SHORT TERM CONTRACT FOR BIOLOGICAL STUDIES (ICCAT GBYP 08/2017-1) OF THE ATLANTIC-WIDE RESEARCH PROGRAMME ON BLUEFIN TUNA (GBYP Phase 7)

Final Report

for:

ICCAT

Scientific coordinator:
Dr. Haritz Arrizabalaga (AZTI-Tecnalia)

Pasaia, February 15th, 2018

PARTNERS:

Fundación AZTI-AZTI Fundazioa

IFREMER

Universitá di Genova

National Research Institute of Far Seas Fisheries

AquaBioTech Ltd.

AquaBioTech Ltd.

Texas A&M University

NECTON

GMIT

ISTANBUL UNIVERSITY

SUBCONTRACTORS and COLLABORATORS:

Dr. Isik Oray

IPMA (Dr. P. Lino, Dr., R. Coelho)

Univ. Cagliari (Dr. P. Addis)

CSIC (Centro de Estudios Avanzados de Blanes)

INDEX:

EXECUTIVE SUMMARY:

The main objective of this project is to enhance knowledge about Atlantic bluefin tuna population structure and mixing, but also to focus on age and other important biological dynamics.

During Phase 7, following sampling protocols agreed in earlier Phases, the consortium sampled a total of 1562 bluefin tuna (612 YOY, 3 juveniles, 205 medium sized fish and 742 large fish) from different regions (461 from the East Mediterranean, 54 from the Central Mediterranean, 180 from the Western Mediterranean, 212 from the Strait of Gibraltar, 30 from the Northeast Atlantic, 384 from the Central North Atlantic, and 241 from the North Sea. In total, 3498 biological samples were taken (1552 genetic samples, 935 otoliths and 1011 spines). The number of individuals collected by the consortium represents 159% with respect to the original plan.

The consortium was also tasked to receive, process and store samples from other ICCAT contracts, including farm operators, the Regional Observer Program and other biological sampling groups. At the time of writing this report, the number of samples that arrived from other contracts was 197% with respect to what was planned. Alltogether (considering the samples collected by the Consortium and those that arrived from other contracts), the consortium handled samples from 3533 individuals (612 YOY, 4 juveniles, 367 medium sized fish and 2550 large fish) from different regions (736 from the East Mediterranean, 489 from the Central Mediterranean, 1441 from the Western Mediterranean, 212 from the Strait of Gibraltar, 30 from the Northeast Atlantic, 384 from the Central North Atlantic and 241 from the North Sea. In total, 6358 biological samples were stored in the data bank (2734 genetic samples, 2154 otoliths and 1470 spines).

During Phase 7, a Shiny application has been developed to facilitate the inspection of available samples in the biological sample bank and to aid sample selection following different criteria to help better design future experiments and analyses. The application allows the user to interactively visualize and filter the database of the

biological samples available at the sample bank held at AZTI, and download the data associated to the selection.

Regarding otolith microchemistry, new carbon and oxygen stable isotope analyses were carried out in 50 otoliths of Atlantic bluefin tuna captured off Morocco, to determine their nursery area. $\delta 13C$ and $\delta 18O$ values measured in otolith cores indicated that these samples were dominated by eastern origin individuals. The comparative analysis with previous Phases suggests that important interannual variations in the mixing proportions can be observed in this area, which warrants year to year monitoring. Different methodologies provide alternative views on the level of mixing, so additional methodological research is further encouraged.

In order to check whether any bluefin could have been born outside the assumed spawning season, some (n=20) of the largest Young of the Year individuals collected early in the 2016 fishing season, together with other large individuals collected later in the season throughout different spawning areas of the Mediterranean were analyzed. Otolith microincrement analysis was used to estimate the age (in days) of the YOY individuals, allowing to backcalculate corresponding birthdates. Estimated birthdates ranged between the 3rd of June and the 13th of July, so within the assumed spawning season. Only one fish was estimated to be born in July, with 19 birthdates estimated within June.

In addition, following specific criteria discussed and agreed with the GBYP coordinator and the SCRS chairman, the consortium selected 2000 otoliths to send to Australia for age reading analyses.

All the objectives of the project have been met. The analyses conducted under this and previous Phases contributed important information that is relevant for Atlantic bluefin tuna management, and thus is used in the context of the bluefin tuna stock assessment and Management Strategy Evaluation (MSE) process. A large biological sample bank is available for additional studies, such as the yearly monitoring of mixing rates in key areas.

1. CONTEXT

On June 27th 2017, the consortium coordinated by Fundación AZTI-AZTI Fundazioa, formed by partners Fundación AZTI-AZTI Fundazioa, IFREMER, Universitá di Genova, National Research Institute of Far Seas Fisheries, AquaBio Tech Ltd., GMIT, Texas A&M University, Istanbul University, Universidad de Cádiz and Necton, with subcontracted parties IPMA, University of Cagliari, CSIC and Dr. Isik Oray, presented a proposal to the call for tenders on biological and genetic sampling and analysis (ICCAT-GBYP 08/2017).

This proposal was awarded and the final contract between ICCAT and the consortium represented by Fundación AZTI-AZTI Fundazioa was signed on July 24th 2017.

According to the terms of the contract, a final report (Deliverable nº 5) needs to be submitted to ICCAT by 15th of February, with a full description of the work carried out during the contract period and taking into account the comments provided by ICCAT on Deliverable nº4. The present report was prepared in response to such requirement.

2. SAMPLING

Task Leader: Igaratza Fraile

Participants:

AZTI: Inma Martin, Naiara Serrano, Ainhoa Arevalo, Goreti Garcia, Haritz Arrizabalaga, Naiara Rodriguez-Ezpeleta, Natalia Diaz, Iñaki Mendibil

UCA: Jose Luis Varela, Antonio Medina

NECTON: Antonio Celona

UNIGE: Fulvio Garibaldi

UNICA: Piero Addis

NRIFSF: Ai Kimoto, Tomoyuki Itoh

ABT: Simeon Deguara

ISTA: Saadet Karakulak

CYPR: Isik Oray

IPMA: Pedro Lino, Rui Coelho

The sampling conducted under this project follows a specific design, aimed primarily at contributing to knowledge on population structure and mixing. As such, the sampling conducted under this project is independent from other routine sampling activities for fisheries and fishery resources monitoring (e.g. the Data Collection Framework). Some of the sampling activities included in this report were conducted under other GBYP contracts and agreements, including alternative contracts for biological samplings in regions different to those sampled by this consortium, contracts with farms to sample in their premises, and agreements with the Regional Observer Program (ROP) to obtain biological samples as part of their activities.

These other contracts required that the samples be sent to AZTI to be merged within the biological tissue bank handled within this contract. Thus, the sampling protocols and forms to collect the data have been amended to include all necessary new codes (e.g. areas or institutions). These new protocols and forms (attached as Appendix 1) have been distributed to all teams involved in biological sampling through ICCAT. The consortium has interacted with these teams to provide appropriate guidelines, as they agreed with ICCAT.

The way ROP samples were to be characterized in the database was discussed within the consortium and also with MRAG, and finally it was decided that each observer would have a different "institution code" within the database, i.e. ROP1, ROP2, and onwards for the first, second, and subsequent observers involved in sampling. Moreover, because some observers were taking samples in farms that were also contracted for sampling themselves, the consortium warned ICCAT to make sure that all the samples arriving to the consortium from different sources were originated from different individuals.

2.1 Sampling accomplished

In this report we include the samples (and associated data) that have physically arrived to AZTI before the 12th of February, so as to allow enough time to be verified. These include all the samples collected by the consortium, except 55 YOY otoliths from Cyprus that, at the time of writing this report, are being dispatched at customs. Among the samples collected out of the consortium, most of the samples have also arrived, but we expect a substantial amount of samples to arrive after the submission of this deliverable, namely: ROP samples that arrived from Murcia the 12th of February, additional ROP samples that are still being collected and will be shipped once the sampling finalizes, ABTL samples from Malta that arrived the 13th of February (otolith samples from these individuals did arrive the 9th of February and are included in the database), and those from University of Bologna collected under a different contract.

A total of 1562 bluefin tuna individuals have been sampled by the Consortium. Table 2.1a shows the number of bluefin tuna sampled by the Consortium in each stratum (area/size class combination), and Table 2.2a provides summaries by main region and size class.

In addition, the Consortium received samples from other teams contracted by ICCAT to conduct biological sampling in farms. Altogether (considering the samples collected by the Consortium and those that arrived from other contracts), the Consortium handled samples from 3533 individuals (Table 2.1b, Table 2.2b and Figure 2.1).

The original plan, according to the Consortium contract, was to acquire samples from 980 individuals. Thus, the current sampling status by the Consortium represents 159% of the target in terms of total number of individuals. The targets for the sampling strategy out of the Consortium were not detailed in the contract, but the Consortium was notified that around 1000 additional individuals would be sampled with other contracts and agreements. This makes an overall target of 1980 individuals for the whole sampling strategy, and the current overall sampling status represents 178% of the original target.

By size class, the consortium sampling objectives for young of the year, juvenile, medium and large fish were accomplished (102%, >100%, 205% and 265% of the target respectively, see Table 2.2). Although the overall target for sampling YOY individuals was met, it needs to be noted that the sampling in most areas that had originally planned to sample YOY (East Sicily and Ionian Sea, Malta, Ligurian and Tyrrhenian Sea) performed below the target, while the sampling in the Levantine Sea was above the target, and interesting samples of YOY in the Atlantic side of the Strait of Gibraltar were obtained. In this region, the sampling of medium size fish was succesful, and additional (unplanned) samples of medium size fish were obtained from both the Western and the Eastern Mediterranean. The sampling of adult individuals was the most productive, especially in the Levantine Sea and the Central Atlantic where the original targets were surpassed, and Norway, where, as in Phase6, several hundred fish were sampled by IMR using their own funds.

Table 2.1. Number of bluefin tuna sampled by area and size class. a) Individuals sampled by the Consortium. Empty cells indicate that no sampling was planned in that stratum. Green cells indicate strata where no sampling was planned but some sampling was finally accomplished. b) Total number of individuals sampled (including those of the Consortium plus the ones sampled under other contracts and stored by the Consortium).

a)

		Age 0	Juveniles	Medium	Large	Total		
		<3 kg	3-25 kg	25-100 kg	>100 kg		Target	%
Eastern Mediterranean	Levantine Sea	358		33	70	461	300	154%
Central Mediterranean	East Sicily and Ionian	52				52	100	52%
	Malta	2				2	100	2%
Western Mediterranean	Ligurian	17	2	29		48	50	96%
	Sardinia		1	34	14	49	50	98%
	Tyrrhenian Sea	83				83	150	55%
Gibraltar	Gibraltar	100		109	3	212	100	212%
Northeast Atlantic	Portugal (Algarve)				30	30	30	100%
Central North Atlantic	Central and North Atlantic				384	384	100	384%
North Sea	Norway				241	241	0	>100%
	TOTAL	**612**	**3**	**205**	**742**	1562	980	159%

b)

		Age 0	Juveniles	Medium	Large	Total		
		<3 kg	3-25 kg	25-100 kg	>100 kg		Target	%
Eastern Mediterranean	Levantine Sea	358		130	248	736	300	245%
Central Mediterranean	East Sicily and Ionian	52				52	100	52%
	Malta	2			435	437	100	437%
Western Mediterranean	Balearics		1	19	887	907	1000	91%
	Ligurian	17	2	29		48	50	96%
	Sardinia		1	80	135	216	50	432%
	Tyrrhenian Sea	83			187	270	150	180%
Gibraltar	Gibraltar	100		109	3	212	100	212%
Northeast Atlantic	Portugal (Algarve)				30	30	30	100%
Central North Atlantic	Central and North Atlantic				384	384	100	384%
North Sea	Norway				241	241	0	>100%
	TOTAL	**612**	**4**	**367**	**2550**	3533	1980	178%

Table 2.2: Number of bluefin tuna sampled by main region and size class. a) Individuals sampled by the Consortium. Empty cells indicate that no sampling was planned in that strata. b) Total number of individuals sampled (including those of the Consortium plus the ones sampled under other contracts and stored by the Consortium).

a)

	Age 0	**Juvenile**	**Medium**	**Large**	**TOTAL**	**Target**	**%wrt target**
Eastern Mediterranean	358		33	70	461	300	154%
Central Mediterranean	54				54	200	27%
Western Mediterranean	100	3	63	14	180	250	72%
Gibraltar	100		109	3	212	100	212%
Northeast Atlantic				30	30	30	100%
Central North Atlantic				384	384	100	384%
North Sea				241	241	0	>100%
TOTAL	612	3	205	742	1562	980	159%
Target	600	0	100	280	980		
% wrt target	102%	>100%	205%	265%	159%		

b)

	Age 0	**Juvenile**	**Medium**	**Large**	**TOTAL**	**Target**	**%wrt target**
Eastern Mediterranean	358		130	248	736	300	245%
Central Mediterranean	54			435	489	200	245%
Western Mediterranean	100	4	128	1209	1441	1250	115%
Gibraltar	100		109	3	212	100	212%
Northeast Atlantic				30	30	30	100%
Central North Atlantic				384	384	100	384%
North Sea				241	241	0	>100%
TOTAL	612	4	367	2550	3533	1980	178%
Target	600	0	100	280	980		
% wrt target	102%	>100%	367%	911%	361%		

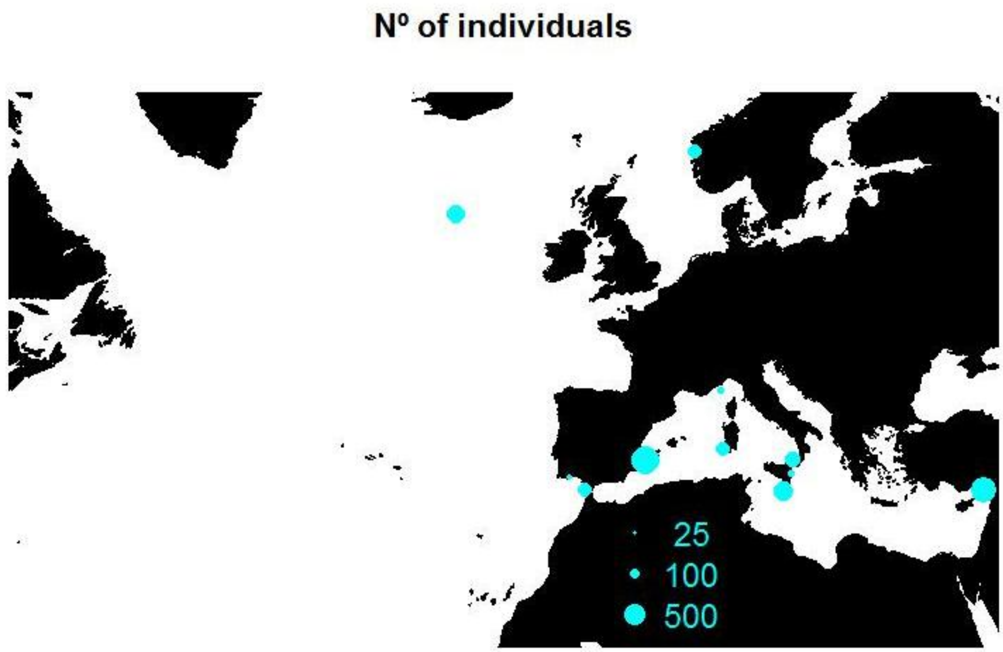


Figure 2.1: Total number of individuals sampled under all GBYP activities in Phase 7 in the Northeast Atlantic and Mediterranean, aggregated by main region. Positions of the dots are averages across all samples by main region.

The overall progress of the project was affected by the late award and signature of the contract, which came after some fisheries had already started or were already closed. Although members of the Consortium tried to keep up with their tasks, the late signature of the contract affected mainly in those cases where travel, purchase and/or subcontracting costs were needed to accomplish the tasks. Yet, most sampling objectives were met.

In the Eastern Mediterranean, 154% of the target number of individuals (YOY and adults) has been sampled. The sampling for YOY in the Levantine Sea was above the original plan, with 358 individuals sampled (out of 200 planned) between July and October, mostly in the area near the Turkish-Syrian border. The sampling of large fish in farms by University of Istanbul was also successful, in number of individuals, with 103 adult individuals sampled (out of 100 planned). Additional 275 adult individuals were sampled through ICCAT observers. Like in previous phases, the success rate of getting otoliths from these fish is very low, due to the way they kill them (bullets use to break them into many pieces). Unfortunately, the genetic samples, that were obtained from most of the fish, were shipped in suboptimal conditions. Some larger vials were not properly sealed, and ethanol was spilled, while many other vials were not large enough to allow the desired sample to ethanol ratio. Thus, upon arrival to AZTI, DNA quality was quickly assessed for a subsample of 4 individuals. Although the assessment indicated evidence of some DNA degradation, the samples could still be useful for some genetic analyses. Thus, all the samples were moved to appropriate vials with enough ethanol and stored.

As for the Central Mediterranean, unfortunately only 27% of the target number of individuals was sampled. NECTON sampled 52 (out of 100 planned) young of the year in East of Sicily and Ionian Sea, between October 2016 and January 2017. In Malta, ABT got the authorization from the Maltese Authorities, but only 2 fish were successfully sampled. The fishermen have lifted the FADs from their deployment area and YOY were not seen around the cages.

In the Western Mediterranean, 72% of the target number of individuals was sampled, including fish from all sizes, but predominantly YOY. The sampling of adult individuals in Sardinia was successful. The individuals were tracked during the processing of their heads in order to sample their otoliths. However, the sampling of YOY in the Tyrrhenian was below the target (83 individuals sampled, out of 150 planned). The sampling of YOY in the Ligurian was also below the target (17 individuals sampled, out of 50 planned), but this was compensated with samples from larger individuals (mostly medium sized).

Under a separate contract, in the Balearics, Taxon S.L., Balfego Group and the ROP sampled 224, 239 and 444 adult individuals of Balearic origin in farms, respectively. As in Phase 6, the percent of otolith samples is very high (430/463) for Taxon S.L. and Balfego Group, but the ROP only provided genetic samples. From these, the percentage of whole otoliths is also high (around 72%), considering the size of the fish and the way they kill them.

In Gibraltar, 212% of the target number of individuals was sampled. The Univ. of Cadiz samples 112 individuals (out of 100 planned), mostly of medium size. In addition, during 2017, unexpected schools of YOY were detected and sampled (n=100) in the Atlantic part of the Strait of Gibraltar, mostly by the Univ. of Cádiz. Three individuals were caught by "Asociación de Amigos del Atún", and sent to AZTI as per indication of the GBYP coordinator. The specimens were sent whole, but arrived in bad status to AZTI, thus only some genetic tissue was preserved in ethanol. The rest of the YOY samples were properly and fully sampled by the Univ. of Cádiz, despite this was not planned originally.

In Portugal, IPMA, in collaboration with observers and Tunipex trap fishermen, conducted the sampling. The objective was to sample 30 whole individuals, and these objectives were met.

In the Central Atlantic, the number of samples is by far beyond the original expectation (n=384 compared to a target of n=100), all belonging to large size fish, which will potentially allow for interesting insights into mixing of stocks and their interannual variability.

Furthermore, as in Phase 6, unexpected samples from Norway were obtained again, since the Institute of Marine Research provided samples from 248 large individuals that were collected using their own funds.

Table 2.3: Number of samples collected by area and tissue type. a) Samples taken by the Consortium. b) Total number of samples (including those of the Consortium plus the ones taken under other contracts and stored by the Consortium).

a)

		Otolith	Spine	Muscle/Fin	Sampler
Eastern Mediterranean	Levantine Sea	314	460	457	ISTA/AZTI(Oray)
Central Mediterranean	East Sicily and Ionian	52	52	52	NECT
	Malta	1	2	2	ABT
Western Mediterranean	Ligurian	48	48	48	UNIGE
	Sardinia	25	48	49	UNIC
	Tyrrhenian Sea	71	83	83	NECT
Gibraltar	Gibraltar	149	55	210	UCA/AZTI
Northeast Atlantic	Portugal	30	30	30	IPMA
Central North Atlantic	Central and North Atlantic	245		382	NRIFSF
North Sea	Norway		233	239	IMR
	Total	**935**	**1011**	**1552**	
			3498		

b)

		Otolith	Spine	Muscle/Fin	Sampler
Eastern Mediterranean	Levantine Sea	314	460	732	ISTA/AZTI(Oray)/ROP
Central Mediterranean	East Sicily and Ionian	52	52	52	NECT
	Malta	436	2	2	ABT
Western Mediterranean	Balearics	430	459	907	BALFEGO/ROP/TAXON
	Ligurian	48	48	48	UNIGE
	Sardinia	192	48	49	UNIC/ABT
	Tyrrhenian Sea	258	83	83	NECT/ABT
Gibraltar	Gibraltar	149	55	210	UCA/AZTI
Northeast Atlantic	Portugal	30	30	30	IPMA
Central North Atlantic	Central and North Atlantic	245		382	NRIFSF
North Sea	Norway		233	239	IMR
	Total	**2154**	**1470**	**2734**	
			6358		

Table 2.4: Number of samples by main region and tissue type. a) Samples taken by the consortium. b) Total number of samples (including those of the consortium plus the ones taken under other contracts and stored by the Consortium).

a)

	Otolith	Spine	Muscle/Fin	TOTAL
Eastern Mediterranean	314	460	457	1231
Central Mediterranean	53	54	54	161
Western Mediterranean	144	179	180	503
Gibraltar	149	55	210	414
Northeast Atlantic	30	30	30	90
Central North Atlantic	245		382	627
North Sea		233	239	472
TOTAL	**935**	**1011**	**1552**	**3498**
Target	980	880	980	2840
% wrt target	95%	115%	158%	123%

b)

	Otolith	Spine	Muscle/Fin	TOTAL
Eastern Mediterranean	314	460	732	1506
Central Mediterranean	488	54	54	596
Western Mediterranean	928	638	1087	2653
Gibraltar	149	55	210	414
Northeast Atlantic	30	30	30	90
Central North Atlantic	245		382	627
North Sea		233	239	472
TOTAL	**2154**	**1470**	**2734**	**6358**
Target	980	880	980	2840
% wrt target	220%	167%	279%	224%

Otoliths

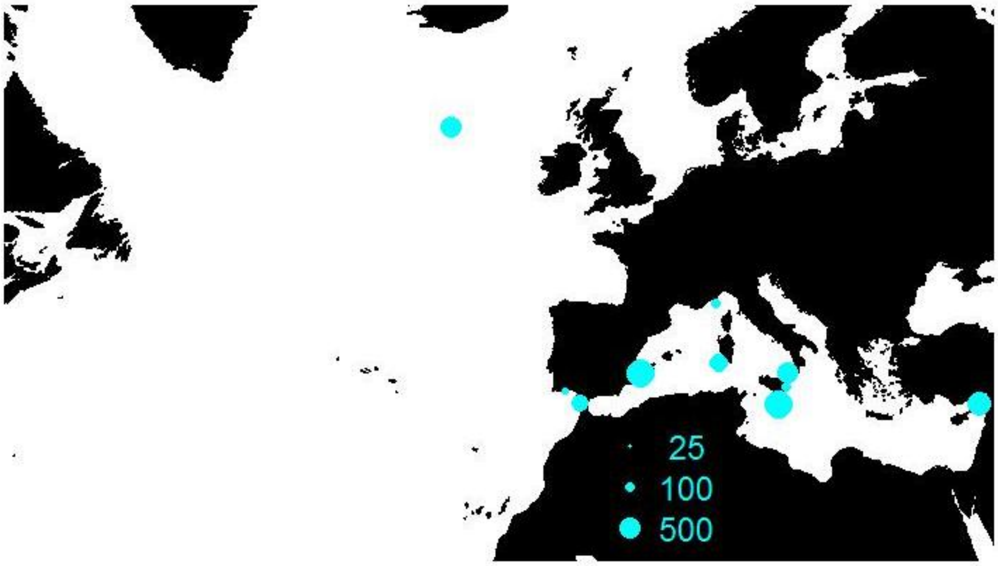


Figure 2.2: Total number of individuals with otolith sampling conducted under all GBYP contracts in Phase 7 in the Northeast Atlantic and Mediterranean, aggregated by main region. Positions of the dots are averages across all samples by main region.

Spines

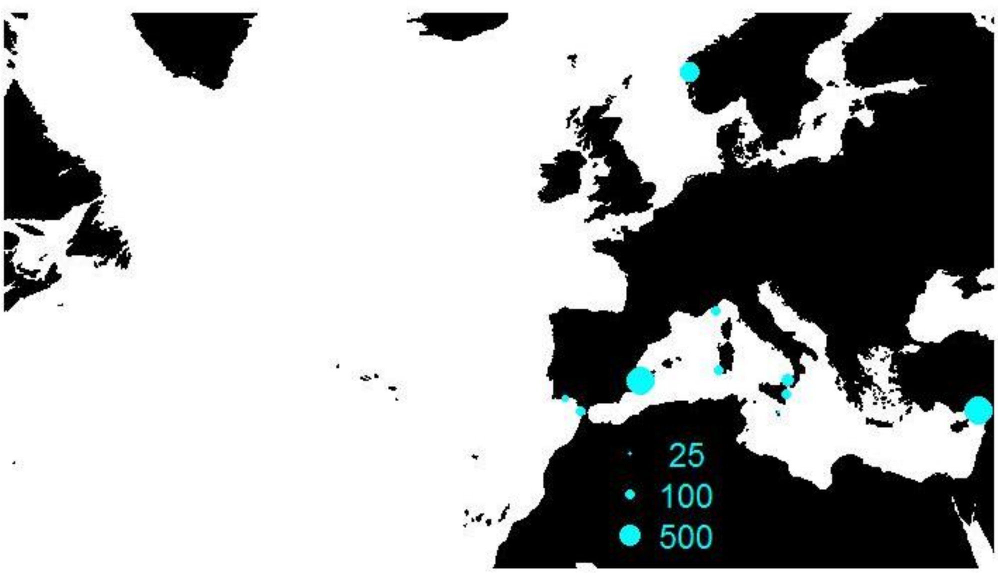


Figure 2.3: Total number of spines collected under all GBYP contracts in Phase 7 in the Northeast Atlantic and Mediterranean, aggregated by main region. Positions of the dots are averages across all samples by main region.

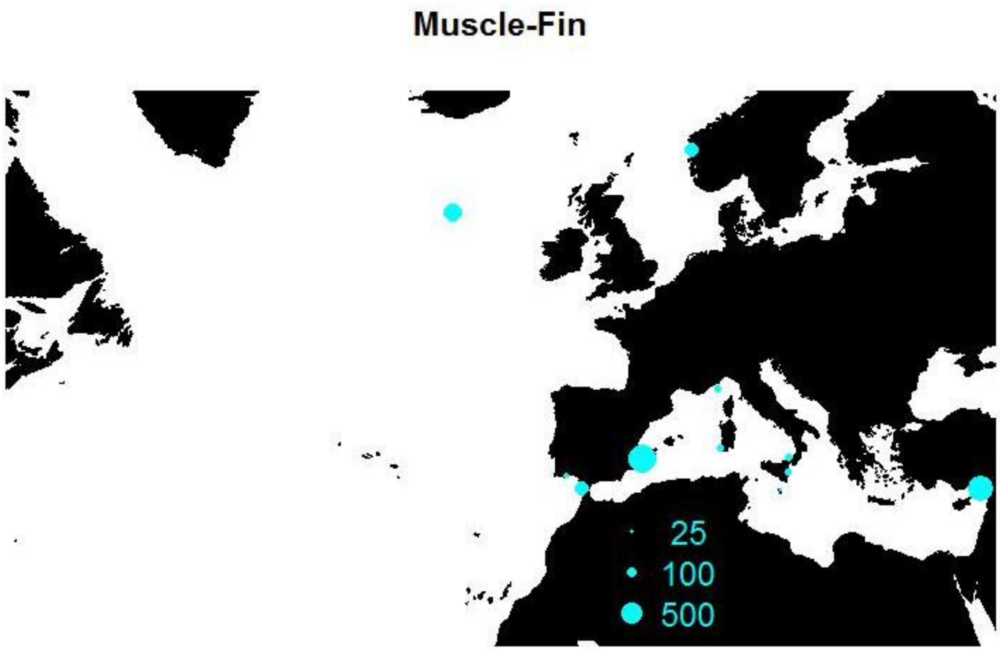


Figure 2.4: Total number of muscle or fin tissue samples collected under all GBYP contracts in Phase 7 in the Northeast Atlantic and Mediterranean, aggregated by main region. Positions of the dots are averages across all samples by main region.

3. SHINY APPLICATION:

Task Leader: María Korta

Participants:

AZTI: Haritz Arrizabalaga, Igaratza Fraile

3.1 Introduction

This application has been prepared on R environment using the "shiny" and several other libraries. Shiny is an open source R package from RStudio that can be used to build interactive web pages with R.

Every Shiny app is composed of two parts: UI (user interface) and Server. UI is a kind of web document – HTML written using functions of Shiny. The file "Server" is responsible for the logic of the app; it's the set of instructions that tell the web page what to show when the user interacts with the page. The Shiny system is designed to simplify the creation of interactive web applications. It provides automatic "reactive" linkage between inputs and outputs: when the user clicks on different selections, the output is re-rendered (Santiago et al. 2017).

3.2 Objective

During Phase 7, a Shiny web application has been developed to facilitate the inspection of available samples in the biological sample bank and to aid sample selection following different criteria to help better design future experiments and analyses (see example in Figure 3.1).

3.3 Characteristics and utility of the Shiny App

The Shiny application builds on the inventory of available samples from Phase 7 and all previous Phases. It allows to interactively subset the sample inventory using the predefined variables (area, year, month, size class and tissue type (namely otoliths, spines, gonads and/or genetic tissue)), and then to plot on the map the number of available samples aggregated by each unique position, with symbol sizes dependent on total sample size. The plotted information can be colored by Year, Month or Size class, using the legend tick box. The map can be refreshed anytime the selection criteria are changed, and the data can be downloaded in a *cvs* file. The downloaded file includes all individual fish (one row for each individual) contained in the final selection made by the user. For each fish, the individual ID number as well as information related to area, catch date, fishing gear, length, weight and tissue availability is included.

The R code is available (Appendix 2), so that the user can run the App in Rstudio, after installing shiny and other libraries. Moreover, this allows to, if desired, modify the code and thus, the App utilities and/or design. Alternatively, the App is also available on a server:

https://aztigps.shinyapps.io/bluefin/

By clicking on the link above, the user can use the App with no need to have installed Rstudio and the required libraries. The current version of the App automatically uploads the dataset thatis provided as part of the files in Appendix 2 to this report. This in principle allows the shiny App to be used by users that do not necessarily have the dataset. To avoid confidentiality issues, the location and catch date data is provided at a resolution of 5º*5º and month. As the final dataset provided in Appendix 3 gets enriched with additional samples (e.g. those that did not arrive on time, or additional samples that will take place in the future), the dataset on which shiny operates will also need to be updated.

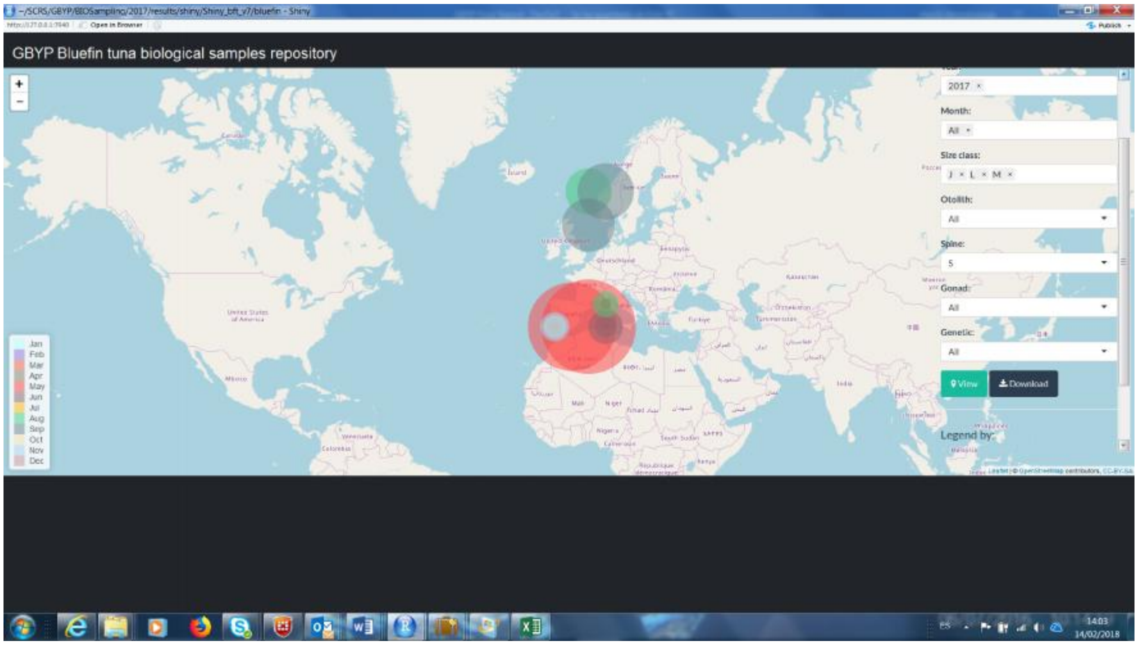


Figure 3.1: Shiny App developed to visualize available biological samples in the ICCAT GBYP Tissue Bank. The dataset can be filtered using the variables in the right column (where none, one, several or all categories can be selected). The map can be refreshed using the "view" button. The plotted information can be colored using different variables specified in the legend, and the data associated to the final subset can be downloaded as a cvs file. In the example, available spine samples collected in 2017 and belonging to size classes "juvenile", "medium" or "large", are shown, coloured by month (specified in the legend).

4. ANALYSES

During Phase 7, only one analysis task (related to the reading and counting of daily rings on YOY to establish their birthdate) was originally funded. Then during the contract amendment, an otolith chemistry task was also agreed. The following sections elaborate on those two tasks.

In addition, following specific criteria discussed and agreed with the GBYP coordinator and the SCRS chairman, the consortium selected 2000 otoliths to send to Australia for age reading analyses, and interacted with Fish Aging Services regarding any clarification around those samples.

5. NURSERY ORIGIN OF BLUEFIN CAPTURED IN MIXING ZONES

Task Leader: Igaratza Fraile (AZTI) & Jay Rooker (TAMU)

Participants:

AZTI: Haritz Arrizabalaga

5.1 Introduction

The results from previous phases suggested that western origin contributions were negligible in the Mediterranean Sea, Bay of Biscay and Strait of Gibraltar, but mixing rates could be important in the central North Atlantic, Canary Islands and western coast of Morocco. To assess the spatial and temporal variability of mixing proportions, otoliths collected in Moroccan coast in 2016 were analyzed for stable carbon and oxygen isotopes ($\delta^{13}C$ and $\delta^{18}O$).

5.2 Material and methods

In this section, we investigate the origin of bluefin tuna collected in the western coast of Morocco using stable $\delta^{13}C$ and $\delta^{18}O$ isotopes in otoliths. Samples utilized for this study (N=50) were collected in May 2016 by Moroccan traps, off the African continent (35ºN, 6ºW approximately).

Otolith handling followed the protocols previously described in Rooker et al. (2008). Briefly, following extraction by GBYP participants, sagittal otoliths of bluefin tuna were cleaned of excess tissue with nitric acid (1%) and deionized water. One sagittal otolith from each bluefin tuna specimen was embedded in Struers epoxy resin (EpoFix) and sectioned using a low speed ISOMET saw to obtain 1.5 mm transverse sections that included the core. Following attachment to a sample plate, the portion of the otolith core corresponding to approximately the yearling periods of bluefin tuna was milled from the otolith section using a New Wave Research MicroMill system. A two-vector drill path based upon otolith measurements of several yearling bluefin tuna was created and used as the standard template to isolate core material following Rooker et al. (2008). The preprogrammed drill path was made using a 500 μm diameter drill bit and 15 passes each at a depth of 50 μm was used to obtain core material from the otolith. Powdered core material was transferred to silver capsules and later analyzed for $\delta^{13}C$ and $\delta^{18}O$ on an automated carbonate preparation device (KIEL-III) coupled to a gas-ratio mass spectrometer (Finnigan MAT 252). Stable $\delta^{13}C$ and $\delta^{18}O$ isotopes are reported relative to the PeeDee belemnite (PDB) scale after comparison to an in-house laboratory standard calibrated to PDB.

Stable isotope signals of mixed stocks were compared with yearling samples from Mediterranean and Gulf of Mexico nurseries revised in GBYP-Phase 3 and presented in Rooker et al. (2014). HISEA software (Millar 1990) was used to generate direct maximum likelihood estimates (MLE) of mixed-stock proportions in each of the mixing zones. HISEA computes the likelihood of fish coming from a nursery area with characterized isotopic signature. MLE estimator is defined as the composition that maximizes the likelihood of the entire mixed fishery sample (Millar 1990). Uncertainty in estimation is addressed by re-sampling the mixed stock data 500 times with replacement. Additionally, individual origin was assigned using Quadratic Discriminant Function Analysis (QDFA). The QDFA method is a common tool for classification analysis that models the likelihood of each class as a Gaussian distribution and estimates the posterior probabilities for a given test point (Hastie et al., 2001). Mixing proportions estimated by QDFA were then compared to those estimated by MLE. Following Fraile et al (2014), individuals with probabilities between 30% and 70% (N=9) were considered non-assigned and were excluded from the proportion estimates.

Results are focused on MLE because the performance is typically superior to individual classification methods such as QDFA. This is so because classification of an individual fish only indicates which stock has the highest likelihood of that fish, while the MLE method uses all the information contained in the likelihood values, including the variability of the reference samples. However, MLE estimator may be biased when some of the stocks in the mixed fishery are low contributors (Millar, 1987, 1990). Thus, results from QDFA estimator are included for comparative purposes.

5.3 Results and Discussion

$\delta^{13}C$ and $\delta^{18}O$ were measured in the otolith cores of bluefin tuna from Atlantic coast of Morocco and compared to baseline populations from the Mediterranean Sea and Gulf of Mexico (Figure 5.1). Otolith $\delta^{18}O$ values correspond well with those measured in yearling otoliths from the eastern and western nurseries, whereas $\delta^{13}C$ values measured in adult bluefin tuna otoliths from the Moroccan coast are more enriched compared to baseline samples (Fig. 5.1). The enrichment of $\delta^{13}C$ has been previously reported in bluefin tuna otoliths (Schloesser et al. 2009, Fraile et al. 2016) and it was attributed to the increase of atmospheric CO_2 derived from the combustion of fossil fuels and deforestation, causing a decrease in atmospheric $\delta^{13}C$ and, in turn, a decrease of $\delta^{13}C$ in biogenic carbonates (Verburg, 2007).

Mixed-stock analyses using MLE procedure indicated that catches in 2016 were comprised entirely by the Mediterranean population (100% of eastern origin fish). Mixing rate estimates in the coast of Morocco using this methodology varied considerably in preceding years, with catches in 2011 and 2014 dominated by the western population and catches in 2012, 2013 and 2015 dominated by the Mediterranean population (Figure 5.2). The results for 2016 confirm that mixing of the two populations occurs at variable rate, but Mediterranean bluefin tuna may be the principal contributors to the fishery in Moroccan traps.

Mixing proportions estimated by QDFA showed a greater mixing of the two populations in this region (80% of eastern fish vs. 20% of western fish), but both methods agree in recognizing that Mediterranean Sea may be the primary source of bluefin tuna contributing to Moroccan trap fishery. It is not surprising that QDFA suggests a larger western proportion, since even some individuals of the Mediterranean baseline are more similar to the western baseline (Rooker et al 2014 and Figure 5.1). Still, it is recommended to conduct further comparative analyses using these and other classification methods to better assess the relative merits under different circumstances, and the possible implications of using one or another methodology.

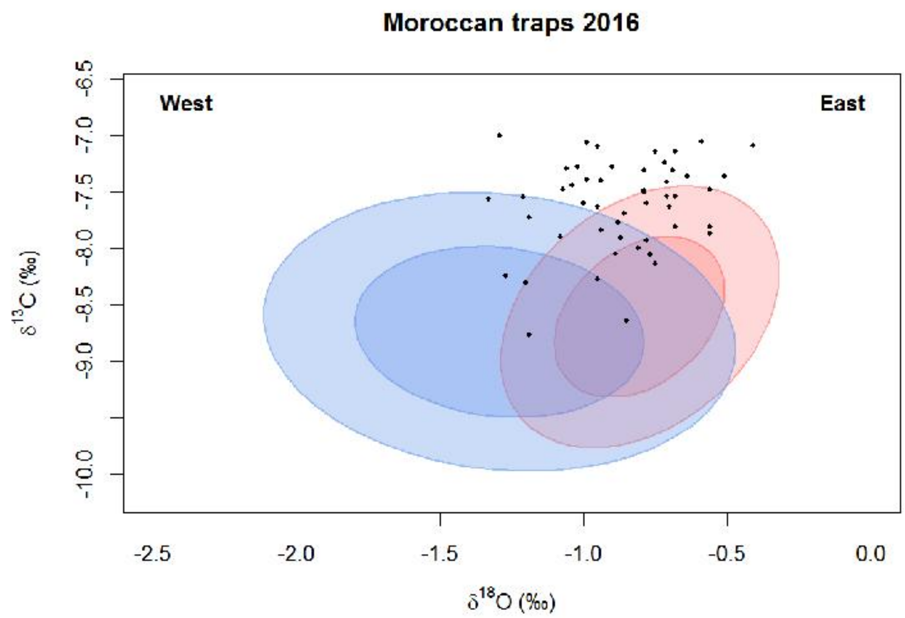


Figure 5.1: Confidence ellipses (1 and 2 SD or ca. 68% and 95% of sample) for otolith δ13C and δ18O values of yearling bluefin tuna from the east (red) and west (blue) along with the isotopic values (black) for otolith cores of bluefin tuna collected from western African coast by Moroccan traps.

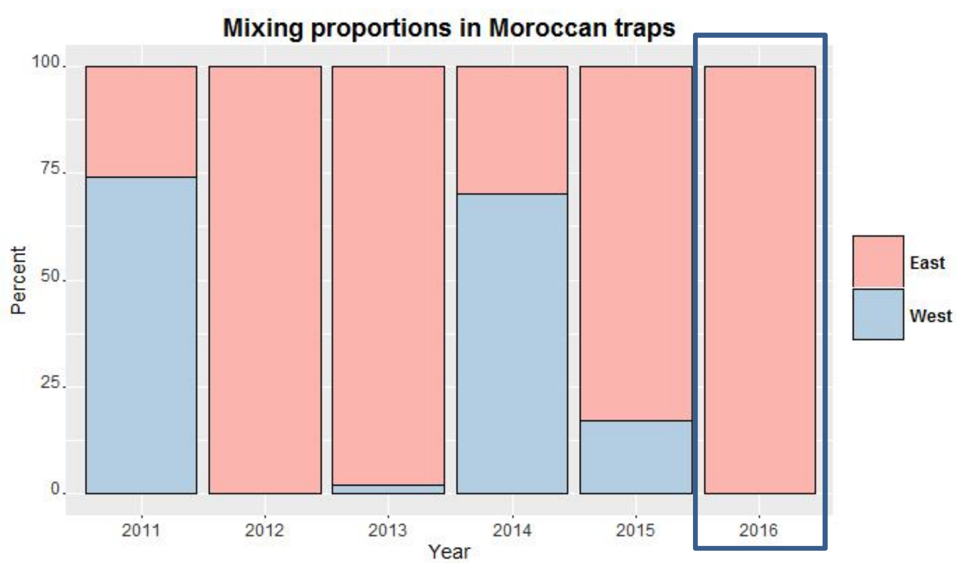


Figure 5.2: Interannual variation of the mixing proportions in the western African coast (Moroccan traps) estimated by Maximum Likelihood Estimator (HISEA program).

Table 5.1: Maximum-likelihood and QDFA predictions of the origin of bluefin tuna from the western coast of Africa analyzed under the current contract. Estimates are given as percentages. The mixed-stock analysis (HISEA program) was run under bootstrap mode with 1000 runs to obtain standard deviations around estimated percentages (± %).

	Mixing proportions by MLE					**Mixing proportions by QDFA**		
Year	**West**	**East**	**SD**	**N**		**West**	**East**	**N**
2016	0%	100%	1%	50		20%	80%	41

6. DAILY AGING

Task Leader: Haritz Arrizabalaga (AZTI)

Participants:

AZTI: Igaratza Fraile

CEAB-CSIC: *Nuria Raventos, Ana Gordoa*

6.1 Introduction

During the 2017 data preparatory meeting, after presentation of document SCRS/2017/040, it was observed that some YOY were larger than usual. The WG recommended to age large YOY individuals fished early in the 2016 season, just to check whether the birth dates corresponded with the assumed spawning season, or they could have been born significantly earlier (working hypothesis). Although a few limited analyses conducted afterwards by CSIC, as well as earlier analyses conducted by AZTI (unpublished data), suggested that this might not be the case, following indications by GBYP, we analyzed a few more individuals (n=20) to completely discard this hypothesis.

6.2 Material and Methods

The original intention was to use largest (>35cm) individuals caught in August-September, with some additional samples from November (>46 cm) and December (>50 cm). The final selection of the YOY individuals was delayed due to some inconsistencies between the date information in the database and the RMA documents (which were originally used for a first sample selection). Once this inconsistency was resolved, the 20 samples that met the original criteria were selected. These included YOY individuals from the Balearic, Tyrrhenian and Levantine seas, caught between 22th of August and 14th of December 2016, straight fork lengths ranging between 35 and 57 cm and round weights between 0.90 and 2.98 kg.

The samples were sent to CSIC premises for analysis, that followed essentially the same procedure as in 2016, where it was concluded that the best way to read daily age was using a transversal section of the otolith. The methodology is laborious, since the section is obtained essentially by sanding the otolith until the daily rings around the nucleus and border of the otolith are visible in a thin section (Figure 6.1). In young individuals this can be obtained in a single plain, but in larger individuals it might require sequential sanding and reading to cover the complete life history of the individual.

Figure 6.1. Transversal section of the YOY otolith with visible daily rings.

Given the large amount of time required to process each otolith, age was read first on a single otolith per individual, using the second otolith only when the first was broken by the nucleus, it was overpolished, or two reads differed by more than 10%, or low confidence in the age estimation.

Each otolith was read at least two times, and sometimes up to 3 or 4 times. Two final values were given for each otolith, and the final age assigned to each individual was the average of the available estimates.

6.3 Results and Discussion

The age estimates for the 20 YOY ranged between 61 and 107 days, with corresponding birthdates ranging between the 3rd of June and the 13th of July. The mean and the median estimated birthdate was the 15th and the 16th of June, respectively, and only one fish was estimated to be born in July, with 19 birthdates estimated within June (Table 6.1).

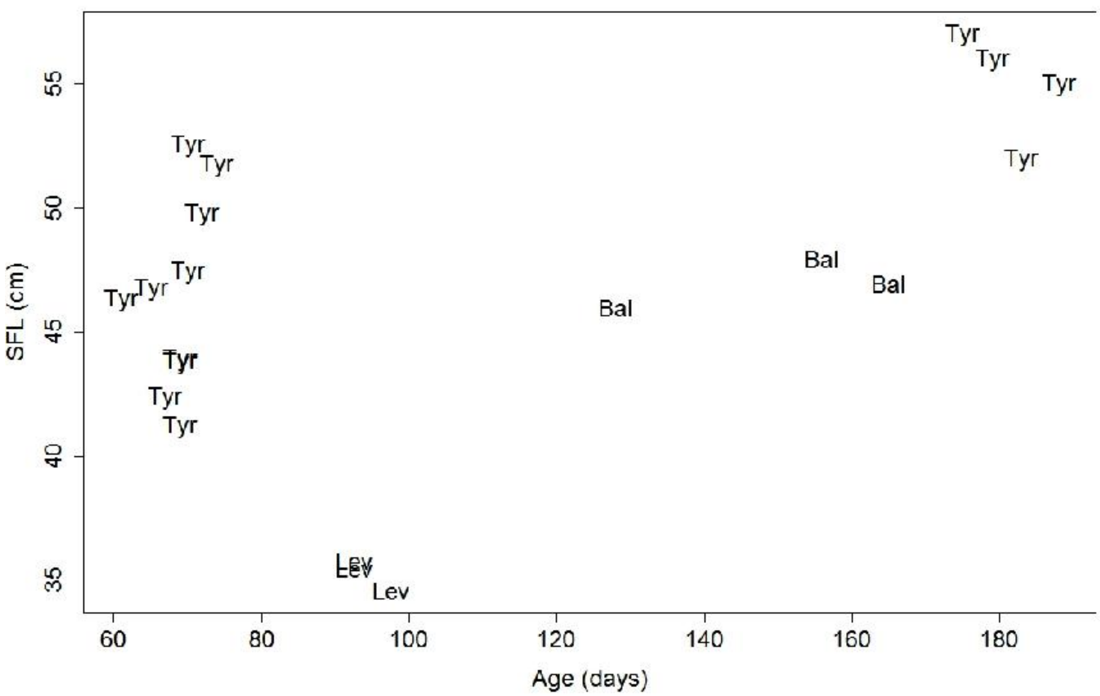
All estimated birthdates are consistent with previous birthdate estimates (AZTI unpublished data) and the current knowledge that bluefin tuna spawn between the 15th of May and 15th of July. So, in principle, and although recognizing the small sample size used, these results do not support the original hypothesis according to which bluefin tuna would be capable of spawning out of that temporal window. Instead, anomalies in the growth pattern during the early months would explain the range of YOY sizes observed in different months.

An exploratory analysis of the age length relationship groups the data into four major groups: those caught in the Tyrrhenian in August, those caught in the Tyrrhenian in December, those caught in the Balearics and those caught in the Levantine Sea (Figure 6.2). For a given age range, considerable variability in length is observed (e.g. among individuals caught in the Tyrrhenian during August). The individuals caught in the Tyrrenian in December show larger lengths, and the individuals caught in the Balearics show intermediate lengths and ages. The individuals caught in the Levantine sea show smaller lengths at larger ages compared to those in the Tyrrhenian. However, with such low sample sizes it is not possible to conduct any group separation and it is not possible to assess on regional growth patterns, which was out of the scope of the analysis.

Table 6.1. Final age (in days) and birthdate estimated for the selected YOY individuals.

Sample ID	Sampling date	Sampling area	SFL (cm)	RW (kg)	Age (days)	Birthdate
UNIB-TY-0-222	22/08/2016	Tyrrhenian Sea	47	2,007	65	18/06/2016
UNIB-TY-0-258	26/08/2016	Tyrrhenian Sea	47	2,094	70	17/06/2016
UNIB-TY-0-256	26/08/2016	Tyrrhenian Sea	50	2,420	72	15/06/2016
UNIB-TY-0-260	29/08/2016	Tyrrhenian Sea	52	2,714	74	16/06/2016
UNIB-TY-0-261	29/08/2016	Tyrrhenian Sea	53	2,843	70	20/06/2016
CYPR-LS-0-427	09/09/2016	Levantine Sea	35	0,896	98	03/06/2016
CYPR-LS-0-426	09/09/2016	Levantine Sea	36	0,947	93	08/06/2016
CYPR-LS-0-425	09/09/2016	Levantine Sea	36	0,955	93	08/06/2016
IEO-BA-0-414	18/11/2016	Balearic Sea	46	2,160	128	13/07/2016
IEO-BA-0-406	18/11/2016	Balearic Sea	47	2,180	165	06/06/2016
IEO-BA-0-413	18/11/2016	Balearic Sea	48	2,220	156	15/06/2016
NECT-TY-0-81	04/12/2016	Tyrrhenian Sea	52	2,300	183	04/06/2016
NECT-TY-0-98	14/12/2016	Tyrrhenian Sea	55	2,750	188	09/06/2016
NECT-TY-0-97	14/12/2016	Tyrrhenian Sea	56	2,980	179	18/06/2016
NECT-TY-0-101	14/12/2016	Tyrrhenian Sea	57	2,980	175	22/06/2016
UNIB-TY-0-244	24/08/2016	Tyrrhenian Sea	44	1,648	69	16/06/2016
UNIB-TY-0-255	26/08/2016	Tyrrhenian Sea	44	1,659	69	18/06/2016
UNIB-TY-0-219	22/08/2016	Tyrrhenian Sea	46	1,954	61	22/06/2016
UNIB-TY-0-241	24/08/2016	Tyrrhenian Sea	41	1,380	69	16/06/2016
UNIB-TY-0-247	25/08/2016	Tyrrhenian Sea	42	1,503	67	19/06/2016

Figure 6.2. Relationship between final age (in days) and straight fork length (in cm) of the individuals collected in the Tyrrhenian, Balearic and Levantine seas used for assignment of birthdate.



7. APPENDICES

Appendix 1: Protocols and forms (see "SAMPLING PROTOCOLS FOR BFT GBYP final 18102016" and "GBYP data form 2017 with macro v2.xls".

Appendix 2: Shiny code (see "Shiny_bft_v7.rar"), includes the shiny database ("mydata.Rdata), based on the database provided in Appendix 3.

Appendix 3: Database as of 15th February 2018 (see "Database_15_Feb_2018.xls). Note that this database is subject to change in the future as new samples are integrated.

8. REFERENCES

Fraile, I., Arrizabalaga, H. and Rooker, J.R. (2015) Origin of Atlantic bluefin tuna (Thunnus thynnus) in the Bay of Biscay. *ICES Journal of Marine Science: Journal du Conseil* 72: 625-634.

Fraile, I., Arrizabalaga, H., Groeneveld, J., Kölling, M., Santos, M.N., Macías, D., Addis, P., Dettman, D.L., Karakulak, S. and Deguara, S. (2016) The imprint of anthropogenic CO 2 emissions on Atlantic bluefin tuna otoliths. *Journal of Marine Systems* 158: 26-33.

Hastie, T., Tibshirani, R., and Friedman, J. (2001) The Elements of Statistical Learning. Springer-Verlag, NewYork.

Millar, R.B., (1987) Maximum likelihood estimation of mixed stock fishery composition. *Canadian Journal of Fisheries and Aquatic Sciences* 44: 583-590.

Millar, R.B., (1990). Comparison of methods for estimating mixed stock fishery composition. *Canadian Journal of Fisheries and Aquatic Sciences*, 47, 2235-2241.

Rooker, J.R., Arrizabalaga, H., Fraile, I., Secor, D.H., Dettman, D.L., Abid, N., Addis, P., Deguara, S., Karakulak, F.S., Kimoto, A., Sakai, O., Macias, D. and Santos, M.N. (2014) Crossing the line: migratory and homing behaviors of Atlantic bluefin tuna. *Marine Ecology Progress Series* 504: 265-276.

Rooker, J.R., Secor, D.H., DeMetrio, G., Kaufman, A.J., Ríos, A.B. and Ticina, V. (2008) Evidence of trans-Atlantic movement and natal homing of bluefin tuna from stable isotopes in otoliths. *Marine Ecology Progress Series.* 368: 231–239.

Santiago, J., Arrizabalaga, H., Merino, G. and Murua, H. (2017) SCRS Annual dashboard: a new tool to complement the management advice to the Commission. *SCRS/2017/101*: 5.

Schloesser, R. W., Rooker, J. R., Louchuoarn, P., Neilson, J. D., and Secor, D. H. (2009) Interdecadal variation in seawater d13C and d18O recorded in fish otoliths. *Limnology and Oceanography* 54: 1665–1668.

Verburg, P. (2007) The need to correct for the Suess effect in the application of d13C in sediment of autotrophic Lake Tanganyika, as a productivity proxy in the Anthropocene. *Journal of Paleolimnology* 37: 591–602.