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Meristic and morphometric variations of critically endangered butter catfish, *Ompok pabo* inhabiting three natural sources

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Abstract

The present study focuses to examine the meristic and morphometric variations of *Ompok pabo* from three populations namely Bergobindapur *baor* (BB), Bhairab River (BhR) and Kopotakho River (KR) in Bangladeshi freshwaters using the landmark-based truss network analysis. Truss protocol used in the present study based on seven general morphometrics and eight landmarks points constructed by interconnecting them to form total 16 truss measurements. Meristic counts were compared among three populations and no significant differences were observed in non-parametric Kruskal Wallis test. Univariate statistics (ANOVA) showed 5 [standard length (SL), post-orbital length (PsOL), maximum body depth (MBD), length of left barbel (LLB) and length of right barbel (LRB)] of seven morphometrics measurement and 8 (2-3, 3-4, 6-7, 2-7, 2-6, 3-6, 3-5, 2-8) of the 16 truss measurements significantly differed to varying degrees ($p<0.05$, $p<0.01$, $p<0.001$) among samples. Cannonical discriminant function analyses were done among the samples and the populations were fully separated. In case of both morphometric and truss measurements, the first and second discriminant function (DF) accounted for 76.3% and 23.7% respectively in group variability explaining 100% of the total among group variability. A dendrogram was drawn for the three populations of BB, BhR and KR based on the morphometric and truss measurements where two clusters were mainly formed in which BB and BhR formed one cluster and KR formed a separate cluster. The preliminary information derived from the present study may be useful to manage and conserve of *O. pabo* populations in three aforementioned ecological niches.

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Introduction

Variation in growth, maturation and development generates a variety of body shapes within a species (Cadrin, 2000). Morphometric is the measurement and quantitative analysis for the morphology or shape (Daly, 1985) and its studies are important to understand the taxonomy and the variations in its features are probably related to the habitat among the variants in the species. Morphometric means, the quantitative study of biological shape, variation of shape, and co-variation of shape with other biotic or abiotic variables or factors (Webster and Sheets, 2010). A meristic count means anything that can be counted while morphometry is external measurement of an organism (Conover *et al.*, 2007). Meristic characters are commonly external, including number of fin spines and fin rays, gill rakers and scales (Waldman, 2005). The morphometric and meristic characteristics are often analyzed together for the purpose of population structure analysis. Study of morphometric and meristic in the same species are vigorous tools for measuring discreteness (Naeem and Salam, 2005) and its study is important for demarcation of diverse population within species in a geographical boundary (Miller *et al.*, 1988) which are basic and useful for the development of the fisheries management strategies that will be helpful in conserving the biodiversity of different species, subspecies, stocks and races (Turan *et al.*, 2005). In stock identification phenotypic variations have an important role among groups of fish (Costa *et al.*, 2003). Use of phenotypic characters is particularly important where the differences are mostly attributable to environmental influences rather than to genetic differentiation (Pinherio *et al.*, 2005). Landmarks are defined as some arbitrarily points which is selected on a fish's body and with the help of these arbitrarily points, the individual fish body shape can be analyzed (Ahammad *et al.*, 2018). Truss network system which is constructed with the help of this landmark points are powerful tools for stock identification. Stocks are random group of fishes that are essentially self-producing, with members of each group having similar life history features (Hilborn and Walters, 1992). Identification of stock is a basic requirement to describe the stock status and to support better stock assessment of fishery (Cadrin *et al.*, 2005).

Different tools, such as meristic and morphometric, traditional tags, parasites as natural tags, otolith chemistry, molecular genetics and electronics tags have been used for the purpose of stock identification, among which the study of morphometric traits is one of the most frequently employed and cost-effective methods (Mir *et al.*, 2013). Morphometric and meristic variations have been used as a method for stock identification for many fish species such as *Trachurus mediterraneus* (Turan, 2004), *Limanda ferruginea* (Cadrin and Silva, 2005), *Clarias gariepinus* (Turan *et al.*, 2005), *Pomatomus saltatrix* (Turan *et al.*, 2006), *Rastrelliger kanagurta* (Jayasankar *et al.*, 2004), *Megalaspis cordyla* (Sajina *et al.*, 2011), *Cirrhinus cirrhosis* (Gain *et al.*, 2017), *Labeo bata* (Mahfuj *et al.*, 2017) etc.

Ompok pabo is a freshwater fish belonging to the family Siluridae of the order Siluriformes (Siddiqua *et al.*, 2000). Body of the fish is bi-laterally compressed. A large superior and oblique mouth is present at the anterior portion. Two pairs of barbels are present below the lower lip and snout rounded. A faint or dark spot with silvery-grayish body is marked on the body of each site (Rahman, 1989). This species is naturally distributed in Bangladesh, Pakistan, Northeast- India and Myanmar (Talwar and Jhingran, 1991; IUCN-Bangladesh, 2000). This species are commonly found in fresh water bodies. Common habitats are rivers, rivulets, streams, *beels*, canals etc. It's found where water body having little depth. It is also found in muddy water. It is omnivorous, feeds on vegetables maters, small fishes (IUCN-Bangladesh, 2000). Presently, the distribution of *O. pabo* has been reduced alarmingly and it is recognized as a critically endangered fish (Mollah, 2015). The present research work has been undertaken to determine the morphometric and meristic variation of *O. pabo* among three populations namely Bergobindapur *baor*, Bhairab River and Kopotakho River in Bangladesh using landmark-truss network analysis.

Materials and methods

Sampling

From May to August, 2018 total 63, *Ompok pabo* were collected from three water bodies, *viz,* Bergobindapur *baor*, Bhairab River and Kopotakho River (Fig. 1).

Then the samples were brought to the laboratory of Department of Fisheries and Marine Bioscience of Jashore University of Science and Technology in Bangladesh for meristic and morphometric measurements. Stock name, sample size, total length and date of collection of each water body were shown in (Table 1).

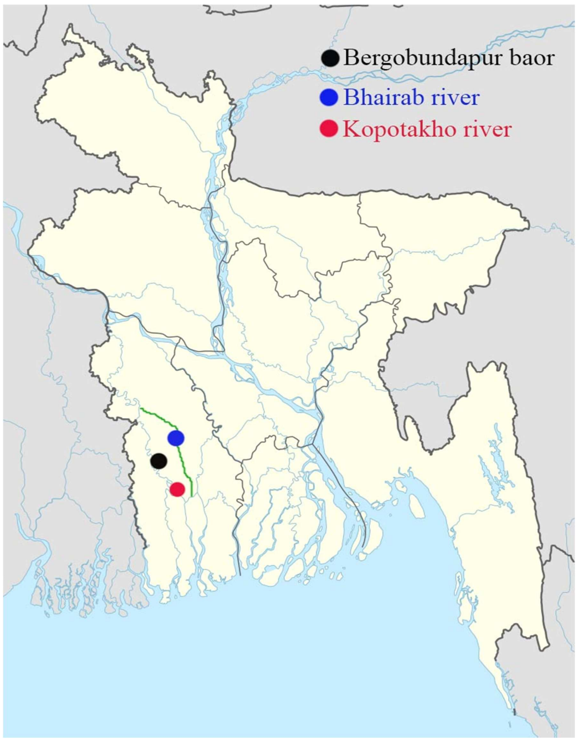

Fig. 1. Map of Bangladesh showing collection sites of *Ompok pabo* from three freshwater sources.

Table 1. Stock name, sample size, total length and date of collection of *Ompok pabo*.

Stock name	Collection site (District)	Sample size	Total length (Mean±SD)	Date of collection
Bergobindapur baor	Jashore	12	11.87±1.05	16/05/2018
Bhairab river	Jashore	21	10.75±0.92	17/06/18
Kopotakho river	Jashore	30	10.85±0.89	25/08/2018

Meristic characters counting

Total 5 meristic characters, namely number of dorsal fin rays (DFR), number caudal fin rays (CFR), number of anal fin rays (AFR), number of pelvic fin rays (PelFR), number of pectoral fin rays (PecFR), were analyzed with the help of needles for easy counting.

Morphometric characters measurement

A total of eight morphometric characters, namely total length (TL), standard length (SL), pre-orbital length (PrOL), eye length (EL), post-orbital length (PsOL), maximum body depth (MBD), length of left barbel (LLB) and length of right barbel (LRB) were measured using software platform tpsDig2V2.1 (Rohlf, 2006) (Fig. 2 and Table 2).

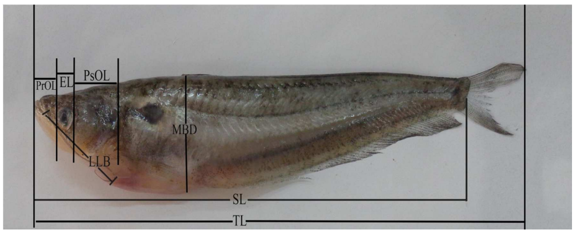

Fig. 2. Eight morphometric characters were used for the analysis of *Ompok pabo*.

Table 2. Description of morphometric characters of *Ompok pabo* fish used for analysis.

Character	Description	Short form
Total length	Distance from the tip of the upper jaw to the longest caudal fin rays.	TL
Standard length	Distance from the tip of the upper jaw to the end of the vertebral column.	SL
Pre-orbital length	Distance from the tip of upper jaw to the front margin of the orbit.	PrOL
Eye length	Distance from the anterior to the posterior rims of the eye in the longitudinal axis.	EL
Post-orbital length	The distance from hind margin of the orbit to the tip of the opercular membrane.	PsOL
Maximum body length	Maximum depth measured from the base of the first dorsal fin ray.	MBD
Length of left barbell	Length of the left barbel.	LLB
Length of right barbell	Length of the right barbel.	LRB

Digitalization of samples

At first the samples were washed in running fresh water. After washing, the water was sucked finely by using soft tissue paper from the body surface of fish. Then the fish was placed on a white paper as a background, which was used for capturing the digital image. Each individual was labeled with a specific code of identification. A cyber shoot DSC-W 300 digital camera (Sony, China) was used to capture the digital images, which provide a complete archive of body shape and allowed a repeat of the measurement when necessary (Cadrin and Friedland, 1999).

Truss distances measurement

Eight landmarks delineating 16 distances were measured on the fish body from left to right side by one person, therefore avoided the biasness (fig. 3). Truss distances from the digital images of specimens were extracted by using a linear combination of tpsDig2v2.1 (Rohlf, 2006). A box truss of 16 lines connecting these landmarks was generated for each fish to represent the basic shape of the fish (Strauss and Bookstein, 1982). Then all measurements were transferred to a Microsoft Office Excel spreadsheet software, 2007 and SPSS 21 version software for subsequent analysis.

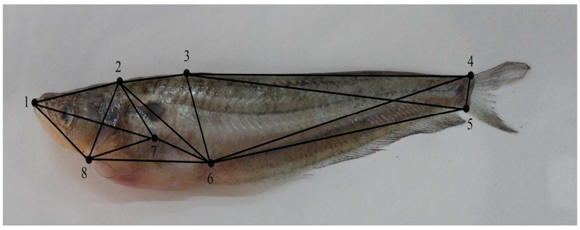

Fig. 3. Location of 16 landmarks for constructing the truss network on fish body illustrated as closed circle and morphometric distance measures between the circles as lines.

Statistical analysis

During analysis, the size effect was eliminated from the data set. The variations were attributed to body shape differences and not to the relative sizes of the fish. In the present study, there were significant linear correlation among all measured characters and the total length (TL) of the fish. So, the elimination of size-dependent variation from all measured characters is necessary. Size dependent variation was corrected by adapting an allometric method as suggested by Elliot *et al.* (1995)

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

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 calculated 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 transformed data were checked for efficiency by testing the significance of the correlation between the transformed variable and the TL.

The degree of similarity between the samples in overall analysis and the relative importance of each measurement was evaluated by using discriminant function analysis (DFA) with cross-validation. A dendrogram of the stocks based on the morphometric and landmark distance data was drawn by the unweighted pair group (UPGMA) and cluster analysis. A univariate analysis of variance (ANOVA) was carried out to test the significance of morphological differences. All statistical analyses were done using SPSS 21 (SPSS, Chicago, IL, USA).

Result

Meristic counts

Meristic counts of all samples ranged from 3-4 for dorsal fin rays, 12-21 for caudal fin rays, 43-64 for anal fin rays, 4-9 for pelvic fin rays, 11-17 for pectoral fin rays in three stocks examined. Meristic counts were compared among three populations [Bergobindapur *Baor* (BB); Bhairab River (BhR); and Kopotakho River (KR)]. No significant differences were observed (Kruskal-Wallis test; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$).

Table 3. Meristic counts of *Ompok pabo* of three different stocks in Bangladesh (BB: Bergobindapur *Baor*; BhR: Bhairab River; KR: Kototakho River).

Meristic Characters	Name of stocks-Mode (Minimum-Maximum)			Kruskal Wallis Test (H- value)	Significance
	BB	BhR	KR		
DFR	4(4-4)	3(3-4)	4(3-4)	1.143	0.565
CFR	17(16-19)	18(12-21)	18(13-19)	0.281	0.869
AFR	56(51-64)	54(43-60)	54(51-64)	3.227	0.199
PelFR	7(6-8)	6(4-9)	7(7-9)	0.101	0.951
PecFR	12(11-13)	14(11-17)	13(11-15)	3.284	0.194

Morphometric and landmark distances

Among three stocks of *O. pabo,* 13 morphometric measurements were found significantly (p<0.01) different in univariate analysis of variance and the ten remaining morphometric measurements, *viz*. PrOL, EL, 1-2, 4-5, 5-6, 7-8, 1-8, 1-7, 4-6, 6-8 were found to be insignificantly different (p>0.05) (table 4).

In discriminant function analysis, two discriminant function (DFs) were produced. The first discriminant function (DF1) accounted for 76.3% of the total variation.

The second discriminant function (DF2) accounted for 23.7% among group variability and mutually they explained 100% of the total among group variability. In case of both morphometric and truss measurement, the populations are clearly separated from each other in the discriminant space (Fig. 4).

Table 4. Univariate statistical (ANOVA) of all twenty three morphometric and truss measurement of *Ompok pabo* from three sources. *P<0.05, **P<0.01, ***P<0.001.

Measurements	Wilks' Lambda	F	Significance
SL	0.724	11.461	0.000***
PrOL	0.950	1.583	0.214
EL	0.961	1.225	0.301
PsOL	0.798	7.615	0.001**
BD	0.830	6.128	0.004**
LLB	0.853	5.170	0.008**
LLR	0.845	5.513	0.006**
1 – 2	0.943	1.804	0.173
2 – 3	0.641	16.798	0.000***
3 – 4	0.721	11.623	0.000***
4 – 5	0.940	1.924	0.155
5 – 6	0.997	.091	0.913
6 – 7	0.868	4.547	0.014*
7 – 8	0.912	2.881	0.064
1- 8	0.918	2.688	0.076
1 – 7	0.962	1.171	0.317
2 – 7	0.865	4.666	0.013*
2 – 6	0.748	10.103	0.000***
3 – 6	0.582	21.553	0.000***
4 – 6	0.997	.096	0.909
3 – 5	0.682	14.015	0.000***
2 – 8	0.770	8.957	0.000***
6 – 8	0.921	2.578	0.084

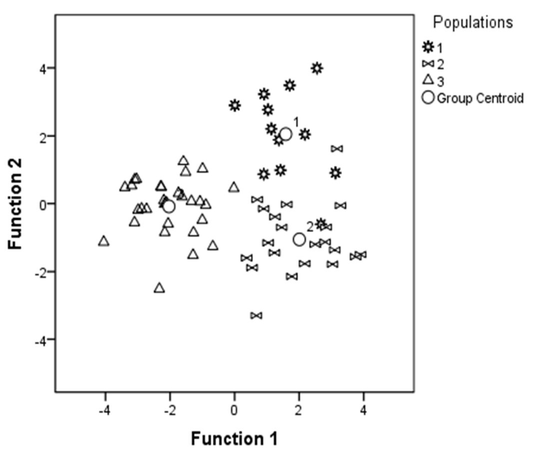

Fig. 4. Discriminant analysis plot with 23 morphometric variables for *Ompok pabo* [1. Bergobindapur *Baor*, 2. Bhairab River, 3. Kopotakho River].

Pooled within group correlation between discriminant variables and DFs informed that thirteen measurements, 3-5, 2-3, 3-4, 2-6,2-8, maximum body depth (MBD), 6-7, 1-8, 6-8, 7-8, 4-5, 1-7 and 5-6 particularly contributed to the DF1 and the rest, 3-6, standard length (SL), length of left barbel (LLB), length of right barbel (LRB), 2-7, post-orbital length (PsOL), eye length (EL), pre-orbital length (PrOL), 1-2, and 4-6 contributed to the DF2 (Table 5).

Table 5. Pooled within-groups correlation between discriminating variables and discriminant function in case of general morphometric characters and landmark distance. (*) denotes the largest absolute correlation between each variable and any discriminant function.

Character/distance	DF1 (76.3%)	DF2 (23.7%)
3-5	0.342*	-0.020
2-3	-0.342*	0.276
3-4	0.312*	0.018
2-6	0.285*	-0.100
2-8	0.270*	-0.081
MBD	0.226*	0.015
6-7	0.195*	0.012
1-8	0.150*	-0.012
6-8	0.147*	-0.016
7-8	0.138*	0.127
4-5	0.125*	-0.043
1-7	0.095*	-0.051
5-6	0.028*	0.002
3-6	0.367	-0.383*
SL	0.232	0.367*
LLB	-0.057	0.359*
LLR	-0.134	0.301*
2-7	0.117	-0.286*
PsOL	-0.208	-0.256*
EL	0.048	0.160*
PrOL	0.074	0.159*
1-2	0.088	-0.155*
4-6	0.023	0.030*

The finding suggested that the population were fully separated. On the basis of morphometric measurements, the percentage of original grouped cases correctly classified is 91.7% for Bergobindapur *Baor*, 95.2% for Bhairab River and 100% for Kopotakho River (Table 6).

Table 6. Percentage of specimen correctly classified into their original population collected from Bergobindapur *Baor* (BB), Bhairab River (BhR), Kopotakho River (KR) using discriminant function analysis (96.8%) of originally grouped cases correctly classified, 73.0% cross-validated grouped cases correctly classified).

		Stock name	Predicted Group Membership			Total
			BB	BhR	KR	
Original	Count	BB	11	1	0	12
		BhR	1	20	0	21
		KR	0	0	30	30
	%	BB	91.7	8.3	0	100
		BhR	4.8	95.2	0	100
		KR	0	0	100.0	100
Cross-validated	Count	BB	6	5	1	12
		BhR	4	14	3	21
		KR	2	2	26	30
	%	BB	50	41.7	8.3	100
		BhR	19	66.7	14.3	100
		KR	6.7	6.7	86.7	100

A dendrogram was developed on the basis of morphometric and truss distances of three stocks BB, BR and KR. The population of the BB and BhR formed one cluster. On the other hand the population of the KR formed separated cluster (Fig. 5).

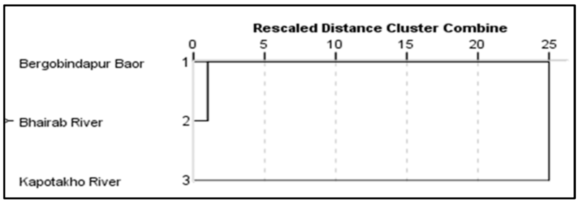

Fig. 5. Dendrogram based on morphometric characters and landmark distances of three populations (BB, BhR and KR).

Discussion

Landmark-based morphometric & meristic variations of *Ompok pabo* collected from three different populations exhibited significant differences in all morphometric & truss network measurements. There were found no significant differences in meristic measurements. As a potential indicator of phenotypic stocks, analysis of morphometric landmarks is a valuable tool that complements other stock identification methods. The identification, discrimination, and delineation of phenotypic stocks are essential for population modeling, which generally assumes homogenous ontogenetic rates within a stock.

In the present study, meristic counts of all samples ranged 3-4 rays for dorsal fin, 12-21 rays for caudal fin, 43-64 rays for anal fin, 4-9 rays for pelvic fin and 11-17 rays for pectoral fin. These results are similar to those reported by Rahman, (2005); IUCN Bangladesh, (2000) for *Ompok pabo*. In Kruskal Wallis test the H-value showed no significant differentiation in terms of meristic characters among the population. Çakmak *et al.* (2010) found no significant differentiation in terms of meristic characters in *Mastacembelus mastacembelus* populations among three stocks.

In the present, highly significant morphological variations were found among the Bergobindapur *baor* (BB), Bhairab River (BhR) and Kopotakho River (KR) of *Ompok pabo* populations and morphometric differences among populations are expected, because they are geographically isolated. Moreover, they may have originated from different ancestors. However, obvious environmental variation exists in these 3 habitats (BB, BhR, and KR). These explanations matched with the previous research accomplished by Hossain *et al.* (2010) in *Labeo calbasu*; Khan *et al.* (2013) in *Channa punctatus*; Gain *et al.* (2017) in *Cirrhinus cirrhosis*; Mahfuj *et al.* (2017) in *Labeo bata*; Mahfuj *et al.* (2019a,b) in *Macrognatus pancalus* and *Xenentodon cancila* respectively. Nevertheless, researchers have been recognized the real phenomena of morphological and physiological alterations (i.e. genetic factors like natural selection, epigenetic inheritance) (Murta, 2000), and effective population size and inbreeding (He *et al.*, 2013) as well which aggregately affected the evolutionary changes of a population. Moreover, phenotypical plasticity and adaptations are completely or partially relied on external forces from the environment (He *et al.*, 2013). Morphology is particularly dependent on environmental conditions during early life history stages (Ryman *et al.*, 1984; Cheverud, 1988). In general, fish shows more prominent changes than other vertebrates in morphological characteristics both inside and between populations, and are more defenseless to naturally incited morphological varieties (Allendorf, 1987; Swain *et al.*, 1991; Wimberger *et al.*, 1992). The studies of morphometrics have been able to identify differences between fish populations and are helpful tools for the discrimination of fish populations (Bailey, 1997; Palma and Andrade, 2002). Besides, morphometric measurements, combined with image analysis, represent a method for improving our understanding about fish stocks structures (Bailey, 1997).

Fish adapt quickly by modifying their physiology and behavior to environmental changes as the phenotypic plasticity of fish is very high. Phenotypic plasticity in morphometric traits may often be adaptive (Robinson and Parsons, 2002). These modifications ultimately change their morphology (Stearns, 1983). Plasticity in trophic morphology induced by diet or feeding mode is usually assumed to result from bone remodeling in response to differences in loading regime (Swain *et al.*, 1991). In a small country, there are possibly very small environmental changes from place to place like Bangladesh. However, due to small environmental differences, the subsequent morphological differences in fish may be small that they may be difficult to recognize with gross morphomeristic characters.

Truss network systems are a powerful tool for identifying stocks of fish species (Turan *et al.*, 2004) and truss network measurements were employed in this experiment. Truss measurements showed significant correlation with total length (TL). In the present study, 8 truss measurements (2-3, 3-4, 6-7, 2-7, 2-6, 3-6, 3-5 and 2-8) of the 16 truss measurements were significantly differed to varying degrees among samples ($^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$).

Conclusion

The result obtained from this study is highly important for balanced exploitation, selection & breeding, habitat restoration, management & conservation of Ompok pabo. The data obtained from this study would be useful for the conservation of the reducing stocks of the *O. pabo* and in designing breeding strategies. It is an essential part to select the genetically superior stocks with better features along with morphometric investigations. More research especially on genetic studies and investigations of the impacts of environmental factors is needed for conservation and mass seed production of selected stocks to pave the way to saving the critically endangered species from extinction in Bangladesh.

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