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REPORT OF THE BLUEFIN TUNA DIRECT AGEING NETWORK (UNDER THE BYP FRAMEWORK)

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SUMMARY

*This document presents the report of the activities carried out by the working group on the direct age determination of bluefin tuna (*Thunnus thynnus*) during its first year. A bibliographic list has been created and an inventory of hard parts and the identification of laboratories with collections were started. A comparative study is also being carried out on the interpretation of age based on vertebra and dorsal spines from the same fish. A sampling protocol of bluefin tuna hard parts has been developed and a collection of images has started, as well as preparations of bone structures, which serve to establish a reading criterion.*

RÉSUMÉ

Le présent document fait état des activités menées à bien par le groupe de travail sur la détermination directe de l'âge du thon rouge (Thunnus thynnus) lors de sa première année de fonctionnement. Une liste bibliographique a été créée, un inventaire des structures calcifiées a été mis sur pied, ainsi que l'identification des laboratoires dotés de collections. Il est également réalisé une étude de comparaison de l'interprétation de l'âge à partir des vertèbres et épines dorsales provenant du même spécimen. Un protocole d'échantillonnage des structures calcifiées de thon rouge a été élaboré et une collection d'images et de préparations de structures squelettiques a débuté, lesquels devraient servir à établir un critère de lecture.

RESUMEN

En este documento se presenta el informe de las actividades desarrolladas por el grupo de trabajo sobre la determinación directa de la edad de atún rojo (Thunnus thynnus) durante su primer año de funcionamiento. Se ha creado un listado bibliográfico, se ha comenzado un inventario de estructuras calcificadas y la identificación de laboratorios con colecciones. También se está realizando un estudio de comparación de la interpretación de la edad a partir de vértebras y espinas dorsales procedentes del mismo ejemplar. Se ha elaborado un protocolo de muestreo de estructuras calcificadas de atún rojo y se ha comenzado una colección de imágenes, de preparaciones de estructuras esqueléticas, que sirvan para establecer un criterio de lectura.

KEY WORDS

Bluefin tuna, Age determination

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1. Introduction

The bluefin tuna direct ageing network (hereafter ageing network) was formally established at the beginning of 2004 following the recommendation of the most recent meeting of the Bluefin Year Program (BYP) Working Group (Appendix 6 of the ICCAT SCRS Report 2002-2003). The participants are listed in **Appendix 1**.

Direct ageing of bluefin catch has been recommended as a superior means of estimating catch at age for the purposes of stock assessment. The catch at age matrix is currently obtained by applying age slicing to length distributions of catches, a method which involves the use of two growth curves, one for each stock, which were estimated over ten years ago. Old and recent studies show the viability of using hard parts for age determination based on age interpretation of spines and vertebrae. Nevertheless, otoliths have also been used successfully for this species.

2. Objectives

The aim of the ageing network is to compare and evaluate various ageing methods in order to develop a standardised protocol for age determination of bluefin tuna.

To achieve this objective, the technique of age reading of calcified structures must be standardised and later, using other methods, validated. This work must be co-ordinated since the migrations of this species make it difficult for a single country to gain access to sampling of all ages of the population. This co-ordination is necessary for the exchange of hard parts, the organisation of reading workshops and the verification and standardisation of age estimation methodology.

3. Work plan

A series of tasks were identified by the ageing network as a starting point. Some of them have been completed and others need more time to be finished.

3.1 Task 1. Bibliography

An updated database with direct ageing references of bluefin tuna was produced. This bibliography contains references based on age estimation from reading otoliths, vertebrae and spines of bluefin tuna. There are also other studies on bluefin tuna growth (from tagging data, length frequency analysis, validation of age reading, etc.); and references of direct ageing of other closely related species such as southern bluefin tuna and other papers on age reading and growth in general. See **Appendix 2**.

3.2 Task 2. Identification of places of storage and inventory of calcified structures

The ageing network needs to know what calcified structures are available to be used for the standardisation of the ageing technique. The group must also know what hard parts are being collected.

For this inventory the following information was considered essential:

3.2.1 Place of storage, *Origin of the fish sampled* (location of the catch)*, Type of calcified structure* (otolith, etc.), *Year* when samples were obtained, *Month* (*) when samples were obtained, *Number* of samples, *Length range* of the specimens sampled (minimum and maximum lengths) and *Main length range* (5 or more specimens sampled by 5 cm length groups).

For the moment few participants have responded to the survey into this historical inventory. The provisional historical inventory appears in **Appendix 3**.

3.2.2 Sampling in 2004

Priority was given to the sampling of three calcified structures (otolith, vertebra and spine) whenever possible with the aim of comparing age estimation from different structures.

3.3 Task 3. Age determination from different calcified structures of the same specimen

In order to standardise the technique of age estimation it is necessary to compare the readings of different hard parts of the same specimen. At the same time, each calcified structure has a different technique of preparation and reading methodology, making it necessary that an experienced person takes the responsibility for the age determination of each calcified structure. The group designated three people, one for each structure, as those responsible: vertebrae: Droplaug Olafsdottir, spines: Enrique Rodriguez-Marín and otoliths. Dave Secor.

The methodology for vertebra and spine reading is more advanced than that of otoliths, for which the technique of preparation and age interpretation is trailing behind. To advance in this latter technique, the methodology of age interpretation from otoliths used for Southern Bluefin Tuna was taken as a reference.

Three collections are currently available:

- Vertebrae and dorsal spines from the same specimen in the samples from Iceland. Sample range 100 to 300 cm fork length.
- Vertebrae, dorsal spines and otoliths in the samples from Spain for juveniles. Sample range 60 to 90 cm fork length
- Vertebrae, dorsal spines and otoliths in the samples from Turkey. Sample range 110 to 170 cm fork length.

The exchange of samples between Spain and Iceland has begun. Comparison of age interpretation from vertebrae and spines is in progress.

3.4 Task 4.- Protocol for extraction and conservation of calcified structures

This task has been completed. See **Appendix 4**.

3.5 Task 5.- Collection of images

The ageing network recognised the need for digital images of the different structures in order to establish the bases and reading criteria and to reach a consensus on age interpretations in exchanges. This task is in progress and some digital images of spine sections are being collected.

4. Other matters

We have contacted the European fish ageing network (EFAN) co-ordinator, Erlend Moksness, so that he is aware of our existence and in order to collaborate.

Some documents produced by the group are posted on the web page of ICCAT and we hope to develop a specific sheet for this working group

5. Future plans

In addition to the work underway already described, we must set about the elaboration of a protocol for the preparation and reading of vertebrae, spines and otoliths.

At the meetings of SCRS of ICCAT, group members shall decide on future tasks.

Table 1. Calcified structure sampling from the same specimen in 2004 (provisional data)

Place of storage	*Origin*	*Year*	*Spine*	*Vertebrae*
Iceland (Marine Resarch)	South of Iceland	2004	Yes	
USA (University of New Hampshire	Gulf of Maine	2004	Yes	
Canada (Fisheries and Oceans)	North Lake	2004	Yes	Yes
Canada (Fisheries and Oceans)	Port Hood	2004	Yes	Yes
Spain (Instituto Español de Oceanografía)	Bay of Biscay	2004	Yes	Yes

Appendix 1

Name	Country	Institution
Cristina Rodríguez-Cabello	Spain	Spanish Institute of Oceanography
David H. Secor	USA	University of Maryland Chesapeake Biol. Lab.
Dennis Lee	USA	NOAA Fisheries
Droplaug Olafsdottir	Iceland	Marine Research Institute
Enrique Rodríguez-Marín	Spain - Coordinator	Spanish Institute of Oceanography
Erin Carruthers	Canada	Marine Fish Division - Biological Station
Isik K. Oray	Turkey	Faculty of Fisheries - University of Istanbul
Jay R. Rooker	USA	Texas A&M University, Galveston
John D. Neilson	Canada	Fisheries and Oceans Canada
Jorge Landa	Spain	Spanish Institute of Oceanography
José Luis Cort	Spain	Spanish Institute of Oceanography
José María Ortiz de Urbina	Spain	Spanish Institute of Oceanography
Julie M. Porter	ICCAT	ICCAT Secretariat
Mario La Mesa	Italy	Institute of Science Marine
Marta Ruiz	Spain	Spanish Institute of Oceanography
Miguel Neves dos Santos	Portugal	IPIMAR - CRIPSul
Molly E. Lutcavage	USA	New England Aquarium Department of Agric. & Fish
Nicoleta Santamaria	Italy	University of Bari
Saadet Karakulak	Turkey	Faculty of Fisheries - University of Istanbul
Sean C. Smith	Canada	Marine Biological Station
Valerie Chosson	Iceland	Marine Research Institute
Víctor Restrepo	ICCAT	ICCAT Secretariat
Vjekoslav Ticina	Croatia	Institute of Oceanography and Fisheries
Xulio Valeiras	Spain	Spanish Institute of Oceanography

Appendix 2

Bluefin tuna age and growth bibliography

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Appendix 3

DORSAL SPINE

NS is the number of samples
Min and Max minimum and maximum fork length
MLR MIN and MLR MAX are the minimum and maximum main length range (at least 5 specimens by 5 cm length groups)

PLACE OF STORAGE	ORIGIN	YEAR	QUARTER	Nº S	MIN	MAX	MLR MIN	MLR MAX
SPAIN								
I.E.O. SANTANDER	?	1979	?	62	75	170	75	149
I.E.O. SANTANDER	?	1980	3	27	61	151	60	69
I.E.O. SANTANDER	?	1981	3	53	51	195	60	124
I.E.O. SANTANDER	ATL W (USA)	1981	3	45	60	92	60	94
I.E.O. SANTANDER	?	1982	3	37	29	177	30	34
I.E.O. SANTANDER	?	1982	4	90	40	98	40	74
I.E.O. SANTANDER	?	1983	2	43	45	202	45	54
I.E.O. SANTANDER	RECAPTURED	1983	2	1	150	150		
I.E.O. SANTANDER	?	1983	3	1	177	177		
I.E.O. SANTANDER	BAY OF BISCAY	1983	3	87	63	167	85	134
I.E.O. SANTANDER	MEDITERRANEAN (ARGELIA)	1983	3	4	51	85		
I.E.O. SANTANDER	MEDITERRANEAN (GARRUCHA)	1983	3	9	24	31	20	29
I.E.O. SANTANDER	BAY OF BISCAY	1983	4	57	58	70	60	69
I.E.O. SANTANDER	BAY OF BISCAY	1983	?	4	60	62		
I.E.O. SANTANDER	GULF OF CADIZ	1984	2	168	135	302	190	264
I.E.O. SANTANDER	GULF OF CADIZ	1984	2	1	WITHOUT FL			
I.E.O. SANTANDER	BAY OF BISCAY	1984	3	195	95	203	95	189
I.E.O. SANTANDER	GULF OF CADIZ	1984	?	27	135	280	245	249
I.E.O. SANTANDER	BAY OF BISCAY	1985	2	147	55	111	55	104
I.E.O. SANTANDER	RECAPTURED	1985	2	1	84	84		
I.E.O. SANTANDER	BAY OF BISCAY	1985	3	347	52	168	55	159
I.E.O. SANTANDER	RECAPTURED	1985	3	2	81	97		
I.E.O. SANTANDER	BAY OF BISCAY	1985	4	122	62	116	65	114
I.E.O. SANTANDER	MEDITERRANEAN (2 SETE,2 VENDRES, 1 TURKEY)	1986	2	5	WITHOUT FL			
I.E.O. SANTANDER	MEDITERRANEAN (VENDRES)	1986	2	1	51	51		
I.E.O. SANTANDER	BAY OF BISCAY	1986	3	397	55	182	55	164
I.E.O. SANTANDER	?	1986	4	2	114	114		
I.E.O. SANTANDER	BAY OF BISCAY	1986	4	187	60	132	60	114
I.E.O. SANTANDER	MEDITERRANEAN (TURKEY)	1986	4	2	39	70		
I.E.O. SANTANDER	BAY OF BISCAY	1987	3	276	50	186	55	154
I.E.O. SANTANDER	BAY OF BISCAY	1987	4	145	61	156	60	154
I.E.O. SANTANDER	BAY OF BISCAY	1988	2	26	75	124	90	99
I.E.O. SANTANDER	BAY OF BISCAY	1988	3	419	54	188	55	169
I.E.O. SANTANDER	Campaña Cimarron/88	1988	3	1	83	83		
I.E.O. SANTANDER	BAY OF BISCAY	1988	4	123	60	140	60	139
I.E.O. SANTANDER	CANARY ISLAND	1988	4	5	44	70		
I.E.O. SANTANDER	BAY OF BISCAY	1989	2	109	55	193	55	159
I.E.O. SANTANDER	GULF OF CADIZ	1989	2	3	195	243		
I.E.O. SANTANDER	BAY OF BISCAY	1989	3	136	54	167	55	159
I.E.O. SANTANDER	BAY OF BISCAY	1989	4	7	64	75	75	79
I.E.O. SANTANDER	BAY OF BISCAY	1990	2	38	59	145	75	99
I.E.O. FUENGIROLA	GULF OF CADIZ	1990	2	116	110	291	180	274
I.E.O. FUENGIROLA	MEDITERRANEAN	1990	2	6	166	271		
I.E.O. SANTANDER	BAY OF BISCAY	1990	3	249	55	190	55	154
I.E.O. SANTANDER	BAY OF BISCAY	1990	4	7	74	75	70	74
I.E.O. SANTANDER	BAY OF BISCAY	1991	3	301	59	202	60	164
I.E.O. SANTANDER	BAY OF BISCAY	1991	4	32	66	118	65	99
I.E.O. SANTANDER	BAY OF BISCAY	1992	2	19	56	100	75	84
I.E.O. SANTANDER	MEDITERRANEAN (ALGECIRAS)	1992	2	1	101	101		
I.E.O. SANTANDER	BAY OF BISCAY	1992	3	180	56	184	55	154
I.E.O. SANTANDER	MEDITERRANEAN (ATMELLA DE MAR)	1992	3	6	50	54	50	54
I.E.O. SANTANDER	RECAPTURED	1992	3	3	103	129		
I.E.O. SANTANDER	BAY OF BISCAY	1992	4	98	63	156	65	124
I.E.O. SANTANDER	MEDITERRANEAN (TURKEY)	1993	1	10	130	178		
I.E.O. SANTANDER	BAY OF BISCAY	1993	2	59	53	157	75	104
I.E.O. SANTANDER	MEDITERRANEAN (TURKEY)	1993	2	4	59	115		
I.E.O. SANTANDER	BAY OF BISCAY	1993	3	205	51	204	55	149
I.E.O. SANTANDER	RECAPTURED	1993	3	2	145	145		
I.E.O. SANTANDER	BAY OF BISCAY	1993	4	5	84	93		
I.E.O. SANTANDER	BAY OF BISCAY	1994	2	12	62	97	80	84
I.E.O. SANTANDER	MEDITERRANEAN	1994	2	2	141	225		
I.E.O. SANTANDER	BAY OF BISCAY	1994	3	110	60	183	60	134
I.E.O. SANTANDER	MEDITERRANEAN	1994	3	5	85	264		
I.E.O. SANTANDER	?	1994	4	1	124	124		
I.E.O. SANTANDER	BAY OF BISCAY	1994	4	96	71	145	70	144

Appendix 3 Cont.

DORSAL SPINE

NS is the number of samples
Min and Max minimum and maximum fork length
MLR MIN and MLR MAX are the minimum and maximum main length range (at least 5 specimens by 5 cm length groups)

PLACE OF STORAGE	ORIGIN	YEAR	QUARTER	Nº S	MIN	MAX	MLR MIN	MLR MAX
SPAIN								
I.E.O. SANTANDER	?	1995	1	1	288	288		
I.E.O. SANTANDER	BAY OF BISCAY	1995	2	27	59	106	80	89
I.E.O. SANTANDER	MEDITERRANEAN	1995	2	5	79	113		
I.E.O. SANTANDER	BAY OF BISCAY	1995	3	144	56	145	55	114
I.E.O. SANTANDER	MEDITERRANEAN	1995	3	26	55	105	55	89
I.E.O. SANTANDER	RECAPTURED	1995	3	1	150	150		
I.E.O. SANTANDER	BAY OF BISCAY	1995	4	75	62	123	65	114
I.E.O. SANTANDER	BAY OF BISCAY	1996	2	30	70	123	75	84
I.E.O. SANTANDER	BAY OF BISCAY	1996	3	298	55	185	55	139
I.E.O. SANTANDER	BAY OF BISCAY	1996	4	67	64	109	65	89
I.E.O. SANTANDER	BAY OF BISCAY	1997	2	15	56	95		
I.E.O. SANTANDER	BAY OF BISCAY	1997	3	197	55	162	55	139
I.E.O. SANTANDER	RECAPTURED	1997	3	2	95	100		
I.E.O. SANTANDER	BAY OF BISCAY	1997	4	186	64	148	65	144
I.E.O. SANTANDER	RECAPTURED	1997	4	2	38	38		
I.E.O. SANTANDER	BAY OF BISCAY	1998	2	28	70	145	95	114
I.E.O. SANTANDER	BAY OF BISCAY	1998	3	326	53	160	50	154
I.E.O. SANTANDER	RECAPTURED	1998	3	1	56	56		
I.E.O. SANTANDER	BAY OF BISCAY	1998	4	53	80	106	80	94
I.E.O. SANTANDER	BAY OF BISCAY	1999	2	41	54	105	70	94
I.E.O. SANTANDER	BAY OF BISCAY	1999	3	129	75	188	75	174
I.E.O. SANTANDER	BAY OF BISCAY	2000	2	32	70	106	95	104
I.E.O. SANTANDER	BAY OF BISCAY	2000	3	160	59	170	60	154
I.E.O. SANTANDER	BAY OF BISCAY	2000	4	55	62	73	65	74
I.E.O. SANTANDER	BAY OF BISCAY	2001	2	16	73	100	75	79
I.E.O. SANTANDER	BAY OF BISCAY	2001	3	120	68	210	75	129
I.E.O. SANTANDER	BAY OF BISCAY	2002	2	23	74	140	75	94
I.E.O. SANTANDER	BAY OF BISCAY	2002	3	104	59	185	60	104
I.E.O. SANTANDER	BAY OF BISCAY	2002	4	16	63	276	65	69
I.E.O. SANTANDER	BAY OF BISCAY	2003	3	91	55	158	70	144
TURKEY								
ISTAMBUL UNIVERSITY	EAST MEDITERRANEAN SEA	2003	2-?	115	100	279	110	169
ICELAND								
MARINE RESEARCH INSTITUTE	SOUTH OF ICELAND	1999-2001	3-4	121	< 100	290		
MARINE RESEARCH INSTITUTE	SOUTH OF ICELAND	2002	3-4	573	160	290		
MARINE RESEARCH INSTITUTE	SOUTH OF ICELAND	2003	?	490				

Appendix 3 Cont.

VERTEBRAE

NS is the number of samples
Min and Max minimum and maximum fork length
MLR MIN and MLR MAX are the minimum and maximum main length range (at least 5 specimens by 5 cm length groups)

PLACE OF STORAGE	ORIGIN	YEAR	QUARTER	Nº S	MIN	MAX	MLR MIN	MLR MAX
SPAIN								
I.E.O. SANTANDER	BAY OF BISCAY	2000	3	30	61	73	65	74
I.E.O. SANTANDER	BAY OF BISCAY	2000	4	20	66	73	65	74
I.E.O. SANTANDER	BAY OF BISCAY	2001	3	48	72	88	75	89
I.E.O. SANTANDER	BAY OF BISCAY	2002	3	20	63	92	65	89
I.E.O. SANTANDER	BAY OF BISCAY	2002	4	15	63	70	65	69
I.E.O. SANTANDER	BAY OF BISCAY	2003	3	36	55	81	60	84
TURKEY								
ISTAMBUL UNIVERSITY	EAST MEDITERRANEAN SEA	2003	2-?	18	100	209	120	139
ICELAND								
MARINE RESEARCH INSTITUTE	SOUTH OF ICELAND	1999-2001	3-4	2070	< 100	290	100	290
MARINE RESEARCH INSTITUTE	SOUTH OF ICELAND	2002	3-4	573				
MARINE RESEARCH INSTITUTE	SOUTH OF ICELAND	2003	?	490				

OTOLITH

NS is the number of samples
Min and Max minimum and maximum fork length
MLR MIN and MLR MAX are the minimum and maximum main length range (at least 5 specimens by 5 cm length groups)

PLACE OF STORAGE	ORIGIN	YEAR	QUARTER	Nº S	MIN	MAX	MLR MIN	MLR MAX
SPAIN								
I.E.O. SANTANDER	MEDITERRANEAN (BALEARIC)	1995	4	3	29	37		
I.E.O. SANTANDER	MEDITERRANEAN (BALEARIC)	1996	4	10	63	67	63	63
I.E.O. SANTANDER	MEDITERRANEAN (BALEARIC)	1997	4	172	26	40	32	40
I.E.O. SANTANDER	MEDITERRANEAN (BALEARIC)	1998	3	25	53	59	55	57
I.E.O. SANTANDER	MEDITERRANEAN (BALEARIC)	?	?	3	31	35		
I.E.O. SANTANDER	BAY OF BISCAY	2000	3	12	61	70	65	69
I.E.O. SANTANDER	BAY OF BISCAY	2000	4	9	66	73	70	74
I.E.O. SANTANDER	BAY OF BISCAY	2001	3	21	74	88	75	84
I.E.O. SANTANDER	BAY OF BISCAY	2002	3	20	63	92	65	89
I.E.O. SANTANDER	BAY OF BISCAY	2002	4	14	63	70	65	69
I.E.O. SANTANDER	BAY OF BISCAY	2003	3	36	55	81	60	84
TURKEY								
ISTAMBUL UNIVERSITY	EAST MEDITERRANEAN SEA	2003	2-?	22	110	169	120	139

Appendix 4

PROTOCOL FOR SAMPLING OF HARD PARTS FOR BLUEFIN TUNA (*THUNNUS THYNNUS*) GROWTH STUDIES

Prepared by M. Ruiz, E. Rodríguez-Marín and J. Landa [2]

1. 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.

2. 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**.

3. 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.

4. Indispensable data

4.1 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 (**Figure 1**).

[2] Instituto Español de Oceanografía, Centro Oceanográfico de Santander, Apdo. 240, 39080 Santander, Spain.

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).

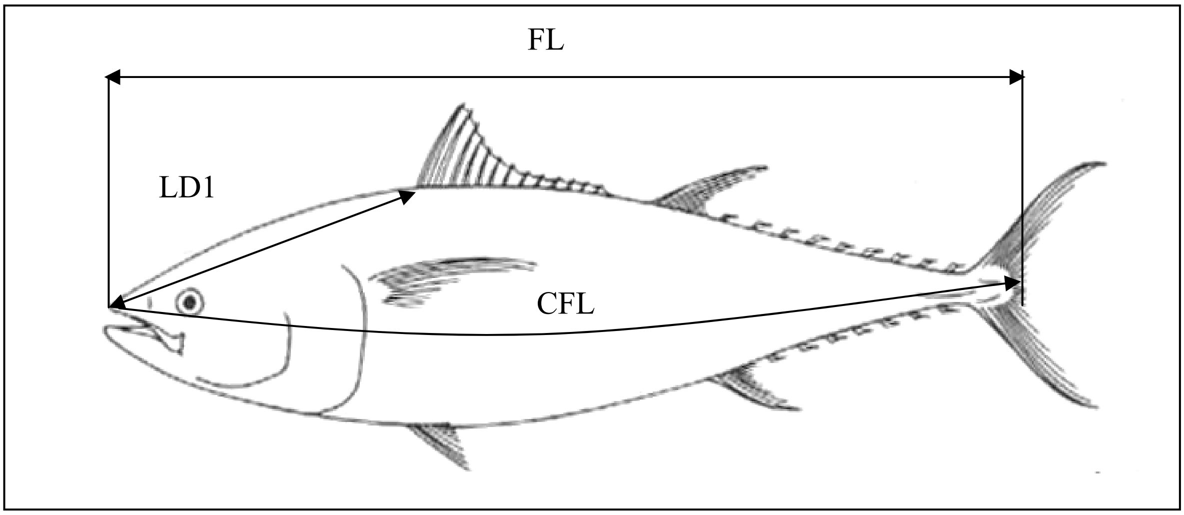

Figure 1. Types of measurements of bluefin tuna: Fork length (FL), First dorsal length (LD1), Curved fork length (CFL).

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

4.3 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.

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

5. Other data

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

6. 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 (see last page).

6.1 Collection of hard parts

6.1.1 Spine sampling

6.1.1.1 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**).

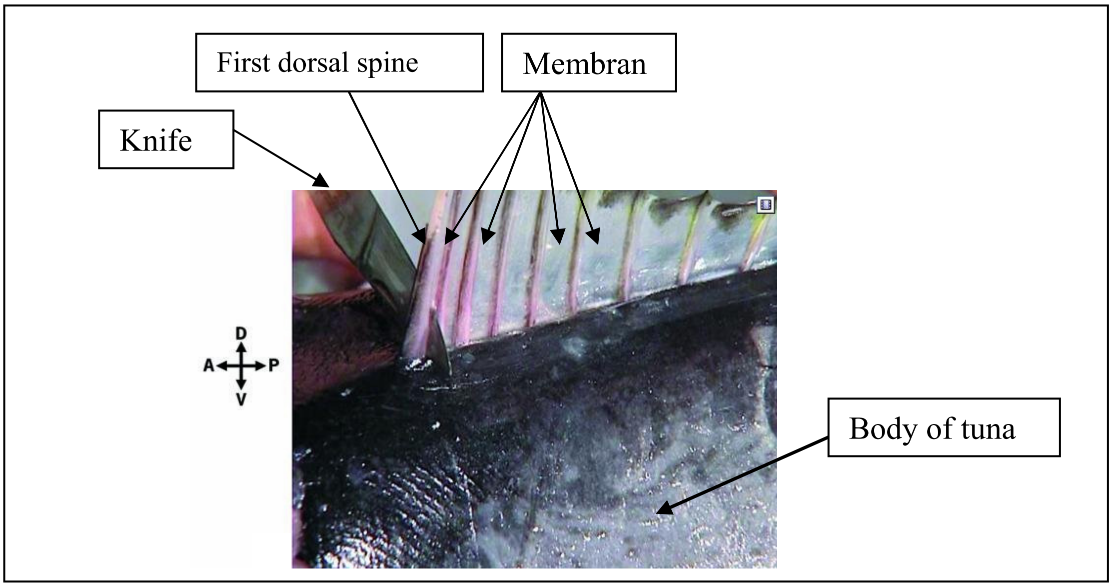

Figure 2. Insertion of the knife into the membrane separating the first two spines of the 1st dorsal fin . (Figure taken from Panfili *et al*., 2002).

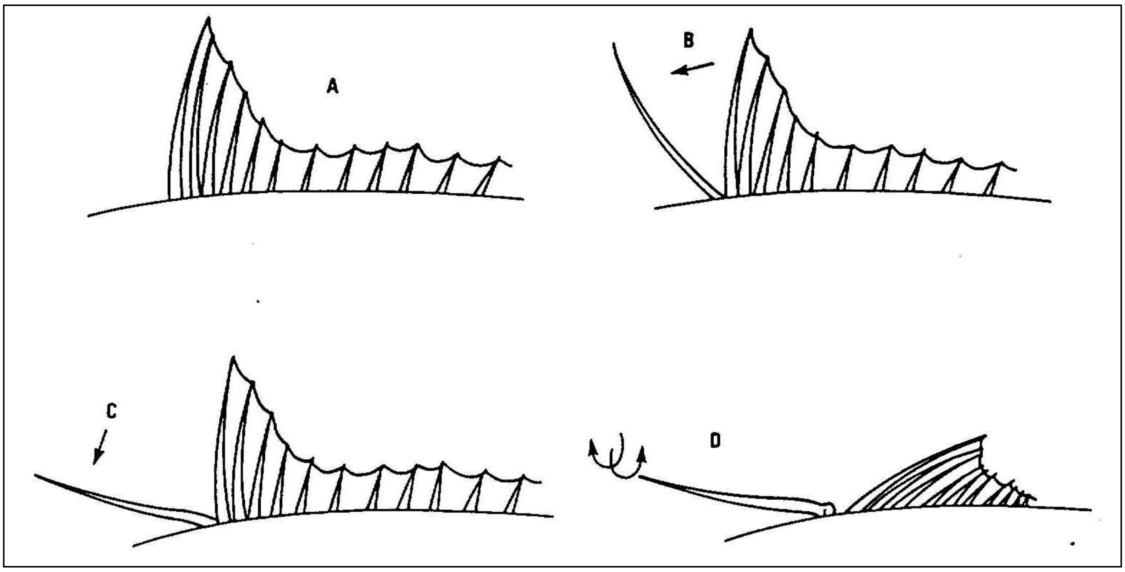

Figure 3. Technique of extraction of the first spine of the bluefin tuna dorsal fin. (Figures taken from Compeán-Jiménez, 1980).

6.1.1.2 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.

6.1.2 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 according to procedure described by Nichy and Berry (1976).

6.1.2.1 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.

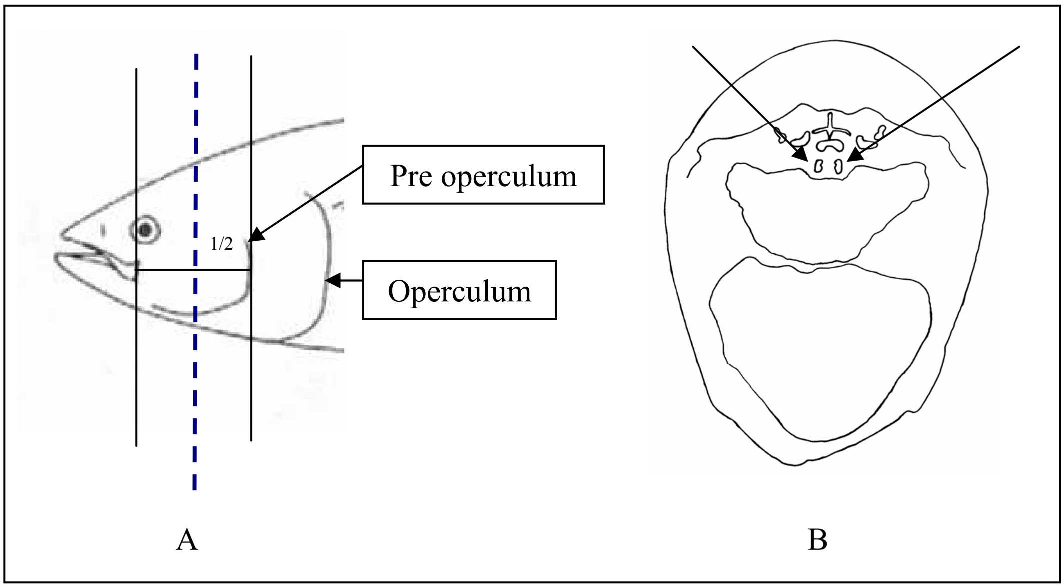

Figure 4. A. Tracing the imaginary line (dotted) along which to make the cut. B. View of the cavities where the pair of otoliths are found in the back of the head.

6.1.2.2 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.

6.1.3 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.

6.1.3.1 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.

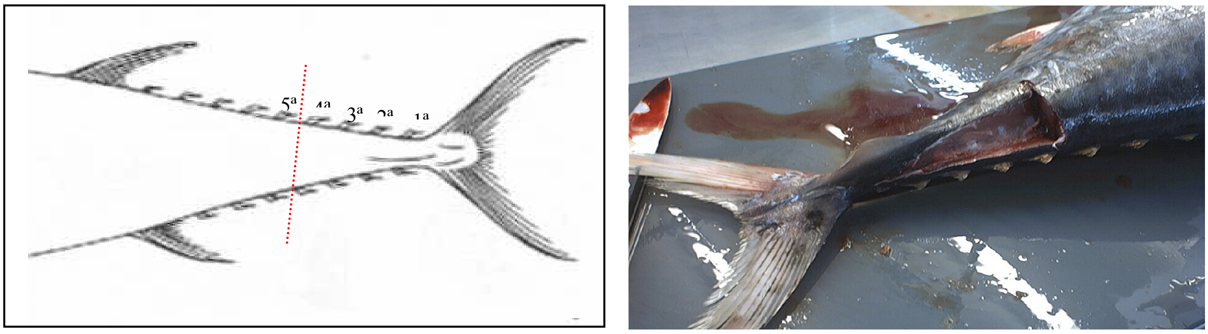

Figure 5. Cutting line to find vertebra 35. The photograph shows the transversal cut and the tail has been peeled to uncover the vertebrae (white marks).

6.1.3.2 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.

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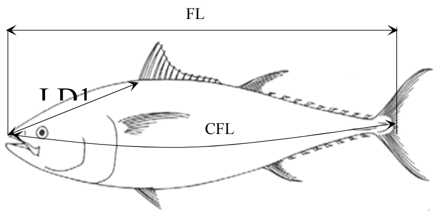
SAMPLING **Atlantic Bluefin Tuna** (*Thunnus thynnus*)

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: ________________________________