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Landmark-Based Morphometric and Meristic Variations of the Critically Endangered Catfish, *Eutropiichthys vacha* from Three Different Populations in Bangladesh

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Abstract: Variation in shape, morphometric & meristic characters of critically endangered catfish Bacha, *Eutropiichthys vacha*, populations from Kaptai Lake, Meghna River &TanguarHaor (wetland ecosystem) were investigated. A total of twenty six fishes (eight from the Meghnariver, eight from KaptaiLake & ten from Tanguarhaor) samples (as this species is rarely found) were collected from March to November, 2012. Eighteen morphometric & four meristic characters were analyzed along with twenty two truss network measurements. Non parametric Kruskal-Wallis test wasused to compare meristic countsand no significant differences were observed among populations. ANOVA showed significant morphometric differences ($p<0.001$) in three (Pectoral fin Length, Snout length, & Eye Diameter) of seventeen morphometric measurements among the populations. None of the twenty two truss network measurements among the populations was significantly different ($p>0.001$). For morphometric & landmark measurements, the first discriminant function (DF) accounted for 97.7% & 99.7% & the second DF accounted for 2.3% & 0.3%, respectively, among-group variability and together they explained 100% of the total among group variability. For the morphometric & truss network measurements, plotting discriminant functions revealed high isolation of the populations. The dendrogram based on morphometric &landmarks distances placed Tanguarhaor&Kaptai Lake in one cluster & the Meghna River in another cluster. Due to similar environmental conditions & less geographical differences all the landmark of truss measurements did not contribute to significant body shape difference among the populations. These all analyses will be an effective tool to describe body shape variation of a fish species.

Key words: Landmark · Critically Endangered · Population · *Eutropiichthys vacha*

INTRODUCTION

Bacha, *Eutropiichthys vacha* [1] is a teleost fish species distributed in Bangladesh, Pakistan, India, Nepal, Myanmar & Thailand. In Bangladesh this species is normally found in the Meghna River, Tanguar *Haor* & Kaptai Lake and some other tidal rivers & lakes. During the 1960's; the species was quite abundant in all the rivers of Bangladesh [2]. But due to overfishing, breeding ground destruction, dam construction and for some other man made causes this species seriously decreased in nature. It is declared as critically endangered in the Red List of IUCN Bangladesh [3]. That's why necessary steps should be taken as early as possible to keep safe and identify pure stock of bacha from the list of extinct.

Meristic & morphometric characters are very much popular to identify different fish races and/or populations [4-8]. But this method is old enough to identify pure stock of fishes. Truss measurements constructed with the help of landmark pointsalong with the measurement of

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morphometric and meristic character are powerful tools which can be used for the stock identification, elucidate relationship among populations and to separate physically similar species of bacha as well as other species too [9, 10]. A landmark can be defined as a point of correspondence of object that matches between and within populations. Nahar *et al*., Begum *et al.* Cheng *et al.* & Hossain *et al.* [9-12] emphasized on the validity of geometric protocol for character selection, the truss network of morphometric characters which enforces systematic coverage of the form and which exhaustively and redundantly archives the landmark configuration.

To sustain the purity and developing breeding program for a sustainable production along with to prevent from extinction it is very necessary to identify the gene pool and best stock from nature of this endangered species. Therefore land-mark based morphometric and meristic analysis was conducted to identify the genetically diversified *Bacha* populations so that recommendationscan be given to minimize the level of threat on this endangered species.

MATERIALS AND METHODS

Collection of Samples: During March to November, 2012, 26 bacha (*E. vacha)* were collected from three different sources Kaptai Lake in Rangamati District, the Meghna River in Laxmipur District, & Tanguar *Haor* in Sunamganj District. The fish were 18.6-28.4 cm in total length(TL) & 36-152 g in weight. The specimens were transported in ice box to the laboratory, where measurements started immediately to avoid shrinkage. Eighteen morphometric measurements were made on each specimen (Table 1). The body parts were measured following standard anatomical reference points [13, 14].

Four Meristic Characters Were Studied: Dorsal fin rays (DFR); pectoral fin rays (PcFR); pelvic fin rays (PvFR); anal fin rays (AFR) were estimated for this study. A magnifying glass was used to count the fin rays & only the principal rays were counted as separate ray.

For measurement of Landmarks distances of the species the truss network system described for fish body morphometric [9, 10] was used to construct a network on fish body. Eleven landmarks determining twenty two distances were produced and measured as illustrated in Figure 1. Data points were arranged in “trusses” around the fish a layout which maximizes the number of measurements & increases the sensitivity of the analysis [15]. Each landmark was obtained from the distances on the graph paper which were measured using Vernier calipers.

Statistical Analysis: One-way analysis of variance (ANOVA) was performed for the comparison of the morphometric data. A multivariate discriminant analysis was used for morphometric data to identify the combination of variables that best separate *E. vacha* species. But before analysis size effects as because most of other variability will occur and that’s why it was

Table 1: Morphometric characters used for analysis of *E. vacha*population variations

SL.No.	Character	Description
1	Total length (TL)	Distance from the tip of the snout to the longest caudal fin ray
2	Fork length (FL)	Distance from the tip of the snout to the middle part of the fork of the tail
3	Standard length (SL)	Distance from the tip of the snout to the end of the vertebral column
4	Head length (HL)	Distance from the tip of the snout to the posterior margin of the opercula
5	Eye Diameter (ED)	Diameter of the eye
6	Pre Dorsal Distance (PDD)	Distance from the tip of the Mouth to the dorsal fin base
7	Pre Pectoral Distance (PPD)	Distance from the mouth tip to Pectoral fin base
8	Pre Ventral Distance (PVD)	Distance from the mouth tip to pelvic fin base
9	Pre Anal Distance (PAD)	Distance from the mouth tip to anal fin base
10	Dorsal fin length (DFL)	Length of the base of dorsal fin
11	Pectoral fin length (PcFL)	Length of the base of pectoral fin
12	Anal fin length (AFL)	Length of the base of anal fin
13	Snout Length (SnL)	Length from mouth tip to eye
14	Inter orbital Length (IoL)	Distance between two eyes
15	Dorsal Spine Length (DSL)	Length of dorsal Spine
16	Pectoral Spine Length (PcSL)	Length of pectoral spine
17	Body Depth (BD)	The vertical distance between the dorsal & ventral margins of the body
18	Head Width (HW)	The distance between the two widest points of the head

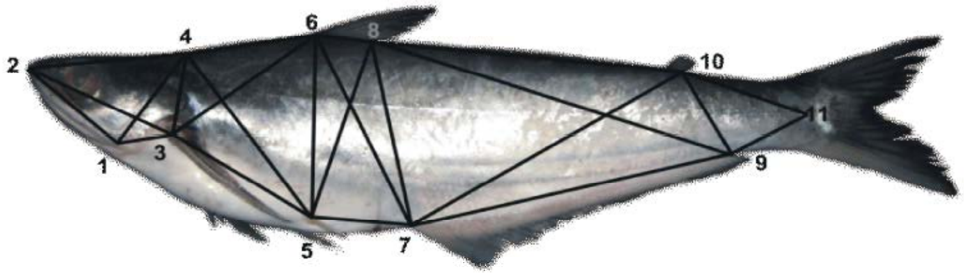

Fig. 1: Locations of 11 landmarks points used for the shape analysis of *E. vacha*

necessary to remove size-dependent variation for all the characters. Using Elliott *et al.* [16] allometric formula variations resulting from allometric growth were eliminated and data were standardized. The formula is given below:

$M_{adj} = M (L_S / L_0)^b$

where M: Original measurement, M_{adj}: Size adjusted measurement, L_o: Total length of fish, & L_s: Overall mean of total length 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 groups. The degree of similarity among samples in the overall analysis & relative importance of each measurement for group separation were assessed by discriminant function analysis (DFA) with cross validation. Population centroids with 95% confidence ellipses derived from the DFA were used to visualize relationships among the individuals of groups. Comparison of meristic characters was done using non parametric Kruskal-Wallis test. All statistical analyses were done using SPSS v 11.5 (SPSS, Chicago, IL, USA).

RESULTS

Meristic Counts: Meristic counts of 26 samples from three different populations showed that there were 8 dorsal fin rays, 14 pectoral fin rays, 6 pelvic fin rays in each sample where anal fin rays ranged from 46-48 (m_e= 47) showed difference among the samples.

In this experiment, meristic counts were compared among three populations (Kaptai Lake, the Meghna River & Tanguar *Haor*). As the number of dorsal, pectoral, & pelvic fin rays showed similar values among the samples from three different populations, so these data were not analyzed. Only anal fin rays showed difference among the samples and was analyzed. The mean number of anal fin rays were not different among fish from three different populations (Kruskal-Wallis test, $p>0.05$).

Morphometric & Landmarks Distance: Among the seventeen transformed morphometric measurements none showed significant correlation with total length. Univariate statistics (ANOVA) showed that four (Pre Pectoral Distance-PPD, Pectoral fin length-PcFL, Snout Length-SnL, Eye Diameter-ED) of 17 measurements were significantly different among samples in varying degrees (Table 2). Univariate statistics (ANOVA) showed that in truss network measurements, among the twenty two transformed measurements only one (3 to 4) measurement was significantly different at $p<0.01$ level&anothertwenty one measurements showed no significant differences (Table 3).

Discriminant function analysis produced two discriminant functions (DF1 & DF2) for both morphometric & landmarks measurements. For both morphometric & landmarks measurements the first DF accounted for 97.7% & 99.7% & the second DF accounted for 2.3% &0.3% respectively, among group variability, explaining 100% of total among groups variability. All the samples were clearly separated from each other in the discriminant space (Fig. 2) with virtually no overlapping. This suggested that there was no intermingling among populations & the populations were separated. In case of morphometric measurements all the samples were clearly separated from each other in the discriminant space.

In truss network system all the populations were also clearly separated from each other in the discriminant space (Figure 3).

Pooled within-groups correlations between discriminant variables & DFs revealed that among the morphometric measurements – pectoral spine length (PcSL), Pre-Pectoral Distance (PPD), & Pre-ventral Distance (PVD) dominantly contributed to first DF & the other fourteen contributed to the second DF (Table 4).

In case of trussmeasurements, among the twenty two measurements first threemeasurements - 3 to 4, 1 to 3, & 1 to 2 dominantlycontributed to first DF & the restnineteen measurements contributed to the second DF (Table 5).

Table 2: Univariate statistics (ANOVA) testing differences among samples from seventeen (17) morphometric measurements

Characters	Wilks' Lambda	F	Sig.
FL	.962	.458	.638
SL	.997	.036	.964
PDD	.984	.186	.832
DFL	.933	.829	.449
DSL	.967	.395	.678
PPD	.693	5.095	.015*
PcFL	.023	483.765	.000***
PSL	.940	.731	.492
PVD	.848	2.054	.151
PAD	.899	1.298	.292
AFL	.986	.161	.852
BDA	.903	1.233	.310
HW	.969	.368	.696
HL	.886	1.484	.248
SnL	.484	12.266	.000***
IoL	.885	1.488	.247
ED	.391	17.879	.000***

*p<0.05, **p<.01, *** p<0.001

Table 3: Univariate statistics (ANOVA) testing differences among samples from twenty two truss measurements

Characters	Wilks' Lambda	F	Sig.
1 to 2	.830	2.359	.117
2 to 3	.916	1.058	.363
3to4	.671	5.646	.010**
4to5	.852	2.000	.158
5to6	.900	1.284	.296
6to7	.842	2.160	.138
7to8	.833	2.301	.123
8to9	.812	2.668	.091
9to10	.948	.633	.540
10to11	.972	.327	.724
9to11	.958	.508	.608
7to9	.942	.704	.505
7to10	.986	.169	.845
8to10	.786	3.127	.063
6to8	.897	1.320	.287
5to7	.877	1.620	.220
5to8	.841	2.178	.136
3to5	.956	.526	.598
4to6	.976	.285	.754
1to3	.803	2.827	.080
1to4	.982	.214	.809
2to4	.856	1.931	.168

*p<0.05, **p<.01, *** p<0.001

A dendrogram based on morphometric &landmarks distance data was shown for the populations of Kaptai Lake, the Meghna River & Tanguar *Haor*, the distances of squared Euclidean dissimilarity were nearest between Kaptai Lake & Tanguar *Haor* populations. From the cluster analysis it seems that both Kaptai Lake & Tanguar *haor* form one cluster. The Meghna River population was in another cluster (Figure 4).

Table 4: Pooled within-groups correlations between discriminating variables & discriminant functions (variables ordered by size of correlation within function)

	Function	
Characters	1	2
PcFL	-.484*	.043
PPD	.050*	.021
PVD	.032*	-.001
ED	.047	-.528*
SnL	.073	-.167*
DFL	.003	-.131*
FL	.001	-.098*
PAD	-.023	.070*
HW	-.009	.067*
IoL	.026	-.054*
AFL	-.004	.051*
BDA	-.024	-.041*
PSL	-.018	-.036*
DSL	-.013	-.035*
HL	.026	-.027*
SL	.002	-.024*
PDD	.009	.023*

*denotes the largest correlation between each variable & discriminant functions.

Table 5: Pooled within groups correlations between discriminating variables & discriminant functions (variables ordered by size of correlation within function)

	Function	
Characters	1	2
3 to 4	-.042*	-.027
1 to 3	-.030*	-.012
1to 2	-.027*	-.002
8 to 10	.024	.373*
4 to 5	-.015	.363*
7 to 8	.019	.347*
8 to 9	.022	.331*
5 to 8	-.019	.322*
5 to 6	-.011	.305*
6 to 8	-.012	-.299*
2 to 4	-.097	-.298*
5 to 7	.018	.245*
9 to 10	.009	.190*
7 to 9	-.011	.186*
4 to 6	.000	.173*
3 to 5	.010	.137*
2 to 3	-.017	-.122*
6 to 7	.025	.080*
1 to 4	-.007	-.072*
7 to 10	.007	.045*
9 to 11	.012	.035*
10 to 11	.010	.010*

*denotes the largest correlation between each variable & discriminant functions

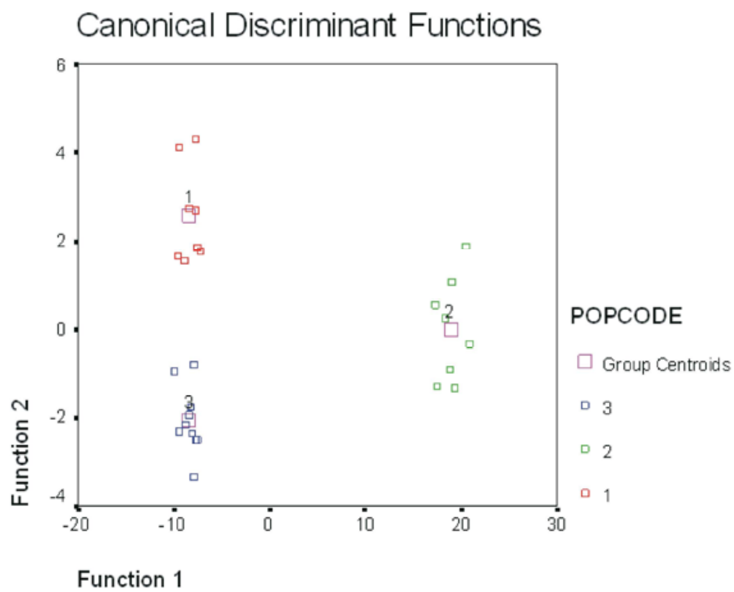

Fig. 2: Sample centroids of discriminant function scores based on morphometric measurements. Samples referred to, 1. Kaptai Lake's population, 2. Meghna River's population & 3.Tanguar*Haor* population

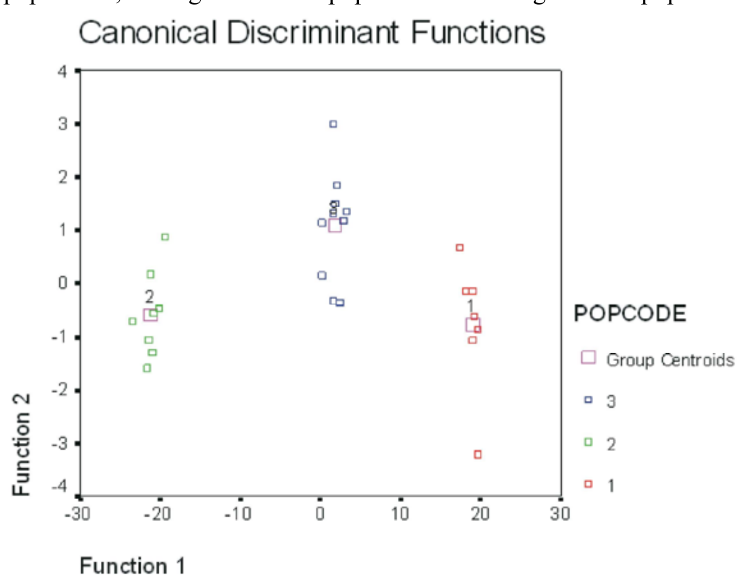

Fig. 3: Sample centroids of discriminant function scores based on truss measurement. Samples referred to, 1. Kaptai Lake's population, 2. Meghna River's population & 3. Tanguar*Haor* population

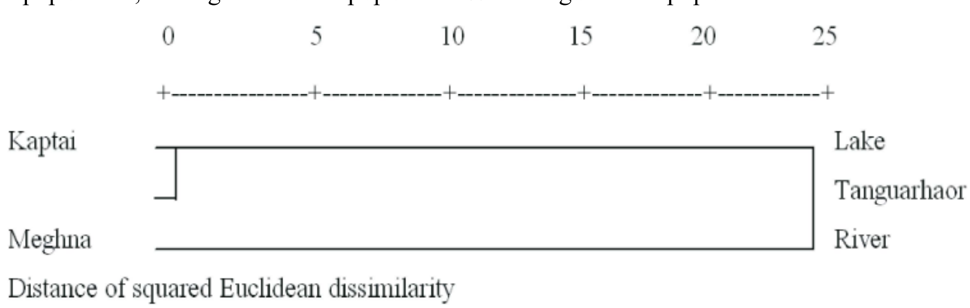

Fig. 4: Dendrogram based on morphometric characters & landmarksdistances Kaptai Lake, the Meghna River & Tanguar*haor* populations

DISCUSSION

In the present study, meristic counts of all samples from three different populations shows 8 rays for the dorsal fin, 14 rays for the pectoral fin, 6 rays for the pelvic fin and 46-48 rays for the anal fin. These results are similar to those reported by Siddiqui *et al*. [2] for bacha. It seemed that fin rays were similar & fixed in number among samples from three different populations. The fairly constant values of fin rays among populations showed similarity with the findings of Reed *et al.* [17] & Hold & Reed [18] that fin rays of same species do not differ much. So, significant differences were not observed in meristic counts.

In early life stage of fish, temperature influences the meristic characteristic of a fish species. Georgakopoulou *et al.* [19] reported that temperature effect from the half-epiboly stage until metamorphosis is enough to permanently alter the meristic counts of many fins in fish juveniles. It can be said that as the temperature of water bodies in Bangladesh is fairly constant, so the observed samples from three different populations showed similarity in case of meristic counts. The present results demonstrated significant differences ($p<0.001$) in three morphometric traits (Pectoral fin Length - PcFL, Snout length - SnL, & Eye Diameter - ED) among the populations of bacha. This morphometric differentiation, however, was not supported by meristic traits. Other morphometric traits did not show differences among populations at the significance level of $p<0.001$. In present study meristic characters were rather more discriminative than morphometric characters

Truss measurements showed no significant difference at the significance level of $p<0.001$. Difference in truss measurements from three different populations have not been occurred due to the similar environmental conditions among three different habitats. So it might be said that there is no shape difference among the populations of three different habitats (Kaptai Lake, Tanguar*Haor*, & the Meghna River).

Cakmak & Alp [20] found no differences in meristic counts & also found significant difference ($p<0.001$) in a number of morphometric traits & the truss network measurements inMesopotamian Spiny Eel, *Mastacembelusmastacembelus* populations from Karakay Reservoir, Tohma Stream & Tigris River in Turkey.

Hossain *et al*. [12] observed Significant differences in four of 12 morphometric measurements, two of 9 meristic counts, & four of 22 truss network measurements in endangered carp, *Labeocalbasu*, from stocks of two isolated rivers, the Jamuna& the Halda and a hatchery in Bangladesh.

Fish are very sensitive to environmental conditions changes (i.e. temperature & food abundance) and quickly adapt themselves by changing necessary morphometrics [21, 22]. Ingeneral, fish show greater variationin morphological traits with in andamong populationsthan anyother vertebrates and are more susceptibleto morphological variations induced by environmental factors [23]. The reason for dissimilarity might be due to environmental variation & also because of genetic variation. In Bangladesh because of less environmental changes fishes may show little morphological changes.

The dendrogram results two major clusters, Kaptai Lake & Tanguar *Haor* populations in one & the MeghnaRiver population in another cluster. The difference between these habitats may be due to environmental variation. The dendrogram based on morphometric & truss distance data was quite understandable. In the dendrogram, it seems that Meghna River population shows greater difference than other two populations (Kaptai Lake & Tanguar*Haor*). It seemed that as the Kaptai Lake & Tanguar *Haor* are lentic water bodies so they form one cluster & showed uniformity. On the other hand, the Meghna River is a lotic water body so it showed more difference than other two populations.

The result of this study may be used as storage of information about bacha & can be used for further studies. It should be emphasized that application of genetic techniques would be very beneficial to confirm the detected phenotypic differentiation. More research is needed, especially on the basis of geneticstudies and research into the causes of the environmental factor for the conservation of a speciesas critically endangered, bacha in Bangladesh.

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