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SAMPLING PROTOCOL FOR SKELETAL STRUCTURES OF NORTH ATLANTIC ALBACORE TUNA (*THUNNUS ALALUNGA*) AND AGEING INTERPRETATION

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SUMMARY

This paper presents a standardized protocol for sampling skeletal hard parts (dorsal fin ray and otoliths), preparation and age interpretation of albacore first dorsal fin ray. Ageing of albacore is focused in interpretation and reading of annual temporal marks (translucent bands) in first ray of dorsal fin. Preparation of fin ray sections (spines) is presented in detail using two different methods. The spines are usually cut individually using a low speed cutter. Depending of the size of spines, a new procedure has been developed for small spines based on encasing spines in a matrix of plastic resin allowing multiple spines cutting. Interpretation of growth marks on spine sections is explained and examples are presented for a range of size of albacore aged using this method.

RÉSUMÉ

Ce document présente un protocole standardisé pour l'échantillonnage des pièces dures du squelette (rayon de la nageoire dorsale et otolithes), la préparation et l'interprétation de l'âge du rayon du premier rayon de la nageoire dorsale du germon. La détermination de l'âge du germon se centre sur l'interprétation et la lecture des marques temporelles annuelles (bandes translucides) du premier rayon de la nageoire dorsale. La préparation des sections du rayon de la nageoire (épines) est présentée en détail à l'aide de deux méthodes différentes. Les épines sont généralement sectionnées individuellement en utilisant un couteau basse vitesse. Selon la taille des épines, une nouvelle procédure a été élaborée pour les petites épines, consistant à enfermer les épines dans une matrice en résine plastique qui permet le découpage de plusieurs épines. L'interprétation des marques de croissance sur les sections des épines est expliquée et des exemples sont donnés pour une gamme de tailles de germon dont l'âge a été déterminé à l'aide de cette méthode.

RESUMEN

En este documento se presenta un protocolo estandarizado para el muestreo de partes duras del esqueleto (otolitos y rayo de la aleta dorsal) y para la preparación e interpretación de la edad del primer rayo de la aleta dorsal del atún blanco. La determinación de la edad del atún blanco se centra en la interpretación y lectura de las marcas temporales anuales (bandas traslucidas) en el primer rayo de la aleta dorsal. Se presenta en detalle la preparación de las secciones del rayo de la aleta (espinas) utilizando dos métodos diferentes. Las espinas suelen cortarse generalmente de forma individual utilizando un cortador de baja velocidad. Dependiendo del tamaño de las espinas, se ha desarrollado un nuevo procedimiento para las espinas pequeñas, que consiste en introducir las espinas en una matriz de resina plástica que permite cortar espinas múltiples. Se explica la interpretación de las marcas de crecimiento en las secciones de espinas y se presentan ejemplos para una gama de tallas de atún blanco cuya edad se determinó utilizando este método.

KEYWORDS

Thunnus alalunga, albacore, first dorsal spiny ray, age estimation, protocol, growth marks, North Atlantic, South Atlantic

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1. Introduction

Different techniques exist for age determination, one of the main ones being the analysis of growth temporal marks (*annuli*), also commonly called growth rings, in the skeletal structures/hard parts of the fish. The formation and biomineralization of these growth bands depend on biotic and environmental factors, such as feeding, both trophic and spawning migrations, water temperature, etc. The rays of the first dorsal fin, also commonly known as spines, and otoliths are the hard parts used in albacore tuna growth studies.

This sampling protocol attempts to explain how to obtain, preserve, prepare and interpret the translucent bands of the calcified structures: dorsal spiny ray to estimate albacore tuna age.

2. Sampling

2.1 Sampling strategy

A number of spines (e.g. 3) must be collected for each 1 cm length class with constant periodicity (every month) throughout the year. Measurements must be taken of sampled specimens: fork length (FL) (e.g. 3 spines from 60 cm FL specimen, 3 spines from 61 cm FL specimen, etc.). Sampling must be carried out on different days throughout the month until the number of spines required to complete the entire length range landed as fully as possible has been collected. Moreover, the samples must come from as many as possible of the different areas where the stock is being caught, thus that coverage is as wide as possible. To accomplish this, sampling of dorsal fin rays must be distributed across different vessels and landing ports on monthly temporal strata.

2.2 Information on specimens sampled

It is necessary to collect the exact biological and fisheries information of the specimen whose hard parts are to be extracted.

Specimen length
Two methods are used to measure the length of the sampled fish. Ideally fork length (FL) measurement following the standard method for tuna species (ICCAT FIELD MANUAL, http//:www.iccat.int) would be taken from fish sampled by means of a calliper or measurement board. However, in the case of specimens of over 1 metre, in which the FL size is difficult to measure, pre-dorsal length is usually taken (LD1). The type of measurement of length used and the unit (cm) must be clearly specified. Fork length (FL) is measured to the lower centimetre (a specimen of 70.9 cm or of 70.1 cm will be recorded as 70 cm); LD1 size is measured to the lower half centimetre (a specimen of 30.4 cm records as 30 cm and one of 30.6 cm as 30.5 cm).

Fork length (FL)
The straight length of the fish taken from the forward most point of the upper jaw (the snout) to the backward most point of the shortest caudal ray (fork of the caudal fin) **(Figure 1).** It is measured using a calliper (the most suitable method), and measuring board or, in their absence, a metric tape measure (trying to keep the tape in a straight line so as to avoid measuring the curved outline of the fish). To measure FL, it is necessary to place the fish on a flat, horizontal surface.

Predorsal length (LD1)
This is the straight length from the forward most point of the upper jaw (the snout) to the base of the first dorsal fin (where the first dorsal fin begins) (**Figure 1**).

Date of capture
Of the specimen (day, month and year)

Fishing area
This refers to the place where the sampled fish was caught. The geographical location of the fishing haul must be established as exactly as possible. Due to the characteristics of albacore tuna fisheries in the area, it is often not possible to determine the exact point of fishing, so the latitude and longitude of an area must be taken (between 44º-45ºN and 5º-7ºW, for example) or a more or less defined geographical area must be defined, such as, for example, the Bay of Biscay.

Name of the *vessel* that caught the specimen and its landing port.
Country to which the samplings correspond and the person responsible for sampling.

The skeletal structures can be coded so that they can be related with the data that have been collected on them.

2.3 Periodic control of the structures collected

The structures collected in each period (month) must be noted on a sampling control sheet. This sheet bears the information of the number of spines collected by length class **(Appendix 1)**.

2.4 Collection and preservation of the skeletal structures

2.4.1 Spines

Spine collection
The first spine of the first dorsal fin must be collected from each specimen. The spine must be extracted whole from the base. The procedure is as follows:

Using a knife, cut the membrane joining the 1st and 2nd rays of the first dorsal fin (**Figure 2, Figure 3A**).

Push the spine gradually forwards (**Figure 3B**) until the ligament breaks (**Figure 3C**). Twist the spine from one side to the other alternately until it comes loose and pull it in order to extract it entirely, taking care not to break it at the base (**Figure 3D**). If the extraction is made from large specimens whose rays are strongly entangled with the tendons at the base of the fin, a small cut can be made in the skin to make it easier to extract without breaking the base of the fin ray.

After extracting the spine, the specimen remains apparently unaffected, so it can be sold perfectly well. For this reason the first dorsal spine is the most commonly used structure for age interpretation in tuna species, hence albacore.

Spine preservation
The spine must be preserved in a dry state within a paper envelope and labelled. It can be kept in a fridge or in a dry place at room temperature. Marked on the envelope must appear the data of the specimen sampled: length, date, port, vessel and fishing trip or the code of the fish sampled.

2.4.2 Otoliths

Sagittal otoliths are small calcified structures in the semicircular cavities of the inner ear at the base of the brain. They are formed by the accumulation of calcium carbonate and protein. The sagittal otolith is the largest of the three calcified structures found in each inner ear of albacore tuna and is the structure used for age interpretation.

Otolith collection
One of the systems for extracting the sagittal otoliths from the albacore tuna consists of making a cut in the upper or dorsal part of the head at the level of an imaginary line established in the following way. An imaginary line is traced perpendicular to the fish's length, which passes through the mid-point of the line between the corner of the mouth and the pre-opercule (**Figure 4A**). The best way of determining this line is to take a ruler and to divide the distance between the two points by two (**Figure 5**) before making a cut in the upper part of the fish along this perceived line. Once the point to be cut has been marked, a metal saw is used in a downward direction to cut the head along this line. The sectioned part of the head contains the otoliths. If a clean cut has been made as described, the otoliths can be found in the cavities under the brain, in the upper part of the head (**Figure 4B**). If they are not to be found there, it may be because the cut has left them in the main part, the body, of the fish. Using fine tweezers and with great delicacy as the otoliths are very fragile, remove each otolith. The otoliths must be taken out of each very fine transparent capsule that covers them. Both otoliths must be collected from each specimen, but if either of them has broken, try to recover the pieces and keep them together. Clean the otoliths with water or 70% alcohol and leave them to dry.

Otolith preservation
Clean with alcohol, once dry stored in empty small tubes labelled with the corresponding code.

3. Preparation of the samples

3.1 Spines

In order that the spine can be read and interpreted a transversal section of the base of the spine must be obtained. The preparation of the spine cuts includes the following processes: cleaning the spine, making the cuts and mounting them.

Cleaning
Having been kept in dry conditions in paper envelopes, the soft muscular tissues at the base of the spines have dried out. These tissues must be carefully removed with a scalpel and tweezers without causing damage to the surface of the base of the spine. In large fishes the epidermis covering the spine must be removed.

Cutting methodology
Making the cuts requires the use of a precision cutter and a diamond saw. The exact point to make the cut is near to the base coinciding with the bulge in the spine (the part with the greatest diameter) and close to the ridge (though this must not be cut), as shown in **Figure 7** (Bard and Compeán, 1980**)**. The cut must have a thickness of approximately 0.5 mm. Two consecutive cuts are normally made in each spine in order to be able to choose the best one of them when making the reading.

The spines are cut individually using the cutter, although two techniques are now used to do so depending on the sze of the spines. A new methodology has been developed for small spines, which consists of encasing several spines in a matrix of plastic resin, making it possible to cut several spines at once.

Cutting methodology for individual spines
Cuts are to be made using a cutter with the following characteristics (**Figure 6**):

ISOMED Low speed Cutter
- Disc speed: 160 rpm
- Width of cut: 75 micrometer units (for a cut of 0.45 mm thickness)

Cutting methodology for spines encased in resin
The following materials are used for the preparation of resin plates:
- Metallic moulds: of 13 x 4 x1.5 cm (length x width x height).
- Chemical material: polyester resin, black colouring, accelerator, spaghetti, Vaseline (**Figure 8**).

Between 5 and 20 spines per plate are introduced depending on the size of the samples. The following are the characteristics of the resin plates: Volume: 50 ml (max. 80 ml); Thickness: 0.8 to 1.3 cm

Cuts are made using a cutter with the following characteristics:

ISOMED 5000 Cutter
- Disc speed: 5000 rpm
- Width of cut: 1400 mm (for a cut of 0.45 mm thickness)
- Cutting disc: Series 15LC Diamond, ISOMED 11-4279. Dimensions: 200 x 0.9 mm.

See **Figure 9** and **Figure 10** for illustration

Mounting methodology (spine blocks mounted or individually mounted)
The sections cut are washed in ethanol at 70%. They are later mounted in labelled on slides of 76 x 26 mm (rounded edges). Mounting must be performed with the concave part facing upwards. Then are embedded in epoxy resin Eukitt highly transparent to fix them (**Figure 11**), and individual spines are covered with a holder of 24 x 50 mm (**Figure 12** and **Figure 13**). The holders are labelled with the code, fish length and the date of capture.

4. Age interpretation

4.1 Spines

Readings of spine sections are made using a NIKON profile projector (**Figure 14**). This magnifier consists of three optical lenses, x10, x20, x50, which transmits the image by light transmitted on the samples through the crystal screen of the projector, where the X and Y axes are drawn. These are for lining up the section and making the corresponding measurements (mm) with the micrometer incorporated in the projector (**Figure 15**). The image is projected onto the screen and allows the differentiation of the translucent bands formed in the structure. A binocular microscope can also be used with a camera incorporated (**Figure 16**) to examine the image of the section on the screen. Both have a zoom-in function to distinguish *annulli* when they are close together.

The criteria used to interpret the pattern of observed translucent bands (*annuli*) formed on the spine sections of albacore, is based on the hypothesis of Bard and Compeán (1980), which assumes that the formation of two translucent bands (*annuli*) per year throughout the life span of North Atlantic albacore corresponds to its migratory behaviour between feeding and spawning grounds (Bard, 1981). Accordingly, the growth bands that form on the spines of albacore combine translucent and opaque bands when observed with the image projector. The translucent bands correspond to periods of long displacement, these *annuli* are narrower as a result of a lower protein supply. The opaque bands form during seasons favourable to growth, such as i.e. summer, when food is plentiful, are wider due to the greater protein supply. Interpreting these growth temporal marks by counting the translucent bands permits the age of the specimen to be estimated. In albacore tuna, two translucent bands (*annuli*) are on average formed each year, corresponding to the two migrations albacore performs every year, one in autumn towards the wintering area and another in spring towards the summer feeding grounds, and two opaque bands (corresponding to the periods of rapid growth). Depending on the migratory behaviour, as many as three translucent bands might be form each year.

Established translucent band (*annulus*) interpretation criteria is needed, since false growth rings can sometimes form, as can secondary growth areas that do not correspond to the year, or not clearly marked rings can be found, whose interpretation is difficult. Attention must be paid to whether the structure follows a pattern in which a number of rings have been formed periodically each year. The translucent bands found are open to different interpretations due to their shape and apparent composition:

- Double or triple *annuli*: formed by two or three fine translucent bands separated by opaque strips, normally considered to be a single *annulus;*
- Single fine *annulus*: little growth in the autumn migration;
- Single thick *annulus*: more active growth than the previous case, but with an absence of protein.

4.1.1 Back-calculation method

As the albacore grows, the first growth translucent band formed in the spine centre can disappear due to re-absorption of skeletal tissue. When ageing large individuals, the first visible *annulus* may not correspond to the first year of age. This means that for older fish specimen ageing it is required to apply the back-calculation method.

Since the fish length and spine diameter relationship can be determined by fitting a linear regression model to measurements done on each specimen pair of variables (fork length and diameter of spine), the estimated allometric relationship is used to back-calculate the length of a given fish at the yearly growth increments (Ortiz de Zárate *et al.* 2005). The correlations between fork length of fish and total width of annuli are used to calculate the age of the first visible ring.

Model formulation for the relationship Fish-Spine is $L_t = a \times D_t^{\ b}$
where:

L_t is Fish length (fork length in cm).
D_t is the diameter of the spine measured (mm).
a, b are the parameters of the linear regression model fit to pair observations.

Based on the underlying assumption that annual increment of fish length (FL) is proportional to annual increment of spine *annuli* (diameter) then the back-calculation is applied.

$FL_x = (Fl \times DA)/DS$

Where,

FL_x = Fish length when its spine diameter was X.
FL= Fish length when it was sampled
DA= Diameter of annulus when the fish was caugth
DS= diameter of the spine.

However direct proportionality does not apply. The known Lee's Phenomenon (Lee, 1912) of "apparent change in growth rate "is a tendency in the back-calculated lengths when considering proportional change between both variables (fish and spine). Years later Lea (1913) demonstrated that part of this observed proportionality was due to a computational artefact. Therefore a correction is need on the back-calculation model used on growth studies. There are several proposed correction procedures to the linear model in the scientific literature (Campana, 1990, Ricker, 1990).

Once the corresponding fish length of a fish to its spine diameter X has been determined by applying the regression model with procedure to eliminate Lee's phenomenon, then the age $\bar{X}$ to which this fish length corresponds can be estimated by length age relationships. Once the value of X is known we can deduce that this first and innermost visible *annulus* on the spine is the one corresponding to age X. In order to find the age corresponding to the spine studied, the number of annual translucent bands (*annulli*) visible on the spine is added to X. In Appendix 2 are presented results of age determination of North Atlantic albacore.

4.1.2 Precision and agreement among readers

The age determination for albacore using spine section readings of translucent bands includes error due to subjectivity of involved readers (Beamish, R. J. and G. A. McFarlane, 1983). To avoid the individual lack of objectivity on the interpretation of *annuli* formation that affects the precision of age estimates or reproducibility of age upon repeated lectures by the same reader, calibration among at least three readers is required (Campana, 2001). A guideline with the tools for age reading comparisons was made by Eltink et al. (2000), and an Excel workbook "AGE COMPARISONS.XLS" (Version 1.0) was produced by Eltink (2000) of reading errors on stock assessments. This method is currently applied on annual bases for ageing albacore obtaining estimates of percent agreement between readers, within reader and coefficient of variation (Ortiz de Zárate *et al.,* 2005).

4.2 Otoliths

Age interpretation is not currently performed using otoliths, since they are difficult to obtain. To extract them a cut must be made in the head, which adversely affects the appearance of the fish and leaves it unfit for sale. It would, therefore, be necessary to buy the specimen, and a small number of samples would require a considerable economic cost. Nevertheless for validation purposes of spine section readings a small sample of fish is being collected for simultaneously sampling of otoliths and first dorsal spine for age 1 class albacore and study both structures.

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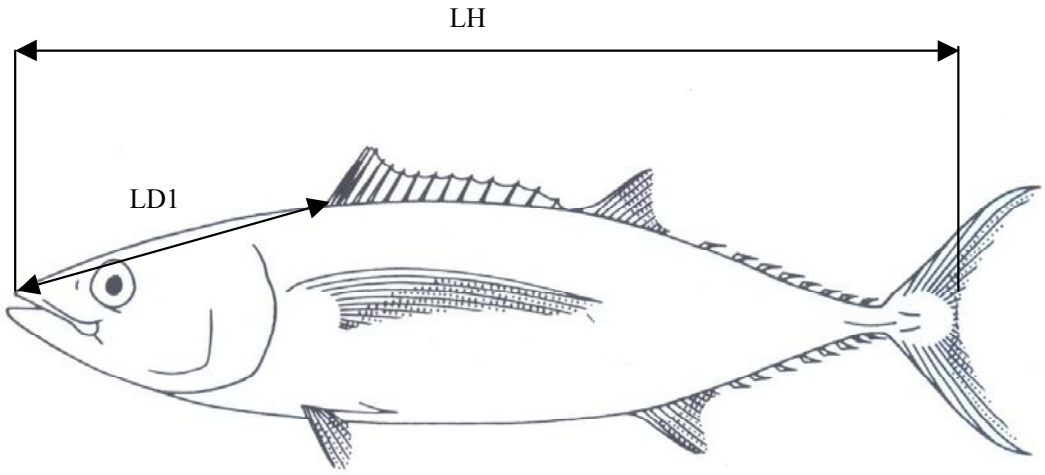

Figure 1. Types of measurements of albacore tuna length: Fork Length (LH), Pre-dorsal Length (LD1).

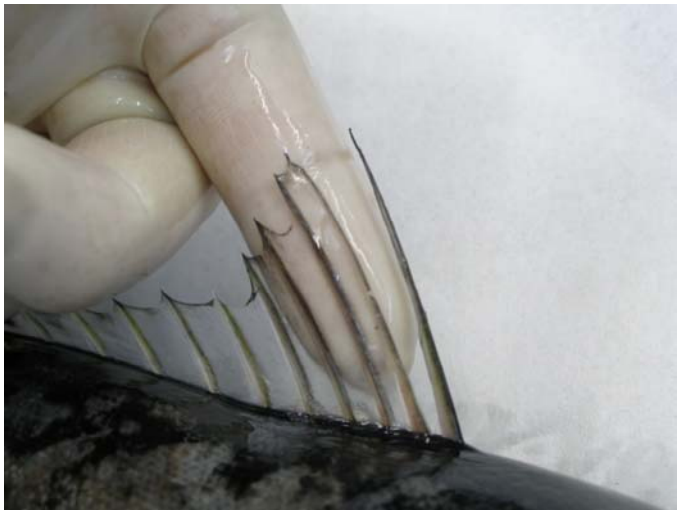

Figure 2. Separating the first two spines of the first dorsal fin. IEO ©

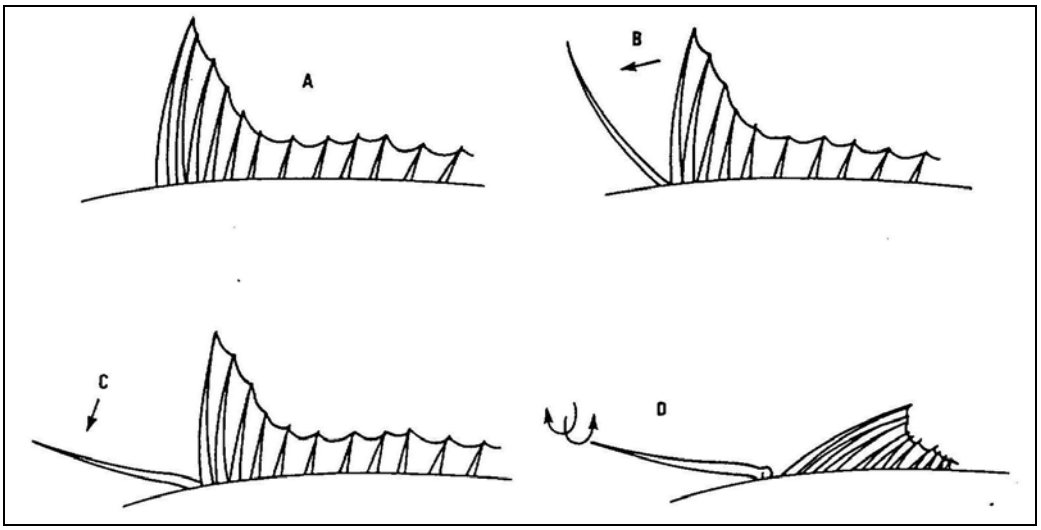

Figure 3. Technique for extracting the first spine of the first dorsal fin (taken from Compeán-Jiménez, 1980).

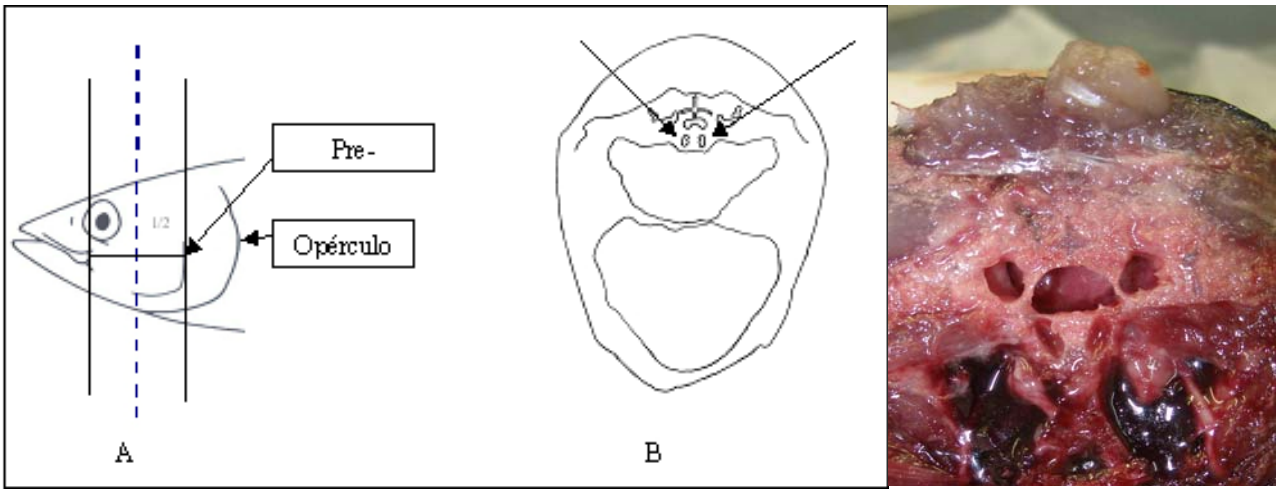

Figure 4.A. Diagram showing how to locate the imaginary (dotted) line to cut along. **Figure 4.B**. View of the cavities where the otolith's pair is found at the back of the skull.

Figure 5. Image of the measurement made to locate the line to transverse cross-cut the head.

Figure 6. Diamond blade slow ISOMET saw cutter.

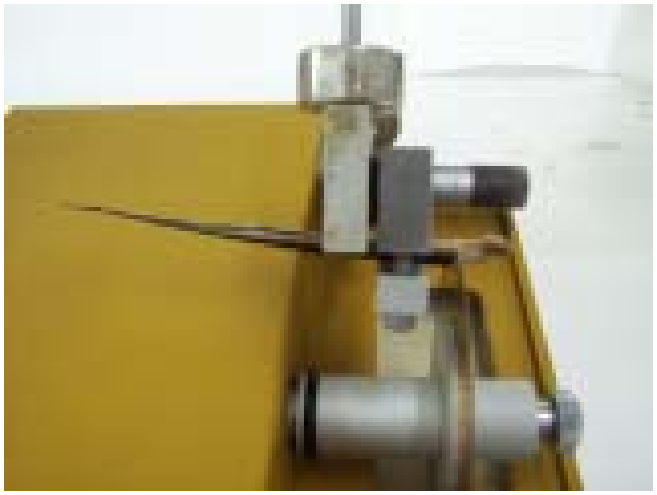

Figure 7. Cutting position of spine to obtain the individual spine sections on each fish.

Figure 8. Material for resin-mould spines preparation. IEO ©

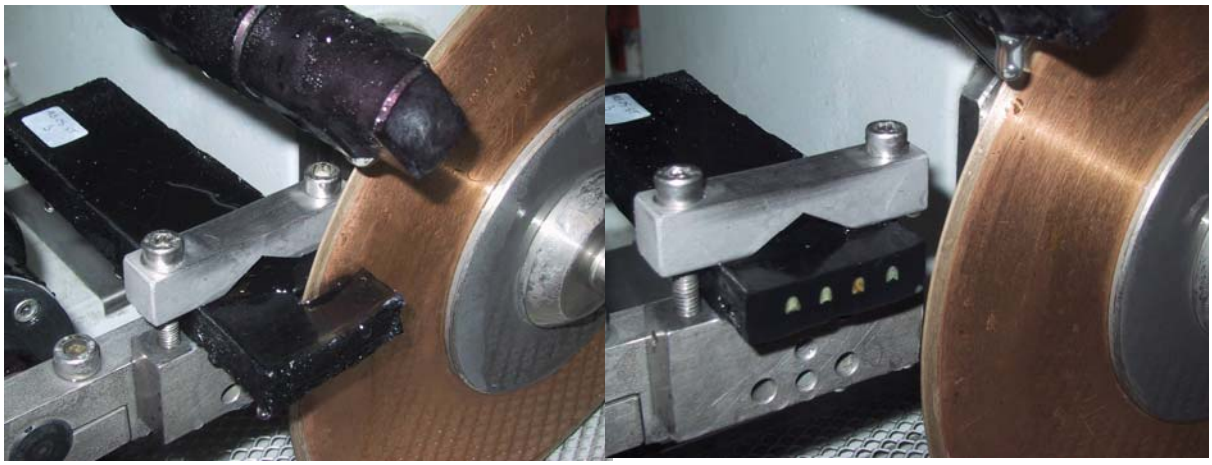

Figure 9 and Figure 10. Cutting a resin-moulded block of spines. ISOMED 5000 cutter. IEO ©

Figure 11. Distribution of resin over the spine sections.

Figure 12. Sections on slide, embedded in epoxy resin. Air-drying.

Figure 13. Blocked spine sections cuts embedded in resin and mounted with epoxy resin. IEO ©

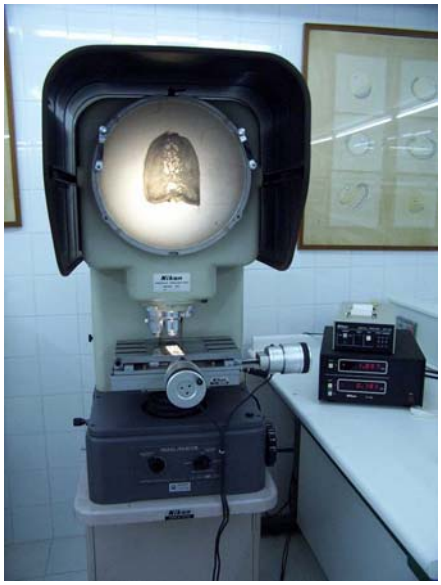

Figure 14. Profile projector.

Figure 15. Micrometer connected to profile projector.

Figure 16. Binocular microscope with camera incorporated for image analysis.

Appendix 1

Form designed to control samples of spines collected

RED DE INFORMACIÓN Y MUESTREO	**IEO Santander**

MUESTREO BIOLÓGICO DE ESPINAS (*1ª espina de 1ª aleta dorsal*)

ATUN BLANCO (*Thunnus alalunga*)
AÑO 2005

Puerto:
Mes:

long. horquilla (cm)	**nº espinas**
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long. horquilla (cm)	**nº espinas**
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118	
119	
120	
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Appendix 2

Examples of age estimation of Atlantic albacore (*Thunnus alalunga*) based on spine readings.

Age 1 Fork Length 56cm 29/07/2003

Age 2 Fork Length 7cm 5/08/2003

Age 3 Fork Length 71cm 06/08/2003

Age 4 Fork Length 94cm 28/08/2003

Age 5 Fork Length 98cm 30/09/2003

Age 6 Fork Length 101cm 15/09/2003

Age 7 Fork Length 100cm 25/08/2003

Age 8 Fork Length 106cm 17/10/2003