Chapter One

INTRODUCTION

The Hilsha Shad (*Tenualosa ilisha)* belongs to the sub-family Alosinae under the family Clupeidae which is characterized by pelagic-neritic anadromous fish species. Hilsha is known to be a fast swimmer, covering 71 km in one day (Pillay *et al.*, 1963). Within a tropical range; 34°N-5°N, 42°E-97°E in marine as well as freshwater ecosystem. It can grow up to 60 cm in length with weights of up to 3 kg. During their breeding season they come toward the fresh water riverine system and the fish is often referred to as the “King of Fishes”. Bangladesh is the global leader, producing 65% of the total Hilsha in the world where India with 10%-15% and Myanmar with 8%-10% of the world’s production (Worldfish Report, 2015). The Hilsha sanctuaries in Bangladesh includes: 100 km stripe in Meghna river from Shatnol to Char Alaxandar, 90 km stripe of Shahbazpur Channel at Meghna estuary in Bhola district, 100 km stripe of Tentulia River in Bhola district, 40 km stripe in Andharmanik River in Patuakhali district, 20 km stripe at lower Padma (Padma confluence) in Shariatpur district 83 km stripe in Meghna River (from Hizla to Mehendiganj) in Barisal district. The fish has two distinct migrations and breeding seasons in a year, one during monsoon and other in late winter according to Hora, 1941; Hora and Nair, 1940; Jones and Menon, 1951; Pillay, 1957; Chandra, 1962; Mathur, 1964. Shafi *et al.* (1978) also reported two separate breeding seasons of this fish.

The measurement of morphometric and meristic characters are powerful tools which can be used for the stock identification, elucidating relationship among populations and to separate physically similar species. Information on the biology and population structure of any species is a prerequisite for developing management and conservation strategies (Turan *et al.*, 2006) and may be applicable for studying short-term and environmentally induced variations, even for the genetic management of the population. Quddus *et al*,. (1984) described meristic and morphometric differences as well as the two types age and growth of *T. ilisha* from Bangladesh coast. Information on the biology and population structure of any species is a prerequisite for developing management and conservation strategies (Turan *et al.*, 2006) and may be applicable for studying short-term and environmentally induced variations, even for the genetic management of the population. Morphometric differences among stocks of a species are recognized as important for evaluating the population structure and as a basis for identifying stocks (Ihssen *et al.,* 1981; Templeman, 1983; Smith and Jamieson, 1986; Turan, 2004a; Turan *et al.,* 2004b; Vishalakshi and Singh, 2008; Randall and Pyle, 2008).

The morphometric characters are classified into genetically (narrow range) controlled, intermediate (moderate range) and environmentally (vast range) controlled characters (Johal *et al.,* 1994). Despite the advent of techniques which directly examine biochemical or molecular genetic variation, the morphometric or meristic methods continue to play an important role in stock identification even today (Swain and Foote, 1999).

Hilsha the national fish of Bangladesh contributes to around 12% of overall fish production in the country Bangladesh, which is equivalent to around 1% of the country's GDP. Bangladesh annually produces 496,417 tons of Hilsha which bring in a substantial amount of foreign currency through exports (Fisheries Statistical Report of Bangladesh 2016-17). Around half a million fishermen are directly and another two million indirectly dependent on Hilsha production for their livelihood. Due to high demand in both home and abroad, excessive fishing in the past couple of decades has led to concerns regarding its endangered existence. To conserve this species, the government has taken several initiatives, one of which is the ban on Hilsha catching during two breeding seasons. The first ban of the year is in March-April, in all sanctuaries except for the one in Andharmanik River. The second ban is in November-January in the Andharmanik sanctuary.

1.1.Objectives of the research:

The objectives of this research are as follows:

- ✓ To investigate different population structure of Hilsha Shad
- ✓ To observe morphometric characters of different Hilsha population

Chapter Two

REVIEW OF LITERATURE

Tenualosa ilisha is known as the national fish of Bangladesh which mainly found in fresh water as well as in marine water. The demand and price of this species is higher than the other fish species inside the country and also outside the country. Many studies have been carried out on biological aspects of different fish species in Bangladesh. This chapter is about a detailed scientific review on the morphological studies carried out in different fish species.

Salini *et al.* (2004) found morphological variation of Hilsha shad in head portion which varied from 81 mm at Khulna and 96 mm at Sylhet. The caudal peduncle height had the highest loading on principal component 1 while body cross section had the peak loading on principal component 2.

The analysis on the morphometric characters between Arctic Char and *Salvelinus alpinus* studied by Doherty and Mc Carthy (2002). In that research they investigated the size and growth rate of western fish is much higher than the northerly population.

González *et al.* (2016) reported that the coefficients of variation of different morphometric characters of wild and cultured *Cichlasoma festae* were not significantly ($p < 0.05$) different between populations, except for Pre-OL, Pre-DL, Pre-AL, AC3, LC2 and LC3. For DFR, RDF, AFR and RAF, have no significant difference ($p < 0.05$) among populations. The coefficients of variation were very low (<7 %) and similar between populations.

The taxonomic variation of Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus cirrhosus*) populations in Bangladesh based on the morphometric and meristic data of the populations studied by Hasan *et al.*, (2007). This research suggested that hatchery populations of Rohu and Mrigal might be deviated from its origin and morphological characters of these species could be used for the determination of purity of the species.

Narejo *et al.*, (2008) investigated that during the year round sampling the two separate populations of *palla*, *T. ilisha* exist. There were no significant differences between male and female. Accordingly, all the six morphometric characters (total length, standard length, fork length, head length, eye diameter and girth) were tested on total length and observed that there was significant difference at 5% level between the types A and B. The type A fish were found larger in size than that of type B.

Mostafa *et al.*, (2010) studied mean numbers of anal, caudal, and pelvic fin rays, and scales below the lateral line, and number of vertebrae did not differ among fish from these stocks (Jamuna, Halda, and hatchery) of endangered Carp and *Labeo calba*su populations (Kruskal-Wallis test, $p > 0.05$), but difference occurred in other characters (d.f. = 2, pectoral fin rays: $H = 6.75$, $p < 0.05$ and scales above the lateral line: $H = 6.61$, $p < 0.05$. By landmark process Among the 11 transformed morphometric and 22 truss measurements, 2 measurements (standard length and post-orbital length) were found to be significantly correlated ($p < 0.05$).

The landmark-based morphometric characters along with truss network measurements and meristic counts to evaluate the population status of the endangered carp, Kalibaus (*Labeo calbasu*) from two isolated rivers (the Jamuna and the Halda) and a hatchery examined by Hossain *et al.*, (2010). He observed significant differences in four morphometric characters (maximum body height, pre-orbital length, peduncle length and maxillary barbell length) of twelve morphometric measurements, two (pectoral fin rays and scales above the lateral line) of nine meristic counts, and four (8 to 9, 3 to 10, 2 to 10, and 1 to11) of twenty two truss network measurements among the stocks.

Saber *et al.*, (2015) showed a morphological segregation of the studied populations (*Caspian lamprey, Caspiomyzon wagneri*) based on the characters pre-dorsal length, interdorsal, interorbital distance, tail length, and first dorsal fin length. The principal component analysis, scatter plot of individual component score between PC1 and PC2, showed the specimens grouped into two areas but with high and moderate overlap between two localities in males and females respectively.

The morphometric variability among three groups (pure progenies, crosses of *Pangasianodon hypophthalmus* and *Clarias gariepinus*) was mainly due to the variation of characters related to fins, and body characteristic by Tosin *et al.*, (2018). The fin characteristic appears to be the most promising index of morphological discrimination.
Hoque and Rahman (1985) reported morphometric characters and their relationship in *Gudusia chapra* and found that these characters were highly correlated with its total

length. While the eyed diameter, snout length and post-orbital head length were highly correlated with the head length of the fish.

Razzaq *et al.*, (2015) describe the fourteen morphological characteristics, the variation between the means of only two morphometric characters such as, first dorsal fin height (D1H) and pelvic fin height (PelFH) of male and female individuals of *Mugil incilus* were found to be significant (t-test; $p<0.05$), thence these two characters could be useful for observing phenotypic variation between male and female sexes of *Mugil incilus*. Fakunmoju *et al.*, (2014) reported the morphometric measurement in *L. goreensis* varied between the stations Lekki and Badagry. Data on the body populations of the specimens from the two stations showed that the ratio of total length to standard length of the fish varied between 62.12 and 69.35 in the lagoon. The ratio of head length to the total length indicated a range from 28.19 – 37.86.

Brraich and Akhter (2015) studied on *Garra gotyla gotyla* (Gray) which showed that out of eighteen characters, ten characters show high values of correlation coefficient indicating that these characters are directly proportional to each other and eight characters show moderate correlation coefficient. In percentage of head length five were genetically controlled and two are intermediate. Three characters show least correlation coefficient and four shows moderate correlation.

The variance results of *Silurus triostegus* from the Tigris and Shatt-al-Arab river showed that all morphometric measurements were significantly different between the samples ($p<0.001$). ANOVA also revealed no statistical differences between males and females for morphometric analysis ($P>0.05$) by Laith *et al.*, (2016).

Bonika *et al.*, (2018) analysis on *Hypophthalmichthys molitrix* under captive conditions. The significant correlations exist in all the morphometric parameters except with caudal length ($p<0.05$). The maximum correlation coefficient of average total length was obtained with fork length having a value of 0.992 and minimum with caudal length with value of 0.323. Akinrotimi *et al.*, (2018) reported the results of the morphometric features of *Sarotherodon melanotheron* from Buguma, Ogbakiri and Elechi Creeks in Nigeria, in the month of April. The result indicated that there were significant differences ($p < 0.05$) in the morphometric characters.

Simon *et al.*, (2010) morphometric and meristic characters were used to differentiate two congeneric archer fish species *Toxotes chatareus* and *Toxotes jaculatrix* inhabiting Malaysian coastal waters.

Farooq *et al.*, (2014) describe that none of the standardized truss measurements showed a significant correlation with the standard length of the fish, indicating that the effect of the body length had been successfully removed by the allometric transformation. Among three populations, mean values of all the truss measurements of *Schizothorax. curvifrons* were found to be significantly ($p<0.001$) different in univariate analysis of variance. The truss characters between two sexes did not differ significantly ($p>0.05$).

Chapter Three

MATERIALS AND METHODS

This section of the thesis involves specific techniques that are adopted in research process to collect, assemble and evaluate data to achieve the objectives of the study.

3.1. Collection of samples

Total 198 samples were collected randomly from different sources for the study of morphometric characters on *Tenualosa ilisha*. Their morphometric characters were measured by using a scale of 4 cm on their body. Then those picture were taken by using a camera to get all morphometric characters of specimen. The descriptions of sampling source, source abbreviation, sample size, total length and total weight are presented in table 01.

Table 01. Collection of Hilsha shad samples from the major migratory routes including sea, estuary and different rivers of Bangladesh.

Source of fish samples	**Source Abbreviation**	**Sample size**	**Total length (cm) Mean±SD**	**Total weight (g) Mean±SD**
Sea at Kuakata	Sea	24	26.12±4.64	312.4±38.4
Meghna Estuary	ME	36	24.63±3.17	298.9±26.8
Meghna River	MR	35	27.12±5.64	326.2±42.5
Lower Padma River	LPR	36	26.67±3.58	318.2±32.6
Upper Padma River	UPR	33	25.26±5.48	286.4±38.46
Upper Jamuna River	UJR	34	27.13±3.73	338.6±28.4

Figure 01 shows that the six regions from where the Hilsha fish species have been collected. The sources were Kuakata (Sea), Meghna Estuary (ME), Meghna River (MR), Lower Padma River (LPR), Upper Padma River (UPR), Upper Jamuna River (UJR). These are the main sources of Hilsha fishes in inland and coastal waters. It can be hypothesized that there may be some morphological differences among the Hilsha fish in this region. So, the aim of this study was to determine the morphometric differences among the six regions of *T. ilisha* populations.

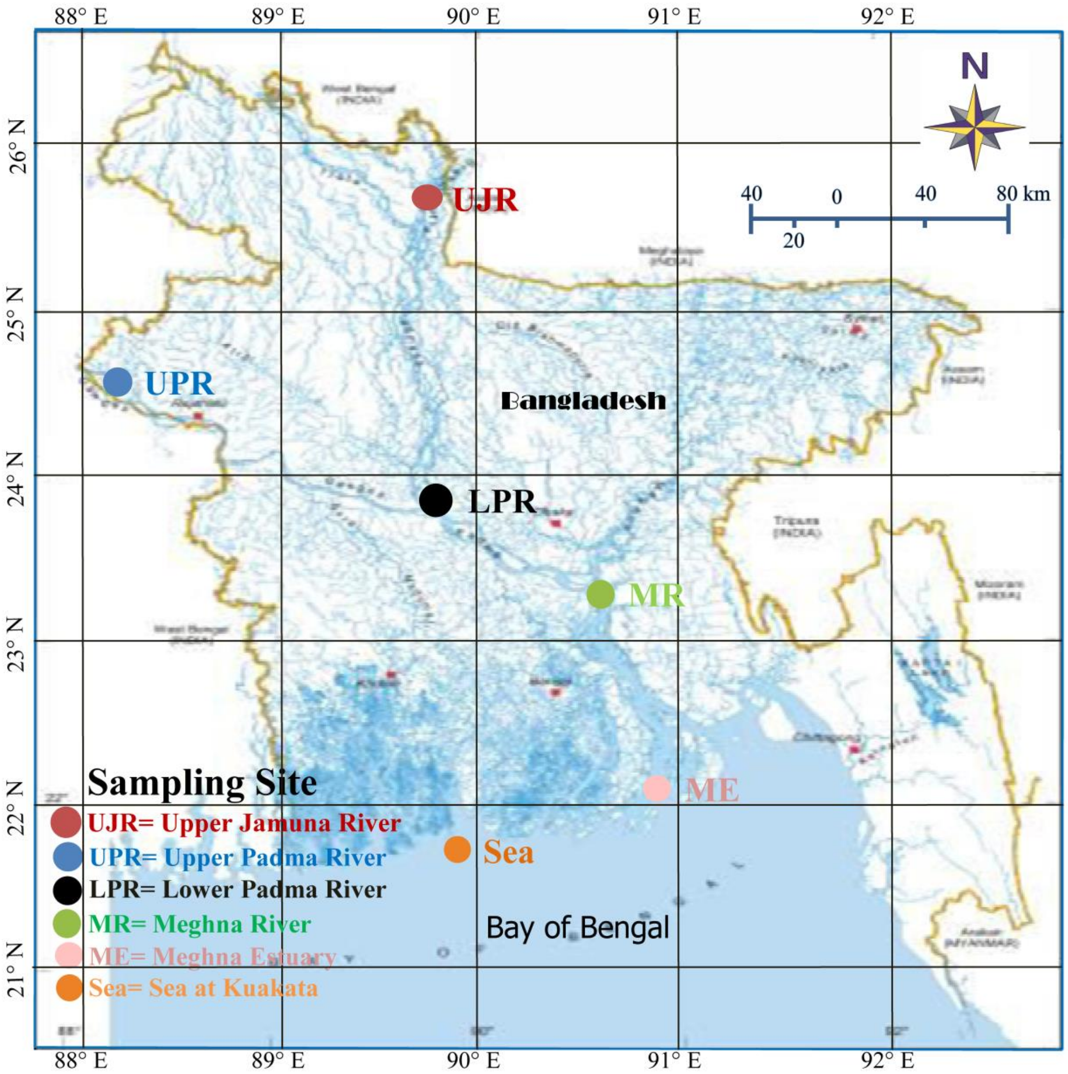

Figure 01: Map of Bangladesh showing sampling sites of Hilsha shad.

3.2. Measurement of morphometric characteristics

Six morphometric measurements and twenty-two truss distances were measured by using the software SigmaScan Pro 5.

Link for details: https://systatsoftware.com/products/sigmascan/

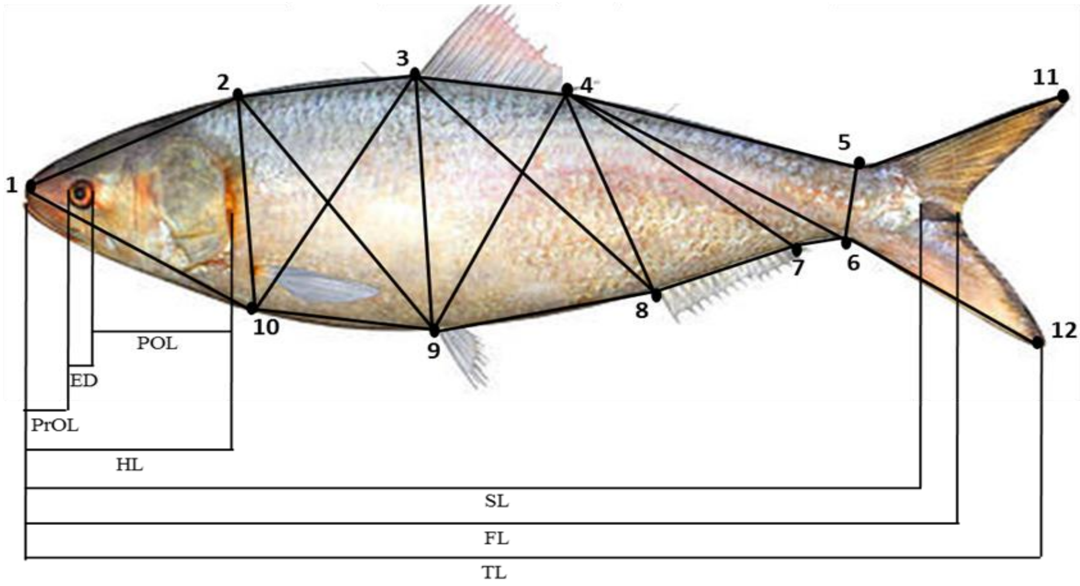

Figure 02. Morphometric characters and locations of the 12 landmarks points used for the shape analysis of anadromous Hilsha shad stock variations.

Table 02: General morphometric characters and their descriptions used for the analysis.

Sl. No.	Characters	Description
01	Standard length (SL)	Distance from the tip of the snout to the end of the vertebral column
02	Fork length (FL)	Distance from the tip of the snout to the middle part of the fork of the tail
03	Head length (HL)	Distance from the tip of the snout to the posterior margin of the opercula
04	Eye diameter (ED)	Diameter of the eye
05	Pre-orbital length (PrOL)	Distance from the tip of the snout to the anterior margin of the eye
06	Post-orbital length (POL)	Distance from the posterior margin of the eye to the end of the operculum
07	D1-2	Tip of the head to posterior margin of the head
08	D1-10	Tip of the head to base of the pectoral fin
09	D2-3	Posterior margin of the head to the anterior of dorsal fin
10	D2-9	Posterior margin of the head to anterior of pelvic fin
11	D2-10	Posterior margin of the head to base of the pectoral fin
12	D3-4	Distance from the anterior of dorsal fin to the posterior of dorsal fin
13	D3-8	Distance from the anterior of dorsal fin to the anterior of anal fin

14	D3-9	Distance from the anterior of dorsal fin to the anterior of pelvic fin
15	D3-10	Distance from the anterior of dorsal fin to base of the pectoral fin
16	D4-5	Distance from the posterior of dorsal fin to the upper anterior of caudal fin
17	D4-6	Distance from the posterior of dorsal fin to the lower anterior of caudal fin
18	D4-7	Distance from the posterior of dorsal fin to the posterior of anal fin
19	D4-8	Distance from the posterior of dorsal fin to the anterior of anal fin
20	D4-9	Distance from the posterior of dorsal fin to the anterior of pelvic fin
21	D5-6	Distance from the anterior of upper caudal fin to the anterior of lower caudal fin
22	D5-7	Distance from the anterior of upper caudal fin to the posterior of anal fin
23	D5-11	Distance from the anterior of upper caudal fin to the posterior of upper caudal fin
24	D6-12	Distance from the anterior of lower caudal fin to the posterior of lower caudal fin
25	D6-7	Distance from the anterior of lower caudal fin to the posterior of anal fin
26	D7-8	Distance from the anterior of anal fin to the posterior of anal fin
27	D8-9	Distance from the anterior of anal fin to the anterior of pelvic fin
28	D9-10	Distance from the anterior of pelvic fin to base of the pectoral fin

3.3. Statistical analysis

Prior to the statistical analysis, size effects from the data set were eliminated. An allometric formula given by Elliott *et al.*, (1995) with slight modification was used to remove the size effects from the data set.

$$M_{adj} = M\ (Ls/Lo)^{b}$$

Here,

M_{adj}: Size adjusted measurement

M: Original measurement

Ls: Overall mean of standard length for all fish from all samples in each analysis

Lo: Total length of fish

Parameter b was estimated for each character from the observed data as the slope of the regression of logM on logL_0, using all fish in all groups. The efficiency of the size adjusted values was then correlated with the TL and the transformed values.

At first, compared among the collected samples of *Tenualosa ilisha* to show the morphological differences among the fishes according to theirs sources and observed the morphological distances. All the morphometric and truss distance data were also standardized and submitted to discriminant functional analysis (DFA). All statistical analysis was carried out using IBM SPSS version 22.0 and MS excel 2014.

Chapter Four

RESULTS

4.1 Analysis of morphometric and landmark distance measurements

The mean and standard deviation of different morphometric and landmark distance have been measured which indicates the variation among the population of different Hilsha stocks. The correlation results revealed that all of the morphometric and landmark variables studied were free from the influence of size. Table 03 shows the different morphometric and truss distance (cm) of the six sampling sites.

Table 03. The standardized six morphometric measurements and twenty-two truss distances (cm) of different Hilsha Shad population

Morphometric characters	**Sampling sites**					
	Sea	**ME**	**MR**	**LPR**	**UPM**	**UJR**
SL	21.24±0.29	21.22±0.26	20.96±0.20	21.27±0.34	20.96±0.57	21.46±0.33
FL	22.57±0.30	22.61±0.27	22.38±0.28	22.78±0.33	22.33±0.51	22.72±0.31
HL	4.75±0.39	5.03±0.24	4.84±0.30	5.24±0.28	4.95±0.47	5.24±0.28
ED	0.96±0.13	1.06±0.11	0.98±0.09	1.08±0.10	1.07±0.11	1.14±0.07
PrOL	0.92±0.09	0.99±0.07	1.03±0.07	1.06±0.08	0.94±0.09	1.01±0.09
POL	3.56±0.19	3.66±0.29	3.52±0.18	3.71±0.14	3.61±0.27	3.73±0.14
D1-2	5.59±0.36	5.63±0.25	5.50±0.31	6.01±0.24	5.49±0.46	5.82±0.25
D1-10	4.98±0.33	5.03±0.22	5.02±0.32	5.19±0.28	4.96±0.41	5.37±0.27
D2-3	3.20±0.23	3.18±0.17	3.33±0.21	2.93±0.21	3.01±0.22	3.11±0.17
D2-9	6.48±0.22	6.33±0.25	6.01±0.22	6.21±0.29	5.83±0.29	6.10±0.23
D2-10	5.40±0.14	5.20±0.19	4.91±0.20	5.25±0.21	4.85±0.25	5.17±0.16
D3-4	2.65±0.17	2.72±0.25	2.31±0.24	2.64±0.16	2.75±0.14	2.68±0.16
D3-8	6.68±0.30	6.40±0.30	6.17±0.29	6.52±0.24	6.07±0.22	6.30±0.25
D3-9	4.93±0.20	4.72±0.22	4.41±0.23	4.65±0.26	4.21±0.23	4.43±0.19
D3-10	6.30±0.24	6.16±0.26	5.99±0.25	5.97±0.28	5.64±0.33	5.87±0.22
D4-5	5.50±0.28	5.33±0.23	5.66±0.37	5.58±0.38	5.28±0.24	5.54±0.28
D4-6	6.30±0.36	5.98±0.24	6.34±0.33	6.32±0.31	5.86±0.24	6.08±0.27
D4-7	4.84±0.27	4.46±0.19	4.81±0.26	4.70±0.28	4.37±0.20	4.56±0.23
D4-8	4.02±0.20	3.74±0.16	3.76±0.21	3.81±0.21	3.41±0.17	3.64±0.17
D4-9	5.63±0.22	5.51±0.25	5.06±0.31	5.25±0.26	4.96±0.23	5.14±0.19
D5-6	2.16±0.17	2.27±0.14	1.81±0.10	2.13±0.12	2.16±0.14	2.20±0.14
D5-7	2.35±0.15	2.63±0.22	2.20±0.17	2.60±0.18	2.48±0.18	2.49±0.23
D5-11	4.45±0.21	4.11±0.28	3.78±0.28	4.16±0.28	4.63±0.45	4.06±0.31
D6-12	4.69±0.21	4.56±0.32	4.83±0.20	4.55±0.30	4.54±0.46	4.28±0.24
D6-7	1.10±0.08	1.27±0.13	1.12±0.06	1.20±0.06	1.14±0.07	1.14±0.07
D7-8	1.62±0.03	1.44±0.25	1.58±0.03	1.59±0.05	1.64±0.05	1.63±0.44
D8-9	4.45±0.21	4.32±0.20	4.40±0.21	4.21±0.18	4.08±0.20	4.15±0.21
D9-10	4.22±0.29	4.17±0.20	4.10±0.20	4.20±0.22	4.04±0.21	4.02±0.25

4.2 Analysis of R-statistical packages

The R-statistical analysis indicates the variation among different variable. Discriminant function analysis of 7 morphometric and 22 truss distance measurements extracted 5 factors with Eigen values>1.0, explaining 100% of the variance (Table 05). The first discriminant function 1 accounted for 47.3% of the variation, discriminant function 2 accounted for 24.6%, discriminant function 3 accounted for 12.7% of the variation, discriminant function 4 accounted for 9.1% of the variation, discriminant function 5 accounted for 6.3% of the variation. These values of variation indicate the variation among the stocks. For this analysis, the characteristics with an Eigenvalue exceeding 0.7 were included. The total cumulative variances for the 5 principal components were 100%. Among they explained 100% of the total variability for morphometric and landmark measurements.

Table 04. Eigenvalues, percentage of variance and percentage of cumulative variance of different morphometric measurements and truss distances of Hilsha shad populations based on the canonical discriminant function analysis by R-statistical packages.

Functions	**Functions/ Dimension**	**Eigenvalue**	**% of Variance**	**Cumulative %**
Discriminant Function Analysis	1	5.225[a]	47.3	47.3
	2	2.713[a]	24.6	71.9
	3	1.398[a]	12.7	84.5
	4	1.010[a]	9.1	93.7
	5	0.700[a]	6.3	100

4.3 Wilks' lambda test

Wilks' lambda is a test statistic used in analysis of variance to test whether there are differences between the means of identified groups of subjects on a combination of dependent variables. Here the value of significance for all the 5 test functions, 1 through 5, 2 through 5, 3 over 5, 4 through 5 and 5 were 0.000 ($p<0.001$). The value indicates that they were highly significant among stock. Results of the Wilk's Lambda values for the all test of function showed the differentiation among them. The value of df, significance indicates that the stocks were highly significant are presented in the Table 05.

Table 05. Results of Wilks' lambda test of different morphometric measurements and truss distances for verifying differences among different populations of Hilsha shad.

Test of Functions	Wilks' Lambda	*Df*	Significance
1 through 5	0.005	145	0.000
2 through 5	0.033	112	0.000
3 through 5	0.122	81	0.000
4 through 5	0.293	52	0.000
5	0.588	25	0.000

4.4 Discriminant function analysis (DFA)

Table 06. Pooled within-group correlations between discriminating variables and standardized canonical discriminant functions of different morphometric measurements and truss distances of Hilsha shad populations based on discriminant function analysis

Variables	Function					Variables	Function				
	1	2	3	4	5		1	2	3	4	5
D5-6	0.452*	0.159	0.169	0.145	0.365	D3-10	-0.102	0.255	0.299	0.390*	0.212
D3-4	0.340*	0.045	0.119	-0.004	0.032	D7-8	-0.109	-0.248	0.207	-0.383*	-0.085
D5-7	0.275*	0.265	-0.123	-0.069	0.131	D8-9	-0.193	0.119	0.193	0.338*	0.077
D4-6	-0.228*	0.132	0.179	-0.194	0.134	HL	0.099	0.090	-0.180	-0.326*	0.324
D6-7	0.127	0.287*	-0.283	0.176	0.092	D2-10	0.062	0.347	0.481	0.026	0.611*
D9-10	0.029	0.177*	0.128	0.052	-0.035	SL	0.074	0.071	0.125	-0.108	0.539*
D3-9	-0.034	0.417	0.507*	0.314	0.366	D5-11	0.275	-0.090	0.359	-0.048	-0.523*
D4-8	-0.192	0.308	0.457*	0.154	0.348	D1-10	0.025	-0.008	-0.065	-0.263	0.457*
D3-8	0.002	0.294	0.425*	0.001	0.303	FL	0.046	0.171	0.059	-0.185	0.403*
PrOL	-0.110	0.175	-0.276*	-0.274	0.214	D2-9	-0.001	0.319	0.376	0.288	0.400*
TL	-0.255	-0.091	0.273*	0.051	0.027	D6-12	-0.182	0.056	0.056	0.0185	-0.383*
D4-7	-0.263	0.078	0.272*	-0.104	0.132	ED	0.163	-0.017	-0.219	-0.253	0.272*
D4-9	0.044	0.334	0.429	0.482*	0.329	POL	0.102	0.065	-0.58	-0.169	0.228*
D2-3	-0.190	-0.108	-0.019	0.465*	0.160	D4-5	-0.163	0.019	0.032	-0.194	0.210*
D1-2	0.048	0.198	0.005	-0.414*	0.340						

The discriminant function analysis (DFA) showed the mostly dominant characteristic among the population. Discriminant function analysis produced five sets of discriminant functions (DF1, DF2, DF3, DF4 and DF5) for all the 7 morphometric and 22 truss distances. Pooled within groups correlation between discriminant variables and discriminant function revealed that the four distances as D5-6, D3-4, D5-7 and D4-6 were dominantly contributed to the first DF, among the 2 distances: D6-7 and D9-10 primarily to the second DF, among the six distances: D3-9, D4-8, D3-8, PrOL, TL and D4-7 mainly contributed to the third DF, among the seven distances: D4-9, D2-3, D1-2, D3-10, D7-8, D8-9 and HL dominantly added to the fourth DF and among the ten distances: D2-10, SL, D5-11, D1-10, FL, D2-9, D6-12, ED, POL and D4-5 shown in Table 06 .

4.5 Original and Cross Validated Count

Table 07. Percentage of specimens classified in each group and after cross-validation for different morphometric measurement and truss distances of Hilsha shad population based on the discriminant function analysis.

		Sampling sites	Predicted group membership						
			Sea	ME	MR	LPR	UPR	UJR	Total
Original	Count	Sea	23	0	0	0	0	1	24
		ME	0	31	1	2	0	2	36
		MR	1	0	34	0	0	0	35
		LPR	0	1	0	35	0	0	36
		UPR	0	0	0	0	31	2	33
		UJR	1	0	0	1	2	30	34
	%	Sea	95.8	0	0	0	0	4.2	100
		ME	0	86.1	2.8	5.6	0	5.6	100
		MR	2.9	0	97.1	0	0	0	100
		LPR	0	2.8	0	97.2	0	0	100
		UPR	0	0	0	0	93.9	6.1	100
		UJR	2.9	0	0	2.9	5.9	88.2	100
Cross validated	Count	Sea	20	2	0	1	0	1	24
		ME	0	29	1	3	0	3	36
		MR	2	0	33	0	0	0	35
		LPR	0	3	0	32	1	0	36
		UPR	0	0	0	0	30	3	33
		UJR	2	1	0	1	3	27	34
	%	Sea	83.3	8.3	0	4.2	0	4.2	100
		ME	0	80.6	2.8	8.3	0	8.3	100
		MR	5.7	0	94.3	0	0	0	100
		LPR	0	8.3	0	88.9	2.8	0	100
		UPR	0	0	0	0	90.9	9.1	100
		UJR	5.9	2.9	0	2.9	8.8	79.4	100

- ✓ 93.05% original grouped population correctly classified.
- ✓ 86.23% cross-validation correctly grouped population classified.

This study helps to identify the source of each species of a stock and overlapped with the species of other stock. The discriminant function analysis for the six sampling site according to their morphometric and truss distance showed that 93.05% original grouped population correctly classify their populations and for the cross-validation test showed that 86.23% properly classify their populations (Table 07).

4.6 Sample Centroids Analysis

Three different discriminant functions has analyzed according to their morphometric distances, truss distances and both of their combination. For figure 3A, 7 morphometric characters are very closely related among the all sources and overlapped to each other. For figure 3B, there were 3 cluster formations. First one was population of MR, secondly the population of LPR, Sea and ME were closely related, and thirdly the population of UPR and UJR were closely related. For figure 3C, only MR was isolated, LPR, Sea and ME were overlapped due to geographical location and UPR, UJR and Sea were in cluster form (Figure 03).

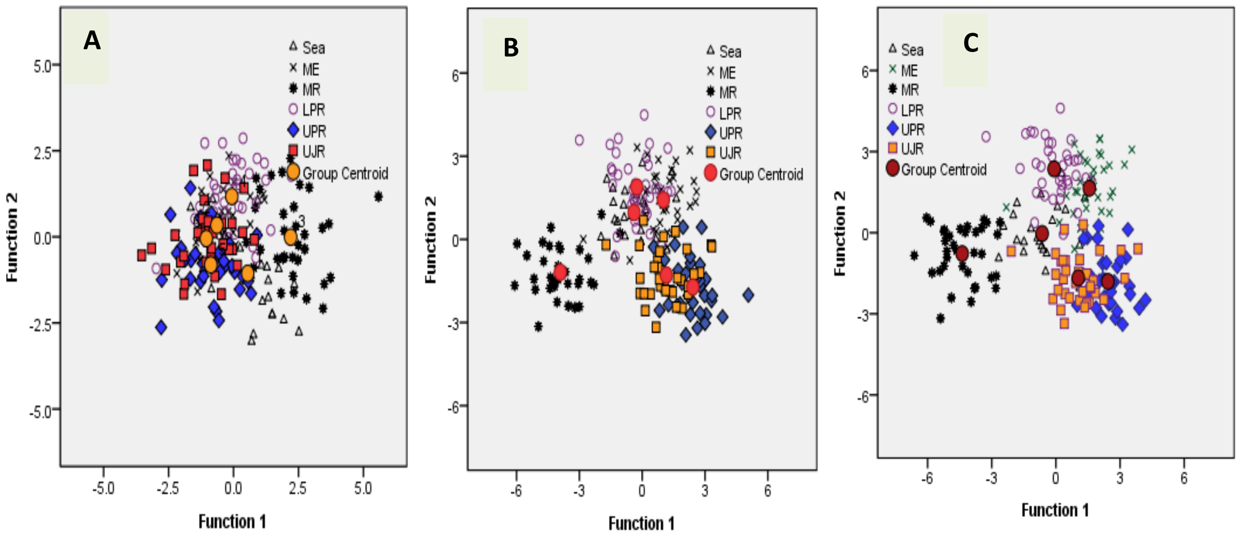

Figure 03. Sample centroids of the discriminant function scores based on the seven morphometric measurement (A), twenty-two truss distances (B) and combining seven morphometric measurements and twenty-two truss distances (C) of different Hilsha shad population.

4.7 Biplot analysis:

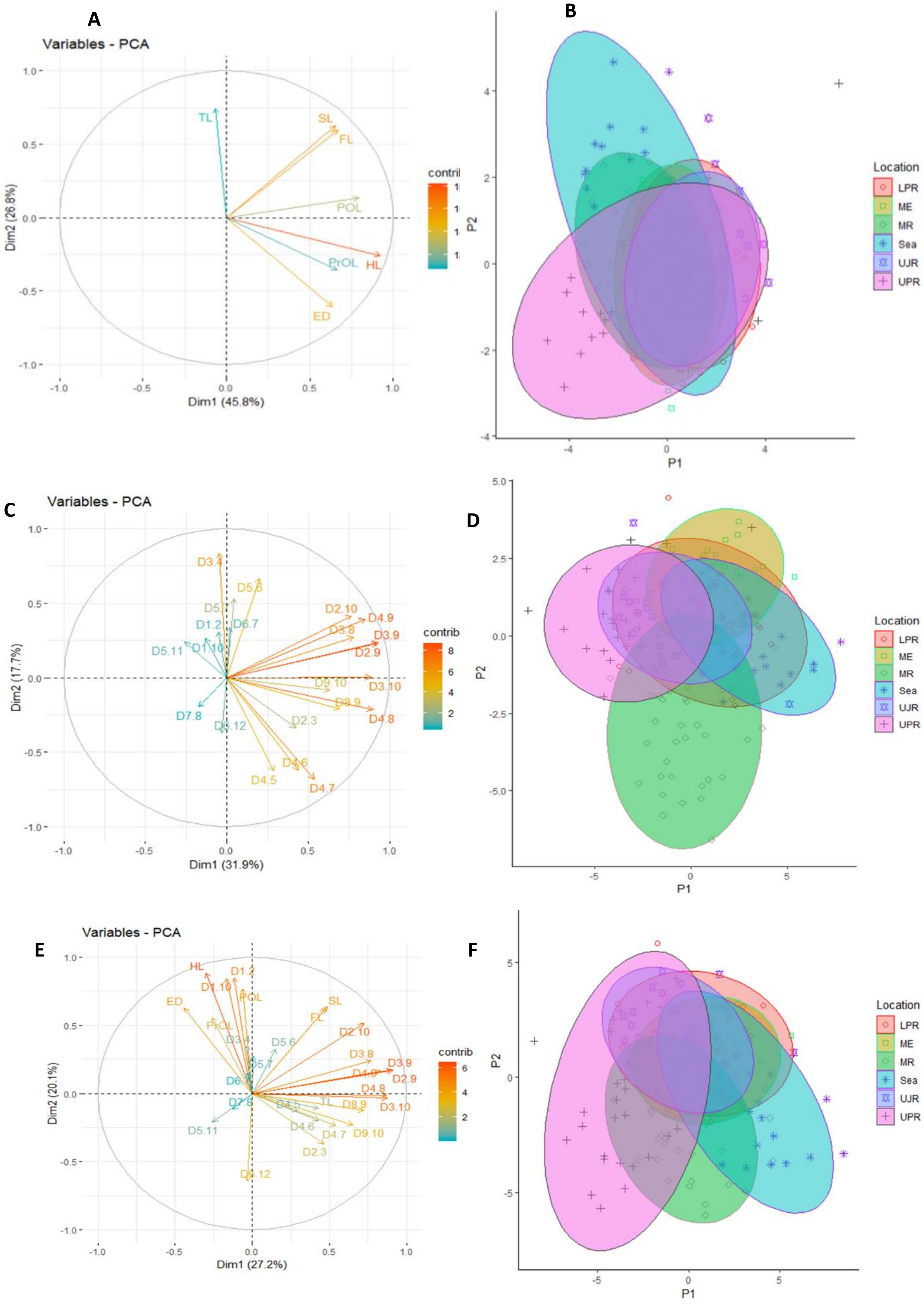

Figure 04. Biplot analysis of the first two principle components of morphometric variables among different Hilsha shad population for seven morphometric measurement (A, B), twenty-two truss distances (C, D) and combined measurement of both seven morphometric measurements and twenty-two truss distances (E, F) using R-statistical package.

Biplot analysis showed that the first two principle components of the morphometric variables and different Hilsha shad population for seven morphometric measurement for figure 4A and 4B. In figure 4B, the population of Sea, UPR showed the more expanding and MR showed less expanding but the other population LPR, ME and UJR were in cluster form. The highest variation in these population were seen in gradual sequence HL>SL> FL> ED>POL>TL>PrOL (Figure 4A). For the twenty-two truss distances (figure 4D) showed the maximum diversified were MR, UPR, Sea and the population of LPR, ME and UJR were in cluster form. In these population the highest variation were seen in gradual pattern as D3-9>D2-9>D4-9>D4-8>D4-7>D2-10>D3-10>D3-4>D3-8>D8-9>D4-6>D5-6>D4-5>D9-10>D2-3>D5-6>D6-12>D5-11 >D7-8>D6-7>D1-2>D1-10 (Figure 4C). For the combined measurement of seven morphometric and twenty-two truss distances (Figure 4F) showed the maximum diversified were UPR, Sea, MR and the population of LPR, ME AND UJR were in cluster form. These population have the highest variation pattern as HL>D3-9>D2-9>D2-10>D1-10>D1-2>D3-10>D4-8>D3-10>D4-9>SL>D3-8>FL>ED>POL>D8-9> D9-10>D6-12>D4-7>D2-3>PrOL>TL>D4-6>D5-6>D3-4>D5-11>D4-5>D5-7>D7-8 >D6 -7 (Figure 4E).

4.8 Contribution of Morphometric variables:

Morphometric variables for the first principal component (Figure 05- left image) showed that the maximum variation among the population were gradually in (D2-9, D3-9), (D3-10, D4-8), D4-9, D3-8, (D8-9, D2-10), D9-10 and D4-7. For the second principal component (Figure 5- right image) of different Hilsha shad population showed that the HL showed higher variation among different regions. Then the variation seen gradually in (D1-2, D1-10), POL, D6-12, SL, ED, FL, PrOL and D2-10 distances among the population of six sources (Figure 05).

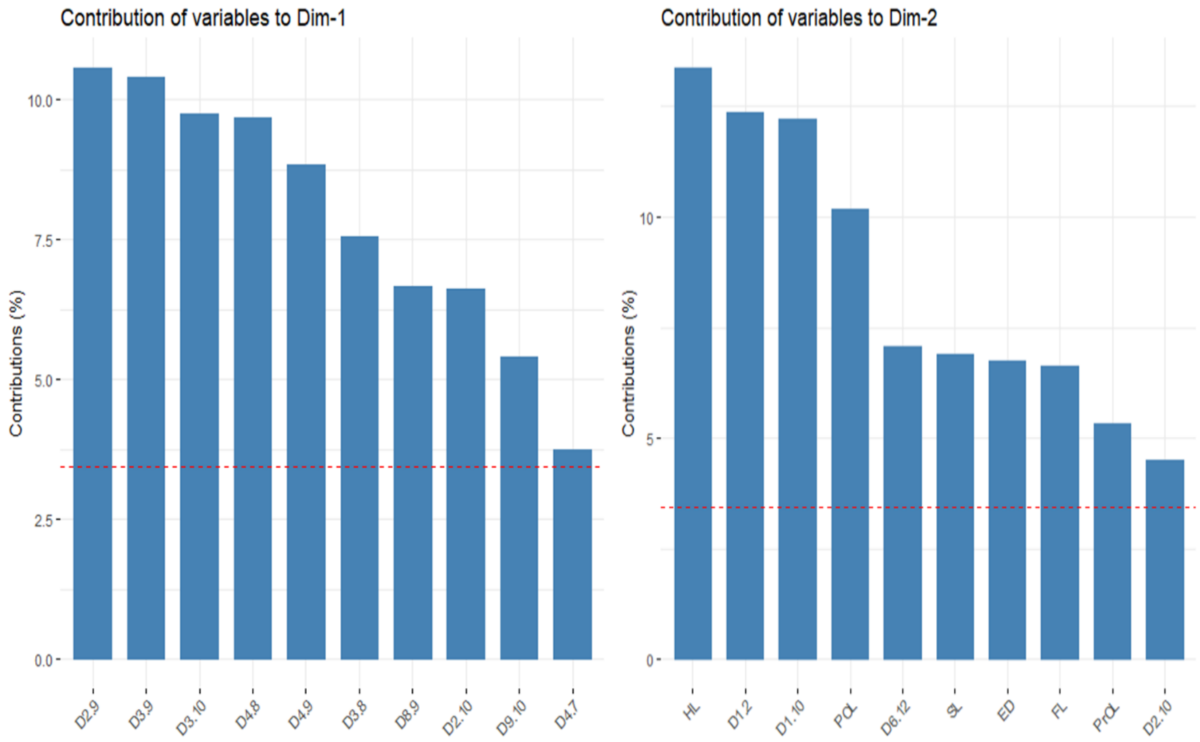

Figure 05. List of the major morphometric variables contributed to the first principal component (left image) and second principal component (right image) of different Hilsha shad population.

4.9 Dendrogram Based Analysis:

The dendrogram based on 7 morphometric measurements and 22 landmark distances of the six sources of Hilsha population. Despite of the geographical distances the individual Hilsha population of ME was the greater homogeneity with LPR and UJR. But the population of others region UPR and MR showed the higher heterogeneity (Figure 06).

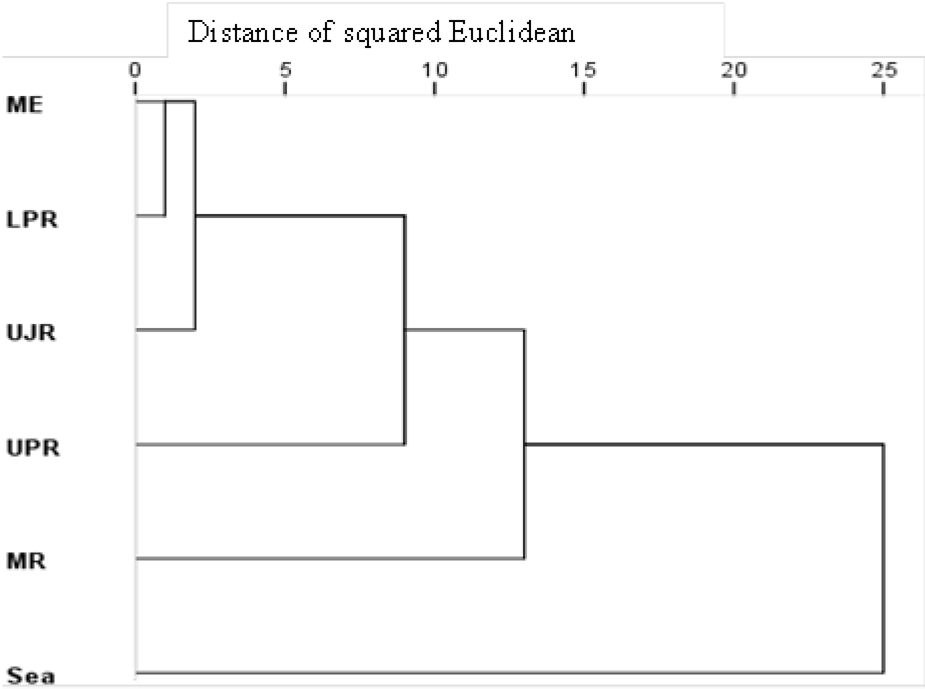

Figure 06. Dendrogram based on seven morphometric measurements and twenty-two landmark distances of the different anadromous Hilsha shad population.

Chapter Five

DISCUSSION

The finding of this research obtained from specimen analysis on morphometric characteristics indicates the morphologically differentiated groups of *Tenualosa ilisha* among the six sources. Distinct morphometric characters suggest that there are some morphological differentiations among the Hilsha due to their geographical distribution. The morphometric characters with truss measurements have been used to analyze the potential differentiation of *Tenualosa ilisha* and the truss network system which is a powerful tool for identifying stocks of fish species (Turan, 2004). According to Austin (1999) the morphological characters are phenotypically plastic and are influenced each year by the physical environment during spawning and early juvenile stages. The identification of morphometric and truss distance has been also successfully utilized to differentiate and identify stock in many fish groups including the tropical shad, Hilsha *Tenualosa ilisha* (Salini *et al.*, 2004); Horse mackerel *Trachurus trachurus* **(**Murta *et al.*, 2008); Japanese threadfin bream *Nemipterus japanicus* (Lim, 2008); Indian major carps (Hossain *et al.*, 2010); Catfish (Parvej *et al.*, 2014, Rahman *et al.*, 2014); and Parassi mullet, *Mugil incilis* (Razzaq *et al.*, 2015). To explain the differences, R-statistical packages, Wilks' lambda test, Biplot analysis, dendrogram analysis, and DFA (discriminant function analysis) were performed in this study.

The morphological variations among the populations of *T. ilisha* due to their different geographical locations, availability of food, difference in various water quality parameters, variation in their habitats etc. The morphological characteristics can show high plasticity in response to differences in environmental conditions (Swain *et al.*, 1991). Difference in body shape are related to body elongation or shortening, thus leads to large difference in body height and caudal peduncle length among the species (Manimegalai, 1999). There were no significant difference in caudal peduncle length and length of dorsal fin base among samples. Georgakopoulou *et al.* (2007) have found that morphometric characters of *Danio rerio* changed with water temperature, which may influence fish metabolism through changes in the concentration of dissolved oxygen (Wimberger, 1992). A similar study was conducted by Mir *et al.* (2013) and reported the variations among the *Labeo rohita* stocks of Ganga basin due to uncommon hydrological conditions among the stocks due to their similar habitat attributes and to environmental impacts. Hossain *et al.*, (2010) observed significant differences ($p<0.05$ or <0.001) in four of 22 truss network measurements in Kalibaus (*Labeo calbasu*) populations collected from the Jamuna, the Halda and a hatchery in Bangladesh. The significant differences ($p<0.05$) were also found in 16 of 25 truss measurements in Anchovy (*Engraulisen crasicolus* L.) in Black, Aegean and Northeastern Mediterranean sea (Turan *et al.*, 2004b).

The canonical discriminant functions in DFA showed an overlapped in all the stocks of *T. ilisha*. In case of morphometric measurement, the first DF accounted for much more (47.3%) of the among group variability than the other four discriminant function. From this both observations, it was obvious that the fifth DF (6.3%) explained much less of the variance than did the first DF and it was much less informative in explaining differences among the stocks. Among the four distances: D5-6, D3-4, D5-7 and D4-6 dominantly contributed to the first DF, among the 2 distances: D6-7 and D9-10 contributed to the second DF, among the six distances: D3-9, D4-8, D3-8, PrOL, TL and D4-7 dominantly contributed to the third DF, among the seven distances: D4-9, D2-3, D1-2, D3-10, D7-8, D8-9 and HL dominantly contributed to the fourth DF and among the ten distances: D2-10, SL, D5-11, D1-10, FL, D2-9, D6-12, ED, POL and D4-5 shown in Table 06. This inter-population variation may be attributed due to separate geographical location as well as the environmental and physiological constraints like salinity, temperature, turbidity, water pressure, current flow and food availability experienced by each population (Allendorf, 1988; Swain *et al.*, 1991). Based on the standardized discriminant function coefficient [7,18] (Tables 3 and 4), thirteen characteristics, Side View: 5to8, 6to7, 7to8, 9to11, 9to12, 10to11, 11to12 and Upside View: 7to1, 7to2, 1to2, 1to3, 2to3 and 2to4 have been identified as the most important variables in discriminating the Persian Gulf (Khuzestan and Bushehr Province) species (Amir *et. al*., 2010).

Wilk's Lambda values showed that the variation 1 through 5, 2 over 5, 3 into 5, 4 over 5 and 5 were 0.000 ($p<0.001$). The significance value indicates that they were highly significant among them. The significance value indicates that they were highly significant among them. Results of the Wilk's Lambda values for the all test of function showed the differentiation among them which shows that there was high degree of variations between two species. Yakubu and Okunsebor (2011) showed significant morphological differences between *Oreochromis niloticus* and *Lates niloticus* where they found the values of Wilk's Lamba was greater than 0.1 in most measurement.

The discriminant function analysis for the six sampling site showed that 93.05% original grouped population correctly classify their populations and for the cross-validation test showed that 86.23% correctly classify their populations (Table 08). Mousavi and Anvarifar (2013) indicated that 58.0 % of the individuals were correctly classified into their original groups on average, demonstrated a high differentiation among the populations of spined loach in the studied areas. Cross-validation with the split-sample method indicated a 97.6% overall success rate whereas 98% of *Oreochromis niloticus* and 97% of *Lates niloticus* were correctly assigned by Yakubu and Okunsebor 2011.

Discriminant function scores of sample centroids for figure 03A, 7 morphometric characters are very closely related among the all sources and overlapped with each other. For figure 03B and 03C there were 3 clusters among the six sources. Only the population of MR was isolated due to their same origin from oceanic region. Muchlisin (2013) stayed that the Depik and Eas species showed considerable low degree of overlap, while Relo was more distant which occurred due to their origin of different geographical location. The overall random assignment of individuals into their original groups was high (71.46%), indicating that these samples are probably divergent from each other (Paknejad *et al*., 2014).

Biplot analysis showed that for first two principle components in figure 4B, the population of Sea, UPR showed the more expanding and MR showed less expanding but the other population LPR, ME AND UJR were in cluster form. These population showed the highest variation in a gradual sequence as HL>SL, FL> ED>TL>PrOL in the figure 4A. For the twenty-two truss distances in figure 4D showed the maximum diversified were MR, UPR, Sea and the population of LPR, ME and UJR were in cluster form. In these populations the highest variation seen in gradually D3-9>D4-9>D4-8>D4-7>D2-10>D3-10>D3-4>D3-8>D8-9>D4-6>D5-6>D4-5>D9-10>D2-3>D5-6>D6-12>D5-11> D7-8>D6-7>D1-2>D1-10 in figure 4C. For the combination of seven morphometric measurements and twenty-two truss distances in figure 4F showed the maximum diversified were UPR, Sea, MR and the population of LPR, ME AND UJR were in cluster form. In these population the highest variation seen in gradual pattern as HL>D3-9>D2-9>D2-10>D1-10>D1-2>D3-10>D4-8>D3-10>D4-9>SL>D3-8>FL>ED>POL> D8 -9>D9-10>D6-12>D4-7>D23>PrOL>TL>D4-6>D5-6>D3-4>D5-11>D4-5>D5-7>D7-8> D6-7 in figure 4E.

Morphometric variables for the first principal component (Figure 5) showed that the maximum variation among the population were maintained the sequential process as (D2-9, D3-9), (D3-10, D4-8), D4-9, D3-8, (D8-9, D2-10), D9-10 and D4-7. For the second principal component HL showed higher variation among the different regions. Then the variation seen gradual pattern as (D1-2, D1-10), POL, D6-12, SL, ED, FL, PrOL and D2-10 distances (Figure 05) among the population of six sources.

Despite of the geographical distances the individual Hilsha population of ME was the great homogeneity with LPR and UJR. But the population of others region UPR and MR showed the higher heterogeneity (Figure 06). Paknejad *et al.*, 2014 showed the Miankale and Sari regions and Rezvanshahr and Anzali regions, morphometric clustering revealed that the individuals of these locations form the same clade with great homogeneity, while Tonekabon and Astara exhibited higher heterogeneity, confirming the results obtained from discriminant analysis for this species. Siddik *et al.* (2016) stayed that the Meghna and Tetulia closed together and far from Baleswar both case of male and female specimen, although they are separated geographically.

CONCLUSIONS

Bangladesh is one of the world's leading fish producing countries with a total production of 4.134 million MT against a demand of 4.0504 million MT in 2016-17, where aquaculture contributes 56.44% to total production. The contribution of Hilsha in GDP is about 1%, which indicates that Hilsha is one of the most important fish species in Bangladesh. Fish provides nutrients and micronutrients that are essential to cognitive and physical development to ensure healthy diet. Biology and population structure is very important for management of Hilsha fisheries. This study has provided significant morphological information which can be used to identify the variation of *Tenualosa ilisha* from different sources. The morphological variation among the Hilsha fish occurred due to their geographical distribution, food availability and various water quality parameters.

The findings of the research will help the policy makers to know the present condition of Hilsha population in Bangladesh and they can use it for the purpose of management and conservation of Hilsha stock. They also can use it for DNA level work as a base of their study. Our government has taken some necessary steps to increase the Hilsha population. The contribution and involvement of local fishermen to implement the rules and regulations related with this fisheries sector will play the major preface in our economy. This research work will be a new dimension to improve the driving Hilsha fisheries sector in Bangladesh.

RECOMMENDATIONS AND FUTURE PERSPECTIVES

Morphometric characters and truss distances are the primary information of different Hilsha stocks. This study provides the information about the variations of various Hilsha stocks of different riverine source of Bangladesh. The study considered some limitations in terms of period of Hilsha catching and limited number of stocks. The variations occurred due to different geographical region, food availability and other water quality parameters.

The findings of the study might be used as a management guideline for future research Hilsha population. To increase the production of Hilsha, peoples should be aware on proper management and following systems might be considered for sustaining Hilsha population in Bangladesh.

- ✓ Identification and proper management of Hilsha sanctuary.
- ✓ Increase awareness and training on Hilsha fish among the peoples.
- ✓ DNA work should be done to know the genetic variations.
- ✓ Protection of breeding ground from pollution and catching fish during ban period.
- ✓ Preservation of sperm which contains high growth rate of Hilsha fishes.
- ✓ Implementation of government rules and regulations.

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APPENDICES

Appendix I. Comparative studies on *T. ilisha* collected from six (06) different habitats for morphometric characters and truss measurements

Group Statistics for Morphometric Characters:

Morphometric characters	Sea Mean±SD	ME Mean±SD	MR Mean±SD	LPR Mean±SD	UPM Mean±SD	UJR Mean±SD
SL	21.24±0.29	21.22±0.26	20.96±0.20	21.27±0.34	20.96±0.57	21.46±0.33
FL	22.57±0.30	22.61±0.27	22.38±0.28	22.78±0.33	22.33±0.51	22.72±0.31
HL	4.75±0.39	5.03±0.24	4.84±0.30	5.24±0.28	4.95±0.47	5.24±0.28
ED	0.96±0.13	1.06±0.11	0.98±0.09	1.08±0.10	1.07±0.11	1.14±0.07
PrOL	0.92±0.09	0.99±0.07	1.03±0.07	1.06±0.08	0.94±0.09	1.01±0.09
POL	3.56±0.19	3.66±0.29	3.52±0.18	3.71±0.14	3.61±0.27	3.73±0.14
D1-2	5.59±0.36	5.63±0.25	5.50±0.31	6.01±0.24	5.49±0.46	5.82±0.25
D1-10	4.98±0.33	5.03±0.22	5.02±0.32	5.19±0.28	4.96±0.41	5.37±0.27
D2-3	3.20±0.23	3.18±0.17	3.33±0.21	2.93±0.21	3.01±0.22	3.11±0.17
D2-9	6.48±0.22	6.33±0.25	6.01±0.22	6.21±0.29	5.83±0.29	6.10±0.23
D2-10	5.40±0.14	5.20±0.19	4.91±0.20	5.25±0.21	4.85±0.25	5.17±0.16
D3-4	2.65±0.17	2.72±0.25	2.31±0.24	2.64±0.16	2.75±0.14	2.68±0.16
D3-8	6.68±0.30	6.40±0.30	6.17±0.29	6.52±0.24	6.07±0.22	6.30±0.25
D3-9	4.93±0.20	4.72±0.22	4.41±0.23	4.65±0.26	4.21±0.23	4.43±0.19
D3-10	6.30±0.24	6.16±0.26	5.99±0.25	5.97±0.28	5.64±0.33	5.87±0.22
D4-5	5.50±0.28	5.33±0.23	5.66±0.37	5.58±0.38	5.28±0.24	5.54±0.28
D4-6	6.30±0.36	5.98±0.24	6.34±0.33	6.32±0.31	5.86±0.24	6.08±0.27
D4-7	4.84±0.27	4.46±0.19	4.81±0.26	4.70±0.28	4.37±0.20	4.56±0.23
D4-8	4.02±0.20	3.74±0.16	3.76±0.21	3.81±0.21	3.41±0.17	3.64±0.17
D4-9	5.63±0.22	5.51±0.25	5.06±0.31	5.25±0.26	4.96±0.23	5.14±0.19
D5-6	2.16±0.17	2.27±0.14	1.81±0.10	2.13±0.12	2.16±0.14	2.20±0.14
D5-7	2.35±0.15	2.63±0.22	2.20±0.17	2.60±0.18	2.48±0.18	2.49±0.23
D5-11	4.45±0.21	4.11±0.28	3.78±0.28	4.16±0.28	4.63±0.45	4.06±0.31
D6-12	4.69±0.21	4.56±0.32	4.83±0.20	4.55±0.30	4.54±0.46	4.28±0.24
D6-7	1.10±0.08	1.27±0.13	1.12±0.06	1.20±0.06	1.14±0.07	1.14±0.07
D7-8	1.62±0.03	1.44±0.25	1.58±0.03	1.59±0.05	1.64±0.05	1.63±0.44
D8-9	4.45±0.21	4.32±0.20	4.40±0.21	4.21±0.18	4.08±0.20	4.15±0.21
D9-10	4.22±0.29	4.17±0.20	4.10±0.20	4.20±0.22	4.04±0.21	4.02±0.25

Average of Mean and Standard Deviation of all Samples:

Source of Samples	Sample Size	Total Length (cm) Mean±SD	Total Weight (g) Mean±SD
Sea at Kuakata	24	26.12±4.64	312.4±38.4
Meghna Estuary	36	24.63±3.17	298.9±26.8
Meghna River	35	27.12±5.64	326.2±42.5
Lower Padma River	36	26.67±3.58	318.2±32.6
Upper Padma River	33	25.26±5.48	286.4±38.46
Upper Jamuna River	34	27.13±3.73	338.6±28.4

Canonical Discriminant Function Coefficients:

Variables	DF 1	DF 2	DF 3	DF 4	DF 5
D5-6	0.452*	0.159	0.169	0.145	0.365
D3-4	0.340*	0.045	0.119	-0.004	0.032
D5-7	0.275*	0.265	-0.123	-0.069	0.131
D4-6	-0.228*	0.132	0.179	-0.194	0.134
D6-7	0.127	0.287*	-0.283	0.176	0.092
D9-10	0.029	0.177*	0.128	0.052	-0.035
D3-9	-0.034	0.417	0.507*	0.314	0.366
D4-8	-0.192	0.308	0.457*	0.154	0.348
D3-8	0.002	0.294	0.425*	0.001	0.303
PrOL	-0.110	0.175	-0.276*	-0.274	0.214
TL	-0.255	-0.091	0.273*	0.051	0.027
D4-7	-0.263	0.078	0.272*	-0.104	0.132
D4-9	0.044	0.334	0.429	0.482*	0.329
D2-3	-0.190	-0.108	-0.019	0.465*	0.160
D1-2	0.048	0.198	0.005	-0.414*	0.340
D3-10	-0.102	0.255	0.299	0.390*	0.212
D7-8	-0.109	-0.248	0.207	-0.383*	-0.085
D8-9	-0.193	0.119	0.193	0.338*	0.077
HL	0.099	0.090	-0.180	-0.326*	0.324
D2-10	0.062	0.347	0.481	0.026	0.611*
SL	0.074	0.071	0.125	-0.108	0.539*
D5-11	0.275	-0.090	0.359	-0.048	-0.523*
D1-10	0.025	-0.008	-0.065	-0.263	0.457*
FL	0.046	0.171	0.059	-0.185	0.403*
D2-9	-0.001	0.319	0.376	0.288	0.400*
D6-12	-0.182	0.056	0.056	0.0185	-0.383*
ED	0.163	-0.017	-0.219	-0.253	0.272*
POL	0.102	0.065	-0.58	-0.169	0.228*
D4-5	-0.163	0.019	0.032	-0.194	0.210*

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions.
Variables ordered by absolute size of correlation within function.
*. Largest absolute correlation between each variable and any discriminant function

Eigenvalues Percentage of Variance and Percentage of Cumulative Variance by R-Statistical Packages:

Function	Eigenvalue	% of Variance	Cumulative %
1	5.225a	47.3	47.3
2	2.713a	24.6	71.9
3	1.398a	12.7	84.5
4	1.010a	9.1	93.7
5	0.700a	6.3	100

a. First 5 canonical discriminant functions were used in the analysis

Appendix II. Comparison among the population based on morphometric characters and their contribution in differences

Wilks' Lambda Test Among Different Populations:

Test of Functions	**Wilks' Lambda**	***Df***	**Significance**
1 through 5	0.005	145	0.000
2 through 5	0.033	112	0.000
3 through 5	0.122	81	0.000
4 through 5	0.293	52	0.000
5	0.588	25	0.000

Coordinates of Variables (Truss Distance):

Variables	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5
D1-2	-0.05330988	0.3048036	0.84068061	-0.1135272	-0.04361621
D1-10	-0.13047399	0.2644470	0.80298390	-0.2909862	-0.16594173
D2-3	0.42373350	-0.3382605	-0.44712347	-0.2187839	-0.47293207
D2-9	0.92356163	0.2296498	-0.04440642	-0.0595992	-0.01225792
D2-10	0.76533542	0.4152853	0.32748453	-0.0962602	0.12232137
D3-4	-0.04436844	0.8354376	-0.16785926	-0.1181192	0.26337193

Coordinates of Variables (Morphometric Measurement):

Variables	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5
TL	-0.06604478	0.7414683	0.5910117	0.02466663	0.30534659
SL	0.65424431	0.6252860	-0.2874924	0.16654144	0.05285930
FL	0.66804845	0.5951129	-0.2821206	0.20136873	-0.13620038
HL	0.92217353	-0.2587892	0.1829510	-0.07740456	0.05869424
ED	0.63474708	-0.6080029	-0.2164229	0.11760268	0.39451028
PrOL	0.66544448	-0.3583440	0.5089677	0.31966576	-0.24429501

Contribution of Variables (Truss Distance):

Variables	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5
D1-2	0.6411953	0.04054950	2.387770	26.28605229	0.104097929
D1-10	0.24289466	1.797336	23.98153539	4.2124597	1.506806741
D2-3	2.56186178	2.940728	7.43563817	2.3813388	12.238941268
D2-9	12.17031626	1.355453	0.07334235	0.1767141	0.008222039
D2-10	8.35744876	4.432465	3.98882421	0.4609824	0.818749350
D3-4	0.02808784	17.938258	1.04798222	0.6941154	3.795642376

Contribution of Variables (Morphometric Measurement):

Variables	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5
TL	0.1359927	29.282144	40.892486	0.1165072	27.4139586
SL	13.3450016	20.824523	9.676184	5.3110179	0.8215395
FL	13.9140839	18.863245	9.317962	7.7645663	5.4543406
HL	26.5133145	3.567057	3.918510	1.1472716	1.0129234
ED	12.5614608	19.689241	5.483497	2.6483025	45.7617110
PrOL	13.8058253	6.839398	30.327162	19.5670520	17.5474814

Values of 22 Truss Distance in Five Dimensions:

Variable	Dimension 1	Dimension 2	Dimension 3	Dimension 4	Dimension 5
D1.2	-0.05331	0.304804	0.840681	-0.11353	-0.04362
D1.10	-0.13047	0.264447	0.802984	-0.29099	-0.16594
D2.3	0.423733	-0.33826	-0.44712	-0.21878	-0.47293
D2.9	0.923562	0.22965	-0.04441	-0.0596	-0.01226
D2.10	0.765335	0.415285	0.327485	-0.09626	0.122321
D3.4	-0.04437	0.835438	-0.16786	-0.11812	0.263372
D3.8	0.778011	0.272226	0.120545	-0.02754	0.313705
D3.9	0.932642	0.234352	9.20E-05	-0.05231	0.076084
D3.10	0.883537	0.001819	-0.18762	-0.09585	-0.1925
D4.5	0.287779	-0.62739	0.411909	0.450382	0.091749
D4.6	0.438568	-0.62031	0.331799	0.464068	0.125067
D4.7	0.538233	-0.6789	0.295349	0.150617	0.24221
D4.8	0.89914	-0.21472	0.167904	0.106666	0.134978
D4.9	0.849924	0.3925	-0.18032	-0.13992	-0.0155
D5.6	0.199986	0.665355	-0.02186	0.398626	0.195026
D5.7	0.045661	0.527665	-0.00356	0.655057	-0.09154
D5.11	-0.2571	0.240338	-0.34734	0.257284	0.628545
D6.12	-0.02936	-0.37214	-0.51725	0.168319	0.264697
D6.7	0.018271	0.338068	-0.03173	0.676252	-0.53408
D7.8	-0.17214	-0.19623	0.052458	-0.3038	0.647573
D8.9	0.694082	-0.21167	-0.16749	-0.33304	-0.17464
D9.10	0.629519	-0.08356	-0.31922	0.1014	0.028671

Values of Biplot Analysis for 7 Morphometric Measurement:

Variable	Eigenvalue	% of Variance	% of Cumulative Variance
Dim.1	3.20746	45.82086	45.82086
Dim.2	1.87751	26.82158	72.64244
Dim.3	0.854179	12.20255	84.84499
Dim.4	0.522236	7.460515	92.3055
Dim.5	0.340106	4.858658	97.16416
Dim.6	0.136954	1.956489	99.12065
Dim.7	0.061555	0.87935	100

Values of Biplot Analysis for 22 Truss Distance:

Variable	eigenvalue	variance.percent	cumulative.variance.percent
Dim.1	7.008578	31.85717	31.85717
Dim.2	3.89088	17.68582	49.54299
Dim.3	2.688665	12.2212	61.76419
Dim.4	2.010063	9.136651	70.90084
Dim.5	1.827484	8.306747	79.20759
Dim.6	1.02153	4.643316	83.85091
Dim.7	0.75992	3.454183	87.30509
Dim.8	0.577623	2.625561	89.93065
Dim.9	0.539831	2.453778	92.38443
Dim.10	0.320308	1.455947	93.84038
Dim.11	0.28546	1.297545	95.13792
Dim.12	0.261765	1.189842	96.32776
Dim.13	0.217721	0.989642	97.31741
Dim.14	0.148282	0.674008	97.99141
Dim.15	0.111413	0.506423	98.49784
Dim.16	0.091534	0.416062	98.9139
Dim.17	0.068046	0.3093	99.2232
Dim.18	0.055789	0.253585	99.47678
Dim.19	0.041342	0.187919	99.6647
Dim.20	0.038132	0.17333	99.83803
Dim.21	0.024842	0.112919	99.95095
Dim.22	0.010791	0.049049	100

Five Principal Components of 22 Truss Distance for All Species:

Number	Location	P1	P2	P3	P4	P5
1	ME	2.383204	2.049333	-0.68678	-0.67846	2.134379
2	ME	0.644948	2.583717	0.213459	0.983127	-3.0958
3	ME	1.750114	-0.08046	1.024835	3.433996	-1.52624
4	ME	-2.01736	2.053201	0.501523	1.561567	-2.28605
5	ME	0.939862	2.120046	0.612808	-0.47085	-3.00287
6	ME	1.777305	3.090972	-1.04951	2.957783	-2.38236
7	ME	2.422293	3.268441	-0.14098	1.59553	-1.80084
8	ME	0.821471	2.754872	-2.40189	0.79733	-3.73547
9	ME	2.449608	3.684845	-1.24712	2.552629	-2.71574
10	ME	0.867995	1.833331	-1.7356	3.011346	-2.07256
11	ME	5.35905	1.899034	0.870513	1.33773	-1.65333
12	ME	1.351383	-0.16914	0.138557	2.649075	-4.41088
13	ME	0.720486	2.070375	-2.73787	1.804122	-2.47101
14	ME	1.750567	1.669275	-3.69192	2.57679	-1.85639
15	ME	0.565369	2.498778	-1.66659	-0.37302	-3.31646
16	ME	2.555652	0.61179	-0.47935	1.239691	-0.38017

17	ME	1.724873	2.229685	-1.47314	-1.19513	-0.24134
18	ME	-1.43217	1.667692	-1.15611	-0.02392	0.048325
19	ME	-0.97921	2.803446	-0.83404	0.715072	-0.04736
20	ME	1.373378	2.36372	-0.89884	1.485835	0.171922
21	ME	0.179069	1.543346	0.796242	-0.84604	0.947034
22	ME	0.795822	1.586665	-2.52113	-1.62881	0.647765
23	ME	-2.68282	-0.32378	0.912695	1.366999	0.443253
24	ME	1.268648	2.628736	-1.54224	-0.31388	-0.5875
25	ME	2.56609	1.883757	-0.09052	-0.95408	0.735641
26	ME	1.538851	0.271326	-1.20541	-1.31471	-1.29722
27	ME	-2.42863	1.067729	-0.11072	1.066701	0.109395
28	ME	1.573305	0.283999	-1.04284	1.68584	0.538058
29	ME	2.259339	1.101911	-1.43313	-1.1331	-0.02391
30	ME	1.792866	1.501959	0.955343	-0.07916	-0.49567
31	ME	1.061876	2.199622	-0.53206	-0.44182	-0.68264
32	ME	0.988841	2.243044	-0.98201	-2.03222	0.800749
33	ME	3.715109	2.237278	-1.74975	0.635918	-1.16468
34	ME	-2.71933	-0.43037	1.158456	-0.24463	-0.71746
35	ME	3.339442	2.151459	-0.2864	0.585819	-0.46694
36	MR	0.718846	-3.20859	-2.75764	0.030474	-2.80676
37	MR	-0.45799	-5.39068	-1.97126	0.184222	-2.34534
38	MR	-1.53713	-1.20826	-2.7435	-2.72432	-2.20492
39	MR	-1.65928	-1.94563	1.755554	-3.65327	-1.23937
40	MR	0.504344	-5.24481	-0.89731	-0.05231	-1.17729
41	MR	0.308799	-2.88202	1.236818	-0.37425	-1.00845
42	MR	-1.26982	-4.05313	1.403708	-0.77521	-1.05349
43	MR	-0.24227	-2.12943	-0.49138	-1.29688	-2.60006
44	MR	4.306505	-0.38494	-1.03942	-1.83072	0.76402
45	MR	2.92954	-3.33907	-2.63495	-1.30698	-1.6953
46	MR	0.528462	-4.85116	-0.97456	-0.82654	-0.40291
47	MR	-1.58467	-4.52746	0.428079	-0.64622	-1.49039
48	MR	-2.11362	-1.91422	-0.02644	1.032053	-0.5515
49	MR	-0.31099	-2.03452	-1.71041	-2.05472	-2.44915
50	MR	-2.30705	-3.45813	0.351824	1.049356	0.179751
51	MR	-0.19372	-1.66867	1.040927	-2.20824	-0.9222
52	MR	-1.11773	-1.77985	-1.04426	-1.37889	-1.49692
53	MR	-2.4767	-0.2956	-0.18774	-2.39419	0.178
54	MR	-1.02798	-4.53609	1.575313	0.684951	0.814846
55	MR	-1.4847	-5.12878	0.12639	1.635754	-0.19517
56	MR	-0.31873	-3.28877	2.625774	2.097676	-0.87296
57	MR	-1.48055	-5.78972	0.77504	1.749659	-0.74324
58	MR	-0.7887	-3.42162	1.595373	-1.48464	0.631262
59	MR	1.614966	-4.56168	-0.66574	1.332901	-1.14232
60	MR	3.671262	-2.97773	-1.34718	-1.92801	-0.55697
61	MR	0.598466	-0.75521	-1.98679	-1.89745	-1.15009
62	MR	-0.54057	-2.8843	0.547069	-1.79826	-0.81117
63	MR	-1.48873	-2.05263	1.448955	-1.66832	-0.07887
64	MR	2.103687	-1.73305	0.629073	-0.52029	-0.57001
65	MR	2.412018	-0.91491	-0.45577	-1.17257	0.760984
66	MR	-1.88837	-2.7606	-0.84424	-1.25916	-1.05964
67	MR	1.603792	-1.74269	-1.86137	-1.0555	-1.41495
68	MR	-4.22472	-1.35906	-0.81276	-2.03311	-0.89122

69	MR	1.050196	-6.57413	-0.31169	1.69698	-0.59464
70	MR	-2.72844	-4.76098	-0.60208	-0.83943	-0.75492
71	MR	0.896495	-4.63981	-1.84437	-0.17937	-1.30005
72	Sea	1.615492	0.638805	2.049596	-3.12899	0.917755
73	Sea	1.152544	0.28585	1.011834	1.012705	0.252606
74	Sea	0.434404	0.624715	-2.172	-1.17699	0.323646
75	Sea	2.843636	0.379498	-0.59738	-1.47958	1.776337
76	Sea	1.269775	1.596393	2.092126	-1.04154	1.294971
77	Sea	4.701673	-0.02325	0.208579	0.197328	1.886872
78	Sea	6.3404	-1.0957	-1.07512	0.505941	1.866703
79	Sea	2.242011	-1.01222	1.727695	1.646698	1.622352
80	Sea	1.640328	-2.13051	1.870599	2.548526	2.72702
81	Sea	3.728888	-0.67754	-0.16994	0.216161	0.372505
82	Sea	1.561855	-0.28245	-0.20911	-0.90682	-0.25887
83	Sea	2.80976	0.709827	0.41243	0.916901	0.139302
84	Sea	7.779893	-0.20037	-3.02242	1.199431	0.557909
85	Sea	0.507345	0.954597	0.653669	-1.46582	1.156302
86	Sea	1.652035	-0.519	-2.17344	0.326147	1.843317
87	Sea	3.383233	0.649087	-1.51321	-1.09473	1.943845
88	Sea	3.186357	0.669759	-2.76222	-1.31278	1.244085
89	Sea	2.943616	1.211047	-0.31235	-2.40486	1.620883
90	Sea	3.880494	-0.98855	-0.20194	-1.06145	2.494562
91	Sea	4.645031	-1.58079	-1.03555	0.05813	1.88245
92	Sea	5.173591	-1.23167	-1.03346	-0.33006	2.310349
93	Sea	0.555732	2.028522	0.097903	-1.90635	-0.21885
94	Sea	3.263793	-1.13368	-2.51343	-1.27267	2.328771
95	Sea	6.363126	-0.91613	-0.96963	-3.10428	0.625422
96	UJR	-3.5149	1.261504	0.692631	0.375749	-0.30128
97	UJR	-2.16863	1.382612	1.479049	2.595364	-0.87266
98	UJR	0.39999	1.023575	0.310743	-2.80225	0.435712
99	UJR	0.025697	1.173672	0.395385	-0.14193	2.298119
100	UJR	-2.06546	0.62695	0.919783	-0.05596	-1.2508
101	UJR	0.418915	-0.53804	1.770587	0.126879	0.243168
102	UJR	0.234066	0.313794	0.748627	-0.80391	0.265606
103	UJR	-0.47581	0.166776	0.82396	-0.49138	0.538807
104	UJR	-2.30581	1.076288	1.112128	-0.29605	-0.41269
105	UJR	-3.75614	0.582252	2.868626	0.19658	-0.50697
106	UJR	-0.02587	1.207804	-0.46345	-0.65267	0.827892
107	UJR	-1.53374	1.692308	0.112195	-1.71251	0.120429
108	UJR	-3.00583	3.648025	0.609372	0.959263	-0.55372
109	UJR	-2.29906	0.901227	2.401446	-1.01431	-0.75383
110	UJR	-1.0139	0.13553	1.126779	0.901476	1.109097
111	UJR	-0.81878	-0.65526	-0.12685	-0.53036	1.114073
112	UJR	0.298109	0.506598	0.357507	0.387228	0.417227
113	UJR	-3.04883	0.076498	-0.00014	0.57031	-0.38548
114	UJR	1.95464	0.330206	-1.73363	0.266568	-1.65816
115	UJR	0.584146	-1.21199	1.393035	1.742272	0.115826
116	UJR	-2.76399	0.739609	2.259965	-3.26205	-0.97403
117	UJR	0.6046	0.87866	-0.37194	-0.14754	0.434205
118	UJR	2.683025	0.374937	-0.04036	-0.014	-1.12364
119	UJR	-3.20381	1.09985	0.524739	-0.20911	-1.04303
120	UJR	-1.48653	-0.89045	2.737266	-1.81974	0.119298

121	UJR	-1.64791	-0.34464	-0.36434	-0.65573	0.510391
122	UJR	-1.1746	0.491958	1.964378	-0.43623	-1.56929
123	UJR	-1.92686	-1.09821	4.714366	0.379684	-1.35939
124	UJR	-1.04033	0.274978	3.672937	-0.91745	0.727439
125	UJR	-0.0167	0.455945	2.784748	0.507	-0.47883
126	UJR	1.16547	1.704325	1.606526	-1.71142	0.313041
127	UJR	5.101227	-2.19674	2.144613	0.470633	-0.2858
128	UJR	-0.44077	1.814037	0.690433	-2.16938	0.545061
129	UJR	0.8835	1.452802	0.298567	0.685878	2.00312
130	UPR	-4.25442	1.424454	1.101008	-0.71669	-0.38103
131	UPR	-6.63208	0.21818	-0.78577	1.494253	2.513305
132	UPR	-0.47139	-0.22246	-2.53028	-1.20366	0.30286
133	UPR	-4.1287	0.5384	-1.39904	-1.62193	0.644974
134	UPR	-3.14834	3.084525	0.666066	-2.35347	0.241727
135	UPR	-3.77512	2.144016	0.733528	-0.12051	-0.32454
136	UPR	-3.24957	-0.0011	-1.45156	-0.10788	0.820519
137	UPR	-4.21912	-0.80273	-3.45832	1.913164	2.177116
138	UPR	-8.49298	0.8107	0.828696	-1.50339	-0.27297
139	UPR	-2.74725	0.266515	-0.38407	-1.16545	0.757273
140	UPR	-4.31927	1.482379	-0.11152	-1.59454	0.197075
141	UPR	-4.54776	-0.49306	-2.75905	0.291065	1.808491
142	UPR	-3.41463	0.485928	-2.16712	2.526325	2.308131
143	UPR	-3.24925	-0.01359	-0.17742	0.123796	1.629101
144	UPR	3.134805	3.489848	3.727362	-2.2507	-3.54577
145	UPR	-3.88995	0.238934	-0.68545	2.291668	0.622315
146	UPR	-4.59312	0.71144	-4.03305	1.19432	0.848156
147	UPR	-3.38882	-0.34471	-3.25685	1.676123	1.914043
148	UPR	0.173966	0.197919	-2.15348	-0.21384	1.20234
149	UPR	-4.04153	0.403348	-1.56731	-0.48449	1.323234
150	UPR	-1.74795	0.024971	-2.1273	1.847724	0.424516
151	UPR	-1.27579	2.975207	0.237669	-0.44649	0.106205
152	UPR	0.082093	-0.76906	-0.02195	-1.2875	1.746589
153	UPR	-0.73899	0.753949	-2.63938	1.154383	0.642357
154	UPR	-2.58321	-1.01894	-0.76411	0.115036	-0.13255
155	UPR	-4.56958	-1.96332	-2.10896	1.683234	1.942121
156	UPR	-0.52739	0.504045	2.660868	-1.77421	0.111127
157	UPR	-2.81001	0.73156	-2.8772	1.423354	2.174452
158	UPR	-5.43939	2.15622	-3.37622	-0.71865	0.183701
159	UPR	-5.46519	-0.41211	-0.88497	0.541507	1.883495
160	UPR	-3.84757	-0.06448	-0.76404	0.763754	-0.65227
161	UPR	-1.60596	-0.5808	-1.4254	0.260238	-1.13782
162	UPR	-2.94139	1.877121	-0.4607	-1.01548	1.381007
163	LPR	1.352108	0.157802	3.70864	0.992189	1.202355
164	LPR	4.132985	-0.27232	3.601647	0.644701	1.400087
165	LPR	2.227848	-0.62638	3.513736	0.858028	1.759157
166	LPR	2.993127	-0.36281	1.014361	-0.48346	-0.14637
167	LPR	3.057167	0.045479	2.364306	1.46838	1.676244
168	LPR	-0.62344	0.750341	1.551579	1.058586	0.794318
169	LPR	3.46246	0.330357	2.232206	0.594412	0.80613
170	LPR	0.376022	1.785838	0.325924	-0.67243	1.066557
171	LPR	-1.36065	1.509705	1.011826	1.88873	1.784479
172	LPR	2.829848	0.603749	0.805802	0.22542	1.210795

173	LPR	-2.61779	-0.32967	0.665001	0.737713	0.445111
174	LPR	1.37607	2.005967	-0.73562	0.878356	1.520155
175	LPR	-0.41719	-0.50214	1.37701	1.777311	1.86068
176	LPR	2.213149	-0.7051	-1.38182	-0.02783	0.08362
177	LPR	1.581038	1.960024	-0.09755	-0.74256	0.967228
178	LPR	2.111715	-2.06468	0.578335	2.248501	1.364405
179	LPR	-3.22941	-1.13325	0.969608	1.833218	0.688403
180	LPR	-3.46738	1.497079	1.554334	0.741642	-0.28807
181	LPR	-2.85356	1.115736	0.570292	1.236993	0.419018
182	LPR	-0.25508	1.61769	0.705613	-1.16075	1.090498
183	LPR	-1.46336	0.590288	0.629581	0.564423	-0.75372
184	LPR	0.946352	1.535855	-0.07103	0.73988	0.000854
185	LPR	-1.51559	0.614772	1.734806	-0.32112	-1.27576
186	LPR	0.86334	1.316042	-0.50541	-0.94277	0.327416
187	LPR	-1.21615	4.462124	1.752565	-0.66702	-1.35655
188	LPR	2.227501	0.056097	1.413679	1.219344	0.016581
189	LPR	0.145816	1.826685	0.743145	1.893882	-0.96569
190	LPR	-1.55372	-0.64312	2.357647	-1.08672	-0.71653
191	LPR	2.386424	0.936113	1.609604	2.889916	-0.11228
192	LPR	2.374518	-0.61759	0.544547	1.13175	-0.02819
193	LPR	2.297208	-2.24831	2.829581	2.630454	0.70195
194	LPR	2.138826	0.393821	2.535312	0.58799	0.135337
195	LPR	2.262698	-0.4464	-1.27112	-0.26071	0.472861
196	LPR	3.562107	0.252918	1.260365	-0.03094	0.072989
197	LPR	-3.75905	-0.98865	2.762548	1.890131	0.64367
198	LPR	1.562568	0.577302	0.153854	1.233065	-1.54043

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