REPORT OF THE 2019 ICCAT WORKSHOP ON SWORDFISH BIOLOGY STUDIES FOR GROWTH, REPRODUCTION AND GENETICS

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SUMMARY

This report describes the June, 2019 ICCAT workshop on swordfish biology studies for growth, reproduction and genetics, hosted by the Instituto Português do Mar e da Atmosfera, Olhão, Portugal . The major objectives of the workshop were to 1) refine sampling and biological data collection protocols, 2) develop protocols and start the sample processing and analysis, and 3) plan for the project future steps. The biological sampling program was established by ICCAT's Swordfish Species Group in 2018, aiming to improve knowledge of the stock distribution, age and gender of the catch, growth rate, age at maturation, maturation rate, spawning season and location and diet. This work will contribute to the next major advance in the assessment of swordfish status, by permitting the development of more spatially and biologically realistic population models used in both Atlantic and Mediterranean populations assessments and within the ICCAT Management Strategy Evaluation (MSE) for North Atlantic swordfish.

RÉSUMÉ

Le présent rapport décrit l'atelier de l'ICCAT tenu en juin 2019 consacré aux études sur la biologie de l'espadon pour la croissance, la reproduction et la génétique, organisé par l'Instituto Português do Mar e da Atmosfera, Olhão, Portugal. Les principaux objectifs de l'atelier étaient 1) d'affiner les protocoles d'échantillonnage et de collecte de données biologiques, 2) d'élaborer des protocoles et de commencer le traitement et l'analyse des échantillons, et 3) de planifier les étapes futures du projet. Le programme d'échantillonnage biologique a été établi par le Groupe d'espèces sur l'espadon de l'ICCAT en 2018, dans le but d'améliorer les connaissances sur la distribution du stock, l'âge et le sexe des prises, le taux de croissance, l'âge à maturité, le taux de maturité, la saison de frai, la localisation et le régime alimentaire. Ces travaux contribueront à la prochaine avancée majeure dans l'évaluation de l'état des stocks d'espadon, en permettant le développement de modèles de population plus réalistes sur le plan spatial et biologique utilisés dans les évaluations des populations de l'Atlantique et de la Méditerranée et dans le cadre de l'évaluation de la stratégie de gestion (MSE) de l'ICCAT pour l'espadon de l'Atlantique Nord.

RESUMEN

Este informe describe el Taller de ICCAT de junio de 2019 sobre estudios de biología de pez espada para crecimiento, reproducción y genética, acogido por el Instituto Português do Mar e da Atmosfera, en Olhão, Portugal. Los principales objetivos del taller eran 1) refinar los protocolos de recopilación de datos biológicos y de muestreo, 2) desarrollar protocolos e iniciar el procesamiento y análisis de las muestras y 3) planificar los pasos futuros del proyecto. El programa de muestreo biológico fue establecido por el Grupo de especies de pez espada de ICCAT en 2018, con el objetivo de mejorar los conocimientos sobre distribución del stock, edad y género de la captura, las tasas de crecimiento, la edad de madurez, la tasa de madurez, la ubicación y temporada de desove y la dieta. Este trabajo contribuirá al próximo gran avance en

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la evaluación del estado del pez espada, permitiendo el desarrollo de modelos de población más espacial y biológicamente realistas utilizados en la evaluación de las poblaciones tanto del Atlántico como del Mediterráneo y en el marco de la evaluación de estrategias de ordenación (MSE) de ICCAT para el pez espada del Atlántico norte.

KEYWORDS

Swordfish, genetics, maturity, ageing

Introduction

The 2019 ICCAT workshop on swordfish biology was held at the Instituto Português do Mar e da Amosfera (IPMA) lab in Olhão, Portugal from 18-21 June 2019. Dr. Rui Coelho (EUPortugal), the Swordfish Species Group rapporteur, opened the meeting and welcomed participants. The group briefly discussed the objectives of the meeting and the relevance of this biological work to the ICCAT swordfish stock assessment and the ongoing north Atlantic swordfish MSE process. The group proceeded to review the Agenda, which was adopted with no changes (**Appendix 1**). The List of Participants is included in **Appendix 2.**

1. Update on progress of the ICCAT swordfish project to date

The group was presented with an overview of the swordfish biology programme's progress to date. In 2018, ICCAT's Swordfish Species Group initiated a sampling programme in the North and South Atlantic and Mediterranean. The aim of the program was to collect biological data that would support research important to the assessment and management of this highly migratory and internationally managed species. In the first year of the programme, an international group of institutions developed a preliminary sampling protocol, collected samples from 1762 fish, developed a database for sample data, and identified strategies for optimizing further sample collection and data analysis. Fish were sampled for size, sex, and maturity; the location and date of capture (and/or landing); calcified parts (anal fin spines and/or otoliths) and/or tissues were collected, processed, and archived for future analysis. The group was presented with an initial overview of length frequencies by sex and stock as well as an analysis of sample spatial-temporal representativeness relative to ICCAT task 2 catch data. Sample data collected for each stock in many cases were representative of the major swordfish catch locations and timing, however, in some locations and seasons, additional sampling coverage is required. These analyses prompted discussion on how best to continue the project so that sample data effectively make contributions to the understanding of patterns of growth, maturity, movement and mixing among the three swordfish stocks under ICCAT management.

2. Revision/update of sampling and biological data collection protocols

The group reviewed the sampling protocol used in the initial year of data collection. For each of the project subject areas (ageing and growth, reproduction and maturity, and genetics) the group added detail to protocols that will add consistency among sampling institutions. Workshop participants made suggestions based on scientific literature and experiences in sampling programs for swordfish and other pelagic fishes in the Mediterranean Sea and Atlantic, Indian and Pacific oceans. The group discussed collection of additional sample materials (otoliths, gonads), and the trade-offs between obtaining a full sample set for each fish and the various challenges of collecting fisheries dependent data at sea or through port sampling. Additional protocols were developed for the collection of otoliths and gonads. Recognizing that there are additional costs associated with collecting additional biological materials, the group discussed the possibility of making available additional compensation for collection of otoliths and gonads. The group discussed best practices for transport of samples between institutions for analysis and archiving and shipping protocols for biological materials were developed. Noting the necessity for strategic spatial-temporal sampling, there was discussion on the need for development of an online tool for rapid update of the programme database with field sample data.

3. Establishing protocols to start sample processing and data analysis

3.1 Ageing, including spines vs. otoliths

There was a presentation on age estimation and growth of swordfish in the Southwest Pacific from Farley et al. (2016). This presentation summarized work to estimate the age and growth of swordfish in the SW Pacific using fin spines and otoliths. The aim of the work was to (i) determine if differences in growth parameter estimates obtained in previous studies of swordfish in the SW Pacific and Hawaiian regions using spines were methodological or due to spatial variation in life-history; and (ii) determine if sectioned otoliths could be used to corroborate age estimates from spines. After undertaking an inter-laboratory age comparison study and re-examining the sectioned spines from the SW Pacific, it was found that methodological differences could explain the different growth parameter estimates obtained in the previous studies in the Pacific. However, the study also found that spine-based ages were only similar on average to otolith-based ages in the youngest age classes. A bias was evident in age classes >7 years for females and >4 years for males, where otolith ages were higher on average compared to spine ages. As expected, the otolith-based growth curves indicate slower growth and a higher maximum age for both sexes compared to the spine-based growth curves. As otoliths are generally considered to be the more accurate structure to age fish than other hard-parts, the study recommended that otoliths should be used to estimate age and growth of swordfish worldwide, particularly for the larger/older individuals. Direct validation of age using otoliths and spines is still required for swordfish.

There was a presentation on the standardization of direct ageing methodology of swordfish using anal fin spines (SCRS/2019/042; to be presented at the September 2019 swordfish Species Group meeting). Different studies have compared different hard structures to age swordfish all around the world. Otoliths and spines have been found as the most suitable options. Despite otoliths being considered as the most realistic hard structure their small size is a problem. On the other hand, the fin spines have some issues too, due to the remarkable bone remodelling in the inner part of the spine. Trying to avoid the issues identified in the fin spines, some comparisons and recommendations have been presented: take particular care when cutting and cleaning the fin so as not to damage outer bony layers; examine the anterior part of the fin and to carefully check if a vestigial first spine exists. Different configurations of the first 3 spines have been found, with one of the types representing around 90% of the samples (**Figure 1**). The 2nd spine of the "type A" configuration is recommended for ageing. Comparative analysis between 0.5 and 1 times distances from the width of the condyle base have been performed. The section that seems to have a lower vascularised area is the section at 1 times the distance from the width of the condyle base. Finally, it was recommended to use the focus line (the reference line which connects the two innermost ends/margins of the lobes) of the structure with perpendicular lines for measurements (**Figure 2**), as the focus line is easy to identify and adaptable to all shapes of the lobes.

A presentation was made reporting the work done in the framework of the Project RECOLAPE (contract MARE/2016/22 – funded by EU DG-MARE), where some tasks were specifically focused on Mediterranean swordfish as a case study. In particular, there was comparison of age-length keys (ALK) in different EU Member States and a relative exploratory analysis and comparison of possible tools to coordinate age-reading. Data available on the swordfish age-sampling strategy in Italy, Greece and Cyprus was evaluated. The precision of the ALKs expressed in terms of coefficient of variation (CV) was estimated for each age group by the means of Principal Component Analysis (PCA). The main results of this analysis confirmed the high variability in the ageing of swordfish. Inside the Mediterranean, two main aspect were found affecting the precision of the age data: age reader experience emerged as a key issue in estimating the age, both in the first-year age group and the oldest age group (>4 years), and the geographical location, with longitude being the most significant factor. Other factors affecting the precision of data were identified, such as age estimation scheme and criteria, hard parts used (otolith or spines), and definition of birthday. In the framework of the same project an exchange exercise based on 79 fish samples collected in the Mediterranean (the Ligurian Sea and the Alboran Sea) was also held. The images of the second anal fin spine sections were prepared and shared among the participants by the means of the SMARTDOTS platform, commonly used by the ICES working groups on age determination. Eight age readers from 4 countries and 5 laboratories participated in this exchange. The preliminary results were already presented and discussed during a Workshop on Swordfish Age-Reading, held in Olhão (Portugal) from the 2nd to the 4th April 2019 and hosted by IPMA Institute. The overall precision was 64.4%, 30.8% and 23%, for Percentage Agreement (PA), Coefficient of Variation (CV) and Average Percentage of Error (APE), respectively. These results could be explained by the difficulty in recognising the first growth increment and the increments in fishes older than 5 years, due to reabsorption of inner bands in the central vascularized area. At the end, a reference set of 11 spines was selected using the sections with a PA $\geq$ 80%. The utility of SMARTDOTS software was discussed and it was pointed out that some improvements are needed in order to make the platform more flexible and useful, such as the implementation of tools for taking measurements.

The need for a common set of methods (preparation of the spines and otoliths) and procedures (aging scheme and criteria) was outlined. The differences between anal fin spines and otoliths was discussed. It is possible that spines, besides the vascularization, can also reabsorb and/or remobilize the increments. Even if the deposition of the increments can be validated by marginal increment analysis of annual rings, there is a possibility that the increments will not remain in the same positions, preventing accurate validation of increment counts. Otoliths do not suffer from these types of remodelling, once the increments are deposited, they remain visible and immobile throughout the fish life.

It was also noted that there are no direct validation studies on increment deposition rate in swordfish hard parts. The work from Farley et al. (2016) tried to validate the location of the first 1-2 increments through daily ageing, however after the first 150 days the increments become diffuse which coincides with the location of the first annual opaque zone. In the current project, this method could be used to validate the distance at which the first opaque zone is deposited, assuming swordfish have a restricted spawning season in the Atlantic and Mediterranean.

Currently, otolith sampling is considered optional in the project, however, participants have collected ~300 otoliths for the Atlantic and ~50 for the Mediterranean (**Figure 3**). It was encouraged that otolith sampling continue to occur. It was discussed that the compensation price would have to be changed for some participants to be able to collect the otoliths as additional sampling materials and effort is required and in some cases the whole fish would have to be bought. It was noted that next year the sample collection could be refined so that areas where no data exists the compensation could be higher in order to obtain all the necessary samples.

As noted by Quelle et al. (2014) there are multiple configurations found in swordfish anal fins. Type A (see **Figure 1**) represents the majority found in nature. As such, it was agreed that at this stage only the Type A second spine should be sent and used for age reading.

3.2 Reproduction and maturity

The group was presented with three main meeting objectives for development of reproduction and maturity studies: 1) refine sampling and biological data collection protocols for gonad collection, 2) coordinate sample processing and analysis logistics, and 3) plan for project future steps. Firstly, it was remarked that the following data and samples are required for maturity studies: gonad weight, gonad tissues and photographs of gonads. Sampling by length classes, taking into account the stock, area, and season should be conducted. It was noted that in some cases, onboard observers may have difficulties identifying sex or maturity stages. To address these challenges, it was proposed that the group create an international maturity guide for swordfish.

A presentation was made to demonstrate new technologies for the evaluation of gamete quality and gonadal maturation in swordfish (SCRS/2019/027). Results from Fourier-transform infrared spectroscopy (FTIR) analysis were presented (Carnevali et al. 2019). FTIR enables macromolecular characterization of oocytes at different developmental stages from oogonia to mature eggs. Such analyses should enable assessment of differences in egg quality related to animal age, size, spatial and temporal distributions, and possibly to identify differences between populations. Secondly, results from an analysis of swordfish maturation using transcriptomics were presented (Gioacchini et al. 2019). These analyses have application for the study of the molecular mechanisms governing puberty onset on swordfish. Finally, in light of uncertainties in the gonad macroscopic evaluation, the importance of histological analysis was emphasized. In this presentation a gonadosomatic index (GSI) validated by histology classification of gonadal stages was presented. To support this maturity work, it was requested that observers involved in the biological sampling also measure the weight of the left gonad and collect samples for histological, FTIR and molecular analyses.

There was a presentation on a study examining the reproduction and maturity of female swordfish in the SW Pacific based on histological analysis of nearly 700 ovaries (Farley et al., 2016). The majority of ovaries had been frozen before a subsample was taken and fixed in 10% formalin, and the quality of the histological sections was suitable for analysis. If analysing frozen gonad tissue, it is recommended that the tissue is fixed while still frozen rather than defrosting the material before fixation. The histological sections were read to assess the ovarian development phase following Brown-Peterson et al. (2011). Additional 'maturity markers' were used to differentiate immature from regenerating females, including muscle bundles, brown bodies (melano-macrophage centres) and encapsulated hydrated oocytes. The study found that the spawning season for swordfish in the SW Pacific is between November and March (austral summer), and that length at 50% maturity is approximately 161 cm orbital fork length (OFL). The estimate of age at 50% maturity was 4.34 years using spine-based age data and 4.42 years using otolith-based age data. The similarity in A_{50} estimates from spines and otolith data is expected given the similar growth rates obtained using these structures for females aged 0-7 years (see section 6.1; Farley et al. 2016).

There was a presentation on the recently initiated Gen&Rec project (Distribution and behaviour of swordfish spawners and juveniles in the vicinity of Corsica). The project began in March 2019, and brings together 5 partners (IFREMER-UMR MARBEC, University of Corsica, IRD-UMR MARBEC, the UMR5175 CNRS-CEFE and the fishing industry, CRPMEM of Corsica) to increase scientific knowledge on reproductive dynamics of swordfish around Corsica. The project aims to determine potential spawning and nursery grounds along with the movements of spawners and juveniles over a period of several months. Reproductive activity will be assessed using macroscopic gonad characteristics, trends of gonadal indexes, sex-ratios for both sexes, oocyte size-frequency distributions and microscopic investigation of oocyte development stages. Environmental DNA techniques will be used to identify potential spawning grounds. The project will investigate large scale vertical movements of juveniles and spawners using 21 pop-up satellite archival tags (PSAT). Longlines instrumented with cameras and sensors will be used to help characterize behaviour of juveniles in the vicinity of the fishing gear.

The group discussed advantages and disadvantages of macroscopic maturity stages and the necessity of using histological examinations of gonads for differentiating between mature and immature individuals. The use of frozen gonads for histological analysis was discussed and the group agreed that frozen gonad are acceptable for histological analysis if fresh material is not available. The procedures for sampling frozen gonads will be added to the sampling protocol. The definitions of undetermined individuals (sex not distinguished by naked eye) and not sexed individuals (fish is not gutted, so the sex in not available) were clarified and incorporated into the sampling protocol. The macroscopic maturity classification for swordfish was improved and it will be used for the participants in the Consortium. The group discussed the importance of developing a histology reference set and suggested a joint aging and histology reference set creation workshop.

3.3 Genetics

A presentation summarising work completed over the last two decades on swordfish genetic populations analysis. Much of this work has been conducted using mitochondrial DNA (mtDNA) and allozyme genetic markers. More recently, microsatellites from nuclear DNA (nDNA) have been used.

These studies illustrated the following: i) substantial differentiation of Mediterranean swordfish from Atlantic populations; ii) lower levels of genetic variation in the Mediterranean population compared to other comparison populations; iii) significant frequency differences between pooled North Atlantic samples and South Atlantic samples (South of 5°N) and finally, iv) inter-oceanic population differentiation of Atlantic, Mediterranean and Indo-Pacific swordfish. In the frame of this presentation, preliminary results obtained by UNIVPM on genetic populations analysis on 298 individuals sampled from 6 different Mediterranean areas, have been reported. In this study it was demonstrated that when using mtDNA as markers, Mediterranean swordfish exhibit low levels of genetic variability compared to those reported for Atlantic and Pacific stocks. No geographical pattern of genetic differentiation was observed between Mediterranean areas estimated using global FST value. Analysing the same samples using 20 microsatellites, 3 different genetic clusters were identified, statistically supported by pairwise FST estimation and DAPC analysis. Distribution of genetic clusters identified by DAPC analysis showed high levels of mixing within the sampling areas.

Variation in results obtained from different genetic variability markers indicate that despite considerable advances in our understanding of Atlantic swordfish genetic population structure, i) mtDNA results are not always in agreement with those obtained by nDNA and ii) that both mtDNA and nDNA studies share several shortcomings due to the limited markers of the tools. Therefore, genome-wide, high-resolution, and more reliable markers are needed to achieve a complete overview of the genetic population of swordfish. Recent technological advances in next generation sequencing (NGS) have dramatically increased sequencing throughput, reduced associated costs, and together with the development of bioinformatics tools, have opened the door for population genomics studies in any species. The study of single nucleotide polymorphisms (SNPs) across the whole genome is a powerful tool for genetic studies. In light of the strengths of the whole genome SNP approach, a set methodologies was presented for analysis of genetic differentiation among North-, South- Atlantic and Mediterranean Swordfish populations.

The first step in the whole genome SNPs analysis is de novo full genome sequencing and assembly. Genome assembly and annotation will be facilitated by the availability of the transcriptome already completed by UNIVPM. This genome will be used as a reference for the study of various genetic populations. Population genetic analysis will be completed using an approach based on the ddRAD technology. The reads will be mapped against the assembled genome and used to perform a variant calling analysis to identify the variants in all the individuals and compare the different populations using dedicated software. It was proposed that in this first phase it will be possible to analyze ~200 samples. The group was receptive this approach and decided that the first priority was to conduct genetic analysis on animals of known stock origin. It was assessed that this first genetic analysis will be

conducted on spawning animals (or young of the year) caught in spawning areas for each of the three stocks in question (e.g. Caribbean Sea, Gulf of Guinea, Mediterranean Sea). Once genetic markers for spawning stocks have been identified, the analysis will focus on identifying stock boundaries and mixing. Priority will be given to animals for which are available spines and/or otoliths for the aging evaluation and gonads for the histological analysis. Regarding Mediterranean populations analysis, it was decided to analyze part of the 298 animals already evaluated by using mtDNA and microsatellites, in order to elucidate the contrasting results obtained and finally to better describe the genetic structure of swordfish populations in the Mediterranean Sea.

Analysis of mixing areas and across the total spatial range of the species, as well as analysis of samples from spawning areas will be undertaken in both current and future projects. We also proposed to analyze samples coming from other Oceans (Pacific and/or Indian) to assess the interocean variability of swordfish genetic populations.

A presentation was made on a large program of work being undertaken by CSIRO (Australia) using next-generation sequencing genetic methods to study a range of tuna, billfish and shark specie in the Pacific, Indian and Atlantic Oceans. SNPs genetic markers were used to investigate population structure/provenance; develop species identification assays; and estimate the absolute abundance of adult and juvenile fish using close-kin mark-recapture and gene-tagging techniques, respectively. Results of population studies for bigeye and yellowfin tuna were highlighted, as well as current work on other species including swordfish in the Pacific and Indian Oceans. The presentation noted several important factors to consider in population structure studies such as sampling design, the importance of sampling spawning adults, larvae or YOY to identify spawning populations, quality control of large datasets to identify poor data, cross-contamination, sequencing platform and batch effects, and finally the use of appropriate analysis approaches & packages.

4. Discussion and plans for future sampling and priority areas for the project

The group identified priority sample analyses for information collected in the initial year of the programme, discussed upcoming research and analysis steps for supporting ICCAT's swordfish assessment and management, and developed recommendations for the swordfish species group and the SCRS. Sampling effort will prioritize filling spatial-temporal and size-sex gaps in the current data set and will add capacity for collection of gonads and otoliths. The ageing and growth project coordinators will continue to process spines and otoliths collected in the initial year of the program and will begin development of an aging reference set. Maturity and reproduction work will focus on development of a macroscopic maturity stage key and the development of histological standards for identification of maturity stage. Genetics analyses will concentration on sequencing the swordfish genome and conduct SNP analysis of spawning or young of the year fish to genetically differentiate between stocks.

5. Recommendations

5.1 General recommendations

Labeling fish that have been used for science (ICCAT Science Certification Label): Fish that have been sampled often can lose value, for example due to slight damage to the carcass (e.g., removal of fins). The Group recommended considering the feasibility of creating an ICCAT Science Certification Label, where fish that have been sampled for science will be recognized. This could increase the value of such fish, as concerned consumers would be assured that such fish were captured and sampled by scientific observers, and that such samples have contributed to the knowledge of the biology of the species and assessments of the stocks, ultimately contributing to the sustainable use of those resources.

Sampling of undersized fish (adopted by the SCRS in 2018, no action from the Commission in 2018): Currently there are Minimum Sizes established for Atlantic swordfish (Recs 17-02 and 17-03) and Mediterranean swordfish (Recs 16-05). Those "minimum sizes" refer to either "taking and landing" or "catching and retaining on board", depending on each specific Recommendation or paragraph. In order to allow the collection of biological samples during commercial fishing operations on undersized swordfish (e.g., vertebrae, tissue, reproductive tracts, stomachs) the SCRS recommends that the Commission considers establishing a new ICCAT Recommendation allowing such procedures. Such permission could be similar to what is already established for no retention shark species (ICCAT Rec. 13-10). The sampling on undersized swordfish could only be carried out if:

- animals are dead at haulback;
- samples are collected by a fishery observer and
- samples are taken in the framework of a research project endorsed and carried out within the priorities of the Swordfish Species group and the SCRS.

5.2 Specific project recommendations

Age validation for swordfish: Direct age validation for SWO has not been carried out anywhere in the world yet. As such, the Group recommends that this is given priority in the future, possibly involving oxy-tetracycline tagging and/or bomb radiocarbon. It is noted that oxy-tetracycline tagging would involve tag and release of many swordfish which would have considerable costs. Additionally, post-release mortality of swordfish is high, which would also create problems in such types of tag-recapture studies. As such, the feasibility of using bomb radiocarbon age validation for swordfish should be explored.

Plan a workshop in 2020 for creating reference sets for reproduction (histology) and ageing (spine and otoliths): Estimated cost: 25.000€. The Group recommended that a 5 day workshop is planned for 2020, with the main objectives of 1) creating ageing reference sets for ICCAT SWO (both spines and otoliths) and 2) creating reference sets for ICCAT SWO reproductive stages (histology).

6. Adoption of the report and closure

The report was adopted during the meeting. The swordfish species group rapporteur thanked participants for their engagement and the meeting was adjourned.

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	1st spine	2nd spine	3rd spine	Description	Photo
A				Short or small 1st ray, a medium length and not branched 2nd one and a 3rd ray larger than the other two and branched.	
B				1st and 2nd rays small and not branched, while the 3rd is larger and branched	
C	?			1st ray was not present; 2nd medium length and not branched, while the third ray is longer and branched.	
D		?		Short or small 1st ray; 2nd and 3rd are of medium/large length and not branched; the 4th is branched	
E	?	?		The 1st and 2nd spines are missing. The first present ray is large and branched.	

Figure 1. Swordfish anal fin spine configurations (Adapted from Vanpouille et al., 2001 and Quelle et al., 2014). Configuration A will be analyzed in this study.

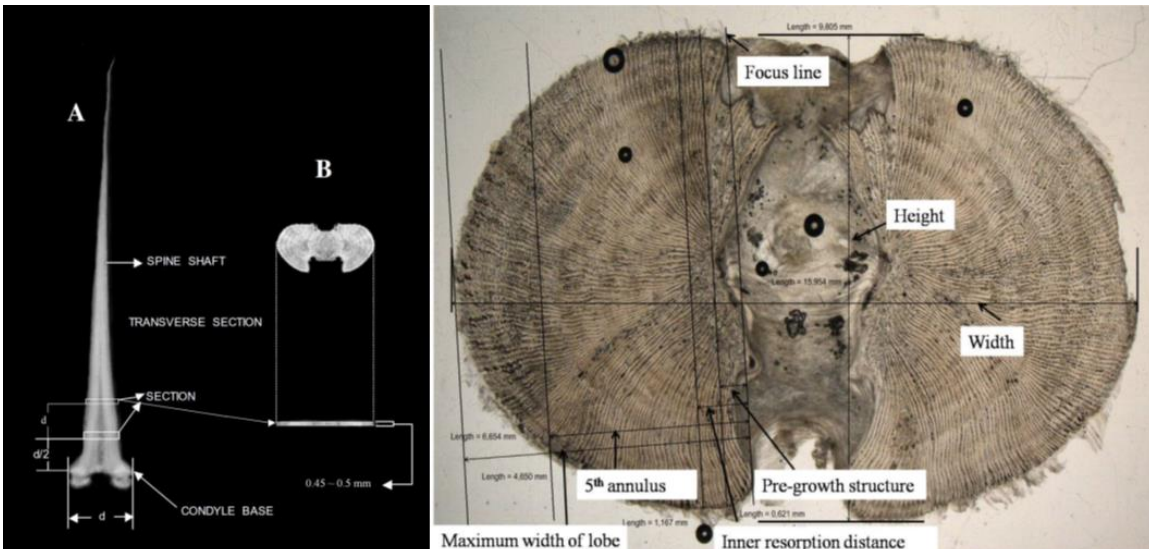

Figure 2. Swordfish second anal spine (left) and spine cross-section (right) cut at one condyle width distance (d) from the condyle base. The spine section (right) is annotated with various measurement points important for ageing, including the focus line (Source: Quelle et al., 2014)

Figure 3. Example of a whole otolith (left), a section otolith for annual ageing (middle) and a spine section (right) at one distance from the condyle base. Images are from samples from the same fish. Image scale differs among images.

Appendix 1

ICCAT swordfish sampling and biology workshop
IPMA-Olhão, Portugal, 18-21 June 2019
AGENDA

Background and objectives
As approved by the SCRS in 2017, the Swordfish Species Group initiated in 2018 a biological sample collection programme to collect biological data for swordfish (SWO), aiming to improve knowledge of the stock distribution, age and gender of the catch, growth rate, age at maturation, maturation rate, spawning season and location and diet. This will contribute to the next major advance in the assessment of swordfish status, by permitting the development of more spatially and biologically realistic population models used in both Atlantic and Mediterranean populations assessments and within the ICCAT Management Strategy Evaluation (MSE) for North Atlantic swordfish. As part of this initiative, a Workshop is planned to take place in 2019, with the major objectives of 1) refining sampling and biological data collection protocols, 2) develop protocols and start the sample processing and analysis, and 3) plan for the project future steps.

Agenda (tentative)
1. Opening
2. Adoption of agenda
3. Nomination of the rapporteurs
4. Status update of the ICCAT swordfish project
4. Revision/update of sampling and biological data collection protocols
5. Establish protocols to start sample processing and data analysis
5.1. Ageing, incl. spines vs. otoliths
5.2. Reproductive biology, incl. macroscopic vs histological scales
5.3. Population genetics, incl. discussion on methods and spatial coverage
6. Discussion and plans for future sampling and priority areas for the project
7. Workshop report and adoption
8. Closure

Appendix 2

List of Participants

Participant	Institute/Country	E-mail
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