

Stock Structure Analysis of Declining Shovel Nosed Lobster *Thenus Unimaculatus* (Burton and Davie, 2007) for Effective Management and Conservation Along the Indian Coast

Rekha Devi Chakraborty (✉ rekhadevi7674@gmail.com)

Central Marine Fisheries Research Institute <https://orcid.org/0000-0002-3857-0231>

Sarada Pengamuck Thanku

CMFRI: Central Marine Fisheries Research Institute

Gyanaranjan Dash

Central Marine Fisheries Research Institute

Rajan Kumar

CMFRI: Central Marine Fisheries Research Institute

Indira Divipala

Central Marine Fisheries Research Institute

Rajkumar Muthusamy

CMFRI: Central Marine Fisheries Research Institute

Sreelakshmy Sivasankaran

CMFRI: Central Marine Fisheries Research Institute

Research Article

Keywords: LDA, Putative stocks, Slipper lobster, *Thenusunimaculatus*, Truss, Population structure.

Posted Date: January 28th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1221819/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Thenus unimaculatus (Burton and Davie 2007) is one of the most important commercially exploited lobster species in India distributed throughout the coast. The declining trends and collapse of the sand lobster fishery has been reported from the northwest coast of India. A number of hypotheses have been proposed to explain the decline, but a lack of basic information on population demographics inhibits hypothesis testing. In this study, morphometric stock structure analysis of *Thenus unimaculatus* was attempted to discriminate the spawning population collected from five locations (673 specimens) during 2017-2019 along the Indian coast. Data was subjected to allometric transformation to remove size effect. The transformed data was subjected to multivariate analysis, such as Principal Component Analysis (PCA) followed by linear discriminant analysis (LDA) using R software. 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. The accuracy (ACC) and misclassification rate (MR) of the linear discriminant analysis (LDA) model performed to optimize separation among different sampling locations were 0.70 and 0.30, respectively, which indicates clear overlapping of the stocks among different sampling locations. The accuracy (ACC) and misclassification rate (MR) of the linear LDA model developed to discriminate between the coasts were 0.74 and 0.26, respectively, indicating no apparent differentiation in the samples between the coasts. The results of this study revealed the presence of a single spawning stock of shovel nosed lobster, *T. unimaculatus* along the Indian coast. The information will aid in the development of better holistic management strategy for the conservation of this declining resource.

Introduction

Lobsters form one of the most valuable crustacean resources, which have been exploited commercially for the past four decades. They have a good market and great demand around the world and thereby fetch foreign exchange for 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 favor the settlement of a wide variety of lobster species (Radhakrishnan *et al.* 2019). Marine lobsters are members of the suborder Macrura Reptantia with 4 infraorders, 6 families, 54 genera, and 260 extant species (including 4 subspecies) (Chan 2019). The lobster fauna of India is so diverse that 38 species belonging to five families and three infraorders (Astacidea, Achelata, and Polychelida) are distributed in the seas surrounding the Indian subcontinent. *Thenus* is the only genus in 7 scyllarid genera that is economically significant (Jones 1990). The scyllarid lobster, *Thenus unimaculatus*, described earlier as *Thenus orientalis* (Chhapgar and Deshmukh 1964) is commonly known as sand lobster, slipper lobster, or shovel-nosed lobster. The species of shovel nosed lobsters available in Indian waters has been confirmed as *Thenus unimaculatus* (Jeena *et al.* 2011). It is one of the important commercially exploited lobster species in India. It is distributed almost throughout the Indian coast and forms an important fishery along the north-west and south-east coasts (Holthuis 1991). The declining trend in catches, as well as the collapse of the fishery in some localities (Deshmukh 2001; Jeena *et al.* 2015; Subramanian 2004) necessitated an assessment of the stock structure to manage this resource sustainably.

From fisheries management perspective, a stock is described as an intraspecific assemblage of finfishes or shellfishes with spatial or temporal stability, that randomly mate with members of each group having identical growth, mortality, and reproductive rates (Hilborn and Walters 1992). Identification of stocks with variable life history attributes such as growth, mortality and reproductive potentials are considered being important for understanding population dynamics and developing sustainable resource management strategy.

Therefore, stock structure analysis is an essential technique to identify and understand the difference among the individuals of the same species exhibiting different growth, mortality and maturation characteristics so that they can be grouped into separate management units for the 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 identification of stocks like meristic, morphometric, parasite as natural tags, otolith study and several molecular markers (Awasthi et al. 2015). It has been observed that variations in morphometric traits do 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 and AnvariFar et al. 2011). Therefore, morphometric traits are more promising for studying short-term environmentally induced variation, which is essential for fisheries management (Begg et al. 1999; Swain and Foote 1999). Several earlier studies have emphasized the utility of morphometric and meristic data in separating the stocks of the 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 way among methods of species identification. Recently, truss networking system has evolved as a new system for morphometric measurement. Truss network systems constructed with the help of landmark points are powerful tools for stock identification. The methodology predicts the measurement of across-body distances connecting two morphological landmarks from a sequential series of connected polygons. This type of landmark-based technique using geometrical morphometrics imposes no restrictions on the direction of variation and localization of shape changes and therefore is highly effective in capturing information regarding the shape of an organism (Cavalcanti et al. 1999). Subsequently, several multivariate techniques, such as principal component analysis and discriminant analysis, are used to discriminate the stocks. In the present study, attempts have been made to describe the stock structure of scyllarid lobster, *Thenus unimaculatus* along Indian subcontinent through morphometric approach so that appropriate management strategy could be developed to rebuild this declining crustacean resource.

Materials And Methods

Sampling

A total of 673 specimens of *Thenusunimaculatus* consisting of 305 males and 368 females were collected from five major fishing harbours along the Indian coast. The specimens were identified as *Thenusunimaculatus* 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 Vishakapatnam (SEZ) along the north-east coast of India (Fig. 1). The descriptive locality, sex ratio and sample size from each location are shown in Table 1. All the specimens procured were in mature condition representing the spawning stock. Breeding season of *Thenusunimaculatus* 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 totally excluded while only matured specimens (carapace length ≥ 6.0 cm) were included in the study.

Table 1

Sampling locality, coast, geographical coordinates, sex and number of specimens collected

Coast	Locality	Latitude, Longitude	Sex	Sample size (n)
West	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
East	Vishakapatnam (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				

Digitization of samples and measurement of truss 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 vertical and horizontal grids of graph paper were used in calibrating the coordinates covering an area of 1 cm². 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 truss distances from the digital images of specimens was conducted using a linear combination of three software platforms: tpsutil, tpsDig2 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 by employing the millimeter grid on the graph paper. A truss box 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 and statistical study

All the morphometric truss 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 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 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

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

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, $\log CL \text{ mean}$ 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 (Samae et al. 2006) and to extract a number of dependent variables for population differentiation (Anvarifaret al. 2011; Kuberan et al. 2020). Bartlett's test of sphericity to ascertain the requisite correlation among the variables, 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 scrutinizing the Eigen values using a scree plot (Kaiser 1960). The variables with significant loadings to 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 the statistical analysis was performed using R statistical software package, Boston, MA, RStudio, Version 1.4.1106, Release name: Tiger Daylily (RStudio Team, 2021).

Result

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 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; $F=7.52$, $p < 0.001$).

Table 2

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

Variables & their interaction	Wilk's lambda	F	D.F.	Numerator D.F.	Denominator D.F.	<i>p</i>
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

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.

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

Measurements	Location		Coast		Sex	
	F	p	F	p	F	p
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

The correlation of individual morphometric measurements with covariate (CL) before transformation and after transformation of morphometric measurements is shown in Fig. 3. All the 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 23 (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 test ($\chi^2 = 18938.07$, $df=496$, $p < 0.01$), multicollinearity test (determinant= 3.5×10^{-13}) and Kaiser-Meyer-Olkin tests (overall MSA=0.81) to measure the sample adequacy. Principal component analysis (PCA) indicated that first eight principal components (PCs) individually explained the variance greater than average variance based on Kaiser Selection criteria (Eigenvalue > 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 eigenvalues, 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.

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

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

Morphometric measurements	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
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								

Bivariate PCA biplots of PC1 against PC2 describing the variations in morphometric measurements between locations, coasts and sexes are shown in Figure 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 principal component analysis to optimize the separation among the groups (locations, coasts, and sexes) is given Table 5 and the pictorial separation has been illustrated as LDA plots in Fig. 10, 11 and 12.

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 indicates 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 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 ($p \leq 0.001$) which indicates that there are significant differences in the frequencies of false positive and false negative during classification

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 preval.	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 preval.	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 preval.	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

Discussion

This present study is the first time investigation in which an attempt has been made to identify any possible variations in sand lobster, *T. unimaculatus* population from Indian waters using truss morphometry techniques. Animals in a population, apart from genetic factors, 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 identical 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 the 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 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 the present study, significant interaction effects ($p < 0.001$) of covariate (CL) with factors, viz., locations, coasts and sexes, were observed, which indicates that the factors are related to covariate. This interaction effect is due to significant variations ($p < 0.001$) in covariate i.e., carapace length (CL) among and along with the factors, i.e., the sampling locations, between the coasts and between the sexes. The above results evidently indicate that there is a size related variation in the morphometric traits for the species and therefore, an allometric correction to remove 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 principal component analysis (PCA), first eight principal components (PCs) 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 that 10 morphometric measurements i.e., T2122, TL, T1920, T2324, T410, T2526, T712, CW, T35 and T911 loaded significantly highest on PC1 and PC2. These morphometric measurements were concentrated more along the abdominal region viz., second to fifth abdominal pleuron characters and anterior portion of cephalothoracic region viz. the antennal region. Most of these measurements (6 out of 10) were distributed perpendicular to 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-ventral compressed shape of the sand lobster. Nevertheless, there was no apparent separation of population, neither among the locations nor between the coasts, which is evident from the PCA biplot using PC1 and 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 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 optimize 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 the 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 sensitivity and precision of the model was relatively high for four of the locations, it was very low for SEZ location, resulting in an overall misclassification rate of 30% for the model.

Similarly, despite a relatively high sensitivity and precision, a misclassification rate of 26% was experienced for the model while attempting to segregate the population between the coasts. These results clearly indicate that there is not adequate evidence based on which the populations can be segregated into separate stocks using the observable variations in morphometric measurements in sand lobster, *T. unimaculatus*. The misclassification rates obtained from DFA clearly indicate the similarity between the populations, which could be attributed to the common environment, genetic origin at an earlier period, and associated with genetic mixing of the stocks, particularly those in the transition zones. The mixing of populations from 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 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 was observed using molecular techniques like RAPD and mitochondrial DNA genes (COI & Cyt b) probably due to higher connectivity among the populations (Jeena et al. 2015).

Lobster have longer pelagic larval period, allowing them longer dispersal and population connectivity to remote locations, which can mask the genetically distinct sub-population (Kough et al., 2013). The present study, based on the morphometric assessment, found no structuring in the population along the Indian coastline when sampling sites were used as factors. However, a closer insight into the specimens grouping in a 2D space created by the linear discriminant 1 and 2 (LD 1 and 2), the samples from southern (SWS, SEC and SEN) and northern localities (SWV and SEZ) were found to be grouped together. But the present assessment cannot conclude that the latitudinal grouping is for genetic structuring or driven by phenotypic responses to ambient environment (physical and biological) which invariably is a

function of latitude. Though previous study (Jeena et al., 2015) demonstrated poor genetic structuring of Indian shovel-nosed lobster populations, a more detailed study using more matrilineal and nuclear gene coupled with tagging experiments is required to have robust information on the population structure (Corrigan et al., 2018) of shovel-nosed lobsters along Indian coastline of over 8000 km.

Conclusion

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

Declarations

Ethics statement

Shrimp samples were directly obtained from the commercial trawl catches and were purchased from the local fishermen. Permission from authorities was not required for the studied sites from where the shrimps were collected as it was outside the protected areas (PAs).

Acknowledgments

The authors are thankful to the Indian Council of Agricultural Research (ICAR), New Delhi for providing the facilities to carry out this work. The authors express their gratitude to the Director, CMFRI for the kind support and encouragement. Thanks are also due to the HOD, CFD for facilitating the research activities. The assistance rendered by all the technical personnel is greatly acknowledged.

Availability of data and materials

Data will be available based on request, and supporting files are also submitted

Author's contribution

Rekha Devi Chakraborty Conceptualization and Planning of work, Writing, overall supervision

Sarada P T Sampling, draft making

Gyanaranjan Dash Software analysis

Rajan Kumar Sampling, draft editing

Indira Divipala Sampling

Rajkumar M Sampling

Sreelakshmy S Digitization of samples, extraction of data

Funding

The research was conducted with the fund support of ICAR Central Marine Fisheries Research Institute

Ethics approval and consent to participate The manuscript is not submitted in any other journal. This manuscript does not involve the use of any live animal or human data or tissue. Shrimp samples obtained from the commercial trawl catches were directly used.

Consent to participate

Not applicable

Consent for publication

The approval for submitting manuscript received from ICAR-Central Marine Fisheries Research Institute.

Competing interests

The authors declare that they have no competing interests.

References

1. AnvariFar H, Khyabani A, Farahmand H, Vatandoust S, AnvariFar H, Jahageerdar S (2011) Detection of morphometric differentiation between isolated up and downstream populations of Siah Mahi (*Capoetacapoeta gracilis*) (Pisces: Cyprinidae) in the Tajan River (Iran). *Hydrobiologia* 673: 41–52
2. Anyanwu AO, Ugwumba OA (2003) Studies on the morphometric, meristic and electrophoresis patterns of *Pseudotolithus* species. *The Zoologist* 2(1): 70–77
3. Awasthi M, Kashyap A, Gupta M, Serajuddin M (2014) Morphometry In Fish Stocks Using Truss Network System. Biodiversity and Environment. In: M. Serajuddin, M. Tripathi, A. Kanaujia, M. Arshad (Eds). Lucknow: Department of Zoology, University of Lucknow, pp 208–211
4. Begg GA, Friedland KD, Pearce JB (1999) Stock identification and its role in stock assessment and fisheries management: an overview. *Fisheries Research* 43: 1–8
5. Burton TE, Davie PJF (2007) A revision of the shovel-nosed lobsters of the genus *Thenus* (Crustacea: Decapoda: Scyllaridae), with descriptions of three new species. *Zootaxa* 1429(1): 1–38
6. Cadrin SX (2000) Advances in morphometric identification of fishery stocks. *Reviews in Fish biology and Fisheries* 10(1): 91–112
7. Cadrin SX, Friedland KD, Waldman J (Eds) (2005) *Stock Identification Methods: Applications in Fishery Science*. Elsevier Academic Press.
8. Cavalcanti MJ, Monteiro LR, Lopes PR (1999) Landmark-based morphometric analysis in selected species of serranid fishes (Perciformes: Teleostei). *Zoological Studies* 38(3): 287–294

9. Chan TY (2019) Updated checklist of the world's marine lobsters. Lobsters: Biology, Fisheries and Aquaculture. In: E.V. Radhakrishnan, B. Phillips, G. Achamveetil (Eds.). Singapore: Springer, pp 35–64
10. Chhapgar BF, Deshmukh SK (1964) Further records of lobsters from Bombay. Journal of the Bombay Natural History Society 61(1): 203–207
11. Corrigan S, Lowther AD, Beheregaray LB, Bruce BD, Cliff G, Duffy CA et al. (2018) Population connectivity of the highly migratory shortfinmako (*Isurus paucus* Rafinesque 1810) and implications for management in the Southern Hemisphere. *Frontiers in Ecology and Evolution* 6:187 DOI: 10.3389/fevo.2018.00187
12. Deshmukh VD (2001) Collapse of sand lobster fishery in Bombay waters. *Indian Journal of Fisheries* 48(1): 71–76
13. Elliott NG, Haskard K, Koslow JA (1995) Morphometric analysis of orange roughy (*Hoplostethus atlanticus*) off the continental slope of southern Australia. *Oceanographic Literature Review* 9(42): 790
14. Hammer O, Harper DA, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4(1): 9
15. Hilborn R, Walters CJ (Eds) (1992) Quantitative fisheries stock assessment, choice, dynamics and uncertainty. Chapman and Hall, London
16. Holthuis LB (1991) Marine lobsters of the World. FAO species catalogue, Vol.13. FAO Fisheries Synopsis, Food and Agriculture Organization, Rome 125 (13): 1–292
17. Iles TD, Sinclair M (1982) Atlantic Herring: Stock Discreteness and Abundance. *Science* 627–633
18. Jeena NS, Gopalakrishnan A, Kizhakudan JK, Radhakrishnan EV, Raj Kumar, Asokan PK (2015) Population genetic structure of the shovel-nosed lobster *Thenus unimaculatus* (Decapoda, Scyllaridae) in Indian waters based on RAPD and mitochondrial gene sequences. *Hydrobiologia* 766(1): 225-236 DOI 10.1007/s10750-015-2458-z
19. Jeena NS, Gopalakrishnan A, Radhakrishnan EV, Lijo J, Joe KK, Basheer VS, Shoba JK et al. (2011) Molecular phylogeny of commercially important lobster species from Indian waters deduced from mitochondrial and nuclear DNA sequences. Book of Abstracts, 9th Indian Fisheries Forum, December 19–23, Chennai, India
20. Jones CM (1990) Morphological characteristics of bay lobsters, *Thenus* Leach species (Decapoda, Scyllaridae), from north-eastern Australia. *Crustaceana* 59(3): 265–275
21. Kaiser HF (1960) The application of electronic computers to factor analysis. *Educational and Psychological Measurement* 20(1): 141–151
22. Khan MA, Miyan K, Khan S, Patel DK, Ansari NG (2012) Studies on the elemental profile of otoliths and truss network analysis for stock discrimination of the threatened stinging catfish *Heteropneustes fossilis* (Bloch 1794) from the Ganga River and its tributaries. *Zoological Studies (Taipei, Taiwan)* 51: 1195–1206
23. Kizhakudan JK (2014) Reproductive biology of the female shovel-nosed lobster *Thenus unimaculatus* (Burton and Davie, 2007) from north-west coast of India. *Indian Journal of Geo-Marine*

Sciences 43 (6): 927–935

24. Kough AS, Paris CB, Butler IVMJ (2013) Larval connectivity and the international management of fisheries. *PloS one*, 8(6): e64970
25. Krumholz LA, Cavanah SH (1968) Comparative morphometry of freshwater drum from two mid western localities. *Transactions of the American Fisheries Society* 97: 429–441
26. Kuberan G, Chakraborty RD, Purushothaman P (2020) Phenotypic variation using truss network system in the deep-sea shrimp *Heterocarpus chani* Li, 2006 (Caridea: Pandalidae) off Arabian Sea and Bay of Bengal. *Indian Journal of Geo-Marine Sciences* 49 (12): 1839–1847
27. Loy A, Genov P, Galfo M, Jacobone MG, Taglianti AV (2008) Cranial morphometrics of the Apennine brown bear (*Ursus arctos marsicanus*) and preliminary notes on the relationships with other southern European populations. *Italian Journal of Zoology* 75(1): 67–75
<https://doi.org/10.1080/11250000701689857>
28. Panikkar NK, Jayaraman R (1966) Biological and oceanographic differences between the Arabian Sea and the Bay of Bengal as observed from the Indian region. *Proceedings: Plant Sciences* 64: 231–240
29. Pinheiro A, Teixeira CM, Rego AL, Marques JF, Cabral HN (2005) Genetic and morphological variation of *Solealascaris* (Risso, 1810) along the Portuguese coast. *Fisheries Research* 73(1–2): 67–78
[doi:10.1016/J.FISHRES.2005.01.004](https://doi.org/10.1016/J.FISHRES.2005.01.004).
30. Radhakrishnan EV, Phillips Bruce F, Gopalakrishnan A (2019) *Lobsters: Biology, Fisheries and Aquaculture*. Singapore :Springer Nature
31. Radhakrishnan EV, Thangaraja R (2008) Sustainable exploitation and conservation of lobster resources in India a participatory approach. In: *Glimpses of Aquatic Biodiversity- Rajiv Gandhi Chair Special Publication*. In book: *Glimpses of Aquatic Biodiversity* (Eds. Natarajan P, Jayachandran KV, Kannaiyan S, Ambat B, Augustine A) Cu Kochi, p 184–192
32. Rohlf FJ (2006) *Tps Dig2*, version 2.1. State University of New York Stony Brook, NY [Internet]. [cited 2018 Dec 5]. Available from: <http://life.bio.sunysb.edu/morph/>
33. Samaee SM, Mojazi-Amiri B, Hosseini-Mazinani SM (2006) Comparison of *Capoetacapoeta gracilis* (Cyprinidae, Teleostei) populations in the south Caspian Sea River basin, using morphometric ratios and genetic markers. *Folia Zoologica* 55(3): 323–335
34. Strauss RE, Bookstein FL (1982) The truss: body form reconstructions in morphometrics. *Systematic Zoology* 31(2): 113-135
35. Subramanian VT (2004) Fishery of sand lobster *Thenus orientalis* (Lund) along Chennai coast. *Indian Journal of Fisheries* 51(1): 111-115
36. Swain DP, Foote CJ (1999) Stocks and chameleons: the use of phenotypic variation in stock identification. *Fisheries Research* 43(1-3): 113–128 [doi:10.1016/S0165-7836\(99\)00069-7](https://doi.org/10.1016/S0165-7836(99)00069-7)
37. Tomovic L, Dzukic G (2003) Geographic variability and taxonomy of the nose-horned viper, *Vipera ammodytes* (L. 1758), in the central and eastern parts of the Balkans: A multivariate study. *Amphibia-Reptilia* 24: 359–377 <https://doi.org/10.1163/156853803322440817>

38. Turan C (1999) A note on the examination of morphometric differentiation among fish populations: the truss system. *Turkish Journal of Zoology* 23(3): 259-263
39. Turan C, Yalcin S, Turan F, Okur E, Akyurt I (2005) Morphometric comparisons of African catfish, *Clarias gariepinus*, populations in Turkey. *Folia Zoologica* 54(1/2): 165-172
40. Veasey EA, Schammas EA, Vencovsky R, Martins PS, Bandel G (2001) Germplasm characterization of *Sesbania* accessions based on multivariate analyses. *Genetic Resources and Crop Evolution* 48(1): 79– 90

Figures



Figure 1

Sampling locations along Indian coast

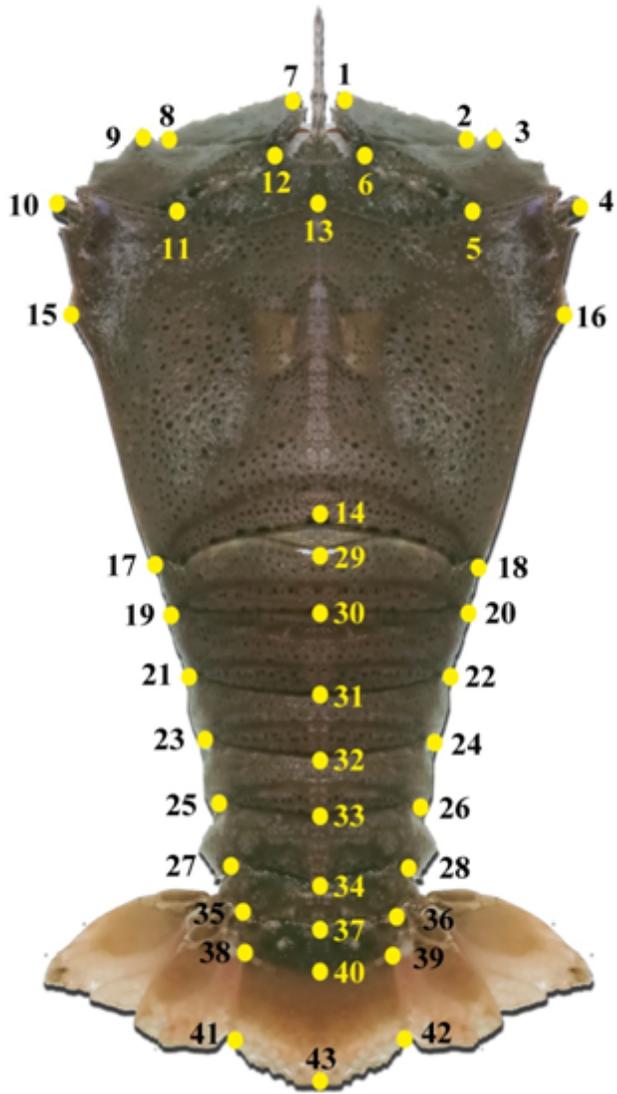


Figure 2

Truss landmark points used for digitization of morphometric measurements of *T. unimaculatus*

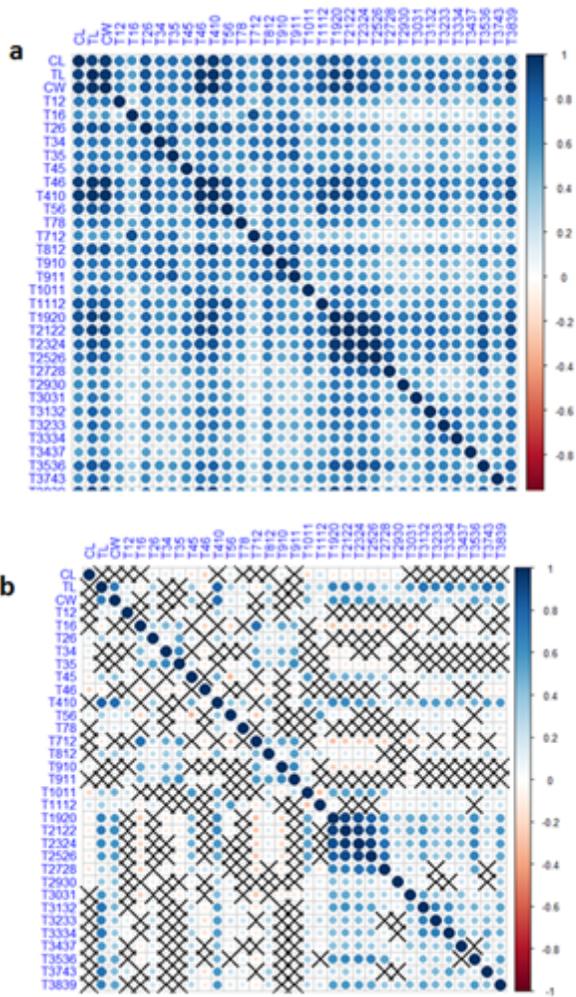


Figure 3

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

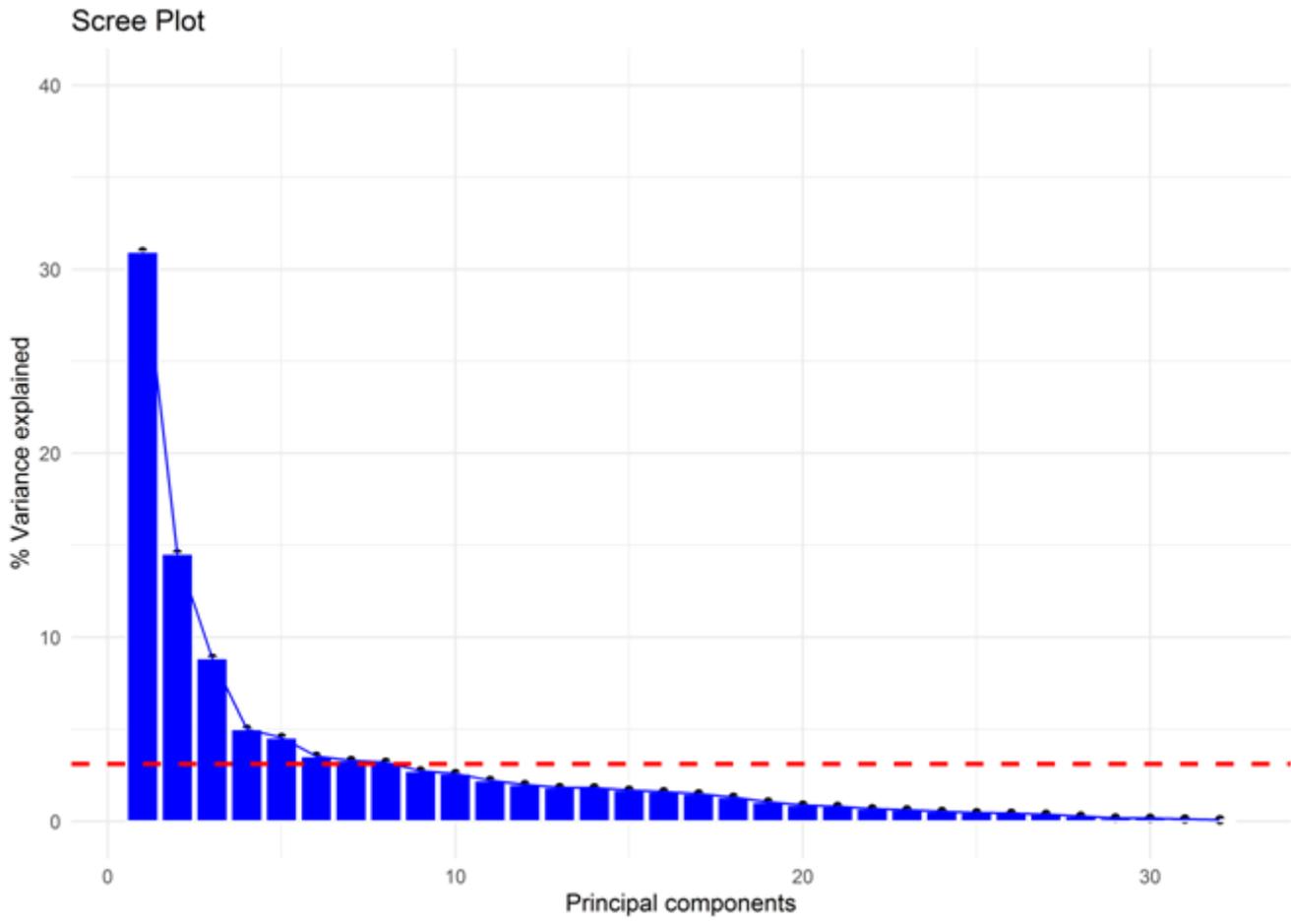


Figure 4

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

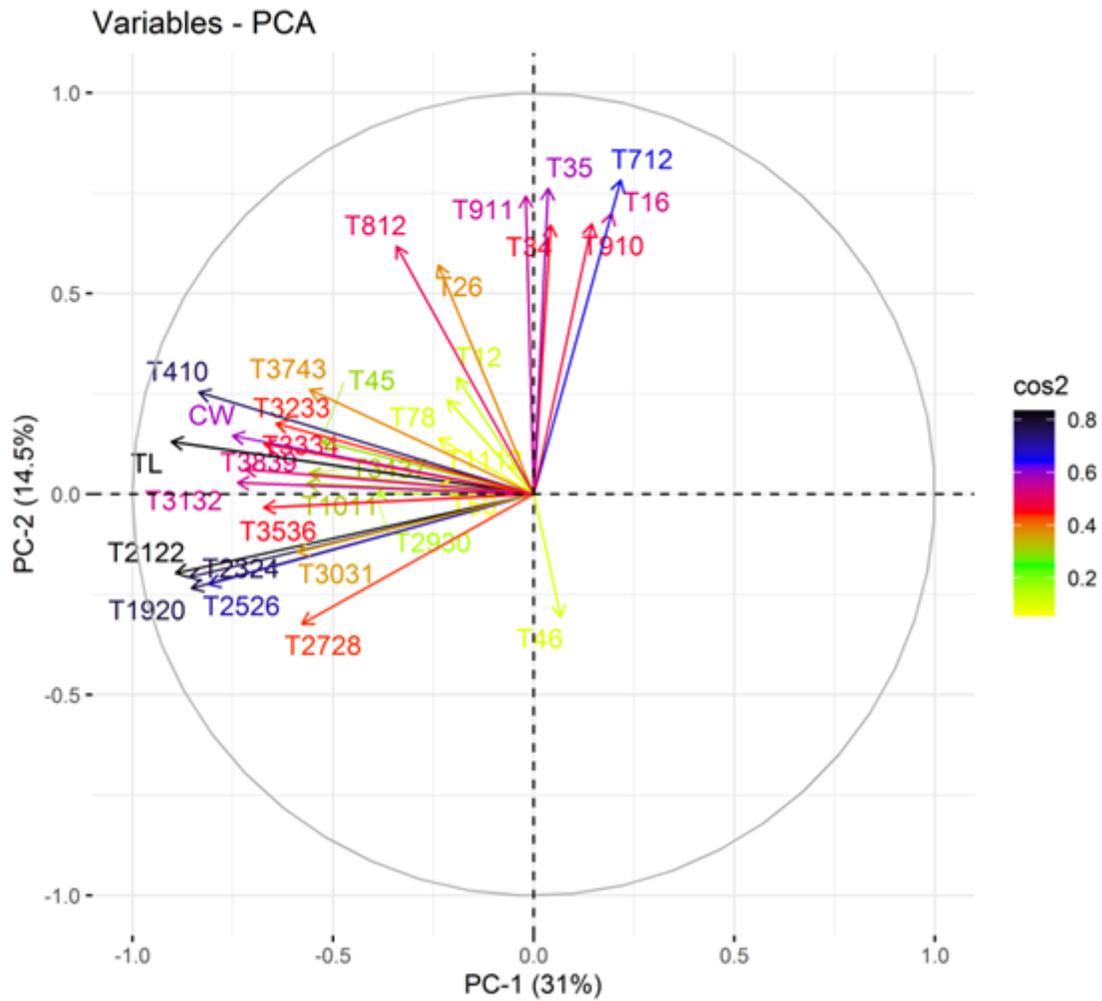


Figure 5

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

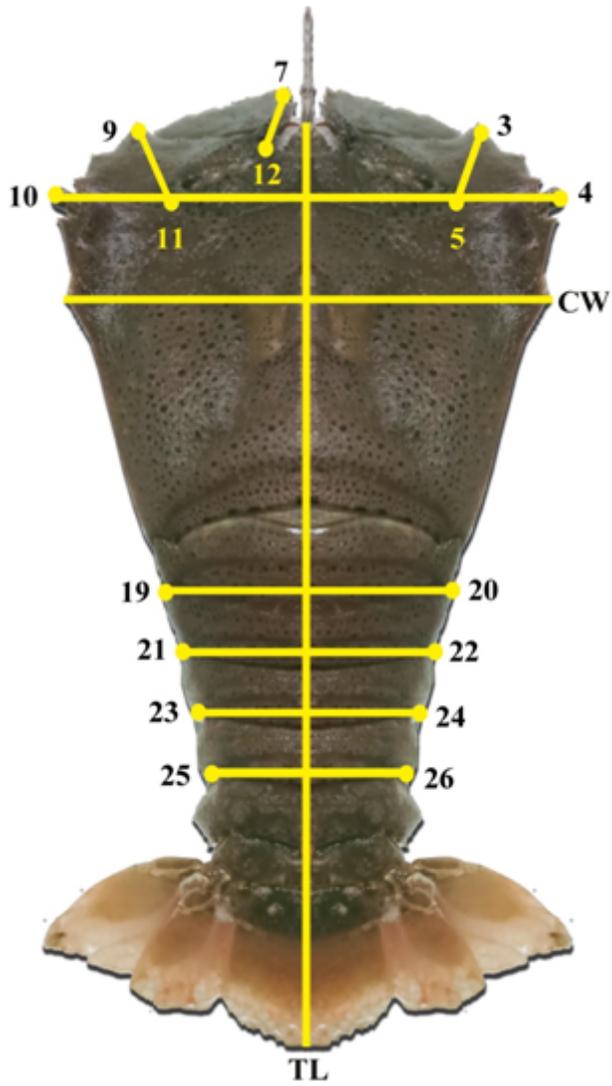


Figure 6

Highlighted morphometric measurements having significant loadings for PC1 and PC2

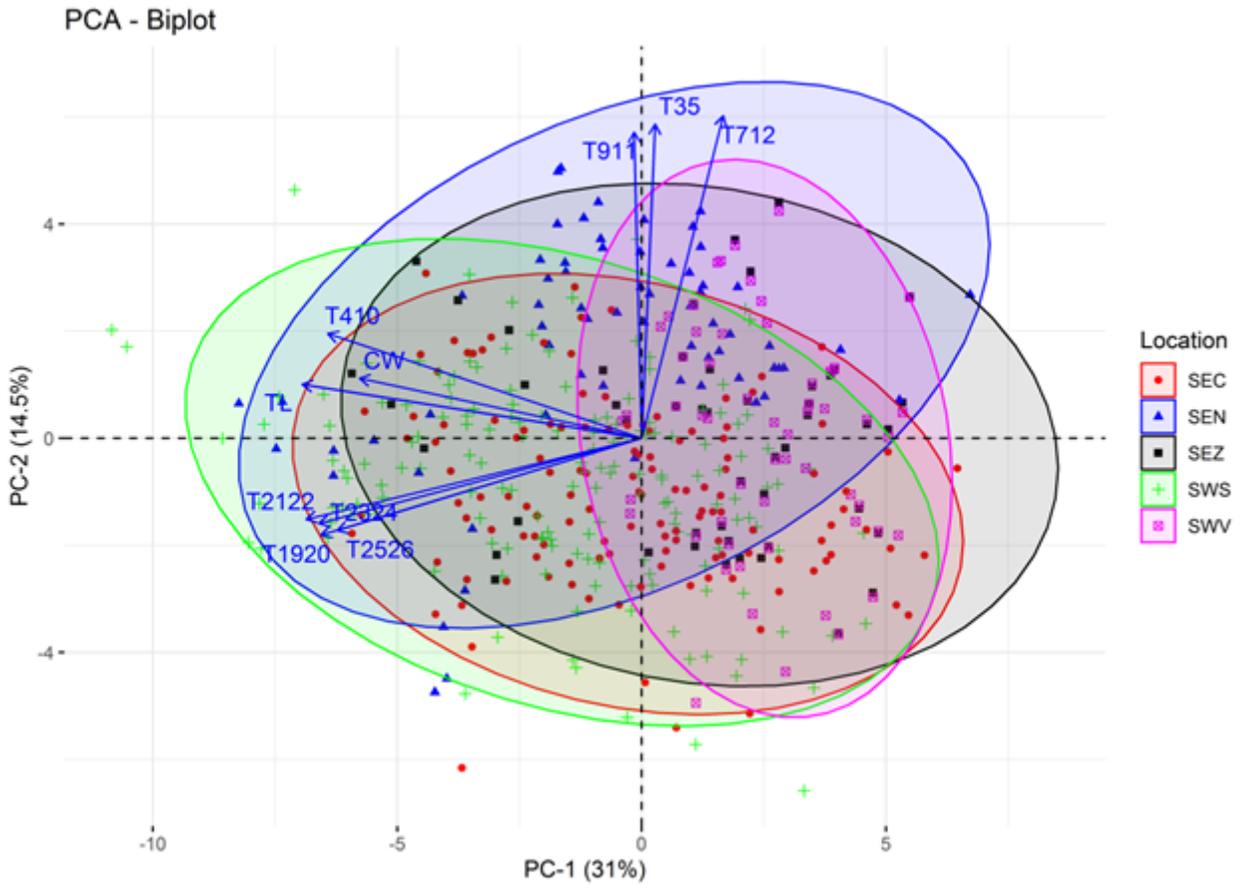


Figure 7

Biplots of PC1 against PC2 describing the variations between locations

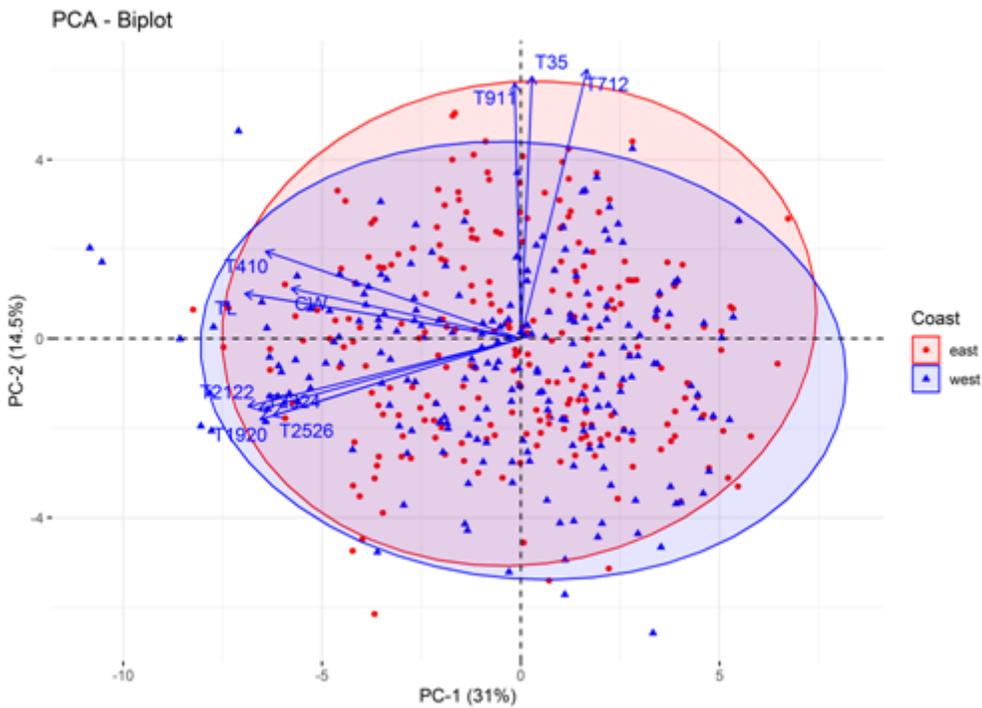


Figure 8

Biplots of PC1 against PC2 describing the variations between coasts

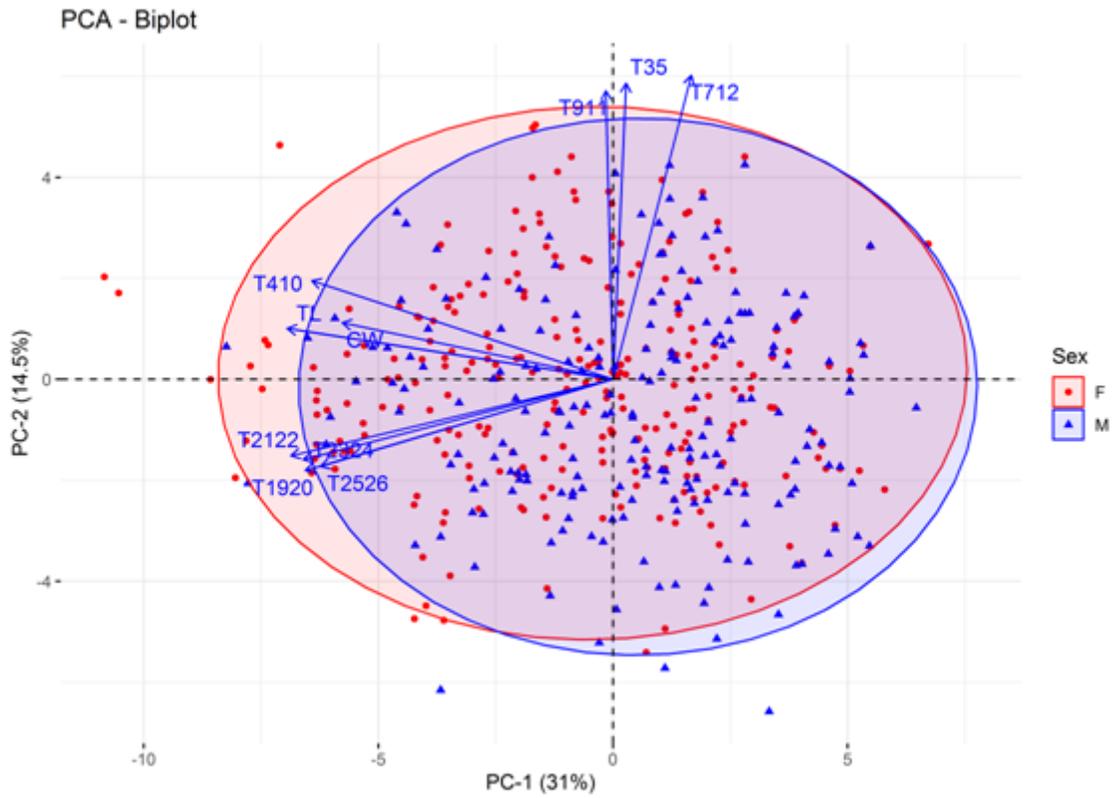


Figure 9

Biplots of PC1 against PC2 describing the variations between sexes

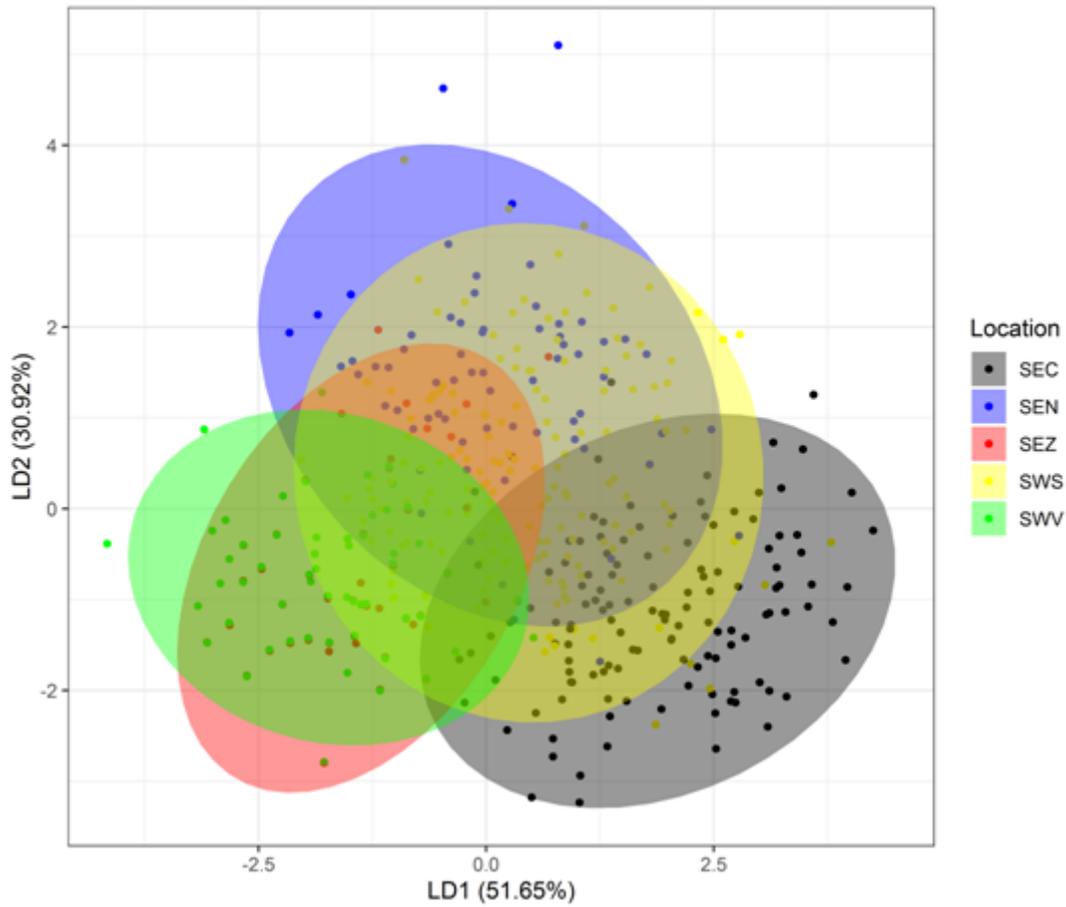


Figure 10

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

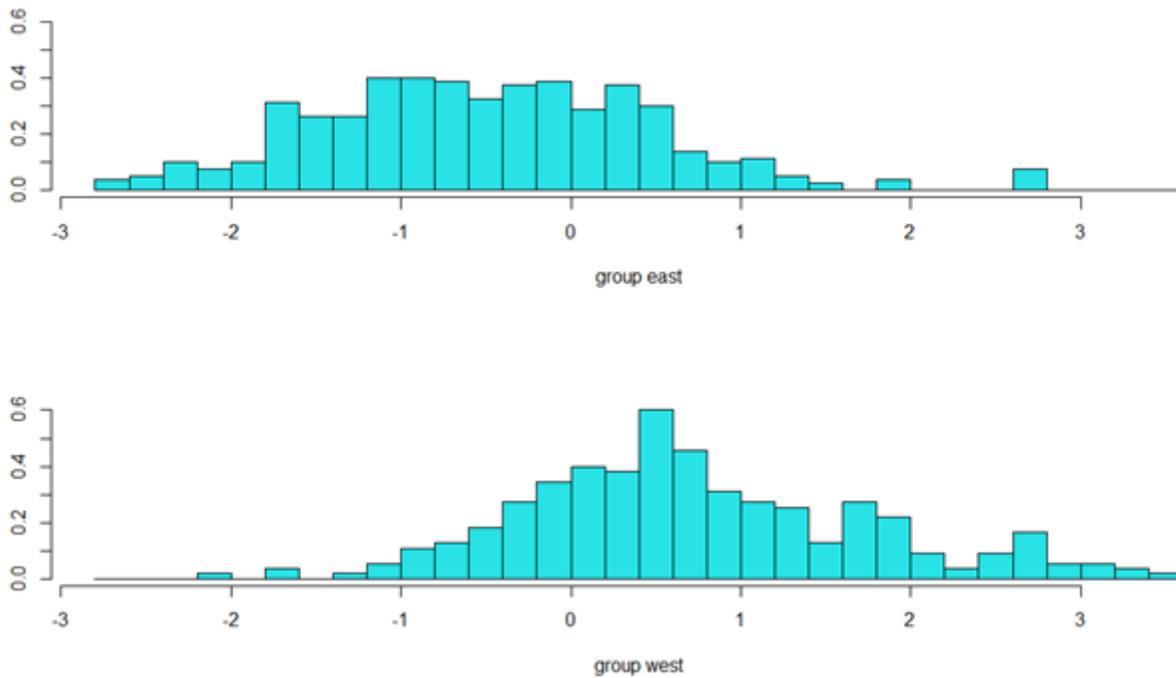


Figure 11

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

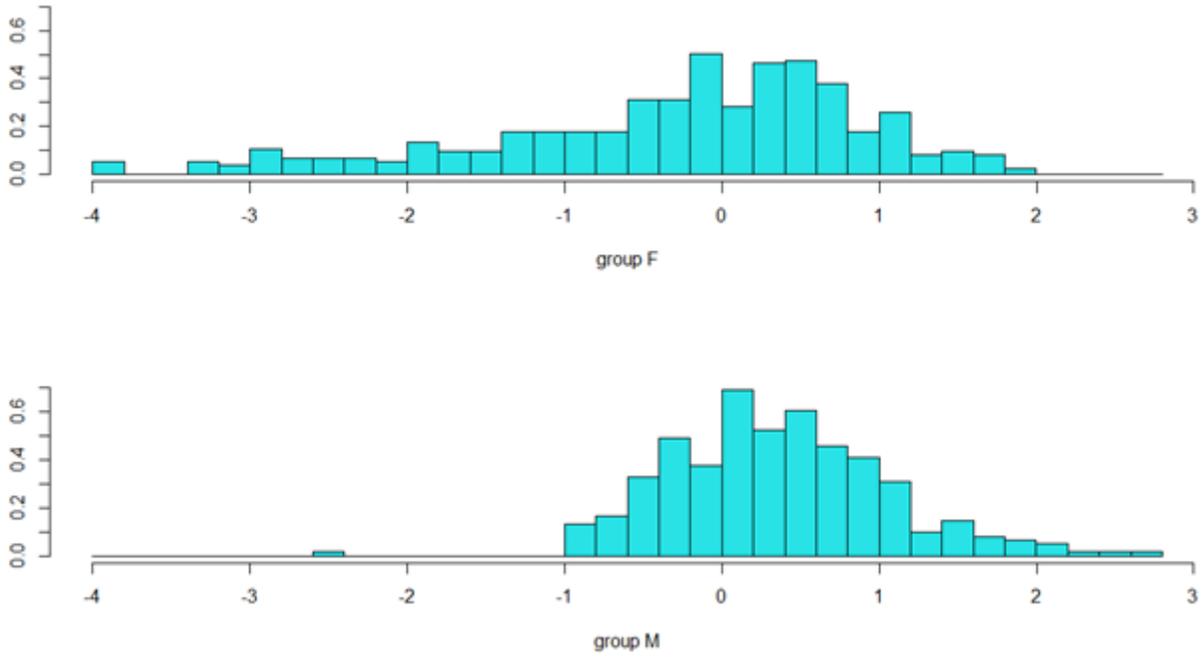


Figure 12

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