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Sex-linked variations in the sagittal otolith biometry of *Nemipterus randalli* (Russell, 1986) from the eastern Mediterranean Sea

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Abstract

Few studies have been conducted on the sagittal otolith shape and morphometry of *Nemipterus randalli*, and none of these studies has examined the effect of sexual dimorphism on the otolith morphology of this species, therefore this study aimed to contribute to knowledge about the otolith morphology of *N. randalli*, an invasive fish species for the Mediterranean Sea. For this purpose, a total of 132 samples (51 female and 81 male) were obtained from İskenderun Bay with the help of commercial fishermen in November 2018. Relationships between otolith measurements and fish size were determined. Shape indices and elliptic Fourier coefficients were calculated. Significant differences were detected between males and females in all analysis. The sexes were separated from each other using both shape indices and elliptic Fourier coefficients. However, shape analysis was more effective in distinguishing sexes than traditional morphometric analysis. Asymmetry in the otolith morphology of sexes has been attributed to differences in the growth and sexual maturity of male and female fish. The results of this study indicated that sexual dimorphism in *Nemipterus randalli* was also reflected in the otolith morphology.

KEYWORDS

Fourier analysis, invasive species, İskenderun bay, otolith shape, sexual dimorphism

1 | INTRODUCTION

Otoliths are small, calcified structures located at the right and left inner ears of all teleost fishes. Since otoliths play a significant role in balance and hearing, they are thought to be biologically vital for teleost fish (Popper *et al*., 2005).

Otolith morphology is species-specific but shows intraspecific variations under the influence of certain variables (Vignon, 2012). Some of these variables are somatic growth (Cardinale *et al*., 2004), ontogeny (Çöl & Yilmaz, 2022), sex and sexual dimorphism (Cardinale *et al*., 2004), reproduction (Carvalho & Corrêa, 2014), genetic factors (Vignon & Morat, 2010), feeding density (Gagliano & McCormick, 2004), habitat use (Jaramillo *et al*., 2014), stock and environmental factors (Cardinale *et al*., 2004). Due to the influences of these complex factors, otoliths have become a useful tool in fisheries biology (Tuset *et al*., 2003).

The morphometric and morphological analysis of otoliths is critical and has been used effectively in many fields, such as stock discrimination (Stransky *et al*., 2008), fish taxonomy (Tuset *et al*., 2006), trophic ecology studies (Bowen & Iverson, 2013), eco-morphological studies (Assis *et al*., 2020) and paleontological studies (Schwarzhans *et al*., 2017).

Nemipterus randalli (Russell, 1986) belongs to the Nemipteridae family and is a demersal fish species. It is generally distributed in sandy and muddy soils between 5 and 80 m in depth. This species is carnivorous and feeds on small fish, crustaceans, molluscs, polychaetes and echinoderms. Its natural distribution areas are the Red Sea, western India, the Persian Gulf and from the coast of East Africa to Durban (South Africa), including Madagascar (Russell, 1990). This species, which entered the Mediterranean Sea with the opening of the Suez Canal, was reported for the first time as mistakenly

Nemipterus japonicus by Golani and Sonin (2006). Later, Lelli *et al*. (2008) reported the species from the Lebanese coast. On the Turkish coasts, it was recorded by Bilecenoglu and Russell (2008) in İskenderun Bay (Mediterranean Sea), by Gökoğlu *et al*. (2009) in Antalya Bay (Mediterranean Sea), by Gülşahin and Kara (2013) in Gökova Bay (Southern Aegean Sea) and by Aydın and Akyol (2016) in İzmir Bay (Aegean Sea). It has been reported that it has also been detected recently on the coasts of Syria (Ali *et al*., 2013) and Cyprus (Iglésias & Frotté, 2015). *N. randalli* has been included in the blacklist of marine invasive species (Otero *et al*., 2013). It is also claimed that after entering the Mediterranean Sea, this species developed a sexual dimorphic character in its body morphology, which is not seen in its natural distribution areas (Uyan *et al*., 2022).

The number of studies on the otolith morphology of the species is very few and the issue of sexual dimorphism was not examined in these studies (Innal *et al*., 2015; SriHari *et al*., 2021; Uyan *et al*., 2019), therefore, in this study, the sexual variations in the otolith shape and morphometry of *N. randalli* were investigated and the association of these variations with the sexual dimorphism in fish morphology was discussed.

2 | MATERIALS AND METHODS

In this study, fish samples were obtained from commercial fishermen. The samples were brought to the laboratory in transport cabinets at −10°C. No experimental studies were conducted on the fish. Animal rights and scientific ethical principles were followed in all transactions.

A total of 132 (51 female and 81 male) samples were obtained from local fishermen in İskenderun Bay in November 2018. The total lengths (TL) of the samples were measured with accuracy of ±1 mm, and their weights (W) were weighed with a scale with an accuracy of ±0.01 g. The sexes were determined by considering the macroscopic examination of the gonads and the morphological characteristics of the samples. Both right and left sagittal otoliths were removed, wiped clean and stored in dry Eppendorf tubes. Finally, the weights of the otoliths (OW) were weighed with an accuracy of ±0.0001 and made ready to be photographed.

All otoliths were placed with their mesial surfaces on top (the side with sulcus acusticus). Otoliths were photographed at 10× magnification with the aid of an Mshot digital camera, Guangzhou, China (resolution 12.5 megapixels (4088 × 3072), pixel size 1 × 1 μm) attached to a Zeiss Stemi 508 binocular microscope, Jena, Germany. Reflected cold light was used to obtain high-contrast digital images and define otolith contours to the finest possible detail. The following morphometric variables were measured with a precision of 0.001 mm according to the main lines in Figure 1 using image analysis software (Mshot Microscope Digital Imaging Analysis Software V1.1.6A): otolith length (OL, mm), otolith height (OH, mm), otolith perimeter (OP, mm) and otolith area (OA, mm^2).

Before each analysis, the data were subjected to normality and homogeneity tests, *i.e*., the Kolmogorov–Smirnov test and Levene's test, respectively. Whether the right and left otolith measurements differ from each other was tested with the paired *t*-test. Since there was no statistical difference between right and left otolith measurements, right otoliths were used in the next analysis.

A power equation ($Y = aX^b$, where Y is otolith dimension, X is fish length, a is the intercept and b is the slope) was used to discover the correlations between otolith measurements and fish size. The parameters a and b were calculated using logarithms in the linear regression analysis, $\log Y = \log a + b \log X$. The analysis of covariance (ANCOVA) was used to determine the statistical variations in regression slopes between the sexes (sex, main factor; TL, covariate).

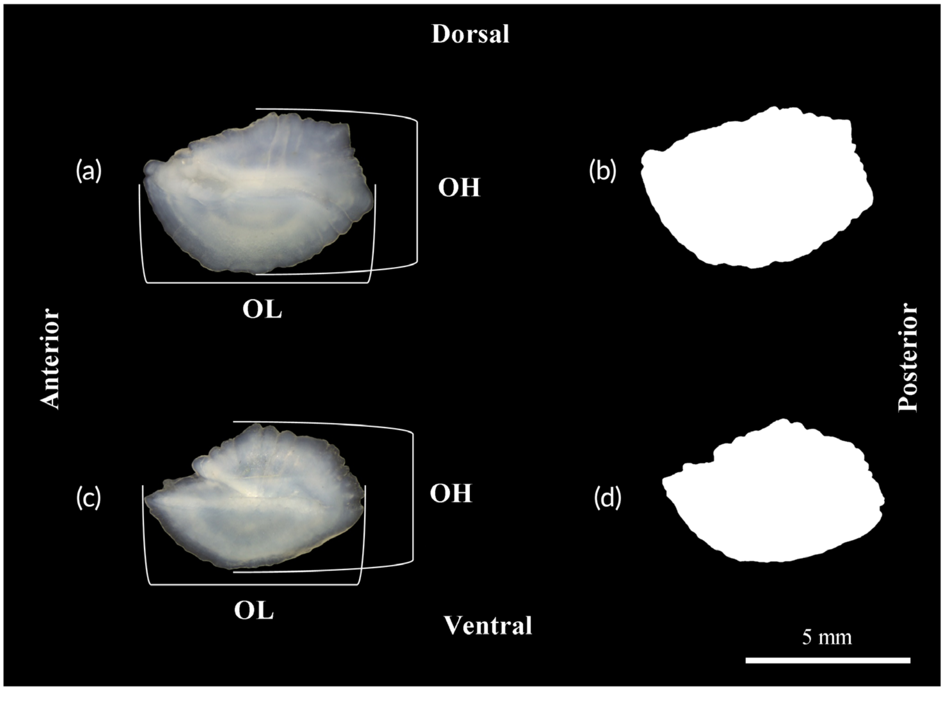

FIGURE 1 The mesial views of right sagittal otoliths of *Nemipterus randalli*: (a) right otolith of female; (b) corresponding binary image of the otolith of females; (c) right otolith of male; (d) corresponding binary image of the otolith of males

Shape indices (Table 1) were calculated using otolith measurements (Bani *et al.*, 2013; Tuset *et al.*, 2003). The effect of fish length on otolith morphological variables should be controlled to make a valid comparison between sexes. For this purpose, the ANCOVA test was applied (Song *et al.*, 2019). In the ANCOVA test, sex and total length were used as the main factor and covariate, respectively. In cases where the 'sex × TL' interaction was not significant, shape indices were standardized with the allometric model. The following equation was used in standardizing the otolith shape indices variables (Lleonart *et al.*, 2000):

$$M_s = M_o\left(\frac{\bar{x}}{x}\right)^b$$

where M_s is the standardized otolith shape indices variable, M_o is the original otolith shape indices variable, $\bar{x}$ is the mean total length of all individuals (15.225 cm), x is the total length of each sample, and b is the slope of the relationship between the otolith shape indices and the fish total length. The PERMANOVA test was performed using standardized data for determining the difference between male and female individuals. Since the shape indices data have multicollinearity, a principal component analysis (PCA) based on a variance–covariance matrix was used to produce a new set of orthogonal variables, principal component scores (PCs), which were then used in a canonical discriminant analysis (CDA) to distinguish between sexes (Agüera & Brophy, 2011; Song *et al.*, 2019). Finally, using the obtained PCs, the CDA was performed to measure the accuracy of sex discrimination.

Elliptic Fourier analysis was used to characterize otolith contours. The elliptic Fourier harmonics for each otolith (20 harmonics) were extracted and normalized with respect to the first harmonic using the program Shape v1.3 (Iwata & Ukai, 2002). The Fourier power spectrum was calculated to determine the minimum number of harmonics required for the best reconstruction of the otolith outline. The first nine of them were used because they are the harmonics that best represent the otolith shape. The first three coefficients (a1, b1, c1) derived from the first harmonic were not considered as they degenerated during the normalization process. In conclusion, a total of 33 ($4 \times 9 - 3$) elliptic Fourier coefficients for each otolith were determined and each was evaluated as an independent variable. PCA was performed to determine the effective elliptic Fourier coefficients. Since multicollinearity was not between elliptic Fourier coefficients, CDA analysis was performed using raw data. The PERMANOVA test was performed using the elliptic Fourier coefficients and differences between males and females were investigated (Agüera & Brophy, 2011). In this study, all statistical analyses were conducted with SPSS 24.

TABLE 1 Shape index formulas calculated for *Nemipterus randalli*

Shape index	Formulae
Form factor (FF)	$(4\pi OA)/OP^2$
Aspect ratio (AR)	OL/OH
Circularity (CIR)	OP^2/OA
Roundness (RD)	$4OA/(\pi OL)^2$
Rectangularity (REC)	OA/(OL × OH)
Ellipticity (E)	(OL − OH)/(OL + OH)
Surface density (SD)	OW/OA

Note: OA, otolith area; OH, otolith height; OL, otolith length; OP, otolith perimeter; OW, otolith weight.

3 | RESULTS

A total of 81 male specimens with an average total length of 14.8 cm (12.0–17.7 cm) and an average total weight of 39.96 g (22.33–64.18 g), and 51 female specimens with an average total length of 15.7 cm (12.0–17.8 cm) and an average total weight of 48.24 g (19.46–66.26 g) were examined (Figure 2). The descriptive statistics and size distribution graphs for otolith measurements are presented in Table 2.

The relationships between the otolith measurements (OL, OH, OA, OP and OW) and fish length (TL) were calculated. The statistical

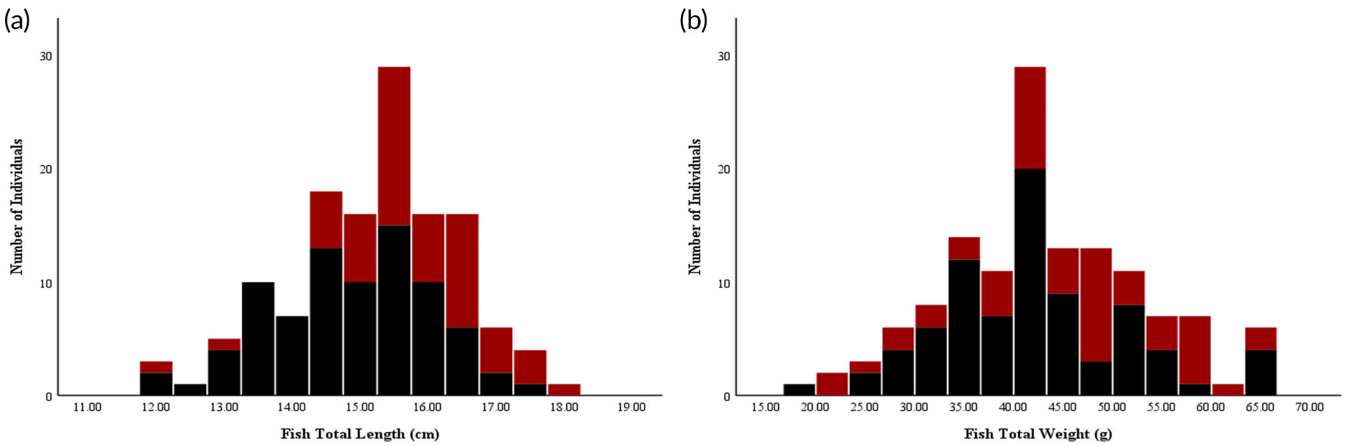

FIGURE 2 The total length and body weight distributions of *Nemipterus randalli* collected from İskenderun Bay (Mediterranean Sea). ■, female; ■, male

TABLE 2 The sagittal otolith measurements in *Nemipterus randalli*

Sex	Variables	*N*	Mean	s.d.	Min	Max
Male	OL	81	6.609	0.544	5.290	7.55
	OH		4.396	0.416	3.748	5.765
	OP		18.656	1.450	14.581	21.279
	OA		19.209	2.799	12.237	24.996
	OW		0.0309	0.007	0.0163	0.0467
Female	OL	51	6.865	0.494	5.039	8.004
	OH		5.073	0.416	3.748	5.765
	OP		20.481	1.557	15.418	23.350
	OA		23.574	3.187	13.527	29.911
	OW		0.047	0.009	0.0191	0.0686

Note: Max, maximum; Min, minimum; *N*, number of samples; OA, otolith area; OH, otolith height; OL, otolith length; OP, otolith perimeter; OW, otolith weight; s.d., standard deviation.

TABLE 3 Regression parameters of the relationships between otolith measurements and fish size in *Nemipterus randalli*

Sex	Relationship	*a* ± s.e.	*b* ± s.e.	R^2
Male	TL x OL	0.519 ± 0.076	0.942 ± 0.055	0.790
	TLxOH	0.383 ± 0.045	0.904 ± 0.045	0.845
	TLxOW	0.022 ± 0.000	2.683 ± 0.134	0.836
	TLxOA	0.157 ± 0.033	1.779 ± 0.077	0.870
	TLxOP	0.149 ± 0.030	1.796 ± 0.149	0.883
Female	TLxOL	0.470 ± 0.076	0.972 ± 0.059	0.848
	TLxOH	0.337 ± 0.098	0.983 ± 0.106	0.639
	TLxOW	0.019 ± 0.000	2.826 ± 0.225	0.763
	TLxOA	0.131 ± 0.046	1.882 ± 0.127	0.813
	TLxOP	1.499 ± 0.343	0.948 ± 0.083	0.727

Note: *a*, intercept; *b*, slope; OA, otolith area; OH, otolith height; OL, otolith length; OP, otolith perimeter; OW, otolith weight; R^2, coefficient of determination; s.e., standard error; TL, total length of fish.

differences in regression slopes between sexes were determined (ANCOVA, $P < 0.05$), therefore otolith measurement–fish length relationships were generated separately according to sex. The values of *a*, *b* and R^2 in these formulae are given in Table 3.

Otolith perimeter (R^2 =0.883 in males) and otolith length (R^2=0.843 in females) were the variables with the strongest correlation with fish length. While all relationships in males were negative allometric except for TL x OL (*t*-test, TL x OL, $t = -1.05$, $P = 0.295$; TLxOH, $t = -2.23$, $P = 0.028$; TLxOW, $t = -2.75$, $P = 0.008$; TLxOA, $t = -2.87$, $P = 0.005$; TLxOP, $t = -2.76$, $P = 0.007$), all relationships in females were isometric (*t*-test, TLxOL, $t = -0.47$, $P = 0.637$; TLxOH, $t = -0.16$, $P = 0.877$; TLxOW, $t = -0.77$, $P = 0.444$; TLxOA, $t = -0.093$, $P = 0.359$; TLxOP, $t = -0.63$, $P = 0.532$). In males, otolith height, otolith weight, otolith area and otolith perimeter were increased slower than fish growth whereas otolith length increased almost at the same rate as fish growth. It can be said that all otolith measurements in females changed at a similar rate with fish growth.

For the shape indices, the 'sex xTL' interaction was found to be insignificant (ANCOVA, Form factor (FF): F = 0.31, P = 0.861; Aspect ratio (AR): F = 0.240, P = 0.625; Circularity (CIR): F = 0.31, P = 0.862; Roundness (RD): F = 0.141, P = 0.708; Rectangularity (REC): F = 0.12, P = 0.902; Ellipcity (E): F = 0.081, P = 0.710; Surface density (SD): F = 0.063, P = 0.801). For these reasons, the shape indices values of females and males were standardized with a common coefficient *b*. In the PCA using shape indices, only one component was obtained and it explained 100% of the total variance. According to this PC, the most effective parameters are circularity (correlation coefficient $R = 0.90$) and aspect ratio ($R = 0.37$). In the analysis made using the PC scores of the shape indices, it was determined that the shape indices of males and females were different from each other (PERMANOVA, Wilk's $\lambda = 0.933$, $P = 0.003$). In the canonical discriminant analysis (CDA), a single function was obtained, and the success of correct classification was 68.2%. The seven analysed shape indices showed that the sagittal otoliths of females were more circular, while males' otoliths were more elongate.

Among the 20 Fourier harmonics extracted to describe otolith contours of 132 specimens of *N. randalli*, the individual Fourier power was calculated and the first nine harmonics explained more than 99.99% of the otolith shape variation for all individual shapes. In the PCA using elliptic Fourier coefficients, only one PC was obtained and it explained 100% of the total variance. The coefficient d1 ($R = 0.99$) was the most effective parameter. The obtained coefficients differed statistically between male and female individuals (PERMANOVA, Wilk's $\lambda = 0.353$, $P = 0.000$). In the CDA-based evaluation, a single function was obtained and the correct classification success was found to be 93.9%. To visualize the heterogeneity between males and females, otolith shapes were reconstructed using elliptic Fourier coefficients and the differences between them were revealed. In females, the dorsal and ventral regions are wider, and the anterior and posterior regions are narrower, while the opposite is true in males (Figure 3).

The shape and morphometry of otolith in *N. randalli* differ between males and females. It was discovered that the otolith height

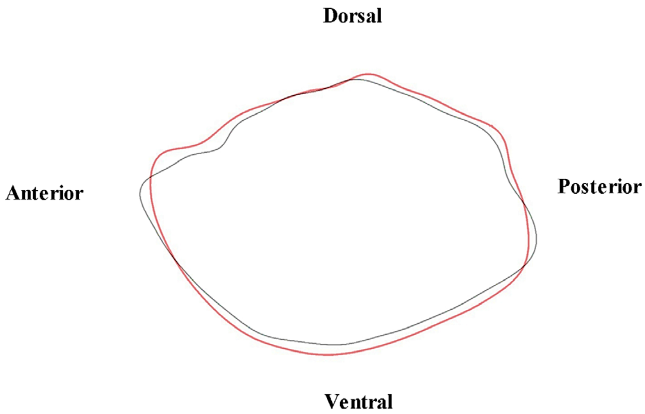

FIGURE 3 Mean otolith shapes based on Fourier outline reconstruction for females and males of *Nemipterus randalli* collected from İskenderun Bay (Mediterranean Sea). ——, male; ——, female

of females had higher than males, and males had longer otoliths. Also, females' otoliths were relatively more circular than males. Both the shape indices and elliptic Fourier analysis point to this conclusion.

4 | DISCUSSION

Otolith measurements are frequently used in fisheries research as an indicator of fish size. In this study, strong relationships were found between otolith size and fish size, and these relationships differed between sexes. Similarly, very strong relationships have been identified in previous research on this species. However, these relationships were not evaluated according to sex (Innal *et al*., 2015; Uyan *et al*., 2019). The determination of strong relationships between otolith sizes and fish size can be based on the assumption that somatic growth affects the growth of the otolith and grows in parallel (Campana & Casselman, 1993; Cardinale *et al*., 2004). However, this relationship may not always be like this. Otolith growth can continue when fish growth slows or stops (Munday *et al*., 2009) Also, Fablet *et al*. (2011) reported that fish body growth slows down more than otolith growth during metabolic times, which may impact how strongly otolith shape and TL are correlated. Additionally, using the equations obtained from these relationships, the length of fish can be calculated back in studies examining predator–prey relationships and in palaeoichthyology studies (Tuset *et al*., 2006). The relationships between otolith sizes and fish size showed negative allometry in males and isometry in females. Here, it was concluded that otolith growth in males was slower than fish growth, and in females, otolith growth was at the same rate as fish growth.

Shape indices showed that the sagittal otoliths were more circular for females and more elongated for males. Otolith shape may vary depending on ontogenetic factors such as fish size and age (Çöl & Yilmaz, 2022; Morat *et al*., 2012). In some studies, it has been found that the otoliths of small individuals are more circular and those of larger fish are more elongated (Curin-Osorio *et al*., 2012). However, the data obtained in this study were not suitable for ontogenetic evaluation according to age or fish size, therefore the effect of ontogeny on otolith shape could not be discussed. There is an asymmetry between males and females in terms of shape indices. A similar situation has been reported for some other fish species (Campana & Casselman, 1993; Mille *et al*., 2015). In this study, males and females were moderately discriminated using otolith shape indices. In some of the studies on discrimination between otolith shape indices of different groups, high discrimination success among groups was achieved by using shape indices (He *et al*., 2018; Pothin *et al*., 2006), but it is seen that shape indices are not very successful in separating groups in most of them (Agüera & Brophy, 2011; Başusta & Khan, 2021; Tuset *et al*., 2003). Additionally, since shape indices are calculated using similar otolith measurements, there may be multicollinearity between the data, which is a negative situation in terms of their usefulness. The fact that shape indices are widely used cannot be denied, but it is recommended that more objective methods such as the elliptic Fourier are used (Pavlov, 2016; Tuset *et al*., 2021).

Elliptic Fourier analysis has been used in many studies and has proven to be valuable in distinguishing between groups (Bose *et al*., 2017; Çöl & Yilmaz, 2022; Popper *et al*., 2005). The elliptic Fourier coefficients showed high success in separating males and females in this study. The high distinctiveness of the elliptic Fourier analysis has also been reported in studies of other fish species (Popper *et al*., 2005; Stransky *et al*., 2008). Elliptic Fourier analysis is more practical and reliable than shape indices in otolith morphology studies. It has many advantages, such as emphasizing objectivity, considering even the smallest detail, and can be applied to many other objects regardless of shape and size. However, the difficulties in determining a fixed shooting position when taking images, the necessity of approaching each sample with the same sensitivity and the problems with lighting are the issues to be considered.

Considering previous studies of otolith morphology, there may be many causes of sexual variation in otoliths. These reasons are discussed below.

According to some researchers, the most obvious change in otolith shape occurs at the beginning of sexual maturity (Gonzalez Naya *et al*., 2012; Tuset *et al*., 2003). Metabolism changes at this stage, sexual maturation affects fish growth and hence has an effect on otolith shape (Morat *et al*., 2012). Females of the species *N. randalli* reach reproductive maturity earlier than males (Taylan & Yapıcı, 2021). Individuals who reach early sexual maturity spend most of their energy on gonadal development and ignore somatic growth (Chen *et al*., 2022). Also, it has also been reported that males and females of *N. randalli* have different growth rates and females grow more slowly than males (Uyan *et al*., 2019; Yapıcı & Filiz, 2019). Both the sexual maturity and the age-related growth rates reported in the literature indicate that the females of the species grow significantly more slowly than the males. Based on the knowledge that the otoliths of slow-growing fish are larger and heavier than the otoliths of fast-growing fish (Reznick *et al*., 1989; Secor & Dean, 1989), the findings of this study confirm these results. However, ontogenetic studies are needed to determine exactly when sexual maturity and thus growth affect the otolith shape and to understand the mechanisms of action of sexual dimorphism.

There may be heterogeneity in otolith morphology due to breeding tactics and vocalization in some fish species (Parmentier *et al*., 2014). It has been reported that especially male individuals increase their vocal intensity during the reproductive period (Parmentier *et al*., 2014; Tellechea & Norbis, 2012) and this tactic can change in muscle activity and otolith (Tellechea & Norbis, 2012). There is no such information about breeding tactics and behaviours in *N. randalli*. However, this hypothesis is thought-provoking for *N. randalli*, as there may be such differences between males and females in many fish species (Bose *et al*., 2017; Thomaz *et al*., 1997), therefore more information is needed about the reproductive behaviour of this species.

In conclusion, this study showed that *N. randalli* has a clear sexual variation in otolith shape, which may be valuable for developing sexual dimorphism. Elliptic Fourier analysis was more effective than morphometric analysis in classifying male and female individuals. The observed dissimilarities in the shape of sagittal otoliths were associated with differences in growth and sexual maturity. In addition, the hypothesis that *N. randalli* faces a shift toward sexual dimorphism in the Mediterranean Sea was supported by this study. It is recommended that researchers focusing on stock discrimination, and paleoichthological and systematic studies on *N. randalli* should shape their research by taking into account the sexes of this species. Finally, the findings of this study can be used in future studies on the trophic ecology of *N. randalli*'s predators and in population studies based on the contour of otoliths of this species.

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