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Morphological Variations in the shapes of the otolith in the freshwater sardine *Sardinella tawilis* and marine sardine *Sardinella lemuru* using Elliptic Fourier Analysis

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ABSTRACT

Elliptic Fourier analysis (EFA) was used to discriminate otolith shapes between the two species of Sardinella. *The freshwater sardine* Sardinella tawilis *from Taal lake, and the marine sardine* Sardinella lemuru *from Butuan bay were investigated to find out whether these two species varied in their otoliths considering the fact that they were from two different aquatic environments. Principal component (PC) scores were obtained from EFA of the left, right, and both otoliths using SHAPE ver1.3 software. Discriminant Function Analysis of PC scores was used to determine the frequency of correctly classified fishes that showed 99.31% correct classification indicating a high discrimination between the two species. While otolith shapes were shown to be highly conserved among the sexes within the species, main variations between the two species were prominent in the dorsal, anterior (i.e. in the antirostrum, excisura, and rostrum) and in the ventral side below the rostrum. This study clearly showed that the shape variations can be quantitatively described using EFA.*

Keywords: Sardinella, Elliptic Fourier, otolith, SHAPE, Discriminant

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INTRODUCTION

Otoliths which are three dimensional structures made of calcium carbonate in aragonite form, have biologically been considered important in teleost fishes since they play a major role in balance and hearing [1-4]. These structures were qualitatively described to differ greatly in sizes and shapes among teleost species [3]. The otolith of a species could be a valuable tool in stock identification [5-10], stomach content of other fish-feeding species [11], and sex and maturing stage during growth [9, 12]. Otoliths lack resorption since minerals that were already deposited in them would not be used again even during the starvation, and the calcified structures grow in a continual way throughout the lifetime of the fish. Thus, it has been a good structure to be used in studying variations within and between species especially those surviving in extremely different environments such as the freshwater sardine Sardinella tawilis [13, 14], and one of the seven marine sardines,

namely *Sardinella lemuru* [15, 16] (Fig. 1). These two species have been socioeconomically and commercially valuable, particularly in developing countries where they serve as a cheap source of animal protein for millions of people [17], and also have been processed for canning, fishmeal, and being dried for human consumption. *Sardinella tawilis* is endemic in Lake Taal in the province of Batangas in the northern part of the Philippines.

In this study, it was hypothesized that the analysis of the otolith shape could be a good parameter in the discrimination of a freshwater and a marine sardine species with the use of Elliptic Fourier Analysis (EFA). EFA is a method which uses Elliptic Fourier descriptors (EFDs)[18] which could describe any shape with a closed outline. EFA describes these outlines in terms of the components called harmonics. The higher the number of the harmonics, the better the accuracy of the shape description [18]. This study described the nature of variations within and between sexes and species based on which, otolith (left, right, or both) would give the highest discriminating power between the two species since most researches regarding otolith have utilized only one side.

METHODOLOGY

Sampling areas, otolith extraction, and image capture

A total of sixty (thirty males and thirty females) individuals of *S. lemuru* from the bays of Butuan City and the same numbers of *S. tawilis* were also collected from Lake Taal in Batangas City (Fig. 1). Gender identification was made though the examination of gonads. Careful extraction of the sagittal otoliths was made through the use of fine forceps to avoid breakage. The samples were dried and kept in individual Eppendorf tubes to separate the left otolith from the right one. Image capture was done through a 2 megapixel USB Digital Microscope connected to a computer, under 20X magnification (Fig. 2).

Fig. 1. Sampling sites for *S. tawilis* from (A) Lake Taal; and S. lemuru from bays of (B) Butuan City, (C) Dipolog City, (D) Pagadian City, and (E) Zamboanga City (Source: **www.googlemaps**.com)

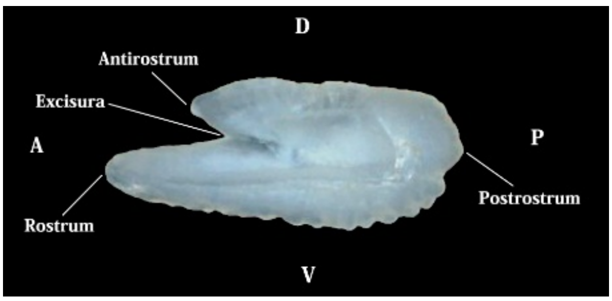

Fig. 2. Sample captured image of sagittal otolith using 2 megapixel USB Digital Microscope under 20X magnification (A – Anterior, P – Posterior, D – Dorsal, and V – Ventral)

Elliptic Fourier Analysis and Statistical Analysis

Captured raw images of the otoliths were initially processed using Adobe Photoshop CS5 to change the background into black, and the shape of the otolith to white, thereby creating binarized images. These images were converted to BITMAP (24-bit, *.bmp) format using Microsoft Paint software. Processing of the left and right otoliths, for male and female individuals was done separately. It was noted that the binarized images of right otoliths were flipped horizontally to be oriented in the same manner with the left otoliths to establish uniformity in orientation prior to using SHAPE v.1.3 [19] software, since during chain coding, contour tracing proceeded in a counter-clockwise direction. Nevertheless, the overall shape and outline of the right otoliths were unaltered.

SHAPE is a software package that contains four programs (ChainCoder, Chc2Nef, PrinComp, and PrinPrint) that are essential for evaluating the contour shapes based on Elliptic Fourier Descriptors (EFDs) which was first proposed by Kuhl and Giardana [18]. EFDs can describe the outlines of the two dimensional images with a closed outline through the use of harmonics. The binarized images of the otoliths were then processed using ChainCoder which records the outline information of the shape using numbers from 0 to 7, these series of numbers have been called chain codes. The chain code output file was then used in ChcNef software which calculates the normalized EFDs from the chain code based on the first harmonic ellipse, using 20 harmonics. This procedure standardized the size and orientation of the shape as well as the starting point for tracing the outline with reference to the major axis. The normalized EFD file was further subjected to principal component analysis using PrinComp. This software can summarize the information in the normalized EFD coefficients. Principal component scores were derived as observed values of the otolith shape features which were subjected to statistical analysis such as MANOVA and Canonical Variate Analysis (CVA), Discriminant Function Analysis (DFA), and boxplot construction through the use of Paleontological Statistics (PAST) software [20]. Contours of the otolith shapes were generated through PrinPrint which provided a proper graphical visualization of the mean shape and the ±2 standard deviation shapes from the mean, according to each respective principal component [21]. These series of steps were done separately for left, right, male, female, and all otoliths

Fig. 3. Summarized procedure of Elliptic Fourier Analysis of all otoliths between S. tawilis and S. lemuru**.**

RESULTS AND DISCUSSION

Canonical variate analysis (CVA) of the significant principal component scores generated from the SHAPE analysis showed significant variations in shapes of the otolith (Wilk's Lambda 0.1018, df 18, F=43.59, P=4.735-98). Kruskall-Wallis test between species and sexes showed significant differences only for the species (Table 1). Significant differences in the distribution of individuals in space were observed between the two species, no sexual dimorphism in otolith shapes was detected (Fig. 4). [22] stated that otolith Fourier shape analysis can be considered as an effective phenotypic discrimination tool if the percentage classification exceeds 75% (Table 2). Thus, the percentages obtained in this analysis were more than enough to justify the species discrimination between *S. tawilis* and *S. lemuru*. The differences in shapes between the species have been shown graphically in Fig. 5

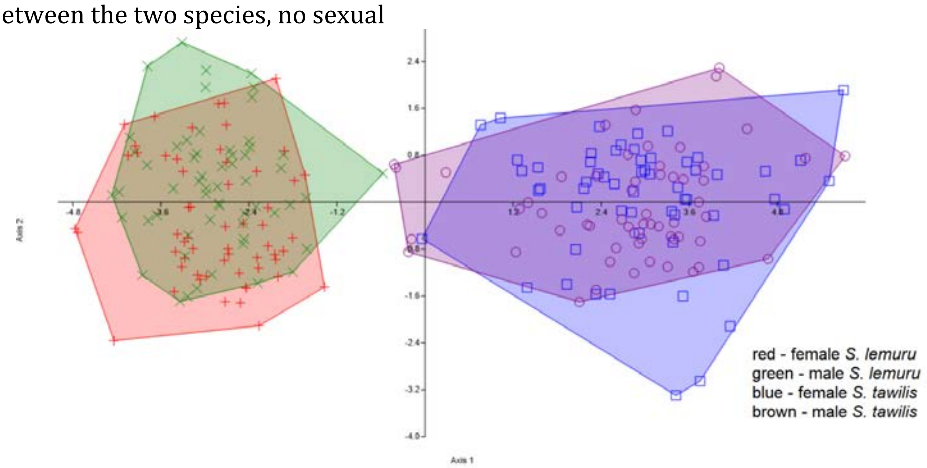

Fig. 4. CVA Scatterplot showing the distribution of individuals based on shapes of the otolith.

Table 1. Kruskall - Wallis Comparison between sexes and two species of *Sardinella*

	Female *S. Tawilis*	Male *S. lemuru*	Male *S. Tawilis*
Female *S. lemuru*	**5.78E-51**	0.144215	6.41E-50
Female *S. Tawilis*	-	**5.45E-51**	0.824163
Male *S. lemuru*		-	**5.08E-50**

Table 2. Confusion matrix between sexes and species of the two Sardinella species.

	Female *S. lemuru*	Female *S. Tawilis*	Male *S. lemuru*	Male *S. Tawislis*	Total
Female *S. lemuru*	38 (65.52)	0	20 (34.48)	0	58
Female *S. Tawilis*	1 (1.72)	36 (62.07)	0	21 (36.21)	58
Male *S. lemuru*	22 (37.93)	0	36 (62.07)	0	58
Male *S. Tawilis*	2 (0.03)	30 (51.72)	2 (0.03)	24 (41.38)	58

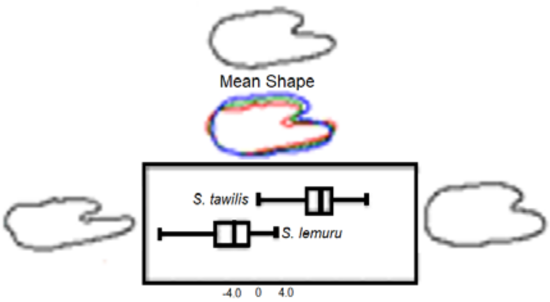

Fig. 5. Graphical presentation of Shape Variations in otoliths between *S. tawilis* and *S. lemuru*

The differences in shapes of otoliths between the two species of *Sardinella* from freshwater and marine environments suggested that the sagittal shape of the otolith maybe influenced by different environmental conditions, food supply, and genetic dissimilarities as suggested by several authors [9, 23, 24]. Taal lake, a freshwater environment where S. tawilis is endemic, is a closed area, and has more stable environmental conditions which may have played a great role in shaping the species' otolith compared to the more open bays of Butuan and other parts of the whole Philippines where S. lemuru can be observed. A number of authors argued that the environmental factors, in a broad sense, have been the most influential determinants of otolith shape, which may also change in response to differences in growth rate [5, 21, 25, 26]. Other elements contributing to differences in otolith shape could include the species behavior [9] which could be linked to changes in food and spatio-temporal roles or niches [27]. Variations in abiotic factors such as salinity and/or temperature [28] or temporary differences in diet characteristics [29] could also have an impact on variations in otolith shapes. The interaction between ontogeny and environmental conditions was possibly mediated by growth rate, that may also have affected otolith shape variations [30]. Faster growth rates usually produce longer and thinner otoliths [31] which may explain the thinner otoliths of *S. lemuru*. *S. lemuru* which thrive in marine environment to reach adulthood earlier, since they live in more open marine waters affected by various environmental factors compared to the more stable freshwater and closed environment in Lake Taal where *S. tawilis* has been found.

CONCLUSION

Results of this study showed the tool of Elliptic Fourier Analysis as an efficient, inexpensive, and more objective method in describing, discriminating, and detecting variations in the otolith shapes within and between the species of *Sardinella*, the freshwater sardine *S. tawilis*, and the marine sardine *S. lemuru*. While no significant variations in otolith shapes between sexes were observed within the species, the differences between species were visibly detected. The differences can be attributed primarily to the differences in habitats of the two species.

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