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Research Article

Discriminant stock structure of *Diplodus annularis* (Perciformes, Sparidae) in Tunisian waters inferred from saccular otolith morphometry and microchemistry

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Abstract

We studied the size dimensions and microchemical structure of the saccular otoliths (sagittae) of two populations of the annular seabream (*Diplodus annularis*) collected from the Djerba and Kerkennah Islands in Tunisian waters. The goals of our study included (1) measuring the otolith's length (Lo), width (Wo), surface area (Ao), and perimeter (Po), and evaluating differences in these characteristics and microchemical elements between individuals of *D. annularis* within each Island, (2) exploring how potential fluctuating asymmetry (FA) in the otolith size affects the stock structure of *D. annularis* in two Islands, and (3) using these findings to recognize if there were distinct stock structures and connectivity between fish within the two Islands, to develop better-guiding policies for managing and governing the fishery within these Islands. The results revealed significant asymmetry in all size dimensions between the left and right otoliths among males and females within the Djerba and Kerkennah Islands and males and females between the two Islands due to FA. In addition, significant differences in otolith contents of K, Cs, and Pb were found between individuals of the two Islands. The significant asymmetry in otolith size dimensions among males and females of the two Islands due to FA and the noticeable difference in otolith microchemical elements between individuals of the two groups were attributed to some factors, such as fish age, environmental conditions, and fish biological traits. Therefore, the results confirmed the discriminant stock structure and limited connectivity between fish within the two Islands, which should be managed separately.

Introduction

Parenti (2019) described that the Sparidae family encompasses about 166 species from 39 different genera. Among these genera, *Diplodus* stands out with 23 species, with the annular seabream, *D. annularis*, being the most prevalent from the Mediterranean to the Black Sea, and the Bay of Biscay to Senegal. According to the literature and FishBase database records, this species a benthopelagic and lives in marine and brackish environments, primarily on *Posidonia* and *Zostera* beds (Harmelin-Vivien *et al*., 1995; Matic-Skoko *et al*., 2004), at a depth up to 90 m (Bauchot and Hureau, 1986). Additionally, Bauchot and Hureau (1986) in their book chapter declared that it is a predatory species that consumes a wide spectrum of invertebrate species, including worms, crustaceans, molluscs, echinoderms, and hydrozoans, and exhibits sexual dimorphism, with some individuals being protandric, capable of changing sex from male to female. In the Mediterranean, including Tunisia, *D. annularis* is a significant catch for fisheries due to its high-quality, white flesh (Gordoa and Molí, 1997). Indeed, no recent data on the total landings of *D. annularia* in Tunisian waters, however, based on the 2006 DGPA report, the Sparidae family contributed 12.7% of the total fish catch in Tunisia, with the genus *Diplodus* accounting for 19 to 20% (Anonymous, 2006), highlighting its economic importance. This species is also a key target for artisanal fisheries and aquaculture, leading to numerous studies on its morphological and biological characteristics in the Mediterranean, including Tunisian waters, and the Atlantic and the Adriatic Sea (for details, see Trojette *et al*., 2015).

Otoliths, also known as the inner ear bones of fish, are integral components of their sensory system, responsible for dealing with sound reception and maintaining equilibrium, both while stationary and in motion (Parmentier *et al*., 2001). Furthermore, these otoliths are key in documenting various aspects of fish life and the impact of environmental conditions from the time of birth, as they continue to grow throughout the fish’s lifespan and are metabolically stable, i.e., their composition is unlikely to change or be reformed (Casselman, 1987). These otoliths are made up of two calcified structures mainly of calcium carbonate, with alternating layers of aragonite (~96%) and a protein matrix (otolin) (Halden and Friedrich, 2008). However, there have been instances of vaterite being present, depending on the level of stress the fish has suffered (Halden and Friedrich, 2008). Additionally, the inorganic part of otoliths can incorporate a broad spectrum of trace elements into their structure, including strontium (Sr), barium (Ba), transition metals, rare earth elements, and heavy metals, such as mercury (Hg) and lead (Pb) (Degens *et al*., 1969). Anatomically, Parmentier *et al*. (2001) mentioned that there are three sets of otoliths located in the inner ear of fish, situated on both sides of the head and connected to the auditory sensory cells, known as the ‘asterisci’ (otoliths of the lagena), ‘lapilli’ (otoliths of the utriculus), and ‘sagittae’ (otoliths of the sacculus). In addition, Schwarzhans *et al*. (2017) described the sagittae or saccular otoliths as the largest, positioned just behind the eyes and nearly perpendicular to them, the lapilli and asterisci are the smallest, found in the utriculus and lagena chambers, respectively, and are continuous with three semi-circular canals. Previous studies on otoliths have indicated that their calcified structure can be influenced by various factors, including fish age and growth, availability of food, as well as environmental conditions, such as water temperature, salinity, depth, and current (Cardinale *et al*., 2004; Capoccioni *et al*., 2011). In terms of its role, it has been extensively confirmed that the ring-like structure of otoliths plays a crucial role in recording and storing data on fish habitats, shifts in both ecological and biological conditions, and exposure to contaminants (Halden and Friedrich, 2008). Additionally, McCormick *et al*. (2010) confirmed that the microstructural changes within otoliths have been a key tool in understanding the relationship between changes in sex and growth history, as once these changes are accurately identified, their size can serve as a proxy for physical growth (Campana, 2001). Furthermore, the fact that otoliths are not metabolically active has led to an increase in their use as indicators of environmental conditions, thanks to technological progress and the creation of various applications, such as stock identification, tracing migration routes, reconstructing histories of temperature and salinity levels, verifying age, detecting changes in migratory patterns, serving as natural tags, and marking for chemical analysis (Campana, 1999).

On the other hand, the size measurements of otoliths offered an approach based on morphometric characteristics for assessing fish traits, as variations in these measurements refer to the potential for adaptation to different geographical areas during a fish’s life cycle (Casselman *et al*., 1981). Therefore, differences in the size measurements of otoliths can be attributed to variations within the fish population (Stransky, 2005). Previous investigations have also shown that the shape, size, and microstructure of otoliths have been significantly influenced by a variety of factors, including developmental stages (Capoccioni *et al*., 2011), genetics (Berg *et al*., 2018), environmental conditions, especially water depth, temperature, salinity, light exposure, and the type of substrate (Ferri *et al*., 2018), feeding behaviors (Cardinale *et al*., 2004), physiological aspects, such as the ability to hear for acoustic communication (Schulz-Mirbach *et al*., 2019), evolutionary history (Torres *et al*., 2000), sex, growth, and maturity stages (Cardinale *et al*., 2004), and the degree of separation between different individuals within the fish populations (Turan *et al*., 2006).

So far, the analysis of otolith microchemistry or elemental signatures has revealed differences across various geographical areas, making it a valuable tool for identifying and characterizing fish groups or populations (Daros *et al*., 2016; Avigliano *et al*., 2017; Soeth *et al*., 2019; Correia *et al*., 2021; Schroeder *et al*., 2022; de Almeida *et al*., 2023, 2024; Ben Ghorbel *et al*., 2024; Hu *et al*., 2024). Moreover, as previously published in the works of Campana (1999), Halden and Friedrich (2008), Carvalho *et al*. (2017), Moreira *et al*. (2018), and de Almeida *et al*. (2024), the microchemistry of otoliths offered insights into environmental conditions and the life history traits of fish throughout their entire lifespan, such as temperature records, detection of migratory behavior, movement patterns, identification of migration routes, age determination, natural markers for reconstructing life histories of species, metabolic indicators, efficient and cost-effective mass tagging, and habitat preferences. Thus, the current study was initiated to 1) evaluate the geographic variation in the size of sagittal otoliths, including length (*Lo*), width (*Wo*), area (Ao), and perimeter (Po), and microchemistry, 2) investigate how potential fluctuating asymmetry (FA) in size dimensions affects the stock structure of *Diplodus annularis* collected from the Djerba and Kerkennah Islands in the Gabes Gulf, Tunisia, and 3) employ these approaches to determine the stock structure and connectivity of *D. annularis* to inform appropriate strategies for fisheries management and governance on these islands.

Materials and methods

Study area

The Islands of Djerba and Kerkennah are situated in the Gulf of Gabes, with Djerba Island (33°48'N, 10°51'E) located in the southeastern region and Kerkennah Island (34°42′N, 11°11′E) in the northeastern region (Fig. 1).

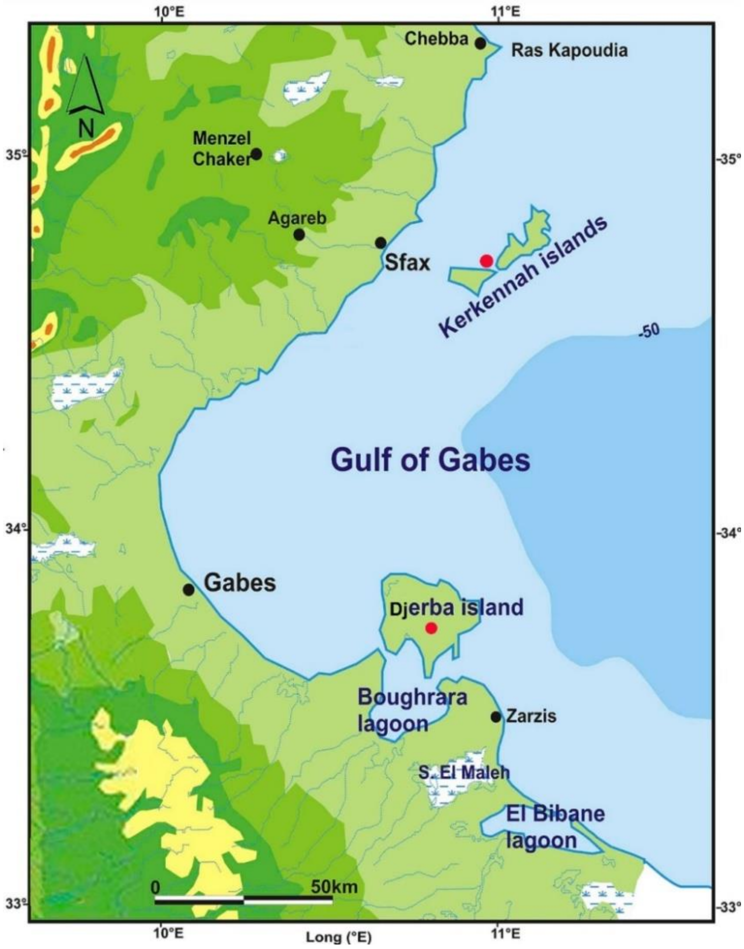

Figure 1: A map showing the geographic sites (●) from which individuals of *Diplodus annularis* were collected from the Djerba and Kerkennah Islands located in the Gulf of Gabes, Tunisia.

Djerba Island has long been recognized as the largest in North Africa, spanning an area of 514 km^2, and has historically been documented as the premier tourist, economic, and commercial hub in Tunisia (Ben Hassen Trabelsi *et al*., 2020). It ranks second among the tourist sites in Tunisia (Ben Abdallah, 2012), with an estimated 1.870 million visitors in 2018, as announced by the Heinrich-Böll-Stiftung. Kerkennah Island, on the other hand, stretches 160 km around and is located about 20 km from Sfax. According to ANGed (2020), Djerba Island faces various forms of pollution from domestic, hotel, and street cleaning activities, with municipal waste being the primary pollutant. Regarding Kerkennah Island, the Heinrich Böll Foundation highlighted in 2018 that the island has been significantly impacted by climate change, due to its long exposure to rising sea levels and increased coastal erosion. Additionally, the presence of plastic and microplastic pollution has become a major concern, posing a threat to the island's marine ecosystem.

Furthermore, Ben Maïz (2001) noted that Djerba Island experiences an average temperature of 23°C and a salinity of 37 psu. Conversely, Kerkennah Island has an average temperature of approximately 21.7°C and a salinity of 37.5 psu (Etienne *et al*., 2012), boasting a rich vegetal cover, including *Posidonia oceanica* and *Cymodocea nodosa* (Mounir *et al*., 2013).

Sampling

The 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 August 14th, 1995), as well as all applicable international, national, and institutional guidelines for the care and use of animals in research. A total of 120 adult specimens of *D. annularis* were collected from September to November 2021 across two Islands, with 60 specimens (30 males and 30 females) collected from each island. These specimens were checked for signs of sexual maturity either by examining them visually or through a microscope for those with small reproductive organs. Next, each individual was weighed for the total body weight (TW) in g using a digital scale with a precision of 0.01 g, and the total length (TL) in mm was measured with an ichthyometer, also with a precision of 0.01 mm. As a rule, it's important to note that for this study, mature individuals with similar sizes and weights were chosen to minimize the impact of size and weight differences on the otolith size dimensions and chemical composition (Longmore *et al*., 2010). Finally, the data was checked for normal distribution and consistency across the two sets of measurements using the Shapiro-Wilk's lambda (λ) test and variance comparison (*F*-test; Snedecor and Cochran, 1989) test, respectively.

Otolith extraction and imaging

Both the right and left sagittae from every fish specimen were removed, rinsed with distilled water, stored in a dry environment for 24 hours, and then placed in Eppendorf tubes for storage. These otoliths were then positioned in the same direction under a high-precision microscope, ensuring the sulcus was facing downwards and the concave side was facing upwards, to prevent any distortion errors during the normalization process. As published by Ben Labidi *et al*. (2020a) and Ben Mohamed *et al*. (2023) digital photographs were taken using a binocular loupe and a Canon IXUS 185 digital camera, capturing images with a resolution of 20 megapixels (Fig. 2A–D). Then, all the photographs were transferred to an image analyzer provided with a built-in millimeter scale.

Measurement of otolith size dimensions

As published by Ben Labidi *et al*. (2020a) and Ben Mohamed *et al*. (2023) the size dimensions of the saccular otoliths, including length (*Lo*), width (*Wo*), area (Ao), and perimeter (Po), were measured using Image J imaging software with a set scale of 1 mm. Dehghani *et al*. (2016) defined *Lo* as the distance between points a and b, and *Wo* as the distance between points c and d (Fig. 2A–D).

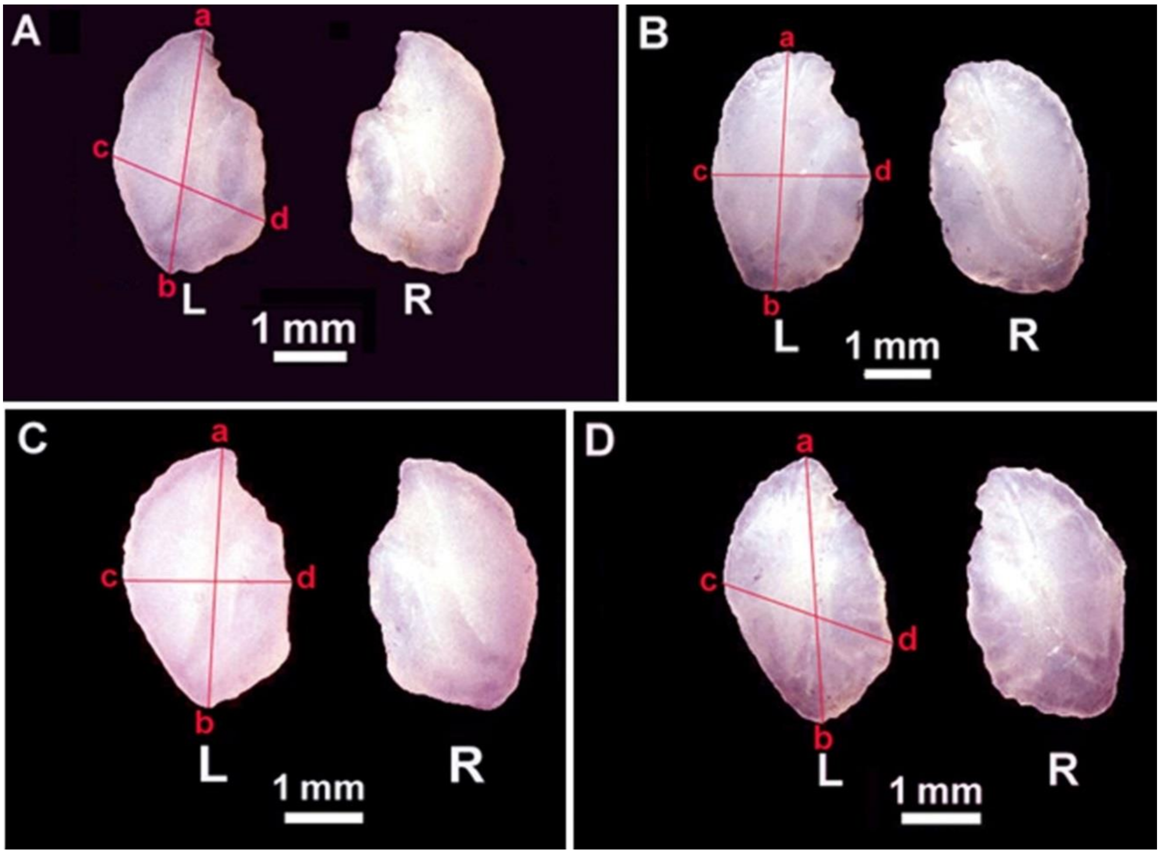

Figure 2: Real images of the left (L) and right (R) otoliths, with otolith length (*Lo*) and width (*Wo*), of (A) *Diplodus annularis* male and (B) *Diplodus annularis* female collected from the Djerba Island, (C) *Diplodus annularis* male and (D) *Dipalodus annularis* female collected from the Kerkennah Island located in the Gulf of Gabes, Tunisia. The *Lo* is the distance between points a and b, while *Wo* is the distance between points c and d.

Before conducting statistical analysis, a one-way ANOVA was applied to ascertain if there were any significant differences in the mean values of *Lo*, *Wo*, Po, and Ao between the left and right sides of the otoliths, both within and between males and females of the two Islands. The values were then examined for homogeneity (equality) and normal distribution through Levene’s and Shapiro-Wilks’ λ tests, respectively.

Additionally, a two-way ANOVA was utilized to investigate if there was any correlation between the otolith size parameters and the individuals of origin for both males and females. Moreover, as published in the work of Ben Labidi *et al*. (2020a) and Ben Mohamed *et al*. (2023) the Student's *t*-test was employed to compare the differences in the four parameters between the left and right otoliths, as well as between the left-left and right-right otoliths of males and females from the two Islands. All statistical analyses were carried out using XLSTAT 2016.

Measurement of fluctuating asymmetry (FA) in otoliths

The fluctuating asymmetry (FA) between the right and left sides of otoliths, measured as the 'FA_i index', was calculated for each otolith dimension per each individual '*i*' for both males and females across the two Islands. This measurement was done by applying the formula detailed by Palmer and Strobeck (1986) and Ben Labidi *et al*. (2020a) in their published work. Next, the differences in FA between the two Islands were examined using the Student's *t*-test, which was illustrated through statistical 'Box plots'.

Analysis of otolith microchemical elements

The analysis of otolith microchemical elements was conducted following the protocol outlined by Ben Ghorbel *et al*. (2024). In this method, 60 otoliths (30 from each side) were ground into a fine powder in a mortar that had been previously cleaned and sterilized with acetone to avoid contamination. The powder was then mineralized by mixing 1 g with 8 mL of a 65% HNO_3 solution and 2 mL of a 2% H_2O_2 solution. This mixture was heated in a hot block to a temperature range of 125±5°C before being dissolved in 50 mL of ultra-pure water (Milli Q). The resulting mineralized solution was analyzed using Flame Atomic Absorption Spectrometry (FAAS) and the Analytik Jena (Novaa350) model. This model facilitated the introduction of acetylene into the spectrometer's flame at a flow rate of 70 L/hour and a burner height of 6 mm. The analysis focused on three trace elements: potassium (K), Cesium (Cs), and lead (Pb), with detection limits at 766.5 nm, 852.1 nm, 228.8 nm, and 283.3 nm, respectively. Subsequently, as cited by Ben Ghorbel *et al*. (2024) a one-way ANOVA was conducted to determine the significance of differences in the mineral content of otoliths among individuals from the two Islands. The mineral content values were then examined for homogeneity (equality) and normality using Levene's and Shapiro-Wilks' λ tests, respectively. Finally, principal component analysis (PCA) and discriminant function analysis (DFA) were utilized to explore the differences and relationships between variations in otolith dimensions (*Lo, Wo,* Ao, and Po) and variations in otolith K, Cs, and Pb contents among individuals from the two Islands.

Results

Differences in total weight (TW) and total length (TL)

In the Djerba population, the combined total weight (TW) and total length (TL) varied from 50.12 to 93.5 g and 133 to 160 mm in males, and from 47.64 to 82.14 g and 130 to 167 mm in females (Table 1).

Conversely, in the Kerkennah population, the combined TW and TL differed from 101.8 to 227.4 g and 215 to 285 mm in males, and from 122.4 to 293.2 g and 190 to 310 mm in females.

Table 1: Range and mean ± SD values of the total weight (TW) and total length (TL) of males (♂) and females (♀) of *Diplodus annularis* collected from the Djerba and Kerkennah Islands located in the Gabes Gulf, Tunisia.

Island (N)	**Sex (N)**	**TW (g)**		**TL (mm)**	
		Range	**Mean ± SD**	**Range**	**Mean ± SD**
Djerba (60)	♂ (30)	50.12–93.5	61.41 ± 8.76	133–160	146.00 ± 7.32
	♀ (30)	47.64–82.14	63.74 ± 8.77	130–167	146.53 ± 7.97
Kerkennah (60)	♂ (30)	47.94–96.74	60.17 ± 10.13	140–192	159.26 ± 10.88
	♀ (30)	49.59–73.48	59.44 ± 6.008	145–177	159.00 ± 9.12

N: number of samples, SD: standard deviation.

Differences in otolith size characteristics

When comparing individuals within the same population, i.e., at the intrapopulation level, the Student's *t*-test of the left and right otolith measurements (*Lo*, *Wo*, Ao, and Po) revealed significant differences ($p<0.0001$) in all aspects in both males and females from the Djerba and Kerkennah Islands (Table 2). Similarly, when comparing the two populations, i.e., at the interpopulation level, the combined analysis of the left and right otolith measurements (*Lo*, *Wo*, Ao, and Po) using the Student's *t*-test showed a significant difference ($p<0.0001$) between males and females from the Djerba and Kerkennah Islands (Table 3).

Table 2: Student's *t*-test analysis of biometric parameters of the left (L) and right (R) otoliths between males and females of *Diplodus annularis* collected from the Djerba and Kerkennah islands located in the Gabes Gulf, Tunisia.

Parameter	**Sex (side)**	**Djerba**			**Kerkennah**		
		Range	**Mean ± SD (*p*-value)**	***p*-value combined**	**Range**	**Mean ± SD (*p*-value)**	***p*-value combined**
Length (*Lo*)	♂(L-R)	4.729–6.372	5.639 ± 0.364 (<0.0001)	<0.0001	3.866–6.338	4.951 ± 0.515 (<0.0001)	<0.0001
	♀(L-R)	4.861–6.513	5.164 ± 0.483 (< 0.0001)		4.310–6.258	5.164 ± 0.482 (<0.0001)	
Width (*Wo*)	♂(L-R)	3.120–3.948	3.562 ± 0.174 (<0.0001)	<0.0001	2.629–3.973	3.194 ± 0.318 (<0.0001)	<0.0001
	♀(L-R)	3.132–4.464	3.135 ± 0.390 (<0.0001)		2.341–3.973	3.135 ± 0.389 (<0.0001)	
Area (Ao)	♂(L-R)	10.171–17.377	14.268 ± 1.494 (<0.0001)	<0.0001	7.555–18.093	11.285 ± 2.362 (<0.0001)	<0.0001
	♀(L-R)	11.798–19.310	12.228 ± 2.328 (<0.0001)		7.415–16.814	12.228 ± 2.327 (< 0.0001)	
Perimeter (Po)	♂(L-R)	13.540–17.494	15.691 ± 0.907 (<0.0001)	<0.0001	11.337–17.877	14.041 ± 1.489 (<0.0001)	<0.0001
	♀(L-R)	12.281–19.397	14.946 ± 1.375 (<0.0001)		11.893–17.001	14.945 ± 1.374 (<0.0001)	

SD: standard deviation.

Table 3: Student's *t*-test combined analysis of biometric parameters of the left (L) and right (R) otoliths between males and females of *Diplodus annularis* collected from the Djerba and Kerkennah Islands located in the Gabes Gulf, Tunisia.

Parameter	Sex (side)	Djerba		Kerkennah		Between islands
		Range	Mean ± SD	Range	Mean ± SD	*p-value*
Length (*Lo*)	♂-♀ (L-R)	4.729–6.513	5.645 ± 0.357	3.866–6.338	5.057 ± 0.508	<0.0001
Width (*Wo*)	♂-♀ (L-R)	3.120–4.464	3.581 ± 0.223	2.341–3.973	3.165 ± 0.355	<0.0001
Area (Ao)	♂-♀ (L-R)	10.171–19.310	14.333 ± 1.624	7.415–18.093	11.756 ± 2.383	<0.0001
Perimeter (Po)	♂-♀ (L-R)	12.281–19.397	15.448 ± 1.242	11.337–17.877	14.493 ± 1.497	<0.0001

SD: standard deviation.

Differences in otolith fluctuating asymmetry (FA)

When examining the average FA values for the right and left otolith measurements among males and females within the two populations, i.e., at the intrapopulation level, the Wilk's λ test indicated a significant FA ($p<0.05$) in the Po only within the Djerba population, and a significant FA ($p<0.0001$) in the *Wo* only within the Kerkennah population. However, when evaluating the combined analysis of the average FA values for the right and left otolith measurements using Wilk's λ test, it was found that there was a significant FA ($p<0.0001$) in the left and right otoliths for all four measurements between males and females from both populations, i.e., at the interpopulation level (Table 4). Moreover, the distribution of the average FA values for the four measurements and the signs of the skewness for males and females from both populations were visually represented by a 'Box plot' chart (Fig. 3).

Table 4: Estimates of the mean values of fluctuating asymmetry (FA) between the left (L) and right (R) saccular otoliths' length (*Lo*), width (*Wo*), area (Ao), and perimeter (Po) within and between males (♂) and females (♀) of *Diplodus annularis* collected from the Djerba and Kerkennah Islands located in the Gabes Gulf, Tunisia.

Population	Sex	Otolith side	Mean FA			
			Lo (in mm)	*Wo* (in mm)	Po (in mm)	Ao (in mm²)
Djerba	♂	R-L	5.639	3.562	15.691	14.268
	♀	R-L	5.164	3.135	14.946	14.946
Wilk's test *p*-value	♂-♀	R-L	0.505	0.308	**0.0402**	0.392
Kerkennah	♂	R-L	4.951	3.194	14.041	11.285
	♀	R-L	5.164	3.135	14.945	12.228
Wilk's test *p*-value	♂-♀	R-L	0.874	<0.0001	0.751	0.0937
Wilk's test *p*-value between populations	♂-♀	R-L	<0.0001	<0.0001	<0.0001	<0.0001

The value marked in bold is statistically significant at $p<0.05$.

Differences in otolith elemental composition

Wilk's λ test indicated significant disparities ($p<0.0001$) in the elemental concentration levels of otoliths across individuals from the two Islands or populations. Specifically, the values for K and Cs (384.64 ppm and 98.62 ppm) were noticeably lower in individuals from the Djerba 'population' compared to those from the Kerkennah population (K=394.12 ppm and Cs=190.48 ppm). On the other hand, the Pb content was notably higher (60.62 ppm) in individuals from the Djerba population than in those from the Kerkennah population (39.27 ppm).

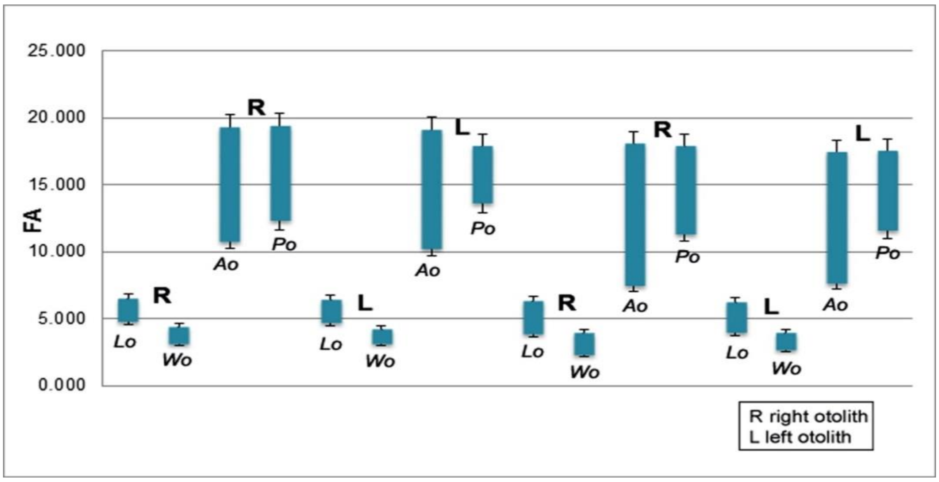

Figure 3: Box plots showing the distribution and signs of skewness of the mean values of fluctuating asymmetry (FA) in the otolith length (*Lo*), width (*Wo*), area (Ao), and perimeter (Po) of the right (R) and left (L) otoliths among males (M) and females (F) of *Diplodus annularis* populations collected from the Kerkennah Island located in the Gulf of Gabes, Tunisia.

Relationship between variations in otolith size and elemental content

As shown in Figure 4, the barycenter projection, derived from PCA using the left and right otolith measurements (*Lo*, *Wo*, Ao, and Po) along with K, Cs, and Pb values across individuals from the two Islands, demonstrated that individuals from both Islands were distinctly separated by the first two principal components (PC1 and PC2), contributing to a total variance of 52.99%.

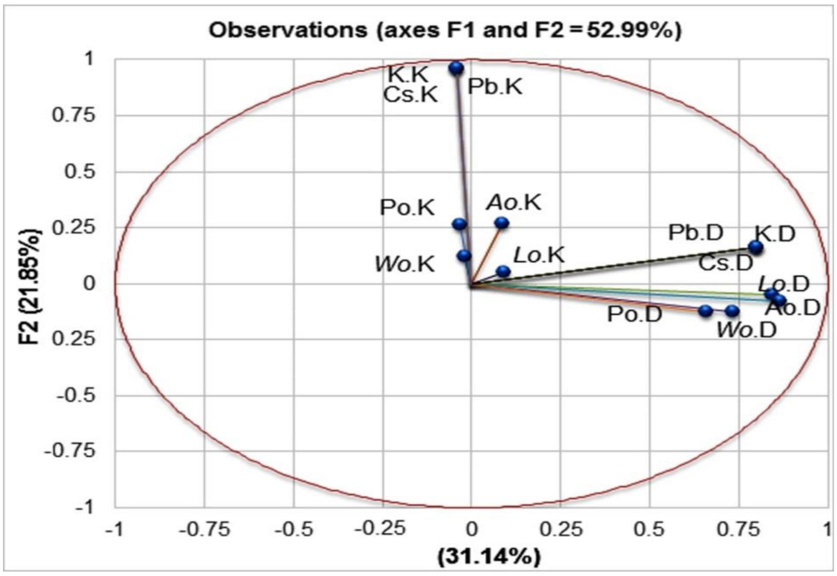

Figure 4: Principal component analysis (PCA) graph (biplot) showing the relationship between the variation in the otolith length (*Lo*), width (*Wo*), area (Ao), and perimeter (Po) and the variation in otolith K, Cs, and Pb contents among individuals of *Diplodus annularis* populations collected from the Kerkennah Island located in the Gulf of Gabes, Tunisia.

Among these two components, PC1 was responsible for 31.14% of the total variance, effectively separating the left and right *Lo*, *Wo*, Ao, and Po and the otolith K, Cs, and Pb contents from individuals of the Djerba Island together with the *Lo* and Ao from individuals of the Kerkennah Island in the positive part and *Wo*, Po, K, Cs, and Pb of the latter in the negative part. Conversely, PC2 accounted for 21.85% of the total variance, further separating the left and right otolith measurements and the otolith K, Cs, and Pb contents from individuals of Kerkennah Island, along with elemental contents of individuals from Djerba Island, in the positive part and all morphometric parameters from individuals of Djerba Island in the negative part.

Moreover, the barycenter projection based on the left and right otolith measurements and the otolith K, Cs, and Pb contents across individuals from the two Islands on the first axes F1 and F2 of the Discriminant Function Analysis (DFA) showed that these two axes fully explained the total variation (Fig. 5). Specifically, the F1 axis alone accounted for 100.00% of the total variance, effectively distinguishing individuals from Djerba Island in the positive part and those from Kerkennah Island in the negative part.

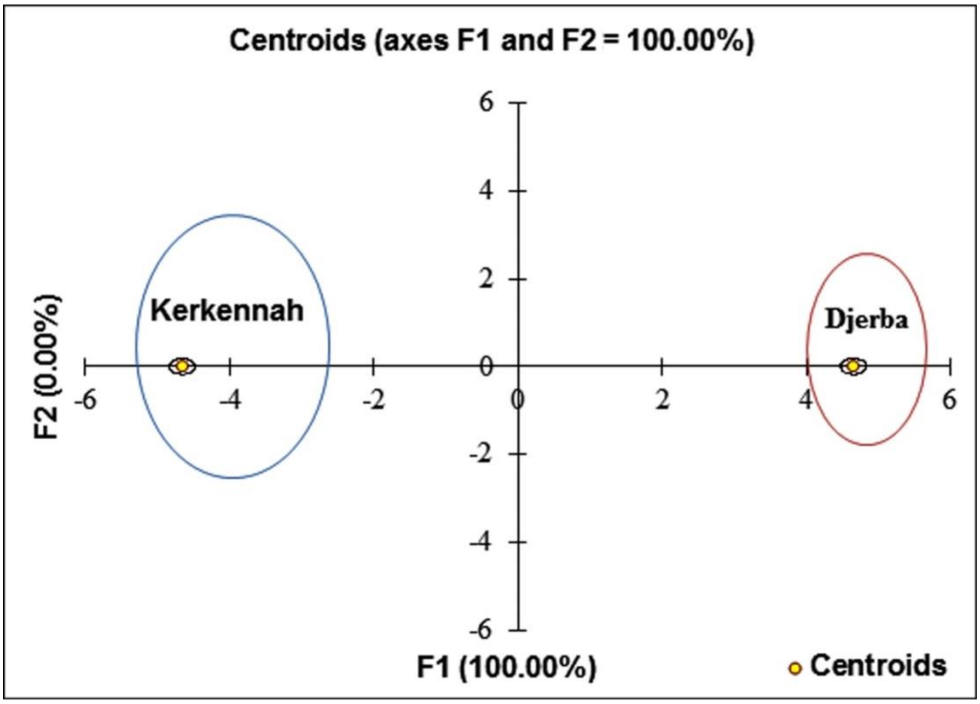

Figure 5: Discriminant function analysis (DFA) showing the barycenter projection and distribution of the otolith length (*Lo*), width (*Wo*), area (Ao), and perimeter (Po) and otolith K, Cs, and Pb contents among individuals of *Diplodus annularis* populations collected from the Kerkennah Island located in the Gulf of Gabes, Tunisia.

Discussion

By combining the study of otolith size with its microchemical composition, the researchers have greatly enhanced their understanding of the structure of fish stocks across different species. For example, this approach has been documented to be effective in studying the stock structure of several fish species, including *Stegastes fuscus* (Daros *et al*., 2016), *Genidens barbus* (Avigliano *et al*., 2017), *Chelidonichthys lucerna* (Ferreira *et al*., 2019), *Chaetodipterus faber* (Soeth *et al*., 2019), *Scomber colias* (Correia *et al*., 2021), *Sardinella brasiliensis* (Schroeder *et al*., 2022), *Eugerres brasilianus* (de Almeida *et al*., 2023), *Pogonias courbina* (de Almeida *et al*., 2024), *Chelon labrosus* (Nzioka *et al*., 2023), *Sarpa salpa* (Ben Ghorbel *et al*., 2024), and *Coilia nasus* (Hu *et al*., 2024). This study, in particular, integrated the analysis of otolith size dimensions and elemental contents signatures to evaluate the stock structure in *D. annularis* populations from the Islands of Djerba and Kerkennah in the Gulf of Gabes, Tunisia. The analysis of otolith size variations, including the differences in left versus right otoliths of males and females, revealed significant asymmetries (p<0.0001) across all parameters at the individual intra and interpopulation levels. Similar results have been observed in other species, such as *Boops boops* (Ben Labidi *et al*., 2020a), *Diplodus vulgaris* (Khedher *et al*., 2021), *Mullus barbatus* (Ben Mohamed *et al*., 2023), *Chelon ramada* (Adjibayo Houeto *et al*., 2024), found in Tunisian waters, as well as in *C. lucerna* (Ferreira *et al*., 2019), *Genidens barbus* (Maciel *et al*., 2021), *C. labrosus* (Nzioka *et al*., 2023), *E. brasilianus* (de Almeida *et al*., 2023), and *P. courbina* (de Almeida *et al*., 2024) in other parts of the world.

Previous investigations indicated that variations in the size characteristics of otoliths within and between species, i.e., intra and interspecies, can be connected to the physical characteristics of the water, particularly its temperature and salinity content, which influence the fish metabolism and growth (Ben Labidi *et al*., 2020a, 2020b; Khedher *et al*., 2021; Mejri *et al*., 2022; Ben Mohamed *et al*., 2023; Bouriga *et al*., 2023; Adjibayo Houeto *et al*., 2024), as well as their absorption of calcium carbonate (Schulz-Mirbach *et al*., 2019). In this context, Etienne *et al*. (2012) noted that the typical temperatures and salinity concentrations are 23°C and 37 psu on Djerba Island compared to 21.7°C and 37.5 psu on Kerkennah Island. Additional factors, including development (Capoccioni *et al*., 2011), hereditary traits (Berg *et al*., 2018), dietary preferences (Cardinale *et al*., 2004), body functions, such as auditory perception and sound signaling (Schulz-Mirbach *et al*., 2019), evolutionary history (Torres *et al*., 2000), sex, growth size, and maturity (Cardinale *et al*., 2004), and geographic separation among populations (Turan *et al*., 2006) have been extensively documented. Given these factors, it has been documented that *D. annularis* is a predatory carnivorous species that consumes worms, crustaceans, molluscs, echinoderms, and hydrozoans, which may show differences in their abundance across the two Islands. Additionally, Bauchot and Hureau (1986) published that the sexes in *D. annularis* are separate although some individuals are potentially hermaphrodites, with some individuals capable of changing sex from male to female, a process known as protandric hermaphroditism. Moreover, the two islands are quite distant, with a distance of 182 km (1° of latitude and longitude) between them. Hence, the notable distinction in *D. annularis* stock across the two Islands can be linked to the minor changes in temperature and salinity levels, variations in the development of the larvae, sex transition, differences in the quality and amount of food, and the geographic separation between the two Islands, which may lead to stock structure difference and limited connectivity of the fish inside these Islands. Similar findings have been reported by other researchers, including Ferreira *et al*. (2019), Maciel *et al*. (2020), Schroeder *et al*. (2022), de Almeida *et al*. (2023, 2024), Nzioka *et al*. (2023), Ben Ghorbel *et al*. (2024), and Hu *et al*. (2024). As for the genetic factors mentioned by Panfili *et al*. (2005), these factors may be responsible for the asymmetry observed on either side of the otoliths. However, it is impossible to discuss this topic here due to insufficient genetic information on *D. annularis* from these islands. Additionally, Jawad *et al*. (2011) cited that this asymmetry could be due to the response of individual fish to stress, a process known as 'asymmetric' stress response.

Moreover, earlier studies have demonstrated that fluctuating asymmetry in the shape and size of otoliths among different populations (Panfili *et al*., 2005) can result from both within and between individuals and between populations variations (Mejri *et al*., 2020). In the current study, examination of FA in the right and left otolith size dimensions between males and females within each population, i.e., at the intrapopulation level, revealed a significant difference only in Po within Djerba Island and *Wo* within Kerkennah Island. However, a significant difference in FA was observed in all size dimensions between males and females of the two populations. Interestingly, Adjibayo Houeto *et al*. (2024) concluded that this pattern of differential FA in both the right and left otoliths between males and females across populations has also been observed in *C. ramada* in Tunisian waters. Indeed, Jawad *et al*. (2011), Ben Labidi *et al*. (2020a), Khedher *et al*. (2021), and Adjibayo Houeto *et al*. (2024) in their publications indicated that this significant difference in right and left otoliths between males and females within and between populations can be linked to adverse environmental conditions, which may induce stress on individuals, leading to asymmetry in the morphometry on either side of the otoliths. In addition, Adjibayo Houeto *et al*. (2024) have suggested that environmental stress may result from the contamination of seawater and sediments with heavy metals, organic matter, and hydrocarbons. Therefore, the contamination levels in the two Islands might play a role in the variation in FA seen in the otolith size between males and females across the intra- and inter-populations.

On the other hand, there were significant disparities ($p<0.0001$) in the otolith contents of K, Cs, and Pb among individuals from the two Islands, with notably lower levels of K and Cs observed in individuals from Djerba Island compared to those from Kerkennah Island, which showed a significantly lower concentration of Pb. These findings contradict the otolith element contents observed in *S. salpa* populations from the same Islands in Tunisian waters, where individuals from Djerba Island had significantly higher levels of K, Cs, and Pb compared to those from Kerkennah Island (Ben Ghorbel *et al*., 2004). Nonetheless, similar significant differences in otolith elemental composition have been documented across various species in different locations, including *S. fuscus* (Daros *et al*., 2016), *G. barbus* (Avigliano *et al*., 2017), *Trachurus picturatus* (Moreira *et al*., 2018), *C. lucerna* (Ferreira *et al*., 2019), *S. colias* (Correia *et al*., 2021), *G. barbus* (Maciel *et al*., 2021), *S. brasiliensis* (Schroeder *et al*., 2022), *C. labrosus* (Nzioka *et al*., 2023), *E. brasilianus* (de Almeida *et al*., 2023), *Coilia nasus* (Hu *et al*., 2024), and *P. courbina* (de Almeida *et al*., 2024). Similar to otolith size, the microchemical elements of otoliths have been affected by the physicochemical properties of the environment and biological traits of the fish, including nutrition, physiology, reproduction, genetics, and developmental history (Clarke *et al*., 2011). Indeed, the chemical composition of otoliths mirrors the water ambient chemistry, and the fish age, along with environmental and physiological conditions, as well as its developmental history, affect the incorporation of inorganic minor and trace elements into the otolith matrix (Campana, 1999). Intriguingly, Fablet *et al*. (2009) reported that temperature indirectly affects the growth of otoliths during fish development.. However, Trojette *et al*. (2015) found that fish are primarily sensitive to temperature changes of about 0.03°C during their life span. Moreover, Nelson and Powers (2020) pointed out that the water salinity content affects the environmental conditions and indirectly affects the otolith chemical composition, and Rebaya *et al*. (2017) declared that this effect leads to changes in the morphology of the otolith. Furthermore, Martin and Wuenschel (2006) established that the variation in otolith chemical composition may be linked to the differences in how individuals respond to changes in salinity, temperature, and the concentration of certain elements, such as Cl, Mg, K, Na, and Ca. Regarding the Djerba and Kerkennah Islands, a significant temperature difference of about 1.3°C and a salinity difference of 0.5 psu were observed between the two Islands. Therefore, we can attribute the significant differences in otolith K, Cs, and Pb contents among individuals from the two islands to variations in fish age, larval development history, environmental conditions, including ambient water chemistry, temperature, salinity, and pollution, and fish biological traits, such as nutrition, physiology, and sex transition.

In studying the relationship between the size dimensions and microelemental content of the saccular otoliths among individuals from the two Islands, the PCA that individuals from both Islands were relatively discriminated from each other by the PC1 and PC2 components, with a total percentage of 52.99% of the overall variation. Additionally, the DFA, based on the otolith size dimensions and microelemental contents analyses, accounted for 100% of the total variation and fully separated between individuals of the two Islands on the F1 axis, with individuals of the Djerba Island separated in the positive part and those of the Kerkennah Island in the negative part. This result agrees with the findings in *S. salpa* populations collected from the same Islands in Tunisian waters (Ben Ghorbel *et al*., 2004), as well as in various other species occurring elsewhere, such as *Genidens genidens* (Maciel *et al*., 2020), *E. brasilianus* (de Almeida *et al*., 2023), *C. labrosus* (Nzioka *et al*., 2023), and *P. courbina* (de Almeida *et al*., 2024). This distinction between *D. annularis* individuals from the two islands suggests a difference in the larvae's inherent traits and life histories that may have been shaped by different environmental conditions and pollution levels within each Island.

Conclusions

This study highlighted the importance of studying saccular otolith size dimensions and analysis of the microelemental contents or fingerprints in determining the stock structure and connectivity of *D. annularis* populations collected from the Djerba and Kerkennah Islands in the Gulf of Gabes, Tunisia. The results revealed significant asymmetry in all size dimensions of the left and right otoliths among males and females within the Djerba and Kerkennah Islands and males and females between the two Islands. In addition, significant FA was found only between the right and left Po among males and females within Djerba Island and in the *Wo* within Kerkennah Island. However, the combined analysis of the mean FA values between the right and left otolith size dimensions demonstrated a significant FA in all parameters among males and females of the two Islands. This observed asymmetry in the left and right otolith size between males and females of the two Islands due to FA was attributed to environmental stress, resulting from the relatively little fluctuation in temperature and salinity, differences in the larvae's developmental stages, sex transition, and geographical isolation. Moreover, there were significant variations in the otolith K, Cs, and Pb contents between individuals of the two Islands, with a significantly lower content of both K and Cs between individuals of the Djerba Island than in the Kerkennah island, which showed a substantially lower content of Pb. Similarly, the variations in otolith microelemental contents between individuals of the two Islands were assigned to factors, such as the fish age, the environmental conditions to which fishes were exposed, including ambient water chemistry, temperature, salinity, and pollution, and the biological traits of the fish, such as nutrition, physiology, and sex transitions. Furthermore, the analyses combining both the otolith size dimensions and microelemental contents between individuals of the two Islands using the PCA and DFA revealed that individuals of the two Islands were limitedly distinguished based on the PCA components, while they were fully separated by the DFA into two distinct clusters corresponding to the Djerba and Kerkennah Islands. Therefore, these findings confirmed the discriminant stock structure and limited connectivity between fish inside the two Islands, indicating that management strategies for these Islands should be considered independently.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work presented in the paper.

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