Growth estimation of *Merluccius merluccius* off the northern coast of Tunisia

by

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Abstract. – Growth parameters of any species are fundamental for biological or dynamic studies. The quality of the estimation for these parameters directly impacts on species stock assessment and, as a consequence, on fisheries management. In order to improve fishery and species stock management, growth analysis, especially by sex, is recommended. In this study, otoliths were used to estimate growth parameters of *Merluccius merluccius* Rafinesque, 1810. Hake is well-known as being a species in which otolith interpretation is extremely difficult. To estimate growth parameters, the first daily increment was validated as the tool for age determination. Otolith interpretation was done according to the last results of ICES cooperative research report. In total, a sample of 1599 *M. merluccius* were examined, consisting in 638 females, 771 males and 190 immature juveniles. They were caught in southwest of Mediterranean Sea, along the northern coast of Tunisia. Transverse sections were used for the interpretation of growth (annual) increments and age estimation. Growth by sex was considered: $TL = 102.850 * (1 – e^{–0.141 * (t + 1.345)})$ for females, $TL = 40.700 * (1 – e^{–0.619 * (t + 0.992)})$ for males and for combined sexes. Validation of the first growth annual increment and knowledge from recent tagging studies have helped in highlighting a different growth model of *M. merluccius* from that previously described. Our study showed that ages previously reported were overestimated, as observed in other regions (Gulf of Lion and Bay of Biscay). Moreover, at defined length and weight, females grow at a faster rate than males. This difference was explained by the difference in performance index (Φ), which is 3.17 for females and 3.01 for males.

Résumé. – Détermination de la croissance du *Merluccius merluccius* sur la côte nord tunisienne.

Les paramètres de croissance d’une espèce sont fondamentaux pour son étude biologique ou dynamique. La qualité de l’estimation de ces paramètres a une répercussion directe sur la précision des estimations du stock et, par conséquent, sur la gestion des pêcheries. Dans le but d’améliorer la gestion des stocks, il est recommandé d’étudier la croissance par sexe. Les otolithes ont été utilisés dans le présent travail pour estimer ces paramètres et ceux du merlu, *Merluccius merluccius* Rafinesque, 1810, sont connus pour être particulièrement difficiles à interpréter. De ce fait, le premier anneau de croissance a été préalablement validé à partir de l’analyse des accroissements journaliers des juvéniles. De plus, l’interprétation a tenu compte des derniers résultats du rapport du CIEM coopération de recherche. Au total, 1599 individus, capturés sur la côte nord de la Tunisie (sud-ouest du bassin méditerranéen) ont été examinés, représentant 638 femelles, 771 mâles et 190 immatures. Des coupes transversales ont été utilisées pour l’interprétation des otolithes et l’estimation d’âge du merlu. La croissance par sexe a été estimée : $TL = 102,850 (1 – e^{–0,141 (t + 1,345)})$ pour les femelles, $TL = 40,7 (1 – e^{–0,619 (t + 0,992)})$ pour les mâles et pour les deux sexes combinés. La validation du premier anneau de croissance et les connaissances issues des études récentes de marquage ont permis de mettre en évidence un modèle de croissance très différent de celui qui avait été décrit antérieurement. Ce travail montre que l’âge observé précédemment a été surestimé comme c’est le cas dans d’autres zones géographiques (golfes du Lion et de Gascogne). De plus, pour une taille et un poids défini, les femelles croissent plus vite que les mâles. Cette différence peut s’expliquer par la différence de l’indice de performance (Φ), estimé à 3,17 pour les femelles et 3,01 pour les mâles.

Merluccius merluccius Rafinesque, 1810, also known as Mediterranean hake, is a species of economic interest and has created large amounts of literature (Casey and Periero, 1995). However, there is still little knowledge about its biology and, in particular, on its age and growth. Growth parameters of any species are fundamental for biological and dynamic studies. Accurate estimation of these parameters directly impacts stock assessment and consequently fisheries management (Bertignac and de Pontual, 2007).

Age estimation of *M. merluccius* constitutes a major problem due to difficult otolith interpretation (Aldebert, 1981; Aldebert and Carries, 1988; Orsi Relini *et al*., 1989; Oliver, 1991; Aldebert and Morales-Nin, 1992; Recasens, 1992; Morales-Nin and Aldebert, 1997; Morales-Nin *et al*., 1998; Morales-Nin and Moranta, 2004; Courbin *et al*., 2007; Ferraton, 2007). Two main reasons could explain this dif-

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ficulty: continuous recruitment of juveniles due to multiple spawning throughout the year (Sarano, 1986; Orsi-Relini *et al.,* 1989); and difficult identification of the first winter annulus due to variable ring patterns related to hatching season (Aldebert and Morales-Nin, 1992). Despite tagging experiments (de Pontual *et al*., 2006; Piñeiro *et al*., 2008; Mellon-Duval *et al*., 2010), age estimation of hake still remains doubtful, and the only result from these experiments confirms that ageing of hake in Atlantic and Mediterranean Sea is overestimated.

In addition to tagging studies, estimation of daily age has been validated. In fact, otolith microstructure provides a useful tool for age determination of juvenile hake on the basis of daily increment deposition. Growth studies of juveniles were carried out in the north (Kacher and Amara, 2005; Otxotorena *et al*., 2010) and south of the Atlantic (Piñeiro *et al*., 2008) and in the north (Morales-Nin and Aldebert, 1997; Morales-Nin *et al*., 1998; Arneri and Morales Nin, 2000; Morales-Nin and Moranta, 2004; Palomera *et al*., 2005; Belcari *et al*., 2006) and south of the Mediterranean sea (Khoufi *et al*., 2012a).

Mediterranean hake is widely distributed throughout Atlantic Ocean and Mediterranean Sea (Pla *et al*., 1991; Roldán *et al*., 1999; Castillo *et al*., 2003; Cimmaruta *et al*., 2005). It is one of the most commercially exploited demersal fish in these areas (Aldebert and Carries, 1989; Martin, 1991; Oliver and Massutí, 1995). In Tunisia, hake is captured along the entire coastline and is a target species for both trawling and artisanal fisheries. 90% of the total removals are done by commercial fisheries (Khoufi *et al*., 2010).

Accurate information lacks regarding age and growth of *M. merluccius*, despite the fundamental roles of these factors in stock assessment. Neither direct or indirect validation of growth estimation was carried out in Tunisia prior the recent study by Khoufi and colleagues (2012a). Growth parameters for this species were brought by Bouhlal (1975) on the basis of annual interpretation of growth rings in the otoliths. In fact, it is necessary to acquire more recent biological data to support hake management in the Tunisian water.

MATERIALS AND METHODS

Sampling

Between January and February 2010, *M. merluccius* were collected in the Northern Tunisian waters, in 53 stations (depths ranging from 50 to 600 m) during a fishery survey of R.V. *Hannibal* from the Fisheries Institute Kelibia (Fig. 1). Additional samples were collected between March and May 2010 during the bottom trawl survey of R.V. *Hannibal* from the National Institute of Science and Technology of the Sea (INSTM) and finally between November 2010 and March

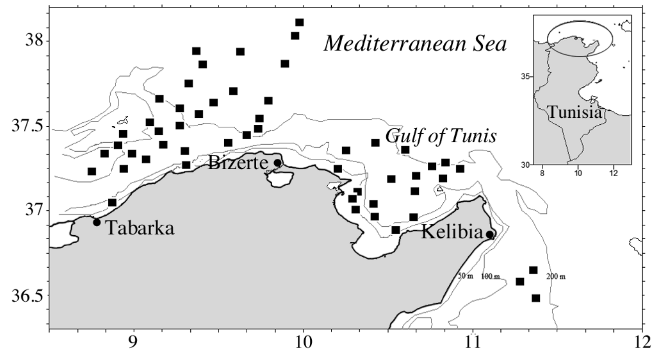

Figure 1. - Location (■) of main sampling areas.

2011 on two commercial benthic trawlers, F.V. *Jaziret Kerkenah* and F.V. *Mostakbel*.

Otolith preparation

The right sagittae were chosen for routine age estimation. A total of 1599 otoliths were cleaned thoroughly using water and stored dry before preparation for sectioning. The method used for sectioning was previously described in an international workshop (Guichet, 1996; Morales-Nin and Aldebert, 1997). Otoliths were embedded in translucent polyester resin, and thin sections of 0.4 mm were obtained using a high-speed sectioning machine. The sectioned otoliths were viewed under transmitted light using a binocular microscope linked to a video camera connected to a computer. Oil was used to clarify growth structures. Each sample was analysed using TNPC (Numeric treatment of calcified pieces, FEI Company, France).

Age estimation

The age estimation of *M. merluccius* is more difficult than for many species because checks or false rings are present, and each growth annulus is characterised by bands of several thinner translucent rings (ICES, 2010). In addition, first annulus identification also created a bias in age interpretation. It is why we performed age validation study by observing daily increment deposition in order to identify the first growth annulus (Khoufi *et al*., 2012a). In fact, age interpretation was done on this result and followed recommendations of ICES group (ICES, 2010) (Fig. 2).

Growth estimation

For all individuals, total length (TL, cm), total weight (W, g) and sex (male, female and undetermined) were recorded. Length-weight relationship was calculated by sex by applying the exponential regression equation $W = a\ TL^b$, where a is the constant and b the allometric coefficient (Ricker, 1975). ANCOVA analysis was applied to test significant difference in length-weight relationship between sexes. Sex ratio was obtained by dividing the number of females by the

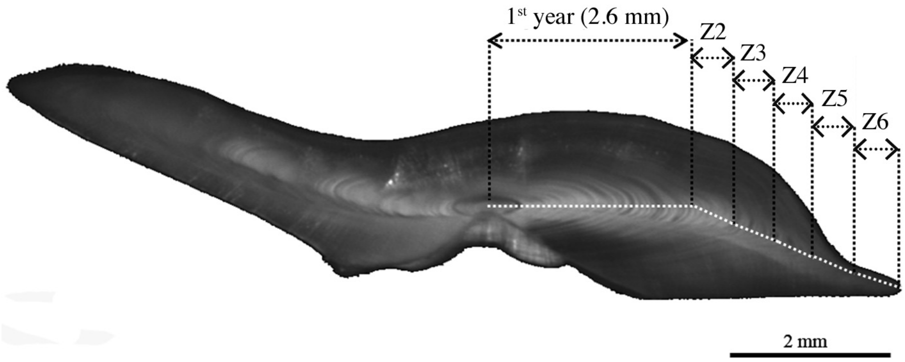

Figure 2. - Age determination of *Merluccius merluccius* from otolith section.

total number of individuals.

To describe *M. merluccius* growth, age and total length were calculated using the von Bertalanffy model (1938) according to the formula:

Length growth: $TLt = TL_\infty (1 - e^{-K (t - t_0)})$,

Weight growth: $Wt = W_\infty (1 - e^{-K (t - t_0)})^3$,

where TLt and Wt are the length and the weight at age t, TL_∞ is the asymptotic length (cm), k is the growth coefficient (year^{-1}), and t_0 is the theoretical age at zero length (year). Growth curves were compared between sexes using ANOVA.

Fish growth was estimated using the growth performance index (φ') (Pauly and Munro, 1984): $\varphi' = \log K + 2 \log TL_\infty$.

For growth comparison, growth performance index was preferred to comparison of TL_∞ and K individually, these two parameters being correlated.

RESULTS

A total of 1599 *M. merluccius* were collected, among which 638 were females, 771 males and 190 were undetermined. Length ranged between 12 and 93.5 cm (24.98 ± 12.58 cm) for females, between 12 and 37 cm (21.60 ± 7.29 cm) for males, and between 8.5 and 21.5 cm for unsexed individuals (14.19 ± 3.78 cm) (Fig. 3). Females were larger than males. The sex ratio evolution by length showed an increasing trend reaching 1:1 ratio to 23 cm. Sampling was primarily made up of females larger than 40 cm (Fig. 3).

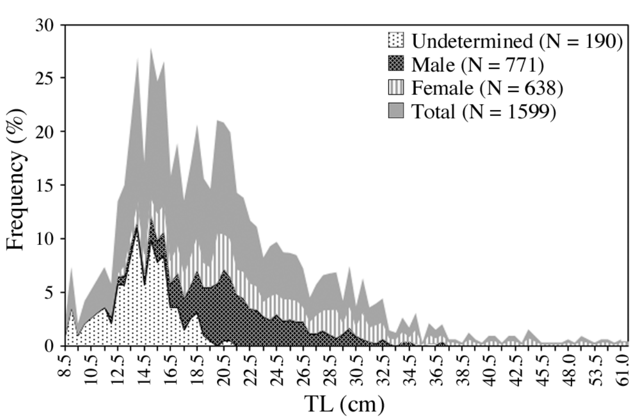

Figure 3. - Length distribution of sample by sex.

Table I. - Length-weight relationship of hake of Tunisian coast by sex and for combined sex. r^2: determination coefficient; N: Individual number.

Sex	Length-weight relationship	r^2	N	P-value
Male	$Wt = 0.004*LT^{3.072}$	0.910	771	0.000
Female	$Wt = 0.003*LT^{3.134}$	0.989	638	0.000
Male + female	$Wt = 0.003*LT^{3.115}$	0.989	1409	0.000

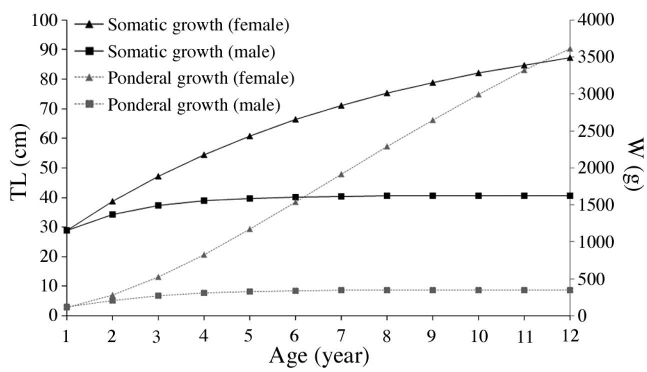

Figure 4. - Ponderal and somatic growth for hake for each sex off the northern coast in Tunisia.

Length-weight relationships by sex and by all individuals showed a positive allometric growth, regardless to sex (Tab. I). The allometric coefficient of regression was significantly higher in females than males (ANCOVA, $p < 0.01$).

Length at age groups and Von Bertalanffy growth parameters for males and females are shown in figure 4 and table II. Females grew to a heavier asymptotic weight (W_∞) and greater length (TL_∞) than males (Tab. II). The Von Bertalanffy growth model for combined sex was calculated by using the data from the complete samples of 1599 individuals:

$$TL(t) = 102{,}850\,(1 - e^{-0.185\,(t + 0.786)})$$

Table II. - Growth model in length and weight of hake off Tunisian coast by sex.

Sex	Growth model in length	Growth model in weight
Male	$TL = 40.700\ (1 - e^{-0.619(t + 0.992)})$	$W = 352.155\ (1 - e^{-0.619(t + 0.992)})^{3.072}$
Female	$TL = 102.850\ (1 - e^{-0.141(t + 1.345)})$	$W = 6072.528\ (1 - e^{-0.141(t + 1.345)})^{3.134}$

Table III. - Parameters of Von Bertalanffy growth (L_∞ and K) and φ' values obtained by various authors.

Authors	Area of study	Sex	L_∞ (cm)	K(year^{-1})	φ' (cm/year)
Present study	North of Tunisia	Male	40.70	0.619	3.01
		Female	102.85	0.141	3.17
Bouhlal (1975)	Gulf of Tunis	Male	59.30	0.190	2.82
		Female	73.00	0.156	2.92
Bouaziz *et al*. (1998)	Algerian coast	Male	100.70	0.124	3.10
		Female	80.64	0.130	2.93
Colloca *et al*. (2003)	Central Mediterranean Sea (Italy)	Male	45.70	0.400	2.92
		Female	93.20	0.130	3.05
Mellon-Duval *et al*. (2010)	Gulf of Lion	Male	72.80	0.239	3.10
		Female	110.70	0.236	3.46
Lucio *et al*. (2000)	Bay of Biscay	Male	80.00	0.181	3.06
		Female	110.00	0.122	3.17
De Pontual *et al*. (2006)	Bay of Biscay	Male	80.00	0.436	3.45
		Female	110.00	0.261	3.50
El Habouz *et al*. (2011)	Eastern Central Atlantic	Male	101.90	0.113	3.07
		Female	114.80	0.129	3.23

Significant differences in the growth parameters were found between sexes (Student's t-test, $p < 0.05$). Based on growth performance index (φ'), females showed higher growth rates than males (Tab. III).

DISCUSSION

Our results showed that length-weight relationship differed significantly between sexes in *M. merluccius* off the Tunisian coast, similar to other studies in the same area (Bouhlal, 1975; Cherif *et al*., 2008; Khoufi *et al*., 2012b) (Tab. IV). Moreover, all studies carried out in the Mediterranean Sea (Campillo, 1992; Arneri and Morales-Nin, 2000; Moutopoulos and Stergiou, 2000; Giacalone *et al*., 2010) and in the Atlantic Ocean (Dorel, 1986; Piñeiro and Sainza, 2003) highlighted that length-weight relationship of *M. merluccius* showed a positive allometric growth, regardless of sex (Tab. IV).

The sex ratio showed that males were only present up to 37 cm TL. Although this length varied between study areas, similar results were reported for hake populations both in the Mediterranean (40 cm, Recasens *et al*., 1998) and in the Atlantic (60 cm, Piñeiro and Saínza, 2003; 45 cm, El Habouz *et al*., 2011; 50 cm, Costa, 2013). This was explained by the difference in growth rate between the two sexes, natural mortality and fishing mortality (El Habouz *et al*., 2011; Piñeiro, 2011).

Use of ageing criteria, established at the hake workshop (ICES, 2010) and off the Northern Tunisian coast, reflected the difference in growth before and after the first year (Fig. 5). For a same age, total length of hake is larger than estimated by Bouhlal (1975) in the Gulf of Tunis. The asymptotic length was underestimated by Bouhlal (1975) because of the lack of old individuals, the largest individual sampled was 56 cm but in our study, the largest hake measured 93.5 cm. Similarly, the total length at one year calculated from age validation in our study (28 cm) was different from the mean length (17 cm) calculated by Bouhlal (1975). Growth index calculated (K = 0.185) was also higher than the value obtained by Bouhlal (1975; K = 0.176). Thus, during fish life, the growth difference between these two studies increased (Fig. 5).

In our study, the values of Von Bertalanffy parameters were compared with those estimated in the Bay of Biscay (de Pontual *et al*., 2006) and in the Gulf of Lions (Mellon-Duval *et al*., 2010), which were the most recent tagging studies. Our study shows that the asymptotic length (L_∞) is higher than for the two other studies. The growth rate (K) of hake in North Atlantic (de Pontual *et al.,* 2006) is higher than in the Mediterranean Sea (Mellon-Duval *et al*, 2010; present study). However, in South Mediterranean Sea (northern Tunisia),

Table IV. - Summary of studies on length-weight relationship realized for hake in the Mediterranean Sea and in the Atlantic.

Citation	Area of study	Sex	LWR
Present study	Tunisian offshore	Females	$W = 0.004 * TL^{3.144}$
		Males	$W = 0.0005 * TL^{3.076}$
Khoufi *et al*. (2012b)	Tunisian North	Females	$W = 0.003 * TL^{3.134}$
		Males	$W = 0.004 * TL^{3.072}$
Chérif *et al*. (2008)	Tunisian Gulf	Females	$W = 0.005 * TL^{3.110}$
		Males	$W = 0.004 * TL^{3.170}$
Bouhlal (1975)	Tunisian Gulf	Females	$W = 0.004 * TL^{3.200}$
		Males	$W = 0.003 * TL^{3.202}$
Giacalone *et al*. (2010)	Gulf of Castellamare (Sicily)	Combined sex	$W = 0.006 * TL^{3.050}$
Arneri and Morales-Nin (2000)	Central Adriatic (Italy)	Combined sex	$W = 0.0004 * TL^{3.072}$
Moutopoulos and Stergiou (2000)	Cyclades (Greece)	Combined sex	$W = 0.036 * TL^{3.200}$
Campillo (1992)	Gulf of Lion (France)	Combined sex	$W = 0.007 * TL^{3.029}$
Piñeiro and Sainza (2003)	Iberian waters (South of Atlantic)	Combined sex	$W = 0.007 * TL^{2.981}$
Dorel (1986)	Bay of Biscay (North of Atlantic)	Combined sex	$W = 0.005 * TL^{3.074}$

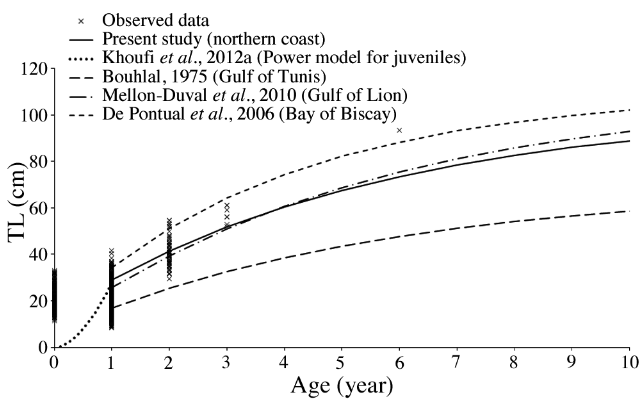

Figure 5. - Von Bertalanffy model for hake combined sex off the northern coast in Tunisia fitted to the data, compared to those observed in the Bay of Biscay and in the Gulf of Lion.

the value of K was close to the value of North Mediterranean Sea (Gulf of Lion) (Fig. 5). To explain growth differences of *M. merluccius* between Atlantic Ocean and Mediterranean Sea, Mellon-Duval *et al*. (2010) proposed three hypotheses: first, genetic factors, confirming the existence of two distinct populations of hake (Cimmaruta *et al*., 2005); second, biotic factors, such as food availability, played an important role in growth and can explain such difference because of the greater productivity of Atlantic (Laborde *et al*., 1999) compared to Mediterranean waters (Lefevre *et al*., 1997; Béthoux *et al*., 1998); third, environmental factors (temperature, salinity, depth, up-welling,…) were the most important. In the Mediterranean Sea, Von Bertalanffy growth model was different (Fig. 5) from the south (present study) and the north (Mellon-Duval *et al*., 2010).

The comparison of Von Bertalanffy parameters between sexes of the studies for different regions from the Atlantic Ocean and the Mediterranean Sea confirmed that growth depends on sex (Tab. III). The performance index of females is higher than that of males, which confirms that the growth rate for female is higher than for males (Tab. III).

In *M. merluccius,* otoliths are difficult to interpret but the validation of first growth increments and knowledge resulting from recent tagging studies have improved age estimation. The result Von Bertalanffy growth model was different from that previously described by Bouhlal (1975) for the Tunisian coasts. Our study confirmed that age estimation was underestimated and supported the hypothesis of the fast-growth of *M. merluccius*, as it is the case in other geographical areas in the Atlantic and Mediterranean Sea.

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