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# Phenotypic disparity in the *Schizothorax richardsonii* of different geographic regions of the Lesser Himalayas

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**ABSTRACT**

The intraspecific variation of *Schizothorax richardsonii* was estimated by analysing the morphometric characters. *Schizothorax richardsonii* is an indigenous Himalayan fish species, generally dwelling in fast flowing snow-fed rivers (8-22°C), streams and lakes and contributes significantly to the cold water fishery in the Himalayan region. Specimens were collected from five sampling sites including four natural lotic water bodies of the state Uttarakhand and one farmed stock of the target fish that is Kosi River, Alaknanda River, Chirapani stream, Gaula River and captive stock of DCFR Farm, Bhimtal. Phenotypic study was performed using Truss Network of 14 landmarks making 31 characters which reported high degree of significant morphometric heterogeneity among the populations with size within landmarks and shape as major factors of divergence in the selected ecosystems. Between-groups Principal Component Analysis revealed that the first and second principal components accounted for 91.2% and 5.4% of variance respectively. 94.5% of original grouped cases were correctly classified each original group for morphometric measurements of *S. richardsonii* and formed separate clusters. The results revealed that the candidate species has significant phenotypic heterogeneity between the stocks. We hypothesize that the marked variation in the stock is supporting the fact that environment plays a vital role in shaping the phenotypic characters.

**KEYWORDS**

Ecosystems, Intraspecific, Lotic, Morphometry, Truss

**HOW TO CITE THIS ARTICLE**

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Snow trout consists of 15 genera and over a hundred species distributed all over the world (Mirza, 1991). Seven genera and about seventeen species of snow trout dwell in the Indian sub-Himalayan region. The major species of the snow trout belong to two genera: *Schizothoraichthys* and *Schizothorax*. These two genera have been broadly differentiated based on the shape of the snout. *Schizothorax sp.* has a blunt snout and suctorial lip whereas *Schizothoraichthys* has a pointed snout without the suctorial lip. Among different species of snow trout, the native *Schizothorax richardsonii* (Gray, 1832) locally known as 'Asela' has a wide distribution in Indian Himalayas. This species alone contributes to 60-70% of the total fish catch from upland riverine systems. *S. richardsonii* is widely distributed in the streams, rivers, and lakes all along the Himalayas. *Schizothorax richardsonii* is an important cold water native fish species of Uttarakhand, India.

It is commonly known as Snow trout. The fish is named as "Snow trout" due to its similarity with trout that is sharp teeth. Snow trout has some favorable cultivable traits such as eurythermal nature, amenable to captive conditions and accepts supplementary feed. *Schizothorax richardsonii*, belongs to the family Cyprinidae, subfamily Schizothoracinae, having a ubiquitous distribution in the Himalayan and sub-Himalayan regions of the Indian subcontinent. They are mostly captured in large numbers from different streams of Indus and Ganga river system across Kashmir to Uttarakhand in India. *Schizothorax sp.* generally prefers to dwell in snow-fed rivers or streams with temperature ranging between 8-22°C. They prefer boulders and mature cobbles covered with slimy algal material as substratum. The identification of the fish stock may be done by different tools like molecular genetics, morphometrics, meristics, otolith chemistry and tagging. The stock distinction within and between population is greatly important for the conservation and management of the fisheries resources. The morphological and meristic characters are influenced by both heritable (genetic) and non-heritable (environment) components.

The phenotypic variation among the stock are generally due to changes in the timing of development events, limited migration and isolation of the stock (Cadrin *et al*., 2005) and is useful for the stock identification (Turan, 2004). The shaping of the morphological characters depends on the evolutionary history and natural selection but the difference in these traits among the species or populations is influenced by the environment (Okumus and Ciftci, 2003) and is also related with the status of the habitat of the fish species, therefore, the morphological study is an important requirement for molecular and genetic investigations. To prevail over the weakness of the traditional method of the study of fish morphometry, truss network system has been widely used for the stock identification. The study of phenotypic characters with the help of truss network system increases the likelihood of the identification of morphometry difference of the species with greater discriminant power thus increasing efficient stock identification ability (Strauss and Bookstein, 1982). Truss is a geometric protocol for character selection and uses the distances between homologous landmarks on the outline of a two-dimensional projection of a form.

The truss network was constructed as described by (Strauss and Booksteins, 1982) and was used to detect differences in shapes of different individuals. The truss network system is to be constructed with the help of landmarks points. Landmarks are the selected points on the fish's body and with the help of these points the fish shape is to be assessed. Truss network analyze the multi-dimensional shape of the fish and has been broadly used for the distinction of the within and between population shape (Strauss and Bookstein, 1982; Rohlf 1990; Bronte *et al.*, 1999). Truss network systems are the commanding way to assess the stock. In this methodology, the two morphological landmarks are connected to form a sequential series of the connected polygon for the measurement of cross-body distances. In this type of geometric morphometrics, there is no restriction on the direction of the variation and is highly useful to gather the information about the shape of the organism (Cavalcanti *et al*., 1999).

The truss network can systematically be used to detect shape differences in oblique, horizontal and vertical directions (Strauss and Bookstein, 1982). Truss network is known to be one of the best methods of morphometric analysis because the configuration of the constructed landmarks is more sensitive to changes and covers the entire fish body with almost no loss of information. The present study gives the comparison of the geographically isolated population of the same species can be useful for understanding the variation in the phenotypic characters and help to separate the stock. It can also provide the data to fishery biologists to further study of this species and helps for better conservation of the species in their natural habitat.

## Materials and Methods

### Sampling

Live specimens of the fish *Schizothorax richardsonii* were sampled from the different lotic water bodies' *viz*. river Kosi, river Alaknanda, river Gaula and Chirapani stream and one farmed stock either by fishing or from commercial catches. A total of 135 individuals of the species were collected from the different sites for the analyses throughout the sampling period from September 2017 to August 2018. Fish specimens were randomly collected from five populations for the study.

The number of sample from each site is presented below in the table 1. The collected specimens were phenotypically analyzed by using truss network system.

**Table 1. Number of fish samples collected from different sampling sites**

| S. N. | Sampling Site | Number of Samples Collected |
|---|---|---|
| 1. | River Kosi | 30 |
| 2. | River Gaula | 27 |
| 3. | River Alaknanda | 23 |
| 4. | Chirapani stream | 30 |
| 5. | Fish Farm, DCFR Bhimtal | 25 |

**Table 2. Particulars of landmarks selected for the study Phenotypic Analyses by Truss Network System**

| Land Mark | Particulars of Land Mark |
|---|---|
| 1. | Tip of snout |
| 2. | End of eye towards mouth |
| 3. | End of eye towards tail |
| 4. | End of operculum |
| 5. | Forehead (end of frontal bone) |
| 6. | Dorsal origin of pectoral fin |
| 7. | Origin of dorsal fin |
| 8. | Origin of pelvic fin |
| 9. | Termination of dorsal fin |
| 10. | Origin of anal fin |
| 11. | Termination of anal fin |
| 12. | Dorsal side of caudal peduncle |
| 13. | Ventral side of caudal peduncle |
| 14. | End of lateral line |

The present study was based on the observations of *Schizothorax richardsonii* specimens collected from different sampling sites which were rendered for morphometric measurements by using truss network measurement. Truss network measurement calculates the series of measurement between landmarks that form a regular pattern of connected quadrilaterals across the body form. The specimens were measured by collecting the X-Y coordinate data for relevant morphological features and can be done by three step process which is discussed below:

**Positioning**

A water-resistant laminated graph sheet was placed on a thin thermocol board with the fish placed on the top. The body posture was stretched to its natural position for measurements on the left side of the specimen.

**Pinning**

A fish was placed on a water-resistant sheet, and measurements were taken by piercing the paper with a needle at corresponding anatomical landmarks. Each specimen was labeled with a specific code to identify it in the image.

**Digitizing**

Freshly caught dead samples were washed properly under running water, drained and wiped dry before taking images. The digital image of each individual was obtained using a Sony (Japan) camera at a fixed distance (Zhang *et al*., 2016). To avoid the human error, a single person took all the images from the same height as well as angle (Mir *et al*., 2013a).

**Measurement of Truss Distances**

The extraction of truss distances from the digital images of specimens was done by using a linear combination of three software platforms *tpsUtil32*, *tpsdig2* (Rohlf, 2006) and PAST software package (Hammer *et al*., 2001). The *tpsUtil32* software converts the 'JPEG/JPG' format to 'TPS' file as *tpsdig2* software works for only in TPS file. The *tpsdig2* software sets the landmarks and measures the truss distances of each captured image. All the calculated measurements were transferred to PAleontological Statistics or PAST software package in a spreadsheet file (Hammer *et al*., 2001) and the coordinate data (X-Y) was transformed into linear distances using the Pythagorean Theorem by the software for subsequent analyses.

14 truss characters were selected to form a truss network using standard truss protocol which was used for further analyses (Strauss and Booksteins, 1982; Winans, 1984; Moore and Bronte, 2001; Mir *et al*., 2013a) and are represented in the figure 1 and table 2. The truss network was constructed by interconnecting the 14 homologous landmarks to form 31 truss distances. The particulars of these characters are presented in table 3. The network extended across the whole body of the fish to depict the full dimension of the body (Mir *et al*., 2013b; Moore and Bronte, 2001).

**Data Analyses of Phenotypic Characters**

The elimination of the size effect is an important stage for the analyses of morphometric data while comparing the different sizes of the fish. Considering allometric growth, before statistical analyses all the measurements were log-transformed to remove the size-effect by employing an allometric approach. A significant correlation exists between the body size and the morphometric variables and hence, the variation in the whole data may discriminate the populations based on the size of fish.

Therefore, each distance was corrected as per (Elliott *et al*., 1995).

$$\mathbf{M_{adj} = M\ (L_s\ /L_0)^{b}}$$

Where,

$M_{adj}$ - The transformed truss measurement
M - The original truss measurement
$L_s$ - The overall mean standard length
$L_0$ - The standard length of fish
b - The within-group slope regressions for plots of M and $L_s$ on logarithmic scale.

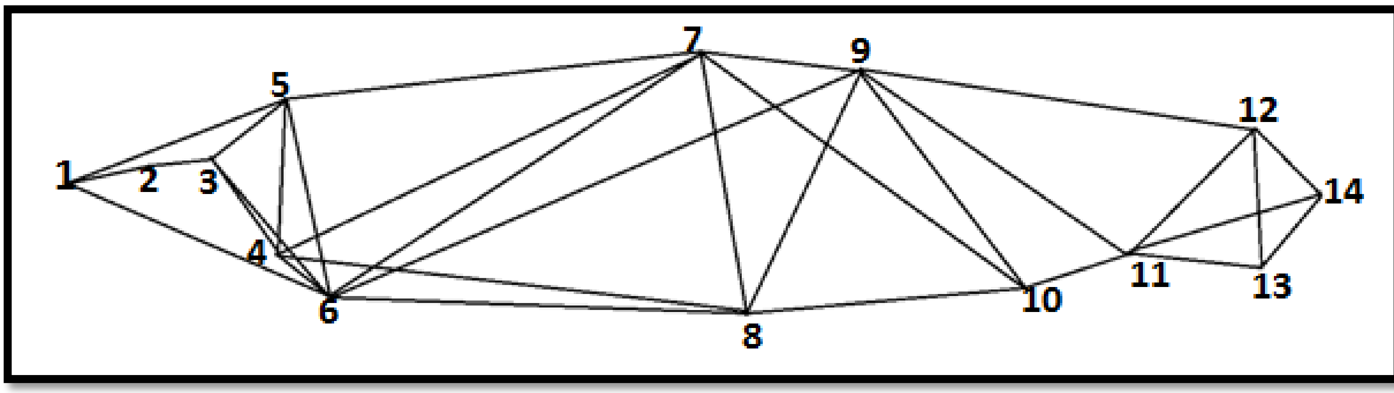


**Fig 1. Consensus of Truss Morphometric Network (TMN) of *Schizothorax richardsonii***

**Discriminant Function Analysis (DFA)**

It is a predictive model for group membership. DFA is composed of discriminate functions that rely on a linear combination of the predictable data. DFA was performed by SPSS software to predict a categorical dependent variable (called a grouping variable) by one or more continuous variables (called predictor variables).

**Results and Discussion**

All the truss distances were analyzed statistically for the interpretations of the results to investigate whether the stocks are geographically isolated from each other or not. Before the analyses, the effect of the body length was removed with the allometric transformation. The correlation coefficient between standard length and truss measurements were close to one before the transformation for size correction. After the transformation, truss measurements did not show significant correlation with standard length of the fish species and were ready for further analysis.

**Kaiser-Meyer-Olkin (KMO) and Bartlett Test**

The phenotypic analysis was done by different statistical analyses such as PCA and DFA. Before proceeding further for phenotypic analysis, the primary tests administer the minimum standards to continue for Factor Analysis. Kaiser-Meyer-Olkin (KMO) and Bartlett's test measures the suitability of the collected data for further statistical analysis and are given in table 4**.** The test analyses sampling adequacy for each character or variable in the model and also for the entire model (Cerny and Kaiser, 1977). The range of KMO statistic was from 0 to 1. The test measures the sampling adequacy. The high value close to one indicates that the data may be useful for factor analysis and if the value is less than 0.50, the factor analysis won't be useful for the data. KMO values for *Schizothorax richardsonii* were calculated and found to be 0.836. According to (Kaiser, 1974), the values of statistic from 0.80 to 0.89 interprets that the sampling is meritorious and 0.90 to 1.00 indicates marvelous sampling. Bartlett's test is used to test the hypothesis of the correlation matrix and indicates that variables are unrelated. The Bartlett's test showed the significance value.

**Table 3. Particulars of truss distances between landmarks**

| S. N. | Landmark Numbers | Truss Distance |
|---|---|---|
| 1. | 1-2 | Tip of snout- End of eye towards mouth |
| 2. | 1-5 | Tip of snout- Forehead (end of frontal bone) |
| 3. | 1-6 | Tip of snout- Dorsal origin of pectoral fin |
| 4. | 2-3 | End of eye towards mouth- End of eye towards tail |
| 5. | 3-4 | End of eye towards tail- End of operculum |
| 6. | 3-5 | End of eye towards tail -Forehead (end of frontal bone) |
| 7. | 3-6 | End of eye towards tail -Dorsal origin of pectoral fin |
| 8. | 4-5 | End of operculum-Forehead (end of frontal bone) |
| 9. | 4-6 | End of operculum-Dorsal origin of pectoral fin |
| 10. | 4-7 | End of operculum- Origin of dorsal fin |
| 11. | 4-8 | End of operculum- Origin of pelvic fin |
| 12. | 5-6 | Forehead (end of frontal bone)- Dorsal origin of pectoral fin |
| 13. | 5-7 | Forehead (end of frontal bone)- Origin of dorsal fin |
| 14. | 6-7 | Dorsal origin of pectoral fin- Origin of dorsal fin |
| 15. | 6-8 | Dorsal origin of pectoral fin- Origin of pelvic fin |
| 16. | 6-9 | Dorsal origin of pectoral fin- Termination of dorsal fin |
| 17. | 7-8 | Origin of dorsal fin- Origin of pelvic fin |
| 18. | 7-9 | Origin of dorsal fin- Termination of dorsal fin |
| 19. | 7-10 | Origin of dorsal fin- Origin of anal fin |
| 20. | 8-9 | Origin of pelvic fin- Termination of dorsal fin |
| 21. | 8-10 | Origin of pelvic fin- Origin of anal fin |
| 22. | 9-10 | Termination of dorsal fin- Origin of anal fin |
| 23. | 9-11 | Termination of dorsal fin- Termination of anal fin |
| 24. | 9-12 | Termination of dorsal fin- Dorsal side of caudal peduncle |
| 25. | 10-11 | Origin of anal fin- Termination of anal fin |
| 26. | 11-12 | Termination of anal fin- Dorsal side of caudal peduncle |
| 27. | 11-13 | Termination of anal fin- Ventral side of caudal peduncle |
| 28. | 11-14 | Termination of anal fin- End of lateral line |
| 29. | 12-13 | Dorsal side of caudal peduncle- Ventral side of caudal peduncle |
| 30. | 12-14 | Dorsal side of caudal peduncle- End of lateral line |
| 31. | 13-14 | Ventral side of caudal peduncle- End of lateral line |

**Table 4. Results of primary tests: Kaiser-Meyer-Olkin Measure of Sampling Adequacy and Bartlett's Test of Sphericity**

| | | |
|---|---|---|
| **Kaiser-Meyer-Olkin Measure of Sampling Adequacy**. | | 0.836 |
| Bartlett's Test of Sphericity | Approx. Chi-Square | 10487.833 |
| | Df | 465 |
| | Sig. | 0.000 |

## Univariate Analysis of Variance (ANOVA)

The selected truss measurements were tested whether there was any statistically significant difference between them in the selected five populations. The results of univariate analysis of variance (ANOVA) showed significant variability ($p < 0.001$) among the selected measurement and are presented in table 5.

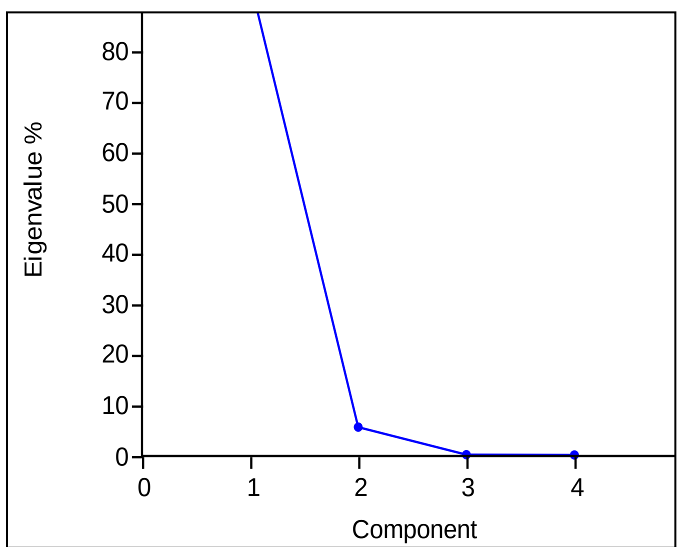


**Fig 2. Scree plot of principal components of *Schizothorax richardsonii* collected from wild and farm environment**

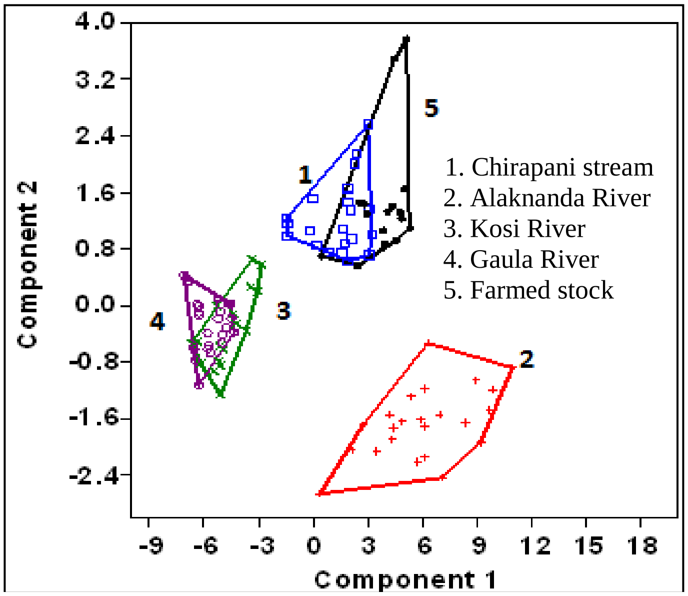


**Fig 3. Scatter plot of between group (inter population) Principal Component Analysis of *Schizothorax richardsonii* collected from wild and farm environment**

## Multivariate Analysis

The multivariate analysis of morphometric data was employed by Principal Component Analysis (PCA) and Discriminant Function Analysis (DFA).

## Principal Component Analysis (PCA)

The PCA was applied to the size corrected normalized measurements to investigate the variability among the stock collected from different geographical locations. The results of between groups PCA are represented in table 6. Phenotypic characteristics based on the truss measurements were evaluated from the body shape and used to evaluate the population structure of the fish species. The component whose Eigen value was more than one was considered for the analysis. The two principal components which have Eigen value more than one were extracted in the results of between groups principal components analysis. The major variation was caused by the first principal components *i.e.* 91.23% followed by second principal components *i.e.* 5.45% of total variance. Together both of these accounted for 96.68% of total variance caused by different variables. The component matrix of the two extracted principal components for 31 truss distances of *Schizothorax richardsonii* collected from wild and farm environment is presented in table 7.

The results of between groups PCA revealed that the size effect on the morphometric characters is more followed by the effect of the shape factor because all the characters in PC1 were significantly positively loaded and equal while in PC2 mostly middle part and the tail region were positively correlated. A scatter plot of PC1 against PC2 using convex hulls showed the clustering of the stocks and is shown in figure 3. The results of the scatter plot revealed that a sufficient isolation between the selected samples of *Schizothorax richardsonii* collected from different geographical regions. The population of river Alaknanda was relatively isolated from other stocks whereas the stock of Chirapani stream is overlapped with the stock of DCFR farm and the stock of river Gaula is overlapped with the stock of river Kosi.

## Discriminant Function Analysis (DFA)

Discriminant Function Analysis was used to measure the percentage of correctly classified fish and cross validation of the classification. The Wilks' λ test was helped to differentiate between all the groups. The results of Wilks' λ are represented in table 8 and showed significant differences in the function 1 through 4, 2 through 4 and 3 through 4 and all the function were highly significant as $p<0.01$. The results indicated significant difference in morphometric characters of selected populations. The results of DFA are given in Table 9. The results of discriminant function analysis revealed that DF1 accounted for 76.8% of the between group variability whereas DF2 accounted for 17% of group variability. DF3 and DF4 accounted 4.3% and 1.9% of total variability respectively. Pooled within-group correlations between discriminating variables are given in table 10 and DFs revealed that twenty body measurements contributed to first discriminant function (DF1). These body measurements were 1-2 (Tip of snout- End of eye towards mouth), 1-5 (Tip of snout- Forehead), 1-6 (Tip of snout- Dorsal origin of pectoral fin), 4-5 (End of operculum-Forehead), 7-10 (Origin of dorsal fin- Origin of anal fin), 3-5 (End of eye towards tail –Forehead), 8-9 (Origin of pelvic fin- Termination of dorsal fin), 5-6 (Forehead-Dorsal origin of pectoral fin), 3-6 (End of eye towards tail - Dorsal origin of pectoral fin), 11-14 (Termination of anal fin- End of lateral line), 4-7 (End of operculum- Origin of dorsal fin), 6-7 (Dorsal origin of pectoral fin- Origin of dorsal fin), 3-4 (End of eye towards tail -

End of operculum), 11-13 (Termination of anal fin- Ventral side of caudal peduncle), 12-13 (Dorsal side of caudal peduncle- Ventral side of caudal peduncle), 12-14 (Dorsal side of caudal peduncle- End of lateral line),13-14 (Ventral side of caudal peduncle- End of lateral line), 5-7 (Forehead- Origin of dorsal fin), 2-3 (End of eye towards mouth- End of eye towards tail) and 4-6 (End of operculum-Dorsal origin of pectoral fin). Almost all the body measurements were loading to the first DF. The body measurements loadings on second DF were 6-8 (Dorsal origin of pectoral fin- Origin of pelvic fin), 4-8 (End of operculum- Origin of pelvic fin) and 7-9 (Origin of dorsal fin- Termination of dorsal fin). The middle portion was concentrated to the second DF. The loadings on DF3 were concentrated on 10-11 (Origin of anal fin- Termination of anal fin) and 11-12 (Termination of anal fin- Dorsal side of caudal peduncle). The fourth DF was concentrated on only one variable 8-9, which is origin of pelvic fin to termination of dorsal fin. The caudal region was concentrated to the third DF. Plot between DF1 and DF2 showed clear morphological differentiation among the selected populations and results were in consonance with the findings of PCA of the samples (figure 4). The results indicated that the stock of river Alaknanda, river Kosi and Chirapani stream was isolated from each other. The farmed stock showed overlapping with Chirapani stock while river Gaula stock showed overlapping with river Kosi stock.

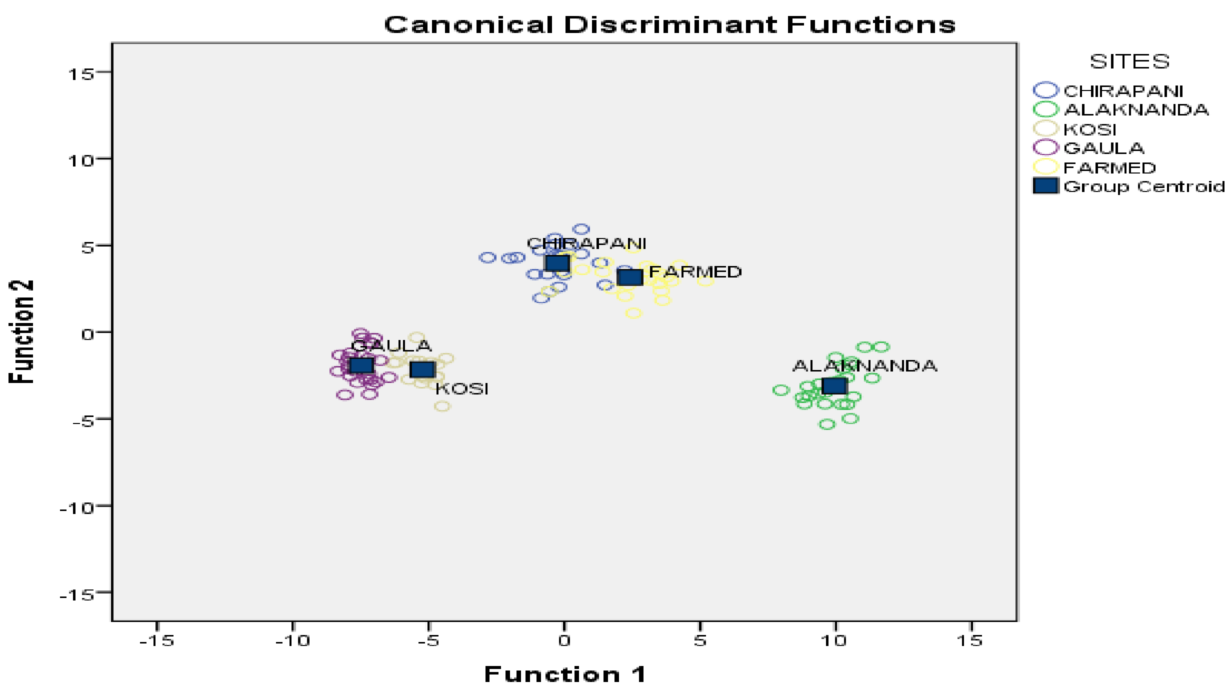


**Fig 4. Results of Canonical Discriminant Function Analysis (CA or DFA) of *Schizothorax richardsonii* populations from collected sites**

## Classification of Individuals

The classification of the collected specimens into their original populations was done by using DFA and 94.5% of original grouped cases correctly classified into their original grouping. The cross validation of the grouped revealed that 91.4% of cross-validated grouped cases correctly classified. The data of percentage wise grouping of *S. richardsonii* of original classified and cross validated classified group are presented in table 11 and 12 respectively.

Predicted group membership revealed that proportion of correctly classified samples was a perfect 100% in river Alaknanda and Chirapani stream. The slight intermingling of farmed stock was observed with Chirapani stock. The farmed stock was 87.5% originally classified and rest 12.5% classified in Chirapani. Another intermingling of the stock was shown by a river Gaula and river Kosi. 91.1 % of total samples were correctly classified of river Kosi and rest 8.9% with river Gaula while 94.5% of total sample were correctly classified of river Gaula and rest intermingled with river Kosi.

**Table 5. The result of univariate ANOVA to examine the difference for each morphometric measurements among the selected five populations of *Schizothorax richardsonii***

| Truss Distances | *p* Value |
|---|---|
| Tip of snout- End of eye towards mouth | 0.000* |
| Tip of snout- Forehead (end of frontal bone) | 0.000* |
| Tip of snout- Dorsal origin of pectoral fin | 0.000* |
| End of eye towards mouth- End of eye towards tail | 0.000* |
| End of eye towards tail- End of operculum | 0.000* |
| End of eye towards tail -Forehead (end of frontal bone) | 0.000* |
| End of eye towards tail -Dorsal origin of pectoral fin | 0.000* |
| End of operculum-Forehead (end of frontal bone) | 0.000* |
| End of operculum-Dorsal origin of pectoral fin | 0.000* |
| End of operculum- Origin of dorsal fin | 0.000* |
| End of operculum- Origin of pelvic fin | 0.000* |
| Forehead (end of frontal bone)- Dorsal origin of pectoral fin | 0.000* |
| Forehead (end of frontal bone)- Origin of dorsal fin | 0.000* |
| Dorsal origin of pectoral fin- Origin of dorsal fin | 0.000* |
| Dorsal origin of pectoral fin- Origin of pelvic fin | 0.000* |
| Dorsal origin of pectoral fin- Termination of dorsal fin | 0.000* |
| Origin of dorsal fin- Origin of pelvic fin | 0.000* |
| Origin of dorsal fin- Termination of dorsal fin | 0.000* |
| Origin of dorsal fin- Origin of anal fin | 0.000* |
| Origin of pelvic fin- Termination of dorsal fin | 0.000* |
| Origin of pelvic fin- Origin of anal fin | 0.000* |
| Termination of dorsal fin- Origin of anal fin | 0.000* |
| Termination of dorsal fin- Termination of anal fin | 0.000* |
| Termination of dorsal fin- Dorsal side of caudal peduncle | 0.000* |
| Origin of anal fin- Termination of anal fin | 0.000* |
| Termination of anal fin- Dorsal side of caudal peduncle | 0.000* |
| Termination of anal fin- Ventral side of caudal peduncle | 0.000* |
| Termination of anal fin- End of lateral line | 0.000* |
| Dorsal side of caudal peduncle- Ventral side of caudal peduncle | 0.000* |
| Dorsal side of caudal peduncle- End of lateral line | 0.000* |
| Ventral side of caudal peduncle- End of lateral line | 0.000* |

**Note:** (*represents significant difference or $p < 0.001$)

**Table 6. Eigen values and percentage of variance accounted by PC1 and PC2 of between groups PCA for *Schizothorax richardsonii* from five selected sites Discussion**

| PC | Eigenvalue | % Variance |
|---|---|---|
| 1 | 28.2824 | 91.234 |
| 2 | 1.69026 | 5.4525 |

The measurements of morphometric characters and their statistical relationships are crucial part of the taxonomy as the origin of the stock and their identification depends on morphometric measurements (Lashari *et al.*, 2004). Results obtained from univariate and multivariate analysis successfully demonstrate that the stocks of that the *S. richardsonii* showed significant phenotypic plasticity among the selected populations and limited overlapping. PCA and DFA could be a useful method to distinguish different stocks of the same species. Fish shows higher degree of variation within and between populations as compared to other vertebrates and are more prone to environmentally influenced morphological variation (Wimberger, 1992). These differences in the morphometry can be attributed to the fact that these subpopulations of *S. richardsonii* are geographically isolated from each other and studies like (Murta, 2000) assumed that these differences may be genetically related or might be associated with phenotypic plasticity in response to different environmental factors of various habitats.

**Table 7. Component matrix of two principal components extracted for 31 morphometric measurements of *Schizothorax richardsonii* collected from five different geographical sites**

| Truss Distances | Component | |
|---|---|---|
| | 1 | 2 |
| Tip of snout- End of eye towards mouth | 0.869 | -0.287 |
| Tip of snout- Forehead | 0.908 | -0.367 |
| Tip of snout- Dorsal origin of pectoral fin | 0.947 | -0.291 |
| End of eye towards mouth- End of eye towards tail | 0.830 | -0.376 |
| End of eye towards tail- End of operculum | 0.916 | -0.206 |
| End of eye towards tail -Forehead | 0.880 | -0.384 |
| End of eye towards tail -Dorsal origin of pectoral fin | 0.956 | -0.170 |
| End of operculum-Forehead | 0.945 | -0.253 |
| End of operculum-Dorsal origin of pectoral fin | 0.700 | 0.030 |
| End of operculum- Origin of dorsal fin | 0.973 | 0.143 |
| End of operculum- Origin of pelvic fin | 0.851 | 0.480 |
| Forehead -Dorsal origin of pectoral fin | 0.934 | -0.246 |
| Forehead - Origin of dorsal fin | 0.963 | 0.152 |
| Dorsal origin of pectoral fin- Origin of dorsal fin | 0.969 | 0.187 |
| Dorsal origin of pectoral fin- Origin of pelvic fin | 0.778 | 0.578 |
| Dorsal origin of pectoral fin- Termination of dorsal fin | 0.932 | 0.118 |
| Origin of dorsal fin- Origin of pelvic fin | 0.942 | -0.145 |
| Origin of dorsal fin- Termination of dorsal fin | 0.826 | 0.175 |
| Origin of dorsal fin- Origin of anal fin | 0.978 | 0.093 |
| Origin of pelvic fin- Termination of dorsal fin | 0.972 | -0.017 |
| Origin of pelvic fin- Origin of anal fin | 0.932 | 0.018 |
| Termination of dorsal fin- Origin of anal fin | 0.936 | 0.154 |
| Termination of dorsal fin- Termination of anal fin | 0.909 | 0.214 |
| Termination of dorsal fin- Dorsal side of caudal peduncle | 0.940 | 0.169 |
| Origin of anal fin- Termination of anal fin | 0.690 | -0.042 |
| Termination of anal fin- Dorsal side of caudal peduncle | 0.916 | -0.023 |
| Termination of anal fin- Ventral side of caudal peduncle | 0.872 | -0.008 |
| Termination of anal fin- End of lateral line | 0.916 | 0.003 |
| Dorsal side of caudal peduncle- Ventral side of caudal peduncle | 0.963 | 0.099 |
| Dorsal side of caudal peduncle- End of lateral line | 0.933 | 0.186 |
| Ventral side of caudal peduncle- End of lateral line | 0.946 | 0.065 |

**Table 8. Results of Wilks' Lambda tests of the Discriminanat Function Analysis (function 1 through 4) of morphometric variables of *S. richardsonii***

| Test of Function(s) | Wilks' Lambda | Chi-square | Degree of freedom | Significance |
|---|---|---|---|---|
| 1 through 4 | 0.000 | 776.299 | 104 | 0.000* |
| 2 through 4 | 0.015 | 411.400 | 75 | 0.000* |
| 3 through 4 | 0.149 | 185.708 | 48 | 0.000* |
| 4 | 0.490 | 69.594 | 23 | 0.000* |

**Note:** (*refers to significant differences or $p < 0.01$)

**Table 9. Results of Discriminant Factor Analysis (DFA) showing the percentage variability with eigenvalues caused by various Discriminant Functions (DFs)**

| Function | Eigenvalue | % of Variance | Cumulative % | Canonical Correlation |
|---|---|---|---|---|
| 1 | 41.206[a] | 76.8 | 76.8 | 0.988 |
| 2 | 9.123[a] | 17.0 | 93.8 | 0.949 |
| 3 | 2.290[a] | 4.3 | 98.1 | 0.834 |
| 4 | 1.042[a] | 1.9 | 100.0 | 0.714 |

**Note:** ([a]denotes that first 4 canonical discriminant functions were used in the analysis)

**Table 10. Contribution to standardized canonical Discriminant Functions (DFs) of truss measurements of *S. richardsonii***

| Structure Matrix | | | | |
|---|---|---|---|---|
| **Truss Measurement** | **Function** | | | |
| | **DF1** | **DF2** | **DF3** | **DF4** |
| 1-2 | .554* | -.138 | .379 | -.489 |
| 1-5 | .453* | -.185 | .025 | -.068 |
| 1-6 | .433* | -.041 | -.044 | .038 |
| 4-5 | .383* | -.009 | -.213 | .139 |
| 7-10 | .377* | .302 | -.133 | .194 |
| 6-9[b] | .369* | .214 | -.223 | -.042 |
| 3-5 | .365* | -.169 | -.236 | .082 |
| 8-9 | .357* | .172 | -.190 | -.047 |
| 5-6 | .328* | .015 | -.091 | .032 |
| 3-6 | .325* | .068 | -.026 | .183 |
| 11-14 | .325* | .234 | -.247 | -.265 |
| 7-8[b] | .283* | .120 | -.160 | .052 |
| 4-7 | .279* | .232 | -.035 | -.030 |
| 6-7 | .274* | .256 | -.041 | -.027 |
| 3-4 | .272* | .037 | -.062 | .181 |
| 11-13 | .266* | .206 | -.224 | -.213 |
| 12-13 | .256* | .191 | -.091 | -.132 |
| 12-14 | .247* | .223 | .069 | -.196 |
| 13-14 | .246* | .123 | -.014 | -.181 |
| 5-7 | .244* | .193 | -.079 | -.087 |
| 2-3 | .244* | -.135 | .051 | .042 |
| 9-10[b] | .214* | .192 | -.006 | .159 |
| 4-6 | .132* | .076 | .010 | .122 |
| 6-8 | .160 | .371* | .207 | -.107 |
| 4-8 | .201 | .366* | .195 | -.073 |
| 9-12[b] | .217 | .236* | -.017 | .088 |
| 7-9 | .207 | .218* | -.028 | -.141 |
| 9-11[b] | .147 | .182* | .135 | .140 |
| 11-12 | .331 | .247 | -.403* | -.153 |
| 10-11 | .145 | .014 | .324* | -.113 |
| 8-10 | .317 | .204 | -.225 | .393* |

**Note:** (*refers to largest absolute correlation between each variable and any discriminant function, [b]marks that this variable not used in the analysis)

**Table 11. Results[a] of percentage of individuals classified in each original group for morphometric measurements of *S. richardsonii***

| **Sites** | **Predicted Group Membership** | | | | | **Total** |
|---|---|---|---|---|---|---|
| | **Kosi** | **Alaknanda** | **Chirapani** | **Gaula** | **Farmed stock** | |
| Kosi | **91.1** | 0.0 | 0.0 | 8.9 | 0.0 | 100.0 |
| Alaknanda | 0.0 | **100.0** | 0.0 | 0.0 | 0.0 | 100.0 |
| Chirapani | 0.0 | 0.0 | **100.0** | 0.0 | 0.0 | 100.0 |
| Gaula | 5.9 | 0.0 | 0.0 | **94.1** | 0.0 | 100.0 |
| Farmed stock | 0.0 | 0.0 | 12.5 | 0.0 | **87.5** | 100.0 |

**Note:** ([a]refers to 94.5% of original grouped cases correctly classified)

**Table 12. Results[b] of percentage of individuals classified in cross validated group for morphometric measurements of *S. richardsonii***

| Sites | Predicted Group Membership | | | | | Total |
|---|---|---|---|---|---|---|
| | Kosi | Alaknanda | Chirapani | Gaula | Farmed Stock | |
| **Kosi** | **89.5** | 0.0 | 0.0 | 10.5 | 0.0 | 100.0 |
| **Alaknanda** | 0.0 | **100.0** | 0.0 | 0.0 | 0.0 | 100.0 |
| **Chirapani** | 0.0 | 0.0 | **100.0** | 0.0 | 0.0 | 100.0 |
| **Gaula** | 8.0 | .0 | .0 | **92.0** | 0.0 | 100.0 |
| **Farmed stock** | 0.0 | 0.0 | 24.5 | 0.0 | **75.5** | 100.0 |

**Note:** ([b]refers to 91.4% of cross-validated grouped cases correctly classified)

The morphometric characters of the fishes which have confined distribution fall under genetically controlled category while the fishes which have wide distribution, their morphometric characters are strongly influenced by environment (Vladykov, 1934). The truss system can be efficaciously used to examine the stock separation within a species. The advantage of truss measurement over traditional method is that it covers the whole body for the analysis and produce reliable information for stock discrimination. According to (Dwivedi and Dubey, 2013), the truss network is more useful and an effective strategy for the descriptions of shape and have a better data collection. The differences may also be attributed to modified swimming performance and mobility of fish (Basaran *et al*., 2007; Hanson *et al*., 2007).

The geographical isolation and environment plays major role in isolation of candidate fish stocks. The findings are in support of (Khan *et al*., 2013) who discriminated the stocks of *C. punctatus* collected from Yamuna, Gomti and Ganga and concluded that environmental conditions play a role in isolation of fish stocks. The environmental factors such as nutrient concentration, and climatic conditions greatly affects the size of the fish species and within the same species, size plays a major role in the variation among the stocks. Haas *et al*. (2010) also reported that the physical characteristics of habitats drive changes in the morphological attributes of native fish populations. Reports of (Slatkin, 1985; Swain and Foote, 1999) suggested that variability among populations is the result of selective regimes leading to environmental discriminations generated by the type of fish habitats, their modification and alteration patterns and their level of human interference. The variation in morphology of the candidate fish may be due to variation in climatic condition as stocks collected from different climatic region.

The univariate statistics of truss measurements study of the Anchovy fish revealed that sixteen of twenty five truss measurements were significantly different from each other among the selected samples (Turan *et al*., 2004). The univariate analysis of selected population of *Clarias batrachus* collected from the Gangetic river system showed that out of thirty characters, twenty four characters showed significant difference (Miyan e*t al*., 2015). The PCA results of other researchers also showed variation in the stock collected from different ecosystems. Kashyap *et al*. (2016) observed that the extracted components PC1, PC2, and PC3 accounted 83.20%, 5.3% and 3.3 % of the total variance respectively while studying Indian Murrel. The four components were extracted while computing PCA on the thirty one truss measurements of Sattar snow trout collected from Kashmir Himalayan region which accounted a total of 80.68% of the variance.

The PC1, PC2, PC3 and PC4 were accounted for 63.44%, 8.35, 5.31 and 3.58, respectively of the total variance (Mir *et al*., 2014). Zhang *et al*. (2016) observed that the WL, ZS and HZB stocks of *Larimichthys polyactis* were relatively isolated while the other stocks showed a significant overlap. The variation in the phenotypic characters of the selected stock may be due to variation in the physico-chemical parameters of the selected water bodies, nutrient concentration, and climatic conditions. Many scientists have stated that local environmental and climatic conditions highly influence particular phenotypes that regulate size and shape (Currens *et al*., 1989; Swain *et al*., 1991). The variation in the stock of *Abramis brama* was observed by (Szlachciak, 1996) using 'truss network' method. The other researchers were also observed variability in the stock on the basis of body shape through truss measurement network (Power and Ni, 1985). Reist and Crossman (1986) have suggested that the body shape variables may be used for distinguishing different population of fishes.

The morphometric characters are subjected to changes in salinity, light, temperature, dissolved oxygen, alkalinity for a long period, manifesting great differences in body size and shape (Ujjania and Kohli, 2011). The stocks of different geographical regions can also show different morphological features because the interactive effects of environment, selection and genetics on individual ontogenies produce morphometric differences within a species (Poulet *et al*., 2005). A Multivariate Analysis Of Variance (MANOVA) found significant differences between the four stocks for the morphometric traits studied to identify stock structure of *Decapterus russelli* collected from East and West coast of India (Sen *et al*., 2011). Similar results were also found by (Mir *et al*., 2013a) while studying the morphometric of *Schizothorax richardsonii* collected from the Indian Himalayas. They analyzed the body measurements and found that fourteen body measurements covering the whole organism contributing to first DF.

Hossain *et al.* (2010) found the morphological differentiation of three populations of *Labeo calbasua* collected from river Jamuna, Halda and hatchery by applying DFA and PCA and concluded that the differences were in the head, abdomen, and caudal peduncle regions. Mir *et al.* (2013a) classified 86.6 % of *S. richardsonii* individuals into their original grouping whereas the proportion of river Mandakini was highest (92.8%) among the selected Himalayan Rivers. The stock of *Decapterus russelli* was analyzed by (Sen *et al*., 2011) using DFA collected from east and west coast of India and observed that 72% stock are originally classified and 28% misclassification was observed in all the four stocks. Kaouèche *et al.* (2017) reported that in White seabream (*Diplodus sargus*) collected from Tunisian coast, the overall classification into their original samples was 95.33%. The fish shows a higher level of phenotypic plasticity. They easily adapt to different environmental condition by changing their physiology and behaviour ultimately resulted change in their morphological characters (Stearns, 1983). The PCA and DFA results supported each other that the stock of Alaknanda isolated from the other stocks. This isolation arises among the population may be due to geographical distances, environmental parameters and different hydrographic features which ultimately result in noteworthy genotypic and phenotypic differentiation of fish stocks (Zhang *et al*., 2016).

The farmed stock overlapped with Chirapani stock while river Gaula stock overlapped with river Kosi stock. The farmed stock overlapped with the Chirapani stock may be due to the similar environment and climatic conditions. According to (Moore and Bronte, 2001) hatchery-reared Trouts retained the physical characteristics of the parental stock. The results are conforming to (Patiyal *et al*., 2014) who also studied the variation patterns of morphometric variations between wild (River Kali) and captive stocks (NBFGR, Lucknow Farm) of endangered *Tor putitora.* The stock of river Gaula showed some similarity with the stock of river Kosi. The reason may be that both the rivers have small geographic distances and these rivers join the river ‘Ramganga’ when enter into tarai region. According to (Murta *et al*., 2008), stock units are spatially distributed and hence the fish stocks from different regions are far apart from each other and cannot be the same stock unit. Phenotypic variations in morphological characters are genetic as well as environmentally induced and the early developmental stages of fish species are greatly influenced by the environmental factors (Pinheiro *et al*., 2005). The variation in body shape of the fishes is also influenced by stream flow, food availability and foraging strategies of fishes (Toline and Baker, 1993).

## Conclusions

The results of the study revealed that the candidate species produces multiple phenotypes as stocks were collected from different geographic regions which showed variation due to different climatic conditions as well as anthropogenic interferences of the selected natural and artificial systems resulting in phenotypic plasticity. The variation in the geographic region and temperature may also affect the variation in the selected species. The abiotic and biotic conditions of an aquatic habitat like temperature, alkalinity, current pattern, food availability and nutrient concentration *etc.* also induce the formation of specific characteristics that may later serve as the base for the isolation of the stocks (Bailey, 1997). The truss protocol revealed a clear separation between the selected stocks, suggesting a need for separate management strategy to sustain stock of the candidate species in each of the selected ecosystems for future development and use.

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