PGCCDBS Guidelines for Otolith Exchanges (update)

7 – 11 February 2011

Vienna, Austria.

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 which may not be applicable in a workshop). 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".

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 small otolith exchange programme is good, a further small otolith exchange programme should be carried out in three years time.

Additionally, PGCCDBS emphasises 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 organisation of an exchange at least once every 3-5 years. The possibility for a workshop should be offered every 5 years.
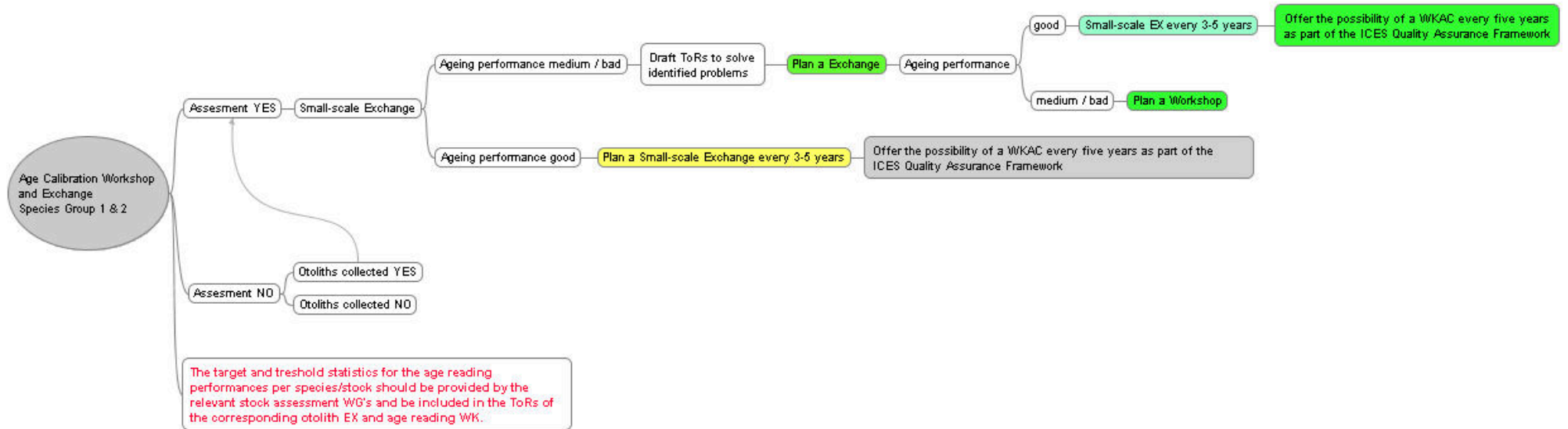
Figure 1. The five-step process for planning age calibration exchanges and workshops.

PGCCDBS highly recommends the use of the **European 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. 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. The use of WebGR needs to be evaluated on the next PGCCDBS after which these guidelines might be updated.

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 2nd 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 PGCCDBS 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 data sets should be.

Identifying Exchange Participants

The 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 by PGCCDBS annually. It is recommended to contact all the age reader coordinators in the first instance to ensure that all interested parties are afforded an opportunity to participate. The exchange can be open to all

interested parties to participate regardless of their level of experience. The exchange should be announced on the European Age Readers Forum also.

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

Generally , it is recommended that two sets of analysis are carried out. Firstly confining the analysis to those readers whose age readings are used for stock or environmental assessments. And secondly reporting the analysis including all readers. The analysis including all readers can be presented in an annex of the exchange report. At the same time he/she needs to inquire how much experience the readers have in age reading this and other stocks. 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 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 standardising 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 sub-sets. Control the effect of variables that are not of interest by standardising 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.

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.

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 specification 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 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 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
- 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 standardising 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 PGCCDBS.

Exchange Checklist

1.	Inform the PG Liaison person/stock coordinator/ or chair of the relevant AWG of pending exchange and look for feedback.	**[ ]**
2.	E mail age reader coordinators (PGCCDBS age contacts list) to establish participation from each country.	[ ]
3.	Establish list of participants and direct them to the European Age Readers Forum (EARF)	[ ]
4.	Using the EARF, agree a circulation schedule for all participants.	[ ]
5.	Establish exchange set – follow PGCCDBS Guidelines on this.	[ ]
6.	All age readings received.	[ ]
7.	Complete analysis – follow PGCCDBS 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 PGCCDBS.	[ ]
12.	Provide an extended abstract to the PGCCDBS.	[ ]
13.	Upload images, or a link to where a set of agreed ages, resulting from the exchange, can be found to the EARF.	[ ]