REPORT OF THE 2021 ICCAT SWORDFISH BIOLOGY WORKSHOP

(Online, 22-26 March 2021)

"The results, conclusions and recommendations contained in this Report only reflect what was discussed by the Group of scientists that participated in the 2021 Swordfish Biology Workshop. Therefore, these should be considered preliminary until the Swordfish Species Group and the SCRS discuss and possibly adopts them at its annual Plenary meeting and the Commission revise them at its Annual meeting. Accordingly, ICCAT reserves the right to comment, object and endorse this Report, until it is finally adopted by the Commission."

1. Opening

The ICCAT Swordfish Biology Program was established in 2018, run by a Consortium that includes scientists from 16 ICCAT CPCs. The Consortium is administered by the Nova Scotia Swordfisherman's Association with organizational oversight provided by Fisheries and Oceans Canada. The 2021 Swordfish Biology Workshop was held online from the 22nd to the 26th, 2021. Dr. Kyle Gillespie (Canada), the Rapporteur for the North Atlantic swordfish stock, opened the meeting and served as Chair.

On behalf of the Executive Secretary, the Assistant Executive Secretary Miguel Neves dos Santos, and the vice-Chair of the SCRS, Rui Coelho, welcomed the participants to the workshop.

2. Adoption of the agenda

The Chair proceeded with the review of the Agenda. The final version is shown in **Appendix 1**.

3. Nomination of the rapporteurs

The list of registered Participants is included in **Appendix 2**. The following served as rapporteurs:

- Section 4: Kyle Gillespie
- Section 5.1: Daniela Rosa, Rui Coelho
- Section 5.2. David Macias
- Section 5.3: Giorgia Gioacchini
- Section 6.1: Dheeraj Busawon, Daniela Rosa
- Section 6.2: David Macias, François Poisson
- Section 7: Kyle Gillespie, Rui Coelho

4. Status update of the ICCAT swordfish biology project

The Project Coordinator (Dr. Kyle Gillespie, Canada) reviewed progress made to date on the project and the core program objectives were presented . The Coordinator recalled the sampling process and the sampling coverage and sample numbers for each phase of the program (See SCRS/2021/119 for detailed sample numbers and sample location maps). To date, 4479 swordfish have been sampled and the sample inventory includes 3467 fin spines, 985 otoliths,

3325 tissue samples, 498 gonads, and 418 stomachs. While phases 1 (2018) & 2 (2019) of the Project largely focused on sample collection and preliminary analysis, the current phase (phase 3 from 2020 to 2021) is focusing on sample processing and analysis and development of standardization procedures among labs. The Coordinator emphasized that the focus of this workshop was on technical aspects of reading for ageing and maturity stage standardization with a view of establishing reference sets.

There were brief comments from the Group on addition of sampling location and fleets, however much of this discussion was deferred to agenda item 7.

5. Update of the sample collection and sample processing

5.1. Ageing (spines and otoliths)

An update of the sample collection and sample processing of both spines and otoliths was presented. Overall, 3467 spine samples and 985 otolith samples have been collected so far, by 15 and 4 institutes, respectively, covering a wide length range for the three stocks. Of these samples, 755 spines and 214 otoliths have been processed for annual age readings and 7 for daily ageing. A batch of spine and otoliths is now being processed and funds are available through this year to process more samples. For detailed sampling metadata, coverage, and maps see SCRS/2021/119.

The Group noted that there are still some important missing areas for the sampling, such as the southern Atlantic areas and the east Mediterranean. The Group also noted that the current project phase is now trying to focus the sampling in those areas where there are still gaps. As in the past, the Project Coordinator emphasized that any scientists/laboratory that wishes to collaborate in this project and has opportunity to collect and provide samples especially from those regions is welcomed to integrate into the Project.

The Group then discussed data confidentially issues. While the Project seeks to obtain samples with associated fine scale spatial-temporal information, the minimum data to be provided would be monthly data reported in 5*5 spatial resolution (10*10 could be considered as well). Having monthly data is important for example to explore age verification methods such as Edge Analysis and/or Marginal Increment Analysis along the year, and a minimum of 5*5 (or 10*10) spatial resolution is needed to identify the location of the samples. There are issues with some CPCs in terms of data-confidentiality, especially the USA, that has around 900 historical spine samples from the late 1990s and early 2000s, but the corresponding data has not yet been provided due to confidentially rules. The Group thanked the USA scientists present at the meeting that are trying to solve this issue, highlighting the importance of integrating those spine samples for the ongoing work. The Project coordinator will continue to work with all CPCs that may have data-confidentiality issues, to see if any type of data sharing agreements can be made.

5.2. Reproduction (macroscopic data and gonad sample collection)

An update of the sample collection and sample processing of gonads and macroscopic maturity data was presented. 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 322 samples of gonads, 262 from the North Atlantic and 62 from the Mediterranean Sea have been processed for microscopic maturity. Some samples from different institutes are going to be sent for processing in a short time. A preliminary analysis of L_{50} comparing macroscopic and microscopic data was conducted in 2020 (SCRS/2020/135), and further analysis will be conducted after increasing the sample size.

The Group noted that there are still some important gaps in the temporal and spatial coverage of the sampling. Improved sampling is needed in the central and western north Atlantic, central and eastern Mediterranean, and non-equatorial areas of the south Atlantic.

Some scientists have offered collaboration to collect and provide samples of those areas where there are still gaps. The Group discussed the problems to cover some spatial gaps in the Mediterranean Sea due to regulations for the Mediterranean swordfish (temporal fishery closure, etc.).

The Group also discussed the sampling collection protocols for sex-ratio and the need to include complete data sets in the database to avoid bias in the analysis (Gillespie et al. 2020).

The Group then discussed the importance of using standardized protocols to collect, preserve and process the samples. The Group noted the difficulties to use a simple protocol due to the different characteristics of the fisheries and constraints to use fixatives onboard.

5.3. Genetics

The Group was presented with an overview of the results regarding genome assembly and comparative genomic analysis done to date. An update on the comparison of the assembled genome with a swordfish genome recently published by a Chinese group (Wu et al. 2021) was provided, highlighting an high percentage of correspondence. In addition, and thanks to the availability of the transcriptome at UNIVPM, the description of the chromosome's genetic features was also possible in this analysis. The authors then discussed the results from their genetic population analysis. Double digest restriction-site associated DNA (ddRAD) sequencing technology was applied to evaluate intra and inter-population differences among 288 samples: 76 North Atlantic (25: BIL 92/94A and 51: BIL 94B 94C), 60 South Atlantic (BIL97) and 152 samples Mediterranean BIL95 (79 CENTRAL MED, 44 EAST MED, 29 WEST MED). The authors showed an overview of the distribution of different characteristics of samples analysed (i.e. N° of specimens per area, maturity stages, sex, month of the year and so on). Genetic analyses were performed using 41277 SNPs examined in ddRAD analysis. Among them 24459 SNPs were found within genes and 16818 SNPs were found within intergenic region. PCA showed a strong genetic diversity (PC1=62.3%) between Mediterranean and Atlantic specimens, the presence of 6 animals caught in area BIL94B clustered as Mediterranean. On the other hand, there was a less marked differentiation between North and South Atlantic (PC2=5.1%). DAPC analysis confirmed the results and in addition showed the genetic link of BIL94B area with all other areas (BIL 92/94a, BIL97 and BIL 95) analysed. The Heatmap representation of the matrix of pairwise genetic distances among all the 288 samples confirmed the genetic distance between Mediterranean and Atlantic specimens, the presence of six animals caught in BIL94B in the Mediterranean cluster, a less marked genetic distance between BIL92/94A and BIL97 and the

wide ranging distribution of specimens caught in BIL94B within other areas. The same results were confirmed by a neighbor-joining cladogram analysis performed both with all samples and with only Atlantic ones.

Fixation index (FST) indicated that the most differentiated specimens are those caught in BIL97 and BIL95 areas, while the less differentiated are those caught from BIL92/94A and BIL94B. Additional statistics analyses revealed that specimens from all the areas showed a lower value of observed Heterozigosity (Ho) with respect to the expected ones, and that specimens from BIL92/94A showed the lowest values of Ho and total allelic richness and the highest value of inbreeding coefficient. The authors further investigated heterozygosity by analyzing only SNPs occurring within genes and the resulting heatmap revealed a completely different pattern of heterozygosity. Most genes with a high degree of heterozygosity in Mediterranean specimens showed a low degree in Atlantic ones, few genes showed the same pattern and among Atlantic specimens there are differences that could be related to North and South Atlantic subpopulation differences.

Finally, genetic structure of different population, in terms of allelic frequencies distribution, was presented. Using all the SNPs available, the most evident differences have been found between Mediterranean and Atlantic populations. The authors showed that using separately intergenic or genic SNPs different and complementary information on genetic structure of sub-population among Atlantic and Med specimens could be obtained. The authors concluded that currently, new analyses are ongoing on additional 384 samples coming from all BIL areas available. In addition, 30 samples (10 NA, 10 SA and 10 Med) will be analysed by WGS (Whole Genome Sequencing) to identify a set of SNPs that can be used to assign an unknown sample to one of the described populations and to identify sex-specific regions or markers for further genetic sex determination of unknown samples.

The Group noted the results are in agreement with results previous obtained and current stock structure. Information has been requested about the gene families expansion on swordfish. The Group asked to focus attention on the Balearic area to confirm the presence of a mixing area by adding new samples coming from this area. The Group discussed the possibility of including new samples coming from different area in Mediterranean and Atlantic. The Group also requested if it is possible to apply ddRAD analyses on historical samples or in bone samples. The Group also noted that so far the genetics do not provide enough evidence to suggest that SWO management boundaries should be redrawn, noting that when filling the sampling gaps this perspective may change.

6. Establishment of reference sets, with regards to

6.1. Ageing (incl. spines and otoliths)

- *Presentations from invited experts*

Invited expert, Freddy Arocha (IOV-UDO), presented the ageing of swordfish from the western North Atlantic in the 1990s. Ageing was based on 4209 samples collected over 10 years (1990-1999) that covered a broad geographical area of the western North Atlantic, from 5°N to 55°N and west of 40°W. The process of the spine sectioning and the age reference spine used in the study were shown in addition to examples of a variety spine section images with different features that made the ageing process difficult and showed the broad variability in size for a

given age. The results of this ageing process led to the estimation of the growth function used in recent stock assessments for the North Atlantic stock.

US and Venezuela scientists will try to locate these historical samples or their images for inclusion in this study. Furthermore, the US indicated that the 900 samples that have been provided for this study (located at SABS Canada) are potentially from the same historical sampling batch and could be reprocessed using current methodologies. It was also noted that it would be useful to compare the historic and current ageing results. There was also some discussion concerning data sharing restrictions by different countries and how it could affect available data.

Jessica Farley (CSIRO) presented an overview of the swordfish age and growth work recently undertaken in the southwest Pacific. The aim of the study was to determine the degree to which differences in growth parameter estimates obtained in the southwest Pacific and Hawaiian regions were methodological or due to spatial variation in life-history. After re-examining sectioned fin spines from an earlier study in Australia, they found that methodological differences did exist between studies in the two regions, and they highlighted the need for consistent reading methods of hardparts among studies. The study also found that otoliths tended to give much higher ages than those estimated from spines in large/old fish. Since otoliths are the preferred structure to age most fish species, the results suggest that swordfish live longer and grow more slowly than previously estimated in the southwest Pacific.

Discussion centered on ageing and age validation. In terms of ageing, questions were asked concerning the ageing of spines vs. otoliths. The expert noted that otoliths are more difficult to age for young specimen but are 'easier' for older specimen and that the reverse is true for spines. The effect of vascularization in spines was also discussed and whether measuring the distance to the 1st increment can help in correcting ageing bias. However, it was noted that even with an adjustment there was still an ageing bias between spines and otoliths and that overall age is being under-estimated for older fish when using spines. It was noted that besides the vascularization the increments that are further away from the core could also be suffering from remobilization, which could account for the underestimation in larger specimens' spines. It was noted that some spines do present a clear pattern and that some had higher counts than otoliths. The Group also inquired about the timing of deposition between the two structures and if different types of gear could introduce ageing bias. For spines the period of formation of the translucent band would be during winter (south Pacific).

In terms of validation, the expert noted that methods that could be considered are bomb-radiocarbon and a tagging program (i.e. OTC mark-recapture). She noted that for tagging you would require a long time at liberty before recapture which might not be ideal. On the other hand, bomb radiocarbon has potential and is something that should be further explored in the future. It was questioned if the small size of the otoliths would be a problem for this analysis, it was noted that this method has been applied successfully in billfish species—otoliths which are similar in size to swordfish otoliths.

Indirect validation through analysis of length frequency distribution was also discussed, the presenter noted that they have not looked at this but that in general smaller fish are missing which would make this method difficult as it would not be possible to track the modes in the data.

Back calculation to estimate missing increments due to vascularization and a method for combining increment measurement with date landed to determine ageing bias were also

mentioned. It was noted that back-calculation has not been attempted with swordfish, but the method has biases that need to be considered.

Kyne Krusic-Golub (FAS – Fish Ageing Services) followed and provided an overview of the technical aspects in the development of the methods for Pacific swordfish otolith preparation and the ageing protocols and interpretation. Initially the three otolith structures (sagitta, lapilli and asteriscus) were investigated for the possibility of providing daily age estimates. Through that process, it became evident that the sagittal otolith was the preferred structure and that while microincrement structure was clearly identified in the nucleus and may provide important information on the juvenile growth, it was considered unlikely that counts of microincrements would provide accurate estimates of annual age. Furthermore, in the process of examination, one large sagittal otolith showed clear annual like structure along the rostrum. Transverse sectioning of that sample provided a preparation that showed clearly alternating opaque and translucent zones and the potential for accurate annual age estimation. In total a further 300 samples were selected for preparation and the annual ageing methods were developed from these samples.

As with other tuna species that have investigated, the first few annuli in the otolith sections were difficult to interpret. Counts of microincrements were used to verify the age and position of the first opaque zone and proxy measurements for the first three annuli were determined from the samples that showed relatively clear increment structure. The approximate age at the first opaque zone was 150 days and the average distance to the first, second and third opaque zone was 0.516mm, 0.715mm and 0.856mm, respectively. The methods developed for Pacific swordfish were then applied to Atlantic swordfish otolith samples that were provided to Fish Ageing Services (FAS). Preliminary results suggest that Atlantic swordfish otoliths are similar to Pacific swordfish otoliths and should be a useful structure in providing annual age. Currently 126 (121 annual and 5 daily/microincrement) otoliths samples from Atlantic swordfish have been processed and aged. A further 208 samples are in the process of being completed and the results will be made available once completed. **Table 6.1** provides the average distance to the first 5 opaque zones used in the estimation of annual age, and **Figure 6.1** provides the image of a reasonably good otolith.

Table 6.1. Average distance to the first 5 opaque zones used in the estimation of annual age.

Opaque increment #	Measurement distance (mm)
1	0.516
2	0.715
3	0.856
4	0.972
5	1.060

Figure 6.1. Example of a reasonably clear to interpret pacific swordfish otolith (arrows show opaque zones).

There was discussion concerning the timing of formation of bands and if it would differ between the hemispheres. FAS took increment measurements for 240 samples and it showed that opaque zone formation was completed at the end of the summer (Pacific). In terms of otolith processing, it was noted that damaged otoliths are still viable as long as the core is present. Age validation using shape analysis was also discussed. Swordfish otoliths would be a good candidate for shape analysis due to high variability in shape, length and weight, as long as this variability is by age class, related to stock growth, and not to differences related to individual traits. It was also noted that swordfish otoliths are very brittle and it is common to get damaged otoliths.

- *Discussion on the age exchange exercise*

Daniela Rosa presented the results of the age exchange exercise. The exchange sample composition was presented, with 25 otolith-spine pairs of varying length classes and both sexes. There were five readers (4 readers for spines and 4 readers for otoliths) from different laboratories in this exercise. For some samples, there was low agreement between readers both in otoliths and spines, underscoring the importance of standardized methods and continued work on a reference set.

The results were discussed in relation to the level of expertise of the readers (i.e., non-experts vs. experts and otolith readers vs. spine readers). For spines, agreement was lower between non-expert readers and expert readers, however the agreement between expert readers was also low indicating that the level of expertise was not the only driving factor in the observed difference. Regarding otoliths, agreement was also low, however in this case only one reader had previous expertise with swordfish otoliths. The results indicated that there was inherent difference in interpreting spines and otoliths: better agreement for young individuals and poorer agreement for older individual in spines while for otoliths the differences in agreement between small and large individuals was not so marked. One reader noted the need for each reader to make 2 readings separate at least 1 week apart, as multiple interpretation might aid in reducing ageing errors.

The Group discussed which structure should be used for ageing and if specific structures should be used depending on age class. The current ageing exercise is important to determine the limitations of each structure and how they should be used. However, the availability of each structure might be a limitation as spine collection is more feasible compared to otoliths (e.g. for some CPCs there is the need to buy the whole fish). It was suggested that new sampling methods could be explored (i.e. using a hole saw method) to expand otolith collection. It was noted that future work on validation is needed. The results of that work should determine sampling prioritization of hard parts.

The Group discussed ageing procedure, methods, and techniques. Expert readers commented that otolith reading might be more consistent if they were read live vs. from images, as one would be able to change magnification, lighting, and focal area. Furthermore, enhancement of

the images was considered a potential to increase readability and agreement between readers, especially for the most difficult to read spines and otoliths. In terms of difference between ageing protocols for reading the various hard structures, experts commented that due to counting opaque bands on otoliths and translucent bands on spines, this might result in a 6-month lag. This should be taken into consideration when comparing zone counts and will be corrected by the age algorithm used to convert zonal counts to age. The results indicated that in general for the older fish the spines seem to be under-estimating ages due to reabsorption, while otoliths from young individuals are hard to interpret and it was suggested that proxy measurements would be quite useful moving forward for otolith-based ageing. Invited expert Kyne Krusic-Golub (FAS) confirmed that the juvenile period in the otoliths is the more difficult to interpret. The spines seem to be much clearer until 2 or 3 years of age, and from then on, the otoliths seem to be more reliable. It was proposed that ageing standardization continue intersessionally.

The presenter noted that there are different configuration of spines and currently only configuration A is being used for the ageing (Gillespie et al., 2020). This is consistent with what has been done in the past, as type A configuration is by far the most common in swordfish and has been used preferentially both historically and currently. The other configurations might be used in the future for comparative purposes. It was further noted that from the Indian Ocean there are also differences in the spine configurations, the Group wondered if there was a genetic basis for different spine configuration. It was noted that the genes for bone formation/growth could be investigated between swordfish with the various spine configurations.

It was mentioned that a third structure (i.e. vertebrae) could be used for ageing comparisons. However, vertebrae samples are problematic in this case as removal from the animal is very invasive and would require purchase of the entire fish but warrants further investigation in future studies.

Potential differences in growth between the Mediterranean and the Atlantic swordfish were mentioned by two readers. It was noted by several participants that stock (or area) specific growth curves will need to be developed (i.e. N-ATL, S-ATL, MED).

Using epigenetics as a validation method was discussed. However, it was pointed out that epigenetic ageing has to be calibrated through direct ages so it cannot be used as a method of validation. Genetic labs were open to attempting other methods to assist with ageing (e.g. FTIR and raman spectroscopies) and also looking into epigenetic ageing.

One participant showed a software that can make comparisons between readers, with estimations of various age bias metrics. It was noted that much of this can also be currently done in R (FSA package – Ogle et al., 2021), and that as this age exchange exercise continues those metrics will be calculated and presented.

For continuing the work, the ICES Smartdots software was proposed as a potential tool for future ageing among readers. This open-source technology is especially appropriate for collaborative age readings. It was originally developed for otoliths, but some recent developments have made it more useful for spines as well.

There was then some discussion on staining of the sections. Invited expert Kyne Krusic-Golub (FAS) noted that this has not been applied to swordfish, however from his previous experiences staining has not helped much with ageing of most species. The Group agreed that for spines the

Quelle et al. (2014) measurement method will be followed. For the otoliths, Kyne Krusic-Golub (FAS) will provide a diagram with the standard measurement method for otoliths.

The Group then carried out the ageing exchange exercise on the subset of structures, which is reflected in **Appendix 3**.

6.2. Reproduction biology (histological scales)

The main topic to consider during the workshop was the establishment of a reference set 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).

A presentation on the reproduction of swordfish from the western North Atlantic in the 1990s was given to the Group by Freddy Arocha. The developmental stages of oocytes (histological and whole fresh) and macroscopic appearance used for maturity classification were based on 2538 samples collected over 5 years (1990-1995) that covered a broad geographical area of the western North Atlantic, from 5°N to 55°N west of 40°W. The process to define the developmental stages of oocytes included a combination of histological and whole fresh oocyte morphological characterization and the construction of size frequency distributions for each developmental stage of the maturation process. The macroscopic maturity stages for males were shown. In addition, the developmental stages of oocytes that were used to define the major spawning grounds in the western North Atlantic were indicated, as well as those used to identify the precise developmental stage of oocytes to produce the fecundity relationship at-size and at-age.

Jessica Farley presented an overview of methods to estimate the maturity status of swordfish in the southwest Pacific. One of the aims of the study was to determine if spatial differences in maturity ogives in the Pacific were due to methodological differences in how females were classified as mature or immature, or whether they were due to spatial variation in life-history. Ovary histology obtained from a previous study in the southwest Pacific was assessed using a modern classification scheme. Immature and regenerating (mature-resting) females were differentiated using 'maturity markers' including muscle bundles, brown bodies and encapsulated hydrated oocytes. The new maturity ogive obtained in the study was similar to that obtained for swordfish in the Hawaiian region, suggesting that methodological differences existed between studies. The presentation highlighted differences in ovary histology from fresh- and frozen-fixed samples but noted that frozen-fixed samples can provide similar results provided readers are aware of whether samples were fresh or frozen and be used for reproductive studies of swordfish.

The Group discussed the importance of differentiating between immature (i.e. juveniles) and regenerating females, as well as between developing female that coming from the immature stage from those mature females at the beginning of the annual reproductive cycle. The Group noted that the use of maturity markers aid to solve these issues.

The Group also noted the importance to provide macroscopic pictures of gonads to establish reference sets for macroscopic maturation. A call for macroscopic images could be useful for complete the gaps in macroscopic maturity images collected by the SWO Biology Project.

The calibration exercise was presented to the Group. Four experts from three different laboratories participated in the exercise. Five sets of microscopic images were used three from the Mediterranean Sea and two from the north Atlantic (n=98). Two different scales for histological classification were used. The reading of each expert and the agreements among readers were presented.

The Group noted that the two maturity classification scales used in the histology staging exercise were very similar and that the maturity status for each fish would be the same using either classification method. The only difference was that the IEO-Malaga scale had an additional stage to specifically differentiate mature-developing from regenerating females. The Group agreed that this scale was clearer and decided to use it for the staging the gonads for the reference set.

The Group discussed the choice of the readers and why there was no data call for readers. The Group noted that all the readers participating in the exercise are experts in histological maturity staging and due to the limited time, readers were selected among the Group. However, any additional inputs from the Group during this exercise at the meeting were welcomed.

The Group also discussed if the number of samples and readers participating in the exercise are sufficient. The task leader noted that in a similar ICES workshop the number of samples per exercise was recommended to be kept to less than 120.

All the microscopic images selected were examined by the Group to identify stages hard to distinguish, resolve interpretation differences between readers and select the images for the reference set.

The Group noted that the quality of the sample is an important parameter. The quality of all the sets was not homogeneous. The staining was irregular and the quality or resolution of the picture could be improved. It is important to get good quality samples in order to stain the tissue in good condition. The Group also noted that doing such exercise from digital images is more difficult than having the slides under the microscope and making the readings directly. It is crucial to be able to cover different areas of the slide and also to zoom in to observe in detail the tissues. Given the current situation the only option was to conduct this workshop online using digital images, but the Group agreed that such exercises should be returned to in-person workshops as soon as possible with the samples and readers together in the same laboratory.

The Group suggested to prepare a grid with all the criteria used to identify the stage (no POFs, early vitelogenic oocytes, etc.) in a way that each reader could tick each column when they observed the structures. The final stage assignment would then be done almost automatically. In addition, this “process” could be used to identify more easily the disagreements between readers.

It was noticed that parasites are present in some gonads and could alter the quality of the slide. It is also possible to confuse such parasites with encapsulated oocytes.

Regarding Sets from North Atlantic, the Group discussed the difficulties for staging histological sections from individuals that spawned a long time ago. Some indicators as gonad weight could aid to avoid stage misclassification. The Group also discussed the issue of skip spawners. Some juvenile SWO could enter in regressing before reaching the final stage of maturation. The Group also discussed the issue of skip spawners and the potential existence/presence of precocious females noted by the observation of younger smaller fish showing signs of maturity markers commonly observed in sexually mature females. Therefore, these fish do not produce eggs in

this reproductive season. Advice from experts from different areas is important as their knowledge of the fishing grounds and ecology of swordfish are crucial to interpret the histological images.

The Group agreed in establishing separate reference sets by stocks (or area) and also reference sets for images coming from frozen samples. These reference sets could take part in a manual that also includes the maturity scales, the description of morphological structures used in staging and, a decision tree or key to facilitate the staging of individuals.

In order to illustrate this "manual", good quality images should be selected among the sets. It was also suggested to keep the ones where the readers did not get consensus in order to highlight potential uncertainties.

Finally, the Group selected a set of images to establish the reference set, and agreed on the need to solve quality issues. These final images are presented in **Appendix 4** of this report.

7. Discussion

7.1 Discussion on any future sampling needs

- *Overview and revision of the program objectives and general observations*

The Group was presented with plans for future sampling needs and project next steps by the project leads. The Coordinator began by reminding the Group that the original goal of this biology program—as envisioned by the Swordfish Species Group—is to conduct sampling and research that supports assessment, MSE, and other SCRS work. Future sampling and research should support the core objectives: generate and compare new growth curves; support knowledge on stock age structure; calculate and compare new maturity ogives; identify spawning areas and timing; identify stock boundaries and mixing areas; and estimate scale of mixing. The Coordinator noted that in many cases, sampling has done a good job of supporting this research, however there are spatial and temporal sampling gaps that are hindering some aspects of the Project. He encouraged the project leads to continue identifying sampling gaps important to addressing their research questions. It was also noted that some port sampling and rules related to carrying chemical fixatives aboard vessels can hinder some sampling plans. To address these gaps, the next phase of the Project may need to direct funds to support sampling in high priority areas. Furthermore, the Group will continue to address data confidentiality regulations that may be preventing inclusion of some samples.

There were no comments specific to the points raised by the Coordinator of the project.

One participant asked about the transparency of the Project participation and on why the current Project has such a small participation in terms of composition of the Consortium leading the project. Both the Project leader and the vice-Chair of the SCRS clarified that the Project is currently composed of 25 institutes from 17 different CPCs, which makes this one of the more collaborative projects in ICCAT. They then added that the Project has been discussed and regularly updated on all SWO Species Group meetings since 2018, and that the results and workplans are shown in the SCRS reports, so all CPCs are fully aware of the state of the Project.

One participant asked about why a particular Italian genetics laboratory that applied to the original data call is not part of the Project. The Project leader explained that the possibility to integrate that institute in the genetic study was made, but there was no further reply. The same

participant also questioned why Morocco was not part of the Project. The Project leader clarified that Morocco is part of the Project. Morocco clarified that there are difficulties because the fleet that operates in that area are mainly small coastal fisheries and it is not possible to take samples. Buying the fish could make sampling possible. Morocco will contact the Project coordinator for follow up on this possibility.

One participant highlighted that the opportunistic sampling that is taking place is important, but to consider that CPCs may have other historical samples already collected through other national programs and projects. As such, it would be advisable to make call for samples in a more official manner, asking CPCs to provide previous samples they may have from other sources. It was clarified by the Project leader that in the original call for the project such a request was made and it was possible to recover some historical samples from other projects. We can make now another recommendation for CPCs with historical samples to provide information to the SCRS on the existence and possibility to integrate historical samples in the project (see recommendations section).

- *Summary of sampling representativeness as related to ageing and growth studies. Identification of sampling gaps related to ageing and growth analysis.*

The following maps provide the spine and otolith spatial distribution (**Figures 7.1 and 7.2**)

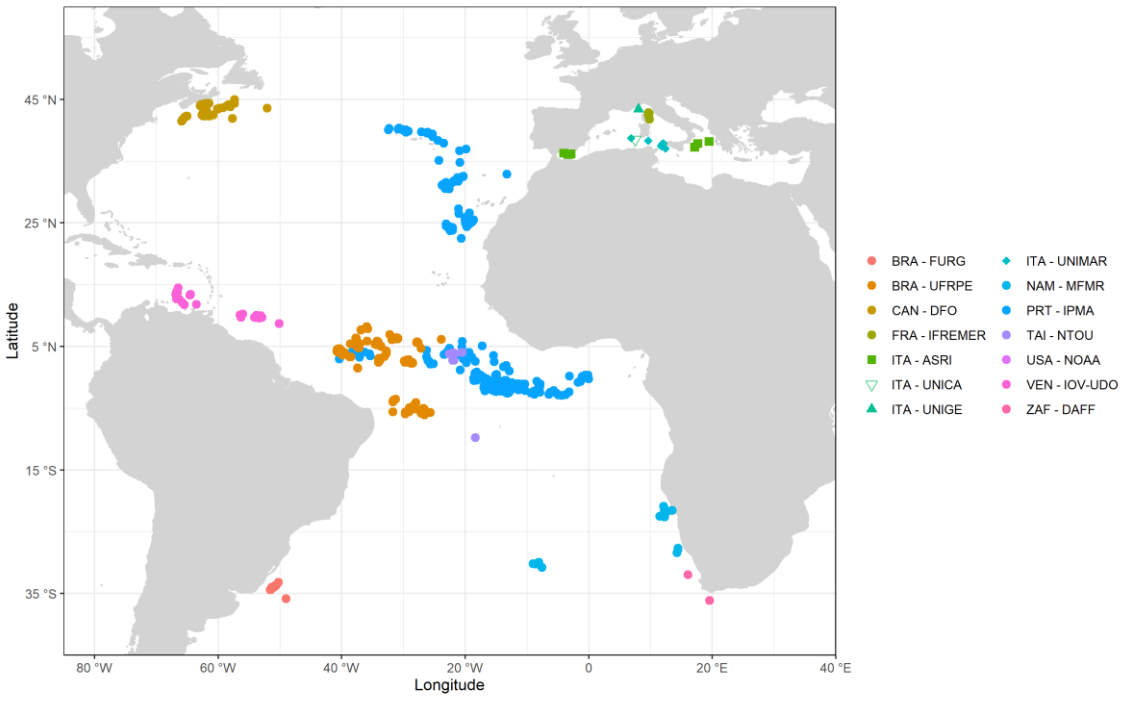

Figure 7.1. Map with the distribution of swordfish spine samples

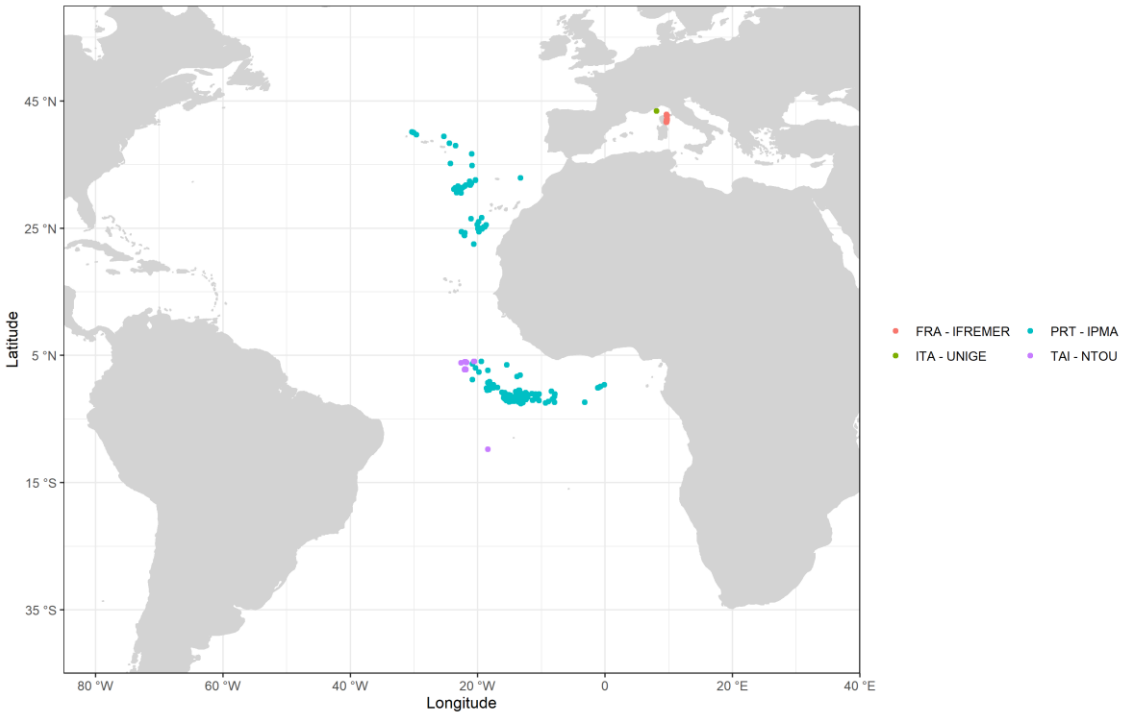

Figure 7.2. Map with the distribution of otolith samples

The following points were noted by the Group:

- The general areas where sample gaps still exist are mainly the East Mediterranean, the western-north Atlantic and the Southern Atlantic in general. It is noted that for some of those areas it is challenging to obtain samples, and as such the option for chartering a vessel for a few days can be considered.
- There are much less otoliths available than spines. As such, an effort should be put into sampling more otoliths from the various regions.
- For the Mediterranean, it is also noted on the need to obtain samples from larger fish, as most samples from there are currently from relatively smaller fish.
- For the western North Atlantic it would be particularly important to get samples from the area between 15°N and 35°N, west of 40°W during the months of November-March. That would be critical for both ageing and reproduction.
- Note that CPCs need to know, as early in the year as possible, about the need for samples so they can plan the sample collection during the year.
- Comment that for the Mediterranean the fishery is stopped between January and March, so in order to sample in those months there would be the need for permission from the Commission.
- Comment that the involvement from Cyprus could be important, noting that there have been some contacts with that CPC for opportunities in sampling in their fisheries.
- The USA commented that besides the spines already provided, they likely also have a collection of historical otoliths that can be shared. The Project leader will contact the USA to coordinate on that. Work is underway to attempt to associate locations with those samples to make the useful for the project.

- *Summary of sampling representativeness as related to reproduction studies. Identify sampling gaps related to reproduction analysis*

The following maps provide the spatial distribution of the macroscopic and microscopic samples (**Figures 7.3 and 7.4**)

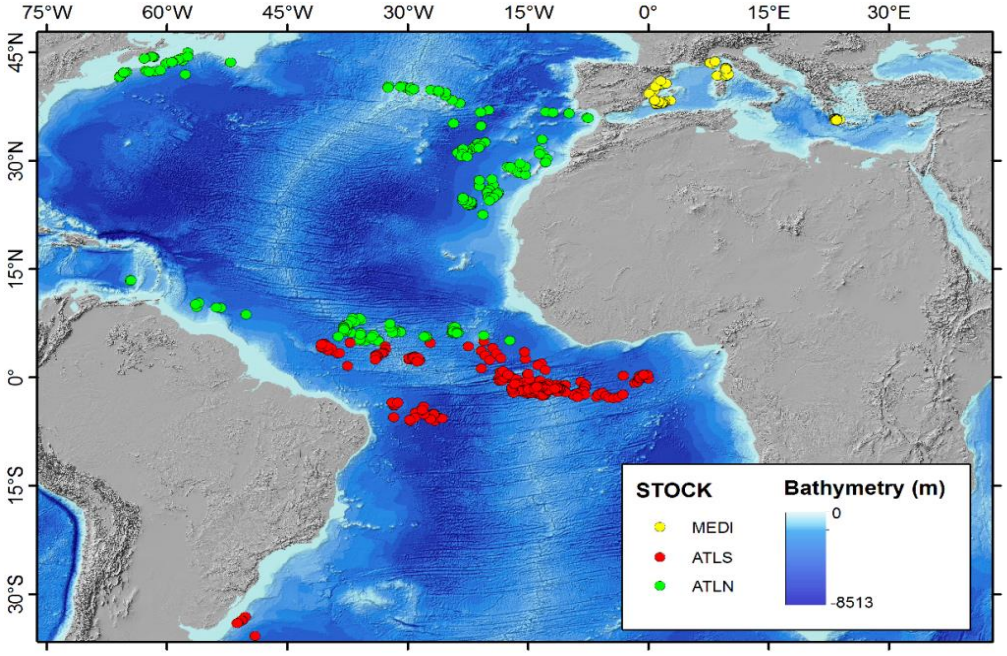

Figure 7.3. Map with the distribution of macroscopic samples

The following points were noted for the macroscopic samples:

- North Atlantic: no samples are available from the central north Atlantic and, low sampling coverage for the western North Atlantic.
- South Atlantic: the majority of the samples comes from tropical areas.
- Mediterranean: the majority of the samples come from Western Mediterranean.

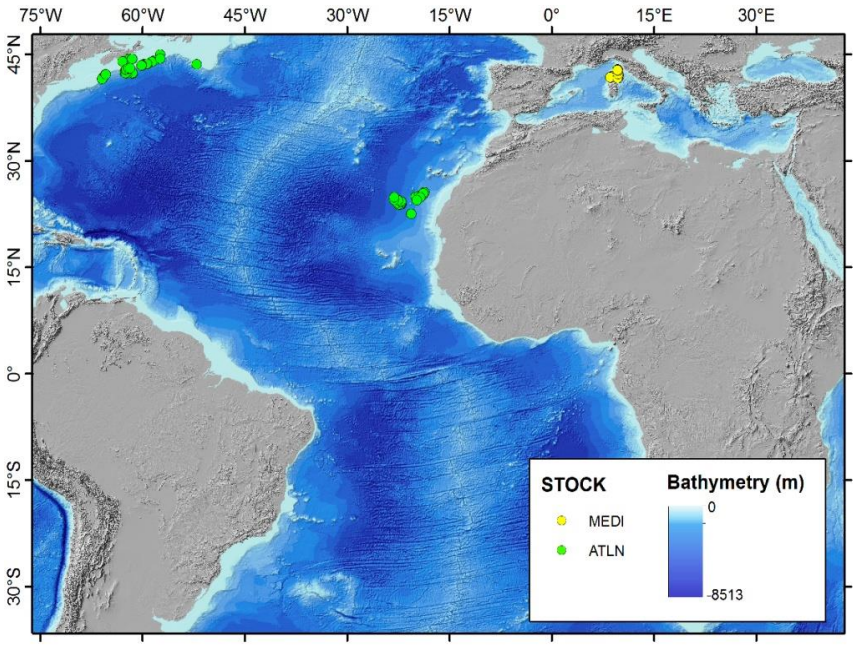

Figure 7.4. Map with the distribution of microscopic samples

The following points were noted for the microscopic samples:

- North Atlantic: Poor spatial coverage. No samples processed from spawning areas.
- South Atlantic. No samples available at the moment.
- Mediterranean: Few samples in general, and all from the Western Mediterranean. There is need for samples from the central and Eastern Mediterranean.
- At the end of the discussion, scientists from Uruguay mentioned they are willing to collaborate in the reproduction and genetics components of the project with samples from the SW Atlantic. They will contact the Project leader and the tasks leaders of those sections about their sample availability and participation.

- *Summary of sampling representativeness as related to genetics. Identify sampling gaps related to genetic analysis*

The following map provide the spatial distribution and needs of the genetic samples (**Figure 7.5**)

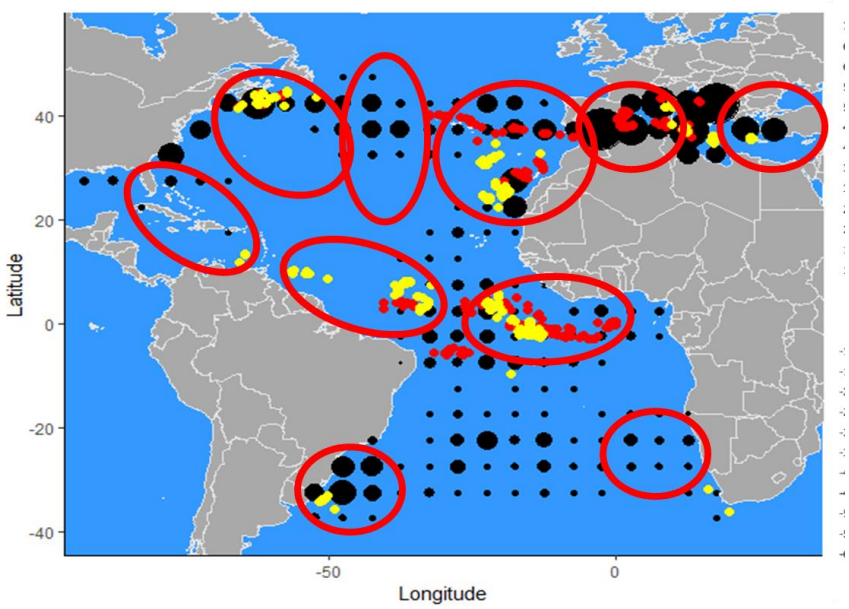

Figure 7.5. Map with the distribution of genetic samples.

The following points were noted with regards to the genetic samples:

- The sample needs for the Atlantic are mainly samples between the equatorial and East Atlantic boundary, samples from the more southern parts of the South Atlantic (BIL 97 and BIL 96) and additional samples from the central North Atlantic (BIL 92).
- For the Mediterranean, more samples are needed for new areas like Malta and Corsica, and samples from the Eastern Mediterranean
- As much as possible we need samples for different periods of the year and that cover the various maturity stages.
- In terms of numbers, the minimum number of samples for population genetics would be 25-30 for each area.
- As in the reproduction section, Uruguayan scientists confirmed they wish to collaborate with samples from the SW Atlantic for the genetics work.
- One main objective for the project mixing areas, it is noted that there are already considerable samples from the Balearic Islands in the Mediterranean and from Portugal and the Canaries Islands from the Atlantic. With regards to the Gibraltar strait, Morocco will contact the Project leader to try to get samples from that region.
- The Group discussed on the need to sample undersized fish from the Mediterranean with an RMA. There is a certain percentage of fish under the minimum size that can be retained, so scientific samples can be retained from those. In that regards, it would be also important to involve the Albacore fishery in the Mediterranean as they may capture young of the year swordfish in the Mediterranean.
- There was also a note that Cyprus has additional observers through the EU/DCF and might be able to collect samples from the east Mediterranean. The Project coordinator will contact after the meeting on that issue.

7.2 Discussion and plans for sample processing completion and data analysis

The tasks leaders briefly commented on the plans for the work until completion of the current contract (30 June 2021):

- For the ageing we will send more samples for processing to FAS and will continue the work on the reference set intersessionaly.
- For the reproduction we are now expecting samples from Italy and Taiwan. Once the samples arrive the analysis will continue with those.
- For the genetics, it was noted that new samples need to be sent to Italy as soon as possible, to be forwarded for processing. A new analysis will be performed for all the samples together (update of the preliminary analysis showed here). Another point is to look into the genes involved in bone formation and differentiation to see if there are genetic differences between Mediterranean and Atlantic as the age readers mentioned phenotypic differences in the structures. There will also investigate on any differences in gonads structures between Mediterranean and Atlantic populations. Finally, they will continue to apply whole genome sequencing to have that available for all the swordfish populations in the Atlantic and Mediterranean.

7.3 Discussion on any other priority areas for the project

The task leaders and the Group discussed on any additional priority areas for each task of the project:

- For the ageing, the main next step will be age validation, especially with bomb-radiocarbon. Current costs will be investigated intersessionaly. Canada and USA have equipment for that type of work and can assist on this, and Jessica Farley can also help. It was noted that the otoliths that will be used for such analysis will have to be destroyed. While originally the method was only used for long lived species, recent developments have also been successful in shorter lived species and recent samples, such as yellow fin tuna. We will work on a research line and budget for this until September to be included in the SWO workplan for 2022.
- For the genetics, it is possible to carry out these types of analysis on historical samples to have also a picture of the past situation to compare with the present. So a next step could be to do DNA analysis of historical samples. Spanish scientists (David Macias) has some historical samples and will provide to Italy, so they can check if it is possible to conduct such analysis. Epigenetic was also suggested as a future next step for complementing the ageing work.

7.4 Final comments from the invited experts

The Group received final comments from the Invited experts. Those are reflected in **Appendix 5** of this report.

7. 5 Recommendations

The following final Recommendations were agreed by the Group:

- *Recommendations related with the ongoing work of the Project*

  - Agreement to use the open-source SmartDOTS platform for the ageing work. The software was developed for otoliths, but the Project is in contact with the developers to make it more useful also for spines.
  - With regards to the maturity stages, the Group agreed and recommended to use the stages from the IEO-Malaga Group (adapted from ICES).
  - For the maturity staging, consider a more standardized way for each reader to provide input in the structures they are observing (e.g., excel table with tick marks for each structure)
  - With regards to genetics, the Group agreed that once the current ageing work is calibrated and finalized, it would be possible to explore ageing work with epigenetics.
  - Consider the possible implications of the project results for the ongoing MSE and especially if further axis of uncertainty need to be considered in the OMs.
  - Coordinate with the Secretariat to create an owncloud space to store digital images from the ageing (spines and otoliths) and reproduction work
  - Establish a set of best practices for saving and archiving images of the structures. The practices that are used by FAS are shown in **Appendix 6**.
  - Coordinate among project collaborators for long term storage of physical samples.
  - Encouragement for reference sets for whole oocytes (preferably fresh) be created and calibrated with histology for potential use in future studies by any CPC with limited resources.
  - The use size frequency distribution of oocytes should also be considered as an ancillary method in the identification of maturity status and developmental phases of swordfish ovaries.
  - Encouragement to create and share videos with the processing and cleaning of the spines and otoliths. Such videos would then be uploaded to the ICCAT owncloud.

- *Recommendations to be passed to the SWO Species Group*

  - Make an official call for CPCs to inform the SCRS on availability of historical samples from other projects that may be available to integrate this study.
  - This research shows the need to have a wide geographical and temporal collection of biological samples for swordfish stocks and the integrated collaboration of scientific institutions and CPCs. It is recommended that the SCRS considers developing a network of institutions/entities available and qualified to centralize, store, process, and maintain biological samples such as otoliths, spines, muscle, gonads, etc. in the correct conservation status that are accessible for current and future research.
  - The Group recommends that CPCs should ensure that their main fisheries should provide regularly a minimum number of biological samples for swordfish. Samples such as hard parts for ageing, reproductive tissues, and other tissues for genetic studies, from the same individual fish. These samples should be collected and stored following guidelines provided by the SCRS and made available for scientific research. A summary of the samples collected so far, either under national programmes or by individual research teams/scientists, should be provided as part of the regular fisheries statistics Task 3 biological samples to ICCAT.

- The Group recognizes that most of the biological sampling comes from commercial fisheries operations, which put restrictions on what the scientific samplers can collect minimizing the damage of the product; alternatively, in some cases, it requires the purchase of the whole fish which substantially limit the number of samples collected. Therefore, it is suggested that the Commission provides a scientific allowance to registered National Scientific Observer programs and/or field sampler for the collection of biological samples, independent of the quotas allocations; implications concerning the prohibition to take on board or land beheaded specimens for the Mediterranean swordfish should be taken into account. The SCRS shall provide in advance the requested scientific allowance based on approved research programs that will make use of such biological samples.
- Consider research cruises for filling important areas with sample gaps and where it is difficult or even impossible to sample from commercial fisheries. A budget will have to be prepared for the SWO Species meeting in September.
- Consider a pilot study for comparing the 3 ageing structures: otoliths, spines and vertebrae. Given that for collecting vertebrae the process is very invasive, we would need to acquire the fish or parts of the fish for collecting the 3 structures. The pilot study should be conducted both for the Mediterranean and Atlantic stocks, with around 30-50 samples for each stock that should cover the size range of the species. It was noted that vertebra are different the various body regions, so that should be considered when designing the study. The Group will prepare a research line and budget for this study to present to the SWO Species Group in July.

References

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Appendix 1 – Meeting agenda

1. Opening
2. Adoption of the agenda
3. Nomination of the rapporteurs
4. Status update of the ICCAT swordfish project
5. Update of the sample collection and sample processing
    5.1. Ageing (spines and otoliths)
    5.2. Reproduction
    5.3. Genetics
6. Establishment of reference sets, with regards to
    6.1. Ageing (incl. spines and otoliths)
    6.2. Reproduction biology (histological scales)
7. Discussion
    7.1. Discussion on any future sampling needs
    7.2. Discussion and plans for sample processing completion and data analysis
    7.3. Discussion on any other priority areas for the project
    7.4. Final comments from the invited experts
    7.5. Recommendations
8. Workshop report and adoption
9. Closure

Appendix 2 – List of registered participants

Original: English/French/Spanish

LIST OF PARTICIPANTS/LISTE DES PARTICIPANTS/LISTA DE PARTICIPANTES

2021 ICCAT Swordfish Biology Workshop (ON-LINE, 22 – 26 March 2021)

Atelier de l'ICCAT sur la biologie de l'espadon de 2021 (RÉUNION EN LIGNE, 22 – 26 Mars 2021)

Taller sobre biología del pez espada de ICCAT de 2021 (EN LÍNEA, 22 – 26 Marzo de 2021)

CONTRACTING PARTIES/PARTIES CONTRACTANTES/PARTES CONTRATANTES

ALGERIA/ALGÉRIE/ARGELIA

Ben Smail, Samia

Bp 67, 11 Bd Colonel Amirouche, Bou-Ismail, Tipaza, 42000

Tel: +213 553 315 469, E-Mail: bensmailsamia@yahoo.fr

Bennoui, Azzeddine

Chercheur au Centre de Recherche et de Développement pour la Pêche et de l'Aquaculture (CNRDPA), 11 Bouevard Colonel Amirouche, 42415 Bou Ismail, Tipaza

Tel: +213 24 32 64 10, Fax: +213 24 32 64 11, E-Mail: bennoui_azeddine@yahoo.fr

Bouhadja, Mohamed Amine

Centre National de Recherche et de Développement de la Pêche et de l'Aquaculture (CNRDPA), 11 Bouleverad Colonel Amirouche, 42004 Tipaza

Tel: +213 24 32 64 10; +213 671 808 052, E-Mail: Bouhadja.amine@gmail.com

Kouadri-Krim, Assia

Chef de Bureau, Ministère de la Pêche et des Productions Halieutiques, Direction du développement de la pêche, Rue des 04 Canons, 16000

Tel: +213 558 642 692, Fax: +213 21 43 31 97, E-Mail: assiakrim63@gmail.com

BELIZE/BELICE

Burgess, Stanley

Keystone Building, Suite 501, 304 Newtown Barracks

Tel: +501 223 4918, Fax: +501 223 5087, E-Mail: stanley.burgess@bhsfu.gov.bz

Howe, Ernie

Keystone Building, Suite 501, 304 Newtown Barracks

Tel: +501 223 4918, Fax: +501 223 5087, E-Mail: ernie.howe@bhsfu.gov.bz

Pinkard, Delice

Senior Fisheries Officer, Belize High Seas Fisheries Unit, Ministry of Finance, Government of Belize, Keystone Building, Suite 501, 304 Newtown Barracks

Tel: +1 501 223 4918, Fax: +1 501 223 5087, E-Mail: sr.fishofficer@bhsfu.gov.bz; delice.pinkard@bhsfu.gov.bz

Robinson, Robert

Deputy Director for High Seas Fisheries, Belize High Seas Fisheries Unit, Ministry of Finance, Government of Belize, Keystone Building, Suite 501, 304 Newtown Barracks

Tel: +501 223 4918, Fax: +501 223 5087, E-Mail: robert.robinson@bhsfu.gov.bz; deputydirector@bhsfu.gov.bz

BRAZIL/BRÉSIL/BRASIL

Alves Bezerra, Natalia

Researcher, UFRPE, Rua Dom Manuel de Medeiros, s/n, Dois Irmãos, 52171-900 Recife, Pernambuco

Tel: +55 819 889 22754, E-Mail: natalia_pab@hotmail.com

Campello, Tiago

Rua Dom Manoel de Medeiros, SN, ZIP-52171900, 500-40090 Recife -Pe Dois Irmãos

Tel: +558199829-5752, E-Mail: tiagohpcampello@gmail.com

CANADA/CANADÁ

Busawon, Dheeraj

Fisheries & Oceans Canada, St. Andrews Biological Station, 125 Marine Science Drive, St. Andrews, NB E5B 0E4

Tel: +1 506 529 5889; +1 506 467 5651, Fax: +1 506 529 5862, E-Mail: Dheeraj.Busawon@dfo-mpo.gc.ca

Duprey, Nicholas

Senior Science Advisor, Fisheries and Oceans Canada - Fish Population Science, Government of Canada, 200-401 Burrard Street, Vancouver, BC V6C 3R2

Tel: +1 604 499 0469; +1 250 816 9709, E-Mail: nicholas.duprey@dfo-mpo.gc.ca

Gillespie, Kyle

Fisheries and Oceans Canada, St. Andrews Biological Station, Population Ecology Division, 125 Marine Science Drive, St. Andrews, New Brunswick, E5B 0E4

Tel: +1 506 529 5725, Fax: +1 506 529 5862, E-Mail: kyle.gillespie@dfo-mpo.gc.ca

Hanke, Alexander

Scientist, St. Andrews Biological Station/ Biological Station, Fisheries and Oceans Canada, 125 Marine Science Drive, St. Andrews, New Brunswick E5B 2T0

Tel: +1 506 529 5912, Fax: +1 506 529 5862, E-Mail: alex.hanke@dfo-mpo.gc.ca

EGYPT/EGYPTE/EGIPTO

Abdelnaby Kaamoush Fetouh, Mohamed Ibrahim

National Institute of Oceanography and Fisheries (NIOF), 14 Aly Abn Aby Taalep, Abo Qir, 11694 Alexandria, Cairo

Tel: +202 279 21341, Fax: +202 792 1339, E-Mail: ma_fotouh@outlook.com

EUROPEAN UNION/UNION EUROPÉENNE/UNIÓN EUROPEA

Aláez Pons, Ester

International Relations Officer, European Commission - DG MARE - Unit B2 - RFMOs, Rue Joseph II - 99 03/057, 1049 Bruxelles, Belgium

Tel: +32 2 296 48 14; +32 470 633 657, E-Mail: ester.alaez-pons@ec.europa.eu

Battaglia, Pietro

Researcher Stazione Zoologica Anton Dohrn (SZN) National Institute of Biology, Ecology and Marine Biotechnology SZN Sicilia Marine Centre Villa Pace, Contrada Porticatello 29, 98167 Messina, Italy

E-Mail: pietro.battaglia@szn.it; battaglia.pietro@szn.it

Biagi, Franco

Senior Expert Marine & Fishery Sciences, Directorate General for Maritime Affairs and Fisheries (DG-Mare) - European Commission, Unit C3: Scientific Advice and data collection, Rue Joseph II, 99, 1049 Bruxelles, Belgium

Tel: +322 299 4104, E-Mail: franco.biagi@ec.europa.eu

Boulila, Nesrine

AquaBioTech Group Central Complex, Naggar Street Targa Gap, MST 1761 Mosta, Malta

Tel: +356 991 02685, E-Mail: neb@aquabt.com

Carnevali, Oliana

Universita Politecnica Delle Marche - Ancona, Department of Environment and Life Science, Via Breccie Bianche, 60131 Ancona, Italy

Tel: +39 338 264 2235; +39 71 220 4990, Fax: +39 071 220 46 50, E-Mail: o.carnevali@staff.univpm.it

Castillo Gutiérrez, Isabel

Instituto Español de Oceanografía, Promontorio San Martin s/n, 39004 Santander, España

Tel: +34 942 291 796, E-Mail: isabel.castillo@ieo.es

Di Natale, Antonio

Director, Aquastudio Research Institute, Via Trapani 6, 98121 Messina, Italy

Tel: +39 336 333 366, E-Mail: adinatale@costaedutainment.it

Durieux, Eric

Université de Corse Pasquale Paoli UMS 3514 Plateforme marine Stella Mare Lido de la Marana, 20620 Corse Biguglia, France

Tel: +33 495 450 698, E-Mail: durieux@univ-corse.fr

Ferreira de Gouveia, Lidia

Técnica Superior, Biologist, Direcçao Regional das Pescas, Direçao Serviços de Investigaçao - DSI, Av. do Mar e das Comunidades Madeirenses, 23 - 1º Esq., 9004-562 Funchal, Madeira, Portugal

Tel: +351 291 203270, Fax: +351 291 229856, E-Mail: lidia.gouveia@madeira.gov.pt

Garibaldi, Fulvio

University of Genoa - Dept. of Earth, Environmente and Life Sciences, Dipartimento si Scienze della Terra, dell'Ambiente e della Vita (DISTAV), Corso Europa, 26, 16132 Genova, Italy

Tel: +39 335 666 0784; +39 010 353 8576, Fax: +39 010 357 888, E-Mail: largepel@unige.it; garibaldi.f@libero.it

Gioacchini, Giorgia

Universita Politecnica delle Marche ANCONA, Dipartimento Scienze della Vita e dell'Ambiente, Via Breccie Bianche 131, 60027 Ancona, Italy

Tel: +39 339 132 1220; +39 712 204 693, E-Mail: giorgia.gioacchini@univpm.it

Macías López, Ángel David

Ministerio de Ciencia, Innovación y Universidades, Instituto Español de Oceanografía, C.O. de Málaga, Puerto pesquero s/n, 29640 Fuengirola Málaga, España

Tel: +34 952 197 124; +34 619 022 586, Fax: +34 952 463 808, E-Mail: david.macias@ieo.es

Males, Josip

Institute of Oceanography and Fisheries, Šetalište I. Meštrovića 63, 21000 Split, Croatia

Tel: +385 214 08065, Fax: +385 213 58650, E-Mail: males@izor.hr

Massa-Galluci, Alexia

AquaBioTech Group Central Complex, Naggar Street, Targa Gap, Mosta, MST 1761, Malta

Tel: +356 996 50785, E-Mail: amg@aquabt.com

Millot, Rémi

Université de Corse Pasquale Paoli UMS 3514 Plateforme marine Stella Mare Lido de la Marana, 20620 Corse Biguglia, France

Tel: +33 420 202 422, E-Mail: millot_r@univ-corse.fr

Muñoz Lechuga, Rubén

Assistant research, Instituto Português do Mar e da Atmosfera - I.P./IPMA, Avenida 5 de Outubro, s/n, 8700-305 Olhão Faro, Portugal

Tel: +351 289 700 504, E-Mail: ruben.lechuga@ipma.pt

Pappalardo, Luigi

Scientific Coordinator, OCEANIS SRL, Vie Maritime 59, 84043 Salerno Agropoli, Italy

Tel: +39 081 777 5116; +39 345 689 2473, E-Mail: gistec86@hotmail.com; oceanissrl@gmail.com

Peristeraki, Panagiota (Nota)

Hellenic Center for Marine Research, Institute of Marine Biological Resources and Inland Waters, P.O. Box 2214, 71003 Heraklion, Greece

Tel: +30 2810 337 830, Fax: +30 2810 337 822, E-Mail: notap@hcmr.gr

Poisson, François

IFREMER -- Centre de Recherche Halieutique, UMR MARBEC (Marine Biodiversity Exploitation and Conservation), Avenue Jean Monnet, CS 30171, 34203 Sète, France

Tel: +33 499 57 32 45; +33 679 05 73 83, E-Mail: francois.poisson@ifremer.fr; fpoisson@ifremer.fr

Romeo, Teresa

Senior Researcher, Stazione Zoologica Anton Dohrn, Sicily Marine Centre, 98100 Messina, Italy

Tel: +39 081 583 3748, E-Mail: teresa.romeo@szn.it

Rosa, Daniela

PhD Student, Portuguese Institute for the Ocean and Atmosphere, I.P. (IPMA), Av. 5 de Outubro s/n, 8700-305 Olhao, Portugal

Tel: +351 289 700 504, E-Mail: daniela.rosa@ipma.pt

Thasitis, Ioannis

Department of Fisheries and Marine Research, 101 Vithleem Street, 2033 Nicosia, Cyprus

Tel: +35722807840, Fax: +35722 775 955, E-Mail: ithasitis@dfmr.moa.gov.cy

Tserpes, George

Hellenic Center for Marine Research (HCMR), Institute of Marine Biological Resources, P.O. Box 2214, 71003 Heraklion, Crete, Greece

Tel: +30 2810 337851, Fax: +30 2810 337822, E-Mail: gtserpes@hcmr.gr

Vázquez Álvarez, Francisco Javier

European Commission DG Maritime B2 Affairs and Fisheries, Rue Joseph II - 99 Room 3/77, 1049 Brussels, Belgium

Tel: +32 2 295 83 64: +32 485 152 844, E-Mail: francisco-javier.vazquez-alvarez@ec.europa.eu

JAPAN/JAPON/JAPÓN

Honda, Hitoshi

Scientist, Reserach Management Department, Highly Migratory Resources Division, Fisheries Resources Institute, National Research and Development Agency, Japan Fisheries Research and Education Agency, 5-7-1, Orido, Shimizu-ward, Shizuoka-city, Shizuoka-prefecture, 424-8633

Tel: +81 54 336 6000, Fax: +81 54 335 9642, E-Mail: hhonda@affrc.go.jp; honda_hitoshi48@fra.go.jp

Miura, Nozomu

Assistant Director, International Division, Japan Tuna Fisheries Co-operative Association, 2-31-1 Eitai Koto-ku, Tokyo 135-0034

Tel: +81 3 5646 2382, Fax: +81 3 5646 2652, E-Mail: miura@japantuna.or.jp; gyojyo@japantuna.or.jp

Nagai, Daisaku

Manager, International Division, Japan Tuna Fisheries Co-Operative Association, 31-1, EITAI 2-CHOME, Koto-ku, Tokyo 135-0034

Tel: +81 356 462 382, Fax: +81 356 462 652, E-Mail: nagai@japantuna.or.jp

Uozumi, Yuji

Adviser, Japan Tuna Fisheries Co-operation Association, Japan Fisheries Research and Education Agency, Tokyo Koutou ku Eitai 135-0034

LIBYA/LIBYE/LIBIA

F. Gafri, Hasan

Director - Libyan Commissioner to ICCAT, Ministry of Agriculture, Livestock and Fisheries, Department of Marine Wealth, P.O. Box 80876, Tajura Tripoli

Tel: +218 916 274 377, E-Mail: gafrihasan@gmail.com; abdusalam.zbida@gmail.com

Abu-Seliana, Ali

Al-Furnaje-Tripoli

Tel: +218 927 941 022, E-Mail: a.abuseliana@uot.edu.ly

Ensair, Hend

Tripoli Tajura

Tel: +218 917 829 192, E-Mail: h.ensair@uot.edu.ly

Nfati, Ahmed

Tajura

Tel: +218 913 204 405, E-Mail: Ahmednfati@yahoo.co.uk

Shafran, Ali

Tajura

Tel: +218 0923 000 268, E-Mail: A.Shafran@uot.edu.ly

Sharif, Ehab A. Tayeb

Tripoli Ain Zahra

Tel: +218 925 053 130, E-Mail: eelshrif@hotmail.com

Showehdi, Mohamed

Tripoli Althahra

Tel: +218 928 312 628, E-Mail: mohamedelshwhdy@hotmail.com

MEXICO/MÉXIQUE/MÉXICO

Ramírez López, Karina

Instituto Nacional de Pesca y Acuacultura - Veracruz, Av. Ejército Mexicano No.106 - Colonia Exhacienda, Ylang Ylang, C.P. 94298 Boca de Río, Veracruz

Tel: +52 22 9130 4520; +52 229 176 8449, E-Mail: kramirez_inp@yahoo.com

MOROCCO/MAROC/MARRUECOS

Ikkiss, Abdelillah

Chercheur, Centre régional de l'Institut national de Recherche Halieutique à Dakhla, Km 7, route de Boujdor, BP 127 bis(civ), HAY EL HASSANI NO 1101, 73000 Dakhla

Tel: +212 662 276 541, E-Mail: ikkiss.abdel@gmail.com

NAMIBIA/NAMIBIE

Shikongo, Taimi

Senior Fisheries Biologist, Ministry of Fisheries and Marine Resources, Large Pelagic Species, 1 Strand Street P.O. BOX 912, 9000 Swakopmund Erongo

Tel: +264 644 101 000, Fax: +264 644 04385, E-Mail: Taimi.Shikongo@mfmr.gov.na; tiemeshix@gmail.com

SOUTH AFRICA/AFRIQUE DU SUD/SUDÁFRICA

Parker, Denham

Stock Assessment Scientist, Department of Environment, Forestry and Fisheries, 9 Martin Hammerschlag Way, 7800 Cape Town

Tel: +27 21 402 3165; +27 82 660 7985, E-Mail: DenhamP@DAFF.gov.za

TUNISIA/TUNISIE/TÚNEZ

Zarrad, Rafik

Chercheur, Institut National des Sciences et Technologies de la Mer (INSTM), BP 138 Ezzahra, Mahdia 5199

Tel: +216 73 688 604; +216 972 92111, Fax: +216 73 688 602, E-Mail: rafik.zarrad@gmail.com; rafik.zarrad@instm.rnrt.tn

UNITED STATES/ÉTATS-UNIS/ESTADOS UNIDOS

Brown, Craig A.

Chief, Highly Migratory Species Branch, Sustainable Fisheries Division, NOAA Fisheries Southeast Fisheries Science Center, 75 Virginia Beach Drive, Miami, Florida 33149

Tel: +1 305 586 6589, Fax: +1 305 361 4562, E-Mail: craig.brown@noaa.gov

Carrano, Cole

737 Hollywood Blvd, Hollywood, FL 33019

Tel: +1 8049725157, E-Mail: cole.carrano@rsmas.miami.edu

Forrestal, Francesca

NOAA Fisheries, Southeast Fisheries Science Center, Sustainable Fisheries Division, 75 Virginia Beach Dr., Miami Florida 33149

Tel: +1 305 903 4535, E-Mail: francesca.forrestal@noaa.gov

Jivanjee, Alejandro

E-Mail: alejandro.jivanjee@rsmas.miami.edu

Keller, Bryan

NOAA Fisheries, 1315 East-West Highway, MD Silver Spring 20910

Tel: +1 301 427 7725, E-Mail: bryan.keller@noaa.gov

Lauretta, Matthew

Fisheries Biologist, NOAA Fisheries Southeast Fisheries Center, 75 Virginia Beach Drive, Miami, Florida 33149

Tel: +1 305 361 4481, E-Mail: matthew.lauretta@noaa.gov

Orbesen, Eric

Research Fish Biologist, NOAA, 75 Virginia Beach Dr., Miami, Florida 33149

Tel: +1 786 368 7560, E-Mail: eric.orbesen@noaa.gov

Schirripa, Michael

Research Fisheries Biologist, NOAA Fisheries, Southeast Fisheries Science Center, 75 Virginia Beach Drive, Miami, Florida 33149

Tel: +1 305 445 3130; +1 786 400 0649, Fax: +1 305 361 4562, E-Mail: michael.schirripa@noaa.gov

URUGUAY

Domingo, Andrés

Dirección Nacional de Recursos Acuáticos - DINARA, Laboratorio de Recursos Pelágicos, Constituyente 1497, 11200 Montevideo

Tel: +5982 400 46 89, Fax: +5982 401 32 16, E-Mail: adomingo@mgap.gub.uy; dimanchester@gmail.com

Forselledo, Rodrigo

Investigador, Dirección Nacional de Recursos Acuáticos - DINARA, Laboratorio de Recursos Pelágicos, Constituyente 1497, CP 11200 Montevideo

Tel: +598 2400 46 89, Fax: +598 2401 3216, E-Mail: rforselledo@gmail.com

VENEZUELA

Bustillos, Freddy

Asesor de la Gerencia de Ordenación Pesquera del Instituto Socialista de la Pesca y la Acuicultura, Ministerio del Poder Popular de Pesca y Acuicultura, 1015 Caracas

Tel: +58 426 913 6036, E-Mail: fxpesca@gmail.com

OBSERVERS FROM COOPERATING NON-CONTRACTING PARTIES, ENTITIES, FISHING ENTITIES/OBSERVATEURS DE PARTIES, ENTITÉS, ENTITÉS DE PÊCHE NON CONTRACTANTES COOPÉRANTES/OBSERVADORES DE PARTES, ENTIDADES, ENTIDADES PESQUERAS NO CONTRATANTES COLABORADORAS

CHINESE TAIPEI/ TAIPEI CHINOIS/TAIPEI CHINO

Cheng, Chun-Ya

National Taiwan Ocean University, No. 2 Peining Rd, 202301 Zhongzheng Keelung

Tel: +886 2 24622192 ext. 5046, Fax: +886 2 24622192, E-Mail: lucky_8043@yahoo.com.tw

Su, Nan-Jay

Assistant Professor, Department of Environmental Biology and Fisheries Science, National Taiwan Ocean University, No. 2 Pei-Ning Rd. Keelung, Zhongzheng Dist., 202301

Tel: +886 2 2462 2192 #5046, Fax: +886-2-24622192, E-Mail: nanjay@ntou.edu.tw

SCRS VICE-CHAIRMAN

Coelho, Rui

Researcher, SCRS Vice-Chairman, Portuguese Institute for the Ocean and Atmosphere, I.P. (IPMA), Avenida 5 de Outubro, s/n, 8700-305 Olhão, Portugal

Tel: +351 289 700 504, E-Mail: rpcoelho@ipma.pt

INVITED EXPERT

Arocha, Freddy

Instituto Oceanográfico de Venezuela, Universidad de Oriente, A.P. 204, 6101 Cumaná Estado Sucre, Venezuela

Tel: +58 424 823 1698, E-Mail: farochap@gmail.com

Farley, Jessica

Senior Scientist, CSIRO Oceans and Atmosphere, 3-4 Castray Esplanade, 7000 Tasmania Hobart, Australia

Tel: +61407255524, Fax: +61362325000, E-Mail: jessica.farley@csiro.au

Krusic-Golub, Kyne

Fisheries Scientist, Fish Ageing Services, 28 Swanston St, Queenscliff Victoria, 3225, Australia

Tel: +61 438 306 179, E-Mail: kyne.krusicgolub@fishageingservices.com

*****

ICCAT Secretariat/ Secrétariat de l'ICCAT/ Secretaría de ICCAT

C/ Corazón de María 8 – 6th floor, 28002 Madrid – Spain

Tel: +34 91 416 56 00; Fax: +34 91 415 26 12; E-mail: info@iccat.int

Neves dos Santos, Miguel

Ortiz, Mauricio

Appendix 3 – Exchange exercise carried out for the ageing component – Spines and otoliths

This will continue to be done intersessionally by the age readers and the final annotated images will be placed in a separate document when finished

Appendix 4 – Exchange exercise carried out for the reproduction task – Histology

This will continue to be done intersessionally by the histology readers and the final annotated images will be placed in a separate document when finished

Appendix 5 – Final comments from the invited experts on the SWO Biology Project

- *Jessica Farley (CSIRO Oceans and Atmosphere)*

Jessica Farley noted that the project is progressing very well, and that the main aspect for its success is the huge collaboration that is taking place in terms of sampling and analysis. Specific for the ageing, she noted that collecting both otoliths and spines is very positive, and that the comparison between the structures will be a key aspect resulting from this project. The development of the reference set with the standardization of readings between laboratories is also a key positive aspect.

One recommendation from Jessica with regards to the age was for the age validation. That is something still missing for swordfish from all Oceans and will be important to resolve in the near future. The best option will likely be bomb-radiocarbon methods. Another alternative could be OTC tag-recapture, but the logistics of the tagging, high mortality of swordfish when captured, and need to recapture, correctly identify it is an OTC tagged fish, and return the hard structures for laboratory processing might complicate the applicability of such method. The Project will consider especially the bomb-radiocarbon method, and will prepare a budget for consideration for the SCRS plenary in 2021.

With regards to the reproduction study, Jessica Farley noted that the classification scheme we are using (IEO-Malaga scale) seems very good and appropriate. She pointed that the standardization exercise between laboratories is also a key positive aspect from this project. One recommendation with regards to the reference set was to provide annotated images, so it is clearer what the readers are interpreting in terms of the various structures.

- *Kyne Krusic Golub (FAS – Fish Ageing Services)*

Kyne Krusic Golub noted it was encouraging for the project to see the general level of agreement among age readers, especially in the otoliths and despite having access to images alone and not physical samples. He made a point that collecting all those samples in extremely valuable, so we need to continue with that but also make sure they are properly curated and stored. He believes that in moving forward, otoliths will likely prove more useful than spines, but continuing with the spines especially for the younger age classes will also continue to be useful. He also fully agrees that age validation on both of those structures needs to be a priority, and that bomb-radiocarbon is probably the best option.

- *Freddy Arocha (IOV-UDO - Instituto Oceanográfico de Venezuela, Universidad de Oriente)*

Freddy Arocha made several comments on the project. A general comment on the sampling of swordfish biological parts for the ageing and reproduction study is that efforts made are commendable, but not exempt of spatial coverage issues common in highly migratory species. The swordfish study undertaken is no exception, but it has overcome and excelled in the sampling of biological parts from the Mediterranean stock and part of the South Atlantic stock. However, samples from the North Atlantic stock are less than desired. Nonetheless, it is expected that a broader coverage in hard part structures for ageing, as well as from tissue samples, will be expanded in the next few months allowing for a broader spatial coverage in all

three stocks as indicated by the coordinator in charge of ageing the hard structures. In the case of reproductive samples for the microscopic analysis it appears to be limited spatially and temporally in all three stocks. It must be acknowledged that sampling for reproductive studies is more complicated and demanding. Therefore, efforts should be increased to collect the necessary and adequate samples across the Atlantic and Mediterranean that would include all known spawning grounds from all stocks to produce robust reproductive parameters (i.e., L50 and Fecundity) to be used in future stock assessments of each stock.

On the ageing section, it is commendable the advances made to produce age readings based on paired hard parts (i.e., spines and otoliths). However, it is recognized that otolith extraction is complicated at sea and the volume of severed head parts may be an issue for some cooperating fleets; thus, efforts should continue in the use of spine sections for ageing. Another issue that should be addressed during the study is age validation. Despite of the age validation conducted for the western North Atlantic on couple of studies, where a ring formation (opaque + translucent band) in anal spine sections represent growth for one year (SCRS/1995/099), new techniques for age validation have become available and should be used to confirm previous findings for the North Atlantic and new ones for the other two stocks.

Regarding the reproduction section, again it is commendable the advances made to produce reference sets for the identification of maturity status and developmental phases from histological analysis of oocytes. It became clear during the exercise that frozen samples to be used in histology are challenging, and reference sets for frozen samples and freshly fixed should be the norm for the Atlantic stocks and the Mediterranean, due to potential differences in the observation of some of the structures in sectioned ovaries. It was considered helpful that the images used is the reference sets be annotated with the key structures that would help define the developmental stage.

It is important to remind the Group that histological analysis is an expensive technique not available to many CPCs, and that there are other techniques less expensive and equally accurate that when properly calibrated with histology will produce robust reproductive estimates in swordfish (SCRS/1995/098). Therefore, it should be encouraged that reference sets for whole oocytes (preferably fresh) be created and calibrated with histology for potential use in future studies by any CPC with limited resources. Also, the use size frequency distribution of oocytes should also be considered as an ancillary method in the identification of maturity status and developmental phases of swordfish ovaries.

Considering the investment made on the collection of biological samples for the present study, and for other similar studies undertaken by the different ICCAT SCRS Species Groups, it should be acknowledged that the storage, conservation, and maintenance of the samples collected should be addressed at the highest level to preserve them as reference collections for future studies. It became evident in the discussions during the Workshop that samples from past studies that would be useful as reference collection are not available due to the absence of a curation protocol by interested parties.

Appendix 6 – Practices for saving and archiving images of structures used by FAS – Fish Ageing Services

Set of practices used by FAS to be considered by this group and updated in later documents