



Otolith Morphology: Aid to Species Variation of Fishes of Family Leiognathidae

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ABSTRACT

Fish otoliths are calcium carbonated structure used as an aid to differentiate allied species from ten species of the family Leiognathidae along the coast of Maharashtra from October 2018 to February 2019. The species *Gazza minuta*, *Karalla dussumieri*, *Karalla daura*, *Nuchequula mannusella*, *Nuchequula nuchalis*, *Eubleekeria splendens*, *Photopectoralis bindus*, *Leiognathus equulus*, *Deveximentum insidiator*, *Secutor ruconius* of seven genera were differentiated on the basis of otolith morphology using otolith atlas and shape indices. Significant difference was observed in circularity, ellipticity, rectangularity, form-factor and roundness ($p < 0.05$) with highest F ratio for Ellipticity and Roundness. The canonical discriminant analysis based on shape indices indicated 100 percent variability which was validated using Jackknife classification. The first two discriminant functions of the CDA performed with shape indices accounted for variance with all the species significantly differentiated except for *Karalla dussumieri* and *Eubleekeria splendens* overlapping. The Jackknife classification ranged from 18.8 % to 94.4 %, with 58.6% correct classification.

Key Words:- Fish, Leiognathidae, Morphology.

INTRODUCTION

Otoliths are largely calcium carbonate structures found within the ears of all Osteichthyes (bony fish). The semi-circular canals and sensory sacs that form the fish ear, overlay the sensory epithelia located in a structure known as the labyrinth organ which detects gravity, acceleration, retardation and hearing. Otoliths or ear stones are dense calcareous structures contained in three chambers associated with the ear in teleost fishes (Popper *et al*, 1988). The sensory epithelia can be sub-divided into the utricular, saccular and lagenar maculae, each of which has an associated otolith. The utricular otolith is called the asteriscus, the lagenar otolith the lapillus and the saccular otolith the sagitta. There are 3 pairs of otoliths (6 in total) in each individual fish in either side of the fish's cerebral hemisphere. The largest is the sagitta otolith, enclosed within the sacculus bag. The sagitta, because of their size and pronounced inter-specific variations in morphology,

is the most widely used of three otoliths in ecological studies (Nolf, 1985). Some otolith size differences are related to fish growth, but otolith in very large fishes can be much smaller than those in very small fish and vice versa. Based on numerous observations, it was said that as compared to slower swimming fishes faster swimming fishes capable of rapid accelerations and turning tend to have smaller otoliths. Also, otolith shape can vary with sex, population and growth rate (Campana, 2004)

Otolith shape analysis is based on the principle that the shape of the otolith varies geographically, even within a species (Campana and Casselman, 1993) and has high morphological specificity (Lowry *et al*, 1990; García, 1995; Hernández *et al*, 2004). Due to these properties, otolith shape is a useful tool for identifying intraspecific relationships and inter generic relationships (Leguá, 2013). Hussy *et al* (2016) studied growth, age at settling and vertical migration pattern using

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Otolith morphology of *Gazza minuta* and *Eubleekeria splendens*

Features	<i>Gazza minuta</i>	<i>Eubleekeria splendens</i>	<i>Karalla dussumieri</i>	<i>Karalla daura</i>	<i>Nuchequula mannusella</i>	<i>Nuchequula nuchalis</i>
Otolith Shape	Discoidal	Fusiform	Oval	broad and oval	Lanceolate	Elliptical, broad, straight
Dorsal margin	Lobate, slightly irregular	Convex anterior, concave, sloppy posteriorly	Irregular and lobate	Irregular, slanted towards the posterior	Irregular, slanted towards the posterior	Irregular anteriorly, straight sloppy posterior process
Ventral margin	Serrated, slightly V- Shaped	Irregular, slightly serrated on posterior side	Dentate, straight	Dentate, convex	Straight, serrated	Curved and serrated
Posterior margin	Round	Flattened	round with supramedial apex	Round	Flattened	Oblique
Excisura major	Short	Blunt and slant	Deep, acute notched	Developed and notched	Not well developed, irregular	Deeply notched
Excisura minor	Circular	Shallow	Lobate	Circular	Irregularly flattened	Blunt
Ostium	Tubular	Bent curved towards the cauda	funnel-like, longer than the cauda	Curved and tubular	Tubular and parallel to cauda	Funnel like and slant
Cauda	Straight, slightly curved	Tubular, slightly curved	Tubular, straight ending far from the posterior margin	Tubular, slightly curved	Tubular, straight	Tubular, straight
Rostrum	Short and pointed towards the anterior side	Short and tip angled	Broad, long pointed	Long and blunt	Longer straight and pointed	Short and pointed
Antirostrum	Well-developed and longer than rostrum	Absent	Short, pointed	Short and comparatively pointed	Absent	Long, well developed and pointed

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otolith microstructure analysis. According to their observation, relationship between fish and otolith size were found to change at settling, with an increase of fish size in relation to otolith size after settling.

MATERIALS AND METHODS

A total of 353 otoliths were extracted using up through the gill method from the specimen belonging to *Gazza minuta*, *Karalla dussumieri*, *Karalla daura*, *Nuchequula mannusella*, *Nuchequula nuchalis*, *Eubleekeria splendens*, *Photopectoralis bindus*, *Leiognathus equulus*, *Deveximentum insidiator*, *Secutor ruconius* and stored in plastic vials. A cut or rip through the gill isthmus was made and the head was bent backwards away from gill and rest of the body. In smaller fishes (TL <100 mm), the bulla portion of the prootic bone (cavity) where otoliths are situated, was located. In larger fishes, the gill arches were cut and the epidermal, connective and muscular tissues from inferior portion of the neurocranium were removed from the cavity. Due care was taken during this process in order to avoid damage to the otolith. Both the left and right otoliths were used in the present study. The extracted otolith was cleaned by brushing with a soft bristled brush and then washed in freshwater to remove the adhering organic tissue (Secor and Dean, 1992).

Otolith images were captured using stereozoom microscope Olyumpus SZX16 with zoom ranging from 1.25X to 4X. Images of both left and right otoliths were captured. Images of the dorsal and ventral view of left and right otolith were identified and described with the help of otolith atlas (Tuset *et al*, 2008). The shape indices could be linked to the fish sizes, therefore, to remove allometric effects of body size in shape analysis, the theoretical equation outlined below were used to scale data (Lleonart *et al*, 2000):

$$Y_i^* = Y_i [(X_0 / X_i)^b]$$

Where,

Y_i^* - is a transformed measurement (it is a theoretical

value that would measure Y_i if the SL was X_0)

X_i - SL of individual i

X_0 - species-wise mean of the standard length

b - within-group slope regressions of the $\log Y_i$ vs $\log X_i$

The transformed variables derived from the above equation were used for subsequent analyses. Differences in shape indices between species were tested by one-way analysis of variances (ANOVA) followed by post hoc comparison (Duncan's multiple range test). A canonical discriminant analysis (CDA) was performed with shape indices, to compare otolith shape variation among the species. The classification success of the discriminant analysis was checked using Jackknife cross-validation (SPSS Inc.,1999).

RESULTS AND DISCUSSION

Otoliths of Ten species was extracted and digitized for the study of morphological characters and shape indices. Different parts of otolith were identified and described with help of otolith atlas (Tuset *et al*, 2008). Otolith morphology in tabular form is given below

Otolith shape indices varied significantly among the species ($p < 0.05$). Among the five shape indices Ellipticity and Roundness showed the maximum F ratio 253.434 and 268.564, respectively, contributing maximum in the discrimination of the species. Significant difference was observed in all the recorded indices of circularity, ellipticity, rectangularity, form-factor and roundness.

The canonical discriminant analysis based on shape indices revealed that indicate first five functions explained 100 percent variability. The first two discriminant functions of the CDA performed with shape indices accounted for 94.15 variance. All the species were significantly differentiated except *Karalla daura* and *Eubleekeria splendens* which shows overlapping around 40 percentage. The Jackknife classification results validate most of the patterns observed in the CDA analysis. _

Otolith morphology of four species of family Leiognathidae.

Features	Photopectoralis bindus	Leiognathus equulus	Secutor ruconius	Deveximentum insidiator
Otolith Shape	Discoïdal	Rectanglar	Discoïdal	Fusiform, ventrally-straight
Dorsal margin	Irregular, convex	Irregular, slant	Irregular, convex	Anterior convex and posterior concave
Ventral margin	Lobed and serrated	Straight and Crenate	Lobed and slightly serrated	Dentate
Posterior margin	Flattened	Blunt	Round	Flattened
Excisura major	Short and notched	Long and notched	Short and notched	Well-developed, peaked
Excisura minor	Angled, circular and well developed	Oblique and finely lobed	Angled with acute notches	Blunt

The Jackknife classification showed classification of the 10 species. Five species showed the classification above 60 percent, two species between the range of 40 to 60 % and three showed below the 40 % and 40 percentages misclassified of two species. The classification ranges from 18.8 % to 94.4 %. The original groups are 58.6 % correctly classified.

Otoliths are useful because their growth is related to increase in fish size and generally follow an allometric increase in dimensions (Chilton and Beamish, 1982). Therefore, differences in the development patterns of otoliths have been associated with variations in growth rate and have been used for separation of stocks (Messieh, 1972). The interplay of environmental, ontogenetic and genetic have a great influence on otolith shape, which obstructs the application of otolith shape for stock discrimination in mixed-stock scenarios (Hussy *et al*, 2016).

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