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Workshop on Age estimation of Blue Whiting (*Micromesistius poutassou*) WKARBLUE2

6-9 June 2017

Lisbon, Portugal

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CIEM Conseil International pour l'Exploration de la Mer

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Executive summary

The workshop on age reading of Blue whiting (WKARBLUE2) took place at IPMA, Lisbon, from the 6th to the 9th of June 2017. The meeting was chaired by Patrícia Gonçalves (IPMA) and Jane A Godiksen (IMR) and included 17 readers from 8 institutes.

The objectives of this workshop were to review, document and make recommendations on current methods of aging blue whiting (*Micromesistius poutassou*).

This workshop was preceded by an otolith exchange, which was undertaken using WebGR in the year prior to the workshop. The actual otoliths were also sent round to all participating institutes. The exchanged otolith collection included 245 images. The overall agreement with modal age of the pre-workshop exercise was 64.1%.There were no clear signs of seasonal misinterpretations, but the Mediterranean and most northern areas (ICES area XIVb and NAFO 1C) proved to be quite difficult.

The main issues during this workshop were identification of the position of the first annual growth ring, false rings and interpretation of the edge. These issues are the same as has been mentioned in previous reports, and thus a reoccurring problem among age readers. A reference collection of images with annotations from the workshop is available in an annex of this report. It will be uploaded to SmartDot as soon as it is up and running on the ICES server. This reference collection of annotated images will hopefully be helpful when running into these issues during future age reading.

Different methods to help age readers determine a zone were discussed during the workshop. The burning of otoliths showed some potential in interpreting the inner ring, but is not to be used as a routine. The sliced technique is time consuming and does not help with interpretation and may introduce misinterpretation of ageing.

During the workshop some of the otoliths from the exercise were polished, to help readers in the cases where the age rings were not so evident, completely absent, or showing a growth pattern different from the expected. The polished results proved useful for ring interpretation and helped during the plenary discussion, although we do not recommend this technique to be used as routine procedure, as it is very time consuming. A Plug-in for ImageJ which can detect variation in opacity in the otolith was presented. Also, a table with possible otolith ring diameters from an IPMA study was tested during the workshop. The table showed potential, but a larger dataset is still needed before it can be adopted as a guideline.

The results from the pre-workshop exchange and from the exercises conducted during the workshop reveal some difficulties on interpreting the blue whiting age rings. Based on those results we further recommend the implementation of daily ring studies and validation of the 1st ring for blue whiting across areas.

1 Terms of Reference

A **Workshop on Age estimation of Blue Whiting** will be established (Co-Chairs: Patrícia Gonçalves from Portugal and Jane A. Godiksen from Norway) and will meet in Lisbon, Portugal, 6–9 June 2017 to:

Review information on age estimations and validation work done so far;

Analyze the results of exchange programme between ageing labs, using a set of otoliths (images);

Clarify the interpretation of annual rings;

Improve the age reading protocols produced during WKARBLUE1

Present and evaluate the results from age validation studies;

Create a reference collection of agreed age otoliths;

Address the generic ToRs for workshops on age calibration (see WGBIOP Guidelines for Workshops on Age Calibration').

WKARBLUE2 will report by 5th of July 2017 for the attention of ACOM, SCICOM and WGBIOP.

Supporting Information

PRIORITY:	Age determination is an essential feature in fish stock assessment to estimate the rates of moralities and growth. In order to arrive at appropriate management advice ageing procedures must be reliable. Otolith processing methods and age reading methods might differ considerably between countries. Therefore, otolith exchanges should be carried out on a regular basis, and if serious problems exist age reading workshops should be organised to solve these problems.
SCIENTIFIC JUSTIFICATION AND RELATION TO ACTION PLAN:	The aim of the workshop is to review the available information on age determination, and validation for blue whiting, to identify the present problems in age determination for this species, improve the accuracy and precision of age determinations and spread information of the methods and procedures used in different ageing laboratories. A number of samples (otoliths or/and images) of otoliths should be circulated among different laboratories to assess the precision of age readers during 2016. Before the workshop, results from the otoliths circulation/exchange will be presented in 2016. Based on the exchange results, in 2016, age validation studies will be established to be conducted by the participants until the workshop. At the workshop, in 2017, results from the exchange and from the age validation studies will be presented and discussed.
RESOURCE REQUIREMENTS:	No specific resource requirements beyond the need for members to prepare for and participate in theexchange and in the meeting.
PARTICIPANTS:	In view of its relevance to the EU Data Collection Framework (DCF), the Workshop is expected to attract interest from ICES Member States.
SECRETARIAT FACILITIES:	None.
FINANCIAL:	Additional funding will be required to facilitate the attendance of the scientists and technicians.
LINKAGES TO ADVISORY COMMITTEES:	ACOM
LINKAGES TO OTHER COMMITTEES OR GROUPS:	WGWIDE,WGBIOP, ACOM, RCMs, all WKACs (Age Calibration Workshops)
LINKAGES TO OTHER ORGANISATIONS:	There is a direct link with the EU Data Collection Framework

2 Agenda and participation

The agenda is presented in Annex 1, and list of participants in Annex 2.

3 Introduction

Blue whiting (*Micromesistius poutassou*) is a pelagic gadoid which is widely distributed in the eastern part of the North Atlantic. The highest concentrations are found during spawning along the edge of the continental shelf in areas west of the British Isles and on the Rockall Bank plateau where it occurs in large schools at depths ranging between 300 and 600 meters but is also present in almost all other management areas between the Barents Sea and the Strait of Gibraltar and west to the Irminger Sea. Adults reach maturation at 1-3 years old (ICES 2013a) and undertake long annual migrations from the feeding grounds to the spawning grounds (Bailey, 1982). Most of the spawning takes place between March and April, along the shelf edge and the banks west of the British Isles. Juveniles are abundant in many areas, with an important nursery area believed to be the Norwegian Sea, at least in times of high abundance. Morphological, physiological, and genetic research has suggested that there may be several components of the stock which mix in the spawning area west of the British Isles. Due to the large population size, its considerable migratory capabilities and wide spatial distribution, the stock composition and dynamics require continued monitoring.

Prior to 1993, for the purposes of assessment, it was assumed that blue whiting had two components, a northern and a southern component. The Northern stock was known to feed in the Norwegian Sea and spawn to the west of the British Isles. The Southern stock was found along the continental shelf off the coast of Spain and Portugal with the main spawning areas towards the Porcupine Bank. The Porcupine Bank was considered a transitional area between the two main stocks (ICES, 1990). In 1993 it was argued that there was no strong evidence to maintain this division between the two stocks. Results from an otolith age reading workshop at that time showed no significant difference in mean annual ring diameter between northern and southern stocks. It was agreed by ACFM in 1993 that the two stocks should be combined for assessment purposes (ICES, 1995). Since then this stock has been assessed as one unit.

Several approaches have been employed to investigate the stock structure of blue whiting. The studies relating to genetics revealed genetic variability between the populations from the Northeast Atlantic and the Mediterranean (Mork and Giæver, 1995; Giæver and Stein, 1998; Ryan *et al* 2005) and from the Celtic Sea and the Bay of Biscay (Was *et al*. 2008). Studies on larval otolith growth patterns (Brophy and King, 2007) revealed differences in growth between Northern spawning areas and the Bay of Biscay. Several studies on the movements of eggs and larvae, and on otoliths shape analysis (Bartsch and Coombs, 1997; Skogenet *et al*, 1999; Pointin and Payne, 2014; Mahe *et al*, 2016; Keating *et al*, 2014) gave results which showed that the southern stock will spawn in an area where the eggs and larvae are likely to drift southwards and the northern stock where the eggs and larvae will drift northwards. Spawning starts earlier in the southern area (by at least one month), with peak spawning occurring later further north and also the existence of two distinct morphology types, from fish occupying distinct geographical distribution areas (northern and southern areas). The most recent studies support the hypothesis of northern and southern components in the blue whiting population which may overlap to varying degrees in the centre of the spawning distribution.

Taking into account those numerous scientific studies suggesting that blue whiting in the North Atlantic consists of multiple stock units, the ICES Stock Identification Methods Working Group (SIMWG) reviewed this evidence in 2014 (ICES, 2014) and concluded that the perception of blue whiting in the NE Atlantic as a single-stock unit is not supported by the best available science. SIMWG further recommended that blue whiting be considered as two units. However, there is currently no information available that can be used as the basis for generating advice on the status of the individual stocks.

4 Review information on age estimations and validation work done so far (ToR a and e)

Little has been done to validate age reading of blue whiting. Only one study on southern blue whiting has been published by Hanchet and Uozumi (1996).

Over the last couple of years, a study using the blue whiting otoliths from the Portuguese coast was conducted. In this study, 67 otoliths from Portuguese Coast were aged, burned and sliced in order to clarify growth rings and improve age readings criteria. Otolith total lengths, widths, thickness, and diameter of the age rings were measured. The main aim was to improve age determination through the correct identification of first annual growth ring. The results of this study are not yet published, but are presented below (Section 4.1).

The data from the pre-workshop calibration exercise (Section 4.2) was used to study and to review the age estimations from the readers. Otolith total length, weight, and also the age ring diameter were measured and the data was analysed and compared with the modal age classifications.

4.1 Portuguese Coast age estimation and 1st ring length validation

Before this workshop a study by IPMA applied to the blue whiting age reading from the Portuguese coast was conducted (see in Section 14: Annex 5 for details – Working Document 1). For this study, a sample of 67 otoliths was selected. From those otoliths age was determined, and the following measurements recorded: otolith length (mm), otolith width (mm), otolith thickness (mm), age ring diameter (mm) and age ring width (mm). One otolith from each pair used in age reading was burned. Digitized images of both otoliths were obtained. Finally, all the 67 otolith pairs (1 burned and 1 not burned) were sliced and digitized images were obtained.

The results show a higher correlation of the modal age with otolith thickness and with otolith length (Figure 4.1.1).

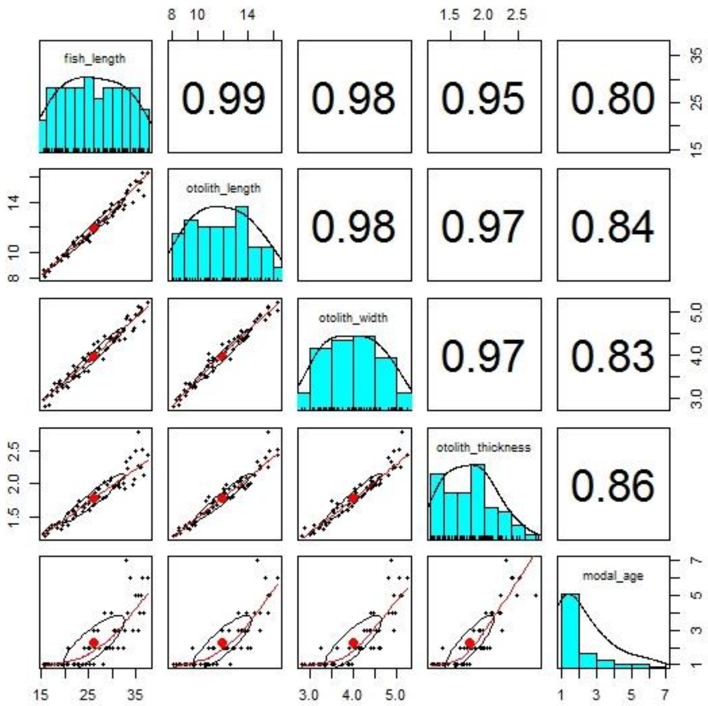

Figure 4.1.1 Relationships obtained between fish length, otolith length, otolith width, otolith thickness and modal age. The numbers represent the R-value from the adjusted model between the variables.

The described relationships between the variables were used to define the equations between them and in this way to predict the variable expected value based on age information, mainly the first annual ring diameter and the otolith total length.

The relationship between otolith total length (Y) and fish length (x) was established in the present work, by the following equation [1]:

$Y=2.796(x)-7.219$ ($R^2=0.99$) [1]

Equation [1] allows the estimation of the total fish length at the time of formation of each annual ring that had been identified by the readers.

Figure 4.1.2 shows the length for each otolith annual ring and the estimated mean fish length for all the annual rings formations through the equation [1].

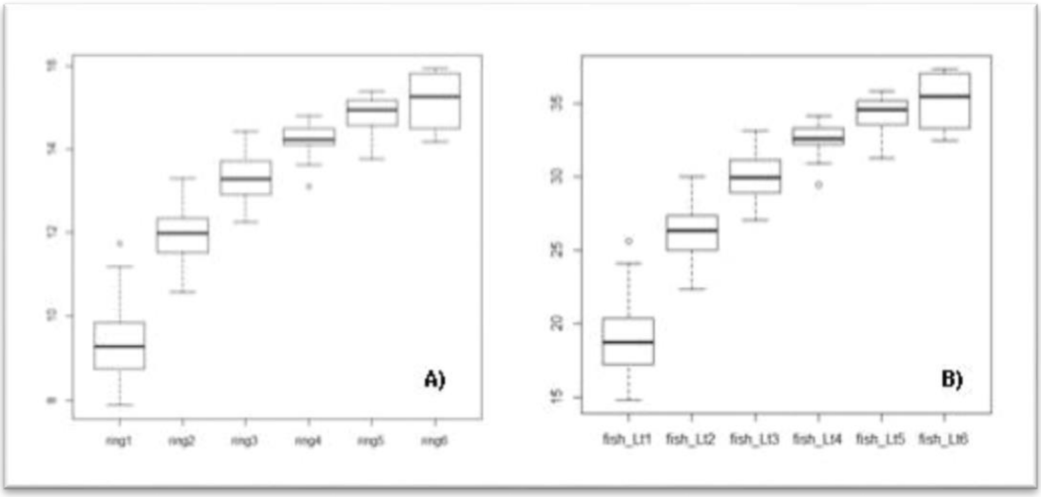

Figure 4.1.2 - A) Boxplot of the length for each annual ring from 67 otoliths; B) Boxplot of estimated mean fish length for all the annual rings formation time.

Based on those results, it was possible to estimate fish length by otolith length, which made possible the comparison between this study results and other studies developed for this species. The international guidelines for blue whiting age reading have as reference for first annual growth ring, an otolith size range of 50 to 56 e.p.u. (corresponding to 8.33 to 9.33 mm) (ICES 2005, 2013b). This reference was established to avoid the Bailey´s zone (Bailey, 1970). However, according to the equation [1], the otolith length established for first year (8.33-9.33 mm), would estimate a fish size of 16.1-18.87 cm which is smaller than the established one in literature (18-23 cm total length fish at age 1).

The differences found in this study may be explained by a different growth rate for first year of blue whiting from the Portuguese coast compared with what is described for other areas.

Different preparation techniques such as burning and slicing the otoliths were also tested. One of the otoliths from each pair used in age reading was burned in order to try to clarify growth rings and improve age reading. As a result, in some otoliths the two inner growth rings previously marked as distinct annual rings by age readers seemed to fuse, apparently forming only one band (Figure 4.1.3).

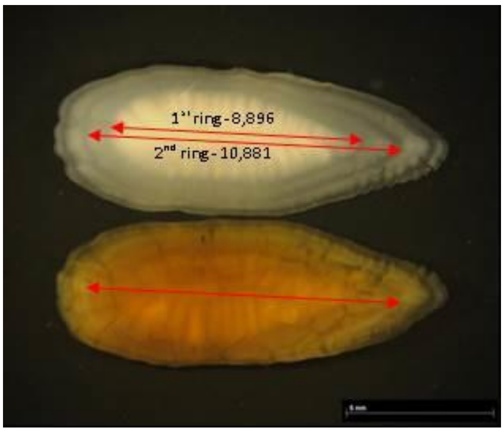

Figure 4.1.3 -Otoliths pair from a 30.7 cm total length fish. The otolith on top is not burned and has two first annual rings length measurements. The otolith below shows where the first two rings apparently have been fused during the burning process.

In the larger otoliths after burning, it is not possible to visualize its internal structure near the nucleus and this is probably the reason why the first annual ring often is larger as the fish increases in age.

The sliced technique did not provide a better ring identification in this particular case and did not improve the age reading. Slicing the otoliths could introduce incorrect age determination since it allows visualization of the entire internal structure of the otolith where numerous false rings are present. This technique was used for European Hake (*Merluccius merluccius*) and was abandoned after a pilot tagging experiment where it was realized that the error in the assignments of ages could be twice the real age (ICES, 2010).

An increase in the otolith first annual ring diameter was observed with age, which could be related with a different application of age reading criteria to otoliths of fish in the different age groups by age readers or with a variation of otolith structure with age that could make the identification of inner annual rings, particularly the first annual ring, more difficult.

4.2 Pre-workshop exchange age estimations

The modal age from the results of the pre-workshop calibration exercise (Section 5.1) were used in order to define more objective criteria that readers could use in order to improve the accuracy and precision of the blue whiting age reading classifications. For each of the 245 otoliths pairs, otolith total length, otolith weight and ring diameter by age were determined. In this analysis the data from the fish total length and the ICES area where fish were caught was used.

This study developed by IPMA (Gonçalves and Dores) comprised two stages: (i) analyse the pre-workshop results and (ii) propose a method with objective criteria's to be used on blue whiting age readings. The results were presented on the first day of the workshop.

4.2.1 Pre-workshop exchange results analysis

The relationship between the modal age with fish length, otolith length and otolith weight was linear (Figure 4.2.1.1).

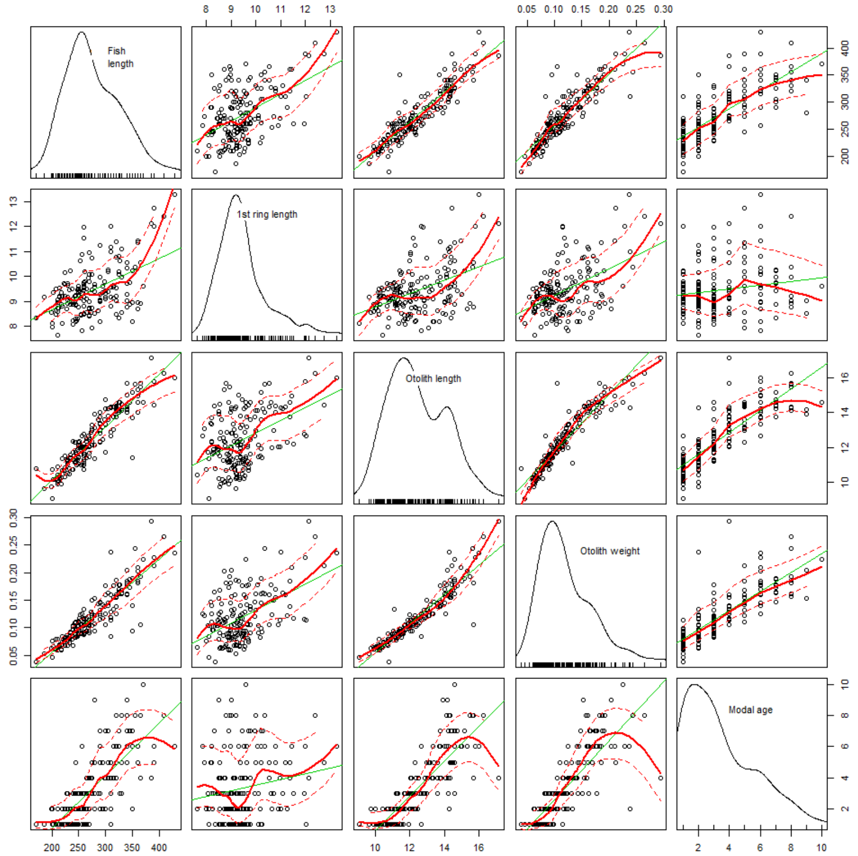

Figure 4.2.1.1.Scatterplot of the relationship between all the variables: fish total length (line 1, column 1); 1st ring diameter (line 2, column 2); otolith length (line 3, column 3); otolith weight (line 4, column 4); and modal age (line 5, column 5).

The modal age presents a linear relationship with otolith weight, otolith length and fish total length. In the 1st ring diameter relationship with all the other variables no clear pattern was evident. Although, the 1st ring diameter shows some increase with the increase in fish total length, otolith length and otolith weight. This increase of 1st ring diameter revealed some problems with interpretation and identification across the ages, which was not expected.

The modal age by fish length and by area was analysed (Figure 4.2.1.2).

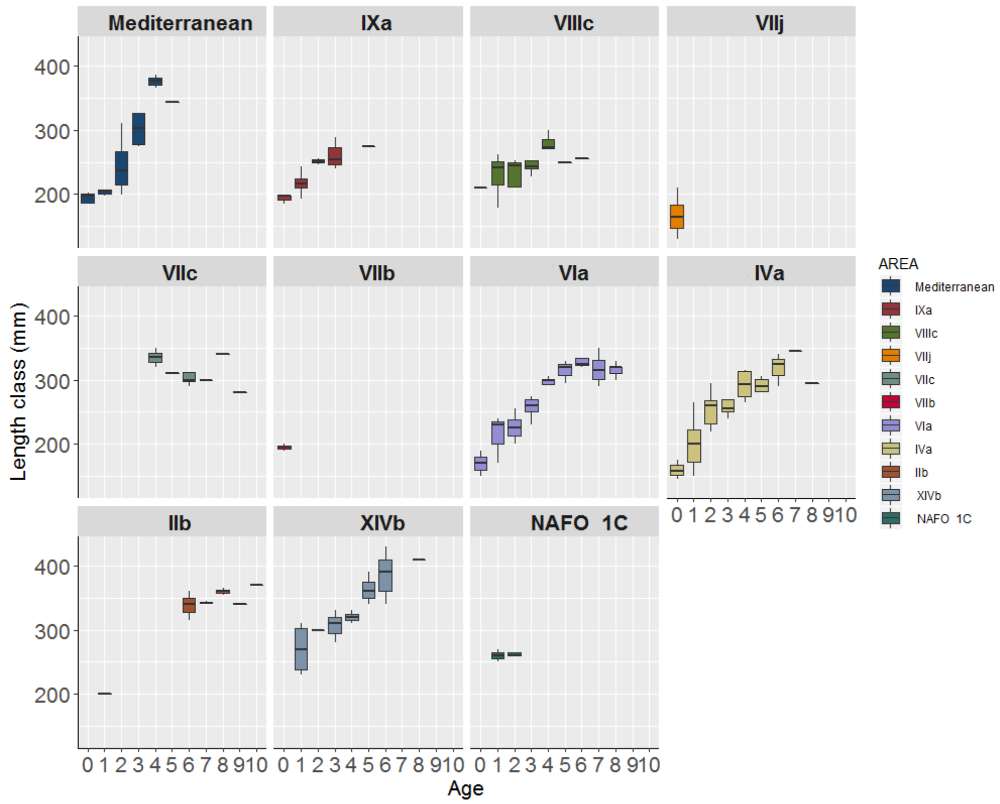

Figure 4.2.1.2 Age-length curve by area: Mediterranean, ICES areas (IXa, VIIIc, VIIj, VIIc, VIIb, VIa, Iva, IIb, XIVb) and NAFO 1C.

The results from figure 4.2.1.2 reveal that there could be some problems in the age classification by area. In some of those areas the age-length curve do not present a shape similar to the von Bertalanffy growth curve model, mainly in VIIIc, VIIc, VIa, IIb and XIVb.

The measurements of each age ring (mm) by area are represented on the next figure (Figure 4.2.1.3).

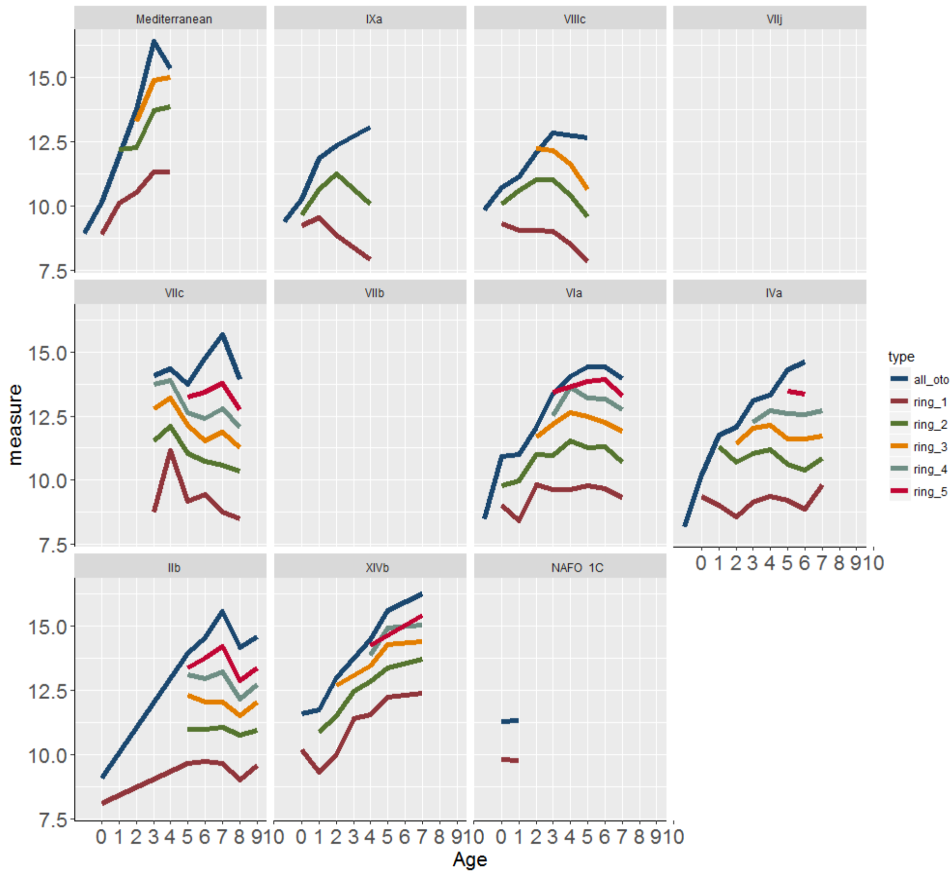

Figure 4.2.1.3. Measurements of otolith length (all_oto) and the each age ring diameter (ring_1, ring_2, ring_3, ring_4, ring_5) by modal age (0 to 10) and by area (Mediterranean, ICES areas (IXa, VIIIc, VIIj, VIIc, VIIb, VIa, Iva, IIb, XIVb) and NAFO 1C).

The age ring diameter seems to change across the ages for the majority of the areas studied, with a decrease observed in the 1st ring diameter mainly in IXa, VIIIc and VIIc, which could indicate that in some cases an inner false ring was classified as a 1st age ring.

4.2.2 Proposal of an age classification criteria

A subset of 215 otoliths from the pre-workshop was used to define objective criteria's to be applied to age reading in routine procedure. From this subset only the otoliths from the Mediterranean and the NAFO 1C areas were excluded as water temperature in those areas are higher and lower which would influence the fish's growth pattern.

Taking into account the growth pattern of blue whiting described for the Northeast Atlantic (Bailey, 1982; ICES, 2016) and the relationship between the age, the fish total length and otolith length a criteria for ageing based on the otolith length has been proposed. The resulting table with proposal named as the IPMA criteria is on table 4.2.2.1.

Table 4.2.2.1. The IPMA criteria describing the correspondence between the otolith ring diameter (mm), fish length (mm) and the expected fish age.

	Fish length (mm)		Ring diameter(mm)	
Age	min	max	min	max
0	177.46	218.30	9.16	10.41
1	221.86	250.22	10.52	11.43
2	253.01	275.18	11.52	12.28
3	277.36	294.70	12.36	13.00
4	296.40	309.95	13.07	13.62
5	311.28	321.88	13.67	14.15
6	322.92	331.21	14.20	14.62
7	332.02	338.50	14.67	15.05
8	339.13	344.20	15.09	15.43
9	344.69	348.65	15.47	15.79
10	349.04	352.13	15.82	16.12

The IPMA criteria were proposed to help readers to achieve the possible fish age based on the otolith length. This proposal is also to help readers interpret the possibility of false rings and split rings. In the cases where the otolith length does not match with the age observed, the readability can be considered weak, i.e. the age quality is classified as AQ2.

The criteria were used during the workshop in an exercise to test its applicability in a routine age reading procedure for blue whiting. During the workshop, a criteria table by area and sex was also been created, but due to the small number of samples available the results were considered not suitable for testing.

Although, the IPMA criteria table seems to work in some cases and help readers to attribute ages, at this stage it is not recommended to apply it routinely. A new criteria table based on more data samples (by area, quarters and sexes) will be proposed and tested during the next blue whiting age reading workshop (WKARBLUE3).

4.3 Marginal increment

An accurate estimate of age structure of a fish population is an important requirement in fisheries stock assessment. Age is usually derived by counting annuli (zones of fast and slow growth) on some calcified structures of fish. For *Micromesistius poutassou*, growth increments are identified by reading annual rings from otoliths. The growth of a large opaque zone is generally observed during the summer (fast growth increment), and a thinner translucent zone is observed during winter (slow growth increment) which gives the reader a good indication for age estimation. The most common semi-direct validation method is marginal increments (MI) analysis which verifies the period and periodicity of growth increments identified by the readers. A large number of otoliths need to be considered for this analysis. In MI analysis, the following measurements are recorded (Figure 4.3.1):

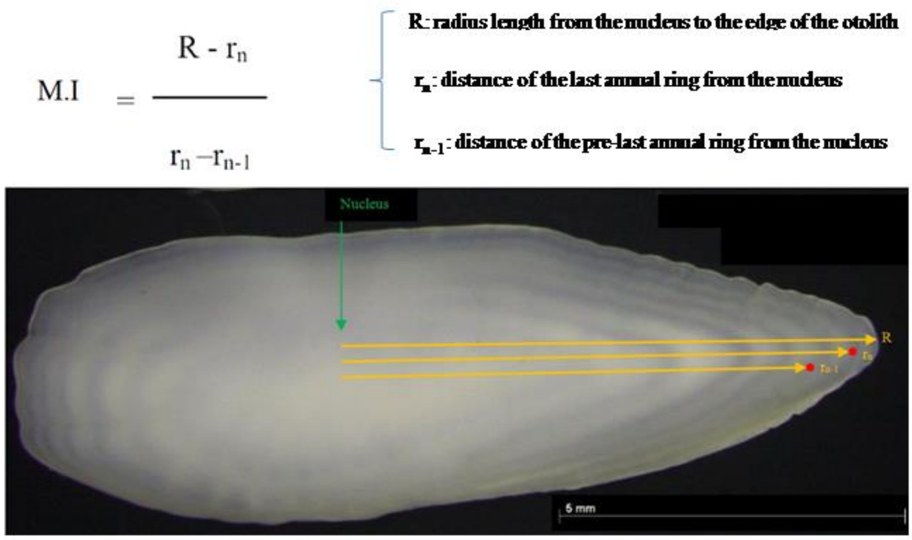

Figure 4.3.1. Marginal increment (MI) equation and the main measurements from the otolith, showed on the entire otolith of blue whiting under reflected light.

Previous MI analysis showed an annual periodicity of increment formation. If rings counted are annual rings, we should observe one peak per year which corresponds to the growth of a large opaque zone (Figure 4.3.2).

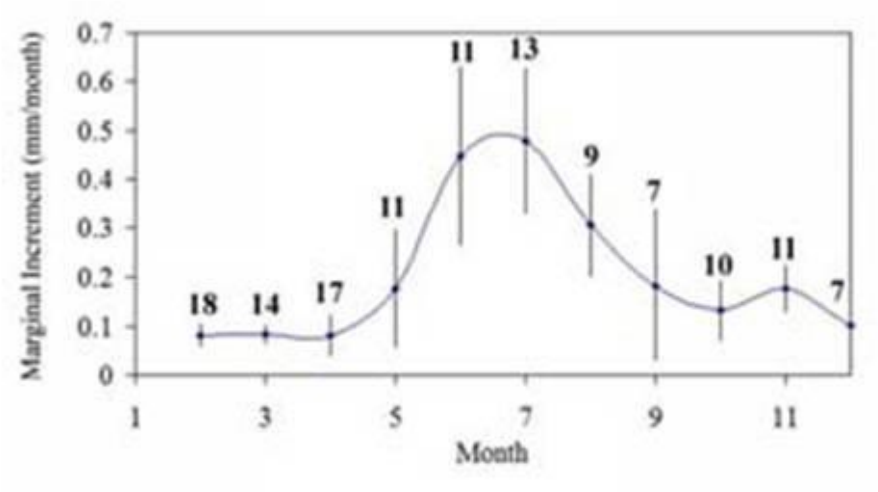

Figure 4.3.2. Monthly trend of average marginal increment (mm.month-1; mean±SD) on the sagittal otolith of the Striped red mullet in the eastern English Channel and southern North Sea (n=128) (*In* Mahé *et al.*, 2013).

During the Workshop, we used data for which all readers have agreed on the age determination during a plenary session. We did not have enough data by month and by area for MI measures. We were forced to combine data from VIIIc and IXa (as south), and the data from IIa, IIb, IVa, VIa, VIIc, VIIj, XIVb (as north). The preliminary resulting figure (Figure 4.3.3) has the mean (MI) data by month for the south, north and the Mediterranean:

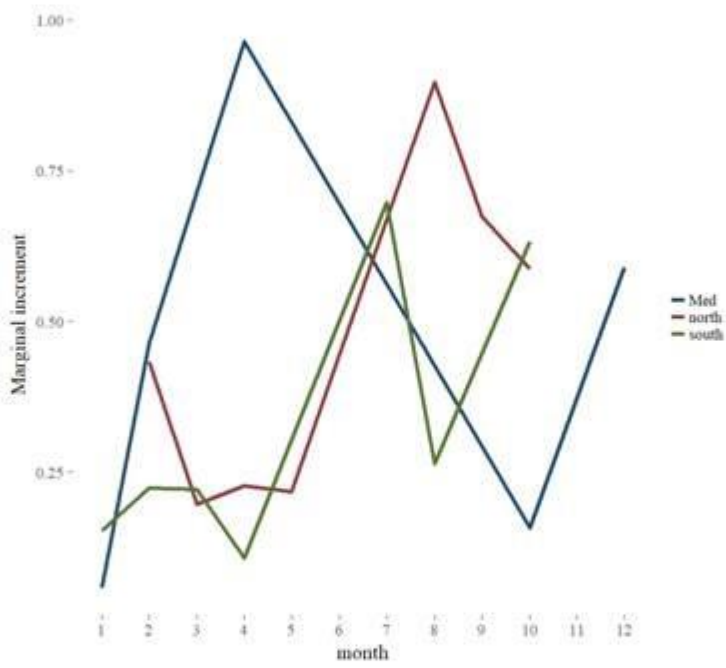

Figure 4.3.3. Mean MI data by month from blue whiting otoliths from the southern and northern ICES areas in the Northeast Atlantic and from the Mediterranean.

One peak per year was observed on the otoliths from the north. However, for Mediterranean and south we observed 2 peaks during the year, which means that the scheme has not been applied for some of the age estimations.

The interpretation scheme remains the most significant factor for the annual pattern of MI (Mahé *et al.*, 2016). These results are still preliminary, since the annual pattern of MI could be influenced by sampling year and area. It would be recommended to do this semi-direct validation method of MI on more samples (with 100% agreement) within the research project, before the next workshop. This would give indications regarding to the importance of following a scheme for a better precision on data assessment.

5 Analysis of the results of exchange programme between ageing labs (ToR b)

5.1 Pre-workshop exercise

Before the workshop, 245 otolith samples from various areas (Figure 5.1.1) and sampled throughout the year (Figure 5.1.2) were annotated by 29 readers from 11 countries. The otoliths were circulated between labs and also made available in WebGR. The readers were asked to read the actual structure and only use WebGR to annotate. That way the results would be as close to the normal way they read as possible.

Modal age was calculated from the expert and intermediate readers. Four trainees took part in the exchange.

The readers were asked to annotate the marked centre and the edge in order to measure the width of the zones. However, WebGR did not cooperate and it was not possible to retrieve the data. Therefore only ages were available for analysis.

The results were analysed in the EFAN Age reading comparison sheet (Eltink 2000).

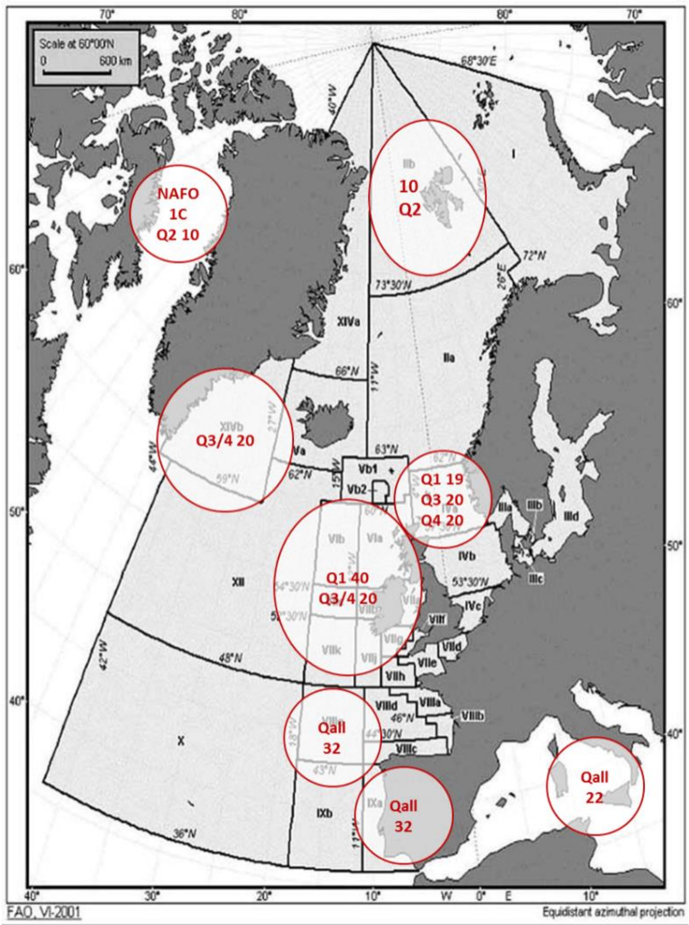

Figure 5.1.1 Map of pre-workshop exchange otoliths. Red circles represent approximate areas and inside are the number of otoliths from each quarter.

5.1.1 Results

The results from the pre-workshop age calibration exercise displayed clear issues in perception of otolith structure. The overall agreement was only 63.7% (ranging between 21 and 100%) with a precision of 50.5% CV (ranging from 0 to 539%). Of the 245 otoliths, 78 (32%) were read with at least 80% agreement (See Annex 4 for figures). For assessment readers the results were a little bit better, with an overall agreement of 68.7% (ranging between 20 and 100%) and a precision of 44.2% CV (ranging from 0 to 447%). Of the 245 otoliths, 100 (41%) were read with at least 80% agreement.

For age readers combined, the relative bias was found to be minimal (-0.07), but for individual age-readers the relative bias varied from -0.96 to +0.58. This shows a significant over and underageing of otoliths by age readers and high bias was found both among experienced readers and trainees. For the youngest fish there was a tendency to overestimate ages among most readers. For older individuals, the majority of the readers either overestimated or underestimated all ages. Very few readers were both over- and underestimating ages of fish over 3 years modal age. The under/over-ageing signifies systematic miss-interpretation of growth structures within the otolith. Wilcoxon inter-reader bias test is presented in Table 15.4, and shows the individual observed bias giving a large degree of significant relative bias among age-readers. Of a possible 406 combinations, only 60 (15%) showed no sign of bias, 22 (5%) showed possible sign of bias, and 324 (80%) showed certain sign of bias. There is also clear bias among the majority of the readers compared to the modal age.

The individual percentage agreement with modal age varied between 50% and 78% for assessment readers, while non-assessment readers had an agreement between 42% and 69%. The highest agreement was, as expected, found among the youngest individuals (>75% for age 0 and 1; 69%< 48% for ages 2-5, with the highest agreement found at 2 years old and lowest among the 5 years old individuals; 44%< 38% for ages 6-10). The agreement to be aimed for is above 80% and none of the readers managed this during this exercise. CV was similar for modal age 3 and above; it was a little higher for 2 and 3 year olds, but extremely high for 0 year olds (up to 539% in cases where all readers except one said 0 years old).

There were no clear trends towards any seasons being more difficult than others, nor were there any signs of under or overestimation of ages over time. It is more reasonable to believe that the small differences seen might be due to one area being more difficult than others. The Mediterranean and northern areas (IIb, XIVb and NAFO) showed rather low agreement. This could be due to many readers not being familiar with otoliths from these areas.

Data on fish length were not available on the image for annotating in WebGR; however, some readers went and found the fish length by searching the WebGR database.

5.1.2 Plenary discussion of otoliths

During the workshop several of these structures with annotations were discussed in plenary. It was not possible to discuss all with disagreement, as this included 95% of the total amount of otoliths. 129 otoliths out of 245 were discussed; 10 were disregarded due to being broken and the 10 otoliths captured in NAFO-area were not discussed, as the German experts were absent and none of the readers present were familiar with the extremely opaque otoliths. 19 were disagreed on and 110 were agreed on during the plenary discussions. Of the 110 agreed otoliths 92 were agreeing with modal age of the pre-workshop exchange.

We discussed the otoliths using the original guidelines from WKARBLUE 2013 and from this a part agreement was usually reached between the readers for most of the otoliths. A disagreement of more than one year was never reached during the discussions. The general issues of disagreement were split rings and the inner ring. For split rings it was often disagreed whether it was a split ring or two rings, and it was difficult to get to an agreement. When looking at the width of the zones it is important to beware of the continuity of the growth. One should expect to see zones becoming narrower and narrower towards the edge. However, the possibility of years with low or high growth needs to be considered as well. This pattern, however, should be possible to see in other specimens from the same area (see section 7 for a detailed description with images).

For the inner zone the size has to be kept in mind. The size of the Baileys zone needs to be taken up for validation. In several of the otoliths we were discussing, the size of the inner zone seemed to be quite variable. Some otoliths were polished down to get rid of the three dimensional structure and get a better view of the zones. Often this gave a good idea of the first annual zone.

5.1.3 Issues

The otoliths were stored in water in Eppendorf vials as they were sent around. The first country read the otoliths in March 2016 and the last received the otoliths in March 2017. It was mentioned by some of the later countries that the zones in the otoliths were more unclear than on the images. Suggesting that this is due to the long time the otoliths has been soaked in water. Some institutes dried the otoliths for a couple of days before soaking in water for 24 hours. This made the otoliths readable again.

An issue arose during the discussion of the individual otoliths. Otoliths of fish, with an opaque edge and no rings marked, were aged differently by the different countries. If a fish was captured before the end of June; Spain, Portugal and France will aged it to be one year, while Norway, Netherlands, Iceland, Faroe Islands will age it to be zero. All countries agree that it is 0 years old, but due to a convention it is placed into a year class older than it actually is. If the fish had been captured in July-December all countries will age it to be 0 year old. The issues were not fully discussed during the workshop, but over email after the workshop it was believed that the reason for the confusion was the scheme used (Figure 5.1.3). The scheme on figure 5.1.3a is common to the majority of species aged under the DCF program on southern countries. During the WKARBLUE1 (Figure 5.1.3b), a scheme was updated to fit with the growth pattern for Blue whiting. However, if readers are using the original scheme, where an opaque edge in June gives a different age than an opaque edge in July, then the aging will be wrong. It is important that the readers use the most updated information for reading. If the readers follow the scheme made specifically for Blue whiting, this shouldn't become an issue in the future. This also shows the importance of using simple ageing schemes, to avoid misunderstandings between readers. If such a scheme could be standardised to apply to all species, this would be an advantage, but it needs to reflect the growth patterns.

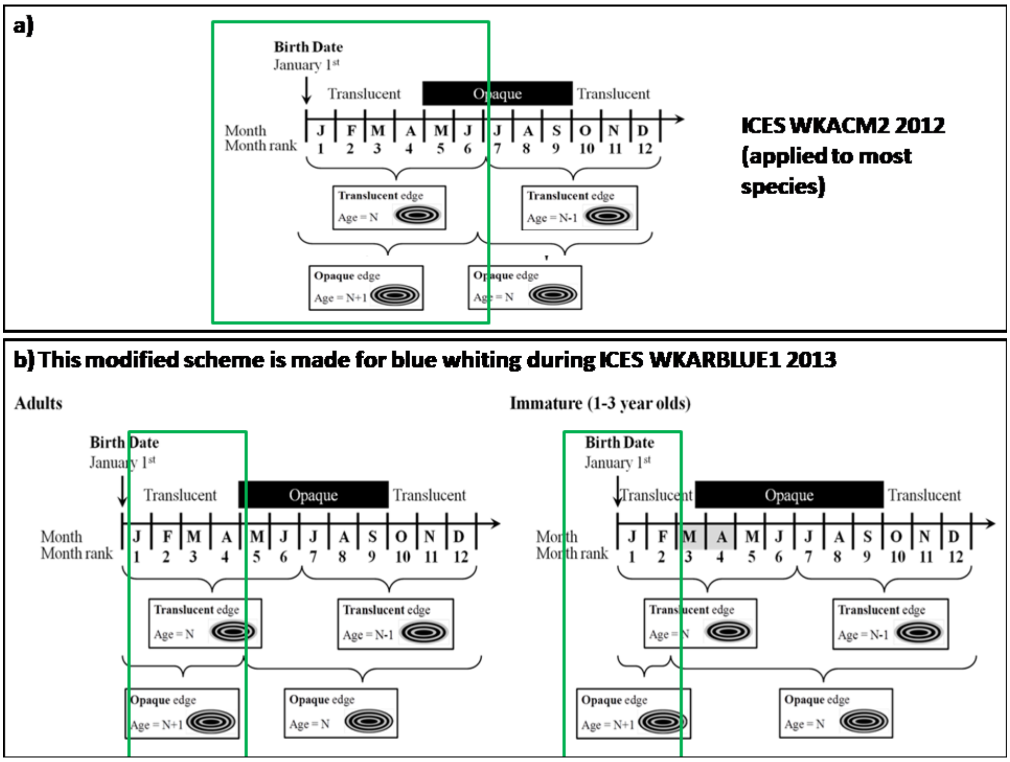

Figure 5.1.3.1. The scheme used for blue whiting ageing: a) applied to the majority of aged species (scheme from ICES WKACM2 2012); and b) proposed for blue whiting on ICES WKARBLUE1 (2013).

5.1.4 Conclusions

The guidelines presented in WKARBLUE 2013 are good and useful – it is, however, not something the reader keeps in front of them as they read, and therefore aging of the pre-workshop exchange was more based on gut feeling than following the guidelines. When using the guidelines during the discussion of the otoliths, we often agreed. A good solution to this could be to introduce a table giving minimum and maximum size of the inner ring. We have decided that this should be done from a larger set of otoliths than the one we have worked with here. We agreed at the workshop that a set of 150 otoliths from each area (Norway, Iceland, Germany, and Portugal) should be sufficient. Portugal will take the lead and conduct the necessary data analysis.

5.2 Workshop exercise using IPMA criteria

An exercise was conducted where 47 otoliths from the pre-workshop exchange otoliths were annotated in WebGR (originally 57, but ten didn't load in WebGR). The otoliths were brought to the workshop, but they had all cracked and it was impossible to use them. Therefore, the readers had to use only the images. A table of otolith ring sizes at ages (from the IPMA validation study) (Section 4.2.) was available to the readers, and they were asked to age the otoliths using this criteria.

5.2.1 Material and methods

The otoliths for the exercise were chosen from the pre-workshop exercise, trying to get a good coverage of ages, areas, sex and season. However, it was important to have enough data from the areas and seasons included, and therefore only Q1 and Q3 were used. Also the Mediterranean and most northern populations were dismissed from the exercise. This was done to ensure a similar growth pattern among the fish. At a later stage more areas should be considered, but for the purpose of this exercise it was enough.

A test of symmetry for determining if significant differences existed between the modal ages by readers and the ages using the IPMA table was applied. The test of symmetry uses a chi-square-type statistical test to determine if the age-agreement table is symmetric or not. If the age-agreement is determined to be asymmetric then it can be concluded that there is a systematic difference in ages observed between readers and ageing using the IPMA table.

Two statistical indicators were used to measure the precision: (i) the average percent error (APE) and (ii) the coefficient of variation (CV). The APE assumes that the standard deviation of the age estimates are proportional to the mean of the age estimates.

5.2.2 Results

The length at age by sex was represented taking into account age classifications by readers (Figure 5.2.2.1a) and using IPMA table criteria (Figure 5.2.2.1b). There are differences in the growth curves, namely the described relation between modal ages (readers) and length is more disperse, this relationship when applying the IPMA table criteria show a more consistent growth pattern along the fish length classes.

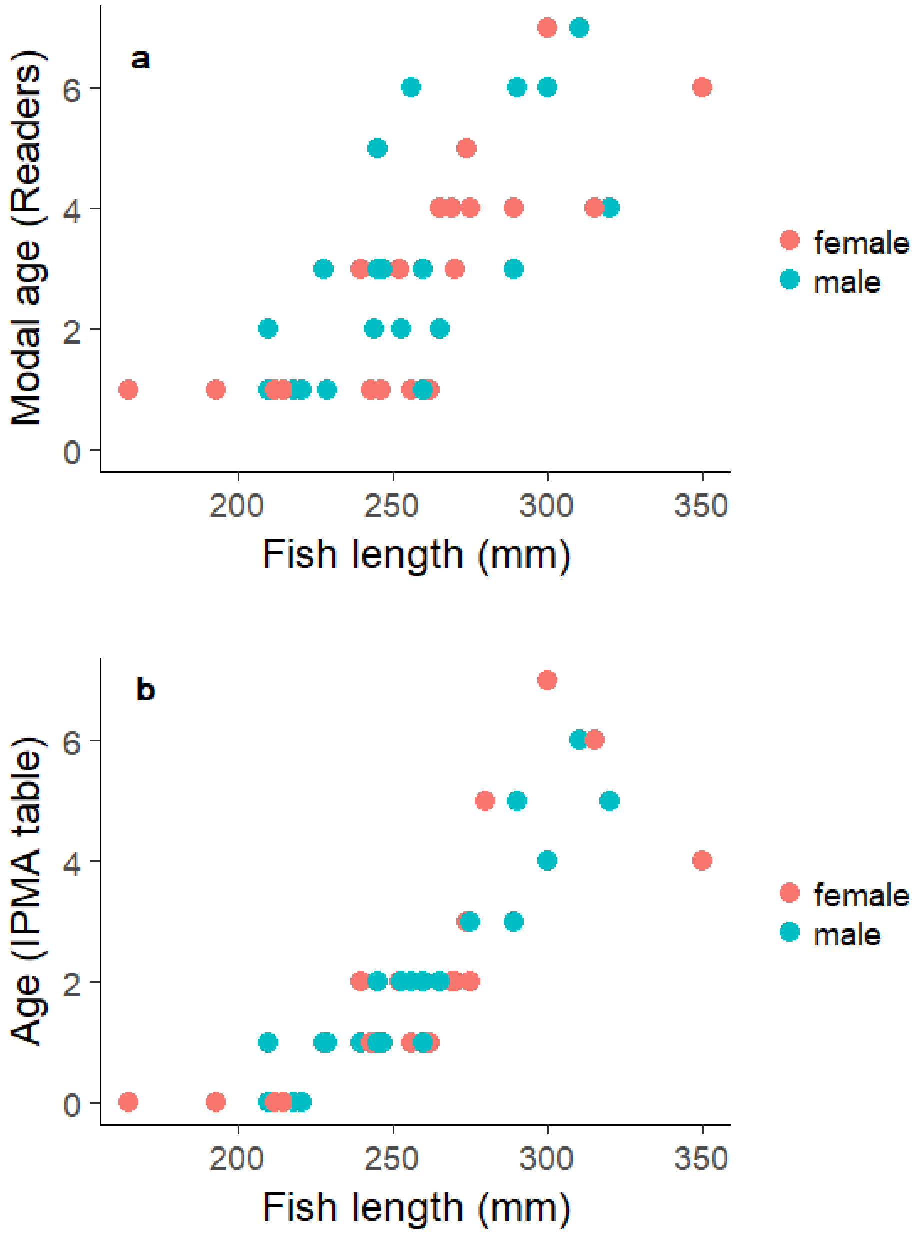

Figure 5.2.2.1. Fish length (mm) by age according to classifications by: a) readers and b) IPMA table criteria.

The Hoenig test of symmetry (p = 4.52e-05) indicates that there are systematic differences in the assigned ages between readers and using IPMA criteria. The ages estimation based on the automatic procedure are overestimated compared with the ages attributed from the readers (Figure 5.2.2.2). The same age was agreed in 11 otoliths, in a total of 47.

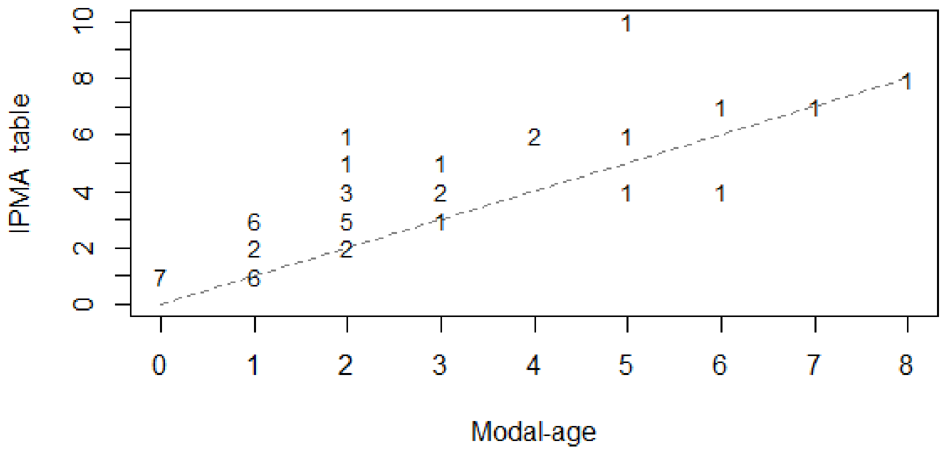

Fig. 5.2.2.2. Age-bias plot of estimations by readers by using the IPMA criteria. The dashed line represents the age-agreement between procedures. The values represent the number of otoliths.

The precision methods reveal a low percentage of agreement (24%) between the two procedures, with CV=47 and APE=33.35.

5.2.3 Conclusions and evaluation of the exercise

When going through the sub-sample of images (n=47) in WebGR it turned out that very few of the otoliths fell into the scheme of the table. It was discussed whether it could be due to different growth patterns between areas and sexes. Although, the group agreed that the concept behind the IPMA table could be a useful tool to help readers ageing by providing objective measurements. It was concluded that this should be based on more data samples.

6 Improve the age reading protocols produced during WKARBLUE1 (ToRd)

6.1 General guidelines for age determination. Ageing manual

Quick guidelines

Preparation	Soak for 24 hours before reading using reflected light.
Identification of nucleus	Beware of the Bailey's zone, and keep in mind the possible size of the first growth zone.
Issues	False or split rings occur. It is important to beware of a reasonable growth pattern in the otolith.
Interpretation of the edge	Growth varies with age, and the two schemes made should always be consulted.
Sexual dimorphism	Females grow faster than males, especially after the age of first maturity.

- Blue whiting **whole otoliths** must be soaked in water 24 hours before reading. The otolith should not be soaked in water for more than 48 hours each time, as it possibly could affect the ring structure due to the composition of freshwater (Anon.1992).
- No other manipulation is needed. It is, however, important to age the fish shortly after sampling, as the otoliths are clearest then.
- Whole otoliths must be **read in water** over a black surface, using **reflected light**.
- **Magnification** of images should always be the same (x 0.64), and a measurement bar needs to be included in all images of blue whiting. This is very important in order to correctly determine the inner zone vs Bailey's zone
- Be aware of which **side of the otolith** is read from. Read from centre to pointy edge, and read on the dorsal side (upside when placed in the inner ear of the fish) (Härkönen, 1986). Often the rings can only be followed from the centre towards one side of the otolith, while they will merge very close to the pointy edge at the other side (Figure 6.1.1).

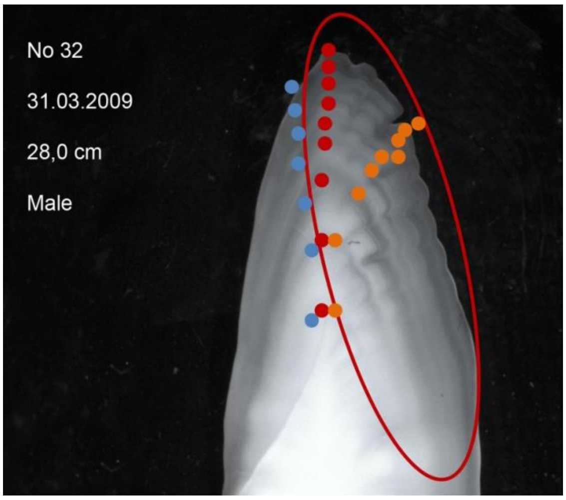

Figure 6.1.1. The red dots indicate the correct direction to read. However, it is important to follow the rings along the side as well. The lines can clearly be followed on the side with the orange transect, but when reading along the blue transect the age goes from 9 to 7 years old. We regard the red circle to be within the area it should be possible to follow the zones to call them annual zones.

- Have in mind which area the fish is captured in. There might be differences in growth pattern between areas, especially for fish captured in NAFO area and the Mediterranean.
- The age is given by the number of translucent (winter) zones in the otolith (Jakupsstovu 1979).
- Correct **identification of annulus** can be induced by measuring the size of the inner ring. It will thereby be possible to avoid including the Bailey's zone (Bailey, 1970) as the first annual ring. Usually a ring diameter in the size range of 48 to 56 e.p.u (corresponding to 8.00-9.33 mm) can be considered the first annual ring (ICES 2005). However, the first annual ring might be found to be wide as 12 mm in diameter (this might be area dependent and individual fish growth).
- **Growth pattern** of the otolith zonation should be reasonable. It should be considered thoroughly if the visual zones are going from narrow to wide when reading from the centre (Figure 5.1.2). It is however, important to look at a group of fish. If several fish with the same measurements show the same pattern, it could be a biological reason to dismiss a normal growth pattern.

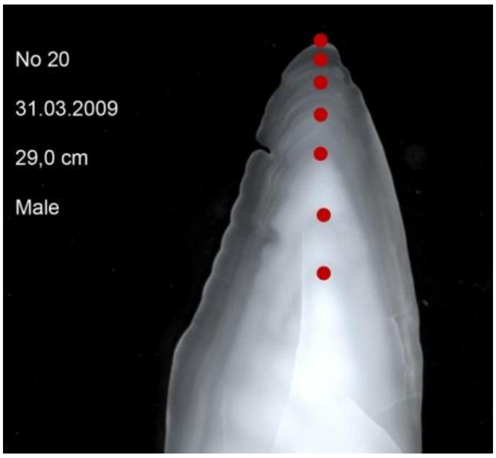

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Figure 6.1.2. The width of the zones should be expected to become smaller with distance from the nucleus. Variation can be expected due to variation in feeding, spawning etc.

- **False rings** are a common issue in blue whiting otoliths. When counting the true annual rings to age the fish, it is important to look at the entire structure of the otolith and follow the sequence of yearly growth. The yearly growth zone increments will most often decrease as the fish get older. When small growth zones are followed by bigger ones, these should be considered as false rings. However, sometimes ring thickness varies within the otolith, and a translucent zone may appear very thin, but is in good sequence, which could be a short winter period and not a false ring.
- A particular case of false rings are the **split rings** (double rings). In many cases they can be easily identified because they merge when you try to follow them around the otolith. It is important to follow the rings as far as possible to the side of the otolith. Zooming out will reduce the possibility of counting split rings. When in doubt check for expected growth pattern (Figure 6.1.3).

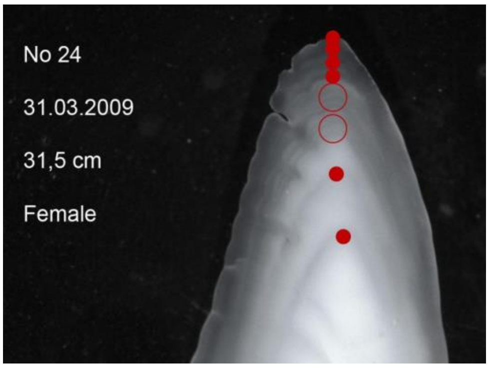


Figure 6.1.3. The empty circles of the image show where there are split rings. Each empty circle should be counted only as one year.

- **Interpretation of the edge** is determined by the time of capture of the fish. By an international convention (Williams and Bedford, 1974), the birthdate of fish has been assigned as January 1st, regardless of hatch date. A fish age-reader must assign a fish to its proper age according to the date it was caught with reference to this birthdate. Criterion of birthday on January 1st must be used to determine when a hyaline ring in the edge must be counted. Growth of immature fish vary from that of adults, as they may feed for a much longer period, and the opaque zone may therefore start forming already in March.

Aging of a fish with an opaque edge present will therefore depend on maturity. Otoliths with translucent edge, sampled from the first half of the year, are aged by counting all translucent annuli, including the edge. Second half of the year, are aged by ignoring a translucent edge if present. This ‘translucent edge’ is the onset of the winter ring. This onset will also vary with time and by geographic location. This scheme must be clarified and validated in the future. This modified scheme is made for blue whiting during the workshop in 2013 using the figure from WKACM2 (Figure 6.1.4).

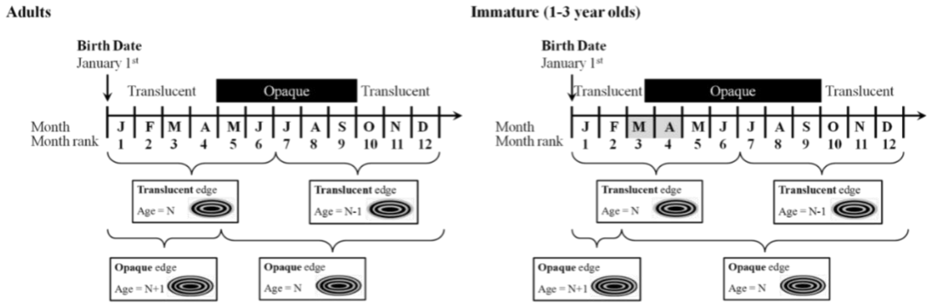


Figure 6.1.4. Modified scheme for Blue whiting.

- Blue whiting age readings **should avoid otoliths classified as unreadable** or very difficult to interpret (0-25% reliability) according to the following 3-point scale of age reading quality that WKNARC (ICES 2011) recommends to be used by all age readers who provide age data for stock assessments:

  AQ1: Easy to age with high precision.

  If a scale of 1-100 is applied, where 100 is when the reader has the highest possible confidence in the age reading and 1 is when the reader has no confidence in the age reading, age quality 1 (AQ1), will apply to approximately the top 25% of the possible quality ratings. AQ1 is an indication that the age data is considered reliable for stock assessment.

  AQ2: Difficult to age with acceptable precision.

  Age quality 2 (AQ2), will apply approximately to age readings within 25 and 75 percentiles of the possible quality ratings. AQ2 is an indication that the age data is sufficiently reliable to be used for stock assessment purposes but improvement is required.

  AQ3: Unreadable or very difficult to age with acceptable precision.

  Age quality 3 (AQ3), will apply to approximately the lowest 25% of the possible quality ratings. AQ3 is an indication that there are serious concerns about the reliability of the age data and/or its value to stock assessment WGs.

- **Reference Collections** should be used as a valuable tool to maintain the accuracy of readers over time.
- **Sexual dimorphism** is present in blue whiting, females grow faster than males, thus are younger in general at similar length to male fish (ICES 2005). This can be observed in the ring patterns in the otoliths with male fish tending to have smaller increments due to slower growth, and is especially seen in fish after

first maturity (Figure 6.1.5).Therefore, knowledge of the sex of the fish may be used as additional factor when ageing.

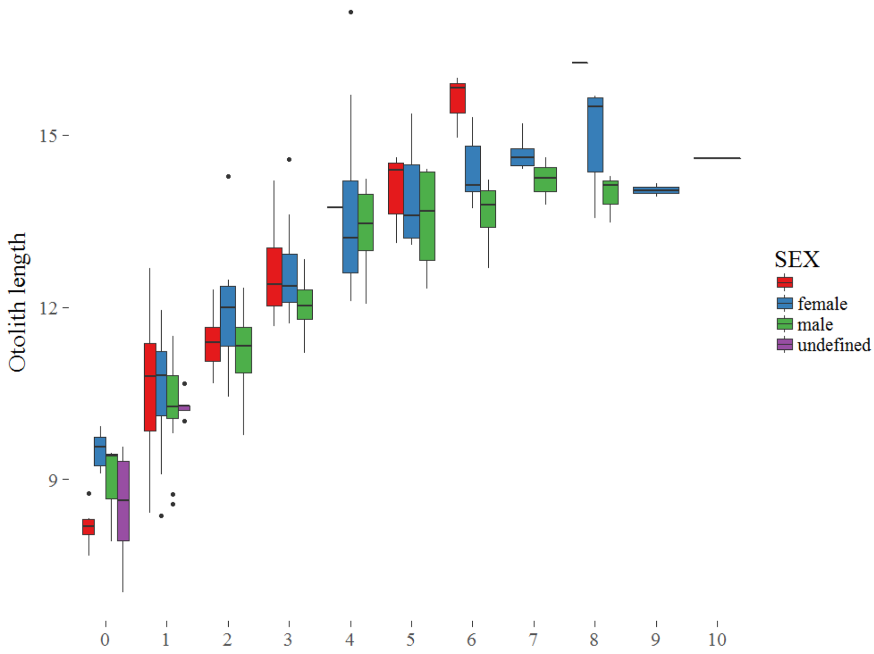


Figure 6.1.5. Otolith length (mm) by fish age and by sex: NA (red), female (blue), male (green) and undefined (purple).

6.2 Additional supporting information on age reading

- The ring called Bailey's zone was first identified by Roger Bailey (Bailey, 1970); In samples of small blue whiting taken by small-meshed trawl in June 1967, Bailey found two distinct modal size groups, one of them around 8-9 cm, the other 13-15 cm. with no clearly defined winter growth rings in the otoliths. However, he considered that to attain a length of 13-15 cm by late June in these areas was unlikely and therefore proposed that, whereas the smaller size group may have been spawned that year, the larger ones were more likely to be 1 year old. He also found that most of the otoliths of the second modal group (13-15 cm) showed a very indistinct ring when viewed in transverse section and this, he thought, may have been a weakly-developed first winter growth check. That zone was called thereafter Bailey's Zone.
- Jakupsstovu (1979) suggested that this zone may be associated with a change of habit or depth; in this case it would be equivalent to the "Bowers Zone" found in whiting otoliths (Gambell and Messtorff, 1964). This first zone is formed when the fish are 4-10 cm in length. Bailey (1982) concludes that it is difficult to explain how the youngest age group of blue whiting could have remained totally unobserved throughout their first winter and spring, especially if one considers their undoubted abundance. On the face of it, therefore, Jakupsstovu's interpretation seems more credible, and for the sake of consistency it is probably wisest at present to follow Jakupsstovu's (1979) interpretation in which the age is given by the number of winter rings on the otolith.
- Mature fish begin to grow later in the year than immature by reason of using the energy resource for gonad maturation cycle vs. using it to the somatic growth only by young fish. New research has shown that blue whiting may mature already around age 1 (ICES 2013a). Thus, when possible, **maturity**

stage should be used as an additional indicator for aging of fish caught during the spawning season.

- **Growth** begins when fish start feeding after the winter period/spawning and finish feeding after the accumulation of enough food reserves. In the last quarter, growth is finished due to enough energy resource for next spawning period, and the next winter ring has started to form. Therefore, stomach fullness can be used as an additional indicator for reading.
- Blue whiting has a wide distribution and a complicated life cycle in Atlantic waters. It can be reflected in all phases of the fish growth, and consequently in the otolith. The distribution is reflected in the landings, as shown in the map that the WGWIDE includes each year (Figure 6.2.1). The map also shows spawning concentrations west of the British Isles (Porcupine and Rockall Bank). That is during winter, where they don't feed and instead spend energy on spawning, and in the pre-spawning and post-spawning migrations. There is almost no otolith calcification so it is marking a translucent ring. After that, eggs and larvae drift mainly northward but also partly southward, recruiting to the nursery areas of the north (mainly Norwegian Sea) and the south (mainly Biscay Bay). At the same time, adult blue whiting migrates to the feeding grounds (in the same areas as the nurseries). They spread all around the Norwegian Sea, and part of the stock distribution is so scattered that it can't be detected by the fisherman or the surveys. That is shown in the 3rd and 4th maps. In the feeding area the fish grow and the otolith is marking a wide opaque ring.
- Another factor that affects the otolith growth is the **strength of recruitment**. Blue whiting stock alternates from periods of high recruitment regime to others of low recruitment. That affects the otolith growth and any other density-dependent characteristic.

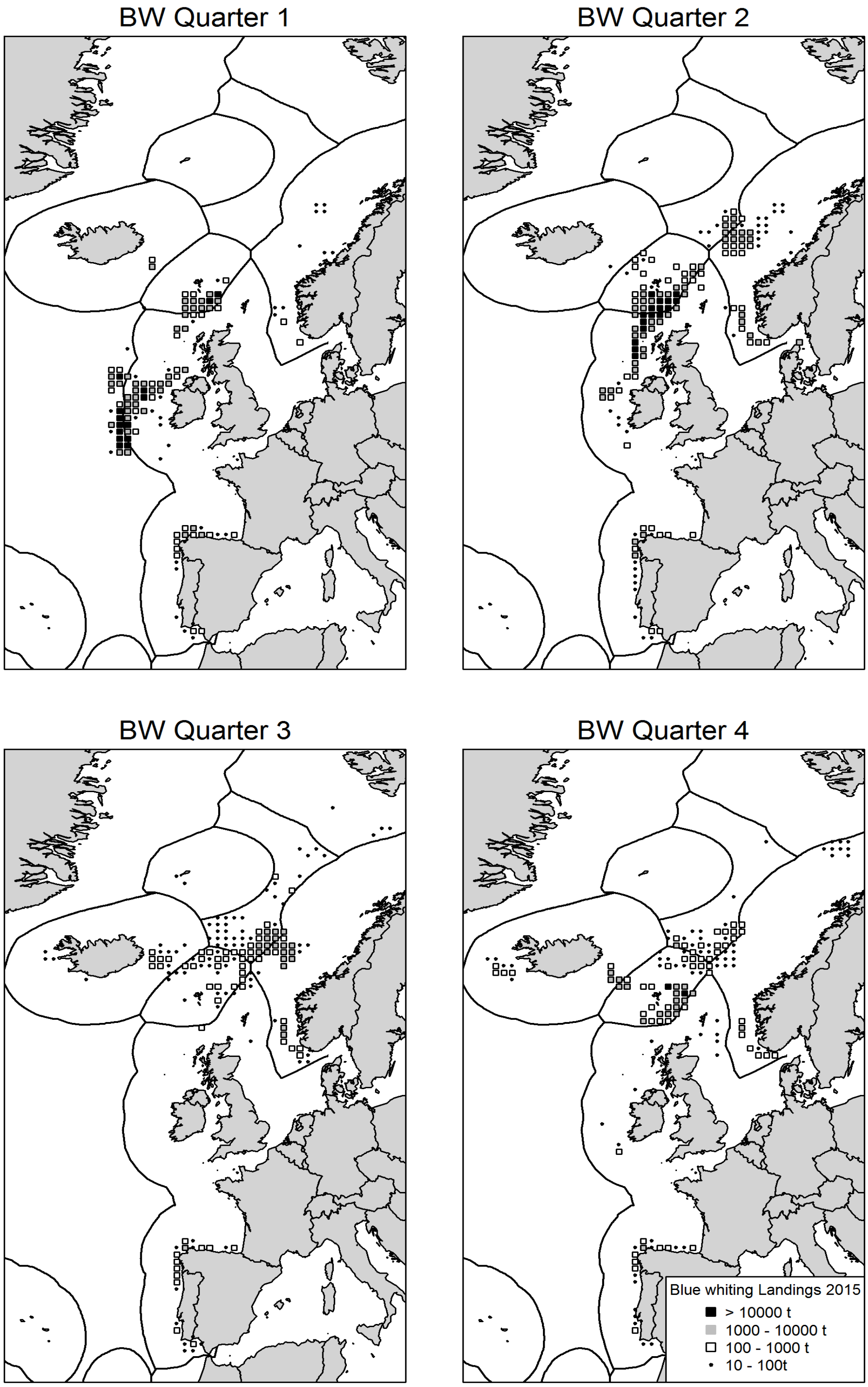


Figure 6.2.1. Blue whiting total catches (ICES estimates) in 2015 by quarter and ICES rectangle. Landings between 10 and 100 tonnes (black dots), between 100 and 1000 tonnes (open squares), 1000 and 10 000 tonnes (gray squares) and exceeding 10 000 tonnes (black squares). The catches on the maps constitute 99.5% of the total catches (ICES 2016).

7 Clarify the interpretation of annual rings (ToRc)

7.1 Issue 1: Faint zone and strange ring pattern

The guidelines are made to guide the readers in getting the same ages across countries. All age readings are based on subjective views and it is important that all readers of the same species have the same view of how to interpret the annual rings of the otoliths. However, sometimes it is not possible to follow all the guidelines, as there might be confounding issues.

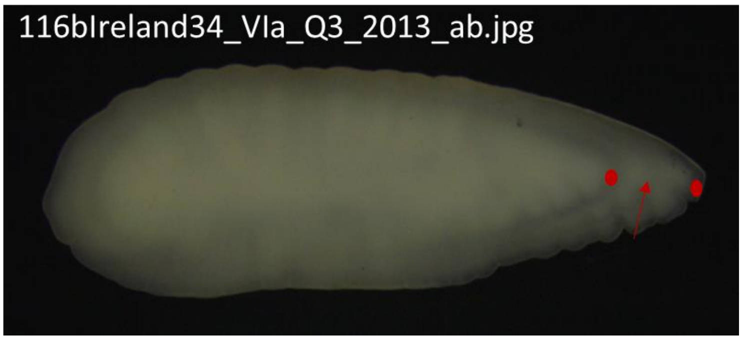
An example is shown below, where the discussion of whether a zone should be counted or not is discussed against growth pattern.

Fish #116 (Figure 7.1.1) is showing a clear inner zone and a clear zone at the edge (marked with red dots). Between the two zones a faint zone can be identified (marked with an arrow). The question arises whether this should be counted as a zone or not. The zone is fainter than the other zones, but you can follow it all round the otolith. The otolith growth pattern, thereby, shows a narrow zone before a larger zone, which, according to the guidelines, is unlikely.

When analysing fish #117 (Figure 7.1.1) a similar zone formation could be seen, but here the growth pattern is more as expected, with zones growing narrower towards the edge.

Fish #118 (Figure 7.1.1) had the same faint zone between the edge and the zone before, though this was a year older than the two other fish. Here it is clear that the zone need to be counted as a year, otherwise the growth pattern would be very unlikely, with very high growth during the last year.

All three fish is captured in the same area and at the same time. This shows the importance of regarding an otolith as a part of a population and not as an isolated individual. When something looks outside the ordinary, it should not be dismissed unless it cannot be found in any other specimens from the same catch/area/time etc. It can be a good idea to scan all the otoliths from the same catch/haul before ageing and then return to the difficult otoliths (AQ2 and AQ3).



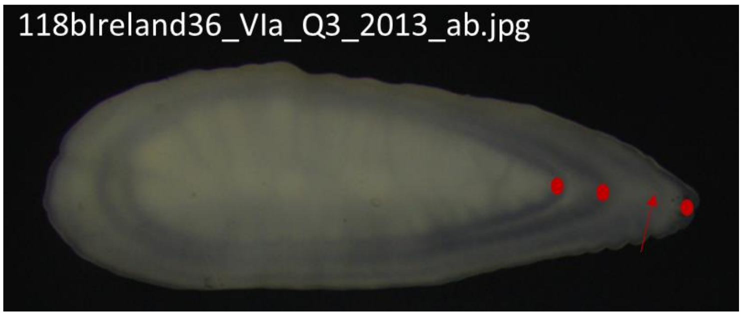


Figure 7.1.1. Three otoliths from the same catch with an unclear zone.

7.2 Issue 2: Size of the first zone

Images below (Figure 7.2.1) exemplify the Baileys zone and the size of the first annual ring. Figures 1a and 79a are images of the otolith as the reader sees them, while figures 1b and 79b are polished down. On both polished images, the inner zone becomes clear with lobes as found on young-of-the-year otoliths. On the unpolished otoliths, this inner zone is only visible in one of the otoliths (1a). According to the rules, the inner zone from fish 1a is too narrow to be counted, and the second zone is also in the lower end of the scale. This zone at 0.79 cm was, however, agreed to be counted as a zone by the members of the workshop. For otolith 79 the first zone most readers annotated during the pre-workshop exercise was by the green arrow (around 1.2 cm), but the polishing reveals two zones inside this, which are in the range above the 8mm.

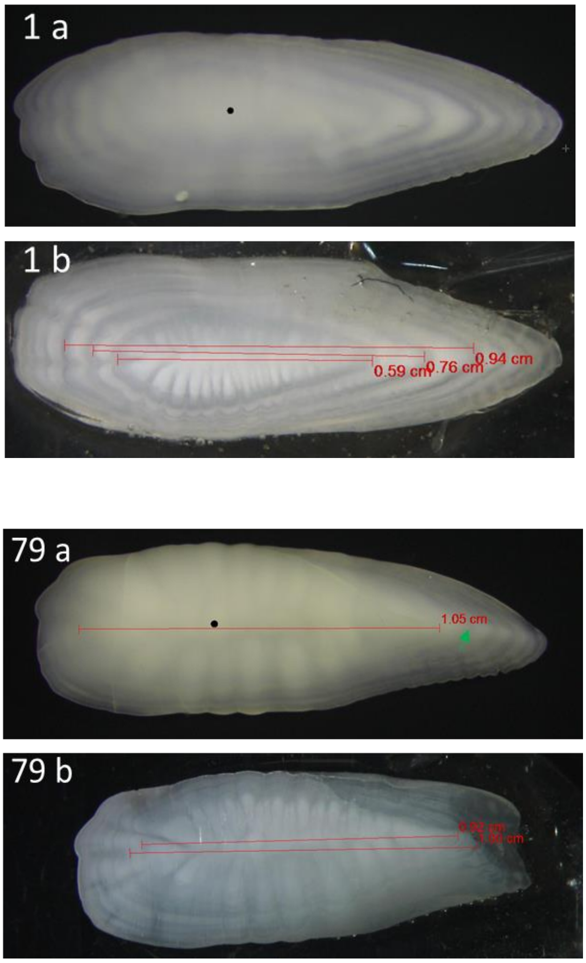


Figure 7.2.1. Otoliths photographed whole in water (1a and 79a), and polished down and photographed in water (1b and 79b).

Otoliths from the NAFO-area were very opaque and zones were difficult to distinguish. After polishing these otoliths, the zones became very clear (Figure 7.2.2).

Figure 7.2.2. An otolith from the NAFO-area whole in water (left) and polished down (right).

7.2.1 Test of agreed otoliths

88 of the otoliths we agreed on during the workshop were annotated in plenary and the radius measured.

Analysis of Variance (ANOVA) confirms that radius of the first ring from Blue whiting otoliths significantly differs between studied areas: Ireland (N=12), Mediterranean (N=6), Norway (N=26), NW Atlantic (N=10), Portugal (N=6) and Atlantic Spanish coasts (N=28) as showed in Table 1.

Table 1. Results of the ANOVA used to test differences in the first ring radius between studied areas.

	Df	Sum Sq	Mean Sq	F value	p-level
area	5	0.04796	0.009592	5.73	$1.51 \cdot 10^{-4}$
Residuals	77	0.12891	0.001674		

Figure 7.2.1.1 shows that the first ring of otoliths from the NW Atlantic is significantly longer (p<0.05) than those from the rest of studied areas, saving Mediterranean Sea. First ring radius of otoliths from the Mediterranean are significantly longer than those from Ireland and Norway, nevertheless there is not significant differences with those otoliths from Portugal and Spain. The results demonstrate that first ring radius depends on geographic distribution, suggesting that Iberian Peninsula is a transition zone among large first rings in the Mediterranean and short ones in the NE Atlantic.

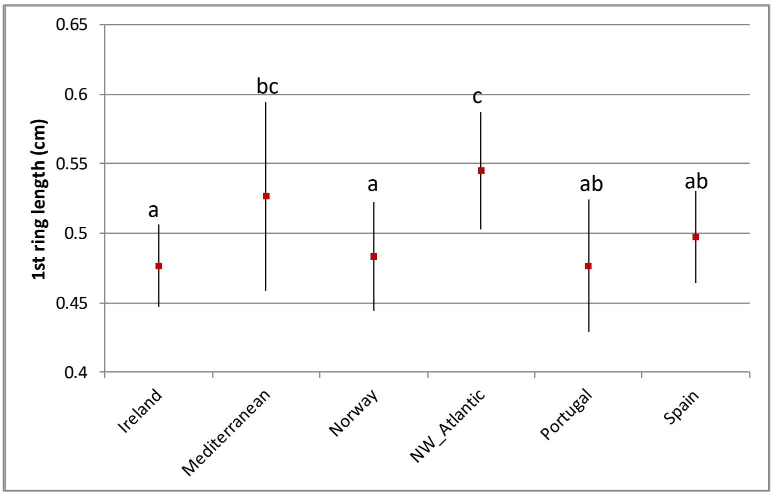


Figure 7.2.1.1. Mean (±SD) radius of the first ring of blue whiting in each studied area. Significant differences (p<0.05) are identified by letters.

Comparison of general growth pattern of otoliths by area shows evidence that geographical differences exist not only regarding the first ring but also along the whole growth of the otolith (Figure 7.2.1.2), although not in the same proportion. Mediterranean otoliths are longer than otoliths from the rest of studied areas at all ages, whereas otoliths from the NW Atlantic (XIVb) present a larger first radius but this difference disappears in the subsequent annual rings. There are few otoliths of fish older than 5 years old, thus recorded results for annual rings corresponding to ages 6, 7, and 8 are not conclusive.

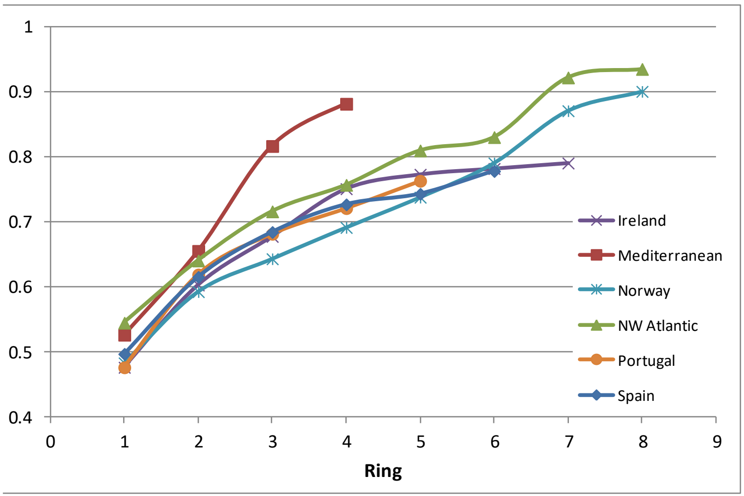


Figure 7.2.1.2.Mean otolith's annual ring radius (cm) in each studied area

Table 2 shows descriptive statistics of the first ring length/diameter (mm) in each studied area. Readers use diameter, when they age the otolith, and therefore this table has been produced to give an idea of the minimum and maximum diameter to be expected in the different areas. This test is made from too few fish to use it as a guideline, but it gives a good idea of the differences between areas.

Table 2. Descriptive statistics of the first ring diameter by studied area

Area	Min.	Quartile 25%	Mean	Quartile 75%	Max.
IIa - IXa	7.83	8.70	9.08	9.38	10.40
XIVb	9.17	9.40	10.32	11.08	11.99
Mediterranean	8.48	9.04	10.15	10.62	12.12

8 Create a reference collection of agreed age otoliths (ToRf)

8.1 Introduction

The aim was to get good coverage of ages, edge development and descriptive otoliths of issues in the reference collection. The otoliths were chosen by the readers and annotated and agreed upon in plenary. The otoliths will be uploaded by the chairpersons to SmartDot as soon as it is up and running and annotated according to the agreement during the workshop. All readers will get access to the collection, and the agreed annotation will be available for all to see.

57 otoliths were chosen to be good enough for the collection (Table 8.1.1). A subsample of these will be included in the online collection.

Table 8.1.1. Otoliths for the reference collection.

ICES AREA	# OF OTOLITHS	SEASONS	AGE RANGE
IIb	2	Q2	1 - 8
IVa	20	Q3, Q4	0 – 6
VIa and VIIc	10	Q1, Q2, Q3	0 – 7
VIIIc	20	Q1, Q2, Q3, Q4	0 – 5
IXa	5	Q1	1 – 5

A collection of otoliths are presented in Annex 4.

9 Image J

IPMA and ISEL (Instituto Superior de Engenharia de Lisboa) have developed a plugin for ImageJ, which can be used as a help for aging otoliths (Gonçalves *et al.* 2017). We were given a presentation by VitorVaz da Silva (ISEL/IPL) on the second day of the workshop. Two useful plugins have been presented: OtoRing (Section 10.1) and 3D Profile (Section 10.2).

9.1 OtoRing

The plugin OtoRing recognises the variation in opacity in the otolith and suggests points with maximum and minimum luminous intensity. The plugin is written in Java and under development, thus future versions can be downloaded from https://github.com/tektonia/otoring where the source code is available. The OtoRing installation follows the normal procedure of packages installation at ImageJ.

In order to use the OtoRing plugin the next steps must be followed:

Select an Otolith image

Select the line tool and Draw a line over the otolith (ROI = Region Of Interest)

Choose on the menu Plugins->OtoRing->OtoRing

If parameters are to be changed use Plugins->OtoRing->OtoRing Parameters

The following figure (Figure 9.1.1) shows an example of the otolith with the line selecting the region of interest, and the result from plugin execution.

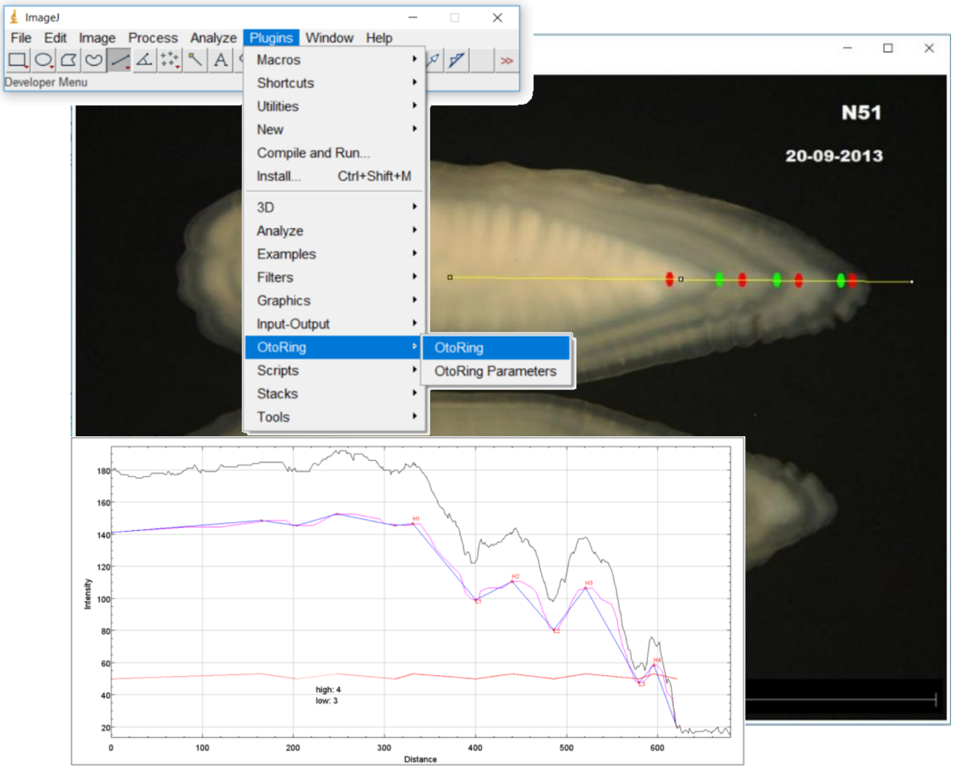


Figure 9.1.1. Profile density plot and corresponding identification marks on the contrasting otolith sections.

The List option on the graph (not shown) opens a window that shows a table with all the points. The last 4 columns of that table, on the right, are the high(x,y) and low(x,y) points. The x is distance and y is intensity. There are other options on the graph namely Save (csv format) that stores the points for other programs.

The OtoRing Parameters definition can be altered to enhance the rings (Figure 9.1.2).

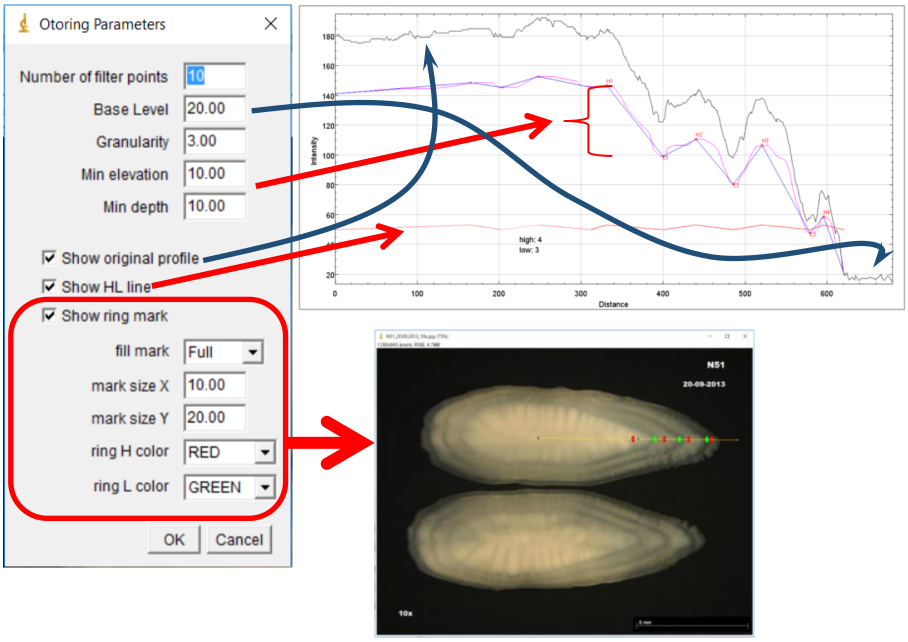


Figure 9.1.2. OtoRing parameters setting definition.

OtoRing parameters setting window (Figure 9.1.2) shows the default setting, used for subsequent images.

9.2 3D Profile

The 3D profile viewer ImageJ plugin is available on the current ImageJ release, but can also be downloaded from https://imagej.nih.gov/ij/plugins/surface-plot-3d.html and installed on other ImageJ version.

In order to use the 3D surface plot plugin the next steps must be followed:

Select an Otolith image

Use one of the tools (polygon, freehand) to select the otolith or area (ROI = Region Of Interest). Otherwise, if no selection is made, the whole image is considered.

Click on the :(Plugins->3D->Interactive Surface Plot) or (Analyse->3D surface plot)

The next figures show (Figure 9.2.1 and 9.2.2) an example of the otolith framed by the polygon tool selecting the region of interest and result.

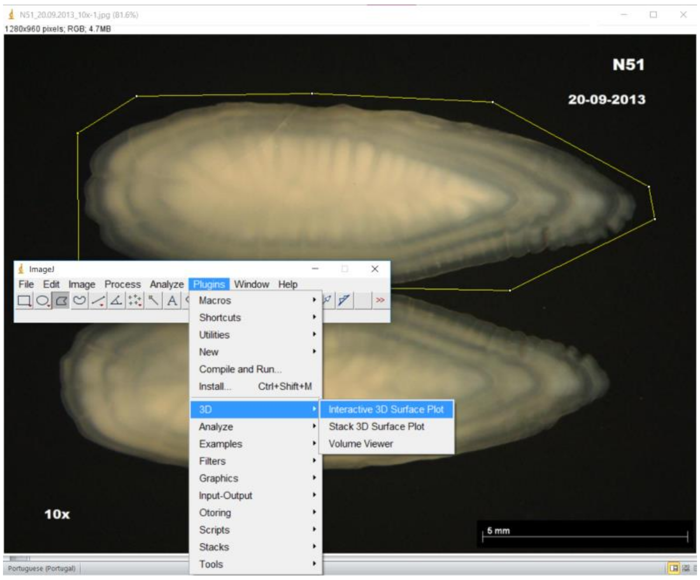


Figure 9.2.1. Otolith framed with the region of interest selected by the polygon tool.

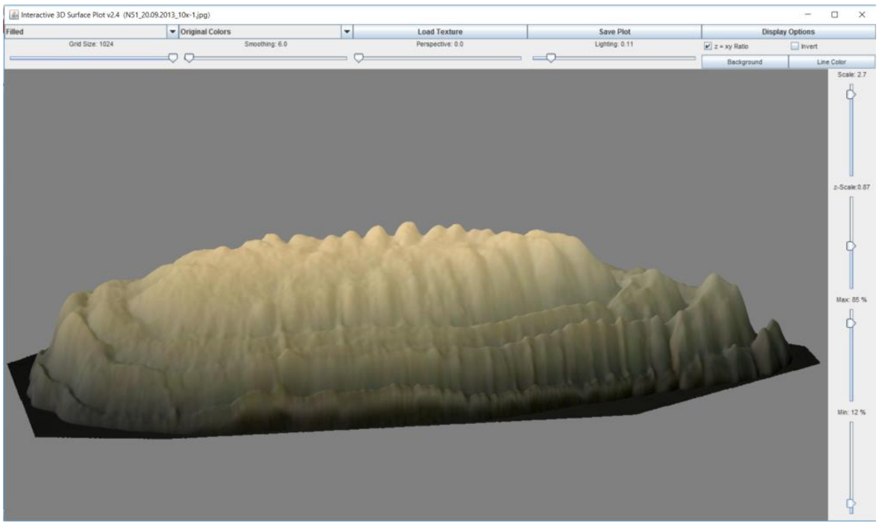


Figure 9.2.2. Otolith selected region after the 3D Profile plugin were executed.

9.3 Links:

ImageJ releases: http://imagej.net

ImageJ plugins:https://imagej.nih.gov/ij/plugins/

OtoRing – source code and plugin: https://github.com/tektonia/otoring

OtoRing – plugin for ImageJ Updater: http://sites.imagej.net/OtoRing

3D – plugins for ImageJ Updater: http://sites.imagej.net/3D/

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Annex 1: Agenda

Tuesday 6th	
10:00 – 10:30	*Welcome and ToRs*
10:30 – 11:00	*Presentation of the results of the pre-workshop exchange (ToR b) and Presentation of issues regarding age readings and age reading protocol (ToR a, c and d)*
11:00 – 12:00	Presentation of validation study by IPMA (ToR e)
12:00 – 13:00	*Go through pre-workshop exchange otoliths in WebGR*
13:00 – 14:00	*Lunch*
14:00 – 15:00	*Go through pre-workshop exchange otoliths in WebGR*
15:00 – 16:00	*Calibration exercise testing validation method by IPMA*
16:00 – 16:30	*Coffee break*
16:30 – 17:30	*Calibration exercise testing validation method by IPMA*
Wednesday 7th	
09:00 –10:00	Presentation of the results of the workshop exchange
10:00 – 11:15	*Create a reference collection of agreed age otoliths (ToR f)*
11:15– 11:45	Coffee break
11:45 – 13:00	Create a reference collection of agreed age otoliths
13:00 – 14:00	Lunch
14:00 – 16:00	*Go through pre-workshop exchange otoliths in WebGR with regard to IPMA study*
16:00 – 16:30	Coffee break
16:30 – 17:00	*Presentation of Image J as tool to age blue whiting (ToR e)*
Thursday 8th	
09:00 – 11:00	*Go through pre-workshop exchange otoliths in WebGR*
11:00 – 11:30	*Coffee break*
11:30 – 13:00	*Go through pre-workshop exchange otoliths in WebGR*
13:00 – 14:30	*Lunch*
14:30 – 18:00	*Go through pre-workshop exchange otoliths in WebGR*
20:00 – ??	Social Dinner *at Adego de Kaïs*
Friday 9th	
09:00 – 10:00	*Update guidelines for age blue whiting in report* *Improve the age reading protocols (ToR d)*
10:00 – 11:00	Going through the draft report
11:00 – 11:30	*Coffee break*
11:30 – 13:00	Go through pre-workshop exchange otoliths in WebGR
13:00 – 13:30	*Sum up and goodbye*

Annex 2: List of participants

Name	Country	Email
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* Did not participate in the workshop, but assisted by presenting or preparing otoliths for the meeting.

Annex 3: Otolith exercise

Table 15.1. Pre-workshop CV and percentage agreement and relative bias against modal age for age-readers.

COEFFICIENT OF VARIATION (CV)

MODAL age	Ferreira Reader 3	Prokhorova Reader 25	Alvarez Reader 18	Pasterkamp Reader 27	Johannsdottir Reader 40	de Lange Reader 10	Wilhelms Reader 12	Beusel Reader 17	Mullins Reader 23	Otero Reader 32	Dufour Reader 33	Resende Reader 45	Rasmussen Reader 30	Mills Reader 37	Sørensen Reader 9	Kolbeinson Reader 13	Pronyuk Reader 35	Thomassen Reader 41	Vestergaard Reader 42	Silva Reader 5	Dores Reader 6	Eriksen Reader 8	Rodriguez Reader 20	Beattie Reader 34	Salvador Reader 15	Sindahl Reader 46	Antolinez Reader 22	van Os Reader 28	Dinis Reader 31	ALL Readers
0	520 %	244 %	0 %	288 %	0 %	0 %	288 %	360 %	360 %	0 %	133 %	244 %	123 %	133 %	0 %	0 %	183 %	244 %	360 %	0 %	144 %	244 %	0 %	360 %	0 %	124 %	520 %	97 %	500 %	228.7%
1	41 %	34 %	42 %	38 %	38 %	45 %	43 %	48 %	73 %	52 %	29 %	54 %	34 %	41 %	37 %	39 %	45 %	50 %	45 %	58 %	34 %	47 %	23 %	56 %	26 %	34 %	50 %	39 %	65 %	38.8%
2	22 %	26 %	32 %	28 %	29 %	29 %	20 %	30 %	35 %	37 %	30 %	43 %	16 %	23 %	17 %	37 %	33 %	21 %	20 %	57 %	24 %	33 %	36 %	45 %	25 %	29 %	19 %	26 %	60 %	29.1%
3	19 %	18 %	13 %	16 %	19 %	18 %	11 %	12 %	23 %	21 %	33 %	36 %	17 %	21 %	20 %	15 %	16 %	21 %	19 %	47 %	15 %	18 %	28 %	27 %	24 %	30 %	30 %	15 %	39 %	22.8%
4	19 %	15 %	23 %	18 %	17 %	9 %	8 %	14 %	19 %	12 %	27 %	45 %	23 %	35 %	17 %	13 %	20 %	17 %	14 %	40 %	20 %	22 %	32 %	27 %	18 %	49 %	21 %	18 %	48 %	24.3%
5	23 %	20 %	24 %	20 %	30 %	21 %	22 %	17 %	34 %	33 %	30 %	35 %	28 %	30 %	41 %	23 %	23 %	28 %	23 %	40 %	17 %	20 %	28 %	40 %	32 %	29 %	18 %	20 %	44 %	24.2%
6	16 %	12 %	12 %	9 %	15 %	8 %	10 %	14 %	23 %	17 %	17 %	25 %	20 %	25 %	20 %	11 %	16 %	11 %	11 %	25 %	18 %	11 %	19 %	22 %	15 %	31 %	17 %	18 %	34 %	20.1%
7	5 %	13 %	19 %	6 %	17 %	8 %	11 %	8 %	21 %	10 %	14 %	15 %	17 %	15 %	15 %	21 %	13 %	19 %	18 %	23 %	11 %	8 %	15 %	18 %	15 %	40 %	22 %	10 %	20 %	20.4%
8	19 %	12 %	17 %	10 %	5 %	9 %	6 %	11 %	16 %	11 %	12 %	18 %	20 %	13 %	29 %	19 %	15 %	14 %	13 %	22 %	16 %	12 %	16 %	20 %	17 %	36 %	19 %	17 %	26 %	24.3%
9	18 %	25 %	6 %	6 %	18 %	13 %	18 %	25 %	10 %	11 %	28 %	29 %	39 %	28 %	6 %	0 %	11 %	12 %	11 %	40 %	28 %	14 %	35 %	6 %	33 %	13 %	35 %	40 %	33 %	21.8%
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	82.5%	**48.6%**	**23.6%**	**52.6%**	**22.9%**	**22.4%**	**51.2%**	**63.0%**	**74.4%**	**27.9%**	**38.8%**	**62.6%**	**34.8%**	**40.0%**	**22.5%**	**23.2%**	**46.2%**	**51.8%**	**62.1%**	**41.3%**	**36.4%**	**52.1%**	**23.7%**	**73.8%**	**20.7%**	**43.4%**	**84.0%**	**33.0%**	**96.1%**	**50.5%**
RANKING	27	17	6	21	4	2	18	24	26	8	12	23	10	13	3	5	16	19	22	14	11	20	7	25	1	15	28	9	29	

PERCENTAGE AGREEMENT

MODAL age	Ferreira Reader 3	Prokhorova Reader 25	Alvarez Reader 18	Pasterkamp Reader 27	Johannsdottir Reader 40	de Lange Reader 10	Wilhelms Reader 12	Beusel Reader 17	Mullins Reader 23	Otero Reader 32	Dufour Reader 33	Resende Reader 45	Rasmussen Reader 30	Mills Reader 37	Sørensen Reader 9	Kolbeinson Reader 13	Pronyuk Reader 35	Thomassen Reader 41	Vestergaard Reader 42	Silva Reader 5	Dores Reader 6	Eriksen Reader 8	Rodriguez Reader 20	Beattie Reader 34	Salvador Reader 15	Sindahl Reader 46	Antolinez Reader 22	van Os Reader 28	Dinis Reader 31	ALL Readers
0	96 %	85 %	100 %	89 %	100 %	100 %	89 %	93 %	93 %	100 %	63 %	85 %	59 %	63 %	100 %	100 %	74 %	85 %	93 %	100 %	67 %	85 %	100 %	93 %	100 %	56 %	96 %	44 %	96 %	86 %
1	77 %	89 %	78 %	75 %	70 %	77 %	75 %	85 %	72 %	75 %	89 %	75 %	78 %	79 %	83 %	87 %	53 %	69 %	77 %	75 %	54 %	66 %	93 %	53 %	93 %	79 %	79 %	31 %	72 %	75 %
2	82 %	75 %	79 %	63 %	68 %	71 %	83 %	68 %	59 %	71 %	68 %	66 %	90 %	88 %	87 %	59 %	70 %	73 %	80 %	34 %	66 %	78 %	60 %	46 %	76 %	70 %	85 %	48 %	46 %	69 %
3	77 %	80 %	82 %	77 %	79 %	64 %	87 %	87 %	67 %	71 %	46 %	46 %	82 %	71 %	82 %	74 %	54 %	64 %	69 %	38 %	79 %	71 %	51 %	61 %	58 %	31 %	54 %	46 %	33 %	65 %
4	69 %	67 %	58 %	56 %	53 %	84 %	89 %	68 %	58 %	78 %	33 %	32 %	59 %	42 %	65 %	74 %	44 %	47 %	63 %	21 %	68 %	44 %	21 %	44 %	50 %	32 %	47 %	67 %	33 %	54 %
5	69 %	69 %	81 %	75 %	56 %	56 %	69 %	50 %	63 %	38 %	38 %	13 %	38 %	31 %	53 %	38 %	38 %	63 %	69 %	19 %	63 %	63 %	25 %	56 %	31 %	13 %	44 %	63 %	6 %	48 %
6	41 %	53 %	61 %	83 %	67 %	72 %	83 %	44 %	44 %	50 %	11 %	6 %	18 %	17 %	65 %	61 %	47 %	72 %	72 %	17 %	56 %	61 %	11 %	39 %	50 %	0 %	33 %	44 %	0 %	44 %
7	89 %	67 %	22 %	78 %	22 %	67 %	44 %	67 %	22 %	56 %	33 %	0 %	50 %	22 %	13 %	33 %	22 %	33 %	56 %	11 %	44 %	67 %	13 %	22 %	33 %	13 %	0 %	22 %	0 %	35 %
8	50 %	40 %	55 %	45 %	82 %	73 %	27 %	10 %	45 %	55 %	0 %	0 %	9 %	0 %	70 %	55 %	30 %	73 %	36 %	0 %	9 %	45 %	0 %	27 %	45 %	0 %	9 %	9 %	0 %	31 %
9	33 %	33 %	67 %	33 %	33 %	0 %	33 %	0 %	33 %	33 %	33 %	0 %	67 %	33 %	67 %	100 %	33 %	0 %	33 %	33 %	33 %	0 %	0 %	67 %	0 %	0 %	0 %	33 %	0 %	29 %
10	0 %	100 %	0 %	100 %	100 %	100 %	0 %	0 %	100 %	100 %	0 %	0 %	0 %	0 %	100 %	0 %	100 %	100 %	100 %	0 %	0 %	0 %	0 %	100 %	0 %	0 %	0 %	0 %	0 %	38 %
	74.7%	**75.6%**	**75.2%**	**72.5%**	**70.6%**	**73.9%**	**77.1%**	**71.7%**	**63.9%**	**70.4%**	**55.3%**	**50.2%**	**65.7%**	**60.1%**	**77.8%**	**71.8%**	**54.1%**	**66.9%**	**73.1%**	**46.5%**	**60.4%**	**66.7%**	**57.4%**	**54.1%**	**69.0%**	**46.2%**	**62.4%**	**42.0%**	**44.0%**	**63.8%**
RANKING	5	3	4	8	11	6	2	10	17	12	22	25	16	20	1	9	23	14	7	26	19	15	21	24	13	27	18	29	28	

RELATIVE BIAS

MODAL age	Ferreira Reader 3	Prokhorova Reader 25	Alvarez Reader 18	Pasterkamp Reader 27	Johannsdottir Reader 40	de Lange Reader 10	Wilhelms Reader 12	Beusel Reader 17	Mullins Reader 23	Otero Reader 32	Dufour Reader 33	Resende Reader 45	Rasmussen Reader 30	Mills Reader 37	Sørensen Reader 9	Kolbeinson Reader 13	Pronyuk Reader 35	Thomassen Reader 41	Vestergaard Reader 42	Silva Reader 5	Dores Reader 6	Eriksen Reader 8	Rodriguez Reader 20	Beattie Reader 34	Salvador Reader 15	Sindahl Reader 46	Antolinez Reader 22	van Os Reader 28	Dinis Reader 31	ALL Readers
0	0.04	0.15	**0.00**	0.11	**0.00**	**0.00**	0.11	0.07	0.07	**0.00**	0.37	0.15	0.41	0.37	**0.00**	**0.00**	0.30	0.15	0.07	**0.00**	0.33	0.15	0.00	0.07	**0.00**	0.52	0.04	0.59	0.04	0.14
1	0.27	0.02	0.17	0.26	0.31	0.15	0.30	0.21	0.20	0.25	0.11	-0.08	0.22	0.25	0.10	0.08	0.60	0.43	0.30	-0.25	0.46	0.33	0.07	0.53	**0.00**	0.21	0.20	0.95	-0.13	0.22
2	-0.03	-0.05	0.26	0.39	0.22	0.07	0.07	0.10	0.49	0.15	-0.17	-0.44	-0.05	-0.10	0.08	0.02	0.46	0.22	0.15	-0.80	0.24	0.27	-0.38	0.49	-0.24	-0.10	0.00	0.65	-0.56	0.05
3	-0.14	0.06	0.13	0.08	0.15	0.21	0.13	0.08	0.49	**0.00**	-0.64	-0.69	-0.21	-0.21	0.21	0.21	0.49	0.46	0.31	-0.97	0.00	-0.03	-0.59	0.45	-0.34	-0.67	-0.41	0.56	-0.79	-0.06
4	-0.13	-0.07	0.26	0.28	0.32	0.16	0.11	**0.00**	0.47	-0.11	-0.89	-1.32	-0.59	-1.00	0.18	-0.05	0.56	0.53	0.16	-1.37	-0.32	-0.06	-1.26	0.06	-0.28	-1.42	-0.37	-0.06	-1.33	-0.27
5	-0.06	0.13	0.50	0.31	0.38	0.56	0.13	-0.19	0.63	-0.50	-1.06	-1.88	-0.88	-1.13	0.80	-0.06	0.88	0.63	0.38	-1.75	-0.38	0.38	-1.31	0.56	-0.75	-1.73	-0.56	-0.44	-2.06	-0.29
6	-0.47	0.29	0.39	0.22	0.44	0.17	0.11	-0.56	0.67	-0.44	-1.11	-1.89	-1.12	-1.17	0.65	-0.17	0.71	0.39	0.11	-1.78	-0.56	-0.17	-1.61	-0.11	-0.39	-2.06	-0.78	-0.72	-2.11	-0.45
7	-0.11	0.56	1.67	0.22	0.89	0.11	-0.11	-0.33	1.56	0.00	-1.00	-1.89	-0.75	-0.44	2.00	0.78	1.33	1.44	1.00	-1.22	-0.67	0.11	-1.25	1.78	-0.33	-2.88	-1.33	-0.89	-2.22	-0.06
8	-0.60	-0.30	0.45	-0.18	0.18	0.18	-0.73	-1.40	0.45	-0.18	-2.09	-3.45	-1.91	-2.18	0.90	-0.36	0.90	0.18	-0.64	-3.36	-1.82	-0.27	-2.60	0.00	-1.00	-4.45	-1.64	-1.82	-3.64	-1.09
9	-0.33	-0.67	0.33	0.67	-0.33	-0.33	-0.33	-2.00	1.00	0.00	-1.67	-3.67	-1.67	-1.67	0.33	0.00	**0.00**	0.33	0.00	-2.67	-1.67	-2.00	-2.33	0.33	-1.33	-4.67	-2.33	-2.67	-3.00	-1.11
10	-2.00	**0.00**	2.00	**0.00**	**0.00**	**0.00**	-1.00	-3.00	**0.00**	**0.00**	-2.00	-4.00	-2.00	-1.00	**0.00**	-2.00	**0.00**	**0.00**	**0.00**	-3.00	-2.00	-1.00	-2.00	**0.00**	-1.00	-7.00	-2.00	-3.00	-6.00	-1.45
	-0.04	**0.05**	**0.28**	**0.22**	**0.26**	**0.15**	**0.10**	**-0.07**	**0.43**	**0.00**	**-0.44**	**-0.84**	**-0.26**	**-0.33**	**0.30**	**0.04**	**0.58**	**0.41**	**0.21**	**-0.94**	**-0.03**	**0.11**	**-0.63**	**0.40**	**-0.27**	**-0.78**	**-0.29**	**0.26**	**-0.96**	**-0.07**
RANKING	4	5	16	11	13	9	7	6	22	1	23	27	14	19	18	3	24	21	10	28	2	8	25	20	15	26	17	12	29	

Table 15.2. Number of otoliths by modal age that achieved over 80% agreement between age readers during the pre-workshop exercise.

Table 7 Agreed collection Criterion 80% agreement	
MODAL AGE	n
0	19
1	32
2	13
3	11
4	2
5	1
6	0
7	0
8	0
9	0
10	0
	78

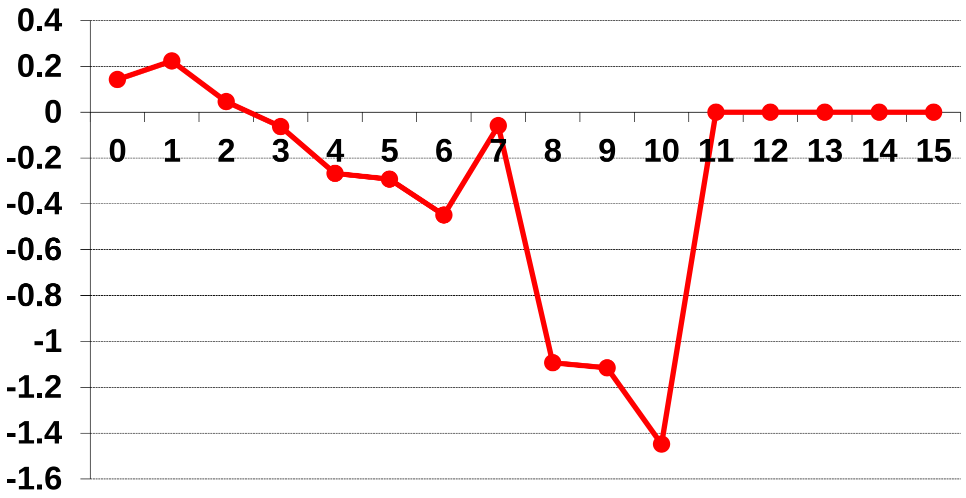


Figure15.3. Relative bias by modal age.

Table 15.4. Agreement by month and area

PERCENTAGE AGREEMENT													
MODAL	1	2	3	4	5	6	7	8	9	10	11	12	Agree-
age	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	ment
0	-	-	-	-	-	83 %	-	79 %	88 %	83 %	-	87 %	86.3%
1	71 %	83 %	-	77 %	76 %	61 %	76 %	94 %	82 %	65 %	91 %	45 %	74.5%
2	78 %	76 %	51 %	64 %	-	52 %	-	66 %	72 %	79 %	-	48 %	69.3%
3	69 %	74 %	58 %	87 %	78 %	-	54 %	56 %	72 %	70 %	-	-	64.8%
4	-	56 %	51 %	72 %	-	-	49 %	48 %	43 %	47 %	-	-	53.9%
5	64 %	29 %	51 %	-	-	24 %	-	55 %	55 %	43 %	-	-	47.8%
6	-	52 %	26 %	40 %	40 %	-	36 %	-	59 %	48 %	-	-	44.3%
7	-	29 %	34 %	43 %	33 %	-	-	-	-	34 %	-	-	35.4%
8	-	31 %	31 %	35 %	26 %	-	-	29 %	-	29 %	-	-	31.0%
9	-	28 %	21 %	-	38 %	-	-	-	-	-	-	-	28.7%
10	-	-	-	-	38 %	-	-	-	-	-	-	-	37.9%
11	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean CV	73.3%	62.2%	49.5%	62.3%	55.6%	57.9%	60.7%	62.4%	76.6%	62.2%	91.1%	79.9%	63.7%
Weighted													

PERCENTAGE AGREEMENT													
MODAL	SAMPLING STRATA												Agree-
age	VIIIc	XIVb	VIa	VIIj	VIIb	VIIc	IVa	IIb	IXa	diterrane	NAFO 1C	L	ment
0	97 %	-	95 %	86 %	100 %	-	89 %	-	83 %	74 %	-	-	86.3%
1	75 %	54 %	77 %	-	-	-	89 %	66 %	81 %	51 %	48 %	-	74.5%
2	82 %	62 %	64 %	-	-	-	77 %	-	54 %	55 %	52 %	-	69.3%
3	72 %	60 %	56 %	-	-	-	58 %	-	79 %	69 %	-	-	64.8%
4	70 %	41 %	51 %	-	-	62 %	52 %	-	-	44 %	-	-	53.9%
5	-	37 %	41 %	-	-	29 %	56 %	-	86 %	24 %	-	-	47.8%
6	48 %	41 %	41 %	-	-	54 %	43 %	40 %	-	-	-	-	44.3%
7	-	-	39 %	-	-	29 %	34 %	33 %	-	-	-	-	35.4%
8	-	38 %	34 %	-	-	31 %	25 %	26 %	-	-	-	-	31.0%
9	-	-	21 %	-	-	28 %	-	38 %	-	-	-	-	28.7%
10	-	-	-	-	-	-	-	38 %	-	-	-	-	37.9%
11	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-	-
Mean CV	74.8%	46.6%	52.2%	86.1%	100.0%	45.4%	69.3%	37.4%	80.3%	59.1%	50.7%	-	63.7%
Weighted													

Table 15.5. The Interreader bias test gives probability of bias between readers and with modal ag.

Inter-reader bias test and reader against MODAL age bias test

	Ferreira Reader 3	Prokhorova Reader 25	Alvarez Reader 18	Pasterkamp Reader 27	Johannsdottir Reader 40	de Lange Reader 10	Wilhelms Reader 12	Beusel Reader 17	Mullins Reader 23	Otero Reader 32	Dufour Reader 33	Resende Reader 45	Rasmussen Reader 30	Mills Reader 37	Sørensen Reader 9	Kolbeinson Reader 13	Pronyuk Reader 35	Thomassen Reader 41	Vestergaard Reader 42	Silva Reader 5	Dores Reader 6	Eriksen Reader 8	Rodriguez Reader 20	Beattie Reader 34	Salvador Reader 15	Sindahl Reader 46	Antolinez Reader 22	van Os Reader 28	Dinis Reader 31
Reader 3																													
Reader 25	–																												
Reader 18	* *	* *																											
Reader 27	* *	* *	–																										
Reader 40	* *	* *	–	–																									
Reader 10	* *	*	*	–	*																								
Reader 12	* *	–	* *	* *	* *	–																							
Reader 17	–	*	* *	* *	* *	* *	* *																						
Reader 23	* *	* *	*	* *	* *	* *	* *	* *																					
Reader 32	–	–	* *	* *	* *	*	–	–	* *																				
Reader 33	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *																			
Reader 45	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *																		
Reader 30	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	*																	
Reader 37	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	–																
Reader 9	* *	* *	–	–	–	*	* *	* *	–	* *	* *	* *	* *	* *															
Reader 13	–	–	* *	* *	* *	*	–	–	* *	–	* *	* *	* *	* *	* *														
Reader 35	* *	* *	* *	* *	* *	* *	* *	* *	*	* *	* *	* *	* *	* *	* *	* *													
Reader 41	* *	* *	*	* *	* *	* *	* *	* *	–	* *	* *	* *	* *	* *	*	* *	* *												
Reader 42	* *	* *	–	–	–	–	* *	* *	* *	* *	* *	* *	* *	* *	–	* *	* *	* *											
Reader 5	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	*	* *	* *	* *	* *	* *	* *	* *										
Reader 6	–	–	* *	* *	* *	* *	*	–	* *	–	* *	* *	* *	* *	* *	–	* *	* *	* *	* *									
Reader 8	* *	–	*	*	* *	–	–	* *	* *	–	* *	* *	* *	* *	*	–	* *	* *	–	* *	*								
Reader 20	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *							
Reader 34	* *	* *	–	*	–	* *	* *	* *	–	* *	* *	* *	* *	* *	–	* *	*	–	*	* *	* *	* *	* *						
Reader 15	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	–	–	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *					
Reader 46	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	–	* *	* *				
Reader 22	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	*	* *	–	–	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	–	* *			
Reader 28	* *	* *	–	–	–	–	*	* *	–	* *	* *	* *	* *	* *	–	* *	* *	–	–	* *	* *	–	* *	–	* *	* *	* *		
Reader 31	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	* *	*	* *	* *	* *	* *	* *	* *	* *	–	* *	* *	* *	* *	* *	* *	* *	* *	
MODAL age	–	–	* *	* *	* *	* *	*	–	* *	–	* *	* *	* *	* *	* *	–	* *	* *	* *	* *	–	*	* *	* *	* *	* *	* *	* *	* *

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

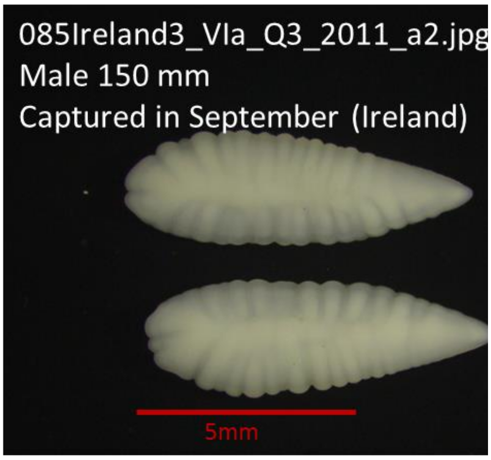
Annex 4: Reference collection

Table 1. 57 otoliths were chosen as useful otoliths for the reference collection.

Stratum	year	no	length	Sex	month	Agreed ages during discussion
IIb	2012	183Norway IIb Q2-2012 24049 1 a.jpg	365	female	5	8
IIb	2012	192Norway IIb Q2-2012 24049 10 a.jpg	200	female	5	1
IVa	2014	160Norway IVa Q3-2014 86611 8 a.jpg	145	U	9	0
IVa	2014	161Norway IVa Q3-2014 86611 9 a.jpg	150	U	9	0
IVa	2014	163Norway IVa Q4-2014 86624 1 a.jpg	155	U	10	0
IVa	2014	164Norway IVa Q4-2014 86624 2 a.jpg	160	U	10	0
IVa	2014	165Norway IVa Q4-2014 86624 3 a.jpg	265	female	10	3
IVa	2014	167Norway IVa Q4-2014 86624 5 a.jpg	270	female	10	2
IVa	2014	169Norway IVa Q4-2014 86624 7 a.jpg	250	male	10	3
IVa	2014	170Norway IVa Q4-2014 86624 8 a.jpg	235	male	10	1
IVa	2009	172Norway IVa Q4-2009 86531 2 a.jpg	265	female	10	1
IVa	2009	177Norway IVa Q4-2009 86531 13 a.jpg	305	female	10	5
IVa	2009	181Norway IVa Q4-2009 86531 23 a.jpg	290	male	10	5
IVa	2009	182Norway IVa Q4-2009 86531 24 a.jpg	290	male	10	6
IVa	2014	143bNorway IVa Q3 2014 8550 1 a.jpg	210	male	8	1
IVa	2014	144bNorway IVa Q3 2014 8550 2 a.jpg	215	male	8	1
IVa	2014	147bNorway IVa Q3 2014 8550 5 a.jpg	225	male	8	1
IVa	2014	149bNorway IVa Q3 2014 8550 11 a.jpg	255	male	8	3
IVa	2014	151bNorway IVa Q3 2014 8550 19 a.jpg	265	female	8	4
IVa	2014	152bNorway IVa Q3 2014 8550 20 a.jpg	245	male	8	3
IVa	2014	156bNorway IVa Q3 2014 8557 11 a.jpg	275	male	8	4
IVa	2014	157bNorway IVa Q3 2014 8557 21 a.jpg	275	female	8	4
IXa	2015	193 PT 1 Q1 IXa a.jpg	165	female	2	1
IXa	2015	194 PT 2 Q1 IXa a.jpg	193	female	2	1
IXa	2015	197 PT 5 Q1 IXa a.jpg	247	male	3	3
IXa	2015	199 PT 7 Q1 IXa a.jpg	260	male	2	3
IXa	2015	200 PT 8 Q1 IXa a.jpg	274	female	3	5
VIa	2011	085Ireland3 VIa Q3 2011 a2.jpg	150	male	9	0
VIa	2011	088Ireland6 VIa Q3 2011 a2.jpg	240	male	9	1
VIa	2011	089Ireland7 VIa Q3 2011 a2.jpg	190	female	9	0
VIa	2011	090Ireland8 VIa Q3 2011 a2.jpg	250	female	9	3
VIa	2011	091Ireland9 VIa Q3 2011 a2.jpg	200	male	9	2
VIa	2011	092Ireland10 VIa Q3 2011 a2.jpg	330	female	9	5
VIa	2013	114bIreland32 VIa Q3 2013 ab.jpg	270	male	3	3
VIa	2012	141bIreland59 VIa Q2 2012 ab.jpg	320	male	4	7
VIIc	2010	107bIreland25 VIIc Q2 2010 ab.jpg	290	male	2	6
VIIc	2010	111bIreland29 VIIc Q2 2010 ab.jpg	320	male	2	4
VIIIc	2015	001Spain1 Q1 VIIIc a.jpg	256	male	2	5
VIIIc	2015	002Spain2 Q1 VIIIc a.jpg	269	female	2	3
VIIIc	2015	003Spain3 Q1 VIIIc a.jpg	228	male	2	3
VIIIc	2015	004Spain4 Q1 VIIIc a.jpg	244	male	2	2
VIIIc	2015	007Spain7 Q1 VIIIc a.jpg	210	male	2	2
VIIIc	2015	009Spain9 Q2 VIIIc a.jpg	186	female	4	1
VIIIc	2015	010Spain10 Q2 VIIIc a.jpg	211	male	4	2
VIIIc	2015	011Spain11 Q2 VIIIc a.jpg	243	male	4	3
VIIIc	2015	012Spain12 Q2 VIIIc a.jpg	299	male	4	4
VIIIc	2015	013Spain13 Q2 VIIIc a.jpg	273	male	4	4
VIIIc	2015	020Spain20 Q3 VIIIc a.jpg	262	female	7	1
VIIIc	2015	021Spain21 Q3 VIIIc a.jpg	289	female	7	3
VIIIc	2015	022Spain22 Q3 VIIIc a.jpg	260	male	7	2
VIIIc	2015	023Spain23 Q3 VIIIc a.jpg	218	male	7	1
VIIIc	2015	024Spain24 Q3 VIIIc a.jpg	253	male	8	2
VIIIc	2015	025Spain25 Q4 VIIIc a.jpg	261	female	10	1
VIIIc	2015	027Spain27 Q4 VIIIc a.jpg	210	female	10	0
VIIIc	2015	029Spain29 Q4 VIIIc a.jpg	242	female	10	1
VIIIc	2015	031Spain31 Q4 VIIIc a.jpg	247	female	10	1
VIIIc	2015	032Spain32 Q4 VIIIc a.jpg	250	male	10	2

Table A4 Examples of otoliths of different ages

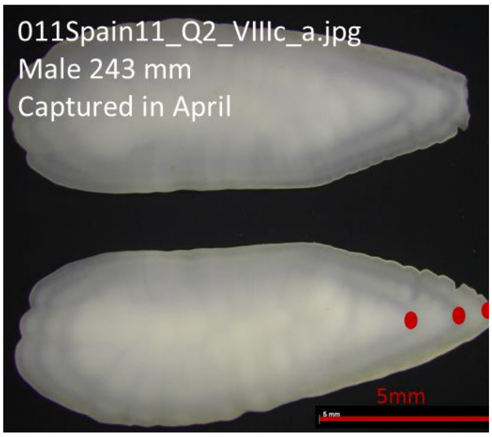
0 Years old

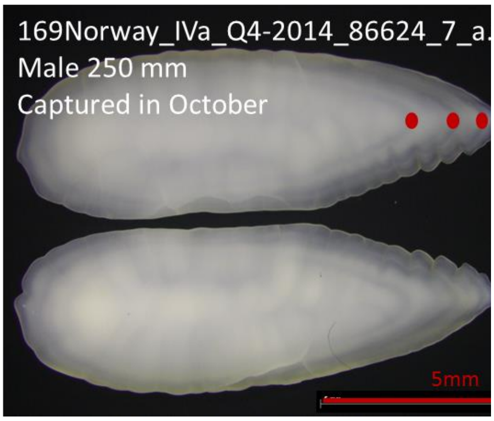


1 year old

2 YEARS OLD

3 YEARS OLD





4 YEARS OLD

7 YEARS OLD

Annex 5: Working Documents

Working Document 1

Age reading of Blue Whiting (*Micromesistius poutassou*) off the Portuguese coast

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Abstract

Blue whiting otoliths interpretation is considered difficult as it reflect in age reading low precision between readers. One of the main problems on age determination is the correct identification of first annual growth ring. In this study, 67 otoliths from Portuguese Coast were aged, burned and sliced in order to try to clarify growth rings and improve age readings criteria. Otoliths total lengths, widths, thickness and also the age rings lengths were measured. The relation between otolith total length (X) and fish length (Y) was established by the equation: Y=2.796(x)-7.219 (R2=0.99) that allows estimate the total fish length at the time of formation of each annual ring that had been identified by the readers, comparing the results from this work with studies previously developed for this species. From the results obtained in this study it should be considered that the measure assumed for first ring in blue whiting age reading criteria may be a false ring.

Keywords: Blue Whiting; Portuguese Coast; Growth; Otolith; 1st Ring; False Ring

Introduction

Age determination on blue whiting otoliths arises some difficulties which reflect on disagreeing results with low precision between readers, obtained during different international age reading calibration exercises and workshops for this species (ICES, 2013).

The correct identification of first annual growth ring is one of the main problems that influence the accuracy of fish age readings. In order to understand the causes of such disagreement, a small exercise was carried out between age readers. This exercise aims also to contribute with improvements allowing defining criteria's for blue whiting age reading.

Material and Methods

For this exercise were used 67 digitized images of otoliths collected from fish samples taken from commercial landings in Portuguese fishing harbours in 2013, on board fishing vessels and also during a research survey held off the Portuguese coast in October 2013.

The age reading procedures followed the described steps:

1. All the otoliths were aged by two readers and the chosen age was discussed and agreed between them.

2. Otoliths total lengths, widths, thickness and also the age rings lengths were measured.
3. One of the otoliths of each pair used in age reading was burned in order to try to clarify growth rings and improve age readings. Digitized images of both otoliths were obtained.
4. All the 67 otoliths pairs (1 burned and 1 not burned) were sliced and digitized images obtained were used to try to improve growth rings and age reading.

Results

The relations between the variables (modal age, otolith thickness, otolith width, otolith length and fish length) were studied (Figure 1).

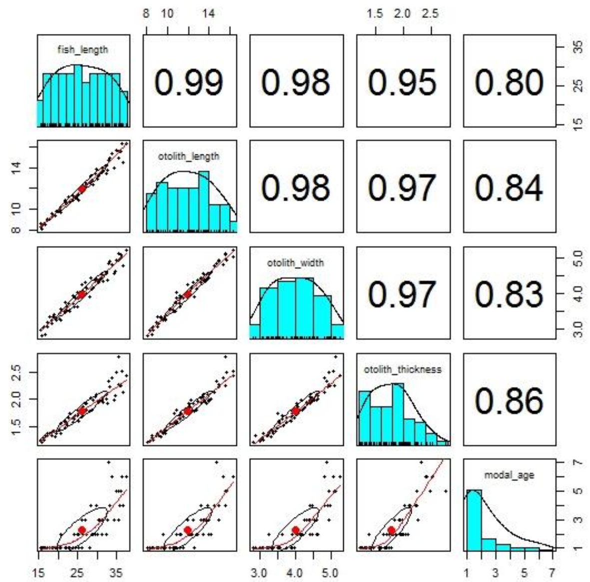


Figure 1 – Relations obtained between fish length, otolith length, otolith width, otolith thickness and modal age. The numbers represent the R-value from the adjusted model between the variables.

From all the relations between all measures and the modal age, otolith thickness shows the better results with higher R-adjusted value (0.86), followed by the otolith length (R= 0.84).

The described relations between the variables were used to define the equations between them and in this way to predict the variable expected value based on age information, mainly the first annual ring length and the otolith total length. Since, one of the main problems on fish ageing is the determination of the first annual ring position.

The linear relation between the otolith length (Y) and the otolith width (x) was established in the present work, by the following equation [1]:

$Y = 3.411(x) - 1.693$ ($R2=0.98$) [1]

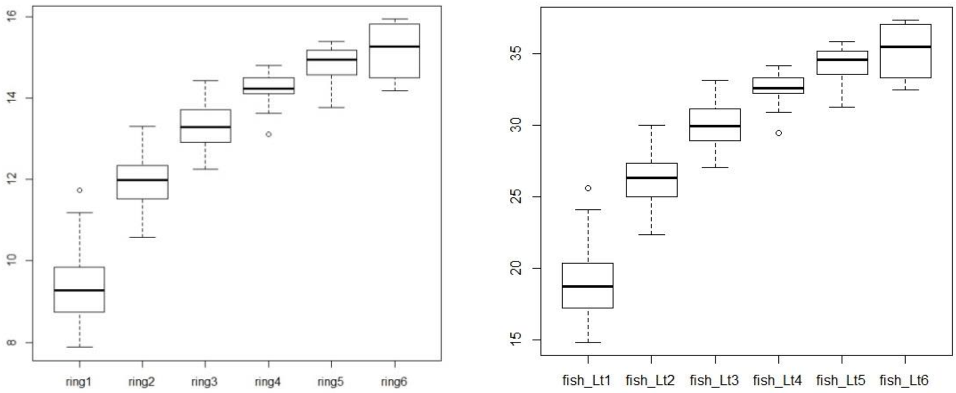
This equation allows estimating length of the otoliths that have broken extremities from their otolith width.

The relation between otolith total length (Y) and fish length (x) was also established through the equation [2]:

$Y = 2.796(x) - 7.219$ ($R2=0.99$) [2]

Equation [2] allows estimating the total fish length at the time of formation of each annual ring that had been identified by the readers.

Figure 2 shows the length for each otolith annual ring and median estimation of fish length for all the annual rings formation time.



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2005, 2013).This reference allows avoiding the Bailey´s zone (Bailey, 1970). Although, according to the equation [2], for the referred otolith length established for first year (8.33-9.33 mm), we obtain an estimating fish size of between 16.1 and 18.87 cm that is smaller than actually the established one, with a fish total length between 18 and 23cm.

The mentioned differences may be explained by a different growth rate for first year of the blue whiting of the Portuguese coast or by a misinterpretation of first annual ring.

Despite observing a well-marked first annual ring clearly distinct from the subsequent ones, its measures are inferior to the estimated for lengths at which supposedly fish reach their first year of life as found by Gjøsæter et al. (1979) for this species off the Norwegian coast. According to this author results, blue whiting may reach a size of 20-25 cm within its first year of life (Figure 3).

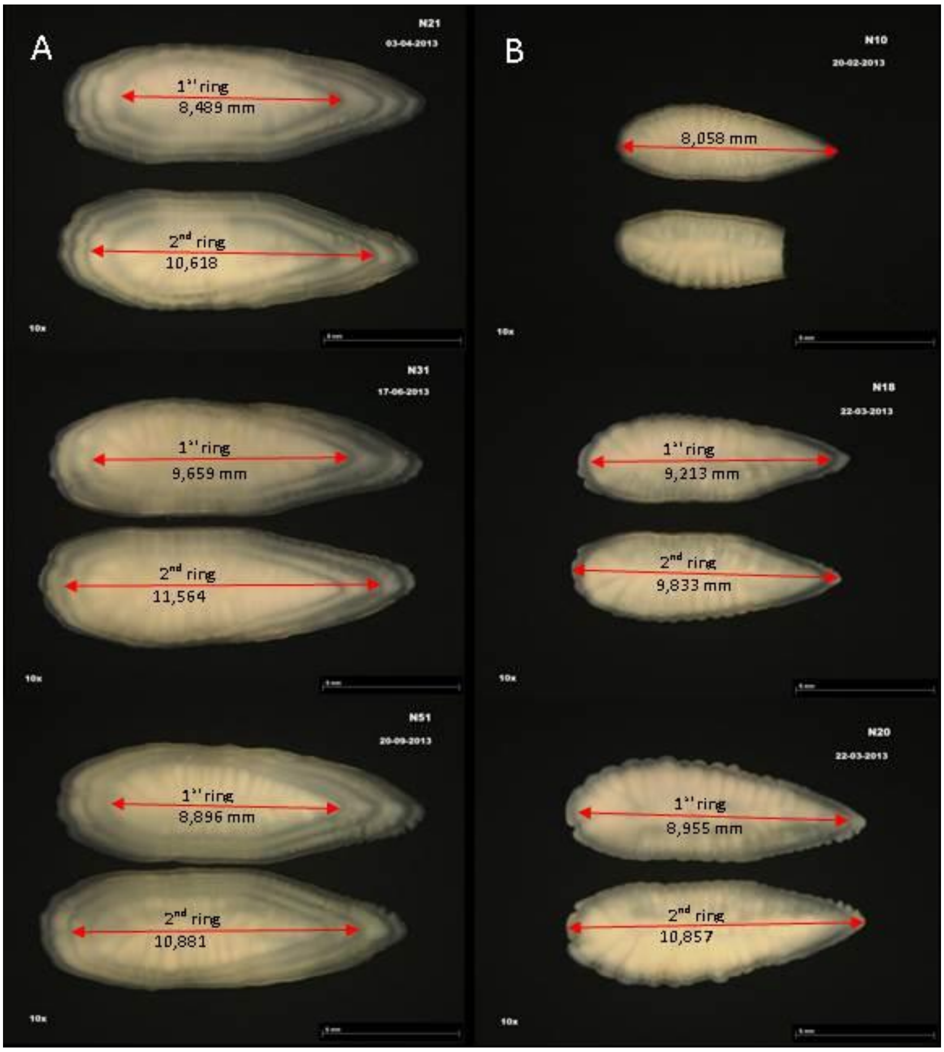


Figure 3. A) Otoliths of fish with 28.4, 32.6 and 30.7 cm total length, showing 1st and 2nd annual growth rings; B) Otoliths of fish with 15.7, 20.5 and 25.2 cm, showing respective annual growth rings, (1st ring and otolith margin). The otolith margin was considered following age reading criteria, as fish was caught during the first semester.

The rings mean length (mm) estimated through equation [1] and the fish mean length (cm) estimated through equation [2] were presented by age in Table 1.

Table 1. Summary of otoliths length range (mm), otolith mean length (mm) and estimated fish mean length (cm) by age group (1-3+).

Reference age group	Nr otoliths pairs	Otolith length range (mm)	Otolith mean length (mm)			Estimated fish mean length (cm)		
			1st ring	2nd ring	3rd ring	1st ring	2nd ring	3rd ring
1	21	7,879 - 10,299	8,988	-	-	17,9	-	-
2	15	7,997 - 10,827	9,432	-	-	19,2	-	-
		10,575 - 12,837	-	11,873	-	-	26,0	-
3	9	8,614 - 9,948	9.255	-	-	18,7	-	-
		10,851 - 12,492	-	11,785		-	25,7	
		12,771 - 13,985	-	-	13,277	-	-	29,9
+3	12	8,520 - 11,727	9,978	-	-	20,7	-	-
		10,763 - 13,306	-	12,009	-	-	26,4	-
		12,257 - 14,428	-	-	13,237	-	-	29,8

From data analysis of Table 1 an increasing trend, for the otolith first ring mean length and the estimated fish mean length, can be observed from the lowest to the highest age groups.

As referred above, one of the otoliths of each pair used in age reading was burned in order to try to clarify growth rings and improve age readings. As a result, in some otoliths the two inner growth rings previously marked as distinct annual rings by age readers, seemed to fuse, apparently forming only one band (Figure 4).

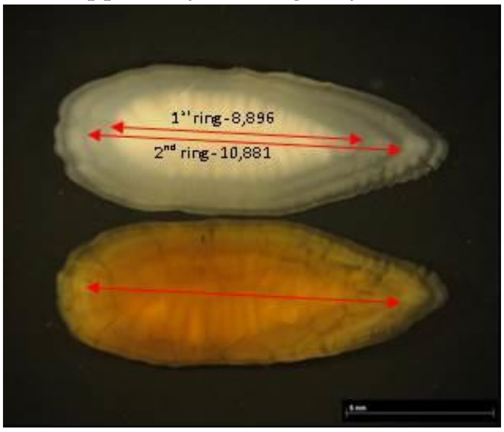
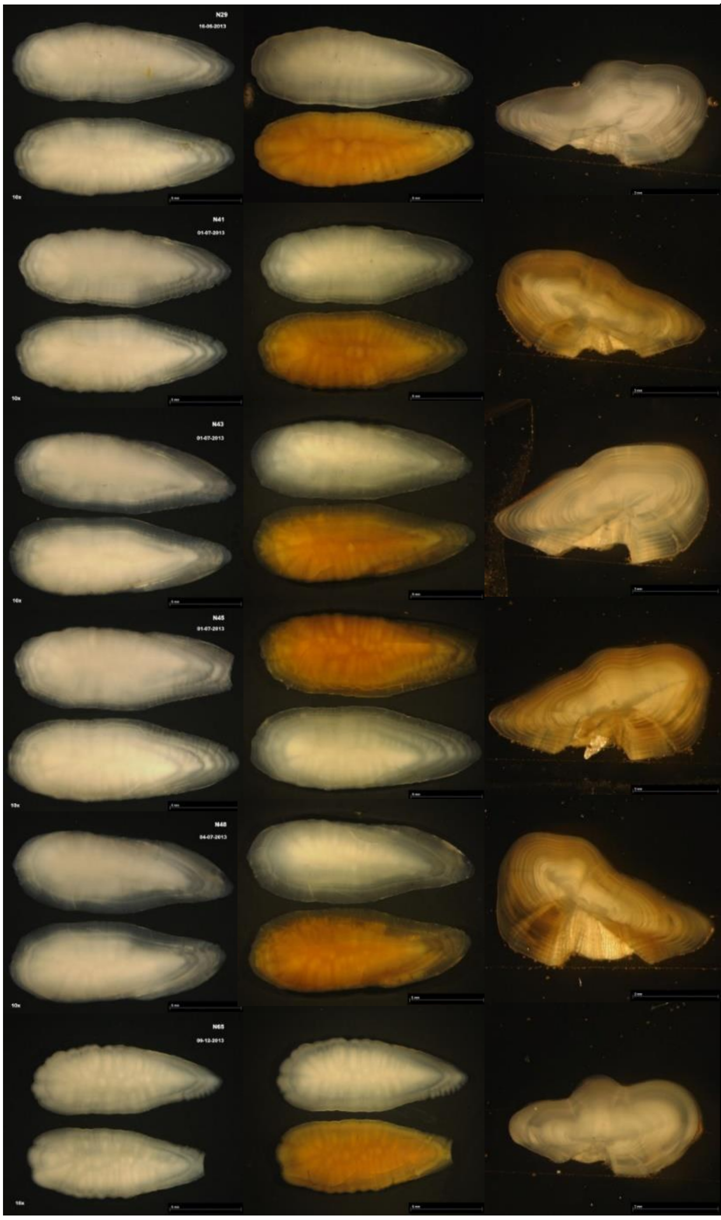


Figure 4. Otoliths pair from a 30.7 cm total length fish. The otolith on top is not burned and has two first annual rings length measurements. The otolith below shows where the first two rings apparently have been fused during the burning process.

Mainly in larger otoliths it is not possible to visualize its internal structure near the nucleus and it is probably that the reason for presenting bigger length for the first annual ring as fish increases in age (Table 1). Also, slicing was tested onthe otoliths in order to try to identify the innermost rings.

All the 67 otoliths pairs (1 burned and 1 not burned) were sliced using Bedford method (Bedford, 1983) and digitized images were obtained (Figure 5).



N029 - June 2013 Fish total length = 35,8 cm Otolith total length = 15,590 mm Otolith total width = 4,967
N041 - July 2013 Male Fish total length = 33,2 cm Otolith total length = 14,906 mm Otolith total width = 4.915
N043 - July 2013 Male Fish total length = 34,9 cm Otolith total length = 15,624 mm Otolith total width = 4,938
N045 - July 2013 Female Fish total length = 37,5 cm Otolith total length = 16,263 mm [illegible]
N048 - July 2013 Female Fish total length = 35,5 cm Otolith total length = 15,561 mm
N065 - December 2013 Female Fish total length = 29,5 cm Otolith total length = 13,409 mm Otolith total width = 4,402

Figure 5. Six otoliths pairs which otolith first ring has more than 10 mm and with an estimated fish length of more than 207 cm. From left to right entire otoliths (not burned), entire otoliths (one burned), sectioned otolith (burned and not burned). On the right side, otoliths and fish data.

The intended growth ring clarification by the burning process was not consistent in all cases, neither in entire nor in sectioned otoliths. Therefore this experience was not conclusive regarding the age readings improvement by these two methods, while in some cases the burned otoliths showed a significant growth ring contrast improvement, in other cases this were not observed. Slicing the otoliths could induce an incorrect age determination since it allows visualizing the entire internal structure of the otolith where numerous false rings are present. This technique was used in European Hake (*Merluccius merluccius*) and was abandoned after a pilot tagging experiment where it was possible to realize that the error in the assignments of ages could be twice the real age (ICES, 2010).

Discussion

There is an increasing trend of otolith first annual ring length within age group increasing (Table 1), which could be related with a different application of age reading criteria to otoliths of fish in the different age groups by age readers or with a variation of otolith structure with age that could difficult the identification of inner annual rings, particularly the first annual ring.

Blue whiting otoliths enlarge in proportion with fish growth. The deposition of organic and inorganic materials in the centre of the otolith as made describing a pyramid shape. It can be compared with an onion growth, with increasing layers around the centre (Panfili et al, 2002). This can explain the fact that for bigger and older fish the first annual ring is larger than in smaller fish. The larger distance from the nucleus of first ring observed in the bigger otoliths could be explained by the thickness increase which may not allow that the inner rings were observed.

According to the results of this exercise and assuming that the estimated fish total length at the 1st annual ring along the fish ages is smaller than what is supposedly reached by this species fish during their first year of life, as stated by Gjøsæter et al. (1979) for the Norwegian coast. Thus, although this species can shows a differentiated growth according to each distribution area, this study results should be considered for age reading criteria, since the first ring may be a false ring.

The possibility of formation of two translucent bands (or rings) in the same year as reported by Bowers (1954) and Jakupsstovu (1979) which may explain the presence of two initial rings in the otoliths with the above measurements and corresponding to fish with total length less than 25.0 cm.

Nevertheless, better knowledge on the growth of blue whiting is needed and therefore more research work must be done for this purpose.

Major priorities for blue whiting age reading research for the Portuguese coast:

1. Implementation of otoliths daily growth rings study of blue whiting on the Portuguese coast for otolith first annual ring validation in this area;
2. Remake the present work with a larger sample including more otoliths of age group 1 with first annual ring completed (opaque border) and also a larger number of age group 3+.

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Annex 6: Acknowledgements

We would like to express our sincerely thanks to Eduardo Santos for polishing otoliths during the workshop. Several otoliths were polished on the dorsal side in order to remove part of the three dimensioned structure of the otolith hoping that the annual zones will become clearer and to clarify the first ring length size.

Annex 7: WKARBLUE3 terms of reference for the next meeting

The **Workshop on Age reading of blue whiting** (WKARBLUE3), co-chaired by Jane A. Godiksen, Norway and Patrícia Gonçalves, Portugal will meet in Torshavn, Faroe Island, May31th – June 4th 2021 to:

a) Review new information from validation study on first annual ring identification from daily increments.

b) Review otolith growth table made by IPMA after WKARBLUE2 for aging of Blue whiting.

c) Clarify the interpretation of annual growth rings (1-3) by sex, quarter and age through image analysis (measurements of ring distances and back calculation).

d) Update on guidelines and common ageing criteria. With emphasis on testing the scheme made by WKARBLUE1.

e) Increase existing reference collections of otoliths and improve the existing data base of otolith images.

f) Analyse the age reading quality from the exchange using the 3-point scale of the image (mentioned in WKNARC)

g) Address the generic ToR's adopted for workshops on age calibration (see 'PGCCDBS Guidelines for Workshops on Age Calibration').

WKARBLUE3 will report by 31.08.2021 to the attention of the ACOM Committee.

Supporting Information

Annex 8: Recommendations

Blue whiting otoliths has proven to be quite difficult to age, and though guidelines have been constructed, the experience of the reader determines the interpretation of the otolith structure. It is therefore recommended to have regular exchanges and workshops in order to improve the agreement between readers.

RECOMMENDATION	ADRESSED TO
1. WKARBLUE3 Workshop in 2021	WGBIOP, ACOM
2. Age validation study on daily growth rings to solve the growth rings interpretation.	WGBIOP, ACOM, WGWIDE
3. Analyse first year growth from different areas using a subset of at lest 150 otoliths per area.	WGBIOP, ACOM, WGWIDE
4. Otoliths Exchange of *M. poutassou* in 2019 covering northern and southern sub-populations. Images and structures to be included.	WGBIOP, ACOM, WGWIDE
5. Update guideline of ageing criteria	WGBIOP, ACOM, WGWIDE