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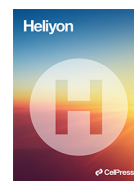
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## Research article

# Chemical signatures in fin spine edge of Atlantic bluefin tuna (*Thunnus thynnus*) can serve as habitat markers of geographically distinct marine environments



Patricia Lastra Luque<sup>a,\*</sup>, Iraide Artetxe-Arrate<sup>a</sup>, Gorka Bidegain<sup>b,c</sup>, Saburo Sakai<sup>d</sup>, Fanny Claverie<sup>e</sup>, Christophe Pécheyran<sup>e</sup>, Igaratza Fraile<sup>a</sup>, Hilario Murua<sup>f</sup>, Jose Luis Varela<sup>g</sup>, Antonio Medina<sup>g</sup>, Haritz Arrizabalaga<sup>a</sup>

<sup>a</sup> AZTI, Marine Research, Basque Research and Technology Alliance (BRTA), Pasaia, Spain

<sup>b</sup> Department of Applied Mathematics, Engineering School of Bilbao, University of the Basque Country (UPV/EHU), Plaza Torres Quevedo 1, 48013, Bilbao, Spain

<sup>c</sup> Research Centre for Experimental Marine Biology and Biotechnology, Plentzia Marine Station PIEUPV/EHU, Areatza Pasalekua z/g, 48620 Plentzia, Spain

<sup>d</sup> Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Biogeochemistry Program, Yokosuka, Japan

<sup>e</sup> Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Pau, France

<sup>f</sup> International Sustainability Seafood Foundation (ISSF), Pittsburgh PA, United States

<sup>g</sup> Departamento de Biología, Universidad de Cádiz, Campus de Excelencia Internacional del Mar (CEI-MAR), Av. República Saharaui s/n, 11510 Puerto Real, Cádiz, Spain

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## ABSTRACT

Chemical fingerprints in otoliths are commonly used as natural habitat markers in fishes. Alternatively, the first dorsal fin spine can provide valuable chemical information and may be more suitable for studying (i) endangered fish species that cannot be sacrificed for their otoliths or (ii) fishes for which otoliths might not be available because of management or commercial reasons. Here, we studied multi-element chemistry of fin spine edges collected from Atlantic bluefin tuna (ABFT; *Thunnus thynnus*) (Linnaeus, 1758) to investigate the utility of the fin spine edge as a natural habitat marker. We determined stable isotopic  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  ratios, as well as concentrations of the tracer elements Mg, Mn, Li, Ba, and Sr, at the edge of ABFT fin spines, and then we used these measures to discriminate ABFT individuals among capture regions (i.e., the eastern Atlantic Ocean or Mediterranean Sea). Isotope ratios and tracer element concentrations, and especially a combined multi-element approach, were able to effectively discriminate individuals by capture region. The Mg, Mn, Li, and  $\delta^{18}\text{O}$  concentrations were the strongest variables driving this discrimination. Overall, our results demonstrate that chemical signatures are consistently retained in the ABFT fin spine edge and support the use of fin spine edges for discerning habitat use. The fin spine chemistry as a minimally invasive sampling method, combined with otolith chemistry, genetic markers, and tagging efforts can help us to reconstruct fish movements, providing a deeper understanding of the spatial population dynamics of this iconic fish species.

## 1. Introduction

Effective fisheries management for marine apex predators, such as tuna, requires an accurate understanding of each species' life history strategies. This information is particularly essential for protecting stocks from overfishing and allowing for the recovery and sustainable management of future stocks (Cadriñ et al., 2013; Kerr and Goethel, 2014). However, data on the movement, population structure, and habitat use patterns of many exploited marine predators are currently insufficient for managerial assessments.

Atlantic bluefin tuna (ABFT) *Thunnus thynnus* (Linnaeus, 1758) is an iconic, highly migratory species that has been exploited by fishermen for centuries. Pronounced population declines in the early 2000s led to the ABFT being classified as globally endangered (Collette et al., 2011). A subsequent implementation of a strict recovery plan by the International Commission for the Conservation of Atlantic Tunas (ICCAT) ended with ABFT overfishing and led the International Union for Conservation of Nature (IUCN) to move its classification to a species of Least Concern (IUCN, 2021). The ICCAT manages the ABFT as two distinct stocks separated at 45° W longitude: (1) the western stock, which mainly

\* Corresponding author.

E-mail address: [plastra@azti.es](mailto:plastra@azti.es) (P.L. Luque).

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spawns in the Gulf of Mexico, and (2) the eastern stock, which mainly spawns in the Mediterranean Sea (Fromentin and Powers, 2005). The eastern and western populations regularly intermix, with tunas crossing the Atlantic Ocean but then returning to their natal areas, the Mediterranean Sea and the Gulf of Mexico, respectively, to spawn (e.g., Graves et al., 2015; Rooker et al., 2019). Spawning occurs in March and April for the western ABFT while there is gradual shift in the ABFT reproductive timing across the Mediterranean basin, with the spawning peak occurs earliest (May) in the Levantine Sea and the latest (July) in the Balearic Sea (Heinisch et al., 2008). Based on sampling on these two spawning grounds, the two ABFT populations have been considered to show distinct reproductive features, which are reflected in an earlier maturation age of the eastern population (age at 50% maturity of 4 years old) (e.g., Corriero et al., 2005) while for western ABFT seems to be more variable, ranging from 8 to 15.8 years (e.g., Diaz and Turner, 2007; Diaz, 2011) and the stock assessment uses a “knife-edge” age at maturity (i.e., all fish reach maturity at the same age). However, recent studies have revealed similarities in reproductive characteristics between the two populations (Knapp et al., 2014) with the western ABFT mature at a younger age than currently assumed (Heinisch et al., 2014).

However, ABFT movement seems to be more complex than stock assessments have assumed particularly for juveniles ABFT (Galuardi et al., 2010; Arrizabalaga et al., 2020) that have showed higher dispersal migratory behavior with individuals commonly displaying distinct structuring across their entire distribution (Arregui et al., 2018; Horton et al., 2020). Besides, recent studies have shown that individuals from both spawning populations may interbreed at a newly discovered spawning ground in the Slope Sea between the Gulf Stream and northeast United States continental shelf (Richardson et al., 2016; Rodríguez-Ezpeleta et al., 2019). Because of this and other discoveries, there is still considerable uncertainty about the population dynamics, mixing patterns, and connectivity of ABFT populations, particularly for juveniles (e.g., Fromentin et al., 2014; Arregui et al., 2018; Arrizabalaga et al., 2019). This knowledge gap hinders the effective conservation of this species and sustainable management of fishing stocks (Kerr et al., 2004; Díaz-Arce et al., 2022).

The tracer element and stable isotopic composition of biomineralized structures, including otoliths (ear stones), fin spines, rays, vertebrae, and scales, can add valuable knowledge of seawater conditions in which these chemical markers were deposited. Biomineral chemical markers thus represent a powerful tool for understanding the movement, population structure, and habitat use of fish species (e.g., Campana, 1999; Cadrin et al., 2013; Madigan et al., 2014; Correia et al., 2021; Martino et al., 2021; Livernois et al., 2021; Rooker et al., 2019; Rooker et al., 2021), as fish grows, these chemical “signatures” are naturally incorporated into the mineral matrix of these hard structures from ambient water and/or diet (e.g., Clarke et al., 2007; Elsdon et al., 2008; Limburg et al., 2010; Gillanders and Munro, 2012; Tzadik et al., 2015; Fowler et al., 2017; Izzo et al., 2018; Avigliano et al., 2019, 2020).

The concentration of different chemical markers in these structures can therefore be used to discriminate among fish that spawned in or inhabited different environments and/or have distinct life histories (e.g., Smith and Whitedge, 2010; Sturrock et al., 2012; Pracheil et al., 2014; Starrs et al., 2016; Willmes et al., 2016; Livernois et al., 2021). However, the elemental composition of biomineralized structures is a function of more than just environmental conditions: physiological regulation of internal elemental composition may lead to active discrimination or preferential uptake of certain elements.

Of the various biomineralized structures in fish, otoliths are considered the most robust indicator of fish life histories because they are metabolically inert and, once accreted, are not reabsorbed (Casselman, 1990; Campana, 1999). Otoliths therefore permanently record lifetime chemical profiles (Campana, 2001; Elsdon et al., 2008; Radigan et al., 2018). However, a growing body of evidence suggests that the internal structure and biomineralization process of otoliths should also be

considered in chemical marker-related studies of fish life histories (Pracheil et al., 2017, 2019). Thus, the specific composition of the otolith can affect the ecological interpretation of otolith trace-element and isotopic results (Walther et al., 2017).

Otolith chemistry in ABFT has been used to explore a broad territory of biological and ecological questions, including natal homing, connectivity (Fraile et al., 2015; Rooker et al., 2008a,b; Rooker et al., 2014), transoceanic movements, and population mixing (Rooker et al., 2019). However, one of the major limitations of using otoliths in this species is that removing the otolith can greatly affect the appearance of a fish, which diminishes the market value of such economically important species, and in some cases, otolith might not be available for sampling during fishing operations. Thus, assessments of whether reliable chemical signatures are retained in other calcified structures such as fin spines could therefore provide a non-invasive, and potentially more informative alternative or complement to traditional otolith chemistry analysis.

Previous studies of freshwater and anadromous fish species have demonstrated that fin spines and rays retain the chemical composition of the aquatic environment where they were grown. Fin spine chemistry therefore provides a useful tracer of the pronounced environmental gradients these fish experience as they travel between euryhaline/estuarine and oceanic marine waters (e.g., variation in salinity, temperature, and/or elemental and isotopic concentrations). Besides it has been used as a *non-lethal* approach particularly useful in endangered fish species (e.g., Clarke et al., 2007; Smith and Whitedge, 2010; Jarić et al., 2011; Wolff et al., 2013; Rude et al., 2014; Phelps et al., 2017; Tzadik et al., 2017; Avigliano et al., 2019, 2020). However, fin spine chemistry in obligate marine fish species, such as tunas, remains largely unexplored. Only the preliminary studies of Luque et al. (2016, 2020) have reported the retention of certain tracer elements and stable isotopic signatures in the fin spine mineral bone matrix (hydroxyapatite), though these studies suggested that the fin spine may be suitable hard structure for chemistry studies in tuna. One challenge with the use of fin spines is that central area (nucleus) in the fin spines of teleost fish species commonly can be reabsorbed or physiologically reworked through a process called “nucleus vascularization,” which destroys early growth increments, alters their primary chemical composition (Prince and Pulos, 1983; Hill et al., 1989), and can thereby limit the use of fin spines as records of the environmental history of fish from birth to death. However, studies have found that this metabolic reworking is potentially minimal (Tillett et al., 2011; Avigliano et al., 2019, 2020). Moreover, nucleus vascularization usually does not occur in recently grown fin spine tissue (Tzadik et al., 2017), and here the chemical signatures are sufficiently stable for use as habitat markers (Avigliano et al., 2019). In addition, the first dorsal fin spine (hereafter “fin spine”) is typically used for ageing small- and medium-sized fish from the eastern ABFT stock (Luque et al., 2014), as they can be rapidly extracted and easily processed, and the eastern stock’s current growth curve is based on estimates using fin spines (ICCAT, 2013). The 2017 ABFT stock assessment reported that otolith sampling produces biased estimates for the ages of juvenile ABFT and estimates for younger specimens remain difficult because of the weak contrast of the earliest annuli and the frequent appearance of numerous, easily distinguishable sub-annual bands that can be misinterpreted as annuli.

In light of these findings, the fin spine may therefore represent a suitable alternative for tracer element and isotope-based reconstructions of environmental conditions, habitat use, and movement among environments. Besides more easily interpretable alternative to otoliths particularly valuable for inferring the habitat use and movement pattern of juvenile ABFT capable of travelling long distances in short period of time (Arregui et al., 2018).

The Mediterranean Sea and eastern Atlantic Ocean have distinct physicochemical characteristics (e.g., salinity and temperature) (LeGrande and Schmidt, 2006; Rogerson et al., 2012; Millot, 2014) isotope concentrations, and metal concentrations that would presumably be reflected in fin spine chemistry. Our aim was to determine whether ABFT

captured within these two regions could be discriminated based on the fin spine edge chemical signatures. As such, here, we measured tracer element (Mg, Mn, Li, Ba and Sr) and stable isotopic ( $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$ ) composition at the edge of the fin spine (i.e., representing most recent habitat and growth) in ABFT. We discuss our results in terms of the potential for using fin spine chemical signatures which, in combination with other approaches such as otolith chemistry, and tagging efforts, has the potential to add knowledge about the habitats and movements of ABFT by gaining a deeper understanding of the spatial population dynamics of this much-admired species.

## 2. Material and methods

### 2.1. Fish sampling

Atlantic bluefin tuna were sampled under the provision of the ICCAT Atlantic Wide Research Programme for Bluefin Tuna (GBYP) as part of the special biological sampling of commercial tuna caught in the Atlantic Ocean. Fish were caught during commercial fishing operations in the northeast Atlantic and the Mediterranean Sea (Figure 1). No live animals were used for this study.

Scientific observers removed individual fin spines from freshly caught ABFT. To reduce potential variability in chemical signatures due to long-term temporal variation in the environment, only ABFT caught in two consecutive years (2016 and 2017) were selected for analysis. To increase the number of individuals per location, data from different sampling locations within the same geographic region (Atlantic or Mediterranean) were combined and treated as a single group. Fish size ranged between 99–244 cm straight fork length (Table 1), and ages were estimated from <2 up to 14 years old based on counts of translucent bands formed annually in the fin spine (Luque et al., 2014).

### 2.2. Fin spine preparation and analyses

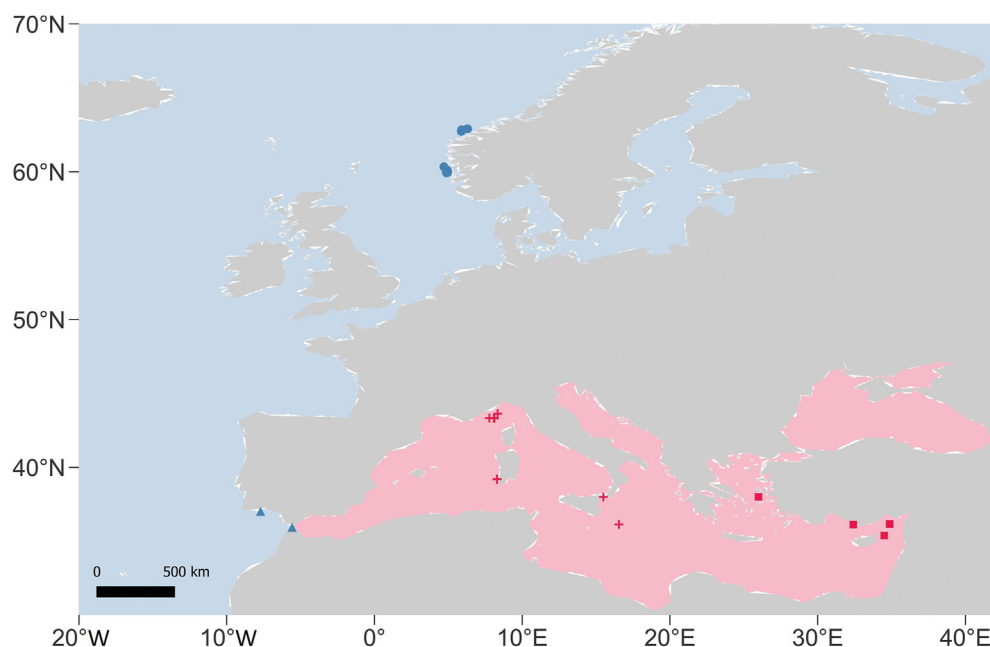
Fin spines were processed and sectioned following Luque et al. (2014). We obtained two consecutive transversal sections, one at 0.7 mm thickness for tracer element and ageing analyses and one at 3 mm thickness for stable isotope analyses (Figure S1). Sections were then cleaned ultrasonically to remove excess organic tissue (Luque et al., 2019).

### 2.3. Tracer element analysis

We measured tracer element composition at the marginal areas of ABFT fin spine sections ( $n = 89$ ) using a high repetition rate (1 Hz–100 kHz) femtosecond laser ablation (fs-LA) system (Alfamet, Novalase, France) coupled to a high resolution inductively coupled plasma mass spectrometer (HR-ICP-MS, Element XR, Thermo Scientific, Bremen, Germany). Analyses were conducted at the Institut des Sciences Analytiques et de Physico-Chimie pour l'Environnement et les Matériaux, Université de Pau et des Pays de l'Adour/CNRS (Pau, France). The laser delivers 360 fs pulses of 1030 nm light and is equipped with a galvanometric scanner beam that allows the small laser beam (17  $\mu\text{m}$ ) to be moved across the surface of the sample.

Each fin spine section was ablated along the fin spine growth trajectory (i.e., from the first inner visible annulus to the fin spine edge). For this analysis, we included the compact cortical bone but avoided the central vascularized area, and we analyzed element composition in the outer  $\sim 150 \mu\text{m}$  of spine edges because this region represents the most recent environment a tuna inhabited before it was caught (Figure S1). Special attention was taken to produce transects that were 50  $\mu\text{m}$  wide and less than 30  $\mu\text{m}$  deep to avoid any inadvertent annulus mixing from in-depth ablation. The particles produced from this ablation process were transported to the HR-ICP-MS with a helium flux of 480 mL/min.

We used the HR-ICP-MS to measure the relative abundances of six elements of interest: magnesium ( $\text{Mg}^{24}$ ), manganese ( $\text{Mn}^{55}$ ), lithium ( $\text{Li}^7$ ), barium ( $\text{Ba}^{138}$ ), strontium ( $\text{Sr}^{88}$ ), and calcium ( $\text{Ca}^{43}$ ), the last of which was used as an internal standard. Tracer element measurements of the blank sample gases were recorded for 20–30 s before each  $\sim 40$  s ablation. Subsequent data processing, including background subtraction, standardization, and concentration determination were performed using an in-lab developed VBA excel program (FOCAL 2.38). National Institute Standards and Technology (NIST) 610 and 612 glass standards with known chemical composition were used for quantitative calibration. Measurement accuracy was determined based on the analysis of a pressed pellet of certified bone powder reference material NIST1486, which contains 265,800  $\mu\text{g}$  Ca per gram of tissue. This CRM is certified for only a limited number of elements in contrast to the NIST glass series. Then, analyzing this bone SRM allowed verifying that the calibration method was accurate for material whose matrices are similar to bones (non-matrix



**Figure 1.** Atlantic bluefin tuna (*Thunnus thynnus*) sampling locations are represented by blue and red marks. Colors represent the two capture regions as defined in this study, either the Atlantic (blue) or Mediterranean Sea (red).

**Table 1.** Number of Atlantic bluefin tuna (*Thunnus thynnus*) fin spine samples used for stable isotope (SI), trace element (TE) and combined stable isotopes and trace element (SI + TE) analyses. Samples are grouped by the two capture regions where ABFT were harvested (i.e., the Atlantic and Mediterranean Sea). Strait fork length (SFL) is given in cm, and age was estimated using the methods described by Luque et al., (2014). All samples were captured in 2016 or 2017.

Region	SFL range (cm)	Age range (years)	Capture year	n (SI)	n (TE)	n (SI + TE)
Atlantic	116–248	4–14	2016 2017	30	61	29
Mediterranean Sea	99–244	2–13	2016 2017	25	28	14

matched calibration) at least for the element certified in NIST 1486. Then we hypothesized that the calibration accuracy was verified for the elements of interest that were not reported in the CRM1486 certificate. To correct for short-term instrumental drift, standards and reference material were measured in triplicate twice each day (i.e., at the beginning and end of each measurement session).

#### 2.4. Stable isotope analysis

For stable isotope analysis ( $n = 55$ ), fin spine sections were micro-sampled following the protocol described by Luque et al. (2020). We used a high-precision, computer-guided micromill system (Geomill326; Izumo Web, Izumo, Japan) to collect micro-samples from the outer  $\sim 150 \mu\text{m}$  of fin spine edges (Figure S1). The resulting powder was vacuumed using a micro-powder collecting device (Kyushu-Danji, Izumo Web Ltd., Japan) to minimize the drilled-powder collection loss.

The oxygen and carbon isotopic composition ( $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ ) of each sample was then determined using an isotope ratio mass spectrometer (GV Instruments IsoPrime, Manchester, UK) with an automated carbonate reaction system (Multiprep) at the Japan Agency for Marine-Earth Science and Technology, Japan. The isotope ratio measurement was calibrated based on repeated measurements of NBS-19, and precision was  $\pm 0.1\%$  (1 SD) for oxygen and carbon isotopes.

#### 2.5. Statistical analysis

We first analyzed tracer element and stable isotope data separately. The  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  data were normally distributed and homoscedastic, so we used Student's t-test for univariate comparisons among regions. Tracer element data (Mg, Mn, Li, Ba, and Sr) did not meet parametric assumptions, so we used the Wilcoxon rank sum test for univariate comparisons among regions. The Wilcoxon rank sum test with a continuity correction was used to compare fish ages among regions. For all comparisons, differences were considered significant at  $p < 0.05$ .

For all multivariate analyses, tracer element and stable isotope data were Z-score standardized. We then performed a permutational multivariate analysis of variance (PERMANOVA) for both stable isotopes and tracer elements to test for differences in the multi-element signature between capture regions. For this analysis, the resemblance matrix was based on Euclidean distance dissimilarities, and the number of unrestricted permutations was set to 999. The test was implemented using the *adonis* function in the R package 'vegan'. Multivariate data were visualized in two dimensions using kernel density plots for stable isotope data, non-metric multidimensional scaling (nMDS) for tracer element data, and canonical analysis of principal coordinates (CAP) for the combined stable isotope and tracer element data (Anderson and Willis, 2003).

We then determined how effectively the fish collected in the two regions could be discriminated based on (1) stable isotope data ( $n = 55$ ), (2) tracer element data ( $n = 89$ ), and (3) combined stable isotope and tracer element data ( $n = 43$ ). The size of the combined data set was limited by the number of individuals for which both stable isotope and tracer element data were available. For this analysis, in the case of stable

**Table 2.** Classification accuracy when assigning Atlantic bluefin tuna (*Thunnus thynnus*) to their region of capture based on (A) stable isotope ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ), (B) trace elements (Mg, Mn, Li, Ba, Sr) and (C) combined multi-element chemistry ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , Mg, Mn, Li, Ba, Sr) measured from dorsal fin spines. Classifications were performed using linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), and random forest (RF) models. Numbers in the table indicate the percentage (%) of individuals that were correctly assigned to their capture region. Overall classification accuracy and Cohen's kappa statistic ( $\kappa$ ) are also presented.

Classification success						
Region	Stable Isotopes $n = 55$			Tracers $n = 89$		Combined $n = 43$
	LDA	QDA	RF	RF	RF	
Atlantic	83%	80%	73%	87%	98%	
Mediterranean	60%	60%	64%	63%	71%	
Overall ( $\kappa$ )	73% (0.44)	71% (0.42)	69% (0.37)	79% (0.51)	90% (0.75)	

isotope data (normally distributed), we compared performance of linear discriminant analysis (LDA), quadratic discriminant analysis (QDA), and random forest (RF) models. However, in the case of tracer element and combined approach data, we only used RF if as data do not meet parametric assumptions of normality as the method is relatively insensitive to imbalanced data (He and Garcia, 2009). Thus, RF was used to compare classification accuracy (Table 2) between elemental approaches used for the analysis.

In all cases, data were randomly split into a training dataset (75% of the data) and a testing dataset (25% of the data), and analyses were repeated 1,000 times to avoid sampling artifacts. Classification success (i.e., the percentage of tunas that were correctly assigned to their sampling region) and mean accuracy values were calculated for each repetition. We also calculated Cohen's Kappa ( $\kappa$ ) statistic, which measures classification accuracy while accounting for agreement occurring by chance alone (Cohen, 1960; Landis and Koch, 1977). The resulting  $\kappa$  value provides a measure of how well the model classifications match the data:  $\kappa < 0$ , less agreement than expected by chance; 0.01–0.20, slight agreement; 0.21–0.40, fair agreement; 0.41–0.60, moderate agreement; 0.61–0.80, substantial agreement; and 0.81–0.99, almost perfect agreement (Landis and Koch, 1977; Titus et al., 1984). All statistical analyses were performed with R statistical software, version 4.0.4 (R Core Team, 2021).

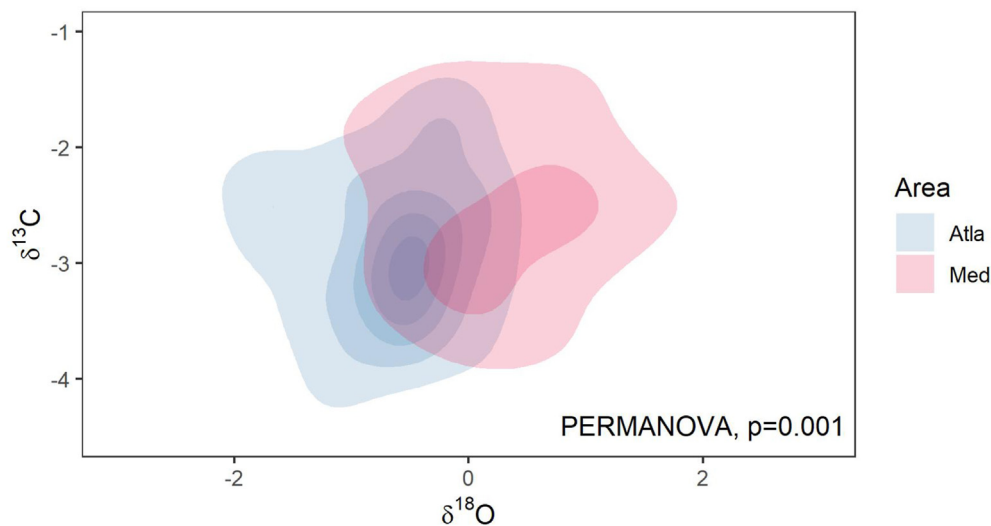
### 3. Results

#### 3.1. Stable isotope analyses

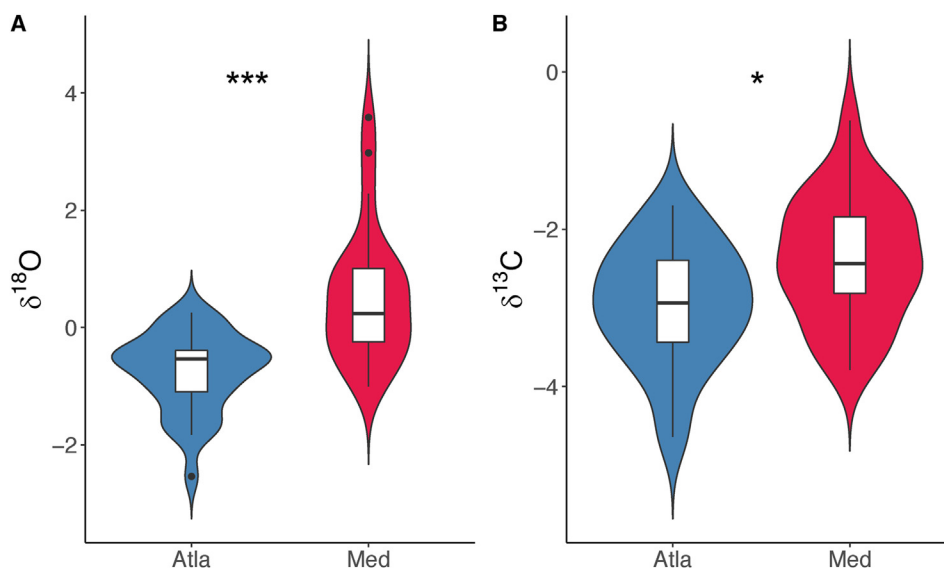
Dorsal fin spine edge stable isotope composition differed significantly between fish captured in the Atlantic and Mediterranean regions (PERMANOVA  $p = 0.001$ ), though  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  signatures overlapped slightly between regions (Figure 2).

The  $\delta^{13}\text{C}$  concentration in fin spine edges from fish captured in the Mediterranean region ( $-2.39 \pm 0.80$ , mean  $\pm$  SD) was significantly higher (t-test,  $p = 0.009$ ) than that of fish captured in the Atlantic region ( $-2.96 \pm 0.76$ ) (Figure 3). The  $\delta^{18}\text{O}$  concentration more strongly differentiated the two regions; like  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$  was significantly higher (t-test,  $p < 0.001$ ) in the spine edge from fish captured in the Mediterranean region ( $0.53 \pm 1.14$ ) than from fish captured in the Atlantic region ( $-0.76 \pm 0.66$ ) (Figure 3).

All the classification algorithms had a fair success rate (69–73%) when classifying ABFT to their capture area based on dorsal fin spine edge stable isotope composition. Overall agreement between the models and data was fair to moderate ( $\kappa$ : 0.37–0.44). Notably, the percentage of correctly classified fish was higher for fish captured in the Atlantic region (73–83%) than for fish captured in the Mediterranean region (60–64%). Classical algorithms (LDA and QDA) performed 7–10% better than RF for



**Figure 2.** Kernel density contour plots for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values measured in the dorsal fin spine edge of bluefin tuna (*Thunnus thynnus*) captured in the Atlantic (blue) and Mediterranean (red) regions. Bivariate kernel density is represented at the 20%, 40%, 60%, 80%, and 100% levels, from dark to light colours.



**Figure 3.** Violin plots and Student's t-test results comparing (A)  $\delta^{18}\text{O}$  and (B)  $\delta^{13}\text{C}$  stable isotope ratios measured in the dorsal fin spine edge of bluefin tuna (*Thunnus thynnus*) captured in the Atlantic (Atla, blue) or Mediterranean (Med, red) regions ( $n = 55$ ). Each violin plot includes a boxplot showing the median (horizontal black line), interquartile range (box), and overall range with outliers removed (thin black lines). The colored regions provide a kernel density estimation representing the distribution of the data. For the Student's t-test between groups, significant differences are represented with asterisks (\*,  $p < 0.01$ ; \*\*,  $p < 0.001$ ; \*\*\*,  $p < 0.0001$ ).

the Atlantic region (Table 2). For the Mediterranean region and the overall data set, differences in classification accuracy between methods were less substantial (2–4%).

### 3.2. Tracer element analyses

Five tracer elements used for analyses were detected above the limit of detection in 100% of samples. Magnesium (Mg) and Strontium (Sr) showed the highest concentrations in ABFT fin spine edge with values ranging from 2.087 to 5.188  $\text{mg g}^{-1}$  and 0.565 to 1.115 ( $\text{mg. g}^{-1}$ ), respectively. While Lithium (Li) showed the lowest concentration values.

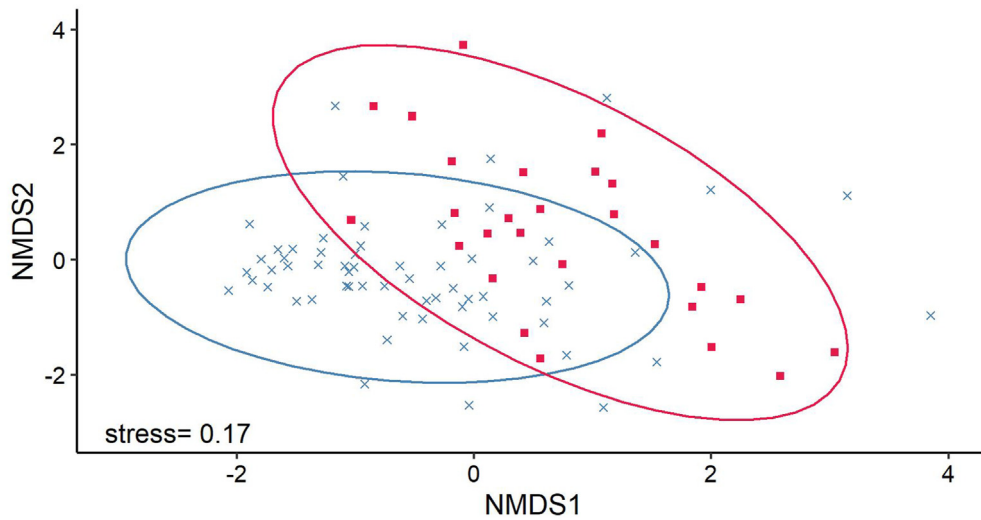
Fin spine elemental chemistry analysis also revealed significant differences in Mg, Mn, Li, Ba, and Sr between fish captured in the two different regions (PERMANOVA  $p = 0.001$ ), although the chemical fingerprint overlapped between regions (Figure 4). Tuna collected from the Mediterranean region had significantly higher Mg, Mn, Li, and Ba concentrations than tuna captured in the Atlantic region, while no significant differences were detected for Sr (Figure 5).

For tracer elements, overall classification accuracy using RF (79%) was higher than the classification accuracy for stable isotope data (69%), and Cohen's kappa showed moderate agreement ( $\kappa: 0.51$ ) (Table 2).

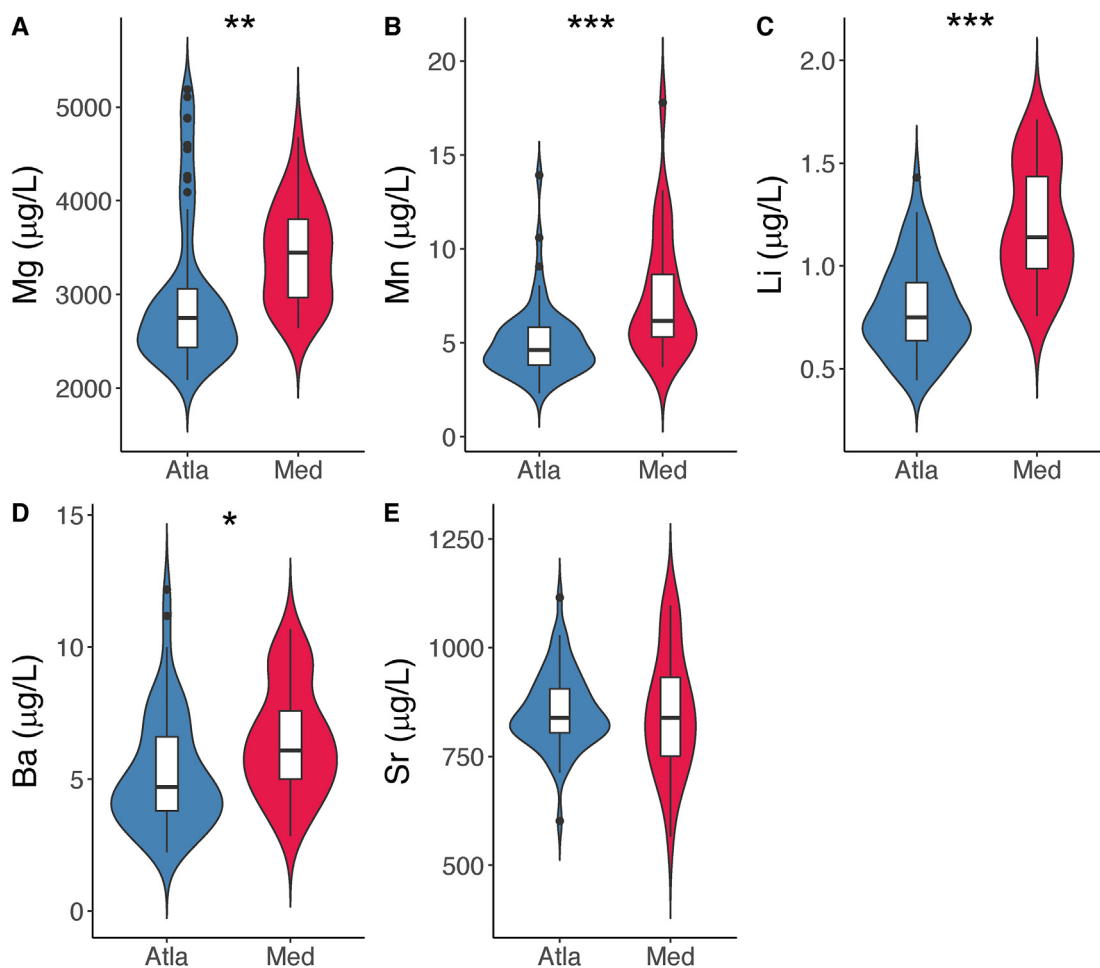
### 3.3. Combined approach

Models were able to differentiate fish by capture region most effectively when both stable isotope and tracer element data were combined ( $n = 43$ ; Table 2). For the combined approach, overall classification accuracy using RF was 90%, showing substantial agreement ( $\kappa: 0.75$ ). The most important elements for discriminating individuals between regions were Mg, Mn, Li, and  $\delta^{18}\text{O}$  (Figure 6; PERMANOVA  $p = 0.001$ ).

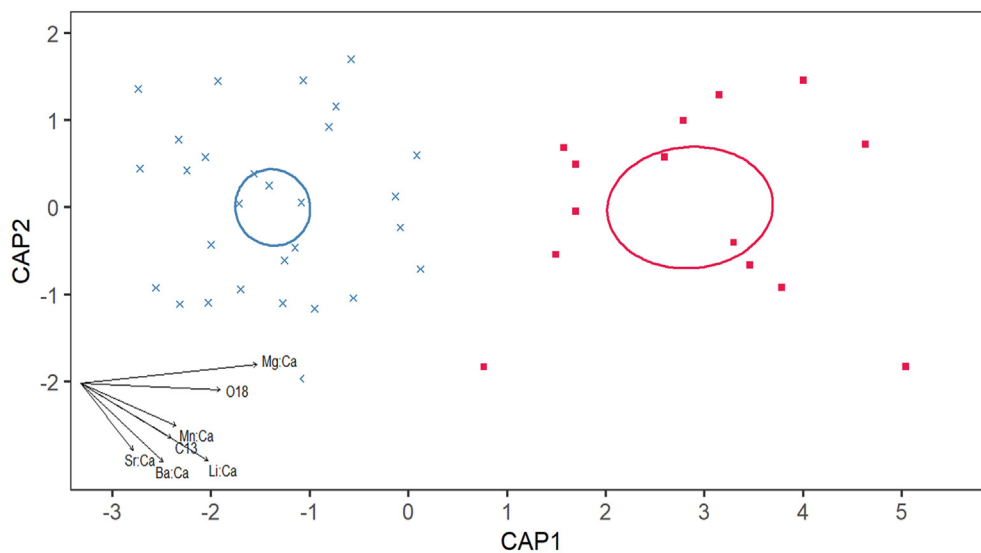
Finally, ABFT captured in the Atlantic region ( $8.2 \pm 2.4$  years, mean  $\pm$  SD) were significantly older than ABFT captured in the Mediterranean ( $6.4 \pm 2.8$  years), as determined based on dorsal fin spine edge annuli (Wilcoxon rank test  $W = 1169$ ,  $p\text{-value} = 0.002$ ). For these mean ages above, the collected micro-sample from the outer  $\sim 150 \mu\text{m}$  of fin spine edges represents  $\sim 3$  months.



**Figure 4.** Non-metric multidimensional scaling (NMDS) plot of tracer element values (Mg, Mn, Li, Ba, Sr) in bluefin tuna (*Thunnus thynnus*) dorsal fin spine edges. Samples were captured in the Atlantic (blue crosses) and Mediterranean (red squares) regions. Ellipses represent 95% confidence intervals around the group mean.



**Figure 5.** Violin plots and Wilcoxon test results comparing tracer element composition, (A) Li, (B) Mg, (C) Mn, (D) Sr, (E) Ba, in bluefin tuna (*Thunnus thynnus*) dorsal fin spine edge captured in Atlantic (Atla, blue) and Mediterranean (Med, red) regions (n = 89). The violin plot shows (i) a boxplot with the median (horizontal black line), the interquartile range (the box) and the rest of the distribution, except for points that are determined to be “outliers” (thin black line), and (ii) a kernel density estimation representing the distribution of the data (each side of the thin black line). For the Student's t-test between groups, significant differences are represented with asterisks (\*, p < 0.01; \*\*, p < 0.001; \*\*\*, p < 0.0001).



**Figure 6.** Canonical variate plots of the multi-element chemical composition ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , Mg, Mn, Li, Ba, Sr) of bluefin tuna (*Thunnus thynnus*) dorsal fin spine edges. Fin spines were collected from tuna captured in the Atlantic (blue crosses) and Mediterranean (red squares) regions. Ellipses show 95% confidence intervals around group means, and the vector diagram in the bottom left shows the direction and weight of individual elements contributing to the sample distribution in two-dimensional space.

#### 4. Discussion

Discrimination analysis indicated that the tracer element and isotopic composition of dorsal fin spine edge was significantly different between ABFT captured in Mediterranean and Atlantic waters. Furthermore, we found that fin spine edge concentrations of Mn, Mg, Li, and, to a lesser extent, Ba were significantly higher for ABFT captured in Mediterranean waters than ABFT in the Atlantic region. Carbon and oxygen stable isotope ratios ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ , respectively) followed the same pattern between regions as tracers, with  $\delta^{18}\text{O}$  providing clearer separation between regions. Findings corroborates that in the ABFT, the fin spine edge retained chemical signatures that can reasonably predict the last habitat used by ABFT individuals and serves as a potentially valuable natural habitat marker. Overall, Mn, Mg, Li, and  $\delta^{18}\text{O}$  were the most effective elements for discriminating ABFT individuals between Atlantic and Mediterranean regions. These signatures were consistently important across all classification algorithms we tested for stable isotopes, and particularly for RF in tracers and the combined multi-element approach. Therefore, results of this study suggest that fin spine chemical signatures retained across growth increments might provide useful information for inferring past ABFT habitats and reconstructing movements through the pronounced environmental gradients experienced when tuna travel between the Atlantic and Mediterranean waters.

Isotopic markers (i.e.,  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) have also been efficient for reconstructing environmental history and delineating of tuna stock population structure (e.g., Rooker et al., 2008a,b; Shiao et al., 2010; Wells et al., 2012; Fraile et al., 2016; Madigan et al., 2018; Rooker et al., 2019; Artetxe-Arrate et al., 2021). The  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values that were recorded in the fin spine edge of ABFT were within the range of values already reported for other marine pelagic fishes in the northeast Atlantic (Moreira et al., 2018; Correia et al., 2021), including previous values for ABFT measured from otoliths (Rooker et al., 2014; Fraile et al., 2015) and fin spines (Luque et al., 2020). We found that fin spine edge  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values were significantly higher in ABFT captured in the Mediterranean than in the eastern Atlantic, which agrees with observed regional differences in seawater  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  concentrations (LeGrande and Schmidt, 2006; McMahon et al., 2013). Even so,  $\delta^{18}\text{O}$  values were able to discriminate ABFT between regions more effectively than  $\delta^{13}\text{C}$  suggesting that  $\delta^{18}\text{O}$  enrichment in fin spines is independent of any physiological or metabolic processes. However, there was some overlap in  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  signatures between regions. Using stable isotopes values, fish captured in the Atlantic region were more likely to be correctly classified to their capture region than those captured in the Mediterranean region. This result suggest that individuals captured in the Atlantic region had

lived in the capture area for more time (in the last months), compared to those captured in the Mediterranean region. ABFT migration dynamics between Atlantic and Mediterranean waters, and even transoceanic transitions, have been largely studied in adults ABFT using e-tagging experiments (Arregui et al., 2018; Block et al., 2020). These findings challenge previous assumptions regarding the seasonality, residency patterns, and annual movements of ABFT within Atlantic and Mediterranean waters.

In contrast, the relationship between sea surface temperature and seawater  $\delta^{13}\text{C}$  is further complex (Cassar et al., 2006; Lorrain et al., 2020) and can vary under climatic and environmental conditions. Differences in fin spine  $\delta^{13}\text{C}$  concentrations had a smaller effect on classification accuracy between regions. Fin spine  $\delta^{13}\text{C}$  values may mirror not only the  $\delta^{13}\text{C}$  of dissolved inorganic carbon in the ambient water ( $\delta^{13}\text{C}_{\text{DIC}}$ ) but also the  $\delta^{13}\text{C}$  in an individual's diet ( $\delta^{13}\text{C}_{\text{diet}}$ ). Thus, the high variability in  $\delta^{13}\text{C}$  values among individuals could be linked to individual differences in behavior, diet, and habitat preference (i.e., for areas with different temperature or productivity regimes). Natural changes in the isotope concentration of the North Atlantic water mass that enters the Mediterranean could introduce additional variability in fin scale isotope measurements by influencing the isotopic signature of individuals that reside in the western part of the Mediterranean Sea (Macías et al., 2008). Lastly, carbon isotope concentrations have been also related to various physiological factors, suggesting that they may have greater potential as lifetime metabolic signatures rather than environmental proxies. Martino et al. (2020) revealed in an experimental study, that energy for basic functioning and activity metabolism, relates to incorporation of  $\delta^{13}\text{C}$  into otoliths of Australasian snapper (*Chrysophrys auratus*) supporting the use of  $\delta^{13}\text{C}$  as a metabolic proxy in field settings. Similar experimental validation studies would be required to advance our understanding of the relationship between fish metabolic rate and to incorporation of  $\delta^{13}\text{C}$  into fin spines as bone like structure.

In addition to isotopic markers, elements such as Magnesium (Mg) was one of the most important tracers for discriminating ABFT between regions and was significantly more abundant in specimens collected in the Mediterranean suggesting that Mg in fin spine edges may be a valuable habitat marker in ABFT. In a laboratory study, Smith et al. (2013) found that Mg concentrations in the vertebrae of round sting rays (*Urobatis halleri*) were tightly correlated to Mg concentrations in ambient seawater. Considering that the sources and pathways of elements to fin rays and spines are believed to be like that of fish vertebrae (Kerr and Campana, 2014), results of this study suggest that the Mg concentration in fin spines can be positively correlated with Mg availability in seawater. Mg is an essential bulk element necessary for life including cellular



metabolism, immune system function, and skeletal growth (Loewen et al., 2016; Martino et al., 2021). Like calcium, Mg is an alkaline earth metal that forms divalent cations in solution (Campana, 1999; Mann, 2001; De Pontual and Geffen, 2002; Allemand et al., 2008), which allows Mg to substitute for and compete with calcium during the mineralization process. This concentration-dependent substitution effect creates Mg-based signatures of migration between different environments, stock discrimination, or habitat use (Crichton, 2008; Omelon et al., 2009). However, the correlation between Mg incorporation and environmental Mg availability seems to be further complicated because Mg concentrations in biomineralized tissues are guided by their own regulatory processes (Crichton, 2008; Omelon et al., 2009).

Furthermore, as average seawater temperature is higher in the Mediterranean Sea than in the Atlantic (Rogerson et al., 2012; Millot, 2014) this factor could be also behind our discrimination results. However, the relationship between temperature and Mg incorporation into biomineralized tissues such as otolith and vertebrae has been found inconsistent among studies. For example, Barnes and Gillanders (2013) found higher Mg/Ca within the otoliths of *Argyrosomus japonicus* with increasing temperature. In contrast, Smith et al. (2013) found this relationship to be negative into the vertebrae of round sting ray. Furthermore, several experimental studies did not find any significant effect of temperature and salinity on otolith Mg/Ca ratios (e.g., Martin and Wuenschel, 2006; DiMaria et al., 2010). In addition, some studies have suggested that Mg is acquired via dietary consumption more than from ambient environmental concentrations (Van Campenhout et al., 2009), albeit it still remained unexplored in fin spines. As such further controlled laboratory studies are essential for further evaluating how environmental (abiotic) and physiological (biotic) factors affect Mg uptake into fin spines.

Mn and Li concentrations were also effective at discriminating ABFT between Atlantic and Mediterranean regions with higher concentrations in tunas collected in the Mediterranean waters. Despite the importance of Mn and Li in this study, the relationship between the environmental concentrations of these elements and their incorporation into calcified structures remains inconsistent across species and environments (e.g., Bath Martin and Thorrold 2005; Miller 2009; Sturrock et al., 2015; Mohan et al., 2018; Reis-Santos et al., 2018; Livernois et al., 2021; Martino et al., 2021). In addition, experimental studies did not find any evidence of a relationship between seawater Mn and Mn in otoliths (see review by Miller, 2009).

In our study, fin spine Mn concentrations were significantly different between our Atlantic and Mediterranean capture locations, indicating that Mn might be a good proxy for habitat use. We found that fin spine Mn concentrations were higher in ABFT collected in the Mediterranean Sea suggesting as for Mg that the Mn concentration in fin spines can be positively correlated with Mn availability in seawater. This result coincides with experimental studies with synthetic hydroxyapatite that indicated the Mn uptake is proportional to the ambient Mn concentration (Mayer et al., 2003). During the precipitation of synthetic hydroxyapatite, Mn can directly substitute for Ca, and Mn uptake and accumulation is also related to calcium metabolism (Pon-On et al., 2008). As such, osmotic regulation of Mn concentrations could alter this relationship in biogenic hydroxyapatites such as fin spines. Therefore, further research on the Mn incorporation into calcified structures should be essential to shed light on this uncertainty.

Mn can be introduced into the water column through terrestrial inputs, upwelling, or microbial oxidation, among other processes (e.g., Klinkhammer and McManus, 2001). Previous studies have provided historical records of hypoxia intensity by demonstrating an association between migrations into deep water hypoxic zones and a higher concentration of Mn in otoliths (Limburg et al., 2011, 2015; Limburg and Casini, 2019). Avigiliano et al. (2019) measured Mn concentrations in the edge of otoliths and fin spines of *Genidens barbatus*, and while the Mn concentrations of the two structures were highly dispersed and only weakly correlated, higher biomineral Mn concentrations were generally

associated with relatively cold waters (i.e., higher probability of hypoxia). In contrast, our results point to an association between fin spine Mn and a higher availability of Mn in the Mediterranean environment that is relatively warmer and less oxygenated than the eastern Atlantic. Even so, the influence of hypoxia on tuna fin spine chemistry remains unexplored. In addition, Mn uptake primarily comes from the diet in elasmobranchs and other vertebrates (Madejczyk et al., 2009; Mathews and Fisher, 2009), and the combination of dietary Mn and uptake from the ambient water might explain the elevated Mn values in ABFT collected from the Mediterranean.

We also found that Li concentrations could effectively discriminate ABFT by capture location areas, but the environmental and physiological factors driving the incorporation of Li into fin spines have not been consistently studied. Li concentration in ABFT fin spines has been shown to increase with age (Luque et al., 2016). This age-dependent accumulation may reflect seawater Li along with a higher degree of physiological modulation (Baumann et al., 2015). Some researchers have also suggested that Li could track salinity (Hicks et al., 2010), but Li has not been directly related to water chemistry (Reis-Santos et al., 2018). These inconsistencies among studies lead to the conclusion that Li concentrations in mineralized tissue may be partially physiologically driven, as natural growth and physiological processes affect the probability of Li atoms being trapped in the fin spine structure.

Sr and Ba were unexpectedly less effective than other elements at discriminating specimens between regions. Sr and Ba uptake in otoliths and fin rays is considered to be an environmental proxy for salinity (McMillan et al., 2017; Livernois et al., 2021), and has therefore been used to reconstruct fish migrations across salinity gradients (e.g., Elsdon et al., 2008; Allen et al., 2009; Avigiliano and Volpedo 2013; Izzo et al., 2018; Rogers et al., 2019). In elasmobranch vertebrae, while Sr increases Ba decreases with increasing salinity (Dorval et al., 2005). However, the limited discriminatory power of Sr and Ba in our study might indicate that the salinity gradient between Atlantic and Mediterranean waters is not strong enough for a differentiated uptake of Sr or Ba (Brown and Severin, 2009; Sturrock et al., 2012).

Both elements may also be influenced by temperature (Smith et al., 2013). Environmental Ba distributions mirror those of other nutrients, with low Ba concentrations in surface waters and substantially higher concentrations in deeper waters (Chan et al., 1977). Ba incorporation into the mineral matrix of fish scales, bone, and teeth has either a positive or non-existent relationship with temperature (Miller, 2009). In our study, Ba concentrations in the fin spines of ABFT might be related to whether fish were obtained from strong, Ba-rich upwelling zones in the Mediterranean (Bakun and Agostini, 2001). However, we found no significant regional differences in the Sr concentration in fin spines, despite assumed differences in temperature and salinity between the Atlantic and Mediterranean. Sr incorporation and temperature were reported to be independent variables in juvenile *L. xanthurus* scales (Wells et al., 2000) common cuttlefish statoliths (*Sepia officinalis*) (Bath et al., 2000), and the otoliths of European eels (*Anguilla Anguilla*) (Marohn et al., 2011). Albeit the physiological role for Sr has not been identified in fishes (Chowdhury and Blust, 2011), our results could indicate that physiological drivers of fin spine chemistry are particularly salient at specific ambient element-to-calcium ratios and fin spine Sr concentrations might reflect and/or respond differently to changing environmental or physiological conditions. Hence, we anticipate that Sr in fin spines is likely not to be a valuable habitat marker in ABFT.

As mentioned earlier, tuna captured in the Atlantic region were correctly classified to their region of capture more often than tuna captured in the Mediterranean region. This difference in classification success rates could be explained by the differences in age between the Atlantic and the Mediterranean ABFT. Because the Mediterranean ABFT in our study were generally younger, the outer ~150 μm of their fin spine edges (i.e., the outermost annulus) could represent a smaller proportion of growth relative to Atlantic ABFT. Samples of a smaller proportion of the annulus growth would likely cover a shorter residence time in the geographic region being sampled and thus reduce our ability to accu-

rately detect the signal of this region. Luque et al. (2016) found that Mg concentrations displayed an increasing pattern with annulus while the concentration of Mn showed a decreasing pattern throughout annuli, with concentrations significantly higher in the opaque bands (i.e., summer bands) regardless of annulus. In our study, ABFT captured in the Mediterranean had higher Mg and Mn concentrations in their fin spine edge but were significantly younger than ABFT captured in the Atlantic indicating that there might not be any association to age-dependent physiological regulation.

Apart from any differences in age between regions, the observed misclassification could also be caused by inherently shorter residency times of ABFT captured in the Mediterranean and therefore had less time to incorporate the Mediterranean chemical signature since ABFT in the Mediterranean arrived recently from the Atlantic. Samples that are more balanced in age and/or have a better age resolution (i.e., to the level of months rather than years) could provide further insight on these two hypotheses.

In addition to extrinsic environmental influences, fin spine chemistry in tuna can also be linked to intrinsic factors (e.g., physiology or metabolic rates) that might affect the incorporation of elements into biomineralized or calcified structures (e.g., Campana et al., 2000; Elsdon et al., 2008; Smith et al., 2013; Kerr and Campana, 2014; Martino et al., 2020). Although the use of calcified structure microchemistry to infer habitat use and life history in fishes has been extremely successful, particularly for migrations between freshwater and salt water (Campana, 1999), persistent limitations in these studies reflect an insufficient understanding of both the factors that determine element presence in biominerals and how ambient calcified structure chemistry relates to environmental conditions (Sturrock et al., 2012, 2014). Organisms control the internal availability of elements either directly (for essential elements such as Mg and Mn) or indirectly (for non-essential elements such as Li) using calcium-dependent uptake mechanisms (Crichton 2008; Loewen et al., 2016). Thus, a deeper understanding of the pathways and rates of element uptake in teleost fin spines is necessary to adequately account for the influence of physiology on fin spine chemistry.

## 5. Conclusions

We demonstrated that the chemical composition of ABFT fin spine edges can effectively discriminate individuals that have recently inhabited different water bodies with different physicochemical characteristics. Combining elemental tracers with stable isotopes provided a more robust discrimination of presumed ABFT capture locations than either suite of variables could provide alone. The fin spine ageing approach also has several advantages over otolith analysis, particularly on juveniles ABFT, especially because growth bands are easier to identify in fin spines. Overall, our results demonstrate the viability of fin spine chemistry as a promising tool for providing retrospective, age-specific reconstructions of fish movement. Future studies should also examine fin spine tracers and isotopes in calcium-rich environments, as these studies will help reveal how phylogeny, ontogeny, stress, temperature, and other factors can affect how different chemical tracers compete with calcium during uptake and biomineralization.

## Declarations

### Author contribution statement

Patricia Lastra Luque, PhD: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Iraide Artetxe-Arrate, PhD; Gorka Bidegain, PhD: Analyzed and interpreted the data; Wrote the paper.

Saburo Sakai, PhD; Fanny Claverie, PhD; Christophe Pécheyran, PhD: Performed the experiments.

Igaratza Fraile, PhD: Conceived and designed the experiments.

Hilario Murua, PhD: Conceived and designed the experiments; Wrote the paper.

Jose Luis Varela, PhD; Antonio Medina, PhD: Contributed reagents, materials, analysis tools or data.

Haritz Arrizabalaga, PhD: Conceived and designed the experiments; Wrote the paper.

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### Data availability statement

Data will be made available on request.

### Declaration of interests statement

The authors declare no conflict of interest.

### Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2022.e11757>.

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