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Ecomorphological patterns and shape indices of otoliths in the *Pagellus acarne* (Actinopterygii, Sparidae) from the Aegean and Marmara Seas

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Abstract: *Pagellus acarne* is found in coastal waters worldwide and it includes important species of commercial and recreational fisheries. In this study, 16 morphometric variables of the sagittal otolith, including six morphometric characters, six shape indices, and four ecomorphological indexes, were investigated for the first time among *P. acarne* stocks collected from the Aegean and Marmara Seas coasts of Turkey. Statistically significant differences were found between the right and left otolith variables of *P. acarne* individuals in both stocks ($p < 0.05$). Similarly, significant differences were observed on the same side between stocks ($p < 0.05$). The right otoliths exhibited higher discrimination power than the left within the two stocks. The PCA showed that only five (31.25%) (otolith area, otolith perimeter, form factor, roundness, and edge complexity index) out of the sixteen variables were quite important characters in the differentiation between stocks. These otolith characters demonstrated a very high rate of accurate discrimination (99.0%) between stocks. The results indicated otolith morphometric characters, shape indices, and ecomorphological indexes can be used as suitable tools to discriminate *P. acarne* stocks. This is the first study to include all otolith characters such as morphometrics, shape, and ecomorphological indices that discriminate between *P. acarne* stocks from these localities.

Key words: Ecomorphological index, otolith shape analysis, *Pagellus acarne*, stock discrimination, sagittae

1. Introduction

Otoliths are three pairs of calcareous structures named sagittae, asteriscus, and lapillus located in the inner ear of bony fishes (Das, 1994). The otolith shape is species-specific and the otolith morphology can help to identify fish species and communities of water-column (Volpedo and Echeverría, 2003; Assis et al., 2020). Otoliths have been used in a wide variety of studies such as age determination, migration, morphometry, and growth pattern (Campana and Casselman, 1993; Secor et al., 1995; Bănaru et al., 2017; Souza et al., 2020). Besides, they have been employed for species and population differentiation (Torres et al., 2000; Bostancı et al., 2015; Yedier et al., 2016; Bostancı and Yedier, 2018; Özpiçak et al., 2021; Yedier, 2021; Yedier et al., 2023a). Many authors have reported that the otolith shape is a species-specific feature resulting from the phylogenetic history of the species (Wilson, 1985; Tuset et al., 2003; Ponton, 2006; Yedier et al., 2019a; Yedier and Bostancı, 2021). The variability in otolith shape has been attributed to many factors related to feeding habits (Nonogaki et al., 2007), substrate type (Volpedo and Cirelli, 2006), ontogenetic shifts (Pérez and Fabré, 2013), and environmental conditions (Lombarte and Lleonart, 1993; Khedher et al., 2021).

The family Sparidae is found in coastal waters worldwide and includes important species of commercial and recreational fisheries. It comprises 38 genera and 158 species all over the world and distributes in marine, fresh and brackish waters (Froese and Pauly, 2023). Six species are belonging to the genus *Pagellus*. These are *P. acarne, P. affinis, P. bellottii, P. bogaraveo, P. erythrinus*, and *P. natalensis*. Three out of these species (*P. acarne, P. bogaraveo*, and *P. erythrinus*) are known in Turkish marine waters, and the most common of them is *P. acarne*. *P. acarne* is one of the commercially important species and is widely distributed in different seas of Turkey (Gül et al., 2021). Despite its commercial high value and abundance along the Turkish coasts, there is a lack of information on the otolith shape and characteristics of this species in the Turkish seas, which contain important fishing areas and many fish species.

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Therefore, the present study was conducted to (i) determine the morphometric features (length, width, area, perimeter, rostrum length, and sulcus area), shape indices (form factor, roundness, aspect ratio, circularity, rectangularity, and ellipticity), and ecomorphological indexes (Edge Complexity Index, E Index, R Index, and S Index) of otolith pairs of *P. acarne* individuals, (ii) compare the otolith characteristics of *P. acarne* individuals between two stocks from the Aegean and Marmara Seas, and (iii) test the usability of otolith characters to discriminate between the two stocks.

2. Materials and methods

A total of 157 fish samples were collected using a commercial bottom trawl net from the Turkish coasts of the Aegean Sea (29.9%) and Marmara Sea (70.1%) (Figure 1). The total length (TL) of each fish sample was measured to the nearest 0.1 cm and weighed with ±0.1 g sensitiveness. The right and left sagittal otoliths were removed and cleaned. The sex status of fish individuals was evaluated according to the codes of sexual maturity in fish criteria (MEDITS, 2007). Sagittal otolith pairs were photographed using a digital camera (Leica DFC 295) and then otolith length (OL), width (OW), area (OA), perimeter (P), rostrum length (RL), and sulcus area (SA) were measured to the nearest ±0.001 mm using a photo processing software (Leica Application Suite, LAS). Different magnifications were used in imaging according to the size of the sample. The right and left otoliths were measured on two axes; the otolith length was named as anterior-posterior axis and the otolith width was the dorso-ventral axis (Figure 2). The morphology of otoliths was described by two different approaches. First, the otolith shape indices were calculated using the following formulas: Roundness (RD) $= 4 * OA/(\pi * OL^2)$; form factor (FF) $= 4 * \pi * OA/OP^2$; circularity (C) $= OP^2/OA$; elipticity (E) $= (OL - OW)/(OL + OW)$; rectangularity (R) $= OA/(OL * OW)$ and aspect ratio (AR) $= OL/OW$ (Tuset et al., 2003; Ponton, 2006). Second, ecomorphological indexes of the otolith were calculated using the following formulas: Edge Complexity Index (ECI) $= OP/(2 * (OA * \pi)^{1/2})$; E Index (EI) $= OA/OL$; R Index (RI) $= RL/OL$, and S Index (SI) $= SA/OA$ (Kalff, 2002; Volpedo and Echeverría, 2003; Volpedo et al., 2008).

Sagittal otolith measurements were standardized to eliminate any size effect (Elliott et al., 1995) using the formula $Ms = Mo * (Ls / Lo)^b$ where Mo = measured character, Ms = standardized measurement, Ls = arithmetic mean standard length for all fish from all samples in each analysis, Lo = standard length of samples, and b was estimated for each character from the observed data by the allometric growth equation. Differences in otolith dimensions of the female and male individuals were compared by the t-test. In addition, the differences between right and left otolith dimensions were tested by the paired-t test. ANOVA was used to determine whether the sagittal otolith characters differ between the Aegean and Marmara stocks. Principal Component Analysis (PCA) was used to evaluate the otolith variables, and PCA variables, which explained more than 1% of the variability, were taken into account to reduce the number of dimensions without loss of information. Canonical Discriminant Analysis (CDA) was also used to determine the differences between the two stocks. The CDA was performed with four groups, i.e. between the right and left otoliths of the two stocks. The performance of the discriminant analyses was assessed using Wilk's lambda (λ) test. In discriminant analyses, the values of λ range from 0 to 1, the closer the λ is to 0, the better the discriminating power of the CDA. The statistical analyses were performed by Minitab v.16, SPSS v.26, and PAST v.2.17.

Figure 1. Sampling sites of *Pagellus acarne* in the Aegean and Marmara Seas.

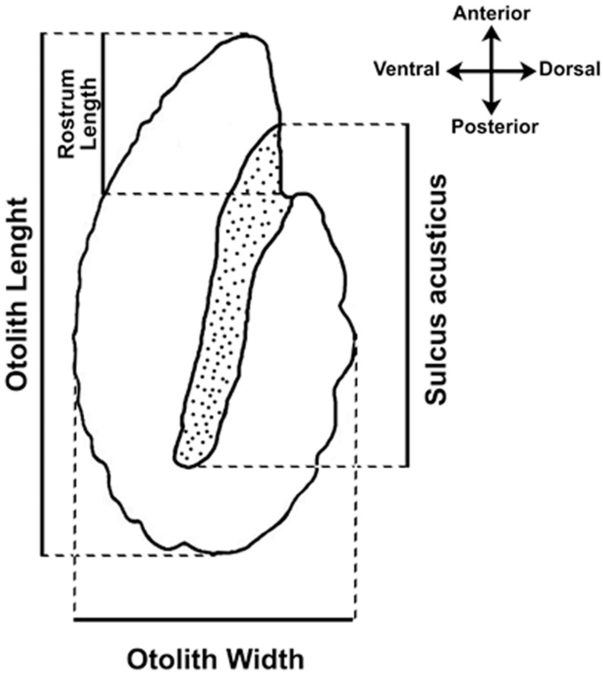

Figure 2. Explanation of morphometric otolith measurements on the proximal otolith surface of *Pagellus acarne*.

3. Results

The mean values of the total length and weight of *Pagellus acarne* individuals sampled from the Aegean Sea (n = 47) were 13.0 ± 0.234 cm and 30.5 ± 1.85 g, while they were 12.8 ± 0.079 cm and 29.4 ± 0.433 g from the Sea of Marmara (n = 110), respectively. Descriptive statistics of the sagittal otolith pairs of *P. acarne* from the Aegean and Marmara Seas are given in Table 1. The results showed no statistical difference between otolith characters among male and female individuals within both stocks ($p > 0.05$). Therefore, the statistical tests were performed on both male and female individuals in each of the two stocks.

The mesial surfaces of the left and right otoliths of *P. acarne* from both the Aegean and Marmara Seas are presented in Figure 3. Analysis of the right and left sagittal otoliths morphometric parameters showed significant differences ($p < 0.05$) in the OL, OW, OA, OP, and SA values among individuals of the Marmara Sea stock and the RL value among those of the Aegean Sea stock ($p < 0.05$) (Table 1). Besides, ANOVA showed significant differences ($p < 0.05$ and $p < 0.001$) in the OP, OW, and SA values between the right and left otoliths of individuals from the Aegean and Marmara stocks (Table 2). Moreover, statistically significant differences were observed in the RD, FF, C, E, R, AR, ECI, EI, and SI values between the right and left otoliths of individuals among the two stocks (ANOVA, $p < 0.001$) (Table 2).

Results of the PCA analysis of the sixteen characters examined in the study showed that only five (31.25%) of them were found useful to discriminate between stocks (Figure 4). The CDA performed on the OA, OP, FF, C, and ECI demonstrated significant discrimination between right and left otoliths ($\lambda = 0.859$, $p < 0.001$) and reclassified 65.5% of the individuals to Marmara Sea stock and 92.5% of the individuals to Aegean Sea stock, and exhibited significant discrimination between the right and left otoliths (Wilk's $\lambda = 0.294$, $p < 0.001$). However, the right otoliths were determined to have a higher discrimination power (95. %) than the left ones (88.2%) in both the Aegean and Marmara stocks. Besides, CDA for either pooled or separate left and right otoliths OA, OP, FF, C, and ECI from individuals of the two stocks showed significant discrimination (99.0%) between the two stocks.

4. Discussion

Otolith morphology is species-specific genetically but also affected by environmental factors (Begg and Brown, 2000; Cardinale et al., 2004). Therefore, otolith morphometrics have been chosen as a potential indicator for species and population discrimination. Several studies were shown that otolith shape analysis allowed population and stock discrimination (Cardinale et al., 2004; Pothin et al., 2006; Mérigot et al., 2007; Vignon and Morat, 2010; Bostancı and Yedier, 2018; Saygın et al., 2020; Khedher et al., 2021;

Table 1. Descriptive statistics of sagittal otolith pairs of *P. acarne* in the Aegean and Marmara Seas and statistical comparisons of left and right otoliths within the stocks.

		Aegean Sea			Marmara Sea		
		Right otolith	**Left otolith**	**P value**	**Right otolith**	**Left otolith**	**P value**
Otolith measurements	**OA**	11.399 ± 0.345 7.225–19.455	11.408 ± 0.361 7.355–20.772	p > 0.05	11.906 ± 0.106 9.325–16.307	11.947 ± 0.106 9.464–15.981	**p < 0.05**
	OP	15.173 ± 0.248 11.901–21.156	15.160 ± 0.233 12.635–21.673	p > 0.05	25.563 ± 0.188 15.484–29.023	24.807 ± 0.176 15.409–28.939	**p < 0.05**
	OL	5.371 ± 0.089 4.170–7.658	5.376 ± 0.089 4.183–7.752	p > 0.05	5.257 ± 0.026 4.552–6.207	5.279 ± 0.026 4.525–6.222	**p < 0.05**
	OW	3.100 ± 0.048 2.605–4.527	3.096 ± 0.054 2.654–4.750	p > 0.05	3.426 ± 0.016 3.046–3.939	3.402 ± 0.016 2.980–3.858	**p < 0.05**
	SA	2.199 ± 0.115 0.894–5.561	2.273 ± 0.102 0.933–4.865	p > 0.05	1.793 ± 0.054 1.419–2.655	1.906 ± 0.042 1.513–2.366	**p < 0.05**
	RL	1.404 ± 0.039 0.710–2.324	1.338 ± 0.037 0.658–2.135	**p < 0.05**	1.389 ± 0.039 1.000–1.833	1.356 ± 0.038 0.898–1.667	p > 0.05
Shape Indices and Ecomorphological Indexes	**RD**	0.525 ± 0.006 0.5442–0.677	0.524 ± 0.007 0.460–0.712	p > 0.05	0.574 ± 0.002 0.508–0.624	0.571 ± 0.002 0.517–0.616	**p < 0.05**
	FF	0.592 ± 0.007 0.475–0.771	0.591 ± 0.006 0.512–0.728	p > 0.05	0.221 ± 0.004 0.176–0.616	0.235 ± 0.003 0.184–0.605	**p < 0.05**
	C	20.403 ± 0.272 15.556–25.231	20.375 ± 0.206 16.473–23.422	p > 0.05	55.158 ± 0.554 19.457–67.999	51.740 ± 0.487 19.816–65.209	**p < 0.05**
	E	0.267 ± 0.005 0.149–0.323	0.268 ± 0.006 0.126–0.322	p > 0.05	0.210 ± 0.001 0.170–0.264	0.216 ± 0.001 0.180–0.268	**p < 0.05**
	R	0.680 ± 0.002 0.630–0.720	0.680 ± 0.002 0.646–0.712	p > 0.05	0.659 ± 0.001 0.629–0.683	0.664 ± 0.001 0.635–0.696	**p<0.05**
	AR	1.735 ± 0.179 1.352–1.955	1.743 ± 0.021 1.288–1.950	p > 0.05	1.535 ± 0.005 1.410–1.720	1.552 ± 0.006 1.440–1.735	**p < 0.05**
	ECI	1.842 ± 0.012 1.610–2.050	1.841 ± 0.009 1.657–1.975	p > 0.05	3.027 ± 0.016 1.800–3.366	2.932 ± 0.015 1.817–3.296	**p < 0.05**
	EI	2.106 ± 0.031 1.732–3.095	2.105 ± 0.034 1.758–3.164	p > 0.05	2.260 ± 0.010 1.978–2.627	2.259 ± 0.010 1.948–2.568	p > 0.05
	RI	0.260 ± 0.004 0.143–0.310	0.248 ± 0.004 0.149–0.287	**p < 0.05**	0.269 ± 0.009 0.182–0.392	0.261 ± 0.008 0.173–0.338	p > 0.05
	SI	0.189 ± 0.005 0.115–0.285	0.197 ± 0.004 0.126–0.259	**p < 0.05**	0.155 ± 0.004 0.118–0.230	0.164 ± 0.004 0.123–0.206	**p < 0.05**

OA: Otolith area (mm^2), OP: Otolith perimeter (mm), OL: Otolith length (mm), OW: Otolith width (mm), SA: Sulcus area (mm^2), RL: Rostrum length (mm), RD: Roundness, FF: Formfactor, C: Circularity, E: Elipticity, R: Rectangularity, AR: Aspect ratio, ECI: Edge complexity index, EI: E index, RI: R index, SI: S index)

Yedier and Bostancı, 2022; Yedier et al., 2023b). In this study, the RD, FF, C, E, R, and AR shape indices and ECI, EI, and SI ecomorphological indices of left-right otoliths of *P. acarne* were statistically different between the Aegean and Marmara stocks. Differences in otolith morphology of fish species of the same species living in habitats with different ecological characteristics can be observed, indicating that ecological indices can be simple indicators of otolith morphology (Mahe et al., 2016). Our results showed that both the shape indices and morphological indexes differentiated clearly between the two stocks. Generally, the shape indices determined the otolith morphology,

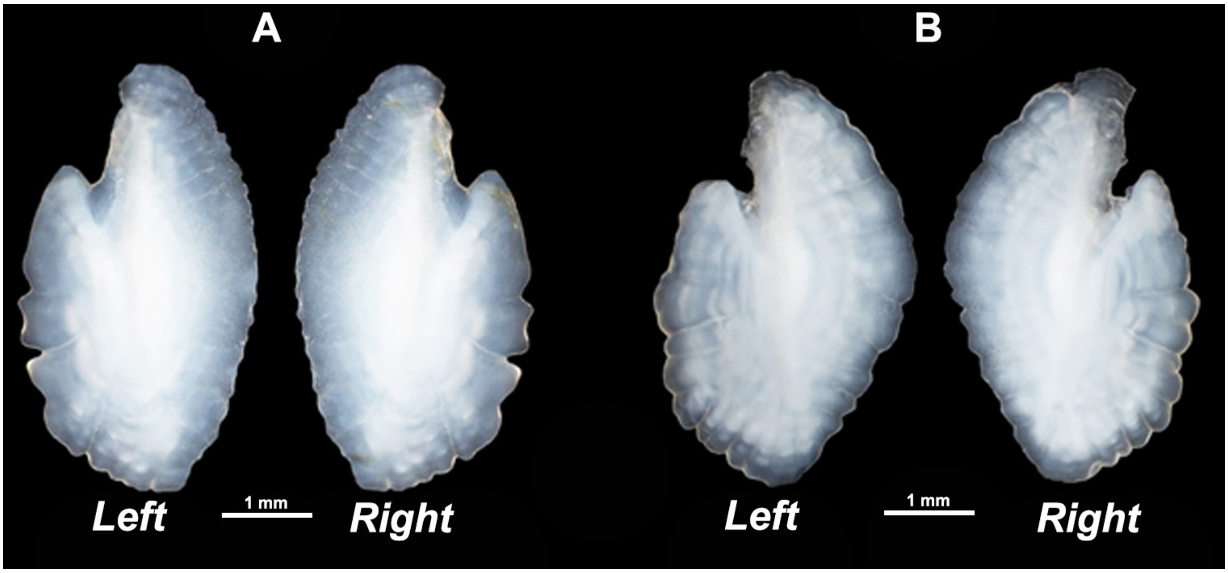

Figure 3. General views of the mesial surfaces of the right and left otoliths of *Pagellus acarne*; (A) Aegean Sea stock, (B) Marmara Sea stock.

whereas the ecomorphological indexes enabled an understanding of the ecological features of populations. In some studies, ecomorphological indexes were used to describe the depth preference and water column used by fish (Volpedo and Echeverría, 2003; Volpedo et al., 2008) and/or to identify the species' ecology in combination with Fourier harmonics (Assis et al., 2020).

Mérigot et al. (2007) reported that left otolith variables were stronger than the right otolith variables in distinguishing *Solea solea* populations in the Mediterranean Sea. In our study, however, it was determined that the right otolith variables had higher discriminating power than the left otolith variables between *P. acarne* stocks from the Aegean and Marmara Seas. In addition, Mejri et al. (2018a) used otolith shape analysis to distinguish between populations of *P. erythrinus* from two different lagoons in Tunisian waters and reported that the right and left otoliths of the two populations showed significant differences within each population, a result that was similar to that found in the present study. Moreover, our results are in agreement with the findings of D'Iglio et al. (2021) who compared three species of *Pagelus*, including *P. acarne, P. bogaraveo*, and *P. erythrinus*, and their populations based on the otolith morphometry and morphology and reported variations in both the otolith morphometry and morphology between populations of the different species as well as within population of the same species from different habitats.

In the current study, it was shown that the OA, OP, FF, C, and ECI discriminated clearly between stocks of *P. acarne* from the Aegean Sea and the Sea of Marmara, with 99.0% correct classifications of individuals to their populations of origin. This percentage is comparable to 83.3% reported by Mejri et al. (2018b) between populations of *P. erythrinus* sampled from Kekennah and Zembra in Tunisian waters. Previous studies have shown that the otolith shape can vary within and among species due to the combined effects of both genetic and environmental factors, such as water temperature, salinity, depth, substrate type, food availability, and pollutants (Torres et al., 2000; Volpedo and Echeverría, 2003; Pérez and Fabré, 2013; Jawad et al., 2020; Mejri et al., 2020; Geladakis et al., 2021; Khedher et al., 2021). Therefore, the current significant differences observed in the sagittal otolith morphology could be attributed to differences in the genetic and environmental factors between these two stocks.

In conclusion, the present study highlighted the use of otolith characters such as otolith measurements (OA and OP), shape indices (FF and C), and ecomorphological index (ECI) as alternative and effective parameters to discriminate between *P. acarne* stocks from the Aegean and the Marmara Seas. However, further studies involving the examination of more populations/stocks of this species, supported by investigation of the environmental, physicochemical, and ecological parameters, will be required to better understand the origin of this variation in the otolith OA, OP, FF, C, and ECI between stocks of *P. acarne* from Turkey.

Table 2. Descriptive statistics of sagittal otolith pairs of *P. acarne* in the Aegean and Marmara Seas and statistical comparisons of left and right otolith values between stocks.

		Right otolith			Left otolith		
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	OL	5.371 ± 0.089 4.170–7.658	5.257 ± 0.0263 4.552–6.207	p > 0.05	5.376 ± 0.089 4.183–7.752	5.279 ± 0.026 4.525–6.222	p > 0.05
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	FF	0.592 ± 0.007 0.475–0.771	0.221 ± 0.004 0.176–0.616	**p < 0.001**	0.591 ± 0.006 0.512–0.728	0.235 ± 0.003 0.184–0.605	**p < 0.001**
	C	20.403 ± 0.272 15.556–25.231	55.158 ± 0.554 19.457–67.999	**p < 0.001**	20.375 ± 0.206 16.473–23.422	51.740 ± 0.487 19.816–65.209	**p < 0.001**
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	AR	1.735 ± 0.179 1.352–1.955	1.535 ± 0.005 1.410–1.720	**p < 0.001**	1.743 ± 0.021 1.288–1.950	1.552 ± 0.006 1.440–1.735	**p < 0.001**
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	EI	2.106 ± 0.031 1.732–3.095	2.260 ± 0.010 1.978–2.627	**p < 0.001**	2.105 ± 0.034 1.758–3.164	2.259 ± 0.010 1.948–2.568	**p < 0.001**
	RI	0.260 ± 0.004 0.143–0.310	0.269 ± 0.009 0.182–0.392	p > 0.05	0.248 ± 0.004 0.149–0.287	0.261 ± 0.008 0.173–0.338	p > 0.05
	SI	0.189 ± 0.005 0.115–0.285	0.155 ± 0.004 0.118–0.230	**p < 0.001**	0.197 ± 0.004 0.126–0.259	0.164 ± 0.004 0.123–0.206	**p < 0.001**

(OA: Otolith area (mm^2), OP: Otolith perimeter (mm), OL: Otolith length (mm), OW: Otolith width (mm), SA: Sulcus area (mm^2), RL: Rostrum length (mm), RD: Roundness, FF: Formfactor, C: Circularity, E: Elipticity, R: Rectangularity, AR: Aspect ratio, ECI: Edge complexity index, EI: E index, RI: R index, SI: S index)

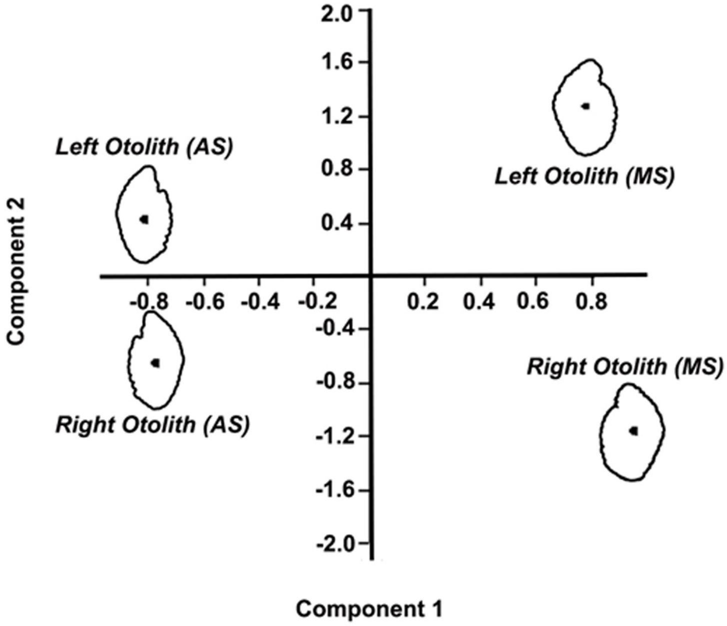

Figure 4. Principal component analysis (PCA) plot showing similarities/ differences between both *Pagellus acarne* stocks and left and right side otoliths: (AS) Aegean Sea and (MS) Sea of Marmara.

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