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Stock identification of Arabian yellow fin sea bream (*Acanthopagrus arabicus)* using shape of otolith in the Northern Persian Gulf and Oman Sea

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Abstract

Otolith shape analysis is one way to identify stocks of different fish species in the marine environment. Length, width, area, perimeter, form factor, aspect ratio, roundness, circularity, ellipticity and rectangularity analyses of otoliths were undertaken to assess patterns of spatial and temporal stock structure of a wide-ranging fish, the Arabian yellow fin sea bream *Acanthopagrus arabicus*. Fish were sampled from 125 stations across the distribution range of the species in the Northern Persian Gulf and Oman Sea from June 2014 to May 2016. Analysis of morphometric parameters of otolith showed the minimum size in Khuzestan waters and the West Strait of Hormuz and the maximum size in the East Strait of Hormuz. In Bushehr waters, form factor showed the lowest and circular factor showed the highest frequency. These findings are in agreement with the irregularity in otolith margin of this area. The highest aspect ratio of otolith indicated higher growth in Khuzestan Waters. The thinner and longer otoliths were identified in the East Strait of Hormuz region. Further, rectangularity ratio factor in the Bushehr and West Strait of Hormuz waters was higher in comparison with other regions and this finding confirmed the quadrilateral otolith shape of this region. There are significant differences among otolith morphometric variables of the *A. arabicus* ($p<0.05$). The result of discriminant analysis on morphometric parameters indicated that 53/8 percent were in their geographic location accurately.

Keywords: Shape otolith indices, Stock identification, *Acanthopagrus arabicus*, Northern Persian Gulf and Oman Sea

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Introduction

Sparidae fishes are coastal water species inhabiting tropical and temperate waters throughout the world. To date 117 species belonging to 15 genera have been ascribed to this family (Chiba *et al*., 2009; Iwatsuki and Heemstra, 2011).They usually live in schools and migrate seasonally. These species move slowly from low depths to deeper waters and vice versa (Kuronuma and Abe, 1972; Al-Yamani, 2004). Otoliths are small calcified structures found in the heads of fish, which assist in detecting sound and are used for balance and orientation (Campana and Neilson, 1985). Morphological otolith shapes of different species are specific and further the size and shape of otoliths are used as one of the assured ways for stocks identification. Surveying the shape of otoliths hasve been done by some ichthyologists. Among them assessing biodiversity of Cod fish is remarkable (Campana, 2004). Differences in geographic distribution of *Sebastes marinus* and *Sebtes mentella* in the north of the Atlantic Ocean was studied based on the analysis of otolith shape (Stransky and MacLellan, 2005). Distinguishing the Norwegian coastal cod from the north polar cod was done by otolith shape investigation (Stransky, 2005). In Atlantic herring, examination of otoliths shape was done as a new method in monitoring the populations of this species (Burke *et al.*, 2008).

Surveying the stock structure of *Diplodus annularis* of Sparidae family in Tunisia was done using otolith shape analysis (Trojjet, 2015).

In recent years geometric morphometrics of otolith was used for recognizing species. Additionally, identification of different stocks of one species is also possible through the mentioned method.

In one research that investigated the otolith morphology of some of the commercial pelagic fishes in the Persian Gulf belonging to the Scombridae family, researchers illustrated that otolith of pelagic fishes are small and thick of medium length. Otolith morphology was also used in and another research to distinguish populations of *Lutjanus johni* species in the Persian Gulf and Oman Sea. Significant differentiation in the shape of the mentioned species were obtained in the areas. According to these results, it can be said that this species has two different stocks (Sadighzade and Tuset, 2012). Comparisons of otolith shape and their morphology in 3 species of Gobiidae in the southern basin of the Caspian Sea in 2013 proved that there were some differences among otolith shapes of each species (Bani *et al.*, 2013). In another research, special characteristics of sagittal otoliths between 13 species of Sparidae were described and morphological parameters of sagittal otolith such as length, width, height and weight were assessed by discriminant

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analysis and results showed obvious differences (Kinacigil *et al*., 2000).

Due to their specific characteristic like size, morphology and compound structure otoliths are helpful for investigation of species, stocks and populations and the knowledge of stock structure of a species is essential for effective stock assessment and fisheries management (Kinacigil *et al*., 2000).

This study was done to identify different stocks of the Arabian yellow fin sea bream *A. arabicus*, the dominant species of *Acanthopagrus* genus in the Northern Persian Gulf and Oman Sea.

Materials and methods

This study was done in the Persian Gulf and Oman Sea from June 2014 through May 2016.

A total of 145 *A. arabicus* were collected from the west to the east from 5 different regions of Khuzestan, Bushehr, West and East Strait of Hormuz and Sistan and Baluchistan by the Research Vessel Stern Trawler of IFSRI in the Northern Persian Gulf and Oman Sea (Fig. 1).

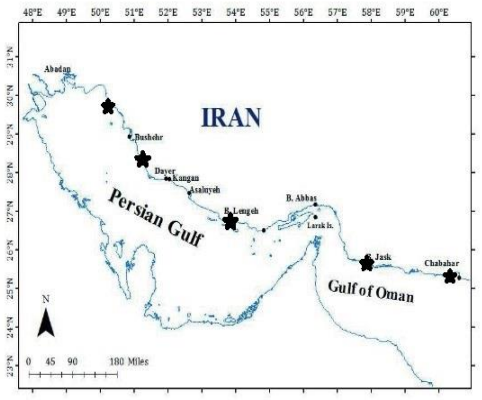

Figure 1: Map of the study area and sampling regions.

Collected species from 125 random stations were preserved in ice boxes and transferred to the laboratory for further biological measurements and otolith investigations. Identification and separation of genus and species of samples were done by using FAO keys (Fig. 2). Standard length of each specimen was measured to the nearest mm (Carpenter *et al*., 1997; Iwatsuki, 2013).

Figure 2: *Acanthopagrus arabicus.*

Moreover, *A. arabicus* species were separated from the total samples for otolith removal.

The same otolith from each individual was used for otolith shape analysis. Left otoliths were used wherever possible to avoid potential confounding effects of differences between left and right otoliths. Indeed, otoliths were removed from the left side of the head by the vertical cut method. They were cleaned, air dried and stored in glass vials for later identification. Photographs of otoliths were taken using Dino- lite software in a dark background (Fig. 3).

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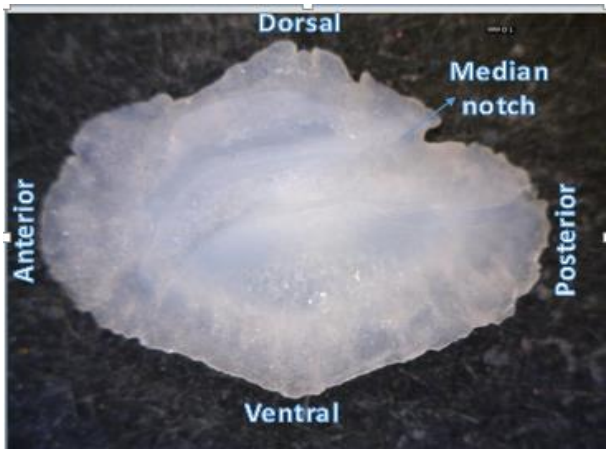

Figure 3: Sagittal otolith of *Acanthopagrus arabicus* (Iwatsuki, 2013).

Morphometric parameters including: otolith length, otolith width, area, and perimeter were measured using Digimizer Software to calculate shape indices (Table 1). Form factor is a parameter to estimate irregularity of surface area. Aspect ratio is the ratio of otolith length to otolith width. Roundness and circularity gives information on the similarity of various features with regard to a perfect circle, and ellipticity indicates whether the changes in the axes are proportional. Rectangularity describes the variations in length and width with respect to the area (Tuset *et al.*, 2003).

Table 1: Morphometric parameters and shape indices with formula.

Form-Factor (FF) = $(4\pi A)/P^2$
Aspect Ratio (AR) = OL/OW
Roundness (RD) = $(4A)/(\pi OL^2)$
Circularity (C) = P^2/A
Ellipticity (E) = (OL-OW)/(OL+OW)
Rectangularity (R) = A/(OL*OW)

Fish measurements (standard length) were examined for normality and homogeneity of variances with parametric analysis. The One way ANOVA test was used to compare fish measurements among different regions of the Northern Persian Gulf and Oman Sea. Duncan test was subsequently applied for comparison of the standard length of this species. Statistical analysis was conducted using SPSS 24. Differences of $p<0.05$ were considered statistically significant. To minimize differences in otolith size as a consequence of variation in fish growth rate, all measurements were normalized to a standard body size. In this study the individual matrices were used and a quadratic discriminant function was calculated. Classification of efficiency-percent was classified correctly and estimates were cross-validated according to the methods (Zar, 1999).

Results

The results revealed the differences in standard length among the five regions in the Persian Gulf and Oman Sea.

The minimum standard length of this species was reported in the Khuzestan waters, while the maximum standard length was observed in the East Strait of Hormuz. Analysis of standard length by Duncan's test showed significant differences between the West and East Strait of Hormuz ($p<0.05$) (Table 2).

Table 2: Standard length (mean±S.E) of *Acanthopagrus arabicus* in the Persian Gulf and Oman Sea.

Morphometric Variables	Khuzestan	Bushehr	West Strait of Hormuz	East Strait of Hormuz	Sistan and Baluchistan	F	df	*p*
S.L(mm)	137.46±2.91	187.54±3.61	149.35±6.52	211.105±7.72	204.61±4.86	41.80	4	<0.05

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This species was categorized into 3 groups according to standard length in the following order: the samples of Khuzestan and West Strait of Hormuz, Bushehr waters, and finally one group in the East Strait of Hormuz and Sistan and Baluchistan Waters (Table 3). These analyses showed significant differences between the West and East Strait of Hormuz regions (Table 3).

Table 3: Comparison (Duncan test) of the morphometric variables (S.L) of *Acanthopagrus arabicus* between the 5 regions in the Persian Gulf and Oman Sea.

			Standard length		
Test	**Region**	**Number**	**1**	**2**	**3**
Duncan [a,b]	Khuzestan	43	137.4651		
	Bushehr	31		187.5484	
	West Strait of Hormuz	34	149.3529		
	East Strait of Hormuz	19			211.1053
	Sistan and Baluchistan	18			204.6111
	Total Number	145	*p*= 0.117	*p*=1.00	*p*=0.391

a. Uses Harmonic Mean Sample Size = 25.892.
b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

There were significant differences among otolith morphometric variables of *A. arabicus*. Investigating morphometric parameters of otolith showed that all the measured parameters such as: length, width, area, and perimeter in the Northern Persian Gulf had the lowest ratio in Khuzestan and in the west Strait of Hormuz waters. The maximum otolith length belonged to the East Strait of Hormuz (Table 4).

Table 4: Morphometric variables (mean±S.E.) of the otoliths of *Acanthopagrus arabicus.*

Morphometric Variables	Khuzestan	Bushehr	West Strait of Hormuz	East Strait of Hormuz	Sistan and Baluchistan	*p*
Area (mm^2)	16.001±0.527	23.817±0.676	17.342±1.024	27.160±1.588	23.728±0.865	<0.05
Length (mm)	5.9351±0.108	7.792±0.179	6.221±0.195	8.175±0.258	7.775±0.138	<0.05
Width (mm)	3.958±0.061	4.743±0.235	3.989±0.115	4.785±0.131	4.467±0.0852	<0.05
Perimeter (mm)	17.728±0.307	22.811±0.458	18.660±0.587	23.828±0.711	21.745±0.423	<0.05

Analysis of shape factors in otoliths of *A. arabicus* species showed that the amount of aspect ratio in otoliths of the East Strait of Hormuz and Sistan and Baluchistan Waters were quite near.

The minimum aspect ratio of these otoliths belonged to Khuzestan and the maximum belonged to Sistan and Baluchistan waters. Moreover, aspect ratio factor of otoliths showed differences between West and East

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Strait of Hormuz in the Northern Persian Gulf and Oman Sea. Investigating shape factors of these otoliths revealed that Ellipticity ratio was maximum in Sistan and Baluchistan and minimum in Khuzestan Waters. Furthermore, form factor and roundness parameters were maximum in otoliths of Khuzestan and Sistan and Baluchistan waters. Circularity factor was maximum in otoliths of Bushehr. The shape indices are considerably different among the 5 regions (Table 5).

Table 5: Otolith shape indices (mean±S.E) of *Acanthopagrus arabicus*

Shape indices	Khuzestan	Bushehr	West Strait of Hormuz	East Strait of Hormuz	Sistan and Baluchistan	*p*
Aspect ratio	1.50±0.012	1.68±0.029	1.56±0.016	1.71±0.028	1.74±0.017	<0.05
Circularity	19.90±0.19	22.23±0.92	20.68±0.36	21.29±0.30	20.06±0.27	<0.05
Ellipticity	0.20±0.004	0.25±0.009	0.22±0.005	0.26±0.007	0.27±0.004	<0.05
Form factor	0.63±0.006	0.58±0.013	0.61±0.010	0.59±0.008	0.63±0.008	<0.05
Rectangularity	0.67±0.002	0.67±0.017	0.68±0.002	0.68±0.005	0.68±0.002	<0.05
Roundness	0.63±0.004	0.60±0.009	0.61±0.010	0.59±0.008	0.63±0.008	<0.05

Results of discriminant analysis on morphometric parameters (length, width, perimeter and Area) of otolith showed that 53.8 percent of *A. arabicus* species were in their accurate places in the sampled regions. Accordingly, 77.8 percent of Sistan and Baluchistan specimens belonged to their original place and had the highest level in comparison with other regions and on the contrary originality of Bushehr species at 45.2 percent was in the lowest level. Overlapping was seen between Bushehr region and East Strait of Hormuz and also to some extent between Khuzestan and West Strait of Hormuz region (Table 6).

Table 6: Results of quadratic discriminant functions on morphometric parameters (length, width, perimeter, and area) to classify *Acanthopagrus arabicus* in the Persian Gulf and Oman Sea.

Region/Number and percentage	Khuzestan	Bushehr	West Strait of Hormuz	East Strait of Hormuz	Sistan and Baluchistan	Total
Khuzestan	25	5	9	1	3	43
Bushehr	1	14	3	7	6	31
West Strait of Hormuz	6	3	16	8	1	34
East Strait of Hormuz	0	4	1	9	4	18
Sistan and Baluchistan	0	1	1	2	14	18
Khuzestan	**58.1**	11.6	20.9	2.3	7.0	100.0
Bushehr	3.2	**45.2**	9.7	22.6	19.4	100.0
West Strait of Hormuz	17.6	8.8	**47.1**	23.5	2.9	100.0
East Strait of Hormuz	0	21.1	10.5	**47.7**	21.1	100.0
Sistan and Baluchistan	0	5.6	5.6	11.1	**77.8**	100.0

Correct classification: **53.8** of originally grouped cases correctly classified

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Discussion

The three measurement analysis methods including: standard length, morphometric parameters and shape factors of otoliths were used for the stock identification of *A. arabicus* in the Northern Persian Gulf and Oman Sea.

Geometric morphometrics is a relatively new tool to fisheries research enabling researchers to categorize fishes to individual stocks cheaply and quickly. Indeed, this tool can be quite practical and effective in identification based on variations in otolith form, most commonly size and surveying the surroundings and numbers of otolith tooth.

According to the results of stock identification of *Lutjanus johni* in the Persian Gulf and Oman Sea, significant differentiation in shape of the mentioned species in the regions was observed and it was considered to be two different stocks (Sadighzadeh and Tuset, 2012).

In another research, special characteristics of sagittal otoliths between 13 species of Sparidae were described and morphological parameters of sagittal otolith such as length, width and weight were assessed by discriminant analysis and results showed obvious differences (Kinaciigil *et al.*, 2000).

Few studies have hitherto focused on stock discrimination and on the fisheries management. The goal of one research was to evaluate the stock structure of *D. annularis* of Sparidae family for two Tunisian insular populations based on the otolith shape, using different statistical approaches. This asymmetry indicated that the two populations belong to different fish stocks. The comparison of the otolith morphology between the two populations showed a clear difference in shape, and a left-right asymmetry of otolith (Trojette *et al.*, 2015).

Studies of numerical taxonomy are more complex, but they give more objective information. The shape indices allow detection of the more significant changes that occur in the otolith of this species. In this study differences were observed in almost all shape indices. Among shape indices, aspect ratio, circularity, and form factor were more efficient than other factors for distinguishing different stocks. The numeric values of the form factor showed that the sagittal shape of *A. arabicus* was geometrically irregular in 5 regions in the Northern Persian Gulf and Oman Sea and the lowest value of form factor of otolith in Bushehr waters demonstrated the most irregular shape in perimeter among the five regions.

The otolith shape of *A. arabicus* in Bushehr waters was more circular indicating equal growth in length and width. Moreover, they had the maximum irregularity in border which is in agreement with the previous theory.

Otoliths of *A. arabicus* mainly grew in length and thus they had the greatest aspect ratio and ellipticity in Sistan and Baluchistan waters. Therefore, otoliths of this region were lengthier and otoliths in Khuzestan with the lowest value of aspect ratio were thinner and these analyses showed significant

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differences in the West with East Strait of Hormuz regions.

Moreover, shape analysis of otolith indicated that the ellipticity factor was more in the West Strait of Hurmuz than in other regions and this finding confirmed that otolith shape of this region is more likely to be elliptic.

The discriminant analysis on otolith morphometric parameters of *A. arabicus* demonstrated that 53.8 of all individuals were correctly classified based on otolith shape in 5 regions of the Northern Persian Gulf and Oman Sea, The same analysis on *Ponticola batyybius* otoliths of Gobiidae family showed 66.7 of all morphometric parameters were correctly classified based on otolith shape in 3 regions such as Miankaleh, Bandar Anzali and Salmanshahr in the Caspian Sea (Tajbakhsh, 2016).

The accuracy percentage for classification of stocks of one species were reported differently in various sources. They were 39% to 58% (Stransky, 2005), 0 to 44 % (Jonsdottir *et al*., 2006) and 18.1 to 44.5% for lower accuracy (Merigot *et al.,* 2007), 63.8 to 68.8 for average accuracy (Tuset *et al.,* 2003) and above 75% for higher accuracy (Friedland and Reddin, 1994).

According to the mentioned explanation, this research with accuracy percentage of 53.8 can be considered near average and this finding obtained by the discriminant analysis, confirmed the normal rate of classification success.

In the present study, otolith investigation of *A. arabicus* revealed that otolith is the accurate factor for identification and distinguishing stocks of this species in five sampling regions in the Northern of Persian Gulf and Oman Sea. The results based on the different analysis of fish standard length, morphometric parameters (length, width, perimeter, area) and discriminant analysis on the mentioned parameters and also shape indices (form factor, aspect ratio, roundness, circularity, ellipticity and rectangularity) showed that otoliths of the East Strait of Hormuz and Sistan and Baluchistan regions are larger and show growth to a greater extent compared to samples of the Persian Gulf. It was attested that *A. arabicus* species has different shaped otoliths in the five regions and changes of otolith shape correlated with geographical regions. It can therefore be concluded that the probable different stocks in the sampled regions are caused by geographical distances.

The observed variations in sagittal shape among the studied species showed that the shape of the otolith had species-specific characteristics. Therefore, differences in otolith shape are attributable to the differences in ecology of the species (Lombarte, 1992; Aguirre and Lombarte, 1999; Paxton, 2000; Volpedo and Echevarria, 2003; Reichenbacher *et al*., 2007).

However, only further investigations on the ecology of this species can elucidate the possible effect of environmental factors on otolith morphology of *A. arabicus.* Other factors such as sound production and hearing ability have been demonstrated

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to cause such differences in the otolith morphology (Cruz and Lombarte, 2004). However other studies such as genetic and otolith microchemistry should be implemented to clarify the stock structure of *A. arabicus*.

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