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A multivariate morphometric investigation to delineate the stock structure of *Labeo calbasu* (Cypriniformes - Cyprinidae)

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Abstract
The present investigation was conducted to delineate the stock structure of *Labeo calbasu* based on morphometric characters. A total of 178 specimens collected from five different locations of river Narmada and Mattur dam of river Cauvery, located in Madhya Pradesh and Tamil Nadu respectively during October 2015 to February 2016. Extracted morphometric data subjected to Univariate ANOVA, discriminate function analysis (DFA), multivariate ANOVA, and principal component analysis (PCA). In DFA, the assignments of individuals 70.78% correctly classified. SDFA classification matrix has been able in showing an accurate classification in 70.7865% cases. It is found that Mettur dam and Khandawa stocks are more similar with 50% accuracy. Six cases of Hosangabad stock (with 85.714% accuracy) predicted as Jabalpur stock and two cases predicted as Baitul stock by the model while Baitul stock and Khandawa stock reveal 61.538% and 78.947% classification accuracy respectively. A scatter plot of canonical scores for Root 1 and Root 2 reveal more discrimination among the populations. The results reveal different stocks of *Labeo calbasu* in the rivers of Narmada and Cauvery.

Keywords: *Labeo calbasu,* Mattur dam, Allometric, stock and population

Introduction
Labeo calbasu (Hamilton, 1822) is one of the major Indian carp widely distributed in India [1]. It is an important food fish and commonly known as “Black rohu / Karnataka labeo/ Orange-fin labeo" in several places [2]. It is an important sports fish in the ponds where it can be cultivate along with other species. It shows better growth in ponds and lakes than in running waters, and also it can tolerate slightly brackish water [3]. It does not breed in ponds and other lentic water bodies; induced bred by hypophysation. It is primarily a bottom feeder and can grow up to 90 cm in length [4]. Morphometric differences among stocks of a species play a vital role in identifying stocks and studying the population structure [5-7]. Morphometric analysis is one of the powerful tools for stock identification, despite problems with interpreting phenotypic characters and the study of morphological characteristics, with the aim of defining or characterising fish stock units, has for some time been of intense interest in ichthyology [8]. ‘Fish stock’ is a local population adapted to a particular environment which has genetical differences from other population and interlinked by a set of ecological and genetic characters [9].

There are several tools has been used for identification of stocks such as meristics and morphometrics, otolith chemistry, molecular genetics, traditional tags, parasites as natural tags, and electronic tags. Among which the study of morphometric traits is one of the most commonly used and cost-effective methods. A system of morphometric measurements called the truss network system [10] has been increasingly employed for stock identification to reduce the inherent weaknesses of traditional morphometric methods. Which essentially discriminates ‘Phenotypic stocks,’ that are groups of individuals with similar growth, mortality and reproductive rates [11]. Morphometrics includes the analysis of body shape or the shape of particular morphological features of various body dimensions or parts. These data are continuous and must be corrected for size differences among specimens. The morphometric expression is under the simultaneous control of genetic and environmental factors [12]. The objective of the study is to differentiate the stocks of *Labeo calbasu* between the river Narmada and Cauvery based morphometric traits by using truss network analysis.

Materials and Methods

Collection of Samples

During October 2015 to February 2016, total 178 specimens (*L. calbasu*) collected from 5 different locations in the state of Madhya Pradesh and Tamil Nadu, India. The fish were ranged 10.51-24.42 cm in standard length (SL). Twelve morphometric characters of the fish were considered for truss analysis (standared length, head length, pre-dorsal length, post dorsal length, post anal-fin length, pre-anal fin length, pre-pectoral fin length, fringed lip length, dorsal fin base length, pelvic fin base length, anal fin base length and pre-anal to pre-pelvic length). Nine landmarks delineating 12distances were measured onthe body (Figure 1). By placing a fish on graph paper, each landmark was obtained, and then landmarks were detected with colored pointers. A linear combination of two software [13] and Paleontological Statistics (PAST) [14] was used to extract morphometric data from the images of each.

Fig 1: An Indicated landmark for extraction of morphometric data

Morphometric Data

Eleven morphometric characters scaled to the standard length of specimens were subjected to descriptive analysis (mean, minimum, maximum, and SD). Variability plots of these traits were also plotted to represent the variables graphically. The significant morphometric difference among the traits was discerned by using. Multivariate analysis of variance (MANOVA) and Statistica (Statistica 12) software operates on both balanced and unbalanced designs was used. A significant source of morphometric variations detected by GLM (general linear models) procedures. The following mathematical model used for quantifying the effect of various factors on morphometric changes on different species.

Yijl = μ+Aijl+Zi+Sj+eijl

Where,

Yijl = observation of lth individual from jth sex and ith species

μ = general mean

Aijl = regression coefficient of standard length on traits

Zi = fixed effect of species

Sj = fixed effect of sex

Eijl = random error associated with Yijl

Factor analysis performed for eight morphometric characters, among them 5 shows differentiation at the threshold value (0.7) so morphometric characters loaded above the threshold value (0.7) were selected for forward stepwise discriminant analysis. Statistica forward (Statistica 12) procedure used for further stepwise selection [15] and determine the combination (discriminant function) of the responses which best described each stock. Each observation was assigned a probability of belonging to a given stock using the distance of its discriminant function of the mean of each class. A total of 5 morphometric characters sorted after factor analysis was used for forward stepwise discriminant analysis and classification matrix and scatter plots generated.

Statistical Analysis

The size effects from the dataset eliminated before analysis. 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 measured characters and the SL of the fish. Data analyses were carried out for morphometric characters since these variables differ with geographical location. Size-dependent variables removed by using an allometric approach [16]. Data transformed using the following formula.

Madj = M (SLmean / SL) ^{b}

Where,

Madj = transformed morphometric measurement

M = original morphometric measurement

SL = standard length of fish

SLmean = combined mean standard length for stocks

B = within group slope of mean regression of log M against log SL

Result

In the present investigation, the mean value of the morphometric variables of samples from all locations was significantly different from each other (Table 1). The canonical value in the discriminant function analysis reflected that there was a significant morphometric variation in between samples of all locations. The general statistical data of morphometric measures comprising maximum, minimum, standard deviation and mean value of variables for each sample of all location presented in Table 1.

The factor loading worth discussed and classified into three categories (a) if factor loading equal or above than 0.3 is considered a significant. (b) If factor loading equal or above than 0.4 is considered more significant, and (c) if factor loading equal or more than 0.5 is considered very significant.

In present investigation factor loading considered > 0.5 which reveals that nine morphometric measures (Table 2) able to discriminate the population of *Labeo calbasu* of Baitul, Mettur dam, Hosangabad, Khandwa and Jabalpur stock So only nine variable selected for stocks discrimination. The selected important traits further taken for Stepwise Discriminate Function Analysis (SDFA) and Principal Component Analysis (PCA).

The relative importance of these integrated variables in the model uttered by their loading on functions (Roots). PoAL/SL have highest factor loadings in both the Roots stresses upon its higher demonstrating ability to recognise or draw fine distinctions in power compared to other variables. Remaining of the incorporated variables showed more or less equal contribution to stock differentiation (Table 2).

Table 1: Descriptive statistics of different morphometric variables of five *Labeo calbasu.*

	Group	SL	HL	PrDL	PoDL	PoAFL	PrAFL	PrPFL	FLL	DFBL	PvFBL	AFBL	PATPPvL
Baitul stock	max.	24.427	5.323	13.243	18.478	21.894	20.364	13.082	1.951	6.510	1.486	2.218	7.778
	mini.	15.728	2.574	8.354	11.872	13.869	12.860	8.836	0.855	4.192	0.614	1.320	4.328
	Mean	20.075	3.983	10.462	15.085	17.903	16.436	11.107	1.343	5.311	0.959	1.839	5.598
	st.dev.	2.662	0.765	1.272	1.865	2.279	2.160	1.218	0.275	0.695	0.225	0.225	1.024
Hosangabad stock	max.	29.801	6.863	16.231	22.063	26.399	24.039	17.529	2.690	8.551	2.084	3.349	8.280
	mini.	16.211	3.368	8.270	12.015	14.460	13.062	9.103	1.035	4.287	0.601	1.563	4.085
	Mean	26.529	5.767	13.499	19.277	23.279	21.283	15.018	1.809	6.650	1.204	2.387	6.513
	st.dev.	2.725	0.638	1.495	2.074	2.381	2.218	1.572	0.349	0.848	0.323	0.440	0.815
Khandwa stock	max.	15.559	3.283	8.000	12.000	13.827	12.928	8.670	0.993	4.656	0.980	1.638	4.522
	mini.	10.512	2.158	5.373	8.096	9.667	8.756	5.793	0.531	3.066	0.474	0.855	3.061
	Mean	13.455	2.681	6.909	10.201	12.002	10.944	7.253	0.803	3.832	0.706	1.286	3.845
	st.dev.	1.236	0.252	0.690	0.942	1.074	1.001	0.692	0.121	0.355	0.115	0.191	0.357
Mettur dam stock	max.	13.856	3.496	7.105	10.697	12.661	11.858	7.898	0.918	3.980	0.898	1.699	4.489
	mini.	11.345	2.332	6.094	8.704	9.898	8.822	5.936	0.482	3.248	0.552	1.106	2.938
	Mean	13.156	2.782	6.746	9.943	11.794	10.783	7.269	0.806	3.747	0.785	1.352	3.769
	st.dev.	0.915	0.323	0.338	0.656	0.991	1.039	0.706	0.134	0.203	0.090	0.170	0.478
Jabalpur stock	max.	14.529	2.954	7.352	10.654	12.556	11.513	7.666	1.056	4.060	0.877	1.621	4.409
	mini.	6.945	1.465	3.501	5.102	6.100	5.564	4.167	0.433	1.786	0.414	0.667	1.489
	Mean	10.959	2.240	5.631	8.172	9.623	8.721	6.031	0.725	2.935	0.703	1.060	2.843
	st.dev.	1.791	0.359	0.918	1.310	1.563	1.421	0.884	0.161	0.484	0.115	0.206	0.605
Baitul stock	max.	24.427	5.323	13.243	18.478	21.894	20.364	13.082	1.951	6.510	1.486	2.218	7.778
	mini.	15.728	2.574	8.354	11.872	13.869	12.860	8.836	0.855	4.192	0.614	1.320	4.328
	Mean	20.075	3.983	10.462	15.085	17.903	16.436	11.107	1.343	5.311	0.959	1.839	5.598
	st.dev.	2.662	0.765	1.272	1.865	2.279	2.160	1.218	0.275	0.695	0.225	0.225	1.024
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	Mean	26.529	5.767	13.499	19.277	23.279	21.283	15.018	1.809	6.650	1.204	2.387	6.513
	st.dev.	2.725	0.638	1.495	2.074	2.381	2.218	1.572	0.349	0.848	0.323	0.440	0.815
Khandwa stock	max.	15.559	3.283	8.000	12.000	13.827	12.928	8.670	0.993	4.656	0.980	1.638	4.522
	mini.	10.512	2.158	5.373	8.096	9.667	8.756	5.793	0.531	3.066	0.474	0.855	3.061
	Mean	13.455	2.681	6.909	10.201	12.002	10.944	7.253	0.803	3.832	0.706	1.286	3.845
	st.dev.	1.236	0.252	0.690	0.942	1.074	1.001	0.692	0.121	0.355	0.115	0.191	0.357
Mettur dam stock	max.	13.856	3.496	7.105	10.697	12.661	11.858	7.898	0.918	3.980	0.898	1.699	4.489
	mini.	11.345	2.332	6.094	8.704	9.898	8.822	5.936	0.482	3.248	0.552	1.106	2.938
	Mean	13.156	2.782	6.746	9.943	11.794	10.783	7.269	0.806	3.747	0.785	1.352	3.769
	st.dev.	0.915	0.323	0.338	0.656	0.991	1.039	0.706	0.134	0.203	0.090	0.170	0.478
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	mini.	6.945	1.465	3.501	5.102	6.100	5.564	4.167	0.433	1.786	0.414	0.667	1.489
	Mean	10.959	2.240	5.631	8.172	9.623	8.721	6.031	0.725	2.935	0.703	1.060	2.843
	st.dev.	1.791	0.359	0.918	1.310	1.563	1.421	0.884	0.161	0.484	0.115	0.206	0.605

Abbreviation: SL: standard length, HL: head Length, PrDL: Pre-Dorsal Length, PoDL: Post-Dorsal Length, PoAFL: Post Anal Fin Length, PrAFL: Pre-Anal Fin Length, PrPFL: Pre-Pectoral Fin Length, FLL: Fringe Lip Length, DFBL: Dorsal Fin Base Length, PvFBL: Pelvic Fin Base Length, AFBL: Anal fin base length, PATPPvL: Pre- Anal to Pre-Pelvic Length

Table 2: Factor structure matrix for the five *Labeo calbasu* stock (pooled within group correlations)

Variable	Factor Loadings (Varimax raw) Extraction: Principal components (Marked loadings are >.500000) Factor 1	Factor 2
HL/SL	0.017697	-0.575163
PrDL/SL	0.514256	-0.074115
PoDL/SL	0.722160	0.504247
PoAFL/SL	0.869950	0.005159
PrAFL/SL	0.838671	-0.149194
PrPL/SL	0.221241	-0.813149
FLL/SL	0.074050	-0.533572
DFBL/SL	0.368365	0.731179
PvFBL/SL	0.092511	0.314579
AFBL/SL	0.157759	0.207290
PATPPvL/SL	0.553274	0.574228
Expl.Var	2.776151	2.565057
Prp.Totl	0.252377	0.233187

SDFA classification matrix has been able in showing an accurate classification in 70.7865% cases. Moreover, revealed that Mettur dam stock (with 50% accuracy) have a more common characteristic with Khandawa stock and Jabalpur stock (with 57.894% accuracy) sharing a common characteristic with all other stock except mettur dam stock (Table 3). Six cases of Hosangabad stock (with 85.714% accuracy) predicted as Jabalpur stock and two cases predicted as Baitul stock by the model while Baitul stock and Khandawa stock reveal 61.538% and 78.947% classification accuracy respectively.

Table 3: Classification matrix generated by SDFA model for five *L. calbasu* stocks.

Group	Classification Matrix Rows: Observed classifications Columns: Predicted classifications Percent Correct	Baitul stock p=.14607	Hosangabad stock p=.31461	Khandwa stock p=.21348	Mettur dam stock p=.11236	Jabalp stoc p=.213
Baitul stock	61.53846	16	2	4	0	
Hosangabad stock	85.71429	2	48	4	0	
Khandwa stock	78.94736	3	0	30	4	
Mettur dam stock	50.00000	0	0	10	10	
Jabalpur stock	57.89474	3	6	6	1	
Total	70.78651	24	56	54	15	

An instance of misclassification between Khandawa stock and Metter dam indicated toward the morphometric propinquity of these stock which is further corroborated by the lower Squared Mahalanobis distance (2.22801) followed by Hosangabad stock and Jabalpur stock by Mahalanobis distance 2.7723. The Hosangabad stock was found to be most distant to Khandawa stock in morphometric terms as the value of squared Mahalanobis distance was recorded maximum for the pair (Table 4). In SDFA Wilks' lambda test revealed major difference in seven morphometric characters in all five stocks and among these seven characters, five characters were highly significant (p<0.00001) (Table 5).

Table 4: Squared Mahalanobis distance between five stocks of *L. Calbasu.*

Stock	Squared Mahalanobis Distances (anil d (B2:FW179)) Baitul stock	Hosangabad stock	Khandwa stock	Mettur dam stock	Jabalpur stock
Baitul stock	0.000000	9.57600	3.44603	5.95136	2.772303
Hosangabad stock	9.576002	0.00000	14.26484	13.84356	6.153437
Khandwa stock	3.446025	14.26484	0.00000	2.22801	4.935308
Mettur dam stock	5.951356	13.84356	2.22801	0.00000	6.175011
Jabalpur stock	2.772303	6.15344	4.93531	6.17501	0.000000

Table 5: Discriminant Function Analysis summary of five stocks *L. Calbasu.*

N=178	Discriminant Function Analysis Summary Step 9, N of vars in model: 9; Grouping: Stock (5 grps) Wilks' Lambda: .15283 approx. F (36,620)=11.209 p<0.0000 Wilks' Lambda	Partial Lambda	F-remove (4,165)	p-level	Toler.	1-Toler (R-Sqr.
DFBL/SL	0.177084	0.863039	6.546247	0.000065	0.234615	0.76538
PATPPvL/SL	0.189816	0.805150	9.982672	0.000000	0.123694	0.87630
HL/SL	0.177627	0.860401	6.692790	0.000051	0.787065	0.21293
PoDL/SL	0.182676	0.836619	8.055578	0.000006	0.155598	0.84440
PrAFL/SL	0.185897	0.822121	8.925119	0.000002	0.091722	0.90827
PrPL/SL	0.175932	0.868689	6.235331	0.000107	0.141446	0.85855
FLL/SL	0.164077	0.931452	3.035683	0.019007	0.812029	0.18797
PrDL/SL	0.160375	0.952955	2.036426	0.091620	0.311967	0.68803
PoAFL/SL	0.157162	0.972440	1.169088	0.326370	0.517853	0.48214

The adequacy of the considered variables in species discrimination and prognostic or assortment power of the model is reflected by canonical scores in the scatter plot where different cases of the stock grouped and isolated from the clusters of the other stock. In present investigation Khandawa stock well isolated from Hosangabad stock and Mettur dam stock reveal overlapping with Khandava stock (Figure 2).

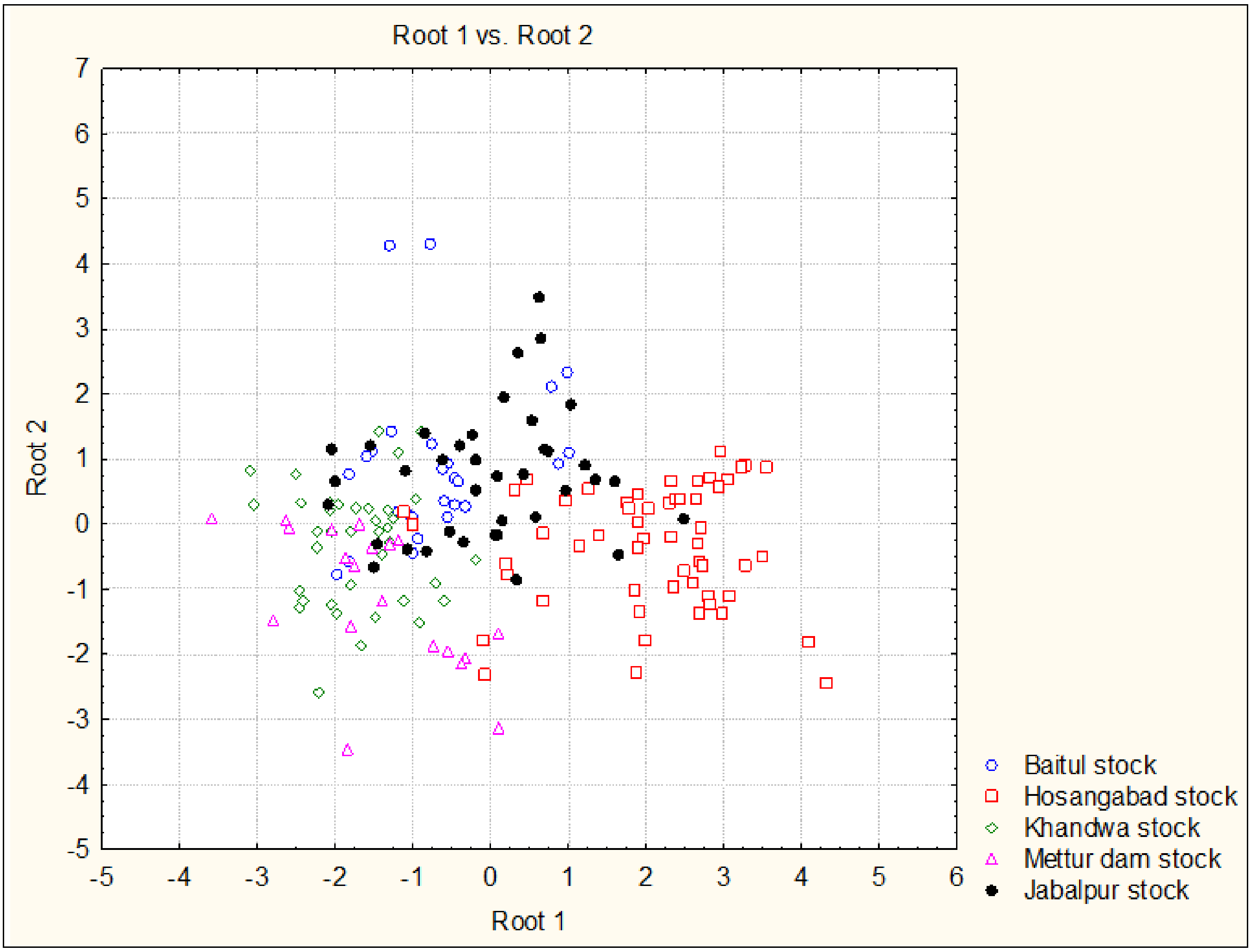

Fig 2: Scatter plot of the canonical score for Root 1 and Root 2 of morphometric variables of five stocks of *L. Calbasu.*

The first principal component (PC1) described 38.459%, and PCA2 described 20.68% variation along with 0.001305 and 0.0007019 Eigenvalue for PC1 and PC2 respectively (Table 6).

Table 6: Eigen value and percentage of variance for principal component analysis of five stock of *L. calbasu.*

PC	Eigen value	% variance
1	0.00130514	38.459
2	0.000701934	20.684
3	0.000452889	13.345
4	0.000245625	7.2379
5	0.000235361	6.9355
6	0.000198729	5.856
7	0.000121246	3.5728
8	7.88658E-05	2.324
9	3.02849E-05	0.89242
10	1.57963E-05	0.46548
11	7.69862E-06	0.22686

The scatter plot has plotted for visual investigation in between PC1 and PC2 score which revealed the relationship between the morphometric variables and it is self-explanatory also (Figure 3). Screen plot also has been plotted (Figure 4) between the components and percentage Eigen value which revealed that component first and component second have maximum value by 38% and 20% on X- axis respectively (Table 6).

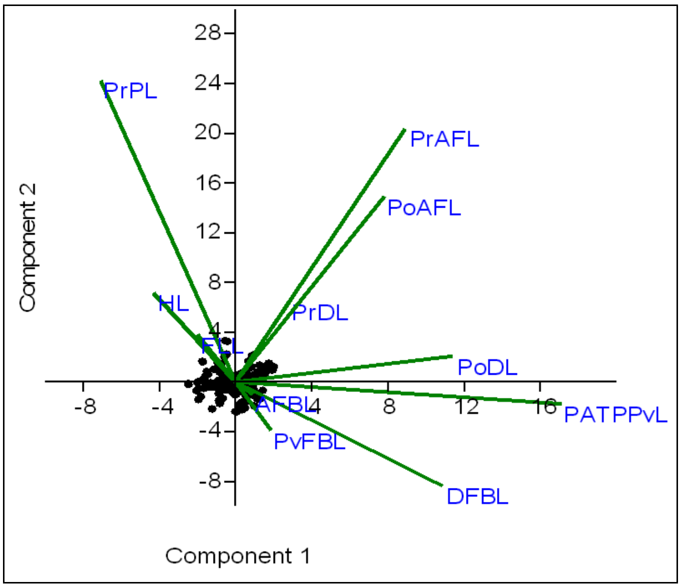

Fig 3: Scatter plot of the score from PC1 and PC2 for morphometric variables of five stocks of *L. calbasu.*

Discussion

One of the important contexts in the fisheries resource management is the identification of discontinuing fish stocks or population, which are defined as self-maintaining population, spatially or temporarily isolated from each other [5]. Morphometric discrimination among stocks expected because they are geographically isolated and may have originated from separate ancestors. Therefore, it is not unconvincing that distinguish environmental variations exist in these five habitats (Baitul, Hoshangabad, Khandawa, Jabalpur stocks of river Narmada, Madhya Pradesh and Mettur dam stock of Cauvery River, Tamil Nadu, India).

In general, fishes reveal higher degrees of difference within and between populations than the other vertebrates and are more sensitive to environmentally-induced morphological variation [17, 18]. Such variation in morphology is generally due to the isolation of fragment of a population within local habitat conditions. Enough degree of separation may result in considered phenotypic differentiation among *L. calbasu* populations within a species, as a basis for discriminate and management of distinct populations [19]. Such separation can occur through different processes. For example, reproductive isolation [20], or by hydro-graphic characters that prevent or reduce migration among areas [21].

It reported that morphological characters can reveal a higher degree of plasticity in response to various environmental conditions, such as temperature and food abundance [22-24]. Because fishes are highly sensitive to environmental fluctuation and they adapt quickly by changing their behaviour to environmental changes and physiology. These changes ultimately modified their morphology [25].

The truss network tools can successfully be used to differentiate morphology among the five stocks of *L. calbasu*. In the present investigation, more significant variability expected because existing fish stock belongs from five isolated stocks from two rivers which reveals complete different habitats, i.e., four stocks from Narmada river of Madhya Pradesh and one stock (Mattur dam) from Cauvery river of Tamil Nadu. Relationships among the five stocks differed according to the root 1 and root two plot were considered (Figure 2). In the classification matrix (Table. 3**),** maximum classification percentage (85.714) in Hoshangabad stock and highest Squared Mahalanobis distance (Table 4) 14.264 in between Khandava and Hoshangabad and fallowed by Mettur dam and Hoshangabad (13.8435) have observed by using morphometric measurements.

With multivariate analysis (SDFA and PCA) the morphometric characters that best separated *L. calbasu* populations of Mettur dam and Hoshangabad stocks were identified. Especially the head size, post dorsal fin, post anal fin, pre anal fin, pre pectoral fin and dorsal fin base length appeared to discriminate the *L. calbasu* populations. Those characters reflect the feeding, swimming and foraging ability of the fish.

The phenotypic variation found in present investigation suggests a direct relationship between the extent of phenotypic divergence and geographic separation, which reveals that geographic isolation, is a limiting factor to migration among stocks. Similar results for *L. calabash* stock from the Hilda Rivers and the Yamuna and a hatchery stock in Bangladesh have been reported [26].

Truss matrix systems are a powerful tool for identifying fish stocks. An unbiased matrix of morphometric characters over a two dimensional outline of a fish removes the need to find the types of traits and a favourable number of characters or morphometric variables for stock discrimination and provides information over the entire fish structure or shape. The truss network tools can efficiently used to discrimination between the stocks.

The experimental phenotypic discrepancies among the *L. calbasu* individuals discovered their existence of five morphologically separated stocks *viz.* Baitul, Hoshangabad, Khandawa, Jabalpur stocks of river Narmada, Madhya pradesh and Metture dam stock of Kaveri River, Tamilnadu India. The phenotypic variations among the samples could be reveals as robust togetherness between the geographic partition and phenotypic disparity that might be an indicator of limited intermingling factor for migration among five populations.

The truss System for morphometric analysis can be successfully use to study stock discrimination within a species, as published for other species in freshwater and marine environments. In this investigation, the truss analysis revealed a distinct separation of *L. calbasu* stock observed from different rivers of India suggesting a need for a separate management plan to sustain the *L. calbasu* stock for future use. The investigation given in the current study can further be confirmed based on biochemical and molecular methods.

The application of genetic markers like mtDNA and microsatellite applications [27-29] along with morphometric investigation would be concrete methods to further analysis the genetic component of phenotypic discontinuity between geographic areas and to facilitate the evolution of management recommendations. The supplemental examination would offer further confirmation of the *L. calbasu* stock resolved in present investigation with the truss analysis. Based on the morphometric investigation, development of suitable guidelines for implementation of suitable mesh size in the river may help to sustain *L. calbasu* resource for the future use. The result of the present investigation is similar to those who reported phenotypic variation in *Macrognathus pancalus* from the Brahmaputra and Ganges river basin of India [30].

Conclusion

The present investigation gives straight forward information about the version of *L. calbasu* populations in the Baitul, Hoshangabad, Khandawa and Jabalpur stocks of river Narmada and Mettur dam stock of Cauveri River. It recommends that use of phenotypic characters generate authentic information for stock differentiation of *L. calbasu*, and fish collected from different sites of the various river in the present investigation belonged to different stocks. The finding of currents tudy in truss analysis revealed significant morphometric differences and thus the presence of five different morphological stocks of *L. Calbasu*. So the consequences of the present study would help as primary information of the *L. calbasu* stock management and enable effective management plan for the distinct stocks of *L. calbasu* populations to make its fishery sustainable and formulate appropriate conservation strategies in coming day.

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