WGBIOP Guidelines for Otolith Exchanges and Workshops

Last updates:

PGCCDBS:

17-21 February 2014

Horta, Azores, Portugal

WGBIOP

7-11 September 2015

Fuengirola, Spain

WGBIOP

10-14 October 2016

Monopoli, Italy

WGBIOP

2-6 October 2017

Cagliari, Italy

WGBIOP

1-5 October 2018

Ghent, Belgium

WGBIOP

7-10 October 2019

Lisboa, Portugal

EXCHANGES

# Introduction

The objective of exchanges of calcified structures is to estimate precision and relative/absolute bias in the age estimations from age readers of the different age reading laboratories, to check that these parameters are still within acceptable level. Also an exchange can be organized to compare ageing methods across laboratories or for other reasons.

The frequency of exchanges and workshops mainly depends on the quality of the age determination and is revised by national age reading coordinators and by expert groups accordingly. Exchange organisers should ensure they have read EFAN Report 3-2000 (Eltink *et al.*, 2000) particularly Section 3.9 “Comparison of sets of different preparation techniques” or of different calcified structures, Section 3.13 “Age reading comparisons” and Section 4.7.2.12 “Age reading of the last set for estimating improvement in age reading”.

WGBIOP has adopted and modified the PGCCDBS approach in implementing otolith exchanges.

Otolith exchanges/workshops are organized using the following instructions:

1. If an analytical assessment for a species is carried out and advice is given, or if otoliths are available and future assessments are being prepared, an otolith exchange programme has to be carried following the benchmark schedule for a given species.
2. If WGBIOP receives a request from other working groups or stock assessors for an exchange on a particular species.
3. If the results of the age reading performance of the otolith exchange is below the expected levels, ToRs must be drafted to solve identified problems and a workshop must be carried out.
4. Workshops consist of a series of discussions and on-spot exchanges designed to resolve the problems identified in the pre-workshop exchange. If the problems are not resolved, the coordinator of the workshop will propose future work (e.g. a smaller exchange on a specific issue).
5. The process could be fast-tracked if there is a case involving species of special conservation or assessment quality concern.

WGBIOP recommends the use of SmartDots and thus the use of images for all exchanges and workshops. Information can be found on [http://ices.dk/marine-data/tools/Pages/smartdots.aspx](http://ices.dk/data/tools/Pages/smartdots.aspx)

The usual procedure for initiating an exchange/workshop is for WGBIOP to draft the proposal based on specific recommendations:

- In the first instance, it is necessary that the identified issue with the age readings is clearly defined.

- It will also be important that an immediate communication occurs between the stock coordinator and the exchange coordinator to decide upon what action is needed. The stock assessor should be informed of the intention to carry out an exchange and should also receive the exchange report and recommendations.

- The chairs of the previous or suggested workshops/exchanges should also be involved in this communication, as there might be some planning already going on, which can be useful to be aware of.

- Planning the exchange in a short time:

1) Identifying exchange participants, those involved in the problem identified;

2) Selecting a representative set of otoliths (only otolith images), so that the results are not biased;

3) Give clear instructions to the participants since the time to perform the exchange will be shorter;

4) Use the SmartDots tool to distribute images for use in the exchange;

5) Analyzing the exchange results;

6) Reporting the results of the exchange to the respective stock assessment coordinator and should be uploaded to the SmartDots web application.

WGBIOP highly recommends the use of the SmartDots tool to streamline the preparation and the implementation of age calibration exchanges and workshops. WGBIOP recommends that future otoliths exchanges and workshops should use SmartDots for the annotation of all exchange images to prevent inconsistency and make collation of results easier.

# Setting up an exchange

Check background documents such as reports from previous exchanges and workshops and where necessary check recommendations from assessment working groups to know what is required for the exchange.

# Selecting otoliths

If carrying out an exchange, the number of otoliths to be included will depend on the aim of the exchange. Be sure to identify the necessary variables required for the exchange. These can be:

* Period (months or quarters)
* Stock
* Age or length structure (e.g. reflected in the assessment)
* Different preparation/reading methods

This is to ensure that the estimated precision and bias are representative for the age readings over the whole year as used for stock assessment purposes.

**Period**

It is important to include samples with translucent edges (e.g. Q1) and a sample of the same size where the otoliths have opaque edges (e.g. Q3/Q4). If two methods are used for age reading, e.g. sectioning and breaking otoliths, there could be also two collections in the exchange. Otoliths should be read by readers using their preferred method.

**Stock**

An exchange should correspond to the stocks used in the assessments. Make sure to include each area of the stock in the exchange. Information on the stocks can be found here [Stock Information Database](http://stockdatabase.ices.dk/Default.aspx)

**Age or length structure (e.g. reflected in the assessment)**

The age/length span in an exchange should, if possible, be from 0 to the maximum possible age/length or may be more interesting for the stock assessor to limit it to the age/length range used in the assessment.

**Preparation/reading methods**

An exchange could include not only images but also samples of calcified structures (CS) as a reading method if requested by the readers. Different preparation methods should also be taken into consideration.

Define strata based on variables that are of interest. An example of stratification by area and quarter can be found in table 1 below.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 7F | | |  | 7G | | | |
|  | Q1 | Q2 | Q3 | Q4 | Q1 | Q2 | Q3 | Q4 |
| age 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| age 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| age 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| age 4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| age 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| age 6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| age 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| age 8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| age 9 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| age 10 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

Table 1: determination of number of otoliths to be used in an exchange based on stratification by area and quarter in function of age structure.

Exclude otoliths you know are poorly prepared or have other obvious reasons why they are different from the rest of the otoliths in the exchange.

# Identifying exchange Participants

The exchange coordinator is required to contact the age reading coordinators of other labs to identify the age readers who will participate in the exchange. The contact details can be found on SmartDots and on the ICES data quality assurance repository, the “material, techniques and preparation methods” file can be found. The exchange can be open to all interested parties regardless of their level of experience. WGBIOP recommends that all age readers who provide ages for the assessment (these are classified as advanced readers), should participate in the exchange.

# Instructions to Participants

It is important to read the exchange otoliths in exactly the same way as they are read for stock or environmental assessment and not to make a special effort to get the best possible result. Participants **must be provided with** the information on area and date of capture for each otolith in the exchange along with the conventional date of birth. Participants should be **strongly encouraged** to familiarize themselves with the sample otoliths before final ageing. In addition to the age estimation, the age quality score (AQ-score) of each age estimation should be assigned. Coordinators should instruct the participants on the use of SmartDots when images are used to be sure to annotate correctly (e.g. annotate along the reference line, not putting a dot in the centre of the otolith, annotations must be approved to be included in the report, …). Where images of otoliths are to be included in the exchange, it is important to standardise the annotations. It is very important to indicate which type of light and which magnification was used when taking the images. Each reader should be requested to mark the outer edge of the translucent zone. These annotated images enable comparisons of how readers derive their age readings and form a valuable record of the exchange that can also be used as a training resource for less experienced readers. All images will be managed through SmartDots, and a user manual is available for all exchange participants.

# Using images of otoliths

Photo quality is crucial and therefore the proper preparation of otoliths is vital in order to obtain good photographs. Avoid over-exposed pictures. Where possible, all images should be processed in the same laboratory in order to standardize the images used. A calibrated scale bar should be visible on each image. The same magnification needs to be used for the whole set of images and for all the sets within the exchange. If the quality of the images is poor, they should not be uploaded. It is highly recommended that a fixed reading line is set on the images so that all readers annotate along the same reading line.

# Managing the exchange when calcified structures are used

One of the major problems in an exchange of calcified structures (CS) is the length of time taken for the successful completion of an exchange scheme. The coordinator of exchange should contact the participating laboratories to find when the readers are available for the most efficient circulation of the exchange otoliths. Once a schedule has been agreed it then becomes the responsibility of the individual age reader to inform the exchange coordinator of any changes necessary to revise the schedule due to other unforeseen work commitments, illness etc., in order to ensure the timely circulation of the exchange material.

The individual age reader is responsible for informing the coordinator when he/she has received the exchange set. Each reader is required to e-mail both the coordinator and the next participant on the exchange schedule before the exchange set is passed on to ensure that the next person on the list is still available to receive the otoliths. If this is not the case the coordinator can arrange for another participant to receive the exchange material. Before sending on the exchange material the age reader must ensure that all the age reading material is present and accounted for. If at this stage any problems with missing material are identified, the individual age reader must inform the coordinator. Participants should ensure the CS are securely wrapped in protective packaging to minimise the risk of damage during shipment to the next laboratory. Caution should be taken to pack the otoliths in a way that the otoliths are safely stored, but still easily handled.

At the end of the planned exchange, the CS can be returned to the reader(s) who were not able to read these at the planned time, before being shipped back to the coordinator. The coordinator should recommend sending the sets by a courier service in order to speed up the exchange and to reduce the possibility of losing one of the sets. The coordinator should return the otoliths to the appropriate age reading laboratories after the exchange.

# Analysing the Exchange Results

SmartDots tool includes an analysis and reporting function, which adheres to the recommendations from [WKSABCAL](http://ices.dk/sites/pub/Publication%20Reports/Expert%20Group%20Report/acom/2014/WKSABCAL/01%20WKSABCAL%20-%20Report%20of%20the%20Workshop%20on%20Statistical%20Analysis%20of%20Biological%20Calibration%20Studies.pdf)2014. SmartDots reporting can easily be used for the exchanges based on images. If calcified structures were also included in the exchange, the Eltink spreadsheet can be used to analyse these data.

In SmartDots two sets of analysis are carried out. Firstly the analysis including all readers and secondly confining the analysis to advanced readers whose age readings are used for stock or environmental assessments.

# Reporting the Results of the Exchange

The coordinator is responsible for the reports of the exchange. Two automated report templates (full and summary) are available on SmartDots, the contents of which was discussed and agreed by WGBIOP 2018.

The coordinator should try to get firm conclusions concerning what preparation techniques or calcified structures to use (aim for standardising methods).

The coordinator might discuss by e-mail the first draft of the reports and incorporate the comments received. Finally, the coordinator should upload the full reports to SmartDots where it can accessed by the participants, relevant stock coordinators, age reading coordinators and chair(s) of the relevant Working Groups. In case an agreed reference image set is one of the outcomes of an exchange, this reference set should be made available to the participants of the exchange. Inform the stock assessor that the summary report are uploaded and available on SmartDots.

# Exchange Checklist

|  |  |
| --- | --- |
| 1. Define the aim and the variables for the exchange. Follow WGBIOP Guidelines on this. | [ ] |
| 1. E-mail National Age Reader Coordinators (WGBIOP age readers contact list, all age reader coordinators regardless of their readers not being immediately relevant for the species or the area of stock in question) to establish participation from each country. | [ ] |
| 1. Establish list of participants. | [ ] |
| 1. Agree a circulation schedule for all participants in the instance calcified structures will still be used | [ ] |
| 1. Set-up the exchange in SmartDots | [ ] |
| 1. E-mail participants and age readers that exchange is open. Give clear instructions on deadlines and on how to annotate and approve their ages. | [ ] |
| 1. Check that you received all age readings at the deadline. | [ ] |
| 1. Complete analysis – follow SmartDots Guidelines on this. | [ ] |
| 1. Produce the full report and summary report | [ ] |
| 1. Upload final and summary report to SmartDots and send summary report to stock assessor | [ ] |
| 1. Provide an extended abstract to the WGBIOP | [ ] |

WORKSHOPS

# Introduction

An exchange has the basic purpose, as part of the quality assurance procedure, to identify sources of errors and inconsistencies among laboratories in stock-specific age estimation, quantify these errors and to address them during subsequent workshops. The main objective of an age reading workshop is to improve accuracy (to decrease the relative/absolute bias) and to improve the precision (reduce CV) of age determinations (their reproducibility) between age readers of the different laboratories. A workshop is thus preceded by an otolith exchange to indicate the errors in age reading. Age validation or verification is often required to improve the accuracy of age determination and such studies are very often recommended as a result age reading exchanges with poor results. In order to avoid a continued cycle of exchanges without improvement in accuracy, a workshop should include a term of reference addressing validation related issues, studies and methodologies (see Vitale *et al.,* 2019).

The results of these exercises, published in ICES reports, have the purpose of reaching both the personnel observing and classifying the biological structures and the scientists involved in the stock assessment. Stock assessment working groups are aware of the potential effect of age reading errors on their results and the use of Age Reading Error Matrices are the right output to be provided by age calibration workshops to stock assessment working groups.

# Problems indicated by the exchange

The exchange should reveal possible problems in age reading such as:

* the age reading methods differ too much (as indicated by statistical tests);
* the precision in age reading is too low for certain age readers;
* precision and/or accuracy differs considerably depending on different preparation methods;
* there is a strong bias in the age readings of young and/or old fish;
* other age reading problems.

At a workshop an attempt should be made to solve the problems indicated by the preceding exchange. The coordinator should also be familiar with the outcomes and recommendations from previous exchanges/workshops on these stocks. It is very important to ensure that the workshop also addresses any issues relating to age reading as highlighted by the relevant assessment working group and endeavour to get feedback on important outcomes that should be achieved from the upcoming workshop.

WGBIOP follows WKSABCAL recommendations on methods/analysis to be run by age calibration workshops (ICES, 2014), and these analysis have been included and automated in SmartDots.

# Points to consider when preparing for a workshop

The following points should be considered:

* Biology of the species;
* The results of previous exchanges and workshops if any;
* If the age reading technique was validated, when and how;
* The sample processing techniques used at the different age reading laboratories;
* Try to standardise the processing techniques of calcified structures;
* Discuss the results from the sets of images or calcified structures read during the exchange and at the workshop and try to agree on the age reading method;
* Determine at the end of the workshop the precision in age reading and the relative bias (if possible the absolute bias);
* Calculate improvement in age reading concerning precision and bias by comparing exchange set and the last set read at the workshop;
* Report the workshop results to the stock assessor;
* Make recommendations on how to improve the age reading quality;

Other topics may be addressed based on the conclusions from the exchange.

# Experimental Design in Age Reading Workshops

Workshops usually compare the performance of readers between the initial exchange set and a smaller exchange set at the end of the workshop. These comparisons need to be planned from the start of the exchange and carried out using the principles of designed experiments. The most important ideas for experimental design are to compare like with like and to control for other variables that affect age reading ability. For example, do not provide otoliths for the exchange from one stock and then read otoliths from a different stock at the end of the workshop.

It is important to avoid running the before and after comparisons on exactly the same set of otoliths. This is necessary if there are small numbers of otoliths and is undesirable as improvements seen in agreement may be from remembering specific cases and not apply in general. The procedure for generating two sets of otoliths for comparison of exchange and workshop results should be the following: Define the relevant variables (see chapter on exchanges) and assign otoliths by variable randomly to either the first or second set. The two sets do not have to be the same size. When the first set is for the exchange and the second set for the end of the workshop it is sensible to make the second set smaller.

All the necessary analysis functionality required for both exchanges and workshops is available in SmartDots or the Eltink spreadsheet.

# Workshop Participants

Whenever possible age readers who participated in the preceding exchange should also participate in the subsequent workshops, to achieve the best results. It is critical that those age readers, whose ages are used in the stock assessment process, participate in both the exchange and the workshop.

# Generic ToR’s and outcomes for ageing workshops

1. Collect information on participating laboratory procedures

Check the tables produced by WGBIOP with information of each lab on sampling, methods, procedures and QC. and procedures. If additional information is required contact age reader coordinators.

1. Classify age reading performance

The ageing performance: WGBIOP recommends that workshop coordinators use the following criteria for classifying age reading performance into 'good', 'medium' or 'bad'.

• **Bad ageing performance:** Where the reliability of the age data is unsatisfactory and well below the agreed targets. Indicators may include poor agreement between age readers, low precision or high bias. Causes may include difficulty in observing/interpreting growth patterns (e.g. disagreement over the location of the first annulus or otolith edge interpretation), missing protocol for preparation/age reading and the use of inappropriate otoliths or preparation methods.

• **Medium ageing performance:** The age data are sufficiently reliable to be used for stock assessment purposes but improvement is required. Indicators may include levels of agreement between age readers that are below a reference target value for the stock/, difficulty in interpreting aspects of growth patterns, protocols for age reading are used but may need revision and the use of less reliable preparation/observation methods.

**• Good ageing performance:** The age data is considered reliable. Indicators may include repeated high levels of agreement between age readers at successive exchanges or workshops. Causes for good performance may include growth patterns that are easier to interpret, good protocols for preparation/age reading and the implementation of QA and/or QC procedures at individual institutes.

1. Resolve interpretation differences between readers and laboratories

Disagreements on the interpretation of annual increments can exist between experienced readers. Usually these differences are resolved when the readers discuss the otoliths jointly (note: annotated images largely simplify this process). However, this is not always the case and then follow-up actions must be formulated.

1. Validation studies on age estimations

Repeated low levels of percentage agreement and high levels of bias seen in exchange results can indicate the need for age validation or verification studies. A review of related studies on the species and applicable methodologies is recommended with follow up actions formulated.

1. Create or update an ageing manual

The agreement on, and production of clear age reading criteria must be a key outcome of any age reading workshop. Therefore, an ageing manual for each species should be produced describing the standardized method that is internationally agreed upon by all experienced age readers. This manual focuses on the interpretation of the structures (e.g. date of birth, interpretation of rings and edges, period of opaque and translucent ring formation).

1. Collate agreed age reference collection.

In case an agreed reference image set is one of the outcomes of a workshop, this reference set should be made available to the WGBIOP community. Collate a set of “agreed age images” during the workshop. These images will have been discussed and the ages agreed during the exchange and/or workshop, and the annuli are to be annotated. This set of images will be an invaluable resource for training or refreshing.

1. Formulate follow-up actions

See the guidelines in the following section

1. Formulate species (and stock specific) target and threshold statistics

As tool for the evaluation of the quality of age readings we recommend that target and threshold statistics are formulated for each species and stock. The statistics refer to the percentage agreement, the CV and the bias. The target value is the value you would like to achieve and know is possible based on exchange and workshop results. The threshold value is the minimum value required before a reader is qualified to supply data to working groups and can if necessary be derived by discussion between expert readers. Implications of the workshop outcome for the assessment of the stock(s) must be discussed and preferably quantified (e.g. age error matrix).

# Reporting the Results of the Workshop

The coordinator is responsible for the report of the workshop. An automated full report template and summary report template for standard analysis is available on SmartDots which can serve as a basis for the reports, the contents of which was discussed and agreed by WGBIOP 2018.

The coordinator might discuss by e-mail the first draft of the report and incorporate the comments received. Finally, the coordinator should upload the reports to SmartDots where it can accessed by the participants, relevant stock coordinators, age reading coordinators and chair(s) of the relevant Working Groups and inform the participants that it can be accessed there.

This summary report should provide sufficient information to enable the assessment working group to judge whether or not the quality of the ageing data (by country) is sufficient to be included the input data set of a quantitative stock assessment.

The extended summary will contain only results of advanced readers:

* Description of sets of calcified structures included in the exchange and/or workshop
* The number of calcified structures in each set
* Composition (age and/or length structure, area)
* Preparation methods
* Images available
* Description of participants (numbers per country etc.)
* Number of readers, laboratories and countries
* Which laboratories provide ageing data to the WG’s
* Which laboratories provide ageing data to the WG’s but were not represented in calibration
* Accuracy and precision estimates, age error matrix
* Summarise currently existing ageing problems, either detected in exchange or not solved in workshop.
* Evaluation of quality of age data provided to WG

# Specific follow-up actions

If ageing problems are not solved within the ageing workshop, then the participants must formulate clear follow-up actions, which will lead to solving the ageing problems. The workshop should recommend who is responsible for coordinating and carrying out the follow-up actions and in what time frame. To aid the workshop coordinator some possible follow-up actions are listed here:

* If validation studies are required the report should include a review of previous studies, recommended methodologies and specific follow up actions and responsible persons.
* In some species in which the contrast between the structures is poorly visible it may be advisable to revise preparation methods.
* When different methods in age structure are used, the following steps should be followed: 1) Compare the different methods, 2) Estimate which is the most accurate, 3) Analyse the benefits of each method, 4) To assess the relative size of sampling and ageing errors when estimating the age structure of populations.
* If one or a few readers are disagreeing with the majority of experienced readers, then small scale regional exchanges and/or meetings can be initiated.
* If age reading protocols are not available for all participants this should be remedied.
* When new age reading criteria are established and agreed at a workshop, these should be implemented by all readers as soon as possible. A small set of images or age reading material can be used with the dual purpose of ensuring that the agreed ageing criteria are adopted by all and providing a format for testing the new criteria.

# Workshop Checklist

|  |  |
| --- | --- |
| 1. Follow WGBIOP Guidelines regarding the design and generic TOR’s for the WK. | **[ ]** |
| 1. E-mail National Age Reader Coordinators (WGBIOP age readers contact list, all age reader coordinators regardless of their readers not being immediately relevant for the species or the area of stock in question) to establish participation from each country. | [ ] |
| 1. Agree a date, and the venue for the WK and any other house-keeping issue around the organisation of the WK. | [ ] |
| 1. Set-up the exchange in SmartDots and Conduct Workshop | [ ] |
| 1. Complete analysis – using SmartDots, or Eltink spreadsheet following WGBIOP Guidelines. | [ ] |
| 1. Produce summary and full reports | [ ] |
| 1. Circulate the draft report of the Workshop to all participants. | [ ] |
| 1. Upload the final reports to SmartDots | [ ] |
| 1. Provide an extended abstract to the WGBIOP. | [ ] |

# Background information on analysis

The ‘Tool for Age Reading Comparisons’, developed by Eltink *et al.* in 2000, has proved an invaluable contribution to Quality Control for fish age calibration. Eltink *et al.*(2000) advised that the precision errors in age readings are best described by the coefficient of variation CV by age group (CV = st. dev/mean age recorded). Although CV is often the preferred statistical tool for this task, the index of average percentage error (APE) is also commonly used (Kimura, D. K., and Anderl, D.M. 2005; Morison *et al.* 2005). The dangers of the percent agreement statistic have long been recognised (Beamish and Fournier 1981; Chang 1982; Campana 2001). More recently Morison *et al.* (2005) reported that responses to a questionnaire to assess current QA and QC practices that was completed by representatives of over 50 fish ageing laboratories worldwide, indicated that percentage agreement was still the most commonly used measure of precision (40% of respondents) despite its limitations and criticisms. Nevertheless, in order to ensure comparability between studies on different species, the CV and/or APE has to be reported as obligatory precision estimate.

# References

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