

**AGE DETERMINATION WORKSHOP FOR SOLE AND PLAICE**  
28-29 JANUARY 2002 OSTEND  
**INTERNAL REPORT**

**PARTICIPANTS**

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**AIMS**

To find out the ageing agreement between countries for sole and plaice.  
To produce a reference collection of sole images for training etc.

**BACKGROUND**

Initially this started out as an exchange of sole otoliths between Ifremer and Cefas. Ifremer have had problems in recent years with ageing sole and this has affected the age compositions provided to the NSSK WG (the reader has since left) and Cefas offered to look at some of their samples to compare results. Rivo and Clo were invited to participate. Rivo have also had problems with sole but these have since been identified to sectioning errors rather than reader ageing errors. The exchange needed to be completed before the 2002 working groups to be useful and so time was limited, however it was also possible to include an exchange of sectioned **plaice** otoliths to Ifremer and Clo. Cefas and Rivo staff had already aged these.

**OTOLITH SAMPLES (see Appendix 1 for methods of preparation)**

**Ifremer soles - 241** aged by all countries before meeting.

**Rivo soles**

**100** aged by Cefas and Rivo **before** meeting. Aged by Ifremer and Clo-dvz **at** meeting.  
**57** aged by Rivo **before** meeting. Aged by other countries **at** meeting.

**Cefas plaice - 112**

Aged by sections on slides by Cefas, Rivo and Clo-dvz **before** meeting.  
Digitised images of whole otoliths (jpg) aged by one Ifremer staff **before** meeting.

## **Day 1**

I had prepared digitised images on CD of 20 otoliths from the Ifremer sole sample. These were projected and discussed. I had also prepared a power point presentation comparing whole, burnt and sectioned plaice otoliths. These were also projected and discussed along with various digitized images from the 112 Cefas plaice otoliths where major disagreements had taken place.

We then had a tour of the Ostend labs otolith processing section.

The 160 soles supplied by the Rivo institute were then aged by the other countries.

## **Day 2**

The sole ages for all countries were input to an age comparison spreadsheet and calculations made.

Mistakes were found on the original Dutch plaice ages. MdeV re-aged these. Bart Maertens aged the Cefas plaice slides. The plaice file was then updated. Various images of sole and plaice otoliths were projected and discussed where major disagreements were found, especially the 2-year-old soles.

## **RESULTS**

The age reading results were analysed using the Excel workbook “AGE COMPARISONS.XLS” by A.T.G.W.Eltink from the Rivo institute.

### **Sole**

THE Rivo sole sample (157 fish) aged by most people at the meeting gave an overall agreement of 90%. This was a distinct improvement on the Ifremer sample (241) that were aged before the meeting (79%). These %'s include the inexperienced people and if they are excluded the % agreement is much higher. It is disconcerting that the largest bias is in the 2 year old age group – one of the most important for the assessment work. This was caused by some of the inexperienced readers **over** ageing this year class by one year. All these fish were subsequently discussed and the experienced readers decided that these fish were **definitely** two year old's and the inexperienced readers had misinterpreted them.

### **Plaice**

Except for one reader all the plaice were aged prior to the meeting and there was not any time to age further samples at the meeting. The French reader aged digitised images of the whole otoliths but the other participants aged the slides which are the main method now used by the other European countries. The overall agreement was only 70% with the experienced readers getting 78% +, reflecting the more difficult interpretation of this species.

There were several problems in the file of the ages originally supplied by Rivo and these will be re-examined.

**The results of the Rivo sole sample are summarized in table 1.**

**The plaice results are summarized in table 2.**

**The full analysis is available if required.**

## CONCLUSIONS

- This workshop was successful in that following the advice and tuition given on the first day a significant improvement of agreement among the beginners was achieved for the Rivo sole sample compared to the Ifremer sample aged before the meeting.
- Rivo will digitize all the 157 soles to provide an agreed collection for training etc.
- Copies of the Cefas plaice and sole images were transferred onto CD's for each country to keep.
- I will send copies of the Cefas plaice otolith edge set to the other countries for use in training etc. I have also promised to make up a similar set for soles. Identification of the edge when the pink ring is forming is a main source of miss-ageing by beginners and a photo set showing the formation of the edge through the year would be very helpful for all species.
- Accurate **mounting** and **sectioning** are just as important as the subsequent age reading ability especially with inexperienced staff who may not realize whether the nucleus ring is missing. Good quality control is needed at all stages to make sure that this does not happen.
- It was agreed that in future any problem samples could be exchanged between countries for advice.
- There are considerable differences of plaice ageing methods between countries. Cefas still use the burnt otolith extensively to age older fish but the other countries do not seem to use this method as much and Ifremer only use whole otoliths. This could lead to under ageing older fish especially from the northern north sea although younger age groups from the southern north sea and the English channel are often just as good or better on the whole otolith or slide than the burnt otolith.
- Sole are regarded as one of the easiest species to age but with inexperience it is still possible to miss identify splits and the edge ring. These 'forums' are an excellent way not only of resolving ageing problems, but also for forging 'networks' of all readers of a species around Europe.
- If possible a similar workshop would be arranged during 2002 but concentrating on **plaice**.

I would like to thank Wim Demare and the Ostend lab for their hospitality and excellent arrangements for this meeting.

M.W.Easey  
12/3/02

## Appendix 1

### OTOLITH PROCESSING AND AGEING METHODS OF EACH COUNTRY

#### SOLE

**Cefas** – Otoliths are mounted in black resin and the sections stained for 20 minutes and then viewed either dry or with water added. A spare section is taken but not stained. This would be used if there were any problem with the main section but it is rarely used and is stored for possible future analysis (DNA etc.). Sections are stored in plastic packets. N.B. after seeing the excellent staining of the Rivo soles we have now extended our staining time to 30 minutes but will carry on ageing them dry or with water added.

**Rivo** – Otoliths are mounted in black resin and the sections stained for 45 minutes. They are then mounted with clear resin onto glass slides. This takes the stain out of the underside of the strip. Thin clear oil (sewing machine) is then painted over the top of the strip. Transmitted light is used. This gives a very good image leaving the pink rings very clear. Long-term storage is more difficult as the surface is very vulnerable. We did notice dust sticking to the surface and it is not known whether the stain fades in time with this oil added. Slides are stored in slide boxes.

**Ifremer** – Otoliths are mounted in opaque resin and stained for 20 minutes. Otoliths are not mounted precisely and two sections are taken to make sure the nucleus is sectioned. They are then stained for 20 minutes and the age reader picks out the best section. They use liquid paraffin on the surface. Sections are stored in paper packets.

**Clo-Dvz** - Otoliths are mounted in black resin and stained for 20 minutes and then viewed **dry or with oil applied?** The processing staff were inexperienced and still learning this work. Otoliths were not all positioned in the same plane to give a consistent transverse cut and the slides we saw were very obviously not all cut through the nucleus. I advised them of positioning the otoliths consistently to get a transverse cut. The cutting machine used by Clo and Rivo is not as accurate as the Cefas one and needs to be constantly repositioned to maintain cutting through the nucleus. They also said that they had experienced difficulties with the staining. They were using old powder stain and I gave them the details of our staining methods and suppliers of the ready-made bottles of stain that give more consistent results.

#### PLAICE

**Cefas** – Two methods:

- 1) Sectioned in black plastic and mounted onto glass slides with clear resin and a glass cover slip. Whole/burnt otoliths examined for any difficult and/or older fish if necessary.
- 2) Only whole/burnt otoliths examined.

**Rivo** - Sectioned in black plastic and mounted onto glass slides with clear resin.  
Whole/burnt otoliths examined if necessary.

**Ifremer** – Only whole otoliths.

**Clo-Dvz** - Sectioned in black plastic and mounted onto glass slides with clear resin.  
Whole otoliths also examined.

**Table 1**

**Dutch sole sample(157 fish)**

**COEFFICIENT OF VARIATION (CV)**

	KG (NL)	PG (NL)	MV (NL)	ME (UK)	IH (UK)	JV (FR)	MLC (FR)	Martine (Be)	Ilse (Be)	Bart (Be)	ALL
	0.02	0.04	0.05	0.01	0.02	0.06	0.14	0.13	0.07	0.03	0.04
ranking	2	5	6	1	3	7	10	9	8	4	

**PERCENTAGE AGREEMENT**

	KG (NL)	PG (NL)	MV (NL)	ME (UK)	IH (UK)	JV (FR)	MLC (FR)	Martine (Be)	Ilse (Be)	Bart (Be)	
	97%	93%	94%	96%	97%	89%	81%	78%	85%	94%	90%
ranking	1	6	4	3	1	7	9	10	8	5	

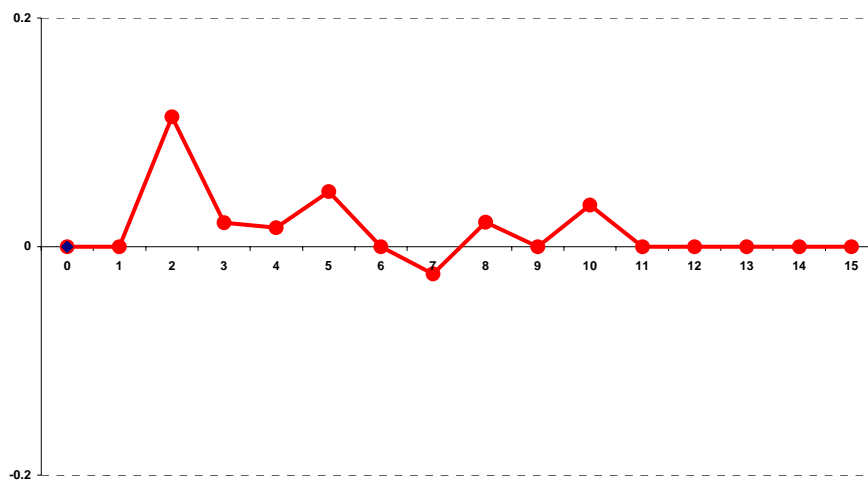
**RELATIVE BIAS**

	KG (NL)	PG (NL)	MV (NL)	ME (UK)	IH (UK)	JV (FR)	MLC (FR)	Martine (Be)	Ilse (Be)	Bart (Be)	
	0.03	-0.01	0.03	0.03	0.01	0.01	0.10	0.20	-0.05	-0.04	0.03
ranking	5	3	6	4	1	2	9	10	8	7	

**Overall ranking**

KG (NL)	PG (NL)	MV (NL)	ME (UK)	IH (UK)	JV (FR)	MLC (FR)	Martine (Be)	Ilse (Be)	Bart (Be)
2	4	5	2	1	5	9	10	8	5

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



**Table 2**

**PLAICE WORKSHOP RESULTS(112 fish)**

**COEFFICIENT OF VARIATION**

	MWE(UK)	MRV(UK)	BH(UK)	IH(UK)	KG(NL)	PG(NL)	MdV(NL)	IM(BE)	MM(BE)	MLC(FR)	BM(BE)	ALL
	0.02	0.08	0.10	0.07	0.10	0.08	0.14	0.07	0.14	0.15	0.06	0.14
ranking	1	5	7	3	8	6	10	4	9	11	2	

**PERCENTAGE AGREEMENT**

	MWE(UK)	MRV(UK)	BH(UK)	IH(UK)	KG(NL)	PG(NL)	MdV(NL)	IM(BE)	MM(BE)	MLC(FR)	BM(BE)	ALL
	97%	84%	82%	83%	9%	78%	63%	83%	26%	76%	89%	70%
ranking	1	3	6	4	11	7	9	4	10	8	2	

**RELATIVE BIAS**

	MWE(UK)	MRV(UK)	BH(UK)	IH(UK)	KG(NL)	PG(NL)	MdV(NL)	IM(BE)	MM(BE)	MLC(FR)	BM(BE)	ALL
	-0.02	0.16	-0.05	0.03	1.19	0.02	0.01	0.07	0.72	-0.21	0.05	0.18
ranking	3	8	6	4	11	2	1	7	10	9	5	

**Overall ranking**

	MWE(UK)	MRV(UK)	BH(UK)	IH(UK)	KG(NL)	PG(NL)	MdV(NL)	IM(BE)	MM(BE)	MLC(FR)	BM(BE)
	1	6	7	3	11	4	8	4	10	9	2

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

