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Landmark-based morphometric and meristic variations of Churru snowtrout *Schizothorax esocinus* from three locations of river Jhelum, Kashmir, India

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Abstract
Landmark-based morphometrics were examined to evaluate the population status of *S. esocinus* to address the taxonomic problems and also to identify the distinctness of populations using truss network amongst the conventional methods. In this context, we have investigated the comparative significance of three morphological methods meristic and truss network system.
Morphometric characters along with truss network measurements and meristic counts were applied. Measurements of body parts were made with the head of fish pointing left. Since meristic characters were independent of size of the fish and did not change during growth the raw meristic data were used in analysis. Significant differences were observed in four of 6 meristic counts and twelve of (1-11, 10-11, 2-10, 3-11, 4-5, 5-8, 5-6, 6-7, 6-8, 3-12, 10-12, 4-6) out of 30 truss measurements. The first DF accounted for 86.0% while the second DF accounted for 14.0 %, respectively explaining 100% of total variability. The cross validation of this analysis revealed that the percentage of correctly classified fishes was 80.7% into their original groups. . The analysis revealed that there is an intermixing of stock between the three populations of *S. esocinus.*

Keywords: landmarks, Meristics, *Schizothorax esocinus*, truss, Kashmir

Introduction
Fisheries and Aquaculture has developed as one of the most vibrant and robust segments of the world. Aquaculture has been designated as "sunrise sector" with diverse resources providing food, nourishment, economy and livelihood to masses around the globe.
Identification of species is a key step towards any research study acting as a key role for the behavioral study. Morphometric measurements and meristic counts are well-thought-out to be as simplest and reliable methods for the identification of specimen which is called as morphological systematics. Morphological measurements, meristic counts, shape and size deliver data valuable for taxonomic status.
The study of morphological characters, whether morphometric or meristic, with the purpose of defining or describing fish stock units, has for some time been of robust interest in ichthyology. Differences in the morphometric and meristic characters have been used widely for stock identification. Fish stock structures have been analyzed for *Catla catla* by (Ujjainia & Kohli (2011) [14], *Megalapsis cordyla* (Sajina *et al.*, 2011) [11], *Rastrelliger kanagurta* (Jayasankar *et al.*, 2004) [4] and *Labeo calbasu* (Hossain *et al*., 2010) [2].
This research aimed at investigating the morphological variations among the populations of *S. esocinus*. This work will contribute to the existing knowledge by acting as a baseline data for carrying out research especially on taxonomy, racial study, morphology and genetic diversity of other fish species

Material and Methods
A total of 180 samples of *S. esocinus* were collected from three sampling sites of river Jhelum namely Srinagar, Anantnag and Baramulla during the period from October 2017 to March 2018. Meristic counts were analysed following the conventional method as described by Hubbs and Lagler (1958) [3]. Six meristic characters were considered for analysis. . These were lateral line scales, dorsal fin rays, pectoral fin rays, pelvic fin rays, anal fin rays and caudal fin rays. Meristic characters were compared using the nonparametric Kruskal-Wallis test. A univariate analysis of variance (ANOVA) was carried out to test the significance of

morphological differences. For truss measurements, digital images of individual fishes were taken immediately after collecting them from landing centers. The truss protocol of *S. esocinus* in the present study was based on twelve landmarks (Figure 1). A truss network was constructed by interconnecting the twelve landmarks to form a total of 30 truss measurements.

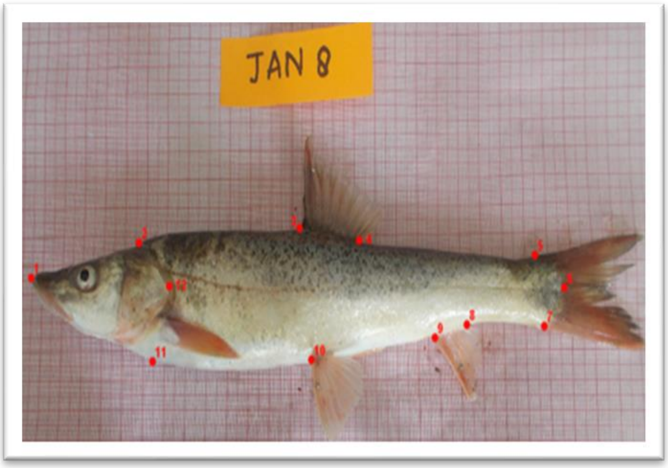

Fig 1: Image of *Schizothorax esocinus* showing the twelve anatomical landmarks

Results

For meristics, the Kruskal Wallis (H) test, presented in Table 1 the number of dorsal fin rays and caudal fin rays were not significantly (p>0.05) different among fish from these stocks and difference occurred in other meristic traits (Lateral line scales: H = 19.58, p<0.01; Pectoral fin rays: H =64.06, p<0.01; Pelvic fin rays: H=27.29, p<0.01; Anal fin rays: H=5.44, p<0.01. In Univariate statistics (ANOVA) similar results were obtained (Table 2). The dorsal fin rays and caudal fin rays showed non-significant difference (p>0.05)

In the PCA of the meristic traits of *S. esocinus*, the first three principal *components* (PCs) together explained 70.74% of total variation. PC1 contributed 35.10%, PC2 contributed 22.05% and PC3 contributed 13.58% of total variation (Table 3). Meristic traits, lateral line scale, showed significant loading on PC1. Pectoral fin loaded significantly to the PC2, and caudal fin rays loaded to the PC3.

The location wise bivariate plot of PC1 and PC3 clearly depicted the separation of Baramulla population along Y-axis, though there was mixing of Srinagar and Anantnag populations along Y-axis, a slight separation between Baramulla and Srinagar was seen along Y-axis and X-axis (Figure 2). The bivariate plot of PC2 and PC3 for different locations revealed slight separation of Baramulla populations along X-axis, though there is mixing of the three populations. (Fig 3). The bivariate plot of PC1 and PC2 depicted slight separation of Baramulla population along X-axis (Fig 4).

For truss morphometry, among the three populations means of 12 (1-11, 10-11, 2-10, 3-11, 4-5, 5-8, 5-6, 6-7, 6-8, 3-12, 10-12, 4-6) out of 30 truss measurements were found to be significantly different (p<0.05). The remaining truss measurements were found to be not different insignificantly (Table 4)

Discriminant function analysis produced two discriminant functions (DF1 and DF2) for truss measurements. The first DF accounted for 86.0% while the second DF accounted for 14.0 %, respectively explaining 100% of total variability. With morphometric measurements 83.3% of Anantnag populations, 80.0% of Srinagar populations, 78.7% of Baramulla populations were correctly classified into their respective groups (Table 5). Among three populations only 80.7% of original grouped cases were correctly classified. (Table 5) while in cross validation 80.0% of Anantnag populations, 58.3% of Srinagar populations, and 72.1% of Baramulla populations were correctly classified. 70.2% of cross-validated grouped cases were correctly classified.

The bivariate plot of the PC 1 and PC 2 extracted from the principal component analysis of the truss network of *S. esocinus* for the three districts *viz* Anantnag, Baramulla and Srinagar indicates no stock separation along the y-axis (Figure 5) Bivariate plot of scores of the two components (PC1 and PC3) extracted from truss measurements of *S. esocinus* by PCA) indicates no stock separation along y-axis. (Figure 6). The bivariate plot of the PC2 and PC3 revealed there is no stock separation along both axis, there is mixing of the three populations. (Figure 7).

Table 1: Test of significance (Kruskal Wallis (H) test) of meristic traits among Srinagar, Anantnag and Baramulla stocks of *S. esocinus*

Meristic Characters	Srinagar Mean ± SD	Anantnag Mean ± SD	Baramulla Mean ± SD	H value	P value
Lateral line scales	101.67±16.38	107.85 ± 15.8	90.72 ±19.7	19.58	<0.01
Dorsal fin rays	7.50 ±0.70	7.73±0.63	7.55±0.87	3.42	>0.05
Pectoral fin rays	9.15 ±1.01	10.77±1.65	11.68± 1.80	64.06	<0.01
Pelvic fin rays	7.87±0.47	8.45±0.85	8.65±0.84	27.29	<0.01
Caudal fin rays	17.77 ±0.65	17.33±0.8	17.95±1.73	6.45	>0.05
Anal fin rays	5.00 ±0.00	5.03±0.37	5.28±0.61	5.44	<0.01

Table 2: Univariate statistics (ANOVA) testing for differences among stocks using meristic measurements

Meristic traits	Sum of Squares	DF	Mean Square	F	P value
Lateral line scales	903.74	2	4516.87	14.89	0.000
Dorsal fin rays	1.81	2	0.91	1.64	0.196
Pectoral fin rays	197.43	2	98.72	42.48	0.000
Caudal fin rays	12.03	2	6.01	4.35	0.014
Pelvic fin rays	19.87	2	9.93	18.06	0.000
Anal fin soft rays	2.87	2	1.43	8.46	0.000

Table 3: Eigen values and proportions of variance contribution to the total variance of meristic traits in *S. esocinus*

Component	Eigen value	Proportion%	Cumulative %
PC1	2.10	35.10	35.10
PC2	1.32	22.05	57.15
PC3	0.81	13.58	70.74

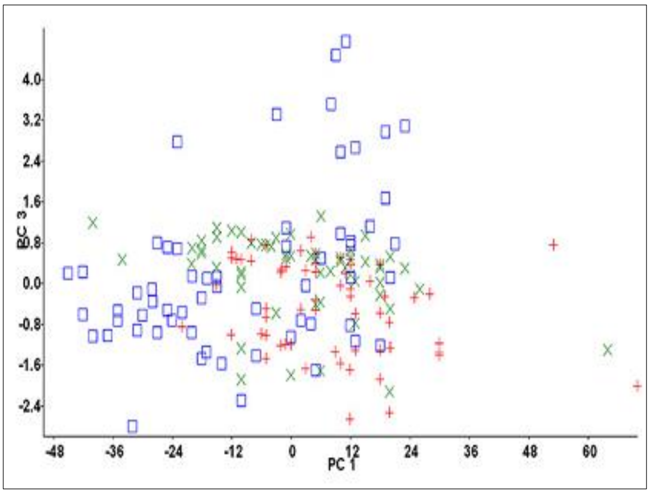

Fig 2: Bivariate plot of scores of the two components (PC1 and PC3) extracted from meristics of *S. esocinus* by PCA
Srinagar (×), Anantnag (+) and Baramulla (□)

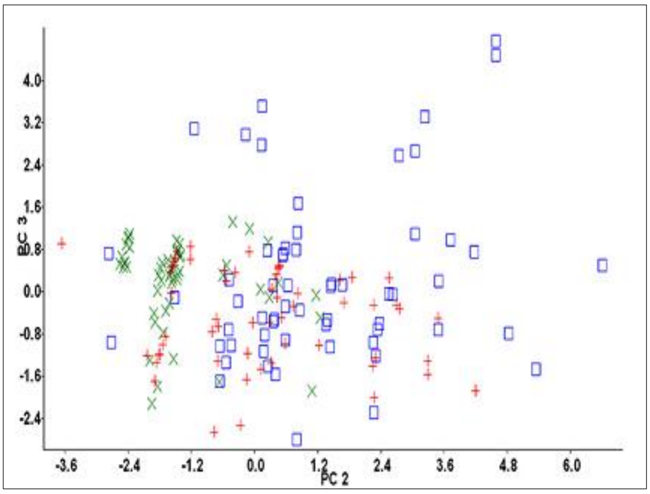

Fig 3: Bivariate plot of scores of the two components (PC2 and PC3) extracted from meristics of *S. esocinus* by PCA
Srinagar (×), Anantnag (+) and Baramulla (□)

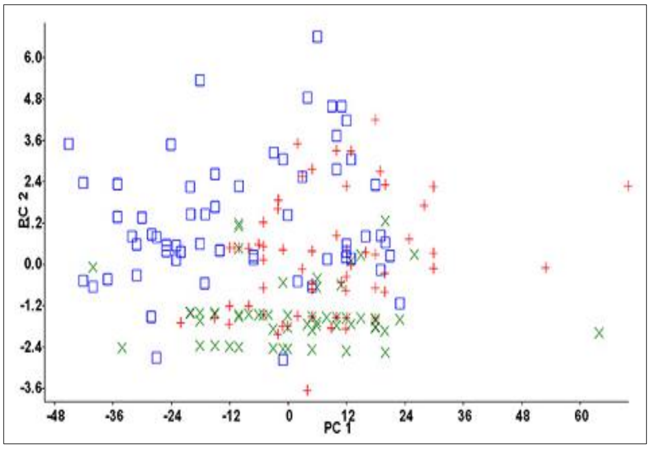

Fig 4: Bivariate plot of scores of the two components (PC1 and PC2) extracted from meristics of *S. esocinus* by PCA
Srinagar (×), Anantnag (+) and Baramulla (□)

Table 4: Univariate statistics (ANOVA) showed thirty measurements

	Wilks Lambda	F	Sig.
1 to 2	0.976	2.193	0.115
1 to 11	0.933	6.407	0.002*
1 to 12	0.979	1.873	0.157
2 to 12	0.979	1.884	0.155
2 to 11	0.983	1.565	0.212
11 to 12	0.996	0.393	0.676
2 to 3	0.964	3.348	0.037
3 to10	0.992	0.680	0.508
10 to 11	0.909	8.875	.0000*
2 to 10	0.801	22.102	0.000*
3 to 11	0.944	5.280	0.006*
3 to 4	0.975	2.252	0.108
4 to 9	0.951	4.600	0.011
9 to10	0.999	0.113	0.893
3 to 9	0.983	1.580	0.209
4 to 10	0.996	0.342	0.711
4 to 5	0.907	7.783	0.0000*
5 to 8	0.887	11.366	0.000*
8 to 9	0.997	0.248	0.781
5 to 9	0.959	3.818	0.024
4 to 8	0.997	0.294	0.746
5 to 6	0.693	39.413	0.0000*
6 to 7	0.883	11.738	0.000*
7 to 8	0.970	2.732	0.068
5 to 7	0.961	3.570	0.030
6 to 8	0.939	5.779	0.004*
3 to12	0.946	5.085	0.000*
10 to 12	0.867	13.607	0.000*
4 to 12	0.950	4.726	0.010
4 to 6	0.895	10.468	0.000*

Table 5: Percentage of specimens classified in each group and after cross validation for truss measurements for *S. esocinus*

Groups			Predicted Group Membership			Total
			Anantnag	Srinagar	Baramulla	
Original	Count	Anantnag	50	7	3	60
		Srinagar	3	48	9	60
		Baramulla	3	10	48	61
	%	Anantnag	83.3	11.7	5.0	100.0
		Srinagar	5.0	80.0	15.0	100.0
		Baramulla	4.9	16.4	78.7	100.0
Cross-validated	Count	Anantnag	48	8	4	60
		Srinagar	8	35	17	60
		Baramulla	3	14	44	61
	%	Anantnag	80.0	13.3	6.7	100.0
		Srinagar	13.3	58.3	28.3	100.0
		Baramulla	4.9	23.0	72.1	100.0

a.80.7% of original grouped cases correctly classified, 70.2% of cross-validated grouped cases correctly classified.

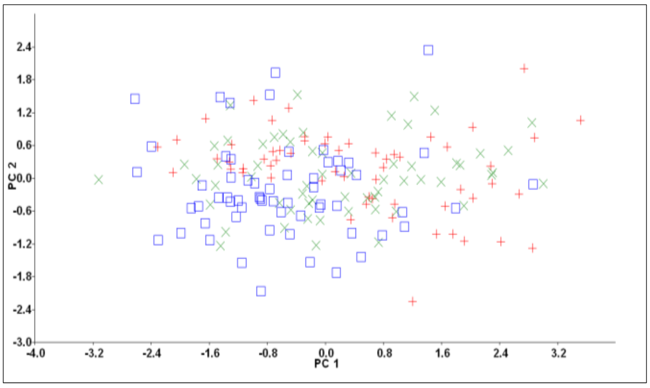

Fig 5: Bivariate plot of scores of the two components (PC1 and PC2) extracted from truss measurements of *S. esocinus* by PCA
Srinagar (×), Anantnag (+) and Baramulla (□)

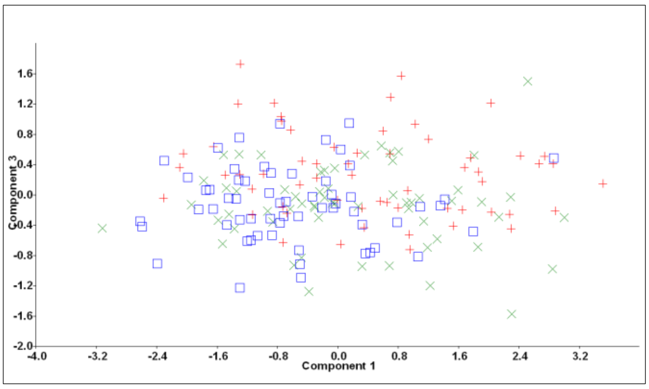

Fig 6: Bivariate plot of scores of the two components (PC1 and PC3) extracted from truss measurements of *S. esocinus* by PCA
Srinagar (×), Anantnag (+) and Baramulla (□)

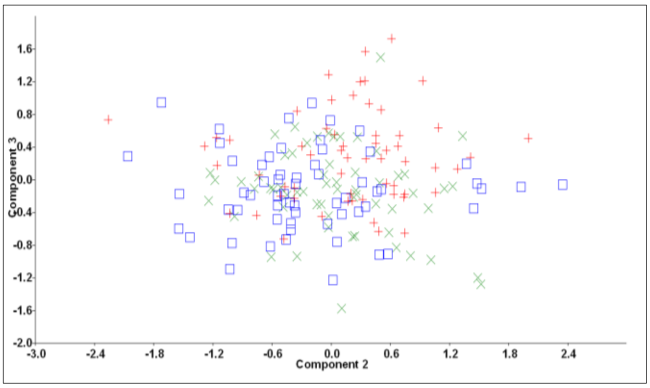

Fig 7: Bivariate plot of scores of the two components (PC2 and PC3) extracted from truss measurements of *S. esocinus* by PCA
Srinagar (×), Anantnag (+) and Baramulla (□)

Discussion

The results of the current study showed a significant variability in 4 of the 6 meristic counts examined. A similar difference in the meristic counts of Japanese charr, *Salvelinus leucomaenis* among the rivers and tributaries has previously been reported by Nakamura (2003) [9]. Variations in meristic counts have recently been reported in *Labeo calbasu* among the stocks of two isolated rivers, the Jamuna and Halda and a hatchery Hossain *et al.,* (2010) [2]. The variability in meristic characters can be attributed to the sensitivity of the fish in response to changes in environmental conditions (temperature and food abundance) as explained by Allendorf *et al*., (1988) [1] and Swain *et al*., (1991) [12].

Principal component analysis of 30 truss measurements extracted from three factors with eigen values > 1, explained 88.83% of the total variance. Similarly, other components showed different degrees of variation. There is mixing of the three populations of *S. esocinus*. In the present study, 80.7% of individuals belonged to their respective groups. All the samples were not clearly separated from the discriminant space. However, a slight variation was seen in Baramulla stock though there may be intermingling among the populations. In case of morphometric measurements three groups are similar. Similar results were obtained by Mir *et al.*, (2013a) [8] in *S. plagiostomus* between Dal lake and river Lidder populations. Mir *et al.,* (2013c) [7] reported 83.4% of individuals belonging to their respective groups by DFA, in *S. curvifrons* indicating slight intermingling among the populations. Mir *et al.,* (2013b) [6] classified 86.6% of the *Schizothorax richardsonii* specimens into their original populations from four different rivers across India. Turan *et al.*, (2005) [13] classified 78% of six populations of *Clarius gariepinus* into their original groups. Similarly, Pollar *et al.*, (2007) [10] reported that the discriminant analysis correctly classified 95.6% of *Tor tambroides*, while the cross-validation testing procedure correctly assigned 93.1% of the fishes into determined populations. Khan *et al.,* (2012) [5] classified *Channa punctatus* from three Indian rivers and lead the conclusion that environmental conditions play an important role in spatial distribution, movement and isolation of fish stocks.

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