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USE OF OTOLITH SHAPE TO DIFFERENTIATE TWO LAGOON POPULATIONS OF *PAGELLUS ERYTHRINUS* (ACTINOPTERYGII: PERCIFORMES: SPARIDAE) IN TUNISIAN WATERS

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Background. The common pandora, *Pagellus erythrinus* (Linnaeus, 1758), is widely distributed in the Black and the Mediterranean seas. Therefore, *P. erythrinus* is a valuable component of the commercial fishery in Tunisia and plays an important role in microeconomics of local areas. The goal of this study was to investigate the stock structure and otolith asymmetry for specimens of the common pandora sampled from two lagoons—Ghar El Melh and Bizerte.

Materials and methods. We collected a total of 119 specimens of *P. erythrinus* from the two lagoons, from May through July 2016. The Elliptical Fourier Analysis (EFA) was determined to evaluate the degree of similarity in the otoliths and detect the reciprocal variability.

Results. The Discriminant Factor Analysis for the sagitta shape clearly demonstrated an asymmetry when comparing otoliths (left–right) ($P < 0.05$) within each population and a difference in shape when comparing the same side between the two populations: between the two sides (right–right and left–left).

Conclusion. The comparison of the otolith morphology of the two populations showed a clear difference in shape and a left–right asymmetry of otoliths between and within populations. This result is probably related to genetic and local environmental factors. In fact, this investigation improves the knowledge on the stock discrimination for *P. erythrinus* and provides useful information for analysing fisheries management of this species in Tunisia.

Keywords: Pagellus erythrinus, sagitta, shape, asymmetry, Tunisia

INTRODUCTION

The common pandora, *Pagellus erythrinus* (Linnaeus, 1758), is widely distributed along the European and African coasts of the Atlantic Ocean (Fischer et al. 1987), from Norway to Angola, and around the Sao Tomé Príncipe and the Canary Islands (Pajuelo and Lorenzo 1995). This species is of particular interest since it represents an appreciated fishery resource occurring throughout the Mediterranean basin (Spedicato et al. 2002). In Tunisian waters, *P. erythrinus* has an important commercial value and it is common in the local fishery, contributing from 8% to 15% of the catches of demersal fishes in the Gulf of Gabès (Ghorbel et al. 1997) and 11% in the Gulf of Tunis (Zarrad et al. 2000).

Various aspects of the biology population dynamics, distribution, reproductive cycle, and feeding ecology of *P. erythrinus* populations were investigated in different geographic areas (Somarakis and Machias 2002, Coelho et al. 2010).

The otolith—the main subject of this study—is Greek for "ear stone" and by definition, is a calcified granule located in the fish inner ear, responsible not only for hearing but also for balance (Popper et al. 2005). Otoliths show phenotypic plasticity as inter- and/or intra-specific and inter- and/or intra-populations variations have been reported (Annabi et al. 2013). Many researchers used the sagittal otolith structures to estimate population growth and mortality or for fisheries management (Cardinale et al. 2004, Tracey et al. 2006, Stransky et al. 2008, Burke et al. 2008, Cañás et al. 2012, Morat et al. 2012). In the context of our work, the otolith serves as fish population discriminator. Its structure, however, can be influenced of many factors, such as sex, growth, maturity, fishery

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exploitation pattern, as well as genetic and environment parameters (Begg and Brown 2000). It is suggested by different authors, that environmental parameters like water temperature, salinity, food availability, depth, and substrate type are the main factors that are most likely responsible for differences in otolith shape among conspecific individuals (Begg and Brown 2000, Torres et al. 2000, Cardinale et al. 2004, Gagliano and McCormick 2004, Mérigot et al. 2007, Hüssy 2008, Morat et al. 2012). Consequently, otoliths have been widely used for determination of fish stock in different environments (Smith et al. 2002, Turan 2006, Vignon and Morat 2010). For example, Morat et al. (2012) examined the otolith for three different species of the Mullidae from the Mediterranean basin and claimed the occurrence of three well distinct groups.

In Tunisia, Trojette et al. (2014) showed an otolith shape discrimination between three populations of *Scorpaena porcus* Linnaeus, 1758 for the marine and insular environment (Rafraf, Hammam Lif, and Djerba). Also, Rebaya et al. (2016) showed, by the otolith shape, the existence of two distinct populations of *Chelon ramada* (Risso, 1827) in two lagoon habitats (Ghar El Melh and Bizerte).

For the species in question, Ghorbel (unpublished*) evaluated the stock of *P. erythrinus* in the Gulf of Gabès (Tunisia) by pseudo-cohort analysis and reported that the stock is already overfished. However, no study has been done on the discrimination of fish populations by the morphology of otoliths in Tunisia.

The aim of this study was to evaluate the stock structure of *Pagellus erythrinus* from two Tunisian lagoons (Ghar El Melh and Bizerte) based on the otolith shape using different statistical approaches.

MATERIAL AND METHODS

Sample collection. The Ghar El Melh Lagoon is a Mediterranean water body, located in northern Tunisia, on the north-western side of the Gulf of Tunis, between Ras Tarf (Cape Farina) and the estuary of the Medjerda River (37°8′0″N, 10°11′30″E) (Fig. 1). It is connected to the Mediterranean Sea through a permanent 70-m wide El Boughaz canal, associated with coastal sand bars (Khemaissia et al. 2013). The salinity reported by Sghaier et al. (2012) was 37‰–45‰[*]. The water temperature displayed a clear annual pattern varying between 14°C and 34°C.

The Bizerte Lagoon (37°11′20.4″N, 9°51′16.2″E) has a double connection with other bodies of water. Through a 7 km long canal in the north, it communicates with the Mediterranean Sea and through the Tinja Wadi in the South, it connects with Ichkeul Lake. The lagoon was subjected to significant environmental changes of both natural and anthropogenic causes. Indeed, according to Dridi et al. (2007), the salinity ranged from 33‰ to 38‰. The water temperatures in the lagoon also presented significant differences ranging from 11.18 to 26.12°C.

During three months, from May through July 2016 we collected a total of 119 specimens of *Pagellus erythrinus* jointly from the two lagoons, Ghar El Melh and Bizerte. All fish were caught using gillnets by coastal boats (5–13 m overall length). For each fish; standard length (SL), total length (TL) to the nearest 0.1 cm, and total weight (TW) to the nearest 0.1 g were respectively recorded (Table 1).

Both sagittal otoliths were removed, rinsed in distilled water, stored in Eppendorf tubes, and finally kept in dry storage for 24 h in order to eliminate the humidity, following the protocol proposed by Trojette et al. (2015). Digital images were captured using, a Nikon Coolpix-S6700 camera (with the resolution of 20.1 megapixels). Otoliths were placed under a dissection microscope and photographed under reflected light. Afterwards, otolith shapes (Fig. 2) were analysed using the program SHAPE v.

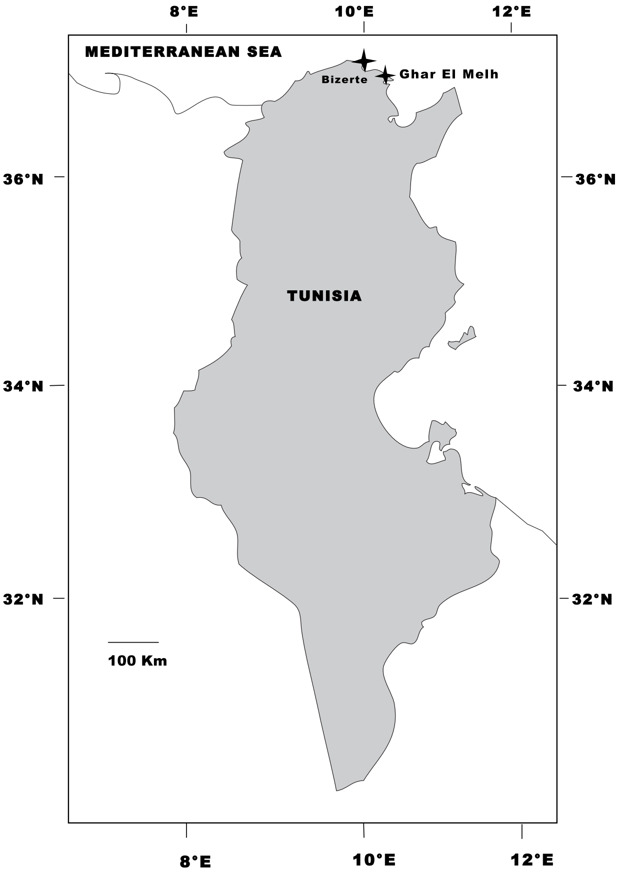

Fig. 1. Sampling sites of *Pagellus erythrinus* in Bizerte and Ghar El Melh lagoons, Tunisia

Table 1

Length and weight of *Pagellus erythrinus* specimens from Bizerte and Ghar El Melh lagoons, Tunisia

Parameter	Sampling site	
	Bizerte	Ghar El Melh
Number of fish	*n* = 55	*n* = 64
Total length [mm]	186.8 ± 17.30	152.2 ± 16.08
Total weight [g]	87.17 ± 21.52	55.19 ± 17.78

Biometric values are mean ± standard deviation.

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[*] In the wake of the growing criticism of the Practical Salinity Scale concept (and especially PSU as a "unit"), Acta Ichthyologica et Piscatoria is in favour of expressing salinity in parts per thousand (‰), regardless if a direct or indirect method was employed to determine the water salinity.

1.3 (Iwata and Ukai 2002). The SHAPE program used a ‘chain coding’ algorithm (Freeman 1974 cited in Iwata and Ukai 2002) first to extract the contours of the otolith outline elliptical Fourier analysis EFDs. Second, the Chain Coder stores the relevant information of the contour as chain-codes and provides the normalized EFD coefficients (NEFDs) through a discrete Fourier transformation of the chain-coded contour.

Statistical analyses. The Elliptical Fourier Analysis (EFA) consists of describing the outline of a specific shape with several components (Fig. 3) (harmonics) with an ellipse as the first approximation step. For each otolith, 20

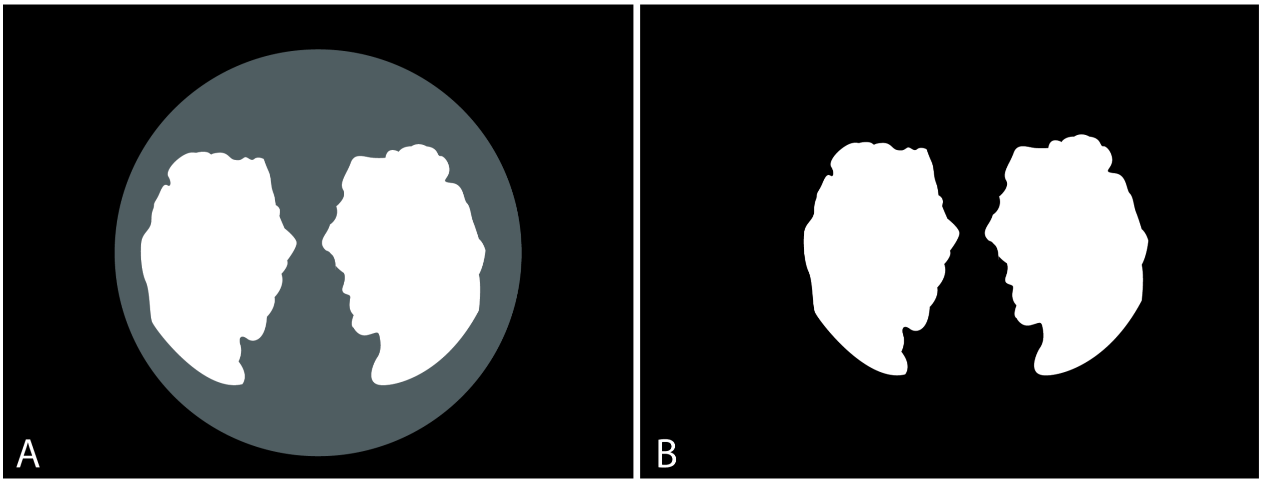

Fig. 2. Otolith images of *Pagellus erythrinus*, from Bizerte and Ghar El Melh lagoons, Tunisia, processed using Photoshop software; (**A**) real image; (**B**) processed image

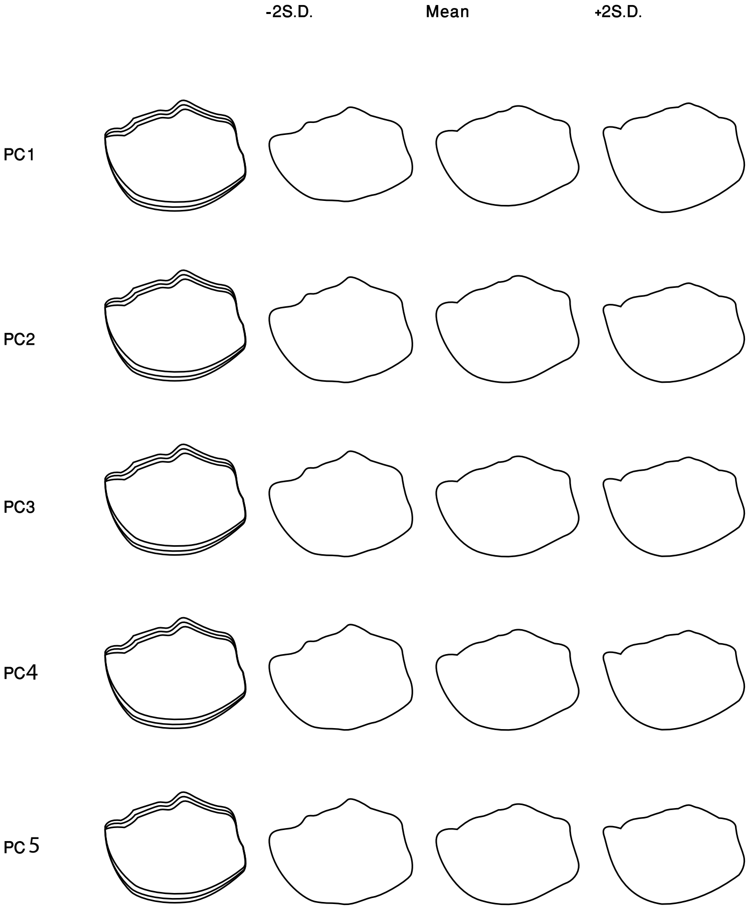

Fig. 3. Effect of five main principal components on otolith shape of *Pagellus erythrinus*, from Bizerte and Ghar El Melh lagoons, Tunisia; the columns show from the right the case where the score takes +2 SD, mean, and –2 SD as presented, and in the left, the last column showing the overlaid drawings of all three cases for the first five main components (PC)

elliptical Fourier harmonics were extracted, each harmonic being characterized by four coefficients (*A*, *B*, *C*, and *D*) and corresponding to the values of the sine and the cosine part of the variation in the *x* and *y* coordinates, resulting in 80 Fourier coefficients per individual.

The four Fourier Descriptors FDs for each harmonic were normalized with respect to the first harmonic to make them invariant to changes in size, location, rotation, and starting point. After this transformation, the three first FDs of the first harmonic were constant and thus not taken into account in the analysis. Each individual was thus represented by the subsequent 77 coefficients.

To determine the required number of harmonics for the best reconstruction of the otolith outline, the Fourier power (FP_n), the percentage Fourier power (FP%), and the cumulative percentage of the Fourier Power (FP*n*% cumulative) were calculated. The respective formulas are provided below:

$$\mathrm{FP}_n = (A^2{}_{\mathrm{n}+}B^2{}_{\mathrm{n}+}C^2{}_{\mathrm{n}+}D^2{}_{\mathrm{n}})^{-2}$$

where A_n, B_n, C_n, and D_n are the Fourier Coefficients

$$\mathrm{PF}\% = 100\mathrm{PF}_n \cdot (\Sigma_1{}^n\mathrm{PF}_n)^{-1}$$

$$\mathrm{PF}_n\% = \Sigma_1{}^n\mathrm{PF}_n\%$$

The relevant number of harmonics was 20 harmonics for a value equal to 99.99% of the cumulative percentage of the mean value of Fourier Power (PF_n%). Therefore, in the multivariate statistical analysis, 77 variables were included.

All harmonics were first tested for normality and homogeneity of variances, using the Shapiro–Wilk normality test (*W*) which showed that the size-corrected FDs were normally distributed ($P > 0.05$) between the two lagoons.

In order to compare the otolith shapes between Ghar El Melh and Bizerte lagoons, several analytical steps were performed. First, to visualize differences in otolith shape between right and left sides, an average otolith shape of each side for each lagoon was reconstructed by the outline reverse Fourier transformation.

Discriminant Function Analysis (DFA) was performed to adjust the XLSTAT model to otolith NEFDs from Bizerte Lagoon ($n = 55$ specimens) and from Ghar El Melh Lagoon ($n = 64$). Results of the FDA were then analysed by different tests including one-way MANOVA Wilks' Lambda, calculations of Mahalanobis distance and Fisher distance and test for significant differences in (*P*-value) the Fisher distance. These tests were conducted in order to test the similarities and differences within each population as well as between populations. In a simple sense, Mahalanobis and Fisher distances were calculated and tested for significant within each population (left–right analysis), and between same-side otoliths of the two populations (left–left, and right–right analyses).

For this analysis, the factorial graphic design allows visualizing individuals and variables. All statistical analyses were performed using XLSTAT 2010.

RESULTS

The Shapiro–Wilk normality test showed that the size-corrected FDs were normally distributed ($P > 0.05$) between the two lagoons. The result from the Multidimensional analysis (Discriminant Function Analysis) adjustment of the XLSTAT model to otolith NEFDs from the Bizerte Lagoon ($n = 55$) and the Ghar El Melh Lagoon ($n = 64$) has been analysed by different tests. The one-way MANOVA Wilks' Lambda test (Table 2) on the DFA showed a significant difference between the two lagoons ($P < 0.0001$).

The Mahalanobis distance (Table 3) describing the distance between left–right otoliths within each population was 24.46 for Bizerte lagoon and 28.50 for Ghar El Melh lagoon (left–right analysis). The Mahalanobis distances calculated between same-side otolith were 14.47 between Bizerte right and Ghar El Melh right (right–right analysis) and 18.18 between Bizerte left–Ghar El Melh left (left–left analysis). These results showed that the distance between (left and right) otoliths within each population was higher than the distance between left–left and right–right otoliths of the two populations. Mahalanobis and Fisher distances gave the same results. Within a population, the Fisher distances (Table 4) between otoliths pooled (left–right analysis) for Ghar El Melh Lagoon and Bizerte Lagoon were 4.03 and 2.92, respectively.

Fisher distances calculated between same-side otolith, i.e., Bizerte right–Ghar El Melh right and Bizerte left–Ghar El Melh left were 1.94 and 2.44, respectively. The values quoted for the distances between the two populations: (right–right and left–left) were close to each other.

Table 2

Wilks' Lambda test between *Pagellus erythrinus* from Bizerte and Ghar El Melh lagoons, Tunisia (Rao approximation)

Parameter	Value
Lambda	0.0803
F (Observed)	2.7106
F (Critical)	1.2013
DDL1	231
DDL2	475
P-value	<0.0001
Alpha	0.05

Table 3

Pairwise Mahalanobis Distances for two sampling sites for *Pagellus erythrinus* from Bizerte and Ghar El Melh lagoons, Tunisia

	GMR	GML	LBR	LBL
GMR	0	28.5027	14.4717	36,3502
GM L		0	52.3941	18.1824
LBR			0	24.4649
LBL				0

LBR = Lagoon Bizerte right otolith, LBL = Lagoon Bizerte left, GMR = Ghar El Melh right, GML = Ghar El Melh left.

P-values for Fisher distances (Table 4) showed that the distance was highly significant ($P < 0.05$) within each population (left–right analysis). In the same way, the result of the comparison of same-side otoliths between populations revealed a significant difference in their shapes (left–left and right–right analyses) ($P < 0.05$).

The Discriminant Factor Analysis (DFA) showed the projection of individuals on the two first axes (F1 and F2) (Fig. 4). The two-discriminant axes represented 56.58% and 29.23% of total variance, respectively, and accounted for 85.81% of the total variance. The first axis F1 separates the otoliths by their side (left–right) of *Pagellus erythrinus* for the two lagoons, the left otoliths being in the right part (positive) of the axis F1 and the right otoliths of both lagoons' being on the left part (negative side) of the F1 axis.

Table 4

Fisher distances between two sides of otoliths for two sampling sites (above diagonal) and *P*-values (below diagonal) for *Pagellus erythrinus* from Bizerte and Ghar El Melh lagoons, Tunisia

	GMR	GML	LBR	LBL
GMR	—	4.0346	1.9481	4.6856
GML	<0.0001	—	6.6670	2.4477
LBR	0.0106	<0.0001	—	2.9222
LBL	<0.0001	0.0011	0.0007	—

LBR = Lagoon Bizerte right otolith, LBL = Lagoon Bizerte left, GMR = Ghar El Melh right, GML: Ghar El Melh left.

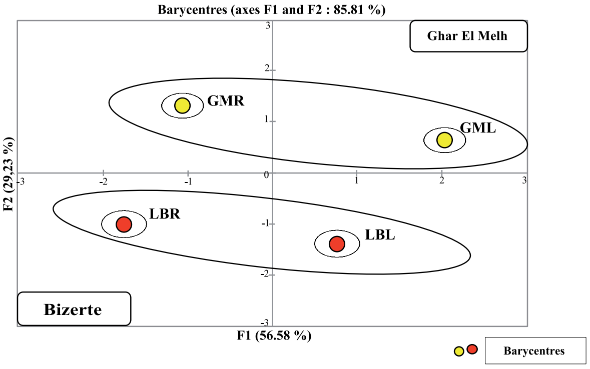

Fig. 4. Discriminant Function Analysis for two sides of *Pagellus erythrinus* from Bizerte and Ghar El Melh lagoons, Tunisia; LBR = Lagoon Bizerte right otolith , LBL = Lagoon Bizerte left, GMR = Ghar El Melh right, GML: Ghar El Melh left

For the axis F2, which discriminate the otoliths of the two populations: the otoliths (left–right) of the Ghar El Melh Lagoon is placed above the axis F2 (positive side), while the otoliths (left–right) of the lagoon of Bizerte are placed below (negative side).

DISCUSSION

The statistical analysis showed a significant asymmetry ($P < 0.001$) (right–left) within each population, and also significant differences between the same sides (left–left) and (right–right) otoliths between Bizerte and Ghar El Melh populations.

Otolith variations within population. The asymmetry detected within each population may be due to genetic variation between individuals. There is usually connectivity between a lagoon and the marine environment (Kraïem et al. 2009), which may suggest the existence of gene flow between marine and lagoon fish populations. Also, the insular environment is characterized by the highest tides in the Mediterranean, a proposed hypothesis to understand the asymmetry (left–right) of otoliths (Trojette et al. 2014).

In fact, according to Smida et al. (2012), the water temperature in the Bizerte Lagoon shows a remarkable variation, between 29°C (August) and 12°C (January). Also, the highest salinity (39.65‰) was recorded in October, while the minimum value (30.90‰) was observed in January. Thus these physicochemical variations may also be related to the characteristics of the lagoon-sea communications (Kraïem et al. 2009) which are subject to large marine influences.

Nourisson et al. (2013) suggested that the Ghar El Melh Lagoon is threatened by two main groups of factors (environmental and anthropogenic) that affect the balance of its natural system. Important fluctuations in physicochemical parameters were also recorded, e.g., the temperatures ranging from 34°C in the summer to 14°C in winter (Sghaier et al. 2012). Moreover, Reinart et al. (2003) found that the detailed picture of the actual conditions of the Ghar El Melh Lagoon and the connected Sidi Ali El Mekki Lagoon revealed a considerable spatial heterogeneity, which was consistent with the human to impacts acting on these ecosystems.

According to Panfili et al. (2005), otolith asymmetry can be explained by a genetic or environmental stress during development or a decrease in specific condition components like growth, fertility, or survival. The two fish populations can thus inhabit potentially stressing thermal conditions in their lagoon and so present high levels of asymmetry in their otoliths.

Otolith variations between populations. The presently reported study provides original data on stock characteristics of *Pagellus erythrinus* by using otolith shape variables. Otolith shape is not easy to interpret biologically since its determinants are only partially understood; however, otolith shape can function as a natural tag for fish stocks because it is a practical tool for stock separation in fisheries management (Begg and Waldman 1999). The presently reported study was intended to answer the question if the species occurring in the two lagoons represent a homogeneous or heterogeneous stock in a local fishery.

The otolith shape differences between the two lagoons (left–left, right–right) revealed that the two populations belong to different fish stock. In our study, we can consider that age and sex can be the factors inducing a strong variation in otolith shape within stocks (Simoneau et al. 2000). Firstly, for the age, this hypothesis is confirmed by the results of Bird et al. (1986) that found that otolith shapes in juveniles are different from the otolith shape in adult herring. As well, for the results of Hüssy (2008), who found that covariance between growth rate and otolith shape parameters in cod. Always in the same context, Reznick et al. (1989) and Secor and Dean (1989) found growth-related differences were responsible for differences in linear otolith morphometrics. This implies a genetic basis for the otolith shape that is closely related to the growth rate in fish. Secondly, in terms of the sex, *P. erythrinus* has been described as showing protogynous hermaphroditism, whereby some larger fish change sex from female to male (Klaoudatos and Klaoudatos 2004). In fact, the otolith shape differences detected between our populations can be explained by the effect of the sexual inversion (hermaphroditism) of some specimens of the population of Bizerte and, therefore, in greater discriminatory success between populations from the two lagoons. Moreover, the effect of the environment is not despicable. So, numerous investigations reported that environmental factors are almost certainly the cause of the variations in the otolith shape (Campana and Casselman 1993, Hüssy 2008, Cañás et al. 2012). Also, Volpedo and Echeverría (2000, 2003) supported this hypothesis.

On the one hand, the type of substrate where the fish is most frequently found and the habitat use (soft substrates/hard substrates/mixed substrates) may affect the different structures as the otoliths (sagittae) (Lombarte and Cruz 2007, Lombarte et al. 2010). In this context, Jaramillo et al. (2014) also described considerable differences in the morphology and the morphometry of otoliths of four benthic fishes: *Scorpaena scrofa* Linnaeus, 1758; *Mullus surmuletus* Linnaeus, 1758; *Uranoscopus scaber* Linnaeus, 1758; *Dagetichthys lusitanicus* (de Brito Capello, 1868) from the coast of Valencia and also associated this result with the type of substrate where the fish is most frequently found and the habitat use.

On the other hand, the salinity is one of the key factors in the marine environments that can directly affect habitat connectivity for a given species and indirectly the chemical composition and shape of their otoliths. Many published otolith articles noted that, the observed variation in otolith composition was probably related with differences between individual responses to the salinity interaction influence on temperature and the concentrations of many of the most common elements (e.g., Ca, Na, K, Mg, Cl) (Elsdon and Gillanders 2002, Martin and Wuenschel 2006).

Among all of the environmental factors, the temperature is probably the most important one for fish, and indirectly otolith, growth (Khemiri et al. 2005, Fablet et al. 2009). Indeed, fish are very sensitive to changes in temperature and can respond to a change of 0.03°C (Bull 1952).

Other factors than age, sex and environmental parameters have effect on otoliths (Torres et al. 2000, Volpedo and Echeverría 2000, 2003, Gauldie and Crampton 2002, Volpedo and Fuchs 2010), such as physiological factors as the hearing capabilities associated with specialization in acoustic communication (Paxton 2000, Lombarte and Cruz 2007); and the phylogeny (Nolf and Tyler 2006).

Different studies attributed the variability of otolith morphology to the effect of the environment; of which we can quote: Trojette et al. (2015), who reported the otolith shape variation for the two insular populations of *Diplodus annularis* (Linnaeus, 1758) along the Tunisian coast to the environmental factors. This result is similar to our results (difference in morphology of otoliths between the two populations and an otolith asymmetry (left–right) for each population). In the same way, Rebaya et al. (2016) revealed the presence of two different populations of the grey mullet between two lagoon stations located in the North of Tunisia (Ghar El Melh Lagoon and Bizerte Lagoon).

Emphasizing the importance of selected factors that influence the otolith variability, we can consider that the imprecision of sexual inversion effect (sexual dimorphism) measured in several hermaphrodite species like the species in question highlights a potential, limitation of otolith morphology analysis. Moreover, it helps to elucidate the different impact of sex and phenotypic effects on otolith morphology, demonstrating where these factors act to determine the observed differences. In the same context, sexual dimorphism in diet composition, particularly at the time of mating, has been demonstrated in several fish species (Casselman and Schulte-Hostedde 2004, Tsuboi et al. 2012) and could thus also explain the otolith shape dimorphism. The information listed above is fundamental if otolith shape is to be used in the future as an effective tool for management of fisheries resources.

In conclusion, the significant differences revealed in otolith (sagittae) morphology between the two populations, demonstrated in this study, were probably related to the result of an unknown combination of multiple factors (age, sex, genetic factors, environmental factors, etc.). In the future, various approaches such as genetic, the micro-chemical of otoliths analyses are necessary for understanding the use of otolith as an indicator of stock differentiations.

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