ISSN: 0001-5113
AADRAY

ACTA ADRIAT.,
59 (1): 97 - 110, 2018

ORIGINAL SCIENTIFIC PAPER

Age, growth and otolith morphometry of Atlantic bonito (*Sarda sarda* Block, 1793) from the eastern Mediterranean Sea

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The otoliths of the Atlantic bonito, Sarda sarda *(Bloch, 1793), were examined with the aim to estimate the age and growth of the species in the eastern Mediterranean Sea and to reveal possible relationships between otolith shape or size and age. All specimens used in this study, ranging from 7.2 to 70.4 cm in fork length and from 20 to 4889 g in total weight, were caught in the Aegean and Ionian Seas during the period 1997-2010. Otolith morphometry was studied using image analysis techniques for all intact sagittae ranging in weight from 0.6 to 11.3 mg and four shape indices were calculated. No statistical significant differences between left and right otolith morphometric variables were found. The age of fish was estimated by counting the pairs of opaque and translucent bands in transversal thin sections of otoliths. The estimated ages ranged from 0+ to 7 years and the von Bertalanffy growth parameters were determined ($L\infty$=79.9 cm, k=0.261 and to=-1.230 years). The examination of the type of growth bands at the outside margin of each otolith per month showed that one translucent band is formed annually during the cold season. The results revealed statistically significant relationships between otolith morphometric variables and fish length or age. Among the variables, otolith weight was the one that showed the highest correlation with age (R=0.77). Therefore, otolith weight could represent a valuable criterion for age estimation in Atlantic bonito that is objective, economic and easy to perform compared to annuli counting method in hard parts.*

Key words: Scombridae, aging, otolith morphology, Aegean Sea, Ionian Sea

INTRODUCTION

The Atlantic bonito, *Sarda sarda* (BLOCH, 1793), is a pelagic, migratory, schooling fish of the family Scombridae that is frequents in coastal and inshore waters in the Atlantic Ocean, the Mediterranean and Black Seas (COLLETTE & NAUEN, 1983). It is a commercially important species and a major target-species in the purse-seine and other artisanal fisheries with substantial landings every year especially in the Eastern Mediterranean Sea, where the maximum size recorded is 96 cm (DE METRIO *et al*., 1998). In response to a growing demand for tunas all over the world during last decades and after the overexploitation of large tuna species in the

Mediterranean and Atlantic Ocean (ICCAT, 2013), attention is expected to be focused on smaller tuna or tuna-like species such as the bonitos. According to the criterion of MUSICK *et al*. (2000), the Atlantic bonito in the Mediterranean Sea is a species with a medium growth rate and might be vulnerable to intensive exploitation. Nevertheless, the lack of complete fishing and life history data make impossible to be achieved reliable stock assessments and successful management proposals for the Atlantic bonito (ICCAT, 2012).

Biological studies on age of fish are very important for stock assessments and fisheries management decisions, as mean length at age data form the basis for the growth, the mortality rates and the productivity estimations. Even small differences in the growth rate estimation of a fish can affect significantly its population analysis which could lead to incorrect fishery management decisions. In numerous cases, wrong age and growth estimations led to overexploitation of a population or a species (CAMPANA 2001). One of the main problems in the attempt to estimate age and growth is the selection of the most suitable calcified structure to age the fish. Comparative analysis of different calcified structures suggested that in large pelagic species it appears that sections made from otoliths and spines are the most reliable whereas the use of vertebrae and scales in a variety of species has been criticized mainly because the ages of older specimens are frequently underestimated (BEAMISH & MCFARLANE, 1983; CARLANDER, 1987; MEGALOFONOU *et al*., 2003; RODRÍGUEZ-MARÍN *et al*., 2007). Until recently, several authors have studied the age and growth of the Atlantic bonito from various places in the Atlantic, the Mediterranean and Black Sea using a variety of methods, such as length–frequency analysis (ZUSSER, 1954; HANSEN, 1989) and calcified structures by counting the growth bands (RODRIGUEZ-RODA, 1981; REY *et al*., 1986; SANTAMARIA *et al*., 1998; ZABOUKAS & MEGALOFONOU, 2007; ATEŞ *et al*., 2008; VALEIRAS *et al*., 2008; CENGIZ, 2013). The differences observed among the above studies in length at age results may be partially attributed to the diversity of ageing methodology used (vertebrae, otoliths and sectioned dorsal fin spines) and the geographic origin of the samples.

In this paper, we estimate the age and growth of Atlantic bonito in the eastern Mediterranean Sea using for the first time otolith sections and compare these with results from previous independent studies that were based on either whole otoliths or dorsal fin spine sections. The hypothesis that otolith size or shape is directly related to fish age, as estimated from otolith growth band counting, is also considered, with the aim of providing strictly objective measurements that could be used to determine age. Hence, the present study examines otolith morphometry using image analysis techniques and determines the relationships between the otolith size and the age or size of fish with the final goal to verify the use of otolith weight and shape for age estimation of Atlantic bonito.

MATERIAL AND METHODS

Sampling

A total of 502 Atlantic bonito were sampled from the catches of the Greek fishing fleets operating with gillnet and purse-seine around the islands of Lesvos and Chios in the Aegean Sea and the Bay of Patras in the Ionian Sea, during the years 1997-2010. For each fish, the fork length (FL) was measured to the nearest millimeter (mm) and the total weight (TW) to the nearest gram (g). The place and date of capture as well as fishing gear used were recorded. When it was possible, sex of fish was determined macroscopically by visual inspection of gonads. Then, until the extraction of otoliths, all samples were frozen at -20° C.

Otolith measurements

The otoliths (saggitae) were removed from the thawed fish with the aid of forceps and insect needles. The dissected sagittae were separated from extraneous organic material and cleaned with distilled water and detersive to take away any tissue residuals. Once dried, they have been weighted to the nearest 0.1 mg and stored dry in small Crystal-grade Polysterene plastic cases with the code number of each specimen. Digital photographs of the otoliths were taken using a binocular stereoscope equipped with an image analysis system (Software Image Pro-Plus 4.1). In a total of 133 specimens, otolith length (L_o), width (W_o), area (A_o) and perimeter (T_o) were measured for each saggita (MEGALOFONOU, 2006). Otolith weight (M_o) was measured to the nearest 0.1 mg in a total of 182 specimens. Broken or visibly damaged saggitae were not measured and were discarded from the analysis.

Through the otolith measurements four shape indexes were calculated. These indexes provide information about the shape of the otolith, and are often used for separation of populations between individuals of the same species (TUSET *et al.*, 2003; MEGALOFONOU, 2006). The indeces Roundness $R=(4*A_o)/(\pi*L_o^2)$ and Circularity $C_o=T_o^2/A_o$ give information about the similarity that otoliths may present to a perfect circle and take minimum values 1.0 and 12.57, respectively. The index Rectangularity $R_o=A_o/(L_o*W_o)$ describes the variation of the length and width to the surface and when the value is equal to 1.0 indicates that otolith shape is a perfect rectangular. Finally, the E_o value $E_o= W_o/L_o$ is the ratio between the width and the length of the otolith and expresses the tendency in the shape of the otolith, circular or elongate (MEGALOFONOU, 2006).

Age and growth estimation

Before the sectioning procedure, the otoliths were observed under a binocular stereoscope in order to define the position of the nucleus. Then, they were embedded in transparent polyester resin inside special silicon cases. From each sagitta three thin transversal sections of 75 μm were made with a low speed saw (Isomet) near the nucleus area (Fig. 1A). The sections were attached to a microscope slide, ground with wet sandpaper and polished with water and aluminum powder (0.3 μm) using a special electric polishing device (Metaserv). To estimate the age of Atlantic bonito, the otolith sections were observed under a binocular stereoscope on a black background. Opaque and transparent zones were distinguished as structural discontinuities and it was assumed that one opaque and one transparent band together corresponds to one year of growth (annulus). Annuli were counted at the largest arm of the otolith section since it was easier to be distinguished (RODRÍGUEZ-MARÍN *et al.*, 2007). To facilitate the identification of the first annulus an average distance d, equal to 0.6 ±0.05 mm, from the core area of the thin section was used. The distance d was measured in a total of 23 otolith sections taken from relatively small specimens with fork lengths ranging from 31.5 to 44 cm. Initially, the measurement of the distance d was taken to smaller specimens and was subsequently applied to larger ones. A total of two readings for each otolith were performed by the main reader (VR_1) independently. In case of disagreement between counts of annuli in the 1st and 2nd reading otolith sections were read for a third time by a second experienced reader (VR_2). If there was a disagreement between the two readers on the counts of annuli on a specific

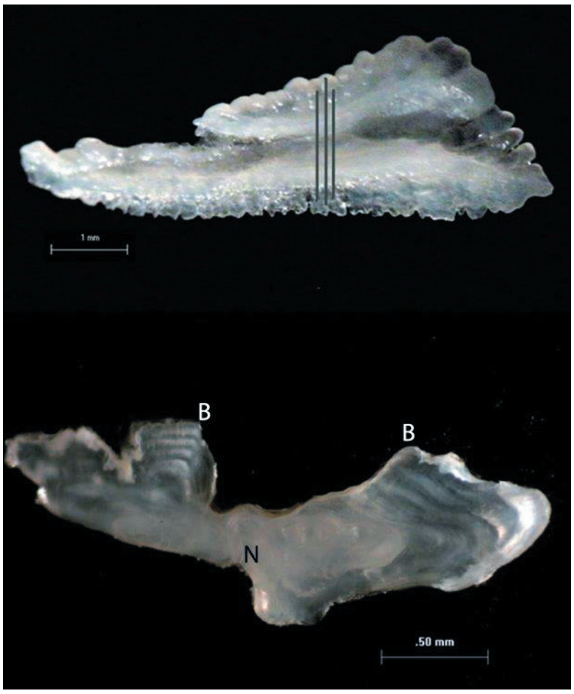

Fig. 1. a) Whole otolith saggita from an Atlantic bonito (FL=59.4 cm) sampled in the Aegean Sea. The lines indicate the area where thin transversal sections were taken. b) Sectioned otolith of an Atlantic bonito (FL=70.4 cm) sampled in the Aegean Sea. N: area of otolith nucleus. B*: otolith “branches”*

otolith section, that section was not included in the analysis.

The von Bertalanffy growth model was used to describe length-at-age of Atlantic bonito and the von Bertalanffy parameters (L_∞, k and t_0) were estimated according to the equation: $L_t = L_\infty[1-e^{-k(t-t0)}]$, t_0 where L_t is the length of fish in cm at age t, L_∞ is the asymptotic fish length in cm, k is the growth coefficient in year^{-1} and t_0 is the theoretical age in years when the fish has zero length. The growth performance index Phi-prime ($\Phi' = \log k + 2\log L_\infty$) was used to compare the growth rate estimated in the present study with those estimated in other similar studies (SPARRE, 1987).

Precision and validation of the ageing method

The precision or the reproducibility of repeated counts of annuli was assessed using the index of Average Percent Error (APE) (BEAMISH & FOURNIER, 1981) and the Coefficient of Variation (CV) (CHANG, 1982) as well. These indexes determine if there are systematic differences in age estimations between the readings of one or more readers. In a total of 90 fish, otolith readings were also made by the second experienced reader (VR_2) from the same laboratory. The readers had agreed in counting criteria prior to the otolith section readings and never had prior access to information on size of fish or date of capture while they were counting growth bands.

The validation of the sectioned otolith ageing method was based on an edge-characterisation analysis (NEWMAN & DUNK, 2003; ZABOUKAS & MEGALOFONOU, 2007). The examination of the type of growth bands (opaque or translucent) at the outside margin of each otolith per month was used to determine the period of growth-band formation and the number of growth bands formatted per year. The frequencies of translucent and opaque zones were plotted per month.

Data analysis

Descriptive statistics were obtained for each measured or calculated variable while frequency histograms were used to present graphically fork length distributions. To compare the mean values among different groups one-way ANOVA was used after the data were tested for normality and homogeneity of variances. When the variables were not normally distributed, the non-parametric Kolmogorov-Smirnov and Kruskal-Wallis tests were used, to determine possible statistical differences among the medians for two or multiple samples, respectively. The null hypothesis that there was no significant difference in the morphometrics from left and right sagitta was tested using paired t-test. Linear ($y=a+bx$) and multiplicative ($y=ax^b$) regression models were used to determine length-length and length-weight relationships, correspondingly. The linear and multiplicative regression models were also used to determine the relationships between otolith morphometric variables and FL or age of fish and between otolith length (L_o) and the other otolith morphometric variables. The values of the constant b (slope) were compared with a Student's t-test while Hypothesis tests were used to detect any significant differences on the relationships. The level of significance was considered at $P \leq 0.05$. All statistical analyses and graphs were done using Statgraphics Centurion XV and Excel (Microsoft Corporation).

RESULTS

Size, sex and length-weight relationship

During the period 1999-2010, a total of 502 Atlantic bonito were collected from the Aegean and Ionian Seas. Their fork length ranged from 7.2 to 70.4 cm and their total weight from 2 to 4889 g. Length frequency distributions (Fig. 2) were significantly different between sexes (Kolmogorov-Smirnov test; $P<0.001$), with males dominating in the smaller length classes (≤33 cm) and females in the larger ones (≥34 cm). Of 502 Atlantic bonito sampled, sex was determined in 377 specimens (237 males and 140 females). Males outnumbered females and the sex ratio was estimated at 1.69 (male: female); significantly deviated from the

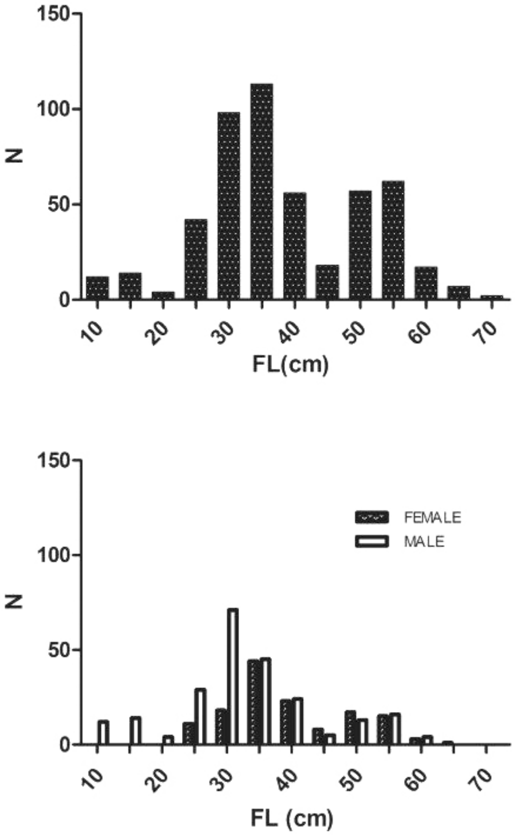

Fig. 2. Fork length (FL) frequency distribution of 502 Atlantic bonito sampled in the eastern Mediterranean Sea during the period 1997-2010

expected hypothetical ratio (1:1). The length–weight relationship was described by the equation $TW=0.0106336*FL^{3.063}$ (R=0.99, n=492) for both sexes (Fig. 3). The slope (b=3.063) of the regression line was significantly different from 3 (P<0.05), indicating a positive allometric growth. No significant differences were observed between the males and females (P<0.05) as well as between the specimens caught in the Aegean and Ionian Sea (P<0.05).

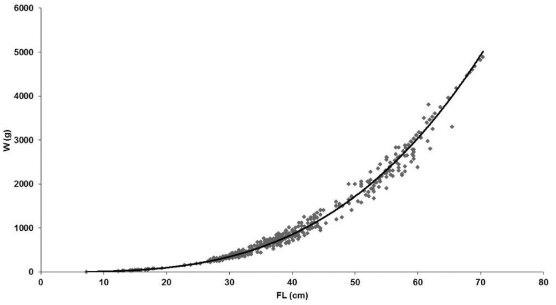

Fig. 3. Length-weight relationship for 492 Atlantic bonito sampled in the eastern Mediterranean Sea during the period 1997-2010

Age and growth estimation

Otolith transversal sections were obtained from a total of 324 Atlantic bonito. Their examination showed that the otolith nucleus (N) is found at the thinner part of the section, between the two "branches" (Fig. 1B). As the fish grows in age and size, the otolith "branches" grow and their ends curve towards the same direction. The annuli appeared as pairs of an opaque (white) and a transparent (dark) band on both the two "branches"; their reading was easier at the largest one (Fig. 1B).

Out of 324 Atlantic bonito only in 24 specimens, that represent 7.4% of the sample, the otolith readings were rejected from the analysis. Overall, the age was estimated in 300 specimens, 87 males, 97 females and 116 of unknown sex. Ages ranged from 0+ to 7 years and the obtained length–age key is presented in (Table 1). The first four age groups included the largest number of fish reaching 91% of the samples (age 0+= 6%, age 1= 43.6%, age 2= 24.3%, age 3= 17%). There were no significant differences (P>0.05) in the mean lengths at estimated ages between male and female bonitos (age 0+: t= 0.360; age 1: t= -1.423; age 2: t= -1.387; age 3: t= -0.042).

The checking of the type of band (translucent or opaque) at the margin of the otolith sections revealed that the higher percentages of the opaque bands are observed in specimens caught during the summer, reaching 86% in July. On the contrary, the higher percentages of the translucent bands are observed in specimens caught during the winter and spring, reaching 90% in March. The plot (Fig. 4) of the monthly categorisation of otolith edge indicated that the lowest frequency of translucent zones was between July and September, increased gradually until March and decreased again reaching the lowest point in July. The frequency of opaque zones showed a reverse pattern of fluctuation.

Table 1. Size–age key of the 300 Atlantic bonito, Sarda sarda, caught in the Aegean and Ionian Seas (Eastern Mediterranean)

FL(cm)	0	1	2	3	4	5	6	7	N
10.1-15	1								1
15.1-20									
20.1-25									
25.1-30	5								5
30.1-35	11	20							31
35.1-40	1	72							73
40.1-45		38	6						44
45.1-50		1	17						18
50.1-55			39	13					52
55.1-60			11	36	7				54
60.1-65				2	10		1		13
65.1-70					2	3	1	1	7
70.1-75							2		2
All									
N	18	131	73	51	19	3	4	1	300
Mean	29.8	38.3	51.6	56.9	61.6	67.8	67.9	70.0	46.1
S.D.	4.9	3.4	3.9	2.4	2.8	1.5	4.0	-	10.4
Females									
N	8	50	26	11	1	-	1	-	97
Mean	31.0	38.3	50.6	57.5	65.5	-	62.0	-	43.6
S.D.	3.4	3.4	4.0	2.8	-	-	-	-	9.1
Males									
N	9	47	20	10	1	-	-	-	87
Mean	30.6	39.0	52.4	57.5	59.4	-	-	-	43.4
S.D.	0.9	3.4	4.2	2.6	-	-	-	-	9.6

The precision of the aging method was estimated using the two readings of the principal reader. The Average Percent Error and the Coefficient of Variation were relatively low (APE=0.77% and CV=0.78%) indicating that differences between readings were minor. Besides, no significant bias was detected between the two readers (ANOVA; P>0.05; n=90). The Average Percent Error and the Coefficient of Variation between the two readers were APE=3.61% and CV=3.61%.

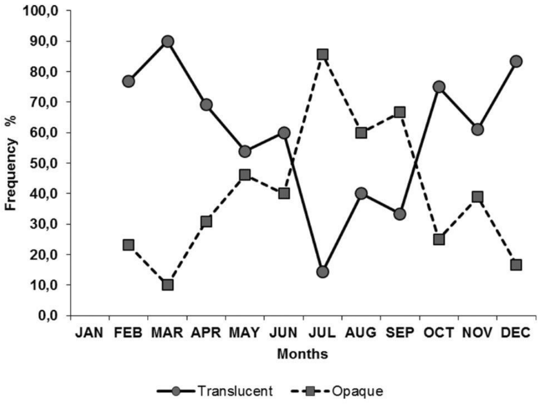

Fig. 4. Categorization of the otolith edges by month for the Atlantic bonito sampled in the Eastern Mediterranean Sea. Lines correspond to the percentages of the opaque and translucent bands observed at the margin of otolith sections

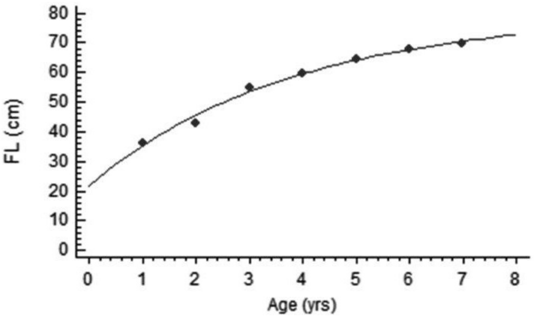

Fig. 5. Von Bertalanffy growth curve for the Atlantic bonito sampled in the eastern Mediterranean Sea. Black dots indicate mean observed lengths at age estimated from sectioned otolith reading

Based on the length-age data the von Bertalanffy growth curve (Fig. 5) and growth parameters were determined (L_∞=79.9 cm, k=0.261 yr^{-1} and t_0=-1.230 yr). The index phi-prime (Φ′) was 3,222.

Otolith morphometry

Otolith weight, length, width, area and perimeter were measured in 133 Atlantic bonitos ranging in FL from 30 to 70.4 mm. No significant differences in morphometric measurements were found between left and right otoliths for 64 pairs of otoliths (paired t test, P>0.05 for all measures), therefore we used the left otolith when it was available and the right otolith when the left otolith was not present or utilizable. Descriptive statistics of otolith morphometry

Table 2. Summary statistics of otolith measurements and otolith shape indexes for Atlantic bonito, Sarda sarda, caught in the Aegean and Ionian Seas (Eastern Mediterranean)

	n	Mean ±S.D.	Range
Otolith measurements			
Weight (M_o, mg)	169	4.4 ± 2.0	0.6-11.3
Length (L_o, mm)	133	5.9 ± 1.4	3.1-9.7
Width (W_o, mm)	133	2.1 ± 0.4	1.3-3.3
Area (A_o, mm^2)	133	7.8 ± 3.2	2.7-17.8
Perimeter (T_o, mm)	133	15.9 ± 3.8	8.9-26.6
Shape indexes			
Roundness (R)	133	0.28 ± 0.02	0.22-0.35
E ratio (E_o)	133	0.35 ± 0.03	0.28-0.42
Rectangularity (R_o)	133	0.61 ± 0.03	0.53-0.70
Circularity (C_o)	133	33.62 ± 4.15	25.37-45.62

measurements, otolith shape indexes and otolith weight are given in Table 2.

Statistically significant relationships were observed between otoliths morphometry measurements and fish somatic growth. The higher correlation (R=0.85) was observed between the fork length of fish and the otolith weight while, the lower correlation between the fork length and the width of the otolith. A high correlation (R=0.84) was also observed between the total weight of fish and the weight of otolith (Table 3). The relationships between the fork length-otolith length and fork length-otolith weight as well as the relationships between the otolith length and the otolith weight, width, perimeter and surface area are shown in Fig. 6.

Statistically significant relationships were also observed between the otolith morphometry variables tested and the age of the fish, which ranged from 0+ to 7 years, as estimated from the annuli counting. A multiplicative or a linear model explained between 0.40% and 0.77% of the variation in age. The higher correlation (R=0.77) was observed between the otolith weight and age of fish while, the lower correlation between the otolith width and age of fish (Table 3, Fig. 7).

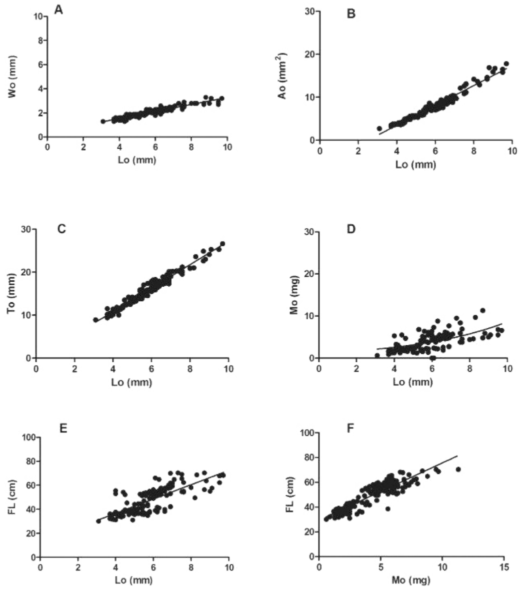

Fig. 6. The relationships between otolith length- otolith width (A), otolith length- otolith area (B), otolith length- otolith perimeter (C), otolith length- otolith weight (D), otolith length- fork length (E), otolith weight- fork length (F) for the Atlantic bonito sampled in the Eastern Mediterranean Sea

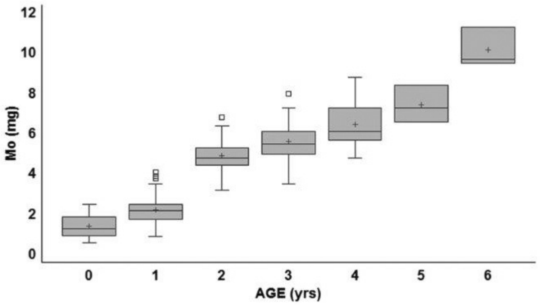

Fig. 7. Box-and-Whisker Plots of otolith weight versus the 6 ages as estimated from the sectioned otoliths of the Atlantic bonito sampled in the Eastern Mediterranean Sea

Table 3. Results of correlations of morphometric characteristics and otolith weight with the length and weight of Atlantic bonito, Sarda sarda, caught in the Aegean and Ionian Seas (Eastern Mediterranean)

X	Y	N	a	b	R	Regression type
FL (cm)	Length (L_o, mm)	133	0.096	1.264	0.57	linear
FL (cm)	Width (W_o, mm)	133	0.028	0.744	0.51	linear
FL (cm)	Area (A_o, mm^2)	133	0.042	1.340	0.55	multiplicative
FL (cm)	Perimeter (T_o, mm)	133	0.5901	0.849	0.64	multiplicative
FL (cm)	Weight (M_o, mg)	169	0.0005	2.305	0.85	multiplicative
AGE (yrs)	Length (L_o, mm)	133	0.704	4.430	0.46	linear
AGE (yrs)	Width (W_o, mm)	133	0.204	1.672	0.40	linear
AGE (yrs)	Area (A_o, mm^2)	133	1.577	4.570	0.41	linear
AGE (yrs)	Perimeter (T_o, mm)	133	2.091	11.657	0.521	linear
AGE (yrs)	Weight (M_o, mg)	169	1.450	1.181	0.77	linear
TW	Weight (M_o, mg)	182	324.79	1.155	0.84	multiplicative

DISCUSSION

This study is the first attempt to estimate the age and growth of the Atlantic bonito by counting the pairs of translucent and opaque growth bands observed in transversal sections of the saggital otoliths. Furthermore, it was the first time that otolith morphometry was described for this species and relationships between various otolith size or shape variables and the age of fish were studied.

The length range of specimens used in the present study was similar to those used in previous studies for the same area (ATEŞ *et al.*, 2008; CENGIZ, 2013; KAHRAMAN, 2014). This is a severe indication that our sampling provided a well representative size distribution of the population. Moreover, the length-weight relationship was statistically significant and the value of allometric coefficient b (3.063) was consistent with the values found in analogous studies (ZORICA & SINOVČIĆ, 2008; CENGIZ, 2013; KAHRAMAN *et al*, 2014; YANKOVA, 2015)

Otolith sections have not been widely used for age estimations of the tuna and tuna-like species because they are extremely time-consuming to collect, prepare and read (FOREMAN, 1996; ITOH *et al.*, 2000; MEGALOFONOU *et al.*, 1995; MEGALOFONOU, 2006). In previous studies, whole otoliths have been used for the ageing of Atlantic bonito caught only in the areas of Marmara Sea and Dardanelles (ATEŞ *et al.*, 2008; CENGIZ, 2013). The method involved a systematic interpretation of the opaque and transparent growth bands observed on whole otoliths against a black background with reflected light. The comparison of length at age results from whole and sectioned otoliths confirmed the view that the whole otolith method most probably underestimates the age of Atlantic bonito (Table 4). Whole otoliths generally require less time to prepare and process than do sectioned otoliths, but annuli can be difficult to detect because of opacity and curvature of whole otoliths. On the other hand, our length at age results based on otolith sections were similar to those based on dorsal fin spine sections (ZABOUKAS & MEGALOFONOU, 2007), (Table 4). These similar results in the mean lengths at ages, obtained in part from the same collection of fish caught in the same area, constitute a preliminary verification of the sectioned otolith ageing method. Nevertheless, more research effort should be directed toward verification studies that will compare

Table 4. Comparison of mean fork length (cm) at age (years) estimated using sectioned various hard parts and growth parameters (L_∞, K, t_0 and Φ) for the Atlantic bonito, Sarda sarda, from the Mediterranean Sea

	Rodriquez-Roda (1981)	Valeiras *et al.* (2008)	Santamaria *et al.* (1998)	Zaboukas and Megalofonou (2007)	Ateş *et al.* (2008)	Cengiz (2013)	Present study
Age	Vertebrae	Dorsal spines	Dorsal spines	Dorsal spines	Whole Otoliths	Whole Otoliths	Sectioned Otoliths
0	-	-	34.8	28.6	23.5-40.5	30.5	29.8
1	42.59	43.7	50.9	40.1	52.5-62.5	52.2	38.3
2	50.51	53.1	57.5	49.2	54.0-67.5	56.3	51.6
3	60.5	57.7	64.8	56.3	68.0-70.5	67.9	56.9
4	64	-	70.4	62.0	-	-	61.6
5	-	-	-	66.4	-	-	67.8
6	-	-	-	69.9	-	-	67.9
7	-	-	-	72.7	-	-	70.0
Von Bertallanfy parameters							
L_∞		62.5	80.6	82.1	68.0	69.9	79.9
K		0.72	0.36	0.24	0.82	0.76	0.26
t_0		-1.21	-1.37	-0.77	-0.39	-0.44	-1.23
Φ		3.45	3.37	3.21	3.58	3.57	3.22

ages assessed independently from different calcified structures from the same fish.

One of the most critical steps of the ageing studies using hard parts is the determination of the number of growth bands that formed per year and the period of formation as well. The monthly categorisation of otolith edge and the pattern of frequencies of transparent and opaque zones at the margins of otolith sections (Fig. 4) confirmed our hypothesis that one transparent and one opaque band are formed per year (annulus) in otoliths of Atlantic bonito, just like in spines. The annual deposition of translucent zones in spines has been already validated by marginal increment analysis (ZABOUKAS & MEGALOFONOU, 2007). In comparison to the monthly frequencies of transparent margin in otolith sections, that showed a pick in March and a low in July, the frequencies of opaque zones showed a reverse pattern of fluctuation. The formation of translucent bands in hard parts has been attributed to various factors that can cause a decreased growth rate in fish, such as low temperatures, reproduction or migration. ZABOUKAS & MEGALOFONOU (2007) showed that the period of translucent ring formation in bonito spines coincides with the period of low temperatures in the Aegean Sea. Similar results were obtained in our study for otoliths since the higher frequencies of translucent zones at the margins of otolith sections were between December and March. Considering the above we attribute the formation of the translucent bands in the bonito otoliths to slow growth during winter-spring and the wider opaque bands to rapid growth during summer.

Our length-at-age estimates showed that successive age groups present wide range and overlap. This may be due to errors generated in the reading technique or may reflect real variability in the growth rate of this species. Actually, at such a rapid growth rate, especially during the first years of life, small changes in individual growth rates can produce significant differences in absolute size achieved at a certain age. Besides, an extended period of reproduction can produce similar phenomena as already detected in other large pelagic species (MEGALOFONOU *et al*., 2003). The spawning period of Atlantic bonito lasts at least 2-3 months, from mid-May to the end of July in the Aegean Sea (DEMIR, 1963) and from June to August in the Ionian Sea (SANTAMARIA *et al.*, 1998). Consequently, an early-spawned fish can be longer than a late-spawned fish from the same year class, as much as the growth of 2-3 months.

Growth parameter estimates obtained in our study are roughly consistent with what is known about the life history of this species in the Mediterranean. Considering that the largest specimen recorded was 96 cm in fork length (DE METRIO *et al.*, 1998), the L_∞ estimate of 79.9 cm seems realistic, although somewhat low, compared with other studies for the Atlantic bonito (Table 4). In our study, the von Bertalanffy growth parameters were quite similar to those calculated by the method of fin spines (ZABOUKAS & MEGALOFONOU, 2007). Compared with previously published estimates of growth parameters for bonito from the Mediterranean and Atlantic areas (Table 4), some differences are obvious: L∞ is among the higher values and k is in the low range; however, the calculated value of Φ quantity is slightly lower. According to (ZABOUKAS & MEGALOFONOU, 2007) the Φ quantity may reflect the trophic conditions of the Aegean Sea, one of the most oligotrophic regions in the Mediterranean Sea.

Comparing the aging methods, we conclude that the method of the dorsal fin spines is an easier and more effective method compared with the method of otoliths. But, unlike the otolith method, the spine method presents other kind of difficulties which are mainly related to the spine core vascularisation in older age specimens. Although the tendency in verification studies is to assume that the structure providing the higher age estimation is the most accurate, this may not always be the case and has to be proved. Dorsal fin spines of albacore have been shown to contain multiple growth bands, which if interpreted literally, overestimated the age of the fish (MEGALOFONOU, 2000; MEGALOFONOU *et al.*, 2003). Our results demonstrate that otolith sections can be used for age estimation, particularly of older fish, because they can be read with accuracy and precision by calibrated readers as demonstrated by the estimations of APE and CV. There is strong evidence that annual growth marks do appear in otoliths, as it happens in spines, of Atlantic bonito and that they can be used to estimate the age of this species in a reliable way. The factors responsible for their formation are not yet completely known. Further investigation with X-ray analysis to determine the composition of bands in minerals and calcium could be useful for the understanding of their formation process. Moreover, analyses of hard parts from tagged fish in marking experiments and comparison of the various hard part aging methods could provide significant advances in age assessment and validation of bonito.

An important assumption in age and growth studies using 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 otoliths, but without examining this relationship. The strong correlation found in this study between the otolith length (Lo) and fork length (FL) indicates that the otolith grows proportionally to bonito body size. Many studies on various Scombrids have shown that the relationship between the radius of various hard parts and fish length can be expressed satisfactorily by a straight line (MEGALOFONOU, 2006; ZABOUKAS & MEGALOFONOU, 2007; MILATOU & MEGALOFONOU, 2014), while other studies have shown that there is not always such a correlation (RADTKE, 1983; WILSON & DEAN, 1983). In our study the linear model explained the highest percent (R) of the variability.

Various researchers, using the progress achieved in methods of image analysis, studied the morphological characteristics of otoliths such as otolith length, width or shape in relation to fish age and demonstrated a clear relationship between the variables and the age of fish (FOSSEN *et al.*, 2003; TUSET *et al.*, 2003; DOERING-ARJES *et al.*, 2008). Moreover, it has been shown that strong relationships exist between fish age and otolith weight for different species (CARDINALE *et al.*, 2000; ARAYA *et al.*, 2001). MEGALOFONOU (2006), considering the high correlations, proposed that the otolith weight and other otolith morphological variables could constitute a valid alternative to the traditional methods for the age estimation of juvenile Bluefin tuna. The study of otolith morphometry of the Atlantic bonito showed that there are relatively high correlations between otolith morphometric variables and the age of fish. On the top of that, a high correlation between the otolith weight and the age of the fish was revealed. Considering that the otolith ageing method has been validated in our study by an edge-characterisation analysis we propose that the otolith weight could be used as a valid alternative to estimate the age of the species (CARDINALE *et al*., 2000; ARAYA *et al*., 2001; MEGALOFONOU, 2006).

ACKNOWLEDGMENTS

We would like to express our most sincere thanks to Greek fishermen for their cooperation during the samplings of the Atlantic bonito and Mrs Niki MILATOU for her assistance to estimate the precision of the ageing method. We are also indebted to the reviewers for their suggestions to improve the manuscript.

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Received: 27 January 2018
Accepted: 14 March 2018

Starost, rast i morfometrija otolita palamide (*Sarda sarda* Block, 1793) iz istočnog Sredozemnog mora

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SAŽETAK

Kako bi se odredila starost i rast palamide *Sarda sarda* (BLOCH, 1793), koja obitava u Sredozemnom moru, analizirani su njeni otoliti. Isto tako se analizom morfometrijskih parametara otolita palamide pokušala utvrditi korelacija između oblika samog otolita i očitane starosti. Vilična dužina i masa svih analiziranih jedinki palamide, koje su prikupljene na području Jonskog i Egejskog mora u razdoblju od 1997. god. do 2010. god., kretala se u rasponu od 7.2 cm do 70.4 cm odnosno od 20 g do 4889 g. Morfometrijske značajke neoštećenih otolita, čija masa je bila od 0,6 mg do 11,3 mg, su definirane preko četiri parametra oblika istih. Nije utvrđeno da postoji statistički značajna razlika između lijevog i desnog otolita. Starost palamide, dobivena očitavanjem i brojanjem zona prirasta na otolitu, istraživanih jedinki je kolebala od 0+ do 7 godina starosti te su definirani von Bertalanffy parametri rasta ($L\infty$=79.9 cm, k=0.261 and t_o=-1.230 god.).

Analizirajući zone prirasta na otolitu uočeno je da tijekom jedne godine nastaje jedan hijalini (proziran) prsten i to u hladnijem dijelu godine. Utvrđena je statistički značajna korelacija između morfometrijskih parametara otolita i dužine ribe kao i starosti. Osobito značajna korelacija (R=0,77) je uočena između mase otolita i dužine istraživane jedinke, što upućuje na to da bi se u budućnosti navedeni parametar – masa otolita, mogla koristiti kao indikator starosti s obzirom da se radi o metodi koja bi u svakom slučaju bila dosta ekonomičnija i jednostavnija.

Ključne riječi: Scombridae, određivanje starosti, morfologija otolita, Egejsko more, Jonsko more