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Corresponding author:
Adel A. Basyouny Shahin;
Email: adel.shahin@mu.edu.eg

Do eco-geospatial differences induce otolith morphological variations? Assessment in *Chelon auratus* (Mugiliformes, Mugilidae) populations collected from Tunisian and Mauritanian waters

Toumene Deida[1], Mehrez Gammoudi[1], Tahani El Ayari[2], Abderraouf Ben Faleh[1], Lassana Djimera[3], Adel A. Basyouny Shahin[4] and Nawzet Bouriga[1,5]

[1]Laboratory of Ecology, Biology and Physiology of Aquatic Organisms (LR/18/ES/41), Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia; [2]Laboratory of Environment Biomonitoring, Group of Fundamental and Applied Malacology (LEB/GFAM), Faculty of Sciences of Bizerte, University of Carthage, Bizerte, Tunisia; [3]Laboratory of Ecology and Biology of Aquatic Organisms (LEBAO), Mauritanian Institute of Oceanographic Research and Fisheries (IMROP), BP: 22 Nouadhibou, Mauritania; [4]Department of Zoology, Faculty of Science, Minia University, El Minia, Egypt and [5]Higher Institute of Fisheries and Aquaculture of Bizerte, University of Carthage, Bizerte, Tunisia

Abstract

Saccular otoliths (sagittae) have long been shown to be species-specific and exhibit inland geospatial intra- and interpopulation morphological differences with variations in environmental conditions. Here, we analysed inland and outland geospatial variations in sagittae shape, length (*Lo*), width (*Wo*), perimeter (Po), and area (Ao), and fluctuating asymmetry (FA) in *Chelon auratus* males and females collected from Ghar El Melh (Tunisia) and Etoile Bay (Mauritania) stations to assess whether sagittae shape and morphometry differ between these two niches having different environmental conditions. At the intrapopulation level, a significant otolith shape asymmetry was observed between left and right and left–left and right–right otoliths among males and females of the Ghar El Melh (Tunisia) population and a significant symmetry among those of the Etoile Bay (Mauritania) population. At the interpopulation level, a significant asymmetry was found between left and right otoliths' shape among males and females of the two populations. Besides, a discriminant function analysis of otoliths' contour shape separated left and right otoliths among males and females at the intra- and interpopulation levels and also separated those of the two populations. Moreover, differential significant asymmetry in *Lo*, *Wo*, Po, and Ao between left and right otoliths was observed among males and females at the intra- and interpopulation levels. Therefore, the geospatial variations in environmental conditions between the two ecological niches effectively induced differences in otolith morphology. These significant asymmetries were discussed in terms of FA caused by environmental stress conditions resulting from variations in abiotic factors between the two ecological niches.

Introduction

The golden grey mullet *Chelon auratus* (Risso, 1810) belongs to the Mugilidae family and is of great importance due to its high commercial value, particularly for its gonads (D'Iglio *et al*., 2022). It is the most abundant species along the northeast Atlantic Ocean and Mediterranean coasts (Bakhshalizadeh *et al*., 2023) and is found at depths of 10–20 m (Thomson, 1990). Juveniles feed solely on zooplankton, whereas adults feed mainly on trivial benthic organisms and detritus (Bakhshalizadeh *et al*., 2023). Socially, it is a gregarious species and moves in shoals in coastal marine areas (Fehri-Bedoui *et al*., 2002). Although its adult size is smaller than other mullets, it is very efficient in the early stages of growth. Its finer body and fillets are of better quality, making it a compulsive choice for aquaculture due to its ability to thrive in various ecosystems (Quirós-Pozo *et al*., 2023). Besides, it is categorized as a euryhaline and eurythermal species because it tolerates an extensive range of salinities and temperatures, where *C. auratus* enters lagoons and estuaries and rarely migrates to freshwater (Whitfield, 2016). Attaining a length of 59 cm, *C. auratus* plays a crucial role in the food chain, with a trophic level of 2.8 (Kesiktaş *et al*., 2020).

Due to the high economic importance of *C. auratus* as food and bait, several studies have been carried out worldwide, including on its age and growth (Hotos and Katselis, 2011), reproductive biology (Hotos *et al*., 2000; Fazli *et al*., 2008; Daryanabard *et al*., 2009; Ghaninejad *et al*., 2010; Bekova *et al*., 2020; Hasanen *et al*., 2021; El-Shenity et al., 2023), reproductive management (Quirós-Pozo *et al*., 2023), population dynamics and stock assessment (Çiloğlu, 2023), and morphology and morphometry of saccular otoliths (Fortunato *et al*., 2017; Ferri *et al.*, 2018; Çiçek *et al.*, 2020; Kesiktaş *et al.*, 2020; Reis *et al.*, 2023). However, few studies have been conducted on some aspects of this species in Tunisian and Mauritanian waters. In Tunisian waters, these studies have also focused on its age and growth (Fehri-Bedoui and Gharbi, 2005; Abdallah *et al.*, 2012), reproductive biology (Abdallah *et al.*, 2013), determination of the reproduction period and sexual maturity (Fehri-Bedoui *et al.*, 2002), and mortality pattern and yield per recruit (Fehri-Bedoui *et al.*, 2013). In addition, Trabelsi *et al.* (2008) identified its juveniles using the *cytochrome b* gene, whereas Blel *et al.* (2008) evaluated the phylogenetic relationships between five mugilid species, including *C. auratus*, based on the biochemical analysis. Similarly, Blel *et al.* (2009) explored the phylogenetic relationships among these species in addition to *Oedalechilus labeo*, based on polymorphism in mitochondrial rRNA, *COI*, *CytB*, and 12S rRNA, as well as nuclear 5S DNA, rhodopsin gene sequence, and 589 bp of the mitochondrial gene sequence (16S rRNA) (Blel *et al.*, 2013). Moreover, Jmil *et al.* (2019*a*) analysed the otolith shape variation in samples collected from the El Biban and Boughrara lagoons and the Bizerte lagoon (Jmil *et al.*, 2019*b*). Recently, Mejri *et al.* (2022*b*) assessed the interspecific and intersexual variability of saccular otolith shape between *C. auratus* and *Chelon ramada* inhabiting the Boughrara lagoon and Bouriga *et al.* (2023) discriminated among six commercially interesting and ecologically diverse fish species in the Gulf of Tunis by using fatty acid composition and otolith shape analysis.

In Mauritania, however, Ould Mohamed Vall (2004) studied the dynamics of the exploitation systems and reproductive ecobiology of *C. auratus* in addition to *Mugil cephalus* and *Mugil capurrii* and analysed their strategies for occupying Mauritanian littoral sectors and their management possibilities.

On the contrary, saccular otoliths or sagittae, the large pair of otoliths found in the inner ear of teleosts, are metabolically inert, i.e. once fully formed, the otolith chemical material is unlikely to be resorbed or altered (Campana, 1999). Therefore, the otolith shape remains unaffected by short-term changes in fish conditions or environmental variations (Campana and Casselman, 1993). However, Tuset *et al.* (2016) reported that the size of otoliths increases with the increase of the habitat depths down to about 1000 m, and Volpedo *et al.* (2008) indicated that the size decreases in fast swimming fish and is associated with feeding habitats (Lombarte *et al.*, 2010; Tuset *et al.*, 2016). Therefore, saccular otolith morphology has long been employed for the identification of species, i.e. it is species-specific and exhibits high inter- and intra-species local geographic variation in shape and size (Ferri *et al.*, 2018; Ben Labidi *et al.*, 2020*a*; Khedher *et al.*, 2021; Mejri *et al.*, 2022*a*, 2022*b*; Ben Mohamed *et al.*, 2023; Bouriga *et al.*, 2023; D'Iglio *et al.*, 2023; Reis *et al.*, 2023; Adjibayo Houeto *et al.*, 2024). In addition, they have been widely used efficiently to identify local species and populations and to discriminate their stocks in different habitats (Jawad *et al.*, 2018; Ben Labidi *et al.*, 2020*a*, 2020*b*; Khedher *et al.*, 2021; Mejri *et al.*, 2022*a*, 2022*b*; Ben Mohamed *et al.*, 2023; Reis *et al.*, 2023). Moreover, the otolith morphology has been used for fisheries management or to estimate population growth and mortality (Cardinale *et al.*, 2004; Tracey *et al.*, 2006; Burke *et al.*, 2008; Stransky *et al.*, 2008; Cañás *et al.*, 2012; Morat *et al.*, 2012; Bakkari *et al.*, 2020). This is because the otolith morphology and morphometry have been reported to be influenced by many factors, such as sex, growth, maturity, fishery exploitation pattern, and genetic and environmental factors (Begg and Brown, 2000; Volpedo and Echeverría, 2003; Vignon and Morat, 2010). Ecologically, otolith shape and morphometry have long been used also as crucial indicators of the ecological characteristics of fish because they provide a wide range of ecology information, including feeding habitat, mobility, substrate association, and water column positioning of fish (Volpedo and Echeverría, 2003; Assis *et al.*, 2020). In addition, earlier studies have shown that the variability in otolith shape is influenced by many factors, such as substrate type (Volpedo and Cirelli, 2006), feeding habit (Nonogaki *et al.*, 2007; Bouriga *et al.*, 2023), ontogenetic shifts (Pérez and Fabré, 2013), and environmental conditions, including water temperature, depth, and pollution (Lombarte and Lleonart, 1993; Ben Labidi *et al.*, 2020*a*, 2020*b*; Khedher *et al.*, 2021; Mejri *et al.*, 2022*a*, 2022*b*; Ben Mohamed *et al.*, 2023; Bouriga *et al.*, 2023; Adjibayo Houeto *et al.*, 2024).

Among the techniques, elliptical Fourier analysis (EFA) is the most efficient one used for otolith shape analysis, which has been proven to be the most widely used and effective method to describe, characterize, and capture specific information of otoliths outlined in a quantifiable manner (Lord *et al.*, 2012; Mahé *et al.*, 2019).

So far, otolith morphology is poorly studied in fish species of Mauritanian waters, and the variability in the otolith shape is greatly affected by diverse sorts of pressure, such as genetic or ecological influences (Grønkjaer and Sand, 2003). Therefore, this study was undertaken to compare the saccular otolith shape for the first time between males and females of *C. auratus* inhabiting two ecologically different geospatial niches in the Ghar El Melh station (Tunisia) and the Etoile Bay station (Mauritania) to evaluate whether the shape and morphometry of saccular otoliths (sagittae) differ between these two niches having different environmental conditions.

Materials and methods

Study area

The Ghar El Melh station is located in the Ghar El Melh lagoon (32°28′33°45″N, 10°45′10°57″E), which ranks first among the Tunisian lagoons because it has an area of 50,000 ha (Guetat *et al.*, 2012). It is located south of Djerba Island on the southern edge of the Gulf of Gabes and communicates with the seawater of the Gulf of Gabes through the El Kantara in the north-eastern part and the Ajim channels in the north-western part (Figure 1A). The water surface temperature ranges from 11.2°C in winter and 24.7°C in summer (Feki *et al.*, 2013) and the salinity mostly varies between 38 and 43‰ in winter (Sellem *et al.*, 2019), while it oscillates between 42.19 and 53.3‰ in summer (Khedhri *et al.*, 2015). In addition, the pH of the water fluctuates between 7.92 in winter and 8.31 in summer (Ben Aoun *et al.*, 2007). Moreover, the lagoon is polluted with organic discharges through harbour-related activities in its southern part (Guetat *et al.*, 2012), as well as with sewage resulting from the transportation of the surrounding ports and the entry of seawater loaded with phosphorous from the Gulf of Gabes (Sellem *et al.*, 2019).

The Etoile Bay station is situated in the Etoile Bay (21°2′12″N, 17°1′1″W) that lies north of Nouadhibou and is identified as an aesthetic site where a variety of space and ecosystem uses coexist (Amadou, 2009). It is an enclave of Bay Lévrier on the Cap Blanc Peninsula and covers a marine area of 1200 ha, and its width ranges between 100 and 150 m in the loop-shaped downstream area and between 150 and 550 m in the slightly straight middle and upstream part (Figure 1B). The seabed consists of shoals with a submerged sandbank, with a maximum depth of 4.5 m in the central-western part, gradually decreasing around the periphery, and the depth does not exceed 1 m at the pass. The bay experiences constant water renewal because the tidal currents dominate the circulation of water masses and due to the small volume of water (Brêthes and Mayif, 2013). In addition, the Etoile Bay receives several sources of chronic pollution that could be responsible for its eutrophication, including the Nouadhibou

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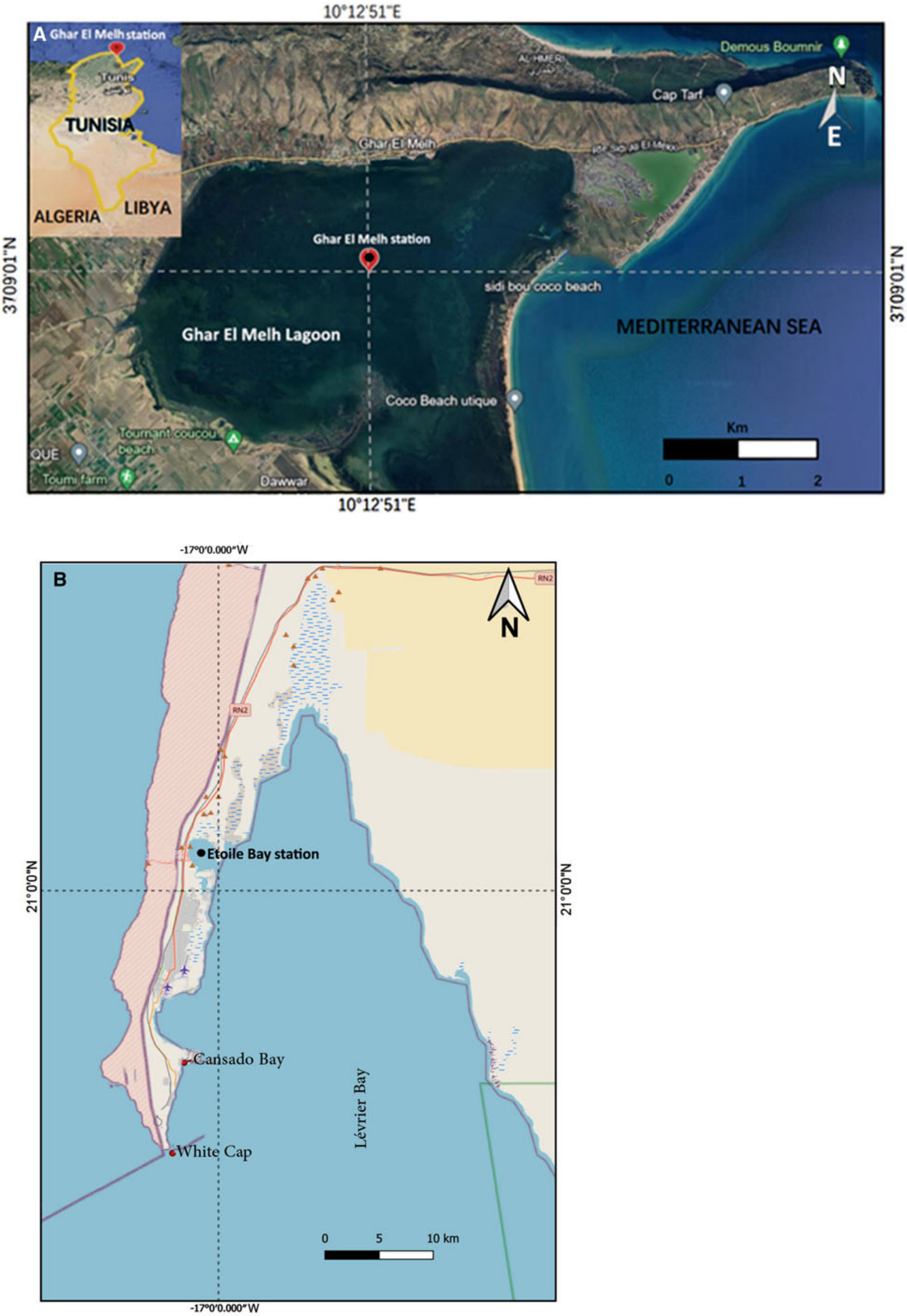

Figure 1. *C. auratus* Risso, 1810: (A) the Ghar El Melh (Tunisia) and (B) Etoile Bay (Mauritania) stations (●) from which the males and females were collected.

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slaughterhouse, the Tarhil districts, the flour factories in Bountiya, the wreckage dump accumulated by the Société hollandaise in Bountiya, the salting, drying, and smoking of fish on the Nouadhibou inlet, as well as domestic discharges from Cabanons (Berque *et al*., 2012). The water surface temperature and salinity are 26.5°C and 37.9‰, respectively, in the warm season (August–October) and 19.4°C and 35.8‰, respectively, in the cold season (January–May), whereas the pH is over 8 in both seasons (Legraa, 2019).

Sampling

A total of 120 adult individuals of *C. auratus* (60 individuals from each station; 30 males and 30 females) were collected between June and October 2023 from the Ghar El Melh station located in northeast Tunisia (37°10′10″N, 10°11′16″E) (Figure 1A) and the Etoile Bay station situated in Mauritania (21°00′15″N, 17°00′45″W) (Figure 1B). All individuals were caught alive by coastal artisanal fishermen using gillnets. Immediately after catching, the sexual maturity status of each individual was examined macroscopically, using the scale of Kesteven (1960) and Treasurer (1990), or microscopically in the case of small gonads, to ensure that all individuals chosen for this study were fully mature. Afterwards, all individuals were weighed for the total weight (TW; g) using a digital balance, and the standard length (*SL*; mm) was measured using an ichthyometer, and the values from both parameters were rounded to the nearest 0.01 (online Table S).

Otolith extraction and imaging

Sagittal otoliths were extracted using a sharp-bladed knife, soaked in distilled water, dried, and stored in an Eppendorf tube. The left and right otoliths were placed on their convex side, more precisely with the grooves positioned upwards and the rostrum below, with an inclination to avoid errors during the normalization process, and digital images were captured using a binocular loupe with a Canon IXUS 185 digital camera, with a resolution of 20 megapixels (Figure 2A, B). After that, all images were sent to an image analyzer equipped with an incorporated millimetre scale.

Otolith shape analysis

The obtained images of the otoliths were processed using Adobe Photoshop CS6 software, which transforms the original picture of the otolith into a binary image. Subsequently, the binary images of the otoliths' shapes were analysed using software SHAPE Ver. 1.3 (Iwata and Ukai, 2002). The outlines of the contour shape of each otolith were evaluated by EFA as previously described by Ben Labidi *et al*. (2020*a*, 2020*b*) and Khedher *et al*. (2021) following the procedures suggested by Kuhl and Giardina (1982).

Statistical data analysis and variable normalization

First, an analysis of variance (ANOVA) was performed to evaluate the significance of differences in the mean values of the *SL* and the *TW* among individuals of the two stations or populations in Tunisia and Mauritania and values were tested for the homogeneity (equality) and the normal distribution using Levene's and Shapiro–Wilks' λ tests, respectively. Second, the differences in the contour shape of otoliths from individuals of the two populations were analysed by discriminant function analysis (DFA). The effect of locations on the elliptical Fourier descriptors (EFDs) was first verified by multivariate analysis of variance (MANOVA); subsequently, all shape variable values were checked for normality; if the values did not follow the normal distribution, a transformation of Box–Cox (Box and Cox, 1964) was performed. Finally, Levene's and Shapiro–Wilks' λ tests were used to evaluate the homogeneity (equality) and the normal distribution of the variance in the values of the variables for the shape of otoliths, respectively. DFA was performed with the normalized elliptical Fourier descriptor coefficients (77 coefficients per otolith) to illustrate the similarities and differences among individuals in the same population or both populations. DFA was used to investigate the integrity of pre-defined groups of individuals belonging to the given geographic population or locality and the percentage of their correct classification by finding linear combinations of descriptors that maximize the value of Wilks' λ. The performance of DFA was verified using Wilks' λ test, which is the ratio between the intra-population variance and the total variance and provides an objective method for calculating the corrected percentage chance for agreement. Moreover, Fisher's (1936) distance was also calculated to characterize the similarity (symmetry) and variability (asymmetry) between the left (L) and right (R) otoliths and between the left–left (L–L) and right–right (R–R) otoliths from the same side within and among males and females of the two populations. The results were interpreted using Wilk's λ test data, and the barycentre projections of the left and right otoliths for both males and females for both populations were displayed on graphs. In addition, MANOVA was used to test the

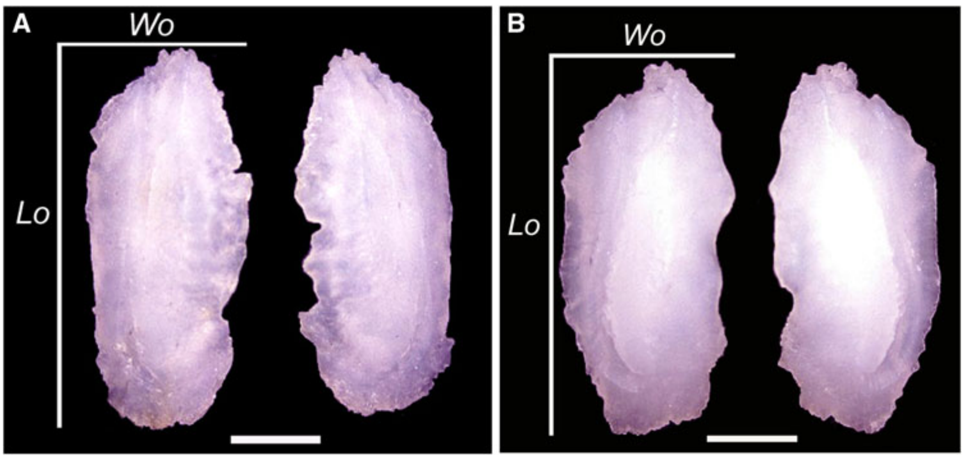

Figure 2. *C. auratus* Risso, 1810: images of the left (L) and right (R) sagittal otoliths showing the length (*Lo*) and width (*Wo*) parameters examined among individuals collected from the (A) Ghar El Melh (Tunisia) and (B) Etoile Bay (Mauritania) stations. Scale bar: 2 mm.

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significance of the left and right otoliths' shape values within and between populations. All analyses were performed by using XLSTAT 2010.

Otolith morphometric analysis

Analysis of morphometric parameters of the otoliths, including length (*Lo*), width (*Wo*), perimeter (Po), and area (Ao), was performed by ImageJ software (Figure 2A, B). Before statistical analyses, a one-way ANOVA was used to determine whether there were any significant differences between the mean values of *Lo*, *Wo*, Po, and Ao for the left and right sides of otoliths within and among males and females of the two populations. In addition, a two-way ANOVA was used to check whether there was a correlation between the otolith's morphometric parameters and the geographic origin of the individuals. Moreover, the Student's *t*-test was used to analyse the differences in the four parameters between the left and right and L–L and R–R otoliths of males and females between the two populations.

Fluctuating asymmetry (FA) measurement and analysis

Statistically, the FA is characterized by a normal distribution of the L_i–R_i, with a mean equal to 0, and the variance of $|l_i - r_i|$ represents a measure of development instability. As depicted by Palmer and Strobeck (1986), FAs are exceedingly subtle in the order of 1% of the character size or less and thus require great care to be detected. The FA between the right and left sides of the otoliths was calculated among males and females of the two populations for each morphometric parameter per individual '*i*' by applying the following formula given by Palmer and Strobeck (1986) and was estimated as the FA_i index:

$$FA_i = \sigma^2 r - l$$

where *r* and *l* are the values of the traits on the right and left sides, respectively.

Results

Total weight (TW) and total length (SL) variation

As shown in online Table S, the TW and *SL* ranged from 106.98 to 234.65 g and 130 to 273 mm in males and from 150.20 to 281.78 g and 173 to 278 mm in females for the Ghar El Melh population (Tunisia). However, the TW and *SL* varied from 160.10 to 450.90 g and 219 to 298 mm in males and from 208.04 to 425.86 g and 231 to 289 mm in females for the Etoile Bay population (Mauritania).

Otolith shape variation

Generally, Shapiro–Wilks' λ test confirmed that all shape variance values were almost distributed normally among individuals in each population with a P value > 0.05. At the intrapopulation level, the Wilks' λ test of the otolith shape values showed that there was a bilateral significant difference ($P < 0.0001$), i.e. there was asymmetry, between the left and right otoliths, as well as between the L–L and R–R otoliths, among males and females of the Ghar El Melh population (Tunisia) (Table 1). However, on the contrary, a significant bilateral similarity ($P > 0.05$), i.e. there was symmetry, was observed between the left and right otoliths and between the L–L and R–R otoliths among males and females of the Etoile Bay population (Mauritania). Similarly, Fisher's (1936) distance matrix of the shape variance also revealed a significant bilateral asymmetry ($P < 0.0001$) between the left and right otoliths within and among males and females of the Ghar El Melh population (Tunisia). However, a significant bilateral symmetry ($P > 0.05$) was found between the left and right otoliths and between the L–L and R–R otoliths among males and females of the Etoile Bay population (Mauritania) (Table 2).

At the interpopulation level, a combined analysis of the left and right otolith shape values between males and females from the Ghar El Melh (Tunisia) and Etoile Bay (Mauritania)

Table 1. Wilk's λ test of the left and right otoliths' shape variance distance approximation values between males and females of *C. auratus* samples collected from the Ghar El Melh (Tunisia) and Etoile Bay (Mauritania) stations, as well as between males and females from the two populations

Population	Wilks' λ	*F* (observed value)	*F* (critical value)	D*F*1	D*F*2	*P* value
Ghar El Melh (Tunisia)	0.0067	2.1408	1.3143	231	115	<0.0001
Etoile Bay (Mauritania)	0.0383	1.0304	1.3076	231	121	**0.4320**
Between populations	0.0034	2.5450	1.1288	539	1088	<0.0001

The value marked in bold is statistically not significant ($P > 0.05$).

Table 2. Combined pairwise Fisher's (1936) distance (*D*) mean values (above diagonal) and their corresponding *P* values (below diagonal) of the left (L) and right (R) otoliths within and between males (M) and females (F) of *C. auratus* samples collected from the Ghar El Melh (GM) (Tunisia) and Etoile Bay (EB) (Mauritania) stations

	GMLM	GMRM	GMLF	GMRF	EBLM	EBRM	EBLF	EBRF
GMLM	–	2.3908	1.2160	2.3325	6.4344	6.3632	6.0423	6.9798
GMRM	<0.0001	–	2.2688	1.1739	6.3574	6.4621	7.0386	6.0121
GMLF	0.1537	<0.0001	–	2.5094	6.6327	5.8881	6.0607	7.1516
GMRF	<0.0001	0.2007	<0.0001	–	5.9278	6.3979	6.6073	6.3078
EBLM	<0.0001	<0.0001	<0.0001	<0.0001	–	1.3200	1.2666	1.7609
EBRM	<0.0001	<0.0001	<0.0001	<0.0001	0.0742	–	1.0883	0.7330
EBLF	<0.0001	<0.0001	<0.0001	<0.0001	0.1091	0.3258	–	1.0264
EBRF	<0.0001	<0.0001	<0.0001	<0.0001	0.2302	0.9356	0.4889	–

GMLM, Ghar El Melh left otolith of male; GMRM, Ghar El Melh right otolith of male; GMLF, Ghar El Melh left otolith of female; GMRF, Ghar El Melh right otolith of female; EBLM, Etoile Bay left otolith of male; EBRM, Etoile Bay right otolith of male; EBLF, Etoile Bay left otolith of female; EBRF, Etoile Bay right otolith of female.

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populations using the Wilks' λ test revealed a significant bilateral asymmetry ($P < 0.0001$) in the left and right otoliths (Table 1). Also, Fisher's (1936) distance matrix demonstrated a significant asymmetry ($P < 0.0001$) in the left and right otoliths, as well as in the L–L and R–R otoliths, among males and females of the two populations (Table 2).

In addition, the barycentre projection based on elliptical Fourier descriptors (EFDs) of the contour shape of the left and right otoliths between and within individuals of the Ghar El Melh population (Tunisia) on the first axes *F*1 and *F*2 of the DFA revealed that the two axes explained 78.69 and 12.13% of the total variation, respectively (Figure 3A). Therefore, the left and right otolith shapes of males and females were separated in the positive and negative parts of the two axes. The two axes accounted for 90.82% of the total shape variance, supporting a significant effect on the variability, and showed the segregation of the left otoliths, as well as the right otoliths, from both males and females either in the positive or negative parts of *F*1 or *F*2. However, in the Etoile Bay population (Mauritania), the first axes *F*1 and *F*2 of the DFA revealed that the two axes explained 54.82 and 31.67% of the total variation, respectively (Figure 3B). The two axes accounted for 86.48% of the total shape variance, and showed as well the segregation of the left otoliths, as well as the right otoliths, from both males and females either in the positive or negative parts of *F*1 or *F*2.

Moreover, the combined barycentre projection of the contour shape of the left and right otoliths between males and females of the Ghar El Melh (Tunisia) and Etoile Bay (Mauritania) populations on the first axes *F*1 and *F*2 of the DFA revealed that the two axes explained 68.78 and 13.09% of the total variation, respectively (Figure 3C). The two axes accounted for 81.86% of the total shape variance, supporting also a significant effect on the variability, and showed the separation of the left and right otoliths of both males and females from the Etoile Bay population (Mauritania) on the positive part and those from the Ghar El Melh population (Tunisia) on the negative part of *F*1. However, the *F*2 axis showed segregation of the left otoliths of males and females from the Ghar El Melh population (Tunisia) with the left and right otoliths of females, alongside the right otoliths of half of the males, from the Etoile Bay population (Mauritania) on the positive part. But the right otoliths of males and females from the Ghar El Melh population (Tunisia) were segregated, alongside the right otoliths of half of the males, with the left otoliths of males from the Etoile Bay population (Mauritania) on the negative part of *F*2 axis.

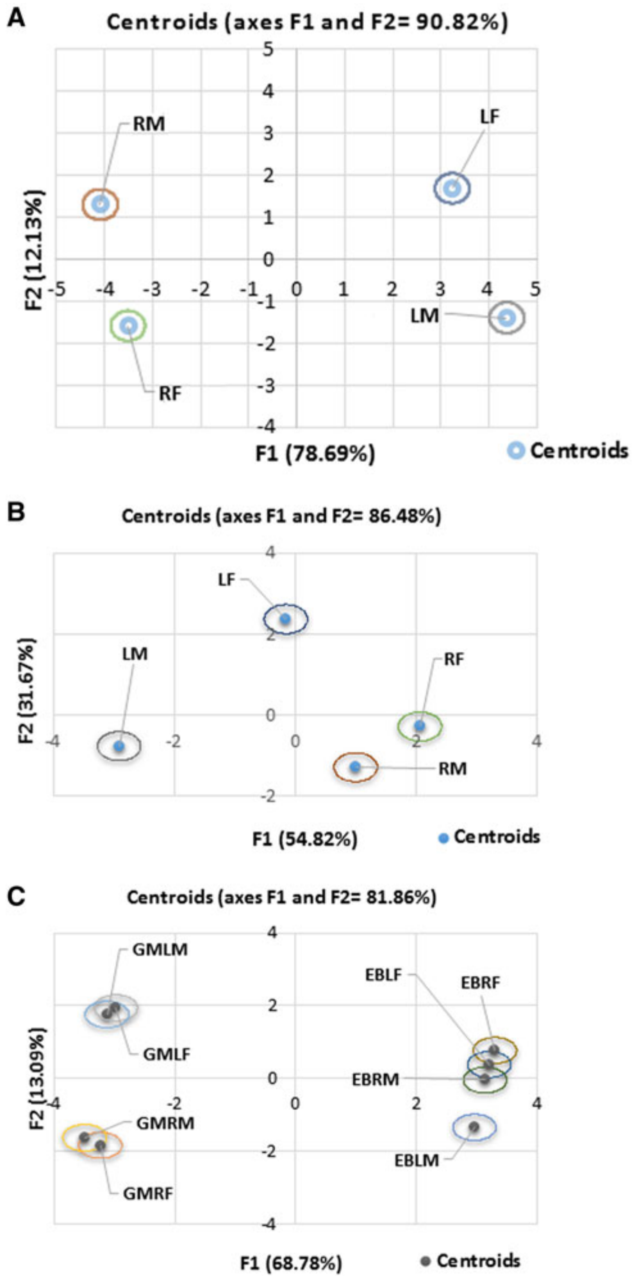

Figure 3. *C. auratus* Risso, 1810: DFA showing the barycentre projection of the left (L) and right (R) shape values of the sagittal otoliths of males (M) and females (F) collected from the (A) Ghar El Melh (Tunisia) ( ) and (B) Etoile Bay (Mauritania) ( ) stations, as well as the two populations combined ( ). GMLM, Ghar El Melh left otolith of male; GMRM, Ghar El Melh right otolith of male; GMLF, Ghar El Melh left otolith of female; GMRF, Ghar El Melh right otolith of female; EBLM, Etoile Bay left otolith of male; EBRM, Etoile Bay right otolith of male; EBLF, Etoile Bay left otolith of female; EBRF, Etoile Bay right otolith of female.

Otolith morphometric variation

At the intrapopulation level, results of the one-way ANOVA and Student's *t*-test of the *Lo*, *Wo*, Po, and Ao showed significant differences, i.e. asymmetry, in *Wo* and Po between the left and right otoliths among males, females, and both sexes combined in the Ghar El Melh (Tunisia) population (Tables 3 and 4). In addition, the Student's *t*-test revealed a significant asymmetry in *Lo* between the left and right otoliths only among females (Table 4). Nevertheless, the paired samples Student's *t*-test indicated a significant asymmetry between the left and right otoliths in *Lo* only among females and *Wo* and Po among males, females, and both sexes combined (Table 5). In the Etoile Bay (Mauritania) population, one-way ANOVA detected significant differences ($P < 0.05$), i.e. asymmetry, in *Wo* between the left and right otoliths among females and both sexes combined (Table 3) and the Student's *t*-test also revealed a significant asymmetry in *Lo* between the left and right otoliths among males, females, and both sexes combined (Table 4). Besides, the paired samples Student's *t*-test designated a significant asymmetry between the left and right otoliths only in *Wo* among males, females, and both sexes combined (Table 6).

At the interpopulation level, the combined analysis of the morphometric measurements of the left and right otoliths between males and females from the Ghar El Melh (Tunisia) and the Etoile Bay (Mauritania) populations using the one-way ANOVA indicated that there were significant differences, i.e. there was asymmetry in the *Lo* only among females, in *Wo* among males, females, and both sexes combined, and in Po and Ao among males and females between the left and right sides of otoliths (Table 3). However, the Student's *t*-test showed a significant asymmetry between the left and right sides of otoliths in *Lo* only among females, in *Wo* among females and both sexes

Table 3. One-way ANOVA of biometric parameters of the left and right otoliths between males and females of *C. auratus* collected from the Ghar El Melh (Tunisia) and Etoile Bay (Mauritania) stations

			Ghar El Melh (Tunisia)		Etoile Bay (Mauritania)		Combined populations	
Parameter	Sex	Otolith side	Mean ± SD	*P* value	Mean ± SD	*P* value	Mean	*P* value
Length (*Lo*)	♂	L	6.679 ± 0.307	0.9576	7.446 ± 0.330	0.7160	7.070	0.8746
		R	6.675 ± 0.340		7.481 ± 0.403			
	♀	L	6.716 ± 0.361	0.3651	7.308 ± 0.448	0.5359	6.972	**0.0055**
		R	6.631 ± 0.361		7.234 ± 0.456			
	♂ + ♀	L	6.698 ± 0.333	0.4730	7.377 ± 0.396	0.8060	7.022	0.6323
		R	6.653 ± 0.348		7.358 ± 0.445			
Width (*Wo*)	♂	L	3.103 ± 0.245	<0.0001	3.833 ± 0.310	0.1028	3.508	<0.0001
		R	3.386 ± 0.295		3.708 ± 0.269			
	♀	L	3.095 ± 0.205	**0.0002**	3.672 ± 0.284	**0.0107**	3.436	<0.0001
		R	3.472 ± 0.221		3.505 ± 0.198			
	♂ + ♀	L	3.099 ± 0.224	<0.0001	3.752 ± 0.305	**0.0055**	3.472	**0.0446**
		R	3.429 ± 0.262		3.607 ± 0.256			
Perimeter (Po)	♂	L	19.661 ± 0.801	**0.0503**	21.091 ± 0.554	0.3971	20.457	<0.0001
		R	20.118 ± 0.962		20.956 ± 0.667			
	♀	L	19.714 ± 0.902	**0.0185**	21.016 ± 0.605	0.1956	20.441	<0.0001
		R	20.230 ± 0.737		20.804 ± 0.649			
	♂ + ♀	L	19.688 ± 0.846	**0.0022**	21.054 ± 0.577	0.1266	20.449	0.1875
		R	20.174 ± 0.851		20.880 ± 0.657			
Area (Ao)	♂	L	18.823 ± 1.246	0.8134	20.581 ± 0.973	0.5181	19.462	<0.0001
		R	18.748 ± 1.195		20.417 ± 0.983			
	♀	L	18.894 ± 1.058	0.9686	19.864 ± 1.179	0.7355	19.351	<0.0001
		R	18.882 ± 1.116		19.764 ± 1.094			
	♂ + ♀	L	18.858 ± 1.146	0.8380	20.223 ± 1.131	0.5151	19.497	0.6032
		R	18.815 ± 1.148		20.091 ± 1.083			

SD, standard deviation.
The values marked in bold are statistically significant ($P < 0.05$).

combined, and in Po only among both sexes combined (Table 4). Besides, the combined analysis between males and females from the two populations, using the paired samples' Student's *t*-test, demonstrated a significant asymmetry in the left and right sides of otoliths in *Lo* only among females, in *Wo* among females and both sexes combined, and in Po only among both sexes combined (Table 7).

FA variation

Estimates of the mean FA values of *Lo*, *Wo*, Ao, and Po between the right and left otoliths within and among males and females are given in Table 8. At the intrapopulation level, a significant FA ($P < 0.05$) was observed only in *Lo* between the right and left otoliths among males, as well as between males and females, i.e. there was a sexual dimorphism, and in Ao among females of the Ghar El Melh (Tunisia). Similarly, significant FA differences ($P < 0.05$) were observed between the right and left otoliths in *Lo* among males, as well as between males and females, and in *Po* between males and females of the Etoile Bay (Mauritania), i.e. there was a sexual dimorphism. However, at the interpopulation level, only significant FA differences were found in *Lo* and *Wo* between the right and left otoliths among males and females. In addition, a box plot chart showing the distribution of the mean values and signs of skewness among males and females of the two populations is given in Figure 4.

Discussion

Both EFA and Fisher's (1936) distance analysis of the otolith contour shape revealed a bilateral significant difference ($P < 0.0001$), i.e. asymmetry, in the left and right otoliths and between the L–L and R–R otoliths only among males and females of the Ghar El Melh population (Tunisia). This asymmetry in the otolith shape is consistent with that found in a range of species inhabiting the Tunisian waters, including *Scorpaena porcus* (Trojette *et al.*, 2014), *Diplodus annularis* (Trojette *et al.*, 2015), *Liza ramada* (Rebaya *et al.*, 2016), *Oblada melanura* (Barhoumi *et al.*, 2018), *Pagellus erythrinus* (Mejri *et al.*, 2020, 2022*a*), *Boops boops* (Ben Labidi *et al.*, 2020*a*, 2020*b*), and *Diplodus vulgaris* (Khedher *et al.*, 2021). Similarly, this asymmetry in the shape of otoliths has also been observed in other species occurring elsewhere outside the Tunisian waters (for details of these species, see Ben Labidi *et al.*, 2020*a*, 2020*b*; Khedher *et al.*, 2021; Mejri *et al.*, 2022*a*; Ben Mohamed *et al.*, 2023).

As regards the causes of asymmetry in the otolith shape, it has been reported that the feeding habits, including food availability (quantity and quality), shifts in diet during ontogenetic

Table 4. Student's *t*-test of biometric parameters of the left and right otoliths between males and females of *C. auratus* collected from the Ghar El Melh (Tunisia) and Etoile Bay (Mauritania) stations

			Ghar El Melh (Tunisia)		Etoile Bay (Mauritania)		Combined populations	
Parameter	Sex	Otolith side	Mean ± SD	*P* value	Mean ± SD	*P* value	Mean	*P* value
Length (*Lo*)	♂	L	6.680 ± 0.307	0.9206	7.446 ± 0.330	0.2743	7.071	0.5770
		R	6.675 ± 0.340		7.481 ± 0.403			
	♀	L	6.716 ± 0.361	**0.0034**	7.308 ± 0.448	0.0596	6.974	**0.0009**
		R	6.631 ± 0.364		7.235 ± 0.456			
	♂ + ♀	L	6.698 ± 0.332	0.0928	7.377 ± 0.396	0.4523	7.022	0.0807
		R	6.653 ± 0.348		7.358 ± 0.445			
Width (*Wo*)	♂	L	3.103 ± 0.245	<0.0001	3.833 ± 0.310	**0.0006**	3.508	0.0594
		R	3.386 ± 0.295		3.708 ± 0.269			
	♀	L	3.095 ± 0.205	<0.0001	3.672 ± 0.284	<0.0001	3.436	**0.0309**
		R	3.472 ± 0.221		3.505 ± 0.198			
	♂ + ♀	L	3.099 ± 0.224	<0.0001	3.753 ± 0.305	<0.0001	3.476	**0.0039**
		R	3.429 ± 0.262		3.607 ± 0.256			
Perimeter (Po)	♂	L	19.662 ± 0.800	**0.0002**	21.091 ± 0.554	0.3546	20.457	0.1013
		R	20.118 ± 0.962		20.956 ± 0.667			
	♀	L	19.714 ± 0.902	<0.0001	21.016 ± 0.605	0.1735	20.441	0.1299
		R	20.230 ± 0.737		20.804 ± 0.649			
	♂ + ♀	L	19.688 ± 0.846	<0.0001	21.054 ± 0.577	0.0997	20.449	**0.0249**
		R	20.174 ± 0.851		20.880 ± 0.657			
Area (Ao)	♂	L	18.823 ± 1.246	0.5907	20.581 ± 0.973	0.2095	19.642	0.2051
		R	18.748 ± 1.195		20.417 ± 0.983			
	♀	L	18.894 ± 1.058	0.9249	19.864 ± 1.179	0.4897	19.351	0.5475
		R	18.882 ± 1.116		19.764 ± 1.094			
	♂ + ♀	L	18.858 ± 1.146	0.6335	20.223 ± 1.131	0.1702	19.497	0.1821
		R	18.815 ± 1.148		20.097 ± 1.083			

SD, standard deviation.
The values marked in bold are statistically significant ($P < 0.05$).

development (Cardinale *et al.*, 2004; Mahé *et al.*, 2019) and the depth at which the fish lives (Fashandi *et al.*, 2019), physiology (e.g. hearing abilities associated with acoustic communication) (Schulz-Mirbach *et al.*, 2019), phylogeny (Torres *et al.*, 2000), sex, growth, and maturity (Cardinale *et al.*, 2004), and spatial isolation between demographic populations (Turan *et al.*, 2006) are among the factors driving differences in otolith shape. Also, Simoneau *et al.* (2000) and Vaux *et al.* (2019) declared that the differences in age and sex among species may lead to a notable difference in the shape of the otolith. Adding to these factors, the asymmetry in the otolith shape has also been attributed to other factors, such as ontogenetic (Capoccioni *et al.*, 2011), genetic (Jawad *et al.*, 2020), and environmental factors, including water temperature, salinity and depth, substrate, light regime and pollution (Panfili *et al.*, 2005; Al-Mamry *et al.*, 2011; Jawad *et al.*, 2012*a*, 2012*b*, 2020; El-Regal *et al.*, 2016; Ferri *et al.*, 2018; Kontaş *et al.*, 2018; Yedier *et al.*, 2018; Mahé *et al.*, 2019; Ben Labidi *et al.*, 2020*a*, 2020*b*; Geladakis *et al.*, 2021; Khedher *et al.*, 2021; Mejri *et al.*, 2022*a*, 2022*b*; Ben Mohamed *et al.*, 2023; Bouriga *et al.*, 2023; Adjibayo Houeto *et al.*, 2024). Besides, Jawad and Al-Sadighzadeh (2013) ascribed the asymmetry between the left and right otoliths to exposure of individuals to environmental stress conditions resulting from changes in some environmental factors, including pollution, severe physical conditions, and habitat quality (Al-Rasady *et al.*, 2010). In addition, Popper *et al.* (2005) stated that the complex shape of otoliths may provide richer information for hearing or balance, whereas Deng *et al.* (2013) suggested that the complex shape of otoliths may alter the dynamics of otolith responses to sounds. Moreover, Gauldie and Crampton (2002) noted that saccular otoliths with more complex shapes are found in hearing-dependent species more often than non-hearing-dependent species.

As far as we know, *C. auratus* is a pelagic neritic species living at a depth of 10–20 m (Thomson, 1990), and adults feed mainly on trivial benthic organisms and detritus (Bakhshalizadeh *et al.*, 2023). Regarding age and sex, the samples studied are adult and sexually mature, with *SL* ranging from 130 to 273 mm in males and 173 to 278 mm in females in the Ghar El Melh population (Tunisia) to avoid the confounding effect of allometric growth (Cardinale *et al.*, 2004) and sexual maturity (Campana and Casselman, 1993) on the otolith shape. Therefore, we can assume that the bilateral asymmetry observed in the left and right and the L–L and R–R otolith shape between males and females can be attributed to the differences in their response to sounds and differences in degrees of hearing and balance, an assumption that needs further investigation. However, Wiff *et al.* (2020) attributed

Table 5. Paired samples Student's *t*-test analysis of biometric parameters of the left (L) and right (R) otoliths within and among males and females of *C. auratus* collected from the Gar El Melh station (Tunisia)

Parameter	Sex	Otolith side	*N*	Range	Mean ± SD	Mean	*P* value
Length (*Lo*)	♂	L	30	6.134–7.408	6.6799 ± 0.3069	6.6776	0.9206
		R	30	6.186–7.291	6.6754 ± 0.3401		
	♀	L	30	6.107–7.644	6.7161 ± 0.3613	6.6735	**0.0034**
		R	30	6.015–7.554	6.6309 ± 0.3609		
	♂ + ♀	L	60	6.107–7.644	6.6979 ± 0.3328	6.6755	0.0928
		R	60	6.015–7.554	6.6532 ± 0.3484		
Width (*Wo*)	♂	L	30	2.605–3.697	3.1032 ± 0.2446	3.2447	<0.0001
		R	30	2.855–3.894	3.3862 ± 0.2954		
	♀	L	30	2.750–3.566	3.0946 ± 0.2054	3.2834	<0.0001
		R	30	3.052–3.947	3.4722 ± 0.2213		
	♂ + ♀	L	60	2.605–3.697	3.0989 ± 0.2239	3.2640	<0.0001
		R	60	2.855–3.947	3.4291 ± 0.2623		
Perimeter (Po)	♂	L	30	18.209–21.735	19.6615 ± 0.8005	19.8899	**0.0002**
		R	30	18.367–21.656	20.1184 ± 0.9616		
	♀	L	30	18.183–21.630	19.7136 ± 0.9019	19.9715	<0.0001
		R	30	19.077–21.630	20.2295 ± 0.7369		
	♂ + ♀	L	60	18.183–21.735	19.6875 ± 0.8459	19.9307	<0.0001
		R	60	18.367–21.656	20.1739 ± 0.8512		
Area (Ao)	♂	L	30	16.094–21.257	18.8232 ± 1.2457	18.7858	0.5907
		R	30	17.051–21.392	18.7484 ± 1.1948		
	♀	L	30	16.760–20.460	18.8936 ± 1.0576	18.8880	0.9249
		R	30	17.081–21.671	18.8824 ± 1.1163		
	♂ + ♀	L	60	16.094–21.257	18.8584 ± 1.1462	18.8369	0.6335
		R	60	17.051–21.671	18.8154 ± 1.1483		

SD, standard deviation; *N*, number of samples.
The values marked in bold are statistically significant ($P < 0.05$).

this bilateral asymmetry to reproductive isolation between individuals that may lead to inter- or even intraindividual variations (Panfili *et al*., 2005) or the possibility of within-individual stress that lead to developmental abnormalities of individuals or poor living conditions for larvae (Ben Labidi *et al*., 2020*a*, 2020*b*; Mejri *et al*., 2020, 2022*a*, 2022*b*; Khedher *et al*., 2021).

Regarding the environmental characteristics of the Ghar El Melh station (Tunisia), the water temperature ranges from 11.2°C in winter to 24.7°C in summer (Feki *et al*., 2013), the salinity mostly varies between 38 and 43‰ in winter (Sellem *et al*., 2019) and 42.19 and 53.3‰ in summer (Khedhri *et al*., 2015), and the pH fluctuates between 7.92 in winter and 8.31 in summer (Ben Aoun *et al*., 2007). In addition, the station is polluted with organic discharges through harbour-related activities in its southern part (Guetat *et al*., 2012), sewage resulting from the transportation of the surrounding ports, and the entry of seawater loaded with phosphorous from the Gulf of Gabes (Sellem *et al*., 2019). In this context, it is worth mentioning that the samples were collected during the period between June and October, and it has previously been confirmed that fish are more sensitive to a temperature change of about 0.03°C (Rebaya *et al*., 2017). Therefore, we can attribute the significant asymmetry in the left and right sides, as well as the L–L and R–R sides, of otolith shape within and among males and females in the Ghar El Melh population (Tunisia) to differences in their susceptibility to differences in these environmental parameters.

On the contrary, both EFA and Fisher's (1936) distance analysis of the otolith contour shape indicated a significant bilateral similarity ($P > 0.05$), i.e. there was symmetry, between the left and right, as well as between the left and L–L and R–R sides, of otoliths shape among males and females of the Etoile Bay population (Mauritania). A similar finding of the bilateral symmetry between the right and left otoliths has been recorded in *B. boops* from the Kelibia station in Tunisian waters (Ben Labidi *et al*., 2020*a*). As previously reported, mullet species, including *C. auratus*, are highly euryhaline and eurythermal and tolerate broad salinities (González-Castro and Minos, 2015). Therefore, this apparent bilateral symmetry recognized here can be explained in terms of the increased developmental stability of individuals in response to environmental stress, resulting from the high water temperature (26.5°C), salinity (35.8‰), pH (8) (Legraa, 2019), and chronic pollution (Berque *et al*., 2012), experienced by the individuals during the warm season (August–October).

In addition, Wilk's λ test of the shape variance between left and right otoliths revealed a significant asymmetry ($P < 0.0001$) between males and females of the two populations. Moreover, the DFA based on EFDs of the otoliths' contour shape noticeably separated between the left and right otoliths among males and females at both the intra- and interpopulation levels. Therefore, we can conclude that the spatial and environmental differences induced apparent effects on the otolith shape between males

Table 6. Paired samples Student's *t*-test analysis of biometric parameters of the left (L) and right (R) otoliths within and among males and females of *C. auratus* collected from the Etoile Bay station (Mauritania)

Parameter	Sex	Otolith side	*N*	Range	Mean ± SD	Mean	*P* value
Length (*Lo*)	♂	L	30	6.633–8.112	7.446 ± 0.3299	7.4637	0.2743
		R	30	6.494–8.301	7.4811 ± 0.4033		
	♀	L	30	6.633–8.211	7.3075 ± 0.4475	7.2712	0.0596
		R	30	6.538–8.394	7.2348 ± 0.4561		
	♂ + ♀	L	60	6.633–8.211	7.3769 ± 0.3960	7.3674	0.4523
		R	60	6.494–8.394	7.3579 ± 0.4445		
Width (*Wo*)	♂	L	30	3.239–4.436	3.8325 ± 0.3095	3.7703	**0.0006**
		R	30	3.169–4.112	3.7081 ± 0.2691		
	♀	L	30	3.197–4.394	3.6724 ± 0.2838	3.5889	<0.0001
		R	30	3.056–3.901	3.5054 ± 0.1984		
	♂ + ♀	L	60	3.197–4.436	3.7525 ± 0.3053	3.6796	<0.0001
		R	60	3.056–4.112	3.6067 ± 0.2557		
Perimeter (Po)	♂	L	30	19.998–21.941	21.0913 ± 0.5541	21.0238	0.3546
		R	30	19.632–21.913	20.9562 ± 0.6668		
	♀	L	30	19.970–21.913	21.0157 ± 0.6052	20.9750	0.1735
		R	30	19.435–21.941	20.8035 ± 0.6493		
	♂ + ♀	L	60	19.970–21.941	21.0535 ± 0.5765	20.9667	0.0997
		R	60	19.435–21.941	20.8798 ± 0.6570		
Area (Ao)	♂	L	30	18.215–21.961	20.5811 ± 0.9734	20.4990	0.2095
		R	30	17.657–21.994	20.4168 ± 0.9830		
	♀	L	30	17.531–21.803	19.8642 ± 1.1794	19.8143	0.4897
		R	30	18.024–21.936	19.7644 ± 1.0944		
	♂ + ♀	L	60	17.531–21.961	20.2226 ± 1.1314	20.1566	0.1702
		R	60	17.657–21.994	20.0906 ± 1.0825		

SD, standard deviation; *N*, number of samples.
The value marked in bold is statistically significant ($P < 0.05$).

and females of *C. auratus* populations collected from the Ghar El Melh (Tunisia) and Etoile Bay (Mauritania) stations.

On the contrary, intrapopulation analysis of the *Lo*, *Wo*, Po, and Ao between the left and right otoliths using one-way ANOVA and Student's *t*-test showed an apparent asymmetry in *Wo* and Po between the left and right otoliths among males, females, and both sexes combined in the Ghar El Melh (Tunisia) population. In addition, the Student's *t*-test revealed a significant asymmetry in *Lo* between the left and right otoliths only among females. Besides, the Student's *t*-test of paired samples indicated a significant asymmetry between the left and right otoliths in *Lo* only among females and in *Wo* and Po among males, females, and both sexes combined. However, in the Etoile Bay (Mauritania) population, the one-way ANOVA detected a significant asymmetry in *Wo* between the left and right otoliths among females and both sexes combined, and Student's *t*-test revealed an asymmetry in *Lo* between the left and right otoliths among males, females, and both sexes combined. In addition, the paired samples Student's *t*-test demonstrated a significant asymmetry between the left and right otoliths only in *Wo* among males, females, and both sexes combined.

Nevertheless, at the interpopulation level, the combined analysis of the morphometric dimensions of the otoliths between males and females from the two populations produced a significant asymmetry in the left and right otoliths in *Lo* only among females, in *Wo* among males, females, and both sexes combined, and in Po and Ao among males and females. In addition, the Student's *t*-test showed a significant asymmetry between the left and right sides of otoliths in *Lo* only among females, in *Wo* among females and both sexes combined, and in Po only among both sexes combined. Moreover, the combined paired samples Student's *t*-test analysis scored a significant asymmetry in the left and right otoliths between males and females from the two populations in *Lo* only among females, in *Wo* among females and both sexes combined, and in Po only among both sexes combined. Similar results of asymmetry in *Wo* have also been found in *P. erythrinus* (Mejri *et al.*, 2020), *B. boops* (Ben Labidi *et al.*, 2020*b*), and *D. vulgaris* (Khedher *et al.*, 2021) in Tunisian waters, as well as elsewhere in *Lo* and *Wo* in *Rastrelliger kanagurta* (Al-Mamry *et al.*, 2011), *Sardinella sindensis* and *Sillago sihama* (Jawad *et al.*, 2012*a*), *Lutjanus bengalensis* (Jawad *et al.*, 2012*b*), *Chlorurus sordidus* and *Hipposcarus harid* (El-Regal *et al.*, 2016), *Merlangius merlangus* (Kontaş *et al.*, 2018), *Trachurus mediterraneus* (Yedier *et al.*, 2018), and *Sarotherodon melanotheron* and *Coptodon guineensis* (Jawad *et al.*, 2020). As is reported, this significant asymmetry in the morphometric dimensions of the otoliths can be interpreted as FA, which Jawad and Al-Sadighzadeh (2013) attributed to the assumption that vulnerable individuals under stressful environmental conditions may develop asymmetry on either side of the otoliths. This assumption has also been confirmed by Ben Labidi *et al.* (2020*b*) and Mejri *et al.* (2020), who reported a direct

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Table 7. Combined paired samples Student's *t*-test analysis of biometric parameters of the left (L) and right (R) otoliths between males and females of *C. auratus* collected from the Ghar El Melh (Tunisia) and Etoile Bay (Mauritania) stations

				Ghar El Melh population (Tunisia)		Etoile Bay population (Mauritania)		Populations combined	
Parameter	Sex	Otolith side	*N*	Range	Mean ± SD	Range	Mean ± SD	Mean	*P* value
Length (*Lo*)	♂	L	60	6.134–7.408	6.6799 ± 0.3069	6.633–8.112	7.446 ± 0.3299	7.0706	0.5770
		R	60	6.186–7.291	6.6754 ± 0.3401	6.494–8.301	7.4811 ± 0.4033		
	♀	L	60	6.107–7.644	6.7161 ± 0.3613	6.633–8.211	7.3075 ± 0.4475	6.9735	**0.0009**
		R	60	6.015–7.554	6.6309 ± 0.3609	6.538–8.394	7.2348 ± 0.4561		
	♂ + ♀	L	120	6.107–7.644	6.6979 ± 0.3328	6.633–8.211	7.3769 ± 0.3960	7.0215	0.0807
		R	120	6.015–7.554	6.6532 ± 0.3484	6.494–8.394	7.3579 ± 0.4445		
Width (*Wo*)	♂	L	60	2.605–3.697	3.1032 ± 0.2446	3.239–4.436	3.8325 ± 0.3095	3.5075	0.0594
		R	60	2.855–3.894	3.3862 ± 0.2954	3.169–4.112	3.7081 ± 0.2691		
	♀	L	60	2.750–3.566	3.0946 ± 0.2054	3.197–4.394	3.6724 ± 0.2838	3.4361	**0.0309**
		R	60	3.052–3.947	3.4722 ± 0.2213	3.056–3.901	3.5054 ± 0.1984		
	♂ + ♀	L	120	2.605–3.697	3.0989 ± 0.2239	3.197–4.436	3.7525 ± 0.3053	3.4758	**0.0039**
		R	120	2.855–3.947	3.4291 ± 0.2623	3.056–4.112	3.6067 ± 0.2557		
Perimeter (Po)	♂	L	60	18.209–21.735	19.6615 ± 0.8005	19.998–21.941	21.0913 ± 0.5541	20.4568	0.1013
		R	60	18.367–21.656	20.1184 ± 0.9616	19.632–21.913	20.9562 ± 0.6668		
	♀	L	60	18.183–21.630	19.7136 ± 0.9019	19.970–21.0157	21.0157 ± 0.6052	20.4405	0.1299
		R	60	19.077–21.630	20.2295 ± 0.7369	19.435–21.941	20.8035 ± 0.6493		
	♂ + ♀	L	120	18.183–21.735	19.6875 ± 0.8459	19.970–21.941	21.0535 ± 0.5765	20.4487	**0.0249**
		R	120	18.367–21.656	20.1739 ± 0.8512	19.435–21.941	20.8798 ± 0.6570		
Area (Ao)	♂	L	60	16.094–21.257	18.8232 ± 1.2457	18.215–21.961	20.5811 ± 0.9734	19.6424	0.2051
		R	60	17.051–21.392	18.7484 ± 1.1948	17.657–21.994	20.4168 ± 0.9830		
	♀	L	60	16.760–20.460	18.8936 ± 1.0576	17.531–21.803	19.8642 ± 1.1794	19.3511	0.5475
		R	60	17.081–21.671	18.8824 ± 1.1163	18.024–21.936	19.7644 ± 1.0944		
	♂ + ♀	L	120	16.094–21.257	18.8584 ± 1.1462	17.531–21.961	20.2226 ± 1.1314	19.4968	0.1821
		R	120	17.051–21.671	18.8154 ± 1.1483	17.657–21.994	20.0906 ± 1.0825		

SD, standard deviation; *N*, number of samples.
The values marked in bold are statistically significant ($P < 0.05$).

Table 8. Estimates of the mean values of FA between the left (L) and right (R) sagittal otoliths' length (*Lo*), width (*Wo*), perimeter (Po), and area (Ao) of *C. ramada* males and females collected from the Ghar El Melh (GM) and Etoile Bay (EB) stations

			Mean FA			
Population	Sex	Otolith side	*Lo* (in mm)	*Wo* (in mm)	Po (in mm)	Ao (in mm^2)
Ghar El Melh (GM)	Male	R–L	**−0.0045**	0.2830	0.4569	−0.0747
	Female	R–L	−0.0851	0.3776	0.5159	**−0.0111**
Wilk's test *P* value	Male–female	R–L	**0.0021**	0.8173	0.8903	0.5584
Etoile Bay (EB)	Male	R–L	**0.0348**	−0.1244	−0.1351	−0.1643
	Female	R–L	−0.0727	−0.1670	−0.2123	−0.0998
Wilk's test *P* value	Male–female	R–L	**0.0218**	0.7487	**0.0457**	0.5898
Between populations	Male–female	R–L	**0.0023**	**0.0243**	0.7243	0.2604

P values marked in bold are significant ($P < 0.05$).

correlation between environmental stress resulting from pollution and asymmetry in the otolith morphology in the species they examined. In addition, the bilateral asymmetry recorded in the otolith morphometric dimensions within and among males and females from the two populations can be explained in terms of abnormal swimming activity and interference with correct sound localization, which results in the incapability of the individuals to integrate with the environment where they live (Helling *et al*., 2003; Lychakov and Rebane, 2005).

In conclusion, according to our knowledge attained from earlier studies on the intra- and interspecific variations in the otolith shape, most of these studies have been carried out on species inhabiting local or inland waters. Therefore, the present study was conducted for the first time on *C. auratus* populations collected from inland and outland waters representing two ecologically different niches, the Gar El Melh (Tunisia) and Etoile Bay (Mauritania) stations, as an attempt to assess whether or not the variations in the environmental conditions between the two niches induce differences in the otolith shape and morphometry. At the intrapopulation level, analysis of the otoliths' contour shape revealed a significant asymmetry between the left and right sides, as well as the L–L and R–R sides, among males and females of the Ghar El Melh (Tunisia) population, and a significant symmetry among males and females of the Etoile Bay (Mauritania) population. At the interpopulation level, a significant asymmetry was also detected between the left and right otoliths' shape among males and females of the two populations. In addition, DFA based on EFDs of the otoliths' contour shape conspicuously separated between the left and right otoliths among males and females at both the intra- and interpopulation levels and also separated between those of the two populations. Moreover, analysis of the otolith morphometric dimensions showed a differential significant asymmetry in the *Lo*, *Wo*, Po, and Ao between the left and right otoliths among males and females at the intra- and interpopulation levels. These significant asymmetries observed at the intra- and interpopulation levels between the left and right otoliths in both contour shape and morphometry were interpreted as FA caused by environmental stress conditions resulting from variations in the water temperature, salinity, pH, and pollution between the two ecological niches. However, the significant symmetry detected between the left and right otoliths, particularly among males and females of the Etoile Bay (Mauritania), was attributed to the increased developmental stability of individuals in response to environmental stress experienced by the individuals during the warm season (August–October). Therefore, the geospatial variations in the environmental conditions between the two ecological niches effectually induced differences in the otolith morphology. The results of this investigation largely contribute to the knowledge of the otolith shape and morphometric data that have long been confirmed as perfect tools for discriminating between species and identifying new species.

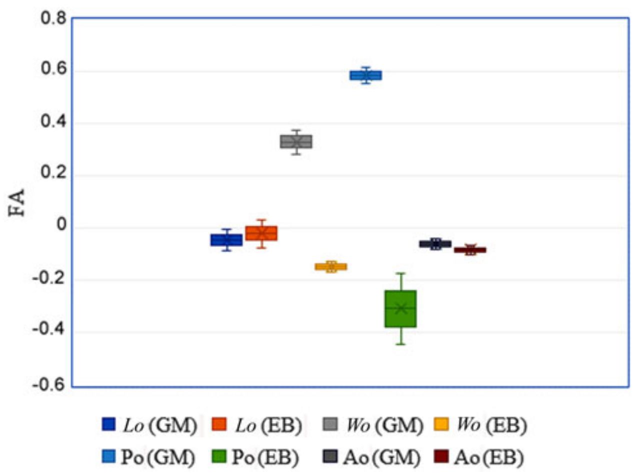

Figure 4. Box plots showing the distribution and signs of skewness of the mean values of FA of the right (R) and left (L) length (*Lo*), width (*Wo*), perimeter (*Po*), and area (*Ao*) of the otoliths between the Ghar El Melh (GM) (Tunisia) and Etoile Bay (EB) (Mauritania) populations. The central line is the median, the boxes indicate the first and third quartiles, and the whiskers indicate two standard deviations. Outliers are those outside the median ± 0.3–0.6 SD.

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Data. Data supporting the findings of this study are available from the corresponding author upon request.

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Ethical standards. The Laboratory of Ecology, Biology, and Physiology of Aquatic Organisms, and Laboratory of Biodiversity, Biotechnology, and Climate Change, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia have approved this research. In addition, all procedures in this study were performed following the guidelines for the Proper Conduct of Animal Experiments outlined by the University of Tunis El Manar, Tunis, Tunisia (No. 1474 certificated on 14 August 1995), as well as all applicable international, national, and/or institutional guidelines for the care and use of animals in research.

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