Protocol for sampling of hard parts for bluefin tuna (*Thunnus thynnus*) growth studies

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Introduction

Different techniques exist to estimate age, one of which is the reading of growth rings in calcified structures. These rings appear with seasonal periodicity, normally growing slowly in winter to form a narrow translucent band and more quickly in summer, giving rise to wider opaque areas, as the season is favourable to growth. The formation and biomineralization of these growth bands depends on metabolic and environmental factors, such as climate, migrations, nutrition, etc. The calcified structures traditionally used in bluefin tuna growth studies are: **spines, otoliths and vertebrae.** Whenever possible it is recommended that these three structures be collected from each specimen.

Sampling strategy

Two strategies are usually used for sampling, either random sampling or stratified by length, in which a certain number of samples are collected for each length group. This latter method is preferable to ensure adequate sampling of the whole length range and from this information estimate the growth curve.

Stratified sampling by length. Every **month** a number of **spines (3)** must be collected **for each 5 cm fork length range** (for example: 3 spines for the 60-64cm length range, 3 spines for the 65-69cm length range, etc.). Sampling should take place on **different days** throughout the month until enough spines have been collected to complete, as far as possible, the length range of landings. Moreover, readings should come from **different catch areas** of the stock under study such that most catch areas are covered as best they can be. To this end sampling must be spread among **different vessels and landing ports**.

Information on specimens sampled

Information must be collected concerning the fish whose hard parts are to be extracted and its capture. There are two types of data, some which are indispensable and others accessory.

Indispensable data.

1) Specimen size; the most commonly used measurement is Fork length.

- Fork length (FL): this is the straight line from the end of the upper jaw (end of the snout) to the posterior of the shortest caudal ray (fork of the caudal fin) (Figure 1). This can best be measured using a **caliper** or alternatively with a tape measure, although it must be kept straight while measuring. The fish should be placed on a flat surface in a horizontal position. In the case of very large specimens in which this measurement is very difficult to make, one of these other two lengths may be used to substitute it:
- First dorsal length (LD1): this is the straight line from the end of the upper jaw (end of the snout) to the base of the first dorsal spine (the start of the first dorsal fin) (Figure 1).
- Curved fork length (CFL): this is the length from the upper jaw (end of the snout) to the fork by an imaginary longitudinal line, with the corresponding fish curvature (Fig. 1).

The type of measurement being used must be clearly specified and the measurement unit (cm). FL and CFL are measured to the lower centimetre (a specimen of 70,8 cm or 70,2 cm would correspond to the 70 cm range), LD1 is measured to the lower half centimetre (a specimen of 30,4 cm measures as 30 cm and one of 30,7 cm corresponds to 30,5 cm).

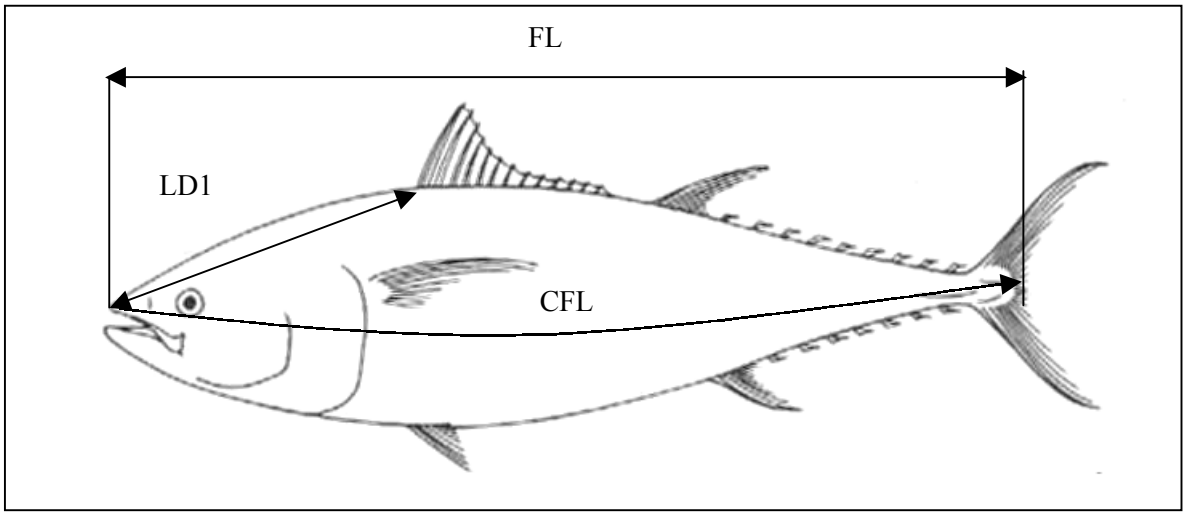


Figure 1. Types of measurements of bluefin tuna: Fork length (FL), First dorsal length (LD1), Curved fork length (CFL).

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

3) Fishing area.
This is the location of the catch from which the sample was extracted, and does not refer to the place where sampling took place. A precise geographical delimitation must be established. The most exact is the **latitude and longitude** where it was caught. As this is not always possible, in the case of sampled specimens captured in different fishing operations, the latitude and longitude of the area (between 44º - 45ºN and 5º - 7º W, for example), or at least a more or less defined geographical area such as the Bay of Biscay or the Alboran Sea, for example, should be noted.

4) The country to which samplings, **organization** and **personnel** responsible for sampling correspond.

Other data
5) Date of sampling (day, month and year)
6) Live and/or gutted specimen weight (kg)
7) Sex
8) Fishing gear used
9) Name of vessel that caught the specimen and the port at which it was landed
10) Other relevant observations: visible presence of ectoparasites on skin, gills, fins, etc.; anomalies or others.

Periodical control of structures collected
The structures collected during each period (month) must be **registered on a statistical sheet** in order to keep a record of both those collected and the number pending collection (Appendix 1).

COLLECTION OF HARD PARTS:

1. Spine sampling

1.1. Spine extraction

The first **spine of the first dorsal fin** shall be collected from each specimen. The spine must be pulled out whole from the base. The operation proceeds as follows:

- Using a knife, cut the membrane joining the 1st and 2nd dorsal fin rays (Figure 2).
- Push the spine forward progressively (Figure 3B) until the ligament breaks (Figure 3C). Twist the spine left and right alternatively until it comes loose and pull to finally extract it (Figure 3D).

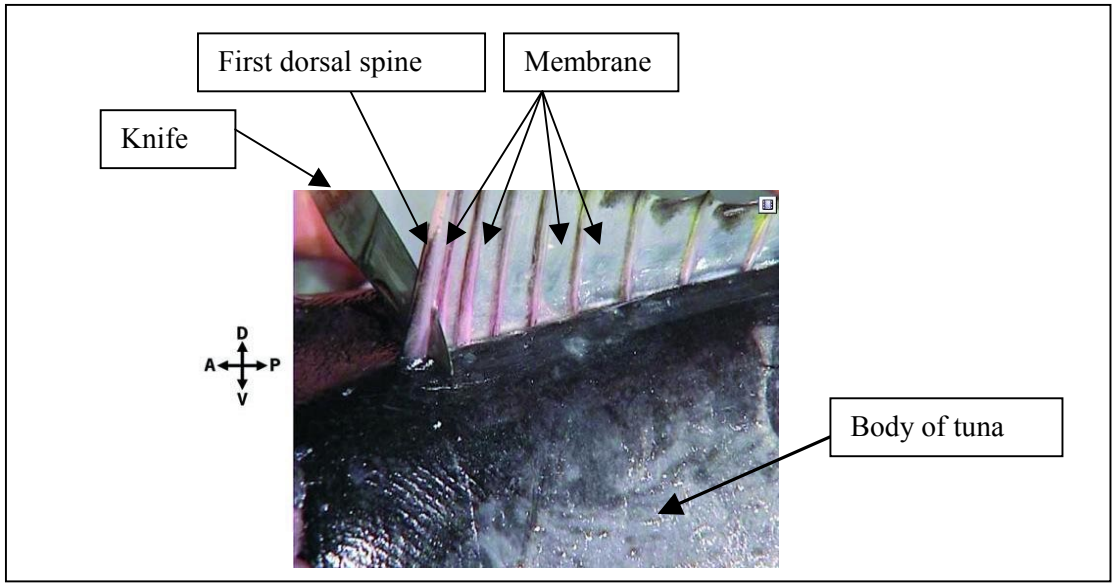


Figure 2. Insertion of the knife into the membrane separating the first two spines of the 1st dorsal fin . (Figure taken from Panfili *et al*., 2002).

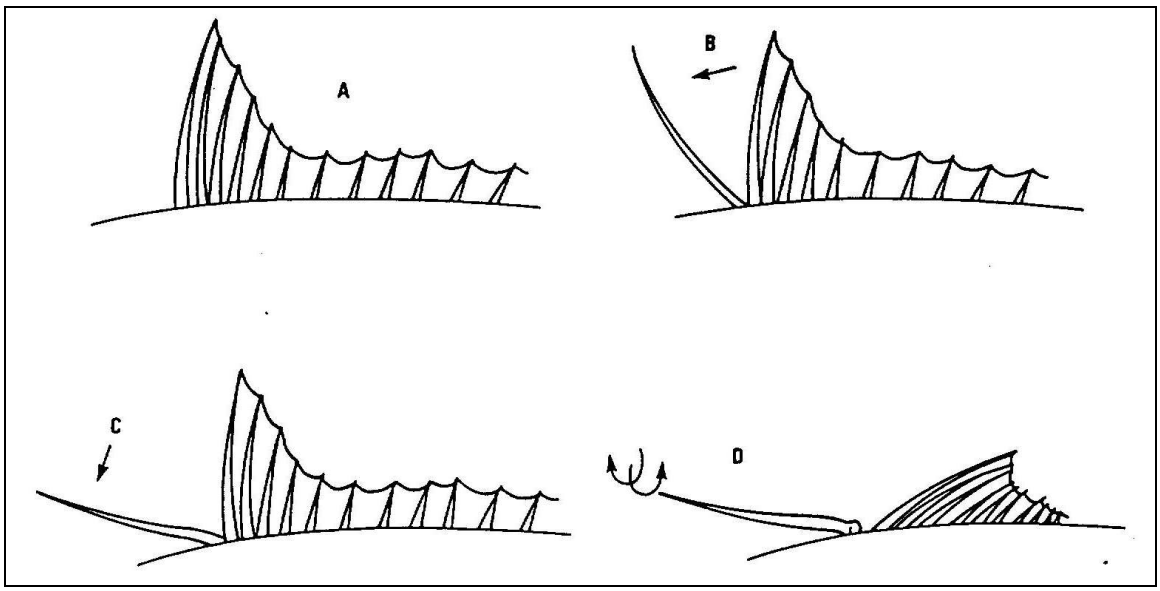


Figure 3. Technique of extraction of the first spine of the bluefin tuna dorsal fin. (Figures taken from Compeán-Jiménez, 1980).

1.2. Spine preservation. Spines are ideally preserved dry in a paper envelope, which should be kept in a cool place (refrigerated). If the spine collected is too large to fit in the envelope, it can be cut in half or even in three pieces and kept in the envelope, remembering that the piece forming the base of the spine is the most important since it is the part used for age interpretation. The data of the specimen sampled or its corresponding code must appear on the envelope.

2. Otolith sampling

Sagittal otoliths are small, calcified structures found in the semicircular cavities of the inner ear, situated 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 otoliths found in each inner ear of the bluefin tuna. There are two main techniques of removal: transverse head section and frontal head section. In the second one a frontal section of the superior part of the cranium is made, passing above the eye and parallel to the mayor axis of the fish. We are going to explain the first technique.

2.1. Otolith extraction

Transverse head section. Consists of making a cut in the upper part or back of the head at the level of an imaginary line which can be traced as follows: Trace an imaginary line perpendicular to the horizontal fish, which passes through the mid-point between the corner of the mouth and the pre-operculum (Figure 4A). For this purpose, the use of a ruler is recommended for dividing this distance in two, and afterwards making a cut in the upper part of the fish which follows this imaginary line. Once the point has been marked to make the cut, use a metal saw and cut down through the head perpendicular to the horizontal axis of the fish.

The sectioned part of the head contains the otoliths. If the above described cut has been made properly, the cavities below the brain in the upper part of the head (Figure 4B) should be searched to find the otoliths. If they are not found here, it may be that they are in the other part of the sectioned fish. Using fine forceps and with great delicacy to avoid breaking these fragile pieces, extract each otolith. They must be taken out of a very fine transparent capsule, which covers them. The otoliths are between 7 and 20 mm in size approximately, and both otoliths must be collected from each specimen. If the otolith has broken, try to recover the pieces and keep them all together. Once extracted, rinse them in water or diluted alcohol and leave them out to dry.

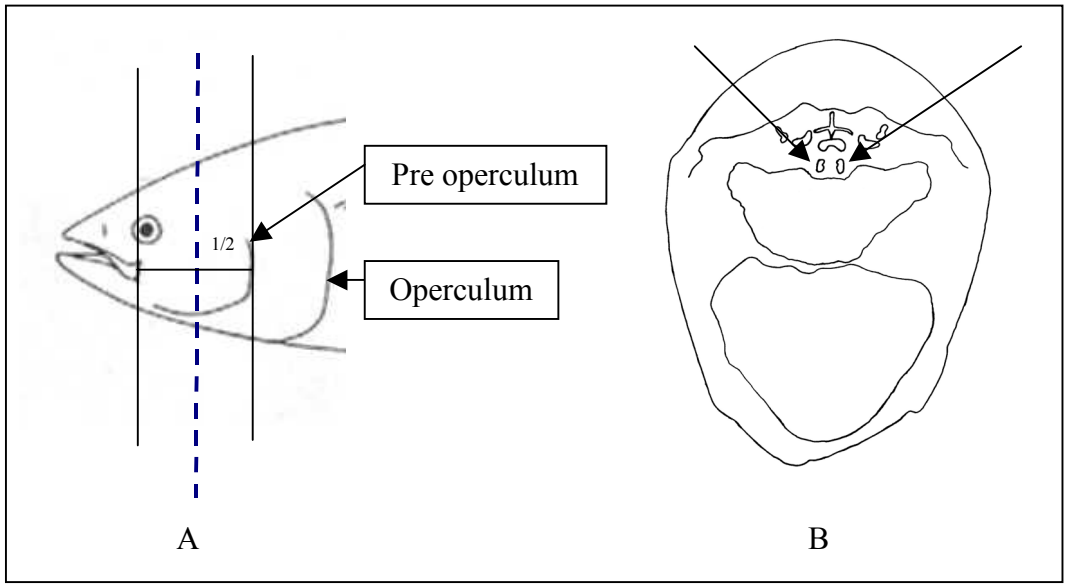


Figure 4. A. Tracing the imaginary line (dotted) along which to make the cut. B. View of the cavities where the pair of otoliths are found in the back of the head.

2.2. Otolith preservation.
Keep dry in a tube or in an envelope. If using an envelope avoid applying pressure that might break them. The data of the specimen sampled or its corresponding code should appear on the envelope or the tube.

3. Sampling of caudal vertebrae

The bluefin tuna has 39 vertebrae, of which 18 are precaudal and 21 caudal. Vertebra 35 is used for the study of growth (Berry *et al*. 1977; Farber and Lee 1981), nevertheless, it is better to collect vertebra 35 and 36 without separating them. Collecting both we have the opportunity of comparing the "whole vertebra" and the "vertebra section" methods. Also, storing vertebrae 35 and 36 attached we preserve the quality of the inner surface preventing dehydration caused by refrigeration. As the surface comes in contact with air, dries up and becomes more difficult to read.

3.1. Vertebra extraction
To find vertebra 35, a transversal cut is made in the caudal area between the 4th and the 5th finlet (counting from the end of the tail forwards, that is to say, there must be 4 more finlets behind the one indicated). On making the cut we find vertebra 35. With luck, the cut coincides with the intervertebral space and the tail will be cut easily. If not, the intervertebral space must be found further forward in the fish. Vertebra 35 is the first found in the part sectioned, and can be separated together with vertebra 36 from the rest of the caudal vertebrae, cleaned and peeled, removed any flesh attached to it.

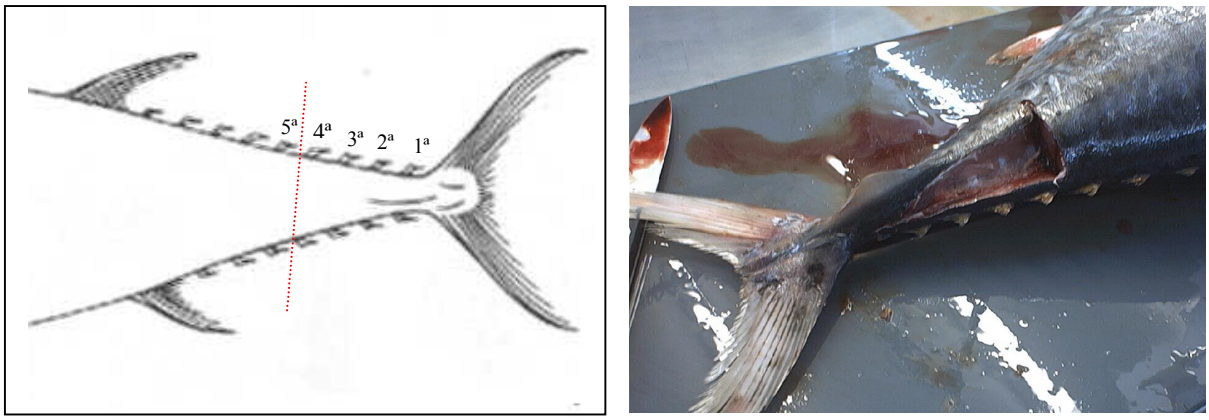


Figure 5. Cutting line to find vertebra 35. The photograph shows the transversal cut and the tail has been peeled to uncover the vertebrae (white marks).

3.2. Preservation of the vertebrae.
The two vertebrae are stored attached and not separated until they are analyzed. They should be stored dry in an envelope and refrigerated (some flesh always remains attached). The vertebrae can be stored together with the spine in the same envelope.

References:

Berry, F. H., D. W. Lee, and A. R. Bertolino. 1977. Progress in Atlantic bluefin tuna ageing attempts. *Collective Volume of Scientific Papers, ICCAT,* 6(2): 305-317.

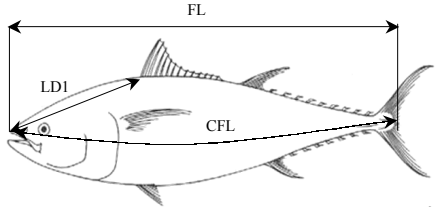
Compeán-Jiménez, G. 1980. Comparaison de techniques de détermination de l´age chez les principales espéces de thonidés atlantiques (Tesis doctoral). Univ. Aix Marseille II, 153 pp.

Farber, M. I., and D. W. Lee. 1981. Ageing western Atlantic bluefin tuna, *Thunnus thynnus*, using tagging data, caudal vertebrae and otoliths. *Collective Volume of Scientific Papers, ICCAT,* 15(2): 288-301.

Panfili, J., H. De Pontual, H. Troadec and P.J. Wright (Editors). 2002. *Manual of Fish Sclerochronology*. Brest, France: Ifremer-IRD coedition, 464 p.

Appendix 1. Monthly statistical sheet for the control of the calcified structure collected.

SAMPLING **Atlantic Bluefin Tuna** (*Thunnus thynnus*)



calcified structure:	☐ 1st dorsal fin ray ☐ vertebrae 35-36 ☐ otoliths
measure system:	☐ **caliper** ☐ tape measure
Country:	

Year:	
Month:	
Latitude-Longitude: Area:	
Collector´s name:	
Organization:	

☐**FL** / ☐ CFL (to lower cm.)	number
40 - 44	
45 - 49	
50 - 54	
55 - 59	
60 - 64	
65 - 69	
70 - 74	
75 - 79	
80 - 84	
85 - 89	
90 - 94	
95 - 99	
100 - 104	
105 - 109	
110 - 114	
115 - 119	
120 - 124	
125 - 129	
130 - 134	
135 - 139	
140 - 144	
145 - 149	
150 - 154	
155 - 159	
160 - 164	
165 - 169	

☐**FL** / ☐ CFL (to lower cm.)	number
170 - 174	
175 - 179	
180 - 184	
185 - 189	
190 - 194	
195 - 199	
200 - 204	
205 - 209	
210 - 214	
215 - 219	
220 - 224	
225 - 229	
230 - 234	
235 - 239	
240 - 244	
245 - 249	
250 - 254	
255 - 259	
260 - 264	
265 - 269	
270 - 274	
275 - 279	
280 - 284	
285 - 289	
290 - 294	
295 - 299	

Total: _____

☐ **LD1 (cm)** (to lower 1/2 cm)	number
26.0 - 27.5	
28.0 - 29.5	
30.0 - 31.5	
32.0 - 33.5	
34.0 - 35.5	
36.0 - 37.5	
38.0 - 39.5	
40.0 - 41.5	
42.0 - 43.5	
44.0 - 45.5	
46.0 - 47.5	
48.0 - 49.5	
50.0 - 51.5	
52.0 - 53.5	

LD1 (cm)	number
54.0 - 55.5	
56.0 - 57.5	
58.0 - 59.5	
60.0 - 61.5	
62.0 - 63.5	
64.0 - 65.5	
66.0 - 67.5	
68.0 - 69.5	
70.0 - 71.5	
72.0 - 73.5	
74.0 - 75.5	
76.0 - 77.5	
78.0 - 79.5	
80.0 - 81.5	
82.0 - 83.5	

Total: _____

Remarks: _____