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).

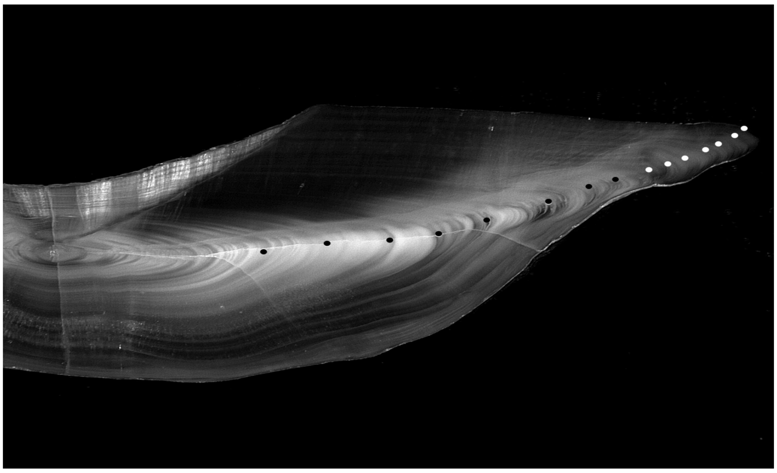

Fig.1. Transverse thin section of otolith of 15 years *Merluccius merluccius* female (880 mm TL). The translucent rings having a yearly meaning are marked.

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).

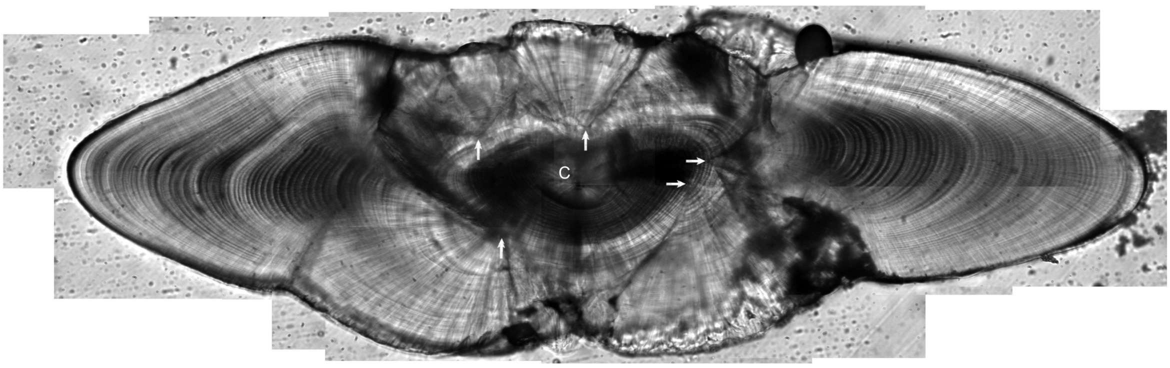

Fig.2. Typical pattern of daily increment in the otolith of young *Merluccius merluccius* (70 mm TL). Arrows point to accessory nuclei.

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).

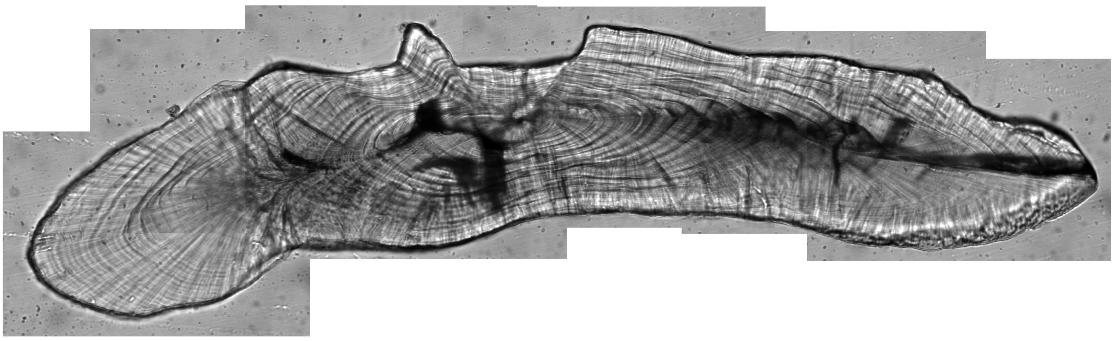

Fig.4. Typical pattern of daily increment in the otolith of young *Mullus barbatus* (67 mm TL). An age of 64 days was estimated.

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

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