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Effect of Season and Environmental Variables on Rotifer Community Structure: Evidence from Two Selected Freshwaters in Northwest Iran

[1]Esmat Khaleqsefat and [2]Reza Malekzadeh-Viayeh
[1]Faculty of Biological Sciences, Shahid Beheshty University, Tehran, Iran
[2]Artemia and Aquatic Animals Research Institute, Urmia University, Urmia, Iran

Abstract: The seasonal patterns of rotifer community structure in response to temporal fluctuations in environmental variables were investigated in two water bodies located in Northwest Iran. Sampling was carried out during October 2007 to November 2008. In total, 45 rotifer species were identified from the two studied sites. In each site, rotifer species diversity differed seasonally. Temporal patterns observed innon-metric Multidimensional Scaling (nMDS) ordinationsrevealed separation of Summer from other seasons due to different diversity and density of the rotifer assemblages. Analysis of Variance (ANOVA) also showed significant difference in the rotifer diversity and abundance among seasons ($F = 4.7$, $p<0.05$). Rotifer species diversity was highly correlatedwith the level of dissolved oxygen and pH in the reservoir Cheshmehgul ($r = 0.92$ and 0.63, respectively, $p<0.001$). However, analysis of the accumulated data of both water bodies revealed that a significant Spearman's correlation coefficient existed between salinity and water temperature and the abundance of the rotifers ($r = 0.54$, $p<0.001$). Canonical Correspondence Analysis (CCA) identified dissolved oxygen and electrical conductivity to be the main environmental factors shaping the rotifer assemblages. According to this study, seasonal variations in environmental factors can influence structure of rotifer communities even in distinct, unrelated ecosystems.

Key words: Rotifer, diversity, abundance, season, environmental variables

INTRODUCTION

Biodiversity in any ecosystem is sustained, to a great extent by the structural diversity of the system. Knowledge of structural organization is important for obtaining a better understanding of the mechanisms of community self-sustention and of the principles that underlie the functioning of freshwater ecosystems (Galkovskaya and Mityanina, 2005). Interactions between biotic factors and the physical features of aquatic environments may be crucial for interpreting community dynamics (Pollard *et al*., 1998).

Rotifers are widely recognized as important components of freshwater ecosystems (Walsh *et al*., 2008). They are pioneer organisms which appear first in newly created water bodies and have the ability to survive a wide range of aquatic environments (Pociecha, 2008). Rotifers make a substantial contribution to zooplankton production in an aquatic system, attaining extremely rapid population growth rates and short developmental times under favourable conditions (Castro *et al*., 2005). High population renewal rates distinguish them as an important link in energy flow and nutrient cycling. Another important characteristic is their high tolerance to changes in environmental conditions (Bonecker *et al*., 2005).

Structuring rotifer communities in any given ecosystem depends greatly on the relationships between rotifer populations and their common environment (Neustupa *et al*., 2009). There are numerous studies attempting to correlate establishment of rotifer populations in aquatic ecosystems with environmental factors (Bonecker *et al*., 2005; Spoljar *et al*., 2005; Silva *et al*., 2009). Several of these studies have elucidated the significant impacts of both biotic and abiotic water parameters on the composition and density of zooplankton (Branco *et al*., 2002; Marton, 2007; Wang *et al*., 2009; Claps *et al*., 2011). It has also been found that ecological barriers have stronger influence on rotifer distribution than geographical isolation thus it has been suggested that the presence of taxa can be as an indicator of specific ecological conditions. Similarly, it has been proposed that rotifer species or assemblages can be used as helpful tools to classify water-bodies based on their trophic level (Branco *et al*., 2002). However, the

Corresponding Author: Esmat Khaleqsefat, Faculty of Biological Sciences, Shahid Beheshty University, Tehran, Iran

Fig. 1: Location of the study sites in Northwest Iran. 1: Pond Haajjamaal (39°11"17.2°N and 45°05"39.5"E); 2: Reservoir Cheshmehgul (37°08"30.4'N and 45°13"26.8"E)

relative role of different ecological conditions may vary among biological systems or within the same system (Devetter, 1998).

Study sites: The two selected water systems are located in North-West Iran (Fig. 1), the first site (Haajjamaal), located at 39°11'17.2"N and 45°05'39.5"E and altitude of about 800 m is a natural pond under the influx of Aras River water. Its water is also supplied by several subterranean springs. The littoral zone of this pond was covered by aquatic macrophytes dominated by *Phragmites* and *Typha* sp. It is far from urban area, not impacted by residences and surrounded by less-fertile lands. The second water system (Cheshmehgul) is an artificial reservoir located between 37°08'30.4"N and 45°13'26.8"E at 1445 m altitude. Its water is supplied by a mountainous stream but is under quite dense impact of human activities including recreation, agriculture and animal husbandry. The reservoir had a muddy bed and was poor of aquatic vegetation.

MATERIALS AND METHODS

Rotifer sampling and identification and water parameters measurements: The rotifers were collected seasonally from November 2007 to October 2008. Sampling of the rotifers was carried out by slowly towing or sweeping an approximate volume of 40 L of water through a fine mesh (30 μm) plankton net. Epiphytic specimens were isolated by rinsing the collected aquatic plants and algae on the net. In each sampling occasion, some main physical and chemical variables including Dissolved Oxygen (DO), pH, temperature, salinity and Electrical Conductivity (EC) were measured. Initial examination of live specimens was accomplished by separating rotifers from other zooplanktonic organisms using a stereomicroscope. Detailed inspection of the rotifers was performed by observing them under a dissecting microscope. Observations were documented by drawing and photomicrography. Taxonomic identifications are based on the keys given by Koste and Shiel (1987), Segers (1995) and De Smet and Pourriot (1997). Abundance of rotifers in each site was estimated by averaging the number of each rotifer species in three subsamples of equal volume and calculating the rotifer abundance in total sampled water (~40 L).

Data analysis: Multivariate community analyses were undertaken using PRIMER 5 (Clarke, 1993; Clarke and Warwick, 1994) to analyze what taxa contribute most to the difference between groups of samples. Non-metric Multidimensional Scaling (nMDS) ordinations, based on

Table 1: Seasonal variation of physico-chemical factors of water in the investigated sites

	Cheshmehgul				Haajjamaal			
Locality season	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
Salinity (ppt)	5.5	1.5	2.0	0.0	6.5	6	7.0	0.00
Temperature (°C)	8.0	7.5	17.5	29.5	9.4	5	22.0	27.00
DO (mgL^{-1})	12.0	5.1	11.5	14.5	12.5	13	14.0	9.25
pH	9.5	8.5	10.0	8.8	9.3	8	8.6	9.30
EC (µs cm^{-1})	247.0	193.0	253.0	205.0	220.0	200	190.0	205.00

Bray-Curtis dissimilarity matrices of the square root transformed abundance data were used to visualize differences in rotifer assemblages between seasons. All analyses were based on total abundance of each species at a corresponding site.

The BIOENV routine in PRIMER was used to identify the environmental factors that best explained the distribution pattern of the rotifer assemblages. The BIOENV analysis examined the match between the normalized-Euclidean environmental matrix and the biotic dissimilarity matrix using Spearman's rank correlation coefficient. The environmental variables used in the BIOENV analysis were water temperature, EC, salinity and DO. EC and DO were log 10 transformed. The difference in diversity of the rotifer assemblages among seasons was tested by one-way ANOVA at $p<0.05$. Prior to the analyses, data were checked for normality by Kolmogorov-Smirnov test by which data showed no normality. The relationships between the rotifer data matrix and the environmental variables were assessed using Canonical Correspondence Analysis (CCA) Table 1.

RESULTS AND DISCUSSION

Rotifer community data analysis: In total, 45 species of rotifers representing 21 genera of 15 Eurotatorian families were identified from the two sites. Table 2 displays the list of the identified taxa observed in each of the sampling sites. The rotifers belonging to the order Ploimahad the highest number of species and abundance among the identified taxa. Brachionidae was the most diverse family represented by 9 species, followed by Synchaetidae and Notommatidae (6 sp. each). Eighteen species were found only in pond Haajjamaal whereas 14 species were identified only in reservoir Cheshmehgul. The members of the subclass Monogononta were abundant in the pond Haajjamaal while the rotifers from the subclass Bdelloidea dominated in the reservoir Cheshmehgul during the sampling period. The two distantly sampled systems carried 13 rotifer species in common.

Seasonality, diversity and abundance of the rotifers were different between the water-bodies. Pond Haajjamaal harbored higher rotifer diversity and abundance with the highest species abundance in Spring and Autumn. In this pond, a dominance of *Synchaeta pectinata* with a density of 250 ind L^{-1} was observed during Autumn and Spring. Three other high density peaks in the pond were for *Lepadella ovalis* and *Colurella uncinata* in Autumn and *Keratella irregularis* in Summer with ca.200 ind L^{-1}. *Polyarthra dolichoptera*, *Cephalodella catellina* and *Proalesfallaciosa* also made large contributions to the total rotifer density in Haajjamaal. At the reservoir Cheshmehgul, the bdelloid *Rotaria rotatoria* was predominant rotifer species in summer with an abundance of 143 ind L^{-1} the remaining rotifer species had densities lower than 25 ind L^{-1}. In general, the rotifer abundance was notably lower in the latter water body. In both the study sites, *Synchaeta*, *Brachionus* and *Euchlanis* were the predominant genera in Autumn while *Polyarthra* dominated in Winter.

According to the, BIOENV analysis, the environmental variables, i.e., salinity, water temperature, dissolved oxygen, pH and EC were found to be relevant in structuring rotifer communities in either of the sites. At pond Haajjamaal all the measured environmental factors were correlated with the rotifer abundance ($r = 0.5$-1, $p<0.001$) while at Cheshmehgulthe density of the rotifershad significant correlation only with dissolved oxygen ($r = 0.92$, $p<0.001$) and pH ($r = 0.63$, $p<0.001$). When data of both sites were analyzed collectively, a significant Spearman's correlation coefficient was found between salinity and water temperature and the density of the rotifers ($r = 0.54$, $p<0.001$).

The nMDS analysis grouped seasons based on similarities in their rotifer assemblage structure. Accordingly, the three groups of seasons in the reservoir Cheshmehgul consisted of Winter, Summer and Spring and Autumn. In Haajjamaal, seasons formed two groups: Summer and remaining three seasons (Fig. 2).

ANOVA showed significant differences in rotifer diversity and abundance among seasons ($F = 4.7$, $p<0.05$) while multiple comparisons uncovered significant difference in the rotifer assemblages of both the water bodies between Autumn or winter and Summer ($p<0.05$) (Table 3). Assemblages built on abundance data appeared to match one another with a significant correlation index ($r = 0.85$, $p = 0.001$).

Table 2: List of the rotifer taxa identified in the investigated sites. The occurrence and absence of the species in each site is shown by + and -, respectively

Family	Species	Haajjamaal	Cheshmehgul
Brachionidae	*Brachionus plicatilis* (Muller, 1786)	+	+
	B. urceolaris (Muller, 1773)	+	-
	B. angularis (Gosse, 1851)	+	-
	B. leydigi (Cohn, 1862)	+	-
	B. quadridentatus (Hermann, 1783)	-	+
	Keratella irregularis (Lauterborn, 1898)	+	-
	K. tecta (Gosse, 1851)	+	-
	Notholca acuminate (Ehrenberg, 1832)	-	+
	N. squamula (Muller, 1786)	+	+
Lecanidae	*Lecane bulla* (Gosse, 1851)	-	+
	L. closterocerca (Schmarda, 1853)	-	+
	L. luna (Muller, 1776)	+	+
Dicranophoridae	*Encentrum saundersiae* (Hudson, 1885)	+	-
Euchlanidae	*Euchlanis dilatata* (Ehrenberg, 1832)	-	+
	E. incise (Carlin, 1939)	+	-
Lepadellidae	*Lepadella acuminata* (Ehrenberg,1834)	-	+
	L. ovalis (Muller, 1786)	+	+
	L. patella (Muller, 1773)	+	+
	Colurella uncinata (Muller, 1773)	+	-
Mytilinidae	*Mytilina mucronata* (Muller, 1773)	-	+
Ituridae	*Itura aurita* (Ehrenberg, 1830)	-	+
Notommatidae	*Cephalodella catellina* (Muller, 1786)	+	+
	C. forficula (Ehrenberg, 1832)	+	-
	C. gibba (Ehrenberg, 1832)	+	+
	C. stenroosi (Wulfert, 1937)	+	-
	Eosphora najas (Ehrenberg, 1830)	+	-
	E. therina (Harring and Myers, 1922)	+	-
Proalidae	*Proales fallaciosa* (Wulfert, 1937)	+	-
Trichotriidae	*Trichotria pocillum* (Muller, 1776)	+	-
	T. tetractis (Ehrenberg, 1830)	+	-
Trichocercidae	*Trichocerca agnatha* (Wulfert, 1939)	+	-
Synchaetidae	*Synchaeta littoralis* (Rousselet, 1902)	+	+
	S. oblonga (Ehrenberg, 1832)	+	-
	S. pectinata (Ehrenberg, 1832)	+	+
	S. tremula (Muller, 1786)	-	+
	Polyarthra dolichoptera (Idelson,1925)	+	+
	P. remata (Skorikov, 1896)	+	+
Filiniidae	*Filinia brachiata* (Rousselet, 1916)	+	-
	F. longiseta (Ehrenberg, 1834)	-	+
	F. terminalis (Plate, 1886)	-	+
Hexarthridae	*Hexarthra bulgarica* (Wiszniewski, 1933)	-	+
Philodinidae	*Philodina. roseola* (Ehrenberg, 1832)	+	+
	Rotaria rotatoria (Pallas, 1766)	-	+
	R. tardigrada (Ehrenberg, 1832)	+	+
	R. neptunoida (Ehrenberg, 1832)	-	+

Table 3: Results of Analysis of Variance (ANOVA) performed to compare rotifer species abundance in different seasons

(I) Treatment	(J) Treatment	Mean difference (I-J)	Standard error	Sig.
Autumn	Winter	68.14	20.72203	0.001**
	Spring	35.46	20.72000	0.091
	Summer	54.48	20.72000	0.010**
Winter	Autumn	-68.14	20.72000	0.001**
	Spring	-32.68	20.72000	0.119
	Summer	-13.66	20.72000	0.512
Spring	Autumn	-35.46	20.72000	0.091
	Winter	32.68	20.72000	0.119
	Summer	19.02	20.72000	0.361
Summer	Autumn	-54.48	20.72000	0.010**
	Winter	13.66	20.72000	0.512
	Spring	-19.02	20.72000	0.361

**The mean difference is significant at $p<0.05$

Results of the CCA analysis are shown in Fig. 3. The two axes of the CCA accounted for 82.3% of total variation, the first for 47.8% and the second for 34.5%. In the biplot of Fig. 3a made by the CCA permutation test, axis 1, the most important environmental gradient was related to increasing DO opposite to increasing EC. The second most-important environmental gradient according to axis 2 was composed by opposite temperature and salinity gradients. Dissolved oxygen and EC were the main environmental variables affecting rotifer abundance of both studied sites, especially in Summer. In Cheshmehgul, Spring and Autumn were associated with high temperature and low salinity. Whereas, in Haajjamaal, Spring was the season with higher water salinity. According to this analysis, the Summer samples plot out at one end and the other samples plot out at theother. The plot of Fig. 3b delineates the occurrence pattern of the rotifer species along the gradient of the estimated

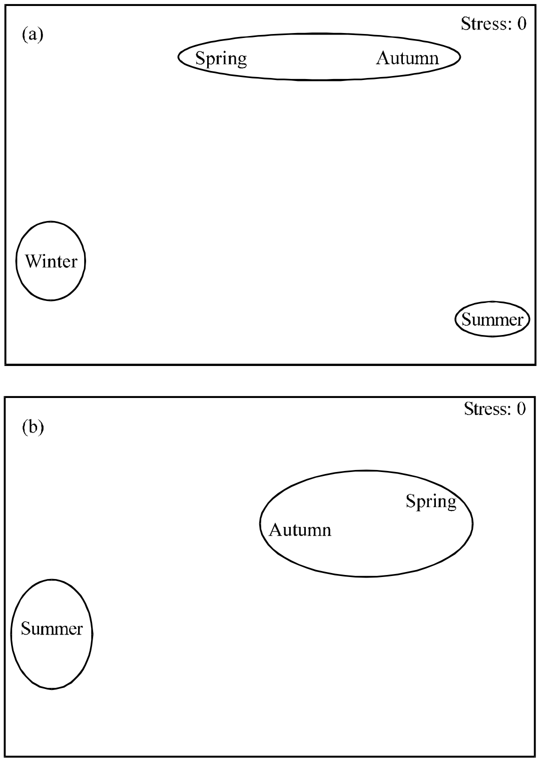

Fig. 2: Temporal patterns of rotifer assemblages defined by non-metric Multidimensional Scaling (nMDS) ordination of reservoir Cheshmehgul and pond Haajjamaal. Circles enclose groups of seasons that exhibited ≤80% dissimilarity in assemblage structure (based on clustering dendrograms. a) Haajjamaal and b) Cheshmehgul

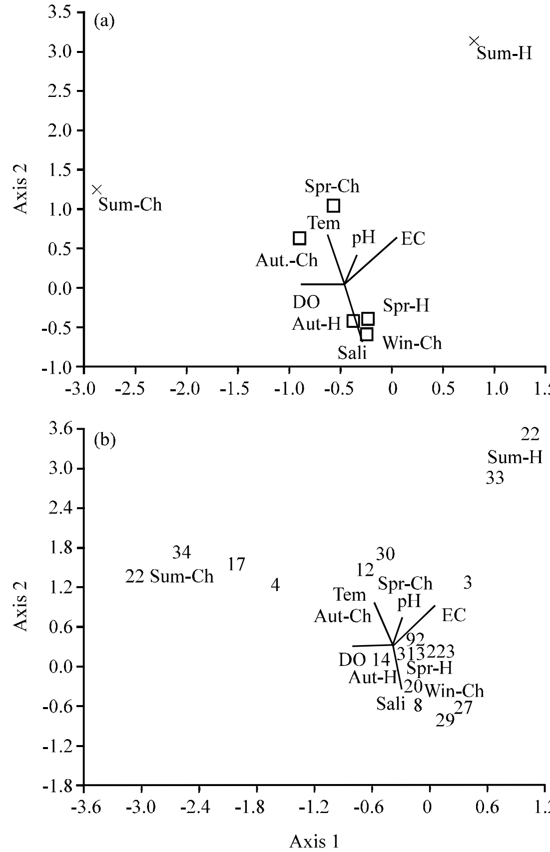

Fig. 3: CCA ordination biplot of site scores and selected environmental variables (represented by multidirectional lines). Only the explanatory environmental variables retained by a forward selection procedure are presented (Sali: water Salinity; DO: Dissolved Oxygen; Tem: water temperature; EC: Electrical Conductivity). H: Haajjamaal; Ch: Cheshmehgul. a) biplot of season (Spr: Spring; Sum: Summer; Aut: Autumn; Win: Winter), site and selected environmental variables; b) biplot of site, environmental measures and species scores. Numbers 1-34 = label of species as follows: 1 = *B. plicatilis*; 2 = *B. leydigi*; 3 = *B. angularis*; 4 = *B. quadridentatus*; 5 = *K. irregularis*; 6 = *K. tecta*; 7 = *N. acuminata*; 8 = *N. squamula*; 9 = *L. bulla*; 10 = *L. luna*; 11 = *E. saundersiae*; 12 = *E. dilatata*; 13 = *L. ovalis*; 14 = *L. patella*; 15 = *C. uncinata*; 16 = *I. aurita*; 17 = *C.catellina*; 18 = *C. gibba*; 19 = *C. forficula*; 20 = *E. najas*; 21 = *P. fallaciosa*; 22 = *T. pocillum*; 23 = *T. agnatha*; 24 = *S. pectinata*; 25 = *S. littoralis*; 26 = *P. remata*; 27 = *P. dolichoptera*; 28 = *F. terminalis*; 29 = *H. bulgarica*; 30 = *C. ornata*; 31 = *R. rotatoria*; 32 = *R. tardigrada*; 33 = *R. neptunoida*; 34 = *P. roseola*

environmental variables. Associated with the higher water salinities were *Notholca squamula*, *P. remata Filinia terminalis* and *Polyarthra dolichoptera* which can also be related to lower water temperatures. *Euchlanis dilatata* and *Hexarthra bulgarica* were the taxa most positively correlated with high water temperature. The incidence of a number of species such as *R. rotatoria*, *R. tardigrada*, *T. agnatha*, *L. bulla* and *B. leydigi* was not directly linked with the environmental factors.

Species richness patterns and biodiversity indicators have widely been used for characterization of freshwater systems. However, a few studies have explored biodiversity variation at different temporal and spatial scales (Steinberg *et al*., 2009). Aquatic habitats are clearly not uniform, showing large variations in food concentration and the level of physical and chemical parameters. Thus, it is not surprising to find that rotifer species are unevenly distributed both temporally and spatially in their habitats.

The two selected sites were chosen for this study as two geographically distant water-bodies belonging to different hydrological systems. Although, they share many abiotic features, they differ from each other in case of some ecological characteristics such as the intensity of vegetation and the source of inflowing waters. Scholl and Kiss (2008) suggested that hydrological conditions of the water bodies can affect both directly and indirectly the structure of planktonic assemblages. They noted that high density rotifer assemblages could develop in water bodies where the intensity of the flow and the frequency of the flow events are not inhibited. The occurrence of some rotifer species in both of the study sites can be at least to some extent because of their cosmopolitan nature and/or tolerant to wide environmental conditions. Fontaneto *et al*. (2007) noted that microscopic organisms such as rotifer are known to have no clear bio geographical patterning. Pond Haajjamaal hosted more rotifer species at higher densities which could be a result of higher vegetation cover and/ or impact of its adjacent riverine system, Aras. The role of macrophyte coverage in establishing diversified rotifer communities has been noted by several researchers (Castro *et al*., 2005; Claps *et al*., 2011). Furthermore, the impact of inflowing waters on the rotifer richness of lentic waters has been documented earlier (Keppeler and Hardy 2004; Bonecker *et al*., 2005). It is predictable that the River Aras which is a large border river between Iran and Republic of Azerbaijan, flushes considerable amounts of nutrients and phytoplanktonic organisms into the neighboring water bodies which in turn can result in increased rotifer diversity and abundance in the waters. The inflowing water from the river can also introduce new rotifer species to the connected water catchments such as that studied here. At least part of these allochthonous rotifer taxa can colonize in the new ecosystem and add the number of resident species.

The correlation coefficient analysis verifies the significant role of the environmental variables in structuring the rotifer communities in aquatic systems. Among the estimated environmental parameters, dissolved oxygen and conductivity found to be the most effective factors on the abundance of rotifers. Positive effect of dissolved oxygen concentration on rotifer abundance was predictable. In addition, since most rotifer species are typical freshwater dwellers, it is no surprise that they favor a lower conductivity for growth and reproduction. Several studies have found higher rotifer population diversity and density at lower EC levels (Neschuk *et al*., 2002; Silva *et al*., 2009; Campillo *et al*., 2011). The direct relationship between the abundance of *B. leydigi* and EC values can be explained by the preference of Brachionus rotifers to live in brackish waters. These rotifers which are known to be tolerant to salinity fluctuations (Silva *et al*., 2009) were found in pond Haajjamaal where higher salinity values were recorded in all seasons. Nevertheless, as the salinity values measured in this study were still in a range attributed to freshwaters, even the higher salinities were unlikely to have negative effects on the diversification and aggregation of the rotifer communities. High pH values of both the studied waters which categorized them as alkaline habitats, could, to some extent, be responsible for their quite rich rotifer assemblages. Alkalinity and pH are often found as variables predicting the zooplankton community composition. These variables are directly influenced by primary production (Devetter, 1998). Conditions of low pH have been suggested to limit the occurrence of many rotifer species (Neustupa *et al*., 2009). However, several species have been found to reach their peak abundances in acidic waters (Deneke, 2000).

Analysis of seasonal data by nMDS was useful for estimation of the patterns of temporal distribution of the rotifer assemblages. According to the analysis, Spring and Autumn shared common features for the diversity and abundance of the rotifer taxa in the both surveyed water bodies. The rotifers showed a high degree of seasonal variations. Besides, ANOVA also confirmed the significant differences in rotifer abundance among seasons. Rotifers seasonality is extremely important in fresh water plankton communities because of their role in aquatic food web and nutrient and energy flow (Holst *et al*., 2002).

CCA is an example of direct gradient analysis where the gradient in environmental variables is known a priori and the species abundances are considered to be a response to this gradient. This analysis allows the exploration of major trends in rotifer community while at the same time, ascertaining relationships between the species data and the explanatory environmental variables (Castro *et al*., 2005). As is evident in the biplot of Fig. 3b, the abundances of most rotifer species were highest in spring. Also, according to the ordination diagram, dissolved oxygen and water conductivity had the highest correlations with rotifer abundances and thus were the most influential factors in constitution of the rotifer communities in the investigated sites. The CCA also discloses the distinct environmental conditions predominated in the two water-bodies in Summer (Fig. 3). One other elucidation resulted from the CCA was the association of taxa such as *E. dilatata* and *H. bulgarica* with higher water temperature (Fig. 3b). The positive correlation between the occurrence of *E. dilatata* and higher water temperatures was discovered by Branco *et al*. (2002).

Dominance of bdelloid rotifers in Cheshmehgul with less vegetation cover suggests that the existence of aquatic vegetation does not necessarily guarantee the colonization of these plant-associated rotifer taxa. It may further stress the fact that the presence and success of an organism or group of organisms depend on a combination of conditions in an ecosystem. In addition to the estimated variables, small-scale differences in factors such as ion composition, food availability and predation pressure may significantly alter the structure of zooplankton communities (Silva *et al*., 2009). An important but not measured, factor must be nutrient levels. In contrary to a quite high degree of the rotifer diversity, comparatively low mean densities of the rotifers in the studied waters may have been the result of low levels of nutrients. Bonecker *et al*. (2005) suggested that food availability is a predominant factor for structuring and dynamics of the rotifer communities. Impacts of the changes in the levels of nutrients such as nitrogen and phosphorus on the diversity and abundance of rotifer were documented by Devetter (1998).

The role of human impact such as periodic depletion and refilling the water for agriculture purpose and also drainage of animal wastes into the reservoir Cheshmehgul may have affected the composition of its rotifer community. This periodic fluctuation in water level may cause an unstable environmental condition and a higher turbidity which could be the other parameters influencing the planktonic communities (Claps *et al*., 2011). However, turbidity has been shown to have minimal role in the regulation of zooplankton population in a similar aquatic system (Pollard *et al*., 1998). On the other hand, pond Haajjamaal which is under periodic influx of Aras River, had a higher rotifer diversity and density. The higher diversity could be attributed to the inflow of nutritive and plankton-rich waters from Aras River and also the existence of macro-vegetation in its littoral area. High density rotifer assemblages could develop in water bodies where the intensity of the flow and the frequency of the flow events are not inhibited (Scholl and Kiss, 2008).

CONCLUSION

Studies on the diversity and ecology of Iranian rotifers are scarce. Nevertheless, existence of diverse climatic conditions and variety of aquatic habitats has made the country a significant biogeographic region for studying the structure of its rotifer communities. The goal of this study was to analysis the rotifer species diversity and abundance in relation with seasonal fluctuations of environmental variables in two typical aquatic systems located in Northwest Iran.

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