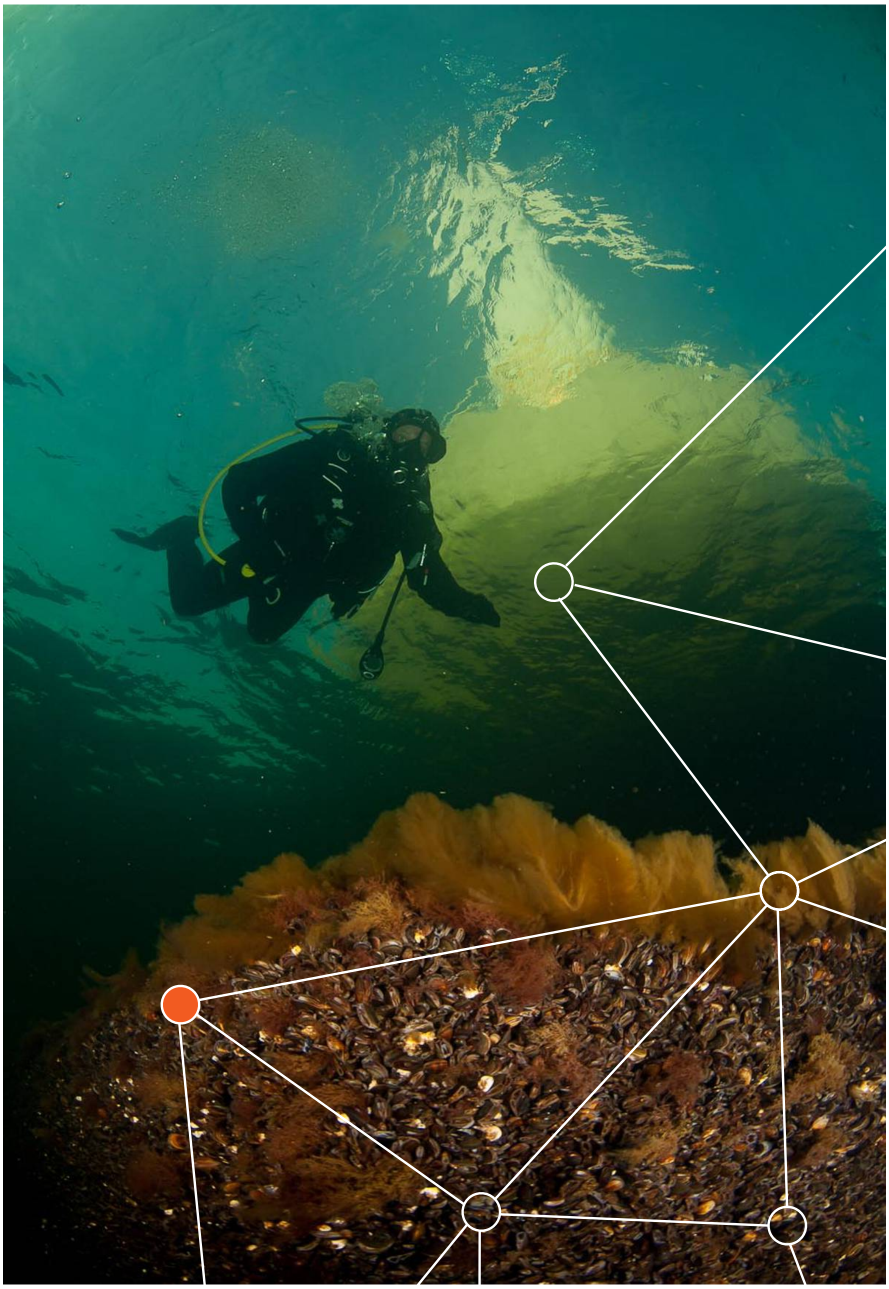
#346 APRIL 2019

ICES CIEM

Handbook of fish age estimation protocols and validation methods

ICES COOPERATIVE RESEARCH REPORT

RAPPORT DES RECHERCHES COLLECTIVES



ICES INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA
CIEM CONSEIL INTERNATIONAL POUR L'EXPLORATION DE LA MER

ICES COOPERATIVE RESEARCH REPORT
RAPPORT DES RECHERCHES COLLECTIVES

NO. 346

APRIL 2019

Handbook of fish age estimation protocols and validation methods

Editors

Francesca Vitale • Lotte Worsøe Clausen • Gráinne Ní Chonchúir

ICES
International Council for the Exploration of the Sea
CIEM
Conseil International pour l'Exploration de la Mer

International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer

H. C. Andersens Boulevard 44–46
DK-1553 Copenhagen V
Denmark
Telephone (+45) 33 38 67 00
Telefax (+45) 33 93 42 15
www.ices.dk
info@ices.dk

Recommended format for purposes of citation:

Vitale, F., Worsøe Clausen, L., and Ní Chonchúir, G. (Eds.) 2019. Handbook of fish age estimation protocols and validation methods. ICES Cooperative Research Report No. 346. 180 pp. http://doi.org/10.17895/ices.pub.5221

Series Editor: Emory D. Anderson

DOI: http://doi.org/10.17895/ices.pub.5221

ISBN 978-87-7482-223-3

ISSN 1017-6195

Contents

Foreword ..... **i**

Acknowledgements ..... **ii**

1 Introduction **1**

1.1 Why do we age read fish? 1

1.2 The history of quality assurance and quality control (QA/QC) 1

1.2.1 Tools available for quality assurance and quality control 3
1.2.2 The concepts of accuracy and precision 4
1.2.3 Validation 5
1.2.4 Direct validation methods 7
1.2.5 Indirect validation methods 7

1.3 Standardization of procedures 10

1.3.1 Why standardization is needed 10
1.3.2 Monitoring performance 10
1.3.3 The benefits of having agreed (validated) manuals 11

1.4 Accreditation 12

1.5 Contributors 14

2 Gadoids **15**

2.1 Introduction 15

2.2 Age estimation methodologies in gadoids 15

2.3 General age estimation methods and problems 17

2.3.1 Saithe (*Polliachius virens*) 17
2.3.2 Whiting (*Merlangius merlangus*) 17
2.3.3 European hake (*Merluccius merluccius*) 18
2.3.4 Cod (*Gadus morhua*) 19
2.3.5 Blue whiting (*Micromesistius poutassou*) 20

2.4 Age validation case studies in gadoids 20

2.5 Application of indirect validation methods 22

2.5.1 Length-based analyses 22
2.5.2 Marginal increment analysis 23
2.5.3 Edge-zone analysis 24

2.6 Application of direct validation methods 24

2.6.1 Tag–recapture 24
2.6.2 Rearing in captivity 26
2.6.3 Daily increment analysis 27

2.7 Future perspectives in gadoids: available tools 28

2.7.1 Back-calculation of size at previous age and hatch date 28
2.7.2 Simulation tools for otolith macrostructure modelling 29
2.7.3 Raman microspectrometry 29
2.7.4 Microchemistry as a supportive tool for age validation 30

2.8 Ongoing and future work 32
2.8.1 Validation of life history events – juvenile check 32
2.8.2 Validation of life history events – spawning check 33

3 Flatfish 36

3.1 Introduction 36
3.2 Age estimation methodologies in flatfish 45
3.2.1 Whole otolith method 45
3.2.2 Break-and-burn method 47
3.2.3 Sectioned otolith method 48
3.2.4 Sectioned and stained 49
3.3 Age estimation problems and features of specific flatfish stocks 49
3.3.1 Plaice (*Pleuronectes platessa*) 49
3.3.2 Sole (*Solea solea*) 50
3.3.3 Turbot (*Scophthalmus maximus*) and brill (*Scophthalmus rhombus*) 51
3.3.4 Lemon sole (*Microstomus kitt*) 51
3.3.5 Megrim (*Lepidorhombus whiffiagonis*) and four-spot megrim (*Lepidorhombus boscii)* 52
3.3.6 Flounder (*Platichthys flesus*) 53
3.3.7 Witch flounder (*Glyptocephalus cynoglossus*) 54
3.3.8 Dab (*Limanda limanda*) 55
3.3.9 Greenland halibut (*Reinhardtius hippoglossoide*) 56
3.4 Age validation case studies 56
3.4.1 Lemon sole (marginal increment analysis) 58
3.4.2 Plaice and sole (mark–recapture) 58

4 Small- and medium-sized pelagic species 59

4.1 Introduction 59
4.2 Summary of age estimation methodologies 59
4.3 Summary of general age estimation methods and problems 66
4.3.1 Anchovy (*Engraulis encrasicolus*) 66
4.3.2 Sardine (*Sardina pilchardus*) 71
4.3.3 Herring (*Clupea harengus*) 73
4.3.4 Sprat (*Sprattus sprattus*) 74
4.3.5 Mackerel (*Scomber scombrus*) 74
4.3.6 Chub mackerel (*Scomber colias*) 77
4.3.7 Horse mackerel (*Trachurus trachurus*) 80
4.3.8 Mediterranean horse mackerel (*Trachurus mediterraneus*) 85
4.3.9 Blue jack mackerel (*Trachurus picturatus*) 89
4.4 Age validation case studies 91
4.4.1 Indirect validation methods 91
4.4.2 Direct validation methods 100
4.5 Future perspectives in terms of validation of age for small and medium-sized pelagic species 102

4.5.1 Tag–recapture and use of chemical agents for otolith marking 102
4.5.2 Validation of life history events 102
4.5.3 Other validation methods 102

5 Deep-water species 103

5.1 Introduction 103

5.2 Background (precision and accuracy) 104

5.3 General recommendations for age estimation of deep-water fish 109

5.4 Identification of the first true annulus 111

5.5 Changing growth patterns along the fish lifespan 112

5.6 Marginal otolith area 112

5.7 Use of supplementary information 113

5.8 Age and growth of individual deep-water species 114

5.8.1 Tusk (*Brosme brosme*) 115
5.8.2 Ling (*Molva molva*) 116
5.8.3 Blue ling (*Molva dipterygia*) 117
5.8.4 Roundnose grenadier (*Coryphaenoides rupestris*) 118
5.8.5 Greater silver smelt (*Argentina silus*) 119
5.8.6 Black scabbardfish (*Aphanopus carbo*) 120
5.8.7 Blackspot seabream (*Pagellus bogaraveo*) 121
5.8.8 Greenland halibut (*Reinhardtius hippoglossoides*) 122
5.8.9 Golden redfish (*Sebastes norvegicus*) 124
5.8.10 Beaked redfish (*Sebastes mentella*) 125
5.8.11 White anglerfish (*Lophius piscatorius*) 126
5.8.12 Black anglerfish (*Lophius budegassa*) 128

6 Statistical handling of uncertainty in age estimations 130

6.1 Introduction 130

6.1.1 Statistical methods for analysing output from calibrations 130
Strengths and weaknesses of each listed method 131
6.1.2 Test of methods on a known dataset 135
6.1.3 Additional analysis based on distances between identified otolith structures 142

6.2 Existing software 145

6.3 Guidelines for data summaries and analysis outputs from calibration workshops 148

6.4 Future perspectives 148

7 References 150

Annex 1: Contributor contact information 171

Editors 171

Contributors 171

Annex 2: Annotated images of the species included in Chapter 2 175

Annex 3: ICES age calibration workshops (1992–2012) **178**

Annex 4: Abbreviations and acronyms used in this publication **181**

Foreword

The Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) 2012 was approached by the ICES Publications and Communications Group (PUBCOM) with the suggestion to combine the existing protocols on the age estimation of fish species within the ICES Area and publish them as an ICES Cooperative Research Report (CRR). This idea was received favourably by PGCCDBS. It was deemed important to (i) summarize the state of knowledge for key species, (ii) scrutinize, by peer review, the work done during the many calibration exercises, and, by doing so, (iii) promote an increase in quality. The aim of the present publication is to provide a comprehensive manual on the methodology of age estimation and validation and represents a collation of the state-of-the-art scientific work on the methods and validated age estimation of commercially exploited fish species across Europe. Having a collation of the latest methodologies by species grouping will also facilitate rapid and quality-assured development of methods suitable for new species.

Acknowledgements

We would like to acknowledge the ongoing work of age readers across Europe and especially their invaluable contributions in chairing and actively participating in a whole host of age calibration exchanges and workshops, the outputs of which have formed the basis for this current concerted research report.

We are also grateful to all of our colleagues from the PGCCDBS who supported the initial idea for the need for a CRR on this subject and whose continued support was much appreciated.

Thank you also to our exceptional chapter editors who invested considerable time and effort to ensure that each chapter is as comprehensive as possible and without whom this publication would not have been possible. It has been a pleasure working with all of you.

We are grateful to the age estimation experts at the Northeast Fisheries Science Center in Woods Hole, MA, USA for their review of this rather comprehensive CRR. We thank Emory Anderson, Claire Welling, Katie Rice Eriksen, Simon Cooper, Celine Byrne, and Søren Lund for their editorial and administrative contributions.

1 Introduction

1.1 Why do we age read fish?

Assessment of individual age through the use of calcified structures (scales, otoliths, opercular bones, fin rays, etc.) has been proven to be very useful in assessing the status of any fish stock. According to Panfili *et al*. (2002), data on age and growth of fish are essential for understanding vital traits of species and populations (e.g. lifespan, age at recruitment, age at sexual maturity, reproduction periods, migrations, mortality) and the study of population demographic structure and its dynamics (e.g. age-based stock assessment). The age profile of a fish stock can be indicative of its general "health", as one will expect to see evidence of a broad range of ages in a healthy population. A lack of young fish may indicate recruitment failure, which will have repercussions in future years, while a lack of older fish can signal overexploitation of the stock. Fisheries scientists are especially concerned with the dynamics of exploited populations, with the view to providing advice about the sustainable harvesting of the resource. In the ICES Area, this task is generally focused on providing a quantitative assessment and forecast on a stock, with age data at its core. Hilborn and Walters (1992) pointed out that the "aim of such studies is not only to assess the state of stocks and fisheries relative to historical states, biological reference points or management targets, but also to evaluate the consequences for both fish stocks and fishermen, of alternative management scenarios." Therefore, it is clear that reliable age–length data are important for the management and sustainable exploitation of fish stocks. The need for reliable data is especially acute in times when stock levels are low and errors in predictions can have devastating effects on the resources.

1.2 The history of quality assurance and quality control (QA/QC)

Improved awareness of ensuring quality and standardization of age estimation has been the main goal for the development of quality assurance and quality control (QA/QC) over time. Quality assurance (QA) can be defined as systematic measurement, comparison with a standard, monitoring of processes, and an associated feedback loop that confers error prevention. This can be contrasted with quality control, which is focused on process output. Thus, quality assurance focuses on the accuracy of the age estimation, i.e. how well is the real, true age estimated and what is the associated precision. The quality control (QC) then is a monitoring system of how well age readers perform in relation to the standards set under QA.

Establishing European-wide control mechanisms is a sensitive issue that requires time to implement as well as a good international working climate. In recent years, an improved awareness of the value of validation and the benefit of evaluating assumptions has developed. However, bridging the gap between highly specific research and the operational QA/QC programmes within various laboratories has been a challenge. Advances in sophisticated research into age estimation and biomineralization processes have not fully translated into the routine age estimation methodologies applied for fishery assessment within European age estimation laboratories. The quality of age data often depends on the individual skills and independent experience of the age reader and does not always incorporate elements of standardization, objective control, and statistical evaluation. Here, we review major milestones in this process.

In December 1997 a Concerted Action, i.e. "European Fish Ageing Network" (EFAN – FAIR PL.96.1304) was started in order to develop a network of laboratories with the capacity to collaborate on research and training. The goal of this network is to ensure that age estimation is a reliable basis for stock assessments and scientific advice on fisheries and environmental resources. By the end of the project in December 2000, EFAN had become a network of 34 university and research institutes representing 16 countries in Europe. EFAN was coordinated through a steering group which collated information and disseminated, via a periodic newsletter, the production of EFAN reports and the maintenance of an internet homepage that is now defunct. This facility provided access to information, reports, and databases, not only for EFAN members, but also for a wider audience. EFAN developed into an important forum for the open discussion of ideas, dissemination of results and access to unpublished material.

The achievements of EFAN were carried further by another concerted Action "Towards Accreditation and Certification of Age Determination of Aquatic Resources" (TACADAR – Q5CA-2002-01891) which was initiated in October 2002. As a starting point, TACADAR used the network developed in EFAN to address the requirements of environmental and fisheries management strategies for data quality, necessary for the timely assessment of living resources. The overall objective was to increase the reliability of age estimation procedures in the European Community and to promote the adoption of procedures that include QA/QC mechanisms. TACADAR developed guidelines and a manual for quality assurance and standardized practices in age estimation to be applied on the European level, including statistical criteria as well as an evaluation of legal aspects of accreditation and certification and implications within the EU.

Under the remit of the International Council for the Exploration of the Sea (ICES), a Planning Group on Commercial Catches, Discards and Biological Sampling (PGCCDBS) was established in 2002 to provide support for the EU Data Collection Framework (DCF). A particular role for PGCCDBS was to develop standards and guidelines for the types of data required by the DCF, principally stock-based biological parameters from sampling of fishery and survey catches (age, growth, maturity, fecundity, sex ratio) and fleet-related variables (discard estimates and length/age compositions of landings and discards). One of the main goals for PGCCDBS was to ensure correct and consistent interpretation of biological material such as otoliths and gonads. The multiannual work plan of PGCCDBS included intercalibration studies to promote agreement among scientists classifying calcified age structures, such as otoliths and gonads of specific species or groups of species, using web-based tools to facilitate this work.

Over the years, it became apparent that more specific workshops were needed to facilitate a pan-European QA/QC of age estimations. Two workshops of National Age Reader Coordinators were established, WKNARC1 in 2011 and WKNARC2 in 2013. These workshops further gauged the quality assurance and the means of dealing with uncertainty in relation to age data in stock assessment. For the purpose of intercalibration between age estimation labs and age readers involved in stock assessment, the WKNARC reviewed preparation methods by species and areas, material and techniques development, methods in image processing, and validation methods.

The most recent development within the ICES community to facilitate and guide QA/QC of age estimations and other biological parameters is the formation of the Working Group on Biological Parameters (WGBIOP). WGBIOP is a newly formed expert group which took over the responsibilities of PGCCDBS on the coordination and implementation of quality assured and statistically sound methods for the provision of accurate biological parameters for stock assessment purposes. However, the focus of WGBIOP is not only on technical aspects of data collection and quality assurance, but also on accuracy in life history parameter estimations to support stock assessment. WGBIOP will review stock-specific life history parameters and monitor potential changes in biological processes, such as growth rate, onset of maturity, maturity and fecundity at size/age, and related causal factors.

WGBIOP is an expert group devoted to all stages of the provision of biological parameters (methodological improvements, implementation, quality assurance, statistical analysis) at a national, regional, and stock level. It provides a bridge between the data collectors and the end users that has often been lacking. The ultimate objective is to stimulate the achievement of a higher level of quality within, and integration among European institutes concerning fish age estimation.

1.2.1 Tools available for quality assurance and quality control

There are several tools available for routine QA/QC, either in the form of a spreadsheet with R-script-based evaluations of reader results, or of web-based tools which facilitate the annotation of images of otoliths, adding further information for evaluation of disagreements. The choice of tool depends on the purpose of the QA/QC.

The more recent recommendations for any calibration are to apply a tool that compares exactly the structures interpreted by the readers (ICES, 2014a). The prevailing tool used in the European Community for many years was WebGR (Web Service for support of Growth and Reproduction studies), which was an open-source software developed in 2008 by a consortium of research institutes and software developers (http://webgr.azti.es/ce/search/myce).

However, many security issues with WebGR were identified and eventually a decision was taken to move to the new tool called SmartDots, developed by the Belgian Institute for agriculture, fisheries, and nutrition (ILVO) and managed by ICES.

The SmartDots age estimation platform is developed to facilitate age estimations based on otolith images. A set of software tools supports the user in managing all of ICES age estimation data. On the one hand the database can manage the metadata related to workshops and exchanges and, on the other hand, the age reader can carry out age estimations by annotating otolith images. All registered data are available in the connected reporting environment. The SmartDots age estimation platform is an open source solution available at http://www.ices.dk/marine-data/tools/Pages/smartdots.aspx.

The coordinator of an age calibration workshop uploads the selected images to the server which stores the images and metadata grouped by species, date, area, etc. All participants of that workshop annotate and assign an age to each individual image without having access to the work of the other participants. When all the images have been annotated and aged, the coordinator arranges access for the participants to all the annotations and aged images. The participants can then compare and discuss each other's annotations and ages to identify sources of disagreement.

SmartDots can contain reference collections with agreed ages, which can be used by inexperienced readers as a self-training tool, where they can access images and compare their annotation of images with those of experts.

SmartDots has been established as the standard tool for managing age calibration exchanges and workshops, across Europe, from 2018.

The Age Readers Forum (ARF) was established by PGCCDBS in 2009, in response to feedback received from those engaged in age estimation across Europe.

ARF was originally envisaged to be a "One Stop Shop" for all those involved in age estimation. 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 estimation manuals, establishing standard operating procedures, and standardising preparation and interpretation methods.

However, this SharePoint site was never widely used in spite of the ageing and maturity communities agreeing that it was a good idea to have such a forum. The past eight years have clearly demonstrated that the current forum does not work as a standalone concept; it must be integrated with the widely used software for managing exchanges and workshops, which will be SmartDots.

Having a central repository of (a) internationally agreed age estimation/maturity staging protocols, (b) contact details for age readers/maturity stagers with the stocks they read and their level of expertise, and (c) a single location for workshop reports and the resulting reference collections of both age and maturity images is still deemed by the community a very positive and necessary resource.

1.2.2 The concepts of accuracy and precision

Errors in age estimation may have two forms not necessarily related (Wilson, 1987; Campana, 2001); one affecting **accuracy**, i.e. the proximity of the age estimate to the true value and the other affecting **precision**, i.e. how close individual measurements on a given structure are to each other, i.e. reproducibility. The latter, related to precision, can easily be detected and described by means of common statistical measures. The most traditional method used to measure interreader precision is the percentage agreement (PA), which is the percentage of structures where there was an agreement by two readers (or by the same reader on two different occasions). However, the average percent error (APE) and the *CV* (coefficient of variation) are commonly deemed more informative indices than PA (Kimura and Anderl, 2005; McBride, 2015):

$$APE_j = 100 \times \frac{1}{R} \sum_{i=1}^{R} \frac{|X_{ij} - X_j|}{X_j} \quad (1)$$

where *Xij* is the *i*th age estimation of the *j*th fish, *Xj* is the mean age of the *j*th fish, and *R* is the number of times each fish is aged (in this case, it coincides with the number of readers). When APE is averaged across many fish, it becomes an index of average percent error.

The use of standard deviation rather than the absolute deviation from the mean age leads to the following equation:

$$CV_j = 100 \times \frac{\sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j} \quad (2)$$

This formula represents the *CV* of the age estimate for a single fish (*j*th fish) and needs to be averaged across fish to produce a mean *CV*. A low *CV* indicates high precision in age estimation.

Both APE and *CV* are more proper indices of precision (actually imprecision) than percent agreement and can either be scaled to percentage or used as proportion. However, *CV* is recognized as having a greater meaning and is easier to interpret (Kimura and Anderl, 2005; McBride, 2015).

It is important to stress that these measures, PA, APE, and *CV*, are independent of the closeness to the true value, i.e. not related to accuracy. The term “absolute bias ” is commonly used to describe age estimation errors related to accuracy and represents the systematic over- or underestimation of age relative to the true age. When the systematic over- or underestimation of age relative to the modal age is determined, the term "relative bias" is used instead (Eltink *et al.*, 2000).

The definition of accuracy is a matter of degree, which measures how close an estimated age is likely to be to the true age (Francis, 1995). It is expected that older individuals are more difficult to read than younger individuals. As a result, accuracy should be measured from the readings of individual age readers across different age groups by estimating how close the estimated ages are to the true ages.

Ideally, validation should be an obligatory step in all age estimation procedures, encompassing two fundamental aspects:

- The age estimation structure has a consistent interpretable pattern of growth increments (i.e. precision).
- The growth increments are laid down with a periodicity that can be related to a regular time-scale (i.e. accuracy).

Accuracy is generally determined by experimental studies that clarify the increment-formation regulatory process, which is linked to environmental cues and life history events. Precision is improved by establishing protocols, repeated readings by the same reader or group of readers, and otolith exchanges among experts.

1.2.3 Validation

Calcified structures (CSs) in fish have the potential to grow throughout the life of the individual and act as a permanent record, documenting episodic patterns of growth at different time-scales. When true, these processes can be related to time, e.g. weights-at-age, fishing mortality by year, maturity-at-age, etc., which are all important parameters for stock assessment. Errors in age estimation will especially affect the estimates of recruitment, fishing mortality, and spawning-stock biomass. Therefore, validation studies are a fundamental part of fish age estimation, allowing the provision of accurate mortality and growth rate estimates for stock assessment.

According to Beamish and McFarlane (1983), age validation is a process of establishing the accuracy of an age estimation method. Validation of an age estimation procedure indicates that the method is sound and based on facts (Kalish, 1993).

In theory, a validation should be made of every population of a given species, since there may be important differences among them (Panfili *et al.*, 2002). Two aspects of validation must be determined: (i) that the increments are laid down with a periodicity that can be related to a regular time-scale (i.e. accuracy) and (ii) that the age estimation structure has a consistent interpretable pattern of increments (i.e. precision).

According to Francis (1995), three levels of validation are possible:

- The first increments are annual, but there are insufficient data to determine the periodicity of the latter increments and to make a quantitative estimate of the accuracy.
- All the increments are effectively annual, but there are insufficient data to make a quantitative estimate of the accuracy.
- The increments are effectively annual, and a quantitative estimate of the accuracy is provided.

Ideally, where finance and time permits, validation techniques should be carried out on all species where the age data are used in stock assessment. Once the ages have been validated for a stock, future age readers can have greater confidence in their readings and the method applied, and the stock assessors will have greater confidence in the data provided.

A comprehensive review of validation methods, including advantages and limits, was presented by Campana (2001), while the status of age validation in Europe was reviewed by Appelberg *et al*. (2005), and a more recent study provides a priority framework for directing future validation studies (Spurgeon *et al.*, 2015).

The methodologies available for validating the frequency of formation of increments can be roughly divided into indirect and direct methods, depending on whether they give support to the growth rates determined in the population or whether they assess the increment periodicity of individual fish. The latter is a population-based technique, which requires the observation of a time-series of growth marks on a large number of individuals. It usually uses otolith-edge evolution through time.

It is noteworthy to also define the term "corroboration", which involves multiple interpretations (obtained after one or more readings) usually using different calcified structures (scales, vertebrae, spines), as a "validation" method.

Several validation methods are described below. Each of the validation methods has its strengths and weaknesses; the choice of which method to apply will depend on a series of circumstances (funding, time, laboratory availability, species, aim of the study, etc.). The species-specific chapters (Chapters 2–5) each have examples of application of the methods for the species where these have been applied. Below, a short list of conclusions for each of the methods is given as a quick overview:

- Length-based methods: estimate the growth rates and age of individual cohort from length frequency data; this has to be compared with other independent methods of age estimation; do not allow the validation of the periodicity in the deposition of growth zones; inexpensive and use data routinely obtained.
- Marginal increment analysis: a successful method to corroborate annual increment formation across large age ranges, but hampered by the difficulty in measuring small increments accurately and the need for high contrast between growth zones; similar alternative: edge-zone analysis; inexpensive.
- Daily increments: a useful tool to (i) identify the first winter ring, (ii) help understand the mechanisms behind observed otolith macrostructure, and (iii) corroborate that an annual growth structure is present; moderately expensive.
- Microchemistry: a tool to link otolith macrostructure features (though not necessarily seasonal structures) with environmental conditions through physiological processes affecting otolith accretion; not useful for age validation, but rather the understanding of otolith features; expensive.
- Tag–recapture: the most direct validation method in use; a highly successful method that validates age directly and should be used if common agreement on age interpretation is not achieved; very expensive; risk of not recapturing the released individuals.
- Rearing in captivity: the only method to validate a fish's true age; expensive and may not mirror conditions in the wild, resulting in otolith macrostructure features that do not correspond to those observed in wild fish.

1.2.4 Direct validation methods

A direct validation method is an individual-based technique which makes use of precise temporal reference marks on a calcified structure relative to other growth marks.

Tag–recapture

Tag–recapture experiments rely on catching, tagging either via a chemical marker like oxytetracycline (OTC) or a physical tag such as a Petersen tag, and releasing a fish in the hope of recapturing it later. The success of a tagging programme relies heavily on the hardiness of the fish because many fish die shortly after capture, either as a result of damage caused in the capture process, shock, or through a higher exposure to predation due to reduced physical capabilities as a result of the tagging process.

Most recaptures happen within a short time after release. However, some are recaptured years later, and it is these examples that are particularly valuable in validation of age estimation. Fish that are tagged at very small sizes, where the age at tagging is virtually certain, can provide strong validation for the whole age structure. Fish tagged at larger sizes can still be useful, providing validated proof of growth rates between the fish length at tagging and that at recapture, assuming the tagging process had no/minor effect on fish growth. If the tagging is performed using both a physical tag and a chemical marker in the otolith, tagging of older fish can potentially give good records of the growth of the calcified structure over seasons (preferably over a whole year), validating the features counted when estimating the age of fish.

This method of validation is described in Campana (2001) as probably the most rigorous validation method for many species because the absolute age of the recaptured fish is known. The major disadvantage of this approach to validation is the time-scale. It can take many years to produce results, given the rather limited recapture successes; however, even a few individuals will form the basis for a validation of the age estimation of any species.

Captive rearing

Compared to all other techniques, rearing of fish in captivity from hatch under laboratory or mesocosm conditions provides a tool for the validation of a fish's true age. The drawbacks of this technique are that the experiments are expensive to set up and run and may not mirror conditions in which a fish lives in the natural environment, resulting in otolith macrostructure features that do not correspond to those observed in wild fish. It can, however, be a useful tool to establish the periodicity of observed rings in juvenile fish.

1.2.5 Indirect validation methods

Indirect validation methods are population-based techniques supporting the growth rates determined by the age estimation. Those methods compare individual age estimates with statistical age estimated from length frequency distributions as well as other age data. They cannot be considered as validation in the sense of Francis (1995), but frequently they are the only available methodology to support age estimation.

Length frequency analysis (LFA)

Length frequency analysis (LFA) is a relatively generalized tool which assumes that each age group has a normal distribution within a length frequency of the whole population and that the modes for each age group occur at a clearly distinct length for each age (differential modal length). Petersen (1891) was the first to identify that the modes of the length composition correspond to age groups. Age 0 corresponds to the smallest mode present in the sample obtained after the spawning period, with subsequent modes corresponding to age 1 and so on. Modal lengths corresponding to age classes can be identified through different methods and then compared to individual length-at-age observed from the calcified structures (Morales-Nin and Panfili, 2002; or more recently Zhu *et al*., 2013). However, it is often only the earliest year classes that can be defined in this way. After first maturity, sexual dimorphism, and individual growth variables, LFA becomes too blurred to identify individual age classes.

Marginal increment analysis (MIA)

Marginal increment analysis (MIA) is the most commonly used validation method and validates increment formation over time. MIA aims to prove that the pattern of alternating opaque and translucent growth zones observed in otoliths is seasonal and predictable (i.e. annual). This method is commonly used and is easy to establish, relying on the type of deposit (opaque or translucent) observed on the edge of the otolith; measuring this by month provides a sinusoidal profile through the year. Thus, the distance of increments (translucent or opaque) can be determined on the otolith edge with monthly periodicity by which the marginal increment can be measured and a growth index estimated. Marginal increment analysis is an indirect validation technique and is quite cost-effective. There are some limitations of MIA, mainly related to the difficulty in measuring small increments accurately. This problem is exacerbated in slow-growing and older individuals, particularly at the edge of the otolith. Difficulties in interpreting opaque/translucent zones can arise, and lighting setup of the microscope may be a factor in this. However, when done properly, this technique is a solid indirect validation technique (Smith, 2014).

Edge-zone analysis

In cases where there is low contrast between growth zones making marginal increment analysis difficult, an edge-zone analysis may be applied (Ross and Hüssy, 2013). This method is also based on the subjective interpretation of whether the edge zone under formation is opaque or translucent.

Daily increment analysis

Daily increment analyses apply measurements of the width of the daily increments to validate an annulus. The method is based on the close relationship between the widths of daily growth increments with the environmental temperature experienced by the fish (Mosegaard and Titus, 1987). The width of daily increments decreases with decreasing temperature, and patterns of successively decreasing/increasing increment widths can, therefore, be used to identify macroscopic growth zones, providing that these occur during specific seasons (and thereby link with environmental temperature) (Hüssy *et al*., 2010). Daily increments are used almost exclusively in the earliest life stages (< 1 year) and are useful in verifying the first winter ring (Rehberg-Haas *et al*., 2012), as well as for corroborating subsequent structures (Hüssy, 2010; Hüssy *et al*., 2010).

Daily increment analysis cannot be considered a true validation technique unless properly validated because it is based on the assumption that the growth increments used to validate a macroscopic structure are formed on a daily basis. Daily increment analysis is, however, a useful tool to help understand the mechanisms behind the observed structures in the otolith macrostructure and to corroborate that an annual growth structure is present.

Microchemistry

Microchemistry analysis is a method to validate the seasonal frequency of otolith macrostructure features through tracking the seasonally varying incorporation rates of different microconstituents and stable isotopes. The elemental composition of otoliths seems to reflect physiological processes which simultaneously induce changes in the otolith macrostructure. This method is, therefore, not useful for independent age validation, but provides insight into the mechanisms behind observed macrostructure features.

Three major assumptions underpin the application of otolith microchemistry to age validation:

- otolith composition reflects seasonal temperature variations;
- temperature variations correspond to visible features;
- variations in microchemical composition validate the seasonal frequency of visible features.

The concentrations of the main microconstituents of the otoliths (Ca, Sr, Na, K, Fe, Mn, and Mg) can be measured using a wavelength dispersive spectrometer (WDS or electron microprobe) to validate the seasonality of visible opaque and translucent (hyaline) bands used for age estimation. Elemental ratios in the otolith calcium carbonate, especially the Sr/Ca ratio, have been hypothesized to vary in response to seasonal changes in water temperature.

Micromilling is used as a tool to extract small samples of biogenic carbonate, with micron-scale resolution, which, in turn, can be analysed to acquire high-resolution $\delta^{13}C$ and $\delta^{18}O$ values and major/minor elemental chemistry.

Isotopes have been used to validate the age estimates of fish. They have mostly been used for long-lived species where the intention has been to "validate" either the younger ages estimated from whole otoliths or older ages estimated from sectioned otoliths. Bomb radiocarbon, based on the fallout from nuclear bomb testing, is one of the most reliable age validation techniques for long-lived species (Kalish *et al.*, 1997; Campana, 1999). Also, radiochemical dating of the otolith core, based on the decay of naturally occurring radioisotopes, is primarily suited for long-lived species (Kastelle *et al.*, 1994; Burton *et al.*, 1999; Cailliet *et al.*, 2001).

In a similar way to the Sr/Ca ratios, dating of the otolith core based on interannual variations in $\delta^{18}O$ is useful to validate the age of short-lived species (Upton *et al.*, 2012). Høie and Folkvord (2006) demonstrated the suitability of stable oxygen isotopes as a validation technique in Atlantic cod (*Gadus morhua*), owing to the link between $\delta^{18}O$ incorporation and environmental temperature.

Thus, otolith element composition holds great potential for validating the total age of fish. For otolith microchemistry to serve as a useful tool for age validation, the mechanisms of element incorporation have to be a function of seasonal patterns in either environment and/or growth and consistent across age classes and years. Hüssy *et al.* (2015) found distinct periodic patterns in the concentration of Cu, Zn, and Rb from the core to the edge of Atlantic cod otoliths that covaried with otolith opacity, with the highest element incorporation during the summer growth season, thus validating age estimations by applying trace elements in the otoliths.

1.3 Standardization of procedures

1.3.1 Why standardization is needed

EU fisheries management relies on data collected, managed, and supplied by EU countries under the Data Collection Framework (DCF; EC, 2008) which was first put into place in 2000 and subsequently reformed in 2008. Under this framework, EU Member States collect, manage, and make available a wide range of fisheries data, including age data, supporting the scientific advice regarding the Common Fisheries Policy (CFP). As a result, different EU Member States and, in many cases, different institutes within the same country dealing with the same stocks are eventually merging their data before the analysis.

Otolith age estimations should not be used indiscriminately by ICES stock assessment groups when there are clear indications of inaccurate age estimations of the species assessed (Reeves, 2003). There is, in fact, a heavy reliance by stock assessors on age data submitted by a variety of countries using different age estimation techniques, not all of which are validated. This, coupled with very low agreement on ages between individual readers and institutes for some stocks, can severely impact the precision of stock assessments. Thus, for stocks for which the responsibility of age estimation resides with multiple institutes, priority should be given to interlab quality checks to ensure that results remain consistent, and common protocols for stocks exploited by different countries have to be agreed and employed to reduce disagreements among age readers.

1.3.2 Monitoring performance

Consistency in age estimation can be monitored by ensuring that (i) the age interpretation by individual age readers does not "drift" through time, introducing bias relative to earlier interpretations, and (ii) the age interpretations by different readers are comparable (Campana *et al.*, 1995).

Growth increment patterns are usually complicated by the presence of "false rings", splits or multiple rings, and growth discontinuities. Therefore, the identification of the "true rings" to use in age estimation is not a simple issue. Discrepancies generally appear in the identification of the first annual increment and in the interpretation of the otolith marginal structures.

Moreover, because age estimation is not an exact science, there is understandably a "drift" among and within readers over time. To avoid this as much as possible, each age estimation laboratory should have protocols in place to ensure that all readers within an institute use an agreed standard age estimation method.

The disagreement in age estimation may be caused not only by different age estimation methods, but also by different preparation methods of the calcified structures (e.g. use of burnt vs. whole otoliths) or by use of different calcified structures (e.g. the use of scales vs. otoliths).

The criteria for interpreting calcified structures are transferred from person to person. With a control collection acting as a reference, subtle shifts in age estimation among age readers can be detected. Past interpretations that are found to be incorrect in the light of new data can be identified and corrected. The use of control collections facilitates the management of age estimation teams, because it can provide early signs of divergence and differences in perception. An evaluation that is perceived to be fair is more likely to elicit positive adjustments among readers.

Each age estimation laboratory may have control collections for each species/stock/season which should be regularly updated, because, over time, environmental factors may affect the micro- and macrostructures displayed within the calcified structures. Analysis of results achieved by different age readers over time, when looking at these control collections, can produce a history of precision and relative bias and show any significant changes that have occurred over time. Such control collections might also be used to test whether an age reader is achieving the expected levels of precision and, for quality assurance, if a threshold is set on the precision that must be achieved before an age reader can become qualified in the age estimation of a certain species (Eltink, 2000; Eltink *et al*., 2000).

At an international level, otolith exchange exercises and age estimation workshops are commonly seen as effective methods of quality control aimed at increasing precision in age estimation. However, high precision cannot replace the lack of accuracy, and validation should be carried out for all species. An exchange programme of calcified structures (otoliths, bones, scales, etc.) can be conducted to test whether significant differences in age estimation methods and age estimations exist among age readers and whether using different calcified structures can lead to significant differences in age estimation results. Thus, an exchange programme is conducted regularly to check whether the precision in age estimation and bias of the age readers is still within acceptable levels.

At the start of an exchange, it is very important to have some idea of the problems that might cause differences in age estimation among the possible participants. These problems generally fall in two categories:

1. The use of different calcified structures or different preparation techniques by age readers.
2. The application of different age estimation methods by the age readers, which might be indicated by the following features:
    - Large differences in growth parameters within the same population.
    - The interpretation of the edge of calcified structures usually causes more problems in age estimation when the calcified structures are collected during the period of fast growth. This can be tested by comparing the age estimation results within sets of calcified structures collected in periods of slow and fast growth.
    - The interpretation of the annual rings in calcified structures might be more difficult because of the occurrence of false rings during the juvenile period. This is indicated by higher *CV*s for the younger ages relative to those for older ages.

Exchange programmes obtain more objective estimations of the precision and bias in age estimation because readers use their own equipment and are not subject to tight time schedules (circumstances which may not be possible in a workshop). The objective of exchanges of calcified structures is to estimate precision (*CV*) and relative/absolute bias in the age estimations by readers from different institutes with an interest in the same species.

1.3.3 The benefits of having agreed (validated) manuals

International committees, through workshops and exchanges, continue to work hard towards age validation or consistency of the interpretation of age, i.e. the repeatability and/or precision of a numerical interpretation that may be independent of age. The basis for this effort is that, although validation of age is of key importance in terms of accuracy, being able to interpret otoliths in a precise and repeatable way is equally important to achieving consistent and useful results. A consistent approach to the processing of otoliths prior to age estimation and the techniques used during the age estimation process are vital. Once a consensus is reached on which techniques produce the best results for a given species, the production of a generic manual for the processing of specific species otoliths and their subsequent age estimation that details the optimum techniques is a valuable step. Such manuals are frequently the outcome of international age estimation workshops in the form of workshop reports (e.g. ICES, 2015a; see also Almeida and Sheehan, 1997, and Matta and Kimura, 2012).

The existence of protocols is essential to define and set up a standard procedure to:

- sample the otoliths (stratified by size range, season, sex, etc.);
- prepare them (sections, clarifying mediums, etc.);
- read them (image-analysis systems, filters, lighting, magnification, etc.);
- establish accuracy and precision.

A manual on age validation should encompass various steps, from otolith sampling to a complete validation of the age estimation procedure. These steps are summarized below in Figure 1.1.

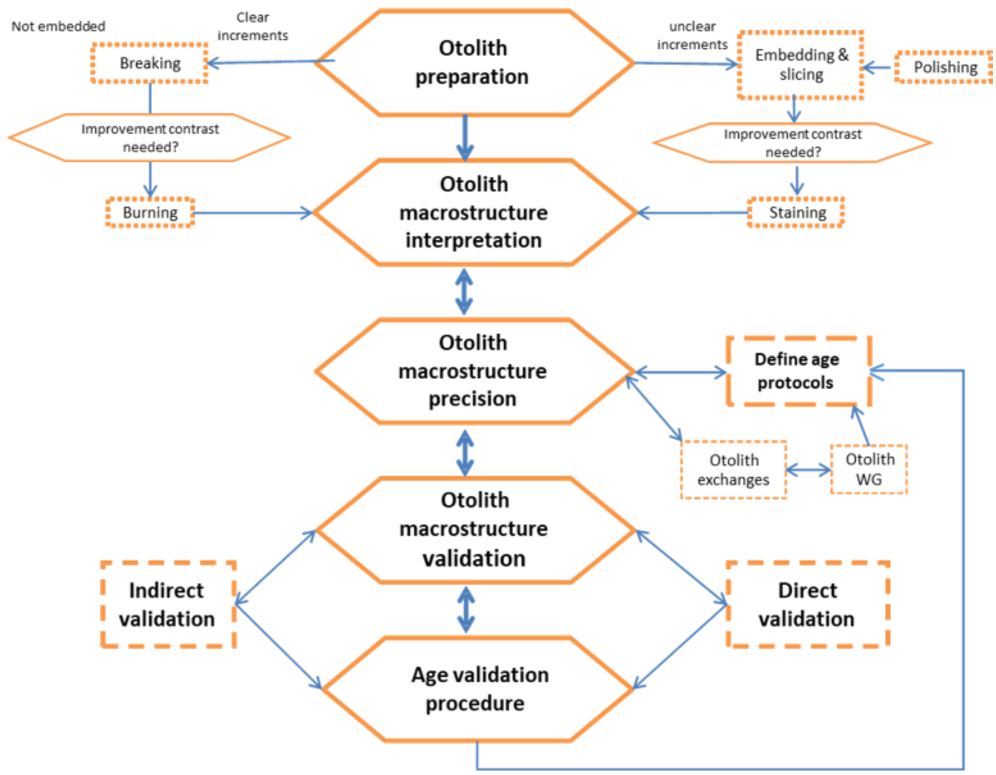


Figure 1.1. Schematic representation of the different steps, from otolith sampling to completed age validation, that should be included in a generic species-specific manual on age estimation.

1.4 Accreditation

The European Commission has stated that "accreditation is essential for the correct operation of a transparent and quality-oriented market." From the scientific perspective, participating in an accreditation scheme can act as a catalyst to raise standards, improve quality, and introduce improved work practices.

EU Member States have established a network of national accreditation bodies, which ensure that the competence of all laboratories and inspection and certification bodies are assessed by the same principles. This is a mechanism for laboratories to demonstrate conformance with internationally agreed protocols and standards of age estimation. For age estimation programmes, it is important to be clear that only the process can be accredited, not the result. This is because for the majority of fish species, validated age material is not available.

Accreditation standards are set by the International Organization for Standardization (ISO), which is based in Geneva, Switzerland. Certification bodies in each EU Member State then apply this internationally agreed standard when auditing individual institutions. Accreditation is then provided by the national accreditation body in the relevant Member State, e.g. the certification body in the United Kingdom is the United Kingdom Accreditation Service (UKAS), and in Ireland it is The Irish National Accreditation Board (INAB). Details on how to achieve accreditation (the processes and documentation that need to be in place) will be provided by these accreditation bodies.

There are many advantages to participating in an accreditation programme; however, in order to be able to accredit a process, there must be clear internationally agreed guidelines and standard operating procedures in place for the preparation of calcified structures as well as established age estimation criteria. Encouraging Europe-wide co-operation and standardization on the issue of age estimation and validation has been one of the key focuses of the PGCCDBS for the past twelve years. Greater consistency in calcified structure collection, storage, preparation, and age estimation has been achieved through an annual schedule of age calibration exchanges and workshops. This work is now continued and developed further in the ICES Working Group on Biological Parameters (WGBIOP).

The objective of these exchanges and workshops is to estimate precision and relative/absolute bias in the age estimations of readers from different laboratories and to check that these readings are still within acceptable levels. The frequency of exchanges and workshops mainly depends on the perceived difficulty of the individual stock whose fish ages are being read and the current quality of the age estimations. Exchanges and workshops can also result from recommendations from ICES assessment working groups or as a result of benchmark reviews. The exchanges and workshops have resulted in the production of agreed age estimation criteria, reference sets of agreed age otoliths, and,in some cases, age estimation manuals that provide an invaluable training resource for all age readers of a particular stock. These age estimation criteria and manuals can prove to be very useful in the pursuit of accreditation.

Working towards accreditation has very clear internal benefits in helping to improve and streamline a laboratory's own processes, and, importantly, it provides a driver to do those things. The process can also highlight why a laboratory does certain things in a particular way and can encourage age readers to investigate alternatives. Accreditation places a very clear emphasis on training programmes and provides the impetus for a structured approach to ongoing training and benchmarking of age readers through interreader checks within laboratories and across Europe. This assures the quality of the agereading process and, by association, the result, i.e. the assigned age estimations, even in the absence of validation.

However, accreditation can be a very demanding process. Achieving accreditation is not the end, but merely the beginning of a rather involved process that places a strong emphasis on constant improvement year after year in order to maintain the accreditation certification. The laboratory or institute must be able to show what they intend to improve, refine, and review next. Furthermore, gaining certification can be costly because it involves fees for the accreditation certification and accreditation visits and will also involve considerable time and effort from a team of people involved in the age estimation process. The cost of getting equipment calibrated annually, if this is not already routinely done, should be factored in because accreditation demands it.

The costs and the administrative burden will invariably increase as the scope of the accreditation widens. Therefore, it is extremely important for a laboratory or institute to understand clearly why they are striving for accreditation in the first place.

For those deciding to embark on the journey towards achieving accreditation, it is wise to begin small and expand as the team becomes more familiar with the demands of the accreditation process. Beginning with one aspect of the age estimation programme should be more manageable as a learning exercise and will allow the team to adjust to the rigors of an accreditation culture. New species or techniques can then be added as knowledge and confidence increases.

1.5 Contributors

This CRR is a product of several contributors; each chapter is edited by experts who have collated all available information. Some of the contributions are derived from workshops. Contact information for the various contributors is provided in Annex 1.

2 Gadoids

Karin Hüssy, Beatriz Morales-Nin, Carmen Piñeiro Álvarez, Hélène de Pontual, Jo-anne Smith, Kélig Mahé, Uwe Krumme, Sally Songer, Yvonne Walther, and Javier Rey

2.1 Introduction

This chapter was written during the Workshop on Age Validation of Gadoids (WKAVGS 2013) which was held 6–10 May 2013 at Imedea in Esporles, Mallorca. The terms of reference for the workshop were to:

1. Review information on age estimations, otolith exchanges, workshops, and validation works done so far on the following species: European hake, cod, pollock, saithe, haddock, whiting, and blue whiting;
2. Assemble and compare the results of different validation methods (i.e. marking and recapture, marking the calcified structure, marginal increment analysis, marginal analysis, modal progression analysis, length back-calculation, etc.);
3. Discuss and propose the most appropriate validation methods of age and growth pattern of calcified structures (CS), for each species and stock;
4. Propose the appropriate validation methods to recognize the growth check as well as the spawning ring, demersal ring, migration ring, etc.;
5. Propose an ICES Cooperative Research Report on: Age Validation Studies for ICES and GCFM Gadoid Stocks to ICES PGCCDBS, using previous studies and the outcome of this workshop;
6. Based on results, conclusions, and recommendations from this workshop to initiate and design an international cooperation project on validation methods (such as on the validation of checks and spawning rings) to commence after the workshop.

2.2 Age estimation methodologies in gadoids

A review of efforts carried out to reach common agreement on the interpretation of otolith growth zones, including an overview of exchange programmes, workshops, and summaries of problem identifications and recommendations, is presented.

The precision of age estimates by different national institutes is improved by means of otolith exchange schemes and age estimation workshops. Several reports on gadoids in ICES waters are available (Easey *et al*., 2005; Worsøe Clausen *et al*., 2005; ICES, 2008a, 2009a, 2013a, 2015a; Mahé, 2009; Piñeiro and Sainza, 2011; Mahé *et al*., 2014). A summary of the results from these workshops can be found in Table 2.1.

Table 2.1. Workshops and exchanges by species. Further information in "WK Ex SG History Master Table by Species 2018" in WGBIOP's Data quality assurance repository (ICES, 2018a, 2018b).

Species	ICES area	*n*	Preparation of the age estimation process	No. of readers	Agreement (%)	*CV*	Workshop/ Exchange
Saithe	Division 4.a Division 6.a	154 137	Sectioned otolith	20 18	95.9 82.8	3.3 5.4	Exchange 2007–2008 (Mahé, 2009)
	Division 2.a Subarea 4 Division 4.a	24 34 237	Sectioned otolith	13	85.9	6.2	Exchange 2013 (Mahé *et al.*, 2014)
	Division 2.a Division 6.a	50 10	Sectioned otolith	10	79.2 reflected light 82.3 transmitted light	3.7 4.6	WKARPV 2015 (ICES, 2015)
Whiting	Various areas around the British Isles	200	Broken otolith Sectioned otolith	11 19	72.6 80.9	16.3 13.7	Exchange 2004 (Easey *et al.*, 2005)
	Divisions 7.d, 4.a, 4.c, and Subarea 6	120	Sectioned otolith	17	80.7	10.3	Workshop 2009 (report not available)
	Division 3.a and subareas 4 and 7	134	Sectioned otolith	16	70	14	WKARWHG2 2016 (ICES, 2017a)
Hake	Divisions 8.a–b, 8.c, and 9.a	104	Sectioned otolith	16	46.3	41.2	WKAEH 2009* (ICES, 2009a)
	Divisions 8.c and 9.a	237	Sectioned otolith	12	62.3	33.1	Exchange 2011 (Piñeiro and Saínza, 2011)
Cod	Divisions 3.b–d		Sectioned otolith				Several workshops and exchanges (summarized by Hüssy *et al.*, 2016)
	Subarea 4	118	Sectioned otolith	21	74.0	39.8	WKARNSC 2008 (ICES, 2008a)
	Divisions 4.a and 4.b	120	Broken/ sectioned otolith	17	66	14.7	Exchange 2010 (ICES, 2011a)

Species	ICES area	*n*	Preparation of the age estimation process	No. of readers	Agreement (%)	*CV*	Workshop/ Exchange
Haddock	Subareas 4 and 6	NA	Sectioned otolith	12	84.2	18	Exchange 2009 (ICES, 2010a)
			Broken otolith	12	85	7.5	
Blue whiting	Division 4.a	100	Whole otolith	15	86.5	12.2	Workshop 2005 (Worsøe Clausen *et al.*, 2005)
	Divisions 4.a, 4.b, 2.a, and 5.a	189	Whole otolith	21	46.4	17.1	Exchange 2010–2011 (Mehl *et al.*, 2012)
	Subdivision 5.b.1	158	Whole otolith	19	57	13.4	WKARBLUE 2013 (ICES, 2013a)
	Mediterranean/ ICES divisions 9.a, 8.c, 7.j, 7.c, 7.b, 6.a, 4.a, 2.b, and 14.b / NAFO 1C	245	Whole otolith	29	68.7	44.2	WKARBLUE2 2017 (ICES, 2017b)

* The age estimation method was invalid.

2.3 General age estimation methods and problems

In this section, the general age estimation methods for gadoids are described by species. A series of images exemplifying the relevant otolith structures to analyse when estimating the age of a particular gadoid species are shown in Annex 2.

2.3.1 Saithe (*Polliachius virens*)

Difficulties of interpretation: Differences could be explained by the position of the first ring and identification of increments representing ages older than eight years. However, this species is generally considered to be relatively easy to read.

Recommendations: It was recommended to compare the two methods of preparation (sectioning and breaking). It is still necessary to present a direct or indirect validation of the formation of the rings (one ring per year).

2.3.2 Whiting (*Merlangius merlangus*)

Difficulties of interpretation: For some fish there was confusion over the first annual zone because of splits and the wide range of growth that can occur during the first year. Indecision over zone formation at the edge of the otolith could lead to differences of one year between reader ages. The wide difference in growth rates between fish caught in the same area also adds to the problem of interpreting the ring structure, as does the fact that the ring structure is only suitable for age estimation on limited parts of the otolith.

Recommendations: There was no significant difference in the results between the two age estimation methods of broken otoliths or sections. Each method has its own advantages and disadvantages. The workshop concluded that both age estimation methods were acceptable for whiting. "Humphries shadow" is a feature that is present on most otoliths, although not in every year and, as such, has only limited use in the interpretation of the ring structure.

2.3.3 European hake (*Merluccius merluccius*)

In the Northeast Atlantic, northern and southern ICES hake stock assessments have been based on age structure from 1992 to 2010. To that effect, age data have been demanded routinely from different research institutes, with many attempts at improving the precision of otolith age estimations through successive age estimation calibration exercises, such as exchanges and workshops (for more details, see Table 2.1.1 in Piñeiro *et al.*, 2009). During the 1980s, when different preparation techniques were used, scientists undertook several exchanges and workshops to agree on standardized preparation techniques and age estimation methods. The main outcome of this decade was the adoption of a common preparation technique (transversal sections of otolith) and the identification of the main sources of discrepancies among readers, i.e. the location of the first annulus, difficulty in discerning differences between annulus and other checks, and the interpretation of otolith edge type. During the 1990s, several workshops and calibration exercises resulted in common age estimation criteria suitable for fish up to age 5, according to the accepted slow-growth model at that time. These criteria were internationally adopted and applied by all readers from institutions involved in hake stock assessments. However, age estimation of hake still presented problems for older ages, which was a limiting factor for assessments. In 2004, an ICES otolith workshop focused on older fish in an attempt to deal with these problems. The results indicated that the precision of age estimations dropped from 0–5 to 0–3 years old. This was a consequence of the difficulty in using non-validated age estimation criteria in hake otolith reading, especially after the presentation of the first tagging results indicated that the age estimation criteria in use at that time were not accurate (de Pontual *et al.*, 2003, 2006). As a consequence of these results, another workshop was organized in 2009 using a reference collection of 104 OTC-marked otoliths. Eight research institutes (AZTI [Spain], IPIMAR [Portugal], Cefas [UK], MI [Ireland], Ifremer [France], IEO [Spain], AFBI NI [Northern Ireland], and VTI-DF [Germany]) participated in the evaluation of age estimation errors (accuracy and precision).

<u>Difficulties of interpretation</u>: Otoliths are difficult to interpret due to the complexity of the macrostructure and growth variability that has been related, among other reasons, to the long spawning season. The internationally agreed age estimation criteria are based on a concentric pattern of translucent and opaque rings/bands around the nucleus of otolith sections. The growth pattern presents several translucent rings per year that probably correspond to short environmental and/or physiological events, and the difficulty in interpreting such otoliths often increases with fish size. The classification of the edge type tends to be complicated since translucent edges appear year-round indicating a high incidence of checks (> 60 %), particularly in summer (Piñeiro and Saínza, 2003). Recently, blind interpretation of marked hake otoliths at the last workshop (ICES, 2009a) demonstrated with tagging material that the internationally agreed age estimation criteria are neither accurate nor precise and provide overestimation of age. This raises concern about the use, for stock assessment, of age–length keys that were inaccurate (ICES, 2010b). At this time, a replacement age estimation method with sufficient precision and accuracy is not available (de Pontual *et al.*, 2006; Piñeiro *et al.*, 2007).

<u>Recommendations</u>: The main results (ICES, 2010b) demonstrated that the age estimation method was not only imprecise, but also inaccurate and led to an overestimation of age (by a factor of two). The age estimation of European hake remains complex, and

further work is needed for both age-related assessment and ecological studies. Therefore, the age estimations used as input for the ICES Working Group on the Assessment of Southern Shelf Stocks of Hake, Monk and Megrim (WGHMM) should be suspended until new validated/accurate criteria are available. Considering the age estimation results obtained in the last workshop (ICES, 2009a) and the recent advances on hake age validation (tagging and recapture experiments, daily growth; de Pontual *et al*., 2006; Piñeiro *et al*., 2007, 2008), it was concluded that there are currently no reliable age estimation criteria. These overall findings led to substantial changes in the assessment conducted by ICES (2010b), that is now carried out using length-based models instead of the age-based model XSA previously used. A better understanding of the complex otolith growth pattern of this species might be achieved through a better knowledge of fish behaviour (migrations, feeding activity, etc.) and differences in individual life histories. Approaches coupling validation methods (e.g. otolith structures and DST tagging, otolith microchemistry, otolith modelling) should be promoted.

2.3.4 Cod (*Gadus morhua*)

Difficulties of interpretation: The interpretation of the first annulus can be confused with a first translucent band most likely deposited at the time the juvenile cod moves from the pelagic to the bottom zone. This confusion can be avoided by considering that the first annulus is wider than the first translucent band, ca. 2 and 1 mm in diameter, respectively. Another difficulty is the interpretation of age 1 cod captured during quarter one, when otoliths have a rather wide opaque-edge growth. Some readers estimated these fish at 2 years old because they assumed the translucent band was deposited after the New Year (1 January), and the opaque edge represented a summer growth period. The agreed interpretation is that the translucent band is deposited in autumn (New Year), and the opaque-edge growth is deposited during winter in quarter one. A third difficulty of interpretation is the occurrence of split rings. Some of the translucent annuli can consist of several thinner translucent bands that can be misinterpreted as true annuli, which leads to overestimation of fish age. These bands can be identified as being thinner than true annuli and with less distance between them.

Contrary to most other cod stocks, the eastern Baltic cod stock (subdivisions 25–32) is subject to extensive age estimation problems. The interaction of various factors (e.g. different hydrographic conditions on the vertical and horizontal scale, successive onset of spawning from west to east) result in unclear growth-ring formation. Age estimation of Baltic cod is presently performed with broken (Denmark, Sweden), broken and burnt (Estonia, Latvia, Poland, Lithuania, Russia), or sectioned (Germany) otoliths. The key problems with age estimation are: (i) identification of the first winter ring, (ii) timing of the winter ring formation, and (iii) interpretation of the edge. The interpretation of growth zones varies widely among countries and institutes, and even among readers within a given institute. To improve the precision of age estimation, a reference collection of otoliths was compiled in 1995–1996. The purpose of the reference collection was to have a set of otoliths as reference material for calibration and training of new and established readers and to reach consensus on the interpretation of the otolith characteristics. As the quality of the otoliths deteriorates with frequent handling, images of each otolith were digitized and the image collection distributed among all countries. Details of the age estimation methods, problem descriptions, and the reference collection can be found in the revised "Manual for Baltic Cod Age Reading" (ICES, 2000). Despite 30 years of effort to standardize preparation techniques and interpretation of growth zones with numerous workshops and otolith exchanges, precision in age estimates is still very low. As a result, the age distributions of catches vary alarmingly by country (ICES, 2012a; specifically section 2.4, Figure 2.4.1a–d), which may negatively influence the quality of the assessment (Reeves, 2003). Since 2013, the traditional age-based stock assessment has been abandoned because of the extensive age estimation problems.

Recommendations: The workshop concluded that sectioning of the otolith is the preferred method to use (vs. the broken method). The various life history traits of North Sea cod may differ within the North Sea, and knowledge of this is highly important for age readers. In addition, all age readers would benefit from more information on the formation of otolith structures in North Sea cod, especially the formation of split rings. Thus, the group recommended the inclusion of general studies on otolith formation and, in relation to this, North Sea cod physiology, growth, and behaviour as part of the training and updating of all North Sea cod age readers. Owing to the poor readability of eastern Baltic cod otoliths, there is still no consensus on the interpretation of growth zones of these two cod stocks. Consequently, neither the age estimation manual nor the reference collection have been updated in recent years. A tag–recapture study for the entire Baltic Sea is urgently required to generate material for validation of fish age and to assess growth, movement, and exchange processes within and between stocks.

2.3.5 Blue whiting (*Micromesistius poutassou*)

Difficulties of interpretation: The first difficulty of interpretation is the position of the first ring where the Bowers zone is clear. This is often seen in younger individuals as the otolith is thinner and the structures therefore clearer. The second difficulty of interpretation arises when some readers choose to omit specific rings identified as splits, while other readers identify the same rings as true annuli. This becomes more problematic after the second year of growth.

Recommendations: Inclusion of general studies on otolith formation and, in relation to this, blue whiting physiology, growth, and behaviour.

2.4 Age validation case studies in gadoids

In gadoids, only a limited number of validation methods described in the literature (Campana, 2001; Appelberg *et al.*, 2005) have been applied. A summary, therefore, is given in Table 2.2, which is a modification of Campana's table, with a column showing the gadoid species that were studied and which technique was used.

Table 2.2. Summary of age validation methodologies, modified from Campana (2001), highlighting the methods used for gadoids. DGI = daily growth increments. N/A = not available.

Method	Annual/DGI	Age	Advantages	Limitations	Gadoids in which this validation technique has been employed
Released marked fish	Annual and DGI	All	Validate absolute age and periodicity	Source of fish with known age, recaptures of old fish are null	N/A
Mark–recapture chemically tagged fish	Annual and DGI	All	Validate periodicity post release	Low recaptures, some markers may affect survival	Hake (de Pontual *et al.*, 2003; 2006; Piñeiro *et al.*, 2007; Mellon-Duval *et al.*, 2010; ICES, 2010b)
Captive rearing from batch	Annual and DGI		Validate absolute age and periodicity	Differences with wild fish	N/A
Microstructure	Annual	1 year	Validation of 1st year	Daily periodicity assumed	Hake, cod (Morales-Nin and Aldebert, 1997; Arneri and Morales-Nin, 2000; Morales-Nin and Moranta, 2004; Belcari *et al.*, 2006; Hidalgo *et al.*, 2009; Hüssy, 2010; Pattoura *et al.*, 2011)
Most likely age (MLA)	Annual and DGI	0–5 years	Validation of ages 1–2	No overlapping length modes, no length-based migrations	N/A
Marginal increment analysis	Annual	All	Validate periodicity	Not so straightforward in slow-growing/older individuals. Needs adequate sample size by month.	Cod, saithe, whiting, haddock, hake (Mahé *et al.*, 2016)
Radiochemical dating	Annual	Plus 5-year-olds	Validate absolute age of old fish	Can only distinguish between divergent estimates	N/A
Bomb radiocarbon	Annual	All	Validate absolute age and periodicity	Very old fish needed	N/A

In the following, the validation studies carried out on gadoids referred to in Table 2.2 will be described in detail. The methods are reported according to whether they are indirect or direct validation methods.

2.5 Application of indirect validation methods

2.5.1 Length-based analyses

Length frequency analysis has been applied successfully on some gadoid species (Table 2.3), especially European hake (Aldebert and Morales-Nin, 1992) and hake in the western Mediterranean Sea, obtaining an indirect validation of the first three age classes and confirming the first winter ring of Baltic Sea whiting (Ross and Hüssy, 2013).

In the case of European hake in the Mediterranean Sea, the formation of the translucent zone corresponds generally to winter months (Colloca *et al.*, 2003), and the frequency distribution of the ring distances from the nucleus shows two principal peaks for each annual ring (Figure 2.1), in agreement with the presence of two spawning periods (spring–summer and autumn–winter; Arneri and Morales-Nin, 2000; Belcari *et al.*, 2006). Consequently, in this case, two spawning groups of individuals should be recognizable at the time of the first hyaline ring formation: those that hatched in summer (age 0+ group) and those that were born the previous winter (age 1 group). This pattern also appears for subsequent age groups.

Table 2.3. Summary of species where length frequency analysis (LFA) has been applied.

Species	Method	Area	Age/size range
Whiting (Ross and Hüssy, 2013)	Mode progression + daily increments	Western Baltic	0–1 years
Hake (Aldebert and Morales-Nin, 1992; Arneri and Morales-Nin, 2000; Belcari *et al.*, 2006)	LFA	Tyrrhenian Sea	0–3.5 years
Hake (Aldebert and Morales-Nin, 1992)	LFA + otolith daily growth increments	Gulf of Lion	7.5–30 cm

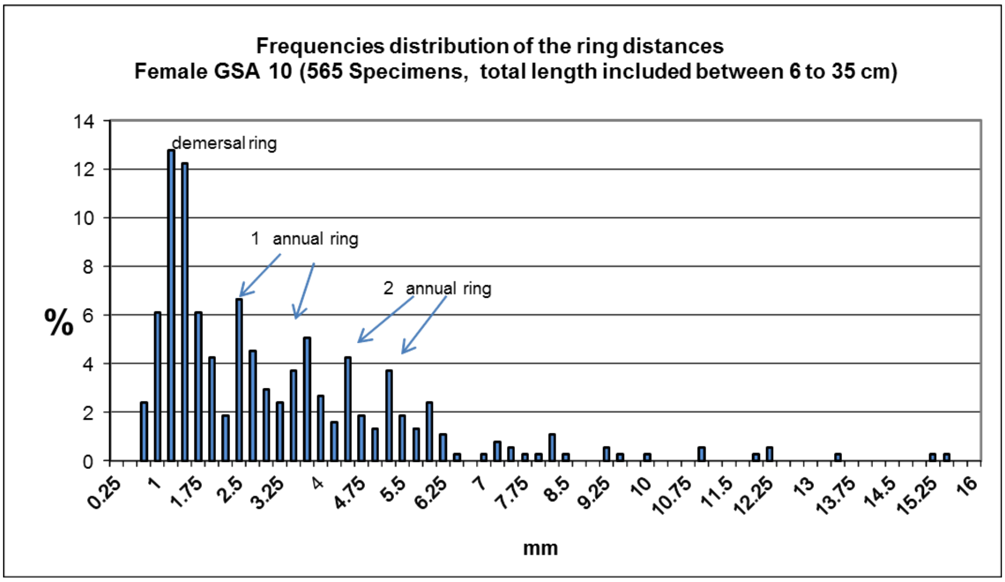


Figure 2.1. Frequency distribution of the distance between the nucleus and specific growth zones in European hake. The peaks represent different age classes, indicated with arrows.

In general, this technique verifies the growth rates associated with each age class by comparing them with another independent method of age estimation. This method is easily applicable because it is based on data gathered on a routine basis in fishery studies (i.e. length frequencies). However, this procedure does not allow validation of the periodicity in the deposition of hyaline rings, and it is difficult to apply when an overlap between modes is present. For this reason, if used for species with a relatively long spawning period (e.g. European hake), it provides reliable growth rate verification only for the first few age classes.

2.5.2 Marginal increment analysis

This method has been carried out on a number of gadoid species (Table 2.4).

Table 2.4. Summary of species where marginal increment analysis (MIA) has been applied. TL = total length (Mahé *et al.*, 2016).

Species	Method	Area	Time-series	Age/size range
Cod	Sectioned	Divisions 7.f–j and 8.a–b	Jan 2011–Jan 2012	2–9 years
Saithe	Sectioned	Division 6.a	Jan 2011–Jan 2012	2–14 years
Whiting	Sectioned	Subareas 4–6 and divisions 7.a–d	Jan 2011–Jan 2012	2–14 years
Whiting	Sectioned	Divisions 7.f–h	Jan 2011–Jan 2012	2–8 years
Haddock	Sectioned	Divisions 7.f–j and 8.a–b	Jan 2011–Jan 2012	2–8 years
Hake	Whole	Tyrrhenian Sea	Mar 1997–Feb 1998	2–16 years
Hake	Sectioned, burnt	Gulf of Lion	Jan 1989–Dec 1990	6–94 cm TL

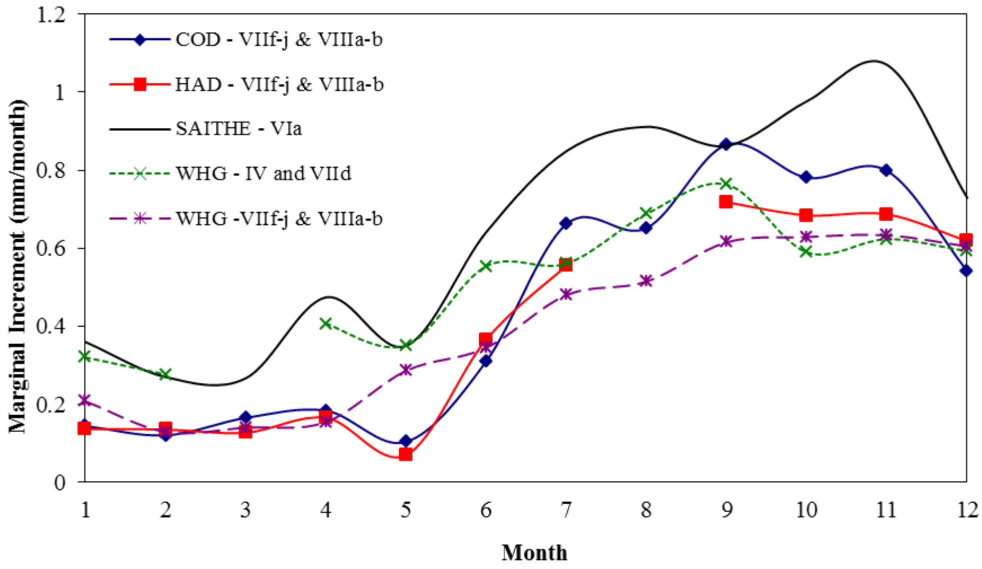


Figure 2.2. Marginal increment analysis of cod, haddock, saithe, and whiting from the North Sea to the Bay of Biscay (source: Mahé *et al.*, 2016).

For the gadoid species discussed at the Workshop on Age Validation Studies of Gadoids (WKAVSG) and listed in Table 2.4 above, the use of MIA proved to be a successful method to corroborate annual increment formation, using sectioned otoliths in fish across large age ranges. Figure 2.2 shows the application of MIA for a range of species.

2.5.3 Edge-zone analysis

See Section 1.2.5 on indirect validation methods.

2.6 Application of direct validation methods

2.6.1 Tag–recapture

Tag–recapture is one of the direct methods to validate the intrinsic age information of otoliths. Upon capture, individual fish are usually marked externally (e.g. with T-bar or spaghetti tags to ensure recovery) and internally (to produce a mark in the otolith at the time of capture). Different chemical marker substances can be used, e.g. fluorescent compounds such as oxytetracycline, that may fade over time but have also been shown to remain visible in cod otoliths 40 years after tagging (Krumme & Bingel, 2016). A chemical mark like strontium chloride is stable over time, but requires a scanning electron microscope to detect it in the otolith (Geffen, 1992). The combined use of injections with tetracyclin and strontium chloride may therefore provide a long-term solution (Stötera *et al.*, 2018).

Information on the suitability of alternative chemical markers for certain species is often poor, so the choice of which otolith marker to use and which concentration to apply in validation studies must often be made without evidence from robust experiments. The material and methods sections of published studies are often not sufficiently detailed to allow the experiments to be reproduced (e.g. it is unclear exactly which chemical substances were used, how they were prepared and how applied). Sometimes, it is not clear whether failure in marker experiments was caused by real biological or other reasons, or whether the chemical concentrations were wrong.

It is, therefore, necessary for large-scale tag–recapture studies, aimed at age validation of otoliths, to be preceded by robust experiments that allow determination of the suitability of different chemical agents at different marker concentrations. Such experiments should ascertain adverse effects on the tagged fish (increased mortality; Morales-Nin *et al*., 2011), perceptibility of the artificial time-mark in the otoliths at different marker concentrations, and visibility of the mark over time. The use of control groups is essential. Stötera *et al.* (2018) provide an example of a systematic approach to assessing the effect of different tetracycline concentrations on the fluorescent band quality of Baltic cod.

A pilot tag–recapture experiment was carried out for hake in the Bay of Biscay in 2002. A specific protocol was developed, including a modified gear designed to optimize fish survival. Recoveries showed much higher growth than previously expected (de Pontual *et al*., 2003).

Analysis of marked and recaptured fish and their otoliths showed that the previous underestimation of growth (by a factor of ~2) could be clearly related to an overestimation of age (de Pontual *et al*., 2006; Figure 2.3).

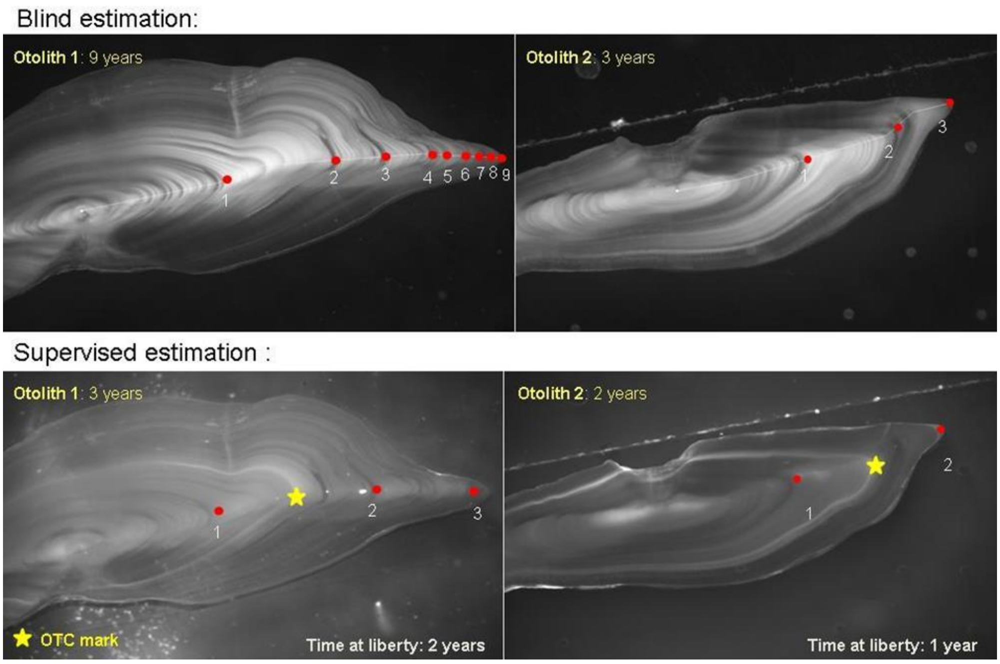


Figure 2.3. Comparison between blind and supervised interpretation of chemically marked (oxytetracycline [OTC]) European hake otoliths.

The approach was extended to the Iberian Peninsula (Piñeiro *et al*., 2007) and the Mediterranean Sea (Mellon-Duval *et al*., 2010), and sustained tagging effort was maintained in the Bay of Biscay during 2004–2007. In this region, 27 690 fish were tagged, chemically marked, and released. A total of 1199 fish have been recovered to date, leading to a new growth model being established as well as new insights on migrations and mortality. An overview of the tagging programmes carried out to date is given in Table 2.5.

The 104 marked otoliths resulting from this tagging programme were analysed in an international exchange and workshop (ICES, 2010a). Supervised otolith interpretation (taking into account oxytetracycline [OTC] mark position and fish data) showed that the internationally agreed age estimation method was neither accurate (bias of a factor ~2) nor precise. Even the otolith-supervised interpretation remained difficult (73.9% agreement among hake readers, with substantial differences in ring positions). It was strongly recommended to stop producing annual age–length keys for use in ICES assessment of European hake.

These overall findings led to substantial changes in the assessment conducted by ICES (2010b) that is now carried out using a length-based model (e.g. stock synthesis 3 [SS3]) instead of the previously used age-based model (XSA).

Table 2.5. Summary of tagging programmes carried out on European hake.

Tagging experiment			Recapture results				
Location	**No. of fish released**	**TL range at release (cm)**	**Max time at liberty (days)**	**TL range at recapture (cm)**	**No. of tagged fish recovered**	**Recaptured (%)**	**Reference**
SW Ireland	78	28.9	255	40.6	1	1.3	Belloc (1935)
Southern Bay of Biscay	152	56	24	60	1	1.9	Lucio *et al.* (2000)
Bay of Biscay	1 307	21–40	1 066	24–67	36	3.1	de Pontual *et al.* (2006)
Bay of Biscay	27 690	9–84	1 555	19.2–78.9	1 199	4.33	de Pontual *et al.* (2013)
Mediterranean Gulf of Lion	4 277	15–40	717	16–57	280	6.5	Mellon-Duval *et al.* (2010)
NW Iberian Peninsula	527	29–36	466	31–56	6	1.3	Piñeiro *et al.* (2007)
NW Iberian Peninsula	2 725	28–46	466	31–56	27	1	C. Piñeiro (pers. comm.)
Balearic Islands	675	10–44	-	-	-	-	E. Massuti (pers. comm.)

The "tag–recapture" programme ended the controversy over the growth rate of European hake. Results invalidated the otolith-based age estimation method that was being used, and it was concluded that there are currently no reliable age estimation criteria. A better understanding of the complex otolith growth pattern of this species might be achieved through better knowledge of fish behaviour (migrations, feeding activity, etc.) and differences in individual life histories. Approaches that couple validation methods (e.g. otolith structures and DST tagging, otolith microchemistry, otolith modelling; see Section 2.7 "Future perspectives") should be promoted.

2.6.2 Rearing in captivity

Rearing European hake in captivity from wild eggs has been done several times. In an experimental environment in Norway, European hake were reared from eggs up to 245 days in temperature- and salinity-controlled stable conditions. The lapillus and sagitta of one of these fish were examined for microstructural features. The age derived from increment counts support the daily nature of the hake sagittal increments that start forming on day 8, probably related to the start of exogenous feeding. The lapillus showed a later increment formation.

2.6.3 Daily increment analysis

See Section 1.2.5 (Indirect validation methods). Table 2.6 summarizes studies where this technique has been applied specifically on gadoid species.

Table 2.6. Summary of species where daily increment analysis has been applied.

Species	Method	Area	Time-series	Age range
Cod	Sectioned	Baltic Sea, subdivisions 22–24 Baltic Sea, Subdivision 25	2009 2001	0–1 years < 3 years
Whiting	Ground in sagittal plane	Baltic Sea, subdivisions 22–24	2009–2011	0–1 years
Hake	Ground in sagittal plane	Ionian Sea	?	0 group
Hake	Ground in frontal plane	Adriatic Sea	1992–1997	0–1 years
Hake	Ground in frontal plane	Tyrrhenian Sea	2001	0–1 years
Hake	Ground in sagittal plane	Gulf of Lion, Balearic Sea	1997, 2004	0–1 years

Validation of the first winter ring

This analysis is based on the enumeration of daily increments from hatch to capture, where individuals are sampled repeatedly from the same cohort before, during, and after the formation of the first winter ring. This approach was successfully used in European hake from the Ionian Sea (Pattoura *et al*., 2011), Adriatic Sea (Arneri and Morales-Nin, 2000), Tyrrhenian Sea (Belcari *et al*., 2006), Mediterranean, Gulf of Lion, and Balearic Islands (Morales-Nin and Aldebert, 1997; Morales-Nin and Moranta, 2004; Hidalgo *et al*., 2009). Similarly, the enumeration of daily increments from hatch to capture can be used to identify the timing of growth zones by linking the occurrence of translucent checks to the time of occurrence.

Subsequent winter rings

Cyclical patterns with daily increments forming a bell-shaped pattern separated by periods without visible increments were linked with seasonal temperature cycles based on cod tagged with data storage tags and strontium chloride (Hüssy *et al*., 2009, 2010). Comparison of daily increment patterns revealed inconsistencies between winter zones identified by the lack of visible increments with the formation of translucent zones (Figure 2.4; Hüssy, 2010).

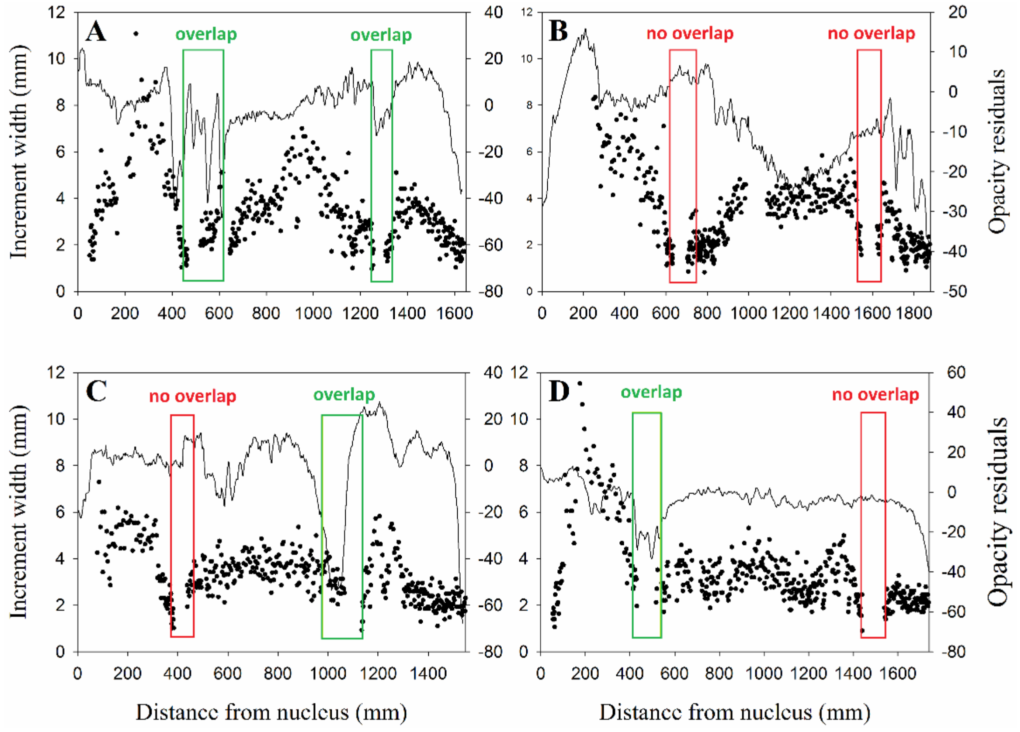


Figure 2.4. Four examples of 3-year old cod with different daily increment (dots) and opacity (lines) patterns over two consecutive years: (a) overlap in both years, (b) no overlap, (c) overlap in the second, but not the first year, (d) overlap in the first, but not the second year (modified from Hüssy, 2010).

2.7 Future perspectives in gadoids: available tools

2.7.1 Back-calculation of size at previous age and hatch date

Back-calculation is an important method of obtaining estimates of the length-at-age prior to capture of an individual fish (Panfili *et al.*, 2002). Specifically, the dimensions of one or more marks in some hard structures of the fish, together with its current body length, can be used to estimate a fish's length at the time of formation of each of the marks. The hard parts used are otoliths, opercular bones, vertebrae, fin rays, or spines (Francis, 1990). The marks are often annual rings associated with growth checks, but back-calculation has also been used in association with marks caused by the stress of liberation of hatchery fish (Davies and Sloane, 1986) and tetracycline injections in tagged fish (Panfili and Tomàs, 2000).

In order to carry out a back-calculation correctly, three main assumptions have to be met:

1. The size of the calcified structure (CS) mark remains unchanged from the time of formation (no resorption or degeneration).
2. The assumed time of formation is correct.
3. The back-calculation method accurately relates body size to CS size for each fish.

Back-calculation of previous size-at-age is an inexpensive tool, but cannot stand alone. This method should preferably be coupled to a length frequency distribution from a survey carried out in the period of the hyaline ring formation or size distributions of individual age classes.

2.7.2 Simulation tools for otolith macrostructure modelling

Improving the reliability of otolith-based individual and population data is critical to population dynamics and ecology. In this respect, the numerical model of otolith formation recently developed and validated by Fablet *et al*. (2011) deserves particular attention. Based on a general bioenergetic theory, it disentangles the complex interplay between metabolic and temperature effects on otolith growth and opacity patterns, resolves controversial issues, and explains poorly understood observations of otolith formation. It represents a unique simulation tool to improve otolith interpretation and applications. Scenario-based model simulations (where temperature and food series are forcing variables) are of primary interest to interpret and predict otolith characteristics in response to environmental features (e.g. Figure 2.5). Besides, they provide new means for the discrimination of seasonal vs. non-seasonal otolith structures, a crucial issue for the improvement of the accuracy of individual age estimation.

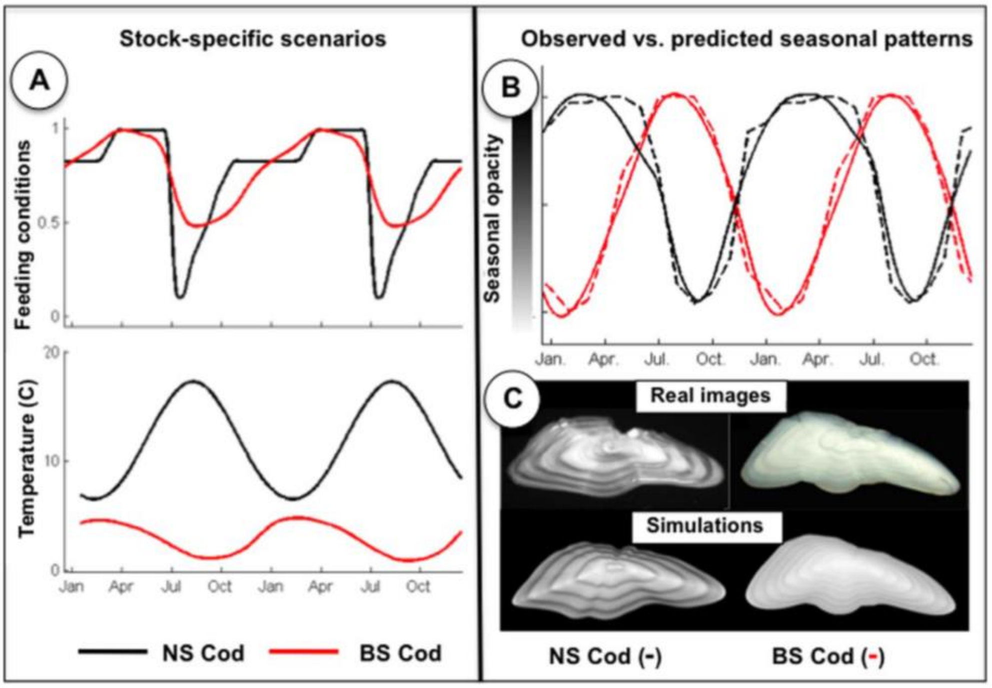


Figure 2.5. Resolving the non-synchronous seasonality of opacity patterns of Barents Sea (BS) and southern North Sea (NS) cod otoliths: feeding and temperature conditions (A) that explain otolith opacity patterns observed for southern North Sea (NS, black lines) and Barents Sea (BS, red lines) cod (B and C). Observed seasonal patterns (dashed lines), given as the relative proportions of opaque edges in the monthly sampled otolith sets (21), are compared to normalized simulated opacity patterns (solid lines). Model simulations reproduce both the opposite seasonal opacity patterns (B) and the remarkable differences in the contrast of the otolith images of the two populations (C). From Fablet *et al.* (2011).

2.7.3 Raman microspectrometry

Raman microspectrometry could be another useful tool to achieve a better understanding and validation of otolith growth patterns. This technique allows a quantitative characterization of the mineral and organic fractions of otolith structures (micro- and/or macro-structures). Recent studies (Jolivet *et al*., 2008, 2013) have provided new insights into understanding otolith biomineralization mechanisms, as well as for the interpretation and discrimination of otolith macrostructures.

2.7.4 Microchemistry as a supportive tool for age validation

Age estimation for most stocks is hampered by the fact that the process is based on counting annual growth increments (rings or annuli), whereas it is not certain that one ring is formed each year. The timing of the deposition of annuli is not verified and pseudoannuli, i.e. rings that can appear at any time during the year, induced possibly by stress, are a common problem. Using corroborative methods for validation of annual rings, such as elemental or isotopic cycles, could potentially support the counting of rings. This would be a correlative way of corroborating the age of the fish, primarily by confirming the identity of true annuli (Campana, 2001). It is necessary to be aware that the use of microchemistry as a supportive tool for age validation requires fundamental knowledge about the chemical environment of the species. While growth rate and metabolism can affect the deposition of chemical elements in the otolith, recent studies show that uptake directly from surrounding water is a main source of certain chemical elements found in otoliths (Walther and Thorrold, 2006).

Techniques commonly used for microchemistry analysis (elemental fingerprint) include:

- Laser ablation inductively coupled mass spectrometry (LA-ICPMS)
- Electron probe microanalysis (EPMA)
- Proton-induced X-ray emission (PIXE)
- Scanning X-ray fluorescence microscopy (SXFM)

Although microchemistry is not widely used for the purpose of validating age, the field has the advantage of being in rapid development (Campana, 2005). Methods have matured and are now more widely used for identifying natural tags. This also means that methods are becoming more affordable to use on a routine basis. The concept of elemental fingerprinting in otoliths is very attractive, telling a story about how to interpret the life history of fish and constructing environmental history. The use of elemental fingerprints is becoming more widely used for separation populations, migration, stock mixing, and map connectivity between habitats (Gillanders, 2005). The various uses of elemental finger printing implies that more effort will be invested in making elemental maps of otoliths, something that can also prove useful for age estimation purposes.

In a recent study of cod and hake (DGXIV Study Project 96-075), it was shown that opaque and translucent zones were generally different in composition during the early stages of development, although the variation declined toward the edge of the otolith. Sr/Ca ratios were generally higher in translucent zones than in opaque zones. Na/Ca ratios were inversely related to Sr/Ca ratios. The decreasing variations in Sr/Ca ratios between translucent and opaque bands towards the otolith edge could be a result of either the decreasing width of the bands or an ontogenetic effect. Because there was such a close coupling of the visual pattern of otolith zone formation and the chemical composition, it seemed unlikely that simple cyclic seasonal temperature fluctuations were responsible for all of the variations in the Sr/Ca signal. Therefore, it was not possible to use the Sr/Ca variations to validate which zones correspond to annual otolith increments in many of the hake otoliths. There is increasing evidence in the literature that the Sr/Ca ratio in fish otoliths responds only indirectly to ambient temperatures. The elemental ratio may be more of a reflection of physiological processes, and these may simultaneously induce visual changes in the otolith. Thus, the Sr/Ca ratio is not independent of otolith growth and cannot be used as an independent validation tool.

Chemical elements suitable for corroboration of age will be highly variable, depending on species and even on stock level, and will reflect the environment. Investigations show that elemental fingerprints in cod otoliths based on the elements Li, Mg, Mn, Sr, and Ba are physically stable. The elemental ratio Sr:Ca is mainly connected with changes in salinity and is popular for tracking anadromous fishes (Walther and Limburg, 2012). Other elemental ratios that have shown variation in otoliths in offshore and coastal waters are Ba:Ca and Mg:Ca (Thorrold *et al.*, 1997). Additional ratios recently identified as promising in exhibiting seasonal patterns are Mg:Ca, Zn:Ca, Cu:Ca, and Rb:Ca (Hüssy *et al.*, 2015; Limburg et al., 2018).

Experiments were made with the Sr:Ca content in cod and hake; however, this elemental ratio proved not to be useful in the marine environment where the variation in concentration is connected to temperature and is not independent of otolith growth.

Another example is the findings from microchemistry analysis of cod in the Baltic Sea. An experiment hypothesized that the incorporation of Mn:Ca and Sr:Ca showed a potential of being related to seasonal events (Limburg *et al.*, 2011). Mn:Ca was found to have a strong correlation with hypoxic events (Itai *et al.*, 2012), which was also found in the experiment of Limburg *et al.* (2011).

Figure 2.6 maps variations in otoliths over a temporal scale of four historical periods of time. A seasonal pattern was detected when the ratio Mn:Ca was high in the summer months of young fish (1–2 years), which can be related to dwelling in shallow, relatively warmer nursery areas and with exposure to seasonal hypoxia. This pattern disappeared in later life when Sr:Ca was elevated (Figures 2.6 and 2.7). Sr:Ca is related both to temperature and salinity, and cod are known to migrate to deeper, more saline and colder water after their juvenile period to either feed or spawn.

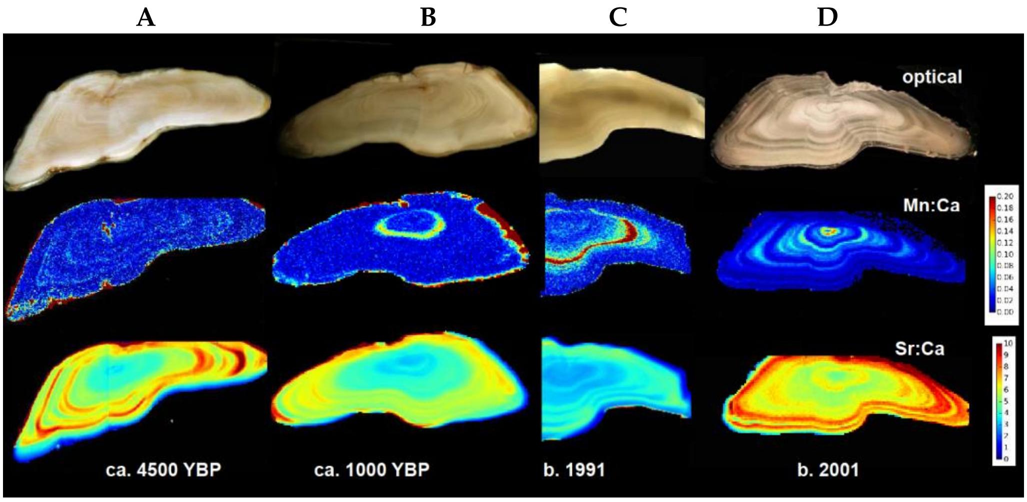


Figure 2.6. Transverse sections of eastern Baltic Sea cod otoliths from four different time periods: the Neolithic (A), late Iron Age/Middle Age (B), early 1990s when the areal extent of hypoxia was low (C), and 2001 when it was high (D). Regions in the core correspond to the juvenile stage. Note that Mn is high in the Neolithic and Iron Age otoliths in small cracks as well as along the edge, where it was in contact with soil pore water. The 1991 otolith is portrayed in a partial map. (Scale bar: 1 mm. Reproduced from Limburg *et al.*, 2011.)

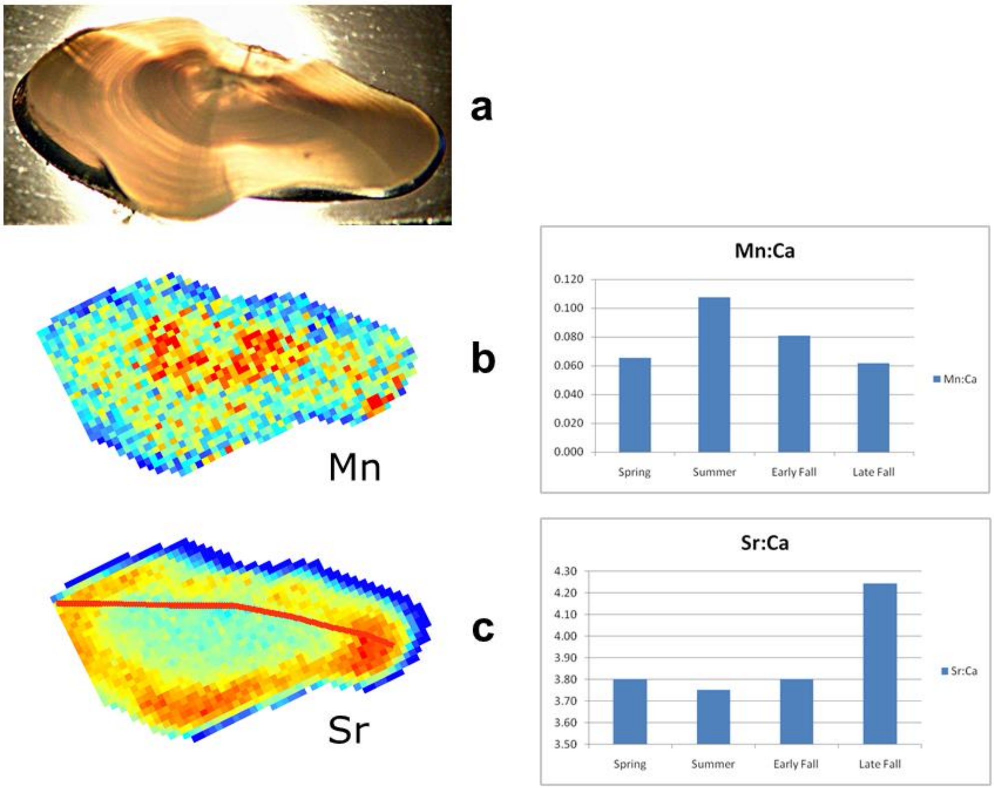


Figure 2.7. Otolith of a young-of-the-year eastern Baltic cod, length = 100 mm, captured 17 November 2008 in a special trawl survey. Scale bar = 1 mm. (a) Photo of a transverse section through the otolith. (b) Manganese elemental map (left) and Mn:Ca ratios (× 103, right) parsed out by season. (c) Strontium elemental map (left) and Sr:Ca ratios (× 103, right) parsed out by season. The red line represents transects of data that were extracted by GIS for preparing the graphs. Examination of daily increments (not visible) indicated the fish was born in May (Images: K. Limburg).

2.8 Ongoing and future work

For anglerfish (*Lophius piscatorius*) and hake (*Merluccius merluccius*), a European project (EASME/EMFF/2016/1.3.2.7/SI2.762036, Validating age-determination of anglerfish and hake) will investigate if calcified structures (otoliths and illicium) contain seasonal otolith microchemistry patterns that can be used for age estimation. A combination of analytical approaches (LA-ICPMS, IR-MS, SIMS) are employed to establish the most effective method for measuring age-related maxima and minima in elements and isotopes. The results will be used to develop the tools for otolith macrostructure modelling.

For Baltic cod (*Gadus morhua*), the seasonality of chemical element patterns occurring in otoliths is being validated through a large-scale tagging project "Tagging Baltic Cod" (TABACOD), funded by BalticSea2020. For further information visit the TABACOD home page at: http://www.tabacod.dtu.dk/

2.8.1 Validation of life history events – juvenile check

In some demersal species, several of the macrogrowth increments in the central part of the otolith confuse the attribution of the first seasonal increment. The growth of these increments during the early life phases may be related to one or several factors, such as changes in habitat (i.e. from pelagic to demersal settlement), feeding patterns (changes in diet), or metamorphosis (i.e. termination of cutaneous respiration and ossification). Any of these changes can result in changes in otolith formation, leading to changes in otolith shape and increment pattern. Changes in optical density and spacing of daily growth increments may also accompany metamorphosis. The most frequent change in the otolith shape observed in gadoids is due to the formation of accessory growth centres (Figure 2.8). In a study of European hake settlement (Arneri and Morales-Nin, 2000), the duration of the pelagic life phase was established at approximately 2 months, which was validated in other studies.

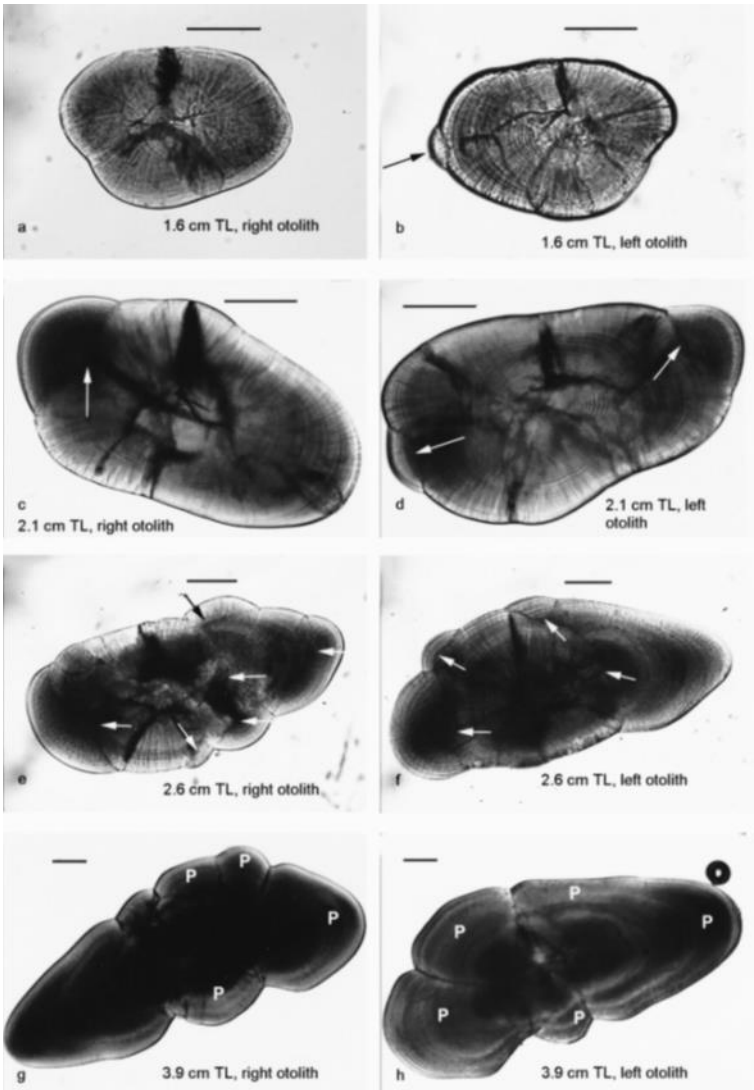


Figure 2.8. Juvenile hake otoliths, showing the changes in shape and increment structure (Arneri and Morales-Nin, 2000).

2.8.2 Validation of life history events – spawning check

Background

Age, size, and growth at the onset of maturation are important stock assessment parameters (Godø and Haug, 1999). So-called spawning checks or "spawning zones" in otoliths may form an integral part of these otolith readings, especially in gadoids (Rollefsen, 1935). Spawning zones in Northeast Arctic cod were first described by Rollefsen (1933, 1935), and were later used to estimate age at maturation and to construct maturity ogives for individual cohorts in the time-period 1946–1981, although gonad staging is normally the main source of information in these types of analyses (ICES, 2001). At present, the Institute of Marine Research (IMR) in Norway uses otolith spawning checks for saithe north of 62°N (*Pollachius virens*) to determine the proportion of mature vs. immature saithe in assessments (ICES, 2005). The background here is that this survey cruise takes place in autumn, i.e. at a time of the year when gonad staging is not helpful in adequately separating maturing from immature individuals. In assessments of Northeast Arctic haddock (*Melanogrammus aeglefinus*), spawning checks are also currently being used, but only to confirm maturation stages already determined by gonad inspection during the so-called "winter survey". Moreover, otolith spawning checks may also be useful to studies on evolutionary changes in maturation-at-age related to commercial fishing, which may be of particular interest to institutions with large historical otolith archives.

Previous work

Despite many years with use of spawning checks in otoliths, direct validation of these assumed spawning indicators has only been brought to our attention in recent years. Previous work to validate "spawning zones" in cod otoliths began with the NFR (Research Council of Norway) project "Timing and determination of fecundity and skipped spawning" (2006–2009), followed by a second project to include haddock ("The occurrence of skipped spawning and its importance for population dynamics in Northeast Arctic gadoids", 2009–2012). The first project included isotope and chemical comparative analyses to validate or corroborate visible "spawning zones" and to compare these in fish with and without gonad indicators of spawning (post-ovulatory follicles [POFs]; Witthames *et al*., 2010; Skjæraasen *et al*., 2012). The second fecundity project addressed, among other things, growth trajectories of fish before and after the "spawning zones" as a further method of validating these features.

The rationale for incorporating data from otolith composition analysis into the determination of fecundity projects was to determine whether the physiological processes during gonad development would cause permanent, detectable features in the otoliths. There were two objectives:

1. to validate the so-called spawning checks by comparing these, in appropriate samples, with the presence of POFs; and
2. to use the isotope and element data to distinguish among individuals with regular or irregular annual reproductive patterns (skip spawners).

Future work

It is still unclear when spawning checks are being recorded in gadoid otoliths (e.g. which months) relative to the actual time of spawning. It is also necessary to investigate whether spawning checks are recorded in the otoliths of individuals that skip spawning after one or several years of spawning. An experimental method of direct validation of spawning checks is now being conducted at Matre Research Station, Institute of Marine Research, Norway. Cod will be reared from hatching to age 3 under two different feeding regimes (low and high), whilst individual growth, time of first maturation, and spawning will be monitored. Half-way through the experiment, 50% of the fish from each feeding regime will be switched to the other feeding regime. During the experiment, otoliths will be regularly stained by alizarin to determine the timing of spawning checks relative to time of spawning. The results of this experiment are not yet ready. Otolith growth and spawning checks will finally be analysed in transversal sections, using a fluorescence microscope to detect the alizarin stain marks. A disadvantage to such an experimental approach is the potential deviations that may occur in the otolith pattern from wild fish, although the husbandry protocols are designed to reflect the natural environment as far as possible.

Nevertheless, it should be expected that the appearance of spawning checks is species- and stock-dependent, as spawning behaviour, migrations, and environment may vary among different gadoid fish stocks. Unfortunately, for some gadoid fish stocks, spawning checks may be difficult, if not impossible, to detect by conventional age estimation methods. Deciding which species are suitable for future validation studies, and the importance of such studies to the respective stock assessment should, therefore, be discussed. Direct validation of age or at least high agreement in age estimation among involved age estimation institutions is a recommended prerequisite before undertaking further studies of spawning-zone validation in any specific gadoid stock. If individual age-at-maturation is not known, this type of spawning validation study has less value in the aspect of stock management.

The use of post-ovulatory follicles (POFs) is another good candidate for validating spawning. POFs persist for about a year in gadoid ovaries post-spawning, i.e. they can be used to justify the presences of an otolith spawning check produced within the last year. In an ongoing study at IMR (Norway), otoliths and ovarian samples from Northeast Arctic saithe captured along the Norwegian coast in October 2010, 2011, and 2012, are being analysed. Otolith transversal sections are being analysed for spawning checks, and gonad samples are being histologically processed (in resin) and stained with periodic acid-Schiff stain (PAS), before being analysed under a microscope for POF prevalence as well as ovarian morphology (method: see Witthames *et al*., 2010). So far, results show good agreement between spawning zones and the presence of POFs among older individuals that had spawned more than once. However, little correspondence was found between POFs present and spawning zones in the otoliths of younger individuals. As an example, no spawning zones were found among individuals between two and four years old in 2012. Nevertheless, half of these ovarian samples showed evidence of POFs, 40% had POFs defined as “almost certain”, and only 10% had no POFs. Hence, our data suggest that Northeast Arctic saithe spawn at a younger age than expected from traditional otolith analyses.

3 Flatfish

Mark Etherton, Sally Songer, Joanne Smith, and Barbara Bland

3.1 Introduction

Flatfish form a large part of both the commercial fisheries and the stock assessment effort within ICES. The majority of category 1 stocks (defined as those stocks where a full analytical assessment and short-term predictions can be made) have age-based assessments. Accordingly, it is vital to obtain ages that are as accurate and precise as possible.

At least 18 flatfish species, comprising numerous stocks, have current age estimation programmes in marine institutes around Europe (Table 3.1). Many of these species are common in most sea areas within the Northeast Atlantic, Baltic, and Mediterranean, with a number of institutes doing age estimations. For others there is little age estimation effort; in some cases only a single laboratory has a flatfish age estimation programme. A list of the known flatfish species with age estimation programmes as covered by the EU Data Collection Framework is given in Table 3.2. A total of 22 institutes across Europe have flatfish age estimation programmes. Common sole (*Solea solea*) and European plaice (*Pleuronectes platessa*) are the most commonly read species and probably those that have the greatest commercial importance.

Table 3.1. Flatfish species with age programmes in European institutes and the number of institutes involved in each species.

Common name	Scientific name	No. of institutes
Common sole (Dover sole/Black sole)	*Solea solea*	10
European plaice	*Pleuronectes platessa*	10
Turbot	*Scophthalmus maximus*	9
Brill	*Scophthalmus rhombus*	7
Flounder	*Platichthys flesus*	7
Megrim	*Lepidorhombus whiffiagonis*	7
Dab	*Limanda limanda*	6
Lemon sole	*Microstomus kitt*	5
Witch (Witch flounder)	*Glyptocephalus cynoglossus*	2
Greenland halibut	*Reinhardtius hippoglossoides*	2
Long rough dab (American plaice)	*Hippoglossoides platessoides*	2
Scaldfish	*Arnoglossus laterna*	2
Four-spot megrim	*Lepidorhombus boscii*	1
Adriatic sole	*Pegusa impar*	1
Atlantic halibut	*Hippoglossus hippoglossus*	1
Sand sole	*Pegusa lascaris*	1
Solenette	*Buglossidium luteum*	1
Thickback sole	*Microchirus variegatus*	1

Within the framework of ICES, there have been a number of flatfish age calibration workshops and exchanges in recent years. Most have included analysis of interlaboratory comparisons of a set or sets of otoliths. Table 3.3 summarizes the available results from these workshops and exchanges.

Table 3.2. Summary of age estimation methodologies by institute.

Species	Area	Institute	Whole	Break/burn	Break/polish	Sections	Section and stain	Notes
Adriatic sole	W. Mediterranean	CNR–IAMC, Italy	✔					
Atlantic halibut	Subarea 4	DTU Aqua, Denmark			✔			
Brill	Subarea 4 and divisions 7.a–7.d	ILVO, Belgium					✔	
	Subarea 4	DTU Aqua, Denmark	✔		✔			
	Subarea 4	Ifremer, France				✔		
	Subarea 4	Thünen Institute,					✔	
	Subarea 4	Imares, Netherlands		✔			✔	
	Subareas 4 and 7	Cefas, England					✔	Currently read by ILVO
	Subarea 7	AFBI, Northern Ireland				✔		
Dab	Division 3.a and Subarea 4	Thünen Institute, Germany	✔			✔		Only large fish are sectioned
	Subareas 4 and 7	Cefas, England					✔	
	Subareas 3 and 4	DTU Aqua, Denmark			✔			
	Subarea 5	MRI, Iceland	✔					
	Subarea 7	AFBI, Northern Ireland				✔		
	Subarea 4	Imares, Netherlands		✔		✔		

Species	Area	Institute	Whole	Break/burn	Break/polish	Sections	Section and stain	Notes
Flounder	Subarea 3	BIOR, Latvia		✔				
	Subareas 4 and 7	Cefas, England					✔	
	Subarea 3	DTU Aqua, Denmark			✔			
	Subarea 3	FGFRI, Finland					✔	
	Subarea 3	Fishery Service, Lithuania				✔		
	Subarea 4	Imares, Netherlands		✔		✔		
	Subarea 3	SLU-Aqua, Sweden					✔	
Four-spot megrim	Divisions 8.c and 9.a	IEO, Spain	✔					Both otoliths used
Greenland halibut	Subareas 2 and 4	IMR, Norway	✔				✔	
	Subareas 8 and 9	IPIMAR, Portugal		✔				
Lemon sole	Subareas 4 and 7	Cefas, England		✔				
	Subarea 4	DTU Aqua, Denmark	✔		✔			
	Subarea 4	Ifremer, France	✔					
	Subarea 5	MRI, Iceland	✔					
	Subarea 4	Imares, Netherlands					✔	
Long rough dab	Subarea 5	MRI, Iceland	✔					
	Subareas 8 and 9	IPIMAR, Portugal			✔			

Species	Area	Institute	Whole	Break/burn	Break/polish	Sections	Section and stain	Notes
Megrim	Subareas 7–8 and Division 9.a	IEO, Spain	✔					Both otoliths used
	Subareas 4 and 6	Marine Institute, Scotland	✔					
	Subarea 7	Cefas, England	✔	✔				Method changing to
	Subareas 7 and 8	Ifremer, France	✔					
	Subarea 7	Marine Institute, Ireland	✔					
	Subareas 7 and 8	AZTI, Spain	✔					
	Subarea 7	AFBI, Northern Ireland				✔		
Plaice	Division 3.a and Subarea 4	Thünen Institute, Germany	✔			✔		Only large fish are sectioned
	Subarea 4 and Division 7.a	ILVO, Belgium	✔			✔		Sectioned only when in doubt
	Subareas 4 and 7	Cefas, England	✔	✔		✔		Stock-specific method
	Subareas 3 and 4	DTU Aqua, Denmark	✔		✔			
	Subarea 4	Ifremer, France	✔					
	Subarea 5	MRI, Iceland	✔					
	Subarea 7	Marine Institute, Ireland	✔					
	Subarea 4	Imares, Netherlands	✔	✔				
	Division 3.a and Subarea 4	SLU-Aqua, Sweden	✔			✔		Only old fish are sectioned
	Subarea 7	AFBI, Northern Ireland				✔		
Sand sole	W. Mediterranean	CNR–IAMC, Italy	✔					
Scaldfish	Subarea 4	Imares, Netherlands					✔	
	Subarea 7	AFBI, Northern Ireland				✔		

Species	Area	Institute	Whole	Break/burn	Break/polish	Sections	Section and stain	Notes
Sole	Division 3.a and Subarea 4	Thünen Institute, Germany					✔	
	Subarea 4 and Division 7.a	ILVO, Belgium					✔	
	Division 3.a	SLU-Aqua, Sweden					✔	Currently read by ILVO
	Subareas 4 and 7	Cefas, England					✔	
	Subareas 3 and 4	DTU Aqua, Denmark		✔				
	Subareas 4, 7, and	Ifremer, France				✔		
	Subarea 7	Marine Institute, Ireland				✔		
	W. Mediterranean	CIBM, Italy	✔					
	Adriatic Sea	Coispa, Italy				✔		
	Subarea 4	Imares, Netherlands		✔			✔	
	Subarea 7	AFBI, Northern Ireland				✔		
Solenette	Subarea 4	Imares, Netherlands					✔	
Thickback sole	Subareas 7 and 8	IEO, Spain				✔		
Turbot	Subarea 3	BIOR, Latvia		✔				
	Subarea 3	RKTL, Finland						Very rare in the area
	Subarea 4 and Division 7.a	ILVO, Belgium					✔	
	Subarea 4	Ifremer, France				✔		
	Subarea 4	Thünen Institute,	✔					
	Subarea 4	Imares, Netherlands		✔			✔	
	Black Sea	NIMRD, Romania	✔					
	Subarea 3	SLU-Aqua, Sweden					✔	
	Subareas 4 and 7	Cefas, England					✔	Currently read by ILVO

Species	Area	Institute	Whole	Break/burn	Break/polish	Sections	Section and stain	Notes
Witch	Subarea 4	DTU Aqua, Denmark	✔					Mainly read by Sweden
	Subarea 5	MRI, Iceland	✔					
	Subarea 7	IEO, Spain	✔					
	Division 3.a and Subarea 4	SLU-Aqua, Sweden	✔					
	Subarea 4	Marine Institute, Scotland	✔					

Table 3.3. Flatfish workshops and exchanges since 2000.

Year	Species	W/E	Area	No. of fish	Preparation	No. of institutes	No. of readers	Agreement (%)	*CV*
2002	Sole	Workshop		157		4	10	90	4
	Plaice	Workshop		112	Section	4	11	70	14
2003	Plaice	Exchange	Division 7.d	245	Section	6	19	61.6	23
		Exchange	Division 7.d	245	Whole	3	7	74.1	19
		Workshop	Division 7.d	81	Section	6	14	86.8	12
		Workshop	Division 7.d	81	Whole	6	11	86.0	8
2004	Megrim	Workshop	Subareas 7 and 8	39	Whole	6	8	54.9	21.5
2006	Flounder	Exchange	Subarea 4 and Division 3.a		Whole	NA	4–6	62.5	19.7
		Exchange	Subarea 4 and Division 3.a		Section	NA	4–6	53.0	21.6
2008	Flounder	Workshop	Subarea 4 and Division 3.a		Section and stain	10	17	70.4	-
	Turbot	Workshop	Subarea 4	110	Section and stain	6	13	82.8	-
		Workshop	Division 3.a	96	Section and stain	6	13	71.6	-
2010	Dab	Exchange	Subarea 4	160	Whole	6	12	79.3	12
	Plaice	Exchange	Subarea 4	112	Section (experienced readers only)	9	20	88	5
		Exchange	Subarea 4	112	Whole (experienced readers only)	9	20	84	8
		Exchange	Division 3.a	92	Section (experienced readers only)	9	20	73	12
		Exchange	Division 3.a	96	Whole (experienced readers only)	9	20	76	7
2011	Sole	Exchange	Subarea 8	120	Section, and section and stain	3	5	88.65	4.7
2015	Dab	Workshop	Divisions 4.b and 4.c	50	Whole	6	8	79	12
		Workshop	Divisions 4.b and 4.c	50	Section	6	8	63	10
2016	Sole	Exchange	Subarea 4	160	Section and stain	9	16	90	3

Year	Species	W/E	Area	No. of fish	Preparation	No. of institutes	No. of readers	Agreement (%)	*CV*
	Plaice	Exchange	Subdivision 22	50	Whole	4	7	68.7	19.6
		Exchange	Subdivision 22	50	Section	4	7	73.2	19.9
		Exchange	Subdivision 23	48	Whole	4	7	73.2	16.7
		Exchange	Subdivision 23	48	Section	4	7	64.3	21.8
		Exchange	Subdvisions 24	50	Whole	4	7	67.5	17.8
		Exchange	Subdvisions 24	50	Section	4	7	77.2	13.9
		Exchange	Subdivision 25	45	Whole	4	7	79.1	10.3
		Exchange	Subdivision 25	45	Section	4	7	78.8	14.3
		Exchange	Subdivision 26	30	Whole	4	7	70.7	16.7
		Exchange	Subdivision 26	30	Section	4	7	79	13

NA = not available.

3.2 Age estimation methodologies in flatfish

Otoliths can be read using three different preparation methods (ICES, 2010c):

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

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

Staining sectioned otoliths is a variation on method 3 and is employed by some institutes for some species.

In fact, most of these methods have lab-specific variations, mostly minor in nature. Generic descriptions, advantages, disadvantages, and variations noted in the age estimation manuals of specific institutes are found below. Figure 3.1 illustrates different interpretations of age of the same individual as a result of different preparation methods.

3.2.1 Whole otolith method

This method has the advantage of speed of preparation and the versatility of being able to manipulate the otolith: turning it, lifting one side, etc. to change the angle and lighting. Different liquids have been experimented with and are currently used, including water, alcohol, baby oil, propylene glycol, and glycerine. Some institutes also read whole otoliths fixed in clear resin between two glass slides. This, however, deprives readers of manipulating otoliths under the microscope to find the optimum angle for interpretation. Otoliths are generally observed under a microscope using reflected light against a dark background. Some readers have remarked that immersion in liquid for up to 48 h (commonly 24 h) prior to reading helps to "clear" the otolith, making age estimation easier. This clearing effect is noted to vary with the length of immersion and the type of liquid used. However, other readers have noted that clarity actually reduces with prolonged immersion and that underageing can result. Species that are regularly age estimated from whole otoliths include megrim (*Lepidorhombus whiffiagonis*), dab (*Limanda limanda*), plaice (*Pleuronectes platessa*), and witch flounder (*Glyptocephalus cynoglossus*).

Procedure

The otoliths are generally stored in paper packets, envelopes, or plastic trays, preferably with the two sagitta otoliths from each fish. Otoliths are removed from the packet with forceps and placed on the microscope stage in a small black dish filled with a liquid. The otolith can be manipulated using the forceps to the ideal position for estimating the age, which will generally be with the concave (distal) side uppermost. Some tissue may remain on the otolith which may appear as a brown residue on the surface (Figure 3.2). This can be removed carefully using the forceps.

The otolith is viewed using reflected light under low-power magnification. Sometimes, a blue filter is used in the path of the beam of light to remove the yellow cast from the tungsten lamp and to aid interpretation of the otolith. The general rule is that the magnification should be such that the whole otolith is visible through the eyepieces at all times because this allows rings to be followed around the otolith. The otolith can be turned, flipped over, and freely rotated using the forceps to provide the reader with many angles of view.

If there are two otoliths from the same fish in the packet, the second otolith can be viewed under the microscope to verify what the reader has seen on the first otolith or provide clarification if the first otolith proved difficult to interpret. As a general rule, the otolith with the asymmetrical nucleus should be viewed first because it provides the longest axis of growth and, therefore, should provide the clearest rings. This is not always the case, however, so caution should be used.

In some instances, otoliths are aged using photographs, with the possibility of routinely marking each image using Photoshop for later reference, rather than using the physical calcified structures. However, using only pictures without the associated physical specimens for reference requires high-quality photographs and is not recommended.

Figure 3.1. Otoliths from a 14-year-old flounder caught in June, showing how easy it is to misinterpret whole otoliths when compared with stained sections. The “cliff-edge effect”, where the axis of growth changes in older fish, cannot usually be seen in the whole otolith, leading to underestimation of age. This fish was aged as part of an exchange, and no reader gave an age greater than 9 from the whole otolith. When it was sectioned and stained, the true age was much clearer.

Figure 3.2. A megrim (*Lepidorhombus whiffiagonis*) otolith viewed whole in water with attached membrane visible at the top.

3.2.2 Break-and-burn method

In this method, the whole otolith is broken transversally across its nucleus, and the broken surface is gently burned in a small flame. The flame has traditionally been from a spirit lamp, but in more recent times, tea-lights have been used due to their low temperature flame, ready availability, and built-in metallic casing for safety. In this method, the annual protein bands are burned, producing a thin brownish–black line at the end of each translucent zone (Figure 3.3). This method is commonly used for species such as plaice, common sole, lemon sole, and megrim.

Procedure

The margin between the opaque and translucent bands becomes dark, sometimes black after burning, making it easier to identify and count the annual rings. Often, a large number of very thin, unclear lines can be seen within the area bounded by two strong darker bands. These are not annuli and should be discounted. If the flame is not applied long enough or if insufficient heat is used, the bands will not show up. If the otolith is burnt for too long or using a flame that is too hot, the otolith will disintegrate.

Once burnt, the otolith section is then mounted on a piece of adhesive gum on the microscope stage, and the burnt surface is viewed using reflected light. A thin layer of oil or water is used to clear the surface and aid in ring identification. Water is recommended because the result is similar to oil; if the otolith then needs more burning, it can still be done, whereas with oil, further burning is impossible. This method requires practice, and inexperienced readers can produce quite variable burning, making age estimation more difficult.

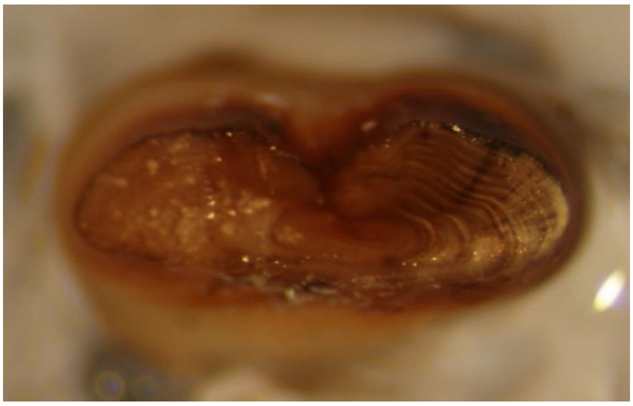


Figure 3.3. A broken and burnt lemon sole (*Microstomus kitt*) otolith.

3.2.3 Sectioned otolith method

Otoliths are embedded in either black or clear resin. A thin slice is then cut transversally through the nucleus, producing thin strips of resin containing several otoliths. The strip of otoliths is read with either reflected or transmitted light, or more likely a combination of both. The advantages of this method are that reading time is reduced, otoliths can easily be viewed in relation to each other, and different lighting techniques can give better overall understanding of ring patterns. Great care must be taken during the sectioning process to ensure that the centre of the nucleus is cut, because a cut to the side of the nucleus could lead to misinterpretation of later rings, and missing the nucleus altogether will result in underageing by one or more years. Many flatfish stocks are read in this way, although some also have the additional process of staining after the sectioning.

Procedure

The otoliths in this method are presented on very thin, narrow strips of cured resin. These strips are very fragile, so great care is needed when handling and storing them. The strips may be mounted on a glass slide and covered with a clear resin, and a glass coverslip is applied over the top, providing a stable, robust storage method. The reader places the strip on the microscope stage and reads from left to right on each strip. Either reflected (Figure 3.4) or transmitted light can be used to age estimate the otoliths. Transmitted light shone from underneath the strip will have the effect of making the opaque zones dark and the translucent zones bright, a reverse of what is seen under reflected light.

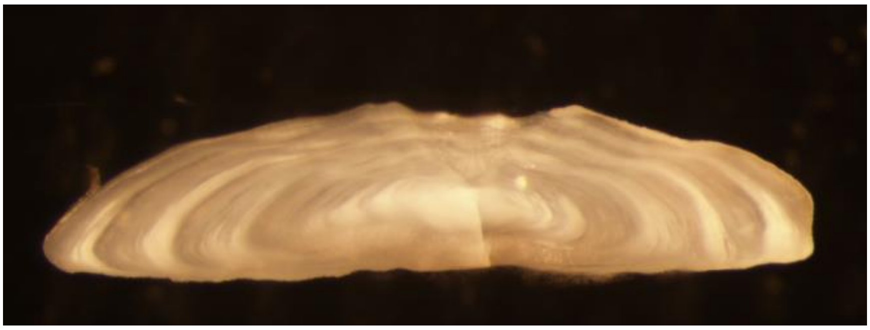


Figure 3.4. A sectioned plaice *(Pleuronectes platessa)* otolith viewed under reflected light.

3.2.4 Sectioned and stained

The process for sectioned and stained otoliths is the same as for sectioned otoliths, with the addition of a stain being applied to the otolith strips after the section has been taken (Figure 3.5). Neutral red stain is often used, although others have been used for specific projects and experiments. Strips are stained for 5–30 min depending on species and stock. The section is then rinsed in tap water and placed in a fume cupboard to dry. During the staining process, a decalcification takes place on the surface of the otolith and the protein bands are stained dark pink or red, making interpretation of the otolith structure easier in a similar way that darkening the protein bands does with a flame when using the break-and-burn method. The stained sections are then viewed under a microscope, using either transmitted or reflected light or, more likely, a combination of both. Common sole and dab are the main species for which this technique is used. The technique is described in detail by Easey and Millner (2008).

The method for reading sectioned and stained otoliths is the same as identified for sectioned otoliths above.

Figure 3.5. A sectioned and stained common sole *(Solea solea)* otolith.

3.3 Age estimation problems and features of specific flatfish stocks

The problems and descriptive features mentioned in this section are derived from the age estimation manuals supplied by participating institutes.

3.3.1 Plaice (*Pleuronectes platessa*)

Most plaice stocks have few issues in the age estimation process. However, fish from the northern North Sea have a much slower growth rate than more southerly fish and exhibit split rings as well as narrow annuli.

There can be considerable variation in the size of the first year's growth. This can cause problems when sectioning the otoliths because a very small nucleus can occasionally be missed by the blade, with the result that the otolith seems to lack a nucleus when viewed, making accurate age estimation almost impossible.

Plaice otoliths are prone to split rings, and the reader should gauge whether inclusion of a split as a true age ring maintains the integrity of the normal growth of the otolith with the age of the fish. A split is often repeated each year, and this is usually seen at the same position between the two annuli each time.

Normally, a plaice otolith shows a good amount of growth in year 1, the largest growth in year 2, and then diminishing growth in subsequent years. At around age 4 or 5, the annual growth becomes uniform. The new annulus ring (the opaque zone) is formed once the feeding season starts and generally occurs around March or April (plaice in ICES divisions 3.a and 7.e and in Subarea 4; Figure 3.6).

Younger fish tend to begin laying down the opaque zone of new growth earlier than older fish.

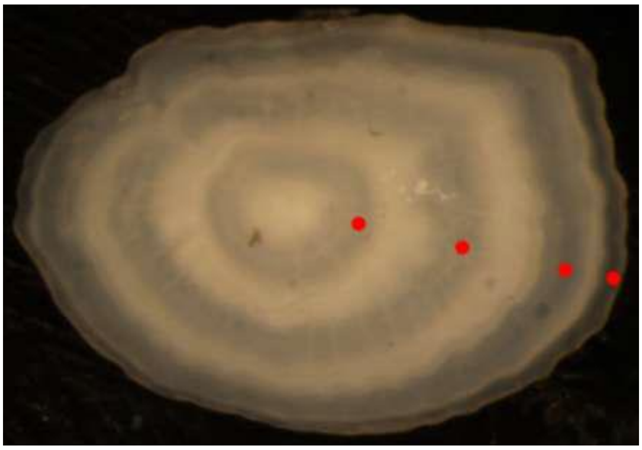


Figure 3.6. Whole plaice otolith (estimated age: 4, month of capture: April – western English Channel). A straightforward fish to age estimate, having a small nucleus with two strong and a third, smaller opaque growth zones. It is likely that the third, smaller opaque zone coincides with the first maturity of this fish. An opaque edge can just be seen forming on the edge of the otolith as the next summers' growth begins after spawning in the spring.

3.3.2 Sole (*Solea solea*)

Growth rates vary widely between sexes. Spawning time also varies among areas, and this affects the timing of the new "annulus" ring being laid down. This species can reach ages of ca. 40 years and as a result, high-powered optics and eyepieces are necessary to age otoliths accurately because the stained rings can be very close together.

Sole otoliths from different sea areas absorb the red stain differently. Those otoliths that absorb the most stain generally require the application of a drop of water over the surface of the otolith. This can leech out any excess stain and make the otolith easier to read. Sole otoliths on slides should be handled with care because they are quite delicate structures (Figure 3.7).

Otoliths on slides should be placed on the microscope initially and viewed using reflected light and 16× magnification eyepieces.

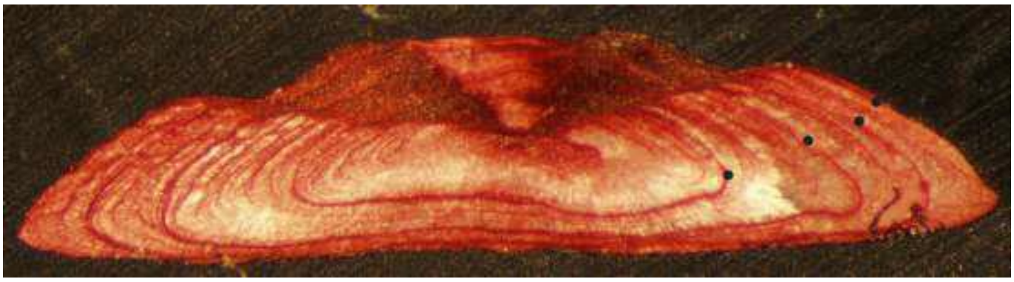


Figure 3.7. Sole otolith (estimated age: 4, month of capture: April – location unknown). A wide nucleus and first growth zone can be seen, with a slowing-down in growth in the final year.

Sole otoliths are prone to split or false rings (Ian Holmes, pers. comm.), and the reader should gauge whether inclusion of a split as a true age ring maintains the integrity of the normal growth of the otolith, considering the age of the fish. Often, a split is repeated each year, and this is often seen at the same position between the two annuli each time. Splits occur more often in fish that live in sea areas, with less variation in water temperature between seasons (Ian Holmes, pers. comm.). Generally, this is more likely to be the case the farther south the fish was living. Readers should be aware that, depending on the width of the section, the nucleus can be clearly visible on one side, but can sometimes be missing on the other. It must be remembered that when the nucleus is visible on both sides of the section, it will differ in size, depending on which side is viewed.

A sole otolith normally shows a reasonable amount of growth in year 1, the largest growth in year 2, and then diminishing growth in subsequent years. At ca. age 4 or 5, annual growth becomes uniform. The new annulus is formed once the feeding season begins, generally around April or May for ICES Division 7.e sole. When this new annulus first becomes visible on the otolith, it will not be seen entirely around the edge of the otolith, but only at the extremes of the otolith at the dorsal and ventral edges.

Younger fish tend to lay down the beginning of the opaque zone much earlier than older fish.

3.3.3 Turbot (*Scophthalmus maximus*) and brill (*Scophthalmus rhombus*)

Turbot and brill otoliths usually have a large nucleus and exhibit regular, strong opaque growth in the first two years. Thereafter, growth slows, and opaque zones narrow considerably. Due to the prolonged spawning season, nucleus size can be variable (Figure 3.8). The otolith takes the neutral red stain well, and good definition of annuli can be seen.

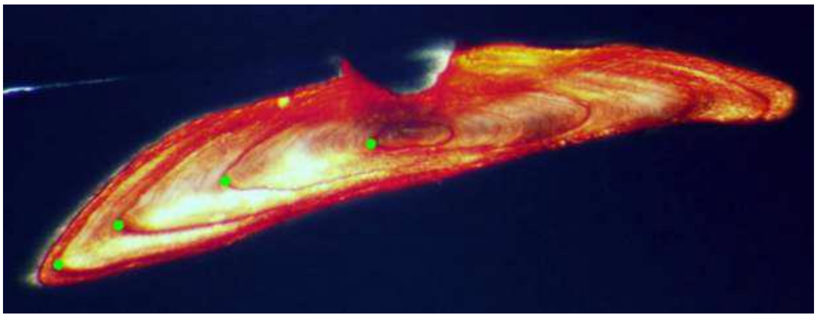


Figure 3.8. Preparation of a turbot otolith (estimated age: 4, TL: 48 cm, sex: male, month of capture: December).

3.3.4 Lemon sole (*Microstomus kitt*)

The otoliths of lemon sole are some of the smallest that are routinely aged. This poses difficulties in the breaking of the otolith prior to burning, and also in the determination of the annual rings because they are narrower than for many other species. Care should be taken when considering the first ring. Lemon sole often lay a false ring which is more circular in shape than the usual oval pattern of a true ring. In addition, lemon sole sometimes lay a "shadow ring", a false ring deposited soon after the true ring (Joanne Smith, pers. comm.), which is much fainter than a true ring (Figure 3.9).

Problems have been noted with the nucleus due to the extended spawning season, leading to a range of sizes of nucleus (or first zone). Some fish appear to have a small, almost circular first hyaline ring, whilst others show a more typically shaped first hyaline ring surrounding a larger nucleus. Lemon sole otoliths seem to fall in two main groups: one with a small, circular first hyaline ring (assumed to be a fish spawned later in the season), and the other with a larger first hyaline zone which contains the opaque "bump" followed by more opaque growth (possibly from fish spawned earlier in the season). The circular "bump" in the centre is considered to be part of the first year's growth (i.e. age 0) that may be linked to some stage in metamorphosis rather than being a full year.

Differences have been found in the formation of annual rings in fish from different sea areas. Zone formation in some areas can be quite blurred, whereas in others, the zones are very well defined and more easily read. It is theorized that this could be caused by different feeding patterns.

The otoliths from juvenile (ages 0–4) female fish can be read whole in water, while for most of the older female and all of the male ones, burning is necessary to establish a reliable age. The formation of the rings is a lot clearer when they are burnt. The burnt otoliths are read mounted in plasticine, either under water or brushed with water. When using oil, the glare from the reflected light impedes the interpretation of the rings. Burning the otoliths for 3–4 s seems quite sufficient for clear ring definition; any additional burning will make them crumble.

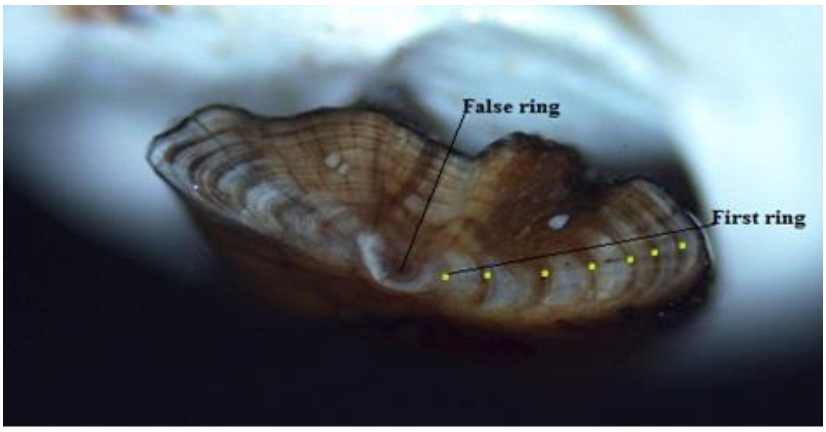


Figure 3.9. Otolith from lemon sole (estimated age: 7, TL: 33 cm, sex: female, month of capture: September – North Sea). This species often lays false first rings and shadow rings; these should not be counted as true rings when carrying out age estimations.

3.3.5 Megrim (*Lepidorhombus whiffiagonis*) and four-spot megrim (*Lepidorhombus boscii*)

Megrim can live to over 16 years of age, while four-spot megrim are shorter lived and do not grow to the same size. Otoliths from both species are routinely read whole. Split rings are relatively common, but pose no significant issue in interpretation. Otoliths from larger fish can become thick and difficult to interpret when whole (Figure 3.10).

When possible, both otoliths should be viewed because the ring structure is not always clear in all parts of the otolith. The asymmetrical otolith generally provides the best chance of accurate age estimation because it has the longest axis of growth. However, this should not always be relied upon since the longest axis of growth is not always the "best" area on the otolith to estimate the age. Splits can, in fact, be more prevalent in this area of the otolith. The opaque zone of new growth can be observed to form earlier in fish from more southerly areas. The opaque zone also forms earlier in younger fish, and is wider and faster growing than in older fish.

New growth on the edge is not uniform, and big differences are observed in different parts of the otolith, making it vital that the otolith is viewed as a whole and that age is estimated from several axes. An exchange of megrim whole-otolith images took place between 2010 and January 2011 with the participation of seven institutes around Europe. The overall percentage of agreement was relatively low and decreased with increased modal age, reflecting the difficulty of the interpretation of megrim otoliths.

Whole otoliths need to be studied carefully as annual rings close to the edge can be packed closely together, making them harder to interpret as the otolith thickens. It was thus recommended that alternative methods of reading (e.g. breaking and burning) should be investigated, especially for ages 6+. A new exchange using the break-and-burn method should be carried out.

Megrim usually mature at age 2 for males and at age 3 for females (Mark Etherton, pers. comm.). After this time, growth slows, which can be quite dramatic, particularly in fish from deeper waters. Since growth rates vary not only between sexes, but also vary quite widely geographically (Mark Etherton, pers. comm.), care should be taken with age estimation samples that may stem from trips spread over a wide area as growth rates could then be extremely problematic to interpret.

Figure 3.10. Otolith from megrim (estimated age: 6, TL: 32 cm). This fish is 6 years old and shows a good first translucent winter ring, followed by a split-opaque summer growth.

3.3.6 Flounder (*Platichthys flesus*)

The diameter of the first annulus (first ring) varies considerably. One explanation is that settlement dates vary between individual fish. Early settlers will experience a longer growth season than late settlers. The length of the first summer growth may affect the width of the first winter growth (Figure 3.11).

Another problem is the infrequent visibility of a very small innermost ring. This ring has sometimes been labelled as the "metamorphosis" ring and is attributed to the transfer to the demersal stage of the life cycle.

The age should be estimated from more than one axis of reading.

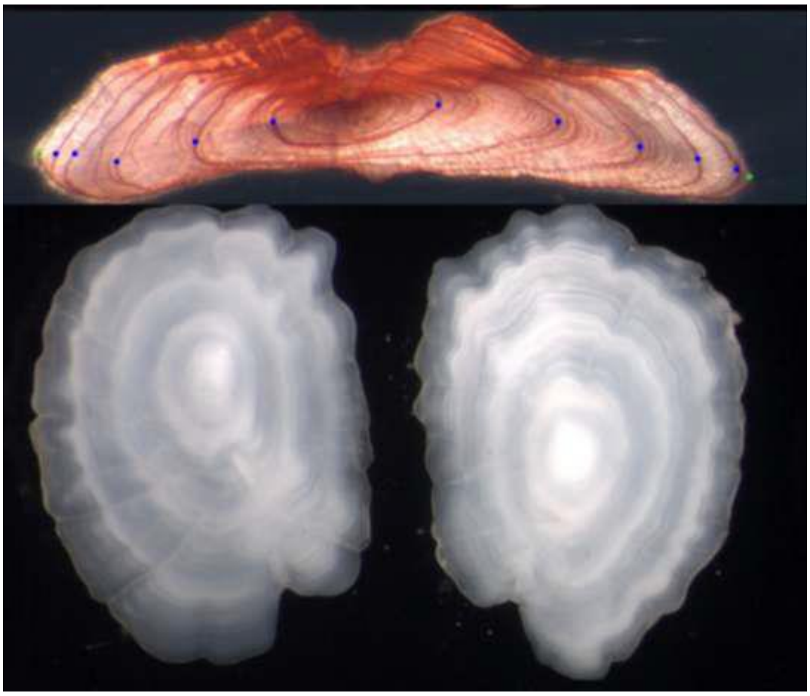


Figure 3.11. Flounder otolith (estimated age: 6, month of capture: June – location unknown). The stained section can clearly be seen to be age 6, whereas the whole otoliths can give ages of 4, 5, or 6, depending on location of the interpretation on the otolith or choice of otolith. This highlights the advantage of sectioned and stained otoliths for the age estimation of flounder.

3.3.7 Witch flounder (*Glyptocephalus cynoglossus*)

Performing age estimation on witch flounder can be quite challenging. There are three main reasons for this:

1. The thickness of the nucleus.
2. Older fish often show a broad hyaline edge in which several rings can be disguised.
3. If the otoliths are dry, the hyaline rings are not discernable.

Several techniques have been attempted to find the optimal one, including grinding the otolith whole, sectioning with or without staining, burning and breaking, as well as reading the otolith whole and wet preferably straight after removal from the fish. The best result was obtained by using a combination of two techniques, namely, reading the otoliths right after the removal from the fish and, if need be, grinding them (Figure 3.12).

The core of the otolith is asymmetrical (as in all flatfish), and usually the rings are easier to make out on the otolith with the central nucleus.

As the core of the otolith is relatively thick and the first ring is sometimes difficult to discern, grinding has proved to be useful in revealing the inner ring. This inner ring has been verified by collecting witch flounder of the 0-group and comparing the distance from nucleus to edge/first ring.

When collecting otoliths from witch flounder, it is recommended to place them in containers with water where they can be kept moist. If the otoliths are stored dry, one can try soaking the whole otoliths for a day in a 0.9% saline solution or in freshwater. This method gives satisfying results, although one should bear in mind that if the otoliths are kept in water for a long time, grinding the surface is usually needed.

Witch flounder otoliths are best read directly after removal from the fish.

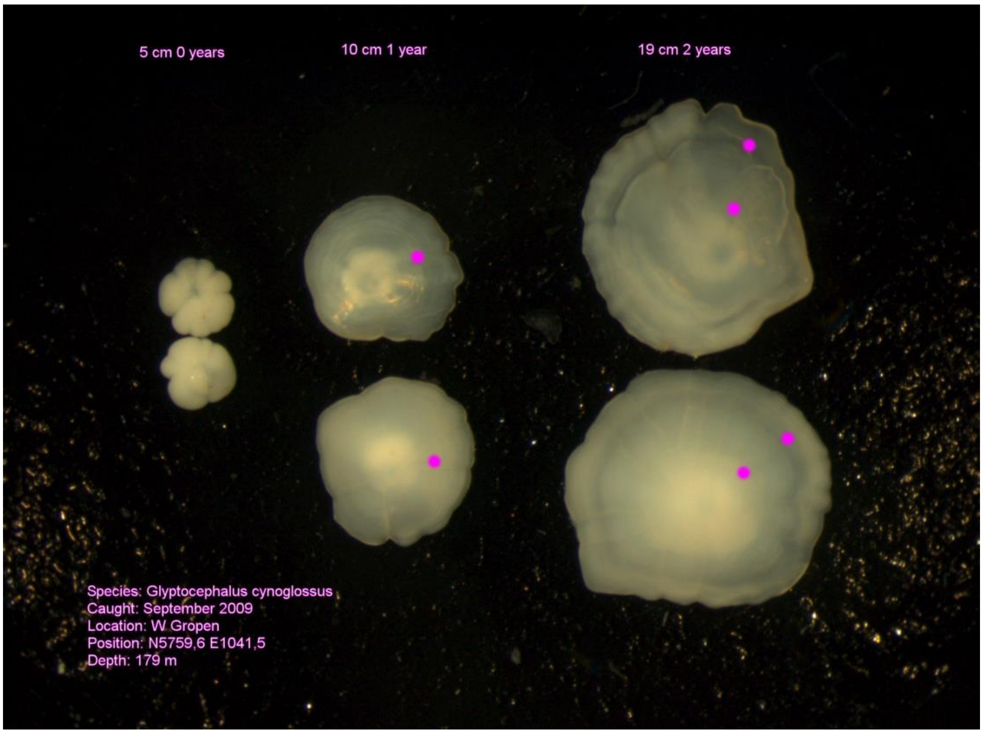


Figure 3.12. Whole witch flounder otoliths of different ages (0, 1, and 2), all caught during September 2009.

3.3.8 Dab (*Limanda limanda*)

The first ring causes problems with some readers, with widely different growth observed in the first year. This may be due to the prolonged spawning of dab and differing growth rates in different sea areas (Figure 3.13).

It is possible for very old individuals to exhibit the cliff-edge effect in the otoliths. This occurs when growth changes from the x-axis to the y-axis, with new growth beginning to manifest itself as a thickening rather than a widening of the otolith. This can lead to difficulties with interpretation and may potentially result in underageing.

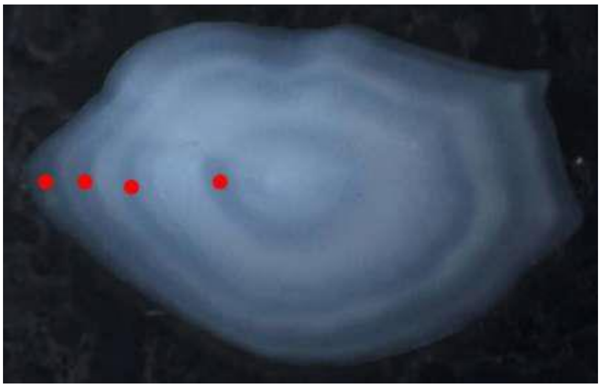


Figure 3.13. Dab otolith (estimated age: 4, TL: 22 cm, sex: female, month of capture: April – location unknown). A relatively small nucleus is followed by a strong first summer of growth. Subsequent growth is slower.

3.3.9 Greenland halibut (*Reinhardtius hippoglossoide*)

Growth is slow, and interpretation of the otoliths can be challenging. A number of reading methods have been investigated, with sectioned otoliths considered to offer the best chance of accurate and precise age estimation (ICES, 2011b).

3.4 Age validation case studies

Several studies have used marginal increment analysis (MIA) to validate the age estimation methodology, including Smith (2014) for lemon sole in the North Sea, English Channel, and Celtic Sea, and Rodriguez and Iglesias (1985) and Landa and Piñeiro (2000) for megrim in Subarea 7 and in divisions 8.a–c and 9.a. Etherton (2015) studied the results of mark–recapture experiments on plaice and sole in the 1970s and 1980s. Table 3.4 provides a summary of the validation studies performed on flatfish species.

Table 3.4. Summary of age validation methodologies, modified from Campana (2001), with methods used for flatfish.

Method	Annual/DGI	Age	Advantages	Limitations	Flatfish for which this technique has been employed	References
Released marked fish	Annual and DGI	All	Validates absolute age and periodicity	Source of fish at known age. Recaptures of old fish are null.	Sole, plaice	Etherton (2015)
Mark–recapture chemically tagged fish	Annual and DGI	All	Validates periodicity post release	Low recapture rates, some markers may affect survival.	N/A	Cappo *et al*. (2000); Quinn *et al*. (1991)
Captive rearing from batch	Annual and DGI		Validates absolute age and periodicity	Differences with wild fish.	Yellowtail flounder	Dwyer *et al*. (2003)
Microstructure	Annual	1 year	Validation of first year	Daily periodicity assumed.	N/A	Lee and Byun (1996)
MLA	Annual and DGI	0–5 years	Validation of ages 1–2	No overlapping length modes. No length-based migrations.	Yellowtail flounder	Dwyer *et al*. (2003)
Marginal increment analyses	Annual	All	Validates periodicity	Not straightforward in older/slower growing individuals. Needs adequate sample sizes by month.	Lemon sole	Smith (2014)
Daily increment analyses	DGI	All	Validates daily formation	As above.	Sole	Lagardere and Troadec (1997)
Radiochemical dating	Annual	Plus 5 years	Validates absolute age	Can only distinguish between divergent estimates.	Sole, halibut	Kalish (1993)
Micromilling	DGI and annual		Validates periodicity	Specialized equipment and skills needed. Once drilled, otoliths cannot be used for age estimation.	Plaice	Geffen (2012)

3.4.1 Lemon sole (marginal increment analysis)

Calculating the age of lemon sole has always presented otolith readers with difficulty. False first rings and shadows are frequently observed and can lead to over/underestimation of ages if misinterpreted.

Smith (2014) carried out marginal increment analysis on the otoliths from lemon sole collected from the North Sea, English Channel, and Celtic Sea. No incremental growth was observed between January and March for any size class. Incremental growth generally commenced earlier in younger fish (April for ages 3–5) and later in older fish (May for ages 6–10, June for ages 11+). The rate of growth increased gradually throughout the year, with completed increment growth by most fish in December. The results clearly demonstrate that a single annulus represents one year of growth.

3.4.2 Plaice and sole (mark–recapture)

In Etherton (2015), plaice and sole were physically tagged and released in the North Sea, subsequently recaptured, and analysed. Specimens were selected to conform to a set of criteria:

- Fish were tagged at a size small enough to ensure they were all the same age.
- All the samples came from the same geographical area and the same month.
- Each studied fish was at liberty for at least two years after release.

For each fish, an age was calculated using the "known" age at release and the date of recapture. Readers then assigned an age to them with knowledge only of the final length of the fish and the month of recapture. The assigned ages were then compared to the "known" age to determine the accuracy of the age estimations. For sole, there was an almost perfect match in the ages among all four readers and the "known" age, thus validating the methodology used (section and stain). For plaice, there was a variable result. Two readers who were accustomed to the method used in the study (sections) obtained good results, while the other two readers who were familiar with the break-and-burn method had poor results. It was thus concluded that employing the best method for each species/stock is vital to a successful age estimation programme. Furthermore, the familiarity of the method to the reader is equally important. Being accustomed to a preparation technique strongly influences both precision and accuracy (ICES, 2011c).

4 Small- and medium-sized pelagic species

Begoña Villamor, Pierluigi Carbonara, Pablo Abaunza, Naroa Aldanondo, Loes Bolle, Gertrud Delfs, María Teresa García Santamaría, Tomas Gröhsler, Carmen Hernandez, Alba Jurado-Ruzafa, Maria Rosario Navarro, Eduardo Soares, Fernando Ramos, Isabel Riveiro, Norbert Rohlf, Jorge Tornero, Jens Ulleweit, Andres Uriarte, Lotte Worsøe Clausen and Francesca Vitale

4.1 Introduction

This chapter presents a summary of the age estimation procedures used in European waters (Atlantic and Mediterranean areas) for some of the main commercial small- and medium-sized pelagic species: anchovy *Engraulis encrasicolus*, sardine *Sardina pilchardus*, herring *Clupea harengus*, sprat *Sprattus sprattus*, Atlantic mackerel *Scomber scombrus*, chub mackerel *Scomber colias*, horse mackerel *Trachurus trachurus*, Mediterranean horse mackerel *Trachurus mediterraneus* and blue jack mackerel *(Trachurus picturatus)*. It provides information about the age estimation criteria and interpretation difficulties. A summary of the information related to the age accuracy, validation, and corroboration of each species is also presented, as well as that related to the age precision, quality control, and verification. The procedures included in this chapter are derived from the age estimation protocols of some European institutes and from the cooperation among institutions through the ICES exchanges and workshops about age interpretation; additional procedures can be found in relevant literature.

4.2 Summary of age estimation methodologies

Considerable efforts have been made by international committees to standardize age interpretation in European waters. During these exchanges and workshops, the samples used are rarely validated; therefore, the "true age" is not known. In this way, the calibrations demonstrate the precision of age estimation among readers, but not the accuracy (Secor *et al*., 1995; Panfili *et al*., 2002). More than 30 reports on small- and medium-sized pelagic species in ICES and GFCM waters are available in the ICES Data Quality Assurance Repository (ICES, 2018a). A summary of the results from the latest workshops or exchanges for estimation of annual age (ICES, 2008b, 2010b, 2010c, 2011c, 2011d, 2014b, 2015b, 2016a, 2017c, 2018c) and daily age (ICES, 2013b, 2018c; Villamor *et al*., 2013) can be found in Tables 4.1 and 4.2.

Table 4.1. Summary of the last annual growth workshops and exchanges by species. GSA = Geographical Sub-Area of the General Fisheries Commission for the Mediterranean (GFCM).

Species	WK/ Exchange	Area	Mode of preparation	Agreement (%)*	*CV**	APE
Anchovy	Exchange 2014/ WKARA2 (ICES, 2016b)	Bay of Biscay (ane.27.8)	Whole otolith, in resin	74.3/80.8/90.9	45.1/22.4/11.4	-
		English Channel (ane.27.7)		66.7/80.4/-	127.6/73.9/-	-
		Gulf of Cadiz and Portuguese coast (ane.27.9a)		68.5/76.4/75.7	49.1/34.7/33.0	-
		Alboran Sea (GSA 01)		58.9/63.5	58.7/71.1	-
		Western Mediterranean Sea (GSA 06)		60.9/59.6/-	49.9/59.2/-	-
		Gulf of Lion (GSA 07)		73.4/75.1/-	31.3/30.3/-	-
		Southern Tyrrhenian Sea (GSA 10)		62.9/62.0/67.3	67.2/86.7/58.1	-
		Strait of Sicily (GSA 16)		58.5/59.9/85.6	78.7/73.8/11.2	-
		Western Ionian Sea (GSA 19)		61.9/60.2/73.5	60.9/73.3/55.3	-
		Aegean Sea (GSA 22)		70.0/78.3/97.1	55.7/42.8/6.7	-
	Exchange 2018 (ICES, 2018a)**	Bay of Biscay (ane.27.8)	Whole otolith, in resin	71.1/82.9/90.7	41.1/25.6/9.3	
		Strait of Sicily (GSA 16)		56.1/59.2/96.3	58.5/56.5/8.8	
Sardine	WKARAS 2011 (ICES, 2011d)	Bay of Biscay North (Division 8.a)	Whole otolith, in resin	73.1	17.3	-
		Portuguese coast (Division 9.a)		76.5	18.1	-
		Gulf of Cadiz (Division 9.a)		77.4	10.9	-
	Exchange 2018 (ICES, 2018a)**	Northeast Atlantic (divisions 8.a–c and 9.a)		Between 60 and 80	Between 20 and 60	Between 10 and 35
		Mediterranean Sea (GSAs 01, 03, 06, 07, 09, 16, and 22)		Between 60 and 80	Between 40 and 100	Between 30 and 60

Species	WK/ Exchange	Area	Mode of preparation	Agreement (%)*	*CV**	APE
Herring	WKARBH 2008 (ICES, 2008b)	Baltic Sea	Whole otolith, in resin	86.9	6.4	-
	Exchange 2008 (ICES, 2008b)		Stained otolith slices	76.3	8.7	-
	Exchange 2005 (ICES, 2008b)	Atlantic	Whole otolith, in resin	83.3	7.9	-
	Exchange 2016 (ICES, 2017c)	Baltic Sea (Subdivision 26)	Whole otolith, in resin	88–94 (sample S1)	1.9–7.5 (sample S1)	-
				52–85 (samples S2 and S3)	1.9–20 (samples S2 and S3)	-
		Baltic Sea (subdivisions 30 and 32)	Stained otolith slices	87–96 (sample S4)	4.0–8.1 (sample S4)	-
Sprat	WKARBS 2008 (ICES, 2008c)	Baltic Sea	Whole otolith, with nail polish	76.1	17.1	-
	Exchange 2016/WKARSPRAT, 2016 (ICES, 2017d)	Division 3.a	Whole otolith, in resin	68.6/67.8/-	22.8/22.3/-	16.9/16.9/-
		North Sea (divisions 4.b–c)		81.5/81/-	15.4/21.7/-	20.4/16.2/-
		Celtic Sea (divisions 6.a, 7.b, 7.g, and 7.j)		94.9/94.4/-	12.1/12.5/-	7.9/9.3/-
	Exchange 2016 (ICES, 2018a)	North Sea and Celtic Sea	Whole in alcohol	79.6	21.7	16
Mackerel	Exchange 2014 (ICES, 2015b)	Northeast Atlantic (Subarea 2 and divisions 4.a–b, 6.a, and 7.b)	Whole otoliths, fixed in resin/loose submerged in water (images only)	68.2/75.5	15.4/-	
	Exchange 2018 pre-workshop (ICES, 2018a)	Northeast Atlantic (divisions 2.a, 4.b–c, 5.a–b, 7.b, 7.j, 7.d, 8.b–c, and 9.a)		59.4/65.2	37.3/17.6	-
	Exchange 2018 WKARMAC2 (ICES, 2019a)	Northeast Atlantic (divisions 2.a, 4.b–c, 5.a–b, 7.b, 7.j, 7.d, 8.b–c, and 9.a)		66.5/73.2	30.4/16.4	-

Species	WK/ Exchange	Area	Mode of preparation	Agreement (%)*	*CV**	APE
Chub mackerel	Exchange 2015 pre-workshop (ICES; 2016a)	Division 8.c	Whole otoliths fixed in resin (images only)	53.5	27.4	-
		Division 9.a		55.3	22.8	-
		Western Mediterranean Sea (GSA 06)		62.1	35.2	-
	Exchange 2015 WKARCM (ICES; 2016a)	Division 8.c	Whole otoliths, fixed in resin/loose submerged in water (images only)	66.7	36.2	-
		Division 9.a		55.6	37.3	-
		CECAF-Mauritania		60.2	41.6	-
		Western Mediterranean Sea (GSA 06)		65.3	29.3	-
		Ligurian and North Thyrrenian Sea (GSA 09)		46.4	64.6	-
		Southern Adriatic Sea (GSA 18)		68.2	65.8	-
	Exchange 2017 (ICES; 2018a)	Division 8.c		56.6/65.5/-	61.7/24.1/-	-
		Division 9.a		56.8/62.4/-	35.6/31.3/-	-
		CECAF-Canaries		70.3/80.3/-	68.0/24.3/-	-
		Ligurian and North Thyrrenian Sea (GSA 09)		52.4/63.4/-	111-3/67.8/-	-
		Aegean Sea (GSA 22)		64.7/70.5/-	35.3/28.1/-	-
		Northwest Atlantic		51.7/52.1/-	39.6/34.6/-	-

Species	WK/ Exchange	Area	Mode of preparation	Agreement (%)*	*CV**	APE
Horse mackerel (*Trachurus trachurus*)	Exchange/ Workshop 2012 (ICES, 2016c)	Ireland waters (Subarea 7)	Sectioned otolith	36.4	26.9	-
		North of Spain (divisions 8.c and 9.a)		53.2	42.3	-
		Portuguese waters (Division 9.a)		44.7	54.7	-
		South of Spain (Division 9.a South)		43.9	43.8	-
		Western Ireland		46.4	21	-
	Exchange/ Workshop 2015 (ICES, 2015b)	Division 7.d	Sectioned otolith	55.7	16.8	-
		Division 7.h		63.8	25.9	-
		Northern Alboran Sea (GSA 01)	Whole otolith	50.1	69.7	-
		Corsica Island (GSA 08)		44.6	32.1	-
		Corsica Island (GSA 08)	Sectioned otolith	44	28.9	-
	Exchange/ Workshop 2018 (ICES, 2018c)	Division 8.c	Sectioned otolith	50.05	19	
		Division 7.d				
		Division 8.c	Whole otolith	49.45	31.9	
		Division 7.d				
		Ligurian and North Thyrrenian Sea (GSA 09)				

Species	WK/ Exchange	Area	Mode of prepara-tion	Agreement (%)*	CV*	APE
Mediterra-nean horse mackerel (*Trachurus mediterra-neus*)	Exchange 2012	Mediterranean Sea (Italian waters)	Whole otolith	56.6	28.7	-
		North of Spain (divisions 8.c and 9.a)		57.5	30.5	-
	Exchange/ Workshop 2015 (ICES, 2015b)	Division 8.c	Sectioned otolith	39.3	40.2	-
		Division 9.a		41.2	41.7	
		Southern Adri-atic Sea (GSA 18)		53.6	46.7	-
	Exchange/ Workshop 2018 (ICES, 2018c)	Division 8.c	Whole otolith	48.35	66.35	
		Division 9.a				
		Ligurian and North Thyrre-nian Sea (GSA 09)				
Blue jack mackerel (*Trachurus picturatus*)	Exchange/ Workshop 2015 (ICES, 2015b)	Azores	Sectioned otolith	35.3	36	-
		Tenerife		60.1	89.3	
		Southern Adri-atic Sea (GSA 18)		79.3	168.8	-
	Exchange/ Workshop 2018 (ICES, 2018c)	Tenerife	Whole otolith	56.8	69.85	

*All readers/Expert readers/Stock readers.
** Preliminary results.

Table 4.2. Summary of daily growth workshops and exchanges by species.

Species	WK/exchange	Area	Live stage	Mode of preparation	Agreement (%)	*CV*	APE
Anchovy	Exchange 2013/WKMIAS 2013 (ICES, 2013b)	Bay of Biscay (Subarea 8)	Larvae & juveniles	Whole and sectioned otolith	-	15.3	10.4
		Western Mediterranean	Larvae	Whole otolith	-	18.6	14.1
		Strait of Sicily	Juveniles	Sectioned otolith	-	34.9	23
		Adriatic Sea	Larvae & juveniles	Whole and sectioned otolith	-	24	18.9
		North Aegean Sea	Larvae & juveniles	Whole and sectioned otolith	-	9	7.8
		Overall	Larvae & juveniles	Whole and sectioned otolith	-	18.9	13.3
Sardine	Exchange 2013/WKMIAS 2013 (ICES, 2013b)	Bay of Biscay (Subarea 8)	Larvae	Whole otolith	-	14.5	10.7
		Atlantic Iberian waters	Juveniles	Sectioned otolith	-	18	13.5
		Western Mediterranean	Larvae	Whole otolith	-	11.7	8.6
		Adriatic Sea	Larvae & juveniles	Whole and sectioned otolith	-	14.3	11.6
		North Aegean Sea	Larvae & juveniles	Whole and sectioned otolith	-	9.4	7.4
		Aquaculture	Juveniles	Sectioned otolith	-	24.6	12.2
		Overall	Larvae & juveniles	Whole and sectioned otolith	-	13.7	10.6
Herring	Exchange 2018 (ICES, 2018a)	North Sea and Western Baltic	Juveniles	ground and polished	82		

4.3 Summary of general age estimation methods and problems

4.3.1 Anchovy (*Engraulis encrasicolus*)

Annual age estimation criteria

The procedure for age estimation of European anchovy is the one adopted in WKARA 2009 (ICES, 2010d) and prior workshops according to the available validations by Uriarte *et al.* (2007, 2016), Aldanondo *et al.* (2016), and ICES (2010d). Anchovy otoliths are presently aged whole, immersed in a clarifying medium (seawater, glycerin, alcohol) with reflected light against a black background. The otoliths are also read mounted in individual cavities on black plastic slides, using a transparent resin as glue. For analysis the otolith is placed with the distal surface face up and the proximal surface (*sulcus acusticus*) face down. The method is based first on the interpretation of otoliths according to prior biological knowledge on the annual growth pattern of the anchovy otoliths, on the seasonal growth of otolith edge by age, and on the most typical checks. The selected interpretation would be the one that best complies with prior biological knowledge. According to the interpretation achieved, the number of past winter (translucent) zones is used to apply the rules for allocating ages according to the assumed birthdate for the population being studied. Base knowledge on the anchovy growth pattern is described in detail in the latest workshop report (WKARA2 – ICES, 2016b). A set of opaque and hyaline zones corresponds to an annual growth zone (annulus), as reported in Uriarte *et al.* (2016) for anchovy in the Bay of Biscay (Figure 4.1, top panel) and in ICES (2010d) for anchovy in Alboran Sea (Figure 4.1, bottom panel). The younger the fish, the earlier in the year the opaque growth is resumed; in spring, the edge of age 1 otoliths is typically opaque, while it is hyaline for older ages. Readers should *a priori* be aware of the expected type of edge, monthly and by age.

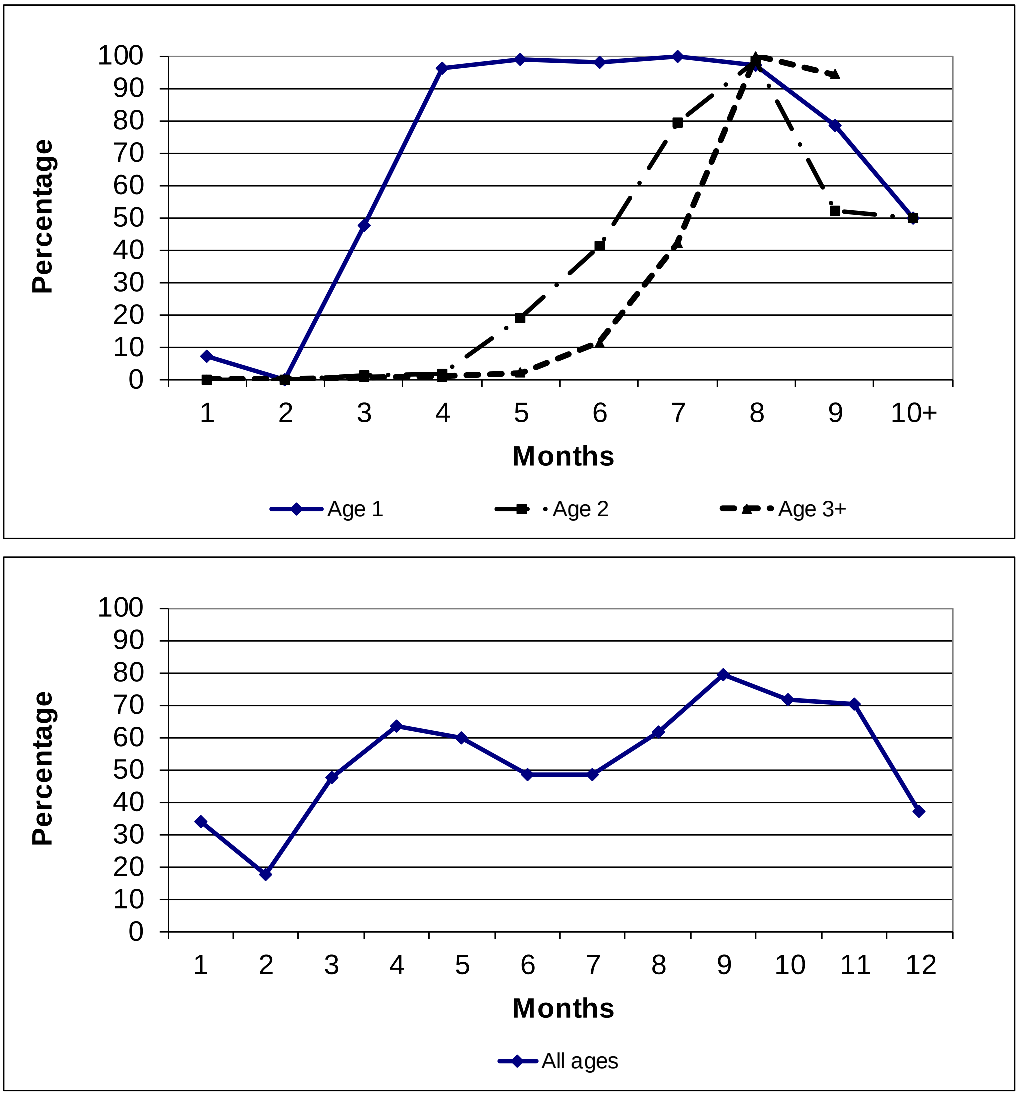


Figure 4.1. Percentages, by age, of otoliths from the Bay of Biscay showing an opaque edge formation (Uriarte *et al.*, 2016 – top panel) and in the Alboran Sea (ICES, 2010d – bottom panel).

The date of birth is conventionally assumed to be 1 January in all Atlantic areas and in the Gulf of Lion, and the fish is assigned to a year class on this basis. In the Mediterranean Sea, the date of birth is 1 June or 1 July, based on reproductive traits such as gonad development cycle and the gonado-somatic index followed throughout the year.

The age estimation scheme is shown in Figure 4.2 and described in detail in WKARA2 (ICES, 2016c).

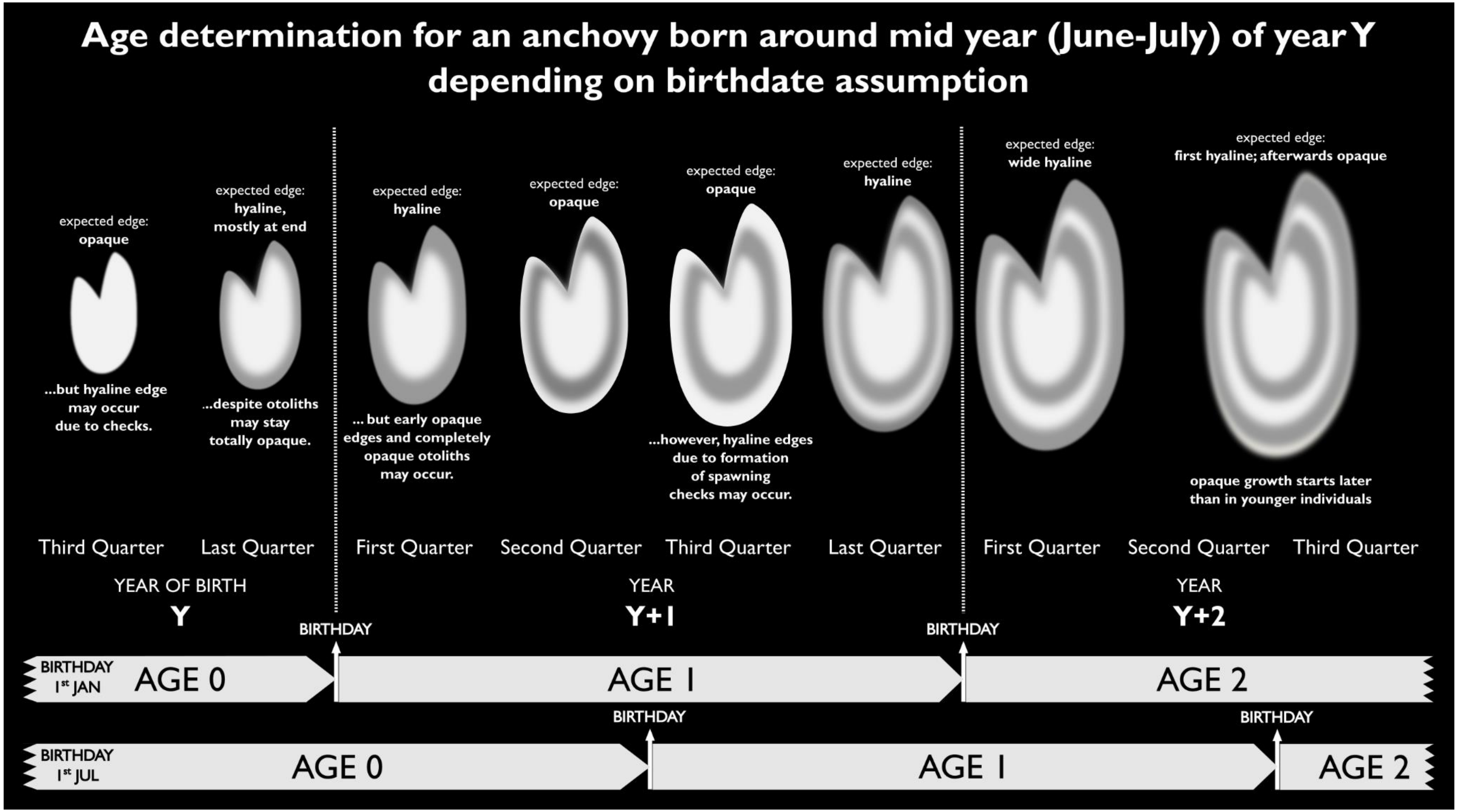


Figure 4.2. Synoptic representation of the anchovy otolith development in time and the different age allocation according to the two conventional birth dates at 1st of January and at 1st of July.

Annual age-interpretation difficulties

Interpretation difficulties are often explained by: (i) the first annulus position; (ii) the otolith edge identification (opaque or hyaline); or (iii) the presence of false growth increments (checks). In general, anchovy otoliths are not difficult to interpret, although there are geographic areas where growth pattern structures are more difficult. However, across areas/stocks, the main problem reported is to determine the position of the true first annual ring. Since anchovy is a short-lived species, faulty allocation to year class will influence the estimate and quantification of the recruitment. High precision in estimating recruitment is important in determining the status of these stocks, since the stocks are mainly composed of juveniles and 1-year-old fish.

Except for anchovy in the Bay of Biscay, precision in age estimation for anchovy is quite low (Table 4.1). This difference may be related to the fact that otoliths from the Bay of Biscay appear to have the clearest structures with a high percentage of easily readable otoliths (ICES, 2010d, 2016b). However, it is also worth noting that, contrary to other areas/stocks, exchanges and workshops have been conducted in this area since 1990, and there are sufficient criteria for the interpretation of anchovy otoliths. In the same time period, only one or two exchanges and workshops have taken place in the other areas.

In terms of structures, a well-defined true hyaline ring is determined by the following features (ICES, 2010d):

1. it must be continuous all around the otolith;
2. it remains clearly visible even when the focus changes;
3. the relative distances between adjacent rings is proportional to the expected growth pattern in the otoliths from that particular area.

The first annulus (opaque and transparent ring) consists of a wider opaque zone (sometimes interrupted by a false ring) compared to the successive annuli. Following this, the distances between the rings decrease with age (Figure 4.3).

Around the nucleus before the first winter ring (ICES, 2010d, 2016b), a false ring is frequently observed at a distance of about 0.8 mm to the core. This ring appears less marked than the true annual zones; it is thus fairly easy to omit it from the age estimation (Figure 4.3, panels a, f, and g).

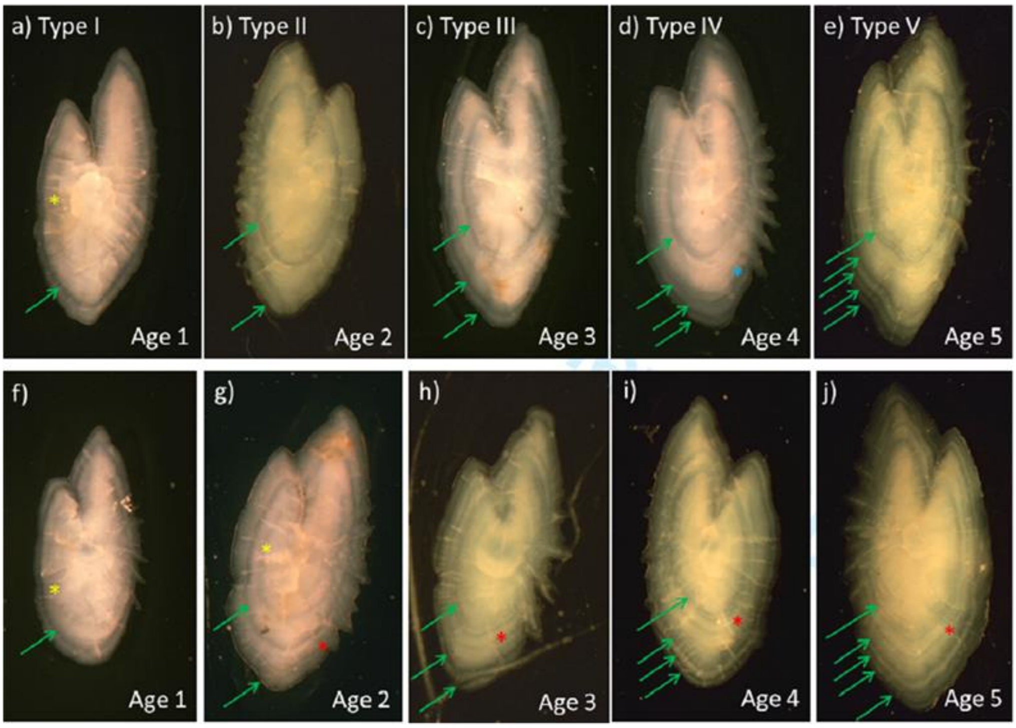


Figure 4.3. [Top panels a–e] Images of typical anchovy otoliths (types I–V) found in spring (May) with increasing numbers of white opaque growth zones: (a) Type I (age-1, caught 29 May 1990); (b) Type II (age-2, caught 31 May 1985); (c) Type III (age-3, caught 23 May 1985); (d) Type IV (age-4, caught 15 May 1986); and (e) Type V (age-5, caught 6 May 1987). [Bottom panels f–j] Images of otoliths showing the most typical checks. (f) age-1 showing check C08 (05 July 1990); (g) age-2 showing checks C08 and C12/15 (22 May 1991); (h) age-3 showing a double first hyaline zone (split ring) and check C12/15 (11 April 1985); (i) age-4 showing a double first hyaline zone (split ring) and check C12/15 (22 May 1986); and (j) age-5 showing check C12/15 (22 April 1987). Arrows indicate successive winter hyaline zones; different coloured stars indicated different checks: yellow, C08; red, C12/15; blue, C18. Scale: the area of the images is 2447 × 4344 µm. (From Uriarte *et al.*, 2016.)

Daily-increment-definition criteria

For daily increment interpretation, two different criteria have been suggested. Using what is commonly referred to as "the group band reading (GBR)" criterion, the reader counts every repetitive cyclic set of growth bands (usually two, but occasionally more) as a single daily increment, assuming that they are subdaily marks. In the other method, known as "individual mark reading (IMR)", each increment, regardless of its appearance, is counted as a single daily increment. According to Cermeño *et al*. (2008), the GBR criterion is the most reliable method for age estimation of European anchovy. For the Bay of Biscay, western Mediterranean, and northern Aegean Sea, it has been agreed to apply the GBR method for anchovy, irrespective of the season and geographical area (Morales-Nin *et al*., 2010; SARDONE, 2010; ICES, 2013b). Contrary to this, no agreement has been reached on interpretation criteria in the Strait of Sicily and Adriatic Sea (ICES, 2013b).

Daily-increment-interpretation difficulties

Diverging counts of daily increments are often rooted in two main disagreements: (i) difficulties in the interpretation of subdaily increments, double structures, or band zones; and (ii) preparation flaws such as unclear images where it is difficult to interpret correctly the daily growth pattern caused by under- or overpolishing, poor image acquisition, or calibration problems. Thus, effort should be put into achieving the best possible images for any reading of daily increments in anchovy (Nava *et al.*, 2018).

4.3.2 Sardine (*Sardina pilchardus*)

Annual age estimation criteria

Sardine age estimation criteria were recommended at the 2011 ICES sardine age estimation workshop in Portugal (ICES, 2011d). The method can be summarized as follows: (i) a set of opaque and hyaline zones corresponds to an annual growth zone (annulus); and (ii) the date of birth is conventionally assumed to be 1 January and the fish is aged on this basis (if an otolith is collected during the first semester, the age corresponds to the number of hyaline zones; if the otolith is collected from a fish caught during the second semester, the hyaline edge is not considered). In cases where the edge is opaque in the first semester, which mostly happens around June when the deposition of the opaque zone has started, the age corresponds to the number of annuli (opaque zone and transparent ring). Estimating age of otoliths with an opaque edge collected in the second semester follows the same rule and is equal to the number of annuli..

A reference diameter of ≈2 mm (radius ≈1 mm) should be used to guide the identification of the first annual ring. This reference should be used in a flexible way, since the diameter of the first annual ring is proportional to the fish growth up to its formation.

Annual age-interpretation difficulties

The main discrepancies in sardine age estimation occur in the identification of the otolith edge type and the first annulus. Two problems related to the edge type were discussed at the 2011 workshop: (i) difficulty in identifying the edge type (hyaline or opaque); and (ii) variation in the seasonality of the edge type.

Also for the sardine, a false ring may be deposited before the first winter at a distance of about 0.5 mm from the core on the postrostrum side (Figure 4.4). The sardine and anchovy otoliths have similar growth patterns, with a big opaque zone in the first annulus. A further characteristic of a true winter ring is that it can be found on the entire perimeter of the otolith, i.e. it can be followed all the way around the outline of the otolith (Figures 4.5 and 4.6).

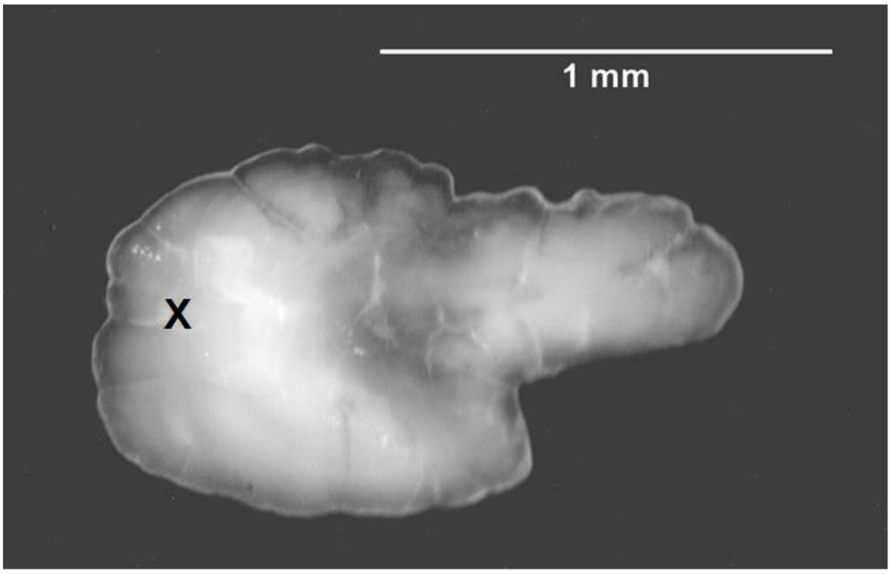


Figure 4.4. Otolith of *S. pilchardus* (TL: 8 cm, sex: F, month of capture: June). X indicates the false ring (Carbonara and Follesa, 2018).

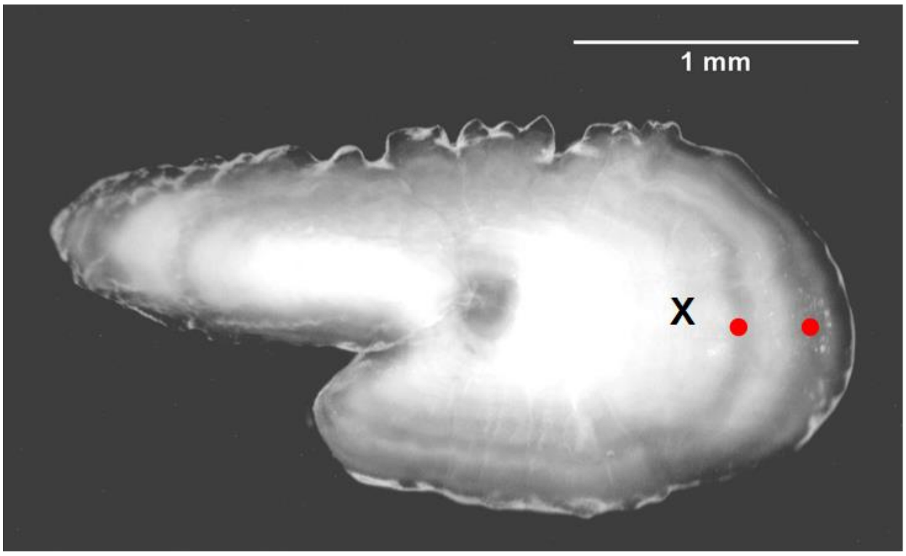


Figure 4.5. Otolith of *S. pilchardus* (TL: 17 cm, sex: F, month of capture: August). The red dots indicate the winter rings, X indicates the false ring (Carbonara and Follesa, 2018).

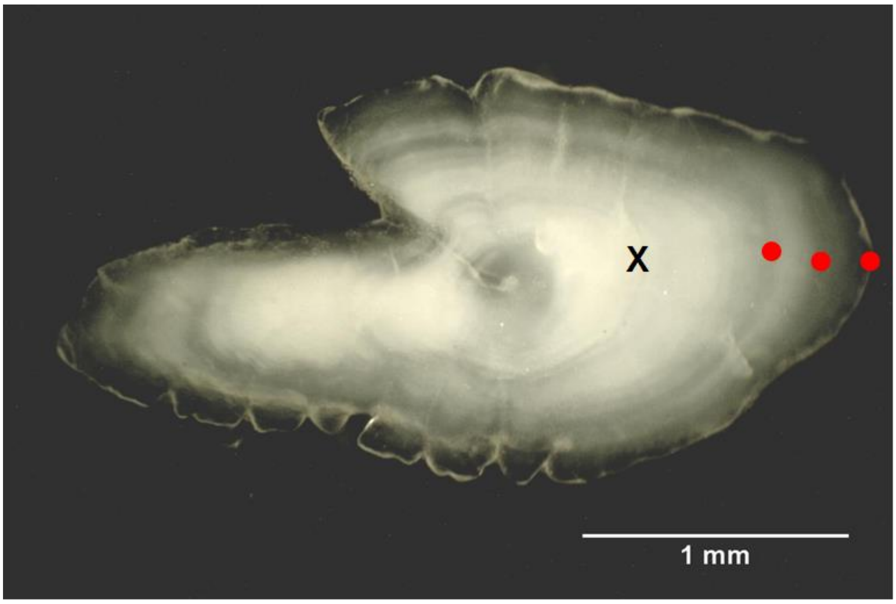


Figure 4.6. Otolith of *S. pilchardus* (TL: 18 cm, sex: F, month of capture: January). The red dots indicate the winter rings, X indicates the false ring (Carbonara and Follesa, 2018).

Daily increment-estimation criteria

For daily increment interpretation of sardine otoliths, the same recommendations are followed as those suggested for anchovy by ICES WKMIAS (ICES, 2013b; see Section 4.3.1). In the Bay of Biscay, Atlantic Iberian Peninsula, western Mediterranean, and the northern Aegean Sea, the agreement was to apply the GBR criteria for sardine, irrespective of the season and geographical area (Morales-Nin *et al.*, 2010; SARDONE, 2010; ICES, 2013b), except for the Adriatic Sea where IMR is adopted (ICES, 2013b).

Discrepancies in interpretation of daily increments

Discrepancies in the interpretation of daily increments are most often along two lines: (i) difficulties in the interpretation of subdaily increments, double structures, or band zones; and (ii) unclear images where it is difficult to interpret correctly the daily growth pattern due to under-or overpolishing, poor image acquisition, or calibration problems. Thus, effort should be put into achieving the best possible images for any reading of daily increments.

4.3.3 Herring (*Clupea harengus*)

Age estimation criteria

The herring age estimation criteria were recommended in the most recent workshops for Atlantic and Baltic stocks (Raitaniemi and Halling, 2005; ICES, 2008c). A birth date of 1 January is assumed for all herring for which age is reported; however, the date of capture and spawning type affiliation must be known. One year's growth is defined as one opaque zone and one hyaline zone. For younger fish, the age structures are visible over the entire perimeter; for older fish, they are mostly discernable in the rostrum. This makes the rostrum the most preferred part for age estimation.

Age-interpretation difficulties

In general, herring otoliths are relatively easy to read, and the annuli can easily be recognized. Misleading false rings caused by starvation, diseases, spawning, or abrupt changes in environmental conditions, etc. are not frequently observed. However, problems are encountered by mixing of stocks, which spawn at different times of the year and may cause bias in age estimations. Given the quite broad spawning period for herring, where some populations/stocks spawn during autumn, some during spring, and others during winter, the appearance of the otoliths and the age structures herein also vary. Herring spawning later in the second half of the year produce larvae, which by definition should be 1 year old, once they have grown into January of the following year. These larvae would, therefore, be of the same age as young fish which have been spawned early in the year and are considerably larger. Thus, from the same year class, some individuals will form a winter ring during their first winter while others (spawned later) do not. Because of this, herring are classified as "ringers", i.e. the total number of annuli is not set equal to years, but to the number of rings.

4.3.4 Sprat (*Sprattus sprattus*)

Age estimation criteria

The sprat age-estimation criteria were recommended in the last ICES sprat age estimation workshops for both Atlantic and Baltic stocks (Torstensen *et al*., 2004; ICES, 2008b, 2017d). The hyaline zones in the otolith are counted as age structures (= winter rings). For sprat older than two years, the first two inner annuli at least should be traceable throughout the whole otolith. For older individuals, the rostrum is the primary structure for age estimation; however, readers tend to try to find the age structure throughout the otolith.

Age-interpretation difficulties

In general, sprat otoliths are not easy to read and the annuli cannot be easily recognized. False rings are not frequently observed, but differences in growth patterns between populations/stocks are often given as the cause for diverging age interpretations. This is particularly a problem in the Baltic where fast-growing sprat from the western Baltic mix with slower-growing sprat from the more eastern areas (east of the Arkona Basin). The otoliths of sprat from the east are generally more difficult to read because of the slower growth and the higher age at a given size.

4.3.5 Mackerel (*Scomber scombrus*)

Annual age estimation criteria

Mackerel age estimation criteria were recommended by ICES (2010e, 2019a). The method, adopted explicitly in the Workshop on Mackerel Otolith Reading in 1995 (ICES, 1995), is based on the age validation of this species by reading otoliths of known age (obtained from a tagging programme). The date of birth is conventionally assumed to be 1 January, and age is estimated on this basis (as described for anchovy and sardine).

Otoliths of mackerel are observed whole, under a binocular microscope with a reflecting light against a black background. However, the preparation technique differs by laboratory. In some laboratories (Portugal, Spain, Germany, Ireland, Norway, Iceland, the Netherlands, and England) the otoliths are mounted in transparent resin inside on black trays. In other laboratories (Scotland, Denmark, Greece, Greenland, and recently also Iceland) otoliths are observed loose immersed in fresh water or alcohol. The orientation for the analysis is with the distal surface up and the proximal surface (*sulcus acusticus*) down. The age structures appear clearer in the post-rostrum compared to the remaining otolith areas. However, ring continuity should be checked on the anterior part of the otolith (*rostrum* and *antirostrum*) and, where possible, on the dorsolateral edge.

The timing of the formation of the opaque zone on the edge of the otolith is heavily dependent on the area from which the sample was taken; the opaque zone is formed earlier in southern areas (ICES divisions 9.a and 8.c).

Annual age-interpretation difficulties

As with many other migrating fish, the age structures of individual mackerel can vary quite a lot. The duration of the period with opaque zone formation in the first year of life may differ among areas; in some areas, a false ring is formed during this first year of growth (Figures 4.7 and 4.8). Onset of maturity may also introduce false annuli (Figure 4.9) and, as growth diminishes in older individuals, the opaque and translucent zones become increasingly difficult to distinguish (Figure 4.8). The interpretation of the otolith edge often differs among readers, one of the main causes of the low percentage of agreement in calibration exercises.

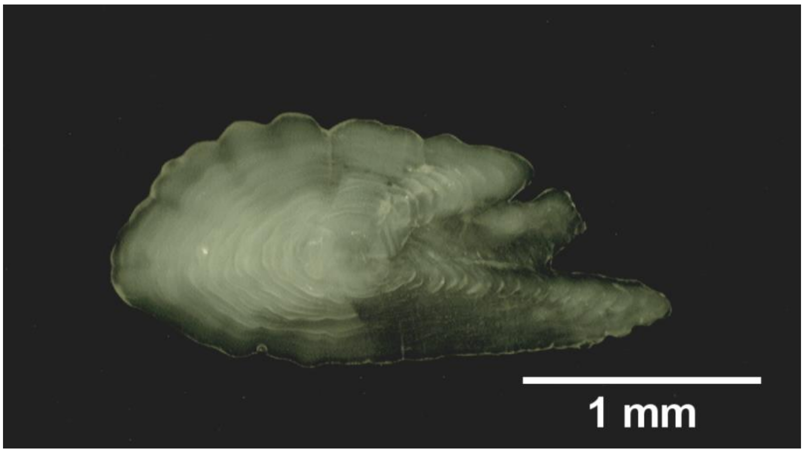


Figure 4.7. Otolith of *S. scombrus* (estimated age: 0, TL: 17 cm, sex: F, month of capture: November) (Carbonara and Follesa, 2018).

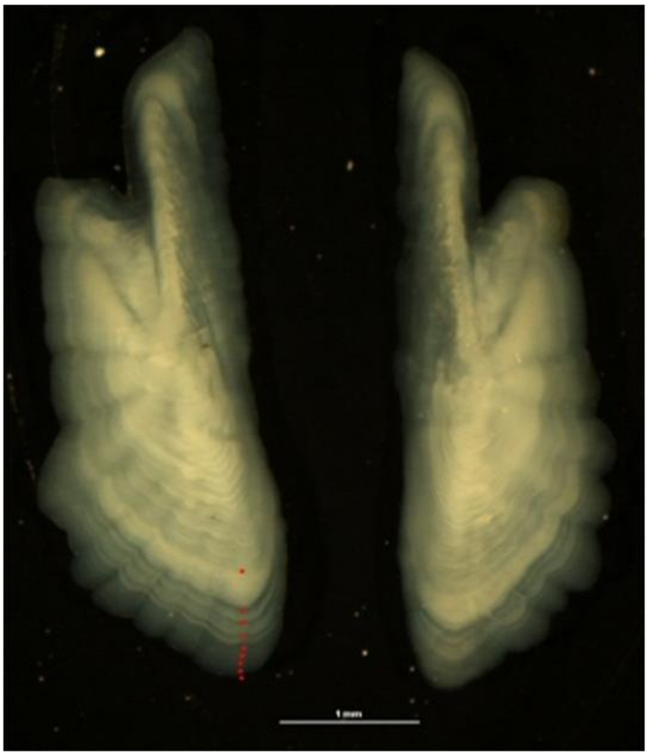


Figure 4.8. Otolith of *S. scombrus* (estimated age: 9, TL: 40 cm; sex: F, month of capture: March). The red dots indicate the winter rings.

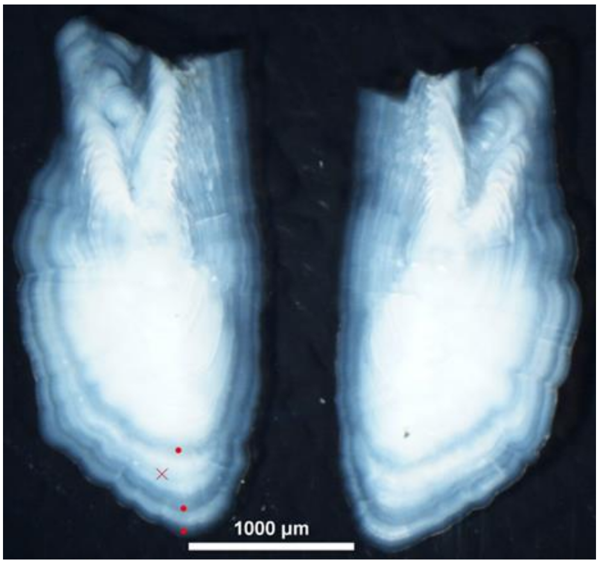


Figure 4.9. Otolith of *S. scombrus* (estimated age: 3, TL: 32 cm; sex: F, month of capture: January). The red dots indicate the winter rings; X indicates the false ring.

Daily age estimation criteria

Enumeration of daily increments should start at the hatch check. Daily age estimations are not frequently done on mackerel. However, the deposition of daily growth increments in mackerel larvae, post-larvae, and juveniles has been validated by Migoya (1989) and D'Amours *et al*. (1990) in the Northwest Atlantic, and by Mendiola and Alvarez (2008) in the Northeast Atlantic.

Daily age-interpretation difficulties

Such difficulties could be explained by difficulties related to the definition of subdaily increments, double structures or band zones, and difficulties in the interpretation of intermediate areas without growth increments in juvenile otoliths.

4.3.6 Chub mackerel (*Scomber colias*)

Age estimation criteria

The criteria for the age estimation of *Scomber colias* in European waters are those recommended by the Workshop on age reading of chub mackerel, WKARCM (ICES, 2016a).

Chub mackerel otoliths have an irregular shape (Fig. 4.10), which is more accentuated in otoliths of older individuals. This shape differs slightly between individuals.

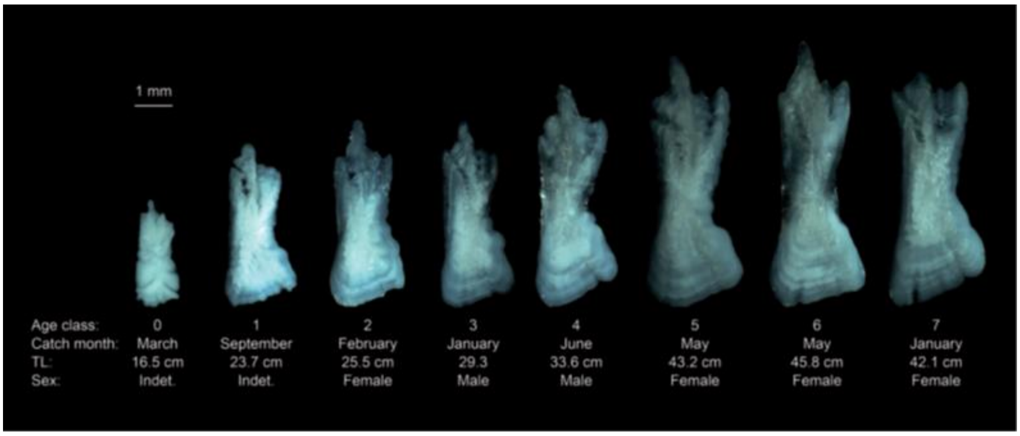


Figure 4.10. Shape of chub mackerel otoliths; differences by age (Photo from Jurado-Ruzafa *et al.*, 2017).

For age estimation, chub mackerel otoliths are orientated with the distal surface turned up and the proximal surface (*sulcus acusticus*) turned down. Annuli are more clearly observed in the post-rostrum and the edge near the rostrum areas. Unlike for Atlantic mackerel otoliths, the rostrum sometimes offers little help in age estimation of chub mackerel otoliths, specially for older individuals, whose annuli are usually not clear in this area (Figure 4.10.). It is recommended that a complete observation is made before an otolith is rejected, as some otoliths with a high presence of false rings may offer an area where interpretation is possible, even when this is not possible in other areas.

Figure 4.11. ***Scomber colias*** **otoliths. The green circles show areas where the annuli are best observed; the red ones show areas where the annuli are usually less clear (WKARCM 2015 – ICES, 2016a).**

Otoliths are observed whole under a binocular microscope with reflected light against a black background. However, the preparation technique differs by laboratory. In laboratories from Portugal and Spain (the northeastern and central-eastern Atlantic and the western Mediterranean), otoliths are mounted in clear resin inside black plastic slides covered with a transparent resin. In laboratories from Italy and Greece, otoliths are observed loose immersed in seawater.

One annulus includes one opaque zone and one hyaline zone, this being counted as a year. Annuli width decreases with age, being more evident in the first three years of life. Checks or false rings are frequent during the first years, which can be identified following this pattern of width decrease (checks does not follow the pattern). The mean radius of the first annuli for Bay of Biscay otoliths is 1.2 mm (ICES, 2016a). This measurement is presently studied in the other areas.

The adopted birth date is 1st January for all Atlantic and western Mediterranean areas (Figure 4.12A). In the Ligurian and Adriatic Sea as well as in the central and eastern Mediterranean, the adopted birth date is 1st July (Figure 4.12B).

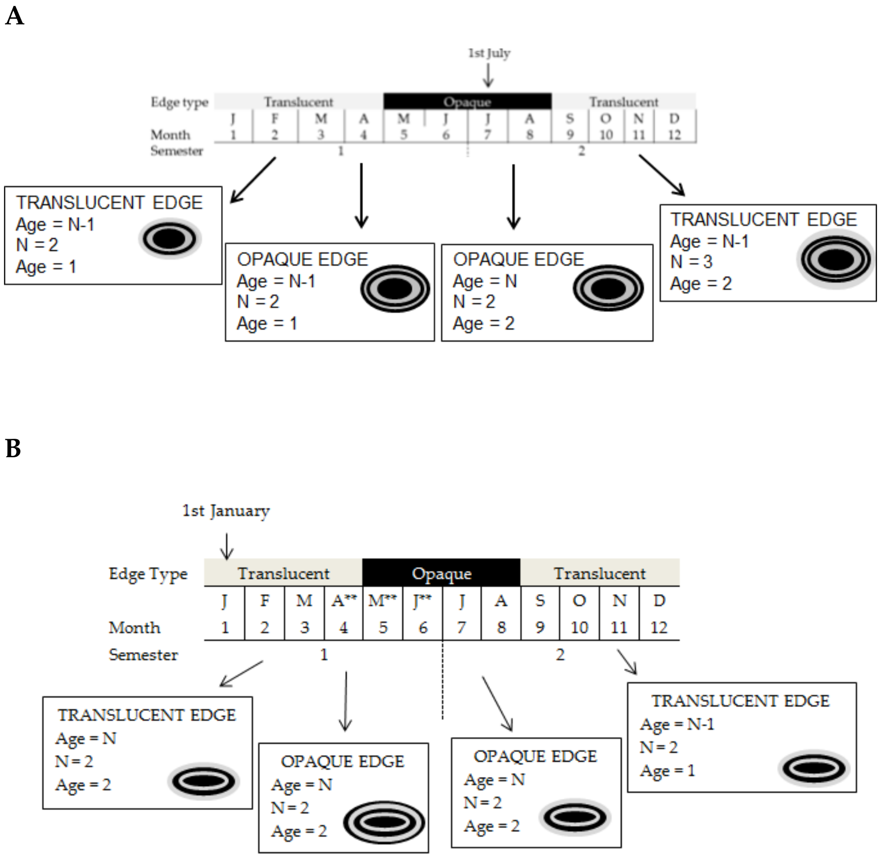


Figure 4.12. Approach of chub mackerel age from otoliths reading in A) Atlantic and western Mediterranean areas and B) Ligurian and Adriatic Sea and central and eastern Mediterranean. N indicates the number of translucent areas. Conventionally, the birth date is fixed at the 1st January as the birth date for all individuals (**), as explained in the main text. (From WKARCM 2015 – ICES, 2016a.)

Interpretation difficulties

The most frequent reasons for difficulties in age estimation of this species are (i) identification of the first annulus (Figure 4.13); (ii) difficulties in differentiating between true annuli and false rings (checks); (iii) insufficient annual growth pattern recognition; and (iv) disagreement in interpretation of otolith edge nature, depending on season and (v) different growth pattern in otoliths of different areas.

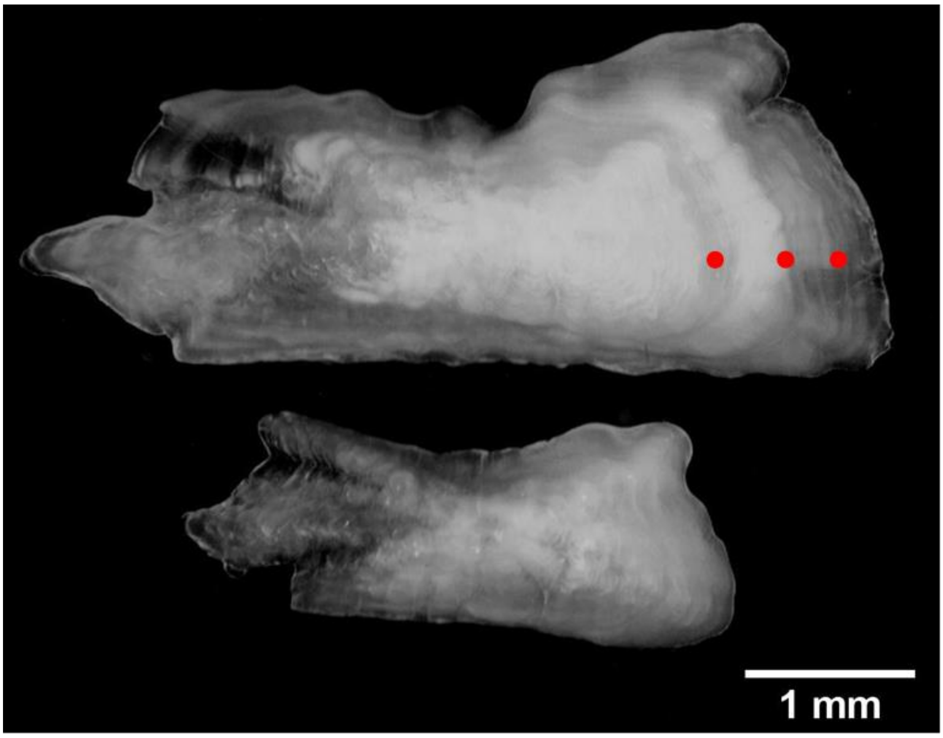


Figure 4.13. Otoliths of an Atlantic chub mackerel caught in November in the Adriatic Sea (central Mediterranean). Upper specimen (age class: 3+, TL: 28.5 cm); lower specimen (age class: 0+, TL: 16 cm). (Carbonara and Follesa, 2018.)

4.3.7 Horse mackerel (*Trachurus trachurus*)

Age estimation criteria

Horse mackerel age estimation criteria have been established by ICES (1999, 2016b, 2018c), based on direct age validation studies (Kerstan and Waldron, 1995) and on indirect validation studies (ICES, 1999; Waldron and Kerstan, 2001; Abaunza *et al*., 2003). Horse mackerel age is presently estimated through sectioned, broken, and burnt or whole otoloths. The interpretation of growth zones varies among readers. Despite efforts to standardize preparation techniques and interpretation of growth zones with numerous workshops and otolith exchanges, precision in age estimates is still very low. This may negatively influence the quality of the assessment.

When performing age estimation using a whole otolith, one of the pair of sagittae (usually the left one) is immersed in seawater or alcohol and glycerin as a clarification medium before the analysis. The otoliths are analyzed whilst immersed in the clarification medium, using a binocular microscope with reflecting light against a black background. The orientation for analysis is with the distal surface face up and the proximal surface (*sulcus acusticus*) face down (Figure 4.14).

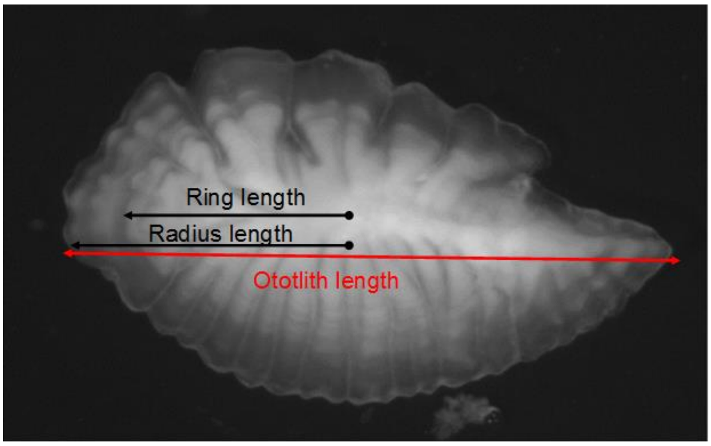


Figure 4.14. Otolith from Atlantic horse mackerel (Carbonara and Follesa, 2018).

In whole otoliths, annuli are counted on the posterior part of the otolith (post-rostrum). However, ring continuity should be checked on the anterior part of the otolith (rostrum) and, wherever possible, on the dorsolateral edge (Figure 4.15).

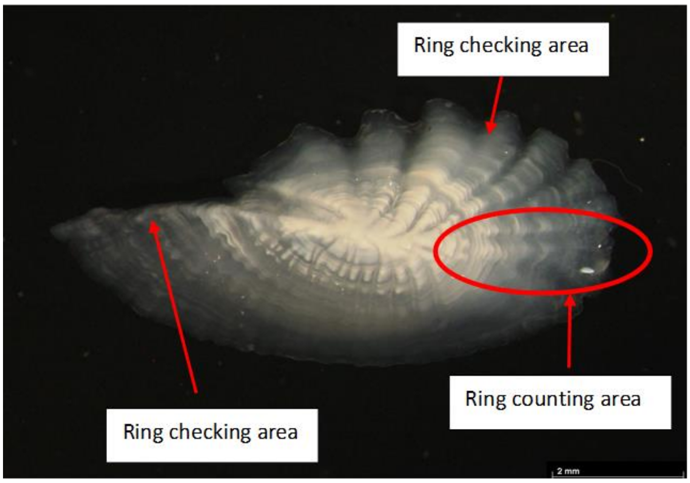


Figure 4.15. Whole otolith of *T. trachurus* immersed in seawater in which the preferred sites for counting rings are shown.

The dark rings are counted as the translucent growth zone (slow growth). The opaque (white – fast growth) zone plus a dark ring is considered as an annual growth (annulus). Age estimation of Atlantic horse mackerel is based on 1 January being the conventional birthday. This is in line with the spawning period (Abaunza *et al*., 2003; Carbonara *et al*., 2012) that is prolonged almost all year, but with a peak during winter. Interpretation of growth structures follows the scheme outlined in Table 4.3; for the specimens caught in the first part of the year, a transparent ring on the otolith edge is counted as an annual ring. If a transparent ring is observed at the edge of the otolith in the second semester of the year, it is not considered an annual ring, and the age is equal to the number of transparent rings excluding the edge (N–1). Opaque zone formation should, in general, have started in June, so in otoliths from individuals caught before June, every structure should be interpreted as being equal to a year. For otoliths collected from the specimens caught in the second part of the year with an opaque edge, the age corresponds to the completed annulus (n).

When performing age estimation on larger individuals, (> 25 cm), the otoliths are embedded in epoxy or polyester resin, and thin sections (about 550 µm) are made along the dorsal–ventral axis through the nucleus. The thin slices are mounted on the glass slide in resin and read under a binocular microscope with reflected light (making transparent rings dark and opaque zones white; Figure 4.16), or with an optical microscope with transmitted light (transparent rings appear light and opaque zones dark). The winter rings are usually counted on the dorsal side.

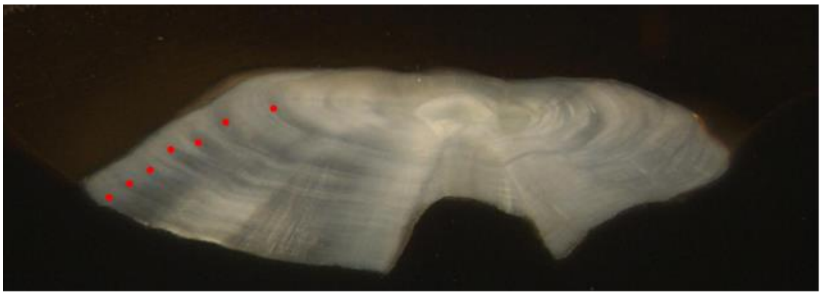


Figure 4.16. Thin section of an Atlantic horse mackerel otolith (estimated age: 7, TL: 34.5 cm, month of capture: September). The red dots represent winter rings.

Break-and-burn technique

Before reading, one of the otoliths is broken transversely across the dorsal–ventral axis through the nucleus. The fractured surface of the anterior half of the broken otolith is polished using wet sandpaper (no. P600). The rostrum is broken off, and the polished part is then burnt over a Bunsen burner for a few seconds while constantly in motion. To clarify the ring structure, these otoliths are carefully charred until darkish brown (Møller Christensen, 1964). The treated otolith is mounted in plasticine and brushed with baby oil on the break. The otoliths are read under a stereomicroscope using direct light, preferably an intensive cold-light source. The translucent rings in the burnt otolith are counted in the large ventral lobe near the *sulcus acusticus* (Figure 4.17).

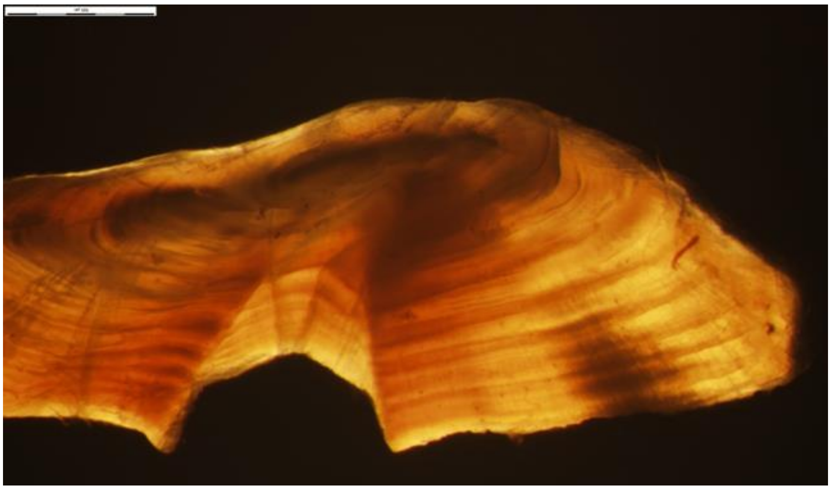


Figure 4.17. Example of a broken and burnt otolith from a horse mackerel (TL: 27.8 cm).

Interpretation difficulties

Horse mackerel otoliths are notoriously difficult to age estimate (Fariña Perez, 1983; Kerstan, 1985; Arruda, 1987; Abaunza *et al.*, 2003), with otoliths from specimens of European waters having particularly complicated ring structures (Karlou-Riga and Sinis, 1997). False rings may be similar in appearance to true annual rings (Karlou-Riga and

Sinis, 1997) and may be erroneously interpreted as annual rings. The potential for inaccurate age estimation of this species is high and can result in a wide variation of age estimates for horse mackerel (Abaunza *et al*., 2003).

In general, the age of horse mackerel otoliths is very difficult to estimate in older fish because the otoliths thicken with age. The first annuli interpretation in both young and older fish appears to be the major cause of differences. The dissimilarity of the false rings and the variety of the true annuli make it difficult to follow the formation of true annuli. Interpretation of the edge causes problems in some otoliths.

The determination of annual increments is difficult because the presence of false rings can mislead the pattern of annuli formation. In fact, this is a major cause of age estimation errors, including the age estimation of *Trachurus trachurus*. The zones are non-seasonal and two major types can be distinguished:

1. <u>False rings</u> appear as translucent zones within an opaque zone. They are common in the first year of life of the fish and in many cases are easily confused with the first annual increment.
2. <u>Split rings</u> are double structures composed of two unusually thin translucent bands separated by a very thin opaque band.

The causes of their formation are not clear, although some factors such as temperature, food intake, other environmental conditions, and developmental transitions have been suggested.

The interpretation of the first annulus is a matter of concern because of the difficulties in distinguishing false juvenile rings from the true seasonal marks (Figures 4.18 and 4.19). In the case of sliced otoliths in *T. trachurus*, examining the whole otolith (the untreated one of the two otoliths) beside the sliced otolith helps in distinguishing this common juvenile false annulus. In addition, the slices are sometimes not made through the centre of the otolith, leading to a modification in the usual perception of the distance between the centre of the otolith and the first true translucent mark. This produces a difficulty in interpreting the first true mark or annual increment in the otolith.

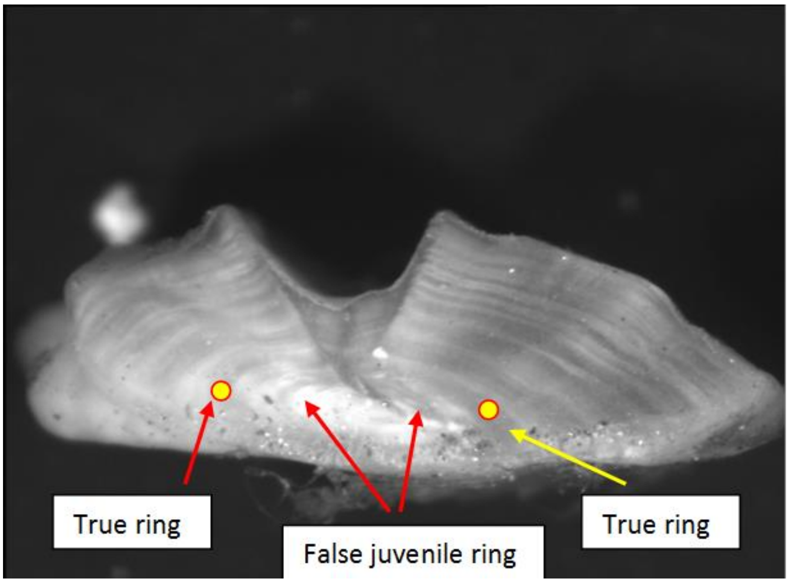


Figure 4.18. A probable false juvenile ring in a thin section from *T. trachurus* (TL: 36 cm, month of capture: February).

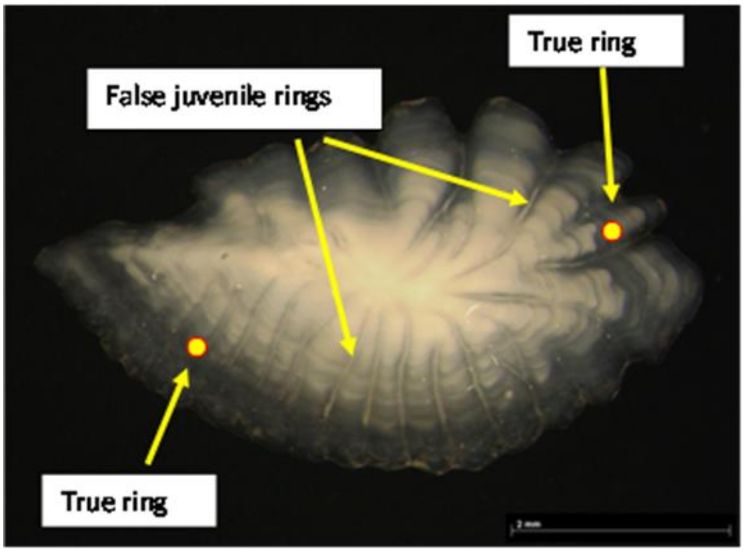


Figure 4.19. True and false juvenile rings in a whole otolith from *T .trachurus* (TL: 18.8 cm, month of capture: August).

These juvenile rings may separate from the first translucent ring or join with it to form a broad translucent zone. The completion of the first translucent zone is usually detected on the rostrum (Karlou-Riga and Sinis, 1997).

Some criteria to help in the identification of these secondary structures (false rings) are described in ICES (1999, 2016b, 2018c):

- Annulus extension in the otolith: A true annulus, ring, or mark should generally be traceable on the whole otolith or the section (ICES, 1999). This is more difficult to observe in the last annuli as the fish is getting older, resulting in a thickening of the otolith.
- Distance between annuli: The widths of consecutive annual growth zones should decrease with increasing age. In horse mackerel, the decrease in the increment widths with age is most obvious between ages 1 and 5. After age 5, the rates of decrease are slow, but rather constant.

- Contrast between seasonal marks: Annual growth zones (annuli, marks) could be distinguished from false rings by their sharper images and the high contrast to the subsequent opaque (= white) increment of the next annual growth zone. Thus, it can be distinguished by the brightest contrast between the preceding translucent and the subsequent opaque zone.

4.3.8 Mediterranean horse mackerel (*Trachurus mediterraneus*)

Age estimation criteria

Age estimation criteria were recommended by ICES (2016b and 2018c), as in the case of horse mackerel. Similarly to *T. trachurus*, the interpretation of the age estimation of *T. mediterraneus* otoliths is difficult, mostly for older specimens where age estimation is particularly imprecise. For the otoliths of *T. mediterraneus*, there are also specific problems in estimating age in younger specimens, particularly in interpreting the first two true annuli (Karlou-Riga, 2000). Indeed, the characteristic of the detection of a ring around the otolith also on the rostrum zone is not always helpful.

Otoliths of Mediterranean horse mackerel are aged whole; one otolith from each pair (usually the left one) is immersed in seawater prior to age estimation. The otoliths do not require a clarification phase before the age analysis except for bigger specimens (> 30 cm), where a very short immersion period in seawater (5–10 min) may be necessary.

The otoliths are analysed in seawater under a binocular microscope using reflected light against a black background. The best otolith orientation for the analysis is with the distal surface face up and the proximal surface (*sulcus acusticus*) face down (Figure 4.20). In this way, the dark rings may be counted in the anti-rostrum area (radius) as translucent growth rings (slow growth). The opaque zone (white – fast growth) plus a dark ring is considered to be an annual increment (annulus).

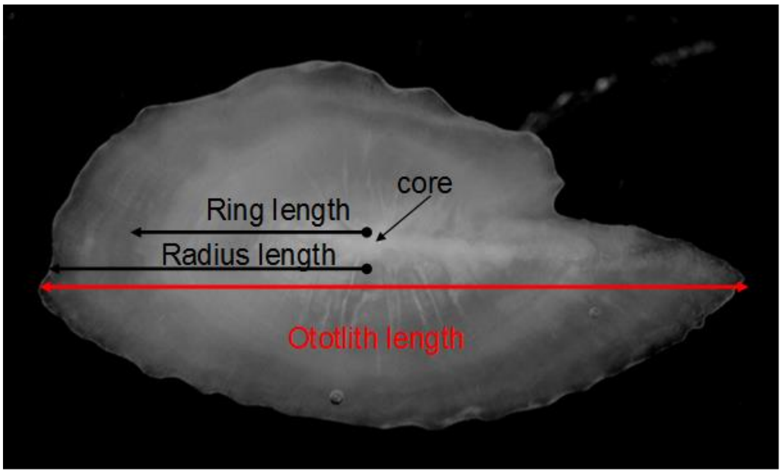


Figure 4.20. Otolith of Mediterranean horse mackerel (Carbonara and Follesa, 2018).

The conventional birthday of horse mackerel in the Mediterranean is set at 1 July, consistent with a spawning period between April and September (Vietti *et al.*, 1997; Karlou-Riga, 2000). The age estimation criteria, as outlined in Table 4.3, take into account the time of annulus formation (generally a transparent ring during winter and spring and an opaque area during summer and autumn), the capture date, the otolith edge, and the spawning period. In individuals caught during the first period, it is highly likely to detect a hyaline edge. In such cases, the age will be the number of transparent rings, excluding the edge (*N*–1). This is also the case if a transparent edge appears in otoliths from individuals caught in the second semester of the year because the otolith can form a transparent ring around December. In these cases, the age should be *N*–1. If individuals caught in July have otoliths with a hyaline edge, this ring corresponds to the growth during the most recent winter; thus, the age will be the number of transparent rings including the edge (*N*). The age in the otolith collected from a fish caught during the second semester with an opaque edge will be the number of completed annulus (*n*). If the opaque edge is present in the first semester, especially around June, the edge should not be counted as an age structure; consequently, the age is equal to the number of the annulus less one (*n*–1).

Table 4.3. The age estimation scheme for *T. mediterraneus* with a birthday of 1 July. *n* is the number of transparent rings, excluding the edge (annulus); *N* is the number of transparent rings, including the edge. For samples taken around July the estimated age is in brackets.

Date of capture	Otolith edge	Age
1 January–30 June	Transparent	*N*–1
	Opaque	*n*–1
1 July–31 December	Transparent	*N*–1 (*N*)
	Opaque	*n*

In the Atlantic areas, the date of birth is 1 January, as described above for *T. trachurus*.

Interpretation difficulties

Mediterranean horse mackerel otoliths are also difficult to interpret, similar to that described in the previous section. However, because of diverging interpretation of the first annulus specific problems with Mediterranean horse mackerel otoliths arise when estimating the age of younger individuals. Karlou-Riga (2000) reported a formation of false rings before the first winter ring. Indeed, otoliths from small specimens (5–8 cm) caught during summer and autumn from the spring–summer spawning often have a transparent edge (Figure 4.21). This is a false ring, probably formed when the juveniles change environment and diet.

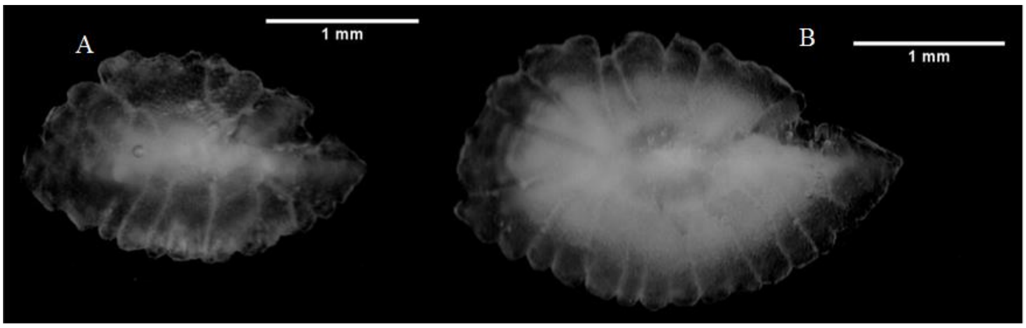


Figure 4.21. Otoliths from small specimens. (A) TL: 5 cm, caught during summer (29/07/2011). and (B) TL: 7.5 cm, caught in autumn (06/10/2011) (Carbonara and Follesa, 2018).

The size of these otoliths is about 2 mm (0.95 mm radius), which corresponds to the false inner ring observed in older specimens (Figures 4.22–4.24).

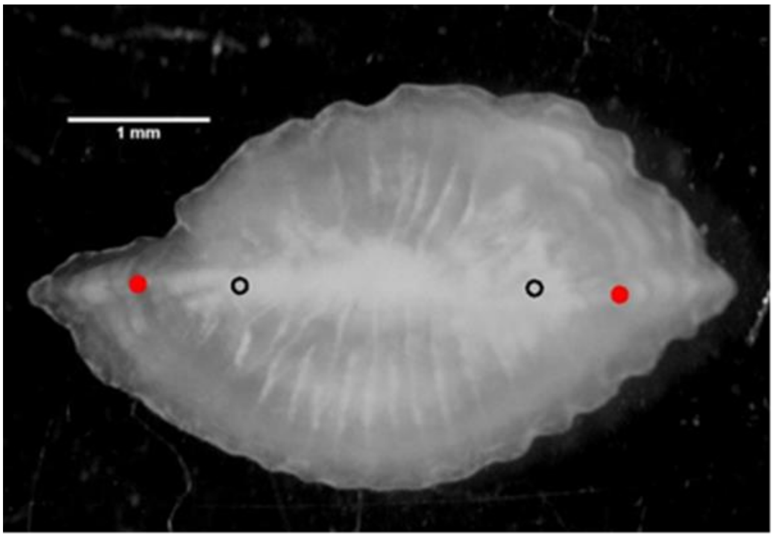


Figure 4.22. Specimen of *T. mediterraneus* (TL: 14.5 cm, caught in summer). The open black circles show a false ring, the red circles the first winter ring (Carbonara and Follesa, 2018).

The first true winter ring follows immediately after the false ring. Specimens caught in winter and early spring and with a total length (TL) of ca. 12–14 cm have otolith edges with a more pronounced transparent ring compared to the early false ring that appears with a radius of ca. 1.5 mm (a whole otolith measures ca. 3.5 mm; Figure 4.23).

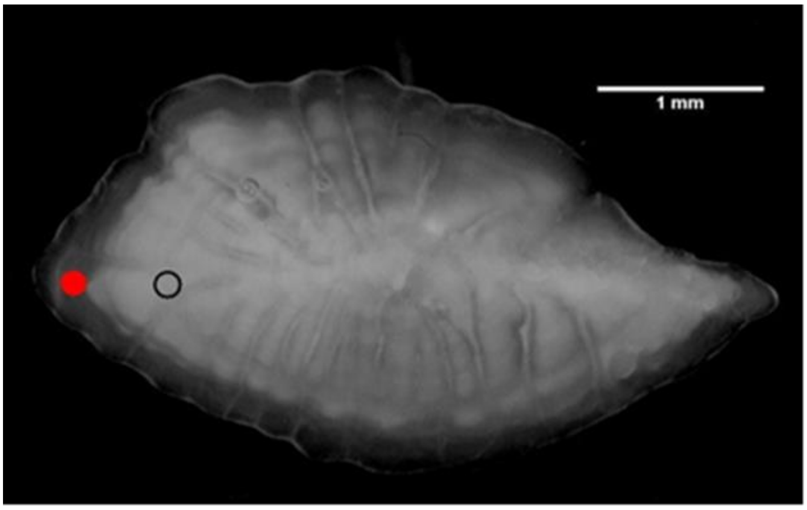


Figure 4.23. Otolith of a *T. mediterraneus* specimen (age: 0, TL: 12.5 cm, caught in early spring). The open black circle is a false ring; the red circle is the first winter ring (Carbonara and Follesa, 2018).

The check before the first true ring sometimes appears joined with the first true annulus in one wide transparent ring or area (Figure 4.24).

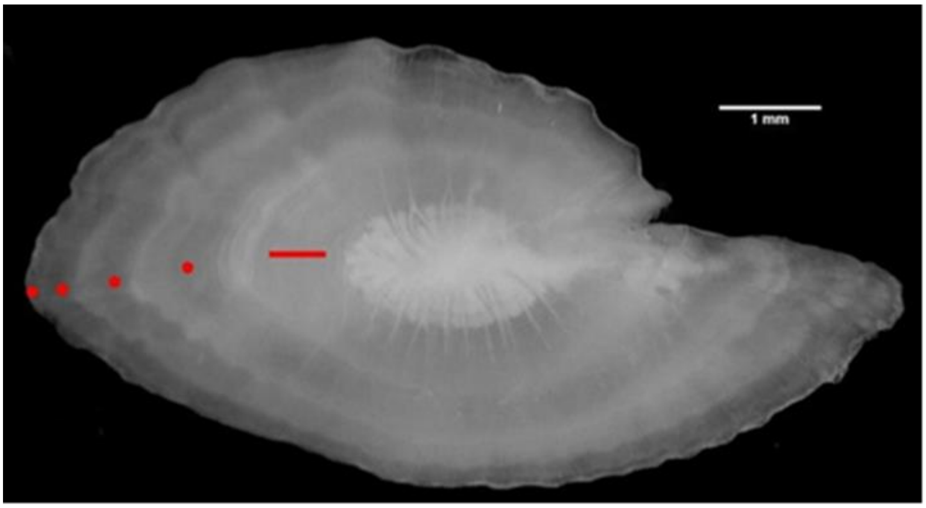


Figure 4.24. Otolith of a *T. mediterraneus* specimen (estimated age: 4, TL: 29 cm, sex: female, month of capture: May). The first winter ring appears as a transparent zone because the false rings are joined with the first true ring. The red dots represent the winter rings; the red line represents the first winter (Carbonara and Follesa, 2018).

After the first winter ring, another false ring may be formed during the second year of life (Figure 4.25). This could be a check related to the first onset of maturity. Vietti *et al*. (1997) reports the onset of first maturity at two years of age, corresponding to a length of 15.6 and 16 cm for smaller mature male and female specimens, respectively, in the northern Adriatic Sea.

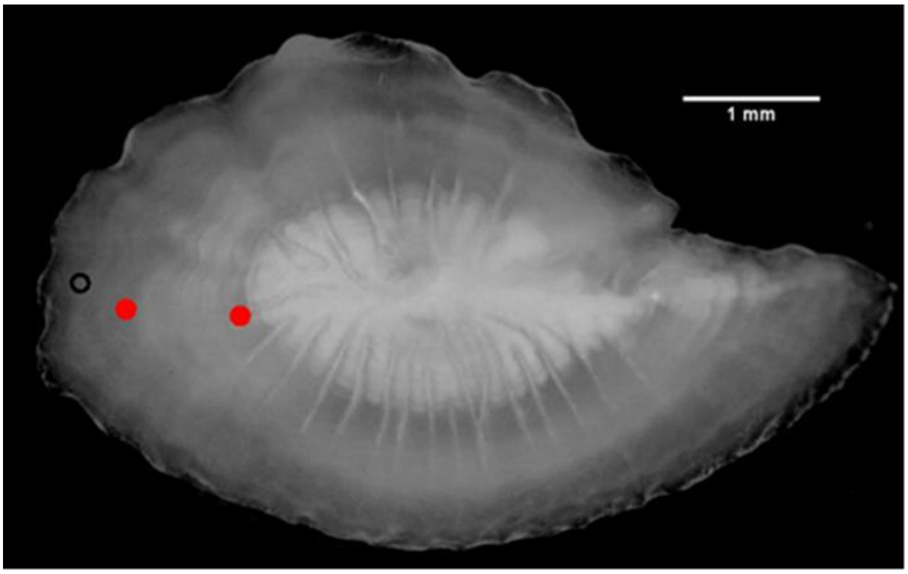


Figure 4.25. Otolith of a *T. mediterraneus* specimen (estimated age: 2, TL: 20.5 cm, caught during early winter) and the gonads in a post-reproductive stage. The open black circle is a false ring, the red circles are the true winter rings (Carbonara and Follesa, 2018).

After the second winter ring, the deposition pattern of the winter band (the transparent–black one) appears regularly, with decreasing distances between rings (Figure 4.26).

Figure 4.26. Otholith of *T. mediterraneus* (estimated age: 9, TL: 35.5 cm, sex: male, month of capture: March). The open black circles represent the false rings, while the red dots represent the true winter rings (Carbonara and Follesa, 2018).

4.3.9 Blue jack mackerel (*Trachurus picturatus*)

The age determination technique for *T. picturatus* utilizes whole otoliths. Annuli are counted preferentially from the nucleus to the posterior margin axis (Figure 4.27). Distilled water was used as clarification medium and the otolith was observed under reflected light and dark background with *sulcus acusticus* placed downwards, so dark (translucent/late summer-winter ring) and white (opaque/spring-beginning summer ring) rings could be seen in alternate positions. The direction of the light relative to the otolith surface also needs to be varied (ICES, 2015b).

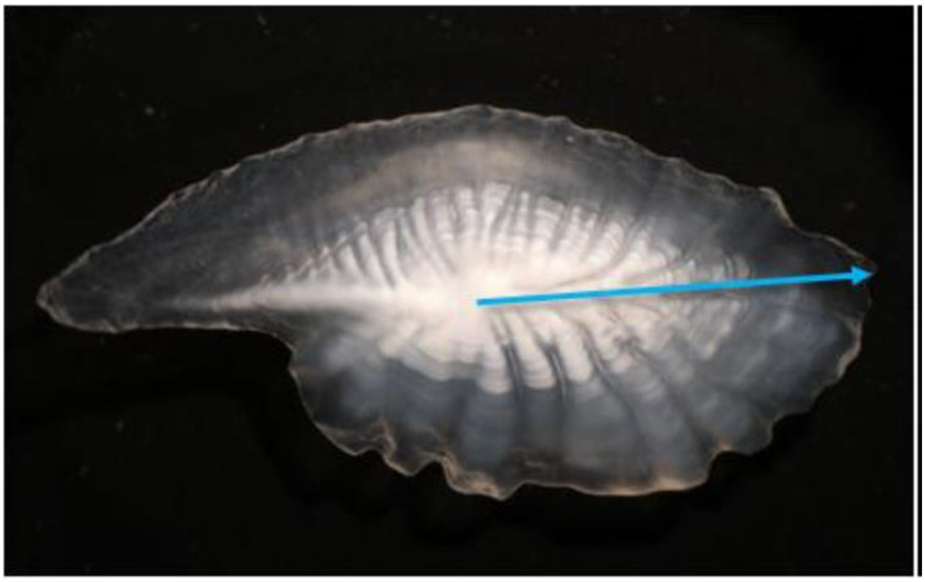
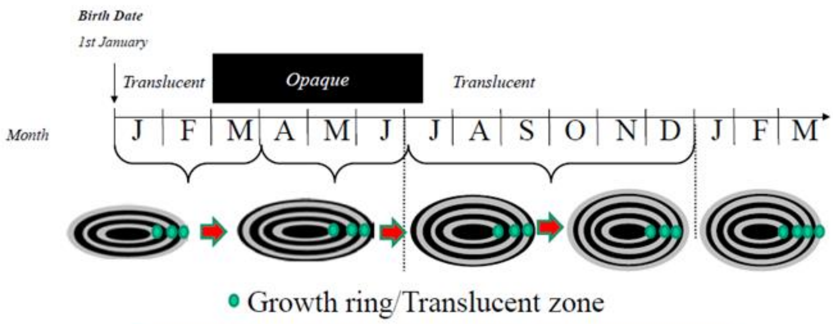


Figure 4.27. Preferred reading axis in the otolith of *T. picturatus*.

It is commonly agreed that one opaque and one translucent zone constitute an annual growth zone (AGZ) in blue jack mackerel otoliths (Vasconcelos *et al.*, 2006; Jurado-Ruzafa and Santamaría, 2018; Garcia *et al.*, 2015).

General adopted criteria for the otolith increments interpretation of *T. picturatus* are:

- Birth date: 1st of January.
- Growth pattern scheme: Age assignment depends not only on the number of annuli, but also on the edge type related to the catch date and the birth date considered. Based on the translucent edge analysis, the pattern annuli deposition is shown in Figure 4.28.



Quarter	Edge of otolith	Age
1	Translucent Opaque	N N-1
2	Translucent Opaque	N-1*
3-4	Translucent Opaque	N-1

Figure 4.28. Scheme of the growth pattern considered for otolith age assignment for *T. picturatus.* (*) is explained in the text.

- For fish caught during the year having an opaque zone on the otolith edge, the age assigned will be equal to the number of rings observed minus one.
- For fish caught in the first quarter having a translucent ring on the otolith edge, the age assigned will be equal to the number of annual rings observed.
- Otoliths with a translucent edge from fish caught in the second quarter of the year (*) have to be examined carefully and assessed by the reader, based on the width of this increment. It has to be determined whether this translucent ring corresponds to the finalization of the annulus of the previous year, or to the new translucent ring of the year.
- For fish caught in the second semester having a translucent otolith edge, the age assigned will be equal to the number of annual rings observed minus 1.

Interpretation difficulties

In the age estimation process, the position of the first annual ring should be the major point of the agreement procedure (FAO, 2002). Especially for the first annulus, AGZ should be traceable on the whole otolith, with the exception of the dorso-medial surface of the rostrum. However, in most cases, it does not occur.

In general, the widths of consecutive AGZ decrease with increasing age. Counting annuli in specimens older than three years (when the growth rate decreases) is more difficult because the annuli overlap each other.

A high frequency of false rings has been observed, mainly in the first annulus. Counting each of these well visible rings will result in overestimation of the age (Figure 4.29).

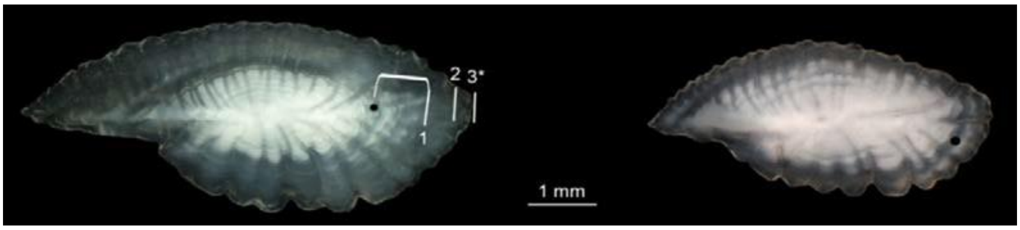


Figure 4.29. Black dots indicate false rings in the first annulus of *T. picturatus* otoliths from the Canary Islands. The otolith on the left is three years old, and the one on the right is one year old.

4.4 Age validation case studies

Several methods exist for the validation of age estimations of calcified structures (Campana, 2001). A summary of age validation methods used for small and medium pelagic species in European waters is shown in Table 4.4.

Table 4.4. Summary of age validation methodologies used for small and medium pelagic species in European waters.

Method	Annual/daily	Pelagic species for which this validation technique has been employed
Marginal increment analysis/ edge-zone analysis	A	Anchovy, sardine, sprat, chub mackerel, horse mackerel, Mediterranean horse mackerel, blue jack mackerel
Progression of strong year classes	A	Anchovy, horse mackerel
Length frequency analysis	A	Anchovy, sardine, chub mackerel, horse mackerel, Mediterranean horse mackerel
Weight frequency analysis	A	Sprat
Daily increments between annuli	A	Anchovy, sardine
Daily increment widths	A	Herring, sprat
Captive rearing	D	Anchovy, sardine, herring, sprat, mackerel

The above studies will be described in detail in the following sections. The methods are classified as either indirect or direct validation methods (Panfili *et al.*, 2002). With respect to the methodologies available for validating daily growth increments, the direct validation methods take into account a precise temporal reference mark in the otolith or a known age (i.e. marking otoliths or rearing experiments), whereas the indirect methods require the comparison of age estimates with statistical age estimated from length frequency distributions or other age data.

4.4.1 Indirect validation methods

Marginal increment analysis and edge-zone analysis

Marginal increment analysis is the most commonly used of the validation methods and is used for validating the periodicity of growth increment formation (Campana, 2001). Two types of studies are possible; one uses qualitative data and the other uses quantitative data (Panfili *et al.*, 2002). The latter, quantitative approach measures the distances between the most recently formed marks at the edge of the otolith. In cases where there is low contrast between growth zones, an edge-zone analysis (qualitative study) may be used to achieve similar, but less accurate, results. Edge-zone analysis does not assign a state of completion to the marginal increment, but instead records its presence as either an opaque or translucent zone (Campana, 2001). Expressing the results in percentage through time is then used as a validation of temporal resolution of the marks observed.

The majority of studies attempting to validate annuli of pelagic species apply the qualitative method, one of the least rigorous methods (Table 4.5). Relative marginal increment analysis (quantitative method) was applied to to anchovy in the Bay of Biscay (Uriarte *et al.*, 2016), sprat in the Baltic Sea (Torstensen *et al.*, 2004), to chub mackerel in the Madeira Islands, Hellenic seas, and in the southern Bay of Biscay (Kiparissis *et al.*, 2000; Vasconcelos, 2006; Navarro *et al.*, 2018), to horse mackerel in the Northeast Atlantic and Hellenic seas (Karlou-Riga and Sinis, 1997; Waldron and Kerstan, 2001), to Mediterranean horse mackerel in the Hellenic seas (Karlou-Riga, 2000), and to blue jack mackerel in the Azores, Madeira, and Canary islands (García *et al.*, 2015; Jurado-Ruzafa and Santamaría, 2018; Vasconcelos *et al.*, 2018).

Table 4.5. Summary of species where marginal increment analysis has been applied.

Species	Area	Method	Time-series	Age/size range	References
Anchovy	Bay of Biscay	Quantitative	2004–2009	Ages 1–4	Uriarte *et al*. (2016) Supplementary material
		Qualitative	1984–1992	Ages 0–3+	
	Galician waters (Division 9.a N)		2015–2016	Ages 0–2+	ICES (2016c)
	Gulf of Cadiz		2005–2008	Ages 1–4	ICES (2010d)
	Alboran Sea		Oct 1989–Dec 1992	All ages together	ICES (2010d)
	Northern Adriatic Sea		Jan–Dec 2007	All ages together/ 10.5–16.5 cm	ICES (2010d)
Sardine	Bay of Biscay	Qualitative	2006–2009	Ages 1–4	ICES (2011d)
	Atlantic Iberian waters		2000–2008	Ages 1–4	ICES (2011d)
			1979–1980	Ages 1–2	Alvarez and Porteiro (1981); Porteiro and Alvarez (1983)
			Jan–Dec 1979	Ages 1–5	Jorge and Costa Monteiro (1980)
Sprat	Skagerrak and Kattegat	Quantitative	Feb 2003–Jan 2004	Ages 0–2	Torstensen *et al.* (2004)

Species	Area	Method	Time-series	Age/size range	References
Mackerel	Portuguese coast	Qualitative	1981	All ages together	Gordo *et al*. (1982)
	North and northwest of the Iberian Peninsula	Qualitative	2013-2017	Ages 0–7+	Villamor *et al*. (2018)
Chub mackerel	North and northwest of the Iberian Peninsula	Quantitative	2011-2012	Ages 0–5	ICES (2016a); Navarro *et al*. (2018)
		Qualitative	2011-2017	Ages 0–5	Navarro *et al*. (2018)
	Portuguese coast		1981–1982	All ages together	Martins *et al*. (1983)
	Azores Islands		1996–2002	All ages together/ 9.6–53.5 cm	Carvalho *et al*. (2002)
	Madeira Islands	Qualitative	2002–2003	Ages 0–4/ 15–37 cm	Vasconcelos *et al*. (2011)
		Quantitative	2002–2004	All ages together / 19–41 cm	Vasconcelos (2006)
	Gulf of Cadiz	Qualitative	1977–1978	All ages together	Rodriguez-Roda (1982)
	Canary Islands		Mar 1988–Jul 1990	All ages together/ 19.2–41.1 cm	Lorenzo *et al*. (1995)
	Southwestern Mediterranean (Alboran Sea)		Oct 2003–Sep 2004	All ages together/ 17–40 cm	Velasco *et al*. (2011)
	Northwestern Mediterranean (Catalan coast)		Apr–Jul 1992 and Dec 1997	All ages together	Perrota *et al*. (2005)
	Eastern Mediterranean (Hellenic seas)	Quantitative	Jan–Dec 1996	Ages 1–3	Kiparissis *et al*. (2000)
Horse mackerel	Northeast Atlantic	Quantitative	Sep 1982–Sep 1984	Ages 0–5	Kerstan (1985)
				Ages 0.6–4.3	Waldron and Kerstan (2001)
	Eastern Mediterranean (Hellenic seas)		Oct 1989–May 1991	Ages 1–5/ 6.5–33.9 cm	Karlou-Riga and Sinis (1997)
	Southern Adriatic Sea	Qualitative	2014–2016	Ages 1–7	Carbonara and Casciaro (2018)

Species	Area	Method	Time-series	Age/size range	References
Mediterranean horse mackerel	Eastern Mediterranean (Hellenic seas)	Quantitative	Aug 1989–Nov 1991	Ages 0–3	Karlou-Riga (2000)
Blue jack mackerel	Azores Islands	Quantitative	1998-2011	Ages 0–18	García *et al.* (2015)
	Madeira Islands		1984–1986	Ages 0–9	Vasconcelos *et al.* (2006)
	Canary Islands		2005–2006	Ages 0–5	Jurado-Ruzafa and Santamaría (2018)
	Canary Islands	Qualitative	2005–2006	Ages 0–5	Jurado-Ruzafa and Santamaría (2018)

Length frequency analysis

Length frequency analysis subsumes a variety of different length-based methods, all of which produce estimates of growth rate. The corroboration occurs when the resulting growth estimate is compared to that of the age estimation method.

The length frequency analysis method has been used for anchovy, sardine, chub mackerel, horse mackerel, and Mediterranean horse mackerel in European waters (Table 4.6). This method was thus applied to validate the otolith interpretation and growth model parameters for anchovy in the northwestern Mediterranean Sea (Pertierra, 1987; Morales-Nin and Pertierra, 1990). In the case of sardine in the same area, Pertierra and Morales-Nin (1989) and Morales-Nin and Pertierra (1990) estimated the age by means of otolith interpretation, which was validated for the younger age classes by length frequency analysis. The method was also applied to chub mackerel of the Madeira Islands at ages 0–5 and of South of the Bay of Biscay (Vasconcelos, 2006; Navarro *et al.*, 2018). The comparison between age estimation and the length frequency distributions of horse mackerel in the Northeast Atlantic confirmed the age estimations of the first years of life (up to age 4: Letaconnoux, 1951; Ramalho and Pinto, 1956; Barraca, 1964; Polonsky and Tormosova, 1969; Sahrhage, 1970; Macer, 1977). In the eastern Mediterranean (Hellenic seas), the first annulus formation of horse mackerel was detected by comparing the progression by month of the smaller modal fish length with the respective otolith appearance during the year (Karlou-Riga and Sinis, 1997). In horse mackerel of the Adriatic Sea, the length frequency analysis method was applied to corroborate the otolith interpretation and growth model parameters (Alegria Hernandez, 1984), and annuli were validated until age 5. Arneri and Tangerini (1984) studied the growth of Mediterranean horse mackerel by otoliths and length frequency in young individuals (ages 0–4) in the Adriatic Sea. The same method was applied for older ages (0–6) in a recent study of this species in the southern Adriatic (ICES, 2018c).

In the Gulf of Salerno (southern Tyrrhenian Sea), an analysis of modal progression in the larval length frequency distribution was used for sardines in the 20–40 mm size range in order to validate larvae growth estimates based on otolith examination (Romanelli *et al.*, 2002).

Table 4.6. Summary of species where length frequency analysis has been applied.

Species	Area	Annual/ daily	Time–series	Age/size range	References
Anchovy	Northwestern Mediterranean Sea	A	Apr 1984–Oct 1985	Ages 0–4/ 5–18.5 cm	Pertierra (1987)
			Jan 1987–Jun 1989	Ages 0–4/ 6.5–20 cm	Morales-Nin and Pertierra (1990)
Sardine	Northwestern Mediterranean Sea	A	Jan 1987–Jun 1989	Ages 0–5/ 6.5–20 cm	Pertierra and Morales-Nin (1989); Morales-Nin and Pertierra (1990)
	Central Mediterranean Sea (Gulf of Salerno – west of Italy)	D	1996–1997	Age 0/ 20–40 mm	Romanelli *et al.* (2002)
Chub mackerel	Madeira Islands	A	2002–2004	Ages 0–5/ 13–41 cm	Vasconcelos (2006)
	North and northwest of the Iberian Peninsula	A	2011–2017	Ages 0–5	Navarro *et al.* (2018)
Horse mackerel	Northeast Atlantic	A	–	Ages 0–4	Letaconnoux (1951)
			Jul 1954–Feb 1955	Age 0	Ramalho and Pinto (1956)
			Jul 1954–Dec 1961	Ages 0–2	Barraca (1964)
			1967–1970	Ages 1–3	Macer (1977)
	Hellenic seas		Oct 1989–May 1991	Age 1	Karlou-Riga and Sinis (1997)
	Adriatic Sea		Jul 1980–Nov 1981	Ages 1–5	Alegria Hernandez (1984)
Mediterranean horse mackerel	Adriatic Sea	A	May–Nov 1982	Ages 0–4	Arneri and Tangerini (1984)
	Southern Adriatic Sea	A	2009–2016	Ages 0–6	Carbonara and Casciaro (2018)

Otolith weight frequency distribution (OWFD) was applied to sprat from the Skagerrak and Kattegat areas (Torstensen *et al.*, 2004) at a meeting in February 2003 on age groups 0–4 (OW 0.22–2 mg). This method is a variant of the length frequency distribution analysis (LFD; Campana, 2001) and assumes that the expected modes of the otolith weight frequency would correspond to the population age classes.

Progression of strong year classes

In a validation based on the tracking of strong and weak year classes, comparison of the interval between yearly samples and the increase in the apparent modal age of a recruitment pulse is determined through annulus counts (Campana, 2001). This method, also considered an "indirect validation" method, indicates that an age estimation method is accurate if the age composition of exceptionally good or weak year classes can be tracked over a long period of time (Panfili *et al*., 2002).

This method has been used only for anchovy and horse mackerel in European waters (Table 4.7). The age estimation criteria of Bay of Biscay anchovy were also corroborated (or indirectly validated) by successive modal lengths in the catches 1982–1992 (Uriarte and Astudillo, 1987; Uriarte *et al.*, 2002) and by tracking year class abundance indices for 1982–1992 in research surveys in the Bay of Biscay (Uriarte *et al*., 2016). In the case of horse mackerel, age estimation criteria were tested by following identifiable year classes through the age compositions of successive years in the catch in number. For the western horse mackerel fishery, the extremely strong 1982 year class could be followed from 1984 to 1996 (Eltink and Kuiter, 1989; Abaunza *et al*., 2003).

Table 4.7. Summary of species where the progression of strong year classes was applied.

Species	Area	Time-series	Age/size range	References
Anchovy	Bay of Biscay	1983–1986	Ages 1–4	Uriarte and Astudillo (1987)
		1982–1992	8–20 cm	Uriarte *et al.* (2002)
		1987–2013	Ages 1–3	Uriarte *et al.* (2016)
Horse mackerel	Northeast Atlantic	1981–1987	Age 5 and older	Eltink and Kuiter (1989); Abaunza *et al.* (2003)

Daily increments between annuli

Daily increment counts between presumed annuli can provide strong corroboration of the frequency of formation of annuli (Campana, 2001). In this method, all increments are examined and counted, presupposing knowledge of dates of hatch and annulus formation.

Based on different daily growth studies, the formation of the first annulus was validated and the position of the first false ring or check was corroborated in anchovy in the Bay of Biscay (ICES, 2013b). The method has also been applied for validating annual increment deposition in the otoliths of young-of-the-year European anchovy (Aldanondo *et al*., 2013). Early anchovy juveniles were maintained in captivity from October 2012 until April 2013, and the first annulus was validated using daily increment counts. According to this study, the first opaque band is completed in October–November, whereas the translucent band is formed by March–April. The position of the first check for anchovy in the Bay of Biscay was also corroborated (Hernández *et al*., 2013). Two methods were used for this purpose: (i) age was estimated by identifying and measuring growth rings formed on sagitta otoliths, and (ii) age corroboration was obtained by means of the otolith microstructure, with fish ages being estimated by daily increment counts.

The position of the first false ring (or check) formed before the first winter ring was identified through micro-increment counts of the otoliths of sardine in the Adriatic Sea (ICES, 2013b) and in Portuguese waters (Silva *et al*., 2012). This study was done with offset in the previous validation of daily increments in these two species (anchovy – Cermeño *et al*. 2003; Aldanondo *et al*., 2008; sardine – Ré, 1984; and larvae and juveniles – Alemany and Álvarez, 1994). See summary in Table 4.8.

Table 4.8. Summary of species where daily increments between annuli were applied.

Species	Area	Method	Time–series	Age/size range	References
Anchovy	Bay of Biscay	Validation of first annulus	Oct 2012–Apr 2013	Age 1/ 8.5–13.6 cm	Aldanondo *et al*. (2013)
		Corroboration of first check	2010–2011	Age 1/ 11.7–20.5 cm	Hernández *et al*. (2013)
Sardine	Atlantic Iberian waters	Corroboration of first annuli	Oct 2008–Apr 2009	Age 1	ICES (2011d); Silva *et al*. (2012)
	Northern Adriatic Sea		Oct–Dec 2012	Age 1/ 11.5–13 cm	ICES (2013b)

Daily increment widths

The method is based on a random sample of daily increment widths along an uninterrupted growth axis of the otolith which, when integrated over the observed length of the growth radius, must yield the daily age of the otolith and fish (Campana, 2001). This can be used to identify the timing of growth zones by linking the occurrence of translucent checks to the time of occurrence.

Studies of microstructures in sprat and herring otoliths (sagittae) have demonstrated structural differences between what are defined as true and false translucent (winter) rings (Mosegaard and Baron, 1999; ICES, 2008c). When the translucent ring is deposited, the width of the daily increments gradually reduces in width (Figure 4.30).

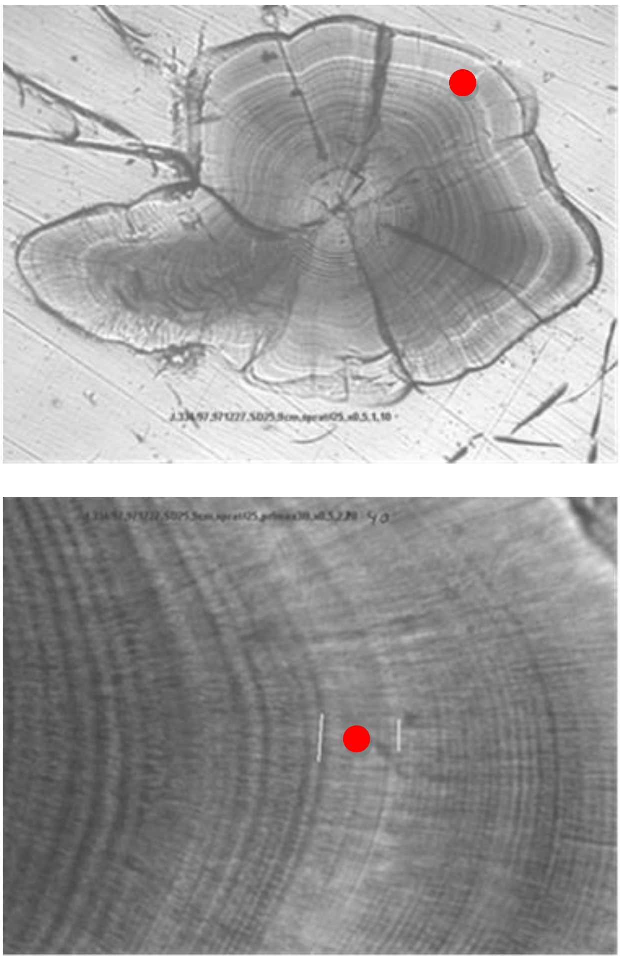


Figure 4.30. A polished sprat otolith (upper panel) where the false winter ring is indicated with a red dot. Analysis of the daily increment structure (lower panel) shows that no decrease in increment width is visible on either side of the transparent zone.

This pattern can be found in true winter rings in the sagittae of sprat aged 0–2 years old (Torstensen *et al.*, 2004). A false winter ring has no gradual reduction in the width of the daily rings, neither in front of it nor immediately after the translucent zone. The characteristic of the winter ring in the sagitae of herring (ICES, 2008c) is illustrated in Figure 4.31. The width of the daily rings decrease prior to the winter ring; no daily ring formation during the winter ring formation is seen, and then progressively wider daily rings appear after the winter ring. These characteristics make it possible to detect false winter rings. Such false rings often appear within the first couple of years as narrow rings close to the center. Thus, in otoliths where the age reader is in doubt as to whether a translucent zone is true or false, the validity of the ring can be examined by reading the otolith microstructure if it is among the first 1–3 rings.

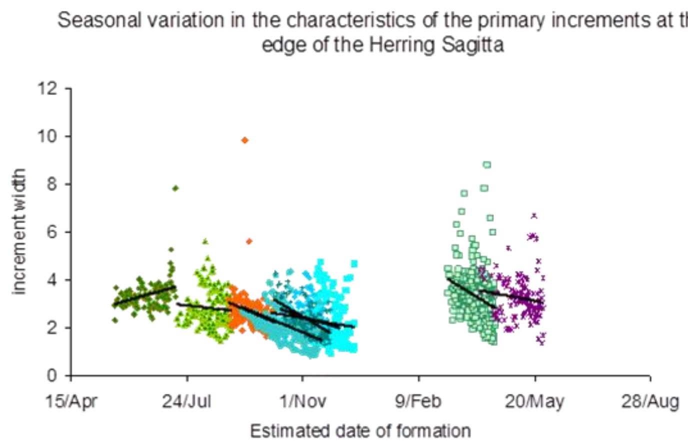


Figure 4.31. Micro-increment validation of wintering formation in Atlantic herring (Mosegaard and Baron, 1999).

Tag–recapture analysis

Tag–recapture analysis is part of a suite of methods that provide growth rate estimates comparable with those derived from annulus counts (Campana, 2001).

Unfortunately, because of the costs associated with these analyses and the usual logistical difficulties (too early recapture of individuals, low return rates, etc.), such studies are very rare.

Other methods

Other validation methods, such as back-calculation of length, should not be considered, neither as validation nor as corroboration (Campana, 2001). Back-calculated length across several age estimation structures merely shows consistency in the interpretation of the sequence of growth increments, independent of whether the interpretation is correct or not.

The back-calculation of length method has, however, been used for anchovy, sardine, chub mackerel, horse mackerel and Mediterranean horse mackerel in European waters (Table 4.9). In the Strait of Sicily, the back-calculation method was applied to anchovy in order to compare results from a growth model (Basilone *et al.*, 2004). Sardine in Atlantic Iberian waters have also been analysed applying these methods (Costa Monteiro and Jorge, 1982; Porteiro and Alvarez, 1983). In the Gulf of Cadiz, Canary Islands, and Madeira Islands, backcalculated length analysis methods have been seen applied for comparison of the otolith interpretation of age and growth model parameters of chub mackerel (Rodriguez-Roda, 1982; Lorenzo *et al.*, 1995; Vasconcelos, 2006). In the eastern Mediterranean (Hellenic seas), the back-calculation method was applied to horse mackerel to compare results from a growth model estimation (Karlou-Riga and Sinis, 1997). The same method was also applied to Mediterranean horse mackerel in the southern Adriatic Sea (Carbonara and Casciaro, 2018).

Table 4.9. Summary of species where back-calculated length analysis was applied.

Species	Area	Time-series	Age/size range	References
Anchovy	Strait of Sicily	May 2000–Oct 2001	Ages 0–3/ 7–16 cm	Basilone *et al*. (2004)
Sardine	Atlantic Iberian waters	1979–1981	Ages 0–7	Costa Monteiro and Jorge (1982)
		1979–1980	Ages 0–6	Porteiro and Alvarez (1983)
Chub mackerel	Canary Islands	Mar 1988–Jul 1990	Ages 1–7/ 19.2–41.1 cm	Lorenzo *et al*. (1995)
	Madeira Islands	2002–2004	Ages 1–4/ 20–40 cm	Vasconcelos (2006)
	Gulf of Cadiz	1977–1978	Ages 0–2	Rodriguez–Roda (1982)
Horse mackerel	Hellenic seas	Oct 1989–May 1991	Ages 1–5/ 6.5–33.9 cm	Karlou–Riga and Sinis (1997)
	Southern Adriatic Sea	2009–2016	Ages 0–6/ 3–38 cm	Carbonara and Casciaro (2018)

4.4.2 Direct validation methods

Captive rearing

This method validates both absolute age and periodicity of growth structures (Campana, 2001).

The daily periodicity of micro-increment deposition was validated in early life stages of European anchovy, sardine, herring, sprat, and mackerel (Table 4.10). These validations were done in rearing experiments; they are thus applicable to the whole species and not only to the respective stock.

As far as anchovy is concerned, validation studies were carried out on individuals from the Bay of Biscay. Daily increment deposition was validated in hatched eggs and larvae reared in the laboratory under different temperature conditions (Aldanondo *et al*., 2008). Analysis of otoliths from wild juveniles, marked by immersion in oxytetracycline hydrochloride (OTC) and reared until reaching adulthood over a period of two years (Cermeño *et al*., 2003), has also been carried out for validation purposes. Furthermore, Aldanondo *et al*. (2008) demonstrated that increment deposition in anchovy starts at hatching.

In the Bay of Biscay, the daily deposition was validated in sagittal otoliths of reared and wild sardine larvae, from hatching to complete yolk-sac absorption (Ré, 1984; Alemany and Alvarez, 1994). Similarly, the validation of daily otolith increment formation was carried out in a mesocosm experiment on wild sardine larvae in the Adriatic Sea (Panfili *et al*., 2012).

The production of daily increments in sagittae of larval sprat has been validated, from 6 to 29 d under laboratory conditions (Alshuth, 1988a). Daily increments have been validated for larval herring under lab, mesocosm, and field conditions (Moksness and Fossum, 1991; Moksness, 1992; Johannessen *et al*., 2000; Fox *et al*., 2004).

The deposition of daily growth rings in larvae, post-larvae, and juveniles of mackerel was validated by Migoya (1989) and D'Amours *et al*. (1990) in several areas in the Northwest Atlantic, and by Mendiola and Álvarez (2008) in the Northeast Atlantic. Migoya (1989) and Mendiola and Álvarez (2008) incubated mackerel eggs in the laboratory and showed that the deposit of the first increment in the otolith occurred on the hatching day and that the increments were formed daily. In addition, D'Amours *et al*. (1990) performed a validation experiment on mackerel juveniles in captivity, marking their otoliths with a fluorescent substance and showing that the increments were deposited on a daily basis.

Table 4.10. Summary of species where captive rearing has been applied.

Species	Area	Rearing conditions	Age/size range	References
Anchovy	Bay of Biscay	Laboratory	Age 0/larvae	Aldanondo *et al*. (2008)
		Laboratory/immersion in oxytetracycline	Age 0/juveniles	Cermeño *et al*. (2003)
Sardine	Atlantic Iberian waters	Field conditions (larval caught every hour during 20 h)	Age 0/larvae	Ré (1984)
		Laboratory	Age 0/larvae	Alemany and Álvarez (1994)
	Northern Adriatic Sea	Mesocosm	Age 0/larvae	Panfili (2012)
Herring	Norwegian Sea	Laboratory and mesocosm	Age 0/larvae (from spring spawning)	Moksness (1992)
		Laboratory	Age 0/larvae (from spring and autumn spawning)	Johannessen *et al*. (2000)
		Laboratory/immersion in alizarin-complexone solution	Age 0/larvae	Fox *et al*. (2004)
Sprat	North Sea	Laboratory	Age 0/larvae	Alshuth (1988a)
Mackerel	Bay of Biscay	Laboratory	Age 0/larvae	Mendiola and Álvarez (2008)

4.5 Future perspectives in terms of validation of age for small and medium-sized pelagic species

4.5.1 Tag–recapture and use of chemical agents for otolith marking

Otoliths from tag–recapture experiments (i.e. the Norwegian programme for mackerel) are potential "philosophers stones" that could iron out many subjective assumptions related to the age estimation of mackerel. It is of the utmost importance that the dimensions and availability of such material is clarified and that efforts are made to reach agreement on potential availability for coordinated validation studies. Alternatively, chemical marker substances can be used in the tag–recapture experiments.

4.5.2 Validation of life history events

Daily ring structures have been validated in otoliths of anchovy, sardine, herring, sprat, and mackerel (Alshuth, 1988b; Moksness, 1992; Alemany and Álvarez, 1994; Johannessen *et al*., 2000; Fox *et al*., 2004; Aldanondo *et al*., 2008; Mendiola and Álvarez, 2008). These studies offer validation of the first years of growth, making standards (L1, etc.), and ruling out double structures in the first years of life.

4.5.3 Other validation methods

Indirect validation methods may be applied to check the accuracy of the age estimation of a given species. For example, catch in numbers of mackerel in the fishery allows the tracing of weak and strong year classes in successive years (ICES, 2013b).

Corroborative methods for validation of annual rings, such as elemental or isotopic cycles, could potentially support age estimation (Campana, 2001). However, this requires knowledge about the chemical environment in which the given species is found. Other age verification methods, e.g. bio-chronology studies of growth increment widths in the otoliths as a supportive tool for age validation, can be useful for long-lived pelagic species such as horse mackerel and mackerel.

5 Deep-water species

Ole Thomas Albert, Christoph Stransky, Jorge Landa, and Rafael Duarte

5.1 Introduction

The assessment of deep-water fish stocks using age-structured models has proven useful in establishing a diagnosis on stock status (ICES, 2013c). However, the approach has several limitations and shortcomings when stock structure, natural mortality, and growth are not adequately known. Age data provided by different countries are often based on age estimation criteria that have not been validated. Therefore, several workshops have been carried out recently on deep-water species in order to conduct a general methodological review, evaluate available information on otolith growth patterns and age estimation issues, and ultimately pave the way for solid input data to age-based assessments. This chapter is based on four ICES workshops on age estimation of deep-water fish (Table 5.1) and describes common problems and agreed guidelines for best practice.

Table 5.1. Recent ICES age estimation workshops on deep-water species.

Workshop	Dates	Location	Chairs	Species
Anglerfish *Illicia*/Otoliths Ageing Workshop (Duarte *et al.*, 2005)	8–12 November 2004	Lisbon, Portugal	Rafael Duarte	White anglerfish *Lophius piscatorius*, black anglerfish *Lophius budegassa*
Workshop on Age Determination of Redfish (WKADR; ICES, 2009b)	02–05 September 2008	Nanaimo, Canada	Fran Saborido-Rey and Christoph Stransky	Beaked redfish *Sebastes mentella*, golden redfish *Sebastes norvegicus*
Workshop on Age Reading of Greenland Halibut (WKARGH; ICES, 2011b)	14–17 February 2011	Vigo, Spain	Ole Thomas Albert and Margaret Treble	Greenland halibut *Reinhardtius hippoglossoides*
Workshop on Age Estimation Methods of Deep-water Species (WKAMDEEP; ICES, 2013c)	21–25 October 2013	Mallorca Island, Spain	Ole Thomas Albert, Beatriz Morales Nin, and Gróa Pétursdottir	Tusk *Brosme brosme*, ling *Molva molva*, blue ling *Molva dypterygia*, roundnose grenadier *Coryphaenoides rupestris*, greater silver smelt *Argentina silus*, black scabbardfish *Aphanopus carbo*, blackspot seabream *Pagellus bogaraveo*
Workshop on Age Estimation Methods of Deep-water Species (WKAMDEEP; ICES, 2019b)	17–21 September 2018	Cadiz, Spain	Ole Thomas Albert, Juan Gil Herrera, and Kelig Mahe	Blackspot seabream *Pagellus bogaraveo*, tusk *Brosme brosme*, greater argentine *Argentina silus*, blue ling *Molva dypterygia*, ling *Molva molva*, greater forkbeard *Phycis blennoides*, black scabbardfish *Aphanopus carbo*

5.2 Background (precision and accuracy)

Accuracy of an age estimation procedure is generally difficult to ascertain. Several traditional validation methods are not applicable to deep-water fish due to the low survival rates after capture as a result of barotrauma. Instead, other approaches, generally referred to as age corroboration or verification methods, are applied for these species. Such methods include modal progression analyses and tracing of strong year classes over many years, but there are also examples of tag–recapture analyses with or without chemical markers.

To date, the focus has mostly been devoted to improving the precision of age estimations of individual species. This includes experiments with different calcified structures, different preparation of samples, different reading axes, and different interpretation of annual zones along those axes. Between-reader comparisons have been conducted both within and between labs with the aim of reaching a common agreement on interpretation of otolith growth zones. Within ICES, the precision of age estimates has generally improved by means of otolith exchange schemes and age estimation workshops, though these have been infrequent for deep-water species in ICES waters. Even with exchanges and workshops, the achieved precision within deep-water species is usually quite low. A summary of the previous results from these workshops is provided in Table 5.2.

The reported precision varied considerably with coefficients of variation (*CV*s), from 8.3% to 22.6%. There is no defined target for this measure, but based on simulations, Powers (1983) found that a *CV* of ≤ 10% would be acceptable when age estimates are used to calculate the population rate parameters (i.e. growth and mortality) needed for stock assessments. Reference to a target *CV* of 10% for the most common age groups is made in Quinn and Deriso (1999). Only one of the previous exchanges listed in Table 5.2 has reported this level of precision.

In addition to these dedicated between-reader comparisons for single species, a small-scale exchange of 50 otolith images for each species was initiated through WebGR (http://webgr.azti.es) prior to the Workshop on Age Estimation Methods of Deep-Water Species (WKAMDEEP; ICES, 2013c). The calibrated images consisted of sagitta otoliths, processed using the protocols employed in individual laboratories. The purpose of the exchange was largely to familiarize all participants with the otoliths from each species to allow everyone to partake in the discussions leading to the agreed recommendations for age estimation protocols. However, owing to the scarcity of precision measures for these species, the results are included here (Table 5.3). For each species, 4–12 age readers interpreted all the otolith images, and the spreadsheet programme by Eltink (2000) was used to compare the age estimates among readers and relative to the modal age for each sample.

In this small-scale exchange, the precision of the age estimates varied considerably between species (Table 5.3). Greater silver smelt was considered the easiest one by all age readers, and the mean *CV* for all 12 age readers was only 7.5%. Also, for ling and roundnose grenadier, the mean *CV* was relatively low compared to many other exchanges of long-lived species. For these three species, the precision is probably high enough to support age-structured analytical assessments.

The mean *CV* was much higher for tusk, black-spotted sea bream, and particularly for black scabbardfish. If this exchange is representative of the present age estimation results of these species, care should be taken when interpreting estimated year-class strength and population rates. However, for some of the age readers, the *CV* was moderate for these species (9.7–12.9%). Only a few of the age readers were trained in age estimation of these species; therefore, it is possible that the *CV* will improve with more training. More exchanges and between-reader comparisons for these species are still needed.

Based on the previous work realized during the WKAMDEEP1 (2013), a second Workshop on age estimation methods of deep-water species (WKAMDEEP2 – ICES, 2019b) was organized. During this workshop, the ageing of several deep-water species were reviewed: blackspot seabream (*Pagellus bogaraveo*), tusk (*Brosme brosme*), greater silver smelt (*Argentina silus*), blue ling (*Molva dypterygia*), ling (*Molva molva*), greater forkbeard (*Phycis blennoides*), and black scabbardfish (*Aphanopus carbo*). The aims of the WKAMDEEP2 were to assemble this group of experts in order to further develop the ageing protocol for all species and to estimate the precision of readings. For each species, a standard ageing protocol was realized with the agreement of all participants and afterwards the exchange of 50 images by species was organized using the SmartDots tool to evaluate the level of precision by species. Results (Table 5.2) showed that, for all deep-sea species, otolith reading is difficult, with a low percentage of agreement between readers and a high coefficient of variation (*CV*) as a consequence of low precision between readers (i.e. results show a difference of several years among readers for the same otolith). The third workshop WKAMDEEP3 is scheduled for 2023 to continue investigations into differences of readings for deep-water species and so to decrease the bias between readers for these difficult species.

Table 5.2. Summary of workshops and exchanges by species.

Species	ICES area	*n*	Preparation of age estimation procedure	No. of readers	Agreement (%)	*CV* (%)	APE (%)	Workshop/ exchange
Ling	Division 5.a	100	Whole in glycerol	3		8		WK 1997 (Bergstad *et al.*, 1998)
	Divisions 3.a, 4.a, and 5.a, and Subarea 6	79	Whole in water			14		EX 2012 (Øverbø Hansen, 2012)
			Sectioned	12	45.7	21.5		WK 2018 (WKAMDEEP2 – ICES, 2019b)

Species	ICES area	*n*	Preparation of age estimation procedure	No. of readers	Agreement (%)	*CV* (%)	APE (%)	Workshop/ exchange
Roundnose grenadier	Division 6.a	64	Transversal slide	11	30	12		EX 2006/ WK 2007 (ICES, 2007)
	Division 6.a	64		6	29	15		EX 2011 (Mahé *et al.*, 2012a)
	Division 3.a	63		7	31	23		WKARRG 2007 (ICES, 2007)
Tusk	Division 5.a	300	Whole in glycerol	3				WK 1997 (Bergstad *et al.*, 1998)
	Division 2.a	50		4	34	21		EX 2010 (ICES, 2013c)
			Whole	11	48.4	11.5		WK 2018 (WKAMDEEP2 – ICES, 2019b)
Black scabbard-fish	Madeira	50	Whole left + right	10		27		EX 1998–1999 (Morales-Nin, 1999)
	Rockall Trough	20	Sectioned	11		22		
	Division 5.b Faroe Ground	50		11	36.7	25.6		WK 2018 (WKAMDEEP2 – ICES, 2019b)
Greenland halibut	Canada (Flemish Cap)	100	Whole in glycerol, whole-baked, or transected			4–18		Treble and Dwyer (2008); ICES (2011b)
		184				11–13		
Golden redfish	Division 5.a; NAFO 3M	90	Break-and-burn, break-and-bake	7–8	12–35	20–26	15–20	EX 2007–2008 (ICES, 2009b)
					42–57	9–18		WKADR 2008 (ICES, 2009b)
Beaked redfish	Subareas 2 and 12, Division 14.b; NAFO 1–2, 3M	273	Break-and-burn, thin sections, break-and-bake	7–8	9–34	15–22	11–16	EX 2007–2008 (ICES, 2009b)
					42–57	9–18		WKADR 2008 (ICES, 2009b)
Blackspot seabream	Mediterranean Sea	50	Whole	12	34.7	30.8		WK 2018 (WKAMDEEP – ICES, 2019b)
Greater argentine	Northeastern Atlantic	50	Whole	12	68.7	8.7		WK 2018 (WKAMDEEP – ICES, 2019b)
Blue ling	Division 5.b and subareas 6 and 7	50	Sectioned	12	34.7	17.1		WK 2018 (WKAMDEEP – ICES, 2019b)

Species	**ICES area**	***n***	**Preparation of age estimation procedure**	**No. of readers**	**Agreement (%)**	***CV* (%)**	**APE (%)**	**Workshop/ exchange**
Greater forkbeard	Division 2a	50	Sectioned	12	54.5	33.9		WK 2018 (WKAMDEEP – ICES, 2019b)
White anglerfish	Divisions 7.g–h, 8.a–b, and 8.d	53	Illicia sections	5	-	-	20	WK 1991 (Anon., 1997)
	Divisions 7.g–h, 8.a–b, and 8.d	45		8	-	-	17	WK 1997 (Anon., 1997)
	Divisions 7.a–b, 7.d, 8.c, and 9.a	147		6–8	-	21–25	16–19	EX 1998; WK 1999 (Anon., 1999)
	Divisions 7.a–b, 7.d, 8.c, and 9.a	86		8	47	25	-	EX 2001; WK 2002 (Landa *et al.*, 2002)
	Subarea 7	50		15	40	21	16	EX 2004; WK 2004 (Duarte *et al.*, (2005)
	Divisions 5.b, 7.b–c, and 7.k	100		11	45	27	18	EX 2002 (Landa, 2011)
	Divisions 7.g–h, 7.a–b, and 7.d	53	Otolith sections	5	-	-	29	WK 1991 (Anon., 1997)
	Subarea 7	50		11	12–15	41–46	33	EX 2004; WK 2004 (Duarte *et al.*, 2005)
	Divisions 5.b, 7.b–c, and 7.k	100	Whole otoliths	12	20	24	22	EX 2011 (Landa, 2011)

Species	**ICES area**	***n***	**Preparation of age estimation procedure**	**No. of readers**	**Agreement (%)**	***CV* (%)**	**APE (%)**	**Workshop/ exchange**
Black anglerfish	Divisions 7.g–h, 8.a–b, and 8.d	54	Illicia sections	5	-	-	21	WK 1991 (Anon., 1997)
	Divisions 7.g–h, 8.a–b, and 8.d	44		8	-	-	13	WK 1997 (Anon., 1997)
	Divisions 7.a–b, 7.d, 8.c, and 9.a	138		6–8	-	18–45	10–36	EX 1998; WK 1999 (Anon., 1999)
	Divisions 7.a–b, 7.d, 8.c, and 9.a	76		7	44	17	-	EX 2001; WK 2002 (Landa *et al.*, 2002)
	Division 9.a	50		13	26	27	22	EX 2004; WK 2004 (Duarte *et al.*, 2005)
	Divisions 7.g–h, 8.a–b, and 8.d	53	Otolith sections	5	-	-	30	WK 1991 (Anon., 1997)
	Subarea 7	50		11	9–13	41–47	34	EX 2004; WK 2004 (Duarte *et al.*, 2005)

Table 5.3. Summary of the small exchanges made before and during the Workshop on Age Estimation Methods of Deep-water Species (WKAMDEEP; ICES, 2013c). ***CV*****: coefficient of variation; PA: percent agreement; RB: relative bias; "Length-at-age 5" etc.: mean length-at-age (in cm) of each species in 5-year increments (up to 20 years), often expressed as a range across age readers.**

Measure	Greater silver smelt	Tusk	Ling	Blackspot seabream	Black scabbardfish	Roundnose grenadier
Mean *CV*	7.5	16.9	10.3	15.3	31.6	10.9
CV per reader	4.2–9.2	12.9–23.7	8.0–14.3	11.1–17.7	9.7–26.0	9.0–11.4
Mean PA	60	37	60	45	33	51
PA per reader	32–86	20–57	28–80	41–49	5–62	32–67
Mean RB	0.09	–0.18	–0.09	0.18	–0.3	–0.54
RB per reader	–0.3 to 0.6	–1.1 to 1.2	–0.9 to 0.4	–0.6 to 0.9	–4.0 to 3.6	–1.5 to 0.9
Length-at-age 5	36–36	43–51	55–67	21–34	101–106	6–7
Length-at-age 10	40–42	54–64	72–103	44–47	96–120	9–12
Length-at-age 15	45–48	–		50–53	101–110	12–14
Length-at-age 20						15–16
No. of age readers	12	10	9	4	6	4

5.3 General recommendations for age estimation of deep-water fish

The preferred methods for age estimation of individual deep-water species are listed in Table 5.4. For routine age estimation of the majority of the species considered in WKAMDEEP, it is often considered sufficient to count annuli on the surface of the whole otoliths. The otoliths should be immersed in distilled water for 24 h prior to observation with a compound microscope. For some species, transversal sections at core level are used for the whole length range, while for others, only for the largest specimens. However, it has been observed that transversal sections do not improve the age interpretation for some species (e.g. ling).

A summary of the age estimation procedure using illicia (extraction, preparation, and the standardized age estimation criteria) for both white and black anglerfish species is available (Duarte *et al.*, 2005). Modifications in the methodology of illicia preparation and in the age estimation criterion for white anglerfish are included in Landa *et al.* (2013).

Table 5.4. Summary of preferred age estimation procedures by species.

Species	Structure	Preparation	Observation	Preferred reading axis	Comments
Tusk	Whole otolith	In water for 24 h	Water	No preferred reading axis	
Ling	Whole otolith	In water for 24 h	Water	Towards rostrum	Difficult for L > 90 cm

Species	Structure	Preparation	Observation	Preferred reading axis	Comments
Blue ling	Transverse sectioned otolith (0.4 mm)	Unclear effects of polishing	Oil	Longest axis and close to the *sulcus acusticus*	Some use TNPC software to guide the interpretation
Greater silver smelt	Whole otolith	In water for 24 h or mounted in Eukitt on black plastic plates	Water	Towards rostrum	
Roundnose grenadier	Transverse sectioned otolith (0.2 mm)		Oil	Start with the longest axis and continue on the *sulcus acusticus* side	Some use TNPC software to guide the interpretation
Blackspot seabream	Whole otolith		Water	Towards rostrum or post-rostrum	Some use Image-Pro Plus software to guide the interpretation
Black scabbardfish	Transverse sectioned otolith (0.5 mm)	In 1:1 glycerin-alcohol for 24 h	1:1 glycerin-alcohol	Start with the ventral axis and bend towards the *sulcus acusticus* side	Some use TNPC software to guide the interpretation
Greenland halibut	Whole right otoliths or sectioned left otoliths	Whole otoliths: stored frozen and read before drying, or dried and submerged in glycerine for 24 h	Whole otoliths usually observed in water; sectioned with oil or water	Either longest growth axis of the whole right otolith, or towards the proximal edge axis of the sectioned left otolith	Some use image analyses software to improve appearance of zones and to make annotations
Golden redfish	Otolith	Break-and-burn, thin-sections	Oil (when break-and-burn)	Start with the longest axis and continue on the *sulcus acusticus* side	
Beaked redfish	Otolith	Break-and-burn, thin-sections	Oil (when break-and-burn)	Start with the longest axis and continue on the *sulcus acusticus* side	
White anglerfish and black anglerfish	Sectioned illicium 0.5 mm	Section fixed in glass microscope slide		No preferred reading axis	Recommended only up to ages 6–8 (< L~80 cm in white anglerfish) due to the presence of multi-checks and opacity that increases with age
	Whole otolith		Water or 1:1 glycerin-water		

In order to avoid age overestimation, it is generally recommended to use the same magnification for otolith reading, irrespective of the otolith size. Not all laboratories use image analysis systems, but it was generally agreed that they are very useful for measuring annuli and for checking precision among readers. WKAMDEEP also recommended that all laboratories should build up a register of calibrated and annotated otolith images, both for documentation purposes and for training of new age readers. With regard to age interpretation of the species considered, it was clear that several issues were common for most, if not all, of the species. A summary of the discussions regarding issues associated with identification of the first zone, the occurrence of transition zones, and the characteristics of the slow growth zones of older specimens is provided below.

5.4 Identification of the first true annulus

For the greater part of the species, the location of the first annulus is a matter of difficulty. Most species have a complex zonation pattern in the central area, with rings that may or may not correspond to life history events (i.e. hatching, settlement marks) that obscure the annuli identification. Due to the lack of knowledge on the early lives of most deep-water fish, the interpretation of these initial rings is usually not clear. Moreover, some species have multiple or extended spawning periods (e.g. greater silver smelt) that may cause different ring patterns, depending on the birth date and environmental conditions.

If feasible, it is recommended to measure otolith dimensions from juvenile fish, preferably down to 0- and 1-groups, and to construct a growth curve of an easily recognizable growth axis (Figure 5.1). By interpolation to the assumed time when the first annulus is formed, the expected size of this annulus is estimated. For species with multiple or extended spawning seasons, it is important to capture the variability in otolith size at juvenile ages. Use of expected size to identify the first annulus may be justified if the frequency histogram of otolith sizes shows clear modal groups attributable to both 0- and 1-groups.

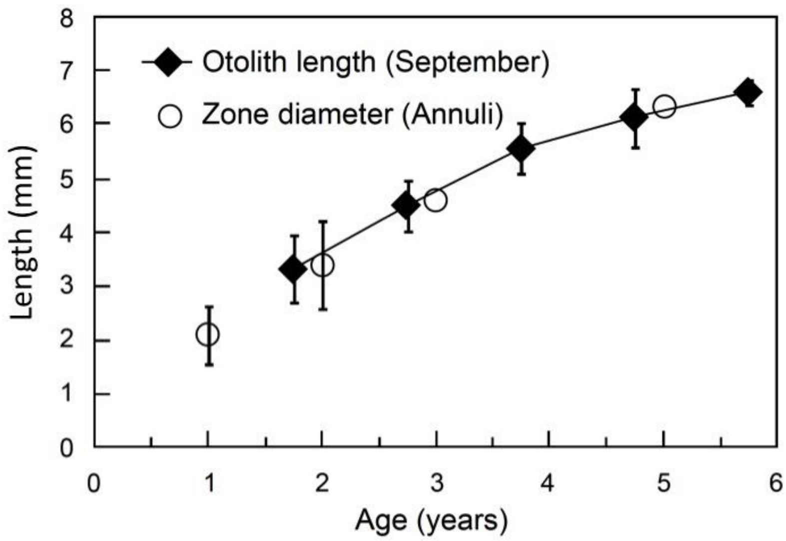


Figure 5.1. Mean otolith length of Greenland halibut at ages 1–5 in September (solid diamonds) and mean zone diameter at ages 1–5 for fish age estimated at more than 10 years (open circles). The bars indicate ± 2 s.d. Age is plotted as a fraction of a year and the annual zones are assumed to be laid down on 1 January (from Albert *et al.*, 2009).

5.5 Changing growth patterns along the fish lifespan

In several species, the otolith growth pattern (increment spacing, increment appearance) may change in the intermediate otolith zone, i.e. in an area consisting of several annual zones between the juvenile fast-growth zones and the often regular, distinct and narrow zones of older ages. The pattern is well described for redfish (ICES, 2009b) and is also recognized for Greenland halibut (Albert *et al.*, 2009; ICES, 2011b; Albert, 2016) and for several of the species dealt with in WKAMDEEP. These changes may be related to sex change (e.g. *Pagellus* spp.), sexual maturity, migrations, and/or diet changes.

In many cases, it is recommended to count annuli along a curved or broken axis, with the bend or kink occurring within or at the end of the intermediate zones. The problematic "checks" are usually more pronounced in the juvenile phase, while in the older years, the problem of zones being vague or discontinuous becomes more serious. Consequently, there is often a danger of overestimating annuli along the first axis or reading direction, and a danger of underestimating annuli along the second axis or reading direction. The actual place where the shift in reading direction occurs is usually not well defined. It is advised that, whenever possible, validation studies with chemical marks should be used to clarify how zones should be interpreted around the inflection point of the reading axis.

5.6 Marginal otolith area

The last annuli formed in old specimens tend to be very narrow and incomplete around the otolith perimeter. Generally, they are first laid down in the area of the longest growth axis. Their narrowness may hinder their identification as annuli; also, they can be confused with checks within the growth zones. For older individuals of some species (e.g. redfish), these zones may become so narrow that they require microscopes of higher quality (or with higher magnification) than those usually available in age estimation labs.

The otolith edge observation and the age-class estimation procedures are summarized in Table 5.5. The age attribution may depend on the spawning period and the time of the opaque zone formation (Morales-Nin and Panfili, 2002). A birth date of 1 January is established for all the studied species. There has been no edge analysis for most species, and the following general rule is considered adequate and is furthermore illustrated in Figure 5.2.

- Captured in quarter 1: A translucent zone is formed at the edge; this should be counted.
- Captured in quarter 2: On very young fish, an opaque zone may be seen at the edge. All translucent zones should be counted.
- Captured in quarter 3: The opaque zone forms and should be visible. If only a thick translucent zone can be seen, it is most likely last winter's growth and should be counted.
- Captured in quarter 4: The opaque zone is mostly formed. The translucent zones can be seen, especially in younger fish. The translucent zone on the edge should not be counted until 1 January.

Table 5.5. Summary of otolith edge and age-class attribution procedures by species.

Species	Spawning period	Opaque edge
Tusk	April–July	No information
Ling	March–August	No information
Blue ling	February–May	No information
Greater silver smelt	Extended or multiple periods throughout the year	No information
Roundnose grenadier	May–November	August–March
Blackspot seabream	Throughout the year	May–September
Black scabbardfish	September–December	July–December (Madeira) April–September (Mainland Portugal)
Greenland halibut	October–December	No information
Golden redfish	March–May	No information
Beaked redfish	March–May	No information
White anglerfish	Varies among areas; mainly November–July	May–September
Black anglerfish	Varies among areas; mainly November–July	May/June–August/September

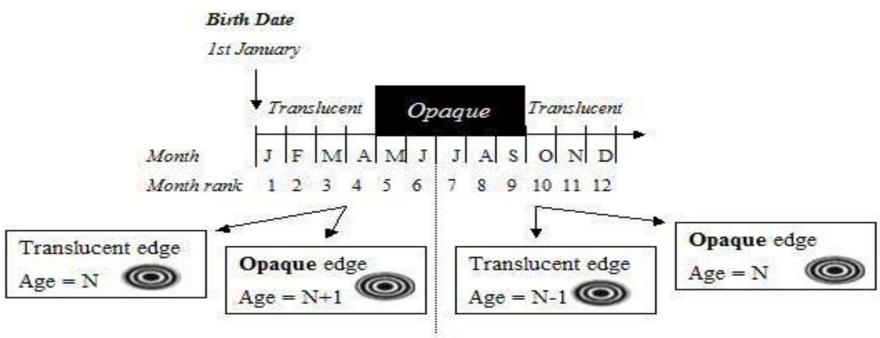


Figure 5.2. Figure describing the general rule on how to count the edge. N = number of complete annual zones (from Morales-Nin and Panfili, 2002).

5.7 Use of supplementary information

The use of daily growth increments (albeit not validated) may help identify the temporal meaning of the first rings and also locate the true annuli. A subsample of otoliths could be prepared, and the enumeration of their daily growth increments may be used to estimate at which age each check was laid down. Once the growth pattern is identified, measuring the distances from the otolith core to the true first annulus could be used to establish an age estimation protocol.

Length frequency analysis is feasible for fast-growing species with short spawning seasons (Pauly, 1983); therefore, its use for deep-water species is limited to the fast-growing juvenile phase. Moreover, in most cases, the small sizes are not present in the landings or surveys, precluding the use of this method. The method may potentially be used to identify the first modal lengths and to clarify whether the first age is correctly estimated.

However, modal analyses of groups of otolith weights could help in identifying more juvenile age classes. Since otoliths tend to grow even when the fish itself does not (Campana and Casselman, 1993; Cardinale *et al*., 2000; FAbOSA, 2002), the cubic root of otolith weight will be linearly related to age for a longer age span than the relationship between fish length and age. Previous studies of otoliths from a wide range of long-lived, deep-water species have shown that the relative weight of an otolith in relation to the somatic weight increases with age (Talman *et al*., 2003). It is, therefore, recommended to study the otolith weights for species where representative sampling of juveniles is feasible. Modal progression analyses may also be applied to the otolith's annuli diameters or radius to assist identification of the true annuli.

When possible, mark–recapture validation experiments are recommended. To solve interpretation issues, it is recommended to have the otolith to hand. For instance, in *Pagellus* mark–release experiments (ICES, 2013c), the length was collected, which helped determine the growth rate, but not the otolith structure. Therefore, it is recommended to mark the fish both externally and internally in order to have a check point on the otolith growth pattern, and to collect the otoliths. This method, although requiring an extensive marking programme, was applied as an aid to solve age interpretation issues in European hake and Greenland halibut (Albert *et al.*, 2009; Mellon-Duval *et al*., 2010; Albert, 2016). For many deep-water species, tagging programmes will only be feasible with the application of new technological solutions. One example is the underwater tagging equipment (UTE), developed by Star-Oddi and applied as a tagging programme for deep-water redfish (Sigurdsson *et al*., 2006).

It is strongly recommended to use radiometric methods (i.e. bomb radiocarbon or lead-radium) to validate the ages interpreted for old fish. These methods have proven useful in validating age interpretation of *Sebastes* species (Andrews *et al*., 2002; Stransky *et al*., 2005a).

The use of image analysis software might help both with interpretations of seasonal zones and in comparisons of individual interpretations. During WKAMDEEP 2013 (ICES, 2013c), some calibrated images from the small exchange with the best percentage of agreement among readers were interpreted with the TNPC software (Figure 5.3; ICES, 2013c). This is an example of several image analysis software packages supporting the interpretation, e.g. by displaying the gray-scale pattern along the reading axis. Such analysis programs can also be used to establish individual growth trajectories and to compare interpretation of annual zones between readers.

5.8 Age and growth of individual deep-water species

For most deep-water species, sufficiently validated age estimation protocols do not exist. Still, for several species, plausible interpretations have been established and agreed upon through exchange programmes and workshops. In many cases, there is supporting evidence from, e.g. length frequency or marginal increment analyses. For a few species, validation of part of the age span has been achieved, while other stocks still lack general validation of a complete protocol. The available corroboration and validation work for each species is reported on here, together with an example of age interpretation with the recommended protocol. Shown is also the resulting length-at-age for selected areas and age groups in tabular form.

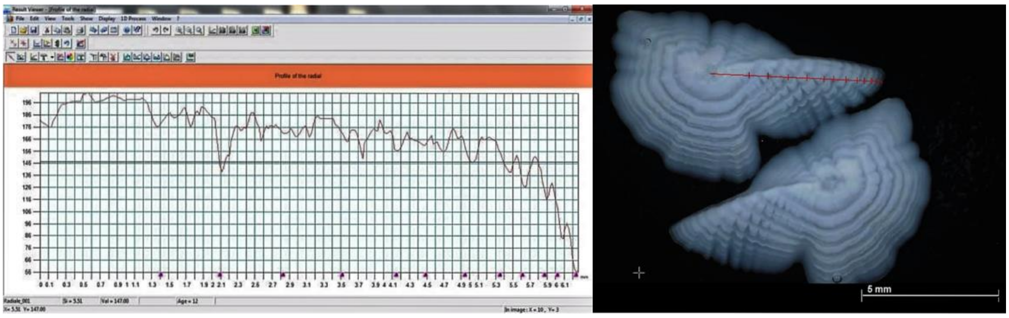


Figure 5.3. TNPC software to help interpret annual zones. The right panel shows the preferred reading axis and interpreted annual zones on a whole otolith of greater silver smelt. The left panel shows the greyscale profile of the image along the chosen reading axis, as well as the position of the annotations.

5.8.1 Tusk (*Brosme brosme*)

There has been no direct validation of age estimation for tusk. Analysis of Icelandic length frequencies, for age groups 2–4 years, showed good correspondence between modes and the length of successive age groups obtained by age estimation of otoliths (Bergstad *et al*., 1998). A mode found at 15 cm represented the 2-group (Bergstad *et al*., 1998). Table 5.6 shows the mean length at various ages in samples taken from three different areas (Iceland, Norway, and the Faroes).

Tusk otoliths are viewed whole and submerged in water for at least 24 h prior to age estimation. They are read directly under a microscope, *sulcus acusticus* side up, using reflected light against a black background (Figure 5.4). The zonation pattern is generally considered blurred and difficult to interpret. Both otoliths are examined, and the one with the clearer annuli is used. The areas of the otolith where the annuli are most distinct are used for counting. A more detailed description of best practice regarding reading axes is not available at present. An illustrated manual for age estimation of tusk otoliths has been assembled by Bergstad and Hareide (1997).

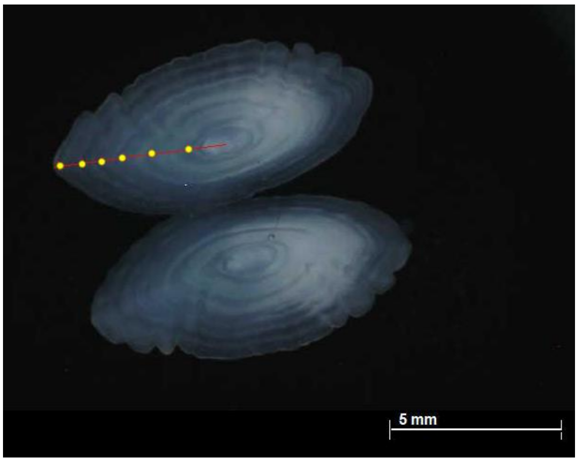


Figure 5.4. Images of otoliths with annotations originating from a tusk 48 cm long, interpreted as being age 6. The first narrow translucent zone is not counted, as recommended by ICES (2013c).

Table 5.6. Mean length (s.d. = standard deviation, *n* = number of observations) of different age groups of tusk. Data compiled by the WKAMDEEP 2013 (ICES, 2013c).

Area	Age 1	Age 5	Age 10	Source
Iceland	12.9 cm (s.d. = 2.3, *n* = 22)	37.0 cm (s.d. = 7.0, *n* = 1 015)	58.7 cm (s.d. = 6.9, *n* = 1 040)	HAFRO
Norway		33.5 cm (s.d. = 3.5, *n* = 2)	57.0 cm (s.d. = 4.4, *n* = 57)	Norwegian IMR
Faroe Islands		36.9 cm (s.d. = 4.0, *n* = 11)	49.0 cm (s.d. = 4.6, *n* = 781)	FAMRI

5.8.2 Ling (*Molva molva*)

There has been no direct validation of age estimation for ling. Based on agreed protocols, growth is rapid, with lengths of 37–62 cm at age 3 and ca. 100 cm at age 10, and it can reach at least 25 years of age (Jónsson and Pálsson, 2013; Table 5.7). Ling otoliths are commonly viewed whole and submerged in water for at least 12 h prior to age estimation. They are read under a microscope, with the *sulcus acusticus* side down, against a black background using reflected light. The zones are difficult to distinguish in older specimens (Figure 5.5).

Table 5.7. Area, mean length of ling at ages 3, 5, and 10 (*n* = number, s.d. = standard deviation), and source. Data compiled by the WKAMDEEP.

Area	Age 3	Age 5	Age 10	Source
Iceland (Division 5.a)	37.5 cm (s.d. = 8.5, *n* = 31)	60.2 cm (s.d. = 12.9, *n* = 619)	101.7 cm (s.d. = 62.6, *n* = 284)	MRI Iceland
Norway (Division 2.a)	62.8 cm	74.5 cm	95.4 cm	Norwegian IMR
Shetland (Division 4.a)	51 cm (*n* = 168)	66 cm (*n* = 140)	102 cm (*n* = 43)	Angus (2011)
Faroe Islands (Division 5.b)	53.9 cm (s.d. = 9.0, *n* = 168)	62.3 cm (s.d. = 6.9, *n* = 140)	99.4 cm (s.d. = 7.9, *n* = 43)	FAMRI Faroe Islands

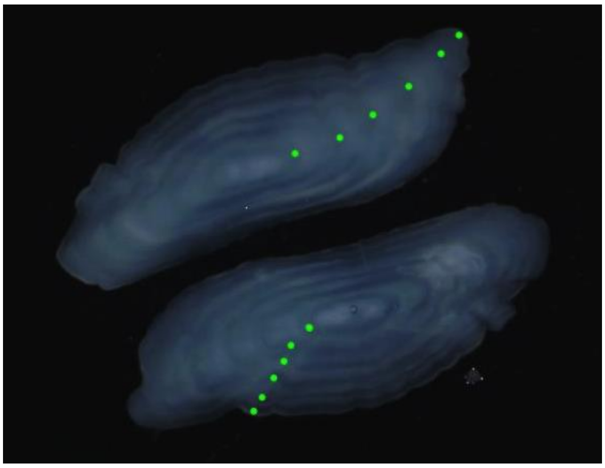


Figure 5.5. Ling otoliths from a fish captured in February and interpreted as being age 6.

5.8.3 Blue ling (*Molva dipterygia*)

There has been no direct validation of age estimation for blue ling. Bergstad *et al*. (1998) carried out an indirect validation with length frequency distribution analysis. The Icelandic data from groundfish surveys conducted every year in March showed modes among the smallest fish (from fish at TL < 60 cm). The 1-group fish in March were mostly < 20 cm, with the 2-group varying between 20 and 40 cm. It was possible to achieve reasonable consistency among readers, but only for otoliths from juveniles of up to 3–4 years old (Bergstad *et al*., 1998). Mean lengths of different age groups from various areas are shown in Table 5.8.

Blue ling has been aged by counting the rings on broken surfaces or thin sections (Ehrich and Reinsch, 1985; Thomas, 1987; Bergstad, 1991; Magnusson *et al*., 1997; Bergstad *et al*., 1998; Magnussen, 2007; Figure 5.6). Transverse, thin, and sectioned otoliths are generally considered the best structures for age estimation of this species.

Table 5.8. Mean length of different age groups of blue ling.

Area	Age 10	Age 15	Age 20	Source
Faroese waters	93 cm	109 cm	127 cm	Ehrich and Reinsch (1985)
ICES Division 6.a	91 cm	132 cm	117 cm	French data 2010
Iceland	87 cm	112 cm	137 cm	Magnussón and Magnussón (1995)

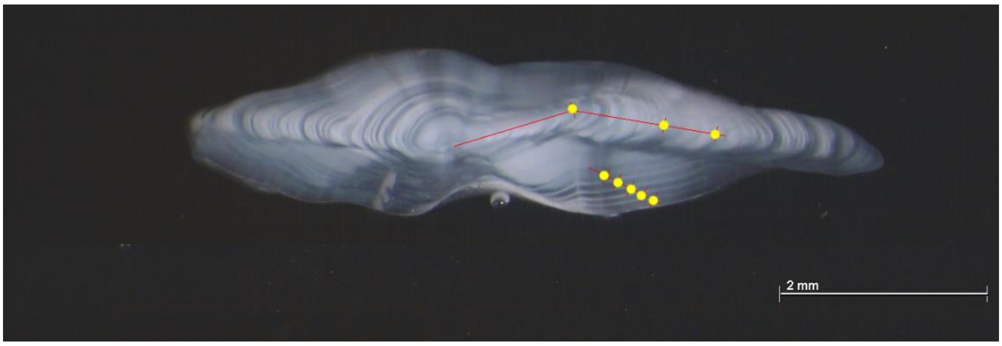


Figure 5.6. Broken interpretation axis for a blue ling otolith, interpreted as being age 8.

5.8.4 Roundnose grenadier (*Coryphaenoides rupestris*)

There has been no direct validation of age estimation for roundnose grenadier, but an indirect validation was carried out with marginal increment analysis which concluded that rings in the otoliths were formed annually (Gordon *et al*., 1996; Gordon and Swan, 1996; Swan and Gordon, 2001).

The preferred structures for estimating the age of the roundnose grenadier are otoliths that are transverse-sectioned through the nucleus (Figure 5.7). Two reading axes are used for the first several annuli and later annuli, respectively. Previously, age estimation of roundnose grenadier was done with whole otoliths, sectioned otoliths, and scales, but Bergstad (1990) indicated that scales may be unsuitable, and Kelly *et al*. (1997) showed that whole otoliths can only be read for very small individuals.

To help identify the first zone, a recent workshop (ICES, 2013c) measured the distance between the nucleus and the end of the first translucent ring in a sample of 40 otoliths. The results showed very little differences among readers, with a mean distance of 1.7 mm (s.d. = 0.2 mm) and a range of 1.1–2.1 mm. Table 5.9 shows the length range at various ages from two different areas.

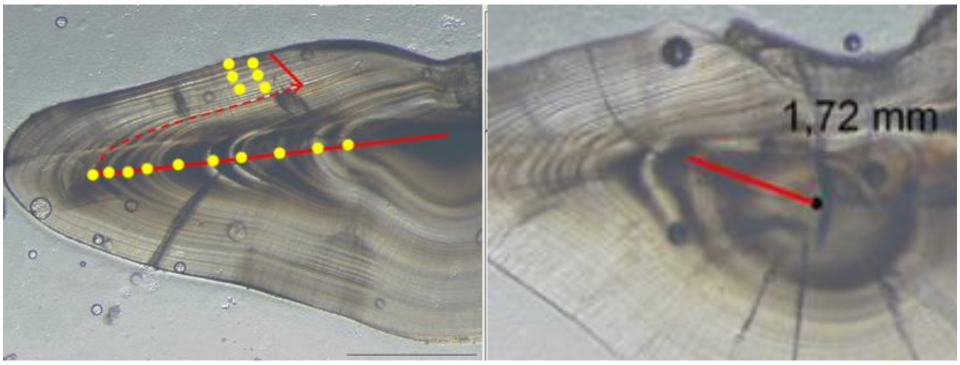


Figure 5.7. Left: interpretation axes for roundnose grenadier otoliths, together with one age reader's interpretation of 16 annual zones. Right: measurement of first radius to help identify the first annual zone in another otolith.

Table 5.9. Length range (pre-anal length, cm) for various age groups of roundnose grenadier.

Area	Age 20	Age 25	Age 30	Source
Division 6.a	12–17.5	13–17.5	15–18.5	Modal age from European exchange in 2011
Division 3.a	14.5–17.5	14.5–19	17–21	Modal age from European exchange in 2011

5.8.5 Greater silver smelt (*Argentina silus*)

There has been no direct validation of age estimation for greater silver smelt. Based on agreed protocols, maximum age can be up to 40 years. Whole otoliths are usually mounted on black plastic plates or submerged in water (for at least 24 h), and interpreted, *sulcus acusticus* side down, using reflected light against a black background. It is best to read the otoliths along the axis from the nucleus to the rostrum (Bergstad, 1995; Figure 5.8). The age estimation is generally considered relatively easy and with good precision. Mean length-at-age for ages 1, 5, 10, and 15 differ by only 4 cm between the institutes of Iceland, Norway, and Faroe Islands (Table 5.10).

The interpretation of the first annual zone, defining the end of the 0-group, varies to some extent between readers. It is believed that due to the prolonged spawning season of greater silver smelt, the first fast-growth zone can vary in size and may sometimes appear small compared with the subsequent opaque zones.

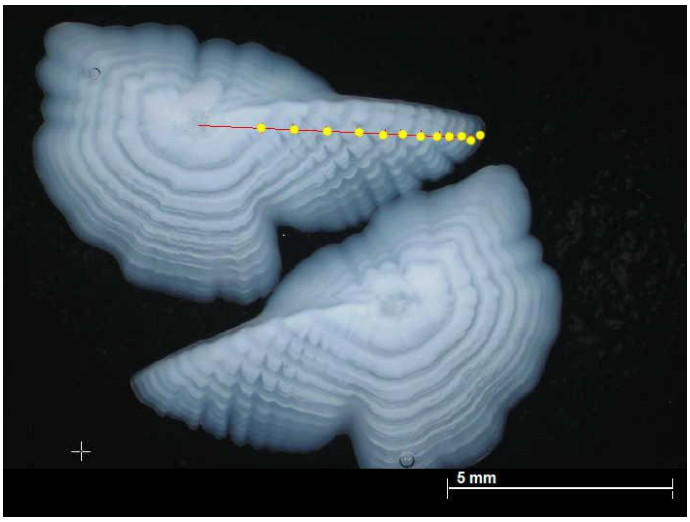


Figure 5.8. Image of greater silver smelt otoliths from a 42-cm long fish caught in February, displaying clear patterns in the translucent and opaque zones. The age is estimated at 12 years.

Table 5.10. Mean length (*n* = number, s.d. = standard deviation) of various age groups of greater silver smelt.

Area	Age 1	Age 5	Age 10	Age 15	Source
Iceland	19.1 cm (s.d. = 3.1, *n* = 239)	33.9 cm (s.d. = 2.7, *n* = 578)	40.5 cm (s.d. = 2.4, *n* = 1 472)	43.9 cm (s.d. = 2.9, *n* = 410)	MRI
Faroe Islands	15.4 cm (s.d. =2.1, *n* = 49)	31.0 cm (s.d. = 2.0, *n* = 146)	38.5 cm (s.d. = 2.3, *n* = 95)	42.3 cm (s.d. = 2.2, *n* = 39)	FAMRI
Norway		29.9 cm (s.d. = 1.9, *n* = 132)	36.1 cm (s.d. = 2.7, *n* = 88)	39.4 cm (s.d. = 3.6, *n* = 43)	Norwegian IMR

5.8.6 Black scabbardfish (*Aphanopus carbo*)

There has been no direct validation of age estimation for black scabbardfish. The annual deposition of increments has been corroborated by marginal increment analysis (Morales-Nin and Sena-Carvalho, 1996; Vieira *et al.*, 2009) showing that, in specimens from Madeira, opaque increments were formed mostly between July and December and that the highest occurrence of opaque margins was in October.

The preferred structure for estimating the age of black scabbardfish is thin sections of otoliths (0.5 mm) through the nucleus (Figure 5.9). The sections are observed under a stereomicroscope using transmitted light, and the best axis for age estimation is along the ventral side (Vieira *et al.*, 2009). In older fish, the growth axis in the last years is oblique to the axis in the first years. The first translucent zone is expected to appear at 0.73 ± 0.01 mm and 0.56 ± 0.02 mm from the nucleus on the ventral and on the dorsal side of the otolith, respectively (Vieira *et al.*, 2009). Table 5.11 shows the length range of selected age groups of black scabbardfish from various localities.

There are many false increments (checks) along the growth axis, mostly in the area corresponding to the juvenile growth area. Hence, the agreed way to identify increments is to consider thicker opaque bands instead of individual rings.

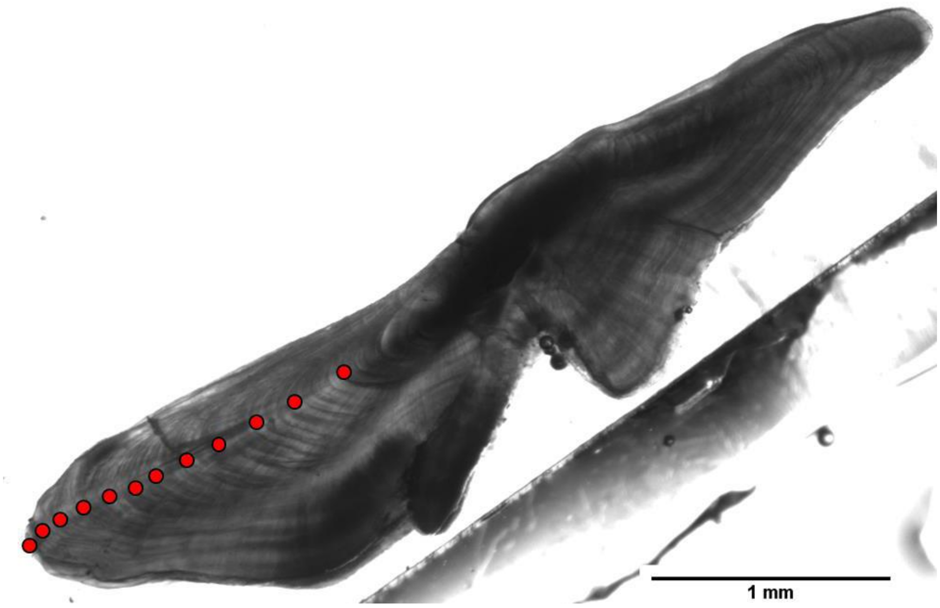


Figure 5.9. Transversal section of the right otolith of a black scabbardfish at 118 cm TL. The estimated age for this specimen is 12 years.

Table 5.11. Length range (cm) of selected age groups of black scabbardfish. NA = not available.

Area	Sex	Age 1	Age 5	Age 8	Age 12	Reference
Mainland Portugal	F	NA	87–97	105–113	120–130	Vieira *et al*. (2009)
	M	NA	85–95	100–115	NA	
Azores	F	NA	NA	175–113	140–130	
	M	NA	NA	85–95	115–120	
Madeira	F	NA	NA	107–112	120–137	
	M	NA	NA	107–115	120–132	
	F	58–70	104–138	114–150	NA	Morales-Nin and Sena-Carvalho (1996)
	M	58–94	100–130	130	NA	
Canary Islands	Both	NA	NA	105	125	Delgado *et al*. (2013)

5.8.7 Blackspot seabream (*Pagellus bogaraveo*)

There has been no direct validation of age estimation for blackspot seabream. A few tag–recapture experiments were conducted along the southern coast of Spain and in the Strait of Gibraltar, giving some guidance to annual length increment of the stock. Daily increment analysis on young individuals was also carried out in order to detect and clarify the position of the first annual ring. The initial results indicated that all specimens in the range of 14–15 cm in length had completed the first annual ring.

Age estimation of blackspot seabream is commonly done by counting the annuli on the whole otolith surface (Ramos and Cendrero, 1967; Sánchez, 1983; Alcazar *et al*., 1987;

Krug, 1989; Castro, 1990; Sobrino and Gil, 2001; Gil, 2006, 2010; Figure 5.10). The otoliths are observed under a binocular microscope with reflected light against a black background. Defined annuli are usually most easily found along the fast-growth axis of the otoliths, particularly along the post-rostrum. Table 5.12 shows the length range of various age groups of blackspot seabream from various localities.

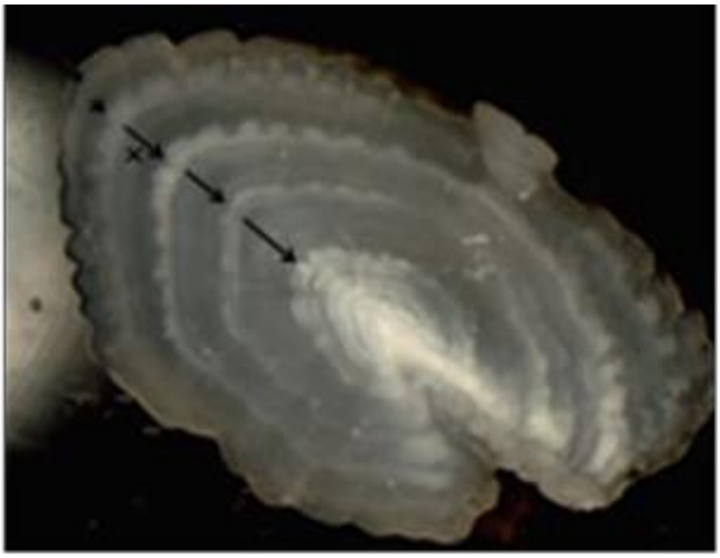


Figure 5.10. Whole otolith of blackspot seabream. The estimated age for this specimen is 5 years.

Table 5.12. Length range (cm) of selected age groups of blackspot seabream.

Area	Sex	Age 1	Age 5	Age 8	Reference
Ionian Sea	M	-	22–27	30–35	Chilari *et al*. (2006)
	F	-	22–28	30–34	
Strait of Gibraltar	All	15–19	29–43	43–54	Sobrino and Gil (2001)
	All	-	29–43	43–51	J. Gil (pers. comm.)
Azores	All	12–18	23–36	34–44	Krug (1989)
Ionian Sea	All	19	29–35	37–39	A. Anastasopoulou (pers. comm.)
Asturian waters	All	15–20	24–38	36–44	Alcazar *et al*. (1987)
Cantabrian Sea	All	8–15	24–36	42–46	Guéguen (1969)

5.8.8 Greenland halibut (*Reinhardtius hippoglossoides*)

Age estimation protocols for Greenland halibut have been partly validated by bomb radiocarbon analyses (Treble *et al*., 2008; Dwyer *et al*., 2016) and by mark–recapture experiments using chemical tags (Treble *et al*., 2008; Treble and Dwyer, 2008; Albert *et al*., 2009; Albert, 2016). The validations are also corroborated from comparisons with modal progression analyses, with growth increments from traditional tag–recapture experiments, and with morphometric analyses (Treble *et al*., 2008; Albert *et al*., 2009; Albert, 2016).

Based on present knowledge, identification of annual zones in Greenland halibut otoliths should preferably be done either along the longest growth axis of the whole right otolith or towards the proximal edge of the sectioned left otolith (ICES, 2011b). In both cases, the age interpretation is often made on digital images, as readers often find it helpful to visualize the growth history to help identify checks from annual zones.

It is essential to use only the right otoliths when reading whole otoliths of this flatfish, and to count the zones along the longest growth axis (Figure 5.11). Analyses of chemically marked individuals have shown that, for older, slow-growing fish, additional growth may not be visible in other parts of the whole otoliths (Albert, 2016). To help identify the narrow zones of older fish, it is further recommended to store the otoliths frozen until imaging and to use transmitted light. For transverse sections, the left otoliths are usually chosen, since they grow more in thickness. Some readers prefer staining, while others find this unnecessary.

The inner edge of the first translucent zone defines a shape with a longest diameter of 2.0 (± 0.5) mm; the following zones usually decrease gradually in zone width. For the slowest-growing fishes, annual zones may be very thin or not visible at all on the surface of the whole right otolith (Albert, 2016). It is still unresolved if these missing zones may be found on the transverse sections of the left otoliths. Table 5.13 shows the length range of various age groups of Greenland halibut from various localities.

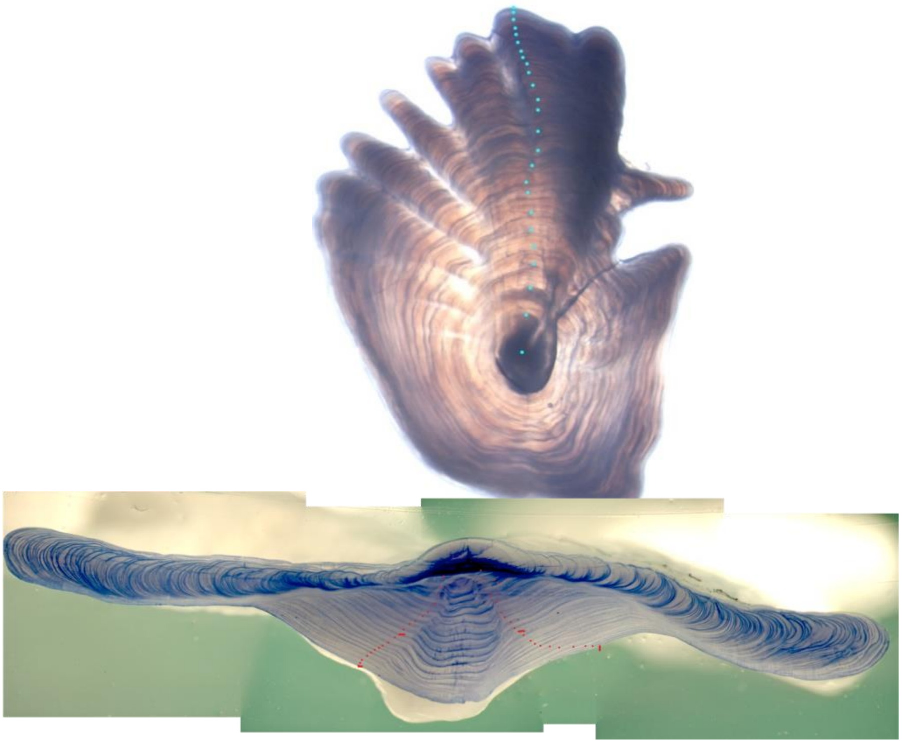


Figure 5.11. Age estimation of the same Greenland halibut individual by two recommended procedures: the sectioned left otolith (top) and the whole right otolith (bottom). The two methods result in an age estimate for this 70-cm fish of 20 and 24 years, respectively.

Table 5.13. Length range (cm) of selected age groups of Greenland halibut.

Area	Sex	Age 1	Age 10	Age 20	Reference
Barents Sea	All	10–19	40–60	58–83	Albert *et al*. (2009); ICES (2011b)
West Greenland	All	11–17			Sünksen *et al. (*2010)
Pacific	All		59–75	66–97	Gregg *et al*. (2006)

5.8.9 Golden redfish (*Sebastes norvegicus)*

Several of the typical growth patterns in redfish otoliths may aid age estimation to a certain degree. WKADR has recommended the identification of reference distances from the nucleus to indicate the position and size of the first annulus (ICES, 2009b); however, these have not yet been established. It is advised that otolith reference collections of known age and with clear annuli patterns are used instead. For redfish the transition zone has been defined as the region of change from juvenile to mature growth (after ca. 10–12 years). The juvenile annual growth zones are relatively larger than those of later adult zones. The reading axis from the nucleus to the transition zone follows the longest axis (distal edge to dorsal or ventral tip) and changes after the transition zone to reading towards the proximal edge, often along the *sulcus acusticus* edge (for details see ICES, 2009b). Old specimens only show thickness growth after the transition zone, requiring careful identification of edge zones with high magnification (100–200×). Though the common preferred otolith preparation method is break-and-burn, other methods such as thin sections (Figure 5.12) and break-and-bake are also being used (ICES, 2009b).

Golden redfish can reach ages of ca. 40 years. Nedreaas (1990) and Saborido-Rey *et al.* (2004) were able to follow strong cohorts in the length distributions and indirectly validate the slow growth of golden redfish. For the Iceland area, Stransky *et al*. (2005b) compared age estimation results that confirmed slow growth and ages up to 30 years.

As part of a radiometric validation study based on radium–lead isotope ratios (Stransky *et al*., 2005a), the age and growth of golden redfish from the Iceland–Greenland area has been validated. Table 5.14 shows the length range for various age groups of golden redfish from various localities.

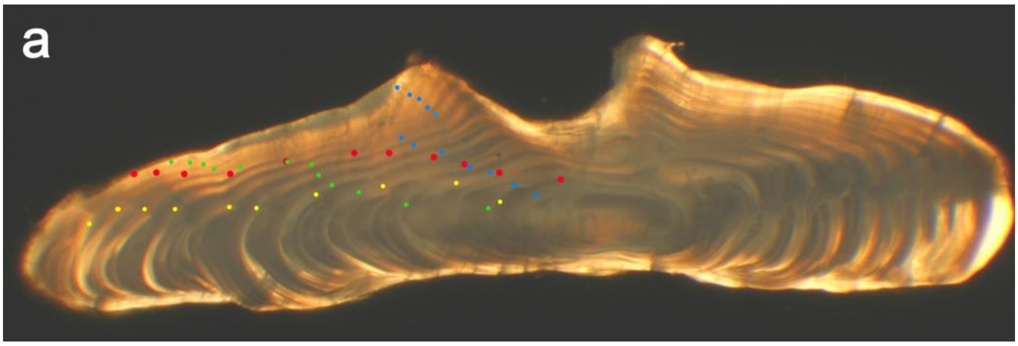


Figure 5.12. Example picture of a golden redfish (*Sebastes norvegicus*, 25 cm TL, caught around Iceland, March 1997) otolith thin section, including age estimation marks of four age readers (10–12 years).

Table 5.14. Length range (cm) of selected age groups of golden redfish (*Sebastes norvegicus*). NA = not available.

Area	Sex	Age 5	Age 10	Age 15	Age 20	Age 30	Age 40	Reference
Norway/Barents Sea	Both	18–22	33–35	38–42	45	48	50	Nedreaas (1990)
Flemish Cap	F	21	30	39	44	51	NA	Saborido-Rey *et al.* (2004)
	M	21	30	37	41	45	NA	
Iceland	Both	16	30	38	42	48	NA	Stransky *et al.* (2005b)
	Both	NA	26–30	NA	46–50	51–55	NA	Stransky *et al.* (2005a)

5.8.10 Beaked redfish (*Sebastes mentella)*

For information on the identification of the first annulus, the transition zone, reading axes, and preparation methods, see golden redfish above. Beaked redfish reach ages of at least 60 years.

Nedreaas (1990) and Saborido-Rey *et al*. (2004) were able to follow strong cohorts in the length distributions and indirectly validate the slow growth of beaked redfish.

As part of radiometric validation studies based on radium–lead isotope ratios (Campana *et al*., 1990; Stransky *et al*., 2005a), the age and growth of beaked redfish has been validated (Figure 5.13). Table 5.15 shows the length range for various age groups of beaked redfish from various localities.

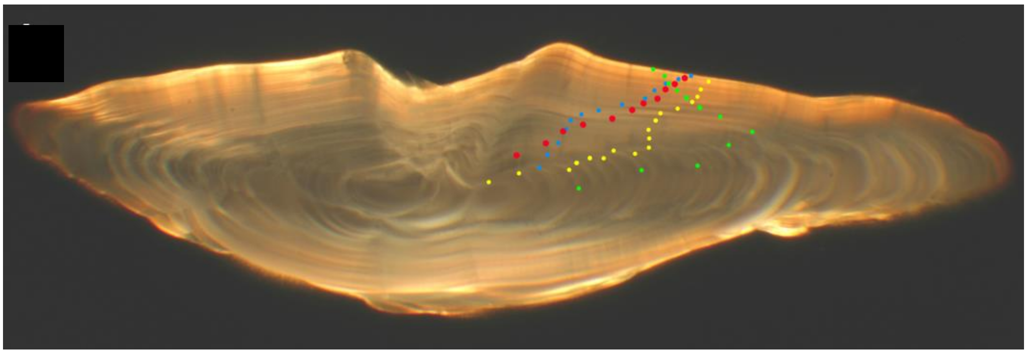


Figure 5.13. Example picture of a beaked redfish (*S. mentella*, 30 cm TL, caught in the Irminger Sea, June 1999) otolith thin section, including age estimation marks of four age readers (11–18 years).

Table 5.15. Length range (cm) of selected age groups of beaked redfish (*Sebastes mentella*). NA = not available.

Area	Sex	Age 5	Age 10	Age 15	Age 20	Age 30	Age 40	Reference
Norway/Barents Sea	Both	17	27	33	38	42	NA	Nedreaas (1990)
Flemish Cap	F	21	30	36	40	42	NA	Saborido-Rey *et al.* (2004)
	M	21	30	36	39	41	NA	
Irminger Sea	Both	23	28	32	34	37	39	Stransky *et al.* (2005b)
	Both	NA	NA	26–30	31–35	36–40	41–45	Stransky *et al.* (2005a)

5.8.11 White anglerfish (*Lophius piscatorius*)

Improving the precision in the absence of accuracy cannot, under any account, guarantee data quality (de Pontual *et al.*, 2006); the age estimation of white anglerfish and black anglerfish may serve as an example of that. Traditionally, age estimation for both species has been performed using two different calcified structures (CS): (a) the illicium (first dorsal fin ray), used by the majority of European readers for stock assessment because the growth pattern is easier to distinguish, and sampling with collection of the CS is also easier (Dupouy *et al.*, 1986; Duarte *et al.*,1997; Quincoces *et al.*, 1998a, 1998b; Landa *et al.*, 2001; García-Rodríguez *et al.*, 2005; Jónsson, 2007; Carlucci *et al.*, 2009; Ofstad *et al.*, 2013); and (b) the sagitta otolith, used only in two countries (Tsimenidis and Ondrias, 1980; Crozier, 1989; Tables 5.16 and 5.17). Several age estimation workshops and exchanges for both species have taken place (Table 5.2) and, in general, illicia have shown better precision (*CV* and APE), agreement, and relative accuracy among readers than otoliths. Even so, illicia have not shown very high precision values when all readers were compared: *CV* (21–27%) and APE (16–20%) for white anglerfish, and *CV* (18–27%) and APE (10–36%) for black anglerfish (Table 5.2). However, in the Anglerfish *Illicia*/Otoliths Ageing Workshop in 2004 (Duarte *et al.*, 2005), illicia expert readers showed a *CV* of ~10% for both species, and their readings were used in the stock assessment. A standardized age estimation criterion based on illicia was also established (Duarte *et al.*, 2002). The presence of multichecks in the otolith, the increasing opacity with age (otolith age estimation was recommended only for specimens ≤ 6 years old; Crozier, 1989), and the lack of a standardized age estimation criterion for the readers (Duarte *et al.*, 2005) have all hindered the use of otoliths as the basis for age estimation in the stock assessment process. Nevertheless, otoliths have been taken into account in the last exchange and workshop (Table 5.2). Despite efforts made to improve precision and agreement, and to have an illicia-standardized age estimation criterion, inconsistencies found in the cohort tracking of the catch-at-age time-series provide evidence that the traditional criterion based on illicia was not accurate for the two southern shelf stocks of each anglerfish species (Azevedo *et al.*, 2008). Since then, both the stock and the species have not been assessed using age estimates based on the traditional criterion, while greater effort has been made to provide more accurate and corroborated growth patterns.

There has been no direct validation of age estimation for white anglerfish, but semi-direct validation has been performed in both CS, using marginal increment analysis (Woodroffe *et al.*, 2003), and in edge-state analysis (Dupouy *et al.*, 1986; Crozier, 1989; Woodroffe *et al.*, 2003; Ofstad *et al.*, 2013). Growth corroboration studies, such as tag–recapture (Laurenson *et al.*, 2005; Landa *et al.*, 2008), microincrement analyses (Wright *et al*., 2002), and length frequency distributions of catches (Fulton, 1903; ICES, 2006; Jónsson, 2007), presented a faster growth rate and were fundamental in proving that the growth pattern estimated using the traditional criterion based on illicia (~19 and 50 cm for ages 1 and 5, respectively) was not accurate (Landa *et al*., 2008) and showed inconsistencies in cohort tracking (Azevedo *et al*., 2008). With the recent modifications in the methodology of illicia preparation and in the traditional age estimation criterion, faster growth has been estimated, enabling good cohort tracking of catch-at-age data (Landa *et al*., 2013; Figure 5.14). The latest studies that use illicia, by Jónsson (2007), Landa *et al*. (2013), and Ofstad *et al*. (2013), have all presented similar growth patterns (~29 and 65 cm for the ages 1 and 5, respectively) in Iceland and in Porcupine Bank and Faroese waters, respectively (Table 5.16). These results are also consistent with growth estimates from length frequency analyses and tag–recapture results. Advances in research on white anglerfish growth, corroborating the illicia age estimates, could allow future use of illicia in the stock assessment process.

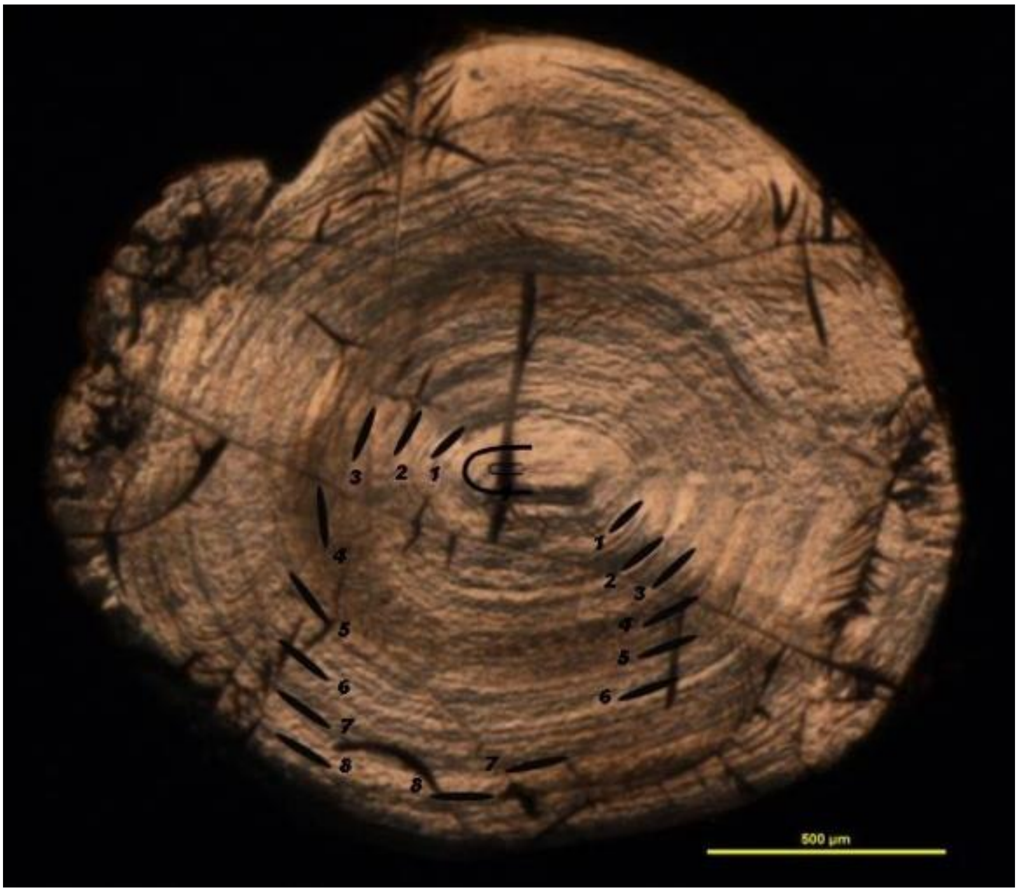


Figure 5.14. Illicium of *L. piscatorius* (89 cm, estimated age 8 years old). The annuli are marked with numbers, and the two structures marked in the central area, lineal and oval in shape, are both considered checks (false annual increment; Landa *et al.*, 2013).

Table 5.16. Mean length (cm) for selected age groups of white anglerfish.

Area	Calcified structure	Sex	Age 1	Age 5	Age 8	Reference
Aegean Sea (Mediterranean)	Otoliths	F	17.5	86.7	102.2	Tsimenides and Ondrias (1980)
Irish Sea	Otoliths	Both	22.5	62.5		Crozier (1989)
Celtic Sea and northern Bay of Biscay	Illicia	F	13.2	53.2	81.1	Dupouy *et al*. (1986)
Southern Bay of Biscay	Illicia	Both	17.5	50.5	71.5	Duarte *et al*. (1997)
Northern Bay of Biscay	Illicia	Both	20.0	50.2	73.0	Quincoces *et al*. (1998b)
Iberian Atlantic waters	Illicia	Both	19.0	46.5	66.7	Landa *et al*. (2001)
Icelandic waters	Illicia	Both	26.7	67.3		Jónsson (2007)
Faroe waters	Illicia	Both	29.1	63.1	85.9	Ofstad *et al*. (2013)
Porcupine Bank	Illicia	Both	31.9	69.4	93.4	Landa *et al*. (2013)

5.8.12 Black anglerfish (*Lophius budegassa*)

There has been no direct validation of age estimation for black anglerfish, but semi-direct validation has been attempted by edge-state analysis in illicia (Dupouy *et al*., 1986; see also the previous section for issues relevant to both anglerfish species). The tag–recapture programme for black anglerfish in Atlantic European waters provided limited evidence on the actual annual growth rate (Landa *et al*., 2002; ICES, 2006). The otolith microincrement analyses in Mediterranean (La Mesa and De Rossi, 2008) and Atlantic waters (Hernández *et al*., 2015) showed faster growth rates in early stages, and the first hypothetical annual increment in illicia that is usually included in the count of annuli is considered not to be really annual. Thus, specimens up to 20 cm found in autumn belong to age class 0 (Hernández *et al*., 2015), and the growth rate for the first age estimated in the studies based on CS seems to be underestimated. García-Rodríguez *et al*. (2005),Carlucci *et al*. (2009) and Landa and Barcala (2017) also obtained higher growth rates using length frequency analysis than those estimated using CS, allowing better cohort tracking of the catch-at-age of black anglerfish in Spanish Mediterranean waters in the last study (Table 5.17). The results of such studies that use alternative techniques to estimate the age of CSs (such as microincrement, length frequency analysis) advance the understanding of the accurate growth pattern of these populations.

Validation of age estimation would be particularly important for black anglerfish, where age estimations between two CSs of the same fish have hitherto not been consistent. Workshop results show that ages from illicia readings are generally higher than otolith readings from the same fish and that this difference is particularly important for black anglerfish (as an example, see Figure 5.15).

Figure 5.15. Illicium and otolith of *L. budegassa* (37 cm TL, Atlantic Iberian coast), estimated at 5 years old based on the illicium (left image, annuli marked in blue) and 4 years old based on the otolith (right image, annuli marked in blue). (Duarte *et al.*, 2005.)

Table 5.17. Mean length (cm) for selected age groups of black anglerfish.

Area	Methodology	Sex	Age 2	Age 5	Age 8	Reference
Aegean Sea (Mediterranean)	Otolith age estimation	Both	15.7	36.4	49.1	Tsimenides and Ondrias (1980)
Celtic Sea and northern Bay of Biscay	Illicia age estimation	F	13.7	31.3	46.4	Dupouy *et al.* (1986)
Portuguese waters	Illicia age estimation	Both	16.4	34.6	48.9	Duarte *et al.* (1997)
Northern Bay of Biscay	Illicia age estimation	Both	15.6	30.7	48.6	Quincoces *et al.* (1998a)
Iberian Atlantic waters	Illicia age estimation	Both	16.6	33.3	48.4	Landa *et al.* (2001)
Spanish Mediterranean	Length frequency analysis	Both	32.4	57.6	73.7	García-Rodríguez *et al.* (2005)
Ionian Sea (Mediterranean)	Length frequency analysis	Both	36.3	55.0	62.9	Carlucci *et al.* (2009)
Spanish Mediterranean	Length frequency analysis	Both	32.7-35.8	58.9-61.1	75.3-76.2	Landa and Barcala-Bellod (2017)

6 Statistical handling of uncertainty in age estimations

Lotte Worsøe Clausen and Ernesto Jardim

6.1 Introduction

Inaccurate age estimations are widespread and negatively impact the accuracy of population dynamics studies and stock assessment outcomes. There are numerous cases in which age estimation errors have contributed to the overexploitation of a population or species (Campana, 2001). Underestimation of age results in overly optimistic estimates of growth and mortality rates, while overestimation of age results in underestimation of growth. Some levels of bias and imprecision can be accounted for in the assessment, but even when possible, this requires measurement of the age estimation error and application of an age estimation matrix within the assessment (Punt *et al.*, 2008).

The nature of whether true age is known affects the terminology used herein. The term "accuracy" is reserved for describing a comparison of the true age with that generated by age readers, but true age is rarely known. More commonly, age estimations are made after testing against a reference collection containing known-age or age-validated samples. As long as the interpretation criteria used in age estimation are the same for all of the samples, the unvalidated sample ages should be accurate, at least on average (Campana, 2001). In the absence of a known-age reference collection, consistency in age estimation is the best that can be achieved (Campana *et al.*, 1995). The "bias" that is often reported from age calibration workshops where validated ages are not available is more an expression of the "skewness" of data around a modal or likely value. The term "precision" is used to describe "agreement", consistency, or variability among readings/annotations of the same specimen by the same or different readers. Reports from age calibration workshops generally give very thorough results and commentary about the accepted interpretation of the age structures of a given species or stock. Stock assessment scientists can use these reports to evaluate the quality of the age distributions available for the assessment, but do they fully do so? And if not, how can these age calibration workshop reports be improved? This is the focus of this chapter: to determine whether these reports reach the right audience, whether the reports include data with appropriate formats, and whether the stock assessment models are prepared in a manner that accounts for the information in the reports.

6.1.1 Statistical methods for analysing output from calibrations

A total of 31 ICES reports published between 1992 and 2012 on age estimation and calibration exercises were reviewed to identify the applied methods for analyses of calibration data (Annex 3). The majority used methods to compare age estimations, such as percentage of agreement (PA), average percentage error (APE), and coefficient of variation (*CV*).

Of the 17 statistical methods reported in the literature used to analyse output from calibrations (Table 6.1), all can be classified as one of the following: (i) identification of bias among age readers or a reference collection; (ii) estimate of precision among or within age readers; (iii) diagnostic of age estimation differences; and (iv) preparation of an age estimation error matrix for use in stock assessment models.

After 2000, the majority of reports used the "Guus Eltink" spreadsheet, i.e. the workbook age estimation comparisons of Eltink (2000) and the guidelines and tools for age estimation comparisons (Eltink *et al.*, 2000). These are useful tools for a general understanding of the uncertainty in age estimations for a stock, or for an age reader. However, these estimates cannot be readily used when age estimation uncertainty is to be incorporated in stock assessments. Different approaches have been taken into account for age estimation error in stock assessments. Several studies compare stock assessment results based on different age scenarios. These scenarios can reflect interreader variability or differences between observed and true ages. Most of the recent studies addressing this issue quantify an age error matrix (AEM) to use in the stock assessment model. The elements of the AEM are the probabilities that a fish of "true age" class is wrongly assigned to one of the observed age classes. The probabilities can be estimated using several functional forms and distribution. The "true age" is usually not really the true age. Examples include simulated true age, known age (based on mark–recapture studies), nearest integer to mean age across readers, "expert" or "consensus" age, modal age, otolith age (with observed age based on other calcified structures), or a preparation method where the observed age is based on other preparation methods. A readability score has been used as a factor in statistical models to estimate the probabilities of the AEM (Candy *et al.*, 2012). Readability score, such as the three-point grading system recommended by PGCCDBS and WKNARC 2011 or the five-class system used in Australia, is a (subjective) variable. It is not a probability or error estimate and, therefore, is not directly applicable in a stochastic assessment model. The readability score can be correlated with age (Candy *et al.*, 2012) and is expected to be correlated with growth rate; thus, a readability score should not be applied as a selection criterion for age data included in the stock assessment because this may cause bias.

Strengths and weaknesses of each listed method

Identification of bias is one of the most important products of an age calibration exercise, as it indicates to what degree age readers differ in their interpretation of the growth increments. Unless one of the age estimations is based on a validated reference collection, it can be difficult to determine which of the age estimations (if either) is more accurate. In Table 6.1, Method 1, the age-bias plot, is a widely used method for visually identifying bias. Methods 2–4 are statistical counterpoints to the age-bias plot. The statistical methods have the advantage of being quantitative, although attention should be paid to the output of the individual test, e.g. will the paired tests fail if there is an overestimation of age at one end, but underestimation of age at the other end of the age range as the differences will cancel each other out (Campana *et al.*, 1995)? The age-bias plot tends to be more sensitive: it represents readings and modal age and can also use "known age" or measured age, but provides a visual, not quantitative, interpretation. Both approaches to estimating bias are useful in an age calibration or quality control exercise; Method 1 is the most cost-effective and thus easily applied as a standard, whereas methods 2–4 provide a quantitatively better estimation of bias. The choice of method will probably depend on the available material and time for the analyses. If limited resources are available, Method 1 is recommended.

Higher precision should be characteristic of experienced age readers, since they tend to be very consistent in their interpretations. There are, however, some grave exceptions, e.g. Baltic cod (Eero *et al.*, 2015). Most of the methods reported in Table 6.1 are measures of precision (methods 5–14). Precision estimates can be expressed by a single number (e.g. $CV = 5\%$; please note that for calibrations where mean and standard deviation have the same units, CV is usually reported as unitless) and are thus easily understood. CV (Method 6) and APE (Method 5) are widely used in the literature, being readily comparable among age calibration exercises. They also have the advantage of being relatively insensitive to the age range in the study, unlike the simple percentage agreement. It is recommended to include at least one measure of precision, either *CV* or APE, as a useful product of an age calibration or quality control exercise.

Table 6.1. Methods which can be used to evaluate age calibration studies; characteristics of the methods, strengths, and weaknesses are given. The colour code highlights methods considered key products of an age calibration study. Yellow: assess bias; orange: assess precision; red: diagnostics; green: output for stock-assessment.

No.	Method	Descriptive statistics	Statistical test	One single number	Visual method	Model-based approach	Precision	Bias	Data requirements	Diagnostics	Strength	Weakness	Observations
1	ABP – age-bias plot			✓	✓			✓	Age estimations		Easily interpreted	Visual, not a statistical test	
2	TS – test of symmetry		✓	✓				✓	Age estimations			Statistical test, not picking up non-monotonic age estimation problems	
3	PTT – paired t-test		✓	✓				✓	Age estimations		Easily interpreted		Parametric test
4	WPRT – Wilcoxon paired ranks test		✓	✓				✓	Age estimations		Easily interpreted		Non-parametric test
5	APE – average percentage error	✓		✓			✓		Age estimations		Easily interpreted		Sensitive to outliers
6	*CV* – coefficient of variation	✓		✓			✓		Age estimations		Easily interpreted		Sensitive to outliers
7	MSD – mean square deviation	✓		✓			✓		Age estimations		Easily interpreted		
8	CCC – concordance correlation coefficient	✓		✓			✓		Age estimations		Easily interpreted		
9	TDI – total deviation index	✓		✓			✓		Age estimations		Easily interpreted		
10	MAD – modal age difference	✓		✓			✓		Age estimations		Easily interpreted		
11	PA – percentage agreement	✓		✓			✓		Age estimations		Easily interpreted	Poor because it is sensitive to range of ages used in the analysis	
12	Rho – average Spearman's rho		✓	✓			✓		Age estimations		Easily interpreted		Just a correlation coefficient
13	W – Kendal's coefficient of concordance		✓	✓			✓		Age estimations		Easily interpreted		

No.	Method	Descriptive statistics	Statistical test	One single number	Visual method	Model-based approach	Precision	Bias	Data requirements	Diagnostics	Strength	Weakness	Observations
14	Tau – average tau		✓	✓			✓		Age estimations		Easily interpreted		
15	MAOI – mode analysis of otolith increments (e.g. with mixed effects models)		✓			✓		✓	Data on individual rings of each otolith as marked by readers are required (WebGR)	✓		Complex output	
16	VAOI – visual analysis of otolith increments (e.g. using Photoshop)				1				Data on individual rings of each otolith as marked by readers are required (WebGR)	✓			Use layers in Photoshop to visualize age estimations and results from the mixed-effects model
17	AOI – Analysis of otolith increments (e.g. using simple statistics)			✓			✓	✓	Data on individual rings of each otolith as marked by readers are required (WebGR)	✓	Summary across readers and otoliths		Requires further development
18	AREM – age reading error matrix					✓	✓		Age estimations				Bridge towards stock assessment; matrix can use observed or modeled data

It is important to note that all of the bias tests (both the age-bias plot and the statistical tests of symmetry) are limited to pair-wise comparisons of age estimations; it is not possible to compare more than two readings at a time. In contrast, the precision measures, diagnostics, and age error matrices can all be based on as many age estimations as are available.

Age readers are often most interested in determining the cause of any systematic differences in age estimation (bias) with other age readers, since it may be possible to correct the error if the source is known. These methods may be referred to as diagnostic methods (methods 15–17). The use of multiple layers in Photoshop (or some other imaging program), with one layer per age reader, allows rapid comparison of growth increment interpretations across multiple age readers. This approach is visual and not quantitative. Alternatively, simple descriptive statistics can be computed and results analysed or mixed-effect models may be applied to growth-increment width data, providing a quantitative diagnostic of systematic growth-increment interpretation differences. Although these methods likely provide too much detail for stock assessment experts, they represent useful products of an age calibration exercise.

Age error matrices (Method 18) provide a quantitative summary of age estimation error. In such matrices, whether empirical or model-based (*sensu* Punt *et al*., 2008), misreading errors are reported as the proportion of an age group that is erroneously misaged as other ages. Empirical age error matrices are readily calculated from age frequency tables, while model-based matrices require specialized software. Age error matrices are of particular value to stock assessment clients, since they can be incorporated into age-structured stock assessment models to correct for systematic and unavoidable age estimation error. Thus, they are an important product of an age calibration exercise.

6.1.2 Test of methods on a known dataset

The quantitative parameters were tested on known datasets of two fish species. A set of haddock age test data was taken from an international age calibration study, involving experts from seven age estimation laboratories and a number of age-validated otoliths. Two subsets of data were used in the examples shown below; the first compares two very similar sets of age estimations (Dataset 1), while the second compares age estimations where one set of age estimations shows substantial bias (Dataset 2). A software tool from NOAA (http://www.nefsc.noaa.gov/fbp/age-prec/) was used to perform this analysis.

In Figure 6.1, the age-bias plot shows no appreciable bias between the two sets of age estimations, although the test age shows a very slight tendency to being underestimated at ages 5–6 and overestimated at ages 7–8. The Bowker (1948) test of symmetry may detect this difference, since the test result is statistically significant ($p = 0.00$). As such, it indicates that this test is highly sensitive to very small differences. The *CV* of 4.75% indicates that there is reasonably good precision between the two sets of age estimations, since *CV* values < 5% are considered relatively precise (Campana, 2001). The APE is not shown, but the *CV* is mathematically equal to 1.41 times the APE.

Figure 6.2 shows the age frequency table that results from this comparison, which is later used to generate the age error matrix. In an additional test of symmetry (the Hoenig test) significant differences also appear between the age estimations, but they are of smaller magnitude than in the Bowker test. The Hoenig test shows a close-to-significant difference between age estimations ($p = 0.057$) and is thus not as significant as the Bowker test ($p = 0.001$). This difference arises because the Hoenig test pools the values along the diagonal, whereas the Bowker test does not pool the cells, so some of the high deviations create rather small *p* values (McBride, 2015). The final product is the age error matrix, which is shown in Figure 6.3. This matrix shows the proportion of each test age (the reference age) erroneously attributed to other ages. Therefore, the sum of each row is 1, equal to 100%.

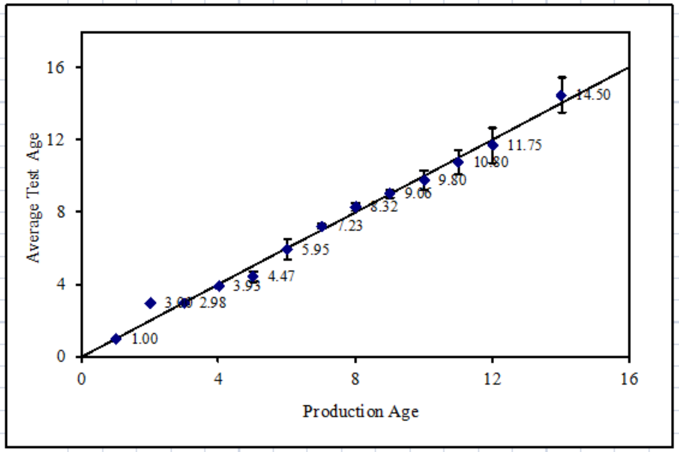
The second test set used data with considerable bias. Figure 6.4 illustrates how the age-bias plot indicates that the test age is substantially underestimated relative to production ages > 5 years. The Bowker test *p* value of 0.00 confirms that age underestimation is significant. The *CV* of 25.21% is also very high, but in this case, a measure of precision is much less important, given the very high bias between the two sets of age estimations. In Figure 6.5, it is evident that the age frequency table as well as the highly significant *p* value of 0.000 from the Hoenig test confirms such bias. Finally, Figure 6.6 shows how the age error matrix from this comparison appears.

Sample Type/year		Species	Haddock		
Aged 2X by ___		Date	10/14/14		
		Age Reader	WHvsLH		
N Aged	**466**			Bowker's test	
N Tested	464	Total CV	4.75%	Chi-sq	56.63
N Agreed	292			d.f.	27
Disagreed	172	%Agreement	62.9%	P-value	0.00
					**

Prod Age	N	N Agreed	%Agrmnt	Ave Age	s.d.	C.I.	95% CI	
0			#¡DIV/0!	0.00		####	#¡NUM!	#¡NUM!
1	3	3	100%	1.00		####	#¡NUM!	#¡NUM!
2	1		0%	3.00	#¡DIV/0!	####	######	######
3	62	61	98%	2.98	0.13	0.03	2.95	3.02
4	89	77	87%	3.93	0.36	0.08	3.86	4.01
5	36	14	39%	4.47	0.88	0.29	4.19	4.76
6	19	5	26%	5.95	1.31	0.59	5.36	6.54
7	60	38	63%	7.23	0.65	0.16	7.07	7.40
8	84	43	51%	8.32	0.85	0.18	8.14	8.50
9	50	36	72%	9.06	0.79	0.22	8.84	9.28
10	30	5	17%	9.80	1.52	0.54	9.26	10.34
11	20	6	30%	10.80	1.54	0.68	10.12	11.48
12	8	3	38%	11.75	1.39	0.96	10.79	12.71
13			#¡DIV/0!			####	#¡NUM!	#¡NUM!
14	2	1	50%	14.50	0.71	0.98	13.52	15.48
15			#¡DIV/0!			####	#¡NUM!	#¡NUM!
16			#¡DIV/0!			####	#¡NUM!	#¡NUM!
Total	464	292						

Omitted Samples

Prod Age	Test Age
	4
	4



Error bars indicate 95% confidence intervals

Test Age	Production Age 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
0																		
1		3																3
2				1														1
3			1	61	9	5												76
4					77	13	2											92
5					3	14	6	1										24
6						4	5	3										12
7							4	38	11	1		1						55
8							1	17	43	6	5							72
9							1	1	24	36	12	3						77
10									4	4	5	3	2					18
11									2	2	2	6	1					13
12										1	4	6	3					14
13											2		1					3
14												1	1		1			3
15															1			1
16																		
Total		3	1	62	89	36	19	60	84	50	30	20	8		2			464

Figure 6.1. Age-bias plot for Dataset 1 without detectable bias.

Sample Type/date		Species	Haddock
Aged 2X by ___		Date	10/14/14
		Testee	WHvsLH

Bowker's Test		**Evans-Hoenig Test**	
Total Chi-sq	56.63	Total Chi-sq	9.16
d.f.	27	d.f.	4
P-value	0.001	P-value	0.057

DIRECTIONS

1) Enter production ages in A and test ages in B, replacing sample ages.
2) Refresh Pivot table (AR1).
3) Edit d.f. value for Hoenig-Evans test. The d.f. is the maximum age difference between the paired ages; the color scale can help in determining this.
4) Fill in labels (species, date, etc.) at top of printout (Cells D1-K3).
5) Save to a distinctive filename before printing.

**For more information, go to
http://www.nefsc.noaa.gov/fbp/age-prec/

This template was created by Sandy Sutherland at the NOAA Fisheries Service

Age-frequency table:

	Production Age																	
Test Age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
0																		
1		3																3
2				1														1
3			1	61	9	5												76
4					77	13	2											92
5					3	14	6	1										24
6						4	5	3										12
7							4	38	11	1		1						55
8							1	17	43	6	5							72
9							1	1	24	36	12	3						77
10									4	4	5	3	2					18
11									2	2	2	6	1					13
12										1	4	6	3					14
13											2		1					3
14												1	1		1			3
15															1			1
16																		
Tota		3	1	62	89	36	19	60	84	50	30	20	8		2			464

Figure 6.2. Age frequency table from the comparison and an additional test of symmetry (Hoenig test) on the data without detectable bias (Dataset 1).

AREM

Test Age(LH) \ Production Age(WH)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
0																		
1		1.00																3
2				0.02														1
3			1.00	0.98	0.10	0.14												76
4					0.87	0.36	0.11											92
5					0.03	0.39	0.32	0.02										24
6						0.11	0.26	0.05										12
7							0.21	0.63	0.13	0.02		0.05						55
8							0.05	0.28	0.51	0.12	0.17							72
9							0.05	0.02	0.29	0.72	0.40	0.15						77
10									0.05	0.08	0.17	0.15	0.25					18
11									0.02	0.04	0.07	0.30	0.13					13
12										0.02	0.13	0.30	0.38					14
13											0.07		0.13					3
14												0.05	0.13		0.50			3
15															0.50			1
16																		
Total		3	1	62	89	36	19	60	84	50	30	20	8		2			

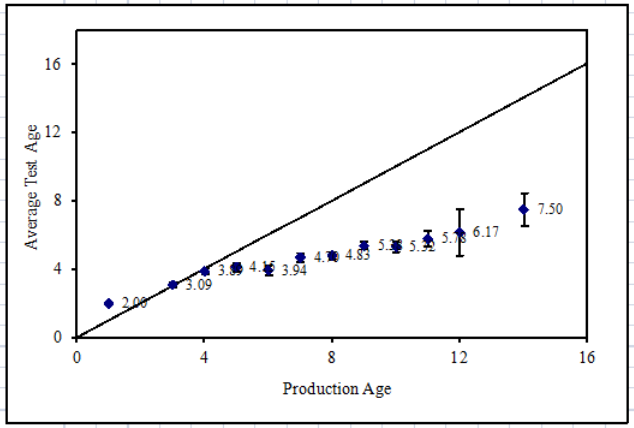
Figure 6.3. The final outcome: the age error matrix on Dataset 1.

Sample Type/year		Species	Haddock		
Aged 2X by ___		Date	10/14/14		
		Age Reader	WHvsLH		
N Aged	**466**			Bowker's test	
N Tested	433	Total CV	25.21%	Chi-sq	271.78
N Agreed	93			d.f.	36
Disagreed	340	%Agreement	21.5%	P-value	0.00
					**

Prod Age	N	N Agreed	%Agmnt	Ave Age	s.d.	C.I.	95% C I	
0			#¡DIV/0!	0.00		####	#¡NUM!	#¡NUM!
1	2		0%	2.00		####	#¡NUM!	#¡NUM!
2			#¡DIV/0!			####	#¡NUM!	#¡NUM!
3	55	37	67%	3.09	0.62	0.16	2.93	3.25
4	85	47	55%	3.89	0.69	0.15	3.75	4.04
5	34	9	26%	4.15	0.74	0.25	3.90	4.40
6	17		0%	3.94	0.56	0.26	3.68	4.21
7	60		0%	4.70	0.93	0.23	4.47	4.93
8	76		0%	4.83	0.93	0.21	4.62	5.04
9	50		0%	5.38	0.78	0.22	5.16	5.60
10	28		0%	5.32	0.82	0.30	5.02	5.62
11	18		0%	5.78	0.94	0.44	5.34	6.21
12	6		0%	6.17	1.72	1.38	4.79	7.54
13			#¡DIV/0!			####	#¡NUM!	#¡NUM!
14	2		0%	7.50	0.71	0.98	6.52	8.48
15			#¡DIV/0!			####	#¡NUM!	#¡NUM!
16			#¡DIV/0!			####	#¡NUM!	#¡NUM!
Total	433	93						

Omitted Samples

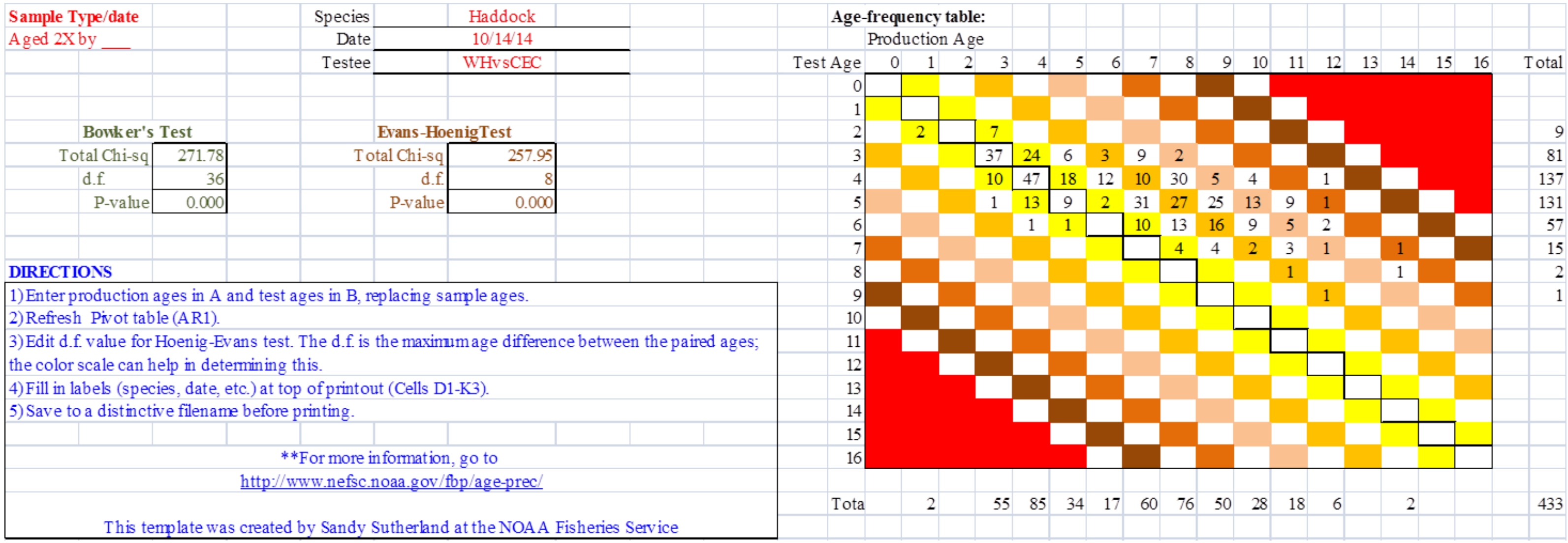
Prod Age	Test Age
	4
10	
	3
12	
8	
3	
3	
	4



Error bars indicate 95% confidence intervals

Test Age	Production Age 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
0																		
1																		
2		2		7														9
3				37	24	6	3	9	2									81
4				10	47	18	12	10	30	5	4		1					137
5				1	13	9	2	31	27	25	13	9	1					131
6					1	1		10	13	16	9	5	2					57
7									4	4	2	3	1		1			15
8												1			1			2
9													1					1
10																		
11																		
12																		
13																		
14																		
15																		
16																		
Total		2		55	85	34	17	60	76	50	28	18	6		2			433

Figure 6.4. Analysis of the dataset with substantial bias (Dataset 2).



Sample Type/date		Species	Haddock
Aged 2X by ___		Date	10/14/14
		Testee	WHvsCEC

Bowker's Test		Evans-Hoenig Test	
Total Chi-sq	271.78	Total Chi-sq	257.95
d.f.	36	d.f.	8
P-value	0.000	P-value	0.000

DIRECTIONS

1) Enter production ages in A and test ages in B, replacing sample ages.
2) Refresh Pivot table (AR1).
3) Edit d.f. value for Hoenig-Evans test. The d.f. is the maximum age difference between the paired ages; the color scale can help in determining this.
4) Fill in labels (species, date, etc.) at top of printout (Cells D1-K3).
5) Save to a distinctive filename before printing.

**For more information, go to
http://www.nefsc.noaa.gov/fbp/age-prec/

This template was created by Sandy Sutherland at the NOAA Fisheries Service

Age-frequency table:

	Production Age																	
Test Age	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
0																		
1																		
2		2		7														9
3				37	24	6	3	9	2									81
4				10	47	18	12	10	30	5	4		1					137
5				1	13	9	2	31	27	25	13	9	1					131
6					1	1		10	13	16	9	5	2					57
7									4	4	2	3	1		1			15
8												1			1			2
9													1					1
10																		
11																		
12																		
13																		
14																		
15																		
16																		
Tota		2		55	85	34	17	60	76	50	28	18	6		2			433

Figure 6.5. Symmetry test on the data with substantial bias (Dataset 2).

AREM

	Production Age(WH)																	
Test Age(CEC)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
0																		
1																		
2		1.00		0.13														9
3				0.67	0.28	0.18	0.18	0.15	0.03									81
4				0.18	0.55	0.53	0.71	0.17	0.39	0.10	0.14		0.17					137
5				0.02	0.15	0.26	0.12	0.52	0.36	0.50	0.46	0.50	0.17					131
6					0.01	0.03		0.17	0.17	0.32	0.32	0.28	0.33					57
7									0.05	0.08	0.07	0.17	0.17		0.50			15
8												0.06			0.50			2
9													0.17					1
10																		
11																		
12																		
13																		
14																		
15																		
16																		
Total		2		55	85	34	17	60	76	50	28	18	6		2			

Figure 6.6. The age error matrix on Dataset 2.

Although the NOAA software tool was used to generate all of the output shown above, it should be possible to generate all of these products from SmartDots or other software after appropriate revision.

6.1.3 Additional analysis based on distances between identified otolith structures

The analysis of growth increments can be very informative about the reasoning behind the attribution of a specific age. As such, the comparative analysis across readers can be used to evaluate the age estimation process and detect severe differences in the interpretation of the otolith. The information generated will be valuable in correcting errors or calling attention to problems detected in bias and precision analysis.

An increment is the difference in distance between successive marks. Marks are potential rings in the otolith, which the reader identifies during the process of reading ages:

$$I_m = D_m - D_{m-1}$$

where I is an increment, D is the distance to the otolith centre, and m indexes the marks. Note that, if readers are correct, the distance between two successive marks represents the otolith growth, which, on average, should be similar across readers and otoliths.

SmartDots stores information about the distance between marks, which constitutes the tool to identify the rings that form the basis for attributing a specific age to an individual. Using such information allows several analyses to be carried out, namely (i) visual analysis of the marks in each otolith using image editing tools; (ii) statistics to extend this analysis and allow a wider assessment of the consistency between readers; and/or (iii) mixed-effects models to test if readers are interpreting the otolith rings in a consistent way. The present chapter is focused on the second analysis; an example of using mixed-effects models can be seen in the report of the Workshop on Scoping for Integrated Baltic Cod Assessment (ICES, 2014c).

Figure 6.7 presents the increments by mark for four readers (in lines) and four otoliths (in panels). This analysis is qualitatively similar to overlaying the images of each reader for a specific otolith to assess their consistency (as illustrated in Figure 6.8). It allows a visual perspective of consistency among readers in interpretation of the structures counted. By comparing the increments as identified by age readers, such an analysis offers a better understanding of the differences detected among age readers when assigning a numerical value as "age" to a given otolith:

$$CV_{rm} = \frac{\sqrt{\frac{1}{n-1}\sum_i^n (I_{rmi} - \bar{I}_{rm})^2}}{\bar{I}_{rm}} \quad (3)$$

with

$$\bar{I}_{rm} = \frac{1}{n}\sum_i^n I_{rmi} \quad (4)$$

where n is the incremental value, r indexes readers, and i indexes otoliths.

The analysis relates to a reader's personal consistency. It shows the consistency of individual readers in the interpretation of rings. In this case, Reader 8 was off on the initial rings/marks while Reader 5 was off on the older ages. The other readers seem consistent and present an increasing precision for older ages (Figure 6.9).

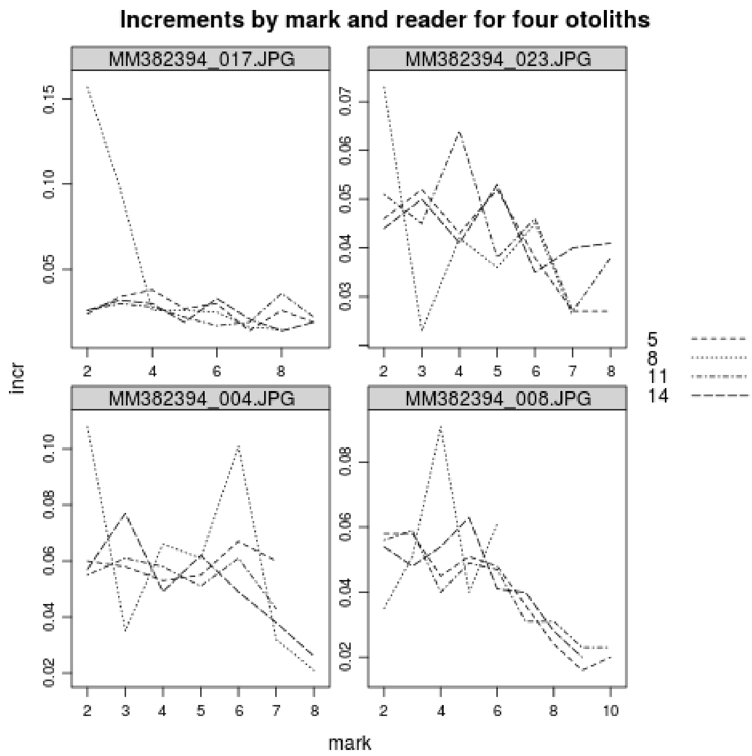


Figure 6.7. Width increments or distance to previous mark (*y*-axis: increments) for four readers (lines) and four otoliths (panels). The plots show the consistency across readers and otoliths in the identification of potential rings (*x*-axis: mark).

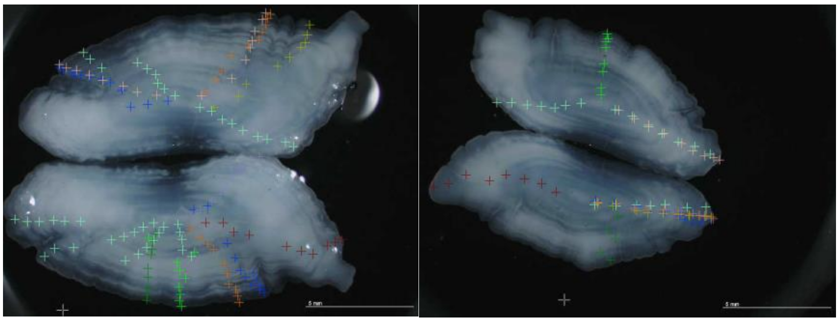


Figure 6.8. Matching otoliths (*Molva molva*) are shown as well as the markings made by each reader, overlaid in one picture.

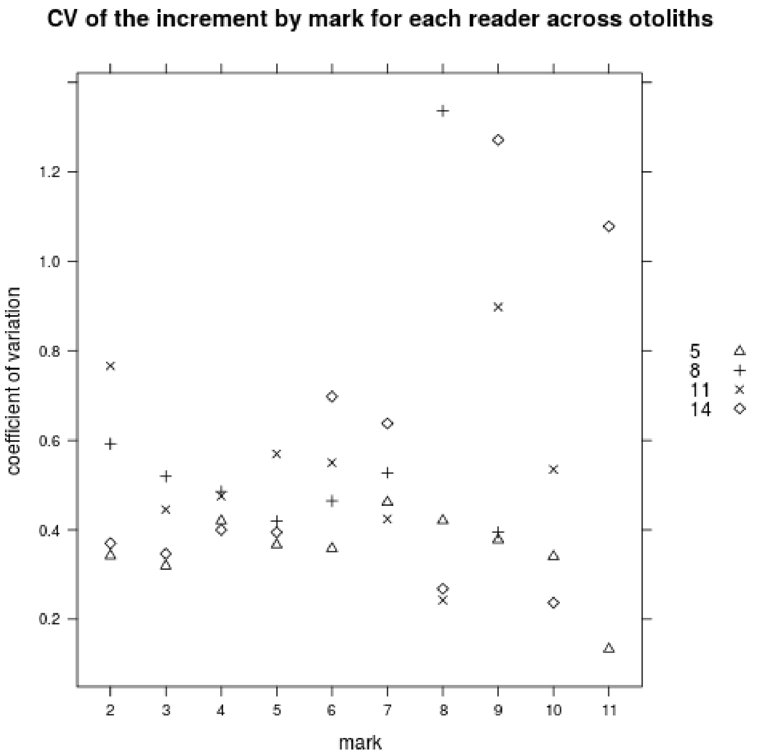


Figure 6.9. Coefficient of variation of the increment by mark (on the *x*-axis, 2–11 that each reader, identified by different symbols (5, 8, 11, and 14), set across otoliths.

It is important to note that this analysis is based on a dataset that is not fully appropriate for such an analysis. To carry out this type of analysis, distances between rings should be measured along the same axis, and this axis should be as close to linear as possible. The present workshops conducted with WebGR do not use this standard, thus increasing the variability by mark. Furthermore, the WebGR design does not use the centre of the nucleus. The first mark is counted as one age, which invalidates the analysis of the first increment, a recognized major source of error. All of these errors can be rectified, however, without having to heavily redesign WebGR.

6.2 Existing software

Direct age estimation studies require specific software tools to be used in calibration exchanges and workshops. Nowadays, most of these studies are based on digital images of calcified structures where readers can annotate their readings. This procedure contributes to the standardization of age estimation interpretation criteria among readers (e.g. the misinterpretation of false checks or the differences in the position of the first annulus) and, at the same time, the annotated images allow for measurement of age increments.

Other tools that have been used are electronic forms or integrated databases that contain sampling information as well as data on assigned ages or growth increments. When non-integrated software is used, these forms have been developed into spreadsheets that are used for further statistical analysis in order to obtain precision, accuracy (relative or absolute), bias analysis, and other outputs. In the case of integrated databases, none of the analysed programs implemented routines for statistical analysis, but all of them have extraction routines that allow data to be exported to statistical programs.

Integrating images and age estimation data analysis in the same software may reduce processing time and avoid data handling errors; for the time being, however, available software is serving both requirements separately.

The software currently available is characterized according to two categories:

(a) imaging software for age or maturity calibration exercises, based on electronic images of calcified structures or gonads;
(b) software for statistical analysis of the results of the calibration exercises.

For category (a), existing software is described in Table 6.2. In all of this software it is possible to calibrate images, integrate annotated layers from different readers into each of the images, and measure true distances between consecutive marks. Table 6.2 also includes information about the interface, open-source availability, integration with databases, measurements of age increments, and, if the software is compatible, with the multilayer-TIF format, which is the format most commonly employed when working with various annotated layers in images.

Table 6.2. Image analysis software for age and maturity calibration exercises. In all of these programs the user can calibrate images, annotate, and measure.

Software	Characteristics					
	Web-based	Type of licence	Main purpose	Integrated database	Multilayer TIF format	Ease for measuring age increments
Smartdots	Yes	Open-source	Calibration exercises	Yes	No	Automatic
Adobe Photoshop	No	Commercial	Photo editor	No	Yes	Time-consuming
PaintShop Pro	No	Commercial	Photo editor	No	Yes	Time-consuming
GIMP	No	Open-source	Photo editor	No	No	Time-consuming
Image J (tree rings)	No	Open-source	Image analysis	Yes	Yes	Automatic
Visilog (TNPC)	No	Commercial	Image analysis	Yes	No	Automatic
NIS-Elements D	No	Commercial	Image analysis	Yes (only marked layers)	No	Automatic
Image-Pro (otolith fish age estimation)	No	Commercial	Image analysis	Yes	Yes	Automatic
Image-Pro (age and shape)	No	Commercial	Image analysis	Yes	Yes	Automatic

The tools for statistical computing and graphics that were known and available at the time of writing this chapter are shown in Table 6.3, with a description of the statistical methods computed by software.

Table 6.3. Software for statistical analysis.

Software	Framework	Source	Data handling	Computed statistics and graphics														
				MSD	CCC	TDI	CP	MAD	PA	APE	*CV*	AREM	Age-bias plot	Symmetry test	Rho	W	Tau	Mixed-effects models
Age estimation comparisons (Eltink, 2000; Eltink *et al.*, 2000 spreadsheet)	MS-Excel		Easy to use, prone to errors	Yes				Yes	Yes		Yes		Yes	Wilcoxon signed-ranks test				
NOAA–NEFSC Excel workbooks (templates for calculating age estimation precision)	MS-Excel	http://www.nefsc.noaa.gov/fbp/age-prec/index.html	Easy to use, prone to errors						Yes		Yes		Yes	McNemar; Evan and Hoenig; Bowker				
Agreement	R-package	http://cran.r-project.org/	Knowledge of R required	Yes	Yes	Yes	Yes											
agRee	R-package	http://cran.r-project.org/	Knowledge of R required		Yes													
irr	R-package	http://cran.r-project.org/	Knowledge of R required		Yes											Yes		
KappaGUI	R-package	http://cran.r-project.org/	Knowledge of R required		Yes											Yes		Yes
FSA	R-package	http://derekogle.com/fishR/	Knowledge of R required					Yes	Yes	Yes	Yes		Yes	McNemar; Evan and Hoenig; Bowker				
nwfscAgeingError (AGEMAT.exe) (Punt *et al.*, 2008)	R-package	https://github.com/nwfsc-assess/nwfscAgeingError/	Knowledge of R required									Yes						
lme4	R-package	http://cran.r-project.org/	Knowledge of R required															

MSD = mean square deviation, CCC = concordance correlation coefficient, TDI = total deviation index, CP = coverage probability, MAD = modal age difference, PA = percentage agreement, APE = average percentage error, *CV* = coefficient of variation, AREM = age reading error matrix, Rho = average Spearman's rho, W = Kendall's coefficient of concordance, Tau = average tau.

6.3 Guidelines for data summaries and analysis outputs from calibration workshops

A calibration workshop has the basic purpose, as part of the quality assurance procedure, to identify sources of errors and inconsistencies among laboratories in stock-specific biological measurements, quantify these errors, and ultimately include them in stock assessments. It is the intention that the results of these exercises, as already published in extensive ICES reports, shall reach both the personnel observing and classifying the biological structures as well as the scientists involved in the estimation of stock biological parameters.

Different output formats from age estimation workshops – from submitted tables of reader raw data over age estimation error matrices to summary statistics of variation and bias – are appropriate, depending on the audience of the report and results.

Age estimation error matrices provide an additional level of detail useful for routine stock assessment exercises. However, disaggregated data at individual fish and reader levels, with additional spatially and temporally resolved covariates, would probably be preferable in many benchmark situations where modelling of data quality is an issue.

It is recommended that the following methods/analyses are run by age calibration workshops:

- To access bias:
  - ABP – age-bias plot;
  - TS – tests of symmetry.
- To access precision:
  - APE – average percentage error; or
  - *CV* – coefficient of variation.
- As diagnostics for problems found by the previous analysis:
  - Analysis of otolith increments, both through image layers and statistically.
- As output to stock assessment groups:
  - AEM – age reading error matrix.

It is important to note that, if validated material is unavailable, a true bias cannot be computed and the analysis is limited regarding its assessment of accuracy. It is thus recommended that regardless of the scope of the calibration workshop, an effort should be made to validate the age estimation of the species/stock under consideration.

With regards to the software packages available, WebGR is the most suitable for running workshops, while FSA (fisheries stock assessment methods) is the most complete for the analysis of age estimation results. None of these packages can run all of the methods recommended here, although they can be further developed to accommodate most of them. WebGR in particular has the potential to develop and implement the methods recommended.

6.4 Future perspectives

Within ICES, the assessment and benchmark working groups have hitherto, in general, been less concerned with explicit age estimation errors, as age estimations were assumed to be unbiased. Defining the best format for age estimation errors to be included in stock assessment and benchmarks has been indicated as a topic for further research.

Of immediate usefulness would be an estimate of the ages that may be confounded. Another modelling advantage would be the possibility to separate age estimation errors from sampling errors in both catches and surveys. However, this separation would also have potential effects on estimates of weight-at-age and maturity-at-age in the stock assessment model fits.

The ability to account for age estimation error is included in several stock assessment programs, such as Stock Synthesis (Methot, 2000, 2007), Coleraine (Hilborn *et al*., 2003), and CASAL (Bull *et al*., 2003). However, although all of these assessment programs include the ability to account for age estimation error, based on an age estimation error matrix, they do not include the facility to internally estimate age estimation error matrices (Punt *et al*., 2008). Also, assessment models are not uniformly structured. For example, in assessment programs used in southern Australia, it is possible to enter age estimation errors per individual reader (Punt *et al*., 2008), whereas Stock Synthesis (Methot, 2000) allows only a single vector of age estimation error as input to the model (Dorval *et al*., 2013).

Use of the age error matrix in age-structured models has been well described. Methods for using and interpreting age error matrices outside of age-structured assessments should be explored. For instance, how age estimation error affects methods for assessing data-limited stocks does not appear to be studied in equal detail. However, methods using the von Bertalanffy growth curve and its parameters L_∞ and K, estimated from reading hard structures from animals, probably suffer from age estimation error.

The link between calibration workshops and stock assessment is very weak and not operational, which makes it very difficult to integrate these error sources. While age estimation error matrices could be the right output for age-calibration workshops to provide to stock assessment working groups, operational integration of age estimation errors and/or maturity-staging errors into stock assessment will require methodological developments of available assessment models and calibration analysis tools.

7 References

Abaunza, P., Gordo, L., Karlou-Riga, C., Murta, A., Eltink, A. T. G. W., García Santamaría, M. T., *et al*. 2003. Growth and reproduction of horse mackerel, *Trachurus trachurus* (Carangidae). Reviews in Fish Biology and Fisheries, 13(1): 27–61. https://doi.org/10.1023/a:1026334532390

Albert, O. T. 2016. Growth and formation of annual zones in whole otoliths of Greenland halibut, a slow-growing deep-water fish. Marine and Freshwater Research, 67(7): 937–942. https://doi.org/10.1071/mf15089

Albert, O. T., Kvalsund, M., Vollen, T., and Salberg, A. B. 2009. Towards accurate age determination of Greenland halibut. Journal of the Northwest Atlantic Fishery Science, 40: 81–95. https://doi.org/10.2960/j.v40.m659

Alcazar, A. J., Corrasco, F. J., Llera, G. E., Menendez de la Hoz, M., Ortea, R. J., and Vizcaino, F. A. 1987. Aportacion al estudio del besugo en el Principado de Asturias. Recursos Pesqueros de Asturias, 4. 88 pp. (In Spanish.) https://doi.org/10.3989/isegoria.2003.i28.508

Aldanondo, N., Cotano, U., Etxebeste, E., Irigoien, X., Álvarez, P., Martínez de Murguía, A., and Herrero, D. L. 2008. Validation of daily increments deposition in the otoliths of European anchovy larvae (*Engraulis encrasicolus* L.) reared under different temperature conditions. Fisheries Research, 93: 257–264. https://doi.org/10.1016/j.fishres.2008.04.012

Aldanondo, N., Cotano, U., Alvarez, P., and Uriarte, A. 2013. Validation of annual increment deposition in the otoliths of young-of-the-year European anchovy. Working Document (WD 01) to the Workshop on Micro Increment Daily Growth in European Anchovy and Sardine (WKMIAS), 21–25 October 2013, Mazara del Vallo, Sicily, Italy. ICES Document CM 2013/ACOM:51. 153 pp. https://doi.org/10.1071/mf15083

Aldebert, Y., and Morales-Nin, B. 1992. La croissance des juvéniles de merlu dans le golfe du Lion: nouvelles méthodes d'approche. Rapport Commission Internationale pour l'exploration scientifique de la Mer Méditeranée, 33. 281 pp. (In French.)

Alegria Hernandez, V. 1984. Observations on the age and growth of *Trachurus trachurus* (L.) in the middle Adriatic. Biljeske-Notes, Institut za Oceanografiju i Ribarstvo, Split, 58. 6 pp.

Alemany, F., and Álvarez, F. 1994. Formation of initial daily increments in sagital otoliths of reared and wild *Sardina pilchardus* yolk-sac larvae. Marine Biology, 121: 35–39. https://doi.org/10.1007/bf00349471

Almeida, F. P., and Sheehan, T. F. (Eds.) 1997. Age Determination Methods for Northwest Atlantic Species. An update of NOAA Technical Report NMFS, 72 (December 1988). Ed. by J. Penttilä and L. M. Dery. http://www.nefsc.noaa.gov/fbp/age-man.html

Alshuth, S. 1988a. Daily growth increments on otoliths of laboratory-reared sprat, *Sprattus sprattus* L., larvae. Meeresforschung, 32: 23–29.

Alshuth, S. 1988b. Seasonal variations in length-frequency and birthdate distribution of juvenile sprat (*Sprattus sprattus* L.). ICES Document CM 1988/H:44. 12 pp.

Alvarez, F., and Porteiro, C. 1981. Growth studies of *Sardina pilchardus* (Walb.) in Galician waters (NW Spain). ICES Document CM 1981/H:29. 11 pp.

Andrews, A. H., Cailliet, G. M., Coale, K. H., Munk, K. M., Mahoney, M. M., and O'Connell, V. M. 2002. Radiometric age validation of the yelloweye rockfish (*Sebastes ruberrimus*) from southeastern Alaska. Marine and Freshwater Research, 53: 139–146. https://doi.org/10.1071/mf01126

Angus, C. 2011. Monitoring changes in exploitation patterns for a data deficient species with decreased quota: northern North Sea ling. Scottish Industry Science Partnership Report, 03/11. 62 pp. Available at: https://www.nafc.uhi.ac.uk/research/fisheries/ling/sisp-ling

Anon. 1997. International ageing workshop on European monkfish, 25–28 June 1991 and 9–11 July 1997, Lorient, France. Internal Document IFREMER. 34 pp.

Anon. 1999. Third International Ageing Workshop on European Anglerfish (Lisbon, March 8–12, 1999). Internal Document IPIMAR, Lisbon. 106 pp.

Appelberg, M., Formigo, N., Geffen, A. J., Hammer, C., McCurdy, W., Modin, J., *et al.* 2005. A cooperative effort to exchange age reading experience and protocols between European fish institutes. Fisheries Research, 76: 167–173. https://doi.org/10.1016/j.fishres.2005.06.001

Arneri, E., and Morales-Nin, B. 2000. Aspects of the early life history of European hake from the central Adriatic. Journal of Fish Biology, 56(6): 1368–1380. https://doi.org/10.1111/j.1095-8649.2000.tb02149.x

Arneri, E., and Tangerini, P. 1984. Biological data collected during the Pipeta expeditions on *Trachurus mediterraneus* (Steindachner) in the Adriatic Sea. *In* GFCM Report of the Third Technical Consultation on Stock Assessment in the Adriatic Sea. 6–10 June 1983, Fano, Italy. FAO Fisheries Report, 290: 127–130.

Arruda, L. M. 1987. On the annual cycle of ring formation in horse mackerel, *Trachurus trachurus*, otoliths off Portugal and its implications on age determination. *In* Proceedings of the 5th Congress of European Ichthyologists, Stockholm 1985. Swedish Museum of Natural History: 229–232.

Azevedo, M., Cardador, F., Costas, G., Duarte, R., Fariña, A. C., Landa, J., and Sampedro, M. P. 2008. Improving the quality of southern anglerfish stocks assessment (ABA). Final report. UE DG FISH/2004/03-22. 131 pp.

Barraca, I. F. 1964. Quelques aspects de la biologie et de la pêche de chinchard, *Trachurus trachurus* (L.) de la côte Portugaise. Notas e estudos do Instituto de Biologia Maritima, 29: 1–21. (In French.)

Basilone, G., Guisande, C., Patti, B., Mazzola, S., Cuttitta, A., Bonanno, A., and Kallianiotis, A. 2004. Linking habitat conditions and growth in the European anchovy (*Engraulis encrasicolus*). Fisheries Research, 68: 9–19. https://doi.org/10.1016/j.fishres.2004.02.012

Beamish, R. J., and McFarlane, G. A. 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society, 112: 735–743. https://doi.org/10.1577/1548-8659(1983)112<735:tfrfav>2.0.co;2

Belcari, P., Ligas, A., and Viva, C. 2006. Age determination and growth of juveniles of the European hake, *Merluccius merluccius* (L., 1758), in the northern Tyrrhenian Sea (NW Mediterranean). Fisheries Research, 78: 211–217. https://doi.org/10.1016/j.fishres.2006.01.006

Belloc, G. 1935. Etude monographique du merlu *Merluccius merluccius* L. (3ème partie). Revue des travaux de l'Institut des Pêches Maritimes, 8: 145–202. (In French.)

Bergstad, O. A. 1990. Distribution, population structure, growth and reproduction of the roundnose grenadier *Coryphaenoides rupestris* (Pisces: Macrouridae) in the deep waters of the Skagerrak. Marine Biology, 107: 25–39. https://doi.org/10.1007/bf01313239

Bergstad, O. A. 1991. Distribution and trophic ecology of some gadoid fish of the Norwegian Deep. Accounts of individual species. Sarsia, 75: 269–313. https://doi.org/10.1080/00364827.1991.10413455

Bergstad, O. A. 1995. Age determination of deep-water fishes: experiences, status and challenges for the future. *In* Deep-Water Fishes of the North Atlantic Oceanic Slope, pp. 267–283. Ed. by A. G. Hopper. Springer, Netherlands. 420 pp. https://doi.org/10.1007/978-94-015-8414-2_10

Bergstad, O. A., and Hareide, N-R. 1997. An illustrated manual for age reading of ling *Molva molva* L. and tusk *Brosme brosme* L. by otoliths. Norwegian fish ageing laboratory (Ald-Fisk). AldFisk Report, 1-97. 15 pp. https://doi.org/10.1006/jmsc.1997.9995

Bergstad, O. A., Magnusson, J. V., Magnusson, J., Hareide, N-R., and Reinert, J. 1998. Intercalibration of age readings of ling (*Molva molva* L.), blue ling (*Molva dipterygia* Pennant, 1784) and tusk (*Brosme brosme* L.). ICES Journal of Marine Science, 55: 309–318. https://doi.org/10.1006/jmsc.1997.9995

Bowers, A. B. 1954. Breeding and growth of whiting (*Gadus merlangus* L.) in Isle of Man waters. Journal of the Marine Biological Association of the United Kingdom, 33: 97–122. https://doi.org/10.1017/S0025315400003507

Bowker, A. H. 1948. A test for symmetry in contingency tables. Journal of the American Statistical Association, 43: 572–574.

Bull, B., Francis, R. I. C. C., Dunn, A., McKenzie, A., Gilbert, D. J., and Smith, M. H. 2003. CASAL (C++ algorithmic stock assessment laboratory): CASAL user manual v2.01-2003/8/01. NIWA Technical Report No. 124. 223 pp.

Burton, E. J., Andrews, A. H., Coale, K. H., and Cailliet, G. M. 1999. Application of radiometric age determination to three long-lived fishes using 210Pb:226Ra disequilibria in calcified structures: a review. American Fisheries Society Symposium, 23: 77–87.

Cailliet, G. M., Andres, A. H., Burton, E. J.,Watters, D. L., Kline, D. E., and Ferry-Graham, L. A. 2001. Age determination and validation studies of marine fishes: do deep-dwellers live longer? Experimental Gerontology, 36: 739–764. https://doi.org/10.1016/s0531-5565(00)00239-4

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series, 188: 263–297. https://doi.org/10.3354/meps188263

Campana, S. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59(2): 197–242. https://doi.org/10.1111/j.1095-8649.2001.tb00127.x

Campana, S. E. 2005. Otolith science entering the 21st century. Marine and Freshwater Research, 56(5): 485–495. https://doi.org/10.1071/MF04147

Campana, S. E., and Casselman, J. M. 1993. Stock discrimination using otolith shape analysis. Canadian Journal of Fisheries and Aquatic Sciences, 50: 1062–1083. https://doi.org/10.1139/f93-123

Campana, S. E., Zwanenburg, K. C. T., and Smith, J. N. 1990. ^{210}Pb/^{226}Ra determination of longevity in redfish. Canadian Journal of Fisheries and Aquatic Sciences, 47: 163–165. https://doi.org/10.1139/f90-017

Campana, S. E., Annand, M. C., and McMillan, J. I. 1995. Graphical and statistical methods for determining the consistency of age determinations. Transactions of the American Fisheries Society, 124: 131–138. https://doi.org/10.1577/1548-8659(1995)124<0131:gasmfd>2.3.co;2

Candy, S. G., Nowara, G. B., Welsford, D. C., and McKinlay, J. P. 2012. Estimating an ageing error matrix for Patagonian toothfish (*Dissostichus eleginoides*) otoliths using between-reader integer errors, readability scores, and continuation ratio models. Fisheries Research, 115–116: 14–23. https://doi.org/10.1016/j.fishres.2011.11.005

Cappo, M., Eden, P., Newman, S. J., and Robertson, S. 2000. A new approach to validation of periodicity and timing of opaque zone formation in the otoliths of eleven species of *Lutjanus* from the central Great Barrier Reef. Fishery Bulletin (US), 98: 474–488.

Carbonara, P., and Follesa, M. C. (Eds.) 2018. Handbook on fish age determination: a Mediterranean experience. Studies and Reviews no. 98. General Fisheries Commission for the Mediterranean, Rome.

Carbonara, P., Casciaro, L., Bitteto, I., and Spedicato, M. T. 2012. Reproductive cycle and length at first maturity of *Trachurus trachurus* in the Central-Western Mediterranean seas. Biologia Marina Mediterranea, 19(1): 204–205.

Cardinale, M., Arrhenius, F., and Johnsson, B. 2000. Potential use of otolith weight for the determination of age structure of Baltic cod (*Gadus morhua*) and plaice (*Pleuronectes platessa*). Fisheries Research, 45: 239–252. https://doi.org/10.1016/s0165-7836(99)00122-8

Carlucci, R., Capezzuto, F., Maiorano, P., Sion, L., and D'Onghia, G. 2009. Distribution, population structure and dynamics of the black anglerfish (*Lophius budegassa*) (Spinola, 1987) in the Eastern Mediterranean Sea. Fisheries Research, 95: 76–87. https://doi.org/10.1016/j.fishres.2008.07.015

Carvalho, N., Perrota, N. G., and Isidro, E. J. 2002. Age, growth and maturity in the chub mackerel (*Scomber japonicus* Houttuyn, 1782) from the Azores. Arquipélago, Ciências Biológicas e Marinhas, 19a: 93–99.

Castro Uranga, R. 1990. Biología y pesca del besugo (*Pagellus bogaraveo B*.). Informes Téchnicos, 30. Servicio Central de Publicationes del Gobierno Vasco. 42 pp. (In Spanish.)

Cengiz, Ö. 2012. Age, growth, mortality and reproduction of the chub mackerel (*Scomber japonicas*, Houttuyn 1782) from Saros Bay (Northern Aegean Sea, Turkey). Turkish Journal of Fisheries and Aquatic Sciences, 12: 1–2.

Cermeño, P., Uriarte, A., De Murguía, A. M., and Morales-Nin, B. 2003. Validation of daily increment formation in otoliths of juvenile and adult European anchovy. Journal of Fish Biology, 62(3): 679–691. https://doi.org/10.1046/j.1095-8649.2003.00056.x

Cermeño, P., Uriarte, A., Morales-Nin, B., Cotano, U., and Álvarez, P. 2008. Setting up interpretation criteria for ageing juvenile European anchovy otoliths. Scientia Marina, 72(4): 733–742. https://doi.org/10.3989/scimar.2008.72n4733

Chilari, A., Petrakis, G., and Tsamis, E. 2006. Aspects of the biology of blackspot seabream (*Pagellus bogaraveo*) in the Ionian Sea, Greece. Fisheries Research, 77(1): 84–91. https://doi.org/10.1016/j.fishres.2005.08.003

Colloca, F., Gentiloni, P., Belluscio, A., Carpentieri, P., and Ardizzone, G. D. 2003. Analysis and validation of annual increments in otoliths of the European hake (*Merluccius merluccius*) in the central Mediterranean Sea. Archive of Fishery and Marine Research, 50(2):174−192.

Costa Monteiro, J., and Jorge, I. M. 1982. Age and growth of *Sardina pilchardus* (Walb.) from Portuguese Coast (ICES Division IXa). ICES Document CM 1982/H:19. 29 pp.

Crozier, W. W. 1989. Age and growth of angler-fish (*Lophius piscatorius* L.) in the North Irish Sea. Fisheries Research, 7: 267–278. https://doi.org/10.1016/0165-7836(89)90060-x

D'Amours, D., Landry, J. G., and Lambert, T. C. 1990. Growth of juvenile (0-group) Atlantic mackerel (*Scomber scombrus*) in the Gulf of St. Lawrence. Canadian Journal of Fisheries and Aquatic Sciences, 47: 2212–2218. https://doi.org/10.1139/f90-245

Davies, P. E., and Sloane, R. D. 1986. Validation of aging and length back-calculation in rainbow trout, *Salmo gairdneri* Rich., from Dee Lagoon, Tasmania. Australian Journal of Marine and Freshwater Research, 37: 289–295. https://doi.org/10.1071/mf9860289

Delgado, J., Reis, S., González, J. A., Isidro, E., Biscoito, M., Freitas, M., and Tuset, V. M. 2013. Reproduction and growth of *Aphanopus carbo* and *A. intermedius* (Teleostei: Trichiuridae) in the northeastern Atlantic. Journal of Applied Ichthyology, 29: 1008–1014. https://doi.org/10.1111/jai.12230

Dorval, E., McDaniel, J. D., Porzio, D. L., Félix-Uraga, R., Hodes, V., and Rosenfield, S. 2013. Computing and selecting ageing errors to include in stock assessment models of Pacific sardine (*Sardinops sagax*). California Cooperative Oceanic Fisheries Investigations (CalCOFI) Report, 54. 13 pp.

Duarte, R., Azevedo, M., and Pereda, P. 1997. Study on the growth of southern black and white monkfish stocks. ICES Journal of Marine Science, 54: 866–874. https://doi.org/10.1006/jmsc.1996.0208

Duarte, R., Landa, J., Quincoces, I., Dupouy, H., Bilbao, E., Dimeet, J., *et al*. 2002. Anglerfish Ageing Guide. Annex 3, pp. 73–112. *In* Report of the 4th International Ageing Workshop on European Anglerfish, 14–18 January 2002, IPIMAR, Lisbon, Portugal. 141 pp.

Duarte, R., Landa, J., Morgado, C., Marçal, A., Warne, S., Barcala, E., *et al*. 2005. Report of the Anglerfish Illicia/Otoliths Ageing Workshop, 8–12 November 2004, IPIMAR, Lisbon, Portugal. 47 pp.

Dupouy, H., Pajot, R., and Kergoat, B. 1986. Etude de la croissance des baudroies *Lophius piscatorius* et *L. budegassa*, de L'Atlantique Nord-Est obtenue a' partir de l'illicium. Revue des Travaux de l'Institut des Pêches Maritimes, 48: 107–131. (In French with an English abstract.) https://doi.org/10.1787/9789264073357-8-fr

Dwyer, K., Walsh, S., and Campana, S. 2003. Age determination, validation and growth of Grand Bank yellowtail flounder (*Limanda ferruginea*). ICES Journal of Marine Science, 60(5): 1123–1138. https://doi.org/10.1016/s1054-3139(03)00125-5

Dwyer, K. S., Treble, M. A., and Campana, S. E. 2016. Age and growth of Greenland Halibut (*Reinhardtius hippoglossoides*) in the Northwest Atlantic: A changing perception based on bomb radiocarbon analyses. Fisheries Research, 179: 342–350. https://doi.org/10.1016/j.fishres.2016.01.016

Easey, M. W. 2003. Preliminary report of the Plaice Age Determination Exchange and Workshop, 13–14 May 2003, Ostend, Belgium. 7 pp. Available at the Data Quality Assurance Repository http://www.ices.dk/community/Documents/PGCCDBS/Plaice_Exchange-Workshop_2003.pdf

Easey, M. W., and Millner, R. S. 2008. Improved methods for the preparation and staining of thin sections of fish otoliths for age determination. Science Series Technical Report, Cefas, Lowestoft, 143. 12 pp.

Easey, M., Henderson, G., and Shanks, A. M. 2005. Report of the Whiting (*Merlangius merlangus*, L.) Otolith Exchange Scheme 2004 and Workshop 2005, Lowestoft, England, 17–20 October 2005. 80 pp. Available at the Data Quality Assurance Repository https://www.ices.dk/community/Documents/PGCCDBS/whg.agewk2005.pdf

EC. 2008. COUNCIL REGULATION (EC) No. 199/2008 of 25 February 2008 concerning the establishment of a Community framework for the collection, management and use of data in the fisheries sector and support for scientific advice regarding the Common Fisheries Policy. Official Journal of the European Union, L 60: 1–12. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32008R0199&from=en

Eero, M., Hjelm, J., Behrens, J., Buchmann, K., Cardinale, M., Casini, M., *et al*. 2015. Eastern Baltic cod in distress: biological changes and challenges for stock assessment. ICES Journal of Marine Science, 72(8): 2180–2186. https://doi.org/10.1093/icesjms/fsv109

Egan, A., Etherton, M., Gomez de Segura, A., Iriondo, A., Marçal, A., Power, A., *et al*. 2004. Report of the 3rd Workshop on Megrim Age Readings, 29 November–1 December 2004, AZTI, Sukarrieta, Spain. 116 pp. Available at the Data Quality Assurance Repository https://www.ices.dk/community/Documents/PGCCDBS/meg.agewk2004.pdf

Ehrich, S., and Reinsch, H. H. 1985. Bestandskundliche Untersuchungen am Blauleng (*Molva dypterygia dypt*.) in den Gewässern westlich der Britischen Inseln. Archiv fur Fischereiwissenschaft, 36(1/2): 97–113. (In German.)

Eltink, A. T. G. W. 2000. Age reading comparisons (MS Excel workbook version 1.0, October 2000). Available at the Data Quality Assurance Repository https://www.ices.dk/community/Pages/PGCCDBS-doc-repository.aspx#others

Eltink, A. T. G. W., and Kuiter, C. 1989. Validation of ageing techniques on otoliths of horse mackerel (*Trachurus trachurus* L.). ICES Document CM 1989/H:43. 15 pp. https://doi.org/10.1006/jmsc.2001.1071

Eltink, A. T. G. W., Newton, A. W., Morgado, C., Santamaria, M. T. G., and Modin, J. 2000. Guidelines and Tools for Age Reading Comparisons. (PDF document version 1.0, October 2000.) European Fish Ageing Network (EFAN) Report 3-2000. 56 pp. Available at the Data Quality Assurance Repository https://www.ices.dk/community/Documents/PGCCDBS/Eltink_etal_2000.pdf

Etherton, M. 2015. European plaice (*Pleuronectes platessa*) and sole (*Solea solea*) indirect age validation using otoliths from mark–recapture experiments from the North Sea. Fisheries Research, 170: 76–81. https://doi.org/10.1016/j.fishres.2015.05.009

Fablet, R., Pecquerie, L., de Pontual, H., Hoie, H., Millner, R., Mosegaard, H., and Kooijman, S. A. L. M. 2011. Shedding light on fish otolith biomineralization using a bioenergetic approach. PLoS ONE, 6(11): e27055. https://doi.org/10.1371/journal.pone.0027055

FAbOSA. 2002. Fish Ageing by Otolith Shape Analysis. Final Report to the European Commission. 204 pp. Available at the Data Quality Assurance Repository https://www.ices.dk/community/Documents/PGCCDBS/fabosa_rapp02_hel.pdf

Fariña Pérez, A. C. 1983. Age and growth of the Galician Shelf horse mackerel (*Trachurus trachurus* L.). ICES Document CM 1983/G:26. 11 pp.

Fox, C. J., Folkvord, A., and Geffen, A. J. 2004. Otolith micro-increment formation in herring *Clupea harengus* larvae in relation to growth rate. Marine Ecology Progress Series, 264: 83–94. https://doi.org/10.3354/meps264083

Francis, R. I. C. C. 1990. Back-calculation of fish length: A critical review. Journal of Fish Biology, 36(6): 883–902.

Francis, R. I. C. C. 1995. The analysis of otolith data. A mathematician's perspective. *In* Recent Developments in Fish Otolith Research, pp. 81–96. Ed. by D. H. Secor, J. M. Dean, and S. E. Campana. University of South Carolina Press, Columbia. 735 pp.

Fulton, T. W. 1903. The distribution, growth, and food of the angler (*Lophius piscatorius*). Fishery Board for Scotland, 21st Annual Report (1902), Part III: 186–217.

García, A., Pereira, J. G., Canha, Â., Reis, D., and Diogo, H. 2015. Life history parameters of blue jack mackerel *Trachurus picturatus* (Teleostei: Carangidae) from north-east Atlantic. Journal of the Marine Biological Association of the United Kingdom, 95(2): 401–410. https://doi.org/10.1017/s0025315414001751

García-Rodríguez, M., Pereda, P., Landa, J., and Esteban, A. 2005. On the biology and growth of the anglerfish *Lophius budegassa* Spinola, 1807 in the Spanish Mediterranean: a preliminary approach. Fisheries Research, 71: 197–208. https://doi.org/10.1016/j.fishres.2004.08.033

Geffen, A. J. 1992. Validation of otolith increment deposition rate. *In* Otolith Microstructure Examination and Analysis, pp. 101–113. Ed. by D. K. Stevenson and S. E. Campana. Canadian Special Publication of Fisheries and Aquatic Sciences, 117. 126 pp.

Geffen, A. 2012. Otolith oxygen and carbon stable isotopes in wild and laboratory-reared plaice (*Pleuronectes platessa*). Environmental Biology of Fishes, 95(4): 419–430. https://doi.org/10.1007/s10641-012-0033-2

Gil, J. 2006. Biologia y pesca del voraz [*Pagellus bogaraveo* (Brünnich 1768)] en el Estrecho de Gibraltar. PhD Thesis, Universidad de Cádiz, Spain. (In Spanish.)

Gil, J. 2010. Spanish information about the red seabream (*Pagellus bogaraveo*) fishery in the Strait of Gibraltar region. A CopeMed II contribution to the SRWG on shared demersal resources. *Ad hoc* scientific working group between Morocco and Spain on *Pagellus bogaraveo* in the Gibraltar Strait area (Málaga, Spain. 22 July, 2010). GCP/INT/028/SPA-GCP/INT/006/EC. CopeMed II Occasional Paper No. 2. 30 pp. https://doi.org/10.1051/alr/2013068

Gillanders, B. M. 2005. Using elemental chemistry of fish otoliths to determine connectivity between estuarine and coastal habitats. Estuarine, Coastal and Shelf Science, 64(1): 47–57. https://doi.org/10.1016/j.ecss.2005.02.005

Godø, O. R., and Haug, T. 1999. Growth rate and sexual maturity in cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*). Journal of Northwest Atlantic Fishery Science, 25: 115–123. https://doi.org/10.2960/j.v25.a10

Gordo, L., Martins, M. M., and Jorge, M. I. 1982. Preliminary study on the age and growth of mackerel (*Scomber scombrus* L.) in ICES Sub-area IX. ICES Document CM 1982/H:16. 28 pp. https://doi.org/10.1093/icesjms/fsm163

Gordon, J. D. M., and Swan, S. C. 1996. Validation of age readings from otoliths of juvenile roundnose grenadier, *Coryphaenoides rupestris*, a deep-water macrourid fish. Journal of Fish Biology, 49: 289–297. https://doi.org/10.1111/j.1095-8649.1996.tb06082.x

Gordon, J. D. M., Merrett, N. R., Bergstad, O. A., and Swan, S. C. 1996. A comparison of the deep-water demersal fish assemblages of the Rockall Trough and Porcupine Seabight, eastern north Atlantic: continental slope to rise. Journal of Fish Biology, 49: 217–238. https://doi.org/10.1111/j.1095-8649.1996.tb06078.x

Gregg, J. L., Anderl, D. M., and Kimura, D. K. 2006. Improving the precision of otolith-based age estimates for Greenland halibut (*Reinhardtius hippoglossoides*) with preparation methods adapted for fragile sagittae. Fishery Bulletin (US), 104(4): 643–648.

Guéguen, J. 1969. Crissance de la dorade, *Pagellus centrodontus* Delaroche. Revue des Travaux de l'Institut des Pêches Maritimes, 33(3): 251–254.

Hernández, C., Villamor, B., Barrado, J., Dueñas, C., and Fernández, S. 2013. Age determination in European anchovy (*Engraulis encrasicolus* L.) otoliths in the Bay of Biscay (NE Atlantic). Working Document (WD 03) to the Workshop on Micro Increment Daily Growth in European Anchovy and Sardine (WKMIAS), 21–25 October 2013, Mazara del Vallo, Sicily, Italy. ICES Document CM 2013/ACOM:51. 153 pp. https://doi.org/10.3989/scimar.2011.75n2227

Hernández, C., Landa, J., Barrado, J., Antolínez, A., and Santos, B. 2015. First estimates of age and growth of juvenile black anglerfish (*Lophius budegassa*), in north-eastern Atlantic waters. Fisheries Research, 161: 269–272. https://doi.org/10.1016/j.fishres.2014.08.002

Hidalgo, M., Tomas, J., Moranta, J., and Morales-Nin, B. 2009. Intrannual recruitment events of a shelf species around an island system in the NW Mediterranean. Estuarine, Coastal and Shelf Science, 83: 227–238. https://doi.org/10.1016/j.ecss.2009.03.037

Hilborn, R., and Walters, C. J. 1992. Quantitative Fisheries Stock Assessment; Choice, Dynamics and Uncertainty. Chapman and Hall, New York. 570 pp.

Hilborn, R., Maunder, M., Parma, A., Ernst, B., Payne, J., and Starr, P. 2003. COLERAINE: A generalized age-structured stock assessment model. User's manual version 2.0. SAFS-UW-0116. 58 pp.

Høie, H., and Folkvord, A. 2006. Estimating the timing of growth rings in Atlantic cod otoliths using stable oxygen isotopes. Journal of Fish Biology, 68: 826–837. https://doi.org/10.1111/j.0022-1112.2006.00957.x

Hüssy, K. 2010. Why is age determination of Baltic cod (*Gadus morhua*) so difficult? ICES Journal of Marine Science, 67: 1198–1205. https://doi.org/10.1093/icesjms/fsq023

Hüssy, K., Mosegaard, H., Nielsen, B., and Worsøe Clausen, L. 2009. Using data storage tags to link otolith macrostructure in Baltic cod (*Gadus morhua* L.) with environmental conditions. Marine Ecology Progress Series, 378: 161–170. https://doi.org/10.3354/meps07876

Hüssy, K., Hinrichsen, H-H., Fey, D. P., Walther, Y., and Velasco, A. 2010. The use of otolith microstructure to estimate age in adult Atlantic cod *Gadus morhua.* Journal of Fish Biology, 76: 1640–1654. https://doi.org/10.1111/j.1095-8649.2010.02602.x

Hüssy, K., Gröger, J., Heidemann, F., Hinrichsen, H-H., and Marohn, L. 2015. Slave to the rhythm: seasonal signals in otolith microchemistry reveal age of eastern Baltic cod (*Gadus morhua*). ICES Journal of Marine Science, 73(4): 1019–1032. https://doi.org/10.1093/icesjms/fsv247

Hüssy, K., Radtke, K., Plikshs, M.. Oeberst, R., Baranova, T., Krumme, U., Sjöberg, R., and Mosegaard, H. 2016. Challenging ICES age estimation protocols: lessons learned from the eastern Baltic cod stock. ICES Journal of Marine Science, 73: 2138–2149. https://doi.org/10.1093/icesjms/fsw107

ICES. 1993. Report of the Blue Whiting Otolith Reading Workshop, 2–6 November 1992, Tórshavn, Faroe Islands. ICES Document CM 1993/H:6. 18 pp.

ICES. 1995. Report of the Workshop on Mackerel Otolith Reading, 8–14 February 1995, Vigo, Spain. ICES Document CM 1995/H:1. 45 pp.

ICES. 1996. Report of the Workshop on Age Reading of *Sebastes* spp., 4–8 December 1995, Bremerhaven, Germany. ICES Document CM 1996/G:1. 34 pp.

ICES. 1999. Report of the Horse Mackerel Otolith Workshop, 15–19 January 1999, Lowestoft, UK. ICES Document CM 1999/G:16. 57 pp.

ICES. 2000. Manual for Baltic Cod Age Reading, Draft II. *In* Report of the Study Group on Baltic Cod Age Reading (SGBCAR), 27–31 March 2000, Karlskrona, Sweden. Addendum in ICES Document CM 2000/H:01. 35 pp.

ICES. 2001. Report of the Arctic Fisheries Working Group (AFWG), 24 April–3 May 2001, Bergen, Norway. ICES Document CM 2001/ACFM:19. 380 pp.

ICES. 2005. Report of the Arctic Fisheries Working Group (AFWG), 19–28 April 2005, Murmansk, Russia. ICES Document CM 2005/ACFM:20. 564 pp.

ICES. 2006. Report of the Working Group on the Assessment of Hake, Monk and Megrim (WGHMM), 10–19 May 2005, Lisbon, Portugal. ICES CM 2006/ACFM:01. 878 pp.

ICES. 2007. Report of the Workshop on Age Reading of Roundnose Grenadier (WKARRG), 4–7 September 2007, Boulogne-sur-mer, France. ICES CM 2007/ACFM:36. 50 pp.

ICES. 2008a. Report of the Workshop on Age Reading of North Sea Cod (WKARNSC), 5–7 August 2008, Hirsthals, Denmark. ICES Document CM 2008/ACOM:39. 71 pp.

ICES. 2008b. Report of the Workshop on Age Reading of Baltic Sprat (WKARBS), 17–20 March 2008, Klaipeda, Lithuania. ICES Document CM 2008/ACOM:37. 28 pp.

ICES. 2008c. Report of the Workshop on Age Reading of Baltic Herring (WKARBH), 9–13 June 2008, Riga, Latvia. ICES Document CM 2008/ACOM:36. 37 pp.

ICES. 2008d. Report of the Workshop on Age Reading of Turbot (WKART), 24–27 June 2008, Oostende, Belgium. ICES Document CM 2008/ACOM:35. 20 pp.

ICES. 2008e. Report of the 2nd Workshop on Age Reading of Flounder (WKARFLO), 26–29 May 2008, Rostock, Germany. ICES Document CM 2008/ACOM:38. 53 pp.

ICES. 2008f. Report of the Workshop on Age Reading of North Sea Cod (WKARNSC), 5–7 August 2008, Hirsthals, Denmark. ICES Document CM 2008/ACOM:39. 71 pp.

ICES. 2009a. Report of the Workshop on Age Estimation of European Hake (WKAEH), 9–13 November 2009, Vigo, Spain. ICES Document CM 2009/ACOM:42. 64 pp.

ICES. 2009b. Report of the Workshop on Age Determination of Redfish (WKADR), 2–5 September 2008, Nanaimo, Canada. ICES Document CM 2009/ACOM:57. 68 pp.

ICES. 2009c. Report of the Workshop on Age Reading of Red Mullet *Mullus barbatus* and Striped Mullet *Mullus surmuletus* (WKACM), 30 March–3 April 2009, Boulogne-sur-Mer, France. ICES Document CM 2009/ACOM:44. 42 pp. https://doi.org/10.1007/s002270050255

ICES. 2009d. Workshop on Age Reading of European and American Eel (WKAREA), 20–24 April 2009, Bordeaux, France. ICES Document CM 2009/ACOM:48. 66 pp.

ICES. 2009e. Report of the Workshop on Age Reading of Greenland Cod (WKARGC), 1–4 September 2009, Reykjavik, Iceland. ICES Document CM 2009/ACOM:55. 60 pp.

ICES. 2010a. Report of the Planning Group on Commercial Catches, Discards and Biological Sampling (PGCCDBS), 1–5 March 2010, Lisbon, Portugal . ICES CM 2010/ACOM:39. 174 pp.

ICES. 2010b. Report of the Benchmark Workshop on Roundfish (WKROUND), 9–16 February 2010, Copenhagen, Denmark. ICES Document CM 2010/ACOM:36. 181 pp.

ICES. 2010c. Report of the Workshop on Age Reading of North Sea (IV) and Skagerrak–Kattegat (IIIa) Plaice (WKARP), 2–5 November 2010, IJmuiden, Netherlands. ICES Document CM 2010/ACOM:45. 65 pp.

ICES. 2010d. Report of the Workshop on Age Reading of European Anchovy (WKARA), 9–13 November 2009, Sicily, Italy. ICES Document CM 2009/ACOM:43. 122 pp.

ICES. 2010e. Report of the Workshop on Age Reading of Mackerel (WKARMAC), 1–4 November 2010, Lowestoft, UK. ICES Document CM 2010/ACOM:46. 62 pp.

ICES. 2010f. Report of the Workshop on Age Reading of Dab (WKARDAB), 16–19 November 2010, Hamburg, Germany. ICES Document CM 2010/ACOM:44. 27 pp.

ICES. 2011a. Report of the Planning Group on Commercial Catches, Discards and Biological Sampling (PGCCDBS), 7–11 February 2011, Vienna, Austria. ICES Document CM 2011/ACOM:40. 168 pp.

ICES. 2011b. Report of the Workshop on Age Reading of Greenland Halibut (WKARGH), 14–17 February 2011, Vigo, Spain. ICES Document CM 2011/ACOM:41. 39 pp.

ICES. 2011c. Report of the Workshop of National Age Reading Coordinators (WKNARC), 5–9 September 2011, Boulogne-sur-mer, France. ICES Document CM 2011/ACOM:45. 175 pp.

ICES. 2011d. Report of the Workshop on Age Reading of European Atlantic Sardine (WKARAS), 14–18 February 2011, Lisbon, Portugal. ICES Document CM 2011/ACOM:42. 87 pp.

ICES. 2012a. Report of the Baltic Fisheries Assessment Working Group 2012 (WGBFAS), 12–19 April 2012, ICES Headquarters, Copenhagen, Denmark. ICES Document CM 2012/ACOM:10. 859 pp.

ICES. 2012b. Report of the Workshop on Age Reading of Red Mullet and Striped Red Mullet (WKACM2), 2–6 July 2012, Boulogne-sur-Mer, France. ICES Document CM 2012/ACOM:60. 52 pp.

ICES. 2013a. Report of the Workshop on the Age Reading of Blue Whiting (WKARBLUE), 10–14 June 2013, Bergen, Norway. ICES Document CM 2013/ACOM:53. 55 pp.

ICES. 2013b. Report of the Workshop on Micro Increment Daily Growth in European Anchovy and Sardine (WKMIAS), 21–25 October 2013, Mazara del Vallo, Sicily, Italy. ICES Document CM 2013/ACOM:51. 153 pp.

ICES. 2013c. Report of the Workshop on Age Estimation Methods of Deep-water Species (WKAMDEEP), 21–25 October 2013, Mallorca Island, Spain. ICES Document CM 2013/ACOM:83. 81 pp.

ICES. 2014a. Report of the Workshop on Statistical Analysis of Biological Calibration Studies (WKSABCAL), 13–18 October 2014, Lisbon, Portugal. ICES Document CM 2014/ACOM:35. 82 pp.

ICES. 2014b. Report of the Planning Group on Commercial Catches, Discards and Biological Sampling (PGCCDBS), 17–21 February 2014, Horta (Azores), Portugal. ICES CM 2014/ACOM: 34. 103 pp.

ICES. 2014c. Report of the Workshop on Scoping for Integrated Baltic Cod Assessment (WKSIBCA), 1–3 October 2014, Gdynia, Poland. ICES Document CM 2014/ACOM:62. 47 pp.

ICES. 2015a. Report of the Workshop on Age Reading of Saithe (*Pollachius virens*) (WKARPV), 26–29 May 2015, Boulogne-sur-Mer, France. ICES Document CM 2015/SSGIEOM:15. 43 pp.

ICES. 2015b. Report of the Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (*Trachurus trachurus*, *T. mediterraneus* and *T. picturatus*) (WKARHOM2), 26–30 October 2015, Santa Cruz de Tenerife, Canary Islands, Spain. ICES Document CM 2015/SSGIEOM:14. 93 pp. https://doi.org/10.1016/j.foodcont.2006.07.014

ICES. 2016a. Report of the Workshop on Age Reading of Chub mackerel (*Scomber colias*) (WKARCM), 2–6 November 2015, Lisbon, Portugal. ICES CM 2015\SSGEIOM:11. 81 pp.

ICES. 2016b. Report of the Workshop on Age Reading of Horse Mackerel (*Trachurus trachurus*), Mediterranean Horse Mackerel (*Trachurus mediterraneus*) and Blue Jack Mackerel (*Trachurus picturatus*) (WKARHOM), 23–27 April 2012, Lisbon, Portugal. ICES Document CM 2012/ACOM:54. 83 pp. https://doi.org/10.1016/j.foodcont.2006.07.014

ICES. 2016c. Report of the Workshop on Age estimation of European anchovy (*Engraulis encrasicolus*) (WKARA2), 28 November–2 December 2016, Pasaia, Spain. ICES CM 2016/SSGIEOM:17. 223 pp.

ICES. 2017a. Report of the Workshop on Age estimation of Whiting (*Merlangius merlangus*) (WKARWHG2), 22–25 November 2016, Lowestoft, UK. ICES CM 2016\SSGIEOM:15. 47 pp.

ICES. 2017b. Report of the Workshop on Age estimation of Blue Whiting (*Micromesistius poutassou*) (WKARBLUE2). 6–9 June 2017, Lisbon, Portugal. ICES CM 2017/SSGIEOM:22. 60 pp.

ICES. 2017c. Report of the Working Group on Biological Parameters (WGBIOP), 2–6 October 2017, Sardinia, Italy. ICES CM 2017/SSGIEOM:08. 129 pp.

ICES. 2017d. Report of the Workshop on age estimation of sprat (*Sprattus sprattus*) (WKARSPRAT), 15–18 November 2016, Galway, Ireland. ICES CM 2016/SSGIEOM:19. 129 pp.

ICES. 2018a. Data Quality Assurance Repository. Online at ICES website: https://www.ices.dk/community/Pages/PGCCDBS-doc-repository.aspx#others

ICES. 2018b. Report of the Working Group on Biological Parameters (WGBIOP), 1–5 October 2018, Ghent, Belgium. ICES CM 2018/EOSG:07. 186 pp.

ICES. 2018c. Report of the Workshop on Age reading of Horse Mackerel, Mediterranean Horse Mackerel and Blue Jack Mackerel (*Trachurus trachurus*, *T. mediterraneus* and *T. picturatus*) (WKARHOM3), 5–9 November 2018, Livorno, Italy. ICES CM 2018/EOSG:28. 186 pp. https://doi.org/10.1016/j.foodcont.2006.07.014

ICES. 2018d. Report of the Workshop on Mackerel biological parameter Quality Indicators (WKMACQI), 15–17 May 2018, IJmuiden, The Netherlands. ICES CM 2018/EOSG 34. 42 pp.

ICES. 2019a. Report of the Workshop on Age Estimation of Mackerel (*Scomber scombrus*) (WKARMAC2), 5–9 January and 22–26 October 2018, San Sebastian, Spain. In preparation.

ICES. 2019b. Workshop on Age Estimation Methods of Deep-Water Species (WKAMDEEP2), 17–21 September 2018, Cadiz, Spain. ICES CM 2018/EOSG:27. 31 pp.

Itai, T., Hayase, D., Hyobu, Y., Hirata, S. H., Kumagai, M., and Tanabe, S. 2012. Hypoxia-induced exposure of isaza fish to manganese and arsenic at the bottom of Lake Biwa, Japan: experimental and geochemical verification. Environmental Science and Technology, 46(11): 5789–5797. https://doi.org/10.1021/es300376y

Johannessen, A., Blom, G., and Folkvord, A. 2000. Differences in growth between spring and autumn spawned herring (*Clupea harengus* L.) larvae. Sarsia, 85: 461–466. https://doi.org/10.1080/00364827.2000.10414595

Jolivet, A., Bardeau, J-F., Fablet, R., Paulet, Y-M., and de Pontual, H. 2008. Understanding otolith biomineralization processes: new insights into microscale spatial distribution of organic and mineral fractions from Raman microspectrometry. Analytical and Bioanalytical Chemistry, 392: 551–560. https://doi.org/10.1007/s00216-008-2273-8

Jolivet, A., Bardeau, J-F., Fablet, R., Paulet, Y-M., and de Pontual, H. 2013. How do the organic and mineral fractions drive the opacity of fish otoliths? Insights using Raman microspectrometry. Canadian Journal of Fisheries and Aquatic Sciences, 70: 1–9. https://doi.org/10.1139/cjfas-2012-0298

Jónsson, E. 2007. Verification of anglerfish (*Lophius piscatorius*) age estimation through comparison of length modes of age read fish (*illicia*) to length modes of large year-classes appearing in the Icelandic stock. ICES Document CM 2007/K:03. 17 pp. https://doi.org/10.6027/9789289335300-10-en

Jónsson, G., and Pálsson, J. 2013. Íslenskir fiskar (Icelandic fishes). Reykjavík, Iceland. 493 pp. (In Icelandic.)

Jorge, I. M., and Costa Monteiro, C. 1980. Preliminary results on age and growth of *Sardina pilchardus* (Walb.) in sub-area IXa. ICES Document CM 1980/H:38. 18 pp.

Jurado-Ruzafa, A., and Santamaría, M. T. G. 2018. Age, growth and natural mortality of blue jack mackerel *Trachurus picturatus* (Carangidae) from the Canary Islands, Spain (NW Africa). African Journal of Marine Science, 40(4): 451–460. https://doi.org/10.2989/1814232X.2018.1550019

Jurado-Ruzafa, A., Hernández, E., and García Santamaría, M. T. 2017. Age, growth and natural mortality of Atlantic chub mackerel *Scomber colias* Gmelin 1789 (Perciformes: Scombridae), from Mauritania (NW Africa). VIERAEA, 45: 53–64.

Kalish, J. M. 1993**.** Pre- and post-bomb radiocarbon in fish otoliths. Earth and Planetary Science Letters, 114: 549–554. https://doi.org/10.1016/0012-821x(93)90082-k

Kalish, J. M., Johnston, J. M., Smith, D. C., Morison, A. K., and Robertson, S. G. 1997. Use of the bomb radiocarbon chronometer for age validation in the blue grenadier *Macruronus novazelandiae*. Marine Biology, 128: 557–563. https://doi.org/10.1007/s002270050121

Karlou-Riga, C. 2000. Otolith morphology and age and growth of *Trachurus mediterraneus* (Steindachner) in the Eastern Mediterranean. Fisheries Research, 46: 69–82. https://doi.org/10.1016/s0165-7836(00)00134-x

Karlou-Riga, C., and Sinis, A. 1997. Age and growth of horse mackerel, *Trachurus trachurus* (L.), in the Gulf of Saronikos (Greece). Fisheries Research, 32: 157–171. https://doi.org/10.1016/s0165-7836(97)00044-1

Kastelle, C. R., Kimura, D. K., Nevissi, A. E., and Gunderson, D. R. 1994. Using Pb-210/Ra-226 disequilibria for sablefish, *Anoplopoma fimbria*, age validation. Fishery Bulletin (US), 92: 292–301.

Kelly, C. J., Connolly, P. L., and Bracken, J. J. 1997. Age estimation, growth, maturity and distribution of the roundnose grenadier from the Rockall Trough. Journal of Fish Biology, 50: 1–17. https://doi.org/10.1111/j.1095-8649.1997.tb01336.x

Kerstan, M. 1985. Age, growth, maturity, and mortality estimates of horse mackerel (*Trachurus trachurus*) from the waters west of Great Britain and Ireland in 1984. Archiv für Fischereiwissenschaft, 36: 115–154.

Kerstan, M., and Waldron, M. E. 1995. Age validation in Horse Mackerel (*Trachurus trachurus*) otoliths from the NE and SE Atlantic Ocean. ICES Document CM 1995/H:23. 16 pp. https://doi.org/10.1006/jmsc.2001.1071

Kimura, D. K., and Anderl, D. M. 2005. Quality control of age data at the Alaska Fisheries Science Center. Marine and Freshwater Research, 56: 783–789. https://doi.org/10.1071/mf04141

Kiparissis, S., Tserpes, G., and Tsimenidi, N. 2000. Aspects on the demography of Chub Mackerel (*Scomber japonicas* Houttuyn, 1782) in the Hellenic Seas. Belgian Journal of Zoology, 130: 3–7.

Krug, H. M. 1989. The Azorean blackspot seabream, *Pagellus bogaraveo* (Brünnich 1768) (Teleostei: Sparidae): age and growth. Cybium, 13: 347–355.

Krumme, U., and Bingel, F. 2016. Tetracycline marks visible in Baltic cod *Gadus morhua* otoliths stored for 40 years. Journal of Fish Biology, 89(4): 2189–2194. https://doi.org/10.1111/jfb.13108

Lagardere, F., and Troadec, H. 1997. Age estimation in common sole (*Solea solea*) larvae: validation of daily increments and evaluation of a pattern recognition technique. Marine Ecology Progress Series, 155: 223–237. https://doi.org/10.3354/meps155223

La Mesa, M., and De Rossi, F. 2008. Early life history of the black anglerfish *Lophius budegassa* Spinola, 1807 in the Mediterranean Sea using otolith microstructure. Fisheries Research, 93: 234–239. https://doi.org/10.1016/j.fishres.2008.05.004

Landa, J. (ed.) 2011. Report of the Anglerfish (*Lophius piscatorius*) *illicia* and otoliths exchange 2011. ICES Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS). Instituto Español de Oceanografía. 61 pp. Available at the Data Quality Assurance Repository https://www.ices.dk/community/Documents/PGCCDBS/Anglerfish%20(Lophius%20piscaorius)%20illicia%20and%20otoliths%20exchange%202011_.pdf; https://doi.org/10.1006/jmsc.2002.1179

Landa, J. and Barcala-Bellod, E. 2017. Corroboration of fast annual growth rate of blackbellied anglerfish (*Lophius budegassa*) in Spanish Mediterranean. Fisheries and Aquaculture Journal, 8:4. 9 pp. https://doi.org/10.4172/2150-3508.1000237

Landa, J., and Piñeiro, C. 2000. Megrim (*Lepidorhombus whiffiagonis*) growth in the North-eastern Atlantic based on back-calculation of otolith rings. ICES Journal of Marine Science, 57: 1077–1090. https://doi.org/10.1006/jmsc.2000.0702

Landa, J., Pereda, P., Duarte, R., and Azevedo, M. 2001. Growth of anglerfish (*Lophius piscatorius* and *L. budegassa*) in Atlantic Iberian waters. Fisheries Research, 51: 363–376. https://doi.org/10.1016/s0165-7836(01)00260-0

Landa, J., Duarte, R., Quincoces, I., Dupouy, H., Bilbao, E., Dimeet, J., Lucio, P., Marçal, A., McCormick, H., and Ni Chonchuir, G. 2002. Report of the 4th International Ageing Workshop on European Anglerfish, 14–18 January 2002, IPIMAR, Lisbon, Portugal. 141 pp. Available at the Data Quality Assurance Repository https://www.ices.dk/community/Documents/PGCCDBS/4th%20Angler%20age%20reading%20Workshop.pdf

Landa, J., Duarte, R., and Quincoces, I. 2008. Growth of white anglerfish (*Lophius piscatorius*) tagged in the Northeast Atlantic, and a review of age studies on anglerfish. ICES Journal of Marine Science, 65: 72–80. https://doi.org/10.1093/icesjms/fsm170

Landa, J., Barrado, J., and Velasco, F. 2013. Age and growth of anglerfish (*Lophius piscatorius*) on the Porcupine Bank (west of Ireland) based on illicia age estimation. Fisheries Research, 137: 30–40. https://doi.org/10.1016/j.fishres.2012.07.026

Laurenson, C. H., Johnson, A., and Priede, I. G. 2005. Movements and growth of monkfish *Lophius piscatorius* tagged at the Shetland Islands, northeastern Atlantic. Fisheries Research, 71: 185–195. https://doi.org/10.1016/j.fishres.2004.08.020

Lee, T. W., and Byun, J. S. 1996. Microstructural growth in otoliths of conger eel (*Conger myriaster*) leptocephali during the metamorphic stage. Marine Biology, 125: 259–268. https://doi.org/10.1007/bf00346306

Letaconnoux, R. 1951. Contribution à l'études des espèces de genre *Trachurus* et spécialement du *Trachurus trachurus* (Linné 1758) de la côte Belge. Notes et Mémoires – Office Scientifique et Technique des Pêches Maritimes, 15: 1–67. (In French.) https://doi.org/10.5962/bhl.title.15581

Limburg, K. E., Olson, C., Walther, Y., Dale, D., Slomp, C. P., and Høie, H. 2011. Tracking Baltic hypoxia and cod migration over millennia with natural tags. Proceedings of the National Academy of Sciences of the United States of America, 108(22): E177–182. https://doi.org/10.1073/pnas.1100684108

Limburg, K. E., Wuenschel, M. J., Hüssy, K., Heimbrand, Y., and Samson, M. 2018. Making the otolith magnesium chemical calendar-clock tick: Plausible mechanism and empirical evidence. Reviews in Fisheries Science and Aquaculture, 26(4): 479–493. https://doi.org/10.1080/23308249.2018.1458817

Lorenzo, J. M., Pajuelo, J. G., and Ramos, A. G. 1995. Growth of the chub mackerel *Scomber japonicus* (Pisces: Scombridae) off the Canary Islands. Scientia Marina, 59(3–4): 287–291.

Lucio, P., Murúa, H., and Santurtún, M. 2000. Growth and reproduction of hake (*Merluccius merluccius*) in the Bay of Biscay during the period 1996–1997. Ozeanografika, 3: 325–354.

Macer, C. T. 1977. Some aspects of the biology of the horse mackerel (*Trachurus trachurus* L.) in waters around Britain. Journal of Fish Biology, 10: 51–62. https://doi.org/10.1111/j.1095-8649.1977.tb04041.x

Magnussen, E. 2007. Interpopulation comparison of growth patterns of 14 fish species on Faroe Bank: are all fishes on the bank fast-growing? Journal of Fish Biology, 71: 453–475. https://doi.org/10.1111/j.1095-8649.2007.01502.x

Magnússon, J. V., and Magnússon, J. 1995. The distribution, relative abundance, and biology of the deep-sea fishes of the Icelandic slope and Reykjanes Ridge. *In* Deep-Water Fisheries of the North Atlantic Oceanic Slope. Ed. by A. G. Hopper. NATO ASI Series (Series E: Applied Sciences), vol. 296: 161–199. Springer, Dordrecht. https://doi.org/10.1007/978-94-015-8414-2_5.

Magnusson, J. V., Bergstad, O. A., Hareide, N. R., Magnusson, J., and Reinert, J. 1997. Ling, blue ling and tusk of the Northeast Atlantic. TemaNord, 535: 1–58.

Mahé, K. 2009. Report of the Saithe (*Polliachius virens*, L.) Otolith Exchange Scheme 2007–2008. Ifremer. 36 pp. http://archimer.ifremer.fr/doc/00225/33631

Mahé, K., Elleboode, R., Øverbø Hansen, H., Román Marcote, E., Marull Hernández, E., Teruel Gómez, J., and Etherton, M. 2012a. Report of the Roundnose grenadier (*Coryphaenoides rupestris*) Otolith Exchange Scheme 2011. Ifremer. 23 pp. http://archimer.ifremer.fr/doc/00063/17436/

Mahé, K., Moerman, M., Maertens, I., Holmes, I., Boiron, A., and Elleboode, R. 2012b. Report of the Sole (*Solea solea*) in the Bay of Biscay Otolith Exchange Scheme 2011. Ifremer. 14 pp. http://archimer.ifremer.fr/doc/00063/17420/

Mahé, K., Sevin, K., Petursdottir, G., Finnbogadottir, G., Wilhelms, I., Beußel, F., *et al*. 2014. Report of the Saithe (*Pollachius virens*, L.) Otolith Exchange 2013. Ifremer. 20 pp. http://archimer.ifremer.fr/doc/00185/29595/

Mahé, K., Etherton, M., Dufour, J. L., Elleboode, R., Sévin, K., de Pontual, H. and Ernande, B. 2016. Marginal increment analysis of fish otoliths: new insights across a range of spe-cies. ICES CM 2016/H:99.

Martins, M. M., Jorge, I. M., and Gordo, L. S. 1983. On the maturity, morphological characteristics and growth of *Scomber japonicas* Houttuyn (1782) of west continental coast of Portugal. ICES Document CM 1983/H:39. 9 pp.

Matta, M. E., and Kimura, D. K. (Eds.) 2012. Age determination manual of the Alaska Fisheries Science Center Age and Growth Program. NOAA Professional Paper NMFS, 13. 97 pp. http://spo.nwr.noaa.gov/pp13.pdf

McBride, R. S. 2015. Diagnosis of paired age agreement: a simulation of accuracy and precision effects. ICES Journal of Marine Science, 72: 2149–2167. https://doi.org/10.1093/icesjms/fsv047

Mehl, S., Meland, E. S., and Høines, Å. 2012. Report of the blue whiting (*Micromesistius poutassou* L.) otolith exchange 2010–2011. 15 pp. Available at the Data Quality Assurance Repository http://ices.dk/community/Pages/PGCCDBS-doc-repository.aspx#age

Mellon-Duval, C., de Pontual, H., Metral, L., and Quemener, L. 2010. Growth of European hake (*Merluccius merluccius*) in the Gulf of Lions based on conventional tagging. ICES Journal of Marine Science, 67(1): 62–70. https://doi.org/10.1093/icesjms/fsp215

Mendiola, D., and Álvarez, P. 2008. Validation of daily increments in the otolith microstructure of Northeast Atlantic mackerel fish larvae. Fisheries Research, 89: 300–304. https://doi.org/10.1016/j.fishres.2007.10.018

Methot, R. 2000. Technical description of the Stock Synthesis Assessment Program. NOAA Technical Memorandum NMFS-NWFSC-43. 56 pp. https://doi.org/10.7755/tmspo.183

Methot, R. 2007. User manual for the integrated analysis program Stock Synthesis 2 (SS2), model version 2.00c. SEDAR 16-AW-05. 84 pp. http://sedarweb.org/docs/wpapers/S16_AW_05.pdf

Migoya, M. C. 1989. Étude de la croissance des larves de maquereau bleu (*Scomber scombrus*, L.) dans le Golfe du St. Laurent, a partir de l'examen de la microstructure des otoliths. M.Sc.Thesis, Université du Québec à Rimouski, Québec. 84 pp. (In French.) https://doi.org/10.1522/030331724

Moksness, E. 1992. Validation of daily increments in the otolith microstructure of Norwegian spring-spawning herring (*Clupea harengus* L). ICES Journal of Marine Science, 49: 231–235. https://doi.org/10.1093/icesjms/49.2.231

Moksness, E., and Fossum, P. 1991. Distinguishing spring- and autumn-spawned larvae (*Clupea harengus* L.) by otolith microstructure. ICES Journal of Marine Science, 48: 66–61. https://doi.org/10.1093/icesjms/48.1.61

Møller Christensen, J. 1964. Burning of otoliths, a technique for age determination of soles and other fish. Journal du Conseil Permanent International pour l'Exploration de la Mer, 29: 73–81. https://doi.org/10.1093/icesjms/29.1.73

Morales-Nin, B. 1999. Black Scabbard Fish (*Aphanopus carbo*) Otolith Exchange (1998–1999). EFAN Report 3-99. 15 pp. Available at the Data Quality Assurance Repository https://www.ices.dk/community/Documents/PGCCDBS/EFAN%20Report%203-99%20(Morales-Nin).pdf; https://doi.org/10.1016/0165-7836(95)00432-7

Morales-Nin, B., and Alderbert, Y. 1997. Growth of juvenile *Merluccius merluccius* in the Gulf of Lions (NW Mediterranean) based on otolith microstructure and length frequency analysis. Fisheries Research, 30: 77–85. https://doi.org/10.1016/s0165-7836(96)00553-x

Morales-Nin, B., and Moranta, J. 2004. Recruitment and post-settlement growth of juvenile *Merluccius merluccius* on the western Mediterranean shelf. Scientia Marina, 68: 399–409. https://doi.org/10.3989/scimar.2004.68n3399

Morales-Nin, B., and Panfili, J. 2002. Age estimation. *In* Manual of Fish Schlerochronology, pp. 91–98. Ed. by J. Panfili, H. Troadec, H. de Pontual, and P. J. Wright. Ifremer-IRD, Brest, France. 463 pp. https://doi.org/10.1111/j.0022-1112.2005.0751d.x

Morales-Nin, B., and Pertierra, J. P. 1990. Growth rates of the anchovy *Engraulis encrasicolus* and the sardine *Sardina pilchardus* in the northwestern Mediterranean Sea. Marine Biology, 107: 349–356. https://doi.org/10.1007/bf01319836

Morales-Nin, B., and Sena-Carvalho, D. 1996. Age and growth of the black scabbardfish *Aphanopus carbo* off Madeira. Fisheries Research, 25: 239–251. https://doi.org/10.1016/0165-7836(95)00432-7

Morales-Nin, B., Aldanondo, N., and Alvarez, I. 2010. Age determination of larval and juvenile small pelagics: the importance of a common protocol. Rapport Commission Internationale pour l'Exploration Scientifique de la Méditerranée, 39: 598.

Morales-Nin, B., Fortuño, J. M., Pérez-Mayol, S., and Grau, A. 2011. The use of energy dispersive X-ray spectroscopy to detect strontium marks in fish otoliths. Scientia Marina, 76(1): 173–176. https://doi.org/10.3989/scimar.03399.16b

Mosegaard, H., and Baron, P. R. 1999. Validation of annual rings by primary increment characteristics in herring and sprat sagitta otoliths. Summary of lecture at EFAN meeting. *In* Report of the Workshop on age estimation of sprat (*Sprattus sprattus*) (WKARSPRAT), 15–18 November 2016, Galway, Ireland. ICES CM 2016/SSGIEOM:19. 129 pp. https://doi.org/10.1006/jmsc.1999.0467

Mosegaard, H., and Titus, R. 1987. Daily growth rates of otoliths in yolk sac fry of two salmonid species at five different temperatures. *In* Proceedings of the Fifth Congress of European Ichthyologist, pp. 221–227. Ed. by S. O. Kallander and B. Farnholm, Swedish Museum of Natural History, Stockholm, Sweden. 500 pp.

Nava, E., Villar, E. I., Clemente, M. C., Rey, J., García, A, Fernández-Peralta, L., Piñeiro, C. G., and Otero, P. 2018. A new digital image tool that enhances otolith microstructure for estimating daily age in juvenile and adult fish. IEEE Journal of Oceanic Engineering, 43(1): 48–55. https://doi.org/10.1109/joe.2016.2644998

Navarro, M. R., Villamor, B., Landa, J., Hernandez, C., and Dominguez, M. R. 2018. Growth and validation of the age estimation of chub mackerel (*Scomber colias*) in the North and Northwest of the Iberian Peninsula. 6th International Otolith Symposium 2018, 15–20 April 2018, Taiwan (Province of China). Theme Session: Life History and Fisheries; 5-Minute Talk Session, No. F-208. Available at the Data Quality Assurance Repository https://www.ices.dk/community/Pages/PGCCDBS-doc-repository.aspx#others

Nedreaas, K. 1990. Age determination of Northeast Atlantic *Sebastes* species. Journal du Conseil International pour l'Exploration de la Mer, 47: 208–230.

Ofstad, L. H., Angus, C., Pedersen, T., and Steingrund, P. 2013. Age and growth of anglerfish (*Lophius piscatorius*) in Faroese waters. Fisheries Research, 139: 51–60. https://doi.org/10.1016/j.fishres.2012.05.011

Øverbø Hansen, H. 2012. Report on otolith exchange of ling 2012. Ling (*Molva molva*) otoliths from Norwegian fishery/reference fleet. IMR report. 11 pp.

Panfili, M. 2012. Ecology of early life stages of small pelagic fish *Engraulis encrasicolus* and *Sardina pilchardus* in the Adriatic Sea. PhD thesis. Universitá Politecnica delle Marche.

Panfili, J., and Tomàs, J. 2000. Validation of age estimation and back-calculation of fish length based on otolith microstructures in tilapias (Pisces, Cichlidae). Fishery Bulletin (US), 99: 139–150.

Panfili, J., de Pontual, H., Troadec, J-P., and Wright, P. J. (Eds.) 2002. Manual of Fish Sclerochronology. Ifremer-IRD, Brest, France. 463 pp. https://doi.org/10.1111/j.0022-1112.2005.0751d.x

Pattoura, P., Lefkaditou, E., and Megalofonou, P. 2011. Age and growth of juvenile *Merluccius merluccius* in the Ionian Sea (Eastern Mediterranean), based on otolith microstructure. 46th European Marine Biology Symposium, September 2011, Croatia. Book of Abstracts, p. 101.

Pauly, D. 1983. Some simple methods for the assessment of tropical fish stocks. FAO Fisheries Technical Paper No. 234. 52 pp.

Perrota, R. G., Carvalho, N., and Isidro, E. 2005. Comparative study on growth of chub mackerel (*Scomber japonicus* Houttuyn, 1782) from three different regions: NW Mediterranean, NE and SW Atlantic. Revista de Investigación y Desarrollo Pesquero, 17: 67–79.

Pertierra, J. P. 1987. Crecimiento del boquerón (*Engraulis encrasicolus*, L. 1758) (Pisces, Engraulidae) de la costa catalana (Mediterráneo noroccidental). Investigation Pesquera, 51(2): 263–275. (In Spanish.)

Pertierra, J. P., and Morales-Nin, B. 1989. Sardine growth in the Catalan Sea (NW Mediterranean) determined by means of otolith interpretation and length frequency data. Scientia Marina, 53(4): 821–826.

Petersen, C. G. 1891. Eine Methode zur Bestimmung des Alters und Wuchses der Fishe. Mitteilungen. Deutscher Seefisherei-Verein, 11: 226–235. (In German.)

Piñeiro, C., and Saínza, M. 2003. Age estimation, growth and maturity of the European hake (*Merluccius merluccius*, Linnaeus, 1758) from Iberian Atlantic waters. ICES Journal of Marine Science, 60: 1086–1102. https://doi.org/10.1016/s1054-3139(03)00086-9

Piñeiro, C., and Saínza, M. 2011. Report on otolith exchange of European hake (2011). Instituto Español de Oceanografía IEO, Vigo, Spain. 13 pp. Available at the Data Quality Assurance Repository http://www.ices.dk/community/Documents/PGCCDBS/Report_on_Hake_Otolith_Exchange_2011.pdf; https://doi.org/10.32800/amz.2011.09.0006

Piñeiro, C., Rey, J., de Pontual, H., and Goñi, R. 2007. Tag and recapture of European hake (*Merluccius merluccius* L.) off the Northwest Iberian Peninsula: first results support fast growth hypothesis. Fisheries Research, 88: 150–154. https://doi.org/10.1016/j.fishres.2007.08.015

Piñeiro, C., Rey, J., de Pontual, H., and García, A. 2008. Growth of Northwest Iberian juvenile hake estimated by combining sagittal and transversal otolith microstructure analyses. Fisheries Research, 93: 173–178. https://doi.org/10.1016/j.fishres.2008.04.001

Piñeiro, C. G., Morgada, C., Sainza, M., and McCurdy, W. J. 2009. Hake age estimation: state of the art and progress towards a solution. ICES Cooperative Research Report No. 294. 43 pp.

Polonsky, A. S., and Tormosova, I. D. 1969. The spawning of the horse mackerel of the North-east Atlantic and the distribution of its eggs and larvae. Trudy AtlantNIRO, 23: 27–48.

de Pontual, H., Bertignac, M., Battaglia, A., Bavouzet, G., Moguedet, P., and Groison, A. L. 2003. A pilot tagging experiment on European hake (*Merluccius merluccius*): methodology and preliminary results. ICES Journal of Marine Science, 60: 1318–1327. https://doi.org/10.1016/s1054-3139(03)00149-8

de Pontual, H., Groison, A. L., Pineiro, C., and Bertignac, M. 2006. Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. ICES Journal of Marine Science, 63: 1674–1681. https://doi.org/10.1016/j.icesjms.2006.07.007

de Pontual, H., Jolivet, A., Garren, F., and Bertignac, M. 2013. New insights on European hake biology and population dynamics from a sustained tagging effort in the Bay of Biscay. ICES Journal of Marine Science, 70: 1416–1428. https://doi.org/10.1093/icesjms/fst102

Porteiro, C., and Alvarez, F. 1983. Determinación del crecimiento de la sardina, *Sardina pilchardus* (Walb.), en aguas gallegas, mediante lectura directa de otolitos. Informes Técnicos Instituto Español de Oceanografía, 14. 19 pp. (In Spanish.)

Powers, J. E. 1983. Some statistical characteristics of ageing data and their ramifications in population analysis of oceanic pelagic fishes. *In* Proceedings of the International Workshop on Age Determination of Oceanic Pelagic Fishes: Tunas, Billfishes, and Sharks, pp. 19–24. Ed. by E. D. Prince and L. M. Pulos. NOAA Technical Report NMFS, 8. 211 pp. https://doi.org/10.1016/b978-0-12-374553-8.00103-9

Punt, A. E., Smith, D. C., Krusic Golub, K., and Robertson, S. 2008. Quantifying age-reading error for use in fisheries stock assessments, with application to species in Australia's southern and eastern scalefish and shark fishery. Canadian Journal of Fisheries and Aquatic Sciences, 65: 1991–2005. https://doi.org/10.1139/f08-111

Quincoces, I., Lucio, P., and Santurtún, M. 1998a. Biology of black anglerfish (*Lophius budegassa*) in the Bay of Biscay waters during 1996–1997. ICES Document CM 1998/O:47. 28 pp.

Quincoces, I., Santurtún, M., and Lucio, P. 1998b. Biological aspects of white anglerfish (*Lophius piscatorius*) in the Bay of Biscay (ICES Division VIIIa, b, d) in 1996–1997. ICES Document CM 1998/O:48. 29 pp.

Quinn, T. J., and Deriso, R. B. 1999. Quantitative Fish Dynamics. Oxford University Press, New York. 542 pp.

Quinn, T. J., Nemeth, R. S., and McIsaac, D. O. 1991. Patterns of homing and straying pattern of fall Chinook salmon in the Lower Columbia River. Transactions of the American Fisheries Society, 120: 150–156. https://doi.org/10.1577/1548-8659(1991)120<0150:haspof>2.3.co;2

Raitaniemi, J., and Halling, F. 2005. Report from The Herring Age Reading Workshop. Archipelago Research Institute, the island of Seili, Finland on 6–9 June 2005. Available at the Data Quality Assurance Repository http://www.ices.dk/community/Pages/PGCCDBS-doc-repository.aspx

Ramalho, A. M., and Pinto, J. S. 1956. Note sur la croissance de *Trachurus trachurus* (L.) pendant la première année. Annales Biologiques, 11: 173–174. (In French.)

Ramos, F., and Cendrero, O. 1967. Notes on the age and growth of *Pagellus cantabricus* (Asso) of Northern Spain. ICES Document CM 1967/G:3. 15 pp.

Ré, P. 1984. Evidence of daily and hourly growth in pilchard larvae based on otolith growth increments, *Sardina pilchardus* (Walbaum, 1792). Cybium, 8(1): 33–38. https://doi.org/10.1016/0165-7836(94)00316-o

Reeves, S. A. 2003. A simulation study of the implications of age-reading errors for stock assessment and management advice. ICES Journal of Marine Science, 60: 314–328. https://doi.org/10.1016/s1054-3139(03)00011-0

Rodriguez, J. L., and Iglesias, S. 1985. Preliminary study of growth of megrim (*Lepidorhombus whiffiagonis*, Walb.) from ICES sub-Area VII. ICES Document CM 1985/G:53. 16 pp.

Rodriguez-Roda, J. 1982. Biologia de la caballa (o estornino), *Scomber (Pneumatoforus) japonicus* Houttuyn (1782) del golfo de Cadiz. Investigaciones Pesqueras de Cadiz, 46: 143–149. In Spanish.)

Rollefsen, G. 1933. The otoliths of the cod. Fiskeridirektoratets Skrifter, Serie Havundersøkelser, 4: 1–14. Directorate of Fisheries, Bergen, Norway.

Rollefsen, G. 1935. The spawning zone in cod otoliths and prognosis of stock. Fiskeridirektoratets Skrifter, Serie Havundersøkelser, 4: 1–10. Directorate of Fisheries, Bergen, Norway.

Romanelli, M., Colloca, F., and Giovanardi, O. 2002. Growth and mortality of exploited *Sardina pilchardus* (Walbaum) larvae along the western coast of Italy. Fisheries Research, 55: 205–218. https://doi.org/10.1016/s0165-7836(01)00286-7

Ross, S., and Hüssy, K. 2013. A reliable method for ageing of whiting (*Merlangius merlangus*) for use in stock assessment and management. Journal of Applied Ichthyology, 29(4): 825–832. https://doi.org/10.1111/jai.12204

Saborido-Rey, F., Garabana, D., and Cerviño, S. 2004. Age and growth of redfish (*Sebastes marinus*, *S. mentella*, and *S. fasciatus*) on the Flemish Cap (Northwest Atlantic). ICES Journal of Marine Science, 61: 231–242. https://doi.org/10.1016/j.icesjms.2003.11.003

Sahrhage, D. 1970. Ein Beitrag zur Biologie des Stöckers (*Trachurus trachurus* (L.)) in der Nordsee. Berichte der Deutschen Wissenschaftlichen Kommission für Meeresforschung, 21: 122–169. (In German.)

Sánchez, F. 1983. Biology and fishery of the red sea-bream (*Pagellus bogaraveo* B.) in VI, VII and VIII Subareas of ICES. ICES Document CM 1983/G:38. 11 pp.

SARDONE. 2010. Improving assessement and management of small pelagic species in the Mediterranean. WP 3 in Final Report of the SARDONE project. UE-FP6-44294. Available at: http://www.ices.dk/explore-us/projects/EU-RFP/EU%20Repository/SARDONE/FP6%20SARDONE%20Final%20Activity%20Report.pdf

Secor, D. H., Dean, J. M., and Campana, S. E. (Eds.) 1995. Recent Developments in Fish Otolith Research. University of South Carolina Press. Columbia, SC. 730 pp.

Sigurdsson, T., Thorsteinsson, V., and Gustafsson, L. 2006. *In situ* tagging of deep-sea redfish: application of an underwater, fish-tagging system. ICES Journal of Marine Science, 63: 523–531. https://doi.org/10.1016/j.icesjms.2005.05.023

Silva, A. V., Meneses, I., and Silva, A. 2012. Determinacao de idade de juvenis de sardinha (*Sardina pilchardus*, Walbaum 1792) a partir de características morfometricas dos individuos e dos otolitos. Relatórios Científicos e Técnicos, Série Digital, 53. 24 pp. (In Spanish.) https://www.ipma.pt/resources.www/docs/publicacoes.site/docweb/2012/Reln53final.pdf

Skjæraasen, J. E., Nash, R. D. M., Korsbrekke, K., Fonn, M., Nilsen, T., Kennedy, J., *et al*. 2012. Frequent skipped spawning in the world's largest cod population. Proceedings of the National Academy of Sciences of the United States of America, 109(23): 8995–8999. 5 pp. https://doi.org/10.1073/pnas.1200223109

Smith, J. 2014. Age validation of lemon sole (*Microstomus kitt*), using marginal increment analysis. Fisheries Research, 157: 41–46. https://doi.org/10.1016/j.fishres.2014.03.011

Sobrino, I., and Gil, J. 2001. Studies on age determination and growth pattern of the red (blackspot) seabream [*Pagellus bogaraveo* (Brünnich, 1768)] from the strait of Gibraltar (ICES IXa/SW Spain): Application to the species migratory pattern. NAFO Scientific Council Research Document, 01/87. 5 pp.

Spurgeon, J. J., Hamel, M. J., Pope, K. L., and Pegg, M. A. 2015. The global status of freshwater fish age validation studies and a prioritization framework for further research. Reviews in Fisheries Science & Aquaculture, 23(4): 329–345. https://doi.org/10.1080/23308249.2015.1068737

Stötera, S., Degen-Smyrek, A. K., Krumme, U., Stepputtis, D., Bauer, R., Limmer, B., and Hammer, C. 2018. Marking otoliths of Baltic cod (*Gadus morhua* Linnaeus, 1758) with tetracycline and strontium chloride. Journal of Applied Ichthyology, https://doi.org/10.1111/jai.13829

Stransky, C., Kanisch, G., Krüger, A., and Purkl, S. 2005a. Radiometric age validation of golden redfish (*Sebastes marinus*) and deep-sea redfish (*S. mentella*) in the Northeast Atlantic. Fisheries Research, 74: 186–197. https://doi.org/10.1016/j.fishres.2005.03.003

Stransky, C., Gudmundsdottir, S., Sigurdsson, T., Lemvig, S., Nedreaas, K., and Saborido-Rey, F. 2005b. Age determination and growth of Atlantic redfish (*Sebastes marinus* and *S. mentella*): bias and precision of age readers and otolith preparation methods. ICES Journal of Marine Science, 62: 655–670. https://doi.org/10.1016/j.icesjms.2005.01.018

Sünksen, K., Stenberg, C., and Grønkjær, P., 2010. Temperature effects on growth of juvenile Greenland halibut (*Reinhardtius hippoglossoides* Walbaum) in West Greenland waters. Journal of Sea Research, 64: 125–132. https://doi.org/10.1016/j.seares.2009.10.006

Swan, S. C., and Gordon, J. D. M. 2001. A review of age estimation in macrourid fishes with new data on age validation of juveniles. Fisheries Research, 51: 177–195. https://doi.org/10.1016/s0165-7836(01)00244-2

Talman, S., Krusic-Golub, K., Robertson, S., and Green, C. 2003. Age estimation of deepwater fish species from the eastern North Atlantic. Final Report to the Bord Iascaigh Mhara (Irish Sea Fisheries Board). Marine and Freshwater Resources Institute, Department of Primary Industries 2003. 78 pp.

Thomas, R. 1987. Biological investigations on the blue ling, *Molva dypterygia* (Pennant 1784 after O. F. Muller 1776), in the areas of the Faroe Islands and to the west of the Shetland Islands. Archiv für Fischereiwissenschaft, 38(1/2): 9–34.

Thorrold, S. R., Jones, C. M., and Campana, S. E. 1997. Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). Limnology and Oceanography, 42(1): 102–111. https://doi.org/10.4319/lo.1997.42.1.0102

Torstensen, E., Eltink, A. T. G. W., Casini, M., McCurdy, W. J., and Worsøe Clausen, L. 2004. Report of the Workshop on Age Estimation of Sprat, 14–17 December 2004, Institute of Marine Research, Flødevigen, Arendal, Norway. 44 pp. Available at the Data Quality Assurance Repository http://www.ices.dk/community/Documents/PGCCDBS/sprat.agewk2004.pdf

Treble, M. A., and Dwyer, K. S. 2008. Report of the Greenland halibut (*Reinhardtius hippoglossoides*) Age Determination Workshop. NAFO Scientific Council Studies, 41: 1–96. https://doi.org/10.2960/s.v41.m1

Treble, M. A., Campana, S. E., Wastle, R. J., Jones, C. M., and Boje, J. 2008. Growth analysis and age validation of a deepwater Arctic fish, the Greenland halibut (*Reinhardtius hippoglossoides*). Canadian Journal of Fisheries and Aquatic Sciences, 65: 1047–1059. https://doi.org/10.1139/f08-030

Tsimenidis, N. C., and Ondrias, J. C. 1980. Growth studies on the angler-fishes *Lophius piscatorius* L., 1758 and *Lophius budegassa* Spinola, 1807 in Greek waters. Thalassographica, 3: 63–94.

Upton, S. A., Walther, B. D., Thorrold, S. R., and Olney, J. E. 2012. Use of a natural isotopic signature in otoliths to evaluate scale-based age determination for American shad. Marine and Coastal Fisheries, 4: 346–357. https://doi.org/10.1080/19425120.2012.675973

Uriarte, A., and Astudillo, A. 1987. The anchovy in the Bay of Biscay: New data and analysis of the fishery 1974–1987. ICES Document CM 1987/H:20. 17 pp.

Uriarte, A., Blanco, M., Cendrero, O., Grellier, P., Millán, M., Morais, A., and Rico, I. 2002. PELASSES Project Report of the Workshop on Anchovy Otoliths from Subarea VIII and Division IXa i**n** AZTI, PASAIA (Spain) from 14 to 18 January 2002. Annex to PELASSES report EU study Project -EC DG XIV Contract nº99/010. Available at the Data Quality Assurance Repository http://www.ices.dk/community/Documents/PGCCDBS/ANCHOVY%20OTOLITH%20WORKSHOP_2002.pdf

Uriarte, A., Rico, I., Villamor, B., Duhamel, E., Dueñas, C., Aldanondo, N., and Cotano, U. 2016. Validation of age determination from otoliths for the European Anchovy (*Engraulis encrasicolus* L.) in the Bay of Biscay. Australian Journal of Marine and Freshwater Research, 67(7): 951–966. https://doi.org/10.1071/mf15092

Vasconcelos, J. 2006. Contribuiçao para o conhecimento da biología da cavala, *Scomber japonicus* Houttuyn, 1782 do Arquipelago da Madeira. PhD Thesis, Universidad da Madeira, Spain. (In Spanish.)

Vasconcelos, J., Alves, A., Gouveia, E., and Faria, G. 2006. Age and growth of the blue jack mackerel, *Trachurus picturatus* Bowdich, 1825 (Pisces: Teleostei) off Madeira archipelago. Arquipélago, Life and Earth Sciences, 23(A): 47–57. https://doi.org/10.1016/j.fishres.2017.02.009

Vasconcelos, J., Dias, M. A., and Faria, G. 2011. Age and growth of the Atlantic chub mackerel *Scomber colias* Gmelin, 1789 off Madeira Island. Arquipélago, Life and Marine Sciences, 28: 57–70.

Vasconcelos, J., Vieira, A. R., Sequeira, V., González, J. A., Kaufmann, M., and Gordo, L. S. 2018. Identifying populations of the blue jack mackerel (*Trachurus picturatus*) in the Northeast Atlantic by using geometric morphometrics and otolith shape analysis. Fisheries Bulletin, 116: 81–92. https://doi.org/10.7755/FB.116.1.9

Velasco, E. M., Del Arbol, J., Baro, J., and Sobrino, I. 2011. Age and growth of the Spanish chub mackerel *Scomber colias* off southern Spain: a comparison between samples from the NE Atlantic and the SW Mediterranean. Revista de Biología Marina y Oceanografía, 46(1): 27–34. https://doi.org/10.4067/s0718-19572011000100004

Vieira, A. R., Farias, I., Figueiredo, I., Neves, A., Morales-Nin, B., Sequeira, V., *et al*. 2009. Age and growth of black scabbardfish (*Aphanopus carbo* Lowe, 1839) in the southern NE Atlantic. Scientia Marina, 732: 33–46. https://doi.org/10.3989/scimar.2009.73s2033

Vietti, M., Giulianini, P. G., and Ferrero, E.A. 1997. Reproductive biology of scad, *Trachurus mediterraneus* (Teleostei, Carangidae), from the Gulf of Trieste. ICES Journal of Marine Science, 54: 267–272. https://doi.org/10.1006/jmsc.1996.0185

Villamor, B., and Meixide, M. 1995. Final Results of the Mackerel (*Scomber scombrus*, L.) Exchange Programme for Otoliths in 1994. Presentation at the Workshop on Mackerel Otolith Reading, 8–14 February 1995, Vigo, Spain. 40 pp. Available at the Data Quality Assurance Repository http://www.ices.dk/community/Documents/PGCCDBS/Mackerel%20Otolith%20Exchange%201994.pdf; https://doi.org/10.1093/icesjms/fsm163

Villamor, B., Basilone, G., and La Mesa, M. 2013. Anchovy and sardine micro increment daily growth exchange results, WD07 p. 81. *In* Report of the Workshop on Micro Increment Daily Growth in European Anchovy and Sardine (WKMIAS), 21–25 October 2013, Mazara del Vallo, Sicily, Italy. ICES Document CM 2013/ACOM:51. 153 pp.

Villamor, B., Navarro, M. R., and Hernandez, C. 2018. Study of seasonal formation of growth rings in the otoliths of the NEA Mackerel (*Scomber scombrus*) in ICES Divisions 8c and 9a North. Working Document presented to the Workshop on Age Estimation of Atlantic Mackerel (*Scomber scombrus*) (WKARMAC2), 22–26 October 2018, Pasaia, San Sebastian, Spain. 96 pp.

https://community.ices.dk/ExpertGroups/WKARMAC2/_layouts/15/start.aspx#/SitePages/HomePage.aspx; https://doi.org/10.1093/icesjms/fsm163

Waldron, M. E., and Kerstan, M. 2001. Age validation in horse mackerel (*Trachurus trachurus*) otoliths. ICES Journal of Marine Science, 58: 806–813. https://doi.org/10.1006/jmsc.2001.1071

Walther, B. D., and Limburg, K. E. 2012. The use of otolith chemistry to characterize diadromous migrations. Journal of Fish Biology, 81(2): 796–825. https://doi.org/10.1111/j.1095-8649.2012.03371.x

Walther, B., and Thorrold, S. 2006. Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. Marine Ecology Progress Series, 311: 125–130. https://doi.org/10.3354/meps311125

Wilcoxon, F. 1945. Individual Comparisons by Ranking Methods. Biometrics Bulletin, 1(6): 80–83. https://doi.org/10.2307/3001968

Wilson, C. A. [Chairman] 1987. Glossary Committee. *In* Age and Growth of Fish, pp. 527–530. Ed. by R. C. Summerfelt and G. E. Hall. Iowa State University Press, Ames, IA. 544 pp.

Witthames, P. R., Thorsen, A., and Kjesbu, O. S. 2010. The fate of vitellogenic follicles in experimentally monitored Atlantic cod *Gadus morhua* (L.): Application to stock assessment. Fisheries Research, 104: 27–37. https://doi.org/10.1016/j.fishres.2009.11.008

Woodroffe, D. A., Wright, P. J., and Gordon, J. D. M. 2003. Verification of annual increment formation in the white anglerfish, *Lophius piscatorius* using the illicia and sagitta otoliths. Fisheries Research, 60: 345–356. https://doi.org/10.1016/s0165-7836(02)00174-1

Worsøe Clausen, L., Power, G., Timoshenko, N., and Tangen, O. 2005. Report of the Blue Whiting Otolith Ageing Workshop, 13–16 June 2005, DIFRES, Hirtshals, Denmark. 36 pp. Available at the Data Quality Assurance Repository http://www.ices.dk/community/Documents/PGCCDBS/whb.agewk2005.pdf

Wright, P. J., Woodroffe, D. A., Gibb, F. M., and Gordon, J. D. M. 2002. Verification of the first annulus formation in the illicia and otoliths of white anglerfish, *Lophius piscatorius* using otolith microstructure. ICES Journal of Marine Science, 59: 587–593. https://doi.org/10.1006/jmsc.2002.1179

Zhu, X., Zhao, Y., Mathers, A., and Corkum, L. D. 2013. Length frequency age estimations of American eel recruiting to the Upper St. Lawrence River and Lake Ontario. Transactions of the American Fisheries Society, 142(2): 333–344. https://doi.org/10.1080/00028487.2012.741554

Annex 1: Contributor contact information

Editors

Francesca Vitale
Swedish University of Agricultural Sciences
Department of Aquatic Resources
Institute of Marine Research, Turistgatan 5
453 30 Lysekil
Sweden
francesca.vitale@slu.se

Lotte Worsøe Clausen
ICES
H. C. Andersens Boulevard 44–46
DK-1553 Copenhagen V
Denmark
lotte.worsoe.clausen@ices.dk

Gráinne Ni Chonchuir
Marine Institute, Ireland
Rinville, Oranmore, Co. Galway H91 R67
Ireland
Grainne.NiChonchuir@marine.ie

Contributors

Pablo Abaunza
Instituto Español de Oceanografia
Subdirección General de Investigacion
Corazón de Maria, 8-28002 Madrid
Spain
pablo.abaunza@ieo.es

Ole Thomas Albert
Institute of Marine Research
P.O. Box 1870 Nordnes, 5817 Bergen
Norway
ole.thomas.albert@imr.no

Naroa Aldanondo
AZTI, Marine Research Division
Herrera Kaia, Portualdea z/g, Pasaia
Gipuzkoa 20110
Spain
naldanondo@azti.es

Barbara Bland
Swedish University of Agricultural Sciences
Department of Aquatic Resources
Institute of Marine Research,
Turistgatan 5
453 30 Lysekil
Sweden
barbara.bland@slu.se

Loes Bolle
Wageningen University and Research Centre (Imares)
Haringkade 1, 1976 CP IJmuiden
Netherlands
loes.bolle@wur.nl

Pierluigi Carbonara
COISPA Tecnologia and Ricerca
Stazione Sperimentale per lo Studio delle Risorse del Mare
via dei Trulli 18–20,
70126 Bari-Torre a Mare
Italy
carbonara@coispa.it

Gertrud Delfs
Institute of Sea Fisheries, Thünen Institut
Palmaille 9, 22767 Hamburg-Altona
Germany
gertrud.delfs@thuenen.de

Rafael Duarte
Inspector-Fisheries,
European Commission
Directorate General for Fisheries and Maritime Affairs
Rue Joseph II, 79 (02/217), Brussels
Belgium
rafael.duarte@ec.europa.eu

Mark Etherton
Centre for Environment, Fisheries and Aquaculture Science (Cefas)
Pakefield Road, Lowestoft, Suffolk
NR33 0HT
UK
mark.etherton@cefas.co.uk

María Teresa García Santamaría
Instituto Español de Oceanografía
Centro Oceanográfico de Canarias
Santa Cruz de Tenerife
Spain
Teresa.gsantamaria@ieo.es

Tomas Gröhsler
Institut of Baltic Sea Fisheries (TI-OF)
Alter Hafen Süd 2, DE-18069 Rostock
Germany
tomas.groehsler@thuenen.de

Carmen Hernandez
Instituto Español de Oceanografia
Promontorio de San Martin, s/n –
39004, Santander
Spain
carmen.hernandez@ieo.es

Karin Hüssy
Technical University of Denmark
National Institute of Aquatic Resources
Kemitorvet, Building 202
DK-2800 Kgs. Lyngby
Denmark
kh@aqua.dtu.dk

Ernesto Jardim
Fisheries Scientist Joint Research Centre (JRC)
Sustainable resources directorate, Water and Marine Resources unit TP 051
Via Enrico Fermi 2749
I-21027 Ispra (VA)
Italy
ernesto.jardim@jrc.ec.europa.eu

Alba Jurado-Ruzafa
Instituto Español de Oceanografía
Centro Oceanográfico de Canarias
Santa Cruz de Tenerife
Spain
alba.jurado@ieo.es

Uwe Krumme
Institute of Baltic Sea Fisheries
Alter Hafen Süd 2
18069 Rostock
Germany
uwe.krumme@thuenen.de

Jorge Landa
Instituto Español de Oceanografia
Promontorio de San Martin, s/n –
39004, Santander
Spain
jorge.landa@ieo.es

Kélig Mahé
Institut français de recherche pour l'exploitation de la mer (IFREMER)
Fisheries Laboratory
150 Quai Gambetta, BP 699
62 321 Boulogne-sur-mer
France
Kelig.Mahe@ifremer.fr

Beatriz Morales-Nin
Mediterranean Institute for Advanced Studies (IMEDEA – CSIC–UIB) –
C/ Miquel Marquès
21 – 07190 Esporles – Islas Baleares
Spain
beatriz@imedea.uib-csic.es

Maria Rosario Navarro
Instituto Español de Oceanografia
Promontorio de San Martin, s/n –
39004 Santander
Spain
charo.navarro@ieo.es

Carmen Gloria Piñeiro Álvarez
Spanish Institute of Oceanography (IEO)
Centro Oceanográfico de Vigo
Subida a Radio Faro, 50
36390 Vigo
Spain
Carmen.pineiro@ieo.es

Hélène de Pontual
Institut français de recherche pour l'exploitation de la mer (IFREMER)
Sciences et Technologies Halieutiques
CS 10070, 29280 Plouzané
France
Helene.De.Pontual@ifremer.fr

Fernando Ramos
Instituto Español de Oceanografia
Muelle de Levante (Puerto Pesquero)
Apdo. 2609, 11006 Cadiz
Spain
Fernando.ramos@ieo.es

Javier Rey
Spanish Institute of Oceanography (IEO)
Centro Oceanográfico de Málaga
29640 Fuengirola
Málaga
Spain
javier.rey@ieo.es

Isabel Riveiro
Instituto Español de Oceanografia
Subida Radio Faro, 50,
36390 Vigo, PO
Spain
isabel.riveiro@ieo.es

Norbert Rohlf
Institute of Sea Fisheries,
Thünen Institut
Palmaille 9, 22767 Hamburg-Altona
Germany
norbert.rohlf@thuenen.de

Joanne Smith
Centre for Environment, Fisheries and Aquaculture Science (Cefas)
Pakefield Road, Lowestoft, Suffolk
NR33 0HT
UK
joanne.smith@cefas.co.uk

Eduardo Soares
IPMA – Algés
Av. de Brasilia 6, 1449-006 Lisbon
Portugal
esoares@ipma.pt

Sally Songer
Centre for Environment, Fisheries and Aquaculture Science (Cefas)
Pakefield Road, Lowestoft, Suffolk
NR33 0HT
UK
sally.songer@cefas.co.uk

Christoph Stransky
Institute of Sea Fisheries,
Thünen Institut
Palmaille 9, 22767 Hamburg-Altona
Germany
Christoph.stransky@thuenen.de

Jorge Tornero
Instituto Español de Oceanografia
Muelle de Levante (Puerto Pesquero)
Apdo. 2609, 11006 Cadiz
Spain
Jorge.tornero@ieo.es

Jens Ulleweit
Institute of Sea Fisheries,
Thünen Institut
Palmaille 9, 22767 Hamburg-Altona
Germany
jens.ulleweit@thuenen.de

Andres Uriarte
AZTI, Marine Research Division
Herrera Kaia, Portualdea z/g, Pasaia
Gipuzkoa 20110
Spain
auriarte@azti.es

Begoña Villamor
Instituto Español de Oceanografía
Promontorio San Martín, s/n Apdo.
240, 39080 Santander
Spain
begona.villamor@ieo.es

Yvonne Walther
County Administrative Board of
Blekinge
Skeppsbrokajen 4
371 86 Karlskrona
Sweden
ymwalther@gmail.com

Annex 2: Annotated images of the species included in Chapter 2

Saithe

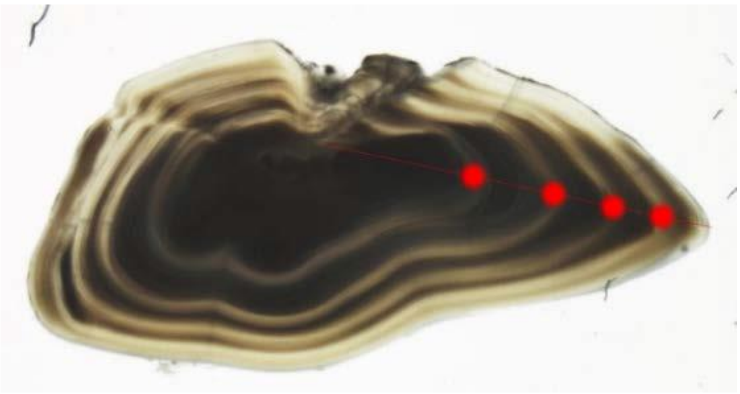


Figure A.2.1. Transversal section of a saithe otolith, viewed with transmitted light and with annotations shown as red circles. Capture location = Barents Sea; sampling date = 10 October 2014; fish size = 46 cm; estimated age = 4 years (Source: ICES, 2015).

Whiting

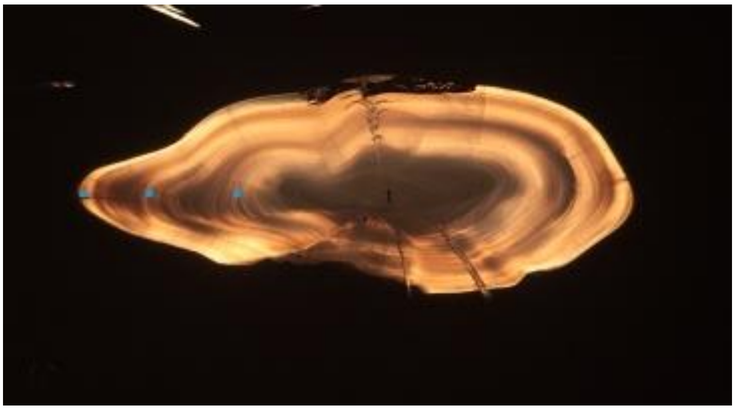


Figure A.2.2. Transversal section of a whiting otolith, viewed with transmitted light and with annotations shown as red circles. Capture location = North Sea; sampling date = 14 May 2010; fish size = 31 cm; estimated age = 3 years (Image: Joanne Smith, CEFAS).

Cod

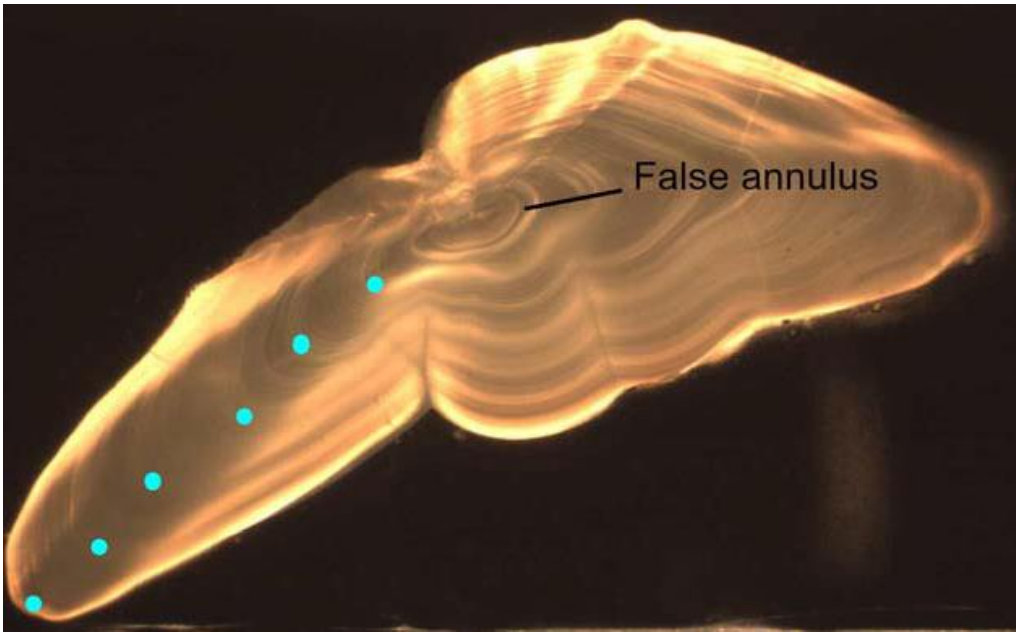


Figure A.2.3. Transversal section of a North Sea cod otolith, viewed with transmitted light and with annotations as blue circles. Capture location = North Sea; sampling date = first quarter 2005/2006; estimated age = 6 years (Source: ICES, 2008a).

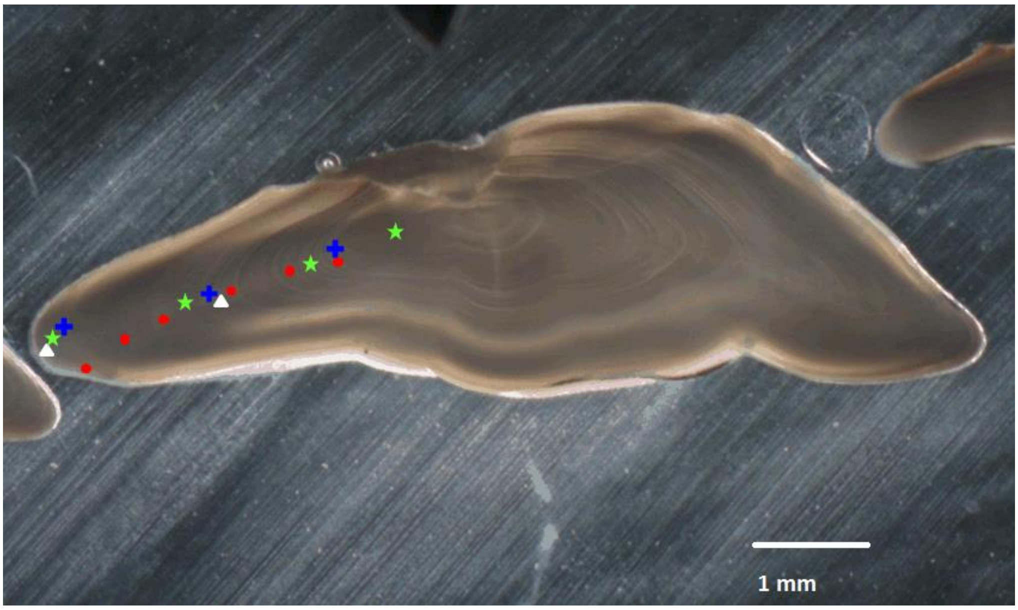


Figure A.2.4. Transversal section of an eastern Baltic cod otolith, viewed with transmitted light and with annotations of four different readers represented by different colours. Capture location = ICES Subdivision 25; sampling date = 11 February 2013; fish size = 35 cm; estimated ages = 2, 3, 4, and 6 years (Image: Karin Hüssy, DTU Aqua).

Blue whiting

Figure A.2.5. Image of a whole blue whiting otolith with annotations of annuli (red circles). Capture location = ICES Subdivision 25; sampling date = first quarter 2009; fish size = 29 cm; sex = male; estimated age = 7 years (Source: ICES, 2013a).

Annex 3: ICES age calibration workshops (1992–2012)

A total of 31 ICES reports on age estimation and calibration exercises, published between 1992 and 2012, were reviewed. Most of them used various methods to compare age estimations, such as percentage of agreement (PA), average percent error (APE), standard deviation (s.d.), and coefficient of variation (*CV*), referring to the guidelines and tools for age reading comparisons (Eltink *et al*., 2000). The age-bias plot is a graphical representation of the comparisons most commonly used among age readers. Since 2012 further calibration workshops have been held, for details please refer to the species-specific chapters. The majority of the calibration workshops were using the methods described in this table. However, more recent workshops (2017 onwards) have made use of SmartDots, applying associated statistical analysis tools. All reports are available on the Data Quality Assurance Repository (ICES, 2018a).

Table A.3.1. ICES age calibration workshops reviewed during the meeting for the use of different analytical methods, software, and diagnostics. Abbreviations used are: ALK = age–length key, ANOVA = Analysis of variance (models), APE = average percentage error, *CV* = coefficient of variation, EDA = exploratory data analysis, EFAN = European Fish Ageing Network, GIMP2.6 = GNU Image Manipulation Program, MAD = modal age difference, MIA = marginal increment analysis, ORACLE = improved version of Eltink *et al.* (2000), OTC = oxytetracycline, PA = percentage agreement, QCapture = imaging software, TNPC = Numerical Treatment of Calcified Structures, WebGR = fish growth and reproduction software.

Workshop	Year	Analysis	Methods, software	Age parameters	Presentation type
Second Workshop on Age Reading of Red Mullet and Striped Red Mullet (WKACM2; ICES, 2012b)	2012	*CV*; MAD; PA; relative bias; precision coefficient; mean age; first/second reading	TNPC software; Eltink *et al.* (2000) spreadsheet	Marginal increment analysis; otolith distances from the nucleus to the edge and to each ring	Histograms, bar plot, linear, box plot; tables; otolith photos; smart arts
Roundnose Grenadier (*Coryphaenoides rupestris*) Otolith Exchange Scheme 2011 (Mahé *et al*., 2012a)	2011	PA; MAD; *CV*; relative bias; s.d.; precision coefficient	Not mentioned	Distance between the nucleus and the first translucent ring	Bar plots, bias plots, linear graphs; otolith photos; tables
Sole (*Solea solea*) in the Bay of Biscay Otolith Exchange Scheme 2011 (Mahé *et al*., 2012b)	2011	PA; MAD; *CV*; relative bias	Not mentioned	Fish length; male/female	Bar plots, bias plots, linear graphs; tables; otolith photos
Report on Otolith Exchange of European Hake (Piñeiro and Saínza, 2011)	2011	PA; *CV*; APE	WebGR; Eltink *et al.* (2000) spreadsheet	Age	Fish length histogram, box-whisker plots; otolith photos; tables
Report of the Workshop on Age Reading of Dab (WKARDAB; ICES, 2010f)	2010	PA; *CV*	Eltink *et al.* (2000) spreadsheet; TNPC software	Otolith radius	Fish length histogram, *CV*, PA, and s.d. were plotted against modal age; otolith photos, reference collection images; tables
Report of the Workshop on Age Reading of North Sea (4) and Skagerrak–Kattegat (3.a) Plaice (WKARP; ICES, 2010c)	2010	PA; *CV*; age bias; APE	WebGR and TNPC software	Age	Reference collection images; age composition histogram; otolith photos; bar plots (type of edge), bias plots; tables

Workshop	Year	Analysis	Methods, software	Age parameters	Presentation type
Report of the Workshop on Age Reading of Red Mullet (*Mullus barbatus*) and Striped Mullet (*Mullus surmuletus*) (WKACM; ICES, 2009c)	2009	Agreement; *CV*; PA; standard deviation by modal age; bias; back-calculation of lengths; the Kruskal-Wallis test; marginal increment analysis	TNPC; Eltink *et al*. (2000) spreadsheet	Age estimation; ring radius; length frequency	Tables; linear plots; histograms; box-and-whisker plots
Workshop on Age Reading of European and American Eel (WKAREA; ICES, 2009d)	2009	Back-calculated growth rates	Eltink *et al.* (2000) spreadsheet	Age estimation; validation of age estimation; fish length	Not available
Report of the Workshop on Age Reading of Greenland Cod (WKARGC; ICES, 2009e)	2009	Modal length progression; deviations from modal age estimates; *CV*; s.d.; PA	EFAN methodology (Eltink *et al*., 2000; Eltink, 2000); the model was coded in Proc NLIN in SAS	Fish size; age estimations; age group is described by three parameters: the mean length (m), the standard deviation (s), and the abundance (a); age–length key	Histograms; age-bias plots, length distribution
Report of the Workshop on Age Estimation of European Hake (WKAEH; ICES, 2009a)	2009	Accuracy and precision; quality control and quality assurance (PA, APE, *CV*); Wilcoxon signed ranks tests (Wilcoxon, 1945); transition matrix	GIMP2.6 software; TNPC V4.1; Excel *ad hoc* Workbook, “AGE COMPARATIONS.XLS” (Eltink *et al*., 2000)	ALKs; age estimation (blind and supervised); OTC validation; assumed a reference age for comparison	Histograms (length frequency distribution); scatterplot; box-whisker plots (for age estimate distribution analysis and ring-to-nucleus distances distribution analysis)
Workshop on Age Reading of European Anchovy (WKARA; ICES, 2010d)	2009	Von Bertalanffy growth equation	Workbook age estimation comparisons of Eltink (2000) and guidelines and tools for age estimation comparisons (Eltink *et al*., 2000).	Microincrement daily growth as validation for first annual ring; age estimation; MIA	Tables; age-bias plots
Report of the Workshop on Age Reading of Mackerel (WKARMAC; ICES, 2010e)	2010	*CV*; PA; bias	Age comparison worksheet (Eltink *et al*., 2000)	Age estimation; otolith weight; length distribution	Tables; age-bias plots
Report of the Workshop on Age Reading of Turbot (WKART; ICES, 2008d)	2008	PA	ORACLE, which is an improved version of the Eltink *et al.* (2000) spreadsheet		Tables, otolith photos
Report of the Workshop on Age Determination of Redfish (WKADR; ICES, 2009b)	2008	PA; *CV*; APE; von Bertalanffy	QCapture software		Age-bias plot, otolith schematic drawing, otolith drawings showing growth timing
Report of the Workshop on Age Reading of Baltic Herring (WKARBH; ICES, 2008c)	2008	PA; *CV*	Eltink *et al.* (2000) spreadsheet; validation (increment width)		The grey tone profile (for validation), otolith photos; age-bias plot
Report of the 2nd Workshop on Age Reading of Flounder (WKARFLO; ICES, 2008e)	2008	PA; s.d.; MAD; *CV*; APE; relative bias; one-sample Wilcoxon rank sum test; t-test	SPSS 15.0	Fish length/age; stained otoliths; male/female	Scatterplots, age-bias plot; otolith photos; tables
Report of the Workshop on Age Reading of North Sea Cod (WKARNSC; ICES, 2008f)	2008	PA; s.d.; *CV*; the Wilcoxon signed ranks test; relative bias; MAD	Spreadsheet software	Marking the identified age structures on an agreed axis on digital images; otolith weight; fish length/age	Otolith photos; linear plots, histogram, Gantt chart; tables

Workshop	Year	Analysis	Methods, software	Age parameters	Presentation type
Report of the Workshop on Age Reading of Baltic Sprat (WKARBS; ICES, 2008b)	2008	PA; *CV*; the Wilcoxon signed ranks test; MAD; inter-reader bias	Not mentioned	Age estimation	Tables; age-bias plot
Report of the Workshop on Age Estimation of Sprat (Torstensen *et al.*, 2004)	2004	PA; *CV*; relative bias; age bias	Not mentioned	Validation (MIA and otolith weight frequency)	Age-bias plots; tables; otolith photos (daily and annual rings); growth plot, histogram
Workshop on Megrim Otolith Age Readings (Egan *et al.*, 2004)	2004	PA; *CV*; APE; exploratory data analysis (EDA)	Eltink *et al.* (2000) spreadsheet	Fish length frequency	Box-whisker plots, age-bias plots, histogram; otoliths photos; tables
Plaice Age Determination Exchange and Workshop, preliminary report (Easey, 2003)	2003	PA; *CV*; relative bias	Eltink *et al.* (2000) spreadsheet	Age	None
Black Scabbardfish (*Aphanopus carbo*) Otolith Exchange (1998–1999) (Morales-Nin, 1999)	1999	Age-bias plot; s.d.; PA; MAD; Bertalanffy curve	Eltink *et al.* (2000) spreadsheet	Fish length vs. age	Linear, box plot, scatterplot, histogram graphs; tables
Horse Mackerel Otolith Workshop (ICES, 1999)	1999	APE; s.d.; *CV*; PA	Eltink *et al.* (2000) spreadsheet	Fish length vs. age	Histogram, age-bias plot, linear, bar plot, notched box plot; graphs; otolith photos
Report of the Workshop on Mackerel Otolith Reading (ICES, 1995)	1995	PA; *CV*; age-bias plot; s.d.; the Wilcoxon signed ranks test; modal age difference	Not mentioned	Marked–recaptured fish length/age; age from the reader/modal age	Regression lines, whisker plots, notched box plot, age-bias plots; tables
Report of the Workshop on Age Reading of *Sebastes* spp. (ICES, 1996)	1995	Age readers comparison; scale/otolith comparison	Not mentioned	Age of differently prepared otoliths	Scatter plots; tables
Final Results of the Mackerel (*Scomber scombrus*, L.) Exchange Programme for Otoliths 1994 (Villamor and Meixide, 1995)	1995	PA; *CV*; APE; s.d.; age-bias plot; the Wilcoxon signed ranks test	Eltink *et al.* (2000) spreadsheet	Fish length vs. age	Linear, box plot, whisker plots, bar plots, histogram graphs; tables
		Average length by age; PA among readers; s.d.; the Wilcoxon signed ranks test	Eltink *et al.* (2000) spreadsheet	Age estimation	Notched box and whisker plot, age-bias plots of each age reader against the modal age, histograms
		PA; *CV*; APE; s.d.; age-bias plot; the Wilcoxon signed ranks test	Eltink *et al.* (2000) spreadsheet	Fish length/age	Linear, box plot whisker plots, bar plots, histogram graphs, age-bias plot; tables
Report of the Blue Whiting Otolith Reading Workshop (ICES, 1993)	1992	PA; s.d.; *CV*	Not mentioned	Fish length/weight; otolith length/height/weight/diameter, ring diameter	Linear, progression plot, box plot graphs; tables
		PA; s.d.; *CV*; ANOVA for differences in the mean ring diameter	Not mentioned	Age estimation; ring diameter; otolith diameter	Tables and graphs (scatterplots, regression line, box plots, linear plot)
		PA; s.d.; *CV* of mean age	Not mentioned	Fish length/weight; otolith length/height/weight/ring diameter	Linear, progression plot, box plot graphs; tables

Annex 4: Abbreviations and acronyms used in this publication

AEM	age error matrix
ALK	age-length key
APE	average percentage error
Bowers zone	A hyaline ring laid down in autumn, followed by a true hyaline winter zone
CFP	EU's Common Fisheries Policy
CS	calcified structure
CV	coefficient of variation
DCF	EU's Data Collection Framework
DGI	daily growth increments
DST	data storage tag
EARF	European Age Readers' Forum
EFAN	European Fish Ageing Network
GIMP software	GNU Image Manipulation Program – open-source software
IMR	Norwegian Marine Institute
INAB	Irish National Accreditation Board
ISO	International Organization for Standardization
LFA	length frequency analysis
LFD	length frequency distribution
MIA	marginal increment analysis
OTC	oxytetracycline
OWFD	otolith weight frequency distribution
PA	percentage of agreement
PGCCDBS	Planning Group on Commercial Catch, Discards and Biological Sampling
POF	post-ovulatory follicle
QA	quality assurance
QC	quality control
s.d.	standard deviation
TACADAR	Towards Accreditation and Certification of Age Determination of Aquatic Resources (EU-funded project)
TL	total length
TNPC software	Traitement Numérique des Pièces Calcifiées (Numerical Treatment of Calcified Structures)

UKAS	United Kingdom Accreditation service
WDS	wavelength dispersive spectrometer (or electron microprobe)
WebGR	Web service for support of Growth and Reproduction studies (open-source software)
WGBIOP	Working Group on Biological Parameters
WKAEH	Workshop on Age Estimation of European Hake
WKAMDEEP	Workshop on Age Estimation of Deep-water Species (2013)
WKARBLU	Workshop on Age Reading of Blue Whiting
WKARNSC	Workshop on Age Reading of North Sea Cod
WKARP	Workshop on Age Reading of Saithe (*Pollachius virens*)
XSA	Extended Survivors Analysis (stock assessment model)