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Truss-based morphometrics of pond cultured Indian major carp, *Labeo rohita* (Hamilton, 1822) from Chitwan District, Nepal

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Abstract

The present study conducted non-parametric test on 22 distance characters of 42 samples of three-month-old *Labeo rohita* from a fish pond in Chitwan district, Nepal. ImageJ software was used to create landmark on the fish samples and established 22 distance characters along the dorso-ventral and antero-posterior body axes. Univariate and multivariate data analysis was used with PAST software. All the 22 distance characters were positively correlated with correlation coefficient greater than zero. Shapiro-Wilk test calculated p-value less than 0.05 for two distance characters. There was high variation in the means and medians of the data based on Levene's test and Kruskal Wallis test. Tukey's pairwise test and Dunn's post hoc test analyzed medians of the data for the significant relationship among the distance characters. Multivariate analysis with Principal Components Analysis (PC1 and PC2) of the 22 distance characters calculated the total Eigenvalue of 19.18944 and 87.2247% of variance under correlation matrix and Bootstrap N: 100. The fish samples varied in external morphology mainly in the body depth shape in dorso-ventral axis which is supposed to be the effect of culture conditions, food and mode of feeding.

Keywords: Morphometrics, *labeo rohita*, quantitative analysis, principal components analysis

1. Introduction

Labeo rohita ('rohu' in Nepali/Hindi) is a soft-rayed teleost and is one of the Indian major carps, which is widely distributed in Nepal, India, Bangladesh, Myanmar, Pakistan and Sri Lanka. The carp is commonly cultured in ponds and is considered the tastiest among the cultivated fishes in Nepal.

L. rohita is diagnosed externally by its elongated and cylindrical body, small or pointed head, sub-terminal mouth, dorsal fin lying midway between tip of snout and base of caudal fin, upper margin of the dorsal fin is concave, caudal fin deeply forked, fairly large cycloid scales, body color with dark blue, reddish tinge and whitish on belly.

Fish are very sensitive to environmental conditions, such as temperature and food abundance, and quickly adopt themselves by changing necessary morphometrics (Allendorf F. W. and S.R. Phelps 1988; Swain *et al.* 1991) ^[1, 17]. Fish show higher degrees of variation within and between populations than other vertebrates and are more susceptible to environmentally-induced morphological variation (Wimberger, 1992) ^[20]. Morphological variation within species level is mainly triggered by environmental factors (Talu, 2012) ^[18]. Pakkasma and Piironen (2000) ^[12], Reis *et al.* (2006) ^[14], Bagherian and Rahmami (2009) ^[3] Mir *et al.* (2013) ^[11] have reported environmentally induced morphological variations in fish.

In geographically isolated populations, pollution of water due to excess use of fertilizers, pesticides can cause genetic drift leading to genetic diversity and morphological variations. Morphometrics is the study of shape variation and its covariation with other variables (Bookstein, 1991; Dryden and Mardia 1998) ^[4, 8]. Morphometrics refers to the quantitative analysis of form that involves measuring the length of, or distances between physical features. Data on morphometric measurements are able to identify differences between fish populations and used to describe the shape of each fish (Pollar *et al.*, 2007) ^[13]. Truss system for morphometric study of fish involves measurement across body distances joining two or more morphological landmarks at specific locations on the body. This method can pin point morphological variations in body shape and size that are not easily detected through traditional forms of measurement or by the naked eyes.

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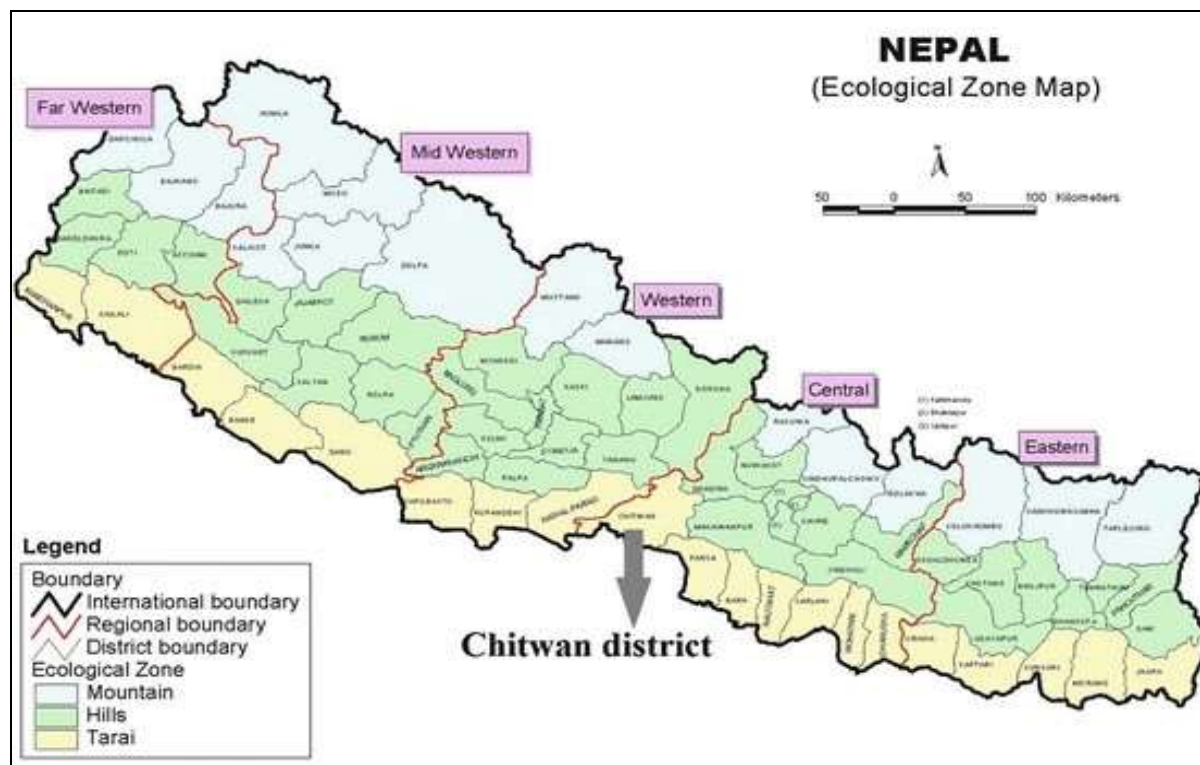
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The study of morphometrics of fish is useful to delimit various populations of the same species and identify populations of fish of the farmed or wild origin. These informations are very useful to fishery biologists and conservation agencies for conservation and management of

the fish species.

2. Materials and Methods

2.1 Study Area



Map 1: Map of Nepal showing Chitwan district

Chitwan district is located in the Tarai region in the Central Development region of Nepal. It covers an area of 2,238.39 km² that includes the famous Chitwan National Park. There are a total of 2073 fish ponds covering a total area of more than 854 ha in Chitwan district. The major fish breeds being cultivated in the district are Chinese carps, Indian major carps (*Labeo rohita*, *Cirrhinus mrigala*, *Catla catla*), Catfish (*Pangasius hypophthalmus*) (Karki, 2016) [10].

2.2 Fish capture and Photograph

A total of 42 samples of *L. rohita* of the same age (3 months) was captured from a pond using drag nets. The fish was photographed with a measuring scale using a Nikon Dslr of model D5200 with 50mm lens fixed in a tripod. A Nikon speed lite was used for sufficient exposure. The photograph had dimensions of 6000 × 4000 at 1/100 sec, f/8 and ISO200.

2.3 Data collection and Analysis

The photograph of the fish was input in software ImageJ (Schneider *et al.*, 2012) [16]. A straight line was drawn over the fish from the tip of the snout to the fork of the caudal fin using freehand line of the software, then the image was scaled from pixels to centimeter.

Using multipoint (+) of the software eleven landmarks were selected at specific locations based on Winans (1987): 1) ventral tip of the operculum, 2) the most distal point of the head, 3) posterior margin of the head, 4) anterior base of the dorsal fin, 5) posterior base of the dorsal fin, 6) dorsal posterior margin of the caudal peduncle, 7) ventral posterior

margin of the caudal peduncle, 8) posterior base of the anal fin, 9) anterior base of the anal fin, 10) base of the pelvic fin and 11) base of the pectoral fin. 22 distance characters were derived from a truss network constructed by interconnecting the eleven landmarks using the freehand line of the software. 1-2 Head length, 1-3 Head depth, 1-11 anterior body length, 2-3 tip of the head to posterior margin of the head, 3-11 base of the pectoral fin to the posterior margin of the head, 3-4 pre-dorsal length, 3-10 posterior margin of the head to base of the pelvic fin, 11-4 base of the pectoral fin to the anterior base of the dorsal fin, 11-10 pre-pelvic length, 4-5 dorsal fin base length, 5-6 dorsal caudal peduncle length, 6-7 posterior caudal peduncle depth, 7-8 ventral caudal peduncle length, 5-8 anterior caudal peduncle depth, 10-4 base of the pelvic fin to the anterior base of the dorsal fin, 10-5 base of the pelvic fin to the posterior base of the dorsal fin, 8-6 posterior base of the anal fin to dorsal posterior margin of the caudal peduncle, 9-6 anterior base of the anal fin to dorsal posterior margin of the caudal peduncle, 9-5 anterior base of the anal fin to posterior base of the dorsal fin, 9-4 anterior base of dorsal fin to anterior base of the anal fin, 8-9 anal fin base length and 10-9 base of the pelvic fin to the anterior base of the anal fin.

The 22 distance characters were tested for normality under Shapiro-Wilk test. One-way ANOVA, Tukey's pairwise test, Kruskal-Wallis test and Dunn's post hoc test were conducted. Multivariate analysis (Principal Component Analysis) was conducted to observe the relationships among the fish samples and the 22 distance characters. The data analysis was performed with PAST v.3.14 (Hammer *et al.* 2001) [9].



Photo 1: A sample of *Labeo rohita* input in ImageJ for 11 landmarks

3. Result

The plotting of the data on the 22 distance characters of the fish samples, a non-normality histogram was obtained (Fig 2). The data analysis in univariate statistics provided coefficient of variation ranging from 9.4% to 31.7% and median ranging from 0.8 to 5.8 (Table 1). The correlation coefficient for all the distance characters was higher than 0 (Fig 2). Analysis of

the relationship between the total length and head length of the fish samples under t test and Mann-Whitney test produced p-value less than 0.05 (Tables 2 and 3).

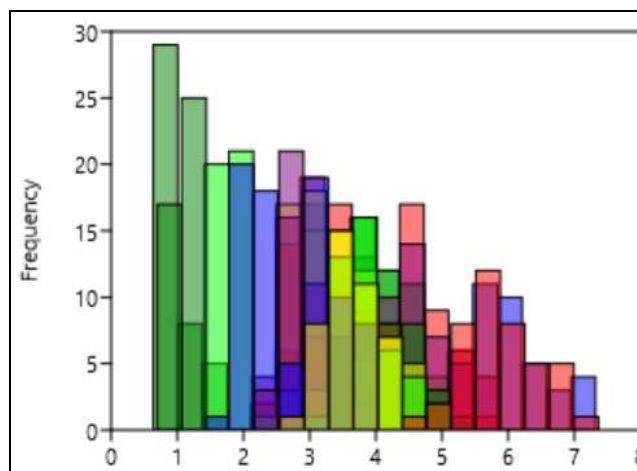


Fig 1: Histogram of the 22 distance characters of the fish samples

Table 1: Summary Statistics of the 22 distance characters from the samples of *Labeo rohita*

	1-2	1-3	1-11	2-3	3-11	3-4	3-10	11-4	11-10	4-5	5-6
No.	42	42	42	42	42	42	42	42	42	42	42
Min.	2.23	2.2	0.63	2.45	2.31	3.12	4.36	3.79	3.04	2.44	3.66
Max.	3.75	3.54	1.7	3.59	3.52	5.11	7.3	5.48	5.1	4.05	5.65
Mean	3.17	2.92	0.94	2.92	2.90	4.03	5.91	4.60	4.13	3.22	4.64
Median	3.14	2.90	0.845	2.91	2.9	3.98	5.85	4.54	4.06	3.21	4.53
Coeff. var	10.13	9.45	31.74	9.67	9.80	11.02	10.51	9.79	10.18	10.81	10.63
	6-7	7-8	5-8	10-4	10-5	8-6	9-6	9-5	9-4	8-9	10-9
No.	42	42	42	42	42	42	42	42	42	42	42
Min.	1.39	1.74	2.78	3.1	2.95	2.34	3.06	2.5	4.45	0.71	2.7
Max.	2.13	2.67	4.31	4.87	4.55	3.57	4.85	3.8	6.93	1.37	4.2
Mean	1.75	2.15	3.52	3.98	3.77	2.85	3.82	3.19	5.79	1.03	3.54
Median	1.77	2.12	3.41	3.91	3.69	2.81	3.79	3.12	5.67	1.02	3.52
Coeff. var	10.95	10.62	10.68	11.13	10.71	10.65	10.88	10.42	10.10	14.32	10.29

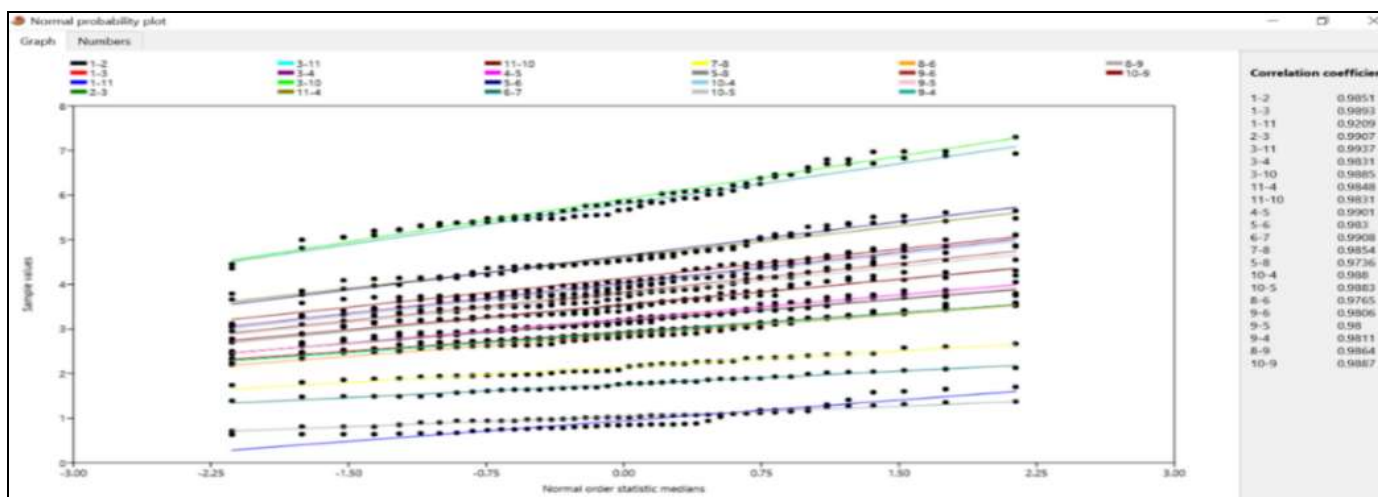


Fig 2: Normal probability plot of the data set showing positive correlation coefficient value of each distance character.

Table 2: Two sample test (t test) of total length and head length of fish samples showing p-value less than 0.05

t test	
t: 55.133	p (same mean): 1.3989E-66
Uneq. var. t: 55.133	P (same mean): 7.4721E-43

Table 3: Mann-Whitney test of total length and head length showing p-value less than 0.05.

Mann-Whitney U: 0	
Z: 7.8864	P (same med.): 3.1095E-15
Monte Carlo permutation:	P (same med.): 0.0001

The Shapiro-Wilk normality test showed that all the data are normally distributed ($p > 0.05$) except the distance characters 1-11 and 5-8.

Table 4: Shapiro-Wilk W test showing p-value less than 0.05 for two characters (1-11, 5-8).

	1-2	1-3	1-11	2-3	3-11	3-4	3-10	11-4	11-10	4-5	5-6
No. of samples	42	42	42	42	42	42	42	42	42	42	42
Shapiro-Wilk W	0.97	0.98	0.84	0.97	0.98	0.96	0.97	0.96	0.97	0.98	0.96
p (normal)	0.39	0.75	3.75E-05	0.48	0.81	0.25	0.62	0.15	0.33	0.70	0.15
	6-7	7-8	5-8	10-4	10-5	8-6	9-6				
No. of samples	42	42	42	42	42	42	42				
Shapiro-Wilk W	0.40	0.96	0.94	0.96	0.96	0.94	0.95				
p (normal)	0.22	0.03	0.037	0.32	0.08	0.13	0.13				

Under one-way ANOVA, the Levene’s test for homogeneity of variance, from means is $P=3.324E-20$ which is less than 0.05. This means there is a difference in the data.

Table 5: Levene's test for the distance characters showing p-value less than 0.05.

One- way ANOVA	
Levene's test for homogeneity of variance, from means	p (same): 3.324E-20
Levene's test, from medians	p (same): 1.615E-16

Tukey’s pairwise test compares the relationship among the 22 distance characters. P- value less than 0.05 shows statistically significant relationship.

Table 6: Tukey's pairwise test calculating significant relationship between the data pairs.

	1-2	1-3	1-11	2-3	3-11	3-4	3-10	11-4	11-10	4-5	5-6
1-2		0.3389	0	0.2629	0.1746	0	0	0	0	1	0
1-3	4.079		0	1	1	0	0	0	0	0.06263	0
1-11	37.73	33.65		0	0	0	0	0	0	0	0
2-3	4.248	0.1691	33.48		1	0	0.00E+00	0	0	4.25E-02	0.00E+00
3-11	4.49	0.4107	33.24	0.2416		0	0.00E+00	0	0	2.36E-02	0.00E+00
3-4	14.56	18.64	52.29	18.81	1.91E+01		0	2.96E-09	1.00E+00	0.00E+00	1.95E-10
3-10	46.32	50.4	84.05	50.57	50.81	31.76		0	0	0.00E+00	0.00E+00
11-4	24.26	28.34	61.99	28.51	28.75	9.701	2.21E+01		5.92E-06	0.00E+00	1.00E+00
11-10	16.32	20.4	54.05	20.57	2.08E+01	1.76E+00	3.00E+01	7.94E+00		0	6.02E-07
4-5	0.9181	4.997	38.65	5.167	5.41E+00	1.36E+01	4.54E+01	2.33E+01	1.54E+01		0
5-6	24.83	28.91	62.55	29.07	2.93E+01	1.03E+01	2.15E+01	5.64E-01	8.51E+00	23.91	
6-7	23.94	19.86	13.78	19.7	1.95E+01	3.85E+01	7.03E+01	4.82E+01	4.03E+01	24.86	48.77
7-8	17.24	13.16	20.48	12.99	12.75	31.8	6.36E+01	41.51	33.56	1.82E+01	4.21E+01
5-8	6.081	10.16	43.81	10.33	1.06E+01	8.48E+00	4.02E+01	1.82E+01	1.02E+01	5.163	18.75
10-4	13.78	17.86	51.51	18.03	1.83E+01	0.7772	32.53	10.48	2.537	1.29E+01	1.10E+01
10-5	10.14	14.22	47.86	14.38	1.46E+01	4.426	36.18	14.13	6.185	9.22E+00	1.47E+01
8-6	5.34	1.26	32.39	1.091	0.8497	19.9	5.17E+01	29.6	21.66	6.258	3.02E+01
9-6	11.09	15.17	48.81	15.33	1.56E+01	3.48E+00	3.52E+01	1.32E+01	5.24E+00	10.17	1.37E+01
9-5	0.4228	4.502	38.15	4.671	4.913	1.41E+01	4.59E+01	2.38E+01	1.59E+01	0.4953	2.44E+01
9-4	44.42	48.5	82.15	48.67	48.91	29.86	1.89E+00	20.16	28.1	4.35E+01	1.96E+01
8-9	36.11	32.03	1.623	31.86	31.62	5.07E+01	8.24E+01	6.04E+01	5.24E+01	37.02	6.09E+01
10-9	6.326	10.41	44.05	10.57	10.82	8.235	4.00E+01	17.94	9.995	5.41E+00	1.85E+01

	6-7	7-8	5-8	10-4	10-5	8-6	9-6	9-5	9-4	8-9	10-9
1-2	0	0	0.003714	0	3.67E-10	0.02798	2.49E-12	1	0	0	0.001764
1-3	0	0	3.26E-10	0	0	1	0	0.1708	0	0	9.64E-11
1-11	0	0	0	0	0	0	0	0	0	1	0
2-3	0.00E+00	0.00E+00	1.41E-10	0.00E+00	0.00E+00	1.00E+00	0	1.24E-01	0	0	4.08E-11
3-11	0.00E+00	0.00E+00	4.17E-11	0.00E+00	0	1	0	7.54E-02	0.00E+00	0	1.15E-11
3-4	0.00E+00	0.00E+00	6.66E-07	1.00E+00	0.1957	0	6.65E-01	0.00E+00	0.00E+00	0.00E+00	1.83E-06
3-10	0.00E+00	0.00E+00	0	0.00E+00	0	0	0	0.00E+00	1.00E+00	0	0.00E+00
11-4	0.00E+00	0.00E+00	0	6.69E-11	0	0	0	0.00E+00	0.00E+00	0	0.00E+00
11-10	0	0	2.19E-10	0.9758	2.72E-03	0.00E+00	0.03613	0	0	0.00E+00	7.28E-10
4-5	0	0	4.29E-02	0	2.73E-08	2.18E-03	3.14E-10	1.00E+00	0.00E+00	0.00E+00	2.36E-02
5-6	0	0	0.00E+00	3.26E-12	0.00E+00	0.00E+00	0.00E+00	0	0	0.00E+00	0
6-7		0.000531	0.00E+00	0	0.00E+00	0.00E+00	0	0	0	0.00E+00	0
7-8	6.70E+00		0	0.00E+00	0.00E+00	0.00E+00	0	0.00E+00	0	0	0
5-8	30.02	23.32		1.49E-05	3.51E-01	1.04E-13	6.15E-02	0.01228	0	0.00E+00	1
10-4	3.77E+01	3.10E+01	7.70E+00		0.5695	0	9.54E-01	0.00E+00	0.00E+00	0	3.73E-05
10-5	3.41E+01	2.74E+01	4.06E+00	3.65E+00		0	1.00E+00	2.79E-09	0.00E+00	0	4.80E-01
8-6	1.86E+01	11.9	11.42	1.91E+01	1.55E+01		0	0.009229	0	0.00E+00	0
9-6	35.03	28.33	5.01E+00	2.698	9.50E-01	1.64E+01		2.58E-11	0	0.00E+00	0.1039
9-5	24.37	17.67	5.658	13.36	9.71E+00	5.76E+00	10.66		0	0.00E+00	0.006214
9-4	6.84E+01	6.17E+01	38.34	3.06E+01	34.29	49.76	3.33E+01	4.40E+01		0	0.00E+00
8-9	12.16	18.86	42.19	49.89	4.62E+01	3.08E+01	47.19	36.53	80.53		0
10-9	3.03E+01	2.36E+01	0.2456	7.46E+00	3.81E+00	1.17E+01	4.76	5.90E+00	38.1	42.43	

Table 7: Kruskal-Wallis test of the data with p-value less than 0.05.

Kruskal-Wallis test for equal medians	
H (chi2):	818.7
Hc (tie corrected):	818.7
p (same):	9.875E-160

Kruskal-Wallis test for equal medians gave P= 9.875E-160. Thus, there is significant difference between the sample medians.

Dunn's post hoc compared medians of the data set for the analysis of statistically significant relationship. P -value less than 0.05 showed statistically different relationship.

Table 8: Dunn's post hoc test of the data set with p-value less than 0.05 indicates significant difference

	1-2	1-3	1-11	2-3	3-11	3-4	3-10	11-4	11-10	4-5	5-6
1-2		0.13	1.30E-09	0.1193	0.1079	5.86E-06	3.56E-17	8.86E-11	6.30E-07	0.789	6.16E-11
1-3	0.1337		4.94E-06	0.9537	0.1942	1.63E-09	3.19E-23	1.41E-15	9.07E-11	0.07716	9.00E-16
1-11	1.30E-09	4.94E-06		6.506E-06	8.212E-06	3.03E-26	1.33E-47	3.86E-36	2.22E-28	2.38E-10	1.93E-36
2-3	0.1193	0.95	6.506E-06		0.9604	1.14E-09	1.78E-23	8.77E-16	6.16E-11	0.06793	5.60E-16
3-11	0.1079	0.91	8.212E-06	0.9604		8.31E-10	1.08E-23	5.84E-16	4.42E-11	0.06078	3.72E-16
3-4	5.86E-06	0.62E-09	3.03E-26	1.135E-09	8.311E-10		9.80E-05	0.05072	0.6523	2.01E-05	0.04458
3-10	3.55E-17	3.19E-23	1.326E-47	1.781E-23	1.078E-23	9.80E-05		0.05219	0.000571	3.38E-16	0.05919
11-4	8.86E-11	1.40E-15	3.863E-36	8.765E-16	5.836E-16	0.05072	0.05219		0.1328	5.05E-10	0.9563
11-10	6.29E-07	9.07E-11	2.217E-28	6.163E-11	4.416E-11	0.6523	0.000571	0.1328		2.43E-06	0.1192
4-5	0.789	0.07	2.378E-10	0.06793	0.06078	2.01E-05	3.38E-16	5.05E-10	2.43E-06		3.56E-10
5-6	6.15E-11	9.00E-16	1.931E-36	5.596E-16	3.716E-16	0.04458	0.05919	0.9563	0.1192	3.56E-10	
6-7	1.42E-06	0.00089	0.2132	0.001097	0.001306	8.48E-21	4.58E-40	1.21E-29	1.08E-22	3.59E-07	6.45E-30
7-8	3.29E-05	0.0079	0.05549	0.009479	0.01094	3.84E-18	2.76E-36	2.00E-26	6.60E-20	9.88E-06	1.11E-26
5-8	0.04037	0.00038	4.778E-16	0.0003088	0.0002547	0.01309	1.81E-10	9.20E-06	0.003368	0.0747	7.12E-06
10-4	1.57E-05	5.94E-09	2.882E-25	4.193E-09	3.103E-09	0.8315	3.99E-05	0.03026	0.5071	5.10E-05	0.02632
10-5	0.00082	1.27E-06	4.935E-21	9.519E-07	7.384E-07	0.2349	3.71E-07	0.00168	0.1013	0.002099	0.001391
8-6	0.05815	0.69	3.012E-05	0.7364	0.7741	1.31E-10	5.65E-25	5.31E-17	6.14E-12	0.03061	3.33E-17
9-6	0.000287	2.96E-07	3.22E-22	2.171E-07	1.661E-07	0.3654	1.58E-06	0.00425	0.1752	0.000783	0.003571
9-5	0.903	0.1049	6.057E-10	0.09302	0.08374	1.04E-05	9.99E-17	1.97E-10	1.17E-06	0.8841	1.38E-10
9-4	8.28E-17	8.61E-23	5.622E-47	4.835E-23	2.941E-23	0.000147	0.9207	0.06547	0.000822	7.66E-16	0.0739
8-9	6.20E-09	1.62E-05	0.798	2.106E-05	2.627E-05	4.53E-25	5.33E-46	9.48E-35	3.71E-27	1.21E-09	4.80E-35
10-9	0.03055	0.00024	1.871E-16	0.0001986	0.0001629	0.01787	3.76E-10	1.55E-05	0.004819	0.05804	1.20E-05

	6-7	7-8	5-8	10-4	10-5	8-6	9-6	9-5	9-4	8-9	10-9
1-2	1.42E-06	3.29E-05	0.04037	1.57E-05	0.000827	0.05815	0.000288	0.903	8.28E-17	6.20E-09	0.03055
1-3	0.000893	0.007994	0.000386	5.94E-09	1.28E-06	0.693	2.96E-07	0.1049	8.62E-23	1.62E-05	0.00025
1-11	0.2132	0.05549	4.78E-16	2.88E-25	4.94E-21	3.01E-05	3.22E-22	6.06E-10	5.62E-47	0.798	1.87E-16
2-3	0.001097	0.009479	0.000309	4.19E-09	9.52E-07	0.7364	2.17E-07	0.09302	4.84E-23	2.11E-05	0.000199
3-11	0.001306	0.01094	0.000255	3.10E-09	7.38E-07	0.7741	1.66E-07	0.08374	2.94E-23	2.63E-05	0.000163
3-4	8.48E-21	3.84E-18	0.01309	0.8315	0.2349	1.31E-10	0.3654	1.04E-05	0.000147	4.53E-25	0.01787
3-10	4.58E-40	2.76E-36	1.81E-10	3.99E-05	3.71E-07	5.65E-25	1.58E-06	9.99E-17	0.9207	5.33E-46	3.76E-10
11-4	1.21E-29	2.00E-26	9.20E-06	0.03026	0.00168	5.31E-17	0.00425	1.97E-10	0.06547	9.48E-35	1.55E-05
11-10	1.08E-22	6.60E-20	0.003368	0.5071	0.1013	6.14E-12	0.1752	1.17E-06	0.000822	3.71E-27	0.004819
4-5	3.59E-07	9.88E-06	0.0747	5.10E-05	0.002099	0.03061	0.000783	0.8841	7.66E-16	1.21E-09	0.05804
5-6	6.45E-30	1.11E-26	7.12E-06	0.02632	0.001391	3.33E-17	0.003571	1.38E-10	0.0739	4.80E-35	1.20E-05
6-7		0.5028	6.33E-12	6.20E-20	3.20E-16	0.003415	2.95E-17	7.65E-07	1.72E-39	0.3227	2.85E-12
7-8	0.5028		5.57E-10	2.44E-17	6.60E-14	0.02397	7.35E-15	1.92E-05	9.69E-36	0.09711	2.70E-10
5-8	6.33E-12	5.57E-10		0.02329	0.1958	8.00E-05	0.115	0.05385	3.45E-10	3.81E-15	0.91
10-4	6.20E-20	2.44E-17	0.02329		0.3295	5.20E-10	0.4887	2.71E-05	6.11E-05	4.08E-24	0.03112
10-5	3.20E-16	6.60E-14	0.1958	0.3295		1.62E-07	0.7774	0.001275	6.24E-07	5.45E-20	0.2378
8-6	0.003415	0.02397	8.00E-05	5.20E-10	1.62E-07		3.38E-08	0.04376	1.59E-24	8.98E-05	4.96E-05
9-6	2.95E-17	7.35E-15	0.115	0.4887	0.7774	3.38E-08		0.000458	2.59E-06	3.82E-21	0.1434
9-5	7.65E-07	1.92E-05	0.05385	2.71E-05	0.001275	0.04376	0.000458		2.30E-16	2.97E-09	0.04124
9-4	1.72E-39	9.69E-36	3.45E-10	6.11E-05	6.24E-07	1.59E-24	2.59E-06	2.30E-16		2.20E-45	7.08E-10
8-9	0.3227	0.09711	3.81E-15	4.08E-24	5.45E-20	8.98E-05	3.82E-21	2.97E-09	2.20E-45		1.54E-15
10-9	2.85E-12	2.70E-10	0.91	0.03112	0.2378	4.96E-05	0.1434	0.04124	7.08E-10	1.54E-15	

Multivariate analysis with Principal Components analysis: Principal Components Analysis PC1 and PC2 of the 22 distance characters calculated the total Eigenvalue of

19.18944 and 87.2247% of variance under correlation matrix and Bootstrap N: 100.

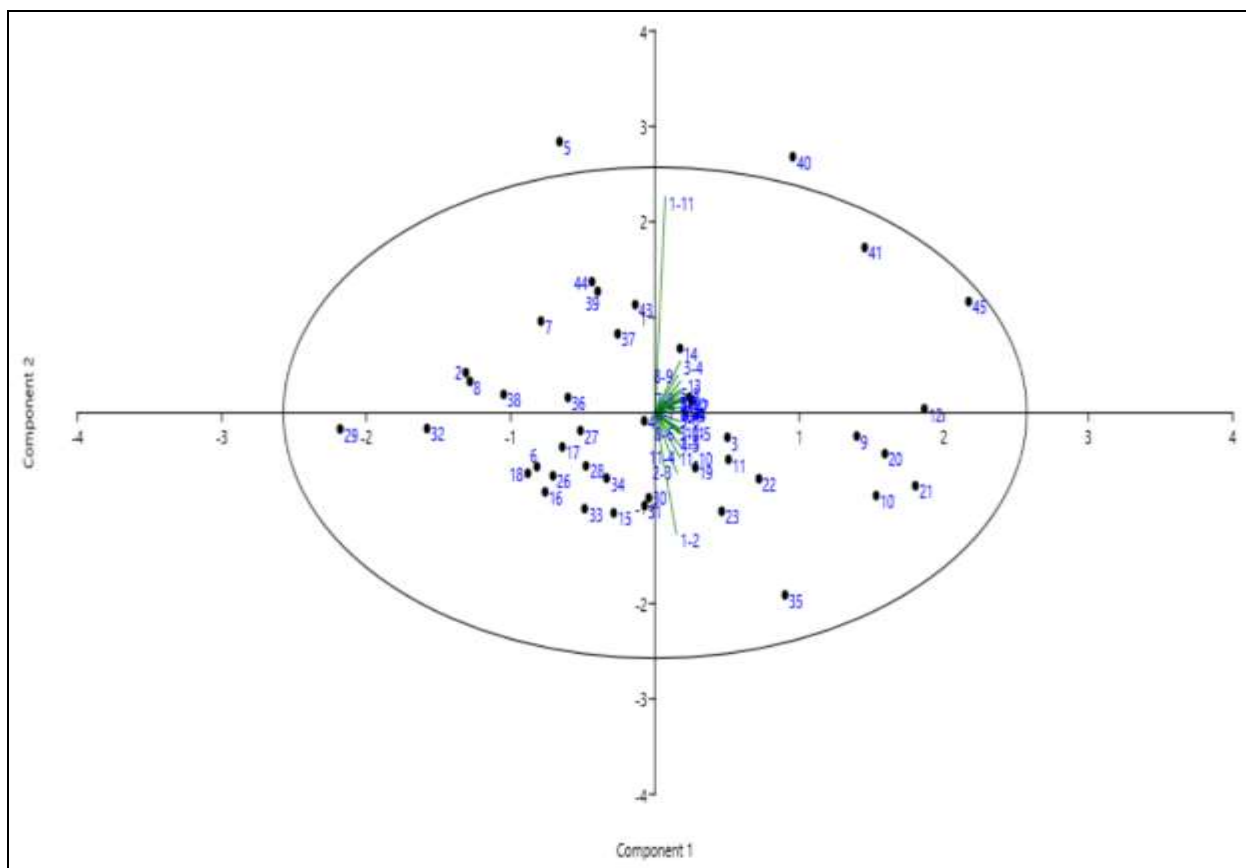


Fig 3: Scatter plot of Principal Components Analysis with Eigenvalue scale, Biplot and 95% ellipses showing positive correlation of the 22 distance characters with the separation of the 42 fish samples into two negatively correlated groups.

Table 9: Principal Components Analysis under Correlation matrix showing PC1 contributing 81% of variance while PC2 contributing only 6% of variance

Principal Components Analysis				
PC	Eigenvalue	% variance	Eig 2.5%	Eig 97.5%
1	17.86	81.22	73.36	85.39
2	1.31	5.99	4.76	9.03

Table 10: Principal Components Analysis showing PC1 with strong loading characters: 1-3, 3-4, 3-10, 4-5, 5-8, 10-4, 10-5, 9-6, 9-5 and 9-4.

Principal Components Analysis		
Distance characters	PC1	PC2
1-2	0.1875	-0.44
1-3	0.2259	-0.059
1-11	0.089	0.7810
2-3	0.1909	-0.2160
3-11	0.2156	-0.031
3-4	0.2208	0.1850
3-10	0.2252	0.073
11-4	0.2181	-0.1620
11-10	0.2173	-0.1187
4-5	0.2244	-0.0698
5-6	0.2154	0.0827
6-7	0.2181	0.0030
7-8	0.2046	0.0477
5-8	0.2267	0.1146
10-4	0.2209	0.041
10-5	0.22989	-0.0730
8-6	0.2128	-0.074
9-6	0.2235	0.020
9-5	0.229	0.055
9-4	0.2320	0.0425
8-9	0.1996	0.1349
10-9	0.2184	0.04180

Thus, PC1 was the most important component contributing to separation among the fish samples. These differences were primarily because of the strong loading of 1-3, 3-4, 3-10, 4-5, 5-8, 10-4, 10-5, 9-6, 9-5 and 9-4 characters. Most of these characters were involved in body depth shape variation (i.e., corresponding shape changes of the dorso-ventral body axis) at the head (1-3), in the middle body (4-10), (9-5) and caudal peduncle (6-7) regions. Strong loading of characters involved in longitudinal body shape changes (i.e., shape changes corresponding to the anterior-posterior body that reflect length changes) are on the dorsal side (3-4), (5-6) and ventral side (10-9).

4. Discussion

The analysis of the data set in the present study rejects null hypothesis of Levene's test and Kruskal-Wallis test. The result of Shapiro-Wilk test showed p-value less than 0.05 for the distances 1-11 and 5-8. This showed that the data is not normal, which is also shown by the histogram. Non-Parametric tests, such as, Tukey's pairwise test and Dunn's post hoc test compared the data for the significant relationship to one another. Based on Tukey's pairwise test, 1-11 distance character has most significant relationship with most of the other distance characters and has the highest value of more than 31% of coefficient of variation.

In general, the body shape of an organism is influenced by both genetic and environmental factors; fishes are recognized as displaying a high degree of environmentally induced morphological variation. Dissimilarities in environmental conditions can be revealed in the phenetic characters of fish populations. Several studies have revealed that body shape in fishes can be altered by culture conditions, such as the quantity of food (Currens *et al.* 1989)^[7], the type of food, or the feeding mode (Wainwright *et al.* 1991, Robinson and Wilson 1996)^[19, 15].

Based on the univariate data analysis (t test and Mann-Whitney test), there is statistically significant relationship between the head and total length of the 42 samples of *L. rohita*. The multivariate data analysis (PCA) exhibited variations in external morphology mainly in 10 distance characters viz. 1-3, 3-4, 3-10, 4-5, 5-8, 10-4, 10-5, 9-6, 9-5 and 9-4. These distance characters are involved in body depth shape variation. Similar result was obtained in crucian carp (*Carassius carassius*) in which body depth was significant in the cultured fish under good food conditions (Bronmark and Pettersson, 1994)^[5]. Principal Components Analysis (PC1) is the most important component contributing to separation between the fish samples (81.225% of the total explained variance). PC1 distinguishes the fish samples into two negatively correlated groups with the samples number 5, 29, 35, 40, 41 and 45 show highest variation. The 22 distance characters are positively correlated with correlation coefficient greater than zero.

PC2 contributes nearly 6% of the total explained variance, which is very less compared to PC1. PC2 presented only three strong loading characters (1-11, 8-9, 5-8) associated with anterior body length and tail region.

5. Conclusion

Morphometrics of the sampled *L. rohita* varies mainly in the body depth, especially in the head, mid body and caudal region. This variation in the body depth is most probably the result of culture conditions, mainly the quantity and type of food and the feeding mode. As the carp is harvested at young

stage, excessive feeding is commonly practiced for fast growth of the fish. Although the study is based on small data set, there is great variance in the means and medians of the data, more than 87% variation based on PCA, positive correlation coefficient value of all the distance characters and significant relationship between the head length and total length of the fish samples.

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