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The use of Fourier analysis as a tool for *Oblada melanura* (Linnaeus, 1758) stock unit separation in the south central Mediterranean Sea

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*For the first time, an otolith shape analysis was used to investigate the stocks of saddled bream (*Oblada melanura*, Linnaeus, 1758) in three fishing zones along the Tunisian coast (Bizerte, Kélibia and Sayada). Otolith shape analysis was used on 30 otoliths for each site, sampled during the spawning period. Using elliptic Fourier descriptors (EFD) the quantization of the shape otolith was investigated by SHAPE and multivariate statistical procedures. Considering the environmental and the genotypic aspects, the preliminary results of the otolith shape analysis showed dissimilarity in silhouette of otoliths of saddled bream stocks collected from the north (Bizerte), the north-east (Kélibia) and the east (Sayada) of the Tunisian coast. Therefore, these three groups could be considered as three sub-units of the Tunisian stock, which should be managed separately.*

Keywords: *Oblada melanura*, otolith shape, elliptic Fourier descriptors (EFD), stock, Tunisia

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INTRODUCTION

According to Gauldie (1991) it can be difficult to define the term 'stock'. Several definitions have been presented in the literature (Gulland, 1969; Jamieson, 1973; Booke, 1981; Ihssen *et al.*, 1981; Gauldie, 1988; Carvalho & Hauser, 1994; Begg & Waldman, 1999), but in the modern definition of a stock it is desirable to incorporate the genotypic and phenotypic components (Cadrin *et al.*, 2013). In fisheries, the delineation of stock is crucial (Waldman, 2007; Cheilari & Rätz, 2009) as an integral component (Tracey *et al.*, 2006) of modern fisheries assessment and management.

Several techniques are used for the discrimination of stocks (Cadrin *et al.*, 2013). Otolith shape analysis is one of the techniques in the morphometric analysis group used as a tool of stock discrimination, because the otolith is influenced by both genetic heterogeneity and environmental factors (Campana & Casselman, 1993; Cadrin & Friedland, 1999; Torres *et al.*, 2000; Cardinale *et al.*, 2004; Swan *et al.*, 2006; Vignon & Morat, 2010).

This technique has been used widely with success in stock identification studies of several species in the Atlantic and the Mediterranean Sea, but has never been used for *Oblada melanura* stock identification studies.

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The saddled bream, *Oblada melanura* (Linnaeus, 1758) belongs to the family of Sparidae, known for its high commercial value, although their importance by weight in catches remains low in the Mediterranean fisheries (Harmelin-Vivien *et al.*, 1995). The saddled bream is also among the species trialled for possible fish farming (Suquet *et al.*, 2009). This species presents a wide geographic distribution that extends in tropical and temperate regions of the Atlantic Ocean and throughout the Mediterranean, the Black Sea (Fisher *et al.*, 1987) as well as in Tunisia in the south central of the Mediterranean Sea from the north to the south.

The saddled bream moves on rocky and sandy seabeds or on seagrass of posidonia or zostera to depths of 30–40 m (Harmelin-Vivien *et al.*, 1995). The recruitment of saddled bream juveniles occurs in rocky micro-habitats with a variable slope, characterized by the presence of overhangs. Afterwards, at the advanced stages, juveniles settle in different deeper zones (Harmelin-Vivien *et al.*, 1995) and adopt gregarious and benthopelagic behaviour.

In the Mediterranean Sea, several studies have researched saddled bream population and stock discrimination using genetic, parasites, otolith chemistry and morphometric analyses (Summerer *et al.*, 2001; Calarza, 2007; Roques *et al.*, 2007a, b; Smrzlić *et al.*, 2012; Gkafas *et al.*, 2013; Nilolioudakis *et al.*, 2014; Caló *et al.*, 2016a, b).

In Tunisia, some studies dealing with the stock assessment and fisheries management of this species are in progress. A stock identification constitutes a first step for fisheries management. For this purpose, we focus our interest in studying the relationship between the otolith shape with geographic distribution for the stock of *Oblada melanura* along the Tunisian coast in three sites.

MATERIALS AND METHODS

Study areas and sampling

In accordance with the distribution of the species, three fishing landing sites: Bizerte (B), Kélibia (K) and Sayada (S) were chosen for the collection of samples and data along the Tunisian coast, in the south central Mediterranean Sea. Bizerte is located at 37°16′N 9°52′E and is at a distance of 188.6 km from Kélibia. Kélibia is located at 36°51′N 11°05′E and is at a distance of 211.4 km from Sayada. Sayada is located at 35°40′N 10°54′E and is at a distance of 255.9 km from Bizerte (Figure 1). A total of 183 individuals were collected (Table 1), of which 90 specimens were used for otolith shape analysis. All specimens were measured in cm (standard length L_{st}; fork length L_f; total length L_t) and weighed (eviscerated fish: W_{ev}) with a precision of 0.1 g (Table 2).

The specimens were sampled between April and June 2014, which corresponds to the reproductive period (May to July). This period allowed minimization of any mixing effects of fish due to migration between spawning areas to reduce the effects of ontogeny; the analysis was performed on a restricted range of fish lengths that included only fish between 13 and 19 cm in standard length (Table 3).

Otoliths, Sagittae left (Figure 2) were extracted, rinsed with distilled water and kept dried in an Eppendorf tube for further treatment.

Image acquisition

To avoid the probable effect of a morphological asymmetry of the pair of otoliths (right and left), we choose the left otolith to

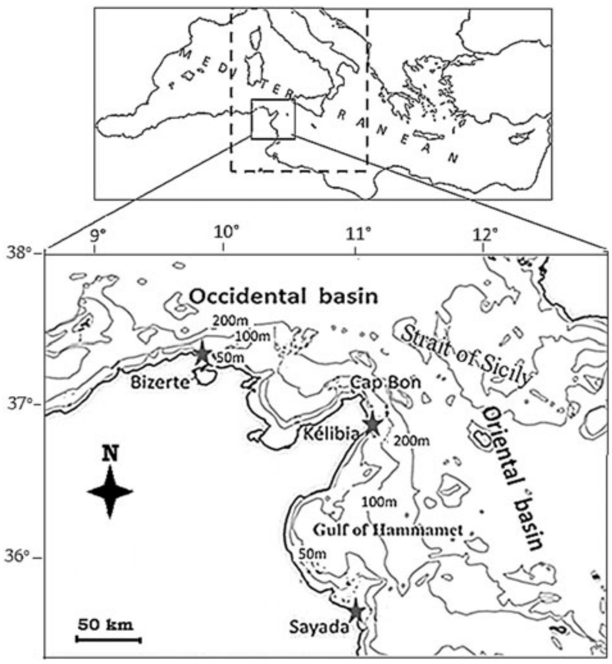

Fig. 1. Map showing the sampling sites (star symbols).

Table 1. Length characterization of specimens collected along the Tunisian coasts.

Length	Sex	*N*	Area	Mean (cm)	SD (cm)	Min (cm)	Max (cm)
L_{st}	Female	53	East	16.309	2.123	11.600	21.500
		21	North	15.319	3.425	13.400	30.000
		18	North-east	13.300	1.125	12.000	15.900
	Male	53	East	16.811	1.746	12.400	22.500
		18	North	16.227	5.291	12.500	32.000
		20	North-east	13.035	0.785	11.800	15.000
L_f	Female	53	East	18.126	2.363	13.400	26.000
		21	North	16.857	3.811	14.500	33.000
		18	North-east	14.516	1.240	12.800	17.500
	Male	53	East	18.586	2.255	12.900	26.000
		18	North	17.816	5.950	13.700	35.500
		20	North-east	14.365	0.988	12.900	16.800
L_t	Female	53	East	20.881	3.830	2.600	29.000
		21	North	19.905	3.817	17.700	36.000
		18	North-east	17.365	1.816	14.300	22.000
	Male	53	East	21.879	2355	16.400	30.000
		18	North	21.011	6.151	16.500	39.500
		20	North-east	16.910	1.179	13.800	19.900

L_{st}, standard length; L_f, Fork length; L_t, total length; SD, standard deviation; Max, maximum; Min, minimum.

conduct the shape analysis. The otolith was placed on a slide on a dark background and the outlines were digitized to minimize the distortion error using a digital camera (type Leica DFC 280) connected to a monitor with Photoshop software. All the otolith images obtained were stored in a database to be treated by the software SHAPE (Iwata & Ukai, 2002). SHAPE is widely used to describe the otolith shape (Turan, 2004; Galley *et al.*, 2006; Megalofonou, 2006; Jemaa *et al.*, 2015; Libungan *et al.*, 2015; Trojette *et al.*, 2015; Mahé *et al.*, 2016).

Shape analysis

The shape evaluation method is based on an elliptic Fourier descriptor (EFD) that is used to delineate the shape with a closed two-dimensional contour as suggested by Kuhl & Giardina (1982). From digital images, the program extracts the contours of the otolith and stores the information as chain code. From the chain-coded contour, the program extracts and normalizes elliptical Fourier harmonics (Hi) with respect to the first harmonic as suggested by the software. Sufficient number of harmonics has been used to reconstruct the otolith outline.

Table 2. Weight characterization of specimens collected along the Tunisian coasts.

Weight	Sex	N	Area	Mean (g)	SD (g)	Max (g)	Min (g)
Wev	Female	53	East	125.708	46.777	297.920	46.360
		21	North	95.136	29.725	203.600	69.450
		18	North-east	64.548	15.991	92.690	44.470
	Male	53	East	137.397	51.576	378.190	47.350
		18	North	105.793	55.862	289.000	56.620
		20	North-east	59.634	9.904	87.540	45.200

Wev, weight of the eviscerated fish; N, number of fish; SD, standard deviation; Max, maximum; Min, minimum.

Table 3. Data on otolith collected for shape analysis.

Sites	Area	Mean L_{st} (cm) SD (min–max)	N otolith
Bizerte (B)	North	14.500 ± 0.452 (13.400–15.600)	30
Kélibia (K)	North-east	17.523 ± 0.738 (16.400–19.300)	30
Sayada (S)	East	15.217 ± 2.320 (11.600–9.500)	30

L_{st}, standard length; SD, standard deviation; Max, maximum; Min, minimum; N, number of otoliths.

To determine the number of harmonics needed to reconstruct the otolith contour, the Fourier Power (PF), the percentage and the cumulative are calculated using formulae (1), (2) and (3).

$$FP = \frac{A_n^2 + B_n^2 + C_n^2 + D_n^2}{2} \quad (1)$$

FP: Fourier power; A_n, B_n, C_n, D_n: coefficients of Fourier.

$$FP\% = \left(\frac{FP}{\sum FP}\right) \times 100 \quad (2)$$

FP%: percentage of FP.

$$FP_n\%_c = \sum_{1}^{n} FP_n\% \quad (3)$$

$FP_n\%_c$: cumulative percentage of FP.

The otolith shape of the studied samples was reconstructed at 100% of the Fourier power corresponding to the first 20 harmonics. Each harmonic was composed of four coefficients, which correspond to values of the projection of the binary image on the two axes (*X*) and (*Y*) (Kuhl & Giardina, 1982). A total of 80 coefficients were allocated to each otolith.

Fig. 2. The left otolith of *Oblada melanura*. L, left.

Statistical analysis

A non-parametric test, the Spearman's rank correlation coefficient (Gibbons, 1985), was carried out between the mean and the maximum and the minimum of length (L_{st}, L_f, L_t) and weight (W_{ev}) to evaluate the effect of sampling on the variability of these different measures.

An analysis of variance test (ANOVA) of the applied General Linear Model (GLM) was carried out to evaluate the significance of differences in mean length (L_{st}, L_f, L_t) and weight (W_{ev}) according to sex and sampling site.

The normality distribution of the three groups was performed using the Shapiro–Wilk's test.

An analysis using covariance test (ANCOVA) was applied to test for significant differences in the length-weight relationship by area.

To determine whether otoliths collected in three sites could be distinguished based on their shapes, two multivariate analyses were used, allowing the visualization of shape variations corresponding to the otolith: (1) a principal component analysis (PCA) (Rohlf & Archie, 1984) and (2) a discriminant factor analysis (DFA) were performed using the variance-covariance matrix of the coefficients.

RESULTS

The analysis of the different fish length measures (L_{st}, L_f, L_t) and weight (W_{ev}) according to sex and sampling site are shown in Table 4. The lengths of the three fish samples showed, statistically, non-significant differences between the mean length and the maximum as well as the mean length and the minimum. However, in weight, a significant effect was observed between the mean weight and the maximum of the eviscerated fish, against significant differences between the mean weight and the minimum.

Given this reason, the analysis of otolith shape was realized for a limited interval to eliminate the sample effect on results for the three sampling sites. The analysis of variance test (ANOVA) of the applied General Linear Model (GLM) showed that the fish mean length differed statistically between the sampling areas but was not affected by the sex (Table 5).

Table 4. Spearman correlation and test of significance between the mean and the maximum and minimum measures.

Different combination of measure	*N*	*R* (Spearman)	*t*(*N* − 2)	*P* level
Mean L_{st} and max L_{st}	6	0.543	1.293	0.266
Mean L_{st} and min L_{st}	6	0.086	0.172	0.872
Mean L_f and max L_f	6	0.522	1.223	0.288
Mean L_f and min L_f	6	0.232	0.477	0.658
Mean L_t and max L_t	6	0.714	2.041	0.111
Mean L_t and min L_t	6	0.314	0.662	0.544
Mean W_{ev} and max W_{ev}	**6**	**0.886**	**3.816**	**0.019**
Mean W_{ev} and min W_{ev}	6	0.543	1.293	0.266

L_{st}, standard length; L_f, Fork length; L_t, total length; W_{ev}, weight of the eviscerated fish.

Table 5. ANOVA test showing the sex and the sampling area effect on different measure.

		SS	df	MS	F	*P* level
L_{st}	Intercept	1380.245	1	1380.245	7771.360	**0.000**
	Sex	0.219	1	0.219	1.232	0.383
	Zone	12.615	2	6.307	35.512	**0.028**
	Error	0.355	2	0.178		
L_f	Intercept	1675.635	1	1675.635	10,819.334	**0.000**
	Sex	0.268	1	0.268	1.731	0.319
	Zone	16.507	2	8.253	53.291	**0.018**
	Error	0.310	2	0.155		
L_t	Intercept	2318.771	1	2318.771	6096.129	**0.000**
	Sex	0.453	1	0.453	1.191	0.389
	Zone	19.915	2	9.957	26.178	**0.037**
	Error	0.761	2	0.380		
W_{ev}	Intercept	68,204,703.295	1	68,204,703.295	865.812	**0.001**
	Sex	57,008.254	1	57,008.254	0.724	0.485
	Zone	39,798,770.491	2	19,899,385.245	252.609	**0.004**
	Error	157,550.815	2	78,775.407		

L_{st}, standard length; L_f, Fork length; L_t, total length; W_{ev}, weight of the eviscerated fish.

For the three groups, the Shapiro–Wilk's test showed that all specimens from Bizerte ($W = 0.978$, $P < 0.778$) and Kélibia ($W = 0.950$, $P < 0.168$) come from a normally distributed population, however, individuals coming from Sayada ($W = 0.923$, $P < 0.032$) do not show a normal distribution.

Moreover, the length-weight relationship between standard length and eviscerated weight was showing a significant difference between the different areas ($P = 0.0177$).

The standardized elliptic Fourier coefficients of 90 otoliths from the three areas were calculated. The mean otolith shape of *Oblada melanura* was then drawn using the mean values of the standardized elliptic Fourier coefficients for each area, showing a variation of the otolith shape (Figure 3).

The 10 principal components provide a summary of the data, accounting for 87% of the total variance (Table 6, Figure 4). The effect of each principal component on otolith shape was visualized (Figure 4A). These reconstructed shapes indicate that the first principal component is good measure of the dome dorsal, the dome posterior ventral and the dome anterior ventral and expresses the depth of notch of the side of the dome anterior ventral parts of the otolith (Figure 4B). The second component is associated with the dome posterior ventral and the dome anterior ventral part of the otolith (Figure 4B). From the third to the 10th component, the variation is not easily explained (Figure 4A).

Table 7 shows the results of the ANOVA performed on the principal component scores of groups as well as the sampling area. The shape variation shown in Figure 3 was significant for the two first, the fourth and the two last principal components, as was confirmed by the discriminant factor analysis (DFA) (Wilk's lambda = 0.002, $F = 1.826$, $P < 0.001$, Figure 5). According to per cent correct classification, 100% of samples were correctly classified indicating that otoliths differed from the northern to the eastern coasts of Tunisia.

DISCUSSION

According to our results, considering the length, the weight and the sex effect on the samples, the shape analysis of the otolith showed three groups within the *Oblada melanura* sampled in the north (Bizerte), the north-eastern (Kélibia) and the east (Sayada) of Tunisian coast.

The analysis was performed for a limit interval of standard length covering 13 and 16 cm corresponding to the adult fraction of *Oblada melanura*. This choice was helpful to decrease the bias introduced by ontogeny impact on the otolith shape analysis as has been demonstrated at earlier stages for deep-sea eels, whose otolith characteristics occur in the adult stage (Hecht & Appelbaum, 1982). Saddled bream adults, in contrast with juveniles, do not exhibit any important migration (Gkafas *et al.*, 2013). Indeed, at an advanced age, juveniles extended their home range vertically into deeper zones, and laterally in more exposed areas (Harmelin-Vivien *et al.*, 1995). Along the coastline, this dispersion is limited to 90 km, only for a short period in the pelagic environment (Calò *et al.*, 2016a). Our analysis, carried out during the spawning period, could support the absence of any eventual populations mixing (Cardinale *et al.*, 2004).

Basing on the genetic structure of *Oblada melanura*, Calò *et al.* (2016b) suggested a local connectivity between protected and unprotected areas (50–100 km) of the west

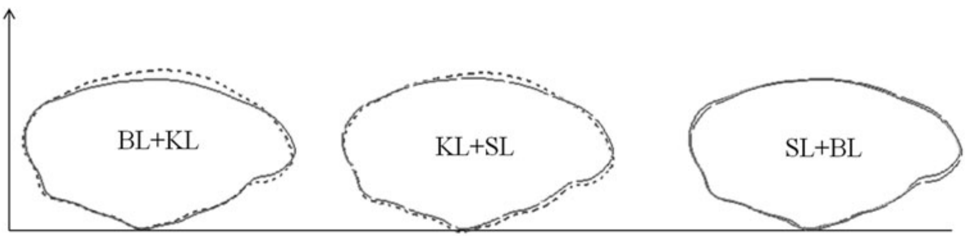

Fig. 3. Mean left otolith shapes of *Oblada melanura* sampled in three areas. B: Bizerte (solid line); K: Kélibia (dotted line) and S: Sayada (dashed line).

Table 6. Eigenvalues and contributions of principal components realized for otolith shape for *Oblada melanura* sampled in three areas.

Component	Eigenvalue	Proportion (%)	Cumulative (%)
1	0.00099	**51.721**	51.721
2	0.00020	**10.287**	62.009
3	0.00011	5.795	67.804
4	0.00009	4.450	72.255
5	0.00006	3.317	75.573
6	0.00006	2.874	78.447
7	0.00005	2.449	80.896
8	0.00004	2.236	83.133
9	0.00004	2.037	85.170
10	0.00003	1.674	**86.845**
Total variance	1.915×10^{-3}		

Table 7. Results of ANOVA for the first 10 principal components (PC) of group coefficients and sampling area.

Components	df error	F	*P* level
PC1	87	21.460	**0.000**
PC2	87	8.566	**0.000**
PC3	87	3.020	0.054
PC4	87	12.435	**0.000**
PC5	87	2.529	0.085
PC6	87	0.838	0.435
PC7	87	1.790	0.173
PC8	87	1.145	0.323
PC9	87	5.020	**0.009**
PC10	87	5.130	**0.007**

Mediterranean. Meanwhile, more distant areas have revealed a genetic clustering. The three sites studied here are far away from each other. Taking into account the environmental factors which impact some inter- and intra-specific otolith differences (Lombarte, 1992) and the genotypic aspects which specify the otolith morphology, Avigliano *et al.* (2015) revealed that the method of otolith shape analysis applied for this study was among the successful tools for stock discrimination (Campana & Casselman, 1993; Burke *et al.*, 2008; Cañás *et al.*, 2012; Avigliano *et al.*, 2014) of different species worldwide (Begg & Brown, 2000; Begg *et al.*, 2001; Brophy & Danilowicz, 2002; Turan, 2004; Galley *et al.*, 2006; Megalofonou, 2006; Mérigot *et al.*, 2007; Duarte-Neto *et al.*, 2008; Renán *et al.*, 2010; Vieira *et al.*, 2014; Jemaa *et al.*, 2015; Libungan *et al.*, 2015; Jawad *et al.*, 2017; Mapp *et al.*, 2017).

Boundaries extraction of otoliths have been carried out with different methods such as Fourier transforms (Begg & Brown, 2000), the Wavelets, the Curvature-Scale-Space (Parisi-Baradad *et al.*, 2005) and the more recent Shapelet transform methods (Hills *et al.*, 2014). However, according to Cadrin & Friedland (1999), Fourier analysis is an efficient method for describing contour shapes. The elliptical Fourier descriptors were successful applied to *Oblada melanura* stock in the south central Mediterranean.

Our findings are consistent with those relating to other species (Campana & Casselman, 1993; Duarte-Neto *et al.*, 2008; Renán *et al.*, 2010; Vieira *et al.*, 2014; Jemaa *et al.*, 2015). Elliptic Fourier descriptors (EFD) and principal component analysis (PCA), using SHAPE, can accurately detect small shape variations and evaluate the shape independently of size (Iwata *et al.*, 1998; Yoshioka *et al.*, 2004).

The morphology of the otolith varies clearly between species and within species from different regions (Campana & Casselman, 1993; Lombarte & Lleonart, 1993; Renán *et al.*, 2010). The sampled *Oblada melanura* specimens in the south central Mediterranean presented a clear discrimination between three different Tunisian areas. This result corroborates with genetic diversity and morphometric analyses conducted in different Mediterranean regions as was reported for the same species in the Aegean Sea (Gkafas *et al.*, 2013) and in the western Mediterranean (Calò *et al.*, 2016b).

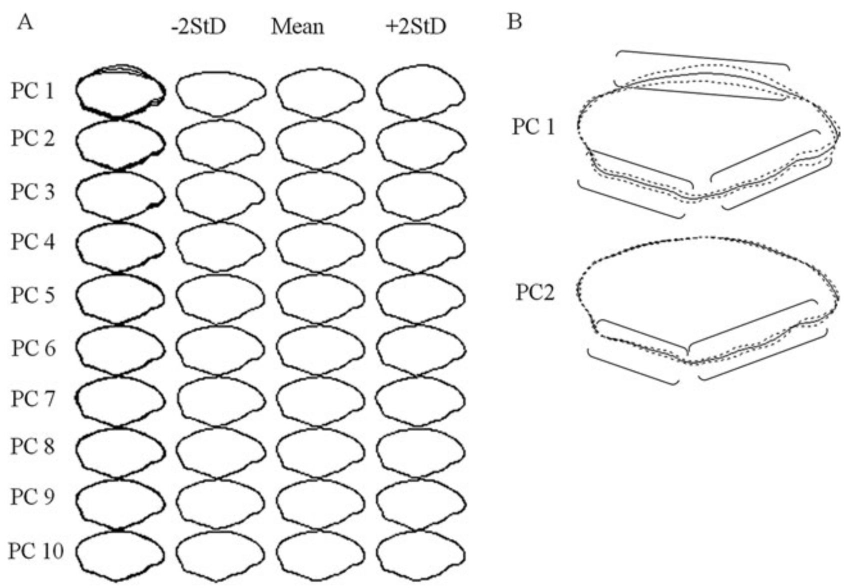

Fig. 4. Effect of each 10 and the two main principal components on otolith shape. (A) The columns show from the right the case where the score takes +2 SD, mean, and −2 SD as presented, and in the left of (A), the last column showing the overlaid drawings of all three cases for each 10 principal component (PC) where the mean is drawn by solid line, −2 and +2 SD (standard deviation) is drawn by dot line. (B) Effect of two main principal components on otolith shape with the localization of shape variation accounted by the principal component.

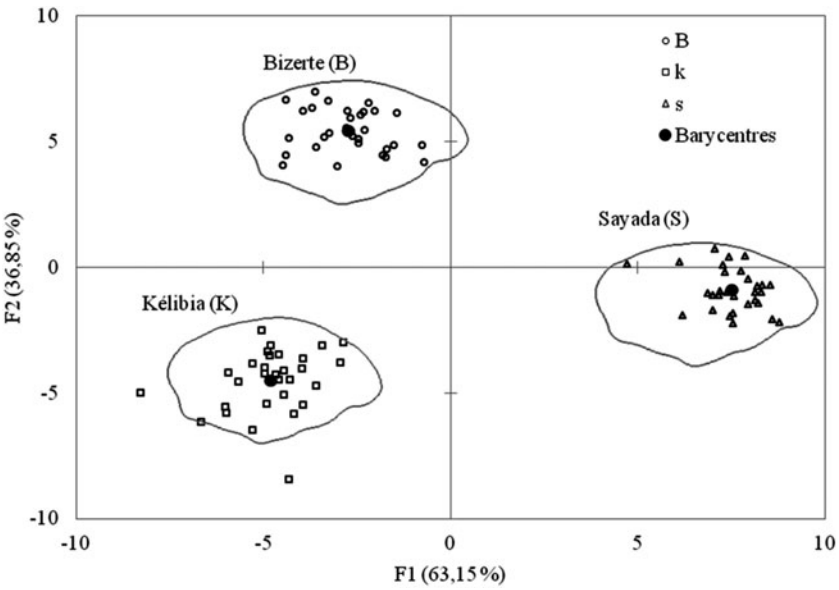

Fig. 5. Graphical representation of discriminant factor analysis for the classification of *Oblada melanura* left otolith according to the sampling areas based on normalized elliptic Fourier descriptor with the mean shape for each region.

Similar findings dealing with high levels of genetic diversity of *Oblada melanura* were observed in gilthead sea bream (*Sparus aurata*) in Tunisia (Ben Slimen *et al.*, 2004) and Italy (Franchini *et al.*, 2011).

Several factors are responsible for the group separation among the species, such as the habitat and the environmental conditions (Cardinale *et al.*, 2004). In fact, Bizerte belongs to the western Mediterranean basin; Kélibia and Sayada belong to the eastern Mediterranean basin. The two basins are separated by the Siculo-Tunisian strait characterized by different climatic conditions, current systems and hydrological regimes (Bethoux, 1979; Borsa *et al.*, 1997; Bas, 2009). *Oblada melanura* is among the species characterized to be sensitive to temperature, salinity and the consumption of oxygen (Antolović *et al.*, 2011). Although the samples from Sayada and Kélibia belonged to the same side, the eastern marine zone, the two groups are different. This could be explained by a second factor – the diet. According to Cardinale *et al.* (2004) the diet may also influence otolith morphology. From Kélibia to Sayada, the food choice of *Oblada melanura* could be different because this species is an opportunistic predator (Pallaoro *et al.*, 2003). The diets of several species have been shown to have an impact on otolith morphology (Ward & Rogers, 2003; Gagliano & McCormick, 2004; Hüssy, 2008). In addition to ecological factors, geneticss can be considered to be a third but not significant factor (Galley *et al.*, 2006) for populations of the same species (Cardinale *et al.*, 2004).

The study of otolith silhouettes using the elliptic Fourier descriptor and multivariable analyses has successfully shown to be a tool for stock management (Campana & Casselman, 1993; Cardinale *et al.*, 2004; Galley *et al.*, 2006; Pothin *et al.*, 2006; Vieira *et al.*, 2014). From north to east of the Tunisian marine zones, shape analysis of *Oblada melanura* otoliths has demonstrated that there exists a significant discrimination presenting a heterogenic population depending mainly on the environmental factors, habitat and on the diet. This result will be very useful for stock management of *Oblada melanura* in the future for the conservation of this resource, by considering the ecological boundaries and not the political limits. In addition, this species is considered among the Sparidae exploited along the Tunisian coast by small-scale fisheries.

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