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Evaluation of Otolith Shape as an Approach for Stock Discrimination of *Sardina pilchardus* off the Moroccan Atlantic Coast

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ABSTRACT

The applicability of the otolith shape analysis of the European sardine, *Sardina pilchardus* was investigated to help in stock discrimination. Discriminant analysis was achieved by an otolith-based morphometrics assignment of otolith shape; 360 specimens of *S. pilchardus* were collected from three localities (Larache, Safi and Dakhla). Otolith shapes were described from otolith measurements and elliptic Fourier descriptors. This analysis showed the presence of three different morphotypes in the studied locations. The existence of three morphotypes is probably related to the variation of oceanographic conditions impacting the feeding regime and fish growth. This study proved that otolith shape analysis could become an accurate marker for *S. pilchardus* population discrimination.

INTRODUCTION

The European sardine is the first species fished in Morocco, with total landings exceeding one million tons per year (**Mounir *et al.*, 2019**). Among the four sardine stocks previously defined by morphometric and meristic studies along the Atlantic coast (**Andreu, 1969; Parrish *et al.*, 1989; Mounir *et al.*, 2022a**), the two most southerly stocks are found off Morocco, the Atlantic western Sahara and extend to northern Senegal (**Mounir *et al.*, 2019**).

A suite of analytical techniques have been used for stock discrimination, ranging from morphometrics and meristic researches (**Mounir *et al.*, 2022a**). However, the absence of invariable and significant phenotypic differences over a large geographic area prevented consensus on the sardine population structure in its range. Only the studies of **Andreu (1969)** and **Parrish (1989)** have been widely accepted and have defined four sardine stocks along the Atlantic: A Saharian stock, from Cape Juby to Levrier Bay in Mauritania, a septentrional Atlantic stock from the North Sea to the norther Iberian Peninsula, a Moroccan stock, from Cap Spartel to Cap Juby, and an Iberian stock, from north of the Iberian Peninsula to the Straits of Gibraltar. This latter stock, located at the southern limit of the distribution area of *S. pilchardus,* is believed to be partly at the origin of the catches of this species in Mauritania and Senegal (**Fréon, 1988; Belvèze & Erzini, 1983**). The Mediterranean stock, from the Gibraltar Strait to the Adriatic, was then identified on the same criteria (**Lee, 1962**).

The usefulness of otolith is a good method to identify fish stocks (**Vieira *et al.*, 2014; Jemaa *et al.*, 2015; Neves *et al.*, 2021; Mounir *et al.*, 2022b**). They are particularly suitable for this type of study because they have two fundamental properties (**Panfili *et al.*, 2002**): they are metabolically inert, and they grow continuously from birth and throughout the life cycle by integrating variations in growth. The otolith is known by high morphological variability which has proved to be an effective tool to discriminate between fish stocks and local populations (**Stransky *et al.*, 2008; Mounir *et al.*, 2022**). The otolith's shape is species-specific, it shows also intraspecific variations (**Torres *et al.*, 2000; Rawat *et al.*, 2017**). These can be explained by the combination of genetics and environment effects; the same fish species with different life histories generally present the morphology variations in otolith (**Vignon & Morat, 2010**). According to **Jemaa *et al.* (2015)**, the otolith studies of the European sardine sampled in the Atlantic and Mediterranean waters showed significant geographical variation.

The physiological disorders or environmental stress induced some changes in fish otolith (**Morales-Nin, 1987**), they caused the morphological anomalies such as the fluctuating asymmetry (**Tuset *et al.*, 2018; Yedier *et al.*, 2018**), affecting the data provided in otolith studies. Therefore, the fluctuating asymmetry (FA) in morphometric and meristic characters paired is related to the environmental disturbances (**Seixas *et al.*, 2016**), and it has been reported for many fish species of both round and flat fish (**Kontaş *et al.*, 2018; Yedier *et al.*, 2018**).

The aim of this study was to compare the morphometric of otoliths' shapes of *S. pilchardus* collected from different Moroccan Atlantic locations to detect the existence of morphotypes or phenotypic stocks of 360 otoliths, collected from three geographically different localities.

MATERIALS AND METHODS

1. Otolith Sampling

Specimens were collected from three different areas with geographical distance between them, and the oceanographic proprieties of the areas have been reported by the Food and Agriculture Organization (**FAO, 2003**) (Fig. 1) during the period between 2017 & 2018 (Table 1). All samples were placed in a specific condition, and each individual was wrapped in plastic film and stored in the freezer at -20°C.

Sagittal otoliths were carefully extracted using fine forceps to avoid breakage, and only the otoliths with no damage were used. Thus, forty otoliths that presented crystallization anomalies were excluded from the analysis.

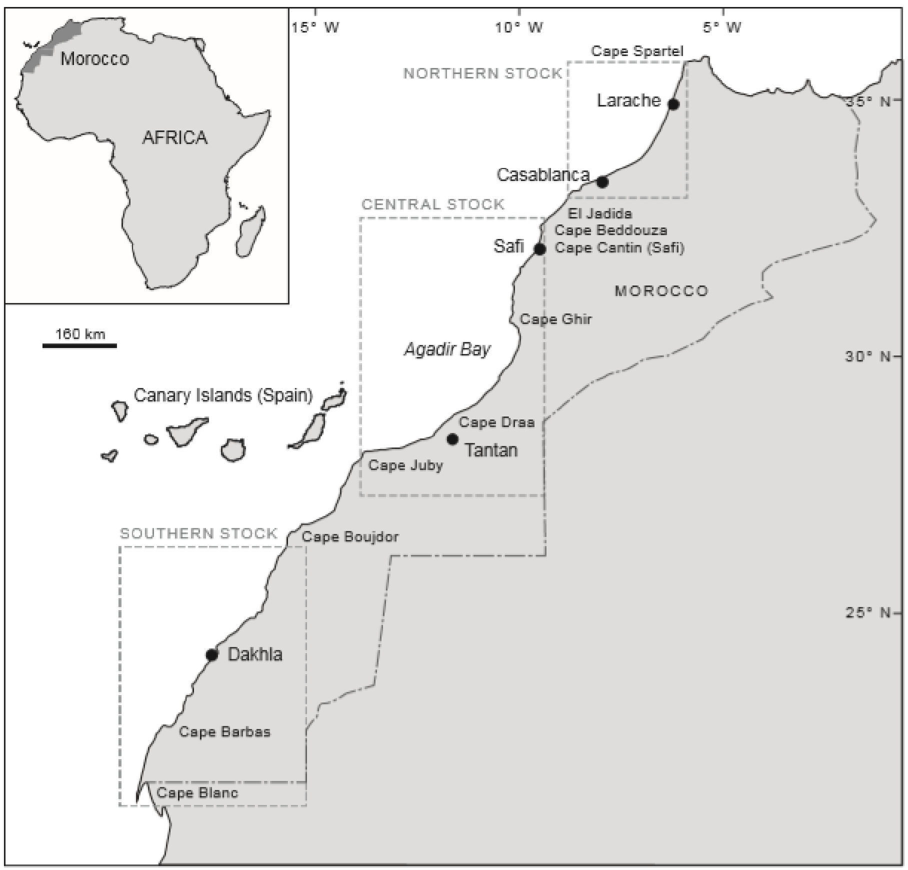

Fig. 1. Sampling areas and delimitation of areas of three stocks of Atlantic Moroccan *S. pilchardus* (points: sites of sampling, dashed boxes: areas of three stocks) (**Mounir *et al.*, 2019**).

Table 1. Summary information about *S. pilchardus* samples

Area	Code	Mean TL (cm) ± SD min–max*	n samples
Larrache	SL	16.55±1.57 (13.4-17.8)	120
Safi	SS	18.52±0.44 (15.9-21)	120
Dakhla	SDk	23.47±0.87 (19-24.9)	120

*TL: Total length; SD: Standard deviation; n: Number of samples per locality, TL: Total length.

2. Otoliths shape analysis

Otolith size was measured by taking the images under stereomicroscope attached to a video camera (Motic image), and the software ToupView (3.7) was used to measure the otolith's width and length. The distance between the midpoint of the rostrum and the posterior edge (A, B) presents the otolith's length, while the otolith's width stands for the length passing on the primordium (C, D) (Fig. 2) (**Javor *et al.*, 2011**).

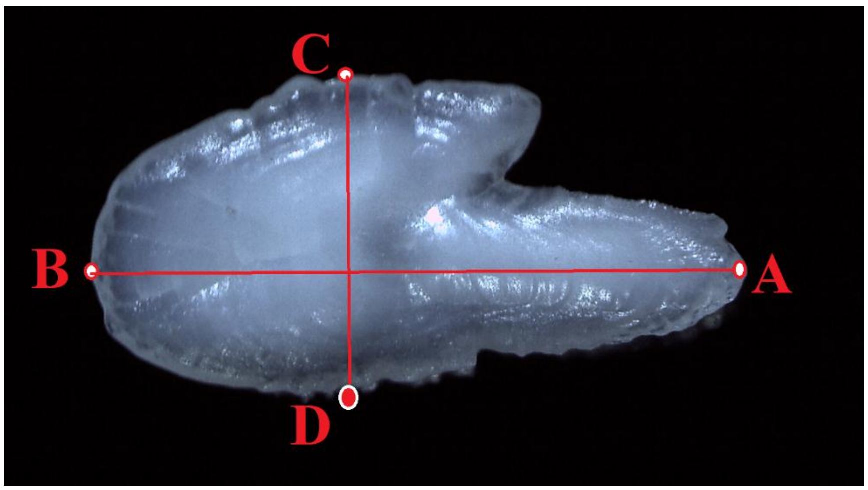

Fig. 2. Otolith of *S. pilchardus*, with distance between points A and B showing otolith's length, and distance between points C and D indicating otolith's width

To describe otolith contour, we have followed the elliptic fourier (**Kuhl & Giardina, 1982; Lestrel, 2008**). To determine the number of elliptic fourier harmonics that is sufficient and necessary for analysis, the SHAPE v1.3 was used to calculate the fourier coefficients (**Lord *et al.*, 2012; Bonhomme *et al.*, 2014**).

3. Data analyses

Relationships between total length (Lt) and otolith length (LO) and otolith width (WO) were studied by the equation: $L_t=a(L)^b$; where, a b are constant coefficients, and L is otolith's length or width. Regression method was used for determining the relation between Lt, LO and WO. Significant differences between area were tested by analysis of covariance (ANCOVA).

Elliptic fourier analysis was performed using the 20 contour coordinates. Multivariate analysis of variance (MANOVA) was used to evaluate the significant differences between contours from different localities. To visualize the differences in shell outlines, the principal component analysis (PCA) was applied to the elliptical fourier analysis (EFA) coefficients. Discriminant function analysis (DFA) was used to assess the discrimination between the three locations, based on otolith's analysis. All the contour analyses were made via the software PAST 3.23 (**Hammer *et al.*, 2001**). The tpsRelw 1.70 (**Rohlf, 2017**) was used to reveal the differences between mean shell contour lines of specimens from the three localities.

RESULTS

1. Otoliths shape analysis

Otolith parameters and total fish length of the sardine were shown in Table (3). Using the allometric transformation method, all otolith variables were free from the influence of body size. The regression equation and coefficients of determination (r^2) for linear (LR) relationship among otolith morphometric variables, between total length and otolith length and width (TL vs. LO and TL vs. WO) in the three locations; Larache, Safi and Dakhla are given in Table (4).

Table 3. Total length, otolith's length and width (cm) of *S. pilchardus* from the Moroccan coast

Location	Total fish length Mean ± SD*	Otolith length Mean ± SD*	Otolith width Mean ± SD*
Larache	16.55±1.57	0.25±0.029	0.11±0.011
Safi	18.52±0.44	0.34±0.024	0.15±0.010
Dakhla	**23.47±0.87**	**0.58±0.028**	**0.19±0.035**

*SD: Standard Deviation

Table 4. Relationship between the total length and maximal otolith length (TL-LO), total length and otolith width (TL-WO), and coefficients of determination (r^2) for *S. pilchardus* from the Moroccan coast

Location	TL-LO		TL-WO	
	Equation	r^2	Equation	r^2
Larache	LO= 0.019TL- 0.267	0.801	WO=0.07TL+0.144	0.711
Safi	LO= 0.031TL-2.247	0.881	WO=0.011TL- 0. 488	0.704
Dakhla	LO= 0.018TL+2.224	0.827	WO=0.023TL-2.631	0.851

The relationships are highly correlated for the three *S. pilchardus* populations. Similar to morphometric characters, the otolith variables revealed a high significant difference among the three locations (MANOVA; Wilks' Lambda $P < 0.005$, and $\lambda < 0.50$) (Table 5).

Table 5. MANOVA test and Wilks´ λ between stocks based on otolith's shape

Area	Wilks' λ	*P*- value
Larache-Safi	< 0.001	0.0001
Safi-Dakhla	< 0.001	0.0001
Larache-Dakhla	< 0.001	0.0001

The first 20 harmonics ensure 99% of the contribution. The principal component values were calculated using the fourier coefficients for the first 20 harmonics, and the two principal components (PCs) account for 68% of the total variance (43% for PC_1, 25% for PC_2). The discriminant function showed clear patterns of otolith shape trait variations, forming three distinct populations that are well separated from each other (Fig. 3). Larache, Safi, and Dakhla samples classified to their original groups were 100%, demonstrating clear separation of these stocks from each other.

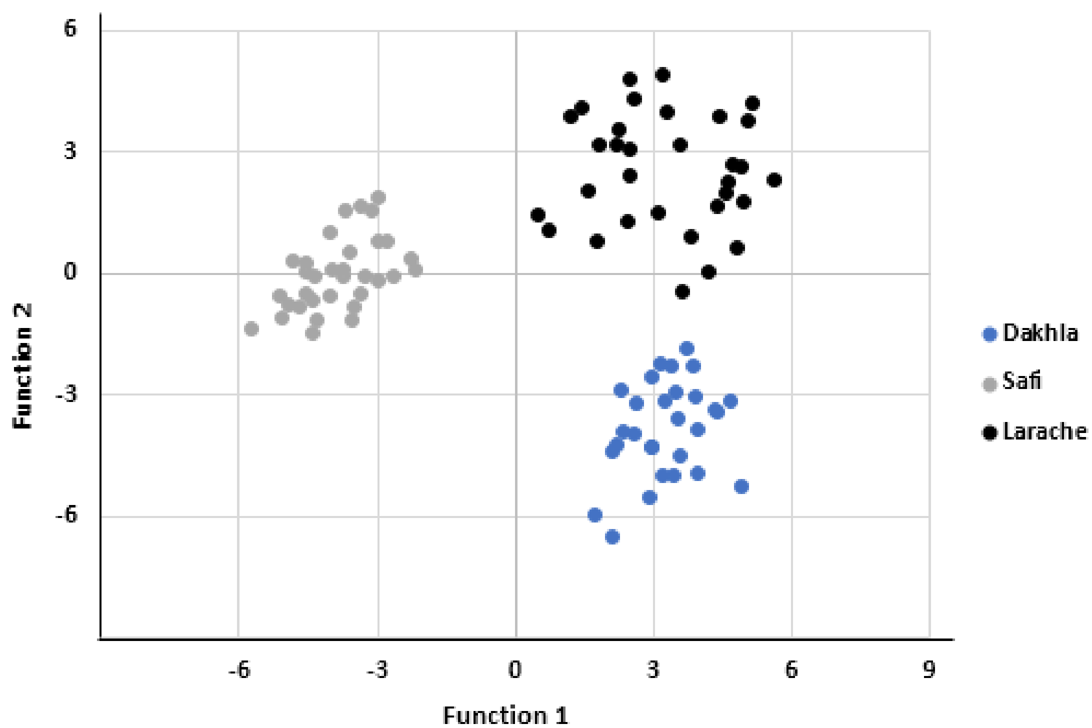

Fig. 3. Discriminant function analysis of *S. pilchardus* populations based on the otolith characters

DISCUSSION

Otolith characters were widely used as a good tool for stock discrimination in fisheries management (**Ihssen *et al*., 1981; Vieira *et al.*, 2014; Neves *et al.*, 2021; Mounir *et al.*, 2022b**). That is why this work focused on the study of the spatial variability of morphometric and otolith shape of *S. pilchardus*, with different ecological characteristics (size, swimming speed, behavior in the water column, etc.) and biogeographical environments along the Moroccan Atlantic coast.

The present work showed a positive correlation between fish length, otolith length and width in all different studied areas. Similar results were reported in the study of **Zorica *et al.* (2010)** for *S. pilchardus* from Adriatic Sea. In fact, different allometric growth of the otolith of sardines from different regions could be explained by the habit, physiochemical environment and food availability (**Javor *et al.*, 2011**). While, other factors could produce a shift in the body size and otolith size relationship including sex composition or large change in age or changes in regulations (**Ma *et al.*, 2010**).

The differences in shapes of otoliths between stocks may be related to environmental conditions and genetic differentiations (**Galley *et al.*, 2006; Doering-Arjes *et al.*, 2008**). Southern stock located in the central canary system is characterized by the presence of upwelling activity almost throughout the whole year, compared to the central and northern stocks. **Vignon (2015)** found that, local environmental conditions provoke an important variation in otolith's shape; the variation in the otolith shape shows that sardines, during their life cycle, partially use different geographic regions. In fact, individuals of the same species living in similar environmental conditions have the same form of otoliths, which explains the difference of groups of the three sites of Atlantic Morocco cost, where the *S. pilchardus* migrate and live with difference in environmental conditions.

Elliptical fourier analysis showed significant differences in the otolith shapes for the individuals of the three studied sites. These results confirm that, the variation in the otoliths shape of *S. pilchardus* is associated with the species' different geographic regions. This is largely in line with the results reported by other studies using the same analysis in the discrimination of stocks and have obtained satisfactory results, in particular, the study on the discrimination of the fish populations from Atlantic and Mediterranean Sea (**Messaoud *et al.*, 2011; Jemaa *et al.*, 2015b; *Neves et al.*, 2021**) by analyzing the forms of the otolith. The current study confirms the results of morphometric studies carried out by **Mounir *et al.* (2019)** on *S. pilchardus* stocks. The discriminant factor analysis also made it possible to separate 3 well-individualized stocks, with each stock belonging to a site. This difference would result on the one hand from differences in environmental and trophic conditions of the different living environments of individuals (**El Mghazli *et al.*, 2020**), and on the other hand, from the migration made by the *S. pilchardus* throughout the year. It is accepted that, the ethology of the sardine is governed by its environment, yet it is still difficult to understand precise determinism of its migrations and to recognize which (s) is (are) the parameter (s) that most condition (s) its behavior. In literature, primacy often goes either trophic causation or looking for a thermal preference. **Binet (1991)** found that movement of sardines in the zone 21-26 °N are synchronous with the planktonic maxima. **Nehring and Holzlohner (1982)** observed that between 21 and 25°N, sardine concentrations are always higher in areas rich in chlorophyll. Variations daily chlorophyll concentrations result in variations in catches per unit effort. **Belvèze and Erzini (1983)** and **Belvèze (1984)** think, however, that migration summer sardines from the Sidi Ifni-cap Juby area around the north are conditioned by a trophic attraction.

In conclusion, the results showed that otolith shapes analysis is a useful tool to discriminate *S. pilchardus* stocks, both locally and on a large scale, and they clearly indicate that the Moroccan Atlantic coast sardines are separated into three stocks, northern (Larache), central (Safi) and southern (Dakhla). The genetic distinctiveness of the sardine of this population might have enhanced the effects of the collapse of the Safi sardine stock (**Atarhouch *et al.*, 2006**). The differentiation of the three morphotypes obtained in this study is in coincidence with the data of **Chlaida *et al*. (2006)**, which showed that the population of Larache is genetically distinct from populations distributed to the south, and with the Safi population also differing from that of Dakhla. The elliptic fourier analysis is an efficient method in discriminating and detecting variations in the shapes of otolith of *S. pilchardus* within and between the Moroccan Atlantic coast fish stocks.

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