FINAL REPORT FOR PHASE FOUR OF THE ICCAT SHORT-TERM CONTRACT: SWORDFISH BIOLOGICAL SAMPLES COLLECTION FOR GROWTH, REPRODUCTION AND GENETICS STUDIES

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SUMMARY

This report details the fourth phase of biological sampling and associated analysis undertaken as part of an international swordfish biology program. The program was established in 2018 and sampling was conducted for swordfish in the North and South Atlantic and Mediterranean. Fish were sampled for size, sex, and maturity. Anal fin spines, otoliths, gonads, and tissues were obtained for ageing, growth, maturity and genetic analyses. These data will be used to inform ICCAT assessment and the ongoing management strategy evaluation process. In this report we examine sampling representativeness relative to spatial and temporal patterns in recent catch data. Samples were obtained from a broad temporal and spatial range, however, some improvements are required in spatial-temporal coverage.

RÉSUMÉ

Ce rapport détaille la quatrième phase de l'échantillonnage biologique et l'analyse associée réalisés dans le cadre d'un programme international sur la biologie de l'espadon. Ce programme a été établi en 2018 et l'échantillonnage a été mené pour l'espadon dans l'Atlantique Nord et Sud et en Méditerranée. Les poissons ont été échantillonnés en ce qui concerne la taille, le sexe et la maturité. Des épines de la nageoire anale, des otolithes, des gonades et des tissus ont été obtenus pour les analyses sur la détermination de l'âge, la croissance, la maturité et les analyses génétiques. Ces données seront utilisées pour apporter des informations à l'évaluation de l'ICCAT et au processus d'évaluation de la stratégie de gestion actuellement en cours. Dans ce rapport, nous examinons la représentativité de l'échantillonnage par rapport aux schémas spatio-temporels des données de capture récentes. Des échantillons ont été obtenus d'une vaste gamme spatio-temporelle mais il convient d'améliorer la couverture spatio-temporelle.

RESUMEN

En el presente informe se detalla la cuarta fase del muestreo biológico y el análisis conexo emprendido como parte de un programa internacional de biología del pez espada. El programa se estableció en 2018 y se realizaron muestreos de pez espada en el Atlántico norte y sur y en el Mediterráneo. Se tomaron muestras de peces para determinar su talla, sexo y madurez. Se obtuvieron espinas de la aleta anal, otolitos, gónadas y tejidos para los análisis sobre determinación de la edad, crecimiento, madurez y genéticos. Estos datos se utilizarán para informar la evaluación de ICCAT y el proceso de evaluación de estrategias de ordenación en curso. En este informe se examina la representatividad del muestreo en relación con los patrones espaciales y temporales en los recientes datos de captura. Las muestras se obtuvieron de un amplio rango espacial y temporal, sin embargo, se requieren algunas mejoras en la cobertura espacio-temporal.

KEYWORDS

*Swordfish, Biological sampling, Growth,
Reproduction, Genetics, Sampling representativeness*

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Executive summary

In 2018, ICCAT's Swordfish Species Group initiated a biological sampling program in the North and South Atlantic and Mediterranean. The aim of the program was to collect biological data that would support research critical to the assessment and management of this highly migratory and internationally managed species. In the first phase of the program, an international group of institutions developed a sampling protocol, collected samples from 1810 fish, developed a relational database for sample data, and identified strategies for optimizing further sample collection and data analysis. In phase two of the program, 1433 samples were collected as well as initial analysis for the three project sub-areas: ageing and growth, reproduction and maturity, and genetics. In phase three of the program, 916 samples were collected and further analysis for the three project sub-areas continued. In this project phase, 498 additional samples were collected and sample analysis was focused on development of growth models, age readings and calibrations, and genetic analysis for stock differentiation. Fish were sampled for size, sex, and maturity; the location and date of capture (and/or landing) were recorded; calcified parts (otoliths and/or anal fin spines) and/or tissues were collected, processed, and archived for analysis. Samples were collected for each stock and in many cases these data were representative of the major swordfish catch locations and timing, however, in some locations and seasons additional sampling coverage is required. In this report we assess where and when additional sampling effort is needed, we provide basic analysis of data collected to date, and we suggest next steps for sample collection and analysis. These data will make contributions to our understanding of patterns of growth, maturity, movement and mixing among the three swordfish stocks under ICCAT management and will be critical for devising management plans that maximize swordfish yield and support stock productivity.

Introduction

Swordfish are an important fisheries resource in the Atlantic and Mediterranean but there remain significant unknowns regarding their basic biology, how the three stocks differ biologically, and the level of mixing between stocks. In 2018, ICCAT's Swordfish Species Group initiated a sampling program to collect biological data for swordfish in the Atlantic and Mediterranean. Sampling continued through. The objective of this ongoing program is to improve knowledge of the stock distribution, age and sex, growth rate, age at maturation, maturation rate, spawning season and location and diet. It is expected that the program 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. This should translate into more reliable advice on stock status for an internationally and collectively managed resource.

Among all phases of this data collection program, 20 institutions from 14 ICCAT CPCs/Cooperating Non-Contracting Parties (**Appendix 1**) collected biological data via existing national fisheries at-sea monitoring programs and through targeted port sampling. This consisted largely of opportunistic collection of anal fin spines, tissues samples, otoliths, size, sex and maturity information. Samples were collected for all three Atlantic swordfish stocks in seven of the eight billfish sampling areas and across all four seasons.

This report provides an overview of the fourth phase of this program, basic analysis of data collected to date and recommendations for next steps for data collection and analysis of samples. We provide a review of sampling methodology and analysis of sample spatial and temporal representativeness relative to fishing effort for each stock. We also provide basic descriptions of length frequencies and sex ratios.

Program objectives

As indicated by the Swordfish Species Group, data collected in this program will support the following objectives:

- Resolve the spatial-temporal distribution of the three known swordfish stocks found within the Atlantic Ocean and Mediterranean Sea using a genetic analysis of tissue sampled from the catch of participating CPCs.
- Resolve the age and size at maturity of the three known swordfish stocks found within the Atlantic Ocean and Mediterranean Sea using samples/measurements provided by participating CPCs.
- Characterize the age composition of the catch and validate the growth curves for each swordfish stock.
- Determine the spawning period and areas of each stock.
- Identify the seasonal and spatial species composition of the swordfish diet using stomach content and/or tissues.
- Develop a protocol/template based on genetic analysis that will allow for the assignment of tissue samples to a particular stock.

- Develop a biological database that links the sample information to the age, stock origin, gender, size, diet and maturity data of each fish.
- Update the ICCAT Manual with new pertinent information.

Methods

Swordfish were sampled in a combination of existing national swordfish sampling programs and targeted port sampling programs (for full methodology see Gillespie *et al.*, 2020). To accommodate data available to port sampling programs (and some at-sea programs), the original sampling requirements were modified in Contract Amendment #1 and Contract Amendment #2 in phase one of this program.

In addition to data required for a full or partial sample, in some cases supplementary data were collected: in some fish, stomach contents were identified and quantified, while in other fish, otoliths were collected. A subset of these otoliths and limited number of anal fin spines were processed and aged by Fish Aging Services, a subcontractor of this project.

Results

Through all phases of this program, 4646 samples have been collected, covering all three stocks. A total of 1810 new and historical samples were sampled from all three Atlantic swordfish stocks in phase one of the program, and another 1433 new and historical samples in phase two of the program (table 1). In phase three, 916 new and historical samples were added to the sample collection. In the current phase, 498 samples were added, with all being newly collected samples. The majority of samples collected in this program are considered "Full" and are associated with an anal fin spine for aging, piece of tissue for genetic analysis, and contain data on fish size, sex, location and date. "Partial" samples lacked some combination of these data but always contained either an anal fin spine or a tissue sample (for sample definitions see Gillespie et al., 2020).

Sample spatial coverage

Samples were collected in several of the major fishing areas in the North and South Atlantic and Mediterranean (figure 1). 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 (figure 2). 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 three 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.

Sampling in the South Atlantic occurred between 5°N and 6°S, stretching from the coast of Brazil to the Gulf of Guinea (figure 3). 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.

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 (figure 4). 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 also required in the eastern Mediterranean in the Ionian and Aegean Seas.

Dedicated scientific research trips were planned for phase 4 of the SWOYP in Canada and Uruguay to fill spatial gaps and to obtain fish from length classes currently under-sampled. These trips have been delayed to Project Phase 5 due to boat availability.

Length frequencies

Length frequencies for fish sampled in this program are plotted by stock, aggregated and disaggregated by sex. The overall size distribution of sampled fish was similar to that of the size distribution estimated by task 2 size data for 2014-2018 (figure 5). Size frequencies for sampled fish in the North and South Atlantic and Mediterranean were roughly similar to estimated size frequencies observed in catch data (figure 6). As expected, females in all stocks were, on average, larger than males. Extrapolating the shape of these length frequencies by sex to overall catch may aid in estimating spawning stock. For a small proportion of sampled fish (~7%), only curved fork length (CFL) or standard fork length (SFL) was available. These lengths are excluded from this analysis, pending an appropriate length-length conversion.

Sex and maturity

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 programs, 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 (see Gillespie *et al.*, 2020). 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.

Progress on the age and growth component of the swordfish biology programme

Progress in this component of the project has continued with sample processing for both spines and otoliths. A preliminary analysis of an age reading for the North Atlantic stock was conducted with multiple readers for 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. These results were presented at the Atlantic swordfish stock assessment meeting in July 2022 in document SCRS/2022/118 (for further details under this task refer to the document). Sampling for under samples sizes and/or areas, processing, and age readings will continue under the program next phase to contribute for the development of new sex-specific growth models for the three stocks.

Reproduction and Maturity

See ICCAT document SCRS/2020/135 (Saber et al., 2020).

A preliminary analysis of L_{50} comparing Macroscopic and Microscopic data was conducted in 2020 (SCRS 2020/135). Altogether, 2434 data on sex and macroscopic maturity for swordfish from North, South Atlantic, and the Mediterranean Sea have been collected to date covering an ample size range (58 to 261 cm LJFL). About 498 gonad samples have been collected from the North Atlantic and the Mediterranean Sea. A total of 767 samples of gonads, 373 from the North Atlantic, 206 samples from the South Atlantic, and 188 from the Mediterranean Sea have been processed for microscopic maturity. Further analysis will be conducted after increasing the sample size. This document also provides 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.

The main topic to consider during the workshop was the establishment of reference sets for microscopic stages. A calibration exercise among experts was performed to reach this objective. For this, the group had the advice of invited experts Jessica Farley (CSIRO) and Freddy Arocha (UDO).

Genetics

The coupling of genome assembly and double digest restriction associated DNA (ddRAD) sequencing was applied to obtain more than 26,324 SNPs for the analysis of genetic differences among 576 Swordfish samples collected from the NA, SA and MED stocks. To quantify the genetic differentiation among stocks, several statistical methods including Principal Component Analysis (PCA), pairwise genetic distances (heatmap matrix), Fixation index (FST), Heterozygosity (both observed and expected), Inbreeding coefficient (FIS) and Allelic richness (both mean and total) were applied. All methods showed considerable genetic differentiation between the Mediterranean and Atlantic stocks and weak differentiation between the North and South Atlantic stocks. In addition, a slight genetic differentiation between the east and west, in both the North and South Atlantic stocks was observed. Furthermore, the allelic richness analysis evidenced that, at the genomic level, the Mediterranean stock is losing allelic richness of important genes involved in detoxification, immune response, vitamin up-take and metabolism and serotonin signaling. Focusing on the stock management boundaries; the South Atlantic population appears to occur north of the adopted 5°N ICCAT management boundary where it mixes with the North Atlantic population. Likewise, the Mediterranean population can occur west of the Strait of Gibraltar management boundary in a Northeast Atlantic mixing zone. Given that specimens belonging to all three stocks are caught in the East-North Atlantic, their presence should be considered when genetic variability is monitored in this area. While these results have been included in a manuscript submitted to an international peer-reviewed journal, ddRAD analyses are in progress for an additional 288 samples to better resolve the weak genetic differentiation between North and South Atlantic stocks.

In addition to the Multivariate and individual-based analyses conducted in the ddRAD study, a focus on Structural variants has been performed to better investigate genetic differentiation among the stocks. This work involved Whole Genome sequencing (WGS) analyses of 30 samples from the 3 stocks (10 from NA, 10 from SA and 10 from MED), where deletions and insertions are the genomic variants (GVs) that most affect DNA gains, losses, or rearrangements and are the basis of genetic differentiation among them. A high genetic differentiation between Mediterranean and Atlantic stocks was supported by a wide distribution of these genomic variants among several chromosomes. While the weak differentiation between the North and South Atlantic stocks depended on GVs that occurred in chromosome 5. A paper based on this analysis is in preparation to be submitted to an international peer-reviewed journal.

Sampling database

Sample data are maintained in an Excel database that has undergone thorough QA/QC.

Sampling recommendations

In many regions, sample data was representative of catch data both spatially and temporally. Despite often good spatial and temporal coverage, we note some gaps that require additional sampling effort. In the North Atlantic, the Gulf of Mexico, Caribbean and mid-Atlantic represent a gap in sampling coverage. In the South Atlantic, additional sampling is required further from the equator, in more southerly regions. In the Mediterranean, sampling coverage is required near the Strait of Gibraltar and in the east, near Greece and Turkey. Amendments to sampling design have added flexibility for port sampling and we anticipate that this will help achieve greater sampling coverage for this species.

Program next steps

As ICCAT stock assessment and MSE processes become more analytically intensive, there is a need for greater accuracy in estimates of biological parameters such as size and age of maturity and stock mixing. These data are critical for devising management plans that maximize yield and support stock productivity. This sampling program is an initial step in reducing uncertainty in important biological parameters. The first four year of biological sampling has produced data that is currently undergoing further analysis in a number of areas: tissue samples for genetic analysis for stock boundary definition and mixing; anal fin spines for aging correction analysis so as to estimate age structure in each stock; sex, size, age, maturity data are being analysed to refine maturity ogives; spatial and temporal abundance data combined with age and size data are helping define movement patterns by age class and are being used to update age-length-sex keys by area. Sample data with greater spatial and temporal coverage, particularly in regions suggested here, will further refine these parameter estimates. While there are some minor spatial-temporal sampling gaps, the primary focus of further work in this program should be analysis of samples collected to date. Significant effort has been invested in collection of samples and there is now a significant volume of samples that ready for analysis

Acknowledgements

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Table 1. Total number of samples realized in this sampling program by stock.

Project Phase	Stock	Total Samples	Full samples	Partial samples	Fin spines	Tissue samples	Stomach contents	Gonads	Otoliths
1	N-Atl	365	184	166	192	348	27	51	17
	S-Atl	894	816	76	887	825	61	0	353
	Med	551	98	441	142	501	101	67	50
	Total	**1801**	**1098**	**683**	**1221**	**1674**	**189**	**118**	**420**
2	N-Atl	524	372	106	467	468	73	127	302
	S-Atl	521	295	173	491	488	145	0	223
	Med	388	0	388	60	388	0	121	24
	Total	**1432**	**667**	**667**	**1018**	**1344**	**218**	**248**	**549**
3	N-Atl	741	271	469	734	301	1	117	0
	S-Atl	66	0	59	66	66	46	46	29
	Med	92	0	90	90	21	71	0	0
	Total	**915**	**271**	**618**	**890**	**388**	**118**	**163**	**29**
4	N-Atl	301	196	84	255	301	0	78	220
	S-Atl	197	149	48	157	195	4	160	135
	Med	0	0	0	0	0	0	0	0
	Total	**498**	**345**	**132**	**412**	**496**	**4**	**238**	**355**
	Grand total	**4646**	**2395**	**2076**	**3534**	**3891**	**529**	**767**	**1351**

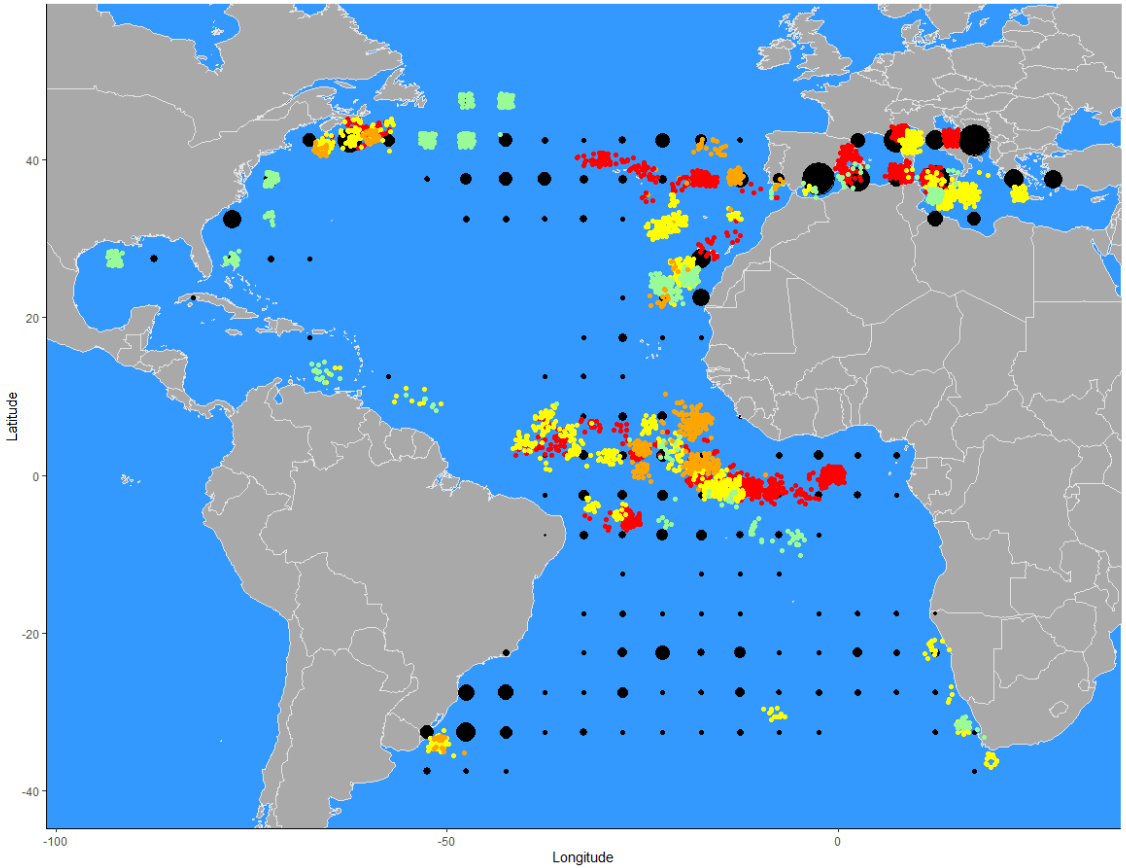


Figure 1. Sampling coverage the North and South Atlantic and Mediterranean. Red dots indicate locations where samples were collected in phase one of the program. Yellow dots indicate sample locations from phase two of the program. Green dots indicate sample locations from phase three of the program. Orange dots indicate sample locations from phase four of the program. Black dots represent swordfish catch for years 2014-2018 for each 5x5 grid cell area, scaled by contribution to the overall catch. Dots for grid cells contributing to less than 0.1% of the total average catch are omitted. Some sample locations have error added to the location data to abide by local privacy laws. For data points that are obscured to the centroid of 5x5 grid cell squares, the points have been jittered to better indicate the scale of sampling in the withing the grid cell—this was done only in the North-West Atlantic and Gulf of Mexico.

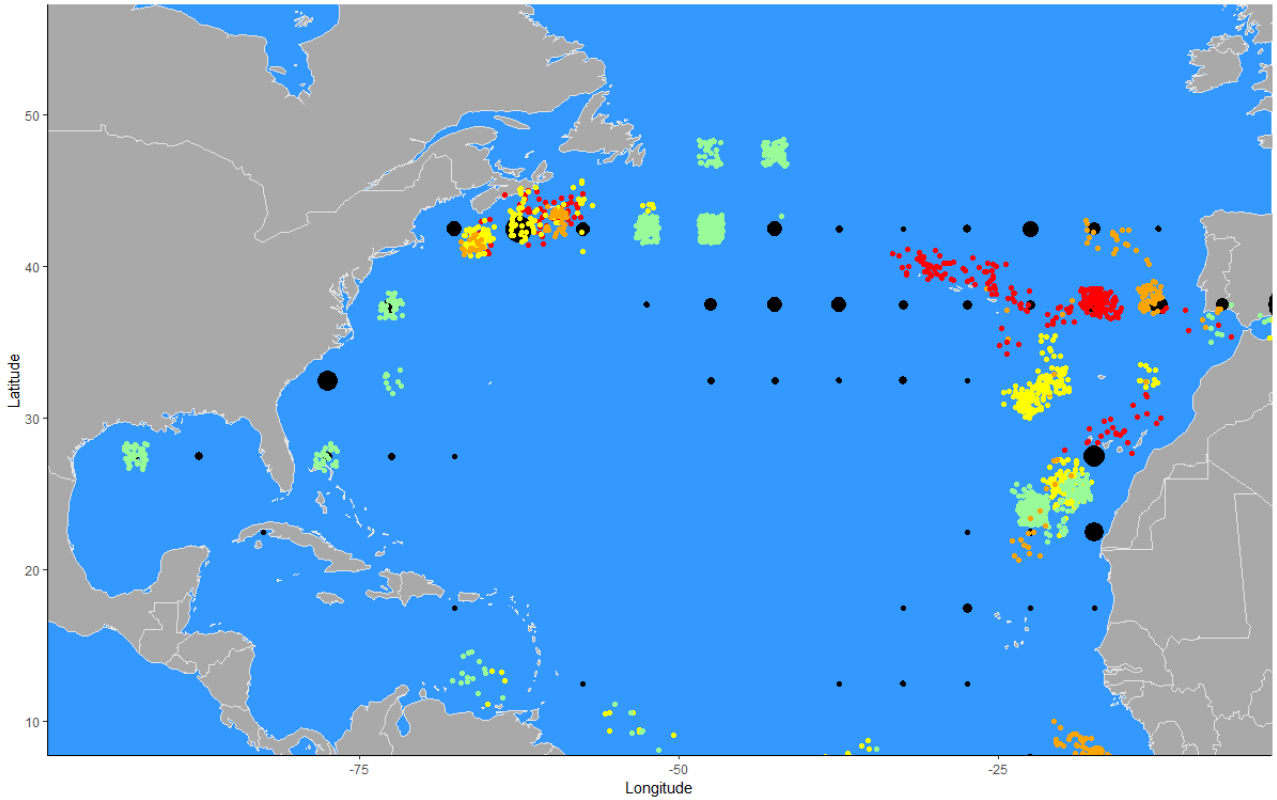


Figure 2. Sampling coverage in the North Atlantic. Red dots indicate locations where samples were collected in phase one of the program. Yellow dots indicate sample locations from phase two of the program. Green dots indicate sample locations from phase three of the program. Orange dots indicate sample locations from phase four of the program. Black dots represent swordfish catch for years 2014-2018 for each 5x5 grid cell area, scaled by contribution to the overall catch. Dots for grid cells contributing to less than 0.1% of the total average catch are omitted. Some sample locations have error added to the location data to abide by local privacy laws. For data points that are obscured to the centroid of 5x5 grid cell squares, the points have been jittered to better indicate the scale of sampling in the withing the grid cell—this was done only in the North-West Atlantic and Gulf of Mexico.

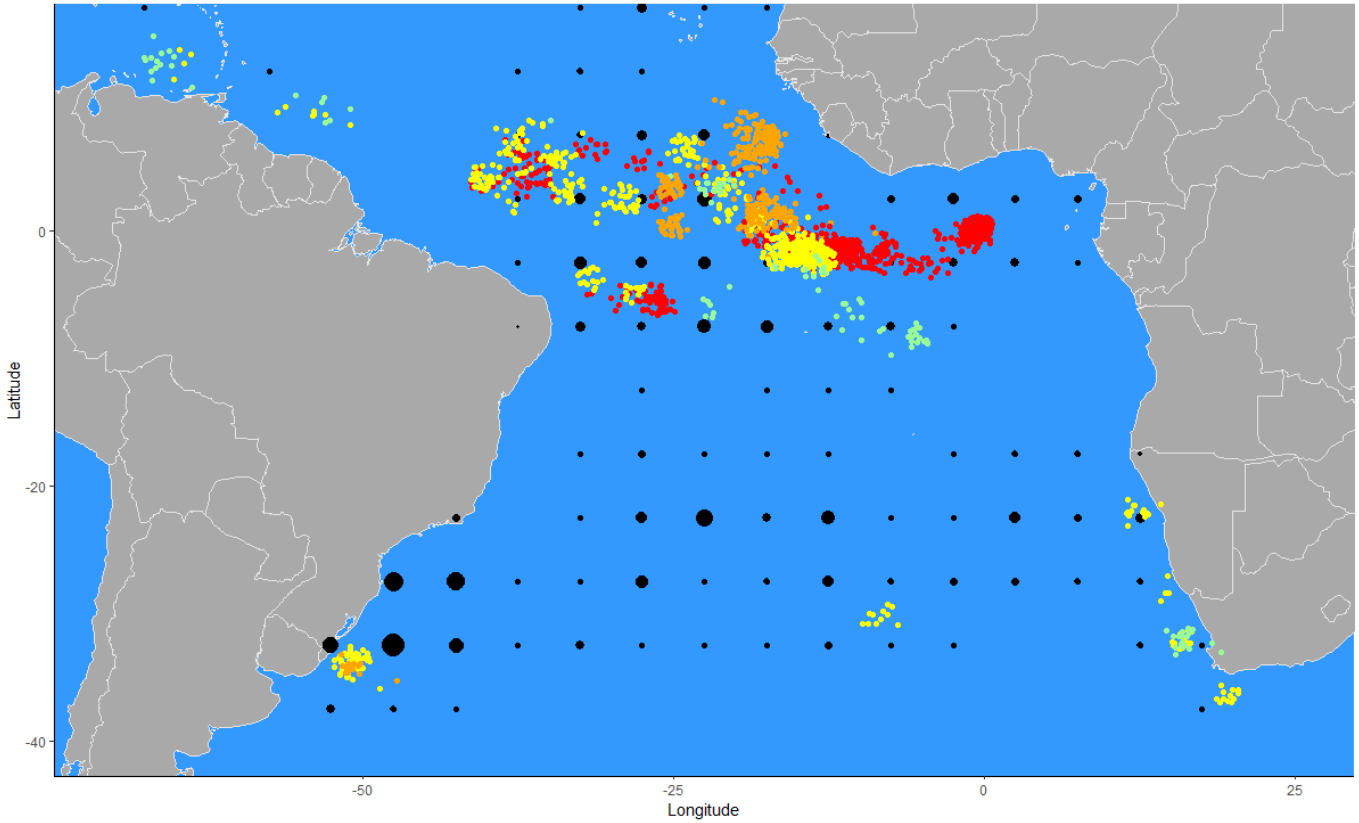


Figure 3. Sampling coverage in the South Atlantic. Red dots indicate locations where samples were collected in phase one of the program. Yellow dots indicate sample locations from phase two of the program Green dots indicate sample locations from phase three of the program. Orange dots indicate sample locations from phase four of the program. Black dots represent swordfish catch for years 2014-2018 for each 5x5 grid cell area, scaled by contribution to the overall catch. Dots for grid cells contributing to less than 0.1% of the total average catch are omitted. Some sample locations have error added to the location data to abide by local privacy laws.

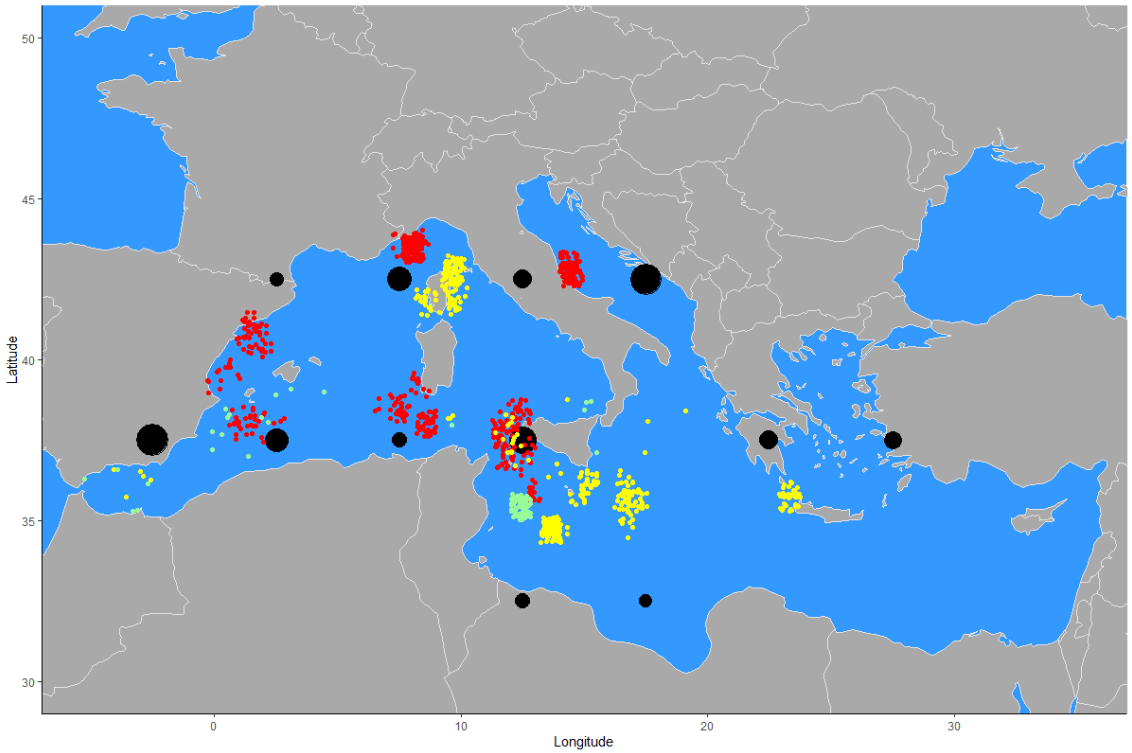


Figure 4. Sampling coverage in the Mediterranean. Red dots indicate locations where samples were collected in phase one of the program. Yellow dots indicate sample locations from phase two of the program. Green dots indicate sample locations from phase three of the program. Orange dots indicate sample locations from phase four of the program. Black dots represent swordfish catch for years 2014-2018 for each 5x5 grid cell area, scaled by contribution to the overall catch. Dots for grid cells contributing to less than 0.1% of the total average catch are omitted. Some sample locations have error added to the location data to abide by local privacy laws.

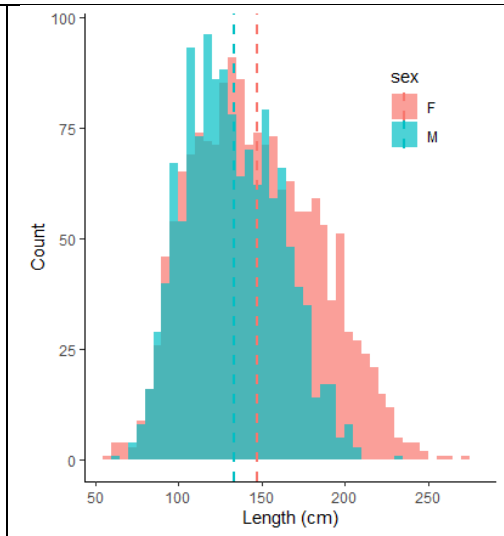
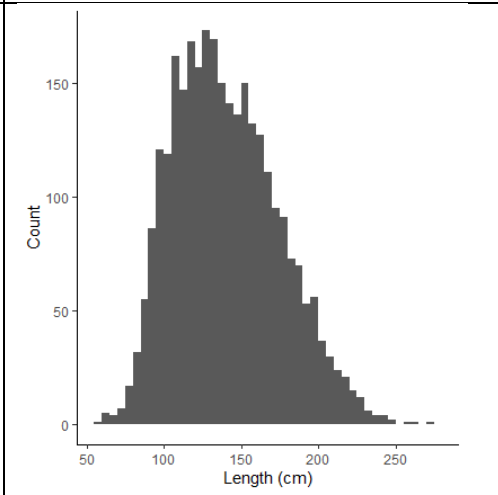
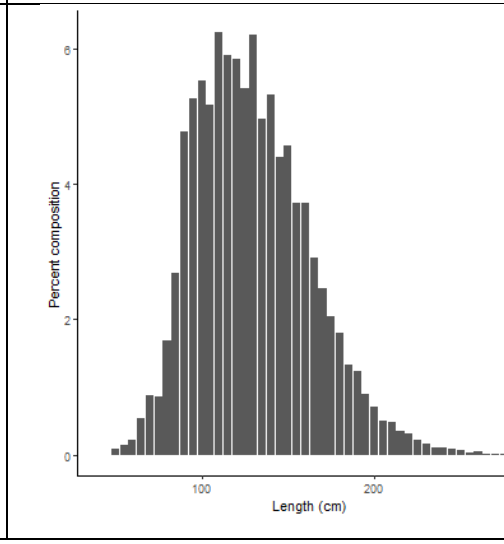
Sample length frequencies, sex disaggregated	
Sample length frequencies	
Catch length frequencies	

Figure 5. Length frequencies for all swordfish sampled in this program disaggregated by sex (top) and non-disaggregated (middle), compared to estimate catch size frequencies from ICCAT task 2 data for 2014-2018 (bottom).

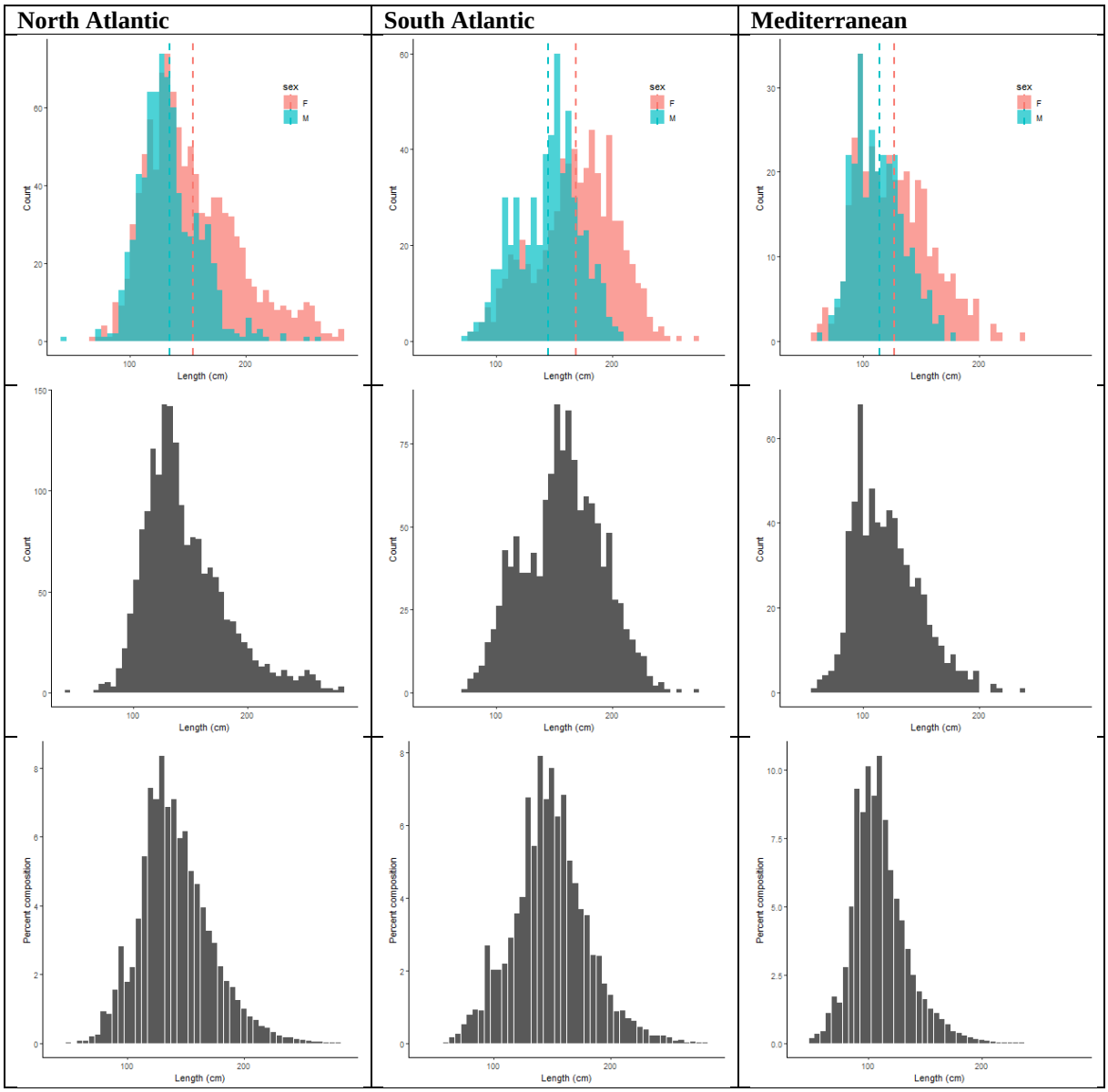


Figure 6. Length frequencies for all swordfish sampled in this program by stock, disaggregated by sex (top) and non-disaggregated (middle), compared to estimate catch size frequencies from ICCAT task 2 data for 2014-2018 (bottom).

Appendix 1

Program contributors

Institute	Flag code
AquaBioTech Group	MLT
AquaStudio Research Institute	ITA
Department of Agriculture, Forestry and Fisheries	ZAF
Fisheries and Oceans Canada	CAN
Hellenic Centre for Marine Research	GRC
Institut Français de recherche pour l'exploitation de la mer	FRA
Instituto Español de Oceanografía	ESP
Ministry of Fisheries and Marine Resources	NAM
National Oceanic and Atmospheric Administration	USA
National Taiwan Ocean University	TAI
Oceanis	MLT
Portuguese Institute for the Ocean and Atmosphere	PRT
National Instutute of Marine Sciences and Technologies	TUN
UNIMAR società cooperativa	ITA
Universidad de Oriente	VEN
Universidade Federal do Rio Grande	BRA
Universidade Federal Rural de Pernanbuco	BRA
Università Politecnica delle Marche	ITA
University of Cagliari	ITA
University of Genoa	ITA