Q5CA-2002-01891

TOWARDS ACCREDITATION AND CERTIFICATION OF AGE DETERMINATION OF AQUATIC RESOURCES (TACADAR)

FINAL REPORT

For the period

1 October 2002 to 31 October 2006

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Abstract

The CA aimed to increase the adoption of procedures for age estimation that include quality assurance and quality control mechanisms, for the improvement of stock assessment and environmental management techniques. The overall objective was increase reliability of age estimation procedures in the European Community, compatible with the possibility of the future establishment of European wide international fisheries laboratories. The ultimate objective was to stimulate the achievement of a higher level of quality within and integration between the partner institutes concerning fish age estimation. Methods for age estimation will be achieved where more than one method is applied in the total data input for a stock assessment or an environmental management model.

The goals have been to strength, through a network of excellence, of the competitive position of European institutions involved in fish ageing, promoting institutional synergy and international co-operation. Improvement of dissemination of information about activities and results to the European institutes of fisheries science and fish ecology engaged in age estimation activities. TACADAR have developed a guideline for manual in Quality assurance and standardised practices in age estimation to be applied on the European level. This has involved statistical criteria as well as an evaluation of legal aspects of accreditation and certification and implications for the EU.

Where has TACADAR brought us so far?

- Production of a guideline for QA and QC manual in age estimation
- Improved awareness and sensitivity towards quality of age reading. It was clear from the beginning that establishing Europe-wide control mechanisms is a very sensitive issue. Imposing them needs time as well as a good international working climate.
- TACADAR has very successfully bridged the gap between science and application.
- Improved awareness that validation is and remains a key issue, and that assumption must be evaluated regularly.
- Identified through recommendations, issues that should be addressed in the future
- Collected various existing procedures related to age estimation of fishes

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1 October 2002 to 31 October 2006

Type of contract: Concerted Action

Total cost: 200.000 ECU **EC contribution:** 200.000 ECU

Commencement date: 1. October 2002 **Duration** 49 months

Completion date: 31 October 2006

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Partners:

#	Name of Institute	Country
0	Institute of Marine Research	Norway
1	Fed. Research Center for Fisheries	Germany
2	I Universidade do Porto	Portugal
3	Instituto de Investigacao das Pescas e do Mar	Portugal
4	Instituto di Ricerche Sulla Pesca Marittima/Consiglio Nazionale delle Ricerche (IRPEM/CNR)	Italy

5	Finnish Game and Fisheries Research Institute	Finland
6	Universidade do Algarve	Portugal
7	Instituto Technilogico Pesquero y Alimentario Txatxarramendi Ugartea, Z/G	Spain
8	Institute of Marine Research	Sweden
9	Institute of Coastal Research	Sweden
10	LASAA, IFREMER	France
11	The Marine Laboratory	UK
12	Aquatic Systems Group	UK
13	Danish Institute for Fisheries Research	Denmark
14	IMEDEA (CSIC/UIB)	Spain
15	CEFAS Lowestoft Laboratory	UK
16	Instituto Español de Oceanografía	Spain
17	Marine Research Institute	Iceland
18	Universita degli Studi di Pisa	Italy
19	IRD - Laboratoire ECOLAG	France
20	National Centre for Marine Research	Greece
21	University of Bergen	Norway
22	Scottish Association for Marine Science	UK
23	University of Liverpool/ University of Bergen	UK/Norway
24	Laboratorio Maritimo da Guia/IMAR	Portugal
25	Universidade do Aveiro	Portugal
26	Research Institute for Fisheries	Hungary
27	Double Delta R&D Ltd	Hungary
28	University of Helsinki	Finland

Section 1 Introduction

Executive summary

The European Commission supported the development of a European Fish Aging 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 harmonize the fish age estimation, mostly carried out by interpreting calcified structures. The data from this are produced on the one hand by the national labs of Europe, as for an example an integral part of the national obligations to contribute to international fish stock assessment programmes, (e.g. ICES, EIFAC, GCFM) and, on the other hand by research labs, mostly belonging to universities.

As a result, the **prime objectives** of both CAs were

- **To improve the quality of the input-data for fish stock assessment**, by
  - (1) initiating steps towards solving pertinent questions with regard to the precision and accuracy of the work of age readers in the different national labs,
  - (2) creating a network of scientists and technicians who meet frequently in the course of the CAs to harmonize the procedures used by European fisheries laboratories by encouraging and recommending age reading workshops,
  - (3) exploring the possibilities of accreditation and certification of the age reading procedures.

- **To coordinate the research activities among the participating partners**, by
  - (1) providing a platform for exchanging research results which may or may not be of direct relevance for the routine age reading procedures,
  - (2) providing a platform to stimulate new research in the context of the network,
  - (3) improving the competitiveness and international visibility of the European science community,

The CAs have been highly productive in forming an effective European network of scientists and technicians. The CAs have been a steering device and at the same time a focal point of the discussions. The regular meetings of the network have been a platform of scientific presentations and exchange of practical experience, apart from forming effectively personal relationships between scientists and technicians who work on the same problems. The meetings worked as mini-congresses and were highly productive. Many projects on Europe and international level were initiated at these meeting by this network (e.g. IBACS, AFISA, MANAGEFISH). Therefore they were decisive instruments to keep the **network functioning**. With the termination of the CAs it can be foreseen that the effectiveness of the network will gradually decrease and will phase out if the working relationships are not supported and strengthened by a structure.

It is clear that the Concerted Actions cannot proceed to keep the network afloat, a kind of central coordinating and integrating figure taking over this function is still needed. There are a number European regulations which require age data, such as the Data Collection Regulation (DCR), Water Framework Directive (WFD) and Regulations concerning biodiversity. In case of the DCR it is recommended that age reading workshops become mandatory, to support the network. The scientific role of the ICES Planning Group PGCCDBS should be strengthened for this purpose. A certain quality assurance of the age readings should also be requested by ICES that could be requested to take over a stronger coordinating role in conjunction with PGCCDBS that could set the standards.

Introduction

To supply high quality input data for fisheries and resource management this CA has developed a guideline for a manual in Quality assurance and standardized practices in age estimation. The TACADAR network worked to improve the efficient dissemination of information about activities and results to the European institutes of fisheries science and fish ecology engaged in age estimation activities. This will lead directly to higher confidence in the data used in living resources assessment and environmental and fisheries management. The structure of TACADAR consisted of one primary activity related to improving quality in age estimations used for the assessment of the living resources. The activities of TACADAR have been carried out through one workpackage, which has focused on defining standards of quality assurance. The work consisted of the following (reference to sections in this report in brackets):

- Develop guidelines for the creation and application of quality assurance and quality control mechanisms to protocols for the determination of the ages of fish from hard tissues (Section 2 and 3).
- Define the framework for the application of quality assurance and quality control mechanisms, to protocols for the determination of fish ages from hard tissues (Section 4).
- Investigate legal aspects and implications of accreditation and certification, including advice from externally invited specialists (Section 4).
- Discuss the evaluation of individual methods of age determination in cases where several methods are used to provide data input for the same stock assessment or environmental management model (Section 5).
- Discuss the levels of precision and accuracy to be used as qualifiers in statistical terms (Section 6; also expansion of Chapter 4 in the Index in Section 2).

Section 2: Index for the Elaboration of the Otolith age determination Quality Manual (QM)

Index

Introduction
1. Field of application (refer to Annex 5 for general procedures for each specific stock)
2. Quality objectives
3. Quality policy and references

Chapter 1 – Structure and management of the QM

1.1. Aims (of the QM)
1.2. Structure (description of the general structure of the QM and related documents/manuals)
1.3. Owners and Distribution (nº copies of the QM)
1.4. Revisions and Updates (who, periodicity, how, etc.)
1.5. Authorised signatures
1.6. References
1.7. Glossary of quality terms
1.8. Acronyms and Abbreviations
1.9. QM ratification (declaration by the administration on the approval of the QM and applicability commitment)

Chapter 2 - Requirements of the quality management system

2.1. Quality management system
2.2. Organization and Management
2.3. Document and Information control
2.4. Analysis of the Order, Proposal or Contract (rules for work requests)
2.5. Procedure sub-contracts
2.6. Rendering of services to the customer
2.7. Procedure non-conformity control (way to non-conformity report)
2.8. Corrective actions
2.9. Preventive actions (e.g. Audits, non-conformity observations, etc.)
2.10. Records (define which actions need to have a record, e.g. data and reading results, meetings, training actions, etc.)
2.11. Internal Audits
2.12. Co-ordinator Review
2.13. Quality system improvement

Chapter 3 – Technical requirements of the laboratory

3.1. General issues
3.2. Personnel
    3.2.1. Responsibilities attribution and description
    3.2.2. Personal file
    3.2.3. Training (see specific instructions for each case specific annex)
    3.2.4. Contracts
3.3. Facilities and physical environment
    3.3.1. Facilities admission
    3.3.2. Health and safety
    3.3.3. Physical environment

3.3.4. Archiving (see specific instructions for each case specific annex)

3.4. Ageing structures, equipment and instrumentation (see specific instructions for each case specific annex)

3.4.1. Storage and tracking of ageing structures

3.4.2. Reagents/chemicals list, material safety data sheets

3.4.3. Equipment list and maintenance

3.4.4. Equipment instructions and calibration

3.4.5. Instrumentation list and maintenance

3.5. Ageing structures reference collections (see specific instructions for each case specific annex)

3.5.1. List and description

3.5.2. Maintenance and improvement

Chapter 4 – Quality Control and Quality Assurance

4.1 Preliminary requirements

4.1.1 Sensitivity analysis procedure

4.1.2 Data management (Database creation and Traceability of data)

4.1.3 Production of known age material (Reference collection; Image libraries; Marking programmes; Controlled rearing)

4.2 Individual procedures assessment

4.2.1 Preparation of age reading material

4.2.2 Observation of age reading material

4.2.3 Interpretation of age reading material

4.2.4 Accuracy analysis (including input and output of sensitivity analysis)

4.2.5 Precision analysis (including input and output of sensitivity analysis)

4.3 Merit evaluation by crosschecking of results

4.3.1 Reader performance assessment (hierarchy of levels)

4.3.2 Procedures performance assessment (efficiency and cost)

4.4 Dissemination of information

4.4.1 Technical reports

4.4.2 Model for Certificates and Reports

4.4.3 Corrections to Certificates and Reports

4.4.4 Results of crosschecking

4.4.5 Centralized facility for storing and distributing information (e.g., error distribution, e.g., for ICES)

Annexes (documents too big to incorporate in the manual, docs that need frequent change, technical aspects)

1-Procedures flowcharts

2-List of procedures implemented by the laboratory

3-Examples on basic excel spreadsheets for statistical analysis

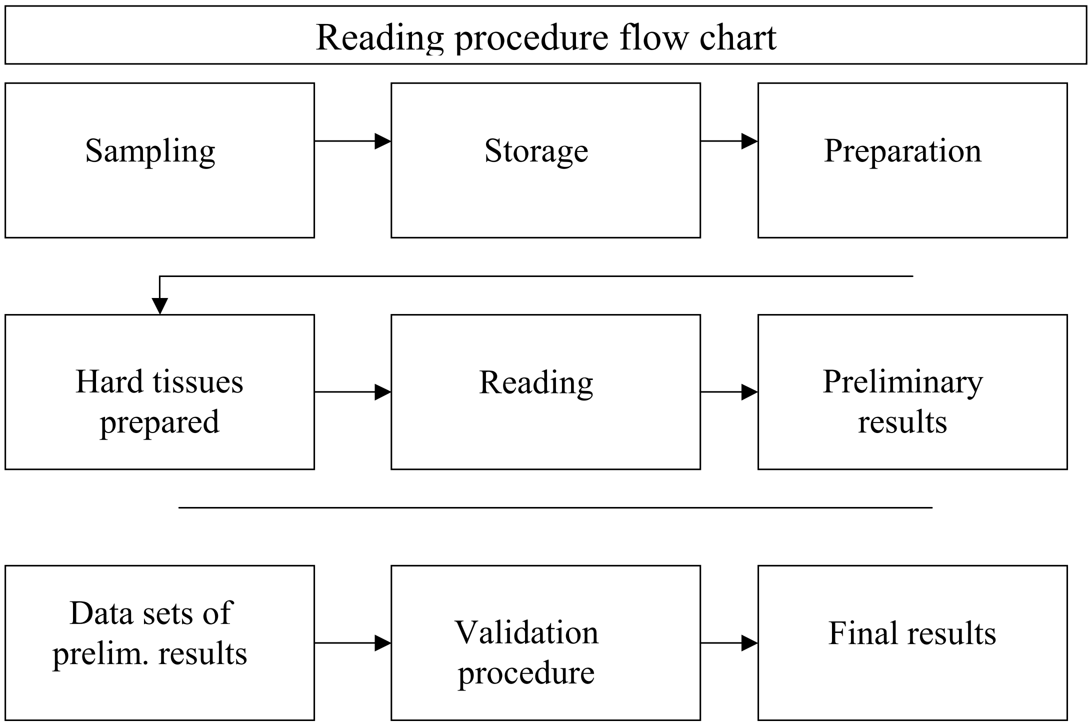
4-Guidelines on minimum hardware requirements for image acquisition and processing

5-Index of a technical annex on procedures

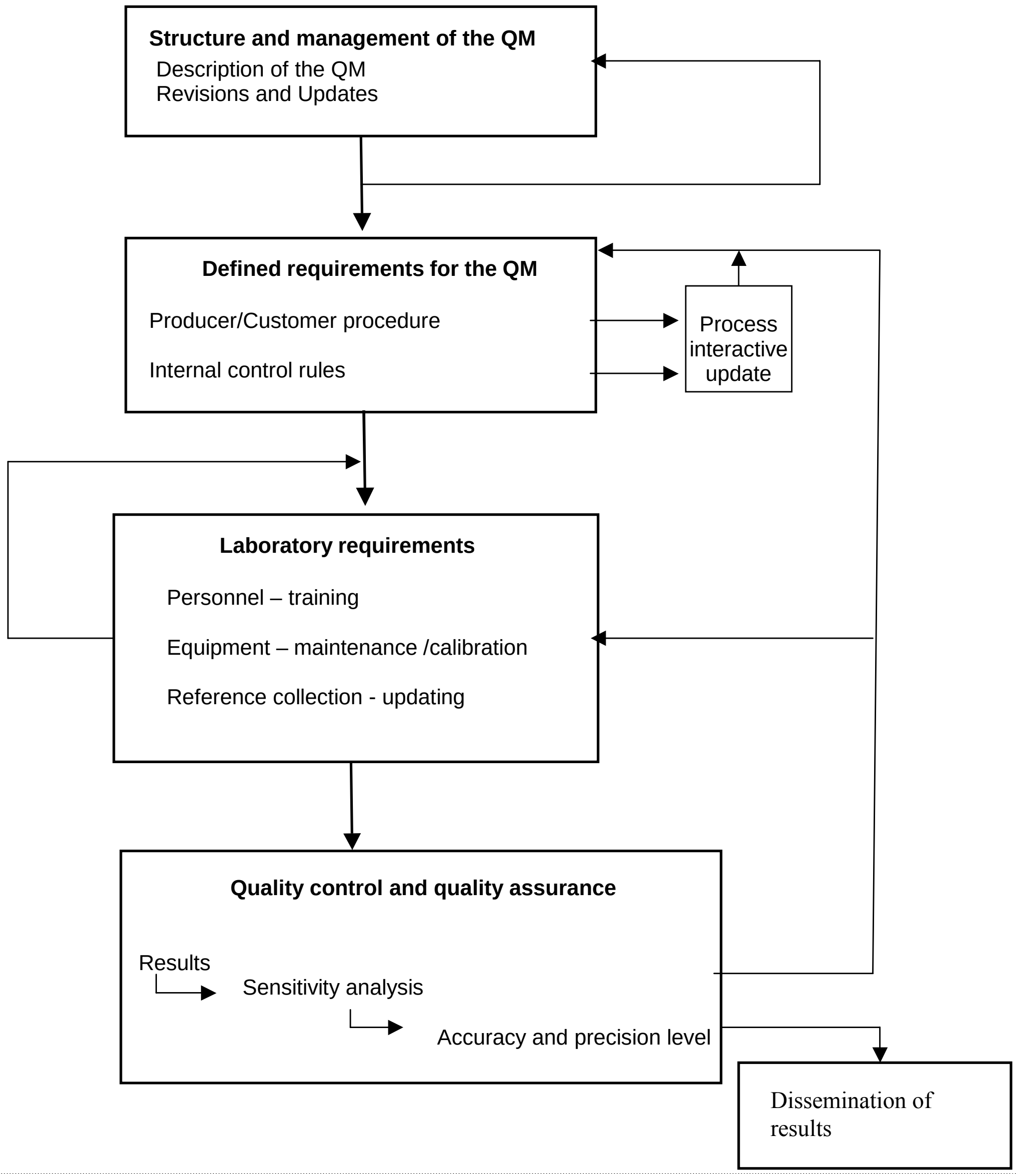
6-Examples of available information (sardine, cod, perch)

Section 3: Annexes to Index

Annex 1: Procedures flowcharts



Flowchart of the main actions in the Otolith age determination Quality Manual (QM)



Annex 2: List of procedures implemented by the participant laboratories

Bellail, R. 2005. "Analyse de la méthode d'estimation de l'âge individuel de la morue en mer celtique (Divisions CIEM VIIe-k)". *Internal Document*

Bellail, R. 2005. "Protocole technique d'estimation de l'âge individuel de la morue en mer celtique (Divisions CIEM VIIe-k)". *Internal Document*

Bellail, R. 2005. "Habilitation des lecteurs de pièces calcifiées pour l'estimation de l'âge individuel de morues de mer celtique (Divisions CIEM VIIe-k)". *Internal Document*

Clausen, L.W., 2002. "Danish manual for age reading – COD section". *Internal Document*

Donato, F., G. Giannetti and E. Arneri, 2003 "Age determination of Sardine (*Sardina pilchardus*) through the Reading of the annual increments". *Internal Document*

Easey, M.W., 1978. "Otolith typing as an aid in the assessment of the contribution of Greenland cod to the Fishery at Iceland". *ICES,CM1978/G:31*

FAO, 1978. "Eastern Central Atlantic Fisheries: Report of the Working Group on standardization of Age Determination of the Sardine (*Sardina pilchardus,* Walb.)". *CECAF/TECH/78/8*

FAO, 2002. "Report of the Sardine (*Sardina pilchardus*) Otoliths Workshop". *Kaliningrad, Russian Federation, 28-31 August, 2001. FAO Fisheries Report Nº 685*

ICES, 1997. "Report of the Workshop on Sardine Otolith Age Reading". *IEO, Vigo, 17-21 February, 1997. ICES CM 1997/H:7*

Newton, A.W., 1999. "North sea cod otolith exchange scheme 1997-1998". *EFAN Report 1-99*

Peturdottir, G., 2004. "Otolith section procedure at the Marine Research Institute of Iceland (MRI)". *Internal Document*

Raitaniemi, J., K. Nyberg & I. Torvi, 2000. "Age and Growth determination of fishes in Finland". *Internal Document*

Raitaniemi, J., 2005. "Ageing of perch (*Perca fluviatilis*) in Finland". *Internal Document*

Reizenstein M., 2005. "Manual on age determination performed at the Institute of the Swedish Board of Fisheries". *Internal Document*

Soares *et al*., 2004. "Report of the Workshop on Sardine Otolith Age Reading". *Lisbon, 28 January-1 February, 2002*

Walter, Y., 2000. "Swedish manual for age reading – COD section". *Internal Document*

Annex 3 Examples on basic excel spreadsheets for statistical analysis

A review of the methods applied in each laboratory led to the identification of the following:
General method: Comparisons of read ages either among readers or within a reader. Percentage agreement and deviation from either mode or previous reading or the 'quality reader' (experienced reader) are the prevailing statistical evaluation methods.

When more than two readers are participating in the calibration, there is a general use of Eltink, A.T.G.W. 2000. Age reading comparisons. (MS Excel workbook version 1.0 October 2000) Internet: http://www.efan.no. This spreadsheet (Fig. 2) gives opportunity to compare readings between many readers and offers many good statistical evaluations (e.g. Age bias plot figures; CV(%), 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; the estimated mean length at age by age reader).

CEFAS has developed a spreadsheet (Fig. 1) by Michael Easey: QC age-check form 2004 (MS Excel workbook version 2004). This spreadsheet compares two readers; the experienced and the new reader. The ages read by the experienced reader are used as 'true ages' and the deviation from these are calculated. The spreadsheet gives a quick overview of differences in interpretation between two readers and the direction of eventual bias. A copy of it was distributed to the WG participants.

2004 QUALITY CONTROL AGE DETERMINATION CHECK RECORD

DATE: SPECIES : SAMPLE NOS.
READER/CHECKER : AREA:
QC PERSON : MONTH:

			GOOD					MODERATE					POOR			
	-2+	-1	Agreed	+1	+2+	-2+	-1	Agreed	+1	+2+	-2+	-1	Agreed	+1	+2+	
AGE 0																0
1																0
2																0
3																0
4																0
5																0
6																0
7																0
8																0
9																0
10																0
11																0
12																0
13																0
14																0
15 +																0
TOTALS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
% AGREED	###	0 %	0 %	0 %	##	##	0 %	0 %	0 %	0 %	###	0 %	0 %	0 %	0 %	

Summary	-2+	#DIV/0!	Total Otoliths Read =	0
	-1	#DIV/0!	Number of unreadable otoliths=	
	Agree	#DIV/0!	Total otoliths=	0
	+1	#DIV/0!		
	+2+	#DIV/0!		

N.B. USE QC PERSONS AGES SO THAT IF THE READER GETS ONE YEAR LESS IT IS RECORDED IN THE MINUS ONE COLUMN

Figure 1. Spreadsheet developed by Michael Easey, CEFAS, UK

Figure 2. Spreadsheet developed by G. Eltink, Netherlands Institute for Fisheries Research, The Netherlands.

Table 1 Horse Mackerel Otolith SET K (WKHMO, Lowestoft, 15-19 January 1999)

RANGE r. 1-15

Stratum	Sample year	Fish no	Fish length	Sex	Landing month	S.Alr NK Reader 1	Nore HG Reader 2	Spain PA Reader 3	Eng PW Reader 4	Germ AC Reader 5	Spain IR Reader 6	Irel EM Reader 7	Irel LB Reader 8	Neth BC Reader 9	Port LS Reader 10	Neth SR Reader 11	Spain OP Reader 12	Germ GG Reader 13	Denm AT Reader 14	Port ANC Reader 15	MODAL age	Percent agreement	Precision CV
Males	-	21	2	14,2	M	10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100 %	0 %
Males	-	19	1	14,9	M	9	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	93 %	24 %
Females	-	21	10	18,9	F	10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	100 %	0 %
Males	-	46	27	19,4	M	10	1	2	3	3	2	2	3	2	3	2	2	2	3	2	2	2	60 %	26 %
Females	-	45	14	19,8	F	10	2	2	3	2	2	2	3	3	2	1	3	2	3	1	2	2	53 %	31 %
Males	-	46	37	20,3	M	10	3	2	3	2	3	3	3	2	2	2	2	3	3	1	2	3	47 %	26 %
Females	-	30	30	20,4	F	10	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	100 %	0 %
Males	-	22	2	20,6	M	9	4	3	4	3	3	3	4	3	3	3	3	4	4	3	3	3	67 %	15 %
Females	-	5	5	20,8	F	4	3	3	3	3	3	3	3	3	3	3	3	4	3	3	3	3	93 %	8 %
Females	-	5	6	20,9	F	4	3	3	3	3	3	4	3	3	3	3	3	3	3	3	3	3	93 %	8 %
Males	-	50	48	21,2	M	10	3	3	3	3	3	4	3	3	3	3	3	3	3	3	2	3	87 %	13 %
Males	-	28	30	21,4	M	10	2	2	2	2	2	3	3	2	2	2	4	2	3	2	2	2	73 %	26 %
Males	-	10	1	21,7	M	4	4	4	5	4	4	4	4	3	3	4	4	4	4	4	3	4	73 %	13 %
Females	-	21	10	21,7	F	9	4	4	4	4	5	5	4	4	4	3	4	4	4	3	3	4	67 %	15 %
Females	-	27	6	21,8	F	10	2	2	2	2	2	3	2	3	2	2	2	2	2	2	2	2	87 %	16 %
Males	-	27	14	22,0	M	10	2	2	2	2	2	3	3	2	2	2	3	3	2	2	2	2	73 %	20 %
Females	-	3	42	22,3	F	3	3	3	3	3	2	3	3	4	6	3	4	2	4	3	3	3	60 %	29 %
Females	-	3	43	22,4	F	3	3	3	3	3	3	3	3	3	3	3	3	3	4	3	3	3	93 %	8 %
Females	-	30	43	22,9	F	10	2	2	3	2	3	3	3	2	2	2	4	4	3	2	2	2	53 %	28 %
Males	-	3	15	23,1	M	3	3	3	3	3	3	3	3	3	3	3	4	3	4	3	3	3	87 %	11 %
Males	-	7	5	23,2	M	4	4	4	4	4	4	4	4	4	4	4	4	4	5	4	4	4	93 %	6 %
Males	-	11	4	23,3	M	4	4	4	5	5	4	4	4	3	4	4	5	4	4	3	4	4	67 %	15 %
Males	-	32	8	23,3	M	10	4	4	4	4	5	5	4	4	4	3	4	4	3	3	3	4	60 %	17 %
Females	-	21	21	23,4	F	9	4	3	4	3	4	4	4	3	4	3	4	3	4	2	4	4	60 %	18 %
Females	-	10	3	23,5	F	4	4	4	4	5	5	5	5	4	5	4	5	5	5	3	4	5	53 %	14 %
Females	-	1	5	23,7	F	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	100 %	0 %
Males	-	7	2	23,8	M	4	4	4	4	4	4	4	4	4	3	4	4	4	4	4	3	4	87 %	9 %
Males	-	4	7	24,0	M	2	4	4	5	4	4	4	4	4	4	4	4	4	4	4	4	4	93 %	6 %
Males	-	48	2	24,1	M	9	5	5	6	5	6	6	6	5	4	5	5	5	5	5	5	5	67 %	11 %
Females	-	2	2	24,3	F	3	5	5	6	5	5	5	5	5	5	5	5	5	5	4	5	5	87 %	8 %
Males	-	33	9	24,3	M	9	5	5	6	6	5	5	7	5	6	5	5	6	5	5	6	5	60 %	12 %
Females	-	1	9	24,5	F	2	4	4	4	4	4	5	4	4	4	4	4	4	3	4	5	4	80 %	11 %
Males	-	7	7	24,5	M	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4	4	93 %	7 %
Males	-	44	4	24,6	M	9	6	6	6	7	5	7	7	6	6	5	6	8	6	6	10	6	53 %	19 %
Females	-	64	4	24,6	F	10	9	8	9	8	8	8	8	8	9	7	8	8	8	8	10	8	67 %	9 %
Males	-	14	14	24,7	M	5	4	4	4	4	4	4	4	5	4	4	4	4	4	5	7	4	80 %	19 %
Females	-	44	11	24,8	F	9	7	6	6	5	6	6	7	6	6	6	6	6	8	6	9	6	73 %	14 %
Males	-	49	2	24,8	M	11	5	5	5	5	5	5	6	5	5	5	5	6	5	6	10	5	73 %	24 %
Females	-	75	5	25,0	F	11	9	8	8	8	8	8	9	8	8	6	7	9	8	8	10	8	60 %	11 %
Males	-	7	1	25,1	M	3	8	9	8	8	8	8	8	8	7	7	8	8	8	8	9	8	73 %	7 %
Males	-	7	6	25.1	M	3	5	5	5	5	5	5	5	5	5	5	5	4	6	5	5	5	87 %	8 %
Males	-	41	9	25,1	M	9	12	9	11	6	9	8	10	9	11	9	9	12	9	8	12	9	40 %	18 %
Females	-	7	18	25,3	F	4	3	4	4	4	4	4	4	4	4	4	5	4	5	6	5	4	67 %	16 %
Females	-	55	19	25,3	F	10	7	7	7	8	7	8	9	7	7	7	7	6	8	8	7	7	60 %	10 %
Males	-	7	7	25,4	M	3	5	5	5	5	5	6	5	5	3	5	5	6	6	6	6	5	60 %	15 %
Females	-	32	21	25,4	F	10	4	4	4	4	4	5	4	5	4	4	4	4	5	4	5	4	73 %	11 %
Females	-	33	9	25,4	F	9	4	4	4	5	5	5	5	4	4	4	4	4	5	5	5	4	53 %	12 %
Females	-	6	1	25,5	F	3	7	7	8	8	7	7	7	8	8	7	7	6	7	10	9	7	53 %	14 %
Males	-	48	12	25,6	M	9	6	7	7	6	7	7	7	6	6	6	8	6	7	8	9	6	40 %	13 %
Males	-	33	15	25,6	M	9	5	5	5	6	6	6	6	6	5	5	5	6	6	7	8	6	47 %	15 %
Males	-	6	3	25,7	M	2	6	6	7	6	6	6	6	7	7	5	6	5	6	6	6	6	67 %	10 %
Females	-	9	5	25,7	F	4	4	4	4	4	4	5	4	4	5	4	4	4	4	4	4	4	87 %	9 %
Females	-	46	8	25,7	F	9	8	6	7	7	7	7	7	6	6	5	7	7	7	7	6	7	60 %	11 %
Males	-	5	6	25,8	M	2	6	6	7	6	6	6	6	6	6	6	6	6	7	6	6	6	87 %	6 %
Males	-	6	10	25,8	M	3	5	5	5	5	6	5	5	5	5	5	6	6	6	6	6	5	60 %	9 %
Females	-	49	7	25,8	F	11	6	5	6	5	6	7	6	6	7	4	6	5	7	6	6	6	53 %	14 %
Males	-	1	17	25,9	M	2	4	4	4	4	4	4	4	4	4	4	4	4	6	4	5	4	87 %	13 %
Females	-	64	10	25,9	F	10	9	9	9	8	9	9	10	9	8	8	8	8	11	9	10	9	47 %	10 %
Males	-	6	16	26,0	M	3	5	5	5	5	5	5	7	6	5	5	5	5	5	5	11	5	80 %	28 %
Females	-	94	12	26,0	F	10	11	8	8	8	8	8	10	9	8	6	8	9	10	11	12	8	47 %	18 %
Females	-	33	17	26,2	F	9	5	5	5	5	5	5	6	6	5	5	11	5	5	6	5	5	73 %	28 %
Males	-	75	21	26,3	M	11	9	8	8	8	9	9	9	8	10	9	9	7	9	9	10	9	53 %	9 %
Males	-	101	11	26,3	M	11	12	10	10	10	8	10	11	10	10	10	9	11	13	10	12	10	53 %	12 %
Females	-	4	12	26,4	F	3	5	5	5	5	5	5	5	5	5	5	4	5	5	6	7	5	80 %	12 %
Males	-	12	2	26,4	M	5	4	5	5	5	4	5	5	4	4	5	5	4	5	6	8	5	53 %	21 %
Females	-	41	16	26,4	F	9	9	9	9	8	10	9	9	8	8	9	10	8	11	9	11	9	47 %	11 %
Males	-	94	9	26,5	M	10	11	8	10	10	9	10	11	9	10	9	9	9	11	11	12	9	33 %	11 %
Males	-	48	15	26,6	M	10	10	9	9	8	9	9	9	8	8	8	8	10	7	8	12	8	40 %	14 %
Males	-	93	10	26,6	M	10	12	10	11	11	11	10	11	10	11	11	10	8	11	12	11	11	53 %	9 %
Females	-	4	11	26,7	F	2	6	5	6	6	6	6	6	7	5	6	6	5	6	6	6	6	73 %	9 %
Females	-	3	7	26,7	F	2	6	6	6	6	6	6	8	6	6	5	6	6	8	7	6	6	73 %	13 %
Males	-	7	2	26,7	M	3	8	8	8	8	9	10	8	7	8	9	9	8	10	10	10	8	47 %	11 %
Males	-	2	4	26,8	M	2	7	8	8	7	8	8	8	6	8	6	8	8	9	10	9	8	53 %	13 %
Females	-	48	4	26,8	F	10	10	9	10	9	10	10	10	9	8	9	9	8	12	9	11	9	40 %	11 %
Females	-	33	22	26,8	F	9	5	6	5	5	5	6	6	5	6	5	5	5	7	7	6	5	53 %	13 %
Females	-	5	2	26,9	F	2	10	10	10	10	10	10	10	10	10	9	10	9	10	12	11	10	73 %	7 %
Males	-	1	14	26,9	M	2	6	6	7	7	6	7	6	6	6	8	6	8	8	7	8	6	47 %	13 %
Females	-	2	7	26,9	F	2	6	6	6	6	7	6	6	6	6	7	7	6	7	6	8	6	67 %	10 %
Males	-	57	10	26,9	M	11	10	10	8	8	10	7	10	10	9	7	10	8	11	8	10	10	47 %	14 %
Females	-	83	22	27,0	F	9	11	11	10	10	11	10	11	9	11	10	11	10	12	11	11	11	53 %	7 %
Males	-	4	5	27,1	M	1	11	10	10	9	8	10	12	9	10	10	8	8	11	11	10	10	40 %	12 %
Females	-	94	17	27.1	F	10	11	12	10	11	11	11	12	11	11	11	11	10	12	12	12	11	53 %	6 %
Males	-	5	7	27,2	M	3	8	7	7	9	7	8	8	5	7	7	7	7	7	9	9	7	53 %	14 %
Females	-	110	12	27,2	F	11	12	12	12	9	10	10	11	11	12	8	10	11	10	12	12	12	40 %	12 %
Females	-	6	15	27,3	F	2	6	5	7	6	6	6	6	6	6	6	6	8	7	7	8	6	67 %	11 %
Males	-	7	5	27,3	M	3	9	8	8	9	9	8	9	8	9	9	9	8	8	9	9	9	60 %	6 %
Males	-	62	8	27,3	M	11	8	7	8	8	8	7	7	9	8	6	8	7	7	9	8	8	47 %	11 %
Females	-	1	1	27,4	F	1	10	12	10	11	10	10	11	8	11	10	10	11	10	11	10	10	53 %	9 %
Males	-	54	13	27,5	M	9	7	-	10	-	7	-	10	8	7	6	7	-	12	10	9	7	36 %	22 %
Females	-	85	18	27,5	F	9	10	11	10	11	10	10	10	9	10	9	10	12	10	11	11	10	53 %	8 %
Males	-	5	11	27,6	M	3	5	5	5	5	5	5	5	4	5	5	5	5	5	6	7	5	80 %	12 %
Females	-	31	21	27,6	F	9	5	4	5	5	5	5	5	4	5	4	5	5	4	5	8	5	67 %	19 %
Males	-	2	8	27,7	M	2	9	9	9	8	8	8	8	8	9	8	8	7	9	8	9	8	53 %	7 %
Males	-	57	23	27,7	M	11	10	11	9	9	10	10	9	7	11	7	9	9	9	11	12	9	40 %	15 %
Males	-	49	13	27,7	M	11	6	5	6	5	6	6	7	7	5	6	5	6	6	7	10	6	47 %	20 %
Females	-	9	2	27,8	F	1	13	13	12	11	13	11	12	11	12	12	12	11	13	12	13	12	40 %	7 %
Females	-	2	4	27,8	F	2	8	8	8	8	8	8	8	8	8	8	8	8	8	9	11	8	87 %	10 %
Males	-	63	6	27,8	M	11	8	8	8	8	9	8	8	8	8	8	8	8	8	9	9	8	80 %	5 %
Females	-	78	6	27,8	F	9	11	9	11	10	11	9	11	9	10	9	7	7	12	11	12	11	33 %	16 %
Females	-	49	17	27,9	F	11	6	6	6	6	7	6	7	7	8	6	7	7	8	9	10	6	40 %	17 %
Males	-	2	4	28,0	M	2	9	9	9	10	9	9	10	8	10	9	8	9	11	11	11	9	47 %	10 %
Males	-	6	20	28,1	M	2	6	5	6	6	6	6	6	6	6	7	8	6	7	7	9	6	60 %	15 %
Males	-	110	22	28.1	M	11	12	12	12	11	11	11	12	10	13	12	12	12	11	12	10	12	53 %	7 %
Females	-	9	11	28,2	F	1	12	12	12	10	12	13	12	11	11	9	12	9	15	10	13	12	40 %	14 %
Females	-	79	23	28,2	F	9	12	11	9	12	9	9	11	7	12	10	11	11	8	12	10	12	27 %	15 %
Males	-	3	6	28,4	M	1	12	12	12	12	13	11	12	11	12	11	10	12	9	13	12	12	53 %	9 %
Females	-	5	14	28,5	F	3	8	8	8	8	8	8	7	7	7	7	7	7	8	9	9	8	47 %	9 %
Males	-	1	3	28,7	M	2	5	5	5	5	5	5	5	6	5	6	6	5	6	7	7	5	60 %	13 %
Females	-	6	3	28,7	F	3	9	9	9	9	9	9	8	8	8	8	9	8	10	9	10	9	53 %	8 %
Females	-	79	20	28,7	F	9	10	10	11	11	12	9	12	10	12	11	9	10	13	13	11	10	27 %	12 %
Males	-	8	23	28,8	M	3	11	10	11	11	10	10	11	7	10	10	13	9	12	12	11	11	33 %	13 %
Males	-	62	17	28,8	M	11	7	8	7	6	7	7	7	7	8	7	7	6	9	7	7	7	67 %	10 %
Females	-	3	13	29,0	F	2	9	9	9	8	9	9	9	8	10	8	9	8	10	8	10	9	47 %	8 %
Males	-	9	17	29,1	M	1	13	13	12	13	13	13	13	11	12	11	13	12	8	13	14	13	53 %	12 %
Females	-	43	17	29,1	F	10	5	5	5	5	5	5	6	6	5	5	5	5	7	6	7	5	67 %	14 %

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Annex 4: Guidelines on minimum hardware requirements for image acquisition and processing

1. Acquisition of CS images
   - High resolution image of the whole otolith (at least the reading zone) (>=1 Mpixels)
   - Preferably non-compressed format (avoid jprg format)
   - Limited noise level (balance between exposure time and gain)

2. Acquisition of CS interpretation
   - Set markers in the image for the position of the interpreted growth rings and possible false rings or checks
   - Display the interpretation as a graphic overlay
   - Store the interpretation (file containing the graphic overlay and the ring positions)

3. Storage and management of database of interpreted CS images
   - Store the images with associated data (species, catch date, CS type,...;)
   - Attach the interpretation (reader, estimated age, interpretation (ring positions))

Guidelines on hardware requirements for image acquisition and processing

1. Workstation for image analysis (recommended configuration)
   - PC 2GHz, 1Gb RAM
   - Equipped with a software for computer-assisted acquisition and interpretation of CS images

2. Acquisition hardware
   - Preferably a digital camera
     - Digital camera (1Mb pixel, squared pixels, exposure time up to 1s)
     - IEEE 1394 connection
   - Controlled stage (recommended for routine ageing, work with microstructures)

Annex 5: Index of a technical annex on procedures for ageing structure extraction and analysis

(it is necessary to develop this annex for each stock and update it regularly)

1. Time planning related to relevant deadlines for submission of data
2. Sampling methods
    2.1. Biological sampling and measurements
    2.2. Ageing structure selection and extraction
    2.3. Ageing structure preservation and traceability
3. Methods of ageing structure preparation
    3.1. Method selection and description
    3.2. Criteria for acceptance/rejection of ageing structure
    3.3. Estimation of uncertainty in ageing structure preparation (degrees of error at preparing the structure)
    3.4. Preparation preservation and traceability
4. Ageing structure observation
    4.1. Equipment, image acquisition and storage
    4.2. Reading equipment set up (magnification, light intensity, etc)
    4.3. Image quality requirements
    4.4. Image analysis and interpretation
    4.5. Interpretation criteria for growth increments
    4.6. Age class attribution
5. Quality control of readings or precision (e.g. Repeated readings)
    5.1. Analytical methodology
    5.2. Standardization
    5.3. Post-reading analysis
    5.4. Applicability
6. Quality Assurance of Results or accuracy
    6.1. Reader performance assessment
    6.2. Validation of methods
    6.3. Validation and verification of ageing
    6.4. Validation of results (e.g. Correlation analysis, data review by statistical sampling, etc.)
7. Validation plan
    7.1. Periodicity and applicability
    7.2. Sensitivity analysis
    7.3. Reference collections
8. Internal data verification and the use of standard protocols
    8.1. Electronic data logging (rules to be sure that data are safe and protected)
    8.2. Traceability of data
    8.3. General issues
    8.4. Specific requirements
9. Reports
    9.1. General
    9.2. Technical reports
    9.3. Results of validation/tests done by external experts (exchange programs)
    9.4. Electronic transmission of results

ANNEX 6: EXAMPLES OF AVAILABLE INFORMATION (Sardine, Cod and Perch)

Aim:

Develop guidelines for the creation and application of quality assurance and quality control mechanisms to protocols for the determination of the ages of fish from hard tissues

From a list of species that were selected by the available information on age and growth, for their relevance and because of the availability of reading control protocols, cod (as an Atlantic species), sardine (as a Mediterranean species) and perch (as a freshwater species) were selected to be used as examples

SARDINE (*Sardina pilchardus*)

Generally otoliths are used but it is very useful to use both structures otoliths and scales to corroborate the assigned age.

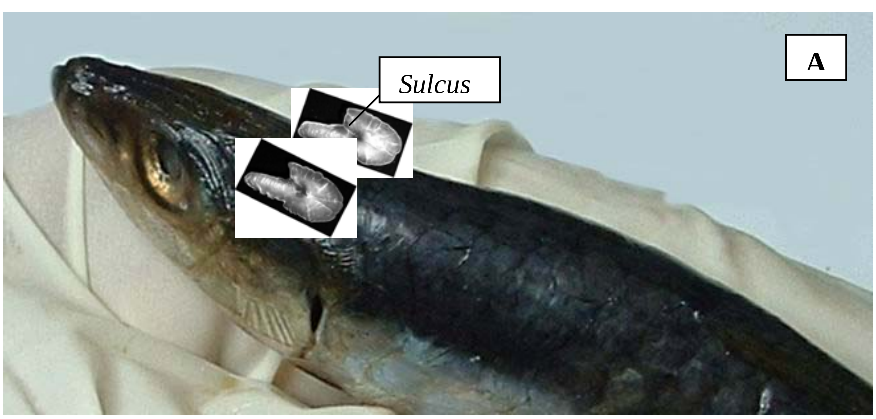
- **Preparation**

- Scales: 4 or 5 scales are removed from the left shoulder of the fish between the head and the dorsal fin; cleaned and mounted between two slides
- Otoliths: 10 individual from each half centimetre length by month of catch should be analysed biologically
- After extraction otoliths are washed, dried, mounted and preserved in black plastic plaques in a synthetic resin (©Eukitt or Entellan)
- It is always advisable to have pairs of whole otoliths available from individual sardine specimens when trying to interpret the ring structure
- Observations of entire otoliths under reflected light are made using a binocular with 20x magnification. Magnification should be increased near the otolith edge to improve the discrimination of narrow translucent rings in older individuals
- Rings are more frequent in the *antirostrum*, so it must be used the *postrostrum* for age determination. However, sometimes it may happen that other areas of the otolith, i.e. the dorsal part, are easier to read. In this case the age reading based on the analysis of these areas can be considered appropriate if the readings prove to be consistent

- **Age interpretation**

- Allocation of birthday: 1st January although the spawning season covers practically the whole year, with lower values in the 3rd quarter

- Scales: it was assumed that any ring which runs round the whole of the anterior edge is a "true ring" and is counted. If this does not happen, the ring is treated as false
- A set of an opaque and a translucent zone corresponds to one annual increment (*growth increments*)
- The basic characteristics to adopt a ring as an *growth increments* are: visibility around the entire otolith body; continuity; thickness. These rule must be applied specially for the first three *annuli*, since in the older specimens growth often slows down to such an extent that translucent rings are very close together
- The width of opaque zones normally decreases from the nucleus towards the periphery as the fish becomes older. Ring width decreases mainly in the first 2 years of life. However, the diminution is not necessarily regular.
- Several rings appear close to the nucleus and the distance to the first true age ring is variable due to the extended spawning season
- In fish there is usually a ring around the nucleus that should not be counted. The first and second translucent zones are normally composed of multiple rings very close together that must be considered as rings. In some cases it was observed that sardines of 6-11 cm in total length caught during the year with 2-5 translucent rings had the age of 60-220 days and these rings cannot be considered as annual but only as false.
- In the age estimation process, the position of the first annual increment should be the major point of the agreement procedure. Generally the first annual increment should have between 0.95 and 1.5 mm of measurement depending on the month of birth of the sardine
- The fish is assigned to a year class based on the date of birth assumed was the 1st of January: If sardine is caught with an opaque zone on the otolith edge the age assigned will be equal to the number of rings observed minus one. If sardine is caught in January-March with a translucent ring on the otolith edge the age assigned will be equal to the number of annual increments observed. Otoliths with translucent edge during second semester are considered to belong to previous age group.
- Both otoliths of an individual must be read and it is recommended that the readings be carried out by at least two readers independently (or at least twice by the same reader). If the readings results are non coincident, a third reader is required. If there is not agreement the otolith must be rejected
- If the difficulties in the age interpretation using otoliths are very persistent then it would be useful to read the scales and compare the results
- The readability of the otolith should be taken into account and if it is low then the otolith should be rejected



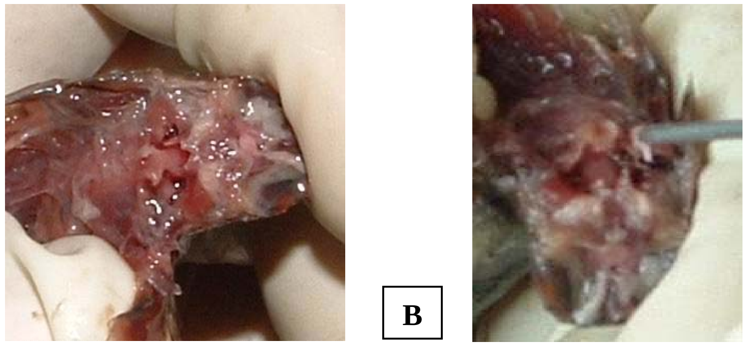


Figure 3. Sardine: Location of the otolith (A) and otolith removal using guillotine method (B)

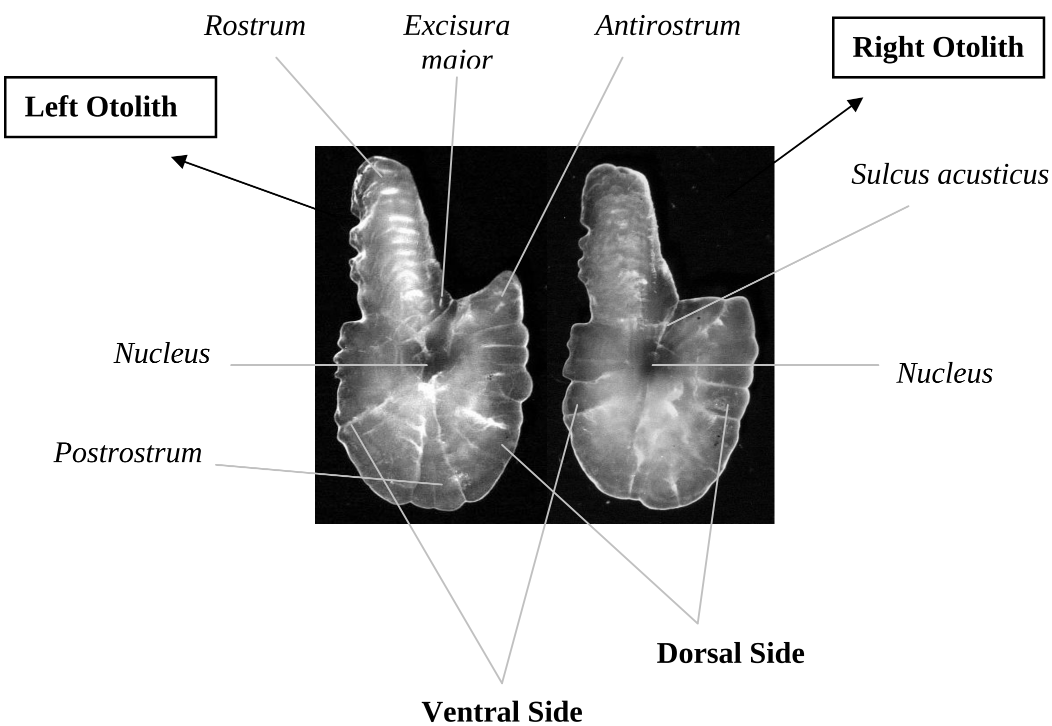


Figure 4. General description of Sardine *Sagitta* Otolith

PERCH (*Perca fluviatilis*)

➢ Preparation

Otolith:

There are two alternative methods:

- Burned in alcohol burner until it turns dark brown and then broken in half through the nucleus
- Cut or ground through the centre to obtain transverse section that is stained with e.g. neutral red (immersion 10–15 minutes)
- The otolith cross sections are studied in a stereo microscope (20-50 x magnification), otolith halves in reflected light and stained thin slices in a compound microscope in transmitted light.

Opercular bones:

- Quickly boiled and cleaned thoroughly with a dry piece of paper and stored in paper bags in a dry place. When the bone is completely dry, it is ready for ageing.
- Can be studied unprepared in a stereo microscope (aprox. 6 x magnification) with reflected or/and transmitted light. In uncertain cases the bones could be placed in a container with clarifying liquid.

Scales:

Sampling perch scales is easy and quick, but the quality and precision of the age determination is limited. The scales must be taken from the same position of the sampled fish.

➢ Age interpretation

(Sagittal Otoliths, Opercula and Scales are used for ageing)

Otoliths (sagitta):

- Allocation of birthday: 1st of January.
- An annual increment consists of an opaque *growth zone*, formed during summer, and a narrow translucent *winter zone* (ring).
- In a burned otolith, opaque zones appear white or light brown on the surface of the cross section, translucent zones appear dark brown. If the cross section is stained with neutral red that attaches onto the protein, winter zones appear as sharp, dark red lines on red or pink background. These sharp lines can also be seen as dark brown stripes in an otolith that is almost burned to ash (but does not cramble).
- The first annual increment is defined by the most inner growth zone (following the nucleus) and the first clear translucent winter zone that circulates the whole surface of the cross section.

- False winter zones, *checks,* are rare but they can occur as weak or interrupted translucent zones, most common on the dorsal part.
- Problem otoliths: There might be problems in observing the most recent winter zone on the otolith edge from fish caught during the first 6–7 months of the year. On the edge of the cross section, fragments can be detected on the dorsal or ventral part of the otolith or near *sulcus*.

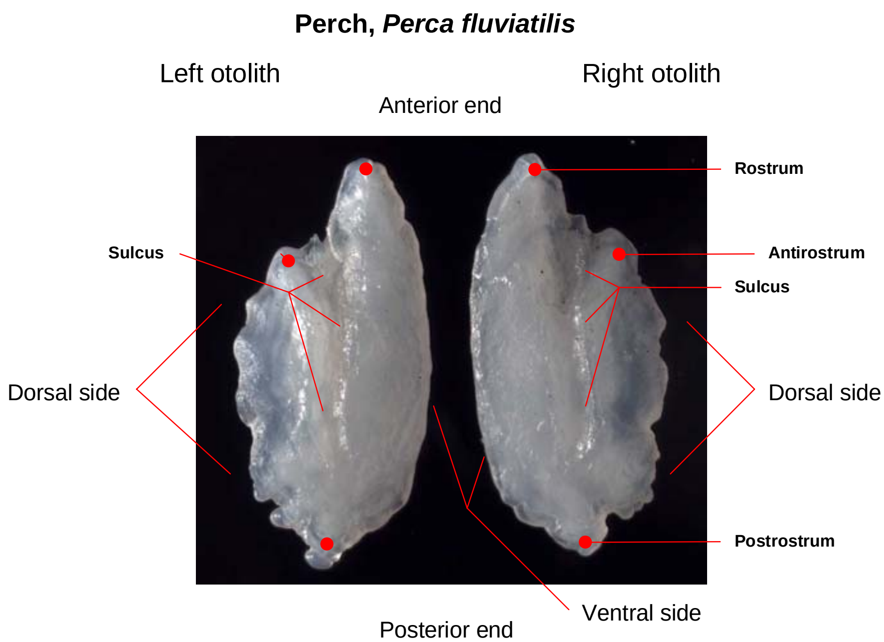
Opercular* bones*:
Are recommendable alternative CS

- Allocation of birthday: 1st of January.
- The annual increments on the operculum are read from the inner to the outer part. The measuring axis starts in the centre of the thin shovel-like part at the highest ridge in the base, and runs in a 90º angle towards the increments to the edge.
- An annual increment consists of a wide opaque *growth zone*, formed during summer, and a narrow translucent *winter zone*. The transition from opaque to translucent zone is often gradual, while the change from translucent to opaque is distinct.
- The first growth zone on the operculum can be more or less transparent, and therefore doesn't appear opaque.
- The first annual increment is defined by the most inner growth zone and the first translucent winter zone. The first winter zone may be vague (see Problem opercula) and it's location might be shown by numerous closely spaced stripes.
- True winter zones are visible over the whole operculum, from dorsal to ventral edge.
- False winter zones are rather common and problematic to interpret. They appear vague and not characteristic. They sometimes don't reach from dorsal to ventral edge or they might not be parallel to the true winter zones. In the comparison to scale ageing of yellow perch, opercular bones are more accurate in slowly growing fish.
- Problem opercula: the first winter zone can be difficult to detect, due to a transparent first growth zone and a gradual transition to the second opaque growth zone. In these cases, the aid of the otolith age and the size of the first annual increment on opercular bones of similar/typical growth pattern are of great importance. On big opercula from large fishes, the bone is so thick that the first annual increment can be hard to detect. In these cases, you need to look at the bone from different angles, i.e. on the backside of the opercula and at the edges as well as with light directed from different directions and use some clarifying liquid.

Scales:

- The measuring axis for annual increments is selected from the anterior field, in which the annual increments are perpendicular to the measuring axis. In the posterior field of a scale, the annual increments are not visible.
- The position of the assumed first growth increment and the radius from centre to edge of the scale, can be examined in relation to the total length of the fish and this information can be used as an aid in locating the first growth increments

- The interpretation of annual increments may be hampered by the condensing of *circuli* of growth formed during the growing season and leaving a false ring in the scale.
- Difficulties: to find the outermost edge of the first annual increment. However, compared with operculum bones, it is often easier to find the first annual increment on scales.



Parts and orientation of sagitta otoliths from perch
Original photograf: Patrik Clevestam

Figure 5. Sagitta otoliths from perch.

COD (*Gadus morhua*)

- **Preparation**

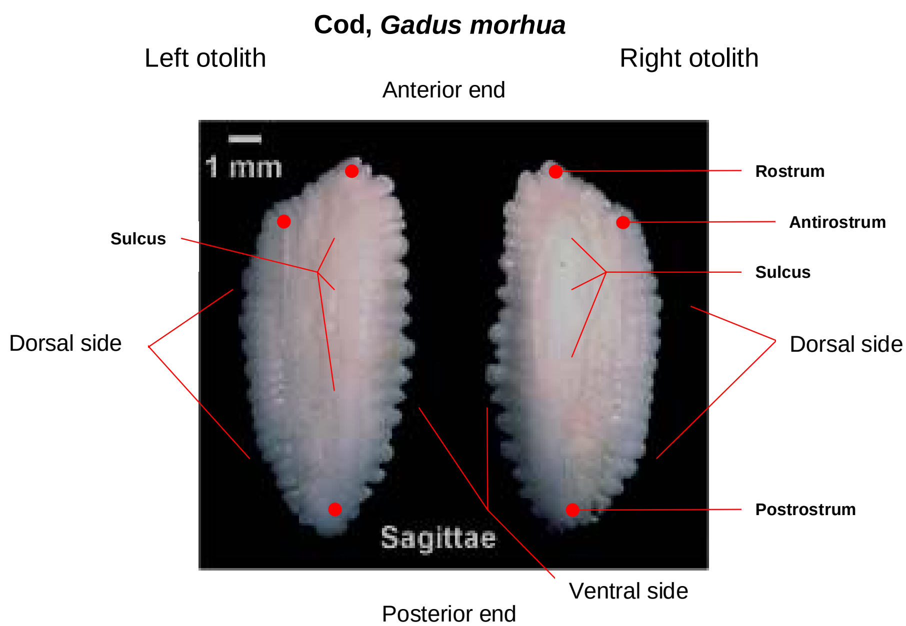
Otoliths (*sagitta*)

Different methods of otolith preparation with different methodologies are described by participant countries in this workshop but in summary:

1. Are removed from the fish and stored dry and prepared for sectioning by embedding in black or transparent polyester resin to obtain thin cross-sections or are broken across the nucleus. The thin section obtained is usually mounted on glass slides for reading.
2. Both preparation method otoliths are examined using a stereo microscope with a magnification from X5 to X10 under reflected or transmitted light
3. A clarifying liquid is applied to the broken surface of the otolith for age reading.

- ***Age interpretation:*** **Otoliths** (*sagitta*)

- The allocation of birthday is the 1st of January and knowledge of spawning season for a particular stock is recommended
- The annuli should be read from the nucleus towards the edge in several radiae always at the lower possible magnification, although unclear areas such as the edge, may be observed at higher magnification. The better axis for reading is the dorso-ventral axis.
- Juvenile zone: Close to the nucleus is often a small, clear and even ring. This is not counted when assigning age
- Translucent zones: in general are regular and concentric but their translucency may vary. Generally they have the same shape as the otolith section. They can sometimes split in certain otolith areas. Also a group of multiple rings, forming a zone, are found and are identified based on their relative spacing.
- False translucent zones: In general a thin often diffuse ring that is not visible around the whole otolith and does not follow the overall pattern of the annual increments of the otolith.
- First translucent zone: Distinct, regular and concentric not to be confused with the smaller juvenile zone.
- Growth zone: Opaque, non-translucent zone. The growth zones of the first years may be broader than the translucent zones.
- Problem otoliths: The first translucent increment is often a double ring especially for fish up to two years. In certain stocks and in older fish the formation of the translucent zone can occur very late in the year and sometimes the reader has to assume the formation of this increment in the edge.



Parts and orientation of sagitta otoliths from cod.
Original picture copied from: http://www.marinebiodiversity.ca/

Figure 6. Sagitta otoliths from cod.

Section 4: Implementing QA and QC in fish ageing

The previous sections 2 and 3 presents protocols and procedures (the QM, quality manual) for QC and QA in fish ageing laboratories. In this section, the process of implementing these procedures is considered. There are many aspects which were considered in order to develop a framework for the application of QC and QA to fish ageing. In this section we present discussions of some of the issues surrounding QA and QC, first by highlighting the importance of the quality of age data, then by examining the critical elements needed for QA and QC as applied to age estimations. This is followed by case studies of the experiences of different institutions, and a discussion of the framework for QA and QC. Finally, recommendations are summarised. The following sub-sections comprise an evaluation of the general process of implementing QA and QC in the context of fish ageing laboratories.

1. Consequences of age reading errors: Literature about the problems of age reading errors related to fisheries institutions and the scientific community were collected and evaluated. These examples are concrete evidence of the existence and impact of age reading problems.
2. Critical elements for QC and QA in fish age estimation: Methods and personnel are critical elements and depend on personal interactions as well as on written protocols. It is also critical in the field of fish age estimation to be able to implement new knowledge and techniques.
3. Examples and case studies: The TACADAR network exists to share information on common problems, and so an important contribution was to collect and disseminate examples from the community on the implementation of QC and QA protocols in the individual laboratories. The costs of the implementation were also included in the discussion of this aspect of the process
4. Framework for QC and QA: Expert advice was gathered on the steps necessary for implementing QC and QA, and the requirements for accreditation for fish age readings. Examples of the general principles, the stages needed, a definition of the customers, and a listing of the critical elements in the process was discussed
5. Recommendations: These need to take into consideration the diversity of the community of age readers, as well as the diversity of the customers for fish age data.

4.1. Consequences of age reading errors

Within the fisheries assessment community, problems with age reading have recently received a higher level of recognition. This is apparent from the reports of ICES working groups, and also of the Planning Group (PGCCDBS; ICES CM 2006/ACFM:18). This planning group is responsible for the quality of fisheries data collected through the DataRegs program, and used by ICES and national assessments. The recognition at such an important level signals the success of EFAN and TACADAR in communicating the importance of reliable age data.

Individual working groups within ICES, the EU, and other organisations have addressed the problems of individual species and stocks. These workshops often provide a quantitative assessment of the variation in age readings, and these results can be used to estimate the impact on stock assessments.

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4.1.1 Baltic cod example

The ICES the Baltic Committee last year (2004) had a Study Group on ageing issues of Baltic cod. The problem related to age determination using otoliths of Eastern Baltic Cod has been identified as follows:

- Otolith growth and ring deposition not known
- Complex hydrographical influence
- Age interpretation not validated
- Inconsistency between age readers.

The consequences have been identified as follows:

- Different age composition between countries
- Smearing of large age-classes
- Inconsistent survival estimates of year-classes
- Uncertain stock assessment

The SG had two meeting with the following issue on the agenda:

1st SGABC meeting in Riga, May 2004 reviewed extent of age inconsistencies (results from previous meetings, publications, age workshop), explored alternatives (fish length, otolith weight) to estimate age proportions, plan for collection of otolith weight data and establishment of a database, and,initiate exchange programs based on otoliths + digital images.2nd SGABC meeting in Klaipeda, May 2005

1. reported on and discussed the administration of otolith weight database,
2. statistical modeling and data exploration, disemination of results from the exchange programs based on otoliths + digital images, and discussed the funding of continuing work.

Recommendations made from this SG, and which have relevance to the work in TACADAR are as follows:

- Compile known-age data, initiate mark-recapture programs.Organise plans and methods for historical reconstruction.Develop exchange programs based on digital images.Establish a permanent WG for age determination problems.The

last recommendation of "Establish a permanent WG for age determination problems" indicates that age problems keep coming back to ICES and therefore should be given a higher priority in the future within ICES. An alternative solution could be to work towards accreditation and certification of age determination of aquatic resources.

4.1.2 Documented problems with age reading errors related to the scientific community: compilation of a sub-set of papers on the effect of age estimate errors on further evaluation/handling/assessments. A sample of papers focusing on the effects of ageing errors is included here.

Campana, S. E. (2001). "Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods." Journal of Fish Biology 59(2): 197-242.

Based on an analysis of 372 papers reporting age validation since 1983, Campana highlights the best available methods for insuring ageing accuracy and quantifying ageing precision. He emphasises the distinction between validation of absolute age and increment periodicity, as is the importance of determining the age of first increment formation. Several of the age validation methods, which have been used routinely, are of dubious value, particularly marginal increment analysis. Random ageing errors can often be corrected using statistical methods. **Through use of quality control monitoring, ageing errors are readily detected and quantified; reference collections are the key to both quality control and reduction of costs.**

Eklund, J., Parmanne, R. and G. Aneer. 2000. Fisheries Research, Volume 46, Issues 1-3, May 2000, Pages 147-154

Eklund et al. 2000 compared age reading of Baltic herring from commercial trap net catches. Agreement between three readers was 70% (72-85%) with the majority of the agreement within +/- 1 year. Despite similar age distributions among readers, bias was present. Highest variability was obtained for the oldest fish, and bias correlated with fish size. **The fishing mortality estimated by population analysis varied among readers by 7-50% for ages 3-10**.

Heifetz, J., D. Anderl, et al. (1999). "Age validation and analysis of ageing error from marked and recaptured sablefish, Anoplopoma fimbria." Fishery Bulletin [Fish. Bull.] 97(2): 256-263.

In a study on ageing errors in sablefish, Heifetz et al. 1999, recognized that use of ageing errors based on known-age samples may help improve stock assessment. Two thirds of the 2-9 years old fish were misaged, most only by one year. Results should be compared between stock assessments that use parameter estimates for different ageing-error matrix. If a sample is obtained that includes older known-age fish, the ageing-error matrix can be estimated for older fish. When known-age specimens are not available variability between readers may be the only data available to assess ageing error. Such data are valuable for evaluating the precision and consistency of ageing criteria applied by different readers. **Estimates derived solely from between-reader variability should be viewed as minimum estimates of ageing error.**

Lai, H. L. and D. R. Gunderson (1987). Effects of ageing errors on estimates of growth, mortality and yield per recruit for walleye pollock (Theragra chalcogramma). Fisheries Research, Volume 5, Issues 2-3, July 1987, Pages 287-302

Lai and Gunderson (1987) investigated the effects of ageing errors on estimates of growth, mortality and yield per recruit for walleye pollock (*Theragra chalcogramma*) by using Monte Carlo simulation. The bias in the von Bertalanffy growth parameters, L_∞ and K, and survival rate, S, were usually < 6%, yet the estimated optimum fishing mortality (F^*) was increased significantly. **Systematic under-ageing error, associated with scale methods, resulted in a substantial bias in most parameters and overfishing would result if the estimated F^* and age at first capture were used in fishery management.**

LeBreton, G. T. O., F. W. H. Beamish. (1999). "Lake sturgeon (Acipenser fulvescens) growth chronologies." Canadian Journal of Fisheries and Aquatic Sciences 56(10): 1752-1756.
LeBreton and Beamish (1999) used annual increments in the pectoral fin rays of lake sturgeon (*Acipenser fulvescens*) to assess growth synchrony. They based the decision criteria on correlation among individual chronologies developed from samples collected in three rivers. Initially, correlations among chronologies were not found significant within these three populations. However, as mean ageing error was reduced, correlations among chronologies increased to significant levels in samples from two rivers, whereas correlations were not significant among consistently aged fish sampled from the third. They concluded that interannual growth variation in lake sturgeon is influenced by population-wide extrinsic factors in some populations. **Their results suggest that both growth synchrony and ageing error should be quantified during the construction of growth chronologies for all organisms.**

Newman, S. J., Cappo, M. and D. McB. Williams 2000. Age, growth, mortality rates and corresponding yield estimates using otoliths of the tropical red snappers, Lutjanus erythropterus, L. malabaricus and L. sebae, from the central Great Barrier Reef. Fisheries Research, Volume 48, Issue 1, August 2000, Pages 1-14
Newman et al. (2000) found that age estimates of *L. erythropterus, L. malabaricus* and *L. sebae* from the central Great Barrier Reef obtained from counts of increments on whole otoliths were consistently much lower and more imprecise, at all ages compared with counts from sectioned otoliths. Precision of counts from whole otoliths declined significantly with increasing fish age. To explore the fishery research and management implications of these biases, age estimates from the two error methods were used to construct comparative growth and mortality parameters and model yield-per-recruit values under contrasting harvest strategies. **While the von Bertalanffy growth functions were not significantly different for the two methods, the underestimates of age from whole otoliths caused serious over-estimation of natural mortality. Serious overfishing could occur if these estimates were applied for fishery management purposes**.

G.R. Power, P.A. King, C.J. Kelly, D. McGrath, E. Mullins and Ole Gullaksen, 2006. Precision and bias in the age determination of blue whiting, Micromesistius poutassou (Risso, 1810), within and between age-readers. Fisheries Research, Volume 80, Issues 2-3, September 2006, Pages 312-321

Power et al. (2006) estimated bias and precision in ageing the heavily exploited combined stock of blue whiting (*Micromesistius poutassou*) in the Northeast Atlantic. Significant linear bias was found to exist between age readers, with age differing, on average, by one year for important year-classes. Within reader precision was found to be higher than between reader precision; however, within reader bias was also evident with two out of three age-readers systematically revising ages downwards when re-ageing otoliths. **Results indicate that differences exist on a limited international level for the age determination of blue whiting. This issue now needs to be addressed by the scientific community.**

Revees, S. A 2003. A simulation study of the implications of age-reading error or stock assessment and management advice. ICES J. Mar. Sci. 60:314-328.

Revees (2003) quantified the effects of age-reading errors on the perception of stock trends and short-term management advice based on Eastern stock of Baltic cod. The results indicate a clear distinction between the performance of the assessment, and the performance of catch forecasts and advice based on that assessment. The ageing error affected the absolute level of estimates of fishing mortality and spawning stock biomass from stock assessment, although overall trend are similar. **Greater problem arose in catch forecasts and advice, for which ageing error led to discrepancies between required and effective fishing mortality and a general tendency for ageing error to lead to advice on TAC that would be too optimistic and, therefore less effective for stock conservation.**

Conclusion: The sample of studies presented here clearly recognise that errors in age reading may be of significant importance in estimating parameter values for stock assessment and other fishery related use. The variety of problems calls for improvement of fish ageing results/harmonisation of methods and protocols, by use of QA and QC.

Other useful references:

Bradford, M. J. (1991). "Effects of Ageing Errors on Recruitment Time Series Estimated from Sequential Population Analysis." Canadian Journal of Fisheries and Aquatic Sciences 48: 555-558.

Brander, K. 1974. The effects of age-reading errors on the statistical reliability of marine fishery modelling. in Bagenal, T.B. (ed) Ageing of Fish, Unwin Brothers Ltd

Polat, N., K. Isik, et al. (1993). "Precision of bony structure-reader for ageing of Biyikli Balik (Barbus plebejus escherichi Steindachner, 1897)." Turkish Journal of Zoology 17((4),): 503-509.

Richards, L. J., J. T. Schnute, et al. (1992). "Statistical Models for the Analysis of Ageing Error." Canadian Journal of Fisheries and Aquatic Sciences 49: 1801-1815.

Worthington, A. J., A. J. Fowler, et al. (1995). "Determining the most efficient method of age determination for estimating the age structure of a fish population." Canadian Journal of Fisheries and Aquatic Sciences.

R.I.C. Chris Francis and Steven E. Campana 2004. Inferring age from otolith measurements: a review and a new approach Can. J. Fish. Aquat. Sci. 61(7): 1269-1284

In 1985, Boehlert suggested that fish age could be estimated from otolith measurements. Since that time, a number of inferential techniques have been proposed and tested in a range of species. A review of these techniques shows that all are subject to at least one of four types of bias. In addition, they all focus on assigning ages to individual fish, whereas the estimation of population parameters (particularly proportions at age) is usually the goal. We propose a new flexible method of inference based on mixture analysis, which avoids these biases and makes better use of the data. We argue that the most appropriate technique for evaluating the performance of these methods is a cost–benefit analysis that compares the cost of the estimated ages with that of the traditional annulus count method. A simulation experiment is used to illustrate both the new method and the cost–benefit analysis.

Conclusion: Need for improvement of fish ageing results/harmonisation of methods and protocols etc.

4.2. Critical elements for QC and QA in fish ageing

There are many elements to consider during the process of implementing QA in an institution. Such elements include the methods, personnel, equipment, material, and even the environment. Methods must be documented before they can be evaluated, and it is essential to do both.

- The Personnel must be trained and provision made for updating of expertise and continued training. It is also important to establish a good cooperation between age readers and lab managers to encourage continued improvement of techniques, and adherence to written protocols. There must also be sufficient resources (time and money) to allow the personnel to develop the protocols, to implement them, and to continue to produce reliable data by following them.
- Equipment must be appropriate, and well maintained.
- Material: is this the reference sets? Or is this the quality of the structures that age readers are expected to read?
- The working environment must be considered, especially with respect to the health and safety regulations of individual countries. The preparation of ageing structures can involve the use of semi-hazardous chemicals (resins, acids, stains, etc.) and also procedures (saws, grinders).

The most important ones of these issues are proper methods, qualified personnel, and equipment with high enough quality. Even a commonly used ageing method may be found inaccurate with some fish species or populations when examined in another way (known aged fish, chemical method, other hard structures, etc.). A deficient ageing method enables the possibility of high precision in quality control but low accuracy. Skilful personnel can be a key element in raising doubts of the reliability of a method generally regarded accurate. Continuous training and search for ways to confirm the reliability of age determinations give the best results in long term. In some cases, the quality of equipment may hinder the reader from interpreting the structures accurately and even noticing the deficiencies in the age determinations.

- How to implement new knowledge and techniques (combination of methods and personnel)
- In implementing new knowledge and techniques, co-operation and exchange of experience with other age readers and laboratories is valuable. Recommendable ways to do this are e.g. intercalibration, exchange of personnel, and workshops.
- It is important that new research into techniques and also into the biological mechanisms are supported and integrated into ageing. The current systems which separate the people involved in research from the people involved in age reading should be broken down. Closer ties and communication will help to encourage research which addresses the problems in ageing and will also encourage age readers to incorporate new findings.
- How would the link actually function? Through in-house workshops? Is it possible to involve the same people, working together?
- This requires investment of time, set aside to encourage cooperation and communication.
- Reports and presentations from attending workshops and exchanges should be made public and circulated in the lab to make everyone aware of problems and new techniques.

<u>Conclusion</u>: Importance of the definition of protocols Methods, Personnel, Equipment

4.3. Case studies of the experience of implementing QA and QC in fish ageing laboratories

4.3.1 CEFAS (Mike Easy)

A Quality Control (QC) programme for assessing the level of consistency in age reading was started in 1999. The aim of the programme is to ensure that all experienced readers have a sample of their age readings checked each year by another experienced reader to identify any problems that might affect the precision of the data supplied to the ICES Assessment Working Groups. The level of agreement between the two readers is calculated, and this can be used as an indicator of the consistency of age reading within CEFAS. There are always some otoliths for which agreement cannot be reached, even with species regarded as being easy to age, while some species that are notoriously difficult to interpret will have a lower level of agreement. Since no validation of the true age is available, the method cannot identify the correct age, but it does quickly indicate if there are serious discrepancies among experienced readers, or if a person's standard has lowered or developed a serious bias, e.g. misidentification of the edge or nucleus. Readers are checked for each species they read and, if a reader ages more than one stock, the most difficult one should be chosen for the QC. If the stocks are similar, each stock can be done on a yearly, rolling basis. Target levels have been set which, if not achieved, could necessitate further checks depending on the severity and species/area involved. Ideally, the QC checks should be completed to the same deadlines needed for the working group data so that any poor results can be investigated and samples re-read if necessary before the data are analysed.

4.3.2 IMR (Erlend Moksness)

At the Institute of Marine Research (IMR) a high number of fish are annually aged to contribute to the assessment of marine fishes. For seven species age procedures are available including an Age Quality manual. The institute are participating in international exchanges and WSs for intercalibration of age for several species.

References

Gjøsæter, H. 1999. Procedure for age estimation of herring (*Clypea harengus*)

Gjøsæter, H. 1999. Procedure for age estimation of capelin (*Mallotus villosus* Muller)

Gjøsæter, H. 1999. Procedure for age estimation of Polar cod (*Boreogadus saida* Lepechin)

Njanger, H, K. Nedreaas, H. Senneset and P. Ågotnes 2000. Prosedure for aldersbestemmelse av torsk, sei og hyse (Procedure for age's estimation of cod, seith and haddock)

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

4.3.3 IFREMER (Jean-Paul Berthome)

Quality analysis started in IFREMER in 2003. As the readers are spread in several laboratories (Boulogne sur mer, Port en Bessin, Brest, Lorient, La Rochelle, Sète, La Réunion-Indian Ocean, St Pierre et Miquelon- NO Atlantic), the first decision has been to built a network co-ordinated by R. Bellail.

The first project has been to write reference documents for age readings involved in stock assessment under the auspices of ICES and NAFO.

At this time, drafts have been written for:

- Cod in Celtic Sea (ICES Divisions VIIe-k): Technical, analysis protocols
- Whiting in Celtic Sea (same Divisions): Technical protocol
- Haddock in VII bc, e-k: Technical protocol
- Saithe in VIa: Technical protocol
- Pilchard in Bay of Biscay (VIIIa,b,d): Technical and analysis protocols
- Anchovy in Bay of Biscay (VIIIa,b,d): Technical and analysis protocols
- Bass in Western Channel and Bay of Biscay: sampling protocol
- Monkfish in Celtic Sea and Bay of Biscay: sampling protocol
- Reader training requirements for Celtic Sea Cod

A trial of sensitivity analysis has been carried out using data from whiting in the North Sea and Celtic Sea. Based on a comparison of ALKs produced by 2 different readers, results of VPA assessments (pseudo-cohort) on the datasets have shown that a few differences between readers can lead to strong discrepancies in the trends of fishing mortalities. Nevertheless, the influence of discrepancies in age reading is moderated in the trends of SSB and recruitment. This trial is only a preliminary investigation of the consequences of discrepancies in age reading. Support of statisticians and members of stock assessment working groups should be used to build a standard method for a sensitivity analysis.

Some other documents are required for the end of 2005, relevant to monkfish and hake from the Southern Shelf and round and flat fish from Eastern Channel and Southern North Sea which are aged in IFREMER

All these documents are also reference documents in the larger project MEQUAPRO of IFREMER which also includes Fisheries statistics, sampling design, and assessment of national stocks. This project has initiated a Quality Action which covers the activities involved in French fisheries.

4.3.4 RKTL (Jari Raitaniemi, Kari Nyberg)

A case study of quality control in Finland, presented in Raitaniemi & Heikinheimo (1998): In 1990's, several readers in the Finnish Game and Fisheries Research Institute were ageing whitefish (*Coregonus lavaretus* (L.)) from both freshwater and brackish water environments, using mainly scales. As it was found that there were discrepancies in the ageing results between different readers, even when they were ageing whitefish from ecologically very similar coastal populations, a comparison of readings was arranged, using two different whitefish populations: a fast growing migratory population and a slow growing stationary population.

Four readers read the samples from both populations, using scales according to their normal practice. In addition, although having little experience on otoliths, three of them read the ages of the same fish also from otoliths. The slowly grown whitefish were estimated older from otoliths than from scales, but two out of three readers estimated the fast grown fish older from scales than from otoliths. The age estimates were also biased between the readers.

During this test, a number of marked and known-aged whitefish from the population with fast growing specimens were recaptured. The calcified structures of the known-aged fish were a key to the correct interpretation of the aged sample from the same population. This examination showed that two out of three readers had overestimated the age of the fish from scales, and this knowledge guided to more correct interpretation of otoliths from both populations, as well.

Thus, the test showed that one of the readers had interpreted the scales of the fast grown fish correctly and gave strong evidence that nobody had been able to age correctly the slowly grown fish from scales. After getting the results the readers continued their work, but the discrepancy still remained between the ageing results of two major readers. As a consequence of this, the reader regarded as the most reliable began to receive more tasks in whitefish ageing and the reader regarded as unreliable began to work with other kinds of duties. In the routine ageing and back-calculation of growth, otoliths have been used since in addition to scales, to give the best possible estimate of age.

Reference

Raitaniemi, J. & Heikinheimo, O. 1998. Variability of age estimates of whitefish (*Coregonus lavaretus* (L.)) from two Baltic populations – differences between methods and between readers. Nordic J. Freshw. Res. 74: 101-109.

4.3.5 A case study: The comparison of two ageing methods of herring from northern Baltic Sea

Jari Raitaniemi, Finnish Game and Fisheries Research Institute, Finland

Herring is usually aged from whole otoliths, in reflected light against a dark background. However, in the brackish water (0.2–0.7 % salinity) of the northern Baltic Sea, the growth of herring is very slow and the rings especially near the edge of the otolith can be blurry. Especially in the otoliths of larger herring, there can be a wide transparent zone at the edge of the otolith, wider than previous clear annual growth zones.

Because of the uncertainties, the staining method with neutral red, introduced by Richter & McDermott (1990) was tested with the otoliths of Bothnian Sea herring, at first by grinding half of the otolith away to get a cross-section through nucleus, and later by sawing thin slices of the otoliths. The more coarse grinding method that in practice made possible only the magnification of 100 times and less, already showed that there was a bias between the methods in the age determination results of herring older than 7–8 years (Peltonen et al. 2002). However, the magnification of 100 times was not enough to differentiate all the thinnest rings near otolith edge. Using thin slices and a compound microscope has essentially improved the possibility to accurately age even the oldest specimens. Thin slices have confirmed that from the age of 6–7, the otolith slice-age of Bothnian Sea herring is on the average higher than the age from whole otolith. In younger otoliths, the differences between methods are smaller and may vary from year to year.

In 2006, parallel age determinations from thin slices and whole otoliths were used in the assessment of Bothnian Sea herring stock for the first time, using the results from both methods from the four most recent years, along with the results of the old method from earlier years (ICES 2006). The change of ageing method affected age distribution as well as the proportion mature fish at age. Determination from otolith slices generally resulted in older ages than from whole otoliths. The numbers at age in the total catch on average differed by about 2%, but ranged from 0.4% to 52% depending on year and age. On average, the proportion of four- to eight-year-olds in the catch was 11% lower, and the proportion of ages 9 and older was 32% higher, when using otolith slices compared to whole otoliths.

The differences in the results of age determination in 2002-2005 (only, so far) had only a small effect on the XSA estimates, compared to output from time series using only ages determined from whole otoliths. The use of thin slices resulted in lower estimates of SSB (at most 8% difference in estimates, in 2005) and recruitment (-1% difference in 2005), and correspondingly, higher estimates of fishing mortality (Fbar 3-7, 0.2% difference in 2005). Neither the size nor the pattern of the residuals changed to any greater extent.

In the Finnish age determinations of herring from Gulf of Finland in 2004, parallel age determination with two methods revealed that the majority of the obvious or most probable year class 2002 was aged erroneously one year younger from whole otoliths, i.e. as if they belonged to the year class 2003.

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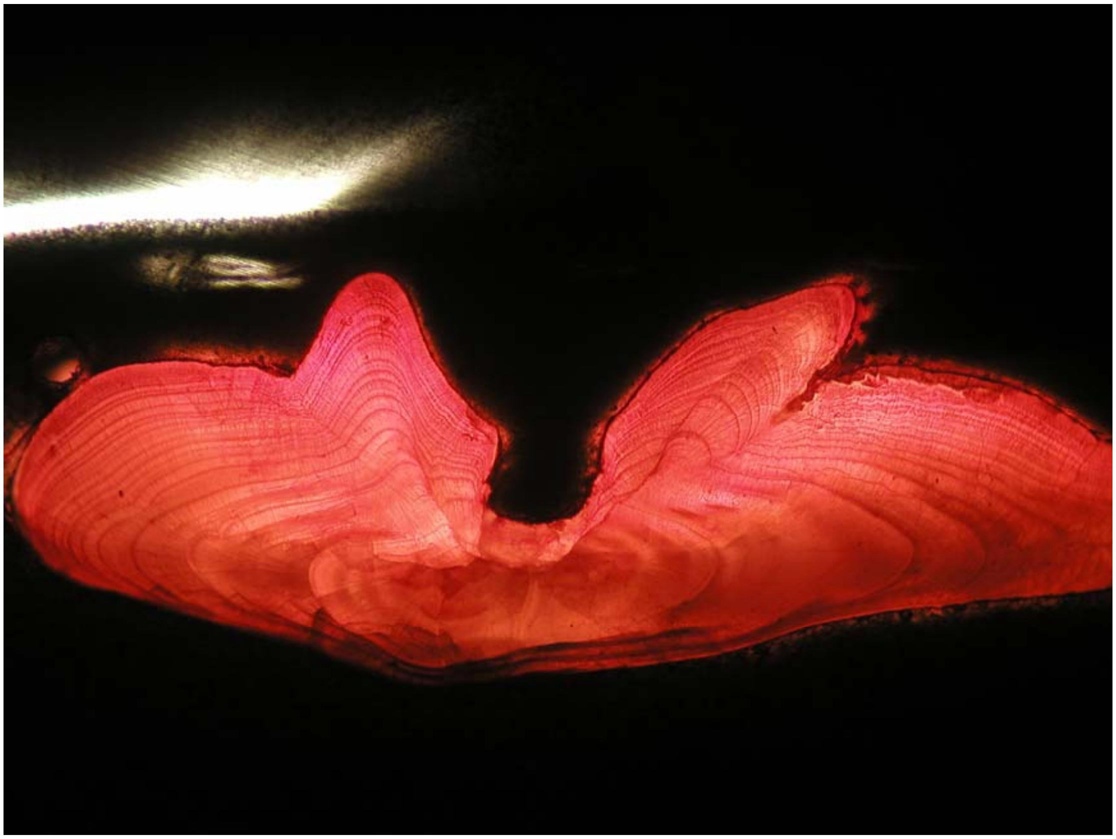

Figure 7. The neutral red stained otolith cross-section of an old herring (age about 23 years) from Bothnian Sea, northern Baltic Sea. Photo by Tarja Wiik

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ICES 2006. Report of the Baltic Fisheries Assessment Working Group. ICES CM 2006/ACFM:24.

Peltonen, H., Raitaniemi, J., Parmanne, R., Eklund, J., Nyberg, K. & Halling, F. 2002. Age determination of Baltic herring from whole otoliths and from neutral red stained otolith cross sections. ICES Journal of Marine Science 59:323-332.

Richter, H. & McDermott, J.G. 1990. The staining of fish otoliths for age determination. Journal of Fish Biology 36: 773-779.

4.3.6 Experiences from implementing QA of fish ageing at the Swedish Board of Fisheries

Magnus Appelberg, Swedish Board of Fisheries, Dept of Research and Development, Sweden

Background

The Department of Research and Development at the Swedish Board of Fisheries (SBF) comprise three geographically separated fishery research institutes and two aquaculture research stations focusing on research on marine, coastal and freshwater fish, fisheries and aquaculture in Sweden. In total, SBF routinely perform fish ageing for 12 marine and 13 freshwater species from different areas and habitats, involving about 20 employees (researchers and technicians). In addition to routine analyses, otoliths are also used for various research purposes.

In mid 1990s it was recognised that accuracy and precision in fish ageing was a common problem among the SBF institutes and that there was a need for QA when data were used in ICES and HELCOM assessments and in other international bodies. Inspired by the work within the EU-CA EFAN (European Fish Ageing Network), a joint project for improving quality in fish ageing in Sweden was initiated. The project later became institutionalised and now forms the Centre for fish Ageing (CfA) at the SBF. A report from the first three years work was included in the final EFAN report, year 2000 (Appelberg *et al*. 2000).

The major objectives of the work have been to increase quality in fish ageing in Sweden and ultimately reach a level of accreditation. A key issue has been to produce a QA manual for fish ageing that should be common for all participating institutes. Other important issues have been the implementation and harmonisation of new methods and techniques for fish ageing at the participating laboratories. Emphasis has also been put on development of personal skill and knowledge in the field of fish ageing. Increased co-operation between age readers for better use of know-how and communication, as well as increased enthusiasm and ownership in the daily work is necessary.

Methods and realisation

Centre for fish Ageing (CfA) is a collaboration of geographically separated laboratories and stations dealing with fish ageing on mainly routine basis Swedish Board of Fisheries; Institute of Marine Research, Lysekil, Institute of Coastal Research, Öregrund, Institute of Freshwater Research, Drottningholm, Aquaculture Research stations at Älvkarleby and Kälarne. Originally the work consisted of a series of workshops, aiming at developing a common manual for fish ageing within the SBF. The manual was the first step towards a future accreditation process. Participants were age readers and researchers working with fish ageing or research dedicated to fish ages and otoliths. Later on, these workshops have evolved to annual meetings where different aspects on age reading, methods and equipment are discussed. Today, CfA is structured as a virtual centre without physical location. The work is co-ordinated through annual meetings and internal newsletters and at present driven mainly by the involved age readers.

Achievements

The achievements after almost ten years of co-operative work are several. Among the most important outcomes is the development a continuously revised quality manual (or "handbook") for fish aging methods and processes used at SBF; "Methodological handbook for fish ageing at the Swedish Board of Fisheries" (Anonymous 2006). This quality manual, covering all fish species routinely aged at SBF, is adopted by all participating institutes. It is revised and updated annually. The handbook comprises five major chapters; 1) Introduction, 2) Definition of age, 3) Sampling, preparation and age determination, 4) Data registration and responsibilities, 5) Archiving and data handling. In addition, 18 addendums define specific routines and methods used at the different laboratories.

A second achievement is a harmonisation of methods and equipment used for routine analyses in fish ageing. The development of the common handbook initialised a harmonisation of methods among institutes, selecting for the most accurate methods. Although harmonisation has not been fully realized, exchange of experiences among the participating age readers have improved methods used as well as increased understanding among age readers. The co-operative work among the group has also been supportive in implementing new equipment and techniques. Among the most important accomplishments is the introduction of otolith saws at all three research institutes. The saw is now routinely used for ageing several marine and freshwater species. Experiences are exchanged among personnel dealing with this.

In the early stages it was realised that there was a need for a uniform use of terms and expressions. This is of special importance during training, inter-calibration exercises and conference readings. For that reason a Swedish glossary for fish ageing was compiled (Anonymous 2000). The glossary is primarily based on ”Glossary for Otolith Studies” (Kalish *et al.* 1995), which was translated into Swedish. The glossary is used all through the methodological description.

A fourth achievement is the implementation of training procedures for new staff. Based on own experiences, as well as by adopting knowledge outside the institutes, procedures for introducing new personnel to fish ageing techniques have been developed and implemented. The procedures used at CEFAS, UK, been an especially important source of inspiration. In addition, the importance of "institutional sustainability" of age reading has been recognized, *i.e.* ensuring the competence of two age readers for all fish species/area aged.

One of the major goals of CfA was to increase the communication between age readers at SBF in order to improve work and results, as well as to increase the enthusiasm for the work. This has been an important outcome of the co-operative work, manifested as a closer contact, increased exchange of ideas and discussions at the national level within the CfA, but also an increased willingness to attend international inter-calibration workshops. The annual workshops and meetings have led to an increased co-operation among age readers involved. It also has increased the self-confidence among readers and promoted an open discussion. The increased communication between age readers and researchers within SBF has gained the overall quality of the work.

Shortcomings and future needs

Although the initial aim to reach a level in QA facilitating national accreditation was not accomplished, the experiences realized so far form an important platform for a future development of the QA at SBF. By keeping the QA process active among age readers, researchers and decision makers, we now believe that the accreditation process itself may be of less importance. Instead, a continuous development of QA and QC, which is an iterative process that needs to be revised on a regular basis, is the major goal. As competence, skill and interest of the age reader is the foundation for the quality in age determination, it has also have been realised that the competence and engagement of the staff involved has to be put in centre in this process.

In recent years it has become increasingly apparent that a strong involvement of research is essential (or even fundamental) for the progress of QA in fish ageing. The scientific needs for accuracy and precision (or needs for alternative methods) as well as new applications of ecological and environmental information based on otoliths are important driving forces for developing new fish ageing methods and techniques. If research is decoupled from routine fish ageing, the evolution of more accurate methods will vanish.

The lack of awareness and expressed needs for QA and QC from “customers” have had a negative impact on the development of QA. In part this is partly is an effect related to the difficulty in defining relevant “customers”. Despite an in depth discussion within CfA on identifying the customers, this still remain an open question.

Repeatedly performed inter-calibrations at intra- and inter-institutional, national and international levels are fundamental to all QA and especially QC in fish ageing. So far, these exercises have not been formalised at an acceptable level. Most often, inter-calibrations are performed *ad hoc* or as the results of a specific problem raised in e.g. ICES or other bodies when problems related to fish ages have been recognised. It would be beneficial if inter-calibrations were performed at a predetermined level, defined by the needs for QC.

It is important that enough time (and means) is allocated to continuous development of QA and QC. However, after the initial years of new experiences in the work, it has become apparent that both means and interest for QA are becoming more difficult to achieve. To continue the QA process at a sustainable level, a number of requirements have to be fulfilled. At first, it has to be recognised that staff involved in the process are encouraged and that time (and money) are spent on the work. QA requires a considerable effort and it is necessary to allocate a realistic amount of time and money to the work. Secondly, engagement from the staff involved in the QA is crucial for a successful result. It is therefore of utterly most important to clarify the advantages by the QA process for employees, and to let everybody involved be genuine part of the work. Thirdly, engagement from managers and researchers is necessary to co-ordinate the work and to take part in the work. To increase the engagement from managers a cost/benefit assessment of the QA-process should be worked out at an early stage. Fourthly, as pointed out above, there is a strong need for a close co-operation between research and age reading.

Costs

Appelberg *et al*. (2000) estimated the annual time spent for the QA work within CfA to about 10 man months the years 1997 – 2000, including time spent at workshops and courses. This would correspond to an annual cost of about 37,000 €. After year 2000 these costs have been slightly increased, however, the focus has now broadened and comprises training, novel methods and techniques as well as other aspects of QA in fish ageing.

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Kalish, J. M., Beamish, R. J., Brothers, E. D, Casselman, J. M., Francis, R. I. C. C., Mosegaard, H., Panfili, J., Prince, E. R:, Thresher, R. E:, Wilson, C. A., Wright, P. J. 1995. Glossary for otolith studies. In: Secor, D. H., Dean, J. M., Campana, S. E. (Eds.) Recent developments in Fish Otolith Research. University of South Carolina Press, Columbia, SC pp. 723-729.

4.3.7 Conclusion

The experiences of implementing QA and QC as demonstrated through these case studies allow for some general observations:

- The use of modal age to evaluate a method is not always desirable, as demonstrated when known age material is compared; thus, it is important to increase the production of known aged material e.g. through mark-recapture studies.
- Costs of implementing QA and QC are considerable, and ongoing. There must be a long-term commitment to the programme.
- The goal of QA and QC must be clear, and it is important to define customers or users of the age data and to continually inform them of the importance of data quality.

4.4. Implementing a Quality Assurance and Quality Control Framework and consideration of accreditation and certification

The previous sections outlined the individual elements which are necessary for a QA/QC approach to ageing. Implementing a formal QA/QC framework requires that these elements are brought together in a more formal management approach.

4.4.1 Considerations for accreditation of age reading

QA and QC are to a large extent performed on a national level. A further possibility is to formalise the process through a system of accreditation. In practice, this means that a laboratory, which may be accredited for one or a number of analyses, undergoes the control of an accreditation board, which controls and accepts the procedures used, and also make repeatedly control inspections to see that there is no deviation from the documented procedures. Whilst there are clearly some benefits from this approach, there are also a number of potential problems in applying accreditation to age reading.

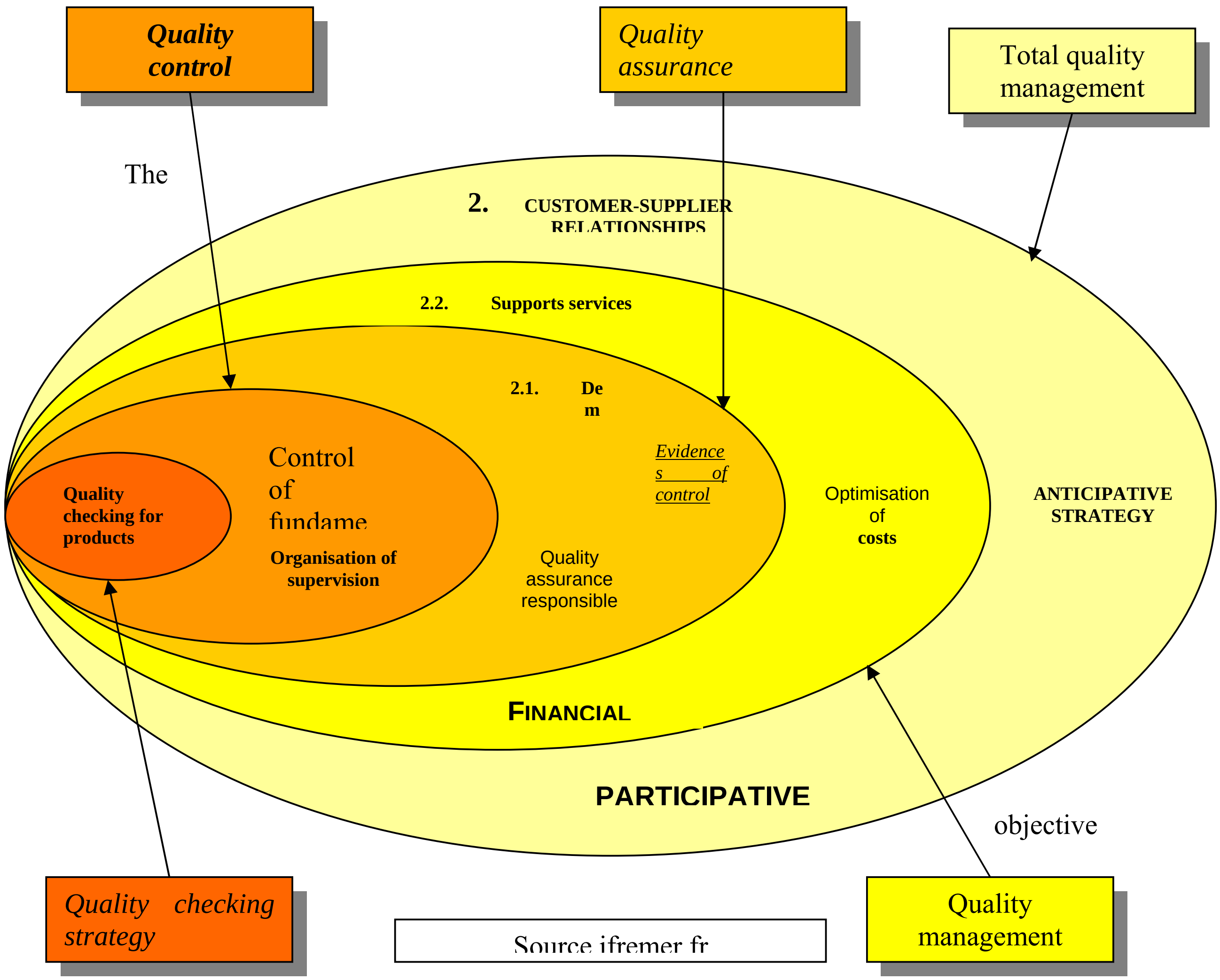


Figure 8. Levels of complexity of QA and QC in the accreditation process.

Some of the main issues are:

- Increased costs involved in accreditation need to be justified by careful cost benefit analysis.
- Accreditation processes are most effectively applied to techniques where the process can be repeated with the same results every time such as weighing chemical samples or carrying out a chemical analysis. This does not apply to ageing where the true age is not normally known and so no absolute standards are available for checking.
- Since many laboratories read more than one species, accreditation would need to be carried out separately for each species, imposing considerable additional costs
- Accreditation would apply not just to the processes but to the staff involved. If expert readers leave, it might be necessary to apply for re-accreditation.

- Since in most situations, there will be no known age material, there could be problems determining a fair arbitration process in cases where accreditation was refused.
- Accreditation may be justifiable in an institute reading large numbers of fish ages but this could disadvantage small research institutes with low numbers of staff

4.4.2 Achieving accreditation

The objective of this section is to explore the implications of accreditation and certification of fish ageing. The general principles of a typical accreditation and certification QA/QC framework developed by IFREMER presented as an example in Figure 8. This suggests that there are a number of levels involved in setting up the full framework. The innermost level involves Quality Checking of individual samples. Quality Checking is itself part of the general Quality Control processes in the laboratory (level 2). At the next level are Quality Assurance procedures (Level 3). Unlike QC which operates at the sample level, these are defined as the "procedural matters that are generally applied lab-wide" (Morison *et al*., 2005). These would include such aspects as preparation of standard operating procedures, staff training and equipment maintenance. However, the present situation is better described by the inner three circles in the same figure, comprising QC and QA. Many fish ageing laboratories operate within these three basic levels. However, a further two levels can be envisaged. The first involving some form of cost benefit optimisation – balancing the additional costs with the improvement in ageing precision. The final level might include an ability to respond pro-actively to customer requirements or could involve development of an accreditation process, if considered appropriate. This is probably a more realistic goal for most fish ageing laboratories.

In addition, the accreditation of fish ageing may act to exclude research laboratories with low numbers of permanent staff. It may also contradict the inclusion of research laboratories from new member states in the field of fish ageing.

It should be noted that development of QC and QA (or accreditation) is only possible difficult when sufficient funds are allocated for it. Since the initial documentation of processes and procedures (protocols, interpretation protocols, together with training etc) is fundamental for all quality assurance it is critical that enough time and resources are dedicated to this these initial steps. This is true no matter what the ultimate goal. It is important that laboratory managers, who are responsible of for the quality of results for customers, support quality actions by providing adapted adequate resources (especially people staff time). The results from modelling exercises may be used for cost benefit analyses.

4.4.2 Costs for QA and QC

The cost for achieving accreditation varies among countries and among laboratories. Concerning the costs of quality assurance in laboratories, experience shows that it is necessary to spend around 10 per cent of worktime of all people involved in, during the phase of implementation of quality assurance (minimum 18 month) and some per cents after (except for quality responsible, depending on size of laboratory but never less than 25 per cent of his time).

Directs costs are links to metrology (buying of reference standards), buying of norms, ring tests and, if hoped, audits by people out of laboratory). In case of accreditation the cost of initial audits are around 10 000 Euros and annual tax of 1 000 euros.

As a general average, experience indicates that it is necessary to spend around 10 per cent of work time of all people involved in, during the phase of implementation of quality assurance (minimum 18 month). In case of accreditation the cost of initial audits are around 10,000 Euros and annual tax of 1 000 Euros.

As a major cost is related to the documentation and description of ongoing procedures, the project recommend that research laboratories set aside the time and personnel well in advance for the preparation of protocols, and establish procedures for the review and updating of documentation on a regular basis.

4.4.3 Definition of customers

At present there is a lack of consistency in definition of the actual customer of fish ageing results. Rather than being many customers there are often many users of fish ageing data. The customer may not be paying for data. However, the TACADAR project recommends defining the customer as "the person or group of people who define the specifications of the final product". Sometimes, the final user (*i.e. decision makers)* who use results for resources management are not able to define the level of quality they need. Scientists themselves often have to define the quality level of their results.

The TACADAR project recommends customers to be educated about the importance of the quality of data they receive. This involves two processes; one is an ongoing exchange of information about the effects of poor data, the other is an effort of the customer to specify the quality of data needed for specific cases.

References

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

4.5. Recommendations for QA and QC in the field of fish ageing

(Conclusions from sub-sections:)

1. An evaluation of the consequences of ageing errors shows that errors in age reading may be significant in estimating parameter values for stock assessment and other fishery related issues. These errors can be quantified and modelled. There are different sources of errors, which calls for improvement of ageing results and harmonization of methods and protocols, through the use of QC and QA.
2. There are different elements that are critical to the implementation of QC and QA. The most important are skilled personnel, the use of reference collections, cross-checking and intercalibration, and the application of statistical methods in

the evaluation of results. Each element needs to be considered and supported with appropriate resources.

3. The experiences of implementing QA and QC as demonstrated through these case studies allow for some general observations: The use of modal age to evaluate a method is not always desirable, as demonstrated when known age material is compared; thus, it is important to increase the production of known aged material e.g. through mark-recapture studies. It is difficult to maintain interest, motivation, and resources in continuing QA and QC after the initial implementation. The application of research to the question of fish ageing is required to ensure improvements and continued interest.
4. In order to have confidence in the ageing data process, it is necessary for a laboratory which participates at the international level to develop appropriate QA/QC procedures. The quality process puts additional and significant costs on the institution and this investment must be supported as part of the institutional strategy. The investment in skilled and expert staff is the most important and often ignored portion of the costs. The steps leading further, to accreditation, may not be necessary.

Section 5: Evaluation of individual methods

Where two or more methods with acceptable methodology are used, the guidance in Section 4 should be applied to determine if the levels of precision and accuracy of any or all of the methods are acceptable for stock assessment or environmental management modeling.

All methods should have acceptable quality assurance (QA), quality control (QC) mechanisms and these should be in accordance with the guidance in Section 4 on the creation and application of QA and QC mechanisms to protocols for the estimation of the ages of fishes from age reading materials.

When evaluating individual methods of age estimation in cases where several methods are used to provide data input for the same stock assessment or environmental management model, it is important to note that variations within an individual method and the application of distinctly separate methods of age estimation, can both affect the quality of the age estimations if they reduce the readability of the growth zones.

Some age reading materials preparations are insufficiently robust to withstand the additional handling and transport that takes place during an otolith exchange. This can lead to a reduced quality of preparation for successive readers and also during 2nd readings at an otolith reading workshop. This can be caused by otoliths not being fully covered by the embedding media (Fig. 9), but it can also be caused by previously unknown incompatibilities between embedding media and some injection-moulded plastic ‘otolith cards’. Where cover-slips are used they should be cleaned and dried before use to ensure a strong bond between the cover-slips and the embedding media.

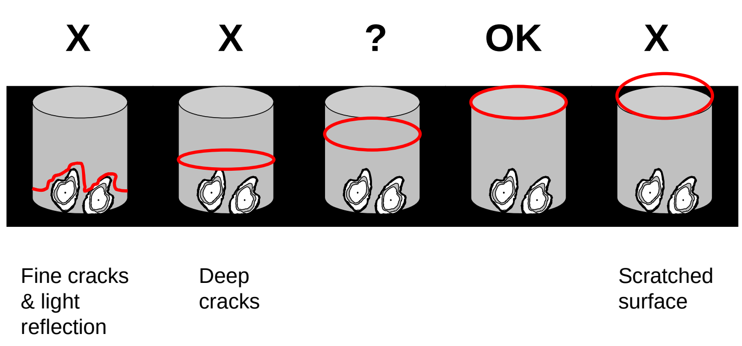


Fig. 9: Correct embedding media fill level in the ‘no cover-slip method’ for black plastic otolith cards.

Discussions with TACADAR members and members of the EFAN Concerted Action, suggest that there is a tendency in some cases for methods of otolith preparation to evolve in response to local needs. Discussions with otolith readers at ICES otolith age reading workshops tended to support this view. Variations of original methods may be driven by perceived improvements on the original methodology and by changes in the availability of materials and equipment used to prepare otoliths for age estimation.

Detailed advice on the selection of appropriate methods of age estimation is provided in the Manual of Fish Sclerochronology, (Panfili *et al*, 2002). The process of estimating the age of an otolith or other age reading material can be broadly divided into three parts:

1. Preparation (Fig. 10)
2. Observation (Fig. 11)
3. Interpretation (Fig. 12)

Significant steps in the preparation, observation and interpretation of the age reading materials where discussed. The processes of sample collection and data storage were not included at this stage. The flow charts in this report were developed from these discussions and are intended to provide guidance on the suitability of individual methods of age estimation.

The group discussions produced a brief overview of the evaluation process. It was noted that it is very difficult to evaluate observation methods without visual access to this part of the age estimation process. While visits to the relevant laboratories would be preferred, video evidence may be acceptable in some cases. It is also difficult to fully define an evaluation process from a QA/QC perspective without knowing the degree of assurance required. This raises certain key questions:

- What precisely does the customer want?
- How much will the customer pay for this service?
- Who should carry out the evaluations?

There are advantages in archiving images of calcified structures in addition to conserving calcified structures for future precision checks, (Fablet, R. TACADAR Ancona Meeting Presentation 2004) and this must also be considered when choosing a particular method of age estimation. A training programme for the age readers must support each age estimation method and providing evidence of this training should be a part of the QC process. The experience of the age readers must also be taken into account. Some age estimation methods require complex preparation of the age reading material, but given appropriate QC measures and effective training, tried and tested simple preparation methods can also consistently produce equally precise results. Each preparation method (and variation within a method) has advantages, disadvantages and constraints. These must all be considered when choosing a particular preparation method.

There are subtle variations in the methods of viewing or observing age reading material for most methods of age estimation, but each observation process must be appropriate for the intended output. The chosen method of observation must produce an image of good quality and there must be evidence that a written procedure is followed correctly.

Figure 10 Preparation of Age Reading Material

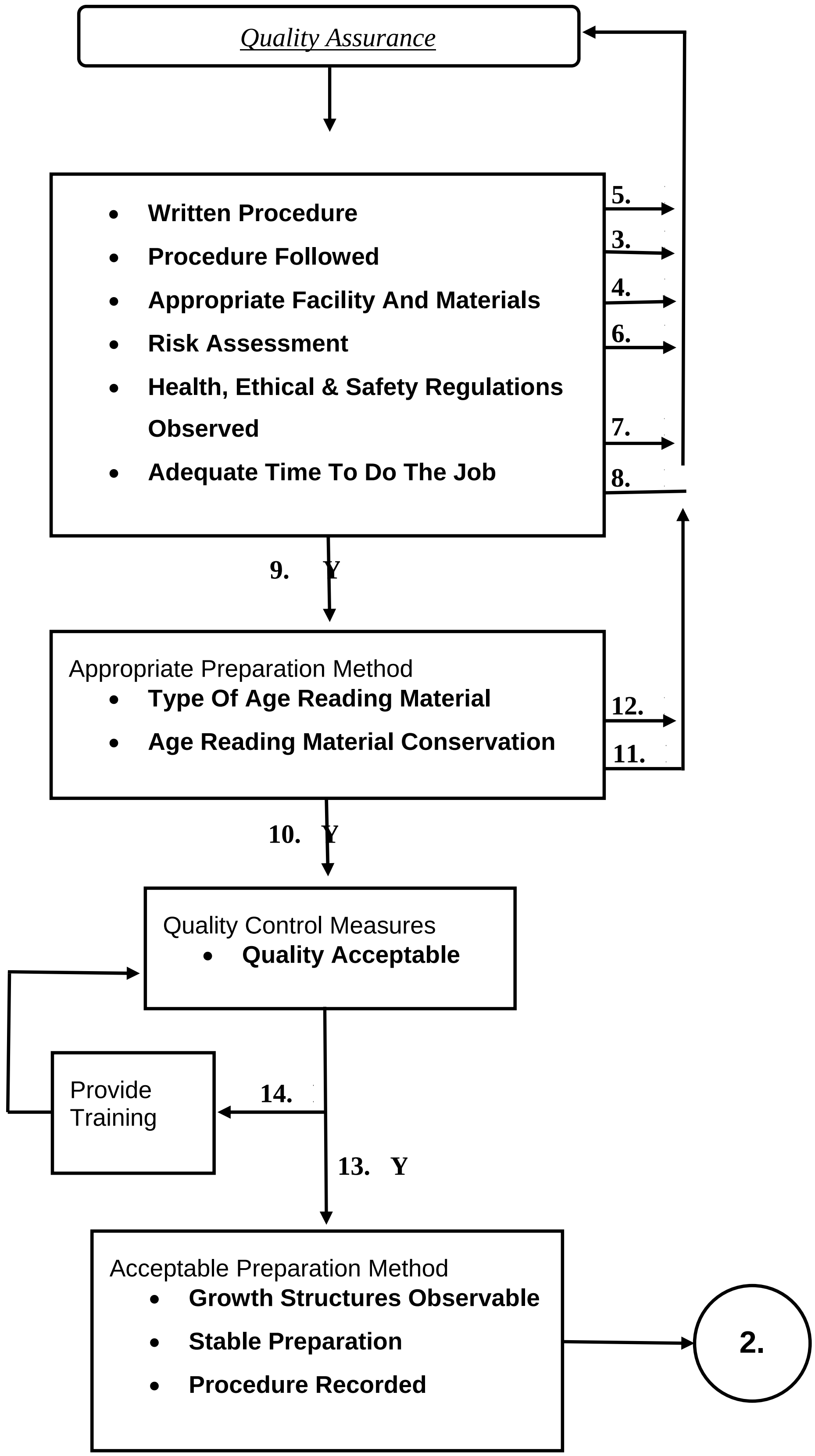


Figure 11. Observation of Age Reading Material

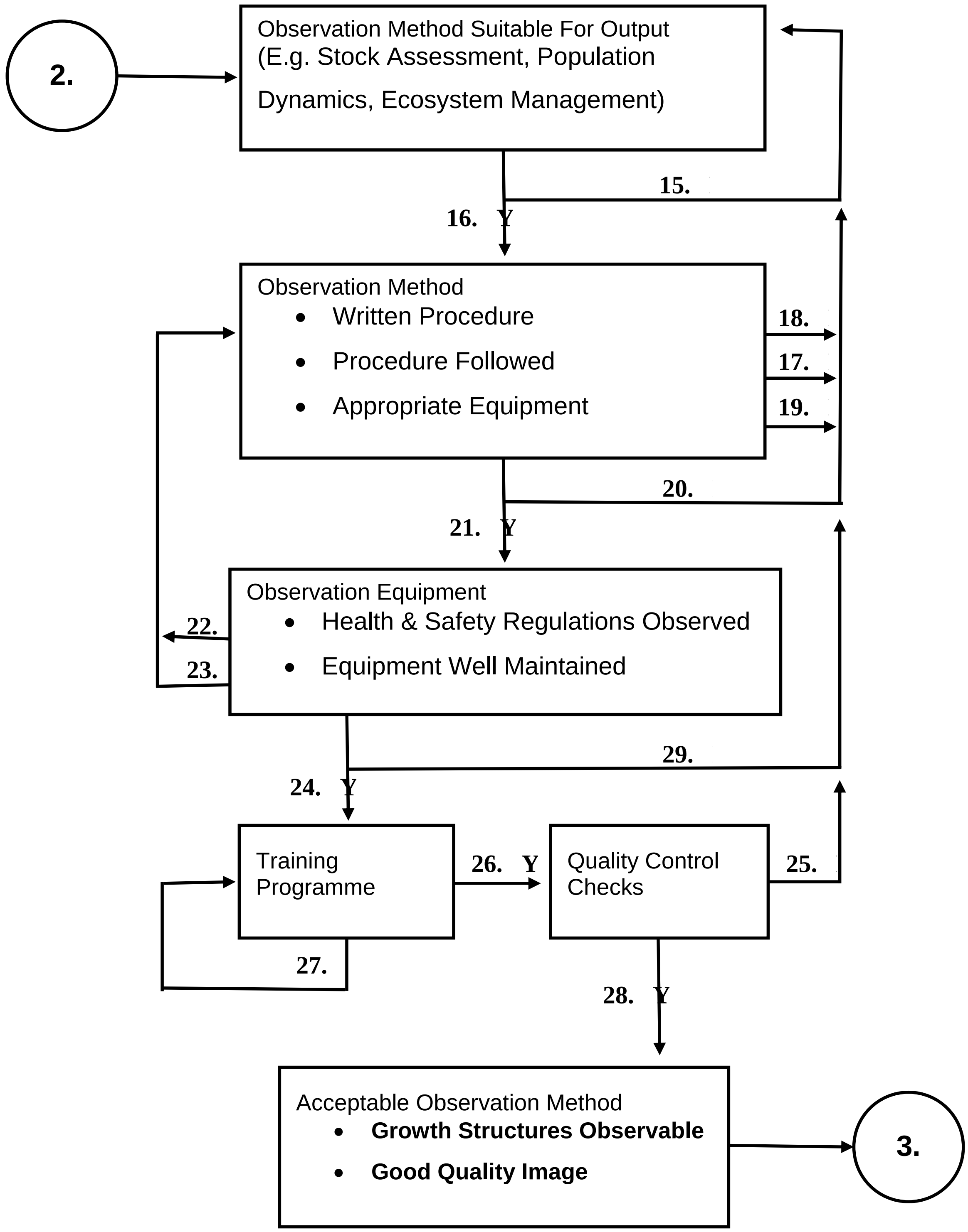
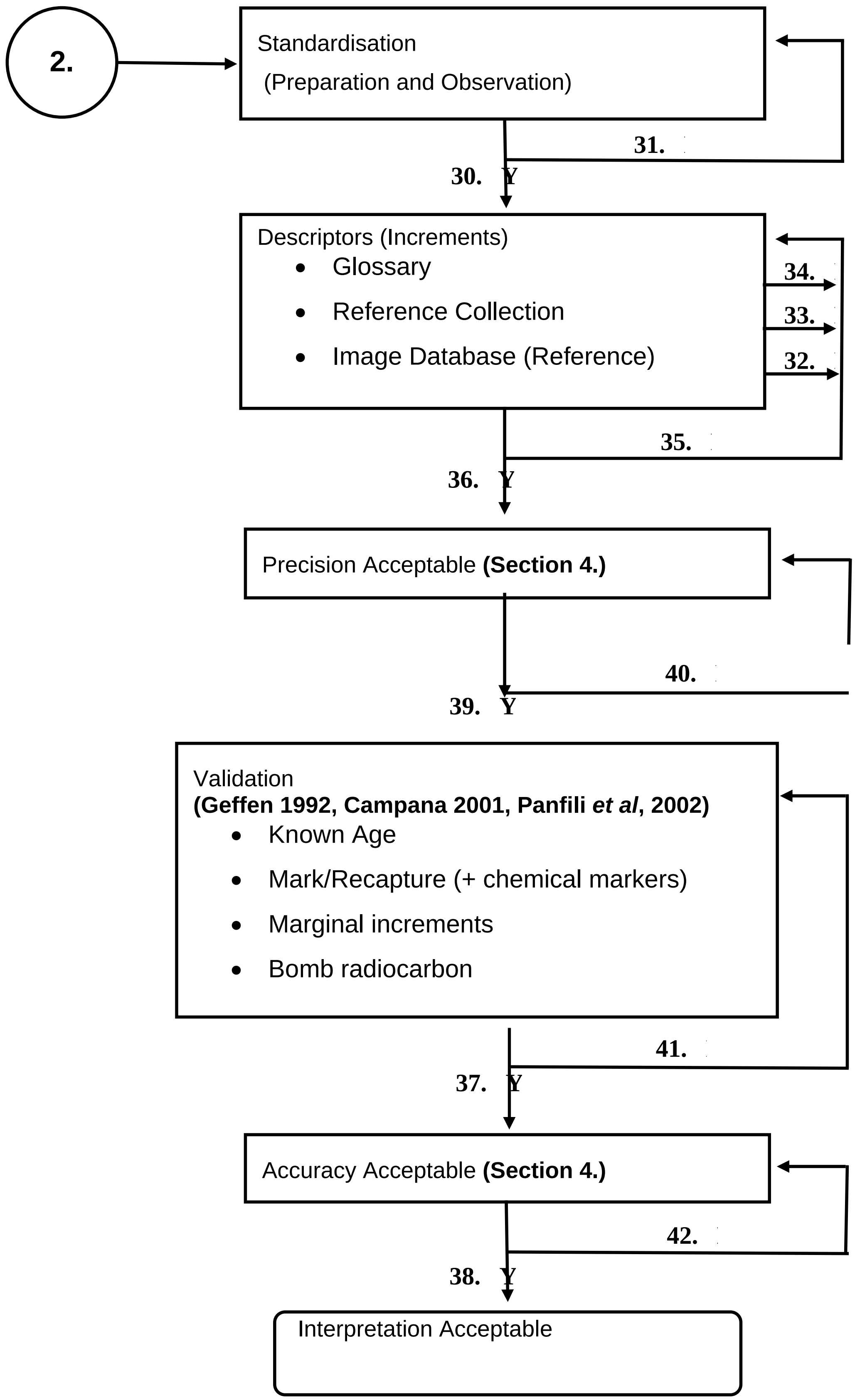


Figure 12. Evaluation Of Methods - Interpretation of Age Reading Material



QA, QC and transparency were recurring themes during discussions on the evaluating individual methods of age estimation. Where it is necessary to examine individual methods in greater detail, an initial inspection could be performed to examine the robustness of the quality of each stage of an age estimation method. It was concluded that this could best be performed by asking a series of 'quality questions', using Guidelines for the Quality Assurance of Fish Age Determination (EFAN Report 5-2000), as a framework for this process. Examples of thee type of questions that could be asked are given in Table 1.

Figures 13, 14 & 15 provide examples of some of the methods currently used for age estimation in sprat, herring and flatfish species.

During the discussions it became clear that there was a need to define what precisely constituted a different method for fish age estimation. Significant aspects of some of the multiplicity of variations within individual methods and some of the definitive features that set other procedures apart as distinct methods were discussed. Three differing groups of age estimation methods, sprat, herring and flatfish species, were chosen to illustrate the relationships between and individual methods and significant variations of those methods, in cases where several methods are used to provide data input for the same stock assessment or environmental management model. These examples were discussed extensively and the conclusions reached are summarised below and in the attached flow charts.

1. Sprat: (Fig. 13)

Custom combination slides that allow simultaneous observation of pairs of sagittae in both transmitted and reflected light, custom black plastic slides, and standard glass microscope slides, were all considered to be variations of the same basic method of age estimation by counting valid annual translucent and opaque growth zones on whole embedded sagittae.

The topographic analysis of the sagitta rostrum surface was considered to be a distinct method of age estimation. This method is preferred for some Baltic stocks where the annual growth zones in the later life history can be closely packed together, making observation of individual translucent and opaque growth zones very difficult.

Figure 13. Sprat age estimation methods

2. Herring: (Fig. 14)

As in the case of sprat sagittae, custom black plastic slides, transparent slides and custom combination slides were all considered to be variations of the same method of age estimation, while sectioning with staining and scale reading were distinctly separate methods of age estimation

The whole otolith observation method was also considered to be distinctly separate method, as the observation of a whole sagitta within a liquid medium, permits both orientation of the light path and the orientation of the sagitta within that light path to obtain the optimum observation of the annual growth zones. It was noted that this method of observation is also used with broken sagittae in the age estimation of whiting.

Otolith grinding was also recognized as a new approach and in herring this can be used to examine the growth increment widths in the early life history to determine the season of spawning. Knowledge of spawning date enables separation of autumn spawning fish of one year from Spring spawning fish of the following year and where this is necessary, only one sagittae should be used to estimate the age of each individual fish in the sample.

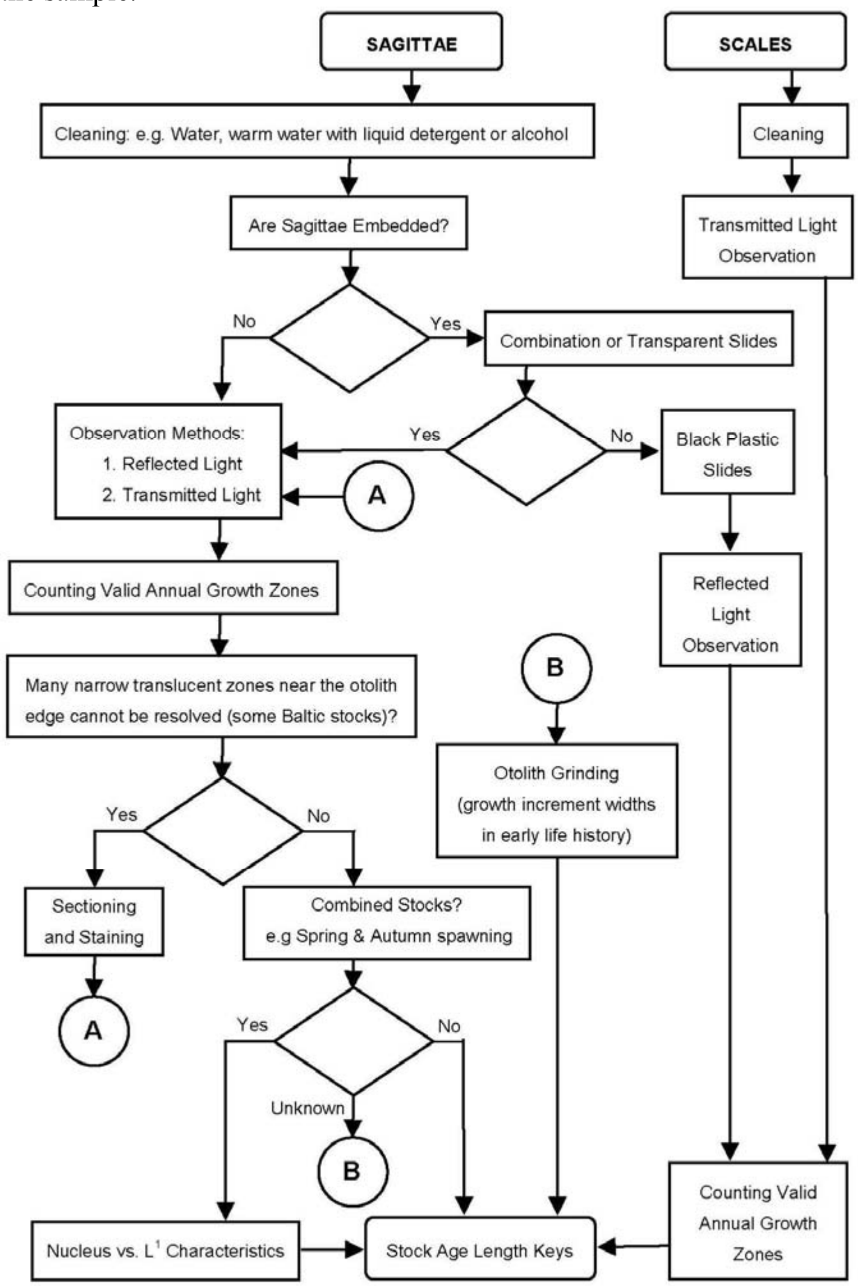


Figure 14. Herring age estimation methods

3. Flatfish Species: (Fig. 15)

Whole otolith observation, burning/breaking etc., sectioning and staining were recognized as four distinctly different methods for the age estimation of flat fish species.

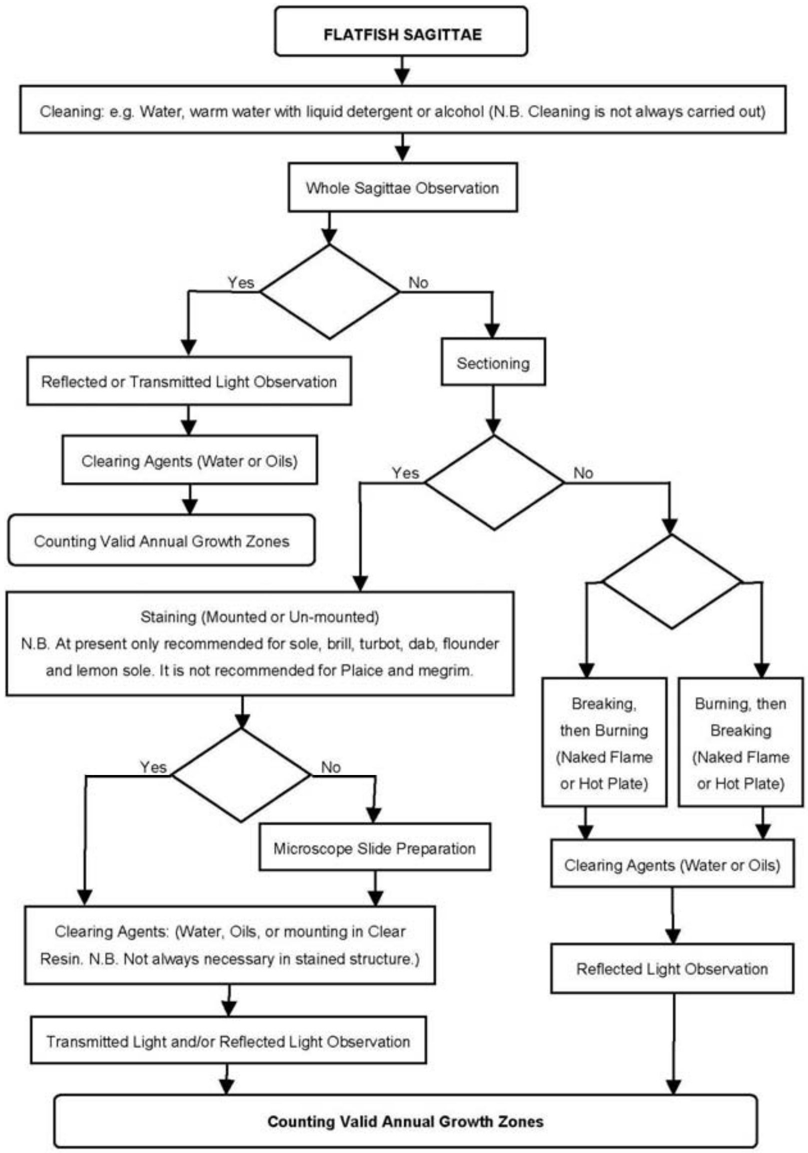


Figure 15. Flatfish age estimation methods

Summary:

It was concluded that the risk of an unacceptable quality of age estimation caused by inappropriate choice of technique was independent of whether that technique was considered to be a variation of a method or a uniquely separate method.

Where individual methods (or variations of methods) of age determination give different results when they are used to provide data input for the same stock assessment or environmental management model, they should be tested using sub-samples from the same batch of calcified structures.

Table 1. Examples of questions that may be asked in order to examine the robustness of the Quality Assurance of an individual method of fish age estimation.

EFAN REPORT 5-200 GUIDELINES	QUALITY QUESTIONS
Fish Sampling at Sea, Research Vessels and Commercial Fishing Vessels	
Survey Design	Is the method part of a multi-partner survey?
	Is there vessel inter-calibration of the vessels?
	Are there protocols for the survey design?
	Are there protocols for the sampling programme?
	How is tow length defined?
Fish Sampling	Is the sample size adequate?
	Does the sub-sampling process reflect the species and size composition of the catch?
	Is there a protocol for measuring fish lengths? Are electronic measuring boards or data capture used?
Fish Sampling at Commercial Fish Markets	
Fish Sampling	Is there a sampling protocol? Does the protocol take fishing gear type and fish size grades into account?
Measurements of fish - the method of measurement must be clearly defined.	Is there a protocol for measuring fish lengths? Are electronic measuring boards or data capture used?
The integrity of the links between the data and the age reading materials must be maintained at all times.	How is this achieved?
All the information required in addition to the measurements themselves, must be specified,	How is this information stored and how are the links between this data and length

e.g. species, area, date, fishing gear, sex, maturity.	measurement preserved?
Selection of Age Reading Materials	
Which age reading materials are to be used?	What are the reasons for the choice of age reading material, e.g. historic use, published method?
The preferred sampling site(s) for scale removal, this may vary with the species and the requirement.	Is this clearly defined within the sampling protocol?
Standards to be shared between institutes working on common stocks	How is this achieved?
Conservation of Age Reading Materials:	
Cleaning method e.g. removing muscular tissue and fats from whole bones and drying.	Is the cleaning method fit for purpose?
Transport and storage must prevent damage and deterioration	What controls are in place to prevent damage and deterioration?
Handling of age reading materials	How is the integrity of the age reading material protected? Will samples be used in age reading exchanges or workshops?
Control of moisture content	Are age reading materials adequately dry before embedding where this is required?
Species Specific Preparation Techniques	
Criteria for acceptance/rejection of age reading materials	Are these criteria clearly defined, e.g. Vaterite otoliths?
Preparation techniques	Are all growth zones revealed? Is grinding necessary for stock separation?
Burning	How is this controlled?
Staining	Is there a risk of over estimating age? Is burning also required?
Grinding	How is over-grinding prevented?
Sawing	Is the method appropriate for the type of age reading material

	How does the protocol deal with sagittae that are not sectioned through the growth centre?
Age Reading Equipment	
Reading equipment set up (e.g. binocular microscope)	Does the age estimation protocol include microscope set up for individual users? Is the working environment safe and comfortable?
Magnification level	Is the magnification suitable for the species and age range of the fish? Is the magnification used suitable for the stock and the expected growth patterns e.g. increments near the edge of the otolith section or whole otolith
Light intensity	Do the light intensity, transmission path and illumination angle used reveal all the growth increments?
Type of illumination	
Equipment quality, define the equipment to be used in each process	Are equipment standards clearly defined?
Confidence in equipment	Are growth zones clearly visible? Is the equipment user friendly?
Training use of equipment	Is training provided?
Image analysis/digitisation	If digital images are used for age estimation, are they conserved in an image database? Does the process reveal and/or generate the required information?
Age Reading Procedure (Manual)	
Time planning for readings and re-readings	Are the otoliths read by more than one person? Is the number of re-readings adequate to meet the quality requirement?
Use of Glossary (1st Otolith Research Symposium, Hilton Head, 1993)	Is this glossary used?
Where to start reading	Where is the growth centre of the otolith or otolith section located?

Definition of ‘rings’	How are valid growth zones defined?
Criteria for genuine/ false ‘rings’	Are the criteria for the acceptance or rejection of growth zones sufficiently objective and robust?
Number and location of reading axes	Are these clearly defined, e.g. is a diagram provided?
Potential age reading problems	How are these addressed in the age estimation protocol?
Double rings	Are the criteria for the acceptance or rejection of growth zones sufficiently objective and robust?
Juvenile growth	If stock separation is required is the protocol sufficiently objective and robust?
Incomplete growth rings in older fish	Does the age estimation protocol deal effectively with discontinuous growth zone patterns, e.g. older whiting?
Edge patterns	How is otolith edge growth identified?
Providing additional information when reading age reading materials	How is unintentional bias avoided if this information used, e.g. is there an initial ‘blind’ reading?
Conference reading	If conference reading is used, are all independent individual age estimations completed before the conference reading takes place?
Recording Age Readings	
Age (and fish) data	Is this information stored in a database? Is the data integrity protected?
Sample ID	
Reader identity	
Data storage	
Ability to trace data edits	Is the data stored securely? Is there a database manager? Is there a quality manager?
Handling and processing the data	
Backup of databases	

Responsibility for the data at different stages	
Training new readers:	Is age reader training an integral part of the age estimation protocol?
Teaching new readers	
Conference reading with skilled reader who has good presentation skills.	How are new readers supported?
Discussion of sample problems with mentor	
Benefits of conference reading	
All age reading materials must be re-read until sample readings reach target agreement level.	How are changes in reader precision identified?
Requirement for basic information on age reading material or other structure	How is this provided?
Requirement for information on the biology of the species	How is this provided, e.g. do age readers participate in biological sampling? Are accurate area and date of capture information provided?
Training log	How are changes in reader precision recorded?
Training course units e.g. preparing age reading materials, age reading material reading, data handling	Is reader training fit for purpose? How is training effectiveness monitored?
Inspection and Control of the process	
Effective validation is mandatory for Quality Assurance,	How are age estimations validated?
Apply EFAN Guidelines for Validation Studies	Are these applied?
Apply EFAN Tools and Guidelines for Age Reading Comparisons	
Reader accuracy checks	How are these used to improve quality control?
Reader precision checks	
Statistics on readings returned to readers	Is feedback to readers effective?
Revision of the Procedures to improve the process	

<table>
<tr><td>Revision of procedures/age reading is initiated by errors found in the control process.</td><td rowspan="2">Are written procedures/protocols controlled quality documentation?
Has the age reading laboratory attained a recognised quality management standard, e.g. ISO 9001?</td></tr>
<tr><td>Revision of Procedures can be initiated by scientific and technical developments.</td></tr>
<tr><td>Revision of procedures should always rely on;</td><td rowspan="4">How are readers informed of new developments?
Are written procedures/protocols controlled quality documentation?</td></tr>
<tr><td>Scientific development within the field</td></tr>
<tr><td>Technological development within the field</td></tr>
<tr><td>Revision should always be performed as specified in the manual</td></tr>
</table>

SECTION 6: ACCURACY AND PRECISION ISSUES IN AGE DETERMINATION AND AGE READING

CONTRIBUTIONS TO CHAPTER 4 OF THE INDEX IN SECTION 2

The final result of this work was a reformulated version of the chapter 4 of the quality manual index dealing with these questions. This version is presented in the next page.

It was also considered advantageous that this contribution to the index was accompanied by a small explanatory text, presenting the basic rationale behind each topic. This text is presented in the following pages.

Finally, a flowchart summarizing the relationships between the different steps of the chapter addressing the accuracy and precision issues is presented.

CHAPTER 4	QUALITY CONTROL AND QUALITY ASSURANCE
4.1	PRELIMINARY REQUIREMENTS
4.1.1	SENSITIVITY ANALYSIS PROCEDURE
4.1.2	DATA MANAGEMENT
4.1.2.1	DATABASE CREATION
4.1.2.2	TRACEABILITY OF DATA
4.1.3	PRODUCTION OF KNOWN AGE MATERIAL (REFERENCE COLLECTION; MARKING PROGRAMMES; CONTROLLED REARING)
4.1.4	PRODUCTION OF AGREED COLLECTIONS
4.2	INDIVIDUAL PROCEDURES ASSESSMENT
4.2.1	PREPARATION OF AGE READING MATERIAL
4.2.2	OBSERVATION OF AGE READING MATERIAL
4.2.3	INTERPRETATION OF AGE READING MATERIAL
4.2.3.1	ACCURACY ANALYSIS (INCLUDING INPUT AND OUTPUT OF SENSITIVITY ANALYSIS)
4.2.3.2	PRECISION ANALYSIS (INCLUDING INPUT AND OUTPUT OF SENSITIVITY ANALYSIS)
4.2.4	CENTRALIZED FACILITY FOR STORING AND DISTRIBUTING INFORMATION
4.3	MERIT EVALUATION BY CROSSCHECKING OF RESULTS
4.3.1	READER PERFORMANCE ASSESSMENT
4.3.2	PROCEDURES PERFORMANCE ASSESSMENT (EFFICIENCY AND COST)
4.4	DISSEMINATION OF INFORMATION
4.4.1	TECHNICAL REPORTS
4.4.2	RESULTS OF CROSSCHECKING

CHAPTER 4 – QUALITY CONTROL AND QUALITY ASSURANCE

The societal valuation of scientific activities should be based on their success in relation to their relevant objectives. Natural science has the implicit aim of increasing our understanding about our universe; whereas fisheries science objectives are often specifically formulated in relation to the exploitation of the living resources, but claims by multiple interests in the maritime sector makes the formulation of objectives ultimately a political issue.

The ICES advice on ecosystems and fisheries management is in a process of evolving towards a more integrated advice serving different clients with a full coverage of the state of the living resources and their environment.

Current EU fisheries regulations are heavily dependent on single species assessments and age structured population analysis appears to provide the most robust and precise stock forecasts, but even when adopting ecosystem based objectives in fisheries science the most efficient analyses of species interactions will rely on methods based on age structure.

TACADAR is aiming at increasing the adoption of procedures for age estimation that include quality assurance and quality control mechanisms, for the improvement of stock assessment and environmental management techniques by increasing reliability of age estimation procedures in the European Community.

The basic requirement for most population dynamics analyses is the age structure of the populations and not individual ages per se.

Methods have been developed that only address the analysis of time series of frequency distributions in the population under the assumption that progressing size modes reflect growth of year classes (Fournier et al. 1990). The often strongly overlapping distributions of the age classes however calls for validation of results or even reparametrisation by other methods that involve age analysis.

New approaches point to more cost efficient use of fish samples by linking size information with age estimation of a sub sample and integrating it with the size information in a larger sample from the population (Francis and Campana 2004, Francis et al. 2005).

The sequence starts with the often expensive production of known age material by either mark recapture experiments, rearing under natural conditions or microchemical analyses of annually varying environmental signals in growth structures. Although known age material itself can be applied directly to calibrate the estimation of age structure in the production sample the expenses of a sufficiently large calibration sample makes the method low in cost efficiency. The addition of a step consisting of a larger less expensive sample where age is interpreted from CS with minimum error may be the most cost efficient way to reach population age structure, thus putting even more focus on the precision and accuracy issues of the involved age estimation methods.

The same reasoning could be applied to other age related vital statistics.

Chapter 4 – Quality Control and Quality Assurance

4.1 - Preliminary requirements

4.1.1 - Sensitivity analysis procedure

This chapter should begin by introducing and summarizing the most common types of errors involved in age related uncertainty.
A conceptual definition of sensitivity analysis must be included; a possible one is: a comparative analysis of the influence of different sources and levels of errors on the estimation of population and environmental parameters (for an application of this type of concept see, for example: Reeves, S. A. 2003. A simulation study of the implications of age-reading errors for stock assessment and management advice. – ICES Journal of Marine Science, 60: 314–328.).
Some information about input and output of sensitivity analysis should be provided, for example, that input to sensitivity analysis is based on the population biology of the species and could include, for example, size structure; that output of sensitivity analysis can be represented by confidence limits on population parameters, determining the required accuracy and precision levels.
A reference should be made to the fact that the outcome of sensitivity analysis may be adjusted iteratively following the procedures described in 4.2.3.1 and 4.2.3.2.
Finally, it should be noted that whether the focus is applied to individual age assessment or population age structure will depend upon the management objective.

4.1.2 - Data management

4.1.2.1 - Database creation

4.1.2.2 - Traceability of data

The first two points of this sub-chapter provide guidance on the common format and on the process of quality control of database creation, to allow full cross analysis of data
The above mentioned guidance includes defining a minimum set of variables that has to be collected. It also implies that, for a subset of data, the minimum set of variables includes interpreted images (see, for example, EFAN REPORT 8-2000 – www.efan.no).

4.1.3 - Production of known age material (Reference collection; Marking programmes; Controlled rearing)

The following point of this sub-chapter deals with known age material. This refers to the entire hard structure, or a defined growth zone produced during a defined period of time (see, for example, EFAN REPORT 4-2000 – www.efan.no).
It also deals with reference collections, which are a physical sample of known age calcified structures with a defined sampling strategy.
It is important that some information is provided concerning the relevance of this kind of material in the context of estimating accuracy.

4.1.4 – Production of agreed collections

The last point of this sub-chapter deals with agreed collections, which are a physical sample of calcified structures of interpreted age, but not known age.
Once again, some information should be given about the importance of this kind of collections in the context of estimating precision, as well as a clear distinction between this type of material and the type of material referred in 4.1.3.

4.2 – Individual procedures assessment

4.2.1 – Preparation of age reading material

4.2.2 – Observation of age reading material

The topics addressed in the first two points of this sub-chapter are covered in Annex of the present manual. For this reason, no further development will be included here about these issues.

4.2.3 - Interpretation of age reading material

4.2.3.1 - Accuracy analysis (including input and output of sensitivity analysis)

The following point in this sub-chapter deals with the interpretation of the age reading material. In this context, interpretation means both the final age estimation and the criteria of estimation (including the actual location of time related features in a given calcified structure).
The first step of this interpretation process is performing an accuracy analysis, which will be done by blind reading of known age material
The tools needed for an accuracy analysis are:

- Age and size structured synthetic population growth model
- Bootstrapping an assessment simulation

Inputs to the sensitivity analysis will be error and co-variation distributions by length group.
Output will be estimates of vital statistics and size distributions of the population.
Goodness of fit relating to bias and thereby perceived growth parameters for the stock may be judged by the correspondence between size frequency modes and age distributions.
Decision on the accuracy level relating to variation may be determined by the width of confidence intervals around F (fishing mortality) and SSB (spawning stock biomass).

4.2.3.2 - Precision analysis (including input and output of sensitivity analysis)

The next step in the interpretation process is performing a precision analysis will be performed on a subsample (e.g. an agreed collection or a control sample).
The tools needed for an precision analysis are:

- Age and size structured synthetic population growth model
- Bootstrapping an assessment simulation

Inputs to the sensitivity analysis will be error and co-variation distributions by lenthg group.
Output will be estimates of vital statistics and size distributions of the population.
Decision on the precision level may be determined by the width of confidence intervals around F (fishing mortality) and SSB (spawning stock biomass); for an example see Reeves, 2003.
Note that precision should be evaluated using information of accuracy to see if an extra effort in accuracy is needed.

4.2.4 – Centralized facility for storing and distributing information

The last point of this chapter is concerned with the creation and maintenance of a web site dedicated to the exchange of information regarding quality control and assurance for the practice of age estimation in fisheries science.
The main feature should be a metadatabase containing information about availability, location and access conditions of the databases (referred in 4.1.2) as well as links to other relevant information (e.g. outputs of accuracy and precision analysis).

4.3 - Merit evaluation by crosschecking of results

4.3.1 – Reader Performance assessment

The evaluation proposed in the first point of this sub-chapter will be done using the results gathered in the accuracy and precision analysis and summarizing them in order to characterize significant sources of error at the individual reader level, the institutional level, the species level, etc.

4.3.2 - Procedures performance assessment (efficiency and cost)

The assessment referred in the last point of this sub-chapter is a comparison of procedure specific costs associated with the reduction in error variance.
This can either be done for the estimation of individual ages from calcified structures or the age structure of the population (e.g. size frequency analysis).
Overall, we have to be aware that additional sampling costs and corresponding errors may affect the overall cost benefit analysis for the specific objectives.

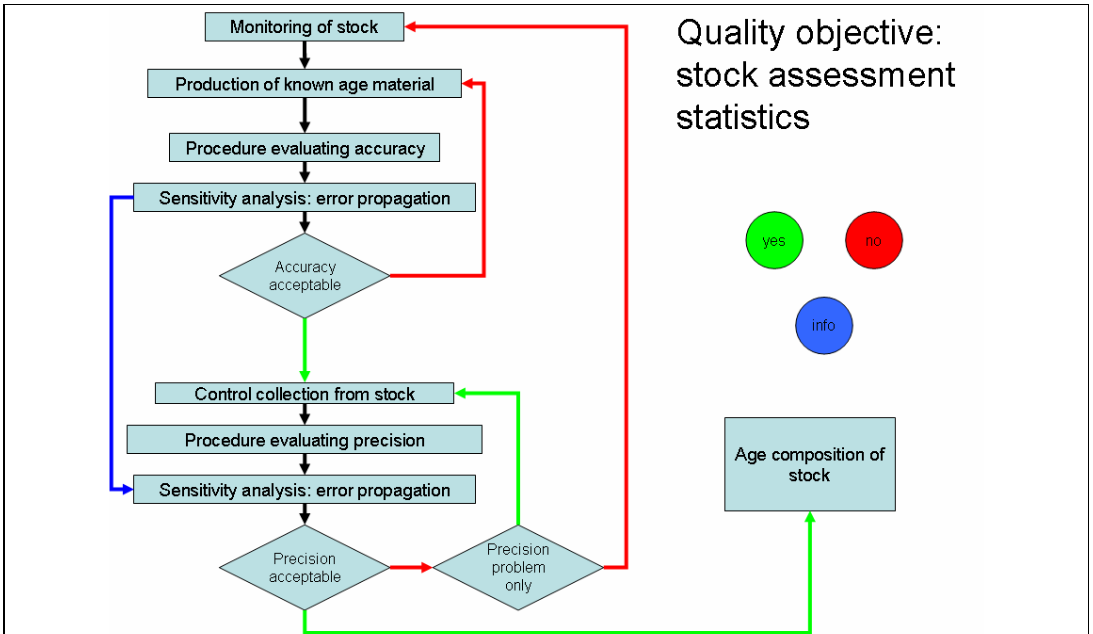
4.4 – Dissemination of information

4.4.1 – Technical reports

Ideally, the dissemination of information referred in this sub-chapter should be done through the central facility referred in 4.2.4.
In can include the publication of technical reports on subjects like procedure specification to ensure reproducibility; synthesis reports of the stock monitoring programs; software manuals; training manuals; etc..

4.4.2 – Results of crosschecking

The last point of this sub-chapter emphasizes the importance of disseminating the results obtained in 4.3.1 and 4.3.2.



Relationships between the different steps of the process of accuracy and precision assessment in age reading and age determination.

Section 7. Impact of accreditation in academic environment on publication prospects

As part of the second Term of Reference (see introduction page 6) (gather information about expectations both within and about the project), the editors of various scientific journals for fisheries, fish biology, and fish ecology were contacted and asked for their views on accreditation in age estimation work. They were specifically asked whether accreditation would influence the 'publishability' of manuscripts sent to their journals. This exercise was one aspect of evaluating the wider impact of developing an accreditation process.

The text of the letter sent to the editors was:
I am working on an EU Concerted Action project called TACADAR, which addresses quality control and protocols for fish age estimation. I am interested in assessing the impact of quality assurance and certification of age readers (those who estimate fish age from scales or otoliths), and for this reason I welcome your opinion on the acceptability of an internationally recognised accreditation scheme for age estimation. In particular, I am interested in the 'credibility' of non-certified researchers when trying to publish their work:

- Would your acceptance of manuscripts be influenced by whether age estimates were done by accredited readers?
- Would your evaluation of reviewers comments be influenced by whether the work was critised for being done without accredited readers?

I hope to present a summary of responses in the next TACADAR report. The project website is http://www.efan.no/tacadar and you can see there more details of the activities.

SUMMARY OF RESPONSES

Thirty-three editors were contacted, spread across 12 countries, representing 18 journals, and these are listed in Table 2. Twelve editors had responded as of May 25, 2003, and several more by August 2003. The responses were summarised and reported at the Enniskillen meeting in 2005.

In general, the issue of accreditation was considered irrelevant, but the issue of following a recognised protocol or defining a repeatable protocol was considered very important. Most editors felt that the review process was sufficient and most appropriate for evaluating work that has been submitted for publication. Several editors felt that the accreditation status of the author would influence the acceptance of a manuscript

Most editors felt that they would not be influenced by reviewer's comments that criticised only the lack of accredited readers, but again would be influenced by criticisms of the methods actually used.

Table 2. List of editors contacted

Journal	Editor	Country	Press
Journal of Fish Biology	RN Gibson	UK	Blackwell
	J Craig	UK	Blackwell
	S Rogers	UK	Blackwell
	J. Johnsson	Sweden	Blackwell
	M. Kaiser	UK	Blackwell
	J Thorpe	UK	Blackwell
ICES Journal of Marine Science	N Daan	Netherlands	Elsevier
	P Pepin	Canada	Elsevier
Fisheries Research	A McIntyre	UK	Elsevier
	Gavin Begg	Australia	Elsevier
	Mike Armstrong	UK	Elsevier
Aquatic Living Resources	B. Milcendeau	France	Elsevier
	A. Eleftheriou	Greece	Elsevier
Journal of Experimental Marine Biology and Ecology	T. Underwood	Australia	Elsevier
Aquaculture	B. Costa-Pierce	USA	Elsevier
Acta Oecologia	R. Arditi	France	Elsevier
Fishery Bulletin	N. Bartoo	USA	NMFS
Transactions of the American Fisheries Society	F. Utter	USA	Allen Press
	D. DeVries	USA	Allen Press
Fisheries Oceanography	S Coombs	UK	Blackwell
	B MacKenzie	Denmark	Blackwell
	D. Checkley	USA	Blackwell
Journal of the Marine Biological Association of the UK	A Pulsford	UK	Cambridge University Press
Canadian Journal of Fisheries and Aquatic Sciences	J. Hutchings	Canada	NRC Research Press
Marine Ecology Progress Series	K. Sherman	USA	Inter-Research
	H. Browman	Norway	Inter-Research
	O. Kinne	Germany	Inter-Research
Journal of Sea Research	J Beukema	Netherlands	Elsevier
	H Veer	Netherlands	Elsevier
Marine & Freshwater Research	M Kingsford	Australia	CSIRO
Sarsia	T Høisæter	Norway	UiB/IMR
Journal of Applied Ichthyology	H Rosenthal	Germany	Blackwell
Environmental Biology of Fishes	D Noakes	Canada	Kluwer

Section 8. Overview of procedures

Technical Protocols:

Title	Responsible	Institute/Country	Version	Last updated
Sectioning otoliths using the accutom 5 cutting machine	M W Easey	CEFAS, UK	1.1	16.11.2001
Sectioning otoliths using the “V4” superior cutting machine	M W Easey	CEFAS, UK	1.1	19.12.2001
Mounting cut sections of otoliths onto glass slides	M W Easey	CEFAS, UK	1.1	19.12.2001
Mounting otoliths in black polyester resin for sectioning	M W Easey	CEFAS, UK	1.1	15.11.2001
Mounting pelagic otoliths in resin for age determination	M W Easey	CEFAS, UK	1.1	19.12.2001
Staining cut sections of otoliths	M W Easey	CEFAS, UK	1.1	19.12.2001
Otolin System for the Embedding Sectioning and Slide Mounting of Demersal Fish Sagittae (otoliths)	W.Mc. Curdy	AFBI, UK	1.4	04.10.2006
Sampling the N. Ireland landings of demersal fish	W.Mc. Curdy	AFBI, UK	1.4	03.10.2006
Sampling the N. Ireland landings of herring	W.Mc. Curdy	AFBI, UK	2.1	04.10.2006
Larval otolith microstructure	P. Re	Guia Mar. Lab., Portugal		Web-based http://astrosurf.com/re/otolith1_bio.html
Otolith preparation: *Coryphaena hippurus*	J. Moanta & B. Morales-Nin	IMEDEA, Spain		
Age determination: Standard methodologies	P. Belcari, A. Ligas & C. Viva	Univ. of Pisa, Italy		
Larval otolith microstructure	J. Panfili	IFREMER, France		
Age determination methods for juvenile plaice *Pleuronectes platessa*	A.J. Geffen	Univ. of Liverpool, UK / Univ. of Bergen, Norway		
Procedures for age determination of anchovy and sardine through the reading of the annual rings at ISMAR	G. Giannetti, F. Donato & E. Arneri	ISMAR, Italy		
Anglerfish ageing guide	Duearte et. al.	Spain, France, Portugal, Ireland		
Procedure for dissecting and preparing of age material on pelagic fish	H. Gjøsæter	IMR, Norway	1.0	Sep. 1999
Manual of analysis at the fishery resources laboratory; Preparation and reading of fish otoliths		AZTI, Spain	2	Jan 2003 Translated
Otolith age estimation of European hake from Iberian Atlantic waters in IEO (ICES Divisions VIIIc and IXa)	C. Piñeiro	Instituto Español de Oceanografía, Spain		Sep. 2003 Translated
Determination of age on the basis of otoliths at the Institut für Seefischerei	G. Gentschow, F. Beußel, S. Cummerow, C. Zimmermann	Germany		Translated

Manual for the preparation and reading of European hake (*Merluccius merluccius*) otoliths	C. Morgado, A. Marçal, M. J. Ferreira, M. de Lourdes Godinho and M. Hortense Afonso	Portugal		Translated
Ageing procedures		Finnish Game and Fisheries Research Institute, Finland		Translated
Age and Growth determination of fishes in Finland	Raitaniemi, J., K. Nyberg & I. Torvi	Finnish Game and Fisheries Research Institute, Finland Internal Document		2000
Otolith section procedure at the Marine Research Institute of Icela (MRI)	Peturdottir, G.	MRI, Iceland Internal Document		2004

Ageing Procedures

Title	Responsible	Institute/Country	Version	Last updated
Age reading procedures for Western Baltic spring spawning herring (*Clupea harengus*)	T. Grøhsler	Institute for Baltic Sea Fisheries (IOR), Germany	1.0	29.10.2003
Age reading procedures for Western Baltic sprat (*Sprattus sprattus*)	T. Grøhsler	Institute for Baltic Sea Fisheries (IOR), Germany	1.0	14.10.2003
Procedure for age estimation of herring (*Clupea harengus* L.)	H. Gjøsæter	IMR, Norway	1.0	Des. 1999
Procedure for age estimation of polar cod (*Boreogadus saida* Lepechin.)	H. Gjøsæter	IMR, Norway	1.0	Des. 1999
Procedure for age estimation of capelin (*Mallotus villosus* Muller.)	H. Gjøsæter	IMR, Norway	1.0	Sep. 1999
Procedure for age's estimation of cod, seith and haddock (In Norwegian)	Njanger, H, K. Nedreaas, H. Senneset and P. Ågotnes	IMR, Norway	1.0	Dec. 2000
Sagittae Preparation and Age estimation of Irish Sea sprat	W.Mc. Curdy	AFBI, UK	1.4	04.10.2006
Age estimation of Irish Sea demersal fish	W.Mc. Curdy	AFBI, UK	1.4	03.10.2006
Analyse de la méthode d'estimati de l'âge individuel de la morue er mer celtique (Divisions CIEM VI k)	Bellail, R.	IFREMER, France		2005 Internal Document
Protocole technique d'estimation de l'âge individuel de la morue en mer celtique (Divisions CIEM VIIe-k)	Bellail, R.	IFREMER, France		2005 Internal Document
Habilitation des lecteurs de pièces calcifiées pour l'estimation de l'âge individuel de morues de mer celtique (Divisions CIEM VIIe-k)	Bellail, R.	IFREMER, France		2005 Internal Document
Danish manual for age reading	Clausen, L.W.	Danish Institute for		2002

– COD section		Fisheries Research, Denmark		Internal Document
Age determination of Sardine (Sardina pilchardus) through the Reading of the annual increments	Donato, F., G. Giannetti and E. Arneri	ISMAR-CNR, Italy		2003 Internal Document
Ageing of perch (Perca fluviatilis) in Finland	Raitaniemi, J.	Finnish Game and Fisheries Research Institute, Finland		2005 Internal Document
Manual on age determination performed at the Institute of the Swedish Board of Fisheries	Reizenstein M.	Sweden		2005 Internal Document
Swedish manual for age reading – COD section	Walter, Y.	Institute of Marine Research, Sweden		2000 Internal Document

Quality Control Procedures

Title	Responsible	Institute/Country	Version	Last updated
Quality control of age determination	M W Easey	CEFAS, UK	1.1	01.04.2003
Procedure for quality assurance of age determination of fish	H. Gjøsæter & K. Nedreaas	IMR, Norway	1.0	June 1999

Training Procedures

Title	Responsible	Institute/Country	Version	Last updated
Training in age determination	M W Easey	CEFAS, UK	1.1	01.05.2003

Guidelines

Title	Code	Institute/Country	Version	Last updated
Quality management systems Fundamentals and vocabulary	ISO 9000/2000			
Laboratory Quality Manual				
Guidelines for QC in the Analytic Laboratory	NATA	Australia		Oct. 1995

Published materials

Title	Authors	Year	Journal
Quality issues in the use of otoliths for fish age estimation.	Morison, A.K., J. Burnett, W. J. McCurdy, and E. Moksness.	2005	Marine and Freshwater Research, 56: 773-782.
A cooperative effort to exchange age reading experience and protocols between European fish institutes.	Appelberg, M., N. Formigo, A. J. Geffen, C. Hammer, W. McCurdy, J. Modin, E. Moksness, H. Mosegaard, B. Morales-Nin, H. Troadec and P. Wright.	2005	Fisheries Research, 76: 167-173.

Section 9. Recommendations

(1) The **EFAN-Homepage** (http//www.efan.no/ or http//www.imr.no/efan) should further be maintained for the existing network as an information source and exchange platform.

(2) Currently different programmes for the use in **otolith image analysis** are developed in different labs. None of them are fully operational for the daily use and the routine lab works. Many of them are designed primarily for research with adaptations for the routine reading. An EU-project to develop (or agree upon) a programme for routine age reading and output formats is ongoing. The recommendations of the project should be taken into consideration in the future and the results spread in the Age-reading network.

(3) Based on the analyses developed in EFAN & TACADAR it is recommended to **define age reading standards** as prerequisites for the submission of data in the context of the DCR, similar to the minimum sampling requirements defined for the DCR. The required standards should rise gradually and be used as a starting point towards accreditation and certification.

(4) **Accreditation and Certification** of the age readings in the national labs was a leading theme through EFAN and TACADAR. However, it turned out that full implementation is premature. Accreditation and certification in the sense of adhering to ISO/EN norms would require substantial financial input either by means of national governments or of the Commission via the DCR. Moreover, it is questioned, if the concept as such is appropriate, since biological material is by nature variable and interpretation of structure subjective. This will remain, no matter whether an institute, a lab or reader are accredited or certified. Before moving too quickly into this direction, a full cost benefit analysis will need to be carried out in view of the costs that this process imposes upon institutions. Alternatively, more input should be directed towards quality assurance within the national labs to define and meet pan-European standards.

(5) EFAN developed an age reading workshop-manual and analysis procedure (**Guidelines** and Tools for Age Reading Comparisons, EFAN-report 3-2000) which had been applied in a number of cases. They were also used by PGCCDBS (ICES 2006 CM/ACFM: 18) recently with some suggestions for refinements. The recognition of this in the scientific world is a considerable achievement of EFAN. TACADAR has now produced a guideline for quality assessment and quality control for fish aging. It is recommended that PGCCDBS continues refining these guidelines.

(6) **Calibration and validation** are the basis of harmonization and standardization and are critical elements for quality assurance and quality control. It is recommended to support initiatives (projects) for validating age structures, which includes the underlying research on the biological basis of

formation of age structures. It is also recommended to support training of staff by networks and by exchange workshops.

An integral part of calibration and validation is, for instance, done by following a cohort through the years, and is therefore an exercise demanding long-term input. EFAN has developed guidelines for this (Guidelines for validation studies, Eltink et al. 2000 in EFAN-report 4-2000) and it is recommended to support validation initiatives, taking also into account that such initiatives may occur on short notice due to usually unforeseen occurrences of extraordinary strong year classes.

(7) With regard to deciding on lab methods and equipment for age determination, it is recommended to refer to EFAN/TACADAR-procedures as elaborated in several reports (e.g EFAN Reports 3-2000, EFAN Reports 4-2000 and EFAN Reports 5-2000). National labs should establish a quality assurance section on their homepages. Here method descriptions for each species dealt with should be made public. These homepages should be linked to the EFAN-homepage.

(8) Quality assurance and control are not static but a process that requires continuous development. It is recommended that there be support for relevant research and active links between researchers and age readers to communicate and exchange development needs and research results.

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List of participants at the four TACADAR meetings

Name	Country	Tacadar No	Budapest	Ancona	Enniskillen	Palma
Erlend Moksness	Norway	0	x	x	x	x
Tor Birkeland	Norway	0		x		
Cornelius Hammer	Germany	1	x	x		x
Nuno Formigo	Portugal	2	x		x	x
Ana Moreno	Portugal	3	x	x	x	x
Enrico Arneri	Italy	4	x	x	x	x
Gianfranco Giannetti	Italy	4		x	x	
Jari Raitaniemi	Finland	5	x	x	x	x
Jose Pedro Andrade	Portugal	6	x	x	x	x
Paulino Lucio	Spain	7	x	x		
Inaki Quincofces	Spain	7	x			
Jon Ruiz	Spain	7			x	x
Fredrik Arrhenius	Sweden	8	x	x	x	
Barbara Bland	Sweden	8	x			
Birgitta Krischansson	Sweden	8	x		x	x
Rajlie Sjøberg	Sweden	8	x			
Carina Jernberg	Sweden	8	x	x		
Yvonne Walter	Sweden	8		x	x	x
Annsofie Ågren	Sweden	8	x	x		
Magnus Appelberg	Sweden	9	x	x	x	x
Jenny Palmkvist	Sweden	9	x			
Carin Ångstrøm	Sweden	9	x	x	x	x
Anne Odelstrøm	Sweden	9		x	x	x
Helene de Pontual	France	10	x	x	x	x
Ronan Fablet	France	10	x	x	x	x
Robert Bellail	France	10			x	
Jean-Paul Berthome	France	10			x	
Francis Neat	UK	11	x	x	x	
Peter Wright	UK	11		x		
Jane Mills	UK	11		x		
William James Mc Curdy	UK	12	x	x	x	x
Henrik Mosegaard	Denmark	13	x	x	x	x
Lotte A. Worsøe Clausen	Denmark	13	x	x	x	x
Beatriz Morales-Nin	Spain	14	x	x	x	x
Javier Tomás	Spain	14	x	x		
Michael Easey	UK	15	x	x	x	x
Richard Millner	UK	15		x		x
Carmen Piñeiro Alvarez	Spain	16	x	x		x
Teresa Garcia Santamaria	Spain	16			x	x
Groa Petursdottir	Iceland	17	x	x	x	x
Paola Belcari	Italy	18	x	x		x
Claudio Viva	Italy	18		x		
Jacques Panfili	France	19	x	x		x
Anastasopoulou Aikaterini	Greece	20	x	x	x	
Vassilopouplou Vassiliki	Greece	20			x	
Arild Folkvord	Norway	21		x		
Audrey Geffen	Norway	21	x	x	x	x
John Gordon	UK	22	x	x	x	x
Pedro Re	Portugal	24	x			x
Jose Eduardo Rebelo	Portugal	25	x	x		x
Peter Lengyel	Hungary	26		x	x	
Olga Bukovskaya	Hungary	27	x	x		x
Kari Nyberg	Finland	28	x	x	x	x
Helen McCormick	Ireland		x	x	x	x
Eva Bergstrand	Sweden			x	x	x
Magnus Kokkin	Sweden			x		
Alex Tidd	UK			x		
Maja Reizenstein	Sweden				x	
Peer Doering-Arjes	Germany					x
Toni Lombarte	Spain					x

ROLE OF PARTICIPANTS

Participants in TACADAR are European Universities and Research Institutes. A total of 28 Institute were registered as partners in TACADAR. Each of the partners participated with one or more participants in the activity of TACADAR and contributed to the reports. More participants joined TACADAR during the course of the Concerted Action, but were not formally included as partners in the project. Area of interest of original participants of TACADAR is listed below.

Name	Methodologies and procedures	Information processing	Information, exchange and training	Validation	Research and application
Erlend Moksness	X		X		X
Cornelius Hammer	X		X	X	X
Nuno Formigo			X	X	X
Ana Moreno		X			
Enrico Arneri		X			X
Jari Raitaniemi	X				X
Jose Pedro Andrade				X	
Paulino Lucio			X		
Fredrik Arrhenius	X			X	X
Magnus Appelberg	X				
Helene de Pondual					X
Peter Wright					X
Willie McCurdy	X				
Henrik Mosegaard		X	X	X	X
Beatriz Morales-Nin		X		X	
Richard Millner	X				
Carmen G. Piñeiro	X				X
Groa Petursdottir	X				
Paola Belcari	X				
Jacques Panfili					X
Vassiliki Vassilopoulou				X	
Arild Folkvord				X	X
John Gordon					X
Audrey Geffen					X
Pedro Ré				X	
Jose E. Rebelo					X
Peter Lengyel	X				
Olga Bukovskaya	X				
Kari Nyberg	X				

Section 11: Appendix

APPENDIX 1: GUIDELINES FROM THE ICES PLANNING GROUP PGCCDBS

Reference: ICES CM 2006/ACFM:18, section 3.1, page 6.

Experimental Design in Age Reading Workshops

Several of the 2005 age workshop reports made comparisons between different methods and comparisons in reading ability between the start and end of the workshop. PGCCDBS advise that these comparisons need to be planned from the start of the exchange and carried out using the principles of designed experiments (see for example, Heath (1995)). We draw attention to the large amount of work on age reading available from the concerted actions EFAN (Anon. 2001b) and TACADAR (http://www.efan.no/tacadar/). PGCCDBS aims to contribute to this work by promoting the current protocols and providing further developments on how to incorporate experimental design into the age reading workshops.

The most important ideas for experiment design are to compare like with like and to control for other variables that affect age reading ability. For example, do not provide otoliths for the exchange from one area then read otoliths from a different area at the end of the workshop. This comparison could show increased agreement in ageing due to increased ability gained at the workshop or due to the 2nd area being easier to read and it will be impossible to separate the two effects. Similarly, avoid running the before and after comparisons on exactly the same set of otoliths. This is necessary if there are small numbers of otoliths but otherwise is undesirable as improvements seen in agreement may be from remembering specific cases and not apply in general.

Exchange organisers should ensure they have read EFAN Report 3-2000 (Eltink et al., 2000) particularly Section 3.9 "Comparison of sets of different preparation techniques" or of different calcified structures, Section 3.13 "Age reading comparisons" and Section 4.7.2.12 "Age reading of the last set for estimating improvement in age reading".

Building on the guidance in the EFAN report we suggest the procedure for generating two sets of otoliths for comparison should be:

1. Exclude otoliths you know are poorly prepared or have other obvious reasons why they are different from the rest of the otoliths in the exchange.
2. Identify variables that you suspect influence ability to age.
3. For variables that are not of interest control their effect by standardising them, for example, keep laboratory procedures consistent.
4. For variables that are of interest or cannot be fixed, define strata based on these variables, for example: month and fish length group. (We suggest strata based on fish length group to help balance the age distributions in the first and second set.)
5. Then for each group defined by the strata, randomly assign otoliths to either the first or second set. The two sets do not have to be the same size. When the first set is for the exchange and the second set for the end of the workshop it is

sensible to make the second set smaller. If the age workshop coordinator can specify changes in reading bias or CV that are biologically meaningful to detect then sample size calculations can be carried out to help decide how big the data sets should be.

APPENDIX 2: PROJECT MANAGEMENT AND COORDINATION

Project management

Overall project co-ordination was undertaken by Research director Erlend Moksness, of the Institute of Marine Research (IMR), Norway.

TACADAR was directed by a Steering Committee chaired by E. Moksness (Institute of Marine Research, Flødevigen Marine Research Station, Norway) and consisting of experts in different fields. The CA project coordinator was formally responsible for the fulfilment of the objectives. The Institute of Marine Research, Flødevigen Marine Research Station, Norway, played the role as coordinator and keeper of central functions such as the TACADAR Homepage, databases and libraries. The participating laboratories were updated on new developments through the «TACADAR Newsletter» which was circulated by e-mail.

TACADAR Steering Committee

Chairman: Erlend Moksness, (Institute of Marine Research, Norway)
Cornelius Hammer (Federal Research Centre for Fisheries, Germany)
Nuno Formigo (Universidade do Porto, Portugal)
Henrik Mosegaard (Danish Institute for Fisheries Research, Denmark)
Beatriz Morales-Nin (CSIC-UIB, Spain)
Audrey Geffen (University of Bergen, Norway)
William James Mc Curdy (Department of Agriculture and Rural Development Aquatic Systems Group, Northern Ireland, UK)
Magnus Appelberg (Institute of Coastal Research National Board of Fisheries, Sweden)

APPENDIX 3: UPDATED LIST OF MILESTONES (31.11.06)

Milestones	Delivery month	Status
4x TACADAR Newsletter	Year 1	Completed
Plenary meeting in Hungary	6	Completed
Annual progress report	12	Completed
4 x TACADAR Newsletter	Year 2	Completed
Plenary meeting in Italy	18	Completed
Annual progress report	24	Completed
4 x TACADAR Newsletter	Year 3	Completed
Plenary meeting in Ireland	30	Completed
Annual progress report	36	Completed
4 x TACADAR Newsletter	Year 4	Completed
Plenary meeting in Spain	42	Completed
Annual progress report	48	Completed
Final report	50	Completed

APPENDIX 4: DESCRIPTION OF THE WORKPACKAGE

WP2.	**Work package description**

Work package number	**0. Management**
Start date or starting event	Month 1
N° of the partner responsible	1
N°s of other partners involved	All participants
Person-months per partner	3.5

Objectives
The objective is to manage the project to facilitate collaboration on fish ageing research and methods and thereby to improve the management of fish stocks and to bring European research to the cutting edge in this field.

Description of work
The Institute of Marine Research, Norway, will serve as the coordinating body for the Concerted Action by maintaining the TACADAR Internet homepage, publishing TACADAR Newsletters and organising annual plenary meetings and workshops.
TACADAR will be organised in one workpackage

Deliverables
- Publish 4 TACADAR Newsletters per year
- Establish TACADAR Internet homepage, incorporating the EFAN pages
- Organize four annual plenary meeting
- Maintain and update TACADAR databases
- Prepare and submit annual progress report

Milestones and expected results

- Year 1: 4 x TACADAR Newsletter
- Month 6: Plenary meeting in Sweden
- Month 12: Annual progress report
- Year 2: 4 x TACADAR Newsletter
- Month 18: Plenary meeting in Italy
- Month 24: Annual progress report
- Year 3: 4 x TACADAR Newsletter
- Month 30: Plenary meeting in Ireland
- Month 36: Annual progress report
- Year 4: 4 x TACADAR Newsletter
- Month 42: Plenary meeting in Spain
- Month 48: Annual progress report
- Month 50: Final report

WP2.	**Work package description**

Work package number	1 **Standards of quality assurance and control**
Start date or starting event	Month 1
N° of the partner responsible	1
N°s of other partners involved	All participants
Person-months per partner	3.06

Objectives
To stimulate the achievement of a higher level of quality within and integration between the member institutions of TACADAR, concerning fish age determination.

Specific objectives:
Increase the adoption of working procedures that include quality assurance and quality control mechanisms, for the improvement of stock assessment and environmental management techniques

Description of work

- Define the framework for the application of quality assurance and quality control mechanisms, to protocols for the determination of fish ages from hard tissues.
- Discuss the levels of precision and accuracy to be used as qualifiers in statistical terms.
- Discuss the evaluation of individual methods of age determination in cases where several methods are used to provide data input for the same stock assessment or environmental management model.
- Develop guidelines for the creation and application of quality assurance and quality control mechanisms to protocols for the determination of the ages of fish from hard tissues.
- Investigate legal aspects and implications of accreditation and certification, including advice from externally invited specialists.

Deliverables
A reviewed manual for the quality assurance of age determination protocols for fish species from hard tissues

Milestones and expected results

- Month 28 Completion of the initial draft of the quality assurance manual
- Month 38: Completion of the final draft of the quality assurance manual
- Month 46: Review of quality assurance manual
- Month 49 Submission of quality assurance manual including legal aspects to the EU

APPENDIX 5: EXPLOITATION AND DISSEMINATION ACTIVITIES

Stock assessment and subsequent TAC and Quota determinations rely on accurate age determination of fish populations. The formulation of standard procedures for age reading and interpretation of fundamental step towards improved stock assessment and management of living resources to achieve a sustainable exploitation of the stocks and an improved basis for natural conservation measures. This will, certainly, favour the maintenance of a stable employment framework, with socio - economic implications.

The present programme has facilitated an increase in the number and skills of trained technicians. The establishment of standardised procedures has encouraged the less developed regions to incorporate technological know how available in the more developed ones. The project has developed guidelines for the creation and application of quality assurance and quality control mechanisms to protocols for the determination of the ages of fish from hard tissues, and the establishment of standardised procedures for age determination to be used in all fisheries laboratories and age estimation activities.

APPENDIX 6: ETHICAL ASPECTS AND SAFETY PROVISIONS

The project is not expected to have any adverse environmental implications. The institutions involved are all well known government institutions which adhere strictly to national and EU laws and safety procedures.

The CA involves:

Human embryos or foetus	Yes☐	No X
Use of human embryonic or foetal tissue	Yes☐	No X
Use of other human tissue	Yes☐	No X
Research on persons	Yes☐	No X
Use of non-human primates	Yes☐	No X
Use of trans-genic animals	Yes☐	No X
Use of other animals	Yes☐	No X
Genetic modification of animals	Yes☐	No X
Genetic modification of plants	Yes☐	No X

Since the project did not deal with genetic modifications, deliberate release or use of infected material no special safety provisions were required.

APPENDIX 7: CONTENT OF THE ENCLOSED CD

- Papers
- Pictures Budapest
- Pictures Ancona
- Pictures Enniskillen
- Pictures Palma
- Posters
- Presentations
- TACADAR Reports
- TACADAR News Letters