REPORT ON THE 2020 ICCAT WORKSHOP ON SMALL TUNAS BIOLOGY STUDIES FOR GROWTH AND REPRODUCTION

S. Saber[1*], R. Muñoz-Lechuga[2], D. Macias[1], J. Ortiz de Urbina[1],
P.G. Lino[2], N'G C. Diaha[3], P. Pascual[4], F. Ngom Sow[5], D. Angueko[6], G. Hajjej[7],
S'A. Baibbat[8], K. Benounnas[9], A. Medina[10], P. Quelle[11], G. da Silva[12], J. Viñas[13], F. Lucena Frédou[14]

SUMMARY

This report describes the 2020 ICCAT workshop on small tunas biology studies for growth and reproduction, hosted by the Instituto Español de Oceanografía, Málaga, Spain. The major objectives of the workshop were: 1) starting the creation of ageing and reproduction reference sets and, 2) providing more training for the ongoing sample collection and processing to the teams involved in these studies. As approved by the SCRS in 2017, the Small Tuna Species Group intersessional meeting decided to prioritize the collection of biological samples aiming at growth, maturity and stock structure studies on three species: little tunny (Euthynnus alletteratus), Atlantic Bonito (Sarda sarda) and wahoo (Acanthocybium solandri), based on their economic importance and the lack of knowledge on their biology. This work will contribute to the next major advance in the assessment of these three species.

RÉSUMÉ

*Le présent rapport décrit l'atelier de l'ICCAT tenu en 2020 sur les études de la biologie des thonidés mineurs pour la croissance et la reproduction, organisé par l'Instituto Español de Oceanografía, à Malaga, en Espagne. Les principaux objectifs de l'atelier étaient les suivants : 1) commencer à créer des ensembles de référence sur la détermination de l'âge et la reproduction et 2) fournir une formation plus poussée sur la collecte et le traitement des échantillons aux équipes participant à ces études. Tel qu'approuvé par le SCRS en 2017, lors de la réunion intersessions du Groupe d'espèces sur les thonidés mineurs, il a été décidé de donner la priorité à la collecte d'échantillons biologiques visant à étudier la croissance, la maturité et la structure des stocks de trois espèces : la thonine commune (Euthynnus alletteratus), la bonite à dos rayé (*Sarda*) et le thazard-bâtard (*Acanthocybium solandri*), sur la base de leur importance économique et des connaissances lacunaires sur leur biologie. Ces travaux contribueront à la prochaine grande avancée dans l'évaluation de ces trois espèces.*

RESUMEN

Este informe describe el taller de ICCAT de 2020 sobre estudios de biología de pequeños túnidos para crecimiento y reproducción, acogido por el Instituto Español de Oceanografía en Málaga, España. Los principales objetivos del taller eran: 1) empezar la creación de conjuntos de referencia de determinación de la edad y reproducción y 2) facilitar más formación a los equipos involucrados en estos estudios para la recopilación de muestras y procesamiento en

*Corresponding author
[1] Instituto Español de Oceanografía, Centro Oceanográfico de Málaga, Spain
[2] Instituto Português do Mar e da Atmosfera, Portugal
[3] Centrer of Oceanology Research,Côte d'Ivoire
[4] Instituto Español de Oceanografía, Centro Oceanográfico de Canarias, Spain
[5] Centre De Recherches Oceanographiques de Dakar, Senegal
[6] Direction General des Peches et de l'Aqualculture, Gabon
[7] National Institute of Marine Science and Technology, Tunisie
[8] Laboratoire des Pêches (Dakhla), Morocco
[9] Chercheur au Centre National de Recherche et de Développement de la Pêche et de l'Aquaculture, Tipaza, Algérie
[10] Universidad de Cádiz, Dpto. de Biología, Puerto Real, Cádiz, Spain
[11] Instituto Español de Oceanografía, Centro Oceanográfico de Baleares, Palma de Mallorca, Spain
[12] Universidade Federal Rural do Semiárido, Dpt.of Animal Sciences, Mossorro, Brazil
[13] Laboratori Ictiologia Genètica, Departament de Biologia, Universitat de Girona, Spain
[14] Departamento de Pesca e Aquilcutura – Universidade Federal Rural de Pernambuco, Brasil

*curso. Como aprobó el SCRS en 2017, en la Reunión intersesiones del Grupo de especies de pequeños túnidos se decidió priorizar la recopilación de muestras biológicas con miras a estudios de crecimiento, madurez y estructura del stock de tres especies: bacoreta (*Euthynnus alletteratus*), bonito (*Sarda sarda*) y peto (*Acanthocybium solandri*), basándose en su importancia económica y la falta de conocimientos sobre su biología. Este trabajo contribuirá a avanzar en la próxima evaluación de estas tres especies.*

KEYWORDS

Small tuna, reproduction, aging, Atlantic bonito, little tunny, wahoo

Introduction

The 2020 ICCAT workshop on small tunas' biology was held at the Instituto Español de Oceanografía (IEO) laboratory in Málaga, Spain, from 17-21 February 2020. The Director of IEO Málaga Dr. Jorge Baro opened the meeting and welcomed participants. The group briefly discussed the objectives of the meeting and the relevance of this biological work to the ICCAT Small Tunas group. The group proceeded to review the Agenda, which was adopted with no changes (**Appendix 1**). The list of participants is included in **Appendix 2**.

1. Update on progress of the ICCAT SMT project to date

The coordinator of the SMT project for the years 2018 and 2019, Dr. Jordi Vinãs, presented the most updated information of the data collection for the three species (*Euthynnus alletteratus* (LTA), *Sarda sarda* (BON) and *Acanthocybium solandri* (WAH)) obtained by the different members of the consortium. This data collection comprised the material for growth studies (spines and otoliths), reproduction (gonads) and population structure (tissue). During the meeting some update of the data collection were also carried out.

Afterwards, the main gaps in terms of size for the three species were identified in order to maximize the effort of data collection for the 2019 project but also to help the construction of 2020 project in order to prioritize the gaps of the sampling.

2. Revision/update of sampling and biological data collection protocols

During the WS the protocol for the collection of gonads was updated (see **Appendix 3**).

A practical laboratory session on *Euthynnus alletteratus* otolith and spines extraction was carried out in order to transfer the methodology to those partners who requested this practical support.

3. Establishing protocols to start sample processing and data analysis

3.1 Reproduction and maturity

There were four presentations on maturity:

There was a presentation from IEO researchers about the difficulties in macroscopic maturity staging and the impact that has on the estimation of maturity ogives and length at 50% maturity (L_{50}), and potentially on future stock assessment of small tuna species. These problems are found throughout the spawning season, but have the most impact at the beginning and in the end of the spawning period, when immature specimens can be mistaken for mature. This is mainly due to the subjectivity in interpreting sizes and colours of gonads.
The specific actions proposed to reach agreement on a common maturity scale for small tuna species and the identification of the limits of the macroscopic maturity staging found was also presented. Maturity staging exercises were prepared and explained to explore these issues.

There was a presentation entitled "How to stage the gonadal cycle in (small) tunas. Histological criteria" presented by the invited expert on maturity from the University of Cádiz. Four approaches are commonly used to determine the reproductive state of fishes: 1) gross anatomical criteria; 2) gonadosomatic index; 3) in the case of females, mean diameter of the most advanced class of oocytes; and 4) histological analysis. While the macroscopic appearance of gonads is the fastest and easiest method, histological classifications are superior to all other methodologies as the histological structures of gonads reliably reflect their physiological state. Yet, histology requires long time for preparation of samples as well as the appropriate equipment and training. For an accurate visual monitoring of the gonadal cycle, one of the central goals of this meeting is to associate the macroscopic appearance of the gonads with the actual physiological status across the reproductive cycle in both females and males. It is, therefore, essential to get familiar with histological techniques and interpretations, and adopt a practical classification system for visual gonadal classification for small tunas. We presented routine laboratory protocols that have been successfully implemented in tunas, including tissue sampling, fixation, paraffin wax embedding, and microtome and staining of sections. Also, basic features of the gametogenetic phases are described under light and electron microscopy as the basis for histological classifications. Different classification schemes using macroscopic and histological criteria (e.g., Schaefer, 1998; Itano, 2000; Brown-Peterson et al., 2011; Ashida, 2020) are discussed within the scope of the biological assessment of small tunas.

There was a presentation from CRO researcher on the studies on small tuna biology with the objective to provide to the commission the appropriate data for a stock assessment. So, within the scope of the small tuna project, gonads of Atlantic bonito and little tunny from Ivory Coast were taken for histological analyses. Also, the gonads of little tunny and Atlantic bonito were photographed in order to highlight the macroscopic stages of sexual maturity, and then put in block, for the microscopic stages.

There was a presentation from IEO researcher on the sampling work and all activity made during the last period of the project. The Spanish Oceanographic Institute has in Canary Islands a sampling and observer net in the main ports of the islands. La Restinga´s observer buys wahoo specimens and sends to Tenerife the samples. After landing the catches, all information of specimens and spines, head or gonads samples are taken by local observer. All samples are stored in a good condition before send to Tenerife´s laboratory. In this laboratory, the otoliths were extracted for each wahoo´s head. All samples were stored in Tenerife until be sent to the coordinator laboratory of the project. Canary laboratory extracted 192 wahoo otoliths with around 35% of it damaged. The lack of experience with this species and the fragile nature of these otoliths could explain this bad result. In another hand, many photos were made for gonads and otoliths. The photos of the gonads were processed by image software to create a reference collection. Some preliminary results for male and female of wahoo were presented by IEO laboratory in this workshop.

The Group discussed the need to improve the seasonal sampling in order to cover the full reproductive cycle in the different areas.

The macroscopic maturity classification was improved mainly for Atlantic bonito after doing the exercises proposed in the present workshop. However, it should be noted that only images for BON and LTA from the Mediterranean and Northeast Atlantic were used for the calibration exercises. The group discussed advantages and disadvantages of macroscopic maturity stages and the importance of histological analysis of gonads for differentiating between mature and immature individuals. The identification of the sex was also discussed and the definitions of undetermined individuals (sex not distinguished by naked eye) and not sexed individuals (not observed sex) were clarified.

Since different maturity scales are used by the participants, the Group discussed the importance of standardization of maturity determination criteria. The group also discussed the importance of creating a macroscopic image reference set and suggested a joint macroscopic workshop for other small tuna species and areas not covered in this workshop. Finally, it should be noted that oocyte maturation thresholds differ among the several experts on maturity present in the WS; therefore a histology workshop in order to agree on common microscopic maturity scale was also suggested. The procedures for sampling gonads and for taking macroscopic images will be added to the sampling protocol (see **Appendix 3; Figures 1 and 2**).

3.2 Ageing, including spines vs. otoliths

The invited expert on Age and Growth, Pablo Quelle from IEO, carried out a presentation on the most common structures used in age and growth in tuna and the posterior processing. The spine is the easier structure to obtain. The processing is easy and different distance options were showed and explained the importance of the morphology of the spine. The development of a standardized methodology was recommended. The otolith

morphology was presented and the relative position in the head was explained. The location of the rostrum, postrostrum, antirostrum and nucleus was detailed. The processing of the otoliths was explained according to the Report of the ICCAT GBYP International Workshop on Atlantic Bluefin Tuna Growth (Rodriguez-Marin et al. 2019). Finally, an option for the smaller otoliths (Farley et al. 2016) methodology was explained. Vertebrae sampling was also explained.

Guelson Silva from UFERSA (Brazil), carried out a presentation on the progress of the wahoo (*Acanthocybium solandri*) sampling by the Brazilian team and the preliminary results from otolith preparations. Previous studies on age and growth for the wahoo were discussed and the protocols to collect the wahoo samples in the Brazilian fishing companies were explained such as the sampling areas and the gathered information (size, date, and weight). It was also presented the preliminary results on the preparation of wahoo otoliths, by both daily and annual approach. In the end, the possible studies based on wahoo otoliths were described, such as otolith shape analysis, microchemistry, stable isotopes, radiocarbon, etc.

The invited expert on Age and Growth, Pablo Quelle from IEO, carried out a presentation explaining the criteria of the interpretation of annual bands and the different validation methodologies. Some examples from otolith and spines were discussed. The vascularized area in the spines was exposed as a great problem in ageing. The methodology used to make the measurements in spines and otoliths was indicated and a suggestion for BON whole otoliths measurement was made. Some methods to validate the increments were shown. The replicability in the measurements between bands was considered a good criterion to identify each band individually. The identification of the first annulus through the daily growth increments was recognized as the best method to identify the first annulus. The chemical tagging, was considered a good method to identify the periodicity of the bands (validation). The edge seasonality of the edge formation and the MIA were presented as an option if the sampling is along the whole year. Back-calculation and comparison between different structures were also shown. References to the importance of the precision and accuracy between the readers were made too. The radiocarbon bomb was presented as a good method to validate readings and annual bands. Classical tag-recapture could be useful to compare growth rates obtained thru the different methods. Finally, some advantage of each structure was presented and evaluated in order to find the best for this project.

Finally, a presentation from IPMA researchers described the status of the consortium age and growth component and the preliminary advances in age and growth of BON, LTA and WAH. More than 1800 spines and 1000 otoliths were collected between 2018 and 2019. After the workshop, an increase in the number of otolith samples is expected. Size distributions of age structures individuals were showed and missing size samples were discussed for sampling in the future. Different works on age and growth have been published, but there is no specific protocol for these species. For this reason, it is essential to create a specific methodology for age structure analysis in order to develop new studies. The preliminary results of the spine analysis were presented and the methods of analysis for otoliths in these species were discussed. In addition, other aspects were proposed to improve the knowledge about stocks and biological parameters, such as to carry out a separate analysis of samples if there are stock differences, create a processing group to analyze samples or to carry out complementary analysis with other techniques such as shape analysis, otolith microchemistry or stable isotopes.

At the time of the meeting it was pointed out that otolith sampling was substantially lower than spine sampling. It was encouraged that an increase of otolith sampling after workshop should occur. It was discussed to focus the collection of samples on those size gaps, by species and by area, to complete the size distribution (**Table 1**). It was discussed that the compensation price would have to be changed for some participants to be able to collect the samplings of large sizes as the whole fish would have to be bought.

Despite the existence of various age protocols of other species such as bluefin or swordfish, it is not possible to apply them to small tunas, therefore the need to create new specific protocols was debated, both for the analysis of spines and otoliths of small tunas. It was argued that the study of age parameters for three different species could be too much for the IPMA laboratory. Guelson Silva from Brazil volunteered to carry out the analysis of the wahoo samples.

The utility of SMARTDOTS software was discussed as a way to carry out online inter-calibration studies. It was commented that SmartDOTS (https://ices.dk/data/tools/Pages/smartdots.aspx) was designed for otoliths but it would be interesting to make the platform more flexible and useful, such as the implementation of tools for taking measurements in spines.

4. Training for age readings and maturity stage assignation to the teams involved in these studies

Reproduction and maturity

During the workshop on the sexual maturity staging of small tuna species, training exercises were done on Atlantic bonito and little tunny. These maturity staging exercises were carried out taking into account the recommendations given for the WGBIOP Guidelines for Workshops on Maturity Staging Calibration of the International Council for the Exploration of the Sea (ICES).

The calibration exercises were trialed on 77 specimens of Atlantic bonito (54 females and 23 males) by 17 participants with different levels of experience (basic, n = 9 and advanced, n = 8) and on 25 specimens of little tunny (14 females and 11 males) by 15 participants, again with different levels of experience (basic, n = 9 and advanced, n = 6).

The exercises highlighted across-the-board difficulties in stage identification. The two main problems in macroscopic maturity staging of gonads that emerged were: (a) difficulties in differentiating between immature and developing and, (b) difficulties in differentiating between developing and spent (post-spawning). It is important to note that the histological examination of gonads showed that ovaries identified as developing and spent when using visual staging method (macroscopic criteria) were subsequently found to be immature by microscopic examination.

5. Discussion and plans for future sampling and priority areas for the project

The group identified priority sample analyses for information collected in the initial year of the programme, discussed upcoming research and developed recommendations for the small tunas group and the SCRS. Sampling effort will prioritize filling spatial-temporal and size-sex gaps in the current data set and will add capacity for collection of gonads and otoliths. The ageing and growth project coordinators will continue to process spines and otoliths collected in the initial year of the program and will begin development of an aging protocol and reference set. Maturity and reproduction work will focus on development of a macroscopic maturity stage key and the development of histological standards for identification of maturity stage.

6. Recommendations

6.1 General recommendations

Target specific sizes of the three species in order to optimise the sampling effort and minimising the costs aiming at providing the best estimates of growth, reproduction and population parameters.

6.2 Specific project recommendations

Reproduction and maturity

In order to estimate the maturity ogives as well as reproductive traits, spawning season and spawning location should be known. If this information is unknown, a minimum of samples should be collected throughout the year, to allow the determination of temporal and spatial patterns in maturity. Furthermore, the sampling needs to ensure that the immature and mature individuals are well represented. An additional effort needs to be made to collect samples throughout the year and covering the whole distribution areas for the three small tuna species.

It is recommended that a platform (e.g. an equivalent to SmartDots for reproduction) is used for future photograph exchanges and workshops. It is also recommended a maturity staging workshop in 2022 for calibration and adopting internationally agreed macroscopic and microscopic maturity scales.

Age and Growth

Promote the collection and aggregation of the age structures still held by the CPCs and create a common storage and database. This could be used to promote online calibration workshops.

An additional effort needs to be made to collect the size gaps needed to create a full age-length key.

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Table 1. Details of the size range of samples for growth that should be provided according to the gaps, by species and area.

Species	Area	Size range needed
BON	MED; BIL95	< 30 cm and > 60 cm
	AT-NE; BIL94	< 30 cm and > 60 cm
	AT-SE; BIL97	< 35 cm and > 50 cm
LTA	MED; BIL95	> 70 cm
	AT-NE; BIL94	> 70 cm
	AT-SE; BIL97	< 35 cm and > 55 cm
WAH	AT-NE; BIL94	< 90 cm and > 150 cm
	AT-SE; BIL97	< 75 cm and > 110 cm
	AT-SW; BIL96	Increase samples for all size range

Appendix 1

ICCAT Small Tunas sampling and biology workshop

INSTITUTO ESPAÑOL DE OCEANOGRAFÍA

Centro Oceanográfico de Málaga, Spain, 17-21 February 2020

Agenda

1. Opening

2. Adoption of agenda

3. Nomination of the rapporteurs

4. Revision/update of sampling and biological data collection protocols

5. Revision/update of protocols for reproductive samples processing and data analysis

    5.1. Reproductive biology, incl. macroscopic vs histological scales

6. Revision/update of protocols for ageing samples processing and data analysis

    6.1. Ageing, incl. spines vs. otoliths

7. Initial steps for the establishment of reference sets

8. Training for age readings and maturity stage assignation to the teams involved in these studies

9. Workshop report and adoption

10. Closure

Appendix 2

List of participants

ALGERIA

Benounnas, Kamel
Chercheur au Centre National de Recherche et de Développement de la Pêche et de l'Aquaculture, 11 boulevard le colonel Amirouche, Bou-Ismail, Tipaza, Algérie.
Tel: +213 24 32 64 10, E-mail: kamel_benounnas@yahoo.fr

BRAZIL

Da Silva, Guelson
Universidade Federal Rural do Semiárido, Dpto.of Animal Sciences, Av. Francisco Mota, 572, Bairro Pres. Costa e Silva, Mossorro / RN, CEP: 59625-900
Tel: +55 85 985032723, E-Mail: guelson@ufersa.edu.br

Lucena Frédou, Flávia
Universidade Federal Rural de Pernambuco, Dpto. de Pesca e Aquicultura, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, CEP: 51020-180 Recife/Pernambuco
Tel: +55 81 3320 6514, E-Mail: flavialucena@hotmail.com

CÔTE D'IVOIRE

Diaha, N'Guessan Constance
Chercheur Hydrobiologiste au Centre de Recherches Océanologiques, Ministère l'enseignement supérieur et recherche scientifique, 29, Rue des Pêcheurs - B.P. V-18, Abidjan 01
Tel: +225 2135 5880, Fax: +225 2135 1155, E-Mail: diahaconstance@yahoo.fr; constance.diaha@cro-ci.org

EUROPEAN UNION

Ciércoles Antonell, Cristina
Ministerio de Ciencia e Innovación, Instituto Español de Oceanografía. Centro Oceanográfico de Málaga, Puerto Pesquero s/n, 29460 Fuengirola, Málaga, España
Tel: +34 952 197 124, Fax: +34 952 581 388, E-Mail: cristina.ciercoles@ieo.es

Godoy Garrido, M. Dolores
Ministerio de Ciencia e Innovación, Instituto Español de Oceanografía. Centro Oceanográfico de Málaga, Puerto Pesquero s/n, 29460 Fuengirola, Málaga, España
Tel: +34 952 197 124, Fax: +34 952 581 388, E-Mail: lola.godoy@ieo.es

Gómez Vives, María José
Ministerio de Ciencia e Innovación, Instituto Español de Oceanografía. Centro Oceanográfico de Málaga, Puerto Pesquero s/n, 29460 Fuengirola, Málaga, España
Tel: +34 952 197 124, Fax: +34 952 581 388, E-Mail: maco.gomez@ieo.es

Lino, Pedro Gil
Instituto Português do Mar e da Atmosfera - I.P./IPMA, Avenida 5 Outubro s/n, 8700-305 Olhão Faro, Portugal
Tel: +351 289 700504, E-Mail: plino@ipma.pt

Macías López, Ángel David
Ministerio de Ciencia e Innovación, Instituto Español de Oceanografía. Centro Oceanográfico de Málaga, Puerto Pesquero s/n, 29460 Fuengirola, Málaga, España
Tel: +34 952 197 124, Fax: +34 952 581 388, E-Mail: david.macias@ieo.es

Medina Guerrero, Antonio
Universidad de Cádiz, Dpto de Biología, Puerto Real, Cádiz, España
Tel: +34 952 197 124, Fax: +34 952 581 388, E-Mail: antonio.medina@uca.es

Muñoz Lechuga, Rubén
Instituto Português do Mar e da Atmosfera - I.P./IPMA, Avenida 5 Outubro s/n, 8700-305 Olhão Faro, Portugal
Tel: +351 289 700 504, E-Mail: ruben.lechuga@ipma.pt

Ortiz de Urbina, Jose María
Ministerio de Ciencia e Innovación, Instituto Español de Oceanografía, C.O de Málaga, Puerto Pesquero s/n, 29640 Fuengirola Málaga, España
Tel: +34 952 197 124, Fax: +34 952 463 808, E-Mail: urbina@ieo.es

Pascual Alayón, Pedro José
Ministerio de Ciencia e Innovación , Instituto Español de Oceanografía, C.O. de Canarias, Vía Espaldón, Dársena Pesquera, Parcela 8, 38180 Santa Cruz de Tenerife Islas Canarias, España
Tel: +34 922 549 400; +34 686 219 114, Fax: +34 922 549 500, E-Mail: pedro.pascual@ieo.es

Quelle, Pablo
Ministerio de Ciencia e Innovación, Instituto Español de Oceanografía. Centro Oceanográfico de Baleares,Muelle de Poniente, s/n 07015 Palma de Mallorca, España
Tel: +34 971 133 720 Fax: +34 971 404 945, E-Mail: pablo.quelle@ieo.es

Rioja Garay, Pilar
Ministerio de Ciencia, Innovación y Universidades, Instituto Español de Oceanografía. Centro Oceanográfico de Málaga, Puerto Pesquero s/n, 29460 Fuengirola, Málaga, España
Tel: +34 952 197 124, Fax: +34 952 581 388, E-Mail: pilar.rioja@ieo.es

Saber Rodríguez, Sámar
Ministerio de Ciencia, Innovación y Universidades, Instituto Español de Oceanografía. Centro Oceanográfico de Málaga, Puerto Pesquero s/n, 29460 Fuengirola, Málaga, España
Tel: +34 952 197 124, Fax: +34 952 581 388, E-Mail: samar.saber@ieo.es

Viñas de Puig, Jordi
Universitat de Girona, Departament de Biologia, Laboratori d'Ictiologia Genètica, C/ Maria Aurélia Capmany, 40, 17003 Girona, España
Tel: +34 629 409 072, E-Mail: jordi.vinas@udg.edu

GABON
Angueko, Davy
Chargé d'Etudes du Directeur Général des Pêches, Direction Générale des Pêche et de l'Aquaculture, BP 9498, Libreville Estuaire
Tel: +241 0653 4886, E-Mail: davyangueko@yahoo.fr; davyangueko83@gmail.com

MOROCCO
Baibbat, Sid'Ahmed
Chef de Laboratoire des Pêches, Centre régional de DAKHLA, Institut National de Recherches Halieutiques (INRH), 2, BD Sidi Abderrahmane, ain diab., 20100 Dakhla
Tel: +212 661 642 573, E-Mail: baibat@hotmail.com

SENEGAL
Sow, Fambaye Ngom
Chercheur Biologiste des Pêches, Centre de Recherches Océanographiques de Dakar Thiaroye, CRODT/ISRA, LNERV - Route du Front de Terre - BP 2241, Dakar
Tel: +221 3 0108 1104; +221 77 502 67 79, Fax: +221 33 832 8262, E-Mail: famngom@yahoo.com

TUNISIA
Hajjej, Ghailen
Maître assistant de l'Enseignement Supérieur Agricole, Laboratoire des Sciences Halieutiques, Institut National des Sciences et Technologies de la Mer (INSTM), Port de pêche, 6000 Gabès
Tel: +216 75 220 254; +216 972 77457, Fax: +216 75 220 254, E-Mail: ghailen3@yahoo.fr

ICCAT Secretariat
Neves dos Santos, Miguel
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

Appendix 3

Protocol for the gonad samples collection

Labeling procedure and codes

The same code to label all samples collected for each fish must be used.

Materials

Containers 60 ml assembled with yellow srew cap
Surgical scissors, tweezers, knife (or scalpel blades)
Fixative liquid (Bouin or 4% formaldehyde)
Ethanol 70%
Gloves

Sampling procedure

Extract the gonads from the peritoneal cavity and weight them.
For histological analysis it is preferable to take and fix fresh gonads. Immediately after recording the gonad weight, a 1-2 cm cross-section from the central part of the right or left lobe should be taken. If gonads are small, the whole gonads or cross-sections from both can be collected. For large gonads a representative sub-sample should be taken, including the area from the *tunica albuginea* to the ovarian lumen (**Figure 1**). Three fixative liquids can be used, formaldehyde, Bouin's fluid or alcoholic Bouin's.

a) The collected gonad sample will be fixed in 4% formaldehyde (10% formalin) in the ratio 1:10 for at least 10 days. After 10 days it can also be stored in 70% ethanol.
b) The collected gonad sample will be fixed in Bouin's fluid for four hours and then preserved in 70% ethanol. The sample to fixative ratio can be 4:10.
c) The collected gonad sample will be fixed in alcoholic Bouin's fluid for 48 hours. The sample to fixative ratio can be 4:10.

Shipping: put the containers with gonads in a storage box, they must be well close to avoid the formaldehyde or the ethanol spills. In order to avoid the liquid (formaldehyde or ethanol) spills, a scotch tape can be used to wrap the screw cap.

Recommendations for the macroscopic images

The maturity scale used for each species should be consistent across the laboratories, so in order to use precise criteria for the classification of maturity each scientific institution will provide a minimum of 30 detailed photos (macroscopic photos) of the all different maturity stages of each species. These images will be an important information for future workshops to assess the differences between laboratories and to validate the maturity staging though microscopic evaluation of gonads.

- ✓ The images have to be clear and reflect the macroscopic differences between maturity stages of different species.
- ✓ The identification number, catch date, sex, maturity and gonad weight should be included in a label. Include an ictiometer or similar as a size reference.
- ✓ It is recommended to take two images. An example is shown in **Figure 2**.

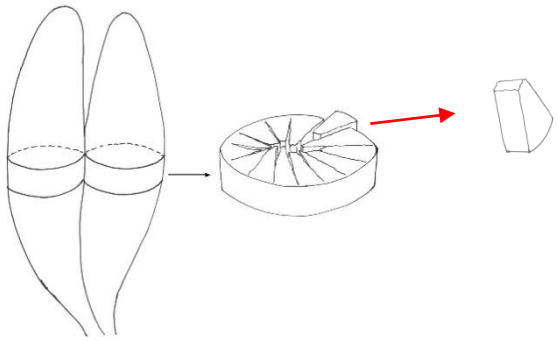

Figure 1. Picture of female gonads showing the full cross section from the central part of one of the lobes. The red arrow points a small portion (sub-sample that includes the *tunica albuginea* to the ovarian lumen) to be taken for large gonads.

Figure 2. Atlantic bonito female. Gonad inside the cavity of fish (upper photograph) and gonad outside (lower photograph).