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Report of the Workshop on Age Estimation of Atlantic Mackerel (*scomber scombrus*) (WKARMAC2)

22–26 October 2018

San Sebastian, Spain



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Executive summary

A Workshop on Age Reading of Atlantic mackerel (*Scomber scombrus*) otoliths were recommended by WGBIOP 2015 to be carried out in 2016-2017. Due to the difficulty of finding a chair time passed and the workshop was recommended again by WGBIOP 2017 to be carried out in 2018. This workshop (chaired by Jens Ulleweit, Germany, and Rosario Navarro, Spain), was held in San Sebastian (Spain) on 22–26 October 2018. 12 European countries took part in this workshop (Portugal, Spain, The Netherlands, Germany, Denmark, Norway, UK, Ireland, Faroe Islands, Iceland, Greenland and Greece), with a total of 23 participants from 14 laboratories. Most ICES Divisions of Northeast Atlantic mackerel distribution were analysed, as well as a bit of the Mediterranean Sea.

The workshop achieved quite a lot in terms of ironing out, through on-screen discussion of difficult and/or old otoliths and calibration, some of the differences in age interpretation between readers. Last workshop (WKARMAC 2010) ageing guidelines were revised and the modifications agreed between the participants. The participants agreed to employ the revised ageing guidelines in their age estimations.

The overall result of the workshop exercise shows an improvement in the agreement between readers (66.8% agreement, 31.4% CV), and especially Expert readers (73.2% agreement, 16.4% CV), regarding the exercise carried out before the workshop, which shows the usefulness of the on-screen discussion of difficult otoliths previous to the workshop exercise. However, the agreement between readers for otoliths with older ages (from age 6) continues to be very low (40-58% all readers; 53-71% Experts).

Both exchanges, the one previous to the workshop and the one carried out during the workshop, were performed via SmartDots, the web application developed by ICES to facilitate the setup of Exchanges, Workshops and Training. As this is a new application, for most readers this was the first time using the program but once all readers became familiar with the use of the tool it proved to be very useful. The exclusive use of images has the disadvantage that the readers find more difficult to identify the nature of the otolith edge, which in some cases, can make the age interpretation more difficult. In addition, the use of a standardized reading line for all readers in each otolith image, even though it makes the comparison between readings easier, sometimes this complicate marking the annuli on the otolith when there are better growth rings observed in another area of the otolith. However, the use of images allows a better comparison between the readers' estimations and a better identification of the problems in locating false rings, as well as to speed up the process. The use of SmartDots was especially useful for a posterior discussion on screen of the most significant otoliths during the workshop.

In addition, a Small exchange with Norwegian otoliths from tag-recaptured experiments was carried out during the workshop with the results being discussed after completion. Images of these otoliths were also discussed during the workshop, which proved to be very interesting due to the importance of these otoliths of known age.

An update of the known changes of mackerel biology was presented during the workshop, as well as a study of the seasonal formation of growth rings in mackerel otoliths from ICES Div. 8c and 9a, which showed a temporal delay in opaque-zone formation with age.

An image collection of agreed age otoliths will be found in the workshop ICES SharePoint and the Age Forum site. Such otolith collection includes the otoliths with > 80% agreement between Expert readers from the WKARMAC2 calibration exercise. In addition, the images of the otoliths from the Small exchange with Norwegian otoliths from the tag-recapture experiments will also be included in the reference otolith collection.

1 Terms of reference

A Workshop on Age Estimation of Atlantic Mackerel (*scomber scombrus*) (WKARMAC2), chaired by Jens Ulleweit, Germany and Maria Rosario Navarro, Spain, was established and taken place on 22– 26 October 2018 in San Sebastian, Spain to:

- a) Review information and results on age estimations and recent otolith exchanges, follow up on the previous workshop in 2010 (WKARMAC) and validate the work done so far.
- b) Summarize the ageing protocols currently in use and improve them where possible.
- c) Address the low agreement between age readers of this species, particularly in fish over the age of 6 years, with group exercises and reading sample sets.
- d) Create a reference collection of agreed age otoliths.
- e) Address the generic ToRs adopted for workshops on age calibration (see 'WGBIOP guidelines for workshops on age calibration').

WKARMAC2 will report by 8 December 2018 for attention to ACOM, SCICOM and WGBIOP.

2 Agenda and participation

The workshop agenda is presented in Annex 1.

A total of 23 participants (Figure 2.1) attended the present Workshop from 14 laboratories of 12 European countries. The list of participants is presented in Annex 2.



Figure 2.1. WKARMAC2 participants, from left to right at the back: Vasiliki Papantoniou, Charo Navarro, Poul Vestergaard, Selene Hoey, Mererte Kvalsund, Jens Ulleweit, Ørjan Sørensen, Jens Arni Thomassen, Camilla Wentzel, Maria Jarnum, Andreia Silva, Tim Huijer, Eilert Hermansen, André Dijkman, Athanasios Spetsiotis; from left to right in front: Iñaki Rico, Naiara Serrano, Gudrun Finnbogadóttir, Gitta Hemken, Gertrud Delfs, Michelle Inglis, Kate Downes and Deirdre Lynch.

Only 11 WKARMAC2 participants took also part in the Small Scale Otolith Exchange in 2014. Seven of them also attended last workshop (WKARMAC) in 2010. There has been a change of mackerel readers since then. 16 WKARMAC2 participants also performed the otolith exchange carried out a few weeks before the workshop. Two more readers, Jane Mills (Marine Lab, Scotland, UK) and Delfina Morais (IPMA, Portugal) could not attend the workshop but participated in the workshop calibration exercise from their labs.

3 Update on known changes to the biology of mackerel

A summary of the biology of mackerel is given in the last workshop report (ICES WKARMAC 2010). During the 2018 workshop a presentation was given on the biology of mackerel with special emphasis on the changes in distribution in stock sizes during the last decade.

Mackerel is a species of commercial importance and also a game fish. A pelagic, ocean and coastal dwelling species, it has a depth range of 0 – 1000 m. Occurring between 25°N – 70°N and 77°W – 42°E, the mackerel is considered a temperate fish species that inhabits the eastern Atlantic, southwestern Baltic Sea, Mediterranean and Black seas, and the western Atlantic from Labrador to Cape Lookout (North Carolina). Mackerel are abundant in cold and temperate shelf areas, but not found in water colder than 6°C (Nottestad *et al.* 2015). The species forms large schools close to the surface with densities up to 9 fish/m³. They overwinter in deeper waters, but move closer to shore in spring, when water temperatures range between 11 °C and 14 °C (Froese & Pauly, 2018; Muus *et al.*, 1997; Muus and Nielsen, 1999). Mackerel do not have a swimbladder and can therefore quickly change depth without suffering from pressure differences.

Its reported maximum size is 70cm (Navarro *et al.*, 2012), the maximum weight 3-4kg. Picture 3.1 shows a 56 cm mackerel with a weight of 2.1kg and an estimated age of 18 years caught in ICES area 6a. Mackerel mature at around the end of age 3 and at a length of ca. 30 cm.



Picture 3.1: 56cm mackerel caught in ICES area 6a

Mackerel is considered to be a determinate batch spawner meaning that all eggs to be spawned are present as oocytes in the female ovary prior to spawning. Spawning occurs near the surface mostly in a temperature range between 10.5 – 13.5°C (Ibaibariaga *et al.* 2007), the minimum temperature for spawning is 8.5°C (ICES 2018a). Mackerel are producing 1000 – 1400 eggs/g body weight (ICES 2017b) or 200.000–450.000 eggs/female in total. Egg diameter is around 1.2 mm diameter and the eggs have an oil globule. Depending on the water temperature, larvae hatch within 6 days. Both, eggs and larvae are pelagic. Mackerel show a very fast growth within the 1st year reaching 22 cm at the end of the year in which they were born, age group 0 (Villamor *et al.*, 2004a).

Mackerel exhibit diurnal feeding activity. The diet of the adults consists of zooplankton and small fish, juveniles mostly feed on copepods, euphausiids, crustacean larvae, and other zooplankton (Olaso *et al.*, 2005; Cabral *et al.*, 2002). The availability of copepods of the genus *Calanus* seems to be crucial for first-year survival (Jansen 2016). Mackerel itself are preyed upon by sharks (porbeagle shark, spiny dogfish), cod, large pelagics like bluefin tuna and

swordfish as well as whales (harbour porpoise, orca) and seals. Known parasites on mackerel include monogenean, trematodes and nematodes (e.g. *Anisakis simples*) (Abaunza *et al.*, 1995).

Mackerel is a temperate fish species which inhabits the Eastern Atlantic, the SW Baltic Sea, the Mediterranean and Black Sea as well as the Western Atlantic from Labrador to Cape Lookout. ICES currently uses the term “Northeast Atlantic mackerel” to define the mackerel present in the area extending from ICES Division 9a in the south to Division 2a in the north, including mackerel in the North Sea and Division 3a. Within this area mackerel is divided into three spawning components according to the main spawning areas:

- Western spawning component (ICES areas 6, 7, 5, 8abde)
- Southern spawning component (ICES areas 8c, 9a)
- North Sea component (ICES areas 4, 3a)

Post-spawning, mackerel migrate from their spawning to feeding grounds and a prespawning migration with halts (=overwintering). Juveniles do not migrate as fast as adults (Uriarte *et al.* 2001). Larger and older fish reach furthest to the North and West during the feeding migrations in summer.

Recent studies show for the North Sea spawning component, which was highly abundant in the 1950s/60s but diminished in the 1970s, that these shifts might also be due to decreasing temperatures in the North Sea and not only related to overfishing (Jansen 2013).

For the western and southern spawning component, spawning commences in February off the Iberian Peninsula and continues in the Bay of Biscay, the Celtic Sea, Porcupine Bank and West Scotland. Spawning continues until July when it ends in the northern areas. However, these migrations patterns are not static but changing through time. This applies to times of peak spawning within the year as well as with regards to the area of spawning, feeding and overwintering. (ICES 2017a,b, ICES 2018a,b).

In recent times, spawning has dramatically increased northwest of Scotland and is widely spread into the open ocean, westerly off Rockall. Parts of the mackerel stock are also following new post-spawning and feeding migrations (paths along the Southern coast of Iceland to Greenlandic waters). The feeding grounds in the Nordic Sea are far more widely spread into Western and Northern regions than a decade ago. All these changes are discussed in relation to temperature changes (Bruge *et al.* 2016, Berge *et al.* 2015, Brunel *et al.* 2017), prey availability (Pacariz *et al.* 2016, Berge *et al.* 2015, Brunel *et al.* 2017) and stock size meaning density-dependent mechanism (Pacariz *et al.* 2016). Additional information on mackerel distribution shifts can be found in the report of the ICES workshop on Fish Distribution Shifts (ICES 2017a). In Aegean Sea, spawning season begins at the end of Spring-early summer.

These changes in migration patterns are mirrored by the development of the commercial catches over time. Since 2007, the importance of the traditional fishing grounds has declined and an expansion of mackerel fisheries in Faroese, Icelandic and Greenlandic waters has taken place. However, the spawning-stock biomass of mackerel which has increased in the late 2000s to a maximum in 2011 is presently decreasing. The stock is estimated to be below the biological reference point $MSY B_{trigger}$ in 2018, for the first time since 2007 (ICES 2018b).

4 Review information on age estimations, otolith exchanges, workshops and validation work (ToR a)

The frequency of workshops and exchanges on age reading of mackerel in the past is far from impressive. The first reported workshop on mackerel ageing was held in Lowestoft in 1987 and following that only two workshops has been held (in 1995 in Spain and 2010 in Lowestoft). Exchanges were carried out in 2002, 2008 and 2014 respectively. All previous workshops and exchanges have had an outcome stating the overall agreement to be somewhat low but fair, but also skewed towards having a higher agreement on the younger ages. All workshops discussed and made an effort to standardize age reading methods by preparing a manual and a reference collection of agreed age otoliths.

4.1 Exchanges and Workshops

The first exchange and workshop held in 1986 and 1987 respectively, had as the first priority to assess the agreement level on the older mackerel, as the assessment working group on mackerel at the time wanted to review the applied plus-group (11+). The participants read through two collections of otoliths, one consisting of otoliths covering all age groups and one holding particularly older individuals for comparison.

The workshop had access to a small number of known-age otoliths which proved very valuable in ironing out discrepancies in the interpretation of the appearance of the edge (opaque/translucent) and timing of the age-structures. This appeared to be area specific within the same season.

The overall agreement was calculated using a different method than what has been used in later workshops; however, the agreement percentage was in the better range (0.3 in a range of 0.0 being perfect agreement and 0.83 being total disagreement). Of particular interest was that the agreement on the set of otoliths comprised of older individuals did not differ significantly from the agreement on the 'normal' set of otoliths. The conclusion of the workshop was thus that the age estimation of older individuals was not associated with a higher variation between readers than age reading of younger individuals and the workshop concluded that the plus-group in the assessment could be expanded to be 15+.

The second workshop, held a decade later, in 1995 (ICES 1995) had as objectives to evaluate a preceding exchange (Villamor and Meixide, 1995), discuss and standardize age reading methods by preparing a manual and a reference collection and give advice on which age groups valid age reading could be achieved. The participants worked with extensive material, no less than 6 sets of otoliths were read prior to and during the workshop, differing in various ways concerning the area and age-range of the otolith set.

Similar to the workshop in 1987, the readers had access to a number of known-age otoliths from a Norwegian tag-release program, and again this set of otoliths proved very valuable in discussions and aided in the creation of age reading criteria for mackerel.

The readers participating in the workshop reached an overall agreement of around 70%, depending upon the sample. The sampling area significantly influenced the degree of agreement and contrary to the findings in the workshop in 1987; the older fish had a tendency to be underestimated compared to modal age. For the known-age set; the agreement was 76%.

In conclusion; the age-reading technique was validated up to age 8 (as bias was observed in the ages of older fish). The workshop recommended the plus-group for the assessment of mackerel to be 12+. The workshop concluded that an appropriate measure of precision would be 2.00 for 2stddev from the modal and assigned age.

A small-scale exchange of mackerel otoliths was completed in 2002. The objectives of the exchange were to monitor the precision of age readings, following the protocols established by EFAN (FAIR concerted action PL96/1304), and to collate a reference database of otolith images from the exchange material. Only 6 institutes participated in the exchange and thus the scale was somewhat smaller than other exchanges.

The conclusion from the exchange was that the precision drops significantly after age 4, and although two institutes did show an improvement in precision from the previous workshop in 1995, the remaining readers showed little improvement. The project concluded that further exchanges and workshops on mackerel were highly warranted.

After the exchange of 2002, veteran readers departed and new readers were recruited. A new exchange in 2008 was carried out to ensure consensus between these new readers. Overall agreement was 67.6%, overall CV was 23.8% and relative bias in age determination by individual experts ranged between -0.59 and +0.45. There were 12 otoliths with complete agreement on age between the 15 experts. These otoliths tended to be among the youngest individuals. The large discrepancies between age determinations highlighted the need to hold a workshop.

This workshop was held in 2010 (ICES 2010). The objectives were to assess the level of agreement between readers and labs, to analyse the differences in age reading interpretation of otolith spatial patterns, to explore the usage of metric measurements of otolith structures as a solution to minimize divergence in age estimation, and for the first time to test image-based reading and OMAP as a new tool for aging workshops. 100 otolith images (ICES div. 4 and 7), sampled by IMARES and photographed by DTU-AQUA were used. The readers aged the otoliths by marking winter rings on the digital images viewed in OMAP v.1.3 (Jansen, 2010 871/id). The exercise was run twice, one a few days before the workshop (part I) and one during the workshop (part II). Overall agreement was low (25%), substantially lower than the exchange of 2008 (67.6%). This may be due to several factors, the most important being that this study did not take into account neither the experience level, nor continuity of the participants in mackerel age estimation. Also, the rather low % agreement could be a result of poor image quality and the unfamiliarity, by the majority of readers, to age mackerel otoliths exclusively using images.

The poor level of agreement between readers obtained in the exchange carried out during the workshop and the need to put into practice the new set of age determination criteria that were established at the workshop highlighted the need for another exchange post-workshop. The exchange had a short timescale and was completed shortly after the workshop (December 2010). A total of 11 institutes took part in the exchange. The software used to analyse the results was the ORACLE (Otolith Reading Age Comparisons) spreadsheet, developed by Cefas from the Eting *et al* (2000) "Age Comparison Worksheet". 248 otolith images from 5 ICES divisions (2a, 5b, 4, 7f and 8) were used in the exchange. The results were better than the pre-workshop exchange. Overall agreement was 78.1%. Agreement rates with the modal age ranged from 71.7% to 85.1%. The bias scores showed a range from -0.30 to +0.22, with an overall bias of +0.01.

The last exchange between European mackerel otolith reading institutes took place in 2014 (Ulleweit, 2014). A set of 164 images of mackerel otoliths were selected and uploaded for analysing using the WebGR application. 16 mackerel age readers participated in the exchange.

Overall agreement was 68.2%. Good agreements were reached for age 1 and 2 (93 and 92%, resp.), for age 3 and 4 agreements were between 74 and 76%, agreement for age 5 is 61% and for age 6 and 7 57%. Only very low agreement was found for the older ages 8 to 14 (between 47% for age 8 and 31% for age 13).

Two additional analyses were performed: Analysis only done with the expert group showed a higher overall agreement of 75.5%, analysis referring to experts and intermediate (14 readers)

showed an overall agreement of 70.4%, still slightly higher than the agreement between all readers.

Overall conclusion was that there was certainly room for improvement both in terms of consistency and agreement between readers. More effort needed to put into the age determination for older mackerel.

4.2 Validation work

The existing material of such work is rather limited, particularly related to the actual yearly age structures of mackerel otoliths.

Captive rearing

This method validates both absolute age and periodicity of growth structures (Campana, 2001). The deposition of daily growth rings in larvae, post-larvae and juveniles of mackerel was validated by Migoya (1989) and D'Amouset *et al.* (1990) in several areas in Northwest Atlantic, and by Mendiola and Álvarez (2008) in Northeast Atlantic. Migoya (1989) and Mendiola and Alvarez (2008) incubated mackerel eggs in the laboratory and showed that the deposit of the first increment in the otolith occurred on the hatching day and that the increments were formed daily. In addition, D'Amours *et al.* (1990) performed a validation experiment on mackerel juveniles in captivity, marking their otoliths with a fluorescent substance and showing that the increments were deposited on a daily basis. These studies give the potential for validating the first years of growth, making standards (L1, etc.) and ruling out double structures in the first years of life. Knowing that the microstructure is daily, it may be possible through analysis of the combined transparency and width of the daily rings on the edge of juveniles over the season to validate the formation of the first and potentially following 2-3 age structures.

Marginal Increment Analysis

The Marginal Increment Analysis (MIA) is the most commonly used of the validation methods, and it is used for validating the periodicity of growth increment formation (Campana, 2001). Two types of studies are possible, one that uses quality data and other that uses quantitative data (Panfili *et al.*, 2002). Only a qualitative analysis in mackerel otoliths has been performed by Gordo and Martins (1982) off the Portugal Coast. During the workshop a study of the seasonal formation of growth rings in otoliths of NEA mackerel in ICES Div. 8c and 9a (Villamor *et al.*, presentation to WKARMAC2 2018) was presented (see section 6).

Mark-Recapture experiments

The Norwegian Mark-Recaptured experiments have provided otoliths which are potentially the golden stones and could iron out many subjective assumptions related to the age estimation of mackerel from this area (and potentially other areas). During the current workshop otoliths from the Norwegian Mark-Recapture experiments were available and used for an exercise. These otoliths are from mackerel which were tagged between 20–28cm and recaptured after a known number of years. Results of the exercise are given in section 5 of this report.

5 Resolve interpretation differences between readers and laboratories; most recent exchange and workshop exercise (ToR b)

5.1 Pre-workshop exercise

An otolith exchange was carried out a few weeks before the workshop took place. As the last exchange was performed four years before (2014), a number of otolith readers had been replaced by new ones and this exchange would provide more accurate information about the level of agreement of current readers before the workshop. Also, this exchange would provide otolith images with the participants' readings to be discussed during the workshop as the program used in last exchange, WebGR, is no longer available, not even for the otolith reading discussion.

The exchange was carried out via SmartDots (<http://www.ices.dk/marine-data/tools/Pages/smarddots.aspx>), the web application developed by ICES to facilitate the setup of Exchanges, Workshops and Training events. A total of 135 otolith images from the main areas of mackerel distribution were included in the exchange. Following the recommendations of WKMACQI (Workshop on Mackerel biological parameter Quality Indicators) (ICES 2018c), it was attempted that the spatial and temporal coverage, as well as the length and age range, of the mackerel otoliths of the exchange corresponded with the coverage in the assessment, (ICES Div. 2a, 4bc, 5ab, 7bjd, 8bc, 9a, 14b). Otolith images from areas 2b, 4a, 6a and 6b were also requested to the laboratories that work with otoliths of these areas but were not provided on time.

18 participants from 10 countries (11 laboratories) participated in the exchange. They were ranked as Experts and Trainees considering the years of experience estimating the age of Atlantic mackerel. Expert readers were considered those participants with more than four years of experience. Moreover, Expert readers coincided with the readers involved in mackerel assessment in their countries (Table 5.1.1).

Table 5.1.1. Participants of pre-WKARMAC2 exercise.

Reader No	Name	Laboratory	Country	Reading level
R1	Eilert Hermansen	IMR	Norway	Expert
R2	Iñaki Rico	AZTI	Spain	Expert
R3	Deirdre Lynch	Marine Institute	Ireland	Expert
R4	Charo Navarro	IEO	Spain	Expert
R5	Gertrude Delfs	Thünen-Institut	Germany	Expert
R6	Gudrun Finnbogadóttir	MFRI	Iceland	Expert
R7	Maria Jarnum	DTU Aqua	Denmark	Expert
R8	Naiara Serrano	AZTI	Spain	Expert
R9	Orjen Sorensen	IMR	Norway	Expert
R10	Merete Kvalsund	IMR	Norway	Expert
R11	Delfina Morais	IPMA	Portugal	Expert
R12	Athanasios Spetsiotis	FRI	Greece	Trainee
R13	Kate Downes	CEFAS	UK	Trainee
R14	Clara Dueñas	IEO	Spain	Trainee
R15	Gitta Hemken	Thünen-Institut	Germany	Trainee
R16	Selene Hoey	Marine Institute	Ireland	Trainee
R17	Tim Huijjer	WMR	The Netherlands	Trainee
R18	Andreia Silva	IPMA	Portugal	Trainee

Age readings results were analysed using the GussEltink spreadsheet (Eltink, 2000). Although SmartDots application can generate an automatic analysis of the results, due to the limited time

available to obtain the results before the workshop and that the application still has some limitations when selecting the options of the analysis, it was decided to use the Eltink spreadsheet for the analysis instead.

In addition to estimating the age of the otoliths included in the exchange, readers were asked to assign the quality to each reading according to the “3 points grading system” (AQ1, AQ2, AQ3) recommended by WKNARC (ICES, 2011). Readings with AQ3 were not taken into account in the analyses. Analyses were performed for the total of areas and all readers and Expert and Trainee readers separately. Additional analyses were performed by each of the four areas of mackerel distribution: Southern component (ICES div. 9a, 8c), Western component (ICES div. 8b, 7bjd), North Sea component (ICES div. 4bc) and Northern distribution (ICES div. 2a, 5ab, 14b). A summary with the overall agreement, CV and bias of all analysis are shown in Table 5.1.2.

Table 5.1.2. Summary of % agreement, CV and bias obtained in the analysis of Atlantic mackerel readings of pre-WKARMAC2 exercise.

Analysis	% agreement	CV (%)	Bias
All	59.4	37.3	-0.05
Experts	65.2	17.6	-0.07
Trainees	56.5	36.4	0.28
Southern component	61.3	54.4	0.11
Western component	58.1	35.9	-0.08
North Sea component	77.9	34.5	-0.01
Northern distribution	48.2	20.8	-0.24

Overall agreement was 59.4%, considerably lower than last exchange of 2014 (68.2%). The best agreement was obtained for otoliths of age 0 (96%) and ages 1–3 (79, 75 and 73%, respectively). Ages over 6 had less than 50% agreement. Overall CV was 37.3%, higher than last exchange (15.4%).

The Expert readers’ analysis showed better results, with 65.2% of agreement (75% agreement in last exchange in 2014). The best agreement was obtained for otoliths of age 0 (100%) and ages 1–3 (84, 75 and 77% respectively). Ages over 7 had 50% or less agreement. CV was 17.6%, better than all readers’ analysis but still higher than last exchange (9.3%).

The Trainee readers’ analysis showed worse results, with lower agreement (56.5%) and higher CV (36.4%) than All readers’ analysis.

By component, the best result was obtained in the North Sea component analysis, with 77.9% agreement, followed by the Southern component analysis with 61.3% agreement. The worst result was obtained in the Northern distribution analysis, with only 48.2% agreement.

The exchange was carried out using the SmartDots application, which made the whole exchange process quite easy. As this is a new application, for most readers this was the first time using the program but once all readers became familiar with the use of the tool it proved to be very useful, though some readers did not know how to use all the potential offered by the application, such as the selection of brightness of the otolith images, which would have helped in the age estimation. Also, the exclusive use of images has the disadvantage that the readers find more difficult to identify the nature of the otolith edge, which can make the age interpretation more difficult in some cases. In addition, the use of a standardized reading line for all readers in each otolith image, even though it makes the comparison between readings easier, sometimes this complicate marking the annuli on the otolith when they are better observed in

another area of the otolith. However, the use of images allows a better comparison between the readers' estimations and a better identification of the problems in locating false rings, as well as speeding up the process. The use of SmartDots is especially useful for a posterior discussion on screen of the most significant otoliths during the workshop.

When comparing this exchange results with the previous exchange (2014), there has been a drastic decrease in the level of agreement, both for all readers and Experts (Table 5.1.3). This could be due to the change of readers produced since last exchange (2014). Only 9 readers of the present exchange also participated in the previous exchange. From them, only 3 remain as Experts, most expert readers in the present exchange participated as Intermediate and Trainee in the previous one. All Trainee readers of the present exchange are new readers and did not participated in last exchange.

Table 5.1.3. % Agreement and CV for all readers' and Expert readers' analysis of the Pre-WKARMAC2 exercise (2018) and the Small Scale Otolith Exchange (2014).

Analysis	2014		2018	
	% agreement	CV (%)	% agreement	CV (%)
All	68.2	15.4	59.4	37.3
Experts	75.5	9.3	65.2	17.6
Experts+Intermediates	70.0	13.9		

From the 135 otoliths of the exchange, 36 otoliths had an agreement of more than 80%, with modal age from 0-4. From these, only 4 otoliths had 100% of agreement, 3 of them with modal age 0 and one with modal age 3. There were also 10 otoliths with an agreement of 94% (modal age 0-3). All otoliths with modal age 5 or more had less than 80% agreement, also, those with less than 30% agreement had modal age from 4-11. The otolith with the lowest agreement (22%) had a modal age of 9. Most otoliths with more than 80% agreement are from ICES divisions 4c, 8b, 8c and 4b.

To sum up, the overall agreement was low with a drastic decline of the % agreement and CV regarding the previous exchange in 2014. There has been a change of readers since last exchange which has been more drastic considering the last Workshop in 2010, which demands a recalibration of the readers. 36 otoliths from the 135 otoliths of the exchange have more than 80% agreement, and only 4 of them have 100% agreement. Otoliths with modal age of 5 or more had the lowest agreement (all otoliths with less than 30% agreement had modal age from 4-11).

The results of this exchange were discussed during the Workshop on Age Reading of Atlantic Mackerel (*Scomber scombrus*) (WKARMAC2).

5.2 Workshop exercise: WKARMAC2 calibration exercise

After discussing, during the workshop, the results of the previous otolith exchange, with an on screen discussion of most representative otoliths, and a revision of the age estimation criteria, a new otolith exchange was performed during the workshop. 22 readers from the 23 WKARMAC2 participants participated in the exchange. In addition, two more readers who could not assist to the workshop participated in the exchange from their computers at their labs. The two readers from Greece participated as one as they shared the computer during the exercise. Readers were ranked as Experts and Trainees considering the years of experience estimating the age of Atlantic mackerel. Expert readers were considered those participants with more than four years of experience. Moreover, Expert readers coincided with the readers involved in mackerel assessment in their countries (Table 5.2.1).

Table 5.2.1. Participants of WKARMAC2 calibration exercise and their reading level.

Reader No	Name	Laboratory	Country	Reading level
R1	Jane Mills*	Marine Lab	Scotland-UK	Expert
R2	Eilert Hermansen	IMR	Norway	Expert
R3	Iñaki Rico	AZTI	Spain	Expert
R4	Deirdre Lynch	Marine Institute	Ireland	Expert
R5	Charo Navarro	IEO	Spain	Expert
R6	Gertrud Delfs	Thünen-Institute	Germany	Expert
R7	Gudrun Finnbogadóttir	MFRI	Iceland	Expert
R8	Maria Jarnum	DTU-Aqua	Denmark	Expert
R9	Jens Arni Thomassen	FMRI	Faroe Islands	Expert
R10	André Dijkman	WMR	The Netherlands	Expert
R11	Poul Vestergaard	FMRI	Faroe Islands	Expert
R12	Naiara Serrano	AZTI	Spain	Expert
R13	Orjen Sorensen	IMR	Norway	Expert
R14	Mererte Kvalsund	IMR	Norway	Expert
R15	Delfina Morais*	IPMA	Portugal	Expert
R16	Selene Hoey	Marine Institute	Ireland	Trainee
R17	Athanasios Spetsiotis**	FRI	Greece	Trainee
R18	Gitta Hemken	Thünen-Institute	Germany	Trainee
R19	Kate Downes	CEFAS	England-UK	Trainee
R20	Tim Huijer	WMR	The Netherlands	Trainee
R21	Michelle Inglis	Marine Lab	Scotland-UK	Trainee
R22	Andrea Silva	IPMA	Portugal	Trainee
R23	Camilla Wentzel	GINR	Greenland	Trainee

* Not present at WKARMAC2, participated from their labs.

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5.2.1 Material and methods

The exchange was carried out in the same way as the previous exchange, via SmartDots (<http://www.ices.dk/marine-data/tools/Pages/smartdots.aspx>), the web application developed by ICES to facilitate the setup of Exchanges, Workshops and Training events. A total of 143 otolith images from the main areas of mackerel distribution were included in the exchange, 134 of them were the ones used in the previous exchange (as there was no time to ask participants for new otolith images), to which 9 more images from the Mediterranean Sea were added. Following the recommendations of WKMACQI (Workshop on Mackerel biological parameter Quality Indicators) (ICES 2018c), it was attempted that the spatial and temporal coverage, as well as the length and age range, of the mackerel otolith of the exchange corresponded with the coverage in the assessment (Table 5.2.1.1).

Table 5.2.1.1. Spatial and temporal coverage of the otoliths used in the WKARMA2 calibration exercise.

Component	ICES Area	subarea	N° images		Length range (cm)	Institute providing data
			Sem 1	Sem 2		
Southern component	8c	8cE	5		35-38	AZTI (Spain)
			2	6	17-41	IEO (Spain)
		8cW	6	6	20-39	
	9a	9aN	3	2	21-38	IPMA (Portugal)
		9aCN	5	5	25-42	
Western component	7	7b	9		31-42	MI (Ireland)
		7j	10		33-40	
		7d	5	5	25-44	
	8abde	8b	5		34-38	AZTI (Spain)
				5	14-21	IEO (Spain)
		4	4b	5	5	16-36
North Sea component		4c		10	23-35	Thünen-Institute (Germany)
	Northern distribution	2	2a	5		14-42
				5	34-39	MFR (Iceland)
5		5a	5	5	32-38	MFR (Iceland)
		5b	5	5	32-39	
14	14b		5	35-40		
Mediterranean Sea				9	20-31	FRI (Greece)
TOTAL			70	73		
			143			

Preliminary results were analysed using R and commented during the workshop. A thorough analysis of the exchange results was carried out after the workshop using the Guus Eltink spreadsheet (Eltink, 2000). Although SmartDots application can generate an automatic and complete analysis of the results, due to the limited time available to obtain the results for the workshop report preparation and also that the application still has some limitations when selecting the options of the analysis, it was decided to use the Eltink spreadsheet for the analysis instead.

5.2.2 Results

A table with the participants' readings can be found in Annex 5 (Table 5.1). In addition to the estimation of the age of the exchange otoliths, readers were asked to assign the quality to each reading according to the "3 points grading system" (AQ1, AQ2, AQ3) recommended by WKNARC (ICES, 2011). Readings with AQ3 were not considered in the analyses. Analyses were performed for the total of areas and all readers and Expert and Trainee readers separately. Additional analyses were performed by each of the four ICES areas of mackerel distribution: Southern component (ICES div. 9a, 8c), Western component (ICES div. 8b, 7bjd), North Sea component (ICES div. 4bc) and Northern distribution (ICES div. 2a, 5ab, 14b), for all readers and Expert readers. The Mediterranean otoliths were included only in the first analysis (all areas, all readers), all analyses after that were with ICES divisions otoliths only. In all Expert analyses were included only Expert readers that also attended the workshop, due to the bias observed between the readings of the 2 Expert that not attended the workshop with the other Expert readers, probably because they could not participate in the previous discussion of relevant otoliths carried out during the workshop. A summary with the overall agreement, CV and bias of all analyses are shown in Table 5.2.2.1. The figures and tables showing the results of each analysis can be found in Annex 5.

Table 5.2.2.1. Summary of % agreement, CV and bias obtained in the analyses of Atlantic mackerel readings of WKARMAC2 calibration exercise.

Analysis	% Agreement	CV (%)	Bias
All (ICES div.+Mediterranean)	66.8	31.4	-0.03
All (ICES divisions)	66.5	30.4	-0.03
Experts*	73.2	16.4	0.01
Trainees	63.6	26.5	0.04
Southern component_All	68.5	41.3	0.05
Western component_All	68.7	37.1	-0.11
North Sea component_All	80.6	23.8	-0.04
Northern distribution_All	53.8	14.5	-0.01
Southern component_Experts*	72.9	24.0	0.05
Western component_Experts*	78.1	17.3	0.03
North Sea component_Experts*	88.4	6.90	0.00
Northern distribution_Experts*	59.4	12.2	-0.09

* Only Experts attending WKARMAC2

Overall agreement was 66.5%, much better than the previous exchange (59.4%), but yet a bit lower than the exchange in 2014 (68.2%). The best agreement was obtained for otoliths of modal age 0 (98%), and modal ages 1–3 (86, 77, and 83%, respectively). Otoliths with modal ages >4 had less than 60% agreement, being the otoliths with the least agreement those with modal age 10 (40%) and 9 (48%). Overall CV was 30.4%, lower than the previous exchange (37.3%), but yet higher than the exchange in 2014 (15.4%). CV peaked at 34.3% for modal age 1. Lowest values were obtained for modal age 7 (10.4%). The CV value of 239.8% for modal age 0 is probably obtained due to the difficulty of the Eltink sheet application to calculate the CV for modal age 0 when one or more readers have a different estimation (Annex 5, Table 5.1.1).

The Expert readers' analysis showed better results, with overall agreement of 73.2% (65.2% in the previous exchange; 75% in 2014 exchange). The best agreement was obtained for otoliths of modal age 0 (98%), and modal ages 1-4 (91, 85, 84 and 78%, respectively). The worst agreement was obtained for otoliths with modal age 10 (53%) and 8 (55%). Overall CV for Expert readers was 16.4% (17.6% in last exchange; 9.3% in 2014 exchange). CV values for otoliths of all modal ages but 0 were <20%, being the lowest value for otoliths of modal age 7 (7.5%) (Annex 5, Table 5.2.1).

The Trainee readers' analysis showed worse results, with lower agreement (63.6%) than All readers' analysis, although the CV value was also lower (26.5%).

In All readers' analysis by component, the best result was obtained in the North Sea component analysis, with 80.6% agreement, followed by the Western and Southern components with almost a tie (68.7 and 68.5% respectively). The worst result was obtained in the Northern distribution with only 53.8% agreement.

In the Experts' analysis by component, the results were better than in All readers' analysis by component. The best result was obtained in the North Sea component analysis, with 88.4% agreement, followed by the Western component with 78.1%. The worst result was obtained in the Northern distribution with 59.4% agreement.

The modal age range was 0–10 for the whole set of otoliths. By component, the modal age range were 0-8 (Southern component), 0–10 (Western component), 1–3/5–6/10 (North Sea component) and 1-10 (Northern distribution).

The results of the inter-reader bias test and reader against modal age bias test are shown in Table 5.1.2 (Annex 5). Only readers 2, 5, 8, 9, 17, 19 and 20 showed no bias against the modal age (in all readers' analysis). Reader 15 showed bias with all the other readers. Also, readers 1, 4, 6 and 22 showed bias with most readers. Readers 8, 19 and 21 had better results in the inter-reader bias test. By component, the best results of the inter-reader bias test and reader against modal bias test were obtained for the North Sea component, with almost no bias between readers, especially between Expert readers (Annex 5, Table 5.4.3.2), followed by the Western component, where reader 15 stands out showing bias with all the other readers, while the other readers practically showed no bias between them (Annex 5, Table 5.4.2.2) and Southern component, with readers 4 and 15 showing bias with most readers and also readers 6, 11, 22 and 23 showing bias with some other readers (Annex 5, Table 5.4.1.2). Worst results were obtained for the Northern distribution with more bias between readers (Annex 5, Table 5.4.4.2).

Figure 5.1.2 (Annex 5) shows age bias plots with the mean age recorded and the standard deviation of each age reader and all readers combined plotted against the modal age. Reader 1 showed underestimation according to the modal age, which was more pronounced for Reader 15. Readers 4, 6, 16, 22 and 23 showed underestimations in older ages regarding the modal age. Readers 8 and 20 also showed less prominent underestimation in older ages. Readers 19 and 20 showed light overestimation in younger ages followed by light underestimation in older ages regarding the modal age; this pattern was more pronounced for reader 17. Readers 10, 13, 14 and 18 showed light overestimation in all ages regarding the modal age, which was more pronounced for reader 11. The rest of the readers showed a more accurate estimation according to the modal age. As the overall agreement between readers is lower with older ages, the standard deviations are also mostly higher for the older ages for all readers combined (Annex 5, Figure 5.1.1). Similar trends are shown for Experts and Trainees separately (Annex 5, Figures 5.2.1, 5.2.2, 5.3.1 and 5.3.2).

5.2.3 Conclusions and evaluation of the exercise

The exercise was carried out using the SmartDots application, which made the whole exchange process quite easy. This was the second time using this application for most readers, who were more familiar with it. However, some of the problems showed in the pre-WKARMAC2 exercise persisted in the present exercise, mainly the position of the reading line, as sometimes the annuli would be better observed in other areas of the otoliths and the difficulty of observing the nature of the edge in the images. However, once again, the use of images allowed a better comparison between the readers' estimations and a better identification of the problems in locating false rings, as well as to speed up the process. Also, the use of SmartDots was especially useful for a posterior on screen discussion of the most significant otoliths during the workshop.

Average percentage of agreement (66.8%) and CV (31.4%) for all components and readers were much better than the pre-WKARMAC2 exercise (59.4% agreement and 37.3% CV), but still slightly lower than the previous exchange in 2014 (68.2% and 15.4%, respectively). The results of the Expert readers were much better than the results of all reads (73.2% agreement, 16.4% CV), but once again, still slightly worse than the previous exchange in 2014 (75.5% agreement, 9.3% CV); whereas the results of the Trainee readers were slightly worse than the results of all readers (63.6% agreement, 26.5% CV). But in general, there was a good improvement in the results regarding the pre-WKARMAC2 exercise (Table 5.2.3.1).

Table 5.2.3.1. % Agreement and CV for all readers', Expert readers' and Trainee readers' analysis of the last three exchanges (2014 and 2018).

Analysis	Exchange 2014		pre-WKARMAC2 exercise		WKARMAC2 calibration exercise	
	% Agreement	CV (%)	% Agreement	CV (%)	% Agreement	CV (%)
All	68.2	15.4	59.4	37.3	66.8	31.4
Experts	75.5	9.3	65.2	17.6	73.2	16.4
Trainees	-	-	56.5	36.4	63.6	26.5

From the 143 otoliths of the exchange, 44 otoliths had an agreement of more than 80% (Annex 5, Table 5.1), with modal age from 0–5. From these, 9 otoliths had 100% agreement, four of them with modal age 0, three with modal age 2, one with modal age 1 and another with modal age 4. There were also 20 otoliths with 90–99% agreement (modal age 0–4). Also, there were 15 otoliths with 80–89% agreement (modal age 1–5). Most otoliths with 50% or less agreement have modal age 5 or older.

When considering only the Expert readers, there were 61 otoliths with more than 80% agreement and modal age 0–9. From these, 31 otoliths had 100% agreement (modal age 0–6 and 9); 16 otoliths had 90–99% agreement (modal age 0–5, 7 and 9) and 14 had 80–89% agreement (modal age 1–7). There were seven otoliths with less than 40% agreement, all of them with modal age 6–10.

To sum up, there has been an improvement in the overall agreement comparing with the pre-WKARMAC2 exercise, in both group of readers (Experts and Trainees), reaching similar values, although slightly lower, than the previous exchange in 2014; with 44 otoliths with more than 80% agreement for all readers (nine of them with 100% agreement), and 61 otoliths with more than 80% for Expert readers (31 of them with 100%). However, the agreement for otoliths with modal age 5 and older remains quite low (otoliths with 30% or less agreement had modal age 5–12, in all readers' analysis), which seems to have a difficult solution as the estimation of the age in these older otoliths is quite subjective and each reader seems to have a different interpretation of the growth pattern in these older otoliths, which seems to persists even after the on-screen discussion of these otoliths during the Workshop.

5.3 Small exchange with Norwegian otoliths. A little experiment.

5.3.1 Introduction

Without the existence of otoliths with a known age, the evaluation of the quality of age readings is based on the agreement and bias between age readers for the same species with regards to the modal value of all age readings. The real age is normally not known.

The existence of otoliths from the Norwegian Mark-Recapture experiments were long seen as potentially the golden stones which could iron out many subjective assumptions related to the age estimation of mackerel from this area and potentially other areas. These tagging data provide a known age between tagging and recapture reducing the unknown time span from hatching to the time of capture for tagging.

5.3.2 Material and methods

All together 28 otoliths from tagged and recaptured mackerel were provided by IMR Norway.

Age at time of tagging was estimated by length measurements and otoliths readings of Norwegian mackerel sampling data from the 1950ies to present. Tagged fish were between 21 and 28cm in time of release. According to the length-age distribution (Figure 5.3.2.1), fish between 21 and 24cm were assigned to age 1, fish between 26 and 28cm to age 2.

The age between release after tagging and recapture was then calculated taken the exact dates into account. Total age of each mackerel was then calculated by adding up the age at tagging and the full years between release date after tagging and recapture date. Table 5.3.2.1 shows an overview on all data.

The whole otoliths were kept on a black plastic slide and embedded in transparent resin. During the workshop participants were asked to read the otoliths under a microscope. Age readings were then put into the Eltink spreadsheet for the comparing of the results. Results were discussed on screen on the bases of digital images of the otoliths during the workshop.

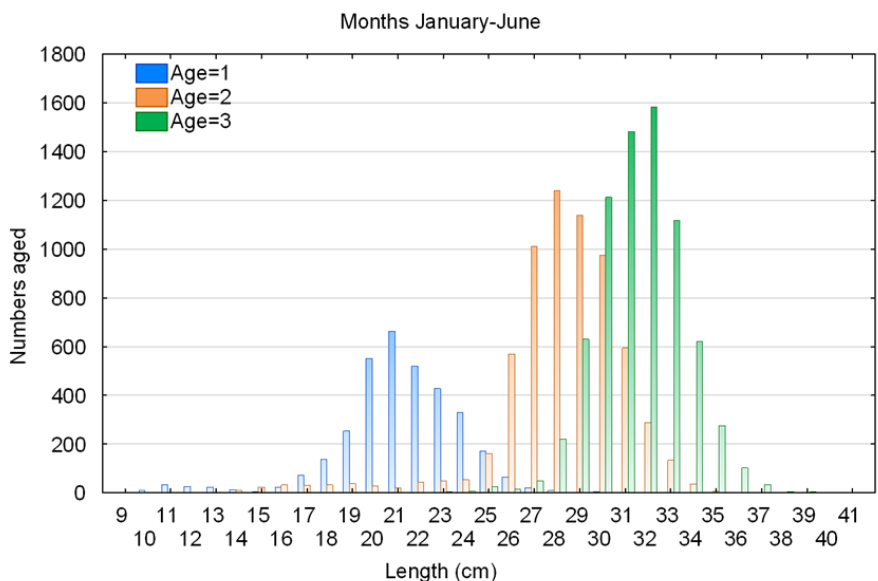


Figure5.3.2.1: Age length distributions of mackerel age 1 – 3, based on Norwegian sampling data

Table 5.3.2.1: Overview on mackerel data from the Norwegian Mark Recaptures

Sample ID	Fish number	tagging	Catch date	Fisk length at release (cm)	Fish length at recapture (cm)	1st time reader after recapture	calculated age*:
1	1	May 2003	06.09.2004	27	31	3	3
4	1	May 2006	24.09.2008	27	30	3	3
5	1	May 2006	06.10.2008	27	35	4	4
7	1	May 1994	06.10.1995	28	32	3	3
10	1	May 1995	29.01.1997	28	31	3	4
16	2	May 1994	20.09.1998	28	34	6	6
21	2	May 1997	25.01.2000	28	35	5	5
22	1	May 1996	29.09.2000	28	38	7	6
23	1	May 1997	13.09.2002	28	37	6	7
24	2	May 1989	01.02.2003	28	42	16	16
25	1	May 2003	28.09.2004	28	35	3	3
26	2	May 2003	05.10.2005	28	36	4	4
27	1	May 2003	30.09.2006	28	36	5	5
28	1	May 2006	22.10.2007	28	33	3	3
30	6	May 1990	25.01.1996	21	33	7	7
31	7	May 1993	08.03.1999	21	37	7	7
33	3	Aug 1988	28.01.1997	24	42	10	10
34	2	May 2002	26.10.2003	24	32	2	2
36	1	Aug 1988	07.10.1995	26	39	8	8
37	2	May 1994	23.10.1995	26	33	3	3
38	1	Aug 1988	26.02.1997	26	40	10	10
40	2	May 1990	05.10.1998	26	40	9	9
41	1	May 1992	02.11.1999	26	38	6	7
42	1	Aug 1988	13.11.2003	26	46	18	16
44	6	May 1994	04.03.1997	27	33	5	5
46	2	May 1992	21.01.2000	27	41	10	10
47	6	May 1989	23.01.2000	27	42	13	13
51	3	May 2004	13.01.2006	28	31	5	5

* age estimation from length at tagging plus years between release and recapture

5.3.3 Results

Overall age reading results are shown in table 5.3.3.1. Age readings were undertaken by all age readers for all 28 otoliths.

Overall agreement with the calculated / validated age is 52.4% (Table 5.3.3.1). Analysis only done with the expert group (13 readers), shows a slightly higher overall agreement of 59.9% and analysis referring to trainees only (9 readers) shows an overall agreement of 41.7%.

Taking only ages 0-5 into account agreements increases distinctly to 74.4% for all readers, 79.1% for the experts and to 67.5% for the group of trainees. Taken only older fish into account (≥ 6 years) a much lower agreement could be reached: only 30.5% for all readers, 40.7% for the group of experts and only 15.9% for the group of trainees.

Table 5.3.3.1: Results of the age reading exercise from the Norwegian Mark Recaptures

Sample ID	Fish number	Released Length	Recovered length	Calculated age	Modal age all readers	Modal age experts	Modal age trainees	agreement all readers*	agreement experts*	agreement trainees*
1	1	27	31	3	3	3	3	91%	100%	78%
4	1	27	30	3	3	3	3	86%	85%	89%
5	1	27	35	4	4	4	4	77%	92%	56%
7	1	28	32	3	3	3	3	91%	100%	78%
10	1	28	31	4	4	4	4	82%	92%	67%
16	2	28	34	6	6	6	6	64%	69%	56%
21	2	28	35	5	5	5	4	55%	62%	44%
22	1	28	38	6	6	6	3	32%	38%	22%
23	1	28	37	7	7	7	6	36%	54%	11%
24	2	28	42	16	13	12	4	14%	23%	0%
25	1	28	35	3	3	3	3	50%	62%	33%
26	2	28	36	4	4	4	4	73%	77%	67%
27	1	28	36	5	5	5	5	82%	85%	78%
28	1	28	33	3	2	2	3	45%	38%	56%
30	6	21	33	7	6	7	6	45%	62%	22%
31	7	21	37	7	7	7	8	36%	38%	33%
33	3	24	42	10	8	10	8	23%	38%	0%
34	2	24	32	2	2	2	2	77%	77%	78%
36	1	26	39	8	7	7	7	14%	23%	0%
37	2	26	33	3	3	3	3	86%	85%	89%
38	1	26	40	10	10	10	9	41%	54%	22%
40	2	26	40	10	10	10	9	41%	62%	11%
41	1	26	38	7	7	7	6	36%	46%	22%
42	1	26	46	16	10	10	10	9%	15%	0%
44	6	27	33	5	5	5	5	64%	69%	56%
46	2	27	41	10	12	12	8	9%	15%	0%
47	6	27	42	13	13	13	13	27%	31%	22%
51	3	28	31	5	5	5	5	82%	85%	78%
Overall agreement*								52.4%	59.9%	41.7%
Overall agreement* ages 0-5								74.4%	79.1%	67.5%
Overall agreement* ages >6								30.5%	40.7%	15.9%

*agreements are given with regards to the calculates/validated age

5.3.4 Conclusions and recommendations

Taking all otoliths into account the results show a quite low agreement for all readers, getting only slightly better when only looking at the group of experts. By splitting the otolith readings into fish younger and older than 6 years, it can be clearly seen that the agreement drops dramatically from 79.1% (experts) and 67.5% (trainees) for the younger fish to 40.7% and 15.9% for fish older 6, respectively.

Looking at individual age readings (Annex 6, Table 1) it can be seen that for the experts the age readings of two of the otoliths coincides with the calculated age. Also, 14 of the 28 otoliths differ only by one year from the calculated age, whereas for the trainees this proportion is lower (12 from 28 otoliths). Highest variations can be found for the oldest fish.

The discussion during the workshop on the images of the Norwegian otoliths revealed a tendency in underestimating older ages. It seems that in older fish more split rings can be seen. These rings were identified by many readers as false rings during the exercise but were in fact real year rings. However, it was not possible to derive a clear rule for identifying real and false split rings other than that the rule that a year ring should always be identified at more than one location on the otolith might not be true for mackerel.

It has to be noted that the number of otoliths used in this exercise were quite low (only 28) and only otoliths from the northern area of the mackerel distribution were used. Therefore, the results might not be generalized. It would be useful to carry out similar experiments with more otoliths and otoliths from other areas.

5.4 Recommended actions for resolving interpretation differences between readers

As these last otolith exchanges show, there is good agreement between readers for the age estimation of otoliths up to 5 years old, especially between Expert readers, whose estimations are used in mackerel assessments. However, the agreement between readers, even Experts, for the age estimation of otoliths over 5 years old remain very low, which has been observed during all previous workshops and exchanges.

One of the recommended actions for trying to improve this low agreement for older ages is the assistance of the readers to the workshops, especially those whose age estimations are used in mackerel assessments, where the different interpretation between readers can be discussed together, on-screen with images of especially difficult and/or old otoliths. The improvement reached in the level of agreement between readers in the calibration exercise carried out during the workshop seems to support this recommendation.

Another recommended action of great importance is to continue carrying out tag-recapture experiments. The information obtained from these experiments as well as the realization of calibration exercise between readers such as the one carried out during the workshop, is of great value in order to validate the age estimation in this species. The information obtained from older otoliths with this kind of experiment would be of great importance in order to help in a more accurate interpretation of older ages in mackerel otoliths.

6 Resume of the studies of the formation of the growth zones in otoliths of different distribution areas presented during the workshop

Study of seasonal formation of growth rings in the otoliths of the NEA Mackerel (*Scomber scombrus*) in ICES Divisions 8c and 9a North. By: Villamor, B.; Navarro, MR.; Hernández, C.; Dueñas-Liaño, C.; Antolínez, A. Presentation to WKARMAC2, San Sebastian, Spain, 22-26 October 2018.

One of the recommendations of the last workshop on age determination of NEA mackerel, WKARMAC (ICES, 2010) was to study the formation of the growth zones in otoliths of the different distribution areas, since the appearance of the otolith edge in a given area and season appears to have changed in recent years, causing the majority of the disagreements between the readers of mackerel otoliths. Attention should be paid to the younger individuals and the appearance of the edge over the season depending on the area.

The periodic formation of growth zones in otoliths has been extensively applied worldwide in age determination of fish. The formation of the opaque or hyaline zones has been attributed to various factors, such as seasonal temperature cycles, light conditions, fish feeding and reproductive cycles (Beckman and Wilson, 1995). In the case of NEA mackerel there is only one study in the literature, about the nature of the edge in the waters of Portugal (Gordo and Martins, 1982).

A work was presented at WKARMAC2 where the periodic formation of the growth zones in the otoliths of the Southern Component of NEA mackerel (ICES Divisions 8c and 9a North) was analyzed. As a preliminary step to find out seasonality in the formation of rings, the edge of the otolith was monitored and the possible relationship between the formation of rings and the temperature of the environment was discussed, as well as other biological parameters of the species as the condition factor (CF) and the gonad somatic index (GSI).

Nature of the otolith edge

Monthly samples were collected between January 2013 and December 2017 from commercial catches and from spring and autumn research surveys. The highest percentage of hyaline edge occurs between January and June, with a maximum in May every single year, except for 2017 which peaked in June. The minimum occurred between August and October. (Figure 6.1).

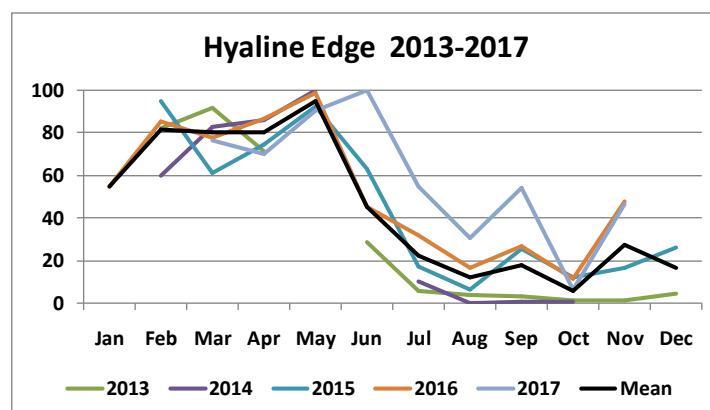


Figure 6.1: Evolution of hyaline edge formation (%) by year and for the whole study period, in Divisions 8c-9a North.

The same results are obtained for each area (8c and 9a North) as for the total area (8c + 9a North) for the whole period studied. (Figure 6.2)

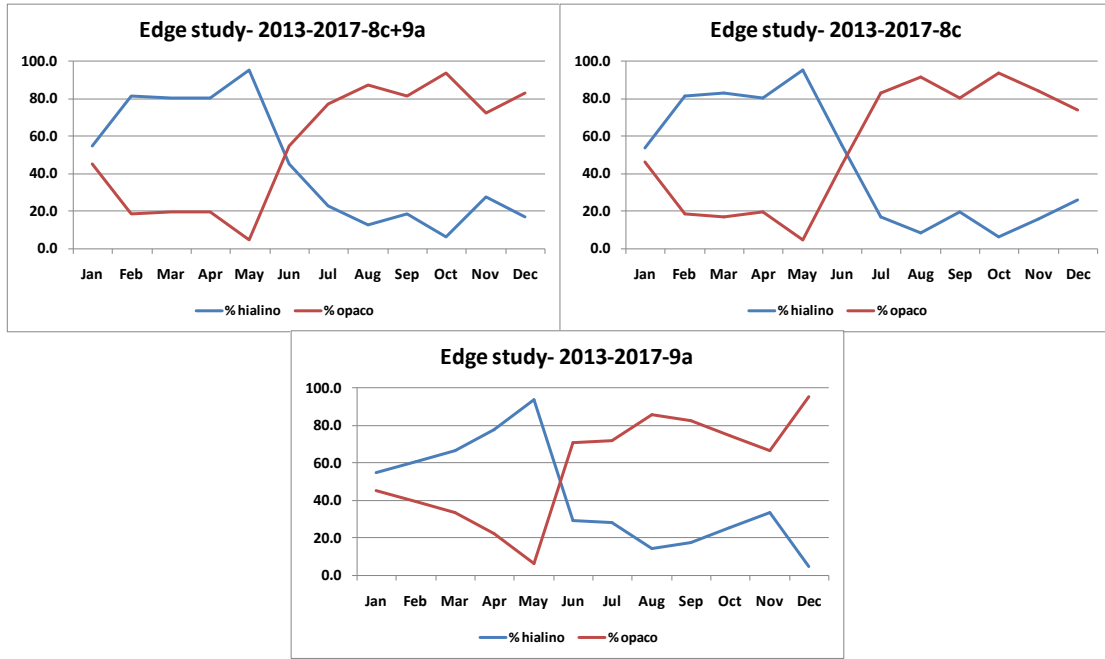


Figure 6.2. Evolution of percentage of hyaline and opaque edge formation by area (8c and 9a North) and for the total area (8c-9a North), for the whole period studied (dashed line without data).

Mackerel Seasonal growth pattern of otoliths by ages

The variation in the proportion of hyaline edges was gradual over months and so was the delay in the formation of the opaque edge with age; in general, the minimum proportion of hyaline edges was observed around April at age 1, June at age 2 and August at ages 3 and older. The temporal delay in opaque-zone formation increase with age, the growth of the younger mackerels of age 1 (all immature) resumes usually during March, mackerels of age 2 (reached maturity) start laying down the marginal opaque growth by May-June, and for mackerels of age 3 (totally matures) and older, it is in June when individuals start showing marginal opaque growth. (Figure 6.3).

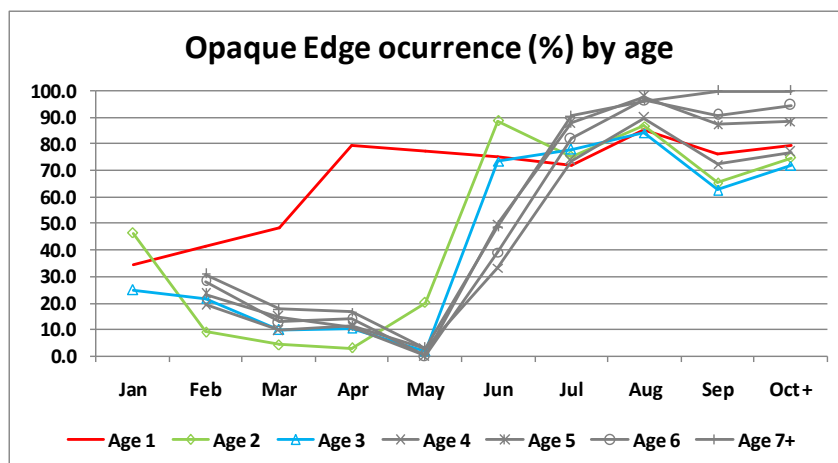


Figure 6.3. Opaque edge occurrence (%) by age for the total area (8c+9a North) and the whole period.

From these results, we must take into account the delay in opaque edge formation with age for the Southern area (ICES Division 8c and 9a); therefore it would be desirable to analyse the seasonality of the edge in other areas more thoroughly and to find out alternatives to the use of otolith edge type in age determination.

Edge relationships with Temperature, GSI and CF

The average temperature of the seawater, gonad somatic index (GSI) and Condition Factor (CF) were also calculated in order to get a yearly pattern for these parameters too. Several Temperatures were compiled with the aim to identify the drivers of mackerel otolith growth, since mackerel can occupy the entire water column. In the studied area, the mackerel can be located on the continental shelf near the marine bottom, below 30 m, appearing in the form of schools or aggregations of individuals (Iglesias *et al.*, 2005; Carrera *et al.*, 2017) but also, adults can be found near the sea surface (30 m approx.), especially in good weather, they would be driven by the weather conditions (Carrera, 2017). Most of the juveniles have the most preferred habitat in the areas closest to the bottom, between the coast and 200 m deep (Jansen *et al.*, 2014). Therefore the monthly averages of the sea temperature from 30m to the bottom for the years 2013 to 2017 were calculated, from the monthly temperature series in the station E55A of Santander Standard Section (southern Bay of Biscay ,43°64'N, 3°78'W) representative of the area at 180 m deep (Radiales Project: www.seriestemporales-ieo.com). Monthly Sea Surface Temperature (SST) was also calculated (upper 30 m) in the same station.

The ring formation of mackerel otoliths seems to be linked to the temperature and food resources (CF), with the fast growth of the fish (Figures 6.4 and 6.5). Based on temperatures it could be assumed that relatively high seasonal temperature is the driving force behind the opaque band formation in the NEA mackerel in the Southern area (ICES Divisions 8c and 9a). Fish feeding is another factor influencing the opaque edge formation in the otolith. NEA mackerel main feeding season starts in summer and ends in autumn (Olaso *et al.*, 2005), at the time of opaque otolith growth of this work. Opaque otolith band at the end of spring is laid down during a period of rapid growth and feeding.

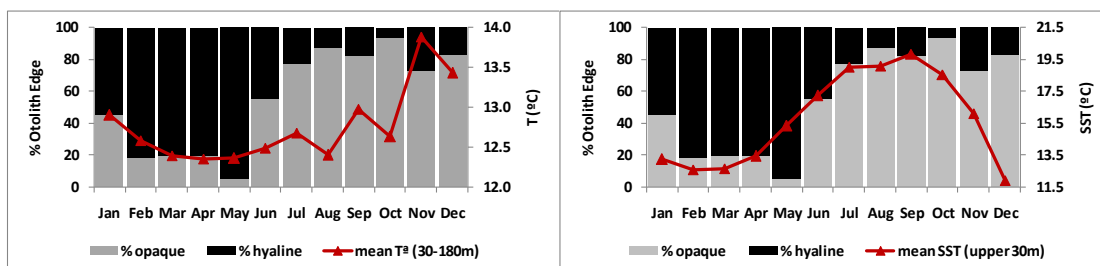


Figure 6.4. Monthly evolution of percentage of otolith edge formation and average monthly seawater temperature 30-180 m deep (top panel) and SST (bottom panel) for the whole study period.

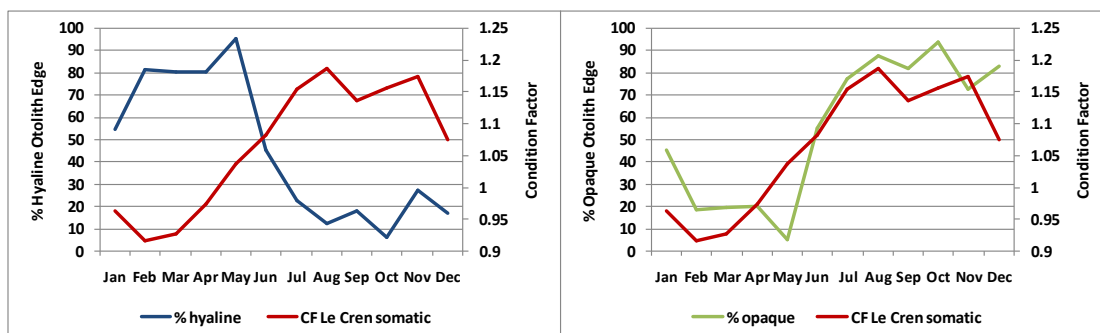


Figure 6.5. Evolution of percentage of hyaline edge formation (left panel) and opaque edge formation (right panel) and Condition Factor (CF) for the entire study period.

The formation of the otolith zones in relation to reproductive activity is controversial. In this work spawning occurs when the translucent zone is well into the process of formation. The

maximum of the gonad somatic index (GSI) coincides when the majority of mackerel individuals are forming the hyaline edge, except for the younger individuals of age 1 (all immature) that are forming the opaque edge (Figure 6.6).

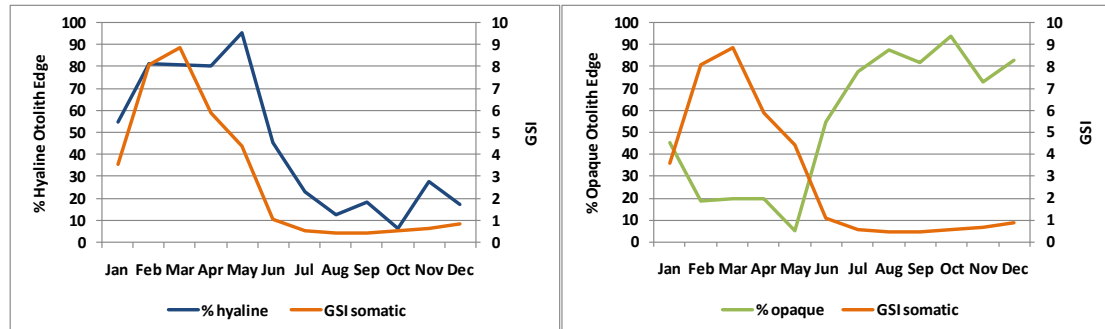


Figure 6.6. Evolution of percentage of hyaline edge formation (left panel) and opaque edge formation (right panel) and gonad somatic index (GSI) for the entire study period.

Remarks and further works

The season of formation of opaque and translucent zones in otoliths may change during the fish development and in relation to geographical distribution, as in the Atlantic cod (Høie *et al.*, 2009) and Sebastes in the Pacific coast (Pearson, 1996). In our study, no geographical differences are found between areas 8c and 9a N, and nor with the one performed on the Portuguese coasts (ICES Subdivisions 9a Central-North and South) by Gordon and Martins (1982). All these areas belong to the same Southern Component of the NEA mackerel. It is advisable to make this type of study for all distribution areas of mackerel in the Northeast Atlantic, from the south of the Iberian Peninsula to northern Europe (Norwegian and Icelandic coasts) to test whether or not there are seasonal differences in the formation of opaque-hyaline zones in otoliths and to study the factors influencing the variation of the opaque edge formation in mackerel otoliths.

7 Compilation of an agreed manual for age estimation of mackerel (part of ToR e)

7.1 Introduction

This section consists of two main parts, a methodology section describing the various approaches to storing, mounting and viewing the mackerel otoliths by all participating laboratories, and secondly an agreed set of ageing criteria made by the WKARMA2 which is an update of all previously used ageing criteria, bridging across differences in perception between readers. The more recent changes in mackerel behaviour in terms of timing of spawning and migration patterns thus call for additional validations of otolith structures, however, the manual can be applied as of now bearing these changes in mind. Table 7.1.1 shows the precedence of mackerel samples of each laboratory.

Table 7.1.1. Precedence of mackerel samples of each laboratory.

Institute	Sampling areas	Quarter	Origin
Denmark (DTU)	4a, 4b, 2a	Q1, Q2, Q3, Q4	surveys, commercial catches
Faroese (MRI)	2, 4a, 5b, 6a	Q1, Q3, Q4	surveys, commercial catches
Germany (TI-SF)	6, 7, North Sea	Q1, Q3, Q4	surveys, observers
Greece (FRI)	Aegean, Ionian Sea	Q1, Q2, Q3, Q4	commercial catches, market samples
Greenland (GINR)	East Greenland and international zone in Norwegian Sea	June-October	surveys, commercial catches
Iceland (MRI)	4a, 5b, 2a, 14b, 12a	Q2, Q3, Q4	surveys
Ireland (MI)	6, 7, 4a	Q1, Q3, Q4	surveys, commercial catches
Netherlands (WMR)	4, 7, 9	Q1, Q2, Q3, Q4	commercial catches
Norway (IMR)	2b, 4a, 4b, 6a, 7b, 7j	Q1, Q2, Q3, Q4	surveys, commercial catches
Portugal (IPMA)	9a	Q1, Q2, Q3, Q4	surveys, commercial catches
Spain (AZTI)	8c, 8b	Q1, Q2	surveys, market sampling
Spain (IEO)	9aN, 8c, 8b	Q1, Q2, Q3, Q4	surveys, commercial catches
UK (England, CEFAS)	7	Q1, Q2, Q3, Q4	market samples, surveys
UK (Scotland, MSML)	4a, 6a	Q1, Q2, Q3, Q4	research & industry surveys, observers, market sampling, commercial catches

7.2 Methods and preparation

Various methods of preparation of otolith samples are used by mackerel otolith reading institutes. Details on each of these are listed in table 7.2.1.

Firstly, the otoliths are extracted from the fish. Mackerel otoliths are removed by making a horizontal cut to the head above the eye from the posterior end of the operculum to the snout. Then a second lateral cut on the head's dorsal side at right angles to the first cut, to remove that

piece of flesh. This exposes the otic capsule and then both otoliths are removed from the grooves they lie in. For this, straight tipped watch-makers forceps should be used. Care should be taken to ensure the otoliths are kept whole as these structures are very fragile. Alternatively, one horizontal cut in the mackerel head can be made in the shape of an 'M' which exposes the otoliths.

All institutes have procedures for cleaning the otoliths immediately after extraction. This is required to remove any blood or membrane attached to the otolith. If these are not removed, they can dry and create difficulties when the otoliths are read.

An aspect of otolith preparation common to all institutes is the collection and initial storage. Otoliths are collected and put into wells on black plastic trays. It is important the trays are black in order to maximise the contrast between the background and the structures.

The subsequent preparation methodology prior to ageing varies between institutes.

This can be broadly divided into two categories, those that fix the otoliths to the slides and those that keep them loose.

For the fixed method transparent resin is used to cover the otoliths. This has the effect of creating a permanent refractive index surrounding the otolith once the resin has hardened.

Alternately, otoliths can be read loose in the wells. For this method, a transparent liquid of appropriate refractive index, most commonly ethanol, is added to the wells.

Both methods have benefits and drawbacks, which are listed in tables 7.2.3 (fixed otoliths) and 7.2.4 (loose otoliths).

It is recommended by most institutes that the otoliths are viewed with a binocular microscope, using bright reflected light, preferably from a fibre optic light source, with a magnification of between 15x and 40x depending on the size of the otoliths, (ICES, 1995).

Table 7.2.1: European research institutes: overview on otolith preparation techniques used and storage facilities.

Institute	Calcified structure	Fixed/loose	Cleaning process	Preparation method	slide description	Storage
Denmark (DTU)	whole otolith	loose	alcohol	loose, read in water		stored in small plastic bags
Faroes (MRI)	whole otolith	fixed	water	embedded in transparent resin (Entellan)	one pair otoliths per tray	
Germany (TI-SF)	whole otolith	fixed	mild soap solution	embedded in transparent resin	glass slides with black background, 200 pairs otoliths per slide	secure glass storage-for example: card file box
Greece (FRI)	whole otolith	loose	water	loose, read submerged in fresh water	one pair otoliths per phial	stored in pvc phials
Greenland (GINR)	whole otolith	loose	water	loose, read submerged in ethanol (approx. 40%)	1 to 4 pairs otoliths per slide	
Iceland (MRI)	whole otolith	loose		loose, read in alcohol		
Ireland (MI)	whole otolith	fixed	water	embedded in transparent resin (Histokitt), covered in oil when reading		
Netherlands (WMR)	whole otolith	fixed	water/alcohol	embedded in transparent resin	slides with no cover, 25 pair of otoliths per slide	slides are stored in carton boxes
Norway (IMR)	whole otolith	fixed	water	embedded in transparent resin (Entellan)	black plastic slides, no cover, 25 pairs otoliths per slide	
Portugal (IPMA)	whole otolith	fixed	water	embedded in transparent resin (Entellan)	black plastic slides, no cover, 10 pairs of otoliths per slide	Slides are stored in paper boxes (20 slides/box)
Spain (AZTI)	whole otolith	fixed	water	embedded in transparent resin (Eukitt)	black plastic slides, no cover, 10 pairs otoliths per slide	slides are stacked held together with a rubber band, first slide is covered to avoid dust
Spain (IEO)	whole otolith	90% fixed	fresh water	embedded in transparent resin (substitute of xylene) + a drop of fresh water when reading	black plastic slides with cover, 10 pairs otoliths per slide	slides with cover stored in cardboard boxes (12 slides per box)
		10% loose	fresh water	loose, read submerged in fresh water	black plastic slides with cover, 10 pairs otoliths per slide	slides with cover and sellotaped, stored in cardboard boxes in horizontal position
UK (England, CEFAS)	whole otolith	fixed	no water/alcohol used	embedded in transparent resin	slides with cover, 25 pair otoliths per slide	
UK (Scotland, MSML)	whole otolith	loose	fresh water	loose, read in distilled water	tray with a lid, 60 pair otoliths per tray	

Table 7.2.2 Pros and cons of using fixed otoliths in transparent resin as method of age estimation (institutes self-estimation).

Institute	Pros	Cons
Faroës (MRI)	if you put entellan on the cracks, it can solve the problem (see cons)	the resin on the trays will crack after some years
Germany (TI-SF)	otoliths are securely fixed and permanently protected, resin does not deteriorate, easy storage after ageing, otoliths cannot be manipulated under the microscope	a fume cupboard must be used when using resin due to health risks, time consuming task, otoliths cannot be used for any other process (eg: microchemistry, microstructure)
Ireland (MI)	fast to read, easy to transport	resin cracks when old, bubbles can form obscuring otoliths, otoliths on their side or back (wrongly mounted)
Netherlands (WMR)	easy storage/well preserved, fast reading per 25	resin can cause reflection, cracks or bubbles
Norway (IMR)	clear zones for reading	after some years the entellan becomes cracked, xylene fumes in entellan is a poisonous gas.
Portugal (IPMA)	the image is clear and we can see the rings well, when the preparation is done it's easy to use for reading at any time	entellan is toxic and there are some constraints in the lab, it can only use otolith for readings and not for other studies (eg. Microchemistry), time consuming task
Spain (AZTI)	easy to storage and difficulty of getting lost through use	over time resin can be damaged, by touching the surface of the resin it remains marked
Spain (IEO)	easy to manipulate, easy to storage, rings are better observed under microscope, slide with cover gives protection	time consuming, otoliths not available for future studies (microchemistry, etc)
UK (England, CEFAS)	many otoliths can fit on one slide, long term storage is easy as the otoliths are fully protected from the elements by resin and cover slip	it's a time consuming process to mount and set otoliths in resin

Table 7.2.3: Pros and cons of using loose otoliths submerged in water/alcohol as method of age estimation (institutes self-estimation).

Institute	Pros	Cons
Denmark (DTU)	easy and fast way to clean them, read them and store them, reading them in water is a non toxic way	to read them in water: are we missing small otolith structures by not using resin? Should we investigate other ways of reading them? The way we store them can easily break the fragile otoliths.
Greece (FRI)		sometimes the quality of photos is bad
Greenland (GINR)		
Iceland (MRI)	otoliths can be moved around, no bubbles or tilted otoliths	
Spain (IEO)	otoliths available for further studies	time consuming, rings are worse observed under microscope
UK (Scotland, MSML)	water makes rings clearer, able to move otoliths around in tray to see them at different angles	at risk of being damaged/lost due to handling

7.3 Agreed criteria for ageing mackerel

7.3.1 Viewing the otoliths

There are two ways of reading mackerel otoliths. The most commonly used is using a binocular microscope, with a reflected light source and a magnification of between x15 and x40, depending on the age of the mackerel. Alternatively, the age can be estimated reading digital images (section 7.3.1.1).

7.3.1.1 Age estimation applying digital images

Reading digital images and reading directly onto the images using an image analysis system is an alternative to reading the otoliths under a binocular microscope. Applying this method, the preservation of both reference materials (digitised images of otoliths) and the interpretations of the age structures (annotations done by the reader) can benefit.

It must be borne in mind that a digitised image does not hold the same 'information' for the human eye and the computer. The reader would obviously prefer the best possible image mirroring what is seen in the microscope (i.e. showing all structures of the otolith) whereas the computer just records an image as a matrix of numbers. The latter does allow a wide span of post-processing, e.g. improving image quality, extracting structures, making measurements, etc.

Holding all otoliths in an image database first and foremost preserves all collected material as the pictures do not deteriorate like biological material (scales, otoliths), thus all information shown in the pictures are kept for good. The images facilitate a number of things:

- -Re-estimation of the age (repeatability of the reader)
- -Sharing otoliths with other readers
- -Storing information about the readings (traceability)
- -Quantitative measurements (growth curves, back calculation, statistical processing, etc.)
- -Potential improvements of the original image to make the structures more visible

The quality of the digital image obviously must be as good as possible, thus attention should be paid to light setting, magnification, etc. It is highly recommended that the quality of both the microscope and the camera used is as good as possible. The pixel capacity and the light sensitivity of the camera are also important factors to consider.

Making sure the image is of the highest quality implies several things:

- -Good preparation of the sample (each species has its own specific method).
- -Special attention should be paid to the background and the light, the goal is to have a strong contrast between the opaque and translucent zones (avoid overexposed images).
- -The digitised image must be as close as possible to the image you have under the microscope.
- -The images must be calibrated (using a micrometre as a reference) with the maximum of precision.
- - All images should be stored in a database that is linked to the biometric data for each otolith sample.

When making age estimations directly on the digitised image, the age structures should be marked applying simple image analysis software. This facilitates a few post-processing measures, as e.g. achieving average distances between rings, comparison of the growth curve

of a specific otolith with the overall growth curve for the otoliths of a particular sample/quarter/etc. It also makes back-calculation of length and additional statistical analysis possible.

The system, by which otoliths are read directly from digitised images and not using a microscope, was fully implemented at Ifremer, France. Here the otolith reader estimates the age of an individual twice, annotates the age structures on the image and checks the entire sample for outliers after finishing the reading exercise. The length of the individual fish is unknown to the reader while doing the age estimation. This information is used as a post-process check using the age-length keys produced for each sample. This technique has been tested by the French institutes where readings performed with 'live' otoliths under a microscope were compared with readings performed directly on digitised images. The percentage of agreement for all species was more than 98%, testing on less complicated otoliths as plaice. However, if trained properly and having a suitable set-up in terms of camera, etc., this technique may be as solid as the more traditional age reading process.

At present only Belgium has also adopted this system and the Netherlands are intending to do so (pers. com. Loes Bolle). In the Netherlands this will be a phased transition. At the moment only herring age reading is based on images analysis alone but from next year more species will be included.

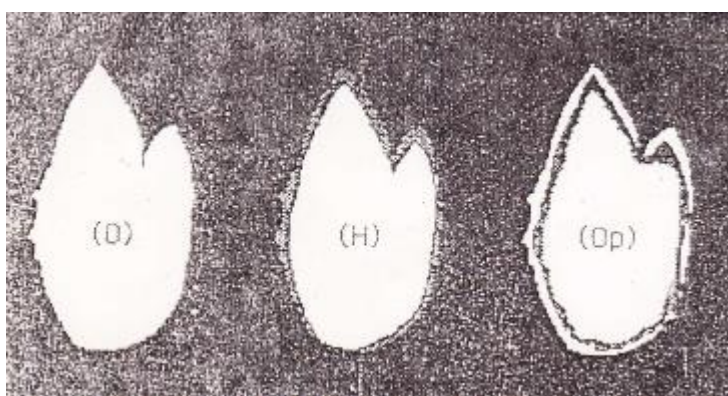
7.3.2 Age determination criteria

It is essential that all otoliths readers are aware of the age determination criteria that should be applied before age determination is attempted (ICES, 1994). The age determination criteria for mackerel are as follows:

- 1) The date of birth is assumed to be 1st January and the fish is assigned to a year class on this basis. In Mediterranean waters the date of birth is assumed to be the 1st July. Therefore, the date of capture of the sample should always be available.
- 2) One opaque zone and one translucent (hyaline) zone constitutes one year of growth (annulus).
- 3) The timing of the formation of the opaque zone on the edge of the otolith is heavily dependent on the area from which the sample was taken. When allocating the fish to a year class therefore, the area of capture should also be known.
- 4) The summer increment (opaque zone) should be continuous around the otolith (the "ring" should be visible in at least two areas; however, especially in old specimens it might only be visible at the rostrum).
- 5) The relative widths of each ring should progressively be smaller as the otolith grows. Although conditions affecting the life history of the fish can create unexpected relative width proportions between annuli.
- 6) For mackerel caught in the 1st and 2nd quarter of the year, all winter rings and the translucent (hyaline) edge are counted. The translucent (hyaline) edge is always counted as one winter ring, even if nothing or very little is visible. However, as the study of seasonal formation of growth rings in the otoliths of the NEA Mackerel in ICES div. 8c and 9a reveals, the proportion of hyaline edges varies gradually over months and so is the delay in the formation of the opaque edge with age (see Section 6). According with this study, 50% of otoliths with opaque edge in one-year-old specimens occur at the end of March, whereas for two-years-old this occurs at the end of May and so on (Figure 6.3). This new opaque ring is usually thin, so is usually easily identified. Therefore, in young fish from ICES div. 8c-9a, this new ring is not counted (Figure 7.3.2.1). Thus, the area of capture is very important in this quarter and similar studies should be carried out in the other areas. For otoliths caught from

1st January to 30th June the reader should count all translucent (hyaline) rings and for those otoliths caught from 1st July to 31st December the reader should assume that the last hyaline ring is not fully formed and therefore not count it. However, if this last ring is thick, then it is probably from last year. It has been noted that a narrow opaque zone is seen at the edge of some otoliths and may be due to a change in the summer growth pattern. The translucent (hyaline) zone that appears before this opaque zone should be counted. This could be especially tricky for otoliths of 3-years-old or older specimens captured in July in some areas (i.e. ICES div. 8c and 9a). Therefore, studies on the otolith edge in all distribution areas are recommended to clarify this.

First semester of the year



Opaque

Hyaline

New opaque

1 year

Figure 7.3.2.1. Different kind of 1 year old mackerel otoliths that are found during the first semester of the year in ICES Div. 8c and 9a. Azti-IEO. (Paulino Lucio, pers. comm.)

- 7) The edge of the otoliths. As explained in the previous paragraph, the timing of the opaque ring formation on the edge of the otolith differs considerably from one area to the other. Therefore, it is useful to collect information regarding which months the opaque edge and the translucent (hyaline) edge on the otolith is laid down for each area and age of the fish. For example, the opaque ring formation is earlier in young fish and more southern areas. This information should help otolith readers with the interpretation of the edge of the otolith.
- 8) Following the recommendations of WKNARC (ICES, 2011) and last workshop (WKARMAC 2010), readers should register the confidence level they have in their otolith readings, reflecting the quality of the data. Most readers should use a scale of 3 levels of quality:
 - Rings can be counted with certainty: 1
 - Rings can be counted, but with difficulty and some doubt: 2
 - Rings cannot be counted; the otolith is unreadable: 3

However, Cefas uses a 4-level key: G (Good), M (Moderate), P (Poor) and 99 (Unreadable). This system is detailed in their accreditation under ISO 17025 and therefore cannot be changed. For the purposes of mapping the 4-level key to the 3-level key, M and P are analogous to level 2.

7.3.3 Other available information

Other information may be available about the fish including length and maturity. There is a school of thought that believes that length information may influence the decision of the reader when assigning an age. While this may be true, sometimes knowing the fish length can help in the age interpretation in older and/or difficult otoliths. Anyway, any experienced otolith reader will know the approximate length of the fish purely from the features of the otolith. Also, it could be an advantage to have the length available when reading samples of otoliths that are mounted together in large numbers. It is often possible to identify whether otoliths have been mixed up during sampling or preparation. Therefore, even if it is recommended to estimate the age of the otolith without knowing the fish length, it would be helpful if this information is available when reading difficult otoliths or in case of doubt.

7.3.4 Otolith interpretation

It is always preferable to have the pair of whole otoliths available when trying to interpret the ring structure. Mackerel otoliths can vary in appearance and therefore it is important to remember that there is no one “correct” position where to count rings. Ideally, the translucent (hyaline) rings should be counted and usually the preferred areas include the rostrums and the posterior regions. As many locations as possible on the otoliths should be examined where the ring structure is clear, and the annual rings are visible. This usually involves counting at the rostrum and the posterior region until the reader is satisfied that consistent interpretation has been achieved. However, it is sometimes possible that other areas of the otolith are readable, e.g. the anti-rostrum, and interpretation of appropriate parts of the otolith should be considered, especially if one of the otoliths is broken, missing or crystalline.

Conflicting ages may be achieved if several parts of the otoliths are examined (usually in older fish). If this happens, the oldest age is probably the correct one, as examination of tagged fish otoliths of known minimum age has demonstrated that the highest age is more consistent with the information on the history of fish (ICES, 1987). Therefore, if in doubt about the interpretation of the rings assign the fish to the highest age but take the length of the fish into account.

7.3.5 False or Split Rings

It is always difficult to define the appearance of false or split rings on otoliths for any species. Usually these are properly identified only after much experience has been gained for a species. False or split rings are usually considered to be those rings that are not as well defined as annual rings. The reason for the deposition of false or split rings is not certain, but they might be caused by aberrant temperature, feeding or spawning conditions, stress or disease. One way to identify false rings is the observation of the ring growth pattern. Usually the annuli width decreases with the age and the presence of rings that not follow this pattern indicates that that ring is false. Once again, this can be difficult for older otoliths and the identification of false rings mostly depends on the experience of the reader.

7.3.6 Factors affecting annual ring formation

7.3.6.1 Formation of the first winter ring

Mackerel spawn from January to April in (Division 9a), from February to May in southern Biscay (Division 8c), March to July in the Celtic Sea and to the west of Ireland, from June to August in the North Sea (Divisions 4b and 3a) (Section 3). Therefore, the amount of time available for growth and the formation of the opaque zone in the first year will vary within and between areas. It is therefore reasonable to expect a large amount of variation in the length of the L1 (first year growth on the otolith) and this should be borne in mind when interpreting the first opaque and translucent zones. In addition to the variation in L1 between areas, it is also been demonstrated that there is considerable variation in the L1 between years for the Celtic Sea and the North Sea (Dawson, 1991). The reader, therefore, when interpreting the ring structure should be aware of sources of variation that may affect the nature of annual ring formation in the first year.

7.3.6.2 Age at maturity

The majority of mackerel otoliths that have been examined show a change in the pattern of ring formation that is presumably associated with the onset of maturity. Usually, growth slows down when the fish diverts much of its energy into gonad maturation. The resultant effect on the otolith is that for juvenile fish a large amount of opaque growth is produced between much narrower translucent rings. After maturity, growth slows down and both the opaque and the translucent rings become narrower and therefore closer together.

The above description is only a guide to the pattern of ring formation and obviously there is much variation in the age at maturity within an area as well as between areas. It is also possible that this change in the pattern of ring formation associated with maturity is not present. Sometimes otoliths may be observed to have very regular, clearly defined ring formation with only a linear decline in growth rate.

7.3.6.3 Reduced growth in very old fish

In most young and middle-aged fish, the growth pattern is well defined on the otolith with clear contrasting opaque and hyaline zones. However, in old fish, growth often slows down to such an extent that the opaque and translucent (hyaline) zones become confused and more difficult to distinguish. That portion of the otolith will have a greyish appearance. When this type of ring formation is observed, the reader usually finds that the translucent (hyaline) rings are very closed together and difficult to identify. However, usually each narrow translucent (hyaline) ring and opaque ring represents one year's growth.

8 Collation of a set of agreed age otoliths (part of ToR e)

An otolith reference collection will be available for all mackerel readers at the workshop ICES SharePoint and the Age Forum site. This collection will include those otoliths images with >80% agreement between Expert readers. Also, this reference collection will include the images of the otoliths used during the Small exchange with Norwegian otoliths carried out during the workshop.

9 Recommendations for further cooperation, exchanges, workshops and other actions in relation to the age estimation of Mackerel (part of ToRe)

9.1 General recommendations

The workshop achieved quite a lot in terms of ironing out, through discussion and calibration, some of the major problems in ageing mackerel otoliths. The group reached agreement in the interpretation of many difficult otoliths discussed during the workshop. Also, the group revised and completed the ageing protocol/guidelines approved in last workshop with the aim to employ these guidelines to eliminate some of the problems with e.g. split rings in the otolith structures. The group strongly recommends that all ageing laboratories processing mackerel should include the guidelines revised during the workshop in their ageing manuals. If possible, the ICES system should facilitate the distribution of these guidelines to all relevant laboratories. For the sake of continuity, it is highly recommended that new readers are trained by experienced readers prior to delivering data to the assessment on mackerel. The workshop exercise clearly showed a difference in the level of agreements between experienced readers and those with less or none experience. Also, it is strongly recommended that all readers whose estimations are used in mackerel assessment attend these workshops on age estimation of mackerel, as it is through the discussion of different interpretations of the age of difficult and/or old otoliths that most agreements are reached.

During last workshop it became apparent that the various life-history traits for the mackerel have changed recently and that knowledge of this is highly important for the age readers. In addition, all age readers would benefit from more information on the formation of otolith structures in mackerel, especially the formation of split rings and the seasonally dependent appearance of the otolith edge. Thus, the group recommends the inclusion of such studies on otolith formation in general for mackerel.

Below are some general recommendations by the group for further action.

9.1.1 Manual

The age reading manual produced during last workshop (WKARMAC, 2010) and updated at this workshop should be maintained and further developed in the future.

9.1.2 Standardised reading within laboratories (section taken from last workshop report, WKARMAC 2010).

It is essential that otolith readers, whether fully trained or otherwise, have their work quality controlled. There are two main reasons for this. The first is that by conducting quality control, extremely valuable evidence of the precision of an age determination programme can be obtained. It is vital that the ages assigned to otoliths that are used in assessments are assigned the "best" age, given the methods at our disposal. As the actual age of the fish is unknown, age determination experts need to ensure the age provided is as close to the actual age as possible and that the ages given are repeatable if the determinations are redone. By having two experts independently ageing the otoliths, we can give assurances about the reliability of the data.

Secondly, even the most experienced readers are capable of drifting away from their training over time and another reader looking at a sample of their reading to check consistency will ensure no drift occurs. This effort to ensure consistency of interpretation is further enhanced with the use of reference collections. The quality controller (QC) will be a very experienced reader in the species and probably the stock concerned. In this way, the effectiveness of the age determination programme to produce consistent results can be assessed and assured.

When a reader has determined the age of the fish, whether by being checked or not, the QC should be given approximately 150 otoliths from the 2nd and 3rd quarters to re-age. These should be selected by someone else (the Co-ordinator of Age Determination), who selects the otoliths to be quality controlled to ensure that the QC does not influence the results by their selection and that the whole of the length and age ranges are represented. The middle two quarters are chosen as they cover the main growth period and therefore the areas of likely uncertainty over the age. The otoliths should be read by the QC without knowledge of the ages previously assigned. The results are then compared, and any discrepancies notified to the reader to check.

It is anticipated that the agreement on this check will be at least 90%. Where QC agreement rates fall significantly below this level, the Co-ordinator of age determination should investigate the reasons with the reader and QC to see if there are any resolvable problems with the otoliths concerned. The QC should have instructions to follow in the event of a failure to make the agreed quality control agreement level and / or if the APE is greater than 3%. These can be seen in Table 8.1.2.1.

When the Co-ordinator of Age Determination is consulted at the end of this process, they should review the paperwork, the otoliths and the ages with both the reader and the QC, providing advice and guidance, attempting to resolve any unresolved ages with them. It is envisaged that by this point, enough of the differences can be resolved to ensure the agreement levels are reached. If this is not possible, the Co-ordinator of Age Determination will be the final arbiter of the age.

Table 9.1.2.1. Actions to be taken by the QC if agreement rate falls below the target.

Action required if QC result falls below target:	
% below target	Action required
- 2.0	Review disagreements with the reader and establish an agreed age. If this proves impossible, contact the Co-ordinator of Age Determination for advice.
2.1 – 5.0	Check for obvious errors the reader may have made (skipping fish or rows etc), establish the pattern in reading (under-ageing, over-ageing, edge problems etc). Review disagreements with the reader and establish an agreed age. If this proves impossible, contact the Co-ordinator of Age Determination for advice.
5.1+	Contact the Co-ordinator of Age Determination immediately.
Action required if the APE is greater than 3%:	
% Agreement	Action required
Above QC target	There may be a problem with the readers' interpretation of younger fish. Review disagreements with reader.
Below QC target	Contact Co-ordinator of Age Determination immediately as it may be necessary to conduct more checks than a standard QC.

9.1.3 Quality control between labs.

Quality control of age estimations between national laboratories can be achieved in at least two ways;

- The standard exchange programme and workshops under the ICES programme. Readers read a set of otoliths and are compared with a modal age. This provides a snapshot of the agreement between readers, both expert and trainees, for the year in which the exchange or workshop takes place. It is particularly useful as many laboratories can take part and results compared. It can be used to confirm the validity of a laboratory's ageing criteria and interpretation or show problems. Exchanges and workshops can be expensive, slow to organise and therefore results are slow in arriving. However, the use of otolith images at otolith exchanges during the last years has accelerated the process.
- A small-scale, ad hoc exchange or workshop between two or more laboratories looking at a specific stock or substock of the species, incorporating just those countries that have a direct interest in the stock. This type of approach is particularly useful to ensure that ages submitted to the stock assessment process are comparable. This type of exchange or workshop is inexpensive and can be done quickly to address the issues.

9.1.4 Regular workshops

The past frequency of workshops has been approximately decadal, which certainly does not cover the requirement for intra laboratory quality control of the age estimation of mackerel. Several factors need to be considered when deciding upon the frequency of workshops; there is the constant changing in behaviour of mackerel, which heavily influence the otolith morphology and thus the patterns to interpret. Also, there is a flow of age readers through the laboratories, which need to be considered. It should be ensured that all labs do have at least one age reader acquainted with the agreed guidelines for age estimations of mackerel. Additionally, there is always a need to update the flow of information between age readers, data collectors and end-users. Thus, during the last workshop (WKARMAC 2010) it was recommended the inclusion of both assessment experts and age readers in future workshops. It has proved very valuable for previous workshops, WKARMAC (2010) and WKARMAC2 (2018), to have a co-chair with experience in mackerel assessment present at the workshops during discussions of results and their potential consequences.

Also, it is recommended that the readers whose age estimations are included in mackerel assessment attend the workshops in age estimation of mackerel.

As a recommendation from WKARMAC2 participants, it would be good that during the next workshop exercise the otoliths would be also available in situ, so the readers can observe them under the binocular microscope, in conjunction with the image analysis.

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Annex 1: Agenda

Meeting agenda

Monday, October 22nd, 2018

10.00 – 10.30 Start of the meeting; Introduction round; Presentation of the agenda; Local and network arrangements; Brief overview of ToRs

10.30 – 11.15 Review information on age determination, otolith exchanges and validation techniques of this species done so far (ToR a);
Summary of the different techniques of chub mackerel otoliths preparation by laboratory.

11.15 – 11.30 Coffee break

11.30 – 12.00 Presentation of the biology of mackerel (ToR a);
Study of the otolith edge development (ToR a)

12.00 – 13.00 Presentation and discussion of the otolith exchanges results (Small exchange 2014 and pre-WKARMAC2 exercise), comparison of precision against modal age and bias; evaluation of levels of agreement among readers and laboratories (ToRs a and b)

13.00 – 14.30 Lunch break

14.30 – 15.45 Recap on the agreed manual for age estimation of mackerel; Identification of problems and difficulties in age estimation of mackerel, including on screen discussion of relevant otolith readings from exchanges (ToRs b and c)

15.45 – 16.00 Coffee break

16.00 – 17.30 Identification of problems and difficulties in age estimation of mackerel, including on screen discussion of relevant otolith readings from exchanges (ToRs b and e)

Tuesday, October 23rd, 2018

09.00 – 11.00 Identification of problems and difficulties in age estimation of chub mackerel, including on-screen discussion of relevant otolith readings from each area (ToRs b and e)

11.00 – 11.15 Coffee break

11.15 – 13.00 Identification of problems and difficulties in age estimation of chub mackerel, including on-screen discussion of relevant otolith readings from each area (ToRs b and e)

13.00 – 14.30	Lunch break
14.30 – 16.00	Identification of problems and difficulties in age estimation of chub mackerel, including on-screen discussion of relevant otolith readings from each area (ToRs b and e)
16.00 – 16.15	Coffee break
16.15 – 17.30	Recap on the agreed age reading criteria; Discussion on the validity of the current mackerel age reading manual. Final Report structure and assignment of responsibilities among participants (ToR e).
Wednesday, October 24 th , 2018	
09.00 – 11.00	WKARMAC2 calibration exercise (via SmartDots) (ToRs a,b,c,d,e)
11.00 – 11.15	Coffee break
11.15 – 13.00	WKARMAC2 calibration exercise (via SmartDots) (ToRs a,b,c,d,e)
13.00 – 14.30	Lunch break
14.30 – 16.00	WKARMAC2 calibration exercise (via SmartDots) / Final Report draft elaboration (ToRs a,b,c,d,e)
16.00 – 16.15	Coffee break
16.15 – 17.30	WKARMAC2 calibration exercise (via SmartDots) / Final Report draft elaboration (ToRs a,b,c,d,e)
Thursday, October 25 th , 2018	
09.00 – 11.00	Presentation of the preliminary results from the WKARMAC2 calibration exercise; comparison of precision against modal age and bias; evaluation of levels of agreement among readers and institutes (ToRs a,b,c,d,e)
11.00 – 11.15	Coffee break
11.15 – 13.00	Presentation of the preliminary results from the WKARMAC2 calibration exercise; comparison of precision against modal age and bias; evaluation of levels of agreement among readers and institutes (ToRs a,b,c,d,e)
13.00 – 14.30	Lunch break
14.30 – 16.00	On-screen discussion of relevant otolith readings from the WKARMAC2 calibration exercise; Identification of persistent problems and difficulties in age estimation of mackerel otoliths (ToR c)
16.00 – 16.15	Coffee break

16.15 – 17.30	On-screen discussion of relevant otolith readings from the WKARMAC2 calibration exercise; Identification of persistent problems and difficulties in age estimation of mackerel otoliths (ToR c); Creation of a reference collection (ToR d)
Friday, October 26 th , 2018	
09.00 – 11.00	Creation of a reference collection (ToR d) / Recommendations based on the Workshop results
11.00 – 11.15	Coffee break
11.15 – 13.00	Recommendations based on the Workshop results / Planning of future activities for enhancing quality in mackerel age determination
13.00	End of meeting

Annex 2: List of participants

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Annex 3: Recommendations

Recommendation	Adressed to:
1 WKARMAC2 recommends that all national databases are adapted according to the agreed mackerel age reading manual. This includes the introduction of a field for the edge structure of the otolith (opaque / hyaline) and the application of the reading grading system / quality indicators 1-3.	Workshop participants, WGBIOP
2. WKARMAC2 recommends that all ageing laboratories use the manual agreed by WKARMAC2.	Workshop participants, WGBIOP
3. WKARMAC2 recommends that exchanges and workshops on age reading of NEA mackerel will be held regularly. A exchange should be scheduled for 2020, a workshop should be scheduled for 2022.	WGBIOP
4. WKARMAC2 recommends that readers whose age estimations are used in assessment attend the workshops on age estimation of mackerel and participate in the exchanges	National Mackerel Age Coordinators, WGBIOP
5. WKARMAC2 recommends to record the nature of the otoliths edge and a study of the otolith edge formation by area.	Workshop participants, WGBIOP
6. WKARAMAC2 recommends the continuity of the Norwegian experiments of tag-recapture of mackerel, especially in order to validate older ages (> 5 years old).	Workshop participants, WGBIOP
7.WKARMAC2 recommends the realizaton of validation/corroboration studies of age estimation of NEA mackerel in all distribution areas (L1 width, length–frequency analysis, etc.)	National Mackerel Age coordinators, WGBIOP

Annex 4: Contributions to the Workshop. Presentations and Working Documents.

During the workshop a total of 6 presentations were performed. They all can be downloaded from ICES SharePoint:

https://community.ices.dk/ExpertGroups/WKARMAC2/_layouts/15/start.aspx#/SitePages/HomePage.aspx

The list of presentations is the following:

- **PRESENTATION 1:** Review information of the information on age determination, otolith exchanges and validation techniques of Atlantic mackerel (*Scomber scombrus*). By: Navarro, M.R. Instituto Español de Oceanografía (IEO). Santander. Spain. Presentation to WKARMAC2, San Sebastian (Spain), 22–26 October, 2018. Presented by Maria Rosario Navarro.
- **PRESENTATION 2: Otolith preparation techniques.** By: Navarro, M.R. Instituto Español de Oceanografía (IEO). Santander. Spain. Presentation to WKARMAC2, San Sebastian (Spain), 22–26 October, 2018. Presented by Maria Rosario Navarro.
- **PRESENTATION 3: Biology of mackerel.....try of a summary.** By: Ulleweit, J. Thünen Institute of Sea Fisheries, Hamburg, Germany. Presentation to WKARMAC2, San Sebastian (Spain), 22–26 October, 2018. Presented by Jens Ulleweit.
- **PRESENTATION 4:** Study of seasonal formation of growth rings in the otoliths of the NEA Mackerel (*Scomber scombrus*) in ICES Divisions 8c and 9a North. By: Villamor, B.; Navarro, M.R.; Hernández, C.; Dueñas-Liaño, C.; Antolínez, A. Instituto Español de Oceanografía (IEO). Santander, Spain. Presentation to WKARMAC2, San Sebastian (Spain), 22–26 October, 2018. Presented by Maria Rosario Navarro.
- **PRESENTATION 5: Results of the Small Scale Otolith Exchange for North East Atlantic Mackerel 2014.** By Ulleweit, J. Thünen Institute of Sea Fisheries, Hamburg, Germany. Presentation to WKARMAC2, San Sebastian (Spain), 22–26 October, 2018. Presented by: Jens Ulleweit.
- **PRESENTATION 6: Pre-WKARMAC2 exchange.** By: M.R. Navarro, Ulleweit, J. Instituto Español de Oceanografía (IEO). Santander, Spain. Presentation to WKARMAC2, San Sebastian (Spain), 22–26 October, 2018. Presented by: Maria Rosario Navarro.

(cont.)

Area	Sample code	Fish length	Landing month	JM	EH	IR	DL	CN	GD	GF	MJ	JT	AD	PV	NS	OS	MK	DM	SH	TS	GH	KD	TH	MI	AS	CW	MODAL
				R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R23	age
27.2.a	096_MAC_2a_Q3	380	8	-	6	6	6	5	4	7	-	5	-	7	6	8	7	5	5	6	-	7	6	-	-	8	6
27.2.a	097_MAC_2a_Q3	340	8	-	5	5	6	6	7	4	5	6	6	6	5	8	7	4	5	5	7	7	7	6	5	8	5
27.2.a	098_MAC_2a_Q3	350	8	-	5	5	4	-	5	5	6	4	5	5	4	7	6	4	5	6	7	7	4	8	4	5	5
27.2.a	099_MAC_2a_Q3	390	8	-	7	10	8	8	6	11	-	8	-	9	9	8	9	6	7	-	11	10	9	9	6	9	7
27.2.a	100_MAC_2a_Q3	370	8	7	8	8	7	7	7	8	7	7	8	8	7	9	8	6	8	7	8	8	7	7	7	7	7
27.2.a	101_MAC_2a_Q2	140	5	1	0	0	0	1	1	1	1	1	0	1	1	1	1	0	0	0	1	0	1	1	1	0	1
27.2.a	102_MAC_2a_Q2	320	5	3	3	3	3	3	3	3	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
27.2.a	103_MAC_2a_Q2	300	5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
27.2.a	104_MAC_2a_Q2	240	5	2	2	2	2	2	2	2	2	1	1	2	2	2	2	2	2	2	2	2	2	2	2	1	2
27.2.a	105_MAC_2a_Q2	420	5	-	13	-	12	-	-	-	-	-	-	-	-	-	-	10	-	-	-	11	-	-	-	12	
27.4.b	106_MAC_4b_Q1	160	2	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
27.4.b	107_MAC_4b_Q1	190	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	
27.4.b	108_MAC_4b_Q1	190	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
27.4.b	109_MAC_4b_Q1	180	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
27.4.b	110_MAC_4b_Q1	360	2	-	10	10	10	10	11	10	9	10	-	11	-	11	9	8	9	9	8	8	9	8	8	9	10
27.4.b	111_MAC_4b_Q3	350	7	5	5	5	5	5	6	4	5	5	5	5	6	5	5	5	5	5	6	5	4	4	6	5	3
27.4.b	112_MAC_4b_Q3	270	8	2	2	3	3	2	-	2	2	3	3	3	2	2	3	3	4	2	3	3	2	2	2	2	3
27.4.b	113_MAC_4b_Q3	360	8	-	6	6	6	6	5	6	5	6	-	6	6	6	6	5	6	6	6	6	6	5	5	6	6
27.4.b	114_MAC_4b_Q3	260	8	1	1	2	2	2	1	-	2	1	2	2	2	1	1	2	2	2	1	1	2	2	1	1	2
27.4.b	115_MAC_4b_Q3	210	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
27.7.j	116_MAC_7j_Q1	330	2	3	3	3	3	3	3	3	3	3	3	3	3	3	2	3	3	3	3	3	4	2	3	3	3
27.7.j	117_MAC_7j_Q2	350	4	4	4	4	4	4	4	4	3	4	4	3	4	4	4	3	4	4	4	4	4	3	4	4	4
27.7.j	118_MAC_7j_Q1	340	2	5	4	4	4	4	4	4	5	4	4	5	4	5	5	3	4	-	4	5	5	4	5	4	4
27.7.j	119_MAC_7j_Q1	370	2	6	7	7	5	6	5	6	6	6	7	7	6	6	6	3	-	5	7	6	6	5	6	6	
27.7.j	120_MAC_7j_Q1	330	2	-	5	5	4	5	4	5	5	9	5	5	4	5	5	3	5	-	5	4	5	4	3	3	5
27.7.j	121_MAC_7j_Q1	370	2	7	7	8	6	8	7	7	8	7	7	7	8	7	6	7	7	7	9	7	7	8	6	7	7
27.7.j	122_MAC_7j_Q1	370	2	9	9	9	8	9	9	9	8	9	9	9	8	9	7	9	8	9	9	9	7	8	9	8	9
27.7.j	123_MAC_7j_Q1	370	2	-	9	9	8	8	8	8	8	8	9	9	8	8	9	6	7	7	9	8	8	8	7	8	8
27.7.j	124_MAC_7j_Q1	370	2	-	-	9	10	-	7	9	-	7	9	10	10	8	5	10	6	8	-	9	7	8	6	9	9
27.7.j	125_MAC_7j_Q2	400	4	10	-	11	10	10	9	10	10	10	-	10	10	11	9	7	11	-	10	6	10	-	-	8	10
27.7.b	126_MAC_7b_Q1	310	3	3	2	2	2	2	2	2	3	2	2	2	2	2	2	2	3	2	2	3	2	2	2	3	2
27.7.b	127_MAC_7b_Q1	310	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
27.7.b	128_MAC_7b_Q1	320	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
27.7.b	129_MAC_7b_Q1	330	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
27.7.b	130_MAC_7b_Q1	350	2	5	5	6	6	-	5	6	6	4	5	6	5	6	5	4	5	5	6	5	5	6	5	6	5
27.7.b	132_MAC_7b_Q1	370	3	-	-	9	9	9	9	9	9	9	9	9	9	10	7	7	-	9	8	8	9	-	10	9	8
27.7.b	133_MAC_7b_Q1	380	3	-	8	8	7	7	7	8	7	8	9	8	8	9	8	6	8	8	7	7	7	8	7	7	8
27.7.b	134_MAC_7b_Q1	390	2	7	-	9	9	-	7	7	8	8	7	8	8	11	6	8	6	7	8	7	8	-	7	8	8
27.7.b	135_MAC_7b_Q1	420	2	-	-	-	9	-	-	-	-	9	-	9	-	-	7	10	-	-	-	-	-	-	-	7	9
22	136_MAC_MED_Q4	311	12	-	-	-	-	2	1	-	2	1	-	-	2	2	-	2	2	2	1	2	-	1	1	-	2
22	137_MAC_MED_Q4	202	12	-	1	1	-	1	1	-	1	-	1	-	1	1	-	0	1	1	0	0	1	1	0	1	1
22	138_MAC_MED_Q4	273	12	-	1	1	-	1	1	-	1	-	1	-	2	2	-	1	1	-	0	1	1	1	1	-	1
22	139_MAC_MED_Q4	230	10	-	1	2	-	1	1	-	1	-	1	-	2	1	-	1	2	1	1	-	-	1	2	1	1
22	140_MAC_MED_Q4	222	10	-	1	1	-	1	1	1	1	-	-	0	1	1	-	1	2	1	1	-	-	1	2	-	1
22	141_MAC_MED_Q4	273	12	-	1	1	-	1	1	-	1	-	-	0	1	3	-	1	2	-	0	-	1	1	1	-	1
22	142_MAC_MED_Q4	298	12	-	2	3	-	3	2	-	3	-	-	2	3	4	-	3	3	2	2	2	2	1	2	-	2
22	143_MAC_MED_Q4	259	10	-	1	1	-	1	1	-	2	-	-	0	2	2	1	1	1	1	1	1	1	1	1	-	1
22	144_MAC_MED_Q4	204	10	-	1	1	-	1	1	-	1	-	-	0	1	1	1	1	1	-	0	0	1	1	1	-	1

5.1. All readers' analysis.

Table 5.1.1. Summary of the average percentage of agreement, CV and relative bias by age for all readers.

Modal Age	% agreem.	CV (%)	Bias
0	98	239.8	0.02
1	86	34.3	0.05
2	77	20.7	0.07
3	83	14.0	0.01
4	68	13.3	0.04
5	57	15.6	0.02
6	58	14.3	0.09
7	56	10.4	-0.01
8	51	11.3	-0.15
9	48	12.5	-0.45
10	40	13.5	-0.52

Table 5.1.2. Inter-reader bias test and reader against modal age bias test (all readers' analysis).

	JM R1	EH R2	IR R3	DL R4	CN R5	GD R6	GF R7	MJ R8	JT R9	AD R10	PV R11	NS R12	OS R13	MK R14	DM R15	SH R16	TS R17	GH R18	KD R19	TH R20	MI R21	AS R22	CW R23
R1 JM	**	**	**	—	**	—	**	**	**	**	**	*	**	**	**	—	**	**	**	**	**	—	—
R2 EH	**	**	—	**	—	**	—	—	—	*	**	—	**	—	**	*	—	*	—	—	—	**	*
R3 IR	**	—	**	**	*	**	—	—	**	—	*	**	—	—	**	**	*	—	—	—	—	**	**
R4 DL	—	**	**	**	**	—	**	**	**	**	**	*	**	**	**	*	**	**	**	**	**	**	—
R5 CN	**	—	*	**	**	**	—	—	—	**	**	—	**	*	**	*	—	*	—	—	—	**	*
R6 GD	—	**	**	—	**	**	**	*	*	**	**	—	**	**	**	—	*	**	**	**	**	**	—
R7 GF	**	—	—	**	—	**	—	—	*	*	—	**	—	*	**	**	—	—	—	—	—	**	**
R8 MJ	**	—	—	**	—	*	—	—	—	—	—	—	*	—	**	*	—	—	—	—	—	**	*
R9 JT	**	—	**	**	—	*	*	—	—	**	**	—	**	**	**	—	—	**	*	—	*	**	—
R10 AD	**	*	—	**	**	**	*	—	**	—	—	*	—	—	**	**	—	—	—	—	—	**	**
R11 PV	**	**	*	**	**	**	—	*	**	—	—	**	—	—	**	**	—	—	—	*	—	**	**
R12 NS	*	—	**	*	—	—	**	—	—	**	**	—	**	**	**	—	—	**	*	*	**	—	—
R13 OS	**	**	—	**	**	**	**	*	**	—	—	**	—	*	**	**	—	—	—	*	—	**	**
R14 MK	**	—	—	**	*	**	—	—	**	—	—	**	*	—	**	**	—	—	—	—	—	**	**
R15 DM	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
R16 SH	—	*	**	*	*	—	**	*	—	**	**	—	**	**	**	—	*	**	**	*	**	*	—
R17 TS	**	—	—	*	—	*	—	—	—	—	—	—	—	—	**	*	—	—	—	—	—	*	*
R18 GH	**	*	—	**	*	**	—	—	**	—	—	**	—	—	**	**	—	—	—	—	—	**	**
R19 KD	**	—	—	**	—	**	—	—	*	—	—	*	—	—	**	**	—	—	—	—	—	**	**
R20 TH	**	—	—	**	—	**	—	—	—	—	*	**	*	—	**	*	—	—	—	—	—	**	*
R21 MI	**	—	—	**	—	**	—	—	*	—	—	**	—	—	**	**	—	—	—	—	—	**	**
R22 AS	—	**	**	—	**	—	**	**	**	**	**	—	**	**	**	*	*	**	**	**	**	**	—
R23 CW	—	*	**	—	*	—	**	*	—	**	**	—	**	**	**	—	*	**	**	*	**	*	—
Modal Age	**	—	*	**	—	**	*	—	—	**	**	*	**	*	**	*	—	*	—	—	*	**	**

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

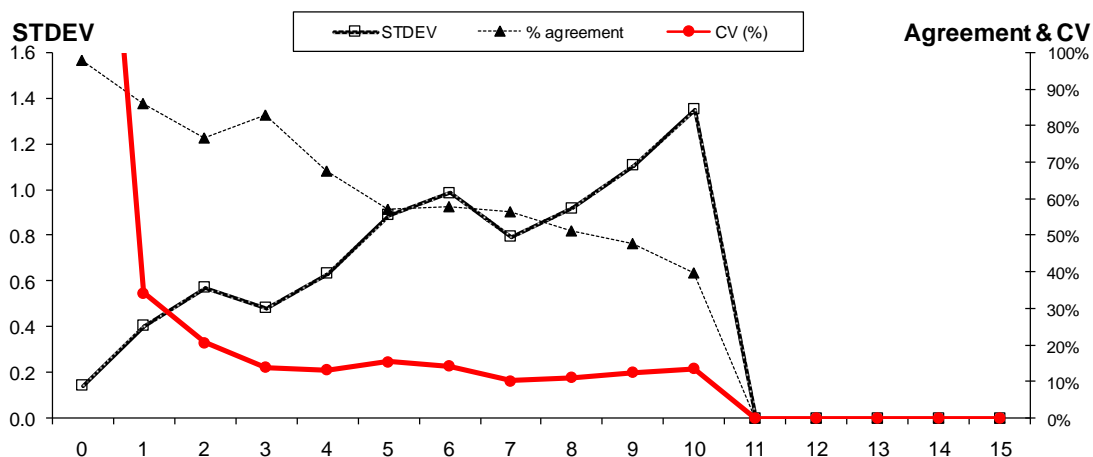


Figure 5.1.1. Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age (all reader's analysis).

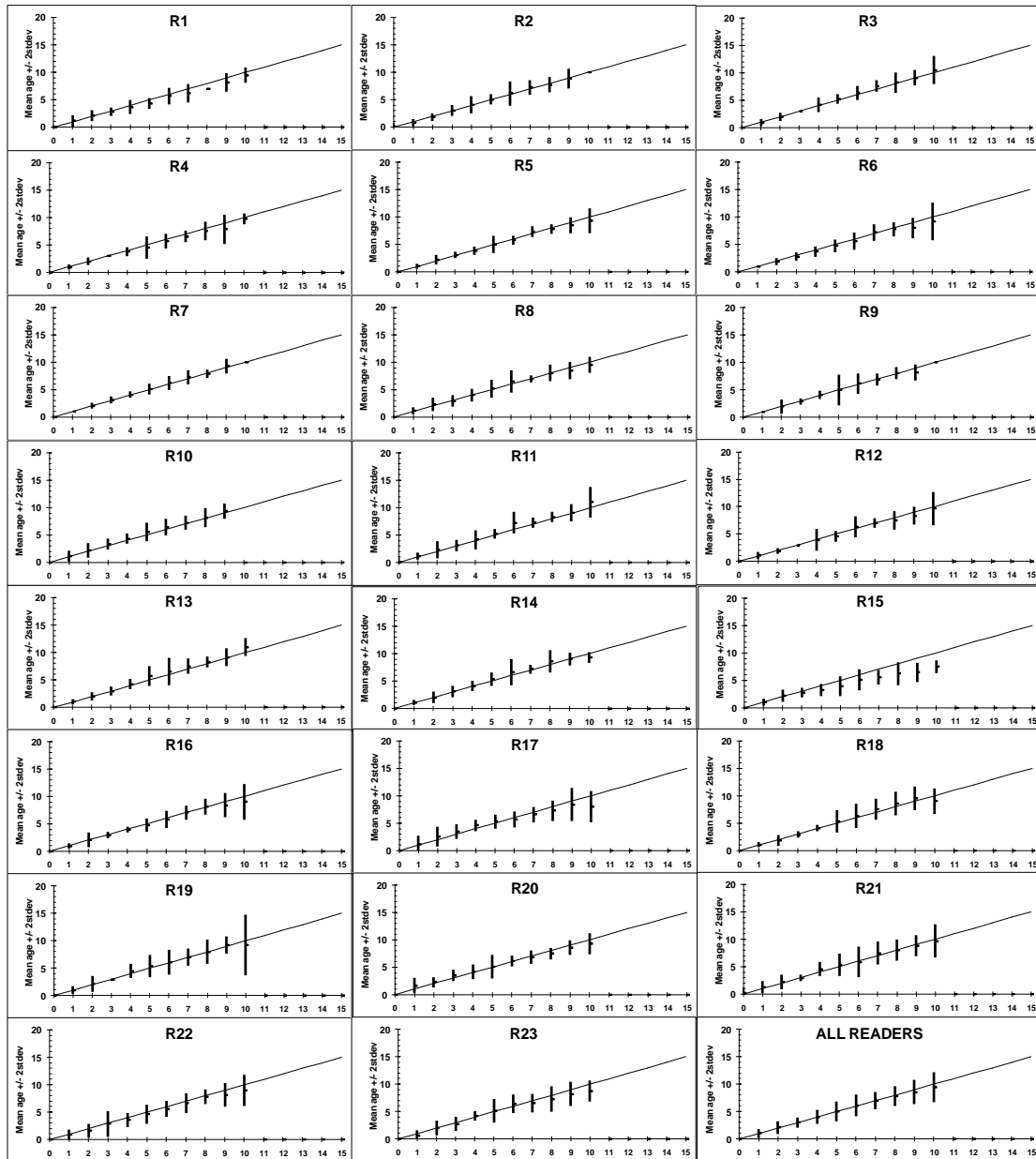


Figure 5.1.2. Age bias plot with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (all readers' analysis).

5.2. Expert readers' analysis.

Table 5.2.1. Summary of the average percentage of agreement, CV and relative bias by age for Expert readers.

Modal Age	% agreem.	CV (%)	Bias
0	98	90.1	0.02
1	91	17.3	0.04
2	85	13.0	0.08
3	84	10.7	0.09
4	78	8.9	-0.02
5	68	14.0	-0.05
6	62	12.2	0.08
7	71	7.5	0.09
8	55	9.1	-0.07
9	61	9.7	-0.21
10	53	9.9	-0.03

Table 5.2.2. Reader against modal age bias test (Expert readers' analysis).

	EH R2	IR R3	DL R4	CN R5	GD R6	GF R7	MJ R8	JT R9	AD R10	PV R11	NS R12	OS R13	MK R14
Modal Age	—	*	**	—	**	—	—	—	**	**	*	**	—

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

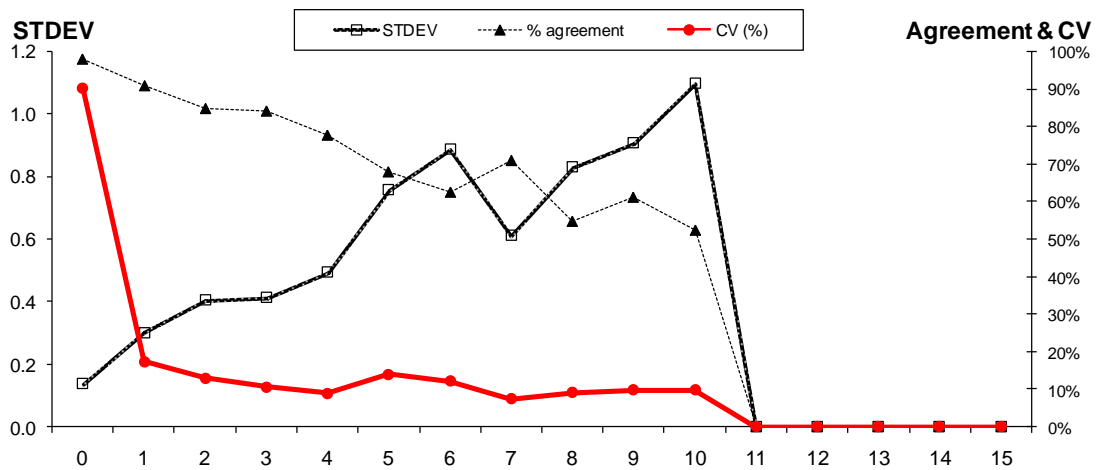


Figure 5.2.1. Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age (Expert reader's analysis).

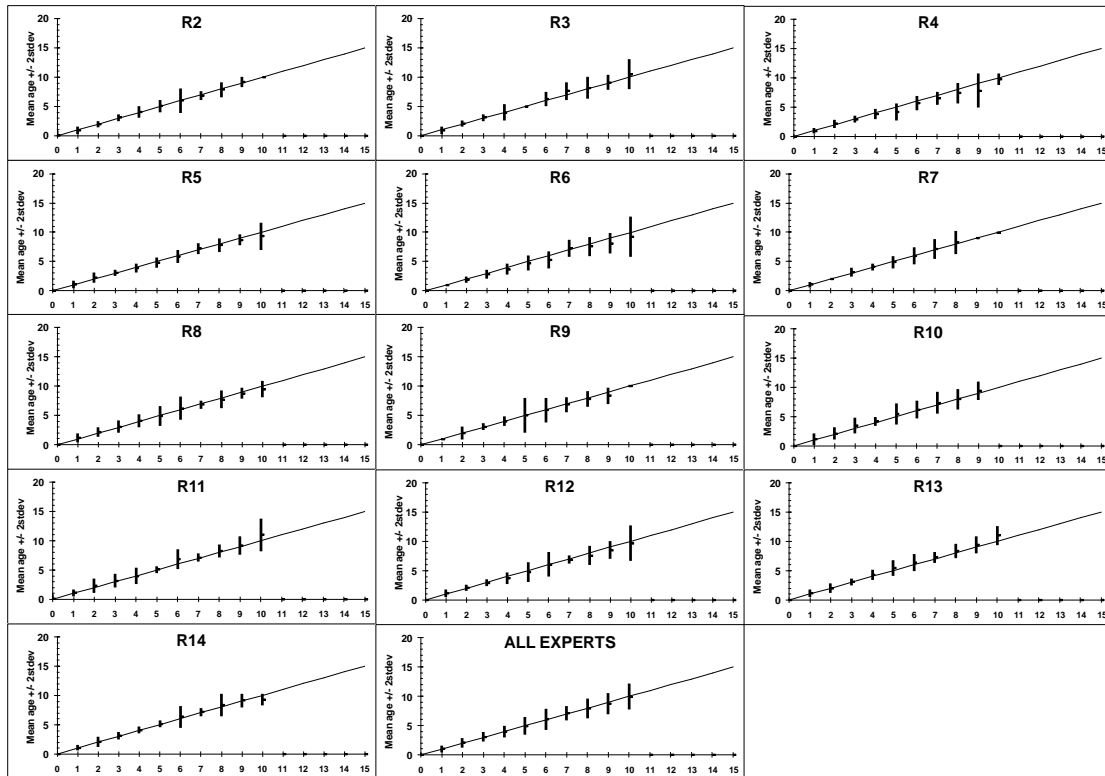


Figure 5.2.2. Age bias plot with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (Expert readers’ analysis).

5.3. Trainee readers’ analysis.

Table 5.3.1. Summary of the average percentage of agreement, CV and relative bias by age for Trainee readers.

Modal Age	% agreem.	CV (%)	Bias
0	92	74.7	0.08
1	77	43.3	0.11
2	77	21.1	0.03
3	76	20.2	-0.01
4	67	13.7	0.13
5	55	15.4	0.1
6	57	10.6	0.32
7	57	11.1	-0.04
8	50	10.7	-0.01
9	49	10.2	-0.34
10	45	16.1	-0.27

Table 5.3.2. Reader against modal age bias test (Trainee readers' analysis).

	SH R16	TS R17	GH R18	KD R19	TH R20	MI R21	AS R22	CW R23
Modal Age	—	—	**	*	—	**	**	—

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

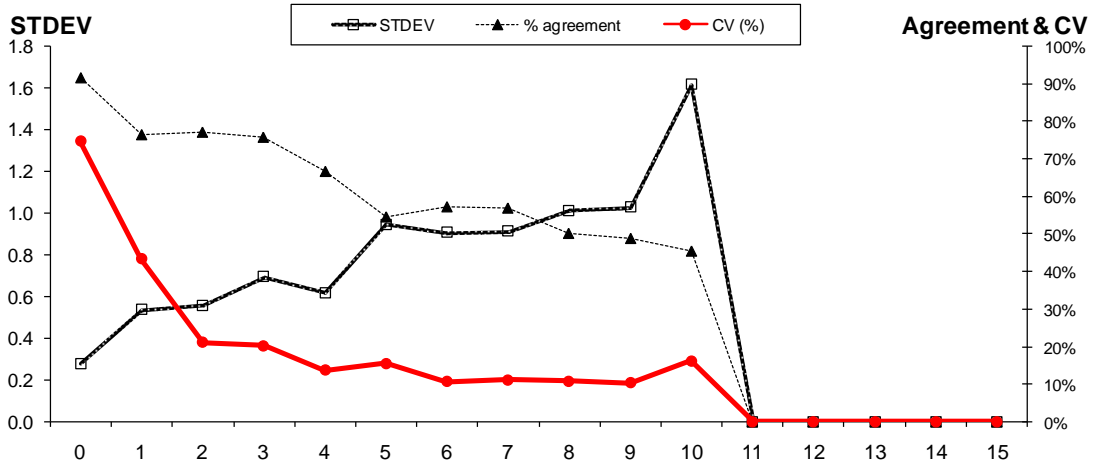


Figure 5.3.1. Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age (Trainee reader's analysis).

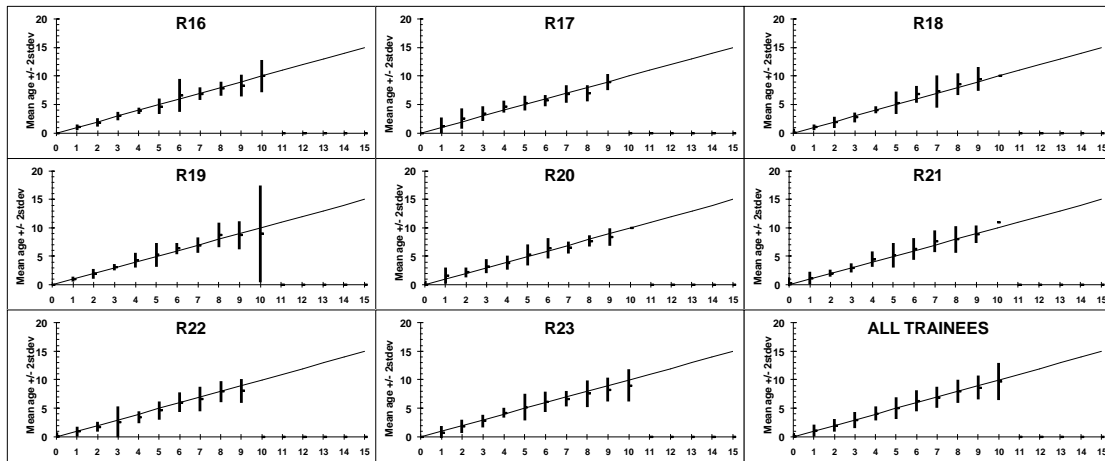


Figure 5.3.2. Age bias plot with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (Trainee readers' analysis).

5.4. Analysis by area (All readers).

5.4.1. Southern component analysis (All readers).

Table 5.4.1.1. Summary of the average percentage of agreement, CV and relative bias by age for All readers (Southern component analysis).

Modal Age	% agreem.	CV (%)	Bias
0	97	319.7	0.03
1	80	34.8	0.18
2	77	17.0	0.23
3	83	14.1	0.06
4	57	16.8	0.03
5	50	20.3	-0.55
6	52	17.7	0.2
7	55	10.4	-0.28
8	56	-	-0.15

Table 5.4.1.2. Inter-reader bias test and reader against modal age bias test (All readers, Southern component analysis).

	JM R1	EH R2	IR R3	DL R4	CN R5	GD R6	GF R7	MJ R8	JT R9	AD R10	PV R11	NS R12	OS R13	MK R14	DM R15	SH R16	TS R17	GH R18	KD R19	TH R20	MI R21	AS R22	CW R23	
R1 JM		-	-	-	-	-	-	-	-	*	**	-	-	*	**	-	-	-	-	-	-	-	-	
R2 EH	-		-	*	-	-	-	-	-	-	**	-	-	-	**	-	-	-	-	-	-	-	-	
R3 IR	-	-		**	-	*	-	-	-	-	*	-	-	-	**	-	-	-	-	-	-	*	*	
R4 DL	-	*	**		**	-	*	**	**	**	**	**	**	**	*	-	*	*	*	**	**	-	-	
R5 CN	-	-	-	**		-	-	-	-	*	*	-	-	-	**	-	-	-	-	-	-	*	-	
R6 GD	-	-	*	-	-		-	*	*	**	**	*	**	**	**	-	-	-	-	-	*	-	-	
R7 GF	-	-	-	*	-	-		-	-	*	*	-	*	*	**	-	-	-	-	-	-	*	-	
R8 MJ	-	-	-	**	-	*	-		-	-	-	-	-	-	**	-	-	-	-	-	-	**	*	
R9 JT	-	-	-	**	-	*	-	-		-	*	-	-	-	**	-	-	-	-	-	-	**	*	
R10 AD	*	-	-	**	*	**	*	-	-		-	-	-	-	**	*	-	-	*	*	-	**	**	
R11 PV	**	**	*	**	*	**	*	-	*	-		*	-	-	**	**	-	*	*	*	-	**	**	
R12 NS	-	-	-	**	-	*	-	-	-	*	-		-	-	**	-	-	-	-	-	-	*	*	
R13 OS	-	-	-	**	-	**	*	-	-	-	-	-		-	**	-	-	-	-	-	-	**	*	
R14 MK	*	-	-	**	-	**	*	-	-	-	-	-	-		**	*	-	-	*	-	-	**	**	
R15 DM	**	**	**	*	**	**	**	**	**	**	**	**	**	**		**	**	**	**	**	**	**	-	*
R16 SH	-	-	-	-	-	-	-	-	-	*	**	-	-	*	**		-	-	-	-	*	-	-	
R17 TS	-	-	-	*	-	-	-	-	-	-	-	-	-	-	**	-		-	-	-	-	-	-	
R18 GH	-	-	-	*	-	-	-	-	-	*	-	-	-	-	**	-	-		-	-	-	-	-	
R19 KD	-	-	-	*	-	-	-	-	-	*	*	-	-	*	**	-	-	-		-	*	-	-	
R20 TH	-	-	-	**	-	-	-	-	-	*	*	-	-	*	**	-	-	-	-		-	**	-	
R21 MI	-	-	-	**	-	*	-	-	-	-	-	-	-	-	**	*	-	-	*	-		**	**	
R22 AS	-	-	*	-	*	-	*	**	**	**	**	*	**	**	-	-	-	-	*	**	**		-	
R23 CW	-	-	*	-	-	-	-	*	*	**	**	*	*	**	*	-	-	-	-	-	**	-	-	
Modal Age	-	-	*	*	-	-	-	-	-	**	**	-	*	*	**	-	-	-	-	-	*	*	-	

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

5.4.2. Western component analysis (All readers).

Table 5.4.2.1. Summary of the average percentage of agreement, CV and relative bias by age for All readers (Western component analysis).

Modal Age	% agreem.	CV (%)	Bias
0	98	191.8	0.02
1	78	-	0.13
2	65	30.3	0
3	89	10.6	-0.02
4	71	11.4	0.04
5	70	13.6	-0.03
6	64	12.0	-0.13
7	67	10.6	-0.2
8	44	-	-0.19
9	56	-	-0.4
10	45	13.8	-0.79

Table 5.4.2.2. Inter-reader bias test and reader against modal age bias test (All readers, Western component analysis).

	JM R1	EH R2	IR R3	DL R4	CN R5	GD R6	GF R7	MJ R8	JT R9	AD R10	PV R11	NS R12	OS R13	MK R14	DM R15	SH R16	TS R17	GH R18	KD R19	TH R20	MI R21	AS R22	CW R23	
R1 JM		-	*	-	-	-	-	*	-	-	-	*	-	-	**	-	-	-	-	-	*	-	-	
R2 EH	-		-	*	-	*	-	-	-	-	-	*	-	-	**	-	-	-	-	-	-	**	-	
R3 IR	*	-		*	-	**	-	-	-	-	-	**	-	-	**	-	-	-	-	-	-	**	-	
R4 DL	-	*	*		*	-	-	-	-	-	*	-	**	-	**	-	-	-	-	-	-	-	-	
R5 CN	-	-	-	*		-	-	-	-	-	-	-	-	-	**	-	-	-	-	-	-	-	-	
R6 GD	-	*	**	-	-		**	*	-	*	**	-	**	*	**	-	-	*	-	-	*	-	-	
R7 GF	-	-	-	-	-	**		-	-	-	-	-	-	-	**	-	-	-	-	-	-	**	-	
R8 MJ	*	-	-	-	-	*	-		-	-	-	-	-	-	**	-	-	-	-	-	-	*	-	
R9 JT	-	-	-	-	-	-	-		-	-	-	-	-	-	**	-	-	-	-	-	-	-	-	
R10 AD	-	-	-	-	-	*	-	-		-	-	-	-	-	**	-	-	-	-	-	-	**	-	
R11 PV	*	-	-	*	-	**	-	-	-		-	*	-	-	**	-	-	-	-	-	-	**	*	
R12 NS	-	*	**	-	-	-	-	-	-	-		*	-	*	-	**	-	-	-	-	-	-	-	
R13 OS	*	-	-	**	-	**	-	-	-	-	-		*	-	**	*	-	-	-	*	-	**	*	
R14 MK	-	-	-	-	-	*	-	-	-	-	-	-		-	**	-	-	-	-	-	-	*	-	
R15 DM	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
R16 SH	-	-	-	-	-	-	-	-	-	-	-	-	*	-	**	-	-	-	-	-	-	-	-	
R17 TS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**	-	-	-	-	-	-	-	-	
R18 GH	-	-	-	-	-	*	-	-	-	-	-	-	-	-	**	-	-	-	-	-	-	*	-	
R19 KD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**	-	-	-	-	-	-	**	-	
R20 TH	-	-	-	-	-	-	-	-	-	-	-	-	*	-	**	-	-	-	-	-	-	-	-	
R21 MI	*	-	-	-	-	*	-	-	-	-	-	-	-	-	**	-	-	-	-	-	-	*	-	
R22 AS	-	**	**	-	-	-	**	*	-	**	**	-	**	*	*	-	-	*	**	-	*	-	-	
R23 CW	-	-	-	-	-	-	-	-	-	-	*	-	*	-	**	-	-	-	-	-	-	-	-	
Modal Age	-	-	-	-	-	**	-	-	-	-	-	-	*	-	**	-	-	-	-	-	-	**	-	

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

5.4.3. North Sea component (All readers).

Table 5.4.3.1. Summary of the average percentage of agreement, CV and relative bias by age for All readers (North Sea component analysis).

Modal Age	% agreem.	CV (%)	Bias
0	-	-	-
1	93	28.6	0
2	77	28.4	-0.17
3	71	20.9	-0.07
4	-	-	-
5	67	11.3	-0.11
6	75	8.0	-
7	-	-	-
8	-	-	-
9	-	-	-
10	30	11.0	-

Table 5.4.3.2. Inter-reader bias test and reader against modal age bias test (All readers, North Sea component analysis).

	JM R1	EH R2	IR R3	DL R4	CN R5	GD R6	GF R7	MJ R8	JT R9	AD R10	PV R11	NS R12	OS R13	MK R14	DM R15	SH R16	TS R17	GH R18	KD R19	TH R20	MI R21	AS R22	CW R23
R1 JM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	**	-	-	-
R2 EH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	*	-
R3 IR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	*	-
R4 DL	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	*	-
R5 CN	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	*	-	*	*	**	-
R6 GD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	*	-
R7 GF	-	-	-	-	-	-	-	*	-	-	-	-	-	-	*	-	-	*	-	-	*	*	*
R8 MJ	-	-	-	-	*	-	*	-	-	-	-	-	-	-	-	-	*	-	-	**	-	-	-
R9 JT	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	*	-
R10 AD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	**	-	-	-
R11 PV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	-	-	*	*
R12 NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	*	-
R13 OS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	*
R14 MK	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	*	-	*	-
R15 DM	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	*	-	-	**	-	*	-
R16 SH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	*	*	**	-
R17 TS	*	-	-	-	-	-	-	*	-	*	-	-	-	*	*	-	-	*	-	*	-	*	*
R18 GH	-	-	-	-	*	-	*	-	-	*	-	-	-	-	-	*	*	-	*	-	**	-	-
R19 KD	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	*	-	*	-
R20 TH	**	*	*	*	*	*	-	**	*	**	-	*	-	*	**	*	-	**	*	*	**	**	**
R21 MI	-	-	-	-	*	-	*	-	-	-	-	-	-	-	*	*	-	-	**	-	**	-	-
R22 AS	-	*	*	*	**	*	*	-	*	-	*	*	*	*	*	**	**	-	*	**	-	-	-
R23 CW	-	-	-	-	-	-	*	-	-	-	*	-	*	-	-	-	*	-	-	**	-	-	-
Modal Age	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	*	-	*	*	**	-

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

5.4.4. Northern distribution (All readers).

Table 5.4.4.1. Summary of the average percentage of agreement, CV and relative bias by age for All readers (Northern distribution analysis).

Modal Age	% agreem.	CV (%)	Bias
0	-	-	-
1	61	-	-0.39
2	93	9.2	-0.07
3	96	6.9	0.04
4	76	-	0.04
5	48	16.8	0.29
6	58	12.0	0.16
7	46	10.1	0.38
8	53	-	-0.14
9	44	12.7	-0.47
10	40	15.0	-

Table 5.4.4.2. Inter-reader bias test and reader against modal age bias test (All readers, Northern distribution analysis).

	JM R1	EH R2	IR R3	DL R4	CN R5	GD R6	GF R7	MJ R8	JT R9	AD R10	PV R11	NS R12	OS R13	MK R14	DM R15	SH R16	TS R17	GH R18	KD R19	TH R20	MI R21	AS R22	CW R23
R1 JM	**	**	**	-	**	*	**	**	*	**	**	-	**	**	*	*	*	**	**	**	**	**	*
R2 EH	**	-	*	-	-	-	-	-	-	-	-	*	**	-	**	-	-	**	*	-	*	-	-
R3 IR	**	-	-	**	-	*	-	-	**	-	-	**	*	-	**	**	-	**	*	-	-	-	-
R4 DL	-	*	**	-	*	-	**	*	-	**	**	-	**	**	**	-	-	**	**	**	**	*	-
R5 CN	**	-	-	*	-	-	*	-	-	-	*	*	**	*	**	*	-	**	**	-	-	-	-
R6 GD	*	-	*	-	-	-	*	-	-	*	**	-	**	**	**	-	-	**	**	*	**	-	-
R7 GF	**	-	-	**	*	*	-	**	-	-	**	-	-	**	**	-	**	*	-	-	-	-	-
R8 MJ	**	-	-	*	-	-	-	-	-	-	-	*	-	-	**	*	-	*	*	-	-	-	-
R9 JT	*	-	**	-	-	-	**	-	-	**	**	-	**	**	**	-	-	**	**	*	**	-	-
R10 AD	**	-	-	**	-	*	-	-	**	-	-	**	-	-	**	**	-	-	-	-	-	-	*
R11 PV	**	-	-	**	*	**	-	-	**	-	-	**	-	-	**	**	-	*	-	-	-	-	*
R12 NS	-	*	**	-	*	-	**	*	-	**	**	-	**	**	**	-	-	**	**	**	**	-	-
R13 OS	**	**	*	**	**	**	-	-	**	-	-	**	-	**	**	**	**	**	-	-	**	-	**
R14 MK	**	-	-	**	**	**	-	-	**	-	-	**	**	-	**	**	-	**	**	-	-	-	*
R15 DM	*	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
R16 SH	*	-	**	-	-	-	**	*	-	**	**	-	**	**	**	-	-	**	**	*	**	-	-
R17 TS	*	-	-	-	-	-	-	-	-	-	-	-	**	-	**	-	-	**	**	-	*	-	-
R18 GH	**	**	**	**	**	**	**	*	**	-	*	**	-	**	**	**	**	**	-	**	*	**	**
R19 KD	**	*	*	**	**	**	**	*	**	-	-	**	-	**	**	**	**	**	-	**	**	-	**
R20 TH	**	-	-	**	-	*	-	-	*	-	-	**	**	-	**	*	-	**	**	-	-	-	-
R21 MI	**	*	-	**	-	**	-	-	**	-	-	**	-	-	**	**	*	*	-	-	-	-	**
R22 AS	**	-	-	*	-	-	-	-	-	-	-	*	-	**	**	-	-	**	**	-	-	-	-
R23 CW	*	-	-	-	-	-	-	-	-	*	*	-	**	*	**	-	-	**	**	-	**	-	-
Modal Age	**	-	-	**	-	*	*	-	*	*	*	**	**	*	**	*	-	**	**	-	*	-	-

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

5.5. Analysis by area (Expert readers).

5.5.1. Southern component analysis (Expert readers).

Table 5.5.1.1. Summary of the average percentage of agreement, CV and relative bias by age for Expert readers (Southern component analysis).

Modal Age	% agreem.	CV (%)	Bias
0	97	120.2	0.03
1	83	32.2	0.14
2	85	11.4	0.15
3	80	14.1	0.03
4	61	14.1	-0.14
5	53	18.4	-0.21
6	58	16.1	0.32
7	69	7.8	-0.05
8	70	6.8	-0.03

Table 5.5.1.2. Reader against modal age bias test (Expert readers, Southern component analysis).

	EH R2	IR R3	DL R4	CN R5	GD R6	GF R7	MJ R8	JT R9	AD R10	PV R11	NS R12	OS R13	MK R14
Modal Age	—	—	**	—	*	—	—	—	*	**	—	*	—

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

5.5.2. Western component analysis (Expert readers).

Table 5.5.2.1. Summary of the average percentage of agreement, CV and relative bias by age for Expert readers (Western component analysis).

Modal Age	% agreem.	CV (%)	Bias
0	98	72.1	0.02
1	81	18.3	0.19
2	91	21.1	0.13
3	79	9.2	0.18
4	81	7.4	0.04
5	87	10.1	0.05
6	68	7.8	-0.13
7	81	6.0	0.04
8	49	10.0	0.19
9	69	-	-0.06
10	57	-	-0.33

Table 5.5.2.2. Reader against modal age bias test (Expert readers, Western component analysis).

	EH R2	IR R3	DL R4	CN R5	GD R6	GF R7	MJ R8	JT R9	AD R10	PV R11	NS R12	OS R13	MK R14
Modal Age	—	—	—	—	*	—	—	—	—	—	—	*	—

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

5.5.3. North Sea component (Expert readers).

Table 5.5.3.1. Summary of the average percentage of agreement, CV and relative bias by age for Expert readers (North Sea component analysis).

Modal Age	% agreem.	CV (%)	Bias
0	-	-	-
1	99	3.0	-0.01
2	78	13.4	0.02
3	88	10.5	0.12
4	-	-	-
5	77	9.5	-0.08
6	82	-	-0.18
7	-	-	-
8	-	-	-
9	-	-	-
10	55	-	0.09

Table 5.5.3.2. Reader against modal age bias test (Expert readers, North Sea component analysis).

	EH R2	IR R3	DL R4	CN R5	GD R6	GF R7	MJ R8	JT R9	AD R10	PV R11	NS R12	OS R13	MK R14
Modal Age	—	—	—	—	—	—	—	—	—	—	—	—	—

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

5.5.4. Northern distribution (Expert readers).

Table 5.5.4.1. Summary of the average percentage of agreement, CV and relative bias by age for Expert readers (Northern distribution analysis).

Modal Age	% agreem.	CV (%)	Bias
0	-	-	-
1	69	-	-0.31
2	92	10.2	-0.08
3	96	4.5	0.04
4	96	3.5	-0.04
5	59	16.0	0.02
6	59	12.0	0.05
7	-	-	-
8	52	9.5	-0.16
9	56	10.8	-0.29
10	38	17.0	-

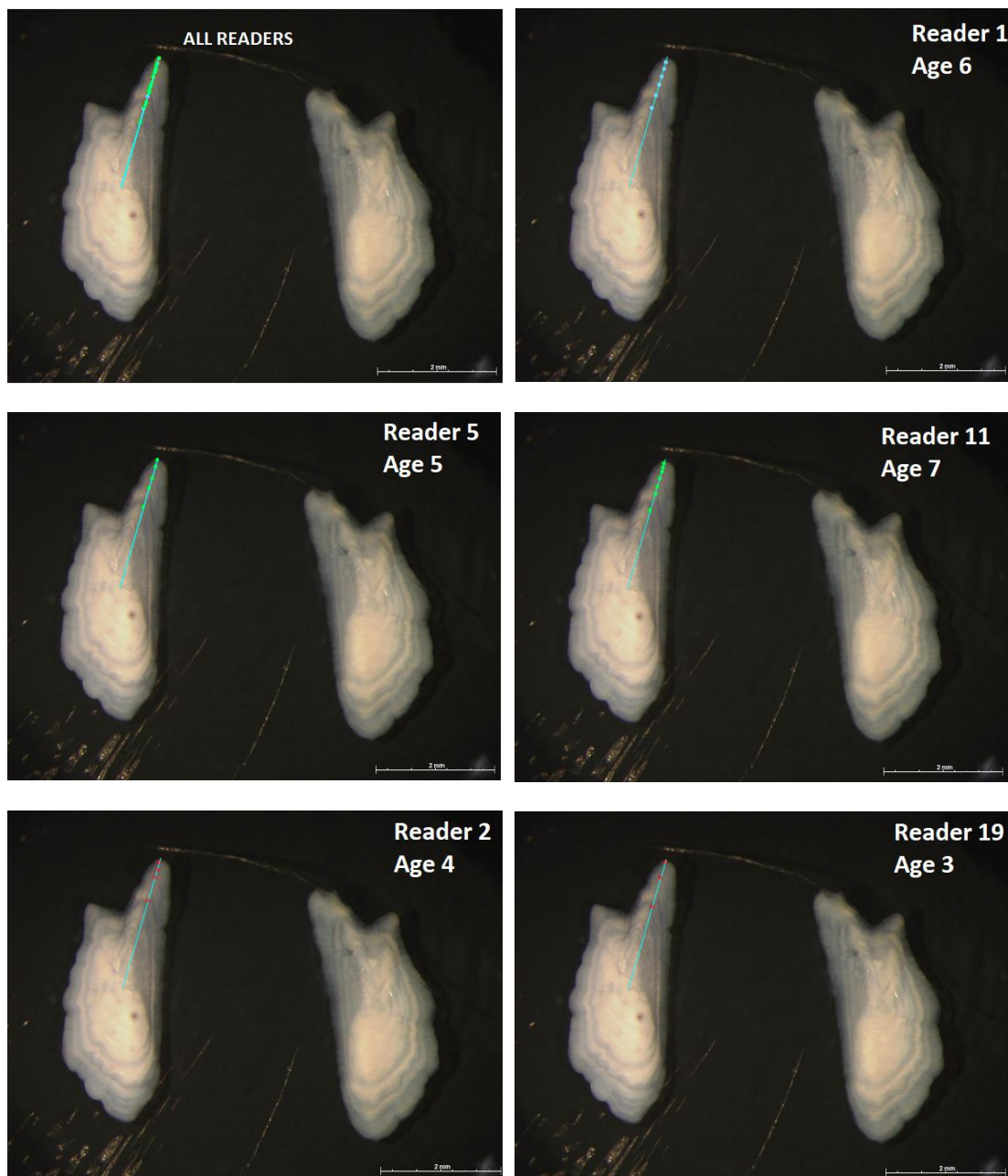
Table 5.5.4.2. Reader against modal age bias test (Expert readers, Northern distribution analysis).

	EH R2	IR R3	DL R4	CN R5	GD R6	GF R7	MJ R8	JT R9	AD R10	PV R11	NS R12	OS R13	MK R14
Modal Age	-	-	**	-	**	-	-	**	-	*	**	**	-

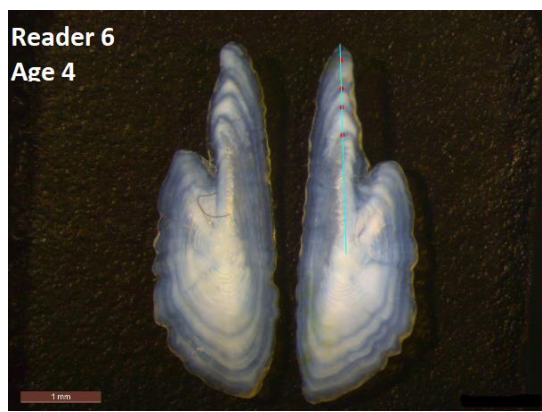
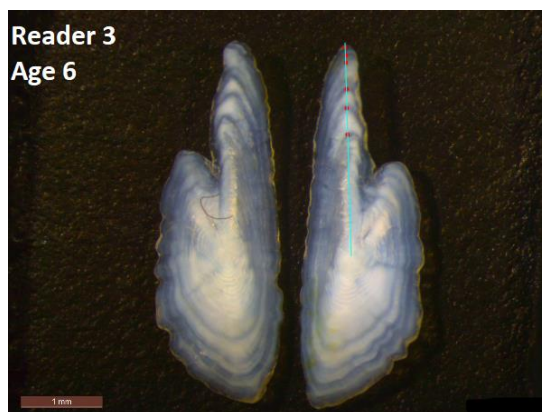
- ** certainty of bias (<0.01)
- * possibility of bias (0.01<p<0.05)
- no sign of bias (p>0.05)

5.6. Relevant otoliths with low agreement.

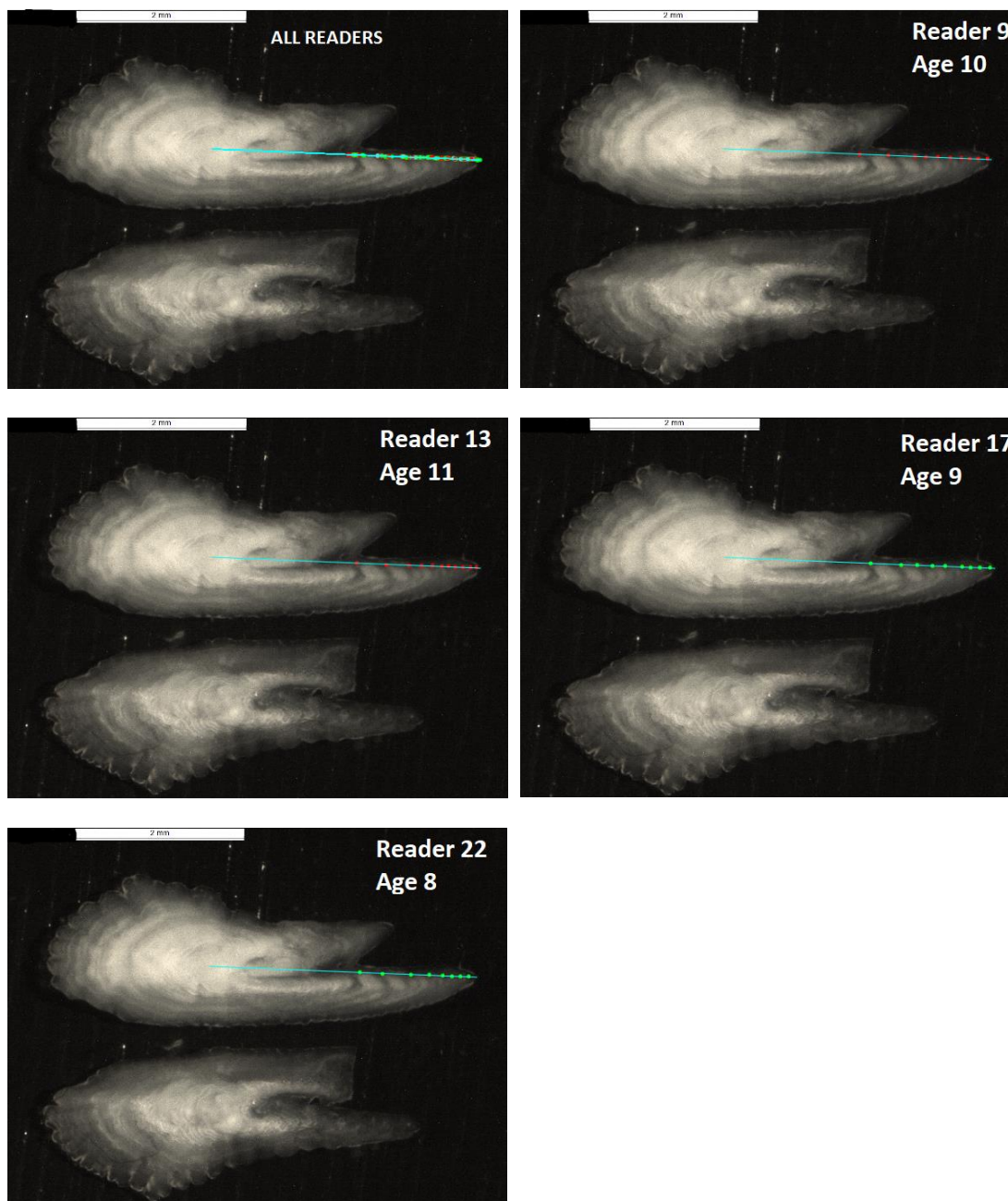
015_MAC_9aCN_Q2. ICES Div.: 9aCN; Month of catch: April; Fish length: 324mm; Modal age 6; Agreement: 28% (Experts' modal age: 6; Agreement: 40%).



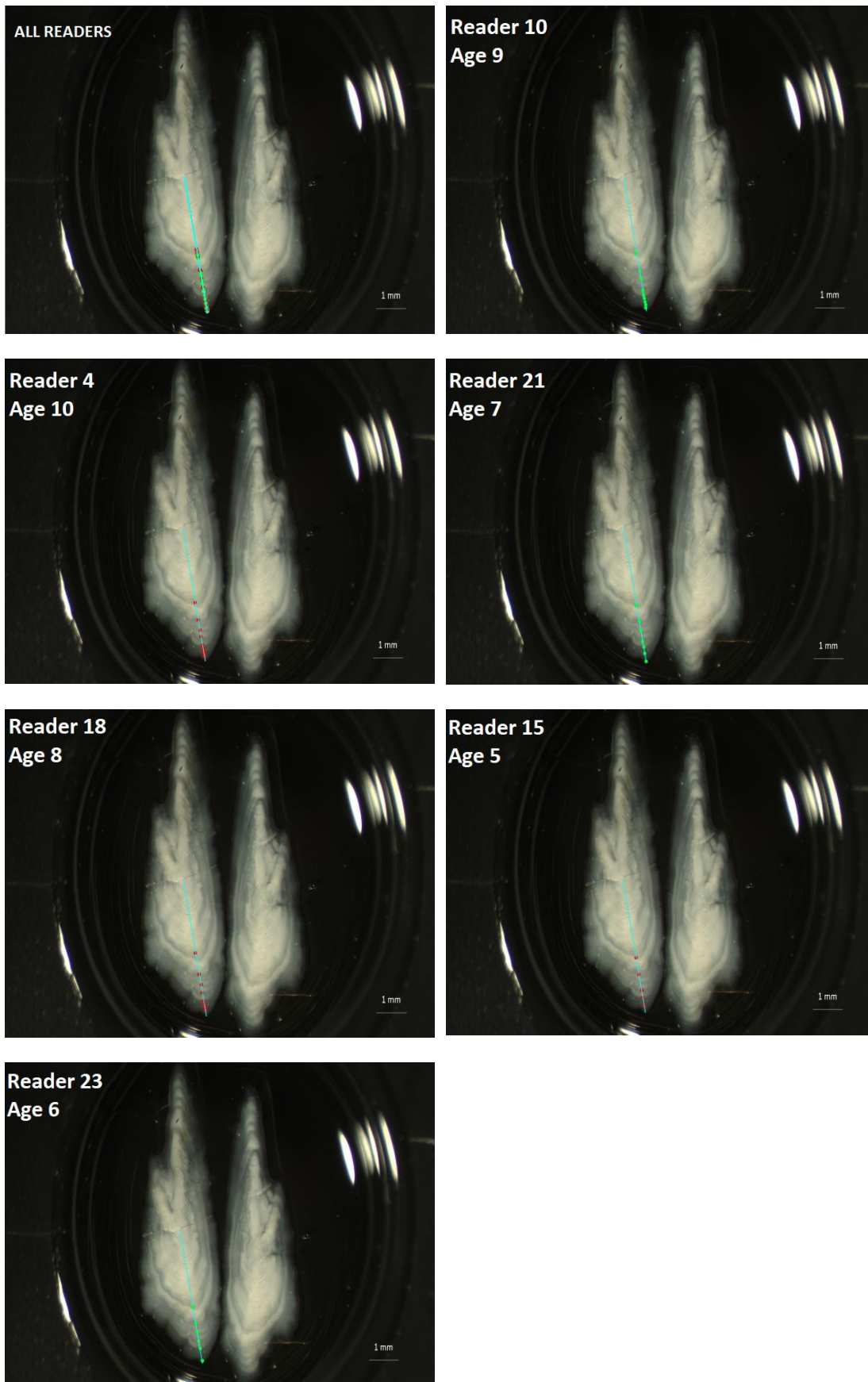
097 MAC 2a Q3. ICES Div.: 2a; Month of catch: August; Fish length: 340mm; Modal age: 5;
Agreement: 30% (Experts' modal age: 6, Agreement: 46%).



110 MAC 4b O1. ICES Div.: 4b; Month of catch: February; Fish length: 360mm; Modal age: 10;
Agreement: 30% (Experts' modal age: 10; Agreement: 55%).



124_MAC_7j_O1. ICES Div.: 7j; Month of catch: February; Fish length: 370mm; Modal age: 9;
Agreement: 28% (Experts' modal age: 9; Agreement: 40%).



Annex 7: Report of the Pre-WKARMAC2 exercise (*Scomber scombrus*).

Pre-WKARMAC2 exercise of *Scomber scombrus* otoliths. Coordinated by María Rosario Navarro and Jens Ulleweit. 3–18 October 2018.

1. Introduction

Atlantic mackerel (*Scomber scombrus*) is a pelagic species of high commercial importance in European waters. Age estimation of mackerel otoliths is an important factor in mackerel assessment and carried out by several laboratories throughout Europe using internationally agreed ageing criteria.

A Workshop on Age Reading of Atlantic mackerel (*Scomber scombrus*) otoliths was recommended by WGBIOP 2015 (ICES 2015) to be carried out in 2016-2017. It was not possible to enlist a chair for the workshop in this timeframe and so the workshop was recommended again by WGBIOP 2017 (ICES 2016) to be carried out in 2018.

The last exchange was completed in 2014, and several otolith readers had been replaced by new readers during that time. Therefore, it was decided to carry out a new otolith exchange a few weeks before the workshop. This exchange would provide more accurate information about the level of agreement of current readers. Additionally, the exchange would provide otolith images with the participants' readings to be discussed during the workshop as the program used in last exchange, WebGR, is no longer available, not even for the otolith reading discussion.

2. Material and Methods.

The exchange was carried out via SmartDots (<http://www.ices.dk/marine-data/tools/Pages/smardots.aspx>), the web application developed by ICES to facilitate the setup of Exchanges, Workshops and Training events. A total of 135 otolith images from the main areas of mackerel distribution were included in the exchange. Following the recommendations of WKMACQI (Workshop on Mackerel biological parameter Quality Indicators) (ICES 2018), it was attempted that the spatial and temporal coverage, as well as the length and age range, of the mackerel otoliths of the exchange corresponded with the coverage in the assessment (Table 2.1).

Table 2.1. Spatial and temporal coverage of the otoliths used in the pre-WKARMAC2 exercise.

Component	ICES Area	subarea	Nº images		Length range (cm)	Institute providing data
			Sem 1	Sem 2		
Southern component	8c	8cE	5		35-38	AZTI (Spain)
		8cW	2	6	17-41	IEO (Spain)
		8cW	6	6	20-39	
	9a	9aN	3	2	21-38	IPMA (Portugal)
		9aCN	5	5	25-42	
Western component	7	7b	10		31-42	MI (Ireland)
		7j	10		33-40	
		7d	5	5	25-44	WMR (Netherlands)
	8abde	8b		5	34-38	AZTI (Spain)
				5	14-21	IEO (Spain)
North Sea component	4	4b	5	5	16-36	DTU-Aqua (Denmark)
		4c		10	23-35	Thünen-Institute (Germany)
	2	2a	5		14-42	DTU-Aqua (Denmark)
Northern distribution	2	2a		5	34-39	MFR (Iceland)
			5a	5	5	32-38
	5b	5	5	32-39		
	14	14b		5	35-40	
TOTAL			71	64		
			135			

Otolith images from areas 2b, 4a, 6a and 6b were also requested to the laboratories that work with otoliths of these areas but were not provided on time.

18 participants from 10 countries (11 laboratories) participated in the exchange. Two more readers (from The Netherlands and Greece) started doing the exchange but only managed to mark a few otoliths, so their readings were removed from the exchange. Another two readers (from Scotland) found problems to install the program in their computers and were not able to do the exchange on time. Readers were ranked as Experts and Trainees considering the years of experience estimating the age of Atlantic mackerel. As Expert readers were considered those participants with more than four years of experience. Moreover, Expert readers coincided with the readers involved in mackerel assessment in their countries (Table 2.2).

Table 2.2. Readers of pre-WKARMAC2 exercise and their level of expertise.

Reader No	Name	Laboratory	Country	Reading level
R1	Eilert Hermansen	IMR	Norway	Expert
R2	Iñaki Rico	AZTI	Spain	Expert
R3	Deirdre Lynch	Marine Institute	Ireland	Expert
R4	Charo Navarro	IEO	Spain	Expert
R5	Gertrude Delfs	Thünen-Institut	Germany	Expert
R6	Gudrun Finnbogadóttir	MFRI	Iceland	Expert
R7	Maria Jarnum	DTU Aqua	Denmark	Expert
R8	Naiara Serrano	AZTI	Spain	Expert
R9	Orjen Sorensen	IMR	Norway	Expert
R10	Merete Kvalsund	IMR	Norway	Expert
R11	Delfina Morais	IPMA	Portugal	Expert
R12	Athanasios Spetsiotis	FRI	Greece	Trainee
R13	Kate Downes	CEFAS	UK	Trainee
R14	Clara Dueñas	IEO	Spain	Trainee
R15	Gitta Hemken	Thünen-Institut	Germany	Trainee
R16	Selene Hoey	Marine Institute	Ireland	Trainee
R17	Tim Huijer	WMR	The Netherlands	Trainee
R18	Andreia Silva	IPMA	Portugal	Trainee

Age readings results were analysed using the GussEltink spreadsheet (Eltink, 2000). Although SmartDots application can generate an automatic analysis of the results, due

to the limited time available to obtain the results before the workshop and that the application still has some limitations when selecting the options of the analysis, it was decided to use the Eltink spreadsheet for the analysis instead.

3. Results.

A table with the participants' readings can be found in Annex 7.2 (Table 7.2.1). In addition to the estimation of the age of the exchange otoliths, readers were asked to assign the quality to each reading according to the "3 point grading system" (AQ1, AQ2, AQ3) recommended by WKNARC (ICES, 2011). Readings with AQ3 were not included in the analyses. Analyses were performed for the total of areas and all readers and Expert and Trainee readers separately. Additional analyses were performed by each of the four areas of mackerel distribution: Southern component (ICES div. 9a, 8c), Western component (ICES div. 8b, 7bjd), North Sea component (ICES div. 4bc) and Northern distribution (ICES div. 2a, 5ab, 14b). A summary with the overall agreement, CV and bias of all analyses are shown in Table 3.1. The Figures and Tables showing the results of each analysis can be found in Annex 7.2.

Table 3.1. Summary of % agreement, CV and bias obtained in the analysis of Atlantic mackerel readings of pre-WKARMAC2 exercise.

Analysis	% agreement	CV (%)	Bias
All	59.4	37.3	-0.05
Experts	65.2	17.6	-0.07
Trainees	56.5	36.4	0.28
Southern component	61.3	54.4	0.11
Western component	58.1	35.9	-0.08
North Sea component	77.9	34.5	-0.01
Northern distribution	48.2	20.8	-0.24

Overall agreement was 59.4%, considerably lower than last exchange in 2014 (68.2%) (Ulleweit, 2014). The best agreement was obtained for otoliths of age 0 (96%) and ages 1-3 (79, 75 and 73%, respectively). Ages over 6 had less than 50% agreement (Annex2, Table 2.1.1). Overall CV was 37.3%, higher than last exchange (15.4%). CV peaked at 45% for modal age 1. Lowest values were obtained for modal age 10, 16% (only one otolith), and modal ages 3, 4, 6 and 9 with values between 17-19% (Annex 7.2, Table 7.2.1.1).

The Expert readers' analysis showed better results, with 65.2% of agreement (75% agreement in last exchange in 2014). The best agreement was obtained for otoliths of age 0 (100%) and ages 1-3 (84, 75 and 77% respectively). Ages over 7 had 50% or less agreement. CV was 17.6%, better than all readers' analysis but still higher than last exchange (9.3%) (Annex 7.2, Table 7.2.2.1).

The Trainee readers' analysis showed worse results, with lower agreement (56.5%) and higher CV (36.4%) than All readers' analysis.

By component, the best result was obtained in the North Sea component analysis, with 77.9% agreement, followed by the Southern component analysis with 61.3% agreement. The worst result was obtained in the Northern distribution analysis, with only 48.2% agreement.

The modal age range was 0-11 for All readers and Expert readers. For Trainee readers the modal age range was 0-10. By component, the modal age ranges were 0-8 (South component), 0-10 (West component), 1-9 (North Sea component) and 1-11 (North distribution).

The mean length-at-age obtained from the readings of all readers is shown in Table 7.2.1.3 and Figure 7.2.1.1 (Annex 7.2). Mean length-at-age 0 is 18cm for all readers. Mean length-at-age 1 is 23cm, at age 2 is 29-30cm, at age 3 is 31-32cm and at age 4 is 34cm for most readers. The value is more variable between readers for ages 5 and older. Reader 16 showed lower values of mean length at all ages compared with the other readers and Reader 12 showed higher values at some ages compared with the other readers. This trend is a bit different for mean length-at-age obtained in the analysis of each component (Annex 7.2, Table 7.2.4.3, Table 7.2.5.3, Table 7.2.6.3 and Table 7.2.7.3).

The results of the inter-reader bias test and reader against modal age bias test are shown in Table 7.2.1.2 (Annex 7.2). Only readers 2, 3, 6, 8, 15 and 17 showed no bias against the modal age (all readers' analysis). Most readers showed no bias with some of the other readers, but in general there was quite a bit of bias between readers. Readers 12, 13 and 16 showed bias with all the other readers. The results from Expert readers

and Trainee readers' analysis were similar (Annex 7.2, Table 7.2.2.2 and Table 7.2.3.2). When performing the inter-reader bias test and reader against modal age bias test by component, the best results were obtained for the North Sea component where only reader 16 showed bias against the modal age and all the other readers, while the other readers showed no bias between them or against the modal age (Annex 7.2, Table 7.2.6.2). In the bias test of the Southern component, readers 2, 3, 5, 6, 7, 8, 14 and 15 showed no bias against the modal age. Readers 9, 10, 11 and 16 showed bias with most of the other readers, while the other readers showed bias only with some of the other readers (Annex 7.2, Table 7.2.4.2). In the bias test of the Western component, readers 1, 2, 5, 6, 8, 15 and 17 showed no bias against the modal age. Readers 7, 10, 11, 12, 13, 14, 16 and 18 showed bias with most readers (Annex 7.2, Table 7.2.5.2). Last, in the bias test of the Northern distribution, readers 1, 2, 3, 4, 8, 8, 15 and 18 showed no bias against the modal age. Readers 6, 7, 11, 12, 13, 14 and 16 showed bias with most readers (Annex 7.2, Table 7.2.7.2).

Figure 7.2.1.2 (Annex 7.2) shows age bias plots with the mean age recorded and the standard deviation of each age reader and all readers combined plotted against the modal age. Readers 2, 4, 1 and 15 showed a more accurate estimation according to the modal age. Readers 5, 6, 7, 8, 10, 14, 17 and 18 showed underestimations in older ages regarding the modal age. Reader 3 showed a bit overestimation of ages 5-7 and then underestimation of older ages regarding the modal age. Readers 9 and specially reader 16 showed overestimation of all ages regarding the modal age. Last, readers 11, 12 and 13 showed underestimations of most ages regarding the modal age. As the overall agreement between readers is lower with older ages, the standard deviations are also mostly higher for the older ages for all readers combined (Annex 7.2, Figure 7.2.1.3). Similar trends are shown for Experts and Trainees separately (Annex 7.2, Figures 7.2.2.1, 7.2.2.2, 7.2.3.1 and 7.2.3.2). This trend is shown also by component, with a few differences. For example, for the Southern component, reader 1 showed over estimation in most ages regarding the modal age. Also, reader 6 showed a more marked underestimation of older ages regarding the modal age (Annex 7.2, Figure 7.2.4.2).

4. Discussion.

The exchange was carried out using the SmartDots application, which made the whole exchange process quite easy. As this is a new application, for most readers this was the first time using the program but once all readers became familiar with the use of the tool it proved to be very beneficial, though some readers did not know how to use all the applications within the software, such as the selection of brightness of the otolith images, which would have helped in the age estimation. Also, the exclusive use of images has the disadvantage that the readers find it difficult to identify the nature of the otolith edge, which can make the age interpretation problematic, in some cases. In addition, the use of a standardized reading line for all readers in each otolith image, makes the comparison between readings easier, it can complicate marking the annuli on the otolith when they are better observed in another area of the otolith. However, the use of images allows a better comparison between the readers' estimations and a better identification of the problems in locating false rings, as well as speeding up the process. The use of SmartDots is especially useful for a posterior discussion on screen of the most significant otoliths during the workshop.

Average percentage of agreement (59.4%) and CV (37.3%) for all components and all readers is far from satisfactory. The results of the Expert readers were better than the results of all readers (65.2% of agreement, 17.6% CV), whereas the results of the Trainee readers were slightly worse than the results of all readers (56.5% of agreement, 36.4%

CV. The results by component seem to be much better for the North Sea component (77.9% of agreement, 34.5% CV), whereas worst results were obtained for the Northern distribution (48.2% of agreement, 20.8% CV) (Table 3.1).

When comparing this exchange results with the previous exchange (2014), there has been a drastic decrease in the level of agreement, both for all readers and Experts (Table 4.1). This can be due to the renovation of readers produced since last exchange (2014). Only 9 readers of the present exchange participated as well in the previous one. From them, only 3 remain as Experts, most expert readers in the present exchange participated as Intermediate and Trainee in the previous one. All Trainee readers of the present exchange are new readers and did not participated in last exchange.

Table 4.1. % Agreement and CV for all readers' and Expert readers' analysis of the Pre-WKARMAC2 exercise (2018) and the Small Scale Otolith Exchange (2014).

Analysis	2014		2018	
	% agreement	CV (%)	% agreement	CV (%)
All	68.2	15.4	59.4	37.3
Experts	75.5	9.3	65.2	17.6
Experts+Intermediates	70.0	13.9		

This decline of the % agreement and CV made it more important the carry out of the Workshop on Age Reading of Atlantic Mackerel (*Scomber scombrus*) [WKARMAC2].

From the 135 otoliths of the exchange, 36 otoliths had an agreement of more than 80% (Annex 2, Table 2.1), with modal age from 0-4. From these, only 4 otoliths had 100% of agreement, 3 of them with modal age 0 and one with modal age 3. There were also 10 otoliths with an agreement of 94% (modal age 0-3). All otoliths with modal age 5 or more had less than 80% agreement, also, those with less than 30% agreement had modal age from 4-11. The otolith with the lowest agreement (22%) had a modal age of 9. Most otoliths with more than 80% agreement are from ICES divisions 4c, 8b, 8c and 4b.

To sum up, the overall agreement was low with a drastic decline of the % agreement and CV regarding the previous exchange in 2014. There has been a renovation of the readers since last exchange which has been more drastic considering the last Workshop in 2010, which demands a recalibration of the readers. 36 otoliths from the 135 otoliths of the exchange have more than 80% agreement, and only 4 of them have 100% agreement. Otoliths with modal age of 5 or more had the lowest agreement (all otoliths with less than 30% agreement had modal age from 4-11).

The results of this exchange were discussed during the Workshop on Age Reading of Atlantic Mackerel (*Scomber scombrus*) [WKARMAC2], which took place the 22-26 October, in San Sebastian, Spain.

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Annex 7.1: Participants' List.

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Annex 7.2: Figures and Tables of pre-WKARMAC2 exercise.

Table 7.2.1. Overall readings (pre-WKARMAC2 exercise).

ICES Div.	Sample code	Fish Landing length month	EH NO	IR	ES	DL	LE	CN	ES	G5	DE	GF	IS	MJ	DK	NS	ES	OS	NO	MK	NO	DM	PT	ASP	GR	KD	GB	CD	ES	GH	DE	SH	IE	TH	NL	AS	PT	R18	HODAL	Percent agreement	Precision CV													
																																										R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13
27.4.c	MAC_001_4c_Q3	230	7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	89%	29%

7.2.1. All readers' analysis.

Table 7.2.1.1. Summary of the average percentage of agreement, CV and relative bias by age for all readers (pre-WKARMAC2 exercise).

Modal Age	Otolith No	%Agreement	CV	Bias
0	8	96	-	0.05
1	18	79	45.0	0.18
2	16	75	26.8	0.15
3	14	73	18.0	0.07
4	21	56	19.2	0.18
5	10	50	21.6	0.15
6	13	51	17.3	-0.07
7	10	41	21.6	-0.28
8	13	34	21.6	-0.74
9	10	39	18.9	-0.81
10	1	50	16.0	-
11	1	22	31.0	-
Total	135	59.4	37.3	-0.05

Table 7.2.1.2. Inter-reader bias test and reader against modal age bias test (pre-WKARMAC2 exercise; all readers' analysis).

	EH NO	IR ES	DL IE	CN ES	GD DE	GF IS	MJ DK	NS ES	OS NO	MK NO	DM PT	ASP GR	KD GB	CD ES	GH DE	SH IE	TH NL	AS PT
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18
R1	EH NO	**	*	-	**	**	**	-	*	*	**	**	**	**	-	**	**	**
R2	IR ES	**	-	*	**	-	**	-	**	**	**	**	**	**	-	**	-	**
R3	DL IE	*	-	*	**	-	**	-	**	**	**	**	**	**	-	**	-	**
R4	CN ES	-	*	*	**	**	**	-	**	**	**	**	**	**	-	**	**	**
R5	GD DE	**	**	**	**	-	**	**	**	**	**	**	**	**	**	**	*	-
R6	GF IS	**	-	-	**	-	**	*	**	**	**	**	**	**	-	**	-	**
R7	MJ DK	**	**	**	**	**	*	**	**	**	-	**	*	-	**	**	**	-
R8	NS ES	-	-	-	**	*	**	*	**	**	**	**	**	**	-	**	-	**
R9	OS NO	*	**	**	**	**	**	**	*	-	**	**	**	**	**	**	**	**
R10	MK NO	*	**	**	**	**	**	**	-	*	**	**	**	**	**	**	**	**
R11	DM PT	**	**	**	**	**	-	**	**	*	*	**	*	**	**	**	**	**
R12	ASP GR	**	**	**	**	**	**	**	**	**	*	*	**	**	**	**	**	**
R13	KD GB	**	**	**	**	**	*	**	**	*	*	*	*	**	**	**	**	**
R14	CD ES	**	**	**	**	**	-	**	**	**	**	**	**	*	**	**	**	-
R15	GH DE	-	-	-	**	-	**	-	**	**	**	**	**	**	*	**	-	*
R16	SH IE	**	**	**	**	**	**	**	**	**	**	**	**	**	**	*	**	**
R17	TH NL	**	-	-	**	*	-	**	-	**	**	**	**	**	-	**	*	-
R18	AS PT	**	**	**	**	-	**	-	**	**	**	**	**	-	*	**	-	*
MODAL AGE	**	-	-	**	**	-	**	-	**	**	**	**	**	**	-	**	-	**

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

Table 7.2.1.3. Mean length-at-age (pre-WKARMAC2 exercise, all readers' analysis).

Age	EH NO	IR ES	DL IE	CN ES	GD DE	GF IS	MJ DK	NS ES	OS NO	MK NO	DM PT	ASP GR	KD GB	CD ES	GH DE	SH IE	TH NL	AS PT	ALL
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	R13	R14	R15	R16	R17	R18	
0	189	189	189	189	189	189	188	189	189	183	188	185	183	189	183	180	187	189	187
1	231	240	232	223	247	236	235	226	228	234	241	265	238	237	242	199	226	231	235
2	296	303	295	293	314	302	286	274	275	278	311	304	297	300	303	258	288	302	294
3	324	318	326	309	318	317	327	319	315	318	321	344	339	335	326	312	326	324	325
4	334	349	346	343	351	346	348	339	332	328	347	363	365	348	354	313	349	348	346
5	349	352	348	353	348	349	368	348	352	353	375	380	366	359	355	330	362	354	355
6	360	361	355	362	365	369	362	361	351	352	381	360	369	369	358	350	359	370	363
7	360	369	370	369	369	371	375	365	364	369	375	-	363	376	373	359	367	397	369
8	368	382	373	366	385	365	380	387	377	366	-	-	380	389	369	362	374	370	373
9	374	385	396	387	377	390	-	372	381	380	-	-	380	385	369	376	376	376	380
10	392	375	410	390	360	400	-	400	380	398	-	-	-	420	388	381	449	385	390
11	360	-	-	-	-	-	-	-	383	400	-	-	-	-	400	375	-	-	381
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	350	-	-	350
13	-	-	380	-	-	-	-	-	-	-	-	-	-	-	-	400	-	-	390
14	-	-	-	-	-	-	-	-	383	380	-	-	-	-	-	380	-	-	381
15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

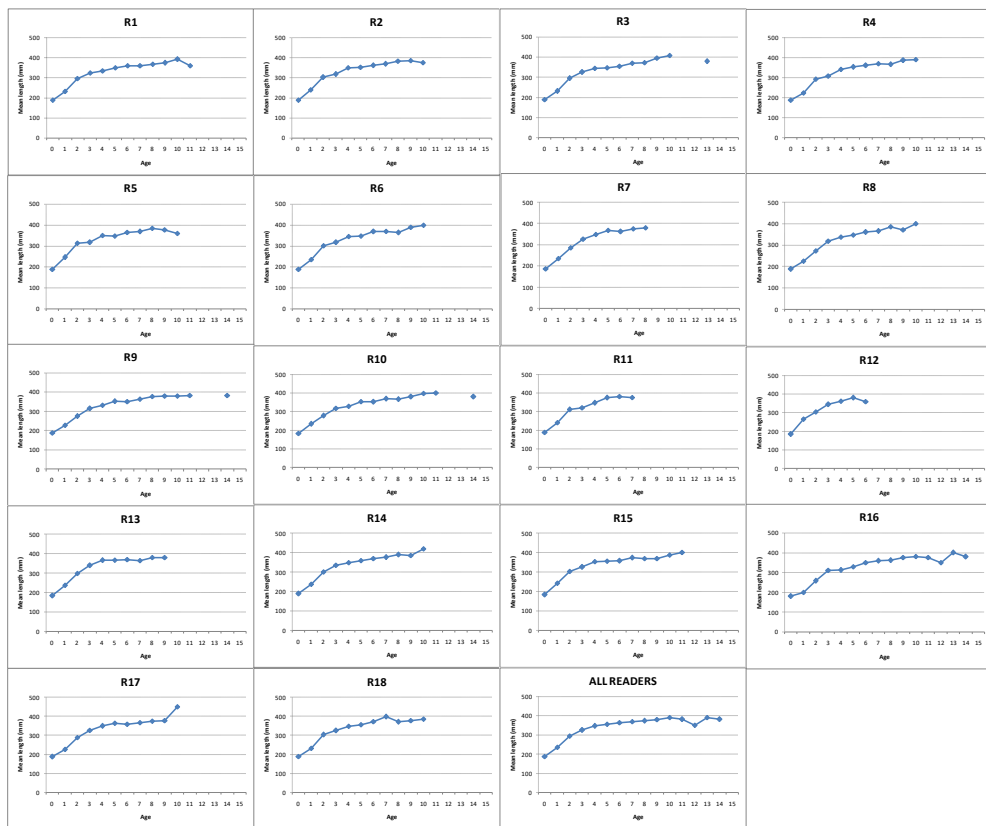


Figure 7.2.1.1. Mean length-at-age (pre-WKARMAC2 exercise, all readers' analysis).

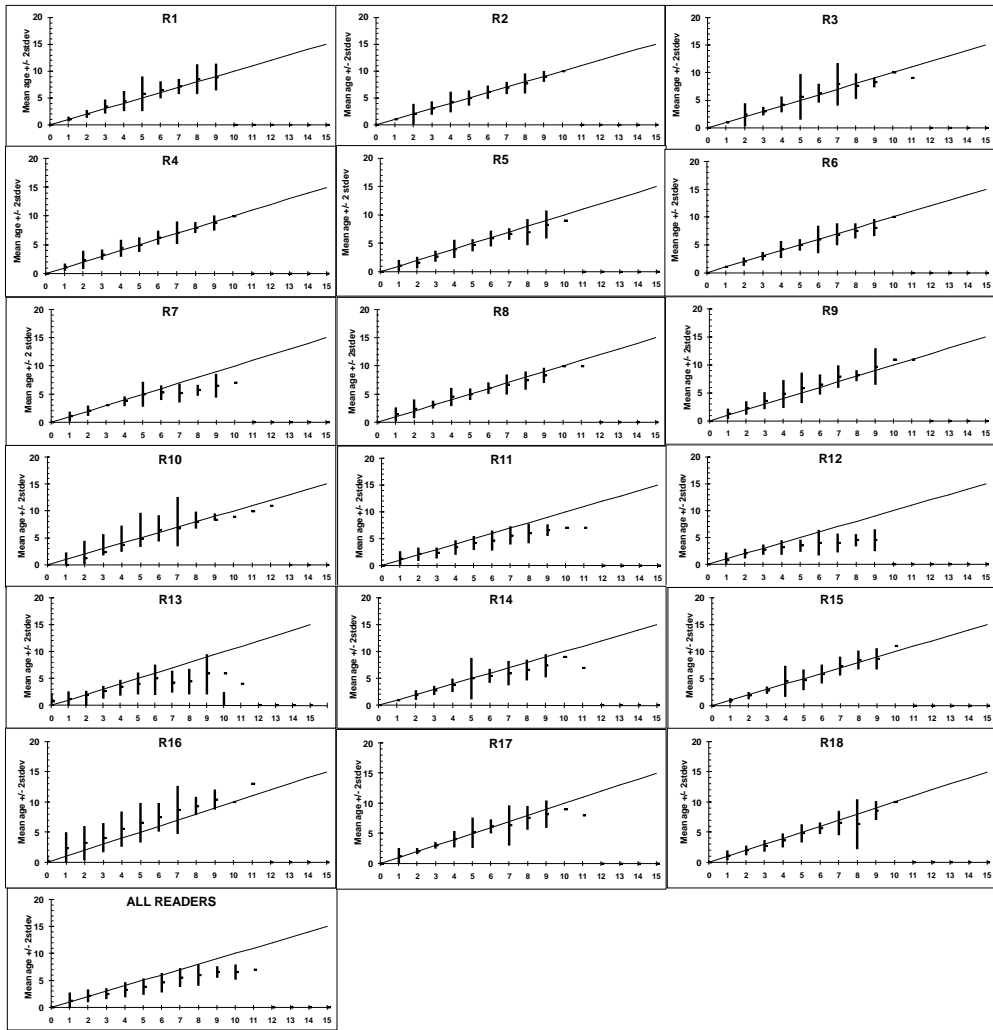


Figure 7.2.1.2. Age bias plot with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (pre-WKARMAC2 exercise; all readers' analysis).

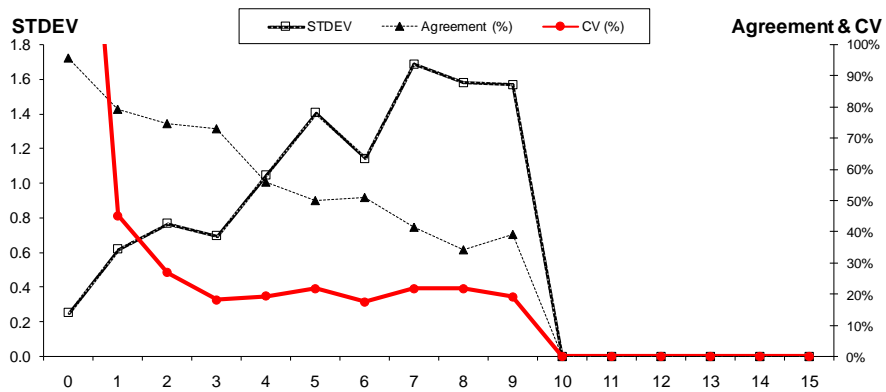


Figure 7.2.1.3. Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age (pre-WKARMAC2 exercise, all readers' analysis).

7.2.2. Expert readers' analysis (pre-WKARMAC2 exercise).

Table 7.2.2.1. Summary of the average percentage of agreement, CV and relative bias by age for Expert readers (pre-WKARMAC2 exercise).

Modal Age	Otolith No	%Agreement	CV	Bias
0	8	100	0.0	0
1	18	84	30.9	0.14
2	15	75	22.5	0.07
3	13	77	16.5	0.02
4	20	66	14.8	0.11
5	10	57	17.6	-0.05
6	12	58	14.2	-0.13
7	14	47	17.7	-0.12
8	13	48	15.6	-0.51
9	9	49	13.7	-0.59
10	2	50	14.0	-1.13
11	1	40	17.0	-
Total	135	65.2	17.6	-0.07

Table 7.2.2.2. Inter-reader bias test and reader against modal age bias test (pre-WKARMAC2 exercise; Expert readers' analysis).

	EH NO	IR ES	DL IE	CN ES	GD DE	GF IS	MJ DK	NS ES	OS NO	MK NO	DM PT
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11
R1 EH NO		**	*	—	**	**	**	—	*	*	**
R2 IR ES	**		—	*	**	—	**	—	**	**	**
R3 DL IE	*	—		*	**	—	**	—	**	**	**
R4 CN ES	—	*	*		**	**	**	—	**	**	**
R5 GD DE	**	**	**	**		—	**	**	**	**	**
R6 GF IS	**	—	—	**	—		**	*	**	**	**
R7 MJ DK	**	**	**	**	**	**		**	**	**	—
R8 NS ES	—	—	—	—	**	*	**		**	**	**
R9 OS NO	*	**	**	**	**	**	**	**		—	**
R10 MK NO	*	**	**	**	**	**	**	**	—		**
R11 DM PT	**	**	**	**	**	**	—	**	**	**	
MODAL AGE	*	—	—	—	**	**	**	—	**	**	**

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

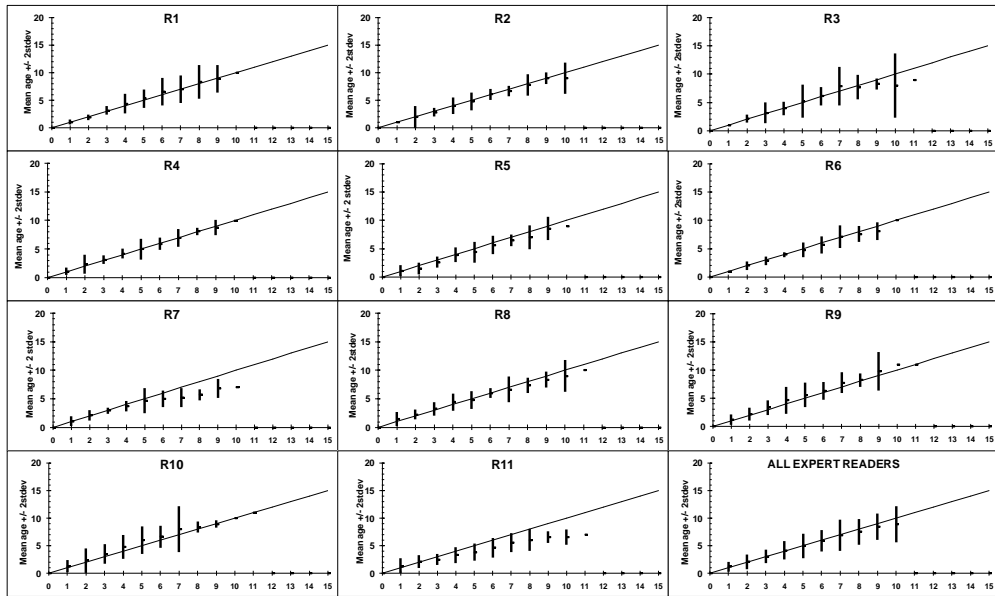


Figure 7.2.2.1. Age bias plot with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (pre-WKARMAC2 exercise; Expert readers' analysis).

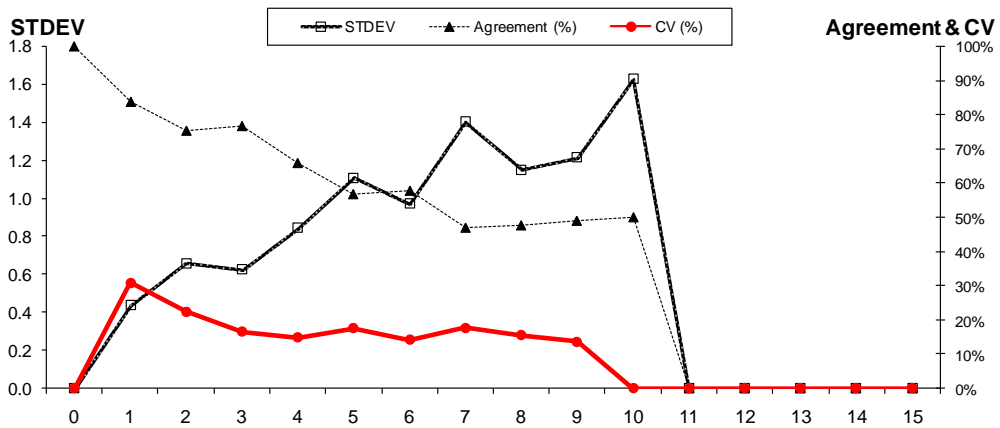


Figure 7.2.2.2. Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age (pre-WKARMAC2 exercise, Expert readers' analysis).

7.2.3. Trainee readers' analysis (pre-WKARMAC2 exercise).

Table 7.2.3.1. Summary of the average percentage of agreement, CV and relative bias by age for Trainee readers (pre-WKARMAC2 exercise).

Modal Age	Otolith No	%Agreement	CV	Bias
0	8	88	-	0.13
1	16	75	55.2	0.20
2	20	72	29.5	0.27
3	24	61	23.8	0.57
4	19	53	24.6	0.61
5	13	45	22.3	0.49
6	11	46	20.8	0.23
7	13	40	-	0.09
8	4	30	-	-0.30
9	5	38	18.9	-0.50
10	2	33	-	-2.50
11	-	-	-	-
Total	135	56.5	36.4	0.28

Table 7.2.3.2. Inter-reader bias test and reader against modal age bias test (pre-WKARMAC2 exercise; Trainee readers' analysis).

	ASP GR R12	KD GB R13	CD ES R14	GH DE R15	SH IE R16	TH NL R17	AS PT R18
R12 ASP GR		**	**	**	**	**	**
R13 KD GB	**		**	**	**	**	**
R14 CD ES	**	**		**	**	**	-
R15 GH DE	**	**	**		**	-	*
R16 SH IE	**	**	**	**		**	**
R17 TH NL	**	**	**	-	**		-
R18 AS PT	**	**	-	*	**	-	
MODAL AGE	**	**	-	**	**	**	-

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

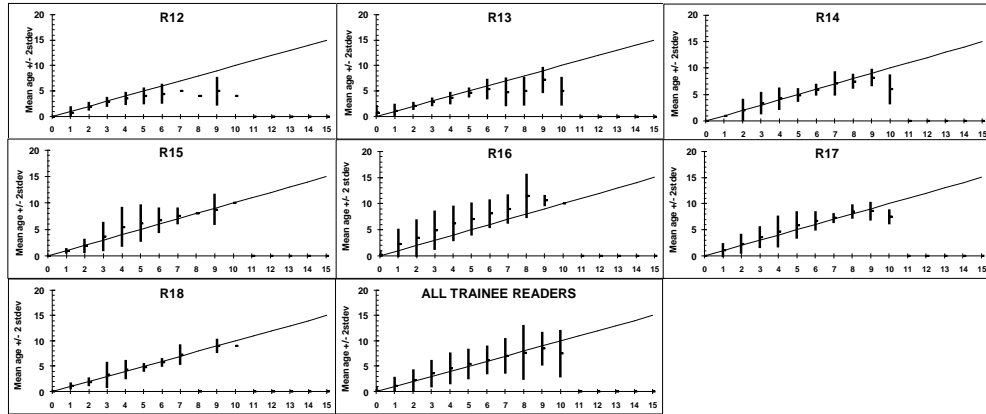


Figure 7.2.3.1. Age bias plot with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (pre-WKARMAC2 exercise; Trainee readers' analysis).

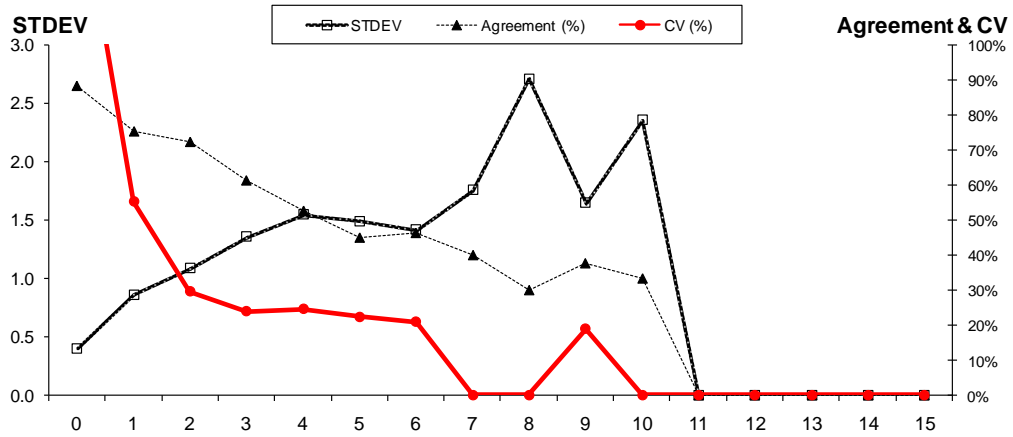


Figure 7.2.3.2. Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age (pre-WKARMAC2 exercise, Trainee readers' analysis).

7.2.4. Southern component analysis (pre-WKARMAC2 exercise).

Table 7.2.4.1. Summary of the average percentage of agreement, CV and relative bias by age for all readers (pre-WKARMAC2 exercise, Southern component analysis).

Modal Age	Otolith No	%Agreement	CV	Bias
0	3	94	-	0.06
1	6	71	39.0	0.36
2	7	76	22.6	0.20
3	8	61	24.0	0.14
4	4	59	19.5	0.44
5	4	39	25.5	0.28
6	3	45	17.3	-0.21
7	1	50	-	-0.39
8	4	38	24.2	-0.81
Total	40	61.3	54.4	0.11

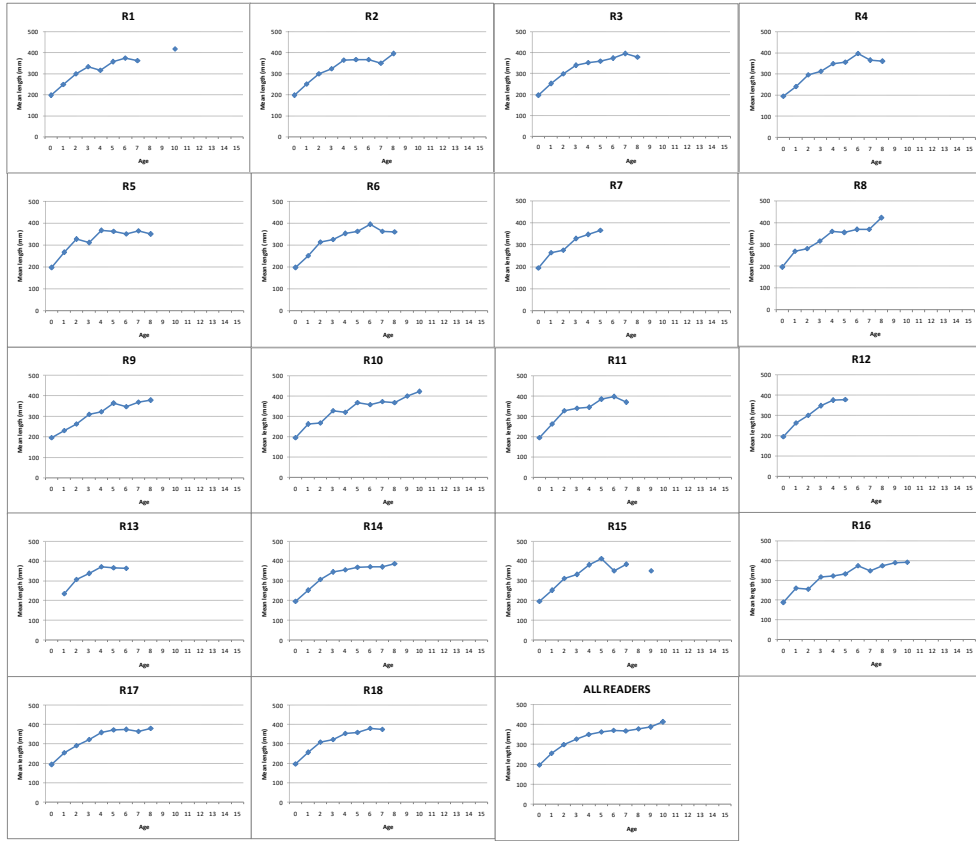


Figure 7.2.4.1. Mean length-at-age (pre-WKARMAC2 exercise, Southern component analysis).

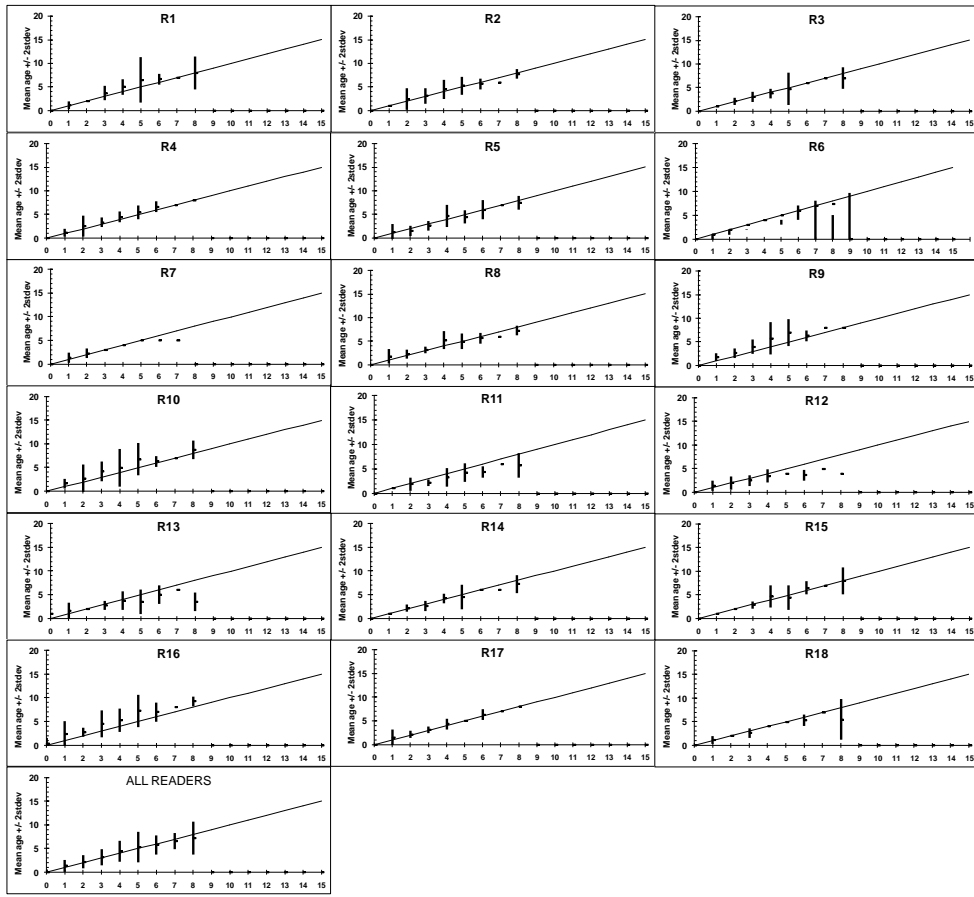


Figure 7.2.4.2. Age bias plot with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (pre-WKARMAC2 exercise; Southern component analysis).

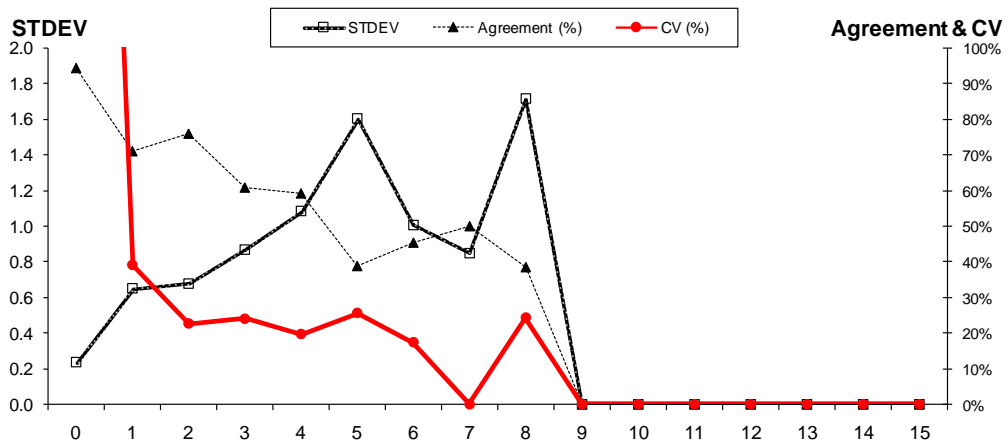


Figure 7.2.4.3. Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age (pre-WKARMAC2 exercise, Southern component analysis).

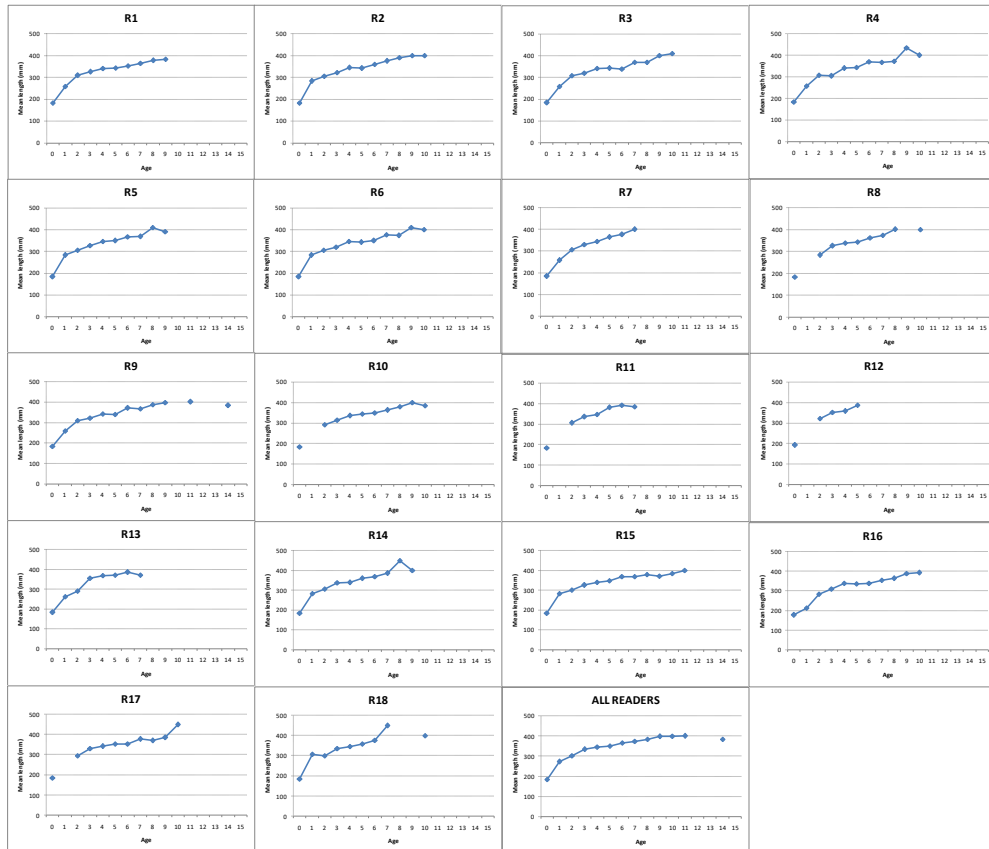


Figure 7.2.5.1. Mean length-at-age (pre-WKARMAC2 exercise, Western component analysis).

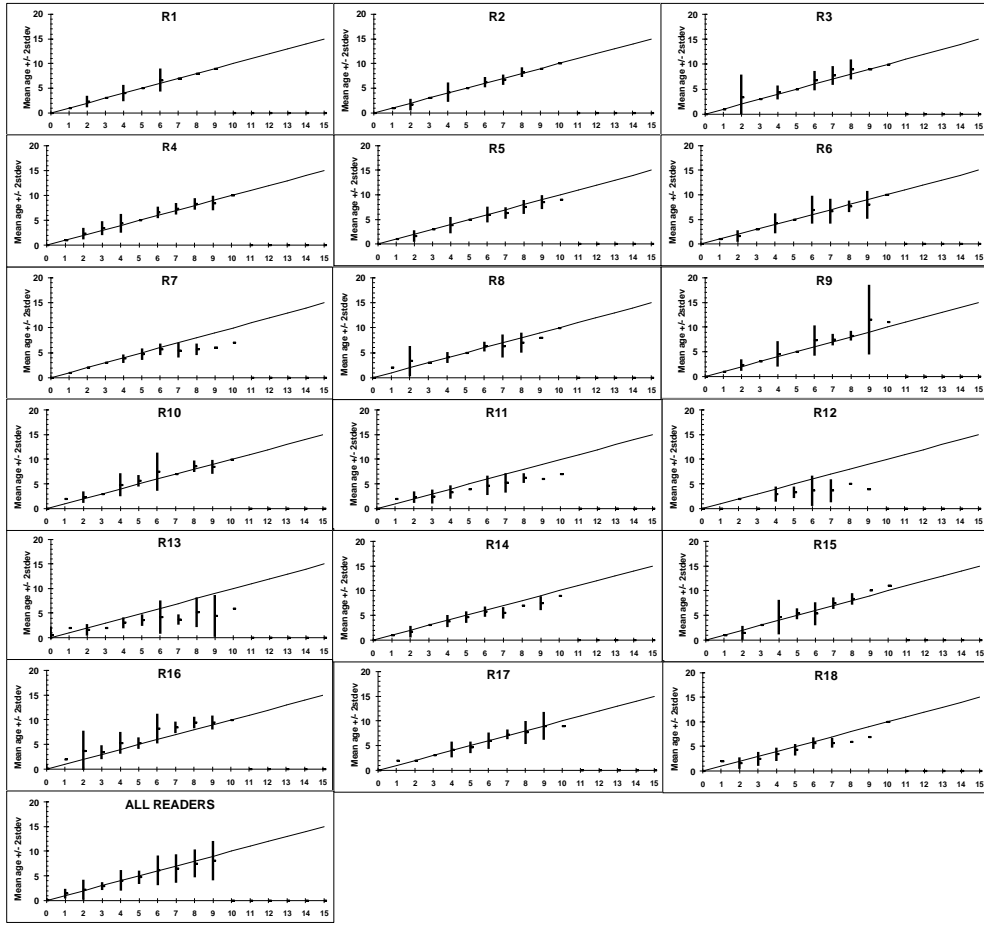


Figure 7.2.5.1. Age bias plot with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (pre-WKARMAC2 exercise; Western component analysis).

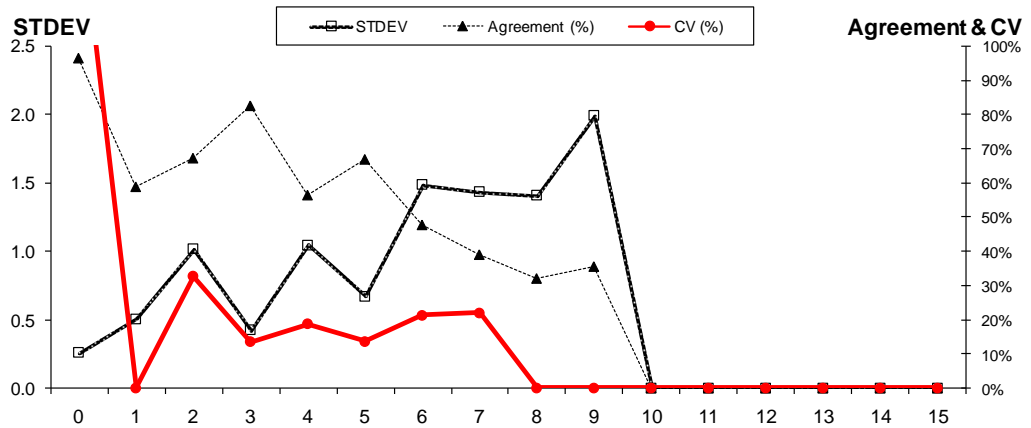


Figure 7.2.5.2. Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age (pre-WKARMAC2 exercise, Western component analysis).

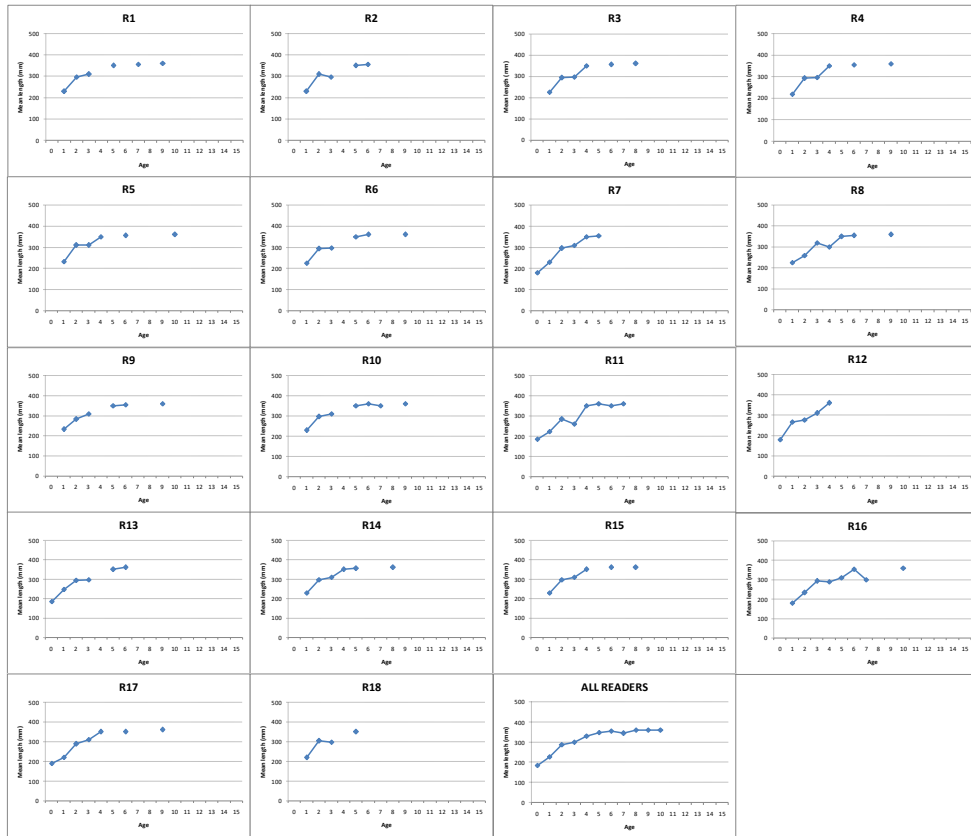


Figure 7.2.6.1. Mean length-at-age (pre-WKARMAC2 exercise, North Sea component analysis).

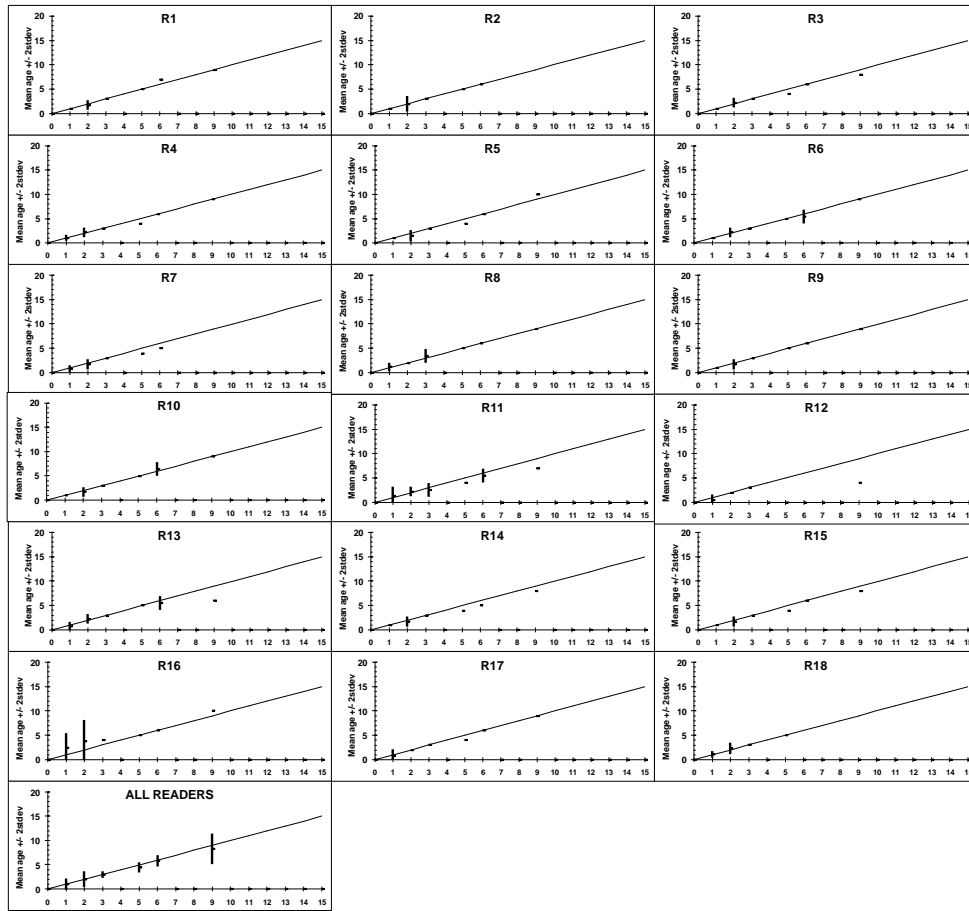


Figure 7.2.6.1. Age bias plot with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (pre-WKARMAC2 exercise; North Sea component analysis).

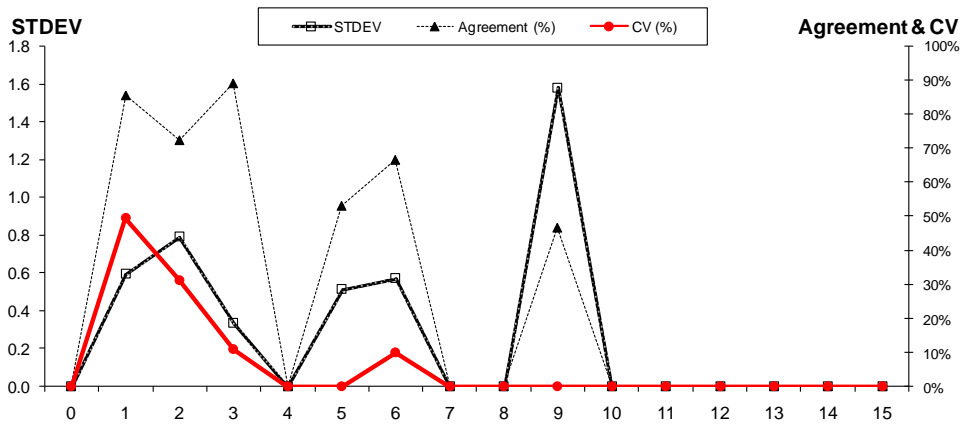


Figure 7.2.6.2. Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age (pre-WKARMAC2 exercise, North Sea component analysis).

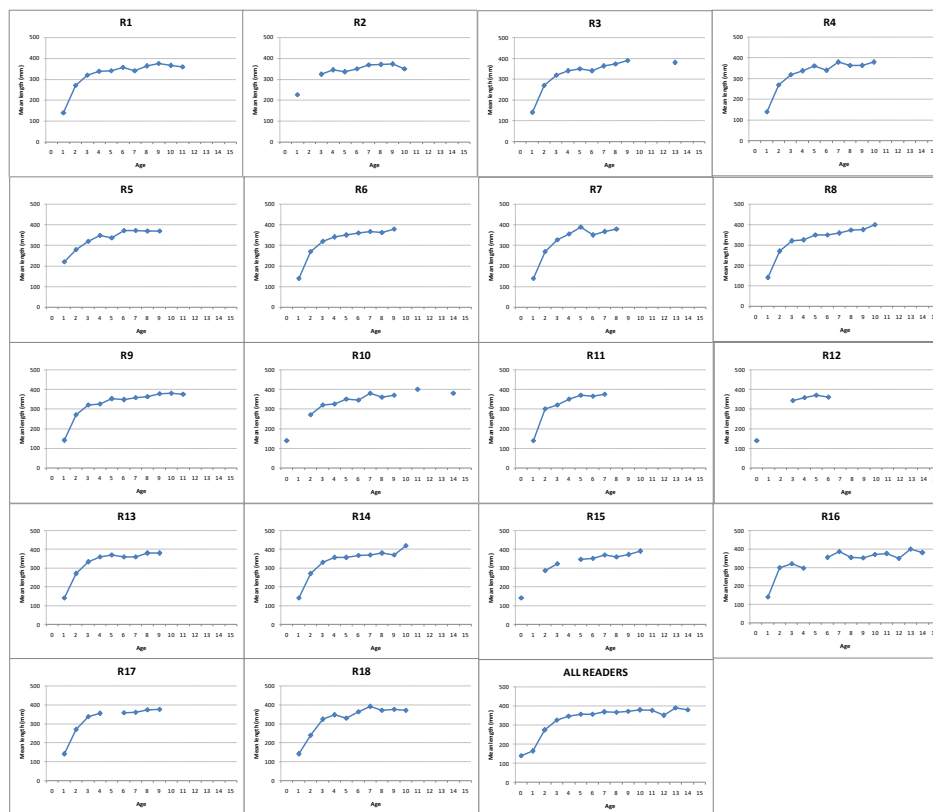


Figure 7.2.7.1. Mean length-at-age (pre-WKARMAC2 exercise, Northern distribution analysis).

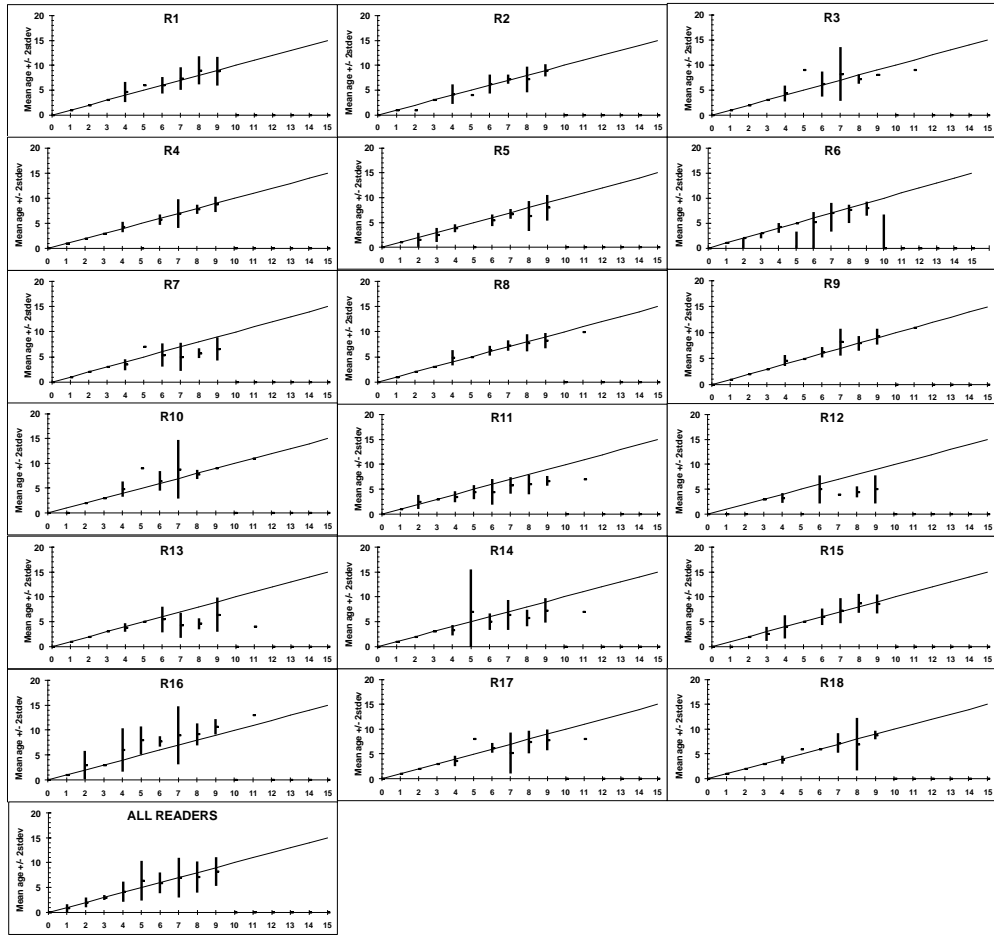


Figure 7.2.7.1. Age bias plot with the mean age recorded +/- 2stdev of each reader and all readers combined and plotted against the Modal Age (pre-WKARMAC2 exercise; Northern distribution analysis).

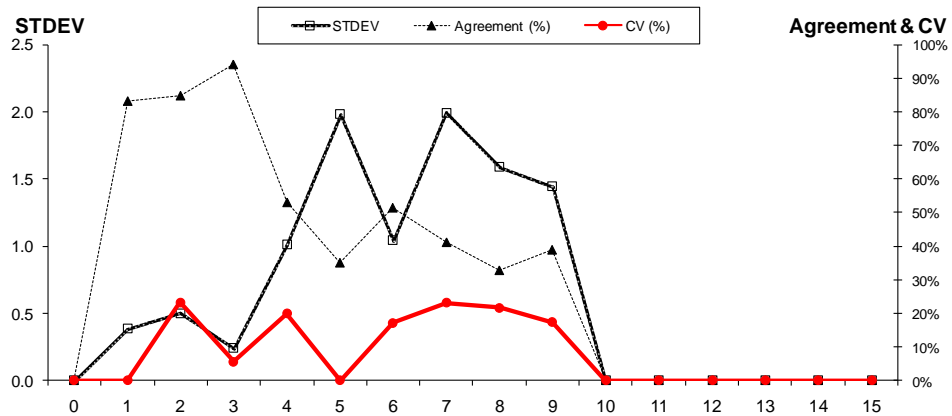


Figure 7.2.7.2. Coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) plotted against Modal Age (pre-WKARMAC2 exercise, Northern distribution analysis).