

## Age estimation of the Atlantic bonito in the eastern Mediterranean Sea using dorsal spines and validation of the method

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**SUMMARY:** Growth parameters were studied in 397 specimens of the Atlantic bonito caught in the eastern Mediterranean Sea (Aegean and Ionian Seas) ranging from 7.2 to 72.5 cm in fork length and from 2 to 5400 g in total weight. The study of the growth bands in spine sections showed that one translucent ring is formed annually during the cold season. The growth parameters estimated ( $L_{\infty} = 82.99$  cm,  $K = 0.24$ ,  $t_0 = -0.77$ ) express the growth of the species more realistically than previous studies in the Mediterranean Sea and the Atlantic Ocean.

**Keywords:** *Sarda sarda*, age and growth, validation, marginal increment analysis, Mediterranean.

**RESUMEN:** ESTIMACIÓN DE LA EDAD DEL BONITO DEL ATLÁNTICO EN EL MEDITERRÁNEO ESTE USANDO LAS ESPINAS DORSALES, Y VALIDACIÓN DEL MÉTODO. – Se estudiaron los parámetros de crecimiento en 397 especímenes de bonito del Atlántico capturados en el Mediterráneo Este (mares Egeo y Jónico) entre 7.2 y 72.5 cm de longitud total y de 2 a 5400 g de peso total. El estudio de las bandas de crecimiento en secciones de espinas mostró que anualmente, durante la estación fría, se forma un anillo translúcido. Los parámetros de crecimiento estimados ( $L_{\infty} = 82.99$  cm,  $K = 0.24$ ,  $t_0 = -0.77$ ) expresan el crecimiento de la especie de manera más realista que en previos estudios en el Mediterráneo y el Atlántico.

**Palabras clave:** *Sarda sarda*, edad y crecimiento, validación, análisis de incremento marginal, Mediterráneo.

### INTRODUCTION

The Atlantic bonito, *Sarda sarda* (Bloch, 1793), is an epipelagic, schooling scombrid found throughout tropical and temperate waters of the Atlantic Ocean, including the Mediterranean and the Black Sea (Collette and Chao, 1975). Despite the fact that the Atlantic bonito is one of the most important commercial species of scombrids exploited in the Mediterranean Sea, relatively little is known about its population biology and stock structure.

Several authors have estimated the age of the Atlantic bonito in different geographical areas using

both length frequency analysis and counts of rings in various hard parts. Rodríguez-Roda (1966) estimated the age of the Atlantic bonito on the Atlantic coast of Spain using both length frequency analysis and counts of rings in the vertebrae. Hansen (1989) studied growth in the Atlantic bonito from Argentina using only length frequency analysis. Rey *et al.* (1986) used vertebrae, spines and scales to estimate age and growth of Atlantic bonito from the western Mediterranean and the Atlantic side of Gibraltar, while Santamaria *et al.* (1998) used spines and vertebrae in the NW Ionian, in the eastern Mediterranean. In the Black Sea and the Sea of

Marmara several studies were performed using length frequency analysis (Zusser, 1954; Numann, 1955; Nikolskii, 1957; Tkacheva, 1958; Turgan, 1958; Mayorova and Tkacheva, 1959; Demir, 1963) and only Kutaygil (1967) used otoliths. None of these authors has validated the annual formation of translucent zones in the hard parts studied.

The growth rate of fish is an essential component of models used in stock assessment of fish populations and small variations in growth rates can have a significant impact on the outcomes of the population analysis. As Campana (2001) stated, there are many instances in which ageing error has contributed to the serious overexploitation of a population or species (Smith *et al.*, 1995; Chilton and Beamish, 1982; Campana *et al.*, 1990). Many authors suggest the use of validation methods (Chilton and Beamish, 1982; Beamish and McFarlane, 1983; Campana *et al.*, 1990; Smith *et al.*, 1995) such as the determination of the age of first increment formation and the verification of the increment periodicity across the entire range of interest. The main goals of the present study were to estimate age and growth using dorsal fin spines and to validate the use of spines by marginal increment analysis.

## MATERIALS AND METHODS

### Sampling scheme

Atlantic bonito ( $n=397$ ) of all size categories were obtained from the Greek commercial fishing fleets operating in the east Aegean Sea, west Aegean Sea, Patraikos Gulf and Korinthiakos Gulf (Fig. 1). These specimens were caught using either purse seines with a mesh size in the cod end of 40 mm (full mesh) targeting small tuna species during the day or purse seines with a mesh size in the cod end of 16 mm (full mesh) targeting small pelagic species during the night. Few individuals were caught from other artisanal fisheries using long lines and drifting nets. Sampling was carried out during the period 1997-2003, throughout the year.

For each fish, the first spiniform ray of the first dorsal fin was collected, and fork length ( $L_F$ ), total weight ( $W$ ), sex when possible, as well as date and place of capture were recorded. Sex was determined macroscopically by visual inspection of gonads according to Nikolsky (1963) and Rey *et al.* (1984).

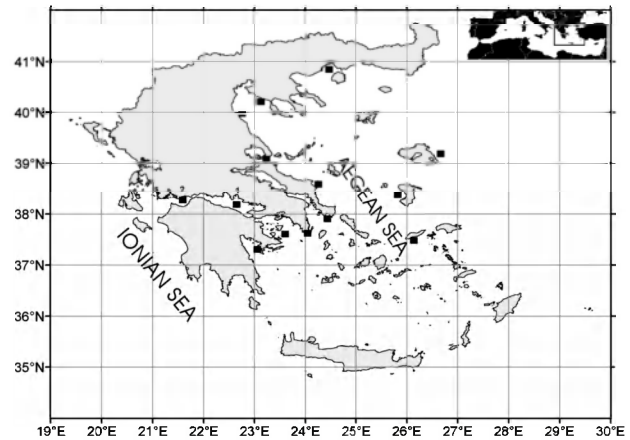


FIG. 1. — Sampling area for the Atlantic bonito, *Sarda sarda*, in the eastern Mediterranean Sea (1 = Korinthiakos Gulf, 2 = Patraikos Gulf).

### Age estimation

The spines were fixed in Epoxini R 800 synthetic resin. Three cross-sections of *ca.* 0.7 mm thick were obtained from each spine at the point near the condyle (González-Garcés and Fariña-Perez, 1983; Megalofonou, 2000) using a low-speed saw and diamond wafering blades. The sections were mounted with a synthetic resin on glass slides and viewed under transmitted light using a Zeiss, Axiolab microscope equipped with an Image Analysis Pro Plus 3.1 image analyser. Growth bands observed under the above optical conditions were distinguished. Translucent zones, which were assumed to be indicative of slow growth, were separated by opaque zones, which were assumed to represent fast growth. Translucent bands were continuous around the perimeter of the spine section (Megalofonou, 2000), but in many specimens were more visible at the rear or at the edge of the section. The total number of translucent bands in spine sections was recorded in order to assign an estimated age to each bonito and build a size-age key. One reader made two readings of each spine independently. When there was disagreement between counts of translucent bands, spines were read for a third time. If agreement could not be reached, those spines were not included in the analysis. The correct identification of the first and second annuli in older fish is the common problem encountered when using spines. In many species the spine nucleus may be reabsorbed and replaced by a hole (vascularisation), which may eliminate the first rings (Casselman, 1983; Kohli, 1989; Mc Farlane and King, 2001). If the first annulus is not identified correctly, fish age

will be underestimated leading to an overestimation of the growth and natural mortality coefficients with drastic implications in fish stock management (Leaman and Nagtegaal, 1987; Casey and Natanson, 1992). Therefore, we followed the suggestion of Beamish (1981) and Chilton and Beamish (1982) to use the mean diameters of the first and the second annuli in spine sections of young fish to localise their position in older fish. If annuli near the centre of the spine were not visible, due to loss of material by vascularisation, the vascularisation area was measured. Linear regression analysis was performed to obtain the equation between fork length and radius of the first dorsal spine (R).

### Validation

The approach to validating the ageing method was based on marginal increment analysis. Observations on the type and frequency of growth zones occurring on the outside margin of each spine per month were made (Cayre and Diouf, 1981; Megalofonou and De Metrio, 2000). Moreover, marginal increment ratio (MIR) analysis was performed to determine the time of band formation using the following equation (Natanson *et al.*, 1995):

$$\text{MIR} = (\text{SD} - D_n) / (D_n - D_{n-1})$$

where SD is the spine diameter,  $D_n$  is the diameter to the last complete band and  $D_{n-1}$  is the diameter to the penultimate complete band. The mean MIR ( $\pm$  S.D.) was plotted monthly in order to locate periodic trends in band formation. An analysis of variance (ANOVA) was used to detect significant differences throughout the course of the year and Tukey tests were performed to demonstrate which months differed from the others. The edge-characterisation analysis was used to determine the period of growth-band formation (Newman and Dunk, 2003). The frequency of translucent zones (MIR = 0) and opaque zones (MIR > 0.4) was plotted monthly. In order to examine the possibility of a link between water temperature and annuli formation we used the data of Vlahakis and Pollatou (1993) from 21 sites in the Aegean and calculated the mean monthly temperature.

### Growth

Student's *t* test was performed to compare the mean length at age between female and male boni-

tos. The VONBER program (Sparre, 1987) was used, allowing parameters to be estimated without transforming the data into linear form. The Von Bertalanffy growth function (VBGF) (von Bertalanffy, 1938) was fitted to data:

$$L_t = L_{\infty} (1 - \exp[-K(t-t_0)])$$

where  $L_t$  = the predicted length at age *t*,  $L_{\infty}$  = the mean asymptotic fork length, *K* = the growth constant, and  $t_0$  = the theoretical age at which the fish would have been zero length. The quantity  $\Phi = \ln k + 2 \ln L_{\infty}$  was computed (Sparre *et al.*, 1989) to compare Atlantic bonito parameters to various studies.

## RESULTS

### Age estimation and growth

Fork length and mass were recorded for 397 bonitos ranging from 7.2 to 72.5 cm  $L_F$  and 2 to 5400 g mass (Fig. 2). Sex was determined in 240 specimens. 157 specimens were immature, 118 males and 122 females. The smallest specimens identified as male and female were 29 cm in  $L_F$ . There was a strong linear correlation between the radius of the first spine of the first dorsal fin and fork length (Fig. 3). Age estimations from spine sections were obtained from a total of 386 bonitos. Only 11 spines that represent 2.85% of the sample were considered unreadable and rejected from the analysis. Spine sections were eliminated because the bands were too broad and diffuse to determine their number and location accurately. Mean rings diameters are presented in Table 1. In the 92 cases where the diameter of the vascularisation area was higher

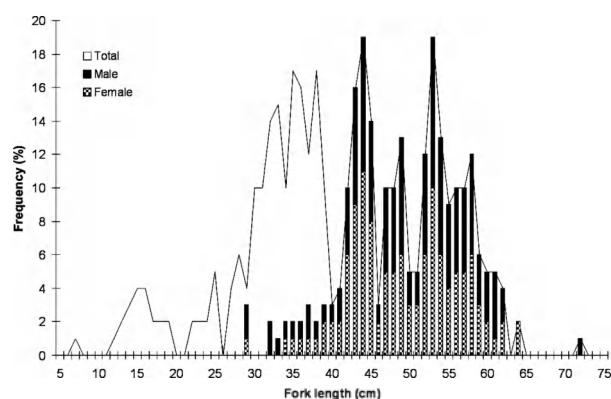


FIG. 2. – Length ( $L_F$ ) frequency distribution of the Atlantic bonito, *Sarda sarda*, sampled in the eastern Mediterranean Sea.

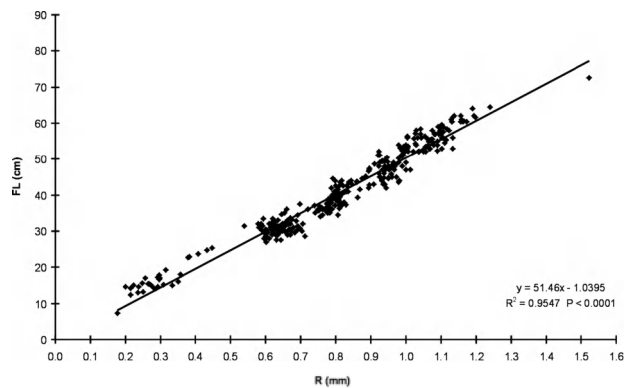


FIG. 3. – Length ( $L_F$ ) regressed on the radius of the first dorsal spine ( $R$ ) in the Atlantic bonito, *Sarda sarda*, in the eastern Mediterranean Sea ( $N = 397$ ).

TABLE 1. – Number of observations ( $N$ ), mean, standard deviation (s.d.), maximum (max) and minimum (min) diameter of translucent rings on the first spine of the first dorsal fin of the Atlantic bonito, *Sarda sarda*, from the eastern Mediterranean.

Ring	mean (mm)	max (mm)	min (mm)	s.d. (mm)	N
1	1.2905	1.4069	1.1131	0.0723	211
2	1.6154	1.7350	1.5030	0.0408	214
3	1.8567	1.7510	1.9570	0.0367	134
4	2.0616	1.9730	2.2130	0.0640	71
5	2.3136	2.2420	2.3830	0.0427	10
6	2.4406	2.4406	2.4406	0.0000	1
7	2.8532	2.8532	2.8532	0.0000	1

than the mean diameter of the first annulus, an additional annulus was counted. In the 5 cases where the diameter of the vascularisation area was higher than the mean diameter of the second annulus, two additional annuli were counted.

The smallest estimated age was 0+ and the highest 7+. Age groups 2 and 3 were the dominant ones in the sample (Table 2). The length-age key of all individuals by age class and length class is presented in Table 2. No significant differences were found in the mean lengths at estimated ages between male and female bonitos (Mann – Whitney, d.f. = 239,  $P > 0.05$ ). The von Bertalanffy growth model was fitted to mean lengths at estimated ages. The von Bertalanffy equation for the theoretical growth in length for both sexes is presented in Figure 4.

## Validation

MIR was calculated in 307 specimens after exclusion of 90 juveniles that had no translucent zones in their spines sections. MIR was lowest in March and highest in October (Fig. 5). ANOVA detected significant differences during the year

TABLE 2. – Age-length key of 386 Atlantic bonitos, *Sarda sarda*, from the eastern Mediterranean;  $N$  total number.

$L_F$ (cm)	Age class								N
	0	I	II	III	IV	V	VI	VII	
7-9	1								1
9-11									
11-13	1								1
13-15	5								5
15-17	8								8
17-19	4								4
19-21	2								2
21-23	2								2
23-25	4								4
25-27	5								5
27-29	10								10
29-31	14								14
31-33	24								24
33-35	24	2							26
35-37	16	9							25
37-39		36							36
39-41		19							19
41-43		27							27
43-45		5	10						15
45-47			9						9
47-49			20						20
49-51			17	1					18
51-53			11	6					17
53-55			14	21					35
55-57				9					9
57-59				23					23
59-61				8	3				11
61-63				9	4				13
63-65					2				2
65-67									
67-69									
69-71									
71-73								1	1
Sum	120	98	81	77	9			1	386
Mean	29.2	38.9	49.2	57.2	62.1			72.5	40.4
s.d.	5.9	2.3	3.3	2.8	1.5			0.0	12.1
%	31.1	25.4	21.0	19.9	2.3	0.0	0.0	0.3	100

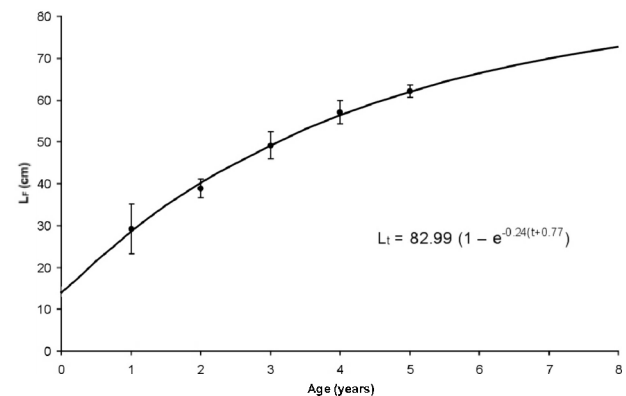


FIG. 4. – Von Bertalanffy growth curve for the Atlantic bonito, *Sarda sarda*, from the eastern Mediterranean Sea. Black dots indicates mean observed length and bars indicate standard deviation.

(d.f. = 306  $P < 0.05$ ). Tukey's test showed that the MIR in January, February, March, April and May was significantly lower than in July, August, September and October. Furthermore, monthly cate-

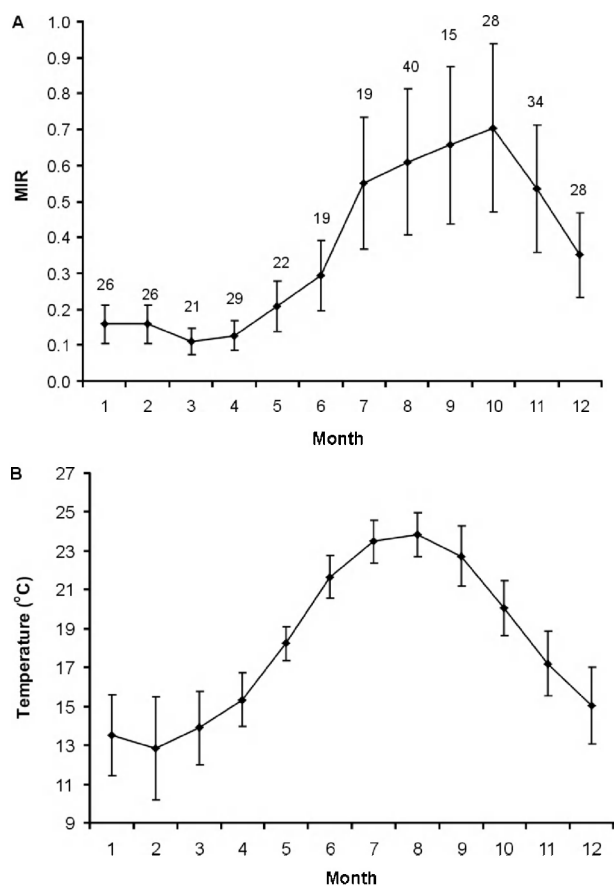


FIG. 5. – A, mean marginal increment ratios (MIR  $\pm$  95% CI) by month for the Atlantic bonito, *Sarda sarda*, from the eastern Mediterranean; B, surface water temperature (Mean  $\pm$  95% CI) in the Aegean Sea.

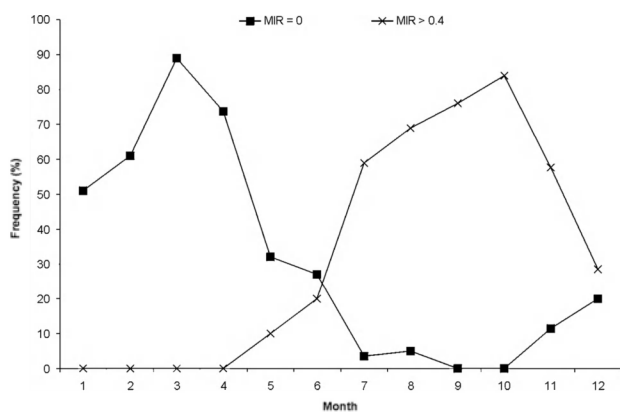


FIG. 6. – Categorization of edges by month for the Atlantic bonito, *Sarda sarda*, from the eastern Mediterranean. Lines correspond to MIR=0 (translucent zones) and MIR > 0.4 (opaque zones).

gorisation of spine edge (Fig. 6) indicated that the highest frequency of translucent zones was low between July and October, increased gradually until March and decreased gradually after March. On the other hand, the frequency of opaque zones showed a reverse pattern of fluctuation.

## DISCUSSION

In this study, age and growth of the Atlantic bonito were estimated using spines of the first dorsal fin and, for the first time, the ageing method was validated with marginal increment analysis.

An important assumption in growth studies using various hard parts is that the size of fish and the size of hard parts are closely related throughout the entire life cycle. Many attempts have been made to estimate the age of Atlantic bonito using skeletal hard parts, but without examining this relationship. The strong correlation found in this study between the radius of the first dorsal spine and  $L_F$  indicates that the spine grows proportionally to bonito length and allows for back calculation of  $L_F$  when insufficient length data are available. Many studies on Scombrid species have shown that the relationship between the radius of various hard parts and fish length can be expressed satisfactorily by a straight line (Berkeley and Hood, 1983; Cayré and Diouf, 1983; Compeán-Jimenez and Bard, 1983; González-Garcés and Fariña-Perez, 1983; Megalofonou and de Metrio, 1989), while other studies have shown that there is not always such a correlation (Radtke, 1983; Wilson and Dean, 1983).

The relatively small percentage of spines that were rejected as illegible shows that, if the problem of dissimulation of rings because of vascularisation were solved, the estimation of bonito age with the use of spines could be an effective and relatively practical method in comparison with the use of otoliths and vertebrae that involve damage to the fish. Beamish (1981) compared the spine method with the otolith and scales methods in the Pacific cod, *Gadus macrocephalus* Tilesius, and the albacore, *Thunnus alalunga* (Bonnaterre), and concluded that spine method had an advantage over the two others. Stequert and Conand (2004) noted that the results obtained in the bigeye tuna, *Thunnus obesus* (Lowe), using spines and otoliths are comparable until the age of 3 years, but spines are not suitable for larger fish. Scales could also be used as a method for age estimation of Atlantic bonito without damaging the fish. This species, however, has scales only in the lateral line and in the corslet. Moreover, Mills and Beamish (1980) and Shirvel (1981) observed that the annual rings are more apparent in spines than in scales, especially in older fish. Megalofonou *et al.* (2003) found that scales are suitable for the estimation of the albacore's age but there is a probability of underestimation at high ages.

Determining the time and period of ring formation is one of the most critical steps of ageing fish using hard parts. In the present work, annual deposition of translucent rings was confirmed by marginal increment analysis. The low MIR values from January to April which coincide with the highest frequency of MIR=0 is strong evidence that the Atlantic bonito in the Greek seas forms one translucent zone per year. Ehrhardt *et al.* (1996) attributed the formation of the translucent areas in rays of fishes to periods of slow growth and the wider dark areas to periods of rapid growth. X-ray analysis has shown that the opaque area in the hard part of fishes is rich in minerals in contrast to the translucent area (Casselman, 1974; 1978) and that the calcium content is reversely correlated with transparency.

The formation of translucent bands has been attributed to various factors that can cause a decreased growth rate in fish, such as reproduction, migration and low temperatures. As reproduction of bonitos takes place from the end of spring to the beginning of summer, the formation of translucent bands cannot be attributed to reproduction. As far as migration is concerned, the data on the bonito are limited. Seasonal migration between the Aegean and the Black Sea has been observed and verified with tagging experiments (Demir, 1963). The migration from the Aegean to the Black Sea begins at the end of April and lasts until the beginning of June, while the opposite migration begins in September and lasts up to the end of November. None of these periods coincides with a translucent ring formation. Moreover, Yoshida (1980) believes that there are schools of bonito that remain in Greek waters all year round. Additionally, in the present study mature bonitos were collected from Greek seas. Hence, migration does not seem to cause the translucent ring formation. The period of translucent ring formation in the bonito coincides with the period of low temperatures in the Aegean. According to Vlahakis and Pollatou (1993), the average temperature of surface waters in the Aegean during the period January-April ranges from 12.8 to 15.3°C. Temperature considerably influences growth rate in fish. It has been found that the metabolic rate of organisms increases dramatically with the increase in temperature (Hemmingsen, 1960; Kleiber, 1932). We can conclude that the low growth rate during the cold period causes formation of the translucent ring in the spines of the Atlantic bonito.

The value of  $L_{\infty}$  that was found in the present study seems to be reliable. It is higher than those of

TABLE 3. – Mean length at age and growth parameters ( $L_{\infty}$ , K,  $t_0$  and  $\Phi$ ) for the Atlantic bonito, *Sarda sarda*, from the Mediterranean Sea and the Atlantic Ocean.

Age	Mean length (cm)			
	Santmaria <i>et al.</i> , 1998	Rey <i>et al.</i> , 1986	Hansen, 1989	Present study
0	34.8	37.0	42.1	28.6
1	50.9	51.7	49.7	40.1
2	57.5	57.0	54.0	49.2
3	64.8	63.15	57.8	56.3
4	70.4	71.0	61.6	62.0
5			65.2	66.4
6			68.8	69.9
			71.7	72.7
Von Bertalanffy parameters				
$L_{\infty}$	80.6	80.87	74.61	82.99
K	0.36	0.35	0.22	0.24
$t_0$	-1.37	-1.70	-2.74	-0.77
$\Phi$	3.37	3.36	3.09	3.21

other researchers (Table 3) and closer to the maximum length values recorded. According to the FishBase (Froese and Pauly, 2005), the largest known individual was 91 cm long and was caught in the western Atlantic. In the Mediterranean the largest known individual was 85 cm long and was caught in the Black Sea. The slightly lower  $\Phi$  quantity (Table 3) perhaps reflects the trophic conditions of the Aegean Sea, one of the most oligotrophic regions (Ignatiades, 1998) of the overall oligotrophic Mediterranean (Estrada, 1996). The closer to zero  $t_0$  in the present study shows that the growth equation estimated expresses more realistically the growth of the species in comparison with previous studies (Table 3), probably due to the presence of a lot of small individuals in the sample.

In response to a growing demand for tuna, and after the overexploitation of large tuna species, attention has been focused on smaller tuna or tunalike species such as the bonitos (Yoshida, 1980). According to the criterion of Musick *et al.* (2000), the bonito in the eastern Mediterranean is a species with a medium growth rate and might be vulnerable to intensive exploitation. Thus, the growth parameters presented in this study may prove to be valuable information to be taken into account in future management measures in the area.

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