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Patterns of variations in populations of *Parupeneus forsskali* (Fourmanoir and Guézé, 1976) of the Red Sea, Gulf of Suez and the Mediterranean Sea as reflected by otolith shape using ShapeR

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ABSTRACT

Population structure and differentiation of *Parupeneus forsskali* (Fourmanoir & Guézé, 1976) was studied by otolith shape variation using the ShapeR package. The quantitative shape analysis was used for studying otolith shapes. The outlines were analyzed with univariate and multivariate methods of Wavelet and Fourier transformations. There were significant differences (P<0.05, ANOVA) between geographically distant populations of *P. forsskali* collected from the Red Sea, Gulf of Suez and the Mediterranean Sea. The differences between populations were 85 % for the first discrimination. Additionally, the classification success with cross- validation for the three populations was 65%. The misclassification error for the linear discrimination analysis was 0.353. The observed morphological differences were supposed to reflect environmental effects or might be attributed to differences in life-history strategies. Studying Population structure and differentiation is important for designing appropriate regulations for effective fisheries management.

INTRODUCTION

Parupeneus forsskali belongs to family Mullidae (Goatfish) which is the most commercially and economically important species along the northern parts of the Red Sea coast, Egypt. *Parupeneus forsskali* is a migrant species, moving through Suez Canal from the Red Sea to the Mediterranean Sea. This species is well established in the eastern areas of the Mediterranean Sea **(Çinar *et al*., 2006; Bariche *et al.*, 2013; Mehanna *et al.*, 2016; Farrag *et al*., 2018; Özvarol & Tatlıses, 2018; Evagelopoulos *et al.*, 2020).**

Many fish species showed effective intraspecific variation in their morphology, both among and within populations, which is frequently induced by environmental factors or may be attributed to behavioral influences **(Turan, 2006; Kocovsky *et al.* 2013; Serdar & Bostanci, 2020).**

Otoliths are hard structures found in the inner ear of fish. Otolith is the most important part to distinguish between fishes and has been widely used in age determination. Moreover, it is functionally associated with hearing and the sense of balance, acoustic communication, feeding strategy, swimming and spatial distribution **(Popper & Lu, 2000; Gauldie & Crampton, 2002; Ramcharitar *et al*., 2006; Lombarte & Cruz, 2007; Volpedo *et al.*, 2008;**

Lombarte *et al.*, 2010; Sadighzadeh *et al.*, 2014; Schulz-Mirbach *et al*., 2019; Wiff *et al*., 2019; Yedier & Bostanci, 2020).

Otolith shapes are considered as species-specific **(Stransky & MacLellan, 2005; Osman *et al.*, 2020).** Geographic variations in shapes of otolith could be attributed to stock differences **(Campana & Casselman, 1993; Begg & Brown, 2000; Stransky, 2005; Sadeghi *et. al.*, 2020; Osman *et al.*, 2021)**.

The analysis of otolith shape has been widely used in stock identification of several marine fish species with high gene flow, such as cod fish **(Campana & Casselman, 1993)**, haddock (**Begg *et al.*, 2001**), horse mackerel (**Turan 2006; Stransky *et al.*, 2008**), anchovy (**Bacha *et al.*, 2014**), and herring (**Burke *et al.*, 2008; Libungan *et al.*, 2015**) . Otolith shape is population specific, and also shows intra-specific geographical variations related to environmental factors (**Cardinale *et al.*, 2004; Vignon 2012; Libungan *et al.*, 2015; Sadeghi *et. al.*, 2020; Osman *et al.*, 2020 ; Osman *et al.*, 2021**).

The present study aimed to evaluate otolith shape efficiency as a convenient morphological marker to study fisheries biological, ecological and geographical differences of *P. forsskali* populations in the Red Sea, Mediterranean Sea and Gulf of Suez.

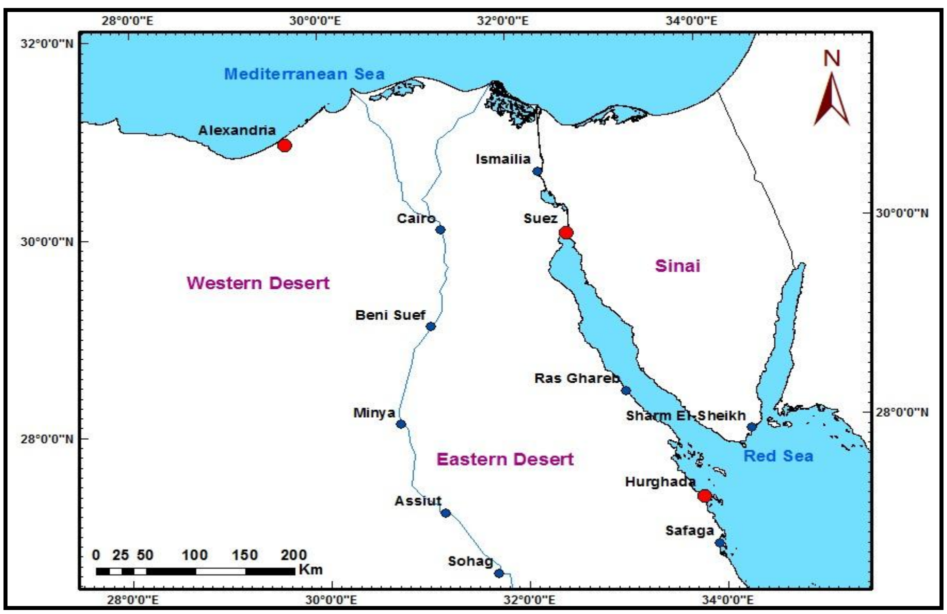

Fig. 1. A map showing sampling sites of *Parupeneus forsskali* from Mediterranean Sea , Red Sea and Gulf of Suez.

MATERIALS AND METHODS

Samples of *P. forsskali* were collected from commercial catch of three separate regions (Fig.1); the Mediterranean Sea, the Red Sea and the Suez Gulf. Biological measurements were recorded, including body length (TL= cm), weight (g) and sex. The sagittal otoliths were extracted and washed using clean water, and then stored either in plastic tubes or paper bags.

Otolith image and shape analysis

A stereoscope (Carl Zeiss Discovery v20 connected to Axio Cam ERc5s camera with Zeiss software) with reflected light and Black background, as well as magnification of microscopy (25x) was utilized for capturing a digital image of every otolith (Fig. 2). Statistical analysis was conducted using the shape R packages **(Libungan & Pálsson, 2015).** Various scenarios were discussed to explain the pattern of otolith shape differentiation and population structure.

Outline analysis

The otolith images were read on basis of the Shape R package which analyzes the shape of the otolith by extracting outlines from digital images of the otoliths and avoiding pixel noise (**Stransky, 2013**; **Libungan & Pálsson 2015).**

A matrix of x and y coordinates was estimated from all otolith outlines by following the rotation of all otoliths horizontally along their longest axis. This was followed by drawing equally spaced radii from the centroid of the otolith to its outline. The radii length acts as a univariate shape descriptor. By using the Wavelet and Fourier transformation on these radii, the Wavelet and Fourier coefficients, respectively, were extracted from the digital images using the Wavethresh 4.6.8 package.

Otolith shape analysis

The average otolith shape of every population was plotted by utilizing the Wavelet coefficients. Mean shape coefficients and their standard deviation of all combined otoliths were plotted against the angle of the outline using Wavelet transform by the package g plots 3.0.3 in order to evaluate which areas of the otolith shape indicated the most variation between populations (**Warnes *et al.*, 2014; Libungan & Pálsson, 2015)**. The variation proportion among groups (the intraclass correlation, ICC), was calculated along the otolith outline to quantify the differences among populations.

Multivariate shape analysis

The standardized Wavelet coefficients were transformed into principal coordinates and subjected to canonical analysis (CAP: Canonical Analysis of Principal coordinates) as a mean for examining the variation in the shape of otolith between populations (**Anderson & Willis, 2003**). The function capscale with the package Vegan 2.5–6 was then used for comparing the outcome between populations (**Oksanen *et al.*, 2013**). Additionally, Wavelet coefficients were used for cluster visualization of the CAP results in two discriminating axes CAP1 and CAP2.

Classification of individuals to their sampling origin

Linear discriminant analysis (LDA) represents a useful statistical technique for dimensionality reduction for pattern recognition using linear combinations of features that are separated linearly. In the present study, linear discriminant analysis (LDA) was applied to the standardized Wavelet coefficients using the LDA function in the MASS package in R for demonstrating the classification of individuals to their origin based on population variation at the three studied locations (**Ripley *et al.*, 2020**).

A cluster analysis was determined based on the values of the CAP1 and CAP2 analysis of the three studied populations of *P.forisskali* using the Euclidean distance as a measure of intra-specific variations using Past version 2.17 software (**Hammer *et al.*, 2001**).

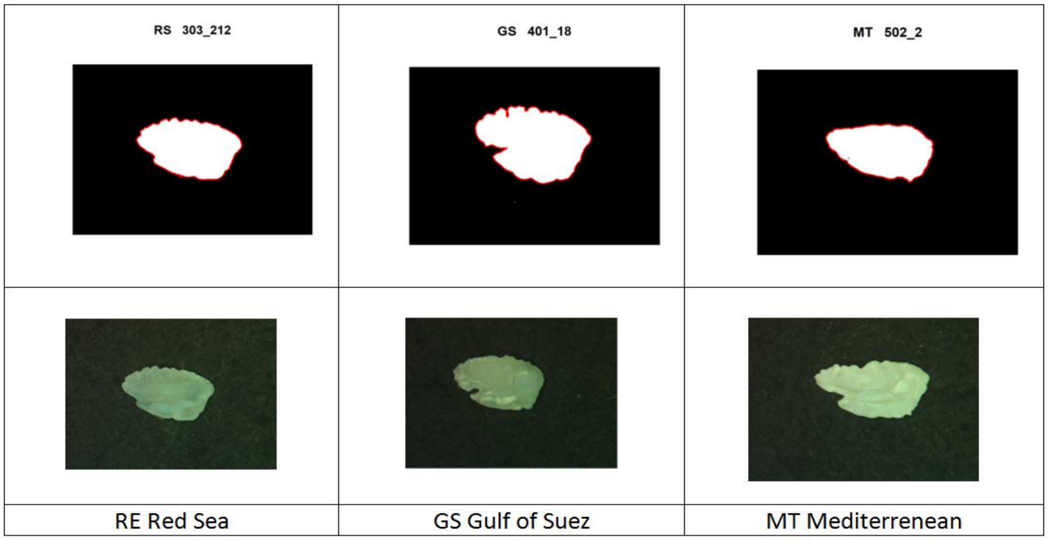

Fig. 2. Sagittal otolith of *Parupeneus forsskali* collected from Red Sea (RS), Mediterranean Sea (MT) and Gulf of Suez (GS); red line marks otolith shape outline.

RESULTS

In the present study, samples of *P. forsskali* collected from three regions (MT, RS and GS) were studied. Table (1) shows number of individuals (males, females) and total length and weight. ANOVA-like permutation test (Table 2) using the radii revealed no significant differences between sexes within every sampling site (P > 0.05). Accordingly, samples (males and females) from all sampling sites were set for further analyses.

Statistical analysis

ANOVA-like permutation test revealed that, the variation in otolith shape among *P. forsskali* populations based on 1000 permutations was large (P<0.05) showing a significant difference. Univariate analysis of otolith shape using the F and P value among populations indicated that otolith shapes of the three populations were correlated with geographical distances between sampling sites. (Table 2).

Table 1. Length(cm), weight(g) and number of *Parupeneus forsskali* collected from three sampling areas, RS; Red Sea, MT; Mediterranean Sea, GS; Gulf of Suez.

Location	Lat.	Lon.	No.			Fish Length (cm)		Fish Weight (g)	
			F	M	C	Min.-Max.	Average ± ST	Min.- Max.	Average ± ST
Red Sea	27.2579°N	33.8116°E	19	28	47	13.8- 24.1	18.38 ±2.48	26.74- 151	76.86 ±33.87
Mediterranean	28.9793°N	32.9006°E	14	26	40	12.1- 23.2	16.17 ±3.52	18.90- 151.50	55.44 ±44.20
Gulf of Suez	31.2001°N	29.9187°E	43	11	54	11.7- 19.0	15.61 ±1.61	14.30- 74.30	43.34 ±12.75

Table 2. Variations in otolith shape among populations of *Parupeneus forsskali* based on 1000 permutations . df, degree of freedom; Var.,variance among populations; F-value; P value. P< 0.05 shows significant effect.

Variables	Df	Var	F	P
GS *v.* MT	1	9.646	12.124	0.001***
GS *v.* RS	1	6.195	4.7974	0.001 ***
MT *v.* RS	1	4.617	5.2239	0.001 ***

Outline analysis of otolith shape

By using the outline analysis, the boundary shapes can be quantified, and consequently the patterns of shape variation within and among groups can be evaluated based on a large number of independent variables. In the present study, the outline analysis using the Shape R package was used for quantifying otolith shape variation among populations of *P. forsskali* from three sampling areas (Fig.3).

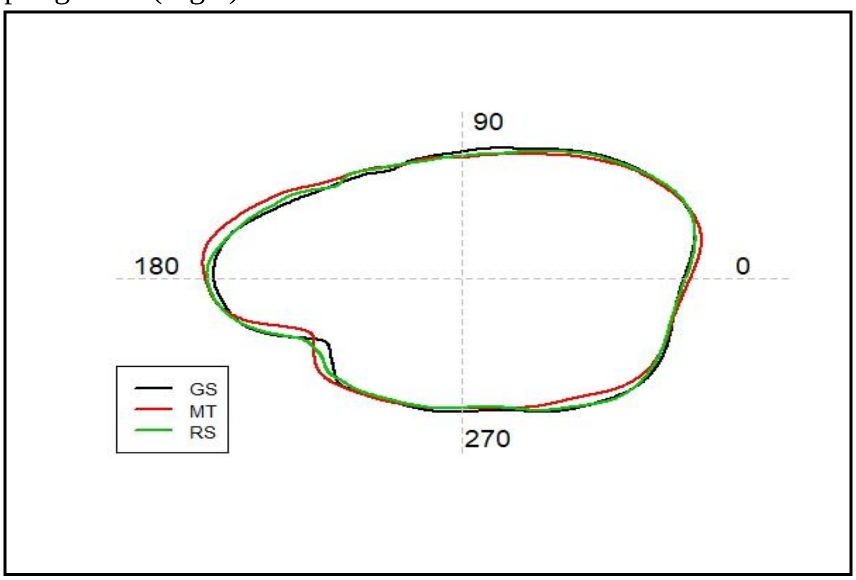

Fig. 3. Mean shapes of the otoliths of *Parupeneus forsskali* from the three sampling areas; Suez Gulf (GS, n= 47), Mediterranean Sea (MT, n=40), Red sea (RS, n=54), as revealed by Wavelet reconstruction. The rosterum (R), excisura major (E) and excisura minor (EM). The numbers 0, 90, 180 and 270 indicate angle in degrees (°) on the outline.

Reconstruction

The quality of the Wavelet and Fourier reconstructions can be estimated by comparing how they deviate from the otolith outline. To avoid the allometric growth effect on otolith shape, a normalization technique based on regression was applied; the Wavelet and Fourier coefficients were scaled with length. Those coefficients resulted in significant interaction between populations and length (P<0.05) were automatically removed from the analysis. Whereas, the remaining coefficients were directly imported into the statistical packages in the software R. The quality increases as expected with the number of Wavelet/Fourier coefficients used. In the present study, the Wavelet transform was more useful than Fourier transform (Fig.4).

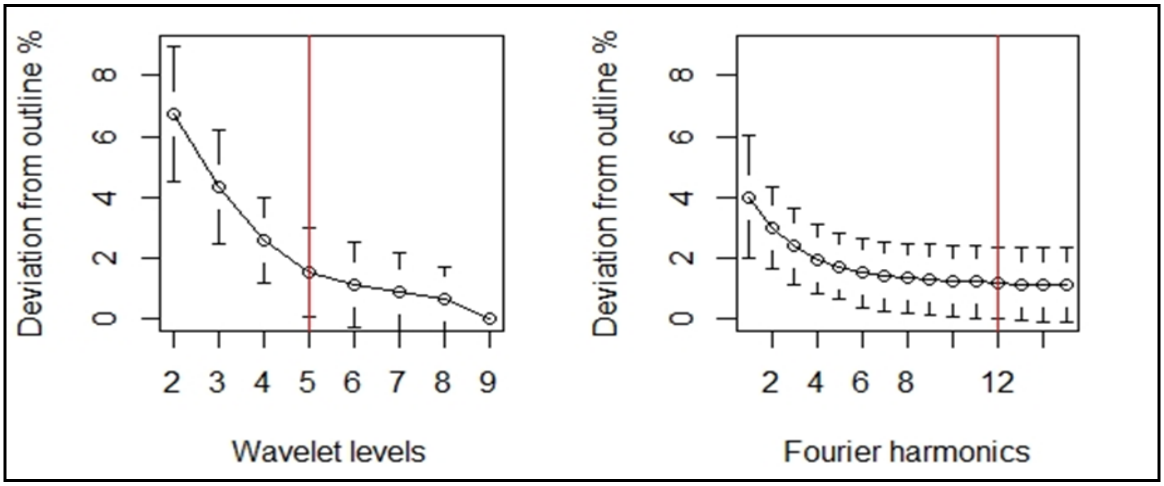

Fig. 4. Quality of Wavelet and Fourier outline reconstruction. The red vertical lines show the level of Wavelet and number of Fourier harmonics needed for a 98.5% accuracy of the reconstruction.

Differences of mean otolith shapes among populations based on Wavelet transform analysis

The CI plot (from the g plots package) was used for plotting the mean and standard deviation of the coefficients against the angle. The proportion of variation between groups and the intraclass correlation (ICC) provides more information about the partition of the variation along the outline. According to the patterns in Fig. (5), it is obvious that the majority of the variation between groups can be traced by two areas of the otolith, angles 140-170 and 200-220, corresponding to the rostrum and excura major respectively (see also Fig.3).

Multivariate analysis of otolith shape

The canonical analysis of principal co-ordinates (CAP), using the CAP scale function in the vegan package in shape R, was used for comparing Wavelet coefficients among populations. Ordination of the population averages along the first two canonical axes was examined graphically with shape descriptors of otoliths. Differences in otolith shape were observed among all populations (Fig. 6). The first discriminating axis showed 85.5% of the variation between populations, where otlith from MD highly deviate from otolith forms of RS and GS. While, the second axis showed 14.5%, where RS otolith form slightly deviated from GS otolith from. The results of CAP analyses using the Wavelet coefficients showed differences among *P.forsskali* populations that correlated with their geographical distances.

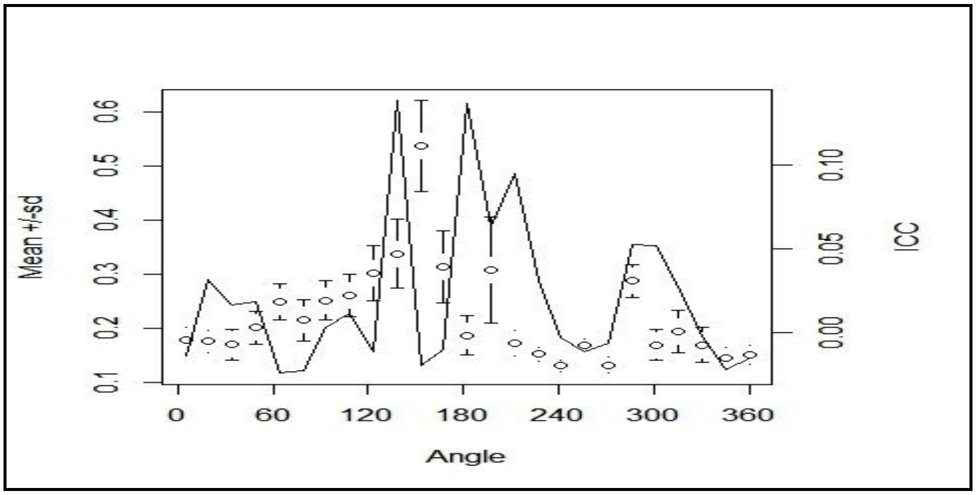

Fig. 5. Mean and standard deviation (sd) of the Wavelet coefficients for all combined otoliths and the proportion of variance of *P. forsskali* populations of the three sampling areas or the intraclass correlation (ICC, the solid line). The horizontal axis shows angle in degrees (°) based on polar coordinates.

Classification of individuals to their sampling origin

Cross-validation estimation was used for evaluating the classification of individuals to their sampling origin. Linear Discriminant Analysis (LDA) was applied on the standardized Wavelet coefficients to show classification of individuals to original populations with cross-validation estimation using the function of LDA in MASS packages. The overall classification success with a leave-one-out cross-validation estimation, based on all samples from the three populations, was 65%, and the highest classification success was recorded for Mediterranean sea (80%) (Table 3). The misclassification error based on the LDA analyses was 0.353.

A dendrogram of hierarchical cluster analysis was drawn using the values of the CAP1 and CAP2 analysis, in addition to using the Euclidean distance as a measure of dissimilarity. This dendrogram shows two main clusters; the Gulf of Suez and the Red Sea in one cluster, and the Mediterranean Sea in the second one (Fig. 7).

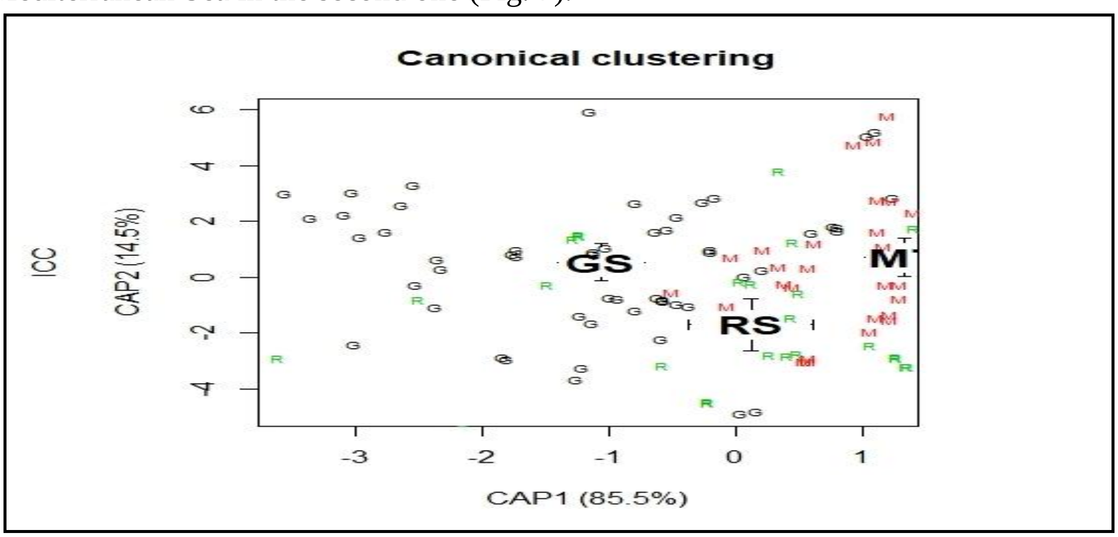

Fig. 6. Canonical scores on the first two discriminating axes CAP1 and CAP2 for the three *Parupeneus forsskali* populations using Canonical analysis of Principal Coordinates with the Wavelet coefficients.

Fig. 7. A cluster analysis showing relationships between *Parupeneus forsskali* populations based on otolith shape data from the three locations.

Table 3. Linear Discriminant Analysis (LDA) of the standardized Wavelet coefficients of *Parupeneus forsskali* populations . Rate of overall correct classification, 65% .

Predicted	**Observed**		
	Red Sea	Gulf of Suez	Mediteranean
Gulf of Suez	4	16	8
Mediteranean	1	2	13
Red Sea	19	0	0
	45%	74%	75%

DISCUSSION

The Red Sea goatfish, *P. forsskali* (Fourmanoir & Guézé, 1976) is an Indian Ocean species and is native to the Gulf of Aden and the Red Sea (**Randall, 2004**). It is a migrant species through Suez Canal, and it is now established in the eastern parts of the Mediterranean sea (**Çinar *et al.*, 2006; Bariche *et al*., 2013; Gurlek *et al.*, 2016; Mehanna *et al.*, 2016; Farrag *et al*., 2018; Osman *et al.*, 2020**).

Based on morphological charateristics, otolith shape analyses have become a valid tool for estimating the contribution of geographical differences caused by environmental effects for the same species.

All the performed analyses indicated significant differences among the studied *P.forsskali* populations. Both multivariate and univariate methods were used for testing the shape differences among populations. It is worth mentioning that, the univariate method is based on using radii length as a shape descriptor for comparing shape within populations. While, the multivariate method uses the scaled Wavelet coefficients for comparing shape among populations as separate stocks. Additionally, linear discriminant analysis and cluster analysis show that populations inhabiting geographically neighboring areas exhibit more similar otolith shape than those further apart.

Otolith shape analysis was used in population identification and differentiation of several marine fish species in the study of **Yu *et al.* (2014)** who addressed five gobiid species from the northern Chinese coastal seawaters. In addition, **Farias *et al.* (2009)** used the elliptic Fourier descriptor analysis and deduced that, there were significant variations in otolith shape between males and females for the *trichiurid Aphanopus carbo* from the Portuguese coast. Moreover, **Sadeghi *et al.* (2020**) detected significant differences among *I. ornatus* populations. While, **Ghanbarifardi *et al.* (2018)** revealed that, the populations of *Periophthalmus waltoni*, along the northern coasts of the Persian Gulf and Oman Sea, are divided into two well-separated clades, the Persian Gulf clade and the eastern clade. Furthermore, the study of **Ghanbarifardi *et al*. (2020),** examined the morphometric data of *Scartelaos tenuis* that revealed significant differences between the corresponding populations of the Persian Gulf and the Gulf of Oman. In addition, **Sadighzadeh *et al.* (2014)** showed that the otolith shape in *Lutjanus johnii* (Bloch, 1792**),** exhibited a geographically structured dissimilarity between the two gulfs. A similar output was reported in the study of **Teimori *et al.* (2018)** for the *Aphanius dispar* species group.

In conclusion, the observed dissimilarity in the otolith shape is sometimes related to environmental factors and genetic heterogeneity.Finally, the pattern of variation between stocks by using otolith shape is very easy and useful to get the differentiation between habitats.

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