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 an 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 MSE 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 Macias, *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. Four 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; a third in 2023 to progress ageing protocols, age calibration, age validation, and development of a reference set; and a fourth in 2024 to develop a longer-term strategic research plan for SWOYP. Electronic tags have been used to support movement and habitat use studies in the North-west Atlantic and in a possible mixing area in the North-east Atlantic. The ATL-N MSE, was initiated in 2018 is being conducted by a core technical team and an outside contractor. A management procedure (MP) was selected by the Commission in 2024 and the technical team will continue technical work on an exceptional circumstances protocol (ECP) and robustness tests related to Climate Change and minimum size limits. In 2024, efforts were largely focused on sample processing. CPC scientists who regularly contribute samples were mostly focused on working through a backlog of sample processing and analysis of materials collected as part of SWOYP in previous years. Additional bomb radiocarbon sample processing was completed in this project phase.

Sample collection and coverage

Through all phases of this programme, 4,712 samples have been collected , encompassing all three stocks. The majority of samples collected consist of an anal fin spine for ageing, 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 ageing and/or a piece of gonad for reproductive analyses.

Over all phases of the SWOYP, samples were collected in many of the major fishing areas in the North and South Atlantic and Mediterranean. Sampling 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 will be of particular relevance in future genetic analyses to understand

mixing between Atlantic and Mediterranean stocks. In Phase 3 of the program, 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. The Consortium is attempting to partner with institutes in these areas to increase spatial coverage.

Since 2018, sampling in the South Atlantic has 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 south of Brazil and off the coast of South Africa and Namibia. The South coast of Brazil and stretching east along the 30°S parallel is a major area for swordfish catch but was not sampled by this program. CPCs with fleets fishing in this area have been approached by the SWOYP in efforts to obtain samples from this area. These CPCs have declined to support sampling efforts. In Phase 6, 51 swordfish muscle tissue samples were provided for genetic analysis. These samples were collected between 2016 – 2019 and will greatly help to resolve the spatial dynamics of swordfish in the South Atlantic stock.

Mediterranean sampling has occurred in three regions during the SWOYP so far: the Balearic Sea, in the western Mediterranean; the Tyrrhenian and Adriatic Seas, in the central Mediterranean; and the Greek Islands. Sampling coverage of these sea appears somewhat representative of 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 required in the eastern Mediterranean in the Ionian and Aegean Seas. CPCs fishing these areas have been approached and have thus far declined to support sampling efforts.

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 many 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*., 2020). 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*. (2020) for a preliminary analysis of the samples collected to date, and recommendations on next steps for data and sample collections. 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 EU-Portugal have been processed. Histological analysis of these samples is ongoing.

Further calibration and exercises are needed to increase capacity within the Group to analyze 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 3542 spine samples (1396 males, 1783 females, 365 specimens with undetermined sex) were collected for this study from the North, South Atlantic and Mediterranean Sea. A total of 1359 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 (Enhanced Program for Billfish Research, EPBR) and small tunas (Small tuna Year Program, 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 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.

In this study, a well-established bomb ^{14}C chronology of the North Atlantic was used to test the validity of age estimates for swordfish by comparing ^{14}C values - measured using novel gas - accelerator mass spectrometry (AMS) technology at ETH Zürich - from otoliths of aged fish with regional ^{14}C references. The alignment or misalignment of ^{14}C values in time, calculated as hatch dates from each otolith age reading estimate, was used to either establish or refine protocols. In some cases, the offset of some otolith ^{14}C values from the reference chronology was used as a basis for re-examining the otolith section for alternate age interpretation scenarios, which was often coupled with use of otolith mass and fish length (for smaller fish) to elucidate problems with age interpretations in the otolith sections. For many of the original age estimates, the calculated hatch years led to a temporal placement of the measured ^{14}C value (from the otolith core) within the expected range of ^{14}C reference values. The greatest sources of error were identified as, (1) poorly defined growth zones in otolith sections, (2) consistent counting protocol that included the earliest growth (first annulus), (3) possible problems with some sample extractions, and (4) alignment of values from older collection dates. The extraction of the otolith material was challenging and approached the limits of what can be assessed from gas-AMS, but mostly because control of the extraction target was difficult and was a work in progress that improved with time. Overall, age reading up to the mid to late teens for swordfish was supported from post-peak bomb ^{14}C dating, although the age reading protocols are clearly in need of refinement for difficult-to-read otolith sections, as well as determining the location of the first annulus used to begin the counting used to estimate age.

The findings of this study provide a validated basis for continued use of annual growth zone counting in thin-sectioned otoliths with room for improvement for swordfish of the North Atlantic. Alignments for many of the measured samples led to good agreement with what was expected from the North Atlantic bomb ^{14}C chronology, but some did not align and led to a range of observations that were attributed to either experimental or environmental complications - consistent control of core sample extractions from the very small otoliths and possible differences in source ^{14}C levels for the earliest otolith growth. Based on the analysis of swordfish in this study, there is currently a follow up series of 20 measurements that are being made on additional samples provided by IPMA with ICCAT to further investigate estimates of age. These samples cover a similar range to the existing data set in terms of fish size, otolith mass, and estimated age to gain more insight on the ^{14}C uptake pattern of hatch year otolith material over time. In addition, a daily increment analysis of the earliest growth has been performed to provide a strong basis for the location of the first annulus, which will be coupled with recent findings that allow a decimal age to be assigned because of otolith growth zone structure. Further refinement of the age reading protocols is why the results presented here are necessarily provisional and will later be adjusted as needed when more information is acquired, which will provide an age-validated basis for a publishable manuscript.

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

Stock differentiation, identification of boundaries and mixing rates among the three swordfish stocks (NATL, SATL and MED) is a critical uncertainty in Atlantic swordfish science and management. The SWOYP has employed a variety of nextGen genetic techniques to clarify some of these Species Groups' knowledge uncertainties. During previous phases of the project, the coupling of the results obtained by analysing 635 samples using Single Nucleotide Variants (evaluated by Double digest restriction-site associated DNA (ddRADseq)) and 30 samples using structural variants (evaluated by Whole Genome Sequencing (WGS)) let determine stocks differentiation (in terms of genetic structure and diversity, fitness and evolutionary potential and identify stock boundaries and genetic admixing among North-, South-Atlantic and Mediterranean stocks). In this Phase of the project (6) the coupling of ddRAD and WGS analyses let identify starting from the 32,459 SNPs used for genetic population analysis, a minimum set of variants (SNPs) required to discriminate between MED and ATL stocks and between ATL-N and ATL-S stocks. Two machine learning models have been developed to discriminate the stocks.

The first model was created to discriminate between MED and ATL stocks. For this purpose, genotyping obtained from the STRUCTURE model with two ancestral populations (K=2, G1 representing SWO-AT and G2 representing SWO-MD) has been used. The second model was created to discriminate between NATL and ATL-S stocks. For this purpose, genotyping obtained from the structure model with three ancestral populations (K=3, G1, G2 and G3) (from Phase5 of the project) has been used. Admixture and Mediterranean samples (G3) have been discarded. To create these two machine learning models, the pairwise FST in both datasets have been calculated, only selecting the SNPs with the highest FST index.

To discriminate MED from ATL it was calculated the pairwise FST index of all samples and then 18 SNPs with a FST score higher than 0.65 have been selected. To discriminate NATL from SATL it was calculated the pairwise FST index of samples included in the second model and then 14 SNPs with a FST score higher than 0.55 have been selected.

Both datasets have been divided in training and test data and the different machine learning algorithms have been tested to predict the origin of swordfish samples: linear discriminant analysis, classification and regression trees, k-nearest neighbors, support vector machines with a linear kernel and random forest. The two models performed very well, an accuracy of 100% was obtained with samples used for test.

Epigenetic ageing exploratory study

Swordfish ageing and growth analyses face numerous challenges given the small size of otoliths, the difficulties obtaining otoliths, and vascularization of fin spines which could otherwise provide age readings. 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 continue the pilot study started during phase 5 to assess viability of these techniques in swordfish. At this purpose total DNA was extracted from 40 samples belonging to ATL stock and have been analyzed by Reduced representation bisulfite sequencing (RRBS) approach. In particular, these samples have been sequenced and soon as the results will be available and after quality check, they will be used to develop BAM that will be mapped against the genome. At this purpose, BEDGRAPH files with methylation values, Plots with the methylation profile across genes/TSS/promoters or specific regions of interest will be carried out. Finally, mCpG associated with aging will be identified also using preliminary analysis conducted on zebrafish and focusing on the mCpG already selected in phase 5 of the project.

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. In 2024, 20 tags were deployed in two SWOYP tagging campaigns: 10 in the northwestern Atlantic and another 10 in the northeastern Atlantic. Priority areas for further tag deployments have been identified for 2025: the South Atlantic, the Gulf of Guinea, and the northeastern Atlantic in a possible stock mixing area.

Management Strategy Evaluation (MSE) in the North Atlantic

Following several years of technical development, the Commission is adopted a MP in 2024 (*Recommendation by ICCAT on conservation and management measures, including management procedure, for North Atlantic swordfish* (Rec. 24-10).

2025 Plan and activities

Sampling

SWOYP task leaders have identified priority locations and size classes requiring additional sampling. 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 2025 with processing and imaging of gonads. 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 2025. Additional samples are required before this work can be initiated for the South Atlantic. Preliminary work will begin in 2025 to estimate fecundity by stock.

Ageing and growth

The ageing and growth component of the SWOYP will include the following in 2025: continued age readings from spines and otoliths, growth 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 2025 will continue the population analysis of tissues samples coming from new areas (Brazil, North African coast, North Central Atlantic Ocean, South Africa and Strait of Gibraltar) 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 2025.

Tagging

Tagging work will continue in 2025 in priority areas (South Atlantic, stock mixing areas in the Northeast, and in the Gulf of Guinea. 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 (MSE)

Following adoption of an MP, in 2025, the work will continue, mostly related to development of an ECP 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.

References

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