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AGE ESTIMATION AND GROWTH OF EUROPEAN HAKE, *MERLUCCIUS MERLUCCIUS* (LINNAEUS, 1758), IN THE NORTHERN TYRRHENIAN SEA

Abstract - Aim of the present study is to estimate the growth rate of the European hake, *Merluccius merluccius* (Linnaeus, 1758) (Osteichthyes; Merlucciidae), in the northern Tyrrhenian Sea (NW Mediterranean), through otolith annual ring analysis. Left *sagittae* of specimens collected by means of trawl surveys, samplings on board and at the auction, were embedded in a synthetic resin, sectioned and analysed under a dissecting microscope connected to an image analysis software. The maximum observed age was 13 years (age class XIV). The von Bertalanffy parameters of males were L_∞ = 53.3 cm, k = 0.224, t_0 = -0.974 years, and those of females were L_∞ = 92.2 cm, k = 0.131, t_0 = -0.615 years. Growth rates of males and females did not differ until age class V; males were very scarce from this age class onwards.

Key-words - *Merluccius merluccius*, age determination, growth, Tyrrhenian Sea.

Riassunto - *Determinazione dell'età e accrescimento del nasello,* Merluccius merluccius *(Linneo, 1758), nel Mar Tirreno Settentrionale*. L'obiettivo del presente lavoro è di stimare la dinamica di accrescimento del nasello, *Merluccius merluccius* (Linneo, 1758) (Osteichthyes; Merlucciidae), nel Mar Tirreno Settentrionale, attraverso l'analisi degli otoliti. Gli otoliti sinistri appartenenti ad esemplari di nasello catturati nel corso di campagne sperimentali di pesca a strascico, o durante campionamenti a bordo di motopescherecci professionali o presso i punti di sbarcato, sono stati inclusi in resina, sezionati ed analizzati utilizzando uno stereo microscopio collegato ad un programma di analisi di immagine.
La massima età osservata nel campione è stata di 13 anni (classe di età XIV). Per i maschi, i parametri della curva di sono risultati L_∞ = 53,3 cm, k = 0,224, t_0 = -0,974 anni; per le femmine L_∞ = 92,2 cm, k = 0,131, t_0 = -0,615 anni. I tassi di accrescimento di maschi e femmine non hanno mostrato differenze significative fino alla classe di età V; da questa classe in poi, esemplari di sesso maschile sono piuttosto rari.

Parole chiave - *Merluccius merluccius*, determinazione dell'età, accrescimento, Mar Tirreno.

Introduction

The European hake, *Merluccius merluccius* (Linnaeus, 1758) (Osteichthyes; Merlucciidae) represents a basic component of the demersal communities and its high market value ranks it among the most commercially important species in the Mediterranean Sea. *M. merluccius* is exploited by multigear fisheries: bottom trawl net mainly affects the juvenile portion of the population, while gillnet and long-line affect the adult fraction. Given its great ecological and economic value, the European hake is one of the most widely studied species. Though many studies have been carried out and the biological parameters of this species are quite well known (Olivar *et al.*, 2003; Alvarez & Cotano, 2005; Palomera *et al.*, 2005), most knowledge is obtained by scientific projects without any continuity in time; therefore, stock assessment and management are rather difficult.
Age determination constitutes an important tool to estimate growth, and to assess population structure, longevity and mortality. Though during last years many scientific efforts have been devoted to study the growth of *M. merluccius* in the Atlantic and in the Mediterranean Sea by means of otolith reading, information on growth rate and longevity of the species is still uncertain.
In spite of the existence of consistent literature coming from other areas of the Mediterranean and the Atlantic, the present study is the first attempt to provide information about growth rate and longevity of the European hake in the northern Tyrrhenian Sea by means of the analysis of otolith annual rings.
An estimation of age and growth rate of European hake adults is instrumental in providing information for adequate management measures. In the northern Tyrrhenian Sea, the adult fraction of *M. merluccius* is exploited by bottom trawl fishery, as well as by a consistent amount of small scale fishery, catching specimens up to 90 cm using gill-nets (Sbrana *et al.*, 2007).

Materials and methods

European hake specimens were collected in the northern Tyrrhenian Sea (Fig. 1), in the period January 1992-October 1993 by means of several approaches: bottom trawl surveys, samplings on board gillnet vessels and monthly samplings at the auction of Porto Santo Stefano (Tuscany, Italy), the main landing point of the area. A total of 1618 otoliths were collected. An amount of 330 otoltihs collected in 2006 was added.
Fish total length (*TL*) and total weight (*W*) were measured to the 0.5 cm below and to the 0.1 g below, respectively; sex was assessed through the macroscopic analysis of the gonads. These data were used to estimate the length at first maturity (L_{50}) in both sexes by fitting a logistic curve, according the following model:

$$\% \; Matures = 1 / (1 - e^{(-r \cdot (L - L_{50}))})$$

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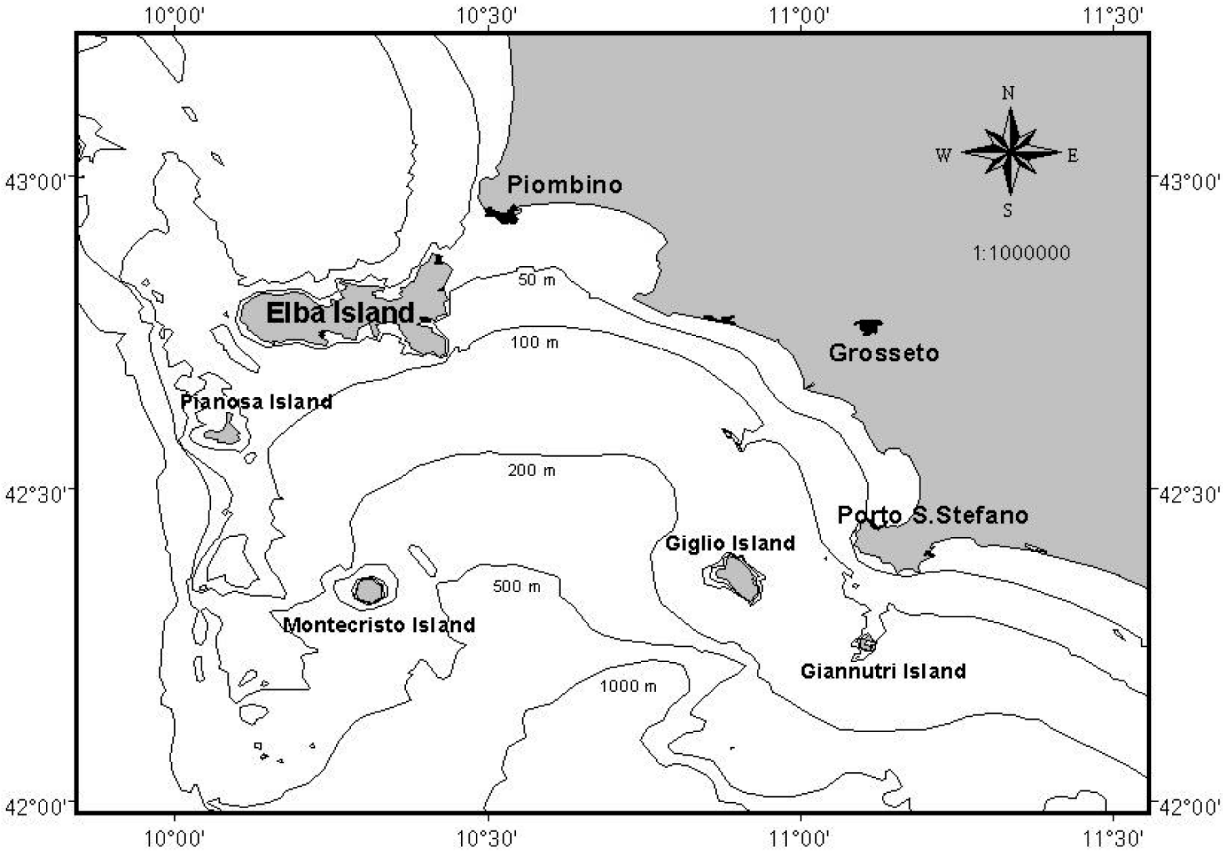

Fig. 1 - Northern Tyrrhenian Sea.

where, r is the slope of the curve, and L_{50} is the length at first maturity.

Otoliths (*sagittae*) were removed from a sub-sample of specimens, cleaned and stored dry in vials. To standardize procedures and analyses, only left otoliths were used.

Otoliths were embedded using a synthetic resin in histological silicon-rubber moulds and stored in a thermostatic stove (42°C) for 15-20 minutes. The resin blocks were cut along the dorso-ventral plane of the otolith using an automatic high-speed cutting machine (MICROMET) provided with two diamond disks in order to obtain thin transversal sections (0.8 mm) including the centre of the otolith. The sections were ground using wet sandpaper (800 μm) and polished using alumina slurry (0.1 μm). Otoliths were regularly observed under the dissection microscope to check surface quality. Sections were read out using a dissection microscope under reflected light; the microscope was connected to an image analysis software (Optimas 6.2) through a video camera. Age determination was carried out counting annual rings (*annuli*) from the centre of the otolith to the ventral edge. Counts of winter rings that appeared as dark narrow bands (translucent), preferably on the ventral region, were used to estimate ages. Age readings were converted in ages (months) using the formula $Age_{months} = 12 \cdot N + (rank_C - rank_B)$, where N is the number of winter rings, $rank_C$ is the number corresponding to the date (month) of capture, and $rank_B$ is the number corresponding to the date of birth (which is considered the 1st January). Ages in years were computed from ages expressed in months. Age-length key (ALK) was obtained for males, females and sexes combined; mean length at each age class was calculated. The Kruskall-Wallis test was applied to compare growth between males and females in the same age range. The growth of *M. merluccius* was estimated computing the von Bertalanffy growth formula (VBGF) $L_t = L_\infty \cdot (1 - e^{(-k \cdot (t-to))})$, where L_t is the mean total length at age t, L_∞ is the asymptotic length, k is the instantaneous growth coefficient, t is age (years), t_o is the age at length zero. The VBGF parameters (L_∞, k and t_o) were calculated from age-length data using the package FiSAT II. In order to compare L_∞ and k with those estimated by different authors, the phi-prime ($\Phi' = \log k + 2 \log L_\infty$) was computed.

For each section, the radius of the otolith (measured along the dorso-ventral axis, from the *core* to the ven-

tral edge), the distance from the *core* to the first well marked check or «false ring» around the nucleus, and the radius of each annual ring were measured using a digitalized measuring tool. The fish size at each year was estimated using the non-linear back-calculation formula (BCF) $L_i = (S_i / S_c)^v \cdot L_c$, where L_i is the fish total length at age i, S_i is the radius at age i, L_c is the fish total length at capture, S_c is the radius at capture, v is the slope of the linear regression of fish total length on otolith radius ($L = d + v \cdot S$). The back-calculation of fish size was used as an indirect validation method.

Results

A total of 8875 European hake specimens were collected in the framework of the sampling activities carried out in the northern Tyrrhenian Sea. The curves fitted to sex and maturity data are summarized in Figure 2. A length at first maturity of about 36 cm TL was estimated for females, while the males reach the gonad maturity at a length of about 22 cm TL.
A total of 1918 otolith sections were used for age determination. The mean length at age for females, males and sexes combined are summarized in Table 1. The maximum age observed was 13 years (age class XIV) for a female of 91.0 cm; for males, the maximum age observed was 6 years (Age class VII), corresponding to a specimen of 51.0 cm *TL*. The Kruskall-Wallis test was carried out on the specimens until age class V: males were not consistently represented from age class VI onwards. The test did not outline any significant difference in growth between males and females. The von Bertalanffy parameters of males, females and sexes combined were summarized in Table 2. The growth curve is shown in Figure 3. According to the fitted curve, males reached a mean total length at age 1 of about 19.0 cm. The phi-prime value (Φ') computed from the VBGF parameters (k and L_∞) fitted to the total sample (F + M + I) was 3.03. A similar value (3.04) was computed from female data, while a value of 2.80 was obtained from male data. Table 3 summarizes the results of the present study and the estimations obtained by previous studies.
The mean radius of the otolith resulted equal to 2.24 (± 0.79 SE) mm. Table 4 summarizes the mean radius of each annual ring estimated from otolith analyses and the mean total length at each year computed from the back-calculation formula.

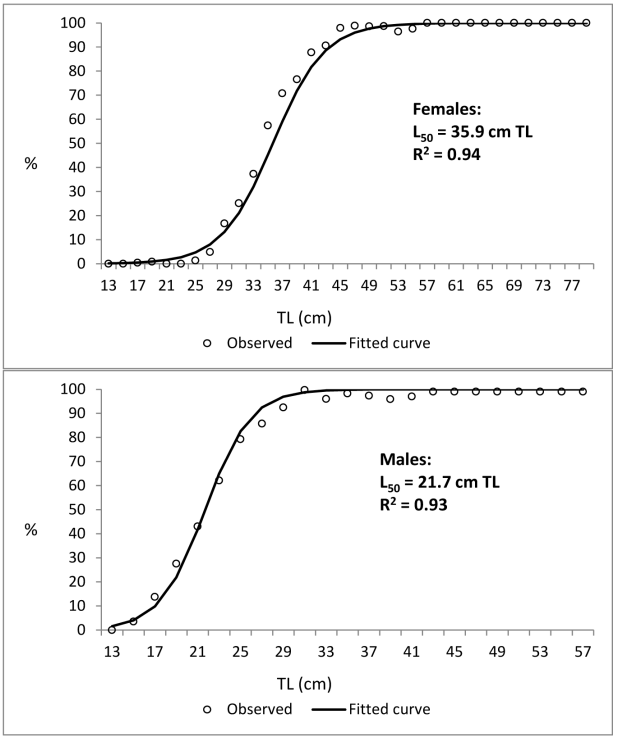

Fig. 2 - Maturity curve computed for European hake females and males.

Discussion

Studies on age determination based on the reading of calcified structures should be supported by validation aimed at check age estimate accuracy. Direct methods to validate age assessment in the European hake are still in progress (de Pontual *et al.*, 2003; 2006; Morales-Nin *et al.*, 2005; Piñeiro *et al.*, 2007), while indirect validation methods, based on the length-frequency analysis, are difficult to use due to the long spawning period of the species (Morales-Nin & Aldebert, 1997).
The results of the present study were validated by means of the size-at-age back-calculation. The ageing methods and criteria adopted allow the interpretation of otoliths of specimens up to 50 cm of *TL*; over this size the readout procedure is impractical due to the high incidence of false rings, which are related to biological or seasonal environmental changes (Piñeiro & Saínza, 2003), and to the ring width decrease (Lombarte *et al.*, 2003; Piñeiro & Saínza, 2003). Further difficulties lie in the identification of the first annual ring: the growth of *M. merluccius* is characterized by the formation of several false rings during the first year of life (Morales-Nin & Aldebert, 1997; Morales-Nin, 2000; Lombarte *et al.*, 2003). The application of the ageing criteria presented here demonstrates that otolith sections can be used for a reliable estimate of hake's age. Therefore, otolith reading techniques, standardized to international level and supported by validation methods, could constitute an accurate tool to improve the knowledge of those aspects still largely debated among fishery biologists.
The oldest fish found in this study was 13 year old; this observation supports the idea that *M. merluccius* is a long-living species (Morales-Nin *et al.*, 1998). The comparison between males and females conducted for specimens up to age class V do not show significant differences in growth rate, in agreement with observation from other authors (Piñeiro & Saínza, 2003).
From 30.0 cm *TL* onwards, the relative number of females rapidly increases to reach 100% in fish larger than 50.0 cm *TL*. A similar pattern had already been

Tab. 1 - Mean total length at age (*TL*; cm); S.E. = standard error; *n* = number of specimens; F = females; M = males; I = undetermined specimens.

	F			M			F + M + I		
Age class	***TL***	**S.E.**	***n***	***TL***	**S.E.**	***n***	***TL***	**S.E.**	***n***
I	17.1	0.2	167	17.4	0.2	223	15.7	0.1	567
II	22.8	0.3	277	22.6	0.3	301	22.6	0.2	594
III	29.1	0.4	174	28.7	0.4	162	28.9	0.3	342
IV	35.7	0.5	86	34.9	0.6	53	35.5	0.4	146
V	45.5	1.0	73	38.4	1.8	12	43.8	0.9	85
VI	52.4	0.9	60	33.0	-	1	52.4	0.9	61
VII	58.9	0.8	57	51.0	-	1	58.8	0.7	59
VIII	63.2	1.0	37	-	-	-	63.4	0.9	37
IX	69.0	1.4	18	-	-	-	69.5	1.4	18
X	74.8	2.7	5	-	-	-	74.8	2.7	5
XI	90.0	-	1	-	-	-	90.0	-	1
XII	79.0	-	1	-	-	-	79.0	-	1
XIII	80.0	-	1	-	-	-	80.0	-	1
XIV	91.0	-	1	-	-	-	91.0	-	1
Total			**958**			**753**			**1918**

Tab. 2 - Von Bertalanffy parameters for males (M), females (F) and sexes combined (M + F + I).

	n	L_∞	**S.E.** (L_∞)	***k***	**S.E.** (***k***)	t_0	**S.E.** (t_0)	R^2
F	958	92.2	2.1	0.131	0.011	-0.615	0.065	0.925
M	753	53.3	6.3	0.224	0.054	-0.974	0.214	0.807
F + M + I	1918	90.7	2.9	0.131	0.007	-0.645	0.049	0.923

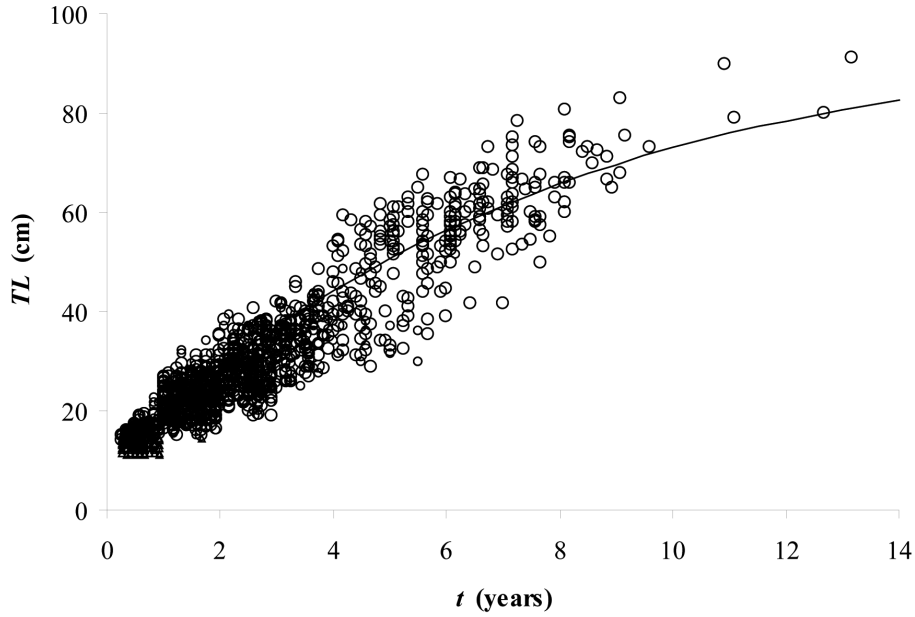

Fig. 3 - Von Bertalanffy fitted growth curve (sexes combined).

Tab. 3 - Parameters of von Bertalanffy growth curve equation (k and L_∞) and phi-prime values (Φ') obtained by different authors.

Author	Sex	Method	Area	k	L_∞	Φ'
Uçkun *et al.*, 2000	sexes combined	otolith reading	Aegean Sea	0.08	81.70	2.73
Garçía-Rodríguez and Esteban, 2002	males females sexes combined	otolith reading	Gulf of Alicante	0.20 0.21 0.21	93.00 108.00 108.00	3.24 3.42 3.39
Colloca *et al.*, 2003	males females	otolith reading	Central Tyrrhenian Sea	0.40 0.13	45.70 93.20	2.92 3.05
Piñeiro and Saínza, 2003	males females sexes combined	otolith reading	Iberian Atlantic waters	0.18 0.13 0.13	70.00 88.70 88.00	2.95 3.01 3.00
de Pontual *et al.*, 2006	sexes combined	tagging	Bay of Biscay	0.36	89.90	3.46
Present study	males females sexes combined	otolith reading	northern Tyrrhenian Sea	0.22 0.13 0.13	53.30 92.20 90.70	2.80 3.04 3.03

Tab. 4 - Total mean lengths back-calculated; n = number of observations; S = otolith mean radius (mm); ΔS = increment in otolith radius; L_i = mean total length (cm); ΔL = increment in fish total length; S.E. = standard error.

Annual ring	n	S_i	S.E. (S)	ΔS	L_i	S.E. (TL)	ΔL
1	402	1.4	0.0	1.4	17.6	0.2	17.6
2	332	2.1	0.0	0.7	26.5	0.2	8.9
3	250	2.6	0.1	0.5	34.4	0.4	7.9
4	166	3.0	0.1	0.4	41.3	0.5	6.9
5	120	3.4	0.1	0.4	47.4	0.5	6.1
6	78	3.7	0.1	0.3	52.7	0.7	5.3
7	42	4.0	0.1	0.3	57.4	1.0	4.7
8	15	4.3	0.2	0.3	61.4	1.3	4.0

observed in studies conducted in the Mediterranean Sea (Uçkun *et al.*, 2000; Colloca *et al.*, 2003). Differences in age composition between males and females were observed in the central Tyrrhenian Sea (Colloca *et al.*, 2003). The dominance of females beyond a particular size could suggest that the natural mortality rate of adult males is higher than that of females; this situation, however, can be related to other circumstances, such as the different behaviour and consequently the different depth distribution of the two sexes that determine the different exposure to fishing gear (Colloca *et al.*, 2003; Piñeiro & Saínza, 2003). Also the different sexual maturity patterns shown by males and females could be at the basis of this phenomenon. Males reach the sexual maturity before females, and some biological aspects, such as growth and longevity, can be influenced by the energy demand requested by the sexual processes (Piñeiro & Saínza, 2003). Sexual inversion and partial or successive hermaphroditism have never been observed in this species (Murua & Motos, 2006; Recasens *et al.*, 2008; El Habouz *et al.*, 2011).

As outlined by the Φ' comparisons, the VBGF parameters estimated in the present study fall within the range of the estimates obtained from other areas. As far as t_0 is concerned, the negative values (-0.974 years for males, -0.615 years for females) obtained in the present study are similar to those estimated by Piñeiro & Saínza (2003) in the Atlantic. Negative values of t_0 are associated with rapid growth dynamics, in particular during the first year of life. As a matter of fact, the present study describes a fast growth, with a fish size at the end of the first year of life of about 18 cm *TL*.

The findings obtained from the northern Tyrrhenian Sea, together with the results of recent studies (Morales-Nin & Moranta, 2004; Belcari *et al.*, 2006), strongly support the hypothesis that growth dynamics of *M. merluccius* in the Mediterranean Sea are faster than those previously estimated, and are close to those reported from the Atlantic (Piñeiro & Saínza, 2003; Kacher & Amara, 2005). These findings should be taken into account for the planning of future management strategies of this resource in the Mediterranean basin (Bertignac & de Pontual, 2007).

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