Iranian Journal of Fisheries Sciences 20(6) 1757-1774 2021
DOI: 10.22092/ijfs.2021.125491

Research Article

Inter-population morphological variability of the round sardinella (*Sardinella aurita* Valenciennes, 1847) in the Algerian Coast based on body morphometric, meristic and otolith shape

Ferhani Kh.[1, 2*]; Bekrattou D.[1]; Mouffok S.[1]

Received: March 2021 Accepted: October 2021

Abstract

Several studies on the round sardinella, *Sardinella aurita* (Valenciennes, 1847) have focused on the identification of stock composition and boundaries, using one method of discrimination. In this study, three approaches (body morphometric, meristic character (vertebrae number) and otolith shape) were applied on the population of round sardinella along the Algerian Coast in the Mediterranean Sea in order to obtain useful information for the management of this resource. 627 specimens were collected from nine locations from December 2018 to January 2019. The linear discriminant analysis (LDA) applied on both body morphometric characters and the otolith shape which indicated significant difference between areas with a low rate of classification success (47% for body morphometric analysis and 37.5% for otolith shape analysis), the misclassification percentage for each sampling location was explained by the proximity of the sites. Grouping samples into three regions according the Algerian coastline division reduced the misclassification rate; the overall random assignment of individuals into their original sample obtained by LDA was 70% for the morphometric characters, and 55% for the otolith shape. However, no significant difference was found between areas using the analysis of the number of vertebrae. No significant sexual effects were observed on the body morphometric characters, on the otolith shape and on the number of vertebrae.

Keywords: Round sardinella, Stock identification, Body morphometry, Meristic characters, Otolith shape, Algerian Coast

1- LRSE/Department of Biology, Faculty of Natural Sciences and Life, University of Oran 1, Ahmed BENBELLA, Oran, 31000, Algeria
2 -National Center for Research and Development of Fisheries and Aquaculture (CNRDPA), Bou-Ismail, Tipaza, 42415, Algeria
*Corresponding author's Email: ferhanikhadra@gmail.com

[DOR: 20.1001.1.15622916.2021.20.6.11.0]

Introduction

The small pelagic fishes constitute the bulk of the landings in Algeria, to implement a fishery management strategy of this resource, it is important to identify the management unit or "stock". The lack of knowledge about the population's structure complicates and reduces the effectiveness of fisheries management plans. Round sardinella (*Sardinella aurita* Valenciennes, 1847) is a small pelagic fish which has great commercial and economic importance in Algeria, it represents around 20% of landings after sardine (*Sardina pilchardus*) in 2018 (DGPA, 2019). This fish is a species of ray-finned fish in the genus *Sardinella* in the family of Clupeidae, widely distributed in the Atlantic Ocean and the Mediterranean Sea (Munroe *et al.*, 2015). It is a coastal, pelagic, species preferring clear saline waters, usually with maximum temperature below 24°C (Bianchi *et al*., 1999), its population size, and structure are strongly tied to environmental conditions (Binet and Servain, 1993).

For the sustainable management of this resource, the identification of stocks is necessary. According to Welch *et al*. (2015), the integration of different approaches must be used in stock identification studies because the results of each method used, maximize the likelihood of correctly defining stocks. In addition, the context of multidisciplinary, the use of results from various approaches such as morphometric characters, meristic and otolith shape provide information on groups of individuals with similar reproductive rates, growth, and mortality (Booke, 1981; Cadrin, 2014).

Body morphometry is one of the methods used in the stock identification in order to assess variation on phenotypic characters. Phenotypic stock definition or morphological traits is less conservative than the genetic stock definition, because it permits for more mixing of genetic material among stocks, but partial isolation is enough that geographic differences persist (Cadrin, 2014). Meristic characters analysis (e.g., fin rays, gill rakers, vertebrae…) is another approach in discrimination studies, count data are discrete, thus facilitating statistical analysis, meristic characters are partially determined by genetics and partially by environmental conditions early in development stage (Swain *et al*., 2005). Many papers were published on stock discrimination of small pelagic fishes in the Mediterranean Sea and Atlantic Ocean, while morphometric and meristic characters have been most frequently used in identifying and classifying stocks (Arrignon, 1966; Quignard *et al*., 1973, Djabali *et al*., 1990; Silva, 2003; Bouaziz, 2007; Kristoffersen *et al.*, 2008; Erdoğan *et al*., 2009; Traina *et al*., 2011; Karahan *et al*., 2014; Geladakis *et al*., 2018).

The otolith shape analysis is an often-used method for stock discrimination of fish. It is a cheaper and an efficient method to differentiate fish stocks or populations (Stransky *et al*., 2008; Cañás *et al.*, 2012; Benzinou *et al.*, 2013; Libungan and Pálsson, 2015). The otoliths continue to grow during the fish

[DOR: 20.1001.1.15622916.2021.20.6.11.0]

life and their shapes are highly dependent on age, sex, heritage and environment, because of this, otolith shape analysis is an important tool in stock discrimination (Khemiri *et al*., 2014; Karahan *et al*., 2014; Bacha *et al*., 2014; Mille, 2015; Jemaa *et al*., 2015; Bacha *et al*., 2016; Ider *et al*., 2017).

Unlike the other two small pelagic fishes (*Sardina pilchardus*, and *Engraulis encrasicolus*), the discrimination of the stock of round sardinella in the south-west of the Mediterranean Sea has not been widely studied. A few studies are conducted using mitochondrial DNA (Chikhi *et al*., 1997), number of vertebrae (Bouaziz, 2007), amino acid composition (Riveiro *et al*., 2011), scale shape (Bräger *et al*., 2016), and helminth parasites (Feki *et al*., 2016).

The aim of this paper is to examine the differences among local populations of round sardinella sampled from different locations in the Algerian basin based on their body morphometric, meristic character (vertebrae) and otolith shape in order to contribute to the understanding of round sardinella stock structure.

Material and methods

Study area and sampling

Round sardinella was sampled from the Algerian waters at the southwestern Mediterranean Sea between 35° 05′ N - 02° 12′ W and 36° 56′ N - 08° 38′ E. Algerian coastline is divided into three regions: west, center and east (Fig. 1), samples were collected from the western region at 4 locations (TLM, ANT, ORA and MOS), from the central region at one location (TPZ) and from the eastern region in 4 locations (BEJ, JIJ, SKK and ETF).

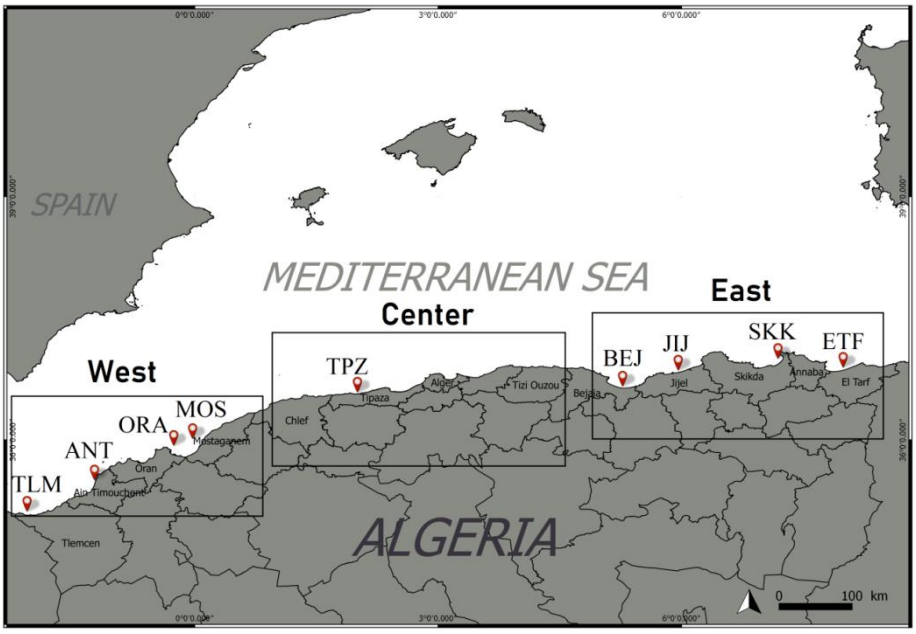

Figure 1: Sampling locations of round sardinella. The rectangles indicate the sample groupings into three regions.

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Samples were caught with a pelagic trawl during two months (December 2018 and January 2019) with R/V Belkacem Grine. At each site, surface water temperature and salinity were measured continuously using a thermosalinograph (Model SBE 21) and the salinity-temperature profile is determined by a CTD (SBE 25). The sampling was done during the same months to avoid the seasonal effect on the result if exists. A total of 627 specimens were collected (Table 1), the left side of each fish was photographed in fresh with a ruler to obtain scaling information by using a digital camera, then was put individually into plastic bags and were kept frozen (-20°C) until transportation to the laboratory. In the laboratory, the sagittal otoliths and the vertebral columns were extracted; sex was determined by macroscopic examination of the gonads whenever possible.

Table 1: Summarized information on sampling locations of round sardinella samples.

Region	*Sampling Area*	*Area Code*	*Date*	*Depth m*	*Surf T°c*	*Surf S‰*	*Depth T°c*	*Depth S‰*	*Sample size*	*Range of TL (cm)*	*Mean TL (cm)*
West	Tlemcen	TLM	20/12/2018	37.5	16.82	36.52	16.43	36.83	49	10.9-20.9	13.5±0.50
	Ain Temouchent	ANT	21/12/2018	37.7	16.75	36.56	16.58	36.57	87	10.8-21.7	15.5±0.51
	Oran	ORA	23/12/2018	79.5	16.77	36.51	16.44	37.02	53	12.5-15.3	13.9±0.19
	Mostaganem	MOS	24/12/2018	69.5	16.63	36.46	16.26	37.29	67	9.8 - 18.5	13.7±0.31
Center	Tipaza	TPZ	26/12/2018	38	16.83	36.57	16.63	36.62	72	10.2-22.1	12.9±0.21
East	Bejaia	BEJ	17/01/2019	40	15.64	36.78	15.58	36.87	91	8.9 - 15.3	12.2±0.33
	Jijel	JIJ	18/01/2019	41	15.47	36.58	15 .60	36.84	64	9.8-18.8	15.8±0.28
	Skikda	SKK	20/01/2019	55.8	14.60	36.47	15.82	36.91	51	13.2-18.2	15.1±0.35
	Eltarf	ETF	21/01/2019	37	15.34	36.84	15.75	36.93	93	10.3-17.5	15.3±0.24

Body morphometry

Ten landmarks were defined to create a set of body lengths forming a "Truss Network" (Strauss and Bookstein, 1982), plus the horizontal eye diameter (Fig. 2). All morphometric measurements were recalculated and standardized to eliminate body size effect following the method of Thorpe (1975) and Lleonart *et al*. (2000) for each fish as:

$$\bar{Y}_i = Y_i \ (X_0/X_i)^b$$

Where; $\bar{Y}_i$ is the size-adjusted measurement, Y_i is the original morphometric measurement, X_0 is the mean of total length for all fish from all samples, X_i is the total length of fish and the parameter *b* is the slope of the regression of log Y_i and log X_i using all specimens.

To test the effects of sex and area, a MANOVA was performed with all samples of round sardinella. A linear discriminant analysis (LDA) was applied to discriminate groups and to

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predict the classification of individuals. The interpretation of the results of the discriminant analysis was done through the Wilks' lambda test. It indicates the discrimination power and it varies from zero to one: Lambda approaches to zero if the groups are well separated, and to one, if the groups are confused when it is difficult to discern individuals belonging to different classes (Merigot *et al*., 2007). For further investigations of the relationships between groups, a hierarchical analysis (HCA) was conducted on the average of morphometrics data using Ward's hierarchical algorithm based on Euclidean distances.

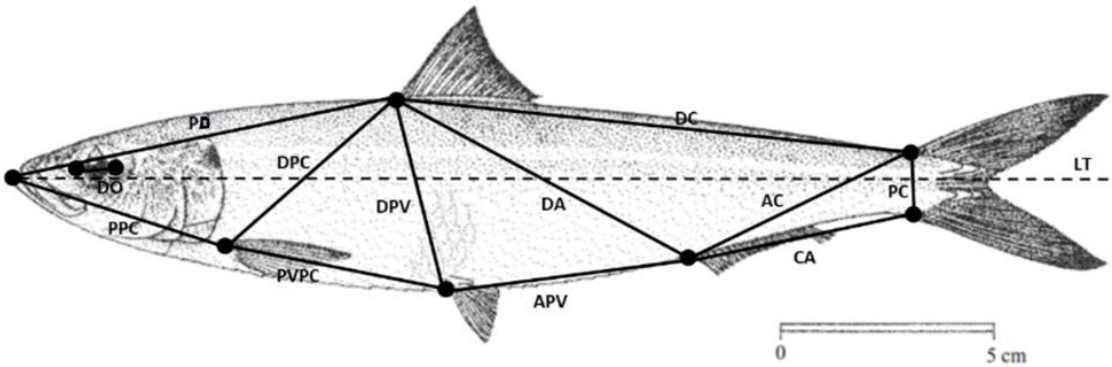

Figure 2: Measurements taken on round sardinella (PD: distance from the end of the muzzle to the beginning of the dorsal fin; DC: distance from the beginning of the dorsal fin to beginning of the caudal fin; PC: between the two insertions of the caudal fin; CA: from the caudal fin to the beginning of the anal fin; APV: distance from the beginning of the anal fin to beginning of the pelvic fin; PVPC: distance from the beginning of the pelvic fin to beginning of the pectoral fin; PPC: from the beginning of the pectoral fin to the tip of the mouth; DPC: distance from the beginning of the dorsal fin to beginning of the pectoral fin, DPV: distance from the beginning of the dorsal fin to beginning of the pelvic fin; DA: distance from the beginning of the dorsal fin to beginning of the anal fin; AC: distance from the beginning of the anal fin to beginning of the caudal fin; DO: eye diameter; LT: total length).

Meristic character

The number of vertebrae was selected for the meristic characters analysis, this character allows a rapid and precise count compared to other meristic characters such as gill rakers (Chase, 2014). Differences in number of vertebrae were tested using Kruskal-Wallis test because data were not normally distributed.

Otolith shape

Sagittal otolith images were captured using a digital camera linked to an Optika loupe stereo. Each otolith (left and right) was positioned with the sulcus acusticus facing down and the rostrum to the left, and photographed under reflected light. Only the right otolith was used in this analysis, however, when the right sagitta was damaged, a mirror image of left otolith was used. Overall, 353 right otoliths, and 167 left otoliths were used in this study.

Otolith shape analysis was based on the Elliptic Fourier Analysis (EFA) (Lestrel, 1997). Using the TNPC software (Digital processing for

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calcified structures, version 7.1), otolith contour (Fig. 3), was detected and the first 99 normalized elliptical Fourier harmonics were extracted with respect to the first harmonic, the resulting Fourier descriptors are invariant with rotation and starting point on the contour (Kuhl and Giardina, 1982). Each harmonic is composed of four (A, B, C and D) coefficients per individual. To select the minimum number of harmonics required to reconstruct the otolith contour, the Fourier Power (PF) was calculated using the formula described in Crampton (1995) and the level of 99.99% accumulated variance was adopted. However, the coefficients A, B, C, and D deriving from the first harmonic were not taken into account because the silhouette of this first harmonic is an ellipse with almost no information on the contour shape.

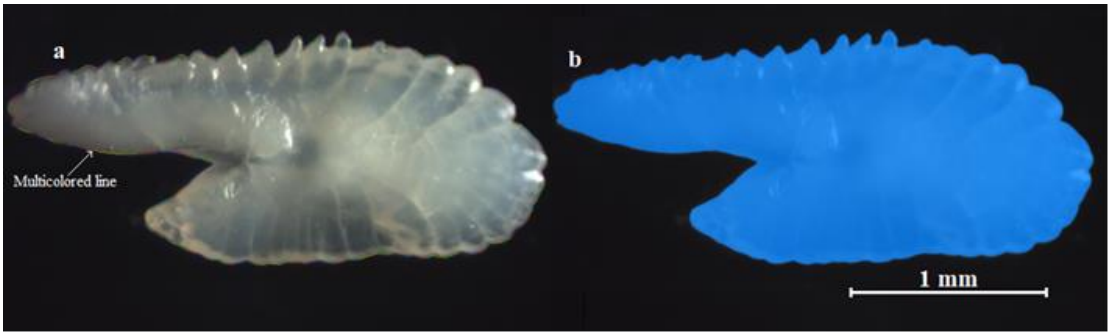

Figure 3: Images of right sagittae from round sardinella (*S. aurita*), a: otolith with the multicolored line representing the contour, b: binarized image of the otolith using TNPC.

Preliminary, a principal component analysis (PCA) was applied on the selected elliptical Fourier descriptors (EFDs) (Rohlf and Archie, 1984); the broken stick model (Karlis *et al*., 2003) was established for decreasing the number of variables. The size effect was statistically removed by using residuals from a PCA in order to ensure an unbiased comparison between groups. Next, the influence of sex, side, and area were tested using the Redundancy analyses (RDA) combined to permutation tests. Again, the LDA was conducted to determine differences between sites and Wilks' lambda was calculated, smaller values of Wilks' lambda indicate greater discrimination. Finally, HCA analysis according to Ward's hierarchical algorithm based on Euclidean distances was performed on the average of the selected normalized Fourier harmonics.

All statistical analyses were performed using the MASS (Ripley *et al*., 2013), ade4 (Dray and Dufour, 2007), vegan (Oksanen *et al*., 2013) and ggplot2 (Wickham, 2016) packages in the statistical environment R version 3.6.1 (R Development Core Team, 2013. http://www.R-project.org).

Results

Total length of specimens collected range from 8.9 to 22.1 cm, the smallest average total length was recorded in Bejaia (12.2±0.33 cm) and the largest in Jijel (15.8±0.28 cm).

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Body morphometry

The MANOVA test does not show any significant difference between males and females (504 specimens) for 12 morphometric distances (Wilks'λ=0.98, p>0.05), unlike, between areas, significant difference was detected (p<0.05), the significance of this variation was proved by Wilks'λ criterion (Wilks'λ=0.23, p <0.05).

The two first components of the discriminant analysis explained 52.6% and 21.3% of variance respectively, the plot obtained with LD1 and LD2 showed that samples are considerably overlapped (Fig. 4a). The overall assignment of individuals into their original sample by LDA was 47% (Table 2). High misclassification percentages were observed within the nine locations.

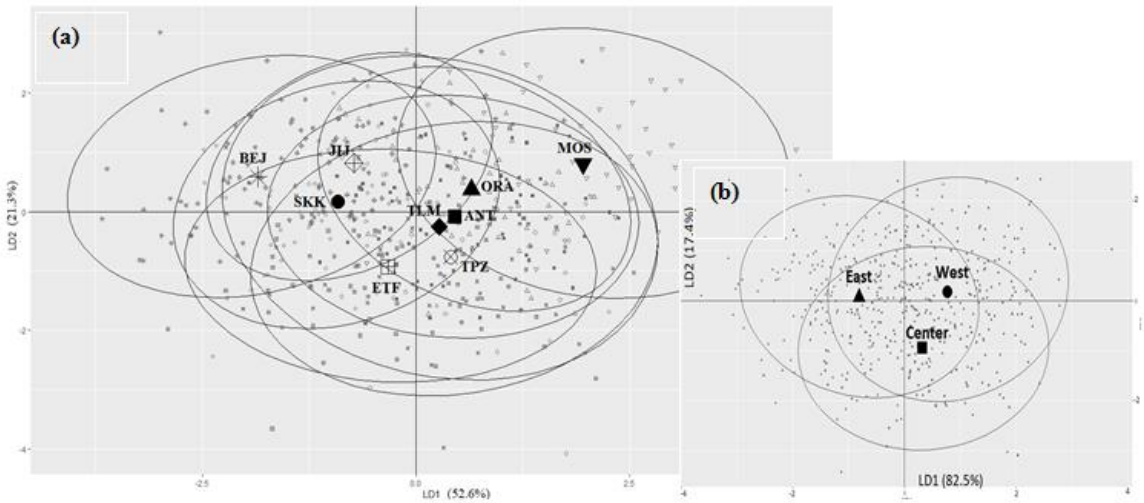

Figure 4: Discriminant analysis plot with 95% confidence ellipses for morphometric analysis of round sardinella. (a) LDA from 09 sampling areas, (b) LDA after grouping samples into 03 regions (west: TLM, ANT, ORA, MOS. center: TPZ. east: BEJ, JIJ, SKK, ETF).

Table 2: Percentage of individuals reclassified in each group in the Linear Discriminant Analysis.

Re-allocation group	Original group (%)									Number of individuals used
	TLM	ANT	ORA	MOS	TPZ	BEJ	JIJ	SKK	ETF	
TLM	**14.3**	12.2	16.3	12.2	12.2	0	8.2	12.2	12.2	49
ANT	4.3	**33.3**	10.1	7.2	21.7	1.4	5.8	7.2	8.7	69
ORA	2	16	**60**	2	6	2	4	2	6	50
MOS	0	4	6	**78**	2	2	2	0	6	50
TPZ	5.2	12.1	5.2	10.3	**34.5**	1.7	0	5.2	25.9	58
BEJ	2	0	6.1	2	2	**65.3**	4.1	14.3	4.1	49
JIJ	2	8	10	2	2	14	**44**	8	10	50
SKK	0	12	6	2	0	12	10	**38**	20	50
ETF	2.5	11.4	5.1	0	10.1	8.9	1.3	5.1	**55.7**	79

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The hierarchical clustering analysis performed on the matrix of averages morphometric lengths of round sardinella identified three clusters of fishes (Fig. 5a). TLM, ANT, ORA, and TPZ were grouped in the same cluster. BEJ and JIJ clustered with SKK and ETF. Sample from MOS was distinguishable from the other areas.

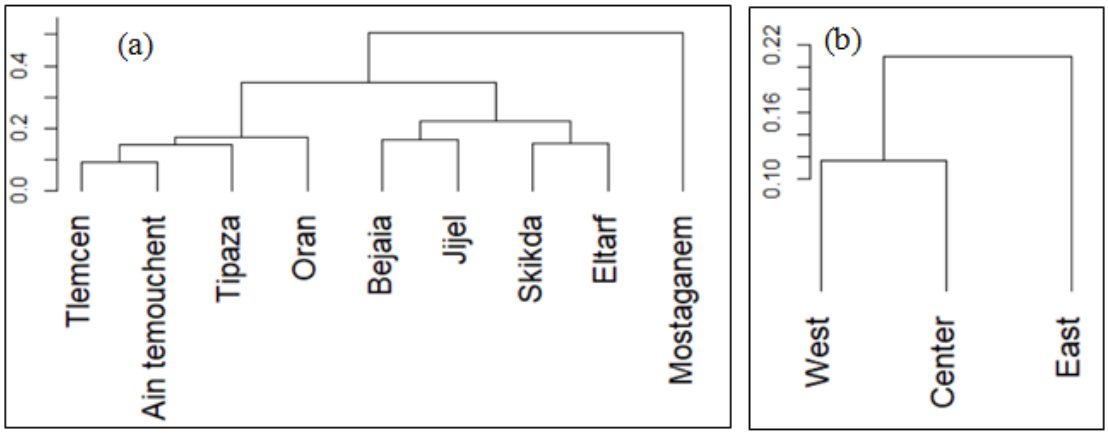

Figure 5: Hierarchical clustering analysis on averages of morphometric lengths of round sardinella. (a) Results obtained from 09 sampling areas. (b) Results obtained after grouping samples into 03 regions (west: TLM, ANT, ORA, MOS. center: TPZ. east: BEJ, JIJ, SKK, ETF).

Given those results, another grouping according to the division of the Algerian coast (west, center, and east) was tested. For the LDA, the percentage of correct classification obtained was 70%. Results observed in the LDA (Fig. 4b) were validated by the hierarchical clustering analysis (Fig. 5b). In general, Despite the considerable overlapping among the samples, the geographical trend is present, three groups can be distinguished, the western group (TLM, ANT, OAR and MOS), the central group (TPZ) and the eastern group (BEJ, JIJ SKK and ETF).

Meristic

The numbers of vertebrae were compared (483 specimens, Table 3), it ranged from 46 to 49 for all samples. Kruskal-Wallis test indicated no-significant difference according to sex (p>0.05), and area (p>0.05).

Otolith shape

The first 32 harmonics extracted to describe otolith contours totalled 99.99% of the cumulated power for both right and left otoliths, thus 128 Fourier coefficients were used for the data analysis. The redundancy analysis (RDA) combined to permutation tests of both otoliths showed no significant difference between right and left otoliths (p>0.05), between males and females. After PCA on the EFDs, only the first five PCs were significant as determined by the broken-stick model. These five PCs explained 81.8% of the total variance.

The LDA showed significant difference among areas (Wilks'λ=0.6, p<0.05)

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using the otolith shape, however, the classification success was low (37.5%) (Fig. 6a). A high overlapping was observed between individuals from neighbouring areas (Table 4).

Table 3: Mean vertebrae numbers of round sardinella in each sampling site.

Area	*Females*	*Males*	*All*	*Sample size*
TLM	46.9±0.195	47±0.261	47.04±0.178	49
ANT	47.17±0.196	47±0.152	47.07±0.114	66
ORA	47±0.171	47.10±0.180	47.06±0.118	50
MOS	47.03±0.212	47.2±0.154	47.1±0.128	50
TPZ	47.2±0.21	47.03±0.203	47.11±0.146	45
BEJ	46.98±0.161	47.08±0.233	46.02±0.141	48
JIJ	46.95±0.164	47.10±0.203	47.04±0.137	50
SKK	47.14±0.227	47.08±0.210	47.12±0.155	50
ETF	46.86±0.168	47±0.165	46.93±0.114	75

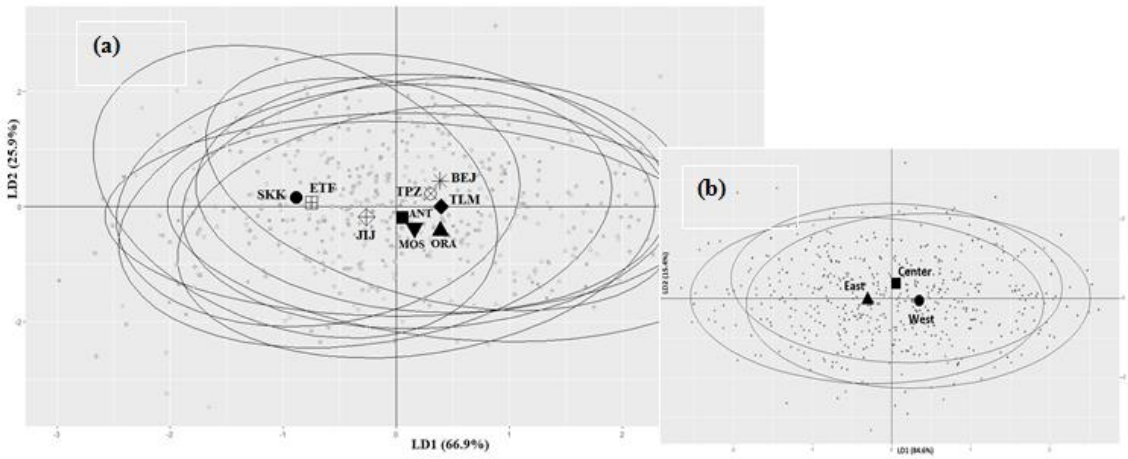

Figure 6: Discriminant analysis plot with 95% confidence ellipses for otolith shape analysis of round sardinella. (a) LDA from 09 sampling areas, (b) LDA after grouping samples into 03 regions (west: TLM, ANT, ORA, MOS. center: TPZ. east: BEJ, JIJ, SKK, ETF).

Table 4: Percentage of individuals reclassified in each group in the Linear Discriminant Analysis based on otolith shape.

Re-allocation group	**Original group**									**Number of otoliths used**
	TLM	**ANT**	**ORA**	**MOS**	**TPZ**	**BEJ**	**JIJ**	**SKK**	**ETF**	
TLM	**27.7**	21.3	8.5	6.4	2.1	17.0	2.1	8.5	6.4	47
ANT	1.7	**31.0**	12.1	8.6	1.7	13.8	6.9	15.5	8.6	58
ORA	2.1	6.3	**39.6**	12.5	0	20.8	4.2	12.5	2.1	48
MOS	5.2	10.3	3.4	**37.9**	0	19	8.6	8.6	6.9	58
TPZ	1.8	7.1	3.6	8.9	**35.7**	26.8	8.9	3.6	3.6	56
BEJ	1.1	4.5	3.4	3.4	2.2	**74.2**	1.1	5.6	4.5	89
JIJ	7.0	3.5	1.8	8.8	0	38.6	**14**	8.8	17.5	57
SKK	0	3.9	2.0	7.8	0	23.5	7.8	**35.3**	19.6	51
ETF	0	8.9	0	8.9	0	33.9	5.4	23.2	**19.6**	56

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The hierarchical cluster analysis identified three clusters of fishes (Fig. 7a). Cluster 1 is composed of round sardinella sampled in ANT, TLM, MOS, and ORA. Cluster 2 grouped TPZ, BEJ, and JIJ. ETF is clustered with SKK.

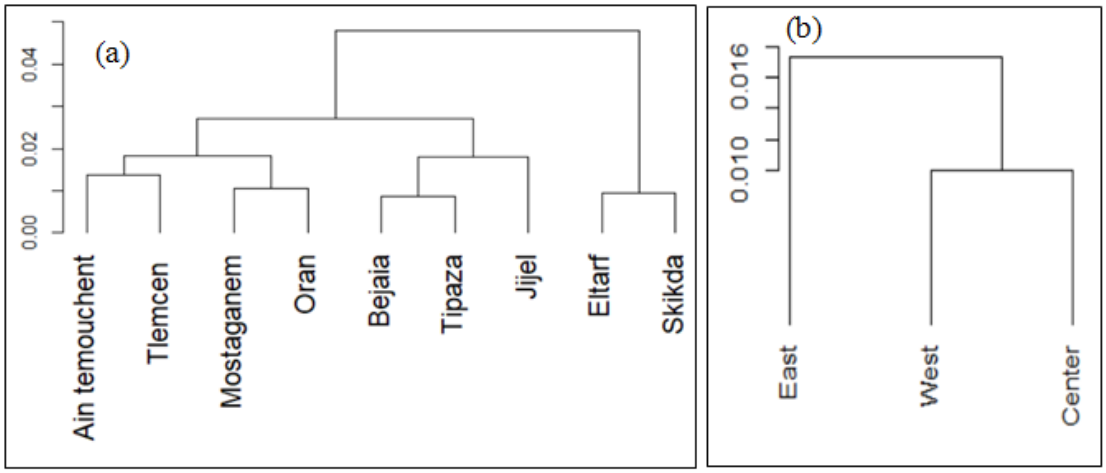

Figure 7: Hierarchical clustering analysis on the mean otolith outline shapes of round sardinella. (a) Results obtained from nine sampling areas. (b) Results obtained after grouping samples into 03 regions (west: TLM, ANT, ORA, MOS. center: TPZ. east: BEJ, JIJ, SKK, ETF).

Because of the high mixing between the nine areas, samples were grouped into three regions (west: TLM, ANT, ORA, and MOS, center: TPZ, east: BEJ, JIJ, SKK and ETF). The overall classification success was 55%, it is still low for a clear separation between the three groups revealed by the LDA or the hierarchical clustering analysis (Figs.6b and 7b).

Discussion

In this research, three approaches of discrimination were applied on the stock of the round sardinella *S. aurita* collected from nine areas in the Algerian waters. Begg and Waldman (1999) noted that a holistic approach to fish stock identification is highly desirable owing to the limitations and conditions associated with any particular method. The results obtained from the analyses of body and the otolith shape indicated a significant difference of round sardinella among areas. However, the analysis of vertebrae number does not show any difference. No significant effect of sex was observed for round sardinella on the body morphometric characters, on the otolith shape and on the number of vertebrae.

The discriminant analysis of morphometric characters and the otolith shape could not separate clearly the nine areas, because there is notable overlapping between the neighbouring populations. Indeed, most of the results observed in the discriminant analysis were validated by the hierarchical clustering analysis.

The proportion of individuals correctly classified into their original area was low

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(47.0% using the body morphometric characters and 37.5% using the otolith shape). This low rate of correct classification found between individuals from different areas, probably is the result from the mixing of round sardinella, which is due to larval dispersion, and migration of adults (Braham *et al*., 2014). For this reason, samples were combined into three regions according to the subdivision of the Algerian waters (west: TLM, ANT, ORA and MOS, center TPZ, east: BEJ, JIJ, SKK and ETF). The overall assignment success rate was 70% using morphometric characters, and 55% using the otolith shape analysis. Typically, species with high rates of dispersal such as round sardinella exhibit low levels of population structure (Stabile *et al*., 1996). Tracey *et al.* (2006) relate sources of misclassification in otolith shape analysis to the individual variability and migration.

The third approach used to discriminate round sardinella stock is the comparison of the vertebrae number; no significant difference between samples was detected. It seems to be in contradiction with study of Bouaziz (2007) in which, a significant difference occurred between samples from west, center, and east of Algeria.

The genetic study on round sardinella based on mitochondrial DNA conducted by Chikhi *et al.* (1997) showed homogeneity in the Mediterranean Sea basin and the existence of some barrier to gene flow between Mediterranean Sea and Atlantic Ocean. However, the phenotypic variability is particularly high in fish, and it is not necessarily associated with high genetic variability (Tudela, 1999). It can explain the prolonged separation of post larval fish in different environmental regimes (Begg and Waldman, 1999).

Body morphometric and otolith shape have been used successfully for distinguish populations at a range of different geographical scales (Cadrin and Friedland, 1999), but are often limited by their possible alteration by both abiotic and biotic environmental parameters (temperature, salinity, prey availability etc.). Vergara-Solana *et al*. (2013) compared the shape of the body and otolith of the Pacific sardine, *Sardinops sagax*, found that the shape of the otolith is least variable compared to the body shape. In addition, variation in the meristic count of a fish has both genetic and environmental components and determined in the early stage of larval development. Actually, importance on the correlation between meristic characters and environmental factors were suggested in previous studies (Vladykov, 1934; Beacham and Murray, 1986; Boglione *et al*., 1993; Chase, 2014).

Recently, several studies revealed that oceanographic properties, such as eddies and fronts could contribute to the random mixing and diffusion of pelagic larvae (Jemaa *et al*., 2015). The Algerian basin is a key-area for the general circulation of the various waters in the west of the Mediterranean Sea (Puillat *et al*., 2002; Aulicino *et al*., 2018), in which, the Atlantic Water (AW) forms

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an unstable current named the Algerian Current (AC), composed mainly by the Modified Atlantic Water (MAW) generating fresh-core coastal eddies that propagate downstream (Taupier-letage *et al*., 2003). Algerian coasts have a complex hydro-dynamism, which explain, on one hand, the lack of the isolated populations (high overlapping) and on the other hand, the heterogeneity among round sardinella inhabiting the coasts of Algeria (local populations).

Bacha *et al*. (2014) associate shape variability of anchovy otoliths with the presence of the Almeria-Oran front, but in the case of round sardinella, it is not possible to consider this barrier because of the overlap between TLM samples in the Alboran Sea and TPZ in the Algerian basin, but it should be noted the existence of a certain geographical coherence from west to east of Algeria in this study. The migratory nature of the round sardinella makes difficult the definition of boundaries in pelagic habitats (Baali *et al*., 2019).

Result of scale shape analysis of round sardinella collected from four different areas in the central and eastern Mediterranean Sea (Adriatic and Aegean Sea), showed that populations of this species from the central and eastern Mediterranean Sea could be separated reliably with an average discrimination rate of 91% (Bräger *et al*., 2016). In the Tunisian water, studies of the spatial variability of helminth parasites in the round sardinella allowed for the identification of two discrete stocks: one in offshore between 60 m and 100 m. and one in inshore waters (Feki *et al*., 2016). Concerning the Spanish Mediterranean coasts, a significant separation between the northern and southern populations of round sardinella is detected (Riveiro *et al*., 2011).

In conclusion, study of the discrimination of the round sardinella in the Algerian coasts using three approaches (body morphometric, meristic characters, and otolith shape) indicated that the geographic trend is present but with high overlap between neighbouring zones. This species is not completely isolated because the separation of population among areas was poor. Stock identification approaches provide different results but complementary information, further studies are needed, such as genetic studies and identification of spawning areas, however, an intensive sampling is necessary over a long period.

Acknowledgments

The authors would like to thank the National Center for Research and Development of Fisheries and Aquaculture (CNRDPA) for the ALPEL acoustic survey, all participants in ALPEL 2017/2018 for collaboration in the collection of samples. Our thanks also go to staff of LRSE/ Department of Biology, University Oran 1. All authors declare no conflict of interest.

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