

SCRS/2016/134

## UPDATED COMPARISON OF AGE ESTIMATES FROM PAIRED CALCIFIED STRUCTURES FROM ATLANTIC BLUEFIN TUNA.

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### SUMMARY

*In this paper we present an updated comparison of age estimates from otoliths and spines from the same specimen, with the intention to analyze whether it is possible to use both structures in obtaining age-length keys for this species. The agreement between otolith and spine age estimates was good for bluefin tuna younger than 14 years old with less than one year difference. Tests of symmetry showed the asymmetrical distribution of ages. However no significant differences were found between the growth parameters estimated from both paired hard parts. It is suggested using both structures readings for constructing age-length keys for bluefin tuna younger than 14 years.*

### KEYWORDS

*Age estimates, direct aging, otoliths, spines, Thunnus thynnus.*

### 1. Introduction

Direct aging in Atlantic bluefin tuna (*Thunnus thynnus*) is made mainly from two calcified structures: otoliths and the first radius of the first dorsal fin (spine). The interpretation of the otoliths age by counting annuli has been validated (Neilson and Campana, 2008), however it was not until recently that otoliths reading criterion has been standardized (Secor et al., 2014; Busawon et al., 2015). Direct aging from spines is standardized and has been shown an annual deposition of translucent and opaque bands forming each growth annulus (Luque et al., 2014), but this methodology has not been validated directly.

Obtaining biological samples of this species is difficult because of the high economic value of each individual and the reluctance of fishermen and traders to the manipulation of fish. This means that it is not always possible to sample otoliths and instead, spines can be more easily sampled. Aiming to indirectly validate the interpretation of age in spines and be able to use either both structures in the direct aging of Atlantic bluefin tuna, paired otolith and spine structures (from the same specimen) have been sampled in several research projects and the research program on bluefin tuna for all the Atlantic (GBYP) from ICCAT. In this paper we present an updated comparison of age estimates from otoliths and spines sampled from the same fish (Rodriguez-Marin et al., 2015), with the intention to analyze whether it is possible to use both structures in obtaining age length keys for this species.

### 2. Material and methods

We analyzed 1064 paired otoliths and spines (532 fish) from sampling of several national and international research projects, including the research program on bluefin tuna for all the Atlantic (GBYP). The size of sampled specimens (n = 532) cover a wide range of lengths, ranging from 22 to 295 cm straight fork length (SFL) (Figure 1).

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Otoliths were prepared and interpreted following the methodology described in Busawon et al (2015), which means that reflected light was used and opaque bands were counted. Spines were prepared and read following the methodology described in Rodriguez-Marin et al. (2012) and Luque et al. (2014), transmitted light was used and translucent bands were counted. Final age was adjusted for both structures to account for the date of capture and the timing of bands formation throughout the year (Luque et al., 2014; ICCAT, 2015). Otoliths and spines were read by experienced readers.

Diagnosis of paired age agreement was evaluated by precision indices through Average Percent Error (APE) and Coefficient of Variation (CV), tests of symmetry and age-bias plots (Campana et al., 1995; McBride, 2015).

A standard von Bertalanffy growth model (von Bertalanffy, 1938, VB) was fitted to length at age data derived from otoliths and spines to compare growth derived from both structures readings:  $L_t = L_\infty \{1 - e^{-k(t-t_0)}\}$ , where  $L_t$  is the LSF at age  $t$ ,  $L_\infty$  is the asymptotic length that  $T. thynnus$  may attain if the fish lived indefinitely,  $k$  is the growth coefficient at which  $L_\infty$  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). VB parameters ( $L_\infty$ ,  $k$  and  $t_0$ ) estimated from both calcified structures were compared using Kimura's (1980) likelihood ratio test.

### 3. Results.

Comparison of age estimates from the two ageing structures within the current study revealed that the disagreement between structures was less than one year for ages 0 to 13 years old (**Figure 2**). The discrepancy between readings from the two structures increased from 13 years old. Precision between paired structures reached CV slightly higher than 10% and symmetry tests detected bias (**Table 1**).

The VB curves fitted using observed length-at-age for otoliths and spines almost overlapped, although spines VB fit showed a greater curvature at the ages 7 to 15 than those of otoliths (**Figure 3**). Results from the Kimura's likelihood ratio test did not show significant differences between growth parameters estimated for both calcified structures (**Table 2**).

### 4 Discussion.

As counts of otolith increments provide an accurate age in years (Neilson and Campana, 2008), the otolith and fin spine counts should be consistent for all ages if the bands we counted on spines were also deposited annually throughout life; and a linear relationship between age estimates from the two structures should be expected (Gunn et al., 2008). Our findings showed that there is a close relationship up to 13 years and an under estimation for older specimens when using spines with respect to otolith readings. Tests of symmetry also showed the asymmetrical distribution of ages. However no significant differences were found between the growth parameters estimated from both hard parts.

Precision indices between both structures were low (CV around 11% and APE around 8%), although it is difficult to establish a threshold. It may be 5% for teleost species and higher, e.g. 10%, for elasmobranches or other species that are more difficult to age (McBride, 2015). A precision level of APE and CV of 10% or lower and no bias would be acceptable to support production ageing in bluefin from otoliths (Busawon et al., 2015).

Considering the results of the comparison, it does not seem unreasonable to use both structures readings for constructing age-length keys for bluefin tuna younger than 14 years.

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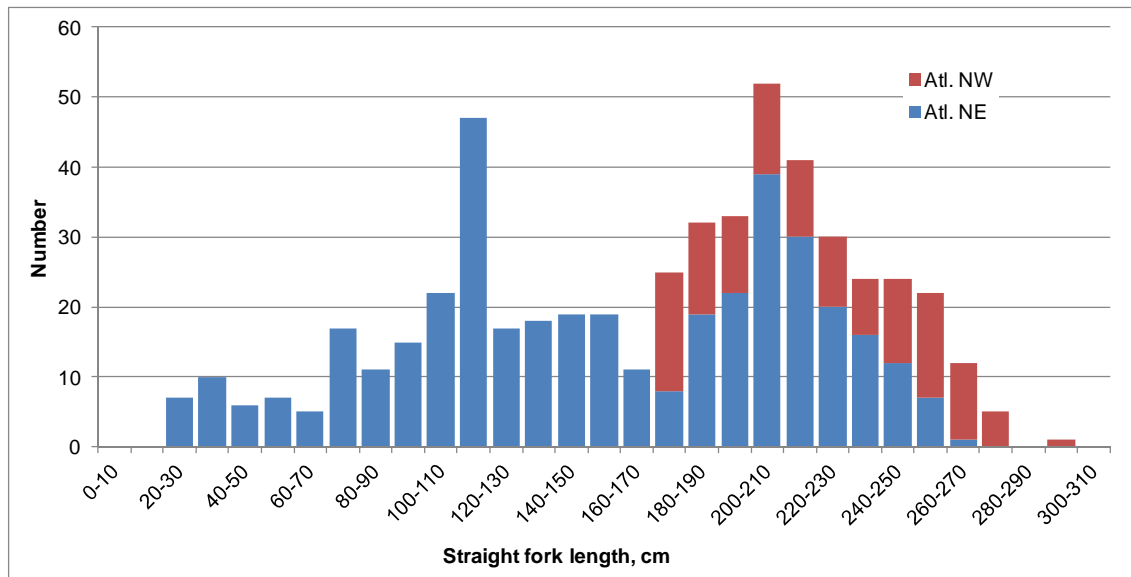
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**Table 1.** Diagnosis of paired age agreement. Precision indices: CV = Coefficient of Variation, APE = Average Percent Error and tests of symmetry.

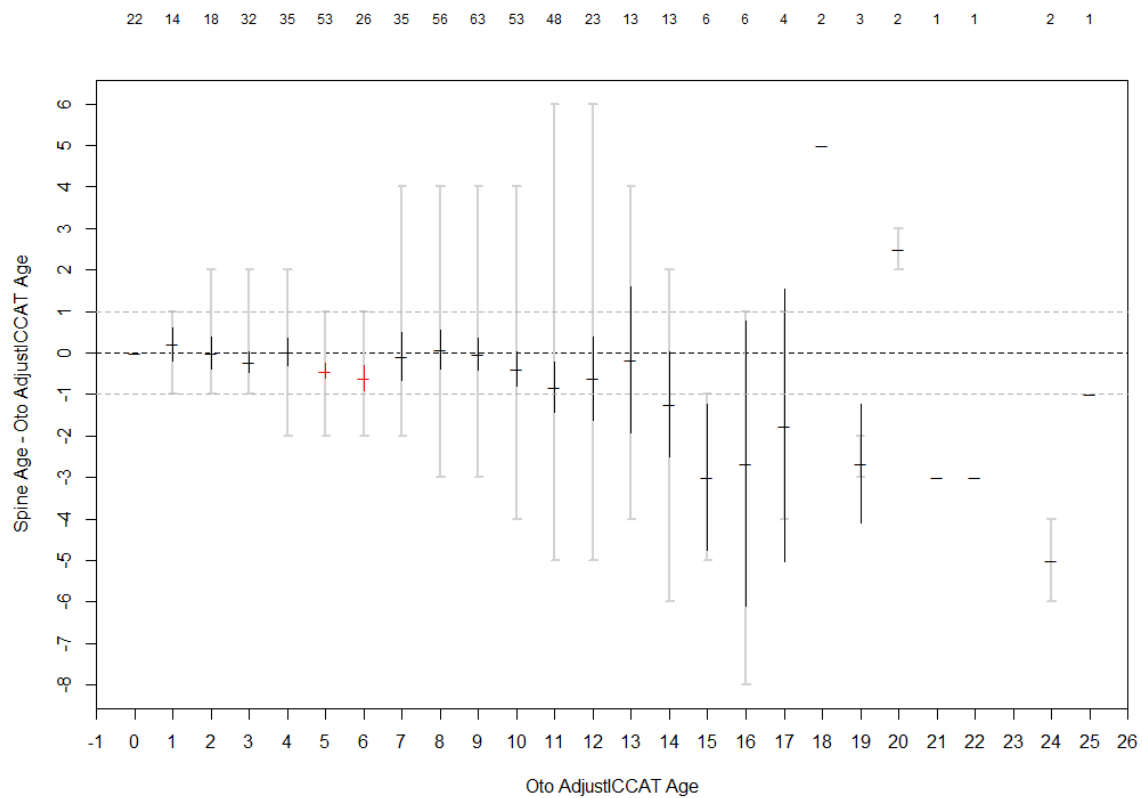
Age comparison	Precision		Symetry test			
	CV	APE		df	chi.sq	p
n= 532	11.32	8.003	Evans Hoenig	7	51.33949	7.88E-09
			Bowkers	66	122.29842	3.13E-05

**Table 2.** Von Bertalanffy growth model parameters estimated by fitting lengths at age from otoliths and spines readings. Statistical significance (P-value) of likelihood ratio test are given, n.s. = non significant.

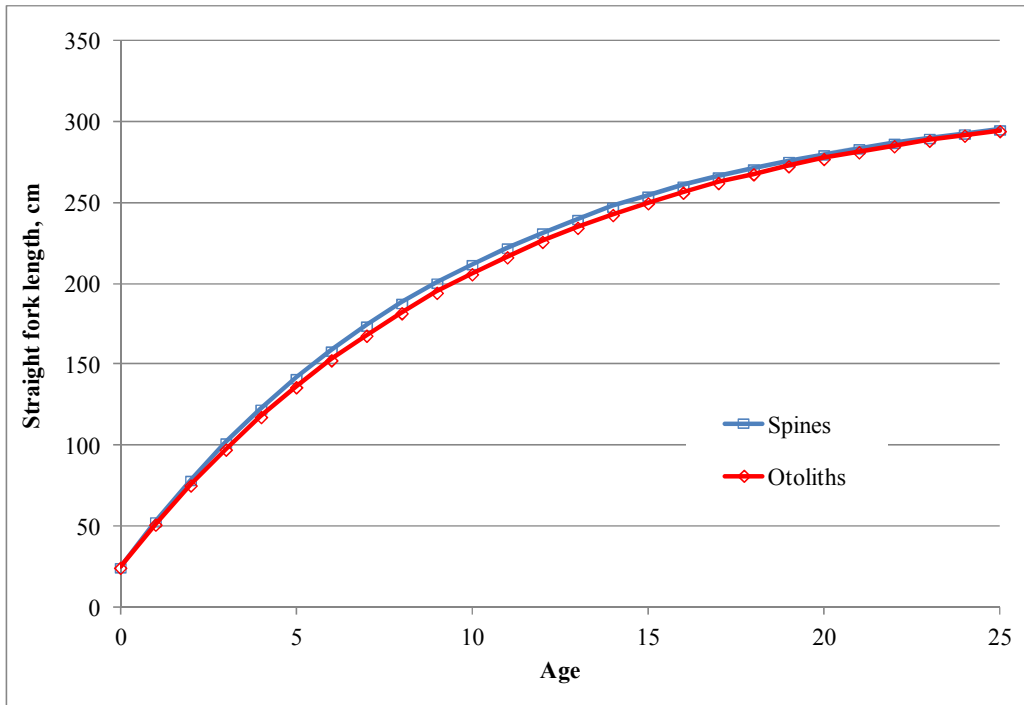
Age range compared	Calcified structure	$L_{\infty}$	k	to	Likelihood Ratio test		
					$L_{\infty} p$	k p	to p
0 - 25	Otoliths	323.4	0.093	-0.853	n.s.	n.s.	n.s.
0 - 24	Spines	318.5	0.101	-0.802			



**Figure 1.** Size distribution of Atlantic bluefin tuna sampled for direct estimation from paired (collected from the same fish) calcified structures: otolith and spines. Numbers of specimens by management unit capture location.



**Figure 2.** Differences in age estimates from otoliths and spines collected from the same fish (n=532). Crosses indicate the average with black lines indicating the 95% confidence intervals and the grey lines indicating the age range. The intervals in red indicate significant differences. The 1:1 equivalence (black dashed) line is also indicated.



**Figure 3.** Von Bertalanffy growth model curve fitted to observed length at age data for otoliths (red) and spines (blue) sampled from the same fish.