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Stock structure analysis of *Johnius borneensis* (Bleeker, 1851) from Indian waters

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Johnius borneensis (Bleeker 1851) contributes substantially to the marine fishery of India. The stock structure analysis of the species is essential for its sustainable management and utilization. The study is based on 411 specimens of the species randomly collected from the commercial landings at four marine fish landing centres in India. A truss network with 28 distance variables, based on 10 landmarks, was developed utilizing the digital images of the specimens, by means of tps Dig2 and PAST software platforms. Multivariate test statistics – Mahalanobis distance, Wilks' lambda and Pillais' test indicated significant difference between the East coast stocks and some extent of mixing among West coast stocks. Truss measurements transformed for allometric variations were subjected to Canonical Discriminant analysis and bivariate plot between the canonical variables showed existence of different morphometric stocks of the species. Major truss distances that contributed to the delineation were that on the head and posterior region of the fish body. The truss morphometric traits, that best discriminated the stocks, were subjected to the discriminant function analysis which appropriately classified 80 % of the specimens to the particular location. The present study is the first account on the stock structure analysis of *J. borneensis* from India and will help in developing policies for the management of the fishery and the sustainable utilization of the resource.

[**Keywords**: Croaker, *Johnius borneensis*, Morphometry, Stock, Truss]

Introduction

The contribution of fisheries sector in India to the overall socio-economic development of the country, through employment generation, food and nutritional security and foreign exchange earnings, is well recognized. In accordance with the global trends in marine fisheries management, the fishery managers in India are also focussing on formulating a successful management system that addresses sustainability issues[1]. Employing uniform management systems to entire Indian coast is not yielding desired results. The managers have realised importance of stock and ecosystem based fisheries management, thus increased the interest in developing area or stock specific management practices which requires the identification of various groups/stocks/populations[2].

Geographical isolation results in adaptive changes supported by natural selection and the genotype by environment interaction leading to the development of dissimilar morphological features among fish populations within a species[3]. A fish stock, a subpopulation, independently responds to the consequences of exploitation, as recruitment, growth and mortality within a particular stock are unique[4]. For a rational and effective fishery management, knowledge on the stock structure of an exploited fish population is inevitable, as each stock must be distinctly managed to optimize the yield[5]. Lack of knowledge on the stock structure of a species and its delineation limits the reliability of stock assessments, and thus the effectiveness of management[6]. This has resulted in high exploitation leading to depletion of fish stocks of many species[7,8]. Various stock identification techniques have been adopted by researchers, including the studies on distribution and abundance of life-history stages, marks and morphological characters, otolith chemistry and numerous molecular markers *viz.*, mitochondrial DNA, microsatellite DNA, protein allozymes, etc. Among them, the analysis of morphological measurements is the most commonly used and cost-effective method[9].

Family Sciaenidae is comprised of at least 270 species under 70 genera, worldwide[10] while in India, there are about 48 species belonging to 27 genera; of which, 34 are commercially important[11]. The sciaenid contribution towards the demersal fish landings in India is showing signs of decline; it was 22 %, 19.2 %, 18 %, 18.94 %, 16.5 % and 15.3 % during 2011, 2012, 2013, 2014, 2015 and 2016, respectively[1,12-16]. The contribution further reduced to 15 % in 2017[(ref. 17)]. One of the important sciaenids contributing, to the fishery in India, is *Johnius borneensis* (Bleeker 1851) (= *Johnius vogleri*), commonly known as the sharp toothed hammer croaker or sharp nose hammer croaker. The species is usually assessed along with other sciaenids like, *Otolithes ruber*, *O. cuvieri*, *Otolithoides biauritus*, *Johnius sina*, *J. glaucus*, *J. carutta*, *Pennahia anea*, *Nibea maculata*, etc. In India, *J. borneensis* is found to mature at a total length (TL) of 14 to 16 cm and attains an average size of 240 mm TL in two years[18]. In 2017, croakers dominated and contributed 33.5 %, 19.67 % and 18.07 % of the demersal catch in Maharashtra, Gujarat and Andhra Pradesh, respectively. *J. borneensis* contributed maximum (27.8 %) to the trawl fishery of Maharashtra in 2017[(ref. 17)]. On the south west coast, the contribution by croakers is less than 5 % of the total demersal landings. In Kerala it has shown a decline of 44 % in 2017 compared to the previous year[17]. In Tamil Nadu, croakers formed > 8 % of the total demersal fish landings[17]. The species is consumed by a large section of the coastal population because of its abundance and availability throughout the year. Despite its importance as a cheap source of protein and significant contribution to Indian marine fishery, knowledge on the stock structure of *J. borneensis* is lacking. Hence, the current study was undertaken to investigate the same using truss network analysis and the results may further help in planning for its sustainable management.

Material and Methods

The samples of *J. borneensis* were randomly picked at four commercial marine fish landing centres in India *viz*, Chennai (Kasimedu) in Tamil Nadu and Kakinada in Andhra Pradesh on the East coast and Mumbai (Versova) in Maharashtra and Veraval (Mangrol) in Gujarat on the West coast (Fig. 1, Table 1). The information on the fishing area was collected from the fishermen to ensure that catch is from the specific location.

The specimens collected were kept in an insulated box, in layers with ice in between and then transported to the laboratory. The samples were washed under running tap water and were arranged in plastic trays, to avoid any disfigurements, and the trays were then kept at -20° C in deep freezer till further use. Later, the frozen samples were thawed under tap water, wiped dry with a cotton towel and positioned on a laminated graph sheet, mounted on a flat platform, to capture the images. The graph sheet was used to calibrate the coordinates of digital images. A sheet of polystyrene was placed beneath the laminated graph sheet to enable pinning of the fins. The fins were placed in erected position and

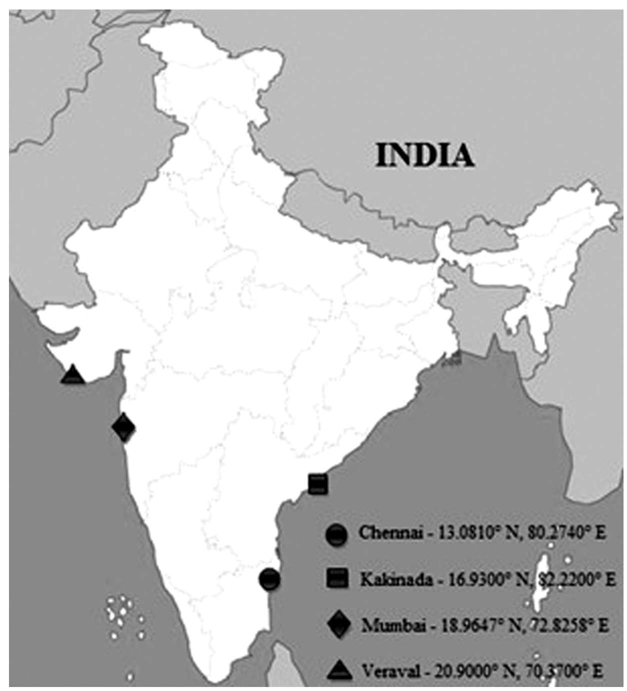

Fig. 1 — Sampling locations along the Indian coast

Table 1 — Sampling details of *J. borneensis* for the present study

Coast (Sea)	Stock	Date	Length range (TL±) (mm)	MSL* ± SE	Sample size	Sex ratio
East (Bay of Bengal)	Chennai	14th October, 2012	154.50 - 222.63	148.93 ± 1.34	92	1.96:1
	Kakinada	27th February, 2013	168.01 - 279.04	195.77 ± 2.09	102	1.14:1
West (Arabian Sea)	Mumbai	8th November, 2012	116.62 -274.96	144.68 ± 2.68	111	1.06:1
	Veraval	21st December, 2012	171.44 - 311.06	196.40 ± 2.70	106	1.5:1
Total					411	1.36:1

±Total Length, *Mean Standard Length

pinned to the laminated graph sheet so that the origin and insertion points were evident. Individuals were labelled with a specific code to identify it in the image for tracking. Digitization of samples was done using a Cyber shot DSC-W300 digital camera (Sony, Japan) mounted on a levelling tripod. Inclination of the tripod and platform were levelled by the bubble level for perfect alignment. After digitization the abdomen portion of each specimen was dissected to identify the sex. The males constituted 48 %, the females 36 % and the indeterminates 16 % of the total number of specimens.

A truss network was developed by interconnecting 10 landmarks for all the 411 specimens and 28 truss measurements were taken from each specimen (Fig. 2). The truss measurements were mined from the digitized samples, utilizing the softwares, tpsDig2 V 2.1[ref. 19] and PAleontological STatistics (PAST)[20].

All truss variables were subjected to logarithmic transformation and tested for normality by means of the PROC UNIVARIATE procedure of SAS[21] and the extreme values, if any, were omitted prior to further analysis. Significant correlations were found between the standard length and truss measurements. To overcome the size dependency, all the variables were transformed as per Reist[22]:

$$M_{adj} = \log M - \beta_1 (\log SL - \log SL_{mean})$$

Where, M_{adj} is the size adjusted truss measurement, M is the original truss measurement, SL is the standard length of fish, SL_{mean} is the overall population mean standard length, β_1 is the coefficient of the overall linear regression of log M against log SL (SL is used instead of TL (total length) to avoid any issues that may arise due to caudal fin distortions).

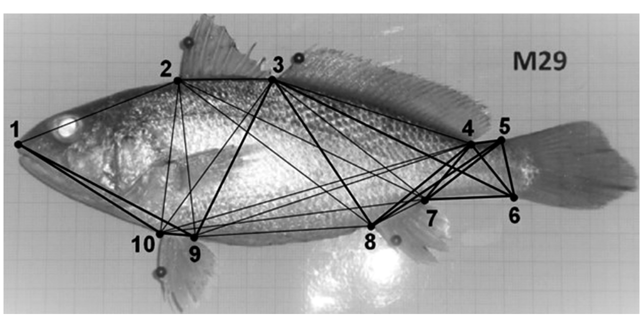

Fig. 2 — Truss network system of *J. borneensis*

To check the effectiveness of the data transformation in eliminating size effect, correlation coefficients between transformed measurements and standard length of the fish were estimated. The size adjusted fish truss measurements were analysed using PROC CANDISC procedure of SAS[21] and canonical coefficients, Eigen values and canonical discriminant plots were produced. Distance statistics included the Squared Mahalanobis distance to find the extent of variation between locations and its significance. The multivariate analysis included Wilks' Lambda to identify the difference between mean scores of various locations considering all the variables simultaneously and Pillais' Trace ($0 \leq P \leq 1$) that calls for rejection of null hypothesis at higher values. Cross validation was done subjecting the variables that had high loadings in canonical analysis, to discriminant function analysis by means of PROC DISCRIM procedure of SAS.

Results

In canonical discriminant analysis, Eigen values of the first and second canonical variables (value >1) were found to be 3.7847 and 1.1658, explaining 73 % and 22.5 % variation, respectively, among the samples, analysed from different locations (Table 2). The pooled within canonical structure had highlighted the significant traits, contributed to the variations (Table 3) that included the measurements mainly on the head portion and those associated with the posterior region of the body i.e, the distances 1-9, 1-10, 3-6, 3-8, 3-9, 4-5, 4-6, 4-7, 4-8, 5-6, 5-7, 5-8, 6-7 and 9-10. These distances are depicted using bold lines in Figure 2. The location wise bivariate plot of Can 1 and Can 2 depicted distinct separation between the Chennai and Kakinada stocks, while some extent of mixing was observed between Mumbai and Veraval stocks (Fig. 3). The Kakinada stock revealed

Table 2 — Canonical correlation and Eigen values depicting effect of location on truss measurements

	Canonical Correlation	Adjusted Canonical Correlation	Approximate Standard Error	Squared Canonical Correlation	Eigen values of Inv(E)*H= CanRsq/(1-CanRsq)			
					Eigen value	Difference	Proportion	Cumulative
1	0.8894	0.8805	0.0104	0.7910	3.7847	2.6190	0.7305	0.7305
2	0.7337	0.7124	0.0235	0.5383	1.1658	0.9350	0.2250	0.9555
3	0.4330	0.3742	0.0414	0.1875	0.2307	0.0000	0.0445	1.0000

a clear vertical separation in comparison to stocks from other locations. The multivariate test statistics and the pair wise Mahalanobis distances between stocks also indicated significant differences between the various stocks ($p < 0.0001$).

Table 3 — Variable loadings in Canonical Discriminant analysis of truss measurements

Pooled within Canonical structure			
Variable	Can 1	Can 2	Can 3
1-2	0.040021	-0.108992	-0.112902
1-9	**-0.310465**	0.230934	**0.402375**
1-10	-0.267198	**0.351153**	0.255667
2-3	0.014323	-0.010301	0.062825
2-7	0.027665	0.104732	0.216244
2-8	0.165946	0.018230	0.201720
2-9	-0.007932	0.219079	0.078020
2-10	-0.112546	0.115174	0.034790
3-4	0.097918	-0.250300	0.299038
3-6	0.277945	-0.089951	**0.405601**
3-7	0.202771	0.169801	0.271682
3-8	**0.383903**	0.102951	**0.312873**
3-9	0.067191	**0.330105**	-0.070483
3-10	-0.079558	0.193243	0.002072
4-5	-0.020869	0.287783	**0.301031**
4-6	0.295017	**0.328147**	0.224100
4-7	0.275830	-0.130830	**0.319741**
4-8	0.113195	-0.087602	**0.484953**
4-9	-0.062516	0.041725	-0.009723
4-10	-0.174450	-0.059920	0.129357
5-6	0.252549	**0.435861**	0.066239
5-7	0.158031	-0.031026	**0.430720**
5-8	0.031142	0.068742	**0.574256**
6-7	**0.310576**	-0.254192	**0.357992**
7-8	-0.235021	0.218782	0.229616
7-10	-0.268174	0.179443	-0.029340
8-9	-0.047106	0.145055	-0.229836
9-10	-0.244818	-0.208242	**0.352698**

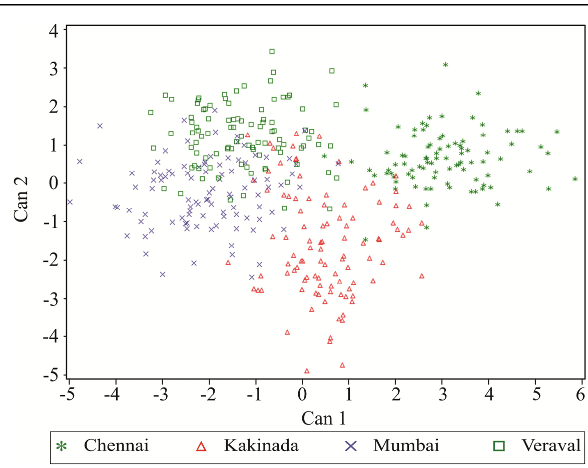

Fig. 3 — Canonical Discriminant plot between first and second canonical coefficients

The Wilks' lambda value of 0.20 for the location wise effect on the specimens from the East coast and 0.44 for the specimens from locations within the West coast also show highly significant difference between the specimens within the coast. The lower Wilks' lambda value for the East coast reveals that the separation of specimens from locations within the coast is more pronounced as compared to the West coast. Similarly, higher value of Pillais' trace for East coast (0.8) and lower value for West coast (0.56) also reveals the same. This is made further clear by the biplots, showing some extent of mixing between the specimens from the two locations in West coast (Fig. 4), but well separated stocks of East coast (Fig. 5). Subset of the major truss morphometric characters that discriminated the specimens from the four locations, selected by the stepwise discriminant function analyses, were utilized to generate a

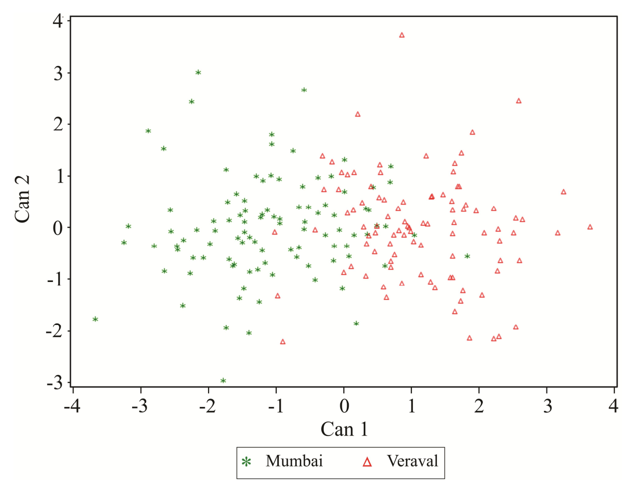

Fig. 4 — Canonical Discriminant plot between first and second canonical coefficients for west coast

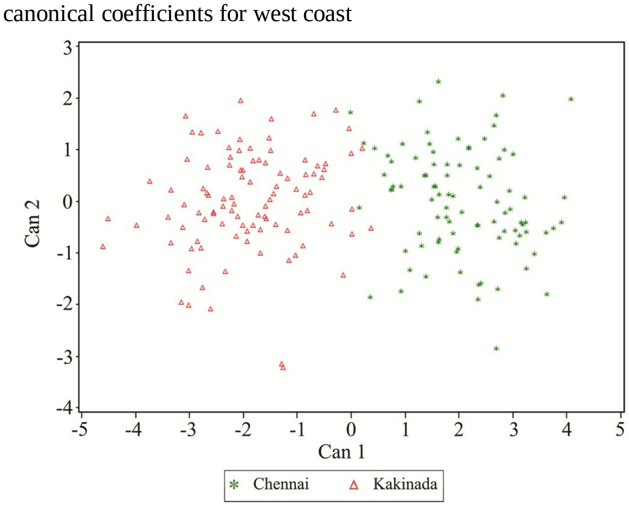

Fig. 5 — Canonical Discriminant plot between first and second canonical coefficients for east coast

Table 4 — Classification of specimens to respective locations based on Discriminant Function analysis (in percentage)

From stock	Chennai	Kakinada	Mumbai	Veraval
Chennai	**95.6**	2.2	0	2.2
Kakinada	8	**75**	3	14
Mumbai	1	4	**74**	21
Veraval	0	5.21	18.75	**76.04**

discriminant function for classifying individuals to different stocks. The selected distances were 1-2, 1-9, 2-9, 2-10, 3-4, 3-6, 3-7, 3-8, 4-6, 4-8, 4-9, 5-6, 5-8, 6-7, 7-8, 7-10 and 8-9. The Discriminant Function Analysis (DFA) of these characters showed an overall classification accuracy of 80 %; however, it was 95.6 % for Chennai, 75 % for Kakinada, 74 % for Mumbai and 76.04 % for Veraval individuals (Table 4).

Discussion

Fish exhibit great variation within and between populations compared to other vertebrates, and are highly susceptible to morphological variations induced by the surrounding environment[23]. These variations among specimens should be attributable to body shape differences, and not related to the relative size of the fish. So, to measure the actual aspect of the body shape and to eliminate any size effect, the truss distances were scaled to standard length of fish. In the present study, after transformation, none of the standardized truss measurements in the data set showed significant correlation with the standard length of the fish, indicating that the transformation effectively eliminated the size effect. No significant difference ($p > 0.05$) was found in the truss variables among different sexes and hence, data for both sexes were combined for all subsequent analysis.

The canonical discriminant plot (Fig. 3) has shown clear separation of the samples from Chennai and Kakinada while some extent of mixing was found between the Mumbai and Veraval samples i.e, higher rate of similarly was seen within the samples from West coast than within East coast. Similar results were observed during the stock structure studies on *Harpodon neherius*[24], *Megalaspis cordyla*[25] and *Decapterus russelli*[26]. Significant difference between the Chennai and Kakinada samples in the East coast is found to be mainly because of the variation in the caudal peduncle region (truss distances 4-5 and 5-6). This may be attributed to the variability in environmental conditions that prevail in both the locations, as the development of morphological traits during early life history stages is dependent on environmental conditions[27,28]. The morphological variation observed might also indicate that the majority of individuals of Bay of Bengal spend their entire life in distinct regions[25]. Environmental factors such as temperature, salinity, food availability, or prolonged swimming prevailing in a location may determine the phenotypic variations among the individuals[29-32]. There may also be different spawning stocks of the species in Chennai and Kakinada as similar findings were reported for horse mackerel from Mandapam and Digha coasts of India[25].

Minimum phenotypic differences among individuals and the incidence of mixing between the populations of the Arabian Sea as an indication of migration of individuals from various locations to adjacent areas representing a unit stock of the species along the West coast was reported earlier[25]. Similarity between individuals from far off locations could be due to the equivalent environmental factors existing in the locations or even by similarities in the genetic pool that persisted during the evolution. As the present study depended on the commercial catches, the mixing of specimens from west coast may also be due to the fishermen, from both Veraval and Mumbai, carrying out fishing operations in same fishing area.

The presence of significant differences of morphometric characteristics among the specimens from east coast indicates limited mixing at spawning time, and the spawning groups can be considered as unit stocks for management purposes[33]. Further studies using molecular methods may be used to find whether true genetic stocks of the species exist. It may also form a base for further studies like stock assessment that will help in determining the kind of management strategies required. Currently, the advanced developments in techniques for detecting specific stocks have been significant with improved possibilities of extending stock specific advice related to fisheries[34]. Future studies on *J. borneensis* by employing other available tools, such as otolith microchemistry, molecular genetics, fatty acid composition, and so on, to substantiate the findings are also advocated. The present study thus recommends for specific management options for the different stocks of the species inhabiting along east and west coasts of India.

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Conflict of Interest

Authors declare no conflict of interest.

Author Contributions

SS: Investigation, Writing - original draft; AKJ: Conceptualization, Supervision, Writing - review & editing; SJ: Supervision - data analysis; GV: Investigation; SKC: Supervision, Writing - review & editing.

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