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A REVIEW OF DIRECT AGEING METHODOLOGY USING DORSAL FIN SPINE FROM ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*).

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SUMMARY

This paper aims to fill the limited literature description of important areas of concern that may influence age estimates of ABFT using spine cross-sections methodology. A total of 3862 ABFT were sampled from the North East Atlantic and the Mediterranean Sea over 21 year period. The first spiniform ray of the first dorsal fin (spine) was removed and cross-sectioned near the condyle base in order to count annulus for age determination. The regression between spine diameter and SFL showed high determination coefficient indicating that the size of the spine and fish body were closely related. Results from edge type and marginal increment analysis confirmed an annual periodicity of annulus formation. In assessing spine nucleus vascularization, approximately half of the total number of inner annulus were missing. A new spine sectioning level showed a clear improvement respect to the "traditional" location reducing the influence of nucleus remodelling.

Mean lengths at age obtained in this and previous studies using spines, show similarities, appearing a small disparity of only one year difference from age 7. Differences were observed when fitting the growth model to observed length at age or mean length at age data, illustrating the sensitivity of parameters estimation to the method employed. The estimated von Bertalanffy growth parameters (L_∞ *=382.7; k=0.070* and t_0=-1.334*) were compared to those reported in the literature, showing some differences. The estimated* L_∞ *is more realistic in relation with the maximum length found in the ABFT catches for the eastern stock and hence more reliable than the currently asymptotic length adopted for this stock. Our growth rate results indicate that spine sections can be used as an alternative to otoliths for ABFT age estimations*.

RESUMEN

El objetivo de este manuscrito es documentar importantes áreas que puedan influir en la estimación de la edad de atún rojo a partir de la de secciones de radios espinosos. Un total de 3862 ejemplares fueron muestrearon procedentes del Atlántico Noreste y el Mar Mediterráneo, durante 21 años de estudio. El primer radio espinoso de la primera aleta dorsal fue extraído y varias secciones se obtuvieron cerca de la base del cóndilo, con el fin de contar los anillos para la determinación de la edad. La relación entre el diámetro de la espina y la SFL mostró índices de determinación ($r)^2$ *altos, indicando que el tamaño de la espina y del pez crecen de forma proporcional. Resultados del análisis del borde y del crecimiento marginal confirman una periodicidad anual en la formación de los anillos. En la cuantificación de la vascularización del núcleo, aproximadamente la mitad del total de anillos estimados se pierden. Se experimentó un nuevo punto de corte, mostrando un claro mejoramiento con respecto al tradicional punto de corte, reduciendo así la influencia de la vascularización.*
Las tallas medias por edades obtenidas en éste y anteriores estudios usando espinas, mostraron similitudes, apareciendo una disparidad de un sólo año de diferencia en la edad 7. Diferencias se observaron en el ajuste del modelo de crecimientos tomando tallas observadas por edad ó

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tallas medias por edad, ilustrando la sensibilidad de la estimación de parámetros al método empleado. Los parámetros de crecimientos obtenidos de la ecuación Von Bertalanffy (L_∞ *=382.7; k=0.070* and t_0=-1.334*) fueron comparados con previos estudios, mostrando diferencias. La longitud asymptótica estimada* L_∞ *es más realista con la máxima talla encontrada en las capturas de ABFT para el stock del Este y por tanto más fehaciente que la adoptada actualmente para éste stock. Nuestros resultados de la tasa de crecimientos indican que las secciones de espinas pueden usarse como alternativa a los otolitos para la determinación de la edad de ABFT.*

KEYWORDS

Atlantic bluefin tuna, age determination, spine nucleus vascularization, growth curves, marginal increment ratio, longevity.

Introduction

Biological studies on age, growth and reproduction of fish are crucial components for describing their life history. Successful fisheries management is based on the knowledge of these species-specific life history data. Age, body length and weight data are important tools in fishery biology since details of species growth and mortality rates, age at maturity and life span can be calculated from such information (Gulland 1983). Current assessment for Atlantic bluefin tuna (*Thunnus thynnus*) (ABFT) is based on age-structured models, therefore the ability to accurately estimate age of ABFT is critical for conducting stock assessment developing management strategies for a sustainable fishing activity. Recently, radiocarbon techniques were used to develop a new growth curve for the western population of ABFT. Results indicate slower growth and older ages than were previously assumed, affecting the calculation of benchmarks of productivity, and by extension, the rebuilding schedules for the resource (Neilson and Campana, 2008; Restrepo *et al.,* 2010). The present stock depletion of this species makes essential to improve all kind of research towards an effective stock assessment (Fromentin and Powers, 2005).

A wide variety of ageing methods have been applied to ABFT including modal analysis of length frequencies, tagging studies as well as the examination of calcified structures (for a revision see Rooker *et al.*, 2007). Concentrating on the later method, several studies have estimated age and growth of Atlantic bluefin tuna directly by counting incremental growth marks present on calcified structures such as scales, vertebrae, otoliths, and spines. In the last ageing workshop for ABFT, a review of previous direct ageing studies using different hard parts was presented showing the advantages and disadvantages of each calcified structure for age interpretation (Rodriguez-Marin *et al*., 2007).

Fin-ray spine cross sections have been used to age fishes (Beamish, 1981; Chilton and Beamish, 1982), notably pelagic fishes such as billfishes and other tuna species (Prince and Pulos, 1983; Kopf *et al*., 2010). Spines are considered the easiest structure to collect compared to other calcified structures such as otoliths which are particularly small in tuna species. Moreover, sampling does not require damage or processing the specimen which is impractical in certain fisheries. In addition, spine preparation method is less consuming time than vertebrae and otolith, as this only requires obtaining transverse sections but staining is not necessary (Stéquert and Conand, 2004; Rodriguez-Marin *et al.,* 2007).While fin spines are considered to provide reliable results for ageing younger fish, age estimation of adults is difficult because of the reabsorption of the first growth marks due to nucleus vascularisation, which increases with age or body length (Panfili *et al.,* 2002; Kopf *et al*., 2010). The reabsorption of the central portion of the spine makes necessary to use the measurements of the first rings of younger specimens, to date the first visible ring of older specimens. This replacement method developed by Hill et al. (1989) has been used in other pelagic fish species such as swordfish (Tserpes and Tsimenides, 1995), sailfish (Chiang et al., 2004) and striped marlin (Davie and Hall, 1990) and has been employed in ABFT since. the first ray of the first dorsal fin was first used to obtain age estimations for this species (Compeán-Jimenez and Bard, 1983).

Nucleus vascularisation of spines in ABFT is considered the mayor limitation in using dorsal fin spines as a calcified structure for ageing purposes and may result in age underestimation or overestimation of growth. This resorption phenomenon has not been quantified in previous studies. Moreover, the methods for processing and interpreting this calcified structure for age estimation have not been clearly defined (e.g. sectioning axis locations) or do not cover a wide age range (Compeán-Jimenez and Bard, 1983; Cort, 1991; Megalofonou and De Metrio, 2000; Santamaría *et al*., 2009). This paper aims to fill the limited literature description of important areas of concern cited above that may influence age estimates of ABFT using spine cross-sections. This include a definition of spine in terms of its biometry relationships, section location, quantification of the spine nucleus vascularization with further definition of the method used to replace the missing annuli, to establish a criteria for identifying and measuring annuli, to assess the annual periodicity in the formation of translucent and opaque bands as well as to estimate spine age reading precision.

Material and Methods

Sampling

A total of 3862 ABFT were sampled from the North Atlantic (n=3164) and the Mediterranean Sea (n=698) over 21 year period from 1990 to 2010, including some spine samples from medium size specimens from 1984 (**Table 1**). This wide sampling period was used with the aim of covering as much as possible the whole size range and months of the year. Sampling was based on port landings from different fisheries and geographic areas in the North East Atlantic and the western Mediterranean including bait boat from the Bay of Biscay, long-line from the south of Island, Gulf of Cadiz and western Mediterranean and Atlantic traps near the Strait of Gibraltar. Straight fork length (SFL) was measured in each tuna sampled to the nearest cm. Specimens caught in the NA ranged from 51 to 291.8 cm length whereas specimens caught in the Mediterranean Sea ranged 26.6 to 251cm (**Table 1**). Date and location of capture were recorded.

Fin spine selection, extraction and conservation

According to Compeán-Jimenez and Bard (1983), growth marks were well evident on the first dorsal spines of bluefin tuna caught from the Atlantic. Consequently, it has been recommended to use them since they were considered a reliable source of age and growth information. The first spiniform ray of the first dorsal fin (hereafter spine) was removed. Spine was extracted complete, including the condyle where the spine inserts in the fish. The sectioning axis location is relative to condyle measurements, hence it is important to extract the entire spine including the base. For small specimens, it was not difficult to remove it but for larger ones, a sharp knife was needed to cut carefully the strong ligaments that support the spine base deep in the fins insertion in body depression. Moreover, care was taken in order not to twist the spine in its base. Spines were cleaned by removing the connective tissue from its base and dried off at room temperature. Spines were stored dry in a paper envelope (for a detailed description of sampling, preparation and ageing interpretation criteria from ABFT dorsal fin spine see Rodriguez-Marin *et al.* (2011).

Fin spine preparation and sectioning axis location

Following Rodriguez-Marin et al. (2007) description of the sectioning axis location, its position was established by measuring the maximum spine diameter called *Dmax* along an imaginary line that passes below the hollows located near the condyle base (**Figure 1**). The cutting axis was allocated by taking half of the *Dmax* from the same imaginary line defined above obtaining the spine section called *S0.5*. We also analysed whether a different sectioning location could improve our age interpretation. Thus, a second cutting axis was established near above, i.e. at 1.5 times the *Dmax,* obtaining a second spine section called *S1.5.* Both sectioning locations differ in the distance from the basal part of the spine, being *S0.5* the "traditional" section used in previous ABFT ageing studies, and *S1.5*, the experimental one proposed in the present study (**Figure 1**).

Spine sections were obtained using a precision rotating diamond saw working at two speeds, 180 rpm for cutting spines of juveniles and 4500 rpm for spines of adults. Section thickness usually ranged from 0.45 to 0.60 mm. Sections were washed in ethanol at 70% and dried out on blotting paper. Spine sections were finally mounted on glass slides and covered with a highly transparent resin named Eukitt Mounting Medium (Electron Microscopy

Sciences, Hatfield, PA), to fix the sections permanently and store them over time. The slides were labelled with an identification code, fish body length and the date of capture.

Age interpretation and reading criteria

In a cross sectional cut of the spine, different optical zones based on their relative translucency appeared as translucent and opaque bands. Sections were examined under transmitted light, appearing the translucent and opaque bands, bright and dark, respectively. Following the assumption considered in previous ageing studies (Mather and Schuck, 1960; Butler *et al.*, 1977; Hurley and Iles, 1983; Compeán-Jimenez and Bard, 1983), in which a translucent band followed by an opaque one were laid down on an annual basis, age was estimated by counting the translucent bands considered annually formed. However, these annual marks were not always a simple bipartite structure and very often multiple translucent and opaque pair banding appeared (Rodriguez-Marin *et al.*, 2007).

In young individuals it is easy to identify the translucent and opaque bands formed on the spine. However, in fish over two years old, the central area of the spine begins to reabsorb and the bands consequently disappear (**Figure 2**). To overcome the problem of reabsorption of rings with age, the translucent band diameters measured from spines without reabsorption (i.e. spines from young specimens) had to be used to assign an age to the first inner visible translucent band in reabsorbed spines. In Figure 2 it is shown how the maximum spine section diameter and translucent bands were measured. A reference table was constructed based on the mean maximum diameter width of the translucent bands by presumed age for the two sectioning axes, *S0.5* and *S1.5* (how to measure translucent band diameters is detailed in Rodriguez-Marin et al., 2011), (**Table 2**). Once the age of the first inner visible translucent band was estimated, final ages were calculated by adding the number of translucent bands estimated to lie within the vascularised area and the number counted between the area of reabsorption and the margin. This technique is based on the assumption of a high correlation between fish length and its maximum spine diameter (Compeán-Jiménez and Bard, 1983; Rey and Cort, 1984; Rodriguez-Marin *et al.*, 2007). Following the Rodriguez-Marin *et al*. (2007) border interpretation criterion, an ABFT tuna with a translucent band formed at the edge of the spine section and caught at the beginning of the year was interpreted as having one year more, although there were still five or six months before its true date of birth (Rooker *et al.*, 2007), whereas when the fish was caught in autumn, this band was not considered as one year more.

Spines direct ageing was carried upon digital images that were captured using a binocular lens magnifier connected by digital camera NIKON. An image analyser (Nis-elements D 3.0 Nikon software) was used to measure the maximum spine diameter as well as diameter for successive growth bands. Spines sections were read at least by two independent readers. For those spines that there was a disagreement between readers, an additional reading was achieved and the final estimated age assigned was the consensus among readers.

Moreover, spine sections were scored according to their quality into one of the four quality grades defined as follows: “Bad quality” which included sections with no clear banding pattern. Nucleus vascularisation was affecting most of the spine section and consequently no annulus measurements could be recorded. Uncertain with age estimation and been likely the rejection of the sample for further analysis. “Regular quality” in which the spine section showed a banding pattern but multiple rings (not annually interpreted) appeared. Slightly uncertain with age estimation. Measurements of rings interpreted as annual do not fit with the mean annulus diameter values given by the reference table. “Good quality” the banding pattern was also present but in contrast with the latter, measurement of annulus fitted with the mean annulus diameter values. Hence, confidence with age estimates. Finally a section was considered “picture quality” when the banding pattern was present and annulus clear in contrast. Meaning sure accuracy in age estimates.

Biometric analysis

Several biometric measures were recorded for each spine in order to analyze the relationship between the growth of the spine and the specimen sampled. This included the total spine length *(Lmax),* the maximum spine diameter *(Dmax)* and distance from the condyle base to the two sectioning axes, i.e. *L0.5* and *L1.5,* respectively (see **Figure 1** for further details). *Lmax* was measured in mm to one decimal whereas *Dmax, LS0.5* and *L1.5* were measured in mm to two decimal places.

The relationship between spine diameter for the two sectioning levels (i.e. S0.5 and S1.5) and the straight fork length (SFL) was determined using standard regressions procedures performed in attempt to verify the proportionality between the spine diameter and the individual body size. Moreover, the relationships between different spine length measurements (i.e. *Lmax*, *LS0.5* and *L1.5*) and SFL were also analysed in order to understand the growth of the spine and to estimate certain lengths from others. Linear and Power regression equations (of the types *SFL= a+b D0.5(1.5)* and *SFL= aD0.5(1.5)b*) were tested for all relationship cited above. The coefficient of determination (r^2) was used as index of the goodness of the estimates.

For medium and giant ABFT it is difficult to extract the entire first spiniform ray and for certain fisheries sampling only allows ray dissection from outside of the tuna body. This procedure involves the loss of the condyle base and thus the reference for section location. With the aim to quantify what do the body embedded portion of the spiniform ray represent in relation to the total length of the ray, the external part of the ray was measured

Timing and periodicity of annulus formation: *Edge type and Marginal increment Ratio (MIR)*

The time of annulus formation was assessed by examining the outermost margin (i.e. edge type) of each spine section. An edge was considered translucent or opaque when the margin of the spine displayed a clearly translucent or opaque band extending along greater than a half of the perimeter of the spine section. The percentage of edge type (i.e. translucent and opaque) was pooled for each month. If growth increments were deposited annually, a single peak could be expected in the monthly frequency of each margin type over a year.

We also analysed the Marginal Increment Ratio (MIR) by month as another measure to support the annual formation of growth increments. The MIR calculates the percentage of annulus completed at the edge by comparing the width of the opaque edge to the width of the previous annulus. The Marginal Increment Ratio (MIR) was estimated as follow:

$$MIR = (S - d_n) / (d_n - d_{n-1}),$$

where S is the spine diameter section, d_n is the diameter of the ultimate (n) translucent band, and d_{n-1} is the diameter of the penultimate translucent band.

MIR was analyzed for three age classes, juveniles (aged 2 to 3 years old), young spawners (from 4 to 8 years old) and adults (older than 8 years old) as well as for all combined age classes, considering the length at sexual maturity for East and Mediterranean ABFT population (Corriero *et al.*, 2005).

Quantifying Spine nucleus reabsorption

Spine nucleus reabsorption was quantified by estimating the number of translucent annulus lost by age using as a baseline the first inner translucent annulus visible (i.e. partially reabsorbed) and the first inner translucent annulus measured (i.e. complete band no reabsorbed). The proportion of readable spine was estimated by analyzing the ratio between the total spine diameter and the diameter of inner annulus that could be measured at the two sectioning levels (i.e. *S0.5* and *S1.5*).

Comparing spine sections at two sectioning locations

The two spine sections (i.e. *S0.5* and *S1.5*), described earlier in the manuscript, were compared using a Chi-square test in terms of the proportion in the number of the inner translucent bands missing due to reabsorption, the quality of spine sections and the number of translucent bands that could be measured. Data were separated in two age groups as follow: group 1 for specimens younger than 7 years old and group 2, for specimens older than 6 years old. Null hypotheses (i.e. no significant differences between sectioning axes) was rejected with a significance level (P-value) <0.05.

Inter-reader comparison: to estimate ageing precision

Five inter-reader comparisons were selected for analysis based on age range, number of readers involved, reading experience and sample size, ensuring that most of the ages were well represented. Readers were scored according to their experience in one of the three following levels: level 1 included experienced reader; level 2, middle experienced reader and level 3 inexperienced reader. Two indices (i.e. statistically sound measures, Campana, 2001), the average per cent error (APE) and the coefficient of variation (CV) were used to estimate spine ageing precision among readers. APE was estimated by using the Beamish and Fournier (1981) recommended formula as follow:

$$APEj = 100 \times \frac{1}{R}\sum_{i=1}^{R}\frac{[X_{ij} - \overline{X}_j]}{\overline{X}_j}$$

where, X_{ij} is the ith age determination of the jth fish, $\overline{X}_j$ is the mean age estimate of the jth fish and *R* is the number of times each fish was aged.

The mean CV was estimated by using the European Fish Ageing Network (EFAN) software (Eltink *et al.*, 2000) following the recommended formula.

$$CV = \frac{100}{n}\frac{\sqrt{\sum_{i=1}^{R}\frac{(Xij - \overline{Xi})^2}{R-1}}}{\overline{Xi}}$$

where, *n* is the number of spines, *R* is the number of readers, X_{ij} is the *j* value of age estimation for the spine *i* *and* $\overline{X}_i$ is the average age calculated for the spine.

Moreover, for testing differences in estimates among readers, an inter-reader bias test was applied using *Statistica V.6* software (variables were treated as dependent). Null hypotheses (no significant differences) was rejected with a significance level P-value <0.05.

Growth parameters

A standard Von Bertalanffy growth function was fitted to lengh at age data derived from spines to characterize the growth of Atlantic Bluefin tuna (von Bertalanffy, 1938).

$$L_t = L_\infty(1 - e^{(-k(t-to))})$$

where *Lt* is the length (SFL) at age t, L_∞ is the asymptotic length that bluefin tuna may attain if fish lived indefinitely, *k* is the growth coefficient at which L_∞ is asymptotically reached and to is the hypothetical age at length 0.

An exploratory analysis of all ages at age showed some inconsistencies in the Mediterranean set, thus only East Atlantic data was used in the growth model. Fish growth equation was estimated from all length at age data instead of using mean length at age values (S. Campana, personal communication). Anyway, this last fitting was using mean length at age was also calculated in order to check sensibility of both models to data. When the mean length at age fitting was used, only ages with at least five reading samples were considered. Age was estimated as interpreted age, plus a correction that takes into account sampling month and the presumed date of birthday of the first of July, since spawning in the western Mediterranean occurs from middle June to middle July (Rooker *et al.*, 2007).

To check the plausibility of the growth equation parameters obtained in the fit of our data to the von Bertalanffy equation, we obtained the maximum SFL of ABFT catches in the study period and estimated the potential longevity. The potential longevity of the species was calculated using Pauly and Munro's formula (1984): $AGE_{max} = 3/k$.

Results

Biometric analysis

Several biometric relationships were analysed. Results of the regression equations are displayed in **Table 3**, showing the parameters for both, the linear and power equations obtained in each relationship. Although, regressions of the two spine measurements (i.e. spine diameter and length) *vs* SFL were best described by power equations, both equations showed good fit indicated by high determination coefficients (r^2) (**Table 3**). The strong relationship between the maximum spine diameter *vs* SFL (**Figure 3**) showed that the fish body length and the size of the calcified structure were closely related throughout the entire life cycle.

Our results show that by sectioning the first spiniform ray at the outside of the tuna body and not extracting the entire spine with its condyle base, a 13% of the total length of the spiniform ray is lost. *L0.5* and *L1.5* section locations represent 10% and 15% of the total spiniform ray length, respectively. These results indicate that only *L1.5* section location is situated within the external part of the ray.

Timing and periodicity of annulus formation: Edge type and Marginal increment Ratio

The monthly proportion of edge type was examined in ABFT spine sections. Results showed that the appearance of the translucent band occurs from September to May, with a 50 % of occurrence between November and April (**Figure 4**). This pattern indicated an annual periodicity in the formation of the translucent bands.

Moreover, to estimate the periodicity of annulus formation, we also analysed the Marginal Increment Ratio (MIR). Results plotted in **Figure 5** showed that the mean monthly MIR described a sinusoidal cycle for the three age groups, with a clear increasing trend from June to October, for juveniles and young spawners, and from July in adults (**Figure 5**). This increasing pattern coincided with the formation of opaque bands that takes place mainly during summer months. Moreover, the mean of MIR increased more sharply in juveniles and young spawner than in adults.

Reabsorption

In attempt to quantify the extent of nucleus vascularization (i.e. reabsorption) present in ABFT spine sections, we estimated the number of missing annulus by age, considering either both the inner translucent annulus partially reabsorbed or the inner annulus with no reabsorption. Results indicated that nearly half of the total number of inner annulus used for assigning final age estimates was lost due to reabsorption (**Figure 6**). Nucleus vascularization represents a loss of approximately 65 to 70% of the total spine diameter.

Comparing the two sectioning axis locations

ABFT spines section locations (i.e. S0.5 and S1.5) were compared by means of the proportion of the number of inner annulus lost, spine section quality and number of annulus that could be measured. Results showed significant differences between the two sections, showing a bigger reabsorption in the section location at *S1.5* than in *S0.5* (**Figure 6 a, b**). However, regarding the quality and the number of annulus measured, sections at *S1.5* were significantly better than *S0.5*, particularly in older specimens (**Figure 6 c, d**).

Inter-reader comparison: to estimate ageing precision

To estimate the reproducibility of fin spine age estimates (i.e. ageing precision) and the relative accuracy, the average percent error (APE) and the Coefficient of Variation (CV) were estimated for the five inter-reader comparisons selected (**Table 4**). Overall, the CV and APE values were low for the five inter-reader comparisons. In four of the five comparisons analysed, the CV values were lower than 5%. Inter-reader bias test was significant in comparisons that involved two readers whom differ in their ageing skills (**Table 4**). Moreover, CV (%) estimated for readers combined in each comparisons did not show a clear trend throughout the age range for all well sampled ages (i.e. from 1 to 10 years old) and no increasing pattern with age was found.

Growth parameters

Estimates of age were made from the spines of 2597 fish that ranged from 53 to 292 cm SFL. The oldest ABFT in our samples was estimated to be 22 years, although ages used predominantly for estimation are from 17 years backwards. Mean lengths at age are presented in **Table 5**. Estimated growth curves fitting the von Bertalanffy model to length at age and to mean length at age are presented in **Figure 8**. Differences were found between both curves showing sensitivity to the different procedure employed. **Figure 9** shows growth curves from the present and previous studies using spines for ABFT direct ageing. Estimated growth parameters are listed in **Table 5**, together with those reported in the literature.

The maximum SFL of the sampled catch for the Eastern Atlantic and Mediterranean stock, after removing the specimens over 400 cm SFL, was 398 and 383 cm for the study period and the last 10 years, respectively. The SFL corresponding to 95% of these maximum sizes was 378 and 364, respectively..

Discussion

Our findings contribute to improve the knowledge of ABFT growth by using dorsal fin spines for direct ageing. We describe the annual periodicity of annulus formation in the calcified structure and assess major limitations for using this structure for ageing, as spine nucleus vascularization, definition of sectioning level and precision and relative accuracy of this methodology. Our growth rate results confirm that spine sections can be used as an alternative to otoliths for ABFT age estimations.

An important assumption inherent in growth studies using calcified structures is that the size of the fish and size of the recording structure used are closely related throughout the entire life cycle (Casselman, 1989). The present study examines the relationship between the spine diameter and the straight fork length for bluefin tuna sampled in the NE Atlantic and the Mediterranean Sea. Results show that there is a strong relationship between both parameters. Similar results were reported in previous studies for ABFT sampled in both areas (Compeán-Jimenez and Bard, 1983; Rey and Cort, 1984; Rodriguez-Marin *et al.,* 2006; Santamaria *et al.,* 2009) as well as other large pelagic fish such as the swordfish (De Martini *et al.,* 2007). Hence, spine sections, at both selected sectioning levels, are good candidates for direct ageing and annuli diameters can be used for back calculating length.

The verification of the periodicity in growth increments deposition is essential for using calcified structures for estimating fish age. There are a variety of validation methods such as bomb radiocarbon, mark-recapture of chemically-tagged fish and radiochemical dating as direct methods, as well as analyses of marginal increment and edge type, which are considered indirect methods (Campana, 2001). Concentrating on the last, our results indicate an annual pattern in the deposition of translucent and opaque bands at the edge of the spine sections, with 50% occurrence of this translucent band between November and April. An entire annual pattern has not been described in previous papers since not a complete sampling throughout the year was available, however, similar deposition pattern was found. Translucent bands have been described to be laid down from November to May and opaque bands from June to October in otoliths, spines and vertebrae (Mather and Schuck, 1960; Butler *et al.,* 1977; Farrugio, 1980; Hurley and Iles, 1983, Lee *et al.*, 1983; Cort 1991; Foreman, 1996). Findings from Megalofonou and De Metrio (2000) are different in time from ours, although their study is only based in very small specimens in a reduced area of the Mediterranean and changes in the seasonal timing of the marginal increment with age or location may contribute.

Marginal increment results together with edge formation might indicate that there is a long period of inhibited growth starting in September to May while growth appeared to resume from June to October. The marginal increment and edge formation are easily observable in young individuals and fast growing fish, but they are more difficult to use for old specimens and slow growing species because of the narrowness of the bands at the margin, which makes difficult to objectively determine marginal growth. Nevertheless, both analysis are considered well suited for determining the month or season of formation of the opaque or translucent band once annulus formation has been validated using an independent means (Natanson *et al.,* 2001). An increased sample size particularly during winter season would be required to confirm our results.

Monthly variation in percent terminal translucent and opaque edges has been used in previous studies to suggest the formation of growth rings once a year (Sun *et al.,* 2000; Cerna, 2009; Griffiths *et al.*, 2010), however the appearance of frequent doubtful or split translucent rings cannot be ignored. In ABFT this sub annual translucent bands are very close together to be considered a year mark and are usually distinguishable within the general pattern of larger summer opaque bands. The formation of sub annual translucent and opaque bands have been related to migrations and different diet regimes and spawning (Compeán-Jiménez and Bard, 1983; Cort, 1991; Tserpes and Tsimenides, 1995, Sun *et al.,* 2002). Translucent ring deposition had also been related to spawning and the use of more energy to produce gametes than for growth (Sun *et al.,* 2000; Stéquert and Conand, 2004; Cerna, 2009), which is not the case of ABFT, since ABFT spawning in the western Mediterranean occurs from middle June to middle July (Rooker *et al.*, 2007). In our results there is a clear pattern of slow growth translucent banding coinciding with cold months and a fast growth opaque banding coinciding with warmer months in the North Atlantic and Mediterranean Sea. This result might suggest that the annual banding is mainly due to physiological changes caused by oceanographic differences between winter and summer in mid latitude areas.

Reabsorption or vascularization of the fin spine nucleus is the mayor disadvantage for using spines in comparison with otoliths for ABFT direct ageing (Rodriguez-Marin *et al.,* 2007). This phenomenon has been reported in several large pelagic fish including billfish and tuna species, and results from an expansion of the vascularised core of the spine during growth of fish (Berkeley and Houde 1983; Panfili *et al.,* 2001; Speare, 2003; Drew *et al.,* 2006). Ageing studies on large pelagic fishes (Hill *et al.,* 1989; Davie and Hall, 1990; Tserpes and Tsimenides, 1995; Chiang *et al.,* 2004) have used the method developed by Hill et al., (1989) that relies on the assumption that the annulus of a particular age class forms at approximately the same radius for each individual in the population. Other studies have overcome the disappearance of earlier growth marks by not counting the annulus but using the method of surface distribution, in which age estimations are based in the relationship between total surface of the spine section in relation with the surface included into the translucent rings (Stéquert and Conand, 2004; Drew *et al.,* 2006). This last method reduces the subjectivity of reader annuli interpretation, but also relies heavily on the relation relationship between the size of the spine and the size of the body and still some interpretation has to be done to identify the complete inner annulus.

To solve the destruction of bone tissue we have used the measurements of annuli of young specimens to infer the number of missing annuli in older specimens that means to use a reference table. This procedure is, to some extent, a deterministic method for age interpretation, however the identification of translucent annulus in juveniles is quite clear and their maximum diameters are distinct with no overlapping confidence intervals until age 10. Previous studies that include information about earlier translucent rings size, show values that are within or very close to the confidence intervals of the values obtained in this study, indicating that there is consistency in the method and the interpretation of the readers of translucent rings, considered as annuals, not subjective. (Cort, 1991; Rodríguez-Marín *et al.,* 2006; 2007; Santamaría *et al.,* 2009).

Reabsorption of ABFT spine nucleus has been described in previous studies (Compeán-Jimenez and Bard, 1983; Cort, 1991 Megalofonou and De Metrio, 2000; Corriero *et al.,* 2005; Santamaría *et al.,* 2009), but was first time quantified by Rodriguez-Marin et al. (2006). This last paper show similar results to ours, which indicate that half of total number of annulus used for assigning final age can be reabsorbed. In general, vascularization is known as bone remodelling. However, very little has been published regarding its histological development throughout individual life cycle, or what physiologic factors are influencing bone formation in relation to environmental features. Further research in this way might help to understand the vascularization process itself and factors that might be involved as has been done in other calcified structures such as otoliths (Campana, 1999).

Section location is an important factor for replacing missing annuli (Kopf *et al.*, 2009) since the appearance and location of annuli may vary between sectioning levels. Annulus counting may vary between different section levels along spines (Buckmeier *et al.,* 2002; De Martini *et al.,* 2007). Studies on other species as billfishes and swordfish have sectioned spines at a level relative to the width of the condyle in order to standardize sectioning methods (Chiang *et al.,* 2004; Cerna, 2009), whereas others simply have used relative proportions of fin spine length (Hill *et al.,* 1989; Speare, 2003). Previous ABFT ageing studies using spines states the section location in an ambiguous point near the condyle base (Compeán-Jimenez and Bard, 1983; Cort, 1991; Megalofonou and De Metrio, 2000; Santamaría *et al.,* 2009). In the present study we have followed Rodriguez-Marin et al. (2007) criterion that seems to be very close to the already cited papers based on annulus measurements. We have also experimented with an alternative section location to reduce the influence of nucleus remodelling and to improve the recognition of annuli. Results show that new section level (i.e. *S1.5*) provides a clear improvement respect to the “traditional” *S0.5* in terms of either better contrast of the bands and higher number of annuli that can be measured, particularly for medium size ABFT, which is crucial whether we wish to use dorsal fin spines for ageing the whole length range of ABFT.

During sampling, the difficulty of extracting the entire first spiniform ray, including its base, for medium and giant bluefin tuna makes it necessary to verify if those spines with the missing condyle base are still useful, since the reference for section location is lost. We estimated the percentage of the total length spine missing in specimens for whom spines were sectioned at the outside of the tuna body and not extracted entire. Results show that approximately 13% of the total spine length is missing by using this procedure, indicating that section *S1.5* is still remaining in the sectioned first spiniform ray. This difficulty in spine sampling of big fish could be solved by changing our criterion for section location to a relative proportion of fin spine length. However, it is quite common that the spines are broken at its apical end and therefore the condyle base width reference is a more accurate method for section location.

A measure of precision is a way of assessing the relative ease of establishing the age of a recording structure, or assessing the reproducibility of an individual´s age determination or just of comparing the skill level between readers (Campana, 2001). Results from the inter-reader comparisons show that in four of the five comparisons analyzed, the CV and APE values are lower than 5.0 % that serves as a reference point for many fishes of moderate longevity and reading complexity (Campana, 2001). These low values for both indexes might indicate an effect of the deterministic method used for age interpretation rather than an age reader effect since readers used a reference table for assigning a final age to the specimen. However, the APE and CV seems to increase with increasing difference in the reading experience, suggesting reading experience might influence in the precision. Nevertheless it should be noted that precision is highly influenced by the species and the nature of the structure and not just the age reader. For example, studies on ageing shark using vertebrae did so with CV values exceeding 10%, while the most frequently reported CV for otoliths was 5%.

According to Chang (1982), in the absence of bias, the CV and APE are equally sensitive to precision differences among readers, although CV is statistically more rigorous and flexible. In the present study, a significant inter-reader bias test was obtained in those comparisons that showed low values for both indexes confirming what was expected since the variation of estimates for each reader by age is small but estimates are significantly different among readers. The lowest is the CV (for each reader by age), the higher is the probability of a significant inter-reader bias test. This might suggest that not only the reading experience and the number of readers involved whom differ in their ageing skills affect but the number of samples of older ages used in the comparison might have influenced. It should be considered that ageing error can be either random or biased, reflecting some combination of process and interpretation error and it can be highly influenced by the nature of the structure. Neither APE nor CV is particularly sensitive to variations in age composition although both tend to decline to asymptotic values as age increases (Campana, 2001). The effect is most pronounced in older age groups since ageing error at a given CV will spread an actual age across more age groups at an older age than at a younger age. However, results of the present study showed that CV values for readers combined in each comparison did not show a clear trend throughout the age range for all well sampled indicating that this index can be considered better for the precision in age BFT reading.

There are general similarities between the mean lengths at age obtained in this and previous studies using spines for ABFT direct ageing. Mean lengths at age classes 2, 10 and 15 showed values of around 80, 205 and 250 cm, respectively, in all studies. A small disparity appears from age 7, in which some studies show differences of only one year difference. In this age range greater than 7, there is a gradient between the values of mean length at age among the various studies, with smaller lengths in Santamaria et al. (2009) and Cort (1991), intermediate in Compeán-Jimenez and Bard (1983) and slightly higher in the present study. These small differences in mean length at age may be related to differences in sampling related to the study areas, year period and months in which specimens were captured.

The von Bertalanffy growth parameters obtained in the present study were compared to those previously reported in the literature. There is some variance in the asymptotic length and growth coefficient among studies. All length at age series do cover similar age ranges but still length at age estimates are not always collected or treated in the same way, either because of sampling bias or differences in length at age estimation methods. These factors affect parameter estimates. All the growth parameters derived from age interpretation on ABFT spines from previous studies are estimated using mean length at age to fit the growth function (Compeán-Jimenez and Bard, 1983; Cort, 1991; Santamaría *et al*., 2009) and is a common practice in other species (Chiang *et al.*, 2004; Cerna, 2009). Conventional von Bertalanffy growth function can produce unrealistic estimates of the growth parameters especially when there are few samples of very large fish (Pauly, 1979) and this argument was also supporting the revision of Western Atlantic ABFT stock growth function (Neilson and Campana 2008; Restrepo *et al.*, 2010). Our results from both fittings, all length at age or mean length at age data, do show differences of L_{∞} and *k*, therefore illustrating the sensitivity of parameters estimation to the method employed because of the scarce number of samples (less than 10) in ages over 14 years in the present and previous studies.

Despite this lack of large fish samples to fit the growth function, and analyzing the asymptotic length results from the present study and the current adopted growth functions for east and west Atlantic stocks (Cort, 1991; Restrepo *et al.*, 2010), the L_{∞} estimation of 383 cm from the present study is more realistic in relation with the maximum SFL found in the ABFT catches for the eastern stock in the study period (398cm) and in recent years (383cm). This difference between the currently asymptotic lengths L_{∞} adopted for both stocks is still more evident for Western catches in which maximum SFL are larger than the ones from the eastern stock. Also the present estimated AGE_{max} is very high in compared with the ones estimated from the current adopted growth functions for both stocks. In this sense it is worth noting that the growth model from Restrepo *et al.*, (2010) is based on specimens older than its maximum predicted age.

Conclusions

The strong relationship between size of the spine and body fish indicate that they are closely related throughout the entire life cycle, indicating that spine cross sections are good candidates for direct ageing and annuli diameters can be used for back-calculating length.

Edge type classification and Marginal increment analysis confirm the periodicity in the formation of annual translucent bands supporting ageing interpretation criterion.

The quantification of the spine nucleus vascularization allowed us to gain a better understanding of the extent of the limitation of using dorsal spines for ageing ABFT. Knowing the limitation, it is more likely to determine what it is the best method for replacing missing annuli.

The new spine sectioning level is recommended since it showed a clear improvement respect to the "traditional" location in terms of age interpretation, because it was found a better contrast of the bands and a higher number of annuli could be measured, thus reducing the influence of nucleus remodelling.

The mean lengths at age obtained in this study show similarities with previous studies using spines for ABFT direct ageing appearing a small disparity of only one year differences from age 7

Differences of L_{∞} and *k* are observed when fitting the growth model to observed length at age or mean length at age data, illustrating the sensitivity of parameters estimation to the method employed mainly because of the scarce number of samples (less than 10) in ages over 14 years in the present and previous studies.

The estimated L_{∞} is more realistic in relation with the maximum length found in the ABFT catches for the eastern stock and hence more reliable than the currently asymptotic length adopted for this stock.

Our growth rate results indicate that spine sections can be used as an alternative to otoliths for ABFT age estimations.

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Table 1. Summary of ABFT sampled for the present study. Specimens were captured in the North Atlantic and the Mediterranean Sea over 21 year period from 1990 to 2010, including some spine samples from medium size specimens from 1984. Straight fork length (SFL) was measured in each tuna sampled to the nearest cm.

	Atlantic		Mediterranean		Total	
Year	n	Size range (cm)	n	Size range (cm)	n	Size range (cm)
1984	183	135,0-287,0			183	135,0-287,0
1990	151	59,0-291,8			151	59,0-291,8
1991	38	66,0-202,0			38	66,0-202,0
1992	48	56,0-184,0	3	50,0-101,0	51	50,0-184,0
1993	31	51,0-204,0			31	51,0-204,0
1994	65	64,0-183,0			65	64,0-183,0
1995	33	58,0-120,0	6	79,0-225,0	39	58,0-225,0
1996	68	55,0-185,0			68	55,0-185,0
1997	375	55,0-162,0	2	38,0-38,0	377	38,0-162,0
1998	369	53,0-160,0			369	53,0-160,0
1999	143	54,0-182,0			143	54,0-182,0
2000	224	59,0-170,0			224	59,0-170,0
2001	179	68,0-271,0			179	68,0-271,0
2002	175	59,0-263,0			175	59,0-263,0
2003	108	55,0-230,0	52	33,0-92,0	160	33,0-230,0
2004	196	63,0-271,0	51	51,0-250,0	247	51,0-271,0
2005	256	57,0-257,0	103	33,0-245,0	359	33,0-257,0
2006	93	59,0-212,0			93	59,0-212,0
2007	96	66,0-210,0	84	48,0-233,0	180	48,0-233,0
2008	130	70,5-279,7	274	49,9-251,0	404	49,9-279,7
2009	122	65,0-196,0	47	26,6-239,7	169	26,6-239,7
2010	81	158,9-257,6	76	27,7-244,0	157	27,7-257,6
Total general	3164	51,0-291,8	698	26,6-251,0	3862	26,6-291,8

Table 2. Reference table used for assigning a final age estimates of ABFT sampled for the two sectioning locations, S0.5 (top) and S1.5 (down). First row provides information of number of measurements taken by age class; second row the mean value of the translucent band diameter by age and the following rows show standard deviation (SD), and 95% confidence limits.

S 0.5

	Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10	Age 11	Age 12	Age 13
Nº measurements	798	1008	602	490	498	495	447	350	260	157	90	55	25
Mean value	**2.44**	**3.55**	**4.75**	**6.02**	**7.18**	**8.26**	**9.32**	**10.30**	**11.20**	**12.06**	**13.02**	**13.74**	**14.53**
SD	0.23	0.25	0.36	0.40	0.37	0.36	0.38	0.34	0.41	0.49	0.48	0.56	0.59
IntConf 95%	0.02	0.02	0.03	0.04	0.03	0.03	0.03	0.04	0.05	0.08	0.10	0.15	0.23
min (95%)	2.42	3.53	4.73	5.98	7.14	8.23	9.28	10.26	11.15	11.98	12.92	13.59	14.30
max (95%)	2.45	3.57	4.78	6.05	7.21	8.29	9.35	10.33	11.25	12.14	13.12	13.88	14.76

S1.5

	Age 1	Age 2	Age 3	Age 4	Age 5	Age 6	Age 7	Age 8	Age 9	Age 10	Age 11	Age 12
Nº measurements	35	87	88	80	110	129	121	92	58	24	11	8
Mean value	**2.31**	**3.35**	**4.48**	**5.63**	**6.66**	**7.69**	**8.67**	**9.46**	**10.29**	**11.06**	**12.07**	**12.90**
SD	0.20	0.33	0.35	0.41	0.40	0.37	0.31	0.36	0.34	0.44	0.52	0.61
IntConf 95%	0.07	0.07	0.07	0.09	0.08	0.06	0.06	0.07	0.09	0.18	0.30	0.42
min (95%)	2.24	3.28	4.41	5.54	6.59	7.62	8.61	9.38	10.21	10.88	11.77	12.47
max (95%)	2.38	3.42	4.56	5.72	6.74	7.75	8.72	9.53	10.38	11.23	12.38	13.32

Table 3. Biometric regression equations for ABFT sampled over the studied period. N: number of specimens; r2: correlation coefficient; P: significance level. S0.5 and S1.5: Spine diameter at the two section locations, Lmax: spine total length, L0.5 and L1.5: Length from the basal part of the spiniform ray to the two sectioning locations. SFL: straight fork length

X	Y	X range	Y range	N	Equation	r2	*Probability*
S0.5 (mm)	SFL (cm)	0.9 - 20	26.6 - 302.3	2749	Y = 16.350 * X +13.959	0,96	p < 0.001
S0.5 (mm)	SFL (cm)	0.9 - 20	26.6 - 302.3	2749	Y = 23.407* X ^ 0.8806	0,98	p < 0.001
S1.5 (mm)	SFL (cm)	2.1 - 14.1	48 - 271	354	Y = 18.655 * X +7.878	0,96	p < 0.001
S1.5 (mm)	SFL (cm)	2.1 - 14.1	48 - 271	354	Y = 21.991* X ^ 0.946	0,97	p < 0.001
Lmax (cm)	SFL (cm)	3.1 - 34.7	26 - 291.8	672	Y = 9.0801 * X -6.9001	0,96	p < 0.001
Lmax (cm)	SFL (cm)	3.1 - 34.7	26 - 291.8	672	Y = 7.7828* X ^ 1.0345	0,98	p < 0.001
Lmax (cm)	L0.5 (mm)	3.1 - 31.9	0.36 - 3.1	282	Y = 0.097 * X - 0.0212	0,94	p < 0.001
Lmax (cm)	L1.5 (mm)	3.1 - 31.9	0.5 - 5.1	282	Y = 0.1573 * X - 0.1012	0,96	p < 0.001

Table 4. Summary of parameters obtained from the five inter-reader comparisons analysed. The table displays coefficient of variation (CV) (%), the Average Per cent Error (APE) (%) and the p significance level of inter-reader bias test. Reader experience was categorized, 1: experienced reader; 2: intermediate; 3: inexperienced.
(- = no sign of bias (p>0.05); * = possibility of bias (0.01<p<0.05); * * = certainty of bias (p<0.01))

Readers	Reading Exp.	n	Study period	Age_range	CV (%)	APE (%)	Inter-reader bias test* (p)
Reader 1_ Reader 2	1-2	778	2001-07	1-11	3.1	2.22	* *
Reader 1_Reader 5	1-2	624	1992,1997,2001-10	1-13	4.4	3.1	* *
Reader 1_Reader 4	2-2	556	1984,1999, 2000-05,07-09	1-14	4.6	3.27	-
Reader 1_Reader 5	1-3	378	2005,07-08	1-13	4.9	3.44	-
Reader 1_ Reader 3_ Reader 5	1-3-3	262	2005,07-09	1-11	8.3	5.84	- R1-R3 - R1-R5 - R3-R5 -

Table 5. Von Bertalanffy growth curve parameters for eastern Atlantic and Mediterranean bluefin tuna (*T. thynnus*) management unit from the present study and from previous studies on ABFT growth using dorsal spine sections ((1): Cort 1991 mean lengths at age estimates from length analysis for ages 1 to 8 and from spine sections for ages 9 to 15).

Author	Compeán-Jimenez & Bard (1983)	Cort (1991)	Santamaria et al. (2009)	Present study		
Sampling Year	1978 - 1979	1975-1986	1998-2005	1984, 1990-2010		
Sampling Month	spring-summer-atumn	summer	-	spring-summer-atumn		
Area	Bay Biscay, Canary Islands, Mediterranean, East Atlantic	Bay Biscay, Gulf of Cadiz, East Atlantic	Central Mediterranean	Bay Biscay, Gulf of Cadiz, East Atlantic		
Growth Model	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy		
Ageing material	Spine sections	Length distribut. & Spine sect. (1)	Spine sections	Spine sections		
Fitting to the model	Mean size at age	Mean size at age	Mean size at age	Size at age	Mean size at age	
Linf	372.2	318.85	373.08	382.65	334.98	
k	0.068	0.093	0.070	0.070	0.088	
to	-1.710	-0.970	-1.760	-1.334	-1.190	
Ages with at least 5 indiv. sampled	1 to 17	1 to 15	1 to 14	1 to 17		
Age class	Mean observed straight fork length (cm)					*n*
1	62.7	53.5	62	64.7		*465*
2	83.1	79.7	79.6	80.8		*694*
3	102.1	100.7	101.4	99.2		*493*
4	119.9	118.8	115.9	118.7		*295*
5	136.5	135.1	134.5	140.1		*149*
6	152.0	150.1	149	157.2		*99*
7	166.5	164.0	161.7	174.4		*77*
8	180.1	177.2	180	190.7		*55*
9	192.8	190.9	186.5	203.2		*51*
10	203.6	206.2	202.6	209.6		*60*
11	215.7	216.1	205.7	219.9		*49*
12	226.1	222.5	223.3	230.3		*28*
13	235.8	232.4	230.9	238.7		*27*
14	244.8	241.6	236.6	247.5		*27*
15	253.3	247.2	252.2	254.3		*13*
16	261.2			265.6		*9*
17	268.5			263.9		*5*
18	275.5					
19	281.9					
20						
21						
22						*1*

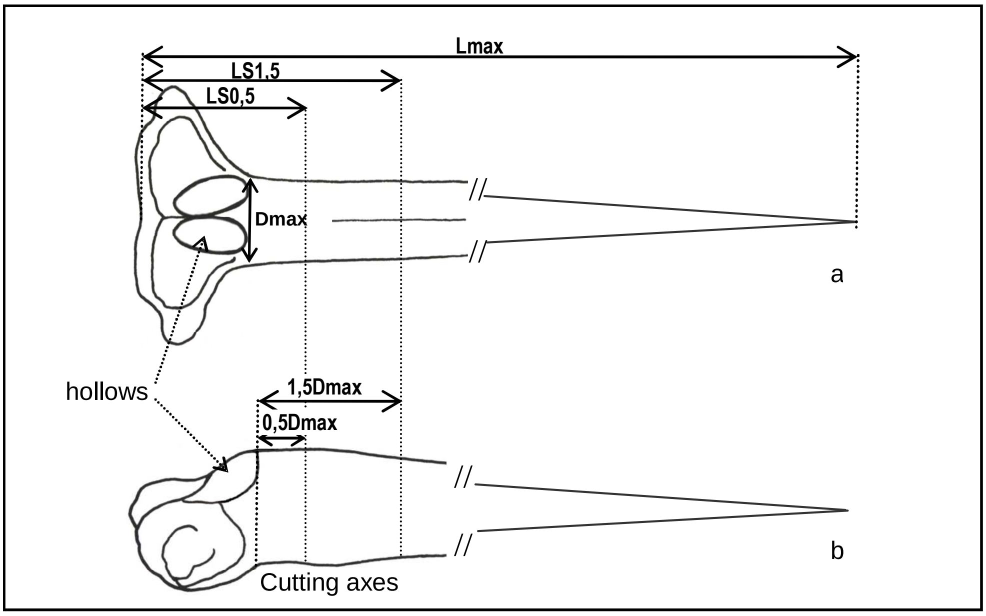

Figure 1. a) Illustration of an anterior view of the first spiniform ray showing the biometric measures recorded. *Dmax:* Maximum spine diameter measured along the imaginary line below the hollows, *Lmax*: total spine length measured from the condyle base to the ape of the spine, *LS0.5* and *LS1.5:* distance from the condyle base to the 1st and 2nd cutting axes, respectively, b) a lateral view of the spine showing the position of the two cutting axes, 0.5Dmax (S0.5 section) and 1.5Dmax (S1.5 section).

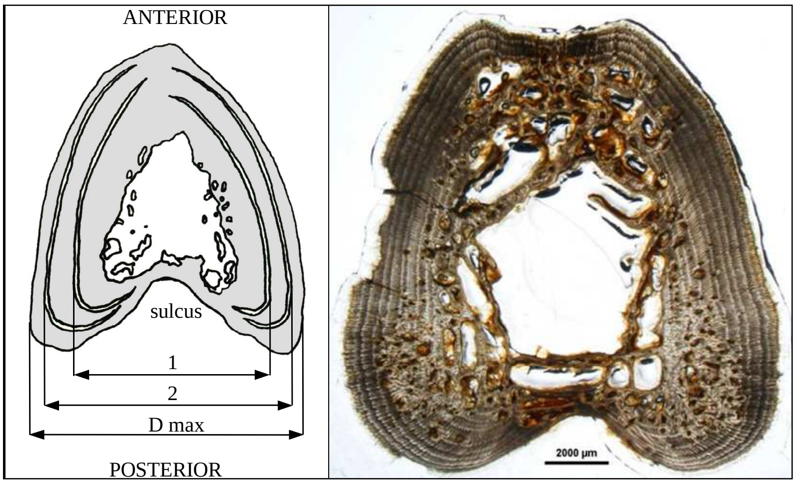

Figure 2. Left: Illustration a spine section showing how to measure the translucent band diameters; right: a digital spine image showing the nucleus vascularisation.

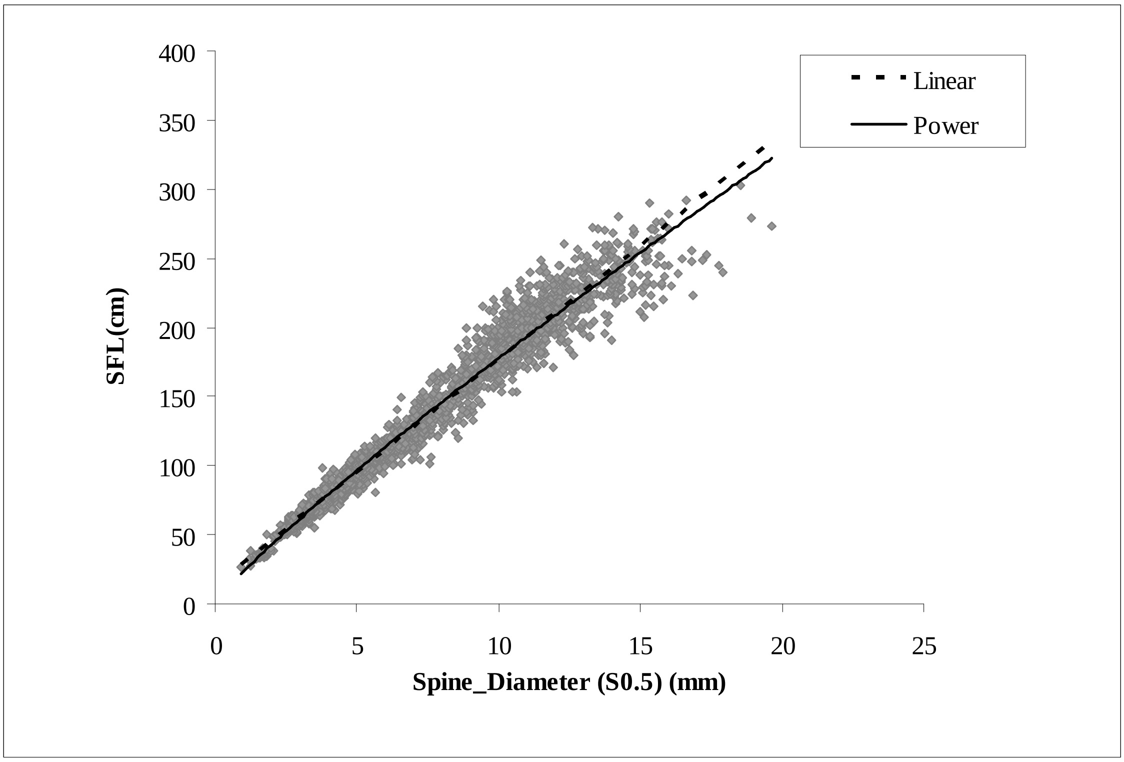

Figure 3. Relationship between the maximum spine diameter at the S0.5 cutting axis and ABFT straight Fork length (SFL) (n=2749).

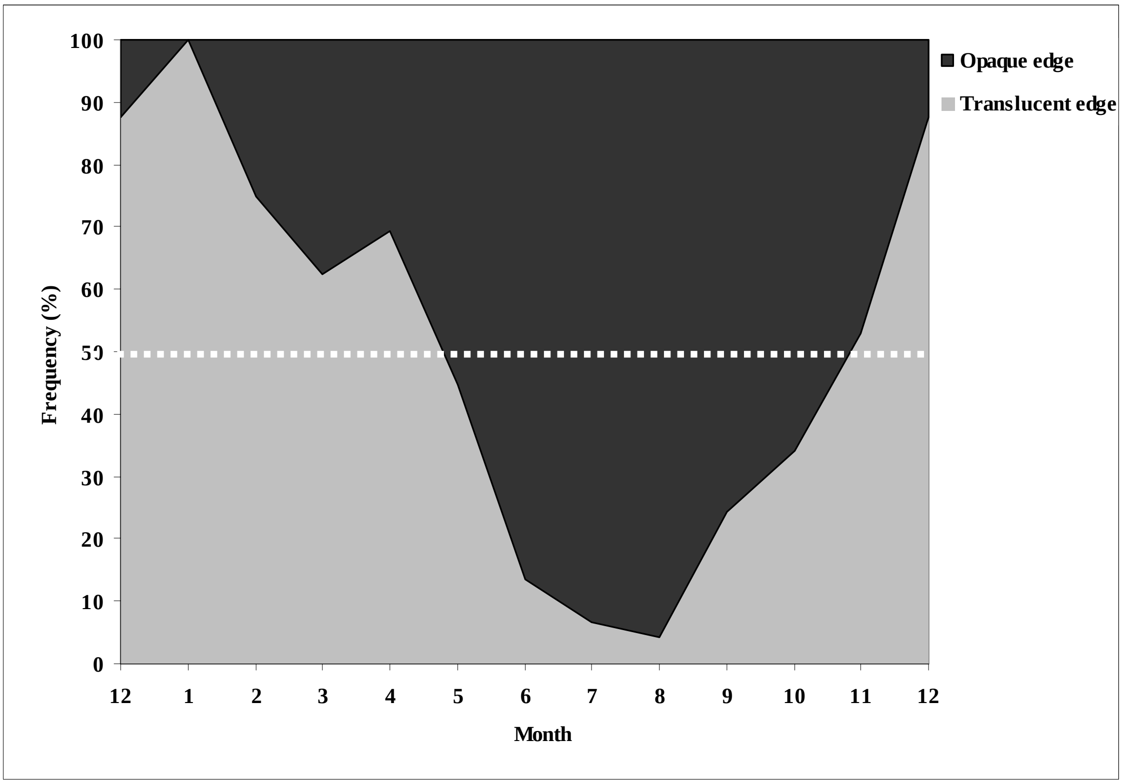

Figure 4. Monthly frequency (%) of edge type examined in spine sections of ABFT (n=2234).

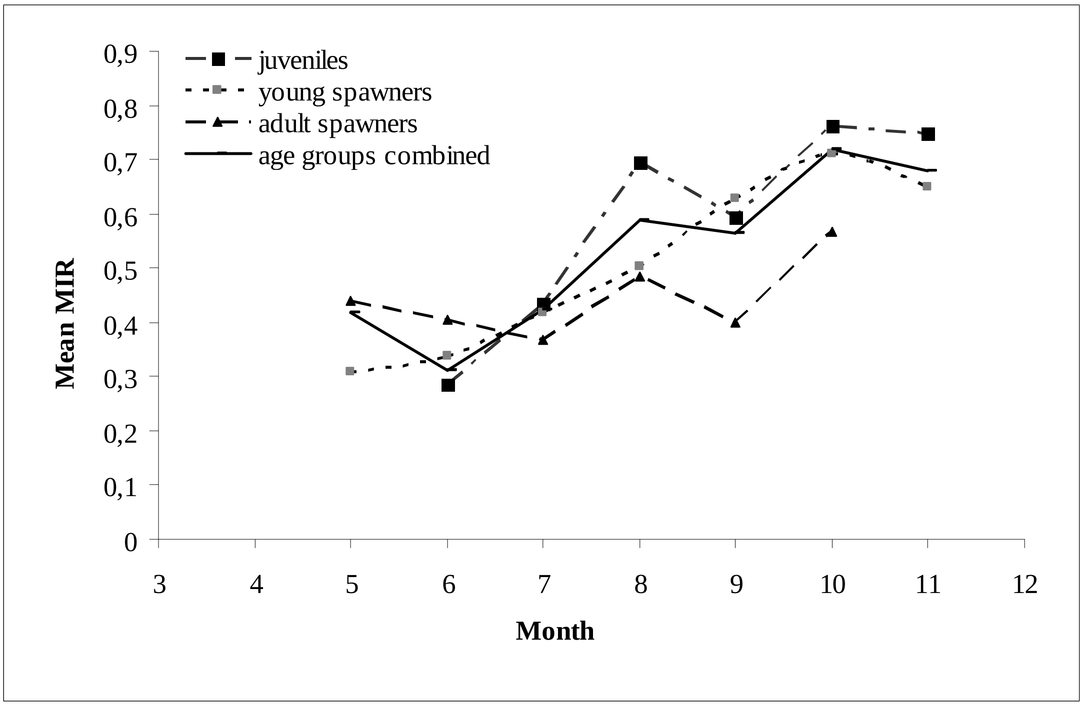

Figure 5. Monthly mean marginal increment ratio (MIR) analysed in spine sections of ABFT (n=948) for three age groups, juveniles: aged 2-3 years old (n=423), young spawners: aged 4-8 years old (n=334), and adults: >8 years old (n=191).

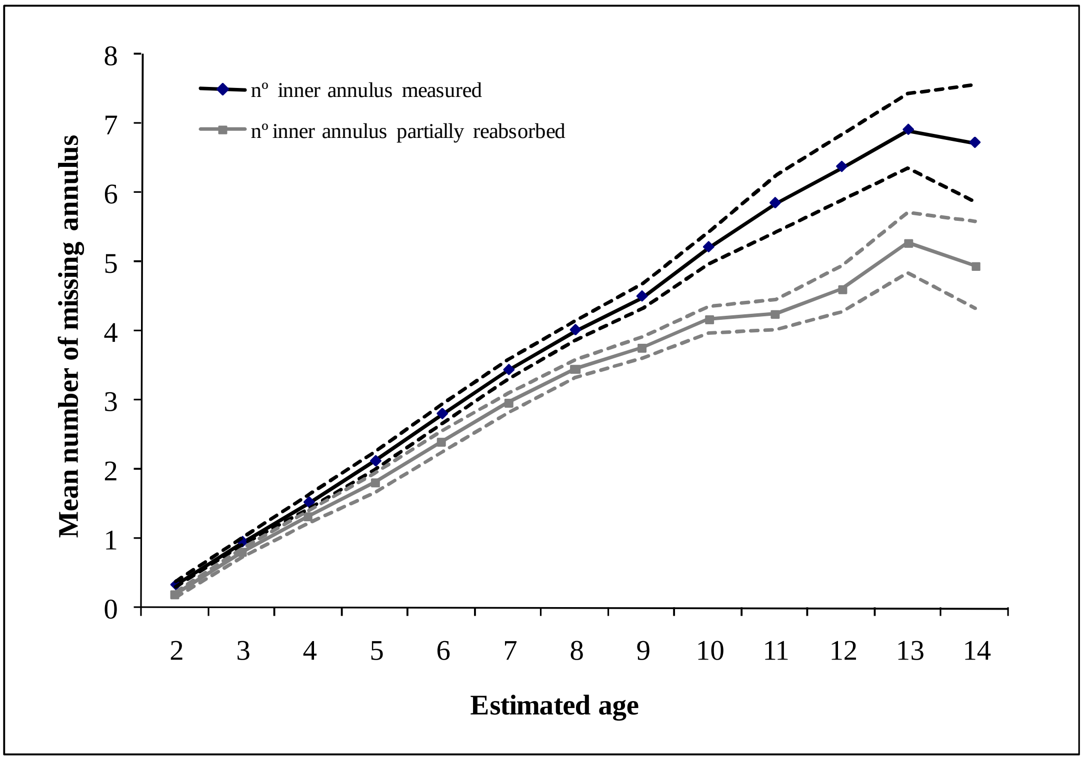

Figure 6. Relationship between the mean number of missing annulus (translucent bands) and estimated age for ABFT (n=2004). Black line considers as a baseline the first inner complete translucent band measured and grey line considers the first inner annulus partially reabsorbed. Mash lines represent the 95%CI in both cases.

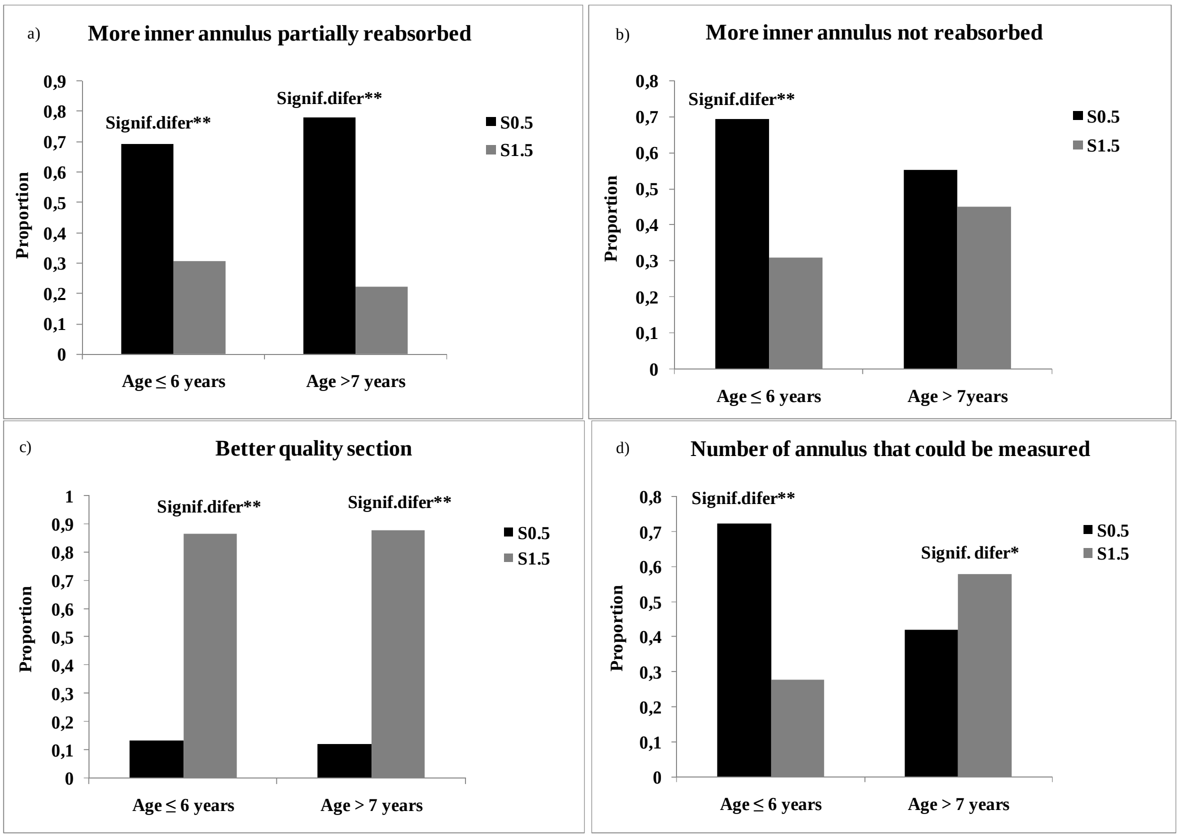

Figure 7. Graphs representing the different comparisons carried out between the two cutting axes, S0.5 in grey bars and S1.5 in black bars. Two age groups were considered: ages from 1 to 6 and older than 6 years old. a) proportion of spine sections that show more inner annulus partially reabsorbed, b) proportion of spine sections that show more inner annulus not reabsorbed, c) proportion of better image quality of the spine sections, d) proportion of spine sections that show more number of annulus that could be measured. Significance level: ** (p<0.01), * (0.01<p<0.05); - no significant (p>0.05).

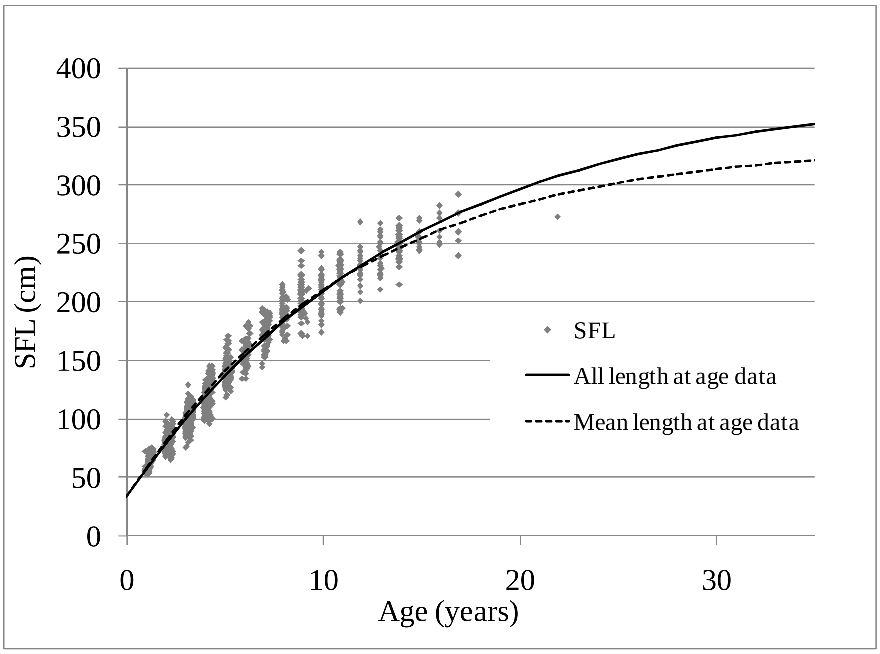

Figure 8. Straight fork length (SFL) versus age estimate from spine section translucent bands counts (n = 2597). The continuous line represents the growth model fitted to observed length at age data and the dashed line represents the adjustment to the mean length at age.

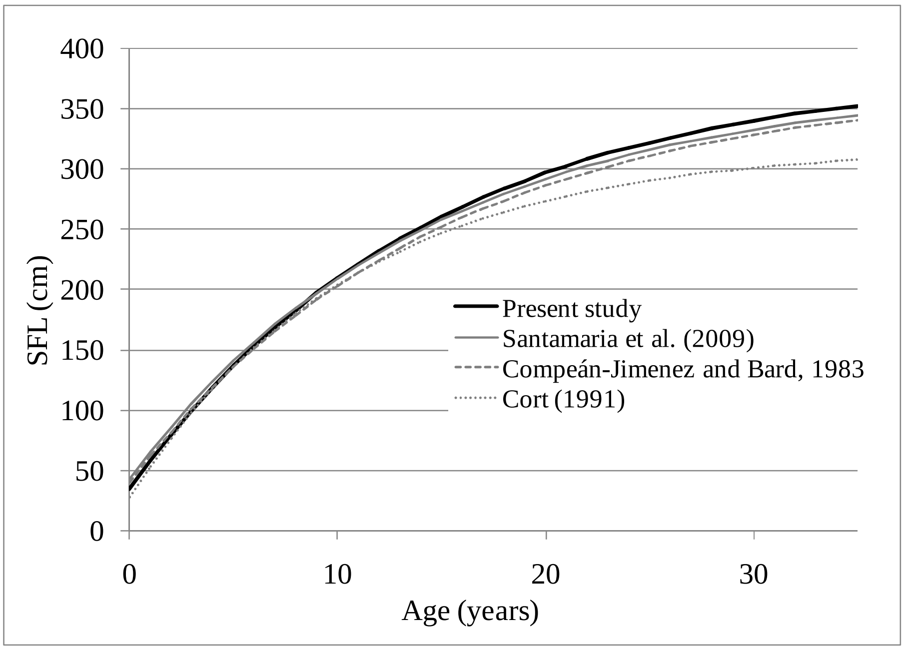

Figure 9. Von Bertalanffy growth functions derived from spines reading from the present (using all length at age data) and previous studies, for eastern Atlantic and Mediterranean ABFT (*T. thynnus*) management unit.