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Intraspecific morphometric variability in the populations of *Barilius bendelisis* (Hamilton) from the Alaknanda basin of Central Himalaya

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Abstract
The present study observed variations in the morphological traits of hill trout, *Barilius bendelisis* (Hamilton, 1807) from Alaknanda basin of Central Himalaya. A total of 124 samples of *B. bendelisis* were collected from three different tributaries, Dugadda Gad, Khankhra Gad and Khandah Gad of Alaknanda river between March 2015 to April 2016. Ninety morphometric characters were measured for each specimen. Statistical tools, univariate analysis of variance (ANOVA), principal component analysis (PCA) and discriminant function analysis (DFA) were used to differentiate the populations of *B. bendelisis*. PCA extracted eight significant morphometric traits explaining 87.9% of total variation among the three populations. DFA revealed that 83.1% specimens were retained into their original groups. Environmental factors were attributed to phenotypic variations among closely related populations. The present study is a first attempt on stock structure of *B. bendelisis* from different tributaries of Alaknanda river. The study will help in future conservation and management of this fish species across Uttarakhand region, India.

Keywords: *Barilius bendelisis*, Hill trout, Intraspecific, Variability.

INTRODUCTION

The study of morphological characters in Ichthyology is of great importance for characterizing fish stocks (Cadrin, 2000). Local fish population (stock) adapts to particular environment and has genetic difference from other stocks (MacLean and Evans, 1981). According to Costa *et al.* (2003), phenotypic characters are more affected by the environmental influence rather than genetic characters. Biologists took interest in understanding the mechanism of phenotypic variations since the advent of Darwin's theory of evolution (Pfennig *et al*., 2010).

In order to overcome the weaknesses of conventional morphometric analysis, the truss network system (Strauss and Bookstein, 1982) has been used at present for the purposes of stock identification, which essentially discriminates 'phenotypic stocks', that are groups of individuals having similar phenotypic traits (Booke, 1981). *Barilius bendelisis* (Hamilton, 1807) has recently got attention as one of the important species for aquaculture (Suresh and Mandal, 2001). It comes under the family Cyprinidae and well distributed in the lotic water bodies of Himalayan region. So far fewer studies have been done on the morphometric and meristic counts of hill trout (Hazarika *et al*., 2011). Truss analysis revealed highly significant difference among 31 morphometeric parameters in the populations of snowtrout, *Schizopyge niger* (Heckel 1838) from Kashmir Himalaya (Mir *et al.*, 2013a). Mir *et al.* (2015) also used truss morphometry to study intraspecific variation in *B. bendelisis* from four rivers of Central Indian Himalaya. Miyan *et al.* (2015) observed significant differences in twenty four morphometric measurements by truss morphometry in *Clarias batrachus*, from Ganga river basin. Kaoueche *et al.* (2016) also found morphometric variations among the populations of white seabream *Diplodus sargus* (Linneus, 1758) populations along the Tunisian coast using 25 truss measurements and 6 traditional measurements. Truss based morphometrics was also effectively used to observe variations in populations of *Danio dangila* (Hamilton, 1822) from different areas of North-East India by Banerjee *et al.* (2017).

Therefore, the present study was aimed at to examine the morphometric variations in populations of *B. bendelisis* from different tributaries of Alaknanda river using truss network analysis. The study will be instrumental for effective conservation and management of the fish stocks in future.

MATERIALS AND METHODS

Sampling: The specimens of *B. bendelisis* were collected from the three different tributaries, Dugadda Gad, Khankhra Gad and Khandah Gad, of Alaknanda before the breeding season and after the spawning season, with the help of local fishermen by using gill net, cast net and traditional Thali method from March 2015 to April 2016. A total 124 fish samples were collected from different spring-fed tributaries, Dugadda Gad (42 specimens), Khankhra Gad (40 specimens) and Khandah Gad (42 specimens). 10% formalin was utilized to preserve the collected samples that were morphometrically analysed. The captured specimens were identified with the help of taxonomic keys of Day (1878), Talwar and Jhingran (1991), Mirza (1991) and Kullander *et al.* (1999).

Digitalization of specimens: After cleaning in running water the fish specimens were placed on a flat platform with graph paper as a back-ground. Fins were erected to make the origin and insertion points visible. Photographs were taken by means of a digital camera (Nikon D3400) from same angle and distance. Specific code was given to each specimen for proper identification.

Measurement of truss distances: The truss protocol used in the present study was based on fourteen landmarks (Mir *et al*., 2013b) and these landmarks were interconnected to get a network of 90 truss measurements (Fig. 2). A set of three softwares, tpsUtil, tpsDig2 v2.1 (Rohlf, 2006) and PAST (Paleontological Statistics; Hammer *et al.,* 2001) was used to measure the truss distances of the digital images.

Statistical procedure: An allometric method suggested by Elliott *et al.* (1995) was used to correct the size dependent variation as "$M_{adj} = M\,(Ls/L0)^b$" where M and M_{adj} represented original and the size adjusted measurements, respectively, L0 and Ls were used for the standard length and overall mean standard length of fish. *b* was slope of the regression of log *M* on log L0 for each character.

The extracted ninety morphometric characters were subjected to a univariate analysis of variance to compare the populations of the different sampled sites of *B. bendelisis*. ANOVA extracted the significant variables (p<0.05) which were later subjected to factor analysis. A maximum likelihood method of factor extraction was used. The significant measurements were also subjected to the discriminant function analysis (DFA) to calculate percentage of correctly classified fishes into their original groups. SPSS vers.20 and Microsoft Excel 2007 were used for statistical analysis of morphometric data.

RESULTS

Ninety variables were obtained by interconnecting the 14 landmarks in truss network system (Fig. 1). 8 significant (p<0.05) characters were extracted by ANOVA from 90 morphometeric measurements. Principal component analysis extracted 3 factors having Eigen-values 3.8, 2.1 and 1.0 respectively from 8 truss measurements, explaining 83.1 % of the total variance (Table 1). The truss distances with meaningful loadings on factor 1 were 1-2, 1-3, 1-4, 1-5 (Table 2), which explained 47.5% of the total variance. The second factor explained 27.0% of total variation from three variables, 1-8, 1-9, 2-8 which showed significant loadings on it. Remaining factor 3 explained 13.4% of the total variance and only one variable 1-10 had significant loading on it (Fig. 2). DFA classified 83.1% of specimens into their original

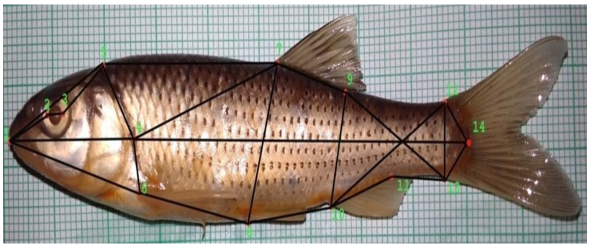

Fig. 1. *Truss network of Barilius bendelisis showing the 14 landmarks: 1 tip of snout; 2 anterior border of eye; 3 posterior border of eye; 4 posterior border of operculum; 5 forehead (end of frontal bone); 6 pectoral fin origin; 7 dorsal fin origin; 8 pelvic fin origin; 9 dorsal fin termination; 10 origin of anal fin; 11 termination of anal fin; 12 dorsal side of caudal peduncle, 13 ventral side of caudal peduncle; 14 termination of lateral line.*

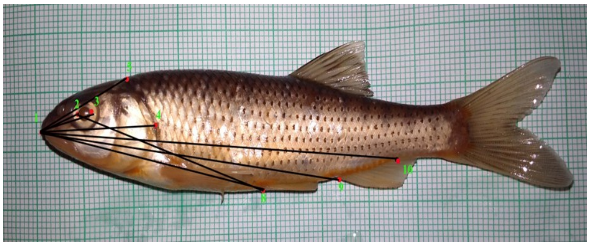

Fig. 2. *Eight morphometric measurements showing significant loadings on three principal components.*

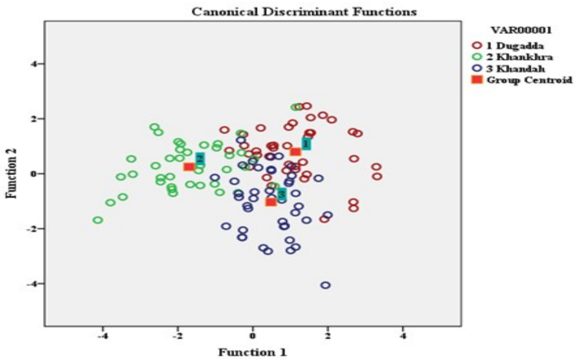

Fig. 3. *Discriminant analysis plot representing distance among stocks of of Barilius bendelisis from three tributaries of Alaknanda river.*

Table1. Three principal components explaining percentage of variance among morphometric measurements of *Barilius bendelisis*.

Component	Initial Eigen values			Extraction sums of Squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
PCA1	3.801	47.507	47.507	3.801	47.507	47.507
PCA2	2.160	27.004	74.511	2.160	27.004	74.511
PCA3	1.079	13.485	87.996	1.079	13.485	87.996

Table 2. Loadings of three principal components (Significant loadings are highlighted).

Component Matrix			
	Component		
	PC1	PC2	PC3
VAR 1-2	**0.955**	0.088	0.056
VAR 1-3	**0.957**	0.106	0.124
VAR 1-4	**0.850**	0.176	0.205
VAR 1-5	**0.900**	0.222	0.102
VAR 1-8	-0.164	**0.935**	0.218
VAR 1-9	0.027	**0.719**	-0.480
VAR 1-10	-0.390	-0.038	**0.846**
VAR 2-8	-0.509	**0.818**	0.117

groups. Wilks' Lambda test also showed highly significant variation in morphometric characters of *B. bendelisis* among three populations of Alaknanda river basin (Table 3). The percentage of correctly classified specimens was highest in Khankhra (85.0%) followed by Dugadda (83.3%) and Khandah (81.0%). These morphometric variables were lying between the snout and anterior end of anal fin. The discriminant function plot obtained by DFA showed the phenotypic heterogeneity among three populations of *B. bendelisis* from Alaknanda River. Small intermingling among the three populations was also found (Fig. 3).

DISCUSSION

Fishes generally show higher degrees of variation within and between populations as compared to other organisms and are also more prone to environmentally-induced morphological variations (Wimberger, 1992). Turan *et al.* (2004) reported that isola-tion may result into phenotypic and genetic differentia-tion among fish populations within a species. The results obtained from the present study indicated phenotypic plasticity among three populations of B. bendelisis from different streams of central Himalaya.

Discriminant function analysis (DFA) has been a useful tool to distinguish different stocks of the same species (Karakousis *et al.,* 1991). In the present investigation, 83.1 % of specimens were rightly classified into their respective groups by DFA. Wilks' Lambda test of DFA also proved significant morphometeric differences or heterogeneity among three populations of *B. bendelisis.* Turan *et al.* (2004) found significant morphometric heterogeneity among different populations of anchovy (*Engraulis encrasicolus)* from different areas of the Mediterranean Sea from DFA. Hossain *et al.* (2010) applied DFA and PCA to three populations of *L. calbasu* from three water bodies and reported environmental factors such as temperature, food availability, and local migration to be the causes of morphological variations among them. Similar findings were reported by Khan *et al.* (2012) in the populations of *Channa punctatus* from three Indian rivers.

The morphometeric variations among three populations of *B. bendelisis* from Alaknanda river could be due to variations in hydrological conditions and consequent phenotypic plasticity. The differences in current pattern, temperatures, turbidity, and land-use patterns among these drainages may result in to these variations. Some similar habitat attributes and their impacts may be associated with the closeness between stocks. Quilang *et al.* (2007) reported similar findings in silver perch *Leiopotherapon plumbeus* from three lakes in the

Table 3. Classification matrix of DFA and Wilks' Lambda based on truss morphometry (83.1% of original grouped cases correctly classified).

Predicted group membership				
Sampling Sites	Alaknanda River			Total
	Dugadda	Khankhra	Khandah	
Dugadda	35	3	4	42
Khankhra	3	34	3	40
Khandah	5	3	34	42
Cross validated results (%)				
Dugadda	83.33	7.14	9.52	100.0
Khankhra	7.50	85.00	7.50	100.0
Khandah	11.90	7.14	80.95	100.0
Wilks' Lambda				
Test of Function(s)	Wilks' Lambda	Chi-square	df	Significance level
1 through 2	0.250	165.650	8	0.000
2	0.620	57.057	3	0.000

Philippines. Poulet *et al.* (2004) also noted that the morphological characters of the fish are determined by environment and genetics, and the interaction between them.

Akbarzadeh *et al.* (2009) used 32 truss morphometric measurements to determine the variation among different stocks of pikefish (*Sander lucioperca)* from the southern Caspian Sea and found 20 significant morphometeric measurements. While in the present study only 8 significant morphometric measurements out of 90 reflected the phenotypic variations in the populations of *B. bendelisis* from the Alaknanda river indicating comparatively less variability in stocks.

The truss system has been effective for stock separation within a species. In the present study, the truss protocol also revealed separation of *B. bendelisis* wild stocks in the three tributaries of Alaknanda river which may be associated with variations in some hydrological conditions. However, it is suggested that the present observations can further be confirmed from molecular and biochemical methods. Molecular genetic markers such as microsatellite and mtDNA applications along with morphometric studies may also be effective methods.

Conclusion

The present study provided information about morphological variability among different populations of *B. bendelisis* from three different springfed tributaries of Alaknanda river of central Himalaya. Truss based morphometrics was found effective to differentiate the stocks. Variation in some of the hydrological conditions in tributaries was attributed to the differences in the stocks. There is a need to frame separate management strategies in order to sustain the stocks for future use.

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