Collect. Vol. Sci. Pap. ICCAT, 79(6): 114-139 (2023)

REPORT OF THE 2021
ICCAT BILLFISH WORKSHOP ON AGE READING

Anon

SUMMARY

This report describes the 2021 ICCAT Billfish Workshop on Age Reading that took place online in October 2021. The major objectives of the workshop were to i) enhance current expertise in the Eastern Atlantic, ii) to standardize processing and reading protocols between laboratories (Eastern & Western Atlantic). The specific growth studies for billfish in the eastern area has been running since 2018 to the present and is part of the Enhanced Programm for Billfish Research (EPBR). During the workshop the sampling and processing protocols were reviewed and updated. Discussion on age validation and verification and readings was initiated. The group proposed 5 recommendations to be passed to the Billfish Species Working Group.

RÉSUMÉ

Ce rapport décrit l'Atelier de l'ICCAT sur la lecture de l'âge des istiophoridés de 2021 qui s'est tenu en ligne en octobre 2021. Les principaux objectifs de l'atelier visaient à : i) renforcer l'expertise actuelle dans l'Atlantique Est, ii) standardiser les protocoles de traitement et de lecture entre les laboratoires (Atlantique Est & Ouest). Les études spécifiques sur la croissance pour les istiophoridés dans la zone Est sont en cours depuis 2018 jusqu'à présent et font partie du programme de recherche intensive sur les istiophoridés (EPBR). Au cours de l'atelier les protocoles d'échantillonnage et de traitement ont été revus et actualisés. La discussion sur la validation, la vérification et les lectures des âges a été engagée. Le Groupe a proposé 5 recommandations qui seront transmises au Groupe de travail d'espèces sur les istiophoridés.

RESUMEN

Este informe describe el Taller de ICCAT sobre lectura de edad de istiofóridos de 2021 que se celebró en línea en octubre de 2021. Los principales objetivos del taller eran: i) mejorar la experiencia y conocimientos actuales en el Atlántico oriental y ii) estandarizar los protocolos de procesamiento y lectura entre los laboratorios (Atlántico oriental y occidental). Los estudios específicos de crecimiento de istiofóridos en la zona oriental se están llevando a cabo desde 2018 hasta la actualidad y forman parte del Programa ICCAT de investigación intensiva sobre marlines (EPBR). Durante el taller se revisaron y actualizaron los protocolos de muestreo y procesamiento. Se inició el debate sobre la validación y verificación de la edad y las lecturas. El grupo propuso cinco recomendaciones para su presentación al Grupo de especies de istiofóridos.

KEYWORDS

Billfish, Enhanced program for billfish research (EPBR), ageing, spines, otoliths, Atlantic

1. Opening

The Enhanced Programm for Billfish Research (EPBR) has been established since 1986, with several objectives aiming to improve biological and fisheries data for billfishes in the Atlantic area. The specific growth studies for billfish in the eastern area has been running since 2018 to the present. This first workshop on ageing is intended to review the existing sampling and processing protocols, for consistency between laboratories, and initiate discussions on age reading protocols. The 2021 Billfish Workshop on Age Reading was held online from the 25th to the 28 October 2021.

On behalf of the Executive Secretary, the Assistant Executive Secretary Miguel Neves dos Santos, and the vice-Chair of the SCRS, Rui Coelho, welcomed the participants to the workshop.

2. Adoption of the agenda

The agenda was reviewed and adopted and the final version is shown in **Appendix 1**.

3. Nomination of the rapporteurs

The list of registered Participants is included in **Appendix 2**. The following served as rapporteurs:

Section 4: Karina Ramirez Lopez

Section 5: Guelson Batista, Bruno Mourato

Section 6a: Dheeraj Busawon

Section 6b: Catarina Santos, Daniela Rosa

Section 7: Bruno Mourato, Guelson Batista

Section 8: Rui Coelho, Daniela Rosa

4. Overview of sampling protocols and data collection database

The workshop chair (Daniela Rosa, Portugal) presented an update on the sampling protocols and the program database for ageing. The background of the ICCAT Enhanced Programme for Billfish Research (ICCAT/EPBR) was presented, emphasizing the objective of the program on collecting biological data (2018-2020) and conducting BIL growth studies in the eastern Atlantic, focusing on three species of BIL: *Makaira nigricans* (BUM); *Kajikia albida* (WHM) and *Istiophorus albicans* (SAI). The presentation referred to the general description of the sampling protocols for both spines and otoliths and the data collection database since 2018. This study has the participation of four institutes (Centre de Recherches Océanologiques (CRO) - Côte d'Ivoire, Direction Generale des Pêches et de l'Aqualculture (GP) - Gabon, Centre de Recherches Océanographiques de Dakar /Thiaroye (CRODT) - Senegal, Instituto Português do Mar e da Atmosfera (IPMA) – Portugal and Instituto Español de Oceanografía (IEO) – Spain), whose activities have focused on conducting collaborative studies, obtaining samples in the industrial and artisanal fleet, as well as collaboration for their processing and analysis. Currently, the collection of the database includes fishing biological information for the years 2018 - 2020, in which 453 spines have been collected (62% of SAI, 25% of BUM and 13% of WHM) and 152 otoliths (43% SAI, 30% BUM and 27% WHM. Regarding the length frequency distribution in lower jaw fork length (LJFL), it was obtained that for BUM most of the sizes were higher and the inverse for SAI and WHM, and that the presence of females has been mostly represented for WHM and BUM, with very few specimens undetermined.

The group expressed interest in the presence of juveniles (<150 cm LJFL), which could be associated with a specific area and fishing gear, as currently there are not many small individuals in the sampling.

The sampling protocol including the extraction of hard parts (spines and otoliths) was reviewed. Finally, a video was presented on the process for the extraction of otoliths, in which the process for frozen brain cases is detailed. The process of extraction of the otolith is also shown and to remove them from their capsules and deposit in plastic vials.

The group agreed that detailed guidelines, preferably with diagrams and images, could be of extreme importance to capacitate more observers and samplers to collect samples of different species.

The method of collecting the brain case was considered useful in cases where it is not possible to collect and freeze the whole head, or it is not possible to collect the semicircular canals onboard. Collecting the semicircular canals has been applied onboard by some observers in the case of swordfish, but it was not possible to do the same with the billfish species due to the semicircular canals being too fragile. The protocol has been updated with more detailed information on the collection of the brain case and on the otolith extraction (SCRS/2022/137).

5. Revision/update of protocols for ageing samples processing

In this section the protocols for processing the ageing structures from billfishes, including spines and otoliths were presented and reviewed. The processing of spines has been done simultaneously at CRODT (Senegal) and IPMA (Portugal), while otoliths are being processed at Fish Ageing Services (FAS).

In the sequence, Admir Sutrovic (FAS) had a presentation entitled "Preparation methods of Atlantic blue marlin, white marlin, and sailfish using otoliths", which included the protocols for the otolith preparation for annual age readings. The presentations started with an image of the whole otolith and another of the transverse section for ageing. The detailed protocol for sectioning by hand grinding was presented using images and videos. The preparation includes several steps of placing the otolith in the correct position on a slide and grinding. With this method thin sections (250-280µm) containing the primordium can be achieved. This thickness was considered deemed for all three studied species. Challenges arise in processing due to the small and fragile nature of the billfishes' otoliths. Furthermore, it was shown the results of the length-weight relationship for the otoliths of the three species plotted by sex.

The Group asked about the conservation of the remaining structure of the otoliths during the process, which could be used posteriorly for the ocean chemistry or radiocarbon analysis. The answer was that the process producing sections for ageing also could be used for milling (i.e. technique for the extraction small portions of the otolith for chemistry studies), however this would have to be done in the twin otolith, as griding leaves no excess material from that same otolith.

It was questioned if it would be possible to section the embedded otolith, the author explained that that process is more appropriate for larger otoliths, while in this case the otoliths are so small that the primordium can be missed, and then it would be lost due to erosion of the blade. By hand grinding it is possible to place the otoliths correctly and even move them at an angle to achieve the desired section.

It was suggested that given the two arms of the otolith are so distinct, joining mostly at the primordium, if it would be possible to separate the arms and section one for the age readings while preserving the other.

The presenter also let the group know that FAS would be attempting to do daily age readings on some of the samples. Having daily age readings could be useful to estimate the age of juveniles and to determine the age at first opaque annuli deposition.

The processing protocol was updated with more detailed information on the processing of otoliths (SCRS/2022/137).

6. Initial guidelines for age reading including:

a) Discussions on age verification and validation

Dr Allen Andrews (University of Hawai, USA) presented work conducted on pelagic fishes for age validation using bomb radio carbon analysis, entitled "Bombs and Fish – Extending the utility of bomb radiocarbon dating to recently collected pelagic fishes". Pelagic fishes of the tropics are aged using a variety of methods including counts of daily increments and annual growth zones in otoliths or other calcified structures. Some species are being aged beyond the typical lifespan estimates derived from daily increments, and otolith-based annual age reading methods have provided estimates approaching and even exceeding 20–30 years. These older ages were supported with bomb radiocarbon (^{14}C) dating for yellowfin (*Thunnus albacares*) and bigeye (*T. obesus*) tuna in the western North Atlantic - Gulf of Mexico. The presented study exemplifies the extended utility of this age validation method by using the post-peak ^{14}C decline to validate ages for recently collected fishes with shorter lifespans. This method harbors great potential for pelagic tropical and subtropical fishes as various ^{14}C records (typically hermatypic coral) are beginning to converge in recent times, as was demonstrated in a fortuitous study of a grander blue marlin (*Makaira nigricans*) in the Pacific (3.7 m and 565 kg) determined to be ~20 years old. At present, the use of the post-peak decline is being explored for tropical tuna of the western and central South Pacific and new coral records across the Pacific support its utility as a temporal reference. This line of work holds promise for many other marine organisms of tropical-subtropical seas where questions of age, growth and lifespan remain unanswered.

It was noted that only a validation study exists for billfishes, specifically for blue marlin in the Pacific Ocean (Andrews et al., 2018). Direct validation is missing for sailfish and white marlin. As such, the group considers this should be a priority for these three species for which an age and growth study is being conducted.

It was highlighted that obtaining samples from juveniles (<150 cm LJFL) to compare to core material from older individuals would be ideal for bomb radiocarbon validation. It is noted that small individuals (<150 cm LJFL) are rarely caught, and it is difficult to define the areas where these would be present with the current knowledge on these species.

It was also noted that in the case of the age validated blue marlin the lapillus otolith was also used to help confirm the age estimates, it would be important to also collect these otoliths when sampling for the sagitta.

Furthermore, it was commented that the presented information/analysis would be relevant, not only for billfishes, but for all ICCAT species lacking validation of band deposition. The group inquired if a reference had been developed for the Mediterranean and if not, what would be required to develop one. The expert mentioned that he had used coral reference from Northern Italy to develop a reference for hake in the Mediterranean (Vitale et al., 2016). However, this work would need to be extended to develop a ^{14}C reference.

Expanding the reference collection for the ^{14}C could be attempted through current sampling programs (e.g. additional coral references, obtaining easy to read otoliths from long lived species that show high site fidelity and obtaining otolith samples from early juveniles).

Future ageing studies could benefit with the application of non-lethal methods to estimate age. Use of DNA methylation rates is a rapidly evolving method that can be used to provide non-lethal estimates of age from muscle tissue (Mayne *et al.* 2019, 2021). To use this method, known age material is needed across all size classes to calibrate the methylation rate; hence, the use of otoliths and an age validation method, like bomb radiocarbon dating, provide a means of defining this tool for a given species.

b) methods to correct for spine vascularization

Dr Constance Diaha (CRO) presented on the methodology for age determination of skipjack in the Atlantic Ocean, with a presentation entitled “Méthodologie de la détermination de l’âge de *Katsuwonus pelamis* de l’Atlantique est à partir de l’épine et validation dépôt”. Age estimates were obtained through reading of spine sections. For sectioning, the condyle width was measured, and sections were taken at a half distance of the condyle width, measured from an imaginary line connecting the largest part of the condyle. The spine was embedded in resin and 2 transversal sections of 350 μm were cut using a low-speed saw. These sections were then mounted on a slide and growth bands were counted using transmitted light. Validation of the deposition rate was conducted by monitoring the formation of the last growth band on the external side of the spine. The quantitative analysis was carried out by monitoring the rate of formation of the translucent band with the calculation of the marginal increment rate (MIR) and the qualitative analysis was based in the classification of the edge type in translucent edge, newly formed opaque edge, and opaque edge. The MIR and the classification of the edge type were analyzed by month in order to examine their evolution during the year. Growth bands ranged from 0 to 8 in individuals with fork length ranging between 30 and 60 cm. In this species, bone reabsorption was observed at the end of the spine.

The authors noted that age estimation of some specimens was problematic due to spine reabsorption and the consequent loss of growth bands. It was also noted that the reabsorption of bands seems to occur in all size classes, including the smaller specimens.

The group commented that this problem was also found in other species (e.g. billfishes). It was mentioned that previous work on billfishes concluded that bone reabsorption occurred in most size classes and was highly variable between specimens of the same size class, as some displayed large areas of reabsorption and others did not. Additionally, previous work investigated if different cutting positions had an influence in the area of the reabsorbed area, however changing the cutting position did not seem to significantly improve the visualization of the age bands.

The group highlighted the importance of data collection and spine measurements in order to model bone reabsorption and perform age corrections. Additionally, it was suggested that otoliths could be used to obtain more accurate age estimations since those structures are not affected by reabsorption.

Dr. Freddy Arocha (Instituto Oceanográfico de Venezuela, Universidad de Oriente) presented a work conducted on billfish on the central-west Atlantic entitled "Ageing of billfish from the central-west Atlantic Ocean". This presentation indicated the period during which anal fins were collected for aging and growth studies of blue marlin, white marlin, and longbill spearfish. The samples were collected by at-sea observers on board Venezuelan longline vessels and from artisanal directed billfish fisheries operating off the central coast of Venezuela. Anal fins were collected between 2003 and 2011, depending on the species. Over 1700 anal fins were collected for each of the marlin species, and under 500 for longbill spearfish. About the same period, anal fin samples were also collected from Brazil and Uruguay. The spatial coverage for blue and white marlin included the eastern Caribbean Sea and the northern area of South America in the Atlantic, as well as a broad area in the Brazilian waters around the Equator and further south. The sample process from Venezuela included cleaning, air dried, and "lightly cooking in oven" the second fin ray from blue marlin and the third fin ray from white marlin and longbill spearfish; the sectioning with a slow-speed saw, after which the sections were read, measured and the information was saved as images and data. Several examples of spine sections images were presented to show the lack of symmetry in spines lobes and the need to define a center or core for initial measurements. Noting that the loss of annuli (rings) due to vascularization over time, a couple of methods from the published literature to estimate missing annuli in the vascular zone were commented. Also noting that the collection of new samples from the west Atlantic may be limited, the use of historical images of spine sections from the collections made in the west Atlantic during the 2000 decade could prove useful to enhance Atlantic-wide estimates on growth for these species, which in the case of the marlins each is considered a single Atlantic-wide stock.

It was suggested by the author that the historical collection of spine images available from the West Atlantic could be included in the current project. It was noted however that the second spine was used for blue marlin. A comparison between both structures would have to be conducted before pulling the samples together. I was noted that in the current project the second anal fin spine might be available or already processed for some specimens, and this could allow for a comparison between both the second and third spines. Additionally, a few (~5/6) of these historical samples could have a corresponding otolith collected. Furthermore, it was noted that it would be important to analyze the historical images available from the West Atlantic since getting new samples would be limited due to the logistics and current restrictions.

The group commented about the importance of smaller specimens (<150 cm LJFL) for age estimation validation. The author mentioned that young of the year (<120 cm LJFL) specimens were very rare and that they possibly occur in the Gulf of Mexico (Yucatán Peninsula).

Kyne Krusic-Golub (FAS) briefly presented SCRS/P/2021/055, showing some of the preliminary results for the three billfish species, highlighting the large variance in age for a given size.

It was questioned if, from the author past experience, there was any difference in band interpretation between tropical and equatorial areas. The author replied that for swordfish in the Pacific no differences were found between areas, and for striped marlin either, however in this case there were only a few samples available so far.

After the review of a preliminary version of reading protocols was reviewed (**Appendix 3**) a small exchange exercise was conducted, by showing pairs of spine and otoliths for the three species (**Appendix 4**).

In general, there were comments about the large proportion of vascularized area of the spines, that prevents reliable age readings, as annual bands are lost due to bone reabsorption. The bands that are observed were considered to be relatively easy to identify. Differences in readers can also arise from where it is considered that it is a vascularized area or not, as in some a translucent band can be seen on a portion of the spine but not in other areas due to reabsorption. Corrections methods will have to be developed for this area to account for lost rings.

It was noted that image enhancement and image analysis could help in achieving better estimates from spines.

Regarding otoliths, from the processed samples only a few were considered "good" to read, which compared to other species can still be classified as "difficult" to read. It was noted that in smaller individuals it was harder to distinguish the annual bands, while in larger individuals it would be easier to count as the pattern of band deposition was better defined. It was also noted that reading would probably be improved if having the sample available to see under a microscope.

For both spines and otoliths, it was considered that readability improves with experience, and that this exercise was very helpful in providing a first overview of available images.

7. Other matters

Dr. Bruno Ferrette (Senckenberg Biodiversity and Climate Research Center) presented his recent work on genetic of sailfish, entitled "Sailfish genomics and population structure". This ongoing study applied a molecular analysis using a genomics approach to better understand the taxonomic and delimitation of genetic stocks of the Istiophoridae. This technique uses PacBio sequencing to assemble high-quality reference genomes and Illumina sequencing to re-sequence the whole-genome of Istiophoridae species. The presentation illustrated that this technique was useful to evaluate the sailfish's stocks. Additionally, preliminary results were presented for white marlin and spearfishes. Among the billfishes, the species *Kajikia albida* and the ones from the genus *Tetrapturus* are difficult for a reliable taxonomic identification in the field that compromises the sustainable management for these species. The topology of a phylogenetic tree constructed using the mitochondrial control region showed that the only species of the genus *Tetrapturus* well resolved is *T. georgii* while all the others are mixed in the tree. This could be due to the wrong identification of the individuals and the consequent deposit of mistakenly identified sequences in the GenBank but also because the actual species could not be valid. Besides, the genus *Kajikia* appears to be well resolved but the genetic divergence is also too low, and that is why there is the need for further investigations with a multilocus approach in order to clarify the validity of both species.

The group noted that the approach applied to sailfish could assist to evaluate other species like the white and blue marlins. The work presented has implication for the ongoing work of the EPBR but also to the general work of the Billfish species group, both for sailfish, indicating there is only one Atlantic stock, and for white marlin, that are misidentified as Tetrapturus species. Given this it was discussed that these results and any further analysis should be presented to the next Billfishes species group meeting. Furthermore, it was suggested the identification keys (e.g., Arocha and Beerkircher, 2012; ICCAT, 2006-2016 see Sections 2.1.7 WHM[1], 2.1.8.3 SPF[2] and 2.1.8.4 RSP[3].) could be improved to help onboard observers distinguish the different species as well as further training in identification for these specific species.

Finally, the group agreed to also discuss in the next species group meeting the possibility to start a specific sampling to attend the ongoing and future analysis using genomic evaluation to evaluate the species delimitation and its genetic stocks. It is important to note that some CPCs already started to collect tissue samples of Istiophoridae that could be used for this study.

A brief protocol on how to sample and store tissue samples for genetic analysis is provided in **Appendix 5**.

7. Recommendations

Age validation with bomb-radiocarbon: The Group recognizes that it is important to conduct age validation when doing age estimation and modelling studies. Within the different possible methods, bomb-radiocarbon seems to be the most appropriate for billfishes. As such, the Group recommends that age validation studies with bomb-radiocarbon start to be developed for the three billfish species within the ageing project (BUM, WHM, SAI). This recommendation has financial implications. The Chair of the BIL Species Group and the task leaders of the ageing project will work on a tentative budget for presenting to the BIL Species Group in their next meeting.

Include historical samples from the West Atlantic*:* Given the Atlantic-wide nature of those species, the Group recommends that any historical samples already available from the West Atlantic are included in the ageing study (e.g.: Venezuela, Brazil, Gulf of Mexico, etc). After all samples (recent and historical) are compiled, the Group can consider expanding the sampling to any other missing regions.

Expansion of the size range of the samples*:* The Group recommends that the widest possible range of specimen sizes are sampled for both spines and otoliths including, as much as possible, both small and large specimens. There may be the need to explore other regions and/or fishing gears with different selectivity, to sample from those additional sizes.

[1] https://www.iccat.int/Documents/SCRS/Manual/CH2/2_1_7_WHM_ENG.pdf
[2] https://www.iccat.int/Documents/SCRS/Manual/CH2/2_1_8_3_SPF_ENG.pdf
[3] https://www.iccat.int/Documents/SCRS/Manual/CH2/2_1_8_4_RSP_ENG.pdf

Simplified/schematic water-proof protocols for onboard sampling*:* The Group recommends preparing simplified (possibly schematic) sampling protocols for onboard observers to quickly follow while sampling the various structures. This can include, for example, collection of spine samples for ageing, collecting brain cases for otolith extraction in the laboratory, tissue samples for genetics, etc.

Genetic sampling*:* Ongoing genetic work has shown that what is usually identified as WHM can probably be a complex of *Kajikia albida* and several *Tetrapturus* spp. The Group recommends that genetic samples are taken regularly from various regions and fisheries across the Atlantic to continue these studies and further improve knowledge on species identification and composition. A simplified protocol for genetic sampling is provided in **Appendix 5** of this report.

References

Andrews, A.H., R.L. Humphreys, and J.D. Sampaga. 2018. Blue marlin (*Makaira nigricans*) longevity estimates confirmed with bomb radiocarbon dating. *Canadian Journal of Fisheries and Aquatic Sciences*, 75: 17–25.

Arocha, F. and Beerkircher, L. 2012. Guide for the identification of Atlantic istiophorids. ICCAT, Madrid, Spain. 4pp. Available at https://www.iccat.int/Documents/SCRS/ISTIOPHORIDS_ENG.pdf

ICCAT. 2006-2016. ICCAT Manual. International Commission for the Conservation of Atlantic Tuna. In: ICCAT Publications [on-line]. Updated 2016. ISBN (Electronic Edition): 978-92-990055-0-7.

Krusic-Golub, K., Sutrovic, A., Nagom Sow F., and Rosa, D. Preliminary ageing of Atlantic blue marlin, white marlin and sailfish using otoliths. ICCAT presentation SCRS/P/2021/055.

Mayne, B., Berry, O., Campbell, D., Farley, J., and Jarman, S. 2019. A genomic predictor of lifespan in vertebrates. Scientific Reports 9, 17866. https://doi.org/10.1038/s41598-019-54447-w.

Mayne, B., Espinoza, T., Roberts, D., Butler, G. L., Brooks, S., Korbie, D., and Jarman, S. 2021. Nonlethal age estimation of three threatened fish species using DNA methylation: Australian lungfish, Murray cod and Mary River cod. Molecular Ecology Resources, 00, 1–9. https://doi.org/10.1111/1755-0998.13440.

Vitale, S., Andrews, A.H., Rizzo, P., Gancitano, S., and Fiorentino, F. 2016. Twenty-five-year longevity of European hake (Merluccius merluccius) from novel use of bomb radiocarbon dating in the Mediterranean Sea. Marine and Freshwater Research 67(7), 1077-1080. https://doi.org/10.1071/MF15376.

Appendix 1

ICCAT Billfish workshop on age reading
Online, 25-28 October 2021

Background and objectives

The Billfish Species Group initiated in 2018 in the framework of Enhanced Programme for Billfish Research (EPBR), a biological sample collection programme on hard parts (spines & otoliths) for three of the four main Billfish Species (Blue Marlin, White Marlin and Sailfish), captured in the Eastern Atlantic, because no previous billfish aging studies have been conducted in this region. This workshop aims to improve knowledge of age and growth rates for the Atlantic billfish main species.

The major objectives are: i) enhance current expertise in the Eastern Atlantic, ii) to standardize processing and reading protocols between laboratories (Eastern & Western Atlantic).

To achieve these goals of the workshop, the respective task coordinators on ageing are urged to have some samples already processed in order to make them available to the group by the time of the workshop.

1. Opening
2. Adoption of agenda
3. Nomination of the rapporteurs
4. Overview of sampling protocols and data collection database
5. Revision/update of protocols for ageing samples processing and
6. Initial guidelines for age reading including:
    a. Discussions on age verification and validation
    b. methods to correct for spine vascularization.
7. Other matters
8. Recommendations
9. Workshop report and adoption
10. Closure

Appendix 2

List of Participants
2021 ICCAT Billfish Workshop on age reading
(online 25-28 October 2021)

CONTRACTING PARTIES

BRAZIL
Leite Mourato, Bruno
Profesor Adjunto, Laboratório de Ciências da Pesca - LabPesca Instituto do Mar - IMar, Universidade Federal de São Paulo - UNIFESP, Rua Carvalho de Mendoça, 144, Encruzilhada, 11070-100 Santos, SP
Tel: +55 1196 765 2711, Fax: +55 11 3714 6273, E-Mail: bruno.mourato@unifesp.br; bruno.pesca@gmail.com; mourato.br@gmail.com

Lopes da Silva Ferrette, Bruno
Universidade Santa Cecília (UNISANTA), Rua Oswaldo Cruz, 277, Boqueirão, Santos, 11045-907 São Paulo
Tel: +55 119 757 8202, E-Mail: brunoferrette@gmail.com

Silva Batista, Guelson
Professor, UFERSA, Av. Francisco Mota, 572 - Bairro Costa e Silva, 59.625-900 Mossoró, Rio Grande do Norte
Tel: +55 859 850 32723, E-Mail: guelson@ufersa.edu.br

CANADA
Busawon, Dheeraj
Fisheries & Oceans Canada, St. Andrews Biological Station, 125 Marine Science Drive, St. Andrews, NB E5B 0E4
Tel: +1 506 529 5889; +1 506 467 5651, Fax: +1 506 529 5862, E-Mail: Dheeraj.Busawon@dfo-mpo.gc.ca

Kraska, Kelly
125 Marine Science Drive, St. Andrews E5B 0E4
Tel: +1 506 529 5891, E-Mail: kelly.kraska@dfo-mpo.gc.ca

CÔTE D'IVOIRE
Diaha, N'Guessan Constance
Chrecheur Hydrobiologiste, Laboratoire de biologie des poissons du Département des Ressources Aquatiques Vivantes (DRAV) du Centre de Recherches Océanologiques (CRO), 29, Rue des Pêcheurs - B.P. V-18, Abidjan 01
Tel: +225 21 35 50 14; +225 21 35 58 80, E-Mail: diahaconstance@yahoo.fr

EUROPEAN UNION
Rosa, Daniela
PhD Student, Portuguese Institute for the Ocean and Atmosphere, I.P. (IPMA), Av. 5 de Outubro s/n, 8700-305 Olhao, Portugal
Tel: +351 289 700 504, E-Mail: daniela.rosa@ipma.pt

Santos, Catarina
PhD Student, IPMA - Portuguese Institute for the Ocean and Atmosphere, I.P., Av. 5 Outubro s/n, 8700-305 Olhao, Portugal
Tel: +351 289 700 500, Fax: +351 289 700 53, E-Mail: catarina.santos@ipma.pt

Battaglia, Pietro
Researcher Stazione Zoologica Anton Dohrn (SZN) National Institute of Biology, Ecology and Marine Biotechnology SZN Sicilia Marine Centre Villa Pace. Contrada Porticatello 29, Messina
Italy
Tel: +39 081 583 3722, E-Mail: piebattaglia@gmail.com, pietro.battaglia@szn.it

GABON
Angueko, Davy
Chargé d'Etudes du Directeur Général des Pêches, Direction Générale des Pêche et de l'Aquaculture, BP 9498, Libreville Estuaire
Tel: +241 6653 4886, E-Mail: davyangueko83@gmail.com; davyangueko@yahoo.fr

LIBYA
F. Gafri, Hasan
Director - Libyan Commissioner to ICCAT, Ministry of Agriculture, Livestock and Fisheries, Department of Marine Wealth, Aldahra Street, P.O. Box 80876, Tajura Tripoli
Tel: +218 916 274 377, E-Mail: gafrihasan@gmail.com; abdusalam.zbida@gmail.com

MEXICO
Ramírez López, Karina
Instituto Nacional de Pesca y Acuacultura, Centro Regional de Investigación Acuícola y Pesquera - Veracruz, Av. Ejército Mexicano No.106 - Colonia Exhacienda, Ylang Ylang, C.P. 94298 Boca de Río, Veracruz
Tel: +52 5538719500, Ext. 55756, E-Mail: kramirez_inp@yahoo.com; karina.ramirez@inapesca.gob.mx

NAMIBIA
Jagger, Charmaine
Fisheries Biologist, Ministry of Fisheries and Marine Resources, National Marine Information and Research Centre (NatMIRC), P.O. Box 912 Swakopmund, 1 Strand street
Tel: +264 64 410 1000, Fax: +264 64 404385, E-Mail: Charmaine.Jagger@mfmr.gov.na

SENEGAL
Sadio, Nfally Sadia Petit
Technicien biologiste, ISRA/CRODT, Pöle de Recherche de Hann, Route du Front de Terre, 2241 Dakar
Tel: +221 776 518 684, Fax: +221 338 328 262, E-Mail: sadiasadio44@gmail.com

Sylla, Mor
Bioilogiste, Pôle de Recherche de Hann, Route du Front de terre, 2241 Dakar
Tel: +221 708 017 623, Fax: +221 338 328 262, E-Mail: msylleu2@gmail.com; msylleu@yahoo.fr

TUNISIA
Zarrad, Rafik
Chercheur, Institut National des Sciences et Technologies de la Mer (INSTM), BP 138 Ezzahra, Mahdia 5199
Tel: +216 73 688 604; +216 972 92111, Fax: +216 73 688 602, E-Mail: rafik.zarrad@gmail.com

UNITED STATES
Allman, Robert
NOAA/NMFS Southeast Fisheries Science Center Panama City Laboratory, 3500 Delwood Beach Road, Florida 32408
Tel: +1 850 235 6541 Ext. 206, E-Mail: robert.allman@noaa.gov

Beckwith, Anna
1907 Paulette Road Morehead City North Carolina, 28557
Tel: +1 252 241 7208, E-Mail: annabarriosbeckwith@gmail.com; anna@pamlicoguide.com

Darrow, Jamie
360 N. New York Rd, Port Republic, NJ 08241
Tel: +1 609 748 2020, E-Mail: jamie.darrow@dep.nj.gov

Golet, Walter
School of Marine Sciences, The University of Maine/Gulf of Maine Research Institute, 350 Commercial Street, Portland, Maine 04101-4618
Tel: +1 207 228 1671, E-Mail: walter.golet@maine.edu

Pacicco, Ashley
NOAA, 3500 delwood beach road, Florida Panama City 32408
Tel: +1 850 234 6541, E-Mail: ashley.pacicco@noaa.gov

Weber, Richard
South Jersey Marina, 1231 New Jersey 109, New Jersey Cape May 08204
Tel: +1 609 884 2400; +1 609 780 7365, Fax: +1 609 884 0039, E-Mail: rweber@southjerseymarina.com

VENEZUELA
Arocha, Freddy
Instituto Oceanográfico de Venezuela, Universidad de Oriente, A.P. 204, 6101 Cumaná Estado Sucre
Tel: +58 424 823 1698, E-Mail: farochap@gmail.com

Bustillos, Freddy
Asesor de la Gerencia de Ordenación Pesquera del Instituto Socialista de la Pesca y la Acuicultura, Ministerio del Poder Popular de Pesca y Acuicultura, 1015 Caracas
Tel: +58 426 913 6036, E-Mail: fxpesca@gmail.com

Castro Duno, Diego
Director General de la Oficina de Integración y Asuntos Internacionales, Ministerio del Poder Popular de Pesca y Acuicultura - MINPESCA, 1020 Caracas
Tel: +58 412 456 3403, E-Mail: castroduno@gmail.com; oai.minpesca@gmail.com

OBSERVERS FROM COOPERATING NON-CONTRACTING PARTIES, ENTITIES, FISHING ENTITIES COLABORADORAS

CHINESE TAIPEI
Cheng, Chun-Ya
National Taiwan Ocean University, No. 2 Peining Rd, 202301 Zhongzheng Keelung
Tel: +886 2 24622192 ext. 5046, Fax: +886 2 24622192, E-Mail: lucky_8043@yahoo.com.tw

Su, Nan-Jay
Assistant Professor, Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, No. 2 Pei-Ning Rd. Keelung, Zhongzheng Dist., 202301
Tel: +886 2 2462 2192 #5046, Fax: +886-2-24622192, E-Mail: nanjay@ntou.edu.tw

COSTA RICA
Pacheco Chaves, Bernald
INCOPESCA, Departamento de Investigación
E-Mail: bpacheco@incopesca.go.cr

OBSERVERS FROM NON-GOVERNMENTAL ORGANIZATIONS

THE BILLFISH FOUNDATION - TBF
Franks, Jim
Senior Research Scientist, University of Southern Mississippi Center for Fisheries Research and Development, 703 East Beach Drive, 39564, United States
Tel: +1 228 369 4301, Fax: +1 228 818 8832, E-Mail: jim.franks@usm.edu

Higgs, Jeremy
The Billfish Foundation, Gulf Coast Research Laboratory, 703 East Beach Drive, MS Ocena Springs 39564, United States
Tel: +1 228 818 8807, E-Mail: J.Higgs@usm.edu

SCRS VICE-CHAIRMAN
Coelho, Rui
Researcher, SCRS Vice-Chairman, Portuguese Institute for the Ocean and Atmosphere, I.P. (IPMA), Avenida 5 de Outubro, s/n, 8700-305 Olhão, Portugal
Tel: +351 289 700 504, E-Mail: rpcoelho@ipma.pt

EXTERNAL EXPERT
Andrews, Allen H.
Age and Longevity Research Lab (SII), Williams California 95987, ESTADOS UNIDOS
Tel: +1 460 708 223 808, E-Mail: astrofish226@gmail.com

Krusic-Golub, Kyne
Fisheries Scientist, Fish Ageing Services, 28 Swanston St, Queenscliff Victoria, 3225, AUSTRALIA
Tel: +61 438 306 179, E-Mail: kyne.krusicgolub@fishageingservices.com

Sutrovic, Admir
Fish Ageing Services, 28 Swanston Street, 3214 VIC, AUSTRALIA
Tel: +61 420 605 882, E-Mail: admir@fishageingservices.com

ICCAT
Neves dos Santos, Miguel
Ortiz, Mauricio
Kimoto, Ai

Appendix 3

Billfish Ageing - Reading Protocol

1. Identification of annuli

i. Spines

- Spine sections were photographed using a dissecting microscope illuminated with reflected light.
- Bands should be marked to the outer edge of each translucent zone (appears dark under reflected light).
- The last translucent band should only be counted if the reader considers that there is at least a small amount of opaque material between the band in question and the spine edge (i.e. being confident that the last translucent band is complete).
- The distance between true annual bands decreases proportionally with age; if the distance between two or three consecutive bands is clearly reduced these might be multiple bands.
- A true band can be tracked along the entire section; false bands or splits will not follow this pattern.
- If possible, provide edge type, where (see **Figure 1**):
    - T = translucent edge (translucent material visible on edge),
    - NO – Narrow opaque edge (opaque material past last translucent band is generally less than 1/3 of previously completed translucent band),
    - WO = wide opaque edge (opaque material past last translucent band is generally greater than 1/3 of previously completed translucent band).

ii. Otoliths

- Otolith sections were photographed at 40x times magnification using a dissecting microscope illuminated with transmitted light.
- Bands should be marked to the outer edge of each opaque band (appears dark under transmitted light).
- The last opaque band should only be counted if the reader considers that there is at least a small amount of translucent material between the band in question and the otolith edge (i.e. being confident that the last opaque band is complete).
- If possible provide edge type, where (see **Figure 2**):

    - O = opaque edge (opaque visible on edge),
    - NT = Narrow translucent edge (translucent material past last opaque band is generally less than 1/3 of previously completed translucent band),
    - WT = wide translucent edge (translucent material past last opaque band is generally greater than 1/3 of previously completed translucent band)

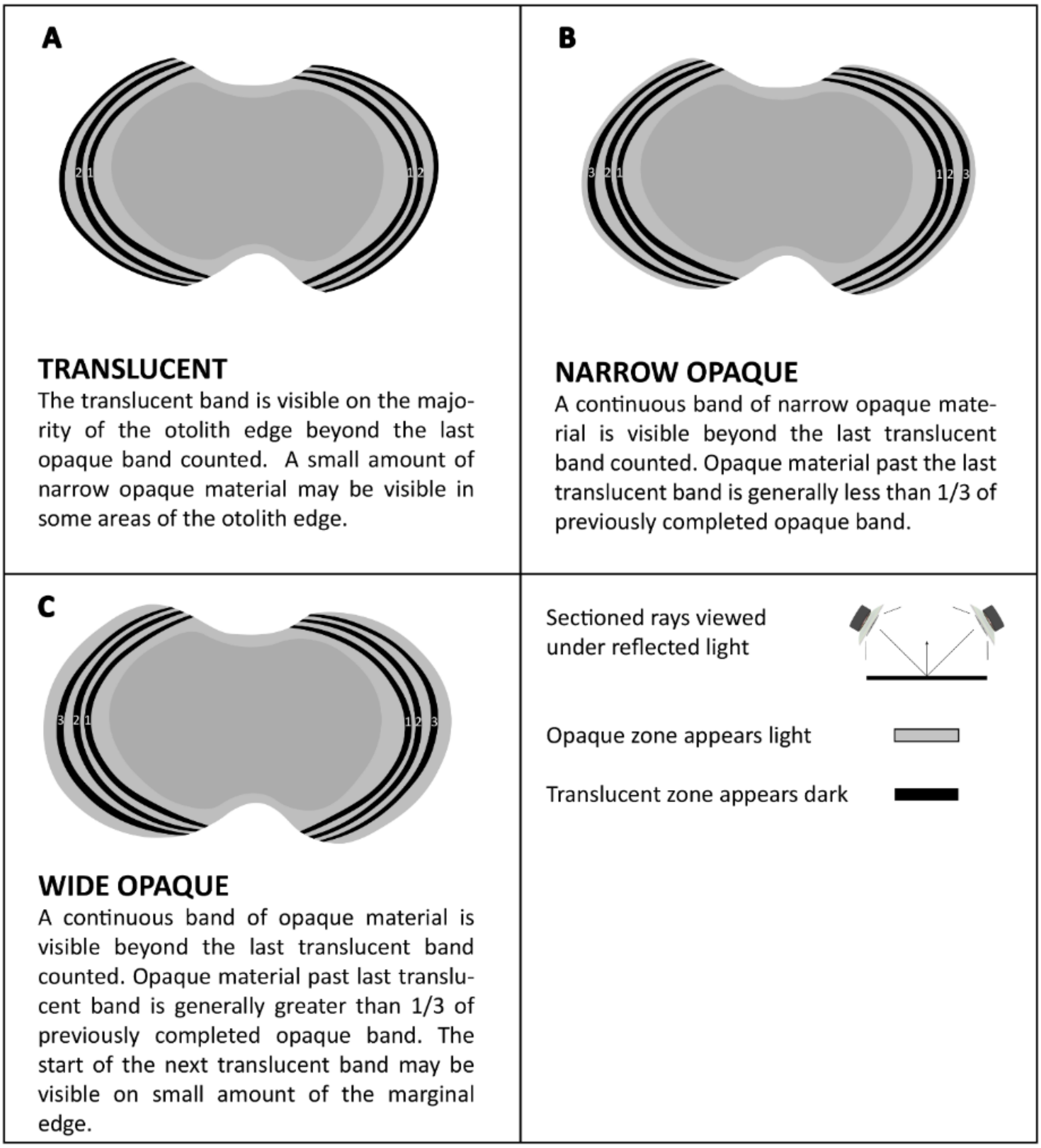


Figure 1. Cycle of band formation and edge classifications for billfish under reflected light (adapted from Farley *et al.*, 2016).

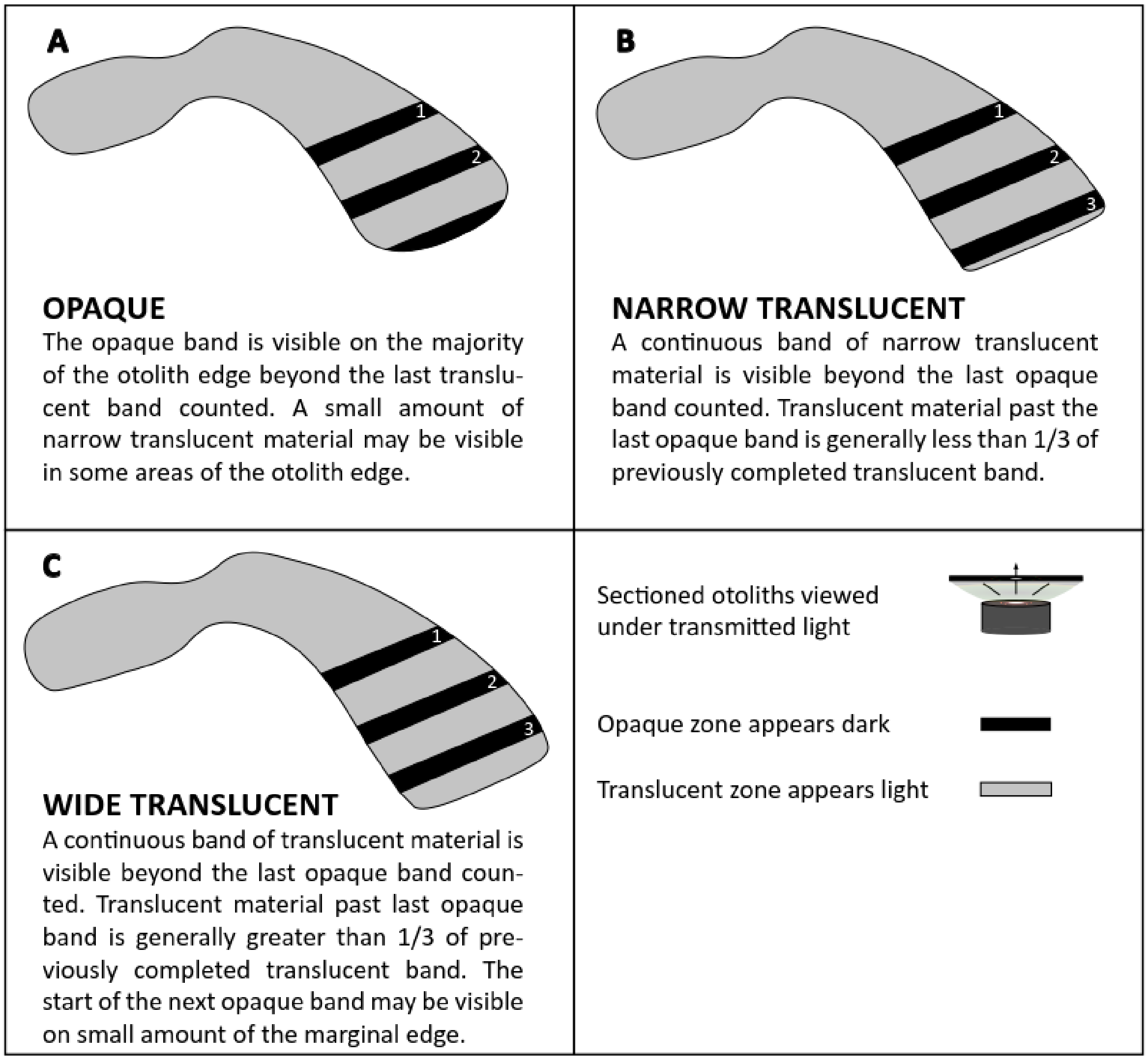


Figure 2. Cycle of band formation and edge classifications for billfish otoliths under transmitted light (source: Gillespie *et al.*, 2021).

References

Farley, J., Clear, N., Kolody, D., Krusic-Golub, K., Eveson, P., Young, J. 2016. Determination of swordfish growth and maturity relevant to the southwest Pacific stock. R 2014/0821: 118 pp.

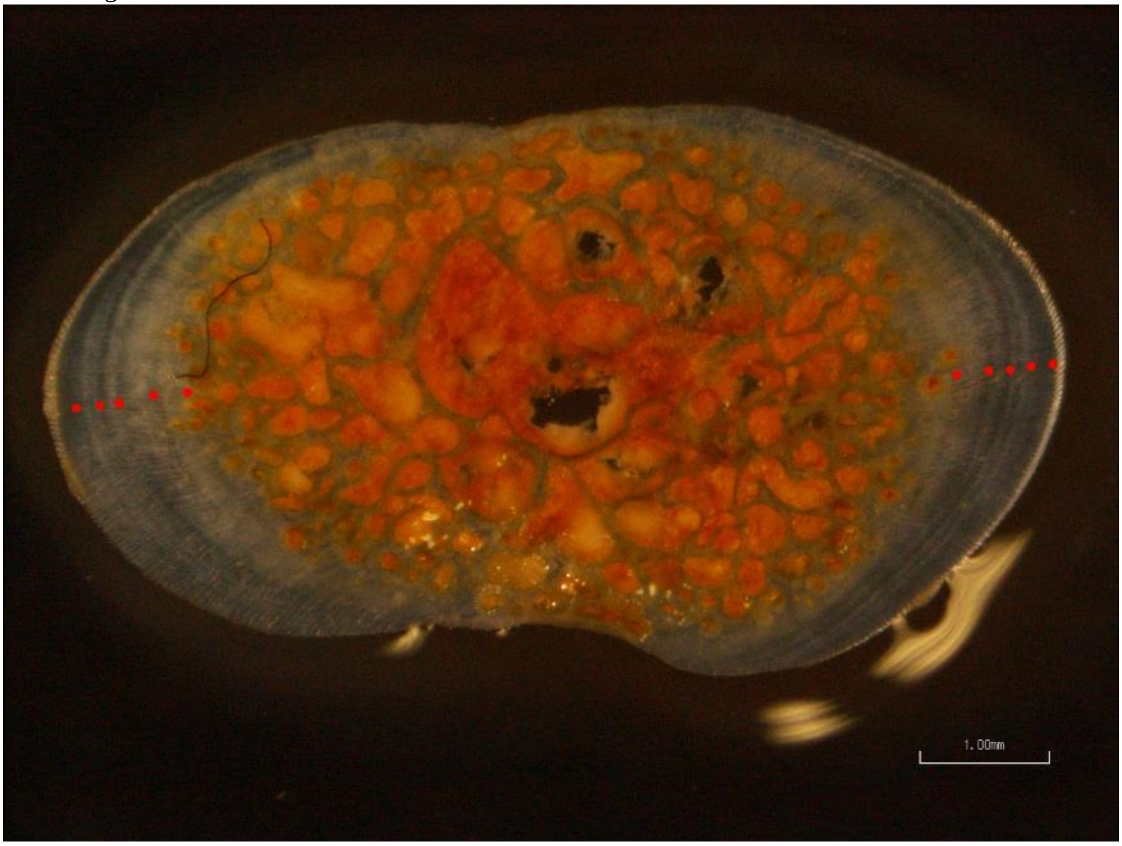
Gillespie K., Hanke A., Coelho R., Rosa D., Carnevali O., Gioacchini G., Macias D. (2021). Final report for Phase three of the ICCAT short-term contract: swordfish biological samples collection for growth, reproduction and genetics studies. ICCAT document SCRS/2021/119.

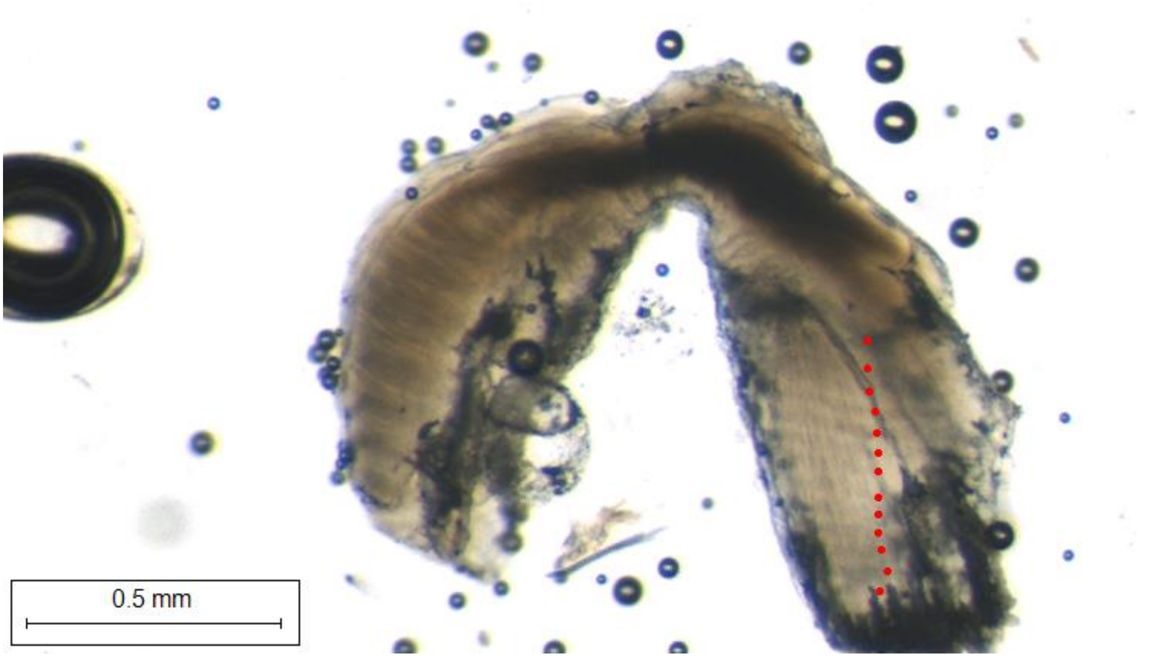
Appendix 4

Billfish spine and otolith images

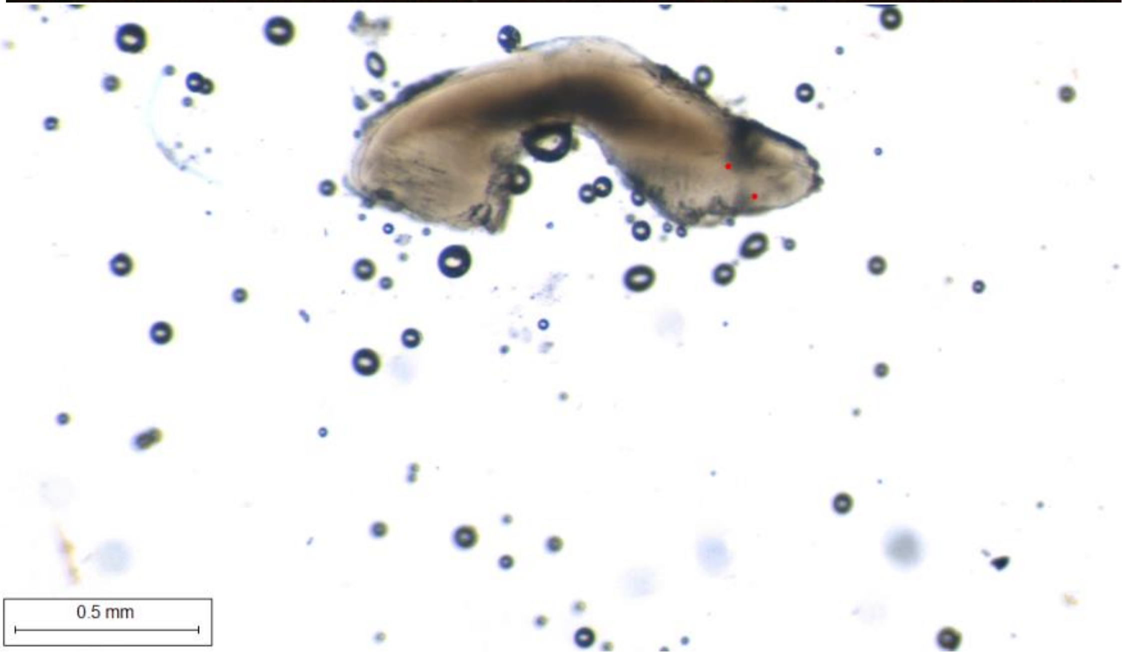
WHM

Pair 1 (162 cm male): Participants had a final reading of 5 bands, if counting the band between the 3rd and 5th point and the two inner bands that are partially in the vascularized zone. The otolith presents 13+ bands, as the edge is not clear enough to count.

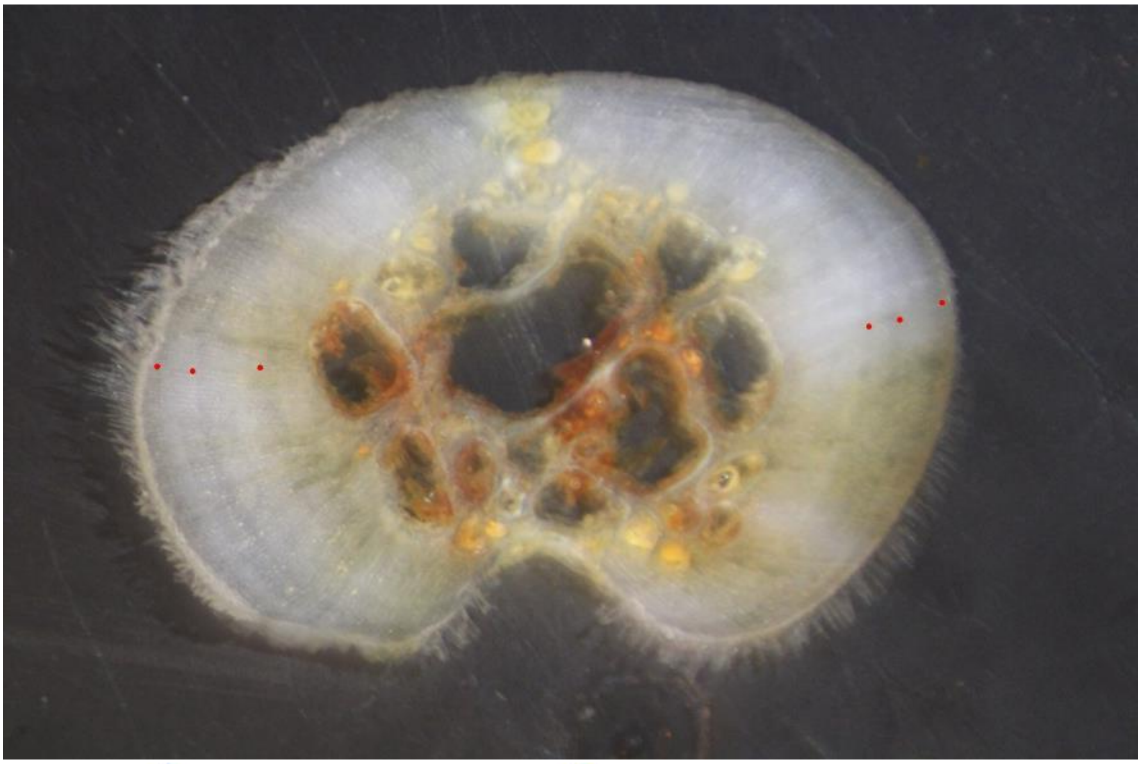


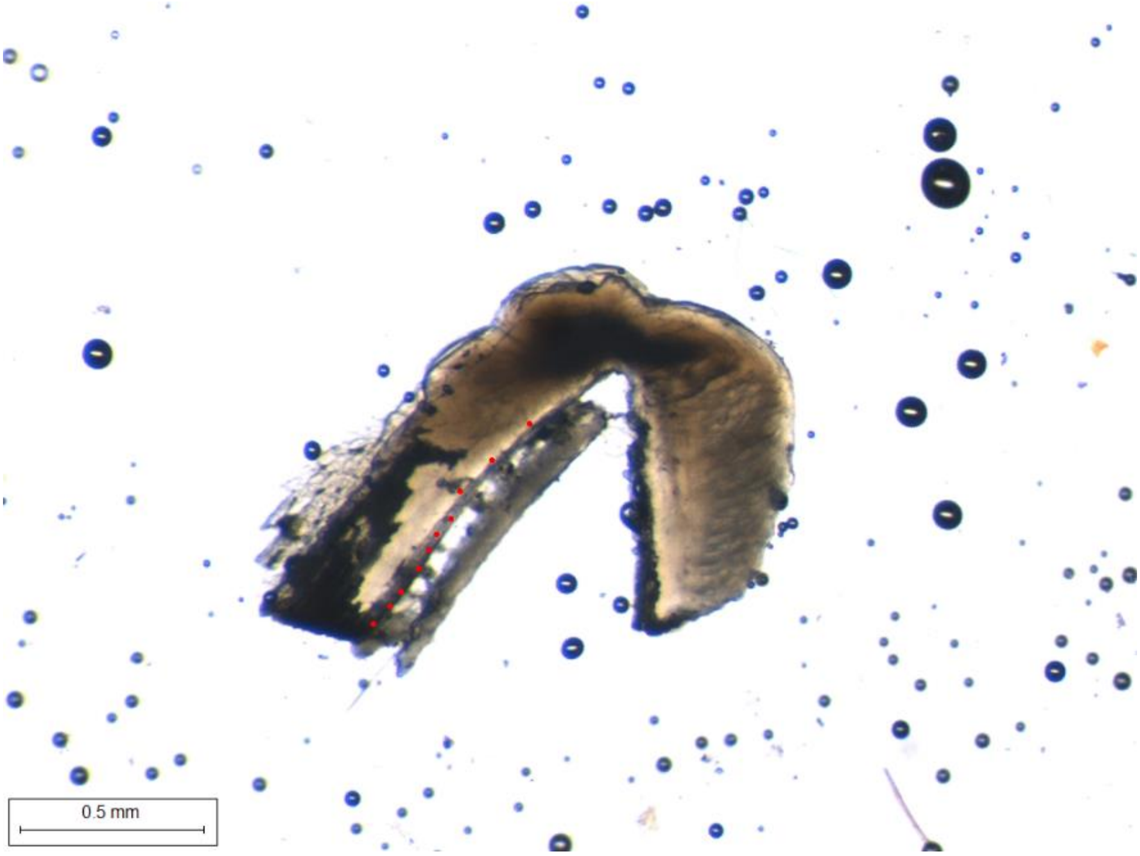


Pair 2 (160 cm female) – A band count of 2 was considered for the spine, one participant highlighted that two further bands could be present (in green), but probably only under the microscope, changing light settings, it would be possible to discern if these should be counted as annual marks. The otolith could be aged 1 or 2 years old, depending if the band closer to the primordium should be considered as the first band. In these cases, having a reference measurement from older individuals could help in deciding when to count the bands or not.



Pair 3 (180 cm female) – There was a consensus among participants that the spine presented 3 growth bands. Regarding the otolith there was difficulty in assigning an age estimate. Admir mentioned that for his preliminary reading he relied heavily on measurement from other individuals to assign an age. In this case it is also difficult to determine the deposition of the first annulus.



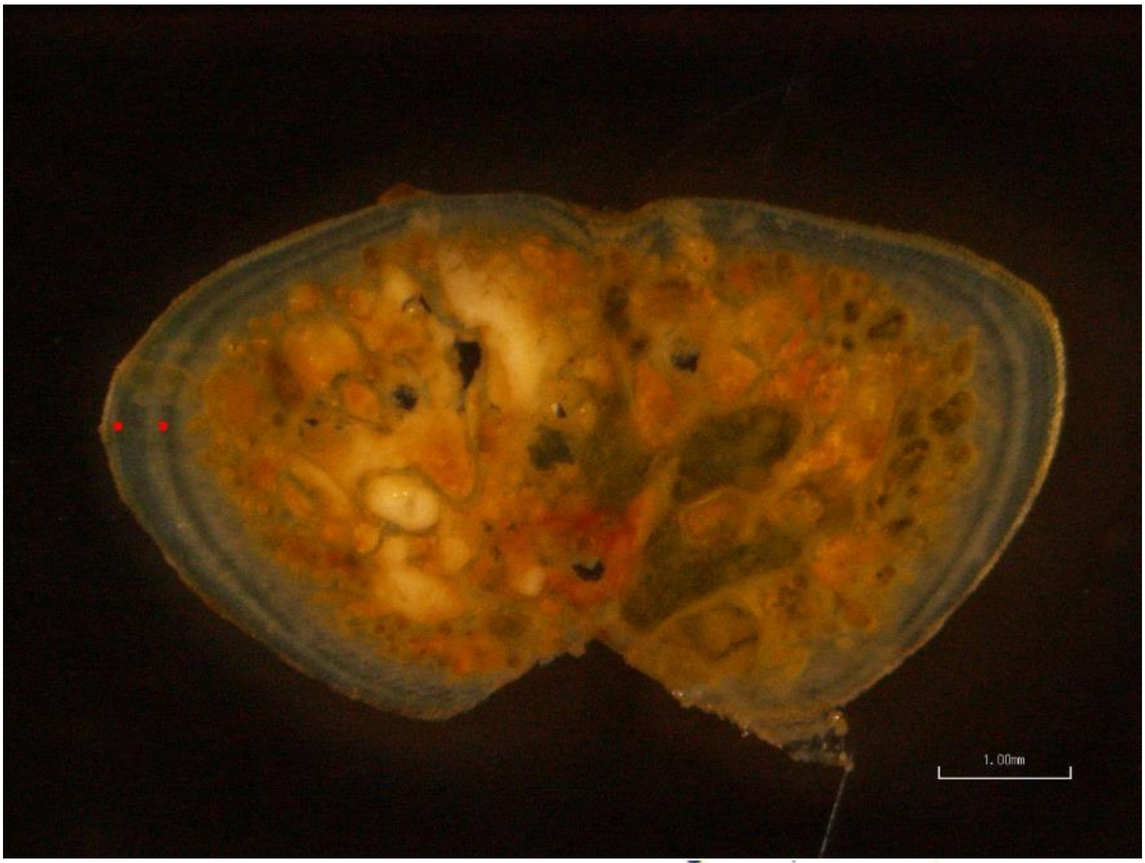


Pair 4 (160 cm female) – In the case of the spine, readers were counting between 2 and 3 growth bands, as in the right hand side lobe the last translucent band, looks completely formed, while on the left hand side lobe it appears the edge is still translucent. Regarding the otolith, there was also some doubts about the band deposition pattern and the location of the first annulus, as the annotated first mark (in red) is closer to the primordium than in other specimens.

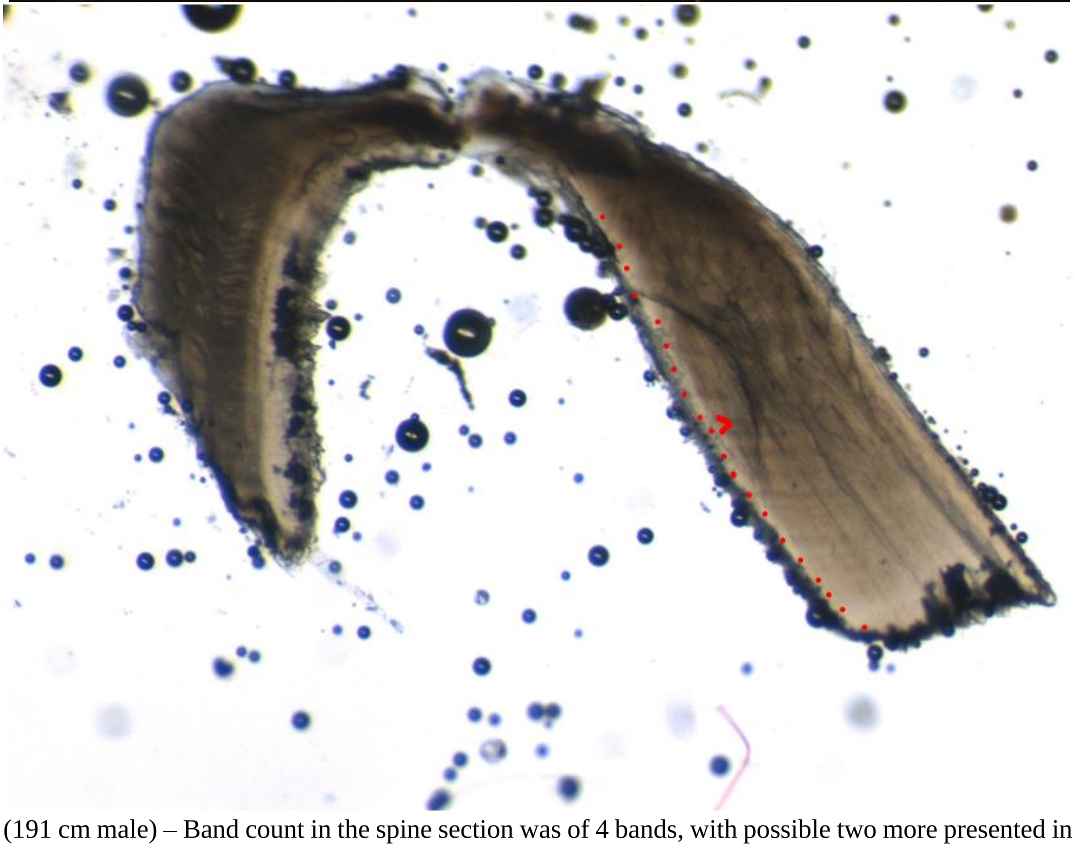
SAI

Pair 1 (168 cm Male) – The participants were counting either 2 or 3 bands, depending on the spine lobe, as on the right hand side the inner band (first red dot) can be counted, but it is missed on the left hand-side. Regarding the otolith, band count varied between 5 or 6 depending if it is considered that the first band should be closer to the primordium.

Pair 2 (180 cm male) – This spine presents two growth marks, and a high proportion of vascularization. The count of 13 (marked in the otolith image) is probably an overestimation, for the preliminary reading Admir was ageing it 7/8 years.



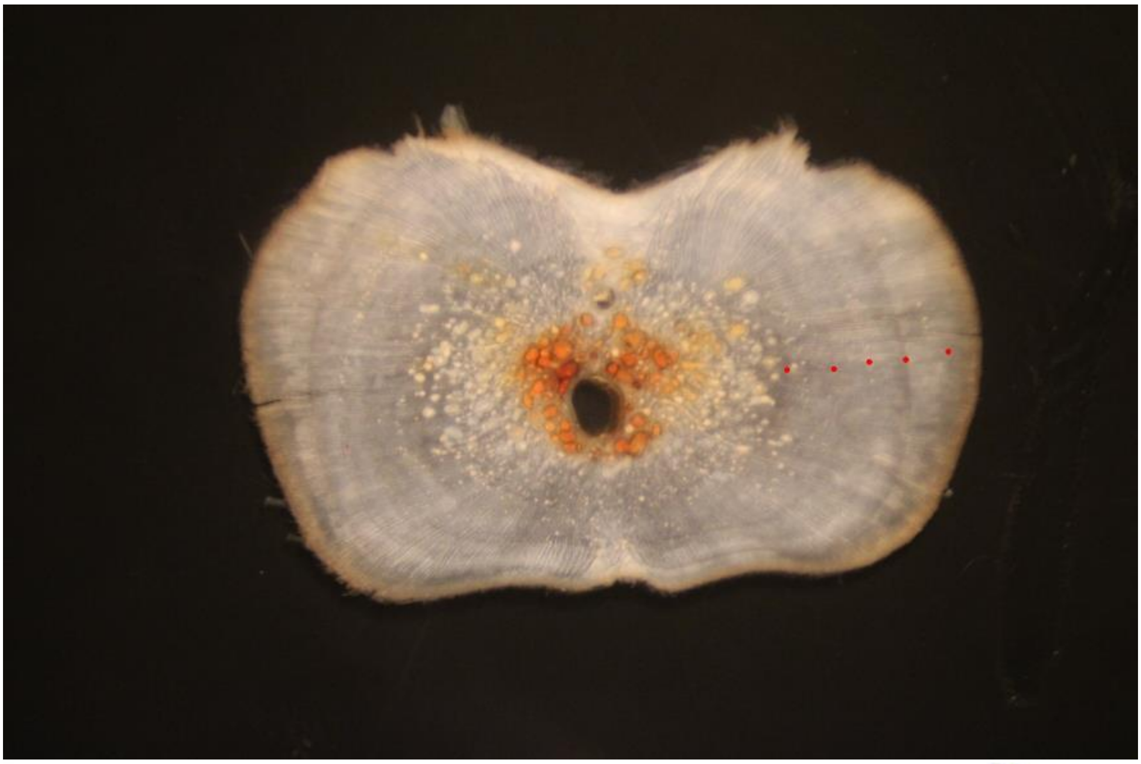
Pair 3 (199 cm female) – This is a highly vascularized spine, vascularization goes almost to the edge, although in the outermost areas some marks can still be counted, specifically 6 bands were counted. The otolith, presented a lot of marks, some of which could be splits or false annuli, considering that the bands in the edge are wider apart, previous bands should not be as close, as distance between bands should decrease with increasing size/age. The represented band count is probably overestimated.



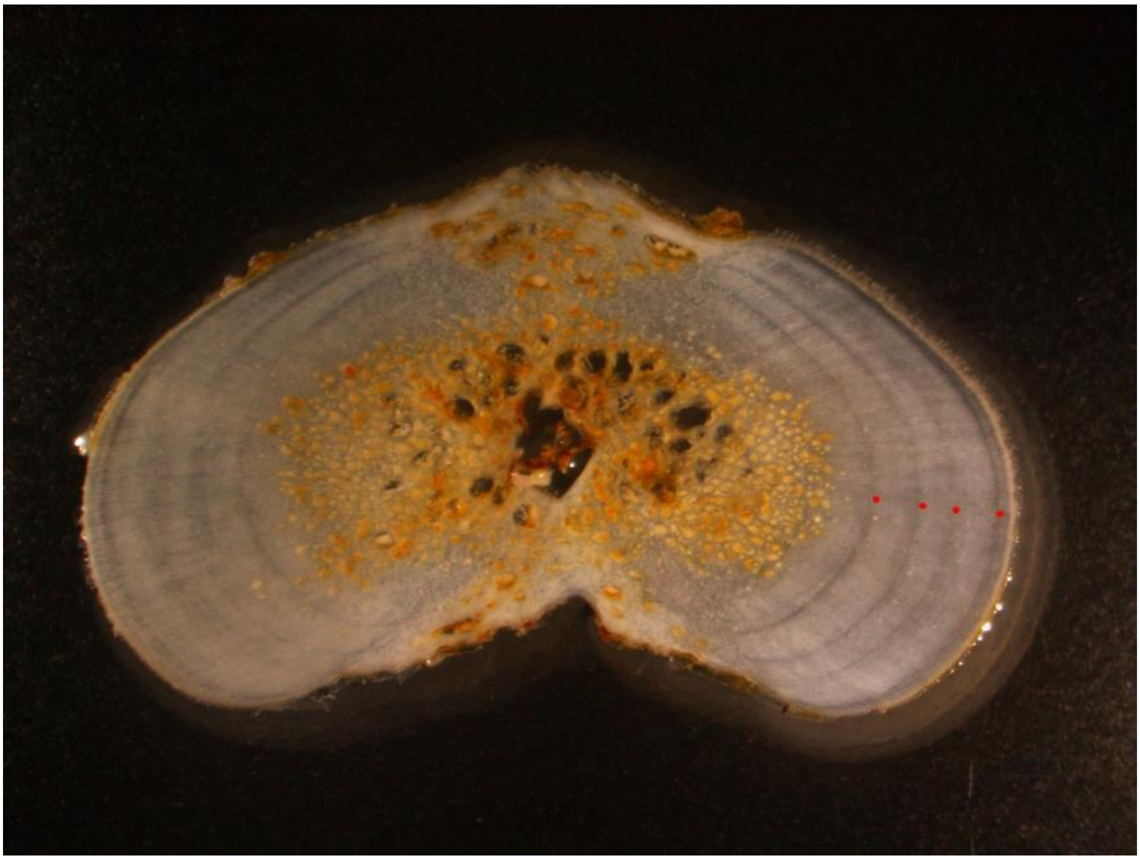
Pair 4 (191 cm male) – Band count in the spine section was of 4 bands, with possible two more presented in green. The corresponding otolith presents 3 growth marks, with one more on the edge, that is not possible to discern if it is a band or an artifact of the image being darker.

BUM

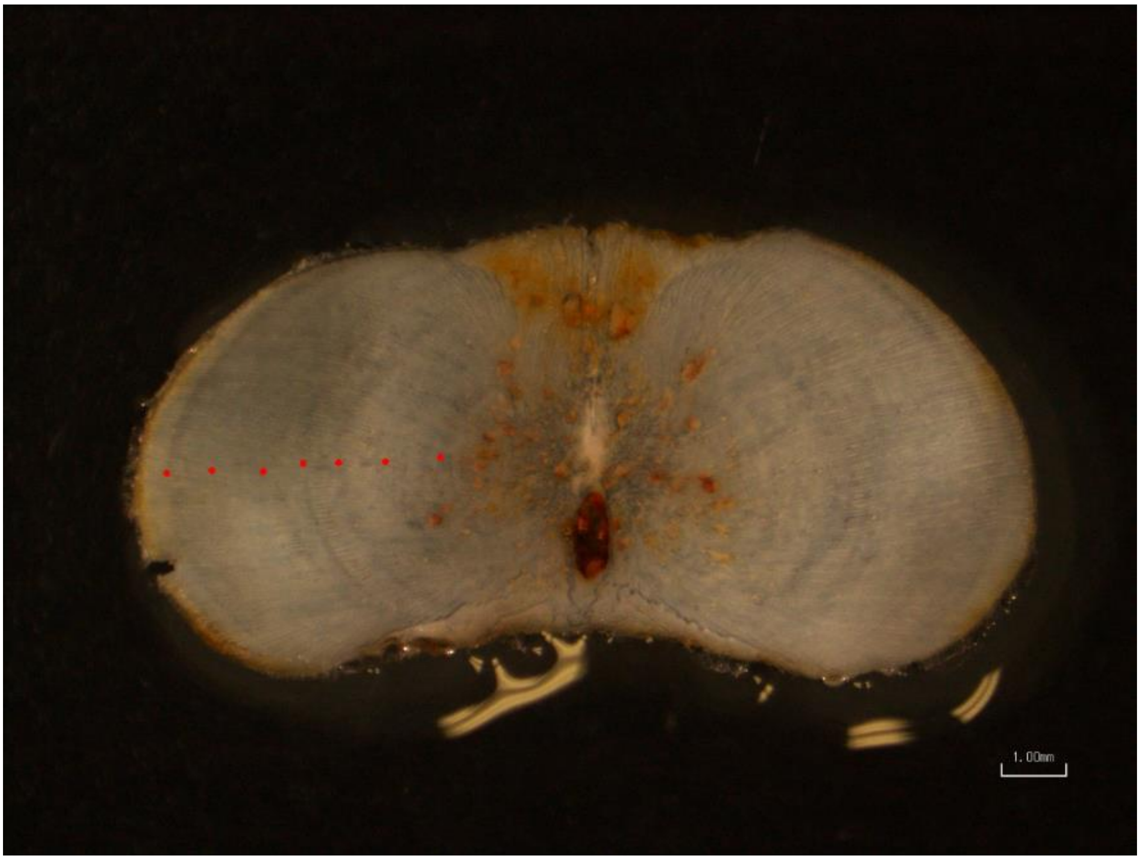
Pair 1 (220 cm female) – The spine band count estimate was of five bands, while the otolith presented only one opaque band.



Pair 2 (220 cm female) – It was noted that this spine presented a clear band deposition pattern, with a band count of four bands. The same count was obtained in the otolith, noting that for blue marlin the first annulus is located at a higher distance from the primordium than for other species.



Pair 3 (174 cm male) – In the case of the spine reading, the estimated band count of seven years is probably overestimated. For the otolith, two zones were marked, although not considered as an annual band, due to the close distance to the primordium.


1.00mm


0.5 mm

Appendix 5

Protocol for genetic sampling

Different tissues and preservation methods should be used depending on the study objective:

- *For population genetics and stocks delimitation:* collect two 1 cm cubic (one sample for genetic analysis, one for backup) of tissue (preferably muscle tissue but can also be large fin clips) and place them in separate labeled 2 ml vials, immersed in 95 % or higher pure absolute (PA) ethanol*. In this case the sample can be frozen before being stored in ethanol.

- *For the assembling of reference genomes:* collect two 1 cm cubic (one sample for genetic analysis, one for backup) of tissue (preferably muscle tissue) and place them in separate labeled 5 ml vials, immersed preferably in RNALater or can also be stored in 95 % or higher pure absolute (PA) ethanol.

- *For genome annotation and/or transcriptomics:* collect two 1 cm cubic (one sample for genetic analysis, one for backup) of different tissues (muscle, heart, liver, pancreas, brain, gonads, etc.) as soon as possible after the animal was capture, and place them in separate labeled 5 ml vials, immersed in RNALater (for this kind of sampling ethanol is not an option). The proportion of RNALater/tissue should be 1 to 5 for the perfect preservation of RNA molecules.

For all the sampling strategies: thoroughly wash the knife before and after collecting each sample. This is important so as to avoid contamination of genetic samples. Write the sample identification number on a small slip of paper and place it in the vial with the tissue sample. Vials should be stored in the freezer at -20°C.

* For population genetics and stock delimitation, other fixatives can also be used successfully, e.g. DMSO buffer.