

*Article*

## **Land-Mark based morphometric and meristic variations in two congeneric hilsha population, *Tenualosa ilisha* and *Tenualosa toli* from Bangladesh water bodies**

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**Abstract:** The morphometric characters are effectively used for the better differentiation among the fish population and sustainable management. The appraisal of the natural population stock and morphological variation within and between two hilsha species (*Tenualosa ilisha* and *Tenualosa toli*) from three different habitat (Coastal, riverine and marine) of Bangladesh, were investigated by applying the land mark based morphometric and meristic variation measurement methods. All data were adjusted and Univariate ANOVA, where discriminant function analysis (DFA) and principal component analysis (PCA) exhibited the divergences in eight morphometric measurements and eight truss network measurements among the three stocks of *T. ilisha*. The 1<sup>st</sup> DFA accounted for 89.8% & 87.4% and the second DFA resolved 10.2% and 12.6%, respectively in morphometric characteristics variation among the group studied. Scattered plotting from PCA and dendrogram from cluster analysis (CA) revealed that, the river habitants were morphologically different from the coastal and marine population. Twelve of fifteen morphometric measurements and thirteen of fourteen truss network measurements showed significant differences between *T. ilisha* and *T. toli* with significant variation in meristic characters. PCA revealed 89.23% and 88.29% in case of morphometric and truss measurement respectively confirmed high degree of variations in morphological characteristics between two species. Overall, our results based on morphometrics with truss measurements together provide useful information about the morphological differentiation which will be helpful for sustainable exploration and effective management for these two species.

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**Keywords:** land-mark; morphometrics; truss-network; population structure; *Tenualosa* spp.

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### **1. Introduction**

Bangladesh is gifted with extensive inland and marine fisheries potential resources enriched with 260 freshwater fish species and 475 marine fish species (DoF, 2013). Hilsha is the national fish of Bangladesh under the family clupeidae, genus *Tenualosa*, which is anadromous in nature and one of the most important tropical commercial fish. Among the three kinds of *Tenualosa* sp. found in Bangladesh, the padma ilish (*T. ilisha*) and the chandana ilish (*T. toli*) are mostly well-known. The production of Hilsha fish is 5.17 MT which contributes 12.09% to the total fish production and 1.15% to the national GDP of the country (DoF, 2018). These species have a wide distribution in the Bay of Bengal and the rivers (Padma, Meghna) of Bangladesh. Being an anadromous fish, Hilsha populations from the Bay of Bengal mainly inhabits in marine water and migrates to the freshwater for spawning and returned to their original habitat (Hossain *et al.*, 2015). Different subset of hilsha population from the Bay of Bengal uses coastal and estuarine habitats for spawning without entering freshwater. On the other hand, small subsets of populations complete their life cycle only within freshwater and does not migrate to sea at any stage of development. Therefore, we hypothesized that, there may be some morphological differences between freshwater, coastal and saltwater inhabitants of hilsha populations. For that, we collected fish from

three different sources in consideration of spawning migration with an aim to determine the morphological differences between hilsha populations using Land-Mark based analysis.

Fish morphological study is significant from various points of view which include evolution, ecology, behavior, conservation, water resource management and stock assessment (AnvariFar *et al.*, 2011). Morphological characters include the measurable or countable characters of fish which are general for all fishes. Morphological characteristics of fish are those authoritative characters which provide relevant way to identify, taxonomic study as well as better understandings of common facts of fishes. The interpretation of morphological structure functions as a stranglehold tool which is applied in practical field during taxonomy and ecological study (Bohlen, 2008). The measuring constituents of fish anatomy such as body parts or fins are widely applied in systematics or taxonomical studies. The variations in shape testing and graphical representation can be easily practiced by applying this utile technique. Landmarks are those considerable points of a fish body which are arbitrarily selected and the individual fish shape can be investigated through the evaluation of these points. Landmark based measurements serve as impactful tools (Hossain *et al.*, 2010) which can be applied for the natural fish stock identification.

It is a prerequisite to understand the life history and population structure of any fish species while attempting the management and conservation strategies (Turan *et al.*, 2006) and may be implementable while studying short-run and environmental impacted variations and even for the genetic management. 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. The differences of morphometric characteristics among the stocks of a particular species are recognized as an important tool to understand and evaluate the population structure and can be served as a basis for identifying new stocks (Turan, 2004; Turan *et al.*, 2004b; Vishalakshi and Singh, 2008; Randall and Pyle, 2008). The Intra-interspecific variations among fishes such as size and shape can be obviously evaluated (Hajje *et al.*, 2011). This conception is considered as a prerequisite in biometric variations of the species that are geographically isolated. The overall pattern of racial, geographic and inter-specific divergence in species has long been evaluated through relative contribution of the size and shape (Gunawickrama, 2007; Hajje *et al.*, 2011) and this concept had effective and successful applications in fish stock identification. Analysis of traditional and geometric morphometric measurements variations which have taxonomic potentials and discriminating powers isolates specific morphometric indices and variants away of the environmental and geographical influences. The meristic characters have also relatable validity in race and species and stock identification for fishery purposes (Turan, 2004). Remarkable application of landmark based morphological studies have been conducted in Bhagna (Ahammad *et al.*, 2018), Gonia (Begum *et al.*, 2013), Kalibaus (Hossain *et al.*, 2010), Rohu (Hasan *et al.*, 2007) and Thai Pangas (Khan *et al.*, 2004) and some other species in Bangladesh. But very limited information is available on *T. ilisha* and *T. toli* comparative morphometry and there have been few attempts to evaluate the population structure using different methods based on morphological aspects. Therefore, the present study deals with the exploration of population structure of *T. ilisha* and *T. toli* from different habitats based on morphometric characters for its sustainable development and management across the Bangladesh.

## 2. Materials and Methods

### 2.1. Ethics approval and consent to participate

This experiment was conducted by following the “animal care and use committee guideline” of Chattogram Veterinary and Animal Sciences University. The ethics issue is not required for the described study in Bangladesh. Samples are collected not from the privately owned or from protected areas and this species are not endangered or protected one.

### 2.2. Sample collection

*T. ilisha* and *T. toli* were collected from the three different habitats (Coastal, riverine and marine) and preserved in ice box with the quick succession of time and brought to the laboratory of Fish Biology and Biotechnology, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Chattogram for morphometric, meristic and landmark studies. Fish samples were collected from all three locations within 3 days considering their capture date and migration time. A total of 16 fresh and healthy fish samples from each group were chosen for further analysis. The descriptions of sampling area, sample size, total length is presented in Table 1.

### 2.3. Measurement of morphometric and meristic characteristics

Sixteen (16) general morphometric characters were measured (Figure 1A) from each sample fish by applying the conventional method described by Hubbs and Lagler (1958). The morphometric measurement was

conducted with an accuracy of 0.05 mm with the help of Vernier calipers and metric scale. The measured morphometric characters used in this experiment for morphological analysis with their descriptions are presented in Table 2. Meristic characters like Dorsal fin rays (DFR), Anal fin rays (AFR), Caudal fin rays (CFR), Pectoral fin rays (PcFR), Pelvic fin rays (PvFR), number of branchiostegal rays and scales on lateral line of each sample were counted from each fish and used for comparative analysis using magnifying glass.

#### 2.4. Measurement of Land-mark distances

Eight landmarks outlining with 14 distances were measured on the body of *Tenualosa* sp (Figure 1B). The selection of landmark points were done to bring the coverage homogeneity of the total body plan in between two species based on the Strauss and Bookstein (1982). Each landmark was obtained by placing the fish sample on a graph paper and the detection of the landmark points were done with colored pointers for enabling accurate and consistent measurements. Finally, the distances found on the graph paper were determined by using centimeter scale. The description of distances between landmark points is presented in Table 3.

**Table 1. Summary of sampling area, sample size, total length and habitats of collected samples of *T. ilisha* and *T. toil* from different water bodies.**

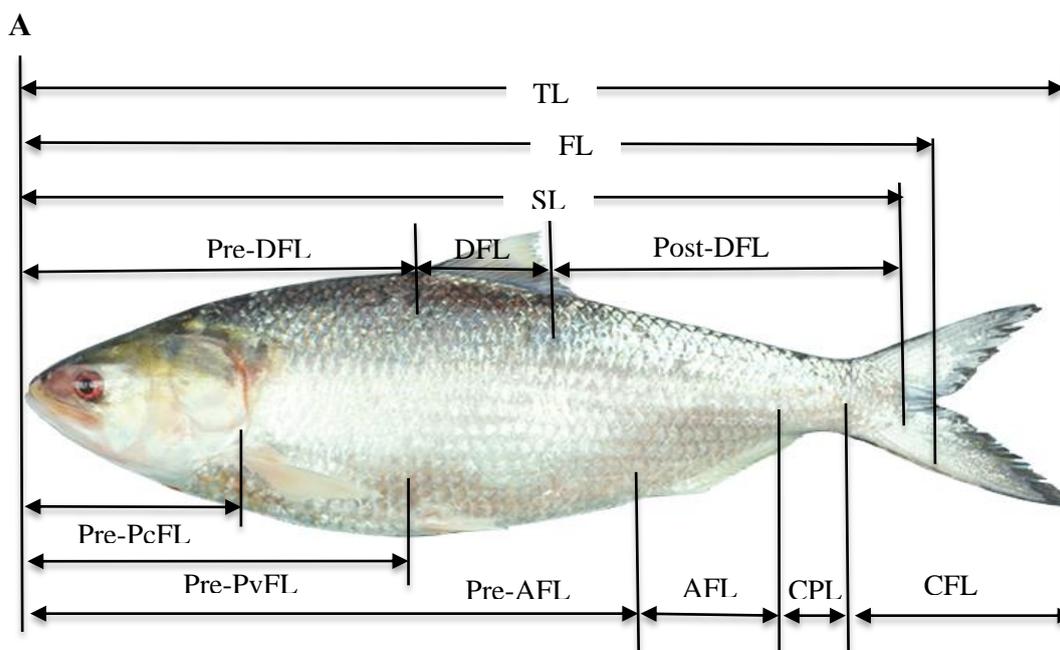
Species	Habitats	Source/location	Total length	Sample size
<i>T. ilisha</i>	Marine	Cox's Bazar <u>21°19N, 91°35E</u>	25.68 ± 1.17	16
<i>T. ilisha</i>	Freshwater	Chandpur 23°12N, 90°37'E	31.65 ± 1.15	16
<i>T. ilisha</i>	Coastal	Chattogram <u>22°11N, 91°37E</u>	26.43 ± 0.77	16
<i>T. toil</i>	Marine	Chattogram <u>22°11N, 91°37E</u>	28.57 ± 1.62	16

**Table 2. General morphometric characters and their descriptions used for the analysis.**

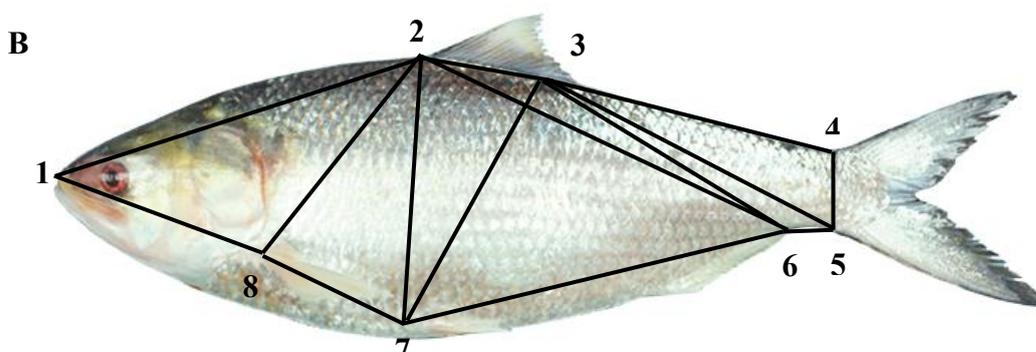
Sl. No.	Characters	Description
01	Standard length (SL)	From the Tip of the snout to the end of the vertebral column
02	Total length (TL)	From the Tip of the snout to the longest caudal fin ray
03	Fork Length (FL)	From the Tip of the snout to the middle part of the fork of the tail
04	Pre-dorsal fin length (Pre-DFL)	From the snout tip to the origin of the dorsal fin
05	Dorsal fin length (DFL)	From base of first dorsal spine to base of last dorsal ray
06	Post-dorsal fin length (Post-DFL)	From posterior base of dorsal fin to the longest caudal fin ray
07	Pre-Pelvic fin length (Pre-PvFL)	Front of the upper lip to the origin of the pelvic fin
08	Pelvic fin length (PvFL)	From base to tip of the pelvic fin
09	Pre-Pectoral fin length (Pre-PtFL)	Front of the upper lip to the origin of the pectoral fin
10	Pectoral fin length (PtFL)	From base to tip of the pectoral fin
11	Caudal fin length (CFL)	From tail base to tip of the caudal fin
12	Pre-anal length (PAL)	Front of the upper lip to the origin of the anal fin
13	Anal fin Length (AFL)	From base of first anal spine to base of last anal ray
14	Highest Body Depth (HBD)	Vertical distance from the anterior part of the first dorsal fin and ventral part of the body
15	Least Body Depth (LBD)	Vertical distance at the end of the Vertebrae
16	Caudal Peduncle Length (CPL)	From the base of the anal fin to the base of the caudal fin

**Table 3. Description of truss network characters used in the study.**

Sl. No.	Character codes	Landmarks points	Description of characters
01	A1	1-2	Anterior tip of snout to the origin of dorsal fin base
02	B1	2-3	Origin of dorsal fin to the end of dorsal fin base
03	C1	3-4	End of dorsal fin base to origin of caudal fin
04	D1	4-5	Upper to lower of caudal fin origin
05	E1	5-6	Origin of lower of caudal fin to end of the anal fin base
06	F1	6-7	Origin of anal fin to origin of pelvic fin
07	G1	7-8	Origin of pelvic fin to origin of pectoral fin
08	H1	8-1	Origin of pectoral fin to the end of snout tip
09	I2	2-7	Origin of dorsal fin to origin of pelvic fin
10	J2	2-6	Origin of dorsal fin to origin of anal fin
11	K2	3-7	End of dorsal fin base to origin of pelvic fin
12	L2	3-6	End of dorsal fin base to origin of anal fin
13	M2	3-5	End of dorsal fin base to lower caudal fin origin
14	N2	4-6	Origin of the upper caudal fin to end of the anal fin base



**Figure 1A. Overview of different morphometric indices of *Tenuulosa* sp.**



**Figure 1B. Randomly selected landmarks points in fish body used in this study. The eight landmarks points refers to (1) anterior tip snout of the upper jaw of mouth (2) base of origin of dorsal fin (3) end of dorsal fin (4) dorsal caudal fin base (5) ventral caudal fin base (6) ending of caudal fin base (7) base of pelvic fin (8) middle base of pectoral fin.**

## 2.5. Data adjustment

The elimination of the size effects from the data set was done before the analytical studies. An allometric formula given by Elliott *et al.* (1995) with slight modification was used to remove the size effect from the data set.

$$M_{\text{adj}} = M (L_s/L_o)^b$$

Where,

$M_{\text{adj}}$ : size adjusted measurement,

M: original measurement,

$L_s$ : overall mean of standard length for all fish from all samples in each analysis

$L_o$ : total length of fish

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

## 2.6. Statistical analysis

In the first level of analysis, we compare among the collected samples of *T. ilisha* to show the morphological differences among habitats. In the second steps, we then compare between the *T. ilisha* and *T. toil* to observe the morphological distances in these two species. Univariate analysis of variance (ANOVA) was conducted to test the significance of morphological differences ( $p < 0.01$ ) on the basis of size adjusted morphological and landmark distance data. Meristic characters among fish groups were compared using non-parametric Kruskal-Wallis test. In addition, all size adjusted morphological and landmark distance data were standardized and submitted to a Discriminant Functional Analysis (DFA) and Principal Component Analysis (PCA). All statistical analysis was carried out using Statistical Packages for Social Sciences (SPSS version 16.0) and Microsoft office excel, 2010.

## 3. Results

### 3.1. Comparative morphometric studies on *T. ilisha* collected from three (03) different habitats:

#### 3.1.1. Analysis of meristic counts

Meristic counts of all samples of *T. ilisha* collected from three different habitats ranged from 17-21 for anal fin rays (Median,  $M_e = 17$ ), 18/19 for dorsal fin rays ( $M_e = 18$ ), 7/8 for pelvic fin rays ( $M_e = 7$ ), 14/15 for pectoral fin rays ( $M_e = 14$ ), and 26-28 for caudal fin rays ( $M_e = 27$ ). Number of branchiostegal rays were fixed in all samples ( $B = VI$ ). Though the number of scales on the lateral line (45-47) and lateral transverse (17-19) varies between species, no significant difference was observed among three habitats. In the Kruskal Wallis (H) test the number of anal fin rays, dorsal fin rays, pelvic fin rays, pectoral fin rays and caudal fin rays were not statistically significantly ( $p > 0.05$ ) among fish from three different habitats. Besides univariate statistics (ANOVA) also showed no significant differences ( $p > 0.05$ ) in meristic characters among fishes from three different regions.

#### 3.1.2. Analysis of morphometric and Land-mark distance measurements

There were no significant correlations between the total length and adjusted morphological values (each  $p > 0.05$ ) among all fishes of *T. ilisha* which indicates that, the size effects were successfully removed with the help of allometric transformations. Therefore, all the morphological and truss-network measurement were considered for Univariate analysis (ANOVA). ANOVA showed that eight [pelvic fin length (PvFL), anal fin length (AFL), caudal peduncle length (CPL), highest body depth (HBD), least body depth (LBD), post-dorsal fin length (Post-DFL), pre-pectoral fin length (Pre-PcFL), pre-pelvic fin length (Pre-PvFL)] of fifteen morphometric measurements were significantly different ( $p < 0.05$  or  $p < 0.01$  or  $p < 0.001$ ) among three groups of populations of *T. ilisha* (Table 4). In case of Land-mark distances, eight (1 to 2, 2 to 3, 3 to 4, 4 to 5, 6 to 7, 8 to 1, 2 to 6, 3 to 6) out of fourteen truss measurements were significantly different among samples in varying degrees ( $p < 0.05$  or  $p < 0.01$  or  $p < 0.001$ ) among three different groups of *T. ilisha* revealed through univariant statistics (Table 5).

First Discriminant function analysis (DFA) resolved 89.8% & 87.4% and the second DFA accounted for 10.2% and 12.6%, respectively of among group variability and together they explained 100% of the total variability for both morphometric and landmark measurements (Table 6). Pooled within-groups correlations between discriminating variables and discriminant functions revealed that among the fifteen morphometric measurements, four measurements of least body depth (LBD), anal fin length (AFL), pre-pectoral fin length (Pre-PcFL), and pre-pelvic fin length (Pre-PvFL) dominantly contributed to the first DF, while the remaining eleven [standard length (SL), fork length (FL), pre-dorsal fin length (Pre-DFL), dorsal fin length (DFL), post-

dorsal fin length (Post-DFL), pelvic fin length (PvFL), caudal fin length (CFL), pre-anal length (PAL), highest body depth (HBD), caudal peduncle length (CPL)] contributed to the second DF (Table 6). In case of truss measurements, among the fourteen measurements two measurements (4 to 5 and 2 to 7) dominantly contributed to the first DF, while the remaining twelve measurements contributed to the second DF (Table 7).

The significant traits (eight morphometric measurements and eight truss measurements) resulted from univariate analysis were further used for principal component analysis (PCA). The PCA based on eight morphometric measurements retained two components with Eigen values  $>1$ , explaining 52.13% of the total variance. The first (PC1) and second (PC2) principal components accounted for 38.31% and 13.87% of the total variance respectively (Table 8). All the eight morphometric measurements had significant loadings on PC1 and on PC2 pelvic fin length (PvFL), highest body depth (HBD), least body depth (LBD) and post-dorsal fin length (Post-DFL) were variant (Figure 2A). The value of KMO for overall matrix was 0.72 and the Bartlett's Test of Sphericity was significant ( $p < 0.01$ ) based on the eight truss-network data of *T. ilisha* from three different habitats. The PCA based on eight truss measurements retained two components with Eigen values  $>1$ , explaining 71.47% of the total variance. The first (PC1) and second (PC2) principal components accounted for 56.19% and 15.35% of the total variance respectively (Table 9). All the eight truss-measurement had significant loadings on PC1 and the most significant loadings on PC2 were 1-2, 2-3, 4-5, 6-7, 8-1 and 2-6 (Figure 2B).

On the basis of morphometric measurement 81.3%, 75.0% and 100% of original group cases were correctly classified in case of coastal, marine and river habitats respectively and a total of 85.4% of original group cases correctly classified for all three groups (Table 10). This suggested that *T. ilisha* of river populations were morphologically dissimilar to others group. But the fish samples from the marine and coastal were not fully separated (Figure 2A) which was revealed by PCA. Based on the truss measurements data, PCA showed the sample stocks were separated from each other specially fish stock of river origin was well separated from the fish stocks of other two sources (Figure 2B). The truss measurement showed 100%, 93.8% and 100% of original group cases were correctly classified in case of Coastal, marine and river populations respectively and a total of 97.7% original group cases were correctly classified for all three habitats (Table 11). This discriminant function scores based on both morphometric and truss measurements suggested that fishes of river origin were isolated from the fish samples of coastal and marine inhabitants (Figure 2).

A dendrogram was drawn based on the land-mark distances and morphological examinations among groups of centroids of *T. ilisha* populations collected from three different habitats. Two main clusters were found based on the Squared Euclidean dissimilarity. The coastal and marine samples show one cluster while the river groups shows a distinct cluster. So that we can say that, the river population is completely separated from the marine habitat (Figure 3).

### 3.2. Comparative morphometric studies *T. ilisha* and *T. toli* from Bangladesh water bodies

#### 3.2.1. Analysis of meristic counts

The number of dorsal fin rays (D17-18;  $M_e = 17$ ), pelvic fin rays (V8;  $M_e = 8$ ), pectoral fin rays (P 14-15;  $M_e = 15$ ), caudal fin rays (C 28-29;  $M_e = 28$ ), scales on lateral line (LL 40-41), scales on lateral transverse (LT 13-14) and the number of branchiostegal rays ( $B = V$ ) in *T. toli* were significantly ( $p < 0.05$ ) different from the *T. ilisha* in the Kruskal Wallis (H) test except anal fin rays ( $M_e = 17$ ). Besides univariate statistics (ANOVA) showed significant differences ( $p < 0.01$ ) between the two fish species (*T. ilisha* and *T. toli*) in case of caudal fin rays.

#### 3.2.2. Analysis of morphometric measurements

Prior to analysis, correlation test between total length and adjusted morphometric characteristics were done for all data to confirm the removal of size effects. Out of fifteen morphometric characters, twelve characters [fork length (FL), pre-dorsal fin length (Pre-DFL), dorsal fin length (DFL), post-dorsal fin length (Post-DFL), pre-pelvic fin length (Pre-PvFL), pelvic fin length (PvFL), pre-pectoral fin length (Pre-PcFL), pectoral fin length (PcFL), anal fin length (AFL), caudal fin length (CFL), caudal peduncle length (CPL), least body depth (LBD)] showed significant difference in univariate analysis (ANOVA) between the populations of *T. ilisha* and *T. toli* in varying degrees ( $p < 0.05$  or  $p < 0.01$  or  $p < 0.001$ ) (Table 12). Univariate analysis (ANOVA) between populations of *T. ilisha* and *T. toli* using fourteen Land-mark distances revealed that, thirteen (1 to 2, 3 to 4, 4 to 5, 5 to 6, 6 to 7, 7 to 8, 8 to 1, 2 to 6, 2 to 7, 3 to 5, 3 to 6, 3 to 7, 4 to 6) out of fourteen truss measurements showed significant difference between the populations of in varying degrees ( $p < 0.05$  or  $p < 0.01$  or  $p < 0.001$ ) (Table 13).

The significant traits resulted from univariate analysis (twelve morphometric measurements and thirteen truss measurements) were further used for PCA. The morphometric characters with an Eigen value above 1 were included and others were excluded in this analysis. In our present study, significant factors considered only those factors with loadings greater than 0.3. PCA for the morphometric measurements of *T. ilisha* and *T. toli*

showed that, the value of KMO for overall matrix is 0.906, and the Bartlett's Test of Sphericity is significant ( $p < 0.01$ ). The results of KMO and Bartlett's suggest that the sampled data is appropriate to proceed with a factor analysis procedure.

The PCA based on 12 morphometric measurements retained two components with Eigen values  $> 1$ , explaining 89.23% of the total variance. The first (PC1) and second (PC2) principal components accounted for 77.42% and 11.81% of the total variance respectively (Table 14). All the twelve morphometric measurements had significant loadings on PC1 and on PC2 caudal fin length (CFL) and caudal peduncle length (CPL) were significant (Figure 4A). PCA for the landmark measurements of *T. ilisha* and *T. toli* revealed that, the value of KMO for overall matrix is 0.887, and the Bartlett's Test of sphericity is significant ( $p < 0.01$ ). The PCA based on 13 truss measurements retained two components with Eigen values  $> 1$ , explaining 88.29% of the total variance. The first (PC1) and second (PC2) principal components accounted for 78.603% and 9.691% of the total variance respectively (Table 15). All the twelve morphometric measurements had significant loadings on PC1 and the most significant loadings on PC2 were 2-6, 3-4, 3-5, 3-6, 4-6, 5-6 and 6-7 (Figure 4B). Based on the Principal component analysis (PCA) in both morphometric and land-mark values it was clearly evident that scatter plots of specimens relating the first and second principal component (PC1 and PC2) revealed a visual differentiation in between two species. Dispersion in PCA plots showed a vast divergence in between *T. ilisha* and *T. toli* (Figure 4).

**Table 4. Univariate statistics (ANOVA) among samples of *T. ilisha* from 15 morphometric measurements from three different habitats. Degree of significance were presented as \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ .**

Characters	Wilks' Lambda	F value	df1	df2	Sig.
SL	0.973	0.632	2	45	0.536
FL	0.887	2.865	2	45	0.067
Pre-DFL	0.985	0.353	2	45	0.704
DFL	0.926	1.793	2	45	0.178
Post-DFL	0.865	3.526	2	45	0.038*
Pre-PvFL	0.868	3.425	2	45	0.041*
PvFL	0.528	20.112	2	45	0.000***
Pre-PcFL	0.835	4.450	2	45	0.017*
PcFL	0.906	2.324	2	45	0.110
Pre-AFL	0.927	1.768	2	45	0.182
AFL	0.669	11.123	2	45	0.000***
CFL	0.986	0.330	2	45	0.721
CPL	0.652	11.998	2	45	0.000***
HBD	0.660	11.612	2	45	0.000***
LBD	0.466	25.781	2	45	0.000***

**Table 5. Univariate statistics (ANOVA) showing the differences among measurement of 14 truss networking (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ) of *T. ilisha* from three (03) different habitats.**

Landmark distance	Wilks' Lambda	F value	df1	df2	Sig.
1-2	0.646	12.333	2	45	0.000***
2-3	0.669	11.110	2	45	0.000***
3-4	0.822	4.887	2	45	0.012*
4-5	0.280	57.751	2	45	0.000***
5-6	0.905	2.354	2	45	0.107
6-7	0.823	4.833	2	45	0.013*
7-8	0.927	1.779	2	45	0.180
8-1	0.822	4.865	2	45	0.012*
2-7	0.996	0.089	2	45	0.915
2-6	0.724	8.585	2	45	0.001**
3-7	0.961	0.907	2	45	0.411
3-6	0.726	8.479	2	45	0.001**
3-5	0.923	1.865	2	45	0.167
4-6	0.903	2.411	2	45	0.101

**Table 6. Pooled within-groups correlations between discriminating variables and discriminant functions in case of general morphometric characteristics.**

Characters	Discriminant function	
	1	2
Least Body Depth (LBD)	-0.416*	0.169
Anal fin Length (AFL)	-0.275*	0.044
Pre-Pectoral fin Length (Pre-PcFL)	-0.174*	-0.010
Pre-Pelvic fin length (Pre-PvFL)	-0.152*	-0.054
Pelvic fin length (PvFL)	-0.284	0.706*
Highest Body Depth (HBD)	0.256	0.349*
Post-dorsal fin length (Post-DFL)	0.111	0.320*
Caudal Fin Length (CPL)	0.269	0.291*
Fork Length (FL)	-0.121	0.206*
Pre-Anal Fin Length (Pre-AFL)	0.085	0.206*
Dorsal Fin Length (DFL)	-0.095	-0.170*
Pectoral fin Length (PcFL)	-0.117	0.137*
Pre-Dorsal Fin Length (Pre-DFL)	-0.022	-0.130*
Caudal Fin Length (CFL)	0.030	-0.110*
Standard Length (SL)	0.055	0.109*

Variables ordered by absolute size of correlation within function.

\*. Largest absolute correlation between each variable and any discriminant function

**Table 7. Pooled within-groups correlations between discriminating variables and discriminant functions in case of landmark distances among the samples of three different habitats.**

Landmark distance	Discriminant function	
	1	2
D1	-0.513*	0.391
J2	-0.194*	0.179
A1	-0.014	0.649*
C1	-0.020	0.406*
H1	-0.050	0.387*
F1	0.078	0.351*
B1	-0.200	0.321*
L2	-0.175	0.281*
G1	-0.022	0.240*
M2	-0.046	0.221*
N2	-0.075	0.208*
E1	0.093	0.143*
K2	0.056	0.097*
I2	0.008	-0.051*

\*. Largest absolute correlation between each variable and any discriminant function. Variables ordered by absolute size of correlation within function.

**Table 8. Component loadings of the first two principal components derived from PCA for the morphometric measurements of *T. ilisha* from three different habitats.**

Morphometric characters	Component	
	PC1	PC2
Post dorsal fin length (Post-DFL)	-0.583	0.529
Pre pelvic fin length (Pre-PvFL)	0.543	
Pelvic fin length (PvFL)	0.564	0.563
Pre pectoral fin length (Pre-PcFL)	0.577	
Anal fin length (AFL)	0.788	
Caudal fin length (CPL)	-0.715	
Highest body depth (HBD)	-0.545	0.347
Least body depth (LBD)	0.592	0.549
Eigen-values	3.065	1.106
% of variance	38.31	13.87
Cumulative variance (%)	38.31	52.13

**Table 9. Component loadings of the first two principal components derived from PCA for the morphometric measurements of *T. ilisha*.**

Landmark distance	Component	
	PC1	PC2
A1 (1-2)	0.612	0.502
B1 (2-3)	0.773	-0.317
C1 (3-4)	0.750	
D1 (4-5)	0.747	-0.458
F1 (6-7)	0.615	0.563
H1 (8-1)	0.740	0.393
J2 (2-6)	0.862	-0.377
L2 (3-6)	0.853	
Eigen-values	4.489	1.228
% of variance	56.118	15.352
Cumulative variance (%)	56.118	71.470

**Table 10. Showing classification results of canonical discriminant function based on all morphometric measurement classification results.**

Original	Count	Species	Predicted group membership			
			Coastal	Marine	River	Total
		Coastal	13	3	0	16
		Marine	3	12	1	16
		River	0	0	16	16
	%	Coastal	81.3	18.8	.0	100.0
		Marine	18.8	75.0	6.3	100.0
		River	0.0	0.0	100.0	100.0

**Table 11. Showing classification results of canonical discriminant function based on all truss measurements classification results.**

Original	Count	Species	Predicted group membership			
			Coastal	Marine	River	Total
		Coastal	16	0	0	16
		Marine	1	15	0	16
		River	0	0	16	16
	%	Coastal	100.0	.0	.0	100.0
		Marine	6.3	93.8	.0	100.0
		River	0.0	0.0	100.0	100.0

a. 97.9% of original grouped cases correctly classified.

**Table 12. Univariate statistics (ANOVA) testing differences among samples from 15 morphometric measurements in *T. ilisha* and *T. toli*. Degree of significance were presented as \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).**

Characters	Wilks' Lambda	F value	df1	df2	Sig.
SL	0.998	0.046	1	30	0.831
FL	0.048	595.656	1	30	0.000***
Pre-DFL	0.041	696.546	1	30	0.000***
DFL	0.499	30.098	1	30	0.000***
Post-DFL	0.182	134.921	1	30	0.000***
Pre-PvFL	0.007	3970.950	1	30	0.000***
PvFL	0.030	953.827	1	30	0.000***
Pre-PcFL	0.036	795.143	1	30	0.000***
PcFL	0.285	75.159	1	30	0.000***
Pre-AFL	0.954	1.455	1	30	0.237
AFL	0.165	151.416	1	30	0.000***

CFL	0.871	4.440	1	30	0.044*
CPL	0.637	17.084	1	30	0.000***
HBD	0.989	0.348	1	30	0.559
LBD	0.056	506.605	1	30	0.000***

**Table 13. Univariate statistics (ANOVA) testing differences among samples from 14 truss measurements between *T. ilisha* and *T. toli*. Degree of varying effects were presented as \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.**

Landmark distance	Wilks' Lambda	F value	df1	df2	Sig.
A1	0.211	112.325	1	30	0.000***
B1	0.896	3.482	1	30	0.072
C1	0.247	91.484	1	30	0.000***
D1	0.026	1107.69	1	30	0.000***
E1	0.710	12.277	1	30	0.001**
F1	0.340	58.135	1	30	0.000***
G1	0.221	105.569	1	30	0.000***
H1	0.040	726.502	1	30	0.000***
I2	0.047	610.882	1	30	0.000***
J2	0.148	172.522	1	30	0.000***
K2	0.058	489.426	1	30	0.000***
L2	0.093	293.826	1	30	0.000***
M2	0.259	85.839	1	30	0.000***
N2	0.379	49.081	1	30	0.000***

**Table 14. Component loadings of the first two principal components derived from PCA for the morphometric measurements of *T. ilisha* and *T. toli*.**

Morphometric characters	Component	
	PC1	PC2
Fork Length (FL)	0.968	
Pre-Dorsal fin length (Pre-DFL)	0.981	
Dorsal fin length (DFL)	-0.742	
Post Dorsal fin length (Post-DFL)	-0.901	
Pre-Pelvic fin length (Pre-PvFL)	0.992	
Pelvic fin length (PvFL)	0.973	
Pre pectoral fin length (Pre-PcFL)	0.981	
Pectoral fin length (PcFL)	0.880	
Anal fin length (AFL)	-0.934	
Caudal fin Length (CFL)	-0.331	0.901
Caudal peduncle length (CPL)	-0.656	-0.650
Least body depth (LBD)	0.974	
Eigen-values	9.290	1.417
% of variance	77.421	11.811
Cumulative variance (%)	77.421	89.231

**Table 15. Component loadings of the first two principal components derived from PCA for the truss measurements of *T. ilisha* and *T. toli*.**

Landmark distance	Component	
	PC1	PC2
A1 (1-2)	0.912	
C1 (3-4)	-0.866	0.444
D1 (4-5)	0.950	
E1 (5-6)	-0.660	0.332
F1 (6-7)	-0.692	0.429
G1 (7-8)	-0.936	

H1 (8-1)	0.983	
I2 (2-7)	0.983	
J2 (2-6)	0.907	0.337
K2 (3-7)	0.978	
L2 (3-6)	0.915	0.337
M2 (3-5)	-0.880	0.413
N2 (4-6)	0.788	0.516
Eigen-values	10.218	1.260
% of variance	78.603	9.691
Cumulative variance (%)	78.603	88.294

A

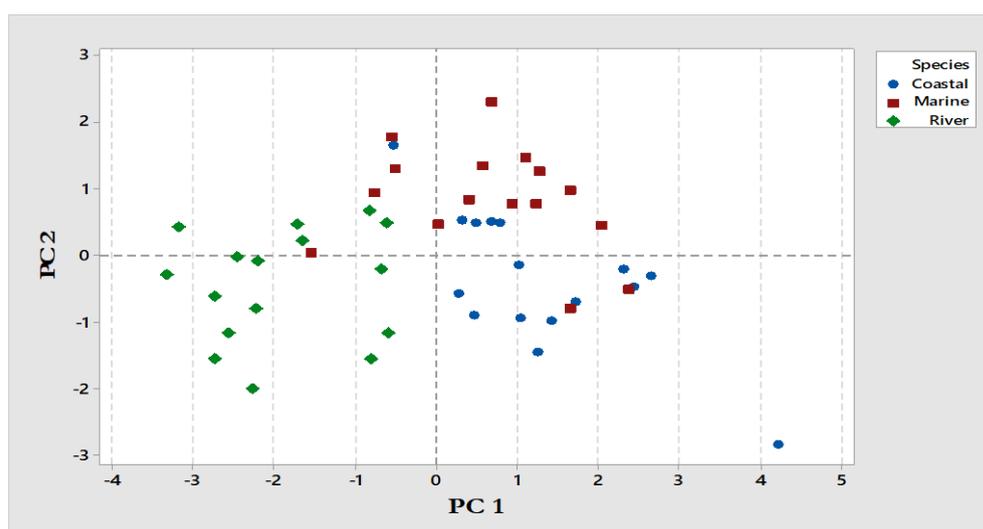


Figure 2A. Principal component analysis (PCA) of morphometric characters of *T. ilisha* collected from three different habitats (coastal, marine and river) of Bangladesh.

B

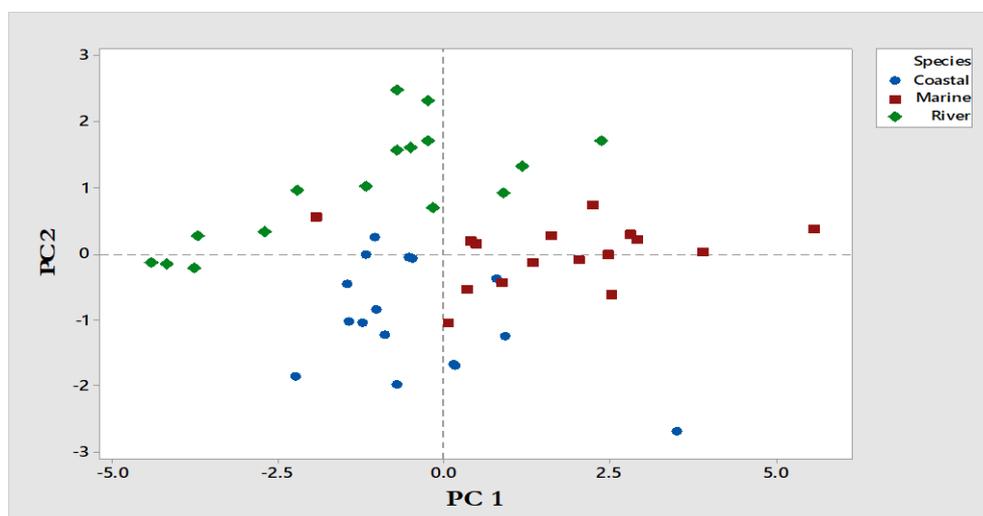


Figure 2B. Principal component analysis (PCA) of truss measurement of *T. ilisha* collected from three different habitats (coastal, marine and river) of Bangladesh.

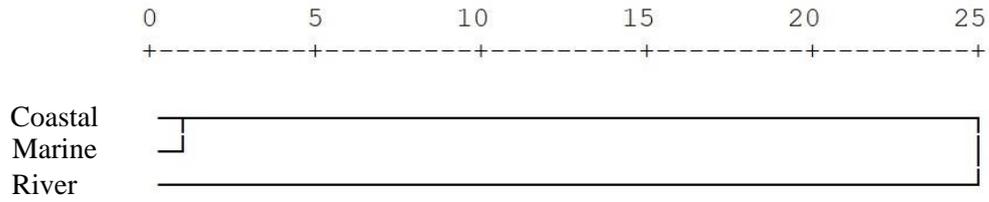


Figure 3. Dendrogram showing the morphometric and landmark distances of *T. ilisha* from three different habitats showed two clusters (one is marine and another one is from river).

A

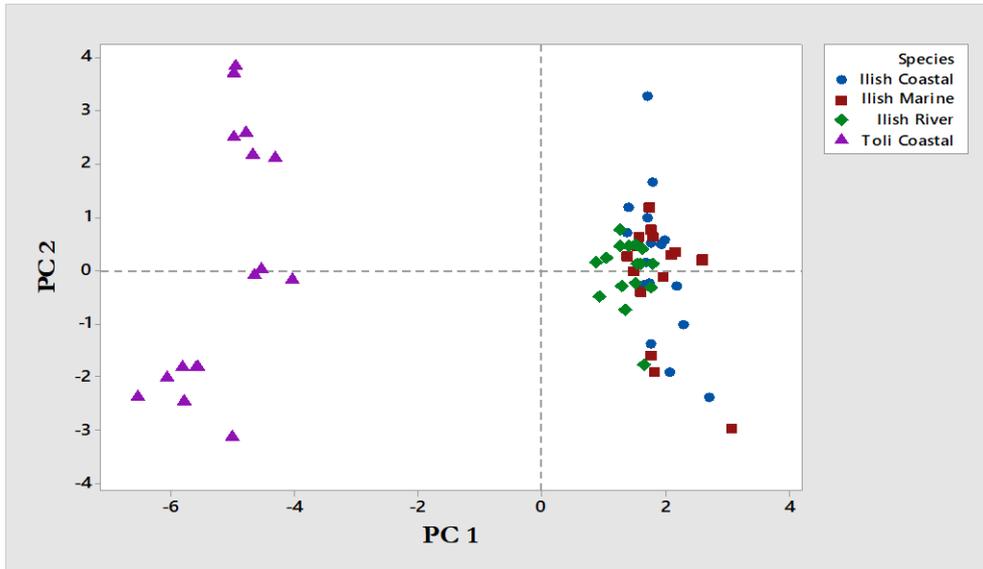


Figure 4A. Principal component analysis (PCA) of morphometric characters of *T. ilisha* and *T. toli* collected from coastal area of Bangladesh.

B

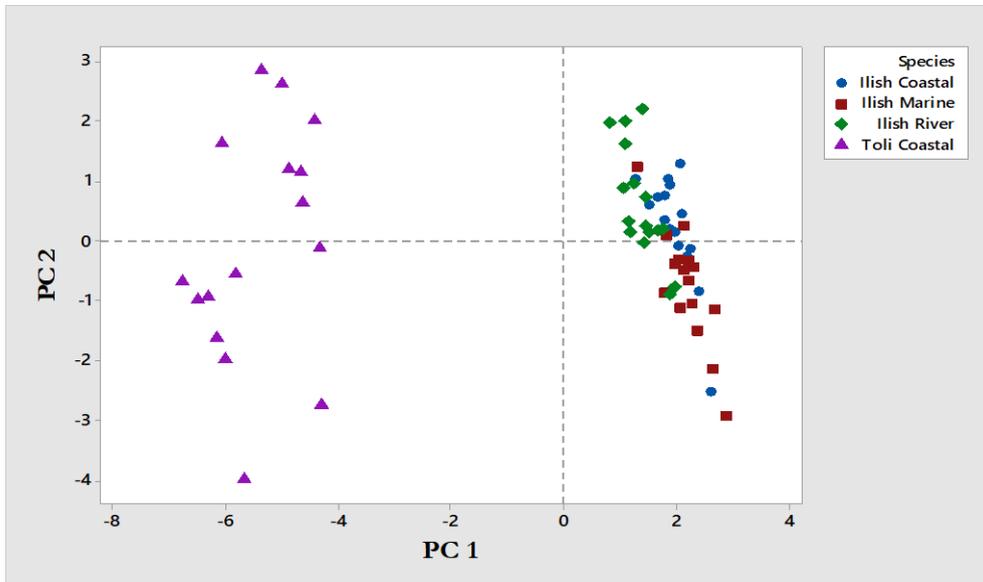


Figure 4B. Principal component analysis (PCA) of truss measurement of *T. ilisha* and *T. toli* collected from coastal area of Bangladesh.

#### 4. Discussion

The phenotypic plasticity is very high in fish (Hossain *et al.*, 2010) and the morphometric and meristic studies provide useful findings to identify the fish stocks (Ihssen *et al.*, 1981). In this experiment, morphometric and meristic characters with truss measurements have been used to analyze the potential differentiation of *Tenualosa* sp. populations collected from different habitats of Bangladesh territory. We apply truss network system as a powerful tool for identifying fish stocks (Turan *et al.*, 2004a). According to Dwivedi and Dubey (2012) the truss network is more implementable and effective strategies for describing the shapes, provides a better way of data collection, enables the data for the application in a diversified manners of analysis in order to discriminate phenotypic stock compared to that of traditional morphometric method because the constellation of the constructed landmarks comprises the entire fish body without losing the information. This method has been also implemented successfully to differentiate and identify stock in many other fish groups including the horse mackerel *Trachurus trachurus* (Murta *et al.*, 2008); Indian major carps (Hossain *et al.*, 2010); mullet (Hossain *et al.*, 2015); catfish (Parvej *et al.*, 2014, Rahman *et al.*, 2014); and gobies (Sabet and Anvarifer, 2013). To elucidate the differences, ANOVA (Analysis of Variance) and DFA (Discriminant Function Analysis) with Principal Component Analysis (PCA) were performed in this experiment. Besides, PCA based on the morphometric measurement of *T. ilisha* from three different habitats showed the value of KMO for overall matrix was 0.685 and the Bartlett's Test of Sphericity also significant ( $p < 0.01$ ). The results of KMO and Bartlett's Sphericity test indicate that the sampled data is appropriate to continue with the factor analysis procedure.

Though no significant difference was observed among the populations of *T. ilisha* from three habitats in case of meristic counts but highly significant variations in morphometric measurements were found among the coastal, marine and riverine populations. ANOVA showed that out of fifteen (15) morphometric measurements, eight (8) morphometric lengths [pelvic fin length (PvFL), anal fin length (AFL), caudal peduncle length (CPL), highest body depth (HBD), least body depth (LBD), post-dorsal fin length (Post-DFL), pre-pectoral fin length (Pre-PcFL), pelvic fin length (PvFL)] were significantly different in varying degrees among these three groups of populations of *T. ilisha* (Table 03). Turan *et al.* (2004a); Hossain *et al.* (2015); Parvej *et al.* (2014); Hossain *et al.* (2010); Rahman *et al.* (2014) also found variations in morphological differences in diverse populations from different habitats in *Liza abu*, *Rhinomugil corsula*, *Eutropiichthys vacha*, *Labeo calbasu* and in *Heteropneustes fossilis* respectively.

The morphological variations found among the populations of *T. ilisha* might be due to their isolated geographical location, high degree of existing environmental variation of their habitats or populations may be originated from different ancestors. Fishes are very responsive to environmental fluctuations and can change their essential morphometrics to adapt with new environmental conditions (Allendorf *et al.*, 1980). It is well known fact that, morphological characteristics can represent high plasticity in response to differences in environmental condition. Therefore, the particular environmental conditions of these habitats may underlie the morphological differentiation among the populations from different locations. Discriminations among six populations of *Capoetacapoeta gracilis* has been reported from the Aras, Sefidrud, Shirud, Tonekabon, Haraz and Gorganrud river systems in Iran (Samaee *et al.*, 2006). Mir *et al.* (2013) reported that the deviations among the *Labeo rohita* stocks of Ganga basin because of unusual hydrological conditions such as differences in pH scale, water flow, temperatures, turbidity and the degree of closeness among the stocks due to their similarities in habitat characteristics and environmental impacts. Dasgupta *et al.* (2014) reported that variation in salinity causes morphometric variation in *Labeo rohita*. Ferrito *et al.* (2007) detailed that, morphological discrimination in several populations were extremely influenced by surrounding habitat characteristics. Morphological variations in *Tenualosa* sp. in this present study may also due to their osmotic physiological variations in three different habitats.

Generally, fishes are subjected to environmentally induce morphological fluctuations and expresses greater variances in external morphological characteristics within and between populations than any other vertebrates (Allendorf *et al.*, 1987; Wimberger, 1992). Due to the higher degree of phenotypic plasticity, fish adjust their physiology and behavior to adapt quickly to environmental alterations which make changes in their morphological structure (Stearns *et al.*, 1983). Therefore, it might be incredible way to detect small morphological differences in fish which are generated due to small environmental fluctuations or physiological adaptations by analyzing only gross morphometric and meristic characters. For this constrains, truss network measurement method was implied in this research. In truss network, eight (1 to 2, 2 to 3, 3 to 4, 4 to 5, 6 to 7, 8 to 1, 2 to 6, 3 to 6) out of 14 distance were significantly different among three populations of *T. ilisha*. Hossain *et al.* (2010) observed significant differences in 4 of 22 truss network measurements in black rohu (*Labeo calbasu*) populations collected from the Jamuna, the Halda and a hatchery in Bangladesh. The significant

differences were also found in 16 of 25 truss measurements in anchovy (*Engraulis crasiolus* L.) in Black, Aegean and Northeastern Mediterranean Sea (Turan *et al.*, 2004b). Parvej *et al.* (2014) found significant differences in 4 of 17 morphometric traits and only 1 of 22 truss network measurements in schilbid catfish (*Eutropiichthys vacha*) populations from Kaptai Lake, Meghna River and Tanguar Haor in Bangladesh.

In this study, characters that were significant at a high level ( $p < 0.05$ ) considered for PCA. To examine the suitability of the data for PCA, Bartlett's Test of Sphericity and the Kaiser–Meyer–Olkin (KMO) measurement was accomplished. The Bartlett's Sphericity tests hypothesized that, the values of the correlation matrix equal zero and the KMO measure of sampling adequacy tests, whether the partial correlation among variables is sufficiently high (Yakubu *et al.*, 2011). The KMO statistics vary between 0 and 1 and the values greater than 0.5 are standard (Yakubu *et al.*, 2011). The morphometric and land-mark distances characters with an Eigenvalue above 1 were included in this analysis. It is noteworthy that, a factor loading more than 0.30 is considered significant, 0.40 is considered more significant, and factor loadings 0.50 or above is considered very significant (Lombarte *et al.*, 2012). In our present study, significant factors considered only those factors with loadings greater than 0.3.

Discriminant function analysis (DFA) could be an appropriate method to differentiate between stocks of the same species, which could provide helpful information in stock management strategies (Karakousis *et al.*, 1991). This discrimination was certified by another multivariate analysis PCA, where pictorial analysis of plotted PC1 and PC2 scores for every specimen was observed in this study. Both DFA and PCA suggested that, the river population of *T. ilisha* have high degree of phenotypic distinction than the coastal and marine populations in case of morphometric and truss measurements. Scatter plotting from PCA endorsed that river populations of *T. ilisha* were isolated from the fish samples of coastal and marine habitats (Figure 2). This inter-population variations may be attained due to separate geographical location as well as the environmental and physiological constrains like salinity, water temperature, turbidity, water pressure, current flow and food availability experienced by each population (Allendorf, 1980; Swain *et al.*, 1991; Wimberger, 1992). Konana *et al.* (2010) applied PCA and demonstrated the notable morphometric variation due to distance and geographical location of rivers the populations of freshwater shrimp *Macrobrachium vollenhovenii* collecting from Côte d'Ivoire Rivers. Paugy and Lévêque (1999) also concluded that populations of same species of different origin from diverse geographical areas were morphologically different. The canonical discriminant functions in DFA showed an overlapped in the coastal and marine stocks of *T. ilisha* whereas the river stocks are totally isolated. In case of morphometric measurement, the first DF accounted for much more (85.4%) of the among group variability than did the second DF (14.6%) and in case of truss measurements the first DF accounted for much more (97.9%) of the among group variability than did the second DF (2.1%). From this both observations, it was obvious that, the second DF explained much less of the variance than did the first DF. Therefore, the second DF was much less informative in explaining differences among the stocks.

The dendrogram that was drawn based on the land-mark distances and morphological examinations among groups of centroids of *T. ilisha* populations collected from three different habitats employed two main clusters: the coastal and marine samples in one and the river groups in another. This demonstrated a high degree of separation of the river population from the marine and coastal habitat. These differences among the habitats might be happened due to environmental as well as genetic variations. A dendrogram based on data of the morphological characters shown in the population of Japanese charr, *Salvelinus leucomaenis* (Nakamura, 2003); Mullet, *Rhinomugil corsula* (Hossain *et al.*, 2015); *Eutropiichthys vacha* (Parvej *et al.*, 2014); *Labeo calbasu* (Hossain *et al.*, 2010) from different habitats revealed separate stocks were possibly due to environmental condition, separate habitat as well as genetic variations.

Besides, this study also demonstrated a comparative morphological difference between *T. ilisha* and *T. tolic* collected from coastal water habitat. We found significant differences in case of morphometric, meristic characters and truss measurements in two species of *Tenulosa*. Meristic characteristics such as dorsal fin rays, pelvic fin rays, pectoral fin rays and caudal fin rays, scales on lateral line, scales on lateral transverse and the number of branchiostegal rays in *T. tolic* were significantly different from the *T. ilisha*.

Twelve (12) morphometric measurement [fork length (FL), pre-dorsal fin length (Pre-DFL), dorsal fin length (DFL), post-dorsal fin length (Post-DFL), pre-pelvic fin length (Pre-PvFL), pelvic fin length (PvFL), pre-pectoral fin length (Pre-PcFL), Pectoral fin length (PcFL), anal fin length (AFL), caudal fin length (CFL), caudal peduncle length (CPL), least body depth (LBD)] out of fifteen(15) morphometric characters and thirteen(13) truss measurements (1 to 2, 3 to 4, 4 to 5, 5 to 6, 6 to 7, 7 to 8, 8 to 1, 2 to 6, 2 to 7, 3 to 5, 3 to 6, 3 to 7, 4 to 6) out of fourteen (14) networking showed significant differences in univariate analysis (ANOVA) between the populations of *T. ilisha* and *T. toli* in varying degrees. The present study has revealed some morphological (i.e., morphometric and meristic) variations between the *T. ilisha* and *T. toli* by using

multivariate techniques as reported for other marine vertebrates and invertebrates also (Bolles and Begg, 2000). The comparative study of two types of palla, (*T. ilisha*) collected from river Indus revealed significant intertype differences in six (6) morphometric measurements (total length, standard length, fork length, head length, eye diameter and girth) and seven (7) meristic characters (total number of scutes, pre pelvic scutes, post pelvic scutes, dorsal fin rays, pectoral fin rays, pelvic fin rays and anal fin rays) reported by Narejo *et al.* (2008).

Wilk's Lambda values were calculated by stepwise discriminant analysis (DFA) and showed greater values in morphometric and truss- networking measurement. The Wilk's Lambda values were found greater than 0.1 in eighteen cases of the total measurement in these two species indicates the higher degree of variations. Yakubu and Okunsebor (2011) showed significant morphological differences between *Oreochromis niloticus* and *Lates niloticus* where they found the values of Wilk's Lambda was greater than 0.1 in most measurement.

In both case of morphometric and land-mark values *T. ilisha* and *T. toli* showed high degree of variations based on the PCA. The PCA with Eigen values >1, shows 89.23% of the total variance. The PC1 and PC2 was 77.42% and 11.81% for the morphometric measurement and the truss measurements revealed 88.29% of the total variance and 78.603% and 9.691% for PC1 and PC2 respectively. This data clearly confirmed the significant differences between these two species. Yakubu and Okunsebor (2011) found morphometric difference between two Nigerian fish species (*Oreochromis niloticus* and *Lates niloticus*) using PCA and DFA. Moreover, scatter plotting from PCA revealed that, *T. toli* also exhibits higher degree of variations from the marine and river habitat of *T. ilisha* in case of both morphometric characters and truss measurements (Figure 04). Pillay *et al.* (1962) reported two separate populations of *T. ilisha* population from studying in rivers and coastal areas in India. Gosh *et al.* (1968) identified three varieties of *T. ilisha*, denoted as sub-populations (slender, broad and broader) from a part of the Gangetic system between Allahabad and Buxar. While Quddus *et al.* (1984) reported meristic and morphometric difference and comparison of age and growth of two types of *T. ilisha* from Bangladesh waters. From the above demonstration it is clearly revealed that, the river populations of *T. ilisha* is morphologically identical than the coastal or marine populations and the *T. toli* is also far more different from the *T. ilisha*.

## 5. Conclusions

Fish plays a central role in dietary patterns, livelihoods and culture in Bangladesh. Fish is by far, the most consumed animal-source food across all population groups around the globe. But this sector is facing an enormous threat due to overfishing, habitat degradation, pollution, indiscriminate use of agrochemicals, introduction of exotic species, lack of suitable habitat, decreased fecundity and so on. The Hilsha fish, called 'Ilish' in Bengali, is of national importance to us. But increased request due to its taste and nutritional profile has led to pressure on the fish species. To fulfill the demand of its increasing pressure sustainable and efficient stock management is necessary. So, we have to save this species from being threatened or extinct. This study has provided important morphological information that can be used to differentiate *Tenuulosa* sp. with more accuracy among groups and species and affords elementary information about the variation of *Tenuulosa* populations in different water habitats of Bangladesh. The outcomes of the study would serve as primary information of the stock management and enable efficient management strategies for the distinct stocks of *Tenuulosa* sp. populations in order to develop sustainable fishery and appropriate conservation plans in near future. The authors hope that the information obtained from the present study will be helpful for fisheries, biologists, and taxonomist concerned with these two fascinating fishes.

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## Conflict of interest

None to declare.

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