

Age Reading Manual of Blue Whiting

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1. Introduction

Blue Whiting is a small meso- pelagic (i.e. middle depth and surface waters) gadoid that reaches an average length of 25-30cm, reaching maturity after 2-4 years. Blue Whiting are an important commercial pelagic species in Northeast Atlantic waters and are a very important component of the pelagic trawl fisheries in Irish waters. (From the wide distribution area follows a very diverse fishery where as many as 13 nations exploit the Blue Whiting population.)

To date, Blue Whiting has been assessed as one stock, extending from Spain to Norway. However, morphological, physiological and genetic research has indicated the presence of two populations, which may mix in the spawning area west of Ireland and the UK.

They form large spawning aggregations off the west coast of Ireland and Scotland between February and March before undertaking extensive migrations up to feeding grounds in the Norwegian Sea. Therefore, there are important spawning and nursery areas off the Irish coast. Blue Whiting is widely distributed in the eastern North Atlantic and perform extensive migrations throughout their distribution as seen in Fig 1 below:

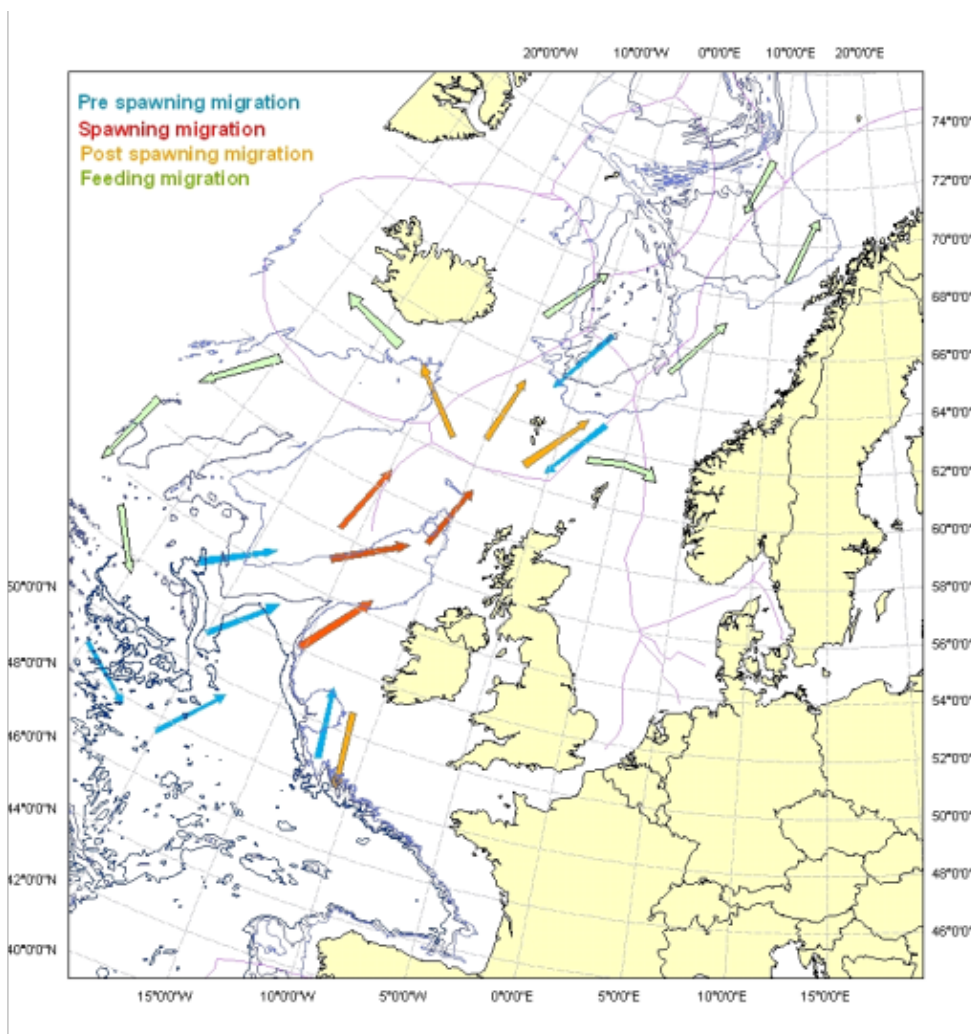


Fig 1: Migration routes for the Blue Whiting in the Northern Atlantic.

2. Preparation methodologies

2.1 Various preparation methods:

Firstly, the otoliths are extracted from the fish. Blue Whiting otoliths are removed by making a transverse cut into the head approx. 1cm behind the eyes. This exposes the otic capsule and then both otoliths are removed from the grooves they lie in. For this, straight tipped watch- maker's forceps should be used. Care should be taken to ensure the otoliths are kept whole as these structures are very fragile.

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

Reading Blue Whiting otoliths are considered easiest when removed from the fish and read immediately. If otoliths are stored dry, it is important to soak the otoliths for 24 hours beforehand and cleaned properly before storage. It is important that otoliths are not soaked in water for more than 48 hours each time as the unstable pH of the water can alter the shape and composition of the otoliths.

2.2 Viewing methods:

Blue Whiting otoliths are read whole immersed in water. Whole otoliths must be read in water over a black surface, using reflected light and magnification X 6/6.4. The magnification and light intensity can be adjusted by the individual reader. The otolith is interpreted by reading up the rostrum area and using the whole otolith pattern as a guide. Usually the clearest pattern is seen when the convex side of the otolith is facing down (sulcus side facing down). However, handling the otolith and turning it in various directions may be a way of assuring the estimated age. With difficult otoliths, it is advised to read both the concave and convex sides of the otoliths to gain a better interpretation of the annuli.

3. Internationally agreed age reading criteria.

It is essential that all otolith readers are aware of the age determination criteria that should be applied before age determination is attempted. The following are age determination criteria for Blue Whiting:

3.1 Birth date of fish:

It is essential that the birth date of the fish is known. The date of birth is assumed to be 1st January and the fish is assigned to a year class on that basis. Therefore, the date of capture of the sample should always be available.

3.2 What makes a year's growth:

Under a microscope, otoliths display a pattern of alternating concentric rings or zones, referred to as hyaline and opaque zones. One opaque zone and one translucent (hyaline) zone constitutes one year of growth (annulus). Fish that are sampled from the first half of the year are aged by counting all the translucent annuli, including the edge, if translucent. Fish sampled from the second half of the year, are aged by ignoring a translucent edge if present. This 'translucent edge' is the onset of the winter ring.

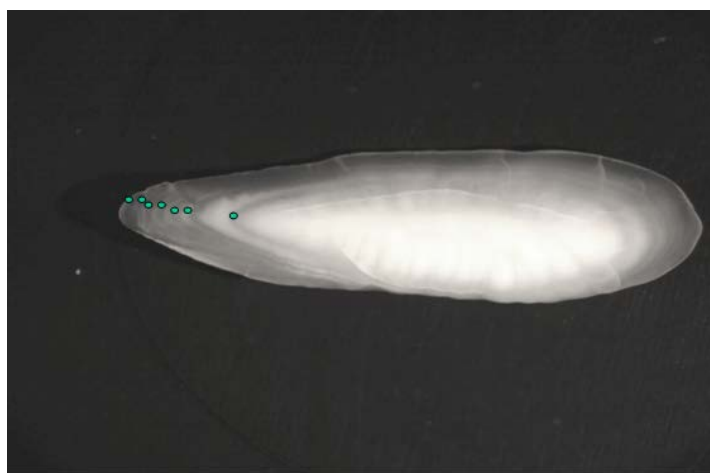
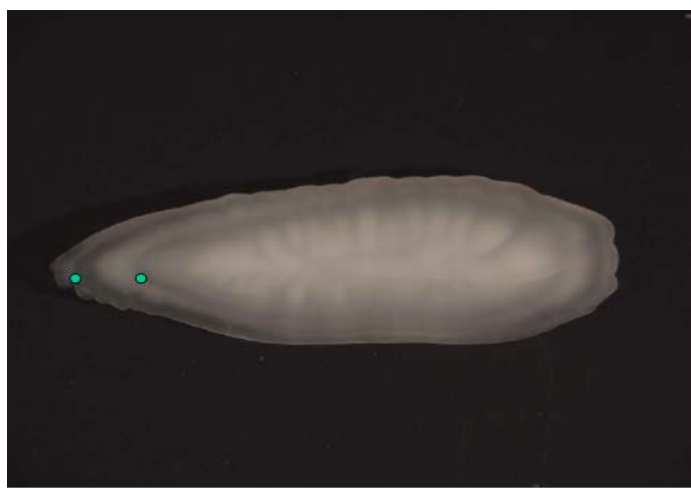


Fig 2: The otoliths from a two year old and a seven year old Blue Whiting. The years observed are marked with a green dot.

3.3 Relative width of the rings:

The relative width of the rings is dependent on the timing of the formation of the opaque zone on the edge of the otolith. This is heavily dependent on the area from which the sample was taken. When allocating the fish to a year class therefore, the area of the capture should also be known.

3.4 Formation of first winter ring:

Blue Whiting spawn gradually later in the season moving from the South to the North. Spawning starts in the South in February and ends sometime during April in the North. Therefore the formation of the first winter ring can occur from October to January.

A 'false' ring, known as the Baileys zone may appear inside the winter ring. 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 winter ring may appear very thin, but is in good sequence, which could be a short winter period and not a false ring.

3.5 Age at maturity:

At the onset of gonad maturation, growth slows down and as a result, mature fish grow later in the year than immature fish. Blue Whiting can mature at age 1 and therefore it is important that the maturity stage should be used as an additional indicator for the aging of fish caught during the spawning season.

3.6 Reduced growth in older fish:

In most of young and middle aged fish, the growth pattern is well defined on the otolith with clear contrasting opaque and hyaline zones. However in old fish, growth often slows down to such an extent that the opaque and translucent (hyaline) zones become confused and more difficult to distinguish. That proportion of the otolith will have a greyish appearance. Therefore, the relative width of each ring should progressively be smaller as the otolith grows.

3.7 Specific details:

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. Sexual dimorphism is present in Blue Whiting with females growing faster than males. As a result, females are younger in general length to male fish and this knowledge of the sex of the fish may be used as an additional factor when ageing. 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 a fish older than 3 years old.

4. Quality control of age readings

4.1 The importance of Quality Control:

Quality control is a critical part of age reading and assesses the level of consistency amongst age reading. The aim of the quality control programme is to ensure that all age readers have a sample of their age readings checked each year. This is done by another experienced reader for a species, in order to identify any problems that might affect the precision of the data supplied to the ICES Assessment Working Groups. The level of agreement between the two readers is calculated, and this is used as an indicator of the consistency of age reading within institutions.

It is important to conduct quality control for two main reasons. Firstly, extremely valuable evidence of the precision of an age determination programme can be obtained. It is vital that the ages assigned to otoliths that are used in assessments are assigned the “best” age, given the methods at our disposal. As the actual age of the fish is unknown, age determination experts need to ensure the age provided is as close to the actual age as possible and that the ages given are repeatable if the determinations are re-done. Secondly, even the most experienced readers are capable of drifting from their training over time and another reader looking at a sample of their reading to check consistency will ensure no drift occurs.

It is equally as important to ensure the quality control of age estimation between national laboratories. One way this can be achieved is through the standard exchange programme and workshops under the ICES programme. Readers read a set of otoliths and are compared with a modal age. This provides a snap shot of the agreement between readers, both expert and trainees, for the year in which the exchange or workshop takes place. It is particularly useful as many laboratories take part and results compared. However, workshops and exchanges can be expensive and slow to organise. Another way of ensuring quality control between national laboratories is through a small scale, ad hoc exchange or workshop between two or more laboratories looking at specific stock or sub stock of the species. This incorporates just those countries that have a direct interest in the particular stock. This type

of exchange is inexpensive and can be done quickly to address the issues. This effort to ensure consistency of interpretation is further enhanced with the use of reference collections.

4.2 Assigning readability, the 3 point scale:

It is essential that otolith readers, whether fully trained or not have their work quality controlled. To ensure quality control measures are followed, every age must be first assigned a level of readability. This indicates the confidence a reader has for a particular otolith. First, it is a good idea to read otoliths in sequence, from month to month as it allows you to see the changes that occur from quarter to quarter. Therefore each quarterly batch of otoliths received from the prep lab should be organised first according to ICES area. These sub batches should then be organised from start to finish of the quarter. This allows the reader to observe in order the sequence of growth and lying down of rings.

The workshop on National Age Reading Coordinators (WKNARC) was established to assess the level of agreement amongst Blue Whiting readers across institutions. WKNARC recommends that the reader has to register the confidence level he/she has in their otolith reading, reflecting the quality of the data. This is done using a scale with 3 levels of quality:

- Rings can be counted with certainty: 1
- Rings can be counted, but with difficulty and some doubt: 2
- Rings cannot be counted, the otolith is unreadable: 3

Using the same 3-point scale for all species – stocks would standardize the quality values so that data users could eventually rely on the quality value attached to the age data, without having to consider the precise nature of the quality assessments used to derive the quality value. WKNARC recommends that Age Calibration Workshops derive descriptors for the three scale points that are applicable to their species. WKNARC recommends that the following 3-point scale of age reading quality be used by all age readers who provide stock assessments.

Age Quality 1 (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.

Age Quality 2 (AQ2): Difficult to age with 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.

Age Quality 3 (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. 3 AQ3 is an indication that there are serious concerns about the reliability of the age data and/or its value to stock assessment WGs.

4.3 The Guss Eltink spreadsheet:

Once ages are assigned a readability level, inter – reader checks must then be performed by using ‘The Guss Eltink spreadsheet’, also referred to as a ‘Tool for Age Reading Comparisons’. This excel workbook was developed for the easy and fast analysis of age reading results. It allows for an intermediate reporting of the results after an exchange/workshop and the results from the analysis on age reading comparisons are easy to understand for the age readers, who have to calibrate their age readings based on these results.

The spreadsheet performs a series of calculations based on the individual age estimations by each reader, which are recorded in the spreadsheet. It then returns tables for relative bias, coefficient variations, percentage agreement (by reader, by month, by modal age) as well as overall estimate for coefficient variation and percentage agreement.

‘The Guss Eltink spreadsheet’ was developed by Eltink *et al.* 2000 has been very valuable for the development of age calibrations. However, being an excel spreadsheet, the tool is rather sensitive to typological errors and destruction of the formulas in the actual cells and thus the spreadsheet is prone to errors caused by unfamiliarity with the set-up, or even ‘ordinary’ mistakes, which can also be made by expert users.

5. Location of reference collections

The otoliths and fish information are uploaded onto the WebGR through a server at AZ-ti (<http://webgr.azti.es/>) and at the European Age Readers Forum (EARF).

First time users:

1. Open the website (<http://webgr.azti.es/>) using Firefox (Internet Explorer and WebGR does not work together. You can download Firefox at [www/Mozilla.org](http://www.mozilla.org)).
2. Register new user (in the menu to the left), and activate the account following the link sent to your email.

3. In the menu to the left, chose "Search" and click on "List all workshops".
4. Send an email to the manager of the workshop of interest for access.
5. The manager will then include the age reader as a participant for the workshop/reading exercise.

From second time and onwards:

1. Open the website (<http://webgr.azti.es/>)
2. Log in.
3. Chose "My calibration exercises" in the menu to the left.
4. Chose "annotate" in the left column of the "Calibration exercise list" under the workshop of interest.
5. Start annotation. Remember to write in the age, this is not done automatically by the programme. Each annotation is ended by clicking "Finalise". This will register the age in the programme. If you press "Save" the annotation will only be available to yourself, and it will be possible to change the annotation later before finalising.
6. Some of the pictures may be small and very dark. It is possible to change the brightness and contrast in WebGR to improve the picture. In the upper right corner is a zoom function.
7. A single wrong placed annotation can be removed using "re-annotating" it. Place the cursor over the annotation to be deleted and when the cross becomes "bold" click on it, and it will disappear. If the annotations have been saved, just remove the annotation and click "Update", and new age will be recorded.
8. In the menu "Help" within WebGR it is possible to download a user manual for WebGR. Page 10 and forwards contain a description of how to annotate.

6. Ancillary information, and training resources

6.1 Otolith training online (OTO):

The rapid developments in Information and Communications Technology (ICT) offer enormous potential as a medium for education and training. OTO is a project that seeks to harness this potential and focuses on the use of otoliths in fisheries science. It uses a combination of text, images and video to provide students and researchers with an engaging and reference tool. The online training programme (OTO) can be found at the following address:

<http://www.marine.ie/home/publicationsdata/data/OtolithTrainingOnline.htm>

6.2 European Age Readers Forum (EARF):

The Planning Group on Commercial Catch, discards and Biological Sampling (PGCCDBS) established the European Age Readers Forum (EARF) in response to feedback received from those engaged in age reading across Europe. The objective was to establish a “One Stop Shop” for all those involved in age reading. It was thought that the forum would provide an important resource for training of new age readers, as well as providing opportunities for sharing and discussing existing age reading manuals, establishing standard operating procedures, and standardising preparation and interpretation methods. The forum is hosted by ICES in the format of a SharePoint site at the following address: <http://groupnet.ices.dk/AgeForum/default.aspx>

Username and passwords can be requested through ICES. At the moment the forum includes the following information:

- The contact details and a mailing list of age reading coordinators as well as those engaged in age reading of fish species in the various European laboratories.
- A calendar of upcoming workshops and also the PGCCDBS meeting details.
- A link to the PGCCDBS documents repository.
- A link to WebGR
- The EFAN Reports
- PGCCDBS guidelines for otolith exchanges and workshops.
- A discussion board.

It is proposed to use the EARF to host any available age reading manuals, resulting from the many ICES age reading exchanges and workshops.

7. References:

- ICES (2005). Report of the Blue Whiting Otolith Ageing Workshop, 13-16 June 2005, Hirtshals, Denmark. 36pp.
- ICES (2013). Report of the workshop on the age reading of Blue Whiting, 10-14 June 2013, Bergen, Norway. *ICES CM 2013/ACOM: 53* 55pp.