SHORT TERM CONTRACT FOR BIOLOGICAL STUDIES - ICCAT ATLANTIC-WIDE RESEARCH PROGRAMME FOR BLUEFIN TUNA (ICCAT GBYP – PHASE 14-2025)

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ICCAT

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CONTEXT

On July 7th, 2025, Fundación AZTI-AZTI Fundazioa, in collaboration with Japan Fisheries Research and Education Agency (FRI), Institut Français de Recherches pour l'Exploitation de la Mer (IFREMER), Instituto Portugues do Mar e Atmosfera (IPMA), Universidad de Cadiz (UCA), National Oceanic and Atmospheric Administration (NOAA) and Institute of Marine Research (IMR), submitted a proposal in response to the call for tenders on biological and genetic sampling and analysis (ICCAT-GBYP Phase 14-2025).

This proposal was awarded and the final contract between ICCAT and the consortium represented by Fundación AZTI-AZTI Fundazioa was signed on August 7th, 2025.

According to the terms of the contract, a revised final report (**Deliverable nº 4**) needed to be submitted to ICCAT by 20th of December. The present report was prepared in response to such contractual requirements.

EXECUTIVE SUMMARY

The GBYP Biological programme is focused on ameliorating scientific advice on the Atlantic Bluefin Tuna (ABFT) through data mining, the understanding of key biological and ecological processes and improving of assessment models. During this Phase-14 efforts have been directed towards 4 main tasks; (1) maintaining the ICCAT GBYP Biobank, (2) gathering new samples in key areas, (3) improving knowledge on stocks mixing and (4) the further development of a public information system on the GB YP Tissue Bank and the results of the analyses carried out with these samples.

The consortium has provided **maintenance and management of the ICCAT-GBYP tissue bank.** The ICCAT GBYP Biobank is a centralized repository created under the Atlantic-Wide Research Programme for Bluefin Tuna (GBYP) to store, catalogue, and manage biological samples (such as otoliths, gonads, spines, and genetic material) collected along all the successive Phases of the program. Its purpose is to ensure long-term availability of standardized samples for genetic, biological, and ecological studies, supporting SCRS advice and ICCAT's management decisions.

During this phase, **sampling** has been accomplished including key areas and life stages across the species distribution range. A total of 551 ABFT individuals have been achieved, consisting of 248 larvae from the Slope Sea and 303 adults, from the Central Atlantic Ocean, the Bay of Biscay, Norwegian waters and off Guinea Bissau. In total, 1003 samples have been collected, consisting of 238 otolith pairs, 239 spines, 501 muscle or fin tissue and 25 gonads, that have already been included in the catalogue.

To further understand the **interbreeding between eastern and western stocks**, genetic analysis on 248 larvae from the Slope Sea have been performed, together with those of 724 reference samples from the two main spawning areas in the Mediterranean Sea and the Gulf of Mexico. The results obtained contribute to support previously formulated hypothesis, reveal relevant aspects for future analysis (such as quality of the samples, levels of kinship found) and highlight the need for further sampling in

the area. Ten samples have been selected based on these results to sequence their whole genomes.

The **stock of origin information** available from genetic and otolith microchemistry assignments of ABFT collected in different feeding areas has been merged into respective complete datasets. The process for estimating proportion of Mediterranean individuals and the standard deviation has been unified between both methods, so that these data can be directly integrated in the Management Strategy Evaluation (MSE) process. Proportion of Mediterranean fish across the strata applied in the MSE was calculated. This exercise is essential for further developing stock assessment models that include mixing among areas and use of biologically realistic operating models for more rigorous management options testing.

Finally, during this Phase-14 development of the online **information system** has continued and does now include a user-friendly module where consolidated results to date can be viewed (https://aztidata.es/BioTuna/). By dynamically linking sample availability to their geographic origin and analytical outputs generated within all GBYP Biological Studies phases, such as age estimations and stock of origin determinations, the application facilitates more efficient planning of future research, supports transparency, enables quick queries in real time for decision making and SCRS meetings, and promotes collaboration among institutions. This new step ensures that the system evolves to support data integration, accessibility, and long-term usability across GBYP components.

Overall, the objectives of the project were met. The outputs provided during this phase of ICCAT GBYP Biological Studies program continue to provide relevant information for a better understanding of the biology and dynamics of Atlantic bluefin tuna, which in turn improves the scientific advice for the stock assessment and management of this valuable species.

1. MAINTAINANCE AND MANAGEMENT OF THE ICCAT-GBYP TISSUE BANK

Task Leader: Igaratza Fraile (AZTI) and Iraide Artetxe-Arrate (AZTI)

Participants:

AZTI: Naiara Serrano, Aitor Baquero

1.1 Introduction

Fundación AZTI-AZTI Fundazioa has taken full responsibility for the maintenance and management of the ICCAT-GBYP tissue bank. The systematic maintenance and management ensures the preservation of high-quality biological samples that can be used for a wide range of bluefin tuna biology related scientific studies under the GBYP research plan. The ICCAT-GBYP tissue bank serves as a repository of otolith, spines, gonads, muscle and/or fin tissues under rigorously controlled conditions to grant their long-term viability and scientific utility.

Effective management of the tissue bank encompasses standardized protocols for collection (see **Annex I**), processing, storage, and retrieval, thereby safeguarding the integrity and reproducibility of samples.

By providing a centralized management, ICCAT-GBYP tissue banks allows collaboration among institutions interested in advancing on bluefin tuna knowledge and fosters future investigations into species resilience under changing oceanic conditions.

1.2 Material and Methods

The consortium has continued to provide appropriate storage for all biological samples collected during previous phases and the ones arrived in the current phase, including otoliths and spines stored in the general store at ambience temperature, gonads preserved in Bouin's or Hollande solution, and muscle and fin tissues, with duplicates in freezers of separate buildings for added security, as well larvae that have not yet being processed, which are also stored in freezers.

The consortium has also been responsible for the reception of samples collected during this Phase-14, which includes the condition and labelling check of the samples received and their re-conditioning when necessary (e.g. change of ethanol or vial relabeling). Each sample associated storage location and metadata has been updated into the ICCAT-GBYP tissue bank catalogue, to guarantee that researchers can trace back any sample when needed. In addition, AZTI has implemented quality control procedures to monitor sample integrity and database accuracy.

1.3 Results

The detailed and updated catalogue of samples stored in the GBYP Tissue Bank can be found as **Annex II** of the present report.

Currently, the catalogue contains information from 34,270 individuals. This includes fish identification related information, as well as sampling information, fish biological data description and sample availability. Since Phase 13, the catalogue also includes consolidated results of analysed samples, such as stock of origin based on genetic and otolith microchemistry data, and age derived from otolith or/and spine readings.

Presently, the tissue bank provides storage of 17,697 otoliths, 8,172 spines, 2,071 gonad samples and 24,266 muscle or fin tissue samples in its original or transformed form (Table 1.1). The tissue bank also provides origin results of 795 fish analysed with the SNP array, origin results of 3,530 fish analysed with the 96 SNP panel, origin results of 2,679 fish analysed with otolith microchemistry, otolith derived age of 4,683 fish and spine derived age of 1,187 fish.

Table 1.1. Number of samples stored in AZTI laboratories as part of the ICCAT-GBYP tissue bank

Region	Area	Otoliths	Spines	Gonads	Muscle/fin
MED	Western Mediterranean	8114	4140	1987	9190
	Central Mediterranean	5600	1377	50	4765
	Eastern Mediterranean	1342	860	7	1691
GOM	Gulf of Mexico	0	0	0	207
SEATL	Gibraltar Strait	1010	482	0	1822
	Madeira and Canary islads	129	0	0	380
	Mauritania and Senegal	205	104	0	130
NEATL	Bay of Biscay	511	601	27	1232
	Norwegian Sea and Skagerrak	207	604	0	1265
CNATL	Central Atlantic (east of 45ºW)	507	2	0	2309
	Central Atlantic (west of 45ºW)	72	0	0	687
NWATL	Gulf Saint Lawrence, Gulf of Maine, New Foundland, Western Atlantic	0	0	0	608
Total		**17697**	**8172**	**2071**	**24266**

1.4 Conclusions

The catalogue of ICCAT-GBYP tissue bank constitutes one of the most comprehensive ABFT tissue banks worldwide. The careful stewardship of these repositories not only preserves invaluable biological material but also strengthens the scientific infrastructure necessary for future studies. Moreover, the current ICCAT-GBYP tissue bank and associated metadata forms an extensive collection of samples with an extensive temporal and spatial coverage. This is essential to enable researchers to identify long-term trends and anticipate future challenges. To date, the samples available in the biobank and the analysis tasks carried out in the different phases of the ICCAT GBYP Biological Sampling Programme have resulted in 18 publications in peer-reviewed journals, and at least 26 working documents presented to the ICCAT SCRS (see **Annex IV**), demonstrating the great scientific potential of this catalogue. Ultimately, the stock-pilling of ABFT samples is a key component for the design of studies that aim to develop conservation strategies that allow the development of informed decision-making for the sustainable fisheries management and conservation policy of the species.

2. SAMPLING

Task Leader: Igaratza Fraile (AZTI)

Participants:

AZTI: Naiara Serrano, Patricia Lastra, Aitor Baquero, Iker Zudaire, Jon Uranga

FRI: Yohei Tsukahara

NOAA: David Richardson and Sarah Glancy

CRODT: Fambaye Ngom

IMR: Leif Nøttestad and Ørjan Sørensen

2.1 Introduction

The biological sampling conducted during this phase of the project continues to follow a flexible, opportunistic strategy aimed at strengthening and expanding the existing ABFT sample bank. This approach is designed to ensure the availability of representative samples across key geographic areas and life stages, supporting studies on stock structure, mixing dynamics, and long-term population trends. Sampling efforts are aligned with the broader goals of the MSE and are particularly relevant in the context of observed shifts in spatial distribution and the origin stock composition of feeding aggregates in the eastern Atlantic Ocean. Besides, during this phase sampling efforts have also been focused on providing new material that has allowed to investigate the role of Slope Sea spawning ground in the ABFT reproductive dynamics.

2.2 Material and Methods

As in previous phases, sampling activities have been carried out in collaboration with local fleets, research institutions, and observer programs, without requiring dedicated campaigns. During this Phase-14 samples have been obtained through collaboration with the Fisheries Resources Institute (FRI) of Japan, the Centre de Recherches Oceanographiques de Dakar (CRODT) in Senegal, which has made an in kind donation of ABFT tissue and otoliths sampled opportunistically from a confiscated catch, the National

Oceanic and Atmospheric Organization (NOAA) in EEUU, the IMR in Norway and ©Itsasbalfegó in the Bay of Biscay.

All sampling protocols, data recording, and shipping procedures have followed ICCAT and GBYP standards (See Annex I), and collected samples have been integrated into the central GBYP repository for future collaborative research (see Task 4).

2.3 Results

In this Phase-14 samples from 551 ABFT individuals have been incorporated to the GBYP Tissue Bank, consisting of 248 larvae, 20 medium sized fish (25-100 kg) and 283 large fish (>100 kg) (Table 2.1). In total, 1003 samples have been collected, consisting of 238 otolith pairs, 239 spines, 501 muscle or fin tissue and 25 gonads (Table 2.2).

Table 2.1: Individuals sampled within the Phase 14, in each area and per each age stratum.

Region	Area	Collaborator	Size class sampled			Total
			Larvae	Medium	Large	
NWATL	Slope Sea	NOAA	248			248
CNATL	Central Atlantic (East and West)	FRI			25	25
NEATL	Bay of Biscay	AZTI		20	97	117
	Norway	IMR			50	50
SEATL	Senegal	CRODT			111	111
Total			248	20	283	**551**

Table 2.2: Detailed number of otoliths, dorsal fin spines, muscle/fin tissue and gonad samples achieved within the Phase 14, in each area.

Region	Area	Collaborator	Tissue sampled				Total
			Otoliths	Spines	Muscle/Fin	Gonads	
NWATL	Slope Sea	NOAA			248		248
CNATL	Central Atlantic (East and West)	FRI	25				25
NEATL	Bay of Biscay	AZTI	59	85	96	25	265
	Norway	IMR	50	50	50		150
SEATL	Senegal	CROD	104	104	107		315
Total			238	239	501	25	**1003**

In the Slope Sea, a total of 248 larvae, both preflexion and postflexion developmental stages, have been sampled. Additionally, 10 more larvae have been received but they were not identified to species level and are pending for genetic species identification before they can also be accounted for or not. Due to unforeseen circumstances derived from the US government shutdown, that kept operations at a standstill for 1.5 months, the obtention of potentially spawning adults from the Slope Sea was not possible within this phase.

In the Central Atlantic, otoliths from 25 large specimens captured by Japanese longliners have been obtained, of which 22 have been captured east of the 45ºW boundary, and 3 west of the 45ºW boundary (Figure 2.1).

In the Bay of Biscay, 59 otoliths, 85 spines, 25 gonads and 96 tissue samples for genetic analysis of medium and large sized individuals have been collected using the experimental fattening facility located near Getaria, in collaboration with ©Itsabalfegó.

In waters off Norway, the IMR has provided 50 otolith pairs, 50 spines and 50 muscle tissue samples from 50 large sized individuals collected by purse-seiners operating in thisregion.

In addition, CRODT, on behalf of Senegalese authorities, has donated biological samples of 111 large adults captured incidentally off Guinea Bissau by a purse seine vessel targeting tropical tunas, from which 104 otolith pairs, 104 spines and 107 muscle and/or fin tissue have been obtained.

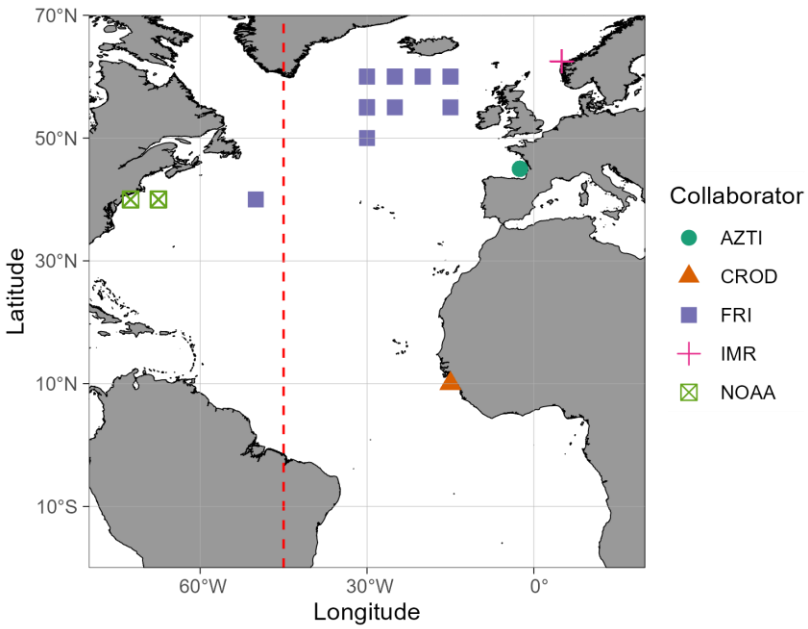

Figure 2.1 Geographical distribution of the ABFT individuals sampled during GBYP Phase 14 by AZTI (green circles), CRODT (orange triangles), FRI (purple squares), IMR (pink crosses) and NOAA (green crossed squares). Note that approximate sampling locations are shown (not actual longitudes and latitudes). Dashed red line indicates the current 45ºW management boundary by ICCAT.

2.4 Conclusions

The strategy of the Phase-14 biological sampling task has been to take advantage of ongoing fishing activities and collaborations, without requiring dedicated sampling campaigns. This flexible and cost-effective approach has proven effective to get samples from areas such as the Central North Atlantic, the Bay of Biscay, Norway and Senegal. This effort contributes to the characterization of medium and large individuals in these highly productive areas where Atlantic bluefin tuna are found in feeding aggregations. Besides, within this phase collaboration with the NOAA has facilitated access to larval samples from the Slope Sea. These samples are particularly valuable for advancing our understanding of the early life history and potential interbreeding between eastern and western bluefin tuna stocks. (see Task 3.1).

3. GENETIC ANALYSES AND STOCK OF ORIGIN ASSESSMENT

Task Leader: Natalia Diaz-Arce (AZTI) and Igaratza Fraile (AZTI)

Participants:

AZTI: Naiara Rodriguez-Ezpeleta, Iraide Artetxe-Arrate, Iñaki Mendibil, Natalia Gutierrez

3.1 Interbreeding between eastern and western stocks

3.1.1 Introduction

The Atlantic bluefin tuna (*Thunnus thynnus*) is a highly migratory species of major ecological and economic importance, traditionally managed as two distinct stocks associated with spawning in the Gulf of Mexico and the Mediterranean Sea. However, the discovery of larvae in the Slope Sea has challenged this paradigm, suggesting the existence of a third spawning area and raising questions about its demographic role and its connectivity with other components of the species' life cycle.

Previous genomic studies have revealed weak but significant differentiation between eastern and western populations and identified the Slope Sea as a mixed spawning ground where individuals of different ancestries interbreed. In particular, Diaz-Arce et al. (2024),reported the presence of a chromosomal inversion associated with outlier SNPs that grouped individuals into three distinct genetic clusters, potentially linked to adaptive variation and introgression. Additionally, analysis of Mediterranean and Gulf of Mexico inherited genomic regions across whole genome sequences of 9 larvae and young of the year collected in 2008 and 2016, respectively, suggest that strong gene-flow from the Mediterranean Sea may have occurred at strong and temporarily variable events (see results from GBYP phase 12). These findings highlighted the complexity of Atlantic bluefin tuna population structure and the need for temporal monitoring to assess the stability of genetic composition in the Slope Sea.

In this study, larvae collected during a dedicated survey in the Slope Sea in 2025 were analyzed to evaluate the persistence of admixture and inversion polymorphism over time. High-density SNP genotyping and comparison with reference individuals from the Gulf of Mexico and the Mediterranean Sea, was used aiming to (i) characterize the ancestry profiles of Slope Sea larvae based on neutral markers and (ii) assess the distribution of

inversion-associated haplotypes using previously identified outlier SNPs. These results provide new insights into the demographic and evolutionary dynamics of this spawning area and their implications for Atlantic bluefin tuna management.

3.1.2 Material and Methods

Obtention and processing of samples from the Slope Sea

Larval samples from specific scientific survey dedicated to collect bluefin tuna larval samples in the Slope Sea (SCRS/2025/216) were sent to AZTI's facilities by collaborators from the NOAA. Given that the survey happened on July 2025, the time required for sample identification and separation, and also due to US government shutdown, which paused public working activities during 1.5 months, larvae arrived to AZTI by mid-November, leaving short time for analysis. Therefore, only 192 larvae, out of the total 248 samples received, were included in the results section of this report. The first batch of 192 samples was sent immediately after receiving them to Xenetica Fontao for genotyping with the ABFT-Array, while the rest of the samples to a total of 248, were sent within a second batch.

Obtention of genotype tables and admixture analysis

Obtained raw cel files were analyzed together with a set of 70 and 26 Mediterranean and Gulf of Mexico reference individuals, respectively, already genotyped in previous GBYP phases, to maximize genetic diversity during genotype calling using the Axiom Analysis Suite software to make it more accurate. The recommended quality filtering parameters were applied and individuals with genotyping rates below 0.97 were excluded. Genotype data was exported to PLINK format and reference individuals included for improved genotyping were excluded.

First, the obtained genotype table was filtered to keep only neutral markers. This genotype table was analyzed using CKMRsim to detect the presence of kin pairs within the dataset. When kin pairs were detected, at least one sample of each pair was removed from the original genotype table in order to prevent bias in population structure analysis.

Then, the original genotype table was split into two tables containing neutral and outlier markers. The table containing neutral markers was merged with genotypes of 593 and 131 larvae and/or young of the year individuals from the Mediterranean Sea and the Gulf of Mexico obtained in previous GBYP phases. Only neutral SNPs with a minimum genotyping rate of 0.99 were kept. Individual ancestry proportions were estimated using the software ADMIXTURE (Alexander et al., 2009) assuming 2 and 3 ancestral

populations. Principal Component Analysis (PCA) was performed using the *adegenet* R package (Jombart, 2008).

The table containing outlier markers was used to perform a PCA to identify the three groups detected in previous studies derived from outlier markers.

Processing of 10 larval samples for obtention of whole genome sequences

Ancestry values (Q scores) estimated from the merged dataset containing neutral markers were used to select a total of 10 Slope Sea larval samples. Samples were also selected based on the amount and quality of available DNA. This set of samples was sent to the CNAG laboratories for sequencing. The resulting sequences are expected to cover the entire genome of the samples at a 12x coverage.

3.1.3 Results

Admixture in the Slope Sea

In total, from the 192 larvae 3 did not pass the quality filtering criteria and one pair resulted to be a replicate and therefore one from the pair was removed from the dataset. Among the remaining 188 larvae, 5 full sibling pairs were found. Besides, 12 half sibling pairs were found, among which two pairs involved the same individual and each of the two larvae involved in one full sibling pair. From this triad, both full sibling individuals were removed from the dataset. The final genotype table contained 5597 neutral markers and 131, 593 and 172 larvae and/or young of the year individuals from the Gulf of Mexico, the Mediterranean Sea and the Slope Sea, respectively.

Population structure analysis based on neutral markers revealed that larvae collected in the Slope Sea in 2025 had genetic profiles closely resembling those from the Gulf of Mexico. However, the Slope Sea samples exhibited a greater overlap than Gulf of Mexico samples with the Mediterranean ancestry distribution (Figure 3.1.1).

These results are similar to those obtained from larvae and young of the year collected in the Slope Sea in 2016, which showed high levels of admixture (Figure 3.1.2), indicating that the Slope Sea continues to act as a meeting point where fish from both stocks interbreed. However, although the high kinship proportions among the sampled individuals could reflect poor representation of the spawning area, the distribution of the genetic profiles of samples collected in 2025 is relatively homogeneous. The heterogeneous pattern observed in 2016 led to the hypothesis that gene-flow from the Mediterranean Sea towards the Slope Sea may not happen at a constant rate. In contrast, the new results

from 2025 could be compatible with a self-sustained population at equilibrium. The results from 2016 (and older) samples could have been influenced by strong gene-flow from Mediterranean individuals on that specific year. Additionally, the reduced number of samples available from that year could have led to high stochasticity, resulting into highly heterogeneous results. Historical genetic data on Slope Sea samples would help to better understand if there is strong variation on annual admixing rates in the Slope Sea.

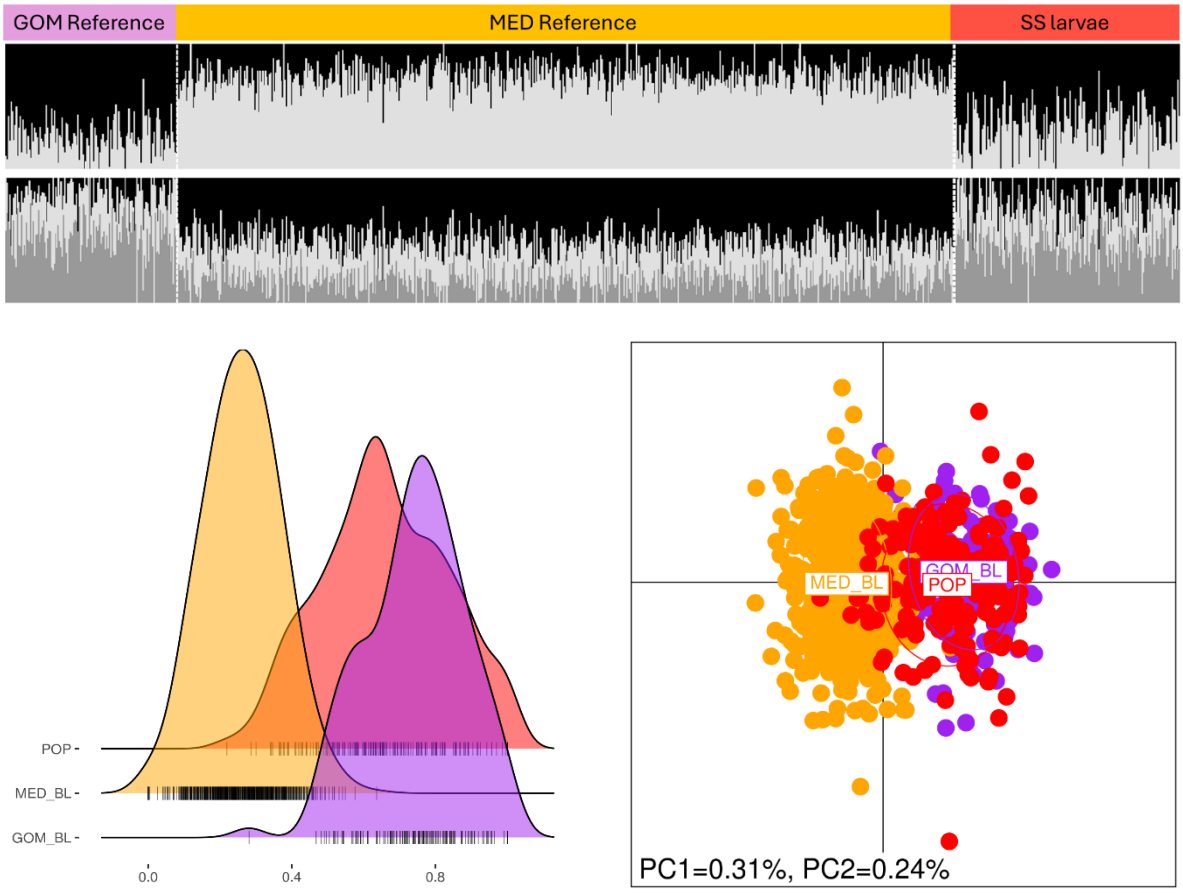

Figure 3.1.1. Population structure analysis based on 5597 neutral genetic markers of 131, 593 and 172 larvae and/or young of the year individuals from the Gulf of Mexico (purple), the Mediterranean Sea (orange) and the Slope Sea (red) respectively. Individual ancestry values estimated using ADMIXTURE (top panel), distribution of individual ancestry values (bottom left panel) and PCA (bottom right panel). Black and grey bars represent individuals' proportions of ancestral genetic components when assuming two ancestral populations.

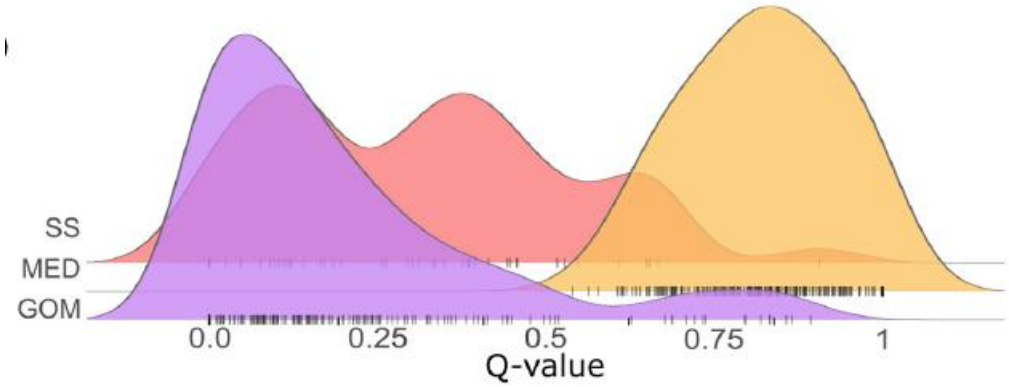

Figure 3.1.2. Figure from Diaz-Arce et al 2024. Distribution of individual ancestry values estimated using admixture for reference individuals (larvae, young of the year and/or spawning adults) from the Gulf of Mexico (purple), Mediterranean Sea (orange) and the Slope Sea (red), which included larvae collected in 2016 and young of the year collected in 2008.

Outlier genetic markers

A total of 172 Atlantic bluefin tuna larvae collected in the Slope Sea during 2025 were analyzed at 98 of the outlier SNPs previously identified by Díaz-Arce et al. (2024) as associated with a putative chromosomal inversion. Chromosomal inversions act as genetic "switches" that facilitate the tracking of population mixing. Three genetic groups were detected, corresponding to the two alternative haplotypes and the heterozygous genotype within this inversion (Figure 3.1.3): Group 1 included 136 individuals (79%), Group 2 representing heterozygotes, comprised 34 individuals (20%) and Group 3, the rarest haplotype combination, included only 2 individuals (1%). These proportions closely match those reported for Slope Sea samples in Díaz-Arce et al. (2024), where Group 3 was nearly absent and Group 2 occurred at intermediate frequency. This supports the persistence of inversion polymorphism and admixture in this spawning area. The predominance of Group 1 suggests continuity in the genetic composition of Slope Sea spawners over time, while the presence of Group 2 indicates ongoing interbreeding between MED-like and GOM-like ancestries. The extremely low frequency of Group 3 remains consistent with its hypothesized Mediterranean origin. These results are consistent with previous observations, suggesting no major changes in mixing patterns between 2016 and 2025.

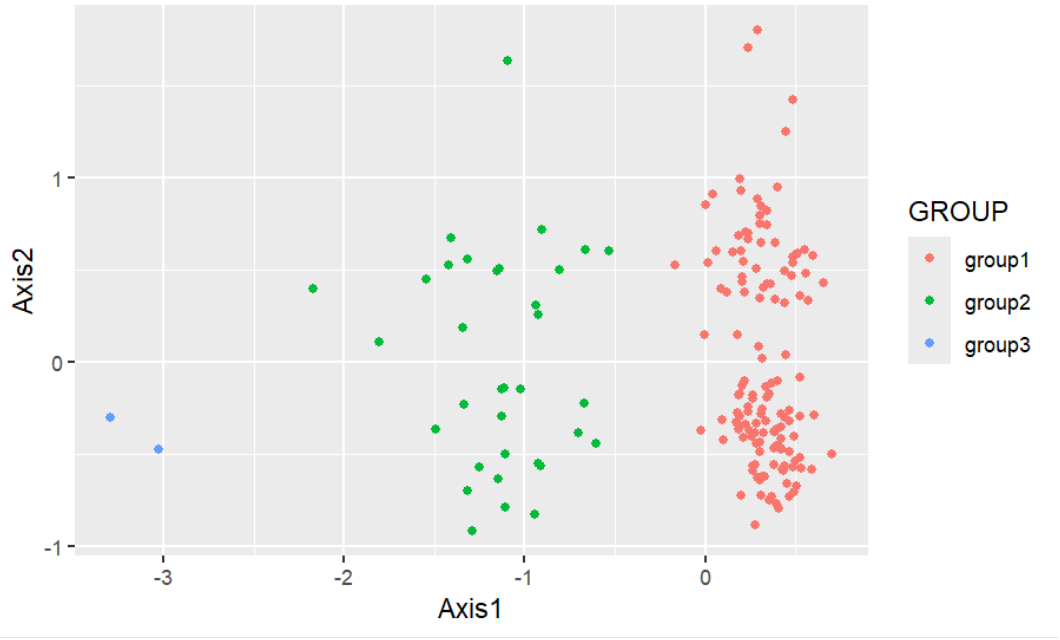

Figure 3.1.3. Principal Component analysis built based on 98 outlier SNPs and 172 larvae collected in the Slope Sea in 2025, where the same three clusters found in Diaz-Arce et al 2024 are formed.

3.1.4 Conclusions

The analysis of 172 Atlantic bluefin tuna larvae collected in the Slope Sea during 2025 confirms the persistence of genetic patterns previously described for this spawning area.

- More than 97% of the larvae were successfully genotyped, demonstrating good preservation of the collected samples. The levels of sibship found among the samples should be considered during sample collection design.
- Neutral markers indicate genetic profiles similar to those of Gulf of Mexico individuals, yet with a broader overlap with Mediterranean profiles than observed in Gulf of Mexico larvae, consistent with ongoing admixture.
- Compared to earlier observations from 2016, the genetic composition of the 2025 samples appears more homogeneous, suggesting that admixture rates may vary across years.
- Outlier SNPs reveal the same three inversion-associated groups as previously reported, with Group 1 dominating (79%), followed by Group 2 (20%) and Group 3 (1%), supporting the persistence of this genetic pattern ongoing admixture.
- Overall, these findings reinforce the role of the Slope Sea as a key area of genetic connectivity between eastern and western stocks and highlight the importance of continued temporal monitoring and integrated approaches to better understand its demographic significance and inform management processes.

3.2 Stock of origin

3.2.1 Introduction

Understanding the natal origin of Atlantic bluefin tuna is essential for accurately characterizing stock composition and informing management strategies. This task aimed to compile and analyze the most recent data on stock mixing using complementary approaches, genetics and otolith stable isotope chemistry ($\delta^{13}C$ and $\delta^{18}O$), to produce standardized and spatially and temporally explicit estimates of mixing proportions. These outputs are intended to support the future reconditioning of Management Strategy Evaluation (MSE) operating models by providing robust, validated information on stock-of-origin patterns.

To achieve this, datasets from previous GBYP phases were integrated with newly generated data to fill gaps in key strata defined by geographic region, season, and age class. Genetic assignments were performed using both high-density SNP arrays and targeted SNP panels, while otolith chemistry analyses were applied to infer origin based on environmental signatures recorded during early life. Comparative analyses between these methods were conducted to assess consistency and improve confidence in assignment results. Finally, mixing proportions were estimated using a Maximum Likelihood framework, incorporating uncertainty and misclassification rates.

The integration of these approaches provides a comprehensive view of ABFT stock mixing dynamics, offering critical insights for refining operating models within the MSE framework. These results will contribute to more accurate projections of stock connectivity and support adaptive management under changing environmental conditions.

3.2.2 Material and methods

This task aimed to characterize mixing rates of Atlantic bluefin tuna across the strata defined by the MSE operational model. To achieve this, new genetic and otolith stable isotope analyses to address existing data gaps (Table 3.2.1) were conducted. In addition, all relevant datasets generated under previous GBYP Biological Studies were compiled and harmonized, integrating them with newly produced data to create a comprehensive and standardized framework for estimating mixing rates across spatial, temporal, and age-class strata.

Table 3.2.1. Number of samples used for additional genetic and otolith chemistry analyses during the current Phase-14.

Years	EATL		NATL		GSL	SATL		WATL	TOTAL	
	GEN	OTO	GEN	OTO	GEN	GEN	OTO	GEN	GEN	OTO
2018								59	**59**	
2019	12		7					35	**54**	
2020	60		25			26			**111**	
2021	39		25			4		13	**81**	
2022	42		25		1	23		55	**146**	
2023			25	22		28			**53**	**22**
2024				3		28	30		**28**	**33**
2025	44	15							**44**	**15**
TOTAL	**197**	**15**	**107**	**25**	**1**	**109**	**34**	**162**	**576**	**70**

Genotyping of new samples

First, a summary of the total number of bluefin tuna samples for which genetic origin had been assigned from each year and geographical area was prepared (Table 3.2.2). Then, a total of 710 adult samples were selected to be analyzed and cover the identified geographical and year gaps when possible (Table 3.2.1). Different age classes and seasons were covered when possible, although sampling availability did not always allow to cover all strata. Samples were prepared and sent for genotyping at Xenetica Fontao laboratories. Originally, it was planned to genotype around 600 samples. However, since the number of samples available for Task 3.1 was lower than originally planned, it was decided to increase the number of samples for Task 3.2 by 110 samples. The last 134 samples were sent in late November in a separate batch and therefore the results are not included in this report.

Individual assignments based on ARRAY results

Genotype tables obtained for individuals captured at different feeding aggregates genotyped with the ABFT-ARRAY from previous GBYP phases (from 10 to 13) were merged into a single genotype table. Additionally, 617 and 174 reference individuals (larvae, young of the year and/or adults captured in the spawning area at the spawning season) from the Mediterranean Sea and the Gulf of Mexico were merged as baseline. The more than 3,000 larvae from the Mediterranean Sea were excluded, since the baseline already included a much higher number of individuals from the Mediterranean Sea than from the Gulf of Mexico, and the high levels of sibship would have biased the results.

Individual ancestry proportions assuming two ancestral populations were estimated for each individual using the software ADMIXTURE (Alexander et al., 2009).

Genetic origin assignment of samples that were genotyped with the ABFT-ARRAY were performed as follows. Distribution of the ancestry values of the individuals from the Mediterranean Sea and the Gulf of Mexico included in the baseline were calculated. Then, the average and standard deviation of both distributions were calculated. Finally, for each individual captured at feeding aggregates, the probability of belonging to each of the two distributions were estimated. Samples showing a probability of being from the Mediterranean Sea higher than 0.9, were assigned as Mediterranean (MED), and if this probability was 0.1 or less, were assigned as Gulf of Mexico (GOM). Intermediate values were labelled as unassigned (UNASS).

Individual ancestry of the newly genotyped samples was analyzed together with the baseline. Individual probabilities of belonging to the MED or GOM distribution and origin assignments were performed using the same procedure.

Individual assignments with the 86 SNP panel

Genetic origin assignments of individuals genotyped with the 96 SNP panel were based on the 86 SNPs in common with the first version of the panel developed in Rodriguez-Ezpeleta et al. 2019 and the new version developed in the GBYP Phase 9. For the baseline, the 94 larvae from the Gulf of Mexico genotyped in GBYP Phase 10 were added the original baseline produced in Rodriguez-Ezpeleta et al. 2019. For those individuals that had been genotyped with both the 96 SNP panel and the ABFT-array only assignments obtained with the second method were considered.

Only individuals successfully genotyped for at least 65 SNPs (>0.75 genotyping rate) were kept. A total of 2,296 samples of unknown origin captured at different feeding grounds in the Atlantic were assigned with GENECLASS2 (Piry et al., 2004) using the Rannala & Mountain (1997) criterion (0.05 threshold) considering results applying 80% threshold for assignment scores to assign samples as MED or GOM.

Stable isotope analyses of new otoliths

A data availability review was carried out to identify gaps in otolith data from previous years and regions. To address these gaps, 70 sagittal otoliths were selected for processing, ensuring a more complete temporal and spatial coverage for subsequent isotopic analysis (Table 3.2.1). Otolith processing followed the protocols described by Rooker et al., (2008) with minor adaptations. After extraction, sagittal otoliths were cleaned of residual tissue

using 1% nitric acid and rinsed with deionized water. One sagittal otolith per individual was embedded in Struers epoxy resin (EpoFix) and sectioned using a low-speed ISOMET saw to obtain transverse sections (approximately 1.2 mm thick) that included the otolith core.

Sections were mounted on sample plates, and core material corresponding to the yearling growth period of ABFT was isolated using a New Wave Research MicroMill system. A standardized two-vector drill path, based on measurements from several yearling ABFT, was applied to ensure consistency. Milling was performed with a 500 µm drill bit, using 12 passes at a depth of 50 µm to obtain powdered core material. Powdered core material was transferred to Eppendorf vials to later analyzed for $\delta^{13}C$ and $\delta^{18}O$ on an automated carbonate preparation device (KIEL-III, Thermo Fisher Scientific, Inc., Waltham, Mass.) coupled to a gas-ratio mass spectrometer (Finnigan MAT 252, Thermo Fisher Scientific, Inc.) at the University of Arizona. Currently, the samples have been sent to the laboratory; however, due to repeated malfunctions of the spectrometer, the results have not yet been received. Once the data becomes available, they will be incorporated into this work.

Individual assignments of existing otolith stable isotope data

Individual assignments of spawning origin were performed using a Random Forest classifier trained on reference baseline data from Brophy et al., (2020). The baseline included mature adults from the Gulf of Mexico (GOM) and Mediterranean (MED) spawning populations, characterized by otolith stable isotope signatures ($\delta^{13}C$ and $\delta^{18}O$). The classifier was implemented in R using the randomForest package and trained with carbon and oxygen isotope values as predictors.

To account for uncertainty in classification, a bootstrap procedure was applied: the baseline dataset was resampled 10,000 times, and a Random Forest model was fitted to each bootstrap replicate. For each individual in the mixed sample, class probabilities (MED vs GOM) were predicted across all iterations, and the median probability and standard deviation were calculated.

Assignments were made using a probability threshold approach: Individuals with MED probability ≥ 0.8 were assigned to MED, individuals with MED probability ≤ 0.2 were assigned to GOM and individuals with probabilities between 0.2 and 0.8 were classified as unassigned (UNASS).

Computation of mixing proportions

Assignments based on the 86 SNPs and the ABFT-ARRAY were combined. After individual assignments using both genetic and otolith chemistry techniques, mixing proportions were estimated using a Maximum Likelihood Estimation (MLE) approach. The likelihood function incorporated: fixed contributions from individuals confidently assigned to MED or GOM and a probabilistic contribution from unassigned individuals based on their predicted MED probability.

$$\log L(\theta) = N_{MED} \cdot \log\big((1-e)\theta + e(1-\theta)\big) + N_{GOM} \cdot \log\big((1-e)(1-\theta) + e\theta\big) + \sum_{i=1}^{N_{UNASS}} \log\big(\theta p_i + (1-\theta)(1-p_i)\big)$$

where:

θ = proportion of tuna of MED origin,

e=misclassification rate for otolith chemistry (Brophy et al., 2020)

$N_{\mathrm{MED}}, N_{\mathrm{GOM}}$ = number of individuals confidently assigned to MED or GOM

p_i = individual probability of being MED for each unassigned fish.

For otolith stable isotope data, a misclassification rate of e = 0.026 (from Brophy et al. 2020) was included to account for classification error. The proportion of MED-origin fish (θ) was estimated by maximizing the log-likelihood using Brent's method in R (optim function). Standard errors were derived from the curvature of the likelihood function (second derivative), and 95% confidence intervals were computed using the normal approximation (Figure 3.2.1).

$$SE(\hat{\theta}) = \sqrt{-\frac{1}{\frac{d^2}{d\theta^2}\log L(\theta)|_{\theta=\hat{\theta}}}}$$

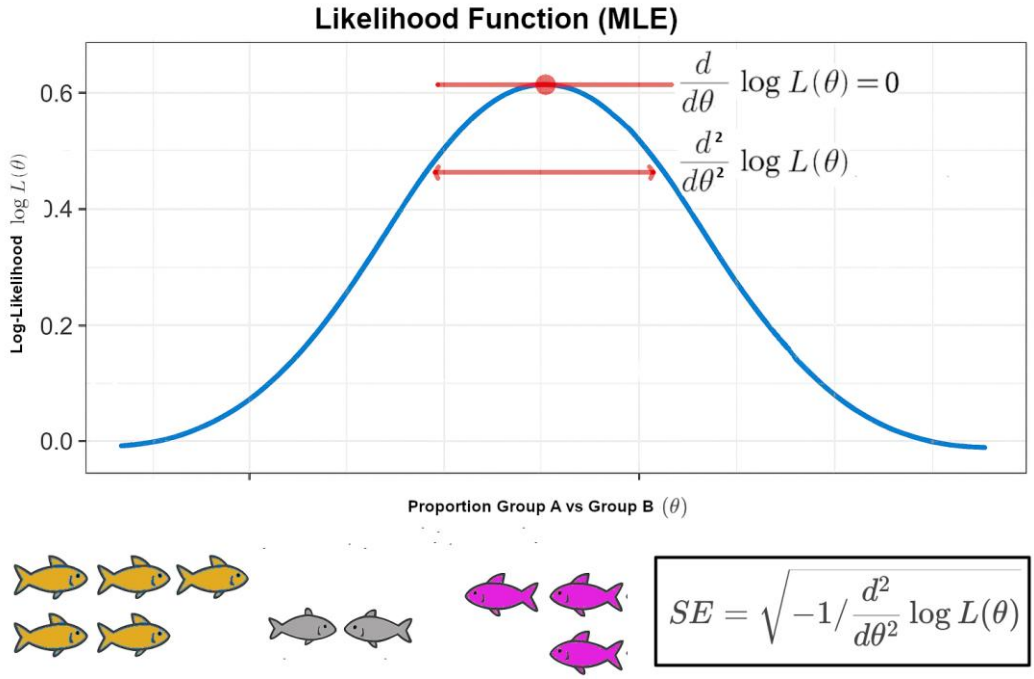

Figure 3.2.1: Likelihood-based estimation of the mixing proportion parameter (θ) using Maximum Likelihood Estimation. The plot shows the log-likelihood function logL(θ) for the proportion of fish from Group A (MED) versus Group B (GOM). The red point marks the maximum likelihood estimate ($\widehat{\theta}$), where the first derivative of the log-likelihood is zero. The curvature at this point, given by the second derivative, determines the precision of the estimate. The standard error (SE) is computed to reflect the uncertainty of $\hat{\theta}$

Mixing proportions were estimated by geographical areas considered in the operational model (Figure 3.2.2), and classified according to catch year, 4 annual quarters (Q1 from January to March, Q2 from April to June, Q3 from July to September and Q4 from October to December) and three age classes (age 0 to 4, age 5 to 8 and age +9).

In total, 3,235 individuals were successfully assigned to their genetic origin, including 2,283 based on the 86-SNP panel and 952 using the ABFT-ARRAY. An additional 710 newly genotyped samples from this phase will be incorporated into this comprehensive database. Furthermore, 3,350 stable isotope measurements generated during various phases of the ICCAT GBYP Biological Studies were included in the analysis.

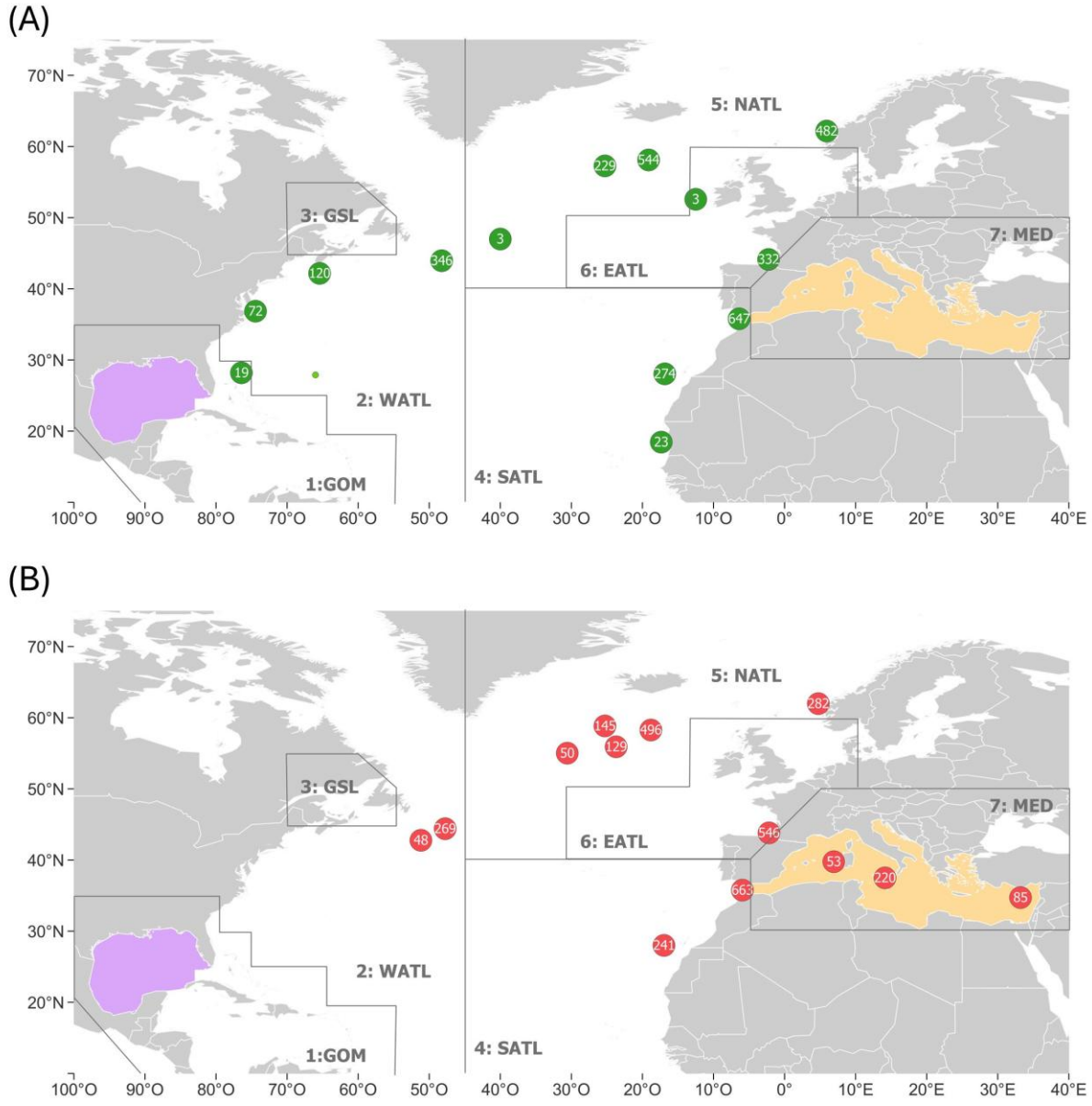

Figure 3.2.2. Map illustrating the seven geographical areas incorporated into the MSE operating model, along with the approximate sampling sites and corresponding sample sizes employed in genetics (upper panel) and the otolith chemistry (lower panel) analyses. Note that fishing positions in the map have been aggregated to preserve privacy requirements

3.2.3 Results

This section presents estimates of Atlantic bluefin tuna stock composition across seven spatial areas defined in the operational model on genetic and otolith chemistry data. Mixing proportions (% MED) were calculated using standardized assignment procedures and summarized at the spatial scale of MSE areas. Confidence intervals, standard errors, and sample sizes are provided to assess the precision of estimates. Results are first presented as overall proportions without stratification by catch-year, season and age-group followed by detailed analyses in subsequent subsections.

Table 3.2.2 summarizes the proportion of Mediterranean-origin fish (% MED) estimated using genetics (GEN) and otolith chemistry (OTO) for each ICCAT MSE area, including 95% confidence intervals, standard errors (SE), and sample counts. Overall patterns are consistent with expectations: very high MED proportions in the Mediterranean Sea and in the Atlantic east of 45°W, and intermediate proportions in the western Atlantic, which is recognized as a mixing zone. In the eastern Atlantic (EATL), both methods show strong agreement, confirming MED dominance in this region. In contrast, in the North and South Atlantic (NATL and SATL), noticeable methodological differences emerge: genetic-based estimates indicate a higher contribution of MED-origin fish compared to otolith-based results. In the western Atlantic (WATL), both methods converge again, indicating intermediate contributions from both stocks and confirming that a substantial proportion of Mediterranean-origin fish crosses the 45°W boundary. Genetic estimates were generally more precise (lower SE), whereas otolith-based estimates exhibited wider intervals.

Table 3.2.2. Estimated mixing proportions (% MED) by ICCAT MSE area, including lower and upper confidence limits, standard error (SE), and sample counts: number of fish assigned to the Mediterranean (N_MED), Gulf of Mexico (N_GOM), unassigned to any source population (N_UNASS), and total sample size (N_TOTAL).

Area	EATL		NATL		SATL		WATL		MED	
	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO
Low_limit	95.9	94.53	97.0	81.82	95.3	86.22	59.3	58.85	-	95.12
% MED	97.5	97.07	97.8	84.43	96.5	88.79	63.3	65.24	-	98.31
Upper_limit	99.0	99.61	98.7	87.04	97.7	91.36	67.2	71.64	-	100
SE	0.78	1.29	0.43	1.33	0.62	1.31	2.02	3.26	-	1.63
N_MED	379	337	1119	700	824	638	347	144	-	180
N_GOM	10	18	25	155	30	97	205	80	-	7
N_UNASS	15	107	115	215	87	139	79	93	-	82
N_TOTAL	404	462	1259	1070	941	874	631	317	-	269

Figure 3.2.2 provides a visual comparison of mixing proportions estimated by genetics and otolith chemistry across MSE areas. Both methods show near-complete MED dominance in EATL (GEN 98%, OTO 97%) and high MED proportions in NATL and SATL, although otolith-based estimates indicate greater GOM contribution than genetics (NATL: GEN 98% vs OTO 84%; SATL: GEN 96% vs OTO 89%). In WATL, both methods converge, showing intermediate mixing proportions (GEN 63% MED vs 37% GOM; OTO 65% MED

vs 35% GOM), confirming that a substantial fraction of Mediterranean-origin fish crosses the 45°W boundary.

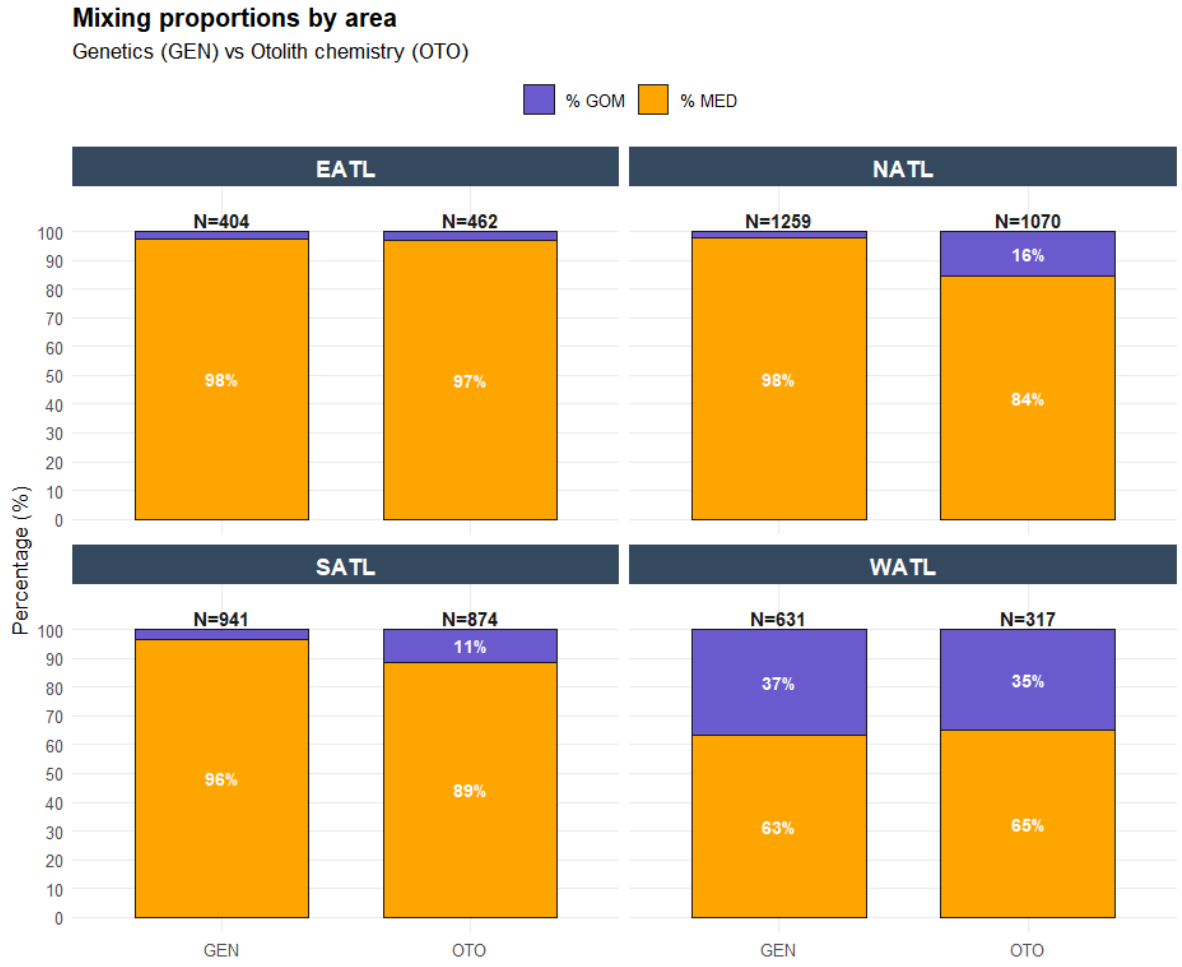

Figure 3.2.2. Comparison of mixing proportions across geographic areas using Genetics (GEN) and Otolith chemistry (OTO) techniques. Each bar represents the proportion of MED (orange) and GOM (purple), which together sum to 100%. The sample size used to estimate each proportion is indicated above the corresponding bar.

The following tables provide a detailed breakdown of data coverage and mixing proportion estimates by catch year, quarter, and age group, based on individual stock-of-origin assignments from genetics and otolith chemistry. Table 3.2.3 highlights temporal coverage across catch years, revealing strong representation for 2011–2017 in both methods, while earlier years (2008–2010) and recent years (2021–2025) show sparse or no data, particularly for otolith chemistry. Table 3.2.4 presents annual mixing proportions, showing consistently high MED contributions (>90%) in EATL and MED areas across

years, while NATL and SATL exhibit greater variability, especially in otolith-based estimates (e.g., NATL OTO ranges from ~53% to 100%). WATL consistently shows intermediate proportions, confirming its role as a mixing zone, but with notable year-to-year fluctuations, likely reflecting sampling heterogeneity and seasonal effects. However, despite these fluctuations, no clear temporal trends in mixing rates were detected across the time series, indicating stable overall patterns in stock composition (Figure 3.2.3).

Table 3.2.3. Data coverage by Catch Year for estimating mixing proportions. Cell shading indicates sample size: red for N < 10, orange for 10 ≤ N ≤ 30, and green for N > 30.

Area	EATL		NATL		SATL		WATL		MED	
Catch-Year	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO
2008	--	--	--	--	--	--	8	--	--	--
2009	--	108	--	3	--	--	--	--	--	--
2010	--	130	--	113	2	19	--	2	--	1
2011	129	172	89	71	158	207	45	23	--	163
2012	81	52	133	153	131	121	51	18	--	56
2013	--	--	102	83	157	103	87	53	--	--
2014	--	--	102	75	47	87	72	104	--	--
2015	--	--	135	72	88	74	89	36	--	19
2016	--	--	279	101	105	93	168	27	--	30
2017	--	--	273	69	133	12	111	29	--	--
2018	--	--	127	71	57	49	--	25	--	--
2019	--	--	19	22	34	98	--	--	--	--
2020	--	--	--	96	1	11	--	--	--	--
2021	21	--	--	40	24	--	--	--	--	--
2022	18	--	--	55	4	--	--	--	--	--
2023	90	--	--	46	--	--	--	--	--	--
2024	62	--	--	--	--	--	--	--	--	--
2025	3	--	--	--	--	--	--	--	--	--

Table 3.2.4: Proportions of MED fish (with SE) by Catch Year. Cell shading indicates sample size: red for N < 10, orange for 10 ≤ N ≤ 30, and green for N > 30. A minimum of 10 observations per year was required to compute mixing proportions.

Area	EATL		NATL		SATL		WATL		MED	
Catch-Year	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO
2008	--	--	--	--	--	--	--	--	--	--
2009	--	93.8 (3.0)	--	--	--	--	--	--	--	--
2010	--	100 (9.9)	--	68.3 (5.1)	50 (35.3)	79.9 (11.4)	--	--	--	--
2011	97.6 (1.37)	96.1 (2.6)	97.6 (1.7)	95.7 (3.4)	96.4 (1.5)	95.7 (2.0)	68.5 (7.6)	38.0 (13.3)	--	92.7 (3.5)
2012	95.0 (2.4)	94.2 (4.1)	98.3 (1.2)	74.9 (4.2)	99.2 (0.8)	92.0 (3.2)	65.1 (6.9)	19.6 (11.3)	--	100 (14.0)
2013	--	--	99.0 (1.0)	52.7 (6.5)	96.7 (1.4)	79.8 (4.7)	57.0 (5.6)	41.0 (8.3)	--	--
2014	--	--	95.9 (2.0)	83.3 (5.3)	97.8 (2.2)	80.2 (5.0)	82.7 (4.9)	90.6 (3.8)	--	--
2015	--	--	95.0 (1.9)	88.3 (5.0)	97.5 (1.7)	74.0 (5.6)	65.1 (5.2)	83.2 (8.6)	--	100 (23.8)
2016	--	--	98.1 (0.8)	89.9 (3.8)	96.9 (1.7)	86.3 (4.5)	59.6 (3.9)	70.3 (10.5)	--	100 (17.5)
2017	--	--	97.9 (0.9)	85.0 (5.4)	96.8 (1.5)	100 (29.7)	60.5 (4.7)	54.8 (10.4)	--	--
2018	--	--	100 (8.5)	86.7 (4.9)	96.0 (2.7)	95.5 (4.0)	--	45.5 (12.3)	--	--
2019	--	--	100 (23.4)	100 (21.8)	79.4 (7.4)	96.1 (2.8)	--	--	--	--
2020	--	--	--	95.6 (2.8)	100 (99.9)	100 (34.0)	--	--	--	--
2021	90.5 (6.4)	--	--	100 (2.7)	100 (20.7)	--	--	--	--	--
2022	100 (23.5)	--	--	100 (14.4)	100 (51.4)	--	--	--	--	--
2023	98.9 (1.1)	--	--	100 (15.7)	--	--	--	--	--	--
2024	100 (12.8)	--	--	--	--	--	--	--	--	--
2025	100 (57.7)	--	--	--	--	--	--	--	--	--

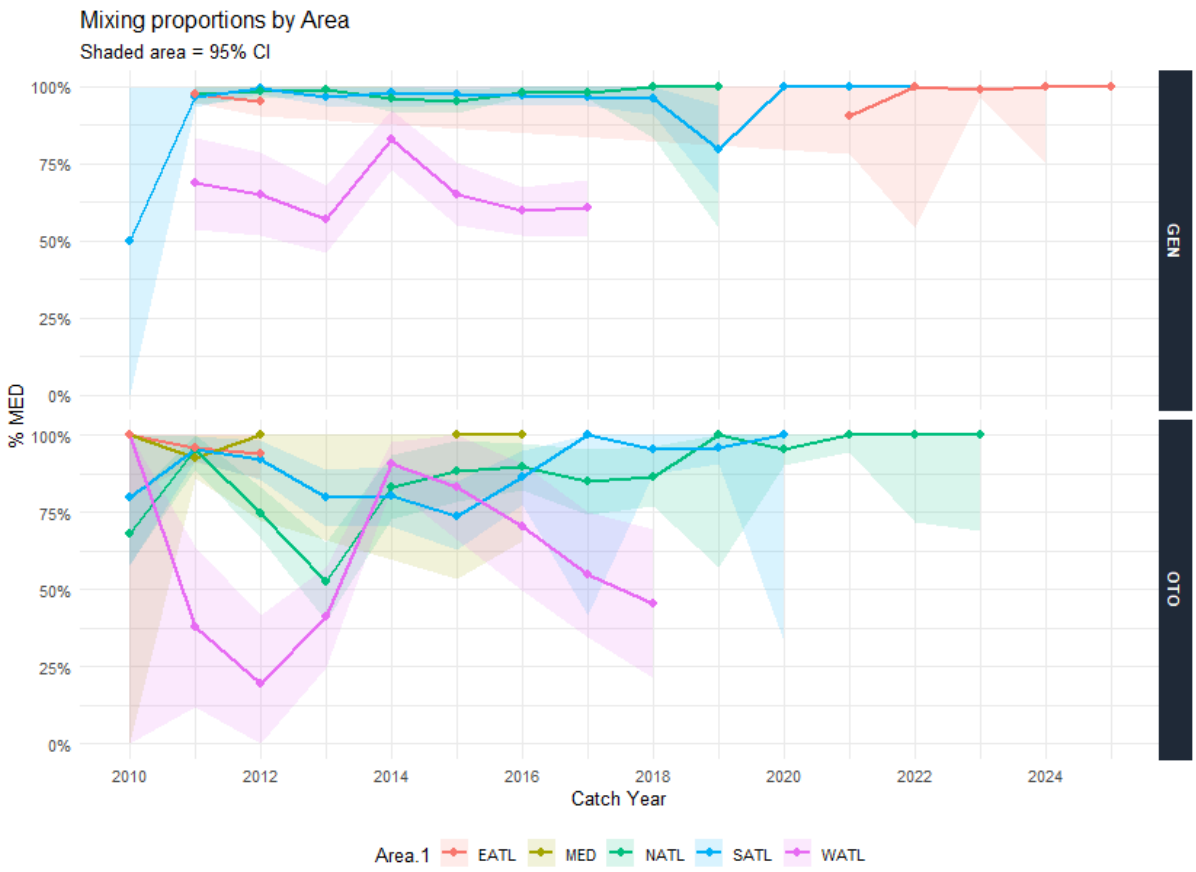

Figure 3.2.3. Temporal trends in mixing proportions by area for two techniques (GEN and OTO). Each panel represents one technique, with colored lines showing estimated proportions for five areas (EATL, MED, NATL, SATL, WATL) across catch years. Shaded regions indicate 95% confidence intervals (CI) around the estimates. The y-axis shows proportion (0–100%), and the x-axis shows catch year (2009–2025).

Quarterly summaries (Tables 3.2.5–3.2.6) indicate that most data were concentrated in Q3 and Q4, with limited coverage in Q1 and Q2, particularly for otolith chemistry. This uneven distribution reflects the seasonal nature of sampling and the operational constraints of collecting otoliths. Mixing proportions by quarter generally mirror annual trends: EATL and MED remain dominated by MED-origin fish (>95%), confirming strong stock fidelity in these regions year-round. In contrast, WATL shows intermediate contributions from both stocks, but with clear seasonal variability: genetic estimates range from ~50% MED in Q1 to >70% in Q4, while otolith-based estimates fluctuate between ~54% and 66%, suggesting that mixing intensity may vary with seasonal migration patterns and feeding aggregations. In NATL and SATL, genetics consistently indicates high MED proportions whereas otolith chemistry suggests greater GOM

presence in some quarters. The differences found between the techniques are highly influenced by the sample selection within the strata. Notably, Q3, the quarter with the largest sample sizes, shows the highest agreement between methods, reinforcing the reliability of estimates when coverage is robust.

Table 3.2.5: Data coverage by Quarter for estimating mixing proportions. Cell shading indicates sample size: red for N < 10, orange for 10 ≤ N ≤ 30, and green for N > 30.

Area	EATL		NATL		SATL		WATL		MED	
Quarter	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO
Q1	67	--	--	--	235	185	26	6	--	--
Q2	53	22	7	--	376	501	62	--	--	161
Q3	234	413	493	295	149	70	400	207	--	108
Q4	50	27	759	775	181	118	143	104	--	--

*Table 3.2.6: Proportions of MED fish (with SE) by **Quarter.** Cell shading indicates sample size: red for N < 10, orange for 10 ≤ N ≤ 30, and green for N > 30. A minimum of 10 observations per quarter was required to compute mixing proportions.*

Area	EATL		NATL		SATL		WATL		MED	
Quarter	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO
Q1	98.5 (1.5)	--	--	--	94.0 (1.6)	83.3 (3.2)	50.1 (10.0)	--	--	--
Q2	96.0 (2.7)	100 (25.9)	--	--	96.8 (0.9)	88.7 (1.7)	56.2 (6.4)	--	--	98.7 (1.7)
Q3	97.0 (1.1)	96.4 (1.4)	98.9 (35.9)	97.7 (1.4)	97.8 (1.2)	92.7 (4.2)	62.2 (2.5)	66.3 (3.9)	--	96.7 (4.0)
Q4	100 (14.1)	100 (21.8)	97.2 (0.6)	78.7 (1.8)	98.2 (1.0)	95.6 (2.6)	72.6 (4.0)	66.4 (5.8)	--	--

Age-group analyses (Tables 3.2.7–3.2.8) reveal that younger fish (0–4 years) are strongly MED in all areas (>96%), while older age classes (9+) maintain high MED proportions in EATL and NATL but show slightly lower values in WATL (GEN: ~61%; OTO: ~66%), consistent with increased GOM proportion in older individuals. Otolith-based estimates for mid-age classes (5–8 years) tend to indicate more GOM contribution than genetics, reinforcing the methodological differences observed in aggregated results.

Table 3.2.7: Data coverage by Age-group for estimating mixing proportions. Cell shading indicates sample size: red for N < 10, orange for 10 ≤ N ≤ 30, and green for N > 30.

Area	EATL		NATL		SATL		WATL		MED	
Age-group	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO
0-4	186	391	--	2	145	41	77	4	--	97
5-8	146	70	284	278	106	94	103	15	--	44
9+	72	--	974	789	690	736	443	294	--	128
NA	--	1	1	1	--	3	8	4	--	--

Table 3.2.8: Proportions of MED fish (with SE) by Age-group. Cell shading indicates sample size: red for N < 10, orange for 10 ≤ N ≤ 30, and green for N > 30. A minimum of 10 observations per age-group was required to compute mixing proportions.

Area	EATL		NATL		SATL		WATL		MED	
Age-group	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO	GEN	OTO
0-4	96.2 (1.4)	98.2 (1.3)	--	--	97.9 (1.2)	99.8 (2.9)	62.3 (5.7)	--	--	96.3 (4.3)
5-8	98.6 (0.9)	91.9 (4.2)	94.6 (1.4)	72.7 (3.2)	98.9 (1.0)	93.6 (3.3)	74.1 (4.6)	65.5 (14.3)	--	95.6 (4.9)
9+	98.6 (1.4)	--	98.8 (0.3)	88.4 (1.4)	95.9 (0.7)	87.4 (1.5)	60.8 (2.4)	65.7 (3.4)	--	99.7 (1.7)

3.2.4 Conclusions

This work demonstrates the effectiveness of a multidisciplinary approach to characterize Atlantic bluefin tuna stock mixing by combining genetic markers (SNP panels and high-density arrays) with otolith stable isotope chemistry ($\delta^{13}C$, $\delta^{18}O$). Harmonizing historical datasets with newly generated data and applying standardized assignment protocols allowed for robust origin classification across spatial, temporal, and age-class strata.

The integration of these complementary techniques provides added value by reducing uncertainty and improving confidence in stock-of-origin estimates. Genetic analyses capture patterns of population connectivity and divergence across evolutionary time scales, reflecting long-term gene flow and historical demographic processes, whereas otolith microchemistry provides fine-scale resolution of habitat use over a life span, thereby offering complementary perspectives on population structure and revealing informative differences in mixing patterns. Together these approaches provide complementary insights that strengthen interpretations of population structure and

supports a more comprehensive understanding of connectivity between Mediterranean and Gulf of Mexico spawning populations.

Key findings include:

- Genetic and otolith chemistry approaches provide broadly consistent estimates of mixing proportions in areas of clear stock dominance, such as the eastern Atlantic (EATL) and the main mixing zone of the western Atlantic (WATL). However, in the North and South Atlantic (NATL and SATL), genetics consistently indicates higher proportions of Mediterranean-origin fish compared to otolith-based estimates. This discrepancy reflects the fundamental difference between the two methods: otolith chemistry captures the environmental signature of the geographic natal origin, whereas genetics reflects ancestral origin, which may, at least in part, explain the differences in the estimates.

- No clear temporal trends were detected overall; however, an apparent increase in MED proportion in NATL based on otolith chemistry likely results from sampling bias, as early samples were collected in the central Atlantic while recent samples originated mainly from the Norwegian coast.

- Seasonal patterns were visible in SATL and WATL, where GOM contributions were higher during the first half of the year, and in NATL during Q4, likely due to fisheries operating closer to the 45°W boundary in that season.

- Age-related differences were minimal, although in SATL a slight increase in GOM proportion was observed in older fish (age group 9+) using both methods, while mixing rates remained stable across age classes in other areas.

These findings underscore the importance of considering spatial, seasonal, and sampling effects when interpreting mixing dynamics and integrating them into MSE operating models. Importantly, the outputs are directly applicable to Management Strategy Evaluation (MSE). Incorporating these standardized, validated estimates into operating models will enhance the realism of stock assessment scenarios, improve projections of stock connectivity, and support adaptive management under dynamic environmental conditions.

4. INFORMATION SYSTEM

Task Leader: Igaratza Fraile (AZTI)

Participants:

AZTI: Iraide Artetxe-Arrate, Ainhoa Orbe

4.1 Introduction

This task seeks continued support to maintain, update, and enhance the online application developed in previous GBYP phases for the visualization and management of biological sample availability and associated metadata (http://aztidata.es/BioTuna). For that, an information system hosted in a public repository and delivered as an interactive Shiny application provides a robust, reproducible framework for visualizing and managing biological sample availability and associated metadata within the GBYP tissue bank. The interface lets users dynamically choose parameters such as geographic areas, time windows, sample types, biological characteristics etc., and explore them through faceted filters and responsive maps, tables and plots.

All visual outputs generated by the application, such as maps, charts, and output graphics, are publicly accessible, and can be downloaded from the online application. However, access to the underlying metadata is restricted. Users wishing to obtain metadata must complete an online request form, which enables the GBYP tissue bank coordinators to evaluate the purpose of the request and grant time-limited access in accordance with ICCAT data-protection procedures.

An advanced information system is essential for maximizing the scientific value of the GBYP tissue bank. By consolidating diverse datasets into a unified, interactive platform, users can efficiently explore the composition of samples and visualize the outcomes of complex analyses. The tool is designed to serve two key user groups: (1) Scientist from various institutions, who require a centralized catalogue of available samples and associated metadata to support diverse research activities, and (2) ICCAT members and fisheries managers, who benefit from a real-time and user-friendly interface to visualize and filter biological parameters essential for stock assessment and management advice.

4.2 Material and Methods

During this phase, the system was updated with consolidated results, including age estimates from otolith and spine readings and mixing proportions from otolith microchemistry, a 96 SNP panel, and the SNP array. A new "Results" tab was added to the Shiny app, while existing tabs like "Map" and "Data distribution" were refined for quicker insights. The "Data" tab was also expanded, introducing a formal request system that standardizes how researchers access data, minimizes ad hoc exchanges that risk errors or noncompliance, and creates an auditable record of requests and conditions.

4.3 Results

The system now includes a filter section and four tabs: "Map", "Data distribution", "Results" and "Data".

Map tab

The "Map" tab of the Shiny application provides a global overview of sample distribution based on the filters selected by the user (Figure 4.1). In the previous version of the application, sample locations were displayed on a 1x1 degree grid, allowing users to visualize individual sampling points. However, due to confidentiality concerns raised by some sample providers regarding the public display of fishing locations, the mapping approach has been revised and edited accordingly. To ensure privacy while maintaining the utility of spatial data visualization, the updated "Map" tab now aggregates sample availability by FAO major fishing areas and sub-areas, using a color-coded system to represent the relative abundance of samples within each sub-area. This new approach preserves the anonymity of exact sampling locations while providing meaningful information on geographical coverage of ABFT samples and individuals.

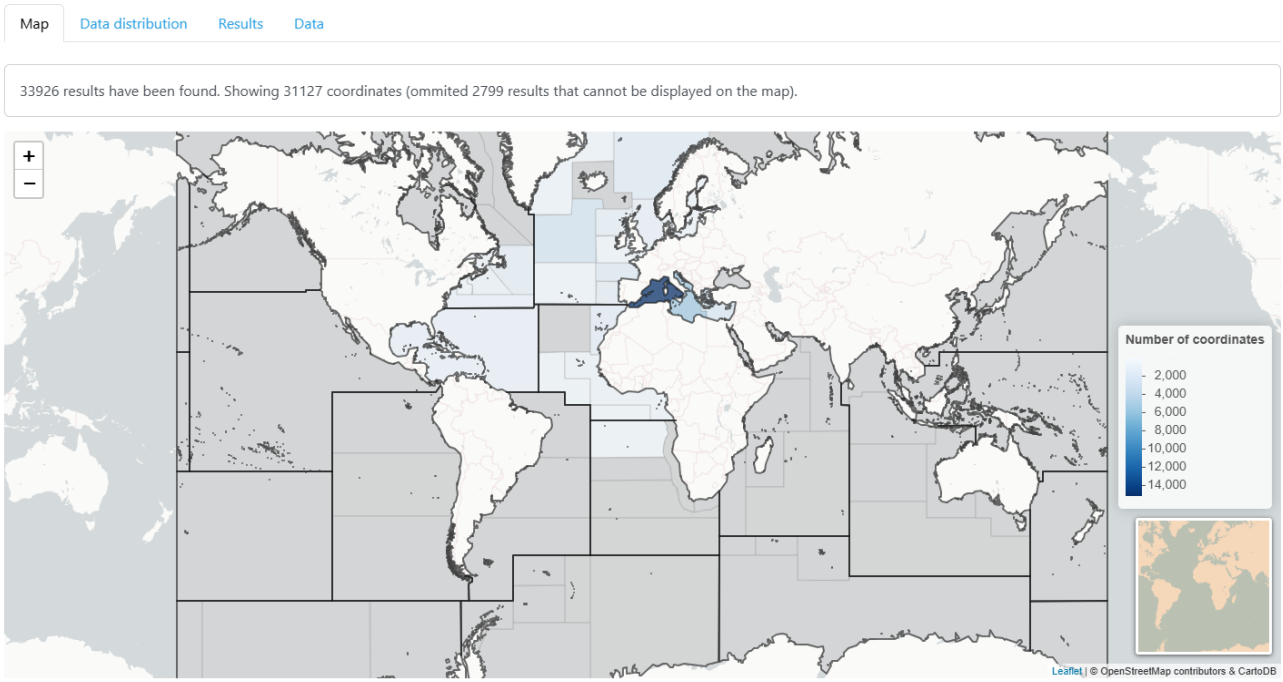

Figure 4.1. Map tab of the Biotuna shiny application, showing geographic distribution of the ABFT data collected under GBYP Biological sampling programme. Blue-scale colour intensity represents relative sample abundance within each sub-area, ensuring confidentiality while providing an informative spatial overview.

In addition to the FAO-level overview, the map also supports zooming into predefined sub-areas, sampling strata pre-defined within FAO regions, offering a more detailed view of sample distribution without compromising confidentiality (Figure 4.2). These sub-areas provide finer spatial resolution and are particularly useful for regional analyses and stock-specific assessments.

The map is fully interactive and responds dynamically to the filters applied on the left-hand panel (see figure 4.2), which include species, catch year, size class, sample type, and other biological or geographic criteria. Once filters are selected, users can click the "Apply" button to update the map view. The "Clean" button resets the search.

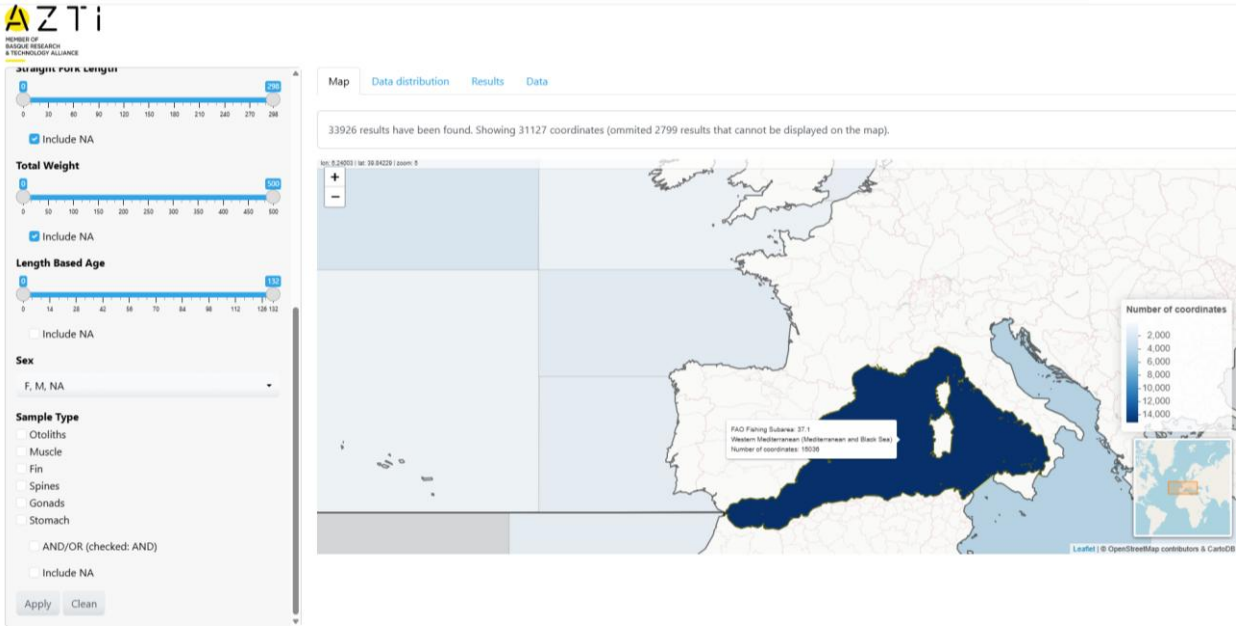

Figure 4.2. Left panel showing applicable filters for map visualization. Right panel is a zoomed view of the Map, illustrating sample availability aggregated by FAO fishing sub-areas. In this example, the Western Mediterranean Sea (FAO Subarea 37.1) is highlighted, showing the number of coordinates (number of fish sampled) that meet the selected filter criteria.

Data distribution tab

The updated "Data distribution" tab has slightly changed from previous versions. It currently presents a set of interactive visualizations intended to facilitate empirical examination of the dataset composition and facilitate data-driven decision-making. This functionality enables users to explore the structure and characteristics of the available fish through five distinct distribution charts:

Length Distribution: Displays the frequency of samples across different straight fork length ranges, offering insights into size composition.

Weight Distribution: Illustrates the distribution of total weight, enabling users to identify patterns in biomass representation.

Age Distribution: Shows the estimated age of individuals based on length-age relationships, which is critical for life history and growth studies.

Distribution by Year of Capture: Highlights temporal trends in sampling effort and availability, useful for understanding interannual variability.

Distribution by Year of Birth: Provides cohort-based visualization, supporting analyses of recruitment and population dynamics.

Each chart is fully interactive and responds to the filters applied. Users can adjust bin sizes to refine the granularity of the visualization, making it easier to detect patterns or anomalies. Additionally, the charts include options to break down distributions by sex or FAO area, adding another layer of detail for comparative analyses (Figure 4.3).

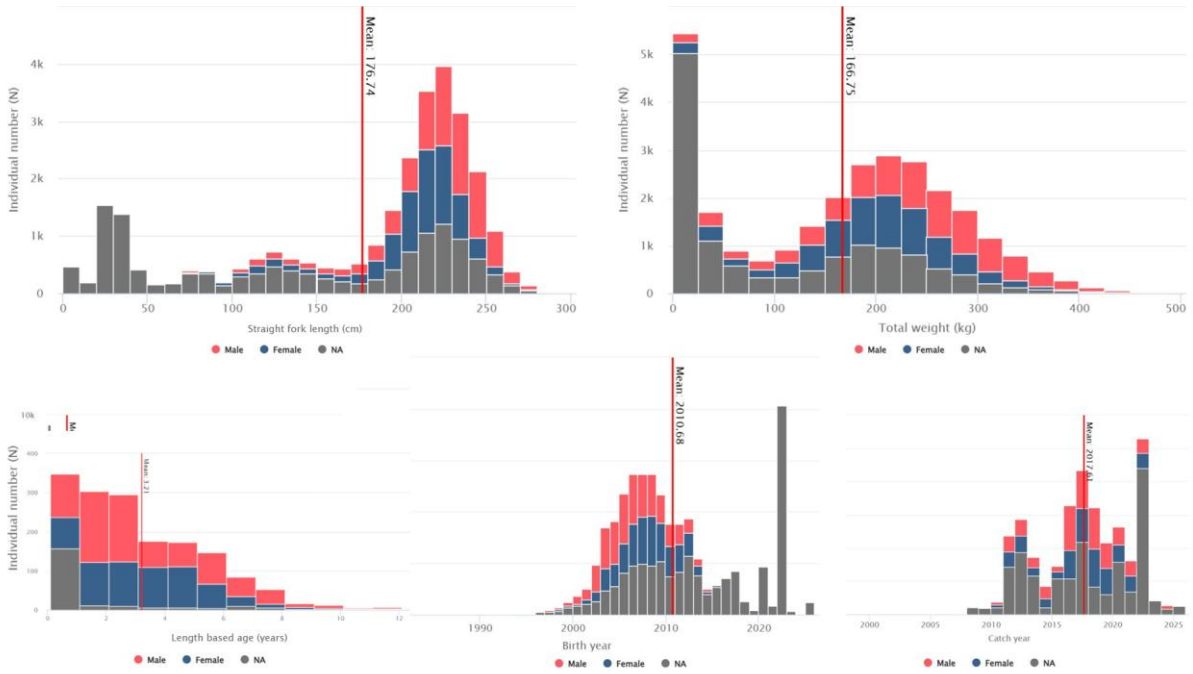

Figure 4.3. Examples of the graphs that can be obtained from the "Data distribution" tab: straight fork length distribution, total weight distribution, estimated age distribution, year of capture distribution, and year of birth distribution. Here data has been showed by sex (male, female or NA). Red vertical line shows mean distribution.

Results tab

The "Results" tab offers real-time visualization of biometric relationships, including: (1) fish length–weight, (2) fish length–otolith weight, and (3) fish weight–otolith weight. These relationships are dynamically calculated according to the selected filters, ensuring that the outputs reflect the subset of data relevant to the user's query. Each plot is accompanied by the corresponding regression equation and the coefficient of determination (R^2), providing an indication of the model fit (Figure 4.4). These relationships are essential for growth studies, age estimation, and stock assessment modelling, as they allow researchers and managers to derive accurate conversions between key biological parameters.

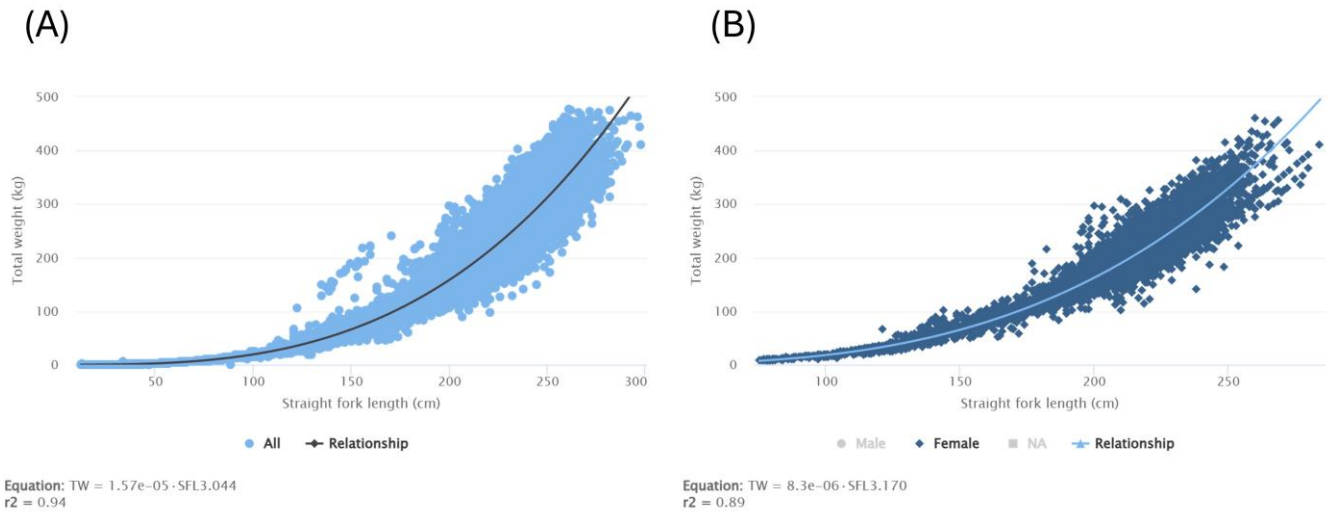

Figure 4.4 Example of length-weight biometric relationship graphs displayed in the "Results" tab; (A) All ABFT lengh-weigth relationship and (B) length-weight relationship filtered for female ABFT only. Relationship equation and R^2 are shown for each graph.

Additionally, the 'Results' tab includes graphical representations of sex ratio (Figure 4.5) and maturity curves (Figure 4.6). As with other visualizations, these charts are automatically updated according to the chosen filters (e.g., species, FAO area, size class, catch year), ensuring relevance to the user's query.

Finally, this section provides an option to visualize population mixing proportions (Mediterranean vs. Gulf of Mexico) for Atlantic bluefin tuna, derived from otolith microchemistry analyses, the 96-SNP panel, and the SNP array (Figure 4.7). These estimates have been calculated following the latest criteria adopted within the GBYP Biological Studies, ensuring consistency with current best practices. For transparency and reproducibility, the details of the methodology used to generate these estimates are fully described in the corresponding section of the final report.

To facilitate reporting and sharing, the application also offers the option to export any chart in multiple formats, including PNG, JPG, PDF, and SVG. This feature allows users to easily incorporate visual outputs into presentations, reports, or scientific publications in real time without additional processing.

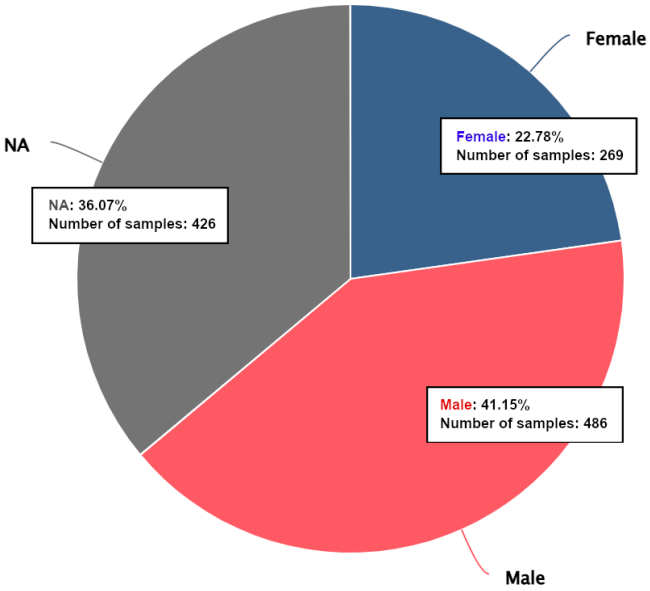

Figure 4.5. Sex ratio distribution within the filtered dataset, showing the proportion of males (41.15%), females (22.78%), and individuals with no available sex information (NA: 36.07%). The interactive pie chart allows users to hover over each segment to view exact percentages and sample counts.

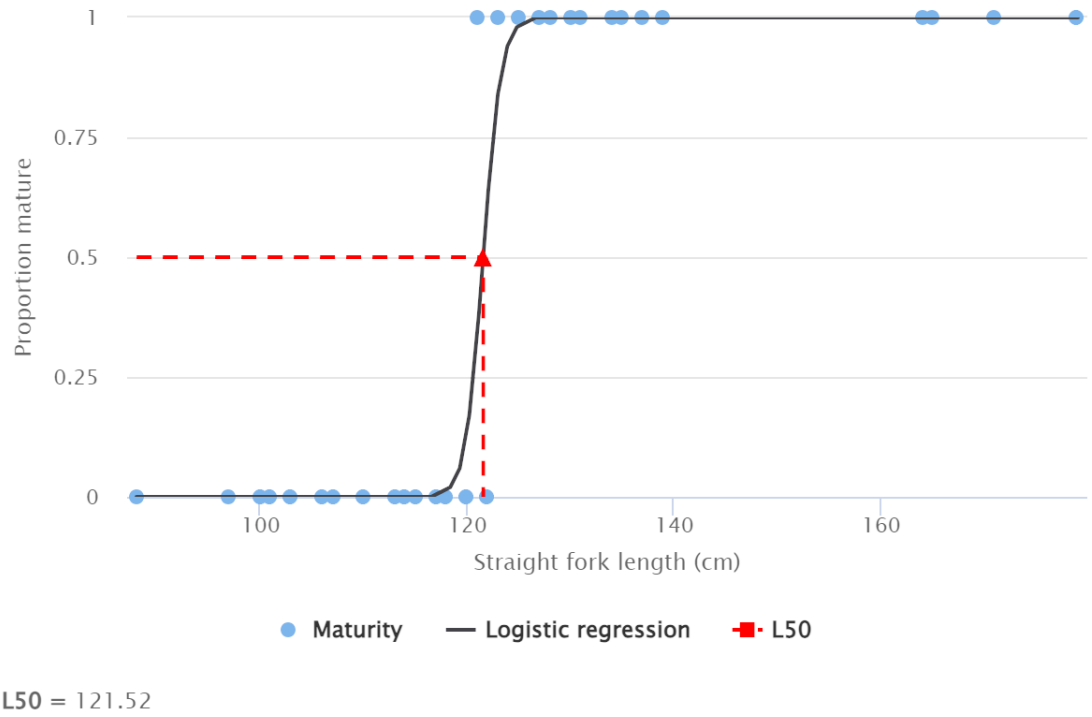

Figure 4.6: Maturity curve for bluefin tuna (Thunnus thynnus) based on logistic regression. The plot shows the proportion of mature individuals as a function of straight fork length (cm). Blue points represent observed maturity data, while the black line indicates the fitted logistic regression curve. The red dashed lines highlight the length at 50% maturity (L_{50} = 121.52 cm), which is a key parameter for reproductive biology and stock assessment.

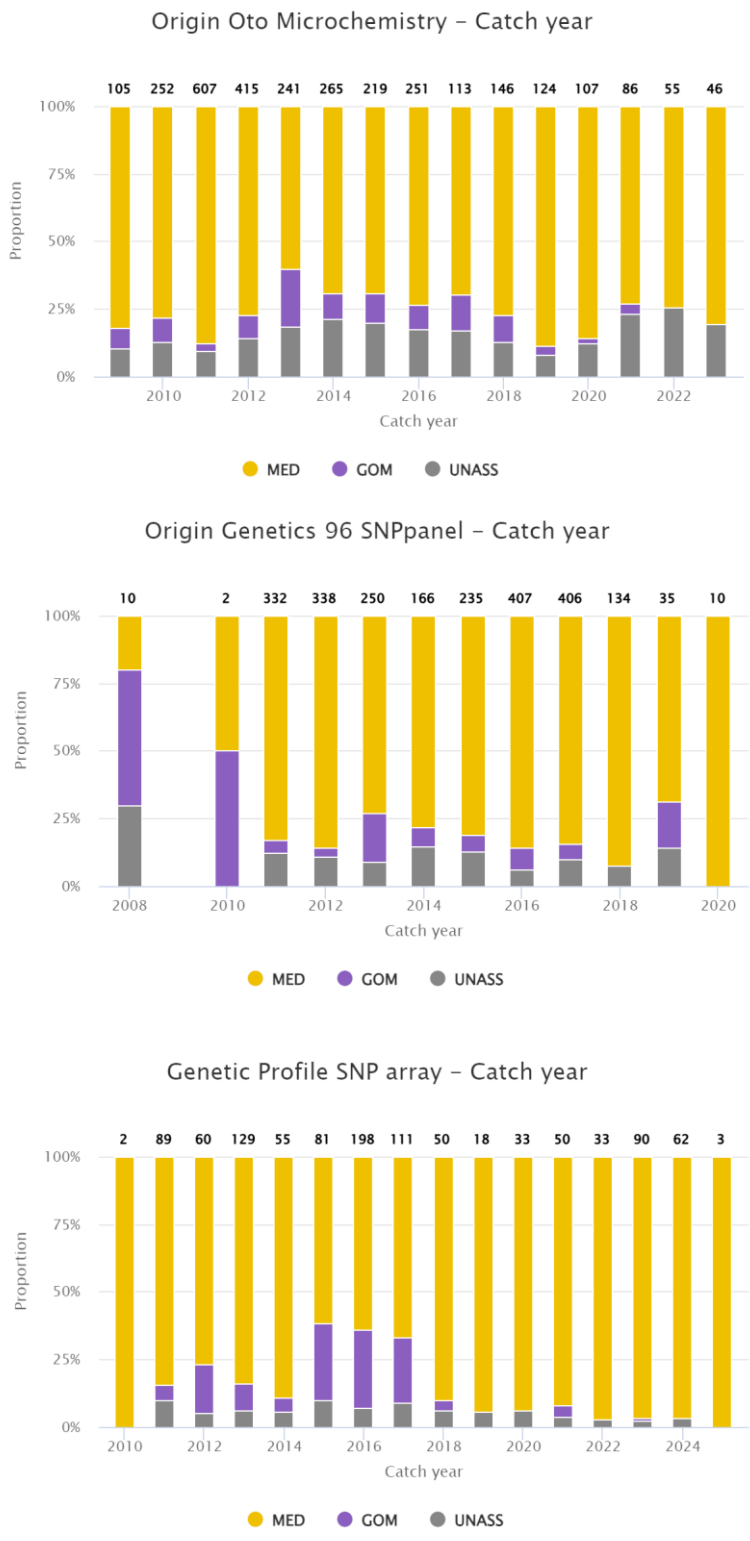

Figure 4.7. Example of stock of origin by catch year based on three analytical approaches. The figure compares annual proportions of Atlantic bluefin tuna assigned to three origin categories: Mediterranean (MED), Gulf of Mexico (GOM), and unassigned (UNASS), using otolith microchemistry (upper panel), a 96-SNP genetic panel (central panel), and a high-density SNP array (lower panel). Numbers above each bar indicate the sample size analyzed per year.

Data tab

Significant improvements have been implemented in the BioTuna platform to streamline and secure access to associated metadata associated with the GBYP tissue bank. While all visual outputs (maps, charts, and aggregated summaries) are publicly accessible, the underlying metadata are not publicly available due to confidentiality and data-protection requirements.

Access to metadata

To access metadata, the new design introduces an online request form that users must complete (Figure 4.7). The form collects essential information needed to evaluate the metadata request, including:

Personal Information: Full name, email, country, telephone, and institution/organization.

Project Details: Project title, main objective, brief description (max. 200 characters), and project duration.

Requested Data: Sampling years, areas, species, and intended use (e.g., research, education, other).

Publication Intent: Indication of whether metadata will be used in publications.

Declaration: Confirmation that the data will only be used for the specified purpose.

Once submitted, the request is reviewed by the ICCAT-GBYP tissue bank coordinators. Approval of the request follows the guidelines of the "*Rules and Procedures for the protection, access to, and dissemination of data compiled by ICCAT*" document, specifically rules described in paragraph 9. (bis), which classifies biological data as medium-risk, non-public data. Consequently, metadata provided will never include confidential information or exact fishing coordinates.

If approved, the user receives temporary, password-protected access to the requested metadata. Access is limited to the purpose and duration specified in the request, ensuring responsible use and full traceability. Users are also informed that the data source must be acknowledged in any resulting outputs.

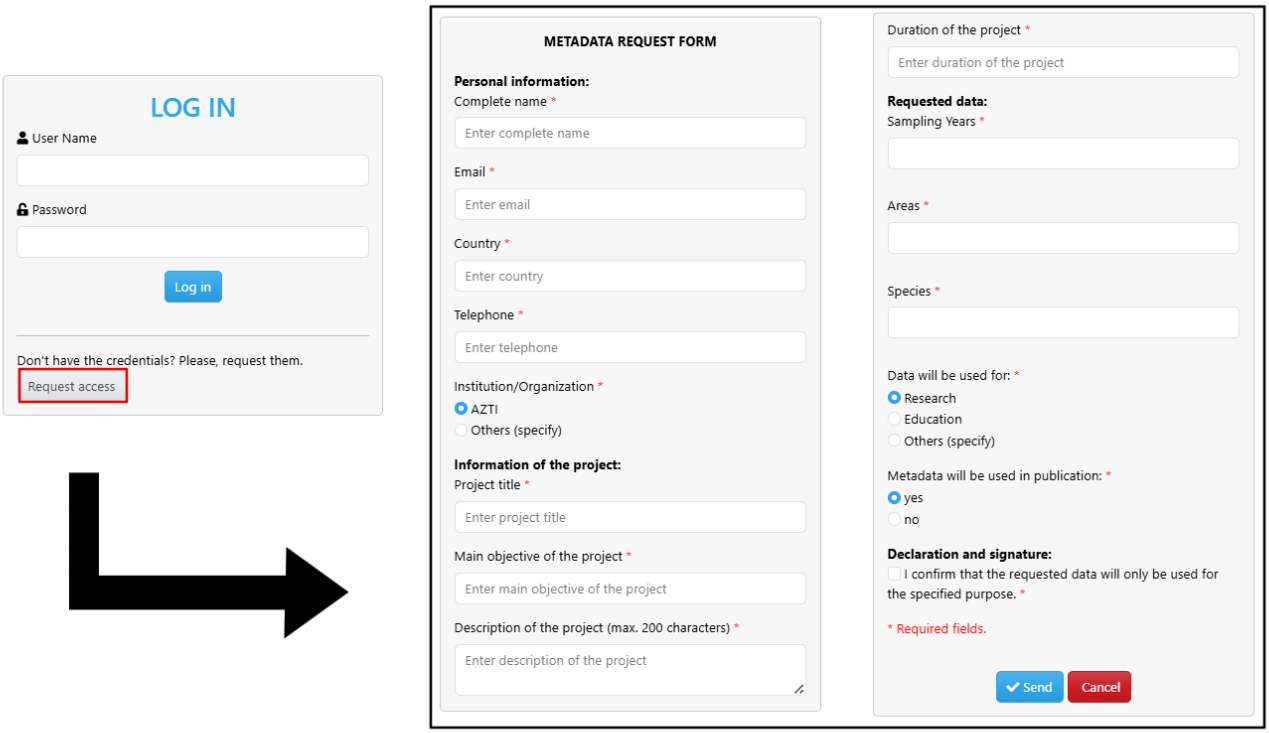

Figure 4.7. Access to metadata request in the BioTuna platform. The left panel shows the login interface for users with existing credentials. For new users, clicking "Request access" opens the metadata request form (right panel), where personal details, project information, requested data, and intended use must be specified. Upon approval by the platform coordinators, temporary credentials are provided for accessing the requested metadata.

Requesting physical samples

The online form does not automatically initiate a request for physical samples. However, users interested in obtaining biological samples can contact the BioBank coordinators, who will connect them with the appropriate contact person to evaluate sample availability and manage the request.

4.4 Conclusions

The information system provided via a shiny page interface enhances accessibility and transparency, enabling dynamic data visualization, improves research planning and informed decision-making, strengthens transparency, and promotes collaboration among institutions. This approach not only accelerates research progress but also ensures that

the tissue bank serves as a robust resource for advancing knowledge in ABFT biology and dynamics. Moreover, the tool plays a critical role in maximizing the scientific value of the GBYP tissue-bank by making **F**indable, **A**ccessible, **I**nteroperable, and **R**eusable (FAIR principles). The continued development of the information system is necessary to incorporate new samples and results, ensure metadata consistency, and adapt the system to evolving analytical needs and data standards. This ensure that information system becomes a central resource for data sharing and knowledge dissemination within the ICCAT GBYP programme.

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7. ANNEX LIST

- ANNEX I: Sampling Protocol.
- ANNEX II: Detailed and updated catalogue of samples stored in the ICCAT-GBYP Tissue Bank, and analyses performed for Phases 1-14.
- ANNEX III: Power point presentation of the main results.
- ANNEX IV: List of scientific contributions derived from ICCAT-GBYP Biological studies