

**SHAPE AND SYMMETRY IN THE OTOLITH OF TWO DIFFERENT SPECIES
MULLUS BARBATUS AND MULLUS SURMULETUS (ACTINOPTERYGII: PERCIFORMES:
MULLIDAE) IN TUNISIAN WATERS**

Wided BAKKARI^{*1}, Marwa MEJRI¹, Samia BEN MOHAMED¹, Abdellah CHALH²,
Jean-Pierre QUIGNARD³, and Monia TRABELSI¹

¹ *Laboratoire d'Écologie, de Biologie et Physiologie des organismes aquatiques, Faculté des Sciences, Université
Tunis El Manar, Tunis, Tunisia*

² *Unité de génétique des populations et ressources biologiques, Faculté des Sciences, Campus Universitaire, Tunis,
Tunisia*

³ *Laboratoire d'Ichtyologie, Université Montpellier II, Montpellier, France*

Bakkari W., Mejri M., Ben Mohamed S., Chalh A., Quignard J.-P., Trabelsi M. 2020. Shape and symmetry in the otolith of two different species *Mullus barbatus* and *Mullus surmuletus* (Actinopterygii: Perciformes: Mullidae) in Tunisian waters. Acta Ichthyol. Piscat. 50 (2): 151–159.

Background. In Tunisian waters, there are only two mullet species, the red mullet, *Mullus barbatus* Linnaeus, 1758, and the striped red mullet, *Mullus surmuletus* Linnaeus, 1758. They are distributed along the coasts of eastern Atlantic from the North Sea to the northern part of West Africa. Therefore, *M. barbatus* and *M. surmuletus* are a valuable component of the commercial fishery in Tunisia. The goal of this study was to investigate the stock structure discrimination and otolith asymmetry for specimens of the mentioned above two species of the family Mullidae from waters of northern Tunisia.

Materials and methods. We collected a total of 120 specimens of *M. barbatus* and *M. surmuletus* from Bizerte waters. The sampling was conducted within three months (March–April–May). The Elliptical Fourier Analysis (EFA) was performed to evaluate the degree of similarity in the otoliths and to determine the asymmetry percentage.

Results. The Discriminant Factor Analysis shows significant results that are clearly demonstrated by an asymmetry when comparing otoliths ($P < 0.05$) within each population and a difference in shape when comparing the same side between the two species: between the two sides (right–right and left–left).

Conclusion. The comparison of the otolith morphology of the two species showed a significant 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 of the stock discrimination for *M. barbatus* and *M. surmuletus* and provides useful information for analyzing fisheries management of these species in Tunisia.

Keywords: otolith, *Mullus barbatus*, *Mullus surmuletus*, shape, asymmetry, Bizerte

INTRODUCTION

The red mullet, *Mullus barbatus* Linnaeus, 1758, and the striped red mullet, *Mullus surmuletus* Linnaeus, 1758 are the two most widely exploited species in Tunisian waters. Indeed, the current annual catch level of *Mullus barbatus* is estimated at 2000 t which represents approximately 55% of the total catch of fishes representing this family and constitutes approximately only 4% of estimated Tunisian demersal catches. (Anonymous 2004).

The striped red mullet, *Mullus surmuletus*, is common species on the Mediterranean shelf. It is distributed along the eastern Atlantic from the North Sea to the northern part

of West Africa and it is a demersal marine fish that inhabits sandy and rocky substrata, usually at depths not exceeding 200 m (Hureau 1986). It is also widely distributed in the Mediterranean and Black seas (Ben Tuvia 1981, 1990).

The spatial distribution pattern of both species was rather complex and, at least to a certain extent, this can be attributed to the different biotic and abiotic conditions prevailing in the same area. Indeed, many studies have shown that certain geological characteristics, such as the structure of the shelf, affect the species distribution. *Mullus surmuletus* prefers rough substrates, while *M. barbatus* is more abundant in muddy bottoms (Hureau 1986, Fischer et al. 1987).

* Correspondence: Dr W. Bakkari, Unité de Biologie marine LR 18 ES 41, Faculté des Sciences de Tunis, Université Tunis El Manar, Tunis, Tunisia, phone: +216 22 445 684, e-mail: (WB) widedispa@yahoo.fr, (MM) mejrimarwa991@gmail.com, (SBM) benmohamedsamia2015@yahoo.com, (AC) abdellah.chalh@gmail.com, (JPQ) jpp2@wanadoo.fr, (MT) atherina2002@yahoo.fr.

Different bony fish otoliths are composed of crystalline calcium carbonate suspended in a protein matrix. In fact, they are considered a true biological and environmental archive with the potential to facilitate reconstruction of environmental parameters (temperature, salinity) and life-history traits of fish (age, growth, reproduction, and migration) (Radhakrishnan et al. 2009).

The otolith shape is species specific (Sadighzadeh et al. 2014). The outer shape of fish otoliths has been used in the identification of the species (Nielsen et al. 2010) and/or fish stock (Cardinale et al. 2004, Ponton 2006). The three otolith pairs in teleost have a large morphological variability potential (Lombarte and Cruz 2007). This variability is especially true for the saccular otolith (sagitta) associated with the inner ear organ, sacculus, in non-ostariophsean fishes (Platt and Popper 1981, Lombarte and Cruz 2007). The morphological differences affect both the size and shape of the otoliths (Paxton 2000).

Sagittal otoliths have some phenotypic plasticity inter and/or intra-specific and inter-and/or intra-populations (Annabi et al. 2013). This property has been used very often in the discrimination of populations evolved in different environmental conditions taking into account possible confounding variables (sex, size) (Volpedo and Vaz-dos-Santos 2015). Therefore, analysis of the morphology of otoliths is one of the powerful tools to identify different fish populations (Furlani et al. 2007). This tool helped to distinguish the difference and origin of the asymmetry between different stocks of fish (Jónsdóttir et al. 2006).

Indeed, the stock is a concept of fisheries biology that corresponds to all individuals of the same species from the same geographical area. The population within a stock is such, that breeding is both possible and more likely between any pair within its distribution area than with individuals from other areas. A part or subdivision of a population, often based on geographical consideration, is a subpopulation. (Mahé et al. 2013). Based on the above-mentioned assumptions, fish stocks may be considered subpopulations of a particular species of fish, for which intrinsic variables (growth, recruitment, mortality, and fishing mortality) are among of significant factors in determining stock dynamics, whereas other factors, particularly immigration and emigration, are considered to have limited effect.

Each stock can have unique demographic properties and unique responses or reconstruction capabilities to exploitation. The biological attributes and productivity of the species may be affected if the stock structure considered by fisheries managers is erroneous (Smith et al. 1991). On the other hand, the otolith shape is also known to vary potentially intra-individually as left otolith shape may not be perfectly symmetrical to right otolith shape one and vice-versa (Díaz-Gill et al. 2015).

The objective of our study is to compare the morphological variability (asymmetry) of left and right otoliths for two mullid species *M. barbatus* and *M. surmuletus* sampled from Bizerte station, north-western Tunisian coast, using different statistical approaches.

MATERIALS AND METHODS

Sample collection. The specimens of *Mullus barbatus* and *Mullus surmuletus* were collected from Bizerte station (as shown in Fig. 1). The sampling was conducted during three months (March–April–May 2018). A total of 120 fishes representing the two species were collected during this period and were distributed as follows: 60 specimens of *M. barbatus* (30 males and 30 females) and 60 specimens of *M. surmuletus* (30 males and 30 females).

All fishes were caught using gillnets by artisanal 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).

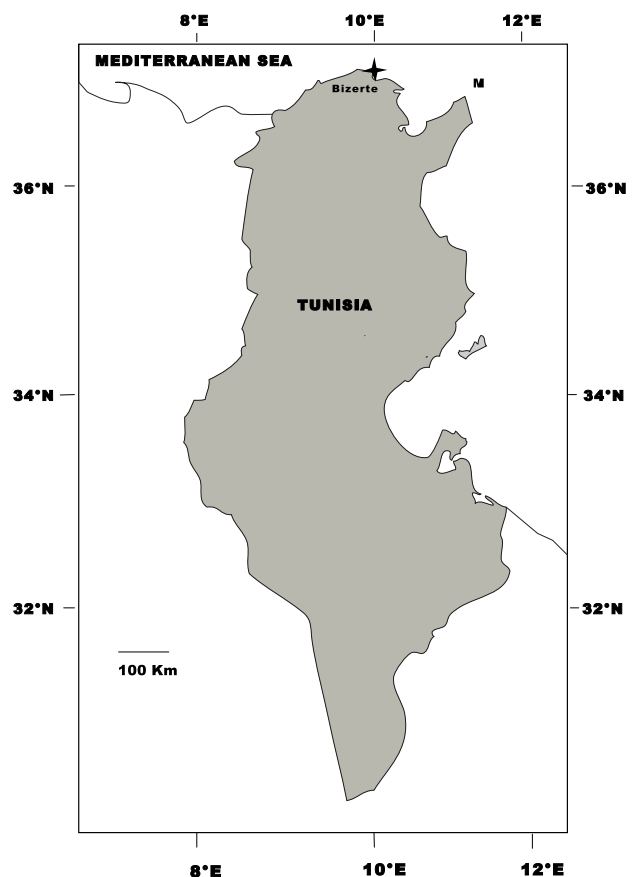


Fig. 1. Sampling site of *Mullus barbatus* and *Mullus surmuletus*, from Bizerte waters (Northern Tunisia)

Otolith extraction. The bony fishes usually have three pairs of otoliths. For our study, we are limited to the extraction of a single pair for each fish, sagitta, which are used to determine the age of fish. In our study, the otolith polymorphism, a total of 60 pairs were extracted, from each species *M. barbatus* and *M. surmuletus*, which were distributed in two lots: 30 pairs of male's otoliths and 30 pairs of female's. The method described for the extraction of the otolith was a cranium section of the fish's head with a knife having a very sharp blade (Fatnassi 2012) after removing the gills. After cleaning the surrounding tissues, the ventral structure of the neurocranial bones appeared. A cut in the external part of the bulla opened the internal ear,

Table 1
Length and weight of *Mullus barbatus* and *Mulus surmuletus* specimens from Bizerte waters (northern Tunisia)

Species	Sex	<i>n</i>	Total length [mm] (Mean ± SD)	Total weight [g] (Mean ± SD)
<i>M. barbatus</i>	M	30	170.87 ± 135.65	61.58 ± 27.89
<i>M. barbatus</i>	F	30	180.24 ± 145.31	69.81 ± 35.20
<i>M. surmuletus</i>	M	30	176.27 ± 141.12	64.04 ± 30.03
<i>M. surmuletus</i>	F	30	183.12 ± 150.10	74.60 ± 40.80

n = number of fish, SD = standard deviation, M = male, F = female.

from which the main otolith (usually the sagittae) can be removed (Panfili et al. 2002). The otoliths were extracted using plastic pliers, after that they were cleaned with distilled water, dried on an absorbent wrapped in cotton wool, referenced, and finally stored in Eppendorf tubes.

The respective otoliths were labeled as follows in the text, female *M. barbatus* right (FBR), female *M. barbatus* left (FBL), male *M. barbatus* right (MBR), male *M. barbatus* left (MBL), female *M. surmuletus* right (FSR), female *M. surmuletus* left (FSL), male *M. surmuletus* right (MSR), male *M. surmuletus* left (MSL).

Image and shape analyses. The photographic method adopted consists in the installation of a digital camera on a dissecting microscope after observation and the analysis of images taken on a computer screen which allows us to evaluate each image and store it in a database. Otoliths' photos were, then, processed, successively, using Adobe Photoshop CS6 software to transform the original otolith images into binary images (Fig. 2).

Afterward, the photos were analyzed by another program, Shape, which will create twenty harmonics for each otolith (each otolith represents an individual). The most popular

method for shape analysis of otoliths is based on Fourier descriptors (Duarte-Neto et al. 2008). Each harmonic is composed by four coefficients, called the Fourier coefficients (A, B, C, and D) corresponding to the values of the projection of the binary image on the axes (*X*) and (*Y*) (Kuhl and Giardina 1982), resulting in 80 coefficients per individual.

Statistical methods. The determination of morphological variation of otoliths in individual fishes was based on statistical analyses of the parameters from the developments in the Fourier series. The mathematical analyses of the otolith shape are performed by Elliptic Fourier Analysis (EFA). This technique describes the silhouette called Harmonic. Each harmonic is characterized by four Fourier Coefficients (*A*, *B*, *C*, and *D*) which calculates the Fourier Power (FP_{*n*}), the percentage of Fourier Power (FP%), and the cumulative percentage of the Fourier Power (FP_{*n*}%). The respective formulas (Crampton 1995) are provided below:

$$FP = (A_n^2 + B_n^2 + C_n^2 + D_n^2)^{-2}$$

$$FP\% = 100FP_n(\sum_1^n FP_n)^{-1}$$

$$FP_n\%c = \sum_1^n FP_n c$$

The cumulative percentage of Fourier Power (FP_{*n*}%) is calculated in order to determine the necessary and sufficient number of harmonics for better construction of the silhouette of the otolith (Crampton 1995). This is obtained for a value equal to 99.99% of the cumulative percentage of the mean Fourier Power (FP_{*n*}%). The results were fed into Microsoft XLSTAT and processed to assess the differences between different batches of otoliths, we performed multivariate analyses that enable the processing of all the otolith parameters at the same time.

The data matrix was subjected to a Discriminant Function Analysis (DFA), to illustrate the differences

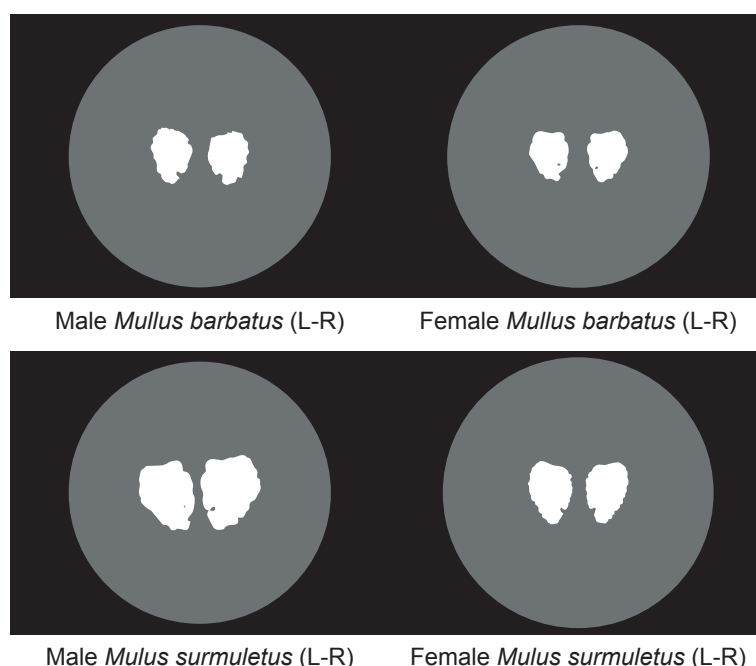


Fig. 2. Photographs of sagittal otoliths (left–right) of the two sexes for *Mullus barbatus* and *Mulus surmuletus*

and similarities between the observed groups and optimize the variability existing between them. The DFA was determined successively, while the factorial graphic designs allow visualizing individuals or variables. Various indicators and tests are also used in order to estimate the reliability of our results. These analyses were performed using 'XLSTAT' (2007) software.

Statistical analyses. The statistical analysis of the otolith shape was performed using the EFA describing the shape of the otolith. To have the most accurate otolith shape, the percentage of Fourier power was calculated to determine the number of necessary and sufficient harmonics. Therefore, this number was fixed at 20 and a total of 180 replicates were obtained (60 observations for each study site). Multivariate analysis (Wilks's Lambda test) was performed for the treatment of all otolith synchronous parameters. To assess the differences between our different batches of otoliths, we performed a multivariate analysis that enables the processing of all the otolith parameters at the same time. In addition, discriminant function analysis (DFA) was performed on shapes indices in order to illustrate the differences and similarities between the observed groups and optimize the existing variability. For this analysis, the factorial graphic designs allow visualizing individuals and variables. Various indicators and tests were also used in order to estimate the reliability of our results. All statistical analyses were performed using XLSTAT (2010) software.

Asymmetry percentage. In this part, we calculated with the Student's *t*-test for paired samples the presence of symmetry or asymmetry between the right and left otolith pairs for each species. Afterward, we determined the percentage of asymmetry using the mean (*M*) of the otoliths pairs with the shape representing an asymmetry

$$M = 100 O_A \cdot n^{-1}$$

where *n* is the total specimen number and *O_A* is the otolith asymmetry number.

RESULTS

Shape analysis. The Wilks' Lambda test (Rao approximation) performed on the otoliths revealed the presence of statistically significant differences between the two populations studied

(*P* < 0.0001) (Table 2). Within each species, the Fisher's distances showed an important distance between the right and left otoliths for females (FBR–FBL; 3.18) and males (MBR–MBL; 1.99), of *M. barbatus*. In addition, the two sides right and left for the species of *M. surmuletus* with 0.69 for females (FSR–FSL) and 1.36 for males (MSR–MSL).

Fisher distances calculated between two-side otolith (R–L), for male (MBR; MSR) and female (FBR; FSR) of the two species *Mullus barbatus* and *Mullus surmuletus* showed an important respective distance of 3.797 and 3.262. Also, a second important distance between the left side for two sexes (MBL; MSL); (FBL; FSL) were 4.282 and 3.947, respectively (Table 3). In the same population, *P*-value of Fisher distances (left–right) was highly statistically significant with *P* < 0.05; asymmetry, for the females (FBR; FBL) and males (MBR; MBL) originating in *M. barbatus*. However, for a symmetry (*P* = 0.173) was showed between females (FSR; FSL) and males (MSR; MSL) originating in *M. surmuletus* (*P* = 0.01). However, for the other population, *P*-value of Fisher distances (left–right) showed a symmetry (*P* = 0.173) between females (FSR; FSL) and males (MSR; MSL) originating in *M. surmuletus* (*P* = 0.01). Moreover, the sexual dimorphism was recorded only in the right side for males and females (FBR; FSR) (MBR; MSR) of the two specimens of *M. barbatus* and *M. surmuletus* showed an asymmetry with (*P* < 0.001). Also, an asymmetry was detected between the left side for male and female (FBL; FSL) (MBL; MSL) of the two species with (*P* < 0.001). and with percentage (6%, 10%) for *M. barbatus* and (9%, 8%) for *M. surmuletus* (Tables 4 and 5).

Table 2

Wilks' Lambda test (Rao approximation) between *Mullus barbatus* and *Mullus surmuletus* from Bizerte waters, Tunisia

Parameter	Value
Lambda	0.305
<i>F</i> (Observed)	2.939
<i>F</i> (Critical)	1.258
DDL1	98
DDL2	1394
<i>P</i> -value	<0.0001
alpha	0.05

Table 3

Fisher distances test between sexes and two sides of otoliths for *Mullus barbatus* and *Mullus surmuletus* (above diagonal) from Bizerte waters, Tunisia

	♀BR	♀BL	♂BR	♂BL	♀SR	♀SL	♂SR	♂SL
♀BR	0	<u>3.187</u>	1.348	2.991	3.262	3.521	3.051	2.849
♀BL		0	2.747	1.909	4.051	3.947	4.701	4.104
♂BR			0	<u>1.993</u>	3.719	4.271	3.797	3.136
♂BL				0	3.623	4.130	5.135	4.282
♀SR					0	<u>0.693</u>	2.280	1.726
♀SL						0	2.787	1.998
♂SR							0	<u>1.362</u>
♂SL								0

BR = *Mullus barbatus*, right otolith, BL = *Mullus barbatus*, left otolith, SR = *Mullus surmuletus*, right otolith, SL = *Mullus surmuletus*, left otolith; the underscored values denote the distance between the two populations; the values in bold indicate the distance within each population.

Table 4

Fisher distances between two sides of otoliths for *Mullus barbatus* and *Mullus surmuletus* (above diagonal) and *P*-values (below diagonal) from Bizerte waters, Tunisia

	♀BR	♀BL	♂BR	♂BL	♀SR	♀SL	♂SR	♂SL
♀BR	1	0.000	0.181	0.000	<u>≤0.0001</u>	<0.0001	0.000	0.001
♀BL	0.000	1	0.001	0.027	<0.0001	<u>≤0.0001</u>	<0.0001	<0.0001
♂BR	0.181	0.001	1	<u>0.019</u>	<0.0001	<0.0001	<u>≤0.0001</u>	0.000
♂BL	0.000	0.027	0.019	1	<0.0001	<0.0001	<0.0001	<u>≤0.0001</u>
♀SR	<0.0001	<0.0001	<0.0001	<0.0001	1	0.780	0.006	0.052
♀SL	<0.0001	<0.0001	<0.0001	<0.0001	0.780	1	0.001	0.019
♂SR	0.000	<0.0001	<0.0001	<0.0001	0.006	0.001	1	<u>0.173</u>
♂SL	0.001	<0.0001	0.000	<0.0001	0.052	0.019	0.173	1

BR = *Mullus barbatus*, right otolith, BL = *Mullus barbatus*, left otolith, SR = *Mullus surmuletus*, right otolith, SL = *Mullus surmuletus*, left otolith; values above the diagonal represent the values of Fisher distance; those with a single underscore indicate distance between the two populations; those with a double underscore indicate the distance within each population.

The Discriminant Factor Analysis (DFA) showed the projection of individuals on the two first axes (F_1 and F_2) (Fig. 3). The two-discriminant axes represented 43.62% and 21.89% of the total variance, respectively, and accounted for 65.51% of the total variance.

The right–left otolith of the two species were separated by the axis F_1 . Indeed, the otoliths of *M. surmuletus* were placed on the positive side. While, the otoliths of *M. barbatus* are placed on the negative side with an important distance (asymmetry) between the two sides (R–L).

For *M. barbatus*, F_2 separate the two sides (R–L) for the two sexes, the right otolith of males and females were placed on the positive side. Indeed, the left otolith of male and female were placed on the negative side. For *M. surmuletus*, separate the two sides for the same sex (MSR; MSL). Indeed, the right and left otoliths of the male were placed on the positive side. However, the right and left sides of female otoliths (FBL; FSL) were placed on the negative side.

DISCUSSION

Stock identification and the knowledge of the population spatial structure, using otolith shape study, provide a basis for understanding fish population dynamics

and achieving reliable assessments for fishery management (Reiss et al. 2009). In our study, the otolith shape analysis was studied to compare morphological variations existing between the two species of *Mullus* (*M. barbatus* and *M. surmuletus*), using different statistical approaches. The results of the comparison between the left and right otoliths for each species revealed an asymmetry between males and females of *M. barbatus*. Moreover, a symmetry was detected between the two sides of otoliths (right–left) from the two sexes of *M. surmuletus*. The statistical analysis showed a significant asymmetry ($P < 0.001$) between the same sides from the two sexes (left–left) and (right–right) of *M. barbatus* and *M. surmuletus* (Table 5). Such asymmetry (sexual dimorphism) can be explained by genetic or environmental stress during development or a decrease in specific condition components like growth, fertility or survival (Panfili et al. 2005).

According to Schwarzahans (1994), the function and role of the observed sexual dimorphism in the social life of the fishes is unknown. For example, within the genus *Neobythites* (Ophidiidae), about half of the species exhibit sexual dimorphism. An obvious explanation would be a specific sound-receiving ability designed to locate mating partners in the deep sea during the reproduction period (Shuster 2009). Indeed, the morphological alteration only occurs in the male otoliths (Schulz-Mirbach et al. 2010), therefore, the change for the male otoliths of fish can easily be evident and recognized (Jaramillo et al. 2014). The otoliths of female fish remain practically unchanged.

Many explanations are suggested based on many assumptions to explain the morphometrical otolith

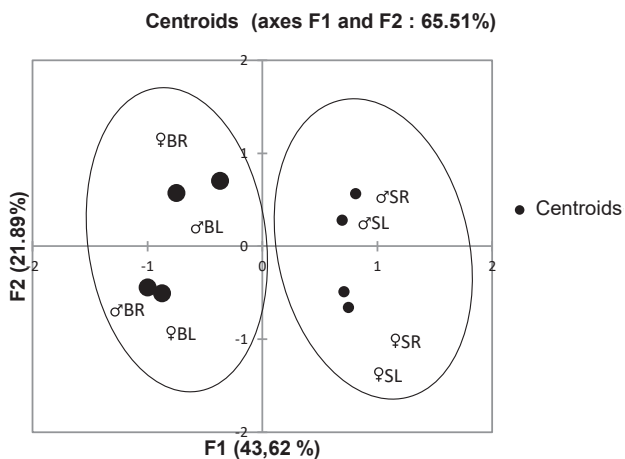


Fig. 3. Discriminant function analysis for two sexes and two sides of otoliths of *Mullus barbatus* and *Mullus surmuletus* from Bizerte waters, Tunisia

Table 5

Proportion test between two species: *Mullus barbatus* and *Mullus surmuletus*

Sex	<i>Mullus barbatus</i>		<i>Mullus surmuletus</i>	
	Asymmetry [%]	Symmetry [%]	Asymmetry [%]	Symmetry [%]
Males	6	24	9	21
Females	10	20	8	20

regularities for both sides and between two sexes for this preliminary work. It was known, however, how the otoliths exhibited some phenotypic plasticity inter- and/or intra-specific and inter and/or intra-population (Vignon 2013).

The asymmetry detected between the right and left otoliths in both species may be related to the physiological and behavioral responses of the two species that appear to be different within the same environment (Paxton 2000, Lombarte and Cruz 2007) results from a sum of factors like the ontogenetic change explained by the diet difference for the two species (Hüssy 2008, Capoccioni et al. 2011, Mille 2015), natural variations (illumination, rhythmic activity, osmotic pressure) and environmental conditions (temperature and salinity) (Gandar 2016). Indeed, according to Belgacem et al. (2013), the salinity ranged from 36.5‰ to 37‰. The water temperatures in Bizerte station also varied from 20.1 to 28.2°C.

As stated by many authors (e.g., Trojette et al. 2015, Rebaya et al. 2016), within a species, the otolith outline may show a discrepancy according to many factors such as the site, depth, etc.

However, the left versus the right symmetry was detected in other species such as flatfish (commonly due to the metamorphosis) (Campana 2004), bluefin tuna, *Thunnus thynnus* (Linnaeus, 1758) (see Megalofonou 2006), and *Chelon ramada* (Risso, 1827) (see Rebaya et al. 2016).

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, Lombarte et al. (2000), who studied the spatial segregation of *Mullus barbatus* and *M. surmuletus* in the western Mediterranean, have also reported that *M. barbatus* shows a clear preference for the areas where the shelf becomes wider, while *M. surmuletus* prefers narrow shelf areas with rocky or sandy bottoms. In addition, ecomorphological studies suggest the existence of adaptive morphological and anatomical characteristics that allow *M. barbatus* to exploit, better than its congener, the resources from muddy and turbid bottoms (Lombarte and Aguirre 1997).

On the other hand, Jaramillo et al. (2014) described a relation between the substrate and the morphologic and morphometric otolith characteristics of four benthic fish species, *Scorpaena scrofa* Linnaeus, 1758, *Mullus surmuletus* (Linnaeus, 1758), *Uranoscopus scaber* Linnaeus, 1758, and *Dagetichthys lusitanicus* (de Brito Capello, 1868) from the coast of Valencia. The analysis showed the significance of the differences in length and width between the right and left sagittae, as determined by the Student's *t*-test. In *Scorpaena scrofa*, no morphological differences between right and left sagittae were observed in the topography of the inner and outer face of the otolith.

This result is similar to our results (left–right symmetry) for marine populations and the author related it to the type of the substrate. *Scorpaena scrofa* is a solitary fish common on hard substrates and in caves, and it may move

to sandy bottom sediments despite its sedentary behavior. According to this study, the differences in otolith shape of *Scorpaena porcus* Linnaeus, 1758 may be not only due to phylogenetic factors but also to environmental and bioecological factors. In this work, the analysis of otolith shape variability has proven to be useful to determine the response of the two species in the same environments.

According to Apostolidis et al. (2009), genetic studies have been carried out in the Mediterranean Sea on both species, the red mullet (*Mullus barbatus*) and the striped red mullet (*Mullus surmuletus*), revealing highly structured metapopulations. A sharp genetic division was detected when comparing striped red mullet originating from the Atlantic Ocean and from the Mediterranean Sea.

In a recent study (Matić-Skoko et al. 2018), three main findings could be observed. At the beginning, there were different levels of diversity indices among these co-occurring species, where *M. barbatus* showed a higher allele richness and higher mean observed and expected heterozygosity in contrast to *M. surmuletus*.

Secondly, the reduced effective population size (N_e) and the sex-ratio values found in both species probably reflects recent demographic changes due to a combination of high fishing pressures (Pinsky and Palumbi 2014), habitat fragmentation (Bijlsma and Loeschke 2012) and naturally occurring fluctuations in the population size (Tserpes et al. 2002).

Thirdly, different patterns of genetic connectivity among populations sampled within the Mediterranean was observed for both species. A higher genetic structure was found for *M. barbatus* in contrast to the more homogenous pattern observed in *M. surmuletus* samples.

The influence of the genetic factors on the growth of the otoliths cannot be disregarded. In other teleosts, differences in growth in small geographic areas (offshore/inshore) were detected for Atlantic cod, *Gadus morhua* Linnaeus, 1758, in the Atlantic Ocean (Imstrand and Jónsdóttir 2003) and for the European anchovy, *Engraulis encrasicolus* (Linnaeus, 1758) in the Mediterranean Sea (Borsa 2002).

Hädar (1970) and Layachi et al. (2007) provided data on the maturity and the reproduction cycle of males of *Mullus barbatus*, *Mullus surmuletus* from the Mediterranean area, but they are in agreement with an anticipation of the spawning phase in males in comparison to females. The observed higher male effort, oriented in the reproductive activity, could explain the growth differences between sexes in *Mullus barbatus*. Indeed, female specimens show higher growth rates compared to male specimens, which accounts for a difference in size of about 2–4 cm of TL for the first two years of life (Tursi et al. 1994, Fiorentino et al. 1998, Voliani et al. 1998).

In addition, through the oocytes' diameter frequency distribution in spawner females, it is possible to define the reproductive behavior of red mullet as a batch spawner, with asynchronous ovary organization (Murua and Saborido-Rey 2003). Results of the presently reported study are in agreement with what has been described by other authors for different areas of the Mediterranean

(Menini et al. 2001, Metin 2005, Layachi et al. 2007). The oocytes' diameter frequency distribution shows a polymodal pattern also in females in the spawning phase. Every mode could correspond to different vitellogenic maturity stages of oocytes (Menini et al. 2001), which would suggest a continuous maturation pattern of oocytes and spawning of eggs in successive batches (Murua et al. 2003, Kokokiris et al. 2014). According to what was reported by other authors (Bougis 1952, Tursi et al. 1994, Metin 2005, Layachi et al. 2007), the males reach sexual maturity at lower sizes in comparison to females. This could be explained by the difference in growth patterns between males and females in *Mullus barbatus* described by several authors (Tursi et al. 1994, Fiorentino et al. 1998, Voliani et al. 1998, Joksimovic et al. 2008).

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 to determine the effect of the environment on otolith morphology.

REFERENCES

- Annabi A., Said K., Reichenbacher B.** 2013. Interpopulation differences in otolith morphology are genetically encoded in the killifish *Aphanius fasciatus* (Cyprinodontiformes). *Scientia Marina* **77** (2): 269–279. DOI: [10.3989/scimar.03763.02A](https://doi.org/10.3989/scimar.03763.02A)
- Anonymous** 2004. *Annuaire des statistiques des produits de la pêche en Tunisie*. Direction Générale à la Pêche et à l'Aquaculture (DGPA), Tunisia.
- Azouz A.** 1974. Les fonds chalutables de la région nord de Tunisie. 2. Potentialité de la pêche, écologie et répartition bathymétrique des poissons. *Bulletin de l'Institut National Scientifique et Technique d'Océanographie et de Pêche Salambô* **3** (1–4): (29–94)
- Belgacem W., Langar H., Pergent G., Ben Hassine O.K.** 2013. Associated mollusc communities of a *Posidonia oceanica* meadow in Cap Zebib (off North East Tunisia). *Aquatic Botany* **104**: 170–175. DOI: [10.1016/j.aquabot.2011.09.012](https://doi.org/10.1016/j.aquabot.2011.09.012)
- Ben Tuvia A.** 1981. Mullidae. [Unpaginated.] In: Fisher W., Bianchi G., Scott W.B. (eds.) *FAO Species Identification Guide for Fishery Purposes. Eastern central Atlantic, Fishing Area 34 and Part of 47. Volume 3. Bony fishes; families Malacanthidae to Scombridae* FAO, Rome and Department of Fisheries and Oceans, Ottawa, Canada.
- Bijlsma R., Loeschke V.** 2012. Genetic erosion impedes adaptive responses to stressful environments. *Evolutionary Applications* **5** (2): 117–129. DOI: [10.1111/j.1752-4571.2011.00214.x](https://doi.org/10.1111/j.1752-4571.2011.00214.x)
- Borsa P.** 2002. Allozyme, mitochondrial-DNA, and morphometric variability indicate cryptic species of anchovy (*Engraulis encrasicolus*). *Biological Journal of the Linnean Society* **75** (2): 261–269. DOI: [10.1046/j.1095-8312.2002.00018.x](https://doi.org/10.1046/j.1095-8312.2002.00018.x)
- Bougis P.** 1952. Recherches biométriques sur le rouget (*Mullus barbatus* L. et *Mullus surmuletus* L.). Centre National de la Recherche Scientifique, Paris, France.
- Campana S.E.** 2004. *Photographic atlas of fish otoliths of the Northwest Atlantic Ocean*. NRC Research Press, Ottawa, Canada. DOI: [10.1139/9780660191089](https://doi.org/10.1139/9780660191089)
- Capoccioni F., Costa C., Aguzzi J., Menesatti P., Lombarte A., Ciccotti E.** 2011. Ontogenetic and environmental effects on otolith shape variability in three Mediterranean European eel (*Anguilla anguilla*, L.) local stocks. *Journal of Experimental Marine Biology and Ecology* **397** (1): 1–7. DOI: [10.1016/j.jembe.2010.11.011](https://doi.org/10.1016/j.jembe.2010.11.011)
- Cardinale M., Doering-Arjes P., Kastowsky M., Mosegaard H.** 2004. Effects of sex, stock, and environment on the shape of known-age Atlantic cod (*Gadus morhua*) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences* **61** (2): 158–167. DOI: [10.1139/f03-151](https://doi.org/10.1139/f03-151)
- Crampton J.S.** 1995. Elliptic Fourier shape analysis of fossil bivalves: Some practical considerations. *Lethaia* **28** (2): 179–186. DOI: [10.1111/j.1502-3931.1995.tb01611.x](https://doi.org/10.1111/j.1502-3931.1995.tb01611.x)
- Díaz-Gil C., Palmer M., Catalán I.A., Alós J., Fuiman L.A., García E., del Mar Gil M., Grau A., Kang A., Maneja R.H., Mohan J.A., Morro B., Schaffler J.J., Buttay L., Riera-Batle I., Tolosa B., Morales-Nin B.** 2015. Otolith fluctuating asymmetry: A misconception of its biological relevance? *ICES Journal of Marine Science* **72** (7): 2079–2089. DOI: [10.1093/icesjms/fsv067](https://doi.org/10.1093/icesjms/fsv067)
- Duarte-Neto P., Lessa R., Stosic B., Morize E.** 2008. The use of sagittal otoliths in discriminating stocks of common dolphinfish (*Coryphaena hippurus*) off northeastern Brazil using multishape descriptors. *Journal of Marine Science* **65** (7): 1144–1152. DOI: [10.1093/icesjms/fsn090](https://doi.org/10.1093/icesjms/fsn090)
- Fiorentino F., Zamboni A., Rossi M., Relini G.** 1998. The growth of the red mullet (*Mullus barbatus*, L. 1758) during the first years of life in the Ligurian Sea (Mediterranean). *Cahiers Options Méditerranéennes* **35**: 65–78.
- Fischer W., Schneider M., Bauchot M.L.** 1987. *Fiches FAO d'identification des espèces pour les besoins de la pêche (Révision 1). Méditerranée et mer Noire. Zone de pêche 37*. FAO, Rome.
- Furlani D., Gales R., Pemberton D.** (eds.) 2007. *Otoliths of common Australian temperate fish; A photographic guide*. CSIRO Publishing, Collingwood, VIC, Australia.
- Gandar A., Jean S., Canal J., Marty-Gasset N., Gilbert F., Lafaille P.** 2016. Multistress effects on goldfish (*Carassius auratus*) behavior and metabolism. *Environmental Sciences and Pollution Research* **23** (4): 3184–3194. DOI: [10.1007/s11356-015-5147-6](https://doi.org/10.1007/s11356-015-5147-6)
- Gharbi H., Abdelkader B., Zaarah Y.** 1986. Résultats des campagnes de prospection par chalutage dans la région nord de la Tunisie (Janvier–Juin 1984). *Bulletin de l'Institut National des Sciences et Technologies de la mer. Océanographie. Pêche, Salammbô* **13**: 123–139.
- Häidar Z.** 1970. L'écologie du rouget (*Mullus barbatus* L.) en Adriatique orientale. *Acta Adriatica* **14** (1): 1–94.

- Hureau J.C., Litvinenko N.I.** 1986. Scorpaenidae. Pp. 1211–1229. In: Whitehead P.J.P., Bauchot M.-L., Hureau J.-C., Nielsen J., Tortonese E. (eds.) Fishes of the North-eastern Atlantic and the Mediterranean, Vol. 3, UNESCO, Paris.
- Hüssy K.** 2008. Otolith shape in juvenile cod (*Gadus morhua*): Ontogenetic and environmental effects. Journal of Experimental Marine Biology and Ecology **364** (1): 35–41. DOI: [10.1016/j.jembe.2008.06.026](https://doi.org/10.1016/j.jembe.2008.06.026)
- Imsland A.K., Jónsdóttir Ó.D.B.** 2003. Linking population genetics and growth properties of Atlantic cod. Reviews in Fish Biology and Fisheries **13** (1): 1–26. DOI: [10.1023/A:1026373509576](https://doi.org/10.1023/A:1026373509576)
- Jaramillo A.M., Tombari A.D., Dura V.B., Rodrigo M.E., Volpedo A.V.** 2014. Otolith eco-morphological patterns of benthic fishes from the coast of Valencia (Spain). Thalassas **30** (1): 57–66.
- Joksimović A., Regner S., Kasalica O., Đurović M., Pešić A., Mandić M.** 2008. Growth of the red mullet, *Mullus barbatus* (Linnaeus, 1758) on the Montenegrin shelf (south Adriatic). Electronic Journal of Ichthyology **1**: 1–7.
- Jónsdóttir I.G., Campana S.E., Marteinsdóttir G.** 2006. Otolith shape and temporal stability of spawning groups of Icelandic cod (*Gadus morhua* L.). ICES Journal of Marine Science **63** (8): 1501–1512. DOI: [10.1016/j.icesjms.2006.05.006](https://doi.org/10.1016/j.icesjms.2006.05.006)
- Kokokiris L., Stamoulis S., Monokrousos N., Doulgeraki S.** 2014. Oocytes development, maturity classification, maturity size and spawning season of the red mullet (*Mullus barbatus* Linnaeus, 1758). Journal of Applied Ichthyology **30** (1): 20–27. DOI: [10.1111/jai.12292](https://doi.org/10.1111/jai.12292)
- Kuhl F.P., Giardina C.R.** 1982. Elliptic Fourier features of a closed contour. Computer Graphics and Image Processing **18** (3): 236–258. DOI: [10.1016/0146-664X\(82\)90034-X](https://doi.org/10.1016/0146-664X(82)90034-X)
- Layachi M., Melhaoui M., Srouf A., Ramdani M.** 2007. Contribution à l'étude de la reproduction et de la croissance du Rouget-barbet de vase (*Mullus barbatus* L., 1758) de la zone littorale méditerranéenne de Nador (Maroc). Bulletin de l'Institut Scientifique, Rabat, Section Sciences de la Vie **29**: 43–51.
- Lombarte A., Aguirre H.** 1997. Quantitative differences in the chemoreceptor systems in the barbels of two species of Mullidae (*Mullus surmuletus* and *M. barbatus*) with different bottom habitats. Marine Ecology Progress Series **150**: 57–64. DOI: [10.3354/meps150057](https://doi.org/10.3354/meps150057)
- Lombarte A., Cruz A.** 2007. Otolith size trends in marine fish communities from different depth strata. Journal of Fish Biology **71** (1): 53–76. DOI: [10.1111/j.1095-8649.2007.01465.x](https://doi.org/10.1111/j.1095-8649.2007.01465.x)
- Lombarte A., Palmer M., Matallanas J., Gómez-Zurita J., Morales-Nin B.** 2010. Ecomorphological trends and phylogenetic inertia of otolith sagittae in Nototheniidae. Environmental Biology of Fishes **89** (3–4): 607–618. DOI: [10.1007/s10641-010-9673-2](https://doi.org/10.1007/s10641-010-9673-2)
- Lombarte A., Recasens L., González M., Gil de Sola L.** 2000. Spatial segregation of two species of Mullidae (*Mullus surmuletus* and *M. barbatus*) in relation to habitat. Marine Ecology Progress Series **206**: 239–249. DOI: [10.3354/meps206239](https://doi.org/10.3354/meps206239)
- Mahé K., Coppin F., Vaz S., Carpentier A.** 2013. Striped red mullet (*Mullus surmuletus*, Linnaeus, 1758) in the eastern English Channel and southern North Sea: Growth and reproductive biology. Journal of Applied Ichthyology **29** (5): 1063–1073. DOI: [10.1111/jai.12266](https://doi.org/10.1111/jai.12266)
- Matić-Skoko S., Šegvić-Bubić T., Mandić I., Izquierdo-Gomez D., Arneri E., Carbonara P., Grati F., Ikica Z., Kolitari J., Milone N., Sartor P., Scarcella G., Tokaç A., Tzanatos E.** 2018. Evidence of subtle genetic structure in the sympatric species *Mullus barbatus* and *Mullus surmuletus* (Linnaeus, 1758) in the Mediterranean Sea. Scientific Reports **8**: e676. DOI: [10.1038/s41598-017-18503-7](https://doi.org/10.1038/s41598-017-18503-7)
- Megalofonu P.** 2006. Comparison of otolith growth and morphology with somatic growth and age in young-of-the-year bluefin tuna. Journal of Fish Biology **68** (6): 1867–1878. DOI: [10.1111/j.1095-8649.2006.01078.x](https://doi.org/10.1111/j.1095-8649.2006.01078.x)
- Menini S., Pirone A., Fabiani O., Volani A., Baino R.** 2001. Observations on gonadic maturity and fecundity of *Mullus barbatus*. Mediterranean Marine Biology **8** (1): 736–740.
- Metin G.** 2005. İzmir Körfezi'nde barbunya (*Mullus barbatus*, L., 1758) balığının üreme özellikleri. [Reproduction characteristics of red mullet (*Mullus barbatus* L. 1758) in Izmir Bay.] EU Journal of Fisheries and Aquatic Sciences **22** (1–2): 225–228. [In Turkish.] DOI: [10.12714/egejfas.2005.22.1.5000156914](https://doi.org/10.12714/egejfas.2005.22.1.5000156914)
- Mille T., Mahe K., Villanueva M.C., De Pontual H., Ernande B.** 2015. Sagittal otolith morphogenesis asymmetry in marine fishes. Journal of Fish Biology **87** (3): 646–663. DOI: [10.1111/jfb.12746](https://doi.org/10.1111/jfb.12746)
- Murua H., Saborido-Rey F.** 2003. Female reproductive strategies of marine fish species of the North Atlantic. Journal of Northwest Atlantic Fishery Science **33**: 23–31. DOI: [10.2960/J.v33.a2](https://doi.org/10.2960/J.v33.a2)
- Murua H., Kraus G., Saborido-Rey F., Witthames P.R., Thorsen A., Junquera J.** 2003. Procedures to estimate fecundity of marine fish species in relation to their reproductive strategy. Journal of Northwest Atlantic Fishery Science **33**: 33–54.
- Nielsen J.R., Methven D.A., Kristensen K.** 2010. A statistical discrimination method using sagittal otolith dimensions between sibling species of juvenile cod *Gadus morhua* and *Gadus ogac* from the Northwest Atlantic. Journal of Northwest Atlantic Fishery Science **43**: 27–45. DOI: [10.2960/J.v43.m667](https://doi.org/10.2960/J.v43.m667)
- Panfili J., de Pontual H., Troadec H., Wright P.J.** (eds.) 2002. Manuel de sclérochronologie des poissons. Coédition Ifremer–IRD, France.
- Panfili J., Durand J.-D., Diop K., Gourène B., Simier M.** 2005. Fluctuating asymmetry in fish otoliths and heterozygosity in stressful estuarine environments

- (West Africa). *Marine and Freshwater Research* **56** (5): 505–516. DOI: [10.1071/MF04138](https://doi.org/10.1071/MF04138)
- Paxton J.R.** 2000. Fish otoliths: Do sizes correlate with taxonomic group, habitat and/or luminescence? *Philosophical Transactions of the Royal Society of London B* **355** (1401): 1299–1303. DOI: [10.1098/rstb.2000.0688](https://doi.org/10.1098/rstb.2000.0688)
- Pinsky M.L., Palumbi S.R.** 2014. Meta-analysis reveals lower genetic diversity in overfished populations. *Molecular Ecology* **23** (1): 29–39. DOI: [10.1111/mec.12509](https://doi.org/10.1111/mec.12509)
- Ponton D.** 2006. Is geometric morphometrics efficient for comparing otolith shape of different fish species? *Journal of Morphology* **267** (6): 750–757. DOI: [10.1002/jmor.10439](https://doi.org/10.1002/jmor.10439)
- Platt C., Popper A.N.** 1981. [Chapter 1] Fine structure and function of the ear. Pp. 3–38. DOI: [10.1007/978-1-4615-7186-5_1](https://doi.org/10.1007/978-1-4615-7186-5_1) In: Tavolga W.N., Popper A.N., Fay R.R. (eds.) *Hearing and sound communication in fishes*. Springer, New York. DOI: [10.1007/978-1-4615-7186-5](https://doi.org/10.1007/978-1-4615-7186-5)
- Radhakrishnan K.V., He W., Liu M., Xie S.** 2009. Otoliths—the biological CD-ROMs of fish. *Current Science* **97** (8): 1121–1122.
- Rebaya M., Ben-Faleh A., Allaya H., Khedher M., Marsaoui B., Chalh A., Quignard J.-P., Trabelsi M.** 2016. Morphological variability of saccular otoliths in two populations of *Liza ramada* (Risso, 1810) (Mugilidae) in Tunisian lagoons (Bizerte and Ghar El Melh). *Cahiers de Biologie Marine* **57** (3): 227–234.
- Reiss H., Hoarau G., Dickey-Collas M., Wolff W.J.** 2009. Genetic population structure of marine fish: Mismatch between biological and fisheries management units. *Fish and Fisheries* **10** (4): 361–395. DOI: [10.1111/j.1467-2979.2008.00324.x](https://doi.org/10.1111/j.1467-2979.2008.00324.x)
- Sadighzadeh Z., Valinassab T., Vosugi G., Motallebi A.A., Fatemi M.R., Lombarte A., Tuset V.M.** 2014. Use of otolith shape for stock identification of John's snapper, *Lutjanus johnii* (Pisces: Lutjanidae), from the Persian Gulf and the Oman Sea. *Fisheries Research* **155**: 59–63. DOI: [10.1016/j.fishres.2014.02.024](https://doi.org/10.1016/j.fishres.2014.02.024)
- Schulz-Mirbach T., Ladich F., Riesch R., Plath M.** 2010. Otolith morphology and hearing abilities in cave- and surface-dwelling ecotypes of the Atlantic molly, *Poecilia mexicana* (Teleostei: Poeciliidae). *Hearing Research* **267** (1–2): 137–148. DOI: [10.1016/j.heares.2010.04.001](https://doi.org/10.1016/j.heares.2010.04.001)
- Schwarzhan W.** 1994. Sexual and ontogenetic dimorphism in otoliths of the family Ophidiidae. *Cybiurn* **18** (1): 71–98.
- Shuster S.M.** 2009. Sexual selection and mating systems. *Proceedings of the National Academy of Sciences of the United States of America* **106** (Suppl. 1): 10009–10016. DOI: [10.1073/pnas.0901132106](https://doi.org/10.1073/pnas.0901132106)
- Smith P.J., Francis R.I.C.C., McVeagh M.** 1991. Loss of genetic diversity due to fishing pressure. *Fisheries Research* **10** (3–4): 309–316. DOI: [10.1016/0165-7836\(91\)90082-Q](https://doi.org/10.1016/0165-7836(91)90082-Q)
- Suquet M., Person'le Ruyet J.** 2001. Les rougets barbets (*Mullus barbatus*, *Mullus surmuletus*): Biologie, pêche, marché et potentiel aquacole. Ifremer, France.
- Trojette M., Ben Falah A., Fatnassi M., Marsaoui B., Mahouachi N., Chalh A., Quignard J.-P., Trabelsi M.** 2015. Stock discrimination of two insular populations of *Diplodus annularis* (Actinopterygii: Perciformes: Sparidae) along the coast of Tunisia by analysis of otolith shape. *Acta Ichthyologica et Piscatoria* **45** (4): 363–372. DOI: [10.3750/AIP2015.45.4.04](https://doi.org/10.3750/AIP2015.45.4.04)
- Tserpes G., Fiorentino F., Levi D., Cau A., Murenu M., Zamboni A., Papaconstantinou C.** 2002. Distribution of *Mullus barbatus* and *Mullus surmuletus* (Osteichthyes: Perciformes) in the Mediterranean continental shelf: Implications for management. *Scientia Marina* **66** (Suppl. 2): 39–54. DOI: [10.3989/scimar.2002.66s239](https://doi.org/10.3989/scimar.2002.66s239)
- Tursi A., Matarrese A., D'Onghia G., Sion L.** 1994. Population biology of red mullet (*Mullus barbatus* L.) from the Ionian Sea. *Marine Life* **4** (2): 33–43.
- Vignon M.** 2013. Démêler et quantifier les sources de variation des otolithes à multiples échelles. Nouvelle méthode de partition de la variabilité morphologique à l'aide d'arbres de classification et de régression. In: Colloque National de Sclérochronologie: Structures dures et/ou calcifiées chez les organismes aquatiques: Leur utilisation en écologie halieutique, 2–4 July 2013, Rennes, France. <https://prodinra.inra.fr/record/282399>
- Voliani A., Abella A., Serena F.** 1998. Problematiche inerenti la valutazione dello stato di sfruttamento di *Mullus barbatus*. [Issues related to the evaluation of the exploitation status of *Mullus barbatus*.] *Biologia Marina Mediterranea* **5** (2): 169–183. [In Italian.]
- Volpedo A.V., Vaz-dos-Santos A.M.** (eds.) 2015. Métodos de estudios con otolitos: principios y aplicaciones; Métodos de estudos com otólitos: princípios e aplicações. Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.

Received: 12 July 2019

Accepted: 28 February 2020

Published electronically: 1 June 2020