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Discriminant inter and intrapopulation variation in sagittal otolith shape and morphometry in *Chelon ramada* (Actinopterygii, Mugilidae) from the Boughrara and El Bibane lagoons in Tunisian waters

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Abstract

Variations in sagittal otolith shape and morphometry, including length (*Lo*), width (*Wo*), area (Ao), and perimeter (Po), were investigated in two populations of *Chelon ramada* collected from the Boughrara and El Bibane lagoons located in southeastern Tunisia. The objective was to assess the geographic variation in the sagittal otoliths' shape and morphometry and the effect of potential fluctuating asymmetry (FA) in morphometry on the stock structure of *C. ramada* in the two lagoons to inform on appropriate management procedures. At the interpopulation level, analysis of sagittal otolith shape showed a statistically significant difference ($P = 0.0001$), i.e. there was a bilateral asymmetry, in the shape of left and right otoliths between individuals of the two populations. In addition, significant FA was found only in *Lo* between the left and right otoliths. At the intrapopulation level, a significant shape difference ($P < 0.0001$), particularly asymmetry, was observed in both left and right otoliths between males and females, indicating sexual dimorphism in shape within the Boughrara lagoon. However, significant shape similarity, i.e. symmetry, was observed in the left and right otoliths among individuals of the El Bibane lagoon. Moreover, a significant FA was detected in *Lo* between the left and right otoliths only among males, as well as between males and females of the Boughrara lagoon. However, a significant FA between the left and right otoliths was found only in *Wo* among males and in all morphometric dimensions among females and *Wo* between males and females of the El Bibane lagoon. Discriminant function analysis of the otolith contour shape confirmed the presence of two separate *C. ramada* stocks, one corresponding to the Boughrara lagoon and the other representing the El Bibane lagoon, which should be managed separately. The possible cause of morphological variation in the sagittal otoliths' shape and morphometry due to FA between individuals of the two populations was discussed in relation to the biotic and abiotic factors.

Introduction

The inner ear of fish is an essential auditory sensor, containing three semicircular canals and paired otoliths. These otoliths are calcareous structures composed primarily of calcium carbonate, trace or vestigial elements, and trace amounts of organic matter (Borelli *et al.*, 2003). These dense otolith structures are the asterisci (otoliths of the lagena), lapilli (otoliths of the utriculus), and sagittae (otoliths of the sacculus) and are closely associated with the auditory sensory epithelium, function in the transmission of sound, and are also involved in maintaining a static and active balance (Parmentier *et al.*, 2001). Of these otoliths, the sagittal otoliths or sagittae are the largest of them, found just behind the eyes and approximately level with them vertically, while the lapilli and asterisci are the smallest and are located within the utriculus and lagena chambers, respectively, which are continuous with three semi-circular canals (Payan *et al.*, 1997; Schwarzhans *et al.*, 2017). Interestingly, the acoustic properties (density and elasticity) of fish tissue are very similar to the surrounding water, and when the fish are exposed to sound, the otoliths function as an accelerometer (Zhang *et al.*, 2021). Indeed, otoliths are denser than water, and their motion changes relative to water, creating a relative movement with auditory hair cells (Schulz-Mirbach *et al.*, 2019). When hair cells deviate with relative motion, neurotransmitters are released in the sensory epithelium to produce auditory responses (Popper and Lu, 2000).

Sagittal otoliths are known to increase in size with increasing habitat depths down to about 1000 m (Tuset *et al.*, 2016) and decrease in size in fish fast-swimming (Volpedo *et al.*, 2008), and are associated with feeding habitats (Lombarte *et al.*, 2010; Tuset *et al.*, 2016). As a result, their morphology has long been used as a characterizing trait of species identification, i.e. species-specific features (Callicó Fortunato *et al.*, 2014; Avigliano *et al.*, 2016; Ferri *et al.*, 2018; Mejri *et al.*, 2022b). In addition, these otoliths have been documented to show variations in morphologies (shape and morphometry) at both the inter and intraspecific levels (Ferri *et al.*, 2018; Tuset *et al.*, 2018; Ben Labidi *et al.*, 2020b; Mejri *et al.*, 2020, 2022b; Khedher *et al.*, 2021; D'Iglio *et al.*, 2023) and their morphometry, in particular, has a close relationship to fish body size (Martucci *et al.*, 1993). In addition, it has been shown that these variations are influenced by several factors, including genetic (Vignon and Morat, 2010; Berg *et al.*, 2018), ontogenetic (Hüssy, 2008; Capoccioni *et al.*, 2011), physiological (Lombarte and Cruz, 2007; Schulz-Mirbach *et al.*, 2019), phylogeny (Campana and Neilson, 1985; Torres *et al.*, 2000), and exogenous factors, such as living depth (Wilson, 1985; Lombarte and Lleonart, 1993), temperature (Lombarte and Lleonart, 1993) and salinity of the water (Capoccioni *et al.*, 2011), and food supplies (Gagliano and McCormick, 2004; Hüssy, 2008; Bremm and Schulz, 2014). Moreover, variations in otolith microchemistry have been used to estimate spatiotemporal migration and feeding behaviour between species (Lord *et al.*, 2011). Therefore, information on the shape and morphometry of fish sagittal otolith has long been considered an appropriate method for studying population structure and stock assessment (Pothin *et al.*, 2006; Gonzalez-Salas and Lenfant, 2007; Duarte-Neto *et al.*, 2008; Rebaya *et al.*, 2016; Bose *et al.*, 2017; Mejri *et al.*, 2018, 2022a; Mahé *et al.*, 2019; Ben Labidi *et al.*, 2020a; Khedher *et al.*, 2021), species identification (Škeljo and Ferri, 2011; Bani *et al.*, 2013; Jawad *et al.*, 2018), assessment of age and growth (Cardinale *et al.*, 2004; Škeljo *et al.*, 2015), diet content (Lilliendahl and Solmundsson, 2006), ontogeny (Capoccioni *et al.*, 2011), spatiotemporal migration (Lord *et al.*, 2011), and fisheries science and management (Vasconcelos *et al.*, 2018; Rani *et al.*, 2019). As the morphological variation of sagittal otoliths is influenced by genetic factors (Vignon and Morat, 2010), external factors, including depth (Lombarte and Lleonart, 1993) and water temperature (Lombarte and Lleonart, 1993; Hüssy, 2008), salinity (Capoccioni *et al.*, 2011), and food supply (Gagliano and McCormick, 2004; Hüssy, 2008) also play a strong role in reshaping of otoliths (Vignon, 2012; Bremm and Schulz, 2014).

Indeed, the study of inter and intraspecific variability in the sagittal otoliths' shape has been performed using several techniques (for details, see Khedher *et al.*, 2021), including elliptical Fourier analysis (EFA), which has been proven to be the most widely used and effective method to describe, characterize, and capture outlines information in a measurable manner (Lord *et al.*, 2012).

As far as is known, mullet species of the Mugilidae family are widely distributed coastal marine species and are widespread in warm waters, including temperate, subtropical, and tropical regions (Kasımoğlu and Yılmaz, 2012; Fortunato *et al.*, 2017), where they live on algae and detritus (Keith *et al.*, 2000). In addition, these species have been assigned as highly euryhaline and eurythermal species and withstand wide-ranging salinities (González-Castro and Ghasemsadeh, 2015; González-Castro and Minos, 2015). Therefore, they inhabit various habitats, including shallow brackish and marine waters adjacent to lagoons, and consume part of their life cycle in coastal lagoons, lakes, and/or rivers, where they use these habitats for feeding and growth, refuge, spawning, and development (Papasotiropoulos *et al.*, 2002; González-Castro and Ghasemsadeh, 2015; González-Castro and Minos, 2015). Mugilids migrate to the Sea to breed after resting and maturing in various variable habitats (González-Castro *et al.*, 2009, 2011; Whitfield, 2015). Some adults return to brackish water after breeding, whereas others remain in marine waters (Whitfield *et al.*, 2012).

In Tunisian waters, Blel *et al.* (2013) identified six species assigned to three genera of the family Mugilidae, including *Mugil cephalus* (Linnaeus, 1758), *Chelon aurata* (Risso, 1810), *C. saliens* (Risso, 1810), *C. ramada* (Risso, 1826), *C. labrosus* (Risso, 1827), and *Oedalechilus labeo* (Cuvier, 1829). Of these six species, the thin-lipped grey *C. ramada* is the most common of these species in Tunisian waters (Rebaya *et al.*, 2016). In addition, it has economic importance because it is one of the target species for commercial fishing along the Tunisian coast (Masmoudi *et al.*, 2001) and its annual catch has increased significantly in recent years (Fehri-Bedoui *et al.*, 2002). According to the last data available by DGPA (2015), the overall annual landings of the Mugilidae species in Tunisia reached 3000 tons, accounting for 9.5% of total coastal landings and 2.26% of total Tunisian fisheries landings, with *C. auratus* alone accounting for around 1350 tonnes, which represents 45% of the total Mugilidae species landings (Romdhane *et al.*, 2019).

Geographically, the Tunisia coastline extends over 1300 km, with about 575 km of sandy beaches (Bounouh, 2010), and various lagoons, including the Boughrara and El Bibane. Similar to Mediterranean lagoons, Tunisian lagoons experience significant seasonal and even daily variations in physicochemical parameters, such as temperature and salinity extremes (Afli *et al.*, 2009). The most frequent group of species in the Boughrara lagoon is represented by the migratory species (sea to the lagoon and vice versa) of the families Sparidae, Soleidae, Moronidae, and Mugilidae. However, the most important species in the lagoon of El Bibane, regularly landing, are essentially those of the family of Moronidae, Sparidae, and Mugilidae. Among these species, the thin-lipped grey mullet *C. ramada* is one of the target fish species of the commercial fishery along the Tunisian coast (Masmoudi *et al.*, 2001).

Because the variability in the sagittal otolith shape has been poorly studied in many species, including *C. ramada*, the present study was conducted for the first time to (1) compare the sagittal otolith shape and morphometry, particularly length (Lo), width (Wo), area (Ao), and perimeter (Po), between populations of this species inhabiting the Boughrara and El Bibane lagoons located in southeastern Tunisia and (2) assess the effect of potential fluctuating asymmetry (FA) in the sagittal otolith morphology (shape and morphometry) on the stock structure of *C. ramada* in the two lagoons to inform on appropriate management procedures.

Materials and methods

Study area

As shown in Figure 1, the Boughrara lagoon (32°28' 33°45''N, 10° 45' 10°57''E) is located south of the Djerba island on the southern edge of the Gulf of Gabes and it ranks first among the Tunisian lagoons because it has an area of 50,000 ha (Guetat *et al.*, 2012). It communicates with the seawater of the Gulf of Gabes through the El Kantara in the northeastern part and the Ajim channels in the northwestern part. The climate of the Boughrara lagoon is dry and sunny, with strong easterly winds that carry particles into the sea (Brahim *et al.*, 2014, 2015) and cause strong wind erosion. The surface water temperature varies strongly with the season, averaging 24.7 °C in summer and 11.2 °C in winter, increasing along a north-south gradient (Feki

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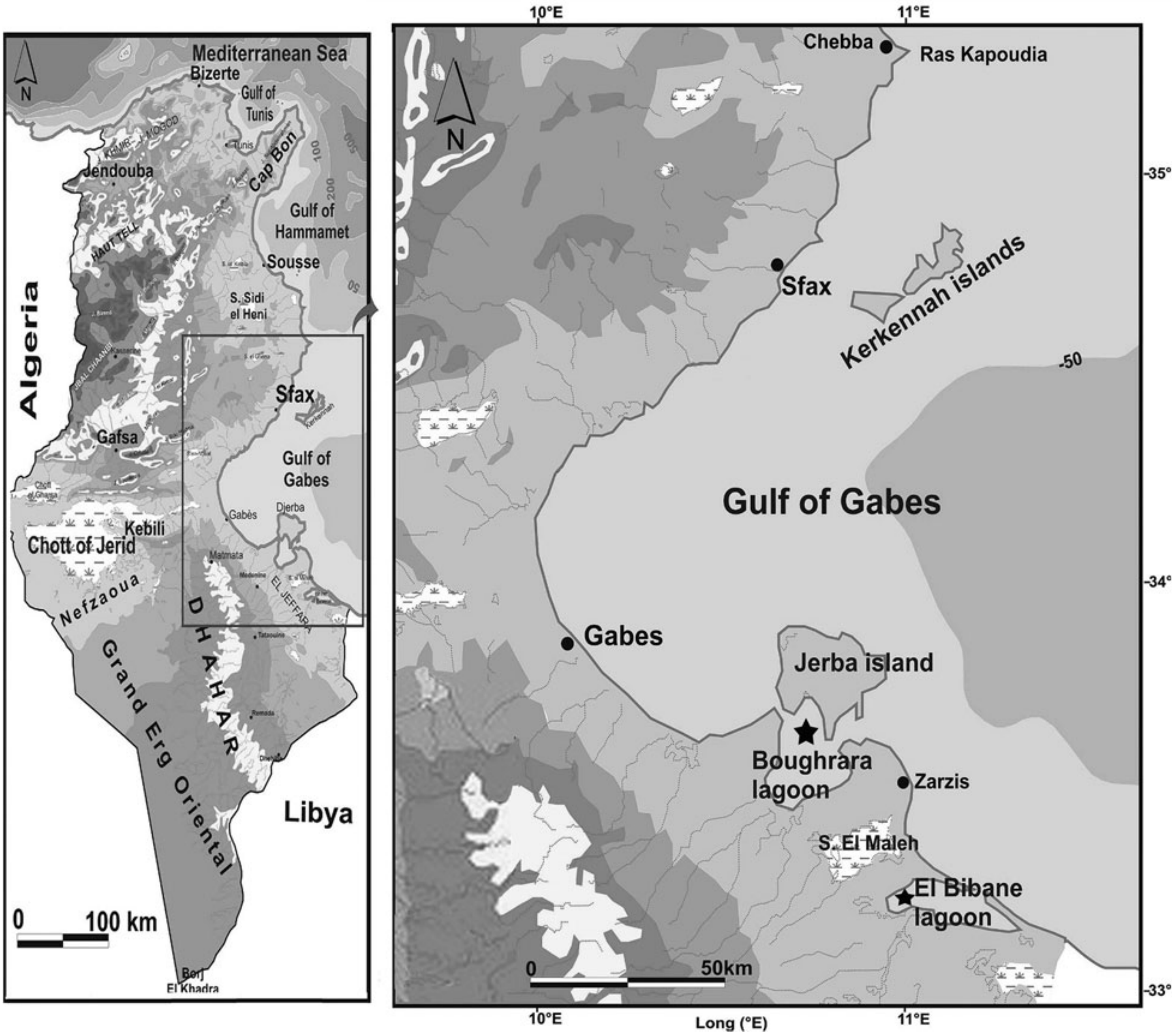

Figure 1. *Chelon ramada* Risso, 1826, the Boughrara and El Bibane lagoons sites (★) from which the individuals were collected in Tunisia.

et al., 2013). The salinity of the lagoon is higher than that of the Sea (Abdenadher *et al.*, 2012) and mostly varies between 38 and 43‰ (Sellem *et al.*, 2019). However, it varies between 42.19 and 53.3‰ during the summer season (Khedhri *et al.*, 2015). The pH of the water fluctuates between an average of 7.92 in winter and 8.31 in summer (Ben Aoun *et al.*, 2007). In addition, the lagoon has in its southern part a distinctive harbour, making it suffers from organic discharges through harbour-related activities (Guetat *et al.*, 2012), as well as the pollution with sewage 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 El Bibane lagoon is situated on the southern coast of Tunisia (33°15"N, 11°15"E). It has an area of 23,000 ha, a surface area of about 230 km^2, and is separated from the sea by a limestone cordon of about 2.5 km long and is divided into nine small islands (Lemoalle, 1986) and it communicates with the sea through a footbridge of about 400 m in length. The surface water temperature is homogeneous but it increases in June in shallow areas and its average is 28 °C in summer and 14 °C in winter (Ben Abdeladhim, 2003). The salinity is relatively high and has an average of 44‰ (Ben Abdeladhim, 2003). Along the central radial, it varies between 39.1 and 46.5‰, with a minimum at the end of winter and a maximum at the end of summer after intense evaporation of water (Zaouali, 1985). The average dissolved oxygen saturation is 109% and can reach 133%, and the pH varies between 8.2 and 8.3 (Ben Abdeladhim, 2003; Akrout, 2012). These characteristics gave it an economic interest in Tunisian waters since fishing and aquaculture have been practiced there for a long time (Zaouali, 1983).

Sampling

A total of 120 adult individuals of *C. ramada* were collected from the two lagoons (60 individuals each) between June and July 2018. All individuals were captured live by gillnets using artisanal coastal boats. Immediately after catching, individuals were examined visually for gonadal maturity, using the scale of Kesteven (1960) and Treasurer (1990), or microscopically in the case of small gonads, to confirm the maturity stage of all individuals. Afterwards, individuals were measured for total length (*TL*) using an ichthyometer, and the values were rounded to the nearest 0.1 mm (Table 1).

Sagittae sampling and processing

Otolith extraction

The right and left sagittal otoliths (*sagittae*) were extracted according to the method of Panfili *et al.* (2002). After extraction,

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Table 1. The number of males and females (N) and mean of total length (TL) ± standard deviation (SD) of *Chelon ramada* examined from the Boughrara (B) and El Bibane (E) lagoons in Tunisia

Lagoon	Boughrara (B)		El Biban (E)	
Sex	Males	Females	Males	Females
Number of samples (N)	30	30	30	30
Mean TL (in mm) ± (SD)	246.67 ± 19.18	229.27 ± 11.41	234.38 ± 9.92	241.46 ± 6.30

they were cleaned with distilled water and then air-dried at room temperature or in a low-temperature oven.

Otolith shape analysis

Otoliths were positioned onto a microscope slide with the sulcus directed down and the rostrum placed in the same direction to minimize distortion errors in the normalization process. Subsequently, each pair of otoliths was photographed under a binocular loupe using a Canon Ixus 185 high-performance digital camera with a resolution of 20 megapixels to obtain images that allow us to trace the outlines of the shape with full accuracy (Figure 2). The obtained images were then processed by Photoshop CS6 software, which transformed the original images of otoliths into binary images. Afterwards, images of the shapes were analysed using SHAPE 1.3 software (Iwata and Ukai, 2002). The contour shape of each otolith was evaluated by EFA as previously described in detail by Ben Labidi *et al.* (2020a, 2020b) and Khedher *et al.* (2021) following the procedures suggested by Kuhl and Giardina (1982).

Morphometric parameters

Morphometric parameters of the otoliths, including length (*Lo*), width (*Wo*), area (Ao), and perimeter (Po), were determined using ImageJ software (Figure 2A, B). Before statistical analyses, one-way ANOVA was used to determine whether there were any significant differences between the mean values of *Lo*, *Wo*, Ao, and Po for the right and left otoliths. In addition, a two-way ANOVA was used to check whether there was a correlation between the otolith's morphometry and the geographic origin of the individuals. The mean values of the four parameters were analysed using the Student's *t*-test to determine the differences between the left and right and left-left and right-right otoliths within and among individuals of the two lagoons.

Fluctuating asymmetry measurements

The FA values between the right (r) and left (l) otoliths were calculated among individuals of the two lagoons for each morphometric parameter per individual (i) by applying the signed formula FA4 given by Palmer (1994) and were expressed here as the FA_i index:

$$FA_i = \text{var}\ (r_i - l_i)$$

where r_i and l_i are the values of the parameter or trait on the right and left otoliths, respectively.

Then, the mean FA values were calculated for the right and left otoliths from all individuals for each morphometric parameter per lagoon. Besides, the inter-population differences in the mean values of the FA between the left and right otolith morphometric parameters between individuals of the two lagoons were tested using multivariate analysis of variance (MANOVA).

Data analysis

First, analysis of variance (ANOVA) was performed to evaluate the significance of differences in the mean values of *TL* among individuals of the two lagoons, and the values were tested for

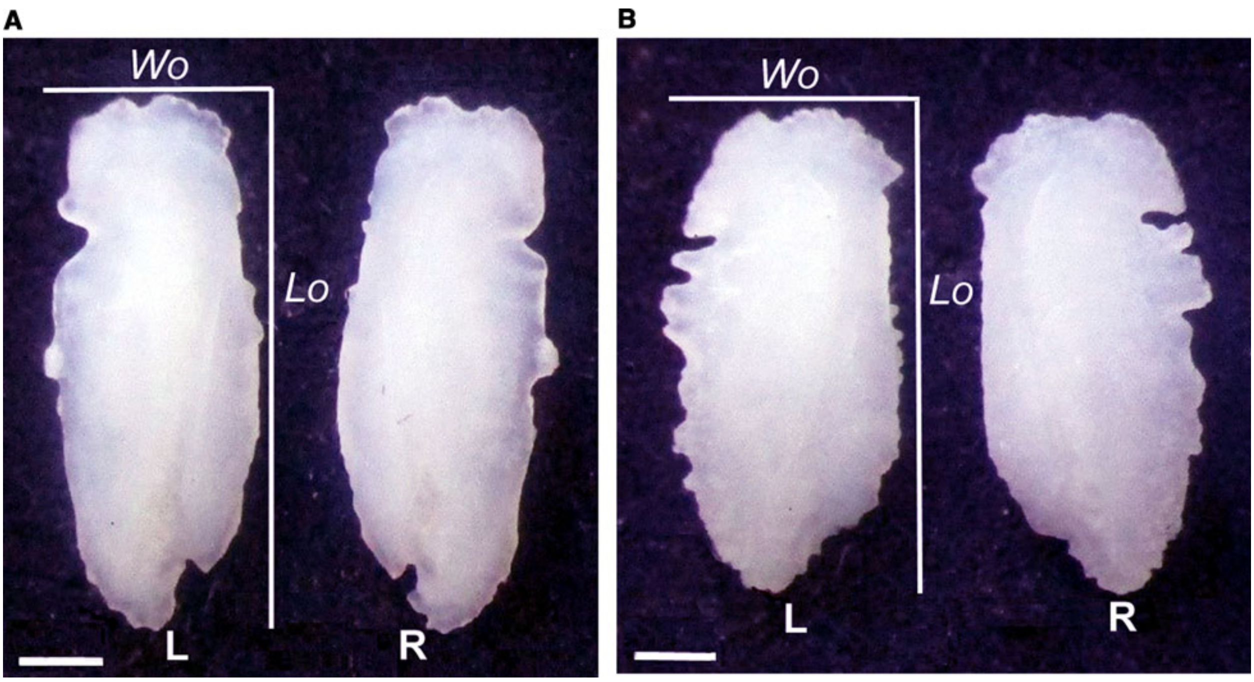

Figure 2. *Chelon ramada* Risso, 1826, images of the left (L) and right (R) sagittal otoliths showing the length (*LO*) and width (*WO*) parameters examined among individuals collected from the (A) Boughrara and (B) El Bibane lagoons in Tunisia. Scale bar: 1 mm.

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homogeneity (equality) and the normal distribution using Levene's and Shapiro–Wilks' λ tests, respectively. Second, the differences in the contour shape of otoliths of all individuals from the two lagoons were revealed using discriminant function analysis (DFA) (Anderson and Robinson, 2003). The effect of individuals on elliptical Fourier descriptors was first tested by MANOVA. Subsequently, all shape variable values were checked for being normal, and if the values did not follow a normal distribution, a Box-Cox (Box and Cox, 1964) transformation was performed. Finally, Levene's and Shapiro–Wilk's λ tests were applied to assess homogeneity (equality) and normality of variance in the variable values of otolith shapes. Afterwards, the DFA was performed by the normalized elliptical Fourier descriptors coefficients (77 coefficients per otolith) to illustrate the similarities and differences within and among individuals of the two lagoons. The objective of the DFA is to investigate the integrity of predetermined groups of individuals of a particular population or lagoon and the percentage of their correct classification by finding linear combinations of descriptors that maximize Wilk's λ value. Wilk's λ test evaluates the performance of discriminant analyses. This statistic is the ratio between intra-population variation and total difference and provides an objective method for calculating the corrected percentage chance of agreement. In addition, the Fisher's (1936) distance was also calculated to describe the differences in the shape of otolith within and among populations of the two lagoons. The results were interpreted using the data of Wilk's λ test, and barycenter projections were shown on graphs for the two lagoons. Besides, the MANOVA was used to test the significance of otolith shape values between individuals of the two lagoons. All these statistical analyses were performed using XLSTAT 2010.

Results

Otolith shape analysis

The Levene's and Shapiro–Wilks' λ tests revealed that all values of the shape variance were equally and normally distributed with a P-value > 0.05. At the interpopulation level, the Wilks' λ test showed a statistically significant shape difference ($P = 0.0011$), i.e. there was a bilateral asymmetry, between the right and left otoliths among individuals of the two populations (Table 2). Similarly, Fisher's (1936) distances also showed significant shape bilateral asymmetry ($P \leqslant 0.05$) between the left and right otoliths, as well as between the left-left (L-L) and right-right (R-R) otoliths among individuals between the two lagoons (Table 3).

At the intrapopulation level, the Wilks' λ test revealed a statistically significant shape difference ($P < 0.0001$), i.e. asymmetry, in the right and left otoliths among males and females of the Boughrara lagoon, i.e. there was a sexual dimorphism (Table 2). Conversely, a significant shape similarity ($P > 0.0001$), i.e. symmetry, was found in the right and left otoliths among the two sexes of the El Bibane lagoon (Table 2). In addition, the Fisher's (1936) distances showed no significant differences ($P > 0.05$) in the left and right otoliths among males and females within each lagoon (Table 3).

Based on elliptical Fourier descriptors (EFDs) of the left and right otoliths contour shape from individuals of the two lagoons on the first two axes F1 and F2 of the DFA, the barycenter projection revealed that the first axis explained 26.91% and the second 18.45%, respectively, of the total variation, showing that otoliths from individuals tended to segregate along the F1 (Figure 3). Therefore, the two axes accounted for 45.36% of the total variance and confirmed the presence of shape variability in the left and right otoliths among individuals of the two populations, i.e. the presence of two distinct main groups of otoliths corresponding to the lagoons of Boughrara and El Bibane. The F1 axis separated the left and right otoliths of males and females of the Bibane lagoon on the positive part and those of the Boughrara lagoon on the negative part. The F2 axis discriminated between the left and right otoliths of males and females of the two populations on both the positive and negative parts (Figure 3).

Morphometric analysis

The Student's t-test confirmed that there were no significant differences ($P > 0.05$), i.e. there was symmetry, between the left and right otoliths in *Lo*, Ao, and Po among males and females in the Boughrara lagoon, i.e. there was no sexual dimorphism (Table 4). However, a highly significant asymmetry ($P = 0.0016$) was detected in *Wo* between the left and right otoliths among males and females, i.e. there was a sexual dimorphism. In the El Bibane lagoon, Student's t-test revealed no significant differences ($P > 0.05$), i.e. there was symmetry, between the right and left otoliths in *Lo*, *Wo*, Ao, and Po among males and females (Table 4).

Fluctuating asymmetry analysis

Estimates of the mean FA values of *Lo*, *Wo*, Ao, and Po between the right and left otoliths among individuals of the Boughrara lagoon showed significant FA ($P < 0.05$) only in *Lo* between the left and right otoliths among males, as well as between males and females, i.e. there was a sexual dimorphism (Table 5). On the contrary, significant FA differences ($P < 0.05$) were observed between the left and right otoliths in *Wo* and Po among males, in all parameters among females, and in *Wo* and Po between males and females of the El Bibane lagoon, i.e. there was a sexual dimorphism. However, at the interpopulation level, only significant FA differences were found in *Lo* between the left and right otoliths.

Discussion

Results of elliptic Fourier analysis (EFA) of the sagitta otolith shape revealed a statistically significant shape difference, i.e. asymmetry, between the left and right otoliths, as well as between the left-left (L-L) and right-right (R-R) otoliths, among populations of *C. ramada* collected from the Boughrara and El Bibane lagoons. Similar findings of the interpopulation variation of the left and

Table 2. Wilk's Lambda (λ) test of the left and right sagittal otoliths' shape variance distance approximation values within and between populations of *Chelon ramada* collected from the Boughrara (B) and El Bibane (E) lagoons in Tunisia

Population	Lambda (λ)	F (Observed value)	F (Critical value)	DF1	DF2	P-value	Alpha
Boughrara (B)	0.0057	2.4072	1.3076	231	121	<0.0001	0.05
El Bibane (E)	0.0506	0.8699	1.3109	231	118	0.8141	0.05
Between populations	0.0351	1.2527	1.1286	539	1095	**0.0011**	0.05

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

Table 3. Pairwise Fisher's (1936) distances (*D*) matrix (above diagonal) and their corresponding *P*-values (below diagonal) of the right (R) and left (L) sagittal otoliths within and between females (F) and males (M) of *Chelon ramada* samples collected from the Boughrara (B) and El Bibane (E) lagoons in Tunisia

	FLB	FRB	MLB	MRB	FLE	FRE	MLE	MRE
FLB	—	3.0133	-	-	1.4679	-	-	-
FRB	0.2282	—	-	-	-	1.7646	-	-
MLB	-	-	—	2.8523	-	-	1.3717	-
MRB	-	-	0.2854	—	-	-	-	1.3243
FLE	**0.0227**	-	-	-	—	1.0199	-	-
FRE	-	**0.0015**	-	-	0.1198	—	-	-
MLE	-	-	**0.0498**	-	-	-	—	0.5801
MRE	-	-	-	**0.0516**	-	-	0.8612	—

-, not examined in this study; FLB, left otolith of Boughrara female; FRB, right otolith of Boughrara female; MLB, left otolith of Boughrara male; MRB, right otolith of Boughrara male; FLE, right otolith of El Bibane female; FRE, right otolith of El Bibane female; MLE, left otolith of El Bibane male; MRE, right otolith of El Bibane male. Values marked in bold are statistically significant ($P < 0.05$).

right otoliths' shape have been previously reported in a wide range of fish taxa for which data are available and occurring either in Tunisian waters or elsewhere worldwide (for details, see Ben Labidi *et al.*, 2020a, 2020b; Khedher *et al.*, 2021; Mejri *et al.*, 2022a). In addition, significant intersexual shape difference, i.e. bilateral asymmetry, was only observed between the left and right otoliths in the Boughrara lagoon. This is consistent with the findings in *Neobythites* spp. (Schwarzhans, 1994), *Salvelinus namaycush* (Simoneau *et al.*, 2000), *D. anularis* (Trojette *et al.*, 2015), *C. ramada* (Rebaya *et al.*, 2017), *P. erythrinus* (Mejri *et al.*, 2018, 2020), and *B. boops* (Ben Labidi *et al.*, 2020a).

Regarding the main reasons for such interpopulation bilateral asymmetry in the otolith shape, numerous investigations have reported that the variability in the otolith shape has been influenced by either genetic (Vignon and Morat, 2010; Berg *et al.*, 2018), ontogenetic (Hüssy, 2008; Capoccioni *et al.*, 2011), physiological (Lombarte and Cruz, 2007; Schulz-Mirbach *et al.*, 2019), phylogenetic (Campana and Neilson, 1985; Torres *et al.*, 2000), or environmental factors, such as living depth (Lombarte and Lleonart, 1993), habitat types (Bautista-Vega *et al.*, 2008), temperature and salinity of water (Panfili *et al.*, 2005; Hüssy, 2008; Capoccioni *et al.*, 2011; Mahé *et al.*, 2019), and food supply (Hüssy, 2008). In addition, Trojette *et al.* (2015) mentioned that fertility, sexual maturity, survival, and growth are among the most important factors that play a role in otolith reshaping. Moreover, Ben Mohamed *et al.* (2019), Jmil *et al.* (2019), Ben Labidi *et al.* (2020a, 2020b), Mejri *et al.* (2020, 2022a), and Khedher *et al.* (2021) attributed the asymmetry in the otolith shape to the instability of development caused either by environmental stress related to the change in water temperature, salinity, living depth, feeding conditions, and pollutants that have led to abnormalities in the ontogenetic development of individuals or due to poor living conditions of larvae in an unfavourable environment. However, the intrapopulation significant shape bilateral asymmetry observed in the left and right otoliths among males and females within the Boughrara lagoon can be attributed to reproductive isolation between individuals (Wiff *et al.*, 2020) that may lead to inter or even intraindividual variations (Panfili *et al.*, 2005) or the possibility of having intraindividual stress that has led to developmental abnormalities of individuals or poor larval living conditions (Ben Labidi *et al.*, 2020b).

In addition to the environmental conditions, pollution can be a relevant factor in the two lagoons, where the Boughrara lagoon receives organic matter discharges through harbour-related

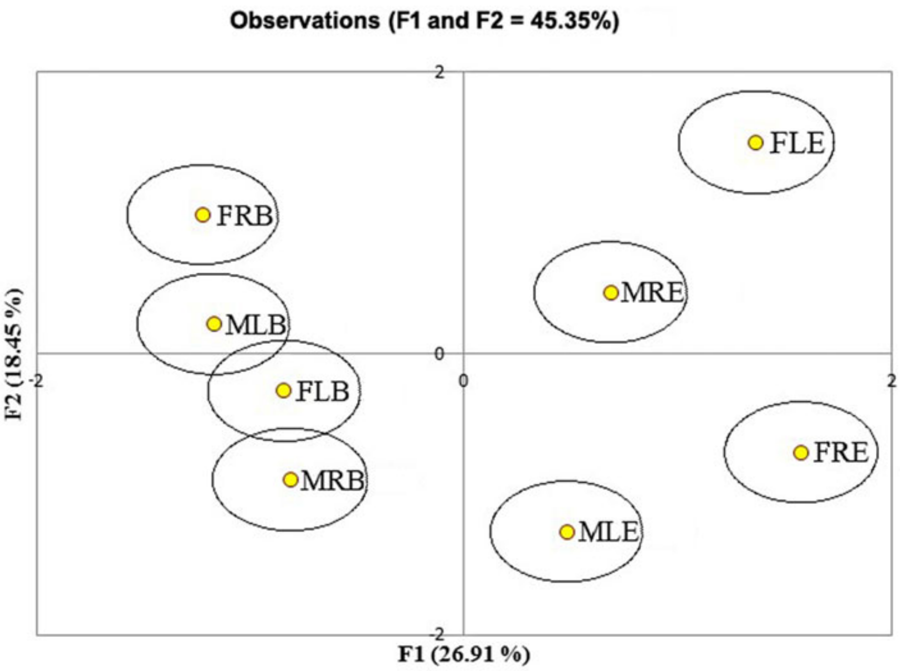

Figure 3. *Chelon ramada* Risso, 1826, discriminant function analysis (DFA) showing the barycenter projection (○) of the left (L) and right (R) shape values of the sagittal otoliths of *Chelon ramada* collected from the Boughrara and El Bibane lagoons in Tunisia: FLB = left otoliths of Boughrara females, FRB, right otoliths of Boughrara females; MLB, left otoliths of Boughrara males; MRB, right otoliths of Boughrara males; FLE, left otoliths of El Bibane females; FRE, right otoliths of El Bibane females; MLE, left otoliths of El Bibane males; MRE, right otoliths of El Bibane males.

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Table 4. Means ± standard deviation (SD) values and Student's *t*-test *P*-values between the right (R) and left (L) sagittal otoliths' length (*Lo*), width (*Wo*), and area (Ao) measurements within and between *Chelon ramada* males and females collected from the Boughrara (B) and El Bibane (E) lagoons in Tunisia

			Mean ± standard deviation (SD)			
Lagoon	Sex	Side	Length (*Lo*) (mm)	Width (*Wo*) (mm)	Area (Ao) (mm^2)	Perimeter (Po) (mm)
Boughrara (B)	Male	R	4.61 ± 0.29	2.31 ± 0.18	7.57 ± 1.01	12.92 ± 0.96
		L	4.59 ± 0.34	2.22 ± 0.18	7.22 ± 1.25	12.64 ± 1.06
	Female	R	4.29 ± 0.16	2.16 ± 0.13	6.53 ± 0.46	11.34 ± 1.98
		L	4.23 ± 0.16	2.06 ± 0.09	6.40 ± 0.51	11.35 ± 0.48
Student's *t*-test *P*-value	Male	R-L	0.71	**0.0016**	0.16	0.50
	Female	R-L	0.53	**0.0016**	0.48	0.46
El Bibane (E)	Male	R	4.27 ± 0.26	2.32 ± 0.64	6.39 ± 0.71	11.67 ± 0.75
		L	4.28 ± 0.26	2.19 ± 0.13	6.51 ± 0.71	11.65 ± 0.76
	Female	R	4.46 ± 0.33	2.30 ± 0.12	7.00 ± 0.82	12.35 ± 0.98
		L	4.44 ± 0.34	2.31 ± 0.16	6.98 ± 0.83	12.33 ± 0.84
Student's *t*-test *P*-value	Male	R-L	0.66	0.88	0.06	0.90
	Female	R-L	0.91	0.93	0.83	0.89

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

activities (Guetat *et al*., 2012), as well as sewage pollution from the traffic flow of the surrounding ports, entry of seawater carried with phosphorus from the Gulf of Gabes (Sellem *et al*., 2019), and industrial discharges from the Ghannouch chemical complex located on the shores of the Gulf of Gabes (DGPA, 2000; SCET-ERI, 2000). Moreover, the lagoon has been subjected to tidal phenomena with quite frequent eutrophication processes, often causing significant damage to the biota (Hamza, 1991, Daly Yahia *et al*., 1994; Daly Yahia and Romdhane, 1996; Romdhane *et al*., 2019; DGPA, 1999; Ben Rejeb Jenhani and Romdhane, 2002). However, the El Biban lagoon receives pollution discharges from fishing and aquaculture activities (Zaouali, 1983). As far as known, fish species are very sensitive to alteration in temperature by about 0.03 °C (Rebaya *et al*., 2017), and the difference in the composition of otolith is associated with differences in fish responses to the effect of salinity reaction on temperature and the concentration of Cl, Mg, K, Na, and Ca (Martin and Wuenschel, 2006). Therefore, minor differences in the temperature, salinity, and pH between the two lagoons may directly affect the habitat and indirectly affect the chemical composition and shape of the otoliths in *C. ramada*. These interpretations are consistent with those of previous studies (Lombarte and Lleonart, 1993; Martin and Wuenschel, 2006; Hüssy, 2008; Capoccioni *et al*., 2011; Ben Labidi *et al*., 2020a, 2020b; Khedher *et al*., 2021; Mejri *et al*., 2022a, 2022b). Additionally, Simoneau *et al*. (2000) reported that differences in the fish's age and sex may lead to a significant difference in the otolith shape in fish stocks. In this study, it is worth noting that the sampling was restricted to adult individuals to eliminate the effect of sexual maturity, which could affect the outline or contour shape of the otoliths (Cardinale *et al*., 2004). This is because it has been reported that the otolith shape is significantly different in juveniles than in adults due to differences in hearing function (Ferri *et al*., 2018), as well as to avoid the impact of confounding factors that can result from allometric growth in the two populations from the larval to the adult stage, where the shape is mostly stable (Santos *et al*., 2017). Moreover, these interpopulation differences in the otolith shape detected here can be attributed to the differences in environmental factors between the two lagoons, such as water temperature and salinity, living depth, type of substrate, and food availability in terms of quantity and quality (Vignon and Morat, 2010; Capoccioni *et al*., 2011; Cañás *et al*., 2012; Sadighzadeh *et al*., 2014; Pavlov, 2016). As reported by Cardinale *et al*. (2004), Galley *et al*. (2006), Stransky *et al*. (2008), Rebaya *et al*. (2016), and Mejri *et al*. (2020), these environmental factors affect metabolism, which in turn affects somatic

Table 5. Estimates of the mean values of fluctuating asymmetry (FA) between the left (L) and right (R) sagittal otoliths' length (*Lo*), width (*Wo*), area (Ao), and perimeter (Po) of *Chelon ramada* males and females collected from the Boughrara (B) and El Bibane (E) lagoons in Tunisia

			Mean FA			
Lagoon	Sex	Side	Length (*Lo*) (mm)	Width (*Wo*) (mm)	Area (Ao) (mm^2)	Perimeter (Po) (mm)
Boughrara (B)	Male	R-L	**0.018**	0.083	0.11	0.08
	Female	R-L	0.29	0.97	0.07	0.09
Wilk's test *P*-value	Male–female	R-L	**0.002**	0.421	0.934	0.578
El Bibane (E)	Male	R-L	0.119	**0.011**	0.115	**0.0003**
	Female	R-L	**0.0244**	**0.0032**	**0.0244**	**0.0218**
Wilk's test *P*-value	Male–female	R-L	0.432	**0.018**	0.568	**0.003**
Between populations	Male–female	R-L	**0.034**	0.964	0.612	0.782

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

growth and thus the amount of material deposited on otoliths. Indeed, it has been declared by Fortunato *et al.* (2017) that the mugilids' diet changes throughout their development, with larvae being planktivorous and juveniles feeding first through the water column, then changing to a benthic diet when they reach a total length of 20 to 30 mm. Regarding feeding behaviour, Rasheed *et al.* (2021) described that *C. ramada* feeds on a wide variety of prey types, including algae (36%), polychaetes (35%), diatoms (16.2%), crustaceans (mainly small prawns, crabs, copepods, and amphipod) (6.2%), sediment (3.4%), foraminifera (2.3%) and fish parts (1%), whose availability may be different between the Boughrara and El Biban lagoons due to the variation in the environmental characteristics, especially water temperature, salinity, and pollutants. Thus, we can suggest that this morphological bilateral asymmetry observed in the left and right otoliths contour shape between the two populations can be attributed either to the effect of the physico-chemical factors or pollutants present in the two lagoons.

On the other hand, examination of the morphometric dimensions of the left and right otoliths between and within individuals of the Boughrara and El Biban lagoons showed only significant asymmetry in *Wo* between the left and right otoliths among males and females within the Boughrara lagoon, i.e. there was a sexual dimorphism. Similar asymmetry in *Wo* has also been found in *P. erythrinus* (Mejri *et al.*, 2020), *B. boops* (Ben Labidi *et al.*, 2020b) and *D. vulgaris* (Khedher *et al.*, 2021). In addition, Wilk's test showed significant FA only in *Lo* between the right and left otoliths among individuals of the two populations. At the intrapopulation level, significant FA was observed only in *Lo* between the right and left otoliths among males and females, as well as among males, within the Boughrara lagoon. However, significant FA asymmetries were found between the left and right otoliths in *Wo* and Po among males, in all parameters among females, and in *Wo* and Po between males and females of the El Bibane lagoon. This differential significant asymmetry observed in the morphometric dimensions of the otoliths, as well as in FA, between and within males and females of the two populations may be largely attributed to the hypothesis that unfavourable environmental conditions may cause stress to the individuals, and thus leading to developing an asymmetry in both otoliths' morphometry (Jawad *et al.*, 2011; Jawad and Al-Sadighzadeh, 2013; Ben Labidi *et al.*, 2020b; Mejri *et al.*, 2020; Khedher *et al.*, 2021). Indeed, environmental stress can arise from the contamination of seawater and sediments in the two lagoons with heavy metals, organic matter, and hydrocarbons discharged from harbour-related activities, as well as sewage pollution from the traffic flow of the surrounding ports and entry of seawater loaded with phosphorus from the Gulf of Gabes in the Boughrara lagoon (Guetat *et al.*, 2012; Sellem *et al.*, 2019) or fishing and aquaculture activities in the El Biban lagoon (Zaouali, 1983). Thus, the status of pollution in the Boughrara and El Biban lagoons may be responsible for the observed differential bilateral asymmetry in the otoliths' contour shape, as well as FA detected in the otoliths' morphometric parameters, within and among the two populations.

In conclusion, at the interpopulation level, analysis of the otolith shape showed a statistically significant difference ($P = 0.0001$), i.e. bilateral asymmetry, in the left and right otoliths' shape between individuals of the two populations. In addition, a significant FA was detected only in *Lo* between the right and left otoliths among individuals of the two populations. At the intrapopulation level, a significant shape bilateral asymmetry was observed in the left and right otoliths among males and females, i.e. there was a sexual dimorphism, within the Boughrara lagoon. However, significant shape symmetry was observed in the left and right otoliths among individuals within the El Bibane lagoon. Moreover, a significant FA was found in *Lo* between the left and right otoliths only among males, as well as between males and females of the Boughrara lagoon. However, significant FA between the left and right otoliths was observed only in *Wo* among males and in all morphometric parameters among females, and in *Wo* between males and females of the El Bibane lagoon. DFA of the otoliths' contour shape confirmed the presence of two separate main stocks, one corresponding to the Boughrara lagoon and the other representing the El Bibane lagoon, which should be managed separately. The possible cause of morphological variation in the otolith shape and morphometry due to FA between the two populations of *C. ramada* can be attributed to the instability of development caused either by environmental stress associated with the variation in water temperature, salinity, living depth, feeding conditions and pollutants that have led to abnormalities in the development of individuals or by the poor living conditions of the larvae in an unfavourable environment. These findings contribute largely to the data on the otoliths' shape and morphometry, which has recently been considered a very important tool for identifying and discriminating fish stocks. In addition, the present study highlights the importance of potential FA in otolith morphometry for identifying fish stocks based on the otolith shape and morphometry and confirms that the *C. ramada* stocks from the two lagoons were discriminated from each other and, thus, should be managed separately.

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