

REPORT ON THE CONTRACT FOR BIOLOGICAL STUDIES-SAMPLING FOR ADULTS (ICCAT GBYP - PHASE 8) OF BLUEFIN TUNA IN THE ATLANTIC-WIDE RESEARCH PROGRAMME



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1. BACKGROUND

Among the ICCAT marked objectives for the management in tuna fisheries is "improve biologic and ecologic key processes for the Atlantic Red Tuna" so in 2011 the "Research Programme of bluefin tuna in the Atlantic wide" started collecting information about principal fisheries of this specie (purse seine fishing, bait fishing, longline fishing, trap, hand line fishing and sport fishing).

The 20th of April of 2018 the company Taxon Estudios Ambientales, S.L. received a informative document by ICCAT GBYP 402/2018 in which we were informed about a Call of the Offer ICCAT GBYP 04/2018 about the mentioned Research Programme (ICCAT-GBYP- Phase 8).

In that call the tasks that the chosen contractor must realize to carry the biologic studies were expose. These consist in adult sampling captured in the Balearic sea, southern Tirreno or southern central Mediterranean in the 2018 fishing campaign and fattened in farms until his sacrifice.

With the decision taken by Taxon about present the offer dated 25 of April, the company Tuna Graso, S.A.U., obtained the authorization with serial number ICCAT: ATEU1ESP00001 and register number ICCAR 01, to be able to sample in the tuna fattening installation which this trade have located off the San Pedro del Pinatar coasts, Murcia, Spain, which owns a DPMT concession formed by 5 floating cages 50 m diameter with exploitation authorization from the "Consejeria de Agua, Agricultura, Ganaderçia y Pesca de la Comunidad Autonoma de la Región de Murcia".

The number of copies to sample was about 300 in any of the three fishing areas mentioned before as it is described in the offer. The data collection and sampling method according to the ICCAT GBYP protocol include biometrics, weight, sex, otoliths and tissue sampling for genetic studies.

The commitment between ICCAT and TAXON to bring about the tasks described before is formalized in June if 2018, starting the working preparations in July of the same year, with coordination meetings between Tuna Graso and TAXON workers.

The sampling process started at the beginning of October, at the same moment that the sacrifice tuna fish started when the fattening process has finished. Thus, 9th of October the first sampling day were tried but finally was cancelled because of the sea weather conditions that impede working in the floating cages. This happened in the San Pedro del Pinatar harbour, which is used as port operation base.

Finally, 10th of October when the weather conditions improved, the sampling process started in the freezer vessel "Paloma Reefer" (serial number ICCAT AT000PAN00032)



because tuna fishes were destined to the frozen market. The next sampling day was made on 16th of the same month and, after this moment and because of diverse grounds as the adverse climatologist conditions, company organization and market demand, the planning was modify making very difficult the coordination for sampling due to the necessity to prepare well in advance and rigor all the necessary material for the sampling moment an also for the laboratory sampling processing or the otolith extraction that requires a white room from Frigorificos de Tunidos, S.L., company that belongs to the Grupo Ricardo Fuentes e Hijos", and to which equally belongs Tuna Graso, S.A.U.. The white room is very demanded caused by the activity carried on by "Frigorificos" and so is very difficult the use coordination and that this room it is unoccupied.

This business group owns another trade called Caladeros del Mediterráneo, S.L., with serial number ICCAT: ATEU1ESP00003 and register number ICCAR 03, that exploit in another DPMT concession facing the Cala de El Gorguel, in T.M of Cartagena, occupying a plot in the polygon "Poligono de Acuicultura de El Gorguel" and where has 8 cages 50 m diameter authorized an 2 of 90 m.

Due to the captures of the red tuna copies in the 2018 campaign that was carried out in the Balearic sea and that all the specimens were transported to both facilities, an inquiry was made to the Secretariat of the GBYP ICCAT to obtain the authorization to sample in Caladeros del Mediterraneo installations, so the rest of the sampling days started again 24th of October of 2018 in the Caladeros del Maditerraneo floating cages, in El Gorguel aboard the vessel Paloma Reefer, ending sampling process 14th of January of 2019.

This report collects a full description of the work carried out during the contract and an executive summary of the final report.

2. GEOGRAPHIC LOCATION

Tuna Graso, S.A.U. belongs to the "**Asociación FARM de empresas de acuicultura de la Región de Murcia**", whose members are SERVICIOS ATUNEROS DEL MEDITERRÁNEO S.L., LEBECHE SPAIN, S.L. (I, II y III), PISCIFACTORÍAS ALBALADEJO, S.L. y BLUE & GREEN PISCIFACTORÍAS DEL SURESTE, S.L.U.

TAXON ESTUDIOS AMBIENTALES, S.L. is the Environmental Operator appointed by



the companies mentioned above. Also is the responsible of the realization of the Environmental Vigilance Program following the technical prescriptions and the information included in the Environmental Impact Statements (DIA) issued by the "Dirección General de Medio Ambiente de la Consejería de Agua, Agricultura y Medio Ambiente de la Comunidad Autónoma de la Región de Murcia".

Nowadays in the Murcia region exist two aquaculture polygons, the one in the north of San Pedro del Pinatar formed by four plots in parallel located and engaged for other companies; being the plot D the one that Tuna Graso S.A.U. has occupied with a surface of 640,000 m2 in seafloor whose deep range between 40 and 43 m. They have other 3 plots separated from those as its appreciable in the map from Figure 1.



Fotografía 1. Floating cages property of Tuna Graso, S.A.U. located 4mn far from coast facing the T.M of San Pedro del Pinatar , Murcia, SE from España.



Figura 1. DMPT concession distribution in the Aquaculture Polygon in San Pedro del Pinatar, Murcia, SE de España.



The biocenosis of coastal detrital bottoms is the one dominating the area where the 7 aquaculture facilities are located. These detrital bottoms extend from the lower limit of the *Posidonia Oceanica* meadow, situated between 26 and 31 meters depth. In these depths the sediments have an important biogenic component and the percentage of mud content is very low, as corresponds with an area where the hydrodynamic is usually high. That's way it can be considered as non-mudded detrital sediments that hold a rich and diverse infaunal community dominated by molluscs and polychaetes.

Towards the north and towards the coast the sediments become finer as result of the terrestrial contributions, catalogued as muddy coastal detrital that has an infaunal community slightly less developed. The muddy coastal detrital bursts into the coastal detrital forming tongues, so that, at the border the two communities intercalate.

Along the entire area the alignments of rocky nature outgrow forming abrupt slopes locally named "barras" as result of the concretion of sandy alignments formed by sedimentation following the mainstream, subsidence processes and sea level rise.

In the north of Cabo de Palos we find the most extensive and wide *Posidonia Oceanica* meadow. Beside the one of Calblanque, it is the largest on the Murcia coast, with an area approximately of 5,000 hectares. It extends from Cabo de Palos to just beyond the province of Alicante, from where it continues toward Guardamar where the influence of the Segura River makes it disappear. The main determinant factor of the wide distribution of the *Posidonia Oceanica* meadow in this area is the width of its continental shelf (ranging from 15, 5 km in Cabo de Palos to 31 km in the provincial boundary) and, therefore, its soft slopes (< 3%). The meadow that covers from the Levante of Cabo de Palos beach till the Mojón Beach, in addition to the extension it is characterized by its high degree of structural development (biomass and cover) and also by its lower limit that reaches a great depth (approximately 31m).



The aquaculture polygon of El Gorguel is located in front of an small beach with the same name, between the Bahia de Portman and the Cabo del Agua, T.M of Cartagena, Murcia, SE from Spain. It is a coast formed by cliffs orient to South with an accused slope and strong currents regime due to the proximity of Cabo de Palos.

In this polygon coexist four plots where there are other companies located and Caladeros del Mediterráneo is one of this, as it is appreciable in the map below.



Fotografía 2: Aquaculture polygon El Gorguel, Cartagena, Murcia, SE from España



Figura 2.DPMT concession map in the Aquaculture Polygon in El Gorguel, in Cartagena, Murcia, SE of España.

3. MATERIAL AND METHODS

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3.1. SAMPLING CHRONOLOGY

During the months of September and October, samples of the slaughtered bluefin tuna for the "fresh" and "frozen" market were collected. In the table below the type of samples collected and its sampling dates can be consulted.

Sampling dates	Sampling type	Market type	Number of fish sampled
09/10/18	Suspended sampling		
10/10/18	Biometry, spine , muscle, sex	Frozen	47
11/10/18	Extraction of otoliths		24
12/10/18	Extraction of otoliths		23
16/10/18	Biometry, spine , muscle, sex	Frozen	52
17/10/18	Extraction of otoliths		20
18/11/18	Extraction of otoliths		32
24/10/18	Biometry, spine , muscle, sex	Frozen	47
25/10/18	Extraction of otoliths		23
26/10/18	Extraction of otoliths		24
06/11/18	Biometry, spine , muscle, sex	Frozen	54
07/11/18	Extraction of otoliths		24
08/11/18	Extraction of otoliths		30
11/12/18	Biometry, spine, muscle, sex. The tuna heads its freezes because the white room was busy.	Frozen	50
27/12/18	Extraction of otoliths		27
28/12/18	Extraction of otoliths		23
20/12/18	Biometry, spine , muscle, sex	Frozen	60
21/12/18	Extraction of otoliths suspended because the heads were lost.		
14/01/19	Biometry, spine , muscle, sex	Frozen	60
15/01/19	Extraction of otoliths		32
16/01/19	Extraction of otoliths		28
			Total number of samples 370

The total number of samples finally was 370. In principle it would be 300.



3.2. METODOLOGHY

A TAXON boat was used to reach the sampling area and access to Tuna Graso working vessels. Prior to start the manoeuvres, TAXON stuff distributed the sampling material over the ship deck.

The sampling and data recording protocols followed where those established by ICCT/GBYP.



Fotografía 3. Taxon boat in the right of the picture and in the left, the maneuvering vessel "Dolfjin" of support and surveillance work. At the bottom of the image the el "Paloma Reefer", the freezer ship.





Figure 3: Freezer vessel "Delame Reefer" and a summer vessel to transmort the tune specimens

• Total weight, in kg



Fotografía 4. Weighing of a specimen on the deck of the freezer vessel.



• **Fork length** (FL), measured in cm with a stainless steel calibre with total length of 300 cm. The calibre measure a maximum of 317 cm.



Fotografía 5. Measured sampling with caliber in the freezer vessel.

• Spine extraction in the deck of a freezer vessel.





Fotografía 6. Spine extraction in the deck of a freezer vessel



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Fotografía 7.Preparation of dorsal spine in the laboratory for further analysis and treatment

• Sex identification





Fotografía 8. Collection of viscera and gonads for "visu" sexing of buefin tuna specimens.

• Sampling tissue for genetic studies on the deck of a maneuvering vessel





Fotografía 9. Sampling process on the deck of the maneuvering vessel.

Otoliths sampling



Fotografía 10. Otoliths extraction in the white room of TG

In order to the otoliths extraction, the tuna fish head are transported to the San Pedro del Pinatar Port and after that to the TG facilities in La Palma (Cartagena).



Fotografía 11: Head cutting with electric chainsaw for the otolith extraction

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Fotografía 12: Otolith extraction following the ICCAT protocol



Fotografía 13. Otoliths without membrane prepared for being conserved.



Fotografía 15. Separation of the otolithic membrane from the otolith before it is washed with deionized water and subsequent immersion in 0.1 %nitric acid for 5^{\prime} according to methodology recommended for Rocker *et al.* (2008)



A total of 370 tunas were sampled. Data of length, weight, otoliths, sex, gonads, dorsal spine and muscle were collected. From 310 of them, with the sampling distribution detailed below:

- The maximum weight of the 370 sampled specimens was 358 kg, the minimum 180 kg, and the average 256,84 kg.
- The maximum length was 260 cm, the minimum 210 cm, and the average 227,78 cm.
- Of the 370 specimens, 306 otoliths samples were obtained. 60 were lost because of a bad management and in 4 specimens there were no otoliths found due to the destruction of the cranial cavity caused by the chainsaw (lupara) projectile
- Of the 370 specimens sampled, the totals were sexed. 213 of them were males and 157 females.
- Gonads were collected from all the specimens, with a maximum weight of 2.807,45 g, and an average of 706,45 g
- The spine and muscle biologic samples were collected from all the specimens.

All these data were entered into the data table (spreadsheet) attached in this report (GBYP data form_2018_TAXON.xlsx), using the model offered by the ICCAT/GBYP Secretariat.

5. DIFFICULTIES

5.1. SAMPLING CONTRAINTS RELATED WITH THE METEOROLOGYCAL CONDITIONS.

As in previous years, more than meteorological adversities this part must be titled "difficulties caused by the quickly decision making". This is about the necessity of fullfil contracts with clients that entails to decide to slaughter or not, waiting for the weather improve that make possible the manoeuvres in the sea and that don't endanger the workers in the farm or in the vessel. This takes 50 people to be waiting expectantly in



their job position (manoeuvre vessels, freezer vessel, sampling workers, trucks drivers...) until the vessel captain and the farm responsible decide what to do, because it is important to remember that the Tuna Graso facilities are 4mn far from coast and without any natural protection against the adverse sea conditions coming from any cardinal point.

5.2. CONSTRAINTS ON OBTAINING COMPLETE AND QUALITY OTOLITHS

About constrains obtaining the otoliths, it should be noted the acquired experience in this sampling process because of this is the third year we do it. In fact, the specimen selection technique has been improved and the chosen were the ones that had the cranial cavity less shattered by the projectile impact when they were uploaded to the freezer vessel, so the results have been more than successfully. Thus the 370specimens, if we don't take into account the 60 heads that wrongly were sent to the flour production, only have not been obtained otoliths in 4 from the total of 310 heads.

Another fact to highlight that has repercussed in the successful sampling results is that the technical worker from Frigorificos de Tunidos has been working with us previous years and have the necessary experience for the chainsaw handling over the cranial cavity.



Fotografía 14: Bluefin tuna specimen raised in the freezer vessel when is appreciable the projectile entrance in the cranial cavity. It is a clean shoot.



5.3. CONSTRAINTS ON OPERATING ON THE DECK OF THE VESSEL.

This year we have had the same constrains and one of them is the high working speed that the deck workers have, also the fact that the collaboration attitude of the crew is not always perfect, so several times he have had to change the specimen because of the lack of time to sample the gonad.

Other difficulties is that not all the specimen are weight separately and it make not possible to chose the head in the best conditions for the future otolith extraction.

Also the weighing cause some constrains, what is tried to solve with the ICCAT observer or the SGPM inspector that is always in the slaughtering vessel. In fact, some times we have had to desist with some specimen.

Not to forget the instability of the deck caused by the cleaning water, blood and fat from tunas that complicate notably the operation capacities. By last, the sharp tools like hooks, electric chainsaw, etc. handle by the crew, what makes it more "exciting".



Fotografía 15. Tuna sampling in the "Paloma Reefer" deck.





Fotografía 16. Gonad and entrails sampling for specimen sex process when it's appreciable the working conditions (water, fat, viscera, knife, etc)

5.4. CONSTRAINTS ON MAKING THE MOST OF RESOURCES.

As it's mentioned in previous sections and as last years happened, on one hand is the quickly decision-making that provoke that after doing the staff and vehicle displacement or boat launching, the sampling process get cancelled and with this, the schedule to the facilities "Frigorificos de Tunidos", what in some occasions induce to stop working to continue two hours later. In that case, the otoliths extraction after head sampling process, which were wrongly sent in a freezer truck to the flour production 60 tuna heads as in last years, so the possibility to obtain the otoliths were lost, but not the biometric, weight, gonad, spine and muscle data.



Fotografía 17: "Frigorificos de Tunidos" White room in otholiths extraction process.

5.5. CONSTRAINTS ON SAMPLING TUNAS FOR THE FROZEN MARKET.

As has been explained in previous report, the slaughtered manoeuvre for the specimens destined to the frozen market is not the same from the fresh market. When tuna is destined to frozen market, at first, about 150 specimen are slaughtered in a single manoeuvre, and from that, groups of 30 to 50 arrive to the freezer vessel in one go and must be processed (Carving-cut into pieces) as fast as possible to introduce the tuna loin in the freezing tunnel at -60° C.

This year because of the acquire experience this difficulties exposed has convert in a chance to choose the specimens with the better shoot in the cranial cavity and that has determined in the successful number of otoliths extracted.

As in last years, the situation gets worse owning the difficulty of working on the deck of a vessel with a layer of blood, fat and water running on it, making it a slippery surface that makes movements difficult and dangerous so the chance of falling to the ground increases.

In addition, the crew more or less collaborative attitude and that the deck is very crowded and there were up to five groups of crew members working on board and handling all kind of cutting tools (knives, hooks, chainsaw, etc.), can make difficult the sample



process to the workers.



Fotografía 18: Manoeuvre vessel "Caladeros III" arriving with tuna specimens to the freezer vessel.

In Murcia, 15th of February 2019

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6. ANNEXES

- Tuna Graso authorization to Taxon to sample in the white room "Frigoríficos de Túnidos".



- Sample shipping receipt to AZTI
- Data tables following the ICCAT format.