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

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SUMMARY

A biological sampling program was conducted for swordfish in the North and South Atlantic and Mediterranean. Fish were sampled for size, sex, and maturity. Anal fin spine samples and tissues were obtained for ageing and growth 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

Un programme d'échantillonnage biologique a été mené pour l'espadon dans l'Atlantique Nord et Sud et en Méditerranée. Les poissons ont été échantillonnés pour obtenir des données sur la taille, le sexe et la maturité. Des tissus et des échantillons de l'épine de la nageoire anale ont été obtenus pour déterminer l'âge et réaliser des analyses génétiques et sur la croissance. Ces données seront utilisées pour documenter l'évaluation de l'ICCAT et le processus d'évaluation de la stratégie de gestion en cours. Dans ce rapport, nous examinons la représentativité de l'échantillonnage par rapport aux modèles spatiaux et temporels dans les données de capture récentes. Des échantillons ont été obtenus à partir d'une large gamme temporelle et spatiale, cependant, certaines améliorations sont nécessaires en ce qui concerne la couverture spatio-temporelle.

RESUMEN

Se realizó un programa de muestreo biológico dirigido al pez espada del Atlántico norte y sur y Mediterráneo. Se muestrearon los peces para obtener datos de talla, sexo y madurez. Se obtuvieron tejidos y muestras de la espina de la aleta anal para análisis de determinación de la edad, crecimiento y genéticos. Estos datos se utilizarán para aportar información a la evaluación de ICCAT y al proceso en curso de evaluación de estrategias de ordenación. En este documento, 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 espaciotemporal.

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 year of the program, an international group of institutions developed a sampling protocol, collected samples from 1762 fish, developed a relational 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) were recorded; calcified parts (otoliths and/or anal fin spines) and/or tissues were collected, processed, and archived for future 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. 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.

In the first phase of this data collection program, 10 institutions from 5 ICCAT CPCs (appendix 2) 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 six of the eight billfish sampling areas and across all four seasons.

This report provides an overview of the first 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. As this the initial phase of an ongoing sampling program, we anticipate more detailed analysis of these data by the Swordfish Species Group.

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.

Sampling contract deliverables

Deliverable	Status	Comments
1. SCRS presentation describing the representativeness of sampling by area, season, and gender (Madrid, 25-28 February 2019)	Complete	A presentation was made at the Swordfish Species Group intersessional meeting (Madrid, 25-28 February 2019). An additional presentation will be made at the Swordfish Species Group meeting in Fall 2019
2. Label anal spines and tissue samples that are to be shipped according to instructions determined by the Swordfish Species Group	Complete	Decisions regarding shipping will be made at the upcoming swordfish biological sampling workshop in Olhão, Portugal in June, 2019
3. Relational database containing the sample data that has undergone thorough QA/QC.	Complete	A draft relational database has undergone thorough QA/QC. A final SQL database is in development in collaboration with the ICCAT secretariat.
4. Processing (cutting) of at least 100 otoliths, together with the provision of photographs or the area being read and the respective aging data.	Complete	A total of 153 spines and 90 otoliths (84 for annual growth and 6 for daily growth) have been processed by Fish Ageing Services, Pty Ltd.
5. Draft final report to be submitted at the latest by 10 May 2019. It shall include: Executive summary; Full description of the work carried out; Detailed description of the sampling that has been realized.	Complete	Submitted to the Swordfish Species Group Rapporteur, May 10, 2019
6. Final report, taking into account the comments provided by the ICCAT Secretariat, the Swordfish Species Group rapporteurs and the SCRS Chair	This document	Submitted to the ICCAT Secretariat and Swordfish Species Group Rapporteur May 15, 2019

Methods

Swordfish were sampled in a combination of existing national swordfish sampling programs and targeted port sampling programs (see appendix 1 for full methodology). 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 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

A total of 1762 fish were sampled from all three Atlantic swordfish stocks (**table 1**). The majority of samples 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 (see appendix 1 for sample definitions).

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. There are notable sampling gaps in areas of high catch levels in North-Central Atlantic (billfish sampling areas 94A and 94C). There is also a lack of samples from the US east coast (billfish sampling area 92), the Gulf of Mexico (BIL91) and the Caribbean (BIL93). 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 that were obtained during this first phase of sampling 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. 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; and the Tyrrhenian and Adriatic Seas, in the central Mediterranean (**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.

Temporal coverage

Samples were obtained in all four quarters of 2018 and the first quarter of 2019 (**figure 5**). In addition, 229 samples were obtained for years previous to the current sampling program. When considering all samples, the first two quarters of the year, January through June, exhibited sampling rates roughly equivalent to Task II catch rates observed in 2012-2016. Relative to Task II, the third quarter of the year is oversampled by approximately eight percentage points, while the last quarter is under sampled by approximately 14 percentage points. In the North Atlantic, the two quarters of the year are oversampled relative to Task II by 23 and 14 percentage points, respectively. The last two quarters are undersampled by 9 and 28 percentage points. In the South Atlantic, sample representativeness is roughly similar to the overall temporal sampling pattern: the first half the year has representative sampling numbers while the last two quarters are over and then undersampled by 16 and 22 percentage point, respectively. In the Mediterranean, the first quarter of the year is heavily undersampled, with only two samples in our data-set while catch from time period makes up nearly 14% of the total annual catch for this stock. The second quarter is undersampled by 7 percentage points, while the last two quarters are oversampled

by a 8 and 11 percentage point, respectively. These data require further analysis alongside age, size, sex, maturity and spatial data to understand patterns of abundance in these stocks across time and space.

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 2012-2016 (**figure 6**). Size frequencies for sampled fish in the South Atlantic and Mediterranean were roughly similar to estimated size frequencies observed in catch data (**figure 7**). In the North Atlantic, however, size frequencies for sampled fish appear to have a greater proportion of 100 - 120 cm fish than would be anticipated based on task 2 data. 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. 88% of samples were assessed for sex, while in the remaining 12% of samples, gonads were not available for assessment or were in a state where sex was ambiguous (**table 2**). 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 North Atlantic, where only 26% of fish were assessed as male. This region also had the greatest level of uncertainty, where sex was unknown in approximately 37% of fish. The large imbalance in sex ratio in the North Atlantic 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. A large proportion of the sampled fish come from more northerly water where female swordfish are known to be at higher abundances. Warmer water regions in the North Atlantic where, presumably, males are found at higher abundances, were not sampled in this first phase of biological sampling. The South Atlantic also had a fairly large difference in the proportion of males versus females sampled but a fewer number of animals where the sex was assessed as "unknown". The Mediterranean had the most even sex ratio among stocks but also had a substantial level of fish labelled as "unknown".

Maturity was assessed on a six point scale (see appendix 1). 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.

Ageing of calcified parts

A limited number of anal fin spines (153) and otolith samples (90) were processed and analyzed for age. These data and images will be used within this project to estimate life-history timing and estimate age structure. They will also contribute to an ageing reference set for use in standardizing age readings across ICCAT Swordfish Species Group member institutions. This project component is important because determination of swordfish age has traditionally been challenging; otoliths are extremely small, are fragile and are difficult to extract from fish and anal fin spines, while relatively simple to extract, are known to provide imprecise age estimates.

Sampling database

Sample data are maintained in an Excel database that has undergone thorough QA/QC. With the guidance of the ICCAT secretariat, this Excel database is being converted into an SQL relational database.

Sampling recommendations

Samples from 1762 fish were collected in this first phase of swordfish biological data collection. In many regions, sample data was representative of catch data both spatially and temporally. Despite often good spatial and temporal coverage, we note several significant gaps that require additional sampling effort. In the North Atlantic, sub-tropical and tropical regions, particularly those along the US east coast, Gulf of Mexico, and Caribbean 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. This is a first attempt at achieving sampling coverage for swordfish on an Atlantic and Mediterranean ocean basin scale—as such we anticipate that gaps will exist until additional partners join this

sampling effort. 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 year of biological sampling has produced data that will undergo further analysis in a number of areas: tissue samples will require genetic analysis for stock boundary definition and mixing; anal fin spines will require ageing and aging correction analysis so as to estimate age structure in each stock; sex, size, age, maturity data will help refine maturity ogives; spatial and temporal abundance data combined with age and size data will help define movement patterns by age class and will be 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.

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

Stock	Total Samples	Full samples	Partial samples	Fin spines	Tissue samples	Stomach contents	Sex	Otoliths
N-Atl	318	172	146	172	318	0	202	17
S-Atl	893	821	70	889	829	101	875	353
Med	551	98	453	142	501	65	368	50
Total	**1762**	**1091**	**669**	**1203**	**1648**	**166**	**1445**	**420**

Table 2. Sex ratios by stock for samples collected in this program.

Sex	Total (n = 1762)	N-ATL (n = 318)	S-ATL (n=893)	MED (n = 501)
Percent male	39.6	25.8	43.2	41.3
Percent female	48.7	37.7	54.8	44.5
Percent sex unknown	11.7	36.5	2.0	14.2

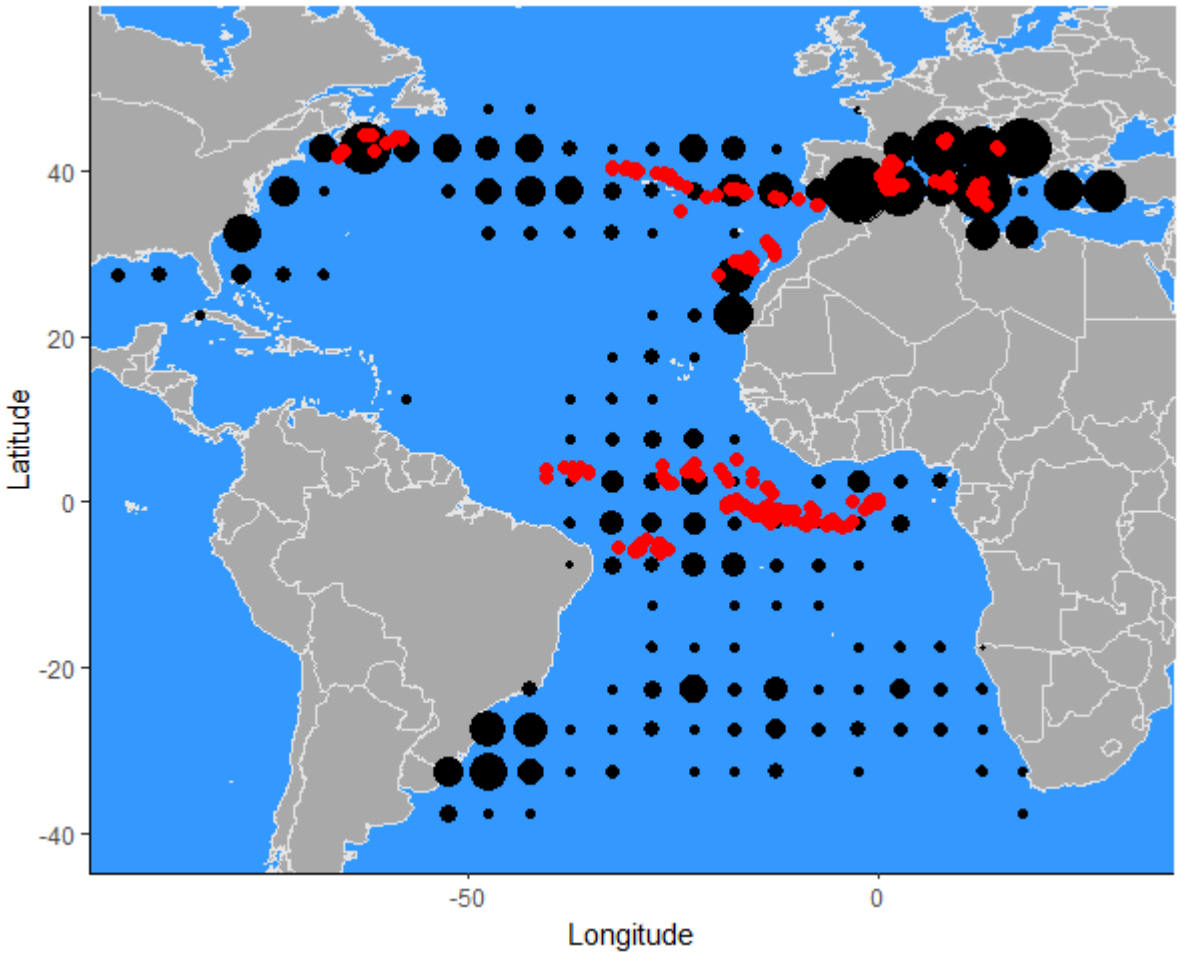


Figure 1. Sampling coverage the North and South Atlantic and Mediterranean. Red dots indicate locations where samples were collected. Black dots represent swordfish catch for years 2012-2016 for each 5x5 grid cell area, scaled by contribution to the overall catch. Dots for grid cells contributing to less than 0.1% of the total average catch are omitted. Some sample locations have been obscured to abide by local privacy laws.

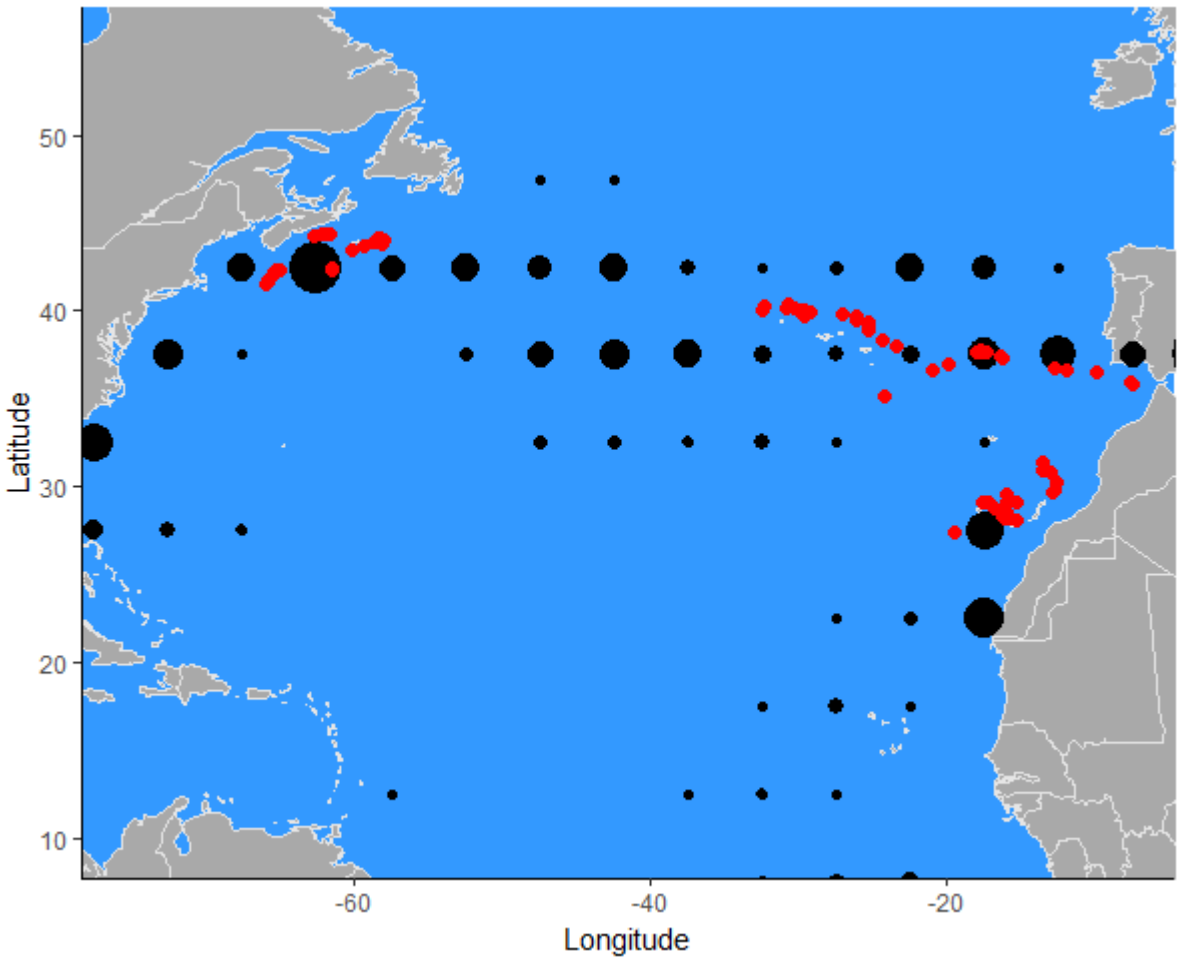


Figure 2. Sampling coverage in the North Atlantic. Red dots indicate locations where samples were collected. Black dots represent swordfish catch for years 2012-2016 for each 5x5 grid cell area, scaled by contribution to the overall catch. Dots for grid cells contributing to less than 0.1% of the total average catch are omitted. Some sample locations have been obscured to abide by local privacy laws.

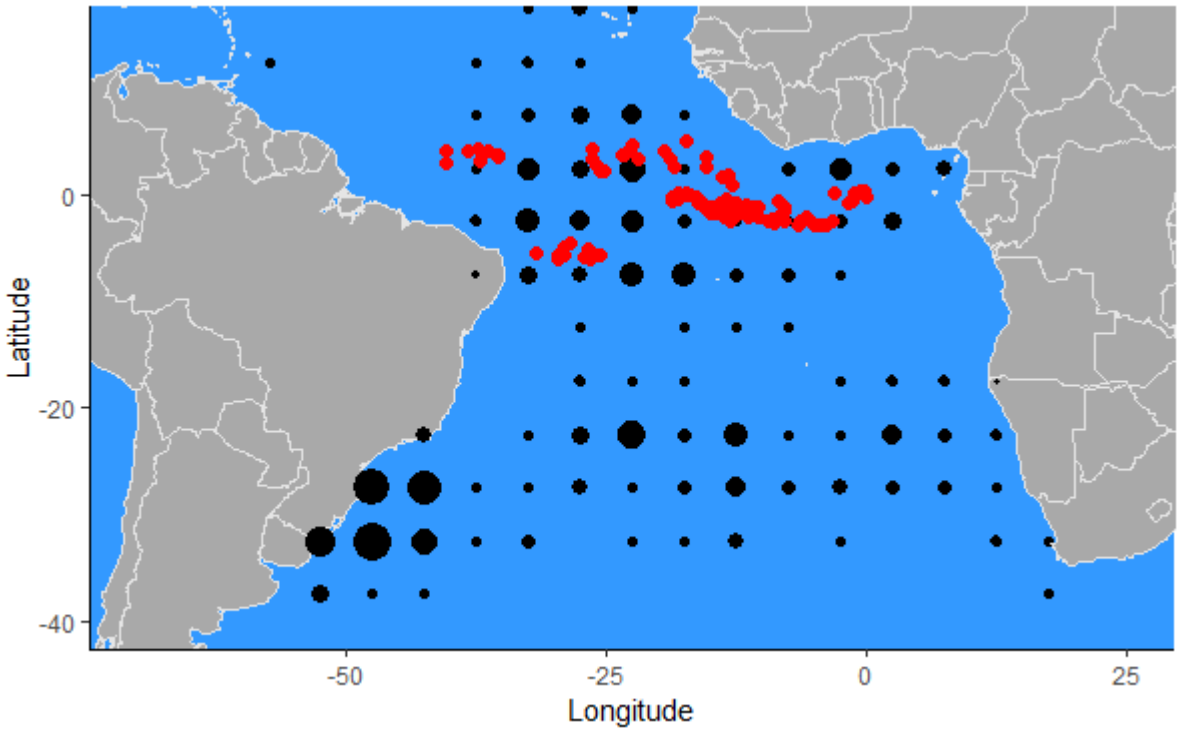


Figure 3. Sampling coverage in the South Atlantic. Red dots indicate locations where samples were collected. Black dots represent swordfish catch for years 2012-2016 for each 5x5 grid cell area, scaled by contribution to the overall catch. Dots for grid cells contributing to less than 0.1% of the total average catch are omitted.

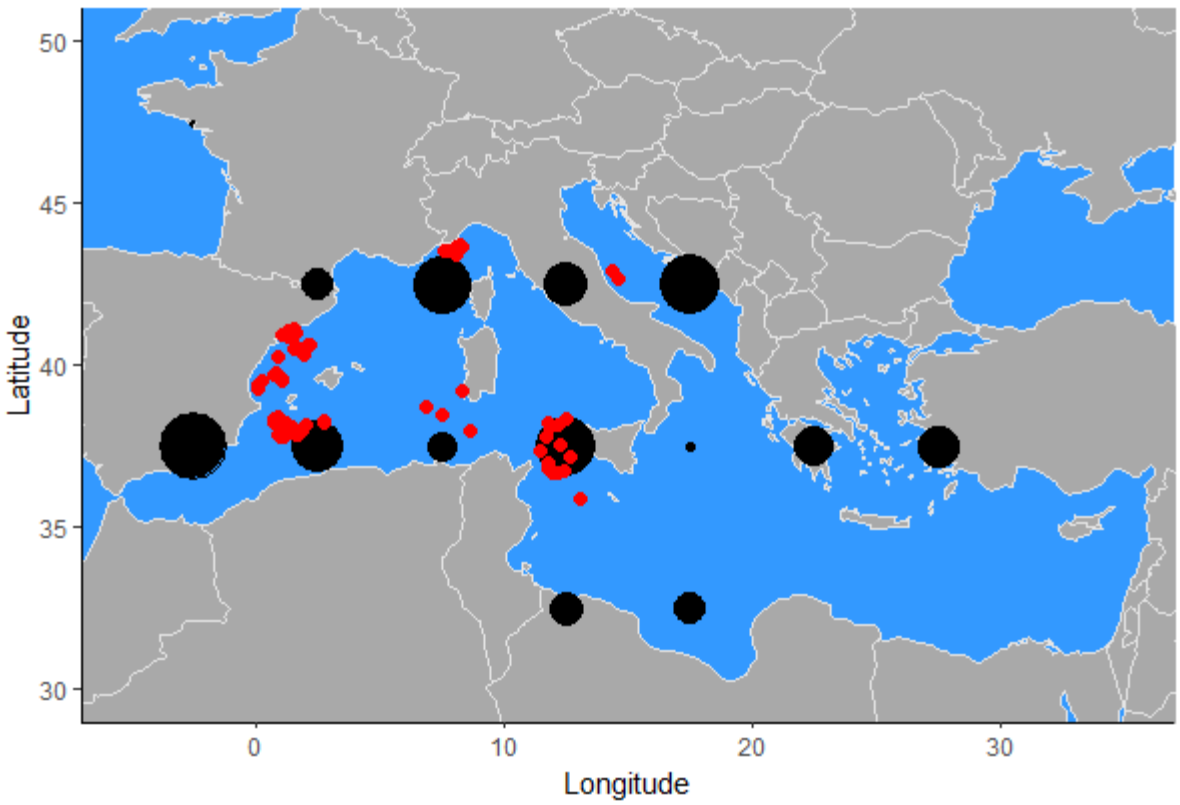


Figure 4. Sampling coverage in the Mediterranean. Red dots indicate locations where samples were collected. Black dots represent swordfish catch for years 2012-2016 for each 5x5 grid cell area, scaled by contribution to the overall catch. Dots for grid cells contributing to less than 0.1% of the total average catch are omitted.

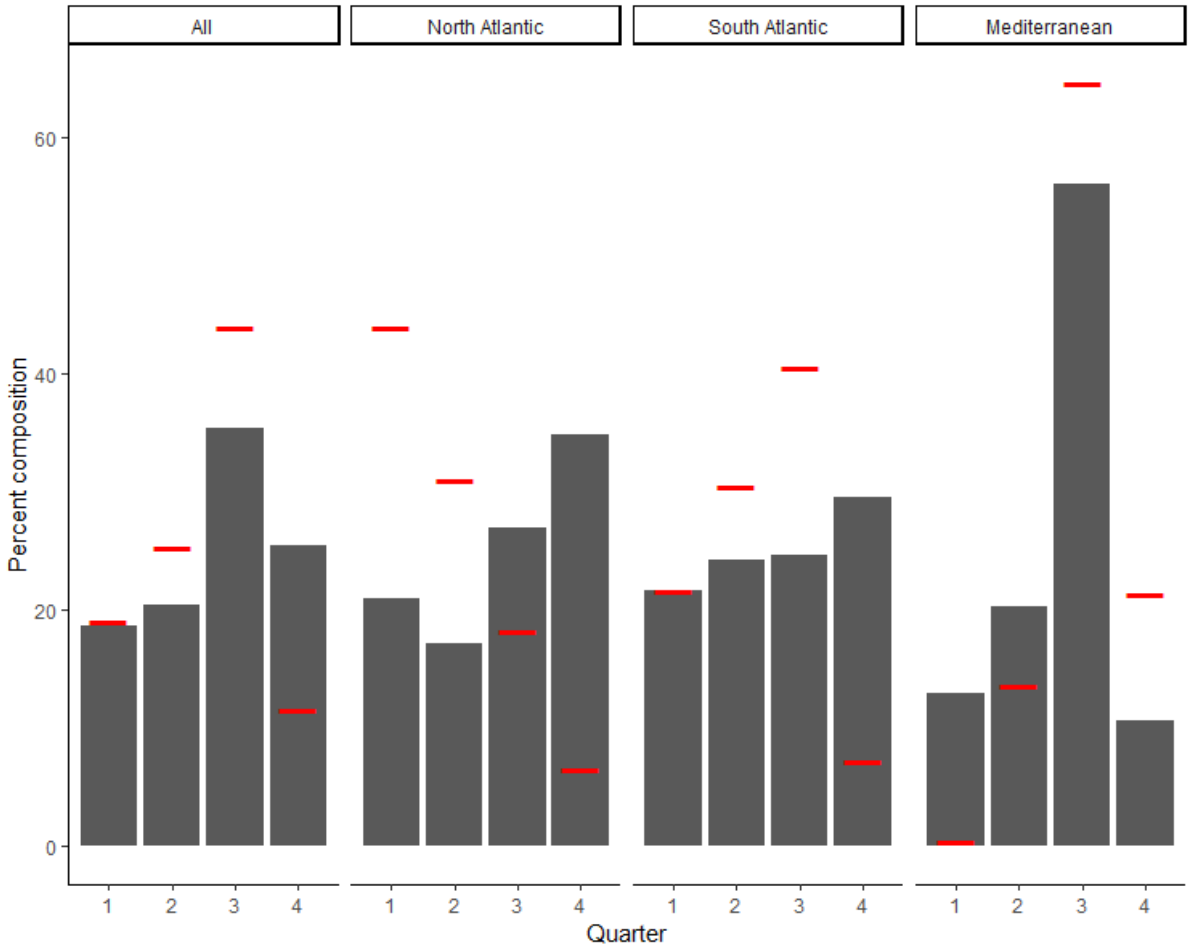


Figure 5. Sampling temporal coverage by quarter and stock. Bars represent the mean proportion of catch obtained in each quarter of the year by stock from Task II data (2012-2016). Red horizontal bars are the percentage of samples in this study obtained in each quarter by stock.

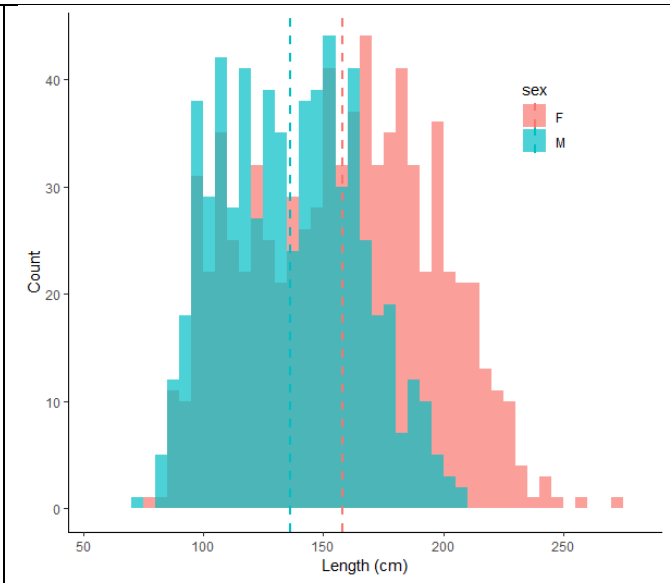
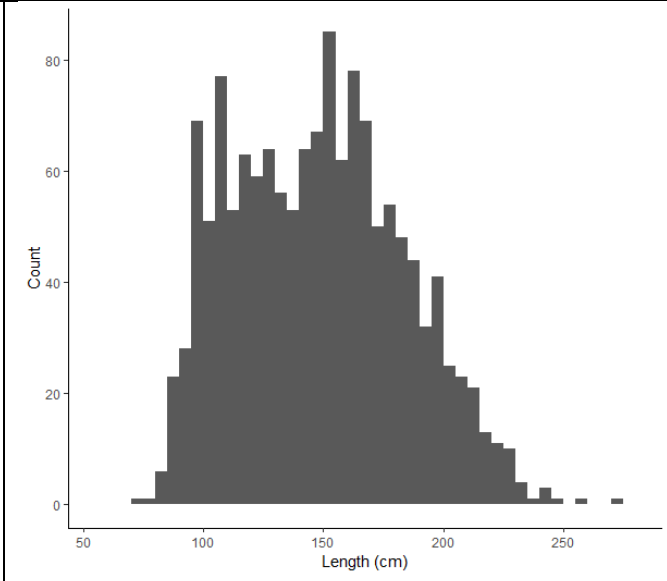
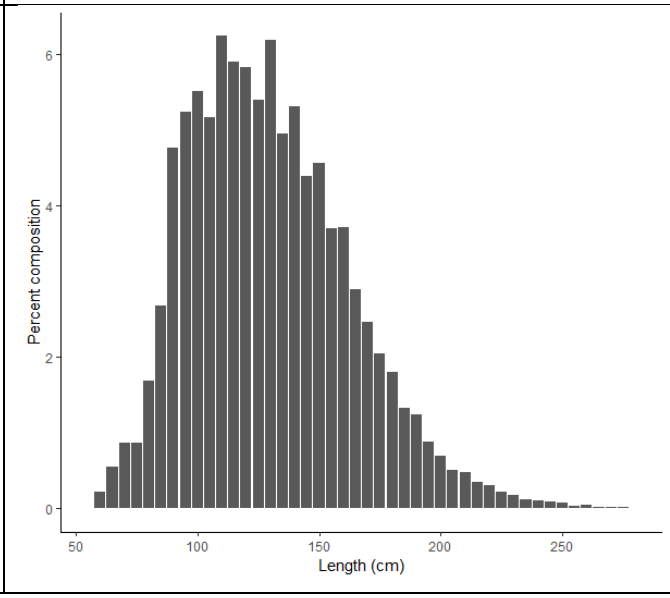
Sample length frequencies, sex disaggregated	
Sample length frequencies	
Catch length frequencies	

Figure 6. 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 II data for 2012-2016 (bottom).

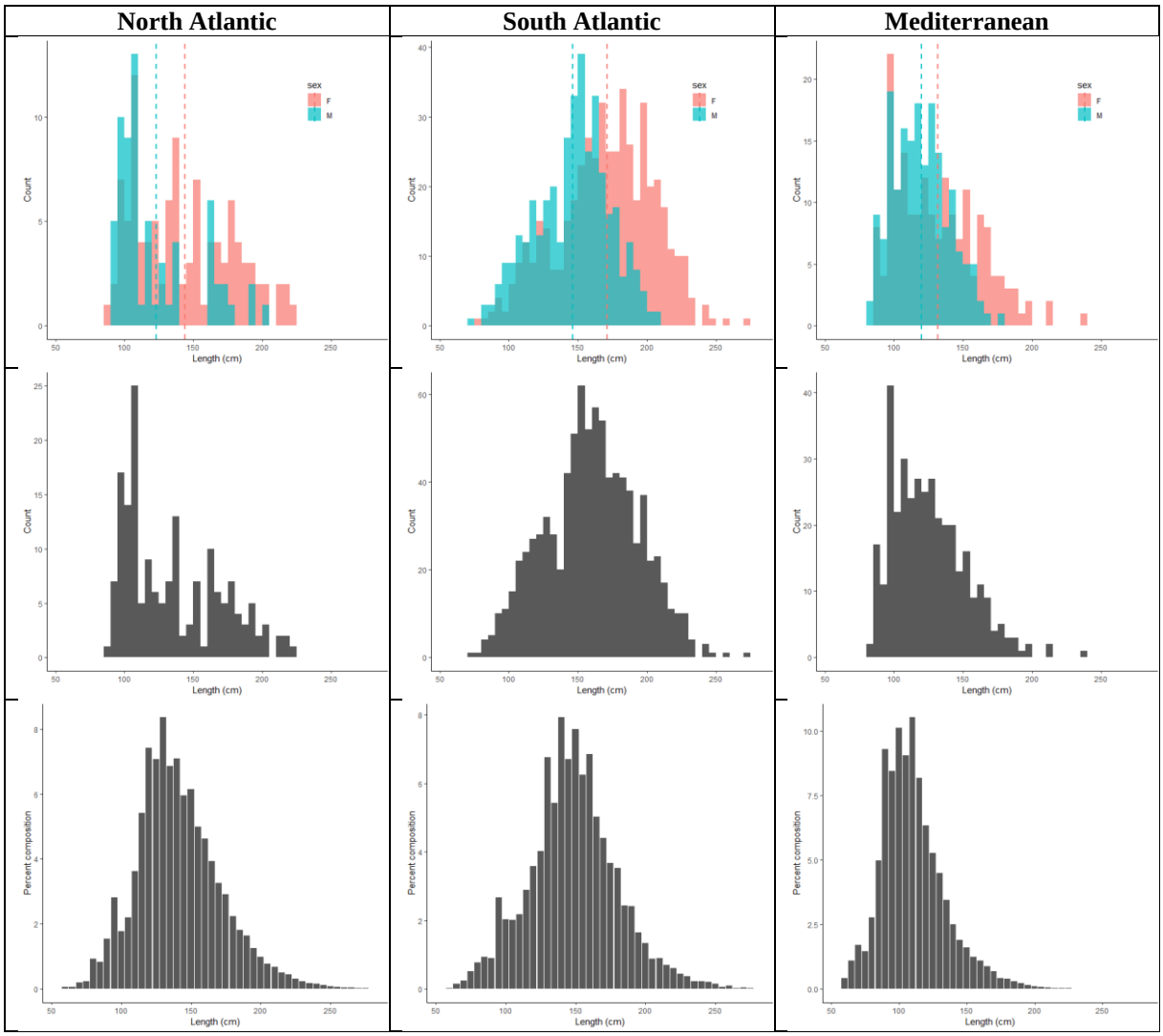


Figure 7. Length frequencies for all swordfish sampled in this program by stock, disaggregated by sex (top) and non-disaggregated (middle), compared to estimate catch size frequencies from ICCAT task 2 data for 2012-2016 (bottom).

Appendix 1 – Sampling protocol

1. Representative sampling

Sampling effort should reflect the spatial, temporal and selectivity patterns of the fishery. Where possible, sample for the duration of the fishing season with increases in sampling effort coinciding with increases in fishing effort. This sampling effort should be representative of the spatial footprint of the fishery. Sample a range of swordfish body sizes reflective of the body sizes found in the catch.

2. Sample completeness

A full set of biological, fisheries and metadata is important to this program, however, we recognize that not every sampling effort will be able to collect all requested data. The following criteria will be used to evaluate sample completeness and determine compensation for samples.

2.1. Complete sample

A complete sample is the full suite of sample metadata, biological data and the properly processed and stored biological materials. A complete sample is of most value to the programme and will be compensated at the maximum rate.

Complete sample; compensation value €20
Source (consortium member name)
SampleID
Date caught
Latitude, longitude caught
Port/Location landed
ICCAT billfish area
Spine (properly processed and stored)
Tissue (properly processed and stored)
Length (and length code)
Sex
Maturity stage code
Gear

2.2. Partial sample

A partial sample lacks information typically available only in a freshly captured, fully intact fish. Spine or tissue samples must be included to count as a partial sample.

Partial sample set, type 1; value €15	**Partial sample set, type 2; value €15**
Source (consortium member)	Sample contains a properly processed and stored fin spine OR a properly processed and stored tissue sample
SampleID	
Date landed	
Port/location landed	Source (consortium member)
ICCAT billfish area	SampleID
Spine (properly processed and stored)	Date caught
Tissue (properly processed and stored)	Latitude, longitude caught or port landed
Length (and length code) OR weight (and weight code)	Length (and length code)
	Gear type

2.3. Incomplete sample

Any sample missing a properly processed, labelled, and stored spine or tissue sample. Value €0.

3. Data and sample management

A sample data sheet for use in the field is provided (this document, appendix 1). Once data is collected, it should be transcribed into the provided Excel workbook (SWO_Biol_Sampling2018_Template.xslx) as soon as possible.

Each fish sampled receives a unique identification code. The sample code should be clearly affixed to spines and the container holding the tissue sample.

IMPORTANT: For sampling in 2018, sample codes should be in the following format: ICCAT flag code followed by a four digit number. For example, the first sample collected by the consortium member in Portugal would be EUPRT0001, the second sample EUPRT0002, etc. CLEARLY INDICATE THE NAME OF THE SAMPLING INSTITUTION ON THE SAMPLE LABEL. In future years, each consortium member will be assigned a set of sample identification numbers.

4. Sampling procedures

4.1. Fish length

The preferred length measure is lower jaw fork length (LJFL). If other measurement types are used, please specify the type using the appropriate length code. LJFL (cm) is measured from the tip of the lower jaw to the fork in the tail following the curvature of the body.

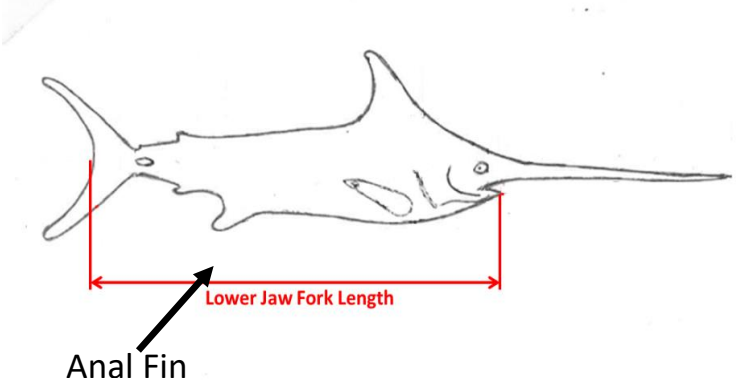


Figure 1: Swordfish with lower jaw fork length and position of anal fin.

4.2. Fish weight

Where possible, record the round weight (weight of the whole animal before processing or removal of any part). Otherwise, record the dressed weight, using the appropriate weight code in the spreadsheet.

4.3. Sex and maturity

Estimate sex and maturity using the following criteria. In the future a photo guide will be provided.

Maturity		**Criteria**	
Stage	**Description**	**Females**	**Males**
0	Undetermined	Gonads are small and ribbon-like. It is not possible to determine sex.	Gonads are small and ribbon-like. It is not possible to determine sex.
1	Immature	Ovaries ~1/3 abdominal cavity, pinkish-translucent, slender and elongated. Sex is obvious.	Testes ~1/3 abdominal cavity and whitish. Testes thin, flattened and ribbon-like. Sex is obvious.
2	Early maturing and recovering	Ovaries ~1/2 abdominal cavity. Individual ova are not visible to the naked eye.	Testes ~1/2 abdominal cavity. Testes triangular in cross section with no milt in central canal.
3	Late maturing	Ovaries ~2/3 abdominal cavity and pinkish – yellowish with granular appearance. Transparent or translucent eggs are visible.	Testes ~2/3 abdominal cavity. Milt flows freely if testes pressed.
4	Mature/Ripe	Ovaries ~2/3 abdominal cavity and orange-pinkish with blood vessels. Eggs large, mature and transparent. Eggs loose in lumen of ovary.	Testes ~2/3 abdominal cavity and creamy white, soft. Milt flows freely.
5	Post spawning/Spent	Ovaries contracted ~1/2 abdominal cavity with loose walls; bloody and flaccid. Ovaries may contain traces of mature, opaque eggs, in resorption which are darkened or translucent ~1.0 mm in diameter.	Testes contracted dull red ~1/2 abdominal cavity with loose walls; bloody/bloodshot and flaccid/flabby. No milt in central canal.

4.4. Anal fin spine

4.4.1. Removal

The whole anal fin (Figures 1 and 2) can be removed by cutting along the body contour, ensuring the bases of the fin spines are remain intact in the fin. Alternatively, the second anal fin spine can be isolated and removed. Spread out the 1st anal fin and cut the membrane joining the three first anal spines using a knife. Then, push the 2nd spine forward and down progressively, then cut and turn it alternately to the right and to the left until the ligament breaks. Finally, the spine must be twisted and pulled out. PLEASE DO NOT SAW OFF THE SPINE. Ensure that the base of the spine (the condyle) is removed along with the rest of the spine. Freeze the anal fin or spine until it is ready for further processing.

Figure 2: Location of swordfish anal fin.

4.4.2. Processing and storage

Excess tissue can be removed from the anal fin and second anal spine by placing the structure in freshly boiled water for 5-10 minutes. Tissue can then be stripped by hand and blunt knife. The second anal spine can appear as either branched or unbranched at the distal end. Using a string or zip tie attach a tag with sample information and leave the spine out to dry. The spine does not need to be frozen.

4.5. Tissue

Collect a 3 cm cube of tissue and place in a labelled vial, immersed in 95 % pure ethanol (denatured ethanol is not good for DNA analysis). Thoroughly wash the knife before and after collecting each sample. This is important so as to avoid contamination of genetic samples. Write the sample identification number on a small slip of paper and place in the vial with the tissue sample. Vials should be stored in the freezer.

4.6 Stomach content

Where possible, cut open the stomach and note whether or not it contains any food items. record the gut fullness using the following codes:

1) Empty
2) Up to ¼ full
3) ½ full
4) ¾ full
5) Full
6) Everted (The stomach has been blown out into the mouth by the pressure.)
7) Regurgitated (The contents have been lost, but the stomach is intact. This can be told from an empty stomach by the possible presence of food in the mouth, or by the fact that the stomach is stretched and flabby, yet empty.)

If there are items in the stomach, attempt to identify the items to the finest taxonomic scale possible and record these items in the spreadsheet

Appendix 2 – Sampling institutions

Sampling group	**ICCAT CPC**
Universidade Federal Rural de Pernanbuco	Brazil
Nova Scotia Swordfishermen's Association*	Canada
Fisheries and Oceans Canada†	Canada
Aquastudio Research Institute	Italy
Department of Environmental and Life Science, University of Cagliari	Italy
Laboratorio di Biologia Marina e Ecologia Animale, Universitá Degli Studi di Genova	Italy
UNIMAR società cooperativa (UNIMAR)	Italy
Università Politecnica delle Marche Home	Italy
Portuguese Institute for the Ocean and Atmosphere (IPMA)	Portugal
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