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Population structure of the blue jack mackerel (*Trachurus picturatus*) in the NE Atlantic inferred from otolith microchemistry



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ABSTRACT

The blue jack mackerel, *Trachurus picturatus*, is an economically important fishery resource of the NE Atlantic, commonly captured around the Macaronesian islands of Azores, Madeira and Canaries, but also along coastal Portugal mainland. Despite this, information regarding the *T. picturatus* population structure is, at present, non-existent. One hundred and twenty individuals of *T. picturatus* were collected in 2013 from six important fishery regions of the NE Atlantic: Azores, Madeira, Canaries and Portugal mainland – Matosinhos, Peniche and Portimão. Elemental and isotopic signatures of whole sagittal otoliths were determined by inductively coupled plasma mass spectrometry and isotope ratio mass spectrometry, respectively. Elemental (Sr/Ca, Ba/Ca, Mg/Ca, Pb/Ca, Li/Ca, Fe/Ca and Mn/Ca) and isotopic ratios ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) were analysed with univariate and multivariate statistics to determine whether these chemical signatures provide insight into regional population structure. Whole otolith signatures gave distinct regional signatures, mainly driven by spatial differences in Sr/Ca, Ba/Ca, Li/Ca and $\delta^{13}\text{C}$, namely between the Portugal mainland and the oceanic islands. Furthermore, the results suggest for the first time that Portugal mainland, Azores, Madeira, and Canaries should be regarded as different population units. The high re-classification success rate (an overall of 81%) for these regions obtained from the quadratic discriminant function analysis supports these findings, and suggests the management of this fishery in the NE Atlantic as different stocks.

1. Introduction

The blue jack mackerel *Trachurus picturatus* (Bowdich, 1825) is a migratory pelagic fish species widely distributed in the NE Atlantic from the southern Bay of Biscay to southern Morocco, including the Macaronesian archipelagos of Azores, Madeira and Canaries, Tristan de Cunha and Gough Islands and also in the western part of the Mediterranean Sea and the Black Sea (Smith-Vaniz, 1986; ICES, 2015). It is an economically important resource around the Macaronesian islands of Azores, Madeira and Canaries, and also in the coastal waters of Portugal mainland. This species is targeted by artisanal fleets using purse-seine nets; in 2015 the reported landings for Portugal reached 3675 t (INE, 2016). The catches in the NE Atlantic have shown regular fluctuations during the last ten years (FAO, 2016), which may be related, at least partially, to natural variations in abundance or recruitment. However, these fluctuations in the landings are difficult to

explain since, at present, studies regarding the population dynamics, stock structure, fish movements and habitat connectivity are not available in the existing literature.

Some attempts to understand the population structure of *T. picturatus* have been done regionally and include studies based on growth and reproduction (Isidro, 1990; Jesus, 1992; Gouveia, 1993; Vasconcelos et al., 2006; Jurado-Ruzafa and Santamaria, 2013; Garcia et al., 2015), fish recruitment (Jurado-Ruzafa and Santamaria, 2011) and the use of parasites as biological markers (Costa et al., 2012, 2013; Vasconcelos et al., 2017). However, studies specifically addressing the existence of distinct population units within the species range have not been published. Growth rate, age at first maturity and reproductive season vary regionally, and, as in closely related species such as *T. trachurus* (Abaunza et al., 2008), the evidences suggest that the species is most likely divided into different population units (Isidro, 1990; Jesus, 1992; Gonçalves et al., 2013; Jurado-Ruzafa and Santamaria, 2013).

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At present, the International Council for the Exploration of the Sea (ICES, 2014) considers that this species is not in need of any special management plan, supported by: the data of commercial abundance; the presence of a stable biomass of juveniles; and an increase in the biomass of adults in the Azores region. Nevertheless, it does recognise that there is no direct surveying of the stock. Marine pelagic fishes, particularly migratory species such as *T. picturatus*, may be erroneously considered a homogenous population unit due to their usually broad geographic distribution, large population sizes and high migratory movements (Gonzalez et al., 2008). However, recent parasitological studies have reported differences between the fish populations of Azores and Canary islands (ICES, 2013), between the fish populations of Madeira and Canary islands (Costa et al., 2013) and among Peniche (located on the Portuguese coast), Madeira and Canary islands (Vasconcelos et al., 2017).

Fish population units, or stocks, can be characterized based on phenotypic characters, (e.g., meristic counts), morphometric measures, comparison of life-history traits, molecular and environmental markers (Begg and Waldman, 1999). Otoliths are regarded as natural tags because: they grow continuously throughout life; they remain chemically inert; and they preserve an uninterrupted record of the environment where fish lived (Campana, 1999). Therefore, otolith-based techniques have proven to be an effective method to assess the population structure in high gene flow systems where environmental heterogeneity exists (Bradbury et al., 2008; Smith and Campana, 2010; Correia et al., 2012b).

The aim of this work was to provide information about the stock structure of *T. picturatus* among four fishery regions – i.e., Azores, Madeira, Canaries, and Portugal mainland – in the NE Atlantic by using an otolith chemistry approach. Moreover, this study used both elemental composition and stable isotopes of whole otoliths (i.e., entire life-history prior to capture) to determine whether distinct chemical signatures were evident.

2. Material and methods

2.1. Fish collection

A total of 120 individuals were collected from four main fishery regions of NE Atlantic: Portugal mainland (three sites: Matosinhos, Peniche and Portimão), Azores, Madeira and Canary islands (Fig. 1).

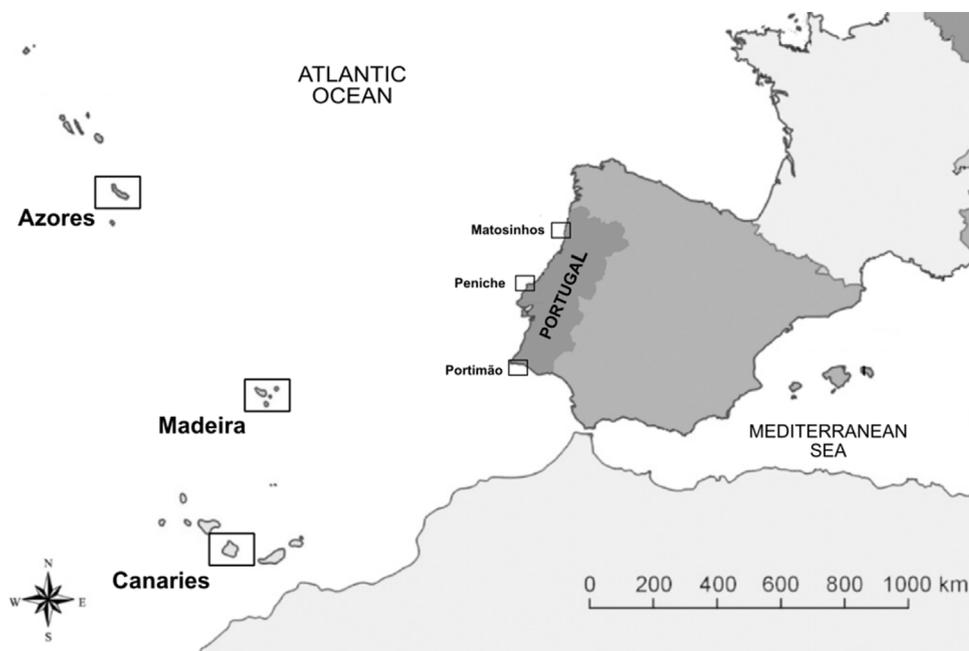


Fig. 1. Sampling location of *T. picturatus* individuals collected in 2013 in the Northeast Atlantic Ocean.

Samples were collected by local fishermen in shallow coastal waters (up to 75 m water depth) using purse-seine nets during May and July 2013. An effort was made to ensure that the fish obtained from each region/site were from the same size class (as a proxy of age).

All fish were stored on ice after landing and transported to the laboratory to be processed. Each specimen was measured for total length (TL, 0.1 cm) and weighed (W, 0.0001 g) (Table 1). Sagittal otoliths were extracted with plastic forceps to avoid metallic contamination, washed with distilled water, dried with lint-free paper, differentiated to left and right otolith (according to the position of the sulcus acusticus and the rostrum), and stored in clean centrifuge tubes.

2.2. Otolith elemental analyses

The chemical compositions of the right whole otoliths were determined using solution based inductively coupled plasma mass spectrometry (SB-ICP-MS). Prior to the analyses, otoliths were cleaned in an ultrasonic bath with ultrapure water (Milli-Q water) for 5 min, followed by an immersion in 3% analytical grade hydrogen peroxide (H_2O_2) for 15 min to remove any remaining biological residue. Thereafter, otoliths were immersed in ultrapure 1% nitric acid (HNO_3) solution for 10 s to remove superficial contamination, followed by a triple immersion in Milli-Q water for 5 min to remove the acid (Rooker et al., 2001). Cleaned otoliths were stored in new decontaminated centrifuge tubes and allowed to dry in a laminar flow fume hood (Patterson et al., 1999). Otoliths were weighed on an analytical balance (0.0001 g) and dissolved for 15 min in 1 mL of 10% ultrapure HNO_3 diluted with Milli-Q water to a final volume of 10 mL (Correia et al., 2011a).

Otoliths were analysed using a double focusing magnetic sector field instrument ICP-SF-MS (Thermo ICP-MS X series, Thermo Electron Corporation). All measurements were performed at the medium resolution setting ($m/m = 4000$) to avoid spectral interferences, particularly on Ni. The instrument was equipped with a microflow nebulizer (PFAAR35-1-C1E, Glass Expansion), operated in the self-aspirating mode (sample uptake rate $\sim 0.93 L min^{-1}$). Quantification of trace elements was based on the external calibration method preparing multi-element standards containing the elements of interest in the expected concentration range (Merck KGaA). To remove the effect of any plasma fluctuations, different nebulizer aspiration rates or sample build-up on the cone orifices, ^{115}In was added at a known concentration to all samples and standards as an internal standard to adjust for instrument

Table 1
 Sampling location, sample size (n), total length (TL), weight (W), otolith elemental (μg element g^{-1} calcium) and isotopic (VPDB, ‰) signatures of *T. picturatus*. Original elemental concentrations and isotopic signatures. Mean values \pm SE.

Regions	Sites	Sampling Location	n	TL (cm)	W (g)	Sr/Ca	Li/Ca	Mg/Ca	Mn/Ca	Ba/Ca	Pb/Ca	Fe/Ca	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$
Azores	-	37°42'6.39"N	20	26.4 \pm 0.3	164.8 \pm 6.0	7444 \pm 121	1.87 \pm 0.08	15.87 \pm 0.65	0.54 \pm 0.03	26.41 \pm 1.13	3.75 \pm 0.23	8.18 \pm 0.76	-6.42 \pm 0.07	1.62 \pm 0.04
		25°29'15.12"W	20	26.2 \pm 0.2	150.5 \pm 2.8	7252 \pm 49	2.57 \pm 0.11	16.09 \pm 0.68	0.53 \pm 0.02	27.87 \pm 1.22	3.46 \pm 0.17	7.41 \pm 0.65	-6.60 \pm 0.08	1.20 \pm 0.01
Madeira	-	16°56'52.75"W	20	25.9 \pm 0.3	142.86 \pm 5.7	6543 \pm 74	1.79 \pm 0.08	21.65 \pm 0.83	0.60 \pm 0.03	29.78 \pm 2.78	4.08 \pm 0.19	8.12 \pm 0.70	-6.35 \pm 0.24	1.09 \pm 0.08
		27°48'42.12"N	20	26.4 \pm 0.2	174.5 \pm 3.9	7796 \pm 231	1.16 \pm 0.07	17.02 \pm 1.11	0.52 \pm 0.04	61.72 \pm 5.52	4.14 \pm 0.28	9.91 \pm 1.00	-5.61 \pm 0.06	1.56 \pm 0.07
Portugal Mainland	-	41°10'49.50"N	20	26.9 \pm 0.2	178.4 \pm 3.2	8463 \pm 51	0.81 \pm 0.03	13.09 \pm 0.75	0.40 \pm 0.02	65.46 \pm 3.83	2.82 \pm 0.16	7.04 \pm 0.79	-5.69 \pm 0.07	1.81 \pm 0.04
		8°42'11.25"W	20	25.5 \pm 0.2	143.8 \pm 3.7	8384 \pm 93	1.05 \pm 0.04	14.22 \pm 0.63	0.46 \pm 0.03	47.52 \pm 3.53	3.46 \pm 0.18	8.40 \pm 0.76	-5.56 \pm 0.11	1.55 \pm 0.09
	Peniche	39°21'51.11"N												
	Portimão	37°6'14.20"N												
		8°31'25.99"W												

drift. Concentrations were calculated by linear interpolation (sum of least squares) based on normalization with the internal standard to account for drift, and on calibration curves derived from the single element standards. The entire set of calibration standards were analysed at the beginning of each session. The matrix of both the blank and the standard solutions was 1% ultrapure HNO_3 .

A preliminary analysis was made to determine the most likely abundant and informative elements (^{88}Sr , ^7Li , ^{24}Mg , ^{55}Mn , ^{138}Ba , ^{208}Pb , ^{54}Fe , ^{63}Cu and ^{60}Ni ; ^{43}Ca provided the internal standard) found in otoliths of *T. picturatus* to serve as environmental tags indicators, considering the elements usually reported in this kind of study (Campana, 2005). Otolith samples were analysed in random order to avoid possible sequence effects. An otolith certified reference material (FEBS-1) was analysed for accuracy quality control (Sturgeon et al., 2005). Elemental concentrations determined in FEBS-1 were within the certified or indicative range, with recovery rate between 92 and 98%. The precision of replicate analyses of individual elements ranged between 2% and 5% of the relative standard deviation (RSD). The limits of detection were calculated from the individual calibration curves using the three sigma criteria and were (in $\mu\text{g L}^{-1}$): ^{43}Ca (4000), ^{88}Sr (400), ^7Li (1), ^{24}Mg (10), ^{55}Mn (0.30), ^{138}Ba (1), ^{208}Pb (0.5), ^{54}Fe (10), ^{63}Cu (2) and ^{60}Ni (4).

2.3. Otolith isotopic analyses

Whole left otoliths were used for isotopic analysis by isotope ratio mass spectrometry (IRMS). For carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotopic determinations, carbon dioxide (CO_2) was extracted from powdered otolith's carbonates in a high vacuum line after reaction with anhydrous orthophosphoric acid for 12 h at 25 °C (Craig, 1957). The released CO_2 was analysed for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ in a double inlet, triple collector (SIRA III) mass spectrometer, using BSC (Borborema Skarn Calcite) as the reference gas. BSC was calibrated against NBS (National Bureau of Standards) -18, NBS-19 and NBS-20. The precision of the analysis was better than 0.1‰ based on multiple analysis of this internal standard. The results are expressed in the notation δ ‰ (per thousand) in relation to international VPDB (Vienna Pee-Dee Belemnite) scale (Epstein et al., 1953).

2.4. Statistical analyses

Concentrations of the trace elements from the SB-ICP-MS analyses, originally in μg element L^{-1} solution, were transformed to μg element g^{-1} otolith and then to μg element g^{-1} calcium. The concentration of ^{63}Cu and ^{60}Ni was consistently below the limit of detection and consequently was not used in the statistical analysis. The raw data for each element and stable isotope was checked for normality, homoscedasticity and homogeneity of variance-covariance matrices prior to statistical analysis. For ^{55}Mn and ^{24}Mg the assumptions were met after log 10 transformation. The relationship between elemental concentration, isotopic ratios and fish size (expressed as otolith mass) was tested with analysis of covariance (ANCOVA using otolith mass as a covariate). To avoid that differences in otolith mass among locations confound any site-specific differences in otolith chemistry, the concentration of elements and isotopic ratios were weight-detrended by subtraction of the common within-group linear slope (Campana et al., 2000; Daros et al., 2016).

One-way analysis of variance (ANOVA) was used to explore the differences in individual elemental fingerprint between regions and sites, followed by a Tukey post-hoc test if significant ($p < 0.05$). Multi-elemental otolith chemistry was tested using a multivariate analysis of variance (MANOVA). For MANOVA the Pillai's trace statistic was reported. Post-hoc multivariate pairwise comparisons between locations were performed using the Hotelling T-square test. The multi-elemental composition of otoliths was analysed with a quadratic discriminant function analysis (QDFA). Classification accuracies of the discriminant

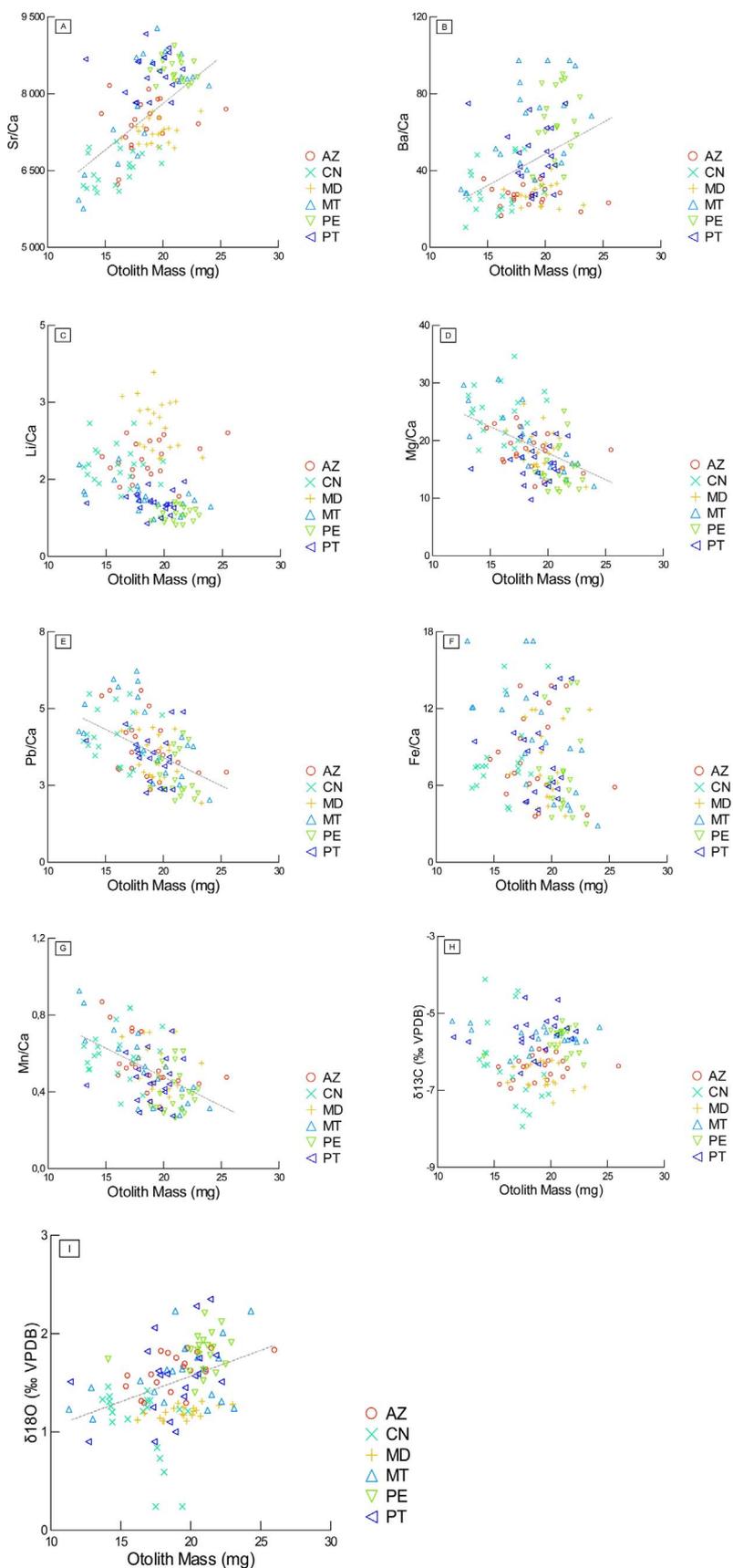


Fig. 2. Element/Ca and isotopic concentrations versus otolith mass (mg) for all sampling locations (Azores–AZ, Canaries–CN, Madeira–MD, Matosinhos–MT, Peniche–PE and Portimão–PT). A trendline was added when a linear relationship ($p < 0.05$) was found between the two variables.

Table 2
ANCOVA for $\delta^{13}\text{C}$ and for $\delta^{18}\text{O}$ values in otolith.

	Source	DF	SS	MS	F-ratio	P-value
$\delta^{13}\text{C}$	Location	5	22.224	4.445	14.843	0.001
	Otolith Mass	1	0.308	0.308	1.029	0.313
	Error	113	33.838	0.299		
	Total	119	56.37			
$\delta^{18}\text{O}$	Location	5	5.462	1.092	14.240	0.001
	Otolith Mass	1	0.457	0.457	5.962	0.016
	Error	113	8.670	0.077		
	Total	119	14.589			

functions for each region and site were evaluated through the percentage of correctly re-classified individuals using jack-knifed (leave-one-out) cross-validation (Correia et al., 2011b). The correlation matrix from the elemental and isotopic data set was analysed by a Canonical Analysis of principal Coordinates (CAP) based on Euclidian distances (Spearman correlation of 55%) (Anderson and Willis, 2003) and the results presented in a two-dimensional biplot (Lo-Yat et al., 2005).

All statistical analyses were performed using Systat (v 12) and PRIMER 6 + PERMANOVA software, with a statistical level of significance (α) of 0.05. Data are presented as mean values \pm standard errors (SE).

3. Results

^{88}Sr and ^{138}Ba concentrations presented positive relationships with otolith mass ($n = 120$, $r^2 = 0.705$, $p < 0.05$; $n = 120$, $r^2 = 0.57$, $p < 0.05$, respectively) (Fig. 2A and B). No significant correlation was found for ^7Li and ^{54}Fe ($n = 120$, $r^2 = 0.774$, $p = 0.100$; $n = 120$, $r^2 = 0.092$, $p = 0.076$, respectively) (Fig. 2C and F). The ^{24}Mg ($n = 120$, $r^2 = 0.443$, $p < 0.05$, Fig. 2D), the ^{208}Pb ($n = 120$, $r^2 = 0.313$, $p < 0.05$) (Fig. 2E) and the ^{55}Mn ($n = 120$, $r^2 = 0.308$, $p < 0.05$) (Fig. 2G) presented negative relationships.

$\delta^{13}\text{C}$ showed no significant relationship with otolith mass (ANCOVA, $p > 0.05$) (Fig. 2H) and 40% of the sum of squares was explained by the location (Table 2). The $\delta^{18}\text{O}$ presented a positive relationship with otolith mass (ANCOVA, $p < 0.05$) and 37% of the sum of squares was explained by location (Fig. 2I). Although otolith mass was significant for $\delta^{18}\text{O}$ it only explained 3% of the sum of squares (Table 2).

There were significant differences among sampling locations (ANOVA, $p < 0.05$) for Sr/Ca, Ba/Ca, Li/Ca, Mg/Ca and Pb/Ca (Fig. 3A–E), but not for Fe/Ca and Mn/Ca (ANOVA, $p > 0.05$) (Fig. 3F and G).

The individuals collected from Portugal mainland (Matosinhos, Peniche and Portimão) presented the highest mean values for Sr/Ca and Ba/Ca ratios (Tukey test, $p < 0.05$; Fig. 3A and B), and the lowest for Li/Ca ratios (Tukey test, $p < 0.05$; Fig. 3C). The Canary islands presented the highest mean values for Mg/Ca ratios being statistically different from Azores, Peniche and Portimão (Tukey tests, $p < 0.05$), but similar to Madeira and Matosinhos (Tukey tests, $p > 0.05$) (Fig. 3D). Two sites in Portugal mainland presented significant differences regarding the ratio of Pb/Ca, namely Matosinhos and Peniche (Tukey test, $p < 0.05$; Fig. 3E).

The otolith isotopic ratios ranged from -7.94‰ to -4.12‰ for $\delta^{13}\text{C}$ and from 0.24‰ to 2.35‰ for $\delta^{18}\text{O}$. The isotopic ratios showed significant differences between the oceanic islands and Portugal mainland for $\delta^{13}\text{C}$ (Tukey tests, $p < 0.05$) (Fig. 3H) and between the Azores/Portugal mainland and Madeira/Canary islands for $\delta^{18}\text{O}$ (Tukey tests, $p < 0.05$) (Fig. 3I).

MANOVA for the multi-elemental signatures (i.e. Sr/Ca, Ba/Ca, Li/Ca, Mg/Ca, Pb/Ca, Fe/Ca, Mn/Ca, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) indicated significant differences among all locations (Pillai's Trace, $F_{45,550} = 8.07$, $p < 0.05$). Pairwise comparisons resulted in significant differences

between all locations (data not shown), except for Peniche and Portimão (Hotelling's T-square, $p < 0.05$). For each pair of groups, Peniche and Matosinhos ($F_{9,106} = 2.80$) and Peniche and Madeira ($F_{9,106} = 56.41$), showed the lowest and highest Mahalanobis distances (Between Group F-Matrix), respectively.

QFDA plot for the multi-elemental signatures discriminated among individuals from the main sampling regions (Madeira, Azores, Canaries and Portugal mainland), but samples from the three Portuguese mainland sites highly overlapped (Fig. 4). The CAP identified four main groups composed of individuals from Azores (Group 1), Madeira (Group 2), Canary islands (Group 3) and Portugal mainland (Group 4) (Fig. 5). The vectors for Mg/Ca and Pb/Ca were aligned with Group 3; Mn/Ca and Li/Ca were aligned with Groups 1 and 2; and Sr/Ca, Ba/Ca, $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ were aligned with Group 4 (Fig. 5). Jack-knifed re-classification accuracy was moderate to high, ranging from 65% for Matosinhos to 95% for Azores, with an overall mean of 81% (Table 3).

4. Discussion

The elemental and isotopic signatures for the otoliths from *T. picturatus* were within the ranges found for other marine fishes (e.g., Correia et al., 2011a; Higgins et al., 2013; Daros et al., 2016). A significant variation in the chemical composition of the whole otoliths among the Atlantic oceanic islands and Portugal mainland sampling locations was clearly observed. This regional variation was largely driven by Sr/Ca, Li/Ca, Ba/Ca and $\delta^{13}\text{C}$. Furthermore, there was a general trend for both elemental and isotopic signatures suggesting a clear discrimination amongst the four main regions: Azores, Madeira, Canary islands and Portugal mainland. Individuals captured in the Portuguese mainland coastal water had, in general, higher concentrations of Sr and Ba, but lower concentrations of Li, compared to the oceanic islands.

Concerning the Sr content in otoliths, a lower concentration was recorded in otoliths from the islands. Additionally, otoliths from Matosinhos also presented unexpected low Sr values when compared to the other Portugal mainland sites. The physico-chemical properties of the environment (e.g., water composition, temperature and salinity), fish physiology (e.g., age, growth and metabolism) and feeding regime are among the factors that can influence the potential incorporation of elements in the otoliths (Campana et al., 2000; Hamer and Jenkins, 2007; Webb et al., 2012). Moreover, Sr is a trace element that is noticeably influenced by its ambient concentration (Bath et al., 2000; Eلسdon and Gillanders, 2004; Walther and Thorrold, 2006). The unexpectedly low Sr content found in the Canaries may be the result of two processes: (1) the higher precipitation and numerous rivers on the central west Africa coast that generate larger volumes of warm (above 24 °C) and low-salinity (less than 35) water (Diop et al., 2014); and/or (2) low-salinity water advected by upwelling filaments generated in the upwelling area off northwest Africa (Barton et al., 1998). Also, the assimilation of Sr into the otoliths seems to be positively influenced by temperature (Bath et al., 2000; Eلسdon and Gillanders, 2002; Martin et al., 2004) and it is well known the existent positive correlation between otolith Sr/Ca and water salinity (Bath et al., 2000; Secor and Rooker, 2000; Eلسdon and Gillanders, 2002). On the other hand, in Matosinhos, the low Sr concentration may relate to the sampling area being adjacent to the Douro River, a major input of freshwater along the Portuguese coast. This area is known to have a low-salinity lens formed by the river discharge and continental run-off extending along the shelf off Northwest Iberia (Otero et al., 2008; Matta et al., 2013).

Regarding Ba, as expected, the values obtained were generally higher in the Portugal mainland sites when compared to the oceanic islands. Upwelling processes and terrestrial sources are often linked to variations in water chemistry (Hamer et al., 2006) and Ba seems to be a reliable indicator of these changes. Thus, the combined effect of the freshwater input from the surrounding rivers (i.e., Douro River for Matosinhos, São Domingos River for Peniche and Arade River for

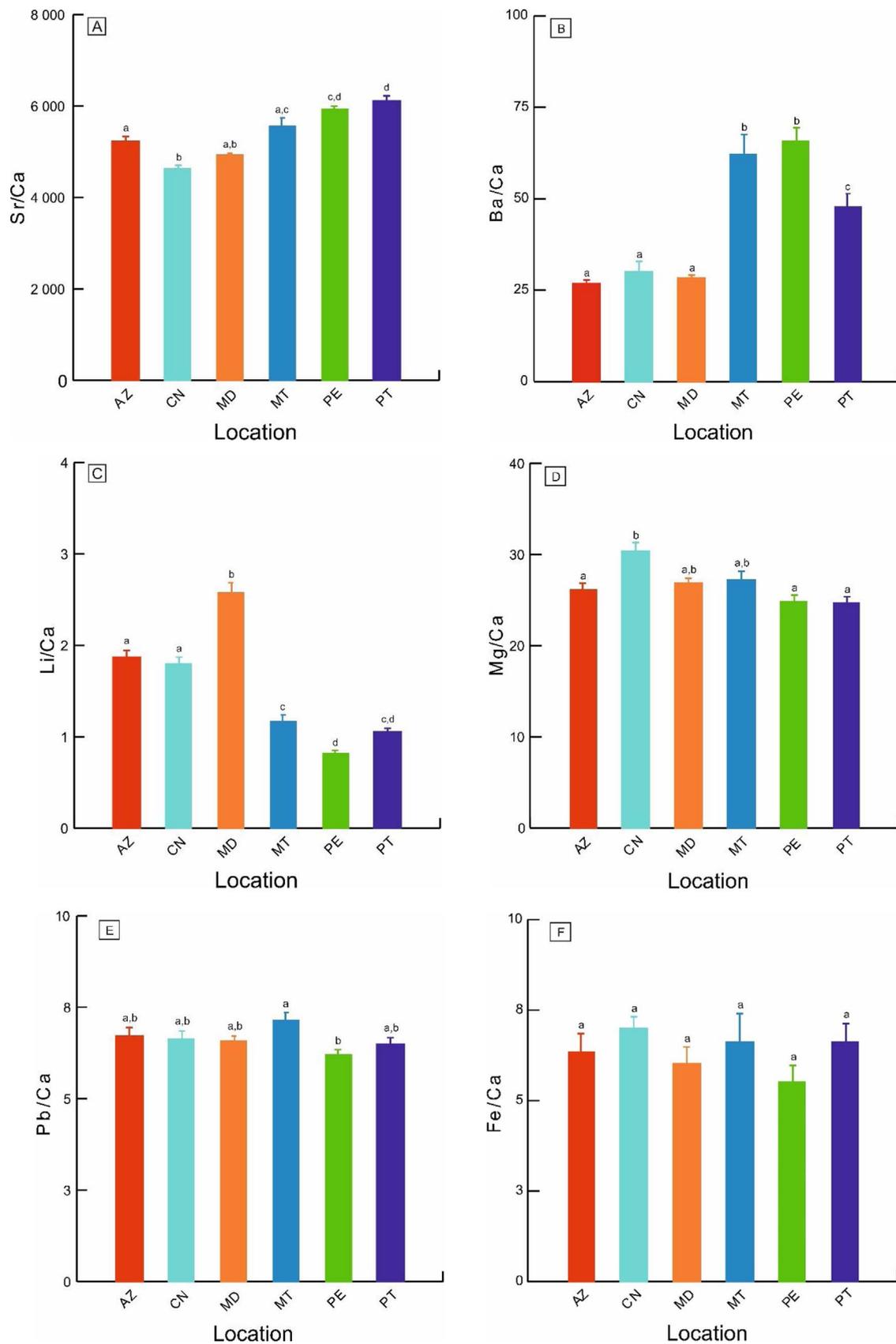


Fig. 3. Element/Ca and isotopic concentrations (Mean \pm SE) recorded in the whole otolith of *T. picturatus* in the six sampling locations (Azores-AZ, Canaries-CN, Madeira-MD, Matosinhos-MT, Peniche-PE and Portimão-PT). Concentrations are given in $\mu\text{g element g}^{-1}$ calcium and VPDB (%), respectively. Different letters in the bars indicate statistically different results ($p < 0.05$). Detrended values for Sr/Ca, Mg/Ca, Pb/Ca, Mn/Ca and $\delta^{18}\text{O}$.

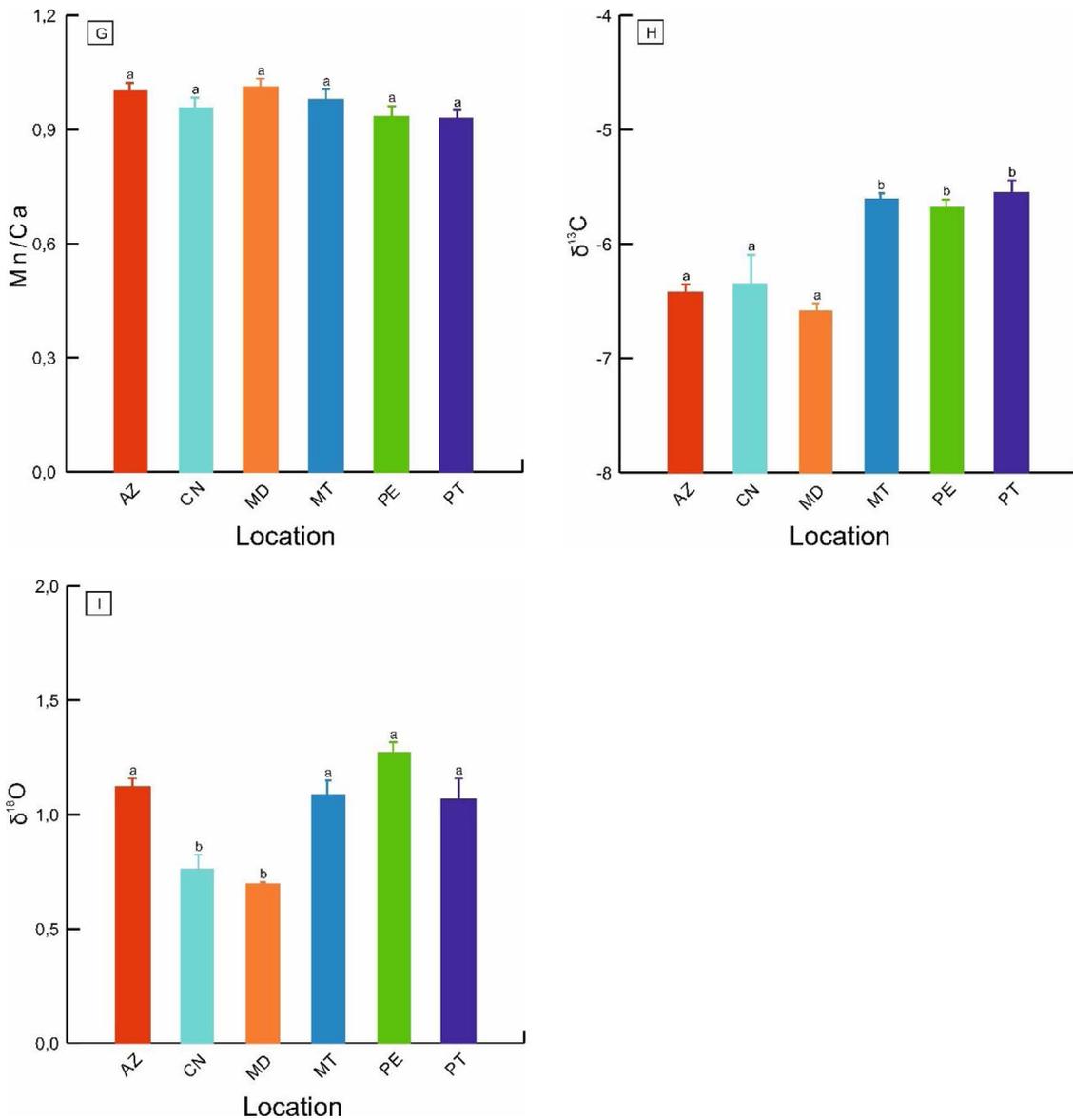


Fig. 3. (continued)

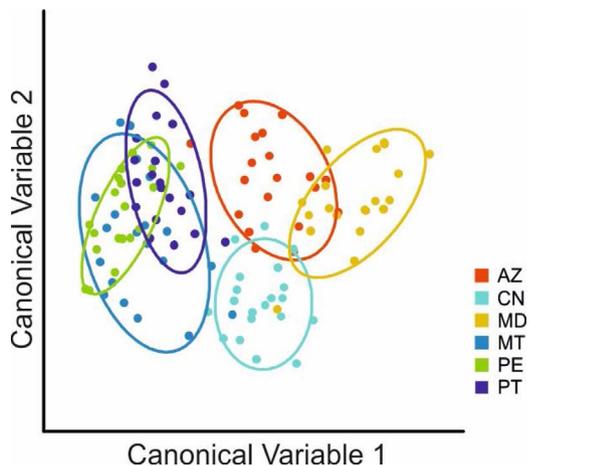


Fig. 4. Canonical variate plot displaying spatial differences in multi-elemental tags in whole otoliths from the six sampling locations in the NE Atlantic [Azores (AZ), Canaries (CN), Madeira (MD), Matosinhos (MT), Peniche (PE) and Portimão (PT)]. Ellipses represent 95% confidence intervals around the data, and data points represent individuals fish.

Portimão) along with coastal upwelling and terrestrial enrichment may explain these results (Elsdon and Gillanders, 2004; Hamer et al., 2006; Hicks et al., 2010). Moreover, previous studies (Fowler et al., 1995; Bergenius et al., 2005) reported a negative relationship between the otolith ratio of Ba/Ca and salinity/temperature consistent with the Ba results found in the present study.

Higher concentrations of Li were observed for the oceanic islands. A recent study by Hicks et al. (2010) reported that, at least under controlled conditions, Li presents a high success rate for distinguishing fish reared at different salinities, as the ratio Li/Ca increases with salinity. However, our results show a clear distinction between the Azores/Canaries and Madeira regions. Even though the Azores and Canary islands are influenced by submarine hydrothermal vents (Desbruyères et al., 2000; Becerril et al., 2014), making the Li concentration in the environment higher and more stable (Chan and Edmond, 1988), these islands are also influenced by the Azores and Canary Currents. These currents are expected to cause some degree of instability and could be the reason for the higher Li concentration in Madeira, when compared to the other islands (Sala et al., 2013).

Although the mechanisms behind the incorporation of elements to otoliths are still not well understood, differences in the concentrations of Mn and Mg have been used successfully to infer the population and

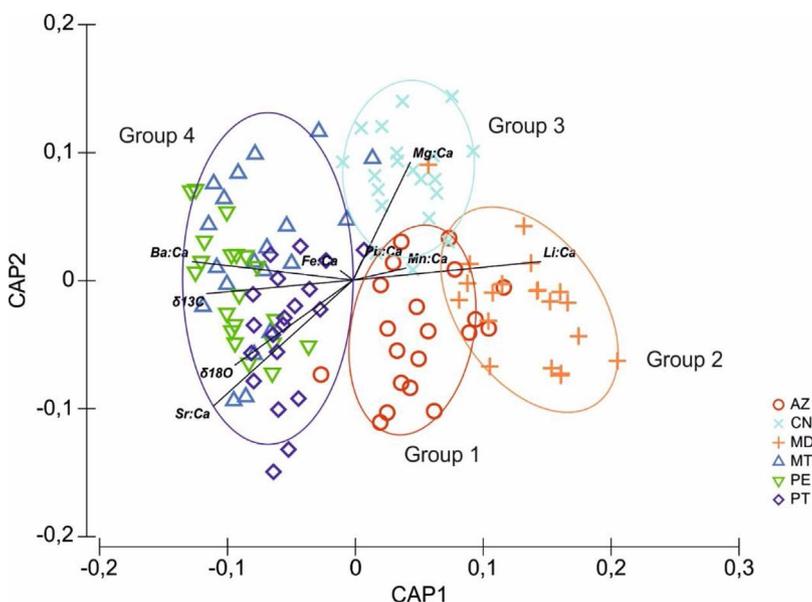


Fig. 5. Canonical analysis of principal coordinates (CAP) plot for the trace elements concentration and the isotopic ratios from *T. picturatus* collected in the six sampling locations of the NE Atlantic [Azores (AZ), Canaries (CN), Madeira (MD), Matosinhos (MT), Peniche (PE) and Portimão (PT)].

Table 3
Jack-knifed classification matrix of *T. picturatus* based in the whole otolith trace elements and isotopes used in quadratic discriminant function analysis for all six locations.

Real Location	Predicted Location						% Correct
	Azores	Canary	Madeira	Matosinhos	Peniche	Portimão	
Azores	19	1	0	0	0	0	95
Canary	1	18	0	1	0	0	90
Madeira	1	2	17	0	0	0	85
Matosinhos	0	1	0	13	3	3	65
Peniche	0	0	0	5	14	1	70
Portimão	0	0	0	3	1	16	80
Total	21	22	17	22	18	20	81

stock structures of important NE fish species (e.g. Silva et al., 2011; Correia et al., 2012a; Higgins et al., 2013). However, in the present study there were no significant differences in the Mn concentrations among the sampling locations. As for the Mg, the only non-significant differences obtained were between Canaries and Madeira/Portugal. In the literature, there is no clear evidence of links between the variation in the otolith concentrations of Mn and Mg and ambient concentrations. Furthermore, these elements are essential for a series of cellular processes, which indicate that there is a high physiological response in their regulation (Hamer and Jenkins, 2007; Barnes and Gillanders, 2013). Combined with this, Mn is highly enriched in the otolith's primordia via maternal transfer, and thus has been used as a natal tag in the reconstruction of environmental shifts for several species (e.g. Ruttenberg et al., 2005; Warner et al., 2009; Clarke et al., 2011).

Concerning the isotopic signatures, $\delta^{18}\text{O}$ showed that similar values between the Canary and Madeira islands were different from Azores and Portugal mainland. It is well known that the oxygen isotopic composition in otoliths is in equilibrium with ambient water $\delta^{18}\text{O}$ being highly influenced by temperature and salinity (Gao et al., 2001; Høie et al., 2004; Correia et al., 2011a). Assuming that in the study area the seawater $\delta^{18}\text{O}$ composition is homogenous, lower values of $\delta^{18}\text{O}$ would mean higher temperatures (Ashford and Jones, 2007). In fact, the $\delta^{18}\text{O}$ values observed across the sampling area followed this expected trend, i.e., presenting higher values in the northern sampling locations (expected lower water temperatures, e.g., Sala et al., 2013) and decreasing towards the south (expected higher water temperatures, e.g., Sala et al., 2013). However, Matosinhos, the site located at a higher latitude in the Portugal mainland, showed a lower value of $\delta^{18}\text{O}$ when compared to

Azores and with the other sites in the Portuguese coast. As mentioned above for Sr, the large input of freshwater from the Douro River is most likely to explain this unexpected low value, as shown by previous studies for other fishes (Correia et al., 2011a; Carvalho et al., 2017).

The higher $\delta^{13}\text{C}$ values were found at the Portuguese coastal sites, while the islands had the most depleted carbon signatures. Ontogenetic changes in trophic levels (i.e., fish diet, growth, and metabolism) are considered the main endogenous factors that affect the incorporation of $\delta^{13}\text{C}$ into the otolith's aragonitic structure (Gillooly et al., 2001; Bastow et al., 2002; Gao et al., 2004). The dissolved inorganic carbon (DIC) in ambient water also seems to be reflected in the otolith concentrations of $\delta^{13}\text{C}$ (Thorrold et al., 1997; Solomon et al., 2006; Daros et al., 2016). The results can be explained by a southward coastal upwelling in summer, parallel to the Iberian coast, as a result of the Portuguese Current (Fúza, 1984). The coastal upwelling is important because it increases food availability, but also enables the retention of larvae and juveniles in the region, which accentuates the higher values of $\delta^{13}\text{C}$ found in the otoliths (Roy et al., 1989; Santos et al., 2001; Sala et al., 2013). However, the Atlantic islands of the Azores and Madeira, exhibit typically low productive (oligotrophic) open ocean waters (Martins et al., 2007), which are probably reflected in the low carbon isotope content in the analysed otoliths.

The multivariate analysis of the otolith elemental fingerprints showed clear separation of the four main sampling regions, i.e., Azores, Madeira, Canaries, and Portugal mainland (Fig. 4). Sr/Ca, Ba/Ca, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ were the most informative elemental and isotopic ratios for the Portugal mainland sites. Mg was informative for the Canaries, Li for Madeira, and Mn for the Azores. Within the fishing region of Portugal

mainland, differences were found mainly between Peniche and Portimão, with samples from Matosinhos overlapping the other sites.

At present, there are several studies on the biology of *T. picturatus* that indicate some degree of differentiation amongst the populations of the NE Atlantic (Shabonev and Ryazantseva, 1977; Isidro, 1990; Jesus, 1992; Gouveia, 1993). One recent study found that individuals caught in the Canary Islands have similar reproductive seasons to those described for Madeira, but different from the Azores individuals (Jurado-Ruzafa and Santamaría, 2013). Moreover, it has been shown that *T. picturatus* can complete the entire life cycle within the Canary waters (Jurado-Ruzafa and Santamaría, 2011) and also within the Azorean waters (Menezes et al., 2006). Until now, the existing information based on the occurrence of parasites indicates the existence of different populations in *T. picturatus* across the NE Atlantic (ICES, 2016). The present work partially agrees with the previously published studies but opens a new debate regarding the possibility of different population units in the Atlantic islands. The data suggest that *T. picturatus* in the NE Atlantic should be regarded as four independent populations units and thus be treated separately in terms of fisheries management.

However the hereby study itself cannot be conclusive about stock structure because of the complexity and requirement for a multi-faceted approach to achieve this. Furthermore, since ICP-MS-SB integrates results across the fish's lives, there is no way of knowing when otolith chemistry differences developed and cannot eliminate the possibility that fish do occupy the same water mass at some time throughout their lives. Nevertheless, there are strong phenotypic differences in terms of otolith chemistry amongst populations, which could provide useful information in terms of life history and demographic processes working towards a better understanding of *T. picturatus* stock structure. For example, is the similarity in otolith chemistry amongst the three coastal sampling locations related to fish movement along the coast between places, or does it mean that the environments offered at the three sampling locations are quite similar to each other? Could be the strong phenotypic differences found between the three offshore island groups interpreted as being three self-replenishing populations, or in alternative that the different populations may be sourced from a common area, through larval advection or juvenile fish movement? More data (e.g., analysis of individuals from different years and analysis of larvae/juveniles) and different approaches (e.g., use of laser ablation ICP-MS and molecular phylogeography of the species) should be considered in the future to complement this information and unravel these questions.

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