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Population structure, movement patterns and habitat connectivity of the blue jack mackerel, *Trachurus picturatus*, in the NE Atlantic: otolith morpho-chemistry, body shape and genetic markers as combined tools for a rational management of fisheries resources

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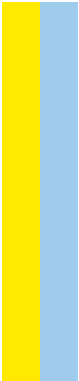


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Declaro que a presente tese é de minha autoria e não foi utilizada previamente noutro curso ou unidade curricular, desta ou de outra instituição. As referências a outros autores (afirmações, ideias, pensamentos) respeitam escrupulosamente as regras da atribuição, e encontram-se devidamente indicadas no texto e nas referências bibliográficas, de acordo com as normas de referência. Tenho consciência de que a prática de plágio e auto-plágio constitui um ilícito académico.

This thesis is composed of six research articles, already published or submitted to be published in peer-reviewed indexed international journals. Since these articles were prepared in collaboration with other authors, I declare that I have led the writing of all the chapters and:

1. In the first and second articles, concerning the molecular analyses, I participated in the fish acquisition, sampling of the specimens, DNA extraction and PCR amplification of the mitochondrial and nuclear markers, treatment and analyses of the data and discussion of the results.
2. In the third and fourth articles, concerning the morphometric analyses, I participated in the fish acquisition, sampling of the specimens, retrieving the measurements of both the body and otoliths, treatment and analyses of the data and discussion of the results.
3. In the fourth and fifth articles, concerning the chemical analyses, I participated in the fish acquisition, sampling of the specimens, preparation of the otoliths for the chemical analysis, treatment and analyses of the data and discussion of the results.

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Resumo

Dados científicos mostram que actualmente várias espécies de peixes são exploradas além do seu limite máximo sustentável, levando a situações de sobrepesca, declínio e esgotamento dos recursos haliêuticos. Para efectuar uma gestão sustentável dos recursos pesqueiros é necessário conhecer para cada espécie a estrutura e dinâmica populacional, padrões de movimento para a postura e alimentação, e conectividade entre as zonas de nascimento/berçário e as áreas de crescimento. Os peixes marinhos são frequentemente estruturados em metapopulações conectadas pela troca de indivíduos na fase inicial de vida, ou aquando juvenis e/ou adultos. É provável que essas subpopulações possuam diferentes características de vida e diferentes características ecológicas e demográficas, mostrando diversidade genética e plasticidade fenotípica e exibindo diferentes formas de lidar com a variabilidade ambiental e a pressão da pesca. Perceber como uma espécie se comporta ao longo do seu ciclo de vida, a dinâmica e as interações entre as populações, irá permitir que os responsáveis pela gestão das pescas verifiquem o impacto que a exploração pesqueira exerce sobre a população e antecipem medidas de gestão eficientes para garantir a exploração sustentável dos stocks pesqueiros. O carapau negrão, *Trachurus picturatus*, é um peixe pelágico migratório, amplamente distribuído pelo Atlântico Nordeste, Mar Mediterrâneo e Mar Negro. Na região Macaronésia dos Açores, Madeira e Canárias esta espécie representa um recurso económico muito importante, sendo uma das espécies de peixes pelágicos mais importantes em termos de capturas. Apesar da sua importância comercial na região, dados científicos sobre esta espécie são, à data, limitados. De facto, essa falta de informação faz com que as organizações nacionais e internacionais, que regulam a actividade pesqueira, considerem que apenas existe uma única unidade populacional em toda a sua distribuição geográfica. No entanto, estudos científicos publicados anteriormente indicam que algumas características do ciclo de vida da espécie, como a taxa de crescimento, idade de primeira maturação e época de reprodução, podem variar regionalmente, o que sugere a existência de diferentes unidades populacionais ao longo da sua distribuição. Além disso, nos últimos anos tem-se verificado uma diminuição nas capturas de *T. picturatus* no Atlântico Nordeste. Este fenómeno pode estar relacionado com oscilações naturais na abundância da espécie e/ou com diferentes taxas de recrutamento. No entanto, a falta de estudos sobre a dinâmica populacional da espécie, estrutura do(s) stock(s), padrões de movimento e conectividade limita a compreensão da sua situação actual. No sentido de colmatar esta lacuna, este trabalho adoptou uma aproximação holística, aplicando três metodologias distintas: análises moleculares, análises morfológicas e análise química de otólitos. A aplicação de marcadores moleculares na Ciência Pesqueira pode ser particularmente útil, uma vez que estes marcadores podem ser aplicados para detectar uma ampla gama de padrões genéticos, como

por exemplo, padrões de isolamento reprodutivo entre espécies. No geral, os resultados obtidos mostram uma ausência de estrutura genética ao longo da área de distribuição geográfica da espécie, com uma elevada conservação espaço-temporal da diversidade genética e altos níveis de endogamia entre as zonas de pesca amostradas. Outra abordagem focou a análise morfológica dos indivíduos. Variações genéticas ou a exposição a diferentes factores ambientais, como temperatura e salinidade, disponibilidade de alimento ou padrões de natação, podem levar a adopção de diferentes estratégias de sobrevivência, dando origem a características morfológicas corporais diferentes entre as populações. No geral, os resultados deste trabalho sugerem que a população de *T. picturatus* no Atlântico Nordeste é fenotipicamente heterogênea no que diz respeito à morfometria corporal. Finalmente, técnicas baseadas em otólitos também provaram ser uma abordagem eficaz para analisar a estrutura populacional de espécies altamente migratórias como o *T. picturatus*, uma vez que os otólitos têm a capacidade de registrar as assinaturas morfológicas e químicas dos peixes ao longo da sua vida. Diferenças geográficas nas propriedades físico-químicas da água, assim como diferentes dietas, podem levar ao desenvolvimento de otólitos com formas morfométricas e composições químicas distintas, o que permite a discriminação entre indivíduos que cresceram expostos a diferentes condições ambientais. No geral, esta abordagem mostrou que a espécie apresenta otólitos com formas e assinaturas químicas distintas, o que sugere a existência de diferentes unidades populacionais ao longo da distribuição da espécie.

Concluindo, os resultados deste trabalho sugerem que a espécie *T. picturatus* no Atlântico Nordeste é formada por uma estrutura metapopulacional, e que esta é dinâmica ao longo do tempo. Os resultados obtidos sugerem que as estratégias de gestão da espécie devem incluir planos de acompanhamento das *cohorts* anuais nas principais zonas de pesca, e enfatizam a necessidade da aplicação de abordagens multidisciplinares para o estudo de *stocks* pesqueiros. Além disso, a complexa estrutura espacial observada nesta espécie recomenda que o recurso seja gerido regionalmente, de forma a poder fazê-lo de uma forma mais racional e sustentável.

Palavras-chave: Gestão de pescas, metapopulação, estrutura dos *stocks*, peixes pelágicos, abordagem multidisciplinar, *stocks* genéticos, *stocks* fenotípicos.

Abstract

Up-to-date scientific information shows that several fish species are currently exploited beyond their maximum sustainable threshold, leading to situations of over-exploitation and depletion of fish stocks. Sustainable management of fisheries resources requires accurate knowledge of the species' population structure and dynamics, spawning and feeding movement patterns, and connectivity between spawning/nurseries and growing regions. Marine fishes are often structured in metapopulations connected by exchange of early life stages, juveniles and/or adults. These subpopulations are likely to have different life traits and demographic characteristics, showing genetic diversity and phenotypic plasticity, and displaying different ways to cope with environmental variability and fisheries pressure. Knowing how a species behaves across its life cycle, and the dynamic and interactions among the populations will allow experts to extrapolate on the impact fisheries have on a population and to forecast efficient management measures to ensure the sustainable exploitation of fish stock. The blue jack mackerel, *Trachurus picturatus*, is a pelagic migratory fish widely distributed across the North-East Atlantic, Mediterranean and Black Seas. In the Macaronesian region of the Azores, Madeira and Canary Islands this species represents a very important economic resource, being one of the most important small pelagic fish harvested. Despite the economic importance of the species, scientific information across its distribution is still limited. In fact, this lack of knowledge leads the national and intergovernmental fisheries science organizations to consider the existence of only one stock across the entire geographic distribution. However, previously published studies indicate that some life-history traits, such as growth rate, age at first maturity and reproductive season, could vary regionally, which suggests the existence of different population units across its distribution. Furthermore, over the last years, there has been a decrease in the captures of *T. picturatus* in the North-East Atlantic. This phenomenon may be related to natural variations in abundance and/or different rates of recruitment. Nevertheless, the lack of studies focusing on the species population dynamics, stock structure, movement and connectivity limit the full understanding of the species present situation. To improve on this gap, a holistic approach to study the population structure of this species was employed by applying different techniques – molecular analyses, morphological analyses and otolith chemical analyses. The application of molecular markers to Fisheries Science can be particularly helpful, since they can be applied to detect an ample range of genetic patterns, e.g. from inter-individual fingerprints to reproductive isolation between species. Overall, the hereby results showed no genetic structure across the species distribution, with high spatio-temporal conservation of genetic diversity and high levels of inbreeding among the sampled fishing grounds. Moreover, another approach focused on the

morphological study of individuals. Genetic variation from different origins or exposure to different environmental factors, such as temperature and salinity, food availability or swimming patterns, can result in individuals following different adaptation strategies, and consequently develop different morphometric features between populations. Overall, results from this work suggested that the population of *T. picturatus* in the North-East Atlantic is phenotypic heterogeneous in terms of body morphometry. Finally, otolith based techniques have also proven to be an effective approach to assess the population structure in species with high migratory rates, such as *T. picturatus*, since otoliths have the ability to record shape and chemical signatures across the life history of the fish. The presence of geographic variation in physicochemical properties of the water, as well as differences in diet regimes, can result in changes in the otolith shape and composition, helping to discriminate among fish that grew up exposed to different environments. Overall, the techniques applied in this work showed that the species has different otolith shapes and chemical signatures suggesting the existence of different population units across the species distribution.

In conclusion, the outcome of this work suggests that *T. picturatus* shows a metapopulation structure in the NE Atlantic and that it is dynamic over time. The results obtained recommend that management strategies include year-classes follow-up of the population units in the main fishing areas and stress the need for holistic methodological approaches for the study of fish stocks. Furthermore, the hereby observed complex spatial dynamic structure of the species demands regional-specific requirement actions to ensure the rational management of the fishery.

Keywords: Fisheries management, metapopulation, stock structure, pelagic fish, multidisciplinary approach, genotypic stocks, phenotypic stocks.

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Objectives

The main goal of this work was to depict if the North-East Atlantic population of *Trachurus picturatus* is a single and homogeneous stock as it is described by the International Council for the Exploration of the Sea (ICES). To do so, indispensable information about the population structure and dynamics, stock(s) identification and delineation, habitat connectivity and migration patterns of the species across the North-East (NE) Atlantic was gathered. Therefore, the objective was to answer the following questions:

1. What is the population structure of the species in the NE Atlantic?
2. Does a panmictic or a metapopulation model fits this species?
3. Are there multiple spawning/nursery/replenishment grounds for this species?
4. What are the implications of this work for the species stock assessment and fisheries management?

Thesis Organization

The first chapter of this thesis (Chapter 1: General Introduction) is focused on explaining why is the management of fisheries resources of extreme importance and why fisheries science is essential for the assessment and identification of fish stocks. The state of the world fisheries is presented, followed by the definition of some important concepts, such as the concept of metapopulation and unit stock, the different components of the stock and the methodologies adopted to identify them.

The main body of this thesis starts by analysing the genetic composition of the species - Chapter 2: Molecular Analyses. Previously, there was no information available regarding the genetic diversity of the species across its western distribution range: Macaronesia (*i.e.*, Azores, Madeira and Canaries) and mainland Portugal. Therefore, the genetic diversity, phylogeography and past molecular demographic signatures of each of the populations in this area, analysed together with the already available data (mtDNA markers) for the Mediterranean Sea, was characterized. To further unravel the population structure and migration dynamics among the different fishing grounds, microsatellites were used for the first time in this species. This allowed us to test the levels of inbreeding and genetic differentiation across the sampled fishing grounds and to infer possible migration patterns.

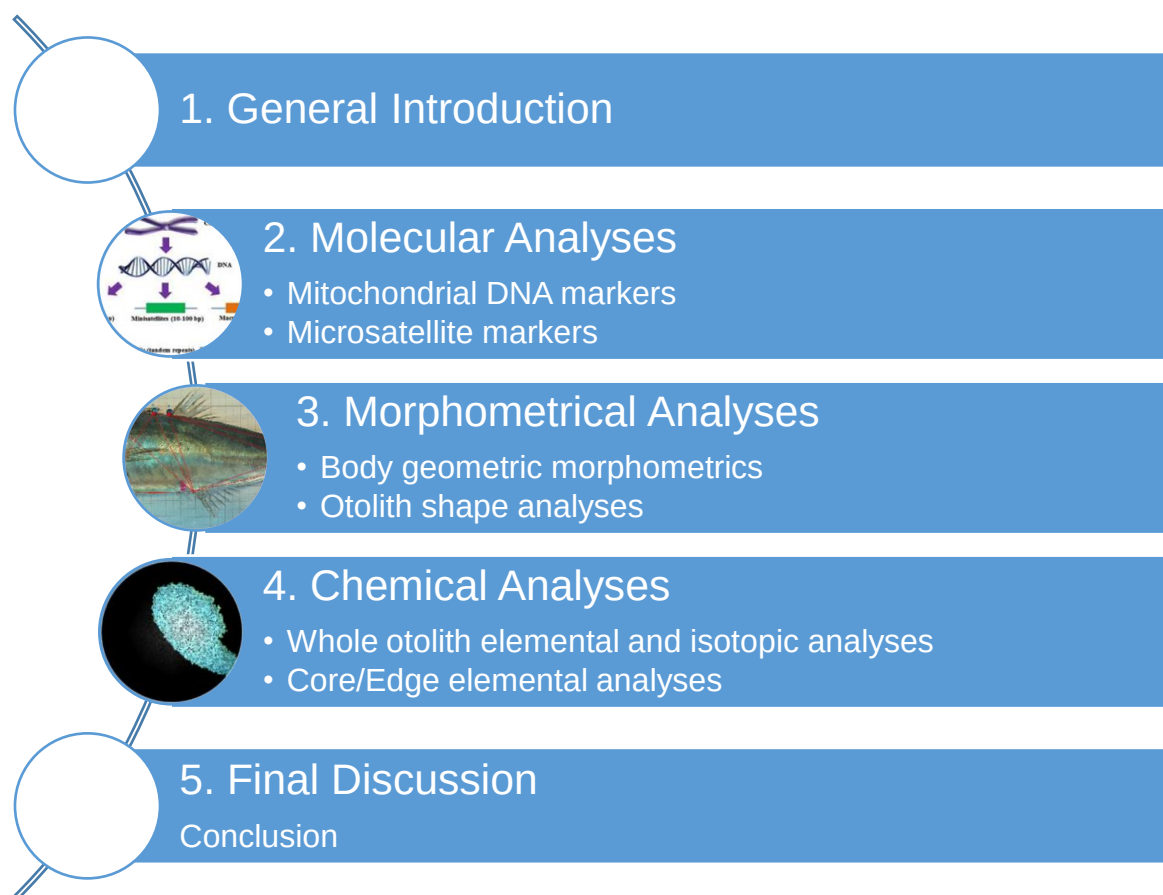
The molecular analyses were followed by the morphological characterization of the species - Chapter 3: Morphological Analyses. Different genetic input and/or exposure to

different environmental conditions can lead to the expression of different features both in the fish body and otolith shape. Specimens and otoliths were characterized across space and time to infer on the population structure of the species, using box truss-measurements (body morphometrics) and shape indices and elliptical Fourier descriptors (for otolith shape analysis).

Finally, the chemical (elements and isotopic ratios) composition of the whole otoliths and otolith's sections (core and edge) were assessed - Chapter 4: Chemical Analyses. The chemical deposition in the otoliths is highly influenced by the environmental properties of the water masses to which the individuals are exposed. Analysing the entire life-history prior to capture (*i.e.*, whole otolith) allowed to determine whether distinct elemental (Element:Ca) and isotopic ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) signatures were regionally present. Additionally, by examining the elemental composition of the otoliths' core (natal origin) and edge (capture origin) of fish belonging to different cohorts and regions, allowed to infer the composition of the natal origin and possible migration patterns.

In the last chapter (Chapter 5: Final Discussion), the hereby results gathered using different natural tags are discussed in the light of the species' current ecological knowledge and fishing situation across the North-East Atlantic and further suggestions to the sustainable exploitation of the *T. picturatus* populations are highlighted.

Organization scheme



Chapter 1

GENERAL INTRODUCTION

*“... to obtain the BEST POSSIBLE utilization of the resource
for the benefit of the COMMUNITY”*

Sætersdal, 1984

1.1 - Overview of the world fisheries

Fishing efforts are known to have a high impact on marine ecosystems, causing the depletion of fish populations and, consequently, disruption of important trophic levels. Not only excessive fishing has reduced stocks to dangerously levels, but the size of individual fishes caught is becoming smaller (King, 2007; Hilborn et al., 2015; Leitão, 2019). While overfishing has been associated with the depletion of a small number of species, e.g. the giant clams in the Pacific Islands (King, 2007), the Pacific salmon in some river systems (King, 2007) or the common angleshark in the Northern Adriatic Sea (Fortibuoni et al., 2016), there are reports of many fisheries that have collapsed to undesirably low levels, e.g. the Atlantic horse mackerel *Trachurus trachurus* (FAO, 2018), or the sardine *Sardinops sagax* (Nilsson et al., 2019). In 2016, the world total marine catch was 79 million tonnes, a decrease when compared to the 81 million tonnes registered in 2015 (FAO, 2018). Moreover, the last worldwide assessment on fisheries suggests that 60% of the fish stocks are at the maximum sustainable yield, with 33% being exploited at biologically unsustainable levels and only 7% are considered underfished (Figure 1; FAO, 2018).

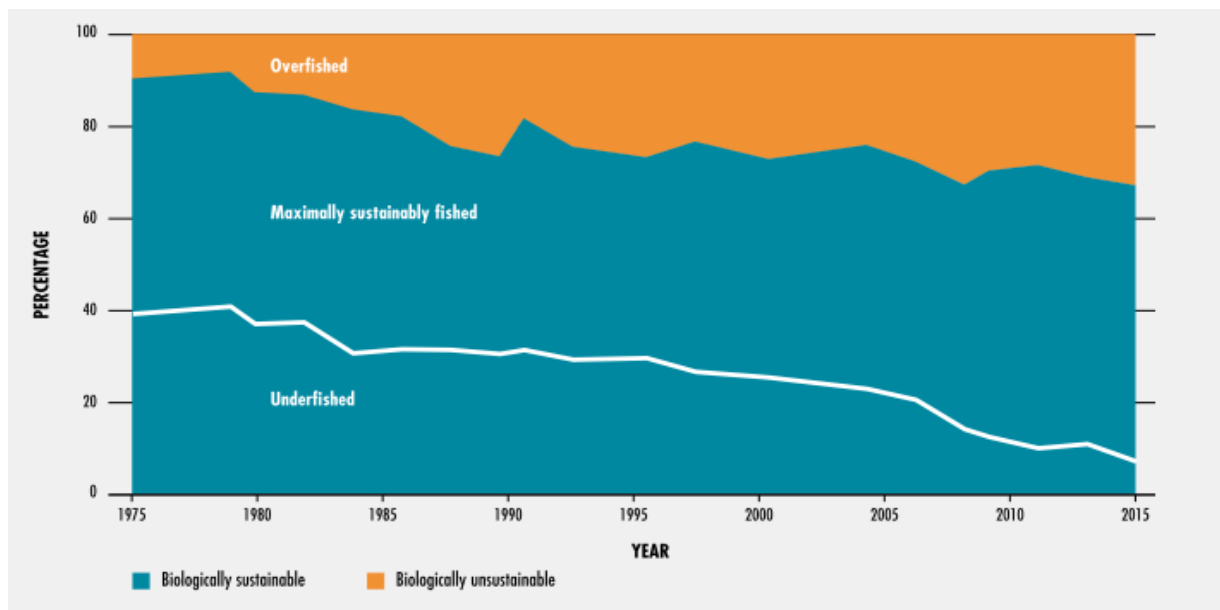


Figure 1: Global trends in the state of the world's marine fish stocks from 1974 to 2015 (FAO, 2018) (license CC BY-NC-SA 3.0 IGO).

In the light of the decreasing number of catches and increasing threatened marine ecosystems, there is a need for responsible fisheries management to ensure the sustainability of aquatic resources, by considering the target species interdependence, other species and

the marine environment (Reiss et al., 2009; FAO, 2018; Link and Watson, 2019). Cases where the implementation of appropriate management actions helped to improve the fishing resource have been reported for some important species in the North-East (NE) Atlantic. The stocks of cod *Gadus morhua* and hake *Merluccius merluccius* have seen a reduction in fishing mortality since recovery plans were set to action (FAO, 2018). But still, there is an abundant number of fish species in need of action plans to ensure their sustainability (Figure 2).

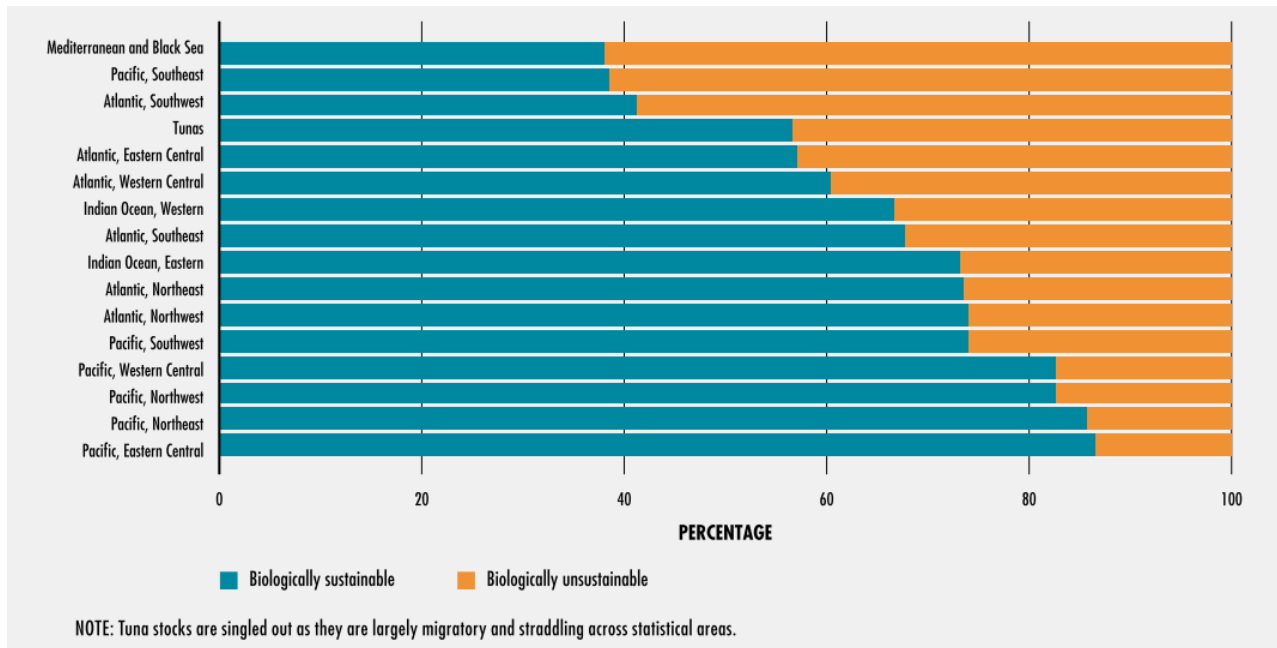


Figure 2: Percentage of stocks fished at biologically sustainable and unsustainable levels by FAO statistical area in 2015 (FAO, 2018; licence CC BY-NC-SA 3.0 IGO).

Depletion of fish stocks is the consequence of several factors, namely the increasing demand of fish for human consumption but also to the growing aquaculture industry. These demands lead to the improvement in the fishing industry and technology to fish longer, harder and farther away from home, contributing to the massive intensification of pressure on fish populations (Tidwell and Allan, 2001). In 2016, a total of 171 million tonnes of fish were captured (including wild and aquaculture production). Of these, 151 million tonnes went directly for human consumption, as a high nutritional value food source, while 15 million tonnes went to the aquaculture industry for the production of fishmeal and fish oil. The remaining 5 million tonnes were used for the direct feeding in aquaculture and raising livestock, as bait, in pharmaceutical uses and for ornamental purposes (FAO, 2018). Moreover, catches are not the only factor that contributes to the stock decline. The fishing gears used inflict extreme changes in the fish's habitats, e.g. destruction of the sea beds by bottom contact gears

(Chuenpagdee et al., 2003). Furthermore, fishing gears are selective for fish size and age groups, which implies changes in the population dynamics, leading to shifts in the fish maturation, reproductive, and recruitment ages and rates (Berkeley et al., 2004; Hsieh et al., 2010; Vasconcelos, 2017). Additionally, these selection pressures can lead to genetic modifications in the populations (Mee et al., 2017; Pauly and Zeller, 2019), as noted for the northern cod *Gadus morhua* (Olsen et al., 2004), two tuna species (skipjack tuna vs. Atlantic bluefin tuna; Mee et al., 2017) and the reef fish *Chrysoblephus laticeps* (Duncan et al., 2019).

It is of extreme importance to gather efforts to improve the correct assessment of fish stocks. Nevertheless, this assessment may be difficult as many fish stocks are composed of species with metapopulation structures.

1.2 - The concept of metapopulation

The concept of metapopulation was first applied by Richard Levins (1969) to terrestrial ecology, where it defined a species survival in balance between local extinctions and recolonizations of currently unoccupied habitat patches (Levins, 1969). Since then, the concept has developed considerably. However, it was only in the early 90's that the theory was transposed to aquatic ecology, and later to marine systems (in the mid '90s) (Sale et al., 2006; Gaggiotti, 2017). This concept widening was probably due to an increasing scientific concern with marine environmental management and conservation (Grimm et al., 2003; Sale et al., 2006), when marine research shifted from questions related to within patch dynamics as a result of isolation to within patch dynamics influenced by replenishment from external sources, which intensifies the application of the metapopulation theory to marine resources (Kritzer and Sale, 2006).

In marine research, the metapopulation concept is fundamentally about ecology at large scales, considering also the spatial arrangement and relationships among local populations (Sale et al., 2006), being defined as:

“a system in which a local population inhabit discrete habitat patches and inter-patch dispersal is neither so low as to negate significant demographic connectivity nor so high as to eliminate any independence of local population dynamics” (Sale et al., 2006).

According to this definition, a metapopulation is a spatial structure set of populations that exhibit some degree of independence among them and are also connected to each other by the dispersal/migration of individuals. Nevertheless, these movements cannot be so strong as to interfere with the dynamics of the receiving population (Figure 3). Therefore, migration is

enough to perpetuate the same gene pool, in such a way that true population isolation is not possible. Several examples of low population genetic differentiation across large water bodies (e.g. the Atlantic Ocean) have been reported for different taxa, including gastropods (De Wolf et al., 2000; Lourenço et al., 2017; Wort et al., 2019), crustaceans (Faria et al., 2013; Quinteiro et al., 2015; Pascual et al., 2016) and fishes (Francisco et al., 2011; Stefanni et al., 2015; Caiafa Hernandez et al., 2017). On the other hand, examples of significant genetic differentiation among populations across the North-East Atlantic have also been reported for gastropods (Sá-Pinto et al., 2008; Marzouk et al., 2017) and fishes (Francisco et al., 2009; González-Wangüemert et al., 2010; Almada et al., 2017).

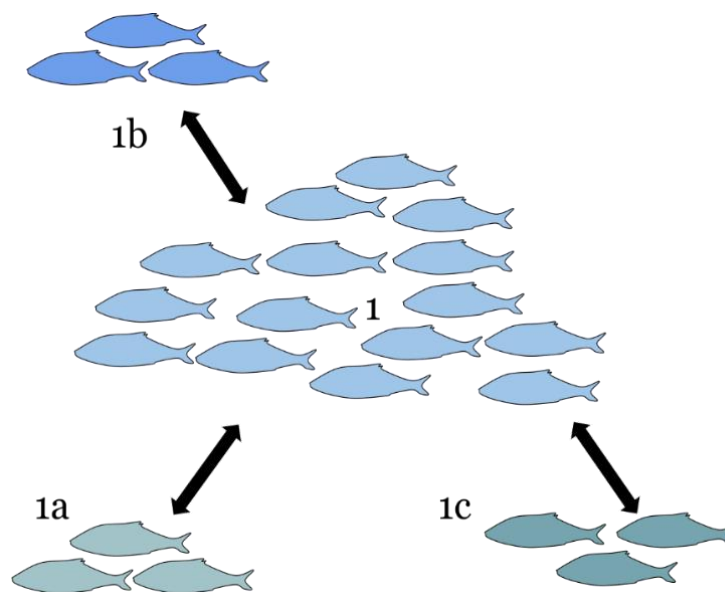


Figure 3: Metapopulations diagram. The local population (1) works as a source for hybridization with surrounding populations (1a, 1b and 1c).

(By Kjspencer12 - Own work, CC BY-SA 4.0, <https://commons.wikimedia.org/w/index.php?curid=69870372>).

The application of the metapopulation theory to marine populations depends essentially on the physical structure of the habitats. However, one of the most distinctive features of the ocean is its vastness, continuous motion and absence of physical barriers. Nevertheless, anthropogenic actions in marine habitats generally result in the intermission, degradation and homogenization of habitats across broad areas, either by coastal pollution, commercial trawling or overfishing. Furthermore, either by the influence of ocean currents, the occurrence of upwelling zones, habitat discontinuities and ecotones between areas with different environmental properties or areas with historical sea level drops (e.g. Stefanni et al., 2015) individuals' free dispersal can be limited or stopped. One example where the physical structure of the habitat can result in the establishment of metapopulations is found in the Macaronesia

region (North-East Atlantic). The specific environmental features of this region, *i.e.* the influence of different oceanic currents, upwelling systems and environmental gradients (*e.g.* temperature and salinity) seem to promote the maintenance of discrete subpopulations of, for example, fishes (Francisco et al., 2009; González-Wangüemert et al., 2010; Almada et al., 2017).

1.3 - The concept of unit stock

Fish stocks represent the exploitable part of the fish population, for which intrinsic parameters, such as reproductive rate, growth, recruitment, natural mortality and fishing mortality, can be estimated (Secor, 2005). Intrinsically, stocks are not ecological entities and, generally, are operationally defined by the geographic extension of anthropogenic effects that act on the population (Hare and Richardson, 2013). In practical terms, a fish stock is a distinct, reproductively isolated population that inhabits within a specific spatial range. Therefore, a particular species may occur within a large geographic area as several unit stocks. Nonetheless, and regardless of the operational definition, there is little doubt that the species population structure is vital to assess and manage resources, and different levels of biological structure can be considered for stock assessment (Figure 4; Secor, 2005).

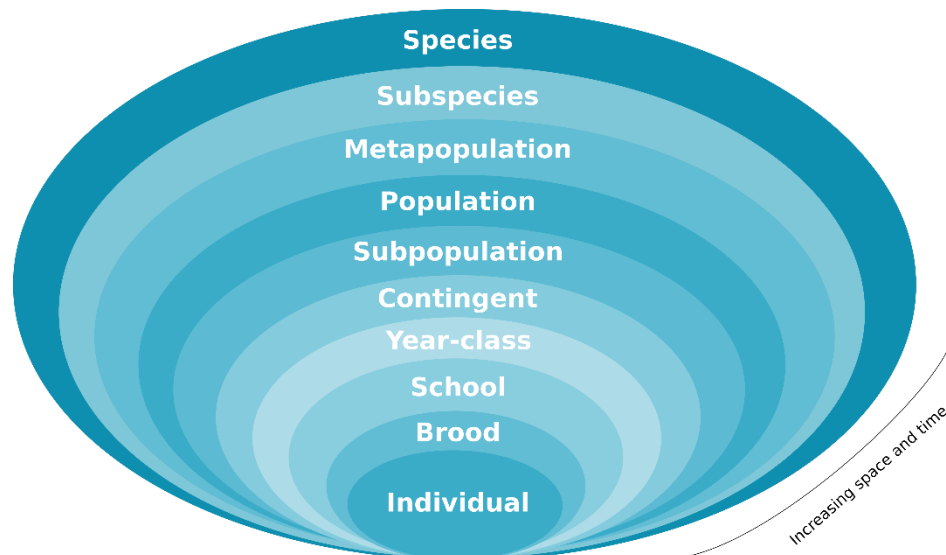


Figure 4: Levels of ecological organization relevant to the unit of stock (diagram concept adapted from Secor, 2014; courtesy of Patrícia Ferreira).

Overharvesting of fish stocks is usually associated with an erroneous choice of management areas (Reiss et al., 2009). Since the spatial delineation of stocks is often based on stationary fish distributions, oceanographic features, and political boundaries or administrative interests (e.g. Pita et al., 2016), the management areas often do not match the biological meaningful entities (Waples and Gaggiotti, 2006; Reiss et al., 2009; Hawkins et al., 2016). Moreover, frequently, factors such as immigration and/or emigration are not considered in stock assessments (Secor, 2005). From a fisheries assessment and management point of view, it is important to determine if two adjacent stocks are sufficiently interactive to be regarded as a single stock or independent enough to be treated as separate stocks (King, 2007).

The concept of fish stock implies that a group of individuals has the same gene pool, is self-perpetuating, and has little contact with adjacent groups of the same species (FAO, 1997). In practical terms, the composition of a fishery stock is, essentially, the result of the recruitment, birth and mortality rate of the species in question (King, 2007). However, what often seems to be an isolated population, might receive recruits from other distant population(s), or, in species with high larval pelagic duration, the larvae can drift and disperse for long periods, thus reaching other distant populations, making the boundaries of the stock very difficult to determine (Gaggiotti, 2017).

Understanding the life history patterns of a species is extremely important to assess fish stocks. However, these patterns are influenced by a complex array of selective forces imposed by the environment experienced by the individuals. Variability in quantitative traits can be originated by demographic, genetic, environmental differences or a combination of these. And any consistent difference between groups can be used to discriminate stocks.

The stock structure should not be seen as static. The natural environmental oscillations, verified in the different habitats, associated with the effect of climate change, imply that the stocks can and will change (Hare and Richardson, 2013; Gaggiotti, 2017). Not only environmental variability can affect stock structure by changing the distribution of individuals within the defined geographical area, but also the connectivity of individuals can change due to alterations in the migration pathways (Hare and Richardson, 2013; Gaggiotti, 2017). Thus, stock assessment needs to consider the evolution of the stocks through time, both in their structure and boundaries (Link et al., 2011). And, to conclude that there is only one stock when individual differences are not observed is a mistake. Failing to reject the null hypothesis does not mean that the hypothesis has been proven to hold true (Abaunza et al., 2013).

1.4 - Different stocks components

Any consistent difference among individuals can be used to discriminate stock units. However, most of the harvest fish stocks are usually composed of two or more populations, morphologically very similar, that overlap in space and time (Dahle et al., 2018). The sustainability of these fisheries depends on the dynamics of each stock. Failure to identify mix stocks and treat them as isolated units can lead to under or over-exploitation of the separate components of the fishery (Allendorf et al., 2008).

The basis for effective fishery management depends on answering three main questions. First, the identification of a group of individuals that share homogeneous internal dynamics and have little exchange of individuals with the surrounding groups; secondly, the geographical boundaries need to be established, including characterization of seasonal movements; and lastly, the stability of the group and their boundaries across time (Secor, 2014). Once these answers are known, the stock can be properly modelled and the managers can define and apply measures for a sustainable fishery.

A species life history patterns are determined by a set of complex selective forces that are applied by the environment. The variability in quantitative traits found among individuals can be originated by demographic, genetic, environmental differences or a combination of these (Begg and Waldman, 1999; Barria et al., 2014). Therefore, different stock units can be identified, accordingly to the different traits analysed. Usually, a fishery stock is defined by two methods: genotypic or phenotypic. A genotypic stock is defined as a group of interbreeding organisms that occupy more or less the same geographic region, while a phenotypic stock is defined as a group of individuals with distinct characteristics, developed due to environmental and/or genetic effects (Begg and Waldman, 1999). However, stocks can be identified by other features, for instance: geographical stocks, when only the species distribution range is considered; political stocks, when political, administrative and/or economic interests are considered; ecological stocks, when the environmental properties of the habitats occupied by the species are analysed; and contingent stocks, when the ontogenetic migrations behaviours are considered (Secor, 1999).

Nevertheless, genetic differences seem to represent a more permanent source of isolation than those originated by demographic or environmental factors, which could change rapidly in time (Cadrin et al., 2014). Nonetheless, whether genetic or phenotypic in origin, life-history traits are vital to stock assessment and or setting reference points (King, 2007).

1.5 - Tools to assess and identify stock units

In order to identify a stock, it is necessary to characterize the connectivity among populations *i.e.*, the exchange and dispersal of individuals among populations. However, migrations can occur at any life stage and can be driven by numerous factors. They can be either passive, with the potential for dispersal during the egg and larval stage, and/or intentional migrations of juveniles from nursery to growing areas and adult migration to feeding or spawning areas (Kerr and Goethel, 2013; Cadrin, 2020).

A number of tools can be used to gain more information for stock assessment, including tagging, geometric morphometrics, otolith chemistry, genetic analysis, studies on early life stages and planktonic dispersal, geostatistics and ecosystem approaches (Hare and Richardson, 2013; Cadrin et al., 2014; ICES, 2018a). These tools focus essentially on the geographic variation and dispersal of individuals, and infer the reproductive isolation or local adaptation, mixing rate and individuals' movement patterns and rates (Cadrin, 2020).

The most common tools applied to assess stock units, focus on the genetic and phenotypic characterization of the populations, leading to the identification of genotypic and phenotypic stocks (Cadrin, 2020). The variability observed in the genetic and phenotypic patterns and the magnitude of these patterns indicate the degree of mixing and/or isolation of fish in different areas.

1.5.1 - Molecular approaches to identify genotypic stocks

The identification of genotypic stocks implies that the populations are reproductively isolated. A number of physical and/or biological reasons may contribute to the establishment of genotypic stocks, including, for example, ocean currents, larval dispersal capabilities, natural selection and adaptation to different environments (Marshall et al., 2010). In fisheries science, both mitochondrial and nuclear genetic markers are commonly employed to infer the level of population structure within a species (Souza et al., 2006; Griffiths et al., 2010; Mansourkiaei et al., 2016).

Among the nuclear markers, allozymes were the first to be applied in fisheries science (Beaumont et al., 2010; Cadrin, 2020). Allozymes are the products of genetic variation at the enzyme-encoding loci and can be identified through the separation of proteins with different molecular sizes and electrical charges by electrophoresis (Okumus and Çiftci, 2003; Berta et al., 2006; Beaumont et al., 2010). These enzymes are associated with cellular functions and so their frequency is highly influenced by selective pressure, making them good markers to infer on the existence of different stocks (Cadrin, 2020). Allozymes were commonly applied in

stock identification, namely of *Trachurus trachurus* (Cimmaruta et al., 2008) and *Engraulis encrasicolus* (Erdoğan et al., 2009). However, these markers present some limitations. Quantification of allozymes needs large quantities of tissues and, after the animals' death, allozymes degrade rapidly (Cadrin, 2020). Moreover, the variation of allozymes is typically lower when compared to other genetic markers (Heist, 1998; Cadrin, 2020).

On the other hand, mitochondrial DNA (mtDNA) markers can be quantified from smaller amounts of tissue and the extracted DNA/mtDNA can be stored for long periods of time. These characteristics lead to a shift from allozymes to mtDNA (Selkoe et al., 2016). Several examples of the application of mtDNA for identification of genotypic stocks include the sardine *Sardina pilchardus* (Atarhouch et al., 2006) and the horse mackerel *Trachurus trachurus* (Comesaña et al., 2008). Moreover, mtDNA is usually maternally transmitted without molecular recombination, usually shows high mutation rates and reflects historical processes (e.g. Pleistocene glaciations) making it more sensitive to genetic drift and population bottlenecks than nuclear markers (Antoniou and Magoulas, 2013).

Another category of nuclear markers has been applied to the identification of genotypic stocks - microsatellites. Microsatellites, or simple sequence repeats, are small segments (1-6 base pair) of repetitive non-coding DNA, characterized by a high degree of length polymorphism (Zane et al., 2002; Li et al., 2018). The application of these markers to infer population genetic structure can provide thousands of independent markers that can be used to assess evolutionary history (Wallis et al., 2016). Moreover, due to the faster mutation rates microsatellites are more sensitive to differences and capable to detect genetic diversity when more conservative markers (e.g. mtDNA) cannot (Wallis et al., 2016). Furthermore, these nuclear markers are co-dominant, biparental inherited and highly variable which makes them good markers to investigate gene flow and genetic structuring over shallow timescales. Microsatellites have been applied to infer fish's population genetic structure for several species, namely the Ambiguous silver pomfret *Pampus argenteus* (Li et al., 2018), the smallmouth bass *Micropterus dolomieu* (Stepien et al., 2017) and the sea bass *Dicentrarchus labrax* (Guinand et al., 2017).

Another category of nuclear markers recently applied for stock identification is the single nucleotide polymorphisms (SNPs). SNPs are single base-pair substitutions and can be located in either coding or noncoding regions of the genome (Mariani and Bekkevold, 2013). Due to their features, SNPs can provide a much larger set of markers from a much lower number of individuals than microsatellites. SNPs provide a greater resolution for studying population genetic structure and are widely applied to infer the existence of genotypic stocks (Zarraonaindia et al., 2012; Rodríguez-Ezpeleta et al., 2016; Lemopoulos et al., 2019).

Even though mitochondrial and nuclear genetic markers can be employed to identify genotypic stocks, the combination of both markers, e.g. mtDNA and microsatellites, can be

very helpful. Additionally, since the markers provide information on the genetic diversity of populations at different time scales and resolutions (Stepien et al., 2017; Lemopoulos et al., 2019), they can be used to address complex processes, such as distribution shifts, population expansion in relation to climatic change or overfishing situations.

1.5.2 - Geometric morphometrics to identify phenotypic stocks

A phenotypic stock is recognized as a group of fish that maintain certain characteristics expressed in one or more ways depending on the type of environment to which they are exposed (Waldman, 2005). The expression of these characteristics can be affected by a number of biological processes, such as ontogenetic development, adaptation to environmental factors or long term evolutionary diversification. The different characteristics can be observed by variations in individuals (e.g. colour differences or body's elongation) or in small parts of the individual's body (e.g. size of the fins or maxilla) (Freshwater et al., 2019; Klingenberg, 2019). Those differences may indicate adaptations to different selective pressures or different responses to the same selective pressure (Donelson et al., 2019). Stable patterns of phenotypic variation can be valuable for stock identification, and one way to recognise these phenotypic variations is through the analyses of the individual's shape (Zelditch et al., 2004).

Different shapes and dissimilarities are perceived when objects are compared to familiar bodies, such as circles or squares. However, when shapes do not resemble familiar objects, comparisons can be vague and misleading (Cortese and Dyre, 1996). To overcome this issue, measurement approaches are applied (Cortese and Dyre, 1996; Zelditch et al., 2004). As a result, a new technique was applied to the field of shape analysis - morphometrics. Morphometrics is a quantitative way of addressing shape comparisons. It describes the shape of an object with a list of numbers or tables, so abstract that we cannot intuitively visualize them as descriptors of shape (Cortese and Dyre, 1996; Cabuga et al., 2019). The way information is extracted from morphometric data involves mathematical operations rather than biological concepts. The tools of geometric morphometrics offer a precise and accurate description of shapes, but also allow for the visualization and interpretation of differences amongst complex shapes (Zelditch et al., 2004).

Traditionally, morphometric data has been related to measurements of length, depth and width. However, these data have little information on the individual's shape and contain far less information than the ones that could be retrieved with the same measurement effort (Cortese and Dyre, 1996). Furthermore, some of the measurements are redundant, many times overlap, and information on the spatial relationship is missing. This method was greatly

improved with the introduction of the box-truss network scheme (Figure 5; Bookstein et al., 1985).

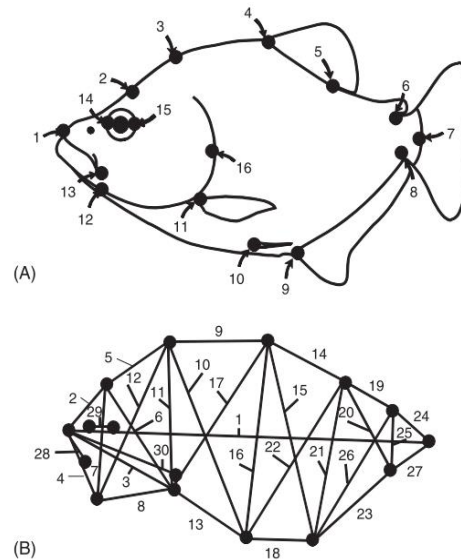


Figure 5: Box-truss network measurements of the external body form of a teleost fish. (A) well-defined landmarks of measurements, (B) the resulting 30 measurements (from Zelditch et al., 2004).

The box-truss measurements include more directions of the organism, and the lengths are more evenly spaced. Additionally, the endpoints of all measurements are biologically anatomical homologous between individual's - landmarks.

The application of geometric morphometrics has proved very useful to describe fish spatial distribution (Kaouèche et al., 2017). Several works have demonstrated morphological divergence in different regions of the fish body for several marine species (Hammami et al., 2013; Kaouèche et al., 2017; Vasconcelos et al., 2018b). The phenotypic variation within a fish species can occur as a result of isolation, environmental variations, thermal reaction, local adaptation and / or a combination of these (Vasconcelos et al., 2018b; Klingenberg, 2019). Exposure to different environments factors, such as temperature and salinity, food availability or swimming patterns, can result in individuals following different adaptation strategies, and consequently develop different morphometric features between populations (Sajina et al., 2011). Quantification of these specific characteristics in an individual or group of individuals can demonstrate the degree of speciation and contribute to the definition of different phenotypic stocks (Bailey, 1997).

1.5.3 - Otoliths to identify phenotypic stocks

Otoliths are regarded as natural tags because they grow continuously throughout life, remain chemically inert, and preserve an uninterrupted record of the environment where fish lived (Campana, 1999). Based on the ability of otoliths to record the life history of the fish, they are extensively used for fish biology studies and fisheries science. Among the applications, otoliths can be used for age estimation purposes (Campana, 2001; Carvalho et al., 2017; Soeth et al., 2019), examining the connectivity of fish populations (Correia et al., 2012a; Calò et al., 2013; Carvalho et al., 2017a), discriminating between fish stocks (Correia et al., 2011; Wang et al., 2018; Harris et al., 2019), tracking fish migration patterns (Elsdon et al., 2008; Reis-Santos et al., 2013; Austin et al., 2019); quantify natal homing behaviour (Avigliano et al., 2017; Prichard et al., 2018; Moll et al., 2019), and assessing larval retention (Jones et al., 2009; Swearer and Shima, 2010; Carvalho et al., 2017b). One of the major strength of this natural tags resides in identifying natal origins, which differentiates this method from other approaches when measuring connectivity, including coupled biophysical modelling and population genetics (Barnett-Johnson et al., 2010; Correia et al., 2012b; Tanner et al., 2014).

Otoliths are paired calcified structures that play an important role in the balance and auditory reception of teleost fish (Pooper and Platt, 1993). The inner ear of fishes is located in the cranium on the other side of the head close to the midbrain and has three semi-circular canals and three otolithic organs filled with endolymph (Figure 6). The semi-circular canals detect angular accelerations, whereas the otolithic organs appear to have a dual function, vestibular and auditory (Popper and Lu, 2000). In the otolithic organs, three different otoliths can be found - the *sagitta*, the *lapillus* and the *asteriscus*. Each of these otoliths has an irregular unsymmetrical shape that is characteristic of every species. The *sagittae* and *lapilli* are generally formed earlier in development than the *asterisci*, which in some species do not develop until after hatching (Green et al., 2009).

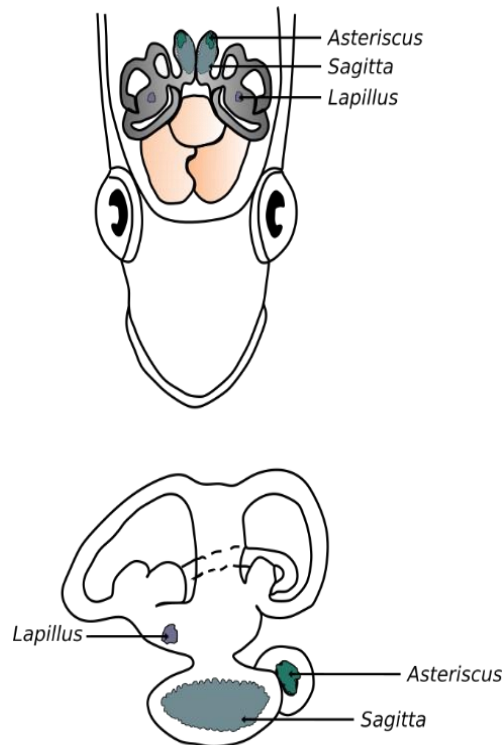


Figure 6: Location of the otoliths within the inner ear of a teleost fish (image adapted from Panfili et al., 2002; courtesy of Patrícia Ferreira).

From the three pairs of otoliths, the *sagittae* are usually the largest and most common otolith pairs used for fish biology studies (Figure 7), and to infer stock composition, through structural, chemical and shape analyses (Volpedo and Vaz-dos-Santos, 2015).

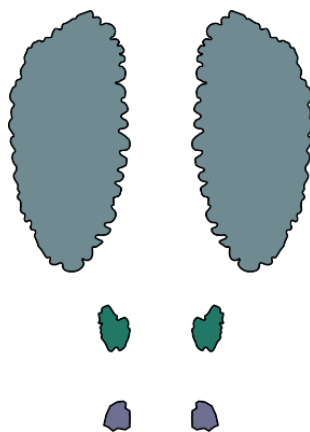


Figure 7: Size comparison of the three paired otoliths found in the inner ear of teleost fish. From the top to the bottom: the *sagitta*, the *lapillus* and the *asteriscus* (courtesy of Patrícia Ferreira).

Otolith microchemistry

Otoliths are acellular and metabolically inert, unlikely to be resorbed or reworked (Campana and Nielson, 1985), which means that any element or compound accreted onto its growing structure is permanently retained. Moreover, its continuous growth implies that the entire lifetime of the fish is recorded (Campana and Nielson, 1985). Otoliths are composed of calcium carbonate (CaCO_3) mainly in the mineral form of aragonite (90%) and of a protein matrix keratin-type, called otolin (10%) (Elsdon et al., 2008).

The otolith calcification process is heavily dependent on the composition of the endolymphatic fluid surrounding the otolith. Although the mechanisms involved in the biomineralization of the otoliths are still poorly understood, it is clear that endolymph composition plays a vital role in the otolith composition (Borelli et al., 2003). During otolith formation, different chemical elements can be incorporated into it (Gillanders and Kingsford, 2003). The elemental composition is dominated by calcium, carbon and oxygen, while other elements occur at lower (> 100 ppm, e.g. Sr, Na, K, N) or even trace (< 100 ppm) levels of detection (Campana, 1999).

The translation of environmental elements into the otolith composition is a complex and still poorly understood process. The inorganic plasma content is mainly derived from the surrounding waters, either by branchial uptake (freshwater fish) or by assimilation through the intestinal epithelium (marine fish). The elemental uptake is dependent on the abiotic conditions of the environment the fish is experiencing, such as pH, salinity, temperature and dissolved oxygen. Along with the uptake from the environment, some elements can be partly supplied by the diet (Cooley and Klaverkamp, 2000). The element's movement from the plasma to the endolymph seems to be regulated by ionic transport that occurs in the saccular epithelium (Panfili et al., 2002). Chemical incorporation processes occurring from the endolymph into the otolith have not been yet clearly established. It is, however, considered that elements are either incorporated within the crystal, absorbed onto its surface or directly bonded to the organic matrix (Panfili et al., 2002).

Several methodologies can be applied to infer the chemical composition of the otoliths depending on the biological question asked. Otolith microchemistry can be analysed in the whole otolith, in sections of the otolith (e.g. core and/or edge), or in specific points of the otolith (e.g. in a transect from the core to the edge by annual rings) (Volpedo and Vaz-dos-Santos, 2015). Analysis of the whole otolith consists in transferring trace elements from the otolith to a solvent followed by the chemical quantification of the elements, a method called otolith solution based by Inductively Coupled Plasma Mass Spectrometry (SB-ICP-MS). The analysis of sections or transects are usually done in the otolith embedded in epoxy resin, sectioned transversely to the core, grounded and polished. The quantification of the elements is done by

Laser Ablation and Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) (Cadrin et al., 2014; Lazartigues et al., 2014; Volpedo and Vaz-dos-Santos, 2015).

The presence of geographic variation in water temperature and chemistry, as well as differences in diet regimes, can result in changes in the otolith composition, suggesting that this structure's microchemistry should help the discrimination among fish that grew up exposed to different environments (Bradbury et al., 2008; Smith and Campana, 2010; Correia et al., 2012a). Thus, otolith microchemistry has been applied for the stock identification of several fish species, such as *Salmo salar* (Reader et al., 2015), *Stegastes fuscus* (Daros et al., 2016) and *Chelidonichthys lucerna* (Ferreira et al., 2019).

Otolith shape analysis

The study of the morphological characteristics of otoliths has been considered an efficient tool for fish stock identification (Ferguson et al., 2011; Jemaa et al., 2015; Tuset et al., 2019). It has proven to be successful in resolving fish stock structure in high gene flow systems, when environmental heterogeneity exists (Bacha et al., 2014), as it can identify groups of fish which may have been spatially or temporally structured at some stage of their life history (Keating et al., 2014). Otolith shape is markedly species-specific, showing nevertheless intra-specific geographic variation in relation to environmental factors and fish growth (Campana and Casselman, 1993; Ferguson et al., 2011; Jemaa et al., 2015).

The use of elliptical Fourier descriptors to describe the shape of otoliths is a rapid, objective, and semi-automated method to obtain significant information to identify populations units (Bird and Eppler, 1986), while the utilization of shape indices has the advantage of being more simple to calculate, representing the growth pattern of otoliths (Tuset et al., 2003). Furthermore, recent improvements in image acquisition and data analysis have allowed to routinely process hundreds of otolith samples, achieving high levels of statistical power (Geffen et al., 2016). The extent to which otolith shape differences are genetically or environmentally induced is not yet clear, albeit influences in the otolith shape have been described in relation to sex, age, year classes, stock and environment (Castonguay et al., 1991; Cardinale et al., 2004; Begg and Brown, 2011). While the environment alters the otolith growth rate, which in turn modifies otolith shape, genetically induced changes can locally affect otolith shape (Cardinale et al., 2004; Vignon and Morat, 2010; Capoccioni et al., 2011). Still, the use of otolith shape analysis to discriminate among fish stocks has been investigated in many small pelagic species, such as the Atlantic horse mackerel *Trachurus trachurus* (Abaunza et al., 2008), the herring *Clupea harengus* (Burke et al., 2008), the European sardine, *Sardina pilchardus*

(Jemaa et al., 2015), and the blue jack mackerel *Trachurus picturatus* itself (Vasconcelos et al., 2018).

1.6 - The blue jack mackerel: fish and fisheries statistics

The blue jack mackerel, *Trachurus picturatus* (Bowdich, 1825), belongs to the genus *Trachurus*, *Carangidae* family. The fishes within the genus *Trachurus* share some common characteristics, including two dorsal fins almost together and an arched lateral line covered with large scales, of which the posterior ones are transformed into strong bony shields. Additionally, *T. picturatus* presents a few distinctive features (Figure 8; Sanches, 1992), namely:

- Elongated and slightly compressed body;
- Colouration ranging from black or grey to bluish-green;
- Scales that form the anterior part of the main lateral line are lower than those of the other *Trachurus* spp;
- Pectoral fins never crossing the lateral line curvature;
- The accessory lateral line terminates below the 6th to 10th soft radius of the dorsal fin.
- A small spot of black colour on the margin near the upper angle of the operculum.

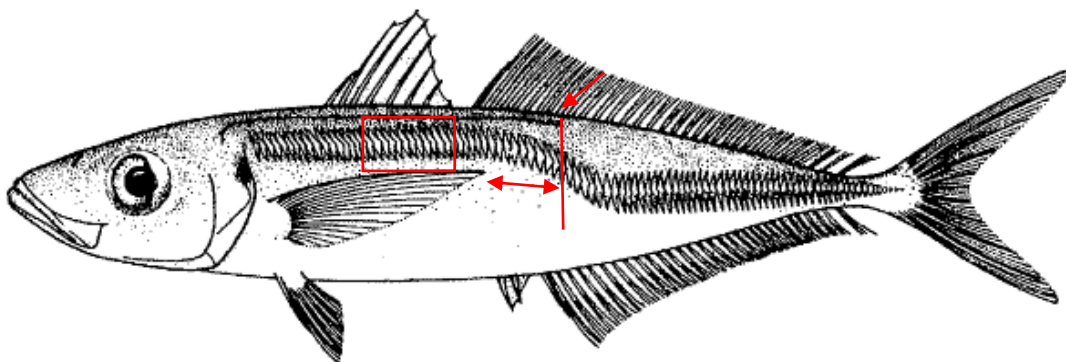


Figure 8: *Trachurus picturatus* (Bowdich, 1825) illustration, with the indication of the species distinctive characters in red (see Sanches, 1992 for more details).

This species is pelagic migratory, with shoal behaviour, that can be found in depths ranging to at least 575 meters (Menezes et al., 2006). It inhabits deeper waters than the remaining

species of the family *Carangidae* and, similarly to other *Carangidae*, descend to greater depths during winter (Benguria and Camiña, 1975). The species is often found in the neritic zones of islands shelves, banks, seamounts and open waters around islands (Smith-Vaniz, 1986). This spatial distribution seems to be related to the fish life stages, *i.e.*, the shelf areas appear to function as nursery or growth zones, while offshore seamounts/banks are preferably a feeding zone, where adult fish predominate (Menezes et al., 2006).

Trachurus picturatus is widely distributed across the North-East Atlantic, from the southern Bay of Biscay to southern Morocco, including the Macaronesian archipelagos of Azores, Madeira and Canary Islands, to Tristan da Cunha and Gough Islands. This species is also found in the Mediterranean and Black Sea (Figure 9; Smith-Vaniz, 1986).

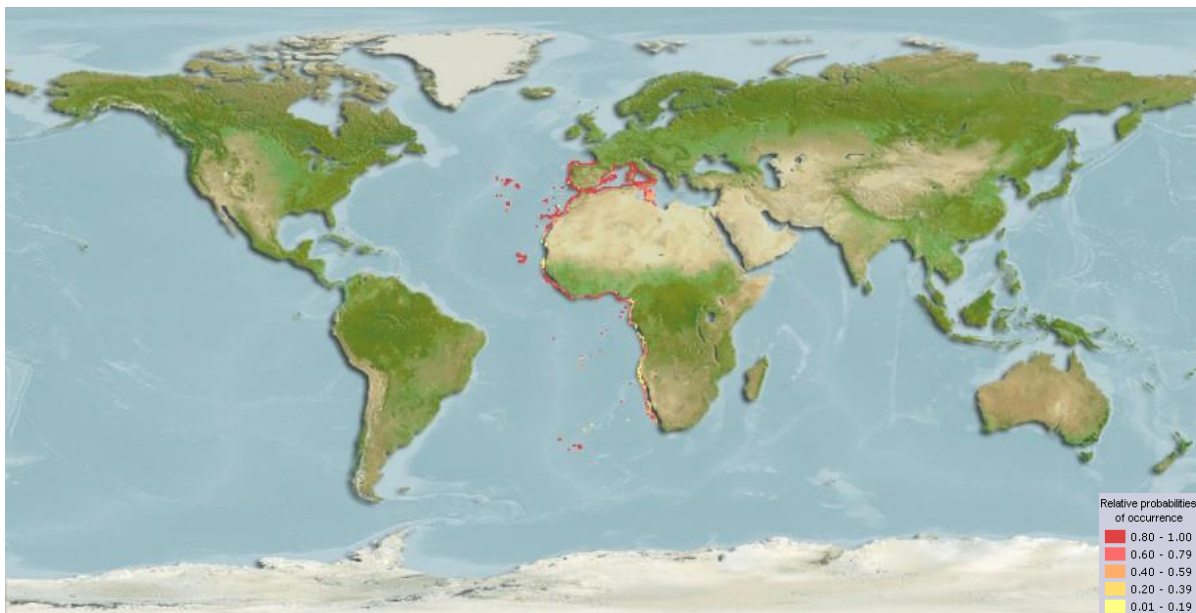


Figure 9: Distribution map of *Trachurus picturatus* (Bowdich, 1825) www.aquamaps.org, version of Aug. 2016. Web. Accessed 20 Sep. 2019.

In the Macaronesian Islands of Azores and Madeira, the blue jack mackerel is an important fishery resource (Vasconcelos et al., 2006; Garcia et al., 2015) being the only species of the genus *Trachurus* that is captured in the Azores (ICES, 2018b). In the Portuguese coast, both *T. picturatus* and *T. trachurus* (Linnaeus, 1758) are present, although *T. trachurus* is the most common (INE, 2017). Due to the phenotypic similarity between the two species, they are often captured together (Gonçalves et al., 2013). *Trachurus picturatus* is essentially captured by local artisanal fisheries using purse-seines (Vasconcelos et al., 2018a). However, in the last decade, fluctuations in the discharges have been reported (Figure 10).

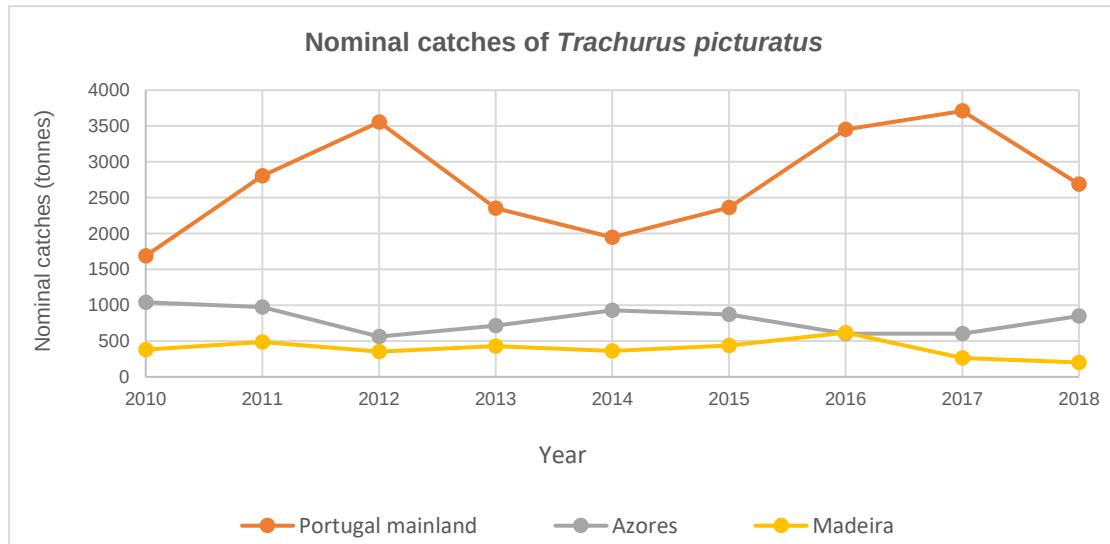


Figure 10: Nominal catches (tonnes) of the blue jack mackerel *Trachurus picturatus* recorded for Portugal mainland, the Azores and Madeira from the year 2010 to 2018 (from INE statistical reports).

During the past years in Portugal mainland, and the Azores and Madeira islands strong oscillations have been registered in the landings of the blue jack mackerel. The pattern of oscillations seems to be periodical, with years of great decline being followed by years of strong fishing (INE, 2018). These fluctuations seem to be related to fish recruitment and not with changes in fishing efforts (ICES, 2018). Nevertheless, in the last two years, Madeira records have shown the highest decrease in landings, from 617 tonnes in 2016 to 201 tonnes in 2018 (INE, 2018), with no apparent recovery in the discharges.

At present, the population of *T. picturatus* is considered stable and categorized as least concerned by the IUCN Red List of Threatened Species (Smith-Vaz, 2015). Moreover, the International Council for the Exploration of the Sea (ICES, 2018) considers this species as one single stock unit, and supports that there is no 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. In recent years, there were a few attempts to fill in the gap, but these studies were done regionally and only focussed on the western part of its distribution. Several authors have studied the growth and reproduction of individuals from the Azores (Isidro, 1990; Garcia et al., 2015), Madeira (Vasconcelos et al., 2006, 2017) and Canaries (Jurado-Ruzafa and Santamaria, 2011; Jurado-Ruzafa and García Santamaría, 2013). These studies found similar reproductive seasons but different growth rates among the sampling locations. Other studies have successfully used parasites as biological markers to study the population structure of *T. picturatus*, identifying different levels of occurrence and

infection in Madeira, Canaries and Portugal mainland (Costa et al., 2012, 2013; Hermida et al., 2015; Vasconcelos et al., 2017b). The use of geometric morphometrics and otolith shape analysis in Madeira and Canaries, including one sampling point in Portugal mainland, revealed three different population units in the region (Vasconcelos et al., 2018). Moreover, previous studies of *T. picturatus* in the Azores region (Menezes et al., 2006) and the Canary Islands have reported the presence of all life stages in different regional habitats (Jurado-Ruzafa and Santamaria, 2011; Jurado-Ruzafa and García Santamaría, 2013), and a pattern of co-existing migratory and resident contingents of *T. picturatus* in the Canary Islands (Tuset et al., 2019).

All this information supports the existence of different stock units across the species distribution. However, information is still missing. For example, there are only genetic studies done for the species Eastern distribution, *i.e.*, in the Mediterranean Sea (Karaïskou et al., 2003, 2004). Therefore, to properly delineate the stock structure of this species it is necessary to employ multidisciplinary approaches, such as genetic markers (*e.g.* mtDNA and microsatellites), geometric morphometrics and otoliths, and to consider not only the spatial distribution but also the evolution of the stock during time.

1.7 - Study area

The Macaronesian region, located in the North-East Atlantic Ocean is comprised of four archipelagos: Azores, Madeira, the Canary Islands and Cape Verde (Figure 11). These islands, just off the coast of Europe and Africa, are formed by volcanic seamounts and were never part of a continent, providing ideal environments for phylogeographic fish studies, since these systems are suitable to apply models to predict the efficacy of pelagic larvae/adult dispersion (Quinteiro et al., 2015; Stefanni et al., 2015). Moreover, the Macaronesia region is characterized by complex oceanic currents and no apparent physical barriers, indicating that dispersal of individuals would depend exclusively on the species' dispersal potential (Selkoe et al., 2016).

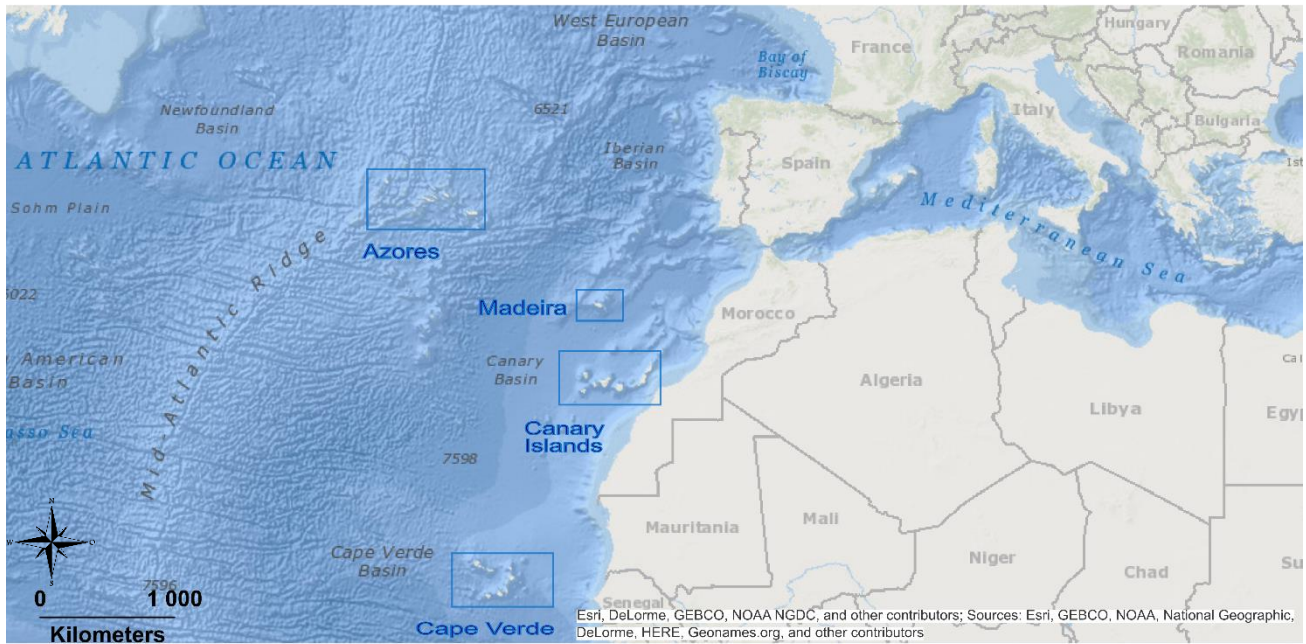


Figure 11: Macaronesian Archipelagos in the North-East Atlantic – the Azores, Madeira, Canary Islands and Cape Verde.

The Macaronesia is separated from the Euro - African mainland by c. 100 – 1900 km, and it is characterized by the presence of several oceanographic surface currents, such as the North Atlantic Current (NAC), the Azores Current (AC) and the Canary Current (CC) (Sala et al., 2013). The AC flows south and eastwards of the Azores islands and splits in two branches: the northern branch flows towards the Gulf of Cadiz, where it joins the CC, proceeding south along the African coast (Johnson and Stevens, 2000); and the south branch that flows southeast passing south of Madeira towards the Canary Islands (Johnson and Stevens, 2000). Additionally, the Portuguese coast is influenced by the Portugal current (PC) that flows between continental Portugal and the Azores and the Portugal Coastal Countercurrent (PCC) which flows poleward during fall, winter and early spring, and southward during summer and late spring (Martins et al., 2002). Furthermore, the PC seems to contribute to the CC (Mason et al., 2006). In the Gulf of Cadiz, the surface currents fed by the northern branch of the AC and the summer poleward PCC, are dominated by the inflow of Atlantic waters into the Mediterranean Sea (Mason et al., 2006).

These currents, together with the apparent lack of physical barriers, can strengthen the potential for long migrations and the development of genetically homogeneous populations. In fact, several phylogeographic studies have reported a low population genetic differentiation in the Macaronesian region for different taxa, including gastropods (De Wolf et al., 2000), crustaceans (Faria et al., 2013; Quinteiro et al., 2015) and fishes (Francisco et al., 2011b;

Stefanni et al., 2015). On the other hand, the Macaronesia archipelagos are also characterised by different upwelling zones, habitat discontinuities and ecotones between areas with different environmental properties and areas with historical sea level drops (*e.g.*, Stefanni et al., 2015). These features seem to be responsible for maintaining discrete subpopulations over a species' range, suggesting stable currents in Macaronesia that promote retention of individuals. Some fish species appear to be isolated in insular populations, namely between the Azores Islands and Portugal mainland (González-Wangüemert et al., 2011), or in regions where upwelling events are stronger, such as in the Atlantic Morocco coast (Atarhouch et al., 2006). Moreover, within Macaronesia, some fish species have shown limited migrations, with the existence of local stocks (Francisco et al., 2009; González-Wangüemert et al., 2010; Almada et al., 2017).

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Chapter 2

MOLECULAR ANALYSES

Article 1. Genetic diversity and population structure of the blue jack mackerel *Trachurus picturatus* across its western distribution.

Article 2. Spatio-temporal microsatellite data suggest a multidirectional connectivity pattern in *Trachurus picturatus* metapopulation from the Northeast Atlantic.

Genetic diversity and population structure of the blue jack mackerel *Trachurus picturatus* across its western distribution

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Abstract

The blue jack mackerel *Trachurus picturatus* is an important economic fish species in the Macaronesian region. Nevertheless, information on its genetic structure is still lacking. Individuals collected at six sampling locations of the North-East Atlantic – Azores, Madeira, Canaries and Portugal mainland (Matosinhos, Peniche and Portimão), and one location in the Mediterranean (Sicily), were used to examine, for the first time, the genetic structure of this species. Three mitochondrial gene regions (cytochrome c oxidase subunit I, cytochrome *b* and control region) were used to study the genetic structure of the species in the Macaronesian region, as well as to compare the genetic diversity of this region with published results from its Eastern distribution. All markers indicated the absence of genetic structure among populations, with high indices of genetic diversity. These results suggest that the species went through a bottleneck event, followed by a recent population expansion. Moreover, the comparison with previously published results from *T. picturatus* Mediterranean distribution suggests the existence of a single panmictic population throughout the species' full range. This was, however, an unexpected result since other methodologies have shown the presence of, at least, three different population-units in the NE Atlantic.

Key words: Carangidae; Macaronesia; mtDNA; Connectivity; Phylogeography

Significance Statement

The present study provides the first insights about the genetic diversity and population structure of *Trachurus picturatus* across its Western distribution range. The mtDNA results obtained suggest that *T. picturatus* suffered a bottleneck event followed by a rapid population expansion, resulting in a single panmictic population across the species' entire distribution. However, this does not necessarily mean that stocks do not exist, as has already been shown by other approaches.

Introduction

The Macaronesian region, located in the North-East Atlantic, is comprised of four archipelagos: Azores, Madeira, Canaries and Cape Verde. These islands, just off the coast of Europe and Africa, are formed by volcanic seamounts and were never part of a continent. This makes the biogeography of the islands quite unique and very attractive for phylogeographic studies (Whittaker, 1998; Quinteiro et al., 2015; Stefanni et al., 2015). The marine environment is very often characterized as having complex oceanic currents and no physical barriers, which greatly increase the species' dispersal potential, promoting high gene flow over large scales (Selkoe et al., 2016). Furthermore, large populations coupled with high fecundity are expected to reduce the effects of drift, slowing differentiation among populations (Palumbi, 1992; Selkoe et al., 2016). The Macaronesia, separated from the Euro-African mainland by between 100 km and 1900 km, is characterized by the presence of several oceanographic currents, such as the North Atlantic Current, Azores Current, and the Canary Current (Sala et al., 2013). These currents, together with the apparent lack of physical barriers, can strengthen the potential for gene flow. In fact, several phylogeographic studies have reported a low population genetic differentiation in the Macaronesian region for different taxa, including gastropods (De Wolf et al., 2000), crustaceans (Faria et al., 2013; Quinteiro et al., 2015) and fishes (Francisco et al., 2011; Stefanni et al., 2015). On the other hand, the Macaronesia archipelagos are also characterized by different upwellings zones, habitat discontinuities and ecotones between areas with different environmental properties, and areas with historical sea level drops (e.g., Stefanni et al., 2015). These features seem to be responsible for maintaining discrete subpopulations over the species' range, suggesting stable currents in the Macaronesia that promote these genetic differences. Some fish species, that exhibit genetic homogeneity in the

transition zone between Atlantic and Mediterranean, appear to be isolated in insular populations, namely between the Azores Islands and the Portuguese mainland (e.g., González-Wangüemert et al., 2011), or in regions where upwelling events are stronger, such as in the Atlantic Morocco coast (e.g., Atarhouch et al., 2006). Moreover, within the Macaronesia, some fish species have shown limited gene flow, with the existence of “local stocks” (e.g., Francisco et al., 2009; González-Wangüemert et al., 2010; Almada et al., 2017).

The blue jack mackerel *Trachurus picturatus* (Bowdich 1825) is a pelagic migratory fish widely distributed in the North-East Atlantic, from the Bay of Biscay to Morocco, and in the Tristan de Cunha and Gough Islands, including the Macaronesian islands (except Cape Verde). Additionally, it also occurs in the Mediterranean and Black seas (Smith-Vaniz, 1986; ICES, 2016). *Trachurus picturatus* is an economically important species, especially in the Azores and Madeira, where is the main pelagic fish being caught by the local artisanal fisheries (Garcia et al., 2015; ICES, 2016). This species has variable landing quantities, with fluctuations in captures for the last five years, ranging from 973 t in 2011 to 603 t in 2016 in the Azores; and from 485 t in 2011 to 617 t in 2016 in Madeira (INE, 2017). Current data indicate that these fluctuations are usually associated with changes in fishing efforts (ICES, 2016). However, these could also be caused by natural variations in abundance and/or recruitment, such as feeding and spawning requirements (Menezes et al., 2006; Vasconcelos et al., 2018).

In recent years, there were several attempts to understand the population structure of *T. picturatus* using different methodological approaches. However, these studies were done regionally and only focusing on the Western part of its distribution. Several authors have studied the growth and reproduction of individuals from the Azores (Isidro, 1990; Garcia et al., 2015), Madeira (Vasconcelos et al., 2006; Vasconcelos et al., 2017) and Canaries (Jurado-Ruzafa and Santamaria, 2011, 2013). These studies found similar reproductive seasons but different growth rates among the sampling locations. Other studies have successfully used parasites as biological markers to study the population structure of *T. picturatus*, identifying different levels of occurrence and infection in Madeira, Canaries and Portugal mainland (Costa et al., 2012, 2013; Hermida et al., 2015; Vasconcelos et al., 2017). The use of geometric morphometrics and otolith shape analysis in Madeira and Canaries, including one sampling point in Portugal mainland, also revealed three different population units in the region (Vasconcelos et al., 2018). Moreover, otolith shape indices and elliptic Fourier descriptors for individuals captured in the Azores, Madeira, Canaries and Portugal mainland (Moreira et al., 2019), also revealed differences among the sampling locations, showing that Azores and Canaries were different from Madeira and Portugal mainland. Finally, otolith elemental and isotopic signatures revealed four different population units in the Azores, Madeira, Canaries and Portugal mainland (Moreira et al., 2018).

Previous studies of *T. picturatus* in the Azores region have reported the presence of all life cycles stages in different regional habitats also supporting the existence of different population units (ICES, 2006; Menezes et al., 2006). Therefore, it is surprising that the genetic structure of *T. picturatus* has never been assessed in the Macaronesia. In fact, until now, its genetic diversity has only been assessed in a partial range of its distribution, *i.e.*, in its Eastern distribution (throughout the Aegean Sea, the Ionian Sea and West Italy), including one site in Portugal mainland (Karaïskou et al., 2003, 2004). Using cytochrome *b* (*cytb*), 16S, and control region (CR) by Restriction Fragment Length Polymorphism, Karaïskou et al. (2003, 2004) reported a lack of genetic variation in this region. This lack of differentiation was explained by the species' high dispersal potential and the apparent lack of physical barriers in the marine environment (Karaïskou et al., 2003, 2004).

Therefore, the present study aims to characterize for the first time the genetic diversity of *T. picturatus* across its Western distribution range - Macaronesia (*i.e.*, Azores, Madeira and Canaries) and Portugal mainland. As mtDNA is often used to provide a first glimpse on a species phylogeographic history (Avice, 2004) and there are previously published *COI* (cytochrome c oxidase subunit I), *cytb* and CR data from the species' Eastern distribution, the same three different mitochondrial DNA regions were used, for obvious comparison purposes.

Material and Methods

A total of 195 individuals from Azores, Madeira, Canaries, Portugal mainland (Matosinhos, Peniche and Portimão) and Sicily were sampled (Figure 1). Individuals were collected by local artisanal fleets and specimens (ranging from 24.5 to 27.2 cm of total length) were acquired in local commercial ports at the sampling sites. Pectoral fins were sampled, stored in 96% ethanol and kept at -20°C until processing.

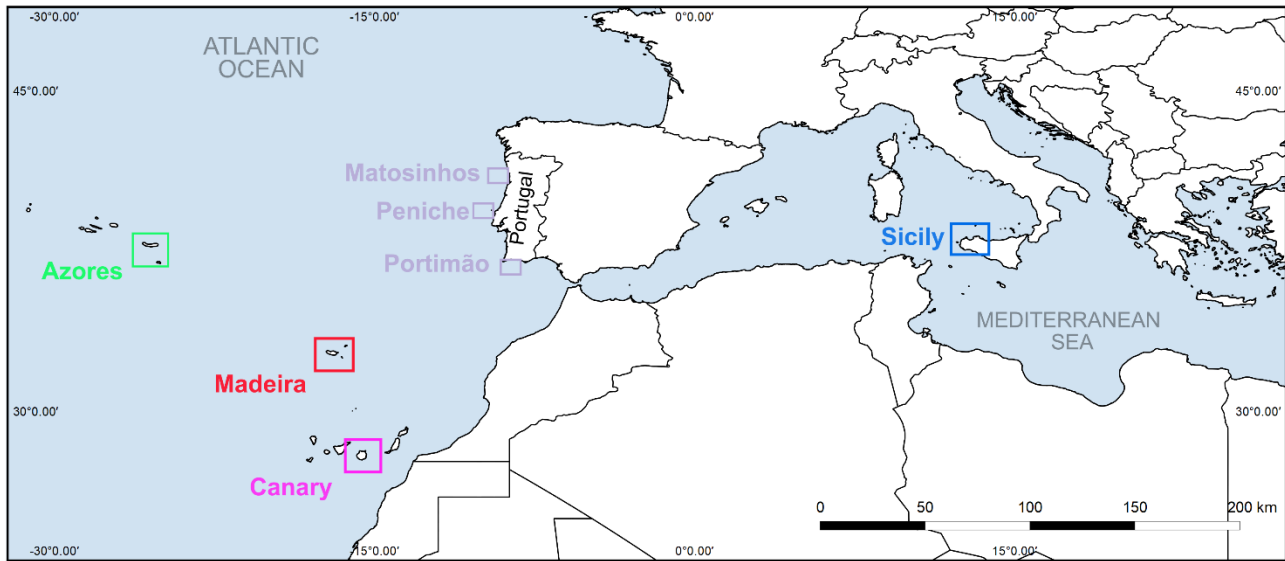


Figure 1: Sampling sites of *Trachurus picturatus* in the north-east Atlantic Ocean and the Mediterranean Sea.

Genomic DNA was extracted by a high-salt protocol (Sambrook et al., 1989) from ~30 individuals per site. Three mtDNA gene regions *COI*, *cytb* and CR were amplified applying the PCR conditions and profiles described in Ivanova et al., 2007 (*COI*), Cárdenas et al., 2005 (*cytb*) and Collette et al., 2006 (CR). PCR products were purified and sequenced (both directions) with the same PCR primers by Macrogen Inc. Chromatograms were checked using Chromas 2.6.2 (technelysium.com.au). The resulting sequences were deposited in GenBank (Supporting Information Table S1). Alignments for each individual gene, *i.e.*, *COI*, *cytb* and CR regions were performed in Bioedit v.7.2.5 (Hall, 1999), including additional sequences from Central and Eastern Mediterranean obtained from GenBank (Supporting Information Table S1). Due to the smaller size of most of the *cytb* sequences available on GenBank (only 239 bp long), a preliminary analysis was performed. Two alignments were obtained, one including all the available sequences (239 bp), the other with 687 bp, but excluding the Eastern Mediterranean samples (n=17).

For each gene, sequences were joined in unrooted networks (95% criterion) in TCS 2.1 (Clement et al., 2000) with gaps treated as missing data. Sequences from the three genes were concatenated using the software SequenceMatrix (Vaidya et al., 2011) and an unrooted network (95% criterion) was done using the same approach. For each gene, the number of haplotypes (*h*), haplotype diversity (*Hd*) (Nei & Tajima, 1981) and nucleotide diversity (π) (Nei, 1987) were calculated in DnaSP v5.10 (Librado and Rozas, 2009). In order to estimate the hierarchical distribution of mtDNA genetic differentiation, within and among populations, an analysis of molecular variance (AMOVA) was performed in ARLEQUIN v.3.5.2.2 (Excoffier and Lischer, 2010), using two group combinations (*i.e.*, Atlantic + Mediterranean; and Macaronesia

+ Portugal mainland + Mediterranean), with Φ_{ST} and 10.000 permutations. Pairwise population differentiation values (Φ_{ST}) were calculated to assess the levels of genetic differentiation among all populations using the same software. Molecular signatures of demographic expansion of each of the populations, were tested by pairwise mismatch-distribution analysis, according to the null hypothesis of constant population size (Rogers and Harpending, 1992), and the Harpending's Raggedness Index, the sum of squared deviations statistics (SSD), Fu's F_s (Fu, 1997) and Tajima's D (Tajima, 1989) were also calculated in ARLEQUIN v.3.5.2.2.

Results

The final alignments consisted of 202 sequences with 455 bp for *COI*; 193 sequences with 678 bp for *cytb* and 210 sequences with 239 bp; and 224 sequences with 647 bp for CR. The final alignment of the concatenated mtDNA dataset consisted of 240 sequences with 1816 bp. Translation to amino acids revealed no stop codons. 49, 70 and 98 polymorphic sites were detected for *COI*, *cytb* and CR, respectively, and the parsimony informative sites were 19, 35 and 67, respectively.

As both unrooted networks constructed for *cytb* resulted in very similar patterns (data not shown) all further analyses were performed with the longest alignment for pictorial purposes. None of the single gene networks revealed any geographic structure (Supporting Information Figure S1). The total number of haplotypes was 48 in *COI* and 70 in *cytb*. 48% (*COI*) and 21% (*cytb*) of all the individuals shared the central haplotype, with the most singletons (*i.e.*, haplotypes represented by a single individual; 186) being found in CR (Supporting Information Figure S1). The network constructed with the concatenated mtDNA dataset also showed no genetic structure among sampling sites (Figure 2). The most common haplotype was shared by only 18 individuals collected from different sampling sites, *i.e.*, Canaries, Atlantic Portuguese Coast, Central and Eastern Mediterranean (Figure 2).

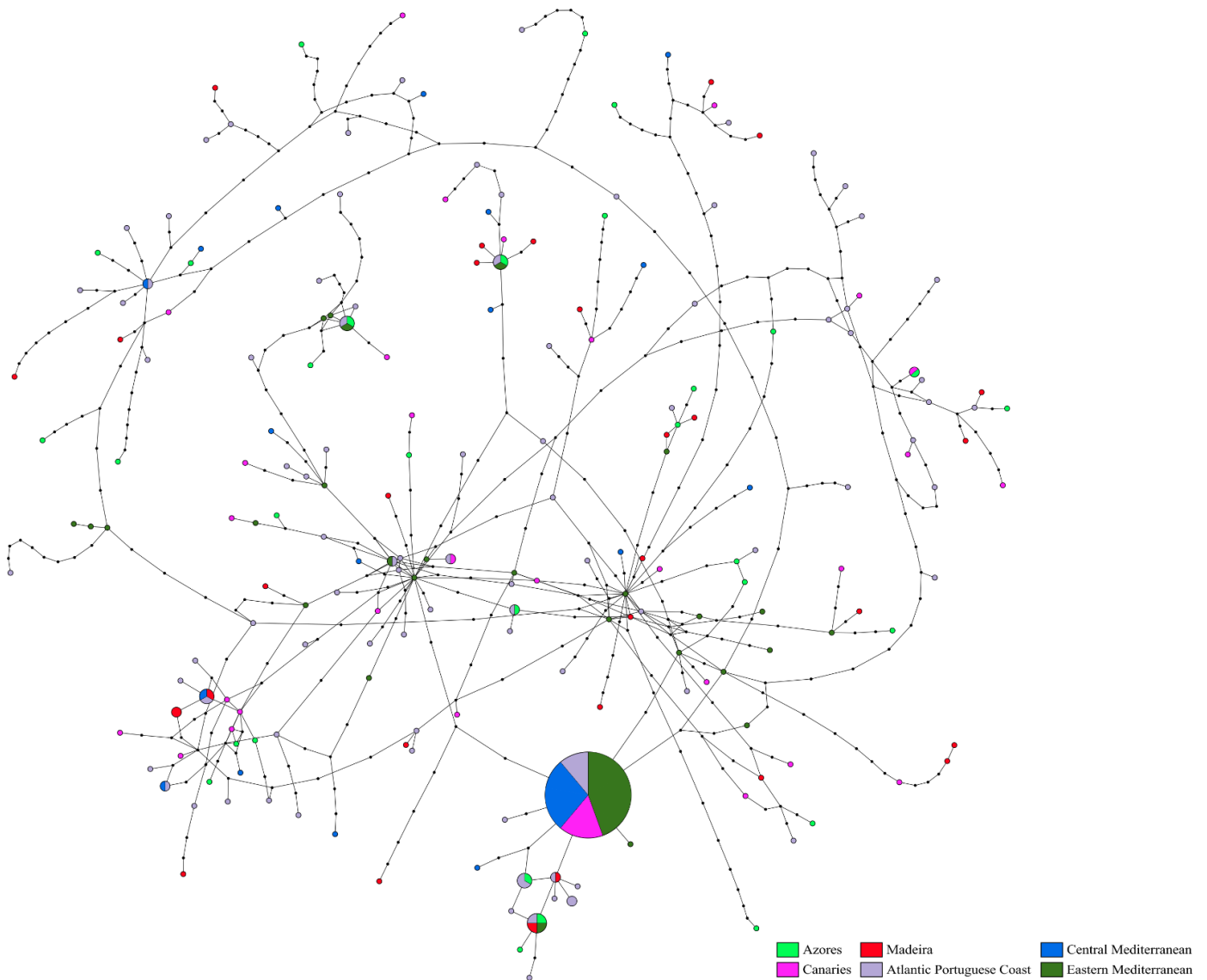


Figure 2: Haplotype (TCS) network of the concatenated gene sequences (*COI*, *cytb* and *CR*) showing the relationships of *Trachurus picturatus* specimens across the species' distribution. Circle size is proportional to the observed haplotype frequencies; ●, unobserved haplotypes and potential intermediate.

The indices of genetic diversity are shown in Table 1. High levels of haplotype diversity and low levels of nucleotide diversity are seen across the species' distribution for all gene regions. AMOVA results for the different structures tested showed that the total variance was always found within populations (~100%). Additionally, for all gene regions, pairwise Φ_{ST} values were very small and only one pair was statistically significant, *i.e.*, the Canaries and Portugal mainland (for *COI*; Supporting Information Table S2).

Tests of demographic expansion yielded mostly significantly negative results for F_s (Fu's) and D (Tajima's) statistics for both *COI* and *cytb* (Table 1) suggesting a rapid

demographic expansion for this species across its distribution. For CR, only the F_s (Fu's) for Portugal mainland presented a significant result (Table 1). The unimodal shape of the mismatch distribution obtained for all populations, for *COI* and *cytb*, indicates a constant population size (Supporting Information Figure S2). For CR, the expected distribution of mutations, according to the null hypothesis of constant population size, does not fit the observed frequencies. The statistics for the Harpending's raggedness index and the sum of squared deviations (SSD) revealed no statistically significant results (Supporting Information Figure S2).

Table 1: Summary of the indices of genetic diversity estimated from COI, *cytb* and CR sequencing data for *Trachurus picturatus* from the Azores, Madeira, Canaries, mainland Portugal, central and eastern part the Mediterranean (see Table S1 for details). Macaronesia represents the Azores, Madeira and Canaries combined.

Sampled Sites	COI						cytb						CR					
	n	h	H _d	π	Fu's F _S	D	n	h	H _d	π	Fu's F _S	D	n	h	H _d	π	Fu's F _S	D
Azores	30	13	0.807	0.00352	-8.584	-1.945*	30	20	0.940	0.00324	-19.351*	-2.110*	30	30	1.000	0.01685	-22.909	-1.180
Madeira	30	12	0.717	0.00354	-6.934	-1.815*	30	19	0.947	0.00447	-13.124*	-2.092*	30	29	0.998	0.01564	-20.340	-1.031
Canaries	27	16	0.892	0.00437	-13.020*	-2.194*	28	19	0.958	0.00057	-16.707*	-2.159*	29	29	1.000	0.0138	-24.599	-1.083
Macaronesia	87	28	0.805	0.00378	-27.701*	-2.236*	88	43	0.944	0.00376	-51.598*	-2.361*	89	81	0.998	0.01540	-33.972	-1.241
Portugal mainland	89	24	0.685	0.00291	-24.125*	-2.395*	87	39	0.933	0.00318	-47.101*	-2.215*	89	81	0.998	0.01500	-99.978*	-1.382
Central Mediterranean	22	11	0.827	0.00439	-5.588*	-1.981*	18	12	0.922	0.00304	-8.280	-1.829*	17	17	1.000	0.01428	-9.618	-0.418
Eastern Mediterranean	4	3	0.833	0.00330	-0.288	-0.754	--	--	----	-----	----	----	29	28	0.998	0.00816	-29.203	-1.120

n = sample size; h = number of haplotypes; H_d = haplotype diversity; π = nucleotide diversity; D, Tajima's D.

*Statistical significance ($p < 0.05$).

Discussion

The present study provides the first insights, based on mtDNA sequencing data, on the genetic diversity of *T. picturatus* across its Western distribution range – Azores, Madeira, Canaries and Portugal mainland. Overall, the results obtained from the three mitochondrial gene regions showed no genetic structure in the NE Atlantic region. This lack of significant variation among populations has previously been observed in the *T. picturatus* Eastern distribution (*i.e.*, Mediterranean Sea; Karaïskou et al., 2004) indicating considerable gene flow among populations and therefore the existence of a single panmictic population.

This pattern of homogeneity was unexpected since the sampling area is extensive (more than 3.000 km). Moreover, recently several different population-units have been revealed in the NE Atlantic, *i.e.*, Azores, Madeira, Canaries and Portuguese coast, using parasite fauna (Costa et al., 2012, 2013; Hermida et al., 2015; Vasconcelos et al., 2017), otolith microchemistry analysis (Moreira et al., 2018), and otolith shape analysis (Moreira et al., 2019). Still, these approaches act at different spatiotemporal scales. Parasites and otoliths, as natural tags, are mainly influenced by ecological factors and therefore retain current connectivity (parasites) and environmental (otoliths) signatures, while mtDNA is influenced by historical processes, *e.g.*, Pleistocene glaciations (Tanner et al., 2014).

This study revealed high levels of haplotype and low levels of nucleotide diversity, a pattern that was already described for the species' Eastern distribution (Karaïskou et al., 2003; Bektas and Belduz, 2008). Typically associated to these frequencies are the star-like topology networks, such as those obtained for *COI* and *cytb*. This pattern suggests that most haplotypes have been originated recently, *i.e.*, the species went through a bottleneck event followed by a recent population expansion (see Ferreri et al., 2011 for a review). The network obtained for the concatenated mtDNA sequences showed a high frequency of singletons, with no high frequency haplotype, also consistent with a lack of historical isolation.

Similar results were obtained in several studies that have employed mtDNA to investigate genetic structuring and demographic history in populations of overexploited fishes. Atarhouch et al. (2006) examined the genetic structure of sardine (*Sardina pilchardus*, Walbaum 1792) using the mitochondrial control region, in the central-eastern and the north-eastern Atlantic Ocean and the Mediterranean Sea reporting high levels of haplotypic and nucleotidic diversity, with no genetic structure between populations from the Moroccan Atlantic coast and the Mediterranean. Furthermore, González-Wangüemert et al. (2010) studied the genetic structure of the white seabream (*Diplodus sargus*, Linnaeus 1758) in the NE Atlantic using the *cytb* gene region and reported similar values of diversity and a network pattern identical to those found in the present work.

The geographical distance between the extremes of the sampling sites (Sicily and Azores are more than 3.000 km apart), the existence of several oceanic currents, *e.g.*, the Azores Current, the Canary Current and the Portugal Current (Sala et al., 2013), and/or the different environmental properties of the water (*e.g.*, temperature, salinity, depth and sediment type; see Selkoe *et al.*, 2016 for a review) should enhance genetic differentiation. However, if currents can act as barriers to gene flow, they can also help surpass the distances and hypothetical physical-chemical blocks, transporting *T. picturatus* larvae and/or adults across long distances (Domingues et al., 2007; Sala et al., 2013). Therefore, the possible gene flow found among the studied populations was unexpected. Nevertheless, the overall genetic variation across the species entire distribution was always found within populations (~100 %) and the pairwise Φ_{ST} values suggest the existence of a panmictic population.

This lack of genetic differentiation found between the Atlantic and Mediterranean has been reported for other marine fish species (Karaiskou et al., 2004; Comesaña et al., 2008; Debes et al., 2008), which suggests that the Almeria-Oran front (transition from the Atlantic Ocean to the Mediterranean Sea) might not be an efficient phylogeographic break for all species. Nevertheless, isolation patterns within the NE Atlantic have been recorded, namely in the Azores region where several marine fishes, *e.g.*, *Coris julis* (Linnaeus 1758; Aurelle et al., 2003), *Lipophrys pholis* (Linnaeus 1758; Stefanni et al., 2006), *Chromis limbata* (Valenciennes 1833; Domingues et al., 2006) and *D. sargus* (Linnaeus 1758; González-Wangüemert et al., 2010), and some invertebrate species of the genus *Patella* (Sá-Pinto et al., 2008) have been reported as having isolated population units.

The pairwise mismatch distribution analysis for *COI* and *cytb*, considering the null hypothesis of constant population size, was consistent with a scenario of constant population size for the species across its distribution, *i.e.*, NE Atlantic and Mediterranean Sea. However, the significant negative results for both Fu's *F_s* and Tajima's *D*, the non-significant values of SSD and raggedness index indicate the species suffered a bottleneck followed by a rapid population expansion event. Additionally, although not significant, CR showed the same tendency of negative results for Fu's *F_s* and Tajima's *D*, low values for SSD and raggedness index, and the pairwise mismatch distribution did not follow the expected distribution of mutations, leading to the rejection of the null hypothesis, *i.e.*, the distribution of mutations was consistent with a scenario of rapid population expansion. These results could be explained by the paleo-biogeographic history of the eastern Atlantic. Changes in the genetic diversity and some local extinctions of Atlantic species have been related to glacial peaks that occur during the Pleistocene glaciations (Almada et al., 2001; Schiebel et al., 2002; Domingues et al., 2006, 2007). Less thermal tolerant species most likely became extinct in some regions, including the western coast of Portugal, the eastern islands of the Canaries and probably to a lesser extent in the Azores (Domingues et al., 2007 and references therein). Recolonization of these regions

may have been possible with individuals from less affected areas, namely from the Mediterranean, Africa and Madeira Islands (Almada et al., 2001; Domingues et al., 2006, 2007). These colonization routes could explain the lack of differentiation found in this study.

Finally, all the mtDNA results suggested that *T. picturatus* comprises a single panmictic population. However, this does not necessarily mean that local stocks do not exist, as has already been shown by other approaches. Although the large scale connectivity of *T. picturatus* seems to be sufficient to prevent the detection of (mtDNA) genetic heterogeneity, other molecular markers with higher mutation rates (e.g., microsatellites) and/or other approaches, such as morphometrics, should be applied to reveal the current population structure of the blue jack mackerel across its entire distribution.

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Supporting Information

Supporting Information may be found in the supplement material of this thesis and in the online version of this paper:

Table S1. GenBank accession numbers for the novel sequences and the additional gene sequences determined in previous studies (*) of *Trachurus picturatus*.

Table S2. Genetic differentiation (Φ_{ST}) for the three gene regions sequenced for *Trachurus picturatus* among the studied populations. Statistical significance ($p < 0.05$) is indicated by *.

Figure S1. Haplotype (TCS) networks for each individual gene showing the relationships of *Trachurus picturatus* specimens across the species' distribution. Circle size is proportional to

the observed haplotype frequencies and black points represent unobserved haplotypes and potential intermediates.

Figure S2. Mismatch distributions for each gene region of *Trachurus picturatus*. Black curves show the expected distribution of mutations according to the null hypothesis of constant population size. The number of pairwise differences and their frequencies is shown on the horizontal and vertical axes, respectively. Colours represent the sampling sites: Azores - green; Madeira - red; Canaries - pink; Portugal mainland - grey; Central Mediterranean - blue; Eastern Mediterranean - dark green. Results for the sum of squared deviation (SSD) and Harpending's Raggedness index are also presented (significant results, $p < 0.05$, are marked with *).

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Spatio-temporal microsatellite data suggest a multidirectional connectivity pattern in *Trachurus picturatus* metapopulation from the Northeast Atlantic

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Abstract

The sustainable management of fisheries requires knowledge on their spatio-temporal delineation as well as on the connectivity pattern within metapopulations. The blue jack mackerel *Trachurus picturatus* is an important fishery in South Western Europe but its population structure and connectivity patterns remain controversial. This study is the first one employing microsatellite markers on *T. picturatus* to unravel the population structure and migration dynamics among fishing grounds sampled in 2013 and 2016 in the Northeast Atlantic and the Mediterranean Sea. Microsatellites show high spatio-temporal conservation of gene diversity ($\bar{H}_E = 0.851 \pm 0.011$) and the absence of genetic differentiation among samples ($\bar{F}_{ST} = 0.0013 \pm 0.0020$) in the whole range. Current genetic data suggest that *T. picturatus* fishery consists of a metapopulation exhibiting high gene flow among the fishing grounds tested so far. These results are consistent with the scenario recently reported with mtDNA genes. Moreover, the Bayesian-computed migration rates and common ancestry suggest a large multidirectional migration ($m \geq 0.30$) across a Northeast Atlantic longitudinal corridor. A directional property of the connectivity pattern is that migration proceeds from Macaronesia to

Portugal mainland in autumn-winter while the way back seems to occur in spring-summer. The information retrieved from life-cycle markers (otoliths, morphometrics, *etc.*) is also discussed in light of the new molecular data. Upcoming scientific challenges for the sustainability of this fishery should focus on the full characterization of the remaining properties of its connectivity patterns and on the contribution of each local recruitment area to the adult fishing grounds.

Key-words: Blue jack mackerel, connectivity patterns, fishery sustainability, genetic structure, migration dynamics.

Introduction

The rational management of fisheries requires knowledge on the spatio-temporal delineation and the genetic background of the harvested population to ensure the sustainability of local stocks (Allendorf et al., 2008). Moreover, one of the main objectives of fisheries management is to ensure the sustainability of aquatic resources by preventing depletion of fish stocks by overharvesting (Reiss et al., 2009; Cadrin et al., 2014; FAO, 2018). However, 60% of the fisheries assessed worldwide are at their maximum sustainable yield, 33% are exploited at biologically unsustainable levels, and only 7.0% are considered underfished (FAO, 2018). Fish stocks are the fundamental management units in fisheries science and their overharvesting can result from a wrong choice of management areas which do not match biological meaningful entities (Waples and Gaggiotti 2006; Reiss et al., 2009; Hawkins et al., 2016), since the spatial delineation is often based on stationary fish distributions, oceanographic features, and political boundaries or administrative interests (*e.g.* Pita et al., 2016).

Traditionally, fish stocks have been viewed as reproductively isolated populations bearing their own internal dynamics so that most efforts to delineate stocks have depended on approaches designed to evaluate reproductive isolation, *e.g.* physical tagging or molecular markers (Reis-Santos et al., 2018). However, harvested fish stocks rarely are biologically homogeneous and closed populations but are better composed of a swarm of reproductive units that come together in space and time, likely bearing a variety of life-cycle histories (Presa et al., 2016; Dahle et al., 2018). Therefore, harvesting mixed stocks as single reproductive units can lead to suboptimal exploitation of separate components of the fishery (Allendorf et al., 2008).

Molecular markers can be particularly helpful in Fisheries Science since their variety can be applied to detect an ample range of genetic patterns, e.g. from inter-individual fingerprints to reproductive isolation between species, including within species evolutionary features, evolutionary lineages, genetic drift, allopatric separation, natal and brood origin, etc. (Tessier et al., 1995). Increasing access to genomic datasets facilitates the identification of loci with high discriminatory power that helps to delineate management units and connectivity patterns in species that typically display low levels of population structure (Kelley et al., 2016; Hemmer-Hansen et al., 2019). Also, population genetic analyses can be helpful at delineating stock boundaries in space and time, and testing the temporal stability of genetic structures and at inferring inter-stock dispersal rates (Reiss et al., 2009; Pita et al., 2014; Dahle et al., 2018). Nevertheless, modelling connectivity patterns in the marine realm is challenging due to the large dispersal potential and gene flow exhibited by most fish populations (Palumbi, 1994). Since early 1990s, highly polymorphic microsatellite markers have been one of the most widely applied markers to study fine stock structures of fishes (Presa et al., 1994; Cuéllar et al., 2016) including those exhibiting a complex biology such as the European sprat *Sprattus sprattus* (Limborg et al., 2012) or the European sardine *Sardina pilchardus* (Kasapidis et al., 2012).

The blue jack mackerel *Trachurus picturatus* (Bowdich, 1825) is a migratory pelagic fish widely distributed in the North-East (NE) Atlantic, from the Bay of Biscay to Morocco, including the Macaronesia Archipelagos, the Mediterranean Sea and the Black Sea (Smith-Vaniz 1986; ICES, 2018). This mackerel is the most exploited pelagic species in Azores and Madeira (Garcia et al., 2015) and a significant decrease of Madeira landings (from 617 t to 263 t) was reported in the last years (INE, 2018). Such dramatic decrease was associated with changes in fishing efforts (ICES, 2018), but no evidence exists to rule out a natural fluctuation in abundance and/or recruitment (Menezes et al., 2006; Vasconcelos et al., 2018). For instance, a population fluctuation could be related to the schooling and migratory behaviour exhibited by species with large population size and high dispersal ability of larvae and adults, as occurs in pelagic fish such as mackerels, sardines, and anchovies (ICES, 2018). So far, there is no published information regarding the location of spawning grounds, pelagic larval duration and migration patterns of juveniles and adults of this species. There are, however, regional studies reporting the presence of all life cycles stages both in Azores and Canaries, which indicated some degree of individual retention on those regions (Menezes et al., 2006; Tuset et al., 2019). Moreover, the Macaronesia region is characterized by habitat discontinuities, different upwelling systems and ecotones between areas with different environmental properties (e.g. Stefanni et al., 2015). These features could act as temporal retention mechanisms to the free movement and dispersal of larvae and/or adults and in some instances, such mesoscale phenomena are responsible for maintaining discrete population units over a species' range (Almada et al., 2017; González-Wangüemert et al., 2010).

Furthermore, similar reproductive seasons were reported for *T. picturatus* in the Macaronesia, but different growth rates were detected among the Azores, Madeira and Canaries (Garcia et al., 2015; Isidro, 1990; Jurado-Ruzafa and Santamaría, 2011, 2013; Vasconcelos et al., 2006, 2017).

The conceptual framework of metapopulations as intraspecific scenarios where the temporal reproductive dynamics of a given subpopulation is reciprocally or unidirectional influenced by the rest of the conspecific subunits is now revealing valid for many fish stocks (e.g. Pita et al., 2016). Modern population genetic analyses allow testing the genetic integrity of metapopulations so current genotyping technologies are useful at delineating stock boundaries in space and time, testing the temporal stability of genetic structures and inferring inter-stock dispersal rates. In this regard, the application of phenotypic tags (*i.e.*, parasites occurrence, body geometric morphometrics, otolith chemical and shape signatures) to *T. picturatus* allowed to suggest the existence of different subpopulation units in the NE Atlantic (Costa et al., 2012, 2013; Hermida et al., 2015; Vasconcelos et al., 2017, 2018; Moreira et al., 2018, 2019a). Moreover, the phenotypic variability observed in *T. picturatus* otoliths from the Canaries has revealed the possibility of co-existence of migratory and resident contingents with different migratory skills (Tuset et al., 2019). Still, as expected for species with large population sizes and high migratory movements, recent mtDNA studies reported a lack of genetic structure for *T. picturatus* from the Mediterranean and the Portuguese coast as well as from Macaronesia (Karaiskou et al., 2003, 2004; Moreira et al., 2019b).

This study employed a panel of microsatellite markers developed from other *Trachurus* spp. genomes to help to clarify the apparent incongruence among studies and markers regarding the genetic structure of *T. picturatus*. In the absence of any stable genetic structure of this fishery, it was also attempted to determine the migration intensity and the connectivity patterns of this species in the NE Atlantic.

Material and Methods

Samples of 306 *T. picturatus* were obtained during May and June 2013 (n = 144) and October to December 2016 (n = 162) from commercial ports at seven fishery grounds in the NE Atlantic (Azores, Madeira, Canaries), in Portugal mainland (Matosinhos, Peniche, Portimão) and in the Mediterranean Sea (Sicily) (Figure 1). All specimens were mature adults of similar size (total length range from 24 to 27 cm) across locations and years. Individual pectoral fins were stored in 96% ethanol and kept at -20°C until processing. Genomic DNA was extracted following a high-salt protocol (Sambrook et al., 1989).

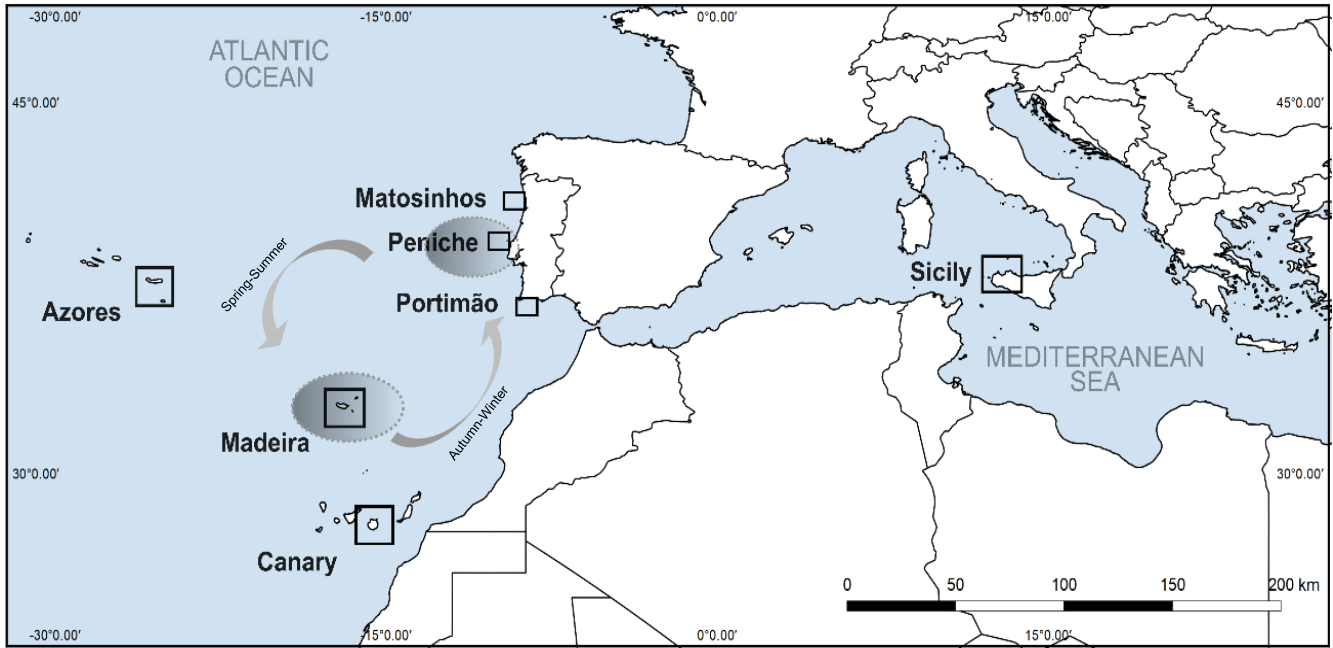


Figure 1: Fish grounds and sample codes of *Trachurus picturatus* sampled in 2013 and 2016 in Macaronesia: Azores (AZ; n = 25; 37°42'6.39"N 25°29'15.12"W); Madeira (MD; n = 25; 32°42'18.79"N 16°56'52.75"W); Canaries (CN; n = 25; 27°48'42.12"N 15°35'6.33"W), in Portugal Mainland: Matosinhos (MT; n = 25; 41°10'49.50"N 8°42'11.25"W); Peniche (PE; n = 25; 39°21'51.11"N 9°23'56.58"W); Portimão (PT; n = 25; 37° 6'14.20"N 8°31'25.99"W) and in 2016 in the Mediterranean Sea: Sicily (SI; n = 17; 37°48'10.8"N 12°25'17.4"E). Putative migration routes indicated by arrows proceeding from Macaronesia to Portugal mainland in autumn-winter and the way back in spring-summer.

Amplification trials of 26 microsatellite primer pairs designed in previous studies on *Trachurus* spp. were carried out on *T. picturatus* following the PCR reaction conditions described originally. Sixteen microsatellites of *T. trachurus* were taken from Cabral (2015; 12 markers, unpublished data) and from Kasapidis and Magoulas (2008; 4 markers). Ten markers of *T. murphyi* were taken from Cárdenas et al. (2009; 1 marker) and from Galleguillos et al. (2012; 9 markers) (Table 1). Sixteen out of the 26 markers amplified in *T. picturatus* but only 12 showed an adequate resolution and polymorphism on a subset of samples. Two hexaplex-PCR reactions were designed and calibrated (upon amplicon size, dye compatibility, primer compatibility, PCR conditions, and co-amplification quality) to improve the genotyping throughput as well as cost-effectiveness in 306 *T. picturatus* individuals. Multiplexed PCR reactions were performed in a DNA Engine Dyad® Peltier Thermal Cycler (Bio-Rad Laboratories) and consisted of a denaturing step at 95°C for 15 min, followed by 9 cycles of denaturation at 95°C for 30 s, 1 min 30 s of 0.5°C touchdown annealing at 58°C and 30 s elongation at 72°C, followed by 31 cycles of denaturation at 95°C for 30 s, annealing at 54°C for 1 min, and extension at 72°C for 30 s. A final extension step was enforced at 60°C for 30 min. A GTTT-sequence 'pig-tail' tag was added to the 5' end of all reverse primers (to reduce

stutter bands, in accordance with Brownstein et al., 1996) and the fluorescent dyes NED, VIC, PET, or 6FAM (Applied Biosystems, CA) were anchored to the forward primers according to the multiplex design (Table 1). Alleles were scored using GeneMapperW v4.0 (Applied Biosystems).

The number of observed alleles (N_A), private alleles (N_{PA}), expected (H_E) and observed (H_O) heterozygosity, the inbreeding coefficient (F_{IS}), Hardy-Weinberg equilibrium (HWE) probability test, allele size range (A_r), and allelic richness (A_R) were estimated with the software GENETIX v4.05.2 (Belkhir et al., 1996-2004). The putative co-segregation of null alleles was tested for each locus using MICRO-CHECKER v2.2.3 (van Oosterhout et al., 2004) and the significance of their frequency was assessed with 1000 permutations. The impact of null alleles on differentiation indexes was assessed by comparing F_{ST} estimates before and after correction for null alleles using the ENA (Excluding Null Alleles) method implemented in FREENA (Chapuis and Estoup, 2007). Null alleles were found to have a negligible impact on F_{ST} estimates, hence all subsequent analyses were conducted on uncorrected data. GENEPOP v4.7.0 (Rousset, 2008) was used to test deviations from the Hardy–Weinberg equilibrium (HWE) with significance being estimated with the Markov Chain Method after 10000 randomization steps. F_{ST} values of genetic differentiation between samples from 2013 and 2016 at each sampling site were calculated with FSTAT v.2.9.3 (Goudet, 1995) and their significance was assessed after 1000 permutations. After this preliminary analyses, fishing grounds from different sampling years could be combined and analysed provided there was no significant genetic differentiation in temporal samples within sampling site. Differences of genetic parameters (H_O , H_E , F_{IS}), among regions and among fishing grounds were tested with 1000 permutations of samples and individuals at the corresponding population level, using the same software. The Analysis of Molecular Variance (AMOVA), implemented in ARLEQUIN 3.5.2.2 (Excoffier and Lischer, 2010) was applied to explore the distribution of the molecular variance at two hierarchical frameworks, *i.e.* the spatial and the temporal.

The method of Luikart et al. (1998) as implemented in BOTTLENECK v.1.2.02 (Piry et al., 1999) was applied to test the excess of heterozygotes that would indicate a recent bottleneck in the NE Atlantic *T. picturatus* population. BOTTLENECK was run under the two-phase mutation model (TPM) which incorporates elements of the stepwise mutation (SMM) and the infinite allele model (IAM). This model is believed to provide the best fit for most microsatellite loci (Di Rienzo et al., 1994) and was run with the proportion of SMM set at 70% and a default variance of 30 among multiple steps. The significance of the heterozygote tests was assessed using Wilcoxon sign-rank test after 10000 iterations.

The inter-individual relationships upon molecular dissimilarity were visualized in a bi-dimensional space using Principal Coordinates Analysis (PCoA) available from GenAlEx 6.5 (Peakall and Smouse, 2012). The most probable number of gene pools (k) in the data set as

well as within years was inferred after 10^7 interactions under the spatial model and the uncorrelated frequency model implemented in GENELAND 3.2.4 (Guillot et al., 2008). The number of gene pools of *T. picturatus* in the NE Atlantic was also explored with the maximum likelihood approach from STRUCTURE v.2.3.4 (Pritchard et al., 2000) by implementing 15 independent runs for each putative k-value (1 to 7), a burn-in period of 10^6 iterations, followed by 5×10^6 Markov Chain Monte Carlo (MCMC) iterations. Correlated allele frequencies and admixed populations were assumed. The most likely k-value was selected upon the best posterior probability of the data.

Migration rates among fish grounds were explored using the MCMC algorithm implemented in BayesAss 3.0 (Wilson and Rannala, 2003). Independent preliminary runs of 5×10^6 iterations, a 2×10^6 burn-in threshold and a 10^3 sampling interval were enforced to adjust mixing parameters (δM ; δA , δF) to the recommended rejection rate. Three larger independent runs of 2×10^7 iterations, a 5×10^6 burn-in window and a 10^3 sampling interval were enforced to test concordance among posterior probabilities as well as convergence among runs. A final larger run was performed per year using 7×10^7 iterations, a 3×10^7 burn-in window and a 10^4 sampling interval.

Results

Genetic diversity

Twelve polymorphic loci were clustered in two hexaplex-PCR resulting in 303 alleles, ranging from 11 (TchM22) to 50 alleles (Tt74) (Table 1). Five loci deviated from HWE towards a heterozygote deficit and three loci showed a significant probability of containing null alleles; those loci with a putative null allele frequency higher than 10% (Tt133 and TchM22) were excluded from further analyses (Table 1).

Comparison of diversity parameters across loci showed no statistical differences among fish grounds or between years, except a marginal HW disequilibrium in the Azores (Table 2). Expected heterozygosity ranged from 0.823 (Sicily) to 0.869 (Azores and Portimão), while observed heterozygosity ranged from 0.812 (Sicily) to 0.880 (Matosinhos; Table 2). The mean inbreeding coefficient varied from -0.008 (Matosinhos) to 0.061 (Madeira; Table 2). Mean allelic richness was similar among sampling sites, with a minimum of 10.827 alleles for Sicily and a maximum of 12.118 alleles for Madeira. Levels of polymorphism varied from 92 alleles (Matosinhos) to 342 alleles (Azores; Table 2). Testing of a putative heterozygosity excess (Wilcoxon test, $p > 0.05$) and three mutation models (IAM, SMM and TPM) revealed no recent Bottleneck events.

Table 1: Characteristics of 12 polymorphic microsatellite loci assayed in two hexaplexes (Mix A, Mix B) on *Trachurus picturatus*. Na, No. of observed alleles per locus; HE, expected heterozygosity; Ho, observed heterozygosity; HWE, Hardy-Weinberg Equilibrium exact test (p-value); NA, null allele frequency (Brookfield method) and its statistical significance: * $p < 0.05$, ** $p < 0.001$.

Locus	Repeat	Primer Sequence 5'-3'	Genbank Acc. No.	Dye	Primer [μM]	Priming T (°C)	MgCl ₂ (mM)	Na	Allele size (bp)	HE	Ho	HWE	NA
Mix A													
TchM04 ^a	(TG) ₁₈	F: AGAGTACGCTCAAAGGTGCAG R: GCCAAGAATAGCACAGGGTG	MK890236	NED	0.4	57	3	21	112-164	0.815	0.816	0.748	-0.006
TchM02 ^a	(AC) ₁₇	F: CTATCCAGGCAATGGGAGAC R: GACATTAATTTAAATGCCCTGTGA	MK890237	NED	0.4	54	3	17	181-213	0.773	0.757	0.599	0.003
TchM03 ^a	(GA) ₁₈	F: CAGAGACAGACAGCTAAAGGGA R: GAGAGCAGCTGTAAGGCCAG	MK890238	PET	0.6	57	3	36	134-206	0.926	0.911	0.164	0.006
TchM12 ^a	(TCA) ₁₉	F: CAGGGACATCTATGGACCAGAT R: TGTCCAGACATGAATGATTAAGAAA	MK890239	VIC	0.4	54	3	21	265-342	0.863	0.893	0.522	-0.015
TmurD112 ^b	(CTAT) ₈	F: CAGAGGACAACCTAACGGAGTC R: GACCTAATGCTGGTTTACAGTG	GU562617	FAM	0.4	57	3	21	230-310	0.923	0.897	0.521	0.016
Tt133 ^c	(AC) ₁₁	F: GATCTCAGACTCTCCACCGTA R: AGCACATCCACACACTTCC	EU200751	VIC	0.5	57	3	29	130-196	0.878	0.299	0.001**	0.325**
Mix B													
TchM05 ^a	(AC) ₁₈	F: AAATGTGAATCCATACATGAGTCC R: AACTCAGCAGGACAGCAACA	MK890240	VIC	0.4	57	3	24	101-153	0.927	0.879	0.347	0.025
TchM08 ^a	(CA) ₁₈	F: TCATGGGACCAAGCTAATCC R: CTGTGTTGGTCAGTCTGTTGG	MK890241	FAM	0.4	54	3	13	95-121	0.722	0.708	0.350	0.004
TchM14 ^a	(TG) ₁₉	F: CTTGTTAAAACACACCAGACCAA R: TTCTATCAGTCCAGCTCTGCTC	MK890242	NED	0.4	57	3	43	143-281	0.910	0.841	0.001**	0.036
TchM18 ^a	(ATC) ₂₁	F: GGACATTTTCAGCCCCAGTT R: TCAGAGGAGGCAACACAACA	MK890243	NED	0.4	54	3	17	92-140	0.870	0.812	0.002*	0.033
TchM22 ^a	(GAG) ₂₃	F: AATGAGACCCCAGGTGAGAA R: CGTCTTCTTCTCCTCGTCTT	MK890244	FAM	0.4	54	3	11	128-158	0.805	0.551	0.001**	0.126**
Tt74 ^d	(AC) ₁₉	F: TGAGCATCAAATGGCAGC R: GACGCAGAAATAGACTGACGG	FJ936110	PET	0.6	57	3	50	125-235	0.967	0.835	0.001**	0.069*

^aMicrosatellite marker from *T. trachurus* (Cabral et al., 2015; PTDC/MAR/117084/2010 BIOCONNECT 2012-2015); ^bMicrosatellite marker from *T. murphyi* (Galleguillos et al., 2012);

^cMicrosatellite marker from *T. trachurus* (Kasapidis and Magoulas, 2008); ^dMicrosatellite marker from *T. murphyi* (Cárdenas et al., 2009)

Table 2: Genetic variation in 10 microsatellites genotyped in six fish grounds of the Northeast Atlantic and one of the Mediterranean Sea *Trachurus picturatus* population in 2013 and 2016. N, number of specimens; N_A, number of alleles per sample; N_{PA}, number of private alleles per sample; H_E, expected heterozygosity; H_O, observed heterozygosity; F_{IS}, inbreeding coefficient; HWE, Hardy–Weinberg probability test (exact p-values); A_r, allelic size range; A_R, allelic richness.

Sample	N	N _A	N _{PA}	H _E	H _O	F _{IS}	p(HWE)	A _r (bp)	A _R
Azores	48	175	7	0.869	0.846	0.038	0.050	95-342	11.853
Madeira	49	185	10	0.856	0.814	0.061	0.113	95-318	12.118
Canaries	49	169	6	0.860	0.824	0.052	0.397	95-321	11.613
Matosinhos	50	179	6	0.864	0.880	-0.008	0.607	92-324	12.057
Peniche	46	162	0	0.860	0.830	0.046	0.555	95-318	11.375
Portimão	47	179	13	0.869	0.838	0.046	0.187	95-321	12.018
Sicily	17	123	4	0.823	0.812	0.045	0.222	98-324	10.827

Genetic differentiation

The pairwise F_{ST} values showed no significant genetic differentiation between years at each fishing ground. Subsequently, the fishing grounds from the different sampling years were combined and analysed together. All pairwise F_{ST} results were between -0.0034 and 0.0038, showing no genetic differentiation among fishing grounds (Table S1). The AMOVA analysis showed that ~99.80% of the variance was allocated within samples and that both variance distribution and fixation index were non-significant at any hierarchical level tested (Table 3). Each of the two main axes of the PCoA plot explained ~4% of the variation among individuals and a large overlap was observed among all samples (Figure 2). The most likely number of gene pools as inferred with the Bayesian methods implemented in GENELAND and STRUCTURE was $k = 1$ for the entire data set as well as within years. In spring 2013, Madeira (MD, Macaronesia) was the unique source exhibiting a significant posterior mean migration rate per generation, *i.e.* $m_{ij} = 28\%$, towards the rest of fish grounds. In autumn 2016, the significant migrant source to the rest of fish grounds was the locality of Peniche (PE), off Portugal mainland (Table 4).

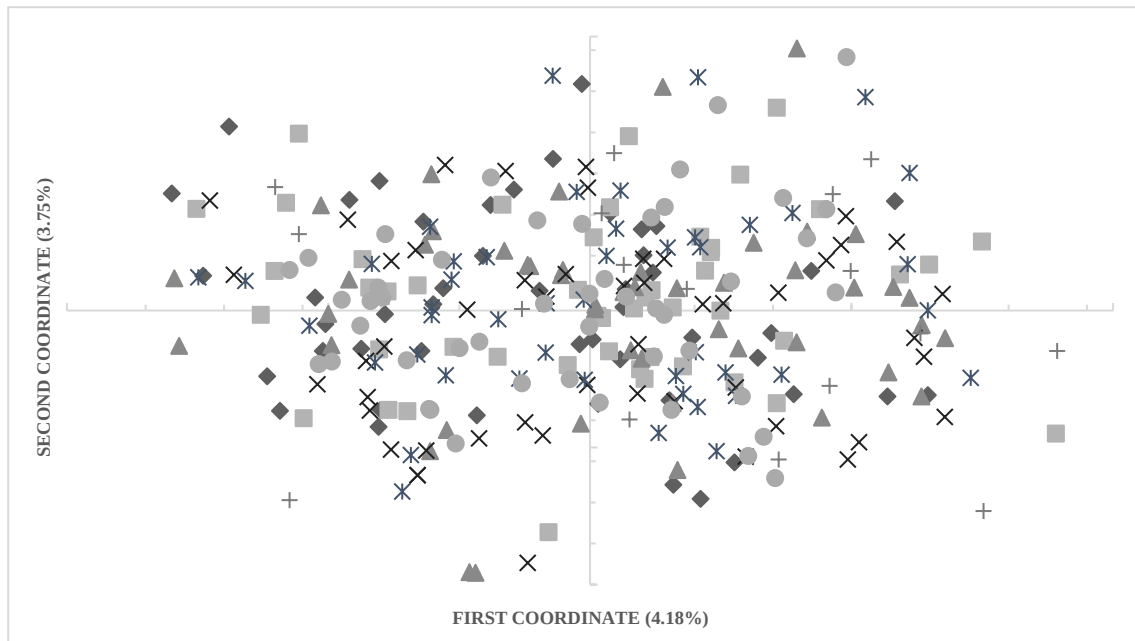


Figure 2: Bi-dimensional PCoA plot of the inter-individual relationships upon their molecular dissimilarity conducted on *Trachurus picturatus* individual gene frequencies. ♦ Azores; ■ Canaries; ▲ Madeira; × Matosinhos; ✱ Peniche; ● Portimão; + Sicily.

Table 3: Analysis of the Molecular Variance (AMOVA) on basins, years and fish grounds of *Trachurus picturatus* from the Northeast Atlantic.

Level	Groups	Source of variation	d.f.	% variation	Fixation indexes ($p \geq 0.05$)
Basins	Atlantic and Mediterranean	Among regions	1	0.20	$F_{CT} = 0.0019$
		Among samples within regions	5	0.04	$F_{SC} = 0.0004$
		Within samples	605	99.76	$F_{ST} = 0.0024$
	Macaronesia, Portugal mainland, and Mediterranean Sea	Among groups	2	0.05	$F_{CT} = 0.0005$
		Among samples within groups	4	0.03	$F_{SC} = 0.0003$
		Within samples	605	99.91	$F_{ST} = 0.0008$
2013	Atlantic Ocean	Among samples	5	0.34	$F_{ST} = 0.0034$
		Within samples	282	99.66	
	Macaronesia and Portugal mainland	Among groups	1	-0.08	$F_{CT} = -0.0007$
		Among samples within groups	4	0.39	$F_{SC} = 0.0039$
		Within samples	282	99.69	$F_{ST} = 0.0031$
2016	Atlantic and Mediterranean	Among regions	1	0.14	$F_{CT} = 0.0014$
		Among samples within regions	5	0.16	$F_{SC} = 0.0015$
		Within samples	282	99.70	$F_{ST} = 0.0030$
	Macaronesia, Portugal mainland, and Mediterranean Sea	Among groups	2	0.04	$F_{CT} = 0.0004$
		Among samples within groups	4	0.16	$F_{SC} = 0.0016$
		Within samples	321	99.80	$F_{ST} = 0.0020$
	Macaronesia and Portugal mainland	Among groups	1	0.00	$F_{CT} = 0.0001$
		Among samples within groups	4	0.20	$F_{SC} = 0.0019$
		Within samples	288	99.80	$F_{ST} = 0.0020$

Table 4: Posterior mean migration rates per generation and standard deviation ($\pm$ SD) of the marginal posterior distribution of the estimate. Sample codes are given in Fig. 1. M-rates indicate the fraction of receptor samples in the first column that are migrants from donor samples in the first row. Diagonal cells contain internal migrants (m_{ii}) with the prior constraint that $m_{ii} \geq 2/3$ ($m_{ij} \leq 1/3$), therefore, the inner connectivity is constrained to be significant and m_{ij} is expectedly underestimated in all cases.

	AZ	MD	CN	MT	PE	PT	SI
2013							
AZ	0.678 $\pm$ 0.011	0.276 $\pm$ 0.022	0.011 $\pm$ 0.011	0.012 $\pm$ 0.0111	0.011 $\pm$ 0.011	0.012 $\pm$ 0.011	-
MD	0.012 $\pm$ 0.011	0.944 $\pm$ 0.023	0.012 $\pm$ 0.011	0.011 $\pm$ 0.0107	0.011 $\pm$ 0.011	0.011 $\pm$ 0.011	-
CN	0.011 $\pm$ 0.010	0.280 $\pm$ 0.021	0.677 $\pm$ 0.010	0.010 $\pm$ 0.0100	0.011 $\pm$ 0.011	0.011 $\pm$ 0.010	-
MT	0.011 $\pm$ 0.011	0.279 $\pm$ 0.021	0.011 $\pm$ 0.011	0.678 $\pm$ 0.0107	0.011 $\pm$ 0.011	0.011 $\pm$ 0.010	-
PE	0.010 $\pm$ 0.010	0.279 $\pm$ 0.022	0.011 $\pm$ 0.011	0.011 $\pm$ 0.0100	0.678 $\pm$ 0.010	0.011 $\pm$ 0.011	-
PT	0.012 $\pm$ 0.011	0.273 $\pm$ 0.024	0.012 $\pm$ 0.012	0.012 $\pm$ 0.0118	0.012 $\pm$ 0.011	0.678 $\pm$ 0.012	-
2016							
AZ	0.677 $\pm$ 0.009	0.010 $\pm$ 0.010	0.011 $\pm$ 0.011	0.010 $\pm$ 0.009	0.270 $\pm$ 0.022	0.011 $\pm$ 0.010	0.010 $\pm$ 0.009
MD	0.010 $\pm$ 0.009	0.677 $\pm$ 0.009	0.010 $\pm$ 0.010	0.010 $\pm$ 0.010	0.271 $\pm$ 0.022	0.010 $\pm$ 0.010	0.011 $\pm$ 0.010
CN	0.011 $\pm$ 0.010	0.010 $\pm$ 0.010	0.677 $\pm$ 0.011	0.011 $\pm$ 0.010	0.270 $\pm$ 0.023	0.011 $\pm$ 0.010	0.010 $\pm$ 0.010
MT	0.010 $\pm$ 0.009	0.010 $\pm$ 0.009	0.010 $\pm$ 0.010	0.677 $\pm$ 0.010	0.271 $\pm$ 0.022	0.010 $\pm$ 0.010	0.010 $\pm$ 0.010
PE	0.012 $\pm$ 0.012	0.012 $\pm$ 0.011	0.012 $\pm$ 0.012	0.012 $\pm$ 0.011	0.929 $\pm$ 0.025	0.012 $\pm$ 0.011	0.019 $\pm$ 0.012
PT	0.010 $\pm$ 0.009	0.011 $\pm$ 0.010	0.010 $\pm$ 0.010	0.010 $\pm$ 0.010	0.272 $\pm$ 0.022	0.677 $\pm$ 0.009	0.010 $\pm$ 0.009
SI	0.014 $\pm$ 0.014	0.014 $\pm$ 0.013	0.014 $\pm$ 0.014	0.014 $\pm$ 0.013	0.249 $\pm$ 0.029	0.014 $\pm$ 0.013	0.681 $\pm$ 0.013

Discussion

This first assessment of the population genetic structure of *T. picturatus* using microsatellites revealed a lack of spatio-temporal genetic structure (constant or sporadic). In this context, the second major contribution of genetic markers is to allow tracking the connectivity patterns in terms of intensity, direction, and frequency (Pita et al., 2016). Despite the weak statistical support induced by the low inter-sample genetic divergence, current data suggest a large multidirectional migration ($m \geq 0.30$) across a NE Atlantic longitudinal corridor. Such high migration intensity would connect Macaronesia islands (mainly the Madeira Archipelago) with Portugal mainland in autumn and *vice-versa* in late spring. Nevertheless, many other properties of the connectivity pattern remain elusive in absence of more appropriate spatio-temporal sampling designs, such as the migration frequency and its timing, the migrants' life-stage and the precise connectivity pathways along the fish life-cycle.

Gene diversity and differentiation

Eighty per cent of the ten polymorphic loci which amplified in *T. picturatus* were described from *T. trachurus*, as expected from their phylogenetic proximity within *Trachurus* genus (Cárdenas et al., 2005). The high genetic diversity of microsatellites was uniformly distributed among locations ($H_E > 0.82$) as has also been observed in other pelagic marine fish such as *Sardina pilchardus* (Ruggeri et al., 2013).

The lack of significant divergence of genetic parameters among fish grounds or between years suggests a high rate of mixing with random mating among sampled sites and excluding any recent population bottleneck in this species. The absence of differential distribution of the molecular variance among hierarchical levels, as well as the low genetic dissimilarity among individuals, suggest that the samples analysed belong to a single panmictic population spread across the NE Atlantic and the Mediterranean. More precisely, the negligible F_{ST} genetic distance between samples ($\bar{F}_{ST} = 0.0013 \pm 0.0020$) as well as the $k = 1$ gene pools depicted with Bayesian clustering approach also reinforce the lack of genetic partitioning in that area. Congruently, the same scenario has been reported for other congeneric pelagic fish such as the horse mackerel *Trachurus trachurus* (Kasapidis and Magoulas, 2008).

The connectivity patterns of the T. picturatus metapopulation

Some species with similar biology to *T. picturatus* have shown signs of isolation patterns either due to past geological events (*i.e.*, glaciations in the Pleistocene) such as the

European anchovy (*Engraulis encrasicolus*) and the European sprat (*Sprattus sprattus*) in the Mediterranean (Magoulas et al., 1996; Debes et al., 2008), to isolation by distance processes such as that shown by the European sprat in the Atlantic, the Mediterranean and the Baltic Sea (Limborg et al., 2012a) or even theoretically to adaptive divergence as reported in the Atlantic herring (*Clupea harengus*) from the North Sea (Limborg et al., 2012b). However, the current assessment of the population genetic structure of *T. picturatus* using microsatellites revealed a lack of genetic structure in space (NE Atlantic and the Mediterranean) and time (2013 and 2016) which is consistent with recent mtDNA observations on the same fish grounds (Moreira et al., 2019b). Such absence of genetic structuring in many marine fishes is commonly associated with two factors, *i.e.* the large effective population size that narrows the impact of the genetic drift (DeWoody and Avise, 2000) and life history features that promote dispersal and high gene flow among populations, *e.g.* passive dispersal of planktonic larvae and/or juveniles, and/or active migration of adults. Like other marine species (*e.g.*, *Merluccius merluccius*, Pita et al., 2016; *Sardina pilchardus*, Correia et al., 2014), *T. picturatus* exhibits those life-history traits and hence an expected metapopulation scenario, *i.e.* a high season-related gene flow among fish grounds which prevents any stable genetic drift among them. Noteworthy, current microsatellite data do not support the existence of previous discrete population units reported within *T. picturatus* from the NE Atlantic, *e.g.* Azores, Canary Islands, Madeira, and Portugal mainland, as depicted with ecological approaches and parasite markers (Costa et al., 2012, 2013; Hermida et al., 2015; Vasconcelos et al., 2017), geometric morphometrics (Vasconcelos et al., 2018) and otolith chemical and shape signatures (Vasconcelos et al., 2018; Moreira et al., 2018, 2019a). Such apparent incongruence among population markers likely relates to the evolutionary time-scale at which a given marker variates. For instance, genetic markers are more conservative at broader spatio-temporal frames than markers based on environmentally-dependent phenotypic traits. Those latter, such as parasitic, morphometric, chemical and physical tagging labels, usually exhibit a high variance provided the various environments experienced by an individual over its lifetime (*e.g.* Elliot et al., 1995; Tanner et al., 2014). For instance, the recently reported pattern of co-existing migratory and resident contingents of *T. picturatus* in the Canary Islands, associated with batch spawning, recruitment and feeding movements (Tuset et al., 2019) is a good scenario illustrating those discrepancies.

The high gene flow assumed among all fish grounds for *T. picturatus*, despite more than 3000 km separating the Azores from Sicily, is congruent with the multidirectional connectivity depicted in this metapopulation with Bayesian-inferred migration rates, *e.g.* from Madeira to Portugal mainland (likely in late autumn) and from Portugal mainland to Macaronesia (likely in late spring). Those population movements could be driven off and/or facilitated by the well-characterized oceanic currents typified in the NE Atlantic, such as the

Azores Current (AC), the Portugal Current (PC) and the Canary Current (CC) (Lamas et al., 2010; Sala et al., 2013). Regarding the AC, one of the branches of this current flows northern in the direction of the Gulf of Cadis, and the other one flows southwards, in the direction of Madeira and Canaries. As for the PC current, one of the branches move from the Portuguese continental coast and the Azores, possibly carrying individuals from the Portuguese coast to Azores and then to the other islands through the AC, while the other branch flows poleward along the Iberian Peninsula during fall, winter and early spring and southward during late spring and summer (Martins et al., 2002). Even though little information is available on the biology of the species, the spawning period of *T. picturatus* is known to occur between January and March/April in all sampling locations (Garcia et al., 2015; Jurado-Ruzafa & Santamaría, 2011, 2013; 2017). This means that individuals (both larvae and/or adults) could be transported by these systems in spring-summer from the Portuguese coast to open waters, reaching the Macaronesia Islands, and in the opposite direction in autumn-winter, which is congruent with the migration patterns suggested in this study. Although experimental evidence is soundly lacking on such synergy.

Regardless the forces determining connectivity among *T. picturatus* fish grounds, the population genetic analyses obtained from mtDNA markers (Moreira et al., 2019b) and the current microsatellites show that restrictions to gene flow are not existent. Obstacles like the Almeria-Oran oceanographic front that act as active barriers to connectivity for some marine invertebrates (e.g. *Mytilus galloprovincialis*, Diz and Presa, 2008) and some fish (e.g., *Diplodus sargus*, González-Wangüemert et al., 2010), or the existence of local eddies that facilitate larvae and/or juveniles/adults retention resulting in the establishment of “local stocks” (e.g. Francisco et al., 2009; Almada et al., 2017) in Macaronesia, are no functional on *T. picturatus*.

Conclusion

The lack of genetic differentiation within the *T. picturatus* metapopulation from the NE Atlantic and the Mediterranean does not provide evidence of any stable structure into meaningful stocks. Moreover, the fact that all *T. picturatus* life stages can be found in Azorean waters as well as in Canarian waters can lead to confounding local spawning aggregations with true stable stocks.

Such a misleading view from an inter-seasonal fading genetic drift occurs when population structuring is inferred from an instantaneous molecular picture on a single sample, better than upon a factorial spatio-temporal sampling design.

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Chapter 3

MORPHOMETRICAL ANALYSES

Article 3. Landmark-based geometric morphometrics analysis of body shape variation among populations of the blue jack mackerel, *Trachurus picturatus*, from the North-East Atlantic.

Article 4. Otolith shape analysis as a tool to infer the population structure of the blue jack mackerel, *Trachurus picturatus*, in the NE Atlantic.

Landmark-based geometric morphometrics analysis of body shape variation among populations of the blue jack mackerel, *Trachurus picturatus*, from the North-East Atlantic.

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Abstract

Phenotypic variation in fish can indicate exposure to different environmental conditions, affecting species growth and maturation rates and/or be a consequence of genetic factors that allow fish adaptation to different environments. Understanding population structure and dynamics is extremely important for the establishment of sustainable fisheries. The blue jack mackerel, *Trachurus picturatus*, is an important fishery resource of the North-East (NE) Atlantic. Fish were captured in six sampling locations in the NE Atlantic - Azores, Madeira, Canaries and Portugal mainland - Matosinhos, Peniche and Portimão. The phenotypic diversity of the species was studied using body landmark-based geometric morphometrics. A truss box method was followed and 30 distance variables were extracted from digital images. Additionally, the temporal stability of the morphological characters was investigated by comparing samples from two different years: 2016 and 2019. The transformed distances were analysed by univariate and multivariate statistics. The Macaronesia region – Azores, Madeira and Canaries – and Portugal mainland displayed a clear phenotypic pattern. Distances related

to the body width and caudal peduncle were the most significant variables explaining the observed phenotypic heterogeneity. Furthermore, this study also showed that *T. picturatus* can exhibit fast phenotypic adaptations to the surrounding environment, observed by the discrimination of Peniche in 2019. Nevertheless, the high overall allocation of individuals to the original location observed for both years (78% in 2016 and 83% in 2019) suggests that the population of *T. picturatus* in the NE Atlantic is phenotypic heterogeneous. These findings corroborate previous studies and have implications for fishery management.

Keywords: Body shape; truss network; morphometric variations; characteristic habitats; phenotypic stocks.

Introduction

Most of the harvest fish stocks are composed of two or more populations of the same species, with very similar traits, that overlap in space and time (Dahle et al., 2018). Mismatch between the biological processes and the realized management actions frequently occur, since a variety of conflicting aspects, such as biological, economic, social and even political factors, need to be considered (Reiss et al., 2009). Currently, management of fish stocks has changed from the simplistic geographical isolation of populations that exhibit identical rates of growth and mortality (FAO, 1998), to include considerations on the complex life traits of a fish (Reis-Santos et al., 2018). Insufficient knowledge of the genetic structure, life history diversity and the biological boundaries of a population will lead to failure in protecting distinct fish stocks (Reis-Santos et al., 2018). Nowadays, stock identification highlights the need for holistic approaches, that consider information across multiple spatial and temporal scales (Cadrin, 2020). Fish stock is generally defined to identify a genotypic and/or a phenotypic group. A genotypic stock implies that individuals are isolated, at least, during the spawning season, and across generations, while a phenotypic stock implies that individual differences occur as a result of genetic or environmental differences during the time when the phenotypic characters are being developed (Hare and Richardson, 2014).

Morphological studies have long been useful to describe fish spatial distributions (Kaouèche et al., 2017). Previous works have demonstrated morphological divergence in the body of several marine fish species (e.g. Hammami et al., 2013; Kaouèche et al., 2017; Vasconcelos et al., 2018). These changes have been associated with the intrinsic specificities of the particular aquatic environment inhabit by the fish (Hammami et al., 2013). Phenotypic variation within a fish species can occur as a result of isolation, environmental variations, thermal reaction, local adaptation and/or a combination of these (Valladares et al., 2014;

Vasconcelos et al., 2018). Different spatial structure within populations can affect the overall dynamics of the species. Growth, maturity, reproduction, recruitment, abundance, and survival of a group of individuals can be greatly influenced by the environmental conditions experienced by these individuals (Abaunza et al., 2008; Cadrin, 2010). Exposure to different environments factors, such as temperature and salinity, food availability or swimming patterns, can result in individuals following different adaptation strategies, and consequently develop different morphometric features between populations (Sajina et al., 2011). Quantification of these specific characteristics in an individual or group of individuals can demonstrate the degree of isolation and contribute to the definition of different phenotypic stocks (Bailey, 1997). A landmark-based technique called the truss network system (Strauss and Bookstein, 1982) has been increasingly employed for the purposes of stock discrimination, essentially describing phenotypic stocks (Sajina et al., 2011). This approach enables covering the entire fish body in a uniform network, with no restrictions on the direction of variation and localisation of shape changes (Rawat et al., 2017). It is a tool highly effective in capturing information about the shape of an individual (Sajina et al., 2011; Kaouèche et al., 2017) and could detect variation among fish populations (Murta et al., 2008; Miyan et al., 2016; Kaouèche et al., 2017).

The blue jack mackerel, *Trachurus picturatus* (Bowdich, 1825), is widely distributed across the North-East (NE) Atlantic, the Mediterranean and Black Seas (Smith-Vaniz, 1986). Although the economic significance of the blue jack mackerel in the Macaronesia islands of Azores and Madeira is recognized, and fluctuations in the catches have been reported (FAO, 2018), the population structure of the species is poorly understood. Furthermore, the International Council for the Exploitation of the Sea (ICES) defined the existence of a single stock for the entire species distribution, simply based on geographical boundaries (ICES, 2018). However, geographical isolation of species with broad distributions, such as *T. picturatus*, can result in the development of different morphological features between fish populations, leading to the discrimination of phenotypic stocks. The genetic structure of the *T. picturatus* was studied by mtDNA in the Mediterranean Sea (Karaïskou et al., 2003, 2004), and both in the Mediterranean Sea and Macaronesia region (Moreira et al., 2019b), suggesting a lack of genetic structure across the species distribution, with high levels of genetic diversity and inbreeding coefficients showing a high rate of mixing. However, regional studies of *T. picturatus* recorded different growth rates among the Azores, Madeira and the Canary Islands (Isidro, 1990; Vasconcelos et al., 2006, 2017; Jurado-Ruzafa and Santamaría, 2011, 2013; Garcia et al., 2015), which could indicate the existence of different population units in the region. Furthermore, other ecological approaches, such as parasites (Costa et al., 2012, 2013; Hermida et al., 2015; Vasconcelos et al., 2017), geometric morphometrics and otolith shape analysis (Vasconcelos et al., 2018), otolith shape analysis (Moreira et al., 2019a), and otolith elemental and isotopic signatures (Moreira et al., 2018), revealed the existence of different

population units in the NE Atlantic. Geometric morphometrics and otolith shape analysis were already applied to study *T. picturatus* populations (Vasconcelos et al., 2018), but as highlighted by the authors, the study area was geographically limited, the collection of individuals did not consider a specific time window, fish were of significantly different size ranges and captured occurred with different fishing methods, which could have somewhat biased the results. To minimize confounding effects of demography, morphometric analyses should be carried out at a time of year when stock mixing is expected to be minimal (e.g. during the spawning season) and the specimens studied should be of similar age or size (Cadrin, 2000; Murta et al., 2008; Heino, 2014). Furthermore, results reported by Moreira et al. (2019a) in a subsequent otolith shape analysis study, revealed that the experimental design set by the previous authors had, in fact, limitations (Moreira et al., 2019a). Therefore, to a better understanding of the population structure of *T. picturatus*, their migration patterns and habitat connectivity, more complete studies are required.

Therefore, the present study aimed to investigate the spatial morphological variation of *T. picturatus* populations in the NE Atlantic using truss network analysis and to assess the temporal stability of the morphological features by comparing two distinct sampling years (2016 and 2019).

Material and methods

Specimens of *T. picturatus* were acquired from commercial fleets at Portugal mainland (Matosinhos, Peniche and Portimão) and at the Macaronesian Islands of Azores, Madeira and Canaries (Figure 1). Fish were caught in shallow coastal waters (up to 75 m water depth) by the artisanal purse-seine fleets to ensure a correct location of the capture. Unfortunately, small fishing boats targeting jack mackerel could suffer several inter-annual constraints, due to adverse weather conditions or by the displacement to other fish species due to market reasons. Anyway, fish were collected for both years (October to December 2016 and February to May 2019) within the main protracted spawning season for the species. Moreover, to minimize confounding demographic effects, individuals of similar length were selected (Table 1). The fish were stored on ice and transported to the laboratory to be processed.

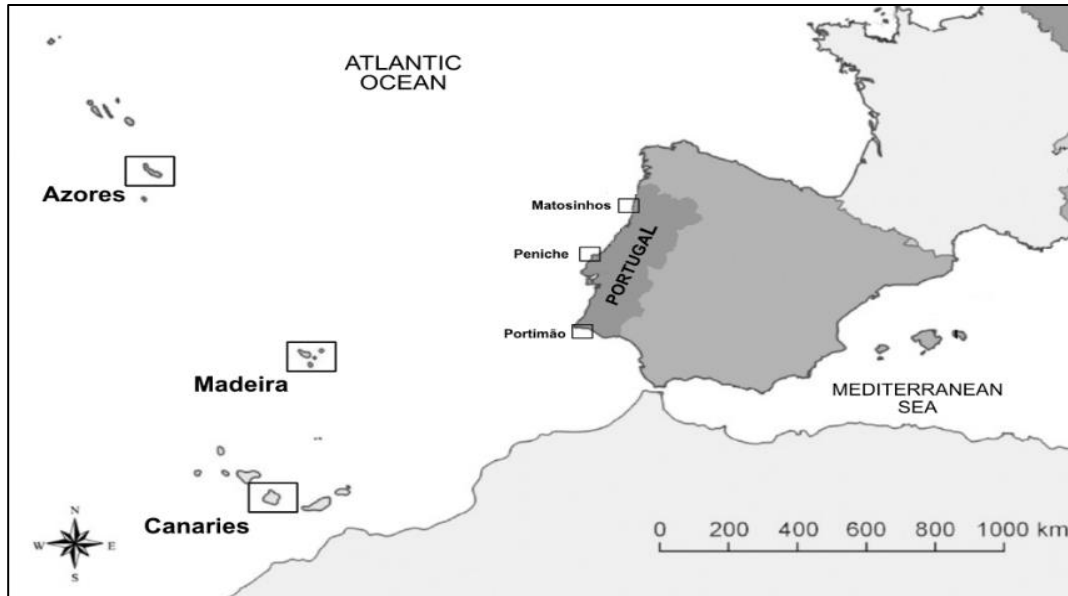


Figure 1: Sampling locations of *Trachurus picturatus* individuals collected in 2016 and 2019 in the Northeast Atlantic Ocean.

Table 1: Sampling locations, year, sample size (n), standard length (SL) of *Trachurus picturatus* used in this study. Values are presented as mean $\pm$ standard error (SE).

Regions	Sites	Sampling location	Year	n	SL (mm)
Azores	-	37°42'6.39"N 25°29'15.12"W	2016	30	21.59 $\pm$ 0.19
			2019	30	23.88 $\pm$ 0.26
Madeira	-	32°42'18.79"N 16°56'52.75"W	2016	30	22.39 $\pm$ 0.03
			2019	30	22.33 $\pm$ 0.20
Canaries	-	27°48'42.12"N 15°35'6.33"W	2016	30	15.23 $\pm$ 0.30
			2019	25	16.66 $\pm$ 0.16
Portugal Mainland	Matosinhos	41°10'49.50"N 8°42'11.25"W	2016	30	24.63 $\pm$ 0.17
			2019	30	24.89 $\pm$ 0.18
	Peniche	39°21'51.11"N 9°23'56.58"W	2016	30	21.69 $\pm$ 0.27
			2019	30	23.06 $\pm$ 0.18
	Portimão	37° 6'14.20"N 8°31'25.99"W	2016	30	23.56 $\pm$ 0.17
			2019	30	22.90 $\pm$ 0.42

For each year and location, a total of 25 to 30 individuals were one-side photographed. The use of this sample size is according to the recommendations for this methodological approach (Reist, 1985), and followed previous works (e.g. Murta et al., 2008; Kaouèche et al.,

2017; Neves et al., 2018). Moreover, it allowed to use the same individuals (namely from 2016) to which we have already otolith's shape analysis (Moreira et al., 2019a) and microsatellites (Moreira et al., 2020) data, allowing a holistic discussion. Individuals of both sexes were analysed together since there is no sexual dimorphism in this species (Vasconcelos et al., 2018), such as in other related species, including *T. trachurus* (Murta et al., 2008) and *T. mediterraneus* (Turan, 2004). Furthermore, a visual inspection of the specimens at the time of sampling did not show any obvious genera morphological differences, like females with protracted abdomens. It means that the existence of any sexual difference was very unlikely in this work. As general rule, including a previous published work on this species (Vasconcelos et al., 2018), for fish bilaterally symmetric, landmarking one side, instead of both, reduces the number of variables, as well as the time and costs of data collection. A total of 16 anatomic landmarks were defined on fish left-side (Figure 2A, Table 2), corresponding to hard structures (e.g. fin insertions points), distributed along the fish body contour (Cadrin, 2000; Murta et al., 2008). Location coordinates of homologous landmarks were digitized using tpsDig (Rohlf, 2018) and used to derive box-truss dimensions (Strauss and Bookstein, 1982; Figure 2B, Table 3). Morphometric measurements correlation with fish standard length was verified using analysis of covariance (ANCOVA). Each distance was corrected to remove the size-effect and possible allometric relationship between variables following Reist (1985) and Kaouèche et al. (2017): $V_{trans} = \text{Log } V - b (\text{Log } SL - \text{Log } SL_{mean})$ where V_{trans} is the corrected measurement, V is the original measurement, b is the within-group slope regression of $\text{Log } V$ against $\text{Log } SL$, SL is the standard length of the individual and SL_{mean} is the overall mean of the standard length for all locations.

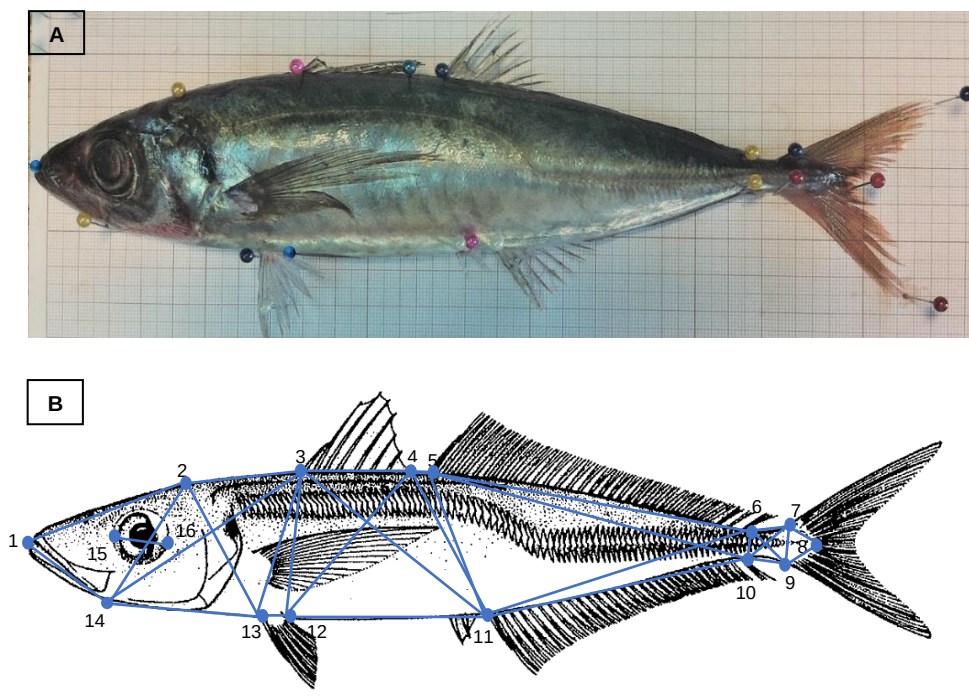


Figure 2: Specimen of *Trachurus picturatus* collected from Madeira in 2019 showing the selected landmarks (A). Illustration of *T. picturatus* (adapted from Sanches, 1992) with indication of the landmarks (1 to 16) and the Truss network represented by the blue lines.

Table 2: Morphometric landmarks of *Trachurus picturatus* used to derive the morphometric distances (see Figure 2).

Landmark	Description
1	Anterior tip of snout
2	Most posterior aspect of neurocranium
3	Origin of dorsal fin
4	End insertion of dorsal fin
5	Origin of second dorsal fin
6	End insertion of second dorsal fin
7	Anterior attachment of dorsal membrane from caudal fin
8	Posterior end of vertebrae column
9	Anterior attachment of ventral membrane from caudal fin
10	End insertion of anal fin
11	Origin of anal fin
12	End insertion of pelvic fin
13	Origin of pelvic fin
14	Posterior most point of maxillary
15	Posterior end of the eye
16	Anterior end of the eye

Table 3: Morphometric distances and associated landmarks for *Trachurus picturatus* (for details see Figure 2 and Table 2).

Truss network Distances	Landmarks	Description
D1	1-2	Head length
D2	1-14	Maxilla length
D3	2-3	Distance from most posterior aspect of neurocranium to 1 st dorsal fin
D4	2-13	Anterior diagonal height of head
D5	2-14	Anterior height of head
D6	3-13	Posterior height of head
D7	3-14	Posterior diagonal head height
D8	13-12	Length of the pelvic fin base
D9	13-14	Distance from maxilla to pelvic fin insertion
D10	3-4	Length of 1 st dorsal fin
D11	3-11	Anterior diagonal of body height
D12	3-12	Anterior body height
D13	4-11	Posterior body height
D14	4-12	Posterior diagonal of body height
D15	11-12	Distance between anal and pelvic fins
D16	4-5	Distance between 1 st dorsal fin and 2 nd dorsal fin
D17	5-6	Length of 2 nd dorsal fin
D18	5-10	Anterior diagonal height of posterior body
D19	5-11	Anterior height of posterior body
D20	6-10	Anterior caudal peduncle height
D21	6-11	Posterior diagonal height of posterior body
D22	10-11	Length of anal fin
D23	6-7	Distance between 2 nd dorsal fin and caudal fin
D24	6-9	Anterior diagonal of caudal peduncle
D25	7-9	Posterior height of caudal peduncle
D26	7-10	Posterior diagonal of caudal peduncle
D27	9-10	Distance between anal fin and caudal fin
D28	7-8	Distance between dorsal insertion of caudal fin and posterior end of vertebrae column
D29	8-9	Distance between ventral insertion of caudal fin and posterior end of vertebrae column
D30	15-16	Eye diameter

One-way analysis of variance (ANOVA) was used to explore the statistical differences of each distance measured in the body shape of *T. picturatus* between the years and among the sampling locations, followed by a Tukey post-hoc test if needed (ANOVA: $p < 0.05$). Multi-distances variations among sampling locations for each year using a permutational multivariate analysis of variance (PERMANOVA) based on the Euclidean distance measure using Monte-Carlo simulations (9999 random permutations). The body truss network was analysed with a stepwise linear discriminant function analysis (LDFA). Re-classification accuracies of the discriminant functions for each location were evaluated using a jackknifed (leave-one-out)

cross-validation. A canonical analysis of principal coordinates (CAP) was performed to detect morphometric differences among locations.

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 Log distances $\pm$ standard errors (SE).

Results

The truss network obtained with the 16 landmarks allowed the calculation of 30 body distances (Figure 2). For both years, all the distances showed a significant correlation with the fish standard length (ANCOVA, $p < 0.05$) and had to be corrected. Correction by allometric transformation successfully removed the effect of body length in all distances (ANCOVA, $p > 0.05$). ANOVA of all the 30 truss measurements showed significant differences between years (ANOVA, $p < 0.05$) and among locations (ANOVA, $p < 0.05$), although with no recognizable pattern (Table 4).

Table 4: Results from the univariate statistics (One-Way ANOVAs, followed by Tukey post-hoc tests, only if $p < 0.05$) applied to the truss measurements of *Trachurus picturatus* for all sampling locations in the year 2016 and 2019. Values are presented as mean and standard errors (SE).

Truss measurements		Azores	Canaries	Madeira	Matosinhos	Peniche	Portimão
2016							
Head Width	D1	4.35 $\pm$ 0.06 ^a	3.39 $\pm$ 0.08 ^b	5.01 $\pm$ 0.11 ^b	4.99 $\pm$ 0.07 ^a	4.59 $\pm$ 0.05	5.02 $\pm$ 0.06
	D2	2.60 $\pm$ 0.03 ^a	2.05 $\pm$ 0.04 ^b	2.90 $\pm$ 0.06 ^{b,c}	2.94 $\pm$ 0.03 ^{a,b,d}	2.66 $\pm$ 0.04 ^{a,b,d}	3.03 $\pm$ 0.04 ^{b,c}
	D5	4.02 $\pm$ 0.06 ^a	2.88 $\pm$ 0.07 ^{a,b}	4.39 $\pm$ 0.08 ^b	4.38 $\pm$ 0.05	4.04 $\pm$ 0.05 ^{a,b,c}	4.38 $\pm$ 0.04 ^{a,b,c}
Body Width	D4	4.82 $\pm$ 0.05	3.10 $\pm$ 0.07	4.86 $\pm$ 0.07 ^a	5.12 $\pm$ 0.04 ^b	4.54 $\pm$ 0.06 ^b	5.09 $\pm$ 0.05 ^a
	D6	4.83 $\pm$ 0.05 ^a	3.01 $\pm$ 0.07 ^b	4.89 $\pm$ 0.08 ^a	5.06 $\pm$ 0.04 ^{b,c}	4.45 $\pm$ 0.05 ^c	5.02 $\pm$ 0.05
	D7	6.68 $\pm$ 0.07 ^a	4.64 $\pm$ 0.11 ^{a,b}	7.13 $\pm$ 0.12 ^b	7.15 $\pm$ 0.06	6.51 $\pm$ 0.09 ^c	7.16 $\pm$ 0.07 ^c
	D11	6.90 $\pm$ 0.06 ^a	4.40 $\pm$ 0.09	6.90 $\pm$ 0.11 ^b	7.78 $\pm$ 0.06 ^{a,c}	6.78 $\pm$ 0.10 ^{b,c,d}	7.27 $\pm$ 0.06 ^{b,d}
	D12	4.72 $\pm$ 0.05 ^a	2.92 $\pm$ 0.07	4.80 $\pm$ 0.08 ^a	4.96 $\pm$ 0.03 ^b	4.35 $\pm$ 0.05 ^b	4.91 $\pm$ 0.05
	D13	5.06 $\pm$ 0.05	3.02 $\pm$ 0.07	4.90 $\pm$ 0.09 ^a	5.44 $\pm$ 0.04 ^a	4.68 $\pm$ 0.06 ^a	5.08 $\pm$ 0.05 ^a
	D14	5.86 $\pm$ 0.06 ^a	3.73 $\pm$ 0.08	6.00 $\pm$ 0.10 ^a	6.34 $\pm$ 0.05 ^b	5.51 $\pm$ 0.07 ^b	6.14 $\pm$ 0.07 ^b
	D18	8.45 $\pm$ 0.09 ^a	5.69 $\pm$ 0.11 ^b	8.54 $\pm$ 0.15 ^b	9.71 $\pm$ 0.08 ^a	8.43 $\pm$ 0.11 ^{a,c}	9.04 $\pm$ 0.08 ^{b,c}
Fin Insertions	D19	4.77 $\pm$ 0.05	2.78 $\pm$ 0.06	4.57 $\pm$ 0.08 ^a	5.01 $\pm$ 0.04 ^a	4.33 $\pm$ 0.06 ^a	4.73 $\pm$ 0.05 ^a
	D3	3.32 $\pm$ 0.05 ^a	2.26 $\pm$ 0.06 ^a	3.42 $\pm$ 0.06 ^{a,b}	3.52 $\pm$ 0.07 ^c	3.15 $\pm$ 0.05 ^b	3.56 $\pm$ 0.06 ^{a,c}
	D8	0.69 $\pm$ 0.02 ^a	0.50 $\pm$ 0.02 ^{a,b}	0.67 $\pm$ 0.01 ^{a,b,c}	0.69 $\pm$ 0.02 ^{a,b,c}	0.67 $\pm$ 0.02 ^{a,b,c,d}	0.78 $\pm$ 0.02 ^{a,c,d}
	D9	4.54 $\pm$ 0.06 ^a	3.10 $\pm$ 0.08 ^{a,b}	4.76 $\pm$ 0.08 ^{a,b}	4.83 $\pm$ 0.05	4.42 $\pm$ 0.07 ^{a,c}	4.80 $\pm$ 0.06 ^c
	D10	3.03 $\pm$ 0.03 ^a	2.02 $\pm$ 0.04	3.10 $\pm$ 0.05 ^a	3.48 $\pm$ 0.05 ^a	3.11 $\pm$ 0.05 ^a	3.32 $\pm$ 0.04 ^a
	D15	5.63 $\pm$ 0.08 ^a	3.88 $\pm$ 0.07 ^{a,b}	5.90 $\pm$ 0.12 ^{a,b,c}	6.69 $\pm$ 0.07 ^{a,c,d}	5.71 $\pm$ 0.11 ^{a,b,c,d,e}	6.14 $\pm$ 0.08 ^{a,b,c,d,e}
	D16	0.75 $\pm$ 0.03 ^a	0.54 $\pm$ 0.01 ^{a,b}	0.79 $\pm$ 0.03 ^{a,b,c}	0.99 $\pm$ 0.03 ^{a,d}	0.83 $\pm$ 0.02 ^{a,b,c,d,e}	0.84 $\pm$ 0.02 ^{a,b,c,d,e}
	D17	8.31 $\pm$ 0.08 ^a	5.62 $\pm$ 0.11 ^b	8.37 $\pm$ 0.15 ^{b,c}	9.56 $\pm$ 0.08 ^{a,d}	8.33 $\pm$ 0.11 ^{a,d,e}	8.94 $\pm$ 0.10 ^{a,b,c,d,e}

	D22	6.90 ± 0.06 ^a	4.82 ± 0.09 ^b	6.96 ± 0.11 ^{b,c}	7.93 ± 0.06 ^{a,d}	6.88 ± 0.09 ^{a,c,e}	7.52 ± 0.06 ^{a,d,e}
	D23	1.14 ± 0.02 ^a	0.83 ± 0.02 ^{a,b}	1.08 ± 0.03 ^{b,c}	1.12 ± 0.02 ^{b,c,d}	0.95 ± 0.02 ^{d,e}	1.05 ± 0.03 ^{b,c,d,e}
	D28	1.30 ± 0.03 ^a	0.84 ± 0.02 ^{a,b}	1.26 ± 0.02 ^{a,b,c}	1.55 ± 0.03 ^{a,b,d}	1.30 ± 0.03 ^{a,b,c,d,e}	1.36 ± 0.03 ^{a,b,c,e}
	D29	1.49 ± 0.03 ^a	1.02 ± 0.03 ^a	1.45 ± 0.03 ^{a,b}	1.75 ± 0.03 ^{a,c}	1.49 ± 0.03 ^{a,b,c,d}	1.58 ± 0.04 ^{a,b,c,d}
Caudal Peduncle	D20	0.88 ± 0.01 ^a	0.58 ± 0.01	0.86 ± 0.01 ^b	0.97 ± 0.01 ^{a,b,c}	0.83 ± 0.01 ^{b,c}	0.95 ± 0.01 ^{a,b,c}
	D21	7.28 ± 0.06 ^a	5.03 ± 0.09 ^b	7.27 ± 0.11 ^b	8.29 ± 0.06 ^a	7.22 ± 0.09 ^a	7.91 ± 0.07 ^a
	D24	1.26 ± 0.02	0.82 ± 0.02 ^a	1.17 ± 0.02 ^a	1.28 ± 0.02 ^{a,b}	1.08 ± 0.02 ^{a,c}	1.17 ± 0.03 ^{a,b,c}
	D25	0.92 ± 0.01 ^a	0.63 ± 0.01 ^{a,b}	0.89 ± 0.02 ^{b,c}	0.94 ± 0.01 ^{b,c,d}	0.80 ± 0.011 ^{d,e}	0.89 ± 0.01 ^{c,d,e}
	D26	1.52 ± 0.02	1.08 ± 0.02 ^a	1.44 ± 0.03 ^a	1.51 ± 0.02 ^{a,b}	1.32 ± 0.02 ^b	1.49 ± 0.02 ^{a,b}
	D27	0.99 ± 0.02	0.68 ± 0.01 ^a	0.90 ± 0.02 ^a	0.94 ± 0.02 ^a	0.84 ± 0.01 ^a	0.92 ± 0.02 ^a
Eye Diameter	D30	1.18 ± 0.02	1.09 ± 0.02 ^a	1.50 ± 0.03 ^a	1.40 ± 0.02	1.41 ± 0.03 ^a	1.58 ± 0.03 ^a

2019		Azores	Canaries	Madeira	Matosinhos	Peniche	Portimão
Head With	D1	5.13 ± 0.06 ^a	4.14 ± 0.05 ^b	5.37 ± 0.07 ^b	5.43 ± 0.05 ^a	5.12 ± 0.06 ^{a,«}	4.95 ± 0.10 ^a
	D2	3.13 ± 0.04 ^a	2.32 ± 0.03 ^a	3.05 ± 0.04 ^a	3.10 ± 0.03	3.08 ± 0.03 ^a	3.04 ± 0.06 ^a
	D5	4.56 ± 0.06 ^a	3.47 ± 0.04 ^b	4.52 ± 0.06 ^b	4.77 ± 0.05 ^{a,b}	4.48 ± 0.05 ^{a,b}	4.31 ± 0.08 ^a
Body Width	D4	5.29 ± 0.07 ^a	3.57 ± 0.04	4.81 ± 0.07 ^{a,b}	5.44 ± 0.04 ^b	5.05 ± 0.05 ^{a,b}	4.88 ± 0.11 ^{a,b}
	D6	5.36 ± 0.08 ^a	3.62 ± 0.05	4.91 ± 0.07 ^a	5.50 ± 0.05 ^{a,b}	5.02 ± 0.05 ^a	4.84 ± 0.12 ^b
	D7	7.30 ± 0.09 ^a	5.09 ± 0.05 ^a	7.01 ± 0.07 ^b	7.68 ± 0.06 ^{a,b}	7.11 ± 0.06 ^{a,b}	6.96 ± 0.13 ^{a,b}
	D11	7.68 ± 0.09 ^a	5.24 ± 0.06 ^b	6.91 ± 0.08 ^c	8.16 ± 0.08 ^{a,b,d}	7.41 ± 0.08 ^{a,b,d}	7.06 ± 0.15 ^c
	D12	5.25 ± 0.07 ^a	3.53 ± 0.05	4.75 ± 0.07 ^{a,b}	5.40 ± 0.05 ^{a,b,c}	4.88 ± 0.05 ^{b,c}	4.71 ± 0.12 ^{b,c}
	D13	5.50 ± 0.07 ^a	3.70 ± 0.05	4.89 ± 0.07 ^b	5.80 ± 0.06 ^{a,c}	5.18 ± 0.07 ^{a,b,c}	4.89 ± 0.12 ^b
	D14	6.53 ± 0.09 ^a	3.35 ± 0.06 ^a	5.83 ± 0.08 ^b	6.70 ± 0.06 ^{a,b,c}	5.92 ± 0.05 ^b	6.01 ± 0.14 ^{a,b,c}
	D18	9.40 ± 0.11 ^a	6.51 ± 0.06 ^{a,b}	8.47 ± 0.10 ^c	9.77 ± 0.08 ^{a,b,d}	8.80 ± 0.09 ^{c,e}	8.66 ± 0.17 ^{c,d,e}
	D19	5.20 ± 0.06 ^a	3.38 ± 0.04 ^a	4.50 ± 0.07 ^b	5.35 ± 0.06 ^{a,c}	4.76 ± 0.06 ^{b,c,d}	4.57 ± 0.11 ^{b,d}
Fin Insertions	D3	3.53 ± 0.06 ^a	2.10 ± 0.04	3.17 ± 0.08 ^{a,b}	3.70 ± 0.04 ^{a,b,c}	3.44 ± 0.06 ^{a,b,c,d}	3.46 ± 0.07 ^{a,c,d}
	D8	0.70 ± 0.01 ^a	0.55 ± 0.01 ^a	0.83 ± 0.02	0.76 ± 0.01 ^a	1.09 ± 0.03	0.68 ± 0.01 ^a
	D9	4.76 ± 0.07 ^a	3.32 ± 0.04 ^b	4.53 ± 0.04 ^{a,b}	5.06 ± 0.07 ^{a,b}	4.63 ± 0.05 ^{a,b}	4.53 ± 0.09 ^{a,b}
	D10	3.44 ± 0.05 ^a	2.26 ± 0.03 ^{a,b}	3.01 ± 0.05 ^{b,c}	3.61 ± 0.06 ^{a,b,d}	3.26 ± 0.05 ^{a,b,c,d,e}	3.24 ± 0.07 ^{a,b,d,e}
	D15	6.38 ± 0.09 ^a	4.50 ± 0.06 ^b	5.89 ± 0.08 ^{a,c}	6.79 ± 0.09 ^{a,b,c,d}	6.05 ± 0.07 ^{a,c,d}	6.17 ± 0.14 ^{a,b,d}
	D16	0.81 ± 0.03 ^a	0.72 ± 0.01 ^b	0.91 ± 0.04 ^{b,c}	1.05 ± 0.04 ^{b,c,d}	0.95 ± 0.04 ^{b,c,d}	0.79 ± 0.02 ^a
	D17	9.30 ± 0.11 ^a	6.42 ± 0.07 ^{a,b}	8.38 ± 0.10 ^c	9.69 ± 0.08 ^{a,b}	8.72 ± 0.08 ^{c,d}	8.56 ± 0.17 ^{c,d}
	D22	7.77 ± 0.09	5.26 ± 0.06 ^a	6.89 ± 0.07 ^b	7.81 ± 0.08 ^{a,b}	7.23 ± 0.08 ^{a,b}	7.10 ± 0.13 ^a
	D23	1.25 ± 0.03 ^a	0.77 ± 0.02	1.13 ± 0.02 ^a	1.28 ± 0.02 ^{a,b}	1.11 ± 0.02 ^b	1.21 ± 0.02 ^{a,b}
	D28	1.11 ± 0.03 ^a	0.68 ± 0.02 ^b	1.02 ± 0.03 ^{a,c}	1.06 ± 0.03 ^{a,b,c,d}	1.04 ± 0.02 ^{a,c,d}	0.93 ± 0.02 ^{b,c,d}
	D29	1.32 ± 0.03 ^a	0.83 ± 0.02	1.25 ± 0.03 ^{a,b}	1.34 ± 0.03 ^{a,b}	1.33 ± 0.02 ^{a,b}	1.18 ± 0.03 ^b
Caudal Peduncle	D20	1.00 ± 0.02 ^a	0.71 ± 0.01 ^a	0.86 ± 0.01 ^b	1.03 ± 0.02 ^{a,c}	0.89 ± 0.01 ^b	0.88 ± 0.02 ^{b,c}
	D21	8.24 ± 0.09	5.54 ± 0.07 ^a	7.26 ± 0.08 ^b	8.30 ± 0.08 ^a	7.64 ± 0.08 ^{a,b}	7.48 ± 0.14 ^a
	D24	1.34 ± 0.02 ^a	0.87 ± 0.02 ^b	1.17 ± 0.02 ^{b,c}	1.31 ± 0.03 ^{b,c,d}	1.11 ± 0.02 ^b	1.20 ± 0.03 ^{a,b,c,d}
	D25	1.02 ± 0.02 ^a	0.69 ± 0.01 ^b	0.89 ± 0.01 ^{b,c}	1.06 ± 0.02 ^{a,d}	0.96 ± 0.01 ^{a,b,c,d,e}	0.91 ± 0.02 ^{b,c,e}
	D26	1.74 ± 0.02 ^a	1.12 ± 0.02	1.52 ± 0.02 ^b	1.82 ± 0.03 ^{a,c}	1.58 ± 0.03 ^{b,d}	1.59 ± 0.03 ^{a,b,c,d}
	D27	1.11 ± 0.02 ^a	0.68 ± 0.01 ^b	0.95 ± 0.02 ^{b,c}	1.13 ± 0.03 ^{a,c,d}	0.91 ± 0.02 ^{b,c}	0.98 ± 0.02 ^{a,b,c,d}
Eye Diameter	D30	1.30 ± 0.02	1.16 ± 0.02 ^a	1.57 ± 0.02	1.45 ± 0.02	1.45 ± 0.02 ^a	1.43 ± 0.03 ^a

PERMANOVA analysis of all the truss measurements resulted in significant differences among all locations for each year (pseudo- $F_{5,174} = 17.917$, p (MC) = 0.0001, and pseudo- $F_{5,169} = 14.857$, p (MC) = 0.0001, respectively for 2016 and 2019).

The stepwise analysis for all sampling locations in 2016 eliminated 5 distances from the analyses - D7, D19, D24, D28 and D29. The plot obtained in the stepwise LDFA showed clear discrimination of the Azores, Madeira and Canary individuals, with a high overlap of individuals from Portugal mainland (Figure 3A). The overall assignment of individuals to their original location was 78%, with values ranging from 60% for Peniche to 97% for the Azores (Table 5). For the year 2019, the stepwise analysis eliminated 9 distances from the analyses - D3, D6, D7, D17, D19, D20, D22, D27 and D28. The plot obtained in the stepwise LDFA showed clear discrimination of Canaries and Peniche, with a high overlap of individuals captured in Matosinhos and Portimão (Figure 3B). The overall assignment of individuals to their original locations was 83%, ranging from 70% for Madeira and Matosinhos to 100% for Canaries (Table 5).

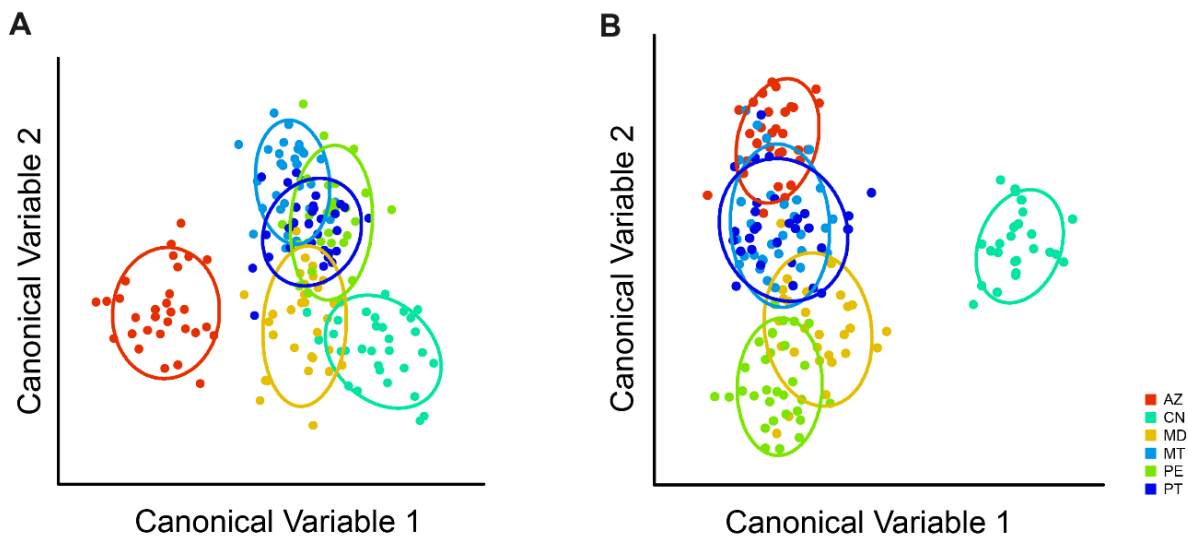


Figure 3: Canonical variable plots displaying spatial differences in the morphometric distances calculated for *Trachurus picturatus* collected in the six sampling locations of the NE Atlantic in 2016 (A) and 2019 (B). Legend: Azores (AZ), Canaries (CN), Madeira (MD), Matosinhos (MT), Peniche (PE) and Portimão (PT). Ellipses represent 95% confidence intervals around the data and data points represent individual fish.

CAP for 2016 identified four main groups, *i.e.* Azores (Group 1), Madeira (Group 2), the Canaries (Group 3), and Portugal mainland (Group 4) (Figure 4A). Distances D4, D6, D12, D14, D24 and D27 allowed the discrimination of group 1 (Azores); distances D3, D5, D7, D9 and D23 allowed the discrimination of group 2 (Madeira); and distances D1, D2 and D30

allowed the discrimination of group 3 (Canaries); all other distances measured overlapped the locations of Portugal mainland, with no clear indication to which distances were more discriminant. CAP for 2019 identified five main groups, i.e. Azores (Group 1), Canaries (Group 2), Madeira (Group 3), Peniche (Group 4), and Matosinhos and Portimão (Group 5) (Figure 4B). Distances D21, D22, D24 and D27 allowed the discrimination of group 1 (Azores); distances D4, D6 and D12 allowed the discrimination of group 2 (Canaries); distances D2, D5, D9, D16 and D30 allowed the discrimination of group 3 (Madeira); distance D8 allowed the discrimination of group 4 (Peniche).

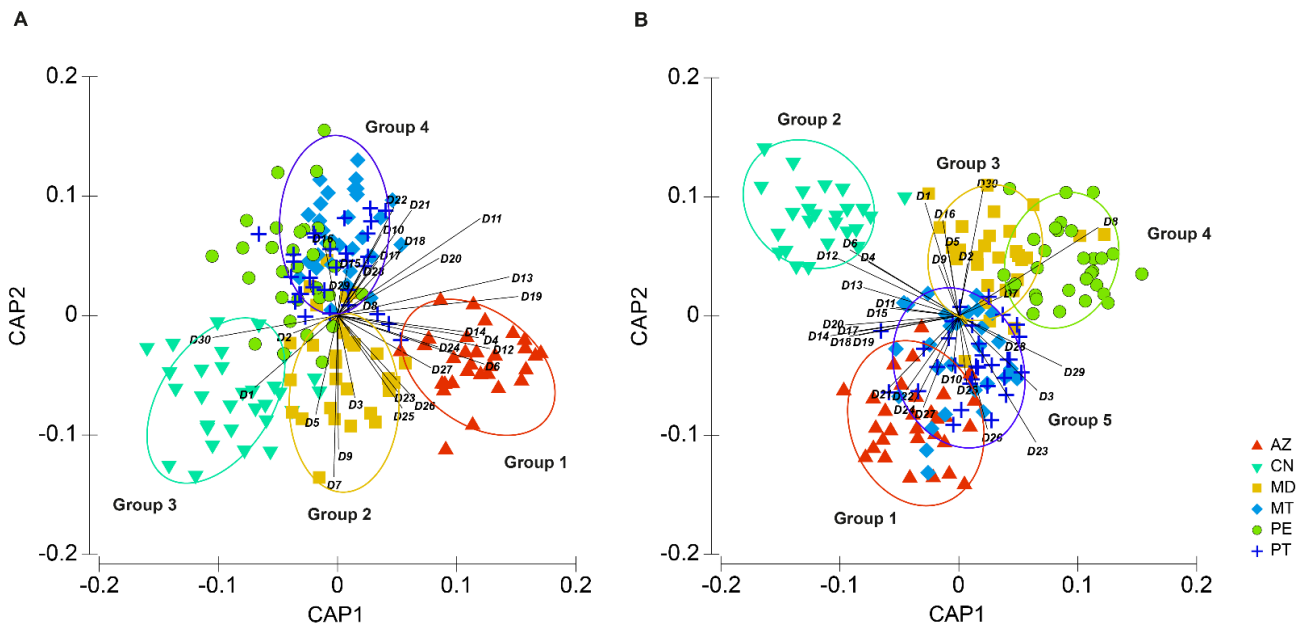


Figure 4: Canonical analysis of principal coordinates (CAP) plots for the morphometric distances calculated for *Trachurus picturatus* collected in the six sampling locations of the NE Atlantic in the year 2016 (A) and 2019 (B). Legend: Azores (AZ), Canaries (CN), Madeira (MD), Matosinhos (MT), Peniche (PE) and Portimão (PT).

Table 5: Jackknifed cross-validation re-classification matrix of the stepwise linear discriminant analysis for the truss measurements of *Trachurus picturatus* for all sampling locations in the year 2016 and 2019.

Original location	Predicted location						
2016	Azores	Canaries	Madeira	Matosinhos	Peniche	Portimão	% correct
Azores	29	0	1	0	0	0	97
Canaries	0	27	2	0	1	0	90
Madeira	1	0	23	0	1	5	77
Matosinhos	0	0	1	23	3	3	77
Peniche	0	0	3	5	18	4	60
Portimão	0	0	2	5	3	20	67
Total	30	27	32	33	26	32	78
2019	Predicted location						
Azores	26	0	0	2	0	2	87
Canaries	0	25	0	0	0	0	100
Madeira	1	0	21	0	4	4	70
Matosinhos	3	0	1	21	0	5	70
Peniche	0	0	1	0	28	1	93
Portimão	0	0	1	4	0	25	83
Total	30	25	24	27	32	37	83

Discussion

In the present study, the differences in the body shape of *T. picturatus* along the NE Atlantic were investigated using truss network morphometrics. It was assumed that genus *Trachurus* shows no sexual dimorphism across its distribution range (e.g. Turan, 2004; Murta et al., 2008; Vasconcelos et al., 2018). The analysis showed that the NE Atlantic *T. picturatus* populations are phenotypically heterogeneous regarding the fish body shape. Furthermore, comparing the morphological characters between two distinct years – 2016 and 2019 – revealed that the species can exhibit fast phenotypic adaptations to the environment (e.g. Heino, 2014). The statistical discrimination of populations was driven by several morphometric distances, but the most significant ones were essential those related to body and caudal peduncle width, a relation that was already reported for other fish species, namely for 92 species of the Sparidae family (e.g. Antonucci et al., 2009) and for *Phycis phycis* (Vieira et al., 2016).

For both years, a clear isolation of the Macaronesian region (*i.e.*, Azores, Madeira and Canaryes) was observed. However, the truss measurements responsible for this regional differentiation were different between the years. For example, the Azores individuals caught in 2016 showed differences for distances related to the anterior body width (D4, D6, D12 and D14), while individuals caught in 2019 showed differences for distances related to posterior body width (D21 and D22). The discrimination of Madeira and the Canaryes was mainly caused by distances related to head morphology, while the Azores region was discriminated by distances related to body height (in 2016) and peduncle region (in 2019). Head morphological variation can be related to different feeding behaviours, while peduncle morphological variation is usually associated with swimming behaviour (Vieira et al., 2016; Kaouèche et al., 2017; Mourir et al., 2019). The diet of the blue jack mackerel is still poorly understood, but crustaceans appear to be the main food item (ICES, 2018). Moreover, different fish size distributions have been observed between the Azores islands shelf and offshore areas, presumably related to different diet preferences (Menezes et al., 2006). The migration of juvenile fish to different adult feeding grounds can result in significant phenotypic variations, accordingly to the new feeding regimes and habitats. Additionally, the correlation between fish length, water depth and diet variations has been reported for several pelagic fish species off the Portuguese coast, such as *Trachurus trachurus* (Cabral and Murta, 2002; Garrido and Murta, 2011) and *Micromesistius poutassou* (Cabral and Murta, 2002). However, if different diets could help explain the differences in the head variables, the morphological peduncle variation is more difficult to clarify. Body form, fin size and location are fish's adaptations for movement and manoeuvrability (Webb, 1984), indicating differences in habitat exploitation (Neves et al., 2018). Indeed, several authors have observed that morphological variations in the peduncle area are mostly associated with different hydrodynamic systems (*e.g.* Sajina et al., 2011; Vieira et al., 2016). The hereby study area (NE Atlantic) is known to be influenced by currents with different hydrodynamic features - *e.g.* the Azores and the Canaryes currents (Sala et al., 2013; Neves et al., 2018) - which could induce morphological variances in body shape, namely in the peduncle area. Furthermore, Azores and Canaryes are volcanic islands with special topographic conditions, such as narrow shelves and steep slopes (Menezes et al., 2006), leading to the formation of regional distinctive habitats. Additionally, the sampling locations are influenced by a sea temperature latitudinal gradient (lower in the Azores and higher in Madeira-Canaryes; Sala et al., 2013; Neves et al., 2018), and individuals from the southern locations, *i.e.*, Madeira and Canaryes, show higher growth rates than those captured in the northern locations, *i.e.*, Azores (Jurado-Ruzafa and Santamaría, 2018). The different regional growth rates described hereby could help to explain some extra phenotypic heterogeneity in fish (Rawat et al., 2017). The potential effect of the asymmetry of ages (not size) of the fish sampled in this broad geographic area cannot be disregarded. Theoretically,

the use of approximate fish sizes over a large geographical area (which is as a common practice in these studies), could impact classification accuracies due to a sampling defect, as result of different regional growth rates. However, a posterior statistical analysis of the same individuals, whose otoliths were still available in the laboratory (only 10 per location, excluding Matosinhos and Portimão) and later on aged with 3 years old, not only confirmed the main scenario but also made it more robust. The overall assignment of individuals to their original location was 95%, with values ranging from 100% for Azores and Canaries, and 90% for Madeira and Peniche, for both years. It means that any fish age effect, to exist, was minimal in this study. However, among the Portuguese coast, differences in morphometric patterns were less evident, although a high percentage of correct assigned individuals was obtained for most locations. Furthermore, in 2019, individuals from Peniche showed an unexpected separation from the other coastal locations. Regarding the Portuguese coast, the continental shelf contributes to a more hydrodynamic stable environment, which is reflected by the existence of a phenotypic homogeneous population among the coastal locations. Nevertheless, seasonal upwelling of nutrient-rich waters in summer, buoyant plumes from river discharge during winter (Santos et al., 2007), along with the existence of several submarine canyons (Aquarone et al., 2008) could be sufficient to create phenotypic patterns in the region. Data from the year 2016 suggest migratory movements among Portuguese coastal sampling locations - Matosinhos, Peniche and Portimão - with an apparent phenotypic homogeneous pattern across the sampling locations. However, individuals captured in 2019 showed clear isolation of Peniche. This location was discriminated by differences in fin related distances, meaning that fish from this location could have adapted for different swimming needs (Webb, 1984). Fluctuations in the upwelling events and/or size of the plumes can result in isolation and consequently local adaptation of these individuals. Additionally, Peniche is located about 40km of the largest canyon found in the Iberian continental shelf - Nazaré Canyon. This canyon has been reported has a clear marine community boundary, creating distinct fish assemblages between north and south (Sousa et al., 2005). Furthermore, considering the different body shape patterns observed between the years, *T. picturatus* seems to exhibit fast phenotypic adaptations to the surrounding environments. The specificities of each environment have been indicated as probable causes for phenotypic heterogeneity in several marine fish, including *T. picturatus* in Madeira, Canaries and Peniche (Vasconcelos et al., 2018), and closed related species, such as *T. trachurus* in the Iberian and North African Atlantic (Murta, 2000) and *T. mediterraneus* in the Mediterranean Sea (Turan, 2004). This phenotypic heterogeneity was also observed in the current study taking into consideration the high overall re-allocation of individuals to the original location for both years - 78% in 2016 and 83% in 2019.

In addition to the environmental factors, phenotypic variation within species is also influenced by the individual genetic input (Cadrin et al., 2010). However, several authors have

shown that heritability of morphometric characters is generally low to moderate (Swain et al., 2005; Cadrin et al., 2010; Costa et al., 2010), suggesting that “local stocks” can be better identified based on phenotypic variations. Accordingly, while some fish species show morphological divergence due to genetic differentiation, others show phenotypic variations that cannot be related to the genetic component (e.g. Domingues et al., 2007; González-Wangüemert et al., 2010; Kaouèche et al., 2017). For instance, Costa et al. (2010) demonstrated that, in reared conditions, the shape of the European sea bass *Dicentrarchus labrax* was primarily influenced by the culturing conditions (*i.e.*, temperature, hydrodynamics, food) and secondly by the parental origins. Moreover, the relationship between morphological and functional traits of fish and ecological niches, *i.e.*, body-shape adaptation to habitat use, has been described by several authors (Price et al., 2011; Farré et al., 2013). This seems to be case for *T. picturatus*, since the genetic component of this species showed a lack of genetic structure across the species distribution (*i.e.*, NE Atlantic and the Mediterranean Sea), high levels of genetic diversity and high rates of mixing (Karaïskou et al., 2003, 2004; Moreira et al., 2019a).

Finally, the present results show the existence of phenotypic heterogeneity across the NE Atlantic *T. picturatus* populations, with the discrimination of four phenotypic stocks – Azores, Madeira, Canaries and Portugal mainland. Adding more sampling locations to the analyses provided a more extensive view of the species’ population structure than the one reported by Vasconcelos et al. (2018). These results are consistent with previous observations achieved with otolith shape analysis (Moreira et al., 2019a), and otolith elemental and isotopic signatures (Moreira et al., 2018). However, data for both years showed that the species can exhibit relatively fast phenotypic adaptations, an approach that was considered for the first time in this work. Nevertheless, results indicate that, even though the species seems to function as a panmictic population, phenotypic stocks seem to exist, as a result of local adaption to different environments. In terms of fisheries management, these stocks should be treated as separate populations, and manage accordingly. Additionally, studies on life-history parameters across all the species distribution should be enhanced and all integrated since information structure is essential to accurately assess and manage fisheries.

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Otolith shape analysis as a tool to infer the population structure of the blue jack mackerel, *Trachurus picturatus*, in the NE Atlantic

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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 its importance for the fisheries industry, information regarding stock delineation for management and conservation purposes is still scarce. This study examines whether otolith shape is efficient to infer about the population structure of the species and if the obtained data is consistent over time. Individuals of *T. picturatus* were collected in 2013 and 2016 from six important fishery regions in the NE Atlantic: Azores, Madeira, Canaries and Portugal mainland – Matosinhos, Peniche and Portimão. Otolith shape indices and elliptic Fourier descriptors were investigated by uni and multivariate statistical analyses. Data showed regional differences in the otolith shape analysis, with differences between the years. Fish sampled in 2013 showed an overall low re-classification rate (43%) with a high overlap of individuals within sampling locations, suggesting a single population unit. However, samples from 2016 allowed the discrimination of three distinct spatial groups with a moderate overall correct re-classification (62%) - group 1 with samples from the Azores; group 2 with samples from the Canaries; and group 3 with samples from Madeira and Portugal mainland. The hereby data suggests that fish population

structure could be dynamic over time, recommends a year-classes follow-up of the population units in the main fishing areas and stresses the need of holistic methodological approaches to study fish stocks. Furthermore, the hereby observed complex spatial dynamic structure of the species demands region-specific requirement actions to ensure a rational management of the fishery.

Keywords: Carangidae; sagittae; otolith shape indices; elliptic Fourier descriptors; fish stocks.

Introduction

The blue jack mackerel *Trachurus picturatus* (Bowdich, 1825) is a migratory fish widely distributed across the NE Atlantic and Mediterranean Sea (Smith-Vaniz, 1986; ICES, 2015). In the Macaronesian islands of Azores, Madeira and Canaries this species represents an economically important resource, being the only species of the genus *Trachurus* caught in the Azores region. Its catches have shown fluctuations during the last five years (FAO, 2017), with recorded landings of 4673 tonnes in Portugal for the year 2016 (INE, 2017). These fluctuations may be related to different fishing efforts and/or to natural variations in abundance or recruitment. Natural variations in abundance of this species are difficult to explain since studies regarding the population structure, fish movements and habitat connectivity are limited. Nevertheless, at present, the International Council for the Exploration of the Sea (ICES) considers that *T. picturatus* is not in need of any special management plans, supported by the data of commercial abundance, the presence of a stable biomass of juveniles and an increase of adult biomass in the Azores region, although there is no direct surveying of the stock. Since all life stages can be found around the Azores, ICES considers this region to be a single biological stock (ICES, 2012). Nonetheless, proper identification of all population-units is of primary importance for successful management and conservation of marine fishery resources.

As other marine pelagic fish (e.g. mackerels and sardines), *T. picturatus* shows schooling and migratory behaviour, large effective population sizes and great dispersal capabilities, both at the larval and adult stages. As result, and in the absence of known oceanographic barriers, these fish populations usually show extensive gene flow and low levels of genetic differentiation. These observations are corroborated by a weak genetic structure across the species distribution range, namely in the Mediterranean Sea and the Portuguese coast (Karaïskou et al., 2003, 2004), but also in the Macaronesian region (Moreira, unpublished results). However, other studies done regionally and addressing the growth rate, age at first maturity and reproductive season, described significant differences between the study areas, suggesting that there are different population units (Isidro, 1990; Gonçalves et

al., 2013; Jurado-Ruzafa and Santamaría, 2013). Furthermore, recently it was found that the otolith chemical signatures were different between individuals from the Macaronesian islands - Azores, Madeira and Canaries - and Portugal mainland (Moreira et al., 2018), supporting other observed regional differences based on the occurrence of parasites (Costa et al., 2012, 2013; Vasconcelos et al., 2017) and on morphometric analysis (Vasconcelos et al., 2018).

Stock discrimination using the morphological, structural and chemical properties of otoliths has been extensively studied by fisheries scientists over the last decades (e.g., Campana and Neilson, 1985; Campana and Casselman, 1993; Campana, 1999). The study of the morphological characteristics of otoliths, in particular, has been considered an efficient tool for fish stock identification (Castonguay et al., 1991; Ferguson et al., 2011; Jemaa et al., 2015). It has proven successful in resolving fish stock structure in high gene flow systems, when environmental heterogeneity exists (Bacha et al., 2014), as it can identify groups of fish which may have been spatially or temporally structured at some stage of their life history (Keating et al., 2014). Otolith shape is markedly species specific, but also shows intra-specific geographic variation in relation to environmental factors and fish growth (Janusz, 1990; Ferguson et al., 2011; Jemaa et al., 2015). The use of elliptical Fourier descriptors to describe the shape of otoliths is a rapid, objective, and semi-automated method to obtain significant information to identify populations units (Bird et al., 1986), while the utilization of shape indices has the advantage of being more simple to calculate, representing the growth pattern of otoliths (Tuset et al., 2003). Furthermore recent improvements in image acquisition and data analysis have allowed to routinely process hundreds of otolith samples, achieving high levels of statistical power (Geffen et al., 2016). However, the extent to which otolith shape differences are genetically or environmentally induced is not clear, and influences in the otolith shape have been described in relation to sex, age, year classes, stock and environment (Castonguay et al., 1991; Begg and Brown, 2000; Cardinale et al., 2004). While environment essentially alters the otolith growth rate, which in turn modifies otolith shape, genetically induced changes can locally affect otolith shape (Cardinale et al., 2004; Vignon and Morat, 2010; Capoccioni et al., 2011). Still, the use of otolith shape analysis to discriminate among fish stocks has been investigated in many small pelagic species, such as the Atlantic horse mackerel *Trachurus trachurus* (Abaunza et al., 2008), the herring *Clupea harengus* (Burke et al., 2008), the European sardine, *Sardina pilchardus* (Jemaa et al., 2015), and the blue jack mackerel *Trachurus picturatus* itself (Vasconcelos et al., 2018). The former study suggested the existence of three phenotypically distinct local populations of blue jack mackerel in the southern NE Atlantic (*i.e.*, Madeira, Canaries and Peniche). However, as highlighted by the authors, the limited geographic study area, the sampling strategy that did not take into account a specific time scale for the collection of the individuals (*i.e.*, January to December 2015), the

use of fish with significant different regional size ranges (*i.e.*, 14 to 37 cm) and the use of different fishing methods (*i.e.*, purse seine and trawl), weakened the obtained findings.

The aim of this work was to perform otolith shape analysis to investigate the population structure of *T. picturatus* covering the main fishery grounds in NE Atlantic (Azores, Madeira, Canaries and Portugal mainland). The temporal stability of otolith shape was also investigated by comparing two sampling years (2013 and 2016).

Material and methods

Fish collection

Individuals were collected from commercial catches landed in the fisheries regions of Portugal mainland (Matosinhos, Peniche and Portimão), and the Macaronesian Islands of Azores, Madeira and Canaries (Figure 1). To ensure that the location of capture was accurate this information was requested from fishermen.

Fish were captured by local fishermen in shallow coastal waters (up to 75 m water depth) by gillnets during May and June 2013 and from October to December 2016. An effort was made to ensure that, for each year, the fish obtained from each location were from the same age/cohort by choosing fish of similar length (Table 1). The fish were stored in ice after landing and transported to the laboratory to be processed.

Each specimen was measured for total length (TL, cm) and sagittal otoliths were extracted with plastic forceps, washed with distilled water and stored dry in plastic tubes.

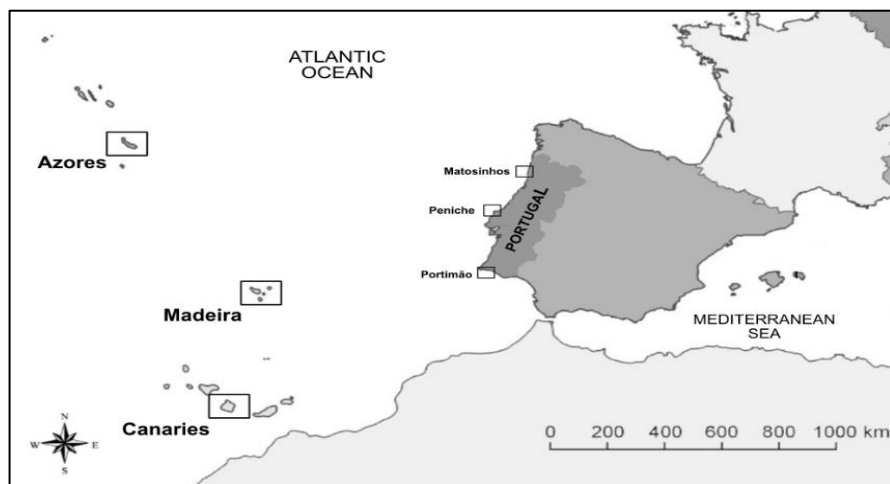


Figure 1: Sampling locations of *Trachurus picturatus* individuals collected in 2013 and 2016 in the Northeast Atlantic Ocean.

Table 1: Sampling locations, year of capture, sample size (n), total length (TL) and otolith length (OL) of *Trachurus picturatus* used in this study.

Regions	Sites	Sampling location	2013					2016				
			n	TL (cm)		OL (mm)		n	TL (cm)		OL (mm)	
				Range	Mean $\pm$ SE	Range	Mean $\pm$ SE		Range	Mean $\pm$ SE	Range	Mean $\pm$ SE
Azores	-	37°42'6.39"N 25°29'15.12"W	29	22.2 - 29.3	26.8 $\pm$ 0.3	6.4 - 7.9	7.38 $\pm$ 0.08	23	23.0 - 26.1	24.7 $\pm$ 0.2	6.2 - 7.2	6.80 $\pm$ 0.06
Madeira	-	32°42'18.79"N 16°56'52.75"W	37	22.5 - 27.0	24.8 $\pm$ 0.2	6.3 - 7.8	7.04 $\pm$ 0.06	23	23.7 - 29.0	26.1 $\pm$ 0.3	6.1 - 8.0	7.25 $\pm$ 0.09
Canaries	-	27°48'42.12"N 15°35'6.33"W	35	22.0 - 29.0	24.8 $\pm$ 0.3	6.4 - 8.1	7.20 $\pm$ 0.07	26	15.4 - 23.1	18.3 $\pm$ 0.4	4.5 - 8.0	5.38 $\pm$ 0.11
Portugal Mainland	Matosinhos	41°10'49.50"N 8°42'11.25"W	34	24.3 - 30.2	26.8 $\pm$ 0.2	6.2 - 8.2	7.29 $\pm$ 0.07	29	25.2 - 28.4	27.2 $\pm$ 0.1	6.5 - 7.6	6.98 $\pm$ 0.06
	Peniche	39°21'51.11"N 9°23'56.58"W	38	25.4 - 32.0	28.6 $\pm$ 0.2	6.8 - 8.4	7.62 $\pm$ 0.06	28	22.0 - 27.4	24.3 $\pm$ 0.2	6.0 - 7.3	6.60 $\pm$ 0.06
	Portimão	37° 6'14.20"N 8°31'25.99"W	38	21.8 - 27.3	24.3 $\pm$ 0.2	6.2 - 7.4	6.91 $\pm$ 0.05	27	24.6 - 28.2	26.2 $\pm$ 0.2	6.5 - 8.0	7.24 $\pm$ 0.08

Image acquisition

Left otoliths were placed ventral margin down, showing the sulcal groove up, and with the rostrum pointing left. Orthogonal two-dimensional digital images of otoliths were captured using a stereomicroscope (Meiji Techno EMZ-13TR) coupled with a USB digital camera (Olympus, SC30) at 10X magnification. High contrast digital images were obtained using reflected light with a dark background, producing bright two-dimensional objects (Figure 2).

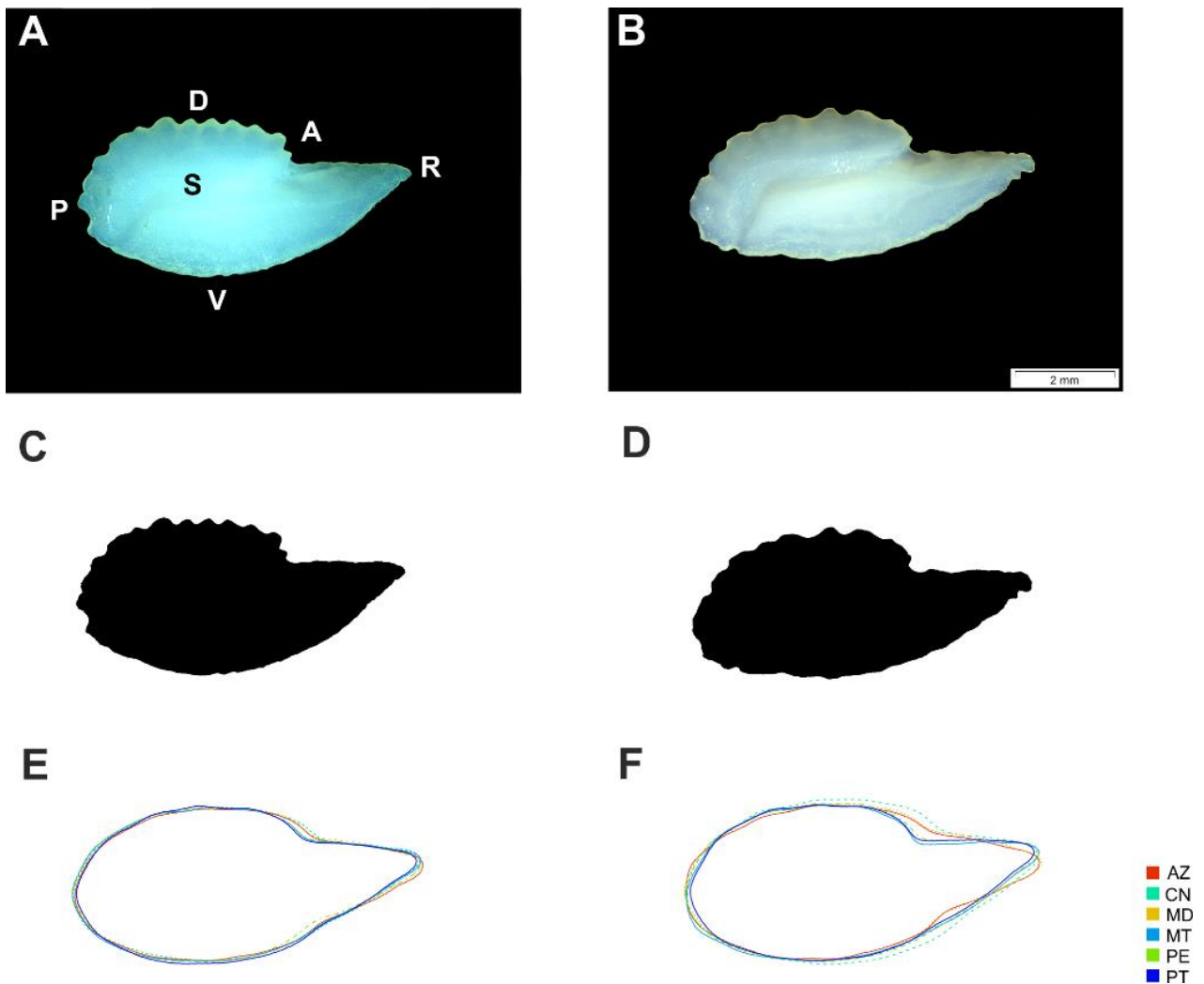


Figure 2: *Trachurus picturatus* inner face of the left sagitta showing the original photograph (A and B), the binary image (C and D) and the otolith averaged outline shape (E and F) from individuals collected in 2013 and 2016, respectively. A/C and B/D are otolith images from Matosinhos individuals with 26.0 and 27.1 cm of TL, respectively. Legend: Dorsal (D) and ventral (V) edges, post-rostrum (P), sulcus acusticus (S), rostrum (R) and anti-rostrum (A), Azores (AZ), Canaries (CN), Madeira (MD), Matosinhos (MT), Peniche (PE) and Portimão (PT).

Shape descriptors

Each otolith was described using (i) otolith shape indices (SIs) and (ii) elliptical Fourier descriptors (EFDs) according to Ferguson et al. (2011). Morphometric measurements were made from each otolith using the program ImageJ v. 1.50i (Rasband, 2009). Measurements of area (A, mm²), perimeter (P, mm), length (L, mm) and width (W, mm) were used to calculate five otolith shape indices: form factor (FF), roundness (RO), circularity (CI), rectangularity (RE) and ellipticity (EL) (Table 2) (Tuset et al., 2003). The elliptic Fourier analysis fits a closed curve to an ordered set of data points, then decomposes the contour into a sum of harmonically related ellipses (Kuhl and Giardina, 1982), suitable for use in statistical comparisons between samples. EFDs for each otolith were estimated using the program Shape v. 1.3 (Iwata and Ukai, 2002). EFDs required to adequately describe the otolith outline were obtained by the Fourier power (FP) (Crampton, 1995). The first 9 harmonics reached > 99% of the mean cumulative power for both years (excluding coefficients c9 and d9), so the Fourier analysis indicates that 34 [i.e. (9 x 4)-2 = 34] EFDs could summarize the otolith shape. The elliptical Fourier harmonics for each otolith were normalized to the first harmonic in the SHAPE program and were thus invariant to otolith size (Kuhl and Giardina, 1982; Pothin et al., 2006), making the first three coefficients (a1, b1 and c1) constant for all outlines, reducing the number of EFDs to 31.

Table 2: Otolith shape indices calculated from the morphometric measurements. A: area (mm²), P: perimeter (mm), L: length (mm) and W: width (mm) (from Tuset et al., 2003).

Shape Indices	Formulae
Form factor	$(4\pi A)/P^2$
Roundness	$(4A)/(\pi L^2)$
Circularity	P^2/A
Rectangularity	$A/(L \times W)$
Ellipticity	$(L - W)/(L + W)$

Statistical analysis

The five SIs were corrected to avoid body size and allometric effects that occur during otolith growth (Lombarte and Lleonart, 1993). The power model, *i.e.*, $Y = aX^b$, was fitted using logarithmic transformation to homogenize the residuals. Each morphometric index was

transformed into Z, according to $Z_i = Y_i (X_0 / X_i)^b$, where Y_i is the morphometric shape index for each individual, X_i is the otolith length of the i-th individual in the sample, and X_0 is the mean total body length (26.0 cm in 2013 and 24.5 cm in 2016), b was estimated for each morphometric index as the slope of the regression between $\log Y_i$ and $\log X_i$ (Thorpe, 1975; Jemaa et al., 2015). After otolith shape indices correction, ANCOVA showed that transformation was effective in removing the otolith size (as fixed co-variable) effect in the data.

One-way analysis of variance (ANOVA) was used to explore the differences in SIs and EFDs between locations, followed by a Tukey post-hoc test if significant ($p < 0.05$). The otolith morphometrics was analysed with a stepwise linear discriminant function analysis (LDFA). Re-classification accuracies of the discriminant functions for each location were evaluated using a jackknifed (leave-one-out) cross-validation. Comparisons between locations were conducted using a permutational multivariate analysis of variance (PERMANOVA) based on the Euclidean distance measure using Monte-Carlo simulations (9999 random permutations). A canonical analysis of principal coordinates (CAP) was performed with SIs and EFDs (pooled) to detect morphometric differences in the otolith shape among locations and years.

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 error (SE).

Results

In the year 2013, the otolith shape indices FF, CI and RE and eleven EFDs (i.e. d2, a3, a4, b4, d4, c5, a6, c6, d7, a8 and d8) showed differences between locations (ANOVA, $p < 0.05$). In 2016, all of the SIs were statistically different among all sampling locations (ANOVA, $p < 0.05$), and the number of EFDs raised to 20 coefficients (ANOVA, $p < 0.05$). The PERMANOVA tests performed on the standardized variables (SIs and EFDs) yielded significant differences between the years and the sampling locations (Table 3).

Table 3: Overall PERMANOVA comparison among the six sampling locations of *Trachurus picturatus* for both otolith shape indices (SIs) and elliptic Fourier descriptors (EFDs) and both years (2013 and 2016).

source	SIs					EFDs				
	df	SS	MS	Pseudo-F	P (MC)	df	SS	MS	Pseudo-F	P (MC)
Years	1	464.56	464.56	146.14	0.0001	1	537.47	537.47	19.403	0.0001
Locations	5	156.56	31.312	9.8495	0.0001	5	662.77	132.55	4.7852	0.0001
Years x Locations	5	87.921	17.584	5.5313	0.0001	5	302.57	60.514	2.1846	0.0004
Residual	355	1128.5	3.179			355	9833.8	27.701		
Total	366	1830				366	11346			

Regarding the year 2013, pairwise analyses for the SIs showed that the Azores was significantly different from Madeira, Matosinhos and Portimão; the Canaries were different from Portugal mainland; and Peniche was different from Madeira and from the other mainland sites (PERMANOVA, $p < 0.05$). For the EFDs, Canaries were significantly different from all other locations and Madeira was significantly different from Peniche (PERMANOVA, $p < 0.05$). The combination of both shape descriptors (SIs and EFDs) resulted in the differentiation between/among: the Canaries and all other locations; Azores and Matosinhos and Portimão; Madeira and Peniche; Peniche and Portimão (PERMANOVA, $p < 0.05$). For the year 2016, the SIs pairwise analyses showed that Azores and Canaries were significantly different from all other locations; Madeira was statistically different from Matosinhos; and Matosinhos different from Portimão (PERMANOVA, $p < 0.05$). The pairwise analyses of the EFDs showed that all locations were significantly different from each other except for Azores and Madeira, and the sites at Portugal mainland (PERMANOVA, $p > 0.05$). The combination of both shape descriptors showed that all locations were statistically different (PERMANOVA, $p < 0.05$), except for the sites at Portugal mainland that were all statistically similar (PERMANOVA, $p > 0.05$).

Using only SIs, the LDFA for the year 2013 showed a low overall re-classification success of 27%. Samples from Madeira and Portimão were allocated to all the sampled locations, with more individuals being assigned to different locations than the originals (Table 4, SIs). The allocation of samples using EFDs improved the overall re-classification to 42% (Table 4, EFDs). The reconstruction of the shape outline using the average harmonics for each location showed similar contour shapes, varying only slightly on the rostrum and anti-rostrum of otoliths for the Azores, Madeira and Canaries regions (Figure 2E). When using both shape descriptors, 43% of the samples were correctly re-classified to the sampled locations (Table 4, SIs and EFDs). Moreover, the best re-classification percentage was obtained for Canaries (60%) and misclassification was more common within the samples from the Azores,

Matosinhos and Portimão (Table 4, SIs and EFDs). Finally, the LDFA showed a high overlap of individuals from all sampling locations for the SIs (Figure 3A), for the EFDs (Figure 3B) and for both combined (Figure 3C). In the CAP, individuals from all locations were concentrated in one large group (Figure 4).

For the year 2016, LDFA for the SIs correctly re-allocated 42% of all samples to the sampled location, again with samples from Madeira and Portimão being more frequently assigned to other locations than the originals (Table 4, SIs). The use of EFDs improved the re-allocation of samples, with an overall allocation of 54% (Table 4, EFDs). However, the reconstruction of the shape outline from the average harmonics for each location showed how the otoliths from Portugal mainland display a steeper slope on the anti-rostrum region leading to a shorter rostrum. Also, the otolith's post-rostrum presented more variation between locations, with otoliths from the Canaries displaying a rounder shape (Figure 2F).

The combination of both shape descriptors resulted in an overall re-allocation of 62% (Table 4, SIs and EFDs). The best classification rate was 83% obtained for the Azores, while the lowest was obtained for Madeira (43%). LDFA for the SIs (Figure 3D) and for the EFDs (Figure 3E) showed a high degree of overlap of individuals from all sampling sites in Portugal mainland and Madeira, with some isolation for the Azores and the Canaries. Both shape descriptors resulted in an LDFA with a clear isolation of the Azores and the Canaries (Figure 3F). Finally, CAP also identified three main groups, i.e. Azores (Group 1), the Canaries (Group 2), Madeira and Portugal mainland (Group 3) (Figure 4), also showing that Canaries were highly discriminated based on RO and RE, while Azores were highly discriminated based on CI.

Table 4: Jackknifed cross-validation re-classification matrix of the stepwise linear discriminant analysis for the otolith shape descriptors (otolith shape indices - SIs, and elliptical Fourier descriptors - EFDs) of *Trachurus picturatus* for all sampling in the year 2013 and 2016.

2013							
Original location	Predicted location						% correct
	Azores	Canaries	Madeira	Matosinhos	Peniche	Portimão	
Azores	7	7	3	1	9	2	24
Canaries	2	16	7	1	4	5	46
Madeira	1	10	3	4	11	8	8
Matosinhos	1	7	6	6	5	9	18
Peniche	2	9	3	1	18	5	47
Portimão	1	6	6	8	10	7	18
Total	14	55	28	21	57	36	27
Original location	Predicted location						% correct
	Azores	Canaries	Madeira	Matosinhos	Peniche	Portimão	
Azores	14	5	1	2	3	4	48

Canaries	5	18	9	1	1	1	51
Madeira	6	3	13	3	4	8	35
Matosinhos	4	4	1	7	8	10	21
Peniche	3	0	3	6	21	5	55
Portimão	2	1	7	11	2	15	39
Total	34	31	34	30	39	43	42

SIs and EFDs		Predicted location					
Original location	Azores	Canaries	Madeira	Matosinhos	Peniche	Portimão	% correct
Azores	8	4	7	2	6	2	28
Canaries	1	21	9	0	2	2	60
Madeira	4	6	13	3	4	7	35
Matosinhos	0	4	3	14	6	7	41
Peniche	1	2	4	5	21	5	55
Portimão	0	4	5	8	7	14	37
Total	14	41	41	32	46	37	43

2016

SIs		Predicted location					
Original location	Azores	Canaries	Madeira	Matosinhos	Peniche	Portimão	% correct
Azores	13	2	0	8	0	0	57
Canaries	2	19	4	1	0	0	73
Madeira	1	3	1	5	5	8	4
Matosinhos	3	2	2	13	8	1	45
Peniche	3	1	2	4	15	3	54
Portimão	1	4	3	6	9	4	15
Total	23	31	12	37	37	16	42

EFDs		Predicted location					
Original location	Azores	Canaries	Madeira	Matosinhos	Peniche	Portimão	% correct
Azores	14	1	2	3	2	1	61
Canaries	1	22	1	0	1	1	85
Madeira	5	3	8	2	1	4	35
Matosinhos	4	0	3	13	5	4	45
Peniche	0	1	2	5	14	6	50
Portimão	1	0	0	4	9	13	48
Total	25	27	16	27	32	29	54

SIs and EFDs		Predicted location					
Original location	Azores	Canaries	Madeira	Matosinhos	Peniche	Portimão	% correct
Azores	19	0	1	2	1	0	83
Canaries	2	19	5	0	0	0	73
Madeira	0	4	10	6	1	2	43
Matosinhos	2	0	1	19	4	3	66
Peniche	0	0	1	5	16	6	57
Portimão	0	0	2	5	6	14	52
Total	23	23	20	37	28	25	62

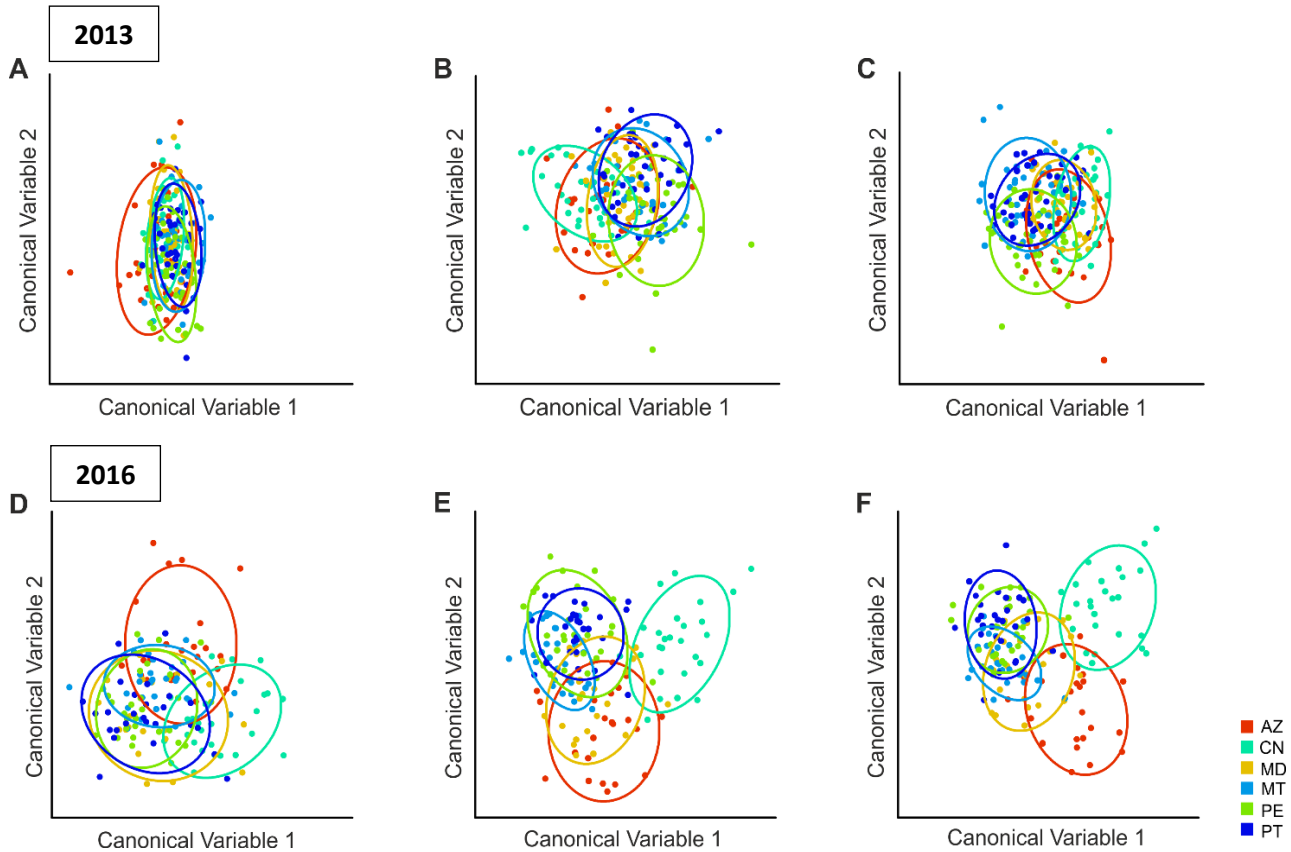
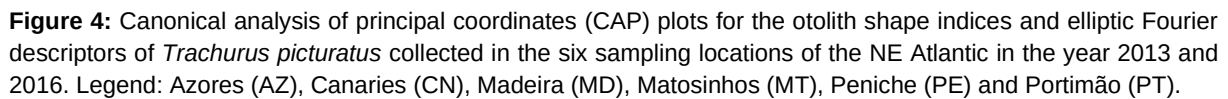


Figure 3: Canonical variable plots displaying spatial differences for the otolith shape indices (A and D), elliptic Fourier descriptors (B and E), and both shape descriptors (C and F) of *Trachurus picturatus* collected in the six sampling locations in the NE Atlantic in the year 2013 and 2016, respectively. Legend: 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 individual fish.



Previous studies on *Trachurus picturatus*, although inconclusive in terms of fish stock delineation, reported regional differences within the NE Atlantic, namely on growth and reproduction (Isidro, 1990; Vasconcelos et al., 2006; Jurado-Ruzafa and Santamaría, 2013; Garcia et al., 2015), recruitment (Jurado-Ruzafa and Santamaría, 2011), parasites (Costa et al., 2012, 2013; Vasconcelos et al., 2017) and otolith microchemistry (Moreira et al., 2018), supporting that the morphology of the otoliths in this species may present spatial differences.

Otolith shape analysis of *T. picturatus* individuals used hereby showed significant variations between sampling locations and years. The EFDs alone proved to be more useful than SIs for discriminating between fish belonging to different sampling locations. The SIs were, however, more valuable for explaining which otolith characteristics varied between locations, namely because EFDs does not encourage intuitive understanding of the reasons for subtle outline differences. Nevertheless, the EFDs for the year 2016, showed significantly different outlines for the island's locations, while the SIs, namely RO and RE, and CI allowed a good discrimination of samples from the Canaries and Azores, respectively. Furthermore, the present study shows that the greater the variables applied in the otolith shape analysis

(e.g. the combination of EFDs and SIs slightly improved the re-allocation success), the better the insight into the variation of the overall shape.

Re-allocation of individuals to the correct original locations was smaller for 2013 (overall 43%) than for 2016 (overall 62%). Data from 2013 showed a high overlap of samples, with no clear distinction between groups, while data from 2016 allowed a separation into three groups: Azores, Canaries and all other locations. This variation between the years can be explained by at least three hypothesis - (1) *T. picturatus* stock structure is dynamic (e.g. migration between regions) showing year-class fluctuations; (2) genetic and environmental differences over time could have affected the shape of otoliths and these differences may be indicative of stock structure; (3) and/or fish size asymmetries in the sampling scheme for 2016 (*i.e.*, smaller individuals caught in the Canaries) could skew the results, although this confounding effect was been theoretically removed using standard techniques. Differences in environmental conditions can have an extensive influence on otolith growth and consequently on the shape of otoliths (Campana and Neilson, 1985). It is well known that sex, age and year classes can influence otolith shape (Castonguay et al., 1991; Simoneau et al., 2000; Cardinale et al., 2004) and growth rate seems to be more important to regional differences than stock origin (Campana and Casselman, 1993; Begg et al., 2001; Hüssy et al., 2016). However, recently it has been shown that for *T. picturatus* there were no interactions between the otolith area and the sex of the fish (Vasconcelos et al., 2018), so for this species, otolith shape can be analysed independently of sex.

The multivariate analysis of both shape descriptors within the year of 2013 showed significant differences between the Canaries and all the other regions, and between the Azores and the sites of Matosinhos and Portimão in the Portuguese coast. Despite those differences, LDFA and CAP plots were unable to clearly isolate different population units. On the contrary, samples from 2016 showed significant differences among the islands studied, and the sites on the Portuguese mainland coast exhibited a high overlap of individuals. For that year, the LDFA and CAP analyses isolated three main groups – Azores, Canaries, and Madeira with Portugal mainland. This geographical separation of samples is most likely related to differences found in the environmental physicochemical properties among regions, such as water chemistry, temperature, salinity and food availability (*e.g.*, Sala et al., 2013). Additionally, the shape of otoliths can also be correlated to the diet of marine fish, which in turn relates to the species growth rate (Mille et al., 2016). Furthermore, variations in the shape of otoliths due to differences in fish metabolism were already found for several other fish species (*e.g.*, Begg et al., 2001; Capoccioni et al., 2011; Hüssy et al., 2016). For *T. picturatus*, different growth rates have been already reported for the Azores, Madeira and the Canaries (Shaboneyev and Ryazantseva, 1977; Faria and Vasconcelos, 2008; Jurado-Ruzafa and Santamaría, 2013). There are known differences between the oligotrophic waters of the Atlantic oceanic islands

and the highly productive Portuguese coast waters (Moreira et al., 2018), partially due to the coastal upwelling in the Portuguese coast (Fiúza, 1984). This issue may affect the food regime of fish, metabolism and consequently the growth rate, contributing to the observed variations in the otolith shape.

Furthermore, for the samples collected in 2013, the multivariate analysis showed that Peniche was significantly different from Portimão, but non-significant from Matosinhos, suggesting that the individuals captured in the Portuguese southern and western coasts were exposed to different conditions. One possible explanation for that specific differences is related to the dominant oceanographic processes along the western coast of Portugal. These processes include a seasonal upwelling of nutrient-rich waters in summer and poleward slope currents carrying warm and saline waters and buoyant plumes from river discharge during winter (Santos et al., 2007). Upwelling in spring and summer promotes the enrichment of the coastal zone with nutrients (e.g. chlorophyll-a) allowing phytoplankton to grow and consequently creating high zooplankton production areas (Santos et al., 2007). This former study showed that along the coast offshore of Matosinhos and Portimão chlorophyll-a was found in higher concentrations, while offshore Peniche chlorophyll-a concentrations were lower, which can improve the availability of fish food. For the year 2016, those differences among sites in the Portuguese coast were not detected. This variation across years could be due to changes in the oceanographic processes (e.g. low coastal upwelling in the years between the sampling), and/or due to the migration of individuals within the coastal area, making the data more homogeneous. Although the effect of allometric growth was removed from the analysis, individuals from the Canaries collected in the year 2016 were smaller than those from the other locations. Since fish age appears to impact classification accuracies for otolith based stock classification (Mapp et al., 2017), the discrimination of that sampling region might be partially due to that sampling defect.

In a recent work, Vasconcelos et al. (2018) used geometric body morphometrics and otolith EFDs to clarify the population structure of *T. picturatus* sampled over the year 2015. The re-allocation success obtained was 89% for Peniche, 66% for Madeira and 62% for the Canaries (73% in overall). Since the present work shares some locations, by applying the same approach of the former work, i.e., by using EFDs and a linear discrimination function analysis in stepwise mode for the same three sampling points, the coherence of results between years was tested and different results were found, namely for 2016. In 2013 the discriminant analysis resulted in an overall re-classification rate of 69% with similar re-classification trends (Peniche 74%, Madeira 70% and the Canaries 63%). However, in 2016, the overall re-classification rate was higher (84%) showing some isolation of the three sampling locations (Canaries - 88%, Peniche - 86% and Madeira - 78%). The differences found between the two studies suggest that there are changes over the years in the environment and/or in the

fish migration patterns (that could also be driven by changes in the environmental conditions) that result in different otoliths shapes. Furthermore, the use of a geographically limited sampling scheme by the previous study, by non-considering all the main fishery NE Atlantic grounds, could overestimate the re-classification rates.

Finally, the influence of genetics and environment on the formation of otolith is still unclear, although there are studies that indicate that environment is more important to its shape than genetics (Cadrin and Friedland, 2005; Capoccioni et al., 2011). It has been already shown that intra-specific genetic variations due to long-time separation influences otolith shape locally, mainly in the rostrum and post-rostrum parts, while overall otolith outline can be reshaped by contrasting environmental conditions that seem to regulate the quantity of material that is deposited during the formation of the otoliths (Vignon and Morat, 2010). Data from the phylogeographic analyses of *T. picturatus* (Moreira et al., unpublished data) revealed a shallow genetic diversity, with no clear separation of populations across the NE Atlantic and the Mediterranean, consistent to the otolith shape analysis results retrieved from the 2013 data. However, when phenotypic differences among regions are significant, phenotypic stocks can still be defined, based on the existence of a partial isolation (Stransky et al., 2008), and for several species otolith shape analysis has proven to be effective in isolating those phenotypic stocks (e.g. Ferguson et al., 2011; Duncan et al., 2018). Nevertheless, a lack of differences in otolith shape for 2013 is not necessarily conclusive since homogeneity in environmental conditions could lead to similar shapes and population structure still be present (Stransky et al., 2008).

In short, the present results showed statistical regional differences in the otolith shape analysis, meaning that otolith shape analysis is able to discriminate if there are different population units in the years sampled. However, differences between the years were found, indicating that the otolith shape for this species may not be consistent over time. In fact, while data from 2013 suggested the existence of a single population unit, data from 2016 allowed the discrimination of three distinct spatial groups. Therefore, the hereby study suggests that holistic approaches should be considered when studying fish stocks, since previous studies (Isidro, 1990; ICES, 2012; Costa et al., 2012, 2013; Gonçalves et al., 2013; Jurado-Ruzafa and Santamaría, 2013; Moreira et al. 2018; Vasconcelos et al., 2018) have pointed out to the existence of distinct population structure scenarios according with the methodologies used. This study also suggests that population structure could be dynamic over time and that a year-classes follow-up of the population units is highly recommended but taking into account all the main fishing areas of the species. Furthermore fish populations with a complex spatial dynamic structure demand region-specific requirement to ensure resource sustainability, such as the use of spatially structured stock assessment models, management strategy evaluation, incorporation of spatial indices into harvest control rules, besides the distribution of catch

among management areas, creation of marine protected areas and nested scales of governance (Kritzer and Liu, 2013).

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Chapter 4

CHEMICAL ANALYSES

Article 5. Population structure of the blue jack mackerel (*Trachurus picturatus*) in the NE Atlantic inferred from otolith microchemistry.

Article 6. Unravelling the spatial-temporal population structure of *Trachurus picturatus* across the North-East Atlantic using otolith fingerprinting.

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.

Keywords: Carangidae, sagittae, elemental and isotopic signatures, fish stocks.

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).

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.

Material and methods

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 (Figure 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.

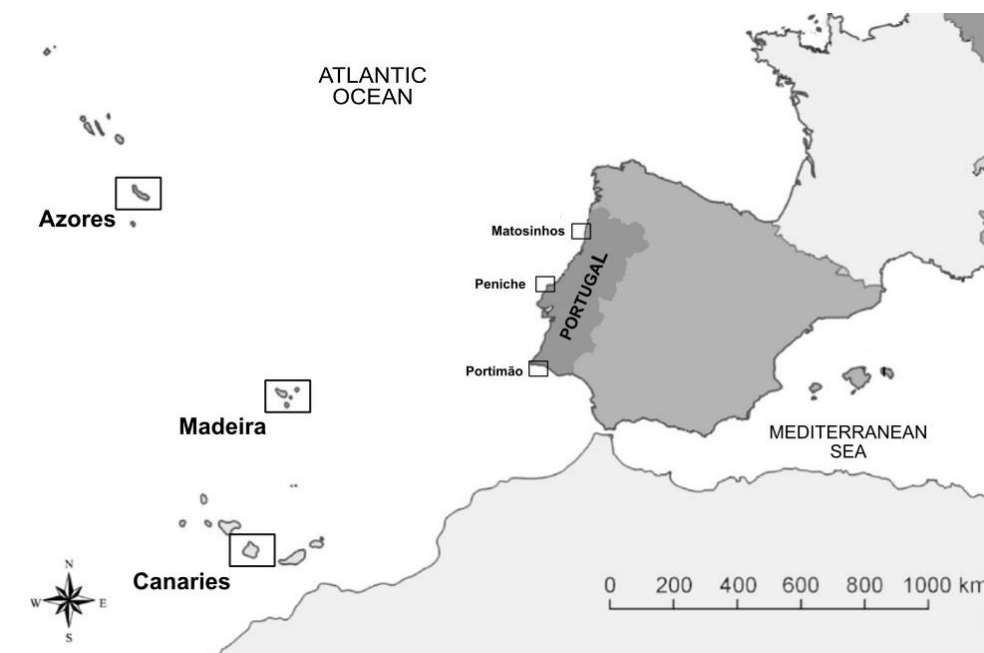


Figure 1: Sampling location of *Trachurus picturatus* individuals collected in 2013 in the Northeast Atlantic Ocean.

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 concentrations and isotopic signatures. Mean values $\pm$ SE.

Region	Site	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 25°29'15.12"W	20	26.4 $\pm$ 0.3	164.8 $\pm$ 6.0	7 444 $\pm$ 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
Madeira	-	32°42'18.79"N 16°56'52.75"W	20	26.2 $\pm$ 0.2	150.5 $\pm$ 2.8	7 252 $\pm$ 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 $\pm$ $\pm$ 0.65	- 6.60 $\pm$ 0.08	1.20 $\pm$ 0.01
Canaries	-	27°48'42.12"N 15°35'6.33"W	20	25.9 $\pm$ 0.3	142.86 $\pm$ 5.7	6 543 $\pm$ 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
Portugal Mainland	Matosinhos	41°10'49.50"N 8°42'11.25"W	20	26.4 $\pm$ 0.2	174.5 $\pm$ 3.9	7 796 $\pm$ 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
	Peniche	39°21'51.11"N 9°23'56.58"W	20	26.9 $\pm$ 0.2	178.4 $\pm$ 3.2	8 463 $\pm$ 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
	Portimão	37° 6'14.20"N 8°31'25.99"W	20	25.5 $\pm$ 0.2	143.8 $\pm$ 3.7	8 384 $\pm$ 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

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 $\sim 0.93 \text{ L min}^{-1}$). 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 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).

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).

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 functions

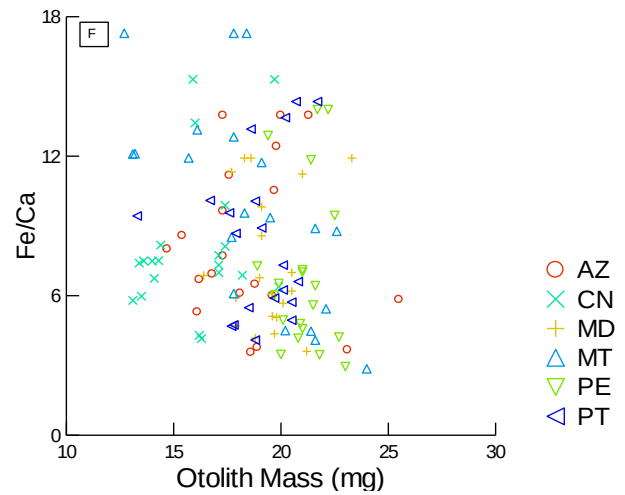
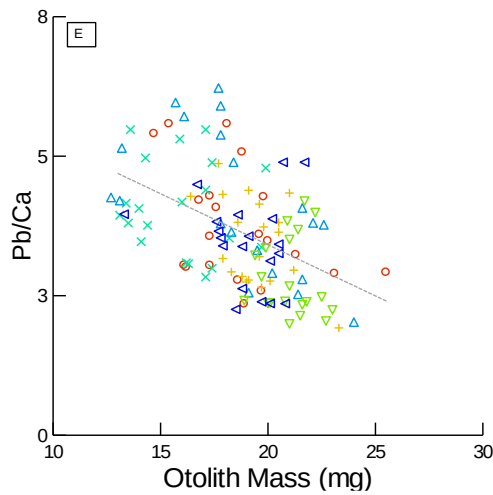
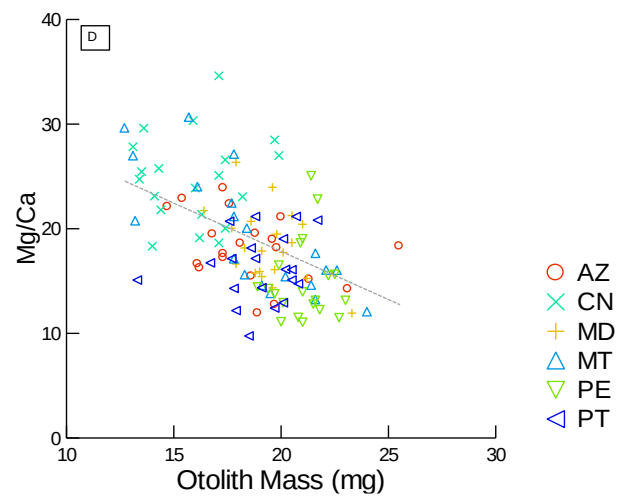
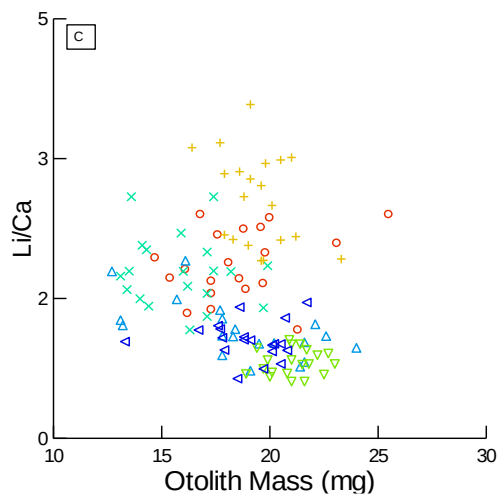
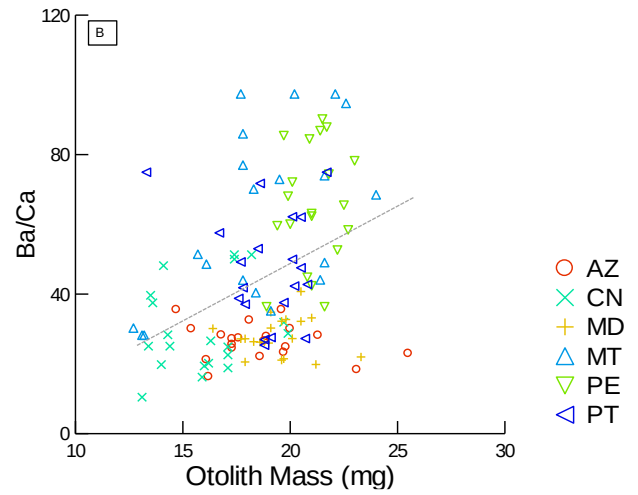
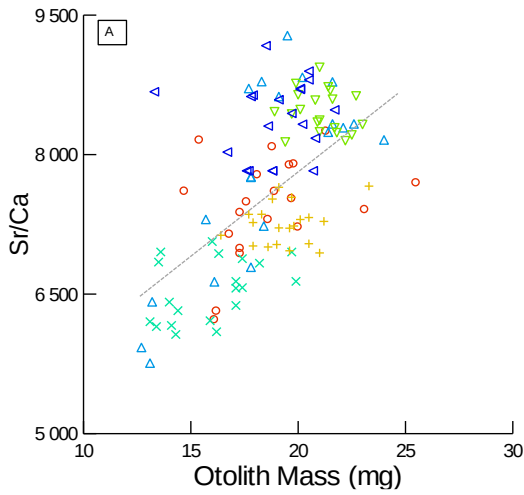
for each region and site were evaluated through the percentage of correctly re-classified individuals using jack-knifed (leave-one-out) cross-validation (Correia et al., 2011b). The correlation matrix from the elemental and isotopic data set was analysed by a Canonical Analysis of principal Coordinates (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).

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) (Figure 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) (Figure 2C and F). The ^{24}Mg ($n = 120$, $r^2 = 0.443$, $p < 0.05$, Figure 2D), the ^{208}Pb ($n = 120$, $r^2 = 0.313$, $p < 0.05$) (Figure 2E) and the ^{55}Mn ($n = 120$, $r^2 = 0.308$, $p < 0.05$) (Figure 2G) presented negative relationships.

$\delta^{13}\text{C}$ showed no significant relationship with otolith mass (ANCOVA, $p > 0.05$) (Figure 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 (Figure 2I). Although otolith mass was significant for $\delta^{18}\text{O}$ it only explained 3% of the sum of squares (Table 2).



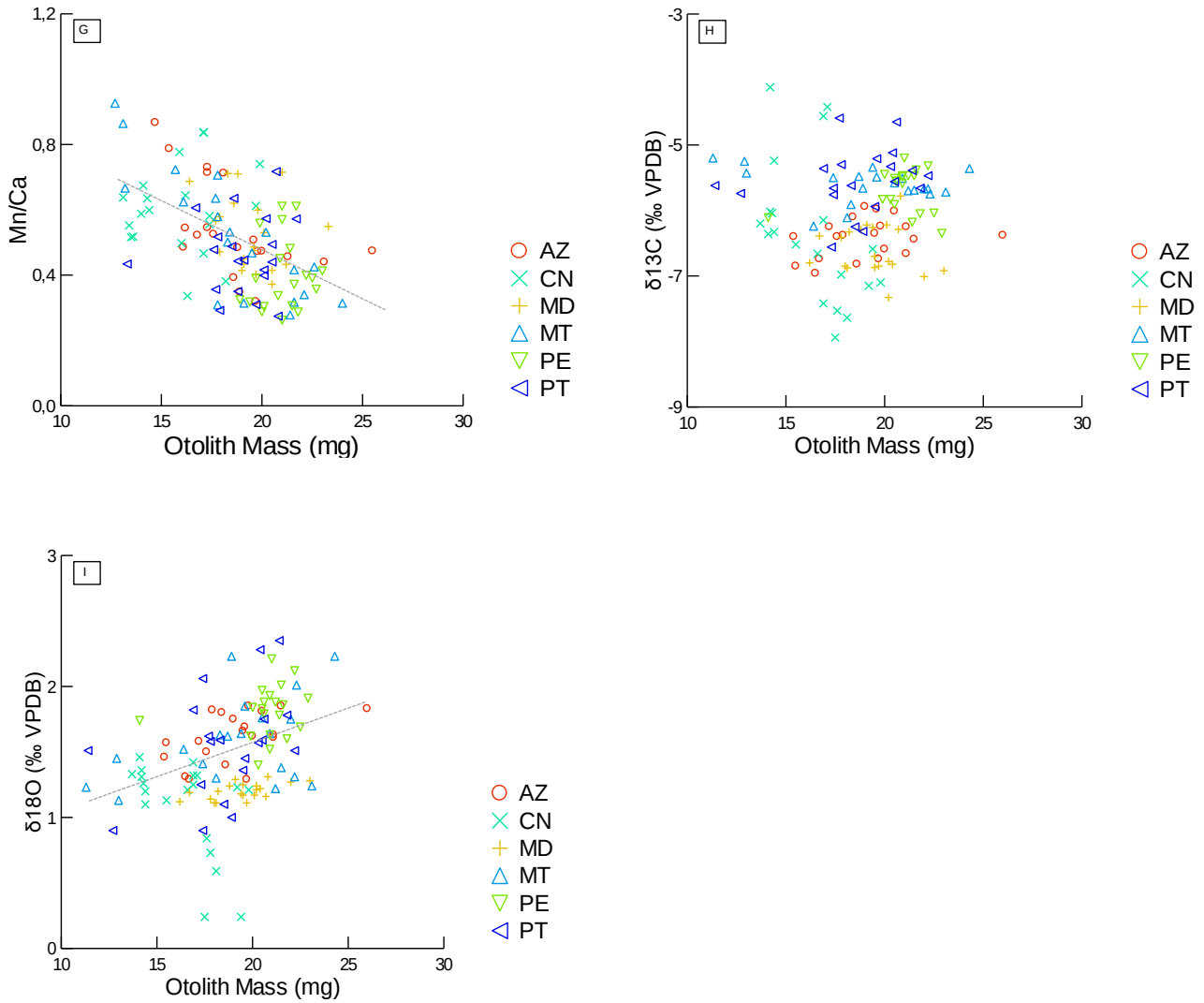


Figure 2: Element/Ca and isotopic concentrations versus otolith mass (mg) for all sampling locations (Azores–AZ, Canaries–CN, Madeira–MD, Matosinhos–MT, Peniche–PE and Portimão–PT). A trendline was add when a linear relationship ($p < 0.05$) was found between the two variables.

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			

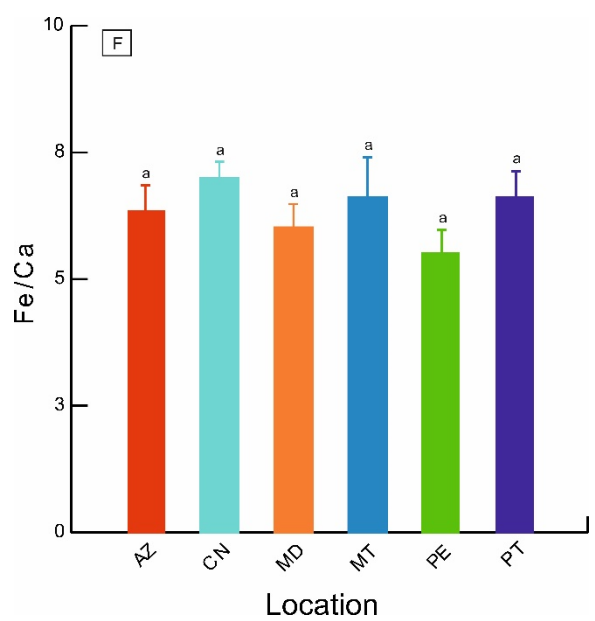
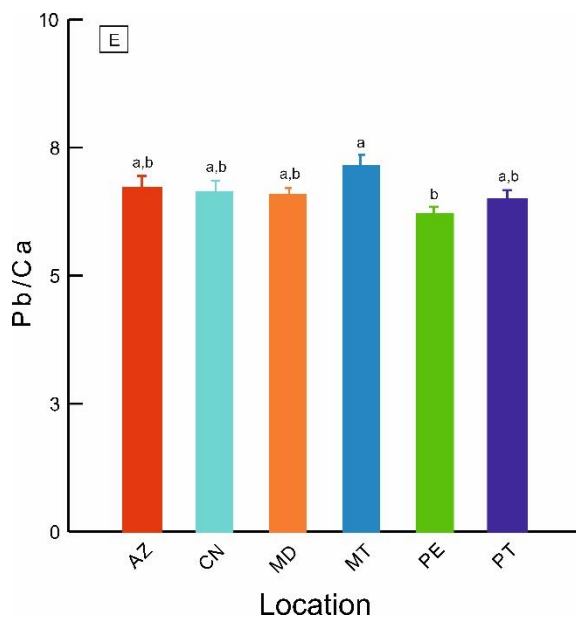
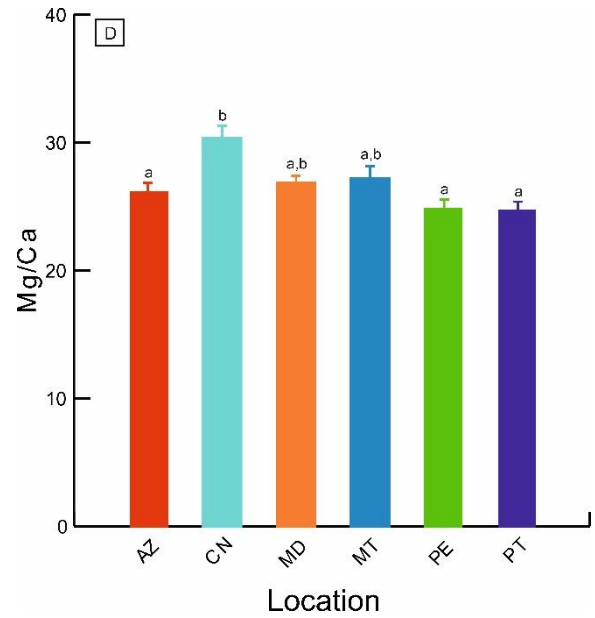
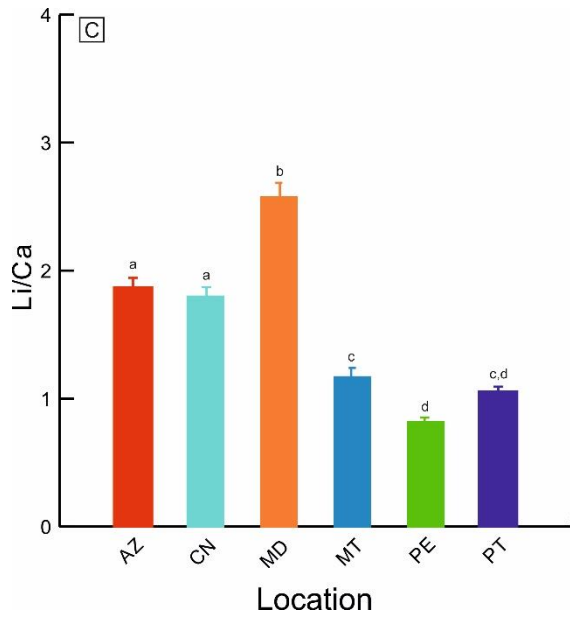
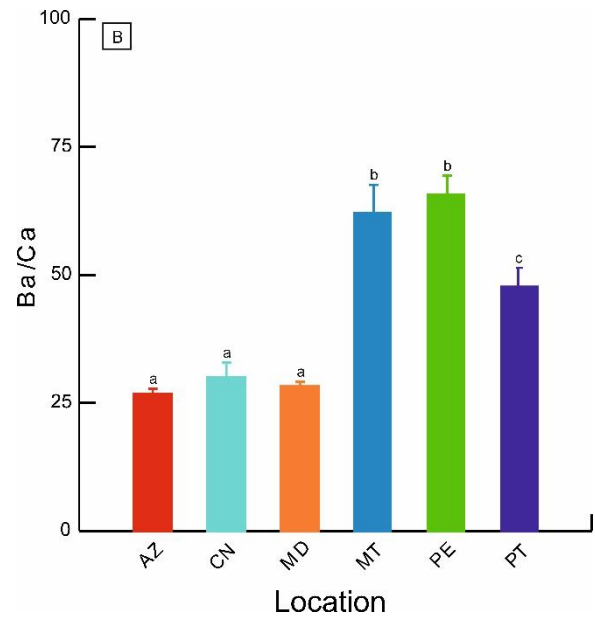
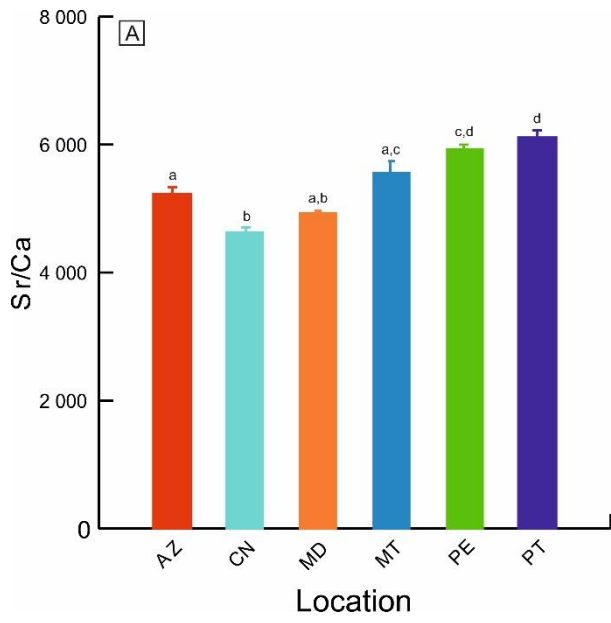
There were significant differences among sampling locations (ANOVA, $p < 0.05$) for Sr/Ca, Ba/Ca, Li/Ca, Mg/Ca and Pb/Ca (Figure 3A - E), but not for Fe/Ca and Mn/Ca (ANOVA, $p > 0.05$) (Figure 3F and G).

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, $p < 0.05$; Figure 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, $p > 0.05$) (Figure 3D). Two sites in Portugal mainland presented significant differences regarding the ratio of Pb/Ca, namely Matosinhos and Peniche (Tukey test, $p < 0.05$; Figure 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, $p < 0.05$) (Figure 3H) and between the Azores/Portugal mainland and Madeira/Canary Islands for $\delta^{18}\text{O}$ (Tukey tests, $p < 0.05$) (Figure 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 (Figure 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) (Figure 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 (Figure 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).



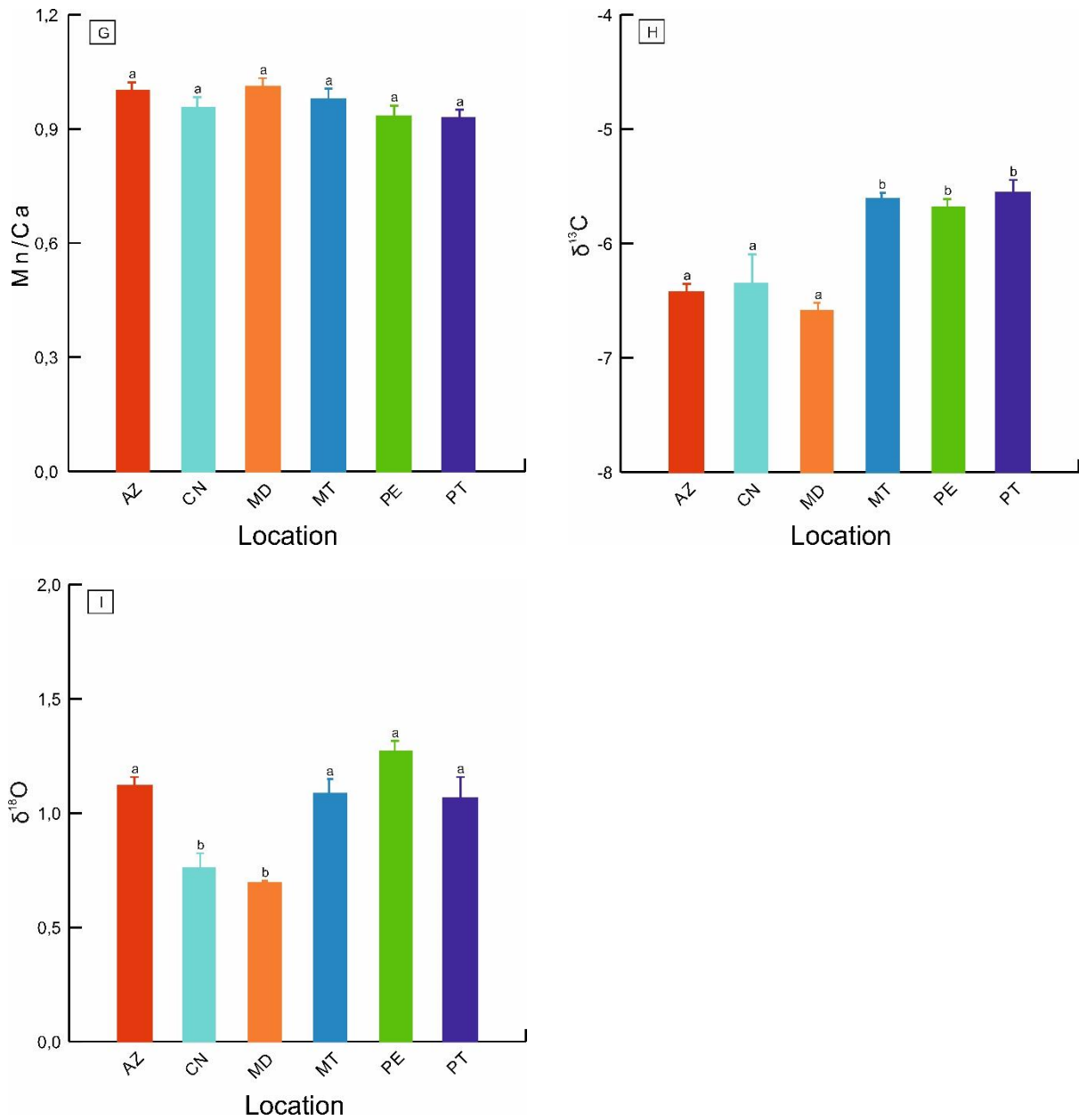


Figure 3: Element/Ca and isotopic concentrations (Mean $\pm$ SE) recorded in the whole otolith of *T. picturatus* in the six sampling locations (Azores - AZ, Canaries - CN, Madeira - MD, Matosinhos - MT, Peniche - PE and Portimão - PT). Concentrations are given in μg element g^{-1} calcium and VPDB (‰), 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 $\delta^{18}\text{O}$.

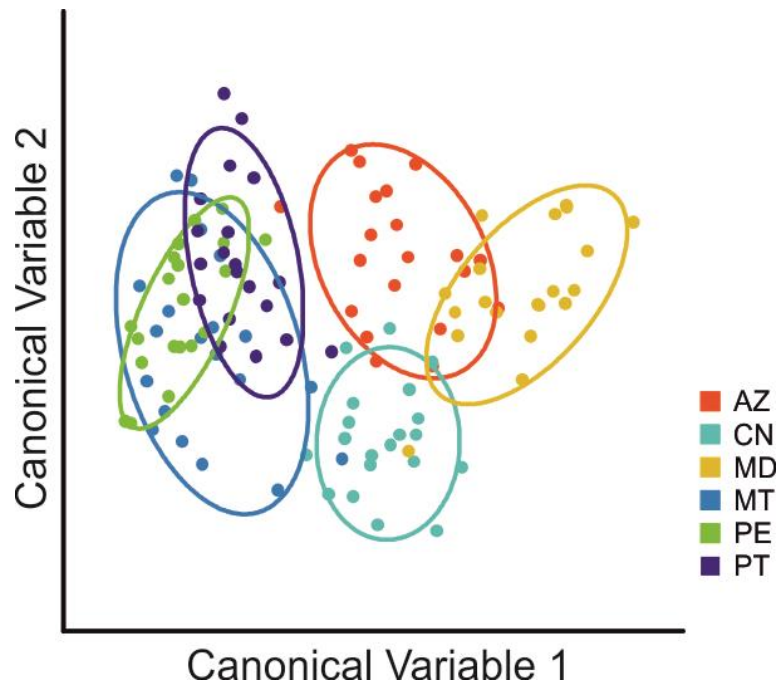


Figure 4: Canonical variate plot displaying spatial differences in multi-elemental tags in whole otoliths from the six sampling locations in the NE Atlantic [Azores (AZ), Canaries (CN), Madeira (MD), Matosinhos (MT), Peniche (PE) and Portimão (PT)]. Ellipses represent 95% confidence intervals around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

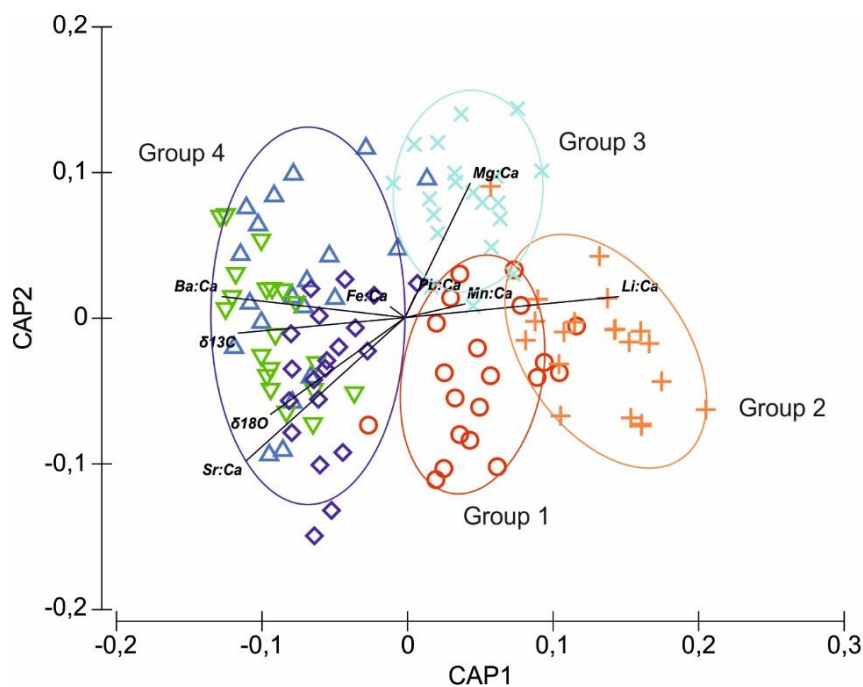


Figure 5: Canonical analysis of principal coordinates (CAP) plot for the trace elements concentration and the isotopic ratios from *T. picturatus* collected in the six sampling locations of the NE Atlantic [Azores (AZ), Canaries (CN), Madeira (MD), Matosinhos (MT), Peniche (PE) and Portimão (PT)].

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

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., 2011; 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; 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 existent 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 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 (Desbruyeres et al., 2000; Becerril et al., 2014), making the Li concentration in the environment higher and more stable (Chang 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, $\delta^{18}\text{O}$ 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., 2011). 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., 2011; 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 (Fiúza, 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 (Figure 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 (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 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 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.

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Unravelling the spatial-temporal population structure of *Trachurus picturatus* across the North-East Atlantic using otolith fingerprinting

In Review in Marine Biology

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Abstract

The blue jack mackerel *Trachurus picturatus* is one of the most important pelagic fish captured in the Macaronesian islands of Azores and Madeira. However, in the last years' landings registered substantial decreases. Fluctuations may be related to oscillations in the cohorts' recruitment rates, although no information regarding the species movements and connectivity is available. The present study applies laser ablation inductively coupled plasma mass spectrometry to investigate the otolith chemical fingerprints (core and edge) of three cohorts sampled off Azores, Madeira, Canaries and Peniche (Portuguese coast) in 2013, 2016 and 2019. Otoliths from juveniles (age 0⁺ and 1⁺) and adults (age 3⁺) were examined to infer the scale of geographical variation among fishing grounds and years. Univariate analyses

showed significant differences among locations based on Li:Ca, Mg:Ca, Cu:Ca, Sr:Ca and Ba:Ca. Multi-elemental analyses allocated 80% (2013), 50% (2016) and 48% (2019) of the individuals to their original location based on core elemental fingerprint, and 61% (2013), 87% (2016) and 84% (2019) based on edge elemental fingerprint. The core signatures suggest that most of the individuals harvested from the fishing grounds were self-recruits. However, subpopulations were still connected by dispersal of larvae/juveniles. Furthermore, discrimination of individuals observed in edge elemental fingerprint suggests that *T. picturatus* populations across the NE Atlantic should be regarded as different population units and manage accordingly, as previously suggested. Future works should focus on identify the natal sources of each fishing ground and assess the impact of environmental changes on the distance and direction of larval dispersal.

Keywords: Otolith microchemistry; laser ablation; natal origin; stock discrimination; blue jack mackerel.

Introduction

Marine fishes are often structured in metapopulations connected by the exchange of early life stages, juveniles and/or adults (Secor, 2005). These subpopulations are likely to have different life traits and demographic characteristics, showing genetic diversity and phenotypic plasticity and displaying different ways to cope with environmental variability and fishing pressure (Silva et al., 2019). However, fishery management still considers fish stocks as reproductively isolated populations, with homogeneous vital rates and closed populations, despite evidence of complex population structures and mixing within and between stocks (Kerr, 2016; Silva et al., 2019; Cadrin, 2020). Moreover, many stock units are still defined accordingly to the species geographic boundaries, economic and/or political importance. Overlooking the complex spatial structure of the populations, their migration routes and connectivity patterns can lead to the overexploitation of the different components of the population (Dahle et al., 2018). Therefore, it is extremely important that stock assessments and fishery management consider the spatial structure and connectivity of populations (Correia et al., 2014; Kerr, 2016; Pita et al., 2016).

Most of the small pelagic fish are characterized as having large population sizes and high fecundity rates, that coupled with the complexity of the oceanic currents and the apparent lack of physical barriers, makes the delineation of connectivity between subpopulations extremely difficult (Hedgecock and Pudovkin, 2011; Selkoe et al., 2016; Silva et al., 2019). For the majority of marine species, dispersal is undertaken during the larval phase, while juvenile

and adult movements are often associated to ontogenetic shifts, i.e. migrations driven by spawning requirements, searching for resources and/or avoiding intra or inter-species competition (Siegel et al., 2008; Vasconcelos et al., 2018; Friesen et al., 2019). Migration of both larvae and adults can be driven and/or facilitated by the geographical characteristics and oceanic features of their habitat. Several authors have reported the retention of larvae in the nursery grounds (Murta et al., 2008; Correia et al., 2014), but other authors shown that migration can occur for large distances, mostly driven by oceanic currents that facilitate the transport of larvae and/or adults (Lipcius et al., 2008; Santos et al., 2018; Silva et al., 2019). Among the tools available for estimating migration and connectivity patterns among spawning, nursery and adults' fishing grounds, otolith chemistry has been one of the most widely applied (e.g. Correia et al., 2012; Fortunato et al., 2017; Bouchoucha et al., 2018). Analysing the elemental fingerprint of otoliths can be used to infer long-distance movements and temporal/spatial separation of fish (Fowler et al., 2017; Moreira et al., 2018; Soeth et al., 2019).

The blue jack mackerel, *Trachurus picturatus* (Bowdich 1825), is a pelagic migratory species that inhabits the North-East (NE) Atlantic, from the Bay of Biscay to Mauritania, including the Macaronesian archipelagos of the Azores, Madeira and Canary, extending to the Mediterranean and Black Seas (Smith-Vaniz, 1986; Jurado-Ruzafa and Santamaría, 2018). The species is one of the most economically important fish in the Azores and Madeira, but in the last year's fluctuations in the landings have been observed, with Madeira showing the strongest decrease in landings (from 617 tonnes in 2016 to 201 tonnes in 2018) (INE, 2019). These oscillations are usually associated with changes in the fishing efforts, however, they can also be due to fluctuations in the recruitment of individuals to the fishing grounds (ICES, 2018). The demographic processes responsible for these oscillations are unknown, reflecting the poor knowledge of the large-scale movement of *T. picturatus* and its influence on regional population dynamics and stock structure. Despite its economic importance in the Macaronesian islands, there are still gaps when it comes to understanding the species life-history traits, namely the location of their spawning grounds and their migratory patterns. Previous studies have shown a lack of genetic differentiation across the Macaronesia and Mediterranean Sea (Karaïskou et al., 2003, 2004; Moreira et al., 2019a), an indication that the populations of *T. picturatus* could share a common spawning ground and/or is able to migrate great distances. However, *T. picturatus* larvae have been observed in Azores, Madeira and Canaries regions during the reproductive season, suggesting that spawning takes place locally (Menezes et al., 2006; Vasconcelos et al., 2017a; Tuset et al., 2019). Furthermore, other works based on ecological approaches, such as parasitology (Costa et al., 2013, 2012; Vasconcelos et al., 2017b), otolith microchemistry (Moreira et al., 2018), otolith shape analysis and geometric morphometrics (Vasconcelos et al., 2018; Moreira et al., 2019b) suggested that the adult populations in the Macaronesia and the Portuguese coast are phenotypically different.

Altogether, these works suggest a metapopulation structure across the species distribution, where subpopulations (usually self-sustained) are connected through the dispersal of individuals and interbreeding. However, Tuset et al. (2019) suggested that the population structure of *T. picturatus* in the Canary Islands fits more within a contingent theory, where migratory and resident individuals coexist within the region and show different capabilities and life-cycle patterns (Tuset et al., 2019).

To further understand the structure and movement patterns of *T. picturatus*, the main goal of this work was to investigate the otolith trace elemental fingerprint of three cohorts sampled in the NE Atlantic in 2013, 2016 and 2019. Otolith laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to infer the scale of geographical variation among the locations, from the analyses of the natal (corresponding to the first months of life) and edge (months prior to capture) signatures of the three cohorts. The temporal stability of the signal for each location was also analysed. These results will help to understand if the replenishment of the stocks is achieved by self-recruitment and/or by immigration, as well as the degree of connectivity among the subpopulations.

Material and Methods

Biological sampling

Specimens of *T. picturatus* were obtained from artisanal fisheries, at the sampling locations of Azores, Madeira, Canaries (Macaronesia) and Peniche (Portugal mainland), in the years of 2013, 2016 and 2019 (Fig. 1). An effort was made to ensure that individuals were from the same cohort by choosing fish with approximately the same length (Table 1). After captured, fish were stored on ice and transport to the lab to be processed. Each fish was measured for standard length (SL, cm) and sagittal otoliths were extracted with plastic forceps, to avoid metal contamination, washed with Milli-Q water, dried and stored in clean centrifuge tubes.

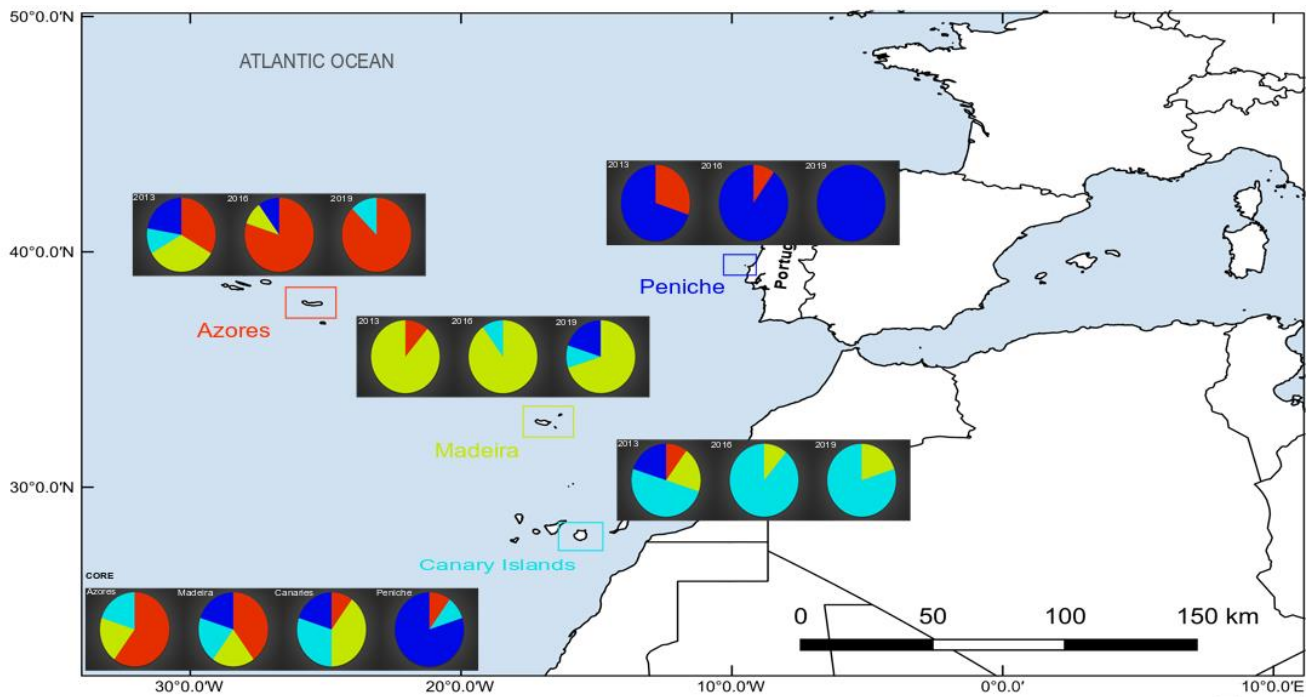


Figure 1: Sampling locations of *Trachurus picturatus* individuals collected in 2013, 2016 and 2019 in the North-East Atlantic Ocean. The proportional stock composition based on the edge multi-elemental fingerprint is indicated by the colours in each pie chart. Allocation of individuals was based on the jackknifed cross-validation matrix. The pie chart for the core multi-elemental fingerprint includes samples with the same birth date (for more details see Material and Methods).

Table 1: Sampling locations, year, sample size (n), standard length (SL), age (years) and birth date of *Trachurus picturatus* used in this study. Values are presented as mean $\pm$ standard error (SE).

Regions	Sites	Sampling location	Collection Year	n	SL (mm)	Age Class	Birth year
Azores	-	37°42'6.39"N 25°29'15.12"W	2013	10	27.06 $\pm$ 2.76	3 ⁺	2010
			2016	10	21.98 $\pm$ 1.09	3 ⁺	2013
			2019	10	23.63 $\pm$ 0.97	3 ⁺	2016
Madeira	-	32°42'18.79"N 16°56'52.75"W	2013	10	24.77 $\pm$ 0.84	3 ⁺	2010
			2016	10	22.68 $\pm$ 1.33	3 ⁺	2013
			2019	10	21.71 $\pm$ 0.83	3 ⁺	2016
Canaries	-	27°48'42.12"N 15°35'6.33"W	2013	10	23.04 $\pm$ 0.69	1 ⁺	2012
			2016	10	14.22 $\pm$ 0.76	0 ⁺	2016
			2019	10	16.23 $\pm$ 0.42	0 ⁺	2019
Portugal mainland	Peniche	39°21'51.11"N 9°23'56.58"W	2013	10	29.35 $\pm$ 1.05	3 ⁺	2010
			2016	10	21.46 $\pm$ 1.34	3 ⁺	2013
			2019	10	22.28 $\pm$ 0.98	3 ⁺	2016

Age reading

Digital images of the whole otoliths were taken using a stereomicroscope (Meiji Techno EMZ-13TR) coupled with a USB digital camera (Olympus, SC30) at 10X magnification, with reflected light against a black background. Otoliths were immersed in a clarification medium (50:50 glycerol:ethanol), with the sulcus acusticus turned down. Images were then used to age the otoliths, following the protocol described by ICES (2015) and Jurado-Ruzafa and Satamaría (2018). Ageing took into consideration the date of birth (1st January following the rules in the northern hemisphere) and the date of capture (Panfili et al. 2002). Age was interpreted three times by the same reader, with a minimum interval of 15 days between readings. Only fish with 100% between-years agreement were used for subsequent chemical analysis. Individuals of age group 3⁺ were selected from each region for further analyses, except for the Canary Islands where the individuals captured were of the age group 0⁺ and 1⁺.

Otolith microchemistry

Sagittae of *T. picturatus* were analysed by laser ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS). Otoliths were cleaned of organic tissues, rinsed with ultrapure water and air-dried. When dried, otoliths were mounted in epoxy resin (Struers) in a sagittal position. The blocks were sectioned near the otolith margins with a low-speed diamond

saw (Buehler, IsoMet), at 6000 rpm, lubricated with Milli-Q water. The resulting blocks were grounded with 500, 1000 and 4000 grade silicon carbide papers (Struers) until the core became visible. Regular optical inspections were done under a metallographic microscope (ML7100, Meiji). Finally, samples were polished using 6, 3, 1 and 0.25 μm diamond pastes (Buehler, Metadi II). The polished sections (~ 0.5 mm) were fixed on microscope slides with a drop of epoxy resin, and once dried, sonicated for 3 min in ethanol, rinsed with ultrapure water, and dried in a laminar flow cabinet before being analysed (Correia et al., 2012).

Trace element analyses were carried out with an ArF Excimer laser ablation system (Photon Machines Analyte G2) coupled to an ICP-QMS Agilent 7700 (Santa Clara, USA). After a preliminary analysis, the isotopes ^7Li , ^{88}Sr , ^{138}Ba , ^{55}Mn , ^{25}Mg , ^{57}Fe , ^{60}Ni , ^{63}Cu and ^{66}Zn along with ^{43}Ca as the internal standard were selected to proceed. The isotopes ^{111}Cd and ^{208}Pb were also quantified, however, these elements were consistently below the detection limit and consequently were not included in further statistical analyses.

A laser spot diameter of approximately 80 μm was used, with a repetition rate of 10 Hz and output energy of ~ 3 J cm^{-2} . One ablation was made on each sagittal section over the otolith core and edge. Otolith surface was pre-ablated prior to actual data acquisition to remove any residual surface contamination. Each laser ablation sample consisted of 15 blank scans of the isotopes, followed by a period of 22 scans. The National Institute of Standards (NIST) 610 and 612 silicate glass reference material (SRM), and the MACS-3 carbonate pellet were used for quantification of elemental concentrations (Pearce et al., 1997). A period of 22 scans was acquired in the beginning and at the end of each session for the three standards. The standard deviation of these blanks was used in the calculation of detection limits. Detection limits are also dependent on the amount of material ablated and were adjusted for each ablation based on ablation yield estimates (Lahaye et al., 1997). Accuracy and precision were estimated on a daily basis for NIST 610, NIST 612 and MACS-3. Precision estimates for individual elements from repeated sampling of all three standards were between 4 and 5% relative standard deviation (RSD) for all elements. Accuracy estimates based on recovery for individual elements were approximately 120% for all elements. Raw data (counts s^{-1}) were converted to elemental concentrations rationed to calcium and presented as molar ratios (element:Ca, $\mu\text{mol element mol}^{-1} \text{Ca}$). The data reduction process was performed by ElementR (see Sirot et al. 2017 for details in data reduction), a package developed in the R language (R Core Team 2018).

Statistical analysis

Otolith concentrations were checked for normality and homogeneity of variance using Shapiro-Wilk and Levene's tests. Assumptions were met after Log10 data transformation. The data from the core and edge samples were treated separately. Since individuals from the Canary Islands were younger (0⁺ and 1⁺) than those captured in the other locations, these samples were removed from the core elemental fingerprint analyses. However, since individuals of the age group 0⁺ collected in 2016 at the Canaries were born in the same year as the individuals captured in 2019 (age 3⁺) in the remaining locations, core elemental fingerprints were analysed together for this samples. Spatial and temporal differences in individual fingerprints were explored by a three-way ANOVA (factors: Year x Spot x Location). Tukey's HSD test was used, if needed ($p < 0.05$), to detect a posteriori pairwise differences among single elements. Permutational multivariate analysis of variance (PERMANOVA), based on the Euclidean distance, measure using Monte-Carlo simulations (9999 permutations), was employed to test multi-elemental differences in otolith core and edge fingerprints among locations for each year. The stability of the cohort's fingerprint signal for each location was tested among years for both otolith core and edge. A Linear Discriminant Function Analyses (LDFA) was used to visualize spatial differences and to examine the re-classification accuracy success (jackknifed procedure) of fishes to this original location. A canonical analysis of principal coordinates (CAP) was performed for core and edge fingerprints to detect elemental differences among locations and years for each location.

Statistical analyses were performed using Sigmaplot (version 11.0), Systat (version 12.02) and PRIMER (version 6.1.12) & Permanova (version 1.0.2). The statistical level of significance (α) for all tests was 0.05. Results are presented as mean $\pm$ standard errors (SE).

Results*Single elemental analysis*

A significant interactive effect between all the factors tested was found for otolith Ba:Ca concentrations (Table 2). For Li:Ca, all factors show significant interactions, except year and year x spot interactions (Table 2). Mg:Ca concentrations showed a significant effect for year x spot interactions, Zn:Ca for year x location interactions and Sr:Ca for spot x location interactions (Table 2). The otolith Zn:Ca concentrations were significantly different among years, while Cu:Ca and Sr:Ca concentrations showed significant differences among locations (Table 2). All the element:Ca ratios were statistically different between core and edge, except

for Fe:Ca concentrations that were similar between the two distinct spots (Table 2). The elements of Sr and Ba were found in significantly higher concentrations on the edge of the otolith, while the other elements had higher concentrations in the otolith core (Fig. 2). Additionally, otolith core and edge Li:Ca concentrations showed a trend between northern and southern locations, with Azores and Peniche (northern locations) showing lower levels of Li:Ca, while Madeira and Canaries (southern locations) showed higher concentrations (Fig. 2, Table 2). Furthermore, otolith edge Ba:Ca concentrations were higher in Peniche and Canaries (Fig. 2, Table 2).

Table 2. Three-way ANOVA dataset for the elemental fingerprint analysis of *Trachurus picturatus*.

Element:Ca	Source of variation	DF	SS	MS	F	P
Li:Ca	Year	2	0.10	0.05	3.04	0.05
	Spot	1	0.50	0.50	31.63	<0.001
	Location	3	5.73	1.91	120.96	<0.001
	Year x Spot	2	0.08	0.04	2.52	0.08
	Year x Location	6	1.04	0.17	10.97	<0.001
	Spot x Location	3	1.50	0.50	31.75	<0.001
	Year x Spot x Location	6	0.42	0.07	4.41	<0.001
	Residual	216	3.41	0.02		
	Total	239	12.78	0.05		
Mg:Ca	Year	2	0.13	0.07	1.43	0.24
	Spot	1	17.89	17.89	391.84	<0.001
	Location	3	1.43	0.48	10.45	<0.001
	Year x Spot	2	0.07	0.04	0.81	0.45
	Year x Location	6	1.18	0.20	4.30	<0.001
	Spot x Location	3	0.17	0.06	1.25	0.29
	Year x Spot x Location	6	0.09	0.02	0.34	0.91
	Residual	216	9.86	0.05		
	Total	239	30.82	0.13		
Mn:Ca	Year	2	0.08	0.04	0.73	0.48
	Spot	1	33.50	33.50	586.32	<0.001
	Location	3	0.18	0.06	1.06	0.37
	Year x Spot	2	0.30	0.15	2.59	0.08
	Year x Location	6	0.49	0.08	1.43	0.20
	Spot x Location	3	0.17	0.06	0.99	0.40
	Year x Spot x Location	6	0.08	0.01	0.24	0.96
	Residual	211	12.06	0.06		
	Total	234	46.82	0.20		
Fe:Ca	Year	2	0.08	0.04	1.73	0.18
	Spot	1	0.00	0.00	0.12	0.73
	Location	3	0.10	0.03	1.46	0.23
	Year x Spot	2	0.00	0.00	0.01	0.99
	Year x Location	6	0.14	0.02	1.06	0.39

	Spot x Location	3	0.00	0.00	0.02	1.00
	Year x Spot x Location	6	0.00	0.00	0.00	1.00
	Residual	216	4.90	0.02		
	Total	239	5.22	0.02		
Ni:Ca	Year	2	0.03	0.02	0.33	0.71
	Spot	1	1.29	1.29	26.34	<0.001
	Location	3	0.23	0.08	1.56	0.20
	Year x Spot	2	0.21	0.10	2.11	0.12
	Year x Location	6	0.51	0.08	1.74	0.11
	Spot x Location	3	0.03	0.01	0.19	0.91
	Year x Spot x Location	6	0.06	0.01	0.21	0.97
	Residual	213	10.42	0.05		
	Total	236	12.80	0.05		
Cu:Ca	Year	2	0.30	0.15	2.09	0.13
	Spot	1	10.73	10.73	148.68	<0.001
	Location	3	0.81	0.27	3.75	0.01
	Year x Spot	2	0.20	0.10	1.39	0.25
	Year x Location	6	0.91	0.15	2.11	0.05
	Spot x Location	3	0.08	0.03	0.36	0.78
	Year x Spot x Location	6	0.21	0.03	0.48	0.82
	Residual	215	15.52	0.07		
	Total	238	28.81	0.12		
Zn:Ca	Year	2	1.68	0.85	5.27	0.006
	Spot	1	36.03	36.03	223.832	<0.001
	Location	3	1.23	0.41	2.55	0.06
	Year x Spot	2	0.43	0.22	1.35	0.26
	Year x Location	6	4.18	0.70	4.33	<0.001
	Spot x Location	3	0.51	0.17	1.05	0.37
	Year x Spot x Location	6	0.88	0.15	0.91	0.49
	Residual	216	34.77	0.16		
	Total	239	79.74	0.33		
Sr:Ca	Year	2	0.04	0.02	2.41	0.09
	Spot	1	1.30	1.30	148.45	<0.001
	Location	3	0.12	0.04	4.50	0.004
	Year x Spot	2	0.01	0.01	0.67	0.51
	Year x Location	6	0.09	0.01	1.69	0.12
	Spot x Location	3	0.07	0.02	2.86	0.04
	Year x Spot x Location	6	0.05	0.01	1.05	0.40
	Residual	216	1.90	0.01		
	Total	239	3.59	0.01		
Ba:Ca	Year	2	0.70	0.35	6.59	0.002
	Spot	1	40.64	40.64	770.81	<0.001
	Location	3	2.24	0.75	14.16	<0.001
	Year x Spot	2	1.30	0.65	12.37	<0.001
	Year x Location	6	2.36	0.39	7.45	<0.001
	Spot x Location	3	2.14	0.71	13.53	<0.001
	Year x Spot x Location	6	1.39	0.23	4.41	<0.001
	Residual	216	11.39	0.05		
	Total	239	62.15	0.26		

Chapter 4: Chemical Analyses

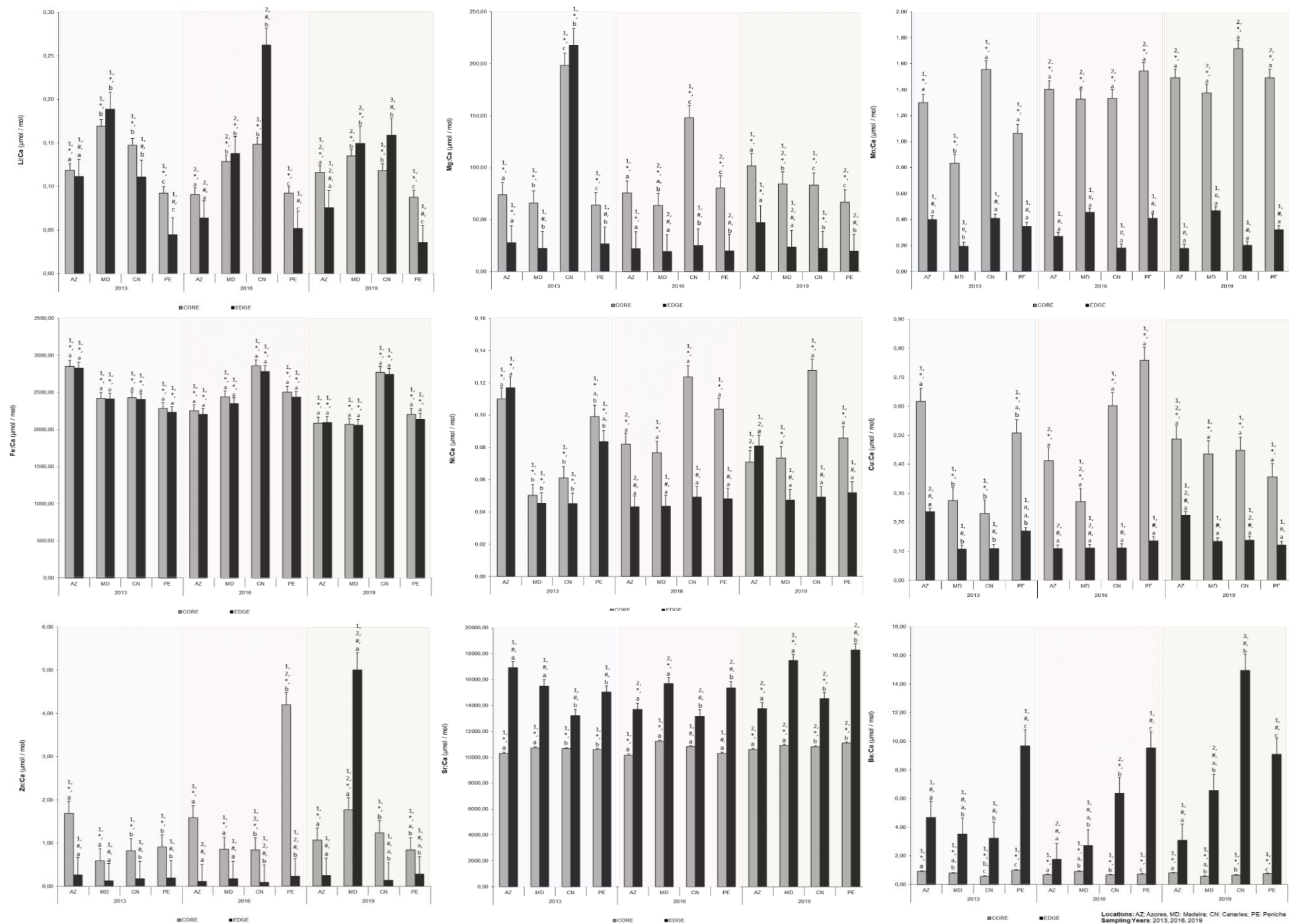


Figure 2: Comparison of element:Ca concentrations (mean $\pm$ SE) from the otolith core and edge of *Trachurus picturatus* harvested in 2013, 2016 and 2019 from the Azores, Madeira, Canary Islands and Peniche. The values marked with different numbers, symbols and letters are significantly different ($p < 0.05$) for year, spot and location, respectively.

Multi-elemental analysis

Core

Multi-elemental analyses for 2013 showed that otolith core fingerprint was significantly different among locations (PERMANOVA, Pseudo-F_{3,29} = 3.02, $p < 0.05$). Significant differences were found between Madeira and Azores and Peniche. The jackknifed re-classification rate of the LDFA was high, with an overall of 80% individuals being correctly assigned to their original location (Fig. 3). Moreover, native individuals seem to contribute more to respective fishing grounds (Table 3). CAP identified three groups, corresponding to the three sampling locations, and showed that Madeira was discriminated based on Li:Ca, while Peniche was discriminated based on Ba:Ca (Fig. 4). For 2016, otolith core fingerprint did not show significant differences among locations (PERMANOVA, Pseudo-F_{3,29} = 1.89, $p > 0.05$). The jackknifed re-classification rate of the LDFA was low (overall 50%) with individuals of Azores being more times assigned to Peniche than to their original location, indicating that the fishing grounds are replenished by a different subpopulation (Fig. 3, Table 3). CAP identified two groups, one with samples from Madeira and another with samples from Azores and Peniche, and showed that Madeira was discriminated based on Li:Ca, Sr:Ca and Ba:Ca ratios, while the other locations were not discriminated by any specific element:Ca concentrations (Fig. 4). Data for 2019 showed significant differences among locations (PERMANOVA, Pseudo-F_{3,29} = 1.97, $p < 0.05$). Differences were found between Peniche and Madeira and Canaries. The jackknifed re-classification rate was low (overall of 48%), with individuals from Madeira being more times assigned to Azores than their original location, and individuals from the Canaries being more times assigned to Madeira than their original location (Fig. 3, Table 3). Furthermore, Peniche population seems to result mainly from self-replenishment, while Madeira appears to have a large contribution of immigrants from the Azores, Canaries seems to have a contribution from all other locations and the Azores shows immigrants from Madeira and Canaries (Fig. 1, Table 3). CAP identified three groups, one with samples from Peniche, a second one with samples from Canaries and the third one with samples from Azores and Madeira overlapped. Madeira was discriminated based on Zn:Ca, while Canaries was discriminated based on Mg:Ca and Ni:Ca, and Peniche based on Mn:Ca and Sr:Ca (Fig. 4).

Edge

Otolith edge fingerprint for 2013 showed high significant differences among locations (PERMANOVA, Pseudo-F_{2,39} = 2.82, $p < 0.05$). Significant differences were found among all locations except Azores and Peniche. The jackknifed re-classification rate of the LDFA was moderate with 61% of the individuals being correctly assigned to their original location (Fig. 3, Table 3). Moreover, native individuals seem to contribute more to the respective fishing grounds, except in the Azores that appears to have a great contribution of immigrants from Madeira (Fig. 1). CAP identified four groups; group 1 with samples from Madeira, group 2 with samples from Peniche, group 3 with samples from the Canaries, and group 4 with samples from Azores (Fig. 4). CAP also showed that Madeira was discriminated based on Li:Ca, the Canary Islands based on Mg:Ca, and Peniche was discriminated based on Zn:Ca (Fig. 4). For 2016, otolith edge fingerprint was highly significant among all four locations (PERMANOVA, Pseudo-F_{3,39} = 3.36, $p < 0.05$). The jackknifed re-classification rate of the LDFA was high, with 87% of the individuals being correctly assigned to their original location (Fig. 3, Table 3), indicating that the fishing grounds are mainly self-replenished (Fig. 1). CAP identified four groups, corresponding to the sampling locations, and showed that the Canaries was discriminated based on Mg:Ca and Fe:Ca, while Peniche was discriminated based on Ni:Ca, Cu:Ca and Zn:Ca (Fig. 4). Data for 2019 also showed significant differences among all locations (PERMANOVA, Pseudo-F_{2,39} = 4 $p < 0.05$). The jackknifed re-classification rate was high with 84% of the individuals being correctly assigned to their original location (Fig. 3, Table 3), again indicating that the fishing grounds are mainly self-replenished (Fig. 1). Again, CAP identified the four groups corresponding to the sampling locations, and showed that the Azores was highly discriminated based on Mg:Ca, Ni:Ca and Cu:Ca, while the Canaries were discriminated based on Li:Ca and Fe:Ca and Madeira based Zn:Ca and Mn:Ca (Fig. 4).

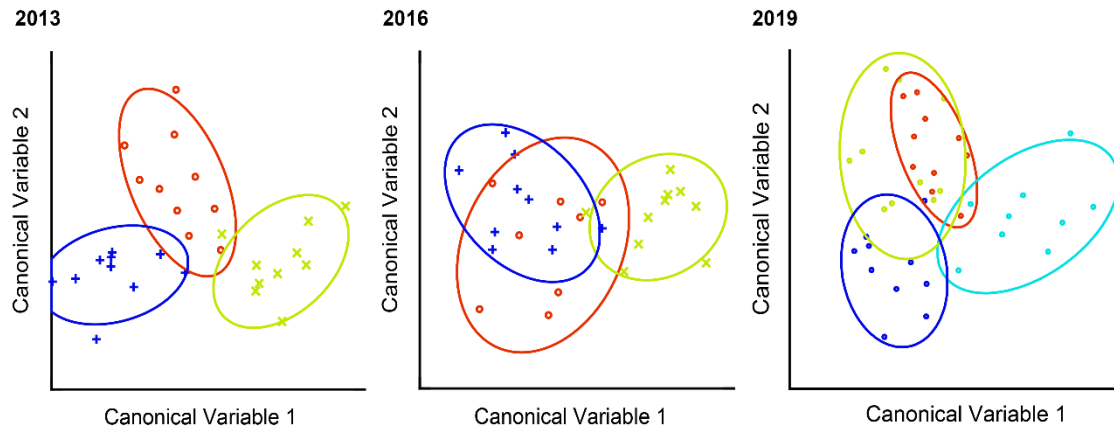
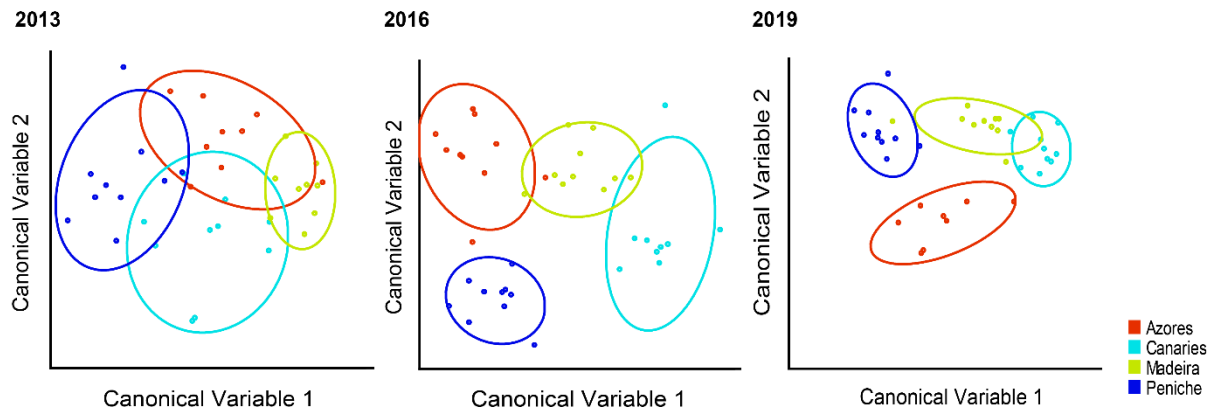
CORE**EDGE**

Figure 3: Canonical variable plots displaying spatial differences in the otolith multi-elemental fingerprints for *Trachurus picturatus* harvested in the four sampling locations of the North-East Atlantic in 2013, 2016, 2019. Core multi-elemental fingerprint for 2019 includes samples from Canaries 2016 (same birth date; for details consult Material and Methods). Ellipses represent 95% confidence intervals around the data, and data points represent individual fish.

Table 3: Jackknifed cross-validation re-classification matrix of the complete linear discriminant analysis for the otolith core and edge multi-elemental fingerprint of *Trachurus picturatus* for each sampling location in the year 2013, 2016 and 2019. For core multi-elemental fingerprint of samples collected in 2019 percentages include data from 2016 samples from the Canaries. For further details, consult Material and Methods.

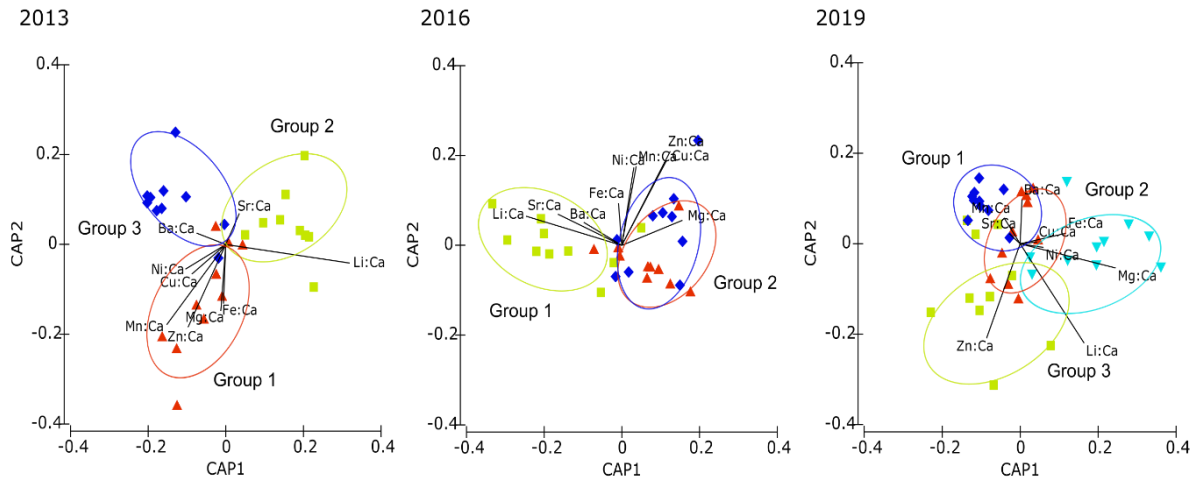
CORE

Original Location	Azores	Madeira	Canaries	Peniche	% correct
2013	Predicted Location				
Azores	7	2	--	1	70
Madeira	1	9	--	0	90
Peniche	1	1	--	8	80
Total	9	12	--	9	80
2016	Predicted Location				
Azores	2	2	--	4	25
Madeira	2	8	--	0	80
Peniche	5	1	--	4	40
Total	9	11	--	8	50
2019	Predicted Location				
Azores	6	2	2	0	60
Madeira	4	2	2	2	20
Canaries	1	4	3	2	30
Peniche	1	0	1	8	80
Total	12	8	8	12	48

EDGE

Original location	Azores	Madeira	Canaries	Peniche	% correct
2013	Predicted Location				
Azores	3	3	1	2	33
Madeira	1	8	0	0	89
Canaries	1	2	5	2	50
Peniche	3	0	0	7	70
Total	8	13	6	11	61
2016	Predicted Location				
Azores	8	1	0	1	80
Madeira	0	9	1	0	90
Canaries	0	1	8	0	89
Peniche	1	0	0	9	90
Total	9	11	9	10	87
2019	Predicted Location				
Azores	7	0	1	0	88
Madeira	0	7	1	2	70
Canaries	0	2	8	0	80
Peniche	0	0	0	10	100
Total	7	9	10	12	84

CORE



EDGE

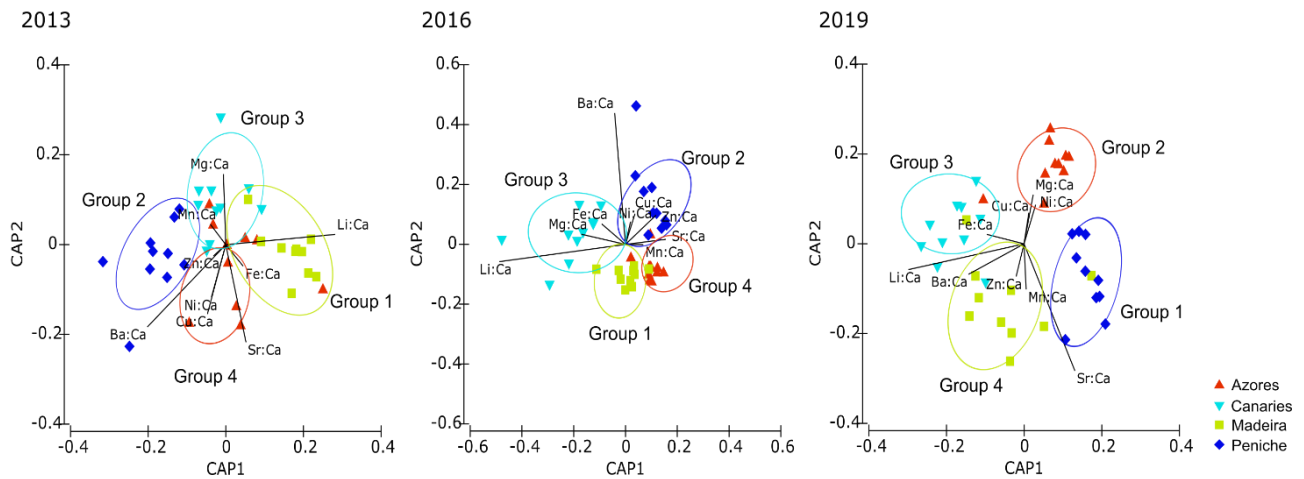


Figure 4: Canonical analysis of principal coordinates (CAP) plots for the otolith multi-elemental fingerprints for *Trachurus picturatus* harvested in the four sampling locations of the North-East Atlantic in the year 2013, 2016, 2019. Core multi-elemental fingerprint for 2019 includes samples from Canaries 2016 (same birth date; for details consult Material and Methods).

Temporal stability within locations

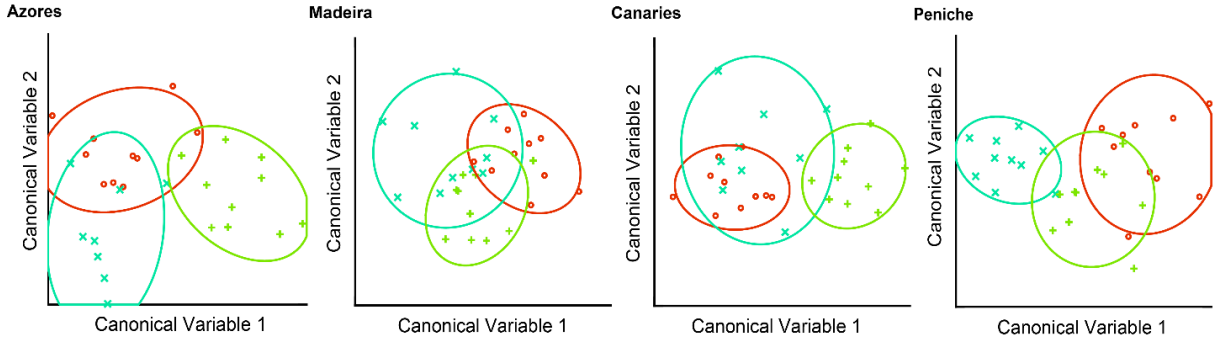
Inter-annual otolith core multi-elemental signatures within the Azores showed significant differences between 2019 and 2013/2016 (PERMANOVA, Pseudo-F_{2,29} = 2.07, $p < 0.05$). The jackknifed re-classification rate was low with 54% of the individuals being correctly assigned to the year of capture (Fig. 5, Table 4). CAP identified two groups, the first with individuals captured in 2019 and the other with individuals from 2013 and 2016 (Fig. 6). Differences among years were driven by Sr:Ca, Mg:Ca and Mn:Ca ratios that contributed to

the discrimination of the samples captured in 2019 (Fig. 6). Within Madeira, the otolith core multi-elemental signatures showed significant differences among the three years sampled (PERMANOVA, Pseudo-F_{2,29} = 2.80, $p < 0.05$). The jackknifed re-classification rate was low with only 43% of the individuals being correctly assigned to the year of capture (Fig. 5, Table 4). CAP identified two groups, one with individuals from 2013 and another with individuals from 2016 and 2019 (Fig. 6). Within the Canaries, there were no significant differences among the sampled years (PERMANOVA, Pseudo-F_{2,29} = 0.73, $p > 0.05$). The jackknifed re-classification rate was low with 52% of the individuals being correctly assigned the year of capture (Fig. 5, Table 4). CAP identified two groups, one with samples from 2019 and another with samples from 2013 and 2016 (Fig. 6). Lastly, otolith core multi-elemental signatures for samples captured off Peniche showed no significant differences among the years sampled (PERMANOVA, Pseudo-F_{2,29} = 0.73, $p > 0.05$). The jackknifed re-classification rate was low with 57% of the individuals being correctly assigned to the year of capture (Fig. 5, Table 4). CAP identified two groups, one with samples from 2016 and another with samples from 2013 and 2019 (Fig. 6).

Otolith edge multi-elemental signatures within the Azores showed significant differences between 2013 and 2016/2019 (PERMANOVA, Pseudo-F_{2,29} = 2.93, $p < 0.05$). The jackknifed re-classification rate was moderated with 67% of the individuals being correctly assigned to the year of capture (Fig. 5, Table 4). CAP identified two groups, the first with individuals captured in 2013 and the other with individuals from 2016 and 2019 (Fig. 6). Differences between years were driven by Fe:Ca, Sr:Ca and Li:Ca ratios that discriminated samples captured in 2013 (Fig. 6). Within Madeira, otolith edge multi-elemental signatures showed significant differences between 2019 and 2013 / 2016 (PERMANOVA, Pseudo-F_{2,29} = 2.99, $p < 0.05$). The jackknifed re-classification rate was moderated with 69% of the individuals being correctly assigned to the year of capture (Fig. 5, Table 4). CAP identified three groups corresponding to the year of capture (Fig. 6). Samples from 2019 were mainly discriminated based on Ba:Ca, Sr:Ca, Ni:Ca, Zn:Ca and Cu:Ca (Fig. 6). Within the Canaries, otolith edge multi-elemental signatures showed significant differences among the three years (PERMANOVA, Pseudo-F_{2,29} = 2.34, $p < 0.05$). The jackknifed re-classification rate was high with 76% of the individuals being correctly assigned to the year of capture (Fig. 5, Table 4). CAP identified three groups corresponding to the year of capture (Fig. 6). Samples from 2013 were mainly discriminated based on Mg:Ca and Mn:Ca, while samples from 2016 were discriminated based on Li:Ca (Fig. 6). Finally, otolith core multi-elemental signatures for samples captured off Peniche showed no significant differences among the years sampled (PERMANOVA, Pseudo-F_{2,29} = 1.28, $p > 0.05$). The jackknifed re-classification rate was low with 47% of the individuals being correctly assigned to the year of capture (Fig. 5, Table 4).

CAP identified three groups corresponding to sampling years, with samples from 2013 overlapping the other two years (Fig. 6).

CORE



EDGE

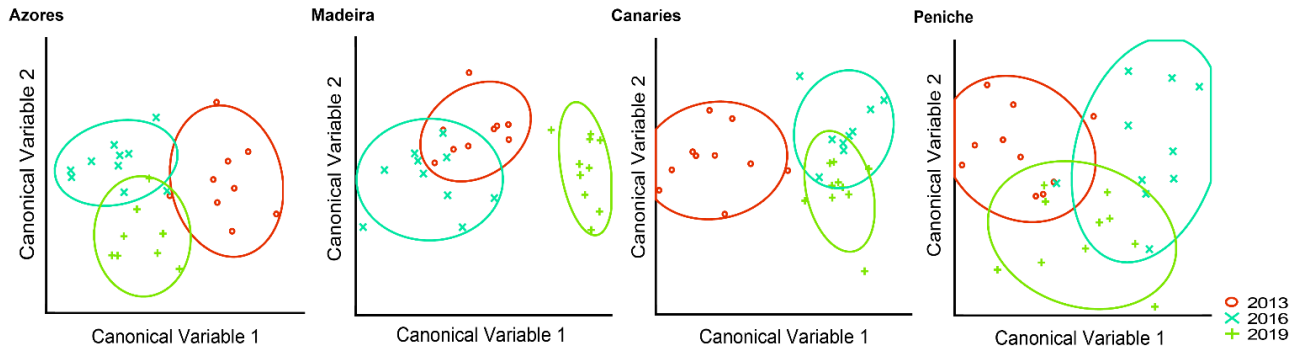
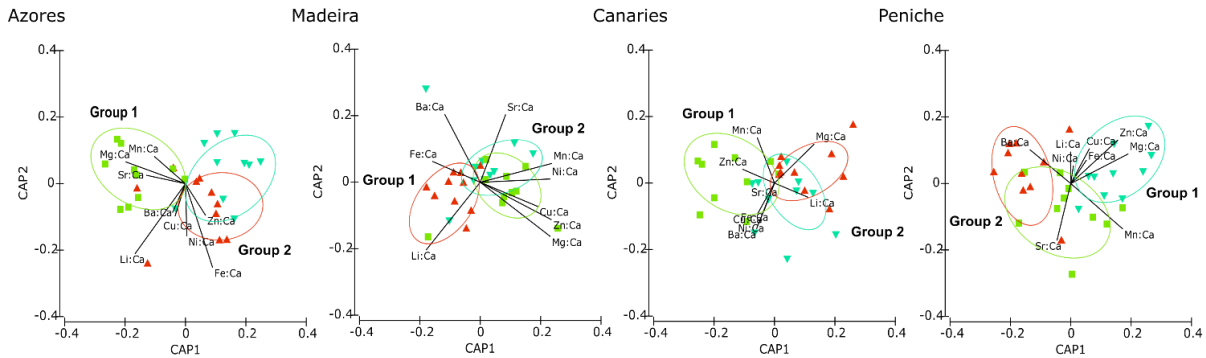


Figure 5: Canonical variable plots displaying temporal differences in the otolith multi-elemental fingerprints for *Trachurus picturatus* harvested in the four sampling locations of the North-East Atlantic in 2013, 2016 and 2019. Ellipses represent 95% confidence intervals around the data, and data points represent individual fish.

Table 4: Jackknifed cross-validation re-classification matrix of the complete linear discriminant analysis for the temporal stability of otolith core and edge multi-elemental fingerprint of *Trachurus picturatus* within each sampling location.

CORE					EDGE				
Original Sampling Year	2013	2016	2019	% correct	2013	2016	2019	% correct	
Azores	Predicted Sampling Year				Predicted Sampling Year				
2013	3	5	2	30	3	1	3	56	
2016	2	5	1	63	5	7	1	70	
2019	2	1	7	70	0	2	6	75	
Total	7	11	10	54	7	10	10	67	
Madeira	Predicted Sampling Year				Predicted Sampling Year				
2013	6	2	2	60	6	3	0	67	
2016	4	3	3	30	5	5	0	50	
2019	2	4	4	40	1	0	9	90	
Total	12	9	9	43	12	8	9	69	
Canaries	Predicted Sampling Year				Predicted Sampling Year				
2013	4	6	0	40	9	0	1	90	
2016	3	3	3	33	0	6	3	67	
2109	0	2	8	80	0	3	7	70	
Total	7	11	11	52	9	9	11	76	
Peniche	Predicted Sampling Year				Predicted Sampling Year				
2013	4	0	6	40	4	1	5	40	
2016	0	8	2	80	2	5	3	50	
2019	4	1	5	50	3	2	3	50	
Total	8	9	13	57	9	8	13	47	

CORE



EDGE

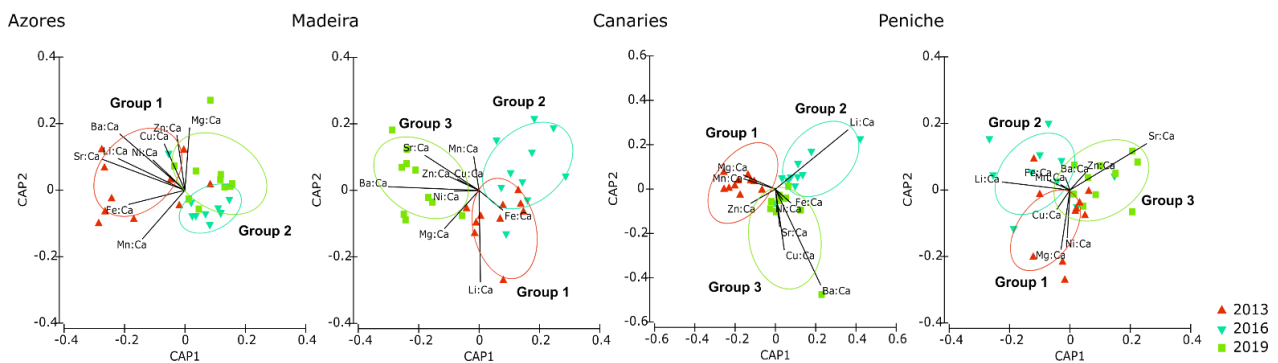


Figure 6: Canonical analysis of principal coordinates (CAP) plots for the otolith multi-elemental fingerprints for *Trachurus picturatus* harvested in the four sampling locations of the North-East in 2013, 2016 and 2019.

Discussion

The good discrimination power for the otolith's edge fingerprints, namely for the individuals collected in 2016 and 2019, suggests that *T. picturatus* populations across the NE Atlantic show high fidelity to the growing, feeding and fishery grounds, and should be regarded as different population units and manage accordingly. These results are congruent with previously published studies that revealed the existence of different population units in the NE Atlantic using different methodological approaches (Hermida et al., 2015; Vasconcelos et al., 2017b, 2018; Moreira et al., 2018, 2019b). Moreover, the overall results of this study showed a moderate assignment of individuals to their natal origin, suggesting that most of the individuals harvested in the sampled fishing grounds are mainly result of self-recruitment, although they could come from different spawning/nursery areas. This is somewhat reported by other studies that recorded the presence of all life cycles stages both in Azores and Canaries (Menezes et al., 2006; Tuset et al., 2019). The degree of connectivity among populations will determine how they replenish themselves, whether they are demographically

open (metapopulation) or closed (self-recruitment), and whether they will persist to intensive harvesting or climate changes (White et al., 2019). The hereby findings suggest that for *T. picturatus*, the populations seem to be open.

The analyses performed showed that there were significant differences among locations for several elemental concentrations, specifically those most likely regulated by environmental features, namely Li, Mg, Cu, Sr and Ba (Webb et al., 2012; Barnes and Gillanders, 2013; Soeth et al., 2019;). The core elemental signature assigned fish to their original location with 80% (2013), 50% (2016) and 48% (2019) average cross-validated accuracy, and the edge elemental signature classified individuals with 61% (2013), 87% (2016) and 84% (2019) average accuracy. These results indicate that *T. picturatus* population in the NE Atlantic fits the metapopulation model, where self-sustainable subpopulations are connected to each other by larval dispersal (Hawkins et al., 2016), albeit small in some years, and most likely related to oceanographic features (e.g., Tanner et al., 2017). This pattern is congruent with the absence of genetic structure observed across the species distribution range, i.e., NE Atlantic and Mediterranean Sea (Moreira et al., 2019a). Similar hypotheses have been used to describe the population dynamics of a variety of pelagic species, such as the anchovy *Engraulis encrasicolus* (Traina et al., 2011) and *E. ringens* (Canales et al., 2018), and the sardine *Sardina pilchardus* (Correia et al., 2014).

The different elemental concentrations observed among locations and between core and edge signatures may be related to variation in water elemental concentrations, as well as the different ontogenetic physiological process, since older individuals have different metabolic costs associated with their age (e.g. feeding, growth, swimming) (e.g. de Pontual et al., 2003; Gibb et al., 2016). The processes involved in the incorporation of trace elements into the otolith are still poorly understood (Limburg et al., 2015; Walther, 2019), but environmental factors, such as salinity and temperature, elements concentration in the water and fish physiology (e.g. growth, metabolic rates) are known to influence the otolith chemical composition (Chang and Geffen, 2013; Thomas et al., 2017; Adelir-Alves et al., 2018). For example, the elemental concentration of Sr and Ba in otoliths is known to be influenced by salinity and temperature, therefore locations with higher salinity are expected to show high levels of otolith Sr:Ca, while higher temperatures and lower salinities are expected to result in high concentrations of Ba:Ca (Barnes and Gillanders, 2013). This relation with environmental features might explain why Azores and Madeira presented higher concentrations for Sr:Ca (locations less susceptible to be influenced by freshwater runoff), and Peniche and Canaries presented higher concentrations of Ba:Ca (locations more susceptible to be influenced by freshwater runoff and anthropogenic effects). Additionally, Mg concentration in the otolith seems to show a positive relationship with temperature, but this element is also likely under biological control, which could explain the higher natal signature (where biological regulation is expected to be lower)

when compared to the moment of capture (Hamer and Jenkins, 2007; Barnes and Gillanders, 2013). Mn incorporation is also poorly understood, but it has been linked to maternal transfer, explaining the much larger natal signatures (Warner et al., 2009; Clarke et al., 2011; Correia et al., 2012). Furthermore, Li incorporation into the otolith is also influenced by salinity, but it seems to occur by direct substitution of Ca, with minimal biological influence (Marriot et al., 2004; Hicks et al., 2010). This suggests that the oscillations observed among locations may be directly related to the environmental concentration of this element. Higher concentrations of Li are expected around Azores and Canary Islands because these locations are influenced by submarine hydrothermal vents (Desbruyères et al., 2000; Becerril et al., 2014), making the Li concentration in the environment higher and, theoretical, more stable around those regions (Chan and Edmond, 1988). However, the results obtained showed that Madeira was repeatedly discriminated based on Li concentrations. Even though Azores and Canaries are expected to present higher levels of Li these regions are also subjected to the influence of the Azores and Canary Currents, respectively, that may cause some instability in the environmental concentrations (Sala et al., 2013). This pattern was already observed for this species in the chemical composition of the whole otoliths (Moreira et al., 2018).

The multi-elemental analyses showed differences among sampling locations for both the otolith core and edge. However, this approach, namely the core fingerprints, does not provide direct information on the accurate location of the different spawning grounds and/or larval sources. To estimate the natal origin of the fish caught at a determined location it is necessary to establish a multi-elemental natal baseline data set for larvae and/or young of the year fish and to make a follow-up of the juveniles and/or adults of the same cohort commercially exploited (Correia et al., 2012). Establishing a baseline sample of pre-dispersal life stages of known origin is complex and laborious since all spawning grounds or nursery areas had to be sampled, which makes this approach difficult to apply (Gibb et al., 2016). Nevertheless, the cluster of individuals observed by the DFA was able to theoretically identify the contribution of an external source to the harvested fishing grounds, and the three cohorts followed in this work showed different levels of contribution to the harvested stocks. In 2013, data suggest that most of the adults who have been harvested result from self-recruitment, with a small contribution of immigrants from the other locations. However, in 2016 there was a large exchange of individuals between Azores and Peniche, where at least half of the adults caught in the Azores showed a similar elemental signal to those of Peniche, and vice-versa. Individuals caught off Madeira and Canaries in 2019 showed an identical pattern of high immigration (half of the individuals showed a similar signal to those found in the Azores and Madeira, respectively). This study was carried out in an extensive geographical area, that is characterized by habitat discontinuities and ecotones between areas with different environmental properties, and complex oceanic currents (e.g. Sala et al., 2013). These

features may contribute to the retention of individuals (larvae and juveniles/adults) in the respective regions, but they can also assist in the long-range dispersal of individuals, a consequence of the several oceanic currents that act in these regions (the Azores Current, the Portugal Current and the Canary Current) (Lamas et al., 2010; Sala et al., 2013). Moreover, the allocation of the individuals accordingly to their birth date showed self-recruitment and migration of individuals across all the sampling locations. Furthermore, both core and edge signatures seem to be temporally unstable between cohorts spaced by three years. These differences have been reported in other studies (Campana et al., 2000; Hamer et al., 2003; Swearer et al., 2003) and are probably related to the natural inter-annual variation in water chemistry expected in regions with such physical characteristics (e.g. Correia et al., 2012; Sala et al., 2103).

Conclusion

The structure detected in this study is common when a metapopulation hypothesis is considered. The results showed that the fishing grounds were self-sustainable, but still connected to the surrounding subpopulations, essentially by the dispersal of larvae/juveniles. Similar structures have been described for several pelagic fish species such as *Trachurus trachurus* (Murta et al., 2008), *Ammodytes marinus* (Gibb et al., 2016) and *Sardina pilchardus* (Silva et al., 2019). Furthermore, although this study only allowed to infer on the possible degrees of mixing, the results agree with previous works and suggest that *T. picturatus* populations in the NE Atlantic should be regarded as different populations units and manage accordingly. Future works should focus on characterizing the multi-elemental baseline data set for larvae or young juveniles in order to identify the different natal sources and their contribution to each adult's fishing ground.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Chapter 5

FINAL DISCUSSION

Recent advances in the application of stock identification methods revealed that most of the marine fish species have a panmictic population structure or numerous distinct populations, with the majority of species exhibiting complex structures within these hypotheses (Reiss et al., 2009; Ciannelli et al., 2013; Kerr, 2016). Very often marine fishes are structured in subpopulations, that are connected by the exchange of early life stages, juveniles and/or adults (Secor, 2015). However, and despite the evidence of a complex population structure and mixing within and between stocks, fisheries management still assume closed stocks for the majority of cases (e.g. Kerr, 2016; Silva et al., 2018). Management of mixed stocks as a single stock unit can potentially lead to overfishing of less productive populations and under fishing of the most productive ones (Kerr et al., 2010; Silva et al., 2018), contributing to the current fishing situation, where 60% of the fish stocks are exploited at the maximum sustainable yield (FAO, 2018).

In marine ecosystems, population structure is determined by larval dispersal and recruitment, and understanding the dynamics of large marine fish stocks continues to be one of the most demanding challenges for fisheries science (Hidalgo et al., 2019; Schunter et al., 2019). It requires the characterization of the connectivity among populations, that in marine fishes can be driven by several features and occur at any life stage (Kerr, 2016; Cadrin, 2020). Connectivity among regions depends on the species pelagic larval duration, reproductive potential, physical barriers, ocean currents or localized eddies and oceanographic retention patterns. Nonetheless, a number of tools can be applied to infer population structure, connectivity, migration and retention patterns, namely genetic, geometric morphometrics and otolith chemistry analyses (Cadrin et al., 2014; ICES, 2018; Cadrin, 2020).

The blue jack mackerel *Trachurus picturatus* is one of the most important pelagic fish species harvested in the Macaronesia Islands of Azores, Madeira and Canaries, with a high economic value for the fisheries industry, namely for the local communities (ICES, 2018; INE, 2018; Tuset et al., 2019). Currently, *T. picturatus* is nationally and internationally considered a single homogeneous stock and managed accordingly (ICES, 2018b). However, scientific information to understand the population structure, individuals' movements and connectivity is scarce. Moreover, the European Commission is requiring the establishment of separate fishing quotas in the Macaronesia Atlantic archipelagos [Council regulation (EU) 2018/120; Tuset et al., 2019], therefore describing the species' population structure across the North-East (NE) Atlantic is mandatory. In the last decade, *T. picturatus* discharge records showed strong fluctuations and authorities considered that these were due to fluctuations in abundance and availability (ICES, 2018). Still, studies specifically addressing why these fluctuations occur are scarce. In view of this lack of information and taking into consideration the economic importance of *T. picturatus*, the main goal of this thesis was to infer the population structure, connectivity and migration patterns across its NE Atlantic distribution. To do so, a holistic

approach was applied using morphometric, and for the first time in the region, otolith chemical and molecular analyses.

Until very recently, *T. picturatus* studies were focused on determine life traits characteristics such as growth, maturation and reproduction in the specific regions they were harvested, *i.e.*, in the Azores, Madeira and Canaries regions, without bearing in mind possible connectivity patterns among regions. Still, these regional studies reported different growth rates between Azores and Madeira (Vasconcelos et al., 2006; Garcia et al., 2015) and, although similar periods of reproduction among the islands have been reported - December to May in the Azores (Garcia et al., 2015), January to April in Madeira (Vasconcelos et al., 2017a) and Canary Islands (Jurado-Ruzafa and García Santamaría, 2013) - the peaks of the spawning season seem to occur in different months for the different locations - February for males and March for females in the Azores; and January - February in Madeira (Garcia et al., 2015; Vasconcelos et al., 2017). These features are an indication that the species may be structured into subpopulations. Moreover, previous studies of *T. picturatus* in the Azores, Madeira and the Canary Islands have described the presence of all life stages, *i.e.*, larvae, juveniles and adults, in these regions (Menezes et al., 2006; Vasconcelos et al., 2017; Tuset et al., 2019). Despite this, the characterization of the species' population structure and movements in the Macaronesia region have only recently started. Parasitic infection rates (Hermida et al., 2015), geometric morphometrics and otolith shape (Vasconcelos et al., 2018) were analysed to infer the population structure of individuals harvested in different fishery regions. However, even though these studies reported the existence of different populations units in the NE Atlantic, supporting the different biological features observed by the regional studies, they were incomplete. There was still a substantial geographic area that the previous authors did not consider, the sampling schemes did not focus on specific life stages, covering a wide range of lengths, and only a small number of the available approaches were applied. To improve this gap, the work included in this thesis focused directly on the study of the species' population structure and connectivity, with the application of a large number of approaches, including the first genetic characterization of individuals captured in the Macaronesia region; with the increase of the geographic range sampled (in relation to the previous studies published), *i.e.*, all of the most important fishing grounds managed by the Portuguese authorities were included; and, for the first time, with temporal analyses of different cohorts in order to determine the temporal stability of the fish' stocks.

The overall results suggest that *T. picturatus* in the NE Atlantic should be treated as a metapopulation. The populations harvested in this study exhibit some degree of independence among them, verified through the identification of different phenotypic stocks, but remain connected to each other by the dispersal of larvae/adults, as was revealed by the lack of genetic structure. These results are in agreement with the population structure described for

other related fish species, namely *Trachurus trachurus* (Abaunza et al., 2008), *Gadus morhua* (Kelly et al., 2009), and *Clupea harengus* (Geffen et al., 2011). The molecular approaches described in Chapter 2 (mtDNA and microsatellite markers) revealed no spatial genetic differences among fishing grounds nor among cohorts. Still, microsatellite markers analyses indicated a weak multidirectional migration of individuals between Madeira and Portugal mainland in autumn/winter and in the opposite direction in spring/summer. Furthermore, the ecological approaches showed clear isolation of the oceanic fishing grounds, albeit there was evidence of connections among sampling locations and fluctuations along the sampling periods, according to the approach applied. Body morphometric analysis (Chapter 3) by truss network and discrimination analysis showed a phenotypic distinction between Azores, Madeira and Canary Islands for both cohorts studied (2016 and 2019). However, the calculated distances that allowed this discrimination were different between years, and the regions' discrimination was more expressive for the last cohort (*i.e.*, 2019), where Peniche was isolated from the other coastal fishing grounds. Otolith shape analysis (Chapter 3) by shape indices and elliptical Fourier descriptors showed different otolith shapes among fishing grounds but also between cohorts. Fish sampled in 2013 showed a low overall allocation rate (43%) suggesting a single population unit, whereas otolith shape from 2016 samples allowed the discrimination of three spatial groups - the Azores, Canary Islands and Madeira/Portugal mainland - with a moderate overall allocation rate (62%). Otolith microchemistry (Chapter 4) showed different chemical and isotopic signatures between Portugal mainland, Azores, Madeira and Canary Islands, with a high overall re-classification rate of 81%. Moreover, otolith core and edge fingerprint (Chapter 4) showed different chemical signatures among the oceanic islands and the Portuguese coast, but it also indicated migration among fishing grounds with different rates of dispersal for the three cohorts.

Overall, for each cohort analysed, the phenotypic approaches employed in this work depict the existence of at least four subpopulations in the NE Atlantic, one subpopulation in the Azores region, another in Madeira, another in the Canary Islands and another in the Portuguese coast. On the other hand, the genotypic approaches showed a lack of genetic structure, even between the Atlantic Ocean and the Mediterranean Sea. The discrimination of these phenotypic subpopulations is most likely related to individuals being exposed to different environmental conditions (Sajina et al., 2011; Mir et al., 2013; Kaouèche et al., 2017). The Macaronesia region is characterized by complex oceanic currents that can have a great effect on the rate and direction of fish species' dispersal, such as the Azores current (AC), the Portugal current (PC) and the Canary Current (CC) (Lamas et al., 2010; Sala et al., 2013). For example, the AC splits into two branches with one flowing north in the direction of the Gulf of Cadis, and the other one flowing southwards, in the direction of Madeira and Canaries, possibly carrying larvae from the Azores to the Portuguese coast and to the other islands. As for the

PC current, one of the branches moves from the Portuguese continental coast to the Azores, possibly carrying individuals from the Portuguese coast to Azores and then to the other islands through the AC, while the other branch flows poleward along the Iberian Peninsula during fall, winter and early spring and southward during late spring and summer (Martins et al., 2002). On the other hand, this region is also known to show habitat discontinuities, different upwelling systems and ecotones between areas with different environmental properties (e.g. Stefanni et al., 2015). The outcome of this study seems to reflect the effect of these oceanic features, where the discontinuities and ecotones are responsible for the retention of individuals, resulting in the establishment of phenotypic stocks, while the existent water currents promote the dispersal of both larvae and adults, leading to the subsequent identification of only one genotypic stock. Moreover, the temporal analyses of the different cohorts showed that *T. picturatus* can exhibit fast phenotypic adaptations to the surrounding environment.

Conclusion

The main goal of this work was to depict if the NE Atlantic population of *T. picturatus* is a single and homogeneous stock as it is described by ICES or if the species is structured in subpopulations, as many marine fish species seem to be (Secor, 2015). To do so, five key questions were raised, and as a result of answering these questions, authorities will be able to understand if the species' fisheries are in need of different management policies.

First, does a self-recruitment or a metapopulation model fit this species? The overall results of this thesis support the existence of a metapopulation structure for the species across the NE Atlantic region. The identification of only one genotypic stock (with the inclusion of samples from the Mediterranean Sea) by mtDNA and microsatellites analyses, together with the identification of different phenotypic stocks depicted by the ecological approaches, support the existence of subpopulations across the NE Atlantic that are still connected by the dispersal and migration of individuals. This pattern seems to be consistent with that described for other marine fish species (Correia et al., 2014; Pita et al., 2016b; Santos et al., 2018), including a closed related species *Trachurus trachurus* (Abaunza et al., 2008).

Secondly, if it is a metapopulation, what is the subpopulations' structure in the North-East Atlantic and the Mediterranean Sea populations? And, what is the magnitude of influence of each subpopulation on adjacent subpopulations? The ecological approaches applied in this work allowed the identification of four subpopulations across the NE Atlantic. Both geometric morphometric analyses and otolith chemical analysis (solution-based and laser ablation techniques) showed clear isolation of Azores, Madeira, Canary Islands and Portugal mainland. Furthermore, the otolith fingerprint analyses indicated that subpopulations are connected to

each other by the dispersion and migration of individuals. However, even though there was a high degree of spatial structure among the islands and the Portuguese coast for each year, the results also showed that this isolation was not stable over time. For most of the ecological approaches used, *i.e.*, landmark-based body morphometrics, otolith shape analysis and otolith laser ablation, differences in the discrimination of fishing grounds were observed. For example, the otolith shape of the samples harvested in 2013 appears to be similar among fishing grounds, while the samples harvested in 2016 indicated clear isolation of Azores, Canary Islands and Madeira/Portugal mainland. Moreover, the signal obtained from the core otolith fingerprint showed different degrees of migration among fishing grounds between the years analysed. Since the identification of phenotypic stocks is strongly dependent on the individuals being exposed to different environmental features (Waldman, 2005), the results obtained suggest that, for the years analysed, the regions were influenced by different environmental factors, therefore conditioning both the dispersal and the development of the individuals. These differences include for instance changes in the current systems, variations on the temperature and/or salinities gradients, stronger or weaker upwelling systems (Kerr, 2016; Kleisner et al., 2016; Kritzer et al., 2016).

Thirdly, are there multiple spawning grounds/replenishment sources for this species? Even though none of the approaches applied can directly provide information on the different spawning grounds, otolith fingerprint allows to infer the number of natal sources contributing to the different fishing grounds (*e.g.* Gibb et al., 2016). Accordingly, results obtained for the otolith fingerprint analyses and discussed in chapter 4 showed that the subpopulations harvested are connected by the dispersal of individuals, apparently with a higher frequency in the larval phase, indicating that there are multiple replenishment sources for the species. Still, replenishment of fishing grounds by immigrants seems to be small and variable among the cohorts analysed. Nevertheless, results support the existence of multidirectional individual dispersal between Azores and Madeira, and between Azores and Peniche. Since dispersal happened essentially in the larval phase, these movements are almost certainly related to the well-characterized oceanic currents found in the NE Atlantic.

Lastly, can the adult fish from different subpopulations be linked to one or several nursery areas? The discrimination of fishing grounds discussed in chapter 3 and 4 indicate that, for the species NE Atlantic distribution range, each of the regions sampled acts as different nursery grounds. Despite the dispersal of individuals observed by all the applied approaches, most of the individuals captured were the result of self-recruitment, *i.e.*, most of the stock replenishment is done by individuals spawned in each of the sampled regions. Moreover, several authors have reported that all the species life cycles can be found in Azores (Menezes et al., 2006), Madeira (Vasconcelos et al., 2017) and Canary Islands (Tuset et al., 2019), indicating that each region has individual nursery areas. Furthermore, Tuset et al.

(2019) have described a pattern of co-existing migratory and resident contingents for *T. picturatus* in the Canary Islands. Still, the present work cannot infer on the possibility that this is the case for the other fishing grounds.

Finally, what are the implications of this work for the species stock assessment and fisheries management? The overall outcome of this work showed that the species has a metapopulation structure, with subpopulations connected by the dispersal of individuals. This means that the current definition of one stock unit across the species distribution range is outdated. Future assessments should consider the new information available and proceed to the application of regional management plans. Moreover, this work also shows that population structure is dynamic and largely influenced by environmental fluctuations, therefore, there is also the need to establish year-classes follow-up of the subpopulation in the different fishing grounds. Sampling larvae or young-of-the-year (YOY) fish in the spawning/nursery areas and subsequent follow up of the cohorts in the fisheries grounds is mandatory to accomplish this task.

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Supplementary Material

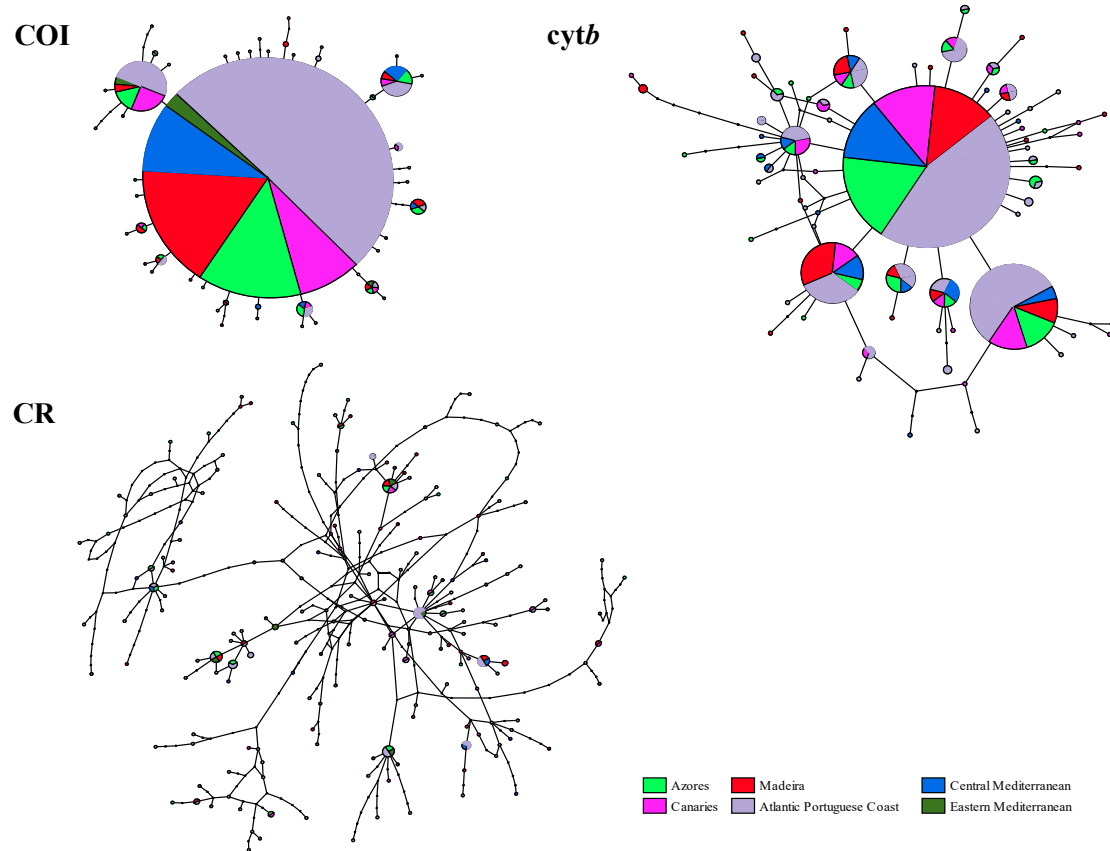
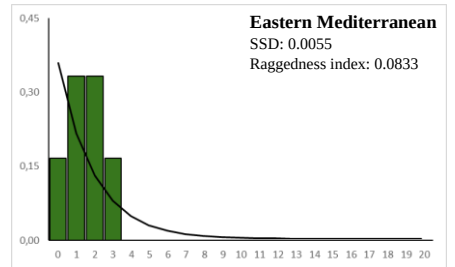
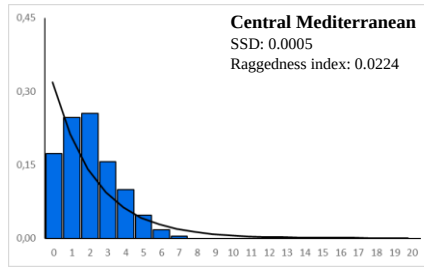
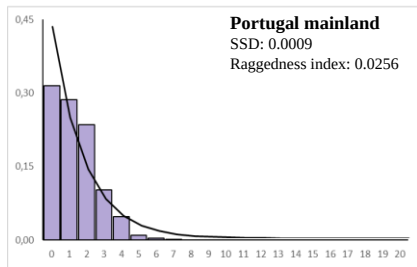
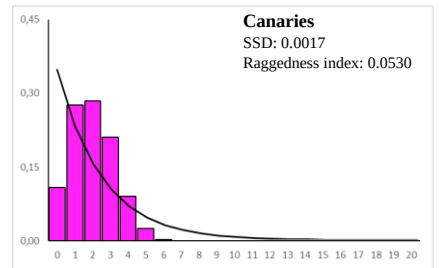
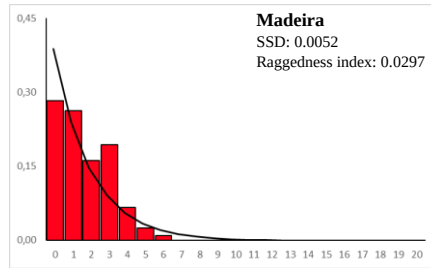
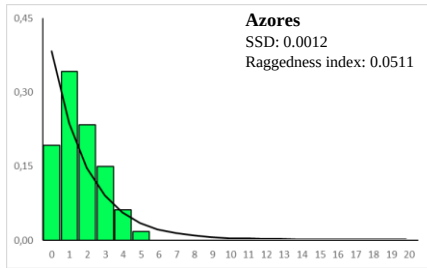
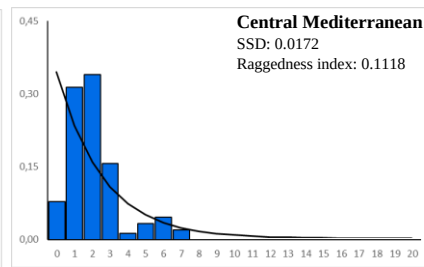
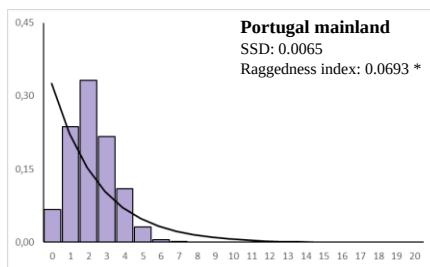
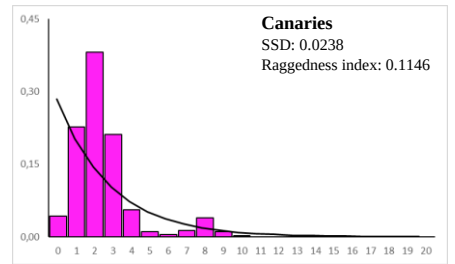
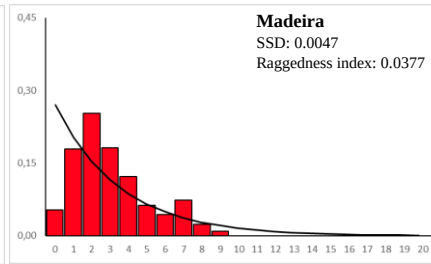
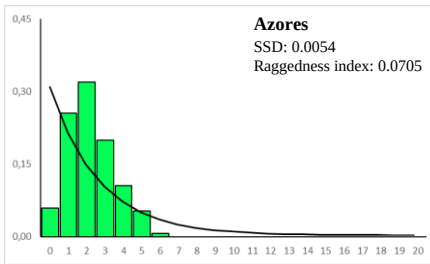


Figure S1: Haplotype (TCS) networks for each individual gene showing the relationships of *Trachurus picturatus* specimens across the species' distribution. Circle size is proportional to the observed haplotype frequencies and black points represent unobserved haplotypes and potential intermediates.

COI



cytb



CR

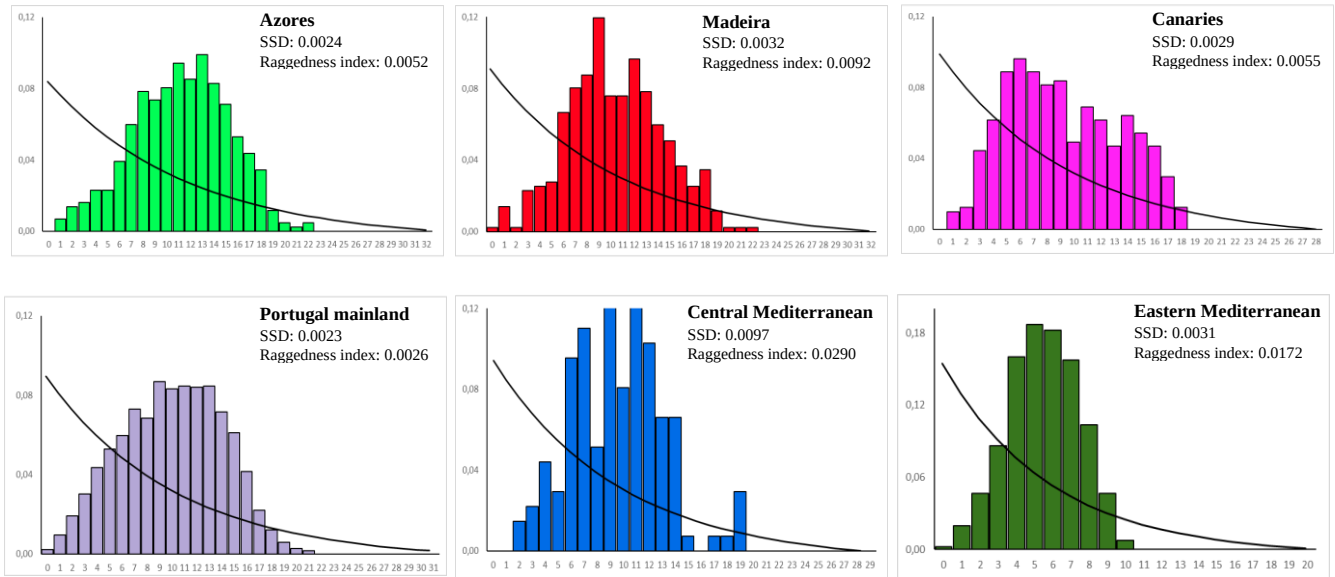


Figure S2: Mismatch distributions for each gene region of *Trachurus picturatus*. Black curves show the expected distribution of mutations according to the null hypothesis of constant population size. The number of pairwise differences and their frequencies is shown on the horizontal and vertical axes, respectively. Colours represent the sampling sites: Azores - green; Madeira - red; Canaries - pink; Portugal mainland - grey; Central Mediterranean - blue; Eastern Mediterranean - dark green. Results for the sum of squared deviation (SSD) and Harpending's Raggedness index are also presented (significant results, $p < 0.05$, are marked with *).

Table S1: GenBank accession numbers for the novel sequences and the additional gene sequences determined in previous studies (*) of *Trachurus picturatus*.

	<i>COI</i>	<i>cytb</i>	<i>CR</i>
Azores	MH191076 - MH191085	MH190978 - MH190987	MH190940 - MH190949
	MK047001 - MK047021	MK047049 - MK047068	MK047253 - MK047272
Madeira	MH191086 - MH191095	MH190997 - MH191006	MH190950 - MH190959
	MK047022 - MK047041	MK047089 - MK047108	MK047273 - MK047292
Canaries	MH191066 - MH191075	MH191027 - MH191035	MH190930 - MH190939
	MK046984 - MK047000	MK047069 - MK047088	MK047234 - MK047252
Portugal mainland	MH191036 - MH191065 MK046924 - MK046983	MH190969 - MH190977	MH190900 - MH190929 MK047175 - MK047233
		MH190988 - MH190996	
		MH191017 - MH191026 MK047109 - MK047168	
Central Mediterranean	MH191096 - MH191105	MH191007 - MH191016 MK047169 - MK047174 AF489423* AY526546 - AY526547*	MH190960 - MH190968 MK047293 - MK047300
	MK047042 - MK047048		
	KJ709658 - KJ709659*		
	FN689060*		
	FN688974*		
Eastern Mediterranean	FN689326*	AF489414 - AF489421* EU246361 - EU246368*	EU246442 - EU246468* AY533477 - AY533478*
	FN689325*		
	KY176679*		
	KX782508*		
	KX782662*		

Table S2: Genetic differentiation (Φ_{ST}) for the three gene regions sequenced for *Trachurus picturatus* among the studied populations. Statistical significance ($p < 0.05$) is indicated by *.

<i>COI</i>	Azores	Madeira	Canaries	Portugal mainland	Central Mediterranean
Madeira	-0.01011				
Canaries	-0.00412	0.03231			
Portugal mainland	-0.00239	-0.00245	0.03483*		
Central Mediterranean	-0.01328	0.00178	0.01427	0.01698	
Eastern Mediterranean	-0.09049	-0.06689	-0.08939	-0.07350	-0.04276

<i>cytb</i>	Azores	Madeira	Canaries	Portugal mainland
Madeira	-0.00037			
Canaries	-0.01155	-0.00630		
Portugal mainland	-0.01060	0.00270	-0.01030	
Central Mediterranean	-0.01368	-0.00938	-0.00795	0.00023

<i>CR</i>	Azores	Madeira	Canaries	Portugal mainland	Central Mediterranean
Madeira	-0.00219				
Canaries	-0.00346	-0.00115			
Portugal mainland	-0.00220	-0.00032	-0.00037		
Central Mediterranean	-0.00196	-0.00078	0.00000	-0.00211	
Eastern Mediterranean	-0.00223	0.00008	0.00004	-0.00033	0.00126