ASSESSMENT AND MONITORING OF THE FISHERY RESOURCES AND ECOSYSTEMS IN THE STRAITS OF SICILY

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MedSudMed

GCP/RER/010/ITA

Report of the Workshop on Standardization of Fish Age Determination Based on Otolith Samples in the MedSudMed Project Area

Mazara del Vallo, Italy, 13 – 17 December 2004

MedSudMed Technical Documents No.9
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Preface

The Regional Project "Assessment and Monitoring of the Fishery Resources and the Ecosystems in the Straits of Sicily" (MedSudMed) is executed by the Food and Agriculture Organization of the United Nations (FAO) and funded by the Italian Ministry of Agriculture and Forestry Policies (MiPAAF).

MedSudMed promotes scientific cooperation among research institutions of the four participating countries (Italy, Libyan Arab Jamahiriya, Malta and Tunisia), for the continuous and dynamic assessment and monitoring of the state of the fisheries resources and the ecosystems in this area of the Mediterranean.

Research and training are supported to increase and use knowledge on fisheries ecology and ecosystems, and to create a regional network of expertise. Particular attention is given to the technical coordination of the research among the countries, which should contribute to the implementation of the Ecosystem Approach to Fisheries. Consideration is also given to the development of an appropriate tool for the management and processing of data related to fisheries and their ecosystems.

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GCP/RER/010/ITA Publications

MedSudMed Project publications are issued as series of Technical Documents (GCP/RER/010/ITA/MSM-TD-00) related to meetings, missions and research organized by or conducted within the framework of the Project.

Comments on this document would be welcomed and should be sent to the Project headquarters:

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Preparation of this document

This document is the final version of the Report of the MedSudMed Workshop on standardization of fish age determination based on otolith samples in the MedSudMed Project area, held in Mazara del Vallo, Italy, from 13 to 17 December 2004.

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ABSTRACT

The Workshop was attended by experts from Tunisia, Italy, Libya and Malta and an international expert on fish age determination, Ms Hélène de Pontual, from the Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER). The main objective of the workshop was to study the possibility of elaborating, a standard protocol on ageing in the MedSudMed Project area, based initially on two target species: *Merluccius merluccius* and *Mullus barbatus*. The current activities in the Project Area and an overview of the methods now used were presented, including existing national and international protocols and their principal technical characteristics regarding materials, methods, sampling design and data processing. Then the problems of standardizing age-reading methodology for the target species were discussed: choice of the hard structure, sampling, otolith extraction, otolith conservation, preparation techniques, accuracy and precision, validation etc. A laboratory exercise of age-reading techniques for *Mullus barbatus* was conducted to check the age-reading agreement and precision among six readers. The average percentage agreement in respect of the modal age across all ages and readers in the comparison was 55.2% and the average coefficient of variation (CV) was 45.5%. The relative bias showed that all the readers tended to underestimate older age-classes. Finally, the participants agreed on a common protocol for age determination of the target species and proposed a future intercalibration exercise to assess the percentage agreement in age determination among readers.

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Section 1: Report of the Workshop on Standardization of Fish Age Determination Based on Otolith Samples in the MedSudMed Project Area

Mazara del Vallo, Italy, 13–17 December 2004

1. Introduction

The Workshop on Standardization of Fish Age Determination Based on Otolith Samples in the MedSudMed Project Area was opened by the Director of IRMA–CNR, Mazara del Vallo, Dr. Sergio Ragonese**,** who welcomed the participants. The Workshop was attended by experts from the countries (Tunisia, Malta, Italy and Libya) participating in the MedSudMed Project and by an invited international expert on fish age estimation, Dr. Hélène de Pontual**,** from the Institut Français de Recherche pour l'Exploitation de la Mer, France (IFREMER); a list of participants is given in Annex A to the present document.

The Director brought to the participants' attention the significance of the possibility of adopting a standard protocol on ageing, based on *Merluccius merluccius* and *Mullus barbatus*, for the MedSudMed participating countries. An agreement on a common protocol among the several countries that are assessing Mediterranean stocks would be a fundamental step in reducing the present differences in the various empirical procedures currently being used.

The MedSudMed Project was presented and the objectives of the Workshop were outlined; the Terms of Reference and the Provisonal Timetable of the Workshop are, respectively, in Annexes B and C to the present report.

Mention was made of the FAO CopeMed[1] initiative[2] which focuses on the standardization of the preparation and interpretation procedures for age/growth estimation based on fish otolith analysis in *Sardina pilchardus*, *Mullus surmuletus* and *Mullus barbatus*.

2. Background presentation

The IFREMER expert was invited to the Workshop in order to convey to the regional experts her experience in the interpretation of fish ageing through the study of otoliths and in recent methodological developments. Various aspects were presented and discussed:

- **Accuracy and precision in age estimation.** Accuracy is the closeness of the measured or computed value of a quantity to its true value, and precision is the closeness to one another of repeated measurements of the same quantity. For a measurement technique that is free of bias, precision implies accuracy. Accuracy and precision were discussed through the case study of *Merluccius merluccius* in the North Atlantic (also referred as Atlantic hake). Regarding this species,

[1] FAO CopeMed Project, ''Advice, Technical Support and Establishment of Cooperation Networks to Facilitate Coordination to Support Fisheries Management in the Western and Central Mediterranean''
[2] COPEMED Rep. Atelier Régional sur la problématique des études de croissance des poissons de la Méditerranée. *A. Carpentier & F. Alemany* – Casablanca (Maroc), 25-30 avril 2004

precision has been increased thanks to several international age-reading workshops (Piñeiro 2000; Piñeiro et al. 2000). However, the results of a recent tagging-and-recapture experiment carried out in the northern Bay of Biscay suggested that the current reading method results in ages that are overestimated and consequently growth is underestimated (de Pontual et al. 2003, 2004). A video was presented to describe the tagging experiment, showing the following components: use of a specially designed codend to catch fish, so as to increase survival; injection of each fish with oxytetracycline; "T-bar" tagging and release of tagged fish. The recovered fish showed somatic growth about twice higher than expected from the current growth models. Underestimation of growth in this way was clearly related to overestimation of age using the internationally agreed ageing criteria. These results highlight the need to develop a new and reliable ageing method based on validated otolith interpretation criteria.

- **Quality assurance and quality control in age estimation.** TACADAR (Towards accreditation and certification of age determination of aquatic resources) is a concerted European-wide action (CA) coordinated by Prof. E. Moksness, IMR, Norway (Tacadar website http://www.efan.no/tacadar/). The CA aims to increase the adoption of procedures for age reading that include quality assurance and quality-control mechanisms, for the improvement of stock assessment and environmental management techniques. The overall objective is to increase reliability of age-estimation procedures in the European Community. The ultimate objective is to stimulate the achievement of a higher level of quality within and integration of the partner institutes concerning fish age determination.

- The **EFAN** (European Fish Ageing Network) website is still active and proposed valuable resources. Guidelines and tools for age reading comparisons by Guus Eltink et al. (2000) were particularly useful for the present Workshop. The guidelines and the programme for age-reading comparisons are an attempt to formalize and advertise the best features currently available in Europe. The guidelines concentrate on reference collections, on exchange schemes and age-reading workshops, on the analysis of the age readings, on the digital-imaging tools and the definition of terms. They can be downloaded from http://www.efan.no under the menu "guidelines".

- **Image analysis software.** Basic principles and operational procedures of TNPC (Traitement Numérique des Pièces Calcifiées) developed by the Laboratoire de Sclérochronologie des Animaux Aquatiques (LASAA; www.ifremer.fr/lasaa) were presented to the participants. This software is dedicated to age and growth determination based on calcified structure (CS) image analysis and is designed to process a large number of data. This kind of software is considered fundamental for implementing and improving age determination and ensures the traceability of age-determination analyses. Dr. de Pontual advised the adoption of such methods, for the sake of standardization.

- **Manual of fish sclerochronology** (Panfili et al 2002). This manual aims to provide an overview of the current theoretical and practical aspects of sclerochronological studies. By providing information on the nature of calcified structures (otoliths, scales, skeleton), their uses in fish research and methods for preparation and examination, the manual constitutes a comprehensive guide for researchers, technicians and students either new to the field or interested in expanding their range of expertise. The manual comprises also a multimedia version (DVD) with videos illustrating the main technical procedures with an alternative navigation mode based on decision trees. The Manual contains in the final part some flow diagrams that show, in a very practical way, all the chronological steps required for a correct approach to growth studies, through the observation of calcified structures in teleosts leading to reliable reading and interpretation of age marks. The videos on the otolith extraction and preparation (cutting, grinding and polishing), as well as the flow diagrams were shown to the participants.

3. Current activities in the MedSudMed Project area

IRMA–CNR (Istituto di Ricerche sulle Risorse Marine – Consiglio Nazionale delle Ricerche, Italy): a brief review was presented of the direct growth studies carried out in the past and in the present on different marine species (29 bony fishes, 4 cartilaginous fishes and 3 cephalopod species), by the technical–scientific staff of the IMRA–CNR Centre in Mazara del Vallo. Particular attention and interest has been placed on European hake, emphasizing the problem of the formation of accessory growth centres and interpretation of growth structures in the relevant hard structures. Experiences in the study of growth rates of *Mullus barbatus* through the observation of the *sagittae* were presented and the advantages of validating the readings through the analysis of the marginal increments were described (though not for *Merluccius merluccius)*; current results are summarized by Rizzo et al. (2004, present report). Growth studies on the red mullet stock in the Gulf of Castellammare show that this stock has a different growth performance from that in the Sicily Channel. The methods used for the preparation and reading of hake otoliths outlined in Rizzo et al. (present report) were discussed and defined during the ad hoc FAO Workshop organized in Palma de Mallorca, Spain, 10–15 April 1989 (Oliver et al., 1991). The protocol currently adopted for routine ageing studies carried out by IRMA (extraction, processing and interpretation) was distributed to all the participants in the present Workshop.

Malta Centre for Fisheries Sciences: a representative of the Malta Centre for Fisheries Sciences explained that his Centre has not been involved directly in such activities, but at the moment they are supported by the laboratories and the technical and scientific staff of IRMA; the MCFS is taking measures to be independent in 2005, however.

INSTM (Institut National des Sciences et Technologies de la Mer), Salammbô, Tunisia: the precise method to obtain reliable age–length key (ALK) data is essential for fishery management and sustainable exploitation. These data are obtained by the analysis of the growth marks in calcified structures (scales, otoliths, opercular bone, fin rays, etc.). The Sclerochronology Laboratory of the INSTM is equipped with a polishing machine, a low-speed saw and an image-analysis system using Optimas 6.5 software. For each species, particularly those for which the individual age is estimated for the first time, the age-determination procedure adopted in the INSTM laboratory is based on the following three steps: (1) choice of the most reliable and suitable calcified structure (CS) and the method of its preparation; (2) validation of the timing of the *annuli* formation; (3) age determination.

Criteria for the choice of the suitable CS and the method of preparation were based on: the distinctiveness, regularity and legibility of the growth marks; and on the ease of collection and cost of preparation. To assess the age-determination methods, statistical tests (paired *t*-test, the Wilcoxon test, the percentage agreement and the average percent error, APE) are performed.

An indirect method is used to validate the timing of the *annuli* formation; it consists in the marginal incremement analysis of the CS by a qualitative and a quantitative analysis (Panfili et al., 2002).

For each specimen, and in order to estimate the age in months, the number of growth zones per year, the total number of growth marks, the date of capture and the mean birth date of the target species are combined. For example, for the round sardinella, *Sardinella aurita*, the mean birth date is 15 August, the number of translucent zones is *n*, the age in months (*Am*) for a given fish is Am = 6*n + P (Gaamour et al., 2001), where *P* corresponds to the period (in months) between the date of capture and:

- August, if the fish is captured between August and February (the latter month is not considered), or
- February, if the fish is captured between February and August (the latter month is not considered).

To date, the INSTM Sclerochronology Laboratory has mainly focused on small-pelagic fish species. The Workshop was informed that age estimation of *Mullus surmuletus* has been performed either by counting scale *annuli* (Gharbi, 1980) or otolith (*in toto*) growth marks (Jabeur, 1999). Not many studies have been carried out in the past on age and growth of *Merluccius merluccius*, and the individual age has been estimated only once on the basis of otolith (*in toto*, or in transversal section) examination (Bouhlal and Ktari, 1975). However, a thesis focusing on hake growth, with particular attention to daily growth, will shortly be initiated at INSTM. Moreover, some studies on the indirect age determination (Bhattacharya method) for *Merluccius merluccius*, *Mullus barbatus* and *Mullus surmuletus* are currently under way. Further studies on growth and the population dynamics of exploited stocks are planned for these three species. Currently, research on growth and the application of research results is focused on round sardinella and anchovy, two species that hold great importance for the Tunisian commercial fishery.

Some sclerochronological results since 2000 were described, including the preferred method for the identification of the most suitable CS for growth studies, and the methods for the validation of growth mark interpretation criteria in CS. The cases of *Mullus surmuletus* and *Merluccius merluccius* were described in detail, with emphasis on the fact that the main problem met in this type of studies is the validation of the criteria of interpretation.

Marine Biology Research Centre (MBRC), Libya: a representative of the Marine Biology Research Centre presented some data on the distribution and abundance of some demersal species, derived from commercial trawl landings and scientific trawl surveys carried out in the past in Libyan waters. The most abundant species, in terms of capture, are *Mullus barbatus*, followed by *Merluccius merluccius* which is captured in smaller amount. The hake minimum and maximum size, in commercial landings, are 10 and 60 cm, respectively. Some data on age estimation from the observation of otoliths are reported, as well as on the sex ratio in the catches and the observed length–weight relationship. Data and results from studies carried out in 1982 were presented. The necessity of carrying on research programmes on these topics was highlighted, owing to the need to remedy the lack of knowledge on biological parameters and stock assessment of demersal fish populations. For this reason the importance of the main objectives and perspectives of this Workshop was stressed, considering that the recommendations derived from this Workshop are an important starting point for joint international research.

4. Discussion of a common protocol

The principal aim of the Workshop was to arrive at a common protocol for the sampling, the extraction and the preparation of otoliths of *Merluccius merluccius* and *Mullus barbatus*, with a view to reading the growth marks in them, and for the interpretation of the resulting data.

4.1 Choice of the hard structure

Preliminary work to verify that otoliths are the most suitable structures for ageing studies in each of the species considered is fundamental. Several specific cases involving extraction, preparation and observation of CSs other than otoliths were considered. Otoliths and, more precisely, *sagittae*, are recognized as the best CS for age determination in hake and red/red stripped mullets.

4.2 Sampling.

All sampling methods currently applied at IRMA were discussed, with a view to improving the sampling criteria to be standardized and adopted by all the participating institutes.

A double-stratified sampling protocol for age-determination studies, with a fixed number of samples for each length-class has been proposed by the IRMA research staff taking into account the positive and negative aspects. The proportional sampling method is recognized as being more complete and thus better able to describe stock variability within each defined length-class; however, the large number of samples required, even from commercial or scientific trawl surveys, is a matter of considerable concern.

Although the best sampling frequency was identified as being monthly, both for scientific and commercial landings, this may not be achievable for logistical reasons (availability of samples, funds etc.); however, a quarterly frequency was recognized as being sufficient to describe seasonal variability.

For the correct choice of a suitable sampling method, it is necessary to separate the direct objectives of age determination from other scientific objectives, such as population dynamics. For instance, regarding North Atlantic *Merluccius merluccius*, ALKs are provided quarterly by IFREMER for the assessment of the northern stock. The sampling rate used therein is 10 specimens for each length-class (1 centimetre). For specific studies (e.g. spatial variability in growth parameters), a double-stratified sampling strategy may be applied (e.g. 10 fish per length-class per geographical unit in the MedSudMed region).

The participants agreed on the adoption a double-stratified sampling method (length-class and geographical unit) for hake and mullets in the MedSudMed Project area.

4.3 Otolith extraction

The procedure described in the document presented to this Workshop (Rizzo et al., 2004), and in accordance with the proposals of Williams and Bedford (1974) and Secor et al. (1991), was discussed and accepted by all participants.

4.4 Otolith conservation

Both otoliths (*sagittae* couples) are dried and stored in special boxes. The importance of acquiring digital pictures of the distal face of each otolith was recommended in order to ensure the provision of suitable material for future morphometric studies. Photographic material for subsequent verification of age determination based on otoliths *in toto* or sectioned was also recommended.

The weighing of each otolith with scientific precision scales was recognized by all participants as being an important tool for providing additional information for age interpretation, especially for problematic species; it was therefore recommended.

The importance of maintaining the correct correspondence between associated biological and morphological data of each specimen and the data set coming directly from otolith processing and observation was recommended. As a general convention, each otolith (both right and left) is observed *in toto*; where necessary, the right otolith is cut and ground while the left one is stored whole.

4.5 Otolith preparation

The whole procedure described in Rizzo et al. (present report), and in accordance with Oliver et al. (1991) and Secor et al. (1991), was discussed and accepted by all participants for hake and mullet otolith processing.

The participants recognized that a transverse section through the otolith nucleus was the most suitable way to obtain a standard cross-section (500 µm thick) for macrostructure observation (e.g. seasonal growth marks) or a thin cross-section (<100 µm thick) for the observation of microstructures (e.g. daily-growth studies, if micro-increments are known to be deposited daily).

The procedure using transverse sections of otoliths for hake specimens bigger than 20 cm TL (total length) was discussed and accepted. This procedure is justified by the difficulty in identifying clearly the first growth ring inside the central region of the otolith, owing to an increase in calcification. However, considering that the calcification can prevent the correct identification of the first growth ring also in small individuals (<20 cm TL), the IFREMER expert suggested the use of transverse sections of otolith for age determination in these individuals as well.

No particular difficulties in the processing of *Mullus* otoliths have been recognized so far, but, in the light of the various expert views of the participants in the workshop, the interpretation of the annuli was seen to be more challenging.

The practice of routine cross-sectioning of otoliths (section 500 µm thick) was judged to be particularly convenient in terms of time-saving and of carrying out multiple-embedding and multiple-cutting. On the other hand, regarding the preparation of thin otolith cross-sections (>100 µm thick), of small-size specimens (less than 22 cm TL), it is necessary to proceed with single embedding, cutting, grinding and polishing to obtain a higher quality cross-section. In the specific case of the hake, it was proposed to cut and process all specimens as described, in view of the particular requirement to correctly identify the first growth ring (first year of age).

4.6 Validation

The problem of validation was recognized as a fundamental starting point for any age and growth studies of marine teleosts. The current situation concerning this problem for *Merluccius merluccius* and *Mullus barbatus* was analysed. The uncertainty of hake age determination based on otolith observation was recognized as being high. The participants identified tagging and recapture experiments as being the best way to obtain a reliable validation of an age determination and strongly recommended their use in the study of hake age and growth. Such experiments and studies not only make it possible to collect reliable information on the growth rate of a species, but also to understand correctly the pattern of growth marks in the otolith.

The possibility to validate the age data and thus provide answers to operative and scientific questions, through other approaches, was discussed, particularly because of the extremely high cost of tagging and recapture experiments.

Captivity experiments were proposed on the basis of the availability of aquacultural field experiments in the MedSudMed Project area. Ongoing experiments are being carried out by IFREMER to rear wild North Atlantic *Merluccius merluccius* in aquaculture infrastructures. Preliminary results are promising. However, this approach cannot substitute for field experiments and is rather seen as complementary, providing knowledge on the relationships between somatic and otolith growth with respect to environmental parameters. Other ways to better understand hake growth were described; for example, the possibility to find significant information from morpho-gravimetric analysis of otoliths and from the relationship between otoliths and fish growth.

4.7 Accuracy and precision

The issues of accuracy (the closeness of a quantitative estimate, or measured or computed value to its true value) and precision (the closeness to each other of repeated measurements of the same quantity) were discussed. Accuracy and precision are considered by means of comparison at different levels.

Accuracy can only be assessed through validation experiments either direct or indirect (see Manual of Fish Sclerochronology). Precision can be assessed through intercalibration exercises at different levels:

a. Single-operator precision; this is strictly linked to the reader's experience.

b. Precision among different readers in the same research institute.

c. Precision among different institutes.

The quantitative analysis of precision among different readers is an extremely important preliminary step. This issue was considered by the participants as the first step towards the construction of a common and standardized protocol for the estimation of age and growth.

Different statistical tools are available for age-reading comparison (see, for example, Guidelines and tools for age reading comparisons; cf. supra). The average percent age error (APE) index (Beamish and Fournier, 1981) and the coefficient of variation (CV) (Chang, 1982) are considered as valid measures of agreement.

As far as hake is concerned, for which the interpretation of growth marks is particularly difficult, in addition to the estimation of the precision in the number of *annuli* counted, the IFREMER expert advised the assessment of the coincidence of *annuli* identified. The high coincidence in this sense is required when growth studies through back-calculation and micro-increment analysis are undertaken. The analysis of *annuli* coincidence represents a higher level for the evaluation of precision as described.

5. Practical exercises

5.1 *Mullus barbatus*

The practical work was done in a suitably equipped laboratory for age determination of fish through the interpretation of growth marks on *sagittae*, and was dedicated to the study of red mullet (*Mullus barbatus* Linnaeus, 1758).

Representatives of each participating institute described the techniques used to determine the age of red mullet. By means of a video camera connected to a microscope, the experts were shown on the monitor whole *sagittae* belonging to three specimens with total lengths (TL) of 13 cm, 16.5 cm and 18 cm, respectively. The interpretation of the growth marks on the whole *sagittae* was discussed and particular attention was paid to the location of the first mark.

In smaller specimens, *sagittae* have an elongated shape, with the core and the pelagic–demersal marks being easily identifiable; the older the specimens become, the more the *sagittae* assume a circular shape and the location of the core and the pelagic–demersal marks becomes more complicated.

Transverse sections of 500 µm thickness through the nucleus of *sagittae* extracted from three specimens of TL 24.5 cm, 17 cm and 16.5 cm, were shown. Moreover, a thin section, less than 100 µm thick, obtained from the *sagitta* extracted from a specimen of 6 cm TL, was shown. Counting techniques for daily marks and the problems connected with the presence of sub-daily rings and multiple *primordia* were discussed.

Because of the greater efficacy of sectioning techniques in exposing growth marks, it is suggested that all *sagittae* extracted from specimens longer than 18 cm TL be transversely sectioned through the nucleus.

A comparison of age reading by six readers was made to check the precision of the age determination. Three different sets of 25 *sagittae* of specimens caught in the Strait of Sicily during scientific trawl surveys performed in 2003 were observed by six readers. Each reader interpreted the *sagittae* and wrote down the estimated age, independently of the other readers. The methodology of otolith interpretation for red mullet was discussed and the scheme proposed in the Manual of Fish Sclerochronology (Panfili et al., 2002; p. 97) was unanimously accepted.

In the comparison of reading results, all ages determined by each reader were recorded in the AGE COMPARISONS.XLS Excel Workbook (Eltink, 2000, Internet http://www.efan.no) which is the companion of the EFAN Programme, the Excel Workbook).

The results obtained by each reader are reported in Table 1. It is important to stress that the readers who participated in this age-reading comparison experiment had different levels of practice and experience in red mullet age determination using otoliths: some of them had good experience in otolith reading; others had good experience in age determination through scale readings and in otolith reading in other species; some had only experience of otolith interpretation; and others had no experience at all in age reading.

The readability of the otoliths was very good: estimation of age was possible from 99.78% of the otoliths read. The average percentage agreement with the modal age across all ages and readers in the comparison was 55.2% and the average coefficient of variation (CV) was 45.5% (Table 1).

Table 1. Comparison of red mullet age estimation by several readers.

Mullus barbatus

RANGE r. 1-15

Sample no	Fish no	Fish length	Sex	Landing month	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	MODAL age	Percent agreement	Precision CV
16	1	16.5	F	9	1	1	1	2	3	2	**1**	50%	49%
16	2	16.0	F	9	2	0	2	2	3	1	**2**	50%	62%
16	4	15.5	F	9	3	3	3	2	3	2	**3**	67%	19%
16	10	17.5	F	9	4	3	3	2	2	2	**2**	50%	31%
16	11	15.5	M	9	2	2	3	2	2	3	**2**	67%	22%
16	12	14.5	M	9	4	3	4	2	4	2	**4**	50%	31%
16	14	15.0	F	9	3	4	4	0	3	2	**3**	33%	56%
16	15	13.5	M	9	5	3	4	0	5	2	**5**	33%	61%
16	20	17.0	F	9	3	3	2	1	5	2	**3**	33%	51%
16	26	15.0	M	9	3	4	5	1	4	2	**4**	33%	46%
16	31	17.0	F	9	5	1	4	2	4	1	**1**	33%	61%
16	32	15.5	F	9	5	2	4	2	4	2	**2**	50%	42%
16	33	17.5	F	9	4	4	4	3	4	3	**4**	67%	14%
16	34	16.5	F	9	2	1	2	1	3	2	**2**	50%	41%
16	36	16.0	F	9	3	3	3	1	4	2	**3**	50%	39%
16	38	15.5	M	9	3	3	3	2	4	3	**3**	67%	21%
16	39	15.0	M	9	2	4	1	2	2	3	**2**	50%	44%
16	40	13.0	F	9	1	1	1	1	2	1	**1**	83%	35%
16	41	13.5	M	9	1	1	1	3	2	2	**1**	50%	49%
16	44	16.0	M	9	3	3	3	2	4	2	**3**	50%	27%
16	45	16.0	F	9	3	3	3	2	3	3	**3**	83%	14%
16	46	14.0	M	9	2	1	1	2	1	1	**1**	67%	39%
16	47	13.0	F	9	1	1	1	2	1	1	**1**	83%	35%
16	48	16.5	F	9	4	4	4	2	3	2	**4**	50%	31%
16	49	14.0	F	9	2	2	3	2	4	2	**2**	67%	33%
19	1	16.5	F	10	3	2	3	6	3	2	**3**	50%	46%
19	2	17.0	F	10	1	1	2	7	2	2	**2**	50%	90%
19	6	15.5	M	10	0	2	1	4	1	1	**1**	50%	92%
19	8	15.5	F	10	3	3	3	4	1	2	**3**	50%	39%
19	9	16.0	M	10	2	3	2	4	3	3	**3**	50%	27%
19	11	17.5	F	10	2	1	2	5	1	2	**2**	50%	68%
19	12	16.5	M	10	2	2	2	5	2	3	**2**	67%	45%
19	14	16.0	F	10	2	3	3	5	1	3	**3**	50%	47%
19	22	15.0	M	10	1	2	1	5	3	2	**1**	33%	65%
19	26	15.0	F	10	2	3	2	5	2	2	**2**	67%	45%
19	27	14.5	M	10	3	2	3	6	1	3	**3**	50%	56%
19	36	13.5	F	10	3	3	3	4	3	2	**3**	67%	21%
19	37	16.5	F	10	2	3	1	5	3	3	**3**	50%	47%
19	39	16.0	F	10	2	2	3	5	2	3	**2**	50%	41%
19	40	16.5	F	10	1	1	1	4	2	2	**1**	50%	64%
19	41	17.5	F	10	1	1	2	5	2	2	**2**	50%	68%
19	42	14.0	F	10	1	1	1	6	1	1	**1**	83%	111%
19	43	17.0	F	10	2	1	2	5	3	4	**2**	33%	52%
19	44	17.0	F	10	2	1	2	5	2	3	**2**	50%	55%
19	45	16.0	F	10	2	2	2	6	2	3	**2**	67%	57%
19	46	16.0	F	10	3	3	2	5	2	3	**3**	50%	37%
19	47	14.0	F	10	1	1	1	4	1	3	**1**	67%	72%
19	48	16.5	F	10	2	2	2	4	2	2	**2**	83%	35%
19	49	17.5	F	10	3	3	3	5	3	2	**3**	67%	31%
19	50	15.0	M	10	1	1	1	5	3	3	**1**	50%	70%
22	1	17.0	F	11	1	0	-	3	3	2	**3**	40%	72%
22	2	17.5	F	11	3	3	3	3	2	3	**3**	83%	14%
22	3	16.5	M	11	1	1	1	3	3	2	**1**	50%	54%
22	4	16.5	F	11	1	1	1	4	2	1	**1**	67%	73%
22	5	16.0	M	11	2	1	1	4	2	2	**2**	50%	55%
22	6	16.0	F	11	2	2	2	3	2	2	**2**	83%	19%
22	8	15.5	M	11	2	2	2	3	3	2	**2**	67%	22%
22	12	15.0	M	11	1	1	1	4	2	3	**1**	50%	63%
22	15	17.5	M	11	3	3	3	4	3	4	**3**	67%	15%
22	17	14.5	M	11	0	1	1	3	2	2	**1**	33%	70%
22	20	18.0	F	11	2	2	3	3	3	2	**2**	50%	22%
22	25	17.0	M	11	2	2	4	3	3	2	**2**	50%	31%
22	38	15.5	M	11	1	1	3	3	2	1	**1**	50%	54%
22	39	15.5	M	11	4	3	3	2	2	3	**3**	50%	27%
22	40	15.0	M	11	4	2	3	3	3	1	**3**	50%	39%
22	41	16.0	M	11	4	1	4	4	3	1	**4**	50%	52%
22	42	16.0	M	11	3	2	3	4	3	2	**3**	50%	27%
22	43	15.0	M	11	2	3	2	4	3	1	**2**	33%	42%
22	44	15.0	M	11	2	2	2	4	2	1	**2**	67%	45%
22	45	14.5	M	11	1	1	1	4	2	1	**1**	67%	73%
22	46	15.0	M	11	2	2	2	3	4	2	**2**	67%	33%
22	47	15.0	M	11	4	3	2	3	3	1	**3**	50%	39%
22	48	15.0	M	11	1	2	1	3	3	1	**1**	50%	54%
22	49	15.5	M	11	2	2	2	4	2	1	**2**	67%	45%
22	50	17.0	F	11	2	2	2	3	3	1	**2**	50%	35%
				Total read	75	75	74	75	75	75		**55.2%**	**45.5%**
				Total NOT read	0	0	1	0	0	0			

The average CV ranged between 33.1% (Reader 2) and 42.6% (Reader 4) (Table 2). The highest mean value of CV in the modal ages 1 and 2 years suggested that most readers tended to be less precise for younger ages. However the precision with age was variable among readers.

Table 2. Coefficient of variation (%) of age reading by modal age and reader.

Coefficient of variation (CV)							
Modal age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	All Readers
0	-	-	-	-	-	-	-
1	64%	42%	37%	88%	32%	47%	**62.2%**
2	32%	30%	40%	36%	44%	36%	**43.8%**
3	22%	35%	50%	23%	27%	30%	**35.3%**
4	11%	15%	48%	12%	41%	35%	**34.9%**
Weighted (by number of age-readings) mean	**35.2%**	**33.1%**	**42.2%**	**42.8%**	**35.2%**	**36.2%**	**45.5%**

The mean agreement among all age readers with the modal age ranged from 57% at age 2 to 33% at age 5. In particular, the mean agreement ranged between 50% and 57% for the first four modal ages, whereas the value decreased (33%) for the last modal age. The maximum mean agreement was for Reader 4 (78.7%) and the minimum, for Reader 3 (16%). Percentage agreements are presented in Table 3.

Table 3. Percentage agreement with modal age by reader and modal age.

Percentage agreement							
Modal age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	All Readers
0	-	-	-	-	-	-	-
1	89%	26%	5%	79%	84%	53%	**56%**
2	67%	56%	22%	85%	56%	56%	**57%**
3	73%	57%	17%	70%	74%	39%	**55%**
4	80%	60%	20%	80%	60%	0%	**50%**
5	0%	100%	0%	100%	0%	0%	**33%**
Weighted (by number of age-readings) mean	**74.3%**	**49.3%**	**16.0%**	**78.7%**	**68.0%**	**45.3%**	**55.2%**

The relative bias is the difference between the estimated mean age and the modal age. The relative bias of the mean values with respect to the modal age, for all six readers, ranged from 0.78 at age 1 to –1.83 at age 5. When the age estimations of each reader coincide with the modal age, the bias value is zero. The best mean relative bias was for Reader 4 (0.04), whereas the highest value was recorded for Reader 3 (1.12) (Table 4).

Table 4. Relative bias with modal age by reader and modal age.

Relative bias							
Modal age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	All Readers
0	-	-	-	-	-	-	-
1	0.26	1.11	2.37	0.16	0.16	0.63	**0.78**
2	0.26	0.48	1.67	0.11	-0.15	0.11	**0.41**
3	-0.23	-0.17	0.30	-0.09	-0.26	-0.61	**-0.18**
4	0.20	-0.40	-1.60	-0.20	-0.80	-2.00	**-0.80**
5	-1.00	0.00	-5.00	0.00	-2.00	-3.00	**-1.83**
Weighted (by number of age-readings) mean	**0.09**	**0.37**	**1.12**	**0.04**	**-0.17**	**-0.16**	**0.22**

The plots of relative bias versus modal age (Fig. 1) showed a difference in the interpretation of growth marks among readers. The solid line represents the equilibrium line.

The visual inspection of bias plots suggested a good agreement among Reader 1, Reader 2 and Reader 3 for age groups 1 to 3, whereas, for Readers 4, 5 and 6, an overestimation of age is observed for the smaller age-groups. However, all the readers tended to underestimate older age classes.

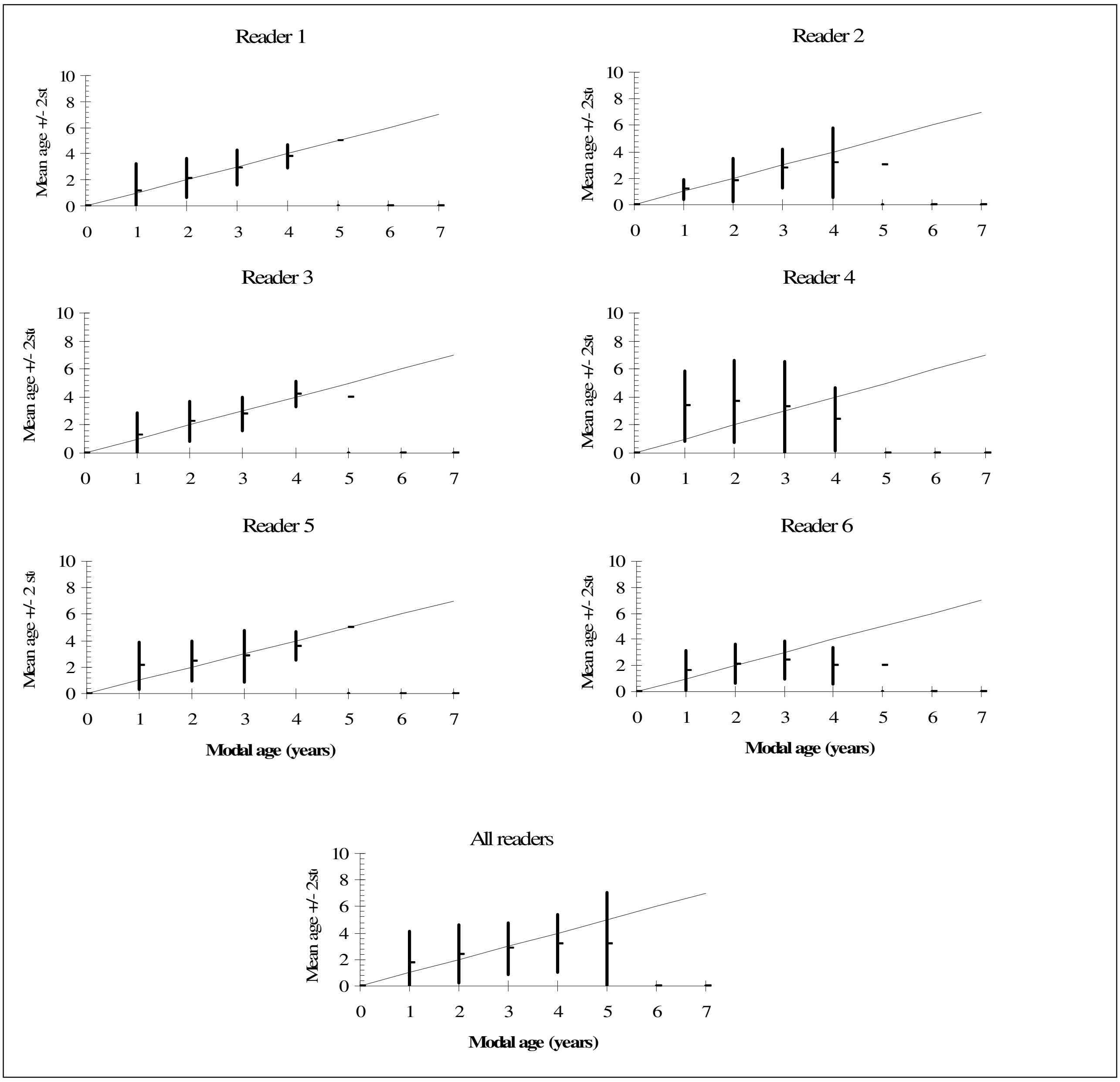

Fig. 1. Age bias plot: mean age +/–2 standard deviations of each reader relative to the modal age. The *solid line* represents the 1:1 relationship between modal age and mean age determination. The mean values for all readers taken together are also shown.

The present comparison outlined the need to improve agreement and precision among all participants in the red mullet age determination. The relatively low level of agreement

(55.2%), precision (CV ~ 45.5%) and the presence of relative bias to the modal age suggested that exchange of otoliths among institutes should be encouraged.

5.2. *Merluccius merluccius*

A representative of each participating institute demonstrated the techniques used to determine the age of hake.

Whole *sagittae* belonging to two specimens, one of 14.5 cm TL and the other of 19.5 cm TL, were displayed on a monitor through a video camera connected to the microscope. As for red mullet, the method adopted by each institute for the interpretation of the marks on the whole *sagittae* was discussed. Particular attention was paid to the problem of the location of the first growth mark, but other problems were highlighted, in particular those related to the interpretation of growth marks in the *sagittae* of hake for the presence of a pelagic–demersal mark, multiple *primordia* and spawning marks.

Cross-sections of the nuclei of three specimens of 38 cm (3 years), 49 cm (5 years) and 55 cm (6 years) TL were inspected and commented.

Owing to the great difficulties that all the participants experienced in the interpretation of growth marks in whole *sagittae* extracted from adult hake, there was general agreement to recommend recourse to transverse sections, 500 µm thick, through the nucleus of the *sagittae* of all specimens longer than 20 cm TL. However, the IFREMER expert, based on the IFREMER experience with North Atlantic *Merluccius merluccius*, recommended the preparation of transverse sections of otoliths also in small individuals (<20 cm TL), in order to better identify the growth increments (especially the first growth increment).

6. Standardized protocols

6.1 *Mullus barbatus*

Action item	Notes
Sampling	Scientific trawl surveys are recommended for the correct standardization within the MedSudMed Project area. Samples taken from the commercial fleet catches are considered complementary and useful. The collection of all data characterizing each haul is recommended.
Collection of ancillary data	The collection of oceanographic data, such as temperature and salinity, is recommended.
Frequency of sampling	The most suitable sampling frequency is recognized as being at monthly intervals, ideally with coverage of the entire year.
Length-class	The length-class is fixed at 1 cm.
Biometric and biological parameters	**a.** Total length: the conversion of the standard length into total length is considered acceptable. **b.** Total weight. **c.** Macroscopic determination of sex for specimens over 10 cm in total length. **d.** Maturity stage referring to the 5-stage maturity scale.
Number of samples for each length-class and each haul	5 males, 5 females and 2 unsexed.
Hard structure chosen for age determination	Otoliths, in particular the two *sagittae*.
Otolith extraction (according to Williams and Bedford, 1974; Secor et al., 1991)	Specimens are dissected along the ventral side with tweezers. Gills are removed. A single cut of the skull base, starting at the beginning of the vertebral column, allows exposure of the vestibular apparatus, where otoliths are located. For specimens less than 50–60 mm total length, the dissection is carried out under a low-magnification light microscope. *Sagittae*, which are easily visible in the *sacculi*, are removed by forceps (Rizzo et al., 2004).
Otolith conservation	*Sagittae* are cleaned in running water, dried and stored in multi-case boxes, with all the details of the corresponding fish recorded beforehand.
Other data on otoliths	**a.** Recording the weight of each *sagittae* is unanimously accepted. **b.** A collection of digital calibrated images of each *sagitta* taken from the distal face is recommended as a suitable tool for use in morphometric analysis.
Observation of *sagittae* for age determination	**a.** Both *sagittae* of each specimen are observed *in toto*. **b.** The right *sagitta* of each fish over 18 cm total length is sectioned. **c.** The right *sagitta* is embedded in highly transparent resin and transversely sectioned through the nucleus (Oliver et al., 1991), using a low-speed rotating precision saw. The section thus obtained, about 500 µm thick, is glued onto a glass slide by thermoplastic cement, ground and polished (Rizzo et al., 2004).
Otolith observation	**a.** *In toto*: with stereomicroscope at 10 x magnification under reflected light. **b.** Section: with stereomicroscope at 10 x magnification under reflected light.
Number of readers	The minimum number of readers is accepted as 5 persons.
Verification of the reader's precision	The use of statistical tests for the assessment of precision is highly recommended. Two methods were assessed: **a.** Average percent error (APE) index (Beamish and Fournier, 1981). **b.** Coefficient of variation (CV): CV can be averaged across a number of fish to produce a mean. CV is statistically more robust than APE and is thus more flexible (Kimura and Lions, 1991). There is no CV threshold value for accepting or rejecting the readings, because these depend on the species and the range of the ages. Laine et al. (1991) suggested a maximum CV value of 5% as the limit for acceptable readings.
Glossary of terms	The glossary of terms presented in the *Manual of Fish Sclerochronology* (Panfili et al., 2002), from Francillon-Vieillot et al. (1990) and Kalish et al. (1995), is considered the most relevant and should be referred to.

6.2 *Merluccius merluccius*

Action item	Notes
Sampling	Scientific trawl surveys are recommended for the correct standardization within the MedSudMed Project area. Samples taken from the commercial fleet catches are considered complementary and useful. The collection of all data characterizing each haul is recommended.
Collection of ancillary data	The collection of oceanographic data, such as temperature and salinity, is recommended.
Frequency of sampling	The most suitable sampling frequency is recognized as being at monthly intervals, ideally with coverage of the entire year.
Length-class	The length-class is fixed at 1 cm.
Biometric and biological parameters	**a.** Total length. The conversion of the standard length into total length is considered acceptable. **b.** Total weight. **c.** Macroscopic determination of sex for specimens over 12 cm in total length. **d.** Maturity stage referring to the 5-stage maturity scale.
Number of samples for each length-class and each haul	5 males, 5 females and 2 unsexed.
Hard structure chosen for age determination	Otoliths, in particular the two *sagittae*.
Otolith extraction (according to Williams and Bedford, 1974; Secor et al., 1991)	Specimens are dissected along the ventral side with tweezers. Gills are removed. A single cut of the skull base, starting at the beginning of the vertebral column, allows exposure of the vestibular apparatus, where otoliths are located. For specimens less than 50–60 mm total length, the dissection is carried out under a low-magnification light microscope. *Sagittae*, which are easily visible in the *sacculi*, are removed by forceps (Rizzo et al., 2004).
Otolith conservation	*Sagittae* are cleaned in running water, dried and stored in multi-case boxes, with all the details of the corresponding fish recorded beforehand.
Other data on otoliths	**a.** Recording the weight of each *sagittae* is unanimously accepted. **b.** A collection of digital calibrated images of each *sagitta* taken from the distal face is recommended as a suitable tool for use in morphometric analysis.
Observation of *sagittae* for age determination	**a.** Both *sagittae* of each specimen are observed *in toto*; in some cases the observation of a transversal section can facilitate the reading. **b.** The right *sagitta* of each fish over 20 cm total length is sectioned. **c.** The right *sagitta* is embedded in highly transparent resin and transversely sectioned through the nucleus (Oliver et al., 1991), using a low-speed rotating precision saw. The section thus obtained, about 500 µm thick, is glued onto a glass slide by thermoplastic cement, ground and polished (Rizzo et al., 2004).
Otolith observation	**a.** *In toto*: with stereomicroscope at 10 x magnification under reflected light. **b.** Section: with stereomicroscope at 10 x magnification under reflected light.
Number of readers	The minimum number of readers is accepted as 5 persons.
Verification of the reader's precision	The use of statistical tests for the assessment of precision is highly recommended. Two methods were assessed: **a.** Average percent error (APE) index (Beamish and Fournier, 1981). **b.** Coefficient of variation (CV): CV can be averaged across a number of fish to produce a mean. CV is statistically more robust than APE and is thus more flexible (Kimura and Lions, 1991). There is no CV threshold value for accepting or rejecting the readings, because these depend on the species and the range of the ages. Laine et al. (1991) suggested a maximum CV value of 5% as the limit for acceptable readings.
Glossary of terms	The glossary of terms presented in the *Manual of Fish Sclerochronology* (Panfili et al., 2002), from Francillon-Vieillot (1990) and Kalish (1995) is considered the most relevant and should be referred to.

7. Final notes

The Workshop was intended to assess the current knowledge of otolith reading in the participating institutions in order to plan further local training activities and to discuss the perspective at regional level, and, in particular, to lay the basis for a standardized protocol for age determination in fish species of main interest to the fishery sector. The Workshop also discussed and adopted a glossary of terms. The experience gained in respect of the North Atlantic *Merluccius merluccius* was taken into consideration in the formulation of both the protocol and the glossary.

During the Workshop the whole procedure for analysing otoliths (from extraction to reading and growth-increment interpretation) was examined and a common protocol to be applied in forthcoming studies in the whole region was agreed upon.

Finally, practical exercises on *Mullus barbatus* and *Merluccius merluccius* were carried out. For *Merluccius merluccius* the practical exercise was aimed at standardizing the procedures for otolith extraction and preparation for reading; the exercise was followed by a discussion on the interpretation of the growth marks. For *Mullus barbatus* the exercise included also the otolith reading by the Workshop participants and the comparison of the age determination among readers. The results of the comparison revealed a relatively low level of precision and agreement, which was mainly attributable to the different know-how of the readers.

This outcome reflects the current level of expertise in the institutions participating in the MedSudMed Project, but it is considered that the aims of the Workshop were met.

8. References

Beamish, R. J. and Fournier D. A. (1981). A method for comparing the precision of a set of age determination. *Canadian J. Fish. Aquat. Sci.* 38:982-983.

Bouhlal M. and Ktari M.H. (1975). Croissance du merlu de la région du Golfe de Tunis. *Bull. Inst. Natl. Sci. Tech. Océanogr. Pêche Salammbô* 4(1):5-47.

Chang W.Y.B. (1982). A statistical method for evaluating the reproducibility of age determination. Can. J. Fish. Aquat. Sci. 39 (8): 1208-1210.

de Pontual, H., Bertignac M., Battaglia A., Bavouzet G., Moguedet P., Groison A. (2003). A pilot tagging experiment on European hake (*Merluccius merluccius*): methodology and preliminary results. *ICES J. Mar. Sci.* 60 (6): 1318-1327.

de Pontual H., Groison A.L., Pineiro C. (2004). Do we need to revise growth estimation of European hake (Merluccius merluccius)? Theme Session on the Life History, Dynamics and Exploitation of Living Marine Resources: Advances in Knowledge and Methodology (K). *ICES CM* No 2004

Eltink, A.T.G.V. (2000). Age reading comparison. *(MS Excel Workbook version 1.0)* Internet: http://www.efan.no

Francillon-Vieillot H., Buffrénil V. de, Castagnet J., Géraudie J., Meunier F.J., Sire J.Y., Zylberbeger L., Ricqlés A. (1990). Microstructure and mineralization of the vertebrate skeletal tissues. In: J.G. Carter (ed.). *Skeletal biomineralization: patterns, processes and evolutionary trends.* Van Nostrand Reinhold. New York, USA. pp471-530.

Gaamour A., Missaoui H., El Abed A., Deniel C. (2001). Age et croissance de l'allache ou sardinelle ronde (*Sardinella aurita* Valenciennes, 1847) dans la région du Cap Bon (Tunisie). *Bull. Inst. Natl. Sci. Tech. Mer Salammbô* (28):9-22.

Gharbi H. (1980). Contribution à l'étude biologique et dynamique des rougets (*Mullus barbatus* Linnaeus, 1758 et *Mullus surmuletus* Linnaeus, 1758) des côtes tunisiennes. Thèse de 3ème Cycle Biologie Marine à la Faculté des Sciences de Tunis. 100pp.

Jabeur C. (1999). La pêche dans le golfe de Gabès : Interactions techniques entre les métiers et exploitation partagée du rouget rouge (*Mullus surmuletus* Linnaeus, 1758). Thèse de Doctorat de l'Université de Bretagne Occidentale. 164pp.

Laine A.O., Momot W.T., Ryan P.A. (1991). Accuracy of using scales and cleithra for ageing northern pike from an oligotrophic Ontario lake. *North Am. J. Fish. Manage.* (11):220-225.

Kalish J.M., Beamish R.J., Brothers E.B., Casselman J.M., Francis C., Mosegaard H., Panfili J., Prince E.D., Thresher R.E., Wilson C.A., Wright P.J. (1995). Glossary. In: (D.H. Secor, J.M. Dean, S.E. Campana (eds.) *Recent developments in fish otolith research.* Columbia, S.C., USA, University of South Carolina Press. pp723-729.

Kimura D.K. and Lyons J.J. (1991). Between-reader bias and variability in the age-determination process. *Fish. Bull.* 89:53-60.

Oliver P., Alvarez F., Morales-Nin B. (1991). Report on the Age-Reading Workshop on Mediterranean Hake and Sardine. *FAO Fish Rep.* 447: 79-84.

Panfili J., de Pontual H., Troadec J.-P., Wright P.J. (eds.) (2002). *Manual of Fish Sclerochronology*. Brest, France: IFREMER-IRD co-edition. 464pp.

Piñeiro C. (2000). Report on Workshop on Hake Otolith Age Reading, Vigo, June 1997. European Fish Ageing Network, EFAN Report, 6–2000.

Piñeiro C., Afonso M.H., Arego S., Bellail R., Labastie J., Loureiro I., Lucio P., Marecos L., McCormick H., Moguedet. P., Morgado C., Sainza M., Santurtún M., Trujillo V.,Watson,T., Woods, F. 2000. Report of 2nd International Workshop on Hake Otolith Age Reading, Vigo, February 1999. European Fish Ageing Network, EFAN Report, 7–2000.

Rizzo P., Gancitano S., Badalucco C., Fiorentino F. (2004). Age estimation from "hard structures" in exploited marine organisms: experiences gathered and procedures adopted in the CNR Centre of Mazara del Vallo. External Document, *Tech. Rep.* 25.

Secor D.H., Dean J.M., Laban E.H. (1991). Manual for otolith removal and preparation for microstructural examination. Electric Power Research Institute and Bell W. Baruch Institute for Marine Biology and Coastal Research. *Tech. Rep.* 91–1.

Williams T. and Bedford B.C. (1974). The use of otoliths for age determination. In: Bagenal T.B. (ed.) *The Ageing of Fish*. Proceedings of an International Symposium on the Ageing of Fish. Reading, UK, 19 July 1973. Old Woking, Surrey (UK): Unwin Brothers Limited, Chessington KT9 2NY, U.K. pp114-123.

Section 2: Description of the ageing activities carried out in the MedSudMed participating Institutes

Summary of age-reading activities at the Marine Biology Research Centre, Tajura, Libyan Arab Jamahiriya

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The activities of the Marine Biology Research Centre (MBRC) included the collection of samples to study the main biological features of fisheries target species including age and growth. For these studies samples were collected during catch-assessment surveys (within-frame surveys). During these surveys fish from trawling hauls were sorted by species, and the individual body length and weight of the important commercial species in the catch were recorded.

Regarding age determination, the species currently being examined in the MBRC, and the corresponding hard parts, are:

1. Hake *(Merluccius merlucciu*s, L.), using the otolith.
2. Pandora *(Pagellus erythrinus*, L.), using the otolith.
3. Round sardinella (*Sardinella aurita,* Val.), using scales.
4. Bluefin tuna *(Thunnus thynnus,* L*.),* using first dorsal spine

In the present paper, only the work on hake is considered.

Hake

Random samples were collected using Italian-type bottom trawl nets, with a cod-end mesh size of 18 mm, in three depth ranges along the Libyan coast: from 50 to 100, from 100 to 200, and from 200 to 300 metres depth

Biological parameters were analysed, such as, individual length and weight, sex, stage of maturity. The *sagittae* (one of the three main types of fish otolith) were collected, measured and weighed. They were washed in running water, dried and stored in small envelopes and identified by relevant information (species, length and weight of specimen, sex, date of collection, etc). Additional details of the fish specimen were recorded on collection sheets. The length-class was based on a 1-cm scale.

Otolith preparation:

1. The number of the *sagittae* collected was 420–600 per year (four seasons), monthly and seasonally.
2. The *sagittae* collected were immediately cleaned with running water and rubbed (by the operator’s fingers) to eliminate any adhering membrane, then dried and stored in small envelopes labelled with all the information related to the fish specimen.

3. The otoliths were slightly burned (over a small flame for 10–15 minutes each), to avoid damage that might impede correct reading; the burning makes the annuli stand out as yellow to light-brown rings.

4. The *sagittae* of the young fish specimens, up to 17 cm in length, were read through a stereo-microscope at low magnification, with reflected light against a black background, and with the distal (concave) side of the *sagittae* upwards. The sample was coated with vegetable oil in a small dish during the examination. The same otolith was analysed by three readers; opaque zones on the otolith appear white and translucent zones appear dark. Otoliths of specimens longer than 17 cm were embedded and then ground carefully on both sides, polished and then examined. The rings became clearer after polishing. However, some information was lost due to the grinding.

5. Annual increments were determined, but no work was done on the daily increment.

In conclusion, it is clear that training in the methodology of age determination in fishes is needed to enable concerned researchers and laboratory technicians to better validate, and discuss, the procedure to be developed by MedSudMed.

Age determination procedure for fishes at the Sclerochronology Laboratory of the INSTM

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Abstract

Reliable age–length data are essential for the management and sustainable exploitation of fish stocks. The precise method to obtain such data is the determination of the age of individual fish specimens based on the analysis of the growth marks on calcified structures (scales, otoliths, opercular bones, fin rays etc.). The ageing procedure used in the Sclerochronology Laboratory of the INSTM is presented. For each species, particularly for those species for which the individual age is being estimated for the first time, the ageing procedure adopted is based on the following three steps: (1) choice of the most reliable and suitably calcified structure and the mode of preparation; (2) validation of the timing of annulus formation; (3) age determination, establishment of the age–length key and study of the fish growth.

Introduction

Age determination is a central part of all work directed to the rational exploitation of a fish stock (Bagenal, 1974; Daget and Le Guen, 1975; Meunier et al., 1979; Mills and Beamish, 1980; Panfili et al., 2002). Knowing the age of a fish provides a clue to its longevity, age at first maturity, age of recruitment, and growth (Summerfelt and Hall, 1987); moreover the age–length key, or age-composition data, allows the development of catch curves from which the annual mortality rates can be calculated. So, ageing fish accurately is indispensable to the understanding of the dynamics of their stocks (Beamish and McFarlane, 1987; Meunier, 1988). An error of one year in estimated age can have grave consequences in fishery management (Beamish and McFarlane, 1996; MacLellan and Fargo, 1996). The age of fish can be estimated indirectly by analysing the length–frequency distribution, from which we can obtain the mean length of each age group; or directly (individual age) by counting the annual growth marks in calcified structures, such as scales, otoliths, opercular bones and fin rays, of each specimen (Daget and Le Guen, 1975; Castanet et al., 1992). The second of these two methods is the more precise and gives more information on the fish population dynamics (Secor et al., 1996). In Tunisia, generally, age determination studies are routinely based on the statistical method or by using scales and otoliths (*in toto*) without a validation of the ageing procedure and age values.

Age determination based on the analysis of the growth marks of calcified structures is the specific aim of the Sclerochronology Laboratory of the INSTM (SLI), which was created in 2000. The present paper describes the methods used in SLI to identify and count growth marks on mineralized structures in fishes and to interpret the corresponding data. Till now, the species of interest to the SLI are small pelagic fishes. Age determination of *Mullus surmuletus* has been performed either by counting scale annuli (Gharbi, 1980) or otolith (*in toto*) growth marks (Jabeur, 1999). However, for *Merluccius merluccius*, the individual age was determined by otolith analysis (both *in toto* and section) (Bouhlal and Ktari, 1975).

General procedure

The hard structures used in the SLI are collected randomly on a monthly basis from commercial and scientific survey catches. For each fish sampled, the following facts are recorded:

- The total, the standard and the fork lengths, to the nearest millimetre
- The total weight, the eviscerated fish weight and the gonad weight to the nearest 0.01 gram
- The sex and the maturity stage on the basis of the macroscopic scale developed by Gaamour (1999)
- The sampling date and area.

Fishes from each area are considered as being aged for the first time (Chilton and Beamish, 1982). For a sub-sample of each species for which the individual age is to be estimated for the first time, both *sagittae*, vertebrae, opercular bone (left one) and fin ray (generally the third dorsal one) were removed, cleaned, dried and preserved in a referenced packet. For each fish, we chose about eight scales from the left side; above the lateral line at midpoint; they were cleaned, dried and mounted between two glass slides.

The different preparation modes for each calcified structure are summarized in Figure 1. Generally, scales are observed under transmitted light, and the other calcified pieces are observed under reflected light in a refractive liquid (water, alcohol, glycerine, Eukitt, cedar oil, botany oil, for example). The Sclerochronology Laboratory is equipped with a polisher, a low-speed saw and an image-analysis unit with the Optimas 6.5 software.

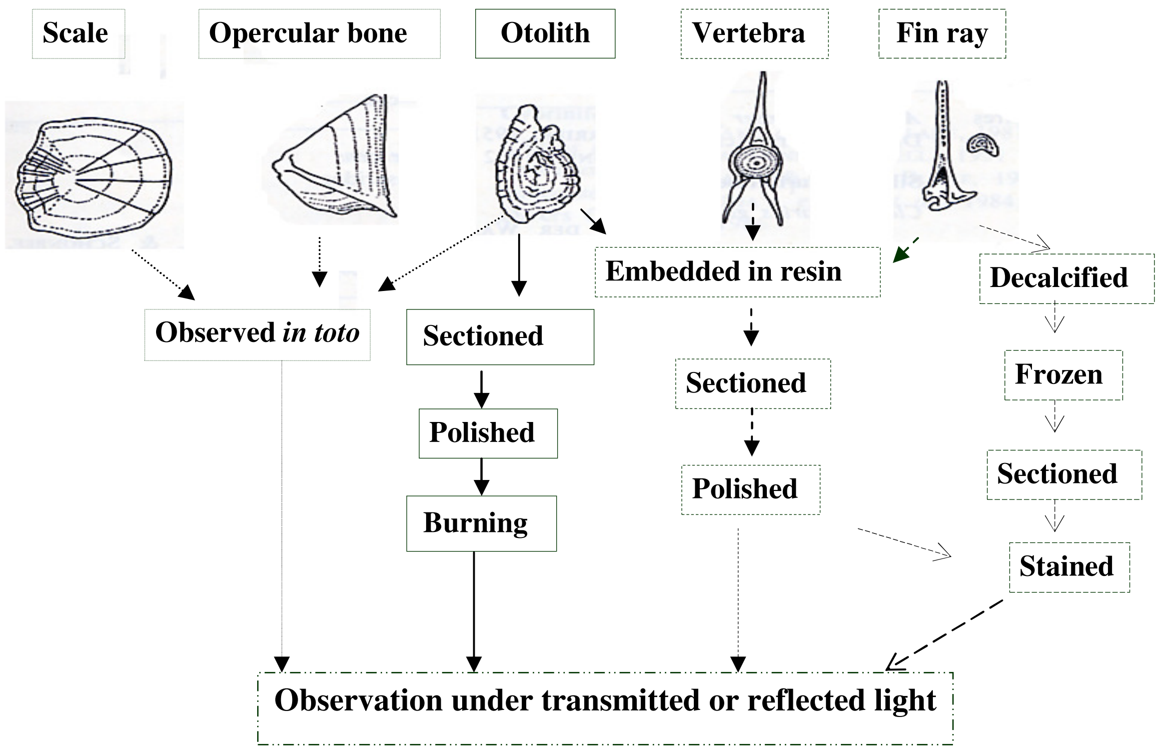

Figure 1. Preparation modes for each hard structure.

Fish age determination requires that growth marks or annuli be identified and counted. For otoliths, vertebrae, opercular bones and fin rays observed under reflected light against a black background, the annulus is identified as the translucent zone and appears as a black band (Baglinière et al., 1992; Panfili, 1992; Beckman and Wilson, 1996; Panfili et al., 2002). For stained preparations, the annuli correspond to the chromophilic rings representing the arrested-growth lines (Castanet et al., 1992). For scales, the annulus corresponds to the area of discordance in the arrangement of circuli or a narrowing between them (Ombredane and Baglinière, 1992). Criteria for the identification of annuli are:

- Their presence in the whole calcified structure
- The decrease in the distance between two successive annual marks, with respect to fish growth, as a fish grows older
- The diminution in the thickness of the faster growth zone as a fish grows older.

For each preparation mode, at least two observations are made by one or more readers. The age determination adopted in the Sclerochronology Laboratory is based on the following three steps:

- Choice of the most reliable and suitable calcified structure and its corresponding preparation mode
- Validation of the timing of annulus formation
- Age determination, establishment of the age–length key, and study of the growth.

Choice of the most reliable and suitable calcified structure

The first step in the age determination was to choose the most reliable and suitable calcified structure and its method of preparation. Criteria for the choice of the appropriate calcified structure are mainly based on the legibility, distinctiveness and regularity of the growth marks (Beamish and Chilton, 1982; Panfili and Loubens, 1992; Panfili et al., 2002). To validate the proportionality between the somatic growth of fish and the calcified structure, we established the relationship between the length of a fish and the radii of its calcified structure (Francis, 1990, 1996; Ricker, 1992).

Some statistical tests (indirect method) were performed to validate the ageing methods (Beamish and Fournier, 1981):

- The paired *t*-test and the Wilcoxon test, to compare the age values from each hard structure
- For each hard structure, the percentage of agreement among readers
- When more than two readings are carried out by the same reader, the average percent error (APE) is used to test the consistency of age values.

These tests were made by using the MS Excel workbook version 1.0 developed by Eltink (2000). More information about the age-reading comparisons can be found in Eltink et al. (2000) and Panfili et al. (2002). If the same precision and bias are found for two preparation modes, we take into account the simplicity and cost of collection and preparation in choosing the most appropriate mode.

For the species of interest along the Tunisian coast, the most reliable and suitable calcified structure are:

- the otolith *in toto*, for sardine (*Sardina pilchardus*) and anchovy (*Engraulis enscrasicolus*)
- the transverse section of otolith for bogue (*Boops boops*)
- the opercular bone for round sardinella (*Sardinella aurita*).

Age validation

The appropriate method to validate (to assess the accuracy of) the age determination is to have fishes of known age (direct method, see Chilton and Beamish, 1982). This method is difficult in practice and can only be applied over a number of years (Beamish and McFarlane, 1987). In the SLI, we use the indirect or semi-direct method to validate the age determination based on analysis of the edge of the chosen calcified structure throughout the year (Baillon, 1992; Panfili, 1992; Beamish and McFarlane, 1987). The timing of annulus development is based on the monthly monitoring of the translucent zone in the annulus: the formation at the growing edge is analysed using quantitative and qualitative techniques (monthly evolution of the frequency of the hyaline margins, FHM, and monthly evolution of the relative marginal distances, RMD). To identify young year-classes, we examine the length–frequency distributions and, if possible, the microstructure of otoliths (Stevenson and Campana, 1992).

Frequency of hyaline margins, FHM = 100*Nhm/Nt, where *Nmh* is the number of hard parts with a hyaline margin; *Nt* is total number of hard parts examined

"Relative marginal distances, RMD = AMD/D_i, i – 1, where AMD is the absolute distance between the last mark (e.g. annulus) and the edge of the hard part; D_i,i–1 is the distance between the two most recent marks (after Campana, 2001).

For example, our observation/analysis indicated that a growth mark corresponding to a translucent zone is formed yearly for sardine, anchovy and bogue, but, for the round sardinella, two hyaline zones are formed yearly.

Age determination, establishment of the age–length key and study of the growth

The age is estimated in months. For this purpose and for each species, we combine the number of hyaline zones per year and the mean birth date with the total number of hyaline zones and the date of capture for each specimen. The Von Bertalanffy growth equation is fitted to the age–length data, using the Statistica software. For each species, the growth curves were established by sex and by area. The mean birth date was determined by analysing the monthly evolution of the gonado-somatic index and the relative frequencies of the macroscopic maturity stages.

The age–length key was also determined by sex, by area and by month. In Tables 1 and 2, below, we give two examples of an age–length key developed in Tunisia for anchovy on the basis of the combined number of hyaline zones on otoliths, the mean birth date and the date of capture; the age-length key was used to estimate growth parameters of the anchovy along the northern coast of Tunisia.

Table 1. Age–length key used in Tunisia: female anchovies, northern coast.

Mois	10	11	12	1	2	3	4	5	12	1	2	3	4	5	6	7	8	12	7	8	9	7	8	9
Lf/ageF	3	4	5	6	7	8	9	10	17	18	19	20	21	22	23	24	25	29	36	37	38	48	49	50
7_7,5																								
7,5_8																								
8_8,5																								
8,5_9			2																					
9_9,5			4		1																			
9,5_10			1																					
10_10,5			1		1			14																
10,5_11								34	1															
11_11,5								22	1															
11,5_12								4	1		3			2										
12_12,5								1			6			3										
12,5_13											1			1										
13_13,5											3													
13,5_14																								
14_14,5														5		13	9		3					
14,5_15																14	13	1	8					
15_15,5																			3					
15,5_16																			3			3		
16_16,5																						1		
Groupe d'âge	Groupe 0:8			Groupe1: 80						Groupe 2: 74									Groupe 3: 20			Groupe4: 4		

Table 2. Age–length key used in Tunisia: male anchovies, northern coast

Mois	10	11	12	1	2	3	4	5	12	1	2	3	4	5	6	7	8	12	7	8	9	7	8	9
Lf/agem	3	4	5	6	7	8	9	10	17	18	19	20	21	22	23	24	25	29	36	37	38	48	49	50
7_7,5																								
7,5_8																								
8_8,5																								
8,5_9			1																					
9_9,5																								
9,5_10								3																
10_10,5			4		2			25																
10,5_11					1			28	2															
11_11,5								10	6		3													
11,5_12								1	6		3													
12_12,5																								
12,5_13											4													
13_13,5											1					1								
13,5_14																		1						
14_14,5														4		15	9		7					
14,5_15																8	6		4					
15_15,5																			2					
15,5_16																						1		
Groupe d'âge	Groupe 0: 5			Groupe 1: 84						Groupe 2: 55									Groupe 3: 13			Groupe 4: 1		

References

Bagenal T.B. (ed.) (1974) The ageing of fish. Proceedings of an International Symposium on the Ageing of Fish. Reading, UK, 19 July 1973. Old Woking, Surrey (UK): Unwin Brothers Limited, Chessington KT9 2NY, U.K. pp114-123.

Baillon N. (1992). Otolithométrie : Revue et problèmes. In: Baglinière J.L., Castanet J., Conand F., Meunier F.J. (eds.) Tissus durs et âge individuel des vertébrés. *Colloques et Séminaires ORSTOM–INRA,* Paris, France. pp21-52

Baglinière J.L., Castanet J., Conand F., Meunier F.J. (eds.) (1992) Tissus durs et âge individuel des vertébrés. *Colloques et Séminaires ORSTOM–INRA,* Paris, France.

Beamish R.J. and Fournier D.A. (1981). Method for comparing the precision of a set of age determination. *Canadian J. Fish Aquat. Sci*. 38:982-983

Beamish R.J. and MacFarlane G.A. (1987). Current trends in age determination methodology. In: Summerfelt R.C. and Hall G.E. (eds.) *The age and growth of fish.* The Iowa State University Press, Ames, Iowa. pp15-42

Beamish R.J. and MacFarlane G.A. (1996). A discussion of the importance of aging errors, and an application to walleye pollock: the world's largest fishery. In: Secor D.H., Dean J.M., Campana S.E. (eds.) *Recent developments in fish otolith research*. University of South Carolina Press, Columbia, S.C. pp545-565

Beckman W. and Wilson C.A. (1996). Seasonal timing of opaque zone formation in fish otoliths. In: Secor D.H., Dean J.M., Campana S.E. (eds.) *Recent developments in fish otolith research*. University of South Carolina Press, Columbia, S.C. pp27-43

Bouhlal M. and Ktari M.H. (1975). Croissance du merlu de la région du Golfe de Tunis. *Bull. Inst. Natl. Sci. Tech. Océanogr. Pêche Salammbô* 4(1):5-47

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

Casselman J.M. (1974). Analysis of hard tissue of pike *Esox lucius L.* with special reference to age and growth. In: Bagenal T.B. (ed.) The ageing of fish. Proceedings of an International Symposium on the Ageing of Fish. Reading, UK, 19 July 1973. Old Woking, Surrey (UK): Unwin Brothers Limited, Chessington KT9 2NY, U.K. pp: 13-22

Castanet J., Meunier F.J., Francillon-Vieillot H. (1992). Squelettochronologie à partir des os et des dents chez les vertébrés. In: Baglinière J.L., Castanet J., Conand F., Meunier F.J. (eds.) Tissus durs et âge individuel des vertébrés. *Colloques et Séminaires ORSTOM–INRA*, Paris, France. pp257-280

Chilton D.E. and Beamish R. (1982). Age determination methods for fishes studied by the Groundfish Program at the Pacific Biological Station. *Canadian Spec. Publ. Fish. Aquat. Sci.* 60:102pp

Daget J. and Le Guen J.C. (1975). Les critères d'âge chez les poissons. In: Lamotte M. and Bourlinière F. (eds.) Problèmes d'écologie : la démographie des populations de vertébrés. Masson et Cie, Paris. pp253-289

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

Eltink A.T.G.W., Newton A.W., Morgado C., Santamaria M.T.C., Modin J. (2000). Guidelines and tools for age readings. (PDF document version 1.0 October 2000). Internet: http://www.efan.no

Francis R.I.C.C. (1990). Back-calculation of fish length: a critical review. *J. Fish. Biol.* 36:883-902

Francis R.I.C.C. (1996). The analysis of otolith data – a mathematician's perspective (What, precisely, is your model?). In Secor D.H., Dean J.M., Campana S.E. (eds.) Recent developments in fish otolith research. University of South Carolina Press, Columbia, S.C. pp81-95

Gaamour A., (1999). La sardinelle ronde, *Sardinella aurita*, dans les eaux tunisiennes : Reproduction, croissance et pêche dans la région du Cap Bon. Thèse de Doctorat de l'Université de Bretagne Occidentale. 246pp

Gharbi H. (1980). Contribution à l'étude biologique et dynamique des rougets (*Mullus barbatus* Linnaeus, 1758 et *Mullus surmuletus* Linnaeus, 1758) des côtes tunisiennes. Thèse de 3ème Cycle Biologie Marine à la Faculté des Sciences de Tunis. 100pp

Jabeur C. (1999). La pêche dans le golfe de Gabès : Interactions techniques entre les métiers et exploitation partagée du rouget rouge (*Mullus surmuletus* Linnaeus, 1758). Thèse de Doctorat de l'Université de Bretagne Occidentale. 164pp

MacLellan S.E. and Fargo J. (1996). Validation of age and growth for English Sole (*Parophrys vetulus*) in Hecate Strait, British Columbia. In: Secor D. H., Dean J.M., Campana S.E. (eds.) *Recent developments in fish otolith research.* University of South Carolina Press, Columbia, S.C. pp341-360

Meunier F.J. (1988). Détermination de l'âge individuel chez les ostéichtyens à l'aide de la squelettochronologie. *Acta Œcologica/Œcol. Gener.* 9(3):299-329

Meunier F.J., Pascal M., Loubens G. (1979). Comparaison de méthodes squelettochronologiques et considérations fonctionnelles sur le tissu osseux acellulaire d'un ostéichtyen du lagon néo-caledonien, *Lethrius nebulosus* (Forskal, 1755). *Aquaculture* (17):137-157

Mills K.H. and Beamish R.J. (1980). Comparison of fin ray and scale age determination for lake whitefish (*Coregonus clupeaformis*) and their implications for estimates of growth and annual survival. *Can. J. Fish. Aquat. Sci.* (37):534-544

Ombredane D. and Baglinière J.L. (1992). Les écailles et leurs utilisations en écologie halieutique. In: Baglinière J.L., Castanet J., Conand F., Meunier F.J. (eds.) Tissus durs et âge individuel des vertébrés. *Colloques et Séminaires ORSTOM-INRA*. Paris, France. pp151-192

Panfili J. (1992). Estimation de l'âge individuel des poissons : Méthodologies et applications à des populations naturelles tropicales et tempérées. Thèse de Doctorat de l'Université de Montpellier II – Sciences et Techniques du Languedoc. 456pp + annexes

Panfili J. and Loubens G. (1992) Starting age study of fishes. Example for *Prochilodus nigricans* (Prochilodidae, Characiformes). *Colloq. Semin. Inst. Fr. Rech. Sci. Dev. Coop.* pp335-340

Panfili J., de Pontual H., Troadec J.-P., Wright P.J. (eds.) (2002). *Manual of fish sclerochronology*. Brest, France: IFREMER-IRD co-edition. 464pp.

Ricker W.E. (1992). Back-calculation of fish length based on proportionality between fish scales and length increments. *Can. J. Fish. Aquat. Sci.* 49:1018-1029

Secor D.H., Dean J.M., Campana S.E. (eds.) (1996). *Recent developments in fish otolith research*. University of South Carolina Press, Columbia, S.C. 735pp

Stevenson D.K. and Campana S. (eds.) (1992). Otolith microstructure examination and analysis. *Can. Spec. Publ. Fish. Aquat. Sci.* 117:126pp

Summerfelt R.C. and Hall G.E. (eds.) (1987). *Age and growth of fish.* The Iowa State University Press, Ames, Iowa. 544pp

Age estimation from "hard structures" of exploited marine organisms in the experience of CNR Centre of Mazara del Vallo: the procedures adopted and the maximum ages estimated

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Abstract

The approaches used in our Centre for ageing marine organisms exploited by fisheries are reviewed, with particular attention to age determination in hake (*Merluccius merluccius*) and red mullet (*Mullus barbatus*). Different kinds of "hard structures" are used, depending on the biological features of each species and the research objectives. Otoliths (*sagittae*), statoliths, spines (i.e. the fin rays) and opercular bones were read for the purpose of ageing bony fish, whereas vertebrae and statoliths were used for selachians and squids, respectively. The estimated maximum age obtained ranged from about 6 months (176 days) in the squid *Illex coindetii* to 30 years in the scorpion fish *Scorpaena elongata*. Regarding hake, whole *sagittae* of specimens up to 190 mm in total length (TL) are routinely examined, whereas thinly sectioned otoliths are used for age determination in larger individuals, giving a maximum age of 15 years. Red mullet otoliths are commonly read in whole. Nevertheless, through the analysis of thinly sectioned otoliths, a maximum age of 10 years was obtained. This experience was applied to the reading of *sagittae* in juveniles of both species, in order to describe the initial growth through the analysis of the daily increments. The main problems in otolith readings in hake and red mullet are briefly discussed.

Introduction

The study of the dynamics of exploited fish populations is mainly based on knowledge of biological processes, such as reproduction, growth, maturity and mortality, strictly linked to age. It is well known that "hard structures" can be very useful in reconstructing the age composition of fish populations and provide basic information to describe and model their growth. Furthermore, reading of hard structures allows estimation of the maximum age; i.e. longevity of a given stock in the wild, which can be related to other relevant population parameters, such as mortality.

Currently, for a successful use of "hard structures" for fish ageing (Williams and Bedford, 1974) the following conditions should be fulfilled:

- That a structural pattern, in terms of succession of opaque and translucent zones, can be recognized in the structure
- That the periodicity of this pattern be known.

Many structures are available for fish ageing, otoliths (*sagittae, lapilli, asterisci*), scales, vertebrae, spines and opercular bones being the most frequently used (Bagenal, 1974; Summerfelt and Hall, 1987; Secor et al., 1995; Panfili et al., 2002).

Regarding the cephalopods (squids), statoliths and the cuttle-bone (*sepion*) are used with good results (Jereb et al., 1991).

In most of the bony fishes, like hake (*Merluccius merluccius*) and red mullet (*Mullus barbatus*), *sagittae* are the hard structures most widely used. On the other hand, vertebrae and statoliths are the most suitable structures for ageing elasmobranchs and cephalopods, respectively.

Our Centre has been involved in the ageing of exploited marine organisms since 1987, within the framework of several Italian and international programmes dealing with stock assessment in the Strait of Sicily (Levi et al., 1998). Up to now, different hard structures of 37 species have been analysed to estimate age (Table 1).

With regard to the demersal resources routinely monitored (CE Regulations 1543/2000, 1639/2001 and 1581/2004), otoliths are the structures regularly chosen for age reading, with the exception of the angler fishes, such as *Lophius budegassa* and *L. piscatorius*, for which an analysis of a thin section of the first ray of the dorsal fin (*illicium*) is used (Landa et al., 2001).

The experience and results reported in this note concern mainly the identification of annual increment in *sagittae* in the two main demersal resource species in the Strait of Sicily (hake and red mullet). Other “hard structures” are studied, depending on the species characteristics and the research objectives in artisanal (Cannizzaro et al., 2000) and in small-pelagic fisheries (Kallianotis et al., 2002; Basilone et al., 2004).

Collection, extraction and conservation methods

Otoliths are currently collected by haul according to a size-stratified sampling design. For each haul, one male and one female, or two specimens if sex is not determined, are collected for each size-class. The size-class range is 2 cm for hake and 1 cm for other bony fishes. In the case of red mullet and striped mullet, two male and two female, or two specimens if sex is not determined, are dissected for otolith extraction for each 1-cm size-class.

The extraction and conservation of the *sagittae* of bony fishes, in general, and of *Merluccius merluccius* and *Mullus barbatus*, in particular, are carried out as follows:

- Specimens are dissected along the ventral side using tweezers. After removing gills, a single cut at the base of the skull, starting at the beginning of the vertebral column, exposes the vestibular apparatus, in which the otoliths are found. This method is suitable for most sizes of hake and red mullet, although those less than 50–60 mm total length are usually best dissected under a low-power microscope
- *Sagittae*, which are easily visible in the *sacculus*, are removed with forceps
- Otoliths are cleaned in running water, dried and stored in multi-case boxes, with the details of the fish specimen: the name of the species, the survey number, the number of the haul and the ordinal number of the specimen.

Otolith preparation methods

<u>Macro-increment analysis</u>: S*agittae* of hake whose total length is less than 190 mm are read in whole for counting the macro-increments assumed to be deposited annually in the *sagittae*. In

larger specimens, the right *sagitta* is routinely embedded in Implex resin and sectioned, with the cross-sectional cut passing through the nucleus (Oliver et al., 1991), using a Buehler Isomet low-speed saw. Then this section, which is about 0.5 mm thick, is glued onto a glass slide by thermoplastic cement, ground with a Remet apparatus, polished and observed.

Sagittae of red mullet specimens are routinely observed in whole, although otoliths of specimens larger than 150 and 190 mm total length (for males and females, respectively), can be sectioned following the same method as that for hake, in order to allow a better counting of macro-increments in the peripheral zone.

Micro-increment analysis: *Sagittae* of juvenile hake and red mullet ranging from 4 to 10 mm in total length are embedded in an Implex resin mould, glued with thermoplastic cement onto a glass slide, ground with a Remet apparatus and polished. The prepared *sagittae* are then analysed for the micro-increments, which are assumed to be laid down daily (Secor et al., 1991).

Reading methods

A glossary for otolith studies (Panfili et al., 2002) is currently followed for otolith nomenclature purposes.

Both sectioned and whole *sagittae* are immersed in a clear liquid (usually water) against a black background and read with a stereomicroscope by two or more readers using the same magnification, under a source of reflected or transmitted light for macro- or micro-increments, respectively.

Readings were repeated at least three times with a reasonable time lapse, and compared later on. In some cases, two readers may analyse the same otoliths together, using a two-seat stereomicroscope, or with the assistance of an image analysis system. Routinely, the observations not matching are not included in the age–length keys, but they are used for assessing precision between readers and consistency over time in age determination (Chang, 1982; Campana et al., 1994).

Some relevant aspects of otolith reading and interpretation in hake and red mullet

The main problems in the interpretation of the growth pattern in hake otoliths are a lack of a clear-cut temporal periodicity in the increment deposition, and the presence of many growth checks, which makes fish age determination very difficult. However, in our Centre, age is determined in an operational way by counting the number of translucent zones; these zones are assumed to be laid down once per year, with the season/period varying, depending on the species. The two inner checks, close to the otolith core, which are more distinct and narrower than the subsequent translucent marks, are considered as a stress-induced mark (pelagic and demersal checks; Morales-Nin et al., 1998; Pinero Alvarez, 2000). In general, the section method allows a clearer identification of macro-increments by emphasizing the corresponding marks better than when the otolith is read *in toto*. In specimens less than 300 mm TL, increment interpretation does not present a major difficulty, since the false rings are discontinuous and less marked than the true ones. In larger fish sizes, the presence of numerous checks with no annual meaning, which can be considered as spawning-induced marks, introduce subjectivity into the counting of annuli. In our experience, up to 300–400 mm TL, no differences are evident between whole and section readings, with concordance in about 80% of cases.

On the basis of thin-section otolith readings of the largest females collected in trawl surveys on the northern side of the Strait of Sicily, a maximum estimated age of 15 years was reported; this age was estimated in a specimen whose total length was 880 mm (Fiorentino et al., 2003; Fig. 1).

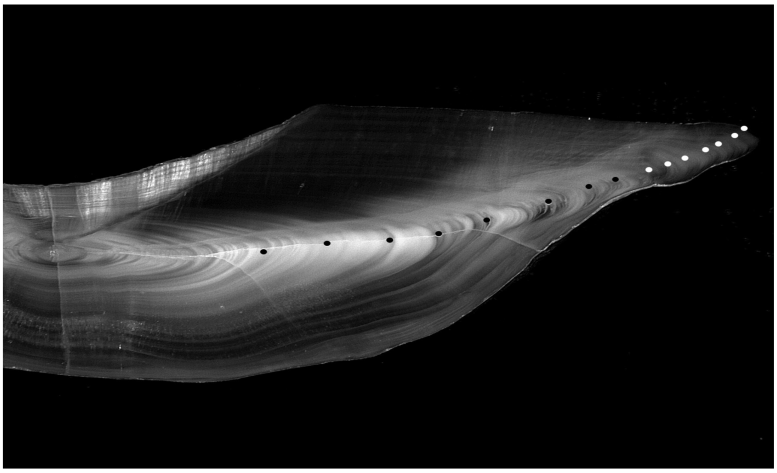

Fig.1. Transverse thin section of otolith of 15 years *Merluccius merluccius* female (880 mm TL). The translucent rings having a yearly meaning are marked.

Daily increment readings in juvenile hake appeared to be difficult, since this species shows many *primordia*, thus biasing the counting of days of life (see Figure 2).

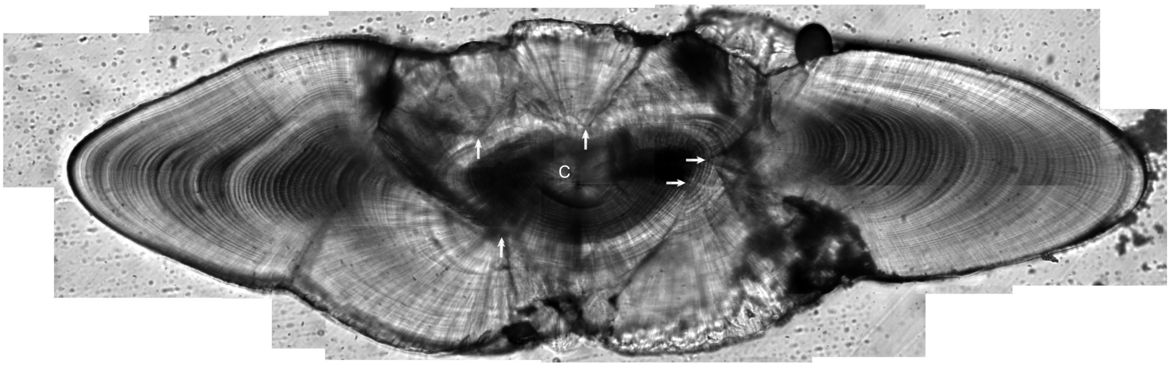

Fig.2. Typical pattern of daily increment in the otolith of young *Merluccius merluccius* (70 mm TL). Arrows point to accessory nuclei.

Generally, otolith reading is easier in red mullet than in hake. In *Mullus barbatus*, the core of the otolith is well defined and easily visible, especially in specimens larger than 120 mm TL. The first annulus after the core is considered a demersal check and is not counted for age estimation. Yearly increments are counted considering the translucent zones. Knowing that they are laid down in winter, one whole year is considered for the count if the border of the otolith is translucent and half a year is added if a wide and opaque border is recognized. Contrary to hake, multiple rings are rarely observed in red mullet. However, some problems have been found in specimens smaller than 120 mm when fully hyaline otoliths can be observed. An example of yearly increments in thin section otolith of red mullet is shown in Figure 3.

Fig.3. Transverse thin section of otolith of 5 years *Mullus barbatus* male (165 mm TL). Arrows point to the translucent rings with a yearly meaning.

Reading of daily increments is easier in juvenile red mullet than in juvenile hake, since a single and well defined *primordium* is found in red mullet (Figure 4).

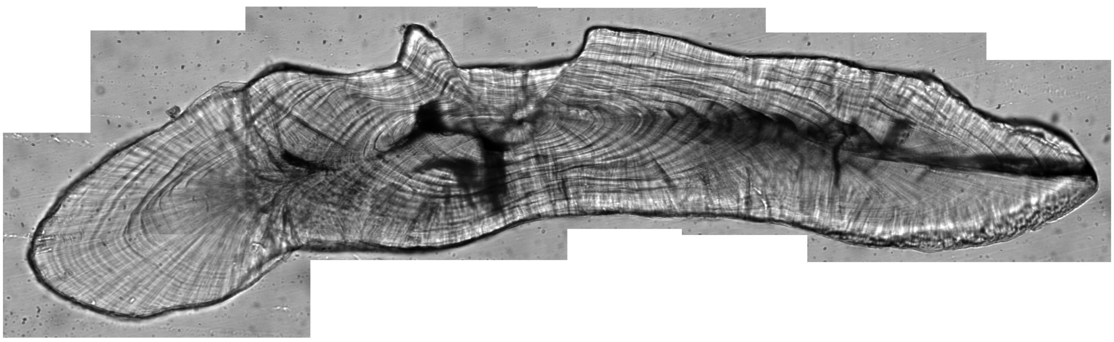

Fig.4. Typical pattern of daily increment in the otolith of young *Mullus barbatus* (67 mm TL). An age of 64 days was estimated.

Conclusions

Although information on the growth of fish species can be derived from analysis of length–frequency distributions, age determination from differential growth zones in hard structures remains a very useful tool, especially in the assessment of the longevity and mortality rates, in the wild, of exploited populations. In this case, the preparation of appropriate thin sections is indispensable for the difficult interpretation of growth increments when a fish is old (Mosegaard et al., 1998).

Another aspect of otolith reading that should be mentioned is the importance of repeated measurements of the same otoliths by different readers and after a given time (precision, see e.g. Gancitano et al., 2006). This kind of quality control should be routinely adopted by all the countries participating in the MedSudMed Project. Furthermore, a standardization of otolith reading could be achieved by delivering standard otoliths to all laboratories involved in the programme.

Finally, further effort has to be made to achieve a good accuracy (defined as the closeness of the estimated age to the true value) in age determination. The necessity of validating the increments in the hard structures is still the most critical point in current reading procedures (Beamish and McFarlane, 1983). At present, in our Centre, two of the available approaches suggested by Campana (2001) are used:

- validation of the correspondence between the observed discontinuities and the time-scale, through the observation of the marginal increments in *sagittae* in seasonal or monthly samples
- progression of a discrete length mode sampled for age determination, mainly for juvenile growth.

Table 1. Fish (teleosts and selachians) and cephalopod specimens studied at the CNR Centre in Mazara del Vallo. For each species analysed, hard structures (*sagittae* = Sg; *lapilli* = Lp; Scales = Sc; *cleithra* = Cl; spines = Sp; *illicia* = Il; statoliths = St; vertebrae = Vr), observational methods (whole otolith = Wh, stained vertebrae = St, and thin otolith section = Ts) and estimated maximum age are reported. *F* = female; *M* = male.

Species	Hard structure	Observational method	Maximum age (years unless otherwise indicated)	Authors & remarks
Merlucciidae				
Merluccius merluccius	Sg	Wh, Ts	15 (F);	Fiorentino et al., 2003
Gadidae				
Phycis blennoides	Sg	Ts	11 (F);	Fiorentino et al., 2003
Micromesistius poutassou	Sg	Ts	10 (F);	Fiorentino et al., 2003
Scorpaenidae				
Helicolenus dactylopterus	Sg	Wh, Ts	10 (F+M)	Ragonese and Reale, 1995
Scorpaena elongata	Sg	Ts	30 (F+M)	Ragonese (pers. com.)
Scorpaena notata	Sg	Wh	9 (F+M)	Not published
Scorpaena porcus	Sg	Wh	11 (F+M)	Not published
Mullidae				
Mullus barbatus	Sg	Wh, Ts	10 (F); 7 (M)	Not published
Mullus surmuletus	Sg	Wh	8 (F); 6.5 (M)	IRMA–CNR, 2002; MaLiRAG, 2003
Sparidae				
Pagellus erythrinus	Sg	Wh	10 (F); 11 (M)	IRMA–CNR, 2002; MaLiRAG, 2003
Diplodus annularis	Sg	Wh	7 (F+M)	Cannizzaro et al., 2000
Diplodus vulgaris	Sg	Wh	10 (F+M)	Cannizzaro et al., 2000
Lithognathus mormyrus	Sg	Wh, Ts	7 (F+M)	Vitale et al., 2003
Sarpa salpa	Sg, Sc, Cl	Wh	9.5 (F+M)	Vitale et al., 2002
Boops boops	Sg	Wh	11.5 (F+M)	Cannizzaro et al., 2000
Carangidae				
Trachurus trachurus	Sg	Wh	14 (F); 13 (M)	IRMA–CNR, 2002; MaLiRAG, 2003
Trachurus mediterraneus	Sg	Wh	7(F); 7 (M)	IRMA–CNR, 2002; MaLiRAG, 2003
Lophidae				
Lophius budegassa	Sg, Il	Ts	9 (F); 6 (M)	IRMA–CNR, 2002
Lophius piscatorius	Sg, Il	Ts	20 (F); 16 (M)	IRMA–CNR, 2002
Soleidae				
Solea lascaris	Sg	Wh, Ts	12 (F); 11 (M)	Gancitano et al., 2006
Solea impar	Sg	Wh	7.5 (F); 6.5(M)	Vitale et al., 2004a
Sciaenidae				
Sciaena umbra	Sg	Ts	26 (F); 17 (M)	Fiorentino et al., 2001
Coryphaenidae				
Coryphaena hippurus	Sg, Lp	Ts	414 (F); 389 (M)	Morales-Nin et al., 1999**.** Age in days.
Trachychthydae				
Hoplostethus mediterraneus	Sg	Wh, Ts	11 (F); 10(M)	Vitale et al., 2004b
Peristaedidae				
Peristedion cataphractum	Sg	Wh, Ts	7 (F); 6.5 (M)	Not published
Balistidae				
Balistes carolinensis	Sg, Sp	Wh, Ts	7 (F+M)	Milazzo et al., 2003
Engraulidae				
Engraulis encrasicolus	Sg	Wh, Ts	3 (F+M)	Basilone et al., 2004
Clupeidae				
Sardina pilchardus	Sg	Wh, Ts	4 (F+M)	Kallianiotis et al., 2002
Triglidae				
Eutrigla gurnardus	Sg	Wh	5 (F); 5 (M)	IRMA–CNR, 2002
Trigla lucerna	Sg	Wh	8 (F); 5 (M)	IRMA–CNR, 2002
Squalidae				
Centrophorus granulosus	Vr	St	5 (F+M)	Rizzo et al., 1993
Squalus blainvillei	Vr	St	8 (F); (8 (M)	Cannizzaro et al., 1995a
Triakidae				
Mustelus mustelus	Vr	St	14 (F+M)	Not published
Rajidae				
Raja clavata	Vr	St	13 (F); 11 (M)	Cannizzaro et al., 1995b
Loliginidae				
Loligo vulgaris	St	Ts	331 (F); 340 (M)	Jereb et al., 1996. Age in days
Loligo forbesi	St	Ts	400 (F); 388 (M)	Jereb et al., 1996. Age in days
Ommastrephidae				
Illex coindetii	St	Ts	176 (F); 191 (M)	Arkhipkin et al., 1999. Age in days

References

Arkhipkin A., Jereb P., Ragonese S. (1999). Checks in the statolith microstructure of the short-finned squid, *Illex coindetii* from the Strait of Sicily (central Mediterranean). *J. Mar. Biol. Assoc. U.K.* 79:1091-1096.

Bagenal T.B. (ed.) (1974) The ageing of fish. Proceedings of an International Symposium on the Ageing of Fish. Reading, UK, 19 July 1973. Old Woking, Surrey (UK): Unwin Brothers Limited, Chessington KT9 2NY, U.K. pp114-123.

Basilone G., Patti B., Mazzola S., Cuttitta A., Bonanno A., Sposito P., Kallianiotis A. (2004). Growth of the European anchovy (*Engraulis encrasicolus*) in the Strait of Sicily. GCP/RER/010/ITA/MSM-TD-05. *MedSudMed Technical Documents* 5:79-89.

Beamish R.J. and McFarlane G.A. (1983). The forgotten requirement for age validation in fisheries biology. *Trans. Am. Fish. Soc.* 112:735-743.

Beltrano A.M., Cannizzaro L., Vitale S., Milazzo A. (2003). Aspetti della biologia di *Diplodus vulgaris* (Pisces: Sparidae). *Biol. Mar. Medit.* 10(2):287-290.

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

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

Cannizzaro L., Rizzo P., Levi D., Gancitano S. (1995a). Age determination and growth of *Squalus blainvillei* (Risso, 1826). *Fish. Res*. 23:113-125.

Cannizzaro L., Rizzo P., Levi D., Garofalo G., Gancitano S. (1995b). *Raja clavata* (Linneo, 1758) nel Canale di Sicilia: crescita, distribuzione e abbondanza. *Biol. Mar. Medit.* 2(2):257-262.

Cannizzaro, L., Garofalo G., Potoschi, A., Kallianiotis, A., Arculeo, M. (2000). Stock assessment of some coastal species caught by artisanal fishery (SACS01). Project No.96/054 - Final Report, Commission of the European Communities, DGXIV, Brussels.

Cannizzaro L., Bono G., Vitale S., Milazzo A. (2001). Age determination and growth of bogue, *Boops boops* (Linnaeus, 1758) in the Strait of Sicily. 10th European Congress of Ichthyology (ECI X, Prague), September 2001.

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

Fiorentino F., Camilleri M., Bono G., Gancitano S., Giusto G.B., Ragonese S., Rizzo P., Rosso B. (2001). On a spawning aggregation of the brown meagre, *Sciaena umbra* L., 1758 (Sciaenidae, Osteichthyes) in the Maltese waters (Sicilian Channel, central Mediterranean). *Rapp. Comm. Int. Mer Medit*. 36: 266.

Fiorentino F., Gancitano S., Giusto G.B., Rizzo P. (2003). Assessing longevity of gadoids in the Strait of Sicily on the basis of otolith microstructure. *Biol. Mar. Medit*. 10(2): 814-818.

Gancitano V., Rizzo P., Gancitano S., Giusto G.B., Cusumano S., Fiorentino F. (2006). Crescita, longevità e mortalità naturale della sogliola dal porro (*Solea lascaris* Risso, 1810; Pisces Soleidae) nello Stretto di Sicilia: informazioni preliminari. *Biol. Mar. Medit*., 13(1):869-871

Kallianiotis A., Mazzola S., Petrakis G., Argyri A., Vidoris P., Basilone G., Patti B., Chilari A., Tsamis E. (2002). The purse-seine landing composition in eastern and central Mediterranean. Project No.99/035 - Final Report, Commission of the European Communities, DGXIV, Brussels.

Jereb P., Ragonese S., Von Boletzky S. (1991). Squid age determination using statoliths. Proceedings of the International Workshop held in I.T.P.P.–C.N.R., 9-14 October 1989 Mazara del Vallo (Italy). *N.T.R.–I.T.P.P. Special Publication* No 1: 127pp.

Jereb P., Ragonese S., Arkhipkin A.I., Bonanno A., Gioiello M., Di Stefano M., Morara U., Bascone M. (1996). Sicilian Channel squid stocks: *Loligo forbesi* Steenstrup, 1856. Project No.93/010 - Final Report, Commission of the European Communities, DGXIV, Brussels.

IRMA–CNR (2002). Il programma CAMP–BIOL 2002 nello Stretto di Sicilia (Mar Mediterraneo): rapporto finale sulle specie demersali. ID/WP/MaLiRAG/3/0103/REL.56pp.

Landa J., Pereda P., Duarte R., Azevedo M. (2001). Growth of anglerfish (*Lophius piscatorius* and *L. budegassa*) in Atlantic Iberian waters. *Fish. Res.* 51:363-376.

Levi, D., Ragonese, S., Andreoli, M.G., Norrito, G., Rizzo, P., Giusto, G.B., Gancitano, S., Sinacori, G., Bono, G., Garofalo, G., Cannizzaro, L. (1998). Sintesi delle ricerche sulle risorse demersali dello Stretto di Sicilia (Mediterraneo centrale) negli anni 1985–1997 svolte nell'ambito della legge 41/82. *Biol. Mar. Medit*. 5(3):130-139.

MaLiRAG (2003). Programma nazionale Italiano per la raccolta di dati alieutici - modulo CAMPBIOL 2003 - Rapporto finale sulle specie demersali nello Stretto di Sicilia (Sub Area Geografica 16; Mar Mediterraneo). ED/WP/MaLiRAG/4/0704/REL.46pp.

Milazzo A., Beltrano A.M., Bono G., Cannizzaro L., Gagliano M.R., Rizzo P., Vitale S. (2003). Age of the triggerfish *Balistes carolinensis* through the reading of the otholith and the first dorsal spine. *Biol. Mar. Medit.* 11(2):603-606.

Morales-Nin B., Torres G.J., Lombarte A., Recasens L. (1998). Otolith growth and age estimation in the European hake. *J. Fish Biol*. 53:1155-1168.

Morales-Nin B., Di Stefano M., Potoschi A., Massuti E, Rizzo P., Gancitano S. (1999). Differences between the sagitta, lapillus and vertebra in estimating age and growth in juvenile Mediterranean dolphinfish (*Coryphaena hippurus*). *Sci. Mar*. 63(3-4):327-336.

Mosegaard H., Morales-Nin B., Weidman C., Geffen A., Arneri E., Millner R., Panfili J., Folkvord A. (1998). Otolith thin-section preparation: some problems and new developments. In: P.J. Wright (ed.). *Otolith preparation and analysis*. Proceedings of European Fish Ageing Network Workshop, University of Bergen, Norway, 18–20 June 1998. EFAN Report 2:11-21.

Oliver P., Alvarez F., Morales-Nin B. (1991). Report on the Age-Reading Workshop on Mediterranean Hake and Sardine. *FAO Fish Rep.* 447: 79-84.

Panfili J., de Pontual H., Troadec J.-P., Wright P.J. (eds.) (2002). *Manual of fish sclerochronology*. Brest, France: IFREMER-IRD co-edition. 464pp.

Pinero Alvarez C. (2000). Report of the Workshop on Hake Otolith Age Reading. Vigo, 23–27 June 1997. EFAN Report 6:31pp.

Ragonese S. and Reale B. (1995). Distribuzione e crescita dello scorfano di fondale, *Helicolenus dactylopterus dactylopterus* (Delaroche, 1809), nello Stretto di Sicilia (Mar Mediterraneo). *Biol. Mar. Medit*. 2(2):269-273

Rizzo P., Gangitano S., Cannizzaro L., Levi D., Mulone S. (1993). Determinazione dell'età e accrescimento in *Centrophorus granulosus* (Schneider, 1801). *Biologia Marina,* Suppl. al Notiziario S.I.B.M.(1), p389.

Secor D.H., Dean J.M., Laban E.H. (1991). Manual for otolith removal and preparation for microstructural examination. The Electric Power Research Institute and the Belle W. Baruch Institute for Marine Biology and Coastal Research, *Technical Report* 91–1.

Secor D.H., Trice T.M., Hornick H.T. (1995). Validation of otolith-based ageing and a comparison of otolith and scale-based ageing in mark-recaptured Chesapeake Bay striped bass, *Morone saxatilis*. Fish. Bull. 93 (1) 186-190.

Summerfelt R.C. and Hall G.E. (eds.) (1987). *The age and growth of fish.* Iowa State University Press, Ames, Iowa. 544 pp.

Vitale S., Cannizzaro L., Bono G., Beltrano A.M., Rizzo P., Milazzo A. (2004a). On the biology of Adriatic sole, *Solea impar* Bennet, 1831 (Pisces; Soleidae), off the SW coast of Sicily. *Rapp. Comm. Int. Mer Méd.* 37:458.

Vitale S., Cannizzaro L., Bono G., Rizzo P., Beltrano A.M., Milazzo A. (2004b). Age determination of silver roughy, *Hoplostethus mediterraneus* (C., 1829) (Pisces; Trachichthyidae) in the Strait of Sicily. *Biol. Mar. Medit*. 11(2):661-665.

Vitale S., Cannizzaro L., Bono G., Beltrano A.M., Milazzo A., Cusumano S. (2003). Maturità sessuale, età e accrescimento della mormora, *Lithognathus mormyrus* (L., 1758) (Pisces; Sparidae) costa sud occidentale della Sicilia. *Biol. Mar. Medit*. 10(2):233-241.

Vitale S., Cannizzaro L., Milazzo A. (2002). Analisi di otoliti, cleithra e scaglie in *Sarpa salpa* (Linneo 1978). *Biol. Mar. Medit*. 9(1):714-716.

Williams T. and Bedford B.C. (1974). The use otoliths for age determination. In: T.B. Bagenal (ed.) *The ageing of fish.* Unwin Brothers Limited, Chessington KT9 2NY, U.K. 114-123.

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Annex B

Terms of Reference[1]

1. Background

The MedSudMed Project is currently implementing a series of activities in the framework of the Project component on "spatial distribution of demersal resources and the influence of environmental factors and fishery characteristics", following a work plan that was discussed and agreed upon by the Project's Coordination Committee. The Expert Consultation organized on this issue (Malta, December 2002), highlighted the need to standardize the data collection and processing methods, in order to produce comparable and valid results at regional level.

The Workshop on Standardization of Trawl Surveys Protocol in the MedSudMed Project Area (Mazara del Vallo, May 2003) was organized in this perspective and was already taken into consideration during recent joint trawl surveys. The agreed indicative work plan adopted during the Expert Consultation also includes the comparison of the procedures used by the participating countries to read the otoliths of priority species identified by the participating experts (see appendix 1). The foreseen target species for the present Workshop are *Merluccius merluccius* and *Mullus barbatus.* Similar activities were implemented within FAO Regional Projects and advantage could be taken from these experiences.

2. Objectives of the Workshop

The Workshop is focused on the preparation and reading of otoliths to get age–length keys and transform length structures into age structures, according to a common protocol to be used in the framework of the MedSudMed activities. The main objectives of the Workshop are therefore to standardize the age-reading methodology and to establish homogeneous age-reading criteria at regional level. The Workshop will provide an overview of the methodologies applied to the targets stocks (presenting existing national and international protocols, giving technical characteristics regarding material, methods, sampling design and data processing). More specifically, the Workshop aims at:

- presenting and discussing the otolith processing methods and the age estimation criteria used by the different research institutes;
- on the basis of the previous, producing a common sampling and processing protocol related to otoliths;
- consequently, planning local training courses on the basis of the common protocol;
- producing a glossary of terms to be used (a draft should be prepared before the Workshop);
- discussing the perspective at regional level.

3. Expected outputs

[1] Notes provided by Othman Jarboui (Institut des Sciences et Technologies de la Mer, Sfax, Tunisia) and Sergio Ragonese (Istituto di Ricerche sulle Risorse Marine e l'Ambiente, Mazara del Vallo, Italy).

The main expected outputs are:

- A common protocol to be used for age-reading at regional level, and in particular for any activity conducted in the framework of the MedSudMed Project.
- An age-reading method which will have been validated and to be used by all four institutes to estimate the age for the species targeted by the MedSudMed Project activities.
- A glossary of terms giving definitions.

4. Participation

The Workshop is open to technicians and researchers belonging to institutes and/or organizations from countries involved in the MedSudMed Project.

An extra-regional expert on age-reading will be invited to chair and supervise the workshop. He should provide a synthetic presentation on the issue in order to update the participants on the latest existing techniques and standards. He should take care of orienting the discussions so that the common protocol produced sticks to international standards.

Invitation will also be made to national programme coordinators and to other regional Projects who might be interested in the issue.

5. Organization

The organization is detailed in the provisional agenda at the end of this document. Each participating institute will present the status and aim of its activities in the specific field of otolith treatment, addressing the points detailed below in a brief document (2–3 pages following the Project's Guide for Authors). Moreover, the extra-regional expert will be invited to prepare an introductory paper giving a general overview of the issue.

These introductory papers will be presented in plenary session; thereafter, a working plan will be agreed upon and, if necessary, different groups will meet in separate sessions to discuss and agree on specific and detailed methodologies for each issue and for each species. A practical session will allow the participants jointly follow the whole process of otolith preparation and reading according to the protocol agreed upon. Eventually, the working groups will present their document to further discussion in plenary session and the meeting will approve the resulting common protocols.

During the Workshop, available glossaries will also be compared and a synthetic annotated glossary will be prepared by the participants.

The items to be discussed are detailed below.

5.0. Choice of the hard structure (otoliths, scales, vertebrae, spines, etc.)

A brief description of the different structures used or potentially usable in the MedSudMed Project area will be given; however, the Workshop will mainly be oriented on otoliths.

5.1. Extracting, cleaning, storing and preparing otoliths

This issue deals with the processing and preparation methods. The following topics summarize the type of information that will be examined:

a. Left, right or both;
b. Storing and labelling;
c. Preparation methodologies (whole surface, whole grounding, break and burnt, thin sections etc.);
d. General procedures (mounting, gluing, resins, bases, cutting, polishing, clearing, etching and viewing,)

5.2. Examining, reading and ageing otoliths

This topic deals with the most critical methodological procedures regarding otoliths. It regards more detailed processing, such as relative and absolute age attribution, and related basic statistics (such as precision of the counts). The issues listed below include also a practical session based on representative otolith samples prepared for one or all target species of the Workshop:

a. Morphometric otolith features (i.e., the primordiun and nucleus) and related measurements;
b. Scaling (morphometric relationships; for example length–weight curve, otolith to somatic growth etc.);
c. Reading and ageing otoliths (classic approach and image analysis application);
d. Checks identification (in particular, those linked to sexual maturation and spawning);
e. Counting axes (i.e., figure out the fast and the slow growth areas);
f. Relative and absolute counts (first growth increment, interpretation of the marginal growth);
g. Relative and absolute age (birthday);
h. Qualitative (i.e., graphing of such percent agreement and age bias plots) and quantitative (i.e., statistical) comparison between readers (precision, average percent error, coefficient of variation, single best increment count, ageing-error matrices etc.);
i. Accuracy and validation (marking, chemical tagging or markers, length analysis, etc.);
j. Preparation of reference collections.

5.3. Individual and length-based growth reconstruction

Age information represents the basis for the estimation of growth, but it is interesting to attempt to clarify the individual trajectories and enlarge the age samples (necessarily small) by using also the length structures routinely collected in the area. The complexity of the issue will allow only a preliminary treatment during the Workshop.

a. Back calculation;
b. Age–length keys (ALK).

5.4. Methods and software to model size (length) at age

The most adaptive procedures and software available to transform size and age data in growth modelling will be analysed. The item, however, is so complex by itself that only a preliminary treatment will be done within the Workshop. Therefore, the issues that will be addressed are:

a. storage of defined information about age and related data;
b. management of input data;
c. exploratory analysis to identify the most suitable model;
d. growth comparison.

6. Date and venue

According to the planned activities, the seminar should have a total duration of 5 days and is scheduled in December 2004. It will be hosted by IRMA–CNR in Mazara del Vallo, Italy.

Annex C

Provisional timetable

1st day

Morning: Presentation of background information on MedSudMed activities
Aims of the seminar and presentation of the participants
Adoption of the agenda and practical organization of the seminar

Afternoon: For each topic, one presentation per research institution (30 min maximum) and by the invited expert is foreseen. An introduction will be made on the glossary, presenting the available information and the expected output.

2nd day

All day: Working groups will be created according to what will have been presented the previous day and according to the number of participants. The issues to be addressed to produce a draft protocol for each topic will be discussed and agreed upon.

3rd day

Morning: Working groups (continued)

Afternoon: Practical session for each species following the whole process from preparation to age determination, under the supervision of the expert. Participants are invited to bring all relevant material (otolith samples, photographs …) that could be useful for the practical session and the drafting of the common protocol.

4th day

All day: Practical session (continued)

5th day

Morning: Discussion of the draft report and definition of the future training activities