SHORT TERM CONTRACT FOR BIOLOGICAL STUDIES (ICCAT GBYP 06/2018) OF THE ATLANTIC-WIDE RESEARCH PROGRAMME ON BLUEFIN TUNA (GBYP Phase 8)

Final Report (Deliverable 5)

for:

ICCAT

Scientific coordinator:

Dr. Haritz Arrizabalaga (AZTI-Tecnalia)

Pasaia, February 10, 2019

PARTNERS:

Fundación AZTI-AZTI Fundazioa

IFREMER

Universitá di Genova

National Research Institute of Far Seas Fisheries

Texas A&M University

GMIT

Universidad de Cádiz

Instituto Español de Oceanografía

University of Cagliari

SUBCONTRACTORS and COLLABORATORS:

Institute of Marine Research

IPMA

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 dynamics.

During Phase 8, following sampling protocols agreed in earlier Phases, the consortium sampled a total of 373 bluefin tuna (112 YOY, 7 medium sized fish and 254 large fish) from different regions (120 from the Strait of Gibraltar, 34 from Portugal, 57 from the Canary Islands, 62 from Norway and 100 from the Central North Atlantic). In total, 862 biological samples were taken (373 genetic samples, 316 otoliths and 173 fin spines). The consortium also received samples from other ICCAT contracts with tagging teams and farm operators. In total, the consortium handled 4571 samples from 2592 individuals.

Regarding otolith microchemistry, new carbon and oxygen stable isotope analyses were carried out in 256 otoliths of Atlantic bluefin tuna captured in the Central North Atlantic, to determine their nursery area. $\delta^{13}C$ and $\delta^{18}O$ 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.

Regarding genetic analyses, we have performed population genetic analyses based on about 10,000 SNPs and 400 reference samples from the Gulf of Mexico, Slope Sea and Mediterranean, and have determined genetic origin of above 1,000 individuals from feeding aggregates based on 96 SNPs that discriminate between Gulf of Mexico and Mediterranean Sea. Our analyses confirm the genetic differentiation of the Gulf of Mexico and Mediterranean Sea; yet, they also show that Mediterranean-like individuals are found in the Gulf of Mexico and that the Slope Sea constitutes a genetically intermediate population. This demonstrates that Atlantic bluefin tuna presents more complex population dynamics than previously thought and calls for additional analyses to determine how genetic differentiation between the two components is maintained and how the “intermediary” population in the Slope Sea is

originated. Concerning the origin of the feeding aggregates, our analyses confirm that samples collected at eastern locations are mostly of Mediterranean origin, and also suggest a larger proportion of Mediterranean origin fish in western locations.

Additional analyses were focused on the integrated approach to stock discrimination. The integrated stock discrimination model developed in GBYP phase 6 combines genotypic (SNPs) and phenotypic (otolith core stable isotopes $\delta^{13}C$ and $\delta^{18}O$) markers of stock origin to discriminate between bluefin tuna from the Gulf of Mexico and Mediterranean spawning populations. In this task, the existing adult baseline was extended to include fish from the western Mediterranean (Balearic Islands). Stable isotope signatures were compared between the adult baseline and the yearling baseline developed by Rooker et al (2014). The resolving power of the integrated model was re-evaluated and compared with single marker approaches. The integrated model was used to assign Bluefin from potential mixing zones in the Atlantic (N=306) to their population of origin and the results were compared with single marker assignments.

Otolith core stable isotope signatures of the extended adult baseline remained more distinct than those of the yearlings. Adult bluefin from the Gulf of Mexico and Mediterranean were classified to their population origin with a mean accuracy of 95.3% compared to a classification accuracy of 82.3% for the yearling baseline. The classification accuracy of the integrated model (97.3%) exceeded that reported in this or previous studies using stable isotopes or genetics, particularly for the Gulf of Mexico population. However, the integrated model did not perform as well when used to assign individuals from the mixing area to their population of origin; 27% of these fish were assigned to the Mediterranean population using genetics and to the Gulf of Mexico population using stable isotopes baseline, and so these individuals could not be assigned to either population using the integrated model. When taken together, the genetic and stable isotope profile of these fish did not match that of the fish in either spawning area. They may represent a third spawning component or a migratory contingent within the Mediterranean population.

A specific objective was to conduct age and genetic analyses on the Norwegian bluefin tuna, to know more about the Norwegian catch composition in terms of cohorts and

origin. In total, 446 individuals collected between 2013 and 2017 were genetically analyzed and the probability to belong to the Mediterranean Sea and Gulf of Mexico populations was estimated. Fin spines of 417 individuals from 2016 and 2017 were used for age reading. Results suggest that the large bluefin tuna individuals that feed in Norwegian waters in summer are predominantly of Mediterranean origin, and similar age classes were observed in 2016 and 2017, ranging between 6 and 14 years old, but mostly of 9 and 10 years old.

Most of the objectives of the Project were met. The analyses already started to provide important information that is relevant for Atlantic bluefin tuna management. As such, project results have continued and will continue to feed the bluefin tuna stock assessment and Management Strategy Evaluation (MSE) process. We compare stock composition percentages in the geographical boxes used in the MSE using different alternative methods and discuss them in relation to the main mixing hypotheses considered in the MSE framework.

1. CONTEXT

On May 28th 2018, 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, GMIT, Texas A&M University, Universidad de Cádiz, University of Cagliari, Instituto Español de Oceanografía, with subcontracted parties IPMA, University of Arizona, SGIKER/IBERCRON and Institute of Marine Research, presented a proposal to the call for tenders on biological and genetic sampling and analysis (ICCAT-GBYP 06/2018).

This proposal was awarded and the final contract between ICCAT and the consortium represented by Fundación AZTI-AZTI Fundazioa was signed on June 27th 2018.

According to the terms of the contract, a final report (Deliverable nº 5) needs to be submitted to ICCAT, incorporating all the suggestions made on the draft final report (Deliverable nº 4). 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

NRIFSF: Yohei Tsukahara, Tomoyuki Itoh, Shuya Nakatsuka

IEO: Enrique Rodriguez Marín, David Macías

IMR: Leif Nøttestad

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 sampling 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.

During GBYP Phase 7, 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. In GBYP Phase 8 the same criteria was followed.

2.1 Sampling accomplished

In this report we include the samples (and associated data), sampled by the Consortium as well as through other ICCAT contracts, that have physically arrived to AZTI before the 28th of February, so as to allow enough time to be verified. These include all the samples collected by the consortium

A total of 373 bluefin tuna individuals have been sampled by the Consortium, with a total of 862 biological samples (316 otoliths, 173 fin spines and 373 genetic samples). Table 2.1a shows the number of bluefin tuna sampled by the Consortium in each stratum (area/size class combination), and table 2.2a shows the number of otoliths, fin spines and genetic samples in each stratum.

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 2592 individuals (Table 2.1b and Figure 2.1), with a total amount of 4571 biological samples (1610 otoliths, 381 fin spines and 2580 genetic samples, Table 2.2b and Figures 2.2, 2.3, 2.4).

The original plan, according to the Consortium contract, was to acquire samples from 330 individuals. Thus, the current sampling status by the Consortium represents 113% 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 1400 additional individuals would be sampled with other contracts and agreements. This makes an overall target of 1730 individuals for the whole sampling strategy, and the current overall sampling status represents 150% of the original target.

By size class, the consortium planned to sample only two size classes, namely young of the year and large fish. The sampling objectives for these two size classes were met at 224% and 91%, respectively, and additional samples were obtained for the medium size class (Table 2.1a). The number of YOY individuals caught in Atlantic side of the Strait of Gibraltar was larger than expected, which will provide additional insights into the origin of these YOY found in the Atlantic (although very close to the Mediterranean). Regarding large fish, the sampling in the central Atlantic and Canarias went as expected, while in Norway the number of samples was below the target due to bad weather conditions that limited fishing activity in that area. The large majority of samples from outside the consortium came from the Balearics, Tyrrhenian and Malta.

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	%
Gibraltar	Gibraltar	112		7	1	**120**	50	240%
Northeast Atlantic	Portugal				34	**34**	30	113%
North Sea	Norway				62	**62**	100	62%
East Atlantic	Madeira, Canary Islands				57	**57**	50	114%
Central North Atlantic	Central and North Atlantic				100	**100**	100	100%
	TOTAL	**112**	**0**	**7**	**254**	**373**	330	113%
	Target	50	0	0	280	330		
	% wrt target	224%		>100%	91%	113%		

b)

		Age 0	Juveniles	Medium	Large	Total
		<3 kg	3-25 kg	25-100 kg	>100 kg	
Central Mediterranean	Adriatic Sea			50		**50**
	Malta			5	499	**504**
	Sicily (East Sicily and Ionian Sea)		50	50		**100**
Western Mediterranean	Tyrrhenian Sea		32	17	466	**515**
	Sardinia			5		**5**
	Gulf of Lion, Catalan				125	**125**
	Balearic			3	849	**852**
Gibraltar	Gibraltar	112		7	51	**170**
Northeast Atlantic	Portugal				34	**34**
East Atlantic	Madeira, Canary Islands				57	**57**
North Sea	Norway				80	**80**
Central North Atlantic	Central and North Atlantic				100	**100**
	TOTAL	**112**	**82**	**137**	**2261**	2592

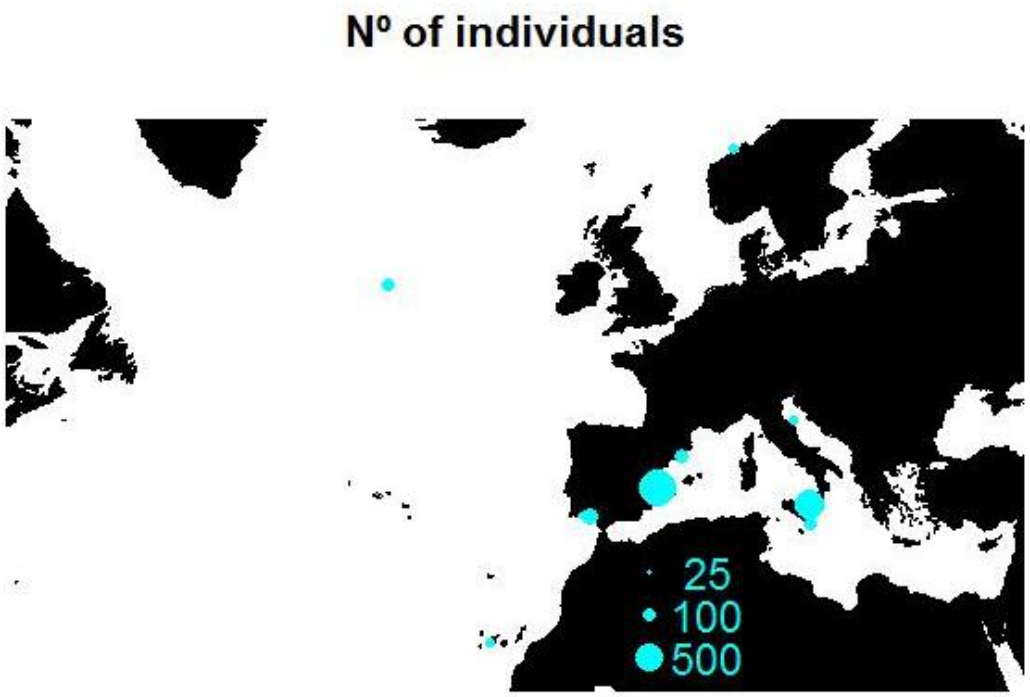

Figure 2.1: Total number of individuals sampled under all GBYP activities in Phase 8 in the Northeast Atlantic and Mediterranean, aggregated by main region. Positions of the dots are averages across all samples by main region.

The overall progress of the project was affected by the late (relative to the timing of some fisheries) signature of the contract, which occurred after some fisheries had already started or were already closed. Yet, most sampling objectives were met.

In the Strait of Gibraltar, 240% of the target number of individuals was sampled, mostly YOY (the original target size), but also a few medium and large fish, all by the University of Cádiz. In Portugal, IPMA, in collaboration with observers and Tunipex trap fishermen, sampled 34 large individuals (113% with respect to the target). In the Canary Islands, 114% of the target number of individuals was sampled by Instituto Español de Oceanografía (IEO), all of large size class (the target size class).

In Norway (Institute of Marine Research, IMR) and the Central Atlantic (National Research Institute of Far Seas Fisheries, NRIFSF), sampling objectives in terms of number of individuals were met at 62% and 100% respectively. Some additional samples were taken by observers in Norway, increasing the total number of samples to n=80 (out

of n=100 originally planned), which will allow for insights on the nature of the bluefin tuna schools visiting Norwegian waters. The samples from the Central Atlantic will allow for additional insights into mixing of stocks and their interannual variability.

Under a range of separate contracts, Aquabio Tech Ltd., the ROP, Taxon S.L., Next Generation Bluefin Tuna Partnership and Aquastudio sampled 965, 675, 370, 197 and 12 samples, respectively, mostly from the western (n=1497) and eastern (n=654) Mediterranean, as well as a few from Gibraltar (n=50) and Norway (n=18). These samples are mostly from large individuals (n=2007), but also medium (130) and juvenile (n=82) individuals.

Table 2.2: 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	Total	Sampler
Gibraltar	Gibraltar	112	81	120	313	UCA
Northeast Atlantic	Portugal	30	32	34	96	IPMA
East Atlantic	Madeira, Canary Islands	51	0	57	108	IEO
North Sea	Norway	23	60	62	145	IMR
Central North Atlantic	Central and North Atlantic	100	0	100	200	NRIFSF
	TOTAL	**316**	**173**	**373**	**862**	
	Target	330	180	330	840	
	% wrt target	96%	96%	113%	103%	

b)

		Otolith	Spine	Muscle/Fin	Total	Sampler
Central Mediterranean	Adriatic Sea	49	50	50	149	NGBFT
	Malta	400	0	503	903	ABTL
	Sicily (East Sicily and Ionian Sea)	97	100	100	297	NGBFT
Western Mediterranean	Sardinia	3	0	5	8	ABTL
	Tyrrhenian Sea	439	58	504	1001	ABTL/AQUA/ NGBFT
	Gulf of Lion, Catalan	0	0	125	125	ROP
	Balearic	306	0	852	1158	ROP/TAXON
Gibraltar	Gibraltar	112	81	170	363	UCA/ROP
Northeast Atlantic	Portugal	30	32	34	96	IPMA
East Atlantic	Madeira, Canary Islands	51	0	57	108	IEO
North Sea	Norway	23	60	80	163	IMR/ROP
Central North Atlantic	Central and North Atlantic	100	0	100	200	NRIFSF
	TOTAL	**1610**	**381**	**2580**	**4571**	

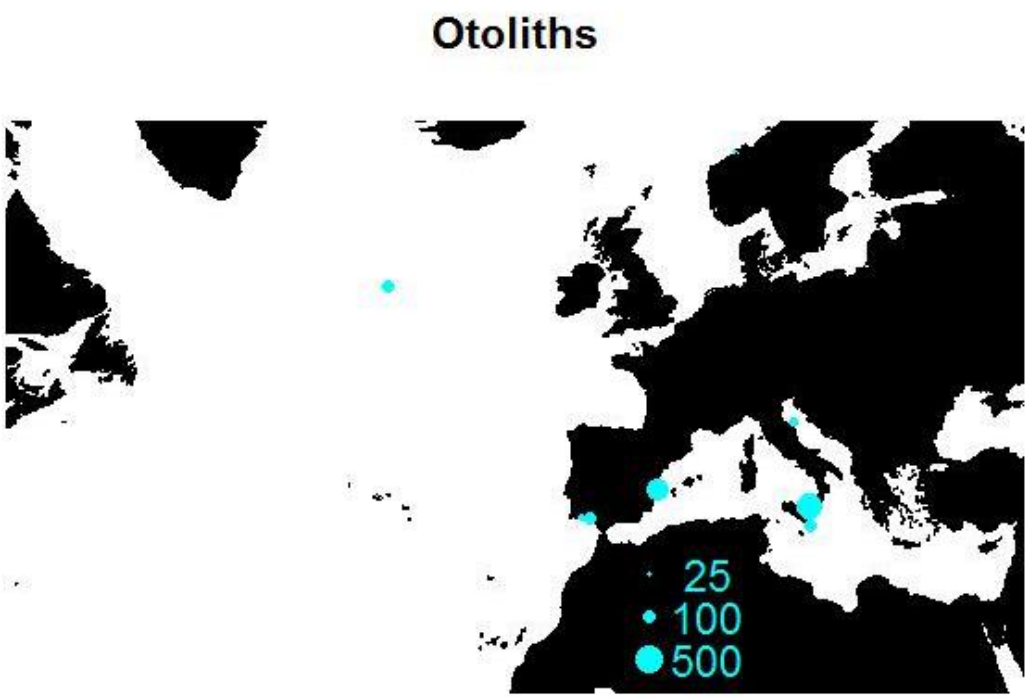

Figure 2.2: Total number of individuals with otolith sampling conducted under all GBYP contracts in Phase 8 in the Northeast Atlantic and Mediterranean, aggregated by main region. Positions of the dots are averages across all samples by main region.

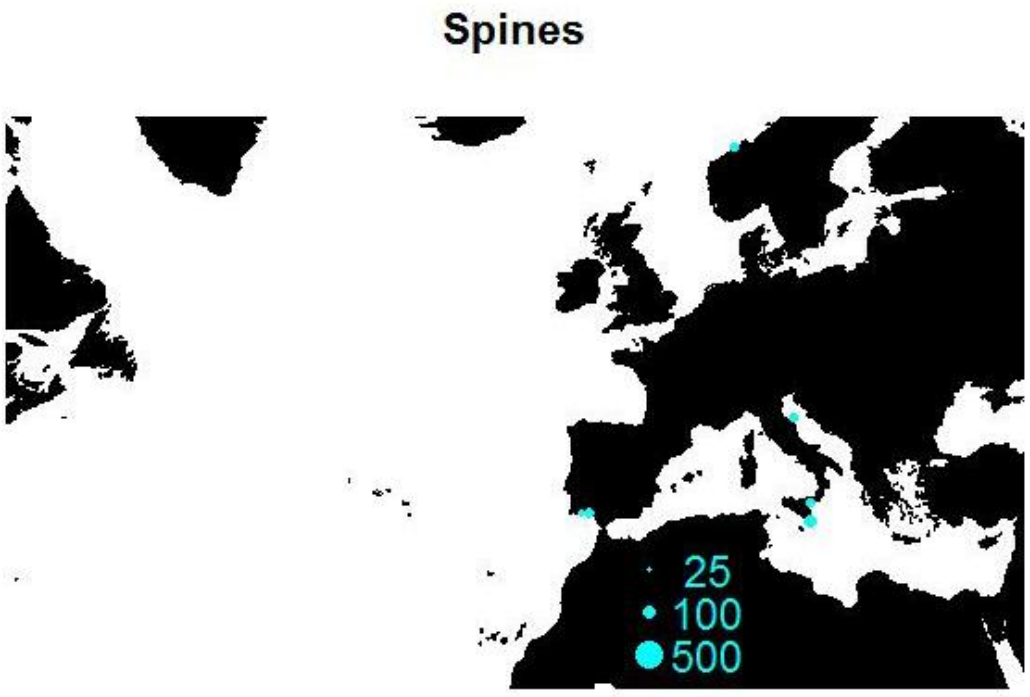

Figure 2.3: Total number of fin spines collected under all GBYP contracts in Phase 8 in the Northeast Atlantic and Mediterranean, aggregated by main region. Positions of the dots are averages across all samples by main region.

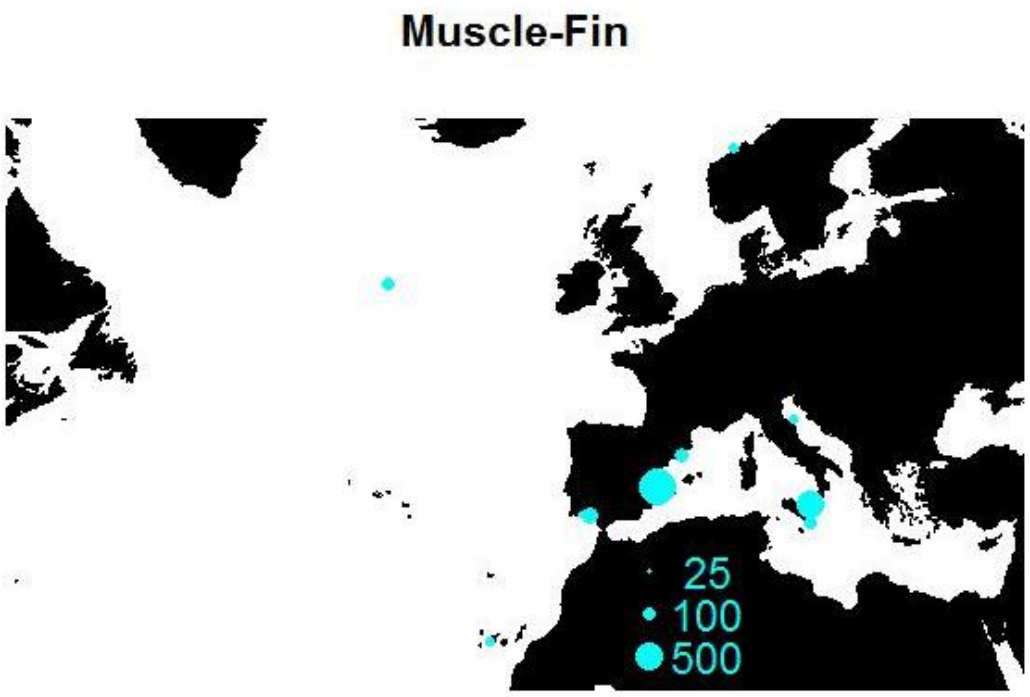

Figure 2.4: Total number of muscle or fin tissue samples collected under all GBYP contracts in Phase 8 in the Northeast Atlantic and Mediterranean, aggregated by main region. Positions of the dots are averages across all samples by main region.

3. ANALYSES

The following sections elaborate on the tasks contracted during Phase 8.

In addition, following specific criteria discussed and agreed with the GBYP coordinator, 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.

4. NURSERY ORIGIN OF BLUEFIN CAPTURED IN THE CENTRAL NORTH ATLANTIC OCEAN

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

Participants:

NRIFSF: Yohei Tsukahara

AZTI: Haritz Arrizabalaga

4.1 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 considerable, in some years, in the central North Atlantic, Canary Islands and western coast of Morocco. To further assess the spatial and temporal variability of mixing proportions, otoliths collected in the central North Atlantic in 2014 and 2015 were analyzed for stable carbon and oxygen isotopes ($\delta^{13}C$ and $\delta^{18}O$).

4.2 Material and Methods

In this section, we investigate the origin of bluefin tuna collected in the central North Atlantic Ocean (east and west of 45ºW), using stable $\delta^{13}C$ and $\delta^{18}O$ isotopes in otoliths. Samples utilized for this study (N=256) were collected from September to November during two consecutive years (2014 and 2015) by Japanese longliners operating in the central North Atlantic Ocean (Figure 4.1).

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 pre-programmed 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.

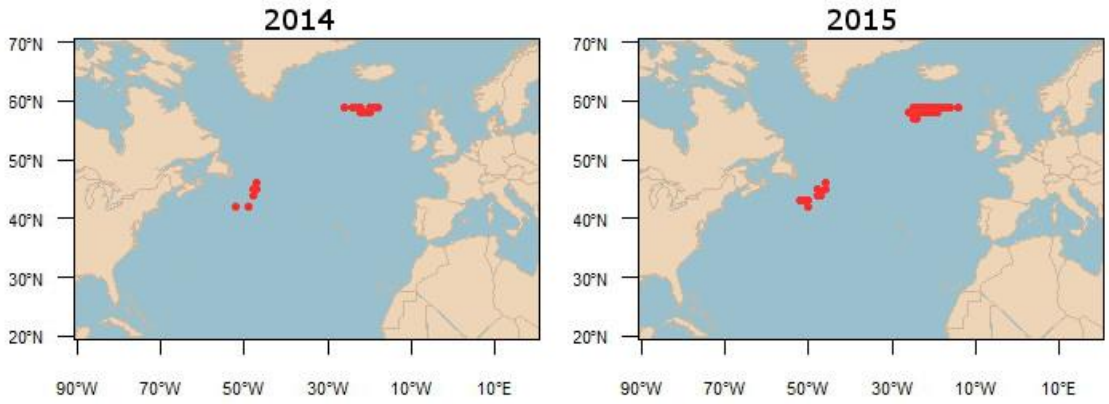

Figure 4.1: Sample distribution. Otoliths were collected by Japanese longliners in 2014 and 2015 in two regions of the central North Atlantic.

4.3 Results and Discussion

$\delta^{13}C$ and $\delta^{18}O$ were measured in the otolith cores of bluefin tuna from the central North Atlantic and compared to baseline populations from the Mediterranean Sea and Gulf of Mexico (Figure 4.2).

Otolith $\delta^{18}O$ values corresponded 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 central North Atlantic were, in general, more enriched compared to baseline samples. 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 2014 and 2015 were comprised largely of the Mediterranean population both east and west of the 45ºW management boundary (Table 4.1). Mixing rate estimates in the western North Atlantic using this methodology varied considerably in preceding years, with catches in 2011 dominated by the Mediterranean population, and in 2012 and 2013 dominated by the Gulf of Mexico population (Figure 4.3). East of 45ºW, catches were usually dominated by the Mediterranean population, although in 2013 a substantial contribution of western migrants was found. The results for 2014 and 2015 confirm that mixing of the two populations occurs at variable rate, but Mediterranean bluefin tuna may be the principal contributors to the Japanese fishery operating in the central North Atlantic.

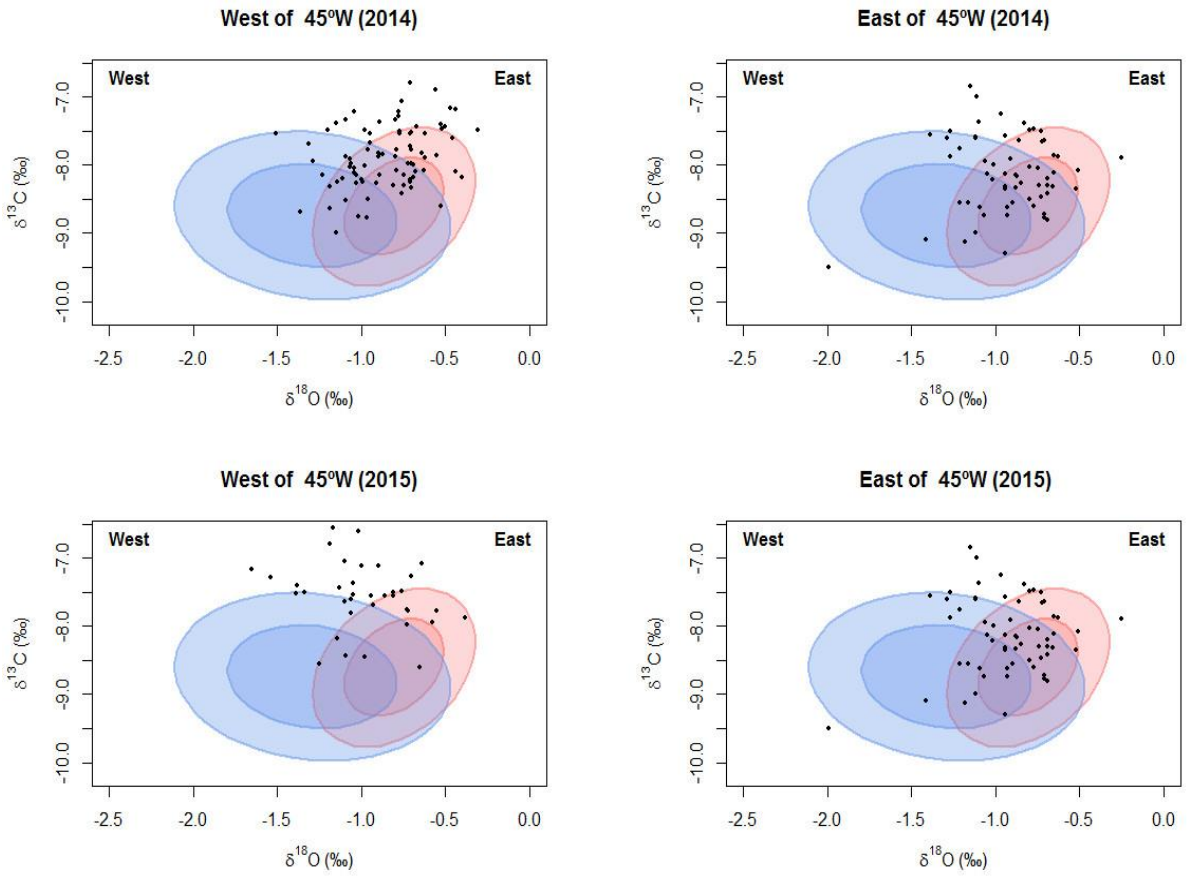

Figure 4.2: Confidence ellipses (1 and 2 SD or ca. 68% and 95% of sample) for otolith $\delta^{13}C$ and $\delta^{18}O$ values of yearling bluefin tuna from the east (red) and west (blue) nurseries along with the isotopic values (black) for otolith cores of bluefin tuna collected by the Japanese fleet east and west of the 45°W boundary in 2014 and 2015.

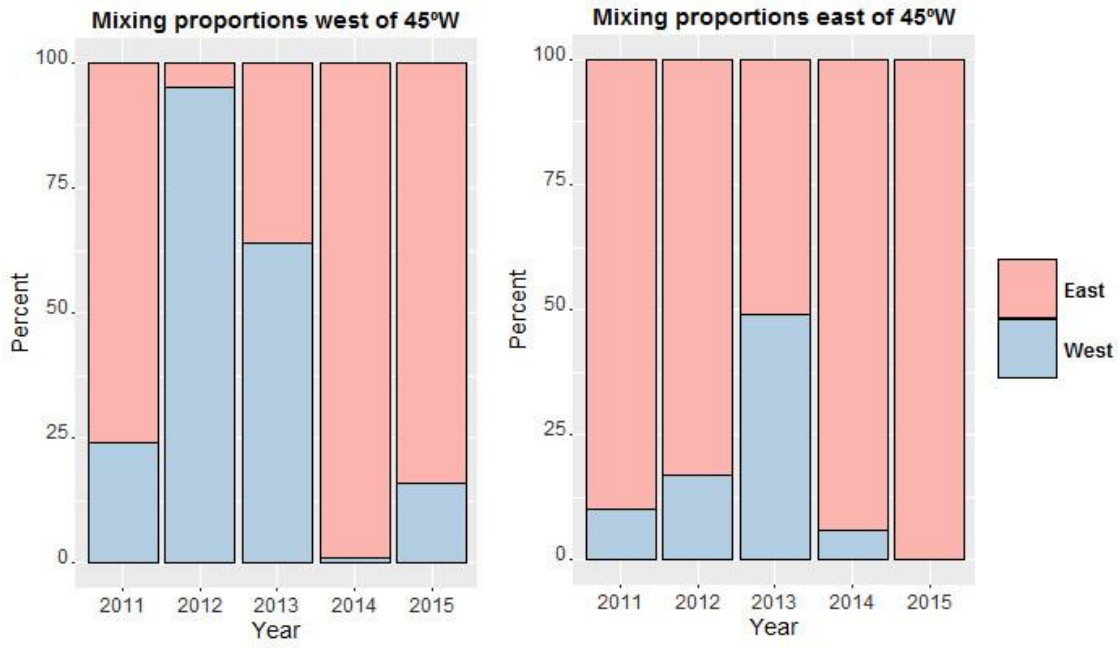

Figure 4.3: Interannual variation of the mixing proportions east and west of the 45°W boundary estimated by Maximum Likelihood Estimator (HISEA program).

Table 4.1: Maximum-likelihood estimates of the origin of bluefin tuna from the central North Atlantic (east and west of the 45°W boundary) 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 west of 45°W					**Mixing proportions east of 45°W**			
Year	**West**	**East**	**SD**	N		**West**	**East**	**SD**	**N**
2014	1%	99%	2%	85		6%	94%	5%	63
2015	16%	84%	12%	36		0%	100%	0%	72

References

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

5. GENETICS

Task Leader: Naiara Rodriguez-Ezpeleta (AZTI)

Participants: Iñaki Mendibil, Natalia Diaz-Arce, Haritz Arrizabalaga

5.1 Introduction

Previous results support the presence of two populations of Atlantic Bluefin Tuna, which, despite the trans-Atlantic migrations of individuals from this species, is maintained through a natal homing behaviour to the two main spawning grounds, the Mediterranean and the Gulf of Mexico (see Phase 6 report). This allowed the development of a traceability SNP panel that assigns individuals to their stock of origin and which is very relevant for ABFT management. Yet, since these analyses were performed, a new study suggested the presence of a third spawning ground within the Slope Sea (Richardson et al. 2016) and controversy exists about the origin of the larvae and young of the year found in this area (Safina 2016; Walter et al. 2016). The presence of a new spawning ground not only requires more in-depth analyses about the reproductive behaviour of ABFT but might also call for the development of a new traceability panel taking a potential "third stock" into account. In this context, two subtasks were envisaged.

i) Complete the population structure of ABFT including Slope Sea individuals based on RAD-seq derived SNPs

ii) Identify additional population structure informative markers based on Pool-seq derived SNPs

Despite the potential new "third stock" that might require developing a new traceability panel in the future, the panel developed on a two-stock model has shown to perform well (see Phase 6 report) and was used to trace 960 fish found in feeding aggregations to their area of birth. During this Phase, this number was expanded to complete the map generated in Phase 6, focusing on the Atlantic, where mixing occurs, and covering

different size classes (juvenile, medium and large size classes) and years. Given that in Phase 6, we focused specially on large fish, in Phase 8 we have focused on medium and juvenile fish, while also enlarging the sample size for adults. In this context, two subtasks were scheduled:

i) Adult individual selection and DNA extraction

ii) Genotype the minimal SNP panel in adults from feeding aggregates

All tasks planned for Phase 8 have been accomplished.

5.2 Material and methods

5.2.1 Complete the population structure of ABFT including Slope Sea individuals based on RAD-seq derived SNPs

RAD-seq libraries from additional 256 spawning adults from the Gulf of Mexico, three Mediterranean locations and from 39 Slope Sea larvae recently generated were merged with the previous RAD-seq data (see report from Phase 6). The merged dataset was analyzed using Stacks 1.44 (Catchen et al. 2013). Quality filtering and demultiplexing was performed with *process_radtags* truncating all reads to 90 nucleotides to avoid the lower quality bases at the end of the read. PCR duplicates were removed applying *clone_filter* to reads whose forward and reverse pairs passed quality filtering. Putative orthologous tags (stacks) per individual were assembled using *ustacks* with a minimum depth of coverage required to create a stack (m) of 3 and a maximum nucleotide mismatches (M) allowed between stacks of 2 and 6. Catalogs of RAD loci were assembled using *cstacks* with a number of mismatches allowed between sample tags when generating the catalog (n) of 6. Matches of individual RAD loci to the catalog were searched using *sstacks*. From each generated catalog, SNPs present in RAD loci found in at least 75% of the individuals under study were selected and exported into PLINK format using populations. Using PLINK version 1.07 (Purcell et al. 2007), SNPs with a minimum allele frequency (MAF) smaller than 0.05, a genotyping rate smaller than 0.90 and which

failed the Hardy Weinberg equilibrium (HWE) test at $p < 0.05$ in at least one area of study were excluded. Only samples with genotyping rate above 0.75 were retained per dataset. Each genotype dataset was exported to Structure and Genepop formats using PGDSpider version 2.0.8.3 (Lischer, Excoffier 2012).

Fst values were estimated using GENEPOP (Rousset 2008), and 95% confidence intervals were calculated for each pairwise comparison based on 10,000 permutations. PCAs were performed using adegenet R package and individual ancestry proportions were estimated using ADMIXTURE (Alexander, Novembre, Lange 2009) using only the first SNP of each tag. ADMIXTURE was run for a 5000 bootstrap, assuming from 2 to 5 (K) ancestral populations. For each analysis, a first exploratory run was launched to check the number of steps necessary to reach the default 0.001 (10-4) likelihood value, so enough number of steps to be fulfilled in each bootstrapped run analysis ("-c" parameter) were set to ensure convergence (from 20 to 100). Cross-validation error estimates were obtained for each assumed number of ancestral populations (K) using the cross-validation procedure implemented in the ADMIXTURE. PLINK format genotype datasets were converted to eigenstrat format, extracting only larvae samples to calculate F3 statistic and Z-score associated values testing for different group admixture scenarios, using convert and qp3Pop functions from ADMIXTOOLS software (Patterson et al. 2012).

5.2.2 Identify additional population structure informative markers based on Pool-seq derived SNPs

Whole genome sequencing data of pools of individuals, grouping reference samples per area and age class, was analyzed. In total, 13 pools of individuals were analyzed, including an average of 40 individuals per location and age class, except for YOY from the Gulf of Mexico and adults from the Slope Sea which were not available. Sequences were filtered using Trimmomatic (Bolger, Lohse, Usadel 2014) removing positions within sliding windows of size 5 when quality score dropped below 25. Only reads with a minimum length of 50 were kept. Sequences were mapped against available reference genome of Pacific bluefin tuna (REF) using BWA MED algorithm (Li 2013). Obtained SAM files were converted to BAM and reads were filtered by keeping only primary alignments and correctly matching read 1 and read 2 alignments using SAMTOOLS (Li et al. 2009). Mapped BAM files were converted to pileup format using SAMTOOLS mpileup tool and to sync file format using Popoolation2 (Kofler, Pandey, Schlotterer 2011) filtering

positions for minimum and maximum coverage of 10 and 60 respectively, minimum count for alternative alleles of 5. Fst were estimated per sliding windows of 5,000 bp and per SNP using Popoolations2. Polymorphic positions with highest 5% F_{ST} estimates for each pairwise comparison were selected, and significance of allele frequency differences were tested using the Fisher exact test implemented in Popoolations2. To screen for potential regions under selection between the three spawning grounds we extracted matching selected positions for all (between the two western Atlantic spawning grounds) or 75% (between Mediterranean and western Atlantic spawning grounds) pairwise comparisons between locations. There were no SNPs passing all the filters in the Mediterranean and Slope Sea, or Mediterranean and Gulf of Mexico pairwise comparisons, and therefore different age class from the western Atlantic were analyzed separately.

To explore population structure derived from whole genome resequencing data, mapped BAM files were merged and converted to Beagle format using ANGSD (Korneliussen, Albrechtsen, Nielsen 2014) only keeping SNPs present in at least the 75% of the pools, with minimum quality score of 20, minimum allele frequency of 0.05, and minimum depth coverage of 10. PCAngsd (Meisner, Albrechtsen 2018) was run to obtain the covariance matrix using genotype likelihoods contained in the Beagle file.

5.2.3 Adult individual selection and DNA extraction

For those samples for which no DNA was already available, DNA was extracted using the Wizard® Genomic DNA Purification kit (Promega, WI, USA) following manufacturer´s instructions for "Isolating Genomic DNA from Tissue Culture Cells and Animal Tissue". The starting material was approximately 20 mg of tissue or whole larvae and after extraction all samples were suspended in equal volumes of Milli-Q water. DNA quantity (ng/µl) was evaluated on the Qubit® 2.0 Fluorometer (Life Technologies) and DNA integrity was assessed by electrophoresis.

5.2.4 Genotype the minimal SNP panel in adults from feeding aggregates

Assignments were performed with GENECLASS2 (Piry et al. 2004) using the Rannala and Mountain (1997) criterion (0.05 threshold) considering two populations (Gulf of Mexico and Mediterranean) as baseline. For each individual, assignment scores, i.e. probability of belonging to each of the baseline populations, were calculated, using the

combined set of 646 reference samples as baseline. Samples with assignments scores lower than either 80% were considered “unassigned”.

5.3 Results

5.3.1 Complete the population structure of ABFT including Slope Sea individuals based on RAD-seq derived SNPs

Results from both catalogs that were built setting M parameter to 2 and 6 were equivalent, and therefore here we only show results for the first catalog. After filtering, the final datasets included 398 individuals and 11,369 SNPs derived from 8,846 RAD loci. PCA and ADMIXTURE, both show differentiation between Mediterranean and Gulf of Mexico individuals and place individuals from the Slope Sea as genetically intermediate (Figures 5.1 and 5.2). Moreover, PCA biologically meaningful differentiation of the individuals was explained by the PC1 (x axis represented in Figure 5.1), and the number of ancestral populations (K) associated with lowest cross-validation error estimate obtained from the ADMIXTURE analysis was 2. Pearson correlation coefficient between PC1 coordinates and ancestral proportion when K=2 was 0.967 ($p<0.01$) showing that both analyses provided with very similar results. Although frequency distributions of estimated ancestry values between locations overlapped, they are statistically different (K-S test $p<0.01$). F_{ST} estimates provided with very low values (Table 5.1), even for a marine fish species for which F_{ST} values are typically low (Ward, Woodmark, Skibinski 1994), but statistically different from 0 ($p<0.05$). This supports that, Gulf of Mexico, Slope Sea and Mediterranean Sea constitute different populations with a weak genetic differentiation.

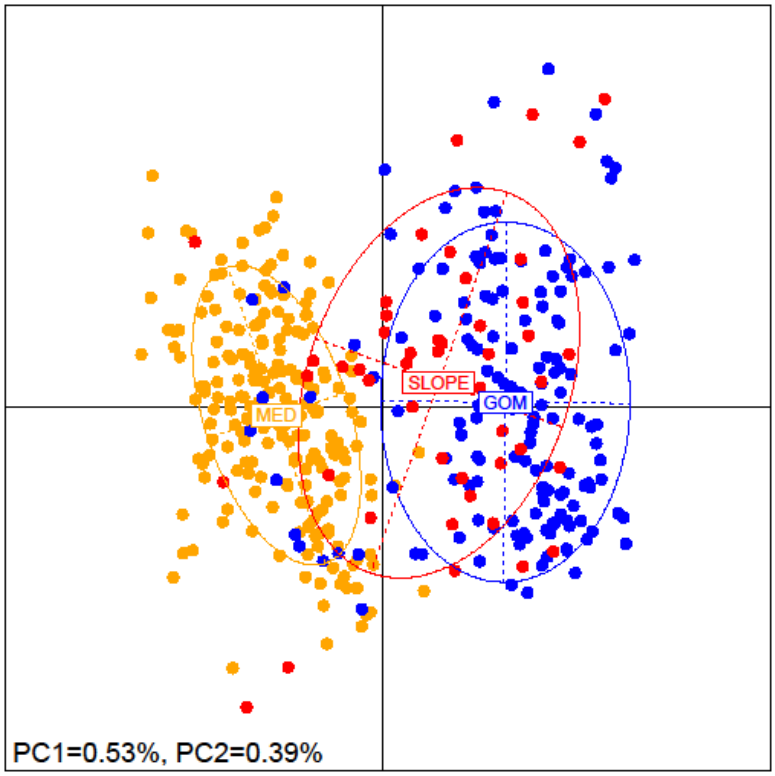

Figure 5.1. Principal Component Analysis of the Atlantic Bluefin tuna RAD-seq derived genotype markers. Each dot represents one sample and colors represent different locations. The Principal Components 1 and 2 explained 0.53% and 0.39% of the variation of the data.

Interestingly, although Gulf of Mexico and Mediterranean form two genetically differentiated populations, our analyses show that some Mediterranean-like individuals can be found in the Gulf of Mexico (Figures 5.1 and 5.2). These are considered "migrants", whose presence can only be compatible with the genetic differentiation between the two spawning components if i) even if they visit the Gulf of Mexico, they do not reproduce, ii) even if they reproduce, they descendants are non-viable or less fit, or iii) other alternative, more complex scenarios.

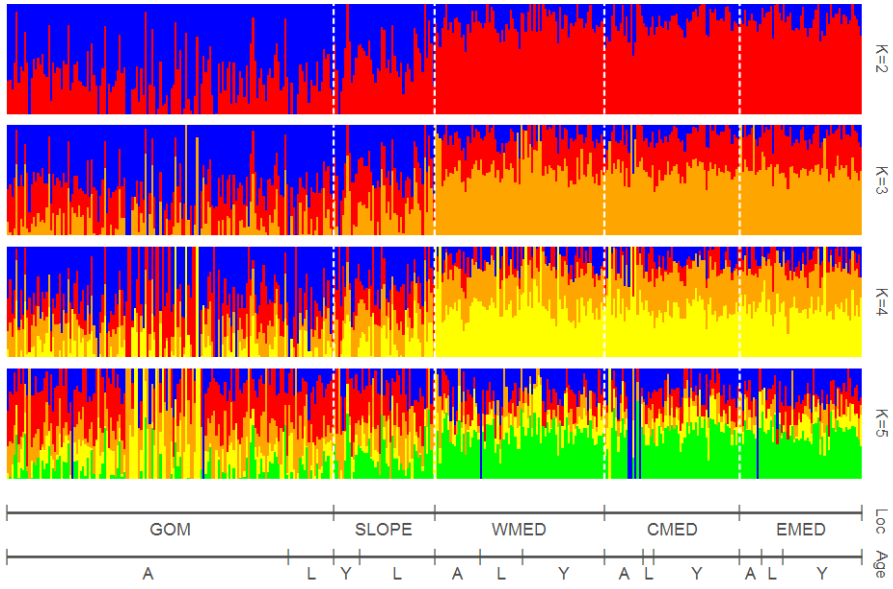

Figure 5.2. Individual ancestral proportions of Atlantic Bluefin tuna inferred using ADMIXTURE. Each color represents one ancestral population. Groups of individuals are identified in the x axis labels divided by location (GOM stands for Gulf of Mexico, SLOPE for Slope Sea and WMED, CMED and EMED for West, Central and East Mediterranean Sea locations respectively) and age group (A, L and Y stand for adult, larvae and young of the year respectively).

*Table 5.1. Pairwise FST values. Values with * and ** were statistically different from 0 for p<0.05 and p<0.01 respectively based on 10,000 permutation analysis.*

F_{ST} values	Mediterranean Sea	Slope Sea
Slope Sea	0.0015 **	
Gulf of Mexico	0.0027 **	0.0002 *

The F3 statistic can be used to test if a population is admixed from other source populations by analyzing correlations between allele frequencies. Negative F3 values support that admixture occurs or occurred in the Slope Sea larvae (and not in the Slope Sea young of the year) from Mediterranean and Gulf of Mexico larvae source populations (Table 5.2).

Table 5.2. F3 statistic testing for different admixture possible scenarios between locations when only larvae are included.

Source 1	Source 2	Target	F3 mean	std. err	Z	SNPs
MED	GOM	SLOPE	-0.0003	0.0003	-1.3230	8846
MED	SLOPE	GOM	0.0036	0.0004	9.2250	8846
SLOPE	GOM	MED	0.0026	0.0003	8.4300	8846

5.3.2 Identify additional population structure informative markers based on Pool-seq derived SNPs

Mapped and filtered sequences from all pools of individuals covered > 99% of the reference genome. Average coverage values per pool varied between 10 and 30. The total number of polymorphic positions obtained was 4,512,902. From those, SNPs under selection where searched in order to determine if they could provide additional population structure information. The number of SNPs potentially under selection varied between groups and need further analyses in order to avoid false positives (Table 5.3).

Table 5.3. Number of SNPs selected as potential markers under selection between spawning grounds. These markers would need to be further screened in order to avoid false positives.

Pairwise Comparison	*Number SNPs*
Slope Sea – Gulf of Mexico	3,574
Mediterranean Sea – Slope Sea Larvae	55
Mediterranean Sea – Slope Sea YOY	141
Mediterranean Sea – Gulf of Mexico Larvae	77
Mediterranean Sea – Gulf of Mexico adults	368

5.3.3 Adult individual selection and DNA extraction

Genomic DNA was successfully extracted from 1151 individuals, which have been genotyped and assigned to origin. Samples from 9 locations were analyzed (Table 5.4): Bay of Biscay (BB), Central Atlantic East (CAE), Central Atlantic West (CAW), Canary Islands (CI), Gibraltar (GI), Gulf of Saint Lawrence (GSL), Morocco (MO), Norway (NW) and Portugal (PO).

Table 5.4. Number of samples per location (rows) and age class (columns) analyzed. See text for location codes.

	0	*J*	*M*	*L*	*Total*
BB	0	47	37	0	84
CAE	0	0	54	167	221
CAW	0	0	10	169	179
CI	0	0	0	127	127
GI	64	11	60	0	135
GSL	0	0	0	20	20
MO	0	0	0	142	142
NW	0	0	0	203	203
PO	0	0	0	40	40
Total	64	58	161	868	1151

5.3.4 Genotype the minimal SNP panel in adults from feeding aggregations

Results show a similar pattern to what we observed before (see Phase 6 report) with samples captured east of the 45ºW being mostly of Mediterranean origin, and samples captured west of the 45ºW meridian being of both origins, with high percentage from the Mediterranean (Figure 5.4).

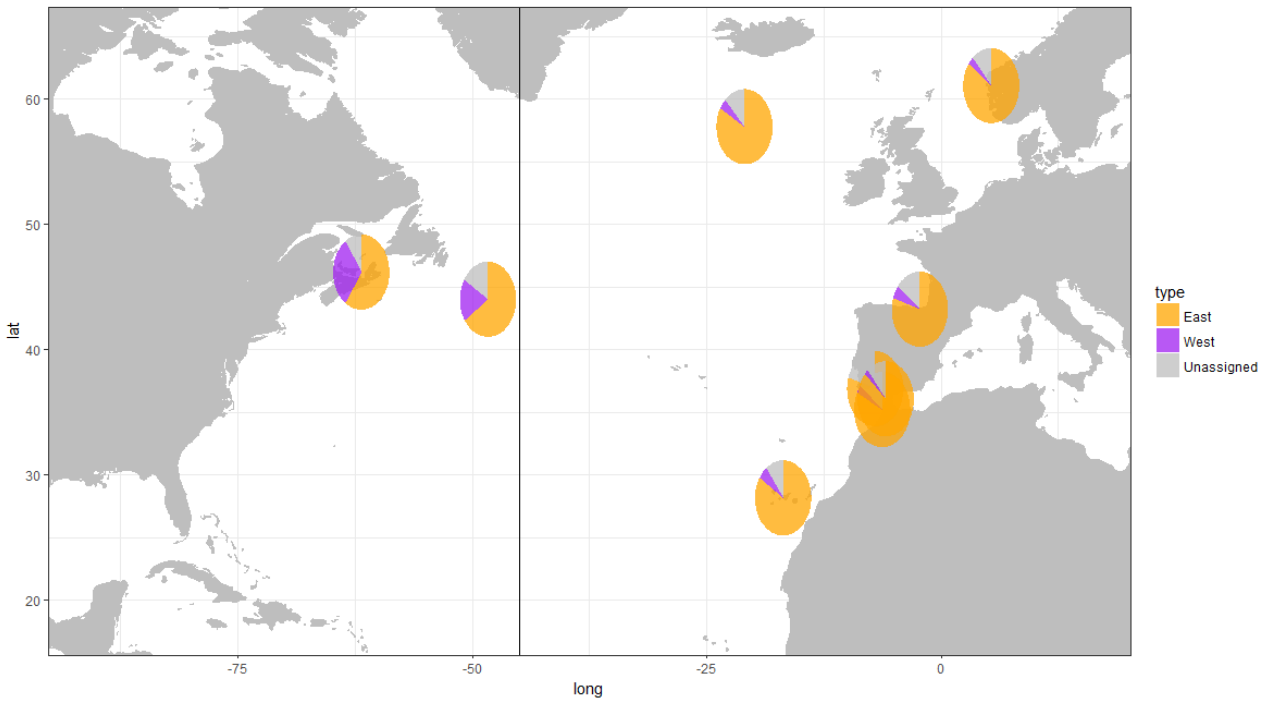

Figure 5.4. Proportion of samples per location assigned to Gulf of Mexico (purple) and Mediterranean Sea (orange). Black line indicates -45º meridian.

Within the eastern locations, all (except Portugal) have a proportion of Gulf of Mexico origin samples, although this proportion is always smaller than 6% overall and smaller than 18% when the analysis is done per year (Tables 5.5 and 5.6). Within western locations, the proportion of Mediterranean origin individuals is high, being almost 66% in the Western Central Ocean and 60% in the Gulf of Saint Lawrence. In the former, in 2013 the proportion of Mediterranean individuals was almost 93%. This proportions are larger than those observed in the samples analyzed in Phase 6 report, were proportion of Mediterranean samples in these locations was less than 50%.

Table 5.5. Percentage of samples assigned to Mediterranean Sea (MED), Gulf of Mexico (GOM), or unassigned (UN) per location.

	MED	GOM	UN
BB	79.76	5.95	14.29
CAE	83.26	4.52	12.22
CAW	65.92	17.88	16.20
CI	84.25	5.51	10.24
GI	86.67	2.22	11.11
GSL	60.00	30.00	10.00
MO	82.39	3.52	14.08
NW	85.22	2.96	11.82
PO	80.00	0.00	20.00

Table 5.6. Percentage of samples assigned to Mediterranean Sea (MED), Gulf of Mexico (GOM), or unassigned (UN) per location and per year.

Location	Year	N	MED	GOM	UN
BB	2011	82	79.27	6.10	14.63
BB	2012	2	100.00	0.00	0.00
CAE	2011	28	78.26	4.35	17.39
CAE	2012	37	85.42	6.25	8.33
CAE	2013	23	60.00	17.50	22.50
CAE	2014	48	72.22	5.56	22.22
CAE	2015	35	80.00	5.71	14.29
CAE	2016	50	72.00	14.00	14.00
CI	2013	20	90.00	5.00	5.00
CI	2017	56	76.79	3.57	19.64
CI	2018	51	90.20	7.84	1.96
GI	2012	13	92.31	0.00	7.69
GI	2013	28	85.71	3.57	10.71
GI	2015	14	87.50	2.50	10.00
GI	2017	80	78.57	0.00	21.43
MO	2011	20	94.44	2.78	2.78
MO	2012	36	96.67	0.00	3.33
MO	2013	30	63.16	10.53	26.32
MO	2014	19	72.22	0.00	27.78
MO	2015	18	73.68	5.26	21.05
MO	2016	19	75.00	5.00	20.00
NW	2017	203	85.22	2.96	11.82
PO	2011	27	77.78	0.00	22.22
PO	2012	13	84.62	0.00	15.38
GSL	2016	20	60.00	30.00	10.00
CAW	2011	18	82.00	8.00	10.00
CAW	2012	21	63.33	23.33	13.33
CAW	2013	20	92.86	0.00	7.14
CAW	2014	40	81.08	0.00	18.92
CAW	2015	50	66.67	23.81	9.52
CAW	2016	30	60.00	25.00	15.00

%MED
100
75
50
25
0

%GOM
100
75
50
25
0

%UN
100.00
75.00
50.00
25.00
0.00

5.4 Conclusions

Our analyses confirm the genetic differentiation of the Gulf of Mexico and Mediterranean Sea. The admixed nature of the Slope Sea suggest that individuals captured in this area could constitute an intermediary population. Our studies also suggest that some

individuals from the Mediterranean Sea can be found in the Gulf of Mexico. In view of these contacts, it is not clear how genetic differentiation between the two components is maintained, nor how the "intermediary" population in the Slope Sea is originated. More analyses are being performed in order to shed light to these questions.

Our analyses confirm previous findings concerning the origin of the catches obtained at different locations within the Bluefin Tuna mixing areas. We confirm that samples collected at eastern locations are almost all from Mediterranean origin whereas samples collected at western locations have larger proportion of Mediterranean origin individuals.

References

Alexander, DH, J Novembre, K Lange. 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome research.

Bolger, AM, M Lohse, B Usadel. 2014. Trimmomatic: a flexible trimmer for illumina sequence data. Bioinformatics Advance Access:1-7.

Catchen, J, S Bassham, T Wilson, M Currey, C O'Brien, Q Yeates, WA Cresko. 2013. The population structure and recent colonization history of Oregon threespine stickleback determined using restriction-site associated DNA-sequencing. Molecular ecology 22:2864-2883.

Kofler, R, RV Pandey, C Schlotterer. 2011. PoPoolation2: identifying differentiation between populations using sequencing of pooled DNA samples (Pool-Seq). Bioinformatics 27:3435-3436.

Korneliussen, TS, A Albrechtsen, R Nielsen. 2014. ANGSD: Analysis of Next Generation Sequencing Data. BMC Bioinformatics 15:356.

Li, H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. eprint arXiv:1303.3997:arXiv:1303.3997.

Li, H, B Handsaker, A Wysoker, T Fennell, J Ruan, N Homer, G Marth, G Abecasis, R Durbin. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078-2079.

Lischer, HE, L Excoffier. 2012. PGDSpider: an automated data conversion tool for connecting population genetics and genomics programs. Bioinformatics 28:298-299.

Meisner, J, A Albrechtsen. 2018. Inferring Population Structure and Admixture Proportions in Low-Depth NGS Data. Genetics 210:719.

Patterson, NJ, P Moorjani, Y Luo, S Mallick, N Rohland, Y Zhan, T Genschoreck, T Webster, D Reich. 2012. Ancient admixture in human history. Genetics:genetics. 112.145037.

Piry, S, A Alapetite, J-M Cornuet, D Paetkau, L Baudouin, A Estoup. 2004. GENECLASS2: A Software for Genetic Assignment and First-Generation Migrant Detection. Journal of Heredity 95:536-539.

Purcell, S, B Neale, K Todd-Brown, et al. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559-575.

Rannala, B, JL Mountain. 1997. Detecting immigration by using multilocus genotypes. Proc Natl Acad Sci U S A 94:9197-9201.

Richardson, DE, KE Marancik, JR Guyon, ME Lutcavage, B Galuardi, CH Lam, HJ Walsh, S Wildes, DA Yates, JA Hare. 2016. Discovery of a spawning ground reveals diverse migration strategies in Atlantic bluefin tuna (Thunnus thynnus). Proceedings of the national academy of sciences 113:3299.

Rousset, F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol Ecol Resour 8.

Safina, C. 2016. Data do not support new claims about bluefin tuna spawning or abundance. Proceedings of the national academy of sciences 113:E4261.

Walter, JF, CE Porch, MV Lauretta, SL Cass-Calay, CA Brown. 2016. Implications of alternative spawning for bluefin tuna remain unclear. Proceedings of the national academy of sciences 113:E4259.

Ward, RD, M Woodmark, D Skibinski. 1994. A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. Journal of Fish Biology 44:213-232.

6. INTEGRATED APPROACH TO STOCK DISCRIMINATION

Task leader: Deirdre Brophy (GMIT) Participants:

GMIT: Deirdre Brophy,

AZTI: Haritz Arrizabalaga, Naiara Rodríguez-Ezpeleta, Igaratza Fraile

6.1 Introduction

Various genotypic (Albaina et al. 2013; Boustany et al. 2008; Carlsson et al. 2007) and phenotypic (Brophy et al. 2015; Dickhut et al. 2009; Fraile et al. 2014; Rooker et al. 2008) population markers have been used to distinguish between bluefin from the eastern and western Atlantic. However, there is a degree of uncertainty associated with each method of population assignment. Genotypic and phenotypic markers provide different information about when in the life cycle and to what extent components in a population diverge. Genetic differences between stocks of a highly migratory fish like bluefin tuna confirms reproductive isolation maintained by natal homing (Boustany et al 2008). Variation in the chemical composition of the otolith core (representing the first 12-18 months of life) has been successfully used to distinguish between bluefin from western and eastern nursery areas. The maintenance of these differences in assemblages of spawning adults indicates that bluefin which spawn in the Gulf of Mexico inhabit the western Atlantic in their first year while those that spawn in the Mediterranean occur at Mediterranean nurseries as yearlings (Rooker et al 2008; Rooker at al 2014). It is assumed that there is little or no migration during the first year and that this separation is indicative of natal homing.

Overall accuracy of stock assignment may be improved by using a combination of population markers in an integrated stock mixture analysis (Smith and Campana 2010), however, the underlying assumptions and limitations of each technique must be considered in the interpretation. As part of GBYP phase 6, an integrated

method of stock discrimination was developed by combining multiple markers of stock origin (otolith stable isotopes, genetics and otolith shape) in a random forest classification model (Brophy et al 2017). The results showed that combining otolith stable isotope signatures and genetic markers can reduce the classification error associated with population assignment of baseline samples (known spawning origin). It was also found that the natal stable isotope signatures of adult fish from the Gulf of Mexico and Mediterranean spawning areas were more distinct than those of yearlings from the same areas (as reported by Rooker et al 2014). Consequently, when discriminating spawning areas based on stable isotopes alone, higher rates of classification success (95%) were achieved using otoliths of adult fish (collected from the spawning areas during the spawning season) than was previously reported using yearling baselines (83%). This may indicate that some transfer of fish from the eastern to the western Atlantic occurs after the natal signature is laid down and before the yearlings were captured (i.e. between 12 and 18 months after hatching), in which case it would be more appropriate to use the adult baselines for stock discrimination or alternatively that there is a third spawning component at nursery areas in the west Atlantic (Brophy et al 2017). However, the adult baseline used in phase 6 did not include samples from the Balearic Islands in the Western Mediterranean. If the natal stable isotope signature of bluefin spawning in this area is less distinct from the Gulf of Mexico baseline than bluefin from other parts of the Mediterranean, this would increase the overlap between the two adult baselines.

In this task, the adult baseline is extended to include fish from the Balearic Islands and otolith core isotope signatures are compared with the yearling baseline developed by Rooker et al (2014). The integrated approach to stock discrimination developed in GBYP phase 6 (combining stable isotopes and genetics) is used to assign population origin to individuals from catches taken in the Atlantic. Assignments are compared with single marker assignments.

Objectives:

- To refine the Mediterranean baseline by combining the Balearic fish with other individuals from the western, central and eastern Mediterranean.
- To assign population origin to individuals of potential mixing zones in the Atlantic using the integrated approach to stock discrimination developed in GBYP phase 6 (combining stable isotopes and genetics).

6.2 Methods

Adult baseline samples

Data describing the Isotope composition ($\delta^{13}C$ *and* $\delta^{18}O$) at the otolith core (representing the first 12 months of life) were available for 107 bluefin (>170cm) collected from the Gulf of Mexico between 2009 and 2014 as part of NOAA sampling programs and for 151 bluefin captured from the central (MA, N=69; SY, N=11) eastern (LS, N=25) and western (SA, N=15; TY, N=1; BA N=30) Mediterranean during the spawning season as part of the GBYP sampling programme in 2011, 2012, 2013 and 2015.

Genetic data from 96 SNP loci were available for a subset of this baseline: 45 bluefin of Gulf of Mexico origin and 105 bluefin of Mediterranean origin (MA, N=58; SY, N=11; LS, N=20; SA, N=15; TY, N=1). This data was obtained from a larger genetic baseline comprising 165 bluefin of Mediterranean origin and 181 bluefin of Gulf of Mexico origin.

Yearling baseline samples

Data describing the isotope composition ($\delta^{13}C$ *and* $\delta^{18}O$) at the otolith core of yearling (12-18 months old) bluefin tuna collected from western (N=115) and eastern (N=150) nursey areas between 1998 and 2011 was obtained from a previously published study (Rooker et al 2014).

Mixed sample

Otolith core isotope data ($\delta^{13}C$ *and* $\delta^{18}O$) were available for 2031 bluefin of unknown spawning origin collected from the central (MA, SY), western (SA, TY,

BA) and eastern (LS) Mediterranean and from the eastern (PO, MO, CI, GI, BB) and central (CA) Atlantic. Genetic data were available for 306 of these fish.

Comparison of adult and yearling baselines

Random forest machine learning algorithms were used to classify the adult and yearling baseline fish based on otolith core $\delta^{13}C$ *and* $\delta^{18}O$ values. Classification error rates were compared between models.

Comparison of genetic, isotope and integrated methods of stock discrimination

Random forest machine learning algorithms were used to classify the adult baseline samples for which both genetic and isotope data were available using three approaches: 1) classification based on genetic data; 2) classification using isotope data; 3) classification using both isotope and genetic data together (integrated model). For the genetic analysis gene frequencies at each of the 96 loci were compared between the two populations using Chi square analysis. Loci which varied between populations were included as categorical predictors in models 1) and 3). Classification error rates were compared between models.

Population assignment of mixed sample

Individuals in the mixed sample for which both genetic and isotope data were available were assigned to their population of origin using the integrated classification model (isotopes and genetics) and the isotope only model. Population assignments were compared with previous assignments based on genetics (Rodríguez Ezpeleta et al, *in review*) and Discriminant Function analysis using the Rooker et al (2014) baseline.

Simulation of population mixtures

The population assignment step provided estimates of the population mixture in each area which varied depending on the method used. The purpose of this step was to compare the observed distribution of otolith $\delta^{18}O$ values in the mixed sample to the distribution that would be expected if the mixture was selected at random from the same populations as the adult baselines.

Three density distributions were generated using the distr package in R by drawing 1000,000 random samples from a two component mixture distribution

with means and standard deviations equal to the Mediterranean and Gulf of Mexico populations in the adult baseline and with mixing coefficients equal to those estimated using 1) the genetic assignment model, 2) the isotope model and 3) the integrated model.

6.3 Results

Comparison of adult and yearling baselines

Adult bluefin collected during the spawning season from the Gulf of Mexico and the Mediterranean had distinct otolith core isotope signatures which showed less overlap than those of yearling bluefin collected from nurseries in the eastern and western Atlantic (Figure 6.1). The addition of samples from the western Mediterranean (Balearic Islands; BA) to the adult baseline did not increase the overlap between the two spawning groups; isotope signatures of the western Mediterranean fish overlapped with those of the central and eastern Mediterranean.

Using random forest, fish from the adult baseline were classified to their population origin with a mean accuracy of 95.3% while mean classification accuracy for the yearling baseline was 82.3% (Table 6.1).

Comparison of genetic, isotope and integrated methods of stock discrimination

Using random forest, bluefin from the adult baseline were assigned to their population of origin with accuracy rates of 95.3% using $\delta^{13}C$ and $\delta^{18}O$ isotope values; 91.1% using three SNP genetic markers (Rad213, Rad26 and Rad35) and 97.3% using a combination of otolith chemistry and genetics ($\delta^{13}C$, $\delta^{18}O$, Rad213, Rad26) (Table 6.2). The difference in accuracy between the three methods was most pronounced for the Gulf of Mexico population (91%, 75% and 99% assignment success for the isotope, genetics and integrated models respectively).

Population assignment of mixed sample

The proportions of fish in the mixed sample that were assigned to each population with a probability of >0.8 using each method are shown in Table 6.3. The yearling and adult baselines (isotopes) produced similar rates of assignment to the western

Atlantic and Mediterranean populations. A lower proportion of fish were unassigned when using the adult baseline. The isotope classification model assigned a higher proportion of fish to the western population than the genetics classification model. Using the integrated model, rates of assignment to the western population were similar to those obtained using the genetics model, but fewer fish were assigned to the Mediterranean population and the proportion of unassigned fish was higher. This reflects a discrepancy between the isotope and genetic profiles of some of the fish in the mixed sample; 83 fish had isotope signatures that were similar to the Gulf of Mexico baseline but were genetically similar to the Mediterranean baseline. When both markers were used together these individuals could not be classified to either population. Otolith core $\delta^{18}O$ values for most of these 83 fish were at the higher end of the range observed in the Gulf of Mexico baseline sample (Figure 6.2).

Simulation of population mixtures

The density distribution of $\delta^{18}O$ values in the simulated population mixtures did not align well with the observed $\delta^{18}O$ values in the mixed sample for any of the estimated mixing rates (Figure 6.3). The distributions suggest that there is a disproportionately high number of fish with $\delta^{18}O$ values of between -1.2 and -1.5 than would be predicted if these samples were randomly drawn from the same populations as the adult baseline. The proportion of fish in the mixed sample with $\delta^{18}O$ values between -1.5 and -2.0 was slightly higher than what would be expected given the predictions from the genetics and integrated models and lower than would be expected given the predictions from the chemistry model.

Distribution of $\delta^{18}O$ values in the full mixed dataset

In the full mixed dataset (N=2031) the distribution of $\delta^{18}O$ values in samples from the west Mediterranean (BA), central Mediterranean (SA, AS, MA, TY), Bay of Biscay (BB) and Gibraltar (GI) aligned well with the distribution of $\delta^{18}O$ values in the adult baseline samples. $\delta^{18}O$ values of between -1.2 and -1.5 were higher than the proportions observed in baseline in samples from the central Atlantic (CA), Canary Islands (CI), Morocco (MO) and to a lesser extent Portugal (PO) and the Levantine Sea (LS).

6.4 Discussion

The addition of individuals from the western Mediterranean to the adult baseline did not increase the overlap between the Gulf of Mexico and Mediterranean spawning groups. The otolith core stable isotope signatures of the adults remained more distinct than those of the yearlings. The isotope data used in this study was collected using the same methods as Rooker et al (2014), the same portion of the otolith was analyzed in both cases using the same techniques and the same machine. Both baselines include a wide range of year-classes so inter-annual variability should be reflected in the isotope signatures. The greater overlap in stable isotope signatures in the yearlings compared to the spawning adults may indicate that some transfer of fish from the eastern to the western Atlantic occurred after the natal signature was laid down and before the yearlings were captured (i.e. between 12 and 18 months after hatching) or alternatively that there is a third spawning component at nursery areas in the west Atlantic. In any case, the results show that bluefin from the Gulf of Mexico and Mediterranean spawning populations can be more accurately distinguished based on stable isotope signatures of the adult baseline rather than the yearling baseline. Individuals in the mixed sample were assigned to their population of origin with higher certainty with adult baseline than with the yearling baseline resulting in lower numbers of unassigned individuals.

Adult bluefin from the Gulf of Mexico and Mediterranean spawning areas were discriminated with the highest degree of accuracy (96.6% and 98.1% for GoM and Med populations respectively) using the integrated model (otolith core $\delta^{13}C$ and $\delta^{18}O$ and two SNP genetic markers). The performance of this method exceeded that of either single marker approach in the current analysis and in previous studies which report accuracy rates of 87% (79% and 92% for western and eastern Atlantic respectively) for stable isotopes (Rooker et al 2008) and 82% (81% and 83% for GoM and Med populations respectively) for genetics (Rodríguez Ezpeleta et al. in review. The integrated model did not perform as well when used to assign individuals in the mixed sample to their population of origin; 29% of these individuals could not be assigned to either population with a probability >0.8,

compared to 4% and 11% for the genetics and isotope models respectively. The reason for this was that 27% of the fish in the mixed sample were genetically similar to the Mediterranean baseline but had stable isotope signatures that overlapped with those of the Gulf of Mexico baseline, reducing their probability of assignment to either population when using the integrated model. When taken together, the genetic and stable isotope profile of these fish does not match that of the fish in either spawning area. The possibility that these fish originate from another spawning group or from a contingent within the known spawning populations must be considered.

The comparison of density distributions showed that mixed samples of bluefin from some areas include a disproportionately high number of individuals with otolith core $\delta^{18}O$ values of between -1.2 and -1.5. The majority of the 83 individuals that were assigned to the Mediterranean based on genetics and to the Gulf of Mexico based on stable isotope signatures also had otolith core $\delta^{18}O$ values that fell within this range. This indicates that mixed aggregations of bluefin are not drawn at random from the same populations as the adult baselines. This could occur if the adult baseline samples are not fully representative of the fish that spawn in the Gulf of Mexico or the Mediterranean in May and June. The baseline samples were collected over four years and from four locations in the Mediterranean and over five years in the Gulf of Mexico. The stable isotope signatures of the Mediterranean baseline align closely with those of adult and juvenile fish (size range 52.5-293cm) collected from many of the sites within the Mediterranean east Atlantic (notably MA, AS, BA, BB, GI) from 2009-2016. It therefore seems unlikely that the isotope signatures of the adult baseline samples are not representative of the two spawning populations.

The fish with $\delta^{18}O$ values of between -1.2 and -1.5 might include fish from a third component that spawns at another location or at a different time of year. Recently, larval collections have provided evidence of a bluefin spawning ground in the Slope Sea, between the Gulf Stream and northeast United States continental shelf (Richardson et al 2016). It has been proposed that bluefin spawning in this area

are from the same population as those spawning in the Gulf of Mexico and that movement of fish between the two spawning grounds is size dependent. However, Rodríguez-Ezpeleta et al (in press) report genetic differentiation between larvae from the Gulf of Mexico and Slope Sea young of the year. The individuals in the mixed sample with $\delta^{18}O$ values of between -1.2 and -1.5 grouped with the Mediterranean baseline samples based on their genetics, so this study found no evidence that these are from a third spawning group. However, a more detailed genetic analysis might reveal genetic differentiation at other loci.

Alternatively, the spawning populations could be comprised of multiple contingents. If the migration pathways taken by fish in each contingent diverge during their first year of life, this could produce differences in their otolith core stable isotope signatures. These differences might be detected when contingents occupy different areas as adults (e.g. when feeding) but may not be evident when they converge in the same areas during spawning time. For example, a contingent of the Mediterranean population with intermediate otolith core $\delta^{18}O$ values might be more likely to be found in the Central Atlantic and some Eastern Atlantic sites. At spawning time the signature from this contingent could be diluted by mixing the rest of the Mediterranean population. Consequently, individuals with intermediate $\delta^{18}O$ values would be present in the Mediterranean adult baseline, but at a much lower frequency than in the feeding areas. This hypothesis is consistent with evidence from electronic tagging studies which suggests that the Mediterranean population comprises a resident and a migratory component (Aranda et al. 2013; Arrizabalaga et al. in press) which both spawn in the western and central Mediterranean (Quílez-Badia et al. 2015) and possibly the eastern Mediterranean (Di Natale et al. 2016).

Although combining stable isotope and genetic data does not resolve the issues around assigning individuals in mixing areas to their population of origin, it does prove useful for identifying individuals of uncertain origin (i.e. genetically similar to the Mediterranean population but with otolith core stable isotope signatures like the Gulf of Mexico population). Given that these fish can be genetically

distinguished from the Gulf of Mexico spawning population, they should not be assigned to that population. However, caution should be exercised before assigning to the Mediterranean population. This group may represent a distinct spawning unit or a contingent of the Mediterranean population with a distinct life history. To ensure that the overall diversity of the stocks is preserved it is important that this sub-group is monitored and that its spawning origin is established. Further analysis is recommended to confirm if these fish can be genetically differentiated from the Mediterranean population. In addition, more detailed analysis of stable isotope composition along otolith transects could help to establish if their migration histories are distinct.

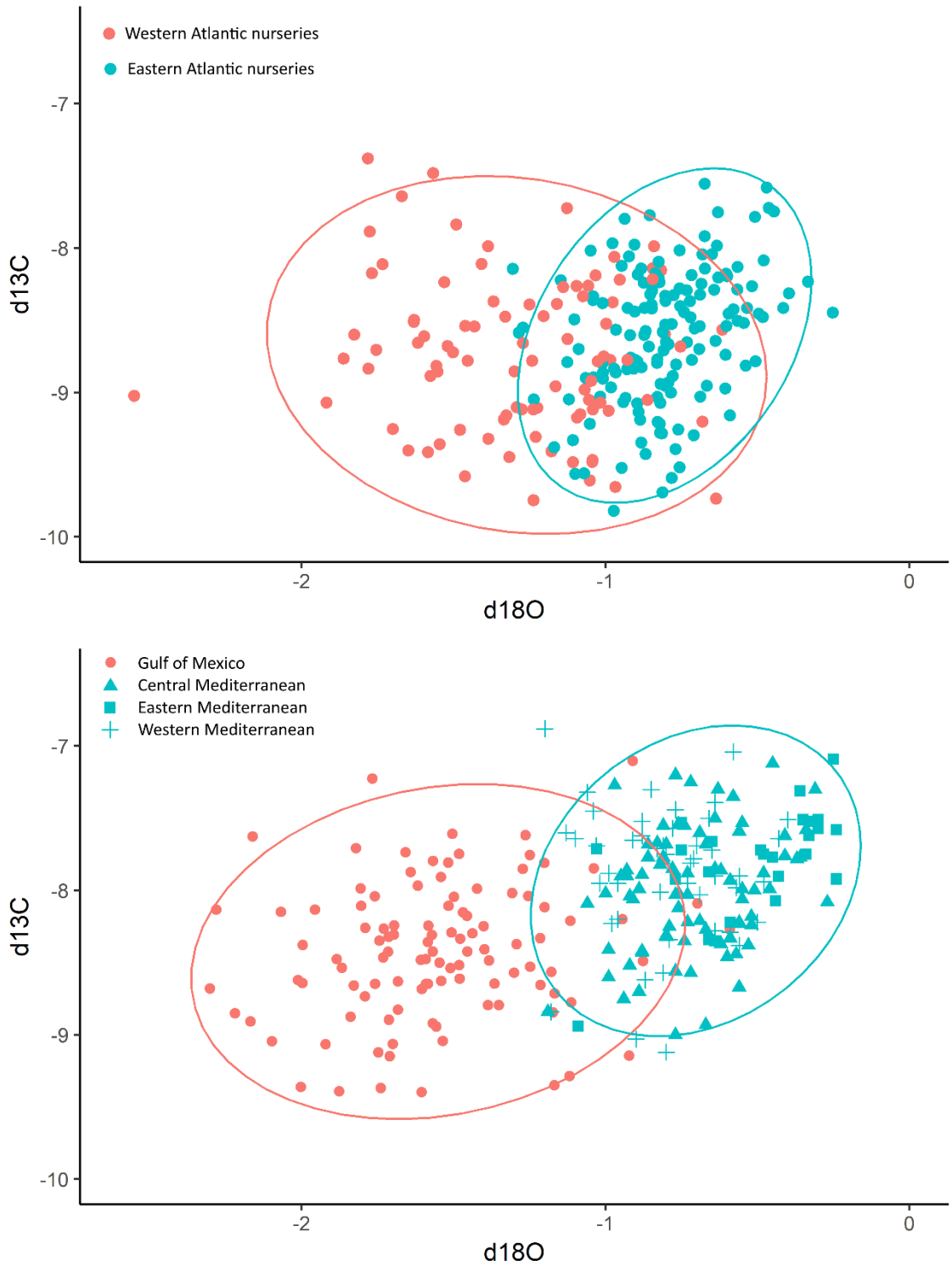

Figure 6.1. Otolith core values of $\delta^{13}C$ and $\delta^{18}O$ for the yearling baseline samples used by Rooker at al (2014) (top panel) and for the adult baseline samples used in this analysis. 95% confidence ellipses are shown for each population.

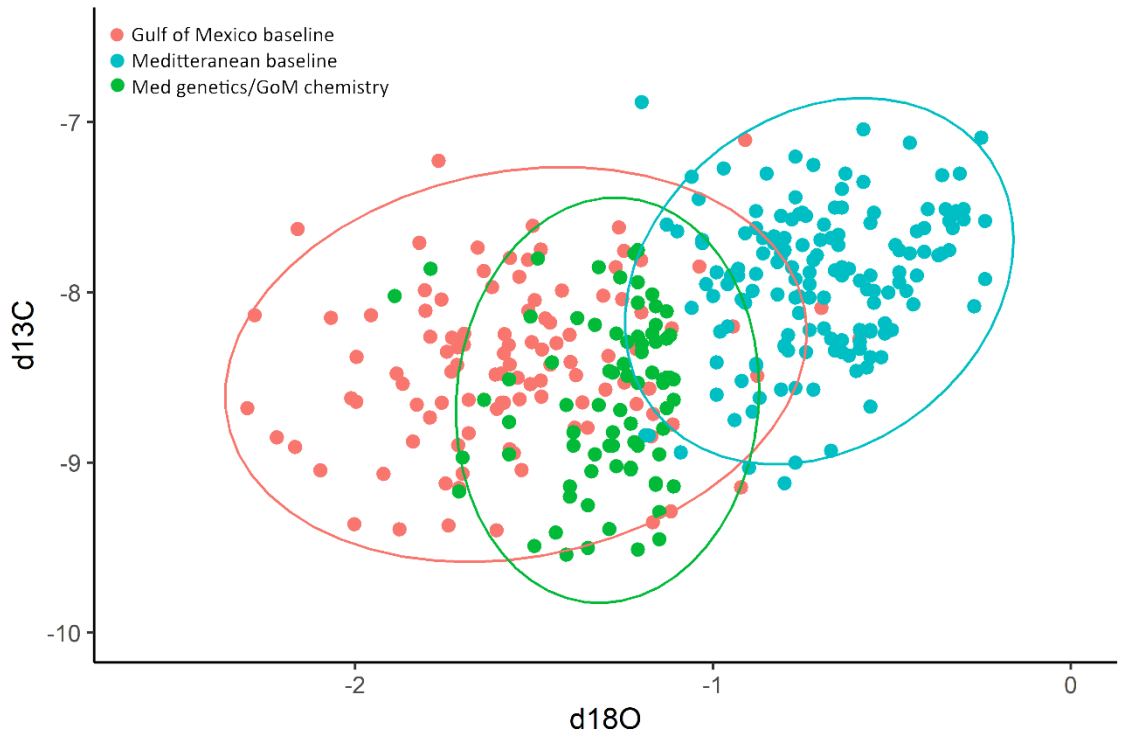

Figure 6.2. Otolith core values of $\delta^{13}C$ and $\delta^{18}O$ for 83 bluefin that were assigned to the Mediterranean population based on their genetics and to the Gulf of Mexico population based on the isotope composition of their otolith cores plotted alongside isotope values for the Gulf of Mexico and Mediterranean adult baselines that were used for the assignment.

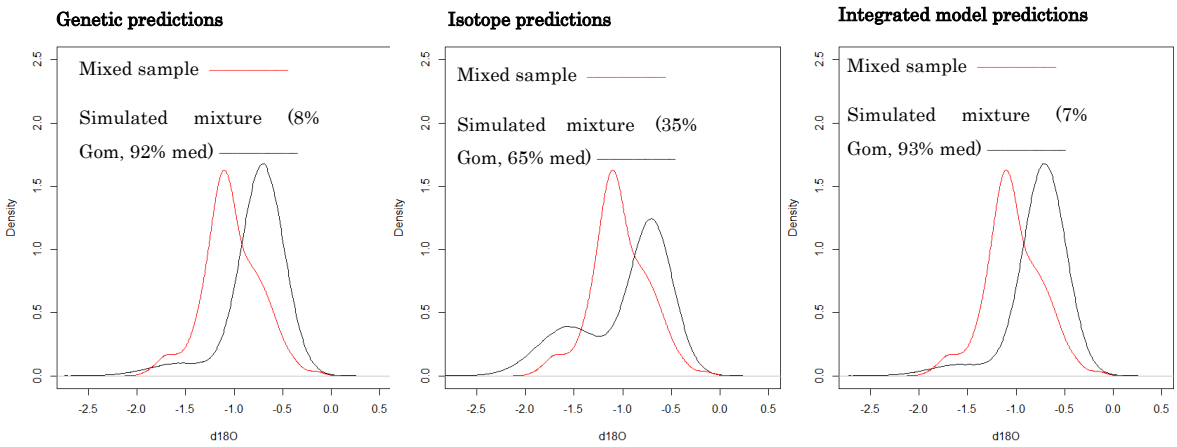

Figure 6.3. Density distributions of otolith core $\delta^{18}O$ values in the mixed sample (N=306; red line) overlaid on predicted density distributions (black line) under the scenario that the mixed sample is randomly drawn from the adult baseline in the proportions indicated by the assignments. Predictions were generated by drawing 1000,000 random samples from a two component mixture distribution with means and standard deviations equal to the Mediterranean and Gulf of Mexico populations in the adult baseline and with mixing coefficients equal to those estimated using the genetic assignment model (left panel), the isotopes model (centre panel) and the integrated model (right panel).

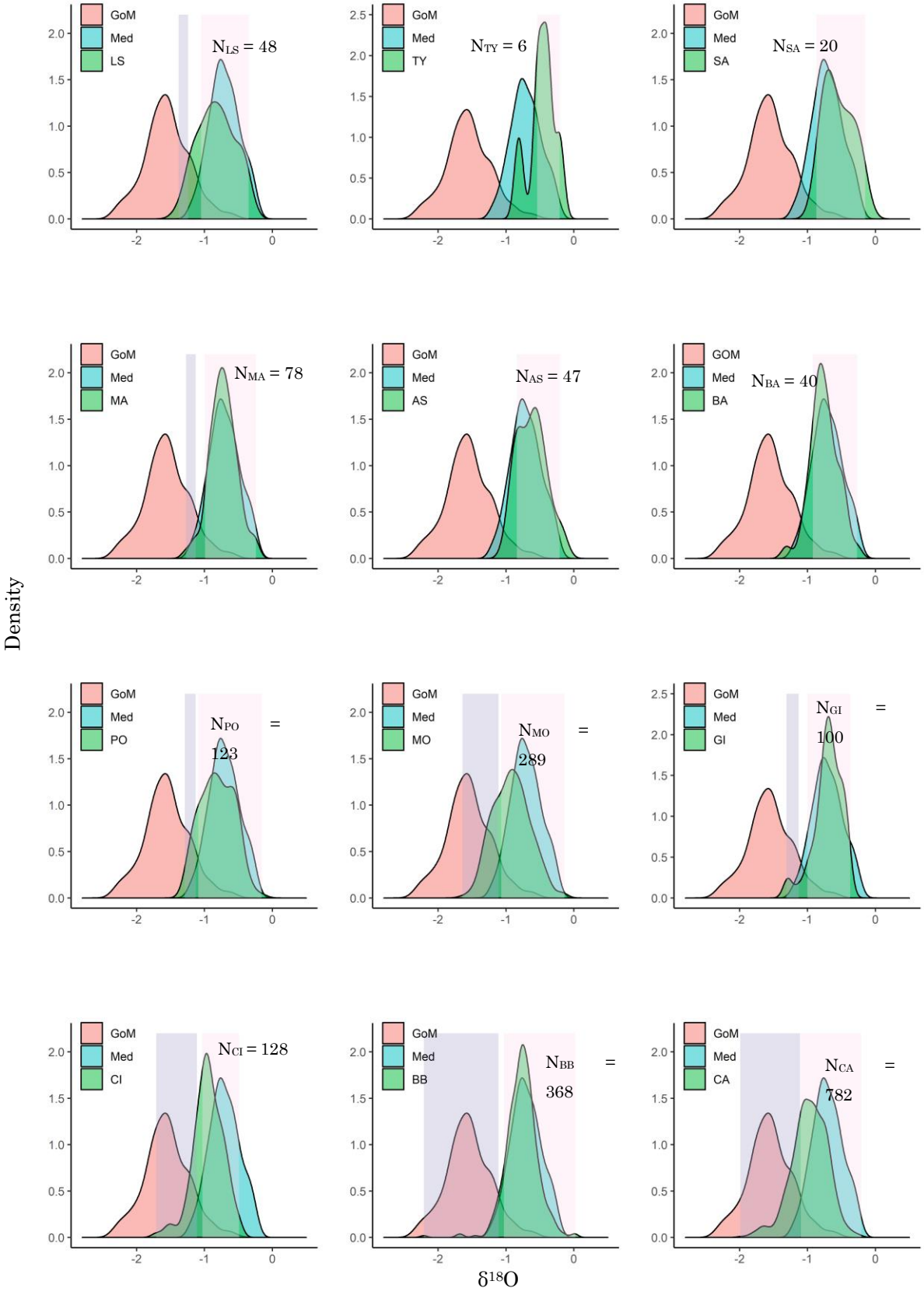

Figure 6.4. Density distributions of $\delta^{18}O$ values in the otolith cores of bluefin of unknown spawning origin collected from the central (MA, SY), western (SA, TY, BA) and eastern (LS) Mediterranean and

from the eastern (PO, MO, CI, GI, BB) and central (CA) Atlantic (green) overlaid on the density distribution of $\delta^{18}O$ values in the otolith cores of adult bluefin from the main spawning areas in the Gulf of Mexico (pink) and the Mediterranean (blue). The shaded areas indicate the range of $\delta^{18}O$ values of the fish that could be assigned to either population with a probability >0.8 (grey shading – GoM; pink shading – Med).

Table 6.1: Confusion matrix from the random forest analysis using $\delta^{13}C$ and $\delta^{18}O$ isotope measurements a) f rom the adult baseline and b) from the yearling baseline samples (Rooker et al 2014)

a)

Isotopes – adult baseline			
	Estimated origin		
True origin	**GoM**	**Med**	**%correct**
GoM	100	7	93.5
Med	5	146	96.7
Total	105	153	95.3

b)

Isotopes – yearling baseline			
	Estimated origin		
True origin	**GoM**	**Med**	**%correct**
GoM	124	26	82.3
Med	21	94	81.7
Total	145	120	82.3

Table 6.2: Confusion matrix from the random forest analysis, using a) $\delta^{13}C$ and $\delta^{18}O$ isotope measurements b) three SNP genetic markers (Rad213, Rad26 and Rad35) and c) a combination of otolith chemistry and genetics ($\delta^{13}C$, $\delta^{18}O$, Rad213, Rad26) to discriminate between adult bluefin tuna (>170cm FL) from spawning populations in the Gulf of Mexico and the Mediterranean. Classifications are based on 150 fish (45 from Gulf of Mexico and 105 from the Mediterranean) which had been analyzed using both methods.

Isotopes – adult baseline			
	Estimated origin		
True origin	**GoM**	**Med**	**%correct**
GoM	41	4	91.1
Med	3	102	97.1
Total	44	106	95.3

Genetics – adult baseline			
	Estimated origin		
True origin	**GoM**	**Med**	**%correct**
GoM	34	10	77.3
Med	3	100	97.1
Total	37	110	91.1

Isotopes and genetics – adult baseline			
	Estimated origin		
True origin	**GoM**	**Med**	**%correct**
GoM	43	2	96.6
Med	2	103	98.1
Total	45	105	97.3

Table 6.3: Proportions of bluefin in the mixed samples assigned to each spawning population using each method of assignment. U: Unassigned; W: West Atlantic; M: Mediterranean.

		Isotopes yearling baseline (Rooker et al 2014)			Isotopes adult baseline			Genetics (Rodríguez Ezpeleta et al in review)			Integrated model (isotopes and genetics)			
Area	**years**	**U**	**W**	**M**	**U**	**W**	**M**	**U**	**W**	**M**	**U**	**W**	**M**	**N**
BB	**2012**	0.25	0.38	0.38	0.13	0.50	0.38	0.13	0.25	0.63	0.50	0.00	0.50	8
CA	**2011, 12,13,14,15**	0.23	0.41	0.36	0.10	0.37	0.52	0.02	0.20	0.78	0.26	0.16	0.58	86
CI	**2013,16**	0.12	0.15	0.72	0.12	0.20	0.68	0.00	0.05	0.95	0.22	0.03	0.75	65
MO	**2012,13,14,15,16**	0.23	0.23	0.54	0.10	0.31	0.59	0.05	0.02	0.93	0.31	0.00	0.69	131
PO	**2012**	0.25	0.44	0.31	0.13	0.38	0.50	0.13	0.00	0.88	0.50	0.00	0.50	16

References

Albaina, A., Iriondo, M., Velado, I., Laconcha, U., Zarraonaindia, I., Arrizabalaga, H., Pardo, M.A., Lutcavage, M., Grant, W.S., and Estonba, A. 2013. Single nucleotide polymorphism discovery in albacore and Atlantic bluefin tuna provides insights into worldwide population structure. Animal Genetics 44(6): 678-692. doi:10.1111/age.12051.

Aranda, G., Abascal, F.J., Varela, J.L., and Medina, A. 2013. Spawning behaviour and post-spawning migration patterns of atlantic bluefin tuna (Thunnus thynnus) ascertained from satellite archival tags. PLoS One 8(10): e76445. doi:10.1371/journal.pone.0076445.

Arrizabalaga , H., Arregui, I., Medina , A., Rodríguez-Ezpeleta , N., Fromentin , J.M., and Fraile, I. in press. LIFE HISTORY AND MIGRATIONS OF MEDITERRANEAN BLUEFIN POPULATIONS. In: B. Block Ed., Bluefin Futures.

Boustany, A.M., Reeb, C.A., and Block, B.A. 2008. Mitochondrial DNA and electronic tracking reveal population structure of Atlantic bluefin tuna (Thunnus thynnus). Marine Biology 156(1): 13-24. doi:10.1007/s00227-008-1058-0.

Brophy, D., Haynes, P., Arrizabalaga, H., Fraile, I., Fromentin, J.M., G., F., Katavic, I., Tinti, F., et al. 2016. Otolith shape variation in bluefin tuna (Thunnus thynnus) from different regions of the North Atlantic: a potential marker of stock origin. Marine and Freshwater Research 67: 1023–1036.

Carlsson, J., McDowell, J.R., Carlsson, J.E.L., and Graves, J.E. 2007. Genetic identity of YOY bluefin tuna from the eastern and Western Atlantic spawning areas. Journal of Heredity 98(1): 23-28. doi:10.1093/jhered/esl046.

Di Natale, A., Tensek, S., and García, A.P. 2016. Preliminary information about the ICCAT GBYP tagging activities in Phase 5. C.

Dickhut, R.M., Deshpande, A.D., Cincinelli, A., Cochran, M.A., Corsolini, S., Brill, R.W., Secor, D.H., and Graves, J.E. 2009. Atlantic Bluefin Tuna (Thunnus thynnus) Population Dynamics Delineated by Organochlorine Tracers. Environmental Science & Technology 43(22): 8522-8527. doi:10.1021/es901810e.

Fraile, I., Arrizabalaga, H., and Rooker, J.R. 2014. Origin of Atlantic bluefin tuna (Thunnus thynnus) in the Bay of Biscay. ICES Journal of Marine Science: Journal du Conseil. doi:10.1093/icesjms/fsu156.

Quílez-Badia, G., Ospina-Alvarez, A., Trápaga, S.S., Di Natale, A., Abid, N., Cermeño, P., and Tudela, S. 2015. The WWF/GBYP multi-annual bluefin tuna electronic tagging program (2008-2013): repercussions for management. .

Rooker, J.R., Secor, D.H., DeMetrio, G., Kaufman, A.J., Rios, 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. doi:10.3354/meps07602.

Smith, S.J., and Campana, S.E. 2010. Integrated stock mixture analysis for continous and categorical data, with application to genetic-otolith combinations. Canadian Journal of Fisheries and Aquatic Sciences 67(10): 1533-1548. doi:10.1139/f10-078.

7. NORWAY CATCH COMPOSITION

Task Leader: Haritz Arrizabalaga (AZTI)

Participants:

AZTI: Patricia Lastra, Naiara Rodriguez Ezpeleta

IEO: Enrique Rodriguez Marín, Marta Ruiz, Elvira Ceballos

UNIGE: Fulvio Garibaldi

IMR: Leif Nøttestad

7.1 Introduction

The Atlantic bluefin tuna (ABFT) catches in Norway occurs near the northernmost distribution area assumed for this species. During the 1950's and beginning of the 1960's substantial catches of BFT reaching up to 15 000 tons were taken along the Norwegian coast, involving up to 470 coastal vessels (Cort and Nøttestad 2007). In 1963, the leading fisheries targeting Atlantic bluefin tuna (*Thunnus thynnus*) in the Norwegian Sea and North Sea quite suddenly collapsed without any warning. Little is known about this collapse and several hypotheses have been put forward, such as changes in migratory routes, recruitment failure, recruitment overfishing or eradication of a sub-population (Fromentin 2009; Fromentin and Powers 2005; Cort and Abaunza 2016). More recent research suggests that the collapse could be linked to environmental effects (Failletaz et al. 2019). In Norway, the mean size of the catches steadily increased, primarily due to repeated annual fishing on a couple of strong year classes, while variability decreased, after the collapse (Nøttestad and Norman 2004).

On top of this, Fromentin (2009) suggested potential links of the Norwegian fishery with Spanish traps and western Atlantic fisheries, but the population of origin of Norwegian fish was never resolved.

During the last decade, ABFT is back in Norwegian waters (Nøttestad et al. 2017), and biological samples of nearly all individuals caught in directed fishery and as bycatch have been provided by IMR since 2016. This provides an excellent opportunity to characterize

the catch composition of ABFT in Norway, both in terms of origin (to see which population they belong to, whether both populations are represented, and if the stock of origin proportions are consistent over time). It will also be interesting to see in the future whether the same cohorts are visiting or living in Norwegian waters over time, suggesting a homing behavior during the feeding period. This could lead to a potential extinction in the future, depending on the fishing pressure and fishing pattern, as we may have experienced in the past).

In this study we assigned origin and age to ABFT individuals collected in Norway during 2017 and compared them to the information obtained previously, to allow for some initial interannual comparison of both origin and age composition of the Norwegian catches.

7.2 Material and Methods

Sample collection

Individual fin spines and genetic samples were collected from fresh Atlantic bluefin tuna (ABFT) caught by commercial purse seine fishing boats in Norwegian waters. Some samples are also taken from by-catches from other small pelagic fisheries, as well as samples taken from ABFT caught inside salmon pens along the coast and fjords of Norway. The sample set analyzed in this case study comprised 446 individuals, with a single individual from 2013 and 26, 200 and 216 individuals from 2015, 2016 and 2017, respectively. Most of these samples were taken in September (n=385), with few samples taken in August (n=52) and April (n=6). From these, 417 fin spines (n=190 and n=227 for 2016 and 2017 respectively) where processed to estimate age and 402 muscle tissue samples were processed to assign genetic origin.

Dorsal fin spine preparation

Fin spine preparation and sectioning procedure were performed following the procedure described by Rodriguez-Marin et al., (2012) and Luque et al., (2014). A cross-section of approximately 1 mm thickness was sectioned at the point 1.5 times the condyle base width with an Isomet low-speed saw (Buehler, Lake Bluff, Illinois, USA). Fin spine sections were examined using an optical microscope and under transmitted light. Age was estimated by counting the translucent bands which are deposited annually (Luque et al., 2014). An annulus is defined as a bipartite structure consisting generally of a wide opaque band followed by a narrow translucent band, presumably formed on a yearly basis. These

annuli were, however, not always a bipartite structure and sometimes multiple opaque and translucent pair banding was observed

Age interpretation

Age interpretation was performed using digital images that were captured with a binocular lens magnifier connected by digital camera. An image analyser was used to measure the diameter of the fin spine section and visible translucent bands.

Overall, fin spine sections were read by at least two independent expert readers, although a total of four readers (PLL, MRS, ECR, FG) from three institutions AZTI, IEO, and Università di Genova, respectively, participated in the ageing of the total data set. For those fin spines for which there was an age disagreement (n=21 in 2016 and n=22 in 2017), an additional reading was conducted by one expert reader (PLL), and the consensus among readers/readings was considered the final age estimation used for further analysis. In addition, final age estimation from fin spines was adjusted by subtracting 1 year to the age when the fish was caught between June and December 31 and the edge of the structure was translucent (Luque et al., 2014).

Origin assignment

Origin was assigned using the same SNP panel used in this project to assign genetic origin of samples caught elsewhere in the Atlantic. The genetic methodology is described in section 5 (Rodriguez Ezpeleta et al 2019). Once the probability to belong to the Mediterranean Sea and Gulf of Mexico populations was estimated, an 80% threshold was used to assign origin to these two areas, while probabilities between 20% and 80% where left unassigned.

7.3 Results and Discussion

Age distribution

The individual straight fork lengths (SFLs) ranged between 188 cm and 262 cm, with a peak around 215-225 cm (Table 7.1 and Figure 7.1). Assigned ages varied between 6 years and 14 years, the majority of individuals being aged 9 and 10 years (Table 7.1 and Figure 7.2). Although in 2016 slightly more age 11 individuals were observed compared to 2017, both SFL and age distributions were quite similar in 2016 and 2017. Thus, the range of ages looks quite variable and it does not so far indicate to be the case that the same age

groups of bluefin tuna visits Norway year after year while becoming older. Nevertheless, it is worth mentioning that practically all samples taken from 2016 originates from one single purse seine catch, whereas samples taken from 2017 were spread out in space and time. Furthermore, we have only so far analysed two consecutive years, which is too short to reach any firm conclusion. Additional light on this issue can be shed in the following years if additional samples are analyzed.

Table 7.1. Norwegian bluefin age-length key built up with samples collected during 2016-2017. Numbers by 5 cm length classes (straight fork length, SFL).

	Estimated Age										
SFL (cm)	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**NULL**	**Total**
185-189	1	1		1							3
200-204			8	4	1						13
205-209		3	8	10	4		1				26
210-214		2	12	29	8	3	1				55
215-219			10	27	32	11	2	1			83
220-224			8	25	29	12	3	1			78
225-229			1	7	26	17		1			52
230-234				6	15	17	4	1			43
235-239				4	10	7	10	2		1	34
240-244				1	5	5	3	3	1		18
245-249					1	1	3	1			6
250-254					2	1	1				4
>255							2				2
Total	**1**	**6**	**47**	**114**	**133**	**74**	**30**	**10**	**1**	**1**	**417**

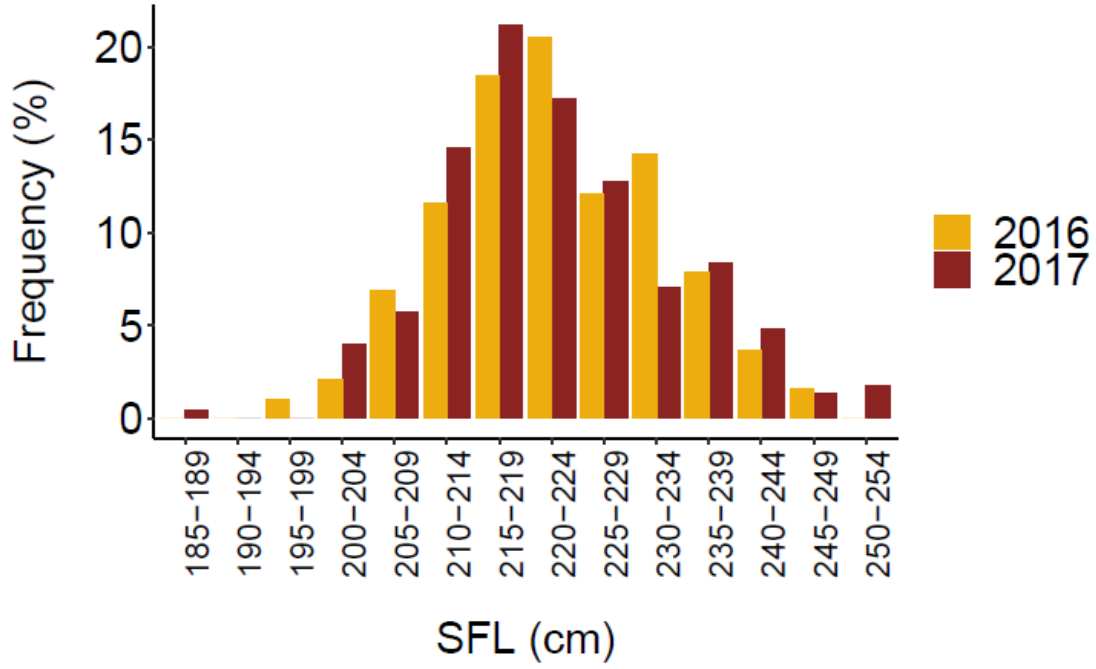

Figure 7.1. Norwegian bluefin tuna straight fork length distribution by year.

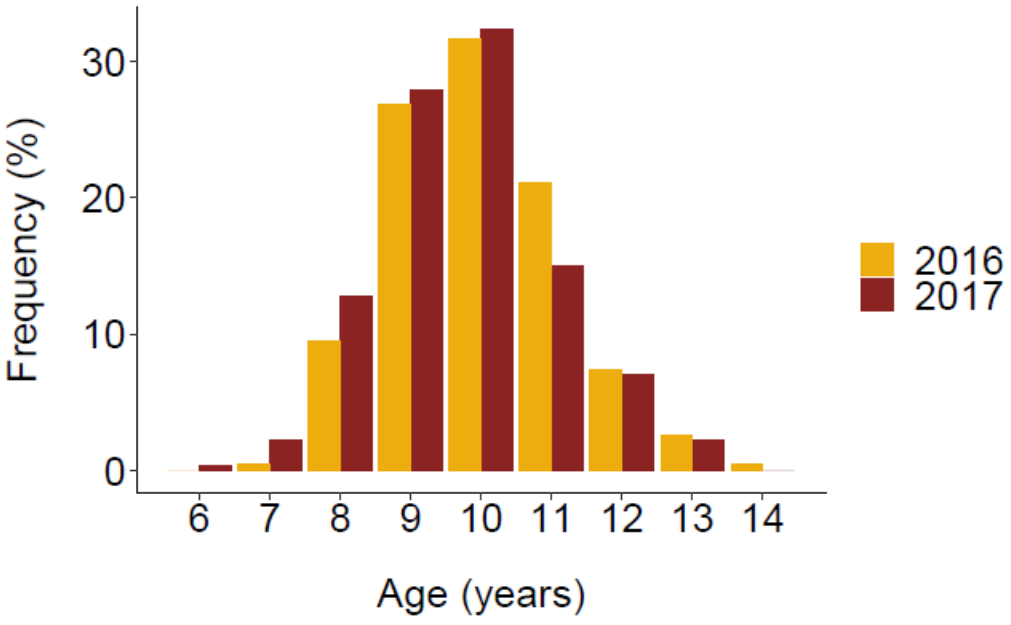

Figure 7.2. Norwegian bluefin tuna estimated age distribution by year.

Following the 80% threshold for assignment, which represents a rather strict threshold so as to have reliable assignments, altogether 335 individuals (83.3%) were assigned to

the Mediterranean population, 15 individuals (3.7 %) to the Gulf of Mexico population and 52 individuals (13%) remained unassigned (Figure 7.3). Thus, the results suggest that the Norwegian feeding grounds are mostly used by the Mediterranean population. The estimated proportion of 3.7% western origin fish is within the genetic method assignment error, thus could just be a result of misassignment and it is difficult to judge whether there is a real, low proportion of western origin fish visiting the Norwegian feeding ground. If these fish were assigned to the western population by error, one would expect them to be randomly distributed in time, age, position, etc. Indeed, Table 7.2 shows that western origin individuals have been assigned in all years and months, as well as in most of the predominant age classes (8 to 13). In these cases, the estimated proportions were small, always below 5% except in two cases (for the year 2015 and for the age 13) with lower sample sizes, where the proportions were slightly larger (around 9%). Figure 7.4 also shows that individuals assigned to the west are not localized in a concise area nor are spatially patchy, instead, they show a relatively spread distribution.

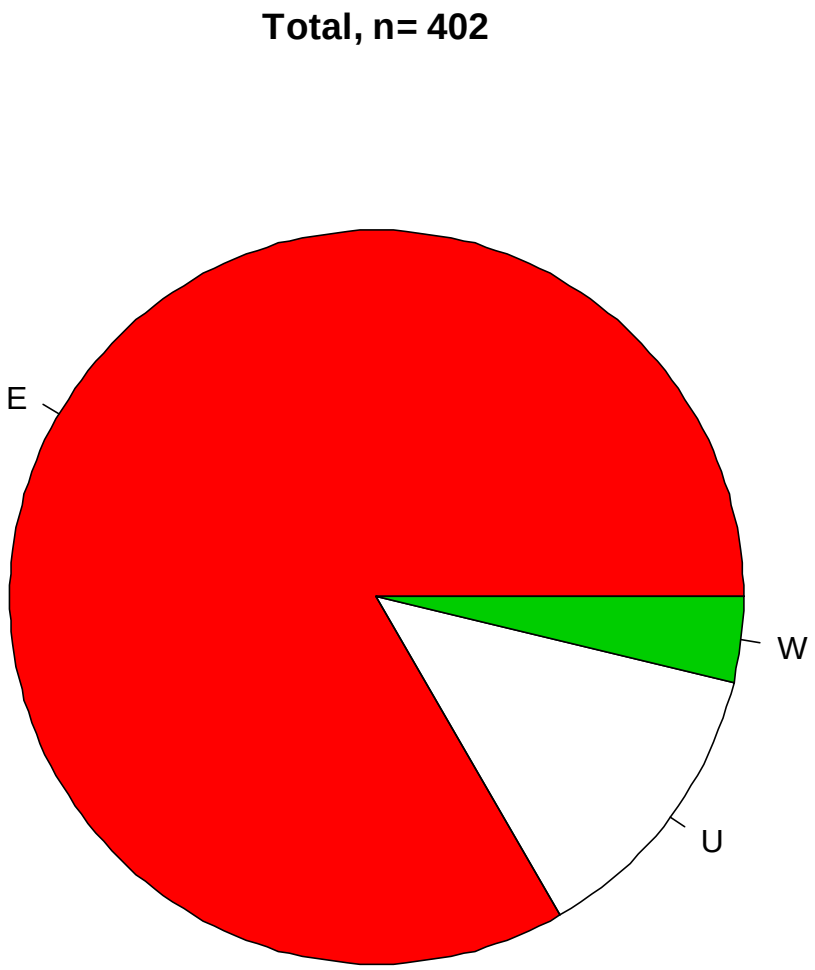

Figure 7.3. Proportion of Norwegian bluefin tuna assigned to the eastern population (red), western population (green) and unassigned (white).

Table 7.2. Proportion of Norwegian bluefin tuna assigned to the western population by year (a), month (b) and age (c).

a)	% West
2015	9.09 %
2016	4.29 %
2017	2.77 %

b)	% West
August	1.92 %
September	4.07 %

c) Age	% West	n
6	0 %	2
7	0 %	5
8	4.88 %	41
9	3.06 %	98
10	4.13 %	121
11	3.64 %	55
12	0 %	21
13	9.09 %	11
14	0 %	1

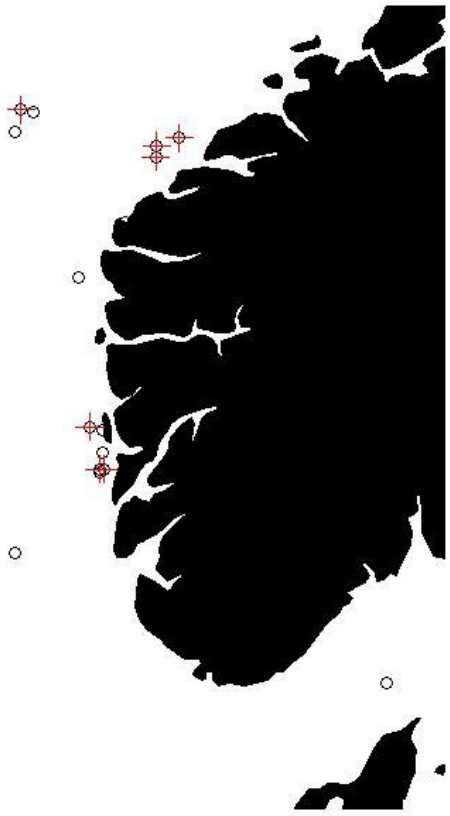

Figure 7.4 Norwegian bluefin tuna catch locations (black circles), and locations where at least one bluefin tuna was assigned to the western population (red crosses).

References

Cort, J. L., and L. Nøttestad 2007. Fisheries of bluefin tuna (Thunnus thynnus) spawners in the Northeast Atlantic. Col. Vol. Sci. Pap. ICCAT, 60: 1328–1344.

Cort, J. L. and P. Abaunza 2016. The impact of massive fishing of juvenile Atlantic bluefin tunas on the spawning population (1949-2010). Standing Committee on Research and Statistics (SCRS) 2016/151, 35 p.

Faillettaz, R., Beaugrand, G., Goberville, E. and Kirby, R.R. (2019) Atlantic Multidecadal Oscillations drive the basin-scale distribution of Atlantic bluefin tuna. Science advances 5: eaar6993.

Luque, P. L., Rodriguez-Marin, E., Landa, J., Ruiz, M., Quelle, P., Macias, D., et al. (2014). Direct ageing of Thunnus thynnus from the eastern Atlantic Ocean and western Mediterranean Sea using dorsal fin spines. J. Fish. Biol. 84, 1876-1903. https://doi.org/10.1111/jfb.12406.

Nøttestad L., og N. Graham. 2004. Preliminary overview of the Norwegian Fishery and Science on Atlantic Bluefin Tuna (Thunnus Thynnus), Presented at the 37th Regular Meeting of ICCAT, New Orleans, USA. 12 p.

Nøttestad, L. Ø. Tangen, K.R. Utne, and J. Hamre. 2017. Distribution, catches and science on Atlantic bluefin tuna (Thynnus thunnus) in Norwegian waters (in Norwegian). Institute of Marine Research, Pelagic Research Group, Bergen, Norway. Scientific report to the Ministry of Trade, Industry and Fisheries in Norway. 35 p.

Rodriguez-Marin, E., Luque, P.L., Ruiz, M., Quelle, P., Landa, J., 2012. Protocol for sampling, preparing and age interpreting criteria of Atlantic bluefin tuna (Thunnus thynnus) first dorsal fin spine sections. Coll. Vol. Sci. Pap. 68, 240–253.

8. MSE HYPOTHESES

Task leader: Haritz Arrizabalaga (AZTI)

Participants:

GMIT: Deirdre Brophy

AZTI:, Naiara Rodríguez-Ezpeleta, Igaratza Fraile

During the course of the contract, substantial interactions occurred with the chair and contractor developing the MSE with regard to how to best use the mixing data. After a first meeting in Madeira, the GBYP dataset was revised to include some information (namely the primary technique used in each assignment), for them to refine the method to integrate this information in the MSE. Subsequently, additional reanalyses on the genetic data have been collected and submitted, namely the reassignment of known origin samples (larvae and YOY from the Mediterranean and the Gulf of Mexico), so as to be able to describe the distribution of the probability to belong to the eastern stock, which was required by the chair and the contractor to develop a new method to use all stock of origin data available (Carruthers and Butterworth 2018). These reanalyses were shared with them together with instructions on their meaning and how to use them in the MSE context. Consequently, the genetic data generated by GBYP has been finally integrated into the MSE approach, as can be confirmed in the MSE Trial Specifications document distributed within the BFT working group.

The MSE discussions in the bluefin tuna working group have evolved relatively quickly during the last weeks/months. Among other things (relevant to this contract), there is a new, simplified, spatial structure adopted for the MSE. On the other hand, there has been some agreement that at least one of the alternative mixing scenarios can consider no presence of western origin fish in the east. This is based mostly on e-tag information, and seems to potentially contradict the otolith chemistry data. In our analyses, we provide ranges of mixing estimates using different methodological options, that might help (further) bracket the possibilities that can be considered in the MSE.

These analyses suggest that variability in estimated stock proportions can be quite substantial (Table 8.1). For each stratum (defined as combination of Year, Quarter, size

class and Area), the proportion of western stock in the sample was computed transforming the probability to belong to the western stock into origin assignment by using a threshold of 0.8. This assigns to the GOM and MED the individuals that have a probability >0.8 and <0.8, respectively, while leaves the other individuals unassigned. The individual probabilities to belong to the western stock were calculated using:

- Genetic methodology described in section 4 of this report
- QDFA on otolith stable isotopes data, using the yearling baseline
- QDFA on otolith stable isotopes data, using the adult baseline
- Random Forest on otolith stable isotopes data, using the yearling baseline
- Random Forest on otolith stable isotopes data, using the adult baseline

Table 8.1. Proportion of western origin individuals by ICCAT area. Summary statistics (minimum, median and maximum) of estimated proportions under each year, season, size class and using a range of methodological alternatives (genetics or otolith chemistry using alternative baselines and alternative individual classification algorithms).

AreaName	min pW	median pW	max pW
WATL	0.08	0.28	0.64
SATL	0	0.1	0.65
NATL	0.02	0.17	0.51
NEATL	0	0.07	0.1
MED	0	0.02	0.11

It is observed that for most areas there is substantial uncertainty around the proportion estimates. The ranges reflect the uncertainty related to natural variations in mixing (e.g. interannual, seasonal and ontogenetic variation), as well as uncertainty due to the methodology used to estimate the proportions. In future, finer scale analyses of these estimates could further inform the BFT MSE process, as well as allow understanding the main factors affecting mixing.

Below is a list of working hypotheses about mixing that have been or are being considered in the BFT MSE:

1. Mixing is reflected by the stock of origin (and other) data

2. No mixing

3. Half the inferred level of stock mixing

4. No western fish in the east

5. Time varying mixing, including up to 150% of inferred levels

Hypothesis 1 is currently the one that is considered in all the Reference set of Operating Models (OMs), as they use the stock of origin (SOO) data compiled by the working group to inform (together with e-tag and the master index) the models about mixing. This has led to some difficulties as substantial proportions of western origin fish in the east have been estimated and are difficult to explain by the spatially structured, two stock model. It is important to note that, while there can be substantial differences in the proportions of origin estimated by different methodologies, currently all data within the data compilation is equally weighted.

Specifically, the data compiled within GBYP across different Phases suggests a higher proportion of western origin fish in the east estimated through otolith chemistry, compared to genetics (Figure 8.1). However, this could be due to both the methodology used and the set of samples analyzed (which differ between methods). In order to shed additional light on the issue, we made the same comparison using only fish that were analyzed with both techniques, and the general picture remains the same, so we can conclude that using otolith chemistry provides the perception that the proportion of western origin fish in the east is higher (Figure 8.2). As a matter of fact, using only fish that were analyzed with both genetics and otolith chemistry, otolith chemistry provided systematically higher proportions of western origin fish in ICCAT Area 4, one of the areas that proved problematic in the past (Table 8.2).

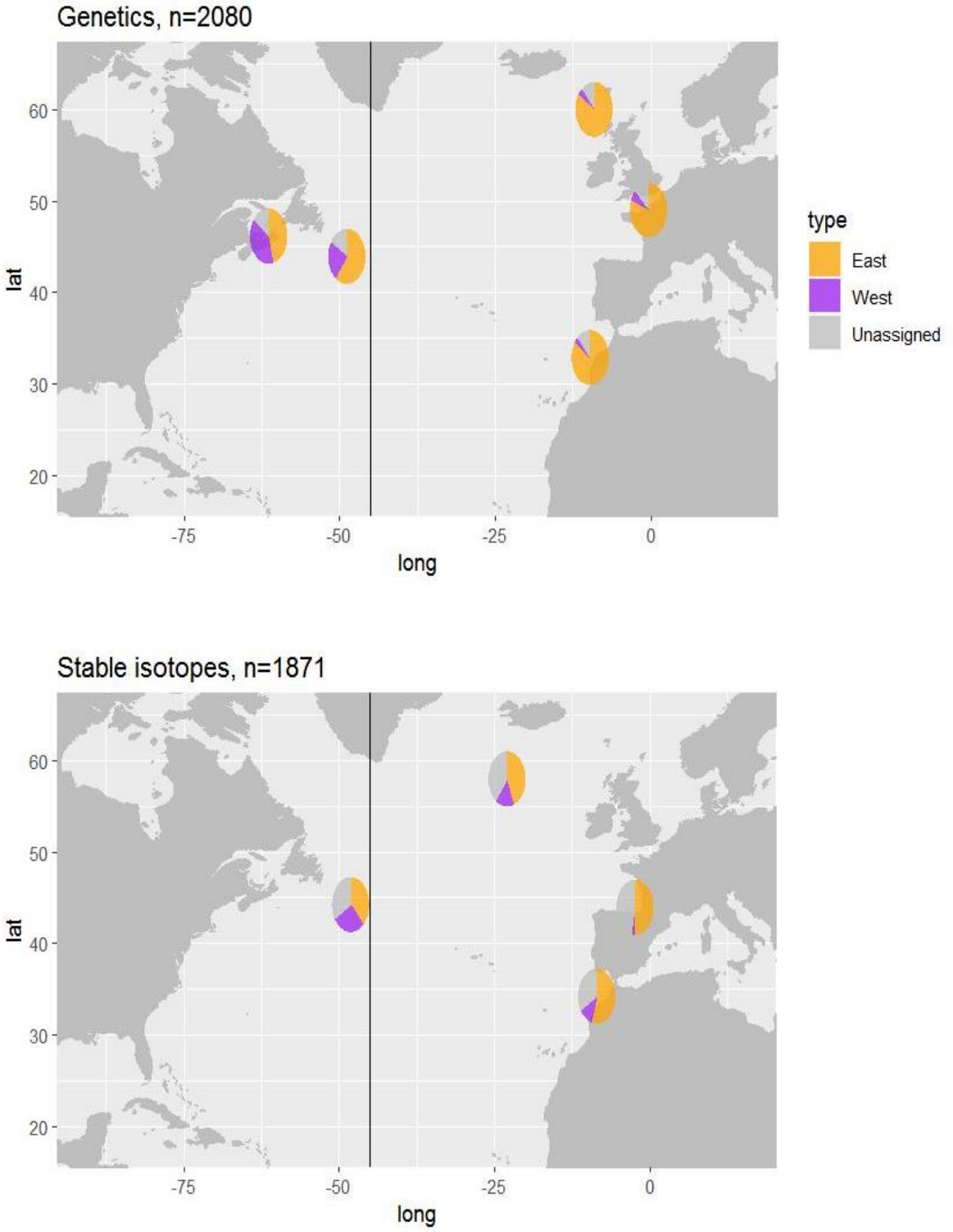

Figure 8.1. Proportion of western origin individuals by ICCAT area, using all the genetic (a) and otolith chemistry (b) analyses conducted within GBYP. In the case of genetics, only RAD-Seq based results are considered.

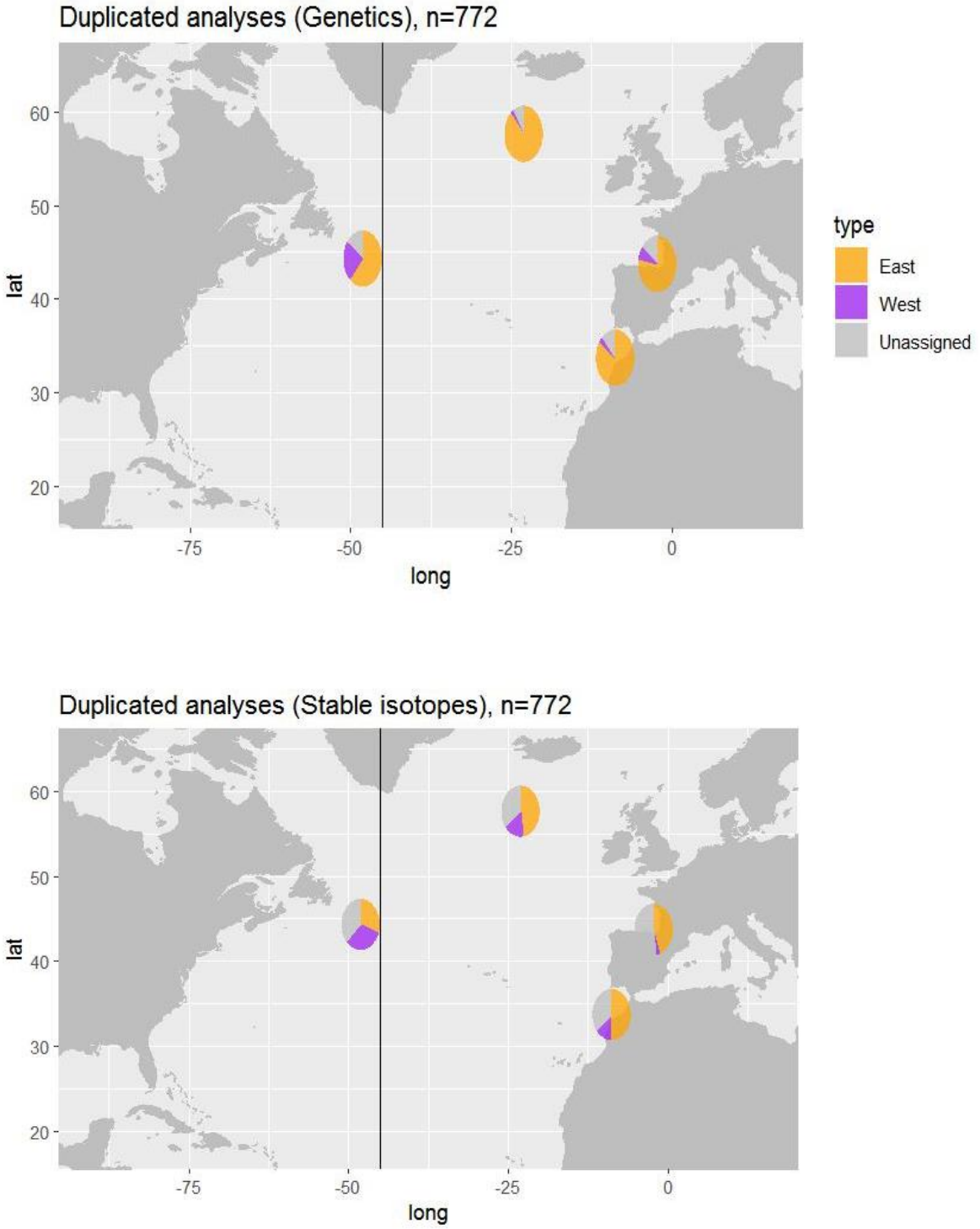

Figure 8.2. Proportion of western origin individuals by ICCAT area, using all the genetic (a) and otolith chemistry (b) analyses conducted within GBYP on exactly the same individuals. In the case of genetics, only RAD-Seq based results are considered.

Table 8.2. Proportion of western origin individuals in ICCAT Area 4, per year, using genetic and otolith chemistry analyses conducted within GBYP on exactly the same individuals (n=384 from Morocco and Canarias). In the case of genetics, only RAD-Seq based results (Phases 6-8) are considered.

	2011	2012	2013	2014	2015	2016
GEN	0	0.03	0.01	0.08	0.02	0.04
CHEM	0	0.13	0.18	0.61	0.2	0.22

In fact, considering the missasignment error rates of the genetic method, the small western proportions in the east observed with genetics can be due to missassignment, thus genetics suggesting that this proportion might be minor (in accordance with e-tags) or at least very difficult to detect.

Hypothesis 2 was considered in the past, on the basis that, depending on the type of analysis conducted, for many strata, one cannot refute the null hypothesis that 100% of the sample belongs to the same population. To illustrate this, we computed proportions of eastern fish across strata using maximum likelihood (Millar 1990) on otolith stable isotope data (Table 8.3). The results suggest that, by analyzing the data in this particular way (using otolith chemistry, which is the majority of the data compiled for the MSE exercise, and the maximum likelihood approach to estimate proportions and standard deviations), in most of the cases, one cannot refute the null hypothesis according to which all fish in the sample are of eastern origin.

Table 8.3. Proportion of eastern origin individuals per year, season and area, estimated with maximum likelihood (Millar 1990) on otolith chemistry data. Standard deviation of the estimated proportions are provided in brackets. White cells indicate the cases where, according to the confidence intervals (two s.d. around the mean), the null hypothesis of single eastern stock contribution cannot be refuted, while yellow cells indicate cases where the alternate hypothesis (that not all are eastern fish) could be accepted.

Mean East Proportion (+-1 s.d)						
		Area				
year	season	EATL	MED	NATL	SATL	WATL
2009	1					
	2					
	3	0.96 (0.02)				
	4					
2010	1					
	2		1 (0)		0.91 (0.07)	
	3	1 (0)				
	4	1 (0)		0.67 (0.08)		
2011	1					
	2		1 (0)		0.92 (0.03)	
	3		1 (0)		1 (0)	
	4		0.99 (0)	0.98 (0.02)		
2012	1					
	2		1 (0)		1 (0)	
	3	0.98 (0.02)				0.16 (0.08)
	4			0.81 (0.06)	0.93 (0.26)	
2013	1				0.81 (0.15)	
	2				0.94 (0.04)	
	3					0.51 (0.12)
	4			0.56 (0.12)		
2014	1				1 (0)	
	2				0.4 (0.12)	
	3					0.99 (0)
	4			0.94 (0.05)		0.88 (0.1)
2015	1				0.78 (0.15)	
	2		1 (0)		0.79 (0.1)	
	3					0.88 (0.16)
	4			1 (0)		0.9 (0.14)
2016	1				0.81 (0.1)	
	2				1 (0.01)	
	3					
	4		1 (0)			

In any case, Hypothesis 2 is currently not considered, as the working group felt that it was too extreme or too wide an envelope, and considered, alternatively, Hypotheses 3 and 4. Hypothesis 3 was considered on the basis that missasignment errors cannot be disentangled from mixing, and thus the mixing rates estimated by fitting to the SOO can be overestimated. Hypothesis 4 was considered on the basis that the compiled e-tag data suggests that western origin fish do not visit eastern areas, which is somewhat concordant with the GBYP genetic data. Currently, the WG, with input from this consortium, is considering merging these two hypotheses and consider them as part of the Reference set of OMs.

References

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

Carruthers T., and Butterworth D.S. A mixture model interpretation of stock of origin data for Atlantic bluefin tuna. SCRS/2018/133

9. APPENDICES

Appendix 1: Protocols and forms (see "SAMPLING PROTOCOLS FOR BFT GBYP final 18102016.doc".

Appendix 2: Database as of 15th March 2019 (see "Database_15_Mar_2019.xls). Note that this database is subject to change in the future as new samples are integrated.