Fishery Technology 52 (2015) : 205 - 212

Meristic and Morphometric Differentiation in Wild Populations of *Barilius bendelisis* (Hamilton 1807) from Kumaon Region of Uttarakhand, India

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Abstract

In this study, differentiation in the morphological traits of an important ornamental cyprinid fish, *Barilius bendelisis* (Hamilton, 1807) was investigated. A total of 134 individuals were collected from River Gaula and Kosi between November 2013 to March 2014 in Uttarakhand region of Central Himalaya; 6 meristic and 24 morphometric characteristics were recorded for each specimen. Principal component analysis (PCA), discriminant function analysis (DFA) and univariate analysis of variance (ANOVA) were used for differentiating the population. 16 significant morphometric variables ($p<0.001$) were considered for multivariate analysis. The principal component (PC) for 16 morphometric variables generated seven components accounting for 71.84% of the total variation between the populations. First principal component alone accounted for 35.24% of total variation. The step wise discriminant analysis retained one factor showing highest variation in body depth, length of pectoral fin, dorsal fin base length, sub orbital width, head length and snout length. Using these variables, 82.7% of individuals were retained into their original groups (82.7% under a 'leave-one-out' procedure). This study hypothesizes that the phenotypic variation between these close populations could be attributed to environmental and genetic factors.

Keywords: Morphometric analysis, descriptive statistics, *Barilius bendelisis*

Received 12 January 2015; revised 03 October 2015; Accepted 12 October 2015

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Introduction

Species characterization is a fundamental step in the field of research. Of late, several methods have been developed to discriminate between species, subspecies and populations, including various molecular methods like mitochondrial and nuclear DNA sequence polymorphisms (Whitfield et al., 2006; Sah et al., 2011). However, molecular methods require relatively expensive laboratory equipment and reagents (Francisco et al., 2008).

Morphometric analysis is most commonly used method for identifying and discriminating the populations because of its practicability and low cost (Francisco et al., 2008). Morphometric analysis methods are generally based upon multiple measurements of various body parts that are assessed across several individuals (Rattanawannee et al., 2012). For measuring discreteness and relationships among stocks, meristic and morphometric characters are considered as powerful tools (Ihssenet al., 1981; Melvin et al., 1992). During early days, descriptive statistics and univariate analysis were independently performed on each meristic and morphometric character, however, they not always yield efficient and accurate results (Surre et al., 1986). Thus, multivariate techniques such as factor analysis, cluster analysis, and discriminant analysis, make morphometric analysis more accurate, precise and practical for discriminating the fishes at population level and have been widely adopted by several authors in the study of population structure of fishes (Hedgecock et al.,1989; Mamuris et al.,1998; Trapani, 2003).

Hill trout, *B. bendelisis* (Hamilton, 1807), has recently drawn attention as one of the potential candidate species for aquaculture (Suresh & Mandal, 2001). It belongs to the family cyprinidae and is commonly

distributed in hilly streams and rivers (25°C) of Himalayan region. As per IUCN red list (2012) this fish has been categorized as least concern (IUCN 2012) but in rivers of Himalayan region it falls under the category of endangered (Kurup et al., 2004). It plays a major role in the capture fishery in several parts of the Himalayan region of Arunachal Pradesh, inhabiting shallow flowing, clear and sometimes lentic water bodies where carps cannot be raised successfully (Sahooet al., 2009). As a potential candidate species for aquaculture, very limited studies have been done for morphometric and meristic count of the hill trout (Hazarika et al., 2011). The present study is, therefore aimed to examine the pattern and extent of morphological and meristic variation in populations of *B. bendelisis* from two rivers of Kumaon region of Uttarakhand using multivariate methods.

Material and Methods

A total of 134 specimens of *B. bendelisis* were collected between November 2013 and March 2014 by using a cast net from Rivers Gaula and Kosi of Kumaon region of Central Indian Himalayas. The choice of these sites was determined by ease of access and the abundance of the species. The geographic coordinates, maximum and minimum length weight and the numbers of individuals used in the present study are given in Table 1. Specimens were identified in the field based on external marking (vertical bands on the body) (Talwar & Jhingran, 1992). Twenty four morphometric and 6 meristic characteristics were measured from the specimen (Fig. 1). All measurements were taken with digital caliper (Mitutoyo digital calliper) to the nearest 0.1 mm and weighed with digital weighing balance (CITIZEN CG2201) up to nearest 0.1g.

Statistical analyses was done using Excel and SPSS 16.0 software. To remove the effect of size and sex all morphometric characters were transformed using allometric adjustment (Reist, 1985; Quilanget al., 2007).

$e = log\ Y - \beta\ (logX - logX_{STL})$ Where 'e' is the adjusted measurement, 'Y' is the original unadjusted measurement, β is the allometric coefficient (the slope of the log Y against log X plot for each population), 'X' is the standard length (SL) of the specimen, 'X_{STL}' is the mean SL of the specimen examined and log is the base 10 logarithm. Total length, fork length and standard length were excluded for multivariate analysis as standard length was used for transformation and total length and fork length are directly related to standard length (Quilang et al. 2007; Miret al. 2014).

Meristic characters were compared using the non-parametric Kruskal–Wallis test. To evaluate the statistical significance of individual morphological characters among the groups, Univariate analysis of variance (ANOVA) was performed for each morphological character.

In the present study principal component analysis (PCA) and discriminate function analysis (DFA) were used to evaluate the variation among population and to test the effectiveness of the characters in predicting different group locations, respectively. Stepwise discriminant analysis was done to reduce the number of variables (Poulet et al., 2004; 2005; Konana et al., 2010). The Wilks' Lambda was used to compare the differences between and among the population. A cross-validation was done to estimate the expected actual error rates of the classification functions (Mir et al., 2013a).

Results and Discussion

Fishes ranging from 8.10-14.90 cm in length and 4.21-36.82 g in weight were used in the study. Descriptive statistics for each of the meristic and morphometric variables is given in Table 2 The morphometric measures from both the sexes do not differ significantly. Hence, both the sexes were pooled for further analysis. Analysis of variance showed that fish samples between the two populations differed significantly ($p<0.001$) in 15 transformed morphometric characters and was non-significant for all the meristic characters (Table 2). Multivariate analysis was done using the significant

Table 1. GPS coordinates altitude (masl; meters above sea level), number of samples, min-max length and weight of *Barilius bendelisis* in Uttarakhand region of Central Indian Himalaya

Parameters	Rivers (Sites)	
	Gaula (Ranibagh)	Kosi (Ratighat)
Latitude ^{0}N	$29^0$17′25′′	$29^0$27′48′′
Longitude ^{0}E	$79^0$32′43′′	$79^0$28′81′′
Altitude (masl)	595	1033
Number of samples	53	80
Min-Max TL (cm)	8.1-14.9	8.5-14.7
Min-Max BW (g)	5.04-25.8	4.21-36.8

Table 2. Definition of meristic counts and morphometric measurements (cm), range mean±standard deviation (in parentheses), and F values (derived from analysis of variance) examined in wild populations of *Barilius bendelisis*

PARAMETER	KOSI RIVER	GAULA RIVER	F Value
Morphometric measurement (cm)			
Total length (TL)	(8.50 - 14.70) 11.10 ± 0.19	(8.10 - 14.90) 10.35 ± 0.21	NI
Fork length (FL)	(1.50 - 13.60) 10.04 ± 0.21	(7.40 - 13.50) 9.41 ± 0.18	NI
Standard length (SL)	(7.00 - 12.90) 9.24 ± 0..17	(6.40 - 12.00) 8.42 ± 0.17	NI
Body depth (Bd)	(1.30 - 3.20) 2.14 ± 0.04	(1.30 - 2.80) 1.88 ± 0.04	16.30*
Body weight (Bwt) (g)	(4.21 - 36.82) 13.40 ± 0.79	(5.04 - 25.86) 10.25 ± 0.68	NI
Snout length (SnL)	(0.40 - 1.40) 0.64 ± 0.02	(0.40 - 1.70) 0.64 ± 0.03	0.55 NS
Head length (HL)	(1.20 - 3.0) 1.82 ± 0.03	(1.00 - 2.60) 1.93 ± 0.04	3.23 NS
Dorsal depth (Dd)	(0.90 - 2.90) 1.86 ± 0.05	(1.00 - 2.90) 1.64 ± 0.05	6.40*
Length of dorsal fin base (LDFB)	(0.70 - 1.80) 1.14 ± 0.03	(0.60 - 1.70) 0.90 ± 0.03	17.79*
Length of anal fin base (LAFB)	(0.60 - 1.70) 1.08 ± 0.02	(0.60 - 2.00) 0.94 ± 0.04	8.10*
Length of pectoral fin (PFL)	(1.00 - 2.50) 1.67 ± 0.04	(1.00 - 2.70) 1.58 ± 0.05	1.77 NS
Length of pelvic fin (PVFL)	(0.60 - 1.90) 1.26 ± 0.03	(0.70 - 2.00) 1.15 ± 0.04	3.70 NS
Straight lateral line length (SLL)	(5.20- 9.40) 6.99 ± 0.12	(5.00 - 9.30) 6.44 ± 0.14	8.26*
Pre dorsal length (PrDL)	(3.60 - 7.00) 5.01 ± 0.09	(3.60 - 6.70) 4.67 ± 0.10	5.25*
Pre pelvic length (PrPVL)	(3.30 - 6.10) 4.58 ± 0.08	(3.10 - 6.10) 4.15 ± 0.09	11.16*
Pre pectoral length (PrPL)	(1.60 - 4.5) 2.42 ± 0.06	(1.60 - 3.40) 2.19 ± 0.05	6.27*
Pre anal length (PrAL)	(4.70 - 8.50) 6.37 ± 0.12	(4.70 - 8.50) 5.86 ± 0.12	7.77*
Depth caudal peduncle (DCP)	(0.50 - 1.50) 0.87 ± 0.02	(0.50 - 1.30) 0.80 ± .02	2.79 NS
Length caudal peduncle (LCP)	(0.70 - 1.70) 1.06 ± 0.02	(0.06 - 2.00) 1.15 ± 0.03	4.27*
Upper jaw width (UJW)	(0.40 - 1.00) 0.53 ± 0.01	(0.40 - 1.90) 0.57 ± 0.04	1.53 NS
Sub orbital width (SOW)	(0.20 - 0.50) 0.35 ± 0.08	(0.20 - 0.70) 0.36 ± 0.16	0.60 NS
Eye diameter (ED)	(0.40 - 0.60) 0.48 ± 0.06	(0.40 - 0.70) 0.46 ± 0.08	2.52 NS
Pectoral to pelvic length (PPVL)	(1.40 - 2.60) 2.03 ± 0.03	(1.50 - 2.50) 1.87 ± 0.03	9.61*
Pelvic to anal length (PAL)	(1.10 - 3.00) 1.80 ± 0.04	(1.20 - 2.20) 1.62 ± 0.03	8.60*
Meristic count			
Lateral line scale (LLS)	(40-44) 41.98 ± 0.12	(40 -44) 41.78 ± 0.22	0.66 NS
Dorsal fin ray (DFR)	(7-8) 7.09 ± 0.03	(7-8) 7.16 ± 0.058	1.69 NS
Pectoral fin ray (PFR)	(12-13) 12.12 ± 0.03	(12-13) 12.09 ± 0.046	0.23 NS
Anal fin ray (AFR)	8.00 ± 0.00	8.00 ± 0.00	0.00 NS
Caudal fin ray (CFR)	(18-19) 18.35 ± 0.05	(18-19) 18.24 ± 0.07	1.60 NS
Pelvic fin ray (PVFR)	(8-10) 8.19 ± 0.06	(8-10) 8.19 ± 0.085	0.01 NS

NS: Not significant, NI: measurement not included for further analysis

* Significant at 5%

variables only. PCA of 15 variables yielded seven components accounting for 71.84% of the total variation. The first principal component accounted for 24.35% of total variance with highest loadings on PrPVL, PrAL, PrPL and SnL. Similarly, second principal component explained 10.89% of total variation with maximum loadings on Bd, SLL, and PrDL (Table 3). It is clear that meristic counts remained same in both the population irrespective of size difference ($p>0.05$; Kruskal-Wallis test).

A multivariate analysis of variance (MANOVA) found significant differences between the two population for the morphometric traits (Wilk's

Table 3. Component loadings of seven principal components for morphometric measurements in *Barilius bendelisis* from wild populations

Morphometric measurement	1 (24.35%)	2 (10.89%)	3 (9.61%)	4 (9.02%)	5 (7.20%)	6 (5.83%)	7 (4.94%)
PrPVL	.797	.250	.048	-.042	.034	.028	.110
PrAL	.791	.144	.152	-.253	-.111	.024	.150
PrPL	.733	-.029	.333	.205	-.122	.278	-.057
SnL	.531	.178	.018	-.167	.429	.290	-.162
Bd	.004	.766	.094	.006	.179	.224	.068
SLL	.115	.600	.271	-.125	-.105	.086	.120
PrDL	.470	.527	.348	.038	.122	-.204	-.058
PVFL	.051	.356	.804	-.195	.031	-.050	-.023
PFL	.264	.264	.650	-.173	-.132	.221	-.068
Dd	.203	-.059	.648	-.034	.118	.129	.159
DCP	-.347	.183	.489	.289	-.314	.271	.422
LCP	-.082	-.029	-.079	.879	-.183	.042	-.016
HL	.051	.310	-.185	.707	.287	-.330	.020
PAL	.242	.326	.350	-.487	-.135	.159	.315
UJW	.070	-.036	-.061	.033	.789	-.015	.096
ED	-.183	.154	.061	-.106	.638	.183	.336
PPVL	.277	.348	-.305	-.307	-.496	-.070	.324
LAFB	.092	.140	-.012	-.043	.191	.813	-.072
LDFB	.123	.009	.289	-.094	-.048	.802	.201
SOW	.129	.064	.075	-.054	.282	.029	.887

Character description is given in Fig. 1

Lambda = 0.56, Chi Square = 74.101, p<0.001). Factor analysis yielded one factor explaining 100% of variation in the samples by using the Varimax rotation. Majority of the values on this factor were positive, indicating the positive correlation between the variables within a factor. This relationship is expected as the variables loading on first factor belonged to the middle portion of the body and these traits grow proportionately. Another reason for positive loadings of variables may be, due to the rotation of the factors which helps to reduce the number of negative loadings to a minimum. The highest variation on this factor was due LCP, Bd, PFL, LDFB, SOW, HL, SnL and UJW (Table 3). Forward stepwise discriminant function analysis showed higher values (82.7%) for the overall allotment of individuals into their original populations and the cross-validation test results were comparable to the results obtained from principal canonical components (Table 4). The percentage of correctly classified fishes was highest in River Gaula (84.9%) with misclassified rate of 15.1%; however,

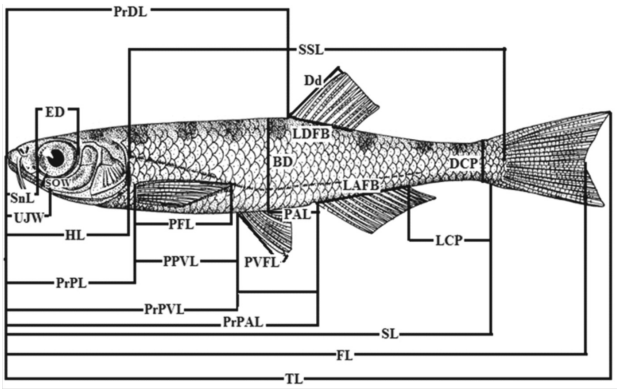

Fig. 1. Morphometric and Meristic characteristics of *Barilius bendelisis*

river Kosi showed 81.2% individuals into original groups and 18.8% were misclassified.

Generally, fish shows high degree of variation among all the vertebrates and are more susceptible to environmentally induced morphological variation (Wimberger, 1992; Mir et al., 2013a). However, the cause of morphological differences between the populations is difficult to explain (Cadrin, 2000). It has been suggested that these variation can be genetically related or might be associated with phenotypic plasticity in response to environmental conditions (Murta, 2000). The results of present study clearly indicate that there are two phenotypically different population of *B. bendelisis* in Kumaon region of Uttarakhand, India. The morphometric variability among these populations was mainly due to the variation of characters related to fins, body depth and body weight since effect of size was successfully eliminated by the allometric transformation and demonstrated by Univariate and Multivariate analysis. This could be attributed to different ecological condition of two rivers due to dam construction over the river and domestic as well as industrial effluent discharge into the river. The discrimination of stock within the water body may be because of the different abiotic and biotic factors like temperature, pH, salinity, food aviability, endemic flora and fauna condition etc which ultimately effect the morphometry of fish (Rohfritsch & Borsa, 2005). Water fragmentation by dam construction is also a commonly contributing factor for phenotypic differentiation of stocks. (Akbarzadeh et al., 2009; Turan et al., 2004). River Gaula receives the effluent of pulp and paper mill, and has a dam constructed over it at Kathgodam which affects fish biodiversity (Valdiya, 1991; Mir et al., 2015). River Kosi has a dam at Lalpur and most of the canal of the district depends on this river for irrigation which ultimately effects the fish diversity and local migration (Kumar & Bahadur, 2009; Mir et al., 2015). Quilang et al. (2007) found similar results in silver perch *Leiopotherapon plumbeus* from the three lakes in Phillipines. Turan et al. (2004) reported that phenotypic and genetic differentiation may occur among fish populations, which may be recognizable as a basis for separation and management of distinct populationns. These results are similar to the findings of Mir et al. (2013 a & b) in *Schizopyge niger*

Table 4. Pooled within-group correlations between the discriminatingvariables and the standardized canonical discriminant functions (DF) variables ordered by size of correlation within function

Morphometric measurement	Function DF1 (100%)
LCP*	.402
PFL*	.355
LDFB*	-.301
SOW*	.282
HL*	.280
Bd*	-.263
SnL*	.235
UJW*	.234
PrPL	.192
PrDL	.145
SLL	.119
PrPVL	.111
PVFL	.110
PrAL	.100
PPVL	.062
LAFB	.062
Bwt	.055
DCP	.051
PAL	-.029
ED	.027
Dd	.017
Eigenvalue	0.784

*Largest correlation between each variable and discriminant function.

Table 5. Percentage of specimens classified in each group and after cross validation for morphometric measurements for *Barilius bendelisis* from wild condition

Water bodies	Gaula River	Kosi River	Total
Original group (%)			
Gaula River	84.9	15.1	100.0
Kosi River	18.8	81.2	100.0
Cross validated (%)			
Gaula River	84.9	15.1	100.0
Kosi River	18.8	81.2	100.0

82.7% of original grouped cases correctly classified, 82.7% of cross validated grouped cases corrected classified

(Heckel, 1838) and *Labeo rohita* (Hamilton, 1822) and attributed this to the difference in the physical and ecological condition of water body. Similarly, Hossain et al. (2010) performed DFA and PCA to three populations of *Labeo calbasu* from the Jamuna and Halda rivers as well as a hatchery and reported morphological discrimination among them because of environmental factors as well as local migration of fish. In a similar line, Khan et al. (2013) studied *Channa punctatus* from three Indian rivers, where the environmental conditions showed a significant role in isolation, distribution and movement of fish stocks. Similar results were obtained in *Chromidotilapia guntheri* from the four tributaries of Tanoe River, Africa which were geographically close to each other (Boussou et al., 2010). Mir et al. (2013 b&c) found similar results in *Schizothorax richardsonii* (Gray, 1832) and *Schizothorax curvifrons* from Himalayan region. Patiyal et al. (2014) found similar result in *Tor putitora*.

Discriminant function analyses could be a useful method to distinguish different stocks of same species (Karakousis et al., 1993). In the present study, 82.7% of the individuals were correctly classified into their respective groups by discriminant analyses indicating intermingling among some of the specimens. This may be due to small geographic distances between these and migration of fish as both the rivers finally draw into the river Ramganga (a tributary of Ganga basin).

In conclusion, the present study provides basic information about the differentiation of *B. bendelisis* population in Uttarakhand region of Central Indian Himalayas using morphometric and meristic characters, which could be attributed to both rearing environment and genetic differentiation. However, the genetic basis of morphometric differences is not explored. Therefore, future studies on determination of population structure may be elucidated using the combined approach which may eventually lead to more precise description of variation among this species and may help in enhancement of natural stock and provide aquaculture sustainability.

Acknowledgements

Department of Science and Technology (DST), New Delhi, is greatfully acknowledged for providing fellowship to the first author in the form of DST Inspire Fellowship to carry-out her research work. We sincerely thank the Director, Directorate of Coldwater Fisheries Research (DCFR) for providing necessary facilities. Thanks are due to the Director, Central Institute of Fisheries Education for valuable suggestions.

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