

Direct ageing of *Thunnus thynnus* from the eastern Atlantic Ocean and western Mediterranean Sea using dorsal fin spines

P. L. LUQUE*†, E. RODRIGUEZ-MARIN*, J. LANDA*, M. RUIZ*, P. QUELLE*,
D. MACIAS‡ AND J. M. ORTIZ DE URBINA‡

*Spanish Institute of Oceanography, Santander Oceanographic Centre, PO Box 240, 39080, Santander, Spain and ‡Spanish Institute of Oceanography, Málaga Oceanographic Centre, PO Box 28, 29640, Fuengirola, Spain

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This study deals with important methodology issues that affect age estimates of eastern Atlantic bluefin tuna *Thunnus thynnus* using dorsal fin spines. Nearly 3800 spine sections were used from fish caught in the north-east Atlantic Ocean and western Mediterranean Sea over a 21 year period. Edge type and marginal increment analyses indicated a yearly periodicity of annulus formation with the translucent bands (50% of occurrence) appearing from October to May. Nucleus vascularization seriously affected specimens older than 6 years, with the disappearance of 40–50% of the presumed annuli by that age. An alternate sectioning location was a clear improvement and this finding is an important contribution to the methodology of using this structure for ageing the full-length range of eastern *T. thynnus*. Finally, there were no significant differences between the coefficients of von Bertalanffy growth model estimated from mean length at age data ($L_{\infty} = 327.4$; $k = 0.097$; $t_0 = -0.838$) and those estimated from the growth curves accepted for the eastern and western *T. thynnus* management units.

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Key words: age; growth; spiniform ray; tuna; vascularization.

INTRODUCTION

The Atlantic bluefin tuna *Thunnus thynnus* (L. 1758) is a long-lived, slow-growing fish species, which makes it vulnerable to fisheries exploitation (Fromentin & Fonteneau, 2001). The International Commission for the Conservation of Atlantic Tunas (ICCAT), the organization responsible for the management of this species, has defined two stocks in the Atlantic Ocean: the eastern and Mediterranean stock and the western stock. Both stocks are in an overfished condition and are subject to multi-annual recovery plans. Current assessments of the status of *T. thynnus* stocks are based on age-structured models, and independent growth curves are applied to each stock (ICCAT, 2013). In this context, life-history studies on age and growth rate of *T. thynnus* are essential components of models used in stock assessment of its population and hence are critical for developing management strategies for sustainable fishing activity on these populations (Fromentin & Powers, 2005; Rooker *et al.*, 2007).

†Author to whom correspondence should be addressed. Tel.: +34 942291716; email: plastralastra@gmail.com

A wide variety of ageing methods have been applied to *T. thynnus*, including modal analysis of length frequencies, tag-recapture studies and counts of incremental growth marks in various hard parts (Rooker *et al.*, 2007) such as scales, vertebrae, dorsal fin spines and otoliths (Mather & Shuck, 1960; Farrugio, 1980; Compeán-Jimenez & Bard, 1983; Lee *et al.*, 1983; Cort, 1991; Santamaría *et al.*, 2009), the last being the only structure that has been directly validated using the bomb radiocarbon method (Neilson & Campana, 2008). The validated growth curve developed for the western stock based on the above otolith-based age estimates for older fish and length frequency data for younger fish (Restrepo *et al.*, 2010) revealed slower growth and older ages than were previously assumed and these values affected the calculation of productivity benchmarks and, by extension, the rebuilding schedules for the resource (ICCAT, 2011).

Fin ray spines have been used to age pelagic fishes such as billfishes and tuna species (Beamish, 1981; Prince & Pulos, 1983; Kopf *et al.*, 2010) mainly because sampling is easy and does not damage the fish, which is impractical in certain fisheries such as the Atlantic traps. In addition, the preparation of spines is less time-consuming than other structures such as vertebrae, as only transverse sections are needed and staining is not necessary (Rodríguez-Marin *et al.*, 2007).

Spines have proven useful in the determination of age, particularly for juveniles and young adult *T. thynnus* (Rooker *et al.*, 2007). Although the spine-based age estimates still remain non-validated directly (Rodríguez-Marin *et al.*, 2012a), results from tracking the exceptionally abundant 1994 cohort in different Atlantic Spanish fisheries did validate indirectly the ageing method at least for juveniles and young adults, as few differences were found between age key lengths (ALK) built by the age interpretation using dorsal spine sections and the growth curve currently used for the eastern *T. thynnus* stock (Rodríguez-Marín *et al.*, 2009).

As the fish grow, the spine nucleus is reabsorbed replacing the earliest annuli by vascular tissue (Rooker *et al.*, 2007). This process, known as nucleus vascularization, is the greatest disadvantage of using this calcified structure for ageing purposes, as it might result in significant age underestimation and growth overestimation (Panfili *et al.*, 2002; Drew *et al.*, 2006; Kopf *et al.*, 2010). Nucleus vascularization has been reported in several tuna species with frequent use of the method developed by Hill *et al.* (1989) to replace lost annuli (Compeán-Jimenez & Bard, 1983; Tserpes & Tsimenides, 1995; Chiang *et al.*, 2004; Zaboukas & Megalofonou, 2007; Duarte-Neto *et al.*, 2012; Farley *et al.*, 2013). This method relies on the assumption that the annulus of a particular age class forms at approximately the same radius for each individual in the population; nevertheless, very little has been published regarding the quantification of obscured annuli due to vascularization in most of these species including *T. thynnus*.

In addition, there are some other concerns with spine analysis that also influence age estimates of *T. thynnus* using spine cross sections that remain unresolved and have not been clearly defined in previous studies including the methods for processing and interpreting data collected from spines for age estimation (Compeán-Jimenez & Bard, 1983; Cort, 1991; Santamaría *et al.*, 2009). In this context, the goal of this study was to expand the limited literature available on important methodological issues including a definition of the spine in terms of its biometric relationships, sectioning location and the quantification of spine nucleus vascularization. Furthermore, the verification of the increment periodicity in spines is also described to indirectly validate the spine-based age estimates. Finally, age estimates by length were used to obtain growth parameters for the *T. thynnus* eastern stock.

TABLE I. Sampling data of *Thunnus thynnus* caught in the north-east Atlantic Ocean and the western Mediterranean Sea over a 21 year period from 1990 to 2010

Year	Atlantic		Mediterranean		Total	
	<i>n</i>	<i>L</i> _{SF} range (cm)	<i>n</i>	<i>L</i> _{SF} range (cm)	<i>n</i>	<i>L</i> _{SF} range (cm)
1984	179	135–287			179	135–287
1990	150	59–291			150	59–291
1991	37	66–202			37	66–202
1992	48	56–184	3	50–101	51	50–184
1993	31	51–204			31	51–204
1994	65	64–183			65	64–183
1995	33	58–120	6	79–225	39	58–225
1996	68	55–185			68	55–185
1997	375	55–162	2	38–38	377	38–162
1998	368	53–160			368	53–160
1999	142	54–182			142	54–182
2000	222	59–170			222	59–170
2001	162	68–271			162	68–271
2002	171	59–263			171	59–263
2003	99	55–220	52	33–92	151	33–220
2004	182	63–236	49	51–250	231	51–250
2005	253	57–257	100	33–245	353	33–257
2006	93	59–212			93	59–212
2007	96	66–210	83	48–233	179	48–233
2008	129	70–279	260	61–251	389	61–279
2009	122	65–196	45	26–239	167	26–239
2010	81	158–257	76	27–244	157	27–257
Total	3106	51–291	676	26–251	3782	26–291

*L*_{SF}, straight fork length; *n*, sample size.

MATERIALS AND METHODS

SAMPLING

In this study, the first spiniform ray of the first dorsal fin (the spine) was collected from *T. thynnus* caught in the north-east Atlantic Ocean (*n* = 2927) and western Mediterranean Sea (*n* = 676) over a 21 year period from 1990 to 2010. Additional spine samples were analysed from medium-sized fish collected in 1984 (*n* = 179) (Table I). This long sampling period increased the likelihood of including samples from all fish sizes and capture months. Sampling was based on port landings from different fisheries and geographical areas, including bait boats from the Bay of Biscay, long-liners from the south of Iceland, Gulf of Cadiz and western Mediterranean, and Atlantic traps near the Strait of Gibraltar (Fig. 1). Fish were measured using straight fork length (*L*_{SF}) to the nearest centimetre. Due to the seasonality of the different fisheries that capture different-sized *T. thynnus*, it should be noted that specimens > 160 cm were collected mainly during the second half of the year, whereas sampling of smaller fish took place during the first half of the year. Date and location of capture were also recorded.

EXTERNAL SPINE MEASUREMENTS

Several biometric measures were recorded for each spine in order to evaluate spine growth. These relationships are necessary due to the aforementioned consequences of the spine reabsorption. The relationships between *L*_{SF} and spine measurements, including total spine

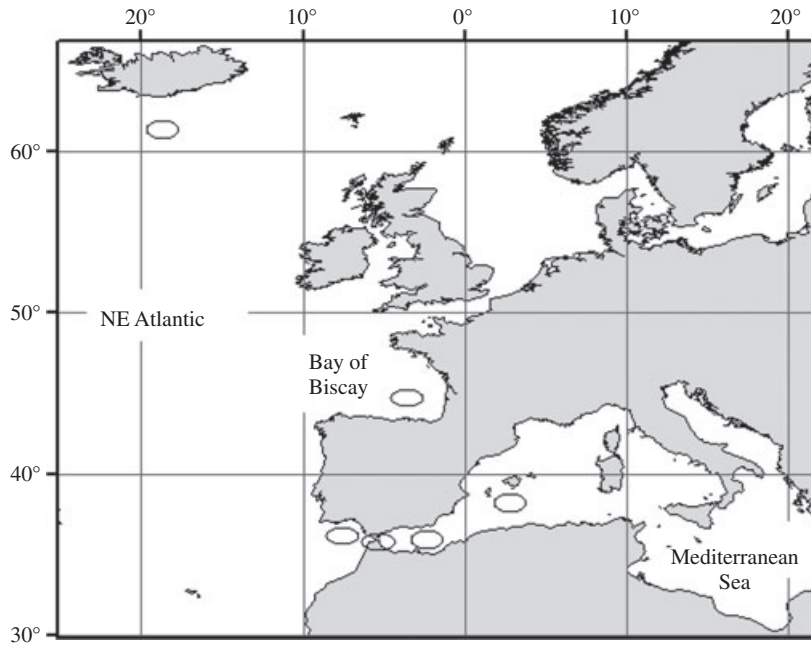


FIG. 1. Study area showing sampling sites (O) of *Thunnus thynnus* caught in the north-east Atlantic Ocean and western Mediterranean Sea.

length (L_{spinemax}), maximum spine diameter (D_{spinemax}) and distance from the condyle base to the two sectioning locations, LS0.5 and LS1.5, respectively [Fig. 2(a)], were analysed using standard regression procedures. In addition, the relationship between spine diameter at the two sectioning locations (*i.e.* S0.5 and S1.5) [Fig. 2(b)] and L_{SF} was analysed. Linear and power regression functions were tested for all relationships using the coefficient of determination (r^2) as a goodness-of-fit index and the F -statistic as a test of the statistical significance of the model ($\alpha = 1\%$).

The external part of L_{max} was measured as the distance from the outside of the tuna body to the tip of the spine. This measurement was taken to determine whether the two sectioning locations L0.5 and L1.5 were located in or out of the embedded portion of the spine. This is a relevant issue because for some ports and especially for old specimens, extraction of the spine in one piece, including the condyle base, is not allowed.

SPINE SECTION PREPARATION

Spines were prepared following the procedure described by Rodriguez-Marin *et al.* (2012b). Spines were sectioned using a precision rotating diamond saw IsoMet Buehler (Buehler; www.buehler.com) at two sectioning locations, the traditional S0.5 ($n = 3782$) and an alternative second cutting axis, S1.5 ($n = 381$), that was established slightly higher along the spine at 1.5 times the D_{spinemax} from the imaginary line passing below the hollows located near the condyle base [Fig. 2(b)]. This sectioning location has been explored for the first time in this study to evaluate whether using a different sectioning location could reduce the influence of nucleus vascularization.

As S1.5 is an experimental sectioning location and it is referred to a sub-sample of the total sample size, the following ageing and growth analyses were based on age estimates using S0.5. Estimates from S1.5 were only used for the comparison analysis between the two sectioning locations.

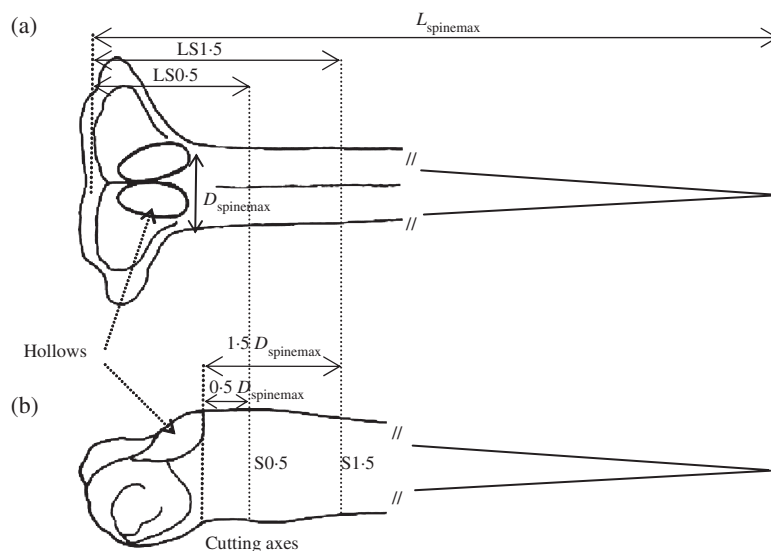


FIG. 2. Illustration of an (a) anterior and (b) lateral view of the first dorsal spine showing the biometric measurements recorded. $D_{\text{spine}\max}$, maximum spine diameter; $L_{\text{spine}\max}$, total spine length; LS0.5 and LS1.5: distance from the condyle base to the first and second cutting axes, respectively, and the position of the two cutting axes $0.5 D_{\text{spine}\max}$ (S0.5) and $1.5 D_{\text{spine}\max}$ (S1.5).

AGE ESTIMATION

In the cross-sectional cut of the spine examined under transmitted light, different visible areas appeared as opaque and translucent bands based on their relative translucency (Fig. 3). An annulus was defined as a bipartite structure consisting generally of a wide opaque band followed by a narrow translucent band, presumably formed on a yearly basis. These annuli were, however, not always a bipartite structure and sometimes multiple opaque and translucent pair banding appeared. Fig. 3 shows a spine section from a *T. thynnus* aged 8 years with an example of a false translucent band (F) after the sixth annulus.

Age was estimated by counting the translucent bands. Age interpretation was performed using digital images that were captured with a binocular lens magnifier connected to a digital camera. An image analyser (Nis-elements D 3.0, Nikon software; www.nikon.com) was used to measure the diameter of the spine section and visible translucent bands.

Overall, spine sections (S0.5 and S1.5) were read independently by at least two readers. For those spines for which there was an age disagreement, an additional reading was conducted and the consensus among readers was considered the final age estimate used for further analysis. If agreement could not be reached, those spines were not included in the analysis. Five readers (A–E) participated in the ageing of spines. Readers were scored into one of the three experience categories: high (A and D), intermediate (B) and low (E). Exceptionally, reader C was scored in two categories, low and intermediate, as the reader gained experience during the study period.

The correct identification of the earliest translucent bands in older fish is a common problem encountered while using spines due to nucleus vascularization that causes their gradual disappearance (Fig. 3). In the current study, a replacement method modified from the one developed by Hill *et al.* (1989) was followed to address the problem. Thus, the mean diameter of the earliest translucent bands in spine sections of young fish with no vascularization was used to identify the first innermost visible translucent band in vascularized spines from older specimens and hence to estimate the number of missing translucent bands that lie within the vascularized zone. As this procedure of measuring advanced, a table that included the mean and s.d. of the translucent annuli for each sectioning location was developed (*i.e.* S0.5 and S1.5; Table II). Thus, when the diameter of at least two of the three innermost visible bands fall within the ranges

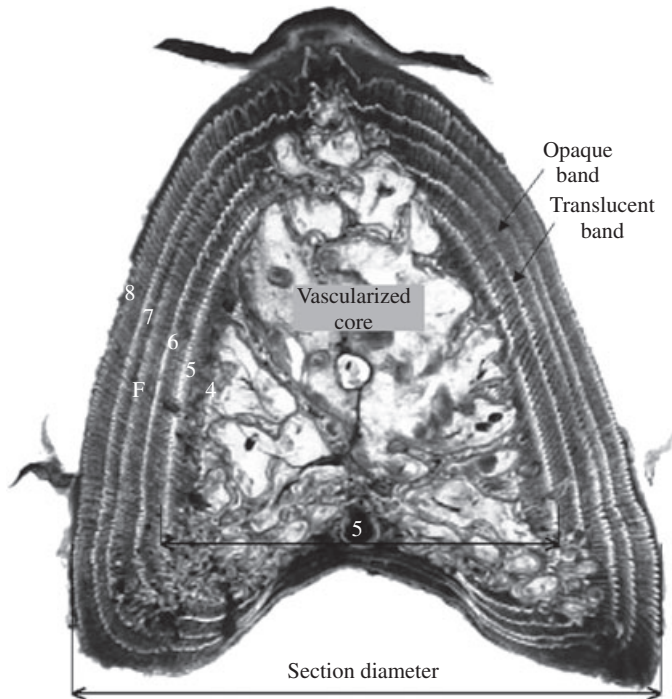


FIG. 3. Spine section image from a *Thunnus thynnus* aged 8 years. Numbers reflect translucent bands counted as annuli and how the maximum width of each annulus was measured. An example of false translucent band (F) appeared after the sixth annulus. The first innermost complete translucent band was assigned as the fifth annulus.

calculated from the s.d. shown in Table II, the corresponding ages were assigned. Once an age was assigned to the first innermost visible translucent band, the age estimate was calculated by adding that estimate to the number of translucent bands count between the vascularized area and the spine edge. In addition, the date of capture and section edge type were considered by readers to assign final age estimates. Thus, *T. thynnus* with a translucent band formed at the edge and caught at the beginning of the year was interpreted as being 1 year older, despite being 5 or 6 months prior to the assumed date of birth, which occurs mid-year (June to July), considering 1 July as the birth date (Rooker *et al.*, 2007). Consequently, when the peripheral translucent band is present and the fish was caught in autumn, this band was not counted as +1 year. A scheme of edge type interpretation was as follow: translucent, first semester age = n and second semester age = $n-1$; opaque, first semester age = n^* and second semester age = n , where n represents number of translucent bands including those estimated due to vascularization (*, unusual edge type).

Spine sections were scored into four readability codes defined as follows: (1) bad quality included sections with no clear banding pattern and nucleus vascularization affected most of the spine section and consequently no presumed annual translucent band could be measured; (2) regular quality sections showed a banding pattern that may contain multiple translucent bands and consequently readers were uncertain of the age estimation and measurements of the translucent bands; (3) good quality, a banding pattern was present and readers were confident of their age estimates despite multiple bands possibly being present; and (4) picture quality section in which the paired banding pattern was clearly present and bands were clearly in contrast, with no multiple bandings resulting in certainty in the age estimates. Only spine sections categorized as (2)–(4) were considered for further growth and ageing analysis.

COMPARING AGE ESTIMATES AMONG READERS

Considering that the five readers did not read the same spines, four cross-reader comparisons were selected for analysis based on number of readers involved, reading experience, age range and sample size, to ensure that most of the ages were well represented. Following Eltink *et al.* (2000), this analysis compared the estimated ages from each reader with the modal age, the latter being the consensus among readers involved. Three indices were used to estimate spine age precision among readers, the average per cent error (APE), the coefficient of variation (C.V.) and the weighted mean percentage agreement (PA) with respect to the modal age. APE was estimated using the formula recommended by Beamish & Fournier (1981). The PA and the mean C.V. were estimated using the formula provided by the European Fish Ageing Network (EFAN) software (Eltink *et al.*, 2000). To test for differences in estimates among readers, an inter-reader bias test was also applied. Moreover, in the absence of calcified structures of known age, the relative accuracy was estimated by the relative bias. This bias is a systematic over or underestimation of age compared to the modal age.

INDIRECT VALIDATION: EDGE TYPE AND MARGINAL INCREMENT ANALYSIS

The timing of band formation was assessed by examining the outermost edge of the spine in section S0.5. The edge was considered to be either translucent or opaque when the pattern was present along more than half the perimeter of the spine section. Marginal increment ratio (R_{MI}) analysis was performed to determine the time of band formation using the following equation (Natanson *et al.*, 1995): $R_{MI} = (S - d_n) (d_n - d_{n-1})^{-1}$, where S is the spine diameter section, d_n is the diameter of the last (n) complete translucent band and d_{n-1} is the diameter of the penultimate translucent band. The percentage occurrence of edge type and the mean $R_{MI} \pm$ s.d. was plotted monthly in order to locate periodic trends in band formation. R_{MI} was analysed for juveniles (ages 2–3 years) and adults (ages > 3 years) as well as for all ages combined.

COMPARISON ANALYSIS AMONG SECTIONING LOCATIONS

Sectioning locations were compared in terms of (1) the number of innermost translucent bands missing due to vascularization, calculated as the estimated age minus number of non-observed bands and (2) the number of translucent bands that could be measured (non-observed observed bands). The readability codes (*i.e.* bad, regular, good and picture quality) of the two sections obtained from the same spines were also compared.

Analysis of deviance for generalized linear models (GLMs) (Hastie & Pregibon, 1992) was applied to examine differences between sectioning locations. The numbers of missing and measured translucent bands were analysed assuming a Poisson error distribution and the linear predictor included the sectioning location and age as categorical explanatory variables. A binomial error distribution was assumed for the readability code response variable, and the linear predictor included age as the variable and sectioning location as the factor. Statistical significance of the reduction in the deviance between nested models was based on the χ^2 statistic (Faraway, 2002; Zuur *et al.*, 2009). All analyses were performed using R software (R Development Core Team; www.r-project.org).

GROWTH MODEL PARAMETERS

A standard von Bertalanffy growth model (VBGM; von Bertalanffy, 1938) was fitted to length at age data derived from spines to characterize the growth of *T. thynnus*: $L_t = L_\infty \{1 - e^{[-k(t-t_0)]}\}$, where L_t is the L_{SF} at age t , L_∞ is the asymptotic length that *T. thynnus* may attain if the fish lived indefinitely, k is the growth coefficient at which L_∞ is reached asymptotically, t is age (year) and t_0 is the hypothetical age at length 0, *i.e.* the point at which the VB curve intersects the x -axis (year).

The VBGM equation was fitted using observed and mean length-at-age values to check sensitivity of both models to the data used. When the VBGM equation was fitted to mean length-at-age data, only those age classes with a minimum sample size of five specimens were

considered for analysis. The age estimates used for fitting the growth curve were adjusted by adding a plus correction that takes into account the sampling month and the assumed date of birth of 1 July [*i.e.* fractional age = estimated age + (sampling month per months of the year) - 0.5], as spawning in the western Mediterranean Sea occurs from mid-June to mid-July (Rooker *et al.*, 2007). To check the plausibility of the growth equation parameters obtained from the adjustment of the data to VBGM, the maximum L_{SF} of *T. thynnus* catches in the study period (ICCAT catch-at-size database; www.iccat.int/en/accesingdb.htm) was obtained and the potential longevity was estimated using Pauly & Munro's (1984): maximum age = 3 divided by k , where k is the growth coefficient.

VBGM parameters (L_{∞} , k and t_0) estimated from the current and previous studies were compared using Kimura's (1980) likelihood ratio test. The test was conducted using equivalent age ranges as recommended by Haddon (2001). The growth parameters were estimated by minimizing the sum of squared residuals. For previous studies, parameters had to be recalculated to be able to compare equivalent age ranges.

For all growth studies of *T. thynnus* including the present, the growth coefficients (k) were plotted against the asymptotic lengths (L_{∞}) to explore the distribution pattern of the estimated values.

RESULTS

EXTERNAL SPINE MEASUREMENTS

The two spine section (S0.5 and S1.5) diameters *v.* L_{SF} and spine total length ($L_{spinemax}$) *v.* L_{SF} showed good fit in the power and linear equations, although the r^2 values were slightly better for the power relationship (Table III). This strong relationship indicated that fish body length and width of the spine were closely related throughout the life cycle of the individual (Fig. 4).

It was estimated that mean \pm s.d. $17 \pm 6\%$ of the total length of the spiniform ray was embedded in the body, which includes its condyle base ($n = 95$, $L_{SF} = 79\text{--}195$ cm). Moreover, the two sectioning locations, LS0.5 and LS1.5, were located at positions that were $9 \pm 1\%$ and $15 \pm 1\%$ of the way up the spine, respectively ($n = 138$, $L_{SF} = 52\text{--}234$ cm), and hence, both sectioning locations were situated within the part of the ray embedded in the body.

AGE ESTIMATION

The mean diameters of the translucent annuli are presented in Table II, for the two sectioning axes, S0.5 and S1.5, and for each age class. Thus, age estimations from spine sections S0.5 were obtained from a total of 3782 fish ranging from 27 to 291 cm L_{SF} , although 115 spines that represented 3% of the total sample were excluded from further analysis as no consensus among readers were achieved after the second round. Age estimations from spine sections S1.5 were obtained from a sub-sample of 381 individuals and only 7% (27) of them were rejected and not used for further analysis. Estimates for *T. thynnus* ranged from 0 to 22 years old, although only ages < 15 years were well sampled (Table II).

COMPARING AGE ESTIMATES AMONG READERS

The c.v. and APE estimates were generally low for the four cross-reader comparisons, with the mean c.v. ranging from 3.1 to 8.3%, which corresponded

TABLE III. The biometric relationships (power and linear equations) between straight fork length (L_{SF} , cm) and several spine measurements (mm) (see Fig. 2)

x	y	x range	y range	n	Equation	r^2	P -value
S0.5	L_{SF}	0.9–20	26–302	2749	$y = 23.407x^{0.8806}$	0.98	<0.001
S1.5	L_{SF}	2.1–14.1	48–271	354	$y = 21.991x^{0.946}$	0.97	<0.001
$L_{spinemax}$	L_{SF}	3.1–34.7	26–291	672	$y = 7.7828x^{1.0345}$	0.98	<0.001
$L_{spinemax}$	L0.5	3.1–31.9	0.4–3.1	282	$y = 0.097x - 0.0212$	0.94	<0.001
$L_{spinemax}$	L1.5	3.1–31.9	0.5–5.1	282	$y = 0.1573x - 0.1012$	0.96	<0.001

x , independent function variables; y , dependent function variables; n , number of specimens.

to an APE between 2.2 and 5.8%. The overall mean PA with respect to the modal age was high in all the comparisons analysed, ranging from 75 to 87% (Table IV).

Overall, the mean relative bias obtained for each of the four cross-reader comparisons was low, with higher values obtained at older ages as expected (see Table IV for further details). The inter-reader bias test was significant in two cross-reader comparisons. Thus, the relative bias was positive in the cross-reader A–B, with an overestimation of age by the reader B (scored as intermediate experienced) whereas, for the cross-reader A–C, the relative bias was negative with the reader C (intermediate) underestimating the age, particularly for ages > 10 years. Nevertheless, both overestimation and underestimation of age were less than 1 year class in most age classes, which indicates low discrepancy between high and intermediate experienced readers. In addition, in the cross-reader A–E–C that included two low experienced readers, the test was not significant and relative values were lower than 0.01, strengthening the reliability of ageing criteria.

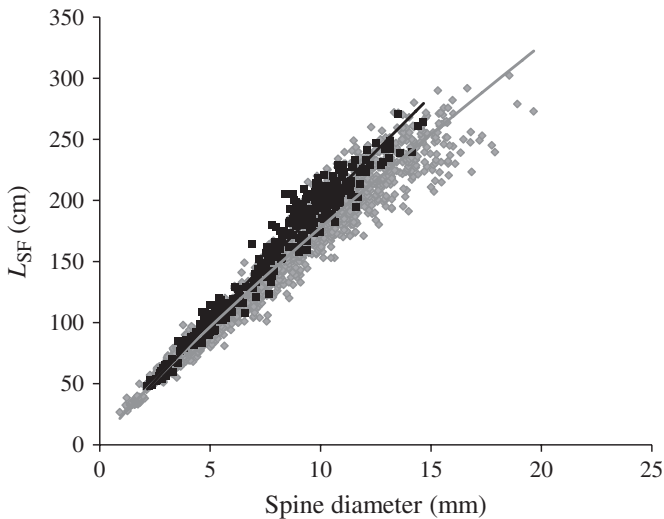


FIG. 4. Relationship between the spine section diameter S0.5 (◆) and S1.5 (■) and straight fork length (L_{SF}) for *Thunnus thynnus* ($n = 2749$) showing the power adjustment (S0.5, —; S1.5, ---).

TABLE IV. Inter-reader comparison results including relative bias, coefficient of variation (c.v.), average per cent error (APE), per cent agreement (PA) and significance level of the inter-reader bias test (*P*). Note that reader C was scored with two categories, low and intermediate, as the reader gained ageing experience during study period

Cross-readers	Reader experience	Spines read by each reader (<i>n</i>)	Years analysed	Relative accuracy			Precision			
				Age range	Mean relative bias	Higher relative bias (age range)	c.v. (%)	APE (%)	PA (%)	<i>P</i>
A-B	High-intermediate	778	2001-2007	1-11	0.070	0.1 (8-11)	3.1	2.22	87.5	<0.01
A-C	High-intermediate	624	1992, 1997, 2001-2010	1-13	-0.010	-0.1(13)	4.4	3.1	81.5	<0.01
A-D	High-high	556	1984, 1999, 2000-2005, 2007-2009	1-14	-0.002	-0.1 to 0.2 (11-14)	4.6	3.27	76.2	>0.05
A-E-C	High-low-low	262	2005, 2007-2009	1-11	0	0.01 (8-11)	8.3	5.84	74.7	>0.05

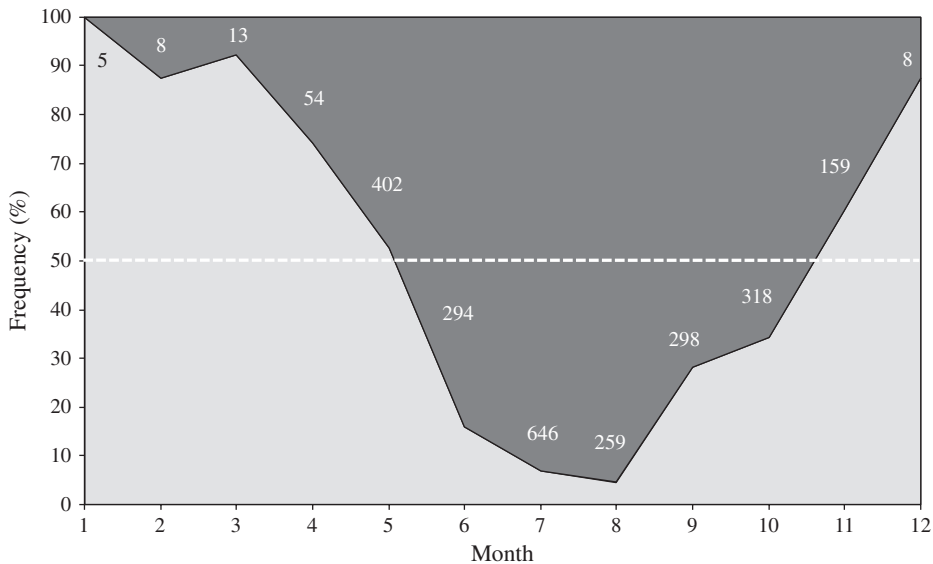


FIG. 5. Categorization of edges (■, opaque edge; □, translucent edge) by month for *Thunnus thynnus* ($n = 2464$). ---, the 50% occurrence of edge type.

INDIRECT VALIDATION: EDGE TYPE AND MARGINAL INCREMENT ANALYSIS

Monthly categorization of spine edge indicated that the highest frequency of translucent bands was low between May and October and then increased gradually during winter months until January and then decreased gradually (Fig. 5). The frequency of opaque bands showed a reverse pattern of fluctuation, increasing during summer months, which suggests annual periodicity in the formation of the translucent bands.

The monthly R_{MI} showed two cycles in juveniles, but it clearly displayed an increasing trend that started in June to October regardless of age class (Fig. 6) that coincided with the formation of opaque bands, which takes place mainly during summer months.

COMPARISON ANALYSIS AMONG SECTIONING LOCATIONS

Deviance analysis for the comparisons of sectioning locations showed significant differences between S0.5 and S1.5 in terms of missing annuli due to vascularization. The age and sectioning location \times age interactions were the main explanatory factors for observed differences (Table V). The number of missing annuli attributed to vascularization increased generally with age for both sections, but it was significantly lower ($P < 0.05$) at S1.5 compared to S0.5, particularly for age class 10 years and older. By that means, in 5 year-old specimens, up to two annuli were obscured due to vascularization. As this trend increased with age, up to five annuli were lost in a 10 year-old specimen (*i.e.* half of the total translucent annuli counted according to the estimated age, were lost), regardless of sectioning location. For specimens aged 10 years and older, however, the number of missing annuli was estimated to be one annulus less at S1.5 than S0.5, *i.e.* in 13 year-old specimens up to six annuli were obscured at S1.5 whereas seven annuli were missing at S0.5 (Fig. 7).

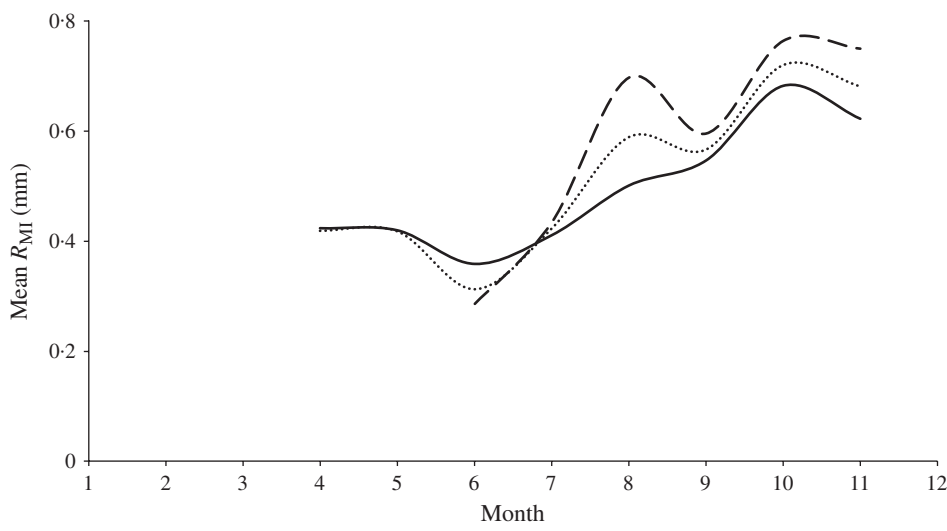


FIG. 6. Mean marginal increment ratio (R_{ML}) by month for *Thunnus thynnus* for juveniles (ages 2–3 years; $n = 423$; —, juveniles), adults (ages > 3 years; $n = 525$; —, adults) and all age classes combined ($n = 948$;, age classes combined).

TABLE V. Summary of analysis of deviance for generalized linear models applied for the comparisons of sectioning locations S0.5 and S1.5. Terms were added sequentially (first to last). Statistical significance $\text{Pr}(>\chi)$ is presented as P -values

	d.f.	Deviance	Residual d.f.	Residual deviance	Deviance (%)	Deviance accumulated (%)	P
Model: Poisson; link: log							
Response: number							
missing annuli							
NULL			1656	3205.9			
Section location	1	28.3	1655	3177.5	0.9	0.9	< 0.001
Age	15	2672.7	1640	504.9	83.4	84.3	< 0.001
Section location \times age	13	4.5	1627	500.3	0.1	84.4	> 0.05
Model: Poisson; link: log							
Response: number							
measured annuli							
NULL			1656	1254.3			
Section location	1	4.6	1655	1249.7	0.4	0.4	< 0.05
Age	15	687.4	1640	562.3	54.8	55.2	< 0.001
Section location \times age	13	15.5	1627	546.7	1.3	56.4	> 0.05
Model: binomial; link: logit							
Response: readability							
scale S1.5 > S0.5							
NULL			19	70.5			
Section location	1	27.9	18	42.7	39.4	39.5	< 0.001
Age	1	22.9	17	19.8	32.4	71.9	< 0.001

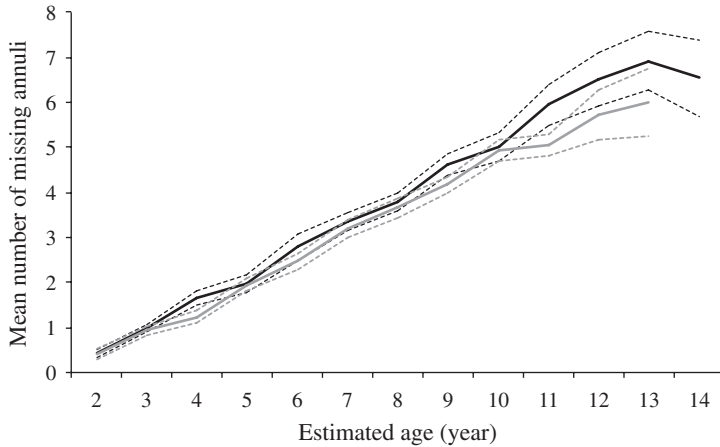


FIG. 7. Mean number of missing annuli attributed to vascularization plotted by estimated age for S0.5 (—) and S1.5 (—). Lower and upper 95% C.I. (----).

The number of annuli that could be measured also differed significantly ($P < 0.01$) with sectioning locations. These differences began at age class 6 years and resulted in one measured annulus more in S1.5 than S0.5 for age class 10 years, and one and a half annuli for age class 13 years (Fig. 8). The age and sectioning location \times age interactions were the main explanatory factors for the observed differences (Table V).

Finally, the readability code showed that S0.5 sections generally received lower scores than S1.5 sections ($P < 0.001$) mainly in older ages [Fig. 9(a)]. The deviance analysis showed that sectioning location and age were the main explanatory factors, with no significant interaction between variables (Table V). These findings indicated

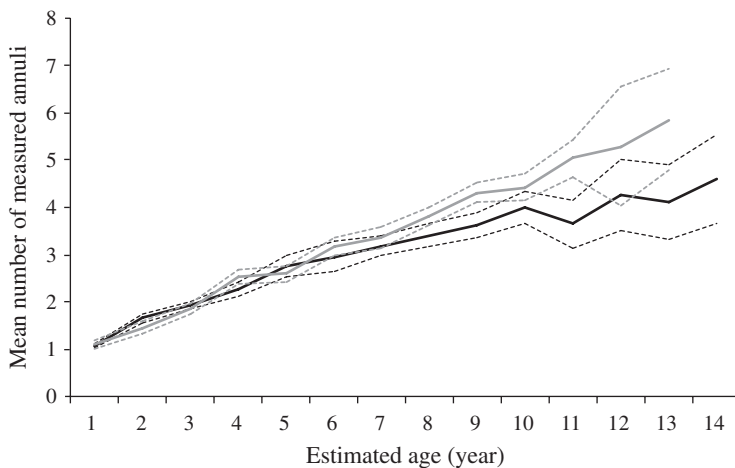


FIG. 8. Mean number of measured annuli plotted by estimated age for S0.5 (—) and S1.5 (—). Lower and upper 95% C.I. (----).

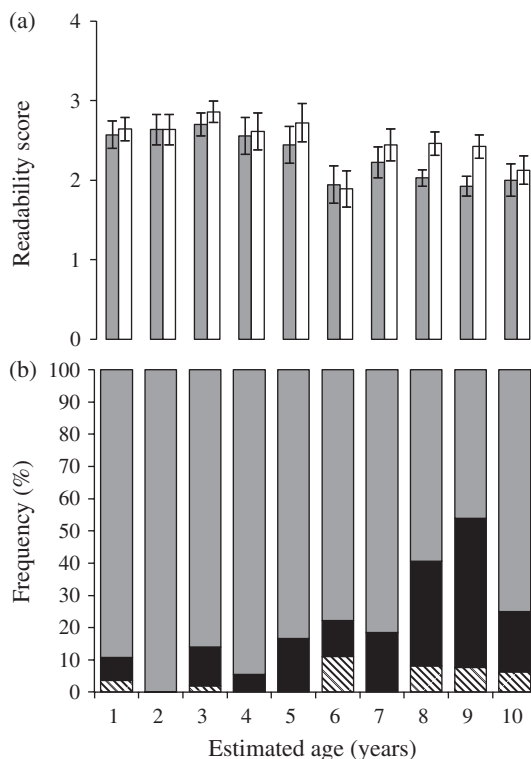


FIG. 9. (a) Readability scores (mean \pm S.E.) defined as 1 = bad, 2 = regular, 3 = good, 4 = picture, for each section (\square , S0.5; \square , S1.5) by estimated age. (b) Per cent frequency of mean readability scores by sectioning location and age (\square , readability score S0.5 = S1.5; \blacksquare , readability score S1.5 better than S0.5; \hatched , readability score S0.5 better than S1.5).

that S1.5 sections had better readability than S0.5 for most age classes, and this trend increased with age [Fig. 9(b)].

GROWTH MODEL PARAMETERS

The age estimates used for growth analysis were obtained from 3667 spine sections (S0.5). The VBGM parameters (L_{∞} , k and t_0) were estimated using both the observed and mean length-at-age data and results of the observed length-at-age data along with those reported in previous studies using the spines for *T. thynnus* direct ageing are displayed in Table VI. Overall, a constant growth rate within the first 7 years was observed, with an inflection at age 8 years, when the growth rate began to decrease gradually (Fig. 10). The potential longevity was estimated at 41 years using the observed length and 31 years for the mean length-at-age data. The VBGM curves fitted using observed length and mean length-at-age data overlapped to age 13 years and beyond this age the VBGM was sensitive to the data used (*i.e.* observed or mean length) (Fig. 11).

Results from the Kimura's likelihood ratio test showed significant differences between growth parameters estimated in this study and those reported by Compeán-Jimenez & Bard (1983) and Santamaría *et al.*, 2009, whereas the test

TABLE VI. von Bertalanffy growth model (VBGM) parameters for eastern Atlantic and Mediterranean *Thunnus thynnus* management units estimated in the present and previous studies using dorsal spine sections for direct ageing of *Thunnus thynnus*. The numbers in parentheses represent the interpreted age plus a correction that takes into account sampling month and the presumed date of birth

	Compeán-Jimenez & Bard (1983)	Cort (1991)	Santamaría <i>et al.</i> (2009)	Current study
Sampling year	1978–1979	1975–1986	1998–2005	1984, 1990–2010
Sampling month	Spring, summer, autumn	Spring, summer	–	Spring, summer, autumn
Area	Bay Biscay, Canary Islands, eastern Atlantic, Mediterranean	Bay Biscay, Gulf of Cadiz, eastern Atlantic	Central Mediterranean	Bay Biscay, Gulf of Cadiz, eastern Atlantic and western Mediterranean
Ageing material	Spine sections	Length distribution and spine sections	Spine sections	Spine sections
Fitting to VBGM				
L_{∞} (cm)	Mean size at age 372.2	Mean size at age 318.85	Mean size at age 373.08	Size at age 381.64
k	0.068	0.093	0.070	0.073
t_0	–1.710	–0.970	–1.760	–1.172
Potential longevity (years)	44	32	43	41
Ages with at least five individuals sampled	1–7, 9, 12–14, 16–17	1–5, 9–15	1–8, 10–14	Mean size at age 327.37 0.097 –0.838 31 0–16

TABLE VI. Continued

Age class	Mean observed L_{5F} (cm)			N
	Compeán-Jimenez & Bard (1983)	Cort (1991)	Santamaría <i>et al.</i> (2009)	
0				117
1	62.69	53.48		670
2	83.05	79.72	62.00	752
3	102.07	100.69	79.60	478
4	119.85	118.84	101.40	312
5	136.47	135.14	115.90	172
6	152.01	150.10	134.50	126
7	166.53	164.04	149.00	162
8	180.10	177.15	161.70	146
9	192.78	190.91	186.50	118
10	203.63	206.21	202.60	81
11	215.71	216.11	205.70	36
12	226.07	222.52	222.58 (10.7)	35
13	235.75	232.38	230.91 (11.8)	31
14	244.80	241.58	238.53 (12.8)	16
15	253.25	247.24	249.44 (13.9)	9
16	261.16		254.35 (14.8)	5
17	268.54		265.58 (15.9)	
18	275.45		263.92 (16.9)	
19	281.90			
20				
21				
<i>n</i> , number of specimens.				1

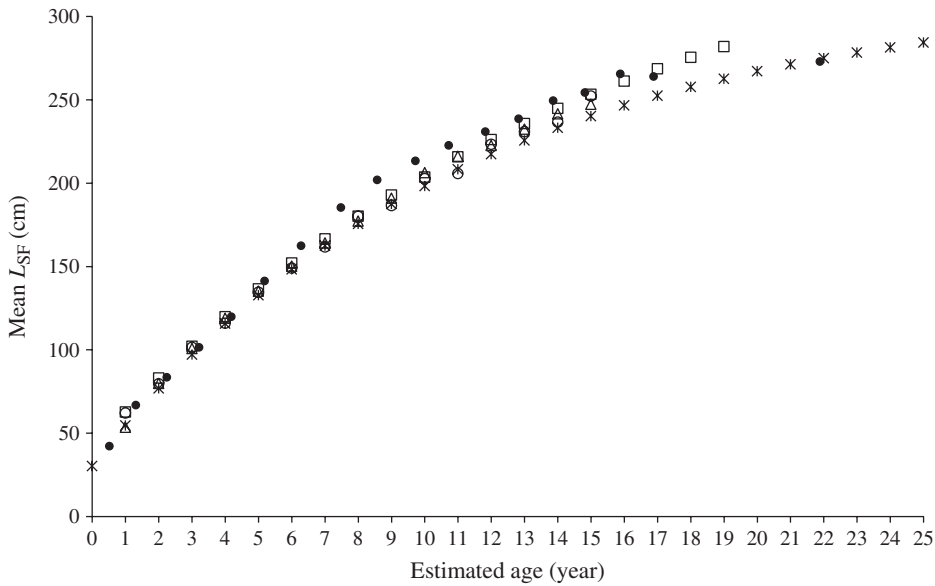


FIG. 10. The mean observed L_{SF} at age data plotted by estimated ages from dorsal fin spine sections of *Thunnus thynnus*. from the current and previous studies (\square , Compeán-Jimenez & Bard, 1983; \triangle , Cort, 1991; \circ , Santamaría *et al.*, 2009; \times , Restrepo *et al.* 2010). The validated otolith based growth curve of Restrepo *et al.* (2010) is also shown (\bullet , current study).

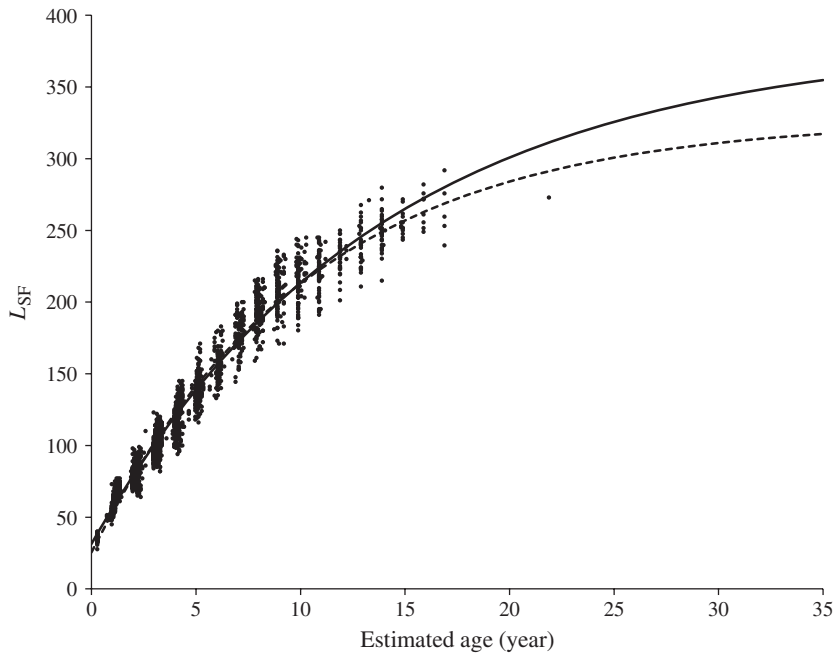


FIG. 11. von Bertalanffy growth model curve fitted to observed length (—) and to the mean length (----) at age data superimposed on the relationship between straight fork length (L_{SF}) and estimated age from spine sections of *Thunnus thynnus* ($n = 3267$).

did not exhibit significant differences when current estimated growth parameters were compared to those reported by Cort (1991) and Restrepo *et al.* (2010), except for the estimated t_0 (Table VII). The plotted relationship between the growth coefficients (k) and the asymptotic lengths (L_∞) obtained in the current and previous growth studies of *T. thynnus* showed dispersion with no clear trends. For example, those estimated values using the same calcified structure and for the same stock fell at both ends of the cloud of dots (Fig. 12).

DISCUSSION

Findings of this study expand existing knowledge of several important methodology concerns that affect direct ageing of *T. thynnus* using dorsal fin spines, including spine nucleus vascularization, the major limitations for using this skeletal structure for ageing purposes, definition of sectioning location, the annual periodicity of annulus formation and evaluation of levels of agreement, precision and relative accuracy among readers are assessed.

An important assumption inherent in growth studies using various hard parts is that the size of fish and the size of hard parts are closely related throughout the life cycle (Casselman, 1987). The strong correlation found in this study between spine diameter at two sectioning locations and L_{SF} indicates that the spine grows proportionally to length and allows for backcalculation of L_{SF} when insufficient length data are available. Similar results have been reported in previous studies of *T. thynnus* sampled in same geographic areas (Compeán-Jimenez & Bard, 1983; Rodriguez-Marin *et al.*, 2007; Santamaría *et al.*, 2009) and hence support the premise that both sectioning axes are good candidates for direct ageing of this species. It is worth mentioning that for some ports and especially for large specimens, extraction of the entire spine in one piece is not allowed and can be difficult consequently the condyle base that is a determinant for allocating the two sectioning location axes is missed. To address this concern, the embedded portion of the spine and location of the two sectioning axes (L0.5 and L1.5) were estimated. By knowing these values and the strong relationship between body fish and spine length, it was possible to determine whether the spine collected was suitable for ageing and to locate the sectioning appropriately without the condyle base as a reference point.

Vascularization of the spine nucleus is the main disadvantage of using this calcified structure for direct ageing. This phenomenon has been described in previous studies (Compeán-Jimenez & Bard, 1983; Cort, 1991; Megalofonou & De Metrio, 2000; Corriero *et al.*, 2005; Santamaría *et al.*, 2009), but very little information has been published regarding its quantification. In this study, it was determined that the phenomenon begins to affect some specimens at 2 years old but seriously affects specimens older than 6 years, with the disappearance of 40–50% of the presumed annuli. The relatively small percentage of spines that were rejected as illegible shows that, if the problem of disappearance of translucent bands because of vascularization was solved, the estimation of age with the use of spines would be an effective and relatively practical method compared with the use of otoliths and vertebrae that involve damage to the fish. To solve the problem of the loss of growth increments due to vascularization, the diameter of earliest translucent bands in spine sections of young fish (with no vascularization) was used to assign an age to the innermost visible translucent band in

TABLE VII. von Bertalanffy growth model parameters estimated by fitting the mean lengths at age from this study and previous studies based on equivalent age ranges. Statistical significance (*P*-value) of likelihood ratio test are given

Age range compared	Current study			Previous studies				<i>t</i> ₀	<i>P</i>	Ageing material	Data
	<i>L</i> _∞	<i>k</i>	<i>t</i> ₀	Authors	<i>L</i> _∞	<i>P</i> -value	<i>k</i>	<i>P</i>			
1–16	324.5	0.10	−0.76	Compeán-Jimenez & Bard (1983)	375.2	<i>P</i> < 0.001	0.07	<i>P</i> < 0.001	−1.73	<i>P</i> < 0.001	Spine
1–15	333.3	0.09	−0.84	Cort (1991)	334.3	<i>P</i> > 0.05	0.08	<i>P</i> > 0.05	−1.17	<i>P</i> < 0.05	Spine
1–15	333.3	0.09	−0.84	Santamaría <i>et al.</i> (2009)	373.1	<i>P</i> < 0.05	0.07	<i>P</i> < 0.001	−1.76	<i>P</i> < 0.001	Spine
1–16	324.0	0.10	−0.76	Restrepo <i>et al.</i> (2010)	314.5	<i>P</i> > 0.05	0.09	<i>P</i> > 0.05	−1.13	<i>P</i> < 0.001	Length distribution and otolith

vascularized spines and hence estimate the number of missing bands. Previous studies that include information about earlier translucent band diameters (Cort, 1991; Farrugia & Rodriguez-Cabello, 2001; El-Kebir *et al.*, 2002; Santamaría *et al.*, 2009) reported values within or very close to the confidence intervals of the values obtained in this study, indicating that the method is robust and strengthens the interpretation of annual translucent growth increments.

A measure of precision is needed to assess the relative ease of establishing the age of a structure, the reproducibility of an individual reader's age determination and the skill level between or among readers. Results from the four inter-reader comparisons performed in this study showed overall low APE and c.v. values. In fact, c.v. for three of the four sets were lower than the recommended reference value of 5% established for fishes of moderate longevity and reading complexity (Campana, 2001). Similar low c.v. results (6.6%) were obtained for age comparison among four readers for Icelandic *T. thynnus* spine samples (Rodriguez-Marin *et al.*, 2007), indicating good precision. In the current study, the highest c.v. and APE values were obtained for the comparison among readers with different ageing experience, indicating that both indices may be affected by differences in ageing skills. PA has been widely used as a measure of ageing precision (Kimura & Lyons, 1991; Campana *et al.*, 1995), which allows comparison among studies. For the four inter-reader comparisons performed in this study, agreement of around 80–90% was obtained for most of the age classes (from 1 to 11 years), and the majority of *T. thynnus* caught belonged predominantly to this age class range.

Determining the time and period of band formation is one of the most critical steps of ageing fishes using hard parts. A variety of direct validation methods exist including bomb radiocarbon analysis, mark-recapture of chemically tagged fishes and radio-chemical dating, whereas indirect validation methods include analyses of marginal increment and spine edge type formation (Campana, 2001). The monthly variation in percent terminal translucent and opaque edges reported in previous studies suggests the formation of growth increments once a year (Sun *et al.*, 2001; Cerna, 2009; Griffiths *et al.*, 2010). The appearance of translucent bands in November and May has been described in *T. thynnus* using several calcified structures such as otoliths, spines and vertebrae (Mather & Shuck, 1960; Butler *et al.*, 1977; Farrugio, 1980; Hurley & Iles, 1983; Lee *et al.*, 1983; Cort, 1991; Foreman, 1996). Identification of an entire annual pattern in edge band formation has not been possible in previous studies because the seasonality of the *T. thynnus* fisheries makes it difficult to obtain samples from November to May. In this work, annual deposition of translucent and opaque bands was confirmed by edge type analysis. This follows a sinusoidal cycle, indicating an annual pattern of deposition of bands. In addition, the increasing trend of the R_{MI} from June to October that coincides with the formation of opaque bands strengthens the expected seasonality in the formation of the growth bands. Nevertheless, the mean R_{MI} displayed a pattern of two cycles in juveniles instead of an expected sinusoidal pattern. This might be an indication of the presence of sub-annual translucent bands observed in some of examined spine sections. The formation of sub-annual bands has been related to migrations or different diet regimes (Compeán-Jimenez & Bard, 1983; Cort, 1991; Tserpes & Tsimenides, 1995; Sun *et al.*, 2002) as well as to spawning events when animals invest more energy in producing gametes than in somatic growth (Sun *et al.*, 2001; Cerna, 2009). Even though analysis of the cause of their formation is beyond the scope of this study, discrimination of false increments from true annuli is a key factor

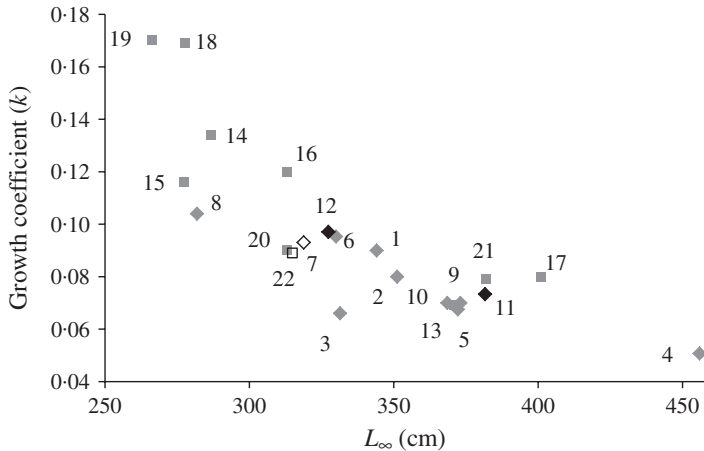


FIG. 12. Relationship between *Thunnus thynnus* growth coefficient (k) and asymptotic length (L_{∞}) estimated in this study (\square) and in previous studies (\diamond), including eastern (\blacklozenge) and western (\blacksquare) management units. Studies included: 1, Rodríguez-Roda (1964); 2 and 3, Farrugio (1980) (length structure and vertebrae, respectively); 4, Arena *et al.* (1980); 5, Compeán-Jimenez & Bard (1983); 6, Hattour (1984); 7, Cort (1991); 8, Rodríguez-Cabello *et al.* (2007); 9 and 10, Santamaría *et al.* (2009) (observed and backcalculated, respectively); 11 and 12, current study (observed and mean length, respectively); 13, Mather & Shuck, 1960; 14 and 15, Butler *et al.* (1977) (males and females, respectively); 16 and 17, Farber & Lee (1981) (mark-recaptured and vertebrae, respectively); 18 and 19, Hurley & Iles (1983) (males and females, respectively); 20, Parrack & Phares (1979); 21, Turner & Restrepo (1994); 22, Restrepo *et al.* (2010).

in ageing analysis and has the potential to be a large source of error (Kopf *et al.*, 2010). Based on observations, these double translucent bands appeared too close together to be considered a year mark and they were usually distinguishable within the general pattern of wide summer opaque bands. This study, and previous studies using spines for *T. thynnus* direct ageing suggest that it is possible to discriminate true annual events from false ones, but age estimates from spines were not validated and thus the problem of ageing remains, for the most part, unresolved. Further research is required in this context for reliable indirect validation of the ageing method using R_{MI} analysis.

Sectioning location is an important factor to be considered because the appearance and location of annuli may vary between sectioning levels (Kopf *et al.*, 2010). In this context, how an alternative sectioning location (S1.5) could reduce the influence of nucleus vascularization to improve recognition of the innermost translucent bands was analysed. Results indicated that the number of missing bands due to vascularization was significantly higher in the traditional S0.5 section than in the S1.5 section, particularly for specimens of > 10 years. Moreover, it was possible to measure more translucent bands in the S1.5 section than in the S0.5 section, particularly for medium-sized specimens aged 6–13 years. Thus, use of this location for sectioning was a clear improvement over the use of the traditional S0.5 location, and this finding is an important contribution to the methodology of using dorsal fin spines for ageing the whole length range of *T. thynnus*.

Overall, the mean length-at-age and the timing of the growth rate inflexion point were similar in this and previous studies that used spines for direct ageing of *T. thynnus* (Compeán-Jimenez & Bard, 1983; Cort, 1991; Santamaría *et al.*, 2009).

In this study, however, the age-at-size estimates were advanced by *c.* 1 year from age 7 years upwards in comparison with data from previous studies. Discrepancies between present results and those from previous studies may be due to the edge interpretation criterion, which is crucial in direct age estimation but it has not been considered in previous studies. Sampling season is also an important factor to consider when comparing different ageing studies and even when comparing ages within the same study.

Furthermore, differences in age interpretation between studies occur mainly in age classes 7–11 years that were poorly sampled in preceding studies. Cort (1991) also used different methods to estimate mean lengths of different aged fish, including length analysis for ages 1–8 years and spine age interpretation for ages 9–15 years. Slight differences in mean length-at-age may not be reflected in the estimated growth curves, but they are important in the construction of age-length keys and therefore in estimating the demographic composition of the catch. Other ageing studies using vertebrae (Olafsdóttir & Ingimundardóttir, 2003) and otoliths (Hurley & Iles, 1983) reported similar results to the present mean length-at-age data within the age range 7–11 years.

In previous studies that used direct ageing of *T. thynnus* spines, growth parameters were estimated using mean length at age to fit the growth function (Compeán-Jimenez & Bard, 1983; Cort, 1991; Santamaría *et al.*, 2009). This is a common practice that has been applied in other species (Chiang *et al.*, 2004; Cerna, 2009). The conventional VBGM, however, can produce unrealistic estimates of growth parameters, especially when there are few samples of very large fish (Pauly, 1979). Because of this argument, the western *T. thynnus* growth stock function was revised (Neilson & Campana, 2008; Restrepo *et al.*, 2010). The present results from fitting observed length to age data and mean length to age data differed after age 13 years, which illustrates the sensitivity of parameter estimation to the method applied due to the lack of fish samples of > 15 years. This fact affects both data sets, but it is more pronounced when using raw data (length-at-age), because there are few samples of older ages in comparison with the previous ages. When mean length-at-age is used, however, all ages are given the same weight in the fit, regardless of the number sampled.

The L_{∞} obtained in the current study using the observed length-at-age data (382 cm) is similar to the maximum L_{SF} found in eastern *T. thynnus* catch-at-size data from the ICCAT for the study period (398 cm) and in recent years (383 cm). It is also similar to the values estimated by Compeán-Jimenez & Bard (1983) (372 cm) and Santamaría *et al.* (2009) (373 cm). Moreover, the growth parameters estimated using mean length at age ($L_{\infty} = 327$, $k = 0.097$) in this study do not differ significantly from those based on the growth functions used by ICCAT for both eastern (Cort, 1991) and western (Restrepo *et al.*, 2010) *T. thynnus* management units, implying that they all follow the same growth pattern. It should be noted, however, that the asymptotic length estimated by Restrepo *et al.* (2010) might be a slight underestimate because it was well below the length of largest specimens found in the catches of the ICCAT database. These giant fish represent a low proportion of the total (22 specimens from the ICCAT database sampled during the study period were 330–398 cm) and therefore do not affect the demographic composition of the population, but they still must be considered.

The L_{∞} and k estimated in the current and previous growth studies of *T. thynnus* plotted in Fig. 12 show that estimates obtained in this study were within

this dot distribution, indicating that the dorsal spine can be considered a suitable calcified structure to use for ageing, at least for small and medium-sized *T. thynnus*. Preliminary results of the comparison of otolith and spine age interpretations coming from the same specimen show a good fit to a linear relationship between both age estimations in age classes ≤ 10 years (Rodríguez-Marin *et al.*, 2013) strengthening the results. This is particularly important for the eastern Atlantic population for which the current growth curve is actually based on estimates using this structure. It appears that spines may underestimate age compared to otoliths in older age classes (*i.e.* > 10 years) (Rodríguez-Marin *et al.*, 2013), thus caution is needed, especially for large fish, until the ageing method is directly validated. Moreover, this study included a wide sampling of the first half of this species' life history and its geographical distribution, but a much broader range of body sizes is needed to accurately describe the growth of *T. thynnus* using this method.

The tag-recapture method has been applied to the eastern Atlantic management unit (Rodríguez-Cabello *et al.*, 2007), although recapture data are subject to several sources of uncertainty, which has led to the use of direct ageing techniques (Restrepo *et al.*, 2010). The potential value of bomb radiocarbon for validating age interpretations in *T. thynnus* using available spine samples has been explored recently (Rodríguez-Marin *et al.*, 2013). Despite being at the very early stages of progress, results indicate that the spine contains radiocarbon at concentrations consistent with expectations and therefore its radiocarbon chronology is consistent with accurate age interpretation, but also consistent with under-ageing. Hence, given the lack of direct validation and lower maximum age obtained compared to western *T. thynnus* (and Pacific bluefin tuna *Thunnus orientalis* ((Temminck & Schlegel 1844) and southern bluefin tuna *Thunnus maccoyii* (Castelnau 1872)), results of this study suggests that the age data obtained for small- and medium-sized fish could be used in integrated growth models, which are crucial for *T. thynnus*. Direct validation is required, however, before ages from large fish can be included.

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