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PRINCIPLE COMPONENT ANALYSIS FOR INTRASPECIFIC VARIATION AMONG THE POPULATIONS OF *Cirrhinus mrigala*

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The intraspecific variation level studies among the populations of *Cirrhinus mrigala* collected from five geographical locations were conducted. The principle component analysis for the degree of variation was done by the Pearson type Principal Component Analysis (PCA) in XLSTAT 2012 version 1.02. Fifty samples from each site were collected and the morphometric parameters of each sample were noted. For the environmental conditions of each geographical location the water analysis was also done and the correlation between the different physicochemical parameters of water viz., water temperature, Dissolved Oxygen (DO), pH, electrical conductivity, total hardness, chlorides, magnesium, carbonates, bicarbonates, total alkalinity, total solids and total dissolved solids were computed by Minitab version 16. The results obtained from the PCA indicated clearly that the increase in the number of factors or components was correlated with the decrease in eigenvalues. The PCA divided the fish populations of five locations on the basis of morphometery into two main components, which all together accounted for 82.56% of cumulative variability. The first group amongst the major two groups accounted for 59.32% of the cumulative variability while the second from these accounted for 23.24% of the cumulative variability.

Keywords: *Cirrhinus mrigala*, PCA, hatchery, natural, morphometric parameters

INTRODUCTION

The *C. mrigala* is grayish silver in colour with plain body, it has 12 to 15 dorsal fin rays, and 39 vertebrae in numbers (Kottelat, 2001). It can obtain maximum length upto 100 cm (Robert, 1997) and the average commonly found individuals are of 40cm (Pethiyagoda, 1991) and maximum reported body weight have been 12.7kg (Talwar and Jhingran, 1991). In the subcontinent the *Cirrhinus mrigala* is commonly known as mrigal, morior, morakhi and it is also recognized as Indian major carp due to their nomenclature and occurrence before the partition amongst the united Indian regions belongs to the ray finned fishes. These fishes are indigenous to the Indus River in Pakistan and Brahmaputra and Ganges Rivers, India. This fish forms one of the top candidates species of the sub-continent aquaculture due to their adaptability in the poly-culture, being herbivorous and harvested record of 463,520 tonnes in India in 2008 (FAO, 2010). Turan and Basusta (2011) studied the level of variation among the twaite shad (*Alosa fallax nilotica*) in the waters of territory of Turkey with the truss morphometric system using Discriminant Function (DFA) and Principal Component Analyses (PCA). Almost forty individuals from the three representative water bodies from three fishing sites viz., Black (Sinop), Aegean (Izmir) and Eastern Mediterranean (Iskenderun) In Discriminant Function Analysis, the ration of correctly classified Eastern Mediterranean sea sample to their original group was highest (90%) with a high overall random assignment of individuals into their original population (78%). Plotting discriminant function 1 (DF1) and discriminant function 2 (DF2) explained 100% of total between group variability and clearly discriminated Eastern Mediterranean sea sample from the Baltic and Aegean sea samples, which were over plotted. PCA revealed that the observed differences were mainly from posterior morphometric measurements of the fish. The patterns of morphological differentiation suggested that there is limited exchange of individuals among areas to homogenize populations phenotypically from the Black and Aegean seas to Eastern Mediterranean sea. Simon *et al.* (2010), used a simple yet useful criterion based on external markings and/or number of dorsal spines which currently being used to differentiate two congeneric archer fish species *Toxotes chatareus* and *Toxotes jaculatrix*. They also studied the other morphometric and meristic characters that can also be used to differentiate these two species. Principal component and/or discriminant functions revealed that meristic characters were highly correlated with pectoral fin ray count, number of lateral line scales, as well as number of anal fin rays. The results indicate that *T. chatareus* can be distinguished from *T. jaculatrix* by having a greater number of lateral line scales, a lower number of pectoral fin rays, and a higher number of anal fin rays. In contrast, morphometric discriminant analyses gave relatively low distinction: 76.1% of fish were ascribed to the correct species cluster. The observed morphometric differences came from the dorsal and anal spines lengths, with *T. chatareus* having shorter dorsal and longer anal spines than *T. jaculatrix*. Overall, meristic traits were more useful than morphometrics in differentiating the two species; nevertheless, meristics and morphometrics together provide information about the morphological differentiation between these two closely related archer fishes. The variation among different species of fishes and other animals from the aquatic as well as teresstrial ecosystems on the basis of morphometric parameters analysis are common (Luthy *et al.*, 2005; Conde-Padín *et al.*, 2007). Therefore, our aim to plan this study was to find variation among the populations of the same species with their occurrence at different geographical locations on the basis of morphometric parameters. The study will be helpful in determining the environmental influence on the differences in the physical appearance of the populations of the same species.

MATERIALS AND METHODS

The 50 samples from each site were collected i.e. were the representatives of hatchery raised and natural populations of the Riverine systems of Punjab viz., UVAS-Fish Hatchery, C-block Ravi campus Pattoki District Kasur, Trimu Barrage at the junction of River Chenab and Jhelum near district Jhang, Taunsa Barrage at River Indus near Tehsil Kot Adu District Muzaffar Garh, Qadirabad Barrage at River Chenab near District Mandi Bahuddin and Baloki Barrage at River Ravi near Tehsil Bhai Phero District Kasur. Data regarding the morphogenetic parameters viz., body weight, fork length, total length and lengths of dorsal, caudal, anal, pectoral and pelvic fins of each individual were recorded. The Pearson type Principal Component Analysis (PCA) was conducted by XLSTAT 2012 version 1.02. For the measurement of water quality parameters to develop correlation matrix between them by Minitab version 16, we followed the manual "Standard Methods for the Examination of Water and Waste Water" by American Public Health Association (1998). From each sampling sites along with the collection of fish morphometric data, water samples for limnological studies were taken in the polythene bottles having 500 ml capacity, the physiochemical characteristics i.e. water temperature Dissolved Oxygen (DO), pH were recorded at the sampling sites. For the remaining physicochemical parameters the samples were taken to the laboratory for analysis of electrical conductivity, total hardness, chlorides, magnesium, carbonates, bicarbonates, total alkalinity, total solids and total dissolved solids.

RESULTS AND DISCUSSION

The results obtained from the PCA indicated clearly that the increase in the number of factors or components was correlated with the decrease in eigenvalues. The values for the different factors showed that the trend reached its maximum at level of second factor (Table 2). The percentage contribution of the each factor to the analysis with transformation showed that as in the squared cosines of the factors, it is clear that the first two factors (F_1 and F_2) have contributed more to the variability and they are indicated by the bold values (Table 3). According to the Kaiser criterion (Kaiser, 1958) based upon the eigenvalues greater than one, the PCA for the morphometric character analysis demonstrated that although the five populations of *C. mrigala* are less distinct, 82.56% of fish were ascribed to the correct species cluster. The first group of parameters which were differentiated as factor one (F1) viz., fish body weight, total length, dorsal fin length, caudal fin length, anal fin length, average length of the paired pectoral fins and average length of paired pelvic fins, accounted for 59.32% of the cumulative variability while the second differentiated group was on the basis of fork length of the fish body determined as factor two (F2) accounted for 23.24% of the cumulative variability (Table 4). Results of these analysis by Pearson correlation showed that the factor one (F1) was positively correlated with the fish body weight, fork length, total length, dorsal fin length, caudal fin length, anal fin length, average length of the paired pectoral fins and also with the average length of the paired pelvic fins. In case of factor two (F2) the correlation with the fish body weight, fork length, total length and dorsal fin length was positive while there was negative correlation of this factor with caudal fin length, anal fin length, average length of the paired pectoral fins and also with the average length of the paired pelvic (Table 5). These results are in accordance with the results of Simon *et al*. (2010), who postulated that PCA account for 60.70% of the total morphological variations while studying the degree of variation among the populations of T. *chatareus* and *T. jaculatrix* on the basis of morphometric parameters. These results are also in accordance with the results concluded by Turan and Basusta (2011) while studying the level of variation among the twaite shad (*Alosa fallax nilotica*) in the waters of territory of Turkey with the truss morphometric system using Discriminant Function (DFA) and Principal Component Analyses (PCA). In their experiment, the ration of correctly classified Eastern Mediterranean sea sample to their original group was highest (90%) with a high overall random assignment of individuals into their original population (78%). On the basis of our results it can be concluded that five populations of *Cirrhinus mrigala* were different from each other on the basis of differences in fish body weight, total length, dorsal fin length, caudal fin length, anal fin

Table 1. Summary statistics for the morphometric parameters

Variable	Minimum	Maximum	Mean	Std. deviation
Weight	204.00	315.00	258.48	27.92
F-Length	20.60	27.60	25.29	1.43
T-Length	24.40	31.50	28.89	1.44
D-Fin	3.20	5.10	4.24	0.42
C-Fin	3.10	5.20	4.07	0.51
A-Fin	2.30	4.90	3.57	0.61
Pe-Fin	2.10	3.90	3.20	0.50
Pl-Fin	1.40	3.40	2.57	0.52

Note: -Weight (Body Weight of fish), F-Length (Fork Length of fish), T-Length (Total Length of fish), D-Fin (Dorsal Fin Length), C-Fin (Caudal Fin Length), A-Fin (Anal Fin Length), Pe-Fin (Average Length of Paired Pectoral Fin) and Pl--Fin (Average Length of Paired Pelvic Fin)

Table 2. Contribution of the each morphometric parameter toward variability (%)

	F1	F2	F3	F4	F5	F6	F7	F8
Weight	13.20	9.52	3.00	10.11	56.80	2.49	4.81	0.07
Fork Length	6.73	34.30	0.74	2.99	1.54	22.77	5.12	25.82
Total Length	10.97	19.06	2.16	21.24	4.34	23.38	0.21	18.57
Dorsal Fin	17.75	0.04	0.13	27.27	14.21	16.43	3.52	20.66
Caudal Fin	12.14	2.31	53.05	0.35	12.36	9.53	0.56	9.71
Anal Fin	12.02	6.51	40.87	7.32	1.33	14.48	0.09	17.39
Pectoral Fin	12.13	15.95	0.04	27.50	1.21	8.45	33.46	1.26
Pelvic Fin	15.06	12.31	0.00	3.23	8.145	2.478	52.248	6.53

Table 3. Squared cosines of the of morphometric parameters

	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Weight	0.63	0.18	0.02	0.04	0.14	0.00	0.00	0.00
Fork Length	0.32	0.64	0.01	0.01	0.00	0.01	0.00	0.01
Total Length	0.52	0.35	0.01	0.08	0.01	0.01	0.00	0.01
Dorsal Fin	0.84	0.00	0.00	0.11	0.03	0.01	0.00	0.01
Caudal Fin	0.58	0.04	0.34	0.00	0.03	0.01	0.00	0.00
Anal Fin	0.57	0.12	0.26	0.03	0.00	0.01	0.00	0.01
Pectoral Fin	0.58	0.30	0.00	0.12	0.00	0.00	0.01	0.00
Pelvic Fin	0.72	0.23	0.00	0.01	0.02	0.00	0.02	0.00

Values in bold correspond for each variable to the factor for which the squared cosine is the largest

Table 4. Eigen values

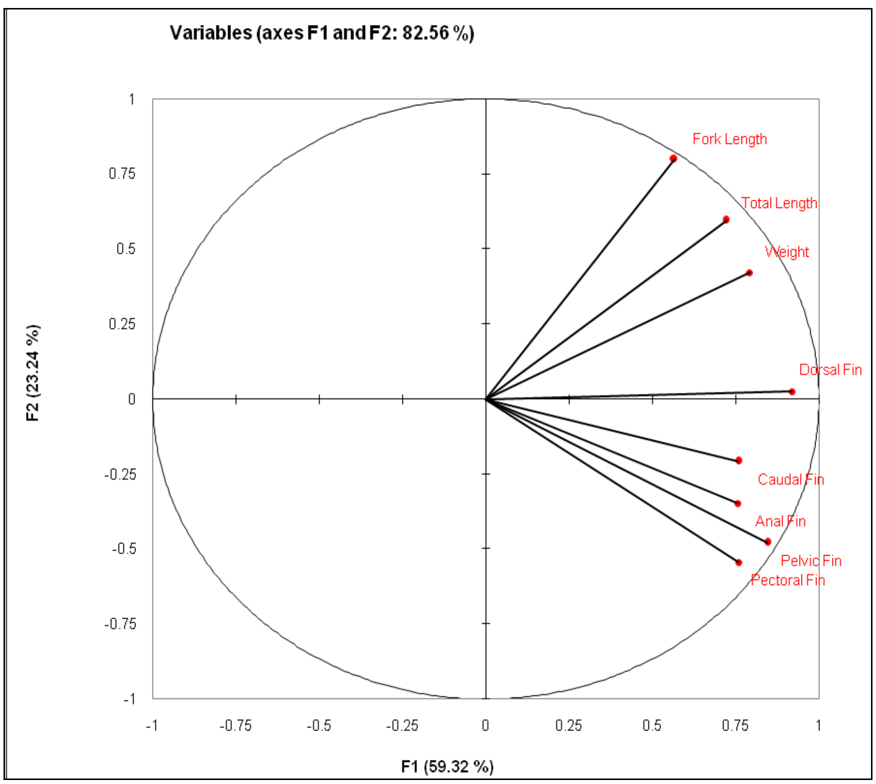
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Eigen value	4.75	1.86	0.64	0.39	0.24	0.05	0.04	0.04
Variability (%)	59.32	23.24	8.06	4.82	2.97	0.65	0.51	0.44
Cumulative %	59.32	82.56	90.61	95.43	98.40	99.06	99.56	100.00

Table 5. Correlations between morphometric parameters and designated as factors

	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Weight	0.79	0.42	0.14	-0.20	0.37	0.04	-0.04	0.01
Fork Length	0.57	0.80	-0.07	0.11	-0.06	-0.11	0.05	0.10
Total Length	0.72	0.60	-0.12	0.29	-0.10	0.11	-0.01	-0.08
Dorsal Fin	0.92	0.03	-0.03	-0.32	-0.18	-0.09	-0.04	-0.09
Caudal Fin	0.76	-0.21	0.59	-0.04	-0.17	0.07	0.02	0.06
Anal Fin	0.76	-0.35	-0.51	-0.17	-0.06	0.09	0.01	0.08
Pectoral Fin	0.76	-0.55	-0.02	0.33	0.05	-0.07	-0.12	0.02
Pelvic Fin	0.85	-0.48	-0.00	0.11	0.14	-0.04	0.15	-0.05

length, average length of the paired pectoral fins and average length of paired pelvic fins and the major difference was on the basis of fork length of the fish. These results are almost according to the findings of Simon *et al.* (2010) who concluded that length of dorsal fin base, depth of caudal peduncle and head length and the first anal spine length and length of soft dorsal ray for *T. chatareus* and *T. jaculatrix* poulations diferrent for the two populations and major share was from the dorsal and anal spines lengths, with having shorter dorsal and longer anal spines. The factor one (F1) determined that fish body weight, fork length, total length, dorsal fin length, caudal fin length, anal fin length, average length of the paired pectoral fins and also average length of the paired pelvic fins of all the populations were positively correlated with each other. Results of these analysis showed that the factor one (F1) was positively correlated with the fish body weight, fork length, total length, dorsal fin length, caudal fin length, anal fin length, average length of the paired pectoral fins and also with the average length of the paired pelvic fins. In case of factor two (F2) the correlation with the fish body weight, fork length, total length and dorsal fin length was positive while there was negative correlation of this factor with caudal fin length, anal fin length, average length of the paired pectoral fins and also with the average length of the paired pelvic. These results are almost like the results of Simon *et al.* (2010), who postulated that almost all the loadings were negative with no clear pattern of the total variance, with length of dorsal fin base, depth of caudal peduncle and head length and the first anal spine length and length of soft dorsal ray.

The graphs generated from the data when subjected to PCA in terms of plots among the variable (Morphometric parameters), observations (all the samples from five sites) and the bi-plot, i.e. variable and observation plot determined the degree of relatedness among the individuals of the different populations from five geographical locations. By the transformation of the data in the PCA the F1 and F2 factors based graph between the variables showed that the cumulative share of these variables was 82.56% while the F1 contributed 59.32% and F2 shared 23.24%. By observing this variables plot it is clear that on the basis of morphometric parameters all the populations lies in the same area (0 to +1.0) with some minute differences (Fig. 1). The observation (samples) plot was also generated on the same share of variability by these two factors. The 20 representative individuals of each population from the total 50 samples of each geographical location were chosen to generate this plot. The observation plot showed that almost 90% of the randomly selected representative samples of the all populations lies in the same region (0 to +5) while the



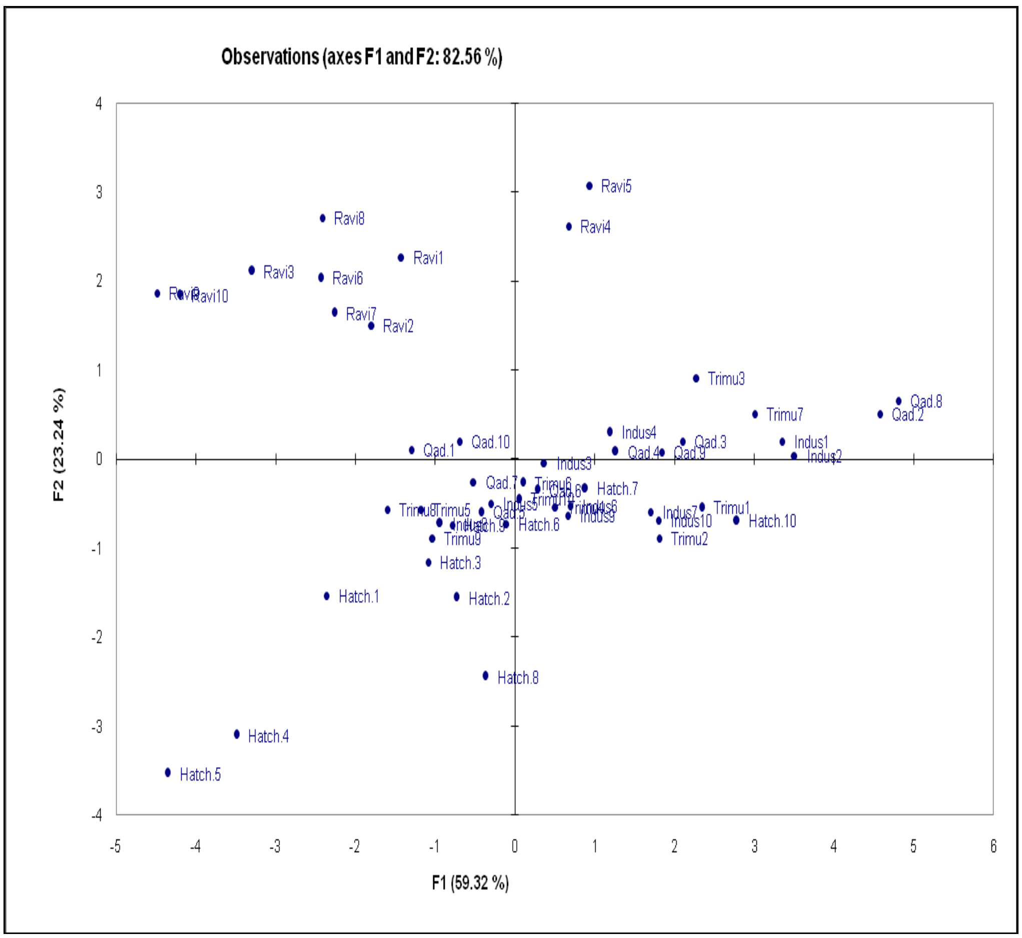
Note: -Weight (Body Weight of fish), F-Length (Fork Length of fish), T-Length (Total Length of fish), D-Fin (Dorsal Fin Length), C-Fin (Caudal Fin Length), A-Fin (Anal Fin Length), Pe-Fin (Average Length of Paired Pectoral Fin) and Pl--Fin (Average Length of Paired Pelvic Fin)

Figure 1. Morphometric parameters (variables) variability plot

remaining individuals lies in the same region (0 to -4.5) (Fig. 2). It means on the basis of PCA analysis the all the geographical representative samples were divided into two major groups and their degree of relatedness varies from 0 to +5 in groups first while the group second was between 0 to -4.5. Bi-plot was also generated with these factors and which shows similar trend in the degree of relatedness as for the variable and observation plot behaves individually (Fig. 3).

The Pearson correlation of the pH with water temperature (r= 0.11) and dissolved oxygen (r = 0.91) was positively non-significant while the correlation with electrical conductivity (r = -0.80), salinity (r= -0.89), total dissolved solids (r = -0.86), total alkalinity (r = -0.74) and total hardness (r = -0.50) was negatively non-significant. The correlation of the dissolved oxygen with water temperature (r= 0.31) was positively non-significant while the correlation with electrical conductivity (r = -0.67), salinity (r= -0.83), total dissolved solids (r = -0.81), total alkalinity (r = -0.93) and total hardness (r = -0.30) was negative but also non-significant as like with the water temperature. The electrical conductivity was positively correlated with all the physico-chemical parameters as with water temperature (r= 0.48), salinity (r= 0.93), total dissolved solids (r = 0.89), total alkalinity (r = 0.45) and total hardness (r = 0.91) and this correlation was non-significant (Table 6).

The salinity amongst the water parameters was correlated positively with water temperature (r = 19), total alkalinity (r = 0.72) and total hardness (r = 0.73) and it was non-significant but with total dissolved solids (r = 0.95) the correlation was also positive but highly significant (P <0.01). The total dissolved solids values observed from the study sites were positively correlated with water temperature (r = 0.17), total alkalinity (r = 0.73) and total hardness (r = 0.66) and this correlation was non-significant. These results are according to the findings of Shinde *et al.* (2011) who found that correlation coefficient showed high significant



Note: -Hatch. (Samples from Hatchery), Trimu (Samples from Trimu Barrage), Indus (Samples from River Indus, Taunsa Barrage), Qad. (Samples from Qadirabad Barrage) and Ravi (Samples from Ravi Barrage)

Figure 2. Samples (observations) variability plot

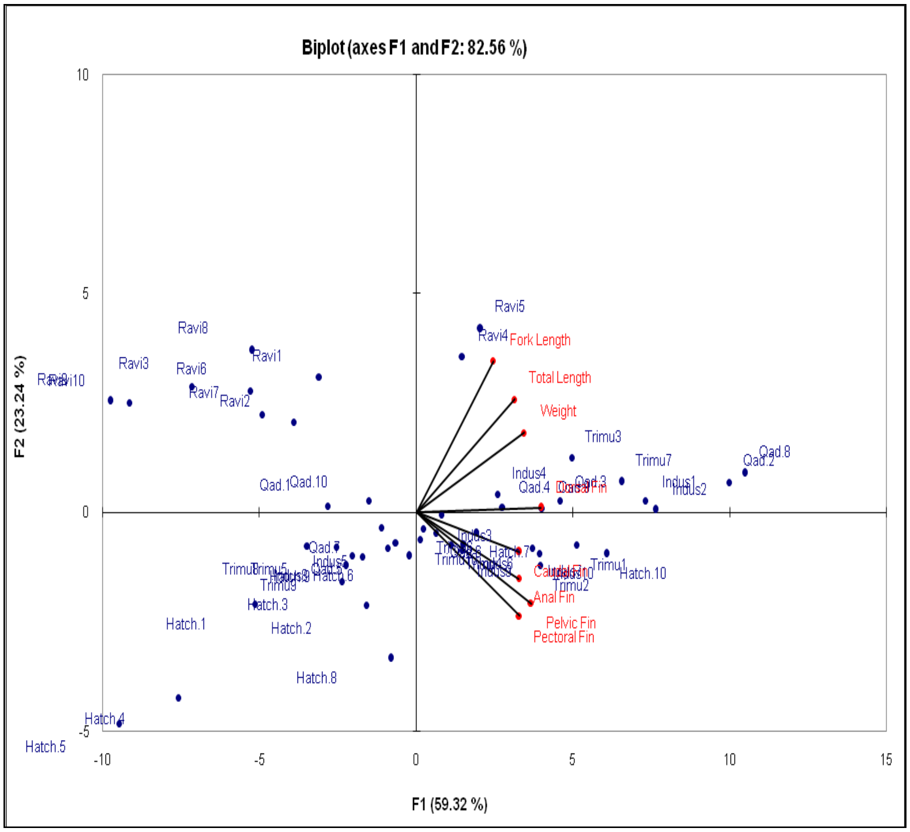


Figure 3. Variables and observations variability Bi-Plot

Table 6. Correlation matrix of physico-chemical parameters of the study sites

	Temp.	**pH**	**D.O.**	**E.C.**	**Sal.**	**T.D.S.**	**T.A.**
pH	0.11						
	0.86						
D.O.	0.31	0.91					
	0.61	0.03					
E.C.	0.48	-0.80	-0.67				
	0.41	0.11	0.22				
Sal.	0.19	-0.89	-0.83	0.93			
	0.76	0.04	0.08	0.03			
T.D.S.	0.17	-0.86	-0.81	0.89	0.99		
	0.78	0.06	0.10	0.04	0.00		
T.A.	-0.48	-0.74	-0.93	0.45	0.72	0.73	
	0.41	0.16	0.02	0.45	0.17	0.16	
T.H.	0.80	-0.50	-0.30	0.91	0.70	0.66	0.05
	0.10	0.39	0.62	0.03	0.19	0.23	0.94

Cell Contents: Pearson Correlation
P-value

positive and negative relationship ($p < 0.01$) while working with the physico-chemical parameters and correlation coefficient of Harsool-savangi Dam, District Aurangabad, India. The correlation between the total alkalinity and total hardness was also positive and non-significant ($r = 0.05$). Most of the physico-chemical parameters were positively correlated with each other which satisfied the water conditions for the presence of fish and these results are in accordance to the findings of Sen *et al.* (2011) while working with the study of some physico-chemical parameters of pond and river water with reference to correlation study.

***Conclusion*:** From the results it was observed that the differentiation is there on the basis of morphometric parameters. So it can be concluded that although all the populations are from the same species but collected from different geographical locations, so the intraspecific variation may be due to the reason of changes in environmental conditions or other evolutionary factors.

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