



**MINISTERU GHALL-AGRIKOLTURA, SAJD  
U DRITTIJET TAL-ANIMALI**

**MINISTRY FOR AGRICULTURE, FISHERIES  
AND ANIMAL RIGHTS**

**MALTA**

*Dipartiment tas-Sajd u Akkwakultura*

*Department of Fisheries and Aquaculture*

**THE ATLANTIC-WIDE RESEARCH PROGRAMME FOR  
BLUEFIN TUNA (GBYP Phase 10)**

**SHORT TERM CONTRACT FOR BIOLOGICAL  
STUDIES - SAMPLING FOR ADULTS  
(ICCAT GBYP 11/2020-A)**

**Final Report (Deliverable #5)**



**Department of Fisheries and Aquaculture  
MINISTRY FOR AGRICULTURE, FISHERIES  
AND ANIMAL RIGHTS**

**Dr. Francesco Lombardo, Scientific coordinator**

**Malta, 10 January 2020**



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## **EXECUTIVE SUMMARY**

The main objective of the ICCAT GBYP is to improve the understanding of key biological and ecological processes for the Atlantic Bluefin tuna (BFT) with the final aim of optimising the sustainable management of this important biological resource.

An agreement between ICCAT and DFA-MAFA of Malta was set up in order to collect biometric data and biological samples of adult BFTs caught in two different fishing grounds of the Mediterranean Sea, the South Tyrrhenian Sea (STS) and the South-Central Mediterranean Sea (SCM) from two different Maltese tuna farms.

During the 2020 BFT harvesting season, nevertheless the COVID-19 pandemic issues and the related difficulties, the biometric data collection (weight, length and sex determination) and the biological sampling (otoliths and muscles) were conducted from mid-October to mid-November 2020 and, to achieve the contracted sampling target of 600 fish in total, a number of fish more than had been requested in the contract were sampled directly on board two different processing vessels (reefers). This was necessary to comply with the aim of sampling a wide size distribution of adult BFTs within each farm and to compensate losses of otoliths caused by both the harvesting process and the fish processing operations.

Therefore, a total of 748 adult BFTs were sampled from different farming cages of two different Maltese tuna farms (Fish and Fish Ltd and Malta Mariculture Ltd) and the BFTs sampled varied in size from 102 cm to 344 cm in Strait Fork Length (SFL) and from 58 kg to 470 kg in Round Weight (RWT). However, only 634 fish were considered as the valid sets of data, i.e. where biometric data (length, weight and sex), muscle samples (duplicate) and otoliths (pair or single) were successfully collected. Within these valid sets of data, a wide size (weight and length) frequency distributions was also achieved as the BFTs sampled varied in size from 136 cm to 344 cm in SFL and from 100 kg to 470 kg in RWT with an overall sex ratio of 48% male and 52% female fish sampled.



## 1. CONTEXT

The main objectives of the ICCAT GBYP is to improve the understanding of key biological and ecological processes for the Atlantic Bluefin tuna (BFT), with the final aim of optimising the sustainable management of this important biological resource.

To this end, on the 23<sup>rd</sup> July 2020, DFA-MAFA with the subcontracted partner OCEANIS Srl presented a proposal to the call for tenders ICCAT GBYP 11/2020 BIOLOGICAL STUDIES – SAMPLING FOR ADULTS - ATLANTIC-WIDE RESEARCH PROGRAMME FOR BLUEFIN TUNA (ICCAT GBYP – PHASE 10).

This proposal, following an amendment communicated to the ICCAT Secretariat on the 07<sup>th</sup> August 2020, which caused the exclusion of the subcontracted partner (OCEANIS Srl), was awarded by the ICCAT Secretariat on the 20<sup>th</sup> August 2020 and the short-term contract provided by ICCAT GBYP (ICCAT GBYP 11/220-A) was signed on the 02<sup>nd</sup> September 2020.

In addition, a request of approval was sent to the ICCAT Secretariat on the 16<sup>th</sup> September 2020 regarding the involvement (without budget allocation) of OCEANIS Srl in the biological sampling activities. This request was confirmed by the ICCAT Secretariat on the 18<sup>th</sup> September 2020.

Ultimately, on the 27<sup>th</sup> November 2020, due to some internal administrative issues, the DFA-MAFA formally requested a second amendment of the contract in order to include OCEANIS Srl as Subcontractor. The ICCAT's approval was received on the 01<sup>st</sup> December 2020.

According to the awarded sampling activities, DFA-MAFA and the Subcontractor OCEANIS Srl are to collect 600 valid and complete sets of biometric data (weight, length and sex determination) and biological samples (otoliths and muscles) from adult BFTs ("L" > 100 kg) which have been fished in two specific area of the Mediterranean Sea (Fig. 1) during the 2020 BFT fishing season and subsequently caged into Maltese farms for their maintenance and fattening until harvest.



**Figure 1.** Approximate catching area of the BFTs from which complete sets of biological samples were collected. The red star indicates the South Tyrrhenian Sea (STS) catching area and the relative BFTs were transferred to and caged at Malta Mariculture Ltd (MML); the yellow star indicates the South-Central Mediterranean (SCM) catching area and the relative BFTs were transferred to and caged at Fish and Fish Ltd (FF).



## 2. THE SELECTED FARMS

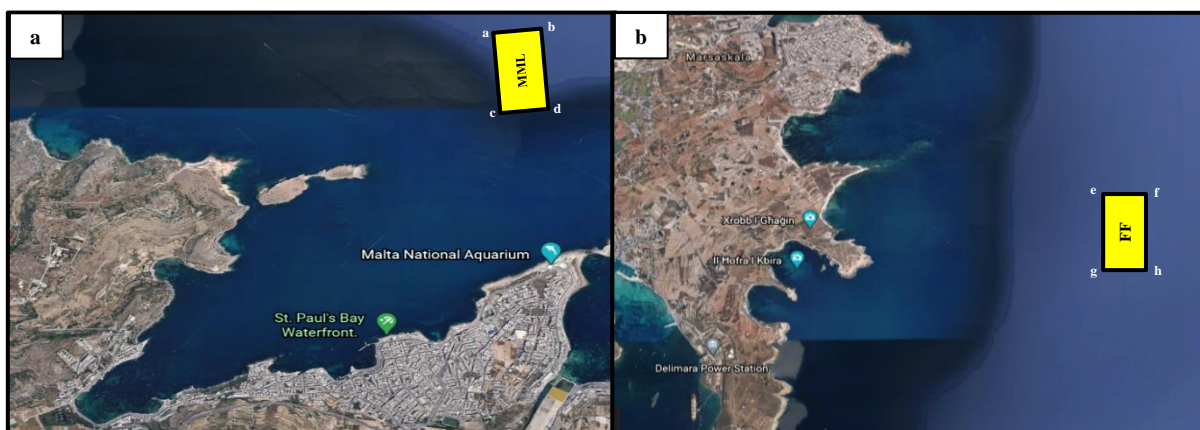
According to the awarded contract, the Maltese tuna farms selected for this sampling project were as follows:

- Mare Blu Tuna Farm Ltd.
- Fish and Fish Ltd.

Prior to board the reefers, due to the current COVID-19 pandemic issues, both farm operators requested to receive negative swab test result against COVID-19 of the personnel involved in the sampling project, 48 hours before boarding the vessel to start the sampling activity. Despite all the medical test results provided (both antibody and swab test), Mare Blu tuna farm Ltd suddenly refused to allow the scientific staff on board its reefer (Princessa Guasimara) to carry out the agreed sampling activity. Therefore, a new Maltese tuna farms was selected to replace Mare Blu tuna farm Ltd and the adult BFTs samples, within this sampling project, were collected from the following tuna farms at last:

- Fish and Fish Ltd, hereafter referred to as FF.
- Malta Mariculture Ltd, hereafter referred to as MML.

The selected Maltese tuna farms are located in specifically assigned Aquaculture Zones, 3nm off the east coast of Malta (Fig. 2) and the geographic coordinates of each polygon are indicated in table 1.



**Figure 2.** Geographic position of the selected tuna farms. **a** shows MML in the North-east Aquaculture Zone and **b** shows FF in the South-east Aquaculture Zone.





**Table 1.** Geographic coordinates (in decimal degrees) of the selected Maltese tuna farms.

Points	Latitude	Longitude	Farm
a	36.006111	14.430556	Malta Mariculture Ltd
b	36.004167	14.435833	
c	36.003056	14.434722	
d	36.006667	14.431667	
e	35.864000	14.643000	Fish and Fish Ltd
f	35.864000	14.660000	
g	35.849600	14.660000	
h	35.849500	14.643000	

### 3. SAMPLING

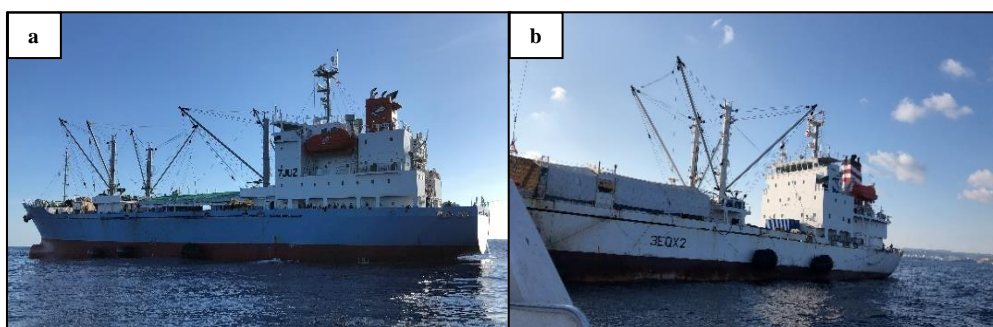
The aim of the following sampling plan was to cover a wide size (weight) distribution of adult BFTs within each farm, from 100 kg up to, but not limited to, 400 kg. The fish to be sampled were selected according to their size in weight with the aim to collect biometric data and biological samples from 5 fish every 5 kg size frequency bin: e.g. 5 fish of 151-155 kg, 5 fish of 156-160 kg, 5 fish of 161-165 kg, etc... Moreover, the head's condition of the fish (Fig. 3) was another criterion used to select the BFT to be sampled.



**Figure 3.** BFT's head condition: **a-b** show how the shoot can compromise the biological sampling and therefore these heads resulted not suitable for otolith's extraction purpose and therefore rejected; **c** shows a relatively good head condition for otolith's extraction.



The biometric data collection and the biological sampling (otoliths and muscles) were conducted from mid-October to mid-November 2020 and were carried out directly on board two different reefers, Gouta Maru for the fish harvested from FF and Lady Tuna for the fish harvested from MML (Fig. 4), during the 2020 BFT harvest season.



**Figure 4.** BFT reefers. **a** shows the vessel “Gouta Maru” used by FF and **b** shows the vessel “Lady Tuna” used by MML.

According to the terms of the contract, the aim of the sampling activity is to obtain from adult farmed BFTs biometric data and biological samples as follows:

- a) Straight Fork Length (SFL) in cm.
- b) Curved Fork Length (CFL) in cm.
- c) Length to the first Dorsal (LD1) in cm.
- d) Round weight (RWT) in kg.
- e) Sex determination (M/F).
- f) Otoliths.
- g) Tissue (muscles) in duplicate.
- h) Additional tissue (muscle) when possible.

#### 4. MATERIAL AND METHODS

The scheme for the Biological Sampling design provided by ICCAT GBYP (Appendix 2 - Last revised: 18 July 2018 - SAMPLING PROTOCOLS FOR THE GBYP BIOLOGICAL SAMPLING) was used as guideline for the biometric data collection and biological sampling carried out within this project.

Briefly, a regular BFT harvesting process involves few procedural and subsequent steps (Fig. 5):

- a) the shooting of the fish inside the farming cage by scuba divers;
- b) the transport of the fish to the reefer by a transport vessel;
- c) the processing of the fish by the reefer’s crew;
  - i. weighing and measuring of the fish;
  - ii. cutting off of the head and tail;





- iii. cutting the fish into loins or fillets;
  - iv. further catting from the heads and pectoral collar;
  - v. freezing of the fish products;
- d) the cleaning of the deck prior of the arrival of the next group of fish.



**Figure 5.** Steps of the BFT harvesting process: **a** shows the shooting of the fish inside the farming cage; **b-c** show the transport of group of fish to the reefer and the lifting up onboard the reefer; **d** shows the weighing and measuring of the fish; **e** shows the cutting off of the head and tail of the fish; **f-h** show the processing of the fish into loins or fillets; **i-j** show further processing of the fish; **k-l** show the sharpening of knives and the cleaned deck.



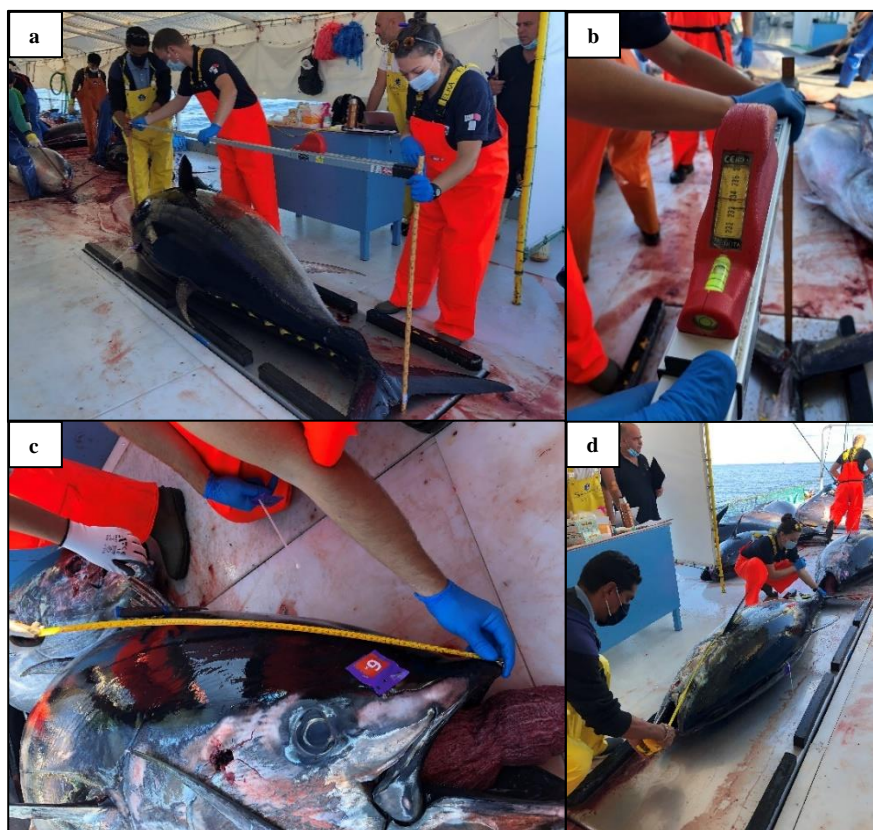
Once the fish has been selected for the purposes of the project, two plastic numbered tags were applied (Fig 6): one tag was applied on the first dorsal spine to easily track the fish for the next step, i.e. the sex determination of the fish, and another tag was applied on the head (either on the eye or on the mouth) for the last step, i.e. the biological sampling.



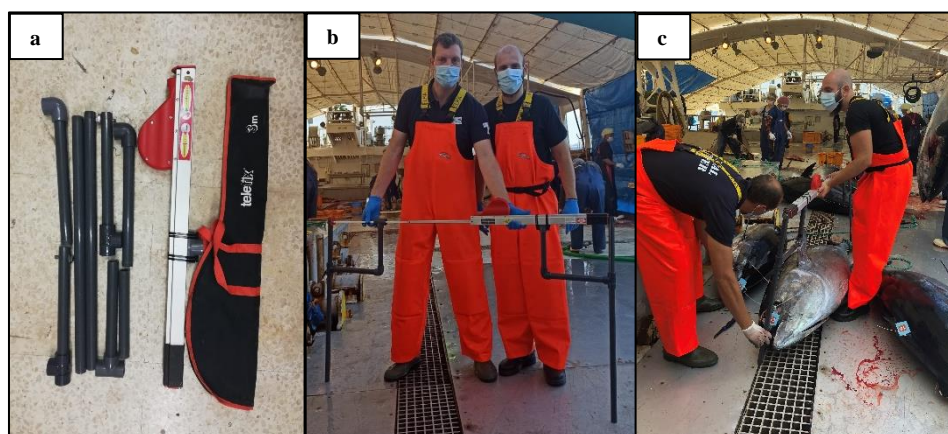
**Figure 6.** Tagging phase of the fish. The black arrows indicate the position of the tags applied on the fish: one tag is applied on the head and one tag on the first dorsal spine of the fish.

The biometric data (SFL, CFL, LD1 and RWT) of the selected fish were obviously determined prior the fish was processed (Fig. 7 and 8); subsequently, the sex determination of the fish (Fig. 9) was macroscopically assessed.





**Figure 7.** Biometric measurements phase: **a** shows the SFL measurement using an a customised calliper; **b** shows the display of the SFL measuring device; **c** shows the LD1 measurement; **d** shows the CFL measurement.



**Figure 8.** Upgraded calliper device (more solid PVC jaws were used to replace the wooden jaws) used for SFL measurements. **a** shows the bag of the device; **b** shows the staff intended to use the device; **c** shows the device in use for the SFL measurement.



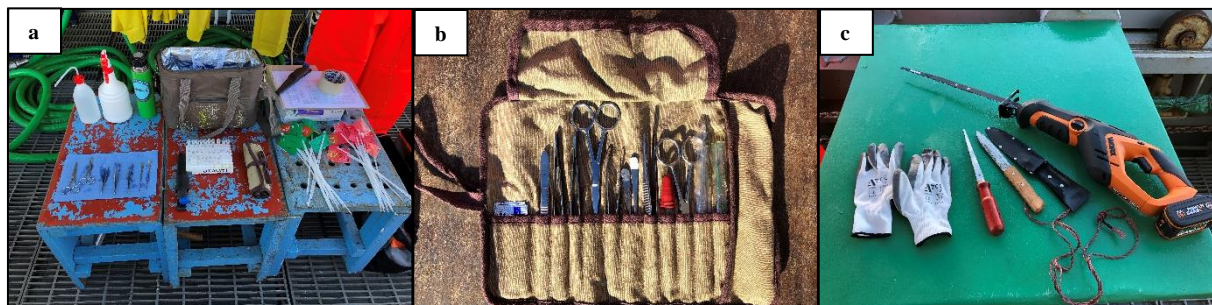
**Figure 9.** Sex determination of the fish. **a** shows some tagged fish placed on one side of the processing area for an easy track of the fish. **b** shows the gill-gutting process of the fish and the black arrow indicates, in this case, the male gonad of the fish.

Consequently, the tagged heads were moved to a different section of the reefer where further processing occurred (Fig. 9). Following completion of the fish processing, the heads were transferred to a dedicated area of the vessel where a field laboratory facility (Fig. 10) was set up to conduct the required biological sampling.



**Figure 9.** Tagged BFT heads awaiting to be further processed prior to proceed with the biological sampling.



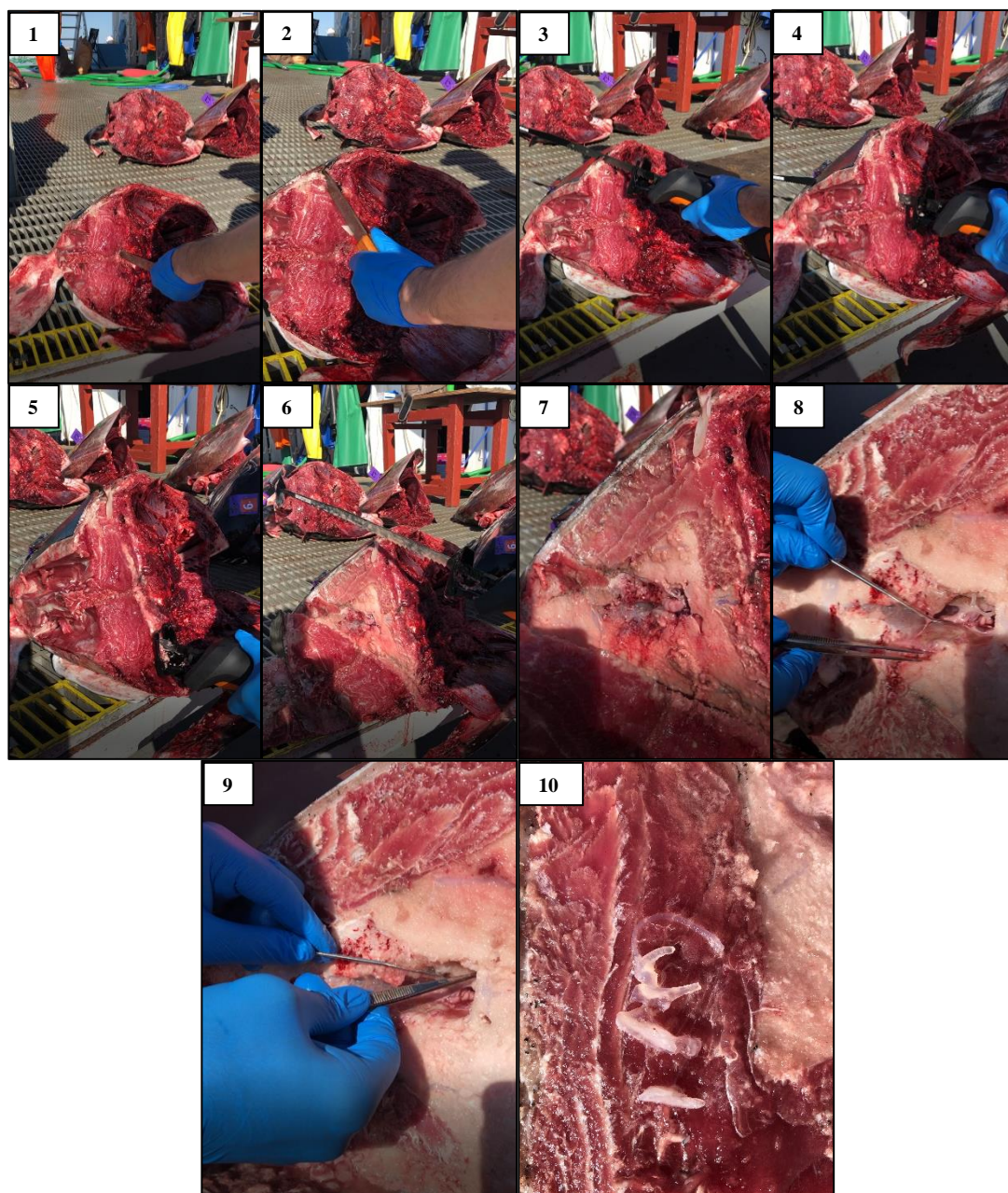


**Figure 10.** Field laboratory facility and equipment: **a** shows the supporting tables for the laboratory equipment; **b** shows the dissection kit; **c** shows the cut-off saw and knives used to cut the BFT head.

One-by-one, the heads were therefore placed on a small table and, by using an electrical saw (WORKX 20V Power Share, equipped with a customised metal blade), a frontal section was performed just top of the spinal cord; the otolith/s were found just below the rear of the brain and the sagittal otolith/s were removed from the left and right otic cavities, using small forceps. From the three pairs of otoliths found in the skull only the largest sagittal pair was collected for aging purposes. Otolith/s were extracted with the otolith membrane still attached and were subsequently gently cleaned to remove the membrane surrounding them and rinsed with deionised water. The otolith/s were therefore stored in in 2ml labelled (O) microtube temporarily filled with deionized water. Figure 11 shows a simple procedure for the otolith's extraction.

When the otolith/s were successfully extracted, two replicates of muscle samples were collected from the head, fixed in 96% Ethanol, stored in 5ml labelled (Ma and Mb) microtubes and kept at 4°C inside a cooler bag. When possible, an additional muscle sample was also collected, fixed in RNAlater, stored in 2ml labelled (Mc) microtube and kept at 4°C inside a cooler bag. Figure 12 shows the sampling process of muscle tissues from the fish's head while figure 13 shows the sampling process carried out on the deck of the reefer by the scientific staff.

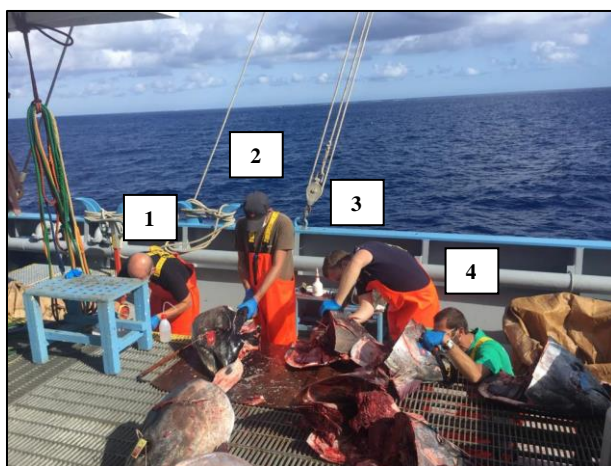




**Figure 11.** Otolith's extraction procedure: **1** shows and indicates an imaginary line just by the spinal cord where to perform the cut; **2** shows a small incision by a knife to facilitate the way in of the blade of the cut-off saw; **3-5** show the cut of the skull; **6** shows the cut off of the top of the fish's skull; **7** shows an ideal cut which removes the top of the skull and reveals the full length of the soft white brain underneath; **8** shows the brain tissue removed to make access to the otolith cavity; **9** shows the otolith extraction and **10** shows the two otoliths extracted with the otolith membrane still attached.



**Figure 12.** Sampling process of the muscle tissues.



**Figure 13.** Sampling process on the deck of the reefer Gouta Maru. **1** shows the personnel preparing the related microtubes; **2** shows the personnel carrying put the cut of the fish's head by using a cut-off saw; **3** shows the personnel carrying out the sampling of muscle tissue; **4** shows the personnel carrying out the otolith's extraction.

At the end of each sampling day, the collected biological samples were examined in the laboratory for the required fixative check and/or fixative/microtube/label replacement. Following these important checks, the muscle samples were stored at  $-20^{\circ}\text{C}$  with the corresponding label, e.g. DFA-SCM-L-001-Ma, DFA-SCM-L-001-Mb and DFA-SCM-L-001-Mc.

The otoliths were placed in small petri dish containing deionized water for further rinse and to remove any biological residues still adhering to the otolith surface. Hence, the otoliths were dried for 24/48 hours and then stored at room temperature, by pairs or single, in plastic 2ml labelled (e.g. DFA-SCM-L-001-O) tubes.



## 5. RESULTS

The BFT biometric data and biological samples were collected from fish caught during the 2020 purse seine fishing season in the following fishing grounds of the Mediterranean Sea:

- South-Central Mediterranean Sea (SCM).
- South Tyrrhenian Sea (STS).

Table 2 shows the statistics of the total number of fish that were sampled within this project and includes all the otoliths extracted from the fish sampled, regardless the status of the otoliths (whole, partially broken or completely broken).

Table 3 shows the statistics of the number of fish that corresponds to the valid and complete sets of biological data collected within this project and includes only the valid and complete sets of data (RWT, SFL, CFL, LD1, Sex, Muscles in duplicate and 1 or 2 whole Otolith/s) for each fish sampled.

The size (RWT, SFL, CFL and LD1) frequency distribution (Fig. 14) and the sex (male/female) frequency distribution (Fig. 15) of the valid sets of data are also provided. Table 4 shows some additional information about the BFTs that have been sampled within this project, e.g. eBCD number, number of the farming cage from where the fish were sampled, the total numbers of samples, the number of valid sets of data and the daily sampling efficiency.

**Table 2.** Overall biometric sampling table related to the sampling activities carried out at FF for the fish caught in the SCM and at MML for the fish caught in the STS.

Area	SCM	STS	Total
Farms	FF	MML	2
Number of fish sampled	367	381	748
SFL range (cm)	141-275	102-344	102-344
SFL average (cm)	218.8	207.9	213.2
CFL range (cm)	160-295	135-283	135-295
CFL average (cm)	239.8	228.8	234.2
LD1 range (cm)	45-85	42-81	42-85
LD1 average (cm)	65.9	64.2	65.0
RWT range (kg)	61-470	58-442	58-470
RWT average (kg)	253.6	227.9	240.5
Sex (M/F)	203M/164F	149M/232F	352M/396F
Muscle sample (Ma)	333	339	672
Muscle sample (Mb)	333	339	672
Muscle sample (Mc)	284	37	321
Otolith/s	325	332	657





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**Table 3.** Biometric sampling table related to the valid sets of data obtained during the sampling activities carried out at FF for the fish caught in the SCM and at MML for the fish caught in the STS.

Area	SCM	STS	Total
Farms	FF	MML	2
Number of fish sampled	308	326	<b>634</b>
SFL range (cm)	148-275	136-344	<b>136-344</b>
SFL average (cm)	221.0	209.8	<b>215.3</b>
CFL range (cm)	160-295	169-283	<b>160-295</b>
CFL average (cm)	241.8	230.7	<b>236.1</b>
LD1 range (cm)	46-85	49-81	<b>46-85</b>
LD1 average (cm)	66.3	64.4	<b>65.3</b>
RWT range (kg)	100-470	100-440	<b>100-470</b>
RWT average (kg)	260.0	232.1	<b>245.6</b>
Sex (M/F)	175M/133F	132M/194F	<b>307M/327F</b>
Muscle sample Ma	308	326	<b>634</b>
Muscle sample Mb	308	326	<b>634</b>
Muscle sample Mc	261	157	<b>418</b>
Otolith (1)	75	81	<b>156</b>
Otolith (2)	233	245	<b>478</b>
Valid sets	308	326	<b>634</b>



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**Table 4.** Additional information about the BFTs that have been sampled within this project, e.g. cage number, the relative eBCD number and the number of fish sampled from each cage. Daily sampling efficiency is also indicated.

Farm	Reefer	Farming Cage Number	BCD	Number of samples collected	Number of valid set	Daily Sampling Efficiency
FF	Gouta Maru	EU.MLT.002.FF	IT20900798-CG01	20	12	60.0%
		EU.MLT.003.FF	IT20900798-CG02	31	28	90.3%
		EU.MLT.004.FF	DZ20900001	61	49	80.3%
		EU.MLT.006.FF	IT20900798-CG03	26	23	88.5%
		EU.MLT.008.FF	TN20900012	37	26	70.3%
		EU.MLT.009.FF	DZ20900004-CG01	39	31	79.5%
		EU.MLT.010.FF	DZ20900002	85	80	94.1%
		EU.MLT.011.FF	TN20900013	68	59	86.8%
Subtotal		8	6	367	308	83.9%
MML	Lady Tuna	EU.MLT.007.MML	FR20900716-CG01	11	6	54.5%
		EU.MLT.010.MML	IT20900930-CG01	27	14	51.9%
			IT20900917			
		EU.MLT.020.MML	IT20900903-LT01-CG01	99	83	83.8%
			IT20900888-LT01-CG01			
		EU.MLT.021.MML	IT20900860-LT01-CG01	140	129	92.1%
			IT20900872-LT01			
			IT20900903-LT01-CG02			
		EU.MLT.022.MML	IT20900916	104	94	90.4%
			IT20900930-CG02			
Subtotal		5	8	381	326	85.6%
Total		13	14	748	634	84.8%





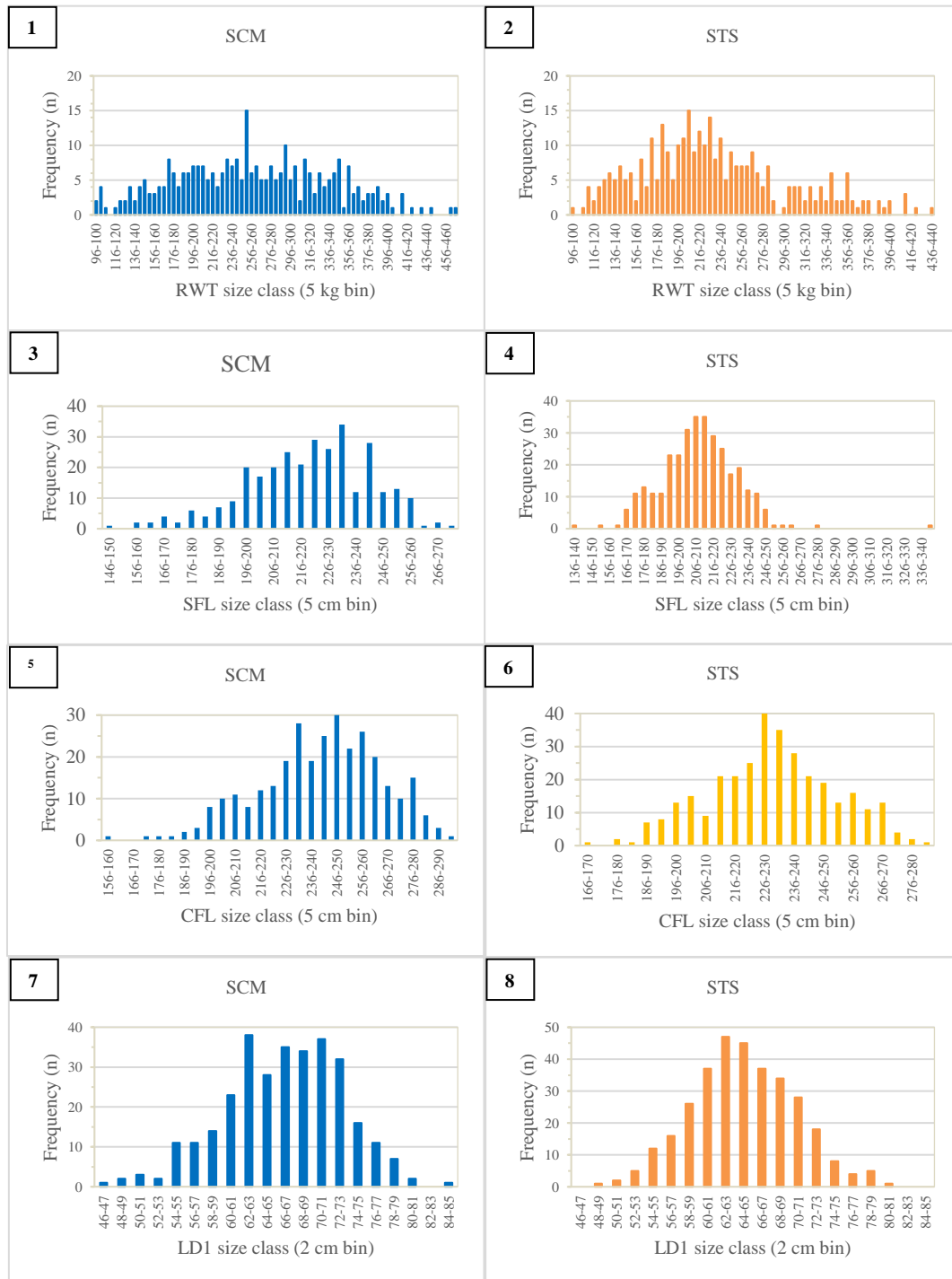
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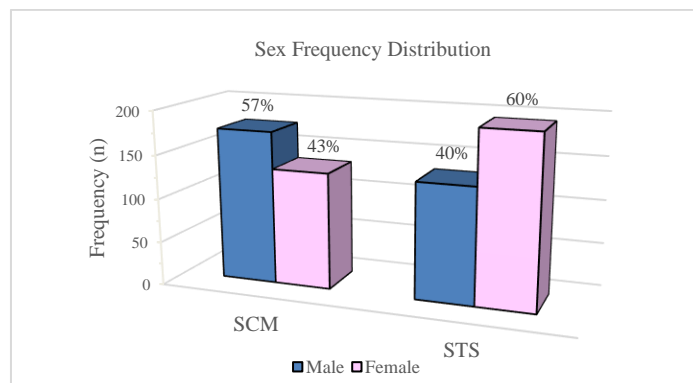
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**Figure 14.** Size frequency distribution of the collected valid sets of data. **1-2** show the RWT frequency distribution, **3-4** show the SFL frequency distribution, **5-6** show the CFL frequency distribution and **7-8** show the LD1 frequency distribution for the valid sets of data of the fish caught in the SCM and STS catching area.



**Figure 15.** Sex (male/female) frequency distribution of the collected valid sets of data for the fish caught in the SCM and STS catching area. The number of fish sampled are indicated on the vertical axis while relative percentage is shown on top of each histogram.

## 6. CONCLUSION

The contract agreement between ICCAT and DFA-MAFA of Malta was signed with the aim to collect biometric data (RWT, SFL, CFL, LD1 and sex determination) and biological samples (otoliths and muscles) from 600 adult BFTs caught in two different fishing grounds of the Mediterranean Sea (SCM and STS) from two different Maltese tuna farms (FF and MML). This project was successfully accomplished in 16 sampling days at sea, from mid-October to mid-November 2020, and according to the terms of the contract from the 748 adult BFTs sampled from two different Maltese tuna farms, only 634 fish were considered the valid sets of data, i.e. where biometric data (length, weight and sex), muscle samples (duplicate) and otoliths (pair or single) were successfully collected. A wide size (weight and length) frequency distribution was also achieved as the BFTs sampled varied in size from 136 cm to 344 cm in SFL and from 100 kg to 470 kg in RWT with an overall sex ratio of 48% male fish sampled and 52% female fish sampled.

Outstanding cooperation was provided by both farm operators and the crew of the reefers during the daily sampling activities, despite the current COVID-19 pandemic issues.

## 7. DIFFICULTIES

The adverse weather conditions experienced at the beginning of October 2020 can be mentioned as the main difficulty encountered during this sampling project because of the delay caused on the initial sampling plan.

The otolith/s extraction was challenging and failed only in some (15%) cases mainly due to the harvesting methodology adopted by the divers of the farm operator, specifically, the central position of the shot in the head to stun the fish prior the removal from the cage: in these cases, the otoliths were found broken/damaged or completely missing.



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Another difficulty encountered was due to the current COVID-19 pandemic situation which caused the desertion of Mare Blu tuna farm from the agreed sampling collaboration, but fortunately another farm operator, MML, was readily to participate with the DFA-MAFA and OCEANIS Srl in this sampling project.

## **8. ACKNOWLEDGMENTS**

This work has been carried out under the ICCAT Atlantic-Wide Research Programme for Bluefin Tuna (GBYP), which is funded by the European Union, several ICCAT CPCs, the ICCAT Secretariat, and other entities (see <https://www.iccat.int/gbyp/en/overview.asp>). The content of this paper does not necessarily reflect ICCAT's point of view or that of any of the other sponsors, who carry no responsibility. In addition, it does not indicate the Commission's future policy in this area.