Major population's separation area for sardine (Sardina pilchardus) and hake (Merluccius merluccius) revealed using otolith geochemistry on the Atlantic coast of Morocco

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Major population's separation area for sardine (*Sardina pilchardus*) and hake (*Merluccius merluccius*) revealed using otolith geochemistry on the Atlantic coast of Morocco

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Abstract :

The Moroccan Atlantic coast (21–36°N) is part of one of the most productive marine eastern boundary upwelling ecosystems worldwide. Nevertheless, information about the structure of its exploited fish populations is scarce. In this study, whole otolith elemental signatures of 111 hake (Merluccius merluccius) and 118 of sardine (Sardina pilchardus), sampled during the period June 2012 - July 2013 in 11 locations along the Moroccan coast, were analysed by solution based ICP MS. These 2 species were selected for their importance in the fisheries, their contrasted life histories, and differences in trophic behavior. Whole otolith comparisons used Ba/Ca, Li/Ca, Mg/Ca, Mn/Ca, Rb/Ca, Sc/Ca, Sr/Ca, to demonstrate the regional population structures. The linear discriminant analysis of whole otolith chemistry data of hake and sardine reached respectively 88 % and 71 % mean adequate classification, and rose to 100 % within the identified groups. The spatial variation in the elemental composition of the otoliths of the two species were significant, and all showed a clear geographic cut, thus identifying a northern and a southern group. The major split for the two species occurred in the same area corresponding to the transition zone between the northern temperate mud-shelf ecosystem and the arid ecosystem at the south (28–29°N). Complex oceanographic processes may act in this area as a barrier to planktonic dispersion favouring isolation processes that lead to distinct populations. These findings are new to hake and confirm previous studies on sardine population structure, and should be considered in fisheries management to better match management units and stock structure.

Keywords : Handled by B, Morales-Nin, Otolith, Microchemistry, ICP MS, Morocco, Hake, Sardine, Population discrimination

1. Introduction

Management and conservation of fisheries resources require more accurate information on the population structure of target species, and renewed findings confirm the need for considering stock complexity in fisheries management (Ying et al., 2011; Izzo et al., 2017; Correia et al., 2021). The fact that distinct populations or populations sub-units may respond differentially to anthropogenic or environmental pressures (Leibold et al., 2004; Kritzer and Sale, 2004; Erauskin-Extramiana, 2019), makes the identification of appropriate management units and persistent spatial patterns within them essential to achieving sustainable fisheries objectives (Hilborn et al., 2022).

Among the approaches using natural tracers for fish stock identification purposes, methods based on the analysis of trace elements in otoliths have been used over several years to trace the movements of fish populations and identify the stock structure or the origin of spawning adults (Kerr et Campana, 2014; Tanner et al., 2016; Artetxe-Arrate et al., 2021). Otoliths are part of the balance organ found in the ear canals of teleost fishes. They grow continually throughout life and are formed by the deposition of calcium carbonate crystals within a protein matrix (Pannella, 1971). Ambient water chemistry and environmental conditions affect elemental incorporation into the otolith (Miller, 2011; Webb et al., 2012; Nazir and Khan, 2019). Variations in the concentrations of selected elements, identified as 'chemical fingerprints' can therefore be used as natural markers to discriminate among groups of fish. However, factors that affect the elemental incorporation into otoliths are not yet completely understood and various factors other than ambient water chemistry may also play important roles in elemental incorporation: dietary sources, physiological processes or genetic variations (Clarke et al., 2011; Sturrock et al., 2015;

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Izzo et al., 2018; Hüssy et al., 2020). The success in the use of the whole otolith chemistry (i.e., entire life-history prior to capture) to investigate fish population structure at large geographic scale (Moreira et al., 2018; Soeth et al., 2019 ; Correia et al., 2021; Khan et al., 2021) allows for an increase in sample size due to a reduction in the time invested in otolith processing procedures and chemical analyses.

The Moroccan Atlantic coast (21–36 °N) in northwest Africa is one of the most productive marine areas worldwide (Carr, 2001). Being a part of the Canary Current Large Marine Ecosystem, these subtropical waters house one of the four major eastern boundary upwelling ecosystems, that support a rich and complex food-web and important fisheries (Kifani et al., 2008). This area is also a transition zone between temperate European waters to the north and tropical African waters to the south, and is the seat of a major biodiversity break in the North Atlantic fish communities (Briggs, 1995; Gislasson et al. 2020). This increases the uncertainties associated with the effects of climate change on fish communities composition and organization (Kaimuddin et al., 2016) and reinforces the need for a better understanding of fish population structuring and biodiversity patterns.

Morocco is considered as the most productive fish producer in Africa with 1.36 million tons in 2018, and reaches the 17th rank globally (FAO, 2020). The European sardine (*Sardina pilchardus*) is the most important species in terms of biomass, reaching 80% of the landings of the fisheries targeting small pelagic fish, which are responsible for 86% of total landings (purse seiners and pelagic trawlers) (DPM, 2018). Bottom trawl fisheries targetting octopus (*Octopus vulgaris*) and hake (mainly *Merluccius merluccius*) are some of the most important in Morocco, alongside small-scale fisheries (Kifani et al., 2008; DPM, 2020). Sardine (*Sardina pilchardus*) and hake (*Merluccius merluccius*) are then two of the most important species for Moroccan fisheries. Hake is a temperate, benthopelagic species with a longevity of 20 years, preying mainly on fish and shrimps (Abdellaoui et al., 2014). Sexual maturity is reached for female at 34 cm (2 years) (El Habouz et al., 2011) and there is a strong habitat segregation between juveniles on the shelf and large adults on the slope. The Saharan bank (21-26°N) is the southern limit of the latitudinal distribution of this species where only large adults persist on the slope. Sardine is a subtropical pelagic filter feeder that consumes phytoplankton and zooplankton with varying proportions between areas and seasons (Garrido & van der Lingen, 2014 ; Gushchin et Corten, 2015;

Bachiller et al., 2020). Two distinct populations were identified along the Moroccan coast (Atarhouch et al., 2007; Chlaida et al., 2009) with a break at 30 °N. The observed maximum age is 6 years, however, the southern individuals grow faster and reach a higher maximum length (Amenzoui, 2010). Sexual maturity occurs during the second year at a length between 14 cm and 16 cm (L50) (Amenzoui et al., 2004).

In Moroccan waters, the information on fish stock identity remains scarce, even though it has been identified as a major concern for the Moroccan fisheries management system (Chlaida, 2006). The aim of our work is to study the population structure and provide information on stock identity for major fish species for Moroccan fisheries. We selected 2 species occurring along the Moroccan coast with contrasted life histories and trophic behaviour: *Merluccius merluccius* and *Sardina pilchardus*. Hake lacks studies on their population structure in Moroccan waters, while different methods (morphometry, allozyme, mtDNA) have been used to study sardine populations (Chlaida et al., 2006, 2009; Atarhouch et al., 2006, 2007; Ouakka et al., 2012).

2. Material and Methods

2.1 Study Area

The Moroccan Atlantic coast located in northwest Africa between 21°N and 36°N (3000 km), is part of the Canary Current Ecosystem and is a highly productive eastern boundary upwelling system (Figure 1) (Vazquez et al., 2022). The coastal geomorphology associated to variable climatic and environmental conditions lead to variable upwelling activities along this coast (Benazzouz et al, 2014). The Saharan bank (21–26°N), which has an intense and permanent upwelling activity, is an extended continental shelf at the border of a hyper-arid landscape. The nearby area located northward (26–29°N), has an arid climate with intense seasonal upwelling activity. The northernmost area (29–36°N) is a mud-shelf ecosystem where the upwelling has low and seasonal activity. The landscape at its borders has a sub-humid climate and houses the most important rivers of the Moroccan Atlantic coast. This latter area is the most impacted by human activities with the largest and most densely populated cities, and the highest rates industrial activity and agriculture.

2.2 Sampling

Fish were sampled in landing's fish markets at 11 localities during two sampling periods in 2012, Assilah to Agadir in June and Sidi Ifni to Dakhla in November (Figure 1). All samples came from small scale fishing units and fishermen that are present during the selling process were interviewed to ensure the local origin of the samples. Species that could not be found in fish markets during these two periods were sampled on board research vessels in July 2013. That was the case for hake at Dakhla, Boujdor, and Tantan, and sardine at Tantan. Nevertheless, sardine were not sampled at Assilah and Boujdor, nor hake at Sidi Ifni. 111 hakes (27–50 cm) and 118 sardines (15–21 cm) were collected. Fish were measured (Total Length (TL, 0.1 cm), weighed (W, 0.01 g) and dissected for sex determination and gonadal maturity stages assignment. Thereafter, both sagittal otoliths were extracted, cleaned from adhering tissues, and stored in plastic microcentrifuge tubes.

2.3 Chemical Analyses

Otoliths from 111 hake and 118 sardine were analysed (Table 1). Multi-elemental composition of whole otoliths (elemental signatures recorded from the fish birth until capture) were determined using solution based inductively coupled plasma mass spectrometry. Sample preparation and analyses were performed at the Pôle Spectrométrie Océan (Plouzané, France). All samples were prepared in a class 10000 clean laboratory. Ultra-pure deionized water (resistivity = 18.2 M.cm) was used for material cleaning and acid dilutions. Nitric acid solutions (commercial grade, Merck) were purified by distillation in sub-boiling silica glass stills (Quartex). All materials (polypropylene centrifuge tubes, disposable pipette tips, etc.) were pre-cleaned using 5% HNO3 and rinsed with ultra-pure deionized water. Otoliths were cleaned of organic material following the method of Warner et al. (2005). Otoliths were bathed in a 50/50 H_2O_2 (30%, Suprapur grade) and NaOH (Suprapur grade, 0.1 mol L^{-1}) solution for 1 hour, sonicated during the last 5 min of the bath, rinsed 5 times with ultrapure water for 5 min, dried under a laminar flow hood (HEPA 100), and stored dry in individual vials until the microchemistry analyses. They were weighted and transferred into a pre-cleaned polypropylene centrifuge tube, dissolved in 2% HNO3, and spiked with a known amount (approximately 7 µL) of a mono-elemental thulium solution (Tm concentration = 77.9 ng g^{-1}). Thulium was used as an internal standard to correct short and long-term instrumental drifts (See Barrat et al., 1996; Bayon

et al., 2009 for detailed information on this method). An external calibration was performed using an in-house multi-element solution prepared from certified stock solutions. This calibration solution was prepared so that it closely matched the calcium carbonate matrix and elemental composition of otoliths.

Elemental concentrations were measured on a Thermo Electron Element2 high-resolution Inductively Coupled Plasma Mass Spectrometer equipped with an ASX 260 auto-sampler (CETAC Technologies). Solutions were introduced via a Teflon nebulizer and a Peltier cooled cyclonic spray chamber. We chose to analyze concentrations in the most commonly used chemical elements, as described in the review by, Chang and Geffen, 2013. Therefore, after preliminary analysis, the following 8 chemical elements were detectable in the whole otolith and were measured with solution based ICP MS: ^{138}Ba, ^{43}Ca, ^{7}Li, ^{25}Mg, ^{55}Mn, ^{85}Rb, ^{45}Sc, and ^{88}Sr. All the concentrations were above the detection limits calculated using the three sigma criteria in μg/L: Ba (0.01), Li (0.01), Mg (0.1), Mn(0.04), Rb (0.002), Sc (0.4), Sr(0.4) Concentrations were calculated using the Tm addition method. Briefly, for each sample, elemental concentrations were calculated using the sample mass, the amount of Tm added, and by calibrating the raw data acquired during the measurement session against the unspiked (no added Tm) in-house multi-element solution, run after every five samples.

Otolith samples were analysed in random order to avoid possible sequence effects. For quality control of the measurements a fish otolith reference material FEBS-1 was analysed (FEBS-1: red snapper *Lutjanus campechanus sagittal* otolith, National Research Council of Canada, Sturgeon et al. (2005)) and the accuracy tested. The elemental concentrations determined were within the certified or indicative range, with recovery rate between 92 and 98%. The precision of replicate analyses of individual elements ranged between 2% and 5% of the relative standard deviation (RSD) for most of the element but reached 15% for Rb and Sc.

2.4 Statistical Analyses

The between species otolith elemental composition was analysed using a PERMANOVA (*adonis2* function in *vegan* package) where species and locations were used as factors. For each species, the effect on otolith elemental composition of sampling location, otolith weight, fish total length, sex and sexual maturity, was analysed using a

PERMANOVA (euclidian distance). The multiple variable for PERMANOVA analyses was the log(x+1) transformed otolith trace element/Ca composition (Ba, Li, Mg, Mn, Rb, Sc, Sr/Ca). Pair-wise analyses were then performed to compare between localities differences in otoliths chemical compositions (function *pairwise.perm.manova* in *RVAideMemoire* package*).*

Prior to the statistical analyses, ANCOVA analyses were conducted (function *anova (*on an *aov* output) from *car* package, using Type III sum of squares) to test the effect of otolith weight among regions on chemical elements, and resulted in significant differences for Hake's Li and Sc and for Sardine's Li ($p < 0.05$). The linear regression of the element against otolith weight appeared to be significant only for hake's Li concentrations ($r^2 = 0.22$, $p < 0.05$). Therefore we decided not to adjust otolith element ratios for otolith weight prior to the statistical analyses, and we tested its effect in PERMANOVA analyses.

Linear discriminant analyses (LDA) with a leave-one-out method were performed (function *discrimin* in *ade4* package) to determine the accuracy at which the combinations of selected elements could be used to reclassify individual fish to their region of collection. The jacknifed matrix was produced using the functions *lda* and *predict* of the package *MASS*. All statistical analyses were performed using R 3.4.4 (R Core Team, 2021).

3. Results

3.1 Comparison of trace metals composition between species

The trace metal composition of the otoliths differed significantly among species (PERMANOVA, $r^2 = 0.631$, $p < 0.01$) and localities (PERMANOVA, $r^2 = 0.131$, $p < 0.01$). These differences between species persisted within localities, which was indicated by the significance of the interaction between the factors, species, and localities (PERMANOVA, $r^2 = 0.075$, $p < 0.01$). Sardine otoliths had higher concentrations of Li than hake (11.6 ± 5.1, 0.94 ± 0.13, respectively), and were also enriched in Sc (1.05 ± 0.48, 0.65 ± 0.35 ppm, respectively). Hake showed higher concentration for all other elements, the most contrasted values being observed for Sr, Rb and Mg that were twice those of sardine (Table.2).

3.2 Spatial variation in hake otolith trace element composition

The elemental composition of hake otoliths showed significant differences among localities (PERMANOVA, $r^2 = 0.547$, $p < 0.001$). This factor explained the main part of the variability, while length, sex, and sexual maturity had no effect on the otoliths trace elements composition. Otolith weight had a significant effect (PERMANOVA, $r^2 = 0.057$, $p < 0.001$) and the variance explained was 1/10th of the variance explained by locations. The pairwise analyses showed no significant differences between most of locations at north of Agadir ($p>0.05$), while all these locations from the north Moroccan Atlantic coast showed highly significant differences ($p < 0.01$) with the locations to the south, from Tantan to Dakhla. Within the southern locations, Dakhla and Boujdor showed no significant differences ($p = 0.703 > 0.05$) while Boujdor and Tantan had no high significant differences ($p = 0.023 > 0.01$).

The LDA resulted in 87.7% adequate classification with Laayoune, Agadir, Boujdor, and Tantan as the best classified localities (>70%). Safi had the lowest classification score (46.6%) with most individuals classified as whiting Assilah and El Jadida. Two groups were then identified being the first one at north: I) Assilah, Mehdya, El Jadida, Safi, Agadir and the second one at south II) Tantan, Boujdor, Dakhla, in addition to Laayoune, which tended to be separate from the latter group (Figure 2). The reclassification occurred exclusively within locations of the same group. The first discriminating factor isolated the identified groups, and was mainly determined by Sc in opposition with Mn and Rb. The Sc concentration was higher in the southern localities of Tantan, Laayoune, Boujdor, and Dakhla, while Mn concentrations were lower in the same localities except for Laayoune (Figure 2).

3.3 Spatial variation in sardine otolith trace element composition

Sardine otolith trace elements composition differed significantly among localities and explained the largest part of the variance (PERMANOVA, $r^2 = 0.615$, $p < 0.001$). Otholith weight had significant effect on otolith chemical composition and explained a weak part of the variance (PERMANOVA, $r^2 = 0.0104$, $p < 0.046$), while the other dependent variables showed no significant effect (length, sex, and sexual maturity) (Table 3). The Li concentration rose rapidly from Mehdya to Essaouira, then slightly between Agadir and Laayoune, and decreased in Boujdor and Dakhla. Samples from the area south

of Sidi Ifni had higher Rb concentrations, while those from Laayoune and Dakhla had higher concentrations in Ba and Sc but were lower in Mn (Figure 2). The pairwise analyses showed three pairs of locations with no significant differences ($p > 0.05$): Safi - El Jadida, Safi – Agadir and Dakhla - Laayoune. Five other pairs located at north of Agadir showed no highly significant differences ($p > 0.01$).

The LDA resulted in 71.2% of adequate classification results based on Ba, Li, Rb, Sr, and Mg. The best classified localities were Ifni, Tantan, and Laayoune (>83%), and the less reliable was Safi (35%), for which individuals were classified in Essaouira, Jdida, and Agadir. The LDA identified two groups from north to south: I) Mehdya, El Jadida, Safi, Essaouira, Agadir, Sidi Ifni, Tantan, and II) Laayoune, Dakhla. The first axis was determined by Ba, Rb, and Sc, and separated clearly the identified groups with no overlapping confidence ellipses (Figure 4). The second axis was mainly driven by Li and separated group II into two subgroups: I.a: Mehdya, El Jadida, Safi, and I.b: Essaouira, Agadir, Sidi Ifni, Tantan, which showed coordinates of opposite signs, and distributed along a North-South gradient revealed by the second axis, with overlapping confidence ellipses.

4. Discussion

The analysis of whole otolith chemical compositions has been used in several studies to characterize the chemical signatures of fish groups at large spatial scales (Moreira et al., 2018; Soeth et al., 2019; Correia et al., 2021). They are mainly used in studies focusing on fish population structure and dynamics (Correia et al., 2014; Hägerstrand et al., 2017; Khan et al., 2021). Although the whole otolith chemical composition integrates the overall fish life history, methods based upon whole otolith chemistry have been successfully used to identify fish stock units with low levels of connectivity (Correia et al., 2021).

In our study, the two selected species differed clearly in their otolith elemental compositions, which may be due to the differences in their physiology, trophic habits, and their habitat types and ranges. Moreover, the incorporation of trace elements in otoliths (in the crystalline matrix, proteanous matrix, or both) can change with the species, but the incorporation process remains largely unknown even if the role of biomineralization has

gained more studies (Loewen et al., 2016; Thomas and Swearer, 2019; Hüssy et al., 2021). These specific accumulations in each species' otoliths have been linked to various environmental factors (e.g., chemical composition, salinity, and temperature) and fish physiology (e.g., age, growth, metabolism, and ontogeny) (Campana et al., 2000; Hüssy et al., 2021). Furthermore, differences in otolith chemistry may have a genetic basis (Clarke et al., 2011; Barnes et Gillanders, 2013) or may be related to phylogeny (Chang and Geffen, 2013). Melancon et al. (2009) revealed that fish otoliths from two species with radically different compositions can be precipitated from endolymph fluids of similar chemistries. Elements could be classified following their affinity with the protein component or the mineral crystalline matrix and thus could be used specifically to reconstruct or to be informative about both endogenous and exogenous processes (Sturrock et al., 2012; Izzo et al., 2015; Thomas et al., 2017). These results suggest different crystallization processes in these species or the presence of different proteins (and/or organic matrices) that selectively influence elemental incorporations in the otoliths and differ for each species. The incorporation of some elements is much more thoroughly documented. The incorporation of Ba and Sr into otoliths is now well known as being influenced by ambient concentrations, temperature and salinity (e.g., Elsdon and Gillanders, 2003; Walsh and Gillanders, 2018). However, Sr incorporation exhibits considerable interspecific differences (Brown and Severin, 2009) and is known to be influenced in some marine fish otoliths by ontogeny (Brown and Severin, 2009; Mc Donald et al. 2020). In our study, the analyses were conducted on narrow size classes of adult individuals and few elements show a significant relationship with otolith weight for both species (Li for Sardine, Li and Sc for Hake). The effect of otolith weight appeared to be significant for both species (PERMANOVA), but explained a weak part of the total variance when compared to the effect of sampling locations. Sampling was conducting during a one year period and seasonal variations in chemical elements incorporation to the otolith is well documented (Sturrock et al., 2014; Hüssy et al. 2020), but these variations are considered to have little effect on the whole otolith chemical composition (Elsdon & Gillanders, 2003; Campana, 2005; Elsdon et al., 2008). Therefore, the spatial patterns revealed from otolith chemical composition can be attributed prominently to the similarity or differences between sampling locations.

In our study, despite the differences between species, they both show a clear cut in the spatial variability and that the species separation can be defined by the differences in Ba, Li, Mg, Mn, Sc and Sr concentrations. The review of Chang and Geffen (2013) compared regional differences in otolith elemental concentrations from 12 species sampled in 16 locations across the Mediterranean, the Baltic, and the northeast Atlantic coasts. They found that five elements, namely, Ba, Li, Mg, Mn, and Sr were the most useful for species differentiation. The trace elements that drove species differentiation are known to characterize the elemental composition of the studied species. This was the case of the high Li values for sardines (Castro, 2007; Khemiri et al., 2014; Hampton et al., 2018). Furthermore, the elemental compositions of hake and sardine otoliths from Morocco are similar to those of the same species from the Atlantic Ocean (Swan et al., 2006; Castro, 2007; Morales-Nin et al., 2014; Correia et al., 2014).

4.1 Hake

The analyses of whole otoliths provide a tag that integrates chemical signatures across the entire life of the fish from the embryonic stages to capture. Thus the chemical signature, integrated over the fish's entire life, characterizes some groups of fish. These signatures remain distinct and identifiable even if the group moves as long as the new material accreted on the otolith remains minimal. So the signatures won't be identifiable if the fish move around a lot throughout their lives (Elsdon et al., 2008). In our study, on the scale of the Moroccan coast (3500Km), the sampled localities for hake split into two groups. The first group brings together locations at the north from Agadir, and the second group are the ones south of Tantan. Scandium, rubidium, and manganese played a major role in this grouping. The structuring of the hake populations in Moroccan waters remains unknown, nevertheless, otolith microchemistry studies conducted in European waters were consistent with the stock limits used for stock assessment purposes (Casey et Pereiro, 1995; Piñeiro and Sainza, 2003) and our study extends the knowledge towards the North African coast. Morales-nin et al. (2005) and Tomás et al. (2006) revealed different chemical signatures from Atlantic and Mediterranean hake. Swan et al. (2006) showed two groups in the European Atlantic that differed from the Mediterranean hake based on the concentrations of Ba, Sr, Mn, and Mg. Furthermore, Tanner et al. (2014) found that coupling genotype and otolith geochemistry for hake in European waters enhanced the

accuracy of population structure studies by complementing the assessment of the early life dispersal stage in populations.

The identified groups in our study split accordingly into the two main ecosystems of the Moroccan waters: the least productive temperate northern ecosystem and the highly productive arid ecosystem at south. A secondary differentiation between locations occurred within the two groups, involving mainly Mg and Mn, and might be related to the local environmental conditions.

4.2 Sardine

The sardine sampled locations were split into three groups at Safi and Tantan. The between groups separation is more noticeable between the southernmost group (Dakhla - Laayoune) and the two others groups which show overlapping confidence ellipses. This pattern may be primarily underpinned by genetic bases such as population identity studies conducted on Moroccan sardine using different genetic methods resulted in the identification of two populations overlapping in the Agadir–Tantan area (Chlaida et al., 2006, 2009; Atarhouch et al., 2006, 2007). Safi was denoted as the seat of a genetic drift for sardine. Our results are supported by other studies linking genetic and trace elements incorporation (Clarke et al., 2011; Barnes and Gillanders, 2013). The southernmost locations experience intense and permanent upwellings. These upwellings are located in the area between Cape Boujdour (26 °N) and Cape Blanc (20.3 °N), with a northward recirculation and mixing of upwelled waters in the Canary Islands-Morocco transition zone (Hernández-Guerra et al., 2017; Vélez-Belchí et al., 2017), where Laayoune is located. Upwelled waters are known to be enriched in trace elements (e.g., Ba, Cu, Cd), which can then be incorporated into calcified structures such as corals (e.g., Lea et al., 1989) and otoliths, as otolith chemistry is primarily the product of water chemistry (Campana, 1999; Bath et al., 2000; Walther and Thorrold, 2006). Previous studies linked the high barium concentrations in sardine otoliths to upwelling activity (Clarke et al., 2007; Kingsford et al., 2009; Lin et al., 2013; Woodson et al. 2013; Wheeler et al., 2016). The differentiation of the two other groups appears to be associated with lithium, and no information from the literature on chemical elements incorporation in the otolith could inspire an explanation.

4.3 Environmental Conditions

The spatial variability in the otolith elemental composition of the two species was significant and showed a clear geographic cut, thus identifying a northern and a southern group with different chemical signatures. The major split for the two species occurred in the same area between Agadir and Tantan, thereby corresponding to the transition zone between the northern temperate mud-shelf ecosystem and the arid ecosystem at south. The identity of these two ecosystems appears to be a major population structuring driver for the two species. The break area located around Sidi Ifni, between Cape Ghir and Cape Juby, which was pointed out as the southern limit of the Lusitanian Province (Briggs, 1995), is also known as the southern limit in the geographic distribution of different bivalve species such as *Litophaga litophaga* (Menioui, 1988) and *Ostrea edulis* (Ranson, 1967; Jaziri, 1990). This area acts as a boundary between different populations of *Mytillus galloprovincialis*, as well (Jaziri and Benazzou, 2002). This basin is bordered on the north by Cape Ghir and on the south by the Cape Juby-Lanzarote strait and exhibits specific geologic and oceanographic features. The Atlas Mountain chain, which is oriented NE-SW, acts as a barrier to southward-blowing winds north of Sidi Ifni, thereby leading to the dominance of trade winds (NE direction) (Barton et al., 1998). South of Cape Ghir, the coastline exhibits a concave shape with a narrow shelf around Sidi Ifni preceding a wide shelf extension until Cape Juby. All these features stimulate considerable temporal and spatial variability in the oceanographic conditions in this area (Van Camp et al., 1991; Sicre et al., 2001).

Upwelling of intense and seasonal activity with summer peaks takes place in this area (Barton et al., 1998; Benazzouz et al., 2014), and Cape Ghir displays high mesoscale and sub-mesoscale activity, including the generation of fronts, eddies, and filaments (Hagen et al., 1996; Hernández-Guerra and Nykjaer, 1997; Barton et al., 1998; Nieto et al., 2012; Anabalón et al., 2014). This region is also an important transition zone for the export of nutrients and plankton from the coast to the adjacent open ocean and the Canaries Archipelago (Mason et al., 2012). These complex oceanographic processes may act as a barrier to planktonic dispersion favouring isolation processes. Oceanographic processes such as upwelling systems, fronts, moving convergences, eddies, and counter currents are widely recognized as influences for the movement of species pelagic stages (Palumbi, 2004; Sponaugle et al., 2002) and may act as barriers to connectivity (Galarza et al., 2009;

Treml et al., 2008; White et al., 2010). This limit also matched with the limit of the spatial distribution of the biogeochemical provinces defined by Longhurst (2007) and Reygondeau et al. (2013) concerning the eastern subtropical North Atlantic gyre and the Canary Current Coastal provinces.

Moreover, the Sahara–Sahel Dust Corridor expelled huge amounts of mineral aerosols into the Atlantic which accounted for 36 %–79 % of the global total dust emission (Almeida- Silva et al., 2013; Wu et al., 2020). Moreno et al., 2006, indicate that potential sources of saharan dust (Hogar Massif) have various geochemical signatures notably in Li. The southern areas from our sampling are under this direct influence which may contribute to explain the different chemical signature of our samples.

Conclusions

In this study, the populations of two different fish species from the Atlantic coast of Morocco were identified and characterized using the chemical signature of the otoliths. Two groups of hake and three groups of sardines along the Moroccan coast were identified. Despite various biological, ecological, and behavioural characteristics, the two species showed a major population break in the same area corresponding at the transition zone between the northern temperate mud-shelf ecosystem and the arid ecosystem at south. Even through further research efforts are still needed to clarify the oceanographic processes involved in population isolations in this area, otolith chemistry of the whole otolith proves to be a powerful tool to distinguish fish populations and homogeneous environmental areas. Despite the possibility of intermixing, hake and sardine populations along the Moroccan coast show 2 different local populations, and should be regarded as different stocks for fisheries management purposes.

CRediT authorship contribution statement

Labonne Maylis: Methodology, Formal analysis, Data curation, Software, Writing - original draft; **Masski Hicham**: Funding acquisition; Project administration, Investigation, Formal analysis, Data curation, Software, Writing - original draft; **Talba Sophia**: Investigation, Formal analysis; **Tai Imane and Manchih Khalid**: Investigation;

Chfiri **Rachid**: Formal analysis; **Lae Raymond**: Funding acquisition, Project administration, Investigation, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Tables and figures

Table 1. Hake (Merluccius merluccius) and Sardine (Sardina pilchardus) samples number (Nb), locations from north to south, date of collection and size (total length (TL): mean, minimum, and maximum) in cm.

	Hake			Sardine		
Location	Month	Nb	TL: Mean (min-max)	Month	Nb	TL: Mean (min-max)
Assilah	Jun. 2012	15	42.5 (27 - 49)			
Mehdya	Jun. 2012	15	36.4 (27 - 48)	Jun. 2012	8	15.3 (15 - 17)
El Jadida	Jun. 2012	15	36.6 (27 - 46)	Jun. 2012	14	19.4 (17 - 21)
Safi	Jun. 2012	15	37.1 (27 - 48)	Jun. 2012	12	18.8 (17 - 20)
Essaouira				Jun. 2012	15	17 (16 - 19)
Agadir	Jun. 2012	10	36 (27 - 44)	Jun. 2012	11	17.2 (16 - 19)
Sidi Ifni				Nov. 2012	13	15.8 (15 - 17)
Tantan	Jul. 2013	12	42.3 (33 - 48)	Jul. 2013	15	18.3 (16 - 20)
Laayoune	Nov. 2012	15	37.1 (27 - 44)	Nov. 2012	15	18.4 (16 - 20)
Boujdor	Jul. 2013	7	38.7 (30 - 47)			
Dakhla	Jul. 2013	7	40 (34 - 49)	Nov. 2012	15	19.1 (18 - 21)
Total		111	37.4 (27 - 49)		118	17.8 (15 - 21)

Table 2. Chemical element concentration (mean (SD); µg element g-1 calcium) in otoliths of hake (*Merluccius merluccius*) and sardine (*Sardina pilchardus*). Means of items with the same letters ("a", "b") are not statistically different (Tukey test). Nb is the number of fish otoliths analyzed per species.

	Hake	Sardine
Nb	111	118
Ba/Ca	4.02 (0.91) a	3.49 (3.18) b
Li/Ca	0.94 (0.13) a	11.6 (5.1) b
Mg/Ca	68.61 (15.64) a	37.44 (13.03) b
Mn/Ca	5.57 (3.20) a	2.88 (1.15) b
Rb/Ca	0.56 (0.58) a	0.27 (0.16) b
Sc/Ca	0.65 (0.48) a	1.05 (0.35) b
Sr/Ca	3908 (295) a	1928 (423) b

Table 3. PERMANOVA analyses for hake (*Merluccius merluccius*) and sardine (*Sardina pilchardus*). Trace elements concentrations (Ba, Li, Mg, Mn, Rb, Sc, Sr) are the multiple dependent variable. The between locations differences appear to be the most important factor in the variation of the chemical composition of the otoliths of both species.

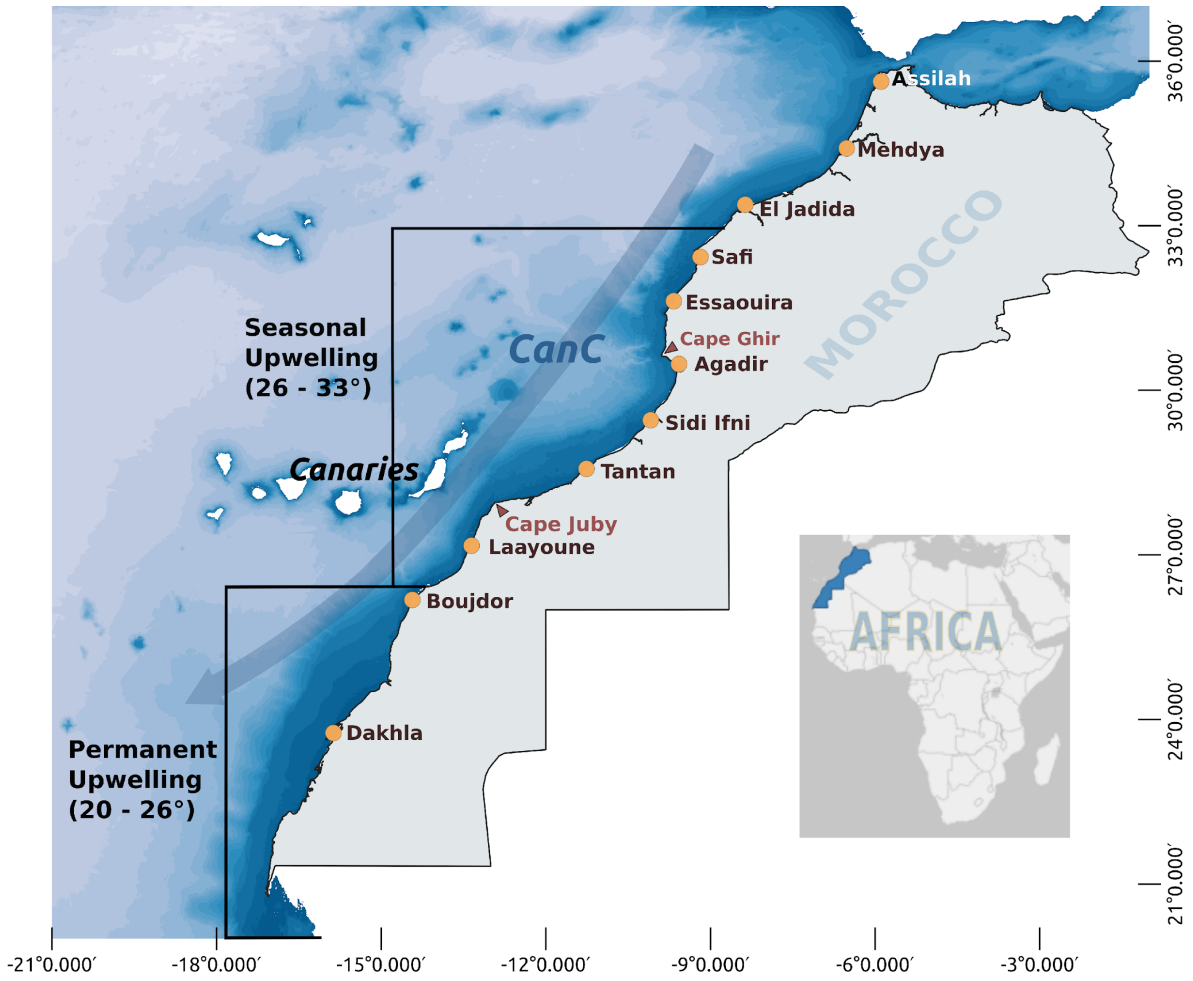|	Hake		Sardine	
	R^2	Pr(>F)	R^2	Pr(>F)
Location	0.50486	0.001 ***	0.61492	0.001 ***
Otolith weight	0.05751	0.001 ***	0.01035	0.046 *
Length	0.12860	0.064	0.00200	0.658
Sex	0.00293	0.455	0.00379	0.826
Maturity	0.01051	0.469	0.00649	0.813
Residual	0.29658		0.36246	

Figure 1. Sampled locations along the Moroccan Atlantic coast. Upweling areas of permanent and seasonal activity are shown. CanC: Canary Current.
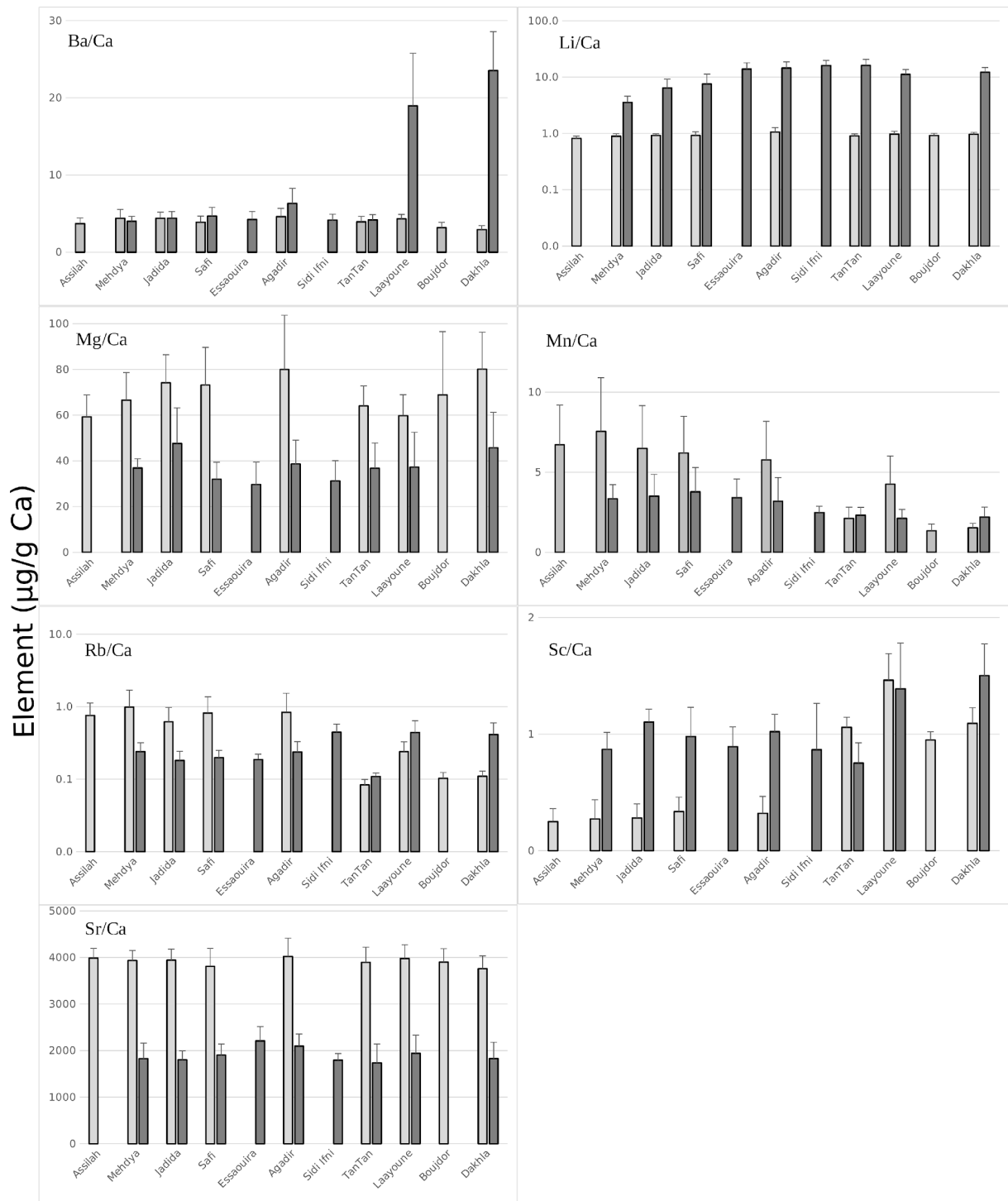
Figure 2. Concentrations (μg element/ g calcium) and standard deviation in otoliths for each species and locations. In dark grey sardine and light grey hake.
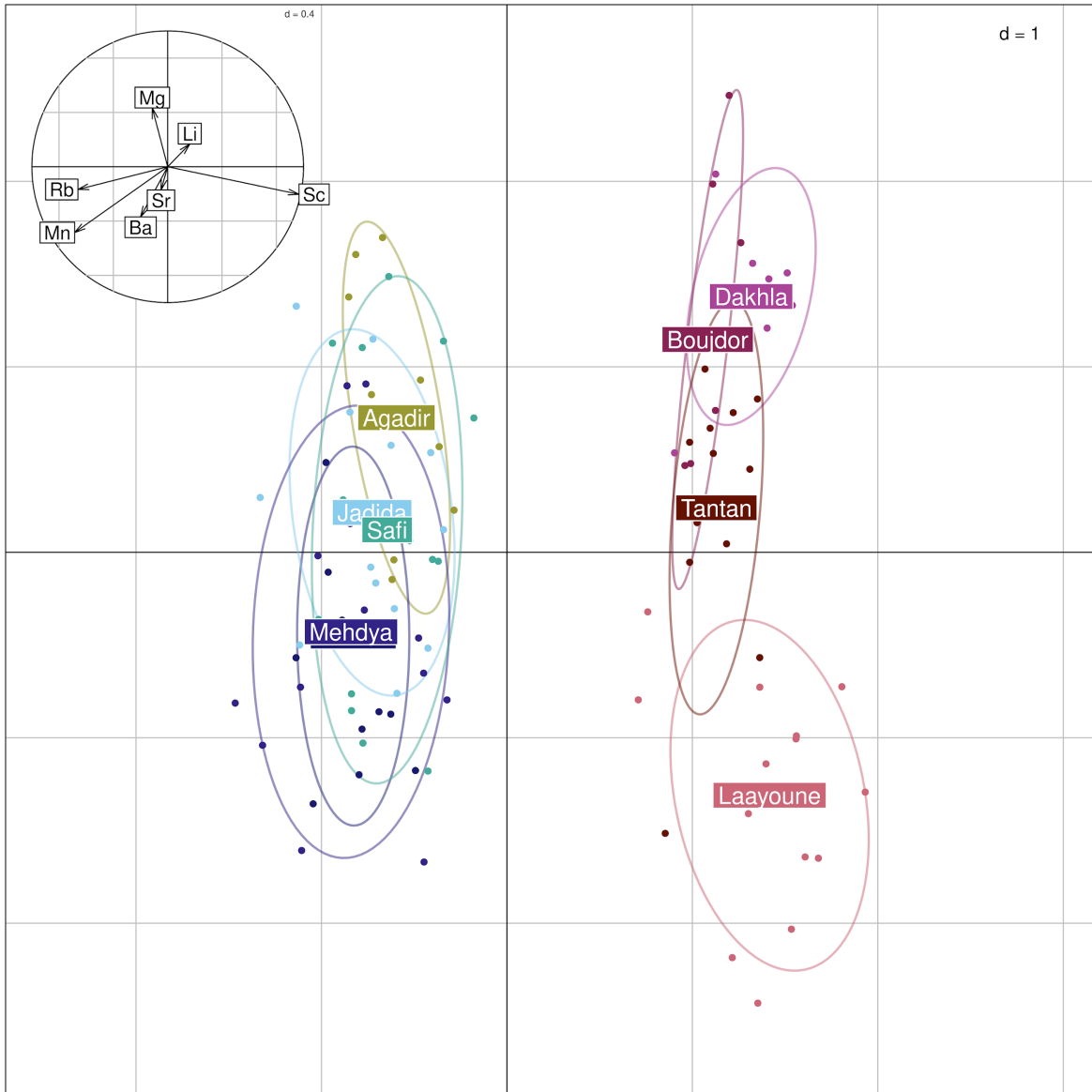
Figure 3. Plot of the first two discriminant functions for hake (*Merluccius merluccius*). The first discriminant factor separate a northern (Assilah - Agadir) and a southern group (Tantan - Dakhla) of sampled location. Each point is an individual fish.
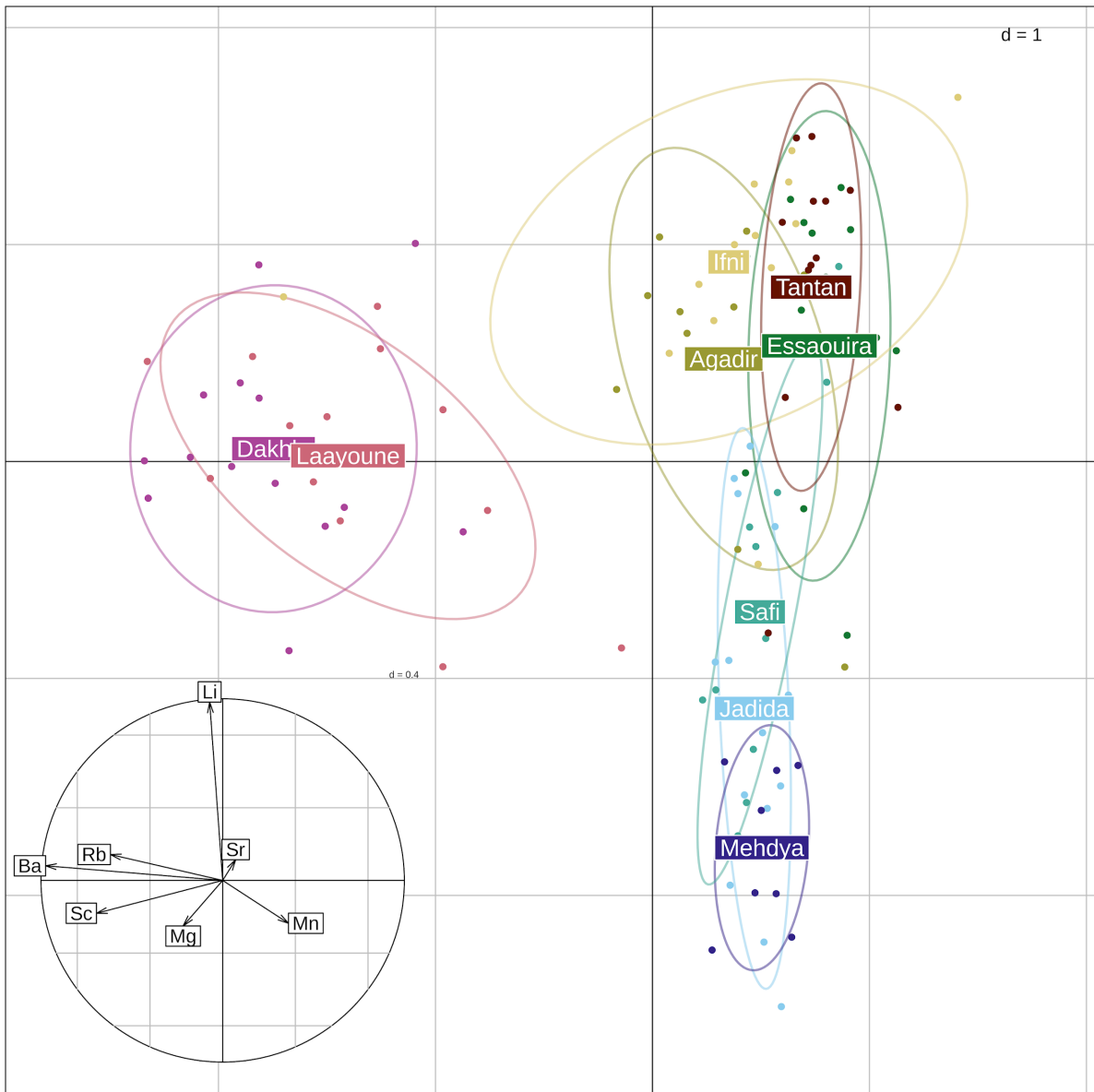
Figure 4. Plot of the first two discriminant functions for sardine (*Sardina pilchardus*). The first discriminant factor separate a northern (Mehdya - Tantan) and a southern group (Laayoune - Dakhla) of sampled location. Each point is an individual fish.