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STANDARDIZED AGE- LENGTH KEY FOR EAST ATLANTIC AND MEDITERRANEAN BLUEFIN TUNA BASED ON OTOLITHS READINGS

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SUMMARY

*This paper presents an age-length key (ALK) based on age estimates from otoliths following a recent standardized reading criterion. Sampling of Atlantic bluefin tuna (*Thunnus thynnus*) otoliths comes from the "Biological and genetic sampling and analysis" (GBYP) project and was complemented with some samples from the Spanish institute of Oceanography. Using 2010 to 2012 data improves length range and monthly sampling coverage. Variability in length at age from ALK seems acceptable and although it was developed from data pooled over three years, the bias is likely to be small. Final age was adjusted to account for the date of capture and the timing of bands formation throughout the year. Monthly formation of edge type, translucent or opaque, was inconclusive and did not allow establishing an annual formation pattern. The confidence interval of the von Bertalanffy growth model curve fitted to ALK data included currently adopted growth function for eastern bluefin stock and western stock function up to age 8. From age 9, this last western function predicts slightly older ages than the present ALK growth model.*

RÉSUMÉ

*Le présent document traite d'une clé âge-taille (ALK) fondée sur des estimations de l'âge à partir d'otolithes en appliquant des critères de lecture récemment standardisés. L'échantillonnage des otolithes de thon rouge de l'Atlantique (*Thunnus thynnus*) provient de l'échantillonnage biologique et génétique et analyses du projet ICCAT-GBYP et a été complété avec quelques échantillons de l'Institut espagnol d'océanographie (IEO). L'utilisation de données de 2010 à 2012 améliore la couverture de la gamme de tailles et d'échantillonnage mensuel. La variabilité de longueur à l'âge de ALK semble acceptable et bien qu'elle ait été élaborée à partir des données regroupées sur trois ans, le biais devrait probablement être faible. L'âge final a été ajusté pour tenir compte de la date de la capture et du moment de la formation des bandes tout au long de l'année. La formation mensuelle du type de bord, translucide ou opaque, s'est avérée peu concluante et n'a pas permis de dégager de cycle annuel de formation. L'intervalle de confiance de la courbe du modèle de croissance de von Bertalanffy ajusté aux données ALK incluait la fonction de croissance récemment adoptée pour le stock de thon rouge de l'Est et la fonction pour le stock de l'Ouest jusqu'à l'âge de 8 ans. À partir de l'âge de 9 ans, cette fonction concernant le thon rouge de l'Ouest prédit des âges légèrement plus âgés que le modèle actuel de croissance ALK.*

RESUMEN

*Este documento presenta una clave edad-talla (ALK) para el stock de atún rojo del Atlántico este y Mediterráneo inferida a partir de otolitos y siguiendo el criterio de lectura estandarizado recientemente. El muestreo de otolitos de atún rojo del Atlántico (*Thunnus thynnus*) procede del "Muestreo biológico y genético y análisis" de proyecto ICCAT GBYP, y se complementó con algunas muestras del Instituto Español de Oceanografía. La utilización de los datos de 2010 a 2012 mejora la cobertura de gamas de talla y del muestreo mensual. La variabilidad de talla por edad de ALK parece aceptable y, aunque se desarrolló a partir de datos agrupados durante tres años, es probable que el sesgo sea de escasa magnitud. Se ajustó la edad final para tener en cuenta la fecha de captura y el momento de la formación de bandas a lo largo del año. La formación mensual del tipo de borde, translúcido u opaco, era poco concluyente y no permitió establecer un patrón de formación anual. El intervalo de confianza de la curva del modelo de crecimiento de von Bertalanffy ajustado a los datos ALK incluía la función de crecimiento adoptada actualmente para el stock de atún rojo del este y la función del stock occidental hasta la edad 8. A partir de la edad 9, esta última función occidental predice edades ligeramente superiores que el actual modelo de crecimiento de ALK.*

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KEYWORDS

Age estimates, age-length key, length-at-age, otoliths, Thunnus thynnus

1. Introduction

The simplest method to obtain estimates of proportions at age is by the standard age-length key (ALK) approach. However to build an ALK it is necessary to use a validated calcified structure and age estimates need to meet a series of quality controls. For Atlantic bluefin tuna (*Thunnus thynnus*) age inferences from otoliths have been validated by the bomb radiocarbon method (Neilson and Campana, 2008) and standardized age interpretation protocols have been established for otoliths through workshops conducted in 2011 and 2013 (Rodriguez-Marin *et al*., 2012; Secor *et al*., 2014; Busawon *et al*., 2015). Furthermore, a reference collection was developed to support routine production ageing of bluefin tuna (Busawon *et al*., 2015). To confirm that independent laboratories were providing comparable interpretations from otoliths, a calibration exchange within the Atlantic wide research programme for bluefin tuna (GBYP) was held in 2013 (SCRS/2014/150). Results from this exchange confirmed that applying the standardized reading criterion for otoliths, an acceptable level of precision was obtained and that readers showed no systematic bias compared to the modal age from expert readers.

In 2013 an ALK was presented based on otoliths sampled in the North East Atlantic and Mediterranean Sea within the project "Biological and genetic sampling and analysis" (GBYP) (Rodriguez-Marin *et al*., 2014). This ALK was produced while the age interpretation criterion of otoliths was still been discussed and when laboratories were using different methodologies to read these structures. Thus, the objective of this paper was to build an age-length key by re-reading these calcified structures applying the recently standardized direct ageing methodology.

2. Material and methods

Otoliths sampling was obtained from specimens caught from 2010 to 2012. Specimens were caught in the eastern, central and western Mediterranean Sea, and in the north-eastern Atlantic in offshore waters of the Iberian Peninsula. Bluefin tuna juveniles were caught by bait boats and adults by longliners, hand line, purse seiners and traps. Otoliths extraction and conservation were carried out following the "Biological and genetic sampling and analysis" GBYP project sampling protocols (adapted from Ruiz *et al*., 2005). Bluefin was measured as straight fork length (SFL) in cm.

A total of 561 otoliths have been used to build the age-length key, of which 524 are from the "Biological and genetic sampling and analysis" (GBYP) project and 37 samples come from the Spanish institute of Oceanography sampling in 2010. Another 6 otoliths were discarded because were unreadable or there was a clear mismatch with bluefin tuna size. Samples were collected from January and from May to November. Otoliths were selected trough length-stratified sampling by month and year, and all years were pooled in order to get a representative sampling by 5 cm SFL (**Table 1**).

Otoliths were sectioned by embedding them in a matrix resin within a mould. Three consecutive sections of 300-400 µm were obtained in the core area of each otolith, using a low-speed diamond cutting saw (Isomet 1000) equipped with four 0.3mm wide diamond impregnated blades with spacer at 0.3-0.4 mm. Encased otolith sections were mounted on glass slides using Eukitt, and then polished using 240-600 grit sandpaper with 0.3 micron polishing compound to improve the contrast of bands before imaging. Polished sections were placed in Petri dish and cover with ethanol or distilled water to improve the contrast of bands. Otolith images were taken using reflected light on a black background. Otoliths direct ageing was carried upon digital images that were captured using a binocular lens magnifier connected by digital camera NIKON.

The age interpretation was performed on digitally enhanced images using Adobe Photoshop. The standardized ageing protocol described in Busawon *et al*. (2015) was followed: 1) prior to production ageing, readers read the reference set (100 images) one time single blind under reflected light type. A precision level of APE and CV of 10% or lower and no bias was considered acceptable to support production ageing. 2) A reference scale was used as a guide to identify the first annulus. 3) Annulus counts were made along the longest (ventral) arm of the sectioned sagittae otolith ("Y" type section). 4) Annuli are a bipartite structure consisting of a translucent and opaque zone; age was estimated by counting the opaque bands. 5) Edge type assignment (translucent or opaque), and quality in terms of readability (1=Pattern present-no meaning, 2=Pattern present-unsure with age estimate, 3=Good pattern present-slightly unsure in some areas, 4=Good pattern-confident with age estimate) were annotated.

Taking advantage of the available consensual readings from the calibration exercise from GBYP (Rodriguez-Marin *et al*., 2014), 89 otolith readings from the modal age estimated by the experts of the exchange were used in the standardized ALK. The rest of the otoliths were read once by three expert readers. Otoliths with low confidence on age estimates were read by a second reader and determined a final consensus increment count. All readings were conducted without reference to the size of fish, date or area of capture, or previous readings.

Final age was adjusted to account for the date of capture and the timing of bands formation throughout the year (Luque *et al*., 2014; Anon. 2015 (*in press*).

Annual variability in length at age was explored by means of length standard deviation by age from annual ALKs and compared with those from western bluefin growth function (Restrepo *et al*., 2010). Furthermore, a standard von Bertalanffy growth model (von Bertalanffy, 1938) was fitted to length at age data derived from present ALK and compared with ICCAT currently adopted growth functions for Atlantic bluefin (Cort, 1991; Restrepo *et al*., 2010).

3. Results and discussion

Standardized ALK by number and by 5 cm SFL is presented in **Table 2.** This table also shows (shaded cells) the age estimates from expert readers' modal age from the exchange exercise held in 2014 (SCRS/2014/150), which provide comparable interpretations with newly read otoliths.

Age-length keys can become unreliable if the spread of age-at-length is large (Gulland and Rosenberg, 1992). To verify whether variability in length at age from the ALK was too large, length at age standard deviation (SD) from present ALK was compared with length at age SD from Restrepo *et al*. (2010) growth function. This comparison is just qualitative since length at age SD from these authors come from a model by minimizing a likelihood function based on length frequency analyses and direct ageing data, thus both SDs are not equivalent. **Figure 1** presents lengths at age SD from present ALK and from Restrepo *et al*. (2010), and differences between both SD by age (only ages with more than 5 samples were used). It can be appreciated that length at age SD increases as age increase with bigger differences between both data series in ages 6 to 8 years old (with a maximum divergence of 15 cm in age 7), but with a general difference of around 7 cm in length (SFL). Therefore, it seems that overall variability in length at age from ALK is acceptable.

The variability in the length at age from present ALK can be due to several reasons, including natural variability from a long lived species which perform large migrations and therefore individual are exposed to different habitat conditions; sex-specific differences in growth (Rooker *et al*., 2007); sampling effects and other. Furthermore, when estimating the catch-at-age from length frequency data, significant bias can be introduced if ALKs are developed from data pooled over several years (Westrheim and Ricker, 1978; Farley *et al*., 2007). The length at age variability from present ALK, measured as SD of length at age, showed almost no differences when years 2010 (n=37), 2012 (n=97) or both, were excluded from the ALK (**Figure 1**). Using 2010 and 2012 data improves length range and monthly sampling coverage and does not contribute to increase length at age variability. We believe the bias if present is likely to be small. However annual ALKs are more appropriate for tracking cohorts than pooled ones (Rodriguez-Marin *et al*., 2009). A compromise needs to be reached between sampling effort and having a representative ALK by month and size. Sampling this small calcified structure is not always possible due to different bluefin fisheries processing practices, length and monthly sampling coverage is difficult to achieve from seasonal fisheries which capture fractions of the population and the large size and price attained by bluefin do not contribute to facilitate sampling. This means that multiannual ALKs may be still useful for this species.

Edge of otoliths interpretation, in relation to date of collection and assigned birth date, remains an unsolved matter for Atlantic bluefin tuna. Translucent bands within increments form annually between May and October (austral winter) in southern bluefin tuna (*Thunnus maccoyii*, SBT) otoliths (Clear *et al*., 2000). Thus translucent bands appearing at the otolith edge are counted or not in accordance with assumed birth date and month of capture for SBT (Farley *et al*., 2007). In contrast to SBT findings, present results in monthly formation of edge type, translucent or opaque, are still inconclusive and do not allow establishing an annual formation pattern (**Figure 2**). This lack of a comprehensible pattern in the assignment of edge type may be due to the difficulty in interpreting the otolith edge and the lack of a clear criterion for the identification of edge type. Inconsistencies in edge type assignment have been previously described (Busawon *et al*., 2014; Rodriguez-Marin *et al*., 2014), and need further investigation trough marginal increment analysis as it was suggested at the 2013 Ageing Workshop (Secor *et al*. 2014) or by chemical marking as have been done for SBT (Clear *et al*., 2000).

Until the timing of opaque band formation in otoliths can be more precisely determined and in order to correctly track cohorts, it was necessary to assign the fish correctly to the year it was born. To do so, a criterion was established in the 2015 Bluefin Data Preparatory Meeting (Anon. 2015 (*in press*)), based on the timing of opaque band formation inferred from monthly formation of edge type in bluefin tuna fin spines (Luque *et al*., 2014) and band formation from chemical tagging in SBT (Clear *et al*., 2000). Both sources coincide in opaque bands forming annually in summer (**Figure 3**). The adopted rule for otoliths is that when counting opaque bands: if the fish is caught between January 1 and the assumed time of the opaque band formation (June 1), then 1 year is added to the age. When counting translucent bands: if the fish is caught between June 1 and 31 of December, then 1 year is subtracted to the age.

Age estimates from otoliths based on a standardized reading criterion is presented**,** as percent by number by length class (SFL, cm), in **Table 3**. The confidence interval of the von Bertalanffy growth model curve fitted to ALK data included currently adopted growth function for eastern bluefin stock (Cort, 1991) and western stock function up to age 8 (Restrepo *et al*., 2010). From age 9, this last western function predicts slightly older ages than the present ALK growth model (**Figure 4**).

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Table 1. Summary of bluefin tuna otoliths used in the standardized age length key by month and by length range. ABFT length was measured as straight fork length (SFL) in cm.

	Month								
SFL (cm)	Jan	May	Jun	Jul	Aug	Sep	Oct	Nov	Total
20-24						6			6
25-29					4				4
30-34						6			6
35-39						1	3		4
40-44							5		5
45-49								1	1
50-54		1							1
55-59		1		5	1			1	8
60-64		1	1	3	5				10
65-69				1					1
70-74			1		1		2		4
75-79		11		1	4		2		18
80-84		2	2	6	10				20
85-89			2			1			3
90-94		2							2
95-99		10		2	2	1			15
100-104		4	1	4	3	2			14
105-109	1		1	1	3	6			12
110-114	1	1	3	5	8	9			27
115-119	2	3	2	7	5	3			22
120-124		1		3		6			10
125-129	1	1	3	3		4			12
130-134		3	6	3	2	2			16
135-139			2	3	1	3		1	10
140-144		1	1	1	1	6	1	1	12
145-149			4	2		1		2	9
150-154			2	1		1		10	14
155-159		1	8					2	11
160-164			5			1		4	10
165-169			2		1	1	3	7	14
170-174		3	1	1			1		6
175-179			6	1			2		9
180-184			13			1	1		15
185-189		4	8		2				14
190-194		3	7				2		12
195-199		1	16		1	1	1		20
200-204		6	16		1		3		26
205-209		3	12	1	1		7		24
210-214		8	9		3		7		27
215-219		2	14		1		5		22
220-224		2	17	1	2		2		24
225-229		2	6		1		1		10
230-234		1	8	1	2	1	3		16
235-239			6	1	1			1	9
240-244		1	7	2	2		1		13
245-249		2	2						4
250-254		1	1				1	1	4
255-259		1					1		2
260-264			1				1		2
265-269									
270-274									
275-279						1			1
Total	5	83	196	59	68	64	55	31	561

Table 2. Number of age estimates by length class (SFL, cm) used in the standardized age-length key based in direct ageing of bluefin otolith sections. Shaded cells represent cells were expert modal age from GBYP exchange exercise is included.

	Age																			
SFL (cm)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total
20-25	6																			6
25-30	4																			4
30-35	6																			6
35-40	4																			4
40-45	5																			5
45-50		1																		1
50-55			1																	1
55-60		7	1																	8
60-65		8	2																	10
65-70		1																		1
70-75		2	2																	4
75-80		4	7	6	1															18
80-85		5	11	4																20
85-90			2	1																3
90-95				2																2
95-100				11	4															15
100-105				8	5	1														14
105-110			1	4	2	2	3													12
110-115				1	12	9	5													27
115-120				1	4	14	2	1												22
120-125					3	6	1													10
125-130					2	6	3	1												12
130-135				2	2	5	4	3												16
135-140					1	1	5	1	2											10
140-145					1	2	2	6	1											12
145-150						2	5	2												9
150-155						3	3	4	3	1										14
155-160						1	2	6	1	1										11
160-165						1	2	3	3	1										10
165-170							1	7	6											14
170-175									3	2		1								6
175-180								3	3	1	2									9
180-185							1	5	7	2										15
185-190								1	4	3	4	1	1							14
190-195									3	4	4	1								12
195-200								2	6	6	2	2	1	1						20
200-205								4	5	5	3	7	1	1						26
205-210								1	5	7	3	5	3							24
210-215								3	1	8	7	4	3	1						27
215-220								2	3	6	5	4	1	1						22
220-225									1	5	6	7	2	2		1				24
225-230										1	3	1	3		1	1				10
230-235									1	3	5	3	4							16
235-240									2	1	1	2	1	1			1			9
240-245										1	2	2	3	1	2		1	1		13
245-250										1			2		1					4
250-255											1	1		1	1					4
255-260													1					1		2
260-265															1				1	2
265-270																				
270-275																				
275-280																	1			1
Total	25	28	27	40	37	53	39	55	60	59	48	41	26	9	6	2	3	2	1	561

Table 3. Otolith-based age length key for bluefin caught in the eastern Atlantic and Mediterranean stock. Numbers represent percent by number by length class (SFL, cm).

	Age																			
SFL (cm)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Total n
20-24	100																			6
25-29	100																			4
30-34	100																			6
35-39	100																			4
40-44	100																			5
45-49		100																		1
50-54			100																	1
55-59		88	13																	8
60-64		80	20																	10
65-69		100																		1
70-74		50	50																	4
75-79		22	39	33	6															18
80-84		25	55	20																20
85-89			67	33																3
90-94				100																2
95-99				73	27															15
100-104				57	36	7														14
105-109			8	33	17	17	25													12
110-114				4	44	33	19													27
115-119				5	18	64	9	5												22
120-124					30	60	10													10
125-129					17	50	25	8												12
130-134				13	13	31	25	19												16
135-139					10	10	50	10	20											10
140-144					8	17	17	50	8											12
145-149						22	56	22												9
150-154						21	21	29	21	7										14
155-159						9	18	55	9	9										11
160-164						10	20	30	30	10										10
165-169							7	50	43											14
170-174									50	33		17								6
175-179								33	33	11	22									9
180-184							7	33	47	13										15
185-189								7	29	21	29	7	7							14
190-194									25	33	33	8								12
195-199								10	30	30	10	10	5	5						20
200-204								15	19	19	12	27	4	4						26
205-209								4	21	29	13	21	13							24
210-214								11	4	30	26	15	11	4						27
215-219								9	14	27	23	18	5	5						22
220-224									4	21	25	29	8	8		4				24
225-229										10	30	10	30		10	10				10
230-234									6	19	31	19	25							16
235-239									22	11	11	22	11	11			11			9
240-244										8	15	15	23	8	15		8	8		13
245-249										25			50		25					4
250-254											25	25		25	25					4
255-259													50					50		2
260-264															50				50	2
265-269																				
270-274																				
275-279																	100			1
Total %	4.5	5.0	4.8	7.1	6.6	9.4	7.0	9.8	10.7	10.5	8.6	7.3	4.6	1.6	1.1	0.4	0.5	0.4	0.2	561

0-20%
20-50%
50-100%

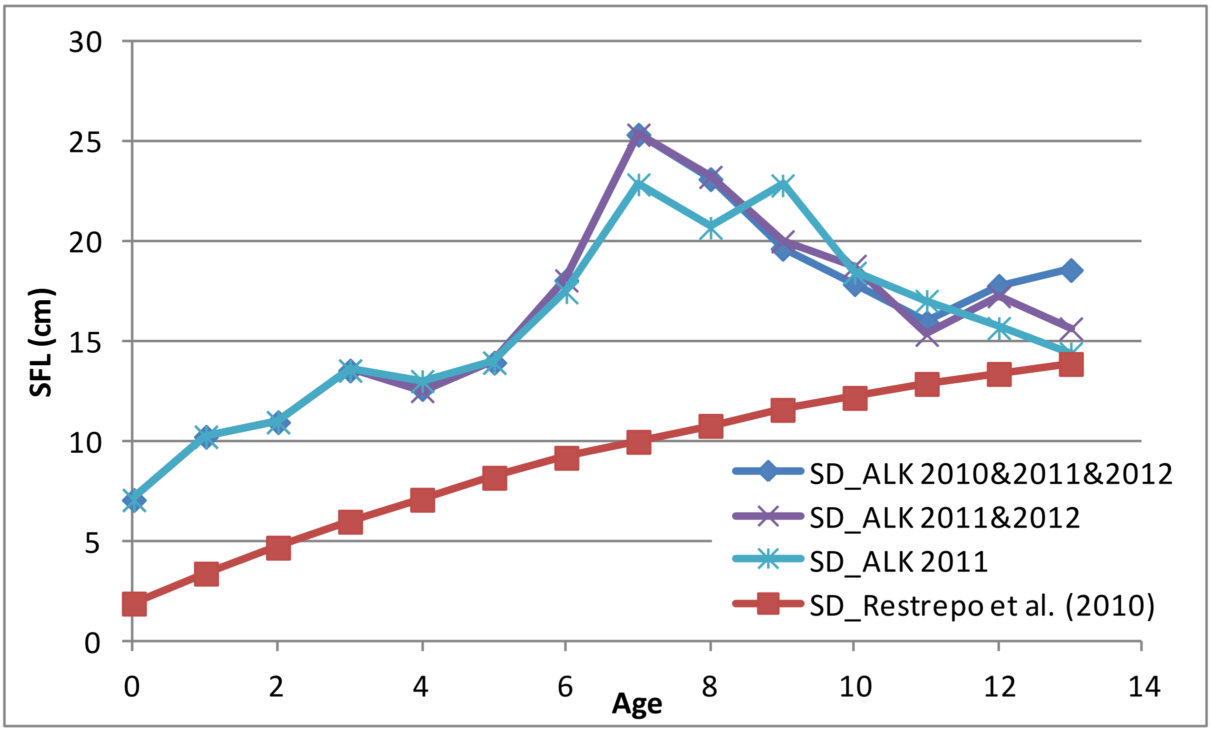

Figure 1. Qualitative comparison among length at age standard deviation (SD) from present age-length key (SD_ALK 2010&2011&2012), ALK without 2010 age estimates (SD_ALK 2011&2012), ALK without 2010 and 2012 age estimates (SD_ALK 2011) and length at age SD from Restrepo *et al*. (2010).

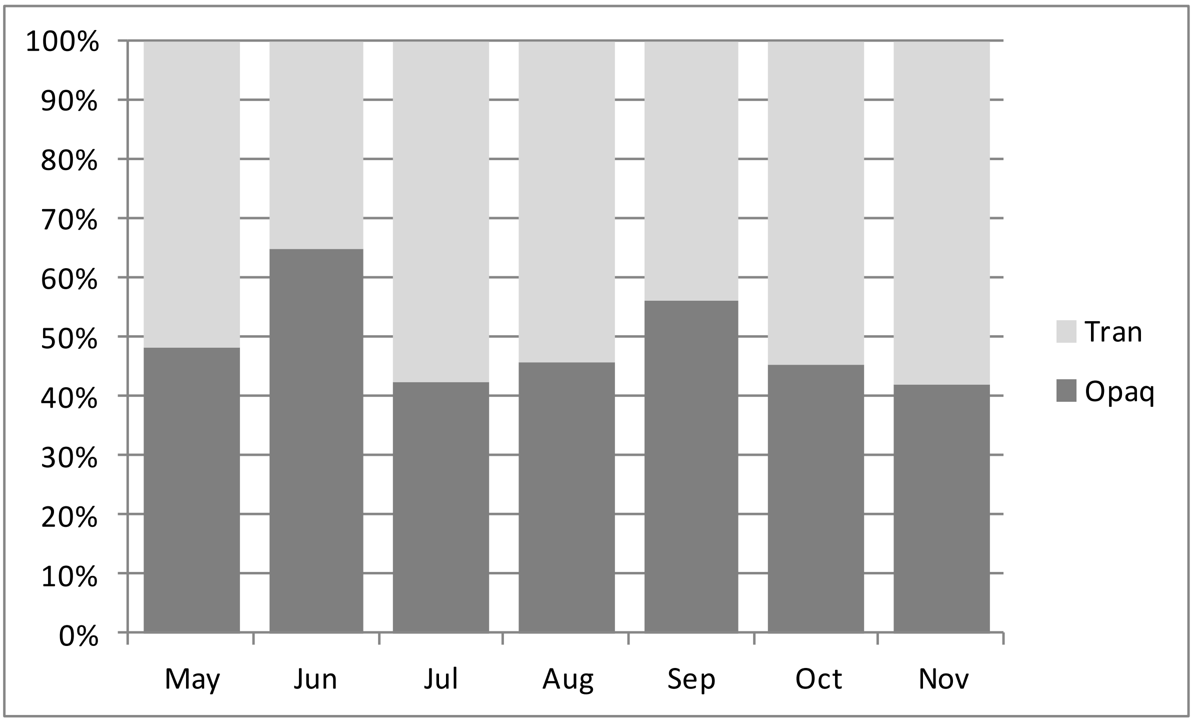

Figure 2. Monthly edge type assignment (translucent or opaque) from the otoliths used to build the age-length key.

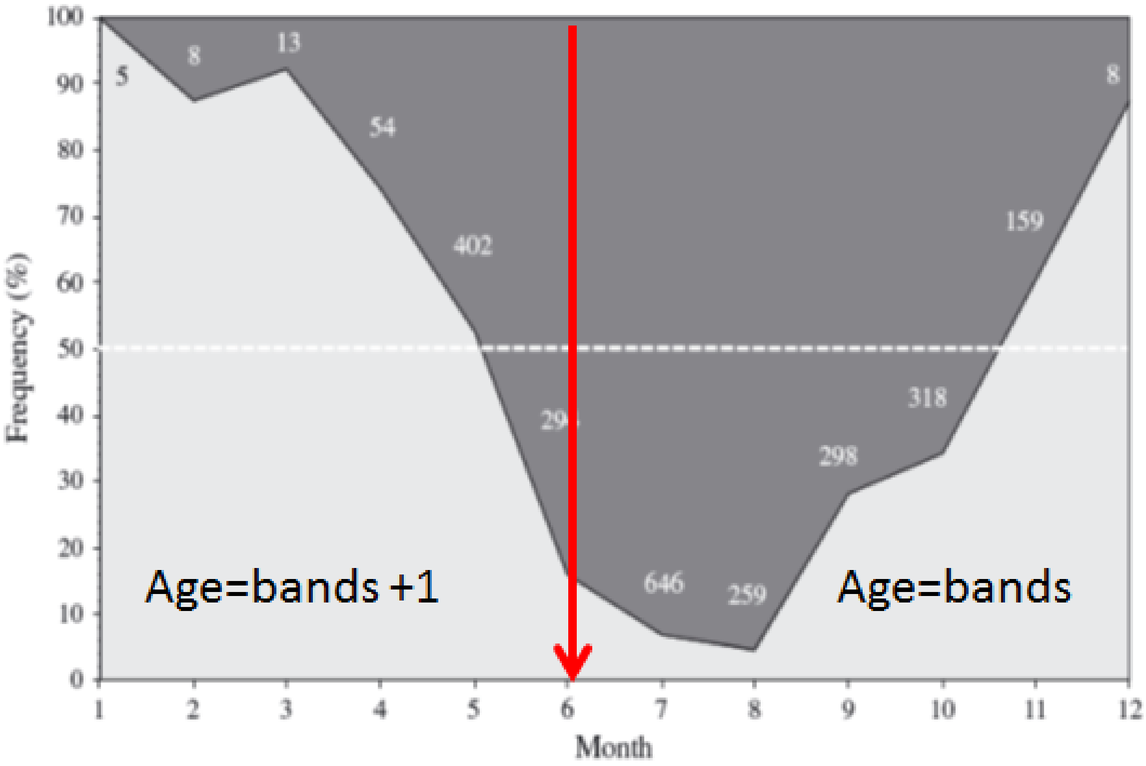

Figure 3. Monthly formation of edge type in bluefin tuna fin spines (reprinted from Luque *et al*., 2014). Dark shading is the opaque edge and light is the translucent edge and the frequency represents the frequency of each edge type. Adjusted age criterion corresponds to opaque bands counting.

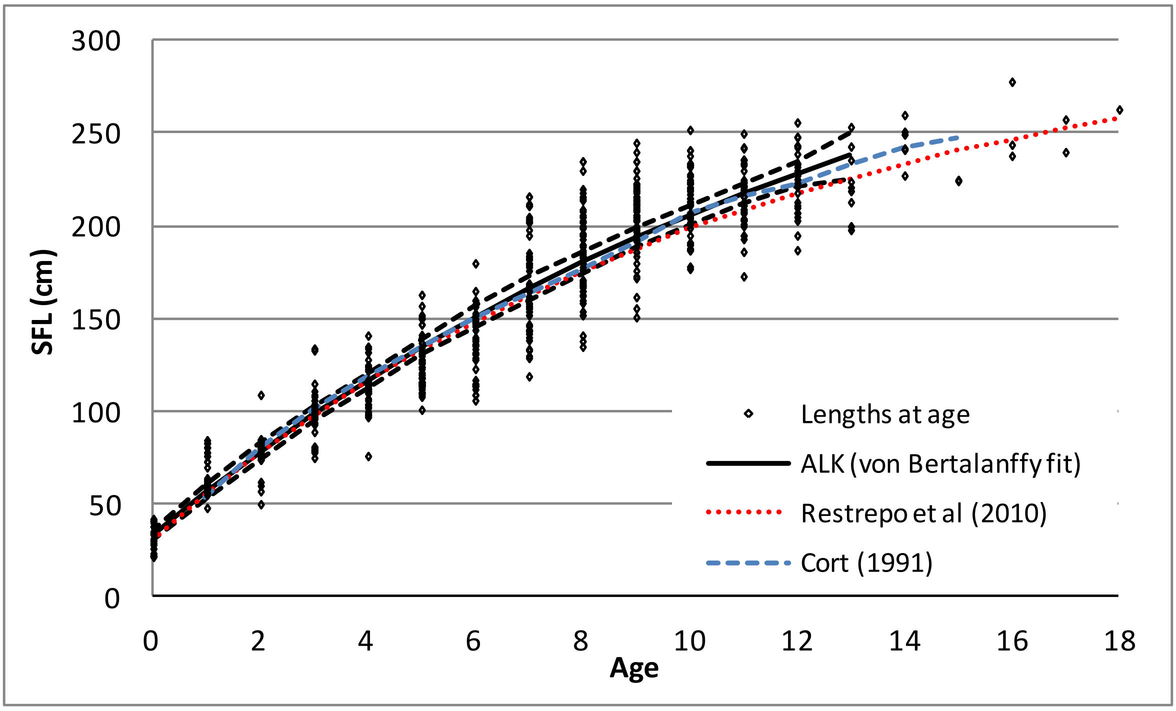

Figure 4. Comparison of length at age from present ALK and ICCAT currently adopted growth functions for Atlantic bluefin tuna stocks. Present ALK von Bertalanffy growth model curve (black line) fitted to observed length at age data (black triangles) with 95% confidence intervals (black dashed lines). Eastern stock growth curve from Cort (1991) (blue dashed line) and western curve from Restrepo *et al*. (2010) (red dashed line).