SCRS/2006/157 Col. Vol. Sci. Pap. ICCAT, 60(4): 1349-1392 (2007)

REPORT OF THE 2006 ICCAT WORKSHOP FOR BLUEFIN TUNA DIRECT AGEING

(*Instituto Español de Oceanografía, Santander, Spain, 3-7 April 2006*)

E. Rodríguez-Marín[1], N. Clear[2], J.L. Cort[1], P. Megalofonou[3], J.D. Neilson[4], M. Neves dos Santos[5], D. Olafsdottir[6], C. Rodriguez-Cabello[1], M. Ruiz[1], J. Valeiras[1]

SUMMARY

This report provides the presentations, discussions and conclusions from the ICCAT bluefin tuna workshop for direct ageing held in Santander, Spain, in April 2006. The report summarizes the ageing criteria used in the past and the agreements on future age determination based on otoliths, vertebrae and spines. Advantages and disadvantages of each calcified structure for ageing and border interpretation were discussed. It was considered that bluefin tuna age interpretation becomes very difficult from age ten onwards using the whole vertebra and the spine sections methods, but this last technique continues to be useful for older ages. Otolith sections can be used for the whole age range. Participants agreed that none of these three structures could be excluded from routine ageing because otoliths are not easily available. Age estimations within the same structure and between different structures of the same specimen were compared for several readers. Better precision was found between spine readers compared to vertebra and otolith readers. Good age agreement was also achieved between readers of spines and vertebrae from the same bluefin for ages less than 12 years. Preliminary results from radiocarbon assays on otoliths were presented at the workshop and gave promising outcomes for bluefin tuna age validation. Also, these suggested that bluefin tuna can live longer than had previously been established and that a review is needed of the currently used asymptotic size and growth rate for both stocks. Another important contribution of the workshop was a manual for age interpretation.

RÉSUMÉ

Le présent rapport recueille les présentations, discussions et conclusions de l'Atelier de l'ICCAT chargé de la détermination directe de l'âge du thon rouge, tenu à Santander (Espagne) au mois d'avril 2006. Le rapport résume les critères employés par le passé pour interpréter l'âge et les accords pour la détermination future de l'âge à partir des otolithes, vertèbres et épines. L'Atelier a discuté des avantages et des inconvénients de chaque structure calcifiée pour déterminer l'âge et l'interprétation du type de bord. On a abordé la difficulté de l'interprétation de l'âge des thons de plus de 10 ans au moyen de la vertèbre entière et des sections des épines, bien que cette dernière méthode continue d'être utile pour les âges avancés. Les sections d'otolithes peuvent être employées pour toute la gamme d'âges. Les participants ont convenu qu'aucune de ces trois structures ne doit être exclue pour l'interprétation de l'âge parce qu'il n'est pas toujours possible d'obtenir des otolithes. On a comparé les lectures de l'âge à l'intérieur de la même structure et entre différentes structures du même exemplaire pour divers lecteurs. On a obtenu une plus grande précision parmi les lecteurs d'épines que parmi les lecteurs de vertèbres et d'otolithes. On a également obtenu un bon accord entre les lecteurs d'épines et de vertèbres originaires du même exemplaire pour les âges inférieurs à 12 ans. Les résultats préliminaires des essais de radiocarbone dans les otolithes ont été présentés à l'Atelier, offrant de bonnes perspectives pour son utilisation dans

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[1] Instituto Español de Oceanografía, Ministerio Educación y Ciencia, Apdo. 240, 39080 Santander, Spain; rodriguez.marin@st.ieo.es

[2] CSIRO Marine Research and Atmospheric Research, P.O. Box 1538, Hobart, Tasmania, Australia 7000.

[3] Dep. of Zoology-Marine Biology, Faculty of Biology, University of Athens. 157 84 Panepistimiopolis, Ilisia, Athens, Greece.

[4] Fisheries and Oceans Canada, 531 Brandy Cove Road, E5B 2L9, St. Andrews, New Brunswick, Canada.

[5] Instituto de Investigaçao das Pescas e do Mar, Centro Regional de Investigaçao Pesqueira do Sul, Av. 5 Outubro s/n, 8700-305, Olhao, Faro, Portugal.

[6] Marine Research Institute, Skúlagata, 4, P.O. Box 1390, IS-121 Reykjavik, Iceland.

la validation de l'âge. Ces résultats indiquent aussi que le thon rouge a une plus grande longévité que ce qui avait été auparavant établi et qu'il est nécessaire de réviser la longueur asymptotique et le taux de croissance actuellement utilisés. L'élaboration d'un manuel aux fins de l'interprétation de l'âge a constitué une autre contribution importante de l'Atelier.

RESUMEN

Este informe recoge las presentaciones, discusiones y conclusiones del congreso de ICCAT para la determinación directa de la edad de atún rojo, celebrado en Santander, España, en abril de 2006. El informe resume los criterios empleados en el pasado para interpretar la edad y los acuerdos para la determinación futura de la edad a partir de otolitos, vértebras y espinas. Se discutieron las ventajas y los inconvenientes de cada estructura calcificada para determinar la edad y la interpretación del tipo borde. Se planteó la dificultad en la interpretación de la edad de atunes mayores de 10 años utilizando la vértebra entera y las secciones de espinas, no obstante este último método continúa siendo útil para edades mayores. Las secciones de otolitos pueden ser empleadas para todo el rango de edades. Los participantes acordaron que ninguna de estas tres estructuras deben excluirse para la interpretación de la edad porque no siempre es posible obtener los otolitos. Se compararon las lecturas de edad dentro de la misma estructura y entre diferentes estructuras del mismo ejemplar para varios lectores. Se obtuvo una mayor precisión entre lectores de espinas comparada con las obtenidas por los lectores de vértebras y otolitos. También se obtuvo un buen acuerdo entre lectores de espinas y vértebras procedentes del mismo ejemplar para edades menores de 12 años. Los resultados preliminares de las pruebas de radiocarbono en otolitos fueron presentados en el congreso, proporcionando buenas expectativas para su uso en la validación de la edad. Estos resultados también indican que el atún rojo es más longevo de lo que se consideraba y que es necesaria una revisión de la longitud asintótica y de la tasa de crecimiento empleadas actualmente. Otra importante contribución del congreso fue la elaboración de un manual para la interpretación de la edad.

KEYWORDS

Bluefin tuna, age determination, ageing comparison,
validation,otoliths, vertebrae, spines

1. Opening of meeting

The Director of the Santander Centre of the Spanish Institute of Oceanography (IEO), Jose Luis Cort, opened the meeting. He welcomed participants and thanked Enrique Rodriguez-Marin for his initiative in establishing the workshop and for its organization. Dr. Cort stressed the importance of producing outcomes and recommendations that would be of use to the ICCAT committee.

The meeting was chaired by the ICCAT Direct Ageing Group Coordinator, Dr. Rodríguez-Marín. Some members of this Direct Ageing Group, such as S. Karakulak, D.H. Secor and V. Restrepo, were unable to attend this workshop due to prior engagements. The adopted Agenda and List of Participants are attached as **Appendix 1** and **Appendix 2**.

The following participants served as rapporteurs for various sections of the report:

Section	*Rapporteurs*
1, 2, 3	E. Rodríguez-Marín
4	N. Clear and J. Neilson
5	E. Rodríguez-Marín and C. Rodríguez-Cabello
6	C. Rodríguez-Cabello and J. Valeiras
7	E. Rodríguez-Marín and J. Neilson
8	J. Neilson and D. Olafsdottir
9, 10, 11	All participants

2. Introduction

The estimation of growth is an essential part of the stock assessment of bluefin tuna (*Thunnus thynnus*), since age-structured models such as Virtual Population Analysis have been used to assess bluefin tuna stocks (Anon., 2003a). Age distribution of the catch is obtained by applying age slicing to length distributions of catches, a method based on the use of two growth curves, one for each stock. This method introduces uncertainties in the catch at age matrix because it fails to consider differences in the relative proportion of year classes in the catch and growth differences over time. Other key life-history parameters such as natural mortality, age at maturity and maximum age also need accurate growth estimation. Although these "growth uncertainties" exist, the Standing Committee for Research and Statistics (SCRS) of ICCAT is now more concerned about the quality of basic fisheries statistics for the east Atlantic and Mediterranean bluefin tuna stock. Catch misreporting and poor size sampling have been identified as the main difficulties in constructing the catch at size (age) matrix (Anon., 2005a; Anon., 2005b).

Two ageing workshops have been held in the past, one in 1977 especially for bluefin tuna, and another in 1982 for large pelagic fishes, including this species (Hunt *et al.,* 1978; Anon., 1983). Since then very few basic growth studies have been carried out. In 2004 a network on direct ageing was initiated following the recommendation of the Bluefin Year Program Working Group (BYP, Anon, 2003b). Some progress has been made since then, and the collaboration within the Direct Ageing Group and the exchange of calcified structures led the Group to realise that agreement on the reading criteria for different structures was needed (Rodriguez-Marin, 2005; Rodriguez-Marin *et al.,* 2005). The 2005 SCRS meeting agreed with the Group's research proposal to hold a workshop in 2006 to assess whether age determinations obtained from different structures offered consistent ages and to obtain a standardized protocol of direct ageing procedure (Anon., 2005b).

The Bluefin Year Program Working Group also agreed on the benefit of participation in the 2006 workshop of a scientist directly involved with age determinations for southern bluefin tuna (SBT, *Thunnus maccoyii*), given that the Commission for the Conservation of Southern Bluefin Tuna (CCSBT) have developed a common method of collection, preparation and interpretation of age structures that is currently used in southern bluefin tuna assessment. Dr. Clear, who plays a key role in CCSBT efforts to develop common ageing techniques and has authored a manual for age determination and a number of southern bluefin tuna age and growth publications, kindly accepted the invitation to participate in the workshop.

3. Objectives

The Direct Ageing Group acknowledged the following for the Workshop:

1. To establish age interpretation criteria for three calcified structures: otoliths, vertebrae and spines.
2. To compare age estimations from different calcified structures of the same bluefin tuna specimens.
3. To write a manual of the preparation of hard structures and age interpretation procedures.
4. To compare currently used growth curves of East and West stocks.
5. To discuss and evaluate validation approaches.

4. Current age interpretation criteria

In order to know the current age estimation methodologies used at various institutes, compare reading structures, introduce and train new readers, agree on reading criteria and border interpretation and, above all, to generate discussion among participants, a series of presentations were organized. Each presentation is summarized below.

4.1 Review of bluefin tuna direct ageing and growth. E. Rodríguez-Marín

This opening presentation summarizes techniques that have been used to estimate ages of bluefin tuna: calcified structures, modal analysis of length frequencies and tagging studies. Ageing studies began 30 years ago but most of the work was done 20 years ago. Growth curves obtained for the East stock have produced a maximum age of 20 years and are based on vertebrae and spines interpretation. For the West stock, ages of more than 30 years were estimated, based mainly on otoliths and vertebrae. When analysing all growth curves from both stocks together, small differences of less than one year were observed, and it is precisely the growth models obtained from tagging data on individuals from the west, Turner & Restrepo (1994) and Parrack & Phares (1979), that represent the upper and lower limits of the remaining growth curves **(Figure 4.1)**.

A review of previous direct ageing studies was presented for scales, otoliths, vertebra and spines, along with the advantages and disadvantages of each hard structure for age interpretation. General ageing interpretation criteria were summarized based on references. Sex and stock growth differences have been found between the eastern Atlantic & Mediterranean and the western Atlantic stocks.

Questions and discussion included: Whether or not to count the peripheral translucent band when present if the fish is caught in autumn. A criterion needs to be established: to count natural years or complete years of life.

A summary of growth parameters and mean length at age for eastern and western Atlantic bluefin tuna from different authors is presented in **Appendix 3**.

4.2 Ageing of southern bluefin tuna **Thunnus maccoyii** *N. Clear*

The author presented a summary of the research on direct ageing of southern bluefin tuna, which began in the mid-1990s. This included the justification for the study, methods researched using various hard structures, the results that otoliths produced the most accurate age estimates and validation to determine this and the implications of clarifying new parameters for life cycle, transferring the techniques to other CCSBT country scientists and application of the techniques developed (Anon., 2002a).

The formation of translucent zones in otoliths peaks in August, but may be formed throughout the southern winter, (May-September).

A participant asked what are some of the advantages and disadvantages of the bomb radiocarbon method? The author responded that one advantage is that the result provides an absolute birth date, not just time-at-liberty obtained from tagging studies. One disadvantage is that the method is most useful only for fish spawned in the 1950s and early 1960s, when the most rapid increase in radiocarbon occurred.

4.3 Bluefin tuna age interpretation from vertebrae. D. Olafsdottir

This presentation described the methods used to collect, prepare (clean, stain, rinse and dry) and read the vertebrae. The author recommends collecting the 35th and 36th vertebrae and storing them frozen by leaving the cones attached together until just before staining.

Challenges in estimating ages from the vertebrae include: 1) Interpreting the 1st ridges and grooves (deposited when fish were very young). 2) From around 8-10 years the ridges and grooves are deposited very close together, making them difficult to interpret.

A workshop participant asked what is the composition and significance of the ridges and grooves? The author replied that ridges form at the end of the growth period (mid-autumn) and grooves in the spring. Participants asked if it would be possible to estimate ages using vertebrae from small fish and otoliths in older fish (since otoliths are harder to collect)? The author responded that this might result in a "gap" in the age range. The possibility that marginal increment analysis could be a method of validation was discussed. It was noted that the fishery is limited to part of the year, making it impossible to collect samples throughout the year.

4.4 Bluefin tuna age interpretation from spines. C. Rodriguez-Cabello

The author discussed the collection, preparation and reading of bluefin tuna spines. She stated that the advantages of using spines rather than other calcified structures are that they are easy to collect, sampling does not damage the fish and hence is a cheaper method as there is no need to buy the fish. A diamond saw is used for sectioning. Spines are sectioned near the base just above the condyle. Sections cut further up the spine may produce underestimates of age.

Spines are viewed under transmitted light. The structures seen in spine sections can be complex, with double and triple rings present. Hyaline (slow growth) rings are formed during winter (November-March), possibly due to lower water temperature and migration to wintering areas. She also described the method used to overcome the problem of resorption of rings with age, because of the close relationship between fish size and spine diameter. Age estimates are calculated by adding the number of rings estimated to lie within the area of resorption (vascularised nucleus) and the number counted between the area of resorption and the margin.

Workshop participants noted that considering tagging as a method of validation, there is evidence that tagging may affect growth and thus confound the validation results. The effects of tagging are apparent in the decreased width of zones on fin spines. Participants then discussed factors that could potentially influence growth including temperature, food availability, and physiology. The Group noted that there is variation in the annual timing of slow/fast growth and formation of translucent/opaque /hyaline zones in each structure. It was suggested that it would be useful to document those discrepancies, perhaps starting by making a table listing the structures and the timing of band formation in each study.

4.5 Bluefin tuna age interpretation from otoliths. P. Megalofonou

The technique for estimating age in days from the otoliths of small fish involves sectioning and then immersion in methylbenzoate for three days. For larger fish, age is estimated by counting annuli in sagittal sections to 280 cm FL (an example shown was a 151 cm LCF fish, estimated to be seven years old). Translucent zones are thought to be deposited during periods of slow growth from December to May. Counts of the translucent zones are made rather than the opaque zones. In younger fish the opaque and translucent zones are apparent in the whole (unsectioned) otoliths. The oldest fish aged from a whole otolith was 270 cm LCF, estimated to be 10 years old. Identifying the 1st increments can be problematic, and examining the form of otoliths from very small fish can help. Megalofonou stated that the annual formation of increments in sagittae needs to be validated.

Workshop participants asked along which axis are increments counted on the whole otolith? The author responded that increments are easiest to read along the rostral and postrostral axes but can be followed all around the face of the otolith, from rostrum to postrostrum. She further noted that for sectioned otoliths, they are cut along a transverse line. The Group asked if spines are an option for age estimates to three years and then otoliths for older fish?

4.6 Spines marginal increment. J.L. Cort

Dr. Cort presented results of his studies of juvenile bluefin tuna (from the Cantabrian Sea) age and growth derived from the examination of dorsal fin spines. He noted that:

- Hyaline rings are winter rings, which are formed between fall and winter (October-March);
- Areas of active growth start forming in spring and conclude their formation in the fall (March-October);
- For younger aged bluefin (age-classes 1 to 3), summer growth is from 3 to 4 times faster in length and from 4.5 to 6 times more in weight than winter growth;
- Winter hyaline rings can be single (thin or thick), or double.

The average increase in length and weight for the different age classes of bluefin tuna in the Cantabrian Sea can be found in Table 8a from Cort (1991).

The Group noted that to examine the pattern of outer margin growth of calcified structures in greater detail, it is important to get samples from other areas and seasons. The Strait of Gibraltar winter fishery was mentioned as a specific example. It was also suggested that electronic tagging in conjunction with otolith studies may offer insight into the effects of fish movement on otolith growth patterns. There was a question of why double rings would not appear in otoliths as in spines. One workshop participant noted that perhaps the physiological pathways determining the growth of otoliths are different compared with other structures. The importance was noted of determining the comparability of the various structures being counted in spines, vertebrae and otoliths. A table indicating timing of formation of different structures was suggested. The question arose of what the mechanism is that governs ring formation. Some participants felt that determining the ultimate mechanism for ring formation was not as important as understanding the timing of formation. For example, the timing of ring formation may influence assignment of year class. Generally, the group agreed on the fundamental importance of studies dealing with periodicity of formation of structures.

4.7 Spine Section Interpretation: Types of Annuli and Growth by Season. J. Valeiras

The author presented examples of bluefin tuna fin spine sections illustrating double and triple rings. In some specimens, both double and triple rings can be observed within the same section. The presenter showed a specimen from the Strait of Gibraltar, caught in February, and the winter ring could be seen.

The Group discussed the process of assigning ages accounting for absorption of the central annuli. It was noted that the older the fish, the greater the source of this potential error. Nevertheless, for fisheries based on younger fish (such as the Spanish bluefin fishery described by Dr. Cort in an earlier presentation), this error may not be a great source of concern.

4.8 Age estimates and information for 17 United States giant bluefin tuna. D. Secor

The workshop reviewed the otolith photographs provided by D. Secor. The first annulus is also beyond the inflection point. Workshop participants commented that it is difficult to age from photographs, and that working with a live image is preferable as this allows the operator to vary lighting and focal point. It was noted that the first annulus can be found after the inflection point of the ventral limb of the otolith. After the second inflection in older animals, the appearance of the annuli becomes quite regular and counting becomes fairly routine.

4.9 General discussion on age interpretation criteria used until now

- It was suggested that it would be useful to have a table of the advantages and disadvantages of each method. For example, one advantage of using spines for age estimation is that they are the easiest structure to collect, but the uncertainty introduced by resorption is a disadvantage.
- Ideally, one structure would be used to estimate age throughout the whole size range, but this may not be possible due to sampling limitations. Nevertheless, the use of a second or even a third structure to estimate age in some sizes of fish would be a practical solution.
- It was suggested it might be possible to sample otoliths from the whole length range in the Mediterranean, possibly from the farms. The effects of potential growth changes due to captivity were discussed. Such captive fish are of limited use for validation studies, held for a number of months. But if so, fish introduced in cages would be better to use. What are the obligations of farms to research and monitoring? Farms must measure 100 fish for every 100t in the farm.
- A comment was made that sampling large numbers of fish for routine annual ageing is not the aim of our research. A good outcome would be two new growth curves updated using current direct age estimates.
- It was suggested that useful workshop outcomes should include:
    - A review of direct ageing techniques;
    - Updated growth curves for east and west bluefin tuna;
    - Recommendations about the best direct ageing method to produce accurate growth curves.
- It was agreed to include in the manual/report the following information:
    - A table of advantages and disadvantages of each direct ageing method
    - An estimate of the months when translucent and opaque zones form on each structure.

5. Agreement on future age interpretation criteria

5.1 Advantages and disadvantages of each calcified structure for ageing

The objective of this analysis of the pros and cons is neither to exclude nor favour one structure or another for routine ageing. This would be impractical, as sampling difficulties in certain fisheries, fish processing or fish markets would prevent from changing to another structure **(Table 5.1)**.

5.2 General assumptions, reading agreements and border band interpretation

Summary of general assumptions about bluefin tuna direct ageing interpretation based on otoliths, vertebrae and spines.

1) Different optical zones based on their relative translucency appear in all these calcified structures. Opaque zones are dark in transmitted light and bright in reflected light and inverse for translucent zones.

2) It is also assumed that adjacent translucent and opaque growth zones are laid down on an annual basis. One year's growth consists of an alternating translucent and opaque zone on otolith and spine sections, or alternating ridges and valleys (grooves) on the vertebral cone surface. (Mather & Schuck, 1960; Butler *et al*., 1977; Hurley & Iles, 1983).

3) Annual marks are not always a simple bipartite structure as described above. Several authors have described double, triple, paired, thin and thick translucent bands, which should not be counted as so many year rings (Sella, 1929; Berry *et al.*, 1977; Cort, 1991). These "false" translucent bands are closer together than normal and, if they coalesce at the edge, they can be counted as part of the same increment.

4) Multiple markings or checks have been related to metabolic changes associated with migrations or reproduction and have been reported as contributing to periods of slow growth and annulus formation in bluefin tuna (Tiews, 1963; Butler *et al.*, 1977; Compeán-Jiménez & Bard, 1983; Lee *et al.*, 1983; Cort, 1991).

5) In young fish, the opaque bands are much wider than translucent ones. As bluefin grows, the opaque zone becomes progressively narrower, whereas the translucent one remains approximately the same (Hunt *et al.*, 1978).

6) The existence of an important slowdown in growth rate in winter has been confirmed clearly for bluefin tuna juveniles (Mather &Schuck, 1960; Furnestin & Dardignac, 1962; Farrugio, 1980; Cort, 1991).

7) Translucent zones are laid down from November to May and opaque growth zones from June to October (Mather & Schuck, 1960; Butler *et al.*, 1977; Farrugio, 1980; Hurley & Iles, 1983, Lee *et* al., 1983; Cort 1991; Foreman, 1996).

8) June 1st is taken to be the date of birth in the East Atlantic, and one month earlier in the West Atlantic.

9) Age is estimated as the number of translucent bands observed and as they are laid down in late autumn and winter, the convention is to count natural years, which means counting winters (Butler *et al.*, 1977; Hurley & Iles, 1983).

10) An Atlantic bluefin tuna with a translucent band formed at the edge of the hard part and caught at the beginning of the year would be interpreted as having one year more, despite there still being five or six months before its true date of birth. Consequently, when the peripheral translucent band is present and the fish was caught in autumn, this band should not be considered as one year more (Mather & Schuck, 1960; Butler *et al.*, 1977; Hurley & Iles, 1983).

5.3 Reference collection

The Direct Ageing Group decided to create a collection of calcified structures that would serve as a reference set for bluefin tuna (*Thunnus thynnus*) age determination. This collection contains samples and preparations of different hard parts (otoliths, vertebrae and spines) from the same specimen. The reference collection can be found at the laboratory of the *Instituto Español de Oceanografía* in Santander, Spain.

6. Calcified structures ageing comparison

The main objective was to compare age estimations obtained from different calcified structures belonging to the same fish. Age comparisons among readers were also made, as well as discussion of criteria interpretation.

Although the Age Working Group had, from the beginning, encouraged all participants involved in the bluefin tuna fishery to join in and participate in collecting hard structures for age interpretation and exchanging information, not many samples obtained for this exercise were available at the time. Four countries provided samples for age comparisons: Canada, Iceland, Portugal and Spain **(Table 6.1)**.

6.1 Material and methods

Several collections of bluefin tuna calcified structures from different areas were available to the workshop. Nevertheless, at the time the workshop was held not all the samples were ready for age reading. For this reason two data sets were used: a) samples from Iceland (East Atlantic), which comprised spines and vertebrae b) samples from Canada (West Atlantic), which consisted of spines, vertebrae and otoliths.

The two data sets corresponded to large bluefin tuna since the fisheries where the samples came from are mainly based on these large fish. The Icelandic samples are 145 cm-270 cm in fish length and those of Canada are between 219 cm and 295 cm **(Figure 6.1)**.

Each hard structure was processed following the respective protocol (section 9. Manual). The spines from Canada were already cut when they arrived at the laboratory since the people in charge of their collection could not extract the whole spine from the fish due to the large size of specimens. For this reason the sectioning line could not be precisely determined (see protocol), however this was considered in the age interpretation.

Three groups were formed and each focussed on one of the three structures **(Table 6.2)**. The data analyses were done using the European Fish Ageing Network (EFAN) software (Eltink *et al.*, 2000). As the guidelines for age reading comparisons point out, the variation coefficient (CV) is much less age dependent than the standard deviation (STDEV) and the percentage of agreement, therefore, CV is a better index for precision in age reading (Eltink *et al.*, 2000) and was chosen for the age estimation comparison.

The mean coefficient of variation (CV):

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

where,
n = number of calcified structures (spines, vertebrae or otoliths)
R = number of readers for each calcified structured
Xij= the j value of age estimation for the calcified structured i
Xi= average age calculated for the calcified structured

6.2 Results

Comparison between ages estimated by several readers from the same structure

Table 6.3 shows the agreement between readers for each structure. The best agreement is found for the readers of spines. The comparison results from different readers are shown for Icelandic samples in **Figure 6.2** for spines and in **Figure 6.3** for vertebrae. In general low values of CV, indicating a good precision, were found for well-sampled ages. Canadian calcified structures comparisons were not plotted because of low number of samples.

Comparison between ages estimated by several readers from different structures of the same specimen

The comparison of vertebrae and spines age interpretation obtained from the Icelandic samples shows a good fit to a linear relationship between both age estimations, indicating good age agreement, particularly from 5 to 11 years old. For older ages more variability exists and spines appear to underestimate age (by 1 year) in relation to vertebrae age estimations **(Figure 6.4)**.

The comparison of the vertebrae and spines age estimations corresponding to the Canadian samples did not give a good fit to a linear relationship **(Figure 6.5)**. In general terms and according to the results, vertebrae underestimate age.

The relationship between Canadian bluefin otoliths and spines showed high variability without any trend **(Figure 6.6)**. Under 12 year-old fish, spines overestimate age with respect to otoliths and the opposite is true for specimens older than 12 years. The comparison between otoliths and vertebrae showed agreement until age 12, and beyond this age vertebrae gave consistently lower ages than otoliths **(Figure 6.7)**.

The comparison of the spines, vertebrae and otoliths from the Canadian samples is shown in **Figure 6.8**. Only the readings of the three most experience readers by structure are plotted.

6.3 Discussion

Data on age and growth of fishes are essential for understanding life characteristics of species (lifespan, age at recruitment, age at sexual maturity, mortality, migrations, etc.) and the study of population demographic structure and its dynamics. These are also one of the main inputs required in age-structured assessment models applied in fishery management.

Comparison of ages obtained from different calcified structures of the same fish do not validate age estimation, but provide a measure of agreement and give some indication of the degree of confidence that can be placed on the interpretations (Campana, 2001).

Although north Atlantic bluefin tuna (*Thunnus thynnus*) has been studied for many years, particularly regarding age and growth, there are not many research papers that compare age estimations from different hard structures (Mather *et al*., 1995; Clay, 1991). Several ageing studies have been based on otoliths, vertebrae and dorsal spines but only one compares the first two structures (Lee *et al*., 1983). According to this study vertebrae tend to underestimate age since the crowded banding at the edge of the centrum beyond age 9 to 10 years leads to unclear age interpretation. Previous analysis of comparisons between vertebrae and spines from the same specimen indicated that there was almost no bias between both structures in ages from 6 to 11 years, however a tendency to estimate one year less in vertebrae than in spines from age 10 was also observed (Rodriguez-Marin *et al*., 2005).

Age and growth of southern bluefin tuna (*Thunnus maccoyii*) has been thoroughly examined and comparisons of direct age estimations from several calcified structures have been performed (Anon, 2002a). The results in this report were rather similar. Age estimates of vertebrae and otoliths matched closely for the first 10 years of life. Nevertheless, in larger fish the counts diverged, otoliths consistently providing higher age estimates (Gunn *et al*., 2007).

Our results indicate that the highest agreement between readers is found between readers of spines, and that the lowest occurs between readers of otoliths, although the degree of readers' experience, ages analyzed and the number of samples used must be taken into account in these comparisons. Moreover, in the case of the readers of spines, these good results may be influenced by the age interpretation methodology, which forces the estimation of the number of rings resorpted.

The good fit between readers of the spines and vertebrae from the Icelandic samples for ages less than 12 years indicates that both structures may be used indistinctly for age determination of these specimens. The low agreement between age readers based on the different structures of the Canadian samples is affected by the following factors: the small number of samples, the advanced age of the specimens analyzed, which tends to increase variability in age estimation, and lastly the state of conservation of vertebrae, the dehydration of which prevented adequate staining and analysis.

Samples of old fish like these are much more difficult for age interpretations, however this workshop not only provided the opportunity to estimate the age of old fish from expert readers but also to compare results from different hard structures. These comparative studies must be continued, with increasing numbers of ages and samples.

7. Currently used east and west growth curves comparison

The growth curves currently used by the ICCAT Standing Committee on Research and Statistics (SCRS) in bluefin tuna assessments were estimated in the early 1990s independently for each bluefin tuna stock, by Cort (1991) for the eastern Atlantic and Mediterranean and by Turner and Restrepo (1994) for the western Atlantic stock. There are differences between these two growth curves, especially for older fish, with an increasing divergence that reaches one year of difference from age 8. The differences in growth between both stocks need to be reviewed to determine whether discrepancies are statistically significant.

During the Workshop no statistical comparison was made of the growth curves currently used for the western and eastern Atlantic stocks. This comparison was postponed until the bluefin tuna assessment meeting in June 2006, for further information see SCRS/2006/079 (Restrepo *et al*., 2006). Nevertheless, the matter was discussed, a summary of which appears below.

In the Western North Atlantic assessments two main growth studies, based on data from tag-recapture experiments, have been used: Parrack and Phares (1979) and Turner & Restrepo (1994). Both growth curves obtained by these authors represent lower and upper values of Cort's growth curve used for the Eastern bluefin tuna stock **(Figure 7.1)** The main difference between these tag-recapture studies is the number of large specimens (over 150 cm) included in the analysis, although the method for estimating the von Bertalanffy growth parameters and the length data set filtering are different.

These different results in the growth parameters show the importance of the length data of large specimens, of over 150 cm straight fork length (SFL), and reveals the scarce number of giant specimens, of over 250 cm SFL, used in the growth curves now used. The study by Hurley & Iles (1983), based on age interpretation by sex from otolith sections, in which large and giant specimens are well represented, the tangent of the resulting growth equation has a lesser slope than that obtained by Cort (1991) and Turner and Restrepo (1994), and therefore a lower asymptotic size and a greater growth rate **(Figure 7.2)**.

The preliminary tests using the bomb radiocarbon method, in which ages estimated by reading 5 otoliths coincide with the age estimated by the radiocarbon assays, support the results obtained by Hurley & Iles (1983), and indicate that asymptotic length may be lower than that now being used for both stocks **(Figure 7.2)**. For further information see Doc SCRS/2006/077 (Neilson & Campana, 2006).

8. Validation approaches

Many attempts have been made to estimate the age of bluefin tuna, and although some of them provide some age validation, none of them achieve this goal for all ages.

8.1 Bluefin tuna ageing validation. J. Neilson

Dr. Neilson introduced various means of validating age interpretation methods for bluefin tuna and discussed the advantages and disadvantages of each method.

Ways for validating age determination methods:

1) Release of marked fish of known age (mark-recapture case).
2) Age validation using chemically tagged fish (number of increments formed between chemical tagging and time at recapture enable interpretation of annuli. Environmental events such as “El Niño” that may cause permanent marking in fish can also be used for the same purpose).
3) Modal analyses (length frequency in populations used to reflect age frequency. Works best for young ages and in fast growing species).
4) Passage of strong or weak year classes (Follow a cohort in the catch over a longer time).
5) Marginal increment analyses (studying the consistency/seasonality in formation of increments).
6) Bomb radiocarbon (Radiocarbon ratio levels in otolith cores compared to known levels in the oceans at each time since radiotesting in the 50’s and 60’s took place. Works best for fish born before 1965).
7) Captive rearing.

Dr. Neilson noted that the ICCAT approach for growth in western bluefin tuna is based on analyses of mark-recapture information (Turner & Restrepo, 1994). The data used in fitting the model was mainly from fish smaller than 150 cm, which may result in L_∞ not being well estimated. Secondly, the model is based on data obtained prior to 1980, and it is unclear if the historic growth patterns still apply.

A pilot validation of age determinations based on otolith readings was completed by Campana and Neilson. Initial trials for radiocarbon assays were performed on Western Atlantic bluefin sampled in 1976. Direct age readings were based on counting of growth increments in otoliths and radiocarbon ratios were measured in otolith cores. The results appear to indicate a lower L_∞ than predicted using the Turner & Restrepo (1994) model. Dr Neilson stressed that these are preliminary results involving five otoliths at this point, and more work is required to examine otoliths of younger fish during the period of rapidly increasing radiocarbon. This material is available, and the authors will be seeking funding to extend the work.

8.2 Age and growth of **Thunnus maccoyii** *southern bluefin tuna, N. Clear*

Dr. Clear described how bomb radiocarbon, tagging and mark-recapture techniques have been used for validating age interpretation methods. She discussed the advantages and disadvantages of these techniques and their application to studies of southern bluefin tuna: During 1990-1996, 20,204 southern bluefin tuna were tagged and injected with strontium chloride. Strontium marks on otoliths revealed that increments form annually in fish aged 1 to 6. The increments beyond age 6 will be validated if more otoliths can be recovered from older fish.

Conventional tagging of young southern bluefin tuna in the Great Australian Bight has provided information on growth and movements of the fish between release and recapture dates. Archival tags that collect information on sea and body temperature, pressure (depth) and light (geographic location) have been deployed in 600 fish off southern Australia since 1994. Data from recaptured tags have so far provided detailed information on the behaviour and physiology of southern bluefin tuna. The information collected in the tags has shown that most fish migrate from the tagging location into the Indian Ocean during winter and that few remain in the tagging area. The data also show that large-scale cyclical migration is common in 2-5 year old fish. Information on daily diving profiles shows that juvenile southern bluefin tuna make short feeding dives at dawn while on the shelf but in the Indian Ocean feeding events are more extended, e.g. spending 9 hours at around 300 m, where the water temperature is 9ºC.

Feeding events can be identified in plots of visceral temperature. Visceral temperature increases as digestion begins and remains above basal core temperature throughout the digestion process, producing a characteristic "feeding" curve. It appears that the fish may go for days to weeks without feeding or may also feed for a sequence of a few days and then not feed for an extended time. Sudden shifts in water temperature at constant depth are observed in the tag data and indicate site fidelity around water temperature fronts.

8.3 General discussion and future

In the discussion following the presentation, Neilson assumed that ageing based on radiocarbon measurements may give a precision of about 3-4 years. Naomi Clear noted that the accuracy may be even larger for fish born around 1955-1965, when C^{14} in the worlds' oceans increased rapidly. There was a question of how one might differentiate a radiocarbon value on the right or left hand limb of the calibration curve. Neilson responded that the interannual values change at different rates, and an analysis of covariance may allow correct assignment.

The group also considered that validation using otoliths might support age inferences from other hard parts. This is particularly true if hard parts are available from the same specimens. Unfortunately, otoliths are only available in the Canadian archived samples.

The work involving radiocarbon validation of western bluefin tuna should continue and be expanded to include a broader range of year classes, including the period of rapidly increasing radiocarbon in the 1960s. Also, scientists from ICCAT member countries should identify the presence of archived otolith collections that could be used for radiocarbon investigations in eastern bluefin tuna.

9. Manual for hard structures preparation and age interpretation procedures for bluefin tuna (*Thunnus thynnus*)

In this section a detailed description on the preparation of calcified structures of bluefin tuna and age interpretation from them is presented. The sampling protocol has already been described in Ruiz *et al.* (2005).

9.1 Manual for direct ageing of bluefin tuna using vertebrae

9.1.1 Sampling and preparation of vertebrae

The protocol of sampling and preparing vertebrae for age reading is based on the procedure given by Berry *et al.* (1977).

Sampling

Two vertebrae (35th and 36th) are collected from each fish (see Ruiz *et al*., 2004). Skin and flesh is removed from vertebrae as fully as possible. The vertebrae are not separated and are stored attached in the freezer **(Figure 9.1.1)**. It is important that the vertebrae do not dry before staining. If the staining procedure can be performed within 48 hrs post mortem the vertebrae may be stored in a cool environment.

Preparation

Vertebrae are thawed at room temperature. Remaining flesh is removed with a knife as thoroughly as possible. The two vertebrae are separated and care must be taken not to damage the connective tissue on the conical edge of the vertebrae. The jelly in the cone is removed and the vertebrae are rinsed in running tap water. The vertebrae are dried with tissue and laid in the dyeing solution (Figure 9.1.1).

Staining

The vertebrae are soaked in stain solution (see stain solutions) for 2-5 hrs. The required time for sufficient staining increases with the size of the vertebrae. The time of staining may be adjusted by interchanging the proportions of the alizarin stock solution and glyzerin in the stain solution.

Note, the stain solution can be used twice but that will at least double the staining time and may give poorer staining results. If vertebra becomes too dark it may be distained in base solution diluted by three parts distilled water for 10 to 20 hrs. After the staining the vertebrae are rinsed in running tab water for 2-20 min and then dried with paper and placed on a piece of paper at room temperature until fully dry.

Stain solutions

Alizarin stock solution (1,5 l)

1) 6,2 g alizarin red (S) is mixed in 100ml distilled water and 100ml concentrated acetic acid and let settle for approximately 20 min.
2) The mixture is added slowly to 300ml glycerol and 1000ml distilled water.
3) Store the solution in opaque, airtight container for up to 2 months.

Base solution (4.3 l)
645 g KOH diluted in 4300ml distilled water or 460 g NaOH diluted in 4300ml distilled water

Stain solution (5 l)
4300 ml base solution
500 ml glycerol
200 ml alizarin stock solution

Storage
The stained vertebrae are stored dry at room temperature.

9.1.2 Age interpretation from whole vertebrae

(Largely based on Berry *et al.*, 1977)

Terms used
Focal side side facing the centre/focus of the vertebra cone
Distal side side facing the outer margin of the vertebra cone
Six features or structures are analysed*: ridges, grooves, stained rings, unstained rings* and *annuli.*

The annual increments in the vertebrae are believed to consist of one *ridge* and one *groove***.** The *ridges* **(Figure 9.1.2)** begin to form around the end of the feeding season in November. The ridge tops are probably formed around New Year and the fish age thus equals the number of ridges (based on birthdays on January 1.). Grooves are probably formed in spring and summer. The ridges are usually pronounced for the first 5 to 10 years. After that the interpretation of ridges may become quite difficult. In some specimens a fine line is located on the middle or slightly towards the distal side of the ridges **(Figure 9.1.3).** The ridge may be interrupted by a small groove **(Figure 9.1.4)**. These grooves are usually less pronounced than the true *grooves* **(Figure 9.1.2)** but may cause confusion in identifying the annuli.

In alizarin stained vertebrae *stained rings* are positioned on the focal side of the ridges and separated by *unstained rings* **(Figure 9.1.2)** positioned on the distal side of the ridges and the focal sides of the grooves. Early in the staining process multiple staining rings are formed. During staining the rings coalesce and form one broad stained ring (sometimes two) within each year's growth or *annuli.*

In some specimens multiple narrow within-year ridges and grooves or lines may be formed. These specimens may be difficult to read and the annuli must be interpreted by jointly studying all the features and structures mentioned above. For difficult specimens with irregular layers some patterns may best be observed in the lateral sides of the cones, especially in the posterior cone.

If difficulties arise in interpreting the first annuli it may help to measure the diameter of the first increments. The diameter of the distal edge of the ridge formed around the first New Year, which represents the first birthday, is about 8-10 mm in the 35th vertebra **(Figure 9.1.5)**.

In old fish age interpretation often tends to be quite difficult. At the age of 10 onwards the ridges become less pronounced and the grooves are usually very narrow. Counting annuli close to the distal margin in large vertebrae may often be easier in the posterior cone. If the surface of the dyed vertebrae is dry and the increments become blurred, a small amount of oil on the surface of the cone may help in making the features in these specimens more visible.

9.1.3 Age interpretation from vertebrae sections

This method is based on counting internal bands in vertebra sections. For a detailed description of the preparation of vertebra sections see the methodology of Prince *et al*., (1985). The internal bands are reabsorbed as the vertebra grows and only the most recently formed layers may be visible. To prepare a vertebra section a cut as thin as possible is made through the posterior - anterior axes of the vertebra centre. The section is mounted on a glass slide and polished to the thickness at which it becomes translucently visible in a stereomicroscope with transmitted light.

Positions of ridges are marked by pen on the slide by comparing them to the remaining pieces of the whole vertebra. Age is assigned by counting the number of ridges (pen marks on the slide) up to the ages when internal bands start to appear. From that point on, the count is continued by counting the internal bands.

The vertebra section method has been recommended for ageing large bluefin based on data from tags recaptured from giant tunas. On the other hand, the method appeared to underestimate the age of young fish (Prince *et al*., 1985). Proper validation of the method by comparison with other methods has not been performed.

9.2 Manual for direct ageing of bluefin tuna using spine sections

9.2.1 Sampling and preparation of spines

Sampling

The spine used for age interpretation is the first ray of the first dorsal spine. The best way to remove it from the fish is to spread the first dorsal fin and cut the membrane between the two first dorsal rays; then, pull it forward and down, cut the ray joint and turn it to the right and to the left, alternately to break the ligament. The spine must be pulled out whole from the base. For more details see Compeán-Jiménez (1980) and Ruiz *et al*. (2005). The ray is kept in an envelope on which the size, weight, date of capture and situation (latitude and longitude) are noted. If the spine is not going to be cut immediately, it is advisable to keep it in a cool place. It can be kept in a fridge or in a dry place at room temperature.

Preparation

The preparation of the spine cuts includes the following processes: cleaning the spine, making the cuts and mounting them.

Cleaning

Having been kept dry in paper envelopes, the connective tissue at the base of the spines has 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

Better discrimination of optical zones in the spine section is found if the spine is completely dry before cutting it, however keeping it in fresh air for some hours is sufficient.

The cross section position on the spine is very important as will be explained later. Previous literature (Compeán-Jiménez & Bard, 1983; Cort, 1990) describes the sectioning methodology but does not clearly establish the distance from the condyle spine base where the section has to be made. We propose a criterion to define the position of the cut, and thus standardise the methodology that has been used to date. **Figure 9.2.1** describes the methodology and spine structures used as a reference. The sectioning position is established by measuring the diameter (width from the anterior spine side **Figure 9.2.1c**) along an imaginary line that passes above the hollows present at the spine base. Then, taking half of this diameter measurement from the same imaginary line, we obtain the axis of the cut to be made **(Figure 9.2.1d)**.

The cuts are made using a low rotating diamond saw. Two consecutive cuts are made with a thickness of approximately 0.5 to 0.7 mm, to obtain a spine cross section. Two sections of the same spine should be done to ensure a good growth banding interpretation.

The spines can be cut individually or encasing several spines in a matrix of plastic resin in order to cut several spines at once **(Figure 9.2.2)**. The lubricant needed for cutting is a mixture of: 25% ISOMET special oil, 25% liquid soap and 50% water. The following materials are used for the preparation of resin plates: metallic moulds, polyester resin, black colouring, accelerator, spaghetti and Vaseline.

Mounting methodology

The sections cut are washed in ethanol at 70%. They are then mounted on labelled slides and covered with Eukitt, a highly transparent mounting resin, to fix them and clarify the calcified sections. A slide cover can be used for individual spines. The slides are labelled with the fish code and the date of capture **(Figure 9.2.3)**.

9.2.2 Age interpretation from spine sections

Age interpretation and measurement of the spine sections can be carried out with a profile projector or binocular lens with a micrometer or connected to an image digitiser.

In a cross sectional cut on the spine two types of zones are visible under transmitted light: a translucent or hyaline band and an opaque one. According to previous studies of this species in this area (Cort, 1990), translucent zones are interpreted as low fish growth while the opaque zones correspond to fast growth. Translucent bands are narrower and are assumed to be annually formed based on marginal increment analysis (Cort, 1991). Thus, the opaque zones will correspond to the active growth period, which for this species in the North East Atlantic would take place from June to October, while the translucent bands are formed between late autumn and winter. These bands are clearly visible in young bluefin tuna (1 to 5 years old), and as the fish gets older there is less contrast in growth increments between summer and winter growth.

We can distinguish three main different situations:

a) Fine and single rings: These indicate that individuals hardly grew during the winter season. From the beginning of the cold season until the fish returned to the active feeding areas (in spring and summer), growth slowed down considerably. (See **Figure 9.2.4)**.
b) Thick translucent rings: These indicate that the bluefin tuna underwent more intensive winter growth than in the previous case. (See **Figure 9.2.5)**.
c) Double ring: In some cases two thin translucent rings separated by an opaque band can be seen, this is called *doublet*. The first ring indicates the beginning of the cold season which may coincide with the outward migration from the Cantabrian Sea to the wintering areas. The opaque band that appears next (between the two rings that make the doublet) is the winter growth. Finally there is the second translucent ring, which can coincide with the return to the active feeding areas during the summer season. (See **Figure 9.2.6)**.
d) Three thin rings: also called *triplet.* The explanation of them is not easy however three translucent bands very thin and close appear, which indicates that all of them correspond to the winter growth period. (See **Figure 9.2.7)**.

In young individuals (up to three years old) it is easy to find all the hyaline and opaque bands formed on the spine. However, in fish over three years old, the central area of the spine begins to reabsorb and the bands consequently disappear. As the nucleus of the spine is resorpted, the translucent band diameters had to be used to assign an age to the first observed band. **Figure 9.2.8** shows a spine cross section and how to measure the translucent band diameters. Therefore, for those fish a reference table with translucent band diameters is needed to estimate the age of the first visible band **(Table 9.2.1)**. This technique is based on the correlation between fish length and its maximum spine diameter (Compeán-Jiménez & Bard, 1983; Rey and Cort, 1984; Rodriguez-Marin *et al.*, 2006). Once the age of the first visible band is determined, the rest of the bands are counted annually. This is the reason why the sectioning line location is so important, since spine diameter decreases from the base to the apical end.

Table 9.2.1 shows mean translucent band diameter values by age. This table is based on bluefin tuna samples from the Atlantic from 1990 to 2003. The first row indicates the mean value of ring diameter; the second row the number of rings measured in each year class and the third row the standard deviation. The top of the column (beg and end) indicates the diameter at the beginning and end of ring formation. Previous and similar values were obtained by Cort (1991) for the same area but for different years.

9.3 Manual for direct ageing of bluefin tuna using otoliths

The otoliths (or ear bones) of fish are small structures located in the semi-circular canals at the base of the brain. They are formed by the daily accretion of a layer of calcium carbonate bound within a protein matrix. In tunas, as in other teleost fish, there are 3 pairs of otoliths. The sagittal otoliths are the largest of the 3 pairs and, in bluefin tuna, are oblong with a pointed rostrum and a deep sulcus **(Figure 9.3.1).**

9.3.1 Sampling and preparation of otoliths

Otolith extraction

There are different ways of removing otoliths from the head, which basically depend on the size of the fish and whether the head can be separated from the body or not. One way is to cut the head with a saw or knife in a horizontal plane above the eyes. Remove the brain and locate the bony auditory capsules at the back of the cranium inside which the semi-circular canals encapsulating the otoliths will be found. Insert forceps into the bony capsules to extract the sagittal otoliths. They may come away separated from or partly inside the semicircular canals. The second technique to remove otoliths is to cut the head at a midpoint behind the eyes but before the gill rakes. More details of this technique can be found in Ruiz *et al.* (2005).

Cleaning, handling and storage of otoliths

The otoliths should be cleaned as soon as possible after extraction as it becomes more difficult after the surrounding tissue has dried. Air-dry the clean otoliths and store them in small plastic vials to protect them.

Preparation

Whole otoliths
Otoliths from juvenile fish, up to 5 or 7 years old, can generally be read whole. If the age estimation is made using whole otoliths, these should be burnt on a hot plate until they turn golden brown; the translucent zones will become more obvious than the opaque ones. View the burnt otoliths under a light microscope, with reflected light and a black background.

Sections of otoliths
Many laboratories prepare thin sections of otoliths for age estimation. The methods and materials used will vary slightly between laboratories but whatever method is used it is important that it results in a section along the correct plane. The ideal sectioning plane should pass through the central core or primordium to ensure that all annual marks are visible in the cross section **(Figure 9.3.1)** (Lee *et al.* 1983). A 700 micron thick section is considered optimum because it is thin enough for increments to be clear but thinner sections can reveal too much sub-annual detail, potentially causing confusion. As well as the section containing the primordium it can be useful to cut one or two sections on either side along the same plane. During routine ageing all the sections can be viewed and assessed while taking into account that the sections that do not pass through the primordium may provide less information.

Clean dry otoliths can be arranged in two or more columns of several otoliths and embedded in clear casting polyester resin (methyl-ethyl ketone peroxide is used as a hardening agent), ensuring that the primordial of the otoliths are aligned. The advantage of making these moulds or blocks of different sizes lies in the high number of cuts that can be achieved in one process. The usual tool for sectioning is a low-speed saw with a diamond-coated blade. The sections are then cleaned in water, rinsed with alcohol and dried. However, some researchers polish the cut sections with aluminium oxide (grit size 0.3 microns). Sections are then mounted on numbered microscope slides using polyester resin and covered with glass cover-slips. More details on this technique can be found in Anon. (2002b) and Gunn *et al.* (2007).

9.3.2. Age interpretations from otolith sections

Otolith sections are most often viewed under a compound, light microscope, with both transmitted and reflected light. An image analysis system attached to the microscope is a useful tool for otolith interpretation; it allows readers to save images of specimens, record their age estimates on the images and facilitates discussion of individual specimens among readers.

An annulus is a bipartite structure consisting of one light and one dark concentric band or zone. "Light" and "dark" refer to the appearance of translucent and opaque zones, respectively, under transmitted light. In some fish species the light and dark areas have been shown to be deposited annually, the result of changes in otolith growth rate and structure each year. In southern bluefin tuna (*Thunnus maccoyii*) narrow, translucent zones are deposited during periods of slow growth each year and in these areas there is more organic material incorporated into the otolith matrix. However, in Atlantic bluefin tuna, the temporal meaning of the structures has not been validated and further studies are recommended.

Before making an age estimate, readers should view the whole specimen. In both arms of the otolith section, the increments appear as alternating light and dark zones. The longer arm is used routinely for estimating age, but the shorter arm is often used to verify counts toward the terminal edge **(Figure 9.3.2)**. For some specimens the shorter arm can provide an age estimate when the reader is confident that increments are obvious throughout the arm. A light and dark band together is considered one year's growth but readers will usually count either the light or the dark band in each increment.

It can be useful to view the increments under a combination of transmitted and reflected light, but for a final age estimate only transmitted light should be used. The external margin of a section can be a guide to where increments occur: in some specimens the margin is scalloped, each indentation indicating an annulus. This may be the result of the cyclical change in otolith growth and structure.

The first annulus can be difficult to identify, and experience plays an important role in locating this feature. A wide dark area close to the primordium provides a reference for growth during the first year of life **(Figure 9.3.3)** and the end of the first annulus is always found after the first inflection **(Figure 9.3.2).** Counts of daily growth increments apparent in sections from very small fish can be used as a guide to the position of the first annulus. Measurements of the position of the 365th daily increment can be used as a reference line corresponding to the expected first year of growth **(Figure 9.3.4.)**

The appearance of the increments is not consistent along the long arm. The first 4 to 5 nearest to the primordium are difficult to interpret, as they are broad and diffuse **(Figure 3)**. On whole (unsectioned) otoliths the translucent zones in the first 4-5 increments are distinct, narrow bands, however. This is not the case in sectioned sagittae. After the first 4-5, the increments become more condensed and slightly easier to read. In otoliths from fish older than about 9 years the distal increments are usually regular, comprising a distinct light and dark zone that can often be traced from one edge of the section to the other.

Suggestions for training readers:

* Start with an experienced tuna otolith reader
* Read otoliths from a reference set
* Read the same otoliths several times, with a delay (several days) between readings. Calculate precision of estimates.
* For the 1st increments read one sagitta whole, one sectioned (or make an age estimate from the whole otolith before sectioning it).

The composition of otoliths is complex, as is obvious when examining sagittal sections, and there are often small checks within the annual growth that can confuse the reader. Generally, the opaque areas are wider than the translucent areas and appear darker under transmitted light. Nevertheless, for the first increments there is an area of transition between opaque and translucent growth. This can give the impression that opaque zones are narrower than the adjacent translucent zones and this effect can be more pronounced if the specimen is viewed under a combination of transmitted and reflected light.

Assigning ages is most difficult during the period when growth is rapid (ages 1-5). At this stage, when increments can be vague or diffuse, it is useful to use the scalloping or indentations on the margin of the ventral (long) arm to guide the placement of the annuli. In general, locating annuli during the first few years of life is sometimes ambiguous. Especially at this stage, having too thin a section may cause problems in age interpretations, as too much detail becomes apparent.

Analysis of the type of growth on the terminal edge can help in validating the annual formation of annuli. For fish caught in June in the Mediterranean, a complete translucent zone can be seen on the edge of the otoliths. Cort (1991) studied the timing of opaque and translucent growth on Atlantic bluefin tuna fin ray sections. He found that opaque areas start forming in spring and are fully formed in autumn (March-October), and that "hyaline rings are winter rings", which are formed between autumn and winter (October-March).

Although not optimal, age estimates can be made from the inspection of electronic images rather than viewing the specimen under a light microscope with an attached image analysis system. The images can be enhanced to increase contrast along the section. Example can be seen in **Figure 9.3.5**.

10. Conclusions

1) An agreement in reading criteria and peripheral translucent band interpretation was achieved.

2) Between reader agreement was higher in spines compared to vertebrae and otoliths. Precision in spines readings, described by coefficient of variation, was 7% (percent of agreement was more than 50%) and lower precision was found for the other two calcified structures, CV was 10% (40% agreement). These results should be revised for otoliths and spines since a bigger sampling is needed.

3) The good age agreement between readers of the spines and vertebrae from the same bluefin, for ages less than 12 years, indicates that both structures may be used indistinctly for age determination of these age ranges.

4) Bluefin tuna age interpretation becomes very difficult from age ten onwards using the whole vertebra method. This increasing difficulty also happens when using spine sections, but this method continues to be useful for older ages. Otolith sections can be used for the whole age range although some difficulties in age interpretation occur in the first five years of bluefin tuna life. Further research for the vertebra section age interpretation method is needed.

5) None of these three structures can be excluded for routine ageing because certain fisheries, fish processing or fish markets would prevent the sampling of some of them. This means that more ageing comparison studies are needed on the calcified structures of the same specimen.

6) Preliminary results from radiocarbon assays on otoliths indicate that bluefin tuna can live more years than previously established and may indicate that currently used asymptotic size and growth rate for both stocks need to be reviewed.

11. Recommendations

1) In order to obtain updated growth curves for east and west bluefin tuna is necessary to support cooperation and coordination for sampling following the established sampling protocol. Other methodologies such as tagging should be encouraged for growth studies.

2) Comparative ageing studies between calcified structures of the same specimen must be continued, with increasing numbers of ages and samples. Enlarge calcified structures sampling from same specimen is advisable.

3) Research involving radiocarbon validation of western bluefin tuna should continue, and be extended to include a broader range of year classes, including the 1960s during the period of rapidly increasing radiocarbon.

12. Acknowledgements

We would like to thank Jorge Landa for his useful comments and to Dave Secor, Valerie Chosson-Pampoulie and Stacey Paul for their collaboration.

Table 5.1 Pros and cons of different calcified structures for age interpretation of bluefin tuna.

	Scales	*Spines*	*Vertebrae*	*Otoliths*
Currently used	No. Used at first in ageing studies	Yes	Yes	Yes
Sampling	Cheap and easy not necessary to buy or process the fish.	Cheap and easy, not necessary to buy or process the fish.	Necessary to buy or process the fish.	Necessary to buy or process the fish.
Removal	Easy to extract but needs to be selected thin and round from special area.	Easy to extract but it is difficult to remove the whole spine from giants.	Easy to take the vertebrae	Difficult to extract, fragile and small size
Preparation of the structure for reading.	Easy, not time-consuming.	Easy, less time consuming compared with vertebrae and otoliths.	Time- consuming in cleaning process, separation and staining. It is important that vertebrae do not dry before staining.	Somewhat time-consuming in moulding and serial cutting. It is important to define the sectioning plane.
Type of preparation	Projected impressions of scales facilitate readings for specimens older than 5 years.	Important to define the cut axis. Transverse sections, no staining necessary	Whole vertebra or vertebra sections.	Transverse sections, no staining necessary
Resorption of the nucleus	No	From age 3 and reducing with age	Nucleus obscured (vascularized tissue in old specimens)	No
Recommended use, based in references	In first ages, up to 4 or 5 years	Up to about 10 years	Up to about 10 years	All ages. First 5 years difficult to interpret.
Relationship between size of hard part and length	Not known	Spine diameter fits a linear relationship, including giants (1,2).	Vertebral cone radius fits a linear relationship, including giants (3,4,5)	Otolith length fits a potential curve, breaking down for giants (5).
Collections available	No	Yes	No	Yes

(1) Compeán-Jiménez & Bard, 1983; (2) Cort, 1990; (3) Rodríguez-Roda, 1964; (4) Farrugio, 1980; (5) Lee *et al*., 1983. References in **Appendix 3**.

Table 6.1. Collections of calcified structures from same specimen.

Calcified structure	Origin collecion	year	number	Main size range (SFLcm)	sent to	number sent	number examined	size range examined (SFLcm)	remarks	Total for workshop comparison
Vertebrae	Iceland. Marine Research Institute	2001-2002-2003-2004		120-292	Origin Lab		142	120-292	42 more are being aged for widening age range	142
Spines				120-292	Spain IEO Santander	266	142	120-292	42 more are being aged for widening age range	142
Vertebrae	Spain, Instituto Español de Oceanografía	2000-2001-2002-2003-2004-2005	261	60-90	Iceland. Mar.Res.Ins.	60	?	60-90	most of them 1 and 2 years old, but also some age 3 and 4 (4 specimens)	?
Spines			261	60-90	Origin Lab		216	60-90		216
Otoliths			200	60-90	Origin Lab		?	60-90		?
Vertebrae	Canada. Fisheries and Oceans	2004-2005	54	190-290	Iceland. Mar.Res.Ins.	20	20	190-290	only 17 vert/spine from same specimen in 2005	17
Spines			48	190-290	Spain IEO Santander	48	48	190-290	only 12 otol/spine from same specimen in 2005	12
Otoliths			23	190-290	Australia CSIRO	18	18	190-290	only 15 otol/vert from same specimen 2004&05	15
Vertebrae	Portugal, IPIMAR - CRIPSul	Years?	150?	150-300?	Origin Lab				They were ready to be sent but we had no time for preparing and reading them	
Spines			150?	150-300?	Origin Lab					
Vertebrae	Turkey, Faculty of Fisheries - University of Istanbul	2003-?	18?	120-140?	Origin Lab					
Spines			115?	110-170?	Origin Lab					
Otoliths			22?	120-140?	Origin Lab					

Table 6.2. Participants and readers for age reading comparisons among different calcified structures (H= high, M= medium, L= low).

Name	Country	Institute	Reader ID	Calcified structure readings			Experience		
				Otoliths	Vertebra	Spines	Otoliths	Vertebra	Spines
Clear, Naomi	Australia	CSIRO	R1	x			H		
Cort, José Luis	Spain	IEO							H
Megalofonou, Persefoni	Greece	Atenas Univ.	R2	x			H		
Neilson, John D.	Canada	Fisher. & Oceans. Biol.I S	R3	x			L		
Neves dos Santos, Miguel	Portugal	IPIMAR - CRIPSul	R4			x			L
Olafsdottir, Droplaug	Iceland	Mar. Res. Institute	R5		x			H	
Rodríguez-Cabello, Cristina	Spain	IEO	R6		x	x		L	H
Rodríguez-Marin, Enrique	Spain	IEO	R7			x			H
Ruiz, Marta	Spain	IEO	R8		x	x		L	H
Valeiras Mota, Julio	Spain	IEO	R9		x	x		L	M
Choson, Valerie	Iceland	Mar. Res. Institute	R10		x			H	

Table 6.3. Summary of the results obtained from age structures and readers comparisons.

North-East Atlantic samples

Hard Structure	Nº readers	Agreement (%)	C.V. (%)	Bias	Nºsamples
Spines	4	64.9	6.6	0.06	151
Vertebrae	2	42.9	7.2	0.07	35

West Atlantic samples

Hard Structure	Nº readers	Agreement (%)	C.V. (%)	Bias	Nºsamples
Spines	5	52.7	7.4	0.11	22
Vertebrae	4	40.8	9.9	0.34	19
Otoliths	3	40.7	10.7	0.26	18

Table 9.2. Diameter measurements of the beginning (beg) and ending (end) of the translucent bands by age (mm). East Atlantic (without the Mediterranean) bluefin tuna spine samples from 1990 to 2003.

East Atlantic bluefin tuna	1		2		3		4		5		6		7		8		9		10	
	beg 1	end 1	beg 2	end 2	beg3	end3	beg4	end4	beg5	end5	beg6	end6	beg7	end7	beg8	end8	beg9	end9	beg10	end10
Mean	2.19	2.50	3.31	3.52	4.55	4.79	5.73	6.04	6.74	7.16	7.81	8.30	8.93	9.33	9.87	10.32	10.64	11.12	11.82	11.80
Number measured	112	184	236	282	127	168	92	121	64	100	50	72	35	58	24	37	9	12	3	4
Standard deviation	0.21	0.22	0.22	0.21	0.37	0.37	0.44	0.47	0.38	0.42	0.41	0.45	0.43	0.45	0.64	0.37	0.50	0.45	0.53	0.72
Confidence interval 95%	0.04	0.03	0.03	0.03	0.06	0.06	0.09	0.08	0.09	0.08	0.11	0.10	0.14	0.12	0.26	0.12	0.33	0.26	0.60	0.70
minimum interval value (95%)	2.15	2.47	3.28	3.49	4.49	4.73	5.64	5.95	6.65	7.08	7.69	8.19	8.79	9.22	9.61	10.20	10.31	10.87	11.22	11.09
maximum interval value (95%)	2.23	2.53	3.34	3.54	4.61	4.84	5.82	6.12	6.83	7.24	7.92	8.40	9.08	9.45	10.13	10.44	10.97	11.38	12.41	12.50

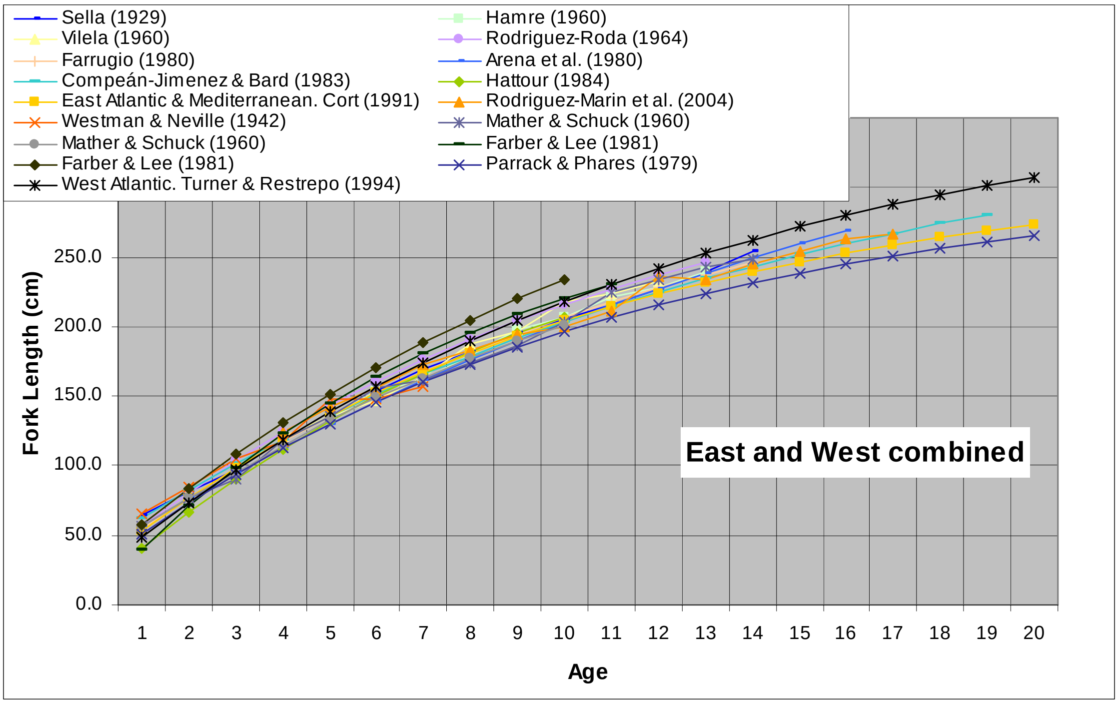

Figure 4.1. Growth curves from East and West Atlantic stock specimens obtained by several authors (references in Appendix).

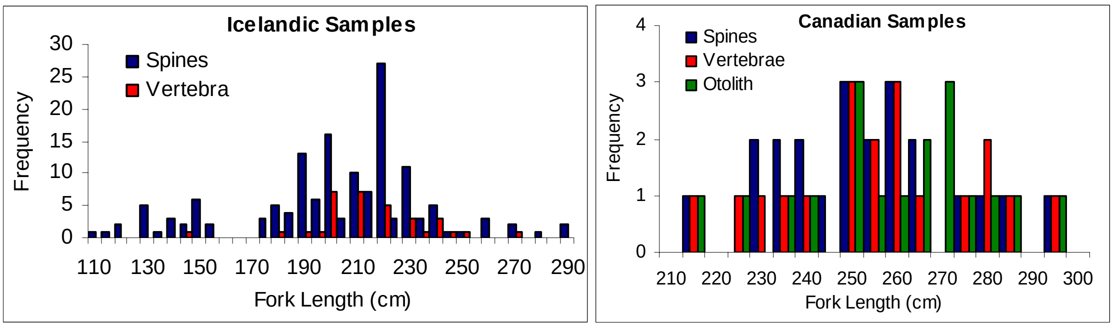

Figure 6.1. Length distributions of fish sampled (spines, vertebrae and otolith) for Iceland and Canadian fisheries.

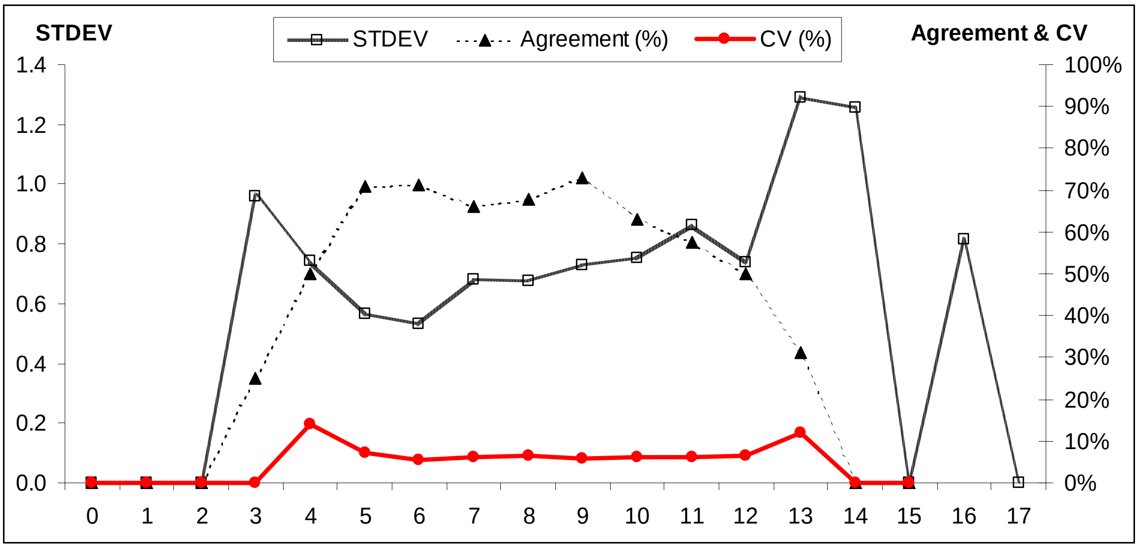

Figure 6.2. Age comparison between spine readers for Icelandic bluefin tuna samples.

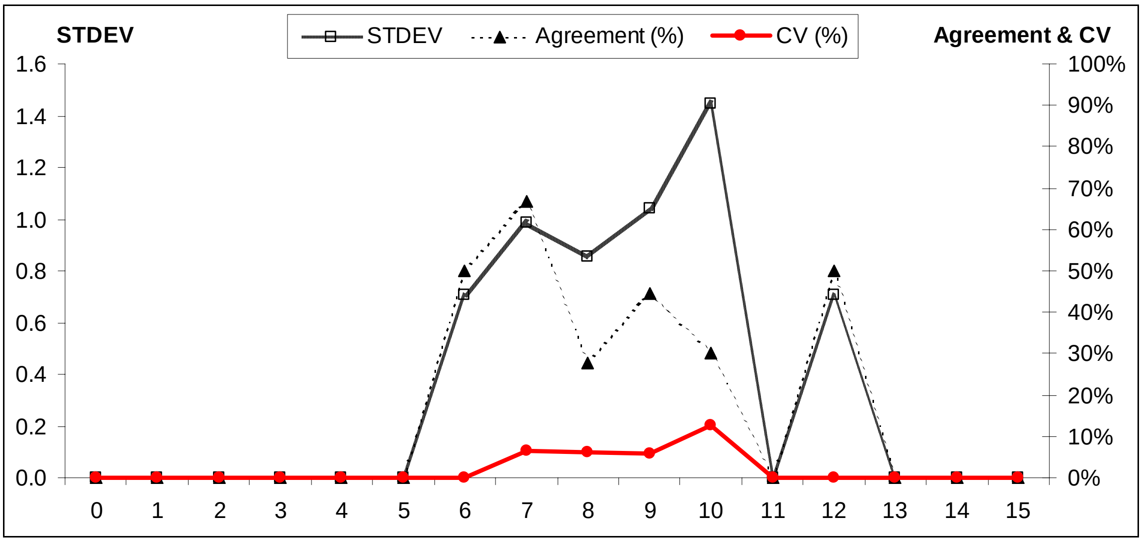

Figure 6.3. Age comparison between vertebrae readers for Icelandic bluefin tuna samples.

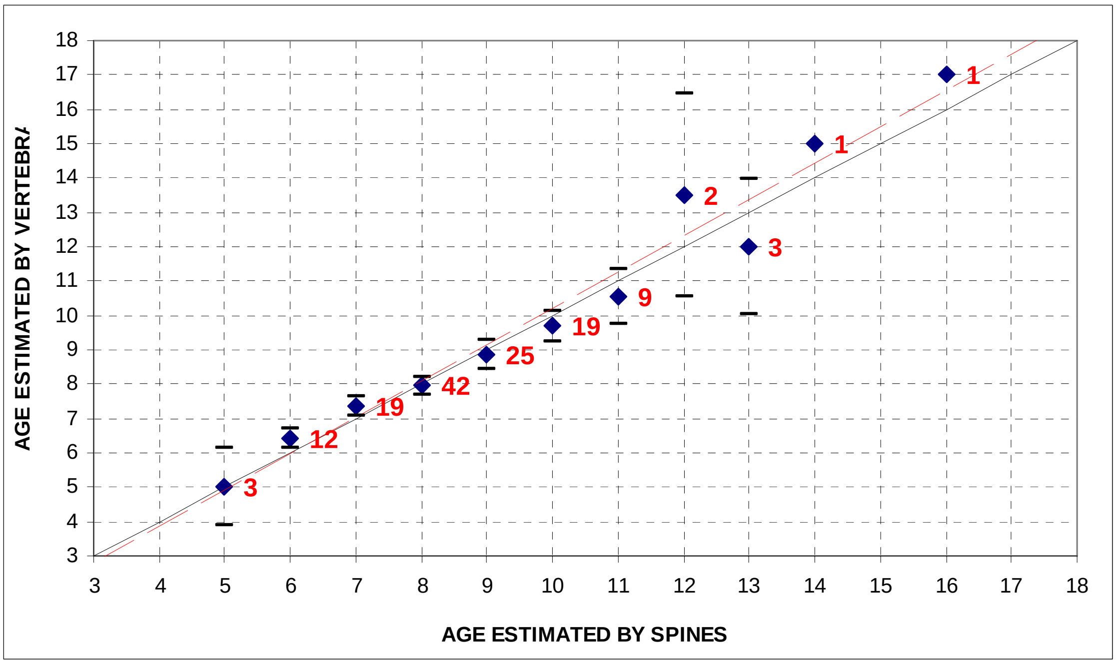

Figure 6.4. Bias comparison between vertebrae and spines from Icelandic samples. Vertebrae age readings are presented as the mean age and 95% confidence interval corresponding to spine consensus age readings (in red, number of calcified structures used).

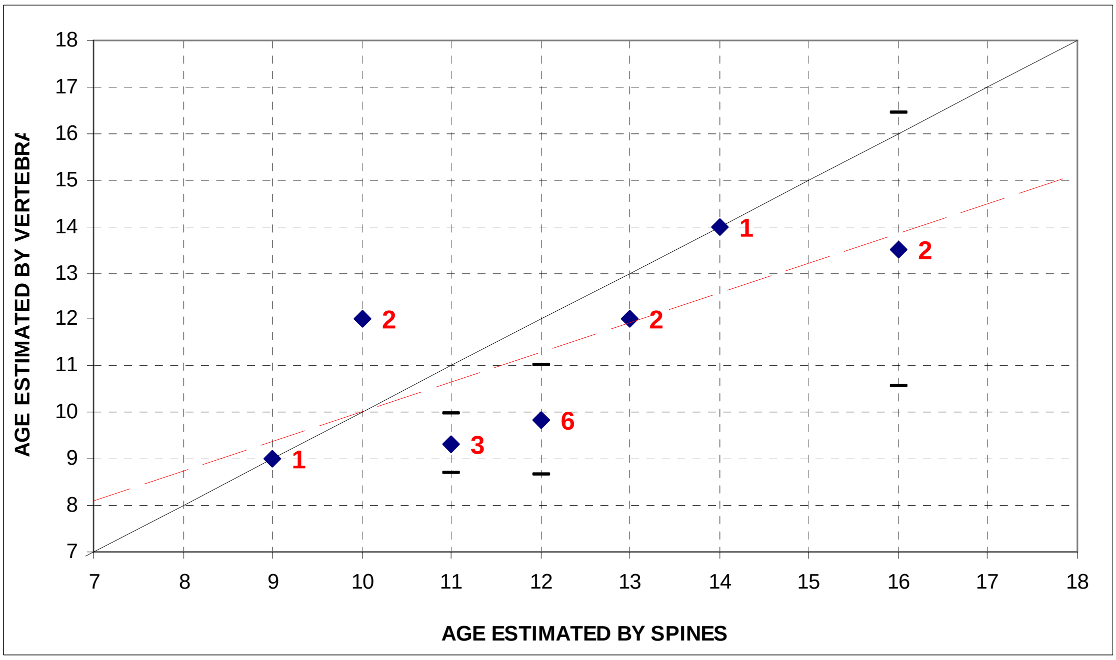

Figure 6.5. Bias comparison between vertebrae and spines from Canadian samples. Vertebrae age readings are presented as the mean age and 95% confidence interval corresponding to spine consensus age readings (in red, number of calcified structures used).

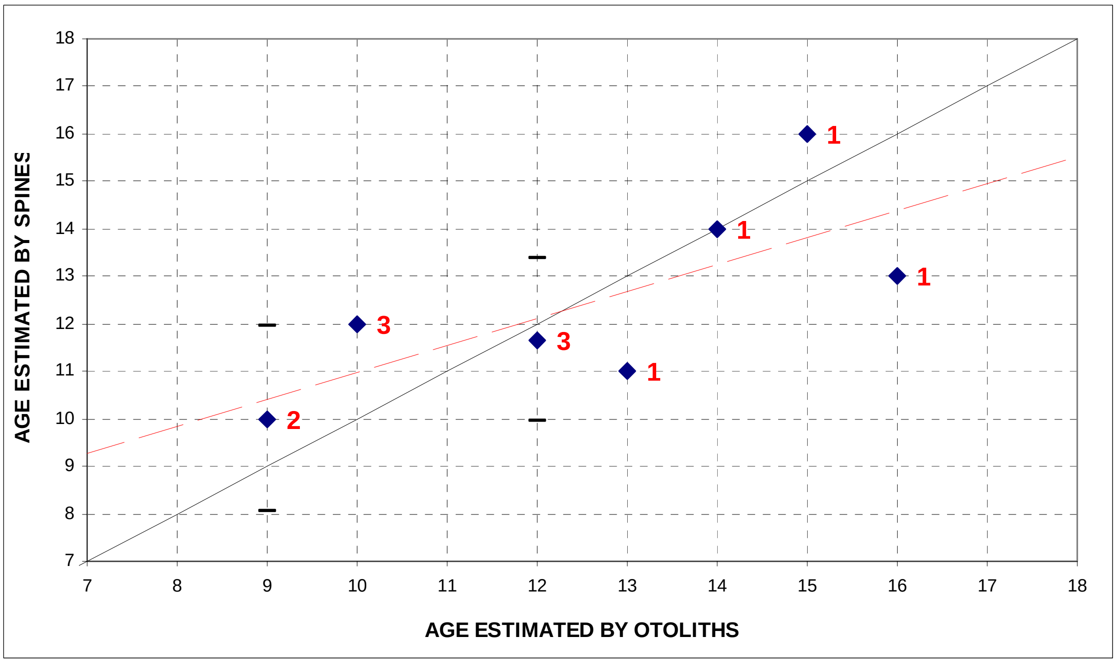

Figure 6.6. Bias comparison between spines and otoliths from Canadian samples. Spine age readings are presented as the mean age and 95% confidence interval corresponding to otolith consensus age readings (in red, number of calcified structures used).

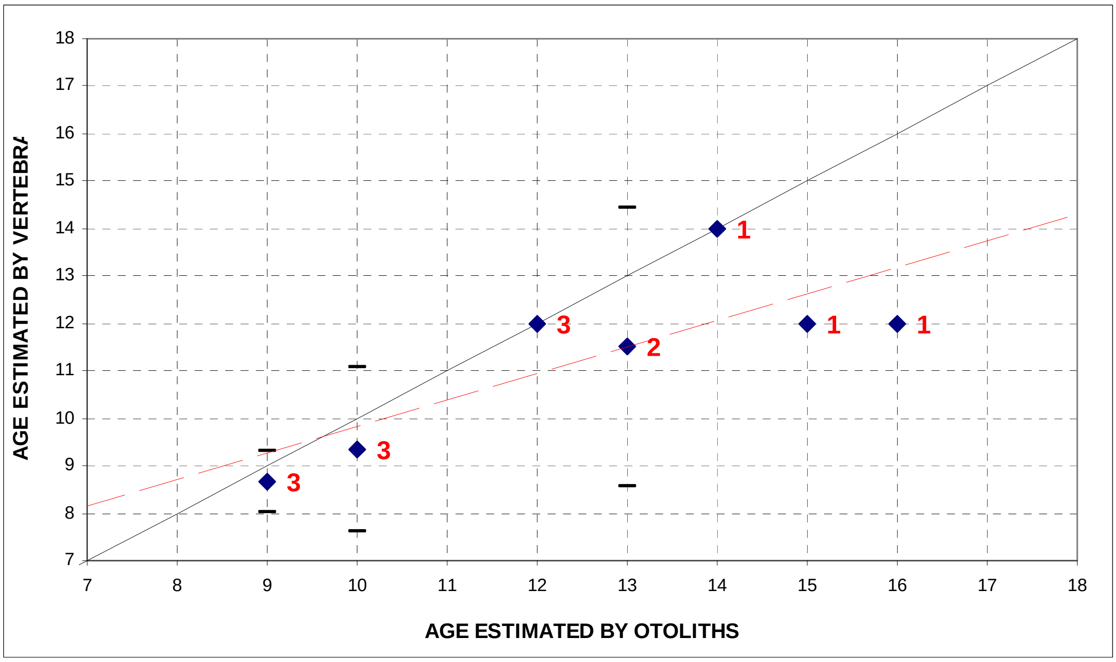

Figure 6.7. Bias comparison between vertebrae and otoliths from Canadian samples. Vertebrae age readings are presented as the mean age and 95% confidence interval corresponding to otolith consensus age readings (in red, number of calcified structures used).

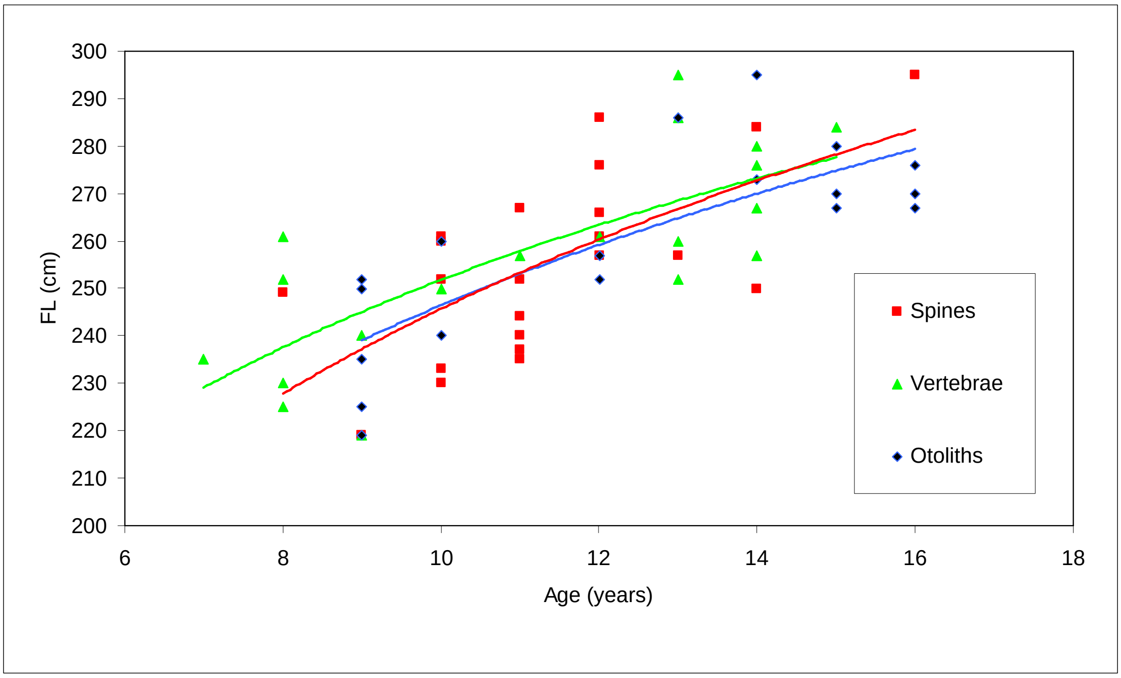

Figure 6.8. Bias comparison between spines, vertebrae and otoliths from Canadian samples (readings of the most experienced readers by each structure).

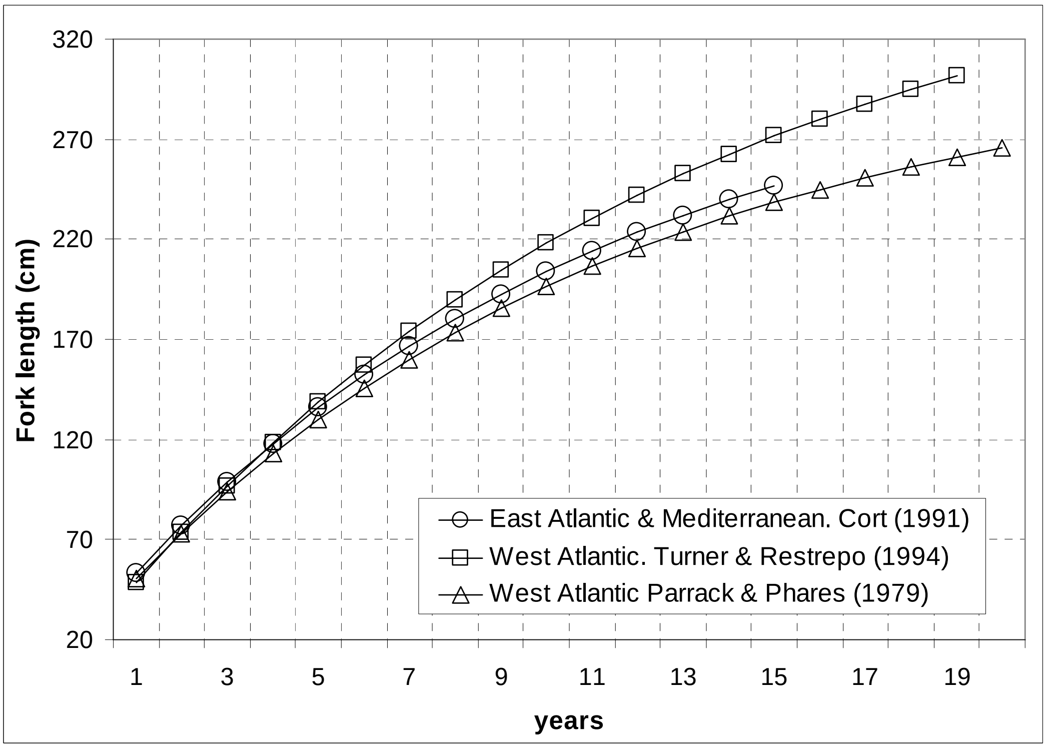

Figure 7.1. Past and currently growth curves used for east and west Atlantic stocks.

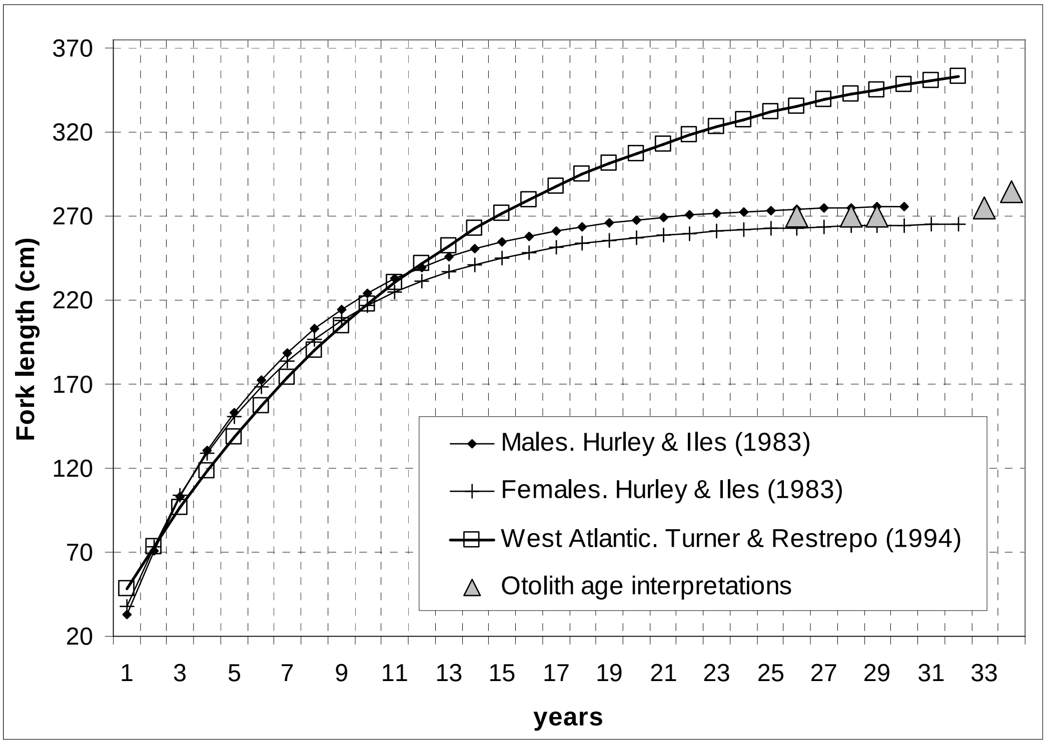

Figure 7.2. Growth curves by sex and combined currently used for West Atlantic bluefin tuna stock.

Figure 9.1.1. Joined 35th and 36th vertebrae, cleaning process, separating vertebrae and staining process.

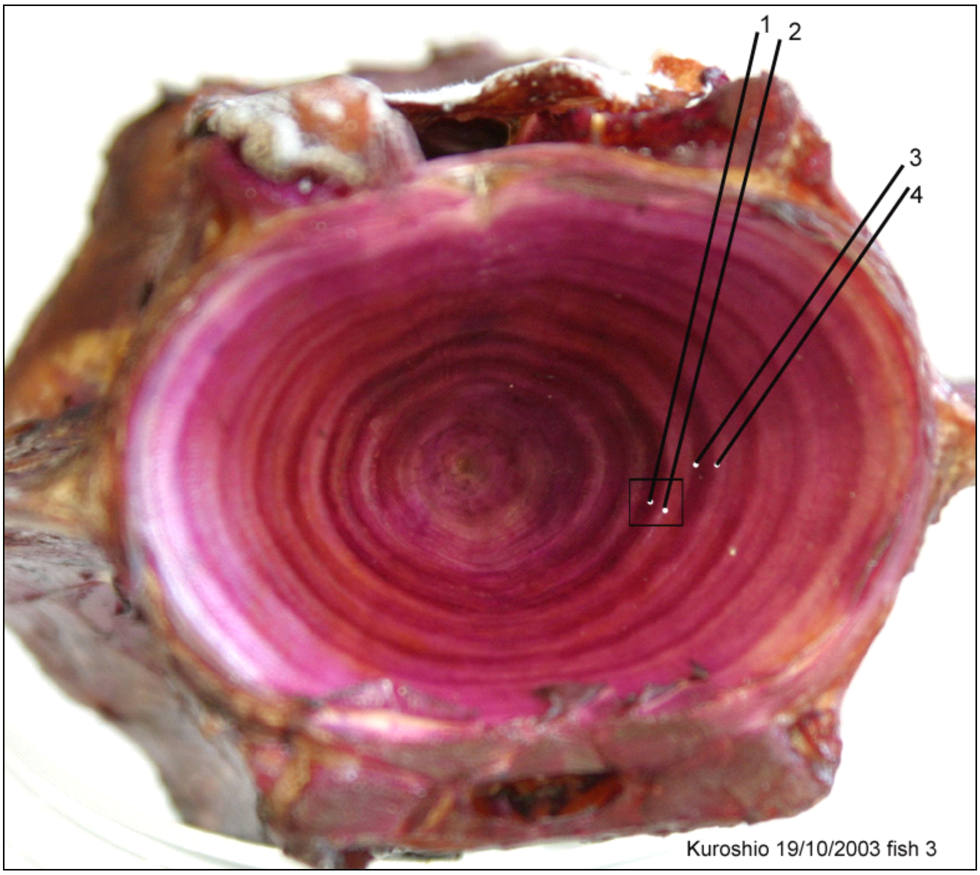

Figure 9.1.2. One year's growth is considered one groove (3) and one ridge (4). Stained rings are generally formed on the focal side of the ridge (1) and unstained on the distal side (2).

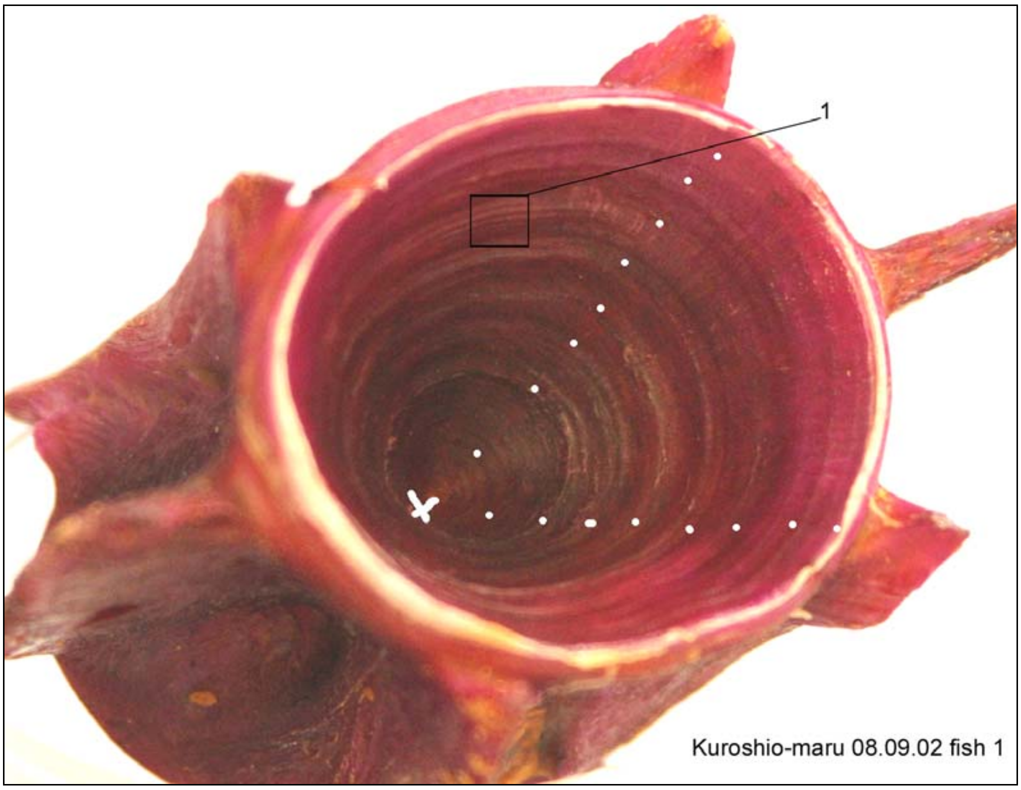

Figure 9.1.3. Fine lines (1), can be observed from the middle to the distal side of the ridge.

Figure 9.1.4. The ridge can be interrupted by a small groove (1).

Figure 9.1.5. Studies of vertebrae of fish from 1 to 3 years old to determine the position and diameter of the first year ring. The white spots are located at the distal side of the ridge.

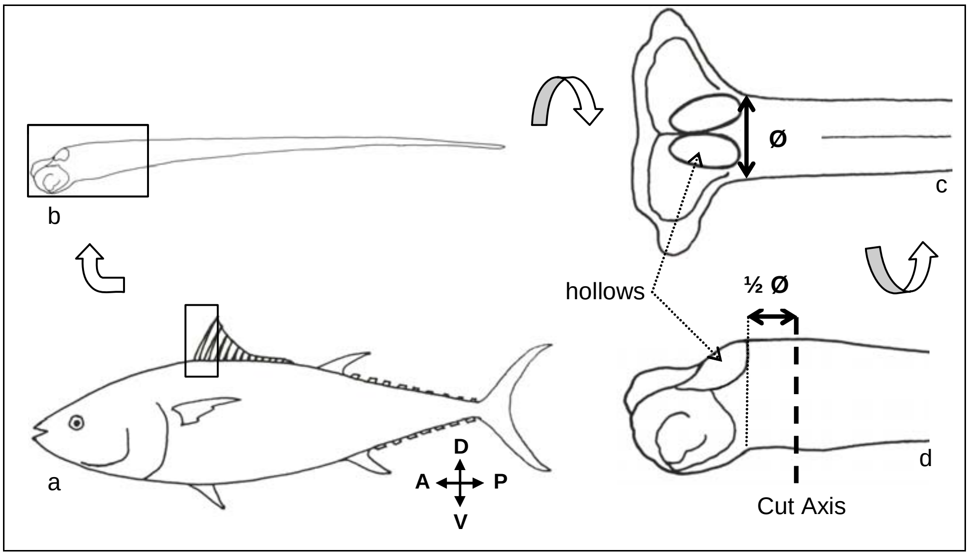

Figure 9.2.1. Description of cutting axis location. (a) and (b) show lateral view of a bluefin tuna and the first dorsal ray, respectively. (c) shows an anterior view of the ray base and the location where the diameter is measured along an imaginary line that crosses the base just above the hollows. (d) shows the lateral view of the ray base with the location of the cut axis, obtained by applying half of the diameter from the same imaginary line.

Figure 9.2.2. Cutting a block of spines.

Figure 9.2.3. Distribution of resin over the spine sections, air-drying and spine cuts embedded in resin and mounted with Eukitt.

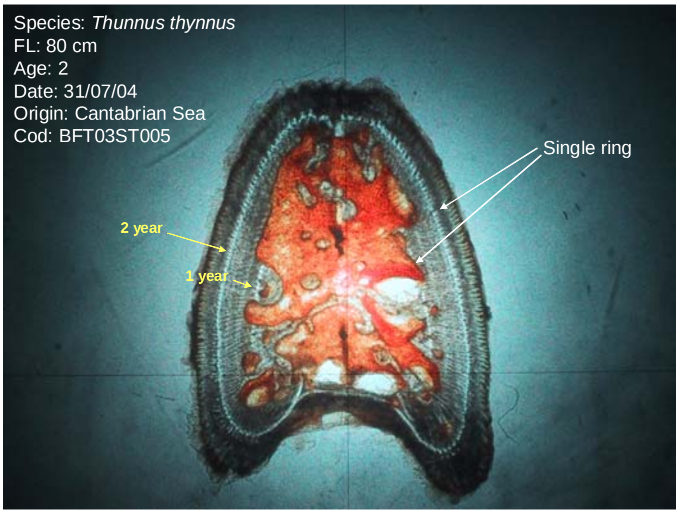

Figure 9.2.4. Spine section of a two year old bluefin tuna.

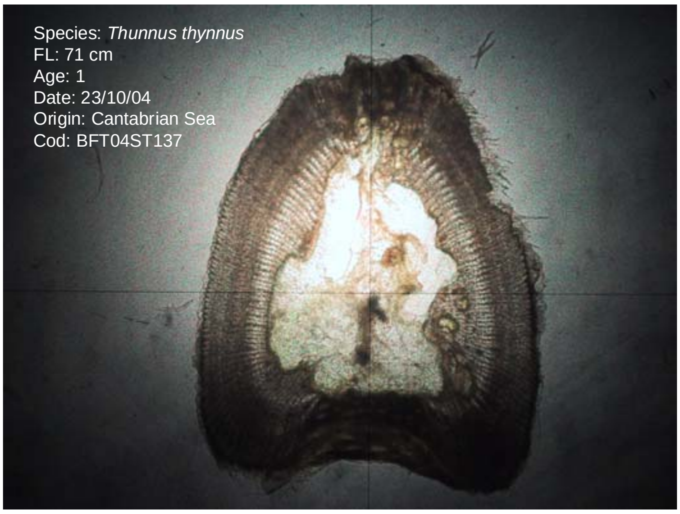

Figure 9.2.5. Spine section of a 1 year old bluefin tuna.

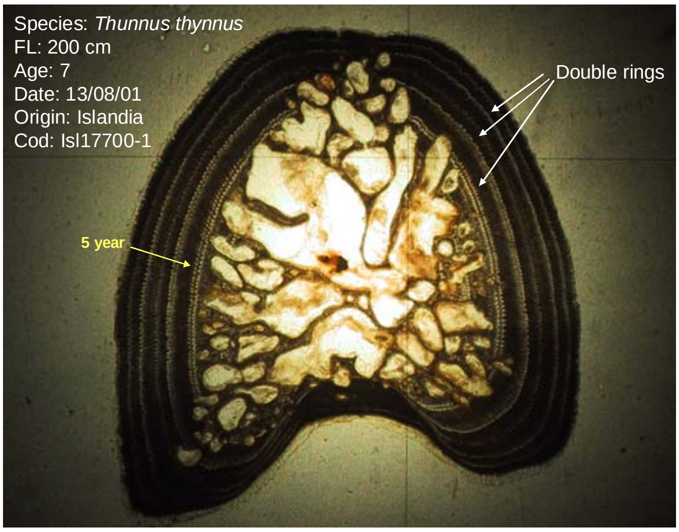

Figure 9.2.6. Spine section of a 7 years old bluefin tuna.

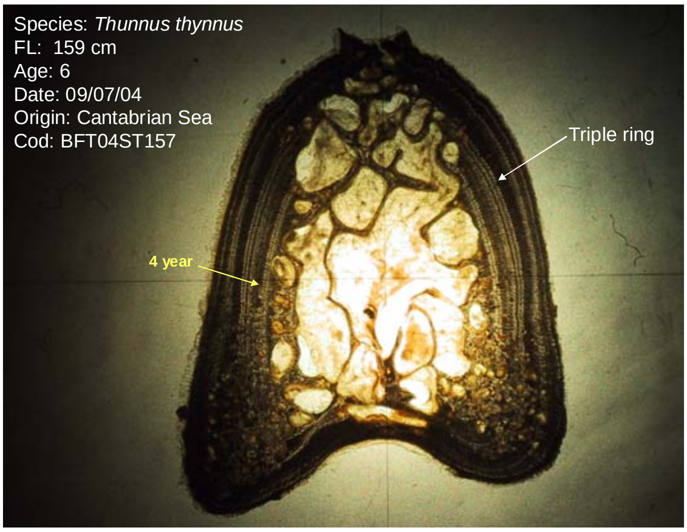

Figure 9.2.7. Spine section of a 6 years old bluefin tuna.

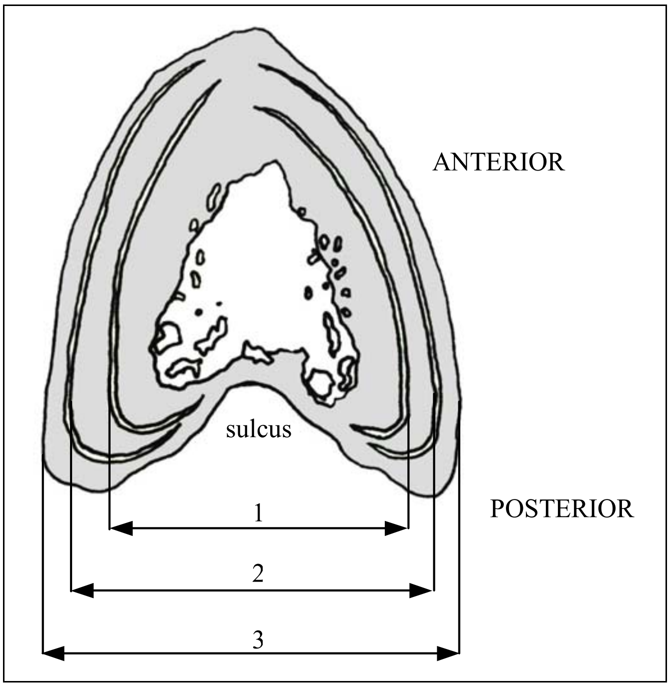

Figure 9.2.8. Spine cross section under transmitted light. 1: maximum diameter of 1st translucent band (ending or external measurement), 2: minimum diameter of 2nd translucent band (beginning or internal measurement), 3: spine diameter. See **Table 9.2**.

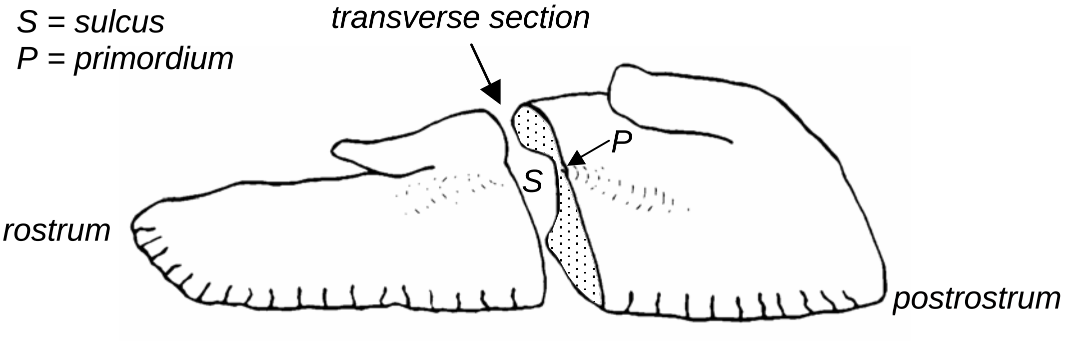

Figure 9.3.1. Left sagittal otolith of a bluefin tuna showing location of a transverse cross section (adapted from Rees *et al*., 1996).

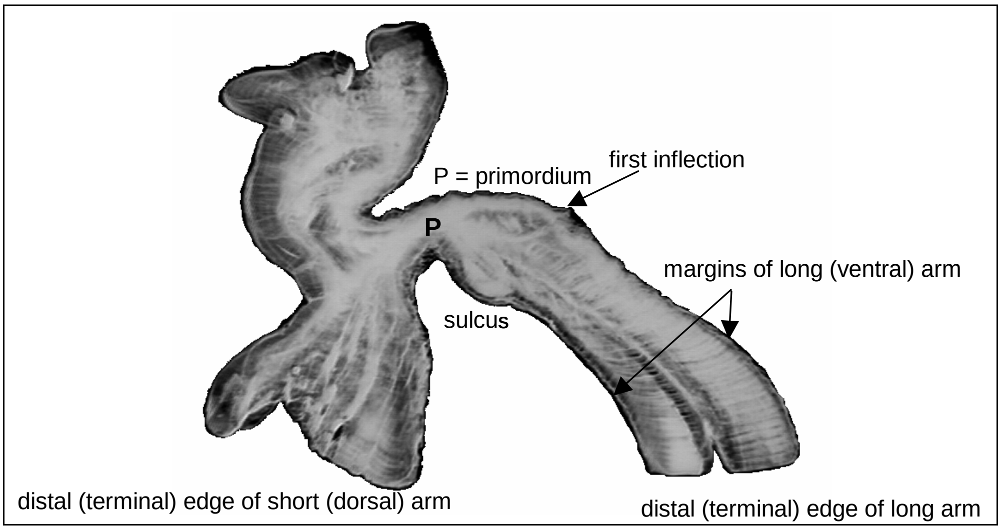

Figure 9.3.2. Transverse cross section through the primordium of a bluefin tuna sagittal otolith.

Figure 9.3.3. Age 16 bluefin tuna otolith showing pattern of increment formation. Note the broadly spaced diffuse increments in ages 1-5 and more regular and discrete increments after age 9.

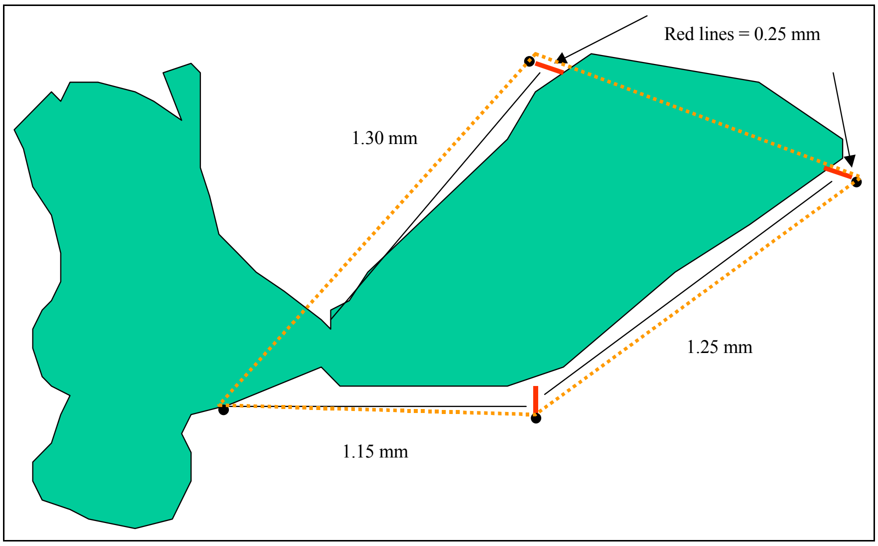

Figure 9.3.4. The approximate size of an otolith from a 1-year-old bluefin tuna (supplied by D. Secor).

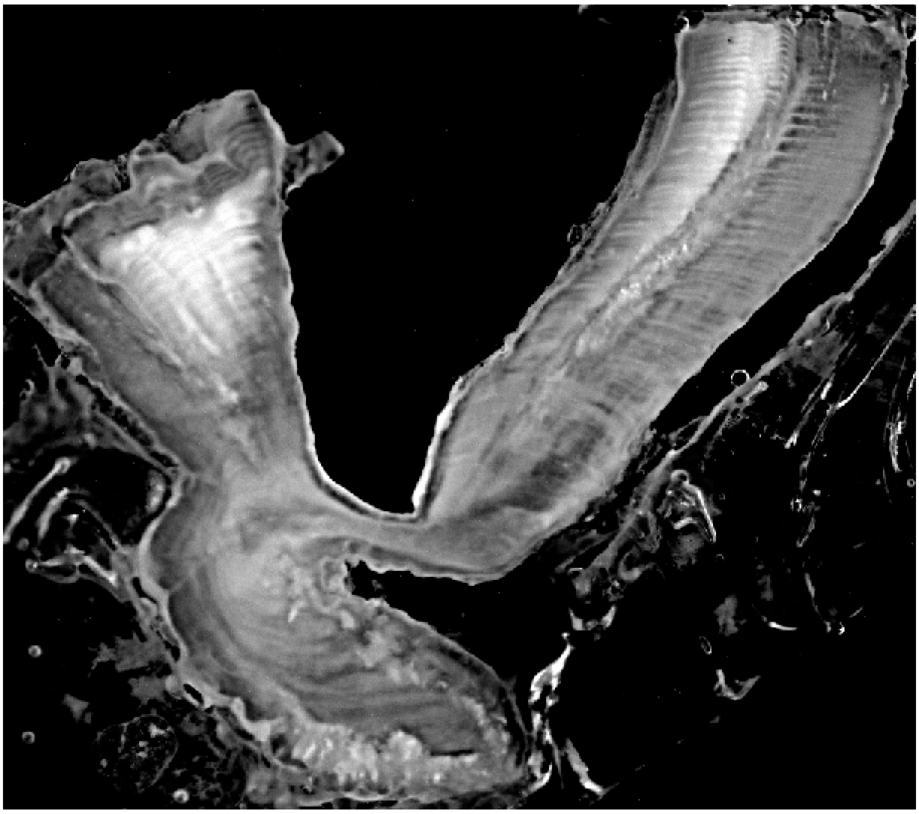

Figure 9.3.5. Image of otolith section from very large fish (404 Kg), obtained from Canadian archives. Age estimates were made from these images. Estimated age by two readers was between 23 and 29 years (supplied by J. Neilson).

Figure 9.4. Selected images of spine sections, vertebrae and otolith sections coming from the same specimen.

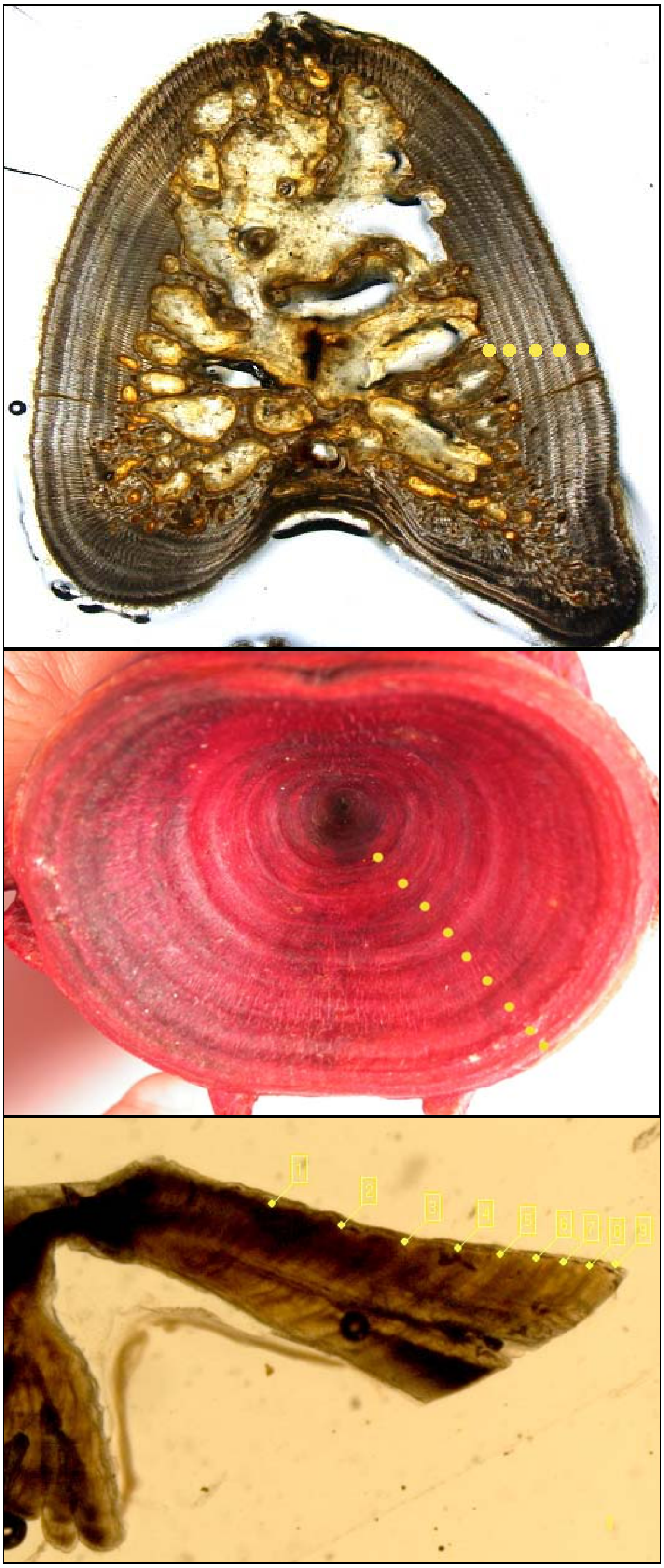

Figure 9.4.1. Image comparison between three calcified structures coming from the same bluefin tuna. An age agreement of nine years was found. The first identified ring in the spine corresponds to age 5.

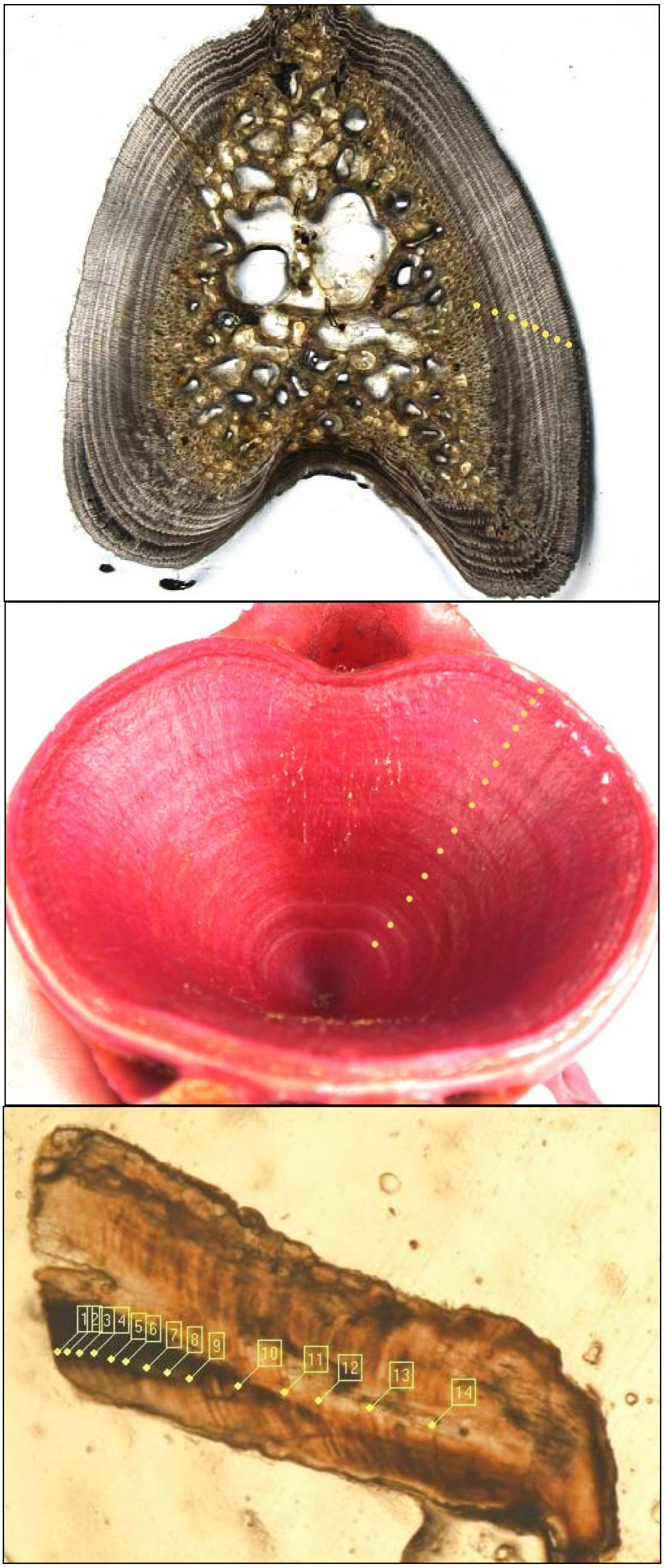

Figure 9.4.2. Image comparison between three calcified structures coming from the same bluefin tuna. An age agreement of fourteen years was found. The first identified ring in the spine corresponds to age 7.

Figure 9.4.3. Image comparison between three calcified structures coming from the same bluefin tuna. An age agreement of twelve years was found. The first identified ring in the spine corresponds to 6 years old.

Figure 9.4.4. Image comparison between three calcified structures coming from the same bluefin tuna. In this case no agreement was reached. Age estimated from spine, vertebrae and otolith were 16, 12 and 15 years respectively. The first ring identified in the spine corresponds to 6 years old.

Appendix 1

Agenda

	Monday 3	Tuesday 4	Wednesday 5	Thursday 6	Friday 7
9:00	**Opening of the meeting.** Introduction of participants. Welcome from Laboratory Director. Adoption of agenda and arrangements for the meeting	Age interpretation criteria employed till now (1)	**Agreement in future ageing interpretation criteria and advantages and disadvantages of each structure.**	**Discuss and evaluate validation approaches** Bluefin tuna ageing validation. J. Nielson	**Manual writing. Hard structures preparation and age interpretation procedures**
9:30	**Age interpretation criteria employed till now**: Review of bluefin tuna direct ageing and growth. E. Rodríguez-Marín.			Sothern bluefin tuna validation N. Clear	
10:00	Ageing of southern bluefin tuna *Thunnus maccoyii*: N. Clear			Discuss and evaluate validation approaches	
10:30	Bluefin tuna age interpretation from vertebrae. D. Olafsdottir.				
11:00-11:30	Coffee break	Coffee break	Coffee break	Coffee break	Coffee break
11:30	Bluefin tuna age interpretation from spines. C. Rodríguez-Cabello	**Same specimen calcified structures ageing comparison and analysis**	Agreement in future ageing interpretation criteria and advantages and disadvantages of each structure.	Same specimen calcified structures ageing comparison and analysis	Manual writing. Hard structures preparation and age interpretation procedures
12:00	Bluefin tuna age interpretation from otoliths. P Megalofonou				
12:30	Age interpretation criteria employed till now (1)				
13:30-15:30	Lunch break	Lunch break	Lunch break	Lunch break	Lunch break
15:30-16:50	Spines marginal increment. J.L. Cort Spine Section Interpretation: Types of Annuli and Growth by Season. X. Valeiras	Same specimen calcified structures ageing comparison and analysis	**East and West stock's growth curves comparison**	Santillana del Mar and Altamira Museum visit	Manual writing. Hard structures preparation and age interpretation procedures
16:50-17:10	Coffee break	Coffee break	Coffee break		Coffee break
17:10-18:30	Age interpretation criteria employed till now (1)	Same specimen calcified structures ageing comparison and analysis	East and West stock's growth curves comparison		Manual writing. Hard structures preparation and age interpretation procedures
21:00			Workshop official dinner		

(1) Exposition and comparison about age interpretation criteria employed till now for each structure. Discussion based on photographs and optical instruments use

Appendix 2

List of participants

AUSTRALIA

Clear, Naomi
CSIRO Marine Research and Atmospheric Research, PO Box 1538. Hobart, Tasmania, Australia 7000
Tel: +61 3 62325222. Fax: +61 3 62325125, E-mail: Naomi.Clear@csiro.au

CANADA

Neilson, John D
Fisheries and Oceans Canada, 531 Brandy Cove Road, E5B 2L9, St. Andrews, New Brunswick
Tel: +1 506 529 5913, Fax: +1 506 529 5862, E-mail: neilsonj@mar.dfo-mpo.gc.ca

EUROPEAN COMMUNITY

Cort, Jose Luis
Ministerio de Educación y Ciencia, Instituto Español de Oceanografía, Apartado 240, 39080 Santander, Spain
Tel: 34 942 291060, Fax: 34 942 27 5072, E-mail: jose.cort@st.ieo.es

Megalofonou, Persefoni
Department of Zoology-Marine Biology, Faculty of Biology, University of Athens. Tel: (030-1)7274620, Fax:7274620. E-mail: Pmegalo@biol.uoa.gr

Neves dos Santos, Miguel
Instituto de Investigaçao das Pescas e do Mar (IPIMAR), Centro Regional de Investigaçao Pesqueira do Sul, Avenida 5 Outubro s/n, 8700-305, Olhao, Faro, Portugal
Tel: +351 289 700 504, Fax: +351 289 700 535, E-mail: mnsantos@cripsul.ipimar.pt

Rodríguez-Cabello, Cristina
Ministerio de Educación y Ciencia, Instituto Español de Oceanografía, Promontorio San Martín, s/n, 39004 Santander, Spain
Tel: 34 942 291 060, Fax: +34 942 275 072, E-mail: c.cabello@st.ieo.es

Rodríguez-Marin, Enrique
Ministerio de Educación y Ciencia, Instituto Español de Oceanografía, Promontorio de San Martín s/n, 39004, Santander, Cantabria, Spain
Tel: +34 942 29 10 60, Fax: +34 942 27 50 72, E-mail: rodriguez.marin@st.ieo.es

Ruiz, Marta
Ministerio de Educación y Ciencia, Instituto Español de Oceanografía, Promontorio de San Martín s/n, 39004, Santander, Cantabria, Spain
Tel: +34 942 29 10 60, Fax: +34 942 27 50 72, E-mail: rodriguez.marin@st.ieo.es

Valeiras, Julio
Ministerio de Educación y Ciencia, Instituto Español de Oceanografía, Promontorio de San Martín s/n, 39004, Santander, Cantabria, Spain
Tel: +34 942 29 10 60, Fax: +34 942 27 50 72, E-mail: rodriguez.marin@st.ieo.es

ICELAND

Olafsdottir, Droplaug
The Marine Research Institute, Skúlagata, 4, P.O. Box 1390, IS-121 Reykjavik
Tel: +354 552 0240; Fax: +354 562 3790; E-mail: droplaug@hafro.is

From left to right: Persefoni Megalofonou, Miguel Neves dos Santos, Julio Valeiras, Jose Luis Cort, Enrique Rodríguez-Marin, Cristina Rodríguez-Cabello, John Neilson, Naomi Clear, Droplaug Olafsdottir and Marta Ruiz.

Appendix 3

Growth review table for North Atlantic Bluefin tuna

Table 1. Annual mean lengths at age and von Bertalanfy growth parameters of *Thunnus thynnus* for east Atlantic and Mediterranean (a) and west Atlantic (b) specimens.

(a) Growth review table for the East Atlantic and Mediterranean stock

Author	Sella (1929)	Hamre (1960)	Vilela (1960)	Rodriguez-Roda (1964)	Farrugio (1980)		Arena *et al.* (1980)	Compeán-Jimenez & Bard (1983)	Hattour (1984)	Cort (1991)	Farrugia & Rodriguez-Cabello(2001)	El-Kebir *et al.* (2002)	Olafsdottir & Ingimundardottir (2003)	Rodriguez-Marin *et al.* (2004)
Sampling Period	summer	1956-1960	1957-1960 spring-summ.	1956-1959 summer	1975-1977		1960-1980	1978 - 1979 spri-summ-atu	1979-1983	1975-1986 summer	1999 spring		1999-2000 autumn	1990 spring
Area	Adriatic Sea, off Libya, Mediter. Sea	Norway. EastAtlantic	Algarve, Portugal, EastAtlantic	Gulf of Cádiz Spain, EastAtlantic	Gul of Lyon, France, Mediterranean Sea		Thyrrenian Sea, Italy Mediter. Sea	Bay Biscay, Canary, Medit East Atlantic	Coast of Tunisia Mediter. Sea	Bay Biscay, Gulf of Cadiz EastAtl	Maltese Islands, Mediter. Sea	Offshore Lybia, Mediter. Sea	South of Iceland EastAtlantic	South west Spain EastAtlantic
Growth Model	Mean length at age	Mean length at age	Mean length at age	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy	Mean length at age	Mean length at age	Mean length at age	Mean length at age
Ageing material	Caudal vertebrae	Caudal vertebrae	Precaudal vertebrae	Precaudal vertebrae	Caudal vertebrae	Length structure	Length structure	Spine sections	Otolith. Back calculated	Length str. & Spine sect.	Spine sections	Spine sections	Caudal vertebrae	Spine sections
Linf				344.1	351.2	331.42	455.89	372.2	330	318.85				
K				0.090	0.080	0.066	0.05	0.068	0.095	0.093				
To				-0.970	-1.087	-2.276	-1.613	-1.710	-0.366	-0.970				
Ages with >3 indiv. sampled	all		6 to 9	1, 4 to 9	1 to 5	1 to 5	all	1-7,9-10,12-18		all	6, 8 to 13	5 to 10	5 to 16	10 to 17
Mean fork length (cm)	straight ?	curved	straight	straight	straight	straight	straight	straight	straight	straight	straight	straight	straight	straight
Data	Observed	Observed	Observed	Observed	Estimated	Observed	Observed	Observed	Observed (1)	Observed (2)	Observed (3)	Observed (3)	Observed	Observed (3)
Age 1	64.0			55.3	54.0	58.5	57.0	62.7	42.0	53.5				
2	81.5			79.0	76.8	77.0	77.0	83.1	69.1	79.7				
3	97.5			116.2	97.9	94.8	97.0	102.1	93.2	100.7			134.0	
4	118.0		120.5	130.1	117.4	111.9	115.0	119.9	113.0	118.8	108.0	127.0	128.0	123.7
5	136.0	135.0	136.0	146.9	135.4	128.3	128.0	136.5	127.0	135.1	133.2	126.6	160.4	142.2
6	153.0	153.0	146.9	165.1	152.0	144.1	145.0	152.0	139.0	150.1	150.7	150.2	171.1	156.0
7	169.0	161.0	163.9	178.1	167.3	159.2	165.0	166.5	151.2	164.0	148.0	152.2	184.0	172.3
8	182.0	180.0	187.2	192.9	181.4	173.7	176.0	180.1	157.0	177.2	197.8	176.5	199.5	182.8
9	195.0	198.0	196.1	206.5	194.5	187.7	187.0	192.8	164.0	190.9	194.2	181.8	205.3	194.0
10	206.0	207.0	217.7	220.3	206.5	201.0	198.0	203.6	183.0	206.2	213.7	183.7	208.8	199.3
11	216.0	221.0	223.0	221.5	217.6	213.9	216.0	215.7		216.1	214.1	217.0	219.8	211.0
12	227.0	228.0	232.8	244.0	227.9	226.2	228.0	226.1		222.5	239.5	215.8	216.1	235.5
13	239.0	239.0		246.0	237.4	238.0	239.0	235.8		232.4	222.7		221.2	233.5
14	254.0				246.1	249.3	250.0	244.8		241.6	256.6	209.9	236.3	245.0
15							260.0	253.3		247.2	258.0		241.7	253.7
16							270.0	261.2				287	247.3	263.5
17								268.5					265.0	267.0
18								275.5						
19								281.9						
20														

(1): Probably calculated at the beginning of the year or during slow growth/translucent band formation.
(2): Estimated from length structure for ages 1 to 8 and observed from spines from 9 to 15 years old.
(3): Mean fork length at age was obtained by applying length distributions of catches to the age length key.

(b) Growth review table for the West Atlantic stock

Author	Westman & Neville (1942)	Mather & Schuck (1960)		Caddy *et al.* (1976) and Butler *et al.* (1977)		Farber & Lee (1981)		Hurley & Iles (1983)		Parrack & Phares (1979)	Turner & Restrepo (1994)
Sampling Period	1938-1941 summer	1950-1959	1941-1959	1975 Summer- autumn		1966-1978	1974-1978	1975 - 1981 summer - autumn		1963-1978	1963-1990
Area	Long Island, NY, USA. WestAtlantic	Cape Cod, USA, WestAtlantic	Cape Cod, Long Island USA, WAtl.	Offshore Canada West Atlantic		Middle Atlantic bight, offshore USA West Atlantic		East coast of Canada & USA West Atlantic		West Atlantic	West Atlantic
Growth Model	Mean length at age	Mean length at age	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy	Von Bertalanffy
Ageing material	Length str. & scales	Scales & caudal vert.	Length str. & caudal vert.	Otolith sect. males	Otolith sect. females	mark - recapture	Caudal vert. Back calculat	Otolith sect. males	Otolith sect. females	mark - recapture	mark - recapture
Linf			371.0	286.6	277.3	313.0	401.0	277.8	266.4	313.0	382.0
K			0.069	0.134	0.116	0.120	0.080	0.169	0.170	0.090	0.079
To			-1.373	-0.328	-0.800	-0.140	-0.920	0.254	0.106	-0.960	-0.707
Ages with >3 indiv. sampled	1 to 4	1 to10, 12,13	all				all	2,4-6,11,13-28	2,4-6,15-30		
Mean fork length (cm)	curved	straight	straight	straight	straight	straight	straight	straight	straight	straight	straight
Data	Observed	Observed	Estimated	Estimated (4)	Estimated (4)	Estimated	Observed	Observed	Observed	Estimated	Estimated
Age 1	65.0	55.9	57.0			40.0	44.8	64.0	64.5	50.6	48.2
2	85.0	76.5	77.0			70.9	67.6	75.5	82.3	73.2	73.5
3	105.2	90.5	95.0			98.3	91.6	77.3	82.5	93.8	97.0
4	117.6	118.8	114.0			122.5	116.2	113.3	130.0	112.7	118.6
5	147.8	135.0	133.0			144.1	138.8	147.1	149.4	129.9	138.6
6	148.3	155.4	149.0			163.2	157.9	146.4	161.1	145.7	157.1
7	157.5	161.6	163.0			180.1	176.3	173.3	175.0	160.1	174.2
8		174.4	177.0			195.2	189.7	253.0		173.3	190.0
9		186.1	190.0			208.5	200.8	223.7		185.3	204.6
10		203.4	201.0			220.3	217.2	234.0		196.3	218.0
11		224.5		223.8		230.8		234.5	229.0	206.3	230.5
12		233.7		231.7				234.5	262.0	215.5	242.0
13		243.3		238.6				256.3	251.5	223.9	252.6
14		248.0		244.6				253.5	252.0	231.6	262.5
15				249.9	233.0			262.9	250.3	238.6	271.5
16				254.5	237.8			262.5	250.1	245.0	279.9
17				258.5	242.1			262.5	252.5	250.8	287.7
18				262.1	246.0			264.0	258.9	256.2	294.9
19				265.1	249.4			267.5	258.0	261.1	301.5
20				267.8	252.5			268.7	259.3	265.5	307.6
21				270.2	255.2			268.3	257.4		
22				272.3	257.6			270.4	259.8		
23				274.1	259.8			270.1	259.1		
24				275.6	261.7			271.5	260.3		
25				277.0	263.4			272.2	260.6		
26								274.4	262.7		
27								274.4	265.0		
28								272.5	256.6		
29								272.7	259.0		
30								273.7	271.1		
31									265.7		
32									268.0		

(4): Growth parameters estimated using ages 1-4 from Mather & Schuck (1960)

Appendix 4

Bluefin tuna ageing bibliography

This appendix includes all cited references in the report and some not cited, but useful, references about this subject.

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