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

C. Moreira a,b, E. Froufe a, A.N. Sial c, A. Caeiro a, P. Vaz-Pires a,b, A.T. Correia a,d,*

a Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR/CIMAR), Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos S/N, 4450-208 Matosinhos, Portugal
b Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto, Rua de Jorge Vierbo Ferreira 228, 4050-313 Porto, Portugal
c Universidade Federal de Pernambuco, Departamento de Geologia, Núcleo de Estudos Geoquímicos e Laboratório de Isótopos Estáveis (NEG-LABISE), Recife, Brazil
d Faculdade de Ciências da Saúde da Universidade Fernando Pessoa (FCS/UFP), Rua Carlos Maia 296, 4200-150 Porto, Portugal

A R T I C L E   I N F O
Handled by Prof. George A. Rose
Keywords:
Carangidae
Sagittae
Elemental and isotopic signatures
Fish stocks

A B S T R A C T
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 population dynamics, reproduction (Isidro, 1990; Jesus, 1992; Gouveia, 1993; Vasconcelos et al., 2006; Jurado-Ruzafa and Santamaria, 2011) and the use of parasites as biological markers (Costa et al., 2012, 2013; Vasconcelos et al., 2017), has been done regionally and include studies based on growth and 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 made 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 (Abauzna 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).

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 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.

http://dx.doi.org/10.1016/j.fishres.2017.08.012
Received 2 May 2017; Received in revised form 26 July 2017; Accepted 16 August 2017
Available online 09 September 2017
0165-7836/ © 2017 Elsevier B.V. All rights reserved.
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).

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 (H2O2) for 15 min to remove any remaining biological residue. Thereafter, otoliths were immersed in ultrapure 1% nitric acid (HNO3) 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 HNO3 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/Δm = 4000) to avoid spectral interferences, particularly on Ni. The instrument was equipped with a microflow nebulizer (PFAAR35-1-CIE, Glass Expansion), operated in the self-aspirating mode (sample uptake rate 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, 115In was added at a known concentration to all samples and standards as an internal standard to adjust for instrument
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 HNO₃.

A preliminary analysis was made to determine the most likely abundant and informative elements (⁸⁸Sr, ⁷Li, ²⁴Mg, ⁵⁵Mn, ¹³⁸Ba, ²⁰⁸Pb, ⁵⁴Fe, ⁶⁶Cu and ⁶⁰Ni; ⁴⁳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 µg L⁻¹): ⁴⁳Ca (4000), ⁸⁸Sr (400), ⁷Li (1), ²⁴Mg (10), ⁵⁵Mn (0.30), ¹³⁸Ba (1), ²⁰⁸Pb (0.5), ⁵⁴Fe (10), ⁶⁶Cu (2) and ⁶⁰Ni (4).

### 2.3. Otolith isotopic analyses

Whole left otoliths were used for isotopic analysis by isotope ratio mass spectrometry (IRMS). For carbon (δ¹³C) and oxygen (δ¹⁸O) isotopic determinations, carbon dioxide (CO₂) 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 CO₂ was analysed for ¹³C and ¹⁸O 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) -19, 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 ⁶⁶Cu and ⁶⁰Ni 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 ⁵⁵Mn and ²⁴Mg 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 (ANOVA 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 T² square test. The multi-elemental composition of otoliths was analysed with a quadratic discriminant function analysis (QDFA). Classification accuracies of the discriminant

| Table 1: Sampling locations (sampling sites), sizes (weight and otolith mass), elemental (µg element g⁻¹ calcium) and isotopic (‰) signatures of *T. picturatus* original elemental concentrations and isotopic signatures. Mean value ± SE. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Regions | Sites | Sampling Location | n | tL (cm) | W (g) | Sr/Ca | Li/Ca | Mg/Ca | Mn/Ca | Ba/Ca | Pb/Ca | Fe/Ca |
| Azores | | | 20 | 26.4 ± 0.3 | 160.6 ± 5.6 | 7.449 ± 0.2 | 121.8 ± 15.8 | 0.08 | 16.09 | 0.68 | 0.53 | 0.02 | 27.89 | 1.22 | 3.46 | 1.17 | 7.41 | 0.65 |
| Madeira | | | 20 | 26.2 ± 0.2 | 193.5 ± 2.8 | 7.352 ± 0.4 | 49 | 2.57 | 0.11 | 16.09 | 0.68 | 0.53 | 0.02 | 27.89 | 1.22 | 3.46 | 1.17 | 7.41 | 0.65 |
| Canaries | | | 20 | 25.9 ± 0.3 | 180.6 ± 5.2 | 5.762 ± 0.3 | 74 | 1.79 ± 0.2 | 0.08 | 21.05 | 0.83 | 0.60 | 0.03 | 27.89 | 1.22 | 3.46 | 1.17 | 7.41 | 0.65 |
| Portugal | Mainland | | 20 | 26.4 ± 0.2 | 174.5 ± 3.9 | 7.296 ± 0.4 | 3.9 | 2.16 | 0.16 | 17.02 | 1.11 | 0.52 | 0.04 | 61.72 | 5.92 | 1.41 | 0.28 | 9.91 | 1.00 |
| | Peniche | | 20 | 26.9 ± 0.2 | 178.4 ± 3.2 | 7.638 ± 0.4 | 3.2 | 1.94 | 0.16 | 17.02 | 1.11 | 0.52 | 0.04 | 61.72 | 5.92 | 1.41 | 0.28 | 9.91 | 1.00 |

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.
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 ± standard errors (SE).

3. Results

88Sr and 138Ba concentrations presented positive relationships with otolith mass (n = 120, r² = 0.705, p < 0.05; n = 120, r² = 0.57, p < 0.05, respectively) (Fig. 2A and B). No significant correlation was found for δ13C and δ18O (Fig. 2C and F). The 24Mg (n = 120, r² = 0.443, p < 0.05, Fig. 2D), the 208Pb (n = 120, r² = 0.313, p < 0.05) (Fig. 2E) and the 54Mn (n = 120, r² = 0.308, p < 0.05) (Fig. 2G) presented negative relationships.

δ13C showed no significant relationship with otolith mass (ANOVA, p > 0.05) (Fig. 2H) and 40% of the sum of squares was explained by the location (Table 2). The δ18O presented a positive relationship with otolith mass (ANOVA, p < 0.05) and 37% of the sum of squares was explained by location (Fig. 2I). Although otolith δ13C was significant for δ18O 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 δ13C and from 0.24‰ to 2.35‰ for δ18O. The isotopic ratios showed significant differences between the oceanic islands and Portuguese mainland for δ13C (Tukey tests, p < 0.05) (Fig. 3H) and between the Azores/Portugal mainland and Madeira/Canary islands for δ18O (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, δ13C and δ18O) indicated significant differences among all locations (Pillai’s Trace, F5,5,59 = 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 (F9,106 = 2.80) and Peniche and Madeira (F9,106 = 56.41), showed the lowest and highest Mahalanobis distances (Between Group F-Matrix), respectively.

QFD 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, δ13C and δ18O 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 δ13C. 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; Elsdon 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; Elsdon and Gillanders, 2002; Martin et al., 2004) and it is well known the existing positive correlation between otolith Sr/Ca and water salinity (Bath et al., 2000; Secor and Rooker, 2000; Elsdon 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

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F-ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>δ13C Location</td>
<td>5</td>
<td>22.224</td>
<td>4.445</td>
<td>14.843</td>
<td>0.001</td>
</tr>
<tr>
<td>Otolith Mass</td>
<td>1</td>
<td>0.308</td>
<td>0.308</td>
<td>1.029</td>
<td>0.313</td>
</tr>
<tr>
<td>Error</td>
<td>119</td>
<td>33.838</td>
<td>0.299</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>56.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ18O Location</td>
<td>5</td>
<td>5.462</td>
<td>1.092</td>
<td>14.240</td>
<td>0.001</td>
</tr>
<tr>
<td>Otolith Mass</td>
<td>1</td>
<td>0.457</td>
<td>0.457</td>
<td>5.962</td>
<td>0.016</td>
</tr>
<tr>
<td>Error</td>
<td>113</td>
<td>8.670</td>
<td>0.077</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>14.589</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 3. Element/Ca and isotopic concentrations (Mean ± 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 μg 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 δ¹⁸O.
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
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, $\delta^{18}O$ 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 $\delta^{18}O$ being highly influenced by temperature and salinity (Gao et al., 2001; Hsie et al., 2004; Correia et al., 2011a). Assuming that in the study area the seawater $\delta^{18}O$ composition is homogenous, lower values of $\delta^{18}O$ would mean higher temperatures (Ashford and Jones, 2007). In fact, the $\delta^{18}O$ 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 $\delta^{18}O$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ (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 (Fíuza, 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 $\delta^{13}C$ 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, $\delta^{18}O$ and $\delta^{13}C$ 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

### Table 3

<table>
<thead>
<tr>
<th>Predicted Location</th>
<th>Azores</th>
<th>Canary</th>
<th>Madeira</th>
<th>Matosinhos</th>
<th>Peniche</th>
<th>Portimão</th>
<th>% Correct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Real Location</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azores</td>
<td>19</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>Canary</td>
<td>1</td>
<td>18</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>90</td>
</tr>
<tr>
<td>Madeira</td>
<td>1</td>
<td>2</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>85</td>
</tr>
<tr>
<td>Matosinhos</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>13</td>
<td>3</td>
<td>3</td>
<td>65</td>
</tr>
<tr>
<td>Peniche</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>14</td>
<td>1</td>
<td>70</td>
</tr>
<tr>
<td>Portimão</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>22</td>
<td>17</td>
<td>22</td>
<td>18</td>
<td>20</td>
<td>81</td>
</tr>
</tbody>
</table>
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 (Shaboneyev 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 Canaries 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 population 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 fishes’ 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 find 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.

Acknowledgments

This research was partially supported by the NORTE-01-0145-FEDER-000035 (MARINFO) and the Strategic Funding UID/Multi/04423/2013 through national funds provided by Foundation for Science and Technology (FCT) and European Regional Development Fund (ERDF), in the framework of the programme PT2020. Cláudia Moreira has also a PhD scholarship from FCT (SFRH/BD/111133/2015). The authors also wish to thank Ana Paixão, João Delgado and Sara Sanz for the assistance in the fish acquisition and S. Sara Sanz for the assistance in the fish sampling. Moreira has also a PhD scholarship from FCT (SFRH/BD/111133/2016). The authors also wish to thank Ana Paixão, João Delgado and Sara Sanz for the assistance in the fish sampling. Moreira has also a PhD scholarship from FCT (SFRH/BD/111133/2016). The authors also wish to thank Ana Paixão, João Delgado and Sara Sanz for the assistance in the fish sampling.