Using the outline analysis of otolith to study stocks of the predominant species of genus *Acanthopagrus* (Sparidae family) in the Persian Gulf and Oman Sea

Doustdar M.[*1]; Seifali M.[2]; Hashemi S.A.[3]

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Abstract

The present study was conducted using geometric morphometric analysis of otolith to discriminate stocks of the predominant species of genus *Acanthopagrus* in the west and east of Strait of Hormuz. Samples were taken from 5 regions in the Persian Gulf and Oman Sea from March 2016 to March 2018. The 145-otolith samples were obtained from the Arabian yellowfin sea bream (*Acanthopagrus arabicus*). The results of this investigation confirmed the existence of two separate populations in the west and east of the Strait of Hormuz.

Keywords: Sparidae, *Acanthopagrus arabicus*, Fish stocks, Fourier analysis, Otolith, Persian Gulf, Oman Sea

1-Iranian Fisheries Science and Research Institute, Agricultural Research Education and Extension Organization, Tehran, Iran
2- Department of Plant Sciences, Faculty of Life Sciences, Alzahra University, Tehran, Iran
3-Offshore Fisheries Research Center, Iranian Fisheries Science and Research Institute, Agricultural Research Education and Extension Organization, Chabahar, Iran

[*]Corresponding author's Email: mastooreh.doustdar@gmail.com

Introduction

The Arabian yellowfin sea bream is found in Bahrain, India, Kuwait, Iran, Oman, Pakistan, Qatar, Saudi Arabia, UAE and Iraq. There is very little information available on this species (Iwatsuki, 2013). The Arabian yellowfin sea bream (*Acanthopagrus arabicus*) has 11 to 12 dorsal spines and 10 to 11 dorsal soft rays. The pelvic and anal fins and the lower caudal-fin lobe are yellow (FAO, 2014). Otoliths are among the most useful anatomical structures for studies that are used not only for ichthyology research but also for studies on nutrition, ecology, animal geography, etc. One of the important uses of otolith is the identification and classification of species, reserves and populations (Doustdar *et al*., 2019).

Recent studies on otolith shape include the study which has done on 4 species Caranjidae species in the Persian Gulf (Fashandi *et al*., 2019). Although ecological studies can reveal the effect of environmental factors on the otolith morphology of the Arabian yellowfin sea bream, other studies such as the genetics and the study of the trace-element distributions in fish otoliths can also clarify the stock structure of this species. As it was found in research, the basis for the growth of otolith is genetic (Swain *et al*., 2005). There is very little information available on this species and due to its characteristics; otolith can help in separating the stock of this species.

Materials and methods

This study was carried out by the R/V Ferdows-1 belongs to Iranian Fisheries Science Reaserch Institute in the Persian Gulf and Oman Sea. In this regard, Specimens of *A. arabicus* were caught from 125 random stations to investigate the otoliths of the specimens in five different regions in the Persian Gulf and Oman Sea (Fig. 1). The gills were cut from their bottom connection points and the otoliths were removed from the inside of the otic capsule. In this study, the left otoliths of all fish were separated. Then, the otoliths were washed and placed in glass containers after drying (Tuset *et al*., 2003).

Figure 1: Location of data collection areas in the Southern Waters (Persian Gulf and Oman Sea).

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For geometric morphometric analysis of otolith, their images were investigated using the Shape Software. At first, 500 points were placed on the perimeter of each otolith using the Shape Software and their x and y coordinates were stored. The outline of each otolith was then studied through the Elliptic Fourier analysis. In fact, this technique describes the otolith perimeter by several components called harmonics. Each harmonic consists of 4 coefficients. Photos taken from otoliths were converted to black and white images. With the use of chain coders, the outlines of the otolith images were extracted and the harmonics of each image were obtained. Using the Chc2Nef software program, Elliptical Fourier Descriptors (EFD) was calculated. Using the PRINCOMP procedure, the Principal Component Analysis (PCA) was performed. PAST 1.97 and SPSS 24 (Iwata and Ukai, 2002) analyzed data (Zare, 1999).

Results

The geometric morphometric of otolith was investigated using the outline analysis of otolith and the Shape Software. At first, 500 points were placed on the perimeter of each otolith using the Shape Software and their x and y coordinates were stored. The outline of each otolith was then studied through the Elliptic Fourier analysis. In fact, this technique describes the otolith perimeter by several components called harmonics.

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Figure 2: Otoliths of the Arabian yellowfin sea bream from 5 regions studied. (a, b, c: Persian Gulf . d , e: Oman Sea).

The samples of the Arabian yellowfin sea bream otolith were described by 20 harmonics, each of which consisted of 4 coefficients (a, b, c, and d) that was related to the x and y coordinates of the points determined and applied (Fig. 3).

The uniformity of variance was investigated using the Levene's test. The difference of data was obtained through Discriminant Function Analysis (DFA). The results of analysis performed otolith samples of the

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Arabian yellowfin sea bream showed that 6 components out of 77 main components indicated 90% of cumulative energy and difference. In fact, this number of components was sufficient to calculate and show the difference (Table 1).

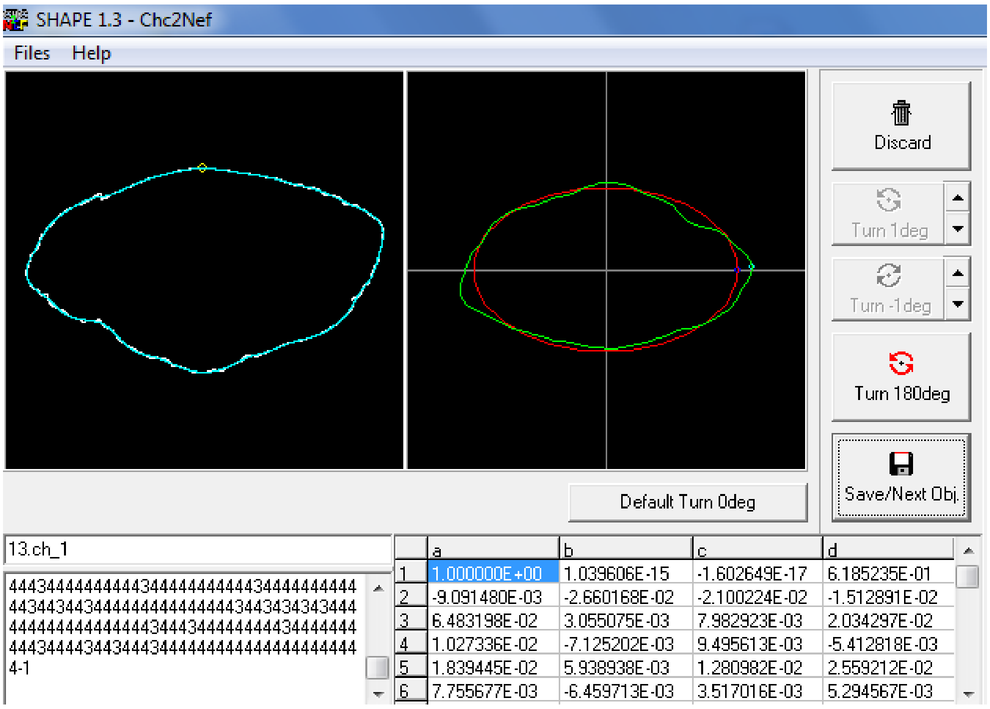

Figure 3: Image code and creation of the elliptical Fourier descriptors for the otoliths of the Arabian yellowfin sea bream.

Table 1: Cumulative percentage of variance of geometric morphometric coefficients of the Arabian yellowfin sea bream otoliths of components extracted from the PCA.

Extracted components	Certain number	Variance (%)	Cumulative percentage
PC1	5.018	74.215	74.215
PC2	4.044	5.981	80.196
PC3	3.163	4.677	84.874
PC4	1.790	2.648	87.522
PC5	1.034	1.530	89.052
PC6	9.339	1.381	90.434

In fact, based on the chain codes of each otolith in different regions, 77 elliptic Fourier descriptors were obtained per sample. The multivariable analysis of descriptors determined the minimum Fourier coefficients needed to reconstruct the average shape of each sample. In this case, six certain numbers were able to justify 90% of the variation in shape of otolith margin in the Arabian yellowfin sea bream. It was also observed that with the increase for number, the shape changes were reduced and the average shape of the otoliths was closer to the real shape. The reconstruction of the shape of the otolith margins was also performed based on the six main components in the image analysis software environment. For this purpose, first, the

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EFD coefficient was calculated and the score of each main component was defined with the mean plus or minus 2 times the standard deviation (Fig. 4).

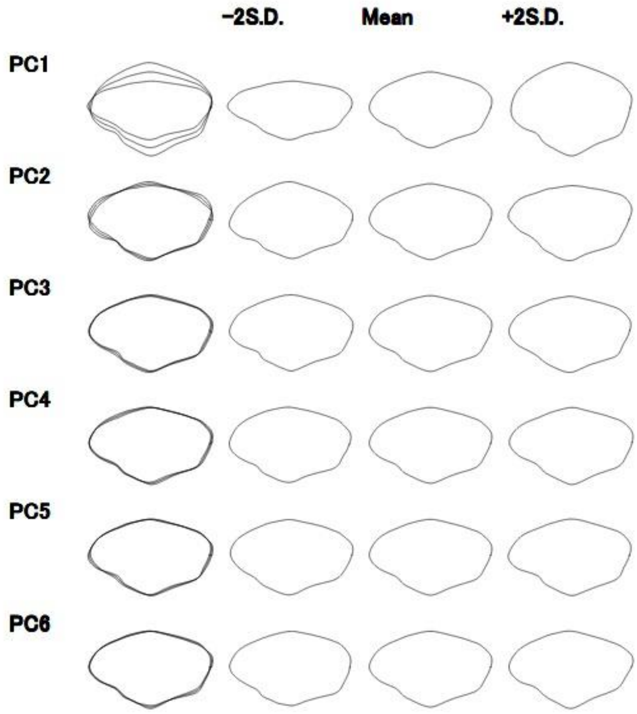

Figure 4: Reconstructed images based on the six main otolith components of the Arabian yellowfin sea bream.

According to the above figure, the shapes of otoliths in the 5 regions were divided into 6 categories. The most changes were observed in the dorsal and ventral regions of the otolith of the Arabian yellowfin sea bream (Table 2).

Table 2: Cumulative percentage of variance and certain number of 6 components extracted from PCA.

Main component	Certain number	Variance
1	0.00501829	82.065
2	0.00040448	6.614
3	0.00031631	5.172
4	0.00017906	2.928
5	0.00010345	1.691
6	9.33973000	1.527

The results indicated that 6 components showed shape changes in otoliths (Fig. 5).

In fact, the harmonic coefficients were normalized by the first harmonic and shape analysis of the Fourier coefficients revealed significant differences among the average shapes of the otoliths in the study area (5 regions). Comparison of the individual

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samples clearly showed the separation of regions in west and east of the Strait of Hormuz (p<0.05).

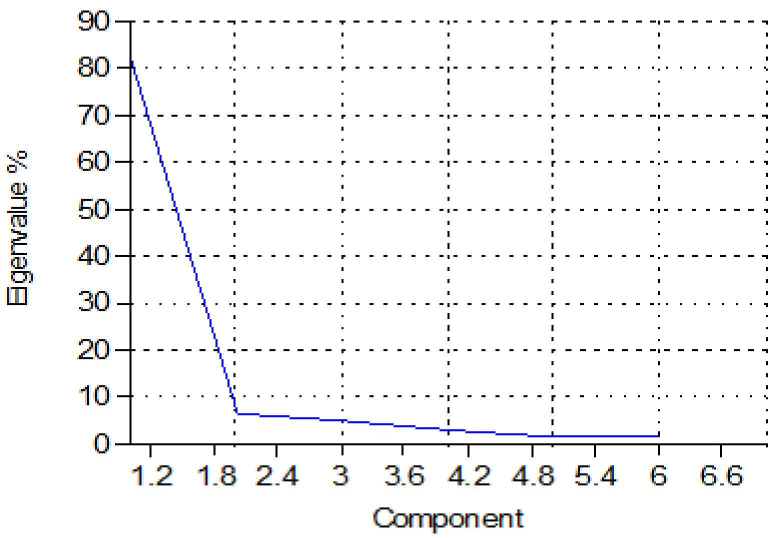

Figure 5: Main components and their total values.

The components of Canonical Discriminant Analysis **(**CDA) with the values of Wilks' Lambda showed variations in the otoliths in the 5 regions. Considering the value of Wilks' Lambda that ranges from 0 to 1and according to previous studies, when the value of Wilks' Lambda reaches zero, it indicates the separation of populations and when the value of Wilks' Lambda shows "1", it indicates that the groups have small differences. In the otoliths samples of Arabian yellowfin sea bream, the values of Wilks' Lambda of the first to fourth components were 0.237, 0.366, 0.538 and 0.746, respectively of which showed that the first and second components with the values of 0.237 and 0.366 were closer to zero and they showed more changes. The third component with the value of 0.538 indicated the middle level of separation and the fourth component with the value of 0.746 was closer to one and represented less separation (Table 3).

Table 3: Results of the Canonical Discriminant Analysis (CDA) with values of Wilks' lambda.

	Wilks' Lambda	Chi-square	df	Sig.
1	0.237	493. 672	308	0.000
2	0.366	344.774	228	0.000
3	0.538	212.693	150	0.001
4	0.746	100.593	74	0.022

The results of the Discriminant analysis of the Fourier coefficients showed that 70.4 % of the species were correctly located in their region. In fact, from the 77 Elliptical Fourier Descriptors for each region, the otoliths of the Arabian yellowfin sea bream were correctly attributed to individuals in their own

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region in the Persian Gulf and Oman Sea and in total with 77.9, 76.6, 1.70, 67.5, 59.7 and 70.4 % accuracy, respectively (Table 4).

Table 4: Estimated classification accuracy based on the Fourier coefficients of the Arabian yellowfin sea bream otoliths in the waters of the Persian Gulf and Oman Sea.

	Region	Region1	Region 2	Region3	Region 4	Region 5	Total
Number	Region 1	46	4	10	9	8	77
	Region 2	3	60	6	1	7	77
	Region 3	2	6	59	7	3	77
	Region 4	9	2	6	52	8	77
	Region 5	4	1	15	3	54	77
Percent	Region1	59.7	5.2	13.0	11.7	10.4	100.0
	Region 2	3.9	77.9	7.8	1.3	9.1	100.0
	Region3	2.6	7.8	76.6	9.1	3.9	100.0
	Region4	11.7	2.6	7.8	67.5	10.4	100.0
	Region5	5.2	1.3	19.5	3.9	70.1	100.0

Accuracy of classification (%): 70.4

There is a clear difference between the otoliths in the Persian Gulf and Oman Sea, because of the first and second discriminant functions in relation to the geometric morphometrics analysis of otoliths based on the analysis of the Fourier coefficients (Fig. 6).

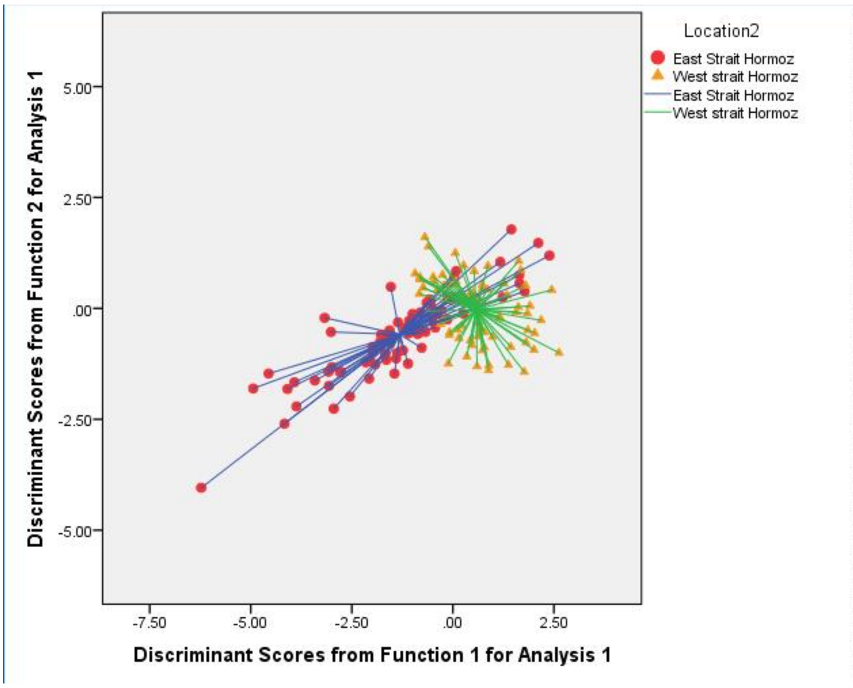

Figure 6: Discriminant Function Analysis (DFA) chart based on the Fourier coefficients of the Arabian yellowfin sea bream otoliths in the Persian Gulf and Oman Sea.

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In the study of the clustering analysis or clustering with the Fourier coefficients of the Arabian yellowfin sea bream otoliths, as it is shown in the figure, the otoliths of the Sistan and Baluchestan region in the east of the Strait of Hormuz and the otoliths of the Khuzestan in the west of the Strait of Hormuz were clearly classified into separate groups. The samples of Sistan and Baluchestan and the east of the Strait of Hormuz were located on the border of less than 5 which indicated the close proximity of samples of these regions. The samples of the east of the Strait of Hormuz and Bushehr were located on the border of less than 10 which indicated the proximity of these regions with each other. Also, the samples of the west of the Strait of Hormuz and Khuzestan were located on the border of less than 10 (Fig. 7).

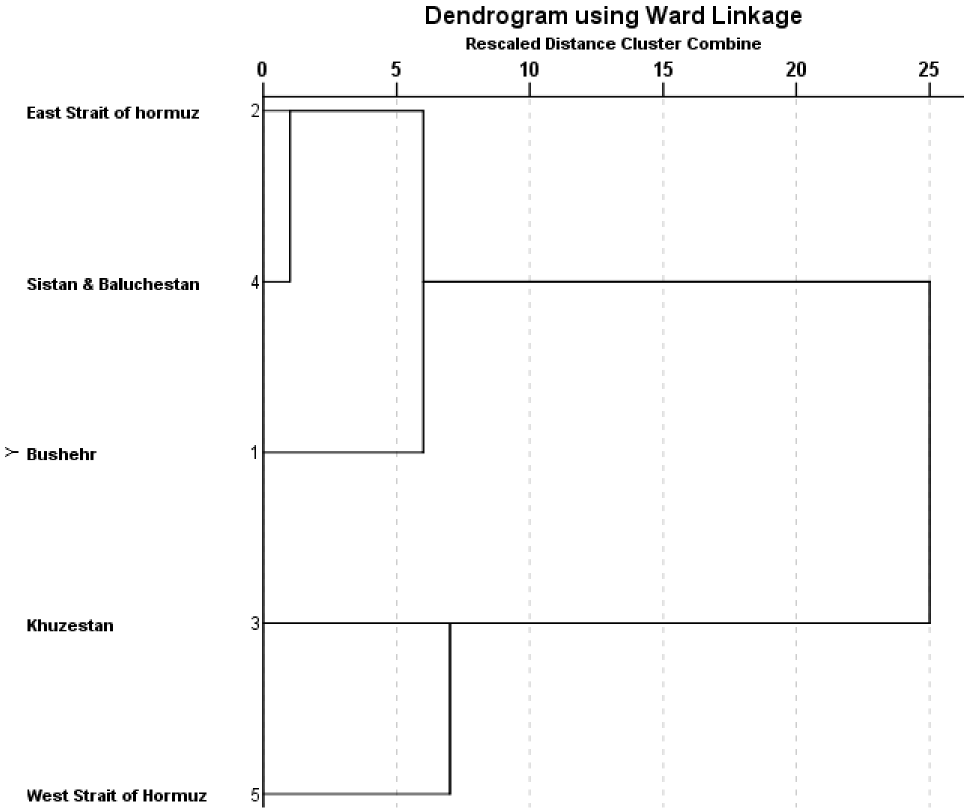

Figure 7: The clustering dendrogram of the Arabian yellowfin sea bream individuals based on the Fourier coefficients of otoliths.

Discussion

In many studies, otolith morphological characters are indicated as informative characters in the identification of fish species and can be used in the differentiation of the species (Sadighzadeh *et al.,* 2012). But recent study has pointed to its value as an indicator of stock identity. Shape analysis of otoliths has been used tool in stock identification studies of marine fish species and this method has never been used in sparidae family stock identification studies.

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Several studies on population structure of fish have focused on the characteristics of the otolith shape. Most studies have been conducted in a variety of ways such as the analysis of shape indicators, outline analysis, fast Fourier analysis, elliptic Fourier analysis and wavelet analysis with the aim of discriminating fish populations (Campana and Gagne, 1995; Anken, 1998). In the present study, it was determined that the otolith perimeter and shape indicators related to the otolith margin can be used as index parameters for distinguishing the Arabian yellowfin sea bream individuals in the Persian Gulf and Oman Sea in 5 regions in the Persian Gulf and Oman Sea. The elliptic Fourier analysis used in this study showed that it could be used as a powerful technique for identifying within-species differences and discriminating fish populations. By the method used in the present study, the Arabian yellowfin sea bream individuals were discriminated in 5 regions.

The Cumulative Distribution Function (CDF) of Fourier coefficients showed that 70.4% of the total population was properly classified in their respective region. According to (Frienland and Reddin, 1994), the percentage of classification above 75% is acceptable criterion for discriminating fish populations. Therefore, the classification accuracy estimated in this study was close to 75%, which indicated the high and acceptable level of discrimination of individuals in 5 regions located in the west and east of the Strait of Hormuz. In addition, individuals in the east of the Strait of Hormuz had better classification accuracy than individuals in the West of the Strait of Hormuz. In fact, despite all the differences among regions, it seems that the differences are due to the diversity of traits in large populations.

The results of the principal component analysis indicated that the six components included the most of the changes in otolith which in fact showed that in terms of shape changes, the otoliths of the regions were classified into six classifications in terms of shape and the most changes in otolith margin were observed in the dorsal and ventral regions of the otolith of the Arabian yellowfin sea bream. In the study by Christina Treinen in 2012 on population discrimination of *Haemulon plumierii*, the average variation in the shape of the otolith margin in different populations was observed in the ventral and Post rostrum regions of the otolith (Treinen-Crespo *et al.*, 2012). Although ecological studies can reveal the effect of environmental factors on the otolith morphology of the Arabian yellowfin sea bream, other studies such as the genetics and the study of the trace-element distributions in fish otoliths can also clarify the stock structure of this species. As it was found in research, the basis for the growth of otolith is genetic (Swain *et al.* 2005). Therefore, in similar environmental conditions like the same temperature and growth conditions, different species have different otoliths (Labee-Lund and Jensen, 1993). However, separate resources with similar genetic features show differences in the shape of otolith, which suggests the effects of environmental factors on them (Cardinale *et al.,* 2004). Some researchers believe that environmental factors have a significant effect on the formation of otoliths, especially in the various periods of ontogeny (Anken, 1998). The external shape of these structures varies in response to differences in growth rates at different stations (Smith, 1992; Campana and Casselman, 1993; Vignon and Morat, 2010). Biological factors such as diet and reproduction, or environmental factors such as temperature, salinity, and pH affect the growth of otolith (Campana and Gagne 1995; Tuset *et al.,* 2003).

Given that otolith is one of the hardest internal structures of the body, is less affected by the environmental factors and is more influenced by the genetic factors and the environmental factors only affect the formation of otolith during the periods of ontogeny, it can be stated that otolith shows the species differences in different populations more accurately than other things. The geometric morphometric analysis of otolith in numerous studies indicated that the shape of the otolith is less affected by aquatic nutrition and spawning and more affected by temperature in similar growth conditions where otoliths can be observed in various shapes (Vieira *et al*., 2014). In some cases, living conditions are uniform and far from environmental stress which can have some effect on the shape of otolith. The present study showed that the otoliths in the east of the Strait of Hormuz had the higher height and more growth than the otoliths in the west of the Strait of Hormuz. Of course, this theory should be evaluated on the larger number of existing species and their differences should be investigated. The results of the present study showed the diversity and power of morphological adaptability of different Arabian yellowfin sea bream individuals and indicated that the morphological characteristics of otolith can discriminate different individuals of this species in its habitat. The results of this study will benefit the fisheries management of this species by giving new contributions to the field.

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