SCRS/2013/080 Collect. Vol. Sci. Pap. ICCAT, 70(2): 321-331 (2014)

# AGE DETERMINATION ANALYSES OF ATLANTIC BLUEFIN TUNA (*THUNNUS THYNNUS*) WITHIN THE BIOLOGICAL AND GENETIC SAMPLING AND ANALYSIS CONTRACT (GBYP)

Enrique Rodríguez-Marín[1], Patricia L. Luque[1], Pablo Quelle[1],
Marta Ruiz[1], Bernardo Perez[1], David Macias[2] and Saadet Karakulak[3]

*SUMMARY*

*This paper presents direct ageing of Atlantic bluefin tuna based on otoliths and dorsal fin spines sampled in the North East Atlantic and Mediterranean Sea, with the aim of estimating the age of the catch of the eastern stock. Six month age-length keys (ALKs) were obtained through length-stratified sampling. Half year ALKs were insufficiently sampled, thus, it was suggested to use annual ALKs with calcified structures from 2011 and 2012. Asymptotic lengths and growth coefficients obtained from ALKs derived from both structures did not present significant differences. Inter-reader precision within each structure, described by Coefficient of Variation and Average Percent Error, was high with low values of both indices.*

*RÉSUMÉ*

*Ce document présente la détermination directe de l'âge du thon rouge de l'Atlantique, basée sur des otolithes et des épines des nageoires échantillonnés dans l'Atlantique nord-est et en Méditerranée dans le but d'estimer l'âge de la capture du stock Est. Des clés âge-longueur semestrielles (ALK) ont été obtenues par le biais de l'échantillonnage stratifié par taille. Des ALK semestrielles n'ont pas été suffisamment échantillonnées ; il a donc été suggéré d'utiliser des ALK annuelles avec des structures calcifiées de 2011 et 2012. Les tailles asymptotiques et les coefficients de croissance obtenus des ALK issues des deux structures n'ont pas présenté de différences significatives. La précision entre les lecteurs au sein de chaque structure, décrite par le coefficient de variation et l'erreur moyenne de pourcentage, s'est avérée élevée avec de faibles valeurs des deux indices.*

*RESUMEN*

*En este trabajo se presenta la interpretación directa de la edad del atún rojo del Atlántico basada en otolitos y espinas de la aleta dorsal muestreados en el Atlántico nororiental y el Mediterráneo, con el objetivo de estimar la edad de las capturas de la población oriental. Se obtuvieron claves talla-edad (ALK) semestrales procedentes de un muestreo estratificado de tallas. Las ALK semestrales fueron insuficientemente muestreadas, por lo que se sugiere utilizar ALK anuales con estructuras calcificadas de 2011 y 2012. Las longitudes asintóticas y los coeficientes de crecimiento obtenidos de las ALK derivadas de ambas estructuras no presentaron diferencias significativas. La precisión entre lectores para cada estructura, descrita por el coeficiente de variación y el porcentaje medio de error, fue alta, con valores bajos de ambos índices.*

*KEYWORDS*

*Direct ageing, Otoliths, Fin spines,*
*Age-length keys, Biometry, Inter-reader precision*

## 1. Introduction

Biological studies on age and growth of fish are crucial components for describing their life cycle (age at maturity, age at recruitment, longevity, etc.). Age determination is an essential feature in fish stock assessment to estimate the rates of mortalities and growth. Assessment of Atlantic bluefin tuna (*Thunnus thynnus*, ABFT) using age structured models has proved useful in establishing a diagnosis of stock status (ICCAT, 2012).

[1] Spanish Institute of Oceanography. C.O. Santander. PO Box 240. 39080, Santander. Spain. rodriguez.marin@st.ieo.es
[2] Spanish Institute of Oceanography. C.O. Málaga. PO Box 285. 29640, Fuengirola, Málaga, Spain.
[3] Istambul University. Faculty of Fisheries, Ordu St. No. 200, 34470 Laleli, Istanbul, Turkey.

Various calcified structures have been used for age estimation of ABFT, including scales, vertebrae, otoliths, and dorsal fin spines (Rooker *et al*., 2007). Of all these structures, the latter two are those which have provided more reliable results (Rodriguez-Marin *et al*., 2007). Otoliths represent an advantage for ABFT direct ageing in relation to fin spines because all ages can be interpreted since there is no nucleus vascularization; conversely, dorsal fin spines (hereby spines) are easier to collect and prepare than otoliths (Rodriguez-Marin *et al*., 2007).

One of the goals of the project "Biological and genetic sampling and analysis" (within the ICCAT Atlantic Wide Research Programme for Bluefin tuna, GBYP) is the estimation of age composition of the bluefin tuna catches in the NE Atlantic and Mediterranean Sea, including age information of the samples used for population structure identification. To estimate the age of the catch we attempt to build half a year age-length keys based on otoliths and on spines. Furthermore, an estimation of age interpretation precision for both structures and calcified structures biometry are also provided.

## 2. Material and methods

### *2.1 Sampling*

Calcified structures sampling was obtained from specimens caught in 2011 and 2012 during the second and third phases of the project, respectively. Samples were collected from May to November in 2011 and from January to November in 2012. In order to adequately represent the seasonal growth and obtain samples throughout the year, 2012 samples were used to improve month and size coverage of the 2011 sampling for both otoliths and spines. This samples selection procedure prevented comparison between years. Six month age-length keys were built through length-stratified sampling.

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. Dorsal fin spines and sagittal otoliths extraction and conservation were carried out following the "Biological and genetic sampling and analysis" GBYP project sampling protocols. ABFT length was measured as straight fork length (SFL) in cm.

### *2.2 Calcified structures biometry*

Several biometric measures were recorded for each structure in order to analyze the relationship between the growth of the hard part and the specimen sampled. Spine diameter and total spine length were measured. For otoliths the longest and widest axes of the sagittal otolith were measured by placing the whole sagittal otolith sulcus side down on a black background (**Figure 1**). Weight was also recorded. Incomplete otoliths were not used for this biometric analysis. Linear and power regression functions were tested for the relationships mentioned above, using the coefficient of determination ($r^2$) as a goodness index.

### *2.3 Calcified structures preparation and age interpretation*

Spine preparation and age interpretation criteria were performed according to Rodriguez-Marin *et al*. (2012). Spine section location was established at 1.5 times the condyle base width. Sections were obtained using a precision rotating diamond saw and mounted on glass slides. It is easy to identify the translucent and opaque bands formed on the spine of young individuals. However, in fish over two years old, the central area of the spine begins to reabsorb and the bands consequently disappear. To overcome the problem of nucleus vascularization with age, the translucent band diameters measured from spines without vascularization (i.e. spines from young specimens) had to be used to assign an age to the first inner visible translucent band in vascularised spines (**Figure 2**). Age was estimated by counting the translucent bands which are deposited annually between November and April (Luque *et al*., 2014). For the interpretation of the border of the spine section we followed Rodriguez-Marin *et al*. (2007) criterion, in which a bluefin 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, 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 analyzer (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 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.

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 (**Figure 1**), 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 to improve the contrast of bands. Otolith images were taken using reflected light on a black background and the same procedure described for spines was used to obtain digital images of otoliths. Age interpretation was performed on digitally enhanced images using Adobe Photoshop and annulus counts were made along the longest (ventral) arm of the sectioned sagittae otolith. Age was estimated by counting the opaque bands. Quality in terms of readability for both calcified structures was annotated.

### *2.4 Comparing age estimates from otoliths and spines*

A von Bertalanffy growth model (VBGM) was fitted to mean length at age data derived from age-length keys (ALKs) based on otoliths and spines readings to compare age estimations from both structures. Only those age classes with a minimum sample size of five specimens were considered for analysis. Growth parameters derived from both structures were compared by Kimura's (1980) Likelihood Ratio test. The test was conducted using equivalent age ranges as recommended by Haddon (2001).

### *2.5 Precision of age estimates*

Comparisons of age estimates between readers for spines and otoliths were carried out. Readers were scored into categories according to their reading experience. Age readings were analysed using the method developed by Eltink *et al*. (2000). This analysis compares the estimated ages from each reader with the modal age, the latter being the consensus among readers. Three indices were used to estimate ageing precision among readers: the average percent error (APE), the coefficient of variation (CV), and the weighted mean percentage agreement (PA).

APE was estimated using the formula:

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

where Xij 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 using the formula in the European Fish Ageing Network (EFAN) software (Eltink *et al*. 2000):

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

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

PA, which indicates agreement with respect to the modal age, was estimated following 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.

## 3. Results

### *3.1 Calcified structures biometry*

The number of otoliths used in biometric analysis was 569. Relationship between otolith size and fish length are described in **Figure 3**. The goodness of fit was high, despite increasing variation in data in all size-length relationships for fish over 180 cm SFL. Regression functions showed better potential than linear fitting with high determination coefficients ($r^2$) for all the relationships between otolith size (length, height and weight) and fish length.

A total of 468 spine samples were used for the biometric analysis. Both linear and power equations fit adequately the spine length and diameter versus fish length relationship (**Figure 4**). The goodness of fit between the spine diameter and SFL showed that the fish body length and the size of the calcified structure were closely related.

### *3.2 Calcified structures age interpretation*

Overall, a total of 525 otoliths and 533 spines were used for age interpretation of ABFT. **Table 1** and **Table 2** show the number of samples from both phases of the project, including the number of samples obtained in 2011 and 2012. Otoliths and spines age length keys (ALKs) by six months time period are displayed in **Table 3** and **Table 4**, respectively. Annual age-length keys for both structures are shown in **Table 5**.

### *3.3 Comparing age estimates from otoliths and spines*

The growth parameters of the von Bertalanffy growth model (VBGM) estimated from annual ALKs using spines and otoliths together with the likelihood ratio test for the growth parameters comparison is shown in **Table 6**. Asymptotic lengths and growth coefficients obtained from both structures did not present significant differences, except for $t_0$. Estimated growth curves that fit the VBGM to the observed mean length at age data from annual ALKs from both structures are presented, together with currently used growth curves for western and eastern ABFT stocks, in **Figure 5.**

### *3.4 Precision of age estimates*

The results of precision analysis for spines and otoliths inter-reader comparisons are shown in **Table 7**. Overall, for each calcified structure, both CV and APE were low, with CV values of 1.9% for spines and 2.2% for otoliths, corresponding to an APE of 1.55% and 1.52%. The overall PA was high for both structures, with 91% and 88% for spines and otoliths, respectively. In addition, the inter-reader bias test was no significant. When analyzing the evolution of the CV and PA by age (**Figure 6**), there was found no pattern with age for the spines inter-reader comparison, while for otoliths the CV increased and the PA decreased as specimens age increases.

## 4. Discussion

This paper presents direct ageing of Atlantic bluefin tuna based on otoliths and spines sampled in the areas of the North East Atlantic Ocean and western, central and eastern Mediterranean Sea, with the aim of estimating the age of the catch of the eastern stock of this species. The age-length keys were obtained through length-stratified sampling instead of through random sampling, because of the wide length range of this species and due to the seasonality of all bluefin tuna fisheries, which mostly capture only a fraction of the population. This approach has been also applied for estimating southern bluefin tuna (*Thunnus maccoyii*) age composition (Anon., 2002).

We used two calcified structures for the age interpretation of ABFT, sagittal otolith and the first dorsal fin spine. None of these two calcified structures can be excluded for routine direct ageing because in certain fisheries, fish processing or fish market practices would hinder the sampling of either structure. Direct ageing techniques using otoliths were verified for ABFT in 2008 by Neilson and Campana (2008), but analogous validation studies are not yet available for spines. Thus, in this GBYP project we have hardly focused upon the comparison of the age interpretation from spines and otoliths from the same specimen as an indirect validation method. Results of the ageing comparisons between paired structures are going to be presented in another document (Rodriguez-Marin *et al*., 2013) with samples from various projects besides the ones from the present project in the framework of GBYP.

During the 2012 ICCAT-GBYP operational meeting it was stressed the need to take into account the seasonal growth and thus to have an ALK with an adequate sampling throughout the year in all the range of sizes. Given these requirements, we improved the sampling annual coverage in the phase 3 of the project, attending to the seasonality of the fisheries that take place mainly between May and November, by splitting the year in two and getting two ALKs by semester and calcified structure, i.e. otoliths and spines. The target objective for sampling 10 specimens by 10 cm length range for the six month ALKs was not fully achieved and there were numerous gaps due to the wide length range of this species. Available samples from the present contract did not allow covering those half year ALKs gaps despite having used the two years of sampling. Number of samples for both calcified structures and half year ALKs was insufficiently represented and first semester comprises mainly the months of May and June and second semester comprises the months from July to November. In view of these results, we recommend using annual ALKs using samples from 2011 and 2012.

No significant differences were found between the von Bertalanffy growth parameters obtained from annual ALKs derived from otolith and spine readings. However the asymptotic lengths are excessively high in both cases. This is because the curves did not converge to the maximum length due to scarcity of samples over 13 years. Both calcified structures growth curves also show similarity with the growth equations currently used by ICCAT for western and eastern stocks (Cort, 1991; Restrepo *et al*., 2010) for adequately represented age classes (0 to 13).

Special care has been taken about the consensus on the methodology of preparation and reading of otoliths with other research institutions in the U.S. and Canada who also conduct ABFT age estimates from otoliths (Center for Environmental Science of the University of Maryland, Panama City Laboratory of the National Marine Fisheries Service, Gulf of Maine Research Institute and Fisheries and Oceans Canada). In this context, the Spanish Institute of Oceanography scientists have participated, together with scientists from other laboratories, in ageing workshops in 2011, 2012 and the present year, in order to standardize important areas of methodological concern that may influence age estimates of ABFT using otoliths. Direct ageing using spines have also been comprehensively reviewed in a paper that is been actually under revision (Luque *et al*., 2014). There are some laboratories from different countries involved in direct ageing standardization, but it is necessary to increase the number of laboratories involved in this task for both calcified structures, especially in the eastern side of the Atlantic.

## Acknowledgements

This work was carried out under the provision of the ICCAT Atlantic Wide Research Programme for Bluefin Tuna (GBYP), funded by the European Community (grant SI2/542789), Canada, Croatia, Japan, Norway, Turkey, United States, Chinese Taipei and the ICCAT Secretariat. The contents of this paper do not necessarily reflect the point of view of ICCAT or of the other funders, which have not responsibility about them. Neither does it necessarily reflect the views of the funders and in no ways anticipate the Commission's future policy in this area. We also would like to thank the following scientific institutions involved in the calcified structures sampling: Spanish Institute of Oceanography (IEO), University of Cagliari, AZTI-Tecnalia, Instituto de Investigação das Pescas e do Mar (IPIMAR), University of Bologna, University of Genova, Istambul University and Federation of Maltese Aquaculture Producers (FMAP).

## References

Anon. 2002. A manual for age determination of southern bluefin tuna *Thunnus maccoyii*. Otolith sampling, preparation and interpretation. In: Report of the Direct Age Estimation Workshop. 11-14 June 2002. Victoria, Australia. Meeting Report of CCSBT. 36p.

Cort, J. L. (1991). Age and growth of the bluefin tuna, *Thunnus thynnus* (L.) of the Northwest Atlantic. Collect. Vol. Sci. Pap. ICCAT, 35: 213–230.

Eltink, A. T. G. W., Newton, A. W., Morgado, C., Santamaria, M.T.G., and Modin, J. 2000. Guidelines and tools for age reading (PDF document version 1.0). Internet: http://www.efan.no. Eltink, A. T. G. W. 2000. Age reading comparison. (MS Excel workbook version 1.0, October 2000). Internet: http://www.efan.no.

Haddon, M. 2001. Modelling and quantitative methods in fisheries. Chapman and Hall/CRC, Boca Raton. 406pp.

Anon. 2013, Report of the 2012 Atlantic Bluefin Tuna Stock Assessment Session (Madrid, Spain, September 4 to 11, 2012). Collect. Vol. Sci. Pap. ICCAT, 69(1): 1-198.

Kimura, D.K. 1980. Likelihood methods for the Von Bertalanffy growth curve. Fish. Bull., 77(4): 765-776.

Luque, P.L., Rodríguez-Marin, E., Ruiz, M., Quelle, P., Landa, J., Macías, D. and Ortiz de Urbina, JM. 2014. Direct ageing of Atlantic Bluefin tuna (*Thunnus thynnus*) from East Atlantic and Western Mediterranean using dorsal fin spines. J. Fish Biol., (under review).

Neilson, J. D., and Campana, S. E. 2008. A validated description of age and growth of western Atlantic bluefin tuna (*Thunnus thynnus*). Can. J. Fish. Aquat. Sci., 65: 1523-1527.

Restrepo, V. R., Diaz, G. A., Walter, J. F., Neilson, J. D., Campana, S. E., Secor, D., and Wingate, R. L. 2010. Updated estimate of the growth curve of Western Atlantic bluefin Tuna. Aquat. Living Resour., 23: 335–342.

Rodriguez-Marin, E., Clear, N., Cort, J.L., Megalofonou, P., Neilson, J.D., Neves dos Santos, M., Olafsdottir, D., Rodriguez-Cabello, C., Ruiz, M. and Valeiras, J. 2007. Report of the 2006 ICCAT Workshop for bluefin tuna direct ageing. Collect. Vol. Sci. Pap. ICCAT, 60(4): 1349-1392.

Rodriguez-Marin, E., Luque, P. L., Ruiz, M., Quelle, P. and Landa, J. 2012. Protocol for sampling, preparing and age interpreting criteria of Atlantic bluefin tuna (*Thunnus thynnus*) first dorsal fin spine sections. Collect. Vol. Sci. Pap. ICCAT, 68(1): 240-253.

Rodríguez-Marín, E., Luque, P.L., Busawon, D., Campana, S., Golet, W., Koob, E., Neilson, J.D., Quelle, P. and Ruiz, M. 2013. An attempt of validation of Atlantic bluefin tuna (*Thunnus thynnus*) ageing using dorsal fin spines. ICCAT SCRS/2013/081.

Rooker, J.R., Bremer, J.R.A., Block, B.A., Dewar, H., Metrio, G.d., Corriero, A., Kraus, R.T., Prince, E.D., Rodriguez-Marin, E. and Secor, D.H. (2007). Life history and stock structure of Atlantic bluefin tuna (*Thunnus thynnus*). Rev. Fish. Sci., 15: 2365-2310 doi: 10.1080/10641260701484135.

**Table 1**. Summary of bluefin tuna otoliths used for age interpretation by length range. ABFT length was measured as straight fork length (SFL) in cm.

| **Otolith samples** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | GBYP-Phase 2 (samples from 2011) | | | GBYP-Phase 3 (samples from 2011 & 2012) | | | | | Total |
| SFL (cm) | 1st semester | 2nd semester | Total | 1st semester | 2nd semester | 2011 | 2012 | Total | Phase 2 & 3 |
| 20-30 | | 10 | 10 | | | | | | 10 |
| 30-40 | | 10 | 10 | | | | | | 10 |
| 40-50 | | 6 | 6 | | | | | | 6 |
| 50-60 | 2 | 7 | 9 | | | | | | 9 |
| 60-70 | 2 | 9 | 11 | | | | | | 11 |
| 70-80 | 4 | 9 | 13 | 8 | 1 | 9 | | 9 | 22 |
| 80-90 | 5 | 16 | 21 | | 1 | 1 | | 1 | 22 |
| 90-100 | 7 | 5 | 12 | 5 | | 5 | | 5 | 17 |
| 100-110 | 2 | 17 | 19 | 5 | 2 | 6 | 1 | 7 | 26 |
| 110-120 | 2 | 28 | 30 | 10 | 10 | 17 | 3 | 20 | 50 |
| 120-130 | 4 | 12 | 16 | 1 | 3 | 3 | 1 | 4 | 20 |
| 130-140 | 11 | 13 | 24 | | 2 | 2 | | 2 | 26 |
| 140-150 | 6 | 11 | 17 | | 4 | 3 | 1 | 4 | 21 |
| 150-160 | 6 | 7 | 13 | 4 | 6 | 6 | 4 | 10 | 23 |
| 160-170 | 4 | 8 | 12 | 3 | 9 | 3 | 9 | 12 | 24 |
| 170-180 | 5 | 3 | 8 | 3 | 2 | | 5 | 5 | 13 |
| 180-190 | 18 | 3 | 21 | 5 | 1 | 1 | 5 | 6 | 27 |
| 190-200 | 17 | 4 | 21 | 6 | 1 | | 7 | 7 | 28 |
| 200-210 | 17 | 3 | 20 | 11 | 10 | 2 | 19 | 21 | 41 |
| 210-220 | 17 | 4 | 21 | 6 | 12 | | 18 | 18 | 39 |
| 220-230 | 17 | 3 | 20 | 6 | 4 | | 10 | 10 | 30 |
| 230-240 | 11 | 9 | 20 | 3 | 3 | | 6 | 6 | 26 |
| 240-250 | 6 | 4 | 10 | 6 | 1 | | 7 | 7 | 17 |
| 250-260 | | 3 | 3 | 1 | | | 1 | 1 | 4 |
| 260-270 | | | | 1 | 1 | | 2 | 2 | 2 |
| 270-280 | | 1 | 1 | | | | | | 1 |
| Total | 163 | 205 | 368 | 84 | 73 | 58 | 99 | 157 | 525 |

**Table 2**. Summary of bluefin tuna spines used for age interpretation by length range. ABFT length was measured as straight fork length (SFL) in cm.

| Spine samples | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | GBYP-Phase 2 (samples from 2011) | | | GBYP-Phase 3 (samples from 2011 & 2012) | | | | | Total |
| SFL (cm) | 1st semester | 2nd semester | Total | 1st semester | 2nd semester | 2011 | 2012 | Total | Phase 2 & 3 |
| 20-30 | | 10 | 10 | | | | | | 10 |
| 30-40 | | 10 | 10 | | | | | | 10 |
| 40-50 | | 6 | 6 | | | | | | 6 |
| 50-60 | 2 | 8 | 10 | | | | | | 10 |
| 60-70 | 1 | 5 | 6 | | 5 | | 5 | 5 | 11 |
| 70-80 | 4 | 8 | 12 | 11 | 4 | 15 | | 15 | 27 |
| 80-90 | 2 | 32 | 34 | | 2 | 2 | | 2 | 36 |
| 90-100 | 7 | 3 | 10 | 5 | | 5 | | 5 | 15 |
| 100-110 | | 16 | 16 | 4 | 2 | 5 | 1 | 6 | 22 |
| 110-120 | 6 | 31 | 37 | 9 | 8 | 14 | 3 | 17 | 54 |
| 120-130 | 10 | 19 | 29 | | 3 | 3 | | 3 | 32 |
| 130-140 | 16 | 12 | 28 | | 2 | 2 | | 2 | 30 |
| 140-150 | 12 | 16 | 28 | | 5 | 5 | | 5 | 33 |
| 150-160 | 10 | 9 | 19 | 4 | 3 | 3 | 4 | 7 | 26 |
| 160-170 | 4 | 8 | 12 | 2 | 3 | 4 | 1 | 5 | 17 |
| 170-180 | 3 | 1 | 4 | 3 | 2 | 3 | 2 | 5 | 9 |
| 180-190 | 20 | | 20 | 5 | 1 | 1 | 5 | 6 | 26 |
| 190-200 | 15 | 2 | 17 | 8 | 2 | 2 | 8 | 10 | 27 |
| 200-210 | 14 | 1 | 15 | 11 | 5 | 5 | 11 | 16 | 31 |
| 210-220 | 14 | | 14 | 7 | 5 | 5 | 7 | 12 | 26 |
| 220-230 | 15 | | 15 | 6 | 7 | 7 | 6 | 13 | 28 |
| 230-240 | 12 | 3 | 15 | 4 | 7 | 7 | 4 | 11 | 26 |
| 240-250 | 1 | 1 | 2 | 6 | 1 | 1 | 6 | 7 | 9 |
| 250-260 | 3 | 3 | 6 | 1 | | | 1 | 1 | 7 |
| 260-270 | | | | 1 | 3 | 3 | 1 | 4 | 4 |
| 270-280 | | | | | | | | | |
| 280-290 | | | | | 1 | 1 | | 1 | 1 |
| Total | 171 | 204 | 375 | 87 | 70 | 93 | 65 | 158 | 533 |

**Table 3**. Six month age-length keys based in age interpretation from Atlantic bluefin tuna otolith sections. Numbers represent percent by number by length class (SFL, cm). Samples include 2011 and 2012.

**First and second quarters**

| | Age class | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Length class | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | n |
| 20-30 | | | | | | | | | | | | | | | | | | | | | |
| 30-40 | | | | | | | | | | | | | | | | | | | | | |
| 40-50 | | | | | | | | | | | | | | | | | | | | | |
| 50-60 | 50 | 50 | | | | | | | | | | | | | | | | | | | 2 |
| 60-70 | | 100 | | | | | | | | | | | | | | | | | | | 2 |
| 70-80 | | | 100 | | | | | | | | | | | | | | | | | | 12 |
| 80-90 | | 40 | 60 | | | | | | | | | | | | | | | | | | 5 |
| 90-100 | | | 8 | 83 | 8 | | | | | | | | | | | | | | | | 12 |
| 100-110 | | | 29 | 71 | | | | | | | | | | | | | | | | | 7 |
| 110-120 | | | | 25 | 58 | 17 | | | | | | | | | | | | | | | 12 |
| 120-130 | | | | 20 | 60 | | 20 | | | | | | | | | | | | | | 5 |
| 130-140 | | | | | 45 | 45 | 9 | | | | | | | | | | | | | | 11 |
| 140-150 | | | | | 50 | 33 | 17 | | | | | | | | | | | | | | 6 |
| 150-160 | | | | | | | 50 | 30 | 20 | | | | | | | | | | | | 10 |
| 160-170 | | | | | | | 14 | 57 | 29 | | | | | | | | | | | | 7 |
| 170-180 | | | | | | 13 | | 13 | 50 | 25 | | | | | | | | | | | 8 |
| 180-190 | | | | | | | | 17 | 48 | 26 | | 9 | | | | | | | | | 23 |
| 190-200 | | | | | | | | 9 | 30 | 35 | 22 | 4 | | | | | | | | | 23 |
| 200-210 | | | | | | | | | 11 | 36 | 43 | 7 | 4 | | | | | | | | 28 |
| 210-220 | | | | | | | | | 0 | 13 | 48 | 35 | 4 | | | | | | | | 23 |
| 220-230 | | | | | | | | | 13 | 13 | 22 | 30 | 22 | | | | | | | | 23 |
| 230-240 | | | | | | | | | | | 29 | 29 | 29 | 14 | | | | | | | 14 |
| 240-250 | | | | | | | | | | | 25 | 25 | 17 | 25 | 8 | | | | | | 12 |
| 250-260 | | | | | | | | | | | | | | | | | | | | 100 | 1 |
| 260-270 | | | | | | | | | | | | | | | | 100 | | | | | 1 |
| 270-280 | | | | | | | | | | | | | | | | | | | | | |
| Total | 1 | 5 | 18 | 19 | 19 | 10 | 9 | 14 | 32 | 32 | 40 | 27 | 13 | 5 | 1 | 1 | | | | 1 | 247 |

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

**Third and fourth quarters**

| | Age class | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Length class | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | n |
| 20-30 | 100 | | | | | | | | | | | | | | | | | | | | 10 |
| 30-40 | 100 | | | | | | | | | | | | | | | | | | | | 10 |
| 40-50 | 100 | | | | | | | | | | | | | | | | | | | | 6 |
| 50-60 | 29 | 71 | | | | | | | | | | | | | | | | | | | 7 |
| 60-70 | | 100 | | | | | | | | | | | | | | | | | | | 9 |
| 70-80 | | 70 | 30 | | | | | | | | | | | | | | | | | | 10 |
| 80-90 | | 35 | 53 | 12 | | | | | | | | | | | | | | | | | 17 |
| 90-100 | | | 40 | 60 | | | | | | | | | | | | | | | | | 5 |
| 100-110 | | | 16 | 58 | 21 | 5 | | | | | | | | | | | | | | | 19 |
| 110-120 | | | | 13 | 47 | 37 | 3 | | | | | | | | | | | | | | 38 |
| 120-130 | | | | | 47 | 53 | | | | | | | | | | | | | | | 15 |
| 130-140 | | | | | 33 | 40 | 20 | 7 | | | | | | | | | | | | | 15 |
| 140-150 | | | | | 7 | 20 | 60 | 13 | | | | | | | | | | | | | 15 |
| 150-160 | | | | | | 31 | 31 | 23 | 15 | | | | | | | | | | | | 13 |
| 160-170 | | | | | | 12 | 6 | 41 | 12 | 18 | 12 | | | | | | | | | | 17 |
| 170-180 | | | | | | | | 60 | 20 | 20 | | | | | | | | | | | 5 |
| 180-190 | | | | | | | | 25 | 25 | 50 | | | | | | | | | | | 4 |
| 190-200 | | | | | | | | | 40 | | 60 | | | | | | | | | | 5 |
| 200-210 | | | | | | | | | 23 | 15 | 38 | 8 | 15 | | | | | | | | 13 |
| 210-220 | | | | | | | | | | 38 | 44 | 13 | 6 | | | | | | | | 16 |
| 220-230 | | | | | | | | | | 14 | 29 | 14 | 14 | 14 | 14 | | | | | | 7 |
| 230-240 | | | | | | | | | | 8 | 8 | 67 | 8 | 8 | | | | | | | 12 |
| 240-250 | | | | | | | | | | | 20 | 40 | | 20 | | | 20 | | | | 5 |
| 250-260 | | | | | | | | | | | | 100 | | | | | | | | | 3 |
| 260-270 | | | | | | | | | | | | | | | | | | | 100 | | 1 |
| 270-280 | | | | | | | | | | | | | | | | | | | | 100 | 1 |
| Total | 28 | 27 | 17 | 21 | 35 | 38 | 18 | 17 | 11 | 16 | 21 | 17 | 5 | 3 | 1 | | 1 | | 1 | 1 | 278 |

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

**Table 4**. Age-length keys based in age interpretation from Atlantic bluefin tuna spine sections. Numbers represent percent by number by length class (SFL, cm). Samples include 2011 and 2012.

| | **First and second quarters** | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Age class | | | | | | | | | | | | | | | | | | |
| Length class | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | n |
| 20-30 | | | | | | | | | | | | | | | | | | | |
| 30-40 | | | | | | | | | | | | | | | | | | | |
| 40-50 | | | | | | | | | | | | | | 0-20% | | | | | |
| 50-60 | | 100 | | | | | | | | | | | | 20-50% | | | | | 2 |
| 60-70 | | 100 | | | | | | | | | | | | 50-100% | | | | | 1 |
| 70-80 | | 7 | 87 | 7 | | | | | | | | | | | | | | | 15 |
| 80-90 | | | 100 | | | | | | | | | | | | | | | | 2 |
| 90-100 | | | | 100 | | | | | | | | | | | | | | | 12 |
| 100-110 | | | | 75 | 25 | | | | | | | | | | | | | | 4 |
| 110-120 | | | | | 67 | 33 | | | | | | | | | | | | | 15 |
| 120-130 | | | | | 10 | 90 | | | | | | | | | | | | | 10 |
| 130-140 | | | | | 6 | 81 | 13 | | | | | | | | | | | | 16 |
| 140-150 | | | | | 8 | 50 | 25 | 17 | | | | | | | | | | | 12 |
| 150-160 | | | | | | 14 | 57 | 21 | 7 | | | | | | | | | | 14 |
| 160-170 | | | | | | | | 83 | 17 | | | | | | | | | | 6 |
| 170-180 | | | | | | | | 17 | 50 | 33 | | | | | | | | | 6 |
| 180-190 | | | | | | | 8 | 24 | 48 | 20 | | | | | | | | | 25 |
| 190-200 | | | | | | | | 13 | 35 | 39 | 13 | | | | | | | | 23 |
| 200-210 | | | | | | | | 4 | 12 | 48 | 24 | 12 | | | | | | | 25 |
| 210-220 | | | | | | | | | | 24 | 52 | 10 | 10 | 5 | | | | | 21 |
| 220-230 | | | | | | | | | | 29 | 29 | 33 | 5 | 5 | | | | | 21 |
| 230-240 | | | | | | | | | | | 31 | 56 | 13 | | | | | | 16 |
| 240-250 | | | | | | | | | | | | 57 | 14 | 29 | | | | | 7 |
| 250-260 | | | | | | | | | | | 25 | 25 | 25 | | | | | 25 | 4 |
| 260-270 | | | | | | | | | | | | | | | 100 | | | | 1 |
| 270-280 | | | | | | | | | | | | | | | | | | | |
| 280-290 | | | | | | | | | | | | | | | | | | | |
| Total | | 4 | 15 | 16 | 14 | 35 | 15 | 21 | 28 | 39 | 32 | 26 | 7 | 4 | 1 | | | 1 | 258 |

| | **Third and fourth quarters** | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Age class | | | | | | | | | | | | | | | | | | |
| Length class | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | n |
| 20-30 | 100 | | | | | | | | | | | | | | | | | | 10 |
| 30-40 | 100 | | | | | | | | | | | | | | | | | | 10 |
| 40-50 | 100 | | | | | | | | | | | | | 0-20% | | | | | 6 |
| 50-60 | 13 | 88 | | | | | | | | | | | | 20-50% | | | | | 8 |
| 60-70 | | 90 | 10 | | | | | | | | | | | 50-100% | | | | | 10 |
| 70-80 | | 8 | 92 | | | | | | | | | | | | | | | | 12 |
| 80-90 | | | 85 | 15 | | | | | | | | | | | | | | | 34 |
| 90-100 | | | | 100 | | | | | | | | | | | | | | | 3 |
| 100-110 | | | | 44 | 50 | 6 | | | | | | | | | | | | | 18 |
| 110-120 | | | | 13 | 67 | 21 | | | | | | | | | | | | | 39 |
| 120-130 | | | | | 41 | 55 | 5 | | | | | | | | | | | | 22 |
| 130-140 | | | | | | 79 | 21 | | | | | | | | | | | | 14 |
| 140-150 | | | | | 5 | 48 | 48 | | | | | | | | | | | | 21 |
| 150-160 | | | | | | 50 | 50 | | | | | | | | | | | | 12 |
| 160-170 | | | | | | 18 | 45 | 27 | 9 | | | | | | | | | | 11 |
| 170-180 | | | | | | | 100 | | | | | | | | | | | | 3 |
| 180-190 | | | | | | | | 100 | | | | | | | | | | | 1 |
| 190-200 | | | | | | | | 25 | 75 | | | | | | | | | | 4 |
| 200-210 | | | | | | | | 17 | 50 | 17 | | 17 | | | | | | | 6 |
| 210-220 | | | | | | | | 40 | 20 | 40 | | | | | | | | | 5 |
| 220-230 | | | | | | | | 29 | 14 | 14 | 43 | | | | | | | | 7 |
| 230-240 | | | | | | | | | 20 | 20 | 30 | 20 | 10 | | | | | | 10 |
| 240-250 | | | | | | | | | | 50 | 50 | | | | | | | | 2 |
| 250-260 | | | | | | | | | | | 33 | 67 | | | | | | | 3 |
| 260-270 | | | | | | | | | | | | | 67 | 33 | | | | | 3 |
| 270-280 | | | | | | | | | | | | | | | | | | | |
| 280-290 | | | | | | | | | | | | | | | | | 100 | | 1 |
| Total | 27 | 17 | 41 | 21 | 45 | 50 | 28 | 10 | 11 | 7 | 8 | 5 | 3 | 1 | | | 1 | | 275 |

**Table 5**. Annual age-length keys based in age interpretation from Atlantic bluefin tuna otoliths (above) and spines (below). Numbers represent percent by number by length class (SFL, cm).

| | Annual ALK based on otoliths (samples from 2011 and 2012) | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Age class | | | | | | | | | | | | | | | | | | | | |
| Length class | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | n |
| 20-30 | 100 | | | | | | | | | | | | | | | | | | | | 10 |
| 30-40 | 100 | | | | | | | | | | | | | | | | | | | | 10 |
| 40-50 | 100 | | | | | | | | | | | | | | | | 0-20% | | | | 6 |
| 50-60 | 33 | 67 | | | | | | | | | | | | | | | 20-50% | | | | 9 |
| 60-70 | | 100 | | | | | | | | | | | | | | | 50-100% | | | | 11 |
| 70-80 | | 32 | 68 | | | | | | | | | | | | | | | | | | 22 |
| 80-90 | | 36 | 55 | 9 | | | | | | | | | | | | | | | | | 22 |
| 90-100 | | | 18 | 76 | 6 | | | | | | | | | | | | | | | | 17 |
| 100-110 | | | 19 | 62 | 15 | 4 | | | | | | | | | | | | | | | 26 |
| 110-120 | | | | 16 | 50 | 32 | 2 | | | | | | | | | | | | | | 50 |
| 120-130 | | | | 5 | 50 | 40 | 5 | | | | | | | | | | | | | | 20 |
| 130-140 | | | | | 38 | 42 | 15 | 4 | | | | | | | | | | | | | 26 |
| 140-150 | | | | | 19 | 24 | 48 | 10 | | | | | | | | | | | | | 21 |
| 150-160 | | | | | | 17 | 39 | 26 | 17 | | | | | | | | | | | | 23 |
| 160-170 | | | | | | 8 | 8 | 46 | 17 | 13 | 8 | | | | | | | | | | 24 |
| 170-180 | | | | | | 8 | | 31 | 38 | 23 | | | | | | | | | | | 13 |
| 180-190 | | | | | | | | 19 | 44 | 30 | | 7 | | | | | | | | | 27 |
| 190-200 | | | | | | | | 7 | 32 | 29 | 29 | 4 | | | | | | | | | 28 |
| 200-210 | | | | | | | | | 15 | 29 | 41 | 7 | 7 | | | | | | | | 41 |
| 210-220 | | | | | | | | | | 23 | 46 | 26 | 5 | | | | | | | | 39 |
| 220-230 | | | | | | | | | 10 | 13 | 23 | 27 | 20 | 3 | 3 | | | | | | 30 |
| 230-240 | | | | | | | | | | 4 | 19 | 46 | 19 | 12 | | | | | | | 26 |
| 240-250 | | | | | | | | | | | 24 | 29 | 12 | 24 | 6 | | 6 | | | | 17 |
| 250-260 | | | | | | | | | | | | 75 | | | | | | | | 25 | 4 |
| 260-270 | | | | | | | | | | | | | | | | 50 | | | 50 | | 2 |
| 270-280 | | | | | | | | | | | | | | | | | | | | 100 | 1 |
| Total | 29 | 32 | 35 | 40 | 54 | 48 | 27 | 31 | 43 | 48 | 61 | 44 | 18 | 8 | 2 | 1 | 1 | | 1 | 2 | 525 |

| | Annual ALK based on spines (samples fron 2011and 2012) | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Age class | | | | | | | | | | | | | | | | | | |
| Length class | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | n |
| 20-30 | 100 | | | | | | | | | | | | | | | | | | 10 |
| 30-40 | 100 | | | | | | | | | | | | | | | | | | 10 |
| 40-50 | 100 | | | | | | | | | | | | | | 0-20% | | | | 6 |
| 50-60 | 10 | 90 | | | | | | | | | | | | | 20-50% | | | | 10 |
| 60-70 | | 91 | 9 | | | | | | | | | | | | 50-100% | | | | 11 |
| 70-80 | | 7 | 89 | 4 | | | | | | | | | | | | | | | 27 |
| 80-90 | | | 86 | 14 | | | | | | | | | | | | | | | 36 |
| 90-100 | | | | 100 | | | | | | | | | | | | | | | 15 |
| 100-110 | | | | 50 | 45 | 5 | | | | | | | | | | | | | 22 |
| 110-120 | | | | 9 | 67 | 24 | | | | | | | | | | | | | 54 |
| 120-130 | | | | | 31 | 66 | 3 | | | | | | | | | | | | 32 |
| 130-140 | | | | | 3 | 80 | 17 | | | | | | | | | | | | 30 |
| 140-150 | | | | | 6 | 48 | 39 | 6 | | | | | | | | | | | 33 |
| 150-160 | | | | | | 31 | 54 | 12 | 4 | | | | | | | | | | 26 |
| 160-170 | | | | | | 12 | 29 | 47 | 12 | | | | | | | | | | 17 |
| 170-180 | | | | | | | 33 | 11 | 33 | 22 | | | | | | | | | 9 |
| 180-190 | | | | | | | 8 | 27 | 46 | 19 | | | | | | | | | 26 |
| 190-200 | | | | | | | | 15 | 41 | 33 | 11 | | | | | | | | 27 |
| 200-210 | | | | | | | | 6 | 19 | 42 | 19 | 13 | | | | | | | 31 |
| 210-220 | | | | | | | | 8 | 4 | 27 | 42 | 8 | 8 | 4 | | | | | 26 |
| 220-230 | | | | | | | | 7 | 4 | 25 | 32 | 25 | 4 | 4 | | | | | 28 |
| 230-240 | | | | | | | | | 8 | 8 | 31 | 42 | 12 | | | | | | 26 |
| 240-250 | | | | | | | | | | 11 | 11 | 44 | 11 | 22 | | | | | 9 |
| 250-260 | | | | | | | | | | | 29 | 43 | 14 | | | | | 14 | 7 |
| 260-270 | | | | | | | | | | | | | 50 | 25 | 25 | | | | 4 |
| 270-280 | | | | | | | | | | | | | | | | | | | |
| 280-290 | | | | | | | | | | | | | | | | | 100 | | 1 |
| Total | 27 | 21 | 56 | 37 | 59 | 85 | 43 | 31 | 39 | 46 | 40 | 31 | 10 | 5 | 1 | | 1 | 1 | 533 |

**Table 6**. Comparison of the estimated parameters of the von Bertalanffy growth model from annual ALKs using otoliths and spines. Likelihood ratio test, n. s.: not significant, ∗ p < 0.05.

| Age range compared | Otoliths | | | Spines | | | Likelihood Ratio test. | | |
|---|---|---|---|---|---|---|---|---|---|
| | L∞ | k | to | L∞ | k | to | L∞ *p* | k *p* | to *p* |
| 0–13 | 392.5 | 0.065 | -1.65 | 380.2 | 0.074 | -1.18 | n.s. | n.s. | ∗ |

**Table 7**. Summary of parameters obtained from the inter-reader comparisons. The table shows Relative bias, Coefficient of variation (CV), the Average percent error (APE), Percent agreement (PA) and p significance level of the inter-reader bias test (n. s.: not significant).

| | | | | | | Relative accuracy | | Precision | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Readers | comparison | calcified structures | Reader experience | n | Age range | Relative bias | | CV (%) | APE (%) | PA (%) | Inter-reader bias test |
| MR_PL | between readers | spine | high | 243 | 1-12 | 0.02 | | 1.9 | 1.55 | 91.4 | n.s. |
| PL_ ER | between readers | otolith | low | 194 | 1-12 | 0.04 | | 2.2 | 1.52 | 88.4 | n.s. |