JOURNAL OF ADVANCED VETERINARY AND ANIMAL RESEARCH
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2019.f321
A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)

March 2019
VOL 6, NO. 1, PAGES 117–124

ORIGINAL ARTICLE

Landmark-based morphometric and meristic variations of freshwater garfish, *Xenentodon cancila* from four natural stocks of South-Western Bangladesh

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ABSTRACT

Objective: The morphometric and meristic variations of *Xenentodon cancila* was studied based on the landmark-based truss network system to assess their phenotypic variations among four different freshwater stocks, *viz*. Boluhorpur *baor*, Jhenaidah (BBJ) (n = 29); Bhairab River, Jashore (BRJ) (n = 34); Arial Khan River, Madaripur (AKRM) (n = 28), and Bohnni baor, Gopalganj (BBG) (n = 25) in Bangladesh.

Materials and methods: Seven meristic characters were counted by using a needle. Eight morphometrics and 28 truss measurements were measured by using tpsDigV.2.1 software. In meristic characters, Kruskal–Wallis test was performed to determine any significant differences, whereas, in morphometrics and truss measurements, univariate statistics and discriminant function analyses were carried out by using SPSS 22 version.

Results: Significant differences were observed in four meristic characters among seven meristic characters in the Kruskal–Wallis test. In univariate statistics, only nine characters were observed significantly different among eight morphometrics and 28 truss measurements. The contribution of three discriminant function analyses (DFA), in which first DFA showed 49.2%, second DFA showed 33%, and third DFA showed 17.8% on behalf of both morphometric and truss measurements. In discriminant space, the four stocks were clearly separated. Two clusters were formed among four stocks, where BBG formed a single cluster, whereas BBJ and BRJ aggregately formed another cluster. Additionally, AKRM formed a sub-cluster with BBJ.

Conclusion: The preliminary information generated from the current study would be beneficial for further genetic studies and in the assessment of ecological impacts on *X. cancila* stocks in Bangladesh.

ARTICLE HISTORY

Received November 11, 2018
Revised December 27, 2018
Accepted December 27, 2018
Published February 22, 2019

KEYWORDS

Morphometric; meristic; garfish; truss network; Bangladesh

Introduction

The investigation of external phenotype features in fishes, by the point of describing fish stocks, takes for quite a while been of solid enthusiasm in ichthyology [1]. The term “fish stock” refers to a neighborhood populace adjusted to a specific domain and devouring hereditary contrasts from different stocks [2]. Albeit hereditary contrasts among stocks remain a state of this delineation, morphological variety still keeps on having an imperative part in stock distinguishing proof among gatherings of fish

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How to cite: Sarower-E-Mahfuj M, Rahman MM, Islam M, Samad MA, Paul AK, Adhikary RK. Landmark-based morphometric and meristic variations of freshwater garfish, *Xenentodon cancila* from four natural stocks of South-Western Bangladesh. J Adv Vet Anim Res 2019; 6(1):117–24.

[3]. Utilization of phenotypic types is especially essential wherever the distinctions stay generally inferable from natural impacts instead of to hereditary separation [4]. Different apparatuses, for example, meristics and morphometrics, parasites as regular labels, otolith science, subatomic hereditary qualities, and electronic labels have been extensively utilized with the end goal of stock recognizable proof, among which the investigation of morphometric characters is a standout and savvy strategy. To understand the inborn shortcomings of customary morphometric techniques, an arrangement of morphological estimation named the truss organize framework. According to Strauss and Bookstein [5], truss organize framework has been progressively utilized on behalf of the motivations behind stock distinguishing proof that basically segregates "phenotypic stocks," thereof gatherings of people with comparable development, death, and regenerative rates [6]. The method is based on the estimation of whole physique separate associating two remarkable morphological points of interest from a consecutive arrangement of associated points. Morphometric contrasts among loads of animal varieties are perceived as essential for assessing the populace structure and as a reason for recognizing distinctive fish races and additionally populaces [7].

External morphological types of fish are quantifiable and countable characters, separately normal to all fishes. Truss estimations alongside the estimation of morphometric and meristic characters are capable devices for stock identification, uncovering comparability and divergence among populaces or races which are developed with the assistance of landmark focuses. Landmarks allude to some self-assertively chose emphases on fish's organization and through the support of these applications, the specific fish body form can be wrecked down and that matches between and inside populaces [8]. Such elective phenotypes seem to have developed generally every now and again in sympatry of freshwater angles possessing postglacial lakes [9,10].

In addition, the extent of occurrence of the *X. cancila* species is 2,17,467.88 km^2 and area of occupancy is 11,856.77 km^2, outperforming the edge estimations of any threatened category. Thus, this species is surveyed as least concern species that occurs in South Asian countries [11,12]. It lives in freshwater waterways, *hoars, baors, beels*, lakes, and between tidal salty water streams and their tributaries in Bangladesh [13,14]. As indicated by IUCN-Bangladesh [15], *X. cancila* is one of the commonest freshwater angle species in the nation. Late faunal review demonstrated that slight decrease in populace; however, there is concrete information to order it under a least concerned category. The overall aim of the existing research is to assess the external morphological variations of *X. cancila* from four different freshwater bodies in Bangladesh for their sustainability and stock status.

Materials and Methods

Sampling

A whole of 119 *X. cancila* were collected from four different freshwater bodies, *viz.* Bhairab River, Jashore (BRJ); Bohnni *baor,* Gopalgonj (BBG); Boluhorpur *baor*, Jhenaidah (BBJ), and Arial Khan River, Madaripur (AKRM) (Fig. 1) and instantly well-kept in the ice container. Then samples were transported to the laboratory for morphometric, meristic studies. The general descriptive characters and studies and date of collection are presented in Table 1.

Measurement of meristic characteristics

In total, seven meristic characters, namely number of teeth on upper jaw (TOU), number of teeth on lower jaw (TOL), number of dorsal fin rays (DFR), number of caudal fin rays

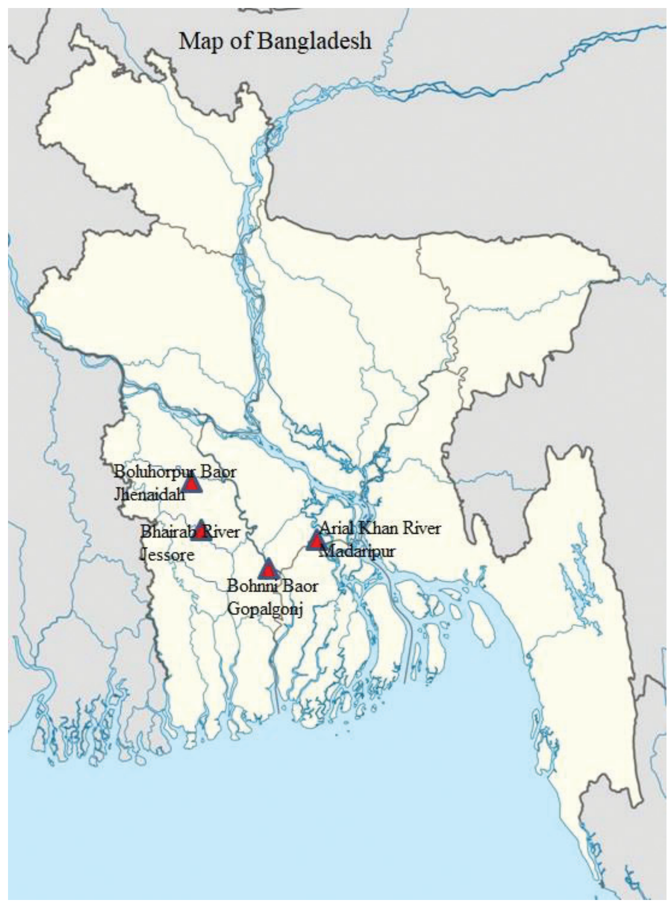

Figure 1. Map of Bangladesh showing collection locations of *X. cancila* from four freshwater sources.

(CFR), number of anal fin rays (AFR), number of pelvic fin rays (PelFR), and number of pectoral fin rays (PecFR) of each sample were counted by using needles.

Measurement of morphometric characteristics

Eight morphometric characters of each sample fish were measured using tpsDigV.2.1. (Table 2).

Image captured for digitization

Firstly, the collected samples were opened from the ice container thawed under the flow of tap water. Then, each of the thawed samples was placed on a white paper with a scale and an identification mark. After that, a digital camera was used to take the digital image and finally, this image was transferred for the measurement [16].

Measurement of truss distances

The extraction of truss distance from the digital images of specimens was conducted using a linear combination of tpsDigV.2.1 software [17]. A box truss of 28 lines connecting these landmarks was generated for each fish to represent the basic shape of the fish [5] (Fig. 2).

Statistical analyses

Firstly, the effects of the size of the dataset were removed. The external discrepancies were ascribed to differences in body feature but not in relation to size of fish. In the contemporary research investigation, significant linear correlations were observed between the total length and other remaining measured features of fish. Hence, to reduce the variation resulting from morphometric characters, at first, they were uniformed that was previously developed according to Elliott et al. [18]

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

where M: new dimension or measurement, M_{adj}: size accustomed dimension, L_o: total length of fish, and L_s: general mean of the whole length for all fish. Factor b was assessed for each character from the experiential data as a slope of the regression of log$M$ on logL_o, using all fish groups. The effectiveness of magnitude modification alterations was weighed by testing the importance of the relationship between a distorted variable and the TL. The amount of resemblance among the samples in general examination and the comparative position of each size for the parting of the cluster were assessed by discriminant function analysis (DFA) with cross-validation studies. Populace centroids with 95% sureness abridgments resulting from the DFA were used to envisage associations among the distinct groups. A dendrogram of three populations based on the morphometric and landmark distances data was drawn by the unweighted pair group (UPGMA) and cluster analysis. Univariate analysis of variance (ANOVA) was conducted to check the significance of morphometric and meristic modifications. All data analyses were performed using SPSS v 22 (SPSS, Chicago, IL).

Table 1. General description regarding on sampling of *X. cancila*.

Source of fish samples	Collection site	Sample size	Total length (Mean ± SD)	Date of collection	Coordinates
Bhairab River	Jashore	34	13.45 ± 1.12	08-10-2017	23.1634° N and 89.2182° E
Bohnni Baor	Gopalgonj	25	13.79 ± 1.14	29-10-2017	23.0488° N, and 89.8879° E
Boluhorpur Baor	Jhenaidah	29	14.01 ± 1.34	21-11-2017	23.5450° N and 89.1726° E
Arial Khan River	Madaripur	28	16.77 ± 1.22	16-12-2017	23.2393° N and 90.1870° E

Table 2. Description of morphometric characters of *X. cancila.*

Character	Description	Acronym
Total length	Distance from the tip of the upper jaw to the longest caudal fin ray	TL
Standard length	Distance from the tip of the upper jaw to the end of the vertebral column	SL
Length of upper jaw	Straight line measurement between the snout tip and posterior edge of maxilla	LUJ
Length of lower jaw	Straight line measurement between the snout tip and posterior edge of mandible	LLJ
Pre-pectoral length	Front of the lower lip to the origin of the pectoral fin	PPCL
Body depth	Maximum depth measured from the base of the first dorsal fin ray	BD
Eye diameter	The greatest crystal like diameter of the orbit	ED
Inter orbital	Distance between dorsal side of both eyes	IO

Results

Counting of meristic characters

Counting of meristic characters in samples was ranged from 14 to 34 in TOU, 12 to 30 for TOL, 12 to 19 for DFR, 15 to 27 for CFR, 14 to 23 for AFR, 5 to 9 for PelFR, and 6 to 11 for PecFR in four stocks examined. Substantial significant differences were observed in TOU, TOL, AFR, and PecFR (Table 3) (Kruskal–Wallis test; $p < 0.05$, $p < 0.01$, and $p < 0.001$).

Morphometric and truss distances

Univariate statistics (ANOVA) showed that nine characters, *viz.* LJL, IO, 2–3, 4–5, 11–12, 13–1, 3–12, 4–12, and 1–3 of seven morphometrics and 28 truss measurements were remarkably dissimilar among samples in fluctuating marks ($p < 0.05$ or $p < 0.01$ or $p < 0.001$) (Table 4).

Three discriminant functions were generated (DF1, DF2, and DF3) in the DFA for morphometric and truss measurements. The first DF accounted for 49.2%, the second DF accounted for 33%, and the third DF accounted for 17.8% separately among group variability elucidating 100% of the entire among group variability. In case of both morphometric and truss measurements, the stock is clearly separated in the discriminant space (Fig. 3).

On the basis of morphometric and truss measurements, 87.9%, 100%, 72.4%, and 85% of original grouped belongings appropriately categorized in case of BRJ, BBG, BBJ, and AKRM (Table 5).

Figure 2. Location of 13 landmarks across the fish body explained as a closed circle, number, and truss measurements among the circles as outlines.

Characters of both morphometric and truss measurements, *viz.* 3–12, 13–2, 11–12, 11–13, 6–7, and body depth BD contributed to first DF in Pooled within-groups parallels. Moreover, interorbital (IO), 1–3, 13–1, 4–10, 2–3, 3–11, 6–10, 1–4, 6–9, 1–2, 10–11, 7–8, 12–13, 6–10, 3–4, and 4–11 contributed to second DF. Length of upper jaw (LUJ), length of lower jaw (LJL), 9–10, 4–12, 5–10, standard length (SL), 4–5, eye diameter (ED), 5–9, 7–9, 8–9, pre-pectoral length (PPCL), 3–13, and 5–6 contributed to third DF (Table 6).

A UPGMA dendrogram developed by using morphometric and landmark measurements data was drawn. Two clusters were formed among four stocks, where BBG formed a single cluster, whereas BBJ and BRJ aggregately formed another cluster. Additionally, AKRM formed a sub-cluster with BBJ (Fig. 4).

Discussion

In the current revision, meristic characteristic of all trials fluctuated 14–34 for teeth on upper jaw, 12–30 for teeth on lower jaw, 12–19 rays for the dorsal fin, 15–27 rays for the caudal fin, 14–23 rays for the anal fin, 5–9 rays for the pelvic fin, and 6–11 rays for the pectoral fin. These outcomes are parallel to those described by Rahman [19] for *X. cancila* as (D. 15–16; P_1. 10–11; P_2. 6; and A. 17–18). In the Kruskal–Wallis test, the H-value significantly differed in four meristic characters among all stocks. Nakamura [20] instigated the alterations in meristic features in Japanese Charr (*Salvelinus leucomaenis*) midst the brook systems and among the tributaries of the Naka River. Alongside, in the current research study, extremely substantial morphological disparities were originated among the stocks. The external shape discreteness suggests a straight correlation between the range of phenotypic deviation and topographical partition, which specifies that geographic separation, is a warning inspiration to voyage among stocks. Turan [7] also observed parallel outcomes for populaces from the three tributaries, *viz.* Orontes, Euphrates, and Tigris Rivers

Table 3. Meristic characters measurements of *X. cancila.* Significance levels indicate the *p* values and asterisk marks indicate the level of significance at *** = $p < 0.001$, ** = $p < 0.01$ and * = $p < 0.05$.

Meristic characters	BRJ	Descriptive statistics of stocksmode (Minimum–Maximum)			Kruskal–Wallis Test(H-value)	Significance
		BBG	BBJ	AKRM		
TOU	22 (14–31)	30 (26–35)	25 (14–34)	27 (20–32)	43.648	0.000***
TOL	15 (12–30)	20 (17–23)	21 (15–30)	20 (15–23)	12.250	0.007**
DFR	17(12–18)	17 (13–19)	17 (13–19)	16 (13–18)	2.377	0.498
CFR	16 (15–27)	17 (15–19)	17 (15–27)	18 (13–20)	6.949	0.074
AFR	17 (14–23)	14 (11–17)	17 (11–20)	14 (11–17)	35.425	0.000***
PelFR	6 (5–9)	6 (6–7)	6 (6–9)	6 (6–7)	4.670	0.198
PecFR	7 (6–11)	7 (7–9)	7 (6–11)	7 (7–11)	9.771	0.021*

Table 4. Univariate statistics of *X. cancila* among samples by using seven morphometric and 28 truss measurements. Significance levels indicate the *p* values and asterisk marks indicate the level of significance at *** = $p < 0.001$, ** = $p < 0.01$ and * = $p < 0.05$.

Characters	Wilks' Lambda	F	df1	df2	Significance
SL	0.963	1.487	3	115	0.222
LUJ	0.950	2.024	3	115	0.114
LJL	0.937	2.557	3	115	0.059*
PPCL	0.971	1.157	3	115	0.329
BD	0.988	0.475	3	115	0.700
ED	0.957	1.736	3	115	0.164
IO	0.772	11.297	3	115	0.000***
1-2	0.964	1.411	3	115	0.243
2-3	0.921	3.303	3	115	0.023*
3-4	0.990	0.369	3	115	0.776
4-5	0.934	2.692	3	115	0.049*
5-6	0.974	1.014	3	115	0.389
6-7	0.961	1.558	3	115	0.203
7-8	0.969	1.220	3	115	0.306
8-9	0.940	2.455	3	115	0.067
9-10	0.969	1.220	3	115	0.306
10-11	0.972	1.087	3	115	0.357
11-12	0.932	2.817	3	115	0.042*
12-13	0.981	0.735	3	115	0.533
13-1	0.835	7.600	3	115	0.000***
13-2	0.871	5.689	3	115	0.001
3-13	0.962	1.518	3	115	0.213
3-12	0.798	9.727	3	115	0.000***
4-11	0.995	0.178	3	115	0.911
5-10	0.958	1.701	3	115	0.171
5-9	0.977	0.901	3	115	0.443
6-9	0.942	2.349	3	115	0.076
4-12	0.911	3.739	3	115	0.013*
4-10	0.913	3.631	3	115	0.015
6-10	0.990	0.371	3	115	0.774
3-11	0.943	2.321	3	115	0.079
11-13	0.963	1.483	3	115	0.223
1-3	0.893	4.598	3	115	0.004**
1-4	0.966	1.351	3	115	0.261
7-9	0.970	1.199	3	115	0.314

in Turkey. Same results are also observed by Ahammad et al. [21] in *Labeo ariza*, Chaklader et al. [22] in *Ompok pabda*, Mahfuj et al. [23] in *L. bata*, Gain et al. [24] in *Cirrhinus cirrhosus*, and Hossain et al. [25] in *L. calbasu*. Morphometric modifications among stocks are probable, as they are

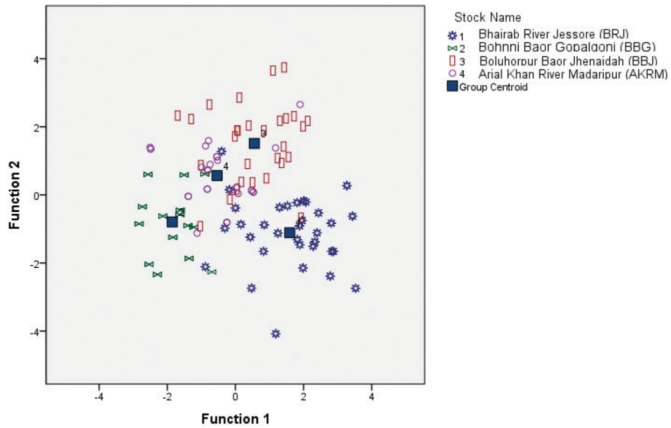

Figure 3. Sample centroid of the discriminant function scores based on morphometric and truss measurements of *X. cancila*.

Table 5. Proper classification of *X. cancila* individuals composed of four freshwater sources.

		Stock Name	Predicted Group Membership				Total
			BRJ	BBG	BBJ	AKRM	
Original	Count	BRJ	29	1	0	3	33
		BBG	0	29	0	0	29
		BBJ	1	1	21	6	29
		AKRM	0	2	2	24	28
	%	BRJ	87.9	3	0	9.1	100
		BBG	0	100.0	0	0	100
		BBJ	3.4	3.4	72.4	20.7	100
		AKRM	0	7.1	7.1	85.7	100

purely detached and may have instigated from different pedigrees. Consequently, it is improbable that clear ecological distinctions exist in these four stocks. Fish and aquatic organisms are highly subtle to ecological vicissitudes and hurriedly acclimatize themselves by shifting vital phenotypes. It is prominent that morphological appendages can exhibit high flexibility in reaction to changes in ecological circumstances, as for instance food richness and temperature [26,27].

Literally, fish prove superior modifications in morphological behaviors both intra and inter populaces than any further vertebrates, as well as more vulnerable to ecologically persuaded morphological disparities [28]. Moreover, the phenotypic flexibility of fish, as well as aquatic organisms is very significant. They adjust rapidly by transforming their composition and performance to ecofriendly fluctuations. These variations eventually alter their phenotypes [26]. Although, for a small country resembling Bangladesh, there are perhaps very minor ecological vicissitudes from

Table 6. Results of discriminant function analysis for both morphometric and truss measurements. Asterisk (*) marks indicate significant difference.

Characters	DF1 (49.2%)	DF2 (33%)	DF3 (17.8%)
3-12	0.370*	−0.068	0.124
13-2	0.273*	0.088	−0.120
11-12	−0.191*	−0.090	0.023
11-13	−0.129*	0.072	0.076
6-7	0.126*	−0.082	−0.090
BD	0.075*	−0.016	−0.059
IO	0.064	0.494*	0.055
1-3	−0.096	0.298*	−0.014
13-1	0.232	0.277*	−0.153
4-10	−0.071	0.259*	−0.109
2-3	−0.051	0.214*	−0.211
3-11	0.052	0.210*	0.083
6-10	−0.161	0.181*	−0.149
1-4	−0.041	0.166*	−0.020
6-9	0.114	0.161*	−0.115
1-2	−0.021	0.144*	0.136
10-11	−0.062	0.126*	0.069
7-8	-0.092	0.121*	0.011
12-13	0.058	−0.091*	0.077
6-10	0.027	−0.084*	−0.014
3-4	0.031	0.082*	0.014
4-11	0.006	0.055*	−0.040
LUJ	0.017	−0.083	0.265*
LJL	−0.125	0.073	0.230*
9-10	0.020	0.017	0.221*
4-12	−0.191	0.086	0.201*
5-10	−0.102	0.039	−0.197*
SL	0.091	−0.016	−0.196*
4-5	−0.094	0.169	−0.185*
ED	−0.126	−0.017	0.166*
5-9	−0.002	−0.079	0.161*
7-9	0.044	0.107	−0.152*
8-9	0.138	0.125	0.142*
PPCL	−0.101	−0.019	−0.137*
3-13	0.077	−0.127	0.128*
5-6	−0.090	0.065	0.109*

ecological niches to niches. In spite of four stocks possessed a sole environment that fluctuates from other streams of Bangladesh. However, owing to trivial ecological dissimilarities, the subsequent morphological changes in fish may be so minor that they might be difficult to distinguish with gross morpho-meristic features [23–25]. Hence, truss network dimensions were engaged in this experimentation. Truss network structures are influential implement for recognizing stocks of certain fish species [7].

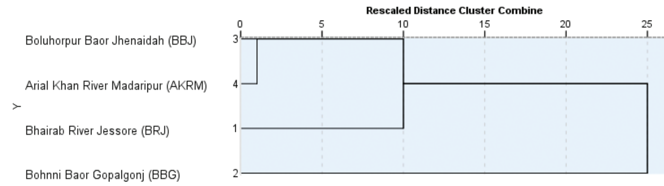

Figure 4. UPGMA dendrogram of four *X. cancila* stocks was developed by using morphometric and truss network measurements.

Environmentally induced phenotypic variations; however, may have rewards in the stock arrangement inquiry of exploited species, particularly when the time is inadequate for a momentous hereditary distinction to gather among inhabitants. Moreover, only genetic indicators might not be enough to perceive current genetic disparity among inhabitants of fish species, and also only a small quantity of DNA is examined by heritable indicators. Associations among the four stocks varied bestowing to whether the first, second, or third DF was measured. The first DF considered for 49.2%, the second DF measured for 33%, and the third DF indicated for 17.8%. It is noticeable that the second DF elucidates much less of the difference than does the first DF. Again third DF elucidates less of the modification than does the second DF. The third DF is, therefore, much less explanatory in elucidating transformations among the stocks. The alteration between the *baor* and river stocks may have been owing to ecological, as well as heritable disparities.

Conclusion

The superior stocks were observed in two stocks, namely BBG and BRJ. However, the remaining stocks BBJ and AKRM were showed intermingled condition according to varying proportion. Hence, the importance of the learning is valuable as baseline evidence of *X. cancila* populations for additional enquiries. This knowledge is very much crucial for both aquaculture and open-water fisheries supervision. However, it is indispensable to choice genetically loftier stocks laterally with healthier features. Plethora of research, specifically genetic readings and inquiries of the impacts of ecological aspects, is desirable for preservation and mass seed fabrication of designated stocks to overlay the way to saving this endangered species from extermination.

Acknowledgments

The authors are grateful to all fishermen during the sample collection from four stocks. Additionally, the authors also wish to acknowledge the laboratory assistant during image collection.

Conflict of Interest

The authors acknowledged no probable struggles of curiosity with respect to the investigation, authorship, and publication of this object.

Authors' Contribution

Md. Sarower-E-Mahfuj, Md. Motiur Rahman, and Monirul Islam implemented the study design. Md. Sarower-E-Mahfuj, Md. Motiur Rahman, and Ripon Kumar Adhikary participated in data collection. Md. Sarower-E-Mahfuj, Monirul Islam, Alok Kumar Paul, and Ripon Kumar Adhikary performed all the tests. Md. Sarower-E-Mahfuj, Monirul Islam, and Alok Kumar Paul drafted; Md. Sarower-E-Mahfuj, Md. Abdus Samad, and Ripon Kumar Adhikary revised the manuscript. Md. Sarower-E-Mahfuj and Md. Motiur Rahman critically checked the article and corrected the script. All authors read and approved the latest version of this manuscript.

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