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Stock structure analysis of *Nemipterus bipunctatus* (Valenciennes, 1830) from three locations along the Indian coast

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Present study was done to identify the occurrence of various stocks of *Nemipterus bipunctatus* along the Indian coast, based on their body and skull shape morphometrics. Fish samples were collected from three locations along the Indian coast viz. Chennai along the East coast and Mumbai and Veraval on the West coast. Twenty truss distances from nine-point truss network of body and twenty-one truss distances from eleven-point truss network of the skull were measured from each fish sample. The canonical discriminant analysis showed that the truss distances belong to the anterior region and caudal peduncle of body and olfactory region of skull were significant in separating the fish stocks. The artificial neural network analysis revealed 91.4 % and 86.14 % well classification of the specimen, based on the truss distances of body and skull respectively. The results from the study indicated that there is a significant difference among the stocks of *N. bipunctatus*.

[Keywords: Body morphometry; Delagoa threadfin bream*; Nemipterus bipunctatus*; Skull morphometry; Stock structure; Truss network analysis]

Introduction

A fish stock can be defined as a subset of species which encompass an intraspecific group of randomly mating individuals with spatio-temporal integrity[1]. As an intraspecific group of individuals, fisheries management measures without considering the stock structure of a fish species may consequently lead to its overexploitation[2]. Hence, it is important to perceive more information on stock structure of a target fish species for the development of well outlined policies and sustainable management measures in order to optimize the yield of its multiple stocks which are differentially exploited[3].

Stock identification studies based on meristics, morphometrics, otolith microchemistry, molecular genetics etc., were practiced in fishes[4-7]. Morphometric analysis offers more efficacious, facile and powerful techniques which essentially discriminates 'phenotypic stocks' that are groups of individuals with homogenous growth, mortality and reproductive rates[8]. A better description of shape is possible with the analysis of morphometric characters using truss network system[9]. Also truss network analysis helps to disclose more complex aspects of shape differences in intraspecific population, in comparison to traditional morphometric methods[10].

Threadfin breams under the family *Nemipteridae* constitute nearly 18 % of the total demersal finfish landings and 4.5 % of total marine landings in India[11]. *N. bipunctatus* is one of the important threadfin bream species available in Indian waters[11]. Considerable landing of this species was started reporting from some maritime states of India such as Maharashtra, Tamil Nadu and Gujarat in recent years[11]. It is a benthic carnivorous species, found on sand or mud bottoms in depths between 18 to 100 m. Studies were conducted on the biology and fisheries of *N. bipunctatus* in India[12,13]. But in spite of its commercial importance, extensive study has not been undertaken to identify stock structure of this species along the Indian coast. The present paper seeks to study the stock structure of *N. bipunctatus* from three sites along Indian coast based on truss network analysis so that the management measures in future can be designed based on assessments from independent fish stocks.

Materials and Methods

Sampling

Samples of *Nemipterus bipunctatus* along Indian coast were collected from three different locations two along the North west coast, Mumbai (Maharashtra State) and Veraval (Gujarat State), and Chennai (Tamil Nadu State) along the South east coast (Fig. 1). The sampling sites were selected as per the information available from the reports of Central Marine Fisheries Research Institute (CMFRI) and the number of sampling sites was limited within the scope of the study. A total of 303 fish Samples were collected randomly during October to December 2012. Fish samples were collected from the commercial landings after obtaining information about fishing area from the fishermen. The sampling period was designed based on the spawning season of the fish, so that the mixing of the putative stock is less during that period[14]. The peak spawning season of *N. bipunctatus* was reported from September to January along the Vizhinjam coast[12] and a prolonged spawning season from October to February off Tuticorin coast[15].

Digitization of fish samples

The samples were first cleaned in running water, wiped with clean thin cotton towel and placed on a flat platform with laminated graph paper, keeping left side up. The graph paper with fixed horizontal and vertical grids was used in calibrating the coordinates of digital images. The fins were kept erect by pinning so as to make the origin and insertion points clear, which is essential while selecting homologous landmarks around the outline of fish form. A block of expanded polystyrene (2 cm thickness) was placed beneath the graph sheet to facilitate pinning. Digital images of each fish sample were captured using a Sony cyber shot DSC-S300 digital camera (Sony, Japan).

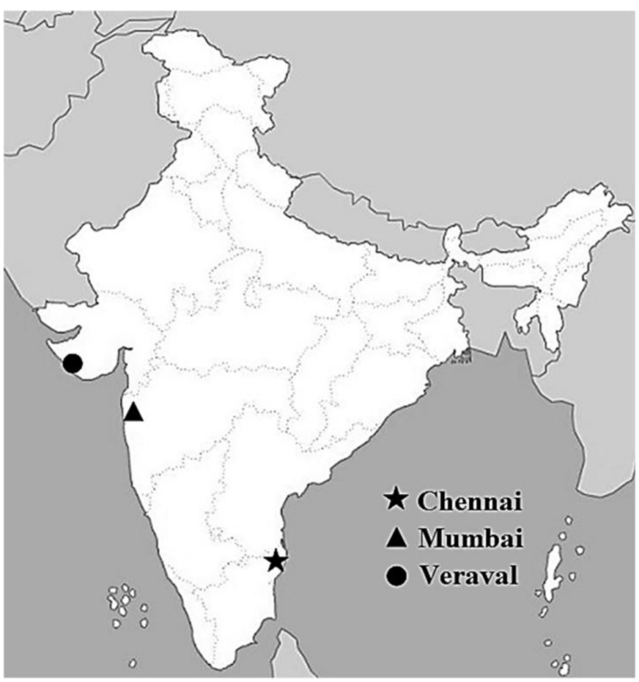

Fig. 1 — Location selected for sampling of Delagoa thread fin bream, *Nemipterus bipunctatus*

Collection and digitization of fish skull

The head of fish samples was lopped after the digitization of fish samples and kept in 95 % alcohol for hardening. It was then boiled just enough to loosen the muscles and washed in a mild jet of water to remove the loosened muscles and skin. The skull was carefully removed and air dried without exposing it to direct heat or sun light. Air dried skulls were transferred to 1 % KOH in distilled water and kept for 24 h. It was then washed thoroughly again in mild pressure of water and air dried properly before staining. A stock dye of Alizarin Red was prepared for staining[16]. Dried skulls were transferred to 1 % KOH and Alizarin Red was added drop wise (3-4 drop). The stained skulls were kept for three days for drying.

Out of 303 fish samples, 231 intact skulls could be obtained, which were used for further analysis. For digitization the stained skull was placed on a flat platform with laminated graph paper in such a way as to obtain the dorsal view of the skull. A light source was provided below and above the laminated plane to improve the clarity of the image. Digital images of each skull were captured using a Sony cyber shot DSC-S300 digital camera (Sony, Japan).

Measurement of truss distances of body and skull

The geometric morphometric analysis called truss network analysis based on a series of truss network measurements that form a regular pattern of connected quadrilaterals, was selected for both body and skull of fish. These measurements were calculated on the basis of morphologically significant anatomical locations on fish body or skull called as 'landmarks'. The landmarks were digitized on the image using the software "tpsdig2 V2.1"[17] and the data were encrypted into the tps files in the form of X-Y coordinates. A total of twenty truss distances were measured along the entire body surface on left side of the fish based on nine landmarks (Fig. 2). For the digitized skull image another truss network was formed by colligating 11 landmarks, forming 21 truss distances (Fig. 3). Paleontological Statistics (PAST) software[18] was used to extract the truss distances between pre-determined anatomical landmarks on each fish and skull specimen.

Analysis of truss morphometric data

The truss distances were first tested for outliers, based on Cook's distance estimates using PROC ROBUSTREG procedure of SAS Version 9.3[19] to

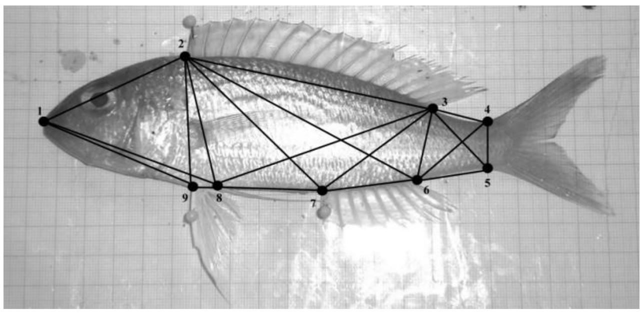

Fig. 2 — Truss network series of body with interconnected landmarks

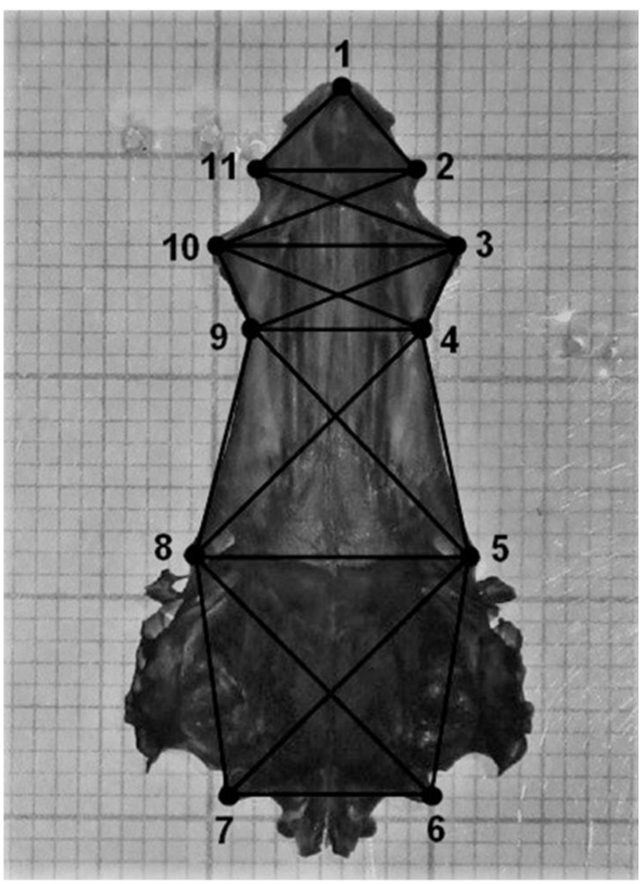

Fig. 3 — Truss network series of skull with interconnected landmarks

remove potential outliers from further analysis. A total of 23 fish samples were excluded from further analysis as they may have led to biased inferences. There were significant correlations between the standard length of fish and truss distances on the body and the standard skull length and truss distances of the skull. To overcome the length dependency, the transformation of absolute measurements into size independent shape variables was carried out by a modified formula originally given by Ihssen *et al*.[1] and Hurlburt and Clay[20]:

$$D_{trans} = D \times \left(\frac{SL_{mean}}{SL}\right)^b$$

where,

D_{trans}: transformed truss distance

D: original truss distance

SL: standard length of fish*

SL_{mean}: mean standard length

b: Within-group slope of the regression oflog D on log SL

* Standard skull length is used for transformation of skull truss measurements

The effectiveness of transformation of truss distances of body and skull was ascertained by estimating the correlation coefficients of transformed truss measurements with the standard length of fish and standard skull length data, respectively. The transformed truss distances from body and skull were subjected to canonical discriminant function analysis using the CANDISC procedure of SAS[19] to extract linear combinations of the canonical variables to obtain the maximum differences between various groups. Cumulative variance explained by the canonical axes and biologically meaningful groupings of the traits loaded on each canonical axis were taken into consideration for grouping the samples.

The truss distances loaded on the first two canonical axes were selected based on the Hatcher's scratching procedure[21]. The Artificial Neural Network (ANN) analysis was employed using NEURAL MODEL option in JMP 8[19] to find out the accuracy of classifications of observations among different locations and coasts on the basis of the first two canonical axes scores. ANN is a nonlinear mathematical structure capable of representing complex nonlinear process that relates the inputs to the outputs of a system[22]. The analysis was separately carried out for transformed body truss distances and transformed skull truss distances. The structure of a neural net consists of connected units referred to as "nodes" or "neurons". Each neuron performs a portion of the computations inside the net. A neuron takes some numbers as inputs, performs a relatively simple computation on these inputs, and returns as an output. Output value of a neuron is passed on as one of the inputs for another neuron, except for neurons that generate the final output values of the entire system. In the present study, we used a single layered feed forward network (SLFN) of 3 hidden nodes with the following specifications (Fig. 4).

1) Input layer: The first two canonical axes scores (Can1 and Can 2)
2) Hidden layer: 3 hidden nodes; H1, H2 and H3
3) Output: Region (Location/coast)
4) Over all penalty =0.01
5) Number of tours = 8
6) Iterations = 500
7) Training data set = 65 % of the observations
8) Cross validation = Random Hold back

Results

The correlation coefficients between size adjusted variables and standard length reduced considerably, indicating that the data transformation was effective in removing the effect of size. In the present study, the mean standard length of all the three populations was significantly different from each other (Table 1). The canonical discriminant analysis on truss distances of body and skull revealed that there is a significant variation between stocks from all the different sampling locations and the variables with high loadings on the first two canonical axes were found useful in distinguishing these samples. The ANN analysis based on first two canonical scores of the body truss measurements differentiated the

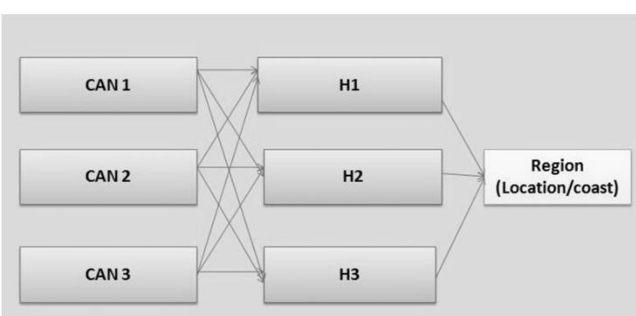

Fig. 4 — Single layered feed forward artificial neural network with 3 hidden nodes (H1, H2 and H3).

individuals to their respective populations with an accuracy of 91.4 % and the accuracy of classification based on skull truss distances was 86.14 %.

Differentiation of the stocks based on truss network analysis of body

Eigen value for first canonical axis was 2.22 and that for the second canonical axis was 0.9238 and they explained 70 and 22 percent of the between group variations, respectively. The pooled within group correlation of canonical axes revealed that the truss distances 1-2, 2-3, 2-6, 2-7, 2-8, 3-4 and 3-5 contributed to the first canonical axis (Can 1) and truss distances 3-7, 3-8, 3-9, 4-5, 4-6, 5-6 and 6-7 contributed to the second canonical axis. The bivariate scatter plot of canonical axes (Can1 and Can2) figured out a better discrimination of the *N. bipunctatus* stocks from three locations with limited mixing among each other (Fig. 5a).

Differentiation of the stocks based on truss network analysis of skull

Eigen value for first canonical axis was 0.9088 with a between group variability of 62 % and the Eigen value for the second canonical axis was 0.5505 with a between group variability of 35 %. In Canonical discriminant analysis for skull the truss distances 1-2, 1-11, 3-4, 3-9, 4-10 and 9-10, which are truss distances from the olfactory region and anterior portion of orbital region of the skull contributed to the first canonical axis (can 1). The truss distances contributed to second canonical axis (can 2) are; 3-11, 4-5, 4-8, 5-6, 5-7, 6-7, 6-8, 7-8 and 8-9 which are on orbital and otic region of the skull. The bivariate scatter plot of the canonical axes (Can1 and Can2) depicted a better discrimination between the fish stocks with limited overlapping (Fig. 5b).

Classification by ANN

The first two canonical axes scores were taken for classifying samples among different stocks. They clearly discriminated fishes to their respective locations, with an accuracy of 91.4 % (Table 1). Similarly, for the skull specimens, accuracy of classification is 86.14 % (Table 1).

Discussion

The canonical discriminant analysis of transformed truss distances of body and skull showed that there is a significant morphometric heterogeneity among *N. bipunctatus* stocks from the three selected locations.

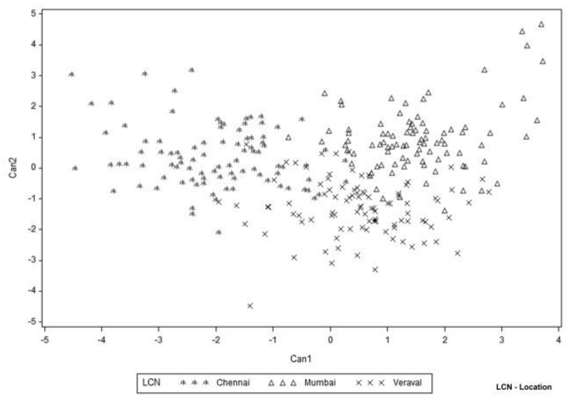

Fig. 5a — Stock wise canonical discriminant plot between first and second canonical coefficient for the truss distances of fish body.

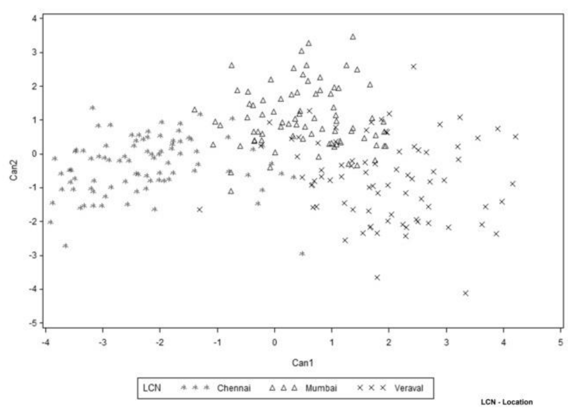

Fig. 5b — Stock wise canonical discriminant plot between first and second canonical coefficient for the truss distances of skull

The classification using ANN has also confirmed the above result. The truss distances are transformed into size independent shape variables during statistical analysis as the morphometric variations should be attributable only to the body shape differences, and not relative to the size of the fish[23]. There are no significant differences between the male and female specimen of the species and that rejected the chance of occurrence of sexual dimorphism in the species. Therefore, all the specimen was clubbed together for the truss network analysis as the shape difference was not evident between male and female specimen.

Morphological difference between the three fish stocks may be attributed to the difference in their location of existence[24]. Organisms of same species ensue morphological variations in wild while experiencing different ecological factors[25]. This may be due to extended use of morphological structures in that particular condition[26] or an important adaptive strategy for populations experiencing inconsistent environments[27,28]. Moreover, phenotypically plastic traits of marine organisms are potentially affected by environmental factors such as temperature and salinity, which preclude adaptation to a particular habitat[29]. Difference in physical and ecological conditions are evident from Bay of Bengal and Arabian Sea situated along the East and West coast of India, respectively[30-33]. These divergent environmental conditions may cause morphological variations to the fish species living in that habitat.

Table1 — Cross-validation of individuals classified by neural network analysis. Percentage of fish from each location or coast (in rows) classified by ANN to their respective groups (in columns) based on body truss and skull truss distances, collected from the respective locations.

Body truss distances							
Location	n	SL*	Veraval	Mumbai	Chennai	Total rate of classification (%)	Total rate of misclassification (%)
Veraval	100	15.45^a	90.5	3.2	6.3		
Mumbai	103	14.41^b	7.7	91.2	1.1	91.4	8.6
Chennai	100	13.59^c	6.4	1.1	92.5		
Skull truss distances							
Location	n	SKL**	Veraval	Mumbai	Chennai	Total rate of classification (%)	Total rate of misclassification (%)
Veraval	71	3.23^a	85.3	9.8	4.9		
Mumbai	75	2.96^b	12.6	81.3	6.1	86.1	13.9
Chennai	85	2.72^c	2.7	5.3	92		

*SL - The mean standard length in cm, n - the total number of samples
**SKL – The mean skull standard length in cm
*means bearing the same superscript within a subgroup are not significantly different from each other ($p \leq 0.01$).

Body Morphometry

The analysis of the shape morphometry of the body revealed that the majority of the variations were associated with the traits of head region and the posterior body portion including the caudal region. The morphometric variations in the anterior body portion and caudal region was reported in the stocks of *Megalaspis cordyla* populations in Arabian Sea and Bay of Bengal based on the truss network analysis[5]. The variations in the head region may be attributed to the change in feeding pattern of the fish[34]. *N. bipunctatus* is a carnivore fish which mainly feeds on benthic crustaceans, finfishes, cephalopods and polychaete worms[35]. The study on the feeding biology from west coast of India more clearly depict that this species is mainly a zoo-benthic feeder and occasionally column feeder[36]. Spatial variation in the feeding pattern of a related species *N. japonicus* is reported along East and West coasts of India[34]. Moreover, the variation of feeding pattern of fishes totally depends on the food availability and environmental conditions at a particular habitat[37].

Head related variations also suggest the impact of diverse ecological conditions to which the populations are exposed[38]. Morphological variation on the caudal region may be attributed to the variation in turbulence of water or the fluctuation in the temperature and salinity of the water[39,40]. The Bay of Bengal is more turbulent than Arabian Sea[41] and other hydrological conditions such as water temperature, salinity, productivity, etc., also vary between the Arabian Sea and Bay of Bengal[42,43].

Skull Morphometry

The analysis of skull shape was exercised widely on many major vertebrate phyla for studying the spatio-temporal variations in the morphology[44-46], but much work was not done on truss network analysis of fish skull. Also, it is clearly pointed out that the skull can be chosen for morphometric investigation because of its presumed ability to reflect any local adaptation in feeding biology with respect to different environment[47]. Here the truss network analysis of skull clearly depicts a significant difference in the anterior and otic regions of skull. As *N. bipunctatus* is a carnivore's fish species, the different feeding strategies in changing ecological conditions may bring changes to skull structures. Various researches also suggest that predatory or carnivore's fishes may show some morphometric variation on the skull as an adaptive strategy for prey capture and drag reduction in different habitats[48,49]. In addition to feeding pattern of fishes, the influence of environmental parameters of the water body may also bring shape variations on the skull[50,51]. The latitudinal and longitudinal variation of environmental parameters are evident from the literatures on Arabian Sea and Bay of Bengal as discussed early. A significant difference in the skull morphometrics of the different stocks of oceanodromous Baltic Sea herring from different spawning locations, across various temperature gradients were also reported[47]. Moreover, the truss network analysis of the entire body of *N. bipunctatus* discloses a significant variation in head region among different stocks. The possibilities of variations in the morphometry of skull due to changes in head region were reported in aquatic animals[52]. The present study using truss network analysis revealed more complex variation in skull structure that can be an evidence for the occurrence of heterogeneous stocks.

Conclusion

Significant variation in the phenotype of *N. bipunctatus* based on the truss network analysis of whole body of fish and skull well elucidate the existence of three different stocks along the Indian coast. The occurrence of same species in multifarious environmental condition may leads to the variation in the phenotypes, which is argued to be the optimal strategy to suit the local environment. Moreover, truss network analysis of the skull is performed for the first time in fish. The result of the present work is useful in developing the preliminary guidelines for the assessment of exploited populations of *N. bipunctatus*. It is mattering much to acquire the necessary knowledge about the stock structure of a commercially exploited species for proper management before exploitation proceeds too far.

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