ANA MOURA

POPULATION STRUCTURE, HABITAT CONNECTIVITY AND MIGRATION PATTERNS OF ATLANTIC MACKEREL (*Scomber scombrus*) IN THE NORTH ATLANTIC USING OTOLITH CHEMICAL AND SHAPE ANALYSES

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Population structure, habitat connectivity and migration patterns of Atlantic Mackerel (*Scomber scombrus*) in the North Atlantic using otolith chemical and shape analyses

Declaração de autoria de trabalho

Declaro ser a autora deste trabalho que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

(Ana Moura)

This work has been already presented in one international congress:

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Abstract

Atlantic Mackerel is a widely distributed fish species in the North Atlantic with two stocks, one in the North Eastern Atlantic (NEA) and other in the North Western Atlantic (NWA). Each stock is composed of different spawning components which present seasonal and spatial dynamics influenced by the environment. The knowledge on the structure of these stocks and its migration patterns are a challenge to scientists, since many environmental factors can influence this species dispersion. Information on fish stocks with economic importance, such as *Scomber scombrus,* is important in order to maintain a sustainable fishery. The use of natural tags, such as the chemical and shape signatures of otoliths have been proven to succeed in providing insights on fish populations and habitat connectivity. Here, six locations were analysed, with a total of 180 individuals, which were caught between January and February of 2018: the two spawning components from the NWA stock, the Canadian Northern Component and the US Southern Component; and the three spawning components from the NEA stock, namely the North Sea, Western and Southern Components, plus, an overlapping area, the Bay of Biscay. Both otolith's chemical and shape signatures had high reclassification percentages in separating the two stocks and the components within each stock, especially when using both tools combined: 100% reclassification for the stocks and the components from the NWA stock, and 82% for the NEA stock components. The Bay of Biscay had a large overlap with the Southern and North Sea Components. Results revealed that these are good tools for population discrimination revealing grouping separation of the stocks and the components. These findings call for the necessity of further investigation and multidisciplinary approach on the assessment of this species and the necessity of revaluating the stocks management, primarily in the NWA stock.

Keywords: Scombridae, Pelagic fish, Otoliths, Natural tags, Stock discrimination

Resumo

A sarda, *Scomber scombrus*, é uma espécie de peixe pelágico, que apresenta uma ampla distribuição no Atlântico Norte, sendo caracterizada por ter uma vasta dispersão migratória. Os seus padrões migratórios sofrem alterações espaciais e temporais de ano para ano, sendo que é uma espécie sensível à temperatura e movimenta-se em função da mesma. No Atlântico Norte existem duas unidades populacionais distintas para fins de gestão pesqueira, uma que habita o Atlântico Nordeste e outra que habita o Atlântico Noroeste, aparentemente sem conexão. Cada uma destas populações é constituída por diferentes componentes reprodutoras. A população do Atlântico Nordeste é formada pela componente Sul, cuja área de reprodução rodeia a Península Ibérica, a componente Este, cuja área de reprodução se estende desde a Baia da Biscaia até às Ilhas Britânicas, e a componente do Mar do Norte, cuja área de reprodução se situa no Mar do Norte. Estas componentes, após reprodução nos seus respetivos locais, migram para norte e juntam-se, no Outono, na zona do Mar da Noruega e águas da Islândia para a fase de alimentação. Após esta fase, dirigem-se ligeiramente para sul e mantêm-se no Norte das Ilhas Britânicas até ao fim do ano. Por fim, voltam a migrar para sul para as zonas de reprodução. A população do Noroeste Atlântico é constituída pela componente Norte, no Canadá, e a componente Sul, nos EUA, que se juntam em águas mais fundas e exteriores à costa do "Mid-Atlantic Bight" no Inverno. No fim do Inverno as componentes separam-se, sendo que a componente Sul se reproduz e alimenta na costa Norte dos EUA e a componente Norte se reproduz e alimenta na costa sul do Canadá. Estas populações têm uma grande importância económica, sendo exploradas em toda a sua zona de distribuição. A população do Nordeste faz parte de uma das maiores pescarias do Norte Atlântico e, em contraste, a população Nordeste encontra-se em declínio crescente. Com as alterações climáticas e outros factores relacionados, a distribuição de ambas as populações tem-se estendido para Norte. Esta extensão torna a Sarda num recurso disponível para novos países e, como tal, explorada pelos mesmos. Estas alterações têm precursões para o estado desta unidade pesqueira que, se não for bem estudada, avaliada e compreendida, pode deixar de ser um recurso com exploração sustentável. Uma vez que a distribuição das componentes de cada população sofre alterações dinâmicas e sazonais com o ambiente, a sua estrutural populacional ainda não se encontra bem definida. Pode haver a necessidade de que as componentes destas duas populações sejam geridas de forma mais particular, caso a separação das mesmas prove ser suficiente para que sejam consideradas populações e unidades pesqueiras distintas. A gestão não informada de recursos pesqueiros pode à levar depleção das mesmas, com graves consequências ecológicas, económicas e sociais. As assinaturas naturais aparentam ter bons resultados na recolha de informação acerca da conectividade e isolamento populacional de espécies marinhas, e aferir sobre a estrutura das mesmas. A assinatura química e de morfologia dos otólitos de um peixe reflectem o ambiente em que este vive, uma vez que são afectadas por questões relacionadas com as massas de água por onde este passa. E, assim sendo, as alterações, nas assinaturas dos otólitos, entre populações pode providenciar informações sobre os seus movimentos e permitir perceber se existe ou não mistura entre elas. Como tal, o objetivo do trabalho foi adicionar informação acerca da Sarda, tentando compreender a sua estrutura populacional utilizando estes marcadores naturais. Adicionalmente também perceber se este tipo de estudos pode ser relevante, ou não, para responder a questões importantes sobre as populações desta espécie. Na análise, indivíduos de 3 anos de idade capturados entre Janeiro e Fevereiro de 2018, de 6 locais diferentes, foram analisados. Foi feita amostragem a indivíduos de todas as componentes de cada população e, adicionalmente, foram adicionados à análise indivíduos do Golfo da Biscaia. O Golfo da Biscaia é uma zona em que a reprodução dos indivíduos das componentes Este e Sul da população do Atlântico Nordeste se sobrepõe e, como tal, os resultados podem permitir tirar conclusões acerca da magnitude desta sobreposição. Nos resultados, comparando as análises, a assinatura química demonstrou melhor sucesso discriminativo na separação dos locais mas, de um modo geral, a junção das duas metodologias teve o maior sucesso discriminativo. Como seria de esperar, houve uma completa discriminação (100%) das populações dos dois lados do Atlântico. Esta diferença parece ser marcada pela diferente influência hidrológica, a nível das principais correntes oceânicas, nas duas costas do Norte Atlântico. Na população do Nordeste Atlântico, apesar de não ter havido completa discriminação, a percentagem de reclassificação das componentes também foi bastante alta (82%). A separação destas componentes pode ter tido influenciada por diversos factores como a temperatura, salinidade e a intensidade de fenómenos de afloramento costeiros. A Baia da Biscaia demonstrou uma maior sobreposição com as componentes Sul e do Mar do Norte. A sobreposição com a componente Sul seria está em conformidade, no entanto, a maior sobreposição com o Mar do Norte e menor sobreposição com a componente Este não seria o resultado esperado. Ainda assim, reflecte a grande dinâmica, a níveis migratórios, que caracteriza esta espécie e a maior conectividade de habitats que esta população apresenta. Para a população do Noroeste Atlântico houve a completa separação dos componentes, usando a junção da assinatura química e morfológica (100%). A separação destas componentes pode ter sido influenciada por factores ambientais como a salinidade, mas também questões fisiológicas, uma vez que os indivíduos de cada componente aparentam ter taxas de crescimento distintas. Este resultado reforça o facto de estas componentes terem uma menor mistura, quando comparadas com as componentes da população do Nordeste Atlântico. Adicionalmente, podem indicar a necessidade de considerar estas componentes como unidades populacionais distintas para fins de gestão pesqueira. Este estudo levantou questões importantes acerca das populações da Sarda, no entanto uma abordagem interdisciplinar é necessária para que a gestão das mesmas possa ser de alguma forma ajustada. Como tal, as conclusões deste estudo demonstram a necessidade de contínua investigação desta espécie, de forma a promover a conservação da mesma.

Palavras-chave: Scombridae, Peixe pelágico, Marcadores naturais, Otólitos, Unidades pesqueiras

Table of contents

Index of figures

Index of tables

List of abbreviations

ANOVA: One-way analysis of variance

ANCOVA: One-way analysis of covariance

CAP: Canonical analysis of principal coordinates

CI: Circularity

EFD: Elliptic Fourier Descriptors

EL: Ellipticity

FAAS: Flame atomic absorption spectrometer

FF: Form Factor

IRMS: Isotope ratio mass spectrometry

NEA: North Eastern Atlantic

NE-S: North Eastern Southern component

NE-BB: North Eastern Bay of Biscay

NE-W: North Eastern Western component

NE-NS: North eastern North Sea component

NWA: North Western Atlantic

NW-N: North Western Northern component

NW-S: North Western Southern component

OA: Otolith Area

OL: Otolith Length

OM: Otolith Mass

OP: Otolith Perimeter

OW: Otolith Width

PERMANOVA: Permutational multivariate analysis of variance

RE: Rectangularity

RO: Roundness

SB-ICP-MS: Solution based inductively coupled plasma mass spectrometry

SI: Shape Indices

SE: Standard Error

TAC: Total Allowable Catch

TL: Total Length

TM: Total Mass

1. Introduction

1.1. Fisheries stock assessment

A fish stock is a semi-discrete group of fish with definable characteristics that are assumed to be a homogeneous unit for fisheries management purposes (Begg and Waldman, 1999). Stock identification is an interdisciplinary field that involves the recognition of self-sustaining components within natural populations (Cadrin *et al*., 2005). To manage a fishery effectively it is important to know the identity of the stock structure of a species in combination with estimation of the degree of exchange between stock members since, in fisheries that can contain several stocks, each stock may have unique demographic properties and distinct responses to exploitation or rebuilding strategies (Begg, 1998; Begg *et al*., 1999; Cadrin *et al*., 2005). Failing to detect the population structure of exploited marine fish species by neglecting that a single stock could be replenished by multiple populations or that multiple stocks could belong to a single population can lead to local overfishing and severe stock decline (Ying *et al.*, 2011). Because stock demographics and the degree of overlap in their spatial range for periods of time are difficult to simultaneously assess, they are often ignored, resulting in false apparent trends in fishery assessments (Cadrin *et al*., 2014).

Ocean circulation patterns, sea-floor topology and other geographic features provide opportunities for species isolation and differentiation, but most of the world's oceans lack obvious barriers (physical and/or oceanographic) (Souza *et al.*, 2006). Several fish species have developed extended pelagic larval stages and high migratory capabilities as larvae, juveniles and adults, resulting in widespread ocean dispersal, which reduce the potential for geographic differentiation between distant populations (Souza *et al*., 2006). In the North Atlantic, commercially important pelagic fish stocks undertake extensive seasonal migrations that are connected to local ecosystem regimes, however, with changing environmental conditions, the spatial and seasonal distribution and life history strategy of the species may vary over time (Trenkel *et al.*, 2014). Furthermore, stock discrimination is imperative for the fisheries management in the North Atlantic, and stock structure information provides a basis for understanding the dynamics of fish populations (Begg, 1998; Begg and Waldman, 1999). Modern fisheries management is moving towards a precautionary approach to ensure a sustainable and rational use of the marine resources, but stock assessment models are mainly based on a single unit stock assumption; however, this assumption is often violated by greater stock complexity (Begg *et al*., 1999).

1.2. Atlantic mackerel

Atlantic mackerel (*Scomber scombrus*) is one of the most abundant and widely distributed pelagic migratory fish species in the North Atlantic, mostly restricted to the cold and temperate coastal regions (Hamre, 1978; Villamor *et al*., 2004; Souza *et al*., 2006). It is highly abundant from Morocco to the North of Norway in the Northeast Atlantic and from North Carolina to the North of Newfoundland in the Northwest Atlantic (Sette, 1950; Jamieson and Smith, 1987).

Figure 1.1 Atlantic mackerel geographic distribution (FAO, 2018).

Scomber scombrus belongs to the scombridae family that aggregates all mackerel and tuna species which normally habit temperate waters (Collette and Nauen, 1983). Its entire life cycle is pelagic: eggs and larvae drift passively with the oceanic currents until being juveniles and adults with a great swimming capability that form large schools, resulting in a great capacity for dispersion (Lockwood, 1988; Jansen and Gislason, 2013). This species lacks swim bladder, meaning that individuals need to swim continually to prevent sinking, but unlike species with swim bladder, fish can change depth rapidly (DFO, 1997). This species is affected by changes in sea water temperature via growth and mortality rates, particularly during the larval stage, which will reflect in its migratory and seasonal distribution patterns (Ware and Lambert, 1985; TRAC, 2010). *S. scombrus* is sensitive to temperature both in terms of physiological and behavioural responses and timing of migration and spawning, standing between minimum temperatures of about 5-6ºC and maximum of 15-16ºC; it can change very easily to shallower or deeper depths in the water column according to their needs; and increases swimming speeds at low temperatures (Sette. 1950; Overholtz and Anderson., 1976; Studholme, 1999; Iversen, 2004). As juveniles they are opportunistic feeders, using both filter and biting behaviour to capture small crustaceans, pelagic fish and invertebrates; as adults the diet includes a variety of planktonic organisms (Studholme., 1999). Predation of this species is the largest component of natural mortality, with a large variety of predators such as larger fish, marine mammals and seabirds (Overholtz., 1991; Overholtz and Waring, 1991). Mackerel plays a key ecological role in oceanic and coastal ecosystems and supports one of the most valuable commercial fisheries in the North Atlantic (Trenkel *et al*., 2014).

Scomber scombrus is a fast growing fish species which begin to mature at age 2. being generally fully mature at age 3; they are multiple spawners and the onset of spawning may be triggered by warm water temperatures (ranging between 13 and 15ºC) that ensure eggs hatch during periods of high zooplankton abundance (ICES, 1993; Studholme, 1999). The adults could reach a maximum length of 70 cm and 3 kg of weight, but these large sizes seem to be decreasing due to overfishing, with maximum sizes being around 60 cm and 2 kg at present (Navarro *et al.*, 2012). The schools of mackerel tend to be composed of identical sized fish because of the close relationship between fish length and swimming speed (increasing with length) (DFO, 1997). Larger and older fish are the first to arrive to the spawning grounds followed by successively smaller individuals, by the end of the spawning season only the younger fish remain (Lockwood *et al*., 1981). Males mature earlier than females, but spawning ends at about the same time for both sexes (the testes seemed to develop earlier, but slower, than the ovaries) (Eltink, 1987). There is no evidence that migration to the spawning areas is carried out at different times for males and females (Villamor *et al*., 2004). Juveniles recruit in nearshore areas along the spawning grounds and the combination of early juveniles originating from all these areas results in the final overall recruitment (Lockwood, 1988; Borja *et al*., 2002).

There are two stocks in the North Atlantic, the Western and Eastern stocks (NWA and NEA, respectively). It is considered that these stocks have no habitat connectivity, being confirmed by molecular and tagging studies: the presence of mitochondrial DNA differentiation suggest a restriction in gene flow at large spatial scales; and tagging studies showed no individuals from Eastern origin being caught in the Western side, or vice versa (Nesbø *et al*., 2000; Uriarte and Lucio, 2001; Iversen, 2002; Tenningen *et al*., 2011). Atlantic mackerel´s stocks are divided in five spawning components, two in the North Western Atlantic (NWA) and three in the North Eastern Atlantic (NEA) (Sette, 1950; ICES, 1996). Geographic distances separate the many grounds of the regional components of mackerel and although it was thought that they followed predictable migration patterns and spawned at regular times and places this does not necessarily occur (Jamieson and Smith, 1987; Jansen and Gislason, 2013). Through the years many interpretations on the mackerel migration and geographic distribution have been done because of its spatial-temporal dynamics due to environmental variations, resulting in lack of concrete knowledge on how these populations are structured (Sette, 1950; Jamieson and Smith, 1987; D'Amours and Castonguay, 1992; Iversen, 2002; Overholtz *et al.*, 2011; Astthorsson *et al.*, 2012; Radlinski *et al.*, 2013; Jansen, 2016).

1.2.1. NEA population

The North Eastern Atlantic (NEA) stock, in terms of fisheries management, is composed of three spawning components: North Sea Component (ICES areas IIIa and IV), Western Component (ICES areas VI, VII and VIII) and Southern Component (ICES areas IX, X and subarea VIIIc), with the North Sea, Celtic Sea and Bay of Biscay as the main spawning grounds, respectively (Hamre, 1980; ICES, 2017). The latitudinal propagation of spawning reflects the increase of sea surface temperatures in the spring, as the migration from the spawning areas follows the Warm Shelf Edge current (Eltink, 1987; Reid *et al*., 1997). Spawning happens from February to May in the Southern component, March to July (with a peak in May) in the Western component and June to August (with a peak at the end of June) in the North Sea (Hamre, 1980; Iversen, 1981, Jorge *et al*., 1982). Fish after spawning in the Southern area migrate northwards, mix with the Western component and enter in the Norwegian Sea for the feeding period where they meet with the North Sea component (Uriarte and Lucio, 2001). Mackerel generally remain in the feeding area until the end of autumn, after which the individuals return south to their overwintering grounds around the North of the British Isles and then migrate back to their spawning grounds in the first half of the year (Punzón and Villamor, 2009; ICES, 2011). The onset of the spawning migration is determined by the rising temperatures in the wintering area, and higher temperatures are related with an early spawning season (Hamre, 1980). Furthermore, the southern pre-spawning migration pattern of the Atlantic mackerel seems to be directed towards areas with low turbulent mixing at spawning time, providing a "stable environment" for egg and larval survival as it seems that the turbulence conditions of pre-spawning and spawning periods have the largest influence on the success of recruitment (Borja *et al*., 2002; Jansen, 2013).

The first establishment of the NEA spawning components was based on tagging experiments and, since then, other natural tags (e.g. parasite infestations, protein polymorphisms, juvenile growth patterns in otoliths, age composition, length at age, genetics) where used to differentiate them, but until now none really supported their spatial isolation (Hopkins, 1986; Jamieson *et al*., 1987; Dawson, 1991; Nesbø *et al*., 2000; Jansen and Gislason, 2013; Levsen *et al*., 2017). The existence of the Southern component, which aggregates mackerel from the Iberian Peninsula, is recent (ICES, 1996). Before that, the individuals from the Southern and Western component were considered to belong to one single component, the Western component alone. Furthermore the mixing of individuals from the southern and western areas throughout most of the year and their cohabitation in the western spawning grounds still raise doubts on the reliability of the assumption of separate spawning components in these two areas (ICES, 2001). The fact that the egg distribution of the Southern and Western components overlaps in the Bay of Biscay, makes it very difficult to define the Northern border of the Southern component and the southern border of the Western component (Iversen, 2002).

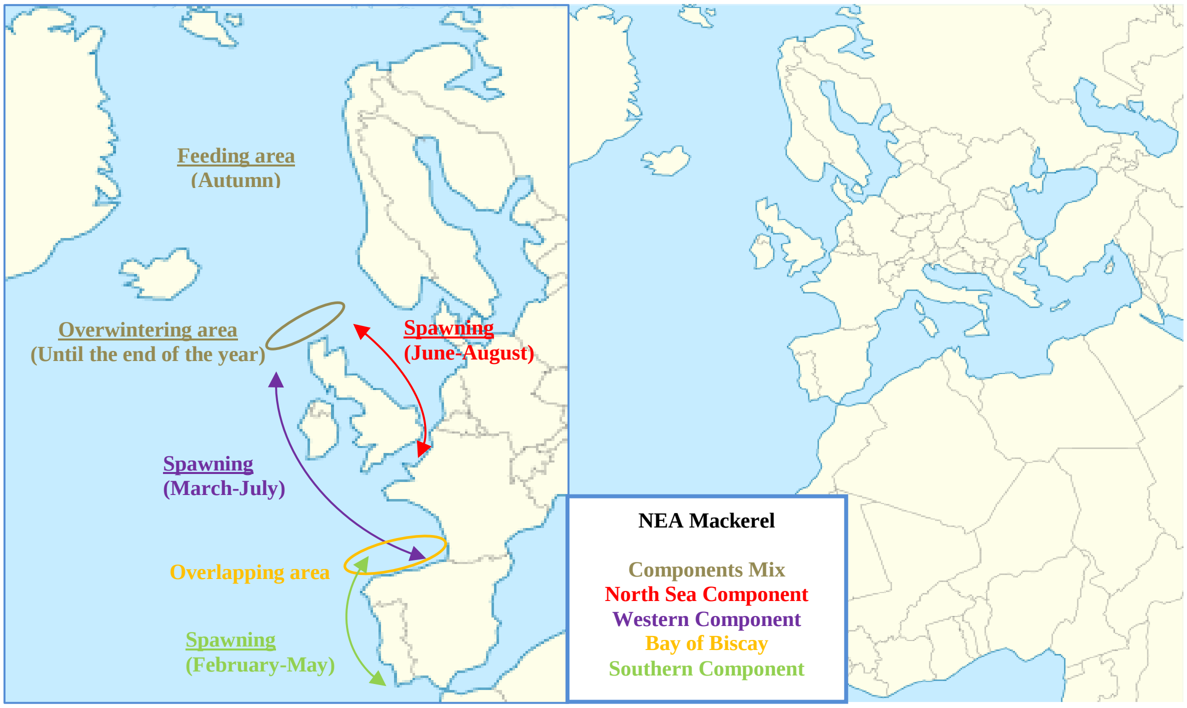

Figure 1.2 NEA Mackerel components distribution.

1.2.2. NEA stock fishery

The stock size and migration pattern of the different spawning components of mackerel in the NEA have changed over time and, as a consequence, also the fishery and its management (Iversen, 2002). This species is widely distributed through the ICES areas and supports one of the most valuable European fisheries, with an estimated catch of 1 155 944 tons in 2017, and world fisheries, being ranked 9th in 2014 (FAO, 2016; CES, 2018). Mackerel is caught by a variety of fleets ranging from open boats using hand lines and purse seines on the Iberian coasts to large freezer mid-water trawlers in the Northern Areas (ICES, 2011). Migration routes are usually known and exploited by local fisheries (Punzón and Villamor, 2009). In the beginning of each year, the stock is mainly fished in the spawning grounds west of the British Isles, France, Spain and Portugal (about ¼ of the total catch); in the summer and autumn, mainly in the north into the Norwegian Sea and around the south of Iceland (about ½ of the total catch); and in the end of the year, the fishery is concentrated around the Shetland Islands in the overwintering area, when the components are migrating back to their spawning grounds (about ¼ of the total catch) (Hannesson, 2012; ICES, 2018).

The Western component is considered to be the largest and the North Sea is considered overfished since 1970´s (ICES, 2011). The collapse of mackerel in the North Sea in the late 1960´s was most likely driven by very high catches and associated fishing mortality; however the lack of recovery is probably associated with unfavourable environmental conditions that led the mackerel to spawn in Western waters instead of in the North Sea (ICES, 2018).

Over the last few years there have been dramatic changes in the mackerel fishery in the Northeast Atlantic. In 2007 the mackerel changed its migratory habits and appeared in large quantities in the Icelandic economic zone, and in 2013 the first records in the Arctic waters of Svalbard were registered (Hannesson, 2014; Berge *et al.*, 2015). The feeding distribution has apparently expanded northward and westward probably related to co-occurring factors, such as a gradual increase in temperature, changes in the feeding conditions, competition with other major pelagic fish stocks in the area, and the relatively good status and age/size structure of the mackerel stock (Astthorsson *et al.*, 2012). Climate change affects zooplankton abundance and distribution, as well as ocean temperature and ocean circulation patterns (Astthorsson and Gislason, 2003). The increasing water temperature opens new and different feeding grounds and in contrast to species with restricted dispersal, migratory species, such as mackerel, might be less constrained in responding to climate change (ICES, 2011; Hughes *et al*., 2014). Especially when, at the time, there were signs of an increased abundance of mackerel with higher recruitment, year-classes and large cohorts suggesting that the expansion of the mackerel stock might also been density driven (Hannesson, 2012; ICES, 2013; Jansen, 2016).

The appearance of mackerel in Iceland has led to some disagreements between the parties involved in its management (Norway, European Union, Faroe Islands North East Atlantic Fisheries Commission), since Iceland did not agreed with the total allowable catch (TAC) offered by the traditional partners and unilaterally set a quota for itself, taking about 20% of the total catch in 2008 (Hannesson, 2012). In response, the Faeroe Islands dropped out of the mackerel agreement, since they thought that their share was small compared to the Icelandic catches (Hannesson, 2014). As result the management agreement was broken, and in 2010 the share of catches taken in the area which covers parts of the Icelandic, Faroese, and Norwegian economic zones, increased to unprecedented levels (Berge *et al.*, 2015). Currently there is no agreement on a management strategy covering all parties fishing mackerel (ICES, 2018). In 2014, three of the Coastal States (The EU, Faroes and Norway) agreed on a Management Strategy for 2015 and the subsequent five years; however, the total declared quotas taken by all parties since 2015 have greatly exceeded the TAC advised by ICES and spawning stock biomass has been decreasing since 2016 (ICES, 2018).

1.2.3. NWA population

The North Western Atlantic (NWA) mackerel is found from North Carolina to Newfoundland and has two spawning components (Sette, 1950). The Northern component that spawns mainly in the Gulf of St. Lawrence, along the Coast of New Scotland and possibly on the Grand Banks of Newfoundland (from May to August); and the Southern component that spawns from the Mid-Atlantic Bight to the Gulf of Maine (from April to June) (Sette, 1950; Berrien, 1982; DFO, 1997). During spring and summer Atlantic Mackerel is found in inshore waters, and from late fall through winter they co-occur deeper in warmer waters at the edge of the continental shelf in the Mid-Atlantic Bight (DFO, 2012). After the overwintering period, the Southern component moves inshore inhabiting the waters between Cape Hatteras and Delaware from March to April, thereafter migrating North to spawn, after which they move North again to feed in the coastal waters of the Gulf of Maine where they stay until autumn (Ware and Lambert, 1985). The Northern component moves shoreward from the Continental Shelf off New England and southern New Scotland in the spring and migrates north to spawn; after spawning the schools disperse north to feed around the Newfoundland coast (Ware and Lambert, 1985). The Southern contingent stays farther inshore, the northern component stays more offshore, but the two may cross paths in late spring or late summer in southern New England and in the Gulf of Maine (Sette, 1950; Studholme, 1999). Similarity to the NEA stock, no significant genetic differences have separated the two components (Souza *et al*., 2006). Also, they seem to be very similar in terms of migratory behaviour change response when faced with different environmental conditions (Overholtz *et al*., 2011; Radlinski *et al*., 2013; McManus *et al*., 2016).

Figure 1.3 NWA Mackerel components distribution.

1.2.4. NWA stock fishery

Atlantic mackerel is part of an important Northwest fishery, the stock is commercially exploited by the USA and Canada, and quota regulated by both (NOAA and DFO respectively) (TRAC, 2010). The two components are managed as a single stock (Northwest Atlantic Fisheries Organization, NAFO, Subareas 2-6) because of the important fishery in their overwintering area offshore along the edge of the Continental Shelf, from Stable Island to Long Island, where they co-occur (Ware and Lambert, 1985; TRAC, 2010). Both populations are also exploited on their spawning grounds in a summer and in a spring fisheries (Overholtz, 1991).

Both US and Canada landings ($^{1}/_{2}$ of the total catch reportedly taken from each component) have been decreasing since 2006 and it is now at a point never seen before, with landings being more than 10 times less from what they used to be (decreased from around 110 000 tons to 10 000 tons) (DFO, 2017). As for the NEA stock, NWA stock distribution has been shifting (e.g, northern and eastern) due to global warming (Overholtz *et al*., 2011; McManus *et al*., 2017). Also, recently, Atlantic mackerel have been seen in more inshore shallower waters of the Northeast Continental Shelf during winter, possibly owing to a general warming pattern in the region (NEFSC 2006; Overholtz *et al*., 2011; Radlinski *et al*., 2013). The availability of the fish to the fishery seems to be highly affected by environmental changes from year to year, since their migration behaviour change in order to follow the optimal conditions (Overholtz, 1991). The stock decline is being caused by levels of fishing mortality much higher than previously sustainable levels, and is also thought that is currently in an overfishing situation (DFO, 2014; Plourde *et al*., 2015). Besides the decreasing trend in catch through the years, other indicators such as the absence of older individuals and recent poor recruitment are solid indicators of a stock that is being overfished and, additionally, it is near its historical minimum (Duplisea and Grégoire, 2014). For the Northern component, a study revealed the possibility of being at its lowest biomass, since the annual catch was being well below the TAC (Grégoire and Beaudin, 2013). Furthermore, there are large and unaccounted catches in recreational and bait fisheries in Canada, since the commercial fishery (primarily seine) is obligated to declare landings but bait and recreational fisheries do not always need to report catches (Duplisea and Grégoire, 2014; VanBeveren *et al*., 2017). Though there is no clear handle on the magnitude of unregulated recreational and bait fishery catches, they may sum to more than the reported commercial fishery catch (Duplisea and Grégoire, 2014). The bait fishery is mainly to bait American lobster and snow crab pots, and mackerel angling is a common summer activity on the wharves, rocky points and recreational boats in Atlantic Canada (VanBeveren *et al*., 2017). As for the Southern component (with a fishery supported by midwater and bottom trawls) the Atlantic mackerel supplied an early-spring recreational fishery along the nearshore region of the Middle Atlantic Bight (when the Southern component moves inshore from the wintering grounds); however, this fishery began to decline in the late 1970´s and early 1980´s (Overholtz, 1991). Landings of Atlantic mackerel in the Middle Atlantic Bight further declined from the 1990´s and, beginning in 2005, the U.S. commercial fishery began to experience difficulty in locating large schools of Atlantic mackerel (NEFSC 2006).

1.3. Otoliths as natural tags

1.3.1. Otolith chemical signature

Otoliths have several specific characteristics that make them excellent natural markers of fish habitat and valuable tools for studies of fish life history and movements (Campana and Thorrold, 2001). They are biogeochemical structures deposited continuously throughout the fish life that grow by the addition of calcium carbonate and by the successive uptake of chemical elements present in the surrounding seawater in a layered manner that preserves the timing of deposition (Thresher, 1999; Elsdon and Gillanders, 2003). Otoliths are accretionary structures located within the inner ear of teleost fish, composed primarily of aragonite deposited on a proteinaceous matrix, accreted within a gel-filled endolymph and thus are isolated from direct exposure to the external water (Campana and Neilson, 1985) That, along with low ratios of surface area to volume and a relatively large size (mm to cm) and being metabolically inert, makes them less vulnerable to post-depositional chemical and structural modification than many other types of biogenic carbonate (Thorrold *et al*., 1997; Ghosh *et al*., 2007).

The uptake of elements into the growing structures usually reflects the aquatic environment where the fish lived (Campana *et al*., 2000). Any relationship between water composition and otolith chemistry will be determined by the kinetics of ion transport from water to the precipitating surface, but will also be a function of the mechanism by which the trace elements are incorporated into otolith aragonite (Bath *et al*., 2000). The physico-chemical properties of the environment (e.g., water composition, temperature, pH and salinity), fish physiology (e.g., age, growth and metabolism), upwelling phenomena and feeding regime are among the factors that can influence the potential incorporation of elements in the otoliths (Campana *et al*., 2000; Gao *et al*., 2001; Elsdon and Gillanders, 2002; Elsdon *et al*., 2008). The concentration of certain elements in the water is known to have a higher effect on the chemistry of the otoliths; the use of combined elemental signatures is likely to enhance the interpretations made (Thorrold *et al*., 1997; Elsdon and Gillanders, 2003; Elsdon and Gillanders, 2004).

Stock discrimination is based on the hypothesis that fish inhabiting different water bodies will incorporate elements into their calcified structures, which combine to form a unique and spatially distinct chemical signature that reflects the length of time that the fish occupied a particular water body (Elsdon *et al*., 2008; Daros *et al*., 2016). The most common application of stock identification is discriminating between separate populations that were previously assumed to be a single one, through quantitative analysis of the micro constituents and trace elements in otoliths which can provide information on population structure, habitat connectivity and the movements of individual fish (Campana *et al*., 2000; Higgins *et al*., 2013; Correia *et al*., 2014; Carvalho *et al*., 2017). A more robust application of whole otolith fingerprints might be one which is targeted at questions of stock mixing or for tracking stock migrations, in which the fingerprints are used as biological tracers of pre-defined groups of fish over short periods of time (Campana *et al*., 1995).

Owing to the potential value of otolith microchemistry to fisheries ecology and management, numerous analytical techniques have been adapted to quantify the elemental concentrations of otoliths (Campana *et al*. 1997; Campana, 1999; Thresher, 1999). One technique of growing importance and widespread use is the inductively coupled plasma mass spectrometry (ICP-MS), probably related to its extremely low detection limits which allow for a wide range of elements to be precisely and accurately quantified (Campana *et al*. 1997; Campana, 1999; Thresher, 1999). Choosing the exact instrument of analysis is important because of the elevated detection limits that might arise from small otolith mass or contamination during storage, preparation and handling, which can prevent the chemical detection by the process (Elsdon and Gillanders, 2003; Ludsin *et al*., 2006).

1.3.2. Otolith shape signature

In the variety of techniques used at present for the study of fish population structure (e.g. genetics, parasitic fauna, body morphology) otolith geometric morphometric is a relatively new tool to fisheries research and it seems promising as a means of enabling researchers to cheaply and quickly categorize fish to individual stocks based on variations in otolith form, most commonly size and shape (Tracey *et al*., 2006; Agüera and Brophy, 2011; Tuset *et al*., 2013). The shape of the otolith would appear to be an ideal natural marker for fish populations, as it is species specific and less affected in growth than fish body growth due to short-term environmental changes (Hopkins, 1986; Campana and Casselman, 1993). Also, measurements made on otoliths have the advantage of being unaffected by short-term changes in fish condition or by preservation, as long as acidic preservatives are avoided (Campana and Casselman, 1993).

Otoliths may show characteristics that are stock specific since geometric outline methods quantify boundary shapes so that patterns of shape variation within and among groups can be evaluated (Cadrin and Friedland, 2005; Pothin *et al*., 2006; Moreira *et al*., 2019; Soeth *et al*., 2019). Although sagittal otoliths have certain morphological features that are laid down early in the ontogeny of the fish, some characteristics (e.g. sulcus area, depth of the sulcus and the sulcus area: otolith area ratio) vary according to ecological factors such as fish length, geographic area, depth, conspecific abundance, food regime, chemical and physical characteristics of the environment (Lombarte and Lleonart 1993; Tuset *et al*., 2003; Cardinale *et al*., 2004).

Sagittae, the most used and bigger otolith's pair, are generally laterally compressed, elliptical on their sagittal plane, compressed on their internal-external axis and present a main axis of growth oriented in the anterior-posterior direction; most morphometric studies of otoliths have concentrated on these characteristics, evaluating size dependent measurements made on their sagittal plane and on size independent shape analysis of contour (Ponton, 2006). The size dependent variables recorded from the otolith will allow for different shape indices to be accounted like, roundness, circularity, rectangularity, ellipticity and eccentricity (Tuset *et al*., 2003; Ponton, 2006). The size independent measurements, most known as Fourier descriptors, are mathematically defined as a series of sinusoids (harmonics) that together give information on the outline of a shape (Gastonguay *et al*., 1991). This technique is considered useful because of the magnitude of the amplitude associated with each harmonic, indicating the contribution of that particular harmonic to the total form, each of them adding increasing detail to the description of the shape (Campana and Casselman, 1993; Ponton, 2006). This method represents a precise way of accommodating significantly complex shapes and efficiently capturing outline otolith information (Tracey *et al*., 2006).

Otolith shape analysis is an efficient tool to assess the stock identity and/or population structure, reflecting the areas which the fish inhabits (Farias *et al*., 2009; Moreira *et al*., 2019; Soeth *et al*., 2019). Age, sex, fish length and year class usually have effect on otolith shape Simoneau *et al*., 2000; Cardinale *et al*., 2004); and, even though for this species sex has showed not to influence, the otolith shape, age and year class seem to have great effect on shape variability and they must be accounted for (Gastonguay *et al*., 1991). Several environmental factors can make the pattern of mackerel growth variable in time and place so length distributions for age assignation is not recommendable and every individual should be aged by otolith reading (ICES, 2001). Also, comparing otoliths of fish from different size ranges can lead to wrong interpretations about the population structure if these differences are not accounted and techniques must be used to control the effect of fish otolith size on otolith shape (Campana and Casselman, 1993; Simoneau *et al*., 2000; Ponton, 2006; Mapp *et al*., 2017).

2. Objectives

Atlantic Mackerel seems to have a complex population structure since its movement patterns and spatial distribution keep changing due to environmental conditions, which results in a fishery that is somewhat uncertain. It becomes urgent to truly understand the differentiation between the spawning components for a correct management of the different stocks. To complement past studies on the population structure, the hereby work tested alternative natural tags allowing the use of a multidisciplinary approach than can help unravel mackerel population dynamics. The purpose of this work was to assess the utility of otolith chemistry and shape analysis to provide information about the population structure, habitat connectivity and migration patterns of *S. scombrus*. The obtained new findings will be made available for decision makers and fisheries agencies to improve the mackerel management in the North Atlantic. Specifically, we aimed to:

1. examine the variations of whole otoliths chemistry (entire life-history prior to capture) and shape characteristics between *S. scombrus* from the NEA and NWA stocks and to evaluate the differences between components within each stock;
2. investigate whether there is a discontinuity or not between Southern and Western components of the NEA stock, including a sampling location in the Bay of Biscay.

3. Methodology

3.1. Fish collection

A total of 300 individuals were collected (50 from 6 different locations) from local fisheries or boat surveys (in general, with seiners and trawlers) from January to February 2018. An effort was made to choose adult individuals from specific component occurrence areas and with similar length. Length (total length, TL, 0.1 cm) and weight (total mass, TM, 0.1 g) of each individual were measured.

Figure 3.1 *Scomber scombrus* individuals from Matosinhos collected at 16 of January 2018 (personal photograph).

Fish samples came from: NWA Northern Component - Corner Brook, Newfoundland, Canada; NWA Southern Component - Belford, New Jersey, USA; NEA Southern Component - Matosinhos, Porto, Portugal; NEA Bay of Biscay (Part of the Southern and Western Components) - Gijon, Oviedo, Spain; NEA Western Component - Saint Kilda, Scotland, United Kingdom; NEA North Sea Component - Isle of Wight, London, United Kingdom (Figure 3.2).

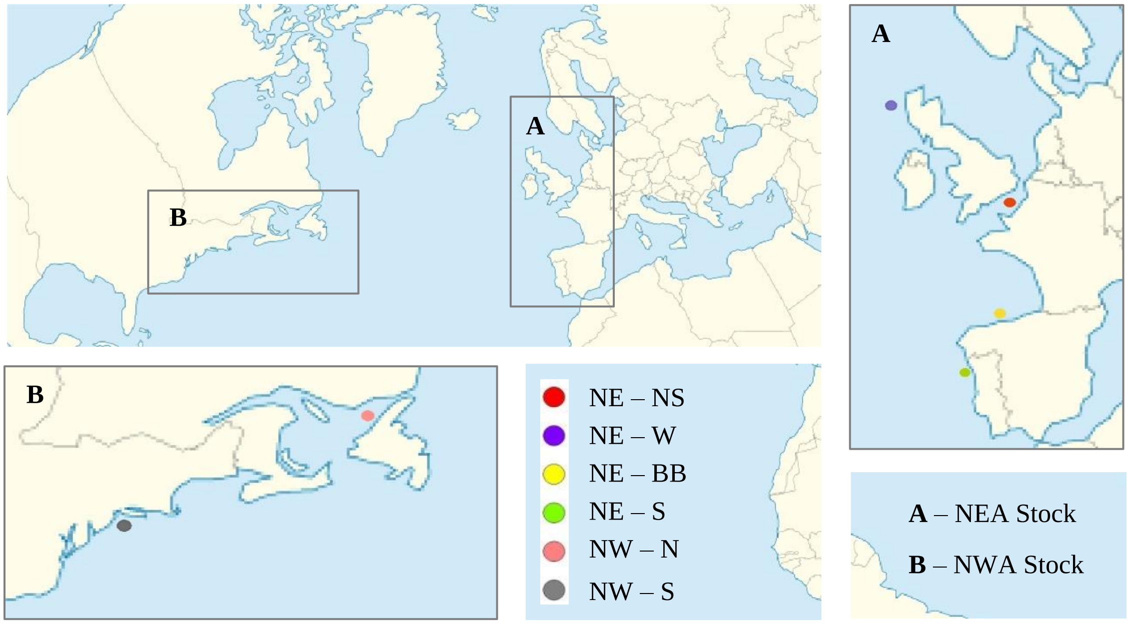

Figure 3.2 *Scomber scombrus* sampling locations of the individuals collected from January to February 2018 (A - NEA stock; B- NWA stock).

3.2. Otoliths extraction, storage and age reading

Each pair of otolith was extracted with plastic forceps to avoid metallic contamination, cleaned with ultrapure water, until all organic tissue was removed, air dried and storage in labelled Eppendorf's tubes.

For mackerel age estimation an existent standard protocol was followed (ICES, 2010). Otoliths were viewed with a stereomicroscope (Meiji Techno, EMZ-13TR) with a reflected light and under black background, sulcus faced down, and immersed in a clearing agent (ethanol and glycerol, 1:1) to enhance their annuli transparency during reading. The annual growth pattern is well defined on the otolith with clear contrasting opaque and translucent bands. The date of birth is assumed to be 1st January and the fish is assigned to a year class on this basis. One opaque zone and one translucent band constitutes one year of growth (annulus). For counting purposes the opaque increment should be continuous around the otolith (the increment should be visible in at least two areas). For mackerel caught in the 1st semester of the year, all translucent increments and the translucent edge are counted. The translucent edge is always counted as one winter ring, even if nothing or very little is visible. Two independent and experienced observers made two blind readings of each otolith and concordance percentage was calculated (% concordance = number of coincident reads/number of total reads) to determine reliability of age estimates. Only fish with 100% concordance of age readings were used.

Figure 3.3 3 year old *Scomber scombrus* left and right otoliths, respectively (individual from Bay of Biscay collected at 24 of February 2018 with a TL of 30 cm). See on left otolith the translucent bands numbered.

The majority of the samples of all the places were 3 years old so that was the age chosen to analyse. Regarding this, it was possible to select 30 individuals from each location for the analysis (with a total of 180 individuals analysed). Furthermore right otoliths were used for the elemental and shape analyses and the left otoliths for the isotopic analysis (Table 3.1.)

Table 3.1 Samples selected for the following analysis and respective sample size (N), fish total length (TL), otolith length (OL) and otolith mass (OM). Values are presented as mean ± SE.

Location	**N**	**Code**	**TL** (cm)	**OL** (mm)	**OM** (mg)
NEA Southern Component	30	NE-S	32.6 ± 0.3	4.21 ± 0.05	2.50 ± 0.05
NEA Bay of Biscay	30	NE-BB	29.6 ± 0.3	3.97 ± 0.05	2.31 ± 0.06
NEA Western Component	30	NE-W	32.9 ± 0.2	3.99 ± 0.05	2.37 ± 0.06
NEA North Sea Component	30	NE-NS	28.1 ± 0.2	3.93 ± 0.05	2.31 ± 0.06
NWA Northern Component	30	NW-N	38.4 ± 0.4	4.50 ± 0.04	3.23 ± 0.06
NWA Southern Component	30	NW-S	29.3 ± 0.4	3.98 ± 0.07	2.14 ± 0.06

3.3. Otolith shape analysis

3.3.1. Shape Indices

Orthogonal two-dimensional digital images of the sagittal otoliths were captured using a stereomicroscope (Meiji Techno, EMZ-13TR) coupled with a USB digital camera (Olympus, SC30) at 1.5 X magnification with the analySIS getIT software (Moreira *et al.*, 2019). Right otoliths were all photographed in the same position, with reflected light and dark background. The photos quality was improved in the paint.NET (v. 4.0.21) program, in order to maximize the differentiation between the otolith and the background and to create a binary image.

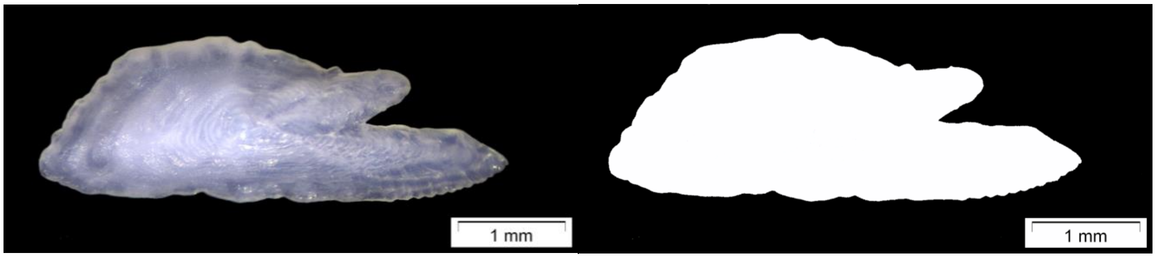

Figure 3.4 Right sagittal otolith photograph and the corresponding binary digital image (individual from Bay of Biscay collected at 24 of February 2018 with a TL of 30 cm), respectively.

Binary otolith images were measured using the program ImageJ (v. 1.50) (Rasband, 2009) to assess the morphometric size parameters, otolith length (OL, mm), otolith width (OW, mm), otolith area (OA, mm^2), and otolith perimeter (OP, mm). With these variables is possible to calculate and assess the Shape Indices (SI) (Form factor, Roundness, Ellipticity, Circularity and Rectangularity) that were used to evaluate and compare the otoliths shape (Tuset *et al.*, 2003).

Table 3.2 Formulas used to obtain the otolith shape indices (Tuset *et al.*, 2003).

Shape Indices (SI)	Formula
Form Factor (FF)	(4𝝅OA)/OP2
Roundness (RO	(4OA)/(𝝅OL2)
Ellipticity (EL)	(OL−OW)/(OL+OW)
Circularity (CI)	OP2/OA
Rectangularity (RE)	OA/(OL×OW)

3.3.2. Elliptical Fourier Descriptors

The Elliptic Fourier analysis fits a closed curve to an ordered set of data points and then decomposes the contour into a sum of harmonically related ellipses (Kuhl and Giardina, 1982). The program Shape (Version 1.3) was used to extract the otolith contour and to determine the number of Elliptic Fourier Descriptors (EFD) required to adequately describe the otolith outline. A level of 95% of accumulated variance was used to select the minimum number of harmonics (each harmonic characterised by 4 Fourier descriptors, a, b, c and d) (Ferguson *et al*. 2011). The first 5 harmonics reached >95% of the cumulative power, excluding coefficients b5, c5 and d5, indicating that the otolith shape could be adequately explained by 17 Fourier coefficients. After normalization to the first harmonic (EFD invariant to otolith size), the first three coefficients (a1, b1 and c1) were constant and excluded (Iwata & Ukai 2002; Pothin *et al.*, 2006), and 14 Fourier descriptors (17-3) used in the subsequent analyses.

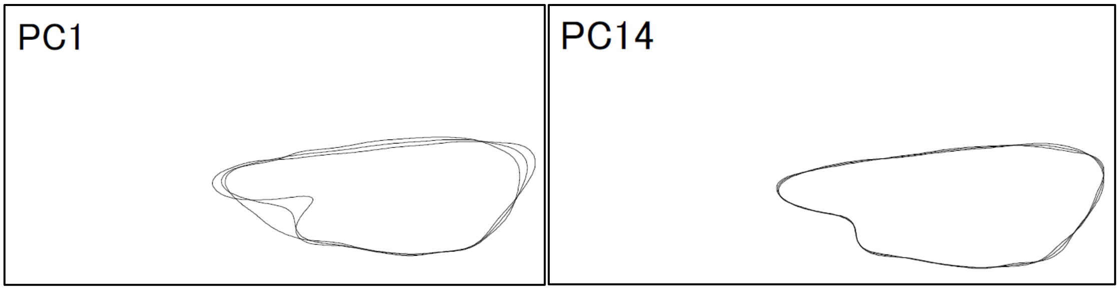

Figure 3.5 EFD Output - Otolith Shape Contour of the 1_{st} Principal component (31% cumulative significance) and of the 14_{th} Principal component (95% cumulative significance), respectively.

3.4. Otolith chemical analysis

3.4.1. Otoliths pre-treatment

Even though the glycerol, from the clearing solution for the age reading, would take 1 to 2 months of immersion to enter otoliths, otoliths pairs were rinsed in ethanol and brushed in Milli-Q water to remove any superficial glycerol contamination (Campana, 1999; Campana *et al*., 2003). Thereafter otoliths were cleaned and decontaminated in an ultrasonic cleaner for 5 min in ultrapure water (Milli-Q water) to remove any adherent biological tissues, followed by immersion in 3% analytical grade hydrogen peroxide (H_2O_2) for 15 min, to remove any remaining biological residues (Correia *et al*., 2011). Otoliths were immersed in ultrapure 1% nitric acid (HNO_3) solution for 10 s to remove any superficial contamination, followed by a triple-immersion in Milli-Q water for 5 min to remove the acid (Rooker *et al*., 2001). The otoliths were stored in new decontaminated Eppendorf micro centrifuge tubes, and allowed to dry in a laminar flow fume hood (Patterson *et al*., 1999; Daros *et al*. 2016). The decontaminated otoliths were weighed on an analytical balance (otolith mass, OM, 0.001 g).

3.4.2. Elemental analysis

Right whole otoliths were dissolved for 15 minutes in 60 μL of ultrapure HNO_3 and diluted with Milli-Q water to a final volume of 3 mL [2% of HNO_3 (v/v) and 0.02% of TDS (m/v)]. The solution was stirred with a vortex and sent to the laboratory (Correia *et al*., 2011).

Multi-elemental analysis was performed by SB-ICP-MS using an iCAPTM Q (Thermo Fisher Scientific, Bremen, Germany) instrument equipped with a concentric glass nebulizer, a Peltier-cooled baffled cyclonic spray chamber, a standard quartz torch and a two-cone interface design (sample and skimmer cones). High-purity (99.9997%) argon (Gasin II, Leça da Palmeira, Portugal) was used as the nebulizer and plasma gas. The equipment control and data acquisition were made through the Qtegra software (Thermo Fisher Scientific, Bremen, Germany). To minimize the effect of any plasma fluctuations or different nebulizer aspiration rates among samples, Indium (^{115}In) was monitored as internal standard. The limits of detection (LoD were calculated as the concentration corresponding to three times the standard deviation of 10 sample blanks. Only the ^{44}Ca was analysed by FAAS - Flame Atomic Absorption Spectrometry instrument (Perkin Elmer, Überlingen, Germany). Otolith samples were analysed in random order to avoid possible sequence effects.

For quality control, precision and accuracy checks, the NRC otolith certified reference material FEBS-1 was also analysed (Sturgeon *et al*., 2005). Elemental concentrations determined in FEBS-1 were within the certified and indicative range, with a value of recovery >95%. The precision of replicate analyses of individual elements ranged between 3% and 4% of the relative standard deviation (RSD). Nine elements) were above the limit of detection (LoD mg/L): ^{44}Ca (0.015 mg/L), ^{23}Na (0.403 μg /L), ^{88}Sr (0.079 μg /L), ^{7}Li (0.002 μg /L), ^{26}Mg (0.044 μg /L), ^{55}Mn (0.019 μg /L), ^{59}Co (0.006 μg /L), ^{60}Ni (0.009 μg /L), and ^{137}Ba (0.007 μg /L). ^{44}Ca provided the internal standard. Data were also collected for ^{9}Be (0.004 μg /L), ^{52}Cr (0.051 μg /L), ^{65}Cu (0.022 μg /L), ^{66}Zn (0.252 μg/L), ^{82}Se (0.354 ug/L), ^{85}Rb (0.024 μg/L), ^{75}As (0.119 μg /L), ^{95}Mo (0.006 μg /L), ^{111}Cd (0.003 μg /L), ^{118}Sn (0.005 μg /L), ^{121}Sb (0.002 μg /L), ^{205}Tl (0.002 μg /L), ^{209}Pb (0.001 μg /L) and ^{238}U (0.0004 μg /L), but their concentrations were consistently below the limit of detection.

The trace elements concentrations, originally in μg element/L solution, were transformed to μg element/g otolith and finally to μg element/g calcium (Correia *et al*., 2011).

3.4.3. Isotopic analysis

δ^{18}O and δ^{13}C were analysed. Otoliths were crushed into a fine powder using a small mortar and pestle. The crushed powder (20–40 μg) was analysed for stable oxygen and carbon isotopic composition using an automated carbonate device (Kiel IV) connected to a Thermo Finnigan MAT 253 Dual Inlet Isotope Ratio Mass Spectrometer (IRMS). Otolith samples were analysed in random order to avoid possible sequence effects. In the carbonate, the otolith powder was reacted with phosphoric acid (H_3PO_4), the formed CO^2 was purified by two traps and transported by vacuum to the IRMS, where $\delta^{18}O$ and $\delta^{13}C$ were measured against a calibrated reference CO^2 gas. Each sequence was carried out with external NBS19 (for both $\delta^{18}O$ and $\delta^{13}C$) and NBS18 (for $\delta^{13}C$ only) reference standards, and in- house NFHS1 standard (for both $\delta^{18}O$ and $\delta^{13}C$) to check for drift. Test measurements were done to check the homogeneity of one sample. Isotopic concentrations measurements (‰ VPDB) were guided and calculations done with Isodat 3.0 (Thermo Scientific) software. The reproducibility of all standards, standard deviation (SD), amounted to 0.1‰ and 0.06‰ for $\delta^{18}O$ and $\delta^{13}C$, respectively.

3.5. Data analysis

To ensure that differences in otolith length and mass among locations did not confound any location specific differences in otolith shape or chemical analysis, the relationship between Shape Indices and otolith length (OL) (as covariate) and between Element:Ca concentrations and otolith mass (OM) (as covariate) was evaluated using analysis of covariance (ANCOVA) (Campana *et al*., 2000; Daros *et al*., 2016). EL and CL showed a positive relationship with OL ($r^2 = 0.530$, n=180, $p < 0.05$; $r^2 = 0.410$, n=180, $p < 0.05$, respectively), opposite to FF, RO and RE that showed a negative relationship with OL ($r^2 = 0.417$, n=180, $p < 0.05$; $r^2 = 0.529$, n=180, $p < 0.05$; $r^2 = 0.167$, n=180, $p < 0.05$, respectively). Mg:Ca and Ba:Ca concentrations showed a negative relationship with OM ($r^2 = 0.417$, n=180, $p < 0.05$; $r^2 = 0.529$, n=180, $p < 0.05$, respectively). The variables affected by the respective covariate were corrected using the ANCOVA slope, the formula used to correct it was $V_{adj} = V - (\beta \times \text{covariate})$, where Vadj is the adjusted sample value, V is the original sample value and β is the slope value (Campana and Casselman 1993; Cardinale *et al*. 2004; Ferguson *et al*. 2011). After the corrections, ANCOVA showed that the corrections were effective in removing the OM and/or OL effect in the data.

Prior to the rest of the statistical analysis, data were checked for normality (Shapiro–Wilk test, $p > 0.05$) and homogeneity of variances (Levene's test, $p > 0.05$). These assumptions were met after $\log_{10}$ transformation of RO and RE in the shape analysis and Sr:Ca and Mn:Ca in the chemical analysis.

All statistical analysis were applied to infer for differences between stocks (NEA vs NWA), differences between locations within each stock (NWA: NW-N vs NW-S and NEA: NE-NS vs NE-W vs NE-BB vs NE-S) and an additional analysis was performed to the NEA stock without the presence of the NE-BB location (NEA: NE-NS vs NE-W vs NE-S).

Univariate one-way analysis of variance (ANOVA) was used for each variable of the shape and chemical analysis. ANOVA was followed by a Tukey post-hoc test if significant differences were found ($p < 0.05$).

Multivariate canonical analysis of principal coordinates (CAP) based on Euclidian distances to examine the reclassification accuracy (leave-one-out cross-validation) was applied to the shape signature, to the chemical signature and to the shape and chemical signatures combined to observe grouping separation of the locations and the variables that most contributed for the discrimination. CAP was followed by a permutational multivariate analysis of variance (PERMANOVA) (*p*-values were generated using 9999 random permutations) to check for significant differences. Models found to be statistically significant were followed by permutational pairwise comparisons ($p < 0.05$).

All statistical analyses were performed using Systat (v. 12) and PRIMER 6+PERMANOVA software with a statistical level of significance (α) of 0. 05.

4. Results

4.1. Shape signature

Less than half of the EFD (a3, c3, a4, c4, d4 and a5) presented differences between the stocks (NEA vs NWA), but all SI differentiated them (ANOVA, $p < 0.05$) (Table 4.1 and 4.2). Regarding the NWA stock, NW-N and NW-S sampling locations did not show significant differences for any EFD (ANOVA, $p > 0.05$) but in contrast showed significant differences for all SI (ANOVA, $p < 0.05$) (Table 4.1 and 4.2). Regarding the NEA stock, essentially, NE-S differed from NE-W in 5 EFD (a2, a3, b3, b4, d4) (Tukey test, $p < 0.05$), as for SI none of the NEA sampling locations showed significant differences (ANOVA, $p > 0.05$) (Table 4.1 and 4.2). ANOVA and Tukey test results with and without the presence of NE-BB location presented identical results.

Table 4.1 Mean ± SE otolith Elliptic Fourier Descriptors (EFD) values for the sampling locations. In EFD* there were significant differences between the NEA and NWA stocks (ANOVA, $p < 0.05$). For each line (EFD) NEA sampling locations sharing the same letter do not show any statistical difference (Tukey, $p > 0.05$). For each line (EFD) NWA sampling locations sharing the same number do not show any statistical difference (ANOVA $p > 0.05$).

EFD	NE-NS	NE-W	NE-BB	NE-S	NW-N	NW-S
d1	0.437 ± 0.005^{a}	0.436 ± 0.008^{a}	0.430 ± 0.005^{a}	0.427 ± 0.006^{a}	0.435 ± 0.006 $^{(1)}$	0.423 ± 0.005 $^{(1)}$
a2	0.028 ± 0.003^{a,b}	0.020 ± 0.004^{b}	0.023 ± 0.005^{b}	0.043 ± 0.005^{a}	0.036 ± 0.005 $^{(1)}$	0.026 ± 0.004 $^{(1)}$
b2	0.067 ± 0.003^{a}	0.060 ± 0.003^{a}	0.062 ± 0.003^{a}	0.072 ± 0.004^{a}	0.062 ± 0.003 $^{(1)}$	0.058 ± 0.003 $^{(1)}$
c2	0.041 ± 0.003^{a}	0.043 ± 0.003^{a}	0.051 ± 0.002^{a}	0.051 ± 0.002^{a}	0.040 ± 0.003 $^{(1)}$	0.040 ± 0.003 $^{(1)}$
d2	0.080 ± 0.004^{a}	0.082 ± 0.004^{a}	0.075 ± 0.003^{a}	0.083 ± 0.003^{a}	0.070 ± 0.004 $^{(1)}$	0.074 ± 0.003 $^{(1)}$
a3*	0.057 ± 0.003^{a}	0.065 ± 0.003^{b}	0.059 ± 0.003^{a,b}	0.053 ± 0.003^{a}	0.047 ± 0.004 $^{(1)}$	0.055 ± 0.003 $^{(1)}$
b3	-0.032 ± 0.003^{a,b}	-0.029 ± 0.003^{b}	-0.031 ± 0.003^{a,b}	-0.041 ± 0.003^{a}	-0.031 ± 0.003 $^{(1)}$	-0.029 ± 0.002 $^{(1)}$
c3*	0.013 ± 0.002^{a}	0.010 ± 0.002^{a}	0.014 ± 0.002^{a}	0.018 ± 0.002^{a}	0.005 ± 0.002 $^{(1)}$	0.002 ± 0.002 $^{(1)}$
d3	0.040 ± 0.002^{a}	0.040 ± 0.003^{a}	0.041 ± 0.002^{a}	0.039 ± 0.001^{a}	0.049 ± 0.003 $^{(1)}$	0.042 ± 0.003 $^{(1)}$
a4*	-0.013 ± 0.003^{a}	-0.014 ± 0.002^{a}	-0.011 ± 0.003^{a}	-0.008 ± 0.003^{a}	0.001 ± 0.004 $^{(1)}$	-0.005 ± 0.002 $^{(1)}$
b4	0.054 ± 0.003^{a,b}	0.047 ± 0.003^{b}	0.052 ± 0.003^{a,b}	0.062 ± 0.003^{a}	0.055 ± 0.003 $^{(1)}$	0.050 ± 0.003 $^{(1)}$
c4*	-0.008 ± 0.002^{a}	-0.008 ± 0.002^{a}	-0.004 ± 0.002^{a}	-0.008 ± 0.002^{a}	0.000 ± 0.002 $^{(1)}$	0.002 ± 0.002 $^{(1)}$
d4*	0.017 ± 0.002^{a,b}	0.010 ± 0.002^{b}	0.013 ± 0.002^{a,b}	0.018 ± 0.002^{a}	0.017 ± 0.002 $^{(1)}$	0.022 ± 0.002 $^{(1)}$
a5*	0.024 ± 0.002^{a}	0.024 ± 0.002^{a}	0.022 ± 0.002^{a}	0.023 ± 0.002^{a}	0.013 ± 0.004 $^{(1)}$	0.017 ± 0.003 $^{(1)}$

Table 4.2 Mean ± SE otolith Shape Indices (SI) values for the sampling locations. In SI* there were significant differences between the NEA and NWA stocks (ANOVA, $p < 0.05$). For each line (SI) NEA sampling locations sharing the same letter do not show any statistical difference (Tukey, $p > 0.05$). For each line (SI) NWA sampling locations sharing the same number do not show any statistical difference (ANOVA $p > 0.05$).

SI	NE-NS	NE-W	NE-BB	NE-S	NW-N	NW-S
FF*	0.824 ± 0.006^{a}	0.839 ± 0.007^{a}	0.826 ± 0.005^{a}	0.844 ± 0.006^{a}	0.883 ± 0.006 $^{(1)}$	0.834 ± 0.005 $^{(2)}$
RO*	0.617 ± 0.004^{a}	0.621 ± 0.005^{a}	0.612 ± 0.004^{a}	0.626 ± 0.002^{a}	0.663 ± 0.004 $^{(1)}$	0.611 ± 0.003 $^{(2)}$
EL*	0.167 ± 0.004^{a}	0.160 ± 0.004^{a}	0.171 ± 0.004^{a}	0.158 ± 0.003^{a}	0.130 ± 0.004 $^{(1)}$	0.179 ± 0.004 $^{(2)}$
CI*	7.662 ± 0.371^{a}	6.887 ± 0.373^{a}	7.421 ± 0.253^{a}	6.575 ± 0.346^{a}	4.383 ± 0.300 $^{(1)}$	7.032 ± 0.277 $^{(2)}$
RE*	0.792 ± 0.004^{a}	0.788 ± 0.005^{a}	0.791 ± 0.003^{a}	0.797 ± 0.004^{a}	0.824 ± 0.005 $^{(1)}$	0.802 ± 0.005 $^{(2)}$

Reclassification analysis comparing the stocks (NWA vs NEA) showed a high reclassification success of 81% with both EFD (a3, c3, a4, c4, d4 and a5) and SI (all) contributing to the discrimination (Table 4.3; Figure 4.1(A)). Furthermore, there were significant differences between the NWA and NEA stocks (PERMANOVA, pseudo-$F = 10.767$; $df = 1, 178$; $p < 0.05$).

The NWA stock sampling locations showed the highest reclassification success (93%) driven by all SI variables (Table 4.3; Figure 4.1(A)). Furthermore, there were significant differences between the NW-N and NW-S sampling locations (PERMANOVA, pseudo-$F = 9.0854$; $df = 1, 58$; $p < 0.05$).

The NEA stock had the lowest overall reclassification success, with an improvement from 48% to 62% when NE-BB is not considered; NE-S location maintained the highest reclassification success (70% and 73% respectively) with EFD (a2, a3, b3, b4, d4) providing the highest contribution (Table 4.3; Figure 4.1 (B) and (C)); the rest of the NEA stock locations showed a high overlap and a lower reclassification success (Table 4.4; Figure 4.1(B) and (C)). There were significant differences for the NEA stock locations in both analysis with (PERMANOVA, pseudo-$F = 2.2837$; $df = 3, 116$; $p < 0.05$) and without (PERMANOVA, pseudo-$F = 2.6454$; $df = 2, 87$; $p < 0.05$) the NE-BB location. Moreover pairwise comparisons were the same with and without the NE-BB sampling locations, differentiating only NE-S from the rest of the NEA stock locations (PERMANOVA, $p < 0.05$).

Table 4.3 The leave-one-out reclassification matrix of the otolith shape signature (Shape Indices and Elliptic Fourier Descriptors analysis) for both stocks, NWA stock, NEA stock and NEA stock without NE-BB location.

Both stocks	**Predicted Locations**			NWA stock	**Predicted Locations**		
Original Locations	NEA	NWA	% Correct	**Original Locations**	NW-N	NW-S	% Correct
NEA	100	20	83	NW-N	28	2	93
NWA	15	45	75	NW-S	2	28	93
Total	115	65	81	Total	30	30	93

NEA stock	**Predicted Locations**						**Predicted Locations**			
Original Location	NE-NS	NE-W	NE-BB	NE-S	% Correct	**Original Location**	NE-NS	NE-W	NE-S	% Correct
NE-NS	10	9	9	2	33	NE-NS	16	9	5	53
NE-W	5	14	6	5	47	NE-W	8	18	4	60
NE-BB	7	5	13	5	43	NE-S	4	4	22	73
NE-S	4	3	2	21	70	Total	28	31	31	62
Total	26	31	30	33	48					

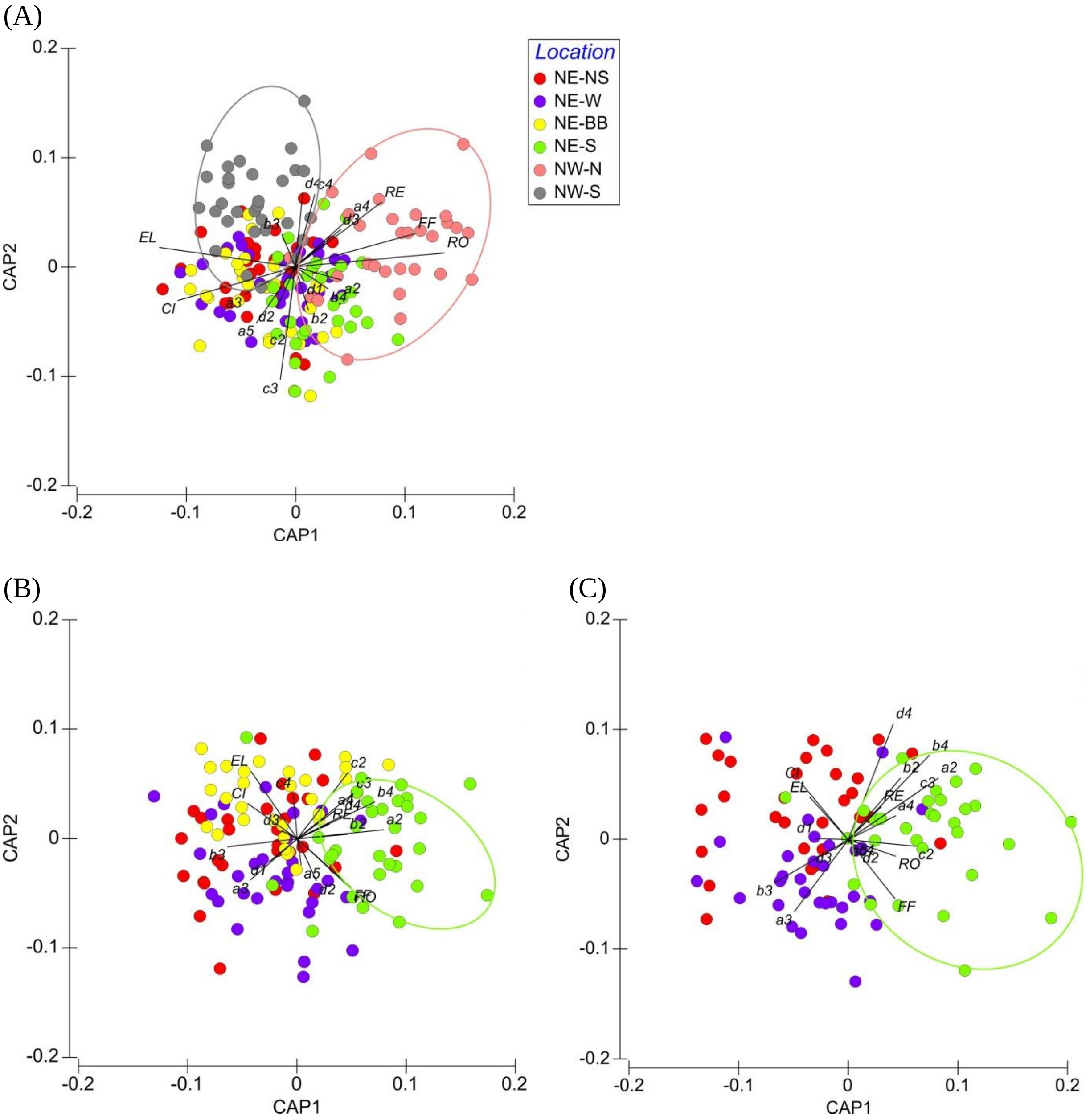

Figure 4.1 Canonical analysis of principal components (CAP) of the otolith shape signature (Shape Indices and Elliptic Fourier Descriptors analysis) for all the sampling locations (A), for the NEA stock (B) and for the NEA stock without NE-BB location (C).

4.2. Chemical signature

From the otoliths detected elements, Sr:Ca, Mg:Ca, Ba:Ca, Li:Ca, Mn:Ca were the ones presenting significant differences, and all of them differentiated the stocks (ANOVA, $p < 0.05$). Additionally, from the isotopic analysis, both $\delta^{18}O$ and $\delta^{13}C$ showed significant differences and both differentiated the stocks (ANOVA, $p < 0.05$). ANOVA and Tukey test results for the NEA stock with and without the presence of NE-BB location presented identical results.

Sr:Ca results, for the differences between the stocks, presented lower concentrations for the NWA stock. Sr:Ca also showed differences in the NEA stock, between NE-W (highest mean value) and NE-NS (lowest mean value) (Tukey test, $p < 0.05$) (Figure 4.2 (A)).

Ba:Ca results presented differences in the NEA stock (ANOVA, $p < 0.05$), with a higher mean value for NE-W and lower for NE-NS, NE-S and NE-BB presented an intermedium mean value (Tukey test, $p < 0.05$). Ba:Ca also showed differences in the NWA stock (ANOVA, $p < 0.05$), with a lower mean value for the NW-S sampling location. When comparing the stocks, NWA stock showed the lowest concentrations (Figure 4.2 (B)).

Li:Ca results, other than differentiating the stocks (lower concentrations for the NWA stock), showed further differences for the NEA stock (ANOVA, $p < 0.05$), in which NE-NS presented the lowest mean value (Tukey test, $p < 0.05$), and for the NWA stock (ANOVA, $p < 0.05$), in which NW-S presented the lowest mean value (Figure 4.2 (C)).

Mg:Ca results other than presenting differences between stocks, also showed differences within each stock (ANOVA, $p < 0.05$). The NEA stock, comparatively to NWA, showed lower concentrations. In the NEA stock, NE-W presented the lowest mean value (Tukey test, $p < 0.05$) and in the NWA stock NW-N presented the lowest mean value (ANOVA, $p < 0.05$) (Figure 4.2 (D)).

Mn:Ca results only presented significant differences between the stocks, with lower concentrations for the NEA stock (Figure 4.2 (E)).

$\delta^{18}O$ results presented contrasting positive values for the NEA stock and negative values for the NWA stock. Additionally, there were differences in the NEA stock (ANOVA, $p < 0.05$), in which NE-NS differentiated (with a lower mean value) from the rest of the sampling locations (Tukey test, $p < 0.05$) (Figure 4.3 (A)).

$\delta^{13}C$ results presented negative values and, additionally to differences between the stocks, there were differences in the NEA stock (ANOVA, $p < 0.05$), in which NE-S differentiated (highest mean value) from the rest of the locations (Tukey test, $p < 0.05$) (Figure 4.3 (B)).

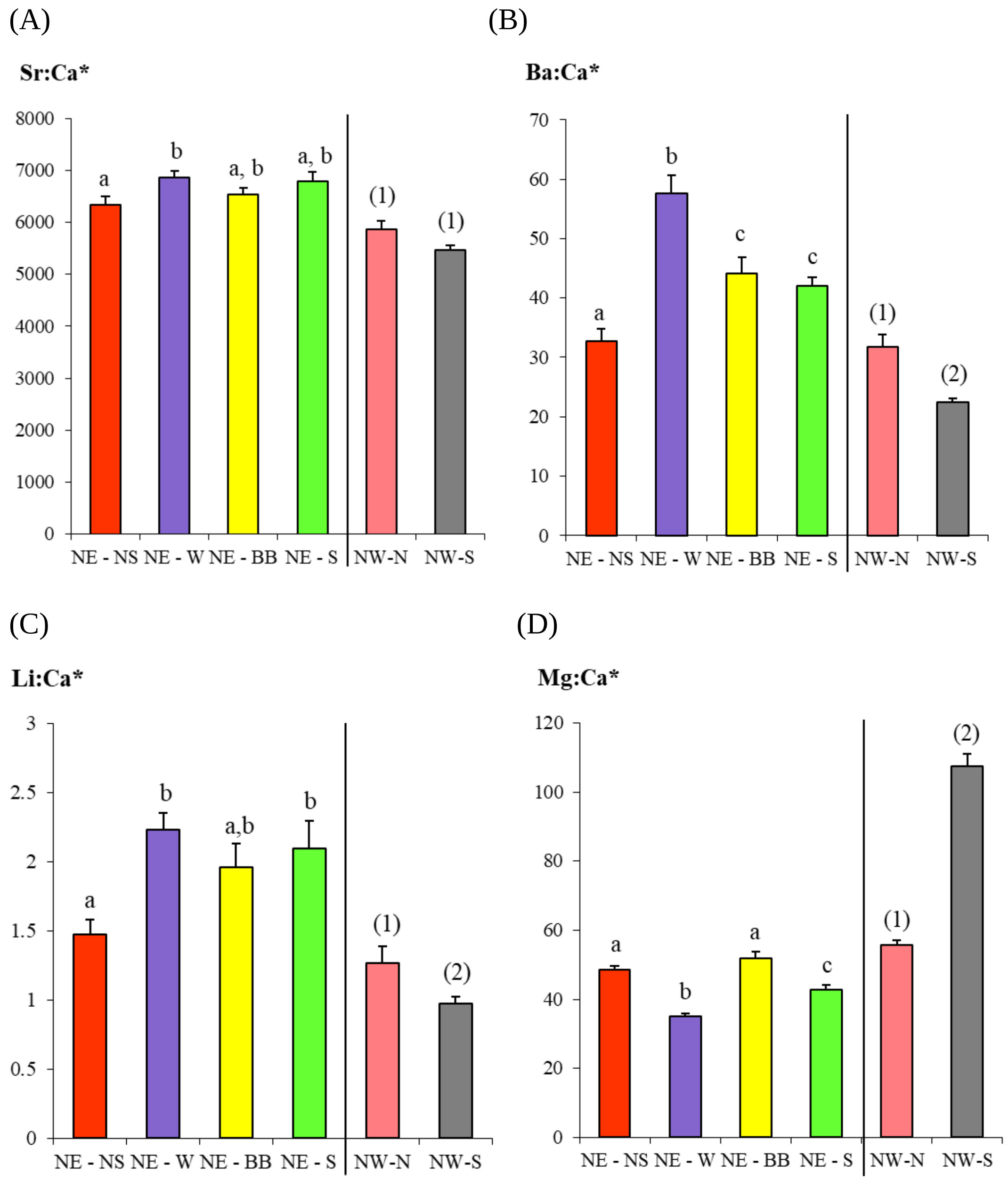

Figure 4.2 Mean ± SE Element:Ca concentration values for the sampling locations. In Element:Ca* there were significant differences between the NEA and NWA stocks (ANOVA, $p < 0.05$). For each Element:Ca, NEA sampling locations sharing the same letter do not show any statistical difference (Tukey, $p > 0.05$). For each Element:Ca, NWA sampling locations sharing the same number do not show any statistical difference (ANOVA $p > 0.05$).

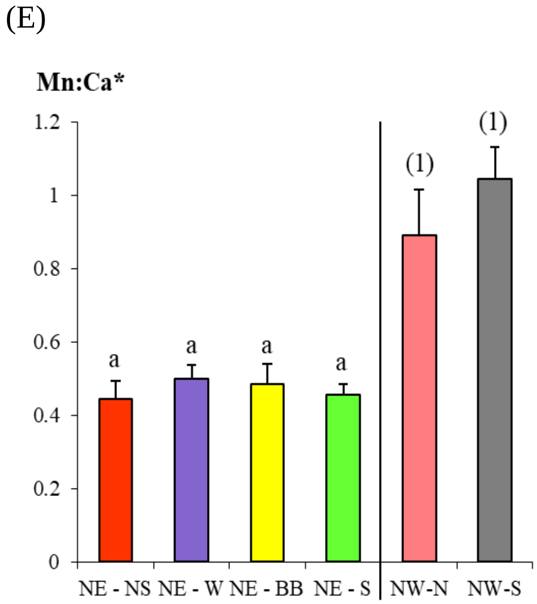

Figure 4.2 (continued)

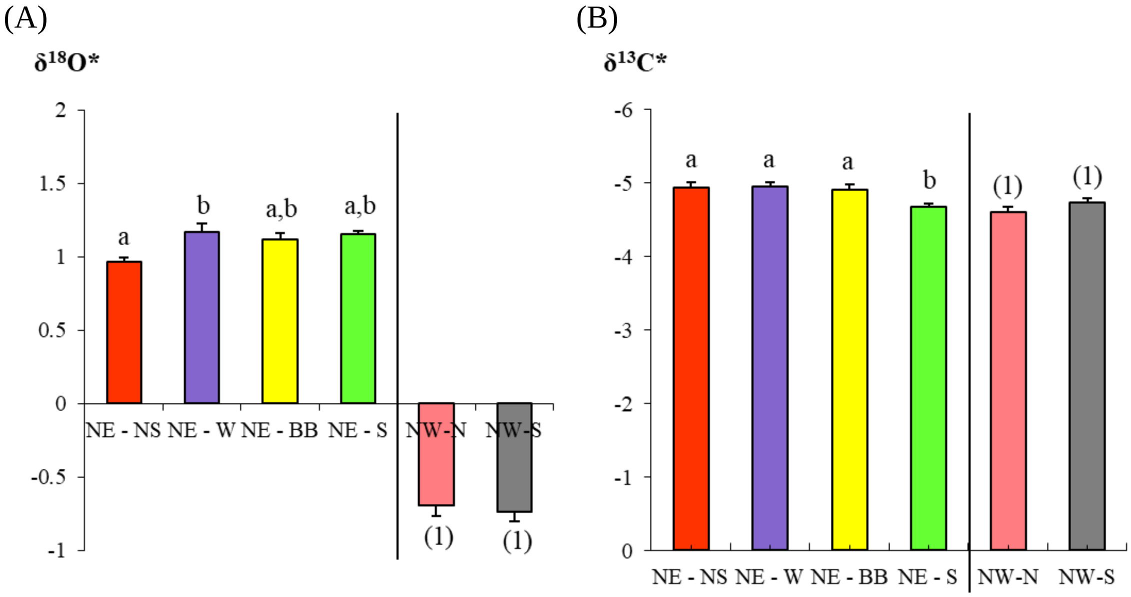

Figure 4.3 Mean ± SE Isotopic Ratio concentration values for the sampling locations. In Isotopic Ratio* there were significant differences between the NEA and NWA stocks (ANOVA, $p < 0.05$). For each Isotopic Ratio, NEA sampling locations sharing the same letter do not show any statistical difference (Tukey, $p > 0.05$). For each Isotopic Ratio, NWA sampling locations sharing the same number do not show any statistical difference (ANOVA $p > 0.05$).

Reclassification analysis comparing the stocks (NWA vs NEA) showed a perfect reclassification success of 100% with both element and isotopic analysis contributing to the discrimination (Table 4.4; Figure 4.4 (A)). Furthermore, there were significant differences between the stocks (PERMANOVA, pseudo-F = 56. 736; df = 1, 178; $p < 0.05$).

The NWA stock sampling locations showed a high reclassification success (93%) mostly driven by Mg:Ca (Table 4.4; Figure 4.4 (A)). Furthermore, there were significant differences between NW-N and NW-S locations (PERMANOVA, pseudo-F = 8.1657; df = 1, 58; $p < 0.05$).

The NEA stock had the lowest overall reclassification success, with an improvement from 65% to 76%, when NE-BB is not considered; NE-W sampling location maintained the reclassification success (80%) and NE-NS and NE-S improved (Table 4.4). The variables that showed to most contribute for the NEA stock grouping discriminations were Ba:Ca, Mg:Ca and δ^{13}C (Figure 4.4 (B) and (C)). The elements such as Ni:Ca and Na:Ca, which did not showed to be statistically significant in the univariate statistical analysis, in the NEA stock seem to still contribute for the discrimination (Figure 4.4 (B) and (C)). There were significant differences for the NEA stock locations in both analysis with (PERMANOVA, pseudo-F = 6.148; df = 3, 116; $p < 0.05$) and without (PERMANOVA, pseudo-F = 8.3465; df = 2, 87; $p < 0.05$) the NE-BB location. Moreover pairwise comparisons were the same with and without the NE-BB sampling location, differentiating all locations from the NEA stock (PERMANOVA, $p < 0.05$).

Table 4.4 The leave-one-out reclassification matrix of the otolith chemical signature (element and isotopic analysis) for both stocks, NWA stock, NEA stock and NEA stock without NE-BB location.

Both stocks	**Predicted Locations**			NW Stock	**Predicted Locations**		
Original Locations	NE	NW	% Correct	**Original Locations**	NW-N	NW-S	% Correct
NEA	120	0	100	NW-N	28	2	93
NWA	0	60	100	NW-S	2	28	93
Total	120	60	100	Total	30	30	93

NE Stock	**Predicted Locations**						**Predicted Locations**			
Original Location	NE-NS	NE-W	NE-BB	NE-S	% Correct	**Original Location**	NE-NS	NE-W	NE-S	% Correct
NE-NS	20	2	3	5	67	NE-NS	24	2	4	80
NE-W	3	24	0	3	80	NE-W	2	24	4	80
NE-BB	6	1	15	8	50	NE-S	5	5	20	67
NE-S	4	1	6	19	63	Total	31	31	28	76
Total	33	28	24	35	65					

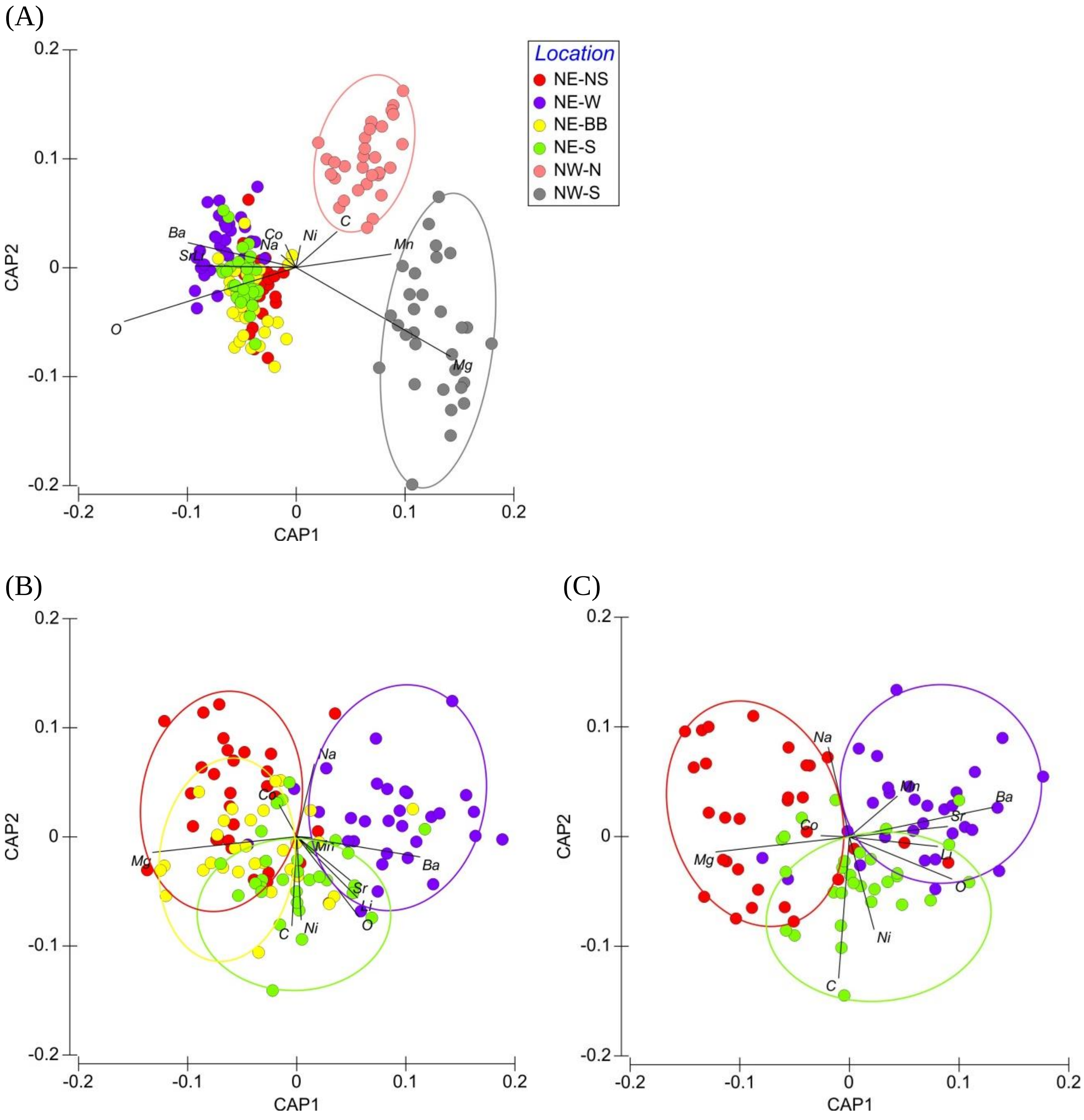

Figure 4.4 Canonical analysis of principal components (CAP) of the otolith chemical signature (elemental and isotopic analysis) for all the locations (A), for the NEA stock (B) and for the NEA stock without NE-BB location (C).

4.3. Shape and chemical signatures

Reclassification analysis comparing the stocks (NWA vs NEA) showed a perfect reclassification success of 100% with both shape and chemical signatures contributing to the discrimination (Table 4.5; Figure 4.5 (A)). Furthermore, there were significant differences between the stocks (PERMANOVA, pseudo-F = 24.437 736; df = 1, 178; $p < 0.05$). Both NWA and NEA stocks locations reclassification percentages improved with chemical and shape signatures combined.

NWA stock presented a perfect reclassification success (100%), mostly driven by Mg:Ca and SI variables (Table 4.5; Figure 4.5 (A)), Furthermore, there were significant differences between NW-N and NW-S (PERMANOVA, pseudo-F = 8.7452; df = 1, 58; $p < 0.05$).

NEA stock presented, again, an improvement in the reclassification success from 65% to 76%, when NE-BB is not considered; NE-BB showed the highest overlap with NE-NS sampling location (Table 4.5; Figure 4.5 (B)). The NEA stock locations discrimination was mostly driven by Ba:Ca and Mg:Ca, $\delta^{13}C$ and a few EFD (Figure 4.5 (C)). There were significant differences for the NEA stock locations in both analysis with (PERMANOVA, pseudo-F = 3.5386; df = 3, 116; $p < 0.05$) and without (PERMANOVA, pseudo-F = 4.4641; df = 2, 87; $p < 0.05$) the NE-BB location. Moreover pairwise comparisons were the same with and without NE-BB sampling location, differentiating all locations from the NEA stock (PERMANOVA, $p < 0.05$).

Table 4.5 The leave-one-out reclassification matrix of the otolith shape and chemical signatures (combined) for both stocks, NWA stock, NEA stock and NEA stock without NE-BB location.

Both stocks	**Predicted Locations**			NWA stock	**Predicted Locations**		
Original Locations	NE	NW	% Correct	**Original Locations**	NW-N	NW-S	% Correct
NEA	120	0	100	NW-N	30	0	100
NWA	0	60	100	NW-S	0	30	100
Total	120	60	100	Total	30	30	100

NEA stock	**Predicted Location**						**Predicted Location**			
Original Location	NE-NS	NE-W	NE-BB	NE-S	% Correct	**Original Location**	NE-NS	NE-W	NE-S	% Correct
NE-NS	20	2	6	2	67	NE-NS	26	1	3	87
NE-W	1	27	0	2	90	NE-W	3	25	2	83
NE-BB	8	1	16	5	53	NE-S	4	3	23	77
NE-S	3	3	2	22	73	Total	33	29	28	82
Total	32	33	24	31	71					

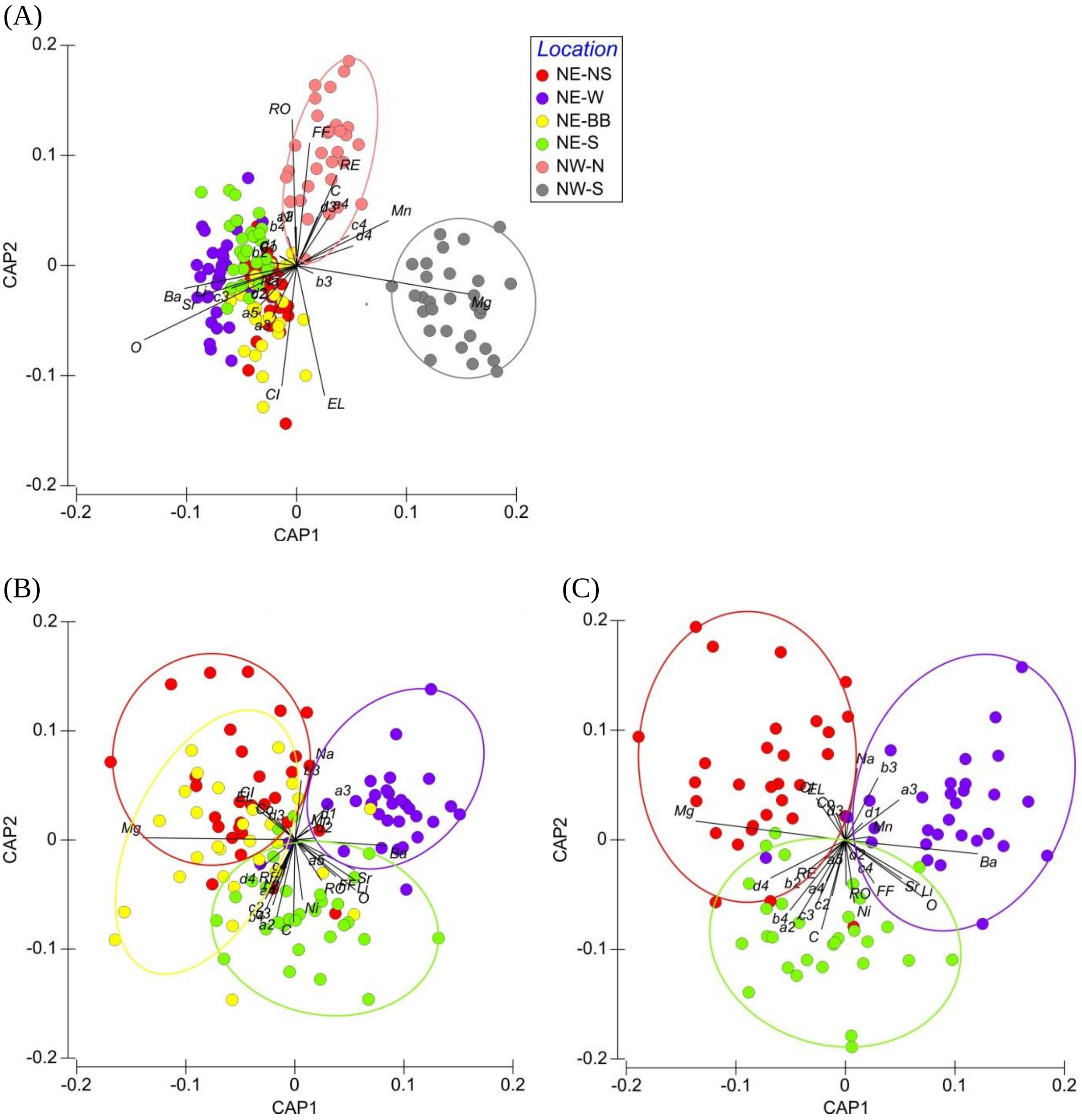

Figure 4.5 Canonical analysis of principal components (CAP) of the shape and chemical signatures (combined) for all the locations (A), for the NEA stock (B) and for the NEA stock without NE-BB (C).

5. Discussion

Annual fluctuations in continental shelf water characteristics include endless factors of variability, such as seasonal heating and cooling, river runoff, coastal hydrology, as well as intrusions of offshore waters affecting fish distributions (Radlinski *et al*., 2013). These characteristics are hard to be all accounted for but can lead to the regional habitat differentiations or similarities between fish populations (Elsdon *et al*., 2008). Natural tags can be used to track groups of fish but not in a simplistic manner, since they are not necessarily stable over long periods due to environmental plasticity (Campana, 2005; Campana *et al*., 1999). Still, whole otolith analysis seem to be particularly good for characterizing groups of fish, since differences indicate that they must have spent part of their life history in different locations, and subsequently infer about the movement and mixture of these groups over short periods (Campana *et al*., 1997; Rooker *et al*. 2001; Elsdon *et al*., 2008). A large number of migratory marine fish species that have broad geographical distributions can exhibit significant levels of population subdivision if they show post-dispersal spawning fidelity to natal areas, a phenomenon known as natal homing (Ruzzante *et al*. 1998; Souza *et al*., 2006). And, even though there is lack of genetic divergence between traditional and phenotypic recognized populations, this appears to be a feature of pelagic species (Jamienson *et al*., 1987). As adaptive genetic differentiation between recently diverged groups or between incompletely isolated groups may not be reflected by neutral molecular genetic differences (Swain *et al*., 2005). A holistic approach is possible and necessary, which will provide more reliable information for resource management and the promotion of an interdisciplinary approach for a better stock identification (Cadrin *et al*., 2005).

The first objective of this work was to assess how successful the use of chemical and shape analysis would be in the separation of the NEA and NWA mackerel stocks. Secondly we intended to evaluate the degree of separation between components within each stock, since the existent studies so far have not been able to fully separate them. Additionally, we tried to obtain new findings on the fish movement and habitat connectivity of the components and to infer if the results would support the current knowledge on the migration patterns of both stocks.

When comparing the distinct natural tags, chemical signature was more successful for the discrimination of the stocks and components within each stock. Even though shape signature presented lower reclassifications it still presented good results, especially for the stocks separation and NWA stock components discrimination. Shape and chemical signatures combined really improved the reclassification percentages, proving to be very effective tools.

Otoliths shape signature presented the highest reclassification success for the NWA stock. The discrimination of the NW-N and NW-S locations was based on all the Shape Indices variables (e.g. Rectangularity, Form Factor, Roundness, Ellipticity and Circularity). Such intra-specific differences in otolith Shape Indices have been attributed to differences in fish growth rate (Ferguson *et al*., 2011). These two components were originally separated in terms of length distributions. In their mixed occurrence areas, there was a bimodal pattern in the length distribution catches that would disappear temporarily (at the time of spawning and feeding separation) and, has this evidence of two distinct groups held throughout multiple years, the components differentiation was created (Sette, 1950). Additionally, in this study, the sample lengths available from the fisheries and the length distribution of the three year old individuals of each component were highly divergent (3 year old individuals TL mean of 38.4 cm for the NW-N component and of 29.3 cm for the NW-S component) which is in accordance with what is described (Sette, 1950). These reportedly differences in the growth rate of each component can explain their otolith shape signature differentiation.

In the NEA stock only the NE-S location differentiated and, contrary to the NWA stock, the discrimination was based on the otolith contour, characterized by the Elliptic Fourier Descriptors. Otolith shape signature has been also correlated to the diet of marine fish (Mille *et al*., 2016). There is a rich upwelling phenomenon in the continental shelf of Galicia and NW Portugal making it an area of high primary production (Cabanas *et al*., 1992; Varela, 1992; Cushing, 1995). The Galician western coast is part of the so called North Western Iberian upwelling system, which is the northernmost limit of the Eastern North Atlantic Upwelling System, characterized by strong and frequent upwelling events during spring and summer (Wooster *et al*., 1976; Fiúza *et al*., 1982 Alvarez *et al*., 2005). Additionally, upwelling does not have the same strength along this area, with a decrease from Portugal (south) to the Bay of Biscay (north) (Alvarez *et al*., 2005). This can explain the southern component location (Matosinhos, Portugal) otolith shape signature differentiation from the rest of the NEA stock sampling locations. Bay of Biscay presented a significant overlap with all the NEA stock components, especially with the North Sea component, which is not in its vicinity as western and southern components supposedly are.

As expected, from what is described for these stocks separation, the NEA and NWA stocks differentiated, with both SI and EFD contributing for the discrimination.

Although not fully understood, the otolith form could provide a phenotypic basis for separating the fish populations, considering that the otolith morphology varies geographically according to the effects of genetic variation and local environmental factors (Cardinale *et al*. 2004). The extent to which otolith shape differences are genetically or environmentally induced is still in question but, in the overall, these differences increase the knowledge on stock structure and can help make assumptions on each stock contribution to a mix stock fisheries (Begg and Brown, 2000; DeVries *et al*., 2002; Cadrin and Friedland, 2005). Additionally, the study of the morphological characteristics of otoliths has been considered an efficient tool for fish stock identification and has proven successful in resolving fish stock structure in high gene flow systems, when environmental heterogeneity exists (Ferguson *et al*., 2011; Jemaa *et al*., 2015; Bacha *et al*., 2014).

Otoliths chemical signature was highly successful in the grouping formation. It completely discriminated the NEA and NWA stocks, and presented high reclassification success within each stock, especially for the NWA stock locations. The elements, Sr:Ca, Ba:Ca, Li:Ca, Mn:Ca and Mg:Ca, which showed significant local differences are also the ones that better reflect their relative environmental abundance on the otolith (Fowler *et al*.,1995; Campana, 1999; Panfili *et al*., 2002).

Sr:Ca discriminated the stocks and presented higher concentrations for the NEA stock. Sr is a trace element that is noticeably influenced by its ambient concentration and presents a positive correlation with water salinity (Secor and Rooker, 2000; Elsdon and Gillanders, 2004; Walther and Thorrold, 2006). The ocean circulation pattern along the NWA continental shelf is dominated by the southward transport of Arctic waters via the Labrador Current (LC) that enter in the Gulf of St Lawrence, which is characterised by cooler, less saline waters (Beardsley and Boicourt, 1981; Loder *et al*., 1998; Fratantoni and Pickart, 2007). In contrast, NEA continental shelf has the great influence of the North Atlantic Current which results from the warm Gulf Stream current (Krauss, 1996) and, additionally, NEA stock individuals follow the relatively warm high/saline eastern Atlantic water flowing northeast and along the continental shelf edge (Jansen, 2013), which could explain the higher Sr content on this stock. Additionally, there were significant differences in the NEA stock, with the North Sea component differentiating and presenting lower Sr:Ca concentrations; this result seems to be also influenced by a lower salinity content in the North Sea sampling area. The North Sea waters are affected, not only, by the warmer North Atlantic Current, that enter the North Sea through the English Channel and between the Shetland Islands and Norway, but also by colder, less-saline waters that come from the Baltic Sea through the Skagerrak (Alexander, 2015). Additionally, large quantities of fresh water also enter in the estuaries of the southern area, in the English Channel (area of sampling in this study), essentially through the Rhine and Thames Rivers (Alexander, 2015).

Ba:Ca also contributed for the stocks differentiation. Upwelling processes are often linked to variations in water chemistry and Ba seems to be a reliable indicator of these changes (Elsdon and Gillanders, 2004; Hamer *et al*., 2006; Hicks *et al*., 2010). Upwelling events are attributed to the actions of the winds along the coast that generate an Ekman drift which is directed offshore in eastern boundary coastal systems (as the NE Atlantic) or onshore (as the NW Atlantic) (Alvarez *et al*., 2005). This result in different upwelling intensities in each side of the North Atlantic coasts, in the NEA coast there are stronger upwelling events characterized by water depletion in the upper layers, the space vacated is replenish by the offshore drift with deep nutrient rich waters which trigger intense phytoplankton blooms (Alvarez *et al*., 2005). This can explain the higher Ba:Ca values for the NEA stock. Moreover, Ba has been showed to be positively influenced with temperature (Elsdon and Gillanders, 2002; Webb *et al*., 2012). The colder waters from the NWA coast could also influence the lower values of Ba:Ca for this stock. Furthermore, differences within each stock were also recorded. In the NEA stock there were contrasting higher values in the western component and lower values in the North Sea component. The higher Ba:Ca values in the western component could be related with salinity. It has been noted the Ba enrichment in saline environments; these richer saline environments can happen via advective exchange between an aquifer (groundwater) and the coastal ocean, and it seems that exceptional high level of saline waters occur in many coastal aquifers of the British Isles (Shaw *et al*., 1998; Webb *et al*., 2012; Darling *et al*, 1997). Additionally, even though not so prominent, NE-W presented the highest values of Sr:Ca also positively influenced by salinity. The lower Ba:Ca values for the North Sea could be related with colder waters in this area. One of the reasons that appear to prevent the rebuilding of the North Sea stock is the decreasing of spawning in the area due to avoidance of cool surface waters in that area (Jansen, 2013). Additionally, the lower content in the North Sea could also contribute to the lower Ba:Ca values in this area. In the NWA stock, there were differences between the components with NW-S presenting lower concentrations. While the Gulf of St Lawrence has the majority effect of the Labrador Current, the southern Scotian Shelf, Gulf of Main, and southwards, are more influenced by the inshore Nova Scotia Current (NS) and the Shelf-Break (SB) current (an extension of the Labrador Current) that originate in the Gulf of St. Lawrence moving south westward along the coast (Loder *et al.*, 1997; Rutherford and Fennel, 2018). The Nova Scotia currents carries low salinity waters (from the St Lawrence River) (Smith, 1989) which can explain the Ba:Ca lower concentrations in this area. Additionally, even though not so prominent, NW-S presented lower concentrations of Sr:Ca also positively influenced by salinity.

Li:Ca showed a similar pattern as for Sr:Ca and Ba:Ca for the locations, this is in accordance with what is described, Li presents a high success for distinguishing fish at different salinities, as the ratio Li:Ca increases with salinity (Hicks *et al.*, 2010; Moreira *et al.*, 2018).

Mn:Ca was successful in the stock discrimination, with NWA stock locations presenting comparatively higher Mn:Ca concentrations. This is in agreement with previous studies suggesting that species with lower Sr levels tend to display higher Mn levels in otoliths, since Mn has a negative relation with salinity (Campana, 199; Hamer and Jenkins, 2007; Soeth *et al.*, 2019). Moreover, while Sr incorporation into otoliths is influenced by water chemistry, Mn appears to be regulated by physiological mechanisms and the influence of environmental factors remains somewhat unclear (Elsdon and Gillanders, 2003; Hamer and Jenkins, 2007). Mg:Ca showed the highest amount of differentiations within each stock. Mg seems to be affected by physical and chemical environment influences even though there is no further knowledge on this (Fowler et al. 1995; Campana, 1999). And, as for Mn, Mg is essential for a series of cellular process, with a high physiological response in their regulation (Hamer and Jenkins, 2007; Barnes and Gillanders, 2013). Although the mechanisms behind the incorporation of the Mn and Mg elements into otoliths are still not well understood, differences in their concentrations have been used to successfully infer the population and stock structures of important fish species (Silva *et al.*, 2011; Correia *et al.*, 2012; Higgins *et al.*, 2013).

δ^{18}O results, which mostly contributed for the stock differentiation, are in agreement with global sea surface δ^{18}O, that is more depleted at more polar latitudes and low salinities areas (Schmidt *et al.*, 1999; LeGrande and Smith, 2006; Torniainen *et al.*, 2017). The negative values in the samplings areas from the NWA should be mostly related to the Labrador Current and in contrast, the NEA positive values should be related with the North Atlantic Current; and these values are in agreement to what is described for the North Atlantic (Thorrold *et al.*, 1997; Frew *et al.*, 2000 LeGrande and Smith, 2006). In the NEA, the North Sea differentiation and low concentration should be related to what has been said before about this area of being of lower salinity when compared with the other NEA sampling locations.

δ^{13}C values are in agreement to what is usually described in marine fishes (Redding, 2017; Moreira *et al.*, 2018). δ^{13}C is normally related with fish diet, with δ^{13}C content reflecting changes in trophic levels (i.e., fish diet, growth, and metabolism) which, along with the dissolved inorganic carbon (DIC) in the water, are considered the main endogenous factors that affect the incorporation of δ^{13}C into the otoliths (Gillooly *et al.*, 2001; Gao *et al.*, 2004; Elsdon *et al.*, 2008). δ^{13}C results discriminated the stocks which is expected since, as seen before, there are different upwelling phenomena between the NEA and NWA coasts which lead to ecosystem differentiation between both and will reflect on different feeding conditions for each stock. Within the NEA stock, the southern component differed from the rest of the sampling locations presenting the highest δ^{13}C content. As already described, there is a higher upwelling content in the southern component sampling location which can explain this result. In the NWA stock, even though the locations did not present significant differences, NW-N presented slight higher δ^{13}C content. The northern component individuals spend the majority of the year in the Gulf of St. Lawrence. This area has particular characteristics such as being similar to an inland sea with partial isolation from the North Atlantic, has estuarine and ocean influence, and a combination of numerous shallow areas and deep troughs; which distinguish this area of being of high biological productivity and diversity (DFO, 2005).

Regarding the Bay of Biscay and its place in the NEA stock dynamics, results showed overlapping with the southern component sampling location (essentially in the chemical signature) as expected, since, reportedly, Bay of Biscay is the main spawning area of the southern component and Matosinhos and Bay of Biscay ae relatively close areas. As for the lower overlap between the western component sampling location and Bay of Biscay, this can be explained by two scenarios. First, from the North Atlantic Current (originated from the Gulf Stream and moving in a north eastern direction) a small part branches out and moves southwards, the Portugal Current being part of that branch (Taylor and Stephens, 1980; Krauss, 1986). This reflects on different hydrodynamic conditions affecting the Iberian Peninsula versus the British

Isles and can explain the low overlap between these areas (since, additionally, the western and southern components sampling areas did not overlap greatly). Secondly, the western component supposedly spawns from the British Isles to the Bay of Biscay, so it would be necessary to also include individuals from different areas along this range in the analysis to really assess the length of differentiation and be able to make conclusions about the degree of overlapping. Bay of Biscay actually showed the biggest overlap with the North Sea component in both chemical and shape signatures alone and combined. Even though the North Sea has been described as the most isolated component from the NEA stock, recently it was showed to considerably mix with the other components and the need of studying this component more deeply was advised (Jansen and Gislason, 2013). Additionally, the surface flow pattern in the Bay of Biscay is likely to be seasonal, depending on whether northerly or southerly influences prevail (Krauss, 1986; Pingree, 1993) which, other than providing a similarity with the southern areas (Matosinhos), can be influenced by more northern areas (North Sea) in terms of water characteristics. The Bay of Biscay region tends to be a relatively stagnant part of the ocean, lying south of the relatively faster northeastward moving waters of the North Atlantic Current, and north of the weaker southeasterly flow of Portugal Current, which only a small fraction goes into the Bay of Biscay creating a slack region with weak flow (Krauss, 1986; Pingree, 1993). The higher isolation of this area, can create similar conditions as the, also isolated area, North Sea.

In the overall, the NEA stock showed higher overlap in the sampling locations when compared with the NWA stock (which components presented completed discrimination). Components distributions in each North Atlantic coast have different dynamics and the NEA stock components seem to mix more extensively, which is in agreement with this study findings. Still, all the components from the NEA stock did differentiate and grouping of each one was visible. The biggest discrimination of the NWA stock components reflect the fact that they spend more time apart (when compared with the NEA stock), since their feeding period is not spent at the same place and they join only in the overwintering period. Additionally, there is the information that NWA mackerel have been seen in more inshore shallower waters of the Northeast Continental Shelf during winter due to a general warming in the region and a decreasing of mackerel in the overwintering area (Overholtz, 1991; NEFSC 2006; Overholtz *et al.*, 2011; Radlinski *et al*., 2013). If these observations prove to be true, there is a possibility that fewer individuals are migrating and mixing in the overwintering grounds.

The successful grouping of the components within each stock may indicate that mackerel show homing behaviour and site spawning fidelity. Other studies have also reached this conclusion but each stock complexity has left that question in doubt (Studholme *et al*. 1999; Nesbø *et al*., 2000; Jansen and Gislason, 2013). Moreover, to more confidently make this assumption based on natural tags, further studies would be necessary, such as determine natal otolith fingerprints of each component of a specific cohort and follow the fish migration of that specific cohort. Furthermore this kind of study would also allow to assess the contribution of each component to the mix fishery when the components are gathered (Correia *et al*., 2014).

As for the stocks the results showing their complete differentiation are in accordance to what would be expected, proving that this type of analysis can provide essential information on marine fish population structure.

6. Conclusion

Otolith chemical signature proved to be more successful on stock and stock components discrimination of Atlantic mackerel, but there was an improvement with both shape and chemical signatures combined for the components discrimination. The natural tags proved to be useful on giving additional information about the Atlantic mackerel population structure. The NWA and NEA stocks completely discriminated and the NWA stock components as well. For the NEA stock, discrimination was also high but some overlapping was visible, probably due to the higher habitat connectivity of the components from this stock when compared with the NWA stock. Bay of Biscay showed an unexpected high overlap with the North Sea component, which can be a reflection of the reported great spatial temporal distribution dynamics of this stock. As for the NWA stock, the complete differentiation of the components calls for a more urgent revaluation of its management, since there is a possibility of a change in its migration patterns (in particular, to the overwintering grounds when the components mix) and it may be necessary for these components to be considered as separated stocks.

These new findings show that natural tags are in fact helpful tools for fish population studies, bringing new insights and valuable knowledge to answer fishery important questions. Although this study alone cannot make complete conclusions on each stock structure, it provides information that, with an interdisciplinary approach, can be used by decision makers and fisheries agencies to improve the mackerel management in the North Atlantic. Further investigation on this species is necessary since it has high economic and social importance in both sides of the Atlantic. Additionally, as the NEA stock supports one of the largest fisheries and the NWA stock it's at the lowest values in history, both populations urgently need to be explored at sustainable levels. Also, accordance and compliance by all the nations that explore this resource are necessary for its conservation.

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