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Landmark-based morphometric and meristic variations of stinging catfish, *Heteropneustes fossilis* (Bloch) among three isolated stocks, the Old Brahmaputra river and the Tanguar haor and a hatchery

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ABSTRACT
The experiment was conducted by observing the landmark-based morphometric and meristic variations to evaluate the population status of three different stocks of stinging catfish or shing, *Heteropnuestes fossilis.* A total of 150 *H. fossilis* were collected from the Old Brahmaputra river, Mymensingh; the Tanguar haor, Sunamganj and a hatchery, Mymensingh during August to September 2011. Six meristic, sixteen morphometric characters and twenty three truss distances were measured. Significant differences were found in three meristic counts ($P<0.05$). But morphometric and truss network measurements after plotting discriminant functions revealed high segregation among three different stocks of *H. fossilis.* Discriminant Function (DF) analysis of seven morphometric measurements of pre-dorsal length (PrDL), anal fin length (AFL), highest body depth (HBD), post-orbital length (PsOL), maximum barbel length (BLmx), mouth gap (MG) and peduncle length (PdnL) were contributed dominantly to the first DF and the rest of the characters of post-dorsal length (PsDL), pre-orbital length (PrOL), head length (HL), standard length (SL), lowest body depth (LBD), minimum barbel length (BLmn), caudal fin length (CFL) and eye length (EL) contributed to the second DF. The twenty three truss measurements, six were leading to the first DF and the rest seventeen contributed to the second DF. The dendrogram showed two major clusters: the Brahmaputra and Tanguar stocks in one cluster and hatchery stock in another cluster. There was a high degree of variation in morphological characteristics among three different stocks of *H. fossilis*.

Keywords: *Heteropneustes fossilis*, meristic and morphometric variations, truss measurement, Discriminant Function Analysis.

1. Introduction

Bangladesh is a blessed with aquatic resources as a land of rivers. The country has most productive freshwater fisheries comprising 6,27,731 ha of closed and 40,24,934 ha of open water body [1]. Freshwater fisheries are very rich in species diversity (ranked as 3rd in position in the south Asia) having more than 260 indigenous fish species [1]. In spite of having vast water resources, per capita annual fish intake only 17.52 kg against the annual requirement of 20.44 kg [1]. This deficient amount (2.92 kg) should be fulfilled through aquaculture production.

Heteropneustes fossilis belongs to the order Siluriformes; a diverse group of ray-finned fish commonly known as stinging catfish is under the family Heteropneustidae, an indigenous species of Indian subcontinent [2] and locally called shing fish. This fish is highly tasty, nutritious, easily digestible and quickly assimilable into the body. It is widely distributed in lentic freshwaters and rarely in brackish waters throughout Bangladesh, India, Pakistan, Nepal, Sri Lanka, Myanmar, Thailand and Laos [3]. It is an omnivorous, demersal and predatory in nature. Generally, during the dry season, *H. fossilis* lives in semi-liquid and semi-dry mud and even when the mud dries up due to having an air sac as an accessory respiratory organ; it takes its body above the bottom of fissures and crevices. It is capable of breeding in the onset of the monsoons in ponds, ditches, beels, canals, haors, baors and rivers when sufficient rain-water accumulates [4]. The production of *H. fossilis* is being decreased gradually from natural water bodies due to different harmful effects. If it would continue for several years, once upon a time, it will become endangered towards to extinct. However, it is a matter of hope that several environmental management programs (fish sanctuary, fishing rules etc.), breeding and culture techniques of *H. fossilis* are successfully being conducted throughout the country [5] to revive the natural stocks. Faster growth rate, easy cultivability and high market price of *H. fossilis* by using supplemental feeds make its popularity to adopt in culture by all fish farmers. All these have drawn attention what is existing status of *H. fossilis* in our country for further research and culture.

Information on the biology and population structure of any species is a prerequisite for developing management and conservation strategies [6] and may be applicable for studying short-term and environmentally induced variations. Morphometric differences among stocks of a species are recognized as important for evaluating the population structure and as a basis for identifying stocks [7, 8, 9, 10]. Morphometric and meristic characters of fish are the measurable and countable characters, respectively common to all fishes. Landmarks refer to some arbitrarily selected points on a fish's body and with the help of these points, the individual fish body shape can be analyzed. In other words, a landmark is a point of correspondence on an object that matches between and within populations [11, 12]. Truss network systems constructed with the help of landmark points are powerful tools for stock identification. A sufficient degree of isolation may result in notable morphological, meristic and shape differentiation among stocks of a species which may be recognizable as a basis for identifying the stocks. The characteristics may be more applicable for studying short-term, environmentally induced disparities and the findings can be effectively used for improved fisheries management [13, 14, 15, 7, 16]. Therefore, it is inevitable to know the morphological, morphometric and meristic variations among the Old Brahmaputra river and the Tanguar haor and a hatchery as a preliminary base line information. There is possibly no information regarding the phenotypical variations of hatchery, the old Brahmaputra river and the Tanguar haor source of *H. fossilis* and there may be few attempts to evaluate the population structure based on genetic aspects. The present study deals with the population structure from a phenotypical point of view to determine the morphometrics among three stocks of *H. fossilis*: the old Brahmaputra river, Tanguar haor and a hatchery source.

2. Materials and methods

2.1 Collection of samples

During August-September 2011, a total of 150 shing, *H. fossilis* were collected live in oxygenated polythene bags from three sources: the Old Brahmaputra river and the Tanguar haor, Sunamganj and Rupali Hatchery Ltd., Khagdahor, Mymensingh and kept temporarily in fiberglass tank at the Wet Laboratory Complex, adjacent to the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. The fish were measured as 18.77-23.07 cm in total length (TL) by a measuring scale and 30-150 g in weight by a sensitive portable electric balance (METTLER TOLEDO, Switzerland). The sample size, mean length and weight were presented in Table 1. The fish was handled with the help of a small piece of cloth and water was sucked finely by blotting paper. Morphometric data were collected by using "Truss network system". Data points were arranged in "trusses" around the fish, a layout which maximizes the number of measurements and increases the sensitivity of the analysis were shown in Fig. 2. In total, 6 meristic characters: branchiostegal rays (BSR), dorsal fin rays (DFR), pectoral fin rays (PFR), ventral fin rays (VFR), anal fin rays (AFR) and caudal fin rays (CFR) were analyzed. All the meristic counts were set up against incoming light direction in the room with the help of needle and small pins for easy counting. Eleven landmarks delineating 23 distances were measured on the body (Fig. 2). Each landmark was obtained by placing a fish on graph paper and then the landmarks were detected with colored pointers for enabling accurate and consistent measurements. Finally, the distances on the graph paper were measured using vernier calipers.

Table 1: Average length (cm) and weight (g) of the samples collected from different sources

Group name	Abbrev.	Sample size	TL (M±SE)	W (M±SE)
Hatchery stock	HS	50	10.79±2.32	6.00±2.27
Old brahmaputra river, Mymensingh	BS	50	18.77±1.04	31.00±4.11
Tanguar haor, Sunamganj	TS	50	24.07±1.67	78.70±13.56

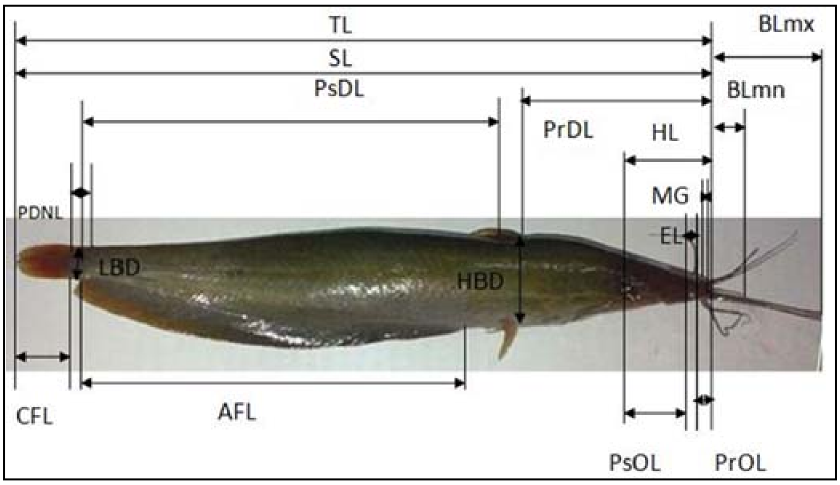


Fig 1: Morphometric measurements of *H. fossilis*

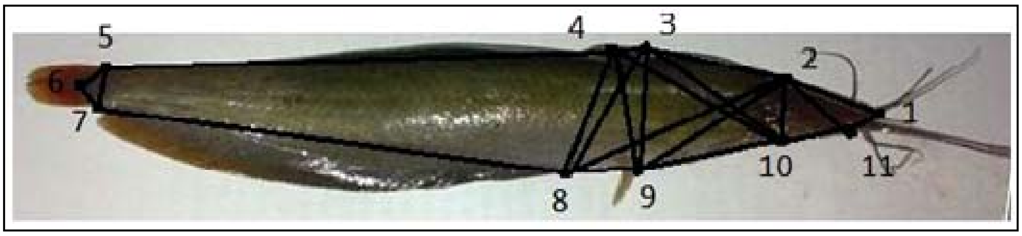


Fig 2: Locations of the 11 landmarks for constructing the truss network on *H. fossilis* illustrated as black dots and morphometric distance measures between the dots as lines.

2.2 Statistical analyses

Prior to analysis, the size effects from the data set were eliminated. Variations were attributed to body shape differences and not to the relative sizes of the fish. In the present study, there were significant linear correlations among all the measured characters and the total length (TL) of the fish. Therefore, it was necessary to remove size-dependent variations from all of the characters. An allometric formula given by [17] with slight modification was used to remove the size effect from the data set:

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

Where M is the original measurement, M_{adj} is the size-adjusted measurement, L_o is the TL of the fish and L_s is the overall mean of the TL for all fish from all samples. 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 stocks. The efficiency of the size adjustment transformations was assessed by testing the significance of the correlation between a transformed variable and the TL. A univariate analysis of variance (ANOVA) was carried out to test the significance of morphological differences. Meristic characters were compared using the non-parametric Kruskal-Wallis test.

In addition, size-adjusted data were standardized and submitted to a discriminant function (DF) analysis. A dendrogram of the populations based on the morphometric and landmark distance data was drawn using the Squared Euclidean Dissimilarity Distance Method. All statistical analyses were done using SPSS software package version 11.5 [18].

3 Results

3.1 Meristic counts

Six Meristic counts with their median values for all samples were shown in Table 2. Among the correlation measurements, DFR was correlated with PFR and AFR with significant difference (P<0.05) and highly correlated with VFR (P<0.01). PFR is highly correlated with VFR and CFR (P<0.01). There were no significant differences among mean number of BSR, pectoral fin rays, caudal fin rays and differences were occurred only in other characters (ANOVA test, df= 2, dorsal fin rays: F_{st}= 7.258, P<0.05; ventral fin rays: F_{st}= 5.256, P<0.05 and anal fin rays: F_{st}=4.355, P<0.05). In the Kruskal-Wallis (H) test, similar results were found (P>0.05) and df= 2, dorsal fin ray: H= 7.258, P<0.05; ventral fin rays: H= 5.256, P<0.05 and anal fin rays: H=4.355, P<0.05.

Table 2: Meristic counts (median) of shing (*H. fossilis*) of different stocks (in the parenthesis indicates minimum and maximum counts)

Characters	Hatchery (Me, mn-mx)	Brahmaputra river (Me, mn-mx)	Tanguar haor (Me, mn-mx)
BSR	15(12-16)	13(12-17)	13(12-15)
DFR	6(5-7)	6(6-7)	7(6-8)
PFR	6(4-8)	6(6-7)	7(5-8)
VFR	5(4-6)	6(5-7)	6(5-6)
AFR	66(55-74)	73(66-83)	74(66-82)
CFR	15.5(15-22)	18(15-23)	16.5 (11-19)

3.2 Morphometric and landmark distances

The efficiency of the allometric formula in removing the size effect from the data was justified by using correlations between the total length and adjusted characters. Among the 15 transformed morphometric and 23 truss measurements, none of them were found to be significantly correlated (P>0.05). Hence, all the measurements were considered for further calculation. A univariate analysis of sixteen morphometric characters shown that all morphometric measurements and truss measurements were significantly differed to varying degrees (P<0.05 and, or P<0.001) Table 3 and Table 4, respectively. The discriminant function (DF) analysis produced 2 DFs (the 1st and 2nd DF) for both morphometric and landmark measurements. For morphometric and landmark measurements, the 1st DF accounted for 83.9%, 91.6% and the 2nd DF accounted for 16.1%, 8.4%, respectively among group variability and mutually they explained 100% of the total among-group variability. All morphometric stocks were different from each other in the discriminant space (Fig. 3, canonical graph) and virtually no overlapping. The canonical graph of hatchery stocks (1), then the Old Brahmaputra river (2) and finally the Tanguar haor (3) were sequentially distributed in cluster-form around their centroid values. In case of all truss measurements, the stocks were differed from each other in the discriminant space (Fig. 4, canonical graph). In the Fig. 3, the canonical graph of hatchery (1) then the old Brahmaputra river (2) and the Tanguar haor (3) were not sequentially distributed. Pooled within group correlations between discriminant variables and DFs revealed that among fifteen morphometric measurements: seven measurements of pre-dorsal length (PrDL), anal fin length (AFL), highest body depth (HBD), post-orbital length (PsOL), maximum barbel length (BLmx), mouth gap (MG) and peduncle length (PdnL) dominantly contributed to the first DF and the rest eight characters: post dorsal length (PsDL), pre-orbital length (PrOL), head length (HL), standard length (SL), lowest body depth (LBD), minimum barbel length (BLmn), caudal fin length (CFL), eye length (EL) contributed to the second DF (Table 5). Among twenty three truss measurements; six measurements of dist 9-10, dist 2-3, dist 10-11, dist 2-11, dist 1-2, and dist 1-11 dominantly contributed to the first DF and the rest seventeen contributed to the second DF (Table 6). A dendrogram (Fig. 4) based on morphometric and landmark distance data was drawn for the stocks of hatchery, the Old Brahmaputra river source, the Tanguar haor. The Squared Euclidean Dissimilarity method distance was close among the Tanguar haor and the Old Brahmaputra stocks and the distance for hatchery stock was more deviated.

Table 3: Univariate statistics (ANOVA) showed that all sixteen morphometric measurements were significantly different among sample in varying degrees

S. No.	Characters	Wilks' Lambda	F	df1	df2	Sig.
1	TL	0.064	196.955	2	27	0.000*
2	SL	0.200	54.006	2	27	0.000*
3	HBD	0.180	61.601	2	27	0.000*
4	LBD	0.336	26.698	2	27	0.000*
5	HL	0.093	132.441	2	27	0.000*
6	EL	0.663	6.867	2	27	0.004*
7	PrOL	0.260	38.459	2	27	0.000*
8	PsOL	0.299	31.640	2	27	0.000*
9	PrDL	0.040	324.711	2	27	0.000*
10	PsDL	0.086	143.705	2	27	0.000*
11	PdnL	0.555	10.819	2	27	0.000*
12	BLmx	0.184	59.965	2	27	0.000*
13	BLmn	0.327	27.842	2	27	0.000*
14	AFL	0.091	134.548	2	27	0.000*
15	CFL	0.171	65.453	2	27	0.000*
16	MG	0.196	55.216	2	27	0.000*

**P*<0.001

Table 4: Univariate statistics (ANOVA) showed that all twenty three truss measurements were significantly different among samples in varying degrees

Distances	Wilks' Lambda	F	df1	df2	Sig.
dist 1-2	0.088	139.540	2	27	0.000*
dist 2-3	0.063	200.241	2	27	0.000*
dist 3-4	0.086	142.696	2	27	0.000*
dist 4-5	0.054	236.533	2	27	0.000*
dist 5-6	0.148	77.566	2	27	0.000*
dist 6-7	0.204	52.729	2	27	0.000*
dist 7-8	0.061	207.117	2	27	0.000*
dist 8-9	0.054	236.709	2	27	0.000*
dist 9-10	0.056	228.472	2	27	0.000*
dist10-11	0.089	138.708	2	27	0.000*
dist 1-11	0.236	43.649	2	27	0.000*
dist 2-11	0.079	158.474	2	27	0.000*
dist 2-10	0.046	281.120	2	27	0.000*
dist 2-9	0.052	248.025	2	27	0.000*
dist 2-8	0.044	291.293	2	27	0.000*
dist 3-10	0.036	357.992	2	27	0.000*
dist 3-9	0.045	285.208	2	27	0.000*
dist 3-8	0.047	276.210	2	27	0.000*
dist 4-10	0.044	294.121	2	27	0.000*
dist 4-9	0.057	224.046	2	27	0.000*
dist 4-8	0.040	319.989	2	27	0.000*
dist 4-7	0.044	291.414	2	27	0.000*
dist 5-7	0.280	34.633	2	27	0.000*

* P<0.001

Table 5: Pooled within group correlations between discriminating variable and standardized canonical discriminant functions (variables ordered by absolute size of correlation within function. * denotes the largest absolute correlation between each variable and discriminant functions)

Characters	DF1	DF2
PrDL	0.697(*)	0.475
AFL	0.533(*)	0.381
HBD	0.386(*)	0.197
PsOL	0.360(*)	0.194
BLmx	0.313(*)	-0.003
MG	0.299(*)	0.052
PdnL	0.073(*)	0.064
PsDL	0.440	0.460(*)
PrOL	0.143	0.448(*)
HL	0.338	0.357(*)
SL	0.262	0.319(*)
LBD	0.158	0.311(*)
BLmn	0.210	0.275(*)
CFL	0.223	0.250(*)
EL	0.203	0.242(*)

Table 6: 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)

Characters	DF1	DF2
dist 9-10	-0.524(*)	0.291
dist 2-3	-0.470(*)	0.274
dist 10-11	0.366(*)	0.210
dist 2-11	0.299(*)	0.280
dist 1-2	0.284(*)	0.261
dist 1-11	0.211(*)	0.073
dist 3-10	-0.048	0.388(*)
dist 4-8	-0.029	0.372(*)
dist 2-10	0.014	0.362(*)
dist 4-10	-0.031	0.361(*)
dist 4-7	0.127	0.349(*)
dist 3-8	-0.033	0.348(*)
dist 3-9	-0.040	0.344(*)
dist 2-8	-0.047	0.340(*)
dist 4-5	0.138	0.338(*)
dist 4-9	-0.019	0.321(*)
dist 7-8	0.041	0.315(*)
dist 8-9	-0.062	0.284(*)
dist 2-9	-0.171	0.281(*)
dist 3-4	0.025	0.262(*)
dist 5-6	0.012	0.127(*)
dist 5-7	0.034	0.058(*)
dist 6-7	0.031	0.035(*)

* P<0.01

4. Discussion

In the present study, meristic counts of all samples varies from 6-8 rays for the DFR, 6-7 rays for the VFR, 55-83 rays for the AFR. The similar results were found for *H. fossilis* [4]. The mean numbers of DFR, VFR and AFR were significantly different ($P<0.05$) among the stocks. The mean numbers of meristic counts were significantly differed ($P<0.05$) among 3 stocks (the Halda, the Jamuna and a hatchery) of kalibaush reported by [19]. Meristic counts in Japanese charr, *Salvelinus leucomaenis* among the river systems (the Naka and the Tone rivers, central Japan) and the tributaries of the Naka River (Ashinagasawa, Akasawa, Ushirosawa and Moto-okashirasawa streams) were significantly different [20]. In this study, significant morphological variations were found among the hatchery, Tanguar haor and the Old Brahmaputra stocks of *H. fossilis*. These phenotypic discreteness due to the habitat condition, their feeding strategies as well as separate geographical location. The similar results were found for anchovy, *Engraulis encrasicolus* in the Black, Aegean and northeastern Mediterranean Seas [8]. Morphometric differences among stocks are expected, because they are geographically separated and may have originated from different ancestors. Thus, it is not unlikely that obvious environmental variations exist among 3 stocks of *H. fossilis*. Fish are very sensitive to environmental changes and quickly adapt themselves by changing necessary morphometrics. It is well-known that morphological characters can show high plasticity in response to differences in environmental conditions, such as food abundance and temperature [21, 22, 23].

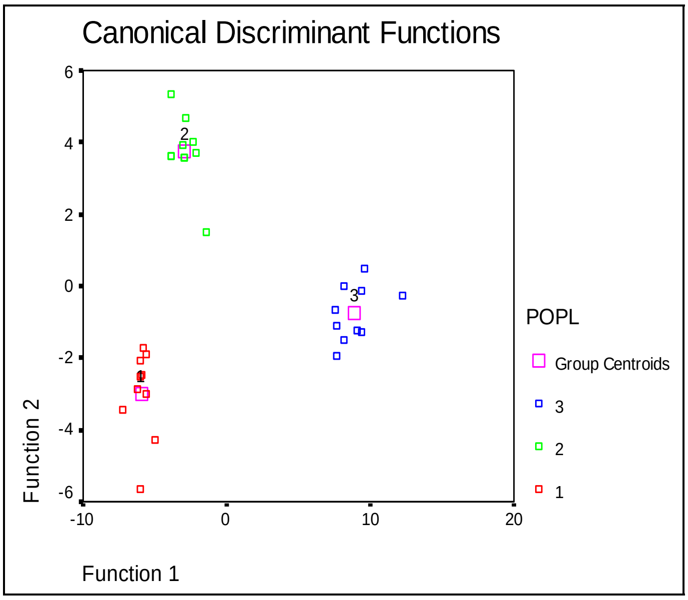


Fig 3: Sample centroids of discriminant function scores based on morphometric measurements. Samples referred to, 1. Hatchery stock, 2. Old Brahmaputra river stock, 3. Tanguar haor stock**.**

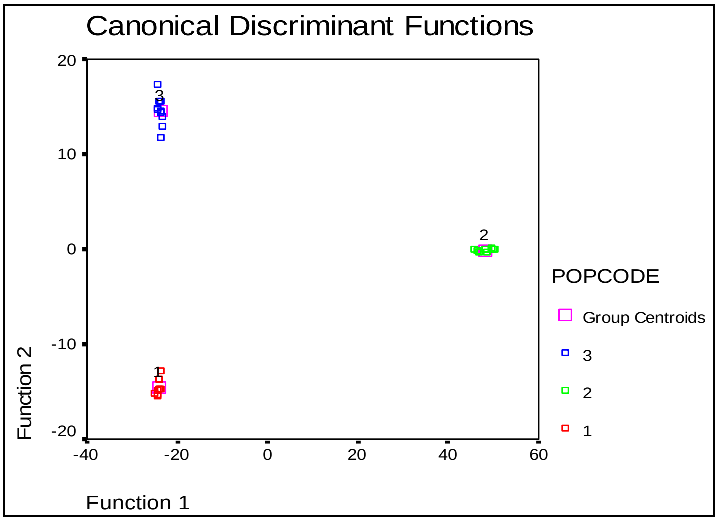
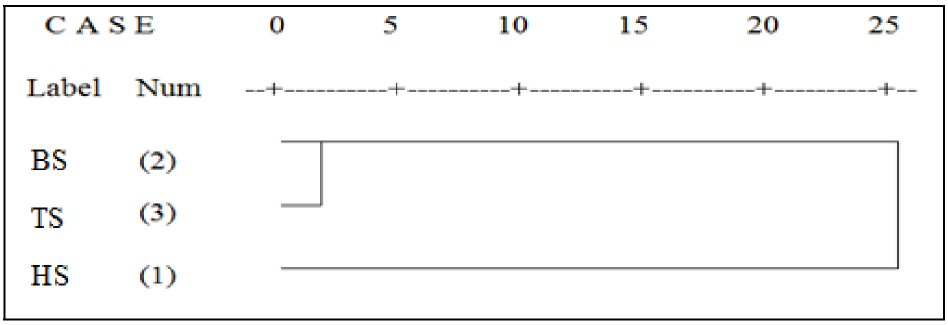


Fig 4: Sample centroids of discriminant function scores based on truss measurements. Samples referred to, 1. Hatchery stock, 2. Old Brahmaputra river stock, 3. Tanguar haor stock



Distance of Squared Euclidean Dissimilarity

Fig 5: Dendrogram based on morphometric characters and landmark distances of three groups: 1, Hatchery stock (HS); 2, Old Brahmaputra river stock (BS) and 3, Tanguar haor stock (TS).

In general, fish demonstrate greater variances in morphological traits both within and between populations than any other vertebrates and are more susceptible to environmentally induced morphological variations [21, 23]. The phenotypic plasticity of fish is very high. They adapt quickly by modifying their physiology and behavior to environmental changes. These modifications ultimately change their morphology [24]. In a small country like Bangladesh, there are probably very small environmental changes from place to place. In spite of this, the hatchery population possesses a unique environment that differs from the Old Brahmaputra River and the Tanguar haor of Bangladesh. However, due to small environmental differences the resulting morphological differences in fish may be so small that they might be impossible to discern with gross morphomeristic characters. Therefore, truss network measurements were employed in this experiment. Truss network systems are a powerful tool for identifying stocks of fish species [8]. An unbiased network of morphometric measurements over a 2 dimensional outline of a fish removes the need to find the types of characters and optimal number of characters for stock separation and provides information over the entire fish form [8]. The truss network system can effectively be used to distinguish between the hatchery and wild stocks. In this case, more-significant differences were expected because of the 2 completely different habitats i.e., an open-water habitat and the other was closed water. Environmentally induced phenotypic variations however, may have advantages in the stock structure analysis of exploited species, especially when the time is insufficient for significant genetic differentiation to accumulate among populations. Genetic markers might not be sufficient to detect existing genetic variation among populations and also only a small proportion of DNA is analyzed by genetic markers.

Relationships among the 3 stocks differed according to whether the 1st and 2nd DF was considered (Fig. 3 and Fig. 4). In the Fig 4 (1st DF), the hatchery stock displayed intermediate characteristics between the Brahmaputra and the Tanguar stocks of *H. fossilis*. As the hatchery owners use the fish as a brood collected from the wild source (open water) in the country, the hatchery stock was shown some proximity to river stocks and haor stocks. In this experiment, DF analysis determined the dissimilarity among the stocks and significant correlations were observed between size and truss measurement characteristics among three stocks of *H. fossilis*. In the Fig. 3, the 1st DF accounted for much more (83.9%) of the among group variability than did the 2nd DF (16.1%). In the Fig. 4, the 1st DF accounted for much more (91.6%) of the among group variability than did the 2nd DF (8.4%). In both Fig. 3 and Fig. 4, it was obvious that the 2nd DF explained much less of the variance than did the 1st DF. The 2nd DF was therefore, much less informative in explaining differences among the stocks. The dendrogram employed in this study resulted in 2 clusters: the Old Brahmaputra river and the Tanguar haor stocks in one and hatchery stock in another (Fig. 4). The differences between the hatchery and river and haor stocks may have been due to environmental as well as genetic variations. Plotting DFs (Fig. 3 and Fig. 4) revealed high isolation in morphometrics among the three stocks. The Jamuna and the Halda stocks were very close, while the hatchery stock clearly differed by DF analysis based on the truss measurements reported by [19]. Based on the 2nd DF, the Jamuna and the Halda stocks, however, broadly overlapped, while the hatchery stock clearly differed. The 1st DF accounted for much more (75.5%) of the among group variability than did the 2nd DF (24.5%). It was obvious that the 2nd DF explained much less of the variance than did the 1st DF. The dendrogram employed by [19] in his study resulted in 2 clusters: the Jamuna and hatchery stocks of *L. calbasu* in one and the Halda stock of *L. calbasu* in another. They concluded that the morphological difference between the hatchery and wild stocks were possibly due to environmental condition, separate habitat as well as genetic variations. A dendrogram based on data of the meristic and morphometric characters shown that the population of Japanese charr, *Salvelinus leucomaenis* of the Tone River was included within the variation detected among the tributary populations of the Naka River reported by [20]. Meristic and morphometric characters of Japanese charr, *Salvelinus leucomaenis* varied not only between river systems but also among tributaries within a river system were possibly due to environmental condition, separate habitat as well as genetic variations. In their study DFs shown isolation in morphology among the stocks of different sources.

The results of this study are useful as preliminary baseline information of *H. fossilis* populations for further studies. More research especially genetic studies and investigations of the impacts of environmental factors is needed for conservation and mass seed production of this indigenous and commercially important aquaculture species, *H. fossilis* to save from being extinction.

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