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UPDATE OF THE AGEING ANALYSIS FOR ATLANTIC BONITO (*SARDA SARDA*) OF THE SMALL TUNA BIOLOGY STUDIES

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SUMMARY

The Atlantic bonito, Sarda sarda, *is a pelagic, migratory, schooling fish of the family Scombridae that inhabit coastal and inshore waters in the Atlantic Ocean, including the Mediterranean and Black Seas. In 2017, ICCAT launched the Small Tunas Year Programme (SMTYP), prioritizing the study of this species due to their economic importance and the deficiency of knowledge of their biology, including the specific component on age and growth. Spines and otoliths were collected and processed for this component from different areas of Atlantic Ocean and Mediterranean Sea. A total of 311 spines and 135 otoliths were analyzed. For 84 fishes, both structures were processed and analyzed for comparative purposes. It was agreed that spines structures were better suited for reading ages of Atlantic bonito. Mean length-at-age and growth parameters based on standard von Bertalanffy growth function were presented in this paper. Further research utilizing both small and large sample individuals is essential to enhance the age and growth component, besides incorporating samples from previously uninvestigated geographical areas.*

RÉSUMÉ

La bonite à dos rayé, Sarda sarda, *est un poisson pélagique, migrateur et grégaire de la famille des Scombridés qui vit dans les eaux côtières et littorales de l'océan Atlantique, y compris en mer Méditerranée et en mer Noire. En 2017, l'ICCAT a lancé le Programme annuel sur les thonidés mineurs (SMTYP), donnant la priorité à l'étude de cette espèce en raison de son importance économique et du manque de connaissances sur sa biologie, notamment sur l'aspect spécifique de l'âge et de la croissance. Des épines et des otolithes ont été collectés et traités pour cette composante dans différentes zones de l'océan Atlantique et de la mer Méditerranée. Au total, 311 épines et 135 otolithes ont été analysés. Pour 84 poissons, les deux structures ont été traitées et analysées à des fins comparatives. Il a été convenu que les structures des épines étaient mieux adaptées pour la lecture de l'âge de la bonite à dos rayé. La longueur moyenne par âge et les paramètres de croissance basés sur la fonction de croissance standard de von Bertalanffy sont présentés dans cet article. Des recherches supplémentaires utilisant à la fois des échantillons de petite et de grande taille sont essentielles pour améliorer la composante d'âge et croissance, en plus d'incorporer des échantillons provenant de zones géographiques précédemment non étudiées.*

RESUMEN

El bonito del Atlántico, Sarda sarda*, es un pez pelágico, migratorio y de cardumen de la familia Scombridae que habita en aguas interiores y costeras del océano Atlántico, incluidos el mar Mediterráneo y el mar Negro. En 2017, ICCAT lanzó el Programa anual de pequeños túnidos (SMTYP), priorizando el estudio de esta especie debido a su importancia económica y la deficiencia en el conocimiento de su biología, incluido el componente específico de edad y*

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crecimiento. Se recogieron y procesaron espinas y otolitos para este componente de diferentes áreas del océano Atlántico y el mar Mediterráneo. Se analizaron un total de 311 espinas y 135 otolitos. Para 84 peces, se procesaron y analizaron ambas estructuras con fines comparativos. Se acordó que las estructuras de las espinas eran más adecuadas para leer las edades del bonito del Atlántico. En este documento se presentan la talla media por edad y los parámetros de crecimiento basados en la función de crecimiento estándar de von Bertalanffy. Es esencial realizar más investigaciones que utilicen tamaños de ejemplares pequeños y grandes para mejorar el componente de edad y crecimiento, además de incorporar muestras de áreas geográficas no investigadas previamente.

KEYWORDS

Small tunas; age and growth; spine; otoliths; growth

1. Introduction

Atlantic bonito (*Sarda sarda*) is a medium sized Scombrid which inhabits a broad distribution range spanning tropical and temperate areas of the Atlantic Ocean, including the Mediterranean and the Black Sea (Sabatés & Recasens, 2001). Atlantic bonito is one of the most important small tuna species targeted in these coastal waters (Majkowski, 2007). In the Atlantic Ocean, small tunas are managed by the International Commission for the Conservation of Atlantic Tuna's (ICCAT), specifically by the Small Tunas Group. In 2017, the ICCAT launched the Small Tunas Year Programme (SMTYP), prioritizing the study of three species based on their economic importance and the deficiency of knowledge of their biology (Viñas *et al*., 2020). These species were: Little tunny (*Euthynnus alletteratus*), Atlantic bonito (*Sarda sarda*), and Wahoo (*Acanthocybium solandri*).

Knowledge of biological information, such as age and growth, is crucial for conducting effective stock assessments. Most of the available growth studies of Atlantic bonito comes from the local areas of the Mediterranean Sea and adjacent areas as Strait of Gibraltar (Rey *et al*., 1986), Spanish coast of the Mediterranean Sea (Valeiras *et al*., 2008), Ionian Sea (Kotsiri *et al*., 2018; Santamaria *et al*., 1998), Aegean Sea (Cengiz, 2013; Zaboukas & Megalofonou, 2007), Black Sea and the Sea of Marmara (Ateş *et al*., 2008; Kahraman *et al*., 2014). Age and growth studies have primarily developed on readings taken from the first dorsal fin spine (Santamaria *et al*., 1998; Valeiras *et al*., 2008; Zaboukas & Megalofonou, 2007), sagittal otoliths (Ateş *et al*., 2008; Cengiz, 2013; Kahraman *et al*., 2014; Kotsiri *et al*., 2018; Rey *et al*., 1986), or vertebrae (Rey *et al*., 1986). In comparison to otoliths and vertebrae, spines have emerged as the preferred structure due to their ease of collection and processing (Carbonara & Follesa, 2019; Rodriguez-Marin *et al*., 2022; Williams *et al*., 2013). However, the vascularization is the greatest disadvantage of using calcified fin spines for ageing purposes given that the spine nucleus may be reabsorbed, which may eliminate the first annuli (Drew *et al*., 2006; Kopf *et al*., 2010). However, there are no studies in the literature for Atlantic bonito that have analyzed and compared age structures as seen in other migratory species (Filmalter *et al*., 2009; Rodríguez-Marín *et al*., 2006).

Marked variations between previous works have been observed in key growth parameters like asymptotic length (L_∞; 62.5 – 82.1 cm) or growth coefficient (K; 0.24 - 0.82). These differences can be attributed to factors such as the number and size range of individuals analyzed, limited study area, hard part structure analyzed, or processing techniques employed. However, marginal increment analysis or edge depositions observed, indicate clearly that growth bands are deposited annually, regardless of the hard part structure analyzed (Kotsiri *et al*., 2018; Zaboukas & Megalofonou, 2007).

In 2017, the ICCAT initiated the Small Tunas Year Programme (SMTYP), which consists on a biological sampling programme. The specific objectives were to collect biological samples for estimating growth parameters, assessing the maturity, and stock structure analysis through populations genetics with the aims of improving biological and population knowledge of small tunas in both the Atlantic and the Mediterranean Sea. This working document presents the progress on the ageing and growth component of the programme for Atlantic bonito, with updates on the number of samples collected and processed, as well as the results of age reading.

2. Methods

Samples were collected in commercial fishing vessels or in port sampling using gillnets, trawls, traps, purse seines, and handlines from technicians of institutions such as Instituto Português do Mar e da Atmosfera - IPMA (Portugal), Instituto Español de Oceanografía - IEO (Spain), AquaBioTech - ABLT (Malta), National Institute of Marine Science and Technology - INSTM (Tunisia), Institut National de Recherche Halieutique - INRH

(Morocco), Centre De Recherches Oceanographiques de Dakar - CRODT (Senegal), Centre de Recherches Océanologiques - CRO (Côte d'Ivoire), and General Directorate of Fisheries and Aquaculture – GDFA (Gabon). Straight fork length (SFL) and total body weight (W) were measured for each individual to the nearest mm and g, respectively. Location, sex, maturity stage and other biological parameters were taken. For age and growth, sagittal otoliths and the first spiniform ray (spine) of the first dorsal fin were removed from each fish. The spine with the complete condyle were removed according to protocol of Muñoz-Lechuga and Lino (2021). Within a selected group of fishes, both structures were processed and analyzed for comparative purposes.

2.1 Spine processing

The processing of spines was conducted at the IPMA laboratory. The first dorsal fin spine of each fish was embedded in polyester resin for sectioning, and three sections with approximately 0.5 mm thick were cut at distances from the spine base equal to one and half times (1.5*CW), one time (1.0*CW), and at half of the condyle width (0.5*CW) according to the standardized method of Muñoz-Lechuga *et al*. (2024). A total of 311 spines were sectioned with an Isomet low-speed cutting machine, using two pro slicer diamond blades in parallel. Three sections from the same spine were mounted on labeled glass slides with a mounting medium to fix the sections permanently. At first, after a preliminary observation of the cut sections, the 1.5*CW distance sections were excluded of readings because the annuli were not clearly visible.

Sections were observed under a Nikon dissecting microscope with a mounted high resolution digital camera, using transmitted white light. Digital images of each cross-section were recorded and then digitally enhanced using the ImageJ software (Schindelin *et al*., 2015). Sections at two different condyle distances (1.0*CW and 0.5*CW) were compared in terms of readability, vascularization, and annulus loss. The optimal cut-off distance (0.5*CW) was selected and all spines processed were aged and measured at this distance three times by the main reader without previous knowledge of the length of each specimen in order to prevent bias while counting the growth bands. Additionally, a fourth reading was carried out for any samples whose first three readings produced three different attributed ages. In certain large individuals, analyzing only the 0.5*CW optimal cut-off distance section, the complete disappearance of the first or second annuli was observed due to vascularization, so they had to be estimated in these cases. To mean position of the first and second annuli were calculated from specimens where distance was measurable. Following this, annuli were assigned to individuals where they had been lost due to vascularization.

2.2 Otolith processing

The otoliths processing of Atlantic bonito was outsourced to a specialized laboratory (FAS - Fish Ageing Services Pty Ltd in Australia). A total of 153 otolith samples were sent for analysis (135 for annual and 18 for daily reading). Samples were sent to process following the criteria of the largest relevant selection of otolith samples that were available in the consortium collection at the time of shipping (October 2020) and project budget available for the analysis. Prior to any preparation one sagittal otolith from each sample was weighed to the nearest 0.0001 g and an image of each whole otolith was captured.

For daily age reading, the best sectioning method was first determined. Two otoliths were selected and one was prepared as thin transverse sections and one was prepared as longitudinal or frontal sections (both approximately 120 µm thickness). Unfortunately, it was not possible to directly compare both preparation methods on the same samples. It was concluded that the longitudinal sectioning method was the preferred method and the remaining samples selected for daily ageing were prepared in this way following methods described by Schaefer & Fuller (2006).

For annual ageing, only the transverse sectioning plane was considered as it has been shown to be the best method for preparing otoliths from a number of other tuna species. It was still necessary however to investigate the preferred section thickness. This was done by selecting the four largest otoliths by weight and first cutting a relatively thick (450 µm) section from the nucleus of the otolith. The sections were ground down in thickness and observed and imaged under reflected and transmitted light each time the thickness was reduced by 40-50 µm. From this process we concluded that sections 280 - 300 µm thick are most suitable for Atlantic Bonito otoliths.

Otolith sections were read at 32x times magnification using a Leica M125 dissecting microscope illuminated with transmitted light. As with other tuna species investigated, the first few annuli in the otolith sections were difficult to interpret. Counts of microincrements in the two samples previously prepared in the transverse plane and used for the daily aging comparison were used to verify the age and position of the first assumed opaque zone and proxy measurements were determined to allow the easier identification of the first zone in structure. For Atlantic Bonito the approximate age was 100-120 days to the first assumed annual opaque zone and the distance was 0.356 mm.

Ages were estimated by counting the opaque zones and the proxy measurement was used to help identify the position of the first zone. Images of the sections were captured and the distance between the primordium and start of each opaque zone was measured.

2.3 Growth modelling

After comparing spine and otolith structures, it was agreed that the spines were better suited for reading ages. Consequently, they were utilized for ageing analysis. For growth modelling, the stock structure results of Atlantic bonito, previously analyzed through genetic evaluation, were considered (Ollé-Vilanova *et al*., 2024). Samples collected from the Mediterranean were analyzed alongside those from Portugal in the Northeast Atlantic (NEATL_MED). In contrast, the samples from the tropical Eastern Atlantic were examined separately (SEATL).

To obtain the growth curve for Atlantic bonito, a growth model was used for the two sexes combined with the optimal cut-off distance (0.5*CW) for both limited areas. Male and female growth curves were not included due to too few sexed individuals. The von Bertalanffy Growth Function (VBGF) (Von Bertalanffy, 1938) was fit to the length at age data derived from spines to estimate the growth parameters for Atlantic bonito following the equation:

$$Lt = L_{\infty}\,[1 - e^{[-k(t - t_0)]}]$$

where Lt is the predicted length at age t, L_{∞} is the asymptotic length, k is the growth rate coefficient, and t_0 is the theoretical age when length is zero.

The model was fit to the length at age data using nonlinear least squares (*nls* function in R) and all plots were created with the package "ggplot2" (Wickham, 2009) in R environment (R Core Team, 2023). For the fitted model, the growth parameters were estimated, along with standard error (SE) and 95% confidence intervals (CIs).

3. Results and Discussion

A total of 1096 spines samples of Atlantic bonito were collected for this study from several areas of the eastern Atlantic Ocean and Mediterranean Sea between 2017 and 2022. A total of 250 spines per species was planned by the group for ageing processing, finally 311 spines of Atlantic bonito were processed and analyzed for age reading.

Spine analyses were carried out for 311 Atlantic bonitos, 200 from NEATL_MED ranging from 14.3 to 71.5 cm SFL and 111 from SEATL ranging from 29.5 to 65.5 cm SFL (**Figure 1**). There was a strong linear correlation between the diameter section of the spine and straight fork length (**Figure 2**). The obtained relationships between SFL and spine diameter (SD) were the following:

NEATL_MED: SFL = 24.069×SD+1.415, r^2=0.867.
SEATL: SFL = 16.664×SD+14.836, r^2=0.649.

Poorly preserved spines or processed sections considered unreadable were rejected from the analysis (n=81). Spine cross-sections were eliminated when the bands were too diffuse or unreadable to determine their number and location accurately. When the diameter of the vascularization area was higher than the mean diameter of the first or second annuli, one or two additional annuli were added.

The estimated ages from NEATL_MED ranging from 0 to 7 and from SEATL ranging from 1 to 6. In a general way, age groups 0, 1 and 2 were the dominant ones in the analyses. However, no individuals at age group 0 were found in the SEATL due to the low number of small-sized individuals sampled.

The absence of samples of small-sized individuals from the SEATL area, made it impossible to compare daily age results between areas. A total of 16 specimens from NEATL_MED could be processed and examined for daily age. Microincrements were counted and the daily age ranged from 70 to 160 days for the Atlantic bonito samples. The daily age analysis results demonstrated a weak correlation with size (r^2=0.14) (**Figure 3)**, likely attributed to the limited number of structures examined and the challenges in interpretation.

Annual ageing was analyzed for 132 otoliths and 3 were rejected due to the low readability (**Figure 1**). The relationship between otolith weight and straight fork length demonstrated a positive correlation (r^2=0.69, P<0.001) and the equation obtained was: SFL (cm) = 0.0002451 × Otolith weight (g) – 0.007363 (**Figure 4**). Zone counts ranged from 0 to 7 for Atlantic bonito ranging in length from 36.8 to 67.8 cm FL. The interpretation of the otolith increment structure was considered difficult but the age estimates were not considered unreasonable for young individuals, however it was noted that the age data should still be used with caution.

A total of 84 pairwise age comparisons between otolith age reading and the spine for consensus age were analyzed (**Figure 5**). When comparing both structure readings the Evans-Hoenig test of symmetry is significant (P-value < 0.05), indicating that there is bias in the estimated ages between the structures, particularly in ages higher than 2 from otoliths there is an underestimation facing spines (**Figure 6**). Percent agreement was of 51.2% and 96.4% within one year, the maximum difference between spines and otoliths was of 2 years. Some differences in the estimation of age between structures could be due to readability or to different growth band deposition of individuals from diverse stock areas. The obtained results agreed that the spines were better suited for reading ages of Atlantic bonito. Besides that, otoliths are more complex to collect due to the difficulty in extracting given its small size and fragility.

The von Bertalanffy equations and curves for the theoretical growth in length for both sexes combined is presented for two areas (**Table 1** and **Figure 7**). Similar results were described in the Mediterranean Sea by other authors when size range sampled of the species was wider (Kotsiri *et al*., 2018; Zaboukas & Megalofonou, 2007). However, variation in age groups estimates among analyzed areas was moderate, with greater differences starting from age group 2. The observed differences could be attributed to variations in growth patterns between stocks (Ollé-Vilanova *et al*., 2024), but further research with small and larger sample sizes, besides incorporating samples from previously uninvestigated geographical areas, are necessary to draw definitive conclusions. It is important to note that these limitations could potentially affect the reliability and generalizability of the findings.

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Table 1. Mean length at age and growth parameters (L_∞, *K* and t_0) for the Atlantic bonito from several authors and areas.

	Rey *et al.* 1986	Zaboukas and Megalofonou, 2007	Ateş *et al.* 2008	Valeiras *et al.* 2008	Kotsiri *et al.* 2018	This paper	This paper
Age BON	Strait of Gibraltar	Aegean Sea	Sea of Marmara and Black Sea	West Mediterranean	East Mediterranean	Northeast Atlantic and Mediterranean	Tropical East Atlantic
0	37.03	28.6			29.8	32.40	
1	51.71	40.1	46.2	49.8	38.3	41.97	41.87
2	57.04	49.2	58.4	56.3	51.6	49.78	47.49
3	63.15	56.3	63.8	59.5	56.9	56.15	52.63
4	71.00	62.0	66.1	61.0	61.6	61.35	57.33
5		66.4	67.2		67.8	65.59	61.63
6		69.9			67.9	69.04	65.56
7		72.7			70.0	71.86	
von Bertallanfy parameters							
L_∞	80.87	82.1	68.0	62.5	79.9	84.34	107.44
K	0.352	0.24	0.82	0.719	0.26	0.204	0.090
t_0	-1.7	-0.77	-0.39	-1.21	-0.44	-2.379	-4.508

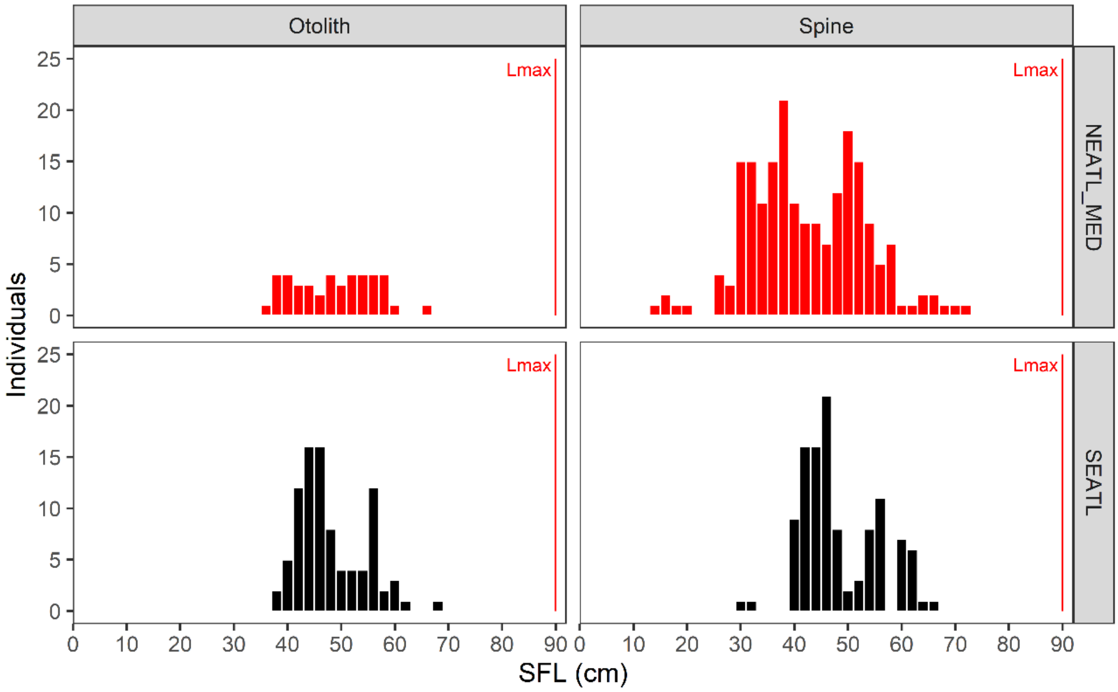

Figure 1. Size (SFL, cm) frequency distribution of Atlantic bonito individuals analyzed for otoliths and spines.

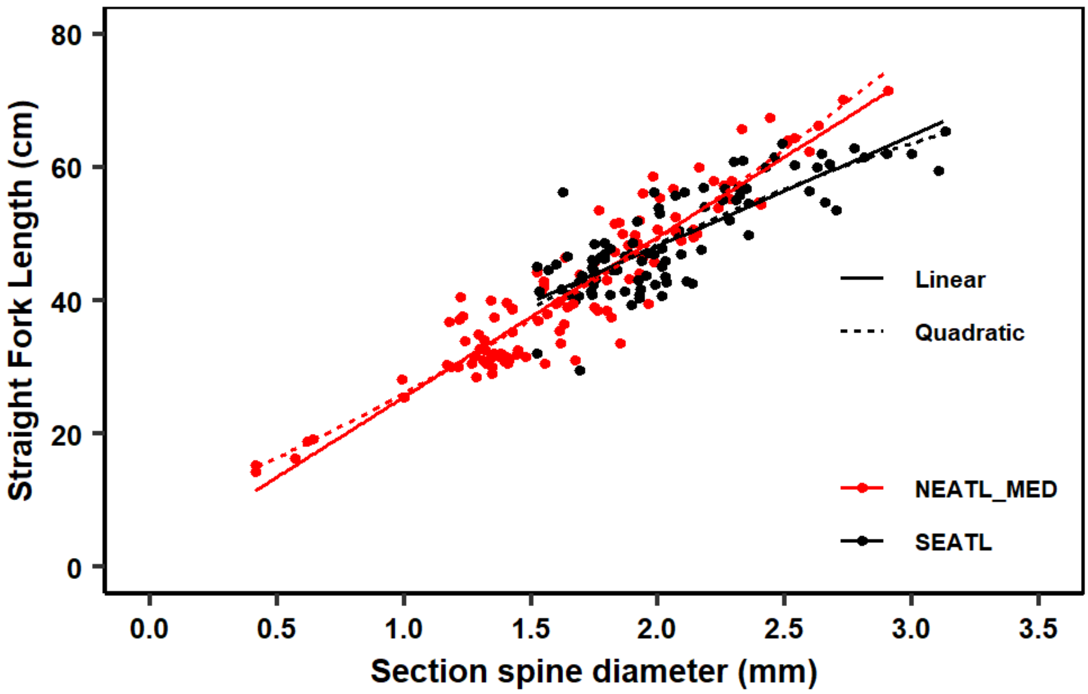

Figure 2. Relationship between the SFL (cm) and transverse sections diameter (0.5*CW) of dorsal fin spine for both areas.

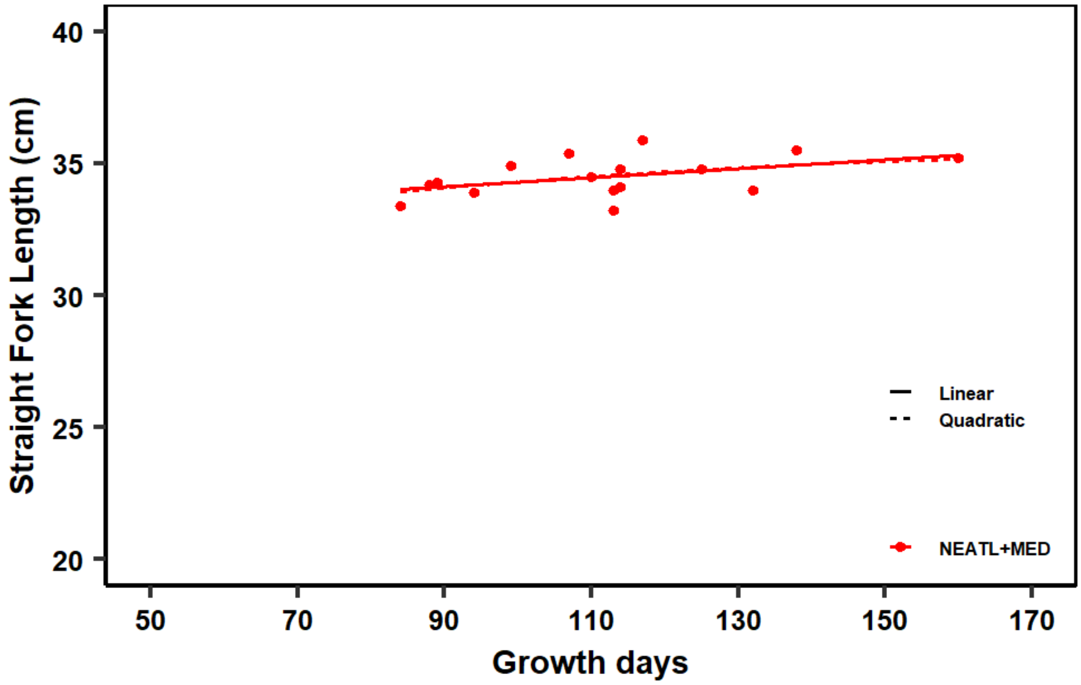

Figure 3. Relationship between growth days of small fish otoliths and SFL (cm).

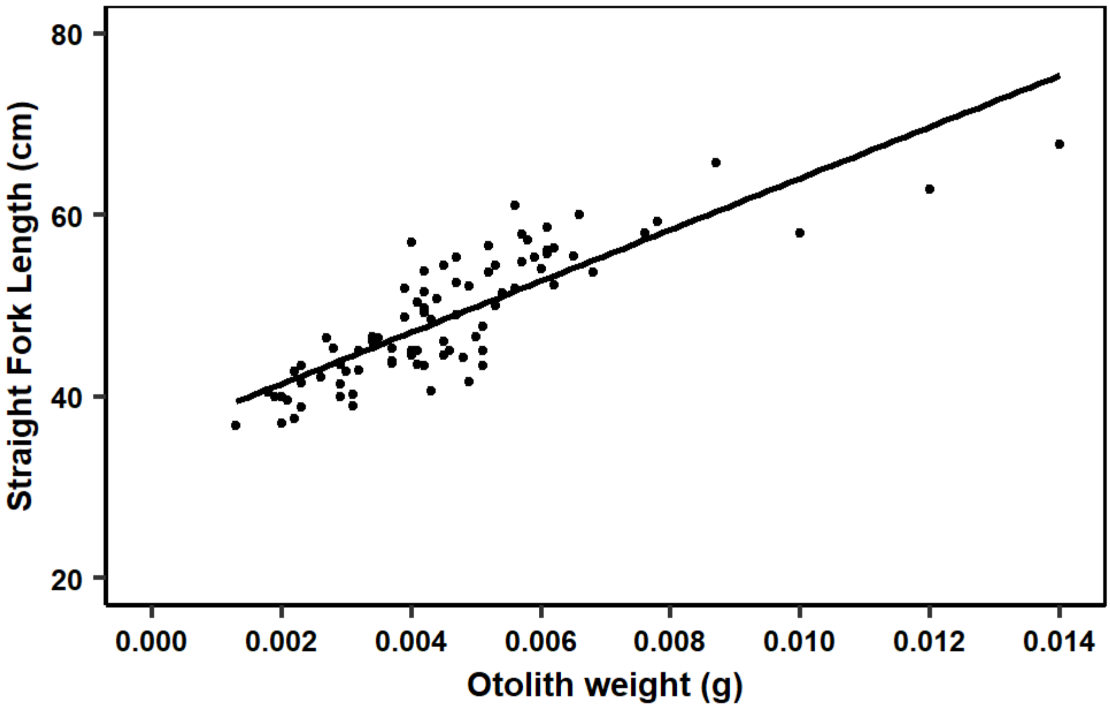

Figure 4. Relationship between otolith weight (g) and SFL (cm).

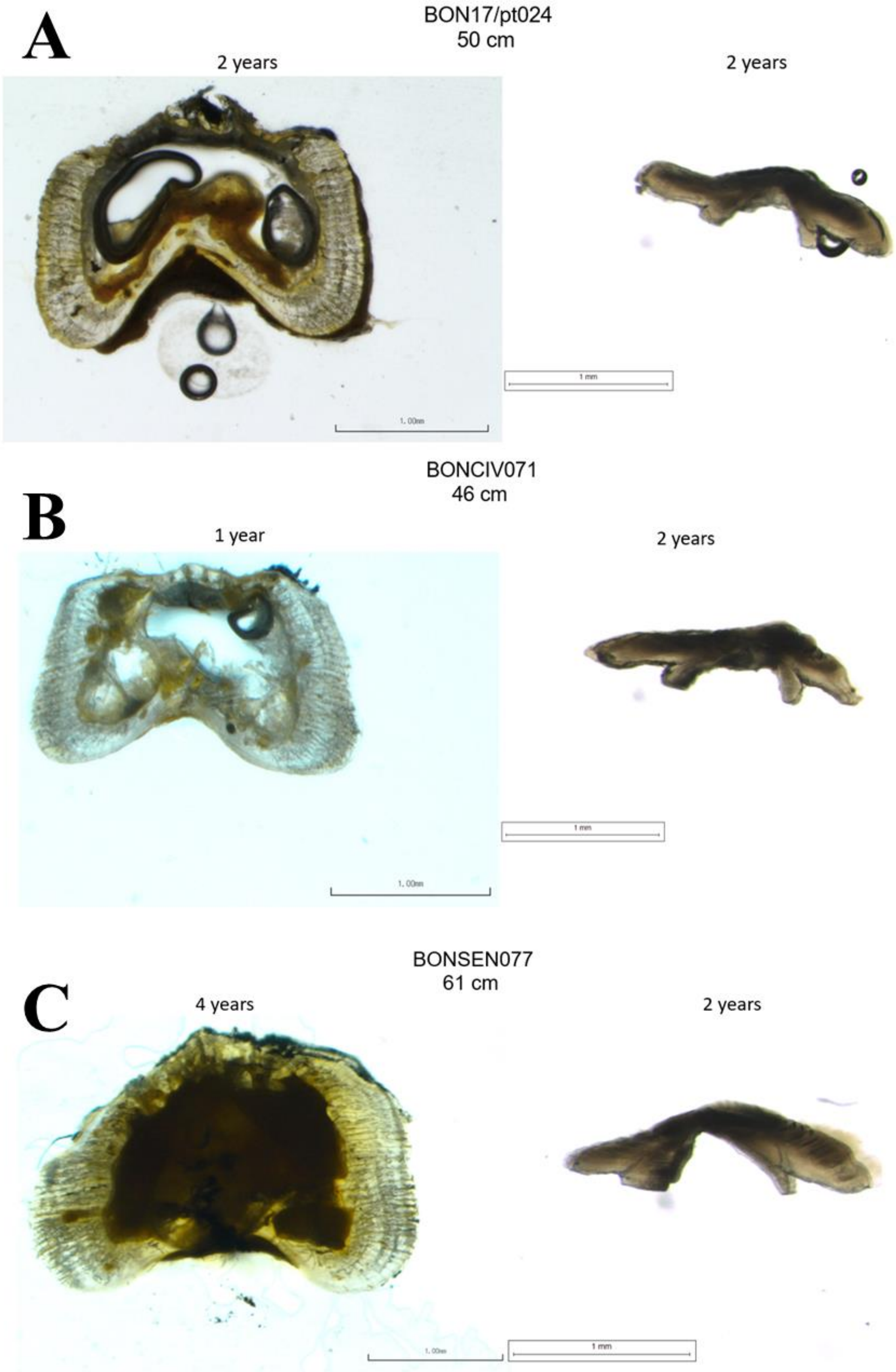

Figure 5. Three examples of pairwise cross-section of spines (left) and otoliths (right) of the same individual. A) The age reading matches for both structures. B) and C) The readings were unequal between age structures.

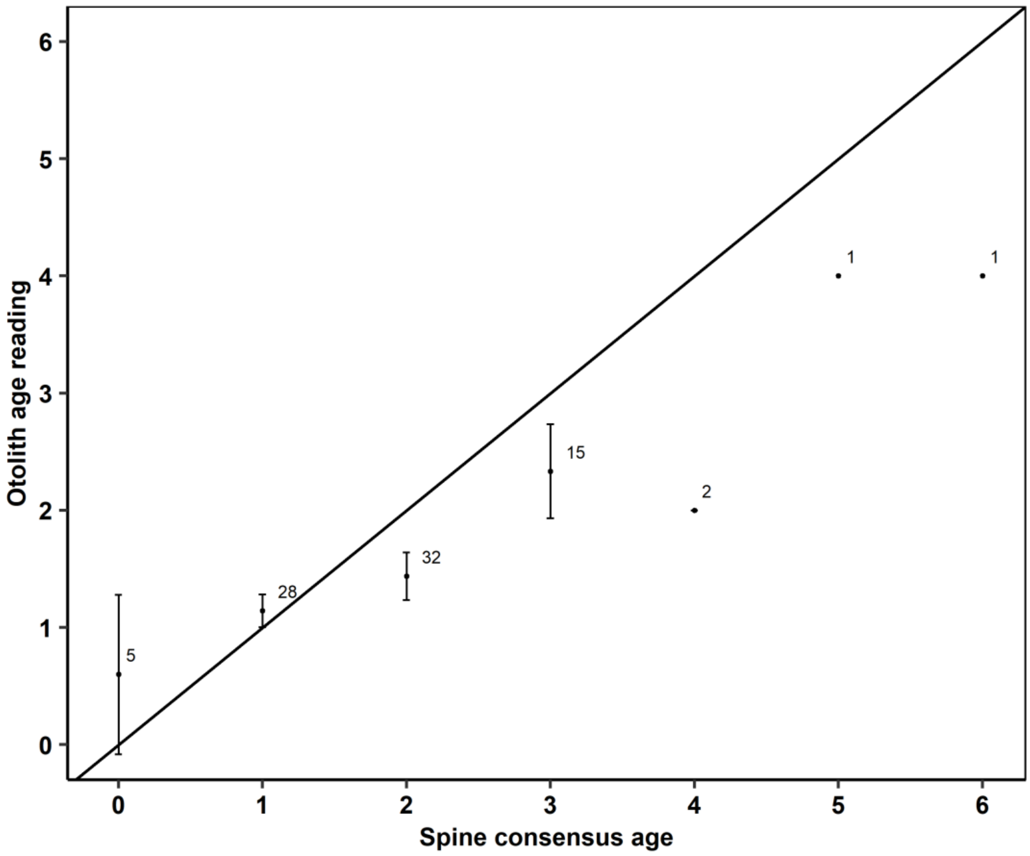

Figure 6. Age-bias plots of pairwise age comparisons (n=84) between spines and otoliths. Circles with error bars represent the mean counts of reading (± 95% confidence intervals). The diagonal line indicates a one-to-one relationship.

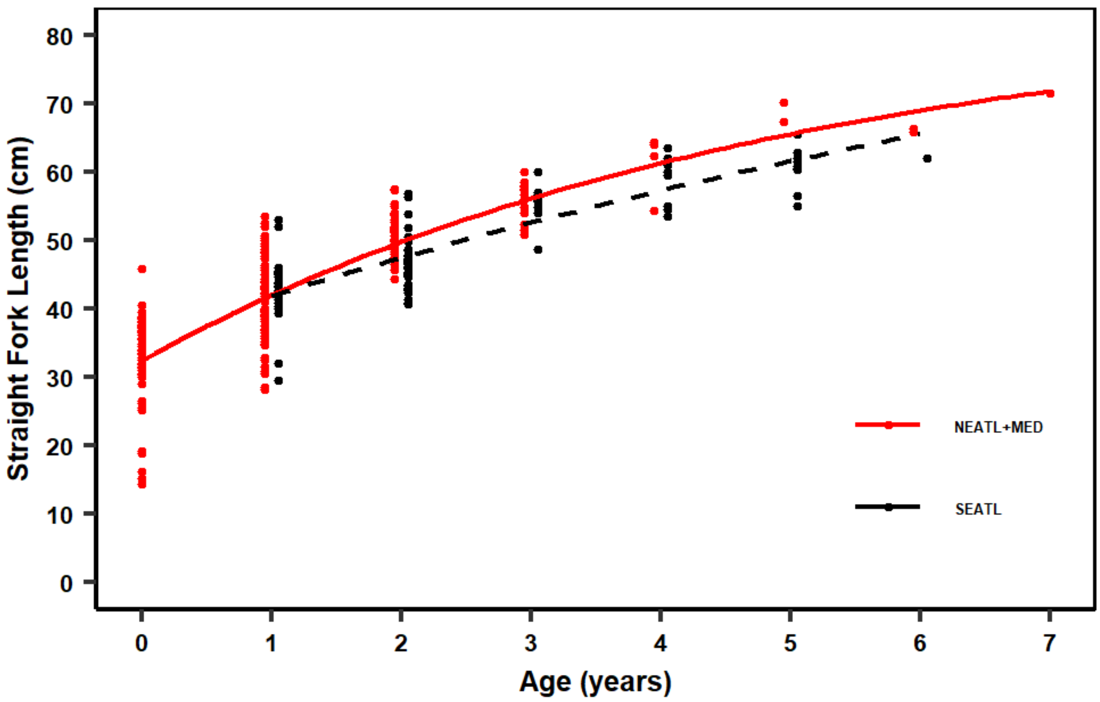

Figure 7. The von Bertalanffy growth function (VBGF) for Atlantic bonito. Dots represent observed data and the lines represent the VBGF for both areas.