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.

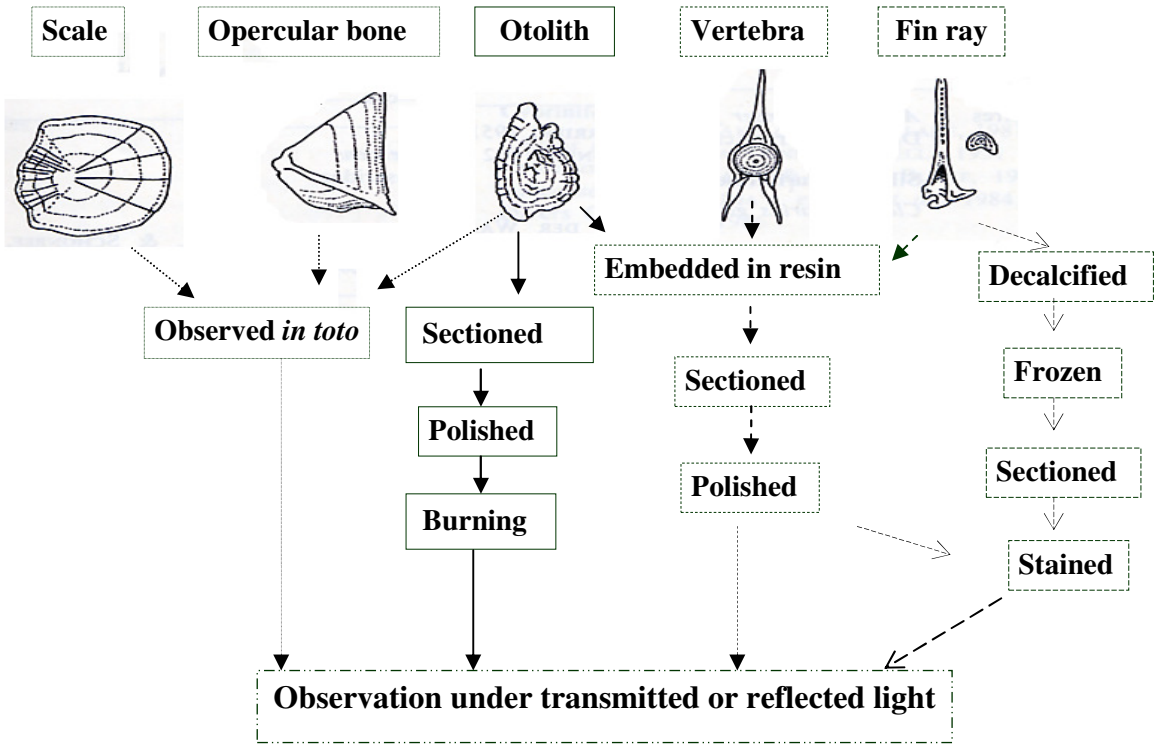

Figure 1. Preparation modes for each hard structure.

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		

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