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STOCK IDENTIFICATION AND CONNECTIVITY OF ATLANTIC BLUEFIN TUNA (*THUNNUS THYNNUS*) WITH OTOLITH CHEMISTRY

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SUMMARY

Geographic natal assignments of Atlantic bluefin tuna, Thunnus thynnus, *captured over the last decade have been carried out using otolith carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotope analyses to examine population structure. Regions included Mediterranean Sea and Gulf of Mexico, as well as several areas within the North Atlantic Ocean. The results provide strong evidence of longitudinal population structuring of bluefin tuna in the North Atlantic Ocean. Mixing of the eastern and western stocks occurs on foraging grounds on both sides of the 45ºW management boundary, although the proportion of Gulf of Mexico origin fish found to cross to the east, is smaller than the proportion of Mediterranean Sea origin fish found to cross to the west. Furthermore, for all regions analysed, mixing rates appear to be non-constant and vary considerably from year to year.*

RÉSUMÉ

Les assignations géographiques natales de thons rouges de l'Atlantique, Thunnus thynnus, *capturés au cours de la dernière décennie ont été réalisées en utilisant des analyses des isotopes stables du carbone (δ13C) et de l'oxygène (δ18O) des otolithes afin d'examiner la structure de la population. Les régions incluaient la mer Méditerranée et le golfe du Mexique ainsi que plusieurs zones de l'océan Atlantique Nord. Les résultats ont fourni des preuves solides d'une structuration longitudinale de la population de thon rouge dans l'océan Atlantique Nord. Le mélange des stocks de l'Est et de l'Ouest a lieu dans les aires d'alimentation des deux côtés de la délimitation de gestion de 45ºO, même si la proportion des poissons originaires du golfe du Mexique traversant vers l'Est est plus faible que la proportion de poissons originaires de la mer Méditerranée traversant vers l'Ouest. En outre, pour toutes les régions analysées, les taux de mélange semblent ne pas être constants et varier considérablement d'une année sur l'autre.*

RESUMEN

Las asignaciones geográficas natales del atún rojo del Atlántico, Thunnus thynnus*, capturado durante la última década se han llevado a cabo utilizando análisis de isótopos estables de carbono (δ13C) y oxígeno (δ18O) de los otolitos para examinar la estructura de la población. Entre las regiones se incluyen el mar Mediterráneo y el Golfo de México, y varias zonas del océano Atlántico norte. Los resultados proporcionan pruebas sólidas de la estructuración*

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longitudinal de la población de atún rojo en el Atlántico norte. La mezcla de los stocks orientales y occidentales se produce en las zonas de alimentación a ambos lados del límite de ordenación de 45º O, aunque la proporción de peces originarios del Golfo de México que cruzan hacia el este es menor que la proporción de peces originarios del mar Mediterráneo que cruzan hacia el oeste. Además, en todas las regiones analizadas, los índices de mezcla parecen no ser constantes y varían considerablemente de un año a otro.

KEYWORDS

Bluefin tuna, origin, mixing; connectivity, otolith $\delta^{13}C$, $\delta^{18}O$

1. Introduction

Atlantic bluefin tuna (ABFT, *Thunnus thynnus*) is a large pelagic migratory species that lives mainly in the temperate ecosystem of the North Atlantic Ocean and adjacent seas. The management plan for ABFT considers two management units (stocks) separated by the 45°W meridian, with the western stock spawning in the Gulf of Mexico and adjacent waters and the eastern stock in the Mediterranean Sea. However, stock overlap in the North Atlantic Ocean makes assignment of catches to stock-of-origin difficult, as the degree of connectivity between the two management units is one of the major uncertainties of the stock assessment models (Carruthers, 2016).

The implications of this mixing and the extent to which it could affect the stock assessment and management advice of ABFT has been further discussed for both eastern (E-BFT) and western (W-BFT) stock assessments (ICCAT, 2022; Maunder, 2021). To this regard, the ICCAT Atlantic-Wide Research Programme for Bluefin Tuna (GBYP, https://www.iccat.int/gbyp/en/), as well as national research programmes, have been supporting ongoing efforts to provide important insights into bluefin tuna stock structure, distribution, mixing and migrations (e.g. Rooker *et al.*, 2014, 2019; Rodríguez-Ezpeleta *et al.*, 2019). Among the different lines of research, otolith carbon and oxygen stable isotope ($\delta^{13}C$ and $\delta^{18}O$) composition have been routinely analyzed over the last decade to investigate the degree of eastern and western population contribution to different feeding grounds in the north Atlantic Ocean. This routine task has provided a database with otolith chemical data for >2500 individuals captured between 2009 and 2021 from different regions of ABFT distributional range. Here we have compiled and analysed all otolith stable isotope data generated within the GBYP framework to date. Particularly, we have investigated the temporal evolution of ABFT mixing proportions over the last decade, with the aim of enhancing our understanding of the spatial dynamics of ABFT that can contribute to the development of more robust scientific management advice.

2. Materials and methods

2.1 Sample collection

To investigate the degree of eastern and western population contribution to different mixing areas in the North Atlantic Ocean, otoliths collected under the GBYP framework (N=2376) and archived otoliths from AZTI's otolith collection (N=212) have been combined and analysed for $\delta^{13}C$ and $\delta^{18}O$. Additionally, otoliths of adult bluefin tuna captured from the Mediterranean Sea and Gulf of Mexico spawning grounds were used as reference datasets to characterize the MED and GOM spawning populations. Reference otolith samples (N=299) were obtained under the provision of the GBYP biological sampling program or from NOAA sampling programs. Reference otoliths were sampled from mature adults (>185 cm FL in the GOM, >135 cm FL in the MED) collected from the spawning grounds during the spawning season: from April to June in the GOM, from May to June in the eastern MED and from June to July in the central and western MED (**Table 1**).

2.2 Otolith preparation

Otoliths were handled, cleaned, and rinsed following the protocol described in Rooker *et al.* (2019). One sagittal otolith from each bluefin tuna specimen was then embedded in Struers epoxy resin (EpoFix) and sectioned using a low speed ISOMET saw to obtain 1.2 mm transverse sections that included the core. Following attachment to a sample plate, the portion of the otolith core corresponding to approximately the yearling period was milled from the otolith section using an automated 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. 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 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) available at the Environmental Isotope Laboratory of the University of Arizona. 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.

2.3 Data analysis

Classification accuracy of the baseline samples was tested using five different classification methods, including machine learning-based classification algorithms: multinomial logistic regression (MLR), random forest (RF), Artificial Neural Network (NNet), naïve bayes (NB) and quadratic discriminant function (QDA). Leave-One-Out validation was used for model fitting. The classification method that scored highest at classification accuracy was then used to predict the origin of 2588 ABFT individuals of unknown origin captured outside spawning areas (**Figure 1**). These methods of classification differ from HISEA (used in previous GBYP phases) in the sense that they allow for estimating the individual probability of belonging to the assigned population of origin as a function of $\delta^{13}C$ and $\delta^{18}O$ values. Assuming that the origin of tuna cannot be perfectly predicted from $\delta^{13}C$ and $\delta^{18}O$ values, individuals were assigned to one of the two populations (GOM or MED) when their probability was >70%, or labelled as unassigned otherwise (UNASS). A test of equal proportions was used (Pearson's chi-squared test statistic) to see whether they were differences in origin proportions between different catch years. Additionally, a test for trend in proportions (Mann Kendall trend test) was performed to see whether there was a linear trend in the proportion of MED origin fish at each region across years.

3. Results and Discussion

The classification accuracy of the reference baseline samples ranged from 92.0% to 94.3% (k value 0.80 to 0.86) depending on the classifier chosen (**Table 2**). Multinomial logistic regression (MLR) was chosen to assign the origin of each fish in the mixed sample and its associate levels of probability to belong to one of the two populations (GOM or MED) based on their otolith $\delta^{13}C$ and $\delta^{18}O$ values.

Geographical natal assignments of individuals captured between 2009 and 2021 were first combined to describe the average spatial distribution of stocks during the last decade (**Figure 3**). When several fish from the same catch locations were analyzed, the mean probability value was used to represent that location. The results suggest a longitudinal population structuring of bluefin tuna within the North Atlantic Ocean: fisheries operating in the eastern North Atlantic dominated by the Mediterranean origin fish, western Atlantic coast dominated by the Gulf of Mexico origin fish, and central North Atlantic catches composed by a mixture of stocks.

Temporal variations in mixing ratios were further investigated in the western, northern eastern and southern North Atlantic areas (named WATL, NATL, EATL and SATL in **Figure 3**). West of the 45ºW boundary, temporal persistence of mixing within WATL zone was found, but the proportion of fish assigned to the MED and to the GOM varied significantly between years (chi-squared test, $p<0.001$ and $p<0.001$, respectively). In 2012, 61% of the catches in the WATL were originated in the GOM, while catches were dominated by the Mediterranean population in five of the eight years investigated (2013, 2014, 2015, 2016 and 2017) (**Figure 4A**). In 2011 and 2018 the stock composition of catches of the mixed-stock fisheries were nearly the same. The proportion of unassigned fish did not vary between years (chi-squared test, $p=0.525$), representing 13.7% to 30.5% of the samples depending on the year.

The catches performed east of 45°W boundary (i.e., NATL, EATL, SATL) were dominated (>60%) by tuna of MED origin, regardless of the year. In the NATL (**Figure 4B**), there were significant differences between years in the proportion of fish assigned to the MED (chi-squared test, $p<0.001$), ranging from 60% to 86%. Western contribution in NATL varied between years (chi-squared test, $p=0.005$), being >10% from 2013 to 2015, and barely being present since 2018. The proportion of fish that were unassigned also varied between years (chi-squared test, $p=0.010$), ranging from 0% up to 25%. In the EATL we observed relatively stable stock composition across the years, as bluefin tuna catches were almost exclusively comprised by the MED origin individuals (>90%) in all years analyzed (**Figure 4C**). A low proportion of fish remained unassigned. Thus, Mediterranean bluefin tuna may be the principal, if not the only, contributor to the fisheries operating in the EATL. In the SATL, mixing levels varied significantly between years (chi-squared test, $p=0.002$). The contribution of the dominant MED population ranged from 74% to 100% over the 12 years investigated (**Figure 4D**). A low proportion of fish (2%

to 11%) was presumably derived from the GOM population all years except in 2017, 2020 and 2021, when the entire sample was of Mediterranean origin. Note that the overall assignment error of the methodology is around 6%, meaning that these percentages may vary markedly from the estimated values. Overall, the presence of western migrants in these two areas, EATL and SATL, was limited or insignificant with no indication of western origin bluefin tuna in some of the years sampled. Based on our results, it appears that these areas are utilized primarily as feeding grounds of the eastern bluefin tuna population, and thus the fisheries are supported by the production of bluefin tuna in the Mediterranean Sea.

Results suggest that individuals originated in the GOM and MED cross the 45°W management boundary, mixing with the other population in feeding aggregates of the Atlantic Ocean at variable rates. However, the proportion of fish from GOM origin found to cross to the east, was smaller than the proportion of MED origin fish found to cross to the west. There may be two explanations for this finding or be a combination of both; (1) fish originating in the GOM tend to move less, and/or (2) being a smaller stock in terms of production, the chances of finding a fish from the GOM origin are lower. Unfortunately, for this study the WATL mixed sample was represented by individuals captured close to the 45ºW management boundary (see **Figure 1**). This work would benefit from including individuals from additional feeding grounds in the WATL and the GSL regions. However, previous studies based on otolith stable isotope composition also showed that most of the individuals caught off the USA coast and the Gulf of Maine were assigned to the MED (Kerr *et al*., 2020; Rooker *et al*., 2008), supporting that the Mediterranean population (E-BFT) is the major contributor to the total catches of this species in the North Atlantic Ocean.

Spatio-temporal trends in stock mixing were examined to investigate stock dynamics and to address key issues in the stock management. Although temporal shifts in mixing proportions were detected in most of the regions, we did not find a general trend in stock mixing in the WATL and SATL (Mann-Kendall trend test, p=0.803 and p=0.188 respectively). This suggest that there is no apparent tendency to increase or decrease immigration of bluefin tuna across these regions. It seems though, that the years in which the relative contribution of MED origin fish was higher in the WATL (i.e., 2013-2017), may coincide with the years of relatively lower contribution of MED origin fish in the SATL (**Figure 5**). In the NATL, the proportion of MED origin fish seems to be slightly increasing over time (Mann-Kendall trend test, p=0.050). It has been shown that since 2012, ABFT have reappeared in Norwegian waters, in parallel with an overall improvement of eastern population status (Nøttestad *et al.*, 2020). To further understand temporal variations and environmental and/or biological drivers behind the observed patterns, stock mixing proportions should be explored as relative rates of movement, considering abundance estimates for each of the mixing areas as well as overall stock sizes.

The overall proportion of fish that remain unassigned was highest in the WATL (18%), followed by the NATL (15%), the SATL (12%) and the EATL (2%). When fish are classified as unassigned with otolith stable isotope data, it is mostly because their otolith $\delta^{18}O$ values fall into the overlapping area of GOM and MED baseline signatures used for assignment (see **Figure 2**). There are some hypotheses about these individuals with intermediate signals. On the one hand, they could be individuals that have left their spawning area before the first year of life, so that their otolith $\delta^{18}O$ concentrations would increase/decrease with respect to their spawning area signal (Brophy *et al.*, 2020). According to the isoscape predictions developed by Brophy *et al.* (2020) it seems also possible that individuals with intermediate levels of $\delta^{18}O$ in the otoliths could have originated from the Slope Sea and Bahamas (Brophy *et al.*, 2020), where evidence of spawning has also been detected (Richardson *et al*., 2016). More detailed investigation of $\delta^{18}O$ across otolith transects, and relations between ABFT otolith $\delta^{18}O$ values and water temperature, could help to clarify better the origin and movements of ABFT.

4. Future research needs

We acknowledge the importance of monitoring the geographic origin of tuna from the feeding aggregates in the central North Atlantic Ocean (east and west of the 45º meridian). To this aim we are seeking collaboration with scientist with available otolith stable isotope data from ABFT captured in the WATL and GSL regions to perform a pan-Atlantic study. Besides, we also recognize the importance of periodic sampling at locations where changes in the specie’s traits have been observed, such as the Bay of Biscay, to detect and monitor spatiotemporal variability in population dynamics. Temporal changes in life-history traits, such as spatial structure and movements, are key consideration for the MSE, and one of the most sensitive parameters of the robustness tests. Since the mixing ratios are not stable over time at any region, and do not seem to follow a clear pattern, it is important to see if there are any environmental or biological drivers related to changes in the observed mixing proportions by using the estimates of stock size in each year to translate the mixing proportions to a rate of movement. Factors such as temperature, or fish' size, among others, play a significant role in shaping the distribution and behavior of ABFT populations (Faillettaz *et al*., 2019; Kerr *et al*., 2020). Besides, it will be necessary to update the baseline dataset with otoliths of spawning adults captured in the Mediterranean Sea and the Gulf of Mexico in the most recent years to continue using this approach in the future. As otolith $\delta^{18}O$ values are inversely related to seawater temperatures (Thorrold *et al*., 1997), the increase of seawater temperatures in recent years due to climate change, and the warmer than usual summers that have occurred in the Mediterranean Sea and Gulf of Mexico, can make the chemical signal of fish that have been born in the last decade no longer to correspond well with the reference set used for assignments. Including reference otoliths of spawning adults from Slope Sea spawning ground could also help in quantifying the contribution of this region to the overall population.

Additionally, otolith $\delta^{18}O$ profiles along the otolith transect are useful to detect movements between water masses of different temperatures and isotopic composition (Kawazu *et al.*, 2020). Previous phases of GBYP have progressed the development of otolith chemistry-based approaches for reconstructing migration histories and determining the timing of important events in the life history of ABFT. To advance in this fieldwork, an estimate of the species-specific temperature-dependent rate of $\delta^{18}O$ fractionation, which determines the relationship between the $\delta^{18}O$ of the surrounding water and that of the otolith, needs to be fully developed.

Finally, otolith $\delta^{13}C$ and $\delta^{18}O$ values have been used with a novel approach an index of individual field metabolic rate (Chung *et al*. 2019). Using otolith chemistry data, we found some evidence that the physiological performance of bluefin tuna in the first year is reduced when exceeding the optimum temperature (Trueman *et al*. submitted).

All in all, information provided by otolith chemistry data, can help to better understand the life history and population connectivity of ABFT. This knowledge is instrumental for developing management strategies that ensure the long-term sustainability of this valuable species.

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Table 1. Details on bluefin tuna included in the reference dataset.

Stock	*N*	*Catch month*	*Catch year*	*SFL range*	*Estimated age range*	*Represented time period*
GOM	87	Apr-Jun	2010-2014	199-281	9-18	1992-2005
MED	212	May-Jul*	2010-2015	136-282	5-18	1993-2008

* May to June in the eastern MED and from Jun to July in the central and western MED.

Table 2. Accuracy of different classification methods in assigning individuals to their area of origin. MLR: Multinomial logistic regression, RF: random forest, NNet: Artificial Neural Network, NB: naïve bayes, QDA: quadratic discriminant function.

Classifier	*Mean accuracy %*	*Kappa index*	*% Correct GOM*	*% Correct MED*
MLR	94.3	0.86	87	97
NNet	94.3	0.86	81	98
QDA	94	0.85	87	97
NB	93.9	0.85	86	97
RF	92	0.8	86	97

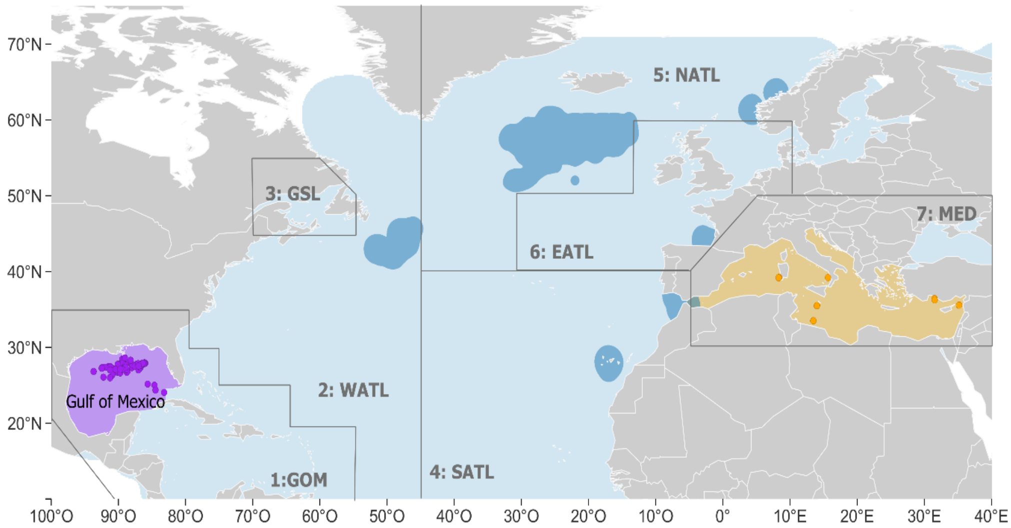

Figure 1. Map of individuals with stable isotope data analyzed in this study. Adults collected from the two main spawning areas, the Gulf of Mexico (GOM, purple area) and the Mediterranean Sea (MED, orange area) are shown as purple and orange dots, respectively. Approximate capture areas of individuals of unknown origin are shown in dark blue, while grey squares represent the spatial definitions of seven areas used in ICCAT MSE: Gulf of Mexico (GOM), West Atlantic (WATL), Gulf Saint Lawrence (GSL), South Atlantic (SATL), North Atlantic (NATL), East Atlantic (EATL) and Mediterranean Sea (MED).

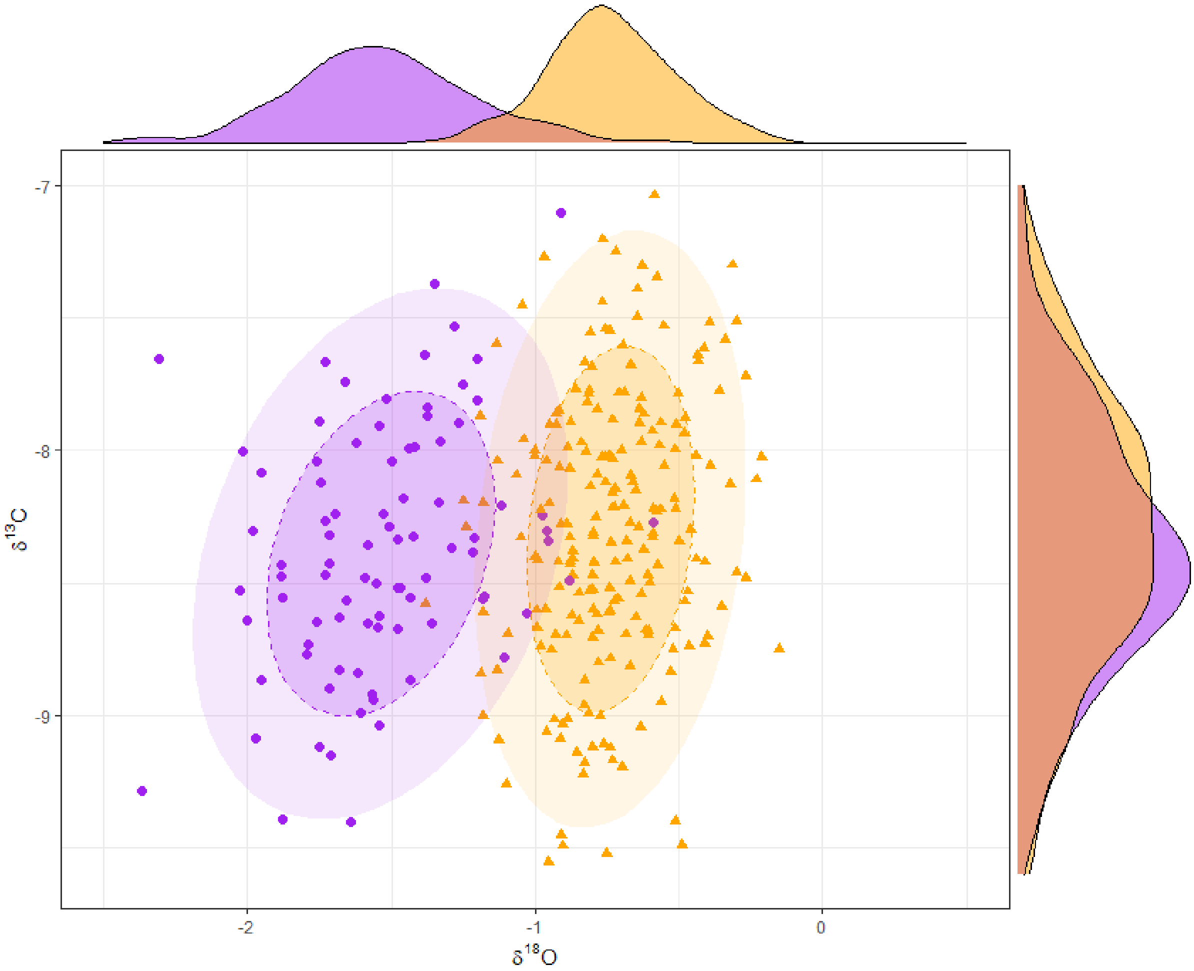

Figure 2. Oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) values in otoliths of adult ABFT captured from the Gulf of Mexico (purple) and Mediterranean Sea (orange) spawning areas and used as a baseline set (N=299). Ellipses show 90% (shaded) and 95% (dashed) confidence intervals for each population.

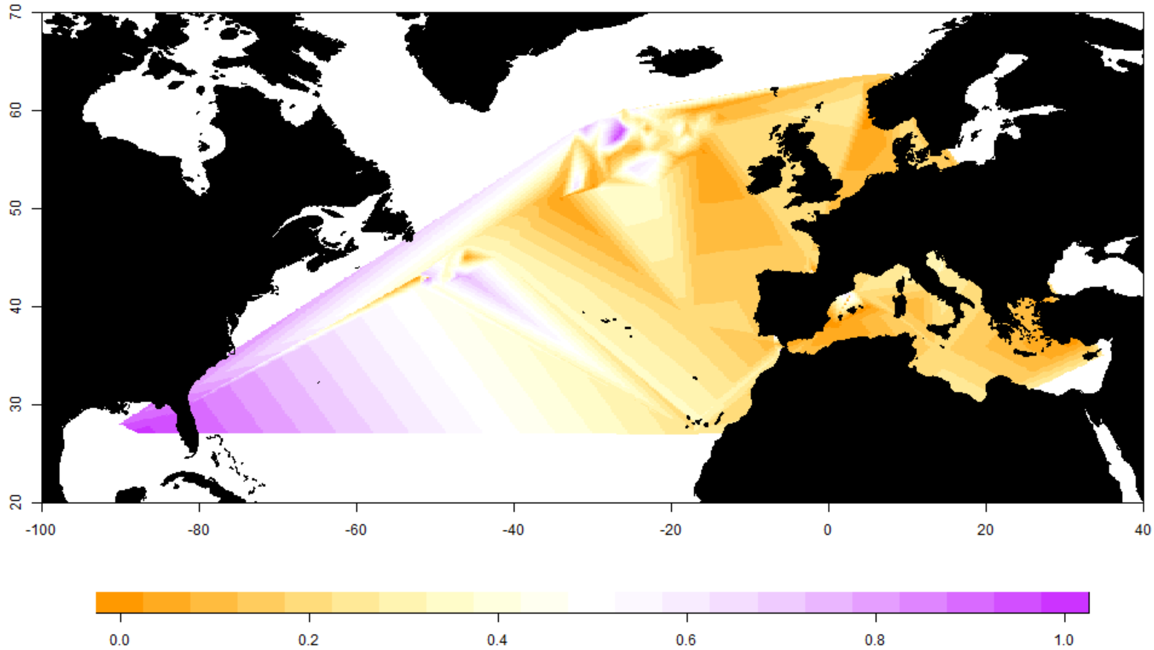

Figure 3. Western contribution estimated by individual origin assignment using Random Forest Discriminant Analysis. Data from 2019 to 2022 were combined and interpolated among the sampled positions (represented by "x"). Mean values were estimated for data from the same catch location. All isolated data points with no adjacent data in the nearby 1x1° cell were removed to reduced the noise.

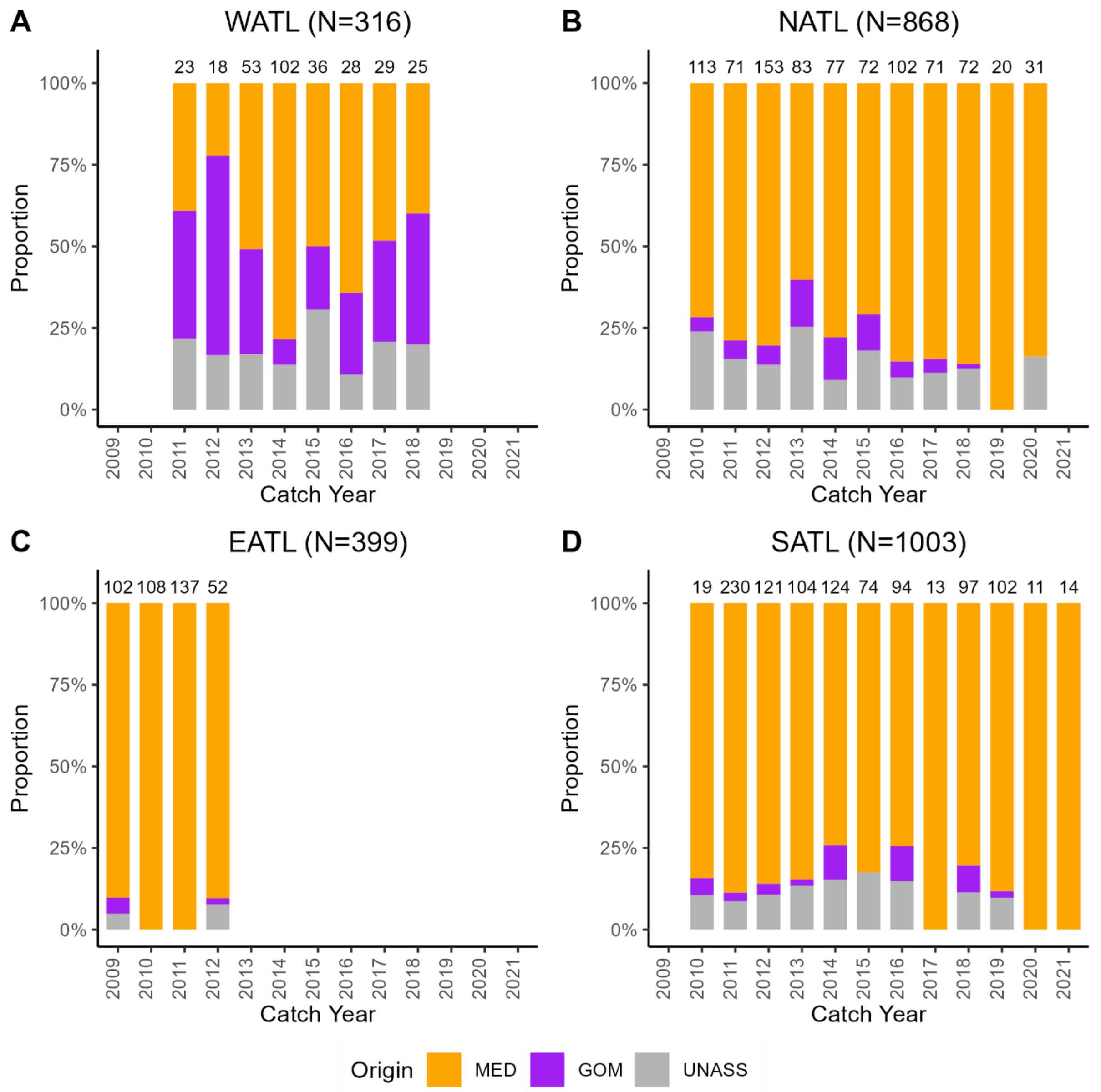

Figure 4. Estimated proportions of MED (orange), GOM (purple) and unassigned (grey) origin of Atlantic bluefin tuna samples collected from 2009 to 2021 in the West (WATL), North (NATL), East (EATL) and South (SATL) Atlantic Ocean. For region geographical delimitation please refer to **Figure 1**. If they were <5 individuals for a given year in a region, they were not accounted for.

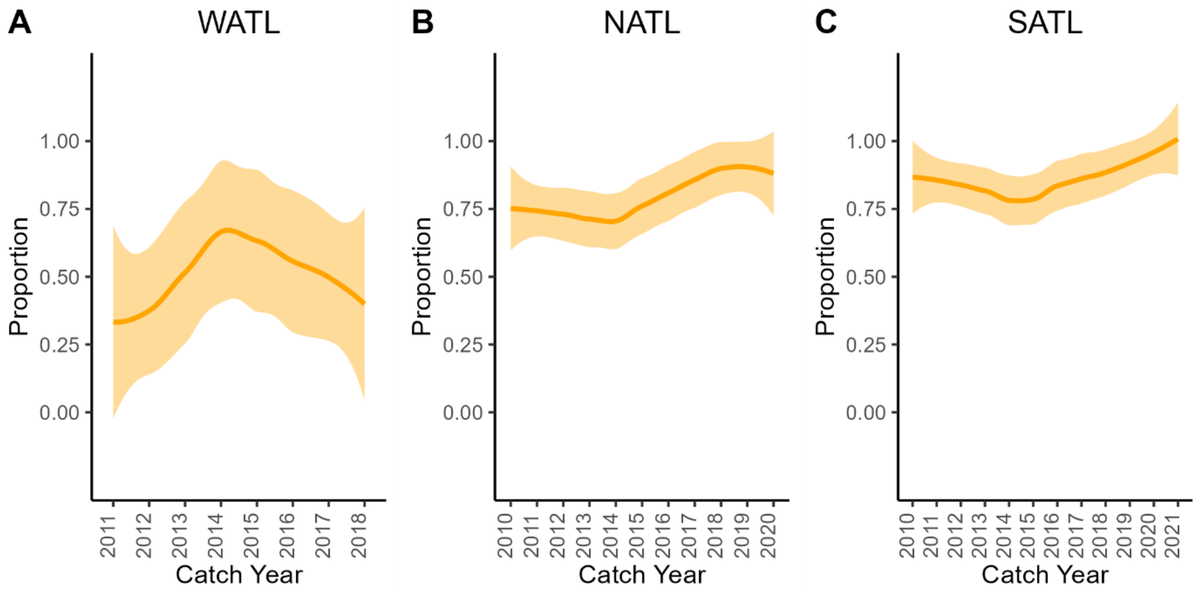

Figure 5. Temporal evolution of estimated proportions of MED origin of Atlantic bluefin tuna samples collected in the West (WATL), North (NATL) and South (SATL) Atlantic Ocean.