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

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

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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.

Keywords: Merlucciidae ; *Merluccius merluccius* ; Tunisia ; Western Mediterranean ; Age estimation ; Growth model ; Otolith

Résumé :

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.

INTRODUCTION

Merluccius merluccius smiridus (Rafinesque, 1810) also known as Mediterranean hake is an important species of economic interest and has created large amounts of literature (Casey and Periero, 1995), but there is very little knowledge abouts its' biology and in particular on its' age and growth estimation. Growth parameters of any species are fundamental for biological and dynamic studies. The quality of the estimation of these parameters directly impacts stock assessment and consequently the management of fisheries (Bertignac and de Pontual, 2007).

For this species, estimation of age constitutes a major problem because of the difficulty in 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). This is explained by two main reasons. Firstly, the continuous recruitment of juveniles is throughout the year due to multiple spawning (Sarano, 1986; Orsi-Relini *et al.*, 1989). Secondly, the identification of the first winter annulus is problematic due to variable ring patterns related to hatching season (Aldebert and Morales-Nin, 1992). Despite tagging experiments (de Pontual *et al.*, 2006; Pineiro *et al.*, 2008; Mellon-Duval *et al.*, 2010), hake age estimation still remains unclear. The only result from these experiments is the confirmation that ageing of hake in Atlantic and Mediterranean Sea is over estimated.

In addition to the 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 on juveniles have been carried out in the north (Kacher and Amara, 2005; Otxotorena *et al.*, 2010) and south of the Atlantic (Pineiro *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 a species widely distributed through the Atlantic Ocean and the 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 Carriès, 1989*;* Martin, 1991*;* Oliver and Massutí, 1995). In Tunisia, hake is captured along the entire coastline (Khoufi *et al*., 2010). It is a target species for both trawling and artisanal fisheries. 90% of the total removals are done by commercial fisheries (Khoufi *et al*., 2010).

There is a lack of accurate information regarding age and growth, despite the fundamental roles of these factors in stock assessment, no direct or indirect validation of growth estimation has been carried out in Tunisia before the study of Khoufi *et al*. (2012a). The last growth parameters for this species were carried out by Bouhlal (1975) on the basis of annual interpretation of the growth rings of hake otoliths. In fact, the need to acquire new biological data to support hake management in the seas around Tunisian water is necessary.

MATERIALS AND METHODS

Sampling

Samples of hake were collected in the Northern Tunisian waters, in depths ranging from 50 to 600 metres. A total of 53 stations were fished (Fig. 1). Samples were collected between January and February 2010, on a fisheries survey on the Research Vessel Hannibal from the fisheries institute Kelibia. Additional samples between March and May 2010 during the bottom trawl survey on board the R.V. Hannibal from the National Institute of Science and Technology of the Sea (INSTM) and finally between November 2010 and march 2011 using two commercial benthic trawlers the F.V. Jaziret Kerkenah and F.V. Mostakbel.

Otolith preparation

The right *sagittae* were chosen for routine age estimation. Otoliths were removed from the fish and were cleaned thoroughly using water. All of the 1599 calcified pieces were stored dry before preparation for sectioning.

The technical method for sectioning used was the same that was described in an international workshop (Guichet, 1996; Morales-Nin and Aldebert, 1997). Otoliths were embedded in translucent polyester resin, and then 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, which was linked to a video camera connected to a computer. Oil was used to clarify of the growth structures. Each sample was then analysed using software TNPC (Numeric treatment of calcified pieces, FEI Company, France).

Age estimation

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

Growth estimation

For all samples, total length (TL, cm), total weight (W, g) and sex (male, female and undetermined) were recorded for all individuals. The length-weight relationship of fish were calculated by sex applying the exponential regression equation $W = a\, TL^b$

Where a is the constant and b is the allometric coefficient (Ricker, 1975). The ANCOVA analysis was applied to test significant difference for the length-weight relationship between sexes. Sex Ratio was obtained by dividing the number of females by the total number of all individuals.

Age and total length data were used to describe hake growth 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 ($year^{-1}$), t_0 is the theoretical age at zero length (year).Growth curves were compared between sexes using ANOVA.

The fish growth was estimated using the growth performance index (φ') (Pauly and Munro, 1984):

$\varphi' = \log K + 2 \log TL_{\infty}$

Growth performance index was preferred for growth comparison rather than comparison of TL_{∞} and K individually, because of the correlation between these two parameters.

RESULTS

1599 hake were collected in total. Of these, 638 were females, 771 males and 190 were unsexed. The length of females ranged between 12 cm and 93.5 cm (24.98±12.58 cm) and between 12 cm and 37 cm (21.60±7.29 cm) for males and between 8.5 cm 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. The Hake sampled was primarily made up of females greater than 40 cm (Fig. 3).

The length-weight relationships by sex and by all individuals are shown in Table I. The length–weight relationship showed a positive allometric growth, regardless to sex. The allometric coefficient of the regression in females was significantly higher than in males (ANCOVA, $P < 0.01$).

The length at age groups and the Von Bertalanffy growth parameters for males, females are shown in Fig. 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:

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

Significant differences in the growth parameters were found between sexes (Student's t-test, $P < 0.05$). Based on the growth performance index (φ'), females showed higher growth rates than males (Tab. 3).

DISCUSSION

Our results showed that length-weight relationship differed significantly between sexes of hake 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; Morales-Nin and Arneri, 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 hake showed a positive allometric growth, regardless of sex (Tab. IV).

The sex ratio showed that males were only present up to the TL of 37cm. Although this length varied between study areas, similar results have been highlighted 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 has been explained because of the difference in growth rate between the two sexes, natural mortality and fishing mortality (El Habouz *et al*., 2011; Piñeiro, 2011).

The use of the ageing criteria established at the hake workshop (ICES, 2010) and the off the Northern Tunisian coast and 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 older individuals, the largest individual sampled was 56 cm but in our study, the largest hake measuring 93.5 cm. Similarly, the total length of one year old from age validation study was very different from the mean length calculated by Bouhlal (1975), which were respectively 28 cm and 17 cm. The growth index calculated (K = 0.185) is higher than the value obtained by Bouhlal (1975; K = 0.176). Thus, during the life of the fish, the growth difference between these two studies increased (Fig. 5).

The values of Von Bertalanffy parameters of our study were compared with those estimated for the Bay of Biscay (de Pontual *et al*., 2006) and for the Gulf of Lions (Mellon-Duval *et al*., 2010) which were most the recent tagging studies. Our study shows the asymptotic length (L_∞) is higher than for the others two studies. The growth rate (K) of hake in the North Atlantic (de Pontual *et al.,* 2006) is higher than that of hake in the Mediterranean Sea (Mellon-Duval *et al*, 2010; present study). However, the value of K in the south of the Mediterranean Sea (northern Tunisia) was close to the value of the north of the Mediterranean Sea (Gulf of Lion) (Fig. 5). To explain the growth differences between the Atlantic and Mediterranean Sea, Mellon-Duval *et al*., 2010 presented several hypotheses. First the genetic factors, confirming the existence of two distinct populations of hake (Cimmaruta *et al*., 2005), second the biotic factors, such as food availability, played an important role in the growth and can explain such difference because of the greater productivity of Atlantic (Laborde *et al*., 1999) compared to that of Mediterranean waters (Lefevre *et al*., 1997; Béthoux *et al*., 1998).

Thirdly, the environmental factors (temperature, salinity, depth, up-welling ...) were the most important. In the Mediterranean Sea, the Von Bertalanffy growth model was different (Fig. 5) from the south (present study) and the north (Mellon-Duval *et al*., 2010).

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

Otoliths of *Merluccius merluccius* are very difficult to interpret but the validation of the first growth increment and knowledge resulted from recent tagging studies has improved the age estimation This has resulted in a Von Bertalanffy growth model very different from that previously described by Bouhlal (1975) for the Tunisian coasts. This work confirmed that age estimation was underestimated and supported the hypothesis of the fast-growth, as is the case in other geographical areas in the Atlantic and Mediterranean Sea for hake.

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Tables

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

Table II -Growth model in length and in weight of hake of 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 phi values (Φ) obtained by different 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
Habouz *et al*. (2011)	Eastern central Atlantic	Male	101.90	0.113	3.07
		Female	114.80	0.129	3.23

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	$W = 0.005 * TL^{3.076}$
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 Castellammare (Sicily)	Combined sex	$W = 0.006 * TL^{3.050}$
Ameri and Morales Nin (2000)	Central Adriatic (Italy)	Combined sex	$W = 0.0004 * TL^{3.072}$
Moutopoulos and Stergiou (2000)	Kyclades (Greece)	Combined sex	$W = 0.036 * TL^{3.200}$
Campillo (1992)	Golfe du 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	$W = 0.005 * TL^{3.074}$

Figures

Figure 1. - Location of main collection areas of samples.

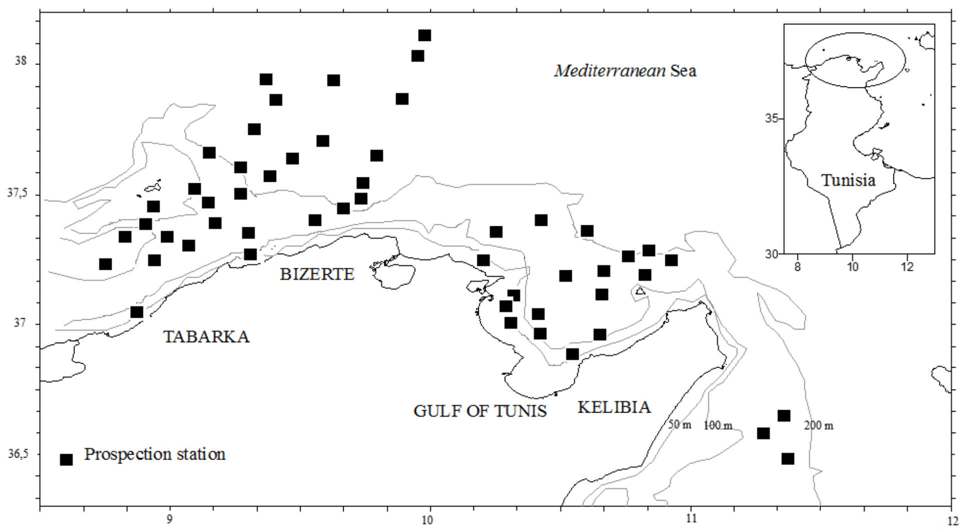

Figure 2. - Age determination from a section of *Merluccius merluccius* otolith.

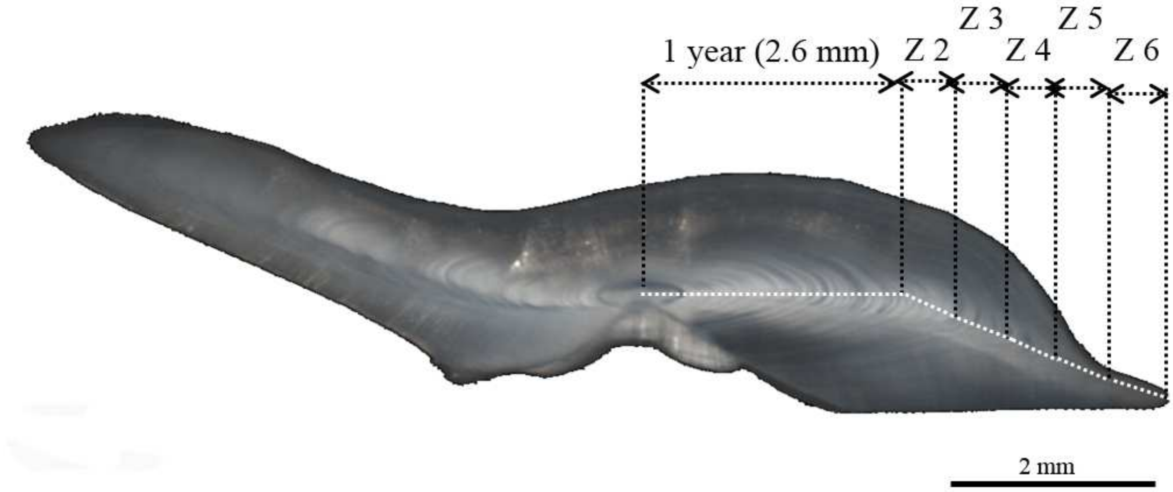

Figure 3. - Length distribution of sample by sex.

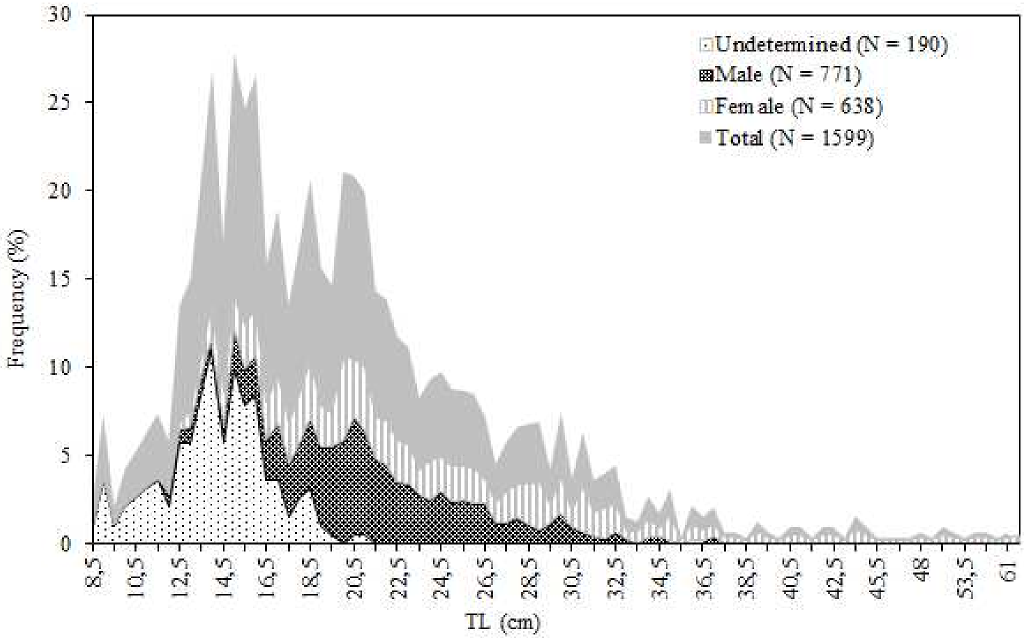

Figure 4. - Ponderal and somatic growth for hake for each sex off the northern coast in Tunisia.

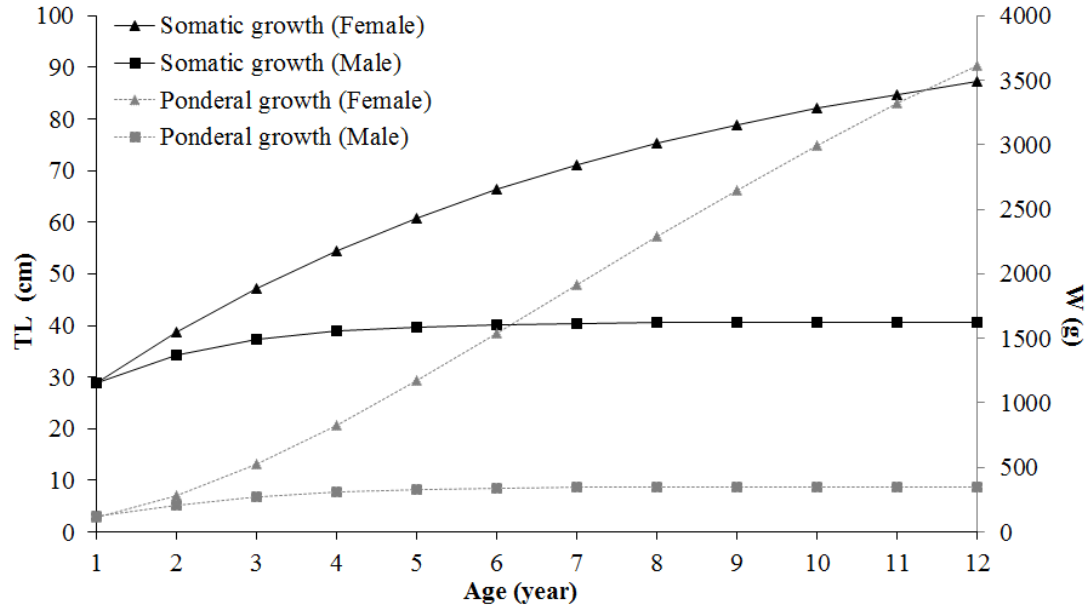

Figure 5. - Von Bertalanffy model for hake combined sex off the northern coast in Tunisia fitted to the data, compared to those observed in the Bay of Biscay and in the gulf of Lion.