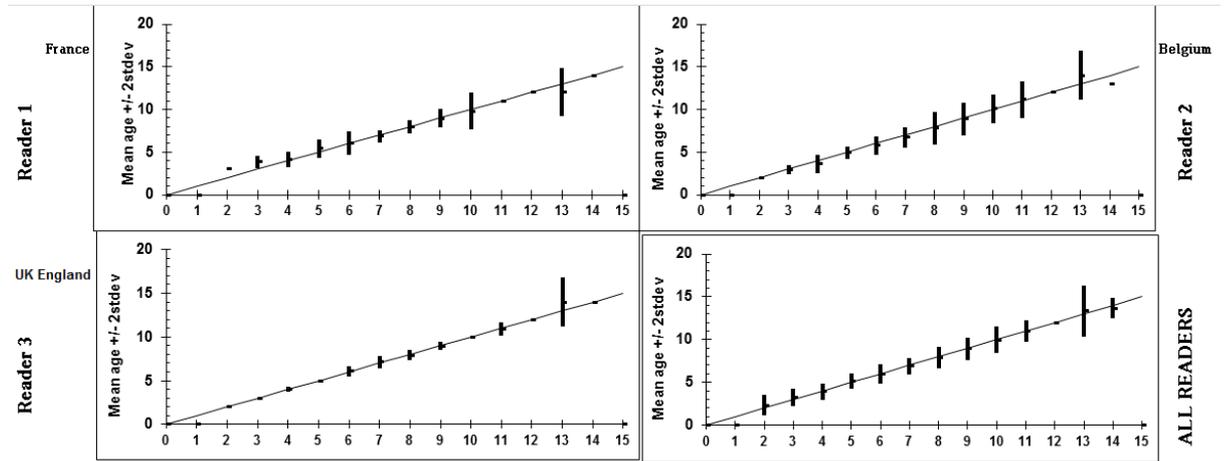
PERCENTAGE AGREEMENT					
MODAL age	France Reader 1	Belgium Reader 2	JK England Reader 3	ALL	
0	-	-	-	-	
1	-	-	-	-	
2	0%	100%	100%	67%	
3	14%	93%	100%	69%	
4	77%	56%	97%	77%	
5	54%	86%	100%	80%	
6	62%	65%	91%	73%	
7	87%	60%	87%	78%	
8	86%	50%	93%	76%	
9	89%	44%	94%	76%	
10	64%	64%	100%	76%	
11	100%	43%	86%	76%	
12	100%	100%	100%	100%	
13	50%	50%	50%	50%	
14	100%	0%	100%	67%	
15	-	-	-	-	
Weighted mean	0-15	58,7%	60,1%	86,1%	68,3%
RANKING		3	2	1	

RELATIVE BIAS					
MODAL age	France Reader 1	Belgium Reader 2	JK England Reader 3	ALL	
0	-	-	-	-	
1	-	-	-	-	
2	1,00	0,00	0,00	0,33	
3	0,86	-0,07	0,00	0,26	
4	0,18	-0,38	0,03	-0,06	
5	0,39	-0,07	0,00	0,11	
6	0,06	-0,24	0,09	-0,03	
7	-0,13	-0,27	0,13	-0,09	
8	0,00	-0,21	-0,07	-0,10	
9	-0,06	-0,11	-0,06	-0,07	
10	-0,18	0,09	0,00	-0,03	
11	0,00	0,14	-0,14	0,00	
12	0,00	0,00	0,00	0,00	
13	-1,00	1,00	1,00	0,33	
14	0,00	-1,00	0,00	-0,33	
15	-	-	-	-	
Weighted mean	0-15	0,17	-0,15	0,02	0,01
RANKING		3	2	1	

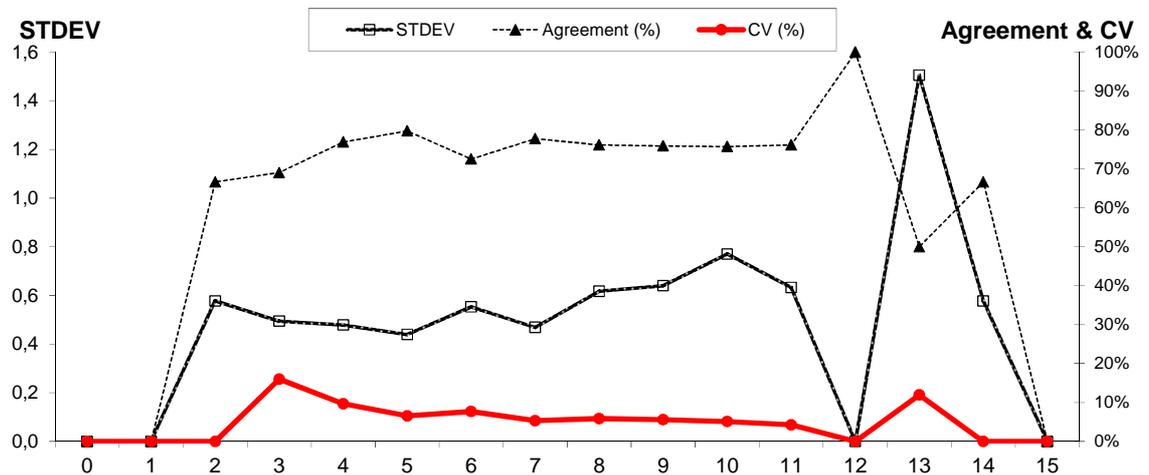
Overall ranking			
	France Reader 1	Belgium Reader 2	JK England Reader 3
Ranking Coefficient of Variation	2	3	1
Ranking Percentage Agreement	3	2	1
Ranking Relative bias	3	2	1
OVERALL RANKING	3	2	1



In the age bias plots below the mean age recorded ± 2 stdev 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.



The coefficient of variation (CV%), percentage of agreement and the standard deviation (STDEV) are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percentage of 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 distribution of the age reading errors in percentage by MODAL age as observed from the whole group of age readers in an age reading comparison to MODAL age. The achieved precision in age reading by MODAL age group is shown by the spread of the age readings errors. There appears to be no RELATIVE bias, if the age reading errors are normally distributed. The distributions are skewed, if RELATIVE bias occurs.

