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The inter and intrapopulation variations in two species of genus *Brachionus* (Rotifera), in Iran: An ecological review

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ABSTRACT

Brachionus is an suitable live foods for fishes larve of Brachionidae family, in the present study inter and intera-population variations of two species of family Brachionidae were investigated. For this purpose 90 individuals of geographical populations of these species were collected from different parts of Arak country and for each habitat five ecological factors were examined. Totally eleven quantitative morphological characters were examined from Lorica. The ANOVA test and also one–sample T-test showed significant difference for some morphological characters. In interpopulation section, 10 individuals were selected randomly for each population. Individuals were separated from each other in PCO, PCA, CA plots and UPGMA tree. Significant correlations negative/positive found between some of morphological characters with studied ecological factors of habitat. In addition, variations in morphological characters were seen between populations and they were separated in CA plot and also UPGMA tree. Ecological factors were different between habitat, Therefore habitats were separated in UPGMA tree and PCO, PCA, CA plots. This subject confirmed effect of different ecological factors on phenotypic plasticity of populations.

Keyword: Ecology, Brachionidae, Species, Populations.

INTRODUCTION

Rotifers as heterotrophic, microscopic, multicellular and diverse are abundance organisms which presence in almost aquatic ecosystem, they play important role as the second link of water food chain [1]. Rotifers are also commonly found on mosses and lichens growing on tree trunks and rocks, also they may be found in rain gutters and puddles, in soil or leaf litter, on mushrooms growing near dead trees, in tanks of sewage treatment plants, and even on freshwater crustaceans as well as aquatic insect larvae [2]. The variation and density of Rotifers in aquatic ecosystems are useful in ecological aspects, water indicator and fishery economy [3]. Difference in the morphological traits which seen in different individuals of a same species is an epigenetic variation that response to various environmental condition and different ecological factors is an aspect of phenotype plasticity [4, 5]. The genus *Brachionus* belongs to Brachionidae family comprising of 20 species in Iran[6]. In present study, in order to compare the effect of different ecological factors on morphological features of these organisms, 90 individuals of nine geographical populations of two species, *Brachionus uceolaris* and *Brachionus quadridentatus*, were examined at inter and intra-population levels.

MATERIALS AND METHODS

The rotifer collection was done with the help of a standard plankton net Hydrobios, Kiel; 55 µm mesh size through vertical and horizontal hauls at about 20 centimeters below the water surface level. Sampling was done from June 2012 to May 2013. The collected samples were preserved in small bottles including 7% glycol–alcohol then

transferred to the biological laboratory of Arak University. The genus *Brachionus* were separated from other genus and were identified based on funesticaly study with the help of Olympus-BX51 microscope, based on valuable and available references such as Ward and Whipple[7] Sharma and Miche[8]. Five geographical populations of the species *Brachionus uceolaris* and four populations of the species *Brachionus quadridentatus* were randomly collected with 10 repetitions. Eleven quantitative morphological characters examined between and within populations. Five ecological features such as longitude (E˚), latitude (N˚), elevation (in meter), average of temperature (in C˚) and pH were examined of each habitat. Longitude, latitude and elevation were calculated with Garmin GPS and averages of temperature for each population were extracted from of meteorology organization of Arak county.

The mean of morphological characters of each populations were standardizes (means=0, variance=1) and used for multivariate analysis including UPGMA (Unweighted Paired Group Using Average Method). Principal Coordinate Analysis (PCA).Analysis of variance (ANOVA) test was performed to assess significant difference in quantitative morphological characters among populations. Pearson's coefficient of correlation was determined between quantitative morphological characters with ecological features of the population's habitat per year to show their possible relationship between them.

RESULTS

In this study, different populations of two species of the genus *Brachionus* namely *B. uceolaris* and *B. quadridentatus* were identified and collected from different environments. This study was done in two levels, among (intra) and between (inter) species. Inter species study carried out at inter and intra-populations levels. Totally five populations of *B. uceolaris* and four populations' *B. quadridentatus* with ninety individuals were examined.

Intrapopulations study of *B. quadridentatus*

Quantitative morphological feature varied between individuals and ANOVA test performed between these characters showed significant difference ($p<0.05$) for all examined features such as lorica length, lorica width, antero midian spin, distance-antero midian spin, antero lateral spin, distance–antero lateral spin, antero intermediate spin, distance-antero intermediate spin, foot length, toes length and occipital median spin. In addition, one- sample T-test showed significant difference for all examined characters (Table 2, 3).

Table1. Habitat Characteristics of studied populations

Populations	Sampling Stations	Longitude (N)	Latitude (E)	Altitude	Abbreviation
1	Baghvahsh Pool	34° 06′98″	49°45′52″	1731m	B.V
2	Karahrod Fountain	34° 034′14″	49°38′44″	1810m	Gh, Ka
3	Kelaleh Dam	34° 03′36″	49°36′55″	1938m	S.K
4	Kellaleh Sinkholes	34° 03′51″	49°36′02″	2020m	A.K
5	Amir kabir Pool	34° 04′87″	49°03′68″	1763m	A.Ka
6	Serahkhomein Pool	34° 04′33″	49°46′33″	1723m	Se.k
7	Daneshjo Pool	34° 05′43″	49°42′28″	1750m	P.D

Table 2.One-SampleT-test of quantitative morpholiogical of *B.quadridentatus*

Morphological characters	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
lorica length	34.105	43	.000	169.35000	159.3361	179.3639
lorica width	34.285	43	.000	140.45682	132.1949	148.7187
antero midian spin	43.060	43	.000	20.11364	19.1716	21.0556
distane-antero midian spin	34.130	43	.000	20.32045	19.1198	21.5212
antero-intermediate midian spin	34.002	43	.000	16.01136	15.0617	16.9610
distance-antero intermediate midian spin	34.268	43	.000	51.97273	48.9141	55.0313
antero lateral spin	37.571	43	.000	17.21818	16.2940	18.1424
distance-antero lateral midian spin	36.194	43	.000	71.66591	67.6727	75.6591
toes length	52.475	43	.000	77.37727	74.4035	80.3510
foot length	41.906	43	.000	5.47386	5.2104	5.7373
occipital median spin	33.013	43	.000	61.36455	57.6159	65.1132

Significant correlations positive or negative occurred between morphological features of individuals with ecological factors of habitat for example a positive significant correlations ($p<0.01$, $r=0.56$) found between lorica length with north distribution, lorica length had significant negative correlations ($p<0.05$, $r=-0.35$) with east distribution and positive significant correlations ($p<0.01$, $r=0.73$) occurred between lorica length with temperature and also positive significant ($p<0.01$, $r=0.56$) width lorica with north distribution width lorica had significant negative with east distribution, positive significant width lorica with temperature ($p<0.01$, $r=0.74$), negative significant antero midian

spin with east distribution (p<0.01, r=-0.52), pH (p<0.05, r=-0.30). A positive significant correlations found between distance-antero midian spin with north distribution (p<0.01, r=0.56), temperature (p<0.01, r=0.74), and also distance-antero midian spin had significant negative with east distribution (p<0.05, r=-0.34), A positive significant correlations found between antero intermediate spin with north distributions (p<0.01, r=0.47), temperature (p<0.01, r=0.64), antero midian spin had negative significant correlation (p<0.01, r=-0.40) with east distribution, A negative significant correlation (p<0.01, r=-0.53) found between distance-antero intermediate spin with east distribution and also positive significant(p<0.01, r=0.53) antero lateral spin with east distribution, distance-antero lateral spin had significant positive correlations with north distribution (p<0.01, r=0.51), habitat elevation (p<0.01, r=0.3), temperature (p<0.01, r=0.74) , also negative significant correlation(p<0.01, r=-0.42) distance-antero lateral spin with east distribution. A positive significant correlation found between foot length with north distribution (p<0.01, r=0.53) and temperature (p<0.01, r=0.72) also had positive significant correlations toes length with temperature (p<0.05, r=0.36), pH (p<0.01, r=0.44), A positive significant correlation occurred between occipital median spin with north distribution (p<0.01, r=0.55) and temperature (p<0.01, r=0.72). also negative significant with east distribution (p<0.05, r=-0.36). Studied individuals were separated from each others in UPGMA tree of morphological features (Fig1). also PCO plat showed high difference between individuals of populations especially in Karahrod Fountain population (Fig 2).

Table3.ANOVA test of quantitative morphological characters of *B. quadridentatus*

Morphological characters		Sum of Squares	df	Mean Square	F	Sig.
Lorica Length	Between Groups	31067.850	3	10355.950	26.585	.000
	Within Groups	15581.600	40	389.540		
	Total	46649.450	43			
Lorica Width	Between Groups	21069.228	3	7023.076	26.292	.000
	Within Groups	10684.840	40	267.121		
	Total	31754.068	43			
Antero Midian Spin	Between Groups	256.710	3	85.570	21.927	.000
	Within Groups	156.102	40	3.903		
	Total	412.812	43			
Distane-Antero Midian Spin	Between Groups	447.575	3	149.192	26.749	.000
	Within Groups	223.096	40	5.577		
	Total	670.672	43			
Antero-Intermediate Midian Spin	Between Groups	251.228	3	83.743	19.901	.000
	Within Groups	168.316	40	4.208		
	Total	419.544	43			
Distance-Antero Intermediate Midian Spin	Between Groups	2884.158	3	961.386	26.198	.000
	Within Groups	1467.889	40	36.697		
	Total	4352.047	43			
Antero Lateral Spin	Between Groups	187.556	3	62.519	11.919	.000
	Within Groups	209.809	40	5.245		
	Total	397.365	43			
Distance-Antero Lateral Midian Spin	Between Groups	5109.648	3	1703.216	29.516	.000
	Within Groups	2308.191	40	57.705		
	Total	7417.839	43			
Toes Length	Between Groups	2159.445	3	719.815	14.732	.000
	Within Groups	1954.373	40	48.859		
	Total	4113.817	43			
Foot Length	Between Groups	8.081	3	2.694	4.452	.009
	Within Groups	24.201	40	.605		
	Total	32.282	43			
Occipital Median Spin	Between Groups	4300.432	3	1433.477	25.636	.000
	Within Groups	2236.631	40	55.916		
	Total	6537.063	43			

Interpopulation study *B. quadridentatu*

In this section average amounts of each morphological character used for examination of variations between populations. Significant correlations positive or negative occurred between average amounts of morphological features with ecological factors of habitat for example a positive significant correlation (p<0.01, r=- 0.99) found between foot length with temperature. Studied populations were different in morphological characters and separated from each other in UPGMA tree as well as CA plot (Fig 3, 4).

Intrapopulations study of *B. uceolaris*

were studied quantitative morphological feature varied between individuals and performed ANOVA test showed significant difference (p<0.05) for all feature such as lorica length, lorica width, antero midian spin, distance-antero midian spin, antro lateral spin, distance–antero lateral spin, antero intermediate spin, distance-antero intermediate

spin, foot length, toes length and occipital median spin. But one- sample T-test showed significant difference for all examined characters (Table 4, 5).

Table4.One-SampleT-test of quantitative morpholiogical of *B. uceolaris*

Morphological characters	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Lorica Length	49.044	54	.000	189.035	181.31	196.76
Lorica Width	53.737	54	.000	141.18000	135.9127	146.4473
Antero Midian Spin	46.843	54	.000	27.80000	26.6101	28.9899
Distane-Antero Midian Spin	53.549	54	.000	28.14545	27.0917	29.1992
Antero-Intermediate Midian Spin	24.570	54	.000	15.67818	14.3988	16.9575
Distance-Antero Intermediate Midian Spin	53.005	53	.000	70.52593	67.8572	73.1947
Antero Lateral Spin	25.180	54	.000	17.04545	15.6883	18.4026
Distance-Antero Lateral Midian Spin	53.280	54	.000	70.54727	67.8926	73.2019
Toes Length	112.093	54	.000	128.40000	126.1035	130.6965
Foot Length	21.920	54	.000	8.62364	7.8349	9.4124
Occipital Median Spin	51.554	54	.000	29.82000	28.6603	30.9797

Table5.ANOVA test of quantitative morpholiogical characters of *B. uceolaris*

Morphological characters		Sum of Squares	df	Mean Square	F	Sig.
Lorica Length	Between Groups	19245.514	3	6415.171	11.700	.000
	Within Groups	21932.982	40	548.325		
	Total	41178.495	43			
Lorica Width	Between Groups	11067.153	3	3689.051	18.840	.000
	Within Groups	7832.289	40	195.807		
	Total	18899.443	43			
Antero Midian Spin	Between Groups	428.646	3	142.882	17.083	.000
	Within Groups	334.558	40	8.364		
	Total	763.204	43			
Distane-Antero Midian Spin	Between Groups	443.605	3	147.868	19.078	.000
	Within Groups	310.022	40	7.751		
	Total	753.627	43			
Antero-Intermediate Midian Spin	Between Groups	162.515	3	54.172	3.657	.020
	Within Groups	592.533	40	14.813		
	Total	755.048	43			
Distance-Antero Intermediate Midian Spin	Between Groups	2819.045	3	939.682	20.052	.000
	Within Groups	1874.496	40	46.862		
	Total	4693.542	43			
Antero Lateral Spin	Between Groups	228.565	3	76.188	3.049	.040
	Within Groups	999.493	40	24.987		
	Total	1228.057	43			
Distance-Antero Lateral Midian Spin	Between Groups	2844.057	3	948.019	19.355	.000
	Within Groups	1959.251	40	48.981		
	Total	4803.308	43			
Toes Length	Between Groups	1033.770	3	344.590	5.346	.003
	Within Groups	2578.462	40	64.462		
	Total	3612.232	43			
Foot Length	Between Groups	201.630	3	67.210	21.252	.000
	Within Groups	126.502	40	3.163		
	Total	328.132	43			
Occipital Median Spin	Between Groups	538.845	3	179.615	19.269	.000
	Within Groups	372.860	40	9.322		
	Total	911.705	43			

Significant correlations positive or negative occurred between morphological features of individuals with ecological factors of habitat for example a negative significant correlation ($p<0.01$, $r=-0.39$) found between lorica length with north distribution, lorica width had significant negative correlations ($p<0.01$, $r=-0.47$) with north distribution and negative significant correlation ($p<0.01$, $r=-0.46$) occurred between antero midian spin with north distribution .A negative significant correlation ($p<0.01$, $r=-0.47$) found between distance-antero midian spin with north distribution and also positive significant antero intermediate spin with north distribution ($p<0.05$, $r=0.32$), also distance-antero intermediate spin had positive significant ($p<0.01$, $r=0.95$) with north distribution, A positive significant correlation ($p<0.05$, $r=0.36$) was found between antero lateral spin with temperature and pH ($p<0.05$, $r=0.32$), foot length had negative significant correlation ($p<0.01$, $r=-0.41$) with temperature , also positive significant correlation with pH ($p<0.05$, $r=0.27$), A positive significant correlation was found between toes length with north distribution ($p<0.05$, $r=0.38$) and pH ($p<0.05$, $r=0.29$) also negative significant with habitat elevation ($p<0.05$, $r=-0.29$),temperature ($p<0.01$, $r=-0.54$), and occipital median spin had significant negative correlations ($p<0.05$, $r=-0.5$) with north

distribution. PCO plot showed high difference between individuals of populations especially in Kellaleh Sinkholes population (Fig 5). Individuals of all populations were placed far from other in the mention diagram. this subject confirmed high variations in individuals morphological characters.

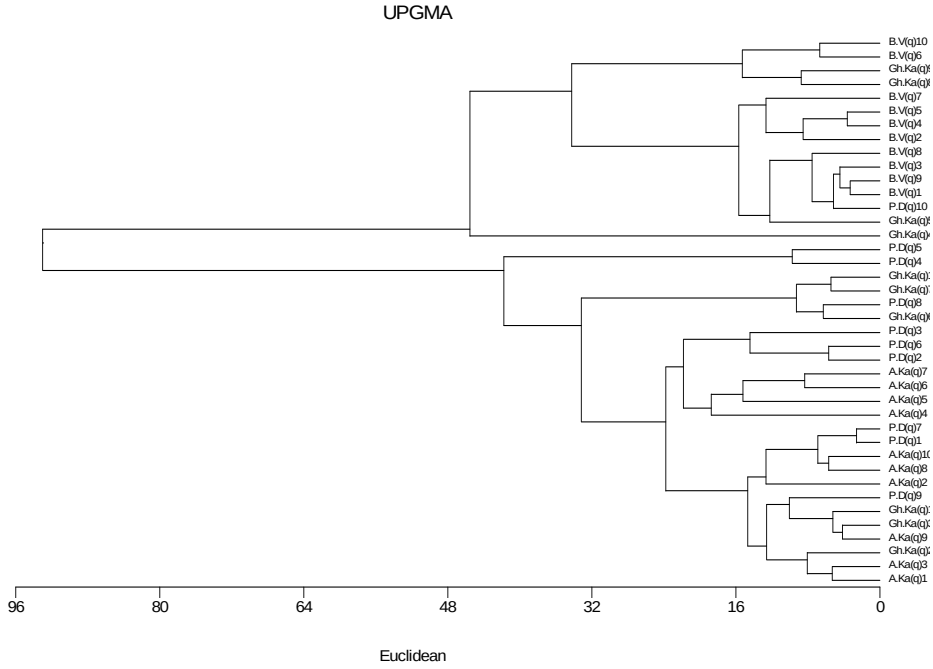

Figure.1.Morphological UPGMA tree of studied individuals populations species o*f B. quadridentatu*s

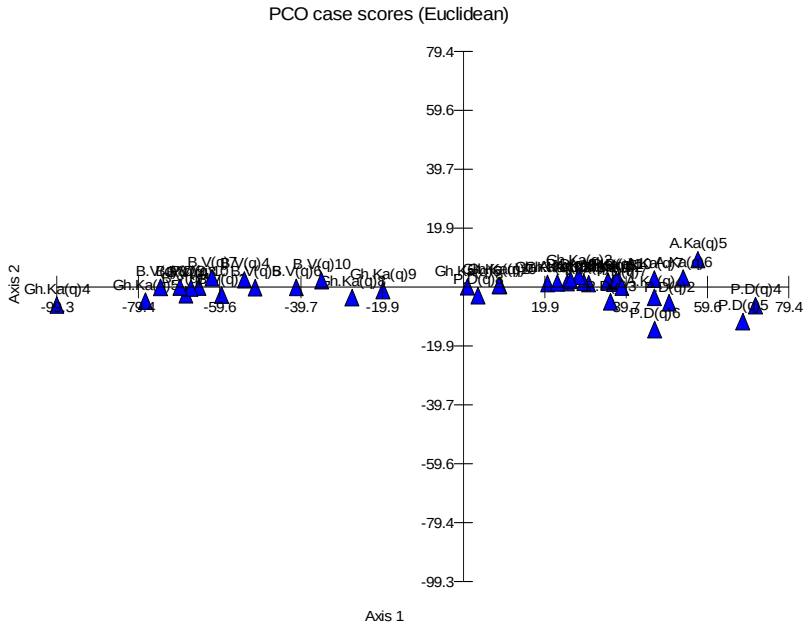

Figure.2. PCO plot of individuals of studied populations species of *B. quadridentatu*s based on morphological characters

Interpopulation study *B. uceolaris*

In this section average amounts of each morphological character were used for examination of variations between populations. Totally eleven qualitative morphological characters were investigated. For example, Amirkabir pool population had shortest lorica length, lorica width, antero midian spin, distance-antero midian spin, distance–antero lateral spin, distance-antero intermediate spin and occipital median spin. No significant differences between average amounts of morphological features with ecological factors of habitat. Studied populations were different in morphological characters and separated from each other in UPGMA tree as well as CA plot (Fig 6 and 7).

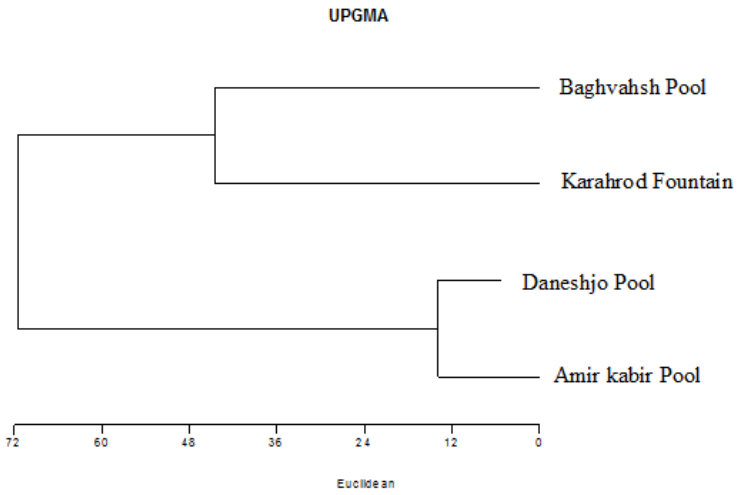

Figure.3. UPGMA tree of populations species of *B. quadridentatus* based on morphological characters

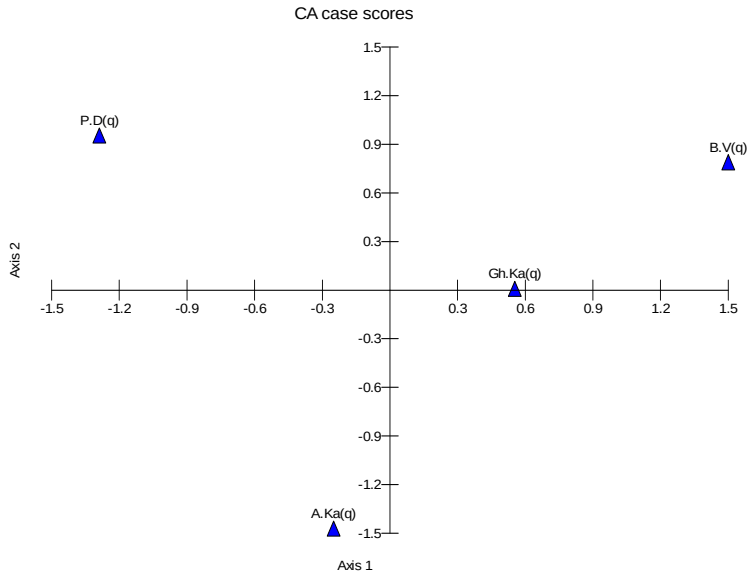

Figure.4 CA plot populations species of *B. quadridentatus* based on morphological characters

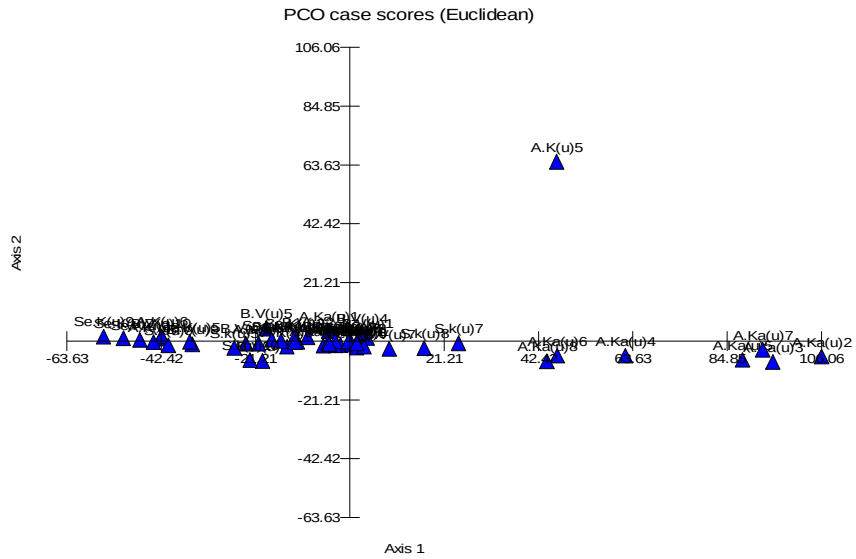

Figure.5. PCO plot of individuals of studied populations species of *B. uceolaris* based on morphological characters

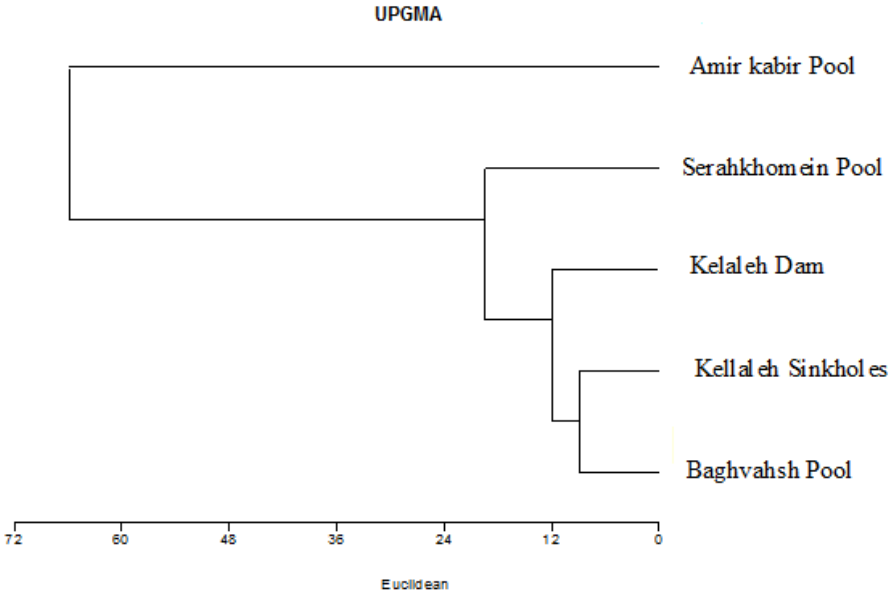

Figure.6. UPGMA tree populations species of *B. uceolaris* based on morphological characters

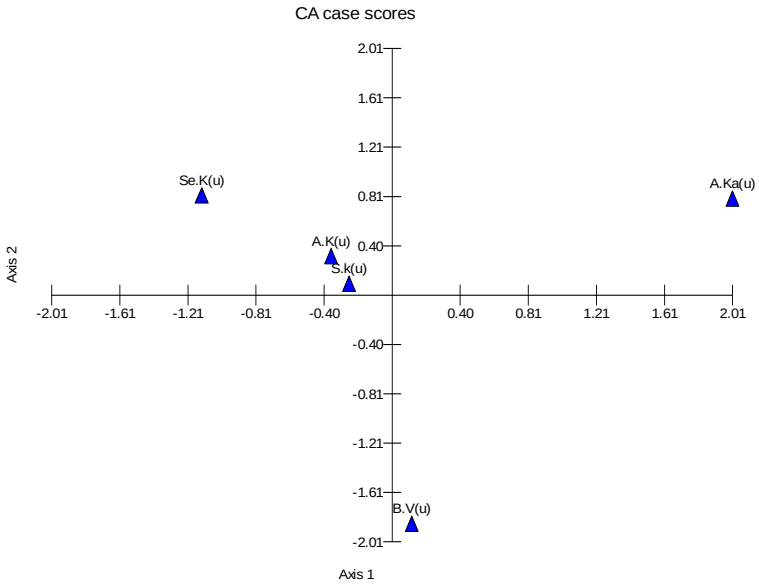

Figure.7. CA plot populations species of *B. uceolaris* based on morphological characters

In addition, in the study, PCO plat as well as CA plot showed high difference between individuals of populations especially in Karahrod Fountain population (figs 9, 10). Individuals of all populations were far from other in the mention diagram this subject confirmed high variations in individuals morphological characters. Variations showed in PCA plot and UPGMA tree between individuals of species *B. quadridentatus* in Karahrod Fountain populations (figs8,9). Also in CA Plot were found Variations between individuals of two species *B. uceolaris* and *B. quadridentatus* over an extended about of interactions inter species (Fig10).

Ecological study

Five environmental factors were examined for each habitat including average temperature, elevation of habitat and their longitude, altitude and pH. The mentioned ecological factors differed between stations and habitat were separated in ecological PCA plot (Fig12) and also UPGMA tree of ecological factors (Fig11). In the mention diagram populations of *B. uceolaris* in Kellaleh Sinkholes habitat and *B. quadridentatus* in Karahrod Fountain habitant were far from each others. This subject confirmed difference and variation in ecological condition of habitats.

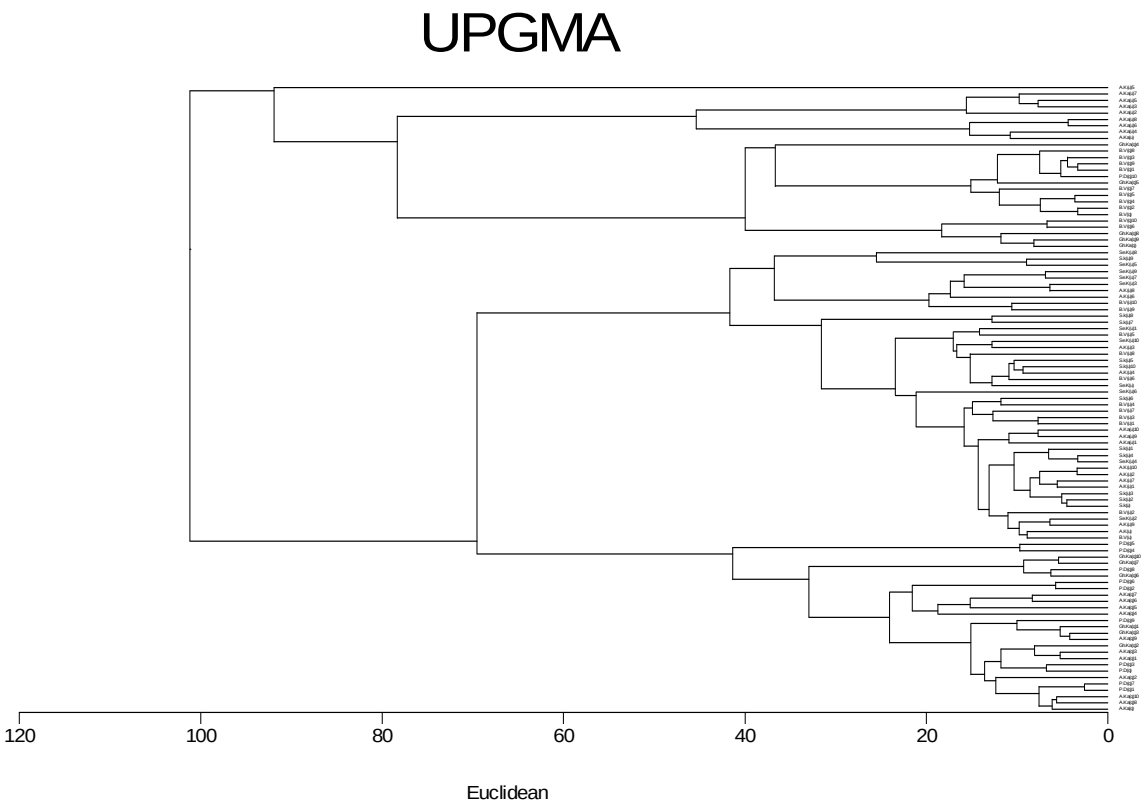

Figure.8. Morphological UPGMA tree of studied individual's populations for two species

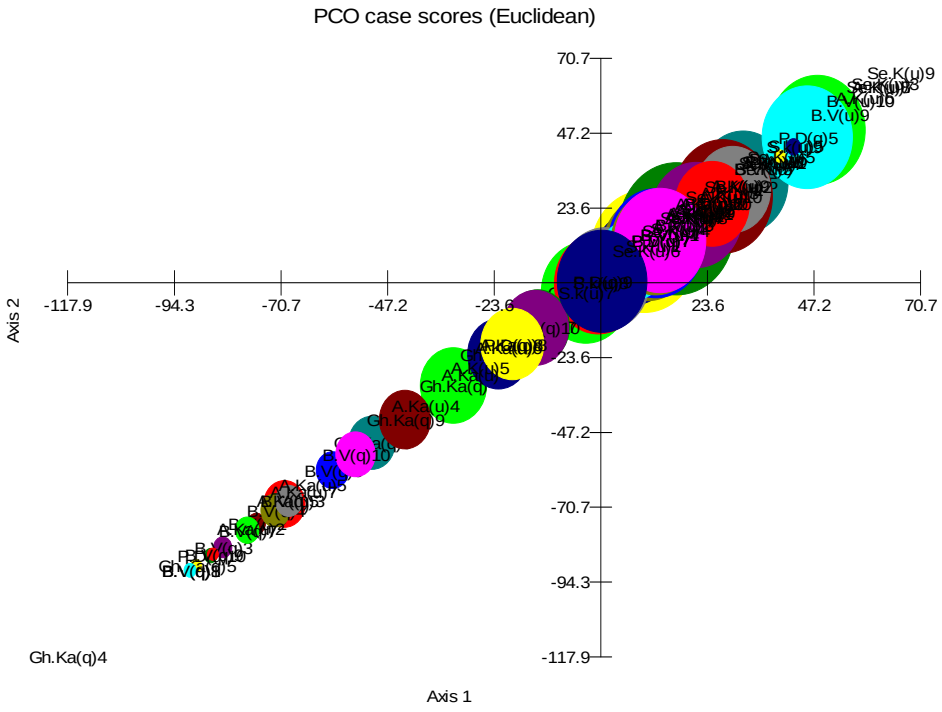

Figure.9. PCA plot of individuals of studied populations based on morphological characters for two species

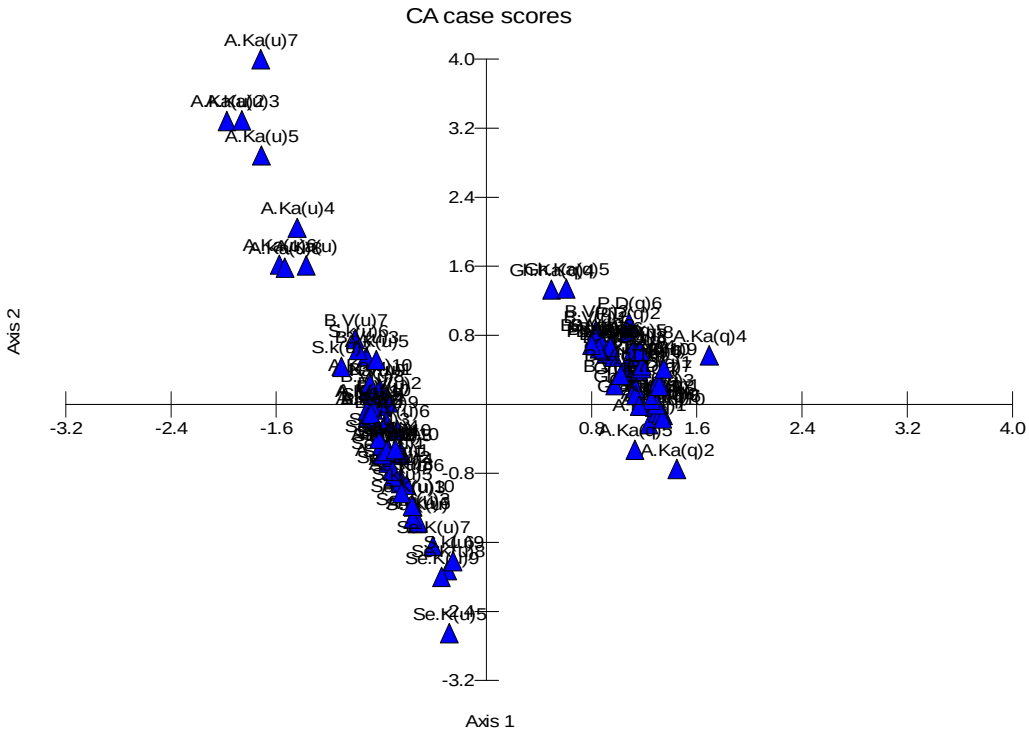

Figure.10. CA plot of individuals of studied populations based on morphological characters for two species

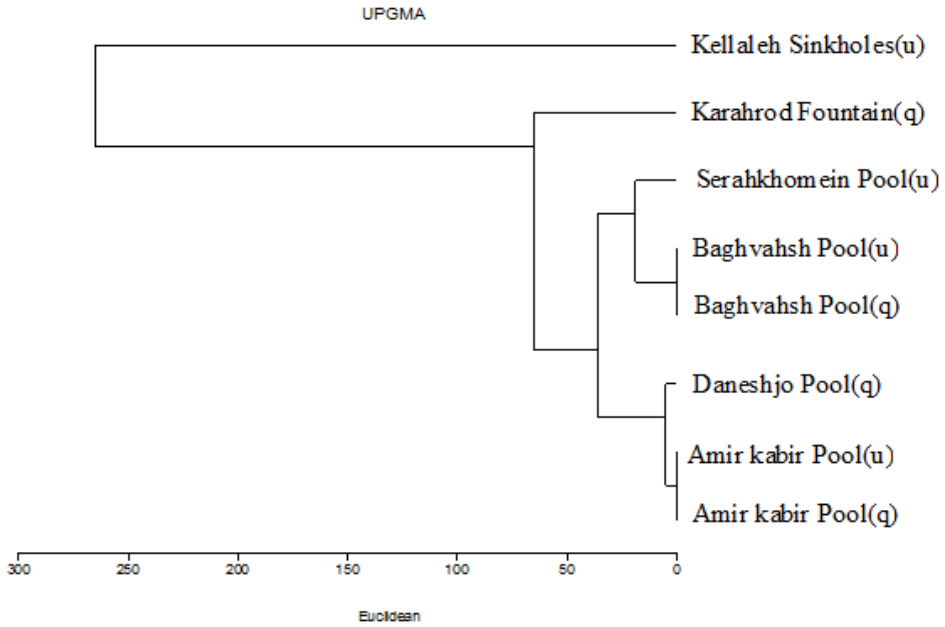

Figure.11. UPGMA tree of habitant ecological factor for two species

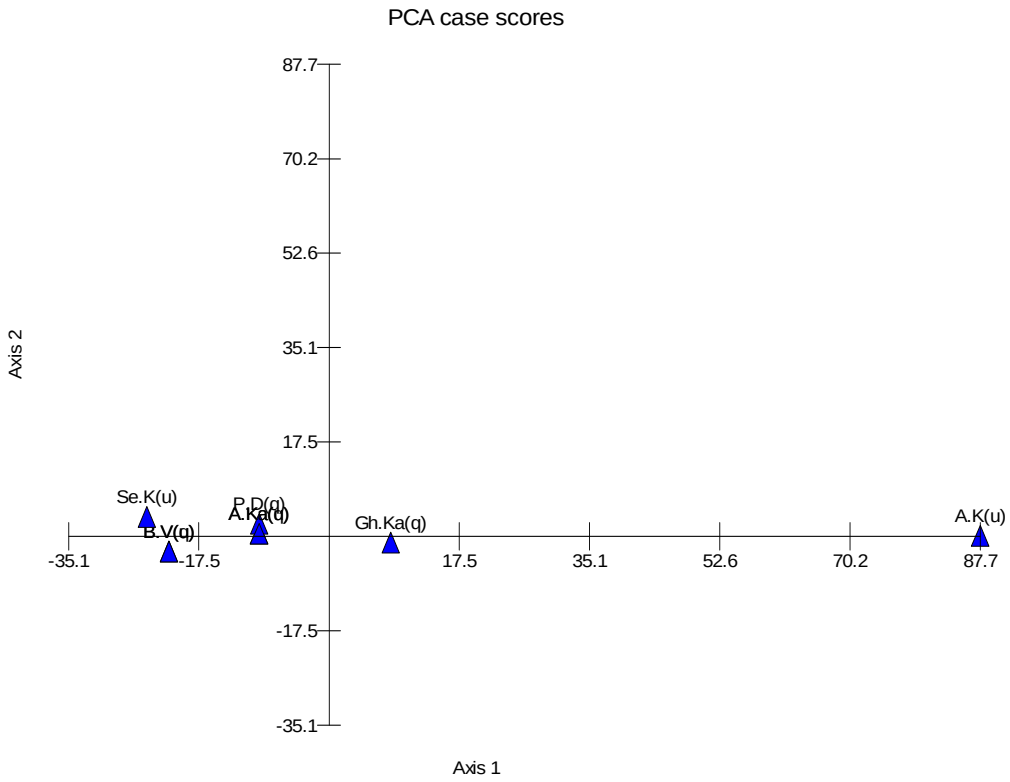

Figure.12. PCA plot of habitant ecological factor for two species

DISCUSSION

Quantitative morphological characters had a significant variation between individuals and populations, the ANOVA test and also T-test confirmed these variations. In addition, significant correlations positive or negative occurred between some morphological features of inter and intra-population with ecological factors of habitat, which this phenomenon confirmed the effect of different ecological characters on populations phenotype about this two species. For example significant different were found in length and width lorica which was important for determination of lorica shape. Amictic females are the life history stage of rotifers that is exposed to natural selective most of time[9], therefore directional natural selection is predicted to have various effects, If natural selection in give population favors individuals with higher values of particular trait, then the population mean value of that trait is predicted to increase from generation to generations[10]. Some fairly obvious but others less so that ought to affect plasticity and Compare species (or population) that vary with respect to ecological factors that might cause variation in how selection views plasticity[11]. Variation were occurred in morphological traits of population's individuals, therefore samples of populations were separated from others and placed separately. For intra-population investigation eleven quantitative morphological characters of ten individuals of four populations of *B. quadridentatus* were studied. Quantitative morphological feature varied between individuals in population Karahrod Fountain. Furthermore inter-populations study of *B. quadridentatus* showed similarity between populations Karahrod Fountain and Baghvahsh Pool. for intra-population investigation quantitative morphological characters of ten individuals of five populations *B. uceolaris* were studied. Morphological traits varied between individuals of populations Kellaleh sinkholes, in the study inter populations of *B. uceolaris* showed similar to between populations Serah khomein, Kellaleh dam, Kellaleh sinkholes and Baghvahsh Pool. In some case, the arrangements of populations in morphological and ecological plots and trees were similar. For example in UPGMA trees of morphological character and ecological factor, populations Kellaleh Sinkholes and Karahrod Fountain were separated from others populations. The phenotypic plasticity is typically induced by environmental heterogeneity or environmental stress [12]. The arrangements of some populations were alike in PCA plot of ecological factors with morphological characters. For example, Kellaleh and Sinkholes populations. Phenotypic plasticity is the ability of an organism to express different phenotypes depending on the biotic (e.g. predation, competition and social interactions) or abiotic (e.g. temperature) environmental in a variety of way and in a wide range of taxa. Single genotypes can change their chemistry, physiology, development, morphology, or behavior or in response to ecological cues [13]. The interpopulation variations may be related to habitat of populations. Therefore, phenotypic variation is due in part to variation in genotype, but it is also due in part to environment and morphological characters enables individuals of populations to establish different habitat and these types of variations provide

material for evaluation of new taxa. Plasticity has also been suggested as a potentially important mechanism facilitating macroevolution [5, 14]. High variations were observed between individuals of two species *B. uceolaris* and *B. quadridentatus*, which this subject signature of reciprocal phenotypic change is the escalation of phenotypes between individuals of two species over an extended about of interactions[15].

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