STOCK DISCRIMINATION OF THE BOGUE, *BOOPS BOOPS* (ACTINOPTERYGII: PERCIFORMES: SPARIDAE), FROM TWO TUNISIAN MARINE STATIONS USING THE OTOLITH SHAPE

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Background. The bogue, *Boops boops* (Linnaeus, 1758), is the most common sparid fish in the Tunisian waters. Despite its economic importance, the stock discrimination of this species is poorly understood up to date. As the otolith shape has long been known to be species-specific and recent studies have shown its value as an indicator of stock identity, the presently reported study was carried out for the first time to investigate the discrimination of the stock structure of *B. boops* samples collected from two sampling sites at the marine stations of Monastir and Zarzis located in Tunisia using the otolith shape descriptors analysis.

Materials and methods. A total of 183 adult samples of *B. boops* were collected between January and August 2019 from two sampling sites at the Monastir and Zarzis marine stations. The outlines of sagittal otolith pairs from fish samples collected from the two sites were digitized and analyzed for shape variation by elliptical Fourier analysis (EFA).

Results. Discriminant Function Analysis (DFA) showed statistically significant differences in otolith shape within and between fish samples in the two sites, i.e., there was an asymmetry. This asymmetry was found between the left and right otoliths within each site, as well as between the same sides (left–left) and (right–right) otoliths between the Monastir and Zarzis stations.

Conclusion. Fish stock samples at the two stations represent two groups or populations of the Tunisian bogue stock and should be managed separately. In addition, the asymmetry in the otolith shape between fish samples from the two stations can be attributed to differences in the rate of growth resulting from local environmental factors such as water temperature, salinity, habitat, and diet. However, the inter-individual or even the intra-population asymmetry between the right and left otoliths can be explained by the possibility of having intra-individual stress that led to abnormalities in the development of the individuals or by the presence of poor living conditions for the larvae, resulting from unfavorable environments.

Keywords: *Boops boops*, elliptic Fourier descriptors, otolith shape, stock discrimination, Tunisia

INTRODUCTION

The bogue, *Boops boops* (Linnaeus, 1758), is the commercially important sea bream and the most dominant species in the Sparidae family (Layachi et al. 2015). This species has a wide geographical distribution that extends from the eastern Atlantic Ocean to the Black and Mediterranean Seas, including Tunisian waters (Amira et al. 2019). In addition, this species is demersal, as well as semi-pelagic, gregarious, and lives on all types of the bottom substrata, including mud, rock, sand, and seagrass beds (Simsek et al. 2018). However, it is commonly found at depths between 50 and 350 m and in coastal waters, it sometimes moves in aggregations, ascending mainly to the surface at night (Ider et al. 2017). Pollard et al. (2014) mentioned that *B. boops* grows rapidly in length during the first year of life, where it reaches 53.49% of its final length of growth and can live up to seven years. However, Khemiri et al. (2005) determined that the sexual maturity of *B. boops* can be reached between one and three years of age at a length of about 15.2 cm. In Egypt, Azab et al.

(2019) stated that its total length ranged from 9.3 to 23 cm and the total weight varied between 7.4 and 133.1 g, while the length–weight relation revealed a tendency towards isometric growth. They also said that its longevity is 4 years and the length at first capture and maturity is 12.5 and 13.2 cm, respectively, while the total mortality rate is 2.26% y^{-1}. Along the Mediterranean coast in Benghazi, El-Maremie, and El-Mor (2015) reported that it feeds on a wide range of prey species, including Crustacea, Porifera, Coelenterata, Seagrasses, Mollusca, and Protozoa, while along the Algerian coast, it is omnivorous and feeds on benthic (Crustacea, Mollusca, Annelida, Sipuncula, Plantae) and pelagic preys (Siphonophorae, Copepoda, eggs) (Derbal and Kara 2008). Jawad (2003) analyzed the asymmetry in some bilateral characters of samples collected from coastal waters near Benghazi City, Libya, and found the highest level of asymmetry in the post-orbital length character, while he observed the lowest level in the eye lens diameter and weight. Also, Ider et al. (2017) analyzed the variability of otolith shape in three populations of *B. boops* collected from the Algerian coast and observed a significant difference between the left and right otoliths and added that the shape is influenced by the fish length and the sampling location, but it is not related to sex and age. In addition, Ferri et al. (2018) studied the variability of the otolith morphology and morphometry in six juvenile specimens collected from the eastern coastal Adriatic region and recorded variations in the sagittae shape, margins, and anterior region among the studied juveniles. Moreover, Mahé et al. (2019) analyzed the directional bilateral asymmetry of otoliths in specimens collected from 11 geographical regions from the Canary Islands to the Aegean Sea using elliptical Fourier descriptors and found a significant otolith bilateral asymmetry at the global scale and non-significant asymmetry at the scale of sampling locations.

In Tunisian waters, however, Anato and Ktari (1986) studied the age and growth of the *B. boops* using otolith and scale reading and found that the age of first sexual maturity is between the thirteenth and fifteenth months after birth and the linear growth and weight of females slightly increase over males during the first four years of life. Similarly, Khemiri et al. (2005) determined the age and growth in four areas along the Tunisian coast by studying signs of growth in cross-sections of otoliths and reported that the hyaline zone deposits annually from November to April and that the increases in length and age fit the von Bertalanffy equations. Additionally, Cherif et al. (2008) analyzed the length–weight relation of individuals in the Gulf of Tunis and found a high degree of a positive correlation between total length and total weight. Moreover, the infection of the gallbladder of *B. boops* by *Ceratomyxa ghannouchensis* and *C. pallida* parasites has been confirmed in the Gulf of Gabes by Thabet et al. (2019).

Otoliths are calcified structures found in the inner ear cavity of all teleost fish and functionally serve as a balance organ and also aid in hearing. Both the left and right inner ear contain three pairs of otoliths. These otoliths grow throughout the life of the fish and, unlike scales and bones,

are metabolically inert, i.e., once deposited, otolith material is unlikely to be resorbed or altered (Campana 1999). Consequently, otolith shape remains unaffected by short term changes in fish condition or environmental variations (Campana and Casselman 1993). Recent studies have shown that the saccular otoliths (sagittae) display high specific inter- and intra-variability in shape and size (Ferri et al. 2018). Thus, they have been widely used as an effective tool to identify fish species and populations and to discriminate their stocks in different habitats (Jawad et al. 2018). In addition, the role of the otolith approach has also been expanded to include ecological research and conservation applications (Miller et al. 2010), ontogenetic processes (Capoccioni et al. 2011), spatial and temporal migrations (Smith and Kwak 2014), and fish age (Škeljo et al. 2015), which has powerful implications for fisheries science and management (Vasconcelos et al. 2018). Besides, the anatomy of the otolith has been shown to play an important role in discriminating the stock based on the life traits of individuals with the evolution of the habitat (Arai et al. 2007). Studies of morphological variability in the otolith shape, structure, and development have shown that the otolith shape is species-specific and is influenced by ontogenetic, genetic, and environmental factors (Fashandi et al. 2019), as well as by sex, growth, maturity, and pattern of fishery exploitation (Begg and Brown 2000), or by individual characteristics, e.g., the individual genotype (Jawad et al. 2020) or the physiological state (Campana and Neilson 1985). But the possible cause of the intra-individual variation, particularly the asymmetry in shape between the right and left otoliths, has been poorly studied (Mille et al. 2015). Under normal conditions, the three orthogonal semi-circular otoliths at both sides of the head are morphologically symmetrical (Panfili et al. 2002), although there are some inter-specific modifications in the size and shape (Popper and Lu 2000) but the weight difference, i.e., mass asymmetry, between masses of the left and right otoliths has been also observed (Yedier et al. 2018).

Indeed, the external contour or shape of the otoliths has been studied by using several techniques, including univariate descriptors such as shape factors that include roundness or circularity (Tuset et al. 2003), geometric morphometrics (Vergara-Solana et al. 2013), wavelet functions (Ferri et al. 2018), growth markers (Benzinou et al. 2013), curvature scale space (Mapp et al. 2017), and geodesic methods (Benzinou et al. 2013). Although, the elliptical Fourier analysis (EFA) remains the most widely used method for describing and characterizing outlines, capturing outline information efficiently in a quantifiable manner has been widely used to describe the variation of the otolith shape between fish species in stock discrimination and analysis of population structure of diverse species (Mahé et al. 2019).

Up to now, some studies have been conducted on the otolith shape variability in some Tunisian sparid species such as *Diplodus annularis* (Linnaeus, 1758) (see Trojette et al. 2015) and *Pagellus erythrinus* (Linnaeus, 1758) (see Mejri et al. 2018) cohabiting different habitats. However, no studies have been carried out on the bogue, *B. boops*. Thus, the presently reported study was carried out for the first time

to investigate the discrimination of the stock structure of *B. boops* samples collected from two sampling sites at the marine stations of Monastir and Zarzis located in northern Tunisia using the otolith shape descriptors analysis.

MATERIALS AND METHODS

Sampling. As the first sexual maturity of *Boops boops* starts at a total length (TL) of about 138 to 152 mm (Bottari et al. 2014), a total of 183 adult specimens of this fish with a TL ranging from 150 to 206 mm were used for this study. Fish samples were caught alive between January and August 2019 from the Monastir (35°46′40″N, 10°49′34″E) and Zarzis (33°30′14″N, 11°06′43″E) marine stations in Tunisia (Fig. 1) by gillnets using coastal boats ranging from 5 to 13 m in length. The geographical positions of the sampling locations at the Monastir and Zarzis stations were determined with the global positioning system (GPS) with coordinates 35°48′27″N, 10°52′28″E and 33°31′37″N, 11°14′13″E, respectively. Immediately after capture, the status of sexual maturity was checked for each sample, TL was measured with an ichthyometer, and total weight (TW) was recorded and the values from the last two parameters were rounded to the nearest 0.01 mm and 0.1 g, respectively (Table 1).

Otolith extraction. The sagittal left and right otoliths from all fish samples were removed, washed with distilled water, stored in Eppendorf tubes, and kept in a dry storage for 24 h to eliminate humidity.
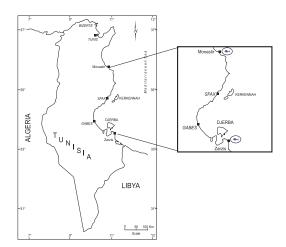

Fig. 1. Sampling sites from which samples of *Boops boops* were collected in the Monastir and Zarzis marine stations, Tunisia

Table 1

Range values of the total length and weight of the *Boops boops* samples collected from the Monastir and Zarzis in Tunisia

Sampling site	n	Parameter	
		TL [mm]	TW [g]
Monastir	91	150–206	28–78
Zarzis	92	157–198	36–70

n = number of fish, TL = total length, TW = total weight.

Otolith shape analysis. Otoliths were positioned onto a microscope slide with the sulcus faced down and the rostrum pointed in the same direction to minimize distortion errors in the normalization process. Subsequently, they were examined under a dissecting microscope at 40× magnification with a black background and photographed by using a digital camera (Samsung HD with a resolution of 16 megapixels). The photos of all otoliths were exanimated by Adobe Photoshop CS6. The contour shape of each otolith (Fig. 2) was assessed by the elliptic Fourier analysis (FDA). The method of elliptic Fourier descriptors (FDs) was used following the procedures suggested by Kuhl and Giardina (1982), where a chain-coding algorithm, based on the projection of the binary contour of each otolith was used, and calculated with SHAPE Ver. 1.3 software (Iwata and Ukai 2002). The chain coder provides the normalized EFDs coefficients through a discrete Fourier transformation (DFT) of the chain-coded contour. The FDs technique describes the outline based on harmonics and generates 20 harmonics for each otolith. Each harmonic was composed of four coefficients (A, B, C, and D), resulting in 80 coefficients per otolith generated from the projection of each point of the outline on axes (x) and (y). The higher number of harmonics, the greater the accuracy of the outline description (Kuhl and Giardina 1982). Each otolith was normalized by the program for size and orientation, which caused the degeneration of the first three FDs derived from the first harmonic to fixed values $A_1 = 1$, $B_1 = 1$, and $C_1 = 0$. Therefore, each sample was represented by 77 coefficients for the shape analysis. The four Fourier coefficients (A, B, C, and D) calculated the Fourier power (FP_n), the percentage Fourier power (FP%), and the cumulative percentage of the Fourier power (FP_n% cumulative). The respective formulas were

$$FP_n = (A_n^2 + B_n^2 + C_n^2 + D_n^2) \cdot 2^{-1}$$

where A_n, B_n, C_n, and D_n are the Fourier coefficients of the **n**th harmonic.

$$FP\% = 100FP_n \cdot (\Sigma_1^n PF_n)^{-1}$$
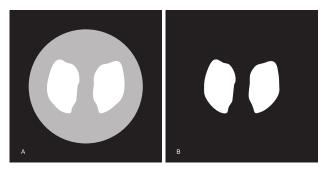
$$FP_n\% = \Sigma_1^n FP_n\%$$

Fig. 2. Photograph of the otoliths of *Boops boops* collected from the Monastir and Zarzis marine stations in Tunisia; (**A**) real image; (**B**) processed image using Adobe Photoshop software

The power of the cumulative Fourier average was then calculated to fix the number of harmonics. The threshold of 99.99% of the total power was chosen to determine the number of harmonics required.

Data analysis. The analytical design was built to detect differences in the contour shape of *B. boops* otoliths collected from the two studied locations through the non-parametric generalized Discriminant Function Analysis (DFA). First, the effect of locations on elliptical Fourier descriptors was tested by multivariate analysis of variance (MANOVA). Second, all shape variable values were firstly checked for normality; if the values do not follow the normal distribution, a transformation of Box–Cox (Box and Cox 1964) was carried out. Finally, the Levene's and Shapiro–Wilks' λ tests were applied to assess the homogeneity (equality) and the normality of the variance in the variable values of otoliths shapes, respectively. The DFA was performed with the normalized elliptical Fourier descriptors coefficients (77 coefficients per otolith) to illustrate the similarity and difference between samples either in the same locality and/or in the two localities. The objective of DFA was to investigate the integrity of predefined groups of individuals belonging to a given geographical location by finding linear combinations of descriptors that maximize the Wilks' λ. The Wilks' λ test assesses the performance of the discriminant analyses. This statistic is the ratio between the intra-group variance and the total variance and provides an objective means of calculating the chance–corrected percentage of agreement. The Mahalanobis generalized distance (D^2) and Fisher distance were also calculated to characterize the differences in the otolith shape between and within stock samples of the two localities. All these statistical analyses were performed using XLSTAT 2010.

RESULTS

The Levene's and Wilks' λ tests confirmed that all values of the shape variance were equally and normally distributed with *P*-value > 0.05. In addition, the Wilks' λ test values showed statistically significant differences ($P < 0.0001$) between the right and left otoliths of the Monastir and Zarzis localities, i.e., there was an asymmetry (Table 2). Similarly, the Mahalanobis (D^2) and Fisher distances also showed significant asymmetry in the otoliths shape within and between fish samples in the two localities. In detail, the D^2 between the left and right otoliths of fish samples within the Monastir locality was 3.90, while that of Zarzis was 3.06. However, the D^2 between the left and right otoliths of fish samples from the Monastir and Zarzis localities were 6.36 and 5.48, respectively (Table 3), whereas the Fisher distances were 1.82 ($P < 0.0001$) and 1.4 ($P = 0.01$), respectively. Nevertheless, by comparing the left and right sides of otoliths between fish samples of the two localities, it was found that the Fisher distances were 2.98 and 2.57 ($P < 0.0001$ for both), respectively (Table 4).

The barycenter projection showed that the two localities were discriminated against by the two axes *F*1 (59.94%) and *F*2 (29.47%) with a total value equal to 89.41%. Indeed, the *F*1 and *F*2 showed the presence of two otoliths groups corresponding to two populations of fish samples from the Monastir and Zarzis localities. The *F*1 axis placed the left and right otoliths of the Monastir fish samples in the positive part and those of the Zarzis in the negative part, however, the *F*2 axis separated the left and right otoliths within fish samples of each locality. In addition, the left otoliths were placed in the positive part, whereas the right ones were positioned in the negative part of the *F*2 (Fig. 3).

Table 2

Wilks' Lambda test between *Boops boops*, from Monastir and Zarzis localities, Tunisia (Rao approximation)

Parameter	Value
Lambda	0.2209
F (Observed value)	2.4328
F (Critical value)	1.1826
DDL1	231
DDL2	859
P–value	< 0.0001
Alpha	0.05

Table 3

Pairwise Mahalanobis Distances (D^2) matrix of the otoliths shape variance between *Boops boops* samples collected from the Monastir and Zarzis localities in Tunisia

	ML	MR	ZL	ZR
ML	—	3.9025	6.3672	6.9938
MR		—	7.3307	5.4876
ZL			—	3.0680
ZR				—

ML = Monastir left, MR = Monastir right, ZL = Zarzis left, ZR = Zarzis right.

Table 4

Fisher distance matrix between the left (L) and right (R) otoliths of *Boops boops* samples collected from the Monastir and Zarzis localities in Tunisia (above diagonal) and the *P*-values (below diagonal)

	ML	MR	ZL	ZR
ML	—	1.8219	2.9888	3.2829
MR	0.0002	—	3.4410	2.5759
ZL	<0.0001	<0.0001	—	1.4480
ZR	<0.0001	<0.0001	0.0161	—

ML = Monastir left, MR = Monastir right, ZL = Zarzis left, ZR = Zarzis right.

DISCUSSION

Elliptic Fourier analysis of the otolith contour shape revealed a clear asymmetry between the left and right sides within and between the fish samples in the two localities. These results are consistent with those previously cited on the sparid *Oblada melanura* (Linnaeus, 1758) (see Barhoumi et al. 2018), *Diplodus annularis* (see Trojette et
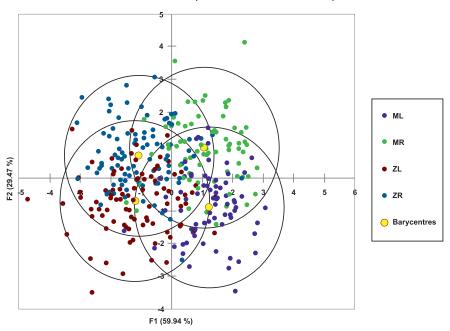
Fig. 3. Samples dispersion of the left and right otoliths of *Boops boops* collected from the Monastir and Zarzis marine stations in Tunisia; ML = Monastir left, MR = Monastir right, ZL = Zarzis left, ZR = Zarzis right

al. 2015), and *Pagellus erythrinus* (see Mejri et al. 2018, 2020), which are cohabiting different habitats in Tunisian waters, as well as on the *B. boops* sampled from three locations along the Algerian coast (Ider et al. 2017) and from the Gulf of Tunis (Mahé et al. 2019). Similarly, the asymmetry of the otolith shape has also been found in other species that live elsewhere outside of Tunisian waters, such as *Gadus morhua* Linnaeus, 1758 (see Campana and Casselman 1993, Hüssy et al. 2016), *Clupea harengus* Linnaeus, 1758 (see Turan 2000), *Lophius piscatorius* Linnaeus, 1758 (see Cañás et al. 2012), *Haemulon plumierii* (Lacepède, 1801) (see Treinen-Crespo et al. 2012), *Sicyopterus* species (Lord et al. 2012), *Xiphias gladius* Linnaeus, 1758 (see Mahé et al. 2016), *Thunnus thynnus* (Linnaeus, 1758) (see Brophy et al. 2016), *Albatrossia pectoralis* (Gilbert, 1892) (see Rodgveller et al. 2017), *Pegusa lascaris* (Risso, 1810) (see Chakour and Elouizgani 2018), and *Engraulis encrasicolus* (Linnaeus, 1758) (see Khemiri et al. 2018).

Additionally, statistical analyses showed a significant difference in the sagittal otolith shape between fish samples in the Monastir and Zarzis locations, and they clearly separated them. This result is in agreement with what has been recorded in some sparid fish from marine environments in Tunisian waters such as *P. erythrinus* that has been examined from the Bizerte, Goulette, Soliman, and Haouaria stations in the Gulf of Tunis (Mejri et al. 2020) and the *Oblada melanura* collected from fishing zones in the Bizerte, Kelibia, and Sayada along the Tunisian coast (Barhoumi et al. 2018). Although the morphological variability in the otolith shape is influenced by genetic factors (Vignon and Morat 2010), exogenous factors such as depth (Lombarte and Lleonart 1993),

water temperature (Hüssy 2008), salinity (Capoccioni et al. 2011), and food supply (Hüssy 2008) also play a strong role in the otolith reshape (Bremm and Schulz 2014). Therefore, this morphological asymmetry between the left and right otoliths in fish samples from the Monastir and Zarzis stations can be attributed either to the effect of the surrounding environment during their lifetime or to a decrease in some factors such as fertility, sexual maturity, survival, or growth (Trojette et al. 2015). In addition, as described by Panfili et al. (2005) and Mahé et al. (2019), the *B. boops* samples from these two stations may have been subjected to different environmental conditions such as temperature and salinity, which may have increased levels of asymmetry in the shape of otoliths. These environmental parameters have also explained the diversity of the shape of the otoliths that have been detected in the sparid fish that live in Tunisian waters such as *D. annularis* (see Trojette et al. 2015) and *P. erythrinus* (see Mejri et al. 2018, 2020).

In this study, the difference in the otolith shape between the same left–left and right–right sides of fish samples from the Monastir and Zarzis localities can be explained by some factors such as fluctuations in the diet, type of swimming activity, life-history traits, and variation in some environmental conditions such as salinity and water temperature. However, some authors have attributed this asymmetry to genetic effects, but this factor cannot be discussed here due to a lack of genetic data on *B. boops* from these two stations. On the other hand, previous studies on the size and morphology of the otoliths have shown that the intra-population variation may reside in inter- and intra-individual and even inter-population variations (Mejri et al. 2018, 2020). Of these variations, Panfili et

al. (2005) pointed out that the intra-individual variation represents the fluctuating asymmetry of the otoliths. In fact, differences in habitats between the Monastir and Zarzis locations, particularly water temperature and salinity, can affect the different structures of saccular sagittae. Moreover, many authors have attributed this difference to the type of substrate which may be either a soft, mixed, or hard substrate and the habitat occupied by the fish (Jaramillo et al. 2014). These two parameters can also be considered herein as key factors supporting the current result of otolith asymmetry between the Monastir and Zarzis localities, where significant differences in substrates and habitats have previously been recognized in these two distant stations (Mejri et al. 2018). Therefore, environmental parameters such as water temperature, salinity, depth, and feeding have been identified as the crucial causes of geographic variation in the shape or appearance of the otolith nucleus, otolith annuli, and variations in the ration of the otolith size to the fish size (Trojette et al. 2015). However, as found herein, the elliptic Fourier analysis can also discriminate between stocks of *B. boops* in Tunisian waters.

Regarding environmental parameters, Fablet et al. (2009) mentioned that temperature indirectly affects otolith growth. Indeed, fish species have been known to be very sensitive to a change of only 0.03°C (Trojette et al. 2015). In the presently reported study, the water temperature varied from 19.6 to 25.8°C in the Monastir (Zaafrane et al. 2019) and from 15.6 to 28.3°C in the Zarzis (Béjaoui et al. 2019). In addition to these environmental factors, some authors have claimed that ontogenetic factors (Gonzalez Naya et al. 2012), physiological factors, where hearing capabilities have been associated with a specialization in acoustic communication, (Lomabrte and Cruz 2007), and phylogeny of fish (Nolf and Tyler 2006), can also affect the shape of the otolith. In addition, it has been documented that salinity has been one of the key factors directly affecting the connectivity of species in marine populations and indirectly affecting the chemical composition and shape of the otoliths (Mejri et al. 2020). Moreover, it is worth noting that many authors have observed a marked difference in the chemical composition of the otoliths, which has been associated with individuals' responses to salinity and water temperature interaction and concentrations of the most common elements such as Cl, Mg, K, Na, and Ca (Mejri et al. 2018). This explanation is likely to support the presently reported result of otolith asymmetry between fish samples in the Monastir and Zarzis localities because the salinity varies between 37‰ in the Monastir (Zaafrane et al. 2019) and 39.5‰ in the Zarzis (Béjaoui et al. 2019). Moreover, the availability of food can change the behavior and morphology of fish, as well as the variability between metabolic rates, which have been linked to the environmental parameters, can affect the shape and growth of sagittae (Lombarte et al. 2010). Indeed, the bogue, *B. boops*, feeds on a wide variety of prey types including Crustacea, Porifera, Coelenterata, Seagrasses Mollusca, and Protozoa (El-Maremie and El-Mor 2015). Thus, we can conclude that the differences in the diet of *B. boops* seem likely to exist between the Monastir and Zazris locations that led to the current difference in the otolith shape between the fish samples from the two localities, a conclusion that needs further investigation. Furthermore, some authors have reported that age and sex may induce a significant difference in the otolith shape in fish stocks (Simoneau et al. 2000). As a result, Ferri et al. (2018) found that the otolith shape is significantly different in juveniles from that in adults due to differences in the hearing function. However, it is noteworthy to mention herein that the sampling was only restricted to adult samples of *B. boops* to eliminate the confounding effect of allometric growth on the otolith form (Mejri et al. 2020). With regard to sex, *B. boops* has been assigned as a protogynous hermaphrodite with a sex change from female to male (Gordo 1995). Therefore, we can suggest that the difference in the otolith shape between fish samples from the Monastir and Zarzis localities can be explained by the effect of the hermaphroditism of some samples from these two locations which can induce higher discrimination in otoliths.

On the other hand, reproductive isolation has been so far known to be one of the main characteristics that could indicate the differentiation among populations and fish stocks (Wiff et al. 2020). Thus, the isolation of *B. boops* in small reproductive units in combination with the exposure to the different environmental conditions in the two localities can act as natural barriers and key factors that could influence stock differentiation. However, genetic analyses will be required to estimate the level of gene flow and may preclude the detection of genetic differences between the Monastir and Zarzis localities.

In conclusion, the comparison of the otolith shape between the bogue, *B. boops* samples collected from the Monastir and Zarzis marine stations showed statistically significant differences within and between the fish stocks of the two localities, i.e., there was an asymmetry. This asymmetry was found between the left and right otoliths within each locality, as well as between the same sides (left–left) and (right–right) otoliths between the Monastir and Zarzis localities. Therefore, our study strongly confirms that fish samples from the two localities were significantly different from each other, i.e., they do not belong to a unique population, and results suggest that they might represent a structured population along the Tunisian coast. In addition, by analyzing the otolith shape, stock discrimination becomes easier and more efficient for *B. boops* in Tunisian waters. Moreover, this asymmetry in the otolith shape may be due to the effect of some environmental factors such as water temperature, salinity, habitat, and diet. However, the inter-individual or even the intra-population asymmetry between the right and left otoliths can be explained by the possibility of having intra-individual stress that led to abnormalities in the development of the individuals or by the presence of poor living conditions for the larvae, resulting from unfavorable environments. Furthermore, these two stations should be considered as two subunits or populations of the Tunisian bogue stock and should be managed separately. In the future, research into the otolith

biochemical analysis will allow us to understand better the further environmental physio-chemical parameters that affect the otolith morphology of the bogue at the two stations. Genetic investigations including mitochondrial DNA such D-loop region would also be necessary to understand better the presently reported results.

COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

MBL, MM, and ARB contributed to the experiment design, conducting experiments, interpretation of data, and writing the manuscript; AABS, JPQ, and MT contributed to the review and editing of the manuscript. All authors made substantial intellectual contributions to the work and are prepared to take accountability for it.

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