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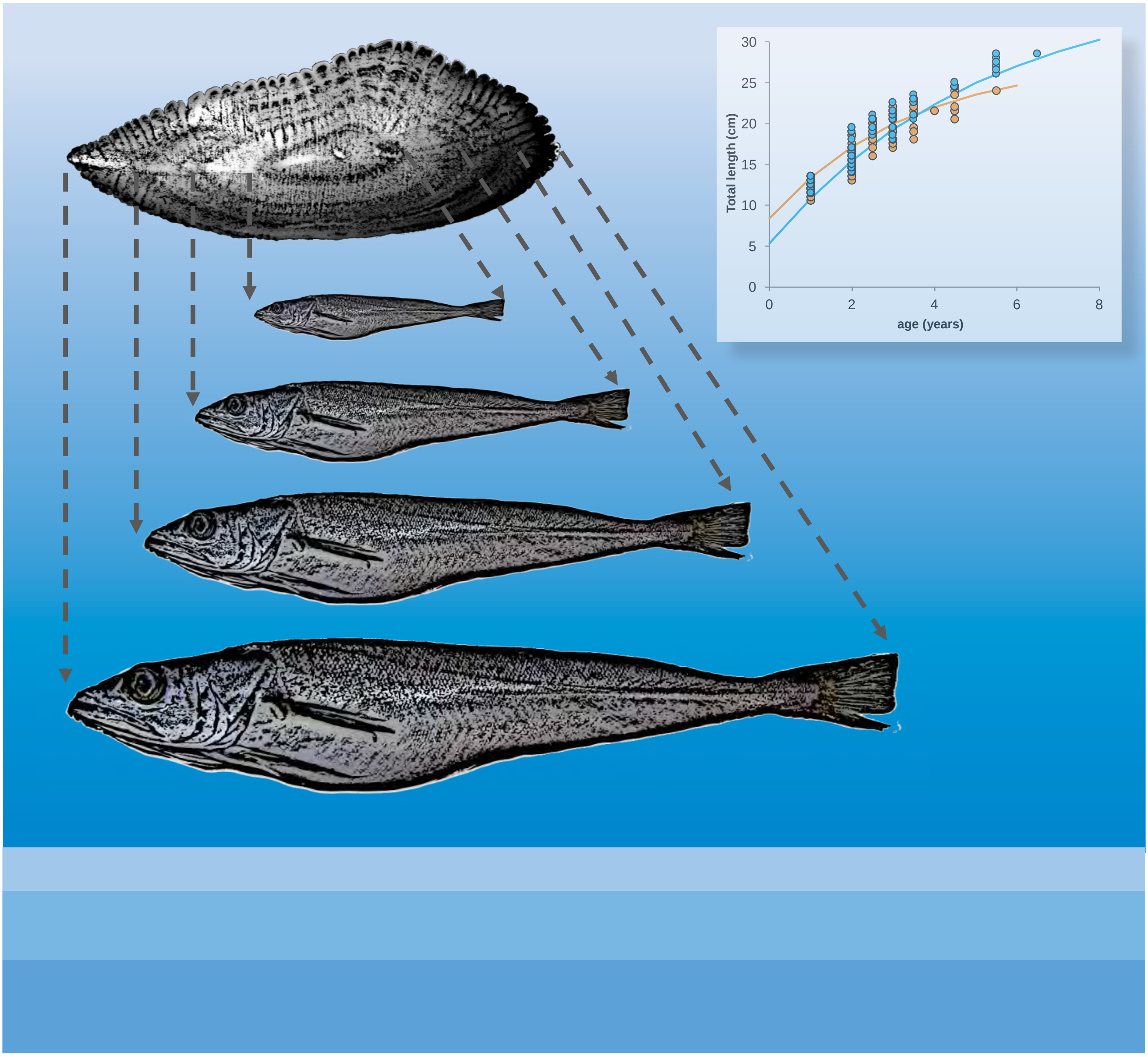
HANDBOOK ON FISH AGE DETERMINATION

a Mediterranean experience



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GENERAL FISHERIES COMMISSION FOR THE MEDITERRANEAN

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HANDBOOK ON FISH AGE DETERMINATION

a Mediterranean experience

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Preparation of this document

This publication is part of the Studies and Reviews series of the General Fisheries Commission for the Mediterranean (GFCM), which focuses on specific aspects of scientific interest for Mediterranean and Black Sea fisheries. This handbook is the fruit of the coordinated work by 23 researchers from different institutes and stems from an experience on fish ageing analysis carried out at the Mediterranean level. It falls within one of the main targets of the mid-term strategy (2017–2020) towards the sustainability of Mediterranean and Black Sea fisheries implemented by the GFCM, which aims, among other things, to enhance knowledge and expertise on fisheries namely by strengthening data collection and information.

Since 1984, several institutes in Italy have been involved in national and international scientific programmes on fisheries, focused on the study of the biology, ecology, population dynamics and assessment of the most important fishery resources, such as *Merluccius merluccius*, *Mullus barbatus*, *Sardina pilchardus* and *Engraulis encrasicolus*. In accordance with regulations, (including within the GFCM Data Collection Reference Framework [DCRF] and, at the European level, the Data Collection Framework [DCF]), biological, environmental, technical and socio-economic data have been regularly collected for the fishing and processing sectors. Since the beginning of data collection, the need for coordination was present and there was general agreement that regional coordination would greatly increase the efficiency of national programmes. In light of this, a working group on fish ageing analysis was created in 2014 under the supervision of the Italian Society of Marine Biology (SIBM). The present volume reports the main results of this working group.

This document was produced with the financial support of the European Union. The views expressed herein can in no way be taken to reflect the official opinion of the European Union.

Abstract

Fish age, among other biological parameters, is one of the most relevant pieces of data in reaching sustainable exploitation of fishery resources. Indeed, most analytical methods used in stock assessment require knowledge of demographic structure according to the age of stocks, as well as to recruitment, growth, maturity, natural mortality, etc., which are strictly linked to information on age and age structure.

The literature on ageing analysis shows some gaps regarding ageing schemes, criteria and methodologies used in preparing calcified structures. These aspects affect both the precision and accuracy of age estimation. One action that could be taken to overcome this gap was to formalize a handbook that clarified approaches to ageing schemes, criteria and preparation methods. Having a common protocol is fundamental to decreasing relative/absolute bias associated with the activities of age determination and to improving the precision (reproducibility and reduction of the coefficient of variation) of age readers from the various laboratories. In the light of these considerations, this handbook aims to be a guideline to standardizing the methods used in fish ageing studies. The document is focused on a description of the general principles on which age analysis relies (assignment of birth date, preparation methods, aging scheme reading and identification of true and false rings). Moreover, common shared analysis methods can enable a high level of calibration among the diverse institutes involved, thus improving the quality and reliability of results.

The volume is subdivided into five main sections: small pelagic species, demersal species, cartilaginous species, large pelagic species and diadromous species. For each section, information on extraction and storage, preparation method, interpretation of age (age scheme) and ageing criteria are provided by species. In total, 30 species were analysed: 6 small pelagic, 12 demersal, 5 cartilaginous, 6 large pelagic and the European eel. These species represent some of the most important fish from an economic and ecological point of view. Thus this volume represents one of the most complete outlooks for fish ageing analysis in the Mediterranean context.

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Acronyms

AA.VV.	autori vari/various authors
AL	anus length
ALK	age–length key
APE	average percent error
BM	birthmark
CAS	catch at size
CFL	curve fork length
CMSY	catch maximum sustainable yield
CS	calcified structure
CV	coefficient of variation
DCF	Data Collection Framework
DCRF	Data Collection Reference Framework
DL	disc length
DW	disc width
EC	European Commission
EDTA	ethylenediamine tetra-acetic acid
EMU	Eel Management Unit
EU	European Union
FAD	fish-aggregating device
FAO	Food and Agriculture Organization of the United Nations
FL	fork length
GFCM	General Fisheries Commission for the Mediterranean
GSA	geographical subarea, sensu FAO-GFCM
G1	group one: including species under EU management or recovery plans or EU long-term multiannual plans or EU action plans for conservation and management and for which assessment is regularly carried out
G2	group two: including species that are important in terms of landing and/or economic values, and for which assessment is not regularly carried out
IA	integrated analysis
IAPE	index of the average percentage error
ICCAT	International Commission for the Conservation of Atlantic Tunas
ICES	International Council for the Exploration of the Sea
LD1	length to first dorsal fin
LJFL	lower jaw – fork length
L/F	length/frequency
LFD	length/frequency distribution analysis
LHead	length of head
LV	length of vertebrae
MEDIAS	Mediterranean Acoustic Survey
MEDITS	Mediterranean International Bottom Trawl Survey

PA	percentage of agreement
PGCCDBS	Planning Group on Commercial Catch, Discards and Biological Sampling (ICES)
RV	radius of vertebrae
SCAA	statistical catch at age
SFL	straight fork length
SIBM	Italian Society of Marine Biology
SPF	small pelagic fish(es)
SS3	stock synthesis
STECF	Scientific Technical and Economic Committee for Fisheries
TL	total length
TW	total weight
VBGF	Von Bertalanffy growth formula
VPA	virtual population analysis
XSA	eXtended survival analysis
YOY	young of the year

1. Introduction

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Since 1984, several institutes in Italy have been involved in national and international scientific programmes on fisheries, focused on the study of the biology, ecology, population dynamics and assessment of the most important fisheries resources, such as European hake (*Merluccius merluccius*), red mullet (*Mullus barbatus*), deep-water rose shrimp (*Parapenaeus longirostris*), giant red shrimp (*Aristaeomorpha foliacea*), blue and red shrimp (*Aristeus antennatus*), sardine (*Sardina pilchardus*), European anchovy (*Engraulis encrasicolus*), swordfish (*Xiphias gladius*), etc. (Relini, 2000; Relini, Carpentieri and Murenu, 2008).

Most analytical methods used in stock assessment – such as virtual population analysis (VPA) and statistical catch at age (SCAA) – require knowledge of demographic structure according to the age of stocks. Moreover, they require information on other biological parameters and processes, such as recruitment, growth, maturity, natural mortality, etc., that are strictly linked to information on age and age structure. Thus it is clear that the availability of accurate information provided by age determination analysis – on age, age structure and growth parameters of exploited stocks – is fundamental to the reliability of scientific advice and the efficacy of the resulting management measures (GFCM, 2017; STECF, 2017).

Fisheries data are commonly collected by length. Length data are generally much easier and cheaper to collect than age data (Hoggarth *et al.*, 2006; Froese, Thorson and Reyes, 2014). The conversion of the length structure of a stock to the age structure required by VPA- and SCAA-based stock assessment models is usually performed by means of age slicing procedures using growth parameters (i.e. the Von Bertalanffy growth formula [VBGF]), or by age–length keys (ALKs) to convert size distribution into age distribution. Despite the availability of models able to perform this conversion internally (e.g. integrated analysis [IA]), this process is usually carried out during data preparation for stock assessment. The use of inappropriate growth parameters or ALKs to convert length distribution into age structure can lead to assessment outputs providing unreliable stock status figures (STECF, 2017). In light of these considerations, it is fundamental that accurate and reliable information on age and growth is made available to end-users and experts involved in stock assessment working groups, such as those carried out under the European Union's Scientific Technical and Economic Committee for Fisheries (STECF) and FAO's General Fisheries Commission for the Mediterranean (GFCM).

It is well known that fish have several calcified structures (CSs) (as do other organisms, such as cnidarians, molluscs, crustaceans, etc. [Panfili *et al.*, 2002]) that can be used for age determination and growth parameter estimation, with the aim of obtaining the age composition of exploited fish populations and stocks. Fish ageing analysis relies on the presence on those CSs with a structural pattern of growth rings in terms of a succession of opaque and translucent zones, as well as on knowledge of the periodicity of that deposition and growth pattern.

In fish, there are several CSs that can be used for ageing analysis: otoliths (sagittae, lapilli, asterisci), scales, vertebrae, spines and opercular bones (Panfili *et al.*, 2002).

So far, about 26 species and taxa are subject to ageing analysis by Italian institutes involved in fisheries data collection and research (i.e. the Data Collection Framework [DCF] in the context of European Union [EU] Council Regulation 1004/2017; GFCM Data Collection Reference Framework DCRF [GFCM, 2018]) (Table 1): bogue (*Boops boops*), tub gurnard *(Chelidonichthys lucerna*), *E. encrasicolus,* grey gurnard (*Eutrigla gurnardus*), blackbellied angler *(Lophius budegassa*), angler (*Lophius piscatorius*), *M. merluccius,* blue whiting *(Micromesistius poutassou*), *M. barbatus,* surmullet *(Mullus surmuletus*), common pandora (*Pagellus erythrinus*), *S. pilchardus,* Atlantic mackerel *(Scomber scombrus*), Atlantic chub mackerel (*Scomber colias*), common sole (*Solea solea*), picarel (*Spicara smaris*), Mediterranean horse mackerel (*Trachurus mediterraneus*), Atlantic horse mackerel (*Trachurus trachurus*), European eel (*Anguilla anguilla*), skates (*Raja* spp.*),* dogfishes nei *(Squalus* spp.*),* Atlantic bluefin tuna *(Thunnus thynnus*), albacore *(Thunnus alalunga*), swordfish (*Xiphias gladius*), Atlantic bonito (*Sarda sarda*), common dolphinfish (*Coryphaena hippurus*).

In bony fish, otoliths (sagittae, in particular) are generally used for age determination of demersal species, with the exception of anglerfish, *L. budegassa* and *L. piscatorius*, in which the thin transverse section of the illicium (first transformed spine of the dorsal fin) is preferred (Landa *et al.*, 2002; Duarte *et al.,* 2005. In large pelagic species (i.e. tuna, swordfish), several CSs can be used, such as otoliths, vertebrae and spines, while in elasmobranchs, a section of vertebrae and/or spines are usually used.

Stock assessment and management need information on an annual basis on fishing effort, total production, size distribution and age composition of catches, etc. from all GSAs in the Mediterranean. The Italian institutes (Table 1) involved in fisheries data collection and research, collect and process biological data, both from commercial landings (EU DCF and GFCM DCRF) and scientific surveys (e.g. the Mediterranean International Bottom Trawl Survey

TABLE 1 – Italian Institutes involved in fish ageing analysis for the Data Collection Framework

Institutes	Area covered	Group(s) of species studied
Centro Interuniversitario di Biologia Marina ed Ecologia Applicata "G. Bacci" – Livorno	GSA 9 – Ligurian Sea and northern Tyrrhenian Sea	Demersal and small pelagic species
Università di Cagliari – Dipartimento di Scienze della Vita e dell'Ambiente	GSAs 11.1 and 11.2 – Sardinian Sea	Demersal and small pelagic species
COISPA Tecnologia & Ricerca – Bari	GSA 10 – southern and central Tyrrhenian Sea GSA 18 – southern Adriatic Sea GSA 19 – western Ionian Sea	Demersal and small pelagic species
Consiglio Nazionale delle Ricerche (CNR) – Istituto per l'Ambiente Marino Costiero (IAMC) – Mazara del Vallo, Trapani	GSA 16 – southern Sicily	Demersal and small pelagic species
Consiglio Nazionale delle Ricerche (CNR) – Istituto di Scienze Marine (ISMAR) – Ancona	GSA 17 – northern Adriatic Sea GSA 18 – southern Adriatic Sea	Demersal and small pelagic species
Laboratorio di Biologia Marina e Pesca – Fano	GSA 17 – northern Adriatic Sea	Demersal and small pelagic species
Università di Bari – Dipartimento di Zoologia	GSA 19 – western Ionian Sea	Demersal and small pelagic species
Università di Roma "Tor Vergata" – Dipartimento di Biologia	All GSAs	European eel
UNIMAR – Rome	All GSAs	Large pelagic species

[MEDITS – AA.VV., 2017b]), covering seven GSAs. Each year, more than 70 000 calcified structures are analysed. In this context, thorough methodological standardization in extracting, preparing and reading CSs is crucial.

Having a common protocol is fundamental in decreasing relative/absolute bias associated with the activities of age determination and in improving the precision (reproducibility and reduction of the coefficient of variation [CV]) of age readers from the various laboratories (PGCCDBS, 2011). In the light of these considerations, this handbook aims to be a guideline to standardizing the methods used in fish ageing studies. The document focuses on a description of the general principles on which age analysis relies (assignment of birth date, preparation methods, ageing scheme reading and identification of true and false rings). Moreover, common shared analysis methods can enable a high level of calibration among the diverse institutes involved, thus improving the quality and reliability of results.

1.1 Sampling methods

Knowledge of the age structure of fish populations can be used to estimate mortality, growth rates, gear selectivity, cohort strength, and other demographic and population dynamics parameters. However, age information is often costly to obtain. High costs force many management programmes to limit the number of fish age-analysed directly, and to rely on ALKs or on age slicing from growth parameters to estimate the age composition of fish stocks.

Proportional subsampling of the catch is desirable as it is based on multiple statistical properties. However, fixed subsampling is frequently used because of improved efficiency in field operations. Instructing field and laboratory staff to collect CSs from a fixed number of fish by length class is much easier than sampling fish by length in proportion to the abundance of each length-class in the catch. The use of ALKs or of age-slicing procedures to provide unbiased age composition of a stock requires that the analysed fish are representative of the whole population. This implies that they are taken with the same gear and in the same season and spatial location as the unanalysed fish (Ricker, 1975; Kimura, 1977). The ability of ALKs to accurately represent the actual age structure of the entire population depends on many factors, such as the sampling strategy (fixed versus proportional subsampling), life span (i.e. short- or long-lived species), exploitation status and recruitment strength (Coggins, Gwinn and Allen, 2013).

The optimum number of otoliths per length class cannot always be provided *a priori*. A description of the optimum sample size for age reading and length measurements dependent on a universal cost function is given in Oeberst (2000). Moreover, according to Mandado and Vásquez (2011), a sample of 20 otoliths per length class is considered the optimum for a species with 30–40 length classes. Coggins, Gwinn and Allen (2013) showed that ten specimens aged per length class (500–1000 fish in total) provide unbiased ALK for both short- and long-lived fish. Negligible benefits were achieved collecting more than ten fish per length class (Coggins, Gwinn and Allen, 2013).

Experiences gathered from the samplings of commercial catches in Italian GSAs evidenced an acceptable coefficient of variation (about 5 percent) when five otoliths per length class (0.5 or 1 cm depending on the species), sex and quarter are collected. The following criteria are taken into account to set the sample size:

- For the smallest size groups, which presumably contain only one age group, the number of otoliths per length class may be reduced.
- In contrast, more otoliths per length are required for the largest length classes (Table 2 provides general criteria).

The combination of data from surveys and landings/discards sampling can contribute to better coverage of a population at sea for growth estimation.

Biological samplings are carried out both at sea, during scientific trawl surveys (i.e. the Mediterranean Acoustic Survey [MEDIAS] and MEDITS), on board commercial vessels, and at landing points (AA.VV., 2017a; GFCM, 2018). Biological sampling of commercial fisheries covers all four quarters of the year, while scientific surveys are usually performed in one season.

1.1.1 MEDITS sampling

In the case of the MEDITS survey, otolith collection and age determination are mandatory for the following species: *M. merluccius*, *M. barbatus* and *M. surmuletus*. Otolith sampling and age determination address several objectives:

- estimate indices of abundance-at-age and monitoring of stock age structure over time;
- monitor spatial distribution of age groups;
- use length-at-age data to estimate growth curves;
- estimate age-based survey indices to be used as tuning information in stock assessment models (i.e. VPA, SCAA, IA); and
- use age data to estimate ecosystem indicators (Barot *et al.*, 2004).

The sampling design adopted is a stratified sampling based on fish size (total length [TL]), in which a fixed number of individuals are randomly collected by length class and sex (Table 2) (AA.VV., 2017b). To avoid samples deriving from only a few hauls, the stratification scheme also includes the haul factor (maximum two pairs of otoliths by length class, sex and haul).

TABLE 2 – MEDITS survey otolith sampling scheme

Species	Length class (cm)	Sample size	Sex
Merluccius merluccius	1	5	M≤14 cm TL
		10	M≥15 cm TL
		5	F≤25 cm TL
		10	F≥26 cm TL
Mullus barbatus	0.5	5	M≤9 cm TL
		10	M≥9.5 cm TL
		5	F≤9 cm TL
		10	F≥9.5 cm TL
Mullus surmuletus	0.5	5	M≤9 cm TL
		10	M≥9.5 cm TL
		5	F≤9 cm TL
		10	F≥9.5 cm TL

Note: M = male, F = female.

For each fish sample, the CSs are usually stored in labelled plastic vials. The code reported on the labels is a combination of both biological (i.e. species name, individual size, sex) and sampling information (i.e. date and haul code).

1.1.2 Sampling of landings/discards of demersal and small pelagic species

The main objective of fisheries data collection is to obtain the demographic structure (by length and age) of the catch (i.e. landings and discards) of each stock. This represents the most important input data for most stock assessment methods (e.g. VPA- and SCAA-based models) for assessing the state of exploitation of the stocks. In each GSA, the sampling design adopted is represented by a stratified random sampling with quarter and *métier* (i.e. fishing technique, such as bottom trawl, pelagic trawl, longline, gill net, trammel net, purse seine, etc.) considered as strata. Species are divided into two main groups: G1 species – which drive the international management process, including species under EU management or recovery plans or EU long-term multiannual plans or EU action plans for conservation and management and for which assessment is regularly carried out – and G2 species – which are important in terms of landings and/or economic values, and for which assessment is not regularly carried out. G1 species are: *M. merluccius*, *M. barbatus*, *M. surmuletus*, *S. solea*, *E. encrasicolus*, *S. pilchardus* and Elasmobranchii. For these species, a fixed number of CSs are randomly collected to achieve a total number of eight CSs (four by sex) for each length class. Length classes are by 1 cm for *M. merluccius*, *S. solea* and Elasmobranchii, and 0.5 cm for *E. encrasicolus*, *S. pilchardus* and *M. barbatus*. G2 species sampling is based on the same protocol with the exception of the *métier* level, which is not considered in the stratification scheme. G2 species are: *B. boops*, *C. lucerna*, *E. gurnardus*, *L. budegassa*, *L. piscatorius*, *M. poutassou*, *P. erythrinus*, *S. scombrus*, *S. colias*, *S. smaris*, *T. mediterraneus* and *T. trachurus*.

Stock management requires information annually owing to the interannual variation in recruitment, which ultimately influences population abundance and age structure. For G1 stocks, collection and analysis of otoliths on an annual basis is mandatory (AA.VV., 2017a; AA.VV., 2017b; ICES, 2015a; GFCM, 2018).

An example of the frequency of sampling for age determination is reported in Table 3 in accordance with the protocol planned in the Italian national programme (AA.VV., 2017a). Data are collected each year, but are provided on an annual basis only for G1 stocks, and every three years for G2 species.

TABLE 3 – Long-term planning of sampling for stock-based variables

MS	Species	Region	RF MO/ RF O/IO	Area/Stock	Frequency	AGE		
						2017	2018	2019
ITA	*Boop boops*	Mediterranean Sea and Black Sea	GFCM	GSAs 9, 10, 11, 16, 17, 18, 19	Q			X
ITA	*Engraulis encrasicolus*	Mediterranean Sea and Black Sea	GFCM	GSAs 9, 10, 11, 16, 17, 18, 19	Q	X	X	X
ITA	*Merluccius merluccius*	Mediterranean Sea and Black Sea	GFCM	GSAs 9, 10, 11, 16, 17, 18, 19	Q	X	X	X
ITA	*Mullus barbatus*	Mediterranean Sea and Black Sea	GFCM	GSAs 9, 10, 11, 16, 17, 18, 19	Q	X	X	X
ITA	*Mullus surmuletus*	Mediterranean Sea and Black Sea	GFCM	GSAs 9, 10, 11, 16, 17, 18, 19	Q	X	X	X
ITA	*Pagellus erythrinus*	Mediterranean Sea and Black Sea	GFCM	GSAs 9, 10, 11, 16, 17, 18, 19	Q			X
ITA	*Sardina pilchardus*	Mediterranean Sea and Black Sea	GFCM	GSAs 9, 10, 11, 16, 17, 18, 19	Q	X	X	X
ITA	*Trachurus mediterraneus*	Mediterranean Sea and Black Sea	GFCM	GSAs 9, 10, 11, 16, 17, 18, 19	Q			X
ITA	*Trachurus trachurus*	Mediterranean Sea and Black Sea	GFCM	GSAs 9, 10, 11, 16, 17, 18, 19	Q			X
ITA	*Micromesistius poutassou*	Mediterranean Sea and Black Sea	GFCM	GSAs 11, 18, 9	Q			X
ITA	*Diplodus annularis*	Mediterranean Sea and Black Sea	GFCM	GSAs 16, 9	Q			X
ITA	*Lophius budegassa*	Mediterranean Sea and Black Sea	GFCM	GSAs 9, 16, 18, 19	Q			X
ITA	*Scomber japonicus*	Mediterranean Sea and Black Sea	GFCM	GSAs 9, 16, 17, 18, 19	Q			X
ITA	*Scomber scombrus*	Mediterranean Sea and Black Sea	GFCM	GSAs 9, 16, 17, 18	Q			X
ITA	*Solea vulgaris*	Mediterranean Sea and Black Sea	GFCM	GSA 17	Q			X
ITA	*Spicara smaris*	Mediterranean Sea and Black Sea	GFCM	GSAs 17, 18	Q			X
ITA	*Thunnus alalunga*	Mediterranean Sea and Black Sea	ICCAT	all areas	Q			X
ITA	*Thunnus thynnus*	Mediterranean Sea and Black Sea	ICCAT	all areas	Q	X	X	X
ITA	*Xiphias gladius*	Mediterranean Sea and Black Sea	ICCAT	all areas	Q			X
ITA	*Galeus melastomus*	Mediterranean Sea and Black Sea	ICCAT, GFCM	GSAs 9, 10, 11	Q			X
ITA	*Raja asterias*	Mediterranean Sea and Black Sea	ICCAT, GFCM	GSAs 9,11	Q			X
ITA	*Raja clavata*	Mediterranean Sea and Black Sea	ICCAT, GFCM	GSAs 9, 11, 16, 18	Q			X
ITA	*Raja miraletus*	Mediterranean Sea and Black Sea	ICCAT, GFCM	GSA 16	Q			X
ITA	*Anguilla anguilla*	Mediterranean Sea and Black Sea	GFCM	all areas	Q			X

Note: Q = quarterly; A = annual.

1.1.3 European eel sampling

Pilot surveys have been carried out under the DCF national programme since 2009–2010, and then on a regular basis since 2011–2013. Currently biological samplings are foreseen for the triennial DCF programme 2017–2019 (AA.VV., 2017a). Samplings are planned for every Eel Management Unit (EMU) – regional administrations in the case of Italy.

Triennial biological surveys are carried out for every EMU in a specific site for each stratum, representative in that EMU in terms of habitat extent and/or amount of eel landings. Eel fishery is still allowed only in the nine regions that presented a management plan (Table 4) and sampling programmes are carried out only in those EMUs.

In each EMU, about 100 individuals for each eel life stage (yellow and silver eel) are randomly sampled every three years from cumulative catches of some days to assess stage composition (reconfirm yellow or silver stage), sex ratio, length and age frequency distributions. Sampling usually takes place in autumn, when eel catches consist of both yellow and silver eels.

From the spatial point of view, for each of the nine EMUs in which eel fishery continues, biological samplings are carried out considering the most relevant sites in terms of eel annual yields (e.g. Comacchio Lagoon in Emilia Romagna, Lake Garda in Lombardia, etc.).

TABLE 4 – Italian administrative regions designated as EMUs (eel fishery is still present in only nine of them)

Administrative region	Code			Stratum	
		River	Lake	Open Lagoon	Managed Lagoon
Valle d'Aosta	VDA			Eel fishery forbidden	
Piemonte	PIE			Eel fishery forbidden	
Lombardia	LOM	np	Y	np	np
Trentino Alto Adige	TAA			Eel fishery forbidden	
Friuli Venezia Giulia	FVG	Y	np	Y	Y
Veneto	VEN	Y	Y	Y	Y
Liguria	LIG			Eel fishery forbidden	
Emilia Romagna	EMR	Y	np	Y	Y
Toscana	TOS	Y	Y	np	Y
Marche	MAR			Eel fishery forbidden	
Umbria	UMB	np	Y	np	np
Lazio	LAZ	Y	Y	Y	Y
Abruzzo	ABR			Eel fishery forbidden	
Molise	MOL			Eel fishery forbidden	
Campagna	CAM			Eel fishery forbidden	
Basilicata	BAS			Eel fishery forbidden	
Puglia	PUG	np	np	Y	Y
Calabria	CAL			Eel fishery forbidden	
Sicilia	SIC			Eel fishery forbidden	
Sardegna	SAR	Y	np	Y	Y

Note: Y = fishery present; np = fishery not present.

Sample processing foresees different procedures depending on the data to be obtained. Annually, length and weight are directly measured on anaesthetized eels, and digital pictures for subsequent specific morphometric measurements are obtained. Samples are released if no other observations are due, or else frozen for further analyses (maturity, ageing analysis, etc.). Every three years, otoliths are collected, but only the left ones are processed for age determination.

1.2 Calcified structure extraction and storage

As the otoliths are located in the saccule (also known as utricle) of the inner ear, specifically in the vestibular labyrinth, their extraction requires the cranium of the animal to be exposed. In order to access the cavities in which the otoliths are enclosed, different cutting methods can be used: i) posterior section ("open the hatch" method); ii) transverse section (guillotine method); iii) longitudinal section ("right between the eyes" method); and iv) "up through the gills" method. In general, the first two methods are used in demersal species. The slicing and cutting tools vary according to the cranium size and robustness, but in general consist of razor blades, scissors and knives. The section must be done with care to avoid damage to the inner ear or to the otoliths. After making the appropriate cut, the otoliths can be removed with stainless steel tweezers.

1.2.1 Posterior section

Holding the fish's head between your thumb and forefinger, a cut at about 30° grade is made on the posterior part of the head (Plate 1).

PLATE 1
Posterior section cut (blue line) in *M. merluccius*, relative to the otolith position (white ellipse)

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Once the skull is opened and the brain moved forward to the anterior part of the fish head, the two largest otoliths (sagittae) are easily detected and can be removed with stainless steel tweezers (Plate 2).

1.2.2 Transverse section

The transverse section cut is performed on the dorsal side of the fish head in correspondence with the preoperculum (Plate 3). Once the skull is opened, the sagittae can be extracted from the posterior part of the head (Plates 4 and 5). This extraction technique is generally used in *M. barbatus*, *M. surmuletus*, *T. trachurus*, *T. mediterraneus* and *A. anguilla*.

PLATE 2
Otolith extraction via posterior section in *M. merluccius*

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PLATE 3
Position of transverse section cut (blue line) in *M. surmuletus*, relative to the otolith position (white circle)

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PLATE 4
Extraction of otolith via transverse head section in *T. trachurus*

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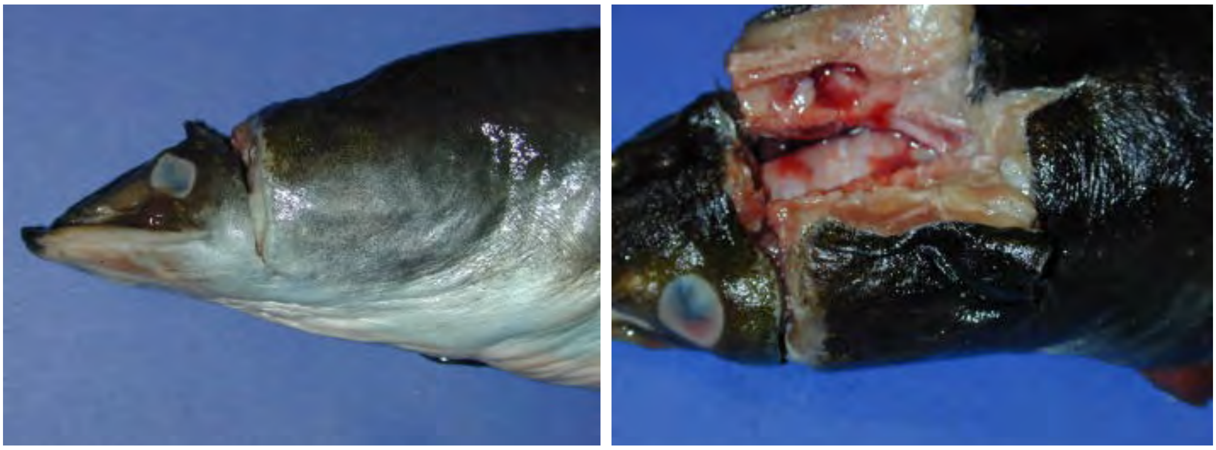
PLATE 5
Extraction of otolith via transverse head section in *A. anguilla*



1.2.3 Removal of illicium

In anglerfish, in addition to the otoliths, the first dorsal transformed spine (fishing filament), also called illicium, is removed with a knife. The illicium is cut at the level of its base and cleaned of soft tissue before storage (Plates 6 and 7). A section of 7–8 cm in length from the base is enough. It is then stored in a plastic vial or an envelope.

PLATE 6
Removal of the illicium in anglerfish (slice in red)

1.2.4 Storage

After extraction, the CSs (i.e. otoliths, illicium) must be cleaned of any residual organic tissue, then washed and dried with paper, and stored in plastic vials or envelopes (subsection 1.2). Plastic tubes (Plate 8) have the advantage of being sufficiently rigid to protect CSs from damage due to handling. When a CS is sampled to estimate its age, it is important to label the vial or envelope with a univocal code to link the CS to a specific specimen.

PLATE 7
Removal of the illicium in anglerfish and storage in envelope

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PLATE 8
Diverse kinds of plastic vial used to store CSsa

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1.3 Ageing scheme

An important point for good practice in ageing analysis is a standardized ageing scheme (ICES, 2013a). This is generally based on several elements: number of translucent rings, theoretical birth date, the pattern of annulus deposition (generally translucent ring during winter/spring months, opaque area during summer/autumn months), date of capture, age resolution (year or half-year) and the edge type (opaque or transparent). A theoretical birth date is set for each species following the reproductive data available in the literature: 1 January for species with the bulk of spawning concentrated during late autumn/winter/early spring, and 1 July for species with a spawning period concentrated in late spring/summer/early autumn.

The age is calculated in year or half-year depending on the lifespan of the species and the possibility of edge discrimination (opaque and transparent).

1.3.1 Species with birth date 1 January

For species with a birth date set at 1 January (i.e. *T. trachurus*, *S. scombrus*, *S. pilchardus, B. boops, C. lucerna*), the translucent rings should be counted, with a 0.5-year resolution for age determination following the ageing scheme reported in Table 5.

TABLE 5 – Ageing scheme for species with a birth date of 1 January

Date capture	Otolith edge	Age
1 January-30 June	Transparent	N
1 July-31 December	Opaque	N + 0.5

Note: N is the number of translucent rings, including those that might be visible on the edge.

Following the scheme in Table 5, specimens caught in the first part of the year (winter/spring) usually have a translucent ring on the otolith edge; this translucent ring is counted as an annual ring and the age is equal to the number of translucent rings (including the edge). In the case of specimens caught in the second part of the year (summer/autumn), with an opaque edge, the age corresponds to the number of translucent rings plus 0.5, which represents the half year already passed.

In some particular cases, this general scheme (Table 5) is not applicable. In fact, an opaque edge can be also present at the beginning and/or the end of the first part of the year (Table 6). The presence of an opaque edge at the beginning of the first part of the year could be due to the fact that formation of a translucent ring has not started yet. In those cases, the age is equal to the number of translucent rings plus 1, because the theoretical birth date has already passed. In contrast, an opaque ring can be present on the otolith edge at the end of the first part of the year owing to an anticipated start of deposition of the opaque ring (Table 6). In those cases, the age is equal to the number of translucent rings (N).

TABLE 6 – Ageing scheme for species with a birth date of 1 January

Months	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec
Deposition pattern	T/O	T	T	T	T	O/T	O/T	O	O	O	O	T/O
Capture date												
Age with edge T	N	N	N	N	N	N	N+0.5					N-0.5
Age with edge O	N+1					N	N+0.5	N+0.5	N+0.5	N+0.5	N+0.5	N+0.5

Note: N is the number of winter rings (translucent); T = transparent edge, O = opaque edge.

In the second part of the year, specimens with a translucent ring at the edge could also be present. As with the presence of an opaque edge in the first part of the year, the presence of a transparent edge in the second part of the year could occur in specimens that have not yet started depositing an opaque ring in early summer (July) or that have already started translucent ring deposition at the end of autumn or early winter (i.e. December) (Table 6). In the first case, age will be equal to the number of translucent rings, including the edge, plus 0.5, which represents the half year already passed. Indeed, the presence or lack of an opaque edge is irrelevant, because the transparent one is counted in the age calculation. If a translucent ring is present at the edge at the end of autumn or early winter (i.e. December), age will be equal to the number of translucent rings, including the edge, minus 0.5, because counting the translucent ring on the edge may overestimate the age by one year, the birth date (1 January) not yet being passed.

1.3.2 Species with birth date 1 July

For *M. barbatus, S. smaris, S. colias* and *E. encrasicolus*, the birth date is set at 1 July. It is commonly accepted that only the translucent rings should be counted, with a 0.5-year resolution. The ageing scheme is reported in Table 7.

Specimens caught in the first part of the year (winter/spring) usually have a translucent ring on the edge, but, according to the scheme, this is not counted as an annual ring as the birth date has not yet passed. Thus the age is equal to the number of translucent rings, including the edge, minus 0.5.

In the specimens caught in the second part of the year (summer/autumn), when an opaque ring is present on the otolith edge, the age is equal to the number of translucent rings (Table 8).

TABLE 7 – Ageing scheme for species with a birth date of 1 July

Date capture	Otolith edge	Age
1 January-30 June	Transparent	N - 0.5
1 July-31 December	Opaque	N

Note: N is the number of translucent rings, including those that might be visible on the edge.

TABLE 8 – Ageing scheme for species with a birth date of 1 July

Months	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec
Deposition pattern	T/O	T	T	T	T	O/T	O/T	O	O	O	O	T/O
Capture date												
Age with edge T	N-0.5	N-0.5	N-0.5	N-0.5	N-0.5	N-0.5	N					N-1
Age with edge O	N+0.5					N-0.5	N	N	N	N	N	N

Note: N is the number of winter rings (translucent); T = transparent edge, O = opaque edge.

The general scheme reported in Table 7 is not applicable when an opaque edge is present at the beginning and/or end of the first part of the year (Table 8), or a transparent edge is present in the second part of the year. An opaque edge can be present in the first part of the year in specimens that have not yet started deposition of a translucent ring or have already started formation of an opaque edge in early summer. In contrast, a transparent edge can be present in the second part of the year in specimens that have not yet started formation of the opaque ring (July), or have already started formation of a translucent ring at the end of autumn or early winter (i.e. December) (Table 8).

When a transparent edge is present after 1 July, the age is equal to the number of winter rings, including the edge (N); when an opaque edge is present before 1 July, the age is equal to the number of winter rings minus 0.5.

When a translucent ring is present in early winter (i.e. before 1 January), the age is equal to the number of translucent rings minus 1, because despite the presence of the winter ring on the edge, the birth date has not yet been reached (Table 8). When an opaque edge is present in the early winter (i.e. January), the age is equal to the number of translucent rings plus 0.5, because, although deposition of the winter ring has not yet started, the birth date has passed. The 0.5 represents the half year that has passed since the birth date.

1.3.3 Ageing scheme for *M. merluccius* and in Elasmobranchii

For Elasmobranchii and *M. merluccius*, the birth date is usually set at 1 January, counting the translucent rings and using a 1-year resolution. The ageing scheme is reported in Table 9.

Following this scheme (Table 9), specimens caught in the first part of the year (winter/spring) usually have a translucent ring on at the edge. This is counted as an annual ring and age will be equal to the number of translucent rings, including the edge. In specimens caught in the second part of the year (summer/autumn) with an opaque edge, the age also corresponds to the number of translucent rings (Table 10).

Table 9 is not always applicable. Indeed, as mentioned in subsection 1.3.1, an opaque edge could be present mostly at the beginning and/or end of the first part of the year (Table 10), as could a transparent edge in the second part of the year (at the beginning and end) (see subsections 3.1 and 3.2). In the second part of the year, specimens with a transparent edge could be present. This could occur in specimens that have already started translucent ring deposition at the end of autumn or early winter (i.e. December) (Table 10). In this case, age will be equal to the number of translucent rings, including the edge, minus 1,

TABLE 9 – Ageing scheme for species with a birth date of 1 January

Date capture	Otolith edge	Age
1 January-30 June	Opaque	N
1 July-31 December	Transparent	N

Note: N is the number of translucent rings, including those that might be visible on the edge.

TABLE 10 – Ageing scheme for species with a birth date of 1 July

Months	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec
Deposition pattern	T/O	T	T	T	T	O/T	O/T	O	O	O	O	T/O
Capture date												
Age with edge T	N	N	N	N	N	N	N					N-1
Age with edge O	N+1					N	N	N	N	N	N	N

Note: N is the number of winter rings (translucent); T = transparent edge, O = opaque edge.

because counting the transparent edge may overestimate the age by one year, the birth date (1 January) not yet having passed.

In the case of an opaque edge present at the beginning of the first part of the year, this may occur in a specimen that has not yet started deposition of a translucent ring. In this case, age will be equal to the number of translucent rings plus 1, because the theoretical birth date has already passed.

1.4 Precision, accordance of readings and preconditioning

In order to minimize the risk of systematic errors due to preconditioning, CS readings should be performed by at least two independent operators with no information on the specimen (i.e. size, sex, etc.). Moreover, readings should be performed at least twice by each reader at an interval of 10–15 days. When readings are in disagreement, the CS should be reanalysed. If no agreement is reached, the CS must be discarded (Goldman, 2005).

The main methods for determining the degree of accuracy of CS readings are: the CV, the index of the average percentage error (IAPE) (Beamish and Founier, 1981; Chang, 1982); and the percentage of agreement (PA), calculated as follows:

$$CV_j = \frac{\sqrt{\sum_{i=1}^{R} \frac{(X_{ij} - X_j)^2}{R-1}}}{X_j} \times 100$$

$$IAPE = \frac{1}{N} \sum \left(\frac{1}{R} \sum \frac{(|X_{ij} - X_j|)}{X_j} \right) \times 100$$

$$PA = \left(\frac{No.agreed}{No.read} \right) \times 100$$

where *N* is the number of samples read; *R* represents the number of readings; X_{ij} is the ith reading of the jth individual; and X_j is equivalent to the average age calculated for the jth individual.

For elasmobranchs and bony fish, CV values of about 10 percent are usually considered acceptable (Bell, 2001). It should, however, be noted that the value of CV is commonly higher by about 40 percent compared to that of IAPE.

As concerns PA, the value for new readers can be considered acceptable when it reaches at least an 80 percent agreement with expert readers. At that point, a new reader can be included in the list of expert readers for a given species.

6. Diadromous species

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6.1 *Anguilla anguilla*

A. anguilla is a diadromous panmictic species (Als *et al.*, 2011) and a shared fishery resource exploited by practically all European and Mediterranean countries. For this species, major problems exist in relation to a continent-wide decline in recruitment observed in the course of the last decades, and to a contraction in adult *A. anguilla* capture fisheries (ICES, 2001; Aalto *et al.*, 2015). *A. anguilla* shows some peculiar features compared with other shared species or other migratory fish. *A. anguilla* exploitation occurs exclusively within national boundaries, in continental waters, without any interaction between economic zones – typical *A. anguill*a fisheries being mainly small-scale. However, spawning takes place in international waters, and all oceanic life stages are unexploited.

Since 2009 the European Union established the Data Collection Framework for Eel (European Council Regulation EC No. 199/2008; EC No. 2017/1004). For the collection, management and use of data in the fisheries sector and support for scientific advice regarding the Common Fisheries Policy. The data collection concerns all *A. anguilla* fisheries in inland and coastal waters, commercial as well as recreational. Moreover the regulation EC No. 1100/2007 required that all member states adopt eel management plans aimed at progressively removing the main causes of *A. anguilla* decline. The plans would guarantee migration towards the sea of at least 40 percent of the silver eel biomass from each catchment basin, with respect to reference conditions defined by the absence of anthropogenic impacts.

The use of sagittal otolith of *A. anguilla* for ageing, rather than other structures, is the most reliable and used method in biological samplings (ICES, 2009b). Knowing age data, among other stock-related variables for a long-live diadromous species such as *A. anguilla*, is particularly important for the modelling quantification of the annual silver eel escapement (Bilotta *et al.* 2011) towards oceanic reproduction.

6.1.1 Otolith extraction and storage

The technique used for otolith extraction in *A. anguilla* is adapted from that described by Moriarty (1973). This technique minimizes loss of and damage to the otoliths and is a quick, clean and efficient method. A primary transverse incision is made behind the eye in two phases using a scissors; first, cutting the skin and flesh and, second, penetrating the cranium through the roof of the mouth and providing access to the cranial cavity.

A. anguilla otoliths, once removed from the heads, are immerged in distilled water, and the attached organic tissues cleaned with the absorbent side of lab bench paper. The otoliths are stored dry in labelled Eppendorf microtubes and left in a heater (at 70 °C) overnight. The microtubes are then closed and stored until otolith examination.

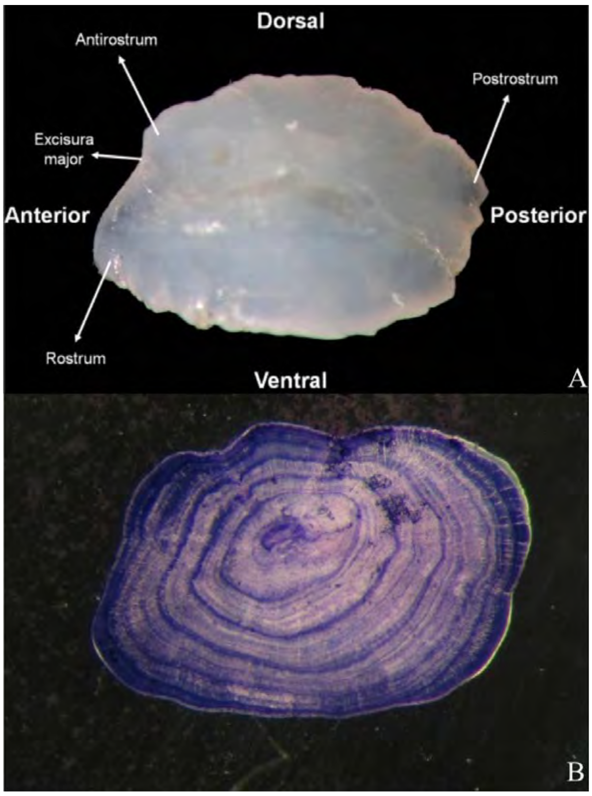
6.1.2 Preparation

The methodology described in this subsection is a modification of the one developed at the Cemagref laboratories (Bordeaux, France) (Capoccioni *et al.*, 2014). Today it is widely used in many laboratories in Italy (including at the Università degli studi di Roma "Tor Vergata"), as it is proposed as the methodology of preference in determining the age of *A. anguilla* of no more than 15 years. With respect to the burning and cracking method, results are easier to interpret and more reliable (ICES, 2009b).

The age of *A. anguilla* is assessed by counting the annuli illuminated by polarized or transmitted light after grinding and polishing (Plate 195).

Each left otolith is placed at the bottom of a numbered mould cavity, with the distal face up. Then drops of an epoxy resin are added to each cavity until the mould is completely filled. Bubbles under the otoliths are gently removed by moving the sample with a needle. Moulds are left to dry overnight until the resin becomes hard. The resin blocks with the embedded otoliths are then removed from the moulds. With the otolith's convex side up, each block is mounted with a drop of Eukitt (transparent glue) onto a histological slide with quick pressure. Slides are labelled with the appropriate code for each otolith (Plate 196).

PLATE 195
Proximal face of an *A. anguilla* right otolith

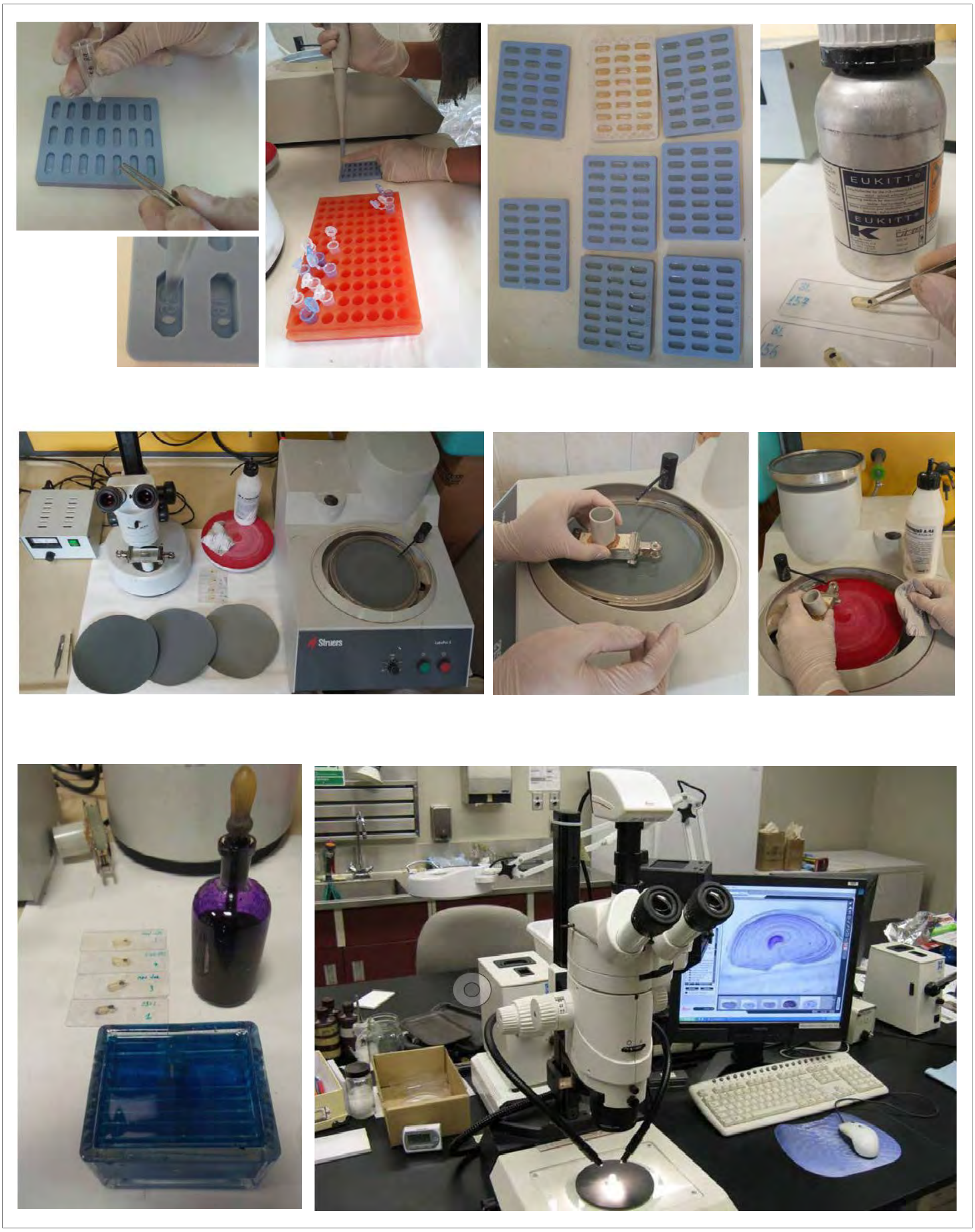


© F. Capoccioni

Note: A – Anterior and posterior regions are aligned with the orientation of the body of *A. anguilla*; B – Example of *A. anguilla* right otolith analysed using the grinding and polishing technique.

The grinding procedure is carried out using a Struers grinding machine (LABPOL-5), beginning with 1 200-grit silicon-carbide sanding papers, and increasing to 5 000 grits until the centre and edge of the otolith are visible. Slides are continually checked to ensure that the grinding is in the right direction and with sufficient force and that the core has not been removed. When the primordium is exposed and is easily recognized as a black point inside the core, the otolith is polished with a jewellery cloth and an abrasive paste (suspension of 1μ alumina) to remove any score lines.

PLATE 196
A. anguilla otolith ageing analysis procedure, with grinding and polishing technique



The sample is now ready for hatching with an acid preparation and then for the staining process. A drop of 5-percent EDTA is applied on each otolith for three minutes and then rinsed with distilled water. Subsequently, a drop of 5-percent toluidine blue is applied to the ground otolith surface. The stained otoliths are left to dry overnight and then immersed in distilled water for an hour.

They are now ready for observation under a binocular microscope with an image acquisition system. Results are recorded in an electronic spreadsheet program database (e.g. an Excel file).

The same reader analyses the otoliths again after three weeks and the 'second opinion' is also recorded in a file as above. After these two sessions, in the presence of contrasting evaluations of age the most frequent evaluations are accepted.

6.1.3 Interpretation

Rings are deposited on otoliths each year, alternating normally one opaque (in summer) and one translucent (in winter). Winter checks are thin and narrow, because the accretion rate varies with the growth of the fish, and during cold months *A. anguilla* metabolism is slow. A year's growth consists of both an opaque and a translucent zone.

Ageing evaluation of *A. anguill*a for the continental phase has traditionally begun from the first clearly marked band outside the nucleus. This 'zero' band (at about a 170 µm radius from the centre) is assumed to be the beginning of the continental growth of *A. anguilla*, and equates to the total length of the glass eel (Moriarty, 1983; Poole, Reynolds and Moriarty, 2004). The next annulus is considered the end of the first year of growth (Plate 197). The conventional birth date is set at 1 January, and, for ageing analysis, the capture date is crucial and must be recorded.

For yellow eel, attention should be drawn to the capture time and local conditions. The appearance of winter annuli on the otolith could vary depending on the starting point and duration of the growing season in the capture location. In the early part of the year, the outermost winter annulus might not be apparent until summer growth begins (in early spring, i.e. 1 April). This will vary by years and by location. Thus an additional year should be added to those eels sampled early in the year after 1 January.

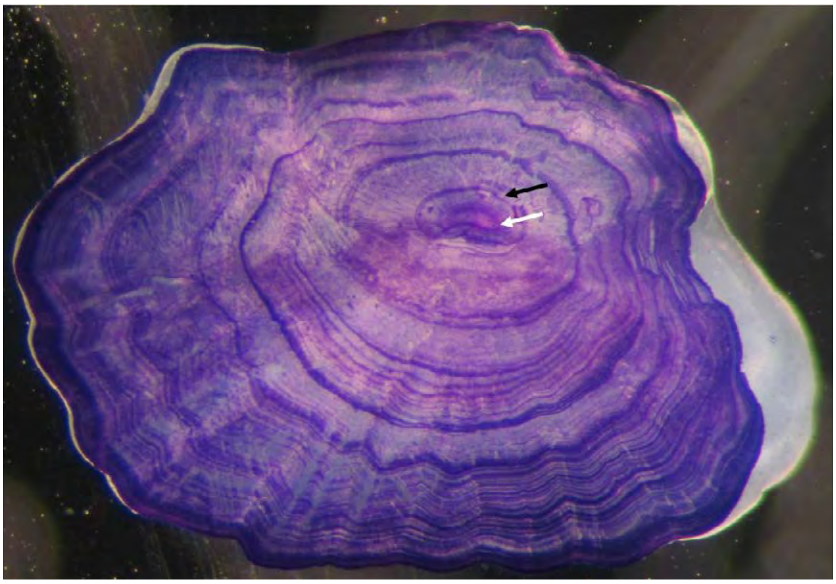
Ageing analysis of silver eel may require flexible interpretation, as their metamorphosis occurs between September and January. They then traditionally migrate to the Sargasso Sea as an annual migration cohort. This cohort receives its age from the following January, as the eels have completed their annual growth period. We assume a putative annulus on the outer margin of the otolith (e.g. silver eels migrating in December 2014 take their age from January 2015) (ICES, 2009b) (Table 21).

The presence of additional checks (false annuli) between two consecutive annual rings (one annual growth period) is also possible. Thus an overestimation of age may occur and, as a result, an underestimation of growth.

High water temperatures and associated low oxygen concentrations in summer or other stress factors in winter can result in the formation of one or more false annuli (Tzeng, Wu and Wickström, 1994; Domingos, Costa and Costa, 2006), as these stressors can produce periods of little or no growth.

The scientific literature (Table 22) and dedicated ICES workshops (Graynoth, 1999; ICES, 2009b, 2011b) have identified several guidelines for discriminating between true and false annuli:

PLATE 197
Ground, polished and stained otolith (sagittal plane) from *A. anguilla*



Source: F. Capoccioni, 2014.

Note: white arrow = nucleus, black arrow = zero band.

TABLE 21 – General ageing scheme for *A. anguilla*

	Date capture	Otolith edge	Age
Yellow eels	1 October – 31 December	Visible opaque ring	N-1
		Thick translucent zone	N
	1 January – 31 March	Visible opaque ring	N
		Thick translucent zone	N+1
Silver eels	30 September – 31 December	Visible opaque ring	N
		Thick translucent zone	N+1

Note: N is the number of opaque rings starting from the first clearly marked band outside the nucleus (0 band).

Table 22 – Criteria for separating winter annuli from summer growth bands and supernumerary checks on *A. anguilla* otoliths

Feature	Summer growth band	Winter annuli	Supernumerary check
Colour	White, often light brown in narrow bands in burnt otoliths, opaque in stained sections viewed using transmitted light	Black or dark brown, dark blue or violet in stained otoliths	Light brown, lighter stain or transpparent in stained otoliths
Width	Much wider than annuli, always >5 µm, usually >15 µm, mean 61 µm, sd 24 µm, *n*=243	Usually 4-18µm, median= 10 µm, *n*=65	Narrow <40% of annuli width, usually <4 µm mu and always < 10 µm
Fine structure	Thin dark lines or slriations occasionally seen	Multiple thin lines visible when stained	Usually one or two thin lines
Continuity	Continuous band	Continuous band	Sometimes broken and not visible on dorsal axis
Position	Uniform spacing	Uniform spacing	Often present adjacent to annulus or nucleus

Source: Graynoth, 1999.

– A clearly visible bold growth check can be considered an annulus.
– In the case of a ground sagittal plane, growth checks should be visible continuously around the otolith to be considered annuli.
– False annuli are usually of lesser strength than annuli, are discontinuous and/or merge with adjacent checks.

- *A. anguilla* growth is highly variable and thus it is difficult to predict 'normal' patterns of annual growth to facilitate identification of false annuli.
- Checks that appear too close to neighbouring checks may be false annuli and should be treated with caution.

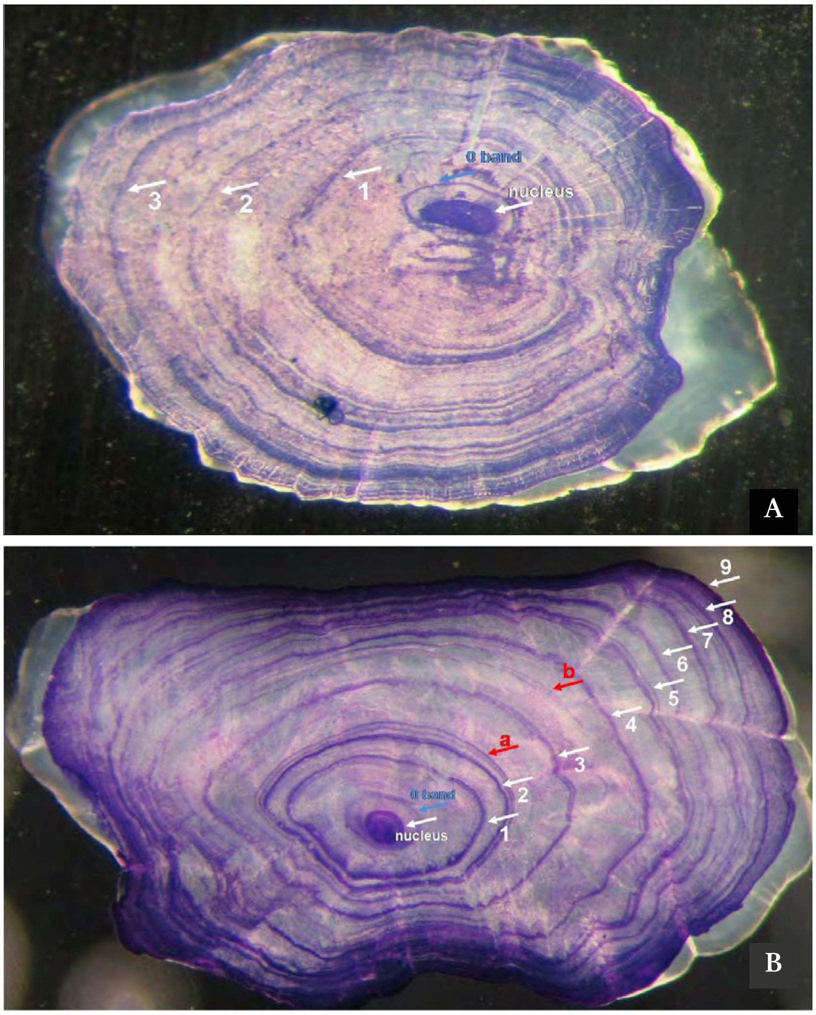
Two examples of aged *A. anguilla* otoliths are shown in Plate 198. In case A, the otolith presents three clear marks. As the specimen has been sampled in winter, the last annulus corresponding to the fourth year is not yet visible, but *A. anguilla* must be correctly aged as an individual of four years old.

In case B, a silver eel, nine years old, presents two typologies of false annulus: the red arrow "a" indicates a false check (false winter check) adjacent to the second-year ring.

The red arrow "b" indicates another kind of false annulus between the third- and fourth-year rings, probably due to a stress factor occurring in summer (false summer check). This event produced the deposition of an additional check on the otolith.

Given the relationship between stress and metabolism and the creation of annual and false check bands within otoliths, it is advisable that readers have additional information on life-cycle and on environmental data on the origin of the specimen in order to assist in correct age interpretation (ICES, 2009b).

PLATE 198
Two examples of aged *A. anguilla* otoliths



Source: F. Capoccioni, 2014.

Note: A – otolith of a 4-year old *A. anguilla* sampled in December; B – silver eel otolith presenting two false checks.

7. Glossary

The morphological description of otoliths is based on the terminology proposed by Secor, Dean and Miller (1995) and Panfili *et al.* (2002) (Plates 199 and 200).

PLATE 199
Views of right sagitta from *T. mediterraneus* with indication of basic structure

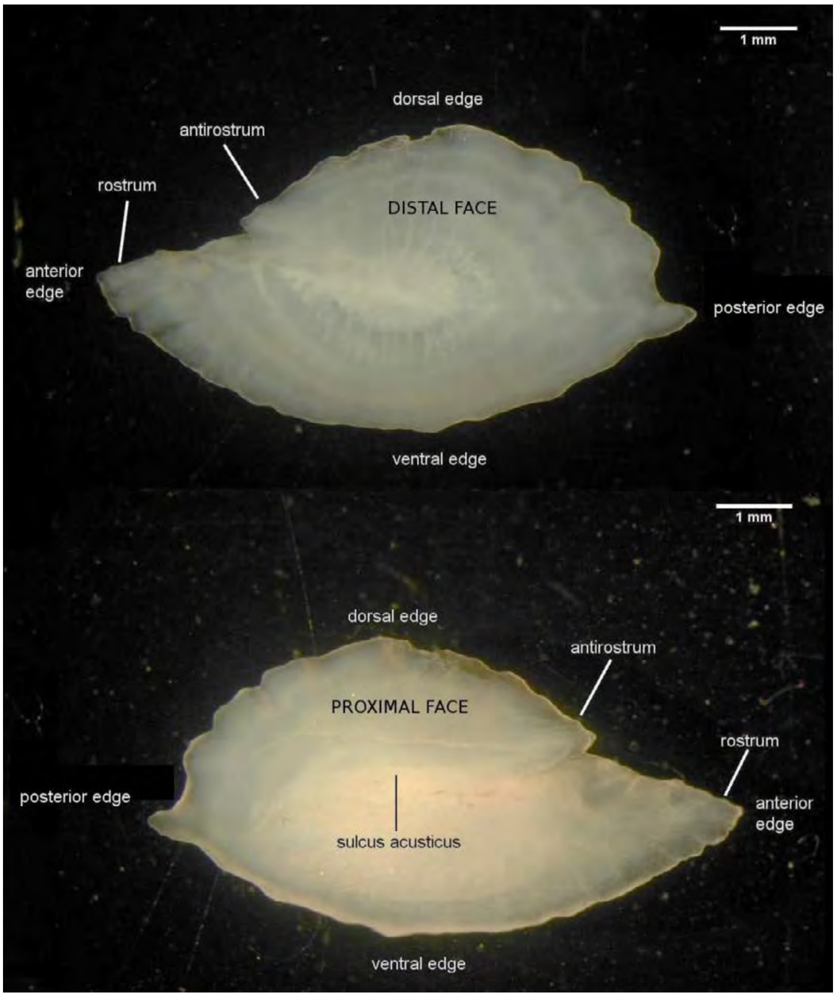


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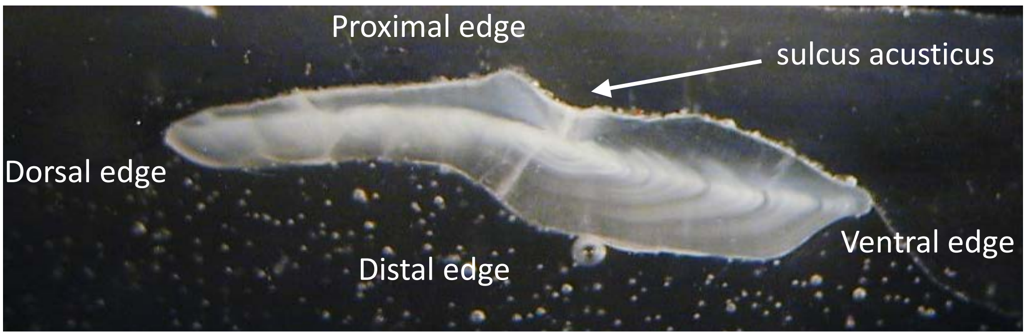
PLATE 200
Transverse thin section through core of *M. merluccius* sagitta with reflected light illumination



© P. Carbonara

Accuracy. The closeness of a quantity estimation (measured or computed value) to its true value.

Age. Age (months, half years, years) is calculated by counting the number of translucent increments, but taking into consideration the date of birth and the date of capture.

Age class. Age class corresponds to number of years. Roman numerals are used for the age class.

Age group. The age group is the number of calendar years after the birth date. The age group to which a fish will be assigned depends on the year in which it was spawned and on the date of capture. Arabic numerals are traditionally used to reflect an age group.

Annulus. One of a series of concentric zones on a calcified structure (CS) that may be interpreted in terms of age. This term usually indicates one transparent ring plus one opaque area/ring. In some cases, an annulus may not be continuous or obviously concentric. The optical appearance of these marks depends on the calcified structure and the species, and should be defined in terms of specific characteristics of the structure. This term has traditionally been used to designate 'year' marks, even though the term is derived from the Latin *anus*, meaning ring, not from *annus*, meaning year. For otoliths, the variations in microstructure that make an annulus a distinctive region are not well understood.

Back-calculation. The back-calculation procedure can be defined as estimating fish size at an earlier time (or times) on the basis of a set of measurements of CS size and fish size, made at a single point in time (usually at capture).

Birth date. The theoretical date when fish hatched; typically, 1 January is used for fish with a spawning period in autumn/winter and 1 July for a spawning period in spring/summer.

Birthmark. A clear variation in the angle of the corpus calcareum. It corresponds to a growth rate acceleration in the individual.

Centra. Central part of vertebral segments.

Check. A discontinuity (e.g. a stress-induced mark) in a pattern of opaque and translucent zones, or microincrements. Checks often appear as an abrupt change in the growth pattern.

Core. The area or areas surrounding one or more primordia and bounded by the first prominent opaque ring.

Corpus calcareum. Load-bearing axis of the vertebral centra. It represents the main structure for counting the annuli.

Corroboration. The measure of the consistency or repeatability of an age determination method.

Double band. A growth mark or check not accepted for annual age determination – also referred to as a growth check or false annulus.

Growth. The change in body or body part size between two points in time.

Hyaline zone. A zone that allows the passage of greater quantities of light than an opaque zone. However, the term hyaline should be avoided; the preferred term is translucent.

Intermedialia. Portion of cartilaginous tissue of the vertebral centra comprised between the arms of the corpus calcareum.

Marginal increment. The region beyond the last identifiable mark at the margin of a structure used for age estimation. Quantitatively, this increment is usually expressed in relative terms, that is, as a fraction or proportion of the last complete annual or daily increment.

Nucleus, kernel. Collective terms originally used to indicate the primordium and core of the otolith. These collective terms are considered ambiguous and should not be used. The preferred terms are primordium and core (see their definitions).

Opaque zone. A zone that restricts the passage of light when compared with a translucent zone. The term is relative, because a zone is determined to be opaque on the basis of the appearance of adjacent zones in the otolith (see 'translucent zone'). In untreated otoliths under transmitted light, the opaque zone appears dark and the translucent zone bright. On the contrary, under reflected light, the opaque zone appears bright and the translucent zone dark.

Precision. The closeness of repeated measurements of the same quantity. For a measurement technique that is free of bias, precision implies accuracy, but the two terms are not equivalent.

Primordium. The initial complex structure of an otolith, it consists of granular or fibrillar material surrounding one or more optically dense nuclei from 0.5 mm to 1.0 mm in diameter. In the early stages of otolith growth, if several primordia are present, they are generally fused together to form the otolith core.

Secondary structure. A term used for all macroscopic zonations that do not appear to conform to the opaque and translucent zones of an annulus. The main examples are false and split or double rings/zones.

Translucent zone. A zone that allows the passage of greater quantities of light than an opaque zone. The term is relative, because a zone is determined to be translucent on the basis of the appearance of adjacent zones in the otolith (see 'opaque zone'). An absolute value for the optical density of such a zone is not implied. In untreated otoliths under transmitted light, the translucent zone appears bright and the opaque zone dark. The term hyaline is also used, but translucent is the preferred term.

Validation. The process of estimating the accuracy of an age estimation method. The concept of validation is one of degree and should not be considered in absolute terms. If the method involves counting zones, then part of the validation process involves confirming the temporal significance of the zones being counted. Validation of an age estimation procedure indicates that the method is sound and based on fact.

Verification. The process of establishing that something is true. Individual age estimates can be verified if a validated age estimation method has been employed. Verification implies the testing of something, such as a hypothesis, that can be determined in absolute terms to be either true or false. See 'corroboration'.

Vertebral focus. The central and innermost part of the vertebral conus.

Zero band. The first growth check of *A. anguilla* outside the nucleus, from where continental age determination begins.

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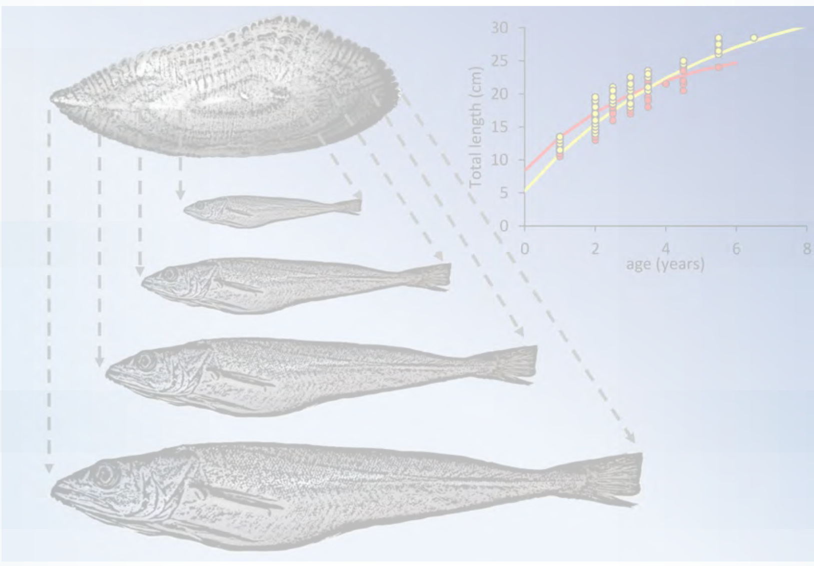
HANDBOOK ON FISH AGE DETERMINATION

This Handbook stems from an experience on fish ageing analysis carried carried out at the Mediterranean level. It aims to provide guidelines to standardize the current methods used in fish ageing studies and it gives an overview of the general principles on which age analysis relies (assignment of birth date, preparation methods, aging scheme reading and identification of true and false rings). The volume provides information on extraction and storage, preparation methods, age interpretation and ageing criteria by species, analysing a total of 30 species. As such, it represents one of the most complete outlooks on fish ageing analysis in the Mediterranean context.

Fish age, among other biological parameters, is one of the most relevant pieces of data in reaching sustainable exploitation of fisheries resources. Indeed, most analytical methods used in stock assessment require knowledge of demographic structure according to the age of stocks, as well as to recruitment, growth, maturity, natural mortality, etc., which are strictly linked to information on age and age structure.

The literature on ageing analysis shows some gaps regarding ageing schemes, criteria and methodologies used in preparing calcified structures. These aspects affect both the precision and accuracy of age estimation. One action that could be taken to overcome this gap was to formalize a handbook that clarified approaches to ageing schemes, criteria and preparation methods. Having a common protocol is indeed fundamental to decreasing bias associated with the activities of age determination and to improving precision in age reading.



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