REPORT OF BIOLOGICAL SAMPLING IN ATLANTIC BLUEFİN TUNA (*Thunnus thynnus* (Linnaeus,1758)) IN AKUA-GROUP FARM, Çeşme, İzmir, TURKEY

(AKUA-GROUP SU URUNLERI A.S.)

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Contents

Abstract

The study was conducted between 24 January 2020 and 7 March 2020 for the sampling of the bluefin tuna sagittal otolith, dorsal fin spine, and muscle tissues in the Aqua Group Inc. harvesting process in cages located in the Gerence Bay (Aegean Sea) - Çeşme, İzmir, Turkey. From a total of 1690 specimens harvested in the period, 463 fish between 141-296 cm and 57-380 kg CFLs and weights respectively were being able to mark and examined in the process. By the end of the study, whereas the sampling of dorsal spines and tissues were done without any missing from all fishes, otoliths could be obtained only 343 specimens.

List of Illustrations

1. Introduction

The purpose of the study to collect various biological tissue samplings from the bluefin tuna (*Thunnus thynnus*) specimens in the period of 2019-2020 harvesting period according to the agreement between the **INTERNATIONAL COMMISSION FOR THE CONSERVATION OF ATLANTIC TUNAS (ICCAT)** and **AKUA-GROUP SU URUNLERI A.S.** which was signed as a short term contract in May 2019.

In the contract's Annex 1 section, it was pointed out that the contractor AKUA-GROUP SU URUNLERI A.S. will obtain six different data and material (namely, straight fork length (SFL) in cm, length to the first dorsal (LD1) in cm, total weight in kg, sex identification, sagitta otoliths, whenever possible and tissue sampling for genetic studies) from 300 bluefin tuna specimens that were going to catch in 2019 summer period and begin for fattening in cages.

The study was carried out with Akua-Group employees;
İrfan ANBAR (general manager),
R. Cenk YURTTAŞ (engineer and manager),
Gökhan GÖKÇEK (engineer and diver)
Okan TABAOĞLU (Computer staff)

2. Material and Methods

2.1. Field studies

All the bluefin tuna specimens used in this study were caught by purse seiners in the summer 2019 fishing season in the eastern Mediterranean Sea around Cyprus and transferred to the transport cages and towed to the facility's fattening cages located in Gerence Bay, Çeşme, İzmir, Aegean Sea. Because the study as a part of the Bft Growth Study in Farm (ICCAT GBYP 09/2019-e), the blufin tuna used here were chosen from the cage "LBY AKU 2019 001".

The samples from specimens were obtained by following the procedure of ICCAT Biological Sampling Protocol Phase 8.

For the purpose, firstly, fish were marked from upper jaws or operculum by using water-resistant marker and photo or thick acetate paper (approximately 6×6 cm) immediately after harvesting on the board of the transferring ship. Besides that, most fishes were weighted, and their curved fork lengths (CFL) measured and recorded in there too. Meanwhile, the 1st dorsal-fin spines were removed by a knife from the base of the fin, including condyle and placed in labeled envelopes and muscle tissues cut from caudal peduncle with sterile surgical blades that are prepared before and changed for every fish. The muscle tissues were fixed in 96% ethanol in 20 ml plastic tubes on the board too.

The marked fish then were transported to the Japanese tuna fishing vessel by transferring ship, and the head of the marked fishes collected in separate gunny bags from other ones and returned after the end of the harvesting day. The heads were stored in ice-filled containers on the land until the dissection for removing otoliths.

If possible (sometimes the harvesting has continued more than one day), the next day, the marked tuna heads were placed on a metal desk on the land. By using a manual or an electrical saw (Bosch® GFZ 16-35), a frontal section performed just top of the spinal cord, and sagittal otoliths were removed from the left and right otic cavities with a plastic or ceramic tip laboratory forceps. The removed otoliths were placed in ice cup trays filled with pure water and cleaned carefully from the remaining tissues and after drying stored in plastic eppendorf tubes temporarily.

2.2. Laboratory studies

Dorsal fin spines have been examined primarily because the epidermis covered them hard to remove if dried and to clean the spine from its residues, not easy. After cleaning, fin spines placed in new envelopes and labeled in the laboratory and stored until shipment.

Then we divided muscle tissues into two parts, which was a requirement for the protocol. For every sampled fish, their muscle tissues taken from the 20 ml cups outside and cut by a surgical blade again and fixed with a fresh 96% ethanol in 5 ml plastic cups in two parts. After labeling them as “a” and “b,” they were ready for shipment.

The sagittal otolith of bluefin tuna specimens were taken out from eppendorf tubes and put in Petri dishes filled with 0.1% nitric acid and waited for 5 minutes as indicated in the protocol and rinsed with deionized water. They have been dried in room temperature for at least 2-3 days before placed and labeled them in new tubes.

3. Results

3.1. Sample size, length, and weight distribution

The sampling studies were started on 24 January 2020 and ended 7 March 2020. In the period, a total of 463 (269 medium and 194 large) specimens examined in 9 harvesting days (Table 1). We have used more than 300 samples owing to maximize the number of otoliths, which is severely affected by the shot in the head method in cages when harvesting process.

Table 1. Sampling dates and the number of specimens in size groups. (M: Medium; L: Large)

	M	L
24 January 2020	-	40
25 January 2020	4	5
30 January 2020	78	31
31 January 2020	33	63
1 February 2020	40	7
8 February 2020	2	13
15 February 2020	4	10
22 February 2020	8	5
2 March 2020	100	20
	269	**194**
Total	**463**	

Curved fork lengths (CFL) of all bluefin tunas were between 141-296 cm in the study, and while the size distribution for medium size fish (≤100 kg) was found between 141-180 cm,

large fish (>100 kg) were found in a greater interval to be in 171 to 296 cm (Fig. 1). The weight distribution of the sampled bluefin tuna is given in Figure 2. It is seen clearly that more than half of the specimens (58.1%) take place in a 50 kg interval in 50-100 kg, which comprises medium size fishes. The remaining 41.9% fish consisted of large individuals and weights of them distributed between 101 and 380 kg, and the mean weight was calculated as 223.78 kg.

	Curved Fork Length (cm)				
	N	Min.	Max.	Mean	SD
M	269	141	180	157,26	7,688
L	194	171	296	227,35	30,760

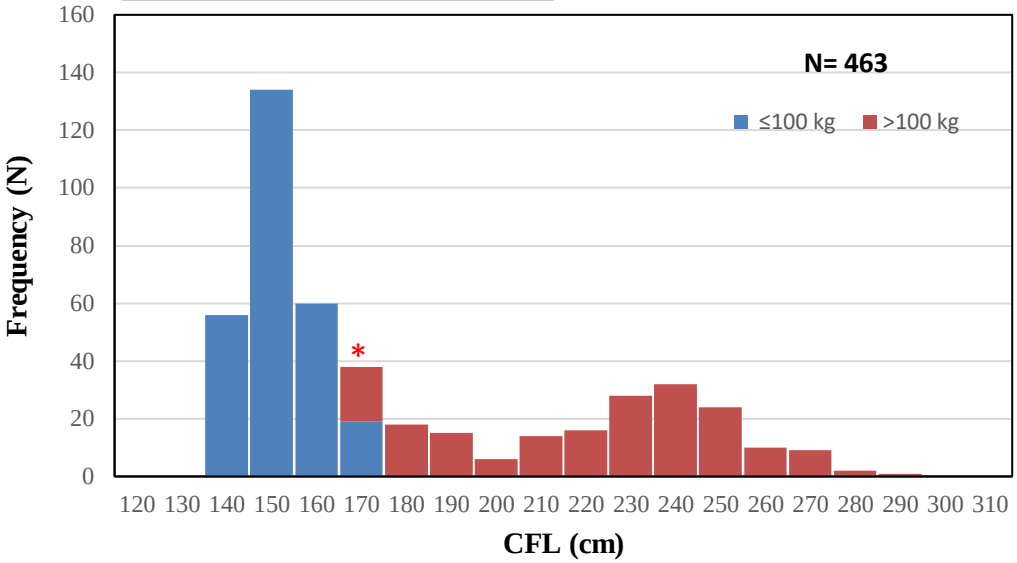

Figure 1. The size distribution of bluefin tuna specimens in the study. (* 170 cm length group includes both medium and large size fish)

	Total Weight (kg)				
	N	Min.	Max.	Mean	SD
M	269	57	100	72,90	10,509
L	194	101	380	223,78	72,607

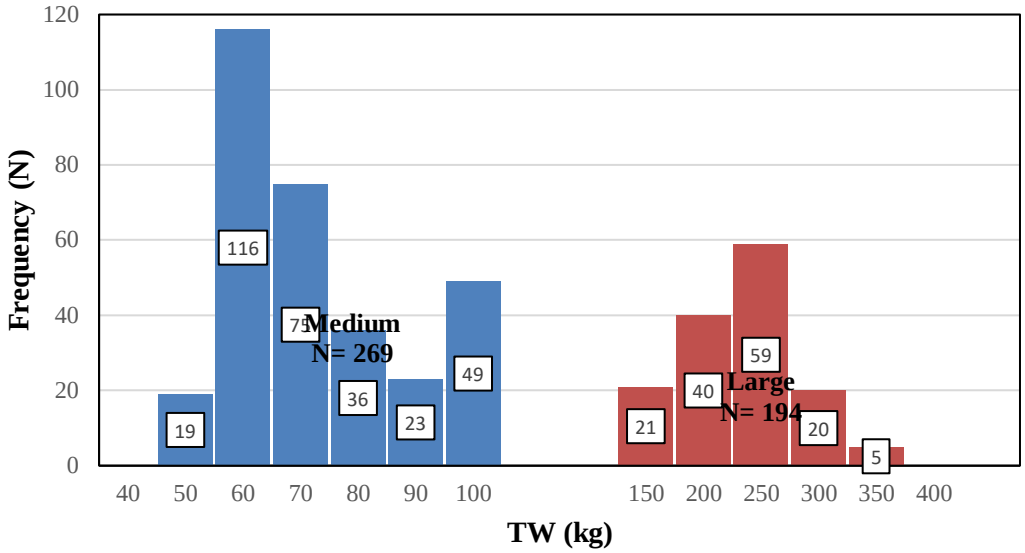

Figure 2. The weight distribution of bluefin tuna specimens in the study.

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3.2. Biological samples

Sagittal otolith

Bluefin tuna sagittal otolith is rectangular to lanceolate in shape, and its shape changes, especially when early growth stages as other fish species (Fig. 3). Its use in fisheries biology mainly for age determination and estimating population differences by shape analysis and to find the origin of hatching areas. Although we have 463 specimens, sagittal otolith of bluefin tuna successfully removed from only 343, which belongs to 215 medium and 128 large size fish. Another limitation is that some of these otoliths not in pairs. As mentioned above, the main reason for this is the harvesting method. If we look at the relationship of the otolith numbers in size groups and the size distribution of fishes, we could observe that in large-size, the missing otoliths were more (32.9%) than as in medium-size (20.8%). It would be because of the difficulty of killing these large individuals by divers, which afforded them make a precise shot to the braincase.

Figure 3. Sagittal otolith pair of a bluefin tuna (CFL: 159 cm; TW: 69 kg)

Dorsal fin spine

In the contract, whereas ICCAT did not demand the collection of dorsal fin spine of the bluefin tuna from AKUA-GROUP, we have collected dorsal fin spines of 425 individuals in harvesting according to the Phase 8 Sampling Protocol (Fig. 4). The number of dorsal spines is 269 and 156 for medium and large sizes, respectively.

Figure 4. Cleaned 1st dorsal fin spine of a bluefin tuna.

Muscle tissue

We achieved to take of the tissue samples from all 463 specimens' caudal fin peduncle and appropriately prepared for shipment to AZTI, as indicated in the sampling protocol (Fig. 5).

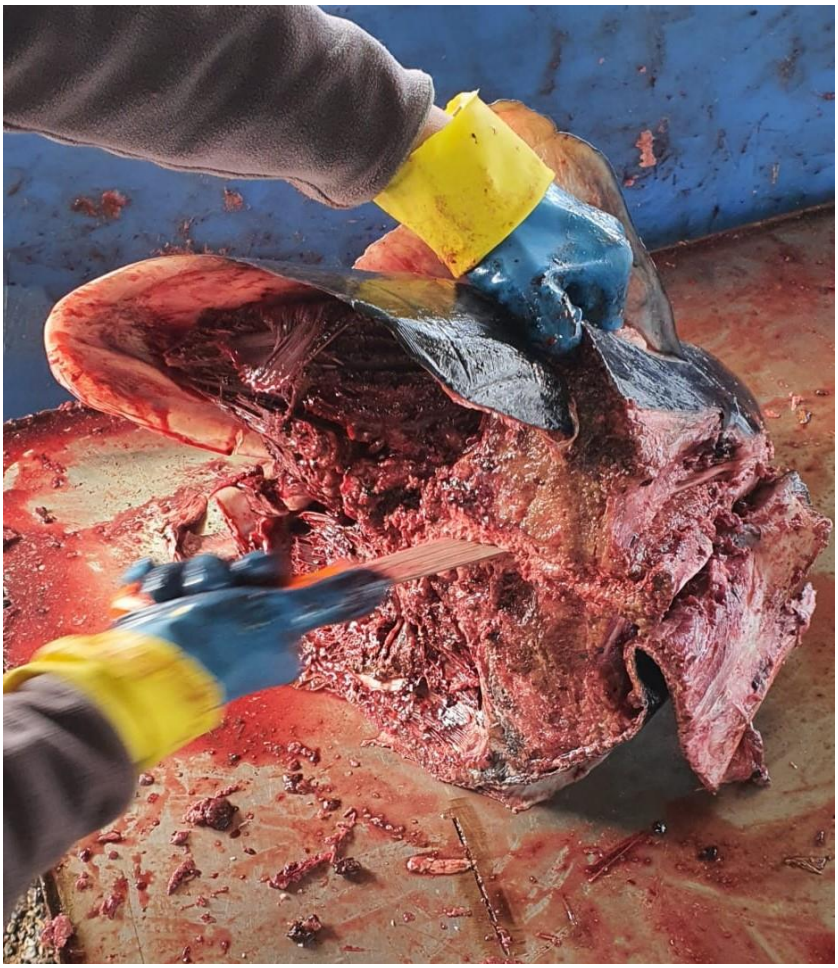
Figure 5. The muscle tissue of a bluefin tuna.

4. Photos from the study

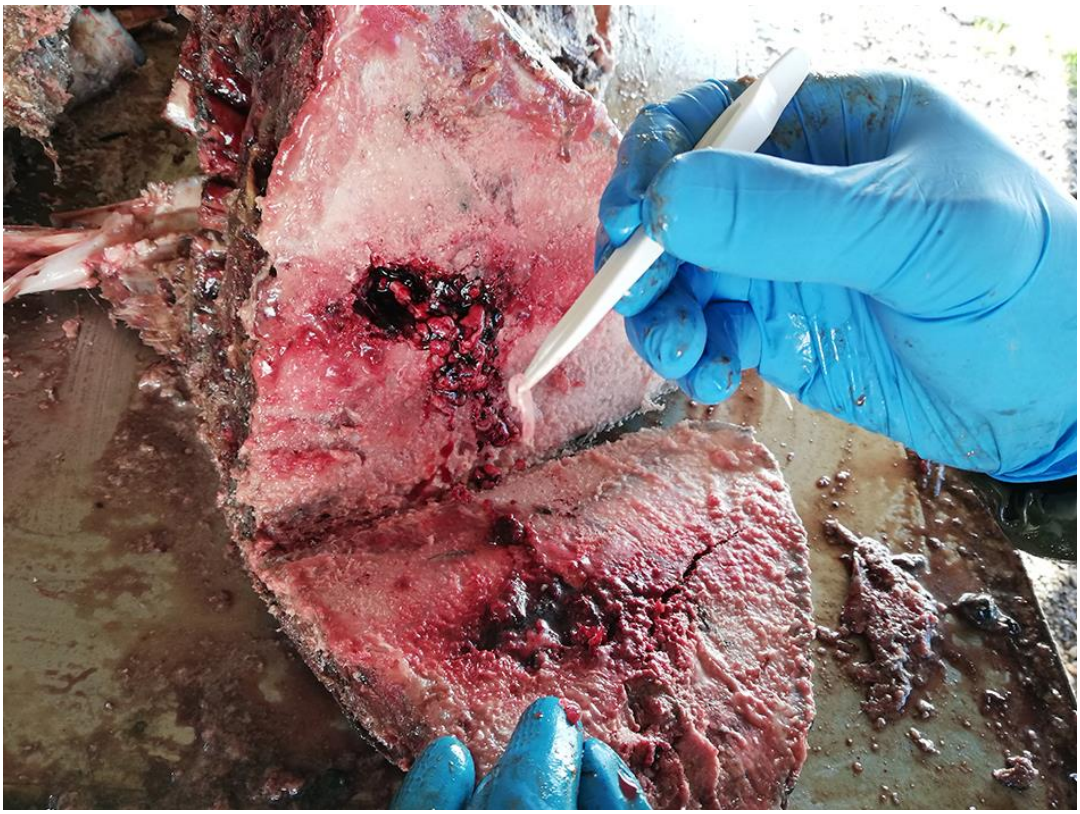
Some materials used in the study period.

A marked bluefin tuna on the board

Dorsal fin spines and muscle tissues were taken mainly on the board.



Bluefin tuna heads were cut by a manual or electric saw on a metal desk.



Sagittal otoliths were removed from the otic canal of the specimens.

Otoliths were cleaned from the gelatinous matrix, covered them, and rinsed with deionized water.

The biological samples (dorsal spine, sagittal otolith and muscle tissue) of the bluefin tuna collected in the study.