WKARP REPORT 2010

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Report of the Workshop on Age Reading of North Sea (IV) and Skagerrak-Kattegat (IIIa) Plaice (WKARP)

2-5 November 2010

Ijmuiden, Netherlands

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Executive summary

The Workshop on Age reading of North Sea (IV) and Skagerrak-Kattegat (IIIa) plaice [WKARP] (Chair: Loes Bolle, The Netherlands) was held 2-5 November 2010 in IJmuiden, The Netherlands. The previous North Sea plaice workshop was held in 2003, and no information is available on when the last workshop was held for Skagerrak-Kattegat plaice. Therefore PGCCDBS called for an otolith exchange (2009-2010) followed by an age reading workshop (2010) for these 2 plaice stocks. The primary aim of the exchange and workshop was to identify and resolve interpretation differences between readers and laboratories.

Nine countries and 20 readers participated in the exchange. The same 9 countries and 14 readers participated in the workshop. The exchange and workshop otolith sets consisted of IIIa and IV otoliths. The readers represented a broader geographical range (III, IV, VI & VII stocks). Some laboratories use whole otoliths, while others use transverse sections for plaice ageing. Therefore both preparation methods were included in the exchange and workshop sets (i.e. a different method was used for each otolith of a pair).

Comparison of pair-wise age readings and overall age compositions showed no bias related to preparation method if age is less than 10. A small sample of 10+ otoliths indicated an underestimation of age in whole otoliths. A larger sample (from different stocks) is required for a better evaluation of preparation methods in older fish (for different stocks).

To identify and resolve interpretation differences, the results and annotated images from the exchange were discussed during the workshop. Differences in interpretation mainly stemmed from whether or not to expect regular growth patterns, i.e. whether or not to apply the rule of the thumb that every annulus is wider than the next annulus. This difference in interpretation was most prominent for the first annulus. Growth increment analyses were carried out to examine this issue in more detail, but the differences were not resolved. Consequently, the agreement between readers did not improve for a new set of otoliths which was read during the workshop. The majority view is not necessarily the correct way to interpret growth structures in plaice otoliths (for all stocks). The only way to prove who is right is to carry out validation studies.

WebGR, the web tool to aid ageing and maturity staging workshops, was used to create an agreed age reference collection with annotations. WebGR was considered to be very useful, but the implementation was hampered because it requires experience in using the tool, which most age readers and coordinators do not have. WebGR training sessions for age readers and age coordinators is proposed. It is furthermore recommended to enlarge the plaice reference collection in WebGR, including both agreed otoliths as well as otoliths subject to interpretation differences.

The group also reviewed calibration work done so far, collated information on national procedures, created an international age reading manual and formulated target/threshold statistics and follow-up actions.

In general, regular workshops (at a 3-5 year interval) are recommended. For plaice, the next exchange is proposed for 2012 and the next workshop for 2013. Goal is to pursue the unresolved issues of the 2010 workshop and to maintain and further enhance international calibration and cooperation between age readers for plaice stocks in ICES areas III, IV, VI and VII.

1 Introduction

1.1 Terms of reference

2009/2/ACOM46 The **Workshop on Age Reading of North Sea (IV) and Skagerrak-Kattegat (IIIa) Plaice [WKARP]** (Chair: Loes Bolle, The Netherlands) will be established and take place in IJmuiden (Wageningen-IMARES), The Netherlands, 2–5 November 2010, to:

a) Review information on age estimations, otolith exchanges, workshops and validation work done so far.

b) Use WebGR for image annotations and data analyses (provisional, depending on the successful implementation of WebGR)

c) Address the generic ToRs adopted for workshops on age calibration (see 'PGCCDBS Guidelines for Workshops on Age Calibration'):

1) Provide information on participating laboratory procedures
2) Identify and resolve interpretation differences between readers and laboratories.
3) Create or update an ageing manual
4) Collate agreed age reference collection.
5) Formulate follow-up actions
6) Formulate species (and stock specific) target and threshold statistics

1.2 Background

The previous North Sea plaice workshop was held in 2003, and no information is available on when the last workshop was held for Skagerrak-Kattegat plaice. Therefore PGCCDBS called for an otolith exchange (2009-2010) followed by an age reading workshop (2010) for these 2 plaice stocks.

1.3 Participants

The participants in the exchange and workshop are listed in Annex 1.

1.4 Agenda

The agenda is presented in Annex 2

2 Review (ToR a)

During an EU project on growth changes in 4 demersal fish species (Bolle & Rijnsdorp 2000, Bolle et al. 2004), age reading and growth increment comparisons were carried between readers from CEFAS (England), ILVO (formerly DSF, Belgium) and IMARES (formerly RIVO, The Netherlands). After an initial calibration exercise consisting of 50 otoliths per species, reader-induced error in growth increment measurements was examined. A total of 211 plaice otoliths were re-aged and re-measured by an independent second reader without prior knowledge of the first results. All analyses were based on sectioned otoliths. Despite some differences at the level of the individual fish, on average the differences were insignificant.

In January 2002, a workshop initially focussing only on sole was elaborated with plaice (Easey, 2002). Eleven readers from 4 countries (England, Belgium, France and The Netherlands) participated in the workshop and a plaice exchange prior to the workshop. The exchange set consisted of 112 North Sea plaice otoliths. No new otolith sets were aged during the workshop. The French reader used digitised images of whole otoliths, the other participants aged sectioned otoliths, which at that time was considered to be the "main method now used by European countries". The results for all readers combined are presented in Table 2.1. The agreement for experienced readers was ≥ 78%.

Table 2.1. Results of the 2001 exchange (112 North Sea otoliths, 11 readers, 4 countries).

Method	Bias range	Overall CV	Agreement range	Overall agreement
Sections (10 readers) Whole (1 reader)	-0.21 – +1.19	0.14	9 – 97%	70%

During the WGNSSK meeting in June 2002, age composition differences were discovered for North Sea plaice related to the country supplying the age data. To rule out errors due to age reading bias, an otolith exchange between England, Denmark and The Netherlands was initiated (Bolle 2002). The otoliths sets included were the previous exchange set (see above) and 2 new North Sea sets (186 otoliths) focussing on the landing year that showed differences in age compositions (2001). This exercise revealed a clear difference in the interpretations by 1 country for the new sets (landing year=2001, Figure 2.1), whereas no inter-reader bias was observed for the old set (landing year=1998). These results show that, although plaice is generally considered to be an easy species to age, interpretation problems do occur and are more prominent in some landing years / year classes than others.

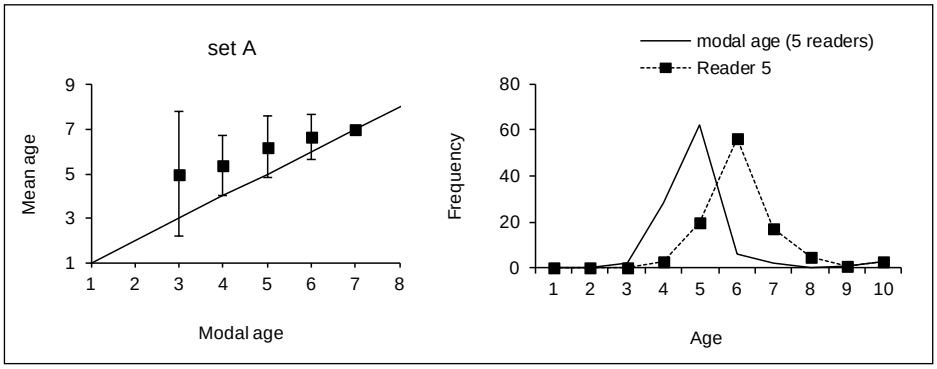


Figure 2.1. Results of 1 of the otoliths sets included in the 2002 exchange (106 North Sea otoliths, 5 readers, 3 countries).

In 2003, a plaice workshop took place at Ostend, Belgium (Easey 2003). Nineteen readers from 6 countries (England, Belgium, France, The Netherlands, Denmark and Ireland) participated in the exchange prior to the workshop. Fourteen readers from the same 6 countries participated in the workshop. The otolith set for the exchange consisted of 245 otoliths from the eastern English Channel (VIId). Both otoliths of a fish were included. One otolith had already been sectioned while the other one was whole (although a few were missing, broken or crystalline). Most readers aged the fish using both methods (independently). A sub-sample of 81 otoliths, which had been selected from the original set, was re-aged at the workshop. The results for all age readers combined are presented in Table 2.2. The precision of the methods cannot be compared based on Table 2.2, because all participants are included in the slide readings whereas 3 participants, 2 of which are inexperienced age readers, did not read the whole otoliths.

The 2003 group concluded that using only whole otoliths could lead to under-ageing older fish especially from the northern North Sea. The burning and sectioning methods both show the annual zones on older fish much clearer than can be seen on the whole otolith. The re-read sample of younger age groups (modal age 1-6) showed very good agreement between the whole otoliths and the sections. However, under-ageing mistakes when using whole otoliths were also observed in younger fish, especially by inexperienced readers.

Table 2.2. Results of the 2003 exchange (245 VIId otoliths, 19 reader, 6 countries) and workshop (81 VIId otoliths, modal age 1-6, 14 readers, 6 countries)

Method	Reading	Bias range	Overall CV	Agreement range	Overall agreement
Sections	1st	-0.07 – +1.41	0.23	7 – 93%	62%
	2nd	-0.12 – +0.68	0.12	43 – 98%	87%
Whole	1st	-0.05 – +0.62	0.19	43 – 96%	74%
	2nd	-0.14 – +0.09	0.08	65 – 93%	86%

The results of the calibration exercises carried out so far show that consistency and interpretation problems may occur in plaice age reading. This corroborates the need of the present exchange and workshop. It furthermore indicates the necessity of validation studies. No true validation studies have been carried out for plaice yet. The validity and precision of back-calculated growth has been examined for North Sea plaice (Rijnsdorp *et al*. 1990), but this study mainly focused on otolith growth compared to somatic growth; it did not validate annual deposition of the structures that are counted in plaice otoliths.

3 WebGR (ToR b)

WebGR, the web tool to aid ageing and maturity staging workshops, was used during WKARP to create an agreed age reference collection with annotations (see section 7).

WebGR was considered to be very useful, but the implementation was hampered because it requires experience in using the tool, which most age readers and age coordinators do not have. The tool was used during a plenary session and was operated by Kélig Mahé who has most experience using WebGR.

WebGR training sessions for age readers and age coordinators is recommended (see section 9).

4 Laboratory procedures (ToR c1)

Plaice otoliths can be aged using 3 different preparation methods:

1) Whole otolith method: Both otoliths are put in a container (black or transparent) filled with a clear fluid (water, oil, alcohol) or embedded in clear resin.
2) Sectioned otolith method: Otoliths are embedded in resin (with or without added black stain) and then sectioned through the nucleus. The thickness of the slides range between 0.5 and 0.6 mm, with some using a glass coverslip as well.
3) Break and burn method: This method involves breaking the otolith in half (as close to the nucleus as possible). The broken halves are burnt until the translucent rings appear dark grey.

The methods used by each country are outlined below.

Belgium

Two otoliths are collected from all fish. Before reading the otoliths are submerged in water in a plastic container for eleven hours. Both otoliths are submerged in water in a blackened embryo dish. The otolith is viewed concave (proximal) side down.

Open the Axiovision program so that a digital camera mounted on the stereomicroscope returns a live image of the otolith on the big screen of the pc. Vary the light intensity until the image is optimal for viewing. Reflected light shows the translucent rings as darker rings. The occular of the microscope has a standard magnification of 10x which is combined with magnification 1x or 0.8x for the objective lens.

Denmark

Two whole otoliths from each fish are placed in wells in a black plastic tray. Each tray has 50 wells (5 rows of 10 wells in each).

Only whole otoliths are used (never sectioned). The whole otoliths are placed in distilled water.

The otoliths are read using a microscope with reflected light.

England

Both otoliths are collected from all fish. Both otoliths are read whole in water. When unable to determine an age confidently, one otolith is broken at the nucleus and burnt. For some stocks, otoliths are embedded in resin, sectioned and then mounted on glass slides with coverslips.

France

Otoliths from certain species of flatfish may be analysed without any previous complicated preparation. This is the case for plaice.

Both otoliths are taken and stored in small paper envelopes. They are then cleaned and read in water.

All otoliths are digitized. The image is used for reading, annotating and for the TNPC software.

Germany (Hamburg lab)

In the case of fish from market sampling both otoliths are removed.

On surveys only one otolith is removed from fish,under 40 centimetres. If the fish is greater than 40 centimetres in length, both otoliths are collected.

The whole otoliths are dried and then one is embedded in clear resin. The otolith which is clearest to read is mounted between two glass slides.

The otoliths are read using transmitted light using a microscope.

Ireland

Otoliths are read whole. Both otoliths are mounted onto black trays with wells using a clear resin called histokitt. They are read using reflected light.

Northern Ireland

Both otoliths are removed from the fish. One is mounted in black resin, which is then sectioned and mounted on a clear glass slide with a glass coverslip.

Sweden

Both otoliths are collected, cleaned carefully and put in plastic trays or in paper envelopes. When reading the whole otoliths they are placed in a black dish in water or alcohol. The otoliths are read with reflected light. If the otoliths are difficult to read or have obvious “cliff edges” issues, they are broken or sectioned.

The Netherlands

Both otoliths are collected from all fish.

Both otoliths from fish collected during the inshore young fish surveys are examined whole in de-ionized water using transmitted light. The sulcus acusticus (proximal side) is placed face down. After examination, otoliths are kept in paper envelopes.

For market sampling and offshore surveys, one otolith of each pair is embedded in black resin and sectioned through the nucleus. The other otolith is stored in paper envelopes. Three resin strips, containing 10 sectioned otoliths each, are glued to a glass slide using clear resin. No coverslip is used. Before reading, the sections are lubricated with a thin layer of oil. The sections are examined using transmitted light.

5 Resolve interpretation differences (ToR c2)

Most work before and during the workshop was related to the 2nd generic ToR: Identify and resolve interpretation differences between readers and laboratories. This work included: collation of otolith sets for the exchange and workshop, digitisation of images, age readings and image annotations, group discussions on interpretation methods, growth increment analyses, and comparison of ageing results using different preparation methods.

Nine countries and 20 readers participated in a pre-workshop exchange. The same 9 countries and 14 readers participated in the workshop. The exchange and workshop otolith sets consisted of IIIa and IV otoliths. The readers represented a broader geographical range (III, IV, VI & VII stocks). Some laboratories use whole otoliths, while others use transverse sections for plaice ageing. Therefore both preparation methods were included in the exchange and workshop sets (i.e. a different method was used for each otolith of a pair).

5.1 Exchange sets

A plaice otolith exchange was carried out prior to the workshop. The protocol for the exchange is presented in Annex 3.

The otolith sets consisted of Kattegat-Skagerrak (IIIa) otoliths contributed by Sweden, and North Sea (IV) otoliths contributed by The Netherlands (Table 5.1.1). One otolith of each fish was sectioned and the other was left whole. For 4 fish in the IIIa set, only 1 otolith was available. A total of 222 fish and 440 otoliths were included in the exchange. All otoliths were sectioned by The Netherlands. The images of whole otoliths were digitised by Belgium and the images of sectioned otoliths by The Netherlands.

Table 5.1.1. Otolith sets in exchange

ICES area	age range	Sections	Whole
IV	0-10	112	112
IIIa	0-10	92	96
IIIa + IV	11+	14	14

Twenty age readers from 9 countries participated in the exchange. The plaice stocks that these readers age, their level of experience and their preferred preparation method are listed in Table 5.1.2. Ten readers aged all otoliths, and 10 readers aged either sectioned or whole otoliths.

The statistics representing age reading performance were calculated for all readers combined and for experienced readers only. They were calculated separately for each area and preparation method (Table 5.1.3). Only otoliths for which modal age (as whole or sectioned otolith) was ≤ 10 were included, to ensure a sufficient number of otoliths per age group. The number of otoliths per age group ranged from 7 to 13 for area IV, and from 5 to 13 for area IIIa except for age group 0 (#1).

As can be expected, agreement was higher and variance (APE & CV) was lower for experienced readers compared to all readers, for each preparation method and stock (Table 5.1.3). Agreement was higher and variance was lower for IV otoliths than for IIIa otoliths, indicating that the IV otoliths are easier to age than IIIa otoliths. In all cases, except experienced readers ageing IV otoliths, the statistics were better for whole otoliths than for sectioned otoliths.

Table 5.1.2. Age readers in exchange

Country	Reader	Experience level	Stock	Preferred method	Sections	Whole
Netherlands	Peter Groot	experienced	IV	sections	√	√
	Kees Groeneveld	intermediate	IV	sections	√	-
	Marcel de Vries	intermediate	IV	sections	√	-
	Philip Nijssen	beginner	IV	sections	√	-
Belgium	Ilse Maertens	experienced	IV	whole	-	√
	Martine Moerman	experienced	IV	whole	√	√
	Christophe Bonje	beginner	IV	whole	√	√
France	Romain Elleboode	experienced	IV & VIId	whole	√	√
Ireland	Susan Beattie	intermediate	VIIa,b,g,j & VIa	whole	√	√
	Marcin Blaszkowski	intermediate	VIIa,b,g,j & VIa	whole	√	√
Northern Ireland	Willie McCurdy	beginner	VIIa	sections	√	√
	Ian McCausland	beginner	VIIa	sections	√	√
England	Brain Harley	experienced	IV & VIId	sections	√	√
	Joanne Smith	experienced	VIIa	whole	-	√
Sweden	Barbara Bland	experienced	IIIa	whole	-	√
	Sofia Carlshamre	intermediate	IIIa	whole	-	√
	Jan-Erik Johansson	intermediate	IIIa	whole	-	√
Denmark	Helle Rasmussen	experienced	IIIa & IV	whole	√	√
Germany	Christine Petersen-Frey	intermediate	IV	whole	-	√
	Cornelia Albrecht	intermediate	IIIc & IIId	sections	√	-

Table 5.1.3. Results of exchange sets

	ICES area IV		ICES area IIIa	
	sections	whole	sections	whole
Number of readers	14	16	14	16
Agreement (%)	77%	81%	64%	70%
CV (%)	12%	10%	15%	12%
APE (%)	8%	7%	10%	9%
Number of experienced readers	5	8	5	8
Agreement experienced readers (%)	88%	84%	73%	76%
CV experienced readers (%)	5%	8%	12%	7%
APE experienced readers (%)	4%	6%	9%	6%

The results for each reader individually are presented (anonymously) in Annex 4. The statistics presented in Annex 4 are based on modal age calculations including all readers.

5.2 Workshop discussions

A selection of the images, which were annotated during the exchange, were discussed at the workshop. This revealed differences between laboratories in reading techniques and interpretation methods.

Reading techniques

Different reading techniques are used by different laboratories. Most laboratories count the translucent rings, but some laboratories count the opaque rings. This difference did not affect the age reading results. Regardless of which method is used, it is important that an age reader is aware of when the opaque and translucent rings are formed. As age is determined relative to January 1st, the number of rings counted deviates from the number rings visible in certain periods of the year. These periods de-

pend on whether opaque or translucent rings are counted. It furthermore depends on regional differences in the timing of opaque/translucent deposition.

The group agreed that, when annotating images, the transition from translucent to opaque should be marked. Firstly because this demarks the onset of the new growing season, and secondly because it avoids confusion between readers using different reading techniques.

It was also brought to the group's attention that individual readers used different light sources, reflected, transmitted or both. As this does not appear to affect the results the reader may use the lighting they prefer.

Interpretation methods

It was discussed whether or not one can expect a consistent growth pattern in the otoliths, i.e. from the nucleus to the edge, the rings should get progressively narrower and more tightly packed. Some countries were of the opinion that if this pattern cannot be seen in the otoliths, some rings should be considered to be false to obtain consistency with the expected pattern. Other countries argued that growth could vary between years due to environmental factors and therefore a consistent growth pattern should not be assumed. The group did not reach an agreement on this issue. This issue can only be resolved by validation studies.

A specific case with regard to regular versus irregular growth patterns is the interpretation of the first annulus and differences between laboratories were most prominent in this case. If a small inner ring close to the nucleus is visible, some readers do not consider this to be a true annulus and count the second more prominent ring as the first annulus. Other readers do count the first visible ring, despite the fact that this may result in the first increment being smaller than the second increment. The first ring debate is illustrated in Figure 5.2.1. It was argued that in northern – slow growing – stocks it is possible that growth during the first year of life is less than during the second year of life. The group agreed to interpret the first annulus differently in otoliths from fast and slow growing stocks (see section 6.6), although no additional information is available to justify this decision. Again, this issue can only be resolved by validation studies.

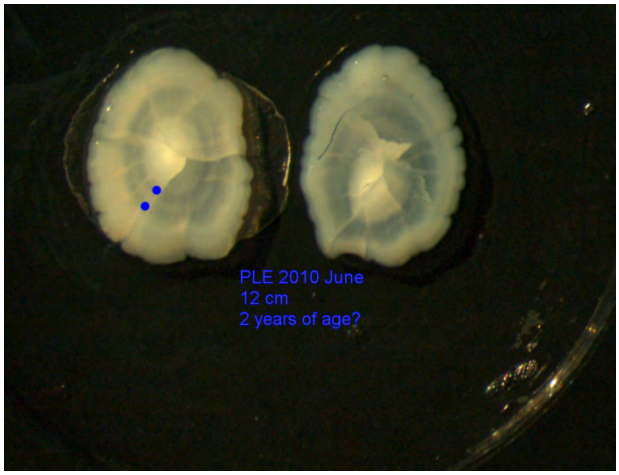


Figure 5.2.1. Plaice otoliths from the Skagerrak (reflected light). The small inner ring is considered to be a false ring by some readers and the first annulus by others. Source: Barbara Bland, Institute of Marine Research, Sweden.

The validity of regular versus irregular growth hypotheses, in general and for the juvenile stages in particular, can only be tested by validation studies. It is recommended that validation studies are carried out and that these primarily focus on the formation of the first annulus in different stocks.

5.3 Workshop sets

New otolith sets were aged by the readers during the workshop. The otolith sets consisted of Kattegat-Skagerrak (IIIa) otoliths contributed by Sweden, and North Sea (IV) otoliths contributed by The Netherlands (Table 5.3.1). One otolith of each fish was sectioned and the other was left whole. For 6 fish in the IIIa set, only 1 otolith was available. A total of 113 fish and 220 otoliths were included in the workshop sets. All otoliths were sectioned by The Netherlands. The images of whole otoliths were digitised by Belgium and the images of sectioned otoliths by The Netherlands.

Table 5.3.1. Otolith sets in workshop

ICES area	age range	Sections	Whole
IV	0-9	51	51
IIIa	0-10	47	41
IIIa + IV	11+	15	15

Fourteen age readers from 9 countries aged the workshop sets. The plaice stocks that these readers age, their level of experience and their preferred preparation method are listed in Table 5.3.2. All age readers aged both sectioned and whole otoliths.

Table 5.3.2. Age readers in workshop

Country	Reader	Experience level	Stock	Preferred method	Sections	Whole
Netherlands	Peter Groot	experienced	IV	sections	√	√
	Marcel de Vries	intermediate	IV	sections	√	√
Belgium	Ilse Maertens	experienced	IV	whole	√	√
France	Romain Elleboode	experienced	IV & VIId	whole	√	√
Ireland	Susan Beattie	intermediate	VIIa,b,g,j & VIa	whole	√	√
	Marcin Blaszkowski	intermediate	VIIa,b,g,j & VIa	whole	√	√
Northern Ireland	Ian McCausland	beginner	VIIa	sections	√	√
England	Joanne Smith	experienced	VIIa	whole	√	√
Sweden	Barbara Bland	experienced	IIIa	whole	√	√
	Sofia Carlshamre	intermediate	IIIa	whole	√	√
	Jan-Erik Johansson	intermediate	IIIa	whole	√	√
Denmark	Helle Rasmussen	experienced	IIIa & IV	whole	√	√
	Frank Hansen	intermediate	IIIa	whole	√	√
Germany	Christine Petersen-Frey	intermediate	IV	whole	√	√

The statistics representing age reading performance were calculated for all readers combined and for experienced readers only. They were calculated separately for each area and preparation method (Table 5.3.3). Only otoliths for which modal age (as whole or sectioned otolith) was ≤ 10 were included. The number of otoliths per age group ranged from 3 to 8 for area IV and from 1 to 11 for area IIIa.

Like in the exchange set, agreement was higher and variance (APE & CV) was lower for experienced readers compared to all readers (Table 5.3.3). Like in the exchange, IV otoliths appear to be easier to read than IIIa otoliths, although the percentage agreement among experienced readers using sections is slightly lower for IV otoliths than

IIIa otoliths. In all cases, except experienced readers ageing IIIa otoliths, the statistics were better for whole otoliths than for sectioned otoliths.

The age reading performance did not improve for the workshop sets compared to the exchanges sets: they deteriorated for IV otoliths and were more or less the same for IIIa otoliths (Table 5.1.3 and 5.3.3). This is partly explained by the fact that the IV otoliths selected for the workshop were considered to more difficult than those selected for the exchange sets. However, major reason is that no consensus was reached on interpretation methods (see section 5.2).

Table 5.3.3. Results of workshop sets

	ICES area IV		ICES area IIIa	
	sections	whole	sections	whole
Number of readers	14	14	14	14
Agreement (%)	73%	76%	69%	71%
CV (%)	13%	9%	18%	15%
APE (%)	9%	7%	12%	11%
Number of experienced readers	6	6	6	6
Agreement experienced readers (%)	75%	82%	76%	76%
CV experienced readers (%)	12%	6%	11%	12%
APE experienced readers (%)	9%	5%	8%	9%

The results for each reader individually are presented (anonymously) in Annex 5. The statistics presented in Annex 5 are based on modal age calculations including all readers.

5.4 Growth analyses

Growth analyses were carried out to examine the previously described interpretation differences (see section 5.2) in more detail. Two experienced readers with opposing views were selected, one reader who ages Kattegat-Skagerrak (IIIa) plaice, the other who ages North Sea (IV) plaice.

The exercise was done using the TNPC software developed by IFREMER. Each annulus was marked and the distance to the nucleus was calculated. The analyses were based on whole otoliths included in the workshop sets. The IIIa reader analysed a selection of 20 otoliths from IIIa and 20 otoliths from IV, which resulted 124 annulus measurements for area IIIa and 117 measurements for area IV. The IV reader analysed the selected otoliths and an additional 49 otoliths, which resulted 166 annulus measurements for area IIIa and 294 measurements for area IV. These data allowed a comparison between 2 areas for the same reader and between 2 readers for the same area.

Growth rates were estimated to be higher in the North Sea (IV) than in Kattegat-Skagerrak (IIIa) according to both readers (Figure 5.4.1). Within each area, the estimated mean annulus size was generally smaller for the IIIa reader than for the IV reader. The difference was small and more or less consistent for IIIa otoliths. The difference was also small, albeit more variable, for IV otoliths up to the 9th annulus. A large difference was observed in 10+ otoliths from area IV, but these estimates were based on only 1 otolith in the case of the IIIa reader and on 3 otoliths in the case of the IV reader.

A consistent difference between 2 readers for all annuli indicates a difference in the interpretation of the first annulus. If the growth curves diverge, interpretation differences occur at a later stage. The results of this exercise tentatively point towards dif-

ferences in the interpretation of the first annulus as the main cause for different growth curves.

Although the differences appear to be small in the growth analyses, the underlying interpretation differences cause clear differences in the age reading results (Figure 5.4.2) and the age composition (Figure 5.4.3).

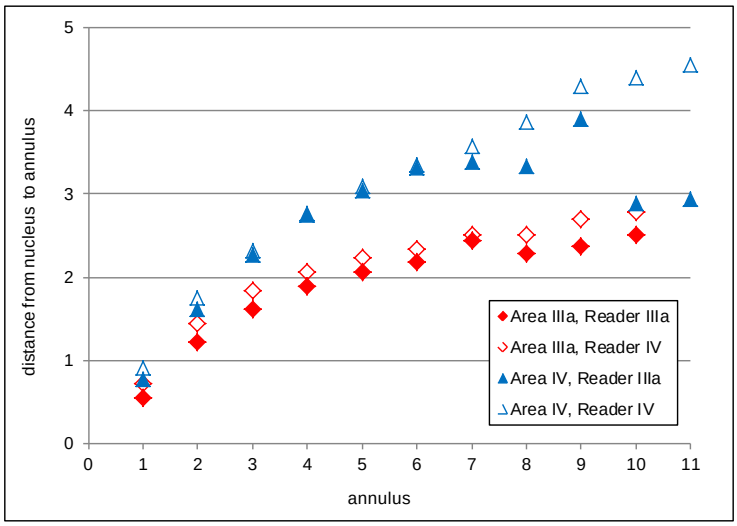


Figure 5.4.1. Distance from the nucleus to the annuli for selected whole otoliths from area IIIa and IV according to 2 different readers (one experienced reader for each area).

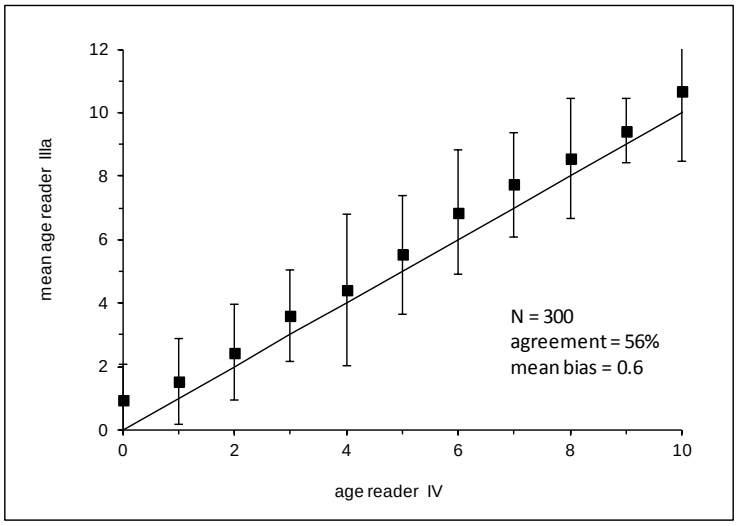


Figure 5.4.2. Age readings of the IIIa reader compared to the IV reader for all whole otoliths (modal age ≤ 10 year) in the exchange and workshop sets.

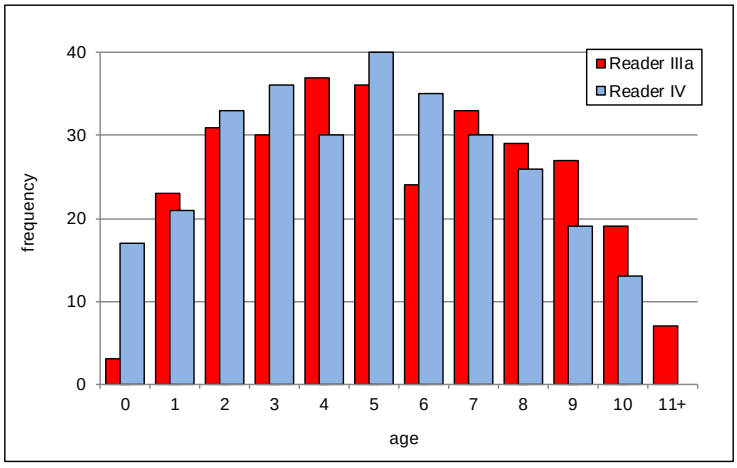


Figure 5.4.3. Age composition according to the IIIa reader and the IV reader for all whole otoliths (modal age ≤ 10 year) in the exchange and workshop sets.

5.5 Whole versus sectioned otoliths

The results of the exchange and workshop sets generally showed higher agreement and lower variance (APE & CV) for whole otoliths compared to sectioned otoliths (Table 5.1.3 and 5.3.3). Apparently whole otoliths were considered to be easier to read by the participants in the exchange and workshop. This is probably because most of the participants are accustomed to whole otoliths and have little to no experience with sectioned otoliths (see section 4).

Agreement and variance represent age reading precision. However, higher precision does not necessarily mean higher accuracy. Age can be over- or underestimated depending on the preparation method used. For many species, sectioning otoliths is considered to be the best way to age fish from a certain age onwards, because age can be underestimated when whole otoliths are used. The cause of this underestimation in whole otoliths is a change in growth direction; older otoliths tend to get thicker instead of longer and wider. The change in growth direction results in a 'cliff edge' in whole otoliths. If there is little to no otolith growth in the horizontal plane then it is difficult to impossible to count the annuli in whole otoliths.

The 2003 plaice workshop examined whole versus sectioned otoliths and recommended sectioning, especially for older fish from the northern North Sea (see section 2). This issue was re-addressed during the 2009-2010 exchange and the 2010 workshop, because several laboratories do not section their plaice otoliths (see section 4).

Comparison of the age composition of the exchange sets based on whole and sectioned otoliths showed no clear differences (Figure 5.5.1). The curves are almost identical up to age 5. Above age 5, there may be an indication for underestimation of age in whole otoliths compared to sectioned otoliths. The number of 10+ otoliths included in the exchange sets was limited.

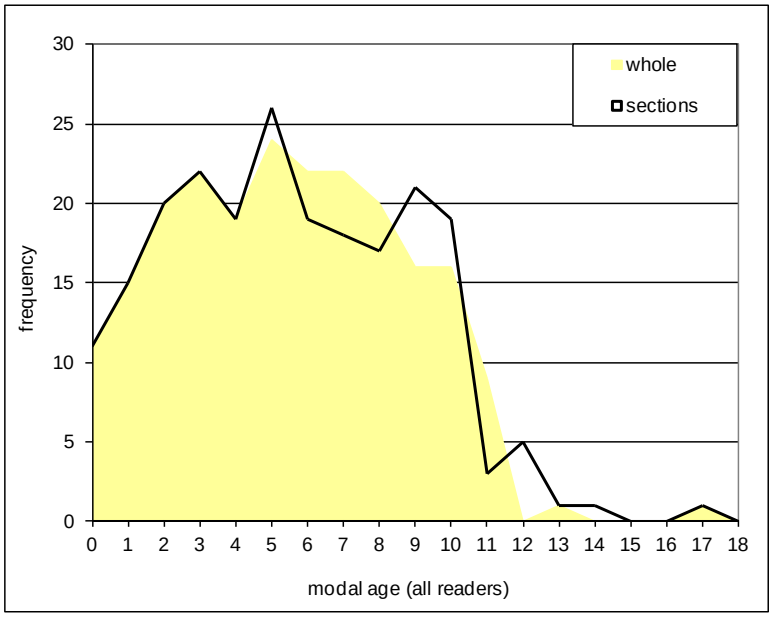


Figure 5.5.1. Age composition of exchange sets based on whole otoliths and sectioned otoliths. Only fish for which both otoliths were available are included (218 otolith pairs, see Table 5.1.1).

Ireland carried out a detailed study on whole versus sectioned otoliths for VIIa, VIIb, VIIg and VIIj plaice stocks and the results were presented at the workshop. Pair-wise comparisons of both otoliths of a fish, 1 sectioned and 1 whole, revealed no age reading bias related to preparation method (Figure 5.5.2). In the majority of the cases where discrepancies in results were observed, the difference was not more than 1 year. Most common problems were low readability, edge interpretation, false rings, splits and checks that caused the under- or overestimation of the ages by 1 year regardless of the preparation method. A 'cliff edge' effect was observed in the older otoliths, but it did not lead to underestimation of the ages while reading whole otoliths. The growth pattern observed on the sections were mirrored in the whole otoliths especially on the nucleus-anterior axis (Figure 5.5.3). However, the number of 10+ otoliths included in this study was limited. It was concluded that for VIIa, VIIb, VIIg and VIIj plaice ranging from 1 to 10 years old, otolith sectioning is not necessary.

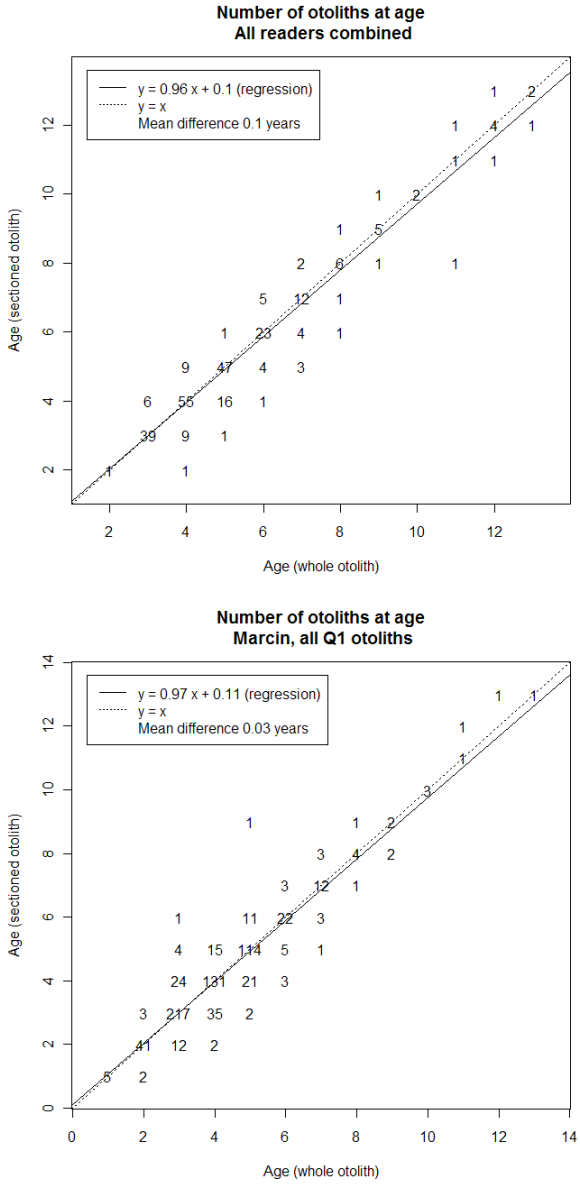


Figure 5.5.2. Pair-wise comparison of whole and sectioned otolith age readings for VIIa, VIIb, VIIg and VIIj plaice. Top panel: 138 pairs and 2 readers. Bottom panel: 893 pairs and 1 reader. Source: Marcin Blaszkowski, The Marine Institute, Ireland.

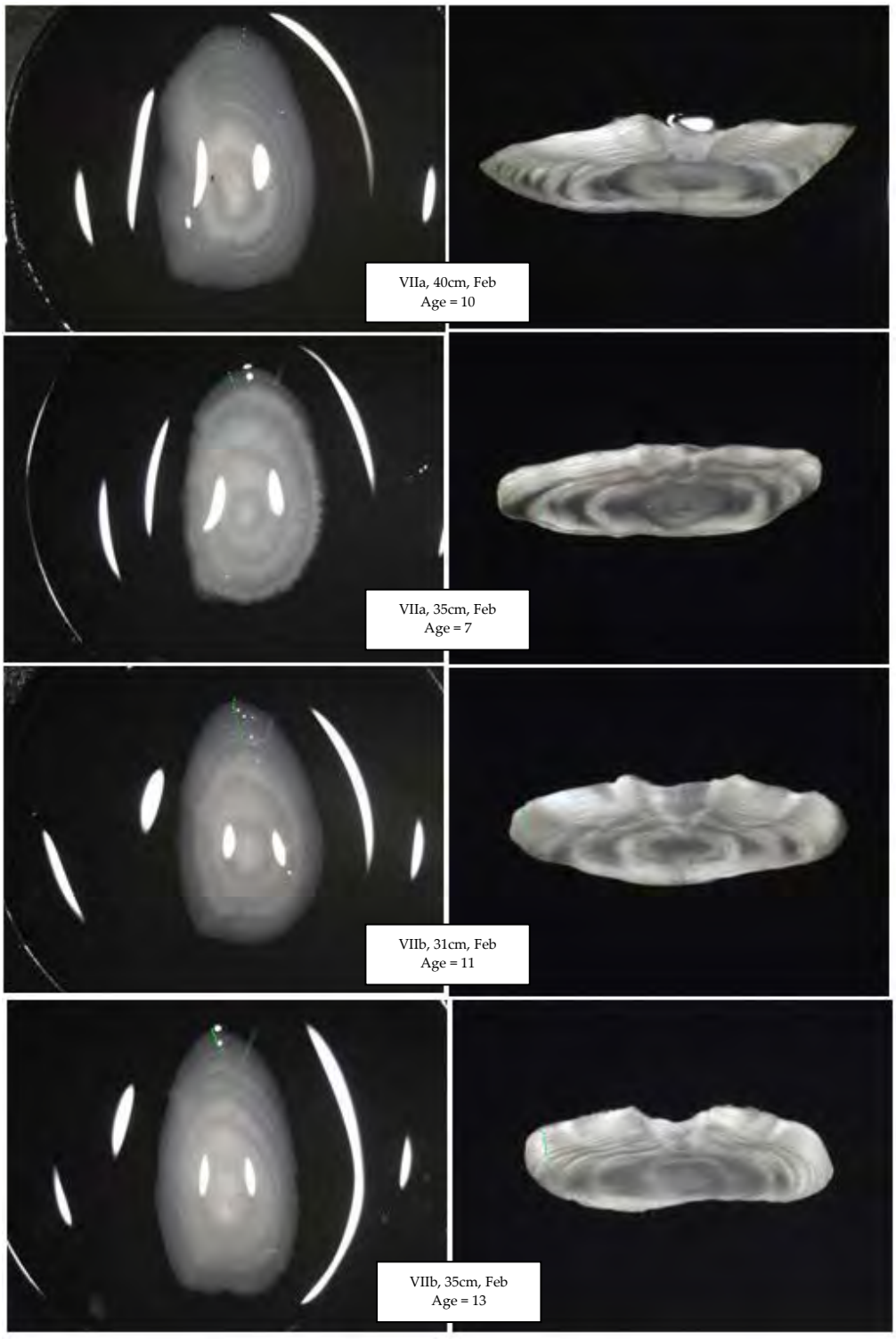


Figure 5.5.3. Pairs of otoliths from VIIa and VIIb which show a 'cliff edge' effect. Source: Marcin Blaszkowski, The Marine Institute, Ireland.

To examine potential bias related to preparation method in older fish, a pair-wise comparison was carried out for all otoliths included in the exchange and workshop sets for which modal age was 11+. Age estimations were significantly higher for sectioned otoliths than for whole otoliths and the fitted line was significantly different from y=x (Figure 5.5.4). This was the case for all readers combined (left panel in Figure 5.5.4) as well as for experienced readers only (right panel in Figure 5.5.4). Although the number of observations was reasonably large (due to the number of readers), the number of otoliths included in this analyses was small (29, see Table 5.1.1 and 5.3.1).

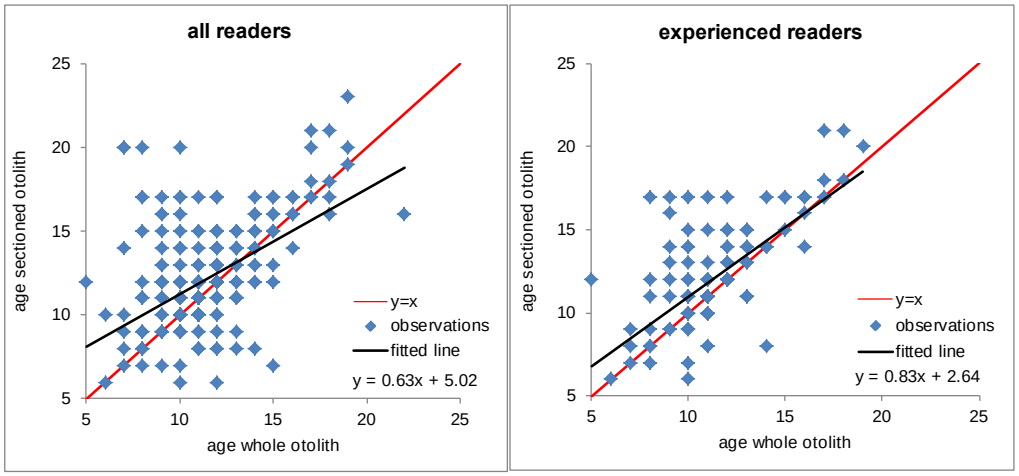


Figure 5.5.4. Pair-wise comparison of whole and sectioned otolith age readings for North Sea (IV) and Kattegat-Skagerrak (IIIa) plaice. Left panel: all readers, 331 paired readings. Right panel: experienced readers, 154 paired readings.

The results presented here suggest that sectioning otoliths is not necessary if age is less than 10, but that sectioning should be done for older fish. However the otolith set for older fish was small. Furthermore, as growth rates differ between areas it is likely that the age at which sectioning is necessary will also differ between areas. Therefore, further examination of whole versus sectioned otoliths in older fish (by area) is recommended.

6 Ageing manual (ToR c3)

6.1 General

Relevant biological data for fish should be available and used in case of doubt when assigning fish age.

Use microscopes with good quality optics. Stick to one microscope that the reader is familiar with. Use generally low magnification, but zoom where necessary.

WKARP advises all laboratories to count translucent rings for consistency, however some laboratories choose to count opaque rings. When annotating otolith images the point between the end of the translucent and beginning of the opaque should be marked.

6.2 Reading techniques

Plaice otoliths can be read using 3 different preparation methods.

1. Whole otolith method: Both otoliths are put in a container (black or transparent) filled with a clear fluid (water, oil, alcohol) or embedded in clear resin / microscopic medium.
2. Sectioned otolith method: Otoliths are embedded in resin (with or without added black stain) and then sectioned through the nucleus. The thickness of the slides range between 0.5 and 0.6 mm, with some using a glass coverslip as well. If sectioned otoliths are not covered with a glass coverslip, the surface of sectioned otoliths is covered with a thin layer of oil before reading.
3. Break and burn method: This method involves breaking the otolith in half (as close to the nucleus as possible). The broken halves are burnt until the translucent rings appear dark grey. The burnt edge is covered with water or oil to view.

Preference of source of light, transmitted or reflected, varies between laboratories. Some use both transmitted and reflected light, others only transmitted or only reflected. Features of the otoliths, especially at the edge, might look different using alternative light settings.

The different preparation methods and light sources are illustrated in Figure 6.2.1.

6.3 Considerations

Consider information from other otoliths caught at the same time and area when interpreting the growth structures.

The clearest axis, generally of major growth, should be used for the age interpretation.

For whole otoliths, usually the distal side is examined but sometimes the observation of the proximal side might be helpful (Figure 6.3.1).

Figure 6.2.1. Otoliths of a plaice (female, 55cm, IVb, July) using different preparation methods and light sources. From top to bottom: (1) whole otolith, reflected light (2) broken-burnt (3) transverse section, reflected light (4) transverse section, transmitted light. The age interpretation is 11 years based on the broken-burnt and sectioned otolith. The age was estimated younger based on the whole otolith (9/10 years). Source: Mick Easey, CEFAS, UK (presented at plaice workshop 2003).

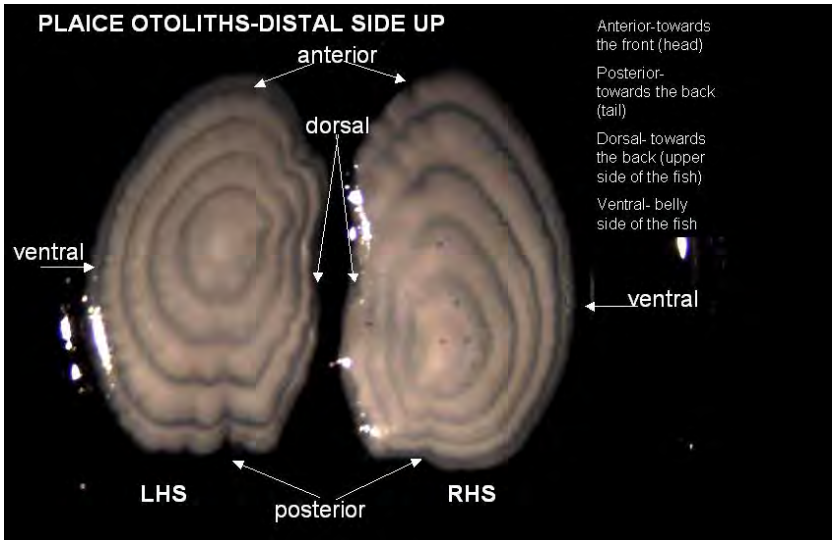


Figure 6.3.1. Description of morphological features of whole plaice otoliths mounted in microscopy medium (Q1, 5 years old). Source: Marcin Blaszkowski, The Marine Institute, Ireland.

6.4 Quality control

New readers should go through an extensive training program lead by an experienced plaice reader.

Fish ages estimated by inexperienced readers should be validated by experienced readers during the first years of training. The experienced readers should also be validated by other experienced readers as a quality control exercise.

6.5 Image capturing

Photographing and annotating images are important for documenting how readers interpret the otolith structures. However, photo quality is very important and proper preparation of otoliths is necessary for obtaining good images. Avoid over exposed pictures as optimum brightness and contrast are essential. Remember to record the image information (resolution in the file name is recommended). Pictures should be saved in Tiff format. Use only one microscope for each stock as there may be microscope specific calibration variance. Recalibrate the microscope setup regularly. The type of light (transmitted or reflected) used for imaging should be recorded on the image.

Recommended camera specifications:

- At least 6 MP
- Good light sensitivity
- High speed connection between camera and computer is recommended.
- Processing pictures can be done with specialized software as TNPC, or more general software as ImagePro, ImageJ, or others. A high resolution screen is important.

6.6 Interpretation

Date of birth

The birthday of all plaice is considered to be January 1st.

First annulus

Skagerrak and Kattegat (IIIa):

IIIa is treated as one stock but could well consist of several subpopulations. Growth rate varies between the Skagerrak and Kattegat. Skagerrak often shows a higher growth rate resembling the North Sea plaice. However, in the Kattegat (particularly in the southwest), one often finds very old but small fish which suggests a slow growth rate. The first annulus is therefore very small and should not be omitted when aging.

North Sea (IV) and eastern English Channel (VIId):

If there is a small ring close to nucleus and the increment is smaller than for the second ring then it is not counted in these fast growing stocks. In that case the second more prominent ring is considered to be the true first annulus.

Irish Sea (VIIa), Celtic Sea (VIIg), ICES Divisions VIIb and VIa:

The size of the first annuli in plaice otoliths can vary considerably. This is probably due to the prolonged spawning period with fish spawned earlier in the season showing larger first annulus and those that spawned later in the season showing smaller first annulus. In some specimens when counting the first annulus, two translucent zones can be very close to each other. In those cases it is generally considered as just one translucent zone and counted as the first annulus.

Otolith edge

The appearance of the otolith edge varies seasonally and between age classes. The translucent zone is deposited in second half of the year and is usually not completed before New Year, except in young fish from fast growing stocks. Older fish deposit the translucent zone over a longer period. It is also important to note that regional differences in timing of otolith zone formation occur; opaque/translucent deposition starts later at higher latitudes. Furthermore annual temperature variations may also affect the timing of opaque/translucent growth.

As age is determined relative to January 1st, the number of rings counted deviates from the number rings visible in certain periods of the year. These periods depend on whether opaque or translucent rings are counted. It furthermore depends on regional differences in the timing of opaque/translucent deposition.

If translucent rings are counted (description seasonality opaque/translucent formation based on Irish Sea, Celtic Sea and VIIb plaice otoliths):

For Q1 fish, at the edge of the otolith there should be a translucent zone. This translucent zone is counted when assigning an age. Therefore the age attributed is n where 'n' is the number of translucent rings.

For Q2 fish, at the edge of the otolith there should be a fully formed translucent zone and there may also be evidence of opaque growth. This translucent zone is counted when assigning an age, therefore the age attributed is n.

For Q3 fish at the edge of the otolith one can expect to see either a translucent zone (possibly incomplete) or an opaque zone.

- Generally a translucent zone at the edge indicates current year's growth and therefore is not counted; the age attributed is n-1.
- However there can be some exceptions in older otoliths where there is a complete translucent zone at the edge with little or no opaque growth. In this case the translucent zone is considered to be last year's growth and is therefore counted, the age attributed is n.
- If there is an opaque zone at the edge of the otolith then all translucent zones are counted and the age attributed is n.

Quarter 3 causes the most confusion, as the age attributed is n or n-1. When assigning an age, one must consider certain factors such as whether the fish is young or old and whether it is early or late in the quarter

For Q4 fish, the edge of the otolith should have a translucent zone. This translucent zone is for the present winter and as the birth date is considered to be the January 1st it is not counted. Therefore the age assigned is n-1.

If opaque rings are counted (description seasonality opaque/translucent formation based on North Sea plaice otoliths):

For Q1 fish, the edge of the otolith should have a translucent zone. The opaque zone has not been deposited yet. Therefore the age attributed is m+1 where 'm' is the number of opaque rings.

For Q2 fish, the edge of the otolith can be opaque or translucent. In young fish and fish from the southern part of the North Sea the edge is usually already opaque. Translucent edges are observed in older fish and dish originating from the northern part of the North Sea.

- If there is a translucent zone at the edge of the otolith then the otoliths are interpreted as Q1 otoliths, i.e. the age assigned is m+1.
- If there is an opaque zone at the edge of the otolith the age assigned is m.

For Q3 fish, the edge of the otolith can be opaque or translucent. In young fish and fish from the southern part of the North Sea the edge is usually already translucent. Opaque edges are observed in older fish and fish originating from the northern part of the North Sea. In both cases the age assigned is m.

For Q4 fish, the edge of the otolith usually has a translucent zone, although in older fish and fish originating from the northern part of the North Sea the translucent zone formation may not have started yet. In both cases the age assigned is m.

The formation of translucent and opaque zones in North Sea and eastern English Channel plaice is illustrated in Figure 6.6.1.

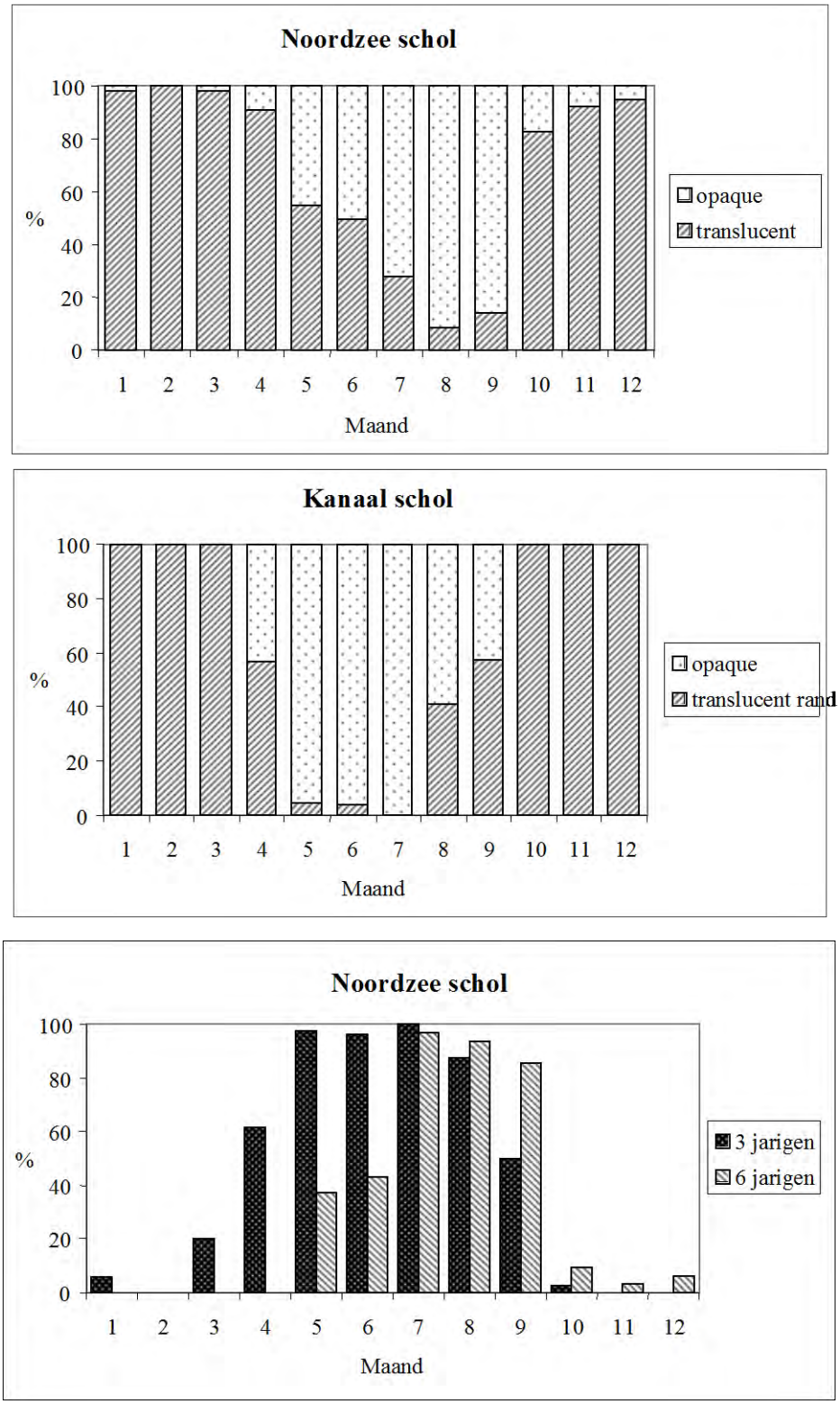


Figure 6.6.1. Proportion translucent/opaque edges by month for North Sea plaice (top panel) and eastern English Channel plaice (middle panel). Proportion opaque edges by age class (3 or 6 year old) and month for North Sea plaice (bottom panel). Source: Loes Bolle, IMARES, The Netherlands.

Split rings

Split rings can be confused with true annuli which leads to age overestimation. Observation of the width of the rings from nucleus to the edge gives a good indication as to whether it is a true ring or not. From nucleus to the edge, rings should get progressively narrower and more tightly packed. However due to environmental factors (i.e. starvation, water temperature fluctuation) it is not always true. The final interpretation of age depends on the experience of the age reader for a given stock. Split rings can also be detected if the ring is not as prominent as the other rings and if it does not appear as a pattern throughout the whole otolith.

7 Reference collection (ToR c4)

The agreed age collection consists of otoliths with a high percentage of agreement between the (experienced) age readers. This set can be used for trainees, to learn age reading, and for all age readers when they are in doubt how to read a certain (difficult) otolith.

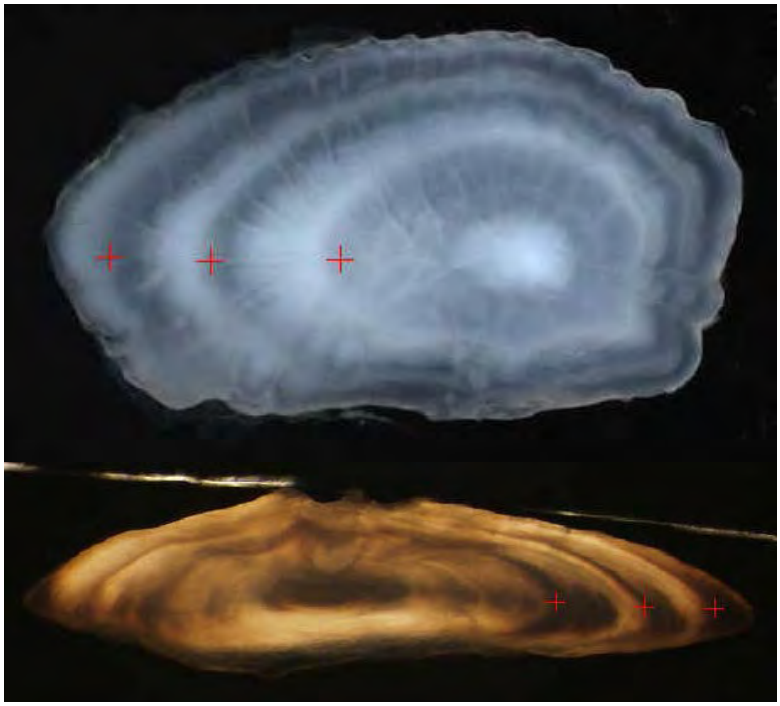
During the plaice age reading workshop in 2010, a start was made with a small set of annotated images of otoliths. This set can be expanded by adding otoliths at any time. For this small set, otoliths were selected from the exchange set 2009-2010. The otoliths are from areas IV (North Sea) and IIIa (Skagerrak-Kattegat), from plaice caught in quarter 3 and quarter 4. The age range consists of 1, 3, 5, 7 and 9 year old fish. Both the sectioned and the whole otolith were included for each fish. The images of whole otoliths were captured using reflected light, the images of sectioned otoliths using transmitted light.

The percentage agreement aimed for in the selected otoliths was at least 85% for area IV, and at least 75% for area IIIa. However, in some cases, it was necessary to choose an otolith with less agreement.

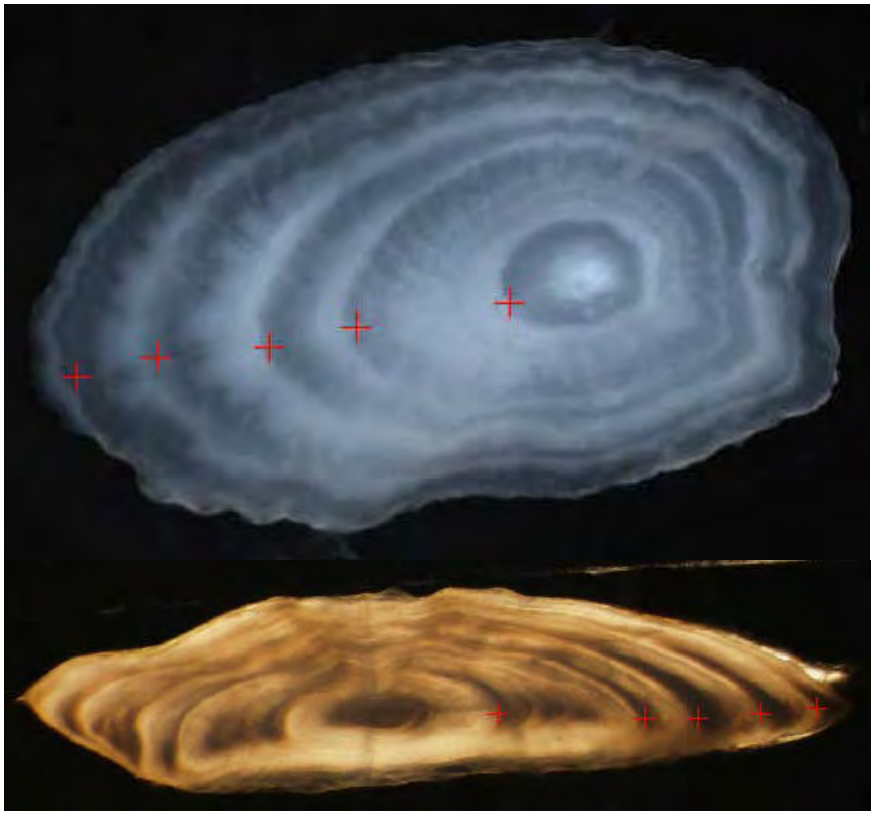
Annotations were made during a plenary session in the workshop using WebGR. Everyone agreed on the final annotations shown in the photos below.

North Sea (IV) set

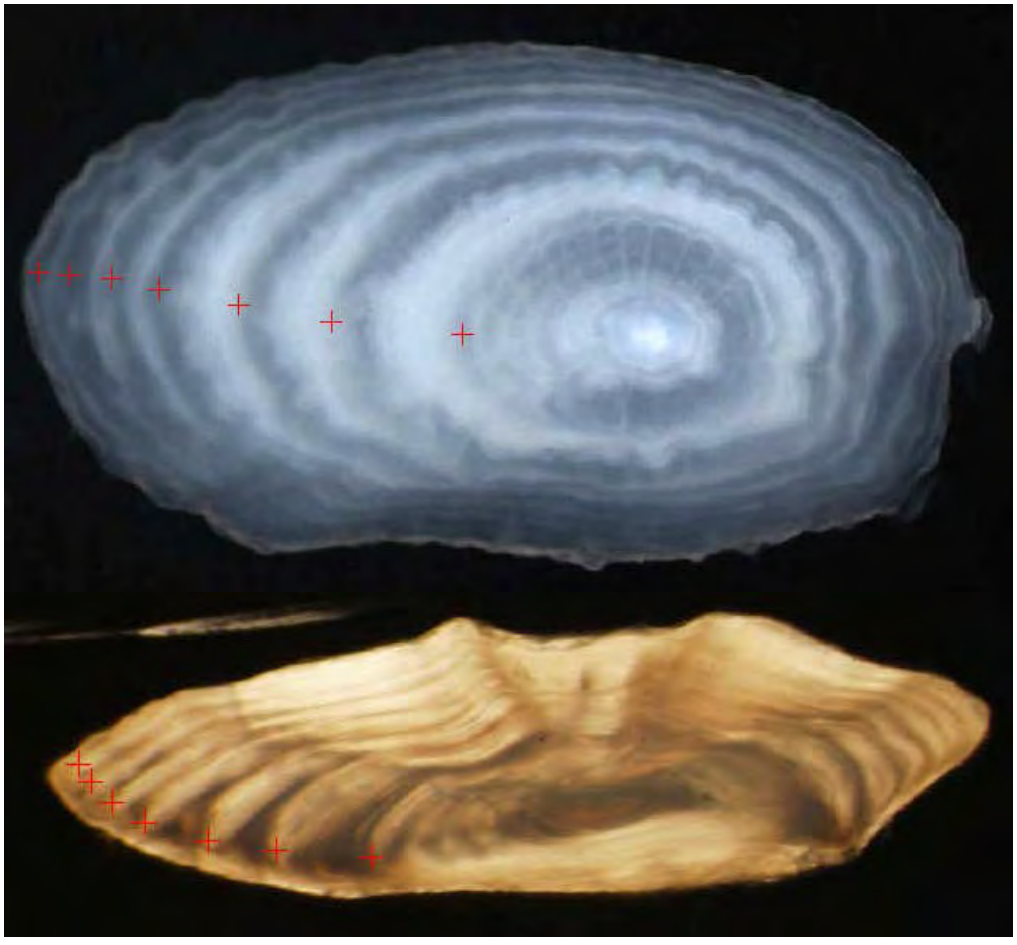
Agreed age = 1 (month = September)



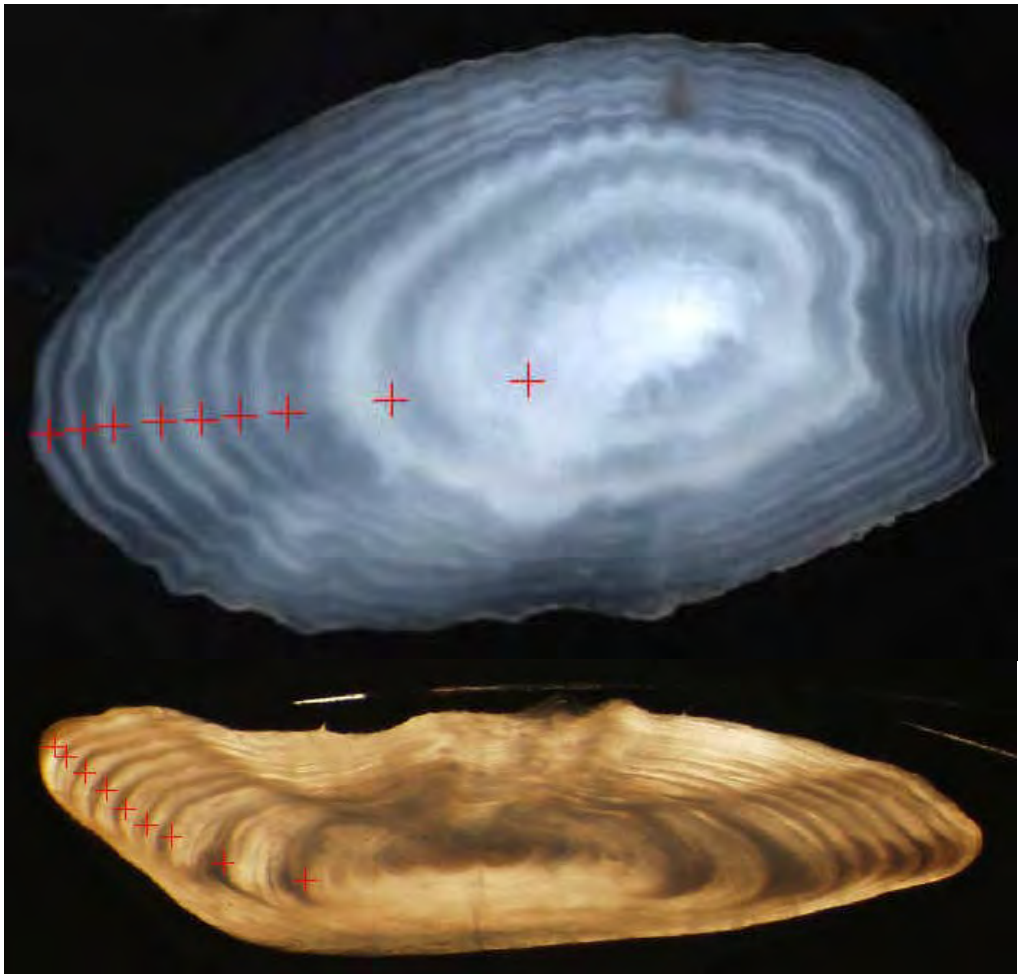
Agreed age = 3 (month = August)



Agreed age = 5 (month = August)



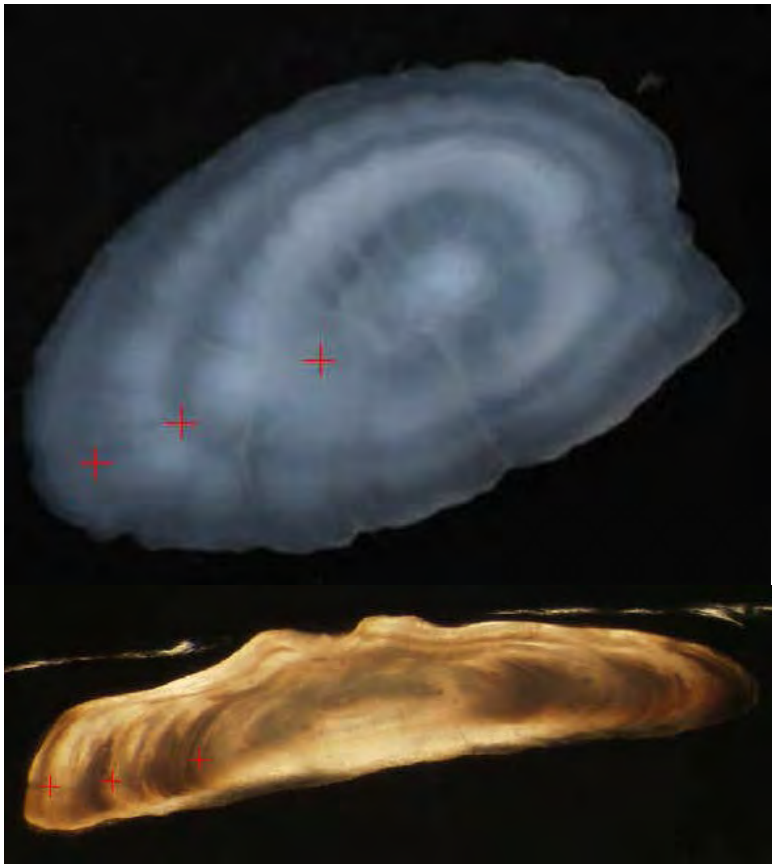
Agreed age = 7 (month = November)



Agreed age = 9 (month = November)

Kattergat – Skagerrak (IIIa) set

Agreed age = 1 (month = August)



Agreed age = 3 (month = September)

Agreed age = 5 (month = July)

Agreed age = 7 (month = November)

8 Target and threshold statistics (ToR c6)

As tool for the evaluation of the quality of age readings, PGCCDBS recommended that target and threshold statistics are formulated for each species and stock (PGCCDBS 2008). The statistics refer to the percentage agreement, a variance estimate (CV or APE) and the bias. The target value is the value you would like to achieve and know is possible based on exchange and workshop results. The threshold value is the minimum value required before a reader is qualified to supply data to assessment working groups. Usually a CV of 5% is set as a threshold for sufficient data quality (Campana 2001)

Percentage agreement, CV and bias were calculated using the "Tool for Age Reading Comparisons" (Eltink *et al* 2000). The average percent error (APE) was calculated according to Beamish and Fournier (1981). These statistics were calculated, by stock and method, for the exchange sets (Table 5.1.3, Annex 4) and for the workshop sets (Table 5.3.3, Annex 5).

Both the exchange and workshop otoliths sets were stratified by age, within the age range 0-10, based on the age readings of the country supplying the otoliths. A few older otoliths were included in the exchange and workshop, but these were excluded from the statistics calculations. The statistics for age range 0-10 years are considered to be relevant because the vast majority of plaice in the North Sea (IV) and Kattegat-Skagerrak (IIIa) are younger than 11 years.

Calculation of both CV and APE poses problems if mean age is close to 0, therefore all observations for which modal age was 0 were omitted from the CV and APE calculations.

If true age is unknown then overall bias based on all (experienced) readers is meaningless. Nevertheless, the bias of an individual reader compared to modal age is a meaningful measure (Annex 4 and 5).

Target and threshold statistics for a specific stock should be based on age readings of experienced readers for that specific stock. The results for experienced readers in Tables 5.1.3 and 5.3.3 were based on all experienced readers, irrespective of the stock they are specialised in. Therefore, for North Sea plaice (IV), the statistics were recalculated including only the experienced North Sea readers. This approach was not possible for Kattegat-Skagerrak (IIIa) plaice, because only 2 experienced IIIa readers participated in the exchange and workshop. Furthermore the agreement between these 2 readers was low (53% based on the combined workshop and exchange sets; whole otoliths).

For North Sea plaice, 4 experienced North Sea readers, who participated in both the exchange and the workshop, were included in the analysis. These 4 readers represented 4 countries. The statistics were calculated for the combined exchange and workshop sets in which modal age was ≤ 10 years. Only whole otolith age readings were included as this is the preferred method for most of these readers. This resulted in a total of 162 otoliths; 5 to 22 otoliths per (modal) age group in the age range 0-10 years. Based on the results of this analysis (Table 8.1), target and threshold statistics for North Sea plaice readers are tentatively proposed (Table 8.2).

Table 8.1. Percentage agreement, CV, APE and bias for North Sea (IV) plaice based on the age readings of 4 experienced North Sea readers (162 whole otoliths, age range 0-10 years).

	ICES area IV			
Overall	4 readers			
agreement	89%			
CV	4%			
APE	3%			
By reader	A	B	C	D
agreement	94%	79%	88%	93%
CV	4%	9%	7%	4%
APE	3%	4%	3%	2%
bias	0.01	0.12	0.10	-0.02

Table 8.2. Proposed threshold and target statistics for North Sea (IV) plaice readers.

	ICES area IV	
	Threshold	Target
Agreement	85%	90%
CV	5%	3%
APE	5%	3%
Bias	0.10	0.05

At present, the proposed threshold statistics are not achieved by all experienced readers. This is related to the unresolved interpretation differences described in section 5.2. If agreement is reached on the issue of regular versus irregular growth patterns (whether or not by means of validation studies), then the proposed threshold statistics are considered to be achievable.

9 Recommendations and follow-up actions (ToR c5)

Follow-up actions and recommendations for further cooperation, exchanges, workshops and other actions in relation to the age estimation of plaice have been formulated.

9.1 WebGR

WKARP 2010 was the first workshop to use WebGR. It was generally agreed that WebGR is a very useful tool. However, the end users (age readers and age coordinators) have had no proper training session with the tool so far. It is felt that, although others from the same institute may have attended one or more of the WebGR workshops, the expertise has not reached the end-users yet. The group recommends WebGR training sessions to be given.

9.2 Manual

The group reached agreement on an ageing manual/guidelines (section 6). The aim is to employ these guidelines to eliminate some of the problems with e.g. split rings in the otolith structures. The group recommends that all ageing laboratories processing plaice 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.

The age reading manual produced at this workshop should be maintained and further developed at future workshops.

9.3 Validation

Through the discussions at the workshop it became apparent that various life history traits for plaice (e.g. growth rate, spawning period) may differ within a stock and most certainly between stocks. The knowledge of the influences of these life history traits on the depositions in otoliths is highly important for age readers. Thus, the group emphasises the necessity of studies on otolith formation in relation to stock specifics, especially with regard to the formation of the first annulus.

To deal with this issue, we ask all participants of the workshop to investigate the possibilities for validation studies focussing on regularity of growth patterns and formation of the 1st annulus in otoliths of plaice. At the time being, we need to find out what studies are possible; we do not ask to do the actual work at present as funding will be required for such studies. We strongly urge all institutes/participants to this workshop to inquire with colleagues and their connections to investigate the possibilities for validation studies on plaice otolith growth. We will report these possibilities to PGCCDBS in 2012.

This work will be coordinated by Loes Bolle.

9.4 Reference collection

The results of the 2009-2010 plaice exchange and the 2010 plaice workshop show more work is necessary for the European institutes involved in the age reading of plaice to come to high quality readings. The quality of age-readings will certainly increase when the age readers can make use of an international agreed reference col-

lection that shows examples of otoliths of all ages per quarter and per stock. The participants to the WKARP feel an agreed reference collection should contain at least 1 otolith per age-group per quarter per stock (or ICES area) because of stock dependent growth patterns and spawning periods. It is not feasible to produce such a large reference collection during a workshop due to time constraints. We therefore recommend to continue this much needed international agreed reference collection prior to the next workshop.

WKARP 2010 proposes to collate an age stratified image set of 50-200 images per stock / ICES area, to whom all countries contribute. Preferably the set will contain both otoliths of a fish, one otolith sectioned the other whole. All images will be taken using reflected light. We propose that in 2012 this set will be used as an exchange set through WebGR to obtain internationally annotated images. These images then form the basis for an international agreed reference collection. After these annotations, we propose a workshop in 2013 to decide on which images from the exchange will be used in the reference collection. This larger set of otoliths images will also provide the possibility to look deeper into the interpretation problems faced in plaice otoliths.

To make this (large scale) otolith exchange work, all institutes that participated to the WKARP 2010 commit themselves to providing their part of the images sets. The aim is to create the set WebGR. This would be facilitated if a WebGR training session was held prior to the exchange (see section 9.1). If not, then the participants will need to find help on the use of WebGR within their own or neighbouring institutes.

No coordinator has yet been appointed for this work.

9.5 Whole versus sectioned otoliths

A recent study from Ireland showed that reading plaice otoliths whole did not increase the possibility of under-ageing fish in comparison to sectioned otoliths, for VIIa, VIIb, VIIg and VIIj otoliths up to age 10 (see section 5.5). A small set of 11+ otoliths included in the present exchange and workshop indicated that underestimation may occur in older fish (see section 5.5). The risk of underestimating age when using whole otoliths increases with age, due to an increase of the 'cliff edge' effect.

As old (10+) plaice are scarce nowadays, these age classes are generally underrepresented in otolith collections. It could be argued that the relevance of ageing precision for these age classes is therefore small. However, some stocks are assessed up to 15+ and age reading precision in older plaice is considered to be important.

WKARP proposes the collation of a set of old otoliths from the North Sea (IV) and Kattegat-Skagerrak (IIIa), containing both otoliths (one sectioned and one whole) from the same fish. We aim at 50 fish per ICES area. The Netherlands and Belgium will work together for the North Sea otolith collection, and Sweden and Denmark for the Skagerrak-Kattegat otolith collection. The whole and sectioned otolith set will be read independently by all participants in the 2012 exchange. The results of this exercise will be discussed at the 2013 workshop.

This work will be coordinated by Annemie Zenner.

9.6 Regular workshops

In general, it is important to have workshops at regular (3-5 year) intervals. Besides international calibration, this would lead to increased focus, continuation of on-going work and a good network of readers for each fish species. It is strongly recommended

that all laboratories contributing age estimates for stock assessments should participate in the workshops and otolith exchanges.

It is furthermore recommended to combine different stocks in age reading workshops and (full) otolith exchanges. Advantage is not only that this will reduce the number of workshops per species, it will also contribute to consistency in procedures and benefit the awareness of age readers with regard to formation of otolith structures. For plaice, it is recommended to combine age calibration exercises for all stocks in ICES areas III, IV, VI and VII.

The next plaice exchange is proposed for 2012 and the next workshop for 2013. If a WebGR training session has not been held prior to the workshop (see section 9.1) then part of the workshop can be used as WebGR training session.

References

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Annex 1: List of participants

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Annex 2: Agenda

Tuesday	
13:00-13:30	Welcome & lunch
13:30-14.00	Opening of meeting: participants, practicalities, ToR's, agenda
14:00-14:30	Presentation on exchange results
14:30-15:00	Presentation by Ireland on whole versus sectioned otoliths
15:00-15:30	Tea break
15:30-17:00	Discuss otolith images from exchange: identify and resolve differences
(17.00-18.00	meeting room available if necessary)
Wednesday	
08.30-08.45	Plenary: progress yesterday & agenda today
08.45-10.00	Continue discussion on otolith images (in 2 subgroups)
10.00-10.30	Coffee break
10.30-12.30	Age readers: read new otolith sets
	Age coordinators: Discuss ToR's, start subgroup work
12.30-13.00	Lunch break
13.00-15.00	Age readers: read new otolith sets
	Age coordinators: subgroup work
15.00-15.30	Tea break
15.30-17.00	Age readers: read new otolith sets
	Age coordinators: subgroup work
(17.00-18.00	meeting room available if necessary)
Thursday	
09.00-09.15	Plenary: progress yesterday & agenda today
09.15-10.30	Discuss ageing manual
10.30-11.00	Coffee break
11.00-13.00	Continue subgroup work
13.00-13.30	Lunch break
13.00-13.30	Discuss results new otolith sets
13.30-14.00	Discuss information on participating laboratory procedures
14.00-16.00	Joint annotations agreed age collection in WebGR
16.00-16.30	Tea break
16.30-17.30	Discuss ageing manual
19.00-	Dinner in Haarlem

Friday

09.00-09.15	Plenary: progress yesterday & agenda today
09.15-10.30	Discuss recommendations & follow-up actions
10.30-11.00	Coffee break
11.00-12.00	Continue subgroup work
12.00-12.30	Closing of meeting
12.30-13.00	Lunch

Annex 3: Protocol exchange

Age reading protocol for the 2009–2010 plaice exchange

Version 18 June 2009 - Loes Bolle, Ineke Pennock, Silja Tribuhl

General

The reference collection of the plaice exchange 2009 consists of 120 otoliths from the ICES subarea IV (North Sea) and 102 otoliths from division IIIa (Skagerrak-Kattegat). Both sets include sectioned and whole otoliths and consist of both young and older fish as well as samples from different quarters. All otoliths have been digitized for age annotations.

Some of the participating countries are thinking of changing their reading method from whole to sectioned otoliths. For this reason we hope that **all** readers are willing to read both methods for a comparison. However, we don't want to force this on laboratories who are not interested in a comparison of methods, in this case the reader can use his/her preferred method. If a reader uses both methods then it is essential that these 2 age determinations are done independently, i.e. first read all otoliths using one method, then read all otoliths using the other method (preferably a few days later) without comparing the results.

The exchange consists of 2 ICES management areas, i.e. division IIIa and subarea IV. We would like to urge **all** readers to read the otoliths from both areas, because otherwise the number of age determinations by expert readers for division IIIa will be insufficient. Please note that the data will be analyzed separately for each area and the expertise of the reader will be taken into account.

CD's containing the otolith images and excel files with data sheets for both sets and methods will be sent to all participating laboratories. Please enter your age determinations in the data sheets and clearly indicate the reader, institute, and whether the ages refer to sectioned or broken otoliths. Please determine the age of the year-class, i.e. assuming January 1st to be the fishes 'birthday'. Provide one age estimate only per otolith and assign an age for every fish, failing to do this will influence the results on precision and accuracy. When ageing is complete please return the age data sheets to the Netherlands by email (ineke.pennock@wur.nl & silja.tribuhl@wur.nl).

Sectioned otoliths

Slides should be positioned for reading, with the label at the top. The otoliths should be read from left to right, working down towards the bottom. There is a white tip-ex mark next to the otoliths included in the exchange. You can find the slide IDs with associated fish numbers in the file **'Data lists PLE exchange 2009 sections.xls'** on the CD. Sectioned otoliths should preferably be examined using transmitted light (as in the images).

When reading the sections please note that the original ID numbers on the slides are not always in sequential order so you have to switch between the slides. (This was caused by the fact that we had to substitute otoliths for which the whole otolith was not available. Apologies for the inconvenience. In the Swedish set four sections are missing, only whole otoliths are present and can be read.

Whole otoliths

Whole otoliths are kept in Eppendorf cups for the North Sea set and in otolith envelopes for the Skagerrak-Kattegat set. Numbers on the Eppendorf correspond to the exchange numbers (**exc. nr.**) in the data list. On the envelopes the exchange number is written on the yellow label at the left top corner. Whole otoliths should preferably be examined using reflected light (as in the images). Every care should be taken to prevent damage to the set, as this will reduce precision in age reading for subsequent readers.

Digitized images

1) All digitised images are on 2 CD's, one containing the images of whole otoliths, the other containing the images of sectioned otoliths. The images of sections are labelled **Ple_set_NL_001.tif** or **Ple_set_SWE_001.tif** respectively. Images of the whole otoliths are labelled **Ple_set_NL_001_whole** and **Ple_set_SWE_001_whole.**

2) All readers are asked to annotate all otoliths (both of the sectioned and the whole otoliths if you are doing both methods). To annotate the annual rings on the images, you will need the program **Paint Shop Pro** (version 7.02 or older). A layer system can be used in this program and each layer will correspond to 1 reader. Please save the pictures as *.psp files. Everyone is asked to save a copy of their interpretation (in a separate layer) on a personal CD and send this CD to the Netherlands. The annotations will be examined prior to the workshop and a selection of the images and annotations will be used for discussion purposes at the workshop, which will be held in 2010.

With regard to the annotations, a dot should be placed on the inside of the opaque ring, corresponding to the onset of the next growth increment. In the case of sectioned otoliths, please mark the rings on the dorsal or ventral side of the otolith (i.e. not in sulcus). In the case of whole otoliths, please mark the rings where in increment structure is clearly visible. Please ensure that the number of dots corresponds to the age estimation in the data sheet. If the ring count is less than age estimation (because of year-class interpretations), please place a dot on the edge of the otolith. At times the digitized images are not as clear as the live image, so you are strongly advised to annotate the images at the same time as you are looking at the otolith live.

Timetable

Period	Country	Insitute	Address (where courier can deliver the otoliths)	to the attention of
15-31 May	Belgium	ILVO	Ankerstraat 1, 8400 Oostende	Annemie Zenner
1-15 June	Netherlands	Wageningen IMARES	Haringkade 1, 1976 CP IJmuiden	Ineke Pennock
16-30 June	Ireland	The Marine Institute	Rinville, Oranmore, Co. Galway.	Gráinne Ni Chonchuir
1-15 July	Northern Ireland	AFBI	Fisheries & Aquatic Ecosystems Branch, Agri-Food & Biosciences Institute, Newforge Lane, Belfast, BT9 5PX, UK	Willie McCurdy
16-31 July	England	CEFAS	Pakefield Road, Lowestoft, Suffolk, NR33 0HT	Mark Etherton
1-15 Aug	Sweden	Institute of Marine Research	Turistgatan 5, S-453 30 Lysekil	Barbara Bland
16-31 Aug	Denmark	DTU Aqua	Charlottenlund Castle, DK-2920 Charlottenlund	Niels Jørgen Phil
1-15 Sep	Germany	vTI Institute for Sea Fisheries	Palmaille 9, 22767 Hamburg	Christine Petersen-Frey
16-30 Sep	France	IFREMER	Centre Manche-mer du Nord, Lab. Ressources Halieutiques, 150 quai Gambetta, BP 699, 62 321 Boulogne Sur Mer	Kélig Mahé
1 Oct.	Netherlands	Wageningen IMARES	Haringkade 1, 1976 CP IJmuiden	Ineke Pennock

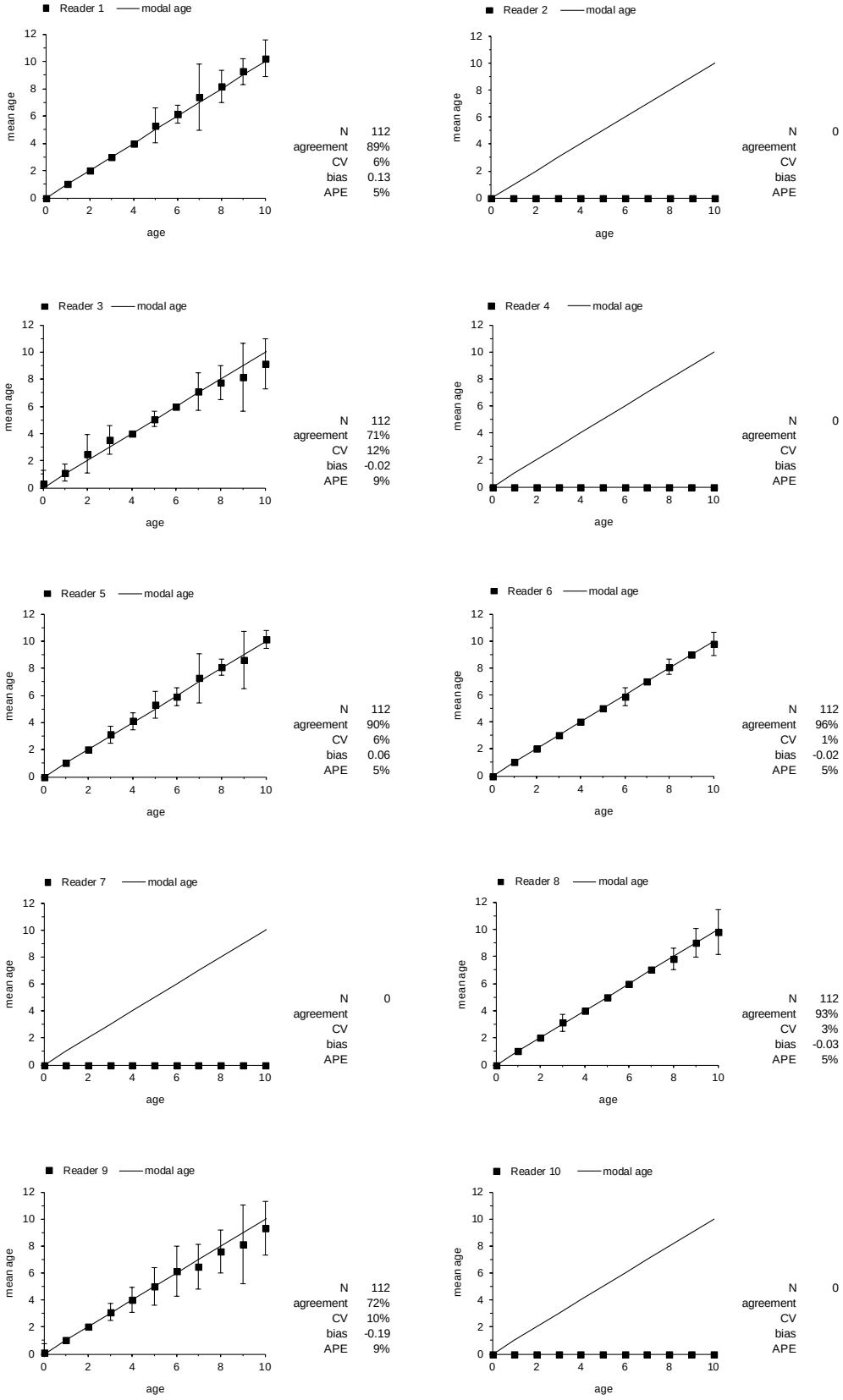
If for any reason, you receive these samples at a time when you are not in a position to read them, please inform us of this and we will come to an alternative arrangement. It would be useful for us if someone could notify us by email when the material arrives at an institute and also when it leaves the same institute, so we have an idea how the reading schedule is progressing.

When the age readings have been completed, please send the samples on to the next institute on the reading schedule and email the completed age data sheets to ineke.pennock@wur.nl & silja.tribuhl@wur.nl. We encourage all participants to read the otoliths and annotate the images as soon as they are received and then send them promptly on to the next institute.

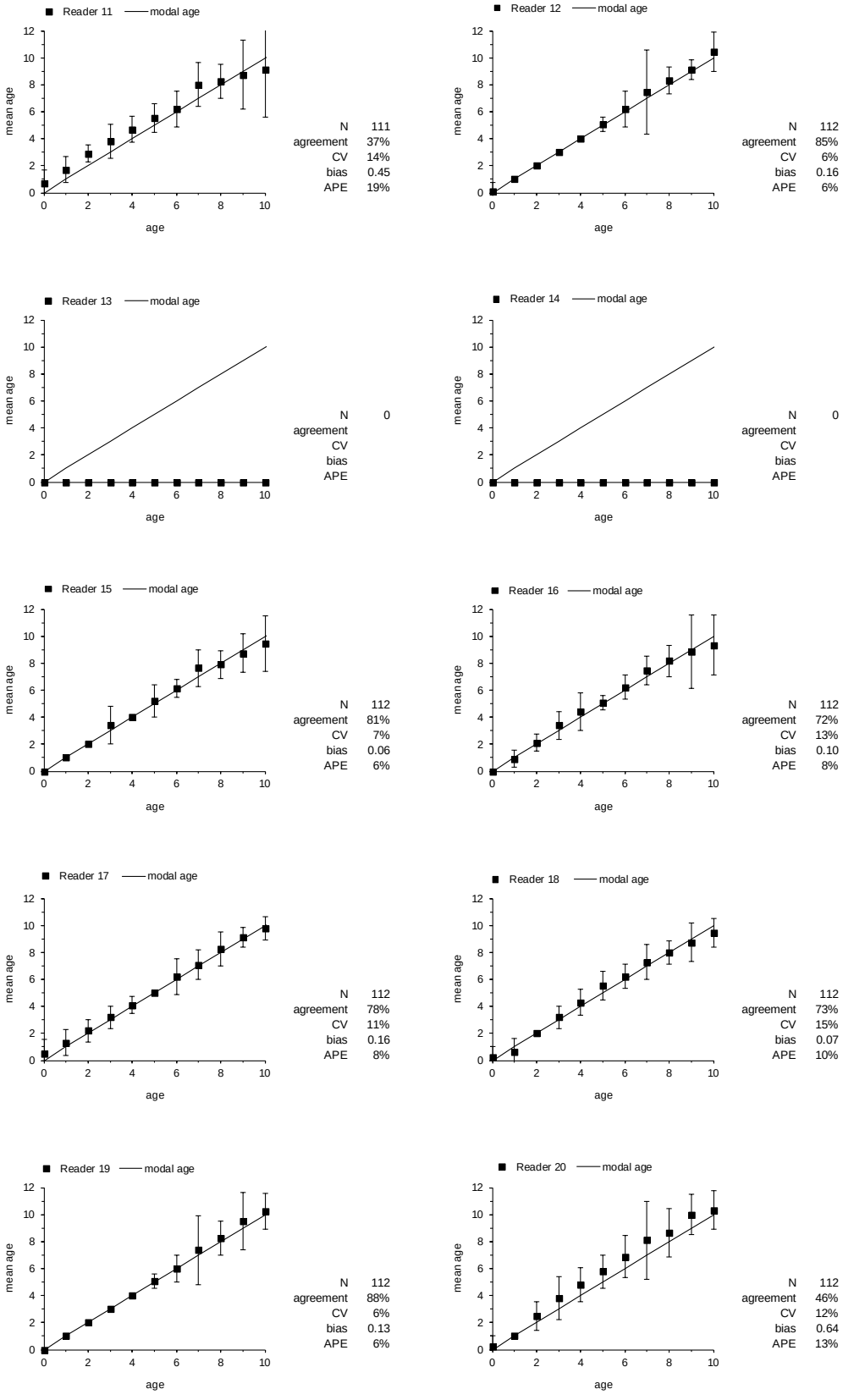
Important note: the samples need to be very well protected when being packaged and should be sent on to the next user by courier, to prevent damage or loss of the material.

Annex 4: Exchange – results individual readers

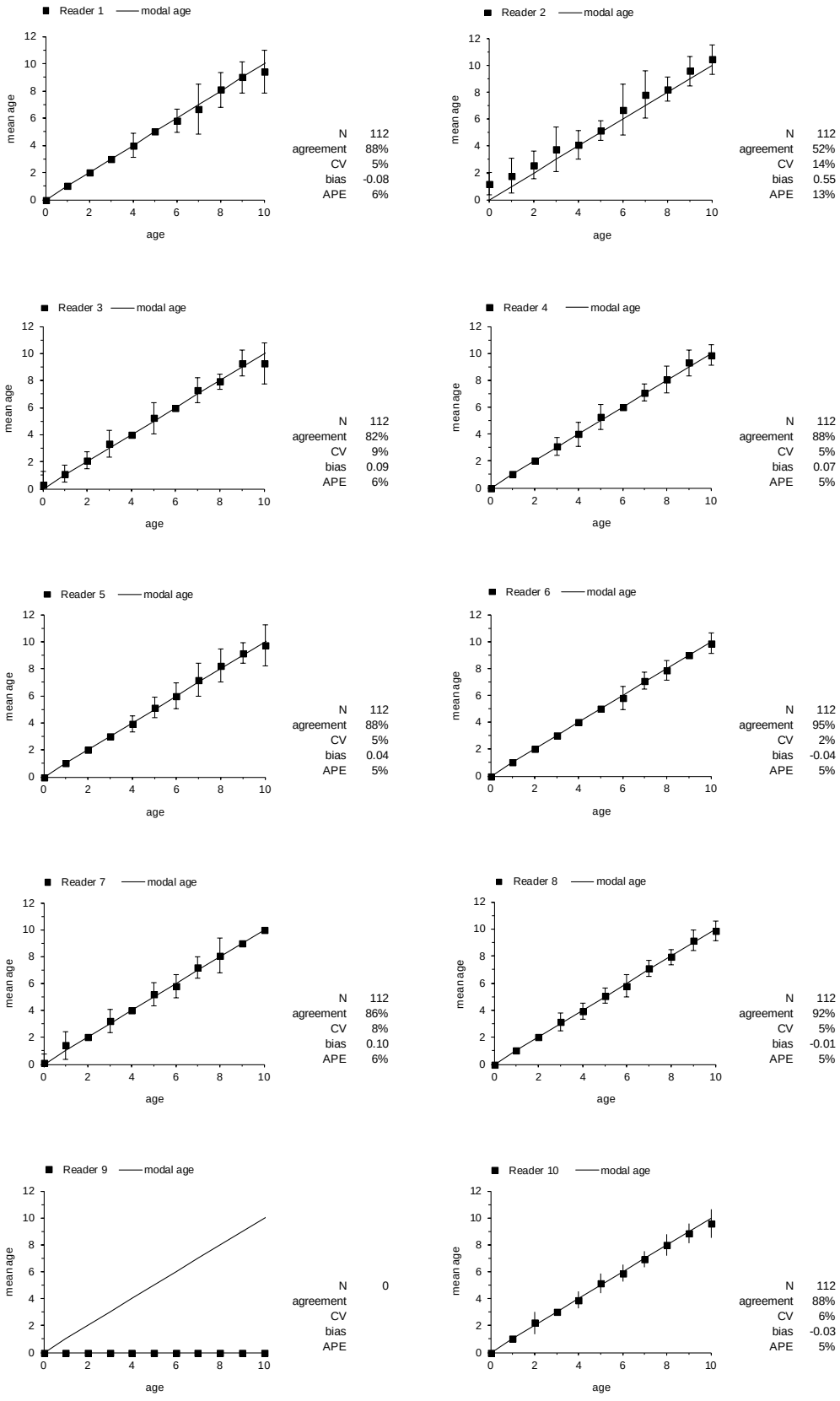
PLE exchange 2009-2010: ICES area IV, sectioned otoliths, modal age ≤ 10



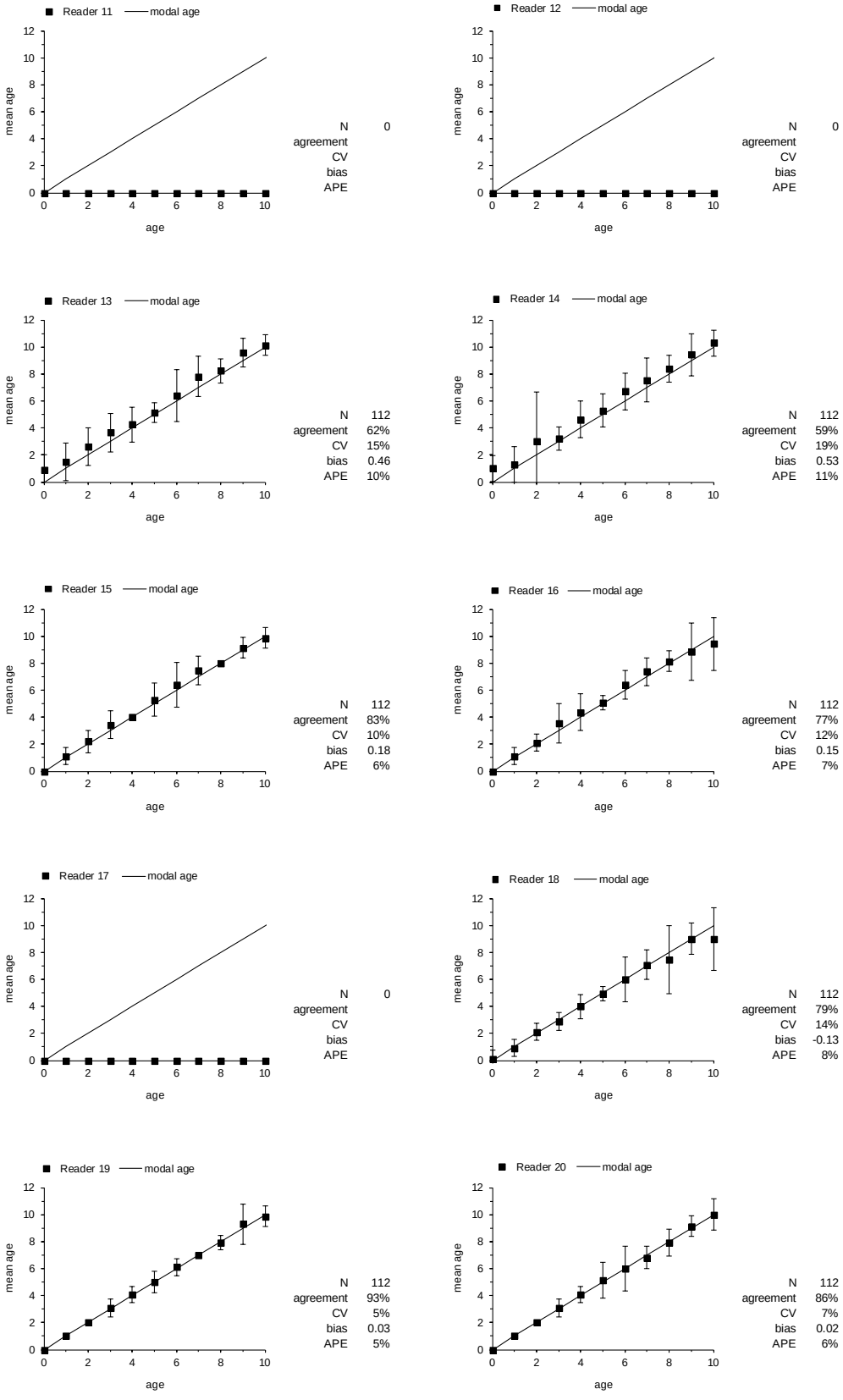
PLE exchange 2009-2010: ICES area IV, sectioned otoliths, modal age ≤ 10



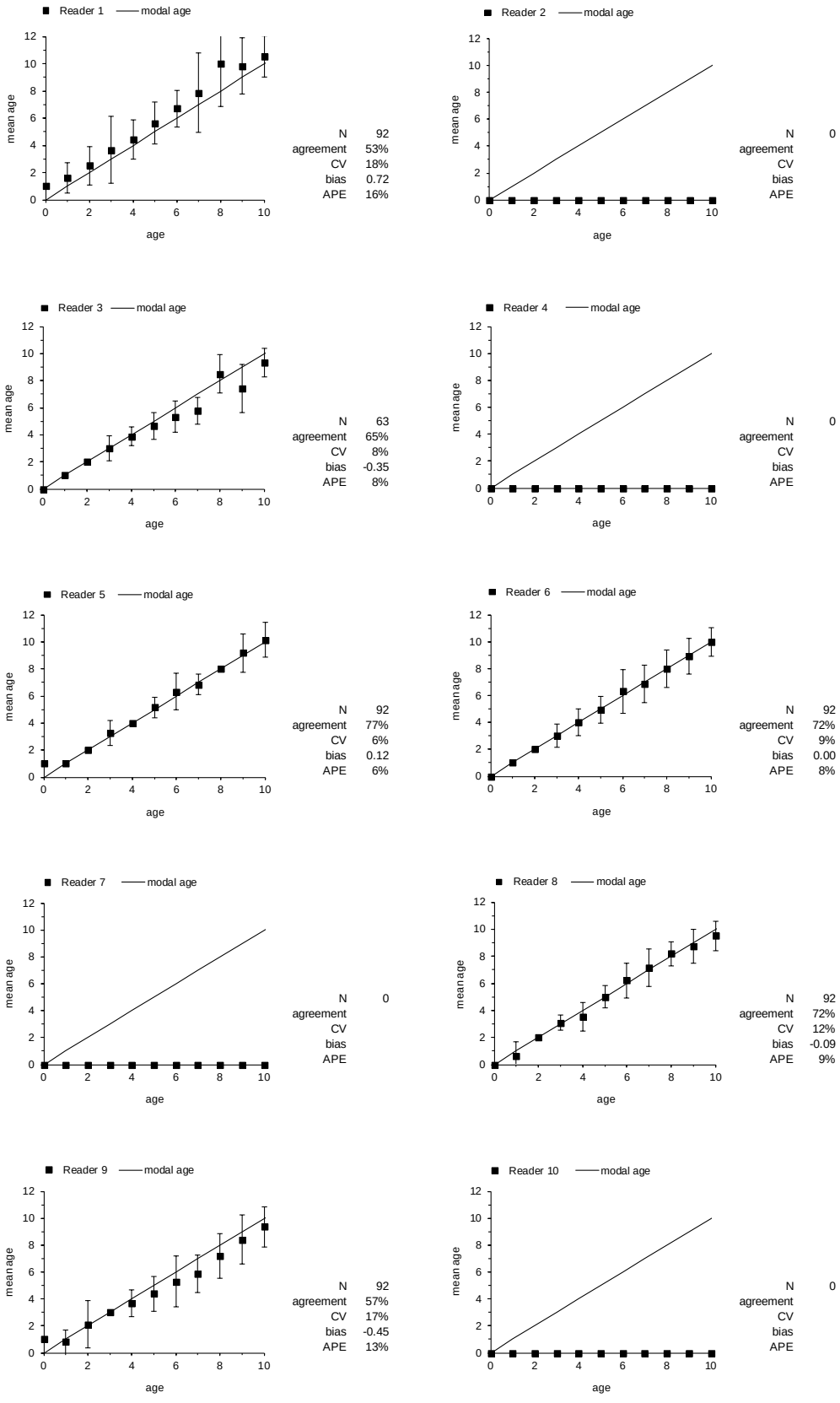
PLE exchange 2009-2010: ICES area IV, whole otoliths, modal age ≤ 10



PLE exchange 2009-2010: ICES area IV, whole otoliths, modal age ≤ 10



PLE exchange 2009-2010: ICES area III, sectioned otoliths, modal age ≤ 10



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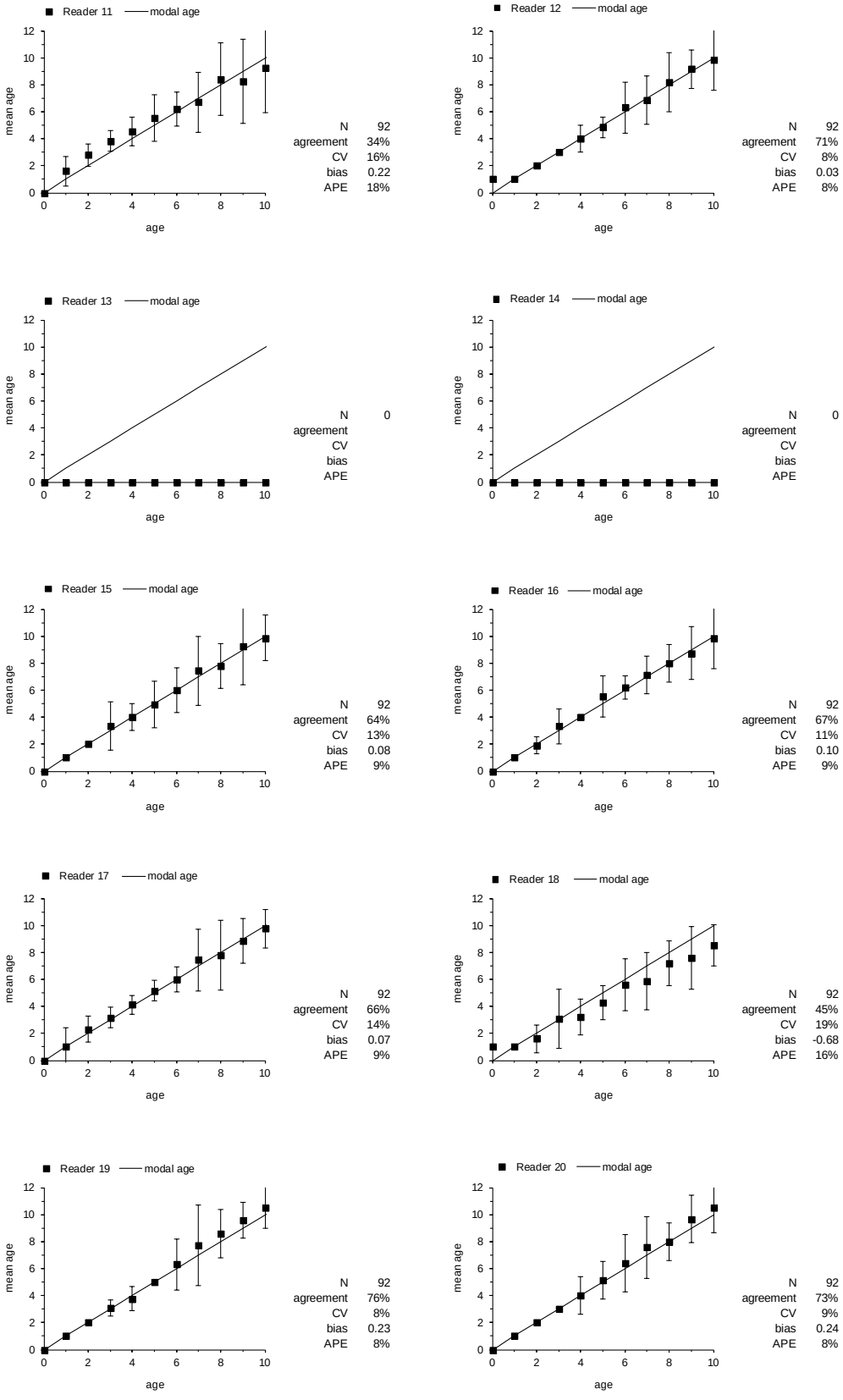
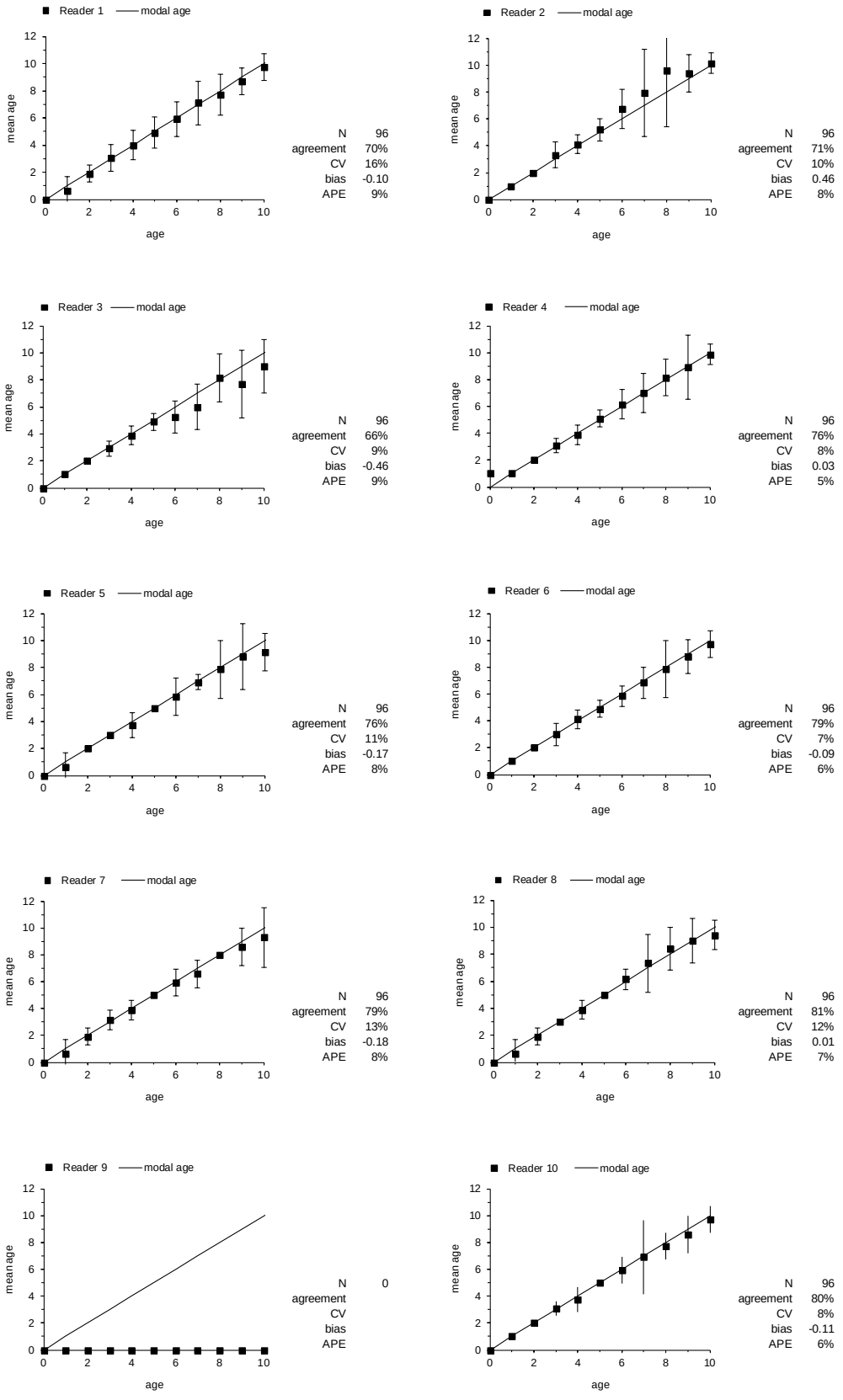


PLE exchange 2009-2010: ICES area IIIa, whole otoliths, modal age ≤ 10



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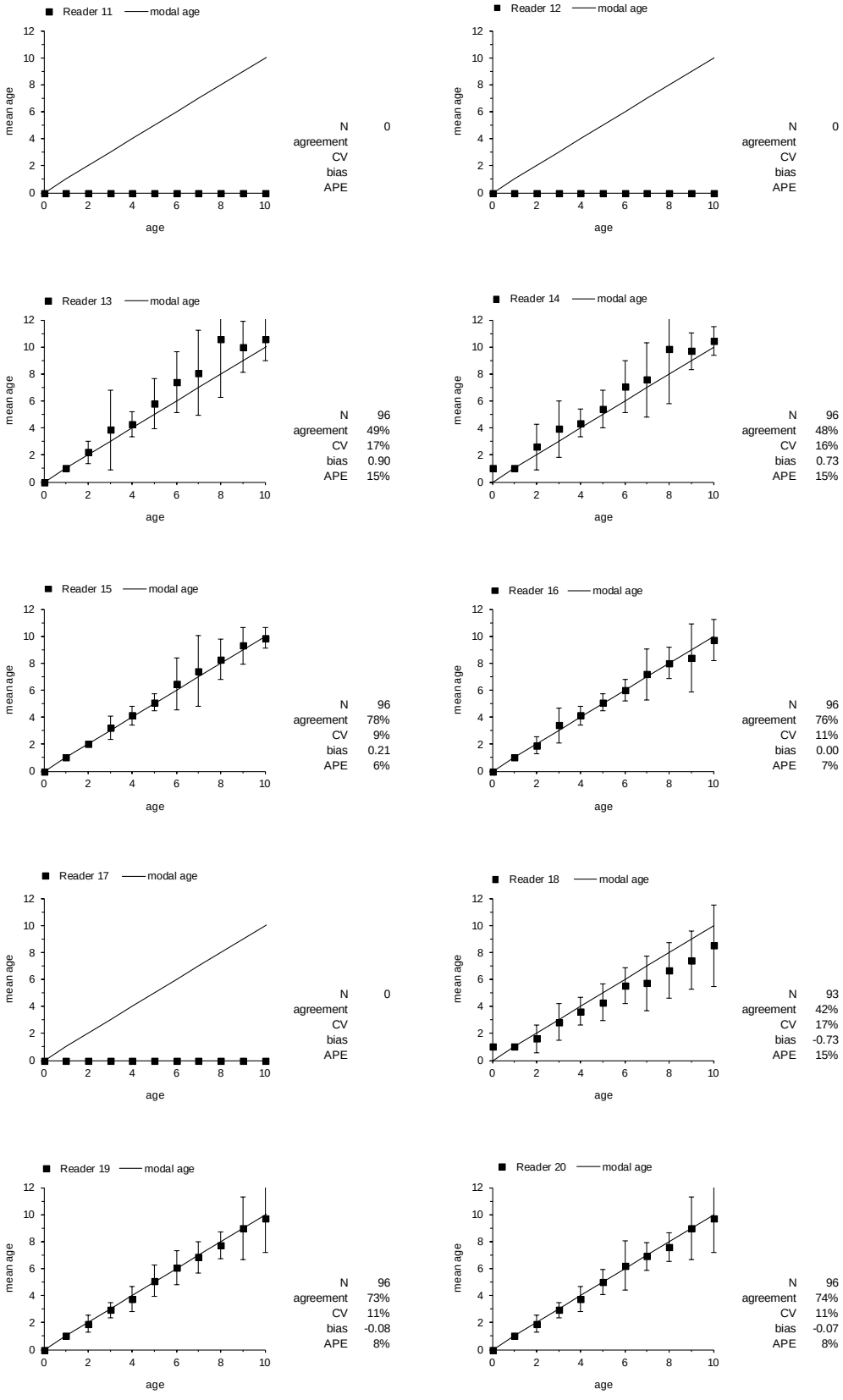
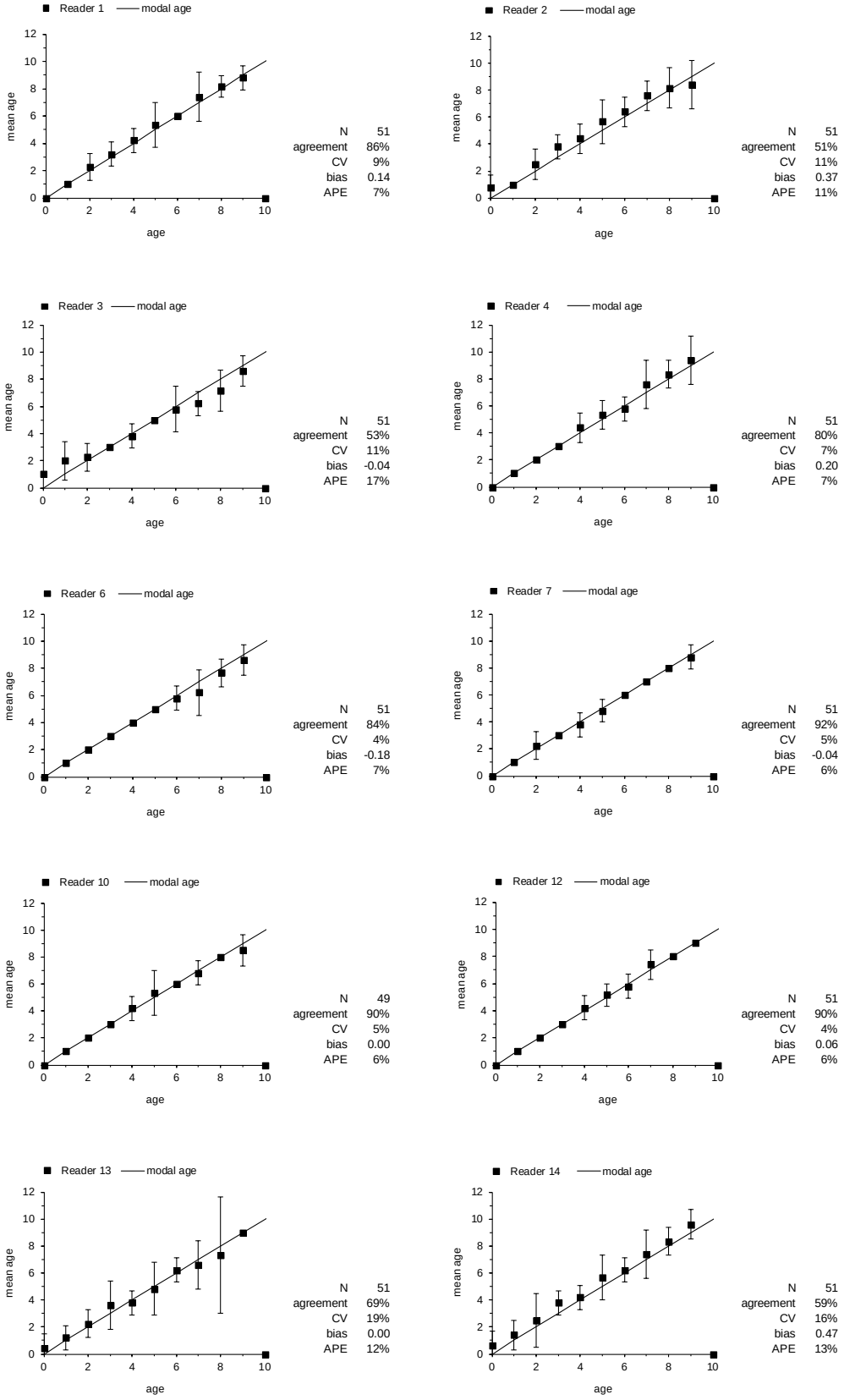
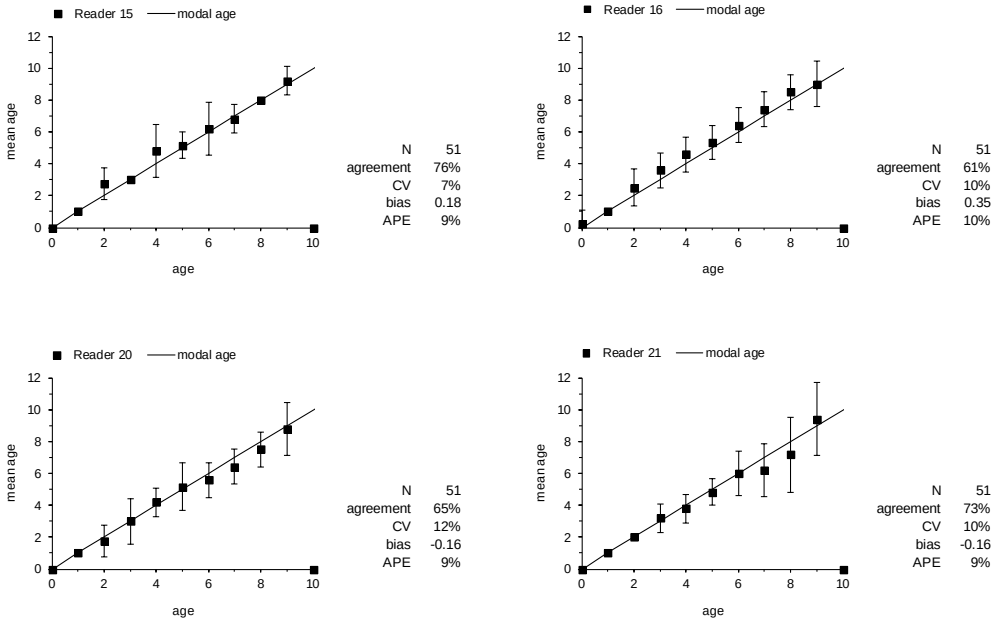


Annex 5: Workshop – results individual readers

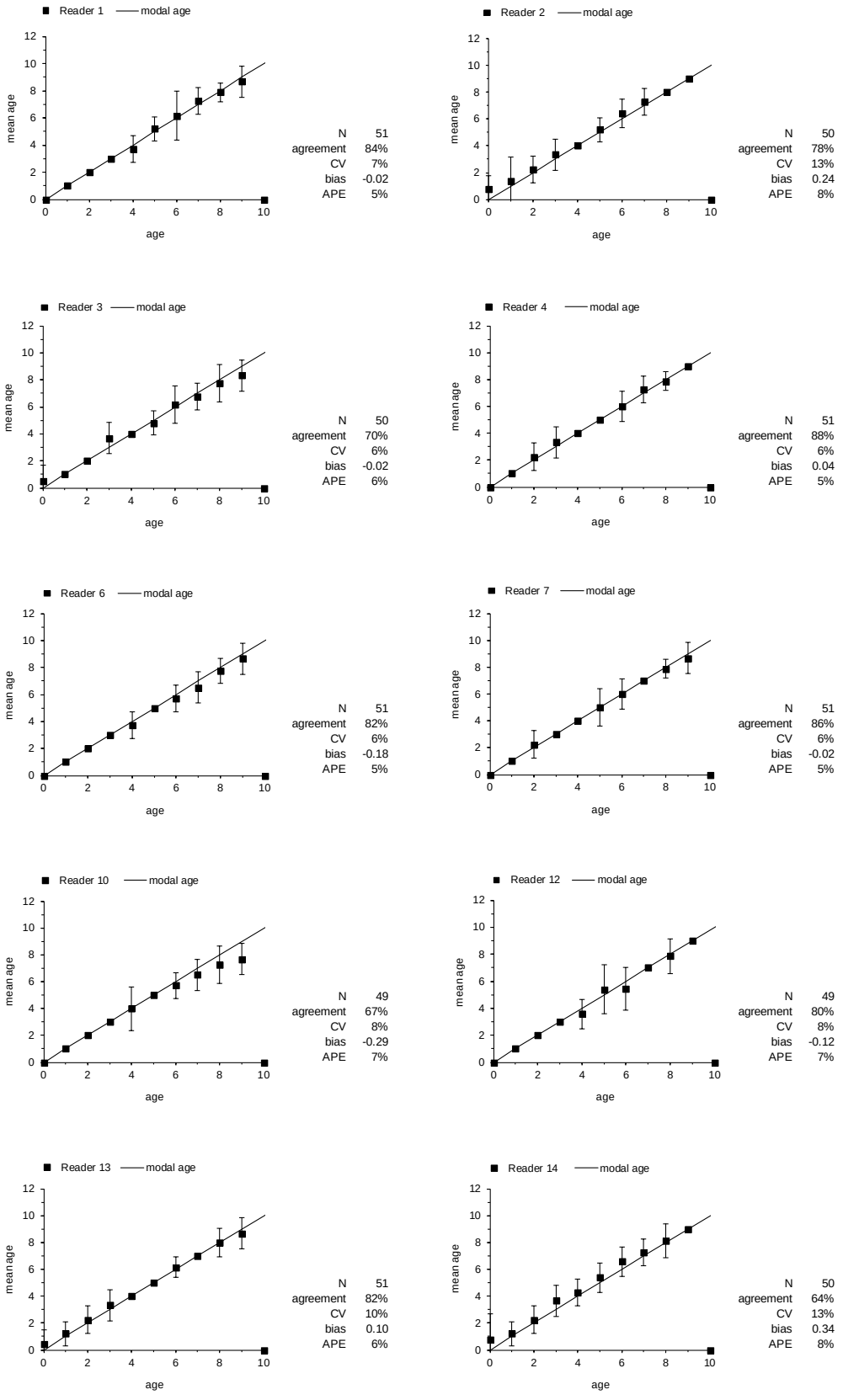
PLE workshop: ICES area IV, sectioned otoliths, modal age ≤ 10



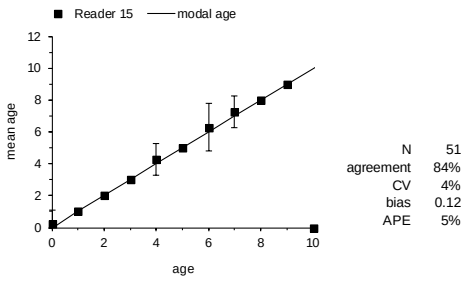
PLE workshop: ICES area IV, sectioned otoliths, modal age ≤ 10



PLE workshop: ICES area IV, whole otoliths, modal age ≤ 10



PLE workshop: ICES area IV, whole otoliths, modal age ≤ 10



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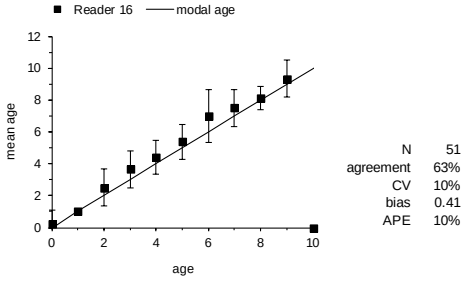
bias	0.12
APE	5%



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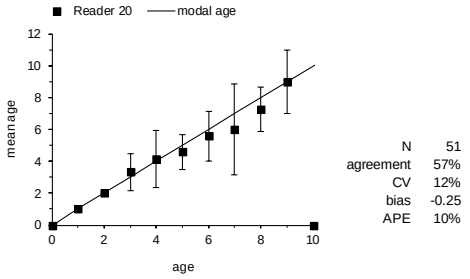

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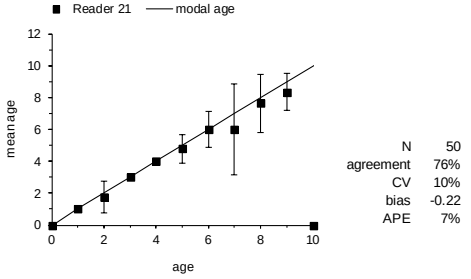
APE	10%



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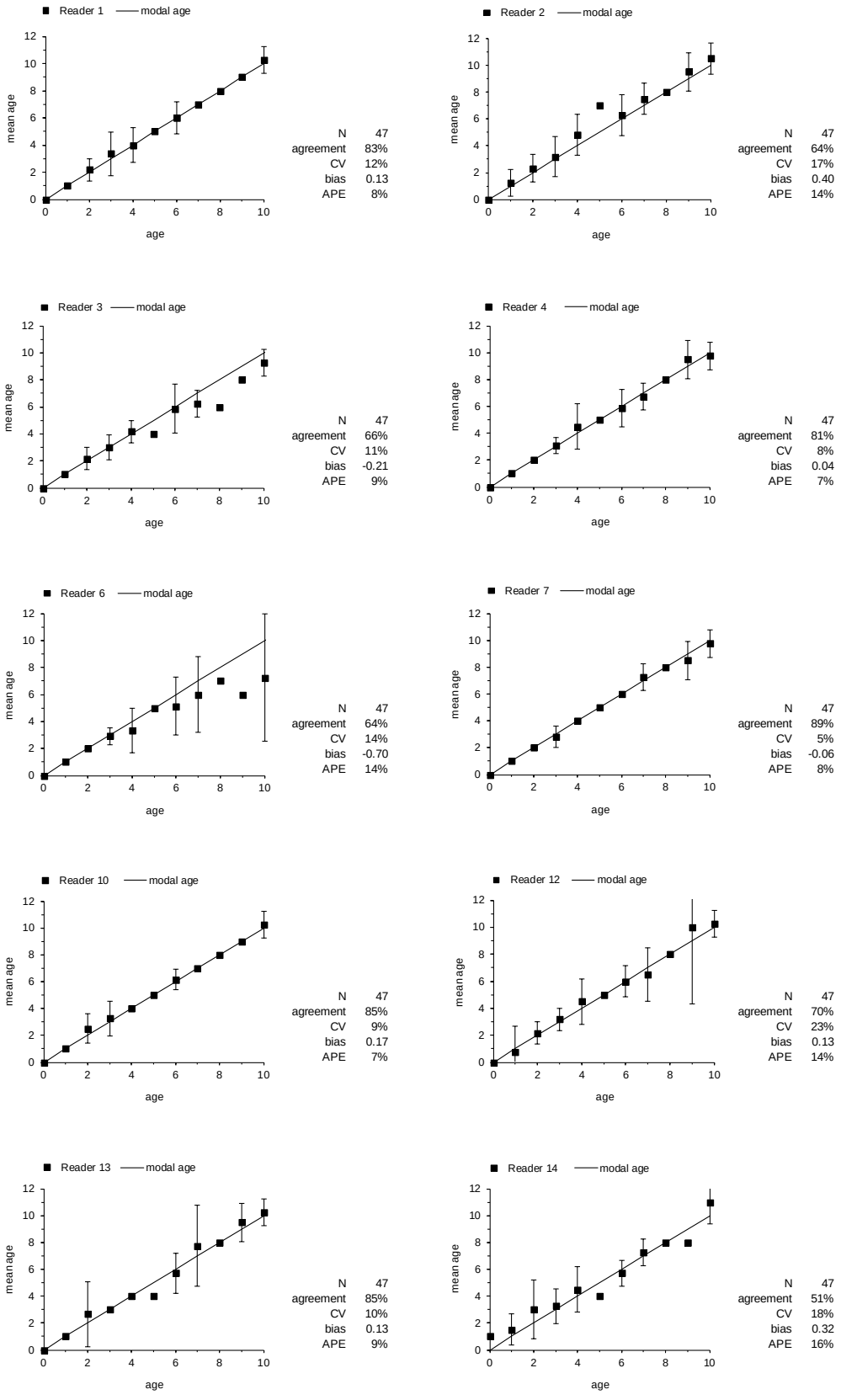
APE	7%

PLE workshop: ICES area III, sectioned otoliths, modal age ≤ 10



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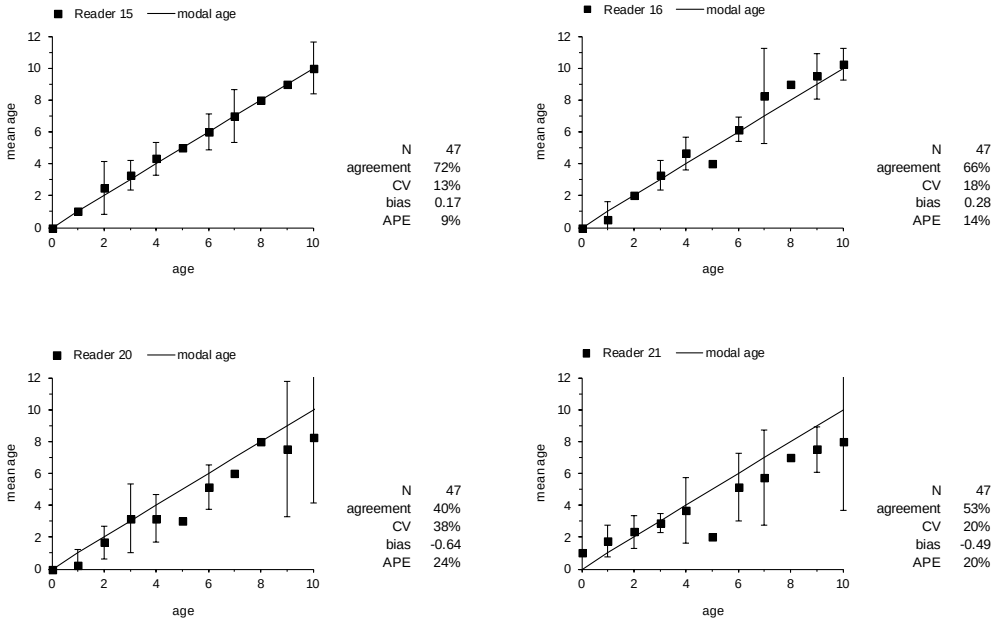

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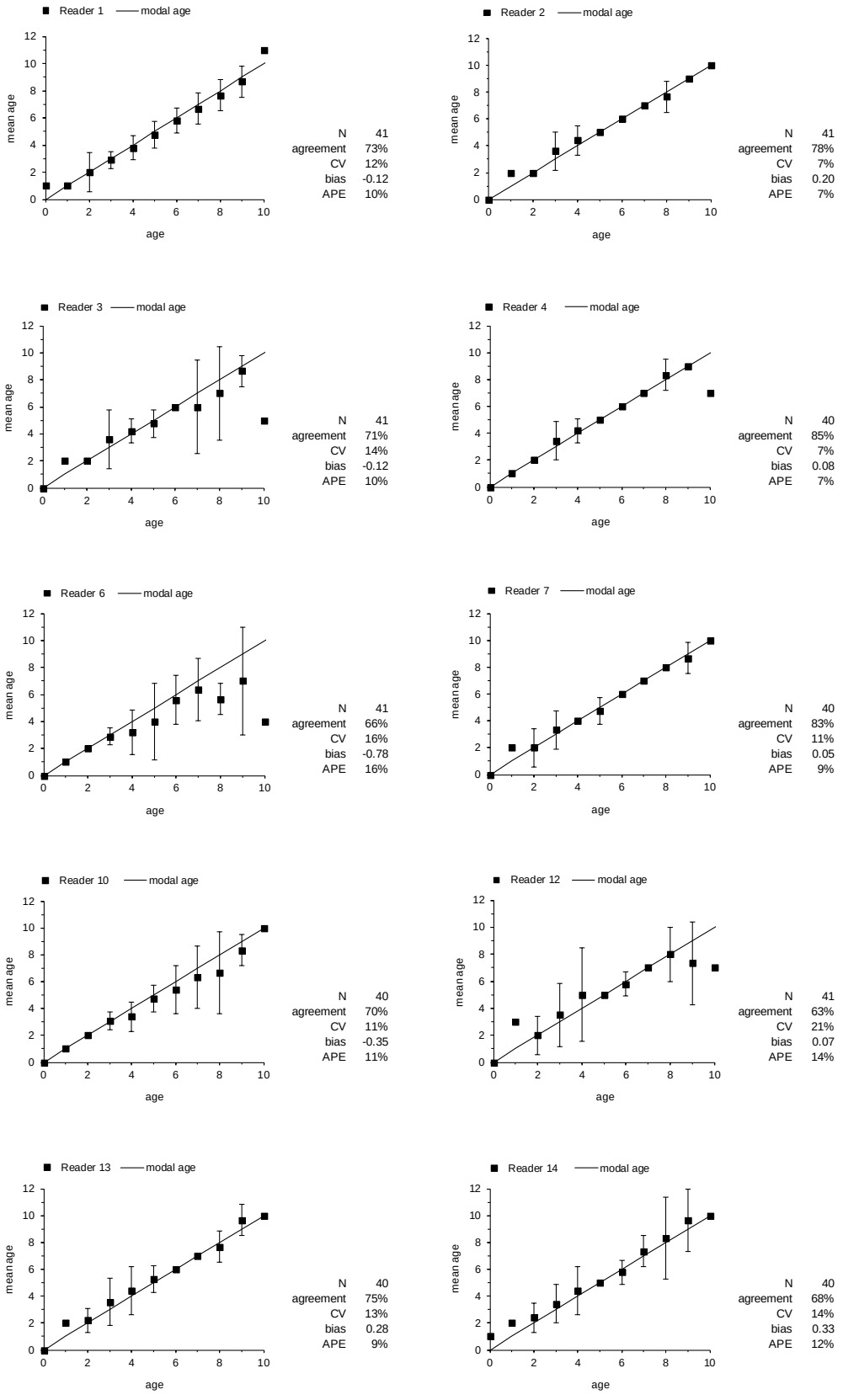
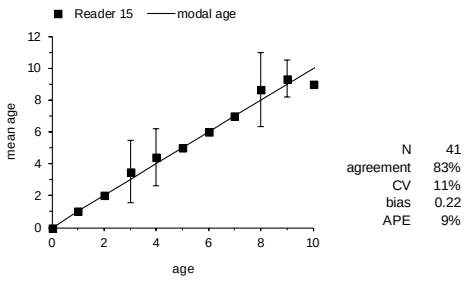


PLE workshop: ICES area III, whole otoliths, modal age ≤ 10



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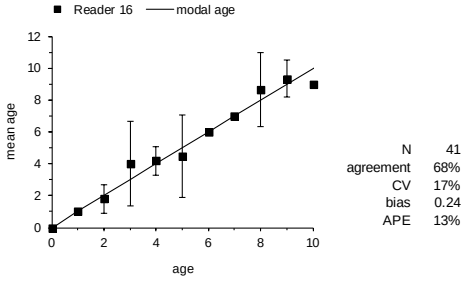
APE	9%



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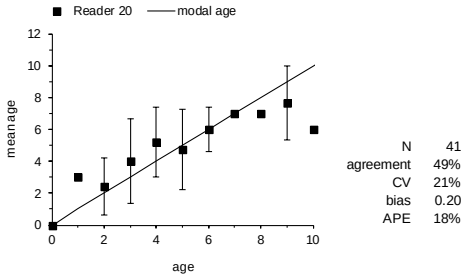
APE	13%



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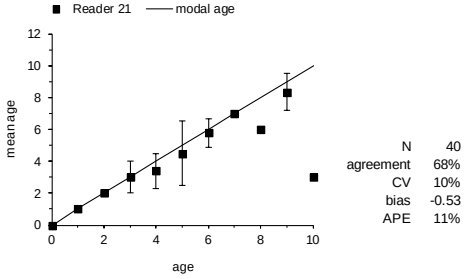
bias	0.20
APE	18%



N	40
agreement	68%
CV	10%
bias	-0.53
APE	11%

Annex 7: Recommendations

The recommendations summarised in this Annex are described in more detail in section 9 of the report.

RECOMMENDATION	FOR FOLLOW UP BY:
1. WebGR training session(s)	PGCCDBS
2. Regular workshops (3-5 year interval) for each species	PGCCDBS
3. Combine stocks in workshops and (full) exchanges	PGCCDBS
4. Next plaice exchange in 2012 and next workshop in 2013	PGCCDBS
5. Implementation of WKARP ageing manual	All plaice reading laboratories
6. Maintenance and further development of ageing manual	WKARP 2013 group
7. Investigate possibilities for validation studies	WKARP 2010 group
8. Compile an otolith collection for the exchange and the reference collection	WKARP 2010 group
9. Compile an old otolith collection for the evaluation of preparation methods	WKARP 2010 group
10. Age reading and image annotations of above otolith collections (= 2012 exchange)	WKARP 2013 group