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Research Article

Stock identification of the Indian halibut, *Psettodes erumei* (Bloch & Schneider, 1801) (Pisces: Psettodidae) using otolith shape in small-scale fisheries

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Abstract

The otolith shape of the Indian halibut, *Psettodes erumei*, from the Persian Gulf, Hormuz Strait and the Oman Sea were studied to discriminate the fish populations in small-scale fisheries. Indian halibut is a commercially valuable flatfish species abundantly caught in the north of the Persian Gulf and Oman Sea. Identifying different stocks is one of the main issues for fishery management programs. In this study, four otolith measurements (surface area, perimeter, length, and width) and five shape indices (from factor, roundness, circularity, rectangularity, and ellipticity) were recorded. The morphological analyses showed a significant asymmetry between the eyed and blind sides of otoliths. The otolith shape was described by shape indices and then, compared using a canonical discriminant analysis (CDA). The shape indices did not display any significantly different mean values among areas. Also, the patterns derived from CDA did not show any separation among populations. The absence of a disparity in the otolith shape indices could be linked to the similar condition of environment and nutrition in the regions and to the ecological behavior of the species. The results of this study may improve the accuracy of decision-making in the fisheries monitoring and management of *P. erumei* in the Persian Gulf and Oman Sea.

Keywords: Stock identification, Indian halibut, Otolith morphometric, Shape indices, Oman Sea, Persian Gulf

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Introduction

Flatfishes constitute a commercially important fishery resource of the Persian Gulf and the Oman Sea because of their abundance and market quality. They are mainly caught with bottom trawl and gill nets (Hensley, 1997). In recent years, the commercial catch of flatfishes in the north of the Persian Gulf and the Oman Sea has fluctuated between 2149 and 4512 tones according to FAO figures from 1997 to 2017 (http://www.fao.org). In particular, the Indian halibut, *Psettodes erumei* (Bloch and Schneider, 1801) —the single species belonging to Psettodidae family that exists in the Persian Gulf and the Oman Sea (Hensley, 1997; Yasemi *et al*., 2008) — is a commercial valuable flatfish species that is abundantly caught in the north of the Persian Gulf and the Oman Sea and composes a high percentage of the commercial shrimp fishery catch (Valinassab *et al*., 2006; Eighani and Paighambari, 2014). In addition, the total biomass of this species was estimated at 174 tonnes in 2017 (Behzadi *et al*., 2024). This species occurs in the Indian Ocean and the southwestern part of the Pacific Ocean, from the Red Sea and eastern Africa to the coasts of Papua New Guinea and Australia (Nelson, 2006).

Generally, the fishing activity in the northern Persian Gulf and the Oman Sea plays an important role in subsistence and trade practices. However, the management and assessment of resources are complex because of unreliable statistics. For instance, official catch data for the Indian halibut in the north of the Persian Gulf and the Oman Sea are not trustworthy because other flatfishes are often recorded as halibut. To our knowledge, there is no real fishery management strategy for the Indian halibut in the region. Successful management of fishery resources depends on knowledge of population structure, as each stock must be managed separately to optimize its yield (Carvalho and Hauser, 1994). Significantly, utilization of the same management for the separated stock is very difficult and fails in many cases, especially when the stocks differ in productivity, which may lead to suboptimal exploitation and ultimately overfishing of some stock components (Begg *et al*., 1999; Heath *et al*., 2013). To avoid overexploitation and mismanagement of the Indian halibut fishery resources, stock identification is necessary for sustainable fisheries in the region.

In recent years, numerous methods have been used for stock identification (Cadrin and Friedland, 2005; Cadrin *et al*., 2014). Some of these include otolith morphology and/or shape analyses (Friedland and Reddin, 1994; Begg and Brown, 2000) which have recently been developed and gained interest among fisheries biologists due to the easy access to the otolith samples (Hüssy *et al*., 2016). Otolith shape is affected by both genetic and environmental factors (Cardinale *et al*., 2004; Vignon and Morat, 2010). So, shape analysis of otolith could be considered a strong tool for discriminating fish stock and consequently for fish stock management purposes (Cardinale *et al*., 2004; Pothin *et al*., 2006; Tracey *et al*., 2006).

So far, a few studies on otolith morphology have been conducted on the Indian halibut from the Persian Gulf and the Oman Sea (Ghanbarzadeh *et al*., 2020). Further research in this area is needed to better understand the stock identification of this commercially important species. Therefore, this study aimed to assess possible differences in the otolith shape of the Indian halibut from the north of the Persian Gulf, Hormuz Strait, and Oman Sea using shape indices to differentiate the stock structure of this species.

Materials and methods

Samples of the Indian halibut were collected from September 2017 to November 2017 from the commercial catches of the local fisheries, which mainly use bottom trawls at depths of 20–50 m in the coastal waters of the Persian Gulf and the Oman Sea (Fig. 1). A total of 94 Indian halibut (n=18 at the Persian Gulf, n=56 at the Hormuz Strait, and n=20 at the Oman Sea) were sampled.

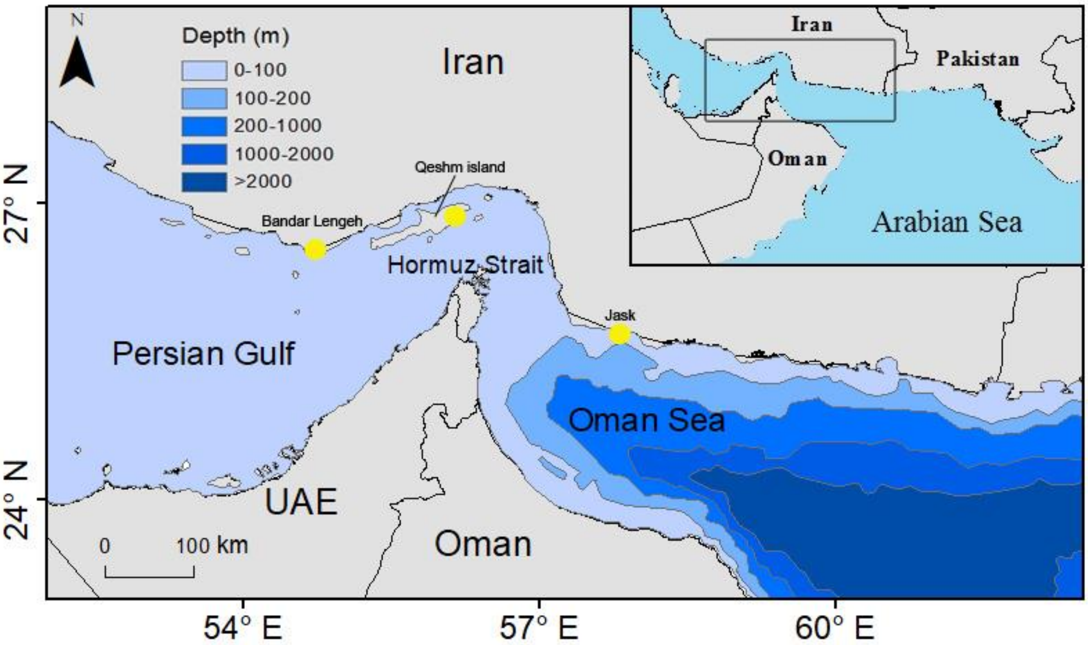

Figure 1: Sampling locations (in yellow) of *Psettodes erumei* along the Iranian coast.

In the first step, dextral or sinistral status was recorded for each specimen. Then, total length (TL), standard length (SL), and body depth (BD, the vertical distance from the dorsal to the ventral margin of the body measured at the base of the pectoral fin where it attaches to the body) were measured to the nearest 0.1 cm and total weight (TW) to the nearest 0.1 g. The sagittae of all individuals were extracted, cleaned of membranes and tissues with 96% C_2H_6O and kept dry in Eppendorf tubes. Both intact eyed-side and blind-side sagittae were used. Prior to imaging, otolith pairs were weighed (W_o) to the nearest 0.001 g. Then, each of the otolith pairs was systematically placed with the sulcus acusticus oriented through the observer and digital images were taken at a magnification of 1x with a stereomicroscope equipped with a digital camera. Measurements of each otolith were extracted and calculated using Image analysis software (Motic Image plus 3.0) on the otolith images, which calculated otolith morphometric parameters including the surface area (A_o), perimeter (P_o), length (maximum measure, L_o) and width (maximum measure, l_o) to the nearest 10^{-2} mm (Fig. 2). These otolith measurements were used to calculate five shape indices (Tuset *et al*., 2003): form factor (F), roundness (Ro), circularity (C), rectangularity (Rt) and ellipticity (E) (Table 1).

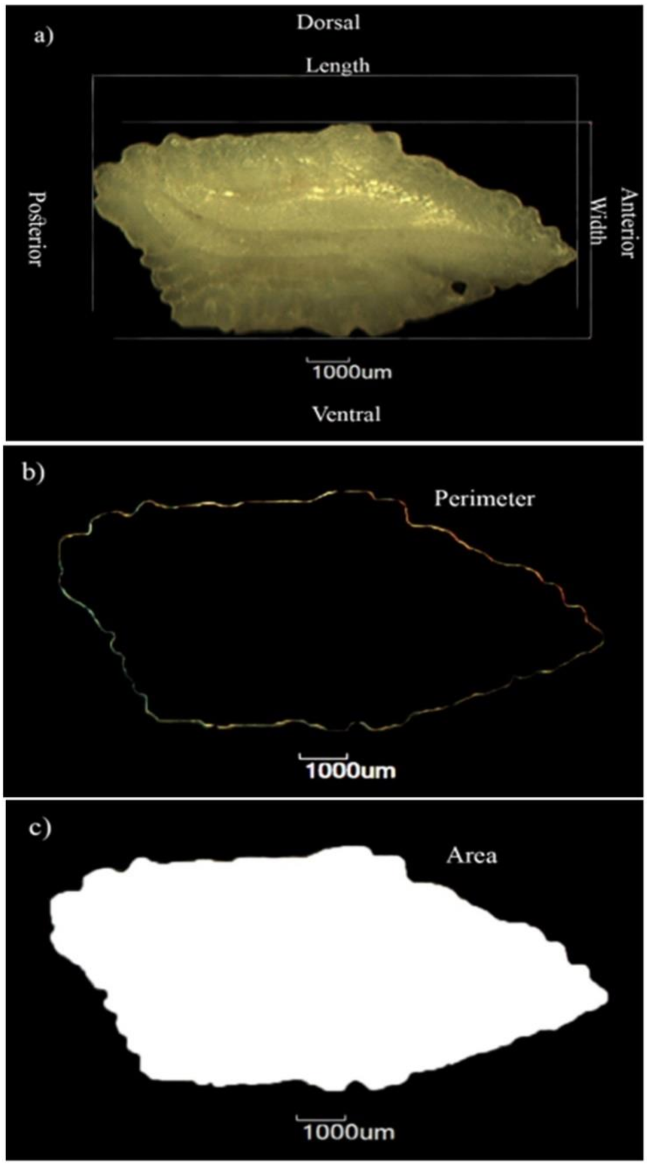

Figure 2: Proximal side of an otolith from *Psettodes erumei* showing (a) otolith length (L_o) and width(l_o), (b) perimeter (P_o) and (c) area (A_o).

Table 1: Shape indices calculated for otoliths of Indian halibut, A_o=otolith surface area, P_o=otolith perimeter, L_o=otolith length and l_o=otolith width (Tuset *et al*., 2003).

Shape index	Formulae	Application
Form factor (F)	$(4\pi A_o)P_o^{-2}$	Estimates the surface area irregularity of otolith (where 1=perfect circle, and <1 irregular)
Roundness (Ro)	$(4A_o)(\pi L_o^2)^{-1}$	Give information on the similarity of different features to a perfect circle (minimum value=1)
Circularity (C)	$P_o^2 A_o^{-1}$	Give information on the similarity of different features to a perfect circle (minimum value=4π)
Rectangularity (Rt)	$A_o(L_o \times l_o)^{-1}$	Illustrate the variations of length and width with respect to the area (where 1=a perfect square)
Ellipticity (E)	$(L_o - l_o)\,(L_o + l_o)^{-1}$	Ellipticity shows if the changes in the axes are proportional

*Units are mm^2 for A_o, and mm for P_o, L_o and l_o.

Statistical analyses were performed in R (Version 3.3.3). Prior to the analysis, all the fish morphometric variables, otolith variables, and shape indices were examined for normality and homoscedasticity using a Kolmogorov-Smirnov test and Levene test, respectively. Results revealed that all the fish morphometric and otolith variables and shape indices followed a normal distribution and showed homogeneity of the variance ($p>0.05$). Statistical differences in fish body size and weight among areas were tested by one-way analysis of variance (ANOVA).

Analysis of covariance (ANCOVA) (with fish size as a covariate and area as a fixed factor) was used to test the effect of fish size on each individual otolith shape index among the Indian halibut from different areas (Zar, 1999). If any significant interaction to be identified between shape index and fish size × area ($p<0.05$), it means that the slope of the fish size/shape index relationship is not consistent between areas. Shape indices that are significantly correlated with fish size should be adjusted using residuals of the common-within group slope (b) (Tracey *et al*., 2006; Burke *et al*., 2008) derived from the relationship between fish size and indices' values. This adjustment successfully removes the significant correlation with fish size.

The differences between eyed-side and blind-side otoliths were evaluated by independent-samples t-test for morphometric variables (A_o, P_o, L_o, l_o, and W_o) and shape indices (F, Ro, C, Rt, and E). Shape indices were compared among areas (Persian Gulf, Hormuz Strait, and Oman Sea) using one-way analysis of variance (ANOVA) to determine if there was any phenotype-based evidence indicating differences among areas.

Lastly, a Canonical Discriminant Analysis (CDA) (Blackith and Reyment, 1971) was carried out to determine differences in Otolith shape among the Indian halibut sampled from different areas. CDA was undertaken using the "MASS" and "ade4" packages in R (Core Team, 2014). This method is a classification approach that investigates the integrity of pre-defined groups (i.e. individuals belonging to a given sample) by finding a linear combination of the descriptors that maximize Wilk's lambda (λ) (Ramsay and Silveman, 2005). This statistic assesses the performance of the discriminant analysis and its values are between 0 (low discrimination) and 1 (high discrimination).

Results

The fish morphometric variables are summarized in Table 2. ANOVAs showed no significant difference in fish total length (TL) among areas (ANOVA, F=2.874, p>0.05), while other parameters (SL, BD, and TW) were significantly different among areas (Table 2).

Table 2: Morphometric measurements (range and mean±sd in bold) ofIndian halibut at the three sampling areas (Persian Gulf, Hormuz Strait, and Oman Sea) and results from the analysis of variance (ANOVA) testing mean differences among areas.

	Persian Gulf (n=18)	**Hormuz Strait (n= 56)**	**Oman Sea (n= 20)**	***F***	***p***
Total length (cm)	38.5-52.0 (44.4±4.5)	36.50-51.40 (43.07±3.60)	37.5-49.5 (45.1±2.7)	2.874	p>0.05
Standard length (cm)	33.0-44.0 (38.0±3.8)	28.00-43.50 (36.03±3.33)	32.0-42.0 (38.5±2.3)	5.483	p<0.05
Body depth (cm)	14.5-22.0 (17.7±2.0)	13.50-19.50 (16.75±1.42)	15.0-21.0 (18.7±1.6)	12.156	p<0.05
Total weight (g)	760.0-2204.0 (1361.9±447.6)	678.00-2119.00 (1140.65±345.95)	855.0-1750.0 (1356.9±250.5)	4.406	p<0.05

Shape indices did not significantly interact with fish size (p>0.05). So, they were tested for correlations with fish size; none showed a significant correlation (Pearson correlation test, p>0.05). All shape indices showed a relationship with fish size and the ANCOVA showed that the relationship has the same shape for each location (Table 3). So, there was no need to adjust the values of shape indices.

Table 3: Mean value of the otolith shape indices of Indian halibut in each area and investigation of the cross effect of fish size × area based on shape indices.

Shape indices	**Form factor**	**Roundness**	**Circularity**	**Rectangularity**	**Ellipticity**
Persian Gulf	0.435	0.433	29.411	0.673	0.329
Hormuz Strait	0.417	0.423	30.852	0.666	0.335
Oman Sea	0.440	0.415	29.186	0.670	0.343
Cross effect of TL and location	F=0.0362 P=0.9645	F=0.3351 P=0.7162	F=0.0521 P=0.9492	F=0.1583 P=0.8538	F=0.3291 P=0.7204

Morphological investigation of sagittae pairs revealed that the blind-side otolith was significantly (p<0.05) larger than the eyed-side in 86% of the examinations. In contrast, eyed-side otolith was larger in 13% of the cases and one pair equivalent in length. Also, the perimeter (P_o) of the blind-side otolith was significantly (p<0.05) higher than that of the eyed-side otolith in 78% of the cases. In comparison, it was higher in eyed-side otolith in 21% of samples, with one pair equivalent in perimeter. Other otolith morphometric variables including surface area, width, and weight did not show any significant difference between eyed-side and blind-side otoliths (*p*>0.05) (Fig. 3).

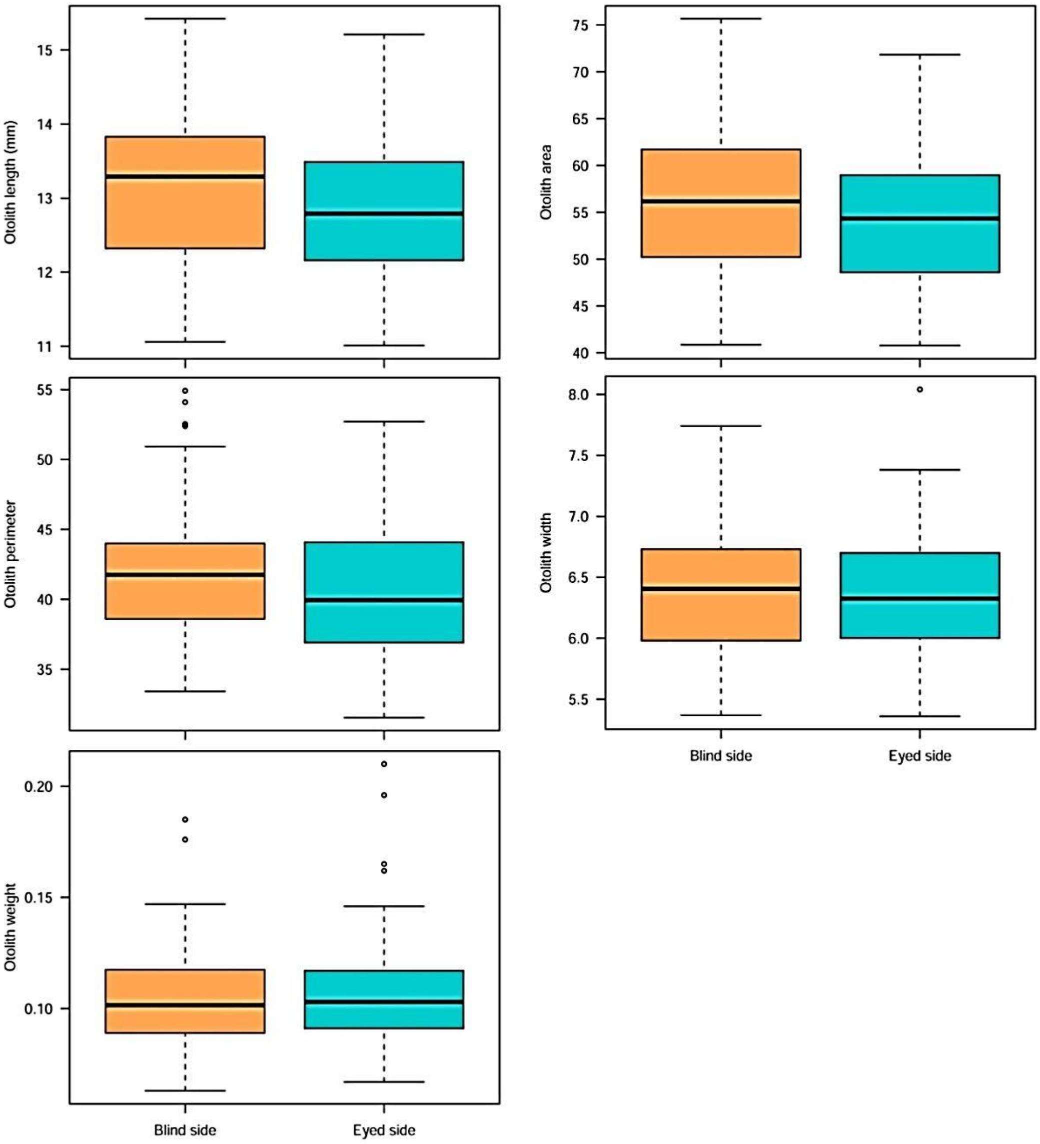

Figure 3: Boxplots of otolith morphometric variables according to their location side, eyed-side or blind-side in *Psettodes erumei*.

The mean values of two of the shape indices (roundness and ellipticity) displayed significant differences (*p*<0.05) between eyed-side and blind-side otoliths whereas the three others did not show any difference (Table 4).

Comparison of the means of the otolith shape indices among areas using one-way ANOVAs showed no significant variation for any of the indices (Table 5). According to Figure 4, each shape indices were generally close to each other and did not vary significantly among areas.

Table 4: Comparison of the mean values (±sd) of the five shape indices between the eyed-side and blind-side otoliths of Indian halibut based on t-test, all areas pooled together.

Shape indices	Eyed-side otolith	Blind-side otolith	*t*	*p*
Form factor	0.425 (0.627)	0.411 (0.640)	1.543	*p*>0.05
Roundness	0.423 (0.306)	0.414 (0.306)	2.028	*p*<0.05
Circularity	30.222 (4.682)	31.413 (5.536)	-1.586	*p*>0.05
Rectangularity	0.668 (0.283)	0.669 (0.289)	-0.102	*p*>0.05
Ellipticity	0.336 (0.322)	0.346 (0.345)	-2.020	*p*<0.05

Table 5: Comparison of the mean values (±sd) of the five shape indices between the areas based on ANOVA.

Shape indices	Persian Gulf	Hormuz Strait	Oman Sea	*F*	*p*
Form factor	0.435 (0.596)	0.417 (0.632)	0.440 (0.632)	1.266	*p*>0.05
Roundness	0.433 (0.361)	0.423 (0.269)	0.415 (0.341)	1.799	*p*>0.05
Circularity	29.411 (4.123)	30.852 (4.854)	29.186 (4.581)	1.274	*p*>0.05
Rectangularity	0.673 (0.302)	0.666 (0.299)	0.670 (0.220)	0.441	*p*>0.05
Ellipticity	0.329 (0.398)	0.335 (0.269)	0.346 (0.373)	1.533	*p*>0.05

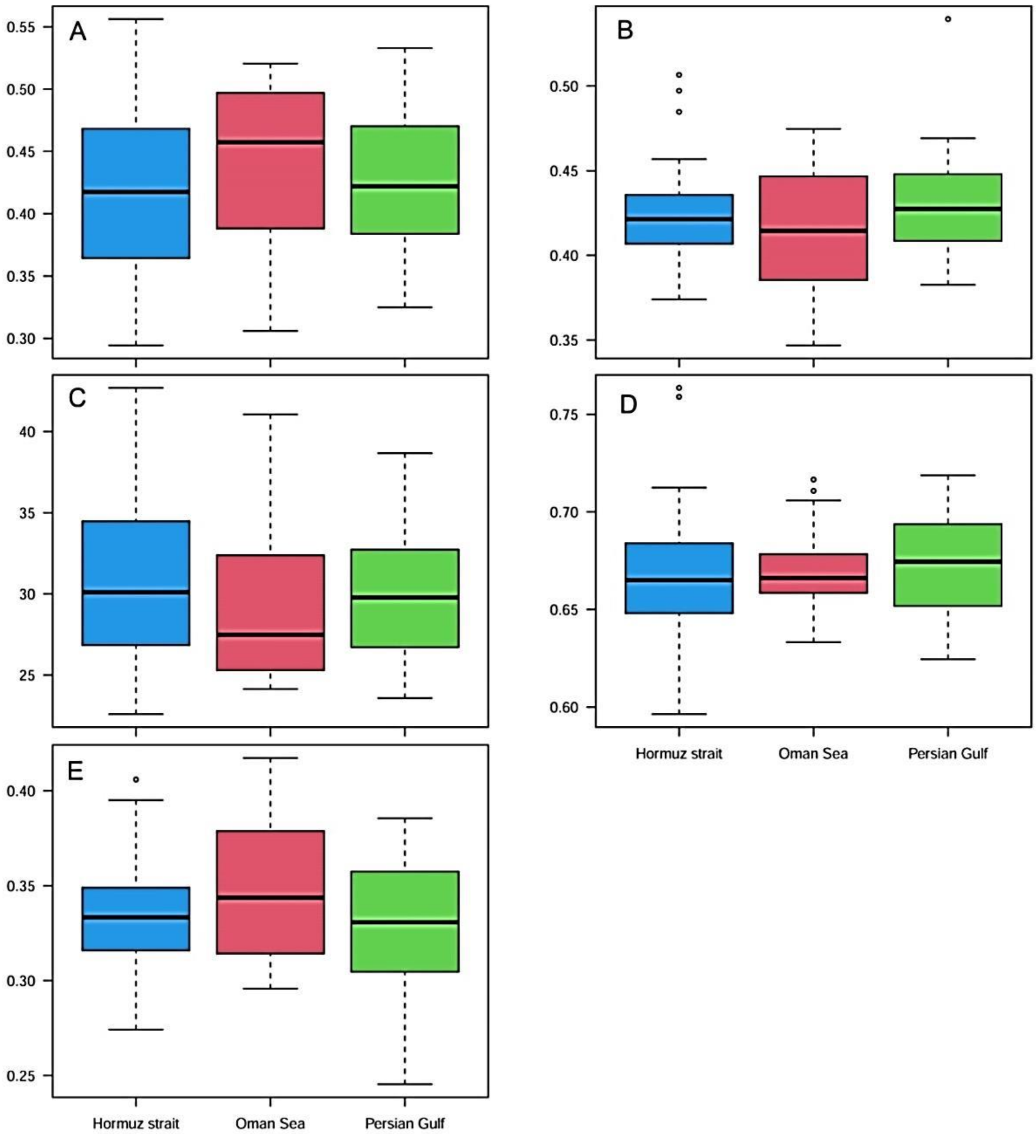

Figure 4: Boxplot of five otolith shape indices of *Psettodes erumei* for each area (A) from factor, (B) roundness, (C) circularity, (D) rectangularity and (E) ellipticity.

The CDA performed with the shape indices of otolith did not indicate any discrimination among the Indian halibut from the different areas. The first and second discriminant functions represented 65.8 and 34.2% of the total variability, respectively, which none of them was significant ($p>0.05$; Table 6; Fig. 5). Notably, all analyses on eyed-side and blind-side otoliths gave the same results. So, only the results on eyed-side otoliths have been mentioned here.

Table 6: Statistics and function coefficients from the discriminant analysis based on the shape indices. (F= Form factor, Ro= Roundness, C= Circularity, Rt= Rectangularity, E=Ellipticity).

	Eigenvalue	Variability in %	Canonical Correlation	Wilks λ	df	*p* value	Standardized canonical coefficients				
							F	Ro	C	Rt	E
Function 1	0.068	65.80	0.25	0.91	10	0.54	1.99	12.11	1.68	-7.46	13.01
Function 2	0.035	34.20	0.18	0.97	4	0.55	-0.41	3.89	-1.01	-1.78	3.31

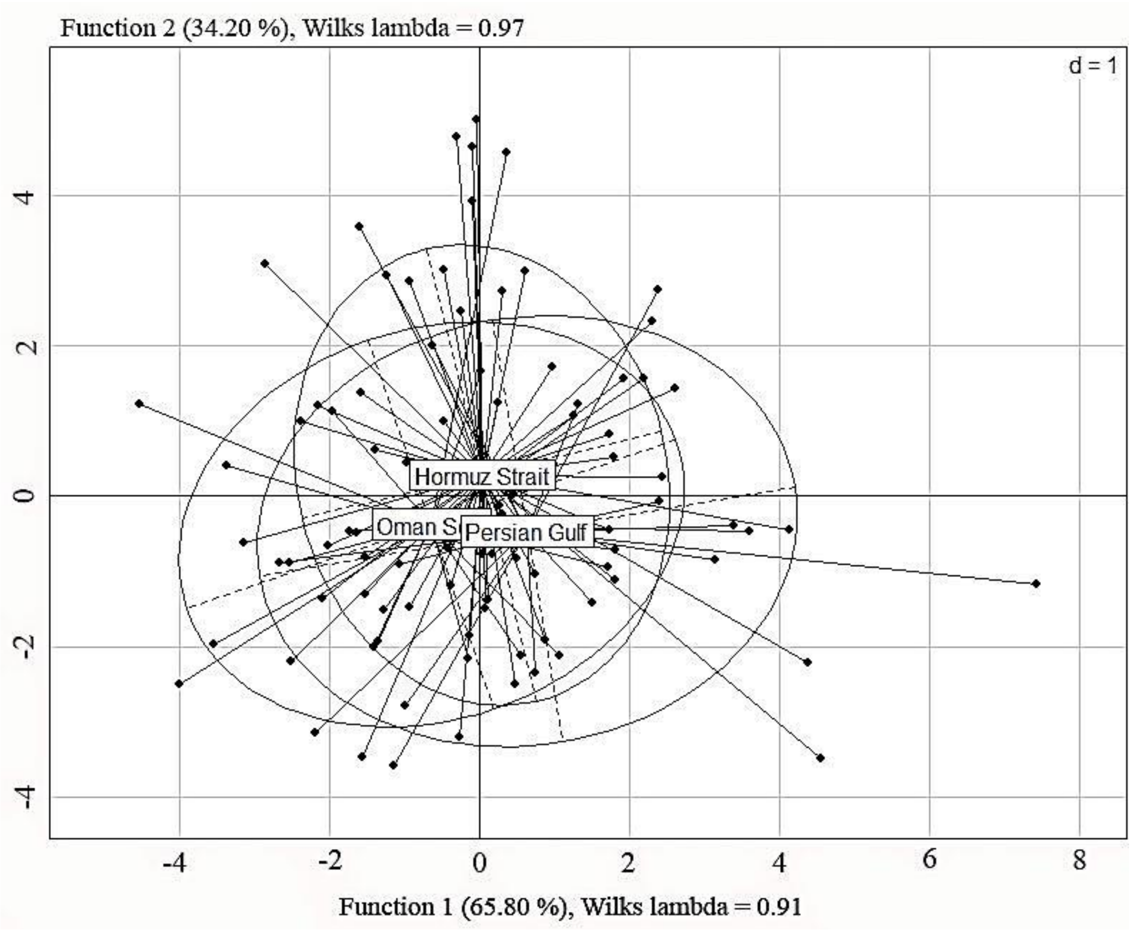

Figure 5: Plot of the canonical discriminant analysis (CDA) performed on the shape indices of the eyed-side otoliths of *Psettodes erumei* from the three sampling areas; Persian Gulf, Hormuz Strait, and Oman Sea.

Discussion

According to the results, we found significant differences between eyed-side and blind-side otoliths for two otolith morphometric variables (otolith length and perimeter) and for two shape indices (roundness and ellipticity). These differences are usually not found in round fish (Lychakov and Rebane, 2005). For flatfish, prior to metamorphosis, the sagittae of larvae are virtually identical, but upon eye migration (after metamorphosis or after settlement on soft bottoms) undergo differential growth rates, which causes changes in mass and shape (Helling *et al*., 2005). For the Indian halibut, as well as other flatfish species, differences in the otolith pair are frequently present and thus they cannot be used interchangeably (Hunt, 1992; Mérigot *et al*., 2007; Jackman *et al*., 2015; Mille *et al*., 2015). Reasons for the morphological asymmetry of flatfish sagittae are speculative, however, the influence of body rotation during the developmental stages, positional control during lateralization processes and adaptation to life in the soft-bottoms have been suggested (Schreiber, 2006). Regardless of the precise reason, a variation in the postural orientation of the Indian halibut during metamorphosis introduces a corresponding asymmetry in the growth patterns of the sagittae that continues to diverge over time (Helling *et al*., 2005). Moreover, some researchers believe that the morphological asymmetry of flatfish sagittae is due to differential accretion of the inorganic constituents ($CaCO_3$) (Kajajian *et al*., 2013).

Shape indices did not display any significantly different mean values among areas. Also, the patterns derived from CDA did not show any separation among populations. Otolith shape is markedly a species-specific feature (Morrow, 1976; Gaemers, 1984; L’Abbe´-Lund, 1988) whose dissimilarity among species (or populations) is known to depend on a combination of genetic and environmental factors (Cardinale *et al*., 2004; Vignon and Morat, 2010). Genetic differences may resolve as different phenotypic traits (such as morphometric variation), which are probably more pronounced among species than populations of the same species (Cardinale *et al*., 2004). Researchers have shown that environmental factors are more influential in determining otolith shape by affecting fish growth rate (De Vries *et al*., 2002). Environmental factors act on metabolism that, in turn, influence the growth of fish and, consequently, the quantity of material deposited on otoliths (Cardinale *et al*., 2004; Galley *et al*., 2006). This phenomenon causes the difference in otolith growth patterns that may translate to different otolith morphology (size and shape). Moreover, otolith shape is related to the biological and ecological behavior of the species (Panfili *et al*., 2002; Cardinale *et al*., 2004) and so differences in food and spatiotemporal niches could lead to the differences in the otolith shape (De Vries *et al*., 2002). Based on our study, it seems that differences in these factors among studied areas are not enough strong to induce shape indices differences. For example, these areas have nearly similar conditions of nutrition and environment (salinity and temperature) — because surface water flows into the Persian Gulf from the northern Hormuz Strait and the Oman Sea (Coles, 1997)—. These hypotheses are consistent with the ecological behavior of some flatfishes that show seasonal and ontogenetic migrations related to feeding. As a result of these migrations and larval transport, flatfishes have a high potential for wide dispersal which could result in population mixing and gene flow among them (Bailey, 1997).

Similar studies have been conducted in the area on different fish species; Sadighzadeh *et al*. (2014) used the otolith shape for stock identification of John's snapper (*Lutjanus johnii*) from the Persian Gulf and the Oman Sea. According to the results of the mentioned study which was based on the identification of the otolith contour changes using wavelet functions, two separate stocks of this species were identified, and it was determined that genetic factors, and not environmental factors, should play a more relevant role in the morphological variability noted. No differences were found among the populations of *Encrasicholina punctifer* in the Persian Gulf, Hormuz Strait and Oman Sea based on the otolith shape analysis which was due to the similar environmental factors and nutrition conditions in the three examined regions (Ataei Daryaei *et al*., 2013).

This study has documented the applicability of otolith shape analysis to identify the stock structure of the Indian halibut in the Persian Gulf and the Oman Sea. The results obtained in this study help us to accurately estimate the vital life history characteristics (*e.g*., growth, mortality, and maturity) of *Psettodes erumei*, and so provide information for fishery management and sustainable exploitation of this species in the small-scale fisheries.

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