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Stock Identification of Critically Endangered Olive Barb, *Puntius sarana* (Hamilton, 1822) with Emphasis on Management Implications

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Abstract

The study was carried out to investigate the stock identification of the olive barb, *Puntius sarana* (Hamilton, 1822) through morphometric characters. A total of 110 sample ranging from 10.00-16.80 cm in total length (LT) and 13.94-63.46 g in body weight (BW) were examined to assess the morphometric variation of *Puntius sarana* from four mighty rivers; the Padma, Meghna, Jamuna and the Halda in Bangladesh. The univariate result showed significantly variation (p<0.05) in seven morphometric characters out of 23 characters among the populations. The discriminant analysis revealed a morphological segregation among the studied populations based on the characters of length of anal base (YZ) and pre-dorsal length (LM). Discriminant function analysis (DFA) showed 55.0% of the individuals were correctly classified into the four regions on the basis of morphological characters. The first principal component (PC I) analysis elucidated 51.56% of total variation whereas PC II and PC III were 10.72% and 8.28%, respectively. The dendrogram was drawn by using morphometric data showed that the Meghna and Halda population make one cluster and the Jamuna and the Padma population form another cluster and the distance between the Padma and Meghna river population were the highest. The canonical graph also revealed all population of the Meghna and Halda were highly overlapped compare to others. The results of the present study would help monitoring the species status in Bangladesh as a bid to take appropriate management measures for its wide geographical distribution.

Keywords: Stock structure; Critically endangered; Conservation; *Puntius sarana*

Introduction

Bangladesh is endowed with vast fisheries resources having ranked third in Asia after China and India [1,2]. However, the country's huge fisheries resources are dominated by 3 major river systems: the Padma, Jamuna, and Meghna. The Halda is another important river in Bangladesh which is geographically isolated from the 3 major river systems but globally recognized as a maiden breeding ground of freshwater fishes [3,4]. There are 260 freshwater fishes in Bangladesh under 145 genera and 55 families, of which 150 species (58%) have been categorised as small indigenous species (SIS) in Bangladesh [4]. *Puntius sarana* is an important component of SIS belongs to the family Cyprinidae commonly known as 'Olive barb' is categorized as critically endangered in Bangladesh [5-7] and vulnerable in India according to conservation status [8]. This species is a delicious and nutritious food item in South Asian countries including Bangladesh, Bhutan, India and Nepal due to rich lipoprotein content and soft bony structure [8,9].

Once *Puntius sarana* was available almost all around the year in ponds, lakes, ditches, floodplains, streams, coastal waters, estuaries, and rivers such as the Padma, Jamuna, Halda, Meghna, and also reported in the Gangetic river system of India and Bangladesh [10,11]. But this species is drastically declined in these water bodies over the years and currently it is on the verge of extinction [5,6,8]. There is an ever declining tendency in this fishery in recent years due to apparent deterioration of the habitat, over-exploitation and indeed lack of proper management [12-15]. The increasing water pollution and destruction of breeding grounds for various reasons also restricted the natural breeding of *Puntius sarana* [11]. In the present situation, it is very crucial to detect the reason of decline and understanding the ecology of the species with a view to managing the species efficiently. As a fishery management tool, morphological characters on fish are crucial from various points of view including evolution, ecology, behavior, conservation, water resource management, and stock assessment and identification [16]. The present study was therefore carried out to identify the stock structure of *Puntius sarana* in four rivers, the Padma, Jamuna, Meghna and Halda based on morphometric characters in Bangladesh.

Materials and Methods

Study area and sampling

A total of 110 samples were collected from the four important rivers in Bangladesh from November, 2013 to September, 2014 (Table 1 and Figure 1). The samples were caught by using the traditional fishing gears like cast net and conical trap. The specimens were moved to the Faculty of Fisheries, Patuakhali Science and Technology University, Bangladesh where all morphometric characteristics were observed following Froese and Pauly [17] method. Digital slide calipers (up to the nearest 0.1 cm)

	Population	Collection site (District)	No. of fish	Date of collection
01	Jamuna river	Sonatala (Bogra)	29	20.11.13
02	Padma river	Pangsha (Rajbari)	26	01.09.14
03	Meghna river	Araihazar (Narayangong)	30	10.09.14
04	Halda river	Hathazari (Chittagong)	25	20.09.14

Table 1: Sources, number of species and date of collection of *Puntius sarana* population from four rivers in Bangladesh.

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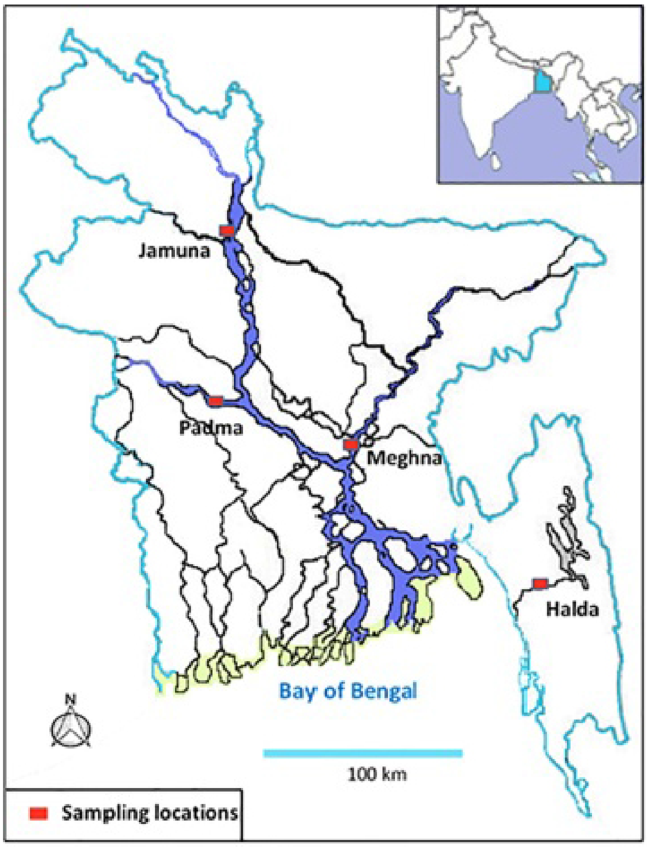

Figure 1: Map of Bangladesh showing the sampling site of four rivers; the Jamuna, Padma, Meghna and Halda.

were used to measure and an electric balance (up to the nearest 0.1 g) was used to weigh the specimen.

Statistical analysis

The morphometric characters of *Puntius sarana* were analyzed by different statistical methods. Univariate and multivariate statistics were used for analyzing character differences among different sources of samples. Most of the variability in a set of multivariate characters is due to size [18]. Thus, shape analysis should be free from the effect of size to avoid misinterpretation of the results [19]. In order to eliminate any variation resulting from allometric growth, all morphometric measurements were standardized according to Elliott et al. [20].

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

Where,

"M" is the original morphometric measurement,

"M_{adj}" is the size-adjusted measurement,

"L_o" is the total length of fish, and

"L_s" is the overall mean of total length for all fish from all samples for each variable.

"b" is the constant value of the equation

The parameter *b* was estimated for each character from the observed data as the slope of the regression of log M against log Lo, using all specimens. Multivariate post hoc Tukey tests were employed to examine the statistical significant differences among mean value of morphometric characters. Discriminant analyses were then applied using stepwise insertion of variables to the size-adjusted traits to test for group membership and to identify discrimination among the populations within the different regions. Then Principal component analysis (PCA) was performed using the same morphological traits because discriminate analysis define the overall morphological variation within the regions [21]. However PCA helps in deduction data dimensions to a few principal components (PC) with identification of meaningful variables based on a combination of the original traits. Varimax rotation was selected for PCA as the rotation minimizes the number of variables with high loading factor. Kaiser [22] recommends that the value of KMO (Kaiser–Meyer–Olkin) less than 0·500 indicates the inadequacy statistic in a PCA for factor analysis. The Wilks' lamda test was performed to compare the differences between and among all groups. Cluster analysis were also applied to determine further the morphometric distances among the individuals of the two groups as a complement to discriminant analysis [23] by adopting the Euclidean distance as a measure of dissimilarity and the UPGMA (unweighted pair group method with arithmetical average) as the clustering algorithm. All statistical analyses were performed using SPSS v16 and SYSTAT v10.

Results

There were twenty three morphometric characters observed from the samples of four river population of *Puntius sarana* (Table 2 and Figure 2). Among which the LT, LF, B↓, LI, EH, IE, SnL, P↓, V↓, A↓, MN, WX, UZ, UL and UJ didn't show significant variation from each other while the head depth (K) and lowest body depth (B) of the Padma, Meghna and Halda river population revealed significant variation compare to the Jamuna river population. The head length (LH) of Jamuna, Padma

Characters	Jamuna	Padma	Meghna	Halda
LT	13.97 ± 0.31^{a}	13.8 ± 0.51^{a}	14.89 ± 0.28^{a}	14.12 ± 0.39^{a}
LS	10.99 ± 0.28^{ab}	10.67 ± 0.40^{b}	11.74 ± 0.20^{a}	11.02 ± 0.38^{ab}
LF	12.24 ± 0.26^{a}	12.14 ± 0.44^{a}	13.01 ± 0.23^{a}	12.33 ± 0.35^{a}
LH	2.32 ± 0.06^{b}	2.47 ± 0.11^{b}	2.83 ± 0.06^{a}	2.55 ± 0.08^{b}
K↓	2.88 ± 0.20^{a}	2.27 ± 0.10^{b}	2.47 ± 0.09^{b}	2.27 ± 0.07^{b}
M↓	4.21 ± 0.10^{a}	4.01 ± 0.22^{a}	4.31 ± 0.15^{a}	4.10 ± 0.12^{a}
B↓	1.68 ± 0.05^{ab}	1.52 ± 0.06^{b}	1.77 ± 0.08^{a}	1.61 ± 0.06^{ab}
LI	0.52 ± 0.02^{a}	0.50 ± 0.01^{a}	0.55 ± 0.02^{a}	0.54 ± 0.01^{a}
EH	1.25 ± 0.05^{a}	1.22 ± 0.05^{a}	1.32 ± 0.06^{a}	1.23 ± 0.02^{a}
IE	0.73 ± 0.02^{a}	0.72 ± 0.04^{a}	0.77 ± 0.06^{a}	0.70 ± 0.01^{a}
SnL	0.85 ± 0.04^{a}	0.78 ± 0.03^{a}	0.79 ± 0.03^{a}	0.78 ± 0.02^{a}
LM	5.32 ± 0.10^{b}	5.25 ± 0.25^{b}	5.85 ± 0.12^{a}	5.39 ± 0.15^{ab}
NS	3.87 ± 0.09^{a}	3.45 ± 0.15^{b}	3.44 ± 0.18^{b}	3.31 ± 0.14^{b}
D↓	2.31 ± 0.07^{b}	2.43 ± 0.08^{b}	2.69 ± 0.08^{a}	2.54 ± 0.09^{ab}
P↓	2.18 ± 0.08^{a}	2.12 ± 0.08^{a}	2.25 ± 0.06^{a}	2.07 ± 0.08^{a}
V↓	1.93 ± 0.06^{a}	1.83 ± 0.07^{a}	1.95 ± 0.05^{a}	1.85 ± 0.07^{a}
A↓	1.67 ± 0.06^{a}	1.69 ± 0.07^{a}	1.79 ± 0.09^{a}	1.69 ± 0.05^{a}
MN	1.88 ± 0.07^{a}	1.77 ± 0.07^{a}	1.96 ± 0.06^{a}	1.76 ± 0.08^{a}
QR	0.46 ± 0.04^{b}	0.53 ± 0.02^{a}	0.59 ± 0.02^{a}	0.56 ± 0.02^{a}
WX	0.59 ± 0.02^{a}	0.61 ± 0.02^{a}	0.61 ± 0.08^{a}	0.59 ± 0.03^{a}
YZ	1.17 ± 0.05^{a}	1.07 ± 0.06a	1.21 ± 0.06^{a}	1.18 ± 03^{a}
JL	0.91 ± 0.02^{a}	0.81 ± 0.04^{a}	0.81 ± 0.04^{a}	0.76 ± 0.03^{a}
JU	0.77 ± 0.03^{a}	0.73 ± 0.03^{a}	0.70 ± 0.03^{a}	0.66 ± 0.03^{a}

Values are mean ± standard error. Mean values in each row bearing different superscripts are significantly different ($P<0.05$).

Table 2: Morphometric characters of *Puntius sarana* population observed in the Jamuna, Padma, Meghna and Halda rivers of Bangladesh.

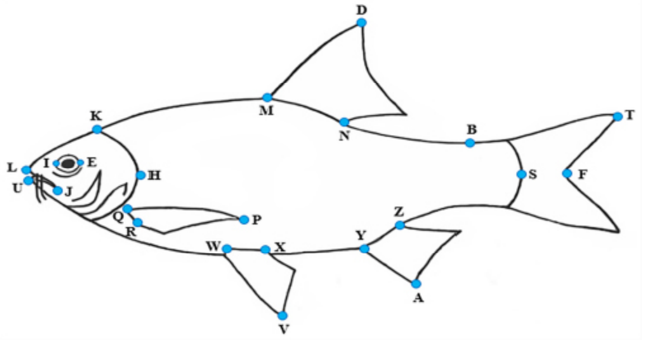

Figure 2: Morphometric measurements of *Puntius sarana.* Total length (LT); Fork length (LF); Standard length (LS); Head length (LH); Head depth ($K_{\downarrow}$); Highest body depth ($M_{\downarrow}$); Lowest body depth ($B_{\downarrow}$); Pre-orbital length (LI); Post-orbital length (EH); Eye diameter (IE); Pre-dorsal length (LM); Post-dorsal length (NS); Height of dorsal fin (D); Height of pectoral fin (P); Height of ventral fin (V); Height of anal fin (A); Length of dorsal base (MN); Length of pectoral base (QR); Length of ventral base (WX); Length of anal base (YZ); Upper jaw length (JL); Lower jaw length (JU).

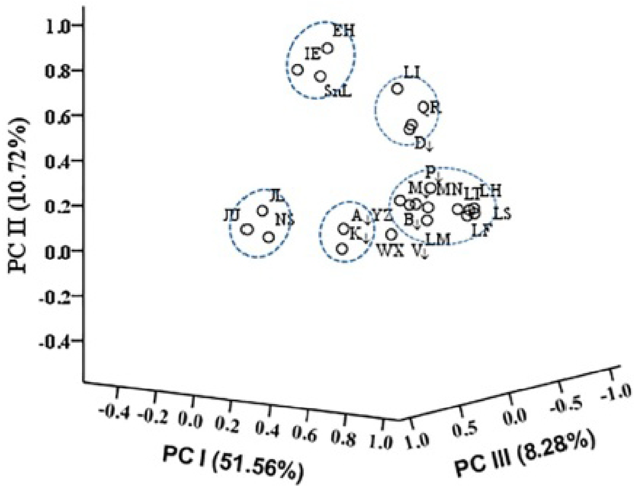

Figure 3: Principal component analysis (PCA) for 23 morphometric characters of *Puntius sarana* collected from four rivers, Bangladesh.

Group	LT:LS	$M_{\downarrow}$: $B_{\downarrow}$	LH:IE	LT: M	LT: $B_{\downarrow}$	TL:LH	LS:LH
Jamuna river	1.27^{ab}	2.33^{a}	3.23^{b}	3.32^{a}	8.37^{b}	6.06^{a}	4.76^{a}
Padma river	1.29^{a}	2.15^{a}	3.51^{ab}	3.52^{a}	9.12^{a}	5.62^{b}	4.34^{b}
Meghna river	1.28^{b}	2.43^{a}	3.89^{a}	3.48^{a}	8.58^{ab}	5.27^{c}	4.16^{b}
Halda river	1.29^{ab}	2.32^{a}	3.65^{ab}	3.45^{a}	8.83^{ab}	5.56^{bc}	4.33^{b}
Vertically, letters a, b and c show statistically significant differences ($p < 0.05$) among the rivers.							

Table 3: Different morphometric proportions of *Puntius sarana* population on four rivers of Bangladesh.

and Halda river population showed significant variation than Meghna river population. During multivariate analysis, an inadequate sample size is a common problem with many fish morphology studies [24]. Many authors performed theoretical works for decades on PCA and DFA recommended that the ratio of N: P (N-number of organisms measured and P-parameters included in the analysis) should be at least 3-3.5 [25]. In the present study, 7 characters were retained and the N:P ratio was 8.57 for all 7 morphometric measurements under these circumstance, suggesting adequate sample size for this study. The contributions of the variables to principle components (PC) were examined to determine the most effective morphometric measurement that discriminates the populations. Bartlett's Test of sphericity and the Kaiser-Meyer-Olkin (KMO) measure was executed to observe the fitness of the data for PCA. Generally the KMO statistics fluctuates between 0 and 1 [24] but Kaiser [22] recommends that values greater than 0.5 are acceptable. The value of KMO between 0.5 and 0.7 are mediocre, between 0.7 and 0.8 are good, and between 0.8 and 0.9 are superb [26]. In this study, the obtained value of KMO for the overall matrix is 0.83 and the Bartlett's Test of sphericity is significant ($P<0.01$). The results (KMO and Bartlett's) estimated from the present study suggest that the sampled data are appropriate to proceed with a factor analysis procedure.

Principal component analysis of 23 morphometric measurements extracted three factors with eigen-values > 1, explaining 70.57% of the variance (Figure 3). The first principal component (PC1) accounted for 51.56% of the variation, second (PC2), third (PC3) for 10.72% and 8.28% respectively. The most significant loadings on PC1 were LT, LF, LS, LH, K↓, M↓, B↓, LI, EH, IE, SnL, LM and NS, JL were on PC II and EH, IE, SnL were on PC III, respectively. Nimalathasan [27] worthy mentioned that factor loading greater than 0.30 is considered significant, 0.40 more important, and 0.50 or greater is very significant. For parsimony, only those factors were considered significant in this study having loadings above 0.50. Visual examination of plots of PC I, PC II and PC III scores showed the most similar loadings were YZ, P↓, B↓, M↓, V↓, LT, LF, LH, LS, WX, and LM (Figure 3).

Different proportions of morphometric characteristics (LT:LS, M↓:B↓, LH:IE, LT:M↓, LT: B↓, LT:LH, LS:LH) of *Puntius sarana* are given in Table 3. There was no significant difference ($p>0.05$) observed in the ratio of M↓: B↓ and LT: M↓ through all four river populations while the ratio of standard length and head length (LS:LH) of Jamuna population was significantly higher ($p<0.05$) than other three river populations (Table 3). Univariate statistics (ANOVA) revealed that 7 morphometric characters such as head length (LH), head depth (K↓), lowest body depth (B↓), post dorsal length (EH), height of dorsal fin (D↓), length of pectoral base (QR) and upper jaw length (UL) of 23 morphometric measurements significantly differed to varying degrees ($p < 0.05$, $p < 0.01$ or $P< 0.001$) among samples (Table 4).

A dendrogram was drawn for all population in four rivers based on morphometric characteristics (Figure 4). The Meghna river populations were nearest to Halda river population compare to two others (Jamuna and Halda) and the Jamuna river population were more adjacent to Padma river population than others (Meghna and Halda) according to the distance of Squared Euclidean Dissimilarity.

Three discriminate function (DF1, DF2 and DF3) produced based on morphometric characters during the analysis of discriminant function. The DF1 was accounted for 83.2%, the DF2 was accounted for 12% and the DF3 was accounted for 4.8% of among groups variability,

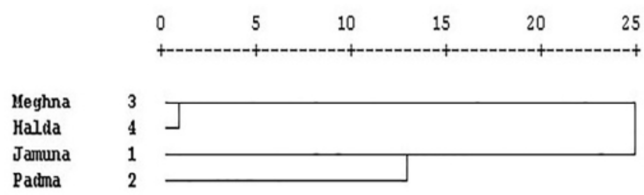

Figure 4: Dendrogram based on morphometric characters of *Puntius sarana* collected from four rivers, Bangladesh.

Characters	Wilks' Lambda	F	df1	df2	Sig.
LT	0.92	1.55	3	56	0.211
LS	0.91	1.95	3	56	0.131
LF	0.93	1.39	3	56	0.255
LH	0.73	7.00	3	56	0.000***
$K_{\downarrow}$	0.78	5.13	3	56	0.003**
$M_{\downarrow}$	0.96	0.73	3	56	0.537
$B_{\downarrow}$	0.87	2.83	3	56	0.047*
LI	0.91	1.78	3	56	0.162
EH	0.96	0.83	3	56	0.483
IE	0.97	0.64	3	56	0.592
SnL	0.94	1.15	3	56	0.335
LM	0.87	2.72	3	56	0.053
NS	0.87	2.86	3	56	0.045*
$D_{\downarrow}$	0.81	4.15	3	56	0.010*
$P_{\downarrow}$	0.94	1.12	3	56	0.350
$V_{\downarrow}$	0.95	0.99	3	56	0.401
$A_{\downarrow}$	0.97	0.57	3	56	0.637
MN	0.90	2.01	3	56	0.123
QR	0.78	5.18	3	56	0.003**
WX	0.99	0.28	3	56	0.844
YZ	0.92	1.62	3	56	0.195
JL	0.84	3.47	3	56	0.022*
JU	0.89	2.33	3	56	0.084

Table 4: Univariate statistics (ANOVA) of morphometric characters of *Puntius sarana* observed in four river of Bangladesh.

explaining 100% of total among group variability as per morphometric measurements. The original grouped case classification in the discriminate function analysis have a high degree of correct classification with 55.0% of all fish being assigned to the correct population. Wilk's lamda tests of discriminant analysis indicated significant differences in first function (Wilk's lambda = 0.411, χ^2 = 49.77, d.f. = 6, P <0·001) of morphometric characters of all populations except the second function (Wilk's lambda = 0.897, χ^2 = 6.06, d.f. = 2, P > 0.05), which were non-significant. There was some intermingling relationship found among all populations and the populations were not separated. The diagram depicts that Meghna were highly dispersed in their morphological parameter compare to other groups of Jamuna, Padma and Halda. The Meghna and Halda river populations were highly overlapped than the Padma and Jamuna river population (Figure 5). The canonical graph for four river populations which was sequentially distributed in cluster form around their centroid value. This recommends that morphological growth trend of them more or less similar.

Discussion

The *Puntius sarana* is one of the most commercially important barb species having great potential for aquaculture not only in Bangladesh but all Southeast Asian countries as well [8,9]. In the recent years, there has been a growing concern about the conservation and sustainable management of this species. Therefore, study on basic biological aspects of *Puntius sarana* is crucial to impose adequate regulations for sustainable fishery management and conservation for this species. In the present study, the morphometric characters of all river population were somewhat similar but some significant variations were observed in HD, HBD, HL (Table 2). A similar study was conducted by Saroniya et al. [28] for different *Puntius* sp and found variation in HD, HBD among all populations of this species. These variation may have been due to the geography, ecology, human activities, genetic diversity and

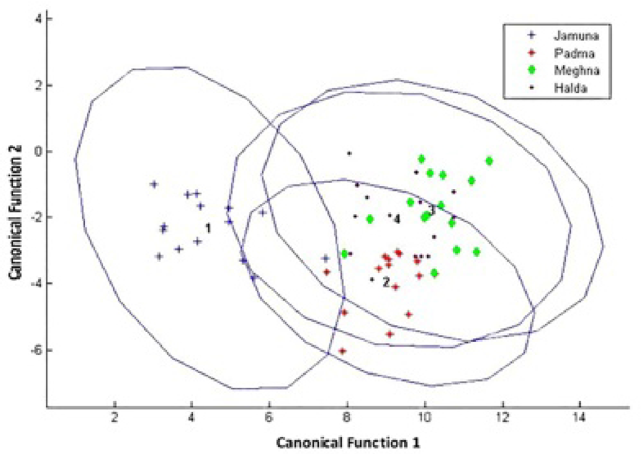

Figure 5: Coordinate plot of two discriminant functions from morphometric measurement of the four river populations of *Puntius sarana*. Samples referred to 1-Jamuna river, 2-Padma river, 3-Meghna river and 4-Halda river population.

experimental error of the population. There are also several studies have conducted on morphometric measurements and meristic counts on different fishes and found variations due to the geography, ecology and human activities [29-31]. However, morphometric variation of all river populations are expected because the specimens were collected from geographically separated location, may have originated from different ancestors and may be due to their adaptation capacity. Fish are very sensitive to environmental changes and adapt quickly by modifying their physiology and behavior to environmental changes [32]. Environmental changes (such as food abundance and temperature) directly affect the fish and the fish quickly change necessary morphometric in order to adapt themselves [19,24,32]. The total length and standard length of *Puntius sarana* populations were 1.27, 3.3-3.5, 8.3-9.1, 5.5-6.0 and 4.2-4.7 times respectively higher than SL, HBD, LBD and HL; and the highest body depth was 2.4-2.6 times higher than the lowest body depth. Again, the head length was 3.2-3.7 times higher than the eye diameter. A similar type of study was conducted by Hossain et al. [11] found that the total length was 5.5-6.0 and 4.0-5.0 times higher than head length and high body depth, respectively for *Puntius pangasius* populations. Moreover, the present findings agree with those of Schreck and Moyle [33] and Kurata [34] who reported that environmental factors (water temperature, pH etc.) considerably affect the morphology of fishes.

The dendrogram which are used in this study resulted in 2 clusters: the Meghna and Halda stocks in one and the Jamuna and Padma stock in another (Figure 4). The Meghna river population had high nearness with Meghna and the Jamuna river with Padma river. This similarity may be due to same genetic structure, environmental and geographical location. Considering DF1, the Jamuna population exhibited some similar characteristics among the Padma, Meghna and Halda river population. The Meghna and Halda river population are broadly overlapped, while the Payra river population clearly differed based on the DF2. In the present investigation, 55.0% of individuals were correctly classified into their respective groups by DFA, indicating intermingling among some the populations. DFA was applied by Turan et al. [35] on the anchovy (*Engraulis encrasicolus*) from different areas of the Mediterranean Sea, and found significant heterogeneity among different populations based on morphometric characters. Chaklader et al. [30] applied DFA for three populations of *Polynemus paradiseus* from the three coastal rivers and reported that environmental parameters and local migration of the fish were influenced the morphological discrimination among them.

The DFA segregation was partly confirmed by PCA, where the graphs of PCA scores for each sample revealed that, among four populations, some features showed overlapping and others were clearly distinct. Among five groups, one group revealed similar loadings of YZ, P↓, B↓, M↓, V LT, LF, LH, LS, WX, LM in four river population (Figure 3), indicated that these may be contributed by geographical location and environmental condition. Hossain et al. [36] applied PCA on *Labeo calbasu* collected from the Jamuna and Halda rivers as well as a hatchery, reported environmental factors and local migration of the fish attributing the morphological discrimination of fish.

Conclusion

The results indicated that *Puntius sarana* still has morphometric heterogeneity among the population from four different rivers of Bangladesh. The observation given in the present study can further be confirmed based on molecular and biochemical methods. The present study contributes baseline biological information that is expected to be helpful in facilitating the development of management strategies in relation to the fishery and conservation of *Puntius sarana* populations in selected rivers. In order to ameliorate the current situation and sustain the fishery stock of *Puntius sarana* in the selected four rivers, it is recommended to:

- Adopt separate management strategies to sustain the stocks from year after year.
- Reduce exploitation rates by implementing appropriate mesh size and gear selectivity to catch the optimum length which gives the maximum possible yield.
- Provide appropriate amenities to allow young individuals to reach the marketable size.
- Implement fisheries and conservation act, imposition ban on breeding time of fish species to sustain this resource for the future use.

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