ICES WKNARC REPORT 2011

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Report of the Workshop of National Age Readings Coordinators (WKNARC)

5–9 September 2011

Boulogne-sur-Mer, France

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

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Executive summary

The Workshop of National Age Readings Coordinators (WKNARC), chaired by Kélig Mahé, France and Willie McCurdy, UK, met in Boulogne-sur-Mer (IFREMER) France, 5–9 September 2011. 17 nations were represented by 27 participants. Age determination is an essential feature in fish stock assessment to estimate the rates of mortalities and growth. Assessment of species/stocks using age structured models has proved useful in establishing a diagnosis of stock status. However, the approach has several limitations and shortcomings such as stock structure, natural mortality and growth. Age data is provided by different countries and are estimated using international ageing criteria which have not been validated.

WKNARC was proposed by the Planning Group on Commercial Catches, Discards and Biological Sampling (PGCCDBS) 2010. Many activities in this group are closely linked to the activities of the Data Collection Framework (DCF). For the purpose of inter-calibration between ageing labs WKNARC reviewed preparation methods by species and areas, material and techniques development, methods in images processing, and the validation methods. WKNARC reviewed tools for the exchanges and workshops (WebGR, PGCCDBS Guidelines for Otolith Exchanges).

The report contains a synthesis of 34 questionnaires (Belgium, Denmark, France, Germany, Iceland, Ireland, Netherlands, Norway, Sweden, UK England, UK-Scotland, UK-Northern Ireland, Slovenia, Spain, Portugal, Malta, Lithuania, Latvia, Greece, Finland, Cyprus, Italy, Romania, Greenland) presenting the species and the techniques of all these institutes. A list of all the publications concerning the age validation studies is provided for ICES and GFCM areas. A summary of the status of quality management for age reading at MS institutes is presented. Good practice for age validations was identified. All Tools for the exchanges and workshops, in particular the WebGR tool, are described in detail. Methods and usage of image processing at MS institutes are presented and an overview of methods in images processing is provided. The report recommends that a 3-point scale of age reading quality be used by all age readers who provide age data for stock assessments and that Age Calibration Workshops derive descriptors for the three scale points that are applicable to their species-stocks.

The report presents the recommendations and the proposals to progress and standardise age estimation within a Quality Control framework.

1 Introduction

1.1 Terms of Reference

2010/2/ACOM46 The Workshop of National Age Readings Coordinators (WKNARC), chaired by Kélig Mahé, France and Willie McCurdy, UK, will meet in Boulogne-sur-Mer (IFREMER) France, 5–9 September 2011 to:

a) Review preparation methods by species and areas,

b) Review material and techniques development,

c) Review methods in images processing,

d) Review of the validation methods,

e) Review possibility of sending otoliths to central labs for processing age reading,

f) Review tools for the exchanges and workshops (WebGR, PGCCDBS Guidelines for Otolith Exchanges, age readers forum),

g) Collate information on the quality status of age reading at MS institutes,

h) The workshop will be preceded by a questionnaire to obtain information on the status of ToRs a, b, c, d, e and g at MS institutes,

i) Address the generic ToRs adopted for workshops on age calibration (see 'PGCCDBS Guidelines for Workshops on Age Calibration')

1.2 Data from MS institutes

Two documents were sent to all the institutes to prepare this meeting.

The first document was a questionnaire with the 36 following questions:

1. Your name:

2. Your country:

3. Your institute:

4. Number of treated calcified structures in 2010:

5. List of your species (Latin name):

6. Your geographical areas:

7. How many laboratories analyse calcified structures in your country?

8. How is the age data used e.g. stock assessment?%, environmental studies?%, other?%, (please specify):

9. Description of your types of calcified structure preparation (e.g broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

10. List of calcified structures that you use:

11. How are calcified structures stored before treatment?

12. How are calcified structure preparations stored?

13. List of your saws (name, manufacturer, description):

14. Description and characteristics of your resin(s):

15. A document presenting the summary of the techniques used (reference):

16. List of bilateral agreements where you receive calcified structures (country name, species, typical number per year):

17. List of bilateral agreements where you sent calcified structures (country name, species, typical number per year):

18. List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

19. List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

20. List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

21. Do you use image capture/analysis software, if so, which?

22. Which type of camera do you use (digital or analogue, description, characteristics)?

23. Which applications is image processing used for (routine, only exchanges, validation studies…)?

24. How many images are made per year on average?

25. Do you calibrate your images?

26. Which measurements do you make on the image (distance between rings…)?

27. What is the format of images?

28. Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species:

29. Do you have an internal quality control (description, references)?

30. Do you have quality management certification e.g. ISO 9001 : 2008?

31. Are you accredited (yes or no, name of accreditation authority)?

32. What age calibration Exchanges or Workshops has your country participated in during the last three years?

33. Are all your age readers aware of WebGR?

34. Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

The second document was a table with the following columns, which would allow a review of methods by species and areas for all institutes:

1. Institute
2. Countries
3. Latin name
4. Area
5. ICES division
6. Calcified structure
7. Preparation

8. Section thickness (mm)

9. Comments

32 institutes have completed these documents (Annex 3: list of Institutes, Annex 7: All questionnaires).

2 Review preparation methods by species and areas (ToR a) and Review material and techniques development (ToR b)

The WKNARC decided to answer these ToR's as one ToR, condensing the preparation methods used by species and eco-region (Annex 4) and giving an overview of the state-of-the-art of the material methods applied in the participating laboratories in relation to storage, preparation and media for age determination of the mentioned species. Based on the questionnaires delivered to WKNARC from MS institutes prior to the workshop, the group reviewed the results and compiled the information about the materials and techniques the different countries use for storage before and after preparation, and the types of resins and saws used.

N.B. The table condensing the preparation method used by species and eco-region from all countries (Review material, techniques and preparation methods by species and areas to fish ageing.xls) is downloadable separately.

An impressive result is that among 34 institutes from a total of 22 MS, 759,403 calcified structures (CS) were processed in 2010. Among the 10 different calcified structures used for ageing, the otolith presents the greatest variety of preparation methods used (about 12). Within a total of 19 eco-regions, otoliths are collected for 112 different species. For 70 species (63%), only one institute in each eco-region analyzes the collected otoliths. While 15 species (13%) are treated using identical procedures by institutes within the same eco-region, 27 species (24%) within an eco-region are treated in a variety of ways that have been developed at the institutes analysing these species.

Thus, a range of different materials for storage and techniques for preparing resins and cutting the calcified structures are applied across species. For several species, the choice of preparation method differs between laboratories within the same eco-region. For example, *Scophthalmus rhombus* in the North Sea is age-estimated in 6 MS institutes using 5 different preparation methods. A good example of agreement of the treatment applied is *Scomber scombrus* for which 11 institutes over 7 ecoregions use the same method, i.e. whole otolith, for age estimations.

The differences in storage, preparation methods and media for analysing the otoliths does not necessarily imply unwanted bias in terms of the precision of the age readings. However, if a particular preparation method implies age reading problems to most readers, it does not necessarily mean that the age estimations using this method are imprecise or biased; being accustomed to a preparation technique, in fact highly influences precision and accuracy. In age calibration exchanges and workshops, special attention should be paid to the participant's familiarity with the calcified structure and/or methods used in the exchange/workshop. Potential unfamiliarity with the exchanged structure/methods could influence the individual age reader's estimation of the structure and thus introduce bias based on the preparation method and not based on the actual perception of the age structures.

The table produced during the WKNARC (Review material, techniques and preparation methods by species and areas to fish ageing.xls) is an essential tool for future alignment or standardisation of the procedures applied for each species.

2.1 Storage and choice of CS

All MS laboratories, which reported back to the WKNARC, use otoliths for age determination. While 35% and 53% of the institutes also use scales and other calcified structures respectively. All the institutes use dry-storage before preparation. However depending on the species, 15% of the MS institutes also use wet storage (distilled water, salted water, alcohol) and 12% store calcified structures frozen.

Depending on the species, institutes permanently store calcified structures in paper envelopes (71% of institutes) or in plastic containers (62% of the institutes). All the institutes that section calcified structures, store the sections on slides.

2.2 Preparation methods applied across MS

Institutes which use a saw for cutting the calcified structures use different resins to prepare for this process. The most frequently used resins are polyester (41% of the institutes) and epoxy (35% of the institutes). In addition, 17% of the institutes use other resins (eukitt/histokitt, acrylic resin and crystal bond). Some institutes have switched from using epoxy to using polyester because polyester is less toxic and does not create bubbles during preparation.

Modelling clays, e.g. Plasticine®, are used to support broken otoliths for age reading at some institutes. Plasticine is oil-based non-drying clay modeling material. Some institutes use Plasticine to prepare broken otoliths for age reading. A plastic lid from a sample jar is filled with Plasticine and broken otoliths are place around the edge of the lid. An arrow drawn on the Plasticine marks the starting point for reading. This makes the reading process more efficient.

24% of the institutes use Buehler saws and 21% use Struers. 38% use saws from other manufactures (Brillant, Conrad, Logitech, Leica, Idnvelar, Pilses, Remet, Extec, Beacon). 56% of the institutes can provide documents presenting the summary of the techniques used.

2.3 Recommendations

The WKNARC strongly recommends that the tables, Review material, techniques and preparation methods by species and areas to fish ageing.xls, that detail the preferred methodology applied for each species within an eco-region, are consulted by the national age reading coordinators to evaluate the preferred methodology by individual institutes in relation to the methods applied by the remaining laboratories in the eco-region.

The WKNARC recommends that where necessary, the methods and techniques are standardised as much as possible. The differences in methods and techniques used may impact the age reading for some stocks. In cases where this is suspected, a comparative analysis should be performed. In cases where such an analysis establishes that the different methods do not affect age reading, standardisation is not necessary.

The WKNARC recommends that during exchanges, attention is paid to the different methods of storage used by the institutes participating in the exchange, as this may impact the appearance of the CS.

3 Review methods in images processing (ToR c)

The ability to readily acquire images of CS (Calcified Structures) with metadata, is essential to ensure that sufficient images of good quality are available to the chairs of age calibration workshops (If these images are regularly uploaded to WebGR, this will make the process of planning and organising WKACs more efficient).

Image processing software can provide a range of data (e.g. grey levels, spacing between growth zones etc.), that is independent of visual perception and that can assist in the age reading of CS that are difficult to interpret. It is preferable that the image processing software can be used to routinely calibrate all acquired images with a scale bar (figure 2). TNPC (Traitement Numérique des Pièces Calcifiées) software (http://www.tnpc.fr/fr/tnpc.html) has been developed specifically to assist with the age estimation of CS and it can be used with a scanner to automatically acquire individual CS images from a batch of CS (e.g. otolith sections). TNPC can also be used to compare growth curves from a selection of CS, to help discriminate between the annual growth zones and other marks on an individual CS.

Other commercial software (e.g. Image-Pro Plus) has been modified for this purpose. Open Source image processing software is also used to interpret CS. There is a growing community of Image J (http://rsb.info.nih.gov/ij/) users who are developing applications and plug-ins (e.g. Object J), that are useful for the treatment of images of CS.

4 Review of the validation methods (ToR d)

For many analytical stock assessments, the lack of validated age data is a major source of uncertainty as to the reliability of the assessment results. Valid age estimates are also important for other stocks, e.g. elasmobranchs and other species that have been recently added to the DCF, since evaluations of vulnerability rely on valid and robust growth models.

Several methods exist for validation of age readings of calcified structures. Some are designed to identify what constitutes a particular seasonal zone, some to confirm the annual deposition of seasonal zones (i.e. marginal increment analysis, marginal analysis, marking calcified structures with oxytetracycline), and some to validate the total age of the structure (marking and re-capture, rearing in captivity, length back-calculated compared with length frequency distribution). An overview of methods and their merits can be found in Campana (2001).

The WKNARC received responses from individual labs on some of the stocks for which validation work has been undertaken

4.1 Tagging studies

de Pontual, H., Groison, A.L., Piñeiro, C., and Bertignac, M., 2006. Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. – ICES Journal of Marine Science, 63 (9): 1674-1681.

Hüssy K., Mosegaard H., Nielsen B. and Worsøe Clausen L., 2009. Using data storage tags to link otolith macrostructure in Baltic cod (*Gadus morhua* L.) with environmental conditions. Mar. Ecol. Prog. Ser., 378: 161-170.

Landa, J., Duarte, R. and Quincoces, I. 2008. Growth of white anglerfish (*Lophius piscatorius*) tagged in the Northeast Atlantic, and a review of age studies on anglerfish. ICES Journal of Marine Science, 65: 72-80.

Mellon-Duval, C., de Pontual, H., Métral, L., and Quemener, L. 2010. Growth of European hake (*Merluccius merluccius*) in the Gulf of Lions based on conventional tagging. – ICES Journal of Marine Science, 67: 62–70.

Piñeiro, C., Rey, J., de Pontual, H., and Goñi, R. 2007. Tag and recapture of European hake (*Merluccius merluccius* L.) off the Northwest Iberian Peninsula: First results support fast growth hypothesis. Fisheries Research, 88:150 – 154.

Raitaniemi, J., Nyberg, K. and Torvi, I. 2000. Age and growth determination of fish (In Finnish). FGFRI, pp. 232

4.2 Growth ring formation studies

Boudaya, L., Neifar, L., Rizzo, P., Badalucco, C., Bouain, A. and Fiorentino, F., 2008. Growth and reproduction of *Chelidonichthys lucerna* (Linnaeus) (Pisces: *Triglidae*) in the Gulf of Gabe` s, Tunisia. J. Appl. Ichthyol., 24:581–588.

Garararibaldi, F., Palandr, G. and Orsi Relini, L., The first mediterranean recapture, useful for growth studies, of tagged swordfish. ICCAT, Coll. Vol. Sci. Pap., 49 (1): 151-152.

Hüssy, K., 2010. Why is age determination in Baltic cod (*Gadus morhua* L.) so difficult? ICES J. mar. Sci. 67, 1198-1205.

Hüssy, K., Hinrichsen, H.H., Fey, D.P., Walther, Y., Velasco, A., 2010. The use of otolith microstructure to estimate age in adult Eastern Baltic cod (*Gadus morhua* L.). J. Fish. Biol. 76, 1640–1654.

La Mesa, M. and De Rossi, F. , 2008. Early life history of the black anglerfish *Lophius budegassa* Spinola, 1807 in the Adriatic Sea using otolith microstructure. Fisheries Research, 93 (1-2): 234-239.

La Mesa, M., Donato, F., Giannetti, G. and Arneri, E., 2009. Age, growth and mortality of juvenile anchovy (*Engraulis encrasicolus*) in the Adriatic Sea. Fisheries Research, 96 (2-3): 275-280.

Landa, J. and Piñeiro, C., 2000. Megrim (*Lepidorhombus whiffiagonis*) growth in the Northeastern Atlantic based on back-calculation of otolith rings. ICES Journal of Marine Science, 57: 1077-1090.

Neilson, J.D. and Campana, S.E., 2008. A validated description of age and growth of western Atlantic bluefin tuna (*Thunnus thynus*). Canadian Journal of fisheries and Aquatic Sciences, 65: 1523-1527.

Panfili, M., Donato, F., Morello, E.B. and Arneri, E., 2010. Growth rates of early stages of *Engraulis encrasicolus* and *Sardina pilchardus* in the Adriatic Sea. Rapp. Comm. int. Mer Médit., 39.

Rijnsdorp, A.D., van Leeuwen, P.I. and Visser, T., 1990. On the validity and precision of back-calculation of growth from otoliths of the plaice, *Pleuronectes platessa* L. Fish Res 9: 97-117.

Sieli, G., Badalucco, C., Di Stefano, G., Rizzo, P., D'Anna, G. and Fiorentino, F. 2011. Biology of red mullet, *Mullus barbatus* (L. 1758), in the Gulf of Castellammare (NW Sicily, Mediterranean Sea) subject to a trawling ban. J. Appl. Ichthyol., in press.

Smedstad, O.M. and Holm, J.C., 1996. Validation of back-calculation formulae for cod otoliths. Journal of Fish Biology 49:973-985.

Stransky, C., Kanisch, G., Krüger, A. and Purkl, S., 2005. Radiometric age validation of golden redfish (*Sebastes marinus*) and deep-sea redfish (*S. mentella*) in the Northeast Atlantic. Fish. Res. 74: 186-197.

4.3 First growth ring studies from the daily increment

Alemany, F. and Álvarez, F., 1994. Formation of initial daily increments in sagital otoliths of reared and wild *Sardina pilchardus* yolk-sac larvae. Mar. Biol., 121: 35-39.

Álvarez, F., 2002. Crecimiento diario de *Sardina pilchardus* y su aplicación al estudio de procesos de reclutamiento. Ph. D. thesis, Univ. Santiago.

Belcari, P., Ligas, A. and Viva, C., 2006. Age determination and growth of juveniles of the European hake, *Merluccius merluccius* (L., 1758), in the northern Tyrrhenian Sea (NW Mediterranean). Fisheries Research, 78: 211-217.

Capoccioni, F., Costa, C., Aguzzi, J., Menesatti, P., Lombarte, A. and Ciccotti, E., 2010. Ontogenetic and environmental effects on otolith shape variability in three Mediterranean European eel (*Anguilla anguilla*, L.) local stocks. Journal of Experimental Marine Biology and Ecology, 397, 1-7.

Dulcic, J., 1997. Growth parameters of sardine, Sardina pilchardus (Walbaum, 1792), and anchovy, *Engraulis encrasicolus* (Linnaeus, 1758), larvae in the Eastern Adratic. Int. Symp. of the Fisheries Society of the British Isles, Galway (Ireland), 8-11 July 1997.

Hernández, C., Villamor, B., Barrado, J., Navarro, C. and Dueñas, C., 2009. Preliminary results on first check validation in European anchovy (*Engraulis encrasicolus*) otoliths. Working Document to ICES Workshop on Age reading of European anchovy (WKARA). Mazara del Vallo, Italy, 9–13 November 2009.

ICES, 1998. Workshop on Otolith Ageing of North Sea Whiting. ICES CM 1998/G:14, 66pp.

Ré, P., 1984. Evidence of daily and hourly growth in pilchard larvae based on otolih growth increments, *Sardina pilchardus* (Walbaum, 1792). Cybium, 8 (1): 33-38.

Rehberg, S., Hammer, C., Hillgruber, N., Hüssy, K. and Temming, A., Validation of the first annulus in juvenile western Baltic cod (*Gadus morhua* L.) using otolith microstructure analysis. In prep.

Serra-Pereira, B., Figueiredo, I., Farias, I., Moura, T. and Gordo, L.S., 2008. Description of dermal denticles from the caudal region of *Raja clavata* and their use for the estimation of age and growth. ICES Journal of Marine Science, 65: 1701-1709.

The list gives a limited overview of the topic, since it does not identify stocks for which the lack of validation is considered to be most problematic.

4.4 Questionnaire to identify the major stocks and species needing age validation studies

During the WKNARC meeting, the principal objective was to identify main species and stocks which are not validated for age determination, with calcified structures formation that are problematic in the consistencies of age readings and for which age validation and calcified structures formation studies should be of high priority for an accurate stock assessment. As this task could not be covered during the meeting with the available information, the WG decide to make the following recommendation to PGCCDBS.

WKNARC recommends that a questionnaire (Annex 5) should be forwarded to each ICES and GFCM stock assessment working group to identify the gaps in age validation and growth formation studies, for stocks that are subject to age structured assessment or require such an approach. The questionnaire will also help to prioritise recommendations for future validation work, using direct recommendations from the assessment working groups and/or the outcome of the most recent age calibration workshops (WKACs) for these stock-species. A table with the questions to be answered by the working groups is attached (Annex 5: Questionnaire: Identification of the major stocks and species needing age validation studies).

Subsequently the WKNARC should examine the results of the questionnaires (in its next meeting in 2013), in order to identify the major stocks and species needing validation studies on calcified structures formation and age determination. Accordingly it should try to suggest a suitable framework for funding these validation studies (either at the EC or more international level) for those identified species and stocks.

In addition, it should identify the species-stocks, for which there is a bad performance in age readings and where a validation method is lacking, to trigger the process (defined in the PGCCDBS Guidelines) to solve the problems. This will be achieved by defining ToRs to solve the identified problems (by carrying out the required validation studies), and by planning subsequent workshops by groups of species, stocks and areas (as appropriate), to disseminate and jointly analyse the results of the validation studies on calcified structures formation and age determination and to improve the quality and consistency of the age determination for these species.

N.B. The PGCCDBS (2011) 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 is 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 pro-

tocol for preparation/age reading and the use of inappropriate CS or preparation methods.

- Medium ageing performance: The age data is 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%). Causes may include 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 that 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 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.

The Questionnaire for preparation of the current WKNARC workshop served to identify the available validation studies. However, there is a need to clearly identify the major gaps and weakness affecting the stocks being assessed in the ICES and GCFM communities. Therefore the WKNARC considered the convenience of carrying out the proposed survey across assessment working groups.

4.5 Good practice for age validations

During the WKNARC meeting, good practice for age validations was identified on regular basis:

- For different species create a reference collection of images (some with OTC marks if possible) of otoliths in WebGR.
- Marginal increment and marginal analysis based on all available biological sampling data.
- Measurements of annual growth increments to allow for:

  searching for patterns of successive year growth increments that may act as a marker to serve to validate the age readings of the species.

  use of back-calculated lengths to verify the potentially correct interpretation of the annual rings, according to their consistency with the length frequency distribution by ages (from surveys, discard projects or other sources).

- Following strong cohorts and using the otoliths of those the modal groups to set the growth patterns and validation of their ageing.
- Assemble and compare the results of different validation methods on the same species-stocks (as i.e. following strong year classes, mark-recapture, back-calculation of lengths etc).

WKNARC recommends that user friendly software to analyse the measurement of growth zones, is established within WebGR .

WKNARC strongly recommends that a Workshop is carried out in 2012 on the validation studies for gadoids using available data.

WKAVSG – Workshop on Age Validation Studies for Gadoids

(WKAVSG), chaired by ?? and ??, will meet at ??, ?? 2013, to:

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

WKAVSG will report by ?? for the attention of ACOM and PGCCDBS.

Supporting Information

Priority:	Age validation is a fundamental need in fish age determination to provide accurate mortality and growth rates estimations for stock-assessment. The model of fish stocks-assessment using age structure population models has proved useful in establishing a diagnosis on stock status. However, the approach has several limitations and shortcomings such as stock structure, natural mortality and growth. Age data is provided by different countries and are estimated using international ageing criteria, many of which have not been validated. Therefore, a WK should be carried out in order to make a general methodological review, evaluate available information on validation of CS (calcified structures) growth pattern, age determination issues and ultimately pave the way for solid input data to age-based assessments which has been subject of concern of EC DCF, PGCCDBS and WKNARC, and make progress towards a solution.
Scientific justification:	The provision of age validation studies for gadoid species is crucial. The stock-assessment is severely hampered by the lack of valid age structured data and the fact that the agreement in the age data supplied to the assessment is very low (as seen in previous exchanges), affected the precision of the diagnosis on stock status. In particular the validation of the annual deposition of seasonal zones (opaque and translucent) and the check (i.e. the spawning ring, demersal ring, migration ring) in the CS represent the focal point to the improve the precision in the fish age determination by the CS. Tagging programs with marking and recapture in order to validate seasonal zones in otoliths (i.e. marking with OTC), cannot easily be applied to all species and stocks. In addition techniques such as marginal

	increment analysis, marginal analysis, length back-calculation may be appropriate to clarify the periodicity of CS growth and the correct interpretation of rings. The aim of the workshop is to identify the state of art of age validation studies conducted so far and to propose appropriate methods for species and stocks and ultimately to promote international cooperation projects on the age validation and CS growth pattern.
Resource requirements:	No specific resource requirements beyond the need for members to prepare for and participate in the meeting.
Participants:	Participants should include a mixture of scientists with expertise in age determination, biology and stock assessment of fish.
Secretariat facilities:	None.
Financial:	Travel costs will be eligible for participants from Member States of the European Union through the EU Data Collection Framework (DCF). Funding for external experts on the age determination methods may be required.
Linkages to advisory committees:	ACOM
Linkages to other committees or groups:	WKNARC
Linkages to other organisations:	There is a direct link with the EU DCF.

4.6 Reference

Campana, S., 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59: 197–242.

5 Review possibility of sending otoliths to central labs for processing age reading (ToR e)

Within the framework of the WKNARC 2011 meeting held in Boulogne-sur-Mer (France), 5-9 September, the possibility of sending calcified structures to central laboratories for processing and age determination was discussed. The general agreement was that some labs could send otoliths or other calcified structures on a voluntary basis to some regional centres that are experienced, capable, and willing to perform the age reading.

The reasons for sending could be calibration, exchange, training, or an extremely low number of specimens caught per year (low importance for the lab). On the other hand, the reasons for receiving calcified structures could be exchange, inter-calibration, training, stock assessment, intensive study of species, publications, economic issues, etc. In this context, particular attention should be paid on the species recently included in the DCF list, such as elasmobranchs (skates and sharks). Institutes already experienced in the age determination of certain species could act as central laboratories. The services offered by a central laboratory could vary from the age reading of calcified structures prepared by the sending laboratory (e.g. where the sending laboratory processes calcified structures, but has no age reading expertise for these species), to complete preparation and age reading of calcified structures (e.g. where the requirement for age data from a species is too low, to permit the economic development of the necessary expertise at the sending institute).

In order to develop the respective cooperation and coordination between national institutes WKNARC produced a questionnaire with attached table (Annex 6: Questionnaire: Review possibility of sending otoliths to central labs for processing), which should provide the necessary information for such actions. The information obtained would serve national institutes to initiate contacts with other institutes as well as for PGCCDBS and RCMs.

WKNARC recommends that national institutes submit the questionnaire to PGCCDBS which could summarise the information and be distributed between RCMs, assessment working groups and national institutes in the framework of the next PGCCDBS meeting that will be held in Rome in 2012. WKNARC considers that this information would be useful for the development of cooperation between national institutes as well as for improvement of age determination quality.

The questionnaire and the table (Annex 6) aim to help the institutes to find the specific information according to their needs. WKNARC believes that this information will help the institutes to plan and organise different bilateral agreements that are needed in order to improve their age determination skills and quality management.

6 Review tools for the exchanges and workshops (WebGR, PGCCDBS Guidelines for Otolith Exchanges, age readers forum) (ToR f)

Because age determination is not an exact science there is understandably a 'drift' between readers and within readers over time. Some species present more problems than others. Within and between age reading laboratories there will inevitably be disagreements between age readers, and a comprehensive exchange scheme of calcified structures between age readers of different age reading laboratories will help to identify differences in age reading. Such an exchange program may identify a problem so serious that a workshop should be held. The following section gives a review of the state-of-the-art for age reading calibration tools currently being used. Very necessary justified developments and recommendations for this are also given.

6.1 Age Readers Forum/guidelines from PGCCDBS

This forum should be the gateway to all exchanges and workshops: http://groupnet.ices.dk/AgeForum/default.aspx

PGCCDBS established the Age Readers Forum (EARF), in response to feedback received from those engaged in age reading across Europe. This forum is now up and running and is being used actively by age readers and those conducting age reading exchanges.

- It can be used as a resource for training, sharing and discussion of all aspects related to age reading (SOPS, age reading manuals, preparation methods etc).
- It holds the contact details and a mailing list of age reading coordinators as well as those engaged in age reading of fish species in the various European laboratories.
- It contains the calendar of upcoming workshops and the PGCCDBS meeting details.
- It contains a link to the PGCCDBS documents repository where all past exchange and workshop reports are stored.
- It contains some key EFAN Reports.
- It contains the PGCCDBS guidelines and checklist for otolith exchanges and workshops.

Two age reading exchanges are currently running through the EARF, one on brill and turbot using WebGR and one on Anglerfish using GIMP.

Regarding the review of guidelines for exchanges and workshops, these were presented at WKNARC, and through discussion it was established that participants had used these when organising exchanges and workshops and had found them useful.

6.2 The EFAN spreadsheet for Age Comparisons; 'The Guus Eltink spreadsheet'

This Excel workbook ("AGE COMPARISONS.XLS") was developed during the EFAN project for an easy and fast analysis of age reading results. It allows for an immediate reporting of the results after an exchange/workshop and the results from the analysis on age reading comparisons are easy to understand for the age readers, who have to

calibrate their age reading method based on these results. Furthermore this tool for age reading analysis is flexible allowing for different types of reference collections, to compare to known age instead of modal age, etc. The spreadsheet performs a series of calculations based on the individual age estimations by each reader, which are recorded in the spreadsheet and returns tables for relative bias, CV's, percentage agreement (by reader, by month, by modal age) as well as an overall estimate for CV and percentage agreement. The spreadsheet also produces illustrative figures of age bias; CV, percentage of agreement and standard deviation plotted against modal age; the distribution of the age reading errors in percentage by modal age; the relative bias by modal age and the estimated mean length at age by age reader.

The 'Tool for Age Reading Comparisons' was developed by Eltink *et al.* 2000 has been very valuable for the development of age calibrations and has played a major part in making these exercises part of the evaluation of the quality of the input of age aggregated data into assessments. It has made the age reader community familiar with the art of quantitative comparisons of qualitative visual interpretations and stands as a milestone for the Quality Assurance/Quality Control of age estimations. However, being an excel spreadsheet, the tool is rather sensitive to typological errors and destruction of the formulas in the actual cells and thus the spreadsheet is prone to errors caused by unfamiliarity with the set-up, or even 'ordinary' mistakes, which can also be made by expert users. Additionally, the spreadsheet does require the user to be alert to how the readers are ranked in the columns, as the weighing of the individual readers is dependent on the column number. This and other inherited features of the spreadsheet make it prone to errors.

The copying and pasting of data into the Excel spreadsheet can lead to transcription errors, and the formulas used in the spreadsheet can be accidentally modified, affecting the results of the analysis. Lack of flexibility with regard to changing the number of readers in an exchange can be problematic. This was found to be a potential problem during various exchanges. Something more automated and more reproducible is required. Comparisons between readings made using different structures can also be problematic, with more than one version of the spreadsheet being required.

6.3 Exchanges of Images

It is very important to include images in exchange exercises, so each reader can annotate the position of each annual translucent zone on every calcified structure. These annotated images enable comparisons among different readers and also allow the identification of how readers derive their age readings. They form a valuable record of the exchange. Annotated images can also be used as a training resource for less experienced readers. All readers are asked to mark every ring on each digitised image.

Different programs are used for exchange exercises:

Paint Shop Pro: A licence for the software must be purchased. There are currently several versions/updates for the program.

It allows working in different layers, so that every reader can use a different symbol/colour to annotate each calcified structure on their uniquely named raster layer for that structure. Subsequently, all the raster layers for an individual image (the interpretation of every reader), can be overlaid on the image of that structure and a direct comparison of the different readings can be performed. This is a great advantage for the image comparisons but on the other hand it presents many disadvantages:

1) It is not free software

2) Every reader should work with the same version of the software. Otherwise compatibility mistakes can appear.

3) Image exchanges can only be made in *psp format (Paint shop pro files) to allow different layers be recognised and plotted at the same time for reading comparisons. *jpg files are not allowed, so exchange files are large and emailing them between institutes can be problematic. Sending a hard copy of the images by mail can sometimes be the only solution to this problem.

4) It is quite a time consuming exercise for the coordinator of the exchange (i.e., preparation of a common image data base for the exchange, communication of instructions about using Paint Shop Pro for the exercise to all readers, assigning colours/labels for each individual and overlaying the annotated layers etc.)

GIMP (www.gimp.org): It presents the same difficulties as the Paint Shop Pro program but it has the advantage that it is free software.

Image J (http://imagej.nih.gov/ij): This is a shareware program which holds a large number of image processing and analysis features. The program is fairly easy to use and matches many of the other available image analysis programs on the market. It does not give the possibility for several layers, but the readers can mark their readings on the image, which then will be saved as X-Y coordinates for analysis in another software (e.g. excel or R).

WebGR: WebGR was developed to support the organisation and data analysis of calibration workshops, both for age and maturity information. The last hake workshop (WKAEH 2009) recommended an intercalibration exchange using WebGR and using the guidelines developed in that workshop (ICES CM 2009/ ACOM: 42). In 2011, a hake otolith image exchange was used to test the effectiveness of the tool WebGR (Piñeiro and Sainza, presentation to WKNARC, 2011). WebGR's ability to run this calibration exercises online was very efficient: Work online is faster and economical; once a workshop is completed, the statistical analysis, reporting and export functions of WebGR were used to create and disseminate reports and there is improvement of Quality Control of age estimation.

However, some issues need to be solved and implemented for future: Statistically miscalculated APE, no distances are recorded for marked growth rings (annuli) and the possibility of overlap the graphical interpretation of the readers, etc.

The new guidelines have not improved hake age estimation as the imprecision has increased and there was high variability in location of the growth rings. However, the tool allowed for analysis of the variability of the interpreted structures for age reading of hake. The complexity of hake age estimation still continues due to the lack of a validated method, which is essential to develop a new interpretation for ageing criteria and it is very important to emphasise the fact: "Precision management in the absence of accuracy cannot, under any account, guarantee data quality" (De Pontual *et al.*, 2006).

6.4 WebGR

WebGR is a free easy to access web service which requires simple registration to activate and use. It is an open source tool that is designed to overcome the problems of previous systems used for age calibration workshops and otolith exchanges.

It stores images and metadata that are grouped by species, date, area etc and is representative of what is required for stock assessment. WebGR has easy to use fields for things like classification, observation and scientist identification etc. Coordinators of a calibration workshop can select a reference collection which is accessible to all participants and can be annotated by several individuals.

The coordinator of an age calibration workshop selects a collection of images for the first age calibration exchange (an age calibration workshop is composed of a series of age calibration exchanges (figure 1), and this collection is made accessible to all invited participants. All the participants annotate each individual image and assign an age to each image without any access to the work of the other participants. When all the images have been annotated and aged, the coordinator arranges access for the participants to all the annotations and aged images. The participants can then compare and discuss each other's annotations and ages to identify sources of disagreement. A term of reference (ToR), is then agreed to try and resolve the identified problem(s) and the coordinator selects another collection of images for the next exchange in response to the new ToR. Each calibration exercise is organised in a sequence of individual and group classifications that can be carried out for as long as necessary and in some cases a workshop may require several group discussions followed by individual exercises to ensure correct interpretation.

The coordinator has great access and as WebGR facilitates the easy collection of results, the coordinator can generate reports with images and statistical analysis which can be quickly disseminated to all participants and other users. By using WebGR workshops and exchanges become efficient and economic.

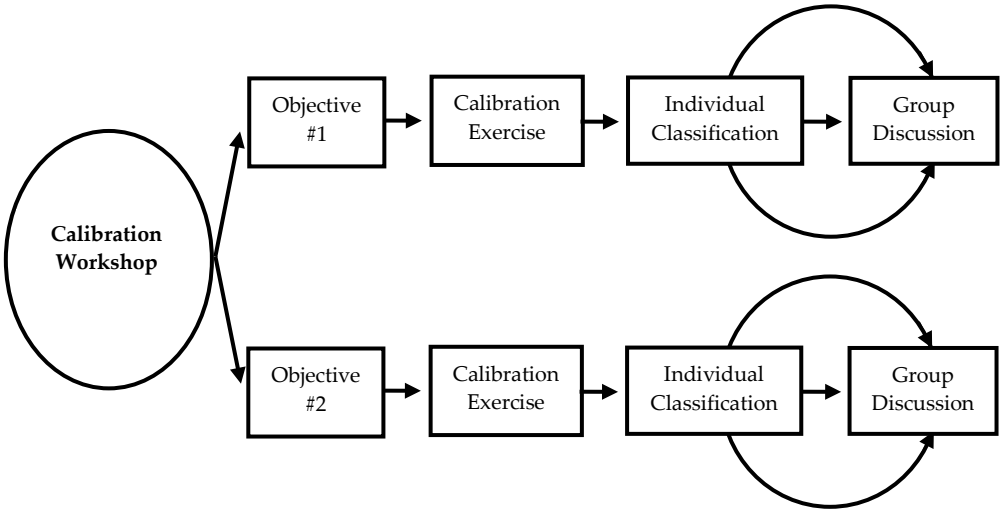

Figure 1: WebGR: Workshop Concept

WebGR aims to improve the reader's precision by allowing readers to gain knowledge of the different ways each reader uses to interpret the growth patterns of the otoliths and can produce a composite image of interpretations which will highlight any disagreements.

Inexperienced readers can use WebGR as a self training tool by accessing images and comparing their annotation of images with those of experts.

Subsequently WebGR can be used to compute catch at age matrices and maturity ogives, both important input parameters to stock assessment. WebGR is also used for

reproduction identification but has the potential for further development into new areas of scientific research such as egg and larvae identification.

WebGR is based more on readers input, which reduces the potential for error caused by copying data from one format to another.

With regard to the overlaying of annotations made by readers on an otolith image, WebGR can be used in a similar way to Paint Shop Pro or GIMP but instead of each reader having to save their own layer in a specified file format, WebGR saves each reader's annotation of each image as a set of xy coordinates that can be mapped on to that image, but the original image and the associated metadata remain unaltered. This makes the collation of this data by the exchange organiser more straight forward. Also in WebGR each reader is assigned a colour for their annotations which they can automatically select on logging in. This reduces the need for communication of the format that each reader should use to make their annotations before the exchange can begin and reduces errors in this area.

WebGR is not just an exchange tool but also has the potential to be a repository for images that could be used to train new readers or as a reference for experienced readers. Images for use in exchanges can be uploaded by the exchange manager in batches and eventually some images already stored in the image repository could be reused, reducing the need for the upload of new images. The planning group reports are stored on the age reader's forum, and it is intended that in future the images associated with any exchanges and workshops described in the reports will be stored on WebGR for reference.

WebGR does not offer a solution to the problem of reading otoliths just from images, something that is not done routinely by all institutes and so may have an effect on results. It is still also necessary to calibrate all images e.g. with a scale bar (Figure 2) and ensure they are consistent externally before they are uploaded to WebGR.

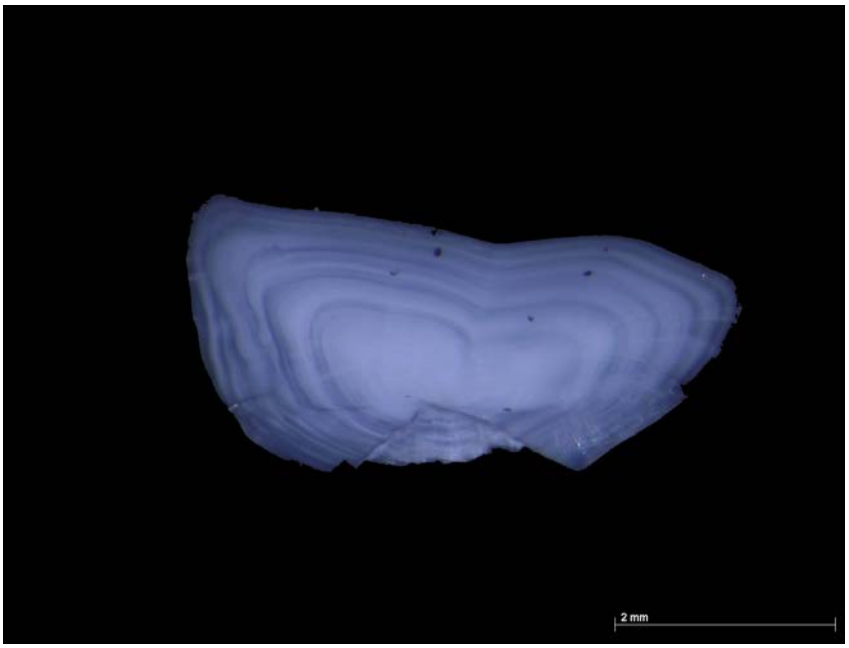

Figure 2: Image of a transverse section of a saithe otolith (*Pollachius virens*) with 5 translucent annuli, photographed in reflected light. This image has been calibrated with a 2mm scale bar to enable age readers in a previous saithe age calibration exchange, to distinguish between small and large otoliths.

It would be helpful for WebGR to include a method not only to compare the results of many readers taking part in an exchange but also to compare the results of two readers for training or internal QC purposes. For this kind of data that is just for use by a specific institute, it would be important to have the ability to use security settings to restrict access. Alternatively, WebGR can also be downloaded and installed locally to manage images and training, or be used for internal QC purposes at an institute.

The current reluctance to use WebGR may be the result of people not being familiar with it or fully understanding its potential uses. Also as WebGR is still in its early stages and is essentially still being tested, people may not be fully confident in its ability to deliver what they require. The fact that the statistical package/reporting is currently not working correctly could be viewed as another reason not to use WebGR at the present time.

6.5 Highly warranted improvements for the immediate future of age calibrations

To strengthen the linkage between the data producers and the end users, the outcomes of calibration exercises should feed directly into assessment models, e.g. by producing a matrix stating the variance or CV around the estimation of a given age and quantifying this into a variance parameter for the age distribution of the stock. This has been tested for Western Baltic Cod applying a matrix with the age bias in a stochastic State-space Assessment Model (SAM; In PGCCDBS 2011 report). The inclusion of the age bias information in the assessment did improve the performance of the assessment model and did increase the quality of the output. Thus the first steps have been taken to feed information from age calibrations directly into assessment and producing a matrix stating the variance or CV around the estimation of a given age and quantifying this into a variance parameter for the age distribution of the stock is within reach, however, it does require effort to be put in to ensure that the statistical/reporting side from age calibrations (preferably via WebGR) can deliver what is required by both age readers and stock assessment scientists and that this data can be delivered in the appropriate format. This would require cooperation between an assessment scientist, an age reading expert and a statistician.

PGCCDBS (2010) recommended that action is taken to create and improve the application of results from exchanges and/or workshops in stock-assessment, by collaboration between the stock-assessment statisticians and the chairs of the calibration workshops and PGCCDBS recommended looking to the approach of the Workshop on the Age Estimation of Hake [WKAEH]. At the WKAEH age readers, growth experts and modelling/assessment experts worked in two parallel sub groups and met in plenary sessions where results that were of interest to both sub groups were presented and discussed.

In the interim, until the statistical package is functioning correctly it is suggested that the Eltink spreadsheet is used in conjunction with WebGR to ensure results produced are as expected.

6.6 Recommendations

PGCCDBS should insist that all age readers and age reading co-ordinators must use the Age Readers Forum and all future age reading exchanges and workshops should be run through the forum using the PGCCDBS guidelines.

WKNARC recommends that future exchanges and workshops should use WebGR for the annotation of all exchange images to prevent inconsistency and make collation of results easier.

PGCCDBS should nominate a WebGR host, who will maintain the application and make any modifications required by users, in similar way to the regional database or FishFrame.

WKNARC recommends that work is put in to ensuring that the statistical/reporting side of WebGR can deliver what is required by both age readers and stock assessment scientists and that this data can be delivered in the appropriate format. This would require cooperation between an assessment scientist, an age reading expert, a skilled programmer, and a statistician. The EFAN/Guus Eltink approach could be used as a basis feeding the results of the age calibrations directly into assessment models, e.g. by producing a matrix stating the variance or CV around the estimation of a given age and quantifying this into a variance parameter for the age distribution of the stock.

WKNARC recommends that precision levels and acceptable 'widths' of confidence bands for age estimates should be evaluated by species, based on simulations with various degrees of disagreement by age.

WKNARC recommends that the WebGR tool should allow for the comparison of various methodologies (preparation, image vs. 'live' otolith) when performing age calibrations. Additionally a plug in for comparison of daily ring results is warranted.

WKNARC recommends that a WebGR training workshop should be held for all interested parties, particularly those involved in the organisation of exchanges and workshops.

6.7 Reference

de Pontual, H., Groison, A. L., Pineiro, C., and Bertignac, M. 2006. Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. ICES Journal of Marine Science, 63: 1674-1681.

Eltink, A.T.G.W., Newton, A.W., Morgado, C., Santamariaand, M.T.G. and Modin, J., 2000. Guidelines and Tools for Age Reading Comparisons. Version 1; October 2000. EFAN Report 3-2000.

7 Collate information on the quality status of age reading at MS institutes (ToR g)

Quality Status Of Age Reading At MS Institutes.

The table (Quality Status Of Age Reading At Institutes.xls) is downloadable separately.

7.1 Internal Quality Management

Three categories of internal quality management for age reading have been identified:

- Quality of the age readings is managed by an individual age reader. CS (calcified structures) preparation and age reading procedures and manuals may not exist for all stocks/species. Typically there is only one age reader for each stock/species and there are no bilateral agreements with readers at other institutes. After an interval of time has passed since the initial readings (usually a minimum of several weeks), the reader re-reads a percentage of each sample of calcified structures. If the readings for an individual CS do not agree and the discrepancy cannot be resolved, that CS is excluded from the assessment. Original CS may not be catalogued and stored in a way that will prevent damage, loss or deterioration. This is the least preferable level of internal quality management, as calcified structures and images can be subconsciously memorised with increasing use, leading to the development of unintentional bias. This situation can be improved by the establishment of reference collections and including some samples from a reference collection in each sample of CS that are re-read, by setting up bilateral agreements and by participation in relevant age calibration exchanges (Ex) and age calibration workshops (WKAC). It is important that reference collections are sufficiently large to avoid frequent use of individual images.
- Quality of the age readings is managed by two or more readers. CS preparation and age reading procedures and manuals exist for all stocks/species. At intervals, each reader re-reads a percentage of the Calcified Structures (CS) read by the other reader(s). If the readings for an individual CS do not agree and the discrepancy cannot be resolved, that CS is excluded from the assessment. There may be bilateral agreements with readers at other institutes. The number of age readers may be low and it is difficult to obtain meaningful results from the analysis of age reader bias. Original CS are catalogued and stored in a way that will prevent damage, loss or deterioration. There is some participation in relevant age calibration exchanges (Ex) and age calibration workshops (WKAC). This situation can be improved by the establishment of reference collections and including some samples from a reference collection in each sample of CS that are re-read, by setting up bilateral agreements with other institutes and increased participation in relevant age calibration exchanges (Ex) and age calibration workshops (WKAC). The management of all documents is controlled and properly managed (unauthorised changes to quality documents are not permitted). It is likely that institutes that meet these requirements may have, or be capable of obtaining quality management certification, e.g. ISO 9001: 2008.

- There are several age readers for each stock/species and bilateral agreements exist with age readers at other institutes who read CS from the same stock/species. CS preparation and age reading procedures and manuals exist for all stocks/species. Reference collections are used for each stock/species and these are updated at intervals with new CS, to reduce the possibility of unintentional bias caused by too frequent exposure to the same CS or (CS image). There are regular scheduled exercises to monitor changes in the age reader performance. There are specified training programmes that require the trainee to be closely supervised for a period of time, before they can enter the cycle of quality management for age readers who contribute data to assessments. A quality manual exists and this defines how reference collections are managed, how training programmes are managed and how quality checks and controls are carried out. The age readers take part in all relevant age calibration exchanges (EX) and age calibration workshops (WKAC). This is the most preferable level of internal quality management. Original CS are catalogued and stored in a way that will prevent damage, loss or deterioration. The reference collections are managed using an image database (N.B. WebGR can be installed locally for this purpose). The management of all documents is controlled and is properly managed, typically by means of an electronic document repository. Unauthorised changes to quality documents are not permitted and printed versions will be watermarked as uncontrolled documents. It is likely that Institutes that meet these requirements may have, or be capable of obtaining quality management certification, e.g. ISO 9001: 2008 and ISO 17025 (accreditation).

7.2 External Quality Management

Accreditation is the highest level of external quality management and two MS institutes currently hold national accreditation, CEFAS (ISO 17025 - UKAS) and IVLO (ISO 17025 - BELAC).

7.3 Study on Harmonised methodology of age estimation Procedures

Most of European fish stocks are assessed using age-based models. In this context, the quality of these data plays a vital role in management of fish resources. Errors in age estimation can be caused by accuracy and/or precision issues (Campana, 2001) and they have to be detected and quantified. Accuracy refers to the closeness between measurements and their true value. Precision is defined as the variability in the age readings. Within and between age reading laboratories there will inevitably be disagreements between age readers.

The European Commission supported the development of a European Fish Ageing Network with two Concerted Actions, i.e. EFAN (European Fish Aging Network) and TACADAR (Towards Accreditation and Certification of Age Determination of Aquatic Resources) from 1997 to 2006. The overall theme of both CAs was to harmonise the fish age estimation, mostly carried out by interpreting calcified structures.

Since 2006, the ICES Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) organises each year of calibration exercises and workshops on calcified structures to estimate precision and relative/absolute bias in the age estimations from readers based in different ageing laboratories.

In 2007, the PGCCDBS meeting decided to produce the first international age length key (ALK) for the sole in the Eastern English Channel (ICES area VIId) in 2008. In the future, the international ALK should develop requiring the harmonisation of the age estimation procedures.

Proposition:

The objective for this proposal is to develop a harmonised methodology and quality assurance process for age estimation procedures for a small region with a group of countries, before trying to achieve general harmonisation of these procedures for all ICES areas. Firstly, we need to identify the region and common species/stocks to be used in this trial harmonisation. The techniques for each step from sampling of the calcified pieces to the storage/archiving then need to be described and compared per country and per species/stocks. Common tools for quality indication and assurance need to be developed for each step of the age estimation procedure. The most qualitative procedures will then be used for optimisation and harmonisation of the protocols (methods of preparation, material etc.) per species/stock and among species/stocks. This will then result in a harmonised quality control mechanism and the development of guidelines for good techniques and good operating procedures. According to these harmonised protocols, we will also need to develop consistent training for new age readers (per species/stock). These new sets of harmonised protocols will give more strength for accreditation processes and will strengthen already accredited labs.

This study was presented during the Regional Coordination Meeting for the North Atlantic (RCM NA) 2010 and during the ICES Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) 2011 meeting.

WKNARC is supporting the idea of this study with a small number of case studies.

7.4 Proposal for the use of a standard grading system by age readers of their own readings to register the confidence level the reader has in their otolith readings, reflecting the quality of the data.

PGCCDBS recommends the use of a standard grading system by the mackerel age reader of his/her own readings (e.g. high, medium, low) be considered during the WKNARC as a standard that could be applied in all age calibration ex-changes and/or WKs (ref. WKARMAC 2010).

It is a recommendation of the WKARMAC 2010 Workshop to register the confidence level the reader has in their otolith reading, reflecting the quality of the data. Most readers should use a scale of 3 levels of quality:

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

WKNARC supports the PGCCDBS recommendation for the use of a grading system by the age reader of their own readings in all age calibration exchanges and/or WKs.

Reading the ages of calcified structures is a subjective process, where the reader uses their acquired knowledge of CS growth zone patterns and the biology of a species-stock, to estimate the age of individual CS that are believed to be from that species-stock. Typically, age readers who provide the age data that are used in stock assessments are experienced in reading the CS of the species-stocks studied at their institutes. Several different scales of different lengths have been used at MS institutes and

currently, 3-point and 4-point scales are used to allow the age readers to record their confidence in their age readings of individual calcified structures. The points on the quality scale of the readings should refer to an age interpretation protocol for the species-stock, to ensure that common principles are used to define the quality of the age readings and minimise the subjective nature of individual age reader quality judgments.

The idea of a grading scale is important, as the readers own assessment of the quality of the ageing structure may well be associated with a bias in interpretation results. E.g. it may well be that 'poor otoliths' are simply otoliths from e.g. slow-growing individuals of the stock, or are from specific parts of the distribution area. Growth is very variable in fish, and age readers should expect variety in the patterns that are used to interpret the age of the calcified structures. It is important to be able to identify such potential bias in the age data. There are many biological, physiological and environmental influences on the growth and some species-stocks may have few calcified structures that are easy to interpret. The use of lowest quality scale value would enable the age reader to give an age estimate, even when the age reader considers the pattern within an ageing structure as poor, knowing that this data will be treated with caution. This in turn enables a revaluation of the poor otoliths and an investigation of which structures the readers should count. The age distribution of the given year/sample can then be updated accordingly and the age reading protocol revised. There is also the risk of introducing bias if only the "good" readings are considered because only a portion of the population is considered, thus the end-users need to be aware of the grading system and what it represents. 'Bad' readings should not be ignored, but it should be examined if a particular pattern lies behind the assignment of 'bad' (growth, migration, stock, etc).

While in most cases the availability of validated CS should eventually increase the readers' confidence in their age readings, there is a lack of validation for many species-stocks. Age data is provided by different countries. Stock assessors and others need an indication of the confidence level that can be applied to the age data that is used to provide a diagnosis of the status of a species-stock. Therefore it is very necessary to have a scale indicating data quality (confidence in the assigned age data), that will enable a common interpretation of the reliability of the age data. Any scale of data quality should take on board both the inherent uncertainty in all age interpretations, as well as the understanding that the individual sense of certainty is related to a given ageing protocol or reading rules that may well be changed should validated calcified structures become more widely available.

The difficulty in interpreting growth zone patterns in CS varies from species to species and from stock to stock. Growth zone features that can be seen relatively clearly in the calcified structures of one species-stock may be very difficult to observe in another species-stock. WKNARC considers that a simple 3-level scale captures the important information.

A major advantage of the 3-point scale is that it can be linked to the PGCCDBS definitions of ageing performance. These are bad ageing performance, where there are serious concerns about the reliability of the age data and/or its value to stock assessment WGs; medium ageing performance, where the age data is sufficiently reliable to be used for stock assessment purposes but improvement is required and good ageing performance, where the age data is considered reliable (see section 4.4 of this report for detailed descriptions of ageing performance.)

Using the same 3-point scale for all species-stocks would standardise the quality values so that data users could eventually rely on the quality value attached to the age data, without having to consider the precise nature of the quality assessments used to derive the quality value. The three scale points can be defined as easy to age with high precision, difficult to age with age with acceptable precision and unreadable or very difficult to age with acceptable precision. Terms like poor, moderate and good quality and/or values relating to the actual growth pattern elements can be used. E.g. clear scale winter rings or otolith translucent annuli that are easily identified, with no unexpected or difficult to resolve growth pattern elements present on the CS. Unreadable CS are classified as difficult to age, as age data from both of these types of CS have the potential to have a detrimental effect on an assessment, if concerns about the reliability of the age data and/or its value to stock assessment WG, are not clearly identified.

WKNARC recommends that Age Calibration Workshops (WKACs), derive descriptors for the three scale points that are applicable to their species-stocks. These can include examples and detailed definitions for these indicators of age reading quality, including quality of the calcified structure and ease of interpretation of the structure.

WKNARC recommends that the following 3-point scale of age reading quality be used by all age readers who provide age data for stock assessments.

AQ1: Easy to age with high precision.

If a scale of 1-100 is applied, where 100 is when the reader has the highest possible confidence in the age reading and 1 is when the reader has no confidence in the age reading, age quality 1 (AQ1), will apply to approximately the top 25 % of the possible quality ratings. AQ1 is an indication that the age data is considered reliable for stock assessment.

AQ2: Difficult to age with age with acceptable precision.

Age quality 2 (AQ2), will apply approximately to age readings within 25 and 75 percentiles of the possible quality ratings. AQ2 is an indication that the age data is sufficiently reliable to be used for stock assessment purposes but improvement is requred.

AQ3: Unreadable or very difficult to age with acceptable precision.

Age quality 3 (AQ3), will apply to approximately the lowest 25 % of the possible quality ratings. 3 AQ3 is an indication that there are serious concerns about the reliability of the age data and/or its value to stock assessment WGs.

7.5 Reference

Campana, S., 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology, 59: 197–242.

Annex 1: List of participants

Name	Address	Phone/Fax	Email
Alessandro Ligas	Centro Interuniversitario di Biologia Marina Viale Nazario Sauro 4 57128 Livorno Italy	Phone +39 0586260723 Fax +39 0586 260723	ligas@cibm.it
Andrés Uriarte	AZTI-Tecnalia AZTI Sukarrieta Txatxarramendi ugartea z/g E-48395 Sukarrieta (Bizkaia) Spain		auriarte@azti.es
Ângela Canha	University of the Azores Department of Oceanography and Fisheries 9901-862 Horta Portugal		angela@uac.pt
Annemie Zenner	Institute for Agricultural and Fisheries Research (ILVO) Ankerstraat 18400 Oostende Belgium	Phone +32 59569823	annemie.zenner@ilvo.vlaanderen.be
Begoña Villamor	Instituto Español de Oceanografía Centro Oceanográfico de Santander P.O. Box 240 39004 Santander Cantabria Spain	Phone +34 942 291 060 Fax +34 942 275072	begona.villamor@st.ieo.es
Francesca Vitale	Swedish University of Agricultural Sciences Institute of Marine Research P.O. Box 4 453 21 Lysekil Sweden	Phone +46 523 18792	francesca.vitale@slu.se
Georgs Kornilovs	Institute for Food Safety, Animal Health and Environment (BIOR) 8 Daugavgrivas Str. Fish Resources Research Department 1048 Riga Latvia	Phone +371 76 76 027 Fax +371 762 6946	georgs.kornilovs@bior.gov.lv
Gróa Pétursdottir	Marine Research Institute PO Box 1390 121 Reykjavík Iceland	Phone +354 5752000	groa@hafro.is
Helen McCormick	Marine Institute Rinville Oranmore Co. Galway Ireland		helen.mccormick@marine.ie
Jari Raitaniemi	Finnish Game and Fisheries Research Institute Turku Game and Fisheries Research Itäinen Pitkäkatu 3 20520 Turku Finland	Phone +358 205 751 685	jari.raitaniemi@rktl.fi

Name	Address	Phone/Fax	Email
Kélig Mahé (Chair)	IFREMER Boulogne-sur-Mer Centre P.O. Box 699 62321 Boulogne Cédex France	Phone +33 321 995602 Fax +33 321 995601	Kelig.Mahe@ifremer.fr
Lise Heggebakken	Institute of Marine Research Institute of Marine Research Tromsø P.O. Box 6404 9294 Tromsø Norway	Phone +47 77 60 97 26	lise.heggebakken@imr.no
Lotte Worsøe Clausen	DTU Aqua - National Institute of Aquatic Resources Section for Fisheries AdviceCharlottenlund SlotJægersborg Alle 12920 Charlottenlund Denmark	Phone +4521362804 Fax +45 33963333	law@aqua.dtu.dk
Mandy Gault	Marine Scotland Science Marine Laboratory P.O. Box 101 AB11 9DB Aberdeen United Kingdom		M.Gault@marlab.ac.uk
Marco Stagioni	University of Bologna V.Le Adriatico 1/N 61032 Fano (PU) Italy		marco.stagioni3@unibo.it
Ole Thomas Albert	Institute of Marine Research Institute of Marine Research Tromsø P.O. Box 6404 9294 Tromsø Norway	Phone +47 77 62 92 32 Fax +47 77 62 91 00	ole.thomas.albert@imr.no
Øyvind Tangen	Institute of Marine Research P.O. Box 1870 Nordnes 5817 Bergen Norway		oyvind.tangen@imr.no
Pierluigi Carbonara	COISPA Tecnologia & Ricerca – Stazione per lo Studio delle Risorse del Mare Via dei Trulli 18/20 70126 Bari-Torre a Mare Italy	Phone +39 080 5433596 Fax +39 080 5433586	carbonara@coispa.it

Name	Address	Phone/Fax	Email
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Tania Zaharia	National Institute for Marine Research and Development "Grigore Antipa" 900 581 Constanta Romania	Phone +40 757 116944 Fax +40 241 831274	tzaharia@alpha.rmri.ro
Tomaz Modic	Fisheries Research Institute of Slovenia Zupanciceva 9 1000 Ljubljana Slovenia	Phone +386 1 224 3400 Fax +386 1 425 5185	tomaz.modic@zzrs.si
Unai Cotano	AZTI-Tecnalia AZTI Sukarrieta Txatxarramendi ugartea z/g E-48395 Sukarrieta (Bizkaia) Spain		ucotano@azti.es
Valodia Maximov	National Institute for Marine Research and Development "Grigore Antipa" 900 581 Constanta Romania	Phone +40 724217409	vmaximov@alpha.rmri.ro
William McCurdy (Chair)	Agri-food and Biosciences Institute (AFBI) Fisheries and Aquatic Ecosystems Branch AFBI Headquarters 18a Newforge Lane BT9 5PX Belfast United Kingdom	Phone +44 28 90 255513 Fax 28 90 2550044	willie.mccurdy@afbini.gov.uk
Yvonne Walther	Swedish University of Agricultural Sciences Institute of Marine Research Utövägen 5 37137 Karlskrona Sweden	Phone +46 10 478 40 50 Fax Cell: + 46 709 35 92 82/+46 76 126 80 41	yvonne.walther@slu.se


WNARC 2011

Annex 2: Agenda

Monday afternoon, 5/09/2011 (Plenary session)

14h00-14h30: Introduction (W. McCurdy and K. Mahé): local info, introduction round, presentation to ToRs, adoption of the agenda

14h30-18h00: Presentation of each laboratory (All participants): 4 or 5 slides per country.

Tuesday morning, 6/09/2011 (Plenary session)

10h00-10h30: ToR a, Review preparation methods by species and areas (W. McCurdy and K. Mahé): summary presentation of reviews preparation methods.

11h00-11h30: ToR b, Review material and techniques development (W. McCurdy and K. Mahé): summary presentation of questionnaire

11h30-12h30: Review material and techniques development for skates and sharks

Tuesday afternoon, 6/09/2011 (Plenary session)

14h00-15h30: ToR g, Collate information on the quality status of age reading at MS institutes

UK England, Accredited laboratory (S. Songer)

Belgium, Accredited laboratory (A. Zenner)

Harmonised methodology of age estimation procedures (A. Zenner, S. Songer and K. Mahé)

15h30-17h30: ToR f, Review tools for the exchanges and workshops (WebGR, PGCCDBS Guidelines for Otolith Exchanges, age readers forum).

European Age Readers Forum (EARF) (H. McCormick)

Guidelines for Otolith Exchanges (L. Worsøe Clausen)

Compile the percentage agreement of recent age reading workshops (A. Zenner)

17h30-18h00: ToR c, Review methods in images processing

TNPC software (K. Mahé)

Wednesday morning, 7/09/2011 (Plenary session)

9h00-10h30: ToR e, Review of the validation methods

11h00-12h30: ToR f, Review tools for the exchanges and workshops (WebGR, PGCCDBS Guidelines for Otolith Exchanges, age readers forum).

WebGR tool (W. McCurdy)

Wednesday afternoon, 7/09/2011 (SubGroups)

14h00: Group picture

14h00-18h00: WebGR: Training session for calibration workshops managers/coordinators, discussion (I. Quincoces or Lucia Zarauz)

Thursday morning, 8/09/2011

9h00-10h00: Plenary session

10h30-12h30: SubGroups

Thursday afternoon, 8/09/2011

14h00-18h00: SubGroups

Friday morning, 9/09/2011 (Plenary session)

9h00-12h30: Draft report and propositions

SubGroups

1) ToR a (Review preparation methods by species and areas) and b (Review material and techniques development)

people: 5

2) ToR c (Review methods in images processing)

people: 2

3) ToR d (Review methods of validation methods)

people: 5

4) ToR e (Review possibility of sending otoliths to central labs for processing age reading)

people: 3

5) ToR f (Review tools for the exchanges and workshops (WebGR, PGCCDBS Guidelines for Otolith Exchanges, age readers forum)

people: 8

6) ToR g (Collate information on the quality status of age reading at MS institutes)

people: 2

Annex 3: List of institutes contributing to the questionnaires

Belgium, ILVO

Cyprus, Fisheries and Marine Research

Denmark, DTU AQUA

Finland, FGFRI

France, IFREMER

Germany, vTI Institute

Greece, Institute of Marine Biological Resources

Greenland, Greenland Institute of Natural Resources

Iceland, The Marine Research Institute (MRI)

Ireland, Marine Institute

Italy, BIOLMAR

Italy, Centro Interuniversitario di Biologia Marina (Liverno)

Italy, CNR (Consiglio Nazionale delle Ricerche)

Italy, COISPA

Italy, Università di Genova

Italy, University of Bari

Italy, University of Rome "Tor Vergata"

Latvia, Institute for Food Safety, Animal Health and Environment (BIOR)

Lithuania, Fisheries service under Ministry of Agriculture

Malta, MRRA

Netherlands, IMARES

Norway, Institute of Marine Research

Portugal, IPIMAR

Portugal, University of the Azores

Romania, National Institute for Marine Research and Development "Grigore Antipa"

Slovenia, Fisheries Institute of Slovenia

Spain, AZTI

Spain, IEO

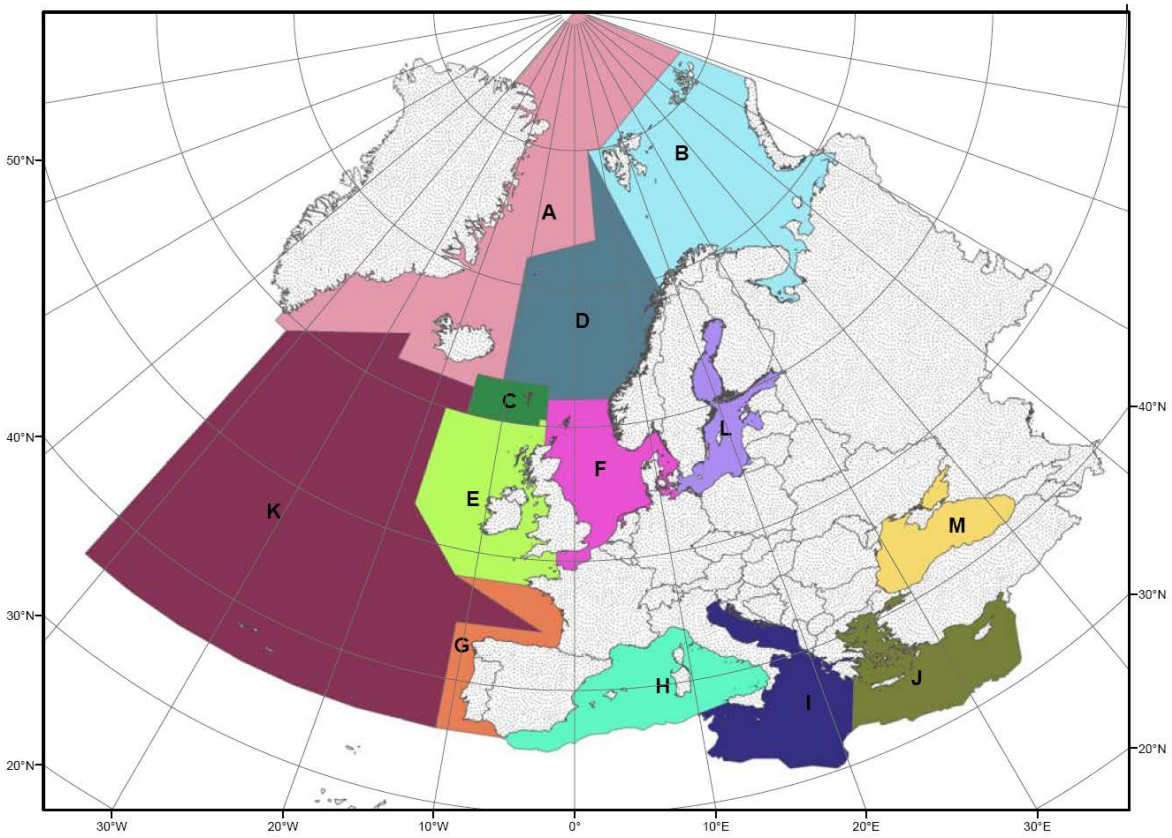
Sweden, Institute of Marine Research

UK England, CEFAS

UK Northern Ireland, Agri-Food & Biosciences Institute (AFBI)

UK Scotland, Marine Scotland

Annex 4: Ecoregions based on ICES Advice ACFM/ACE report (2004) ICES Convention area (FAO area 27) includes regions A–G, L Zones H–J, M are outside the ICES area



A: Greenland and Iceland Seas
B: Barents Sea
C: Faroes
D: Norwegian Sea
E: Celtic Sea
F: North Sea
G: South European Atlantic Shelf
H: Western Mediterranean Sea
I: Adriatic-Ionian Seas
J: Aegean-Levantine Seas
K: Oceanic northeast Atlantic
L: Baltic Sea
M: Black Sea

Annex 5: Questionnaire : Identification of the major stocks and species needing age validation studies

The questionnaire presents the following columns:

- Species
- Areas
- Countries involved
- Institutes involved
- Is the age reading problematic
- % of agreement of last Workshop
- Workshop reference
- Is the ring formation validated for age determination for this stock? and for what range of ages (for Y young, I intermediate, O old or All_all range, None)
- Is the validation borrowed from another stock? Which one?
- Reference of the validation
- Would you recommend a workshop on otolith formation and validation of age determination? (Comments and justifications or TORs)
- Comments?

Annex 6: Questionnaire : Review possibility of sending otoliths to central labs for processing

1. Your name:

2. Your country:

3. Your institute:

Species and geographical areas : follow table (For a correct fulfilling of the table, see the Manual below)

Species or Taxon*	ICES/FAO Area	Country	Institute	Nr. Collected/year	Nr. Aged/year	Level of expertise**	Quality control/Accreditation	Interest in Receiving/Sending
Clupea harengus	III d	Latvia	BIOR	7000	7000	high		receiving
Sprattus sprattus	III d	Latvia	BIOR	4000	4000	high		receiving
Gadus morhua	III d	Latvia	BIOR	4000	4000	high		
Platychtys flesus	III d	Latvia	BIOR	900	900	medium		
Psetta maxima	III d	Latvia	BIOR	300	300	medium		
Salmo salar	III d	Latvia	BIOR	600	600	high		
Salmo trutta	III d	Latvia	BIOR	300	300	high		
Perca fluviatilis	III d	Latvia	BIOR	300	300	medium		
Sander lucioperca	III d	Latvia	BIOR	400	400	medium		
Anguilla anguilla	III d	Latvia	BIOR	200	0	low		sending
Sebastes mentella	NEAFC	Latvia	BIOR	300	0	low		sending
Engraulis encrasicolus	37.2.1	Slovenia	FRIS	1000	700	medium		sending
Sardina pilchardus	37.2.1	Slovenia	FRIS	800	500	medium		sending

4. List of bilateral agreements where you receive calcified structures (country name, species, typical number per year):

5. List of bilateral agreements where you sent calcified structures (country name, species, typical number per year):

6. Does your institute have the capacity of processing calcified structures for other institutes? If yes, please describe what kind of processing you can offer (number of calcified structures, prices, etc.)

7. Does your institute have training capacities for possible students from countries that are sending calcified structures?

8. If sending calcified structures, what kind of information would you like to receive in return? (age determinations, sections, images, ring measurements, etc.)

9. If receiving calcified structures, what kind of information are you able to send in return? (age determinations, sections, images, ring measurements, etc.)

Manual

Column A: use the species list in the Legend sheet. Please, list only the species for which you already collect or would be able to collect calcified structures.

Column B: describe your working area according to ICES or FAO codes.

Column E: number of calcified structures collected per species per year.

Column F: number of calcified structures aged per species per year.

Column G: three levels of expertise: High, age determination performed for more than 10 years; Medium, age determination performed for up to 10 years; Low, beginning or no experience in the age determination.

Column H: specify if the lab is certified or accredited (ISO 9001, ISO 17025, or such), or if there is any internal quality control.

Column I: indicate that the lab is interested that the age determination of the certain species is performed by other lab. Indicate whether your lab has the capacity of performing (receiving) age determination of certain species from other labs.

Column J: specify in what state you would be able to send the calcified structures to other labs (whole, sections, stained, burned, in resin, etc.).

Column K: specify in what state you would like to receive the calcified structures from other labs (whole, sections, stained, burned, in resin, etc.).

Column L: indicate the reason why would you like that age determination of a certain species is performed by other labs, or why would you like to perform age determination on calcified structures from other labs (exchange, intercalibration, training, stock assessment, intensive study of species, publication, not important species, economic issues, etc.).

Column M: indicate if your lab has training capacities or experienced readers who can participate in calibration exercises ("Training"/"Calibration").

Annex 7: Answers of the institutes to the Questionnaire of WKNARC 2011

- Your name: LUCA LANTERI, FULVIO GARIBALDI, ALESSANDRO MANNINI, Cinzia BADALUCCO, Salvatore GANCITANO, Vita GANCITANO, Pietro RIZZO, Sergio VITALE

- Your country: **ITALY**

- Your institute:

BIOLMAR - Centro di Biologia Marina del Mar Ligure (Dip.Te.Ris.), University of Genoa, Corso Europa, 26 – 16132 Genoa, Italy

CNR - National Research Council – IAMC (Institute for Coastal Marine Environment), Mazara del Vallo

- Number of treated calcified structures in 2010 : ABOUT 200 BETWEEN FIN RAYS, VERTEBRAE AND OTOLITHS

- List of your species (latin name) :

Coryphaena hippurus, Thunnus thynnus, Xiphias gladius, Sarda sarda, Thunnus alalunga, Auxis rochei, Prionace glauca, Pteroplatytrygon violacea, Isurus oxryrhincus, Tetrapturus belone, T. albidus

- Your geographical areas : GSAs 9, 15, 16

- How many laboratories analyse calcified structures in your country? Two institutes (as concerns large pelagics)

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify): STOCK ASSESSMENT

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.) :

WHOLE OTOLITHS

SECTIONED OTOLITHS (INPLEX RESIN)

SECTIONED RAY (DORSAL OR ANAL FIN)

SECTIONED VERTEBRAE

GRINDED OTOLITHS MOUNTED ON SLIDES

- List of calcified structures that you use: otoliths (sagittae), fin rays and vertebrae; sagittae or lapillus for Coryphaena hippurus

- How are calcified structures stored before treatment?

Dry in multi-case boxes, deionised water for sagittae and lapillus from Coryphaena hippurus, envelope paper for spine

- How are calcified structure preparations stored? Mounted on slides or in vials; envelope paper for spines

- List of your saws (name, manufacturer, description): Buehler ISOMET 11-1180 LAW SPEED SAW with diamond blade.

Remet Micromet with Diamond disks Norton MDS100 (100x20x0.33x5 mm);

Buehler Isomet low speed saw with Diamond disks Norton MDS101 (100x12.7x0.33x5 mm)

- Description and characteristics of your resin(s) :

Three components INPLEX resin: resin, catalyst, accelerator;

Two component epoxy resin (Araldite AW106; Hardener HV953U)

Eukitt mounting medium acrylic resin

Thermoplastic Quartz Cement (Lakeside N°70C)

Entellan resin

- A document presenting the summary of the techniques used (reference) :

COMPEÁN-JIMÉNEZ, G., BARD. F.X. ,1983. Growth increments on dorsal spines of eastern Atlantic bluefin tuna (Thunnus thynnus (L.)) and their possible relation to migrations patterns. U.S. Dep. Commer., NOAA Tech. Rep. NMFS, 8: 77-86.

CORT J.L., 1990. Biología y pesca del atún rojo, Thunnus thynnus (L.), del mar Cantábrico (Tesis doctoral). Publicaciones Especiales. Instituto Español de Oceanografía, 4 : 272 pp.

STEVENSON, D.K., CAMPANA S.E. [ed]., 1992. Otolith microstructure examination and analysis. Can. Spec. Publ. Fish. Aquat. Sci. 117: 130 pp.

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year) : ---

2009 Malta, Malta Centre for Fisheries Sciences (MCFS), Coryphaena hippurus, 370

2010 Italy, Istituto Cooperativo di Ricerca (ICR mare), Coryphaena hippurus and Thunnus thynnus, 180

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year) : ---

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible) :

GARIBALDI F., PALANDRI G., ORSI RELINI L., 1999. The first mediterranean recapture, useful for growth studies, of tagged swordfish. ICCAT, Coll. Vol. Sci. Pap., 49 (1): 151-152.

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible) : -

GARIBALDI F., PALANDRI G., ORSI RELINI L., 1999. The first mediterranean recapture, useful for growth studies, of tagged swordfish. ICCAT, Coll. Vol. Sci. Pap., 49 (1): 151-152.

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible) : -

- Do you use image capture/analysis software, if so, which? LEICA Q WIN IMAGE PROCESSING & ANALYSIS SOFTWARE

Leica IM50 capture software

Image pro Plus vers. 4.5.0.19 analysis software

- Which type of camera do you use (digital or analogue, description, characteristics)? LEICA IC 80 HD CAMERA MOUNTED ON A STEREOMICROSCOPE LEICA MS5.

Leica DC200 digital colour camera, sensor size 10 mm x 8.7 mm resolution 1.3 M and 2.6 M

- Which applications is image processing used for (routine, only exchanges, validation studies…)? ROUTINE, EXCHANGES

- How many images are made per year on average? IMAGES ARE MADE FOR ALL SPECIMEN.

- Do you calibrate your images? YES

- Which measurements do you make on the image (distance between rings…) ? TOTAL RADIUS, DISTANCE BETWEEN RINGS, ETC.

Radius (nucleus to postrostrum), distance between rings, etc.

- What is the format of images? BMP, JPG

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

Procedure for Large pelagic fishes

The spiniform ray (1st dorsal spine for Thunnus thynnus and other Scombridae, 2nd anal ray for Xiphias gladius) or part of the caudal vertabrae, are removed from each fish (see Compeán-Jiménez e Bard, 1980). Two transversal sections at the point near the condyle base of the ray or a longitudinal section of vertebrae are obtained using a Buehler Isomet law speed saw with diamond blades; each section about 0.5-0.7 mm thick. The sections were mounted with Eukitt resin on glass slides and observed with a binocular lens microscope (Leica MS5) under transmitted and reflected light, connected by digital camera Leica IC 80 HD (Leica) to the image analyser Leica Q Win. Each specimen is photographed and saved as JPEG or BMAP images for image analysis (ring radius, diameter, etc.).

- Do you have an internal quality control (description, references) ?

We have a quality procedure that is written in Italian. This mainly based on:

CAMPANA, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish Biol. 59:197-242.

COMPEAN-JIMENEZ G., BARD F.X., 1980. Age and growth of east Atlantic bluefin tuna as determined by reading of fin rays cross section. ICCAT, Coll. Vol. Sci. Pap., 9 (2): 547-552.

COMPEÁN-JIMÉNEZ, G., BARD. F.X. ,1983. Growth increments on dorsal spines of eastern Atlantic bluefin tuna (Thunnus thynnus (L.)) and their possible relation to migrations patterns. U.S. Dep. Commer., NOAA Tech. Rep. NMFS, 8: 77-86.

CORT J.L., 1990. Biología y pesca del atún rojo, Thunnus thynnus (L.), del mar Cantábrico (Tesis doctoral). Publicaciones Especiales. Instituto Español de Oceanografía, 4: 272 pp.

RODRIGUEZ-MARIN E., RODRIGUEZ-CABELLO C., ORTIZ DE ZARATE V., CORT J.L., 2000. Comparison of three methods to estimate age composition of juvenile east Atlantic bluefin tuna (Thunnus thynnus). ICCAT, Coll. Vol. Sci. Pap., 52: 1215-1225.

RODRIGUEZ-MARIN E., OLAFSOTTIR D., VALEIRAS J., RUIZ M., CHOSSON-PAMPOULIE V, RODRIGUEZ-CABELLO C., 2006. Ageing comparison from vertebrae and spine of bluefin tuna (Thunnus thynnus) coming from the same specimen. ICCAT, Coll. Vol. Sci. Pap., 59 (3): 868-876.

- Do you have quality management certification e.g. ISO 9001:2008? NO
- Are you accredited (yes or no, name of accreditation authority)? NO
- What age calibration Exchanges or Workshops has your country participated in during the last three years? -
- Are all your age readers aware of WebGR? NO
- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? YES

- Your name: Cinzia Badalucco, Salvatore Gancitano, Vita Gancitano, Pietro Rizzo, Sergio Vitale, Alessandro Ligas, Alessandro Mannini, Pierluigi Carbonara, Pesci P., Locci I., Mulas A., Pendugiu A.A., Sabrina Colella, Donato Fortunata, La Mesa Mario, Panfili Monica, Loredana Casciaro; Simona Intini

- Your country: **Italy**

- Your institute:

CNR - National Research Council – IAMC, Mazara del Vallo

CNR - National Research Council – ISMAR, Ancona

University of Cagliari

University of Genoa

CIBM Livorno

COISPA Bari

- Number of treated calcified structures in 2010:

More than 15000 otoliths; 1000 illicia; 100 fin ray.

- List of your species (latin name) :

Aspitrigla cuculus Linnaeus, 1758; Balistes carolinensis Gmelin, 1789; Boops boops Linnaeus, 1758; Centrophorus granulosus Bloch and Schneider, 1801; Chelidonichthys lastoviza Bonnaterre, 1788; Chelidonichthys lucernus Linnaeus, 1758; Coelorinchus coelorhincus Risso, 1810; Diplodus annularis, Linnaeus 1758; Diplodus vulgaris, Geoffroy Saint-Hilaire 1817; Diplodus sargus; Eutrigla gurnardus Linnaus, 1758; Helicolenus dactylopterus dactylopterus Delaroche, 1809; Hoplostethus mediterraneus Cuvier in Cuv. and Val., 1829; Illex coindetii Verany, 1837; Lithognathus mormyrus Linnaeu, 1758; Loligo forbesii Steenstrup, 1856; Loligo vulgaris Lamarck, 1798; Lophius budegassa Spinola, 1807; Lophius piscatorius Linneus, 1758; Merluccius merluccius Linnaeus, 1758; Micromesistius poutassou Risso, 1826; Mullus barbatus Linnaeus, 1758; Mullus surmuletus Linnaeus, 1758; Mustelus mustelus Linnaeus, 1758; Pagellus erythrinus Linnaeus, 1758; Pegusa impar Bennett, 1831; Pegusa lascaris Risso, 1810; Peristedion cataphractum Linnaeus, 1758; Phycis blennoides Brünnich, 1768; Raja clavata Linnaeus, 1758; Sarpa salpa Linnaeus, 1758; Sciaena umbra Linnaeus, 1758; Scorpaena elongata Cadenat, 1943; Scorpaena notata Rafinesque, 1810; Scorpaena porcus Linneus, 1758; Solea solea; Spicara smaris; Scomber scombrus; Scomber japonicus; Squalus blainvillei, Risso 1827; Trachurus mediterraneus Steindacher, 1868; Trachurus trachurus Linnaeus, 1758

- Your geographical areas :

All Italian GFCM Geographical Subareas (GSAs): 9, 11, 10, 16, 19, 18, 17

- How many laboratories analyse calcified structures in your country?

About 10 institutes

- How is the age data used e.g. stock assessment?%, environmental studies ?%, other ?%, (please specify): Stock assessment 90%; environmental studies 10%

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

Whole Otoliths; sectioned otoliths mounted on microscope slides; spine section, illicia for Lophius spp.

- List of calcified structures that you use:

otoliths (sagittae), illicia for Lophius spp.

- How are calcified structures stored before treatment?

Dry in multi-case boxes, or in vials, envelope paper for spine

- How are calcified structure preparations stored?

Mounted on slides, vials or envelope paper for spine

- List of your saws (name, manufacturer, description) :

Remet Micromet with Diamond disks Norton MDS100 (100x20x0.33x5 mm);

Buehler Isomet low speed saw with Diamond disks Norton MDS101 (100x12.7x0.33x5 mm)

Struers dap-V polisher

Struers minitom with diamond blade Struers RS70324 diameter 127 mm thickness 0.4 mm

- Description and characteristics of your resin(s):

Three components INPLEX resin: resin, catalyst, accelerator;

Two component epoxy resin (Hardrock 554 + Hardener)

Thermoplastic Quartz Cement (Lakeside N°70C)

Entellan resin

Crystal Bond resin (Buehler)

Petropoxy 154 epoxide resin (Burnham petrographics)

Two component epoxy resin Buehler EPO-KWICK: resin and hardener.

Entellan Merck resin mounting microscope slides

- A document presenting the summary of the techniques used (reference) :

Rizzo P., Gancitano S., Badalucco C., Fiorentino F. (2005). Age estimation from "hard structures" of exploited marine organisms in the experience of CNR Centre of Mazara del Vallo: the procedures adopted and the maximum ages estimated. GCP/RER/010/ITA/MSM-09.

Carbonara P., Costantino G., Giovine G., Lembo G., Spedicato M.T. (2003) – Some aspects of the life history of Polyprion americanus (Schneider, 1801). Biol. Mar. Medit., 10 (2): 102-112.

IntiIni S., Gaudio P., L. Casciaro, I. Bitetto, M. Donnaloia, W. Zupa, Carbonara P. (2010) Preliminary observations on the growth of Engraulis encrasicolus (Linnaeus, 1758). Biol. Mar. Mediterr. 17 (1): 342-343

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year): ---

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year): ---

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

Vacchi M., Catalano B., Dalù M., Clò S., Mancusi C., Serena F. (2002). Il programma Raja Tag, campagna di marcatura della razza stellata (Raja asterias Delaroche, 1809). Final report.

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

M. La Mesa, F. De Rossi 2008. Early life history of the black anglerfish Lophius budegassa Spinola, 1807 in the Adriatic Sea using otolith microstructure. Fisheries Research, 93 (1-2): 234-239.

M. La Mesa, F. Donato, G. Giannetti, E. Arneri 2009. Age, growth and mortality of juvenile anchovy (Engraulis encrasicolus) in the Adriatic Sea. Fisheries Research, 96 (2-3): 275-280.

M. Panfili, F. Donato, E.B. Morello, E. Arneri GROWTH RATES OF EARLY LIFE STAGES OF ENGRAULIS ENCRASICOLUS AND SARDINA PILCHARDUS IN THE ADRIATIC SEA (ITALY) Rapp. Comm. int. Mer Médit., 39, 2010

M. La Mesa, G. Scarcella, F. Grati, G. Fabi 2010. Age and growth of the black scorpionfish, Scorpaena porcus (Pisces, Scorpaenidae) from artificial and natural reefs in the Adriatic Sea. Scientia Marina, 74 (4): 677-685.

G. Scarcella, M. La Mesa, F. Grati 2011. Age and growth of the small red scorpionfish, Scorpaena notata Rafinesque, 1810, based on whole and sectioned otolith readings. Environmental Biology of Fishes, 91(4): 369-378.

Sieli G., Badalucco C., Di Stefano G., Rizzo P., D'Anna G., Fiorentino F. (2011). Biology of red mullet, Mullus barbatus (L. 1758), in the Gulf of Castellammare (NW Sicily, Mediterranean Sea) subject to a trawling ban. J. Appl. Ichthyol., in press.

Boudaya1 L., Neifar1 L., Rizzo P., Badalucco C., Bouain A., Fiorentino F. (2008). Growth and reproduction of Chelidonichthys lucerna (Linnaeus) (Pisces: Triglidae) in the Gulf of Gabe` s, Tunisia. J. Appl. Ichthyol., 24:581–588.

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

Belcari P., Ligas A., Viva C. (2006) – Age determination and growth of juveniles of the European hake, Merluccius merluccius (L., 1758), in the northern Tyrrhenian Sea (NW Mediterranean). Fisheries Research, 78: 211-217.

Vitale S., Arkhipkin A., Cannizzaro L., Scalisi M. (2011). Life history traits of the striped seabream Lithognathus mormyrus (Pisces, Sparidae) from two coastal fishing grounds in the Strait of Sicily. J. Appl. Ichthyol, 27 :1086–1094.

- Do you use image capture/analysis software, if so, which?

Leica IM50 capture software

Image pro Plus vers. 4.5.0.19 analysis software

tpsDig 2.0; PixeLink and Infinity analysis softwares

Optimas 6.2

Image pro Plus vers. 7.0 Media Cybernetics, Inc.

BELL capture image software

ImageJ

- Which type of camera do you use (digital or analogue, description, characteristics)?

Leica DC200 digital color camera, sensor size 10 mm x 8.7 mm resolution 1.3 M and 2.6 M

Pixelink analysis software, Infinity lite.

2 LEICA digital cameras

Nikon Coolpix 950

BELL DV1300 CMOS Camera

- Which applications is image processing used for (routine, only exchanges, validation studies…)?

Routine, Exchanges, validation studies and formation a reference collection

- How many images are made per year on average? More than 1000--

- Do you calibrate your images?

Yes

- Which measurements do you make on the image (distance between rings…)?

Radius (nucleus to postrostrum), distance between rings, etc.

- What is the format of images?

Jpeg, Bmp, Tiff

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

Otoliths are the structures regularly chosen for age reading, with the exception of some species where other calcified structures i.e. illicium and dorsal spines are used.

Sampling: Otoliths are currently collected by haul according to a size-stratified sampling design.

Preparation: Otoliths are cleaned in running water, dried and stored in multi-case boxes, with the details of the fish specimen: the name of the species, the survey number, the number of the haul and the ordinal number of the specimen. Macro-increment analysis: Sagittae are read in whole for counting the macro-increments or the right sagitta is routinely embedded in Implex resin and sectioned; the section, which is about 0.5 mm thick, is glued onto a glass. Micro-increment analysis: Sagittae of juvenile are embedded in an Implex resin mould, glued with thermoplastic cement onto a glass slide, ground with a Remet apparatus and polished.

Age estimation: Readings were repeated at least three times with a reasonable time lapse, and compared later on. In some cases, two readers may analyse the same otoliths together, using a two-seat stereomicroscope, or with the assistance of an image analysis system. Routinely, the observations not matching are not included in the

age–length keys, but they are used for assessing precision between readers and consistency over time in age determination (Chang, 1982; Campana *et al*., 1994).

Data storage: All data are recorded in database for future computation.

Rizzo P., Gancitano S., Badalucco C., Fiorentino F. (2005). Age estimation from "hard structures" of exploited marine organisms in the experience of CNR Centre of Mazara del Vallo: the procedures adopted and the maximum ages estimated. GCP/RER/010/ITA/MSM-09.

Rizzo P., Gancitano S., Badalucco C., Enajjar S., Mancusi C., Mosteiro Cabañelas A., Saidi B., Sion L. (2006). Contribution to Guidelines for Age Determination of Chondrichthyes fish from the Mediterranean Sea (application to selected species). GCP/RER/010/ITA/MSM-08.

- Do you have an internal quality control (description, references) ?

Yes, we considered the comparison of independent age estimations from the surface of whole otoliths or transverse sections focusing between-readers bias and variability in the comparison among the estimated ages, using standard measurements of precision.

Campana S.E., Annand M.C., McMillan J.I. (1994). Graphical and statistical methods for determining the consistency of age determination. Trans. Am. Fish. Soc. 124:131-138.

Campana S.E. (2001). Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish Biol. 59:197-242.

Chang W.Y.B. (1982). A statistical method for evaluating the reproducibility of age determination. Can. J. Fish. Aquat. Sci. 39:1208-1210.

Morison, A.K., Burnett, J., McCurdy, W.J. & Moksness, E. (2005) Quality issues in the use of otoliths for fish age estimation. Marine and Freshwater Research, 56: 773-782.

Eltink, A.T.G.W., 2000. Age reading comparisons. (MS Excel workbook version 1.0 October 2000) Internet: http://www.efan.no

Report of Workshop on Age Reading of Red mullet Mullus barbatus and Striped mullet Mullus surmuletus (WKACM) 30 March - 3 April 2009 Boulogne sur Mer, France.

Report Workshop on Age estimation of European hake (WKAEH) 9-13 November 2009 Vigo, Spain ICES ADVISORY COMMITTEE ICES CM 2009/ACOM:42 REF.PGCCDBS

Karlou-Riga C. (2000) – Otolith morfology and age and growth of Trachurus mediterraneus (Steindachner) in the Eastern Mediterranean. Fish. Res., 32: 69-82.

Report of the Anglerfish Illicia/Otoliths Ageing Workshop IPIMAR Lisbon, Portugal 8-12 November 2004 ICES Planning Group on Commercial Catch, Discards and Biological Sampling

(PGCCDBS).

Gianetti G., Donato F. (2003) - Age Determination Manual. AdriaMed training course on fish age determination by otolith reading. GCP/RER/010/ITA/OP-08 (AdriaMed Occasional Papers n°8): 13 pp.

- FAO, 1974. Manual of fisheries science Part 2 – Methods of resource investigation and their application. FAO Fish. Tech. Pap., 115 Rev. 1.

FAO, 1992. Determination of growth in bony fishes from otolith microstructure. FAO Fish. Tech. Pap., 322.

Panfili, J., H. De Pontual, H. Troadec and P.J. Wright (Editors), 2002. Manual of Fish Sclerochronology. Brest, France: Ifremer-IRD coedition, 464 p.

- Do you have quality management certification e.g. ISO 9001 :2008? University of Cagliari (Combioma), CIBM and COISPA

- Are you accredited (yes or no, name of accreditation authority)?

University of Cagliari (Combioma); CIBM (Accredia, still in progress)

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

ICES Workshop on Age estimation of European hake (WKAEH) 9-13 November 2009 Vigo, Spain

ICES Workshop on Age reading of European anchovy (WKARA) 2009. Mazara del Vallo, Italy

ICES Workshop on Age Reading of Mullus barbatus and M. surmuletus (WKACM) 2009. Boulogne sur Mer, France

Workshop on AGE READING OF EUROPEAN ANCHOVY AND SARDINE ,24 -28 Jannuary 2011. (EuropeAid/127296/D/SER/TR)".

FAO- Adriamed Workshop on intecalibration of sardine otolith reading, 28-29 June 2011, Split, Croatia.

- Are all your age readers aware of WebGR?

No

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

Yes

- Your name: Sabrina Colella, Donato Fortunata, La Mesa Mario, Panfili Monica, Cinzia Badalucco, Salvatore Gancitano, Vita Gancitano, Pietro Rizzo, Sergio Vitale, Mario Petrillo, Pierluigi Carbonara, Loredana Casciaro, Simona Intini

- Your country: **Italy**

- Your institute:

CNR - National Research Council – ISMAR (Marine Science Institute), Ancona

CNR - National Research Council – IAMC (Institute for Coastal Marine Environment), Mazara del Vallo

University of Genoa, Department for the study of the territory and its resources

COISPA Stazione Sperimentale per lo Studio delle Risorse del Mare

- Number of treated calcified structures in 2010: more than 20000

- List of your species (latin name) :

Engraulis encrasicolus Linnaeus, 1758; Sardina pilchardus Walbaum, 1792

Your geographical areas :

GFCM Geographical Subareas (GSAs) 9, 16, 17, 18, 19

- How many laboratories analyse calcified structures in your country? 4 (as concerns small pelagics)

- How is the age data used e.g. stock assessment?%, environmental studies ?%, other ?%, (please specify):

Stock assessment 70%; environmental studies 30%.

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

Whole Otoliths

- List of calcified structures that you use:

otoliths (sagittae)

- How are calcified structures stored before treatment?

Dry in single box, or polyethylene tubes

- How are calcified structure preparations stored?

Mounted on slides

- List of your saws (name, manufacturer, description):

Remet Micromet with Diamond blades;

Buehler Isomet low speed saw with Diamond blades.

- Description and characteristics of your resin(s):

Three components INPLEX resin

Crystal Bond resin (Buehler)

Petropoxy 154 epoxide resin (Burnham petrographics)

- A document presenting the summary of the techniques used (reference):

AA.VV., 2009. Training course sulla determinazione dell'età di Engraulis encrasicolus e Sardina pilchardus ANCONA, 11-13 MAGGIO 2009. Istituto di Scienze Marine (CNR-ISMAR) & Società Italiana di Biologia Marina

Intini S., Gaudio P., L. Casciaro, I. Bitetto, M. Donnaloia, W. Zupa, Carbonara P. (2010) Preliminary observations on the growth of Engraulis encrasicolus (Linnaeus, 1758). Biol. Mar. Mediterr. 17 (1): 342-343

Giannetti, G. and Donato, F. AGE DETERMINATION MANUAL. AdriaMed Training Course on Fish Age Determination by Otolith Reading. Ancona, 13th – 24th May 2002. AdriaMed Occasional Papers 8: 13 pp.

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year) :

ICES Workshop on Age reading of European anchovy (WKARA) 2009. Mazara del Vallo, Italy

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year): ---

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

References

M. La Mesa, F. Donato, G. Giannetti, E. Arneri 2009. Age, growth and mortality of juvenile anchovy (Engraulis encrasicolus) in the Adriatic Sea. Fisheries Research, 96 (2-3): 275-280.

M. Panfili, F. Donato, E.B. Morello, E. Arneri GROWTH RATES OF EARLY LIFE STAGES OF ENGRAULIS ENCRASICOLUS AND SARDINA PILCHARDUS IN THE ADRIATIC SEA (ITALY) Rapp. Comm. int. Mer Médit., 39, 2010

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

- Do you use image capture/analysis software, if so, which?

Image pro Plus vers. 7.0 Media Cybernetics, Inc.

Leica Application Suite 2.5.0.R1

- Which type of camera do you use (digital or analogue, description, characteristics)?

2 LEICA digital cameras

Digital camera Leica DFC290

- Which applications is image processing used for (routine, only exchanges, validation studies…)? Routine

- How many images are made per year on average? 200

- Do you calibrate your images? Yes

- Which measurements do you make on the image (distance between rings…)?

Radius (nucleus to postrostrum), distance between rings, etc.

- What is the format of images?

Jpeg, Bmp, Tiff

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

Otoliths are the structures regularly chosen for age reading and growth studies.

Sampling: Otoliths are currently collected by landings of commercial fishing fleet and scientific cruises.

Preparation: Otoliths are cleaned in distilled water, dried and stored in boxes.

Macro-increment analysis: Sagittae are read in whole for counting the macro-increments; for some big speciemens (i.e. Merluccius merluccius) the right sagittae is embedded in resin and sectioned. The section, which is about 0.8 mm thick, sometimes is stained in of Neutral Red solution, to highlighted the alternating pattern of

translucent and opaque zones.

Micro-increment analysis: Sagittae of juvenile are embedded in Petropoxy resin onto a glass slide, ground with a Remet apparatus and polished.

Age estimation: Readings were repeated at least three times with a reasonable time lapse, and compared later on. In some cases, two readers may analyse the same otoliths together, using a two-seat stereomicroscope, or with the assistance of an image analysis system.

Data storage: All data are recorded using Office Package and processed by various statistical packages.

- Do you have an internal quality control (description, references) ?

NO

- Do you have quality management certification e.g. ISO 9001 :2008?

NO

- Are you accredited (yes or no, name of accreditation authority)?

NO

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

ICES Workshop on Age reading of European anchovy (WKARA) 2009. Mazara del Vallo, Italy

Workshop on AGE READING OF EUROPEAN ANCHOVY AND SARDINE ,24 - 28 Jannuary 2011. (EuropeAid/127296/D/SER/TR)".

FAO- Adriamed Workshop on intecalibration of sardine otolith reading, 28-29 June 2011, Split, Croatia.

- Are all your age readers aware of WebGR?

No

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

Yes

- Your name: Jari Raitaniemi

- Your country: **Finland**

- Your institute: **Finnish Game and Fisheries Research Institute (FGFRI (or RKTL in Finnish))**

- Number of treated calcified structures in 2010 : FGFRI 15 000–20 000, others several thousands.

- List of your species (latin name): Clupea harengus, Sprattus sprattus, Coregonus lavaretus, Coregonus albula, Sander lucioperca, Perca fluviatilis, Salmo salar, Salmo trutta, Salvelinus alpinus, Anguilla anguilla, Platichtys flesus, other less regular fish species. In addition grey seal (Halichoerus grypus) and ringed seal (Pusa hispida)

- Your geographical areas : northern Baltic Sea (Gulf of Finland, Gulf of Bothnia, Archipelago Sea), lakes and rivers in Finland.

- How many laboratories analyse calcified structures in your country? 8 laboratories in the FGFRI + 7 universities or other educational institutes, some private laboratories.

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify): In FGFRI 70–90% in stock assessments, rest in environmental and other studies.

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

- thin slices of otoliths stained with neutral red

- ground otoliths (to cross-section) stained with neutral red

- broken and burned (or burned and broken) otoliths

- whole otoliths mounted in clear resin (Canada balsam or boat lacquer)

- scale impressions

- bones such as opercular bone or cleithral bone examined dry or moistened with 1,2-propanediol

- List of calcified structures that you use:

Otoliths (mostly sagitta, but also lapillus with e.g. Rutilus rutilus or Abramis brama and asteriscus with e.g. Carassius carassius), scales, and bones (especially opercular bone and cleithral bone)

- How are calcified structures stored before treatment?

In black or transparent well plates (otoliths), paper bags (mostly scales or bones)

- How are calcified structure preparations stored?

Otolith slices on plates, which are kept in boxes with pigeonholes (a pigeonhole/each plate, 100 pigeonholes/box). Mounted otoliths (with clear resin) in clear well plates; scales, scale impressions, and bones in paper bags in dry places.

- List of your saws (name, manufacturer, description): One saw: Accutom 50, Struers. Used to saw 0,4 mm thick slices from hardened epoxy or polyester resin blocks, in which the otoliths are.

- Description and characteristics of your resin(s): Ampreg 21 epoxy resin.

- A document presenting the summary of the techniques used (reference):

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year) :

Bothnian Sea survey common with Sweden, Clupea harengus 800

Gulf of Finland survey common with Estonia, Clupea harengus 800

Platichtys flesus, Dana survey, Denmark, central Baltic Sea, 400.

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year): The same as in 16: half of the sampled specimens from the pelagic surveys in the Bothnian Sea (800) and Gulf of Finland (800) have been aged in Sweden and Estonia, respectively.

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

Tagging of Salmo salar (Carlin tags)

different marking methods have been used in stocking with several species in lakes and coastal areas: Salmo trutta, Coregonus lavaretus, Salvelinus alpinus, S. namaycush, Sander lucioperca

in a part of the studies, the fish can be identified individually, in others not (alizarine marking, colour marking of e.g. left side), but the year of stocking may be known

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

- tagging of Salmo salar (Carlin tags); SGSAD-report from the year 2006

The following specimens in the Finnish ageing guide (Raitaniemi, J., Nyberg, K. & Torvi, I. 2000. Age and growth determination of fish (In Finnish). FGFRI, pp. 232), hard structures of known-aged fish picked from marking and tagging studies:

lake Inari, Salmo trutta, Salvelinus alpinus, Salvelinus namaycush

Gulf of Finland, Coregonus lavaretus

lake Vuokalanjärvi, Coregonus lavaretus

lake Iso-Ruuhijärvi, Sander lucioperca

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible): --

- Do you use image capture/analysis software, if so, which?

Yes, Image Pro Plus 7.0, Cell.

- Which type of camera do you use (digital or analogue, description, characteristics)? Digital cameras: Qimaging Retiga R4000 monochrome, Olympus Color view soft imaging system.

- Which applications is image processing used for (routine, only exchanges, validation studies…) ?

Routine measurement of Atlantic salmon scales, otherwise mostly exchanges or description of otolith morphology, measuring of annual growth zones.

- How many images are made per year on average?

A few thousand images of Atlantic salmon scales, a small number of other hard structures of fish.

- Do you calibrate your images? Calibration is selected for all images of Atlantic salmon scales and calibrations are routinely monitored.

- Which measurements do you make on the image (distance between l rings…) ?

For scales both annual ring distances and inter circuli distances. For otoliths distances between annual rings.

- What is the format of images?

TIFF format, JPG format.

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

the basis is the ageing guidebook (Raitaniemi et al. 2000), and in addition there are some decisions or written procedures on e.g. sampling calcified structures or data storage in special projects

a general procedure for the storage of calcified structures

data storage in a data basis: Clupea harengus, Sprattus sprattus, coastal Sander lucioperca, coastal Perca fluviatilis, coastal Coregonus lavaretus

- Do you have an internal quality control (description, references) ?

Exchange of hard structures between readers, age readings of some samples with different methods as well, intention to have at least two specialists in age determination per species.

- Do you have quality management certification e.g. ISO 9001 :2008?

No

- Are you accredited (yes or no, name of accreditation authority)?

No

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

- herring, Bothnian Sea exchange with Sweden

- Are all your age readers aware of WebGR?

Probably not.

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

We have not done that so far, but the possibility is encouraging.

- Your name: Kostas Efthimiadis, Giorgos Gitarakos, Anna Argyri

- Your country: **Greece**

- Your institute: **Fisheries Research Institute, NAGREF**

- Number of treated calcified structures in 2010: None, because Greece did not do the National program in 2010. The usual number is 2350 otoliths and rays.

- List of your species (latin name): Sardina pilchardus, Encraulis encrasicholus, Boops boops, Trachurus trachurus, Trachurus mediterraneus, Merluccius merluccius, Mullus barbatus barbatus, Mullus surmuletus, Scomber colias, Sarda sarda, Micromesistius poutassou, Lophius budegassa, Mugil cephalus, Solea vulgaris, Xiphias gladius, Thunnus alalunga, Thunnus thynnus.

- Your geographical areas: GRC-G1 - 22401, 22402, 22403, 22404, 22405

- How many laboratories analyse calcified structures in your country? 3

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify): 100% stock assessment

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.): Whole otoliths on custom plastic slides, sectioned rays mounted in clear resin.

- List of calcified structures that you use: Otoliths, spines

- How are calcified structures stored before treatment? Otoliths are cleaned and dried and stored in eppendorf tubes. Spines are preserved dry in a paper envelope, which should be kept in a cool place (refrigerated). If the spine collected is too large to fit in the envelope, it can be cut in half or even in three pieces and kept in the envelope.

- How are calcified structure preparations stored? In eppendorf tubes and paper envelopes.

- List of your saws (name, manufacturer, description): Logitech ICS11 saw, Leica SP 1600 microtome saw

- Description and characteristics of your resin(s): Polyester T-80

- A document presenting the summary of the techniques used (reference): Spines are prepared by taking a cross-section through the basal portion of the spine (where the spine is approximately half the maximum width of the condyle base). These sections are mounted in resin (polyester T-80) and cut with a LEICA sp 1600 microtome saw to obtain a thin section of 0,6 mm. These thin sections can then be mounted on a slide in resin, and can be cleared with 95% ethanol for 5mins, if required. The sections can then be examined under the microscope.

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year): From Cyprus, 60 rays of Xiphias gladius, 60 rays of Thunnus alalunga, 30 rays of Thunnus thynnus

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year) : None

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

- Do you use image capture/analysis software, if so, which? Digital Sight Series by Nikon.

- Which type of camera do you use (digital or analogue, description, characteristics)? Digital, Nikon DS-Fi1, 5.24Mp

- Which applications is image processing used for (routine, only exchanges, validation studies…)? Routine, Validation studies

- How many images are made per year on average? 2000

- Do you calibrate your images? Yes

- Which measurements do you make on the image (distance between rings…)? Distance between centre of the otolith and each ring, total radius

- What is the format of images? Jpeg or/and bmp

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

- Do you have an internal quality control (description, references)? Yes

- Do you have quality management certification e.g. ISO 9001 :2008? No

- Are you accredited (yes or no, name of accreditation authority)?

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

- Are all your age readers aware of WebGR? No

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? No

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- Your name: Helen McCormick
- Your country: **Ireland**
- Your institute: **Marine Institute**
- Number of treated calcified structures in 2010: 54,175
- List of your species (latin name):

Clupea harengus

Gadus morhua

Lepidorhombus whiffiagonis

Lophius budegassa

Lophius piscatorious

Melanogrammus aeglefinus

Merlangius merlangus

Merluccius merluccius

Micromesistius poutassou

Pleuronectes platessa

Pollachius virens

Solea solea

Trachurus trachurus

Scomber scombrusSalmo salar Anguilla anguilla

- Your geographical areas: IV VIa VIb VIIa-k VIIIa
- How many laboratories analyse calcified structures in your country?

One

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify):

100% stock assessment,

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.) :

All sectioned otoliths/illicia are mounted on to 50*75 glass slides

Some are covered with Histokitt resin other are clarified using oil

Whole otoliths are mounted in custom black plastic trays either 10 well or 25 well depending on species.

Break and burn

- List of calcified structures that you use: Otolith, illicia
- How are calcified structures stored before treatment? 25 well plastic boxes, envelopes for illicia

- How are calcified structure preparations stored? All slides stored in metal filing slide drawers. All other trays stored in archive boxes.

- List of your saws (name, manufacturer, description): PILSES high speed sectioning saw

- Description and characteristics of your resin(s): resin is a saturated polyster resin-crystic R115 NTP ,the catalyst is X-8-K1 and the pigment is Oscaldo art powder-Black. Histokitt mounting medium for covering whole mounted otoliths and some slides

- A document presenting the summary of the techniques used (reference): SOPs are available for all processes, mounting whole, mounting for sectioning, sectioning, staining.

FSS - 05 VR 1.0 - Mounting otoliths and illicia.doc

FSS - 06 VR 1.0 - Pilses Sectioning Machine.doc

FSS - 07 VR 1.0 - Staining Cut Sections of Black Sole Otoliths.doc

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year): None

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year): None

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible): None

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible): None

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible): None

- Do you use image capture/analysis software, if so, which? Leica LAS Ez SOP: FSS - 11 VR 1.0 - Otolith Imaging.doc

- Which type of camera do you use (digital or analogue, description, characteristics)? Digital JVC camera Model TK-C148OBE

- Which applications is image processing used for (routine, only exchanges, validation studies…) ? Use the system for Image capturing only or exchanges

- How many images are made per year on average? 1000

- Do you calibrate your images? no

- Which measurements do you make on the image (distance between rings…) ? n/a

- What is the format of images? .PSP or .JPEG

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

FSS - 01 VR 1.0 - Demersal Age Sampling of Plaice, Megrim and Black Sole.doc

FSS - 03 VR 1.0 - Megrim, Plaice and Black Sole otolith extraction.doc

FSS - 09 VR 1.0 - Age Reading of Flat Fish.doc

FSS - 10 VR 1.0 - Training guidlines for Age Determination of Flat Fish Otoliths.doc

FSS - 13 Pelagic - desription of sampling procedure.doc

FSS - 14 Pelagic Sampling Protocols.doc

FSS - 31 Demersal length sampling.doc

FSS - 36 Extraction, cleaning and storing of otoliths.doc

FSS - 37 Anglerfish age sampling.doc

Ageing manuals for Cod Haddock Whiting, Blacksole, Plaice and Blue whiting.

- Do you have an internal quality control (description, references) ? Yes all species have two readers, quality control check are carried out on about 20% of all species. New readers have to reach specific targets in terms of % agreement before they become a primary reader. FSS - 12 VR 1.0 - Quality Control for Otolith Reading.doc

- Do you have quality management certification e.g. ISO 9001:2008? No

- Are you accredited (yes or no, name of accreditation authority)? No

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

WKARP (workshop for age reading plaice) in Inmujden the Netherlands from the 2nd till the 5th of November 2010.

Mackerel workshop, Lowestoft November 2010

WKAEH (Hake) in Vigo 9-13th Nov 2009

Blue Whiting Exchange

Salmon Scale reading Workshop MI Galway 2011

- Are all your age readers aware of WebGR? Not all but some are proficient in the use of Web GR

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? No unless participating in exchange or workshop.

- Your name: Mark Gatt
- Your country: **Malta**
- Your institute: **Agriculture and Fisheries Regulation Division**
- Number of treated calcified structures in 2010 : Still being processed
- List of your species (latin name) : Thunnus thynnus, Xiphias gladius, Coryphaena hippurus
- Your geographical areas : Mediterranean
- How many laboratories analyse calcified structures in your country? One
- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify): Eventually stock assessments
- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

Coryphaena hippurus otoliths are mounted in epoxy resin, cut and polished transversally.

Coryphaena hippurus scales are cleaned and mounted between slides before investigation under a binocular microscope.

Xiphias gladius spines are cleaned and sectioned by a low speed saw before investigation under a binocular microscope.

Thunnus thynnus spines are cleaned and sectioned by a low speed saw before investigation under a binocular microscope.

- List of calcified structures that you use: otoliths, scales and spines
- How are calcified structures stored before treatment?

Otoliths in distilled water and alcohol

Scales stored dry, Spines stored dry

- How are calcified structure preparations stored? Otolith preparations stored on slides, scales stored on slides, spine sections stored dry in paper envelopes.
- List of your saws (name, manufacturer, description): Buehler low speed saw.
- Description and characteristics of your resin(s):

Otoliths mounted in REMET inplex epoxy resin. Sections of otoliths mounted on slides by Crystal bond thermoplastic resin.

Small spines of mounted in REMET hardrock epoxy resin.

- A document presenting the summary of the techniques used (reference):

Otoliths: Manual for otolith removal and preparation for microstructural examination (Secor David H., Dean John M., Laban Elisabeth H.)

Otoliths: Coryphaena hippurus (Joan Moranta & Beatriz Morales-Nin IMEDEA, Esporles, Spain)

Spines: Protocol for sampling of hard parts forbluefin tuna (Thunnus thynnus) growth studies (Prepared by M. Ruiz, E. Rodríguez-Marín and J. Landa, Instituto Español de Oceanografía. Centro Oceanográfico de Santander. Apdo. 240, 39080 Santander, Spain)

REPORT OF THE 2006 ICCAT WORKSHOP FOR BLUEFIN TUNA DIRECT AGEING (Instituto Español de Oceanografía, Santander, Spain, 3-7 April 2006)

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year): not applicable

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year): not applicable

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible): no tagging studies

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible): no studies done at national level

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible): no studies done at national level

- Do you use image capture/analysis software, if so, which? Image capture software from Zeiss.

- Which type of camera do you use (digital or analogue, description, characteristics)? Digital Axiocam microscope camera from Zeiss for image capture

- Which applications is image processing used for (routine, only exchanges, validation studies…) ? No image processing except measurement of seasonal increments for marginal increment analysis.

- How many images are made per year on average? Not applicable.

- Do you calibrate your images? Not yet.

- Which measurements do you make on the image (distance between rings…) distance between seasonal marks (annulus)

- What is the format of images? Saved as jpg

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species : Refer to point 15.

- Do you have an internal quality control (description, references)? No.

- Do you have quality management certification e.g. ISO 9001 :2008? No.

- Are you accredited (yes or no, name of accreditation authority)? No.

- What age calibration Exchanges or Workshops has your country participated in during the last three years? No one.

- Are all your age readers aware of WebGR? Yes

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? Will eventually upload in the future.

- Your name: Tomaz Modic
- Your country: **Slovenia**
- Your institute: **Fisheries Research Institute of Slovenia (FRIS)**
- Number of treated calcified structures in 2010: 1426
- List of your species (latin name):

Sardina pilchardus, Engraulis encrasicolus;

- Your geographical areas: Northern Adriatic sea (GSA 17, FAO 37.2.1)
- How many laboratories analyse calcified structures in your country?

FRIS (only)

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify):

For stock assessment (100%), together with Italian and Croatian data in the Adriamed project;

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

Only whole otoliths in alcohol.

- List of calcified structures that you use:

Otoliths

- How are calcified structures stored before treatment?

Stored dry in small tubes.

- How are calcified structure preparations stored? /
- List of your saws (name, manufacturer, description) /
- Description and characteristics of your resin(s) /
- A document presenting the summary of the techniques used (reference) /
- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year) /
- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year) /
- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible) /
- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible) /
- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible) /
- Do you use image capture/analysis software, if so, which? /

- Which type of camera do you use (digital or analogue, description, characteristics) /

- Which applications is image processing used for (routine, only exchanges, validation studies…) /

- How many images are made per year on average? /

- Do you calibrate your images? /

- Which measurements do you make on the image (distance between rings…) /

- What is the format of images? /

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species:

Biological sampling of the landings accorning to regulation (ES 1639/2001 – appendix XV, sector H) for Slovenia covers only sardine (Sardina pilchardus) and anchovy (Engraulis encrasicolus) caugt by purse seines and pelagic trawlers both targeting strictly small pelagic fish. We are obligated to sample only 2 samples per year according to our yearly landings for the length and once for the age structure.

Every sample covers both species caught by purse seines and pelagic trawlers. Because of the ADRIAMED sampling agreement, we decided to sample both species once each month and that means 2 cases of both species for each fishing gear per month. If the fishing gear is not used or any of the species is not caught in the specific month, then we don't have the sample of that gear or species for that period.

Samples are picked randomly among the landed cases and bought directly from the fishermen.

Instead of measuring minimum of 50 specimens for the length and 25 for age per sample as prescribed in the regulation (ES 1639/2001 – appendix XV, sector H), for better results we measure cca 150-250 specimens per morphometry and cca 40-50 specimens for the age (depending on length classes).

The manual is added as the e-mail attachment!

- Do you have an internal quality control (description, references) no

- Do you have quality management certification e.g. ISO 9001 :2008? no

- Are you accredited (yes or no, name of accreditation authority)? no

- What age calibration Exchanges or Workshops has your country participated in during the last three years? ICES - Workshop on Age reading of European anchovy (WKARA) (Capo Granitola, Italy, November 2009)

- Are all your age readers aware of WebGR? no

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? We plan to photograph our otoliths in the future and have them in the database (.jpg) from 2010 on.

- Your name: Lotte Worsøe Clausen
- Your country: **Denmark**
- Your institute: **DTU Aqua**
- Number of treated calcified structures in 2010 :83,800 aged in 2010
- List of yourspecies (latin name):

Ammodytes marinus

Ammodytes tobianus

Anguilla anguilla

Clupea harengus

Gadus morhua

Glyptocephalus cynoglossus

Hyperoplus lanceolatus

Limanda limanda

Lophius piscatorius

Melanogrammus aeglefinus

Merlangius merlangus

Merluccius merluccius

Micromesistius poutassou

Microstomus kitt

Mullus surmuletus

Platichthys flesus

Pleuronectes platessa

Pollachius pollachius

Pollachius virens

Psetta maxima

Salmo salar

Scomber scombrus

Scophthalmus rhombus

Solea solea

Sprattus sprattus

Trachurus trachurus

Trisopterus esmarkii

- Your geographical areas: North Sea (including very northern and southern parts), Irish and Celtic Seas (boarfish), Skagerrak-Kattegat, Sub.Divs 22-24, and the Baltic

- How many laboratories analyse calcified structures in your country? Only DTU Aqua, but sections in Charlottenlund and Hirtshals

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify): 100% stock assessment and I would guess 80-80 % for scientific purposes (as parts of data material)

- Description of your types of calcified structure preparation (e.g., broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.): We use a range of preparation methods: a) whole otoliths, b) broken and polished, c) broken and burned, d) sectioned otoliths mounted in black resin, e) sectioned otoliths mounted in clear resin on glass microscope slides.

- List of calcified structures that you use: We only use sagittal otoliths for age determinations

- How are calcified structures stored before treatment? We have several storage methods: a) dry in trays, b) frozen in distilled water (individual 'ice cubes'), c) dry in paper envelopes

- How are calcified structure preparations stored? All glass slides are kept in designated boxes. Broken and mounted otoliths (the burned otoliths) are covered with glass slides and kept in boxes. Broken otoliths are kept in paper envelopes

- List of your saws (name, manufacturer, description):

Saw name: OTO-LABCUT 250B BENCH TOP DIAMOND SECTIONING MACHINE

Manufacturer: Benetec Limited, PO Box 472, Edgware, Middlesex HA8 7ZR, United Kingdom

Description: details see www.benetecmetlab.com/OTOLITH SECTIONING

- Description and characteristics of your resin(s):

Resins: PS Poly-Pol PS 230, PS Poly-Pol PS 6, PS Poly-Pol PS 28, PS

Pigment: Polyester Pigmentpasta

Solvent: PEROXAN ME-50 L

Data safety sheets appended.

- A document presenting the summary of the techniques used (reference): N/A regrettably!

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year):

a. Germany

b. Finland

c. Sweden

d. Belgium

e. Ireland

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year):

f. Germany

g. Finland

h. Sweden

i. Belgium

j. Ireland

The sampling are done for length and age of discards and landings, sampling will be carried out in accordance with the respective National Sampling Programme. Levels and coverage of sampling will be as agreed at the annual meetings of the RCM Baltic and RCM North Sea & Eastern Arctic.

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

CODYSSEY

Title: Cod spatial dynamics and vertical movements in European waters and implications for fishery management

Objectives: To study individual migratory behaviour, spatial distribution with particular focus on environmental preference thresholds and habitat utilisation.

Taggingspecifics: Adult cod > 45 cm in size were tagged with electronic data storage tags (DST's) off the coast of Nexø, Bornholm in the Eastern Baltic Sea. 500 individuals were tagged during the years 2003-2006, with a recapture rate of 45%.

Details: http://www.codyssey.co.uk/

References:

Andersen KH, Nielsen A, Thygesen UH, Hinrichsen HH, Neuenfeldt S (2007) Using the particle filter to geolocate Baltic cod with special emphasis on determining uncertainty. Can J Fish Aquat Sci 64:618–627

Neuenfeldt S, Hinrichsen HH, Nielsen A, Andersen KH (2007) Reconstructing migrations of individual cod (Gadus morhua L.) in the Baltic Sea by using electronic data storage tags. Fish Oceanogr 16:526–535

Hüssy K., Mosegaard H., Nielsen B., Worsøe Clausen L., 2009. Using data storage tags to link otolith macrostructure in Baltic cod (Gadus morhua L.) with environmental conditions. Mar. Ecol. Prog. Ser., 378: 161-170.

FEHMARN BELT

Objectives: To estimate the migration rates of juvenile Western Baltic cod and to validate age estimation based on traditional age readings

Taggingspecifics: Juvenile Western Baltic cod < 20 cm were tagged with conventional T-bar anchor tags and chemical marks introduced into their otoliths with SrCl2. 4500 individuals were tagged during autumn 2010, with limited recapture rates so far.

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

Species: Cod (Gadus morhua)

Geographical area: Eastern Baltic Sea

References:

Hüssy, K. 2010. Why is age determination in Baltic cod (Gadus morhua L.) so difficult? ICES J. mar. Sci. 67, 1198-1205.

Hüssy, K., Hinrichsen, H.H., Fey, D.P., Walther, Y., Velasco, A., 2010. The use of otolith microstructure to estimate age in adult Eastern Baltic cod (Gadus morhua L.). J. Fish. Biol. 76, 1640–1654.

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

Baron et al, 1998 on sprat and herring

Rehberg S, Hammer C, Hillgruber N, Hüssy K, Temming A. Validation of the first annulus in juvenile western Baltic cod (Gadus morhua L.) using otolith microstructure analysis (in prep. based on the Master's thesis of S. Rehberg).

- Do you use image capture/analysis software, if so, which? Yes; image analysis software applied are both the ImagePro version 6.3 and ImageJ. For image capture we apply both a set-up by Leica and by Image Pro

- Which type of camera do you use (digital or analogue, description, characteristics)? We use digital cameras (Leica DFS320 digi cam incl. Leica application suite; Q Icam incl. QImaging Fast 1394).

- Which applications is image processing used for (routine, only exchanges, validation studies…)?Routine for some species; routine exchange between national readers, validation studies and other scientific studies.

- How many images are made per year on average?8500 pictures on average pr. year

- Do you calibrate your images? All images are paired with a spatial calibration. No calibration on the illumination, but we aim at constant set-ups.

- Which measurements do you make on the image (distance between rings…)? Distances between rings (both annual and daily rings), otolith shape outline, otolith area and diameter and other specialised measurements (but not for routine)

- What is the format of images? Standard format is 2087X1548 pixels; jpeg file type

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species:

Manual for age determinations; holds a 1-2 page description for the majority of the species aged in the laboratory (12 species)

Specialised manuals for: Whiting, herring, sprat, boarfish, and cod related to age estimations.

Manual for estimating spawning stock identity in herring applying both otolith microstructure and otolith shape

- Do you have an internal quality control (description, references)? We have only the manuals to refer to; no QC scheme has been implemented as of yet.

- Do you have quality management certification e.g. ISO 9001 :2008? No

- Are you accredited (yes or no, name of accreditation authority)? No

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

Workshops: WKAEH (2009), WKARP (2010), WKARMAC (2010), WKARDAB (2010).

Exchanges: Mackerel (2009), Haddock (2009), Plaice (2009), Dab (2009), North Sea cod (2009), Blue Whiting (2009-2010), North Sea sole (2010-2011), North Sea cod (2010-2011), North Sea sprat (2011), Angler (2011).

- Are all your age readers aware of WebGR? Some of them, yes.

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? The intention is to implement WebGR as part of our routine calibration exercises between our readers.

- Your name: Sigbjørn Mehl
- Your country: **Norway**
- Your institute: **Institute of Marine Research**, Bergen
- Number of treated calcified structures in 2010:

75 000

- List of your species (latin name):

Reinhardtius hippoglossoides Walbaum, 1792

Molva dipterygia Pennant, 1784

Lophius piscatorius

Sprattus sprattus Linné, 1758

Brosme brosme Ascanius, 1772

Merlangius merlangus Linné, 1758

Melanogrammus aeglefinus Linné, 1758

Micromesistius poutassou Risso, 1826

Hippoglossoides platessoides Fabricius, 1780

Salmo salar Linné, 1758

Molva molva Linné, 1758

Mallotus villosus Müller, 1776

Scomber scombrus Linné, 1758

Boreogadus saida Lechepin, 1862

Pollachius virens Linné, 1758

Clupea harengus Linné, 1758

Coryphaenoides rupestris Gunner, 1765

Sebastes mentella

Trachurus trachurus Linné, 1758

Ammodytes spp

Gadus morhua Linné, 1758

Sebastes marinus

Argentina silus Ascanius, 1775

Trisopterus esmarkii Nilsson, 1855

Macrourus berglax Lacepède, 1801

Squalus acanthias Linné, 1758

Raja radiata, Donovan 1808

Amblyraja hyperborea

- Your geographical areas :

Barents Sea, Norwegian Sea, Norwegian Coast, North Sea

- How many laboratories analyse calcified structures in your country?

3 + some universities

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify): >90% stock assessment, other: escapement of reared cod, separation coastal cod – NEA cod

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

Whole otoliths, whole otoliths mounted in clear resin on black slides, whole otoliths photographed in water, broken otoliths, burning break, sectioned otoliths mounted on custom microscope slides, scales, whole vertebrae, stained section of vertebrae, whole dorsal fin spines, stained section of dorsal fin spines, whole caudal thorns, stained sections of caudal thorns, sectioned illicium mounted on black resin.

- List of calcified structures that you use:

Otholiths, scales, fin rays, illicium, caudal thorns, vertebrae, fin spines.

- How are calcified structures stored before treatment?

In water, in envelopes, frozen

- How are calcified structure preparations stored?

In envelopes, mounted in eukit on black slides

- List of your saws (name, manufacturer, description):

E300, Mikromekanikk Norway, MELSEC FX2N PLS servo system from Yaskawa Elektric.

IsoMet 5000, linear precision saw, Buehler.

IsoMet low speed saw, Buehler.

- Description and characteristics of your resin(s):

Eukit, epoxy

- A document presenting the summary of the techniques used (reference):

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year) :

Russia	Gadus morhua	200
	Melanogrammus aeglefinus	200
	Mallotus villosus	100
	Sebastes sp	50
Iceland	Sebastes sp	60

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year):

Russia	Gadus morhua	200	
	Melanogrammus aeglefinus	200	
	Mallotus villosus	100	
	Sebastes sp	50	
Iceland	Sebastes sp		60

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

Gadus morhua, Norwegian Coast; Godø, O.R. and Michalsen, K. 2000. Migratory behaviour of Northeast Arctic cod, studied by use of data storage tags. Fisheries Research, 48: 127-140.

Pollachius virens, Norwegian Coast and Norwegian Sea; Jakobsen, T. and Olsen, S. 1987. Variation in rates of migration of saithe from Norwegian Waters to Iceland and Faroe Islands. Fisheries Research. 5: 217-222.

Reinhardtius hippoglossoides, Barents Sea and Norwegian Coast;

Hippoglossus hippoglossus, Norwegian Coast;

Lophius piscatorius, Norwegian Coast, North Sea;

Clupea harengus, Norwegian Coast; Dragesund, O., Hamre, J. and Ulltang, Ø. 1980. Biology and population dynamics of the orwegian spring- spawning herring. Rapports et Procés Verbaux des Réunions du Conseil Permanent Internationale pour l'exploration de la Mer, 177, 1980:43-71.

Scomber scombrus, Norwegian Coast, North Sea; Iversen, S. A. 2002. Changes in the perception of the migration pattern of Northeast Atlantic mackerel during the last 100 years. – ICES Marine Science Symposia, 215: 382–390.

Mallotus villosus, Barents Sea;

Sprattus sprattus, Norwegian Coast;

Salmo salar, Norwegian Coast and Norwegian Sea; Holm, M., Holst, J.C. and Hansen, L.P. 1998. Spatial and temporal distribution of Atlantic salmon post-smolts in the Norwegian Sea and adjacent waters – origin of fish, age structure and relation to hydrographical conditions in the sea. ICES CM 1998/N:15, 8 pp (mimeo).

Scophthalmus maximus, Norwegian Coast; Bergstad, O.A. and Folkvord, A. 1997. Dispersal of tagged juvenile turbot Scophthalmus maximus on the Norwegian Skagerrak coast. Fisheries Research 29: 211-215.

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

Gadus morhua, Norwegian Coast; Smedstad, O.M. and Holm, J.C. 1996. Validation of back-calculation formulae for cod otholiths. Journal of Fish Biology 49:973-985.

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

- Do you use image capture/analysis software, if so, which?

Photoshop

- Which type of camera do you use (digital or analogue, description, characteristics)?

Nikon Digital camera DXM 1200F

- Which applications is image processing used for (routine, only exchanges, validation studies…) ?

Routine, exchange and validation

- How many images are made per year on average?

Reinhardtius hippoglossoides 2000

- Do you calibrate your images?

Yes

- Which measurements do you make on the image (distance between rings…) ?

Diameter for 1st and 2nd ring

- What is the format of images?

JP2 and PSD

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species:

Gjøsæter, H. 1999. Procedure for selection and preparation of age material of pelagic fish.

Gjøsæter, H. 1999. Procedure for age determination of Mallotus villosus.

Gjøsæter, H. 1999. Procedure for age determination of Clupea harengus.

Gjøsæter, H. 1999. Procedure for age determination of Boreogadus saida.

Gjøsæter, H. 2000. Procedure for age determination of Micromesistius poutassou.

Gjøsæter, H. 2000. Procedure for age determination of Scomber scombrus and Trachurus trachurus.

Mjanger, H., Nedreaas, K., Senneset, H. and Ågotnes, P. 2000. Procedure for age determination of Gadus morhua, Melanogrammus aeglefinus and Pollachius virens (in Norwegian).

- Do you have an internal quality control (description, references)?

Gjøsæter, H. and Nedreaas, K. 1999. Procedure for quality assurance of age determination of fish.

Høie. H. 2009. Procedure for quality assurance of age determination of Gadus morhua, Melanogrammus aeglefinus and Pollachius virens at the Institute of Marine Research (in Norwegian).

- Do you have quality management certification e.g. ISO 9001:2008?

No

- Are you accredited (yes or no, name of accreditation authority)?

No

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

Redfish (2008), North Sea cod (2008), Capelin (2009), Mackerel (2010), Greenland halibut (2011), Blue whiting (2011)

- Are all your age readers aware of WebGR? No

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? No

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- Your name: Willie McCurdy
- Your country: **UK Northern Ireland**
- Your institute: **Agri-Food & Biosciences Institute**
- Number of treated calcified structures in 2010: = 11,024

9,592 otoliths of marine species, 432 European eel* otoliths and ≥500 Salmon scale samples# were treated for ageing. Circa 500 herring otoliths are ground and polished each year for stock identification ('daily' ring counting and shape analysis). N.B. *280 yellow eels and 100 silver eels from Lough Neagh have been aged. Work continues on the remaining otoliths. #5-6 scales are aged from each salmon to determine the age, but each has been counted as 1 x CS treatment here.

- List of your species (latin name):

Main Species:

Clupea harengus Linnaeus, 1758

Gadus morhua Linné, 1758

Melanogrammus aeglefinus Linné, 1758

Merlangius merlangus Linné, 1758

Merluccius merluccius Linné, 1758

Sprattus sprattus Linné, 1758

Pleuronectes platessa (Linnaeus, 1758)

Anguilla anguilla Linné, 1758

Salmo salar Linné, 1758

Other Species:

Psetta maxima Linné 1758

Scophthalmus rhomus (Linnaeus, 1758)

Zeus faber Linné, 1758

Molva molva Linné, 1758

Conger conger Linné, 1758

Dicentrarchus labrax Linné, 1758

Pollachius pollachius Linné, 1758

Squalus acanthias Linné, 1758

Ammodytes tobianus (Linnaeus, 1758)

Trisopterus esmarki (Nilsson, 1855)

Trisopterus luscus (Linnaeus, 1758)

Trisopterus minutus (Linnaeus, 1758)

Lophius piscatorius (Linnaeus, 1758)

Solea solea (Linnaeus, 1758)

Lepidorhombus whifflagonis (Walbaum, 1792)

Limanda limanda (Linnaeus, 1758)

Eutrigla gurnardus (Linnaeus, 1758)

Chelidonichthys cuculus (Linnaeus, 1758)

Chelidonichthys lucerna (Linnaeus, 1758)

Trigloporus lastoviza (Bonnaterre, 1788)

Chelidonichthys obscurus (Walbaum, 1792)

Callionymus lyra (Linnaeus, 1758)

Agonus cataphractus (Linnaeus, 1758)

Arnoglossus laterna (Walbaum, 1792)

Salmo trutta Linné, 1758

- Your geographical areas:

Marine: ICES: VIIa (all) and VIa statistical rectangles 39 E1, 39 E2, 39 E3.

Eel, Salmon and Sea Trout: all Northern Ireland Water Framework Directive River basin Districts

- How many laboratories analyse calcified structures in your country?

One

- How is the age data used:

Stock assessment, ≅90%,

Environmental studies ≅9*%, - mostly salmon scales and eel otoliths

Other ≅1%, (e.g. research projects, specimen fish, specialist advice):

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

(a) Gadoid and other demersal otoliths: Otoliths are embedded in black polyester resin using OTOLIN system moulds and are sectioned using the OTOLIN cutting machine. The resin strips are mounted on glass microscope slides (76mm X 50mm 1.0mm) using clear casting polyester resin and glass microscope cover-slips (76mm X 50mm 0.5mm). The otoliths are viewed in transmitted and reflected light.

(b) Clupeoid otoliths: Mounted on pre-drilled black "perspex" (Plexiglas) slides (65mm X 60mm X 3mm), each slide with 25 cavities and glass microscope slide cover slips (54mm X 54mm X 0.5mm). 500 herring otoliths are also ground and polished each year.

(c) Other marine otoliths (including sprat and juvenile 'sprat-sized' herring): whole in water on a black background.

(d) European Eel otoliths: ground and polished.

(e) Salmonid scales: Using tweezers, individual scales are separated and viewed under the scale reader. Any damaged or replacement scales are discarded. When five or six suitable scales have been selected, they are dipped for 30-60 seconds in a 100ml Pyrex beaker containing some 0.1ml NaOH. The scales are rinsed and rubbed dry with tissue paper, removing any tissue or dirt at the same time. The scales are sand-

wiched between two microscope slides and view under a scale reader. The top slide can be pressed to flatten the scales if necessary. A Pasteur pipette can be used to squirt water over scales to prevent. This can aid scale reading and prevents scales drying out and cracking under the heat of the lamp.

- List of calcified structures that you use:

Otoliths

Scales

(2nd) Dorsal spines (for Spurdog)

(N.B. Bones are used for some freshwater fish species, but not for salmon, sea trout or European eel)

- How are calcified structures stored before treatment?

Gadoid and other large otoliths: Repli dishes - clear plastic boxes that are sub-divided into 25 compartments. The lids are taped on in the aerobic position with two strips of masking tape.

Herring otoliths: Custom made black Perspex trays with clear Perspex lids. Each tray had 100 cavities in 4 groups of 25.

Small marine otoliths: 'Nunc' Lidded Microwell dishes – Black dishes with transparent lids.

European eel otoliths: In Eppendorf vials

Scales, cleaned bones: Small paper envelopes

- How are calcified structure preparations stored?

Unaltered/unused calcified structure preparations are stored as in 11 above. Scales are returned to the original envelope after reading.

Ground and polished eel and herring otoliths are stored mounted on slides in slide boxes

Slides of demersal and clupeoid otoliths are stored in plastic tray inserts in 'Bisley' steel cabinets

Herring otoliths Gadoid otoliths

Otolith slide storage cabinets

- List of your saws (name, manufacturer, description) :

OTOLIN cutting machine http://www.benetecmetlab.com/

- Description and characteristics of your resin(s):

Polyester lay-up resin - bottom layer in the mould – also known as 'tacky resin'

Polyester gel-coat resin - top layer in the mould – also known as 'tack-free resin'

Polyester 'Flex' resin should also be added to the lay-up resin and gel-coat resin if available, as this reduces the risk of damage to otolith sections when using a high speed saw.

Clear casting polyester resin.

Methyl ethyl ketone peroxide (MEKP resin catalyst, a.k.a. hardener).

"Ryland's" Black colour paste for polyester resin.

We buy the resins locally, but the original supplier is MB Fibreglass. http://www.mbfg.co.uk/

- A document presenting the summary of the techniques used (reference) :

MARFISH10 The production of slide mounted pelagic fish otoliths, (sagittae) for fish ageing.

MARFISH16 Otolin System for the Embedding Sectioning and Slide Mounting of Demersal Fish Sagittae (Otoliths).

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year) :

None

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year) :

None

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible) :

None

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible): VIIa:

Previously otoliths were sampled from whiting discards and the otoliths from '0'-group fish helped to identify the period of translucent zone formation for this species. Weekly (Fisher) self-sampling of Nephrops and discards from several vessels, now takes place. The samples are analysed at AFBI FAEB. Otoliths collected weekly throughout the year, will be used to identify the periods of translucent zone formation in young fish for these species.

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible) :

None

- Do you use image capture/analysis software, if so, which?

Image J,

EOS Utility

- Which type of camera do you use (digital or analogue, description, characteristics)?

Digital still camera

Digital video camera

- Which applications is image processing used for (routine, only exchanges, validation studies…) ?

To help identify herring spawning stocks

Herring otolith shape analysis to help identify herring stocks

Eel otolith age reading

N.B. The same equipment was also used intensively for fish egg identification for samples from egg surveys.

- How many images are made per year on average?

Circa 500 herring and 300 eel otoliths per year. N.B. Several thousand additional demersal species otoliths, will be imaged each year from 2011/12 onwards – see answer to Q34.

- Do you calibrate your images?

Yes

- Which measurements do you make on the image (distance between rings…) ?

The distance between the growth centre and the 100th ring

Shape analysis

- What is the format of images?

TIFF

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

Marine Fisheries:

MARFISH02 Sampling at sea aboard RV Corystes: pelagic fish

MARFISH03 Sampling at sea aboard RV Corystes: demersal fish

MARFISH06 Sampling at sea aboard RV Corystes: Nephrops

MARFISH07 Sampling at sea aboard RV Corystes: Scallops

MARFISH08 Sampling Nephrops and discards from commercial vessels

MARFISH10 The production of slide mounted pelagic fish otoliths, (sagittae) for fish ageing

MARFISH11 Age determination of Irish Sea demersal fish

MARFISH12 Age determination of Scallops (Pecten maximus L.)

MARFISH13 Age determination of Irish Sea herring

MARFISH16 Otolin System for the Embedding Sectioning and Slide Mounting of Demersal Fish Sagittae (Otoliths).

MARFISH17 Sampling the N. Ireland landings of demersal fish

Freshwater Fisheries:

FWFISHN05 Procedure for Scale Reading: Salmonids.

FWFISHN06 Procedure For Freshwater Fish Processing

FWFISHN07 Procedure for Age Assessment of Coarse Fish by Scale Reading

FWFISHN08 Procedure for Ageing of Coarse Fish by Bone Reading

- Do you have an internal quality control (description, references) ?

No.

- Do you have quality management certification e.g. ISO 9001 :2008?

Yes. We have ISO 9001:2008.

- Are you accredited (yes or no, name of accreditation authority)?

No.

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

Pre-WK Eel otolith exchange

WKAREA

WKAREA2

Pre-WK plaice otolith exchange

WKARP

Pre-WK hake otolith exchange

WKAEH

Haddock otolith exchange

Saithe otolith exchange

Sole otolith exchange

N.B. We will take part in the Brill and Turbot exchanges

- Are all your age readers aware of WebGR?

Yes

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

Yes.

We will get a new Microscope and a PC* with a good graphics card, a 61cm screen, 1TB of internal storage and 1 Tb external back up storage to use with an existing digital 'stills' camera. We will store an image of every DCF otolith that is aged and we will select from these images for upload to WebGR and also for internal training. Regular back-ups of otolith images will also be made to DVDs

*An HP Z400 Workstation or similar is available via our IT Department:

Processor clock speed: 3060 MHz

Processor family: IntelÂ® XeonÂ® Dual-Core

Processor model: IntelÂ® XeonÂ® Quad-Core Processo

Processor socket: Socket 1366

Chipset: IntelÂ® X58 Express

We look forward to the recommendations of WKNARC to help make our final decisions on equipment.

- Your name : ANDRES URIARTE & IÑAKI QUINCOCES
- Your country: **SPAIN**
- Your institute: **AZTI - Tecnalia**
- Number of treated calcified structures in 2010: 9761

Anchovy		3460
Sardine	636	
Horse Mackerel	431	
Makerel		1551
Hake	2200	
Monkfish		900
Megrim	583	

- List of yourspecies (latin name):

Engraulisencrasicolus

SardinaPilchardus

Trachurustrachurus

Scomberscombrus

Merlucciusmerluccius

Lophiuspiscatorius and Lophiusbudegassa.

Lepidorhombuswiffiagonis

- Your geographical areas : Subarea VIII (bay of Biscay)and Subarea VII.
- How many laboratories analyse calcified structures in your country? 3

AZTI, IEO and ICM-CSIC

Foundation AZTI (Basque Country-VIIIc East and VIIIb-),

IEO-Santander (VIIIc East and VIIIb),

IEO-A Coruña (VIIIc West) and

IEO-Vigo (IXa North)

ICM Vigo, ICM Barcelona and Baleares

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify):

In Stock assessment (demography of catches and from surveys), in biological studies for Growth modelling and its relationship with environment.

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

For Anchovy, sardine and mackerel whole otoliths mounted in clear resin in black plastic slides (plaques containing 10 otoliths each)

For horse mackerel, they broken otoliths are read after burning and stored in individual envelops.

No preparation is required for megrim otolith.

For hake, otolith sections stained in a solution prepared with polyester, butanox and black tint.

For monkfish, illicium sections stained in a solution prepared with polyester, butanox and black tint.

- List of calcified structures that you use:

Illicium for monkfish and otoliths for the rest ofthe mentioned species.

- How are calcified structures stored before treatment?

For hake and monkfishcalcified structures are storedin0.5 mm transverselysectionedillicium

For the rest of mentioned species, medium size otoliths in envelops, small otoliths they are directly treated after a night for drying.

- How are calcified structure preparations stored?

Embebed in resin within the black plastic slides (containing 10 otoliths each) (Anchovy, sardine and mackerel), for horse mackerel they are stored in individual envelops. In the case of megrim, they are extracted from the fish and stored in a pipe of test.

For hake and monkfish calcified structures arestuck on reading slides.

- List of your saws (name, manufacturer, description) :

- Description and characteristics of your resin(s) :

Eukit

Cristic 446,Palv. 5Kg , HEGARDT S.L.

Butanox M50 y M60, HEGARDT S.L.

Concentrado negro, 1Kg, HEGARDT S.L.

- A document presenting the summary of the techniques used (reference) :

ICES. 2010. Report of the Workshop on Age reading of European anchovy (WKARA), 9-13 November 2009, Sicily, Italy. ICES CM 2009/ACOM:43. 122 pp.

ICES. 2011. Report of the Workshop on Age Reading of European Atlantic Sardine (WKARAS) , 14-18 February 2011, Lisbon, Portugal. ICES CM 2011/ACOM:42. 91 pp.

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year) :

Not applicable

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year) :

Not applicable

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible) :

Uriarte, A. & P. Lucio. 2001. Migration of adult mackerel along the Atlantic European shelf edge from a tagging experiment in the south of the Bay of Biscay in 1994. Fish. Res., 50 (1-2): 129-139.

Uriarte, A,, P. Alvarez, S. Iversen, J. Molloy, B. Villamor, M.M. Martins & S. Myklevoll. 2001. Spatial pattern of migration and recruitment of North East Atlantic Mackerel. ICES CM200110: 17.40 p.

Report of the Megrim Otolith Age Reading Workshop, 1997

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible) :

For the Anchovy in the Bay of Biscay: Uriarte 2002 (unplished): Descripción y validación de la metodología de determinación de la edad en la anchoa del golfo de Vizcaya mediante el examen de sus otolitos y estudio de su crecimiento anual. ManuscritoInterno AZTI.

See an english summary in Uriarte, A., C.Dueñas, E. Duhamel, P. Grellier, I. Rico1, B. Villamor. 2007: 2006 Anchovy Otolith Workshop. Working Document to the 2007 ICES Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS).

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible) :

- Do you use image capture/analysis software, if so, which? NO

Which type of camera do you use (digital or analogue, description, characteristics)?Not applicable

- Which applications is image processing used for (routine, only exchanges, validation studies…) ?Not applicable

- How many images are made per year on average?Not applicable

- Do you calibrate your images?Not applicable

- Which measurements do you make on the image (distance between rings…) ?Not applicable

- What is the format of images?Not applicable

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species:

Report of the Megrim Otolith Age Reading Workshop, 1997

- Do you have an internal quality control (description, references) ?

- Do you have quality management certification e.g. ISO 9001 :2008?

- Are you accredited (yes or no, name of accreditation authority)?

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

ICES. 2010. Report of the Workshop on Age reading of European anchovy (WKARA), 9-13 November 2009, Sicily, Italy. ICES CM 2009/ACOM:43. 122 pp.

ICES. 2011. Report of the Workshop on Age Reading of European Atlantic Sardine (WKARAS) , 14-18 February 2011, Lisbon, Portugal. ICES CM 2011/ACOM:42. 91 pp.

Otolith exchange workshopfor Megrim in December 2004 in Sukarrieta.

- Are all your age readers aware of WebGR?

Yes in the case of Megrim, in the case of hake only one of them.

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

YES we will intend to do so.

Your name : Annemie Zenner

- Your country: **Belgium**
- Your institute: **ILVO**
- Number of treated calcified structures in 2010: 23787
- List of your species (latin name):

Gadus morhua Linné, 1758

Melanogrammus aeglefinus Linné, 1758

Merlangius merlangus Linné, 1758

Solea solea Linné, 1758

Scophthalmus rhombus Linné, 1758

Psetta maxima, Linné 1758

Pleuronectes platessa Linné, 1758

Raja spp

- Your geographical areas:

North Sea IVabc

Skagerrak & Kattegat IIIa

Sound IIIb

Belt IIIc

Baltic Sea IIId

Irish Sea VIIa(bc)

English Channel VIIde

Celtic Sea VIIfgh(jk)

Bay of Biscay VIIIab

Adriatic Sea 37.2.1

- How many laboratories analyse calcified structures in your country?

1-2

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify):

All of our age readings are meant to be used in assessments if not at the present, then in the "near" future.

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.) :

whole otolith in a blackened dish filled with water

uncoloured otolith sections mounted on a 5.1cm glass microscope slide

coloured otolith sections (not mounted)

- List of calcified structures that you use: Otoliths and vertebrae
- How are calcified structures stored before treatment

Short term in the plastic plates they were collected in (see images below)

long term: dry in a paper otolith envelope.

- How are calcified structure preparations stored?

whole otoliths: dry in a paper otolith envelope

uncoloured slides pasted on a glass slide and wrapped in paper

coloured slides: in a plastic envelope

- List of your saws (name, manufacturer, description) :

OTO-LABCUT 230F, D.R. Bennett Limited (Middlesex, UK): a wet abrasive cutting machine with a 150mm diamond wafering blade for high speed cutting (3600rpm)

Brillant 250X automatic, ATM France Escil (Chassieu Cedex, France): a wet abrasive cutting machine with a 300mm diamond blade for high speed cutting (±3000rpm)

- Description and characteristics of your resin(s) :

Variopox universal epoxy resin base

Variopox universal epoxy resin harder

2 drops of black colouringpaste (Ijmocolor EP191)

- A document presenting the summary of the techniques used (reference) :

Species REFERENCE

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

HAD:(Melanogrammus aeglefinus) Bolle,L.J., Eltink,A.E., Schaap,L., De Vries,M., Bakker,C., Groot,P., Rink,G., Beintema,J., Groeneveld,K., Bol,R., Stoker,M., Jongejans,Y. & Rijs,S. Handboek leeftijdsbepalingen. CVO Rapport 03-010, 1-73. 2003. Netherlands Institiute for Fisheries Research, Ijmuiden.

WHG:(Merlangius merlangus) Easey,M., Henderson,G. & Shanks,A.M. Report of the Whiting (Merlangius merlangus, L) Otolith Exchange Scheme 2004 and Workshop 2005. CEFAS. 2005. Lowestoft, England.

PLE:(Pleuronectes platessa) ICES. 2010. Report of the Workshop on Age Reading of North Sea (IV) and Skagerrak-Kattegat (IIIa) Plaice (WKARP), 2-5 November 2010, Ijmuiden, The Netherlands.ICES CM 2010/ACOM: 45. 65 pp.

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

BLL:(Scophthalmus rhombus) Easey,M.W. & Millner,R.S. Improved methods for the preparation and staining of thin sections of fish otoliths for age determination. Cefas Lowestoft. 143, 0-12. 2008. Cefas Lowestoft. Science series, technical report.

SOL:(Solea solea) Easey,M.W. Age determination workshop for sole and plaice. 2002.ICES. Internal report.

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year) :

Denmark: Turbot (±450 otoliths per year) and Brill (±300 otoliths per year)

UK: Turbot (±40 otoliths)and Brill (±60 otoliths)

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year):

UK: Cod VIIa ranging between 250-1400 per year.

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

All North Sea:

Turbot & Sole: restocking experiment with tagging, turbot otoliths available but not used, use of these turbot otoliths for the 1st ring validation might not be possible due to artificial breeding environment, sole otoliths are likely to be unavailable. Last release of tagged TUR in April 2003. Last release of tagged SOL in June 2001.

Sole & Plaice: Migration (EU FAIR Programme, Project PL96-2079), final report July 2001

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible) :

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible) :

- Do you use image capture/analysis software, if so, which?

At the moment we only use capture software: AxoVision 3.1. Hopefully we will soon start using TNPC 5.0 (awaiting new microscope setup)

- Which type of camera do you use (digital or analogue, description, characteristics)?

Digital camera: Axiocam MRc (Zeiss) http://www.zeiss.com/industry/axiocam_mr_productinfo.pdf

- Which applications is image processing used for (routine, only exchanges, validation studies…) ?

Routine and exchanges.

- How many images are made per year on average?

25.000

- Do you calibrate your images?

Not yet.

- Which measurements do you make on the image (distance between rings…) ?

None

- What is the format of images?

JPG

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

Sampling of the otoliths is a procedure preceding the work in our lab. Our lab deals with the preparation, age estimation, data storage and sample storage. These are described in detail in the following documents:

Beproevingsprocedure OTL001 03 01042011 (general over all species)

Werkvoorschrift WV OTL 001 COD 01 15092010

Werkvoorschrift WV OTL 002 HAD 01 15092010

Werkvoorschrift WV OTL 003 WHG 01 15092010

Werkvoorschrift WV OTL 004 PLE 01 15092010

Werkvoorschrift WV OTL 005 TUR 01 15092010

Werkvoorschrift WV OTL 006 BLL 01 15092010

Werkvoorschrift WV OTL 007 SOL 01 15092010

- Do you have an internal quality control (description, references) ?

Yes.

Preparation: regular checks of the moulds, regular measurements of the section thickness, restricted use in time of the colouring mixture.

Age estimation: Every batch of max 40 otoliths, 1 otolith from our reference collection (agreed age over 2 readers) is also analysed. We have 1 reference collection of about 100 otoliths per species, the age distribution of the reference collections reflects the age distribution of the samples read over the last 4 years. In case the estimated age of the reference does not equal the agreed age, the whole batch needs to be redone. Otoliths of which a reader is uncertain about the age are read again by another reader. In case there is a difference, the otolith is discussed. Turbot and brill have 1 reference otolith per max. 20 samples.

The quality of the reference collection will be enhanced over the next few years (larger number of readers agreeing on the age or even validated otoliths).

Analyses are done with the Guus Eltinck spreadsheet.

Development of database with a quality control system is in progress.

- Do you have quality management certification e.g. ISO 9001 :2008?

ISO17025

- Are you accredited (yes or no, name of accreditation authority)?

Yes, BELAC

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

North Sea cod: WKARNSC 2008 and 2009-2010 exchange

Plaice exchange 2009 and WKARP 2010

Dab exchange 2009

North Sea sole exchange 2010

Sole VIIa exchange 2009

Turbot & Brill: WKART 2008

- Are all your age readers aware of WebGR?

Yes.

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

Yes, we intend to.

- Your name: Charis Charilaou

- Your country: **Cyprus**

- Your institute: **Department of Fisheries and Marine Research (DFMR)**

- Number of treated calcified structures in 2010: 1512

- List of your species (latin name): Boops boops, Mullus barbatus, M. surmuletus, Pagellus erythrinus, Spicara smaris

- Your geographical areas: Mediterranean-GFCM division 37.3.2- GSA25

- How many laboratories analyse calcified structures in your country? 1 (For the period 2005-2010 the structures were analysed by a private laboratory; from 2011 the structures will be analysed by the DFMR)

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify): 100% for stock assessment.

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.) : whole, burning whole.

- List of calcified structures that you use: otoliths

- How are calcified structures stored before treatment? In eppendorf tubes.

- How are calcified structure preparations stored? In eppendor tubes

- List of your saws (name, manufacturer, description): N/A

- Description and characteristics of your resin(s): N/A

- A document presenting the summary of the techniques used (reference) : not available

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year): N/A

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year): N/A

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible): none

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible): none

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible) : none

The DFMR is still in the process of organising the lab for ageing, therefore some of the questions in this section are not applicable for the moment.

- Do you use image capture/analysis software, if so, which? Yes.

- Which type of camera do you use (digital or analogue, description, characteristics)? Digital camera

- Which applications is image processing used for (routine, only exchanges, validation studies…) ? Image processing will be used as routine.

- How many images are made per year on average? It is intended that at least one image will be made for each otolith to be used for ageing (~ 1500).

- Do you calibrate your images? We will calibrate the images.

- Which measurements do you make on the image (distance between rings…) ? Measurements on the axis from the nucleus to the posterior area and on the radius of each ring.

- What is the format of images?

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

For all species that are aged:

- Collection of both otoliths from the age sample. Clean and store dry in eppendorf tubes.

- Use whole (burnt whole for P. erythrinus).

- Place otoliths under the stereomicroscope immersed in water, against a black background, and use reflected light.

- Create digital images for both otoliths (on the concave side) and use the left otolith for age estimation.

- Using the image analysis program perform a blind first reading (only information on the date of capture).

- Measuring axis:

For Mullus use the one joining the sulcus and the nucleus of the otolith, and perform measurements on the axis from nucleus to the posterior area.

For B. boops, P. erythrinus and S. smaris use the diameter and perform measurements on the axis from nucleus to the posterior area.

- Consider 1st of January as the date of birth; in the case of a translucent ring at the edge, consider it as ring if the date of capture is in the first six-months of the year.

- Perform a second reading, considering information from the sample, biological information, results of the first reading, back-calculation and the growth increment between consecutive rings (which should be decreasing).

- Store age estimates in image analysis programme and database.

Note: For the age estimation, the guidelines agreed during the Workshop on age reading of Mullus (WKACM, 2009) are followed.

- Do you have an internal quality control (description, references) ? At the moment no.

- Do you have quality management certification e.g. ISO 9001:2008? No

- Are you accredited (yes or no, name of accreditation authority)? No

- What age calibration Exchanges or Workshops has your country participated in during the last three years? Exchange exercise on Mullus (2007), followed by the workshop on Mullus age reading (WKACM, 2009)

- Are all your age readers aware of WebGR? Yes

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? If requested yes

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- Your name: Kélig MAHE
- Your country: **France**
- Your institute: **IFREMER**
- Number of treated calcified structures in 2010: 34501
- List of your species (latin name):

Aspitrigla cuculus

Clupea harengus harengus

Coryphaenoides rupestris

Dicentrarchus labrax

Engraulis encrasicolus

Gadus morhua

Lepidorhombus whiffiagonis

Lophius budegassa

Lophius piscatorius

Melanogrammus aeglefinus

Merlangius merlangus

Merluccius merluccius

Microstomus kitt

Molva dypterygia

Molva molva

Mullus barbatus

Mullus surmuletus

Pleuronectes platessa

Pollachius virens

Psetta maxima

Sardina pilchardus

Scophthalmus rhombus

Solea solea

Sparus aurata

Sprattus sprattus

Trigla lucerna

Trisopterus esmarkii

- Your geographical areas:

North sea (IVb, c)

Eastern English Channel (VIId)

Western English Channel (VIIe)

Celtic Sea (VIIg, j, h)

VIa

Bay of Biscay (VIIIa, VIIIb)

Golf of Lion (37.1.2)

- How many laboratories analyse calcified structures in your country?

1

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify):

80% stock assessment, 10% environmental studies, 10% stock identification

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

whole otoliths in water

burnt whole otoliths in water

sectioned otoliths mounted on custom microscope slides

whole scale in water

sectioned illicium mounted on custom microscope slides

- List of calcified structures that you use:

Otoliths, scales, illicium.

- How are calcified structures stored before treatment?

Stored dry.

- How are calcified structure preparations stored?

In paper envelopes and in eppendorfs.

- List of your saws (name, manufacturer, description) :

Brillant 250 automatic, ATM France Escil

Brillant 221 automatic, ATM France Escil

- Description and characteristics of your resin(s) :

Crystic R115, polyester resin, Scott Bader society

- A document presenting the summary of the techniques used (reference) :

Mahé, K., Bellail, R., Dufour, J.L., Boiron-Leroy, A., Diméet, J., Duhamel, E., Elleboode, R., Félix, J., Grellier, P., Huet, J., Labastie, J., Le Roy, D., Lizaud, O., Manten, M.L., Martin, S., Metral, L., Nédelec, D., Vérin, Y., Badts, V., 2009. Synthèse française des procédures d'estimation d'âge / French summary of age estimation procedures. http://archimer.ifremer.fr/doc/00000/7294/

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year):

Netherlands, Mullus surmuletus, 250

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year):

None

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

Merluccius merluccius, Bay of Biscay

de Pontual, H., Groison, A.L., Piñeiro, C., and Bertignac, M., 2006. Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. – ICES Journal of Marine Science, 63 (9): 1674-1681.

Merluccius merluccius, Gulf of Lion

Mellon-Duval, C., de Pontual, H., Métral, L., and Quemener, L. 2010. Growth of European hake (Merluccius merluccius) in the Gulf of Lions based on conventional tagging. – ICES Journal of Marine Science, 67: 62–70.

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible) :

Merluccius merluccius, Gulf of Lion

Solea solea, Eastern English Channel

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible) :

Mullus surmuletus, Eastern English Channel (VIId),

The periodicity of the appearance of growth marks on the otoliths has been checked by calculating the growth between the last and the next to last stria or the marginal increment (M.I.).

Mahé K., Destombe A., Coppin F., Koubbi P., Vaz S., Le Roy D., Carpentier A. (2005). Le rouget barbet de roche Mullus surmuletus (L. 1758) en Manche orientale et mer du Nord. http://archimer.ifremer.fr/doc/00000/2351/

- Do you use image capture/analysis software, if so, which?

TNPC software (http://www.tnpc.fr/en/tnpc.html) : Digital Processing for Calcified Structures .

- Which type of camera do you use (digital or analogue, description, characteristics)?

Color CCD - Digital Camera, 1280x960, Sony society

- Which applications is image processing used for (routine, only exchanges, validation studies…)?

Routine

- How many images are made per year on average?

40 000

- Do you calibrate your images?

Yes

- Which measurements do you make on the image (distance between rings…)?

Distance (mm) between rings, nucleus and edge

- What is the format of images?

All formats (im6, tiff, jpeg…)

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

The preparation, age estimation, data storage and sample storage are described in detail in the following document:

Mahé, K., Bellail, R., Dufour, J.L., Boiron-Leroy, A., Diméet, J., Duhamel, E., Elleboode, R., Félix, J., Grellier, P., Huet, J., Labastie, J., Le Roy, D., Lizaud, O., Manten, M.L., Martin, S., Metral, L., Nédelec, D., Vérin, Y., Badts, V., 2009. French summary of age estimation procedures. http://archimer.ifremer.fr/doc/00000/7294/

- Do you have an internal quality control (description, references) ?

Yes

- Do you have quality management certification e.g. ISO 9001?

No

- Are you accredited (yes or no, name of accreditation authority)?

No

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

Workshop on Age Reading of Turbot (WKART)

Workshop on Age Reading of North Sea Cod (WKARNSC)

Workshop on Age estimation of European hake (WKAEH)

Workshop on Age Reading of Dab (WKARDAB)

Workshop on the Age Reading of Mackerel (WKARMAC)

Workshop on Age reading of European anchovy (WKARA)

Workshop on Age Reading of North Sea (IV) and Skagerrak-Kattegat (IIIa) Plaice (WKARP)

Sole (Solea solea) in the Eastern Channel Otolith Exchange 2008

Tusk-otolith exchange

Atlantic mackerel (Scomber scombrus) exchange

- Are all your age readers aware of WebGR?

Yes

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

Yes

- Your name: Susanne Hvass
- Your country: **Greenland**
- Your institute : **Pinngortitaleriffik, Greenland Institute of Natural Resources**
- Number of treated calcified structures in 2010: 5409
- List of your species (latin name) :Gadus morhua
- Your geographical areas: West and east Greenland
- How many laboratories analyse calcified structures in your country? We have 1
- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify): Primarily stock assessment, environmental studies.
- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.) : Broken otoliths
- List of calcified structures that you use: Otoliths
- How are calcified structures stored before treatment? Dry
- How are calcified structure preparations stored? Dry
- List of your saws (name, manufacturer, description): /
- Description and characteristics of your resin(s): /
- A document presenting the summary of the techniques used (reference): None
- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year): None
- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year): None
- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible): Tagging has been done, but growth/age not investigated.
- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible): None
- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible): None
- Do you use image capture/analysis software, if so, which? Software does exist, it is Leica App. Suite, but we do not really use it yet.
- Which type of camera do you use (digital or analogue, description, characteristics)? Leica DFC 280.
- Which applications is image processing used for (routine, only exchanges, validation studies…) ? Were only using for internal purposes – still.
- How many images are made per year on average? /

- Do you calibrate your images? /
- Which measurements do you make on the image (distance between rings…) ? /
- What is the format of images? Tif and jpg
- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species: None, but in progress.
- Do you have an internal quality control (description, references) ? No.
- Do you have quality management certification e.g. ISO 9001 :2008?
- Are you accredited (yes or no, name of accreditation authority)?
- What age calibration Exchanges or Workshops has your country participated in during the last three years? Icelandic/German
- Are all your age readers aware of WebGR? Yes
- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? Not yet.

- Your name: Gróa Thora Pétursdóttir
- Your country: **Iceland**
- Your institute: **The Marine Research Institute (MRI)**
- Number of treated calcified structures in 2010: The number of treated and age-read otoliths and scales in the year 2010 are one hundred and six thousand fishes (106000).
- List of your species (latin name):

Gadus morhua

Melanogrammus aeglefinus

Pollachius virens

Micromesistius poutassou

Clupea harengus

Scomber scombrus

Mallotus villosus

Argentina silus

Sebastes marinus

Sebastes mentella

Anarhichas lupus

Anarhichas minor

Pleuronectes platessa

Hippoglossoides platessoides

Microstomus kitt

Limanda limanda

Glyptocephalus cynoglossus

Brosme brosme

Molva molva

- Your geographical areas: Va
- How many laboratories analyse calcified structures in your country? 1 lab – (two branch laboratories)
- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify):

100% stock assessment – as well studies on growth and maturity of cod ? %.

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):
- List of calcified structures that you use: Otoliths, scales, illicium.

- How are calcified structures stored before treatment?

Stored dry.

- How are calcified structure preparations stored? In paper envelopes and in plastic trays.

- List of your saws (name, manufacturer, description): Iðnvélar ehf. http://www.idnvelar.is/

- Description and characteristics of your resin(s) : Resin CrysticC (SBCRYSTICC).

- A document presenting the summary of the techniques used (reference):

Yes

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year):

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year):

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

- Do you use image capture/analysis software, if so, which? Visilog TNPC : Digital Processing for Calcified Structures .

- Which type of camera do you use (digital or analogue, description, characteristics)? CCD - Digital Camera

- Which applications is image processing used for (routine, only exchanges, validation studies…)? Only exchanges and validation studies.

- How many images are made per year on average? 1 - 5 thousand

- Do you calibrate your images? Yes

- Which measurements do you make on the image (distance between rings…)? Millimetre (mm)

- What is the format of images? *.Im6 and *.tif

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species:

- Do you have an internal quality control (description, references) ? Yes we have an internal quality control – 1-2 times pr. year and use the ("AGE COMPARISONS.XLS") GUIDELINES AND TOOLS FOR AGE READING COMPARISONS by A.T.G.W. Eltink.

- Do you have quality management certification e.g. ISO 9001 :2008? We have quality management in Lotus Notes- Focal Quality handbook.

- Are you accredited (yes or no, name of accreditation authority)? No

- What age calibration Exchanges or Workshops has your country participated in during the last three years? Tusk-otolith exchange (last year) workshop in Iceland on cod otoliths (Greenland, Germany and Iceland) workshop last year in UK on Atlantic mackerel (Scomber scombrus), workshop on Mallotus villosus in Bergen, Norway and Herring otoliths exchange.

- Are all your age readers aware of WebGR? No

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? No

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Otolith section procedure

Otoliths were prepared for sectioning by embedding them in black polyester resin. The resin formula consisted of: 550 ml CrysticC moulding resin (#SBCRYSTICC), 12.5 ml consolidator/hardener, and black pigment past colour (#SBPP0630BK). A metal frame, especially made for embedding otoliths was used. It has a scale from 1 – 20, and in the box is a layer of black polyester resin. A total of 100 otoliths can be placed on the layer of resin in the metal frame so that there are five otoliths in each row. After arranging the first row of otoliths, the gear is automatically moved 1 cm to begin the next row.

After the otoliths have been placed they are covered with the black polyester resin and stored in a fume hood for 12 hours for the resin to harden. The otoliths are then ready for sectioning.

Sections were obtained by cutting the block with a diamond grit blade into 20 sections. To ensure precision this process is automatically steered with software.

The 20 otolith sections were organised on microscope glass slides in groups of 5 slices, with 5 otoliths on each slide. One block, a sample of 100 otoliths, can be put on 4 microscope slides. Clear resin is pored over the slices, then a thin piece of glass is put on top and the slides stored in a fume hood overnight. Finally, after cleaning all excess glue from the slide, the sectioned otoliths are ready for analysis.

- Your name: ALESSANDRO LIGAS

- Your country: **ITALY**

- Your institute: **CENTRO INTERUNIVERSITARIO DI BIOLOGIA MARINA ED ECOLOGIA APPLICATA "G. BACCI", LIVORNO**

- Number of treated calcified structures in 2010: ABOUT 2000 OTOLITHS

- List of your species (latin name):

Merluccius merluccius, Mullus barbatus, M. surmuletus, Boops boops, Trachurus mediterraneus, T. trachurus, Solea solea, Chelidonicthys lucerna, Eutrigla gurnardus, Sardina pilchardus, Engraulis encrasicolus, Pagellus erythrinus, Lophius budegassa, L. piscatorius, Micromesistius poutassou, Spicara smaris (the species included in Group1 and Group2 of the European Data Collection Framework).

- Your geographical areas: GAS9

- How many laboratories analyse calcified structures in your country? ABOUT 10 INSTITUTES

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify):

STOCK ASSESSMENT

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

WHOLE OTOLITHS

SECTIONED OTOLITHS (INPLEX RESIN)

GRINDED OTOLITHS MOUNTED ON SLIDES [Two component epoxy resin (Araldite AW106; Hardener HV953U)]

- List of calcified structures that you use: otoliths (sagittae)

- How are calcified structures stored before treatment? Dry in vials

- How are calcified structure preparations stored?

Mounted on slides, or in vials

- List of your saws (name, manufacturer, description):

Diamond disks Remet MDS100 (100x20x0.33x5 mm)

- Description and characteristics of your resin(s):

Three components INPLEX resin: resin, catalyst, accelerator;

Two component epoxy resin (Araldite AW106; Hardener HV953U)

- A document presenting the summary of the techniques used (reference):

SEE CIBM_GSA9

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year): ---

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year) : ---

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

Vacchi M., Catalano B., Dalù M., Clò S., Mancusi C., Serena F. (2002). Il programma Raja Tag, campagna di marcatura della razza stellata (Raja asterias Delaroche, 1809). Final report.

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

Belcari P., Ligas A., Viva C. (2006) – Age determination and growth of juveniles of the European hake, Merluccius merluccius (L., 1758), in the northern Tyrrhenian Sea (NW Mediterranean). Fisheries Research, 78: 211-217. (see Attachment2 CIBM_GSA9)

- Do you use image capture/analysis software, if so, which? OPTIMAS 6.2

- Which type of camera do you use (digital or analogue, description, characteristics)? CCD Video-camera Sony XC – 77BB/77BBCE

- Which applications is image processing used for (routine, only exchanges, validation studies…) ? ONLY EXCHANGES

- How many images are made per year on average? ---

- Do you calibrate your images? NO

- Which measurements do you make on the image (distance between rings…) ? TOTAL RADIUS, DISTANCE BETWEEN RINGS, ETC.

- What is the format of images? BMP, JPG

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species:

SEE CIBM_GSA9

- Do you have an internal quality control (description, references) ?

We have a quality procedure that is written in Italian. This mainly based on:

FAO, 1974. Manual of fisheries science Part 2 – Methods of resource investigation and their application. FAO Fish. Tech. Pap., 115 Rev. 1.

FAO, 1992. Determination of growth in bony fishes from otolith microstructure. FAO Fish. Tech. Pap., 322.

Panfili, J., H. De Pontual, H. Troadec and P.J. Wright (Editors), 2002. Manual of Fish Sclerochronology. Brest, France: Ifremer-IRD coedition, 464 p.

- Do you have quality management certification e.g. ISO 9001:2008? YES

- Are you accredited (yes or no, name of accreditation authority)? NOT YET; WE ARE INVOLVED IN THE ACCREDITATION PROCEDURE WITH ACCREDIA

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

ICES WKAEH 2009, Vigo, Spain

ICES Workshop on Age reading of European anchovy (WKARA) 2009. Mazara del Vallo, Italy

ICES Workshop on Age Reading of Mullus barbatus and M. surmuletus (WKACM) 2009. Boulogne sur Mer, France

- Are all your age readers aware of WebGR?

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

- Your name: Georgs Kornilovs
- Your country: **Latvia**
- Your institute:**Institute of Food Safety, Animal Health and Environment "BIOR"**
- Number of treated calcified structures in 2010: 15461
- List of your species (latin name):

Clupea harengus membras

Sprattus sprattus balticus

Gadus morhua

Platichtys flesus

Psetta maxima

Salmo salar

Salmo trutta

Perca fluviatilis

Stizostedion lucioperca

- Your geographical areas: the Baltic Sea - ICES III b-d,
- How many laboratories analyse calcified structures in your country?- One
- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify): Stock assessment - 87.6%, the rest 12.4% is collected in the frames of National data collection programme and used for national fisheries management purposes.
- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

i) broken and burn otoliths;

ii) whole otoliths observed on custom microscope slides;

iii) scales observed on custom microscope slides;

iv) gill covers observed on custom microscope slides.

- List of calcified structures that you use:

otoliths, scales, gill covers.

- How are calcified structures stored before treatment? In paper booklets
- How are calcified structure preparations stored? In individual paper booklets
- List of your saws (name, manufacturer, description): none
- Description and characteristics of your resin(s) : none
- A document presenting the summary of the techniques used (reference) : none

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year): none

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year): none

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible): in 1990s the tagging of Salmo salar smolts was performed, the Baltic Sea, ICES area III b-d. The scales of the returned fishes have been stored and used for age determination purposes.

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible) : none

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible): none

- Do you use image capture/analysis software, if so, which?: none in the last years (last time used in 2005-2007 during the cod age reading calibration exercises)

- Which type of camera do you use (digital or analogue, description, characteristics)?: none

- Which applications is image processing used for (routine, only exchanges, validation studies…) ?: only exchanges

- How many images are made per year on average?: none

- Do you calibrate your images?: no

- Which measurements do you make on the image (distance between rings…) ? none

- What is the format of images?: none

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species: We have started to prepare written procedures for age estimation for all the species for which we determine the age (list at point 5).

- Do you have an internal quality control (description, references) ? none

- Do you have quality management certification e.g. ISO 9001:2008? - No

- Are you accredited (yes or no, name of accreditation authority)? -No

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

i) Workshop on Age Reading of Baltic Flounder (2008);

ii) Workshop on Age Reading of Baltic Herring (2008);

iii) Workshop on Age Reading of Baltic Sprat (2008);

iv) Workshop on Age Determination of Baltic Cod (2010)

- Are all your age readers aware of WebGR? - yes

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? – no

- Your name: Romas Statkus
- Your country: **Lithuania**
- Your institute: **Fishery Service, Division of the fisheries research and science**
- Number of treated calcified structures in 2010: 5563 otoliths
- List of your species (latin name):

Clupea harengus Linné, 1758

Gadus morhua Linné, 1758

Platichthys flesus Linné, 1758

Sprattus sprattus Linné, 1758

- Your geographical areas:

Baltic Sea, ICES IIId

- How many laboratories analyse calcified structures in your country?

1 (marine species)

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify):

Age data for main commercial species (C. harengus, G. morhua, S. sprattus)) are used for stock assessment. Less abundant species (e. g. Alsosa fallax, Psetta maximus) for environmental studies and fisheries management

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.) :

Clupea harengus – whole otoliths poured over alkohol for treatment on mounted plastic slides.

Gadus morhua – Broken and burned otoliths for treatment on mounted plastic slides.

Platichthys flesus harengus – whole otoliths poured over alkohol for treatment on mounted plastic slides.

Sprattus sprattus – whole otoliths poured over alkohol for treatment on mounted plastic slides.

- List of calcified structures that you use:

Otoliths only

- How are calcified structures stored before treatment?

Paper packets.

- How are calcified structure preparations stored?

Paper packets.

- List of your saws (name, manufacturer, description) :

We do not use any saws or similar devices

- Description and characteristics of your resin(s):

Not used

- A document presenting the summary of the techniques used (reference):

Clupea harengus Linné, 1758 (ICES. 2008. Report of the Workshop on Age Reading of Baltic Herring (WKARBH), 9–13 June 2008, Riga, Latvia. ICES CM 2008/ACOM:36. 37 pp.)

Gadus morhua Linné, 1758 (ICES, 2005. Report of the Study Group on Ageing Issues of Baltic Cod (SGABC), 17–20 May 2005, Klaipeda, Lithuania. ICES CM 2006/ACFM:02. 32 pp.)

Platichthys flesus Linné, 1758 (ICES, 2007. Report of the Workshop on Age Reading of Flounder (WKARFLO), 20–23 March 2007, Öregrund, Sweden. ICES CM 2007/ACFM:10. 69 pp.)

Sprattus sprattus Linné, 1758 (ICES, 2008. Report of the Workshop on Age Reading on Baltic Sprat (WKARBS), 17-20 March 2008, Klaipeda, Lithuania. ICES CM 2008/ACOM:37. 28 pp.)

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year):

There are no specific agreements on otoliths exchange. Decision on such exchange has been agreed during the relevant workshops

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year):

There are no specific agreements on otoliths exchange. Decision on such exchange has been agreed during the relevant workshops

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

Some experiments were performed with trout smolts (2004-2005) and Atlantic sturgeon (2011 July) for recovery purposes in Lithuanian rivers. No results are available so far

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

Unknown

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

Unknown

- Do you use image capture/analysis software, if so, which?

Motic Image Plus 2.0

- Which type of camera do you use (digital or analogue, description, characteristics)?

Digital

- Which applications is image processing used for (routine, only exchanges, validation studies…) ?

Routine

- How many images are made per year on average?

200

- Do you calibrate your images?

No

- Which measurements do you make on the image (distance between rings…) ?

Distance between rings

- What is the format of images?

jpg

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

We don't have any description of the process

- Do you have an internal quality control (description, references) ?

We have 1 age reader per species. Age reading is performed following instructions in relevant manuals and recommendations from relevant workshops. The quality is assured by age reader himself

- Do you have quality management certification e.g. ISO 9001:2008?

No

- Are you accredited (yes or no, name of accreditation authority)?

No

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

Workshop on Age Reading of Baltic Herring (WKARBH), 9–13 June 2008, Riga, Latvia

Workshop on Age Reading on Baltic Sprat (WKARBS), 17-20 March 2008, Klaipeda, Lithuania

- Are all your age readers aware of WebGR?

Some

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

We did not upload any images yet. If there will be an interest we would do that

- Your name: Loes Bolle / Ineke Pennock
- Your country: **Netherlands**
- Your institute:**IMARES**
- Number of treated calcified structures in 2010:

Otoliths:38079

Scales and fin rays:2500

- List of yourspecies (latin name):

Platichthysflesus

Scophthalmus rhombus

Limanda limanda

Pleuronectes platessa

Scophthalmusmaximus

Soleasolea

Micostomuskitt

Gadusmorhua

Melanogrammus aeglefinus

Merlangius merlangus

Micromesistiuspotassou

Argentina silus

Trisopterusesmarki

Clupeaharengus

Sprattussprattus

Trachurustrachurus

Scomberscombrus

Eutriglagurnardus

Stizostedionlucioperca

Percafluviatilis

Abramisbrama

Rutilusrutilus

Buglossidiumluteum

Arnoglossuslaterna

Merlucciusmerluccius

Echiichthysvipera

Callionymuslyra

- Your geographical areas :

North Sea

(English Channel &Atlantic Ocean< 10%).

- How many laboratories analyse calcified structures in your country?One: IMARES

- How is the age data used

stock assessment:±50%,

environmental studies:± 50%

- Description of your types of calcified structure preparation:

Broken-burnt otoliths mounted in plasticine on glass slide,

Broken,

Whole otoliths in water,

Whole otoliths mounted in clear resin on custom plastic slides (i.e. vulcatene plates),

Transverse sectioned otoliths mounted on glass slide (no cover slip),

Stained transverse sections otolithsmounted on glass slide (no cover slip),

Whole scales,

Polished fin rays

- List of calcified structures that you use:

otoliths, scales, fin rays

- How are calcified structures stored before treatment?

In paper envelopes

- How are calcified structure preparations stored?

Sections and broke-burnt: In boxes for microscopic slides.

Vulcatene plates: in small boxes

Other: back in paper envelopes

- List of your saws (name, manufacturer, description):

EXTEC Diamond wavering blade high concentration nr 12215; EXTEC , 99 Phoenix Avenue, Enfield, CT USA.

- Description and characteristics of your resin(s) :

Assistant Histokitt

- A document presenting the summary of the techniques used (reference) :

Bolle LJ, Eltink AE, Schaap L, de Vries M, Bakker C, Groot P, Rink G, Beintema J, Groeneveld K, Bol R, Stoker M, Y. J, Rijs S (2003) Handboek leeftijdsbepalingen. Netherlands Institute for Fisheries Research, Rapport CVO 03-010 (Dutch)

- List of bilateral agreements where you receive calcified structures

None

- List of bilateral agreements where you sent calcified structures

France – Ifremer–Mullussurmuletus–±200 otoliths

England – CEFAS –Dicentrarchuslabrax– ±200 otoliths

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

Plaice – North Sea

Included in reference below

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

Plaice– North Sea:

Rijnsdorp AD, van Leeuwen PI, Visser TAM (1990) On the validity and precision of back-calculation of growth from otoliths of the plaice, Pleuronectes platessa L. Fish Res 9:97-117

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

None

- Do you use image capture/analysis software, if so, which?

Olympus Cell ^D

- Which type of camera do you use (digital or analogue, description, characteristics)?

Olympus DP 70 microscope digital camera

- Which applications is image processing used for (routine, only exchanges, validation studies…) ?

Exchanges, Backcalculations

- How many images are made per year on average?

Approx. 500

- Do you calibrate your images?

Yes

- Which measurements do you make on the image

Distance between rings, total width otolith (i.e. length of transverse section)

- What is the format of images?

tiff

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species:

Ageing:

Bolle LJ, Eltink AE, Schaap L, de Vries M, Bakker C, Groot P, Rink G, Beintema J, Groeneveld K, Bol R, Stoker M, Jongejans,Y, Rijs S (2003) Handboek leeftijdsbepalingen. IMARES, Rapport CVO03-010

Survey sampling(incl. data storage survey data)

van Damme C, Heessen H, Bolle L, de Boois I, Couperus B, Dekker W, Dickey-Collas M, Eltink G, Grift R, ter Hofstede R, Pastoors M, Piet GJ, Poos JJ, Schaap L, Wieger-

inck H, van Willigen J, Ybema S (2009) Handboek bestandsopnamen en routinematige bemonsteringen op het water IMARES, Rapport CVO 03.011

Market sampling(incl. data storage market data)

Verver SW (2010) Handboek marktbemonstering zeevisserij 2010-2011. IMARES, Rapport CVO 10.011

- Do you have an internal quality control (description, references) ?

Yes. Not documented in report form yet. Will be done in the next version of the ageing manual (called 'Handboek' in Dutch), which is due in 2012.

- Do you have quality management certification e.g. ISO 9001:2008?

ISO 9001: 2008 (certificate nr. 57846-2009-AQ-NLD-RvA)

- Are you accredited (yes or no, name of accreditation authority)?

No

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

Sole exchange 2007-2008

Cod workshop 2008

Turbot workshop 2008

Haddock exchange 2008-2009

Mackerel exchange 2008-2009

Informal (bilateral) blue whiting exchange 2009

Mackerel workshop 2010 (Lowestoft UK)

Plaice exchange 2009-2010

Plaice workshop 2010 (IJmuidenNL)

Dab exchange 2009-2010

Dab workshop (Hamburg DE)

Blue whiting exchange 2010

Cod exchange 2010

Sole exchange 2010-2011

Eel workshop 2011 (FR)

Expected:

Brill exchange 2010-2011

Turbot exchange 2010-2011

Sprat exchange 2010-2011

- Are all your age readers aware of WebGR?

Yes (aware yes, but not capable of using it)

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

Exchange images: yes

All images: probably not, depends on if the time investment is worth the benefits.

- Your name: Eduardo Soares (Sardina pilchardus); Andreia Silva (Sardina pilchardus); Sandra Dores (Merluccius merluccius); Teresa Moura (Centroscymnus coelolepis, Centrophorus squamosus); Marta Gonçalves (Trisopterus luscus); Inês Farias (Aphanopus carbo and Aphanopus intermedius); Maria João Ferreira (Trachurus trachurus); Maria Manuel Martins (Scomber scombrus and Scomber colias); Ana Luísa Ferreira (Micromesistius poutassou); Bárbara Pereira (Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus, Neoraja iberica); Ricardo Alpoim (Hippoglossoides platessoides; Reinhardtius hippoglossoides; Gadus morhua; Macrourus berglax; Sebastes spp.; Lophius spp. (no experience)).

- Your country: **Portugal**

- Your institute: **Instituto de Investigação das Pescas e do Mar (IPIMAR)**

- Number of treated calcified structures in 2010: Sardina pilchardus – 5210 + 250 (microstructural analysis. TL between 7-15 cm); Merluccius merluccius – 2322; Centroscymnus coelolepis and Centrophorus squamosus – approx. 300; Trisopterus luscus - 1261 were collected, but they are stored, waiting to be treated; Aphanopus carbo and Aphanopus intermedius – 1467; Trachurus trachurus – 2075; Scomber scombrus and Scomber colias – approx. 2200; Micromesistius poutassou – 1971; Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – 430; Hippoglossoides platessoides approx. 400.

- List of your species (latin name): Sardina pilchardus (Walbaum, 1792); Merluccius merluccius (Linné, 1758); Centroscymnus coelolepis and Centrophorus squamosus; Trisopterus luscus (Linné, 1758); Aphanopus carbo and Aphanopus intermedius; Trachurus trachurus; Scomber scombrus (Linné, 1782) and Scomber colias (Gmelin, 1789); Micromesistius poutassou; Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica; Hippoglossoides platessoides; Reinhardtius hippoglossoides; Gadus morhua; Macrourus berglax; Sebastes spp.; Lophius spp. (no experience).

- Your geographical areas: ICES Sub-Areas I, II, XII, XIVa, XIVb and IXa, NE Atlantic, Portuguese coast; NAFO area.

- How many laboratories analyse calcified structures in your country?:

Sardina pilchardus – 1: IPIMAR;

Merluccius merluccius – 2: IPIMAR (Lisbon);

Centroscymnus coelolepis and Centrophorus squamosus – 6 or 7; Trisopterus luscus – several, mainly IPIMAR, Interdisciplinary Centre of Marine and Environmental Research (CIIMAR, O'Porto), Faculty of Sciences of Lisbon University (FCUL;

Aphanopus carbo and Aphanopus intermedius – approx. 7;

Trachurus trachurus – 1: IPIMAR;

Scomber scombrus and Scomber colias – 1: IPIMAR;

Micromesistius poutassou – 1: IPIMAR;

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – 1: IPIMAR;

Hippoglossoides platessoides; Reinhardtius hippoglossoides; Gadus morhua; Macrourus berglax; Sebastes spp.; Lophius spp. (no experience) – 1: IPIMAR.

- How is the age data used e.g. stock assessment?%, environmental studies?%, other ?%, (please specify):

Sardina pilchardus – Stock Assessment (80%), Growth studies (20%); Juveniles, improvement of age readings, especially the first annual ring identification (100%);

Merluccius merluccius – Stock assessment (100%);

Centroscymnus coelolepis and Centrophorus squamosus – Stock assessment and biologic studies (100%);

Trisopterus luscus – Stock assessment (100%);

Aphanopus carbo and Aphanopus intermedius – Stock assessment and biological studies (100%);

Trachurus trachurus – Stock assessment (100%);

Scomber scombrus and Scomber colias – growth and geographical distribution studies and population dynamics (100%);

Micromesistius poutassou – Stock assessment (100%);

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – stock assessment (ICES WGEF) and environmental studies (100%);

Hippoglossoides platessoides – 100% stock assessment;

Reinhardtius hippoglossoides – growth studies;

Gadus morhua – no ageing in recent years;

Macrourus berglax – no ageing in recent years;

Sebastes spp. – no ageing in recent years;

Lophius spp. – possible use in stock assessment (ICES benchmark in 2012).

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

Sardina pilchardus – Whole otoliths are mounted in clear resin (Entellan) on custom black plastic plaques (10 otolith pairs mounted in the same number of cavities by plaque); for age reading improvement of juveniles and the first annual ring identification, one of the two otoliths of each pair is mounted in clear resin on a glass slide and polished using 9, 3, 0.3 μm lapping film. The other one is fixed with the resin to a black plastic plaque as usual;

Merluccius merluccius – Sagittae otoliths are collected and stored in individual vials with salted water untill they are included in epoxy resin with black pigment and then sliced into thin sections at the nucleus level. Depending on otoliths sizes, a resin block may have 36, 42 or 48 otoliths. The thin otoliths sections (0,5 mm thick) are mounted on custom microscope slides;

Centroscymnus coelolepis and Centrophorus squamosus – Dorsal spine cross sections are stained and mounted on microscope slides;

Trisopterus luscus – First, otoliths are burned. Then they are embedded in a black polyester resin, sectioned in a high speed saw and mounted in glass slides;

Aphanopus carbo and Aphanopus intermedius – For ageing: otoliths are mounted on resin blocks, sectioned with a high ◎speed diamond b
mounted in glass slides; a test was made with vertebrae: were immersed in a solution of 5% tripsin in phosphate buffer for 1 hour at 50ºC (to remove the muscle) and washed with distilled water; the remaining tissue was brushed out; and finally were immersed in a 1:1 glycerin-alcohol solution fixed with black plastic dough for growth increment observation; For contour shape studies: otoliths are photographed with a stereomicroscope, immersed in a 1:1 solution of alcohol/glycerin or dry and observed under reflected or transmitted light; For laser ablation: whole otoliths are mounted on microscope slides with resin and polished with an automatic polisher;

Trachurus trachurus – For fish with total length up to 26 cm the whole otoliths are submerged in a thymol solution 0.01% for a 24h period. After that they are observed in immersion oil. The otoliths of fish longer than 26 cm are embedded in a black polyester resin and positioned in rows in specially prepared moulds. After dried and hardened the resin, blocks are cut with a high speed diamond grinding disc. All resulting sections are then collected and glued on a glass microscope slide for observation;

Scomber scombrus and Scomber colias – The whole otoliths are mounted in clear resin (Entellan) on custom plastic slides (10 otolith pairs mounted in the same number of cavities by plaque);

Micromesistius poutassou – The whole otoliths are submerged in a thymol solution 0.01% for 24h, and then embedded in immersion oil and observed with a magnifier;

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – whole dermal denticles are cleaned with a trypsin solution, enhanced with EDTA solution and observed directly with a stereomicroscope;

Hippoglossoides platessoides – whole otoliths mounted in clear resin on custom microscope slides and polish;

Reinhardtius hippoglossoides – whole otoliths are soaked in a 50:50 mixture of Glycerin and Thymol for 72 hours. They are then baked in an oven for 30 min. at 200°C. Prior to ageing the otoliths are placed in immersion oil for 24 hours;

Gadus morhua – broken otoliths;

Macrourus berglax – broken otoliths;

Sebastes spp. – broken and baked otoliths;

Lophius spp. – illicia sectioned (sliced).

- List of calcified structures that you use:

Otoliths (Sagittae) – Sardina pilchardus, Merluccius merluccius, Trisopterus luscus, Scomber scombrus, Scomber colias, Trachurus trachurus, Aphanopus carbo, Aphanopus intermedius, Micromesistius poutassou, Hippoglossoides platessoides, Reinhardtius hippoglossoides, Gadus morhua, Macrourus berglax and Sebastes spp.;

Dorsal spines – Centroscymnus coelolepis and Centrophorus squamosus;

Dermal denticles, vertebrae (just for calibration) – Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica.

Vertebrae – Aphanopus carbo;

Illicia – Lophius spp..

- How are calcified structures stored before treatment?:

Sardina pilchardus – After being collected, otoliths are first washed with freshwater, cleaned with absorbing paper and stored for at least 24 hours to completely dry in appropriate plastic containers with several cavities (approx. 100). Aboard, during the research surveys and after being washed with freshwater and cleaned with absorbing paper they are stored in Eppendorf tubes, from which they are collected in the lab ashore to be mounted on custom black plastic plaques;

Merluccius merluccius – Otoliths are cleaned and stored in small vials (16 mm x 50 mm) with salted water;

Centroscymnus coelolepis and Centrophorus squamosus – Dorsal spines (together with fin) are frozen at -20ºC. Trisopterus luscus – Otoliths are cleaned and stored in paper envelopes;

Aphanopus carbo and Aphanopus intermedius – Otoliths were cleaned, dry and stored in paper envelopes or plastic vials; vertebrae were stored frozen;

Trachurus trachurus – Otoliths are cleaned and stored dry in paper envelopes;

Scomber scombrus and Scomber colias – As with Sardina pilchardus, otoliths are first washed with freshwater, cleaned with absorbing paper and stored for at least 24 hours to completely dry in appropriate plastic containers with several cavities (approx. 100). Aboard, during the research surveys and after being washed with freshwater and cleaned with absorbing paper they are stored in Eppendorf tubes, from which they are collected in the lab ashore to be mounted on plaques;

Micromesistius poutassou – Otoliths are stored dry in paper envelopes;

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – dermal denticles removed from the caudal region and vertebrae from the abdominal or caudal regions are stored frozen.

Hippoglossoides platessoides; Reinhardtius hippoglossoides; Gadus morhua; Macrourus berglax; Sebastes spp.; Lophius sp. – otoliths and illicia collected and stored dry in paper envelopes.

- How are calcified structure preparations stored?:

Sardina pilchardus – Plaques with otoliths are stored in appropriate cardboard boxes (each with 25 plaques x 10 otolith pairs = 250 otoliths pairs); the microscopy slides for daily ring readings are stored in plastic boxes, with 100 otoliths each;

Merluccius merluccius – Sectioned otoliths mounted on custom microscope slides are stored in appropriate cardboard boxes;

Centroscymnus coelolepis and Centrophorus squamosus – Dorsal spines are stored in boxes, at room temperature;

Trisopterus luscus – Glass slides with sectioned otoliths are stored in appropriate cardboard boxes;

Aphanopus carbo and Aphanopus intermedius – Slides with sectioned otoliths are packaged in cardboard boxes; slides with otoliths for laser ablation are individually packaged in inert plastic bags; cleaned vertebrae are stored dry in plastic tubes;

Trachurus trachurus – Otoliths are stored dry in paper envelopes and the microscope slides in microscope slide boxes;

Scomber scombrus and Scomber colias – As for sardine, otoliths fixed to black plastic plaques are stored in appropriate cardpaper boxes (each with 25 plaques x 10 otolith pairs = 250 otoliths pairs);

Micromesistius poutassou – Otoliths are stored dry in envelops at room temperature;

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – Dermal denticles and vertebrae are stored dry in plastic tubes;

Hippoglossoides platessoides; Reinhardtius hippoglossoides; Gadus morhua; Macrourus berglax; Sebastes spp.; Lophius sp. – In paper envelopes or in appropriate cardboard boxes.

- List of your saws (name, manufacturer, description):

Merluccius merluccius, Trisopterus luscus, Aphanopus carbo and Aphanopus intermedius - LABCUT 230F - Abrasive/diamond cutting machine with a manually operated cutting head and fixturing for production cutting stock lengths of material. The machine is floor standing; Extec Diamond wafer blade 6'' x 0.020 x 1/2 high cons. (152 mm x 0.5 mm x 12.7 mm) (ref.: 12215).

Centroscymnus coelolepis and Centrophorus squamosus – Accu ◎Edge disposable microtome blades, for cryo sectioning (Sakura, #4810).

- Description and characteristics of your resin(s):

Sardina pilchardus; Scomber scombrus and Scomber colias - Entellan new, rapid mounting medium for microscopy (contains Xylen) (Merck ref.:HX807787);

Merluccius merluccius; Aphanopus carbo; Aphanopus intermedius; Trisopterus luscus; Trachurus trachurus; Lophius spp. - Kit SP 106 Multipurpose Epoxy System: Multi-purpose Epoxy System Resin Mix Multi-purpose Epoxy System Slow Hardener Mix Ratio 5:1, sp106 ref.F510-029; Epoxy PIGMENT Black, sp 106 ref:A 445-004; Honey Wax mould release compound;

Hippoglossoides platessoides - Resin: BUELER THERMOPLASTIC CEMENT Nº 40-81OO A0667.

- A document presenting the summary of the techniques used (reference):

Bedford, B. C., 1983. A method for preparing sections of large numbers of otoliths embedded in black polyester resin. J. Cons. Int. Explor. Mer, 41: 4–12.

Campana, S. E., 1992. Measurements and Interpretation of the Microstructure of Fish Otoliths, p. 59–71. In D. K. Stevenson and S. Campana, S.E. [ed.]. Otolith Microstructure Examination and Analysis. Can. Spec. Publ. Fish. Aquat. Sci., 117.

Campana, S.E., Jones, C.M., 1992. Analysis of Otolith Microstructure Data, p. 73–100. In D. K. Stevenson and S. E. Campana [ed.] Otolith Microstructure Examination and Analysis. Can. Spec. Publ. Fish. Aquat. Sci., 117.

Duarte, R., Landa, J., Quincoces, I., Dupouy, H., Bilbao, E., Dimeet, J., Marçal, A., McCormick, H., Ni Chonchuir, G., 2002. Anglerfish Ageing Guide. In "Report of the

4th International Ageing Workshop on European Anglerfish". IPIMAR, Lisbon (Portugal). 40 p.

Farias, I., Vieira, A. R., Gordo, L. S., Figueiredo, I., 2009. Otolith shape analysis as a tool for population discrimination of black scabbardfish (Aphanopus carbo Lowe, 1839) in Portuguese waters. Sci. Mar. 73S2: 47–53.

Gallagher, M. J., Nolan, C. P., 1999. A novel method for the estimation of age and growth in rajids using caudal thorns. Can. J. of Fish. Aq. Sci., 56: 1590–1599.

Godinho, M. L., 1991. A Method to Help Age American Plaice in Division 3M, NAFO SCR Doc. 91/68, Serial No. N1952.

ICES, 2011a. Report of the Workshop on Age Reading of European Atlantic Sardine (WKARAS), 14-18 February 2011, Lisbon, Portugal. ICES Advisory Committee. ICES, C.M. 2011/ACOM: 42, Ref.: PGCCDBS. 87p.

ICES, 2011b. Report of the Workshop on Age Reading of Greenland Halibut (WKARGH), 14-17 February 2011, Vigo, Spain. ICES CM 2011/ACOM: 41. 38p.

ICES, WKARMAC, 2010. ICES 2010/Acom: 46 (UPDATED VERSION).

Machado, P.B., Figueiredo, I., 2000. A technique for ageing the birdbeak dogfish (Deania calcea Lowe, 1839) from dorsal spines. Fisheries Research 45: 93–98.

Meneses, I. 2003. Estimação de factores que Condicionam a Variabilidade do Recrutamento de Peixes na Costa Atlântica da Península Ibérica. PhD Thesis, INIAP/IPIMAR, 205-260 pp.

Palmer, M., Linde, M., Morales-Nin, B., 2010. Disentangling fluctuating asymmetry from otolith shape. Mar. Ecol. Prog. Ser. 399: 261–272.

Serra-Pereira, B., Figueiredo, I., Farias, I., Moura, T. and Gordo, L. S., 2008. Description of dermal denticles from the caudal region of Raja clavata and their use for the estimation of age and growth. ICES J. Mar. Sci., 65: 1701–1709.

Soares, E., Morais, A., Silva, A., Carrera, P., Jorge, A., Rico, I., Peleteiro, Q., Evano, H., 2002. Report of the Workshop on Sardine Otolith Age Reading (Lisbon, 28 January – 1 February, 2002). Relat. Cient. Téc. IPIMAR, Série digital (http://ipimar-iniap.ipimar.pt) nº14, 16p.

Soares, E., Silva, A., Morais, A., 2007. Workshop on Sardine Otolith Age Reading and Biology. Relat. Cient. Téc. Inst. Invest. Pescas Mar, Série Digital (http://www.inrb.pt/ipimar) nº42, 57p. + Anexos.

Vieira, A. R., Farias, I., Figueiredo, I., Neves, A., Morales-Nin, B., Sequeira, V., Martins, M. R., Gordo, L. S., 2009. Age and growth of black scabbardfish (Aphanopus carbo Lowe, 1839) in the southern NE Atlantic. Sci. Mar. 73S2: 33–46.

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year): - NA

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year): - NA

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible): - NA

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

Sardina pilchardus –

Alvarez, F., Porteiro, C., 1981. Growth studies of Sardina pilchardus (Walb.) in Galician waters (N.W. Spain). ICES, C.M. 1981/H:29. 11 p.

Bravo de Laguna, J., Fernández, M.A.R., Goñi, R., Delgado, A., 1979. Growth studies of Sardina pilchardus (Walb.), done by direct methods and scalimetry, off West Africa. ICES, C.M. 1979/h:52. 20p.

Delgado, A., Fernández, M.A.R., Goñi, R., 1981. Contribución al estúdio de la sardina (Sardina pilchardus, Walb.) en aguas de Africa Occidental. III. Estudio del crecimiento por lectura directa de otolitos y retrocálculo mediante escalimetría de escamas. Bol. Inst. Esp. Oceano. (VI) nº 313: 139-164.

Jorge, I.M., Monteiro, C.C., 1980. Preliminary results on age and growth of Sardina pilchardus (Walb.) in Sub-Area IXa. ICES, C.M. 1980/H:38. 18 p.

Monteiro, C.C., Jorge, I.M., 1982. Age and growth of Sardina pilchardus (Walb.) from Portuguese coast (ICES Division IXa). ICES, C.M. 1982/H:19. 29 p.

Pertierra, J.P., Morales-Nin, B., 1989. Sardine growth in the Catalan Sea (NW Mediterranean) determined by means of otolith interpretation and length frequency data. Scient. Mar. 53(4): 821-826.

Porteiro, C., Alvarez, F., 1983. Determinación del crecimiento de la sardina, Sardina pilchardus (Walb.) en aguas gallegas, mediante lectura directa de otolitos. Inf. Téc. Inst. Esp. Oceanogr. Nº 14. 19 p.

Trachurus trachurus – following Year class of 1982.

Scomber scombrus and Scomber colias –

Martins, M. M.; Jorge, I. M.; Gordo, L.S., 1983. On the maturity, morphological characteristics and growth of Scomber japonicus Houttuyn (1782) of west continental coast of Portugal. ICES C. M. H: 39. 9 pp.

Gordo, L .S., Martins, M. M. B., Jorge, I.M., 1982. Preliminary study on the age and growth of mackerel (Scomber scombrus L.) in ICES Sub-area IX. ICES 1982 C. M. /H 16. 13 pp.

Raja clavata –

Serra-Pereira, B., Figueiredo, I., Farias, I., Moura, T., Gordo, L. S. 2008. Description of dermal denticles from the caudal region of Raja clavata and their use for the estimation of age and growth. ICES Journal of Marine Science, 65: 1701-1709.

This work is a response to a lack of knowledge of the biology of Raja clavata in southern European waters, particularly in terms of age and growth. Two structures were analysed: dermal denticles and vertebral centra. Six types of dermal denticle were identified in the tail. Among those, small thorns were the most suitable for age determination owing to their fixed position, persistence throughout their lifespan, and defined growth-band pattern. Caudal thorns were more accurate than vertebral centra for age determination and were therefore selected as the most appropriate structure for ageing R. clavata. Based on edge analysis, annual band deposition was verified. The birthdate was established as 1 June based on the prevalence of hyaline edges in age-0 class specimens: prevalence peaked in May and June. Both von Bertalanffy and Gompertz growth models were fitted to age-at-length data, but the former was considered more appropriate based on similarity between the estimated L{infty} and the maximum size recorded for the species. No significant differences in growth

parameters were observed between sexes. The estimated growth parameters were Linf= 1280 mm, k = 0.117 year-1, and t0 = -0.617 years. The maximum age estimated for R. clavata was 10 years, for a female of length 835 mm.

Raja undulata (ICES IXa) –

Moura, T., Figueiredo, I., Farias, I., Serra-Pereira, B., Coelho, R., Erzini, K., Neves, A., et al. 2007. The use of caudal thorns for ageing Raja undulata from the Portuguese continental shelf, with comments on its reproductive cycle. Marine and Freshwater Research, 58: 983-992.

The present study focuses on age estimation, with reproductive information contributing to the better understanding of the growth and the biology of Raja undulata. In the age and growth study, two calcified structures were used: caudal thorns and central vertebra. Results of readings showed that there were no significant differences in age estimates between the two structures. Both von Bertalanffy and Gompertz growth models were fitted to size-at-age data by sex and geographical area. No significant differences were found between sexes for the two models but significant differences were found between geographical areas (P = 0.05). The Gompertz growth function was selected as the best model to describe R. undulata growth because it presented the best fit and the most reasonable biological estimates. Reproductive analysis indicates one annual breeding season for R. undulata. The differences found in the estimates of length at first maturity between geographical areas (TL50% = 838 mm in Peniche and 762 mm in Algarve for females and TL50% = 781 mm in Peniche and 736 mm in Algarve for males), together with the regional differences found between growth parameters estimates (P = 0.05), may reflect the existence of different local populations of R. undulata on the Portuguese continental shelf.

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

Sardina pilchardus –

Alemany, F., Álvarez, F., 1994. Formation of initial daily increments in sagital otoliths of reared and wild Sardina pilchardus yolk-sac larvae. Mar. Biol., 121: 35-39.

Álvarez, F., 2002. Crecimiento diario de Sardina pilchardus y su aplicación al estudio de procesos de reclutamiento. Ph. D. thesis, Univ. Santiago.

Dulcic, J., 1997. Growth parameters of sardine, Sardina pilchardus (Walbaum, 1792), and anchovy, Engraulis encrasicolus (Linnaeus, 1758), larvae in the Eastern Adratic. Int. Symp. Of the Fisheries Society of the British Isles, Galway (Ireland), 8-11 July 1997.

Ré, P., 1984. Evidence of daily and hourly growth in pilchard larvae based on otolih growth increments, Sardina pilchardus (Walbaum, 1792). Cybium, 8 (1): 33-38.

Raja clavata –

Serra-Pereira et al. (2008) (see ref. in item 15, above), presented data about the validation of the first growth, but based on newborn observations.

- Do you use image capture/analysis software, if so, which?

Sardina pilchardus – VISIOLOG 6.3, NOESISÒ TNPC 4.1 application;

Merluccius merluccius – NOESISÒ TNPC 4.1 application;

Centroscymnus coelolepis and Centrophorus squamosus – Axio-Vision;

Aphanopus carbo and Aphanopus intermedius – NOESISÒ TNPC 4.1 application.

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – NOESISÒ TNPC 4.1 application.

- Which type of camera do you use (digital or analogue, description, characteristics)?

Sardina pilchardus – digital camera SONY® DFW-SX 910 attached to OlYMPUS® GX 51 microscope for daily age rings image capture;

Merluccius merluccius – digital camera SONY® DFW-SX 910, attached to a stereoscopic microscope - OLYMPUS® SZX9;

Centroscymnus coelolepis and Centrophorus squamosus – AxioCam, ZEISS®;

Aphanopus carbo and Aphanopus intermedius – digital camera SONY® DFW-SX 910;

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – digital camera SONY® DFW-SX 910.

- Which applications is image processing used for (routine, only exchanges, validation studies…)?

Sardina pilchardus – Growth studies and identification of the first annual ring; exchanges and age readings validation.

Merluccius merluccius – A routine work is being initiated;

Centroscymnus coelolepis and Centrophorus squamosus – Routine and basic measurements;

Aphanopus carbo and Aphanopus intermedius – Ageing studies and otolith contour shape studies;

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – Routine ageing and basic measurements.

- How many images are made per year on average?

Sardina pilchardus – 2500;

Centroscymnus coelolepis and Centrophorus squamosus – The studies were not developed under a routine basis. 3 to 6 pictures were taken from each specimen.

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – aprox. 200. The studies are not developed under a routine basis;

Aphanopus carbo – The studies were not developed under a routine basis. Ca. 2000 pictures were taken between 2006 and 2010;

Aphanopus intermedius – The studies were not developed under a routine basis. Ca. 100 pictures were taken in 2010.

- Do you calibrate your images?

Sardina pilchardus – Yes; images are calibrated to a micrometric scale to measure the otolith diameter and radius.

Merluccius merluccius – Yes;

Centroscymnus coelolepis and Centrophorus squamosus – Yes;

Aphanopus carbo and Aphanopus intermedius – Yes;

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – Yes.

- Which measurements do you make on the image (distance between rings…)?

Sardina pilchardus – Increments widths, otolith diameter and radius, first annual ring diameter and radius.

Centroscymnus coelolepis and Centrophorus squamosus – Growth zones width;

Aphanopus carbo and Aphanopus intermedius – Increment widths, otolith radius;

Micromesistius poutassou –

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – distance between rings and proto-thorn margin, dermal denticles length and width.

- What is the format of images?

Sardina pilchardus – JPG and TIFF;

Merluccius merluccius – TIFF or JPG;

Centroscymnus coelolepis and Centrophorus squamosus – JPG;

Aphanopus carbo and Aphanopus intermedius – JPG, BMP;

Micromesistius poutassou –

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja ibérica – JPG and TIFF.

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species:

Sardina pilchardus:

Preparation and reading procedures

For routine age determination, otoliths are extracted from each sampled fish, washed with fresh water, dried and mounted on black plastic plaques glued with resin (Entellan) in individual numbered cavities. Otoliths structure is analyzed using a stereoscopic microscope with a 20X amplification and under reflected light. The diameter or radius of the first hyaline ring is measured (this is the distance up to the end of the previous opaque zone).

For growth studies and identification of the first annual ring based on the observation of daily rings, one of the two otoliths is mounted in clear resin (Entellan) on a glass slide which are put to dry in a stove (37-40ºC) during 12 hours. The otolith diameter and radius are measured using Visiolog software. After that, otoliths are polished using 9, 3, 0.3 μm lapping film. After polishing, the whole otolith is photographed using a calibrated image system, linked to a microscope. Several images covering the different areas of the structure of the otolith are taken using a 200X magnification. These are mounted with a digital image treatment software in order to get a picture of the whole otolith structure. Finally, based on this otolith complete

image microincrements are counted and measured using Visiolog TNPC 4 application.

Sampling

Research Surveys:

Periodicity: annual-spring; Area: coast of Portugal and Gulf of Cadiz; Sampling type: stratified by lengh class and area; Nº by length-class and area: ≤13 cm - 10; 13.5-17.5 cm - 16; 18.0-19.5 cm – 20; ≥20 cm – all; Total per year: 1500.

Ports:

Periodicity: bi-monthly; Area: Portuguese coast – ICES Sub-Div IXa-N (Matosinhos); IXa-CS (Peniche) and IXa-S – Algarve (Portimão); Sampling type: stratified by length class; Nº by length class: 10; Total per year: 5000.

Merluccius merluccius:

Sagittae otoliths are collected, cleaned and stored in individual vials with salted water. Right otoliths are selected and embedded in epoxy resin with black pigment. Depending on otoliths sizes, a resin block may have 36, 42 or 48 otoliths, arranged in 6 rows. From each row, 3 thin sections are sliced at the nucleus level.

Otoliths thin sections (0,5mm) are mounted in glass microscope slides with Fluka (DPX Mountant for Histology) and observed with a stereomicroscope (x20 magnification) under reflected light. The interpretation of hake otoliths is based on the new guidelines established at the last Workshop (Report on WKAEH (ICES CM 2009/ ACOM:42). Data is stored at the PNAB –IPIMAR database ;

Sampling

Research Surveys:

Periodicity: annual - autumn; Area: Portuguese coast

Sampling type: stratified by lengh class and area (3 areas);

Nº by length-class and area: < 20 cm - 10; ≥ 20 cm - 5 male and 5 female; Total per year collected: 1292; Total per year mounted: 784;

Ports:

Periodicity: Quarter; Area: Portuguese coast - ICES Sub-Div IXa-N (Matosinhos/Póvoa de Varzim); IXa-CS (Peniche) and IXa-S – Algarve (Portimão); Sampling type: stratified by length class and ports; Nº by length class and port: 4; Total per year collected and mounted: 1538.

Discards:

Periodicity: Quarter; Area: Portuguese cost

Sampling type: stratified by length class;

Nº by length-class: < 20 cm - 10; ≥ 20 cm - 5 male and 5 female;

Total collected per year: 876.

Centroscymnus coelolepis and Centrophorus squamosus:

Deep-water squalid sharks

First and second dorsal fins of each specimen were left boiling in water for 2 min to facilitate the separation the spines from the flesh.

Spines left in 5% nitric acid for 20 to 40 minutes and after left in distilled water for, at least, 10 minutes.

Cross sections of 60 μm were made using a cryostat.

Cross sections were then stained with Mayer's hematoxylin for 1.30-3 minutes.

Cross-sections were mounted using Entellan and then observed using a microscope.

Growth bands were identified in the cross-sections where the bands were more distinguishable, when linear transepts were impossible to follow.

For age assignment it was assumed that one translucent and one opaque ring corresponds to one growth band, and that these are deposited each year.

For each specimen two readings were made using different cross-sections.

A quality scale on band readability was adopted and only readings with a defined readability score were used for growth modeling.

Aphanopus carbo and Aphanopus intermedius:

Ageing

Whole left otoliths from each pair were immersed in a 1:1 glycerin-alcohol solution, with sulcus acusticus placed downwards under a stereomicroscope with a micrometric ocular and a magnification of 18× using reflected light and a dark background. Right otoliths were prepared for age reading using the thin-sections technique (Bedford, 1983; McCurdy, 1985). They were transversally sectioned with a diamond-tipped saw blade (Labcut 230 Cutting Machine) rotating at 3700 rpm. Slides 0.5 mm thick were mounted in a glass slide with translucent glue, brushed with a 1:1 glycerin-alcohol solution and observed in a stereomicroscope with a micrometric ocular under transmitted light and an 18× magnification;

Vertebrae were immersed in a solution of 5% tripsin in phosphate buffer for 1 hour at 50ºC (to remove the muscle) and washed with distilled water; the remaining tissue was brushed out. For growth increment observation, vertebrae were immersed in a 1:1 glycerin-alcohol solution fixed with black plastic dough in a position that allowed a complete view of the whole surface, and observed under a binocular stereoscope with reflected light and a magnification of 15×. Vertebrae were photographed with a digital camera connected to a computer and the digital image was processed using the TN PC 4.1 software integrated in Visilog 6.3.0.

Otolith shape analysis

Otolith digital images were captured using a SON Y® DFW-SX910 digital camera linked to an Olympus® SZX9 stereomicroscope and the Noesis® TN PC 4.1 image analysis software. Each otolith was previously immersed in a 1:1 solution of glycerine-alcohol, positioned with the sulcus acusticus facing down and the rostrum to the left, and photographed under reflected light using the TN PC 4.1 software integrated in Visilog 6.3.0.

Trisopterus luscus:

30 otoliths of each length class are collected per quarter and stored in paper envelopes. The otoliths are burned at 200ºC for 1 hour. Then they are embedded in a black polyester resin block (42 per block), and sliced into 2mm sections at the nucleus level with a high speed saw. Three sections are made for each otolith, which are mounted onto glass slides with Entalan. Prior to observation at a stereomicroscope, otoliths are soaked into immersion oil in order to enhance the translucent rings.The age determi-

nation is done by attributing one year to each pair of opaque+translucent rings. Data is stored at the PNAB database.

Scomber scombrus and Scomber colias:

Twice a month in Matosinhos (Sub-area IXa Central North) and Peniche ports (Sub-area IXa Central South) samples are collected randomly (if available). For otholits purpose the stratification is made by length class; 10 pairs of otholits are removed in each sample by class (if available). The age determination is done by attributing one year to each pair of opaque+translucent rings. Data is stored at the PNAB –IPIMAR database.

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica:

dermal denticles collected from the skate's tail, and stored frozen before processing;

cleaning the dermal denticles in 5% buffered trypsin solution (pH 7.5) at 50ºC for 20 min;

cleaning with distilled water

band-pattern enhancement with 5% ethylenediaminetetraacetic acid solution (EDTA) for 10 min.

observed with a stereomicroscope at x28 magnification.

storage of dried dermal denticles, inside plastic tubes.

NAFO species – Hippoglossoides platessoides; Reinhardtius hippoglossoides; Gadus morhua; Macrourus berglax; Sebastes spp.:

Samples are randomly collected on board. For otoliths collection purpose the stratification is made by length class (5 pairs by cm class). The age determination is done by attributing one year to each pair of opaque+translucent rings. Data is stored at the PNAB –IPIMAR database.

- Do you have an internal quality control (description, references)?

Sardina pilchardus – Yes, once a year, the age readers on sardine otoliths meet for readings intercalibration and PGCCDBS guidelines are followed:

Eltink A.T.G.W. (2000). Age reading comparisons (MS Excel workbook version 1.0, October 2000). Internet: http://www.efan.no.

- Do you have quality management certification e.g. ISO 9001:2008? Sardina pilchardus – No;

Merluccius merluccius – No;

Centroscymnus coelolepis and Centrophorus squamosus – No;

Trisopterus luscus – No;

Aphanopus carbo and Aphanopus intermedius – No;

Trachurus trachurus – No;

Scomber scombrus and Scomber colias – No;

Micromesistius poutassou – No;

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – No;

Hippoglossoides platessoides; Reinhardtius hippoglossoides; Gadus morhua; Macrourus berglax; Sebastes spp.; Lophius sp. – No.

- Are you accredited (yes or no, name of accreditation authority)?

Sardina pilchardus – No;

Merluccius merluccius – No;

Centroscymnus coelolepis and Centrophorus squamosus – No;

Trisopterus luscus – No;

Aphanopus carbo and Aphanopus intermedius – No;

Trachurus trachurus – No;

Scomber scombrus and Scomber colias – No;

Micromesistius poutassou – No;

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – No;

Hippoglossoides platessoides; Reinhardtius hippoglossoides; Gadus morhua; Macrourus berglax; Sebastes spp.; Lophius sp. – No.

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

Sardina pilchardus – 1 Workshop on Age Reading of European Atlantic Sardine (WKARAS), 14-18 February 2011, Lisbon, Portugal.

Merluccius merluccius – 1 Workshop on age estimation of European hake (WKAEH). 9-13 November, 2009, Vigo, Spain; 1 Hake Exchange otolith reading 2011 (HKEXCH_11).

Scomber scombrus and Scomber colias – 1 Exchange and 1 Workshop;

Micromesistius poutassou: otholits exchanges in 2010;

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – No access to detailed information, but at least 2 per year.

Hippoglossoides platessoides; Reinhardtius hippoglossoides; Gadus morhua; Macrourus berglax; Sebastes spp.; Lophius sp. – 1 Workshop in 14-17 February 2011, Vigo, Spain.

- Are all your age readers aware of WebGR?

Sardina pilchardus – No;

Merluccius merluccius – Yes (it has already been used in a recent intercalibration exercise (HKEXCH_11));

Centroscymnus coelolepis and Centrophorus squamosus – No;

Trisopterus luscus – Yes;

Aphanopus carbo and Aphanopus intermedius – No;

Trachurus trachurus – No;

Scomber scombrus and Scomber colias – No;

Micromesistius poutassou – No;

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – Yes;

Hippoglossoides platessoides; Reinhardtius hippoglossoides; Gadus morhua; Macrourus berglax; Sebastes spp.; Lophius sp. – No.

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

Sardina pilchardus – Yes;

Merluccius merluccius – Yes;

Centroscymnus coelolepis and Centrophorus squamosus – No;

Trisopterus luscus – Yes;

Aphanopus carbo and Aphanopus intermedius – ;

Trachurus trachurus – ;

Scomber scombrus and Scomber colias – ;

Micromesistius poutassou – No;

Raja clavata, Raja brachyura, Raja montagui, Raja undulata, Raja microocelatta, Leucoraja naevus, Dipturus oxyrinchus and Neoraja iberica – Yes;

Hippoglossoides platessoides; Reinhardtius hippoglossoides; Gadus morhua; Macrourus berglax; Sebastes spp.; Lophius sp. – .

- Your name: Alexandra Garcia
- Your country: **Portugal**
- Your institute: **University of the Azores / Department of Oceanography and Fisheries**
- Number of treated calcified structures in 2010: 1659
- List of your species (latin name):

Aphanopus carbo

Aspitrigla cuculus

Beryx spp

Conger conger

Epigonus telescopus

Helicolenus dactylopterus dactylopterus

Lepidopus caudatus

Molva macrophthalma

Mora moro

Mullus surmuletus

Pagellus acarne

Pagellus bogaraveo

Pagrus pagrus

Phycis blennoides

Phycis phycis

Polyprion americanus

Scomber japonicus

Trachurus picturatus

Zeus faber

- Your geographical areas : North Atlantic ICES area X
- How many laboratories analyse calcified structures in your country? 3 (DOP; IPIMAR; DSIP)
- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify): stock assessment (100%)
- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.) :

Depending on the species we perform different types of preparation of their calcified structures, e.g. the whole and the sectioned otoliths were the most common techniques. However, several experiences were already done with broken otoliths.

- List of calcified structures that you use:

Otoliths, exclusively.

- How are calcified structures stored before treatment?

In eppendorf.

- How are calcified structure preparations stored?

Regarding the whole otolith in eppendorfs and then in eppendorf racks, for the sectioned otoliths in paper bags and then in boxes organised by year and species.

- List of your saws (name, manufacturer, description):

BUEHLER® Isomet 1000, low-speed precision saw and diamond wafering blade.

- Description and characteristics of your resin(s):

Usually, epoxy resin and some experiments adding pigment black.

- A document presenting the summary of the techniques used (reference):

When the whole otolith is used for the readings, it is usually placed in a petri dish and immersed in water for posterior reading with binocular microscope. In the case of large otoliths they are immersed in alcohol and/or glycerin. Regarding the sectioned otolith, firstly we immersed the whole otolith in resin and proceed with the longitudinal cut. The resulting surface is observed under binocular microscope with variable magnification depending on the size of the structure.

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year):

A few occasional exchange of both whole and sectioned otoliths occurred in the aim of Workshops attended. In 2000 a Aphanopus carbo otolith exchange with Spain (IEO and IMEDEA), UK (MSS and SAMS) and Portugal (DOP, IPIMAR, FCUL and DSIP).

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year):

In 2008 DOP sent 63 Aphanopus carbo otoliths in collaboration with IPIMAR.

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

Every year, during the demersal species survey, usually in spring, several species are tagged using spaghetti tags. Acoustic tagging is also conducted in coastal species.

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

Not available.

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

Not available.

- Do you use image capture/analysis software, if so, which?

The analysis software currently used is the IM500 from LEICA.

- Which type of camera do you use (digital or analogue, description, characteristics)?

The digital camera used is LEICA DC300.

- Which applications is image processing used for (routine, only exchanges, validation studies…)?

The image processing has been used for routine within the framework of the European Data Collection Regulation.

- How many images are made per year on average?

About 1800 images.

- Do you calibrate your images?

Yes we do.

- Which measurements do you make on the image (distance between rings…)?

Distance from the focus to each ring and to the otolith edge.

- What is the format of images?

The format is jpeg.

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

Not available.

- Do you have an internal quality control (description, references)?

Not available.

- Do you have quality management certification e.g. ISO 9001 :2008?

No

- Are you accredited (yes or no, name of accreditation authority)?

No

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

Please see answer to question 17.

- Are all your age readers aware of WebGR?

Yes

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

Presently, DOP doesn't upload any images in the WebGR database, but in the near future we intend to do so.

- Your name: dr. Valodia MAXIMOV
- Your country: **Romania**
- Your institute: **National Institute for Marine Research and Development "Grigore Antipa", Constanta**
- Number of treated calcified structures in 2010: 300 (only turbot)
- List of your species (latin name):

Black Sea turbot Psetta maxima maeotica

Spat Sprattus sprattus

Withing Merlangus merlangus

European anchovy Engraulis encrasicolus

Horse mackerel Trachurus mediterraneus

Spiny dog Squalus acanthias

- Your geographical areas: Romanian Black Sea coast (M - ICES area)
- How many laboratories analyse calcified structures in your country?

for the marine area: 1

for the freshwater: no data

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify):

stock assessment: 80 %

environmental studies (management of MPAS): 20%

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

whole otoliths

- List of calcified structures that you use: otoliths, scales
- How are calcified structures stored before treatment?

They are mounted whole onto the slides.

- How are calcified structure preparations stored?

In laboratory - no special conditions.

- List of your saws (name, manufacturer, description) : not applicable
- Description and characteristics of your resin(s) : not applicable
- A document presenting the summary of the techniques used (reference):

Radu E., V. Maximov, 2006 - Data processing sampling and fishery statistics guide, Ed. Ex Ponto, ISBN(10) 973-644-561-5 / ISBN(13): 978-973-644-561-3, p. 90

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year):

Not applicable

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year):

Not applicable

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

sturgeons tagging (special national programme for the rehabilitation of the natural populations):

Paraschiv M., Suciu R., Iani M, Maereanu M., 2008 - Coded wire tag use on young sturgeons in Romania- Annual Scientific Symposium of DDNI – Tulcea: 18 – 20 Sept. 2008

Suciu R., 2008 - Sturgeons of the NW Black Sea and lower Danube river countries, Mexico, NDF WORKSHOP WG 8 – Fishes CASE STUDY 5 SUMMARY Acipenser spp., Huso spp. Country – NW Black Sea

fish tagging in the Romanian Marine Protected Areas, 2011 – in progress

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

not applicable

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

not applicable

- Do you use image capture/analysis software, if so, which?

No

- Which type of camera do you use (digital or analogue, description, characteristics)?

Digital

- Which applications is image processing used for (routine, only exchanges, validation studies…)?

Routine

- How many images are made per year on average?

First year of implementatio

- Do you calibrate your images?

No

- Which measurements do you make on the image (distance between rings…)?

None

- What is the format of images?

jpg

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species:

The scales are removed from the place of storage, cleansed and degreased in water, alcohol, 10% NaOH solution, organic solvent etc. and placed between two glass slides, from two to 2-8 pieces, then tightened well and bind at the ends with adhesive bandage. The record numbering system is transferred on the adhesive tape.

Otholites are read directly (if small and transparent) or after prior preparation (degreasing, maintenance in solutions for transparency, roasting, sectioning etc.), according to specific methods

- Do you have an internal quality control (description, references)?

In the institute, is functioning a Internal Advisory Commission.

- Do you have quality management certification e.g. ISO 9001: 2008?

In progress

- Are you accredited (yes or no, name of accreditation authority)?

No accreditation for age reading

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

None

- Are all your age readers aware of WebGR?

Yes

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

We intend to develop this activity in our institute so, we intend to upload routine images to contribute to the WebGR database with calcified structure images.

- Your name: Begoña Villamor, Carmen Piñeiro, Jorge Landa, Juan Gil, Enrique Rodríguez-Marín, Jaime Mejuto.

- Your country: **Spain**

- Your institute: **Instituto Español de Oceanografía, Centro Oceanográfico de Santander, Vigo y Cádiz**

- Number of treated calcified structures in 2010:

30000

- List of your species (latin name):

Engraulis encrasicolus, Sardina pilchardus , Scomber scombrus, Scomber colias, Trachurus trachurus, Trachurus mediterraneus, Micromesistius poutassou.

Trisopterus luscus, Merluccius merluccius, Glyptocephalus cynoglossus, Helicolenus dactilopterus, Conger conger, Molva molva, Molva macrophthalma, Phycis blennoides, Pollachius pollachius, Aspitrigla cuculus, Microchirus variegatus.

Lophius piscatorius, Lophius budegassa, Lepidorhombus whiffiagonis, Lepidorhombus boscii.

Pagellus bogaraveo.

Thunnus thynnus, Xiphias gladius

- Your geographical areas:

ICES Subarea VI, VII; ICES Divisions VIIIa, VIIIb, VIIIc, IXa (North and South)

For large pelagic species also ICES Subarea V, X, XII and ICES Divisions IXb, VIIId, VIIIe and Mediterranean.

- How many laboratories analyse calcified structures in your country?

7 from 9.

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify):

Pelagic Species: 70% stock assessment, 20% environmental studies, 10% others.

Demersal Species: 80% stock assessment, 5% environmental studies, 15% others.

Benthic Species: 90% stock assessment, 5% environmental studies, 5% others (fisheries-induced evolution).

Deep Species: 100% stock assessment.

Large Pelagic Species: 90% stock assessment, 5% environmental studies, 5% others (fisheries-induced evolution).

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.):

Annual Growth:

Whole Otolith

(Engraulis encrasicolus, Sardina pilchardus, Scomber scombrus, Scomber colias): Otoliths are extracted from each sampled fish, washed with fresh water, dried and mounted on black plastic plaques glued with resin in individual numbered cavities. Different resins are used in each laboratory.

(Micromesistius poutassou, Lepidorhombus whiffiagonis, Lepidorhombus boscii): Otoliths are extracted from each sampled fish, and the otoliths are stored in envelopes. The otoliths were placed in water, to read under a microscope.

(Trachurus trachurus, Trachurus mediterraneus <25 cm): Otoliths are extracted from each sampled fish, and the otoliths are stored in envelopes. The otoliths were placed in alcohol and glycerine, to read under a microscope.

(Glyptocephalus cynoglossus, Helicolenus dactylopterus, Molva molva ≤80 cm, Aspitrigla cuculus ≤30 cm): Otoliths are extracted from each sampled fish, cleaned and stored in vials. The otoliths are placed in water, to read under a microscope.

(Pagellus bogaraveo): Otoliths are extracted from each sampled fish, and the otoliths are stored in envelopes. The otoliths were placed in glycerol-thymol solution, to read under a binocular microscope.

Sectioned Otoliths

(Trachurus trachurus, Trachurus mediterraneus >25cm): The whole otoliths are embedded in polyester resin in an aluminium mould. The resin blocks containing the embedded otoliths are removed from the moulds and cut into thin sections (0.5 mm) following the dorso-ventral plane of the otolith. The cutting machine is a high speed saw machine that permits to obtain multiple sections. The resulting sections are stuck in glass slides, with the proper label.

(Trisopterus luscus, Conger conger, Molva molva >80 cm, Molva macrophthalma, Phycis blennoides, Pollachius pollachius, Aspitrigla cuculus >30 cm, Microchirus variegatus, Merluccius merluccius): The whole otoliths are embedded in polyester resin in an aluminium mould. The resin blocks containing the embedded otoliths are removed from the moulds and cut into thin sections (1 mm), following the dorso-ventral plane of the otolith. The cutting machine is a high speed saw machine that permits to obtain multiple sections. The resulting sections are stuck in glass slides with the proper label and observed under a microscope.

(Thunnus thynnus): sections obtained by embedding otoliths in mix epoxy and hardener and sectioning using Low Speed ISOMET Saw. Sections are 250 – 400 μm thick. Sections are polished and placed in Petri dish and cover with ethanol for reading.

Vertebrae (Conger conger): The vertebrae are drawn between the origin of the dorsal and the end of the pectoral fins, boiled and cleaned with a brush, dehydrated in ethanol and dried. The vertebrae from each specimen are attached to a glass slide and covered with the solution (10% glycerine + 90% ethanol), to read directly under a microscope.

Sectioned Illicia (Lophius piscatorius, Lophius budegassa): The illicia are embedded in polyester resin in an aluminium mould. The resin blocks containing the embedded illicia are removed from the moulds and cut into thin sections (around 0.5 mm). The cutting machine is a high speed saw machine that permits to obtain multiple sections. The resulting sections are stuck in glass slides, with the proper label.

Sectioned spines (first ray of the first dorsal fin). (Thunnus thynnus): Spine sections are obtained using a precision rotating diamond saw. Section thickness usually range from 0.45 to 0.60 mm. Sections are washed in ethanol at 70% and dried out on blotting paper. Then sections are mounted on glass slides and covered with a highly transparent resin.

Sectioned spines (Second ray from anal fin). (Xiphias gladius): The anal fins are recollected on board fishery vessels and frozen to posterior analysis in the laboratory. The fins are boiled and the second ray extracted and sectioned in thin sections (around 0.45mm). Isomet low speed saw and Isomet 5000 are used in relation with the size of the specimen/structure. The sections are cleaned and stored in glass slides and covered with Eukkit.

Daily Growth:

Whole Otoliths (Scomber scombrus larvae, in 2000-2001): The larvae and postlarvae were placed on a glass slide with a drop of water. The otolith sagittae were extracted from larvae and postlarvae using fine dissection needles under a binocular microscope 3-5 months after having been preserved. Once the otoliths were extracted, the glass slides with the otoliths were dried carefully with absorbent paper, and the otoliths were covered with a thin layer of nail varnish. Larvae and postlarvae otoliths were mounted as a whole and with the concave side upwards.

Polished Sectioned Otoliths

(Engraulis encrasicolus juveniles, Lophius budegassa): The otolith sagittae extracted from juveniles were polished to obtain a thin section for growth ring interpretation. To obtain daily increment counts otolith were processed along the sagittal plane with respect to the fish. The otoliths were mounted in thermoplastic glue (CrsytalBond) and fixed to a glass slide. Thin sagittal sections were produced by grinding those using automated polishing wheels.

(Scomber scombrus juveniles, in 2000-2001):

The otolith sagittae extracted from juveniles and pre-recruits were cut into sections for growth ring interpretation. Each otolith section was processed on the sagittal plane with respect to the fish. The otoliths were embedded in polyester resin in silicon moulds. These moulds were allowed to dry at room temperature for 24 h before proceeding with sanding and polishing. Both sides of the otoliths were sanded and hand-polished until very fine otolith sections were obtained, after which they were mounted on glass slides. The grades of sandpaper used were 180, 220, 600, 1200 and 1500 and the grades of diamond dust for polishing were 15, 6 and 1 micra.

- List of calcified structures that you use:

Otoliths, Vertabrae, Illicia (= first dorsal fin ray), first ray of the first dorsal spine, second ray of the anal spine.

- How are calcified structures stored before treatment?

The otoliths of Engraulis encrasicolus, Sardina pilchardus, Scomber scombrus and Scomber colias are collected into black plastic moulds covered with a lid and secured with masking tape.

The otoliths of Trachurus trachurus, Trachurus mediterraneus, Micromesistius poutassou, Lepidorhombus whiffiagonis, Lepidorhombus boscii and Pagellus bogaraveo are collected into envelopes.

The otoliths of Trisopterus luscus, Merluccius merluccius, Glyptocephalus cynoglossus, Helicolenus dactilopterus, Molva molva, Molva macrophthalma, Phycis blennoides, Pollachius pollachius, Aspitrigla cuculus, Microchirus variegatus, Lophius piscatorius and Lophius budegassa and Thunnus thynnus are collected into dried vials or in paper envelopes.

Vertebrae of Conger conger are collected into plastic bags and frozen.

Illicia of Lophius piscatorius and Lophius budegassa are collected into envelopes.

First dorsal fin spines were stored dry in a paper envelope.

Anal fin of Xiphias gladius are collected into plastic bags and frozen.

- How are calcified structure preparations stored?

Pelagic Species:

Whole otoliths mounted in Black plastic moulds and polyester resin (Eukitt, Panreac or Entellan). The otolith trays are covered with a lid and stored in cardboard boxes. In the case of Blue Whiting, otoliths are stored dry in envelopes.

Sectioned otoliths mounted on custom microscope slides and stored in specific cases.

Demersal Species:

Whole otoliths are mainly stored in dried vials clearly labelled and set in cardboard boxes (paper envelopes are used specifically for discard surveys samplings).

Sectioned otoliths mounted on custom microscope slides are stored in paper boxes, labelled and set in cardboard boxes.

Vertebrae mounted on glass slides are stored in paper boxes, labelled and set in cardboard boxes.

Benthic Species:

Whole otoliths are stored dry in envelopes.

Sectioned illicia mounted on custom microscope slides and stored in specific cases.

Deep Species:

Whole otoliths are stored dry in envelopes.

Large pelagic species

Otolith sections are stored in vials clearly labelled

Spine sections are mounted on glass slides and covered with a highly transparent resin to fix the sections permanently and store them over time. The slides are labelled with and identification code.

- List of your saws (name, manufacturer, description) :

Annual Growth:

Otolith processing line. OTO-LABCUT 230F. Floor Standing Abrasive Cutting Machine complete with sectioning fixture and diamond wafering blade. Benetec Limited. CO Santander.

Isomet n. 396-IS11935- Buehler S/N221-S385VD. C. O. Vigo.

Electric Cutting Machine for Otoliths ARIEN-OTOCUT 150, with electroplated diamond wafering blade. C. O. Vigo; PILSES S.L., Vigo).

Low speed diamond saw ISOMET (BUEHLER), provided with smaller diamond wafering blades of 102 x 0.30 x 12.7 mm . (Series 15HC; Ref. 11-4244). Variable speed 0-300 rpm. C.O. Santander.

Linear precision saw "ISOMET 5000" provided with a diamond wafering blades of 200 x 0.9 mm (Series 15HC Ref:11-4279). Variable speed 200-5000 rpm. C.O. Santander.

Daily Growth:

Grinder-Polisher (Daily growth) Variable Speed Grinder-Polisher, MetaServ2000, Buehler. CO Santander.

Polishing machine BUEHLER, model PHOENIX BETA, two smoothing-polish stations. Variable speed 30-600 rpm.

- Description and characteristics of your resin(s) :

Annual Growth:

Eukitt. CO Santander and Coruña (Annual growth)

Mounting medium for substitutes of xylene DC (in order to avoid toxicity). 255811.0008 de Panreac. CO Vigo (Annual growth)

ENTELLAN (MERCK). CO Cadiz (Annual Growth)

Pre-accelerated polyester resin, black colorant and catalyst. CO Santander and CO Vigo.

Daily Growth:

Thermoplastic glue: Crystalbond mounting wax 40-8150. CO Santander (Daily Growth)

Mounting medium Modified (acrylic resin dissolved in H Isoparaffin) for substitutes of xylene DC (in order to avoid toxicity). Panreac. C. O. Vigo.

- A document presenting the summary of the techniques used (reference) :

Scomber scombrus:

Villamor, B., P. Abaunza and C. Fariña. 2004. Growth variability of mackerel (Scomber scombrus) off north and northwest Spain and a comparative review of the growth patterns in the northeast Atlantic. Fisheries Research 69, 107-121.

Villamor, B., M. Bernal and C. Hernandez. 2004. Models describing Mackerel (Scomber scombrus) early life growth in the North and Northwest of the Iberian Peninsula in 2000. Scientia Marina, 68 (4): 571-583.

Villamor, B. 2007. La caballa (Scomber scombrus, L. 1758) del atlántico nordeste: estudio biológico y de la población en aguas del norte y noroeste de la península ibérica. Tesis Doctoral (Mimeo), Universidad de Cantabria, Santander (Cantabria), 257 pp

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

Sardina pilchardus:

Silva, A., P. Carrera, J. Massé , A. Uriarte, M.B. Santos, P.B. Oliveira, E. Soares, C. Porteiro, Y. Stratoudakis 2008. Geographic variability of sardine growth across the northeastern Atlantic and the Mediterranean Sea. Fisheries Research 90: 56–69.

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

Engraulis encrasicolus:

Hernández, C., B. Villamor, P. Abaunza and J. Landa. 2009. Age and Growth of European anchovy (Engraulis encrasicolus) in the Bay of Biscay (NE Atlantic), 1994-2008. Working Document to ICES Workshop on Age reading of European anchovy (WKARA). Mazara del Vallo, Italy, 9–13 November 2009.

ICES, 2009. Report of the Workshop on Age reading of European anchovy (WKARA). ICES CM 2009/ACOM: 43, Mazara del Vallo (Sicilia, Italia) 9-3 noviembre 2009.

Trachurus trachurus:

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

ICES, 2006. Report of the Workshop on Age reading of Horse Mackerel. Netherlands, November

Micromesistius poutassou:

ICES, 2005. Report of the Blue Whiting Otolith Ageing Workshop. ICES CM 2005/ACFM, Hirtshals, Denmark 13-16 June 2005.

Merluccius merluccius

Metodología y técnicas usuales en la preparación de otolitos para la determinación de la edad de los peces. 1996. Informes técnicos Instituto Español de Oceanografía. N. 163.

Lepidorhombus whiffiagonis, Lepidorhombus boscii:

Landa, J., Piñeiro, C. and Pérez, N., 1996. Megrim (Lepidorhombus whiffiagonis) growth patterns in the northeast Atlantic. Fisheries Research. 26: 279-294.

Lophius piscatorius, Lophius budegassa:

Duarte, R., Landa, J., Quincoces, I., Dupouy, H., Bilbao, E., Dimeet, J., Marçal, A., McCormick, H. and Ni Chonchuir, G. 2002. Anglerfish Ageing Guide. In "Report of the 4th International Ageing Workshop on European Anglerfish". IPIMAR, Lisbon (Portugal). 40 pp.

Wright, P.J., Woodroffe, D.A., Gibb, F.M. and Gordon, J.D.M., 2002. Verification of first annulus formation in the illicia and otoliths of white anglerfish, Lophius piscatorius using otolith microstructure. ICES Journal of Marine Science., 59, 587-593.

La Mesa, M. and De Rossi, F., 2008. Early life history of the black anglerfish Lophius budegassa Spinola, 1807 in the Mediterranean Sea using otolith microstructure. Fisheries Research, 93(1-2), 234-239.

Pagellus bogaraveo:

Gil, J., 2006. Biología y pesca del voraz [Pagellus bogaraveo (Brünnich, 1768)] del Estrecho de Gibraltar. Tesis Doctoral Universidad de Cádiz.

Gil, J. and I. Sobrino, 2001. Studies on age determination and growth pattern of the Red (blackspot) seabream [Pagellus bogaraveo (Brünnich, 1768)] from the Strait of Gibraltar (ICES IXa/SW Spain): application to the species migratory pattern. NAFO SCR Doc 01/87

Thunnus thynnus:

Rodríguez-Marín, E., D. Olafsdottir, J. Valeiras, M. Ruiz, V. Chosson-Pampoulie and C. Rodríguez-Cabello. 2006. Ageing Comparison from Vertebrae and Spines of Bluefin Tuna (Thunnus thynnus) coming from the Same Specimen. Collective Volume of Scientific Papers, ICCAT, 59(3): 868-876.

Rodríguez-Marín, E., N. Clear, J.L. Cort, P. Megafonou, J.D. Neilson, M. Neves dos Santos, D. Olafsdottir, C. Rodriguez-Cabello, M. Ruiz, J. Valeiras. 2007. Report of the 2006 ICCAT Workshop for bluefin tuna direct ageing. (Instituto Español de Oceanografía, Santander, Spain, 3-7 April 2006). Collective Volume of Scientific Papers, ICCAT, 60(4): 1349-1392.

Ruiz, M., E. Rodríguez-Marín and J. Landa. 2005. Protocol for Sampling of Hard Parts for Bluefin Tuna (Thunnus thynnus) Growth Studies. In: Rodríguez-Marín, E. 2005. Report of the Bluefin Tuna Direct Ageing Network (under the BYP Framework). Collective Volume of Scientific Papers, ICCAT, 58(4): 1414-1419.

Xiphias gladius

Berkeley, S. A., and E. D. Houde. Age determination of broadbill wordfish, Xiphias gladius, from the Straits of Florida, using anal fin spine sections. In Prince, E.D. and L.M.Pulos (eds.). 1983. Proceeding of the international workshop on age determination of oceanic pelagic fishes:tunas, billfishes,and sharks, U.S. Dep. Commer. NOAA Tech.rep.NMFS-8.

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year) :

Lophius piscatorius, Lophius budegassa:

Currently there is not a bilateral agreement, but from 1996 to 2006 IEO (CO Santander, Spain) and IPIMAR (Lisbon, Portugal) had a bilateral agreement for age estimation of Lophius piscatorius and Lophius budegassa from the Southern Stock (ICES Div. VIIIc and IXa). IEO received annually all illicia of L.piscatorius collected by IPIMAR (ICES Div. IXa) and IPIMAR received annually all illicia of L.budegassa collected by IEO (ICES Div. VIIIc). The reference (expert) readers for L.piscatorius were those of IEO (CO Santander, Spain) and aged the illicia of L.piscatorius of whole Southern stock (ICES Div. VIIIc and IXa). The reference (expert) readers of L.budegassa were those of IPIMAR (Lisbon, Portugal) and aged the illicia of L.budegassa of whole Southern Stock (ICES Div. VIIIc and IXa). This agreement supported the specialisation of the readers in the age estimation of each species, due to only the expert readers from each reference institution aged illicia. This specialisation possibly increased the precision in the estimated ages.

The illicia of Lophius piscatorius and Lophius budegassa have not been aged since 2007 due to inconsistencies found in cohort tracking of both species. It was mainly due to the age estimation criterion was biased. When new (and not biased) age estimation criteria are available and the age estimations can begin again for both species, this bilateral agreement could be re-started again between both institutions.

Thunnus thynnus: Vertebrae and first dorsal fin spines from Island and Portugal, Otoliths and first dorsal fin spines from Canada and USA.

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year):

Lophius piscatorius, Lophius budegassa:

Explained in question 16.

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible) :

Scomber scombrus from VIIIc and IXa ICES Divisions:

Villamor, B. 2009. Caballa. Pág. 100-109 en: Instituto Español de Oceanografía, Ministerio de Ciencia e Innovación, editor(es). Estudios de marcado y recaptura de especies marinas (263 pp.). , Madrid (España).

Villamor, B. 2007. La caballa (Scomber scombrus, L. 1758) del atlántico nordeste: estudio biológico y de la población en aguas del norte y noroeste de la península ibérica. Tesis Doctoral (Mimeo), Universidad de Cantabria, Santander (Cantabria), 257 pp

Uriarte, A., P. Alvarez, S. Iversen, J. Molloy, B. Villamor, M.M. Martins and S. Myklevoll. 2001. Spatial pattern of migration and recruitment of North East Atlantic Mackerel. ICES C.M. 2001/O:17. ICES Annual Science Conference. Oslo (Noruega) 26-28/09/2001.

Merluccius merluccius from VIIIc, IXa ICES Divisions:

Piñeiro, C., Rey, J., de Pontual, H., and Goñi, R. 2007. Tag and recapture of European hake (Merluccius merluccius L.) off the Northwest Iberian Peninsula: First results support fast growth hypothesis. Fisheries Research, 88:150 – 154.

Lophius piscatorius:

Pereda, P. y Landa, J. 1997. Recuperación de dos ejemplares de rape blanco (Lophius piscatorius Linnaeus, 1758) en el stock norte (Divisiones VIIIa y b del CIEM) tras ser marcados en el stock sur (División VIIIc). Boletín del Instituto Español de Oceanografía. 13 (1 y 2): 75-78.

Landa, J., Quincoces, I., Duarte, R., Fariña, C. and Dupouy, H. 2008. Movements of black and white anglerfish (Lophius budegassa and L. piscatorius) in the northeast Atlantic. Fisheries Research, 94: 1-12.

Landa, J., Duarte, R. and Quincoces, I. 2008. Growth of white anglerfish (Lophius piscatorius) tagged in the Northeast Atlantic, and a review of age studies on anglerfish. ICES Journal of Marine Science, 65: 72-80.

Lophius budegassa:

Landa, J., Quincoces, I., Duarte, R., Fariña, C. and Dupouy, H. 2008. Movements of black and white anglerfish (Lophius budegassa and L. piscatorius) in the northeast Atlantic. Fisheries Research, 94: 1-12.

Pagellus bogaraveo:

Gil, J., J. Canoura e I. Sobrino, 2009. Besugo. En: Estudios de marcado y recaptura de especies marinas. Temas de Oceanografía, 2 (IEO-MICINN) (ISBN 978-84-95877-47-3).

Gil J., L. Silva and I. Sobrino, 2001. Results of two Tagging Surveys of red seabream [Pagellus bogaraveo (Brunnich, 1768)] in the Spanish South Mediterranean Region. Thalassas, 17.

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible) :

Lepidorhombus whiffiagonis:

Landa, J. and Piñeiro, C. 2000. Megrim (Lepidorhombus whiffiagonis) growth in the North-eastern Atlantic based on back-calculation of otolith rings. ICES Journal of Marine Science, 57: 1077-1090.

Thunnus thynnus:

Neilson, J.D. and Campana, S.E. 2008. A validated description of age and growth of western Atlantic bluefin tuna (Thunnus thynus). Canadian Journal of fisheries and Aquatic Sciences, 65: 1523-1527.

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible) :

Engraulis encrasicolus from ICES Subarea VIII:

Hernández, C., B. Villamor, J. Barrado, C. Navarro, C. Dueñas. 2009. Preliminary results on first check validation in European anchovy (Engraulis encrasicolus) otoliths. Working Document to ICES Workshop on Age reading of European anchovy (WKARA). Mazara del Vallo, Italy, 9–13 November 2009

- Do you use image capture/analysis software, if so, which? Yes

The microstructure analyses were carried out using a light microscope applied to an image analyser (Visilog 6.4- TNPC 4.2, Ifremer, France), at ×100–1000 magnification. Oil immersion was required for the higher magnification. CO Santander

To take measurements for validation studies: Image Analysis Software (NIS-Elements-D 3.0). CO Santander

To take measurements for validation studies: Image Analysis Software (NIS Elements BR Version 2.10). CO Vigo

Image Analysis System OTO v3, software designed by Andersen and Moksness (1988). CO Vigo

Image Analysis Software (TNPC; Fablet and Ogor, 2005). CO Vigo

Only for a certain work of shape analysis: Mª M. Padillo, J. Gil and A. Mª. Juárez, 2011. Morphometric and morphological studies of Red seabream (Pagellus bogaraveo) otoliths from the Strait of Gibraltar: Exploratory analysis of its application for ageing. Working Document (17) presented to the 2011 ICES WGDEEP has been used NIS-Elements AR 3.2 NIKON Software. CO Cádiz

- Which type of camera do you use (digital or analogue, description, characteristics)?

Video Camera. Sony Digital Interface-DFW-SX910. CO Santander

Color Digital Camera. NIKON Digital Sigth.DS-5M.CO Santander

Color Digital Camera. NIKON DS-2Mv. CO Vigo

Color Digital Camera. Sony DFW-SX910. CO Vigo

Color Digital camera NIKON DMX-1200C. CO Cádiz

- Which applications is image processing used for (routine, only exchanges, validation studies…) ?

For daily growth studies routine: VISILOG 6.4- TNPC 4.2. CO Santander

For Validation of annual growth routine: NIS-Elements-D Version 3.06. CO Santander

For otolith/illicia Exchanges: Gimp 2.6; Paint shop Pro 9.

TNPC 4/Visilog 6.3, for routine otolith reading and validation studies. CO Vigo

GIMP 2.6, (free available) for otolith exchange. CO Vigo

For digital image capture and its analysis has been used NIS-Elements AR 3.2 NIKON Software. SHAPE: A Computer Program Package for Quantitative Evaluation of Biological Shapes Based on Elliptic Fourier Descriptors was also been used. CO Cádiz

- How many images are made per year on average?

Around 2735 images.

- Do you calibrate your images?

Always.

- Which measurements do you make on the image (distance between rings…) ?

Daily growth studies: Distance between rings and from the nucleus to the otolith edge

Annual growth studies:

Engraulis encrasicolus: (Validation of first ring and recruitment studies) Total diameter, Nucleus-Postrostrum edge radius, Nucleus-Antirostrum edge radius, Nucleus to L1, L2 and/or checks when possible.

Lophius piscatorius, Lophius budegassa: First false annuli horizontal diameter; first annuli horizontal diameter.

Demersal Species: Total length of otoliths, Total height, Distances from the nucleus to the otolith edge of whole otolith and section otoliths. Distances from the nucleus to every ring considered for annual ageing. Distances from the nucleus to the checks when it is necessary as reference rings (R1, R2 and/or).

Deep Species: Morphometrics variables taken in account are: weigth (precision scale), thickness and curvature (gauge) and others by image analysis as: Area, Maxferet, MinFeret, EqDiameter, Circularity.

Large pelagics: Thunnus thynnus translucent band maximum diameters from spine sections. - Xiphias gladius: distance from focus to the most distal part of the ring and radius of the structure section.

- What is the format of images?

Visilog Files(*.im5,*.im6), TIFF files(*.tif), JPEG files(*.jpg), Also jp2 (jpeg2000) files, JASC PAINT SHOP PRO (*.psp), GIMP files (*.xcf), BMP files (*.bmp), Adobe Photoshop (*.psd).

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

Pelagic Species

Annual Growth:

Engraulis encrasicolus, Sardina pilchardus, Scomber scombrus, Scomber colias:

Otolith sampling by month and ICES Subdivision.

Whole otoliths were mounted on a blackened background, covered with resin, and illuminated by reflected light.

A binocular microscope was used to observe the banding pattern (20x or 40x magnifications, depending on otolith size).

Two experienced readers aged the otoliths. The readings for a given otolith were accepted only if both agreed. When there was a discrepancy between the two readings, a third reading was carried out. Unreadable otoliths were rejected.

Age was estimated by interpreting and counting growth rings on the otoliths. Methodological ageing procedures described in ICES Workshops were followed.

An age length key was elaborated by month, quarter, semester and year. Mean lengths at age and the corresponding standard deviations were calculated.

Trachurus trachurus, Trachurus mediterraneus:

Otolith sampling by month and ICES Subdivision.

<25cm fish length: Whole otoliths were placed in alcohol and glycerine, to read under a microscope and illuminated by reflected light

>25cm fish length: Sectioned otoliths:

Embedding the otoliths in black polyester resin and mounting them for sectioning

Sectioning otoliths using the high speed cutting machine

Mounting cut sections of otoliths onto glass slides.

A binocular microscope was used to observe the banding pattern (20x or 40x magnifications, depending on otolith size).

A specific experienced reader aged the otoliths and each otolith was read twice, on two separate occasions. The readings for a given otolith were accepted only if both agreed. When there was a discrepancy between the two readings, a third reading was carried out. Unreadable otoliths were rejected.

Age was estimated by interpreting and counting growth rings on the otoliths. Methodological ageing procedures described in ICES Workshops were followed.

An age length key was elaborated by month, quarter, semester and year. Mean lengths at age and the corresponding standard deviations were calculated.

Micromesistius poutassou:

Otolith sampling by month and ICES Subdivision.

Whole otoliths were placed in water, to read under a microscope and illuminated by reflected light.

A binocular microscope was used to observe the banding pattern (20x or 40x magnifications, depending on otolith size).

A specific experienced reader aged the otoliths and each otolith was read twice, on two separate occasions. The readings for a given otolith were accepted only if both agreed. When there was a discrepancy between the two readings, a third reading was carried out. Unreadable otoliths were rejected.

Age was estimated by interpreting and counting growth rings on the otoliths. Methodological ageing procedures described in ICES Workshops were followed.

An age length key was elaborated by month, quarter, semester and year. Mean lengths at age and the corresponding standard deviations were calculated.

Daily growth:

Engraulis encrasicolus:

Otolith sampling of juveniles in the second part of the year (recruitment season).

To obtain daily increment counts otolith were processed along the sagittal plane with respect to the fish.

The otoliths were mounted in thermoplastic glue (CrsytalBond) and fixed to a glass slide.

Thin sagittal sections were produced by grinding them using.

The otoliths were observed under a microscope applied to an image analyzer (Visilog 6.4/ TNPC 4.2).

For reading the growth rings were used for the oil immersion objective (x100).

Each otolith was read 2 to 3 times until a consistent age. We measured the otolith radius and counted the number of growth increments and size of each increment.

Demersal Species

Procedures for:

Otolith sampling

Embedding the otoliths in black polyester resin and mounting them for sectioning

Sectioning otoliths using the high speed cutting machine

Mounting cut sections of otoliths onto glass slides.

Training in age determination

Use of IMS for otolith age determination

Age-length key elaboration.

Annual Growth:

Species: Merluccius merluccius1; Glyptocephalus cynoglossus , Helicolenus dactylopterus, Molva molva, Aspitrigla cuculus, Trisopterus luscus, Conger conger, Molva macrophthalma, Phycis blennoides, Pollachius pollachius, Microchirus variegatus,.

Otolith samplings by year and ICES Area (by quarter for Trisopterus luscus, by quarter and ICES Division for Merluccius merluccius1). Vertebrae of Conger conger are also collected in samplings.

Glyptocephalus cynoglossus, Helicolenus dactylopterus, ≤80cm Molva molva, ≤30cm Aspitrigla cuculus specimen length. Whole otoliths are placed on a blackened background, covered with water, to read under a microscope and illuminated by reflected light.

Trisopterus luscus, Conger conger, Molva macrophthalma, Phycis blennoides, Pollachius pollachius, Microchirus variegatus, Merluccius merluccius1, Helicolenus dactylopterus2, >80cm Molva molva, >30cm Aspitrigla cuculus specimens length. Sectioned otoliths:

Embedding the otoliths in black polyester resin and mounting them for sectioning.

Sectioning using the high speed cutting machine.

Mounting cut sections of otoliths onto glass slides.

Vertebrae (Conger conger): Attaching the vertebrae to a glass slide, placed on a blackened background, and covered with a glycerine and ethanol solution, to read under a microscope and illuminated by reflected light.

A binocular microscope is used to observe the banding pattern. Magnification is 10x for Glyptocephalus cynoglossus, and 10x-20x for Helicolenus dactylopterus, depending on otolith size. 20x magnifications are used for sectioned otoliths, 8-10x for vertebrae.

Two experienced readers aged the otoliths and vertebrae. The readings are accepted only if both agreed. A third reading is carried out when there is discrepancy between the previous one. Unreadable structures are rejected.

Age is estimated by interpreting and counting growth rings on the calcified structures.

An age length key is elaborated by year for each species (by semester and year for Trisopterus luscus). Mean lengths at age and standard deviations are calculated.

Daily growth:

Species: Merluccius merluccius1

Otolith sampling of juveniles at the surveys conducted in Spring and Autumn.

To obtain daily increment counts otolith are processed along the transversal plane of the otolith.

Otoliths are embedded in polyester resin.

Transversal sections are produced by using an ISOMET saw machine.

Sections are grounding with sandpaper and a graded series of aluminium between 400 and 1200 μ (BUEHLER PHOENIX Beta 6227-PXB-22251)

The micro increment sequence is counted along the ventral axis from the otolith centre to the edge using a range of magnification (250-400X).

The otoliths are observed under a microscope applied to an image analyzer (Visilog 6.4/ TNPC 4.2).

Each otolith is read 2 to 3 times until a consistent age. Otolith radius and increment size are measured and the number of growth increments are counted.

Benthic Species

Annual growth:

Lophius piscatorius

Illicia sampling by month (or quarter) and stock.

Embedding the illicia in black polyester resin and mounting them for sectioning.

Sectioning illicia using the high speed cutting machine.

Mounting cut sections of illicia onto glass slides.

A microscope or a profile projector is used to observe the banding pattern (at 40-50x magnifications).

A specific experienced reader age the illicia. Each illicium is read twice, on two separate occasions. The age estimation for a given illicium is accepted only if both agreed. When there is a discrepancy between both age estimations, a third reading is carried out. Unreadable illicia are rejected.

Age is estimated by interpreting and counting the supposed annual increments on the illicia. Methodological age estimation criterion is currently in revision.

Lophius budegassa

Illicia sampling by month (or quarter) and stock.

Embedding the illicia in black polyester resin and mounting them for sectioning.

Sectioning, mounting and observation of illicia, and methodological age estimation procedures are currently in revision.

Lepidorhombus whiffiagonis, Lepidorhombus boscii

Otolith sampling by month (or quarter) and stock.

Whole otoliths are placed in water, read under a binocular microscope (at 10-15x magnifications) under reflected light.

A specific experienced reader age the otoliths. Each otolith usually is read twice, on two separate occasions. The age estimation for a given otolith is accepted only if both agreed. When there is a discrepancy between both age estimations, a third reading is carried out. Unreadable otoliths are rejected.

The age is estimated by interpreting and counting growth increments on the otoliths. Methodological ageing procedures described in ICES Workshops are followed.

An age-length-key is elaborated by quarter and/or semester (if there is enough sample number) and year. Mean lengths at age and the corresponding standard deviations are calculated.

Daily growth:

Lophius budegassa

Otolith sampling of juveniles in the second part of the year (recruitment season).

To obtain daily increment counts, otoliths are processed along the sagittal plane with respect to the fish.

The otoliths are mounted in thermoplastic glue (CrsytalBond) and fixed to a glass slide.

Thin sagittal sections are produced by grinding them using automated polishing wheels.

The otoliths are observed under a microscope applied to an image analyzer (Visilog 6.4/ TNPC 4.2).

An oil immersion objective (x100) is used for interpretation of the daily growth increments.

Each otolith is read 2 to 3 times until obtaining a consistent age. We measure the otolith radius and count the number of growth increments and width of each increment.

Deep Species

Annual growth:

Pagellus bogaraveo

Otolith sampling by month (or quarter) and stock.

Whole otoliths are placed in water, read under a binocular microscope (at 10-15x magnifications) under reflected light.

Two experienced readers age the otoliths. Each otolith is read twice, on two separate occasions. The age estimation for a given otolith is accepted only in case of 3 (or more) coincident readings.

An age-length-key is elaborated for the whole year. Mean lengths at age and its standard deviations are calculated.

- Do you have an internal quality control (description, references) ?

Yes, we do periodically intercalibration exercises inter readers

- Do you have quality management certification e.g. ISO 9001 :2008?

No

- Are you accredited (yes or no, name of accreditation authority)?

No

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

Otolith Exchanges:

European Anchovy Otolith Exchange during 2009

Blue Whiting Otolith Exchange 2009

Hake Otolith Exchange Programme in 2009.

Mackerel Otolith Exhange during 2009-2010

Sardine Otolith Exchange during 2010

Megrim otolith exchange in 2010.

Anglerfish (Lophius piscatorius) illicia and otoliths exchange in 2011.

WKAEH 11, currently running by using WebGR.

Otolith Workshops:

ICES Workshop on Age reading of European anchovy (WKARA). Mazara del Vallo, Italy, 9–13 November 2009.

Workshop on Age Estimation of European Hake (WKAEH) in IEO CO Vigo. 2009.

Workshop on Age Estimation of Monkfish (Lophius vomerinus) in IEO CO Santander. 2010.

ICES Workshop on Age Reading of Mackerel (WKARMAC) 1-4 November 2010, Lowestoft, UK

ICES Workshop on Age Reading of sardine(WKARAS) 14-18 February 2011, Lisbon

- Are all your age readers aware of WebGR?

Yes.

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

Only Merluccius merluccius.

- Your name: Magnus Kokkin
- Your country: **Sweden**
- Your institute: **Swedish University of Agricultural Science, Department of Aquatic Resources, Institute of Freshwater Research**
- Number of treated calcified structures in 2010: 1509 (salmon), 301 (sea trout)
- List of your species (latin name): Salmo salar, Salmo trutta
- Your geographical areas: Baltic sea, Swedish westcoast
- How many laboratories analyse calcified structures in your country? 3
- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify):

Stock assessment (85%) , complement to genetic studies (15%).

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.): scales (dry). Mounted on acetate plates or unmounted. Microfiche.
- List of calcified structures that you use: scales
- How are calcified structures stored before treatment? In papers bags in the laboratory
- How are calcified structure preparations stored? In a scales-archive.
- List of your saws (name, manufacturer, description): Struers Accutom-50.
- Description and characteristics of your resin(s): Tacky and non-tacky polyester resin, black.
- A document presenting the summary of the techniques used (reference) : Internal document.
- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year): Finland, Salmon (300 individuals/year)
- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year):
- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible): -
- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible): -
- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible) : -
- Do you use image capture/analysis software, if so, which? Leica Application Suite.
- Which type of camera do you use (digital or analogue, description, characteristics)? Digital camera, Leica DFC295 with a Leica M80 stereo microscope.

- Which applications is image processing used for (routine, only exchanges, validation studies…) ? Routine, exchanges, own reference archive.

- How many images are made per year on average? 300 (salmon and trout)

- Do you calibrate your images? No

- Which measurements do you make on the image (distance between rings…) ? None.

- What is the format of images? TIF or JPG

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

Sampling of the commercial salmon catches, and additionally caught sea trout, in the coastal métier (FPO_ANA_0_0_0) was carried out by fisherman in the Gulf of Bothnia (ICES sub-divisions 30-31). Collected data include length, weight and sex of individual fish. Scales are collected from all fish in the samples to determine age, wild or reared origin as well as use in genetic studies.

Sampling of the recreational salmon and sea trout catches was carried out during the fishing season in two rivers in the Gulf of Bothnia and one river in the Main Basin. The monitored variables include smolt age, sea-age, sex, origin (wild/reared) and size at capture (weight and length). These data are an integral part of the assessment of the spawning run composition and the effects of the fishery. Data on fecundity was collected by a recreational brood stock fishery in River Dalälven, Sub-division 30.

Sampling of the commercial salmon catches, and additionally caught sea trout, in the off shore métier (LLD_ANA_0_0_0) was carried out in the main Baltic (ICES subdivision 25-26). Fish were sampled at landing in harbours and carried out by SBF personal. All fish sampled included collection of scales, length and weight of individual fish. Since all individuals of salmon and trout were gutted off shore, collection of data on sex was not possible.

Age estimation: Shearer. 1992. Atlantic salmon scale reading. Report of the Atlantic salmon scale reading workshop. Internal documents.

Preparation: Mounted (acetate plates) or unmounted (dry) scales.

Data storage: Database (access). Internal document/guide about how to input data.

- Do you have an internal quality control (description, references) ? Under development. At the moment intercalibration between the (2) readers, and some image-exchange with other salmon readers.

- Do you have quality management certification e.g. ISO 9001 :2008?

- Are you accredited (yes or no, name of accreditation authority)? No

- What age calibration Exchanges or Workshops has your country participated in during the last three years? SGSAD 2008, WKADS 2011

- Are all your age readers aware of WebGR? Yes

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? Not at the moment.

- Your name: Francesca Vitale and Yvonne Walther
- Your country: **Sweden**
- Your institute: **Swedish University of Agricultural Sciences, Department of Aquatic Resources, Institute of Marine Research**
- Number of treated calcified structures in 2010:
- List of your species (latin name): ~32000

Gadus morhua

Clupea harengus

Sprattus sprattus

Pleuronectes platessa

Melanogrammus aeglefinus

Trisopterus esmarkii

Pollachius virens

Glyptocephalus cynoglossus

- Your geographical areas : North Sea (ICES SD 20,21,23) and Baltic (ICES SD 24-30)
- How many laboratories analyse calcified structures in your country?
- Within the Swedish University of Agricultural Sciences,

Department of Aquatic Resources there are 3 laboratories dealing with age reading (Institute of Marine Research, Institute of Coastal Research, Institute of Freshwater Research).

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify):

90% Stock assessment and 10% genetic studies (not routinely)

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.) :

Most of the otoliths are read either whole or broken (see excel file)

Scales are read whole

- List of calcified structures that you use:

Otoliths

Scales

- How are calcified structures stored before treatment?

They are stored in a paper bag, at an archive certified by the National Archives of Sweden.

- How are calcified structure preparations stored?

At a certified archive.

- List of your saws (name, manufacturer, description):

Struers Accutom 50

- Description and characteristics of your resin(s):

Norpol Black fillable casting resin with black pigment.

- A document presenting the summary of the techniques used (reference) :

Internal Swedish Board of Fisheries protocol for sampling, age reading and sample storage (CfÅ; in Swedish, to be completed during 2011).

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year):

Denmark: witch flounder (Glyptocephalus cynoglossus) 50-100/year

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year) :

Denmark: Norway pout (Trisopteru esmarkii) 200-300/year

Sole (Solea solea) 100/year

Witch flounder (Glyptocephalus cynoglossus) 50-100/y

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible) : -

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible) : -

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible) : -

- Do you use image capture/analysis software, if so, which?

Yes, not routinely only to assist the age reading.

The software is Corel Paint Shop Pro Photo x2

- Which type of camera do you use (digital or analogue, description, characteristics)?

Leica DFC 320

- Which applications is image processing used for (routine, only exchanges, validation studies…)?

By the time being only exchanges and inter-reader calibration exercises to assist the age reading.

- How many images are made per year on average? Less than 100

- Do you calibrate your images? No

- Which measurements do you make on the image (distance between rings…) ? -

- What is the format of images? Tiff or jpeg

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

Sampling: All the species are sampled as part of the National Programme under the requirements of the EC Data Collection Framework (DCF), both on board of commercial vessels and during ICES internationally coordinated surveys.

Preparation: The otoliths are read either whole or broken:

Whole otoliths: In some species (such as for example, trout and herring / Baltic herring) otoliths can be analyzed without further preparation. This is usually done by placing otoliths in a dish containing a medium (propanediol, ethanol or water) and then examined under the stereo microscope with above lighting. Another method is to fix the otoliths on a microscope slide (for example, with nail polish).

Broken otoliths: otoliths as in cod and haddock are split in half right through the centre with pliers or by hand, the fracture surfaces are made wet and then observed at the stereo microscope.

Data storage: Data are stored in the national database and then submitted to the ICES database.

- Do you have an internal quality control (description, references) ?

At present we only do inter-readers calibration exercises for testing new readers.

An internal quality control program it is planned to start next year.

- Do you have quality management certification e.g. ISO 9001:2008? No

- Are you accredited (yes or no, name of accreditation authority)? No

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

WKARBH 2008; WKARBS 2008; WKARP 2010

- Are all your age readers aware of WebGR?

Yes

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

Not yet, is in the future plans.

- Your name : Sally Songer/ Mark Etherton
- Your country: **UK (England)**
- Your institute: **Cefas**
- Number of treated calcified structures in 2010: 51600
- List of your species (Latin name):

COD COD GADUS MORHUA

HADDOCK HAD MELANOGRAMMUS AEGLEFINUS

EUROPEAN HAKE HKE MERLUCCIUS MERLUCCIUS

POLLACK POL POLLACHIUS POLLACHIUS

SAITHE POK POLLACHIUS VIRENS

SOLE (DOVER SOLE) SOL SOLEA SOLEA (S.VULGARIS)

WHITING WHG MERLANGIUS MERLANGUS

(EUROPEAN) MACKEREL MAC SCOMBER SCOMBRUS

ANGLERFISH (MONK) MON LOPHIUS PISCATORIUS

ANGLERFISH (MONK) WAF LOPHIUS BUDEGASSA

RED MULLET MUR MULLUS SURMULETUS

NORWAY POUT NOP TRISOPTERUS ESMARKI

POOR COD POD TRISOPTERUS MINUTUS

THICKBACK SOLE TBS MICROCHIRUS VARIEGATUS

TUB GURNARD TUB TRIGLA LUCERNA

TURBOT TUR SCOPHTHALMUS MAXIMUS

BLUE WHITING WHB MICROMESISTIUS POUTASSOU

WITCH WIT GLYPTOCEPHALUS CYNOGLOSSUS

WHITING-POUT (BIB) BIB TRISOPTERUS LUSCUS

BRILL BLL SCOPHTHALMUS RHOMBUS

EUROPEAN SEABASS ESB DICENTRARCHUS (MORONE) LABRAX

CONGER EELS COX CONGRIDAE

DAB DAB LIMANDA LIMANDA

FLOUNDER (EUROPEAN) FLE PLATICHTHYS FLESUS

GREY GURNARD GUG EUTRIGLA GURNARDUS

RED GURNARD GUR ASPITRIGLA CUCULUS

STREAKED GURNARD GUS TRIGLOPORUS LASTOVIZA

HERRING HER CLUPEA HARENGUS

EUROPEAN PLAICE PLE PLEURONECTES PLATESSA

JOHN DORY JOD ZEUS FABER

LEMON SOLE LEM MICROSTOMUS KITT

COMMON LING LIN MOLVA MOLVA

MEGRIM MEG LEPIDORHOMBUS WHIFFIAGONIS

- Your geographical areas:

- ICES divisions VII, VI + IV

- How many laboratories analyse calcified structures in your country? 1

- How is the age data used e.g. stock assessment and small amount of work on validation.

- Description of your types of calcified structure preparation Whole otoliths, sectioned otoliths set in polyester resin and mounted on slides, sectioned otoliths set in polyester resin and stained with neutral red, broken and burnt otoliths, whole otoliths set in clear resin on custom plastic slides, whole bass scales.

- List of calcified structures that you use: otoliths and scales

- How are calcified structures stored before treatment? Dry in paper packets or plastic trays.

- How are calcified structure preparations stored? On slides with cover slips on in plastic wallets.

- List of your saws (name, manufacturer, description):

1x Struers Accutom 50

1x Beacon V4

1x Struers Accutom 5

- Description and characteristics of your resin(s) :

Clear casting resin

Black polyester fillable resin

- A document presenting the summary of the techniques used (reference): See documents of Standard Operating Procedure

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year): hake from Scotland ~1000 per annum. VIIa Cod from Belgium ~ 600

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year): brill and turbot to Belgium ~ 200 per annum.

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible): Not aware of any current age determination related tagging programmes.

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible): marginal increment analysis for lemon sole, ongoing.

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible): herring (black water and North Sea), eels.

- Do you use image capture/analysis software, if so, which? Yes – ProgRes CapturePro 2.7, TNPC, GIMP

- Which type of camera do you use (digital or analogue, description, characteristics) Digital, Jenoptik Laser Optic Systeme GMBH. Attached to high power microscope.

- Which applications is image processing used for (routine, only exchanges, validation studies…) exchanges, validation studies, other projects e.g. AFISA. Not used routinely.

- How many images are made per year on average? This last year over 1000 due to lemon sole study.

- Do you calibrate your images? Calibrated at installation. Graticule used to calibrate any measurements made on images.

- Which measurements do you make on the image (distance between rings…) distance between rings, nucleus size.

- What is the format of images? Tif, xcf.

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species: many documents

- Do you have an internal quality control (description, references)? Yes

- Do you have quality management certification e.g. ISO 9001:2008? Yes ISO 17025

- Are you accredited (yes or no, name of accreditation authority)?

Yes - UKAS

- What age calibration Exchanges or Workshops has your country participated in during the last three years? Mackerel, dab, anglerfish, plaice, hake, sole VIId, North sea cod, Celtic and Irish sea cod, flounder, turbot, red mullet, saithe.

- Are all your age readers aware of WebGR? Some are.

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? Currently we do not use images routinely for age determination.

- Your name: Eleonora Ciccotti, Fabrizio Capoccioni, Chiara Leone

- Your country: **Italy**

- Your institute: **Laboratory of Experimental Ecology and Aquaculture - Department of Biology - University of Rome "Tor Vergata"**

- Number of treated calcified structures in 2010: 1400

- List of your species (latin name): Anguilla anguilla

- Your geographical areas: Mediterranean

- How many laboratories analyse calcified structures in your country? One (as concerns Anguilla anguilla)

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify): Growth and Stock assessment

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.) :

Grinding and polishing method (otoliths are embedded in resin, mounted on microscope slides and grinded on the sagittal plane in order to reach the primordium)

Cracking and bourning method (otoliths are cut along the frontal plane, then burned in a flame to reveal the annuli on the broken face of the otolith

- List of calcified structures that you use: sagittae

- How are calcified structures stored before treatment? Otoliths are stored dry in unsealed eppendorf tubes

- How are calcified structure preparations stored? Mounted on slides

- List of your saws (name, manufacturer, description) : surgical blade for B&C method

- Description and characteristics of your resin(s): 3 component resin (Agar Scentific);

- A document presenting the summary of the techniques used (reference):

ICES. 2009. Report of the Working Group on the Age Reading of European and American eel (WKAREA), 20–24 April 2009, Bordeaux, France. ICES CM 2009/ACFM:48. International Council for the Exploration of the Seas, Copenhagen.

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year):

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year) :

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible):

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible):

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible):

No specific validation an intercalibration methods exist at national level. All validation methods are carried out with the international groups involved in the WKAREA workshops.

References

Capoccioni, F., Costa, C., Aguzzi, J., Menesatti, P., Lombarte, A. & Ciccotti, E. (2010) Ontogenetic and environmental effects on otolith shape variability in three Mediterranean European eel (Anguilla anguilla, L.) local stocks. Journal of Experimental Marine Biology and Ecology, 397, 1-7.

- Do you use image capture/analysis software, if so, which?

Image pro Plus vers. 6.0 Media Cybernetics, Inc.

- Which type of camera do you use (digital or analogue, description, characteristics)? LeicaMZ8

- Which applications is image processing used for (routine, only exchanges, validation studies…) ? Calculation of annuli increments, extraction of outline coordinates for shape analysis

- How many images are made per year on average? 1000

- Do you calibrate your images? No

- Which measurements do you make on the image (distance between rings…) ? Radius (nucleus to postrostrum), distance between rings, shape (EFA)

- What is the format of images? JPG and TIFF

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species :

Sampling: Otoliths are currently collected from commercial catches (fyke nets) and scientific surveys.

Preparation: Otoliths are cleaned in distilled water, dried and stored in eppendorf.

Macro-increment analysis: Sagittae are processed with two different methods described above.

Age estimation: Readings were repeated at least three times with a reasonable time lapse by 2 o 3 different operators.

Data storage: All data are recorded using Office Package and processed by various statistical packages.

- Do you have an internal quality control (description, references) ? YES (multiple readings of the same operator after 2or3 weeks, and multiple readers).

- Do you have quality management certification e.g. ISO 9001 :2008?

- Are you accredited (yes or no, name of accreditation authority)?

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

Workshop On Age Reading Of European And American Eel (Wkarea), Bordeaux, France. 20-24 April, 2009.

Workshop On Age Reading Of European And American Eel (Wkarea2), Bordeaux, France 22-24 March 2011.

- Are all your age readers aware of WebGR? No

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? YES

- Your name : Mandy Gault
- Your country : **Scotland**
- Your institute : **Marine Scotland**
- Number of treated calcified structures in 2010 : 40,000
- List of your species (latin name) :

Pollachius virens

Melanogrammus aeglefinus

Melangius merlangus

Gadus morhua

Lepidorhombus whiffiagonis

Lophius piscatorius

Clupea harengus

Scomber scombrus

Sprattus sprattus

Hyperoplus lanceolatus

- Your geographical areas : VI a, VI b, IV a, IV b
- How many laboratories analyse calcified structures in your country? 3
- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify): Stock Assessment
- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.) :

Broken otolith mounted in plastercine, whole otolith in black trays in water and whole otolith in black trays in ethanol.,

- List of calcified structures that you use: Otoliths
- How are calcified structures stored before treatment? Gadoids and Megrims in packets, Pelagic in black trays, Anglers in tubes of water
- How are calcified structure preparations stored? Gadoids on mounts Others in trays.
- List of your saws (name, manufacturer, description) : Beuhler isomet saw.
- Description and characteristics of your resin(s) : Not applicable
- A document presenting the summary of the techniques used (reference) : not applicable
- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year): None
- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year): None

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible): Not applicable

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible): Not applicable

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible) : Not applicable

- Do you use image capture/analysis software, if so, which?

Something we have looked at but are unable to put into action due to staff and time restrictions in current climate.

- Which type of camera do you use (digital or analogue, description, characteristics)?

- Which applications is image processing used for (routine, only exchanges, validation studies…) ?

- How many images are made per year on average?

- Do you calibrate your images?

- Which measurements do you make on the image (distance between rings…) ?

- What is the format of images?

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species: See manuals (to be forwarded at end of month)

- Do you have an internal quality control (description, references) ?

- Yes, it takes the form of a quarterly internal exchange between readers on the four main gadoid species.

- Do you have quality management certification e.g. ISO 9001:2008? NO

- Are you accredited (yes or no, name of accreditation authority)? NO

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

Mackerel Exchange and workshop in Lowestoft England 2010

Small scale haddock exchange 2008

Cod exchange 2009

Currant Angler otolith exchange

- Are all your age readers aware of WebGR? Yes

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images? Possibly in the future.

- Your name: Dr. Christoph Stransky

- Your country: **Germany**

- Your institute: **Johann Heinrich von Thünen Institute (vTI), Institute of Sea Fisheries (SF)**

- Number of treated calcified structures in 2010: 35,000

- List of your species (latin name): Clupea harengus, Engraulis encrasicolus, Gadus morhua, Limanda limanda, Melanogrammus aeglefinus, Merlangius merlangus, Micromesistius poutassou, Pleuronectes platessa, Pollachius virens, Psetta maxima, Sardina pilchardus, Scomber scombrus, Scophthalmus rhombus, Solea solea, Sprattus sprattus, Trachurus trachurus, Trisopterus esmarki

- Your geographical areas: North Sea, North Atlantic

- How many laboratories analyse calcified structures in your country?

All three vTI fisheries institutes (Sea Fisheries, Hamburg; Baltic Sea Fisheries, Rostock; Fisheries Ecology, Hamburg)

- How is the age data used e.g. stock assessment ?%, environmental studies ?%, other ?%, (please specify):

nearly 100% for stock assessment

- Description of your types of calcified structure preparation (e.g, broken otoliths, sectioned otoliths mounted on custom microscope slides, whole otoliths mounted in clear resin on custom plastic slides - specify type of plastic, scale impressions, etc.) :

Whole otoliths mounted on glass plates; thin-sections mounted on glass plates

- List of calcified structures that you use: otoliths only

- How are calcified structures stored before treatment?

dry in envelopes or plastic tubes

- How are calcified structure preparations stored?

mounted on glass plates

- List of your saws (name, manufacturer, description):

ATM Brillant 250 (custom-fit), CONRAD manual mineralogic saw

- Description and characteristics of your resin(s):

polyester resin VossChemie GTS with MEKP hardener

- A document presenting the summary of the techniques used (reference):

For gadoids, see WKARNSC 2008 report (ICES CM 2008/ ACOM:39); manuals available for other species in German

- List of bilateral agreements where you receive calcified structures (country name, species, typical number per year): (none so far)

- List of bilateral agreements where you sent calcified structures (country name, species, typical number per year): (none so far)

- List of tagging studies in your country (species, geographical area, figure, abstract, reference if it is possible): (no recent tagging studies)

- List of validation of the growth ring formation studies (species, geographical area, figure, abstract, reference if it is possible): Sebastes marinus, Sebastes mentella (Stransky et al. 2005, Fish.Res. 74: 186-197)

- List of validation of the first growth ring studies from the daily increments (species, geographical area, figure, abstract, reference if it is possible): (none so far)

- Do you use image capture/analysis software, if so, which? Image J

- Which type of camera do you use (digital or analogue, description, characteristics)? Digital (Leica DFC 420) camera mounted on Leica M80 microscope

- Which applications is image processing used for (routine, only exchanges, validation studies…) ? routine for flatfish

- How many images are made per year on average? several 100s

- Do you calibrate your images? yes

- Which measurements do you make on the image (distance between rings…) ? none

- What is the format of images? jpg

- Please list your written procedures for sampling calcified structures, preparation, age estimation, data storage etc. by species or group of species : apart from WKARNSC 2008 document, sampling manual for commercial observers in German

- Do you have an internal quality control (description, references) ?

reference collections for most stocks

- Do you have quality management certification e.g. ISO 9001 :2008?

No.

- Are you accredited (yes or no, name of accreditation authority)?

No.

- What age calibration Exchanges or Workshops has your country participated in during the last three years?

WKARNSC 2008 (North Sea cod)

WKART 2008 (turbot)

WKADR 2008 (redfish, Co-chair)

WKARFLO 2008 (flounder)

WKARBH 2008 (Baltic herring)

WKARBS 2008 (Baltic sprat)

WKARGC 2009 (Greenland cod, Co-chair)

WKAREA 2009 (eel)

WKARMAC 2010 (mackerel)

WKARP 2010 (plaice)

WKARDAB 2010 (dab, Chair)

WKAREA2 2011 (eel)

- Are all your age readers aware of WebGR?

Part of the age readers

- Do you upload or intend to upload routine images to populate the WebGR database with calcified structure images?

We're prepared to do so.

Annex 8: WKNARC terms of reference for the next meeting

The **Workshop of National Age Readings Coordinators [WKNARC]** (Chair: Ângela Canha and Lotte Worsøe Clausen)will meet in Horta (Portugal), 13-17 May 2013 to:

j) Review and follow up of last WKNARC's recommendations and intersession work;

k) Review progress in preparation methods and material and techniques development;

l) Review progress in tools for the exchanges and workshops (WebGR, PGCCDBS Guidelines for Otolith Exchanges, age readers forum);

m) Review progress in the validation methods and to analyse questionnaires from Assessment WG on the needs for validation studies;

n) Report on the implementation of central labs for processing age reading;

o) Review the success of bilateral agreement;

p) Review progress in the Internal and External Quality Control into institutes;

q) The workshop will be preceded by a questionnaire to obtain information on the status of ToRs b, c, e at MS institutes,

WKNARC will report by 2013 to the attention of the ACOM and PGCCDBS.

Supporting Information

Priority:	The current activities of this Group will lead ICES into issues related to the ecosystem affects of fisheries, especially with regard to the application of the Precautionary Approach. Consequently, these activities are considered to have a very high priority.
Scientific justification and relation to action plan:	Age determination is an essential feature in fish stock assessment to estimate the rates of mortalities and growth. Assessment of species/stocks using age structured models has proved useful in establishing a diagnosis on stock status. However, the approach has several limitations and shortcomings such as stock structure, natural mortality and growth. Age data is provided by different countries and are estimated using international ageing criteria which have not been validated. For the purpose of inter-calibration between ageing labs WKNARC will review preparation methods by species and areas, material and techniques development, methods in images processing, and the validation methods. WKNARC will review tools for the exchanges and workshops (WebGR, PGCCDBS Guidelines for Otolith Exchanges) and will take into account, the recommendations of the EFAN, TACADAR final reports and the report of the EFARO meeting Brest, 2-4 December 2004 (How can otolith research contribute at improving fisheries sciences?), with the purpose of inter-calibration age readers involved in stock assessment. WKNARC will collate information on the quality status of age reading at MS institutes. The aim of the workshop is to identify the current ageing problems between institutes.
Resource requirements:	The workshop will be preceded by a questionnaire to obtain information on the status of Essential.
Participants:	The Group is normally attended by some 30–35 members (National age reading co-ordinators of MS).

Secretariat facilities:	None.
Financial:	No financial implications.
Linkages to advisory committees:	ACOM
Linkages to other committees or groups:	PGCCDBS, ACOM, RCM, all WKACs (Age Calibration Workshops)
Linkages to other organisations:	There is a direct link with the EU DCF There is a link to PGMED

Annex 9: Recommendations

Recommendation	For follow up by:
1. WKNARC recommends that copies of all procedures and manuals for the preparation and age reading of calcified structures are stored on the Age Readers' Forum (EARF) and that these documents are kept up to date by the responsible institutes.	PGCCDBS
2. WKNARC recommends that copies of national quality documents for the preparation and age reading of calcified structures are stored on the Age Readers' Forum (EARF).	PGCCDBS
3. WKNARC recommends that all Institutes that contribute age based data to ICES Expert Groups, actively encourage their age readers to participate in all relevant Age calibration Workshops (WKACs), as these can provide a quality control measure that is external to the individual institutes.	PGCCDBS
4. WKNARC recommends that all Institutes that contribute age based data to ICES Expert Groups, populate the Web GR image database with images and matching meta data, from otoliths that are representative of all the types of growth patterns that are interpreted within species/stocks to provide age based data.	PGCCDBS
5. WNARC recommends that each image of Calcified Structure (CS) is calibrated (e.g. the inclusion of a scale bar to indicate the size of the CS preparation).	PGCCDBS
6. WKNARC recommends that an Image J forum is created within the Age Readers' Forum (EARF) for the community of Image J users to progress applications and plug-ins that are useful for the treatment of images of calcified structures (CS).	PGCCDBS
7. WKNARC recommends that Institutes that contribute age based data to ICES Expert Groups, have a microscope for age reading of CS and a generic camera (a camera that is not specifically designed for use with a microscope), and use image acquisition and processing software.	PGCCDBS
8. WKNARC recommends that future exchanges and workshops should use WebGR for the annotation of all exchange images to prevent inconsistency and make collation of results easier.	PGCCDBS
9. WKNARC recommends that work is put in to ensuring that the statistical/reporting side of WebGR can deliver what is required by both age readers and stock assessment scientists and that this data can be delivered in the appropriate format. This would require cooperation between an assessment scientist, an age reading expert, a skilled programmer, and a statistician. The EFAN/Guus Eltink approach could be used as a basis feeding the results of the age calibrations directly into assessment models, e.g. by producing a matrix stating the variance or CV around the estimation of a given age and quantifying this into a variance parameter for the age distribution of the stock. It will be necessary to analyze the intercalibration tools of developed by the each country.	PGCCDBS
10. WKNARC recommends that precision levels and acceptable 'widths' of confidence bands for age estimates should be evaluated by species, based on simulations with various degrees of disagreement by age.	PGCCDBS
11. WKNARC recommends that the WebGR tool should allow for the comparison of various methodologies (preparation, image vs. 'live' otolith) when performing age calibrations. Additionally	PGCCDBS

a plug in for comparison of daily ring results is warranted.	
12. WKNARC recommends that a WebGR training workshop should be held for all interested parties, particularly those involved in the organisation of exchanges and workshops. All the age reader coordinators are strongly encouraged to learn how to use WebGR tool.	PGCCDBS
13. WKNARC recommends that national institutes submit the questionnaire concerning the possibility of sending calcified structures to central laboratories for processing and age determination, to PGCCDBS which could summarise the information and distribute between RCMs, assessment working groups and national institutes in the framework of the next PGCCDBS meeting that will be held in Rome in 2012. WKNARC considers that this information would be useful for the development of cooperation between national institutes as well as for improvement of age determination quality.	RCMs, PGCCDBS
14. WKNARC recommends that user friendly software to analyse the measurement of rings is obtained with the WebGR.	PGCCDBS
15. WKNARC strongly recommends that a Workshop is carried out in 2012 on the validation studies for gadoids using available data.	PGCCDBS
16. WKNARC recommends that a questionnaire (Annex 5) should be forwarded to each ICES and GFCM stock assessment working group to identify the gaps in age validation and growth formation studies, for stocks that are subject to age structured assessment or require such an approach. The questionnaire will also help to prioritise recommendations for future validation work, using direct recommendations from the assessment working groups and/or the outcome of the most recent age calibration workshops (WKACs) for these stock-species.	PGCCDBS and ICES and GFCM secretariats.
17. WKNARC strongly recommends that the tables (Review material, techniques and preparation methods by species and areas to fish ageing.xls) that detail the preferred methodology applied for each species within an eco-region are consulted by the national age reading coordinators to evaluate the preferred methodology by the particular institute in relation to the methods applied by the remaining laboratories in the eco-region.	PGCCDBS
18. The WKNARC recommends that where necessary, the methods and techniques are standardised as much as possible. The differences in methods and techniques used may impact the age reading for some stocks, In cases where this is suspected, a comparative analysis should be performed. In cases where such an analysis establishes that the different methods do not affect age reading, standardisation is not necessary.	PGCCDBS
19. WKNARC recommends that during exchanges attention is paid to the different methods of storage used by the institutes participating in the exchange as this may impact the appearance of the CS.	PGCCDBS
20. WKNARC recommends that the following 3-point scale of age reading quality be used by all age readers who provide age data for stock assessments. AQ1: Easy to age with high precision. If a scale of 1-100 is applied, where 100 is when the reader has the highest possible confidence in the age reading and 1 is when the reader has no confidence in the age reading, age quality 1 (AQ1), will apply to approximately the top 25 % of the possible quality ratings. AQ1 is an indication that the age data is considered	PGCCDBS and PG Med

reliable for stock assessment. AQ2: Difficult to age with age with acceptable precision. Age quality 2 (AQ2), will apply approximately to age readings within 25 and 75 percentiles of the possible quality ratings. AQ2 is an indication that the age data is sufficiently reliable to be used for stock assessment purposes but improvement is required. AQ3: Unreadable or very difficult to age with acceptable precision. Age quality 3 (AQ3), will apply to approximately the lowest 25 % of the possible quality ratings. AQ3 is an indication that there are serious concerns about the reliability of the age data and/or its value to stock assessment WGs.	
21. WKNARC recommends that Age Calibration Workshops (WKACs), derive descriptors for the three scale points on the scale of age reading quality be used by all age readers who provide age data for stock assessments, that are applicable to their species-stocks. These can include examples and detailed definitions for these indicators of age reading quality, including quality of the calcified structure and ease of interpretation of the structure.	PGCCDBS and PGMed
22. WKNARC recommends that PGCCDBS should nominate a WebGR host, who will maintain the application and make any modifications required by users, in similar way to the regional database or FishFrame.	PGCCDBS

Annex 10: Review material, technicques and preparation methods by species and areas to fish ageing (separate)

This document is available separately as an appendix to the report.

Annex 11: Quality Status of Age Reading at Institutes (separate)

This document is available separately as an appendix to the report.