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Original Article

Otolith shape analysis of *Lethrinus lentjan* (Lacepède, 1802) and *L. microdon* (Valenciennes, 1830) from the Red Sea

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Abstract: Otolith shape and morphology are used to identify fish species and population stocks. The aim of this study was to distinguish the *Lethrinus lentjan* (Lacepède, 1802) and *L. microdon* (Valenciennes, 1830) (family: Lethrinidae) using otolith shape. The analyses apply the ShapeR package in R which enables to extract the outline and otolith morphology from images and for statistical examining of individual variation. Otoliths of 165 individuals from the two *Lethrinus* species were collected during 2019 and 2020. The wavelet levels were examined by using 6 wavelets to collect 63 coefficients. The regression between width and fish length were b = -0.03 (t = 2.6, *P* = 0.01) for *L. lentjan* and was significantly different (t = 2.120, *P* = 0.036) for *L. microdon* (b = 0.018).

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Introduction

The family Lethrinidae is one of the most important groups of fishes in coral reef fisheries in Egypt, which includes 39 species with 29 emperor species of the genus *Lethrinus* (Carpenter and Niem, 2001). The annual catch of this family is around 1,469 tonns representing 3.06% of the Red Sea fishery production (GAFRD, 2020). Generally, the emperors are long-lived, reaching age greater than 20 years, with size less than 20.0 cm FL (*L. variegatus,* Valenciennes, 1830) to 80.0 cm FL (*L. nebulosus* (Forsskål, 1775) (Randall, 1995; Carpenter and Niem, 2001).

Otolith comparison of lethrinids can be challenging due to lack of informative morphological characters to distinguish their species (Carpenter and Allen, 1989; Carpenter, 2002; Carpenter and Randall, 2003). The identification of Lethrinidae based on morphological characteristics could be solved by costly DNA analyses, however, otolith shape analyses may offer a cheap and easily applicable method tin this regard (Libungan and Pálsson, 2015; Libungan et al., 2016; Mehanna et al., 2016; Osman et al., 2020). Otolith shape and dimensions are commonly used to identify fish species but may provide also important information such as stock, age and the growth of the fish during its lifespan (Lecomte-Finiger, 1999; Tuset et al., 2003; Jawad et al., 2017).

ElSherif et al. (2020) estimated the phylogenetic relationships and taxonomy of three species of family Lethrinidae, including *L. mahsena*, *L. nebulusus* and *L. grandiculis* from northern Red Sea, showing that they lack discriminative morphological traits. Therefore, the aim of the present study is to compare *L. lentjan* and *L. microdon* based on their otolith shape to distinguish them and the results could provide a tool to characterize other species of the family Lethrinidae along the Egyptian coast of the Red Sea.

Materials and Methods

Sampling: A total of 165 specimens of *L. lentjan* (n=96) and *L. Microdon (*n=69) were obtained at Hurghada fishing port (27°13'43.32''N, 33°50'33.20''E), in Northern Red Sea, Egypt during 2019 and 2020. The fishes were sampled randomly from the commercial catch of the hook and line fishery. The fish total length (L) was measured to the nearest 0.1 mm; fish weight (W) to the nearest 0.01 g, and also the sex was recorded. Sagittal otoliths were

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extracted, cleaned and dried.

All otolith images were estimated on the distal side using a stereomicroscopic with AxioCam ERc 5s camera (Carl-Zeiss-Promenade 10; 07745 Jena, Germany) and the software of Zeiss. The statistical analysis was performed with Rstudio (R Core Team 2015) using the R packages of ade4 (Dray and Dufour, 2007), pixmap (Bivand et al., 2011), ipred (Peters and Hothorn, 2013), vegan (Oksanen et al. 2013), jpeg (Urbanek, 2014) and shapeR (Libungan and Palsson, 2015). Otolith photos were read into R and the outlines were extracted using the conte function in R (Fig. 1). Feret length and width were measured to the nearest 0.1 mm. Area and perimeters were obtained from the figures using shapeR.

Analysis of species differences and otolith shape: The independence of the different otolith variables was evaluated with Pearson correlation and summarized with descriptive statistics. The difference between the species was analysed for weight, transformed with square-root, and the different otolith characteristics was tested with a linear model taking length and sex into account. A regression line was fitted for both species and the success of these methods in distinguishing species was evaluated by looking at how many individuals of species *L. lentjan* where within the range of *L. microdon* and vice versa.

The shape of each otolith was fitted with a function of independent wavelet shape coefficients, obtained with the wavethresh package in R (Claude, 2008; Nason, 2012; Libungan and Palsson, 2015a). Differences in size among the otoliths were standardized to remove size differences. The number of wavelet coefficients increase by the power of 2 for each wavelet level; 63 coefficients were obtained for each outline using 6 wavelet levels. The quality of the reconstruction rises with the number of wavelet levels (Fig. 6), and the shape of sprat otolith appears to be precisely described (with 98.5% accuracy with respect to the original otolith contour-line) by the sum of the first 5 wavelet levels.

The difference in shape between the species was summarized by plotting the average otolith shape based on normalized wavelet coefficients (Libungan

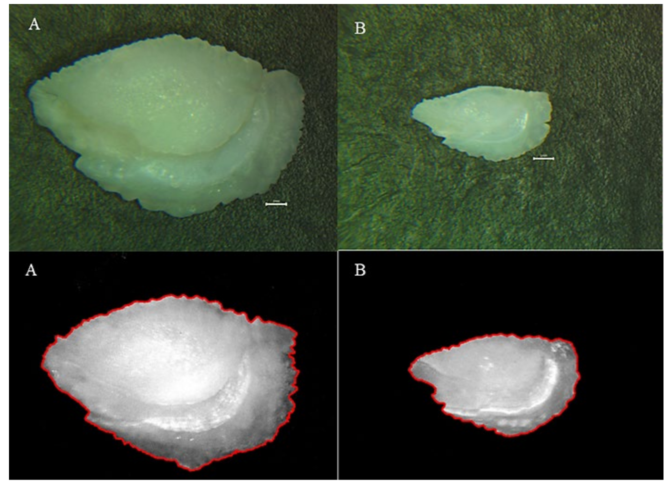

Figure 1. Original otolith shapes and the red outline marks the shape of the otolith which is extracted by shapeR and forms the basis for the analysis of variation within and between the two species investigated. (A) *Lethrinus lentjan* and (B) *L. microdon*, with scale bars (1 mm). Anterior of the otolith is to the left.

and Pálsson, 2015b). To investigate which areas and coefficients on the outline contribute most of the variations in shape, the mean shape coefficients and standard deviation were plotted against the angle of the outline from the coefficients using the plotCI command in the gplots package (Warnes et al., 2014). To determine which region contributed most to the differences between the species, the proportion variation between the species out of total variation (the intraclass correlation ICC), was calculated along the outline of the otolith.

The difference in otolith shape between the two species using length of the fish as a covariate was analysed using Canonical Analysis of Principal Coordinates (CAP) (Anderson and Willis, 2003) using the vegan package (Oksanen et al., 2013) on the standardized Wavelet/Fourier coefficients and tested with PERMANOVA. To classify individuals to their taxonomic classification based on the population variation within the two species, linear discriminant analysis (LDA) was applied to the coefficients using the lda function in the MASS package in R (Ripley et al., 2014), and the misclassification error estimated using cross validation based on bootstrapped samples of the dataset as in Libungan and Palsson (2015).

Results

Morphological measurements: The length

Table 1. Descriptive statistics for *Lethrinus lentjan* and *L. microdon* otoliths. n: sample size, F: females, M: males, TL: total length, BW: weight, OL: length, OH: height; OA: area OP: perimeter.

Species	***L. lentjan***		***L. microdon***	
	Min.-Max.	**Average± SD**	**Min.-Max.**	**Average± SD**
n	96 (66 F, 30 M)		69 (42 F, 27 M)	
TL range (mm)	164-507	286.3±96.9	236-513	368.01±65.70
BW (g)	70-1825	465.741±501.74	134.6-1582.8	643.54±346.71
OL (mm)	6.41-15.3	9.89±2.42	6.36-9.692	8.05±0.84
OH (mm)	4.68-11	6.99±1.57	3.781-5.773	4.78±0.49
OA (mm)	21.08-115.9	49.24±23.52	18.421-37.176	27.20±4.95
OP (mm)	21.76-111	41.6±21.01	20.19-35.485	27.94±4.00

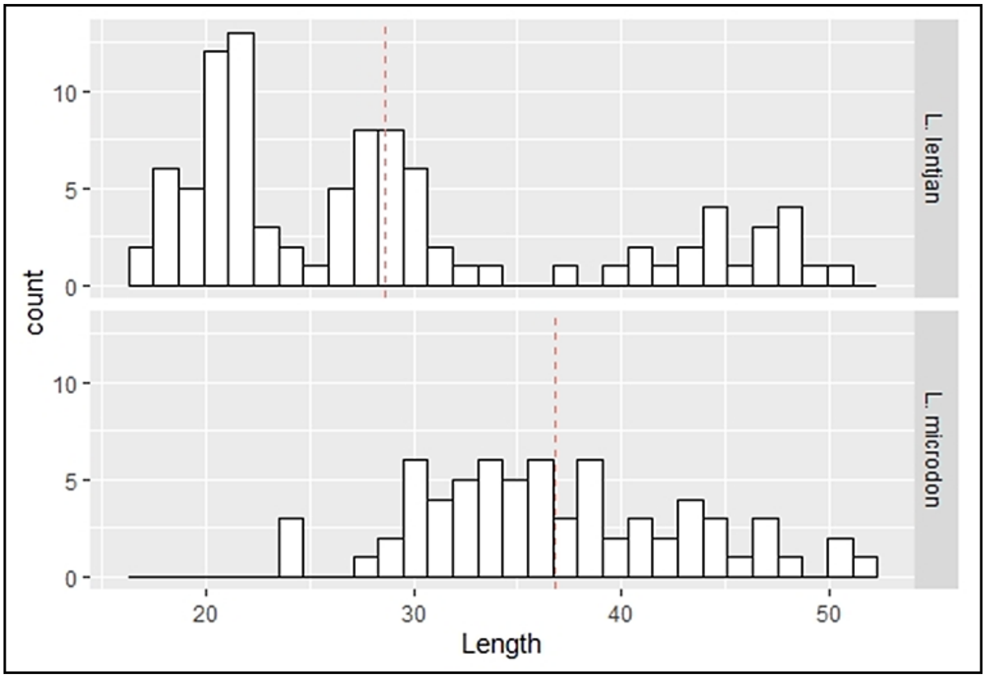

Figure 2. Length distribution of *Lethrinus lentjan* and *L. microdon*; red line presents the mean values for the two species.

distribution of the *L. lentjan* varied from 164 to 507 mm TL, and showed three modes (220, 280 and 450 TL mm) which might present different cohorts, a single unimodal distribution was observed for *L. microdon* which range overlapped with *L. lentjan* (236-513 mm) (Fig. 2, Table 1). The average length for two species was estimated at 286.3±96.0, 368.01±65.70 mm and weight at 465.74±501.74, 643.54±346.71 g for *L. lentjan* and *L. microdon*, respectively.

The correlation coefficient of otolith length, otolith width, otolith area and perimeter for the two *Lethrinus* species were strongly correlated, with *r* varying between 0.88 and 0.97 (Fig. 3). The square root of weight of the fishes could be explained by length and species but was independent of sex (R-squared = 0.987). The square root of the weight increased by b = 1.05 g per cm (t = 107.82, *P*<0.001) for both species, but *L. lentjan* weighted on average 2.97 g more than *L. microdon* (t = 16.49, *P*<0.001) for a given length (Fig. 4). Despite these differences, there is some overlap of the two distributions around the regression lines. Nine *L. microdon* weighted less than the 97.5 percentile of *L. lentjan* and 18 of *L. lentjan* weighted more than 2.5% of the distribution of *L. microdon*.

Separate analyses of variation in length, width, perimeter and area of the otoliths resulted in similar patterns as expected due to their high correlation and

Table 2. Variations in otolith shape between fish sex based on ANOVA-like permutation test based on 1000 permutations.

species	DF	Sum. Sq	F’	*P*-value
L.lentjan v. L.micrododn	1	19.896	102.73	0.001
Fish length	1	0.238	1.23	0.250
Sex	1	0.172	0.890	0.409
Residual	162	31.373		

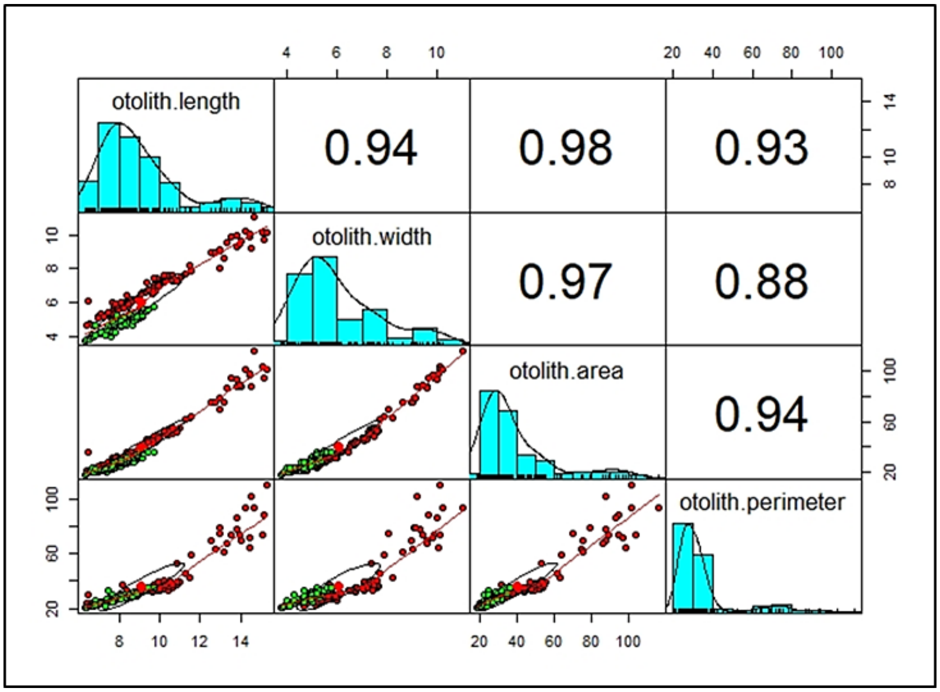

Figure 3. The correlation coefficients of otolith measurements for the two species; *Lethrinus lentjan* and *L. microdon*.

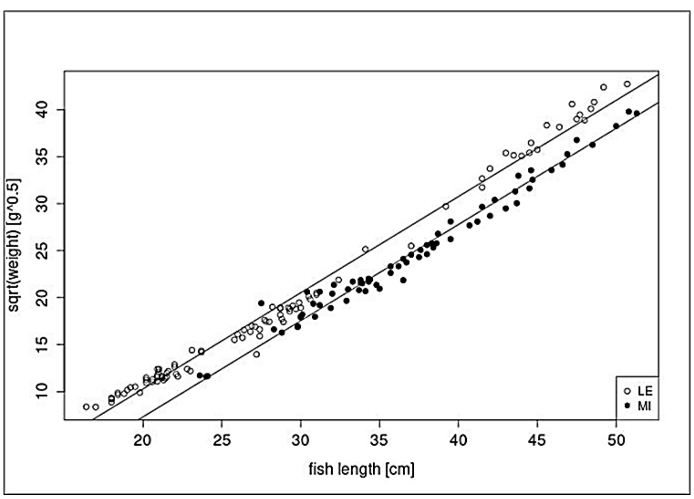

Figure 4. Relationship of weight and length of *Lethrinus lentjan* and *L. microdon*.

presented here just for the width of the otoliths which showed the largest difference between the species (t = 4.19, *P*<0.001), and gave the highest proportion of variation explained by the model (R^2 = 0.45). Differences were found between the species traits in all cases (*P*<0.01 or <0.001), but the difference was smaller with larger fishes as seen with significant differences in the regression slopes and was independent of sex. The regression slopes for width on fish length were b = 0.03 (t = 2.6, *P* = 0.01) for *L. lentjan* and was significantly different (t = 2.120, *P* = 0.036) for *L. microdon* (b = 0.018). However, the variance was much larger for *L. lentjan*, therefore, the significance should be taken with caution (Fig. 5). An inspection of Figure 5 shows the split of the two species but there are about 16 *L. lentjan* individuals with similar width or smaller than the width of *L. microdon*, the overlap was larger for the other traits.

Main shape features: The otolith shape of the two species differs (PERMANOVA F’ = 149.68, *P*<0.001, Table 2) as reflected in the scatter of individual shapes in the ordination plot. The first discrimination axis of the CAP analyses based on the wavelet coefficient showed 98.1% of the differences between the two

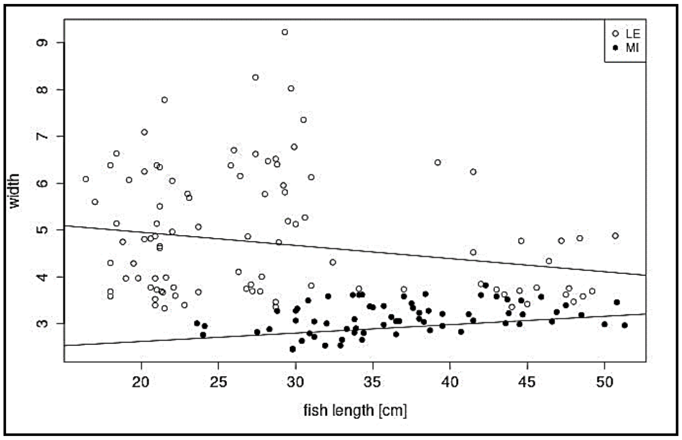

Figure 5. Relationship of otolith width and fish length of *Lethrinus lentjan* (LE) and *L. microdon* (MI).

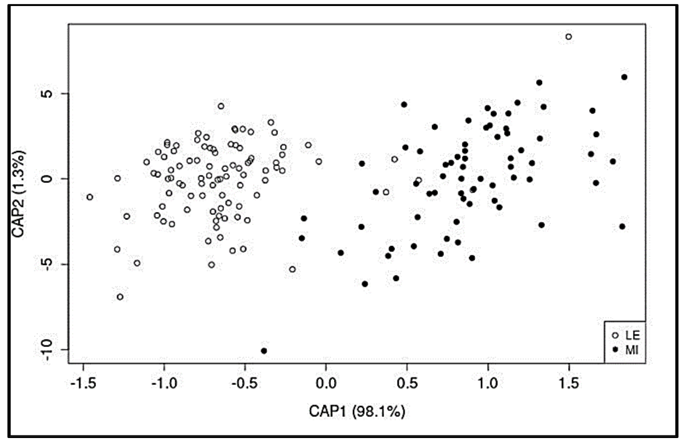

Figure 6. Quality of the Wavelet, the red vertical lines show the level of Wavelet and number of Fourier harmonics needed for a 98.5% accuracy of the reconstruction.

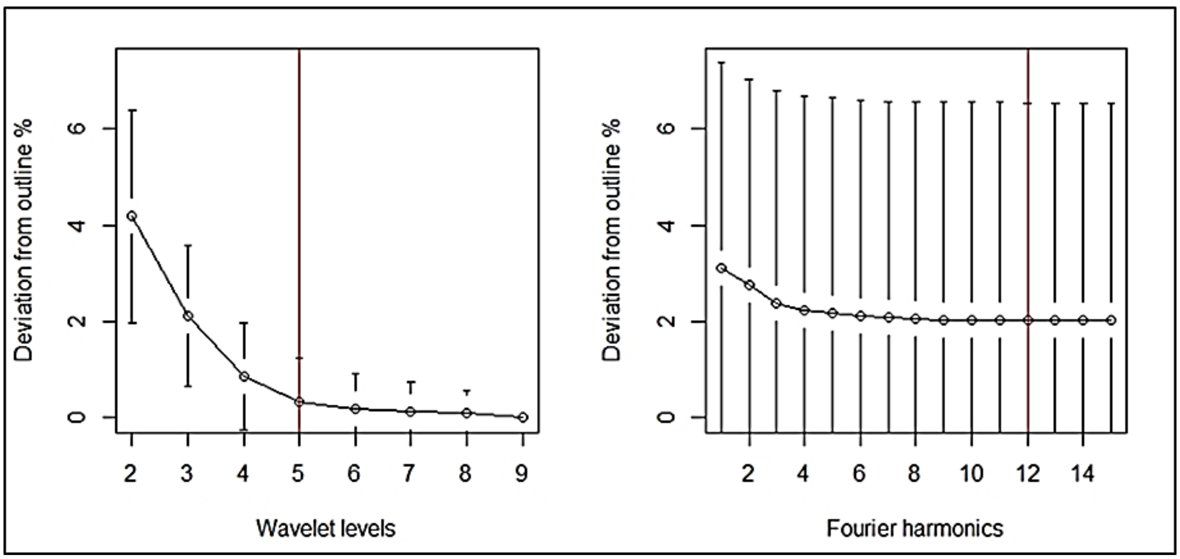

Figure 7. Differentiation of otolith shape of *Lethrinus lentjan* (LE) and *L. microdon* (MI), based on Canonical analysis of Principal Coordinates with the wavelet coefficients. *L. lentjan* and *L. mi-crodon* are indicated by open and filled dots, respectively.

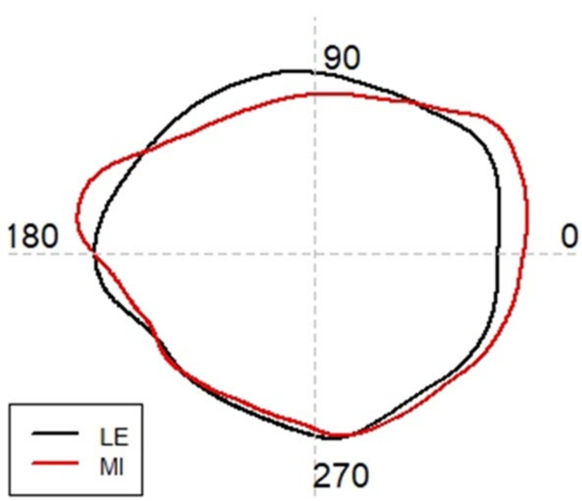

Figure 8. Mean otolith shape based on Wavelet reconstruction for two species *Lethrinus lentjan* (LE, n=96), *L. microdon* (MI, n=69). Numbers represent angles in degrees (°) based on polar coordinates (see Fig. 4). The centroid of the otolith (center of the cross) is the center point of the polar coordinates.

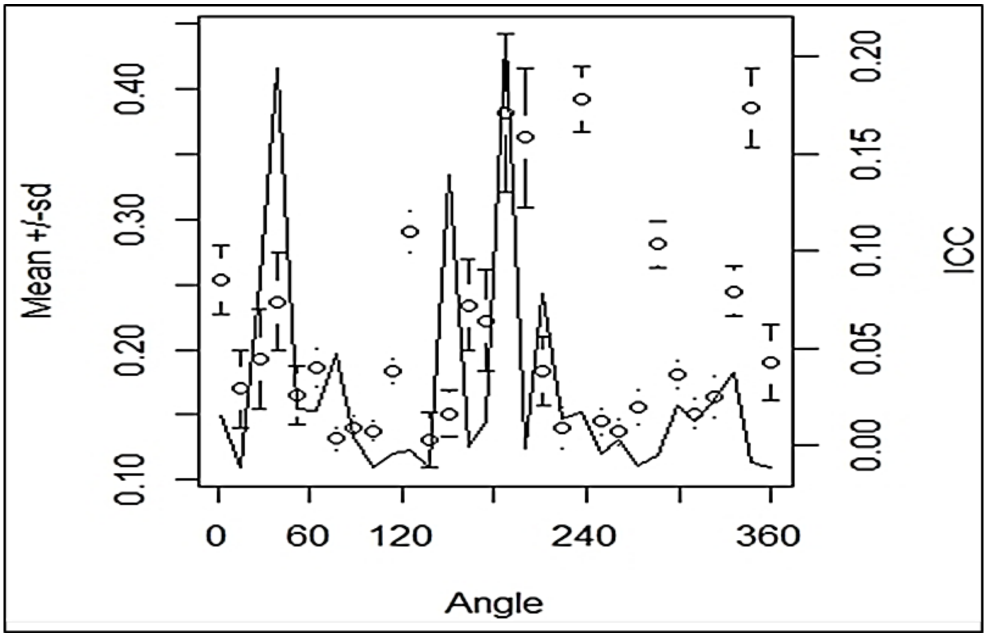

Figure 9. Mean and standard deviation (sd) of the Wavelet coefficients for all combined otoliths and the proportion of variance among groups or the intraclass correlation (ICC, black solid line). The horizontal axis shows angle in degrees (°) based on polar coordinate (see also Fig. 1) where the centroid of the otolith is the center point of the polar coordinates.

species and the second axis 1.3% (Figs. 7 and 8), but the shape of few (~4) individuals of the two species overlapped. The misclassification error based on the LDA-analyses was 2.4%. The differences in the mean shape (Fig. 8) of the two species are mainly at certain regions along the edge of the otoliths, namely at 0-20, 80-140 and 170-190 angles (Fig. 9), and interestingly there is a notable difference in the width. The average shape of otolith varied within species mainly at pararostrum at angle ca 30-40, 140 and 180° counted anti-clockwise from right to left.

Discussions

The relationship between weight and length is very important to estimate the biomass from length and provides information on the condition factors of fish (Moutopoulos et al., 2002; Souza et al., 2019). The otolith morphology may provide better information to comparison between stocks or species as it is independent of conditions and can be used in diverse studies e.g. to characterize fish species in archaeological sites (Aguilera et al., 2013; Souza et al., 2019) or among prey where other information may be lacking. The length frequency may be used to study the age, growth, survival rate, mortality rate and stock differentiation and fisheries management (Jones, 1984; Pauly, 1984; Pauly and Morgan, 1987; Athanassios et al., 2018; Mehanna et al., 2018a, b; Osman et al., 2020; Liang et al., 2020; Froese et al., 2020). The results may be explained by the fact which length of otolith is more sensitive to variation growth rate and relation to changes in fish metabolism (Pawson, 1990; Flecher 1991; Osman et al., 2020).

The otolith measurements of the two species were examined with fish length to get the relationship between the otolith width and fish size. The difference between the species indicate the otolith of *L. lentjan* may be large than *L. microdon*, and the differences in otolith measurement among the species may be due to variation in environmental condition and habitat, as well as water temperature and dissolved oxygen effect on fish growth (Campana and Casselman, 1993; Cardinale et al., 2004; Zischke et al., 2016). Interspecific variation in otolith morphology can reflect live at different depth e.g. fishes live at large depth have generally large otoliths (Tuset et al., 2003a; Baniet al., 2013; Zischke et al., 2016).

The shape of otolith may be estimated with standard statistical methods. In the current study, we used two multivariate methods to distinguish *L. lentjan* and *L. microdon* i.e. canonical analyses of principal coordinates and linear discriminant analyses both revealed clear difference and the latter a high overall score of correct classification.

The wavelet transform may be usefulness in otolith shape analysis for the morphological measurements that estimated by otolith outline (pararsotrum, postrostrum and exicura major) and the most variation between species and among the population. The correlation between species is high for the first canonical analysis. The multivariate method was used to the wavelet coefficient to get shape varietion between species. The ANOVA A-like permutation analyses were significant between two species i.e. the two species differs.

The mainly variation between species at pararostrum at angle 30-40, 140 and 180° counted anti-clockwise from right to left. The wavelet might prove to be better for explaining shape differences, while for others, the Fourier method might be more powerful to distinguish populations. In addition, the evaluation of the applicability of the wavelet, in otolith shape analysis is warranted (libungan et al., 2015; libungan et al., 2016).

The otolith comparison between two species was the first study to estimate the difference between two species and family in the Egyptian coast of the Red Sea. Therefore, this study encourages to more study to compare between the most commercial species of the Red Sea using otolith morphology and shape. Finally, this study considers important by adding more details to food and feeding, stock assessment and paleontology studies.

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