

WGBIOP Guidelines for Otolith Exchanges

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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 this is still within acceptable levels. The frequency of exchanges and workshops mainly depends on the quality of the age determination and will be revised by national age reading coordinators and by expert groups. Exchange programmes obtain more objective estimations of the precision and bias in age reading, since the readers use their own equipment and are not subject to a tight time schedule (criteria that may not be applicable in a workshop). Exchange organizers 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”.

In 2010, PGCCDBS agreed the following 'five-step approach' to be implemented:

- 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, a 'small' scale otolith exchange programme has to be carried out every three years.
- 2) If the age reading performance in the small otolith exchange programme is medium or bad, ToRs must be drafted to solve identified problems and a 'full' scale exchange must be carried out.
- 3) If the age reading remains medium or bad, after this full-scale exchange then, an age calibration workshop must be planned,
- 4) Workshops consist of a series of discussions and exchanges designed to resolve the problems identified in a pre-workshop exchange. If the problems are not resolved or new problems are identified, another full-size exchange must be carried out before a further workshop can take place.
- 5) If the age reading performance in the otolith exchange small programme is good, a further otolith exchange small programme should be carried out in three years time.

Additionally, WGBIOP emphasizes that exceptions to the 'five-step-approach' can be allowed in certain cases, e.g. when species of special conservation concern are involved, it can make more sense to immediately have a second workshop gathering the relevant experts, instead of going through an exchange first. This process is illustrated in a schematic Figure 1.

The frequency of exchanges and workshops mainly depends on the quality of the age determination and will be revised by national age reading coordinators and by expert groups. Even if no age reading issues were revealed in workshops or exchanges, quality assurance requires the organization of an exchange at least once every 3-5 years. The possibility for a workshop should be offered every 5 years.

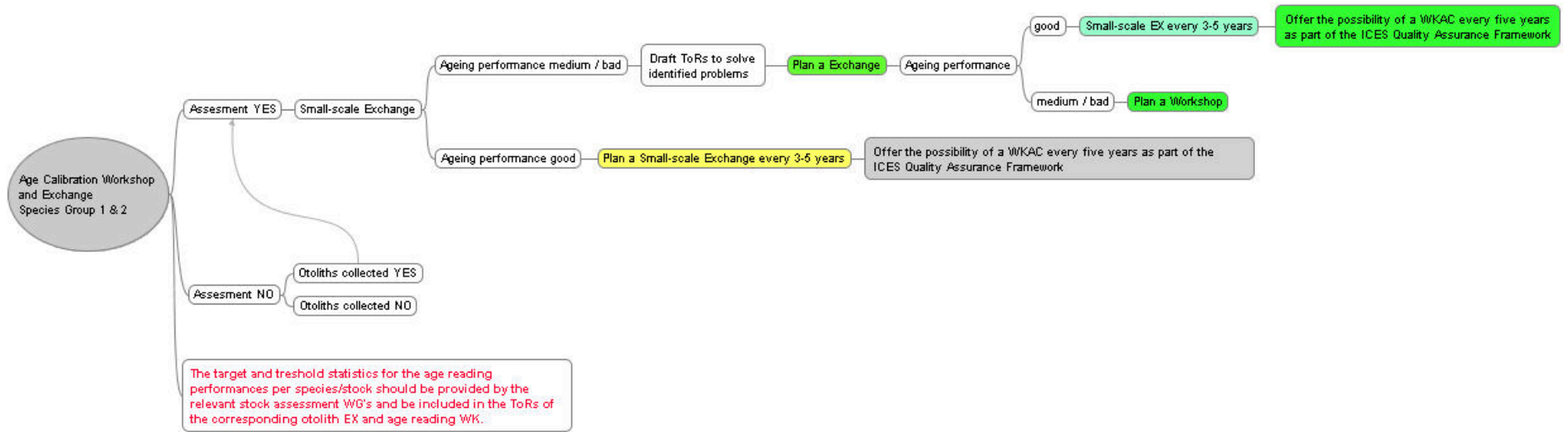


Figure 1. The five-step process for planning age calibration exchanges and workshops.

The usual procedure for starting an exchange/workshop is for WGBIOP to draft the proposal following a recommendation by specific expert groups, this request is subsequently evaluated and decided upon by WGDATA and ACOM/SCICOM. Exchanges and workshops are therefore usually planned at least the year before they are supposed to take place. However, if a stock assessment WG discovers an issue, which needs a sudden action to be taken, it can be necessary to diverge from the normal procedure. In order to be able to promptly react to sudden problems, it will be necessary to have a direct communication between the stock coordinator and WGBIOP, for deciding upon an action. It is important that chairs of previous and suggested workshops/exchanges are included in this communication, as there might be some planning already going on, which can be useful to be aware of.

WGBIOP highly recommends the use of the Age Readers Forum (<http://groupnet.ices.dk/AgeForum/default.aspx>) in tandem with the WebGR tool (<http://webgr.wiki.azti.es/doku.php>) to streamline the preparation and the implementation of age calibration exchanges and workshops. WGBIOP recommends that all future age reading exchanges and workshops should be run through the forum using the WGBIOP guidelines. Furthermore, WGBIOP recommends that future otoliths exchanges and workshops should use WebGR for the annotation of all exchange images to prevent inconsistency and make collation of results easier.

Age calibration exchanges and workshops should be announced and marked on the calendar of the Age Reader Forum. Their reports should also be posted on the forum.

Small-scale exchanges

Images are not required for small-scale exchanges, but could be considered as an option to ease the exchange speed. The suggested sample size for small-scale exchanges is 3-5 recently collected otoliths for each length class, from the period when the otoliths have translucent edges (e.g. Q1) and a sample of the same size from the period when the otoliths have opaque edges (e.g. Q3/Q4). If two methods are used for age reading, e.g. sectioning and breaking otoliths, there should be two collections in the exchange. Otoliths should be read by the preferred method.

The chair of the relevant Assessment Working Group should be informed of the intension to carry out an exchange and should also be circulated the exchange report and recommendations.

Full-scale exchanges

If a full-scale exchange is carried out, it should include both images and samples of calcified structures.

Because comparisons between different methods or comparisons in reading ability between the start and end of a workshop might be required, these possible comparisons need to be planned from the start of the full-scale exchange and carried out using the principles of designed experiments (see for example, Heath (1995)). 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 full-scale exchange from one area to be followed by the age estimation of otoliths from a different area at the end of the workshop. This comparison could show increased agreement in ageing due to increased ability gained at the workshop or due to the second area being easier to read and it will be impossible to separate the two effects. Similarly, avoid running the before and after comparisons on exactly the same set of otoliths. This is necessary if there are small

numbers of otoliths but otherwise is undesirable as improvements seen in agreement may be from remembering specific cases and not apply in general.

Building on the guidance in the EFAN report, the WGBIOP recommended that the procedure for setting up two sets of otoliths for comparison should be by randomly assigning otoliths (described in the paragraph Selecting Calcified Structures (see below)) of each strata defined group to either the first or second set. The two sets do not have to be of the same size. When the first set will be used for the exchange and the second set for recalibration at the end of the workshop, it is sensible to make the second set smaller. If the age workshop coordinator can specify changes in estimation bias or CV that are biologically meaningful, then sample size calculations can be carried out to help decide how big the datasets should be.

Identifying Exchange Participants

The exchange coordinator is required to contact other age reading laboratories to identify the age readers who will participate in the exchange. This is generally done through the Age Reading Coordinators, whose contact details can be found on the age readers contact list updated annually by WGBIOP.

This List of all National age readers coordinators and age readers usually per species (“Age Reader Contact List.xlsx”) is available on the ICES Age Reader Forum ([ARF](#)) and on the [Data Quality Assurance Repository](#). The access must be granted by the ICES Secretariat.

Further useful information can be found in the file “Review material, techniques and preparation methods by species and areas to fish ageing.xlsx”, including the list of all the countries/institutes working on each stock, their preparation methods etc.. This table is also updated by annually WGBIOP and available following the same above mentioned links.

It is recommended to contact all the age reader coordinators (regardless of their readers not being immediately relevant to the species or the area of stock in question as there may be an interest from ‘non relevant’ age-readers to participate to get aligned with the age estimations of the species in common) in the first instance to ensure that all interested parties are offered an opportunity to participate. The exchange can be open to all interested parties regardless of their level of experience. The exchange should also be announced on the Age Readers Forum. The minimum demand for a successful exchange is that all age-readers delivering age data to the assessment participate in the exchange. The chair of the relevant Assessment Working Group should be informed of the intention to carry out a full-scale exchange and should also receive the exchange report and recommendations. Additionally the AWG chair should facilitate the participation of the laboratories delivering age-data to the assessment.

Generally, it is recommended that two sets of analysis are carried out. First confining the analysis to those readers whose age readings are used for stock or environmental assessments and second reporting the analysis including all readers which can be presented in an annex of the exchange report. Participants can be asked to provide a brief statement describing the species that they read (including details on the stock(s)) and the number of years they have been reading these stock(s). This information is also needed to identify the most experienced readers. Participants should also provide a summary of the quality management procedures used at their institute.

Selecting Calcified Structures

Where there is a requirement for an exchange of the same species from areas or different stocks with widely differing growth rates, separate sampling sets must be set up for each area and care must be taken that the sample sets are analysed separately in case appropriate. The exchange set must represent the spatial distribution of the catches of the species with the weight put on the most frequently represented areas in the landings.

The age span in an exchange set of calcified structures (CS) should, if possible, be from age 0 to the maximum age possible (try to exceed the age range as used for stock or environmental assessment purposes).

As a rule of thumb, a minimum of two sets of otoliths from fish caught in the same year are needed for a reliable estimation of CV at age, each with 10 specimens within each age-group, to ensure that the number with translucent edges and the number with opaque edges are representative of the annual distribution (e.g. from January to March and July to September for many Northeast Atlantic continental shelf spp). 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.

Identify variables that you suspect influence the ability to age. The **number of possible age reading problems** that you want to check, **determines the number of sets in the exchange**. Identify variables that you suspect influence the quality of the age readings. Compare years and quarters to look for identifiable features that may reveal faults, e.g. abundant years classes becoming less abundant and vice versa. For variables that are not of interest control their effect by standardizing them. For variables that are of interest or cannot be fixed, define strata based on these variables. The co-ordinator might also decide to assemble a set of calcified structures, which consists of a number of subsets. Control the effect of variables that are not of interest by standardizing them. For example: keep laboratory procedures consistent. Define strata based on variables that are of interest or cannot be fixed. For example: month and fish length group. (We suggest strata based on fish length group to help balance the age distributions in the first and second set.)

The CS for the exchange should be completely representative of the CS used for stock or environmental assessment. Bearing this in mind, the coordinator should try to limit the total number of calcified structures; otherwise, the burden for the age readers will be too much. The co-ordinator should inquire whether calcified structures of known age are available to be included as an extra set in the exchange. He should do his very best to include such a separate set of calcified structures of known age.

- 6) 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.

In the case of a micro-increment daily growth exchange, it is recommended to choose images that are better processed, as the quality of the images greatly influences the interpretation of the micro increments. The set of images have to be accompanied by images presenting the objective micrometre calibration. Increment counts and measures should be reported.

Instructions to Participants

It is important to read the exchange programme 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 area and date of capture for each CS in the exchange. Participants should be **strongly encouraged** to make a **first 'blind' age reading**, for each CS and then make a second reading using the available biological information. Making an initial 'blind' reading can lower unintentional bias in assigning age and may eventually improve reader self-confidence.

Using Images of CS

Where images of CS are to be included in the exchange, it is important to ask each reader to annotate the position of each annual translucent zone on every otolith. 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. The positions of the annual translucent zones are marked on raster layers. The images of the CS should all be prepared at one laboratory. This may either be the co-ordinator's laboratory or another participating laboratory who has agreed to do this work for the co-ordinator.

The coordinator will choose an appropriate value for 'brush size', so that this is not more than 75% of the width of the smallest annual translucent zone and instruct participants to set the brush tool 'hardness' at 100 (no opacity). The coordinator will assign a colour to each age reader at the outset to avoid any duplication. To facilitate the collation of the annotated image data by the coordinator, each participant selects a new raster layer when opening each image and names it with their name or reader identity, before marking the annuli on this layer with their assigned colour and saving it as a '.jpg' image. [See: Report of Irish Sea Celtic Sea Cod Otolith International Exchange scheme 2006 Appendix 1: Instructions for using Paint Shop Pro for more information].

Technical specifications for images

Photo quality is very important and proper preparation of otoliths is necessary for obtaining good photographs. Avoid over-exposed pictures. The same magnification needs to be used for the whole set of images and for all the sets within 1 exchange. Remember to calibrate image, information of resolution in the file name is recommended. Pictures should be saved in Jpeg- or Tiff-format. Use only one microscope for each stock, there might be microscope-specific calibration variance. Recalibrate the setup regularly. The minimum camera specifications are good light sensitivity and a minimum of 6 MP. High speed connection between camera and computer is recommended. Processing pictures can be done with specialized software as WebGR, TNPC, or more general software as ImagePro, ImageJ, or others. A high resolution screen is important. (Based on the Report of the Workshop on Age Reading of North Sea Cod (WKARNSC), paragraph 3.7.)

Use of WebGR

When possible, use WebGR to distribute pictures for use in exchanges and workshops.

WebGR is a European project that aims to develop Open Source software for supporting studies of fish growth and reproduction. In particular, it promotes the usage of online services to organize calibration workshops. The application facilitates the whole workshop and exercise cycle. Multiple images can be uploaded and assigned to an individual fish. The workshop manager uses attribute-based filters to

create a specific image set for an exercise. Each participant annotates the contained image sequence under condition of an appointed key. A group accepted annotation gets a reference state. These reference images will also be used for training purpose.

The Key functions of WebGR are • Set up of workshops and calibration exercises • Make and share annotations (coordinates, text-fields, graphical settings) • Compare annotations • Set reference annotations • Upload images • Manage fish samples • Export lists and tables to process in spread sheet- and statistical software • Training exercises without administrative overhead • Let users choose their expertise coverage • Define different key tables (research standards) • Comprehensive search and filter abilities

Technical details of the WebGR application: • Intranet application, only authorized access • Web browser based • Self registration with e-mail confirmation • Free definable form fields with multiple values and ranges for image search • Free definable value lists for fields • Data validation and filtering • Access control for different roles and actions.

Managing the Exchange

One of the major problems in an exchange of calcified structures is the length of time taken for the successful completion of an exchange scheme. The co-ordinator 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. "Only images"-exchanges possibly in combination with the use of WebGR, will relieve the co-ordinator of these particular problems there the images can/will be available for all participants at the same time.

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 minimize 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 packed, 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 co-ordinator. The co-ordinator should recommend sending the sets **by special courier** in order to speed up the exchange and to reduce the possibility of losing one of the sets.

Analysing the Exchange Results

There are several ways of comparing age readings. However, the best way is by making age bias plots, which are easy to understand for the age readers (ICES, 1994 and Campana *et al.*, 1995). The "Age Comparison Tool" (Eltink *et al.*, 2000) offers an easy tool to analyse the data. The output of this tool is now widely used within

fisheries laboratories in Europe. However, other tools also exist and their use should be examined because the “Age Comparison Tool” by Eltink is not applicable to all species.

Basic statistics are in the output of the WebGR tool.

Reporting the Results of the Exchange

The co-ordinator is responsible for the report of the exchange. Preferably, the report of the age reading exchange contains the following sections:

- Abstract
- Introduction
- Material and methods
- Results
- Discussions
- Conclusions
- Implications for the assessment of the stock/species
- Recommendations.

Valid statistical tests and measures should be used to quantify the conclusions of the exchange. The co-ordinator should try to get firm conclusions concerning what preparation techniques or calcified structures to use (aim for standardizing methods).

The co-ordinator should return the otoliths to the appropriate age reading laboratories.

He/she should discuss by e-mail the first draft of the report and incorporate the comments. Finally he/she should distribute the report to all participants and post the report on the Age Reader Forum so it is available for the whole ICES - age estimation community. 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. Existence of reference sets and their whereabouts should also be specified on the forum.

The coordinator of the exchange should also send a copy of the exchange report and an extended abstract to the chair(s) of the WGBIOP.

Exchange Checklist

1.	Inform the PG Liaison person/stock coordinator/ or chair of the relevant AWG of pending exchange and look for feedback.	[]
2.	Send an e-mail National Age Reader Coordinators (WGBIOP age readers contact list, all age reader coordinators regardless of their readers not being immediately relevant to the species or the area of stock in question) to establish participation from each country.	[]
3.	Establish list of participants and direct them to the European Age Readers Forum (ARF)	[]
4.	Using the ARF, agree a circulation schedule for all participants.	[]
5.	Establish exchange set – follow WGBIOP Guidelines on this.	[]
6.	All age readings received.	[]
7.	Complete analysis – follow WGBIOP Guidelines on this.	[]
8.	Present analysis for age readers contributing to Stock Assessment.	[]
9.	Present analysis for all age readers in the annex of the report.	[]
10.	Circulate exchange results to all participants with draft conclusions.	[]
11.	Forward the report from the exchange to the AWG/stock coordinator and WGBIOP.	[]
12.	Provide an extended abstract to the WGBIOP.	[]
13.	Upload images, or a link to where a set of agreed ages, resulting from the exchange, can be found to the ARF.	[]

WGBIOP Guidelines for Workshops on Age Reading Calibration

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Introduction

The main objective of an age reading workshop is to decrease the relative/absolute bias and to improve the precision (reduce CV) of age determinations (their reproducibility) between age readers of the different age reading laboratories. An exchange of calcified structures must be carried out first to indicate the errors in age reading before a recommendation for an age reading workshop can be made (see previous section).

Problems Indicated by the Exchange.

At a workshop, an attempt should be made to solve the problems indicated by the exchange. The following possible problems in reading might exist:

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

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. The workshop coordinator should endeavour to get feedback from the assessment working group chair on what he/she feels are important outcomes that should be achieved from the upcoming workshop. It is recommended that the chair of the relevant assessment working group should be encouraged to contribute to the workshop as an end-user of the data, either in person where possible or via WebEx etc..

WGBIOP follows WKSABCAL recommendation that the following methods/analysis must be run by age calibration workshops (ICES, 2014):

- To access bias
 - ABP - Age-bias plot
 - TS - Tests of symmetry
- To access precision
 - APE - Average Percentage Error
 - 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
 - AREM - Age Readings Error Matrix

Topics to Consider When Preparing for a Workshop

The following topics can be and all should be considered:

- The biology of the species;
- The results of previous exchanges and workshops;
- When and how the age reading technique was validated;

- The sample processing techniques used at the different age reading laboratories;
- If necessary, try to standardize the processing techniques of calcified structures;
- Agreement on age determination criteria;
- Discuss disagreements in age reading results from the sets of the 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);
- Report (and preferable quantify) the impact of the workshop results on the stock-assessment based on collated age data (e.g. error around SSB estimates);
- Estimate improvement in age reading concerning precision and bias by comparing exchange set and the last set at the workshop;
- Make recommendations on how to improve the age reading quality;
- Indicate which calcified structures can be used for the "agreed collection" and (if possible) produce digitized images.

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

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

- **Bad ageing performance:** When the quality of the data are unknown or there are serious concerns about the reliability of the age data and/or its value to stock assessment WGs. Indicators may include poor agreement between age readers and age data that do not appear to agree with other methods of growth estimation for the stock/species. Causes may include difficulty in observing/interpreting calcified structure (CS) growth patterns, no protocol for preparation/age reading and the use of inappropriate CS 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/species (e.g. VIIa cod - 90%, redfish - 40%), difficulty in interpreting aspects of CS growth patterns (e.g. disagreement over the location of the first annulus or otolith edge interpretation), 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 are considered reliable. Indicators may include repeated high levels of agreement between age readers at successive exchanges or workshops. Causes may include calcified structure CS 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.

Stock coordinators should be aware of levels of percentages of agreement and CV's among stock assessment readers. Age coordinators should recommend achievable percentage agreement and CVs based on the most recent exchange and workshops.

WGBIOP recommends that all future age reading exchanges and workshops should be run through the forum using the WGBIOP guidelines. Furthermore, WGBIOP recommends that future otolith exchanges and workshops should use WebGR for the annotation of all exchange images to prevent inconsistency and make collation of results easier.

Workshop Participants

Everyone who participated in the exchange should also participate in the workshop, and vice versa; no one should participate in the workshop unless they also took part in the exchange.

Experimental Design in Age Reading Workshops

Workshops usually compare the performance of readers between the start and 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 area then read otoliths from a different area 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 but otherwise 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: Define the relevant strata and assign otoliths by strata 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. If the age workshop coordinator can specify changes in reading bias or CV that are biologically meaningful to detect then sample size calculations can be carried out to help decide how big the datasets should be.

The 'Tool for Age Reading Comparisons' was 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 ($CV = \text{st. dev}/\text{mean age recorded}$). 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 recognized (Beamish and Fournier 1981; Chang 1982; Campana 2001), yet despite this Campana *et al.* (1995) reported that roughly 35% of 21 randomly sampled age comparison papers published between 1985 and 1995, used only percent agreement. 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.

Improvements to the original spreadsheet tool have been developed at Cefas, UK. Eltink compared a number of results in the "work table of the bias test" of the original

spreadsheet and the new spreadsheet, which calculates the results of the bias test in the overview table and so far has not found any discrepancies (Eltink pers. com.). Eltink advises that the new spreadsheet is much faster than the original one. The downside is that the new spreadsheet is limited in the number of otoliths as well as in the number of age-readers. The original spreadsheet did not have these restrictions. Eltink concludes that the new spreadsheet cannot replace the original one at this stage, but can be used within these restrictions.

In the case of the micro-increment daily growth comparison, as reference age, it is recommended to apply the mean age rather than the modal age, due to the large number of ages obtained in the daily ring age determination. Although the mean age estimate is not an indicator for the validity of ageing structures, it may provide useful information regarding over- or underestimation of age irrespective of fish size class. Also, it should be compared the increment width reported by reader, in order to know the age structure definition for each reader.

Generic ToRs for ageing workshops

- a) Provide information on participating laboratory procedures
 - Sampling and storing of calcified structures.
 - Equipment and preparation of calcified structures
 - Documentation on processes and protocols (QA)
 - How age determination are being checked within laboratories (QC):
 - availability of reference collections
 - results of age reading comparisons between readers
 - percentage of samples re-read
 - Estimate (relative) accuracy and precision
- b) 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.

- c) Create or update an ageing manual

There should be a standardized ageing manual for each species in a unified format 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). The manuals on preparation of calcified structures are usually created and updated on the national level.

- d) Collate agreed age reference collection.

The output of every workshop should be an agreed age reference collection. Preferably, the agreed interpretation should be annotated (as a separate raster layer – see previous section) in the images. These sets of images could then be made available online to train new age readers or to have as a reference set for experienced readers. If establishing a digital collection on a website is not possible, then information about location of the reference collection and contact person should be available on the website.

e) Formulate follow-up actions

See the guidelines in the following section

f) 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. Usually, a CV of 5% is set as a threshold for sufficient data quality (Campana 2001).

Implications of the workshop outcome for the assessment of the stock(s) must be discussed and preferably quantified (e.g. error distribution around the SSB estimation).

Guidelines for follow-up actions

Dissemination of the results

Dissemination of the results is in principle the responsibility of the coordinator of the exchange and/or workshop. The full report of the workshop should be made available on the Internet, and placed (in pdf-format) in the ICES Data Quality Assurance Repository (<http://ices.dk/community/Pages/PGCCDBS-doc-repository.aspx>). An extended summary of all workshops and exchanges should be submitted to WGBIOP and to the relevant working group/WGBIOP liaison person, and the stock coordinator. This extended summary should provide sufficient information to enable the working group to judge whether or not the quality of the ageing data (by country) is sufficient to include the data in a quantitative stock assessment.

The extended summary should contain the following information:

- 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
- Expertise level of each reader (trainee, intermediate, experienced)
- Which readers provide ageing data to the WG's
- Which laboratories provide ageing data to the WG's but are not represented in calibration
- Accuracy and precision estimates
 - i) Percentage agreement, CV and bias by age-group
 - ii) Only readers providing data to WG's
 - iii) Readers combined

- iv) By reader (anonymous, but lab/country stated)
- v) If relevant, by stratum (spatial and/or temporal differentiation)
- Summarize currently existing ageing problems, either detected in exchange or not solved in workshop.
- Evaluation of quality of age data provided to WG
 - i) Preferably a quantitative evaluation (i.e. in relation to target and threshold statistics)
 - ii) If not possible then a qualitative evaluation
- A list of the expert groups to be informed.

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. If there are no distinct ageing problems, but the workshop thinks the general ageing quality can be improved by follow-up actions than these should be formulated clearly. The workshop should point out who is responsible for coordinating and carrying out the follow-up actions and in what time frame. The required follow-up can differ depending on the species and the problem occurring. To aid the workshop coordinator some possible follow-up actions are listed here:

- Validation exercises must always be encouraged. A continuous comparison of age readings does not always solve the problem (an example to be learned from: the bias in hake ageing).
- In some species in which the contrast between the structures is poorly visible it may be advisable to improve preparation methods.
- If one or a few readers are disagreeing with the majority of experienced readers, then small-scale regional exchanges and/or meetings can be organized.
- If interpretation problems of the first annuli are occurring, then back-calculated growth can provide an indication on the correct interpretation. If samples of '0'-group fish are available throughout the 1st year of life, the period of annual translucent zone may be determined by marginal incremental analysis.
- 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, all readers should be asked to implement the agreed criteria directly after the workshop, using a small set of images or age reading material. This serves the dual purpose of ensuring that the agreed ageing criteria are adopted by all and also provides a format for testing the new criteria.

Workshop Checklist

1.	Inform the PG Liaison person/stock coordinator/ or chair of the relevant AWG of pending exchange and look for feedback.	[]
2.	Inform the PG Liaison person/stock coordinator/ or chair of the relevant AWG of the pending workshop and look for feedback.	[]
3.	Establish list of participants from the exchange and direct them to the European Age Readers Forum (ARF).	[]
4.	Using the ARF, the workshop coordinator should agree a date, and location for the WK and any other house – keeping issue around the organization of the WK.	[]
5.	Follow WGBIOP Guidelines regarding the design and generic TOR's for the WK.	[]
6.	Conduct Workshop.	[]
7.	Complete analysis – follow WGBIOP Guidelines on this.	[]
8.	Present analysis for age readers contributing to Stock Assessment.	[]
9.	Present analysis for all age readers in the annex of the report.	[]
10.	Circulate the draft report of the Workshop to all participants.	[]
11.	Forward the final report from the workshop to ICES, the AWG/stock coordinator and the Chair of WGBIOP.	[]
12.	Provide an extended abstract to the WGBIOP.	[]
13.	Upload images, or a link to where a set of agreed ages, resulting from the Workshop, can be found to the ARF.	[]