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Morphometry based identification of *Nemipterus japonicus* unit stocks from west coast of India

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Abstract

Morphometric analysis of *Nemipterus japonicus* (Bloch) was studied along the west coast of India by using conventional approach. A total 435 fish samples were collected from six geographical locations *viz.*, Veraval, Mumbai, Ratnagiri, Panaji, Malpe and Cochin representing five states along the west coast of India. Total 15 homologous landmarks to get 16 linear distances were used for the stock structure analysis. Multivariate statistical methods of Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA) effectively segregated the fish populations. Both PCA and DFA distinctly identified Ratnagiri and Cochin stocks from the remaining populations. PC1 accounted for 95.25% of total variation. The DFA method correctly classified 92.3% of original grouped cases and 90.5% of cross-validated grouped cases. Thus, morphometric analysis showed the existence of Ratnagiri and Cochin as separate stocks and mixing for Veraval, Mumbai, Panaji and Malpe stocks.

Keywords: *Nemipterus japonicus,* Stock discrimination, morphometric analysis, conventional morphometrics

1. Introduction

Nemipterid fishes commonly known as threadfin breams and whiptail breams are distributed widely in tropical and subtropical waters of Indian and western Pacific Ocean. These fishes are abundant beyond 50 m of depth but show higher concentration at 100-200 m as revealed by the exploratory surveys and experimental fishing [1]. Threadfin breams are one of the most dominant species among the demersal fisheries of India, which are being exploited by commercial trawlers throughout the year [2]. Fishery Survey of India (FSI) had identified threadfin bream resources from Indian waters during the year 1948, but even after introduction of trawling in 1960s, their catch was recorded as trash fish, as trawling operations were mainly shrimp targeted. Threadfin breams from an important demersal fishery resource along the Indian coasts and of late, they have been receiving increasing attention because of their use in manufacture of surami and surami-based products. Almost 90% of the threadfin bream fishery of India is contributed by *Nemipterus japonicus* and *N. mesoprion* [1]. *N. japonicus* (Bloch 1791), the Japanese threadfin bream, has widespread distribution throughout the Indian Ocean and is distributed in the tropical waters [2]. The principal regions supporting *N. japonicus* fishery are the Mediterranean, Red sea, east and west coast of India, Sri Lanka, Andaman, and west coast of Malaysia. The catches of *N. japonicus* populations managed within the judicial limit of Indian EEZ have been fluctuating substantially over the last decades. In many places the catch declined drastically, creating serious economical, social, and ecological problems and accurate assessment of biomass indices are becoming crucial for sustainable harvest of the resource [3]. It is a benthic species, very much abundant in coastal waters with muddy or sandy bottoms; found in 50-80 m depth zone usually in schools [4]. The species has been studied widely for various aspects like maturity, spawning and fecundity [5, 6], biology [7, 8], [9-12], population dynamics [13, 14] and morphology [2], but studies pertaining to morphometerics is scanty. The long-term isolation of populations and interbreeding can lead to morphometric variations between populations, and this morphometric variation can provide a basis for population differentiation. Morphometric differences among stocks of a species are recognized as an important tool for evaluating the population structure and as a basis for identifying stocks [15]. Comparative examination of morphological characters was one of the traditional methods of distinguishing fish taxa and stocks [16]. The multivariate methods like principal component analysis of morphometric traits have been proposed as an efficient tool for stock identification in fishery management programmes [17]. The major objective of the present study was to understand the structuring of population of *N. japonicus* from geographically different environs, using conventional morphometric approach.

2. Materials and Methods

2.1. Sample collection: A total of 424 intact specimens of *N. japonicus* were procured from the six locations along west coast of India (Fig. 1). Sampling was done from each of the six stations during May 2013 and July 2013 such that, no mature fish were included in the sample. The details of samples are given in table 1. All the samples were transported in icebox at 4 °C from sampling station to laboratory for further analysis.

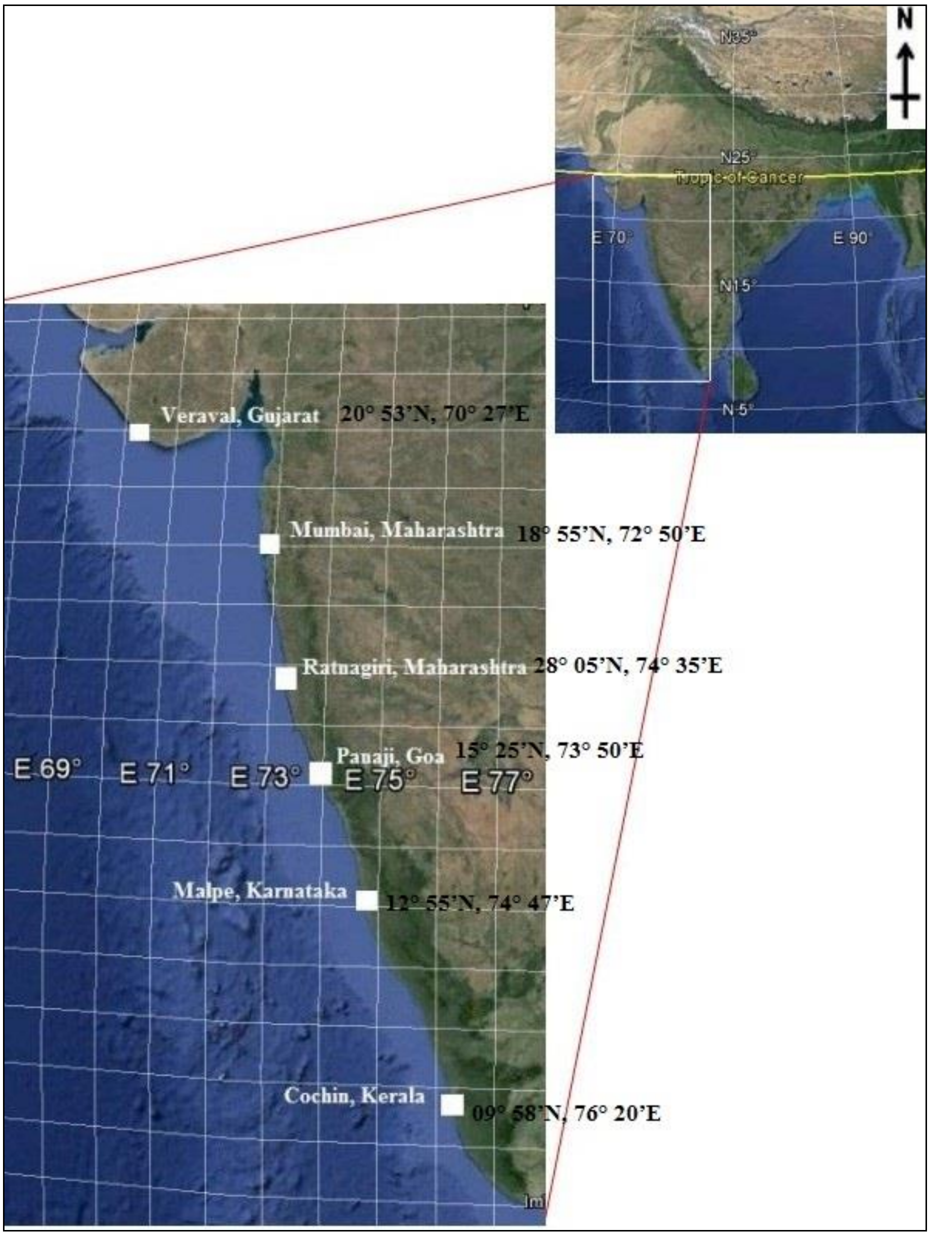

Fig 1: Six sampling locations along the west coast of India

Table 1: Details of samples of *Nempiterus japonicus* collected from west coast of India.

Sampling location	Sample size	Sex ratio(M:F)	MSL±SE
Veraval	107	1:1.2	14.22±0.12
Mumbai	76	1:1.62	13.16±0.14
Ratnagiri	72	1:1	8.47±0.09
Panaji	52	1:1.96	17.01±0.15
Malpae	52	1:1	15.17±0.18
Cochin	65	1:1.03	21.11±0.20
Total	424	1:1.20	14.22±0.12

2.2. Data acquisition: The digitization of the image of samples were taken after thawing the fish under running tap water, wiped well and placed flat platform with vertical and horizontal grids (Fig. 2). The distances between the vertical as well as the horizontal grids were fixed such that, one square unit covered an area of 1cm^2 and used in calibrating the coordinates of digital images. The fins were erected and placed on a platform in such a position that it makes the origin and insertion points clearly visible. Each specimen was labelled with a specific code to identify it in the image. For digitizing images of fishes, a cyber shot DSC-W630 point & shoot (image resolution: 16.1 megapixels) was mounted on a levelling tripod with a bubble level as an indicator of the inclination, and the images were taken. After digitization of the samples, the fishes were dissected to confirm the sex of the fish, as there is no sexual dimorphism known in this fish other than size difference [7].

2.3. Measurement of morhometric data: Photographs were digitized by using a personal computer (Windows®7 operating system). Data was generated by using software tpsDig2 V20.1 [18], Paleontological Statistics (PAST) software [19]. Fifteen homologous landmarks [20] were selected for stock structure analysis (Fig. 2). Description of the landmarks and associated distances are given in Table 2.

Table 2: Morphometric distances and associated landmarks for conventional morphometrics.

Sr. No.	Symbol*	Description*	Distance	Associated Landmark	Description
1	U	Anterior tip of snout at upper jaw	UO	1 – 2	Pre-orbital distance
2	O	Anterior edge of the orbit	UD	1 – 3	Pre-dorsal distance
3	D	Dorsal fin insertion	UD’	1 – 5	Post-dorsal distance
4	D_1	Base of 4th dorsal spine	UT	1 – 6	Total length
5	D’	Dorsal fin termination	UF	1 – 7	Fork length
6	T	Midpoint in line with tip of the lower lobe of caudal fin	US	1 – 8	Standard length
7	F	Membranous tip of caudal fin at fork	UA’	1 – 9	Post-anal distance
8	S	Posterior tip of Urostyle	UA	1 – 10	Pre-anal distance
9	A’	Anal fin termination	UP	1 – 12	Pre-ventral distance
10	A	Anal fin insertion	UP_c	1 – 13	Pre-pectoral distance
11	P’	Pelvic fin termination	UO_p	1 – 14	Head length
12	P	Pelvic fin insertion	UO’	1 – 15	Post-orbital distance
13	P_c	Pectoral fin insertion	OO’	2 - 15	Eye diameter
14	O_p	Point on operculum at maximum width	DD’	3 – 5	Dorsal fin base length
15	O’	Posterior edge of orbit	D_1P'	4 – 11	Body depth
16			A’A	8 – 10	Anal fin base length

*Symbols and description as per Laevastu (1965) with little modifications.

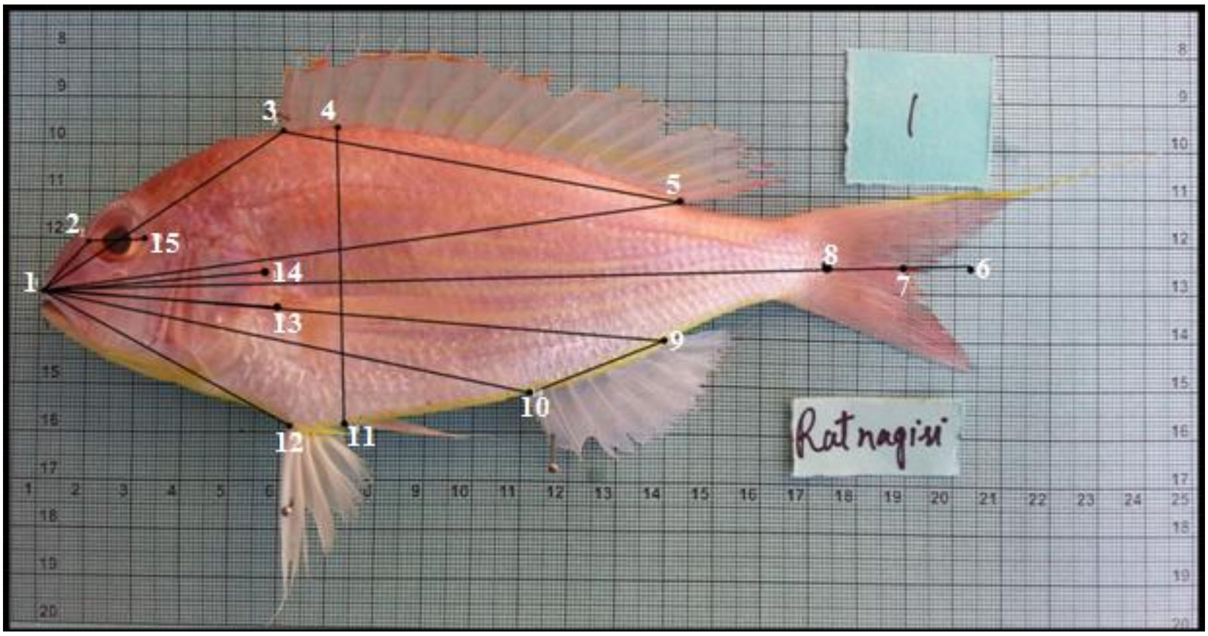

Fig 2: *Nemipterus japonicus* (Bloch). Landmarks (15) and linear distances (16) for conventional morphometric analysis

2.4. Analysis of data: All the measurements were log transformed and transformed data was tested for normality using software PAST (ver. 1.89), and 21 outliers were removed before further analysis. The analysis was carried out to differentiate the stocks within a coast. There were significant correlations between body size and other linear measurements; therefore, the size dependent variation was removed using an allometric approach of Reist [21] with some modification i.e., location wise SL_{mean} was taken in the place of overall mean.

$M_{trans} = \log M - \beta (\log SL - \log SL_{mean})$

where M_{trans} is the transformed measurement, log M is the natural log transform of the original measurement, β is the within-group slope regressions of the log M vs log SL, SL is the standard length of the fish, and SL_{mean} is the location-wise mean of the standard length.

Correlation coefficients between the transformed variables and the standard length of the fish were calculated to check whether the data transformation was effective in removing the size effect in the data. Multivariate analysis of variance (MANOVA) was performed to test for significant differences between the populations of different locations.

The 16 morphometric variables were size corrected by transforming into natural logarithms and were further analyzed by multivariate analysis [22]. Log-transformed data was subjected to Principal Components Analysis (PCA), and Discriminant Function Analysis (DFA) using SPSS (Ver. 21).

3. Results

The total length of the fish samples ranged from 7.26 cm (minimum) from Ratnagiri to 29.57 cm (maximum) from Cochin. Test results of analysis of variance (ANOVA) showed a large variance of 27.022 for mean distance of “UT”, followed by “UF” and “US” distances. The least variance observed was distance “UO”. When the mean morphometric distances of the fishes from all the six locations were compared, the univariate ANOVA showed significant difference at the $p \leq 0.05$ level of significance, leading to the rejection of ‘null hypothesis of ‘no heterogeneity in fish morphology among 6 sampling stations along the west coast of India.

Though there were significant difference in the sample means, it was not clear whether the strength of the relationship was between the different sampling locations (independent variables) or the sample differences (dependent variables). Therefore, the magnitude of effect of the independent variable upon the dependent variables was calculated. A large variance percentage of 0.92 has been accounted for by the sampling locations. Thus 92% of total variance in the samples could be because of differences in locations (location-specific variation) and 8% due to unknown or random factors.

The highest value of correlation coefficient (0.998) among the estimates of coefficients was observed between total length and fork length. Lowest correlation (0.599) was found between pre-orbital distance and eye diameter. In general, the correlation of eye diameter and snout length with other distances, showed relatively low degree of correlation, which was in the range of 0.599 – 0.945. With the exception of distances OO’ and UO, all remaining distances elicited good correlation (0.955 – 0.998) between each other. Thus, it showed that eye diameter and snout length were less associated with other linear measurements and remaining variables showed strong correlation between them.

PCA was carried out factoring the correlation matrix of the conventional morphometric data, between samples of six stations. The first three components accounted for 98.57% of total variance. However, only first component displayed Eigen value greater than one. Results of the screen plot (Fig. 3) also suggested that only the first component was found to be meaningful. The factor loadings of the first component are shown in table 3. Coefficients of the first principal component, *i.e.* PC1 represent overall size. All the variables are equally balanced in PC1, which showed that all sixteen morphometric measurements contribute to differentiate *N. japonicus* populations along the west coast of India. The bivariate scatter plot of component 1 and 2 was not sufficient to outline the morphological heterogeneity existing between populations of *N. japonicus* (Fig. 4). Samples from Ratnagiri and Cochin showed potential heterogeneity from rest of the populations and from each other. Veraval, Mumbai and Malpe samples showed extensive overlapping, thus, making it difficult to segregate into separate groups. Fish samples from Panaji showed an extensive mixing with Malpe and to a lesser extend with Cochin samples. PCA could not clearly discriminate the fish samples from six locations; therefore, DFA was performed.

DFA evoked five discriminant functions or Eigen values. The first function accounted for 82.5% of total variance, 10.9% variance was accounted by second function. The remaining three functions together constituted a variance of 6.7% (0.6% to 3.5). Wilk’s Lambda test showed that all the five functions were significant discriminators. The standardized canonical discriminant functions showed that DF1 was heavily weighted on distance UA’, followed by distance “UT”. DF2 was heavily weighted on distance “UA” followed by distance “US”. Structured matrix for conventional morphometrics showed that DF1 was having largest absolute correlation between each variable and any discriminant function. The loadings of DF1 were in the range of 0.499-0.999. Total length showed highest loading of 0.999 followed by standard length and fork length with values of 0.888 and 0.886, respectively. DF2 showed a negative correlation in three variables while the remaining correlations were less than 0.500 therefore, not significant. The scatter plot of all the six locations (Fig. 5) showed considerable discreteness of discriminant scores for Mumbai, Panaji and Cochin populations. Little segregation was evident in Veraval, Ratnagiri, and Malpe samples. From the scatter plot, it was clear that DFA has successfully discriminated the populations of *N. japonicus* along the west coast of India. The classification results derived in the present study predicted that 100% individuals were classified correctly in Ratnagiri and Cochin groups. In the remaining groups, the prediction membership of individuals sampled ranged from 84.9% to 90.6%. In general, 92.3% of the individuals were correctly classified from original groups (Table 4).

Table 3: Principal Component loadings of linear measurements.

Sr. No.	Variable	PC1
1	UO	0.9146
2	UD	0.9943
3	UD'	0.9961
4	UT	0.994
5	UF	0.996
6	US	0.9964
7	UA'	0.9969
8	UA'	0.9927
9	UP	0.9871
10	UP_c	0.9887
11	UO_p	0.9851
15	UO'	0.9643
13	OO,	0.8883
14	DD'	0.988
15	D_1P'	0.9592
16	A'A	0.9672
	Total	15.6089

Table 4: Conventional Classification Results^{b,c}

		Sample	Predicted Group Membership						Total
			1	2	3	4	5	6	
Original	Count	1	99	3	2	0	7	0	111
		2	5	76	1	1	2	0	85
		3	0	0	74	0	0	0	74
		4	1	1	0	48	3	0	53
		5	2	2	0	4	45	0	53
		6	0	0	0	0	0	65	65
	%	1	89.2	2.7	1.8	.0	6.3	.0	100.0
		2	5.9	89.4	1.2	1.2	2.4	.0	100.0
		3	.0	.0	100.0	.0	.0	.0	100.0

		4	1.9	1.9	.0	90.6	5.7	.0	100.0
		5	3.8	3.8	.0	7.5	84.9	.0	100.0
		6	.0	.0	.0	.0	.0	100.0	100.0
Cross-validated[a]	Count	1	97	3	3	0	8	0	111
		2	7	73	1	1	3	0	85
		3	0	1	73	0	0	0	74
		4	3	1	0	46	3	0	53
		5	2	2	0	4	45	0	53
		6	0	0	0	0	0	65	65
	%	1	87.4	2.7	2.7	.0	7.2	.0	100.0
		2	8.2	85.9	1.2	1.2	3.5	.0	100.0
		3	.0	1.4	98.6	.0	.0	.0	100.0
		4	5.7	1.9	.0	86.8	5.7	.0	100.0
		5	3.8	3.8	.0	7.5	84.9	.0	100.0
		6	.0	.0	.0	.0	.0	100.0	100.0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b. 92.3% of original grouped cases correctly classified.

c. 90.5% of cross-validated grouped cases correctly classified.

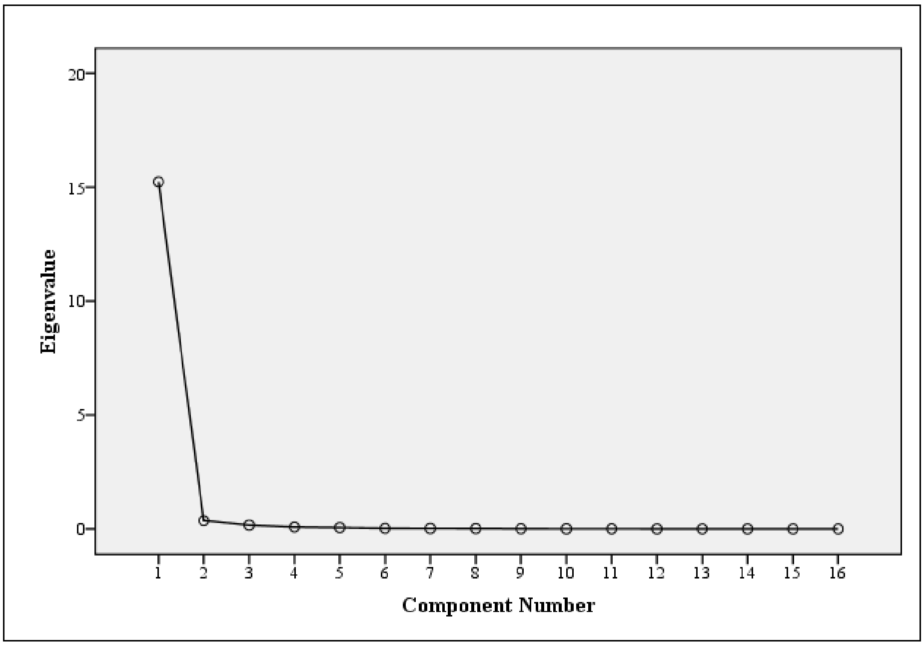

Fig 3: Scree plot of conventional Eigen Values

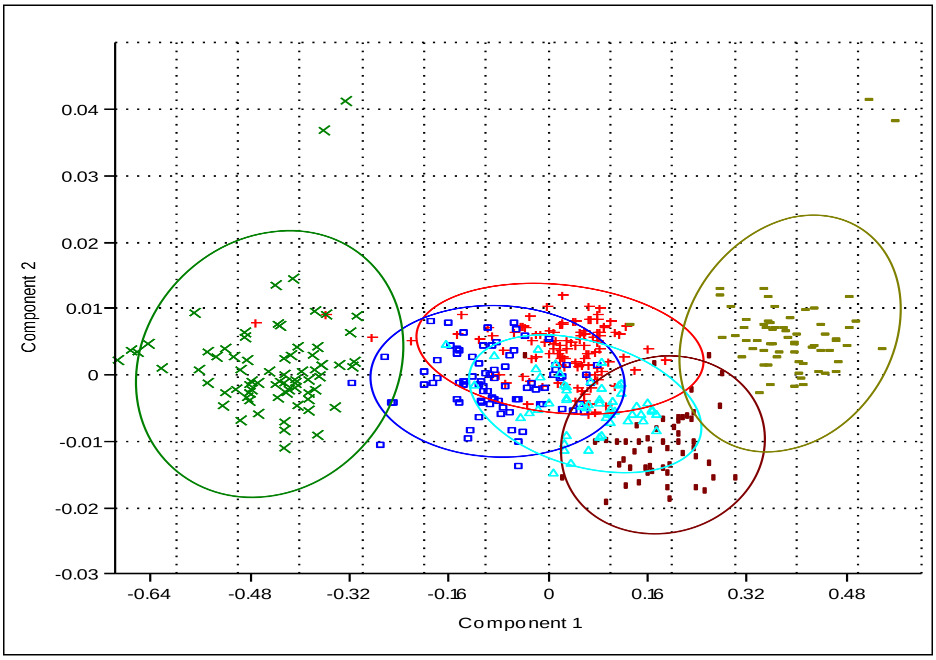

Fig 4: Scatter plot of six locations with PCA

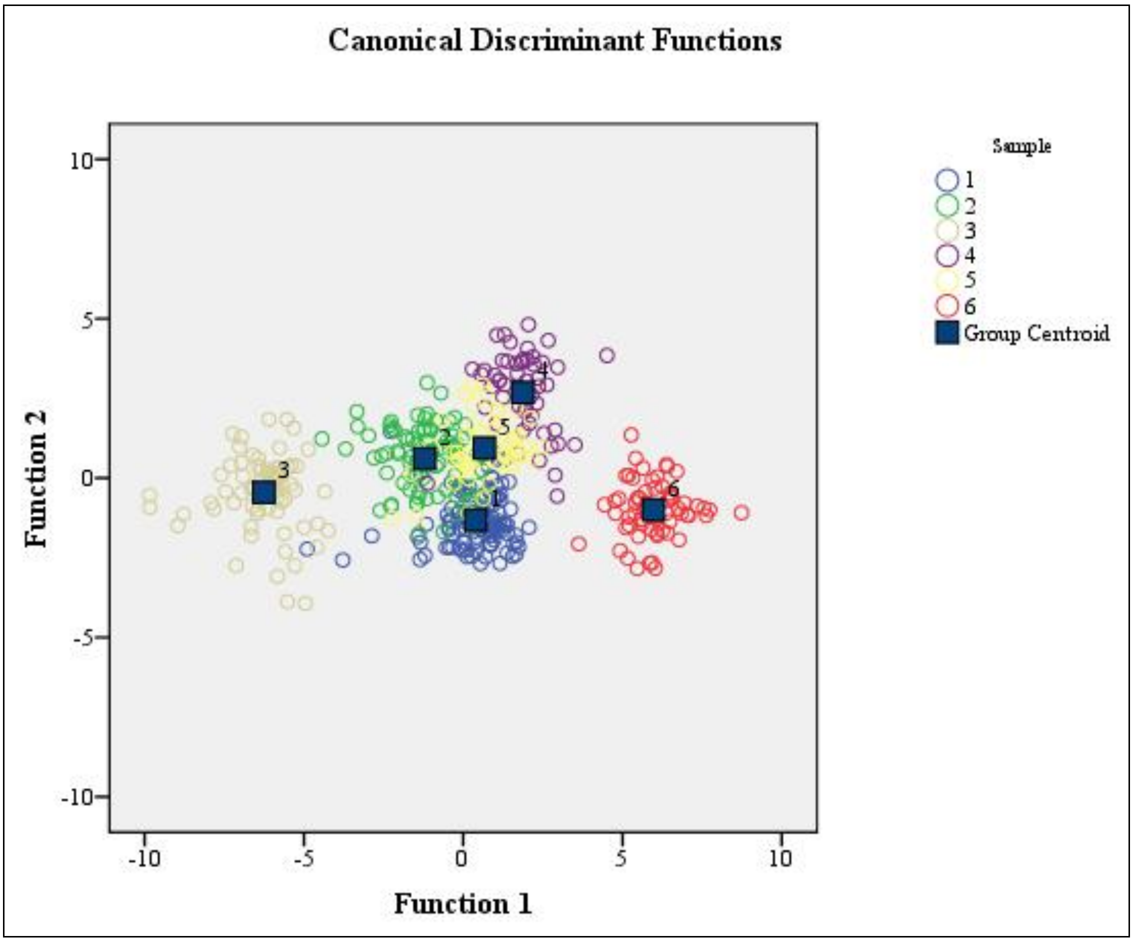

Fig 5: Conventional scatter plot with DFA for all the locations; 1 – Veraval, 2 – Mumbai, 3 – Ratnagiri, 4 – Panaji, 5 – Malpe, 6 – Cochin

4. Discussion

Stock discrimination is a basic need for the scientific management and conservation of a species. The isolated populations within a species, which are genetically distinct, are referred to as stocks. It is believed that a species may undergo micro-evolutionary processes and differentiate into genetically distinct sub-populations or stocks in the course of time, if reproductively and geographically isolated. The detection of stocks would imply that the source group comprises different stocks [23] and should be treated as separate management units as such [23]. Failure to recognize or to account for stock complexity in management units has led to an erosion of spawning components, resulting in the loss of genetic diversity and other ecological consequences [24]. Morphometrical methods like measuring linear distances help discriminate the groups using various statistical techniques. Morphometric and meristic studies, protein and DNA polymorphism studies etc., are the most popular approaches involved in stock identification. Morphological identification of stocks is the traditional approach in stock identification in fishes and the use of modern tools in morphological identification make it more concrete. Multivariate statistical analyses like PCA and DFA are frequently used in stock differentiation. Morphometrics is the analysis of body shape, or the shape of particular morphological feature of various body dimension or parts [25]. Fishes tend to differ in body shape because of interactive effect of environment, selection and genetics on individual ontogeny [26]. A number of researchers in India and abroad have utilized linear measurements for studying the stock or population structure of fishes.

The linear measurements are used as correlated variables for multivariate analysis. However, Cadrin [27] has stated that the geometry of linear distances is ignored by traditional morphometric analysis. Conventional data sets are mainly length, depth, and width measurements. They tend to be repetitious and overlapping and measure the form unevenly [22, 26] Many of the characters are measured as maximum and minimum distances, so their placement of one form might not be comparable to another form of a species. The measurements are selected independently without any strict relationship to one another; the character set generally has no collective or ‘emergent’ geometric properties [27]. However, morphometric characters are continuous variables with meaningful correlations and are therefore appropriate for conventional multivariate analysis [27]. Thus, conventional method of morphometrics was used in the present study.

In general, 11 to 21 morphometric distances have been used by researches to analyze the stock structure of fishes [28-31]. Thus, 16 morphometricial traits used here can be said to be comparable with other works. In PCA a large number of correlated variables are reduced to smaller number of components or factors that show most of the variance in the observed variables. Each component is estimated as being a linear (weighted) combination of the observed variables. As many factors as the number of variables can be extracted but generally, most of them may contribute to small fractions with Eigen values less than unity. Therefore, only first component, which captured most variance, was considered in the present study. The variance of 95.25%, 2.27% and 1.05% from the first, second and third principal components, respectively was noticed in PCA. The meaningful component was PC1 with Eigen value of 14.99. Maximum variation in the samples was contributed by size component. Coelho *et al*., [32] also found a variation of 91.6%, 2.7%, and 1.8% of total variance from the first 3 components while studying stock structure of endangered cyprinid fish. PC1 loadings were all positive supporting the results of Salini *et al*., [33] in hilsa shad *Tenualosa ilisha*; Ruitaisire *et al*., [34] in two *Labeo victorianus* populations of Lake Victoria; Cadrin and Silva [35] in yellowtail flounder. DFA was used in the present study to get a clear understanding of the stock structure. Discriminant functions derived through the analysis were used to classify cases into values of categorically dependent variables. Thus, discrimiannt functions (weighted combinations of the predictors) were used to predict a group (level of the categorical variable) into which a subject falls, based on discriminant scores on the X variables. The DFA analysis of conventional morphometric measurements showed variance of 82.5% and 10.9% for the first two-discriminanting functions. These two functions together accounted for 93.4% of total variance. Remaining three functions contributed to the rest of the variance. Canonical correlation value for the first and the second functions was 0.963 and 0.792, respectively. The percentage variance explained by first two discriminating functions in the studies of Sint *et al*., [36] was 89.1%, 100% and 86.2%, 100% while studying the morphological variation in male and female populations of *Astacus astacus* L. and *Austropotamobius pallipes*, respectively. Mamuris *et al*., [37] reported the variance of 64.24% and 18.34% (combined 82.58%) by first two discriminant functions. The values of discriminators in present study are more or less similar to the studies elsewhere. The classification accuracy of the fish individuals with DFA ranged between 45% to 99% in original count and between 45% to 97% in cross-validated count. In general, 92.3% of original grouped cases and 90.5% of cross-validated grouped cases were classified correctly. Relatively low percentages were found in Malpe and Panaji centres which is an indication of mixing of populations among these centres. Barriga-Sossa *et al*., [38] observed the classification accuracy between 57.9% to 100% for original counts and 66.7% to 100% for cross-validated count. The average percentage of correct classification of 80.43% has been reported by Daud *et al*., (2005) while studying morphometric analysis of Malaysian oxudercine goby, *Boleopthalmus boddarti*. Ouattara *et al*., [39] noticed 88.99% of average classification accuracy, whereas, Abdolhay *et al*., [40] observed 97.3% and 98.3% accuracy of correct classification in their studies. The low percentage of correct classification in the present study for few centres might be attributed to closeness of respective centres with one another thus effecting mixing of reproductive individuals into each other's geographical areas. Second probable reason might be that the fishers of respective locations are fishing in one another's areas and landing the fish in their areas. The misclassification results of discriminant function clearly support this theory.

The scatter plot of discriminant functions (Fig. 5) using conventional measurements clearly separated all the six stocks. However, Malpe population showed a wider distribution by mixing with samples of Veraval, Mumbai, and Panaji. The classification results also revealed the considerable distinctness of the stocks thus supporting the findings of Ihssen *et al*., [41] showing reproductive isolation of individuals with temporal or spatial integrity.

The current research is in aggrement with the work of Mudasir *et al*., [42], who identifed the unit stocks of *N. japonicus* random amplified polymorphic DNA (RAPD). However, a more holistic approach combining the conventional method and more advanced or specific molecular identification of fish stock can prove benifical in divising the management programme.

5. Conclusion

Overall, the present study unambiguously confirmed the existence of Mumbai, Ratnagiri, and Cochin as separate stocks and mixing of Veraval, Panaji and Malpe stocks along the west coast of India. The six populations of *N. japonicus* from Veraval, Mumbai, Ratnagiri, Panaji, Malpe, and Cochin exhibited significant morphological divergence indicating clear population structuring within species. The study emphasizes the need for a management policy based on SMUs (Single Management Units) *i.e.*, stock-specific management programme for the species *N. japonicus.*

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