

## BLUEFIN TUNA BIOLOGICAL SAMPLING

A. Zambetti<sup>1</sup>, P. Pignalosa<sup>1</sup>, F. Lombardo<sup>2</sup>

### SUMMARY

*The objective of a sampling activity is generally to contribute improving the understanding of key biological and ecological processes of a specimen, to support alternative options for fisheries management. This sampling activity was planned with the final aim of optimizing and standardizing the synergic integration of a low-cost sampling activity within, and without hindering, the regular commercial harvesting procedures of the farm operator.*

*With this approach, from 1,616 fish harvested during regular harvesting operations, a total of 144 fish were selected and sampled, of which only 120 were considered as valid sets of data, i.e., where a complete set of biometric data (length, weight and sex) and biological samples (muscle, otoliths and first dorsal spine) were successfully collected from the same fish. The 120 fish sampled varied in size from 120 cm to 264 cm in length and from 43 kg to 411 kg in weight with a sex ratio of 47% male and 53% female. The daily sampling rate achieved during this activity was 9% of the daily harvested fish, with an efficiency of 7.5% and efficacy of 83.3%.*

### KEYWORDS

*Bluefin tuna, sampling, biometrics*

<sup>1</sup> Director of OCEANIS Srl, Via Marittima 59, Ercolano (NA), Italy. Email: [oceanissrl@gmail.com](mailto:oceanissrl@gmail.com).

<sup>2</sup> Chief Scientific Officer at the Department of Fisheries and Aquaculture - Ministry for Agriculture, Fisheries and Animal Rights - Fort San Lucjan, Triq Il-Qajjenza, Marsaxlokk, Malta. Email: [francesco.lombardo@gov.mt](mailto:francesco.lombardo@gov.mt).

## 1. Introduction

Periodically sampling activities of commercially exploited species such as the Atlantic Bluefin tuna (BFT) are a major source of monitoring data for understanding key biological and ecological processes, tracking population trends, and evaluating alternative options for fisheries management. However, most of these scientific activities are constrained by funding availability, uncomfortable working conditions, ongoing of important commercial operations which cannot be hindered, and time, and have to make a trade-off between precision and accuracy of the sampling activity itself. To this end, the following sampling activity was planned with the final aim of optimizing and standardizing the synergic integration of a low-cost scientific activity within, and without hindering, the regular commercial harvesting procedures of the farm operator.

In February 2023, the Maltese tuna farm Fish and Fish Ltd kindly offered the opportunity to carry out a sampling activity on the remaining fish to be harvested for that season. To this end, OCEANIS Srl, with the support of the Department of Fisheries and Aquaculture (DFA) within the Ministry of Agriculture, Fisheries and Animal Rights (MAFA), promptly prepared a sampling plan aimed at collecting all the necessary samples from these fish and contribute therefore to the broader ICCAT-GBYP ATLANTIC-WIDE RESEARCH PROGRAMME FOR BLUEFIN TUNA.

The planned sampling activity allowed the successful collection of 120 valid sets of data from farmed BFTs which have been caught in the Tyrrhenian Sea by Italian and Algerian Purse seine vessels during the 2022 BFT fishing season. The fish were subsequently caged into the Maltese tuna farm Fish and Fish Ltd for their maintenance and fattening (June 2022) until harvest which occurred in February 2023.

This sampling plan was intentionally prepared in order to achieve a significant number of fish sampled in a short time and in synergy with the farm operator and the crew of the reefer, and to cover the sampling of a wide size (weight) distribution of the BFTs within the harvesting operation.

The sampling activity was planned so that, in 5 working days, from the 19<sup>th</sup> to the 23<sup>rd</sup> of February 2023, a sampling target of at least 5% of the daily harvested fish would have been achieved by the sampling team composed of 6 highly skilled professionals, working onboard the processing reefer engaged by the farm operator.

## 2. Materials and Methods

The scheme for the Biological Sampling design provided by ICCAT GBYP (Appendix 2 - Last revised: March 2022 - SAMPLING PROTOCOLS FOR THE GBYP BIOLOGICAL SAMPLING) was used and strictly adopted for the data collected as indicated in table 1.

**Table 1.** Biometric data and biological samples collected from farmed BFTs.

<b>Biometric data</b>	<b>Biological data</b>
Straight Fork Length (SFL) in cm	Muscle samples (in duplicate)
Curved Fork Length (CFL) in cm	Otoliths (pair or single)
Length to the first Dorsal (LD1) in cm	First Dorsal Spine
Round weight (RWT) in kg	
Sex determination (M/F)	

Briefly, a regular BFT harvesting process involves few procedural and subsequent steps:

1. Shooting of the fish inside the farming cage by scuba divers;
2. Transport of the fish to the reefer by a transport vessel;
3. Weighing and measuring of the fish;
4. Fish processing phases by the reefer's crew:
  - a. Cutting off of the head and the tail;

- b. Gill-gutting the fish;
- c. Cutting the fish into loins or fillets;
- d. Further cutting from the head and pectoral collar;
- e. Freezing of the fish products;
- f. Cleaning of the deck prior of the arrival of the next group of fish.

During step 3, the fish was selected by the deployed scientific team and two plastic numbered tags were applied on the fish: one tag was applied on the first dorsal spine and another tag was applied on the head to easily track both fish body parts during the following processing steps. CFL and LD1 were determined using a flexible measuring tape during the tagging process. SFL, instead, was taken using a customised calliper. All length measurements were taken to the nearest cm. RWT was noted directly using the weighing system of the reefer.

Subsequently, during step 4b, the sex determination of the fish was macroscopically assessed and when the processing procedure was completed by the crew of the reefer (step 4c-d), the First Dorsal Spine was extracted from the remaining tuna skeleton using a sharp knife. Soon after, the heads were transferred to a dedicated area of the reefer where a field laboratory facility was set up to conduct the required biological sampling such as Otolith's extraction and muscle samples.

One-by-one, the heads were 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 otoliths were found just below the rear of the brain and the sagittal otoliths were removed from the left and right otic cavities, using small forceps. Otoliths were extracted with the otolith membrane still attached to them which was instantly and gently removed. The otoliths were rinsed with deionised water and stored in in 2ml labelled (O) microtube filled with deionized water.

When the otolith/s were successfully extracted, two replicates of muscle samples were collected from the head, fixed in 96% Ethanol, stored in two 5ml labelled (Ma and Mb) tubes and kept at 4°C inside a cooler bag.

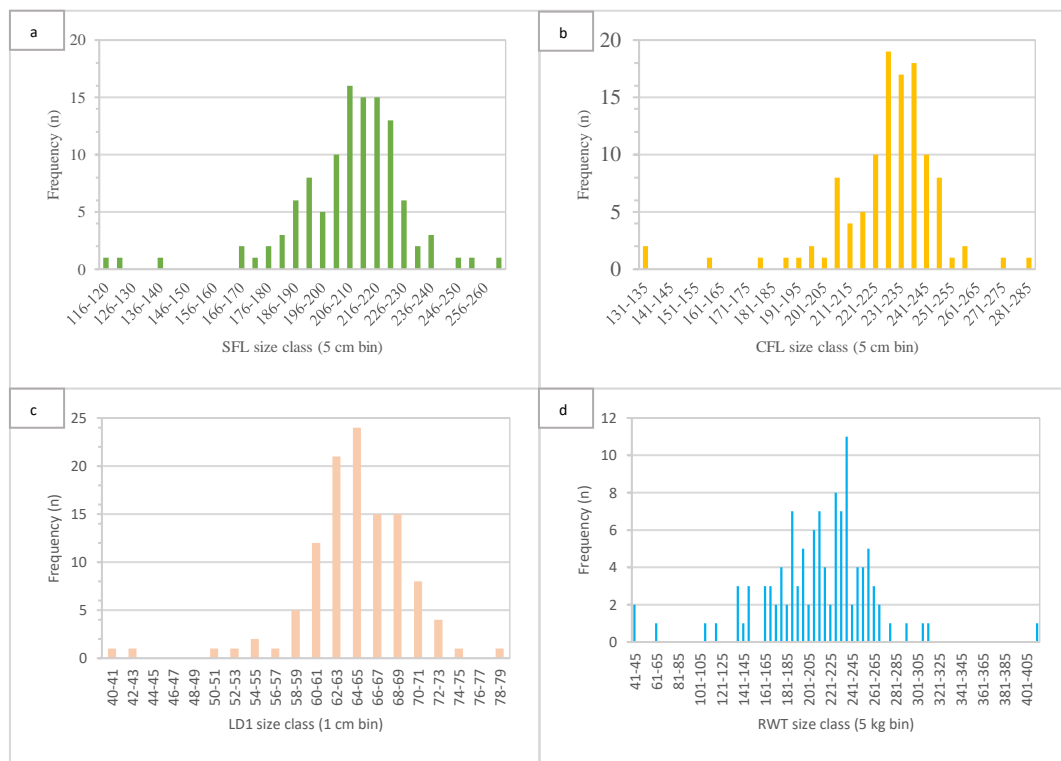
At the end of each sampling day, the collected biological samples were examined in the laboratory provided by MAFA-DFA, for the required fixative check and/or fixative/microtube/label replacement. Following these important checks, the muscle samples were stored at -20°C with the corresponding label (e.g., OCE-TY-L-001-Ma and OCE-TY-L-001-Mb). The otoliths instead, were placed in a 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 at room temperature for 48 hours and then stored at room temperature, by pairs or single, in 2ml labelled (e.g., OCE-TY-L-001-O) microtube.

### 3. Results

The statistics (number, average and range) of the data collected within this voluntary sampling activity are showed in table 2 and the sizes (RWT, SFL, CFL and LD1) frequency distributions are showed in figure 1.

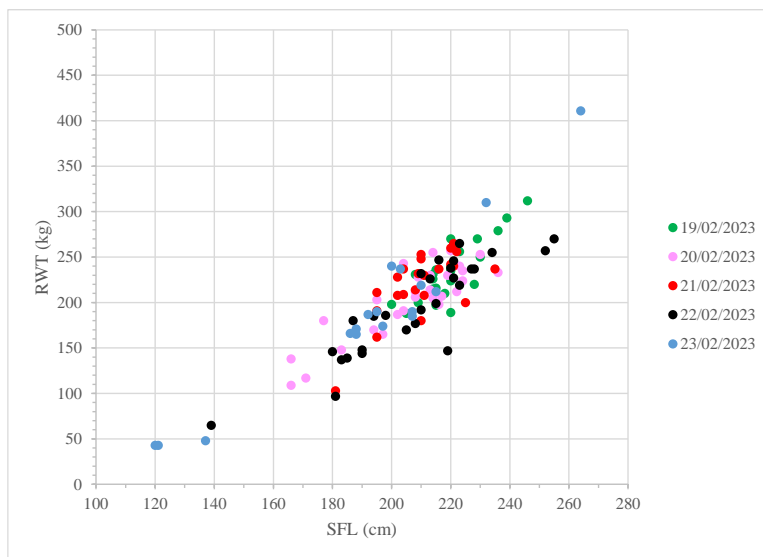
**Table 2.** Statistic table of the data collected during the sampling activities carried out at Fish and Fish Ltd.

Number of fish sampled	120
SFL range (cm)	120-264
SFL average (cm)	208.0
CFL range (cm)	128-312
CFL average (cm)	226.3
LD1 range (cm)	40-78
LD1 average (cm)	63.8
RWT range (kg)	43-411
RWT average (kg)	208.7
Sex (M/F)	57M/63F
Muscle sample Ma	120
Muscle sample Mb	120
Spine	120
Otolith	120



**Figure 1.** Size frequency distribution of the collected data. a) SFL frequency distribution; b) CFL frequency distribution; c) LD1 frequency distribution; d) RWT frequency distribution.

Figure 2 shows the efficacy of the daily sampling activity which covered a wide size distribution of the daily harvested fish, while table 3 shows the range of data of the sampled fish.



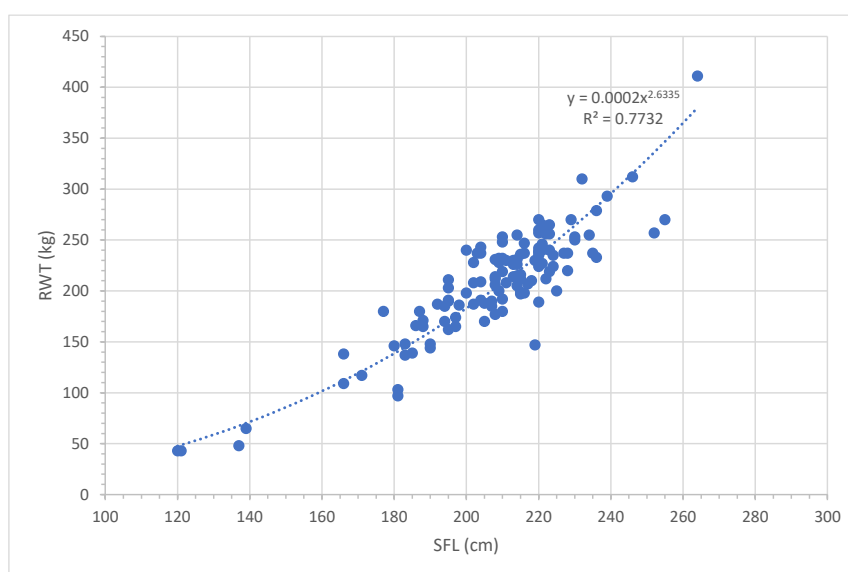
**Figure 2.** Daily size (SFL-RWT) frequency distribution of the collected data.

**Table 3.** Statistic table of the data collected during the sampling activities carried out at F

Sampling date	Fish sampled (n)	RWT (kg)		SFL (cm)	
		Min	Max	Min	Max
19/02/2023	24	188	312	200	246
20/02/2023	28	109	257	166	236
21/02/2023	23	103	265	181	235
22/02/2023	28	65	270	139	255
23/02/2023	17	43	411	120	264

Figure 3 presents the SFL-RWT relationship for this dataset:

$$\text{RWT} = 0.0002 * \text{SFL}^{2.6335} \quad (R^2 = 0.7732).$$



**Figure 3.** SFL RWT relationship ( $\text{RWT} = 0.0002 * \text{SFL}^{2.6335}$ ) for the fish sampled.

Additional information, such as origin of the fish, catching, farming and harvesting data of the BFTs sampled within this voluntary activity is indicated in Table 4 and Table 5.

**Table 4.** Cage number, eBCD number, catch and harvesting date and the farming days of the BFTs sampled.

Cage Number	BCD Number	Catch date	Harvesting date	Farming time
EU.MLT.011.FF	IT22901048	31/05/2022	19/02/2023	264
			20/02/2023	265
			22/02/2023	267
EU.MLT.003.FF	IT22901054	14/06/2022	21/02/2023	252
EU.MLT.016.FF	IT22901048	31/05/2022	22/02/2023	267
EU.MLT.009.FF	DZ22900012	18/06/2022		249
	DZ22900013	20/06/2022	23/02/2023	248
EU.MLT.013.FF	DZ22900012	18/06/2022	23/02/2023	250
	DZ22900014	22/06/2022		246

**Table 5.** Daily harvesting and sampling data.

Date	Cage number	Harvested fish (n)	Sampled fish (n)	Sampling rate (%)	Valid sets (n)	Daily efficiency (%)	Daily efficacy (%)
19/02/2023	EU.MLT.011.FF	294	30	10.2	24	8.2	80.0
20/02/2023	EU.MLT.011.FF	324	30	9.3	28	8.6	93.3
21/02/2023	EU.MLT.003.FF	303	28	9.2	23	7.6	82.1
22/02/2023	EU.MLT.011.FF	340	33	9.7	28	8.2	84.8
	EU.MLT.016.FF						
23/02/2023	EU.MLT.009.FF	355	23	6.5	17	4.8	73.9
	EU.MLT.013.FF						
Total (n)		1,616	144	9.0	120	7.5	83.3
Daily average		323	29		24		

#### 4. Conclusions

OCEANIS Srl and MAFA-DFA, thanks to the kind opportunity and cooperation offered by the farm operator Fish and Fish Ltd, conducted this voluntary sampling activity in February 2023, with the aim to collect valid sets of scientific data from farmed Bluefin tuna. Specifically, the proposed sampling approach achieved a sampling rate of 9% of the fish harvested by the farm operator, with an efficiency of 7.5% and an accuracy of 83.3%.

This voluntary activity was carried out at a very low cost, and it is intended to be considered as a kind contribution to the GBYP tissue bank and in general to the scientific community, in order to improve the efficiency of basic data collection process and contribute therefore improving the understanding of key biological and ecological processes of the Atlantic Bluefin tuna.

No special difficulties were encountered by the sampling team in carrying out this activity onboard the processing reefer, as the experienced and skilled staff coupled with the cooperation of all the parties involved, played a crucial part in maximising the output. The adopted approach is recommended for all concerned CPCs due to its efficiency.

#### 5. Acknowledgments

The scientific staff deployed for this sampling activity is grateful to Cap. Paolo Pignalosa and OCEANIS Srl for have planned, organised, financially supported, and coordinated this activity. A special word of gratitude goes to Mr Bjorn Callus (DG at MAFA-DFA) for have officially authorised this activity and for the unconditioned technical and logistic support and assistance during the entire duration of this activity. The scientific staff, OCEANIS Srl and MAFA-DFA eventually acknowledge special thanks to Dr. Saviour Caruana (CEO at Fish and Fish Ltd) and the crew of Kenta Maru for providing free access to the facility allowing a professional and efficient work.