Plankton composition and environmental parameters in the habitat of the Iranian cave barb (*Iranocypris typhlops*) in Iran

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Abstract

*Plankton composition and environmental parameters in the habitat of the Iranian cave barb (*Iranocypris typhlops*) in Iran.*— The Iranian cave barb (*Iranocypris typhlops* Bruun & Kaiser, 1944) is 'Vulnerable' in the IUCN Red List. It is an endemic species of ray–finned fish of the family Cyprinidae from a single locality in the Zagros Mountains, western Iran. This species is an omnivore that depends on plankton for food. We studied the spatial and seasonal distribution of plankton in the native habitat of the Iranian cave barb between May 2012 and February 2013. We measured various environmental parameters and related these to plankton distribution. The plankton assemblage included 13 genera and five species. Rotifera had the highest number of genera (4) and species (4), followed by Arthropoda (3), Ochrophyta (3), Myzozoa (2), Charophyta (2), Chlorophyta (2), Ciliophora (1) and Cryptophyta (1). In terms of numbers, the dominant species of phytoplankton and zooplankton were *Achnanthidium* sp. and *Lecane* sp. Pearson correlation coefficients showed a low but significant relationship between plankton communities and environmental parameters. Among the environmental parameters, total suspended solids and turbidity seemed to have the most important influence on the temporal distribution of plankton species. We also observed that dissolved oxygen played an important role for most plankton species, as did temperature for most zooplankton species. The diversity and abundance of phytoplankton and zooplankton were low throughout the year in the cave with an annual mean of 96.4 ind./l and they did not show any peaks during the year.

Key words: Iranian cave barb, Endemic, Habitat, Phytoplankton, Zooplankton.

Resumen

La composición planctónica y los parámetros ambientales en el hábitat del barbo cavernícola iraní Iranocypris typhlops.— El barbo cavernícola iraní, *Iranocypris typhlops* (Bruun & Kaiser, 1944) es una especie catalogada como "Vulnerable" en la Lista Roja de la IUCN. Endémica de una única localidad situada en las montañas Zagros, en Irán occidental. Se trata de una especie omnívora que depende del plancton para alimentarse. Se estudió la distribución espacial y estacional del plancton en el hábitat original del barbo cavernícola iraní entre mayo de 2012 y febrero de 2013. Se midieron varios parámetros ambientales y se relacionaron con la distribución del plancton. La comunidad planctónica comprendía 13 géneros y cinco especies. El filo Rotifera tenía el mayor número de géneros (4) y de especies (4), seguido por Arthropoda (3), Ochrophyta (3), Myzozoa (2), Charophyta (2), Chlorophyta (2), Ciliophora (1) y Cryptophyta (1). Por lo que respecta a la cantidad, las especies dominantes de fitoplancton y zooplancton fueron *Achnanthidium* sp. y *Lecane* sp. Los coeficientes de correlación de Pearson pusieron de manifiesto que la relación entre las comunidades de plancton y los parámetros ambientales era baja pero significativa. Entre los parámetros ambientales, el total de sólidos en suspensión y la turbidez parecieron ser los más influyentes en la distribución temporal de las especies de plancton. Asimismo, se observó que el oxígeno disuelto desempeñaba una función importante para la mayoría de las especies de plancton, al igual que la temperatura para la mayoría de las especies de zooplancton. La diversidad y la abundancia de fitoplancton y zooplancton eran bajas durante todo el año en la cueva con una media anual de 96,4 ind./l y no mostraron ningún máximo durante el año.

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Palabras clave: Barbo cavernícola iraní, Endémico, Hábitat, Fitoplancton, Zooplancton.

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Introduction

The Iranian cave barb (*Iranocypris typhlops* Bruun & Kaiser, 1944) is a rare species of the family Cyprinidae endemic to the Zagros Mountains, western Iran (Mahjoorazad & Coad, 2009). The distribution of the species seems to be restricted to a single cave. *I. typhlops* is sympatric with *Paracobitis smithi* (Greenwood, 1976) and both are listed as 'Vulnerable' in the IUCN Red List (IUCN, 2013). As such, Coad (2000), using 18 criteria that focused on distribution and habitat, found this species to be one of the top four threatened species of freshwater fishes in Iran. Zalaghi (2011) estimated the population size of the species at between 353 and 625 individuals. Conservation of this species has received little attention so far. The major conservation objective, perhaps reinforced by legislation, must be habitat restoration and management. Knowledge on this species habitat is poor.

Assemblages of species in ecological communities reflect interactions between organisms and the abiotic environment as well as among organisms (Hughes, 2000). Plankton species are valuable indicators of environmental conditions (Beaugrand, 2004; Bonnet & Frid, 2004) since they are ecological indicators of many physical, chemical and biological factors. On the other hand, the diet of this cave species is extremely dependent on plankton (> 60%), as found in field observations. Food density is a main environmental variable for appearance and abundance of fishes (McNamara & Houston, 1987; Hüppop, 2005). Therefore, information on the species' feeding could prove useful for urgently needed conservation measures, such as breeding programs, stock maintenance or translocation, as well as habitat rehabilitation measures (Kalogianni et al., 2010), similar to those successfully implemented for the conservation of its related species, the native Iberian toothcarp *Valencia hispanica* (Planelles & Reyna, 1996; Risueño & Hernández, 2000; Caiola et al., 2001). A habitat and diet overlap study could improve our knowledge of their effects of such measures on the species, and help develop appropriate management strategies. In this study we outline a one–year study of the plankton community in the habitat, in order to analyse species composition and seasonal dynamic of the plankton community, and to assess their dependence on environmental parameters.

Methods

Study area

The Iranian cave barb's original locality is a water cave, the natural outlet of a subterranean limestone system in the Zagros Mountains. The stream below the cave locality is the 'Ab–e Sirum', a tributary of the Dez River, in Lorestan province. The Dez flows into the Karun River which drains to the head of the Persian Gulf. The cave is located at 33° 04' 39'' N and 48° 35' 33'' E (fig. 1). Recently, this fish has been reported in another locality. The new locality is at 131 km in a direct line from the type locality. The

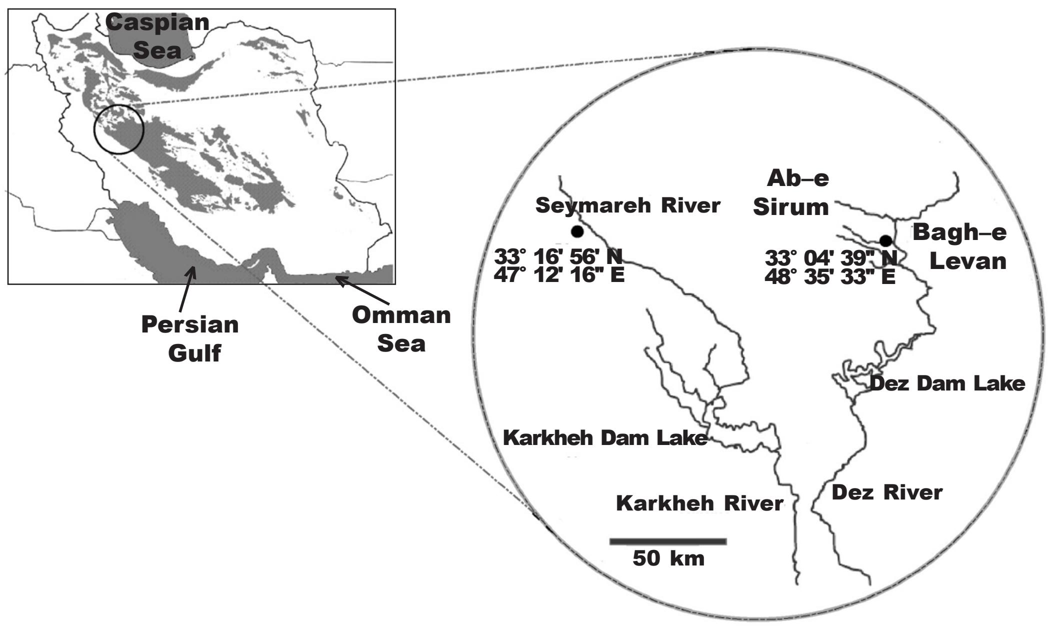

Fig. 1. Location of *Iranocypris typhlops* habitat in Bagh–e Levan and the new locality reported by Mahjoorazad & Coad (2009) in Iran.

Fig. 1. Ubicación del hábitat de Iranocypris typhlops *en Bagh–e Levan y la nueva localidad registrada por Mahjoorazad & Coad (2009) en Irán.*

Table 1. Environmental parameters with their mean, range of variation and statistical difference observed during the study period. Statistical results are for two–way ANOVA (main effects: S. Season, D. Depth; interaction: S x D): **p* < 0.05; ***p* < 0.01.

*Tabla 1. Parámetros ambientales con sus medias, rango de variación y diferencia estadística observados durante el período de estudio. Los resultados estadísticos son para ANOVA de dos factores (efectos principales: S. Estación, D. Profundidad; interacción: S x D): * p < 0,05; ** p < 0,01.*

				ANOVA		
Variables	Mean ± SD	Min	Max	S	D	S × D
Physical variables						
pH	7.61 ± 0.17	7.10	7.90	**		
Electrical conductivity (EC, µs/cm)	458.59 ± 20.38	430.00	506.00	**		
Turbidity (NTU)	0.56 ± 0.07	0.42	0.71	**	**	**
Water temperature (T, °C)	18.14 ± 1.83	15.00	24.00	**	**	**
Dissolved oxygen (DO, mg/l)	5.83 ± 0.85	4.50	7.60	**	**	**
Total suspended solids (TSS, mg/l)	0.53 ± 0.05	0.26	0.80		**	**
Total dissolved solids (TDS, mg/l)	244.46 ± 7.39	226.00	258.00	**		
Metals						
Magnesium (mg/l)	19.48 ± 0.79	17.3	21.18			
Potassium (mg/l)	3.13 ± 0.20	2.70	3.70			
Sodium (mg/l)	19.20 ± 0.94	17.15	22.80			
Calcium (mg/l)	54.68 ± 4.29	46.50	61.50	**		
Iron (II) (mg/l)	00.00 ± 0.00	0.00	0.00			
Total iron (TFe, mg/l)	00.00 ± 0.00	0.00	0.00			
Inorganic (non–metallic) matter						
Chlorine (mg/l)	29.16 ± 1.64	24.80	33.00	**		
Bicarbonate (mg/l)	149.54 ± 3.85	136.00	158.70	**		
Carbonate (mg/l)	0.00 ± 0.00	0.00	0.00			
Total nitrogen (TN, mg/l)	1.30 ± 0.07	1.20	1.45	**		
Nitrite (mg/l)	0.00 ± 0.00	0.00	0.00			
Nitrate (mg/l)	0.51 ± 0.03	0.46	0.60	**		
Total phosphorus (TP, mg/l)	0.59 ± 0.04	0.50	0.68	**		
Phosphate (mg/)	0.34 ± 0.05	0.20	0.45	**		
Total sulfur (TS, mg/l)	88.79 ± 4.63	79.80	99.30	**		
Sulfate (mg/l)	58.57 ± 2.84	50.14	66.00	**		
Organic matter						
Biological oxygen demand (BOD, mg/l)	0.01 ± 0.00	0.00	0.15	**		
Chemical oxygen demand (COD, mg/l)	0.22 ± 0.05	0.01	0.31	**		

construction of a dam on the Seymareh River on the Lorestan–Ilam provincial border, 30 km northwest of Darrehshahr at 33° 16' 56'' N and 47° 12' 16'' E, involved excavation of an intake tunnel for a power house, 11 m in diameter and 1,500 m in length, at 597 m altitude. The tunnel intersected many faults, joints and small karstic features. Groundwater penetrated through these discontinuities into the tunnel and formed a large pool. The tunnel is now encased in concrete and the karst environment is no longer accessible (Mahjoorazad & Coad, 2009).

Sample collection and analysis

The Iranian cave barb's original locality was found to have a depth of 28 m by local divers but for sampling only 5 m from the surface was attainable by a Ruttner sampler. Water samples were seasonally taken from the surface (20 cm) to a depth of 5 m below the cave surface at ten sites of each depth of the cave using a Ruttner sampler (volume of 10 l, 1 l for environmental variables and 9 l for plankton samples) at successive depth intervals of 1 m (Talling, 2003). This sampling

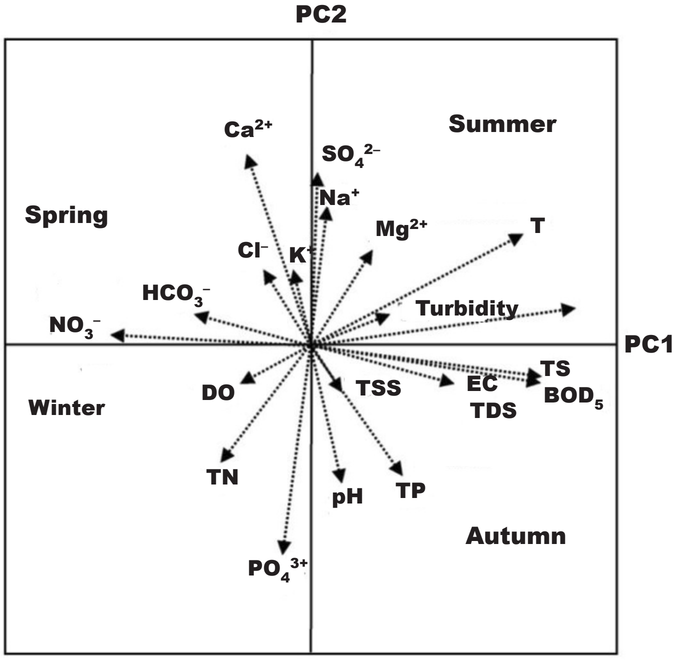

Fig. 2. PCA ordination diagram for the cave seasons and environmental factors. It shows the distribution of environmental parameters over the four seasons during the study period. (The abbreviations used for environmental variables are given in table 1.)

Fig. 2. Diagrama de ordenación mediante análisis de componentes principales de las estaciones y los factores ambientales en la cueva. Se muestra la distribución de los parámetros ambientales en las cuatro estaciones durante el período de estudio. (Las abreviaturas utilizadas para las variables ambientales se indican en la tabla 1.)

took place from May 2012 to February 2013 at 8:00–10:00 a.m. The environmental physicochemical variables are listed in table 1. Inorganic (non–metallic) matter, organic matter, metals and TDS were analyzed in the laboratory according to APHA (2012) methods and DO, T, pH, EC, TSS and turbidity were detected in situ. Plankton samples filtered through a net of mesh size 30 μm. All the concentrated plankton samples (total volume of 100 l) were divided into two parts; 50 ml preserved with Lugol's solution for the enumeration and identification of phytoplankton, and 50 ml preserved with 4% neutral formalin for the enumeration and identification of zooplankton. For each sample (total volume of 50 ml), 20 counts of 1–ml subsamples were counted using an inverted microscope under at 40–600X magnifications. Plankton samples were identified to the lowest taxonomic level possible.

Statistical analysis

We used a two–way ANOVA followed by Duncan's tests to examine the effects of depth and season on environmental variables and plankton densities. Pearson correlations were run between environmental variables and plankton density to distinguish key biotic variables that could affect plankton distribution. Due to low Pearson correlations, we were unable to use an analysis technique to elucidate the relationships between biological assemblages of species and their environment. These analyses were performed with SAS software (SAS Institute Inc., Cary, NC, USA). Principal component analysis (PCA), an indirect gradient analysis technique, was used to detect the main environmental variables in the cave in CANOCO version 4.5 (Braak & Šmilauer, 2002). Data were logarithmically transformed to normalize the distribution prior to statistical analysis. We used the Shannon–Wiener diversity index *H′* to ascertain the structural features of the plankton community.

Results

Environmental variables

The main environmental variables of the water are reported in table 1. Most environmental variables exhibited significant difference between seasons. However, water temperature underwent a typical seasonal trend, with a minimum of 15°C in winter and a maximum of

Table 2. Planktonic species with their mean, range of variation and statistical difference observed during the study period. Statistical results are for two–way ANOVA (main effects: S. Season, D. Depth; interaction: S x D): * $p < 0.05$; ** $p < 0.01$. (For other abbreviations see table 1.)

*Tabla 2. Especies de plancton con sus medias, rango de variación y diferencia estadística observados durante el período de estudio. Los resultados estadísticos son para ANOVA de dos factores (efectos principales: S. Estación, D. Profundidad; interacción: S x D): * p < 0,05; ** p < 0,01. (Para las otras abreviaturas ver tabla 1.)*

Plankton	Mean ± SD (ind./l)	ANOVA S	ANOVA D	ANOVA SxD
Rotifera	9.90 ± 6.69		**	**
Lecane sp.	3.72 ± 3.90			
Brachionus sp.	3.50 ± 3.91		*	
Trichocerca sp.	0.98 ± 1.41		*	*
Philodina sp.	1.70 ± 2.03		**	
Arthropoda	0.21 ± 0.24		**	
Tropocyclops sp.	0.07 ± 0.10		**	
Nauplius sp.	0.09 ± 0.20			
Mesocyclops sp.	0.05 ± 0.08			
Ciliophora	0.06 ± 0.09			
Vorticella similis	0.06 ± 0.09			
Total zooplankton	10.17 ± 6.72	**	**	**
Ochrophyta	62.79 ± 31.47	**	**	**
Melosira varians	19.53 ± 13.52	**	**	
Synedra sp.	20.62 ± 13.60	**	**	*
Achnanthidium sp.	22.64 ± 18.35			
Cryptophyta	0.49 ±0.77			
Cryptomonas sp.	0.49 ± 0.77			
Myzozoa	3.72 ± 3.91		*	
Gymnodinium sp.	0.13 ± 0.26			
Peridinium sp.	3.72 ± 3.90		**	
Chlorophyta	10.14 ± 8.20		**	
Pediastrum boryanum	1.39 ± 1.70		**	
Botrycoccus braunii	8.75 ± 7.94		**	
Charophyta	12.09 ± 12.82	*	**	**
Staurastrum ophiurum	0.11 ± 0.71			
Spirogyra sp.	11.98 ± 12.83	*	**	**
Total phytoplankton	86.16 ± 39.16		**	**
Total plankton	96.39 ± 42.47	**	**	**

24°C in summer. pH was mostly neutral and measured between 7.1 and 7.9. The maximum concentration of DO was recorded in spring (7.60 mg/l) within the surface layer. The predominant anions and cations can be arranged in the following sequence in decreasing order of their average concentration: HCO^- > TS > SO_4^{2-} > Cl^- > T N > TP > NO_3^- > PO_4^{3-} and Ca^{2+} > Mg^{2+} > Na^+ > K^+. Also CO_3^{2-} and NO_2^- concentration in anions and Fe^{+2} and TFe concentration in cations were zero during the study period.

Table 3. Seasonal variation in zooplankton abundance (%): Sm. Summer; Sp. Spring; At. Autumn; Wn. Winter.

Tabla 3. Variación estacional en la abundancia de zooplancton (%): Sm. Verano; Sp. Primavera; At. Otoño; Wn. Invierno.

Zooplankton	Sm	Sp	At	Wn
Rotifera	97.09	97.21	97.47	97.82
Arthropoda	2.21	2.21	2.00	1.64
Ciliophora	0.69	0.58	0.53	0.55

Table 4. Seasonal variation in phytoplankton abundance (%): Sm. Summer; Sp. Spring; At. Autumn; Wn. Winter.

Tabla 4. Variación estacional en la abundancia de fitoplancton (%): Sm. Verano; Sp. Primavera; At. Otoño; Wn. Invierno.

Phytoplankton	Sm	Sp	At	Wn
Ochrophyta	77.41	73.58	68.05	69.31
Cryptophyta	0.54	0.58	0.63	0.49
Myzozoa	0.42	0.35	0.32	5.06
Chlorophyta	11.27	11.10	13.48	11.11
Charophyta	10.36	14.39	17.51	14.03

The PCA of environmental variables showed that most variability (86%) can be explained by two main principal components (fig. 2). Variables most responsible for differentiating samples in the PCA included T, TS, NO^{3-}, Ca^{2+}, PO_4^{3-}, BOD_5, and COD (fig. 2). A PCA biplot clearly indicates the correlation between variables as well as the relative importance of each variable in explaining the overall variability in the environmental data (fig. 2). In general, similar variables clustered together: (i) Ca^{2+}, Mg^{2+}, K^+ and Na^+; (ii) BOD_5 and COD; (iii) TDS and turbidity. The distribution of each parameter over the seasons can be analyzed by the positions of the seasons with respect to the environmental factors. For example, the winter season (lower left quadrant) showed high values for turbidity, DO, TN and PO_4^{3-}, whereas the autumn season (lower right quadrant) tended to be

Table 5. Pearson correlation coefficients between environmental variables and plankton density: * $p < 0.05$; ** $p < 0.01$. (For abbreviations see table 1.)

Tabla 5. Coeficientes de correlación de Pearson entre las variables ambientales y la densidad de plancton: * p *< 0,05;* ** p *< 0,01. (Para las abreviaturas ver tabla 1.)*

Plankton	EC	Turbidity	T	DO	TSS	TDS	NO_3	BOD_5	COD
Rotifera		0.287**	0.189**	0.243**	0.247**		–0.150*	0.166*	
Arthropoda		0.145*	0.146*		0.204**		–0.157*	0.144*	0.196**
Ciliophora			0.140*						
Total zooplankton		0.292**	0.195**	0.245**	0.255**	0.128*	-0.155*	0.172**	0.128
Ochrophyta	0.159*	0.148*		0.166**	0.160				
Cryptophyta				0.142*					
Myzozoa		0.217**	0.181**		0.172**			0.157**	0.142
Chlorophyta		0.182**		0.248**	0.274**				
Charophyta		0.392**		0.471**	0.388**				
Total phytoplankton	0.156*	0.198**		0.227**	0.214**				
Total plankton	0.152*	0.231**		0.239**	0.226**				

associated with higher concentrations of variables such as TDS, TSS, BOD_5, TS and EC (fig. 2). Variables such as Ca^{2+}, Mg^{2+}, K^+ and Na^+ were significantly correlated with axis 2. In contrast, factors BOD_5, COD, TS and T were significantly associated with axis 1. These findings indicate that the second axis is likely related to the cations while the first axis is likely related to the degradation of organic matter, a process that influences the BOD_5 and COD values.

Plankton species composition and density

The plankton species identified in the cave are shown in table 2. The plankton assemblage included 13 genera and cinco species. Rotifera had the highest number of genera (4) and species (4) followed by Arthropoda (3), Ochrophyta (3), Myzozoa (2), Charophyta (2), Chlorophyta (2), Ciliophora (1) and Cryptophyta (1). In terms of numbers, the dominant species of phytoplankton and zooplankton were *Achnanthidium* sp. and *Lecane* sp. with an annual mean of total numbers of 22.64 and 3.72 ind./l, respectively. The seasonal total phytoplankton and zooplankton abundance ranged from 6.40 to 288.20 and from 0.00 to 37.10 ind./l respectively. The seasonal composition of the plankton community is presented in tables 3 and 4. Univariate analysis of variance revealed significant differences between seasons for a few species and between depth layers for most species (table 2). During all seasons, the zooplankton and phytoplankton communities were mainly composed of Rotifer and Ochrophyta, respectively, contributing to the total abundance with a percentage ranging from 97.09% (in summer) to 97.82% (in winter) for Rotifer and 68.05% (in autumn) to 77.41% (in summer) for Ochrophyta (tables 3, 4). The Shannon–Wiener diversity index (*H´*) ranged from 0.84 to 0.98 decits, generally showing lower values in autumn, winter and summer, and higher values in spring.

Influence of environmental variables on plankton

Pearson correlation coefficients between environmental variables and plankton density (table 5) varied between –0.150 and 0.471 for all species, showing a low significant relationship. Among the environmental parameters, TSS and turbidity seemed to have the highest influence on the temporal distribution of plankton species (table 5). Furthermore, DO play an important role for most plankton species and temperature for most of zooplankton species. All the environmental variables correlated positively with plankton except NO_3^-, which correlated negatively with several zooplankton species. Among the other parameters, BOD_5, COD and NO_3^- were important factors for several zooplankton species, but they played a less important role for phytoplankton (table 5).

Discussion and conclusions

Temperate caves are, in general, stable and characterized by a permanent absence of light and temperatures similar to those in the external environment (Ferreira & Martins, 2001; Prous et al., 2004), but tropical and subtropical caves show a great degree of variability in their environmental parameters. A popular misconception about cave environments is that they are always poor in biodiversity and biomass. This misconception stems from the fact that most cave research has been conducted in temperate caves where biodiversity and biomass are rather poor (Romero, 2009).

Population size in fish is limited by food density (McNamara & Houston, 1987). In the present study, the diversity and abundance of phytoplankton and zooplankton in the cave was low. This low food density might account for the small population of the species, estimated to be only 330 and 526 individuals (Zalaghi, 1997).

There are no previous reports providing data on the plankton community of the cave. The cave differed significantly from other nearby aquatic ecosystems as results of its unusual nature: low transparency, low plankton abundance, and low concentrations of nutrients.

Our results provide a spatial and temporal account of the plankton communities in the cave. The highest species diversity was found in the spring, when Cl^- was lowest and pH was highest. We did not observe seasonal patterns in plankton density in the cave. The most abundant zooplankton and phytoplankton were Rotifers and Ochrophyta, respectively.

Compared with the terrestrial environment, the aquatic ecosystem has few physical barriers obstructing the mixing of planktonic species (Prous et al., 2004). In this study, we aimed to develop parameters to predict the relationships between the plankton community and environmental parameters. Although the cave is not a homogametic aquatic ecosystem there is a significant but low relationship between the plankton community and environmental parameters. Our study of the plankton community in relation to environmental parameters in the cave showed that the environmental variables could not have been responsible for the present species composition in the cave.

Numerous studies state the importance of environmental variables on community structure and the plankton community in aquatic ecosystems. In the present study, TSS and turbidity were the most important factors for all plankton species. Nogueira et al. (1999) and Bonecker & Aoyagui (2005) showed that increased turbidity and the consequent decrease in light penetration to the deeper water layers influence plankton density. DO is considered one of the most important abiotic parameters affecting the plankton occurrence and distribution (Zurek, 2006; Vanderploeg et al., 2009; Chen et al., 2011). In the present study, DO played an important role for most plankton species, and the highest significant correlation was between DO and charophyta abundance. Temperature is considered to be a crucial factor that influences many aspects of the biology and ecology of the zooplanktonic organisms (Wetzel, 2001). It has been reported that temperature affects zooplankton occurrence and distribution (Akbulut et al., 2008; Huber et al., 2010), and in the present study temperature played an important role for most zooplankton species. BOD_5, COD and NO_3^- were among the major environmental variables influencing zooplankton in the cave, similar to results reported by others (Arora & Mehra, 2009; Chalkia et al., 2012) NO_3^-, however, was negatively correlated with zooplankton density, a finding also supported by other studies (Tolotti et al., 2006; Chalkia et al., 2012).

Our study presented spatial and temporal variation in environmental variables and plankton species and confirmed that the habitat has low plankton density. The results from this study could be useful for conservation efforts such as habitat rehabilitation and animal translocation programs as well as a basis for future research and monitoring efforts

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