Appendix 11

Report of the Swordfish Year Programme (SWOYP)

Background and programme objectives

Since 2018, the Swordfish Species Group has conducted a research programme to address key uncertainties important for improving the scientific advice for management of the species. The research programme encompasses all three ICCAT swordfish stocks and has been modified each year to respond to new knowledge, priorities and cost estimates. This programme aims to improve knowledge of the stock distribution, age and sex of the catch, growth rates, age at maturation, maturation rate, spawning season and location, stock boundaries and mixing, thereby contributing to the next major advance in the assessment of swordfish status. The SWOYP also encompasses an electronic tagging study to better understand swordfish life cycle and habitat use, and management strategy evaluation (MSE) for the North Atlantic stock to follow the MSE schedule agreed by the Commission collectively. These projects should translate into more reliable advice on stock status for this internationally and collectively managed resource. The Swordfish Species Group has identified this work to be of high priority and will address critical deficiencies in our understanding of the population dynamics and ecology of the stocks. The programme, which has been running on a short-term contractual basis since 2018, was formalized as a ICCAT research programme in 2022.

Overview of activities

The Swordfish Species Group (SWO SG) prioritized the following research topics: an ageing and growth study to improve knowledge of growth patterns among the stocks; a reproductive biology study to improve knowledge on maturity and fecundity; a genetics study to better define stock boundaries and estimate rates of mixing among the stocks; an electronic tagging study to better understand the life cycle and habitat use, and management strategy evaluation to follow the MSE schedule agreed by the Commission. These projects are overseen by a Consortium led by Canada (Dr Kyle Gillespie and Dr Alex Hanke, Fisheries and Oceans Canada) and administered by The Nova Scotia Swordfishermen's Association. Each of the three research areas are overseen by project leaders: ageing and growth (Dr Rui Coelho and Ms. Daniela Rosa, Instituto Português do Mar e da Atmosfera (IPMA)); reproduction (Dr David Macías (Instituto Español de Oceanografía (IEO)); and genetics (Dr Oliana Carnevali and Dr Giorgia Gioacchini (Università Politecnica delle Marche) (UNIVPM)). A total of 21 institutions from 14 ICCAT CPCs are involved in collection and analysis of samples. Three SWOYP biology workshops have been held: the first, in 2019, to refine and standardize sampling methods and sample processing; the second, in 2021 to review study results, and create ageing and histology reference sets and review results from a first calibration exercise; and a third in 2023 to progress ageing protocols, age calibration, age validation, and development of a reference set. Electronic tags have been used to support movement and habitat use studies in data-limited regions. ATL-N MSE, initiated in 2018 is being conducted by a core technical team and an outside contractor. The SWO SG is scheduled to deliver a final set of CMPs to the Commission in 2023.

Sample collection and coverage

Through all phases of this programme, 4,647 samples have been collected from longline fisheries, covering all three stocks. The majority of samples collected consist of an anal fin spine for aging, a piece of tissue for genetic analysis, and include data on fish size, sex, location and catch date. A subset of samples includes otoliths for aging or a piece of gonad for reproductive analysis.

Samples were collected in several of the major fishing areas in the North and South Atlantic and Mediterranean. Sampling in early project phases in the North Atlantic was concentrated in three areas: the Scotian Shelf, in the western Atlantic; along the 39°N parallel, in the eastern Atlantic; and off the western coast of Morocco in the eastern Atlantic. All three of these are major areas for swordfish catch. Samples obtained near the Strait of Gibraltar are of particular relevance to understand mixing between Atlantic and Mediterranean stocks. In later programme phases, a significant number of samples were obtained from the US East coast (billfish sampling area 92), however gaps remain in the Gulf of Mexico (BIL91) and the Caribbean (BIL93). Samples were also added from the coastal waters of Venezuela. In the cases of the Gulf of Mexico and Caribbean, there is relatively little swordfish catch, however, we anticipate that future sampling efforts will include data from these areas.

Sampling in the South Atlantic occurred between 5°N and 6°S, stretching from the coast of Brazil to the Gulf of Guinea. More than half the samples were obtained in this zone which spans two billfish sampling areas (BIL96 and 97). This is an area of significant swordfish catch in distant water fishing fleets. This is also as an assumed mixing area for North Atlantic and South Atlantic stocks. In addition, samples were collected in the waters of Brazil and off the coast of South Africa and Namibia. The South coast of Brazil and Uruguay and stretching east along the 30°S parallel is a major area for swordfish catch but so far have had limited sampling in this programme.

Mediterranean sampling occurred in three regions: the Balearic Sea, in the western Mediterranean; the Tyrrhenian and Adriatic Seas, in the central Mediterranean; and the Greek Islands. Sampling coverage of these seas appears somewhat representative of spatial-temporal patterns in the catch. More samples are required in the very western region of the Mediterranean, in the Alboran Sea and approaching the Strait of Gibraltar where there is suspected mixing between North Atlantic and Mediterranean stocks. Additional sampling is also required in the eastern Mediterranean in the Ionian and Aegean Seas.

Reproductive biology of swordfish in the Atlantic and Mediterranean

The reproductive biology study has the following objectives: a) improve knowledge on the reproduction and maturity for Atlantic and Mediterranean swordfish, b) obtain sex-specific maturity ogives, c) identify spatial and temporal spawning grounds and d) estimate of L_{50} and size/age related fecundity.

The sex of fish was determined via macroscopic observation and through histological analysis. 86.5% of samples were assessed for sex, while in the remaining 13.5% of samples, gonads were not available for assessment or were in a state where sex was ambiguous. Sex data are not typically collected in national sampling programmes, nor are these data required in ICCAT reporting, making it difficult to assess the representativeness of these data. In all regions, females outnumber males in the sample. The most extreme difference in sex ratio was observed in the Mediterranean, where only 30% of fish were assessed as male. This region also had the greatest level of uncertainty, where sex was unknown in approximately 30% of fish. Imbalance in sex ratios may be a result of inherent spatial zonation between sexes or it may be a result of males being classified as "unknown" at higher rates than females. For example, a large proportion of the sampled fish come from more northerly water where female swordfish are known to be at higher abundances.

Maturity was assessed on a six-point scale. Nearly a third of fish sampled had maturity states that were labelled as "undetermined", and these data require further verification. In some cases, histological data are available for samples and in these cases, macroscopic assessments of gonads will be compared to histological data.

A preliminary analysis of L_{50} comparing macroscopic and microscopic data was conducted in 2020 (Saber *et al*., 2020b). Altogether, 2,434 data on sex and macroscopic maturity for swordfish from the North and South Atlantic, and the Mediterranean Sea have been collected covering an ample size range (58 to 261 cm LJFL). About 768 gonad samples have been collected from the North Atlantic and the Mediterranean Sea. Further analysis will be conducted after increasing the sample size. See Saber *et al*. (2020b) for a preliminary analysis of the samples collected to date, and recommendations on next steps for data and sample collections. The descriptions of length frequencies by month/season and by stock of the swordfish sampled for maturity data are also provided.

Fish were classified as either immature (stage 1) or mature (stages 2 - 5). The L_{50} was estimated using the macroscopic maturity data. Sample gonads were sent to the coordinator of the reproductive studies in IEO-Málaga (Spain). Microscopic maturity stating of gonads was based on a modification of the criteria of Schaefer (2001) and Farley *et al.* (2013).

As expected, the analysis of the sex-ratio showed that females were more abundant than males, but further work is needed to verify if the sampling scheme is taking into account both sexes. The estimated L_{50} in the preliminary analysis for the three stocks was consistently lower than those adopted by the SCRS. However, it should be remarked that the significant number of histological sections of ovaries examined showed that females microscopically classified as immature were often incorrectly staged as developing (stage 2, mature) when using the macroscopic criteria. In 2023, an additional 42 samples from Chinese Taipei and 247 samples from Portugal have been processed. Histological analysis of these samples is ongoing.

Further calibration and exercises are needed to increase capacity within the Group to analyse gonad samples. Furthermore, samples are required from hypothesized spawning areas in the Sargasso Sea and the Gulf of Guinea.

Increasing the sampling of swordfish across the Mediterranean Sea and Atlantic Ocean is necessary to collect enough data for the reliable estimation of maturity and other reproductive traits, as is the validation of the macroscopic maturity data using the histological examination of gonads.

Ageing and growth in Atlantic and Mediterranean swordfish

The objectives of the ageing and growth study are to a) develop a standardized methodology for ageing spines and otoliths, b) validate ages through procedures such as bomb radiocarbon, and c) update the sex-specific growth formulas using new sample data and modeling techniques.

A total of 3535 spine samples (1396 males, 1774 females, 365 specimens with undetermined sex) were collected for this study from the North, South Atlantic and Mediterranean Sea. A total of 1352 otolith samples (583 males, 731 females, 38 specimens with undetermined sex) were collected for this study from the North, South Atlantic and Mediterranean Sea.

From the collected spine and otolith samples, 1093 spines, 288 otoliths for annual ageing and 56 otoliths for daily ageing have been processed for the North Atlantic. For the South Atlantic, 979 spines, 500 otoliths for annual ageing and 11 otoliths for daily ageing were processed. For the Mediterranean, 173 spines, 44 otoliths for annual ageing and 6 otoliths for daily ageing were processed.

Sectioning of spines and otoliths is performed at Fish Ageing Services (FAS; Australia). Preparation of spines follows Quelle *et al.* (2014). The second anal fin spine is embedded individually in resin for sectioning, two sections of approximately 0.5 mm were made at one distance of the condyle width (1D) and at half distance of the condyle width (0.5D). Smaller spines were sectioned with a modified gem cutting machine high speed saw, using a single pro slicer diamond blade, while larger spines were sectioned using an Isomet with a diamond wafering blade. Spine sections were preserved in a polyplex clear ortho casting resin and photographed under a dissecting microscope with a digital camera.

Before processing, whole otoliths were measured for length and width and photographed using a Leica M80 with transmitted light and 5x magnification. Otoliths were prepared for annual and daily age readings in thin transverse sections by grinding down the otolith in a 3-step process. Firstly, the otolith was fixed on the edge (end) of a slide using thermoplastic mounting media (Crystalbond 509) with the anterior side of the otolith hanging over the edge. Care was taken to ensure that the primordium was just on the inside of the glass edge. The otolith was then ground down to the edge using 400 and 800 grit wet and dry paper. The slide was then reheated, and the otolith was removed and placed (ground side down) on another slide and Crystalbond was allowed to cool. Once cooled the otolith section was ground horizontally to the grinding surface using varying grades (400, 800 & 1500 grit) of wet and dry sandpaper and finally 5um lapping film. During this process, the otolith preparation was continuously checked for the appropriate thickness (220µm – 250µm for annual readings or 50-80µm for daily readings). Otolith sections were preserved in a polyplex clear ortho casting resin and photographed at a 40x magnification using a Leica M80 dissecting microscope illuminated with transmitted light.

In 2022, a preliminary analysis of an age reading for the North Atlantic stock was completed. Multiple readers read both spines and otoliths and biases were found between readers for both structures. The maximum modal age in spines was 7 years and in otoliths 5 years. The mean length at age from spines was similar to the mean lengths at age from the Arocha *et al.* (2003) study. Sampling, processing, and age readings will continue under the program which will contribute to development of new sex-specific growth models for the three stocks.

During phase 5 of SWOYP a joint workshop for swordfish (SWOYP); billfishes (EPBR) and small tunas (SMTYP) was conducted with the objectives of enhancing expertise among ICCAT scientists by sharing knowledge, standardizing methodologies and reviewing the work already completed and further developing plans for next steps in these research programs.

A new SWOYP ageing and growth project area in 2023 was age validation via bomb radiocarbon analysis. The aim of this component of the age and growth is to use a well-developed reference system for age validation of broadbill swordfish to provide valid otolith age reading protocols and life history characteristics that are essential for sustainable fisheries management. A state-of-the-art method used to address these concerns - known as bomb radiocarbon (14C) dating - has been refined over the last 30 years. Technological improvements, coupled with insight on propagation of the bomb-produced 14C signal in aquatic ecosystems, are now available to resolve age estimation issues for challenging fishes, and specifically for recently collected, fast growing pelagic fishes.

Aged SWO otoliths were selected from archived specimens at the SWOYP (IPMA) and National Oceanic and Atmospheric Administration - Southeast Fisheries Science Center (NOAA-SEFSC) covering the collections made in the 1980s and 1990s to the 2010s and 2020s. From more than 1000 otoliths archived with SWOYP (IPMA), a series of SWO sizes and ages were selected for an analysis of otolith mass as a possible proxy for age that might elucidate maximum ages. This resulted in 30 SWO specimens that had both sagittal otoliths (one aged or to be aged and one intact for bomb 14C analysis) and covered a range of 88-258 cm Lower Jaw Fork Length (LJFL) with otolith masses of 0.234-4.267 mg. Selections were focused on collection years 2018-2019 to cover maximum sized fish available and to best utilize the post-peak 14C decline - young to old fish from a narrow collection period (1-2 years) would progressively trace more elevated 14C levels as calculated hatch years (derived from otolith age reading) extend back in time, toward the bomb 14C peak. Hence, the rate of 14C change recorded in the otolith core of aged fish would agree with the rate of change in environmental 14C. It is anticipated that this work will continue in future project phases.

Genetics, stock delineation, and mixing in Atlantic and Mediterranean swordfish

The objectives of the genetics study are to a) sequence the swordfish genome and identify genetic markers for differentiating between the three stocks, b) evaluate stock boundaries, and c) identify stock mixing areas.

The swordfish genome assembly was completed using a sequencing strategy that combined Oxford Nanopore (MinION) and Illumina (NovaSeq 6000) technologies following standard analysis in a well-established bioinformatics workflow.

By comparing the swordfish genome with that of other 19 fish species, the percentage of swordfish-specific genes and the percentage genes shared was identified. A Gene Ontology Enrichment Analysis (GOEA) was performed on several swordfish-specific orthologous groups to highlight their involvement on Biological Process, Molecular Function and Cellular component. Finally, the new assembled genome was used as a reference genome to guide the double digest restriction-site associated DNA (ddRAD) analysis. Accordingly, the rationale behind this strategy was based on: 1) the better performances (i.e. precision) of the genotyping when guided with a reference genome, and 2) the finer scale of resolution and the expanded set of biological questions that can be addressed when a reference genome is available.

Double digest restriction-site associated DNA (ddRAD) sequencing technology was applied to obtain more than 40,000 SNPs for the analysis of genetic differences among 672 samples collected from the North Atlantic, South Atlantic and Mediterranean stocks. In particular, from the North Atlantic, 322 samples were analyzed, of which 54 samples from BIL92, 12 samples from BIL93, 44 samples from BIL94A, 182 samples from BIL94B and 30 samples from BIL94C. From the South Atlantic a total of 105 samples were analysed of which 11 were from BIL96 and 94 from BIL97. Finally, from the Mediterranean, 243 samples were analysed of which more than 100 were from Balearic Islands. Samples were selected homogeneously not only on the basis of the catch area but also on the basis of gender, gonad maturity, length/weight, and period of catch.

To analyze genetic differentiation among samples, several statistical analyses including Principal Component Analysis (PCA), Discriminant Analysis of Principal Component (DAPC), pairwise genetic distances (heatmap matrix), NEIGHBOR-JOINING Cladogram were applied. Regarding genetic differentiation index such as, Fixation index (FST), Heterozygosity (both observed and expected), Observed heterozigosity related to single codifying genes, Inbreeding coefficient (FIS) and allelic richness (both mean and total) were also calculated. Genetic structure was evaluated quantifying allelic frequencies clusters and their distribution among samples. Two populations were clearly identified among the whole samples analyzed and considerable evidence on the presence of subpopulations within the two populations emerged from the first 288 samples analyzed, and in 2022 an additional 672 samples were analysed.

Also in 2022, Whole Genome Sequencing (WGS) analysis was completed on 30 samples from each stock in order to identify a set of SNPs that can be used to assign an unknown sample to one of the stocks and to identify sex-specific regions to assign sex to an unknown sample.

The coupling of SNPs and WGS analyses with a genome assembly showed that: 1) the Mediterranean stock is strongly genetically differentiated from the two Atlantic stocks; 2) the North Atlantic and the South Atlantic stocks are weakly differentiated, and their differentiation is detectable only with few statistical tests; 3) the coupling of genome-wide SNPs analysis with a genome assembly of the allelic richness is the optimal genetic diversity index to monitor these stocks; 4) the Mediterranean stock is losing allelic richness of important genes associated with detoxification, immune response, vitamin up-take and metabolism and serotonin signaling; 5) in the East-North Atlantic a mixing area for all three stocks was found and the presence of these animals should be considered when genetic variability is monitored in this area; 6) no animals belonging to the North Atlantic stock have been found in the Mediterranean Sea.

Building on work that identified genetic markers for stock differentiation, 200 samples were analyzed in 2023. Previous SWOYP genetic work has identified an area in the North-East Atlantic as a potentially important stock mixing area. Samples were from 150 swordfish caught in the Northeast Atlantic area in which all the three stocks (NA, SA and MD) have been sampled, in addition to 50 samples coming from MD area. These new samples will be analyzed and compared to samples sequenced in previous project phases, using an additional genetic analysis to report “F3 statistics”. This statistical analysis represents an alternative way to measure the allele frequency correlations and relationship among the three stocks (NA, SA and MD) to better evaluate whether admixture exists. The integration of the analysis already carried out using the “Structure” genetic technique will provide further statistical support to the analysis.

New epigenetic techniques have led to advancements in age estimation by examining level of methylation in the genetic material. The goal of this project component was to conduct a pilot study to assess viability of these techniques in swordfish. To develop a pilot study on epigenetic ageing, we identify 1,311 CpG sites that were found to have appropriate methylation levels. These sites were identified as having significant correlation with fish age in other fish taxa (e.g. zebrafish), and therefore are promising for the swordfish age analysis the SWOYP would like to develop. The availability of whole genome sequencing expertise within the SWOYP will allow this programme to identify the conserved CpG sites related to ageing among the swordfish stocks.

This first step provided the SWOYP a swordfish dataset of age-related CpG sites that will be used to define swordfish epigenetic clocks by Reduced Representation Bisulfite Sequencing (RRBS). In the next SWOYP project phase, ten swordfish of different ages previously determined by otoliths analysis and confirmed by radiocarbon analysis, will be analyzed by RRBS.

Tagging

The objective of the swordfish tagging study is to analyse the vertical habitat-use and migration patterns of swordfish and help to delimit the stock boundaries and mixing rate of swordfish between the Mediterranean Sea and the North and South Atlantic. Fifty ICCAT funded tags have been acquired since 2018, when the tagging programme was implemented. To date, a total of 35 miniPAT tags (12 tags have been provided by NOAA) have been deployed in the North (n = 19) and South Atlantic (n = 12) and the Mediterranean Sea (n = 4). Additionally, 5 X-Tag tags have been deployed in the North Atlantic. Data from 10 tags, with deployment days between 67 and 240 days, show that swordfish moved in several directions, travelling considerable distances in both the North and South Atlantic Ocean, while having shorter displacements in the Mediterranean Sea. Regarding vertical habitat use, swordfish spent most of the daytime in deeper/colder waters, and were closer to the surface during the night-time, mostly between the surface and 50 meters in depth. Efforts to include in the analysis historical tags deployed by NOAA and DFO have started in 2023. Updates of this work are regularly provided to the SCRS/SWO SG with the latest one presented in Rosa *et al*. (2022). One dedicated trip for tagging was made in the Northwest Atlantic in 2023. Unfortunately, this trip was not successful. Approximately 20 electronic tags are available for deployment in 2024.

Management Strategy Evaluation in the North Atlantic

The Commission is scheduled to adopt a management procedure in 2023.

Following minor revisions to the OM grid values in 2023, the technical team consulted with ICCAT's Panel 4 on key elements of the MSE framework. Selection of a management procedure requires evaluation of candidate management procedures (CMPs) against predetermined management objectives. The technical team worked with Panel 4 to better define performance metrics, acceptable probability values for those management objectives, and time spans over which those probabilities should be calculated. A variety of model based and empirical CMPs were developed, tuned, and then evaluated for performance. Interactive tools were developed to show trade-offs for among CMPs. A series of Panel 4 engagements, as well as ambassador communications sessions laid the groundwork for managers and stakeholders to understand MSE uncertainties and then provide guidance to the MSE technical team on management priorities as well as priorities for robustness testing.

Based on guidance from Panel 4, in September of 2023, the SWO-N technical team created a shortlist of CMPs for the Panel to consider for adoption. This list includes a variety of harvest control rules, each spanning the performance trade-off space. An Exceptional Circumstances Protocol must be developed in collaboration with Panel 4.

This new MSE framework is a major shift in how the SCRS and the Commission interact for the formulation of management advice. It should be expected that review of this process and the assumption used to model stock dynamics be revisited on a regular basis. In 2023, Panel 4 and the technical team developed a schedule that define when stock assessments and other checks be used to evaluate the performance of the MSE. This collaborative process between ICCAT scientists and managers will require continued engagement between the SCRS and the Commission in coming years.

Expenditures in 2022 and 2023

The total budgets within SWOYP in 2018, 2019, 2020 and 2021 amounted to €199,000, €373,700, €280,614 and €343.480, respectively. The effective expenditures for that period were €149,895, €312,434, €194,734 and 292,134.47, respectively.

In 2022 and 2023, in order to implement the main activities planned in the framework of SWOYP, the total budget of provided by ICCAT amounted to €170,000 and €250,000, respectively.

The detailed fund available for SWOYP during 2022 and 2023 and respective expenditures as of 11 September 2023 are detailed in the table below.

Year	*2022*		*2023*	
Component	*Budget (€)*	*Expenditure (€)*	*Budget (€)*	*Expenditure (€)*
Tagging	10,000	2,640.25	20,000	-
Biological studies	15,000	6,000	5,000	-
Genetics	70,000	28,000	80,000	-
Age and growth	45,000	18,000	25,000	-
Sample collection and shipping	10,000	4,462.43	5,000	-
MSE			100,000	45,000
Workshops	20,000	22,642.89	15,000	
TOTAL	**170,000**	**81,745.57**	**250,000**	**45,000**

2024 Plan and activities

Sampling

The focus of SWOYP has largely shifted to analysis of samples already collected by the programme, however, sampling will continue in 2024, targeting spatial sampling gaps: the Gulf of Mexico, Caribbean, Strait of Gibraltar, the far Eastern Mediterranean, the mid-North Atlantic, southern Brazil and the area stretching East along the 30°S parallel. Additional effort will be invested in collecting gonads and otoliths as these materials have been more challenging to acquire. In addition, otolith-spine pairs in larger fish, will be collected to support the growth curve modelling. Additional CPCs and institutes are welcomed and encouraged to support sample collection and analysis.

Reproductive biology

The reproductive biology component of the SWOYP will continue in 2024 with processing and imaging of gonads. A reproduction, ageing and growth, and genetics workshop in 2024 will focus on creating a reference set of histological images and CPC scientists involved in the study will work to standardize their methods for determining maturity stage. Anticipating increased capacity in the group to evaluate maturity stage, we expect that the preliminary maturity ogives developed in previous project phases will be updated for the North Atlantic and Mediterranean stocks in 2024. Additional samples are required before this work can be initiated for the South Atlantic. Preliminary work will begin in 2024 to estimate fecundity by stock.

Ageing and growth

The ageing and growth component of the SWOYP will include the following in 2024: continued age readings from spines and otoliths, grow modeling, and age validation through bomb radiocarbon analysis. A core team of age readers has prepared a reference set of fin spines and otoliths and have conducted an initial calibration exercise. This Group will continue their readings to increase the number of samples included in the growth modeling. Bomb radiocarbon analysis, initiated in 2023, will continue. This analysis will allow for validation of age readings and will support epigenetic ageing work.

Genetics

Genetics work in 2024 will continue the population analysis of tissues samples coming from new areas (South Africa, Brazil, North Central Atlantic Ocean, Strait of Gibraltar, North African coast) for stock differentiation analysis. In 2023, the genetics team conducted a pilot study on epigenetic ageing, to correlate with otoliths, spines and the bomb radiocarbon study. This work is anticipated to continue in 2024.

Tagging

Tagging work will continue in 2024 with deployment of tags already on-hand. This work will continue to support studies on swordfish distribution, movement, and habitat use. These data will also support ongoing work on the swordfish species distribution model.

Management Strategy Evaluation

The Swordfish Species Group is scheduled to provide the Commission with a final set of CMPs by the end of 2023 for use in management advice for 2024. In 2024, the work will continue, mostly related to development of an Exceptional Circumstances Protocol and further development of robustness tests. The species group will also continue a preliminary simulation study to explore the suitability of MSE in the South Atlantic Stock.