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Latitudinal Variation in the Stocks of *Stolephorus commersonnii* Lacepède, 1803 Revealed by Otolith Shape Indices

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ABSTRACT

The spatial distribution of the stocks of Commerson's anchovy, *Stolephorus commersonnii* Lacepède, 1803 was examined by otolith shape indices. Fishes were sampled across four geographical locations across the distributional range of the species along the Indian coast. Five otolith shape indices were calculated and subjected to multivariate analysis. MANOVA revealed the significant differences between the sampling locations. Ellipticity, rectangularity and roundness of otolith have contributed to shape difference. The re-classification success rate from the discriminant function analysis using otolith shape indices was 99.21%. The results indicated the regional variations of stocks on the East coast and the possibility of connectivity with the closest stock on the West coast. This study validates the uses of otolith shape indices to assess the spatial variations other fish species.

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INTRODUCTION

Anchovies are the small, medium-sized pelagic shoaling fishes that are distributed worldwide along the tropical and sub-tropical Indo-pacific regions. They support a lucrative fishery along the east and west coasts of the Indian coast and contribute about 1.45 lakh tonnes of the total marine landings. Genus *Stolephorus*, which is commonly known as Whitebaits, forms a major fishery (105915 t) among the other genus of anchovies like *Thryssa, Setipinnna* and *Coilia* (CMFRI 2018). The well-being of the marine fishing industry of India is determined to a larger extent by the sustained yields from pelagic fish resources like sardines, anchovies and mackerel. The Commerson's anchovy, *Stolephorus commersonnii* Lacepède, 1803, generally occurs in the geographical range of 27˚ N-24˚S with Indo-west pacific distribution at a depth range of 0-50m (Froese and Pauly 2019).

Stock concept in fisheries has been defined in two different terms harvest and genetic stocks. The genetic stock defines the reproductively isolated unit that is genetically different from other stocks, while the interest to fishery managers is the harvest stock, which is a semi-discrete group of fish with definable attributes and subject to a distinct fishery. Since stocks may respond differently to exploitation, delineation of stocks has significant and crucial importance in stock assessment models and for stock-specific management of fishery resources (Aguera and Brophy 2011).

Otoliths are calcium carbonate structure used in various studies such as age determination, fish growth, population dynamics, to study the relationship between the environment and organisms (Lord *et al*. 2012) and fish population studies (Zengin *et al*. 2015). Morphology of the otoliths have been used widely in identifying the stocks of fish worldwide (Duncan *et al*. 2018, Hari *et al*. 2019, Moreira *et al*. 2019). Various techniques are used to identify the differences in the morphology of the otolith, like Elliptical Fourier descriptors, Shape indices, biorthogonal grids (Tuset *et al*. 2019). Methods that differentiate the stocks based upon their genetic differences will provide a conservative measure of the population as very low level of mixing between the population, which will be difficult for fishery manager to conclude the results (Reiss *et al*. 2009). Hence, phenotypic methods which involve traditional, advanced morphometrics and otolith shape were also used for stock discrimination purposes. The significant advantage of otolith shape over the standard body morphology methods for delineating the stocks is that the former is species-specific, highly sensitive to short-term variations in an environment like feeding, spawning, (Zischke *et al*. 2016, Libungan *et al*. 2016).

Even though there are pieces of literature available on the fundamental biological aspects of the Indian anchovy along the Indian coast, there is no compelling information available on the stock structure of this pelagic species. Therefore, the present study aimed to delineate the stock structure of *Stolephorus commersonnii* from the Indian coast based on the otolith shape indices.

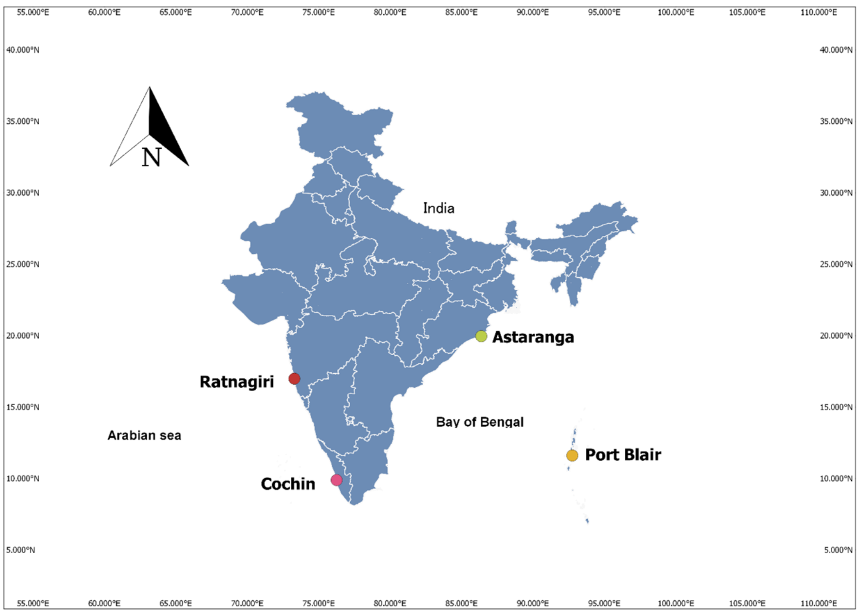

Fig. 1. Map showing the sampling locations of *S.commersonnii* along the Indian coast.

MATERIALS AND METHODS

The samples of *S. commersonnii* were collected from 4 locations along the Indian coast i.e. Port Blair (Andaman and Nicobar Islands), Kochi (Kerala), Ratnagiri (Maharashtra) and Astarang (Odisha) (Fig. 1). The sampling location was selected along the Indian coasts, as the species had a wide distribution along the Indian coast. Specimens were collected from the one-day fishing boats during the period between August 2016 to March 2018. Left and right-side sagittal otoliths were extracted and washed with clean water, air-dried and stored in the plastic vials. For the shape analysis, only left-side sagittal otolith is used.

Images of the left side otolith were taken by stereo zoom microscope SZX16 (Olympus, Inc, Tokyo). All the images were taken at 1 mm scale. Area (Ae), Perimeter (Pe), Length (Le) and Width (We) were calculated with the help of Sigma Scan Pro Version 5.0.0 image analysis software by processing the digitized images of otoliths. Shape indices were calculated following Tuset *et al*. (2003).

The shape indices could be linked to the fish sizes, therefore to remove allometric effects of body size in shape analysis, the theoretical equations outlined below were used to scale data as per Lieonart *et al*. (2000).

$$Yi^* = Yi\ (Xo / Xi)\ b$$

Where, Yi* is a transformed measurement, Xi = SL of individual i, Xo = Overall mean standard length and b is within-group slope regressions of the log Yi vs log Xi.

Multivariate analysis of variance (MANOVA) was used to quantify the shape indices between the stocks. Size-corrected shape indices were subjected to principal components analysis (PCA) to determine the intra-species variations of the otolith. Principal components describe the variance in decreasing order, effectively reducing the variables and identifying the variables responsible for variation (Rohlf and Archie 1984). Linear Discriminant Function Analysis was performed for each site to correctly classify the individuals to their respective locations using jack-knifed classification. All the statistical analyses were performed by using PAST software.

Table 1. Contribution of the principal components in the variation of stocks of *S. commersonnii*.

PC	Eigenvalue	% variance
1	2.86568	57.314
2	1.15822	23.164
4	0.685752	13.715
4	0.279835	5.5967
5	0.010515	0.2103

RESULTS AND DISCUSSION

The MANOVA results showed statistically significant differences in the shape indices between the four regions (MANOVA, $P < 0.05$). The first two principal components accounted for 80.47% of the total variation, where the first and second principal components described 57.31 % and 23.16%, respectively (Table 1). Primarily the stocks were differentiated based on the horizontal axes, which depicts the contribution of PC1 in separation. PC1 was mainly loaded on the ellipticity, rectangularity, roundness, and form factor (Table 2). The Kochi and Ratnagiri stocks form relatively closer clusters when compared with the distinctly separated stocks on the east coast (Fig. 2). The DFA correctly classified 99.21% after jack-knifed cross-validation, validating the use of otolith shape indices for stock delineation of *S.commersonii* in the

Table 2. Correlation coefficient of the otolith shape indices from the principal component analysis.

SI	PC1	PC2	PC3	PC4	PC5
Circularity	-0.42365	0.81786	-0.29335	0.25608	-0.00058378
Ellipticity	0.85137	0.43336	0.2807	-0.061441	0.069285
Rectangularity	0.90962	0.31165	0.26332	0.025058	-0.074233
Form factor	-0.71997	-0.06296	0.44955	0.23612	0.0035539
Roundness	0.78461	-0.4477	-0.17222	0.39256	0.013826

Table 3. Jack-knife classification of *S.commersonnii* based on otolith shape indices from discriminant function analysis.

Stations	Percent correct	Port Blair	Kochi	Ratnagiri	Astaranga
PortBlair	100.0000	30	0	0	0
Kochi	96.5517	0	28	1	0
Ratnagiri	100.0000	0	0	25	0
Astaranga	100.0000	0	0	0	44
Total	99.2188	30	28	26	44

study region (Table 3).

Otolith shape analysis has been used as a reliable marker to differentiate between the fish populations (Hari *et al*. 2019, Deepa *et al*. 2019). The shape of the otolith is linked to the genetic difference between the stocks and the environmental factors prevailing in their respective regions (Vignon and Morat 2010). But the factor which is highly responsible for the formation of otolith is still unclear (Moreira *et al*. 2019). Few studies have indicated that the environment is more influential than the genetic influences (Cappoccioni *et al*. 2011). Studies have suggested that the feeding rate is also a factor responsible for the otolith shape. Further, food availability has variable effects on the growth rate (Hussy *et al*. 2016) and otolith shape is influenced by the growth rate and the increasing differences in the growth rate will increase the discrimination between the stocks. On the west coast of India, the south-west monsoon plays a critical role in triggering environmental features such as temperature, salinity, dissolved oxygen, and nutrient generation, which is responsible for the production

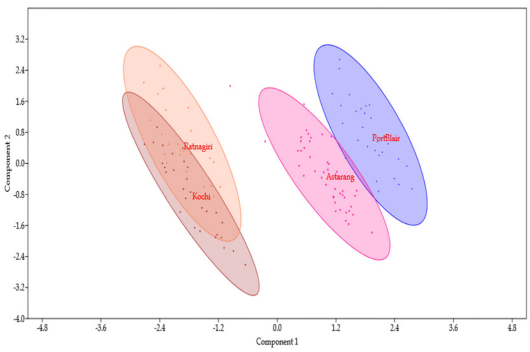

Fig. 2. Principal component plot displaying the differences for the otolith shape indices of *S. commersonnii* collected from four sampling locations in the Indian coast.

of phytoplankton and zooplankton. It can be related to the feeding patterns of *S.commersonnii*, which feeds mainly on copepods, decapods, amphipods and larval bivalves. Otoliths accretion rates also depended upon the salinity (Mazloumi *et al*. 2017). Due to the excessive inflow of freshwater into the Bay of Bengal, the salinity of the Arabian sea is higher than the Bay of Bengal. These salinity differences between the regions might be the factor responsible for the differences in the otolith shape.

Most of the fishes have pelagic eggs and larvae transported over long distances in the open ocean by currents. Due to the absence of geographic barriers, the dispersal will be primarily due to the currents, leading to the transfer of genetic material from one stock to another (Heino 2014). West India Coast Current (WICC) is a surface current, the velocity of which is restricted to the uppermost few hundred meters. The current flows towards the north during January. This may be the cause of the possible mixing of stocks between Ratnagiri and Cochin.

A correctly classified percentage of > 75% is considered acceptable. The overall cross-validated classification of this study revealed 99.21%, which showed that this model has the best discriminatory performance and suggests that the variability in the otolith shape could be a useful tool for the identification of anchovy stocks. Differences among the region and the higher degree of re-classification rates suggest semi-discrete and spatially separated phenotypic stock. These may be due to the separation of post-larval stages in independent environmental regions. The results from this study revealed the separation in the stocks of *S. commersonnii* individuals, indicating the presence of discrete phenotypic local populations and suggest that fishery should be managed as different stocks. Moreover, additional information is needed on the phenotypic variations across temporal scales, and also, the genetic difference between the populations should be considered to complement this study.

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