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Stock structure analysis of the declining shovel-nosed lobster *Thenus unimaculatus* (Burton and Davie, 2007) for effective management and conservation along the Indian coast

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Abstract

The shovel-nosed lobster *Thenus unimaculatus* (Burton and Davie 2007) is one of India's most important commercially exploited lobster species. The declining trends and collapse of the sand lobster fishery have been reported from the north-west coast of India. Several hypotheses have been proposed to explain this decline, but a lack of basic information on population demographics inhibits hypothesis testing. This study attempted morphometric stock structure analysis of *T. unimaculatus* along the Indian coast, using 673 specimens collected from five locations during 2017-2019. Data was subjected to allometric transformation to remove size effect and further subjected to multivariate analysis, including principal component analysis (PCA) followed by linear discriminant analysis (LDA) using R software. The first eight principal components (PCs) cumulatively explained 73.97% of the total variance. The misclassification rate (MR) of the LDA model performed to optimise separation among different sampling locations and coasts were 0.30 and 0.26 respectively, indicating clear overlap of the stocks. The results of this study revealed the presence of a single spawning stock of shovel-nosed lobster along the Indian coast, providing insights for adopting holistic management strategies to conserve this declining resource.

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Introduction

Lobsters form one of the most valuable crustacean resources, commercially exploited for over four decades. They have a good market with high global demand, contributing significantly to foreign exchange earnings of the country (Radhakrishnan and Thangaraja, 2008). The diverse habitats and the physical environment of the southern coast (both the west and east coasts) of India favour the settlement of a wide variety of lobster species (Radkhakrishnan *et al.*, 2019). Marine lobsters belong to the suborder Macrura Reptantia comprising 4 infraorders, 6 families, 54 genera, and 260 extant species (including 4 subspecies) (Chan, 2019). The lobster fauna of India is notably diverse, with 38 species distributed across five families and three infraorders (Astacidea, Achelata and Polychelida) distributed in the seas surrounding the Indian subcontinent. *Thenus* is the only genus among the seven scyllarid genera that holds economic significance (Jones 1990). The scyllarid lobster, *Thenus unimaculatus*, originally described as *T. orientalis* (Chhapgar and Deshmukh, 1964), is commonly known as sand lobster, slipper lobster, or shovel-nosed lobster. Jeena *et al*. (2011) has confirmed that the species of shovel-nosed lobsters found in Indian waters is indeed *Thenus unimaculatus*. This species is a key commercially exploited lobster in India, distributed along the entire Indian coast, supporting significant fisheries in the north-west and south-east coasts (Holthuis, 1991). Declining catch trends as well as collapse of the fishery in Maharashtra (Deshmukh, 2001; Subramanian, 2004; Jeena *et al.*, 2015), necessitates assessment of the stock structure to ensure sustainable management of this resource.

From a fisheries management perspective, a stock is described as an intraspecific assemblage of finfish or shellfish with spatial or temporal stability, where individuals exhibiting identical growth, mortality and reproductive rates randomly mate within the group (Hilborn and Walters, 1992). Identification of stocks with variable life history attributes such as growth, mortality and reproductive potentials are considered important for understanding population dynamics and developing sustainable resource management strategies. Therefore, stock structure analysis is essential to identify and understand differences among the individuals of the same species exhibiting differences in growth, mortality and maturation characteristics so that they can be grouped into separate units for effective and efficient implementation of management interventions (Begg *et al.,* 1999; Turan *et al.,* 2005). Stock identification is an interdisciplinary field that concerns the identification of self-sustaining components within natural populations (Cadrin *et al.,* 2005). There are several techniques for the identification of stocks such as meristic and morphometric characteristics, parasites as natural tags, otolith studies as well as use of molecular markers (Awasthi *et al.,* 2014). Variations in morphometric traits are not always necessarily caused due to genetic variations, as variations in environmental parameters during critical developmental stages of the animal can also significantly influence the morphometric traits (Cadrin, 2000; Pinheiro *et al.,*2005, AnvariFar *et al.,* 2011). Therefore, morphometric traits are more promising for studying short-term environmentally induced variations, which is essential for fisheries management (Begg *et al.,*1999; Swain and Foote,1999). Several studies have emphasised the utility of morphometric and meristic data in separating the stocks of fish living in the same or different environments (Krumholz and Cavanah, 1968; Anyanwu and Ugwumba, 2003; Turan *et al.,* 2005).

Morphometric and meristic methods remain the simplest and most direct for species identification. Recent advancements in data acquisition techniques such as morphological landmark-based geometric methods are highly effective in capturing information regarding the shape of an organism from a sequential series of landmark-connected polygons (Cavalcanti *et al.,* 1999). Subsequently, several multivariate techniques, such as principal component analysis and discriminant analysis can be used to discriminate the stocks. In the present study, an attempt has been made to describe the stock structure of the scyllarid lobster, *T. unimaculatus* along the Indian subcontinent using a morphometric approach to develop an appropriate management strategy to rebuild this declining crustacean resource.

Materials and methods

Sampling

A total of 673 specimens of *T. unimaculatus* consisting of 305 males and 368 females were collected from five major fishing harbours along the Indian coast. The specimens were identified as *T. unimaculatus* according to Burton and Davie (2007). The sampling locations were, Sakthikulangara (SWS) along the south-west coast, Veraval (SWV) along the north-west coast, Chennai (SEC) and Nagapattianam (SEN) along the south-east coast, and Visakhapatnam (SEZ) along the north-east coast of India (Fig. 1). The locality, sex ratio and sample size from each location are shown in Table 1. All the specimens sampled were in mature condition representing the spawning stock. The breeding season of *T. unimaculatus* was selected for the collection of samples to ensure that they belonged to their parent population. The specimens from the west and east coasts were obtained from August to May and June to March, respectively, for a period of 2 years from 2017 to 2019. Male and female specimens were separated according to their sexual dimorphic features. In mature females, the leaf-like endopods of the abdominal pleopods bear long ovigerous setae which are used for attaching spawned eggs until they are hatched, whilst in juvenile females, the pleopods are devoid of setae. Mature males possess a small club-shaped process midway along the inner margin of the endopod of the first pair of pleopods (Kizhakudan, 2014). The total length (TL) is measured as the distance between the notch in the carapace (anterior region) and the posterior margin of the telson and carapace length (CL) is measured as the distance between the notch and the posterior margin of the carapace. Immature and egg-bearing (berried) females were excluded while only mature specimens (carapace length ≥6.0 cm) were included in the study.

Digitisation of samples and measurement of morphological distances

The specimens of *T. unimaculatus* were collected and preserved on-site in an insulated ice box and brought to the laboratory. The specimens were thoroughly cleaned with running tap water, drained and wiped with absorbent paper. The specimens were placed on a flat platform pinned with graph paper for calibrating the coordinates of the digital images. The distances between the

Table 1. Sampling locality, geographical coordinates, sex and number of specimens collected

Locality	Latitude, Longitude	Sex	Sample size (n)
Sakthikulangara (SWS)[1]	8°56'00"N, 76°32'33"E	Male	54
		Female	109
Veraval (SWV)[2]	20°54'19"N, 70°22'53"E	Male	68
		Female	44
Visakhapatnam (SEZ)[3]	17°41'46"N, 83°18'03"E	Male	30
		Female	68
Chennai (SEC)[4]	13°07'44"N, 80°18'03"E	Male	69
		Female	61
Nagapattinam (SEN)[4]	10°75'08"N, 79°84'52"E	Male	84
		Female	86

[1] Kerala, [2]Gujarat, [3]Andhra Pradesh, [4]Tamil Nadu

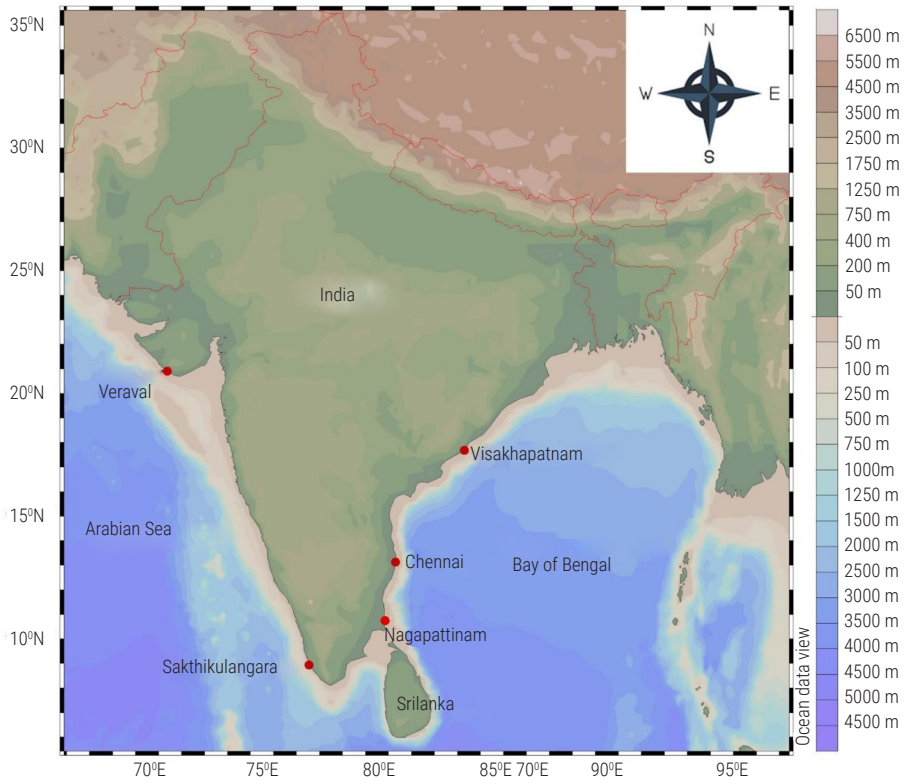

Fig. 1. Sampling locations along the Indian coast

vertical and horizontal grids of graph paper were used in calibrating the coordinates covering an area of 1 cm^2. The lobster specimens were then digitally captured with a camera (Canon G-15, Tokyo, Japan) fixed on a tripod. Further, all the specimens were labelled with a specific code marking the identity of the specimen. The extraction of morphological distances from the digital images of specimens was conducted using a linear combination of three software platforms *viz.,* tps util; tps Dig2 v2.1 (Rohlf, 2006) and Paleontological Statistics (PAST) (Hammer *et al.,* 2001).

Each image was acquired by placing the scale beside it, to have uniformity in all the measurements which were scaled up with tpsDig2 software employing millimetre grid on the graph paper. A total of 43 landmark points was selected to provide a homogeneous coverage of the basic shape of sand lobster (Strauss and Bookstein, 1982) (Fig. 2). All measurements were transferred to a spreadsheet file (Excel, 2007) and the X-Y coordinate data was transformed into linear distances by computer for subsequent analysis (Turan, 1999). Sampling locations, coasts and sexes were used as the class variables to test for significant differences in morphometric characters, if any, between male and female *T. unimaculatus*.

Data analysis

All the morphometric measurements were log-transformed and were tested for normality assumption and outliers were removed before further analysis. Multivariate analysis of covariances (MANCOVA) was performed to examine any significant differences

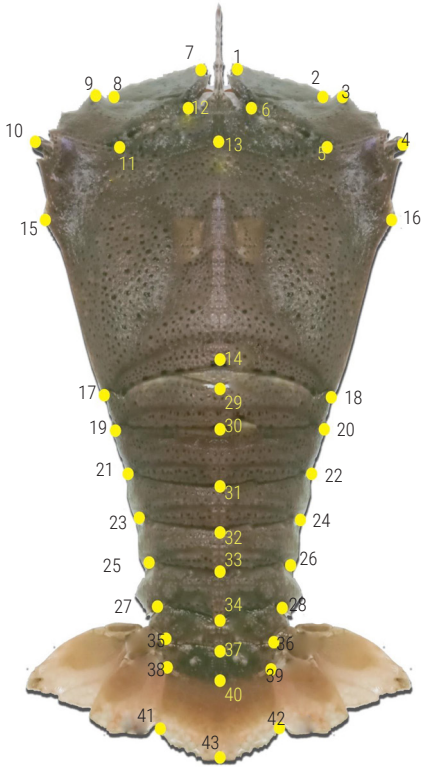

Fig. 2. Truss landmark points used for digitisation of morphometric measurements of *T. unimaculatus*

between the stocks using sampling locations, coasts and sexes as factors, log-transformed carapace length (CL) as a covariate and other log-transformed morphometric data as dependent variables. This statistical model was used to test the effect of covariate (CL) on other dependent morphometric variables and to test interaction effect between factors (sampling locations, coasts and sexes) with covariate (CL). Any size-dependent variation was corrected by adapting an allometric method suggested by Elliott *et al.* (1995) using the formula:

$M_{trans} = \log M - \beta (\log CL - \log CL \text{ mean})$

where, M_{trans} is the transformed morphometric measurement, log M is the log-transformed original measurement, log CL is the log-transformed standard length (*i.e.,* carapace length) of each specimen, log CL mean is the arithmetic mean of the carapace length of the population and parameter β was estimated as the slope by regressing the values of log M against log CL. The correlation coefficient between the transformed variables and the carapace length of the lobster before and after transformation was estimated to examine whether the transformed data were successful in eliminating the size effect (Khan *et al.,* 2012). A univariate ANOVA was performed consequent to allometric correction to test whether there was any statistically significant variance for each morphometric measurement among the sampling sites, between coasts and sexes.

Principal component analysis (PCA) was used for morphometric data reduction (Veasey *et al.,* 2001), in order to decrease redundancy among the 32 morphometric variables (Samaee *et al.,* 2006) and to extract several dependent variables for population differentiation (Anvarifar *et al.,* 2011; Kuberan *et al.,* 2020). Bartlett's test of sphericity to ascertain the requisite correlation among the variables, the determinant test to check multicollinearity and Kaiser-Meyer-Olkin tests to measure the sample adequacy were performed to ascertain that the data follow the assumptions for PCA. The significantly contributing principal components were selected based on Kaiser selection criteria by scrutinising the Eigen values using a scree plot (Kaiser, 1960). The variables with significant loadings to the first two principal components (PC1 and PC2) were selected to examine and describe any possible variations in the morphometric variables among locations and between coasts and sexes.

The coordinate scores of the significant principal components (PCs) were used for discriminant function analysis (DFA) to discriminate the effects of variables into known groups and to explore the effectiveness of variables in predicting factor-wise different groups for locations, coasts and sexes (Tomovic and Dzukic 2003; Loy *et al.,* 2008). Cross-validation technique with confusion (error) matrix was used to calculate classification accuracy. Classification functions were derived from DFA to assign individual specimens to putative stocks. A scatter plot analysis based on linear discriminant analysis (LDA) scores was used for visual observation of groups. All statistical analyses was performed using the R statistical software package, Version 1.4.1106, Release name: Tiger Daylily (RStudio Team, 2021).

Results

Multivariate analysis (MANCOVA) performed to determine the significant difference among the sampling locations, between the coasts (East vs. West) and the sexes (Males vs. Females) using carapace length (CL) as the covariate showed significant variations ($p<0.001$) in morphometric measurements among the sampling locations, between the coasts and the sexes (Table 2). Significant differences in the morphometric measurements were apparent among the sampling locations (Wilk's lambda=0.03; F=26.48, $p<0.001$), between the coasts (Wilk's lambda=0.58; F=14.43, $p<0.001$) and between the sexes (Wilk's lambda=0.73; F=7.52, $p<0.001$). Furthermore, there were significant interaction effects of the covariate (CL) with locations (Wilk's lambda=0.64; F=2.30, $p<0.001$), coasts (Wilk's lambda=0.89; F=2.44, $p<0.001$) and sexes (Wilk's lambda=0.85; F=3.66, $p<0.001$). The carapace length (CL) was also found to vary significantly among the sampling locations (Wilk's lambda=0.91; F=1.95, $p<0.001$); between the coasts (Wilk's lambda=0.77; F=5.81, $p<0.001$) and between the sexes (Wilk's lambda=0.73; F=7.52, $p<0.001$).

The findings of the univariate test (ANOVA) performed to compare the morphometric measurement among the sampling locations, between the coasts (East vs. West), and between the sexes (Males vs. Females) are presented in Table 3. All the morphometric measurements showed a significant difference ($p<0.05$) among the sampling locations. Most of the morphometric measurements except for TL, CW, T26, T78, T1920, T2122, T3132, T3233, T3743 and T3839 showed significant difference ($p<0.05$) when compared between the coasts (East vs. West). Morphometric measurements except for T16, T34, T45, T712, T812, T910, T1011, T1920, T2122, T2324, T2526, T2728, T2930, T3132 and T3437 were found to be significantly different when compared between the sexes.

The correlation of individual morphometric measurements with covariate (CL) before transformation and after transformation of morphometric measurements is shown in Fig. 3. All the

Table 2. MANCOVA tests for effects of sampling site (SS), standard length (SL) (covariate) and their interaction on body morphology of *T. unimaculatus*

Variables and their interaction	Wilk's lambda	F	D.F.	Numerator D.F.	Denominator D.F.	p
Locations	0.03	26.48	4	128	2516.9	<0.001
CL	0.91	1.95	1	32	632.0	<0.001
Location: CL	0.64	2.30	4	128	2516.9	<0.001
Coast	0.58	14.43	1	32	638	<0.001
CL	0.77	5.81	1	32	638	<0.001
Coast: CL	0.89	2.44	1	32	638	<0.001
Sex	0.73	7.52	1	32	638	<0.001
CL	0.79	5.29	1	32	638	<0.001
Sex: CL	0.85	3.66	1	32	638	<0.001

Table 3. Summary of ANOVA performed on 32 morphometric measurements of *T. unimaculatus* collected along India to compare the difference between locations, coasts and sexes

Measurements	Location		Coast		Sex	
	F	*p*	F	*p*	F	p
TL	43.63	<0.001	2.12	>0.1	47.50	<0.001
CW	7.55	<0.001	0.55	>0.1	35.43	<0.001
T12	4.21	<0.001	10.93	<0.001	4.02	<0.05
T16	21.28	<0.001	36.01	<0.001	0.20	>0.1
T26	21.17	<0.001	2.82	>0.1	11.09	<0.001
T34	39.04	<0.001	42.06	<0.001	0.88	>0.1
T35	1.57	<0.001	10.89	<0.01	4.22	<0.05
T45	50.41	<0.001	5.49	<0.05	0.62	>0.1
T46	18.40	<0.001	15.98	<0.001	19.07	<0.001
T410	22.39	<0.001	7.05	<0.01	17.13	<0.001
T56	69.98	<0.001	109.70	<0.001	24.42	<0.001
T78	9.03	<0.001	2.55	>0.1	4.32	<0.05
T712	32.82	<0.001	43.87	<0.001	2.52	>0.1
T812	22.78	<0.001	21.29	<0.001	3.35	>0.1
T910	38.15	<0.001	26.06	<0.001	0.62	>0.1
T911	23.27	<0.001	63.16	<0.001	4.38	<0.05
T1011	57.66	<0.001	5.27	<0.05	2.08	>0.1
T1112	57.37	<0.001	48.64	<0.001	4.16	<0.05
T1920	37.70	<0.001	2.45	>0.1	0.01	>0.1
T2122	43.73	<0.001	0.71	>0.1	2.49	>0.1
T2324	38.49	<0.001	8.57	<0.01	1.50	>0.1
T2526	38.90	<0.001	17.98	<0.001	3.36	>0.1
T2728	27.61	<0.001	9.99	<0.01	3.54	>0.1
T2930	11.18	<0.001	0.48	>0.1	1.44	>0.1
T3031	24.72	<0.001	5.81	<0.05	8.09	<0.01
T3132	67.90	<0.001	2.59	>0.1	3.02	>0.1
T3233	59.93	<0.001	1.13	>0.1	24.70	<0.001
T3334	87.63	<0.001	5.85	<0.05	20.11	<0.001
T3437	40.10	<0.001	4.13	<0.05	2.16	>0.1
T3536	10.17	<0.001	12.42	<0.001	7.08	<0.01
T3743	30.10	<0.001	2.90	>0.1	71.81	<0.001
T3839	20.78	<0.001	1.10	>0.1	8.22	<0.01

morphometric measurements showed high to very high level of significant correlation ($p<0.01$) with CL. The correlation coefficient (r) varies from a minimum of 0.53 (for T3437) to a maximum of 0.95 (for CW and T410). However, after allometric correction, the correlation coefficient was drastically reduced from a minimum of 0.001 (for T3334) to a maximum of 0.26 (for T46). After allometric transformation, most of the morphometric measurements except for T26, T34, T35, T45, T46, T1011, T1112, T1920, T2122, T2324, T2526, T2728, T2930 were not found to be significantly ($p>0.01$) correlated with CL.

The data was found suitable for PCA as it passed the Bartlett's test of sphericity (χ^2=18938.07, df=496, $p<0.01$), multi-collinearity test (determinant=$3.5x10^{-13}$) and Kaiser-Meyer-Olkin test (overall MSA=0.81) to measure the sample adequacy. PCA indicated that first eight principal components (PCs) individually explained the variance greater than average variance based on Kaiser selection criteria (Eigen value >1.0). The first eight PCs cumulatively explained 73.97% of the total variance, wherein the first two PCs together explained 45.50% of the total variance (Fig. 4; Table 4).

The respective loadings of the morphometric measurements to the first eight principal components along with the eigen values, explained variance (%) and cumulative explained variance (%) is shown in Table 4. Morphometric measurements having significant loadings for PC1 and PC2 are shown in variables PCA biplot (Fig. 5).

Top ten morphometric measurements with the highest significant loadings on PC1 and PC2 are T2122, TL, T1920, T2324, T410, T2526, T712, CW, T35 and T911. The corresponding morphometric measurements have been highlighted and presented in Fig. 6.

Bivariate PCA biplots of PC1 against PC2 describing the variations in morphometric measurements between locations, coasts and sexes are shown in Figs. 7, 8 and 9 respectively. No clear separation was observed among the locations, between the coasts or between the sexes, as the overlapping in PCA scores was apparent.

The confusion matrix and statistics to measure the performance of linear discriminant analysis (LDA) models developed consequent to PCA to optimise the separation among the groups (locations, coasts and sexes) is given in Table 5 and the pictorial separation is illustrated as LDA plots in Fig. 10, 11 and 12.

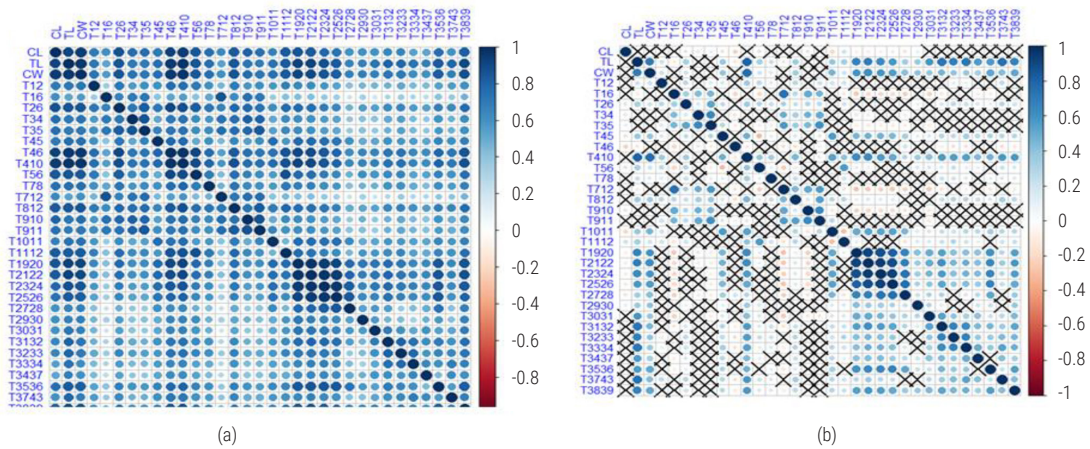

Fig. 3. Correlation of (a) untransformed and (b) transformed morphometric variables with covariate (CL), their degree of correlation (r) and significance of correlation (p value). Cells marked 'X' do not correlate significantly (p<0.01)

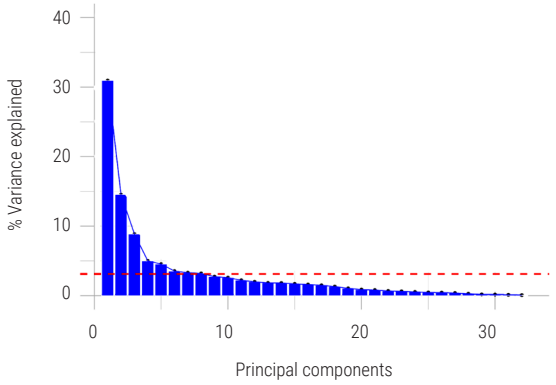

Fig. 4. Scree plot indicating the variance explained by individual principal components (PCs)

The accuracy (ACC) and misclassification rate (MR) of the LDA model for discriminating among different sampling locations were 0.70 and 0.30 respectively. Location-wise sensitivity (True Positive Rate, TPR) and precision (Positive Prediction Value, PPV) were less than 0.90 and 0.80, respectively for all the sampling locations with the lowest values estimated for SEZ (TPR=0.20 and PPV=0.39). Furthermore, the kappa coefficient that measures the agreement between classification and truth values was found to be low (Kappa= 0.62). Therefore, no clear separation was observed among any of the sampling location (Fig. 8). The accuracy (ACC), misclassification rate (MR), sensitivity (TPR) and precision (PPV) of the LDA model for discriminating between the coasts were 0.74, 0.26, 0.84 and 0.75, respectively. The Kappa coefficient for the model was also low (Kappa=0.45) and therefore, clear horizontal overlapping was observed between the coasts, which indicate

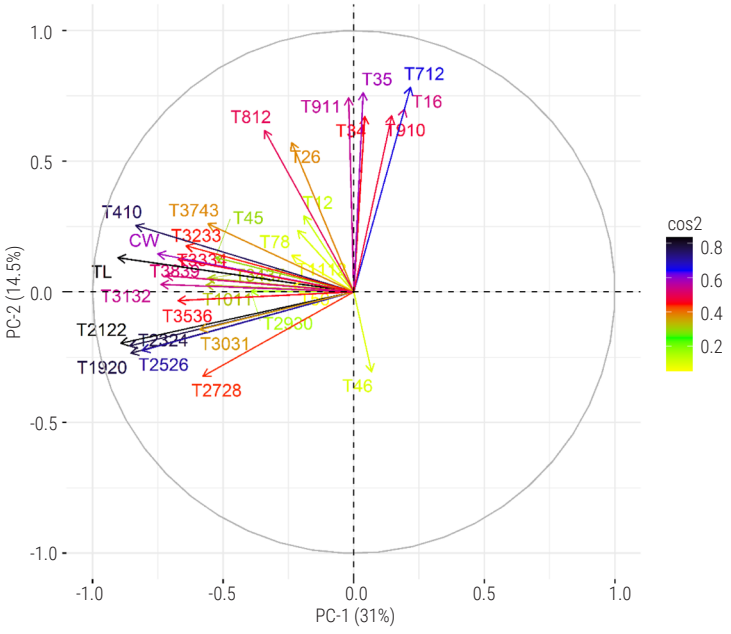

Fig. 5. Variables plot of morphometric measurements having significant loadings for PC1 and PC2

Table 4. Morphometric measurements showing their respective loadings and eigen values to the first eight principal components

Morphometric measurements	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
TL*	-0.90	0.13	-0.26	0.07	-0.08	0.09	-0.02	0.02
CW*	-0.75	0.15	0.04	-0.20	0.09	0.16	0.14	-0.08
T12	-0.19	0.29	-0.23	-0.06	0.67	0.00	-0.11	-0.11
T16	0.19	0.70	0.24	0.00	-0.28	0.17	0.23	0.12
T26	-0.24	0.57	-0.19	-0.06	0.11	-0.03	-0.06	0.59
T34	0.04	0.67	0.16	-0.14	-0.25	-0.19	-0.22	-0.22
T35*	0.04	0.76	0.22	-0.02	0.00	-0.26	-0.17	0.24
T45	-0.53	0.13	0.37	0.33	0.33	-0.03	0.11	0.30
T46	0.07	-0.31	0.16	-0.28	0.18	-0.27	-0.45	0.26
T410*	-0.83	0.25	-0.19	-0.09	0.23	0.06	0.10	-0.03
T56	-0.23	0.02	-0.67	-0.48	0.14	-0.05	-0.14	-0.07
T78	-0.21	0.23	-0.23	0.05	0.17	-0.29	0.45	0.27
T712*	0.22	0.78	0.27	0.03	-0.13	0.18	0.04	0.00
T812	-0.34	0.62	-0.04	0.02	0.16	-0.02	0.02	-0.40
T910	0.14	0.67	0.14	-0.28	-0.16	0.03	-0.12	0.03
T911*	-0.02	0.74	0.35	-0.12	0.14	-0.06	-0.16	-0.22
T1011	-0.57	0.03	0.44	0.18	0.45	0.11	0.01	-0.15
T1112	-0.24	0.14	-0.69	-0.33	0.00	-0.17	0.05	-0.08
T1920*	-0.85	-0.23	0.20	-0.15	0.00	-0.02	-0.02	0.07
T2122*	-0.89	-0.20	0.23	-0.07	-0.06	-0.03	0.00	0.01
T2324*	-0.86	-0.21	0.32	-0.08	-0.07	-0.06	-0.04	-0.03
T2526*	-0.81	-0.22	0.37	-0.09	-0.06	-0.10	0.00	-0.06
T2728	-0.58	-0.32	0.51	-0.21	0.01	-0.08	-0.10	-0.01
T2930	-0.39	0.00	0.05	-0.53	-0.09	0.49	0.18	0.15
T3031	-0.59	-0.14	-0.17	-0.04	-0.21	0.20	-0.28	0.12
T3132	-0.74	0.03	-0.17	0.18	-0.29	-0.19	-0.10	0.03
T3233	-0.64	0.18	-0.34	0.29	-0.22	-0.21	-0.04	-0.04
T3334	-0.67	0.13	-0.22	0.43	-0.16	-0.12	-0.09	-0.07
T3437	-0.56	0.05	-0.11	0.25	-0.05	0.36	-0.41	0.09
T3536	-0.67	-0.03	0.20	-0.19	-0.14	-0.37	0.25	-0.08
T3743	-0.56	0.26	-0.31	0.30	0.11	0.22	-0.01	-0.04
T3839	-0.72	0.06	-0.07	-0.09	-0.21	0.07	0.19	0.00
Eigen value	9.91	4.65	2.84	1.60	1.46	1.13	1.06	1.03
Variance explained (%)	30.96	14.54	8.86	5.01	4.55	3.54	3.30	3.20
Cumulative variance explained (%)	30.96	45.50	54.37	59.38	63.92	67.46	70.76	73.97

* Top ten morphometric measurements with the highest significant loadings on PC1 and PC2

that the samples were not different between the coasts (Fig. 9). The lowest accuracy (ACC), highest misclassification rate (MR), lowest sensitivity (TPR), precision (PPV) and Kappa coefficient of 0.58, 0.42, 0.65, 0.61 and 0.15, respectively were observed for the LDA model developed to discriminate between the sexes which was also apparent from the horizontal overlapping between the sexes (Fig. 10). Moreover, the Mcnemar's test p values for the LDAs of locations and coasts were significant ($p \leq 0.001$), indicating significant differences in the frequencies of false positives and false negatives during classification.

Discussion

This study is the first to investigate possible variations in the sand lobster (*T. unimaculatus*) population from Indian waters using morphometric techniques. Apart from genetic factors, animals in a population experiencing different physical, biological and ecological factors such as geographic variation, salinity, temperature, photoperiodicity, essential food availability and fishing intensity may differentiate into separate stocks with differential growth, mortality and maturation characteristics (Panikkar and Jayaraman, 1966; Iles and Sinclair, 1982). Therefore, it is essential to identify and understand the differences among the stocks so that they can be grouped into separate management units for effective and efficient management interventions (Turan *et al.,* 2005).

In the present study, multivariate analysis (MANCOVA) performed to determine the significant difference among the factors, *i.e.,* sampling locations, between the coasts and between the sexes, showed significant variations ($p<0.001$) in morphometric

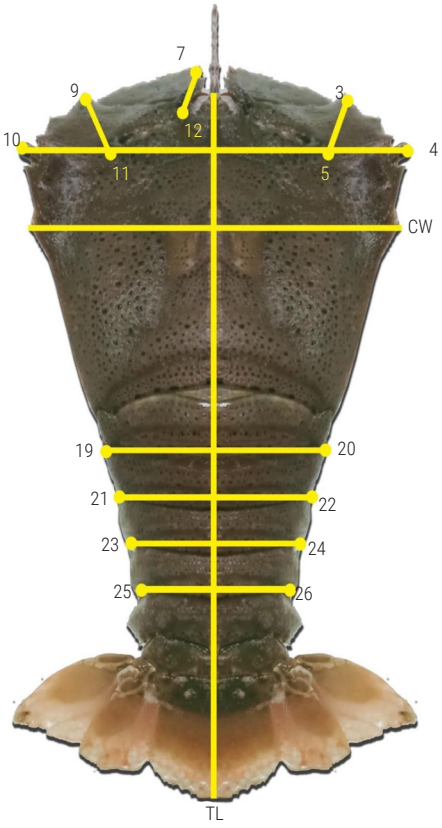

Fig. 6. Highlighted morphometric measurements having significant loadings for PC1 and PC2

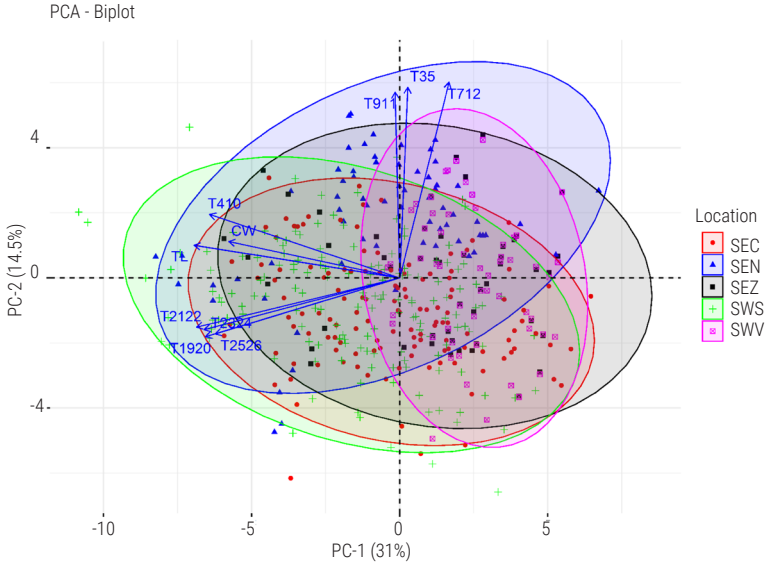

Fig. 7. Biplots of PC1 against PC2 describing the variations between locations

measurements for all the factors. MANCOVA is an essential multivariate statistical tool which reduces the error by controlling the effect covariate (s) (here carapace length) on the relationship between the categorical independent variables or factors (here, locations, coasts and sexes) and continuous dependent variables (here, morphometric measurements). In this study, significant interaction effects ($p<0.001$) of the covariate (CL) with factors, *viz.*, locations, coasts and sexes, were observed, which indicates that the factors are related to the covariate. This interaction effect is due to significant variations ($p<0.001$) in covariate *i.e.*,

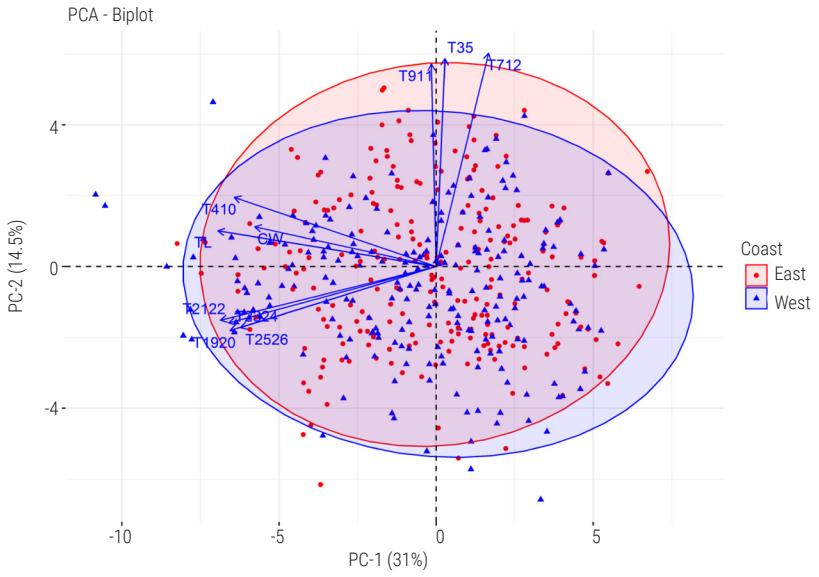

Fig. 8. Biplots of PC1 against PC2 describing the variations between coasts

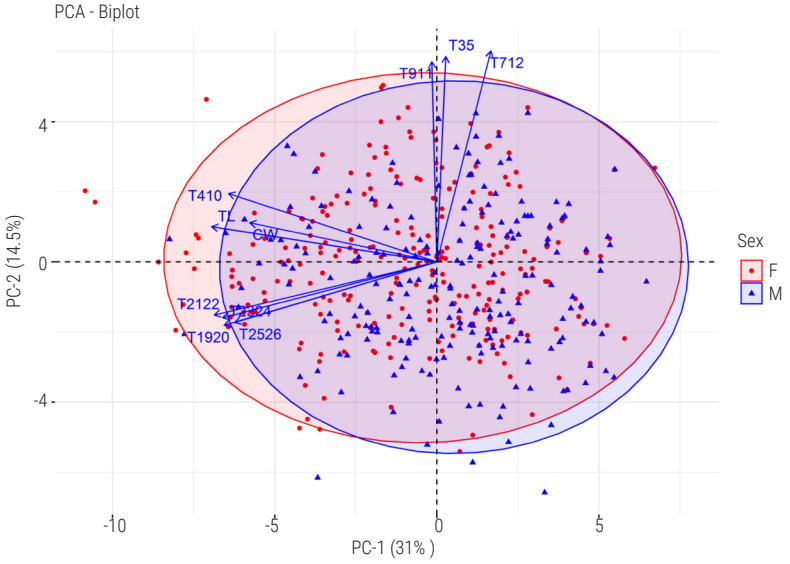

Fig. 9. Biplots of PC1 against PC2 describing the variations between sexes

carapace length (CL) among and along with the factors, *i.e.,* the sampling locations, between the coasts and between the sexes. The above results indicate that there is a size-related variation in the morphometric traits for the species and therefore, an allometric correction to remove the size effect is essentially required before further analysis. The allometric transformation suggested by Elliott *et al.* (1995) to address the size effect successfully reduced the size effect, as the correlation coefficients of individual morphometric measurements with covariate (CL) before transformation were significantly reduced for most of the morphometric measurements after transformation. Interestingly, after allometric correction, all the morphometric measurements for sampling locations, most of the measurements between the coasts and even some of the measurements between the sexes, exhibited significant variation as obtained from univariate analysis, *i.e.,* ANOVA.

From PCA, first eight principal components with cumulatively explained variance of 73.97% were selected based on Kaiser selection criteria (Kaiser, 1960) as each one of them individually explained the variance greater than average variance. Out of eight, first two PCs were further investigated to evaluate the loadings of the variables which elucidated those 10 morphometric measurements *viz*', T2122, TL, T1920, T2324, T410, T2526, T712, CW, T35 and T911 loaded significantly highest on PC1 and PC2. These morphometric

Table 5. Confusion/Error matrix and statistics to measure the performance of linear discriminant analysis (LDA) models developed to investigate the separation among the sampling locations and between the coasts and sexes

Actual cases (Locations)								
	Locations	SEC	SEN	SEZ	SWS	SWV	Predicted total cases	
Predicted cases (Locations)	SEC	115	12	2	17	4	150	
	SEN	3	133	14	20	6	176	
	SEZ	4	6	20	7	14	51	
	SWS	8	19	6	117	4	154	
	SWV	0	0	56	2	84	142	
	Actual total cases	130	170	98	163	112	673	
Statistics	Sensitivity (TPR)	0.89	0.78	0.20	0.72	0.75	Statistics	Location
	Specificity (NPR)	0.94	0.92	0.95	0.93	0.90	ACC	0.70
	Precision (PPV)	0.77	0.76	0.39	0.76	0.59	MR	0.30
	NPV	0.97	0.93	0.88	0.91	0.95	NIR	0.25
	Prevalence	0.19	0.25	0.15	0.24	0.17	*p* (ACC>NIR)	<0.001
	Detection rate	0.17	0.20	0.03	0.17	0.13	Kappa	0.62
	Detection prevalence.	0.22	0.26	0.08	0.23	0.21	*p* (Mcnemar)	<0.001
	Balanced ACC	0.91	0.85	0.58	0.82	0.82		
Actual cases (Coasts)								
	Coasts	East	West				Predicted total cases	
Predicted cases (Coasts)	East	333	110				443	
	West	65	165				230	
	Actual total cases	398	275				673	
	Statistics	Coasts	Statistics				Coasts	
Statistics	Sensitivity (TPR)	0.84	ACC				0.74	
	Specificity (NPR)	0.60	MR				0.26	
	Precision (PPV)	0.75	NIR				0.59	
	NPV	0.72	*p* (ACC>NIR)				<0.001	
	Prevalence	0.59	Kappa				0.45	
	Detection rate	0.50	*p* (Mcnemar)				<0.001	
	Detection prevalence	0.66						
	Balanced ACC	0.72						
Actual cases (Sexes)								
	Sexes	Male	Female				Predicted total cases	
Predicted cases (Sexes)	Males	238	152				390	
	Females	130	153				283	
	Actual total cases	368	305				673	
	Statistics	Sexes	Statistics				Sexes	
Statistics	Sensitivity (TPR)	0.65	ACC				0.58	
	Specificity (NPR)	0.50	MR				0.42	
	Precision (PPV)	0.61	NIR				0.55	
	NPV	0.54	*p* (ACC>NIR)				<0.04	
	Prevalence	0.55	Kappa				0.15	
	Detection rate	0.35	*p* (Mcnemar's)				0.21	
	Detection prevalence	0.58						
	Balanced ACC	0.57						

ACC: Accuracy; MR: Misclassification rate (1-Accuracy); NIR: No information rate, i.e., largest proportion of observed classes; Sensitivity: True positive rate (TPR); Specificity: True negative rate (TNR); Precision (PPV): Positive prediction value; NPV: Negative prediction value

measurements were concentrated more along the abdominal region *viz*., second to fifth abdominal pleuron characters and anterior portion of the cephalothoracic region *viz*., the antennal region. Most of these measurements (6 out of 10) were distributed perpendicular to the longitudinal axis (*i.e.,* TL) of the sand lobster and therefore, the variation explained by the analysis is concentrated more along the width of the lobster, probably for the dorso-ventrally compressed shape of the sand lobster. Nevertheless, there was no apparent

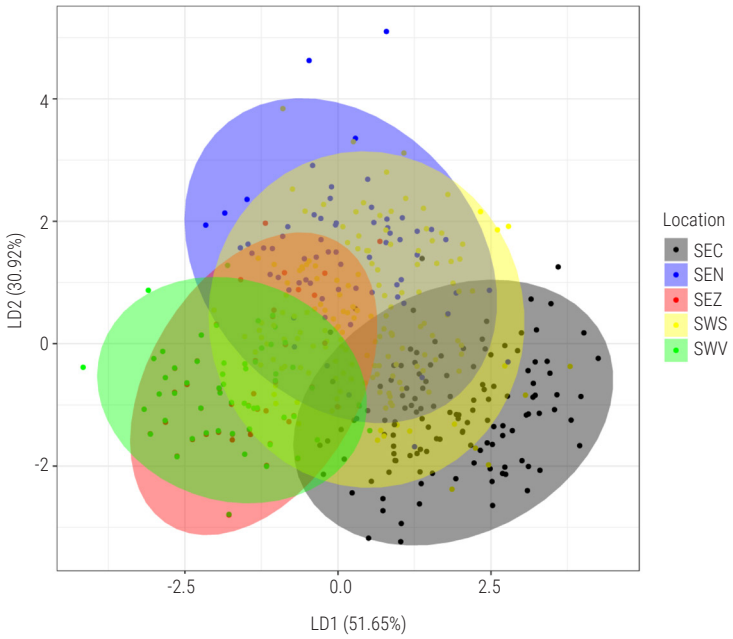

Fig. 10. Linear discriminant analysis (LDA) plot illustrating the separation among the sampling locations

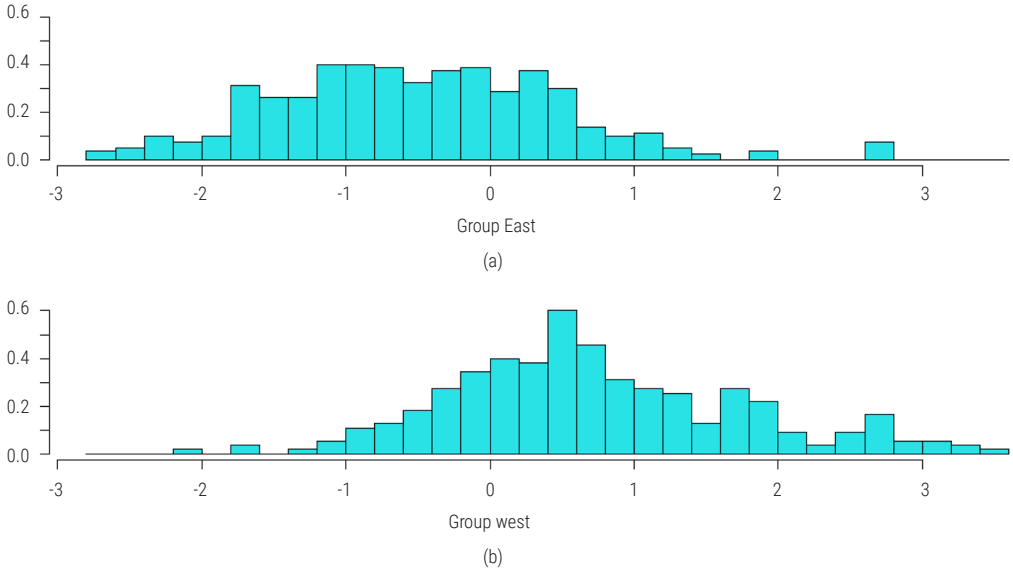

Fig. 11. Linear discriminant analysis (LDA) plot illustrating the separation between the coasts

separation of population, neither among the locations nor between the coasts, which is evident from the PCA biplot using PC1and PC2. It must be noted that with all the morphometric measurements taken into account, both PC1 and PC2 cumulatively explained only 45.50% of the total variance which appears to be low and also gives a hint to the fact that the significant variations in the morphometric measurements obtained by univariate analysis (ANOVA) might not be adequate enough to segregate the population of sand lobster into separate stocks. Like-wise no clear separation was observed between the sexes.

Discriminant analysis performed to optimise the separation of the sand lobster population based on different factors, *i.e.,* sampling locations and between coasts and also to discriminate any variations in morphometric traits between sexes failed to elucidate any clear separation. The linear discriminant analysis (LDA) model developed to separate between the sexes showed a lower sensitivity with low precision and highest misclassification rate of 42% which clearly indicates that significant variations in some of the morphometric measurements between the sexes is not adequate enough to segregate them into two separate groups. Among the locations, though the sensitivity and precision of the model were relatively high for four of the locations, it was very low for the SEZ location, resulting in an overall misclassification rate of 30% for the model.

Despite relatively high sensitivity and precision, the model experienced a misclassification rate of 26% while attempting

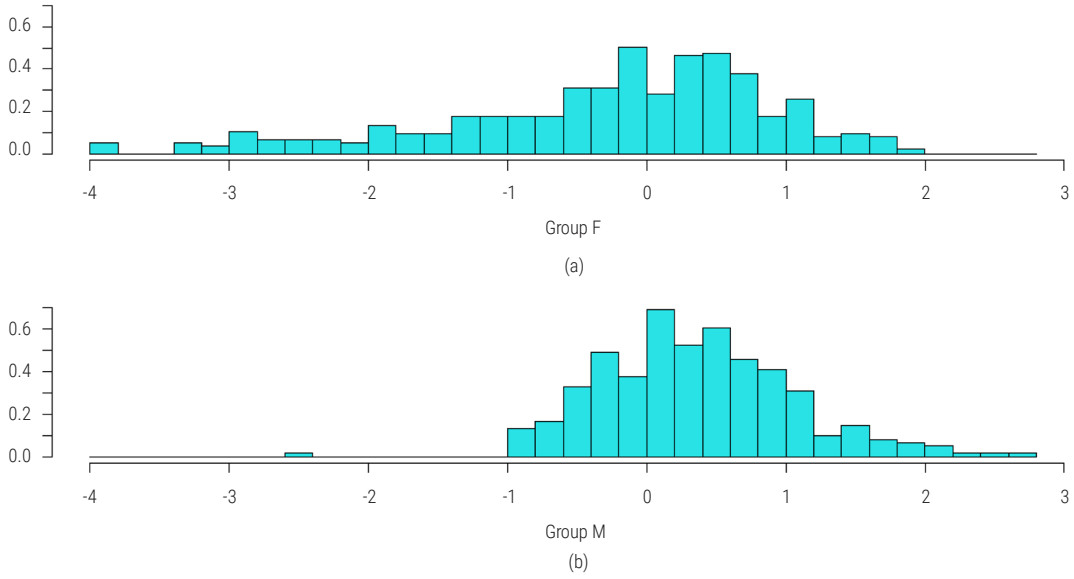

Fig. 12. Linear discriminant analysis (LDA) plot illustrating the separation between sexes

to segregate the population between the coasts. These results clearly indicate that there is insufficient evidence to segregate the populations into separate stocks based on observable variations in morphometric measurements in *T. unimaculatus*. The misclassification rates obtained from DFA indicate the similarity between the populations, which could be attributed to the common environment, genetic origin at an earlier period, and associated genetic mixing of the stocks, particularly those in the transition zones. The mixing of populations from the east coast and west coast could be attributed to the presence of panmictic stocks almost on the same latitudes lying on either side of the coast and due to the absence of any natural geographical impediment. The information derived from the present morphometric study further supports the inference drawn by an earlier molecular study where low levels of genetic differentiation among shovel-nosed lobster populations were observed using molecular techniques like RAPD and mitochondrial DNA genes (COI and Cyt b) probably due to higher connectivity among the populations (Jeena *et al.,* 2015).

Lobsters have longer pelagic larval period, allowing them longer dispersal and population connectivity to remote locations, which can obscure genetically distinct sub-populations (Kough *et al.,* 2013). The present study, based on morphometric assessment, found no population structuring along the Indian coastline when sampling sites were used as factors. However, a closer examination of specimen grouping in a 2D space created by the first two linear discriminants (LD 1 and 2), revealed that the samples from southern (SWS, SEC and SEN) and northern (SWV and SEZ) localities tended to cluster together. However the present assessment cannot definitely conclude whether this latitudinal grouping is due to genetic structuring or driven by phenotypic responses to the ambient environment (physical and biological), which are invariably influenced by latitude. Though a previous study (Jeena *et al.,* 2015) demonstrated poor genetic structuring of Indian shovel-nosed lobster populations aligning with the findings of the present study, a more detailed investigation using matrilineal and nuclear genes coupled with tagging experiments as suggested by Corrigan *et al.* (2018) might be necessary to generate robust information on the population structure of shovel-nosed lobsters along the Indian coastline of over 8000 km.

The findings of the study supplement the present scientific understanding about the shovel-nosed lobster population in India, which would greatly aid in their better management and conservation. As a single population distributed along the Indian sub-continent, a unified management strategy should be adopted, with a specific focus on implementation of minimum legal size (MLS) of capture, strictly preventing the capture of sand lobsters below the size at first maturity and ensuring the release of berried female lobsters back to the sea. Such measures would help to rebuild the declining stock and ensure sustainable management of this valuable resource.

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