DETERMINANTS ON THE STRUCTURE OF AN AQUATIC INVERTEBRATE COMMUNITY IN A NEOTROPICAL LIMESTONE CAVE

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ABSTRACT

Caves are commonly stable environments and hold a unique biodiversity. The importance of water as a vehicle of food resource input and as the habitat of many aquatic organisms is well known. This importance is even greater in subterranean ecosystems. However, there is a lack of studies concerning the subterranean aquatic invertebrate fauna in Brazil. The objective of this study was to characterize the aquatic environment and fauna of a limestone cave located in the state of Minas Gerais (Southeastern Brazil). The relations between the benthic invertebrate fauna and the aquatic environmental characteristics were also analyzed. All the data was obtained in a single sampling event in the rainy season. The stream was divided in 8 sampling stations in a limestone cave *"Gruta do Retiro"* (Brazil). The diversity (Shannon Index) and similarity (Bray-Curtis) among the stations were analyzed, as well as the aquatic fauna and its relation with environmental variables. The physical-chemical characteristics analyzed were: temperature, dissolved oxygen, electric conductivity, pH, velocity of water flow and percentage of organic matter present in the sediment. To evaluate the influence of the abiotic data over the richness of invertebrates, a non-parametric test (Spearman) was performed. A total of 4,490 organisms distributed among the Arthropoda, Annelida, Mollusca, Nematoda and Platyhelminthes taxa were identified. The Arthropoda was the most representative taxa with species from the Insecta, Arachnida and Crustacea groups. Dissolved oxygen, temperature and electric conductivity presented significant relation with the distribution of some aquatic organisms.
Keywords: cave, benthic fauna, aquatic invertebrates, Pains, Brazil.

1. INTRODUCTION

Caves may be formed in an array of lithologies. The distinguish characteristics of each rock type may lead to different environmental features. Most of the known caves are formed in carbonatic rocks due to their high solubility and exposure to water flows (Ginés and Ginés 1992; Gillieson 1996). The chemical nature of the rock where a cave is formed promotes different environmental characteristics on the aquatic system. The alkaline nature of carbonate rocks in karst areas may cause hardness of underground freshwater.

The hardness observed in karstic watersheds is usually caused by the high levels of dissolved $CaCO_3$ in these areas through dissolution of the carbonate rocks (Zhang et al 2000). The precipitation of calcite may occur through natural processes of water softening, such as enhancement of velocity of water flow and outgassing of CO_2 at the

surface (Zhang et al 2000). It is then noticeable the mosaic of environmental conditions that a watershed in a karst area may provide in the epigean and hypogean environments.

The subterranean systems are usually divided into three distinct zones: photic, disphotic and aphotic. Each of these zones has particular environmental characteristics due to the sunlight influence. Deeper zones of caves are usually aphotic (Poulson and White 1969). Such characteristic leads to lack of primary photosynthetic production, which is usually the basis of most food webs. Thus, the hypogean food web must rely on allochtonous food resource or chemoautotrophic production (Sarbu et al 1996; Vlasceanu et al. 2000; Porter 2009). Rivers and water discharges may import a large amount of organic matter and cause passive colonization by the epigean fauna into the cave. The import of organic matter is an important factor for the maintenance of the hypogean fauna, since many cave-dwelling invertebrates rely on this allochtonous resource to survive (Souza-Silva et al 2003).

The benthic invertebrates are among the groups that may colonize the hypogean system either by passive or active mechanisms. They inhabit the bottom of aquatic habitats in lentic or lotic environments (Hauer and Resh 1996; Merrit and Cummins 1996). These organisms are important for the energy flow, nutrient cycling and revolviment of sediment in aquatic environments (Callisto and Esteves 1995; Cummins et al 1989). The importance of the benthic fauna might be even greater in subterranean environments, since the benthic community often plays a key role on the availability and maintenance of energy flow in many systems. This maintenance is usually provided by the fragmentation of organic matter by different groups of benthic organisms (Graça 2001, Tundinsi 2008).

Some benthic groups are also widely used as bioindicators of water quality due to their ecological relations and response to environmental changes (Alba-Tercedor 1996). It is important to highlight that some important human activities may pose serious impacts to the subterranean ecosystem (Hobbs and Gunn 1998; Souza-Silva et al 2003). The use of karst areas for agriculture and cattle may cause serious damage to the subterranean aquatic and terrestrial systems (Hoke and Wicks 1997; Nicod and Solomon 1999; Neil et al 2003; Souza-Silva et al 2003).

Although there are relevant studies on the Brazilian aquatic cave fauna, most of this information have focused on fish taxonomy and ecology (Mattox et al 2008; Bichuette and Trajano 2003; Bichuette and Trajano 2006, Bichuette and Trajano 2008). There are almost no studies on the cave invertebrate benthic fauna in Brazil (Bichuette and Santos 1998; Souza-Silva 2003; Silva 2008). This lack of information of the aquatic environment and the benthic fauna jeopardizes the understanding of this habitat and its communities. Considering the use of areas for agricultural activities coupled with the risks of contamination of karstic aquifers (Nicol and Solomon 1999; Neil et al. 2004), it is clear the urge to consider different aspects of the cave biodiversity aiming to diminish the impacts. The Brazilian karst has been increasingly used in the past decades for agriculture and pasture of living stock. Therefore, we believe that more studies aiming a better understanding of the subterranean ecosystem is of great urgency. Studies including different groups of organisms, such as the benthic communities are extremely important for a better understanding of the cave fauna and its dynamics.

The objective of this study was to characterize the aquatic environment and fauna of an epigean stream running through a karstic area and flowing into a limestone cave. The relations between the benthic invertebrate fauna and some aquatic environmental variables were also analyzed.

2. MATERIAL AND METHODS

Study area

The study was carried out in *Retiro* cave (*Gruta do Retiro*: UTM 23 K 437554,21E/ 7741129,32S). This limestone cave has 204-meter linear development, and is located in the municipality of Pains (Minas Gerais state, Brazil). The surrounding epigean area is used as pasture for cattle.

Retiro cave has a stream that flows downstream from a lake in the epigean system near the entrance into the cave. This water flow into the subterranean system occurs through a small sinkhole at the entrance of the cave and it only springs out at about 40 meters from the entrance of the cave, where after flowing along a couple of meters it flows into inaccessible places under the rocks and fallen blocks. The stream only springs out again approximately 20 meters beyond this site. From this point on, the stream is accessible up to a siphon at the end of the cave. The total accessible length of this stream is approximately 120 meters (Fig.1). The samplings were carried out in the hypogean stretch of this stream.
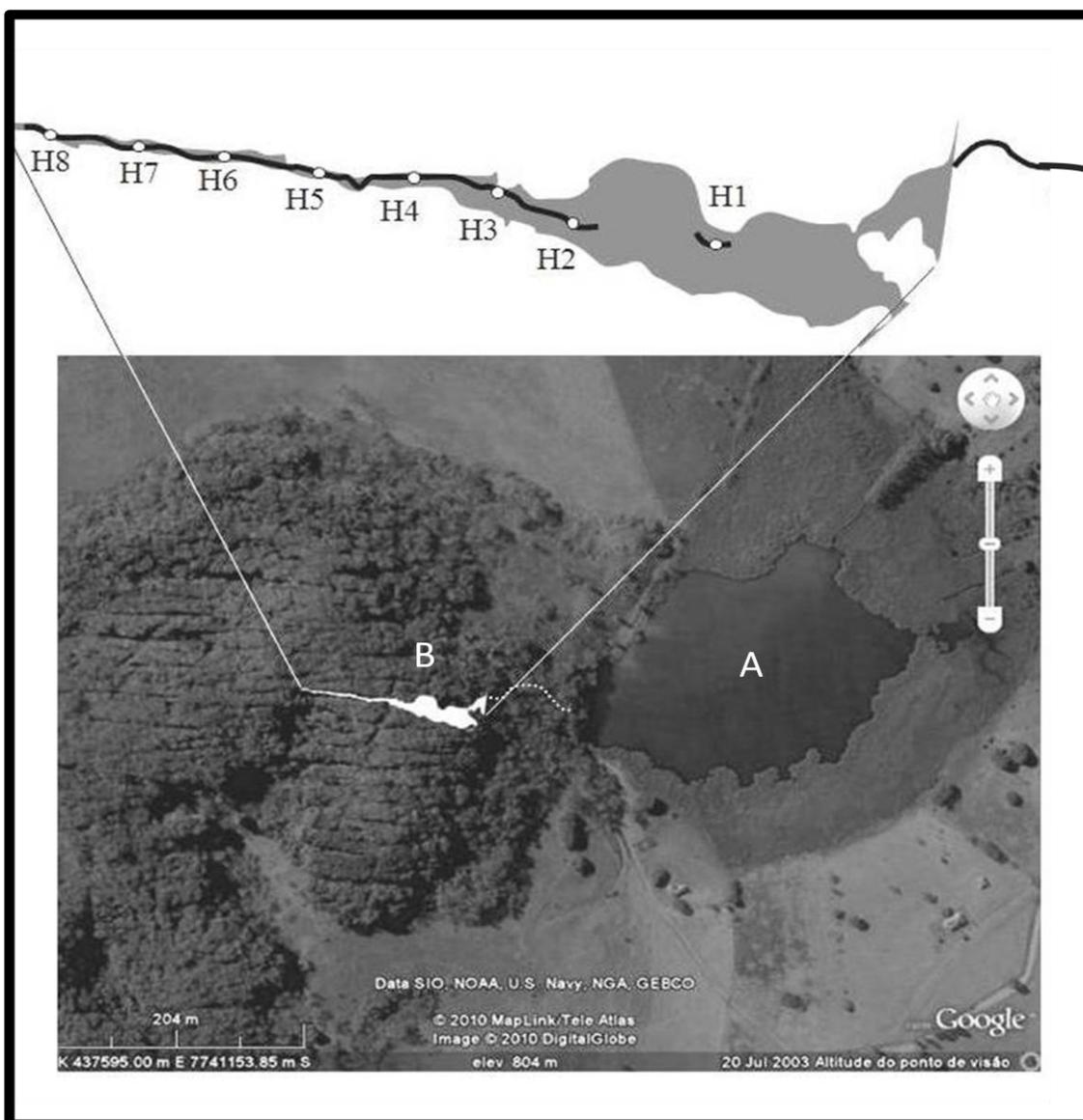
Figure 1 Map of Retiro Cave (Gruta do Retiro) and its location in the area. Dotted line represents the stream in the epygean system flowing downstream towards the cave entrance. (A) Lake where the stream

forms and flows downstream into the cave and (B) cave location. Above a scheme of the stream course inside the cave with the sampling stations (H1-8) plotted (adapted from Googleearth).

3. METHODS

The data were obtained in a single sampling event (December 9, 1999). The hypogean stretch of the stream was divided in 8 sampling stations (H1-H8), which were 13 meters equidistant. The first sampling station is located in the first chamber of the cave (aphotic zone) and presents a large deposit of bat guano and vegetal debris partially covering terrestrial and aquatic systems (Fig. 1).

The physical-chemical variables analyzed were: temperature (T), dissolved oxygen (DO), electric conductivity (EC), pH, velocity of water flow (V) and percentage of organic matter in the sediment (OM). These variables were obtained in all sampling stations. More details are given bellow, in the *abiotic data* section.

Biotic data

The benthic invertebrates were collected through visual sampling method coupled by the use of a Surber sampler (250mm X 250 mm, mesh size 250 μm). They were preserved in plastic bags containing alcohol (70%) and formalin solution (5%) for further identification. All the material sampled was sorted in the laboratory after being washed in sievers (2mm, 1mm, 0.500mm and 0.250mm) and preserved in recipients containing 70% ethanol. A stereomicroscope and a microscope were used for the identification of organisms up to the possible taxonomic level.

Abiotic data

All the abiotic data were sampled before the invertebrate collection at each sampling station. Three values were obtained for each variable in each sampling station. They were then used to obtain a final mean value to be used in the statistical analyses.

A total of 3 samples per station were collected for the analysis of percentage of organic matter. These samples were first dried at 100°C for 24h, weighted and incinerated at 500°C for 3 hours. After incineration, the material was weighted once more to obtain the percentage of organic matter through the difference of weight before and after this process.

The pH, temperature and conductivity were obtained with the aid of a pHmeter, an electronic thermometer and a conductivimeter at each station. This procedure aimed to minimize the effects of sediment suspension on the readings obtained with the respective equipments.

The velocity of water flow was measured by the time spent by a small (3cm) polystyrene ball to flow 1 meter. The data was then mathematically corrected to values in m/s. This was also performed before sampling the biotic data to avoid interference of sediment revolviment in the water flow.

Data analysis

The similarity between the sampling stations was obtained through the Bray-Curtis analysis and the diversity was calculated through the Shannon-Wiener Index. To

evaluate the influence of the abiotic data over the richness and abundance of invertebrates, a non-parametric test (Spearman) was performed. The influence of the environmental variables on each group was tested through a linear regression. For these analyses, the Statistica 9.0 and the PAST programs were used.

4. RESULTS

Environmental data

The pH, dissolved oxygen, electric conductivity, temperature and organic matter content did not vary highly among the sampling stations. High pH values were registered, with the maximum value registered in H7 (pH= 9.81) and the minimum in H5 (pH= 9.43). The minimum dissolved oxygen value was registered in H5 (DO= 6.90 mg/L) and the maximum in H7 (DO= 7.24mg/L). Electric conductivity varied from 250.90 µS.cm^{-1} (H3) to 275.90 µS.cm^{-1} (H1). The temperature varied little, with the maximum value (24.8°C) being registered in H2 and the minimum value (23.4°C) registered in H1. The percentage of organic matter present in the sediment varied from 10.85% (H5) to 13.84% (H1). The velocity of water flow was the only variable that varied considerably, from 3.43m/s (H7) to 9.10m/s (H5) (Table 1).

Significant relations between some variables were observed. The temperature presented a significant and negative relation with the electric conductivity ($F_{(1,7)}=38.91$; R=-0.92; p=0.000) and the percentage of organic matter had a significant and positive relation with the electric conductivity ($F_{(1,7)}=19.37$; R=0.86; p=0.003). A positive and significant relation between the pH and the dissolved oxygen was also observed ($F_{(1,6)}=6.47$, R= 0.72, p < 0.044) (Fig.2).

Table 1 Environmental data per sampling station; H1-8 (sampling stations), pH, DO (dissolved oxygen), EC (electric conductivity), V (velocity of water flow), T (temperature), OM (percentage of organic matter on the sediment), *S* (richness), *H* (diversity).

Sampling station	pH	OD (mg.L^{-1})	EC (µS.cm^{-1})	V (m/s)	T (°C)	OM (%)	*S*	*H*
H1	9.50	7.03	275.90	5.16	23.4	13.84	12	1.007
H2	9.60	6.93	250.97	5.07	24.8	10.50	12	1.044
H3	9.50	7.10	250.90	3.75	24.6	9.79	14	1.500
H4	9.60	6.92	252.07	4.83	24.2	10.85	11	1.502
H5	9.43	6.90	253.70	9.10	24.0	8.67	11	1.387
H6	9.68	7.13	253.33	4.35	23.9	10.31	15	1.733
H7	9.81	7.24	253.87	3.43	23.8	10.57	14	1.773
H8	9.69	7.17	261.10	4.96	23.5	10.26	4	0.994
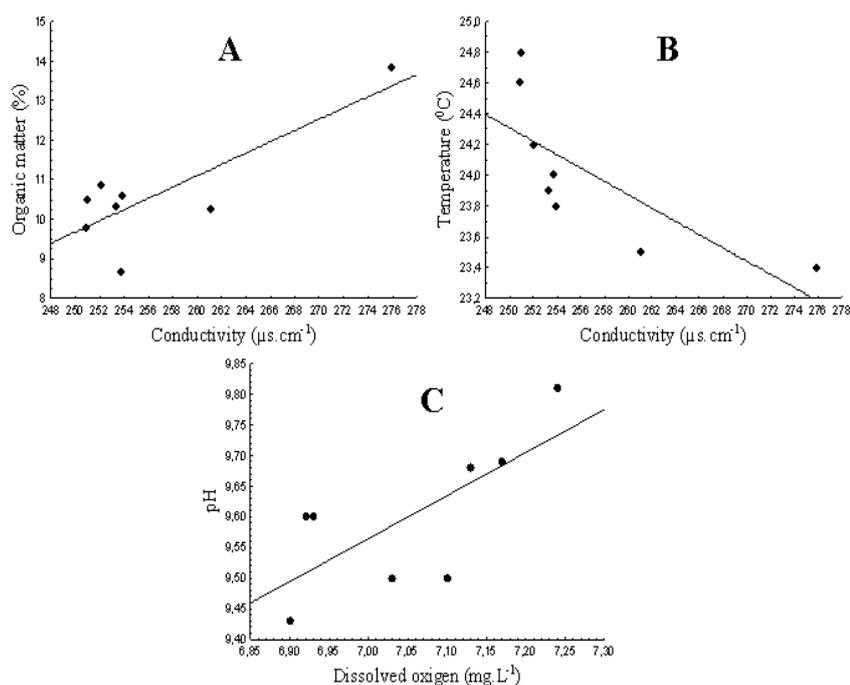
Figure 2 Significant (p<0.05) relations between environmental variables; (A) organic matter and electric conductivity, (B) temperature and electric conductivity, and (C) pH and dissolved oxygen.

5. FAUNA DATA

A total of 4,490 organisms distributed among 22 species of the Arthropoda, Annelida, Mollusca, Nematoda and Platyhelminthes taxonomic groups were identified. The Arthropoda was the most representative taxon with species from the Insecta, Arachnida and Crustacea (table.2).

Insecta was certainly the most representative group in number of species. A total of 11 species were identified. They were distributed among the orders Diptera (Chaoboridae, Simuliidae and *Thienemanniella* sp), Coleoptera (Elmidae), Trichoptera (*Chiamarra* sp), Hemiptera (*Ambrysus teutonius* and *Rhagovelia pachymeri*), and Ephemeroptera (Baetidae).

A total of three Mollusca species were found. They are distributed among Bivalvia (Sphaeridae) and Gastropoda (Ancylidae and Melaniidae). The Melaniidae (*Melanoides tuberculata;* Müller, 1774) was the species that presented the highest abundance. The Crustacea was represented by four species (from Ostracoda and Cyclopoidea). The Plathyhelminthes (*Dugesia* sp), Nematoda and the Arachnida (Trombidiforme) groups were represented by only one species each.

Table 2. Abundance of aquatic fauna separated per taxonomic group and sampling stations (H1-H8).

Taxa	H 1	H 2	H 3	H 4	H 5	H 6	H 7	H 8
Gastropoda								
Melanoides tuberculata	34	380	186	440	296	324	131	24
Ancylidae	3	9	6	94	49	75	47	-
Bivalvia								
Sphaeriidae	-	12	-	15	16	8	-	-
Hemiptera								
Ambrysus teutonius	1	9	7	-	1	3	4	5
Rhagovelia pachymeri	1	-	-	-	-	-	-	-
Coleoptera								
Elmidae sp1	236	76	453	147	117	128	194	9
Elmidae sp2	1	1	13	43	28	23	80	-
Elmidae sp3 (larvae)	27	8	214	31	13	54	72	-
Elmidae sp4 (larvae)	3	-	5	-	-	11	1	-
Ephemeroptera								
Baetidae	3	1	9	3	2	12	13	1
Trichoptera								
Chiamarra sp	6	7	10	-	3	1	2	-
Diptera								
Chaoboridae	2	-	-	-	-	-	-	-
Simuliidae	-	-	-	-	1	-	-	-
Chironomidae								
Thienemanniella sp	-	-	-	-	1	-	-	-
Annelida								
Oligochaeta	-	-	10	1	10	18	10	-
Planariidae								
Dugesia sp	-	-	4	-	-	-	2	-
Arachnida								
Trombidiforme	-	1	-	2	-	1	7	-
Ostracoda								
sp1	-	14	-	46	-	9	1	-
sp2	3	6	65	4	-	20	-	-
sp3	-	-	-	6	-	2	-	-
Copepoda								
Cyclopoidea	-	-	-	-	-	1	1	-
Nematoda	-	-	1	-	-	-	-	-

Most of the sampling stations presented a high similarity, with the values varying from 0.847 (between H5 and H6) to 0.076 (between H8 and H3). The only station that differed greatly was H8, which presented low values of similarity, varying from 0.076 (between H8 and H3) and 0.194 (between H8 and H1) (Fig.3). The diversity varied from 0.994 (H8) to 1.773 (H7).

The environmental variables analyzed did not present any significant relation with the richness of aquatic invertebrates. However, some variables presented a

significant relation with specific groups. The dissolved oxygen presented a significant and negative relation with the abundance of *M. tuberculata* ($F_{(1,7)}$=11.95, R=-0.79, p=0.01), and Sphaeridae sp1 ($F_{(1,7)}$=8.87, R=-0.75, p=0.02). The temperature presented a significant and positive relation with the abundance of *M. tuberculata* ($F_{(1,7)}$=59.88, R=0.95, p=0.0001) and with Sphaeridae sp1 ($F_{(1,7)}$= 37.08, R=0.92, p=0.0005). The conductivity presented a significant and negative relation with the abundance of *M. tuberculata* ($F_{(1,7)}$=22.25, R=-0.87, p=0.002).
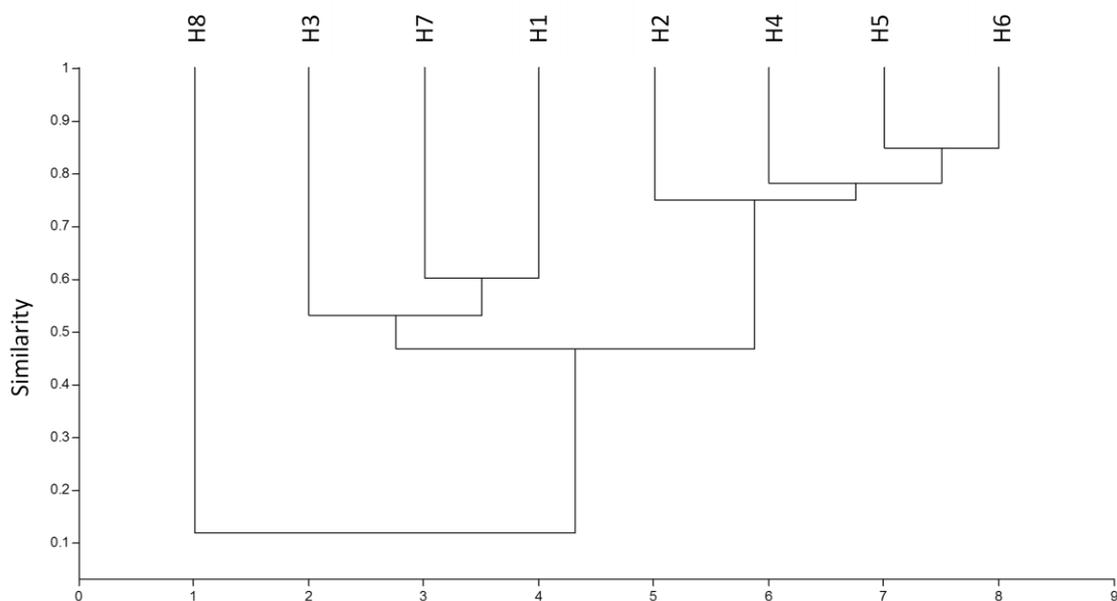
Figure 3 Similarity cluster (Bray-Curtis) between sampling stations (H1-H8)

6. DISCUSSION

Physical-chemical parameters

According to Esteves (1998), one of the main variables that influence the distribution of aquatic fauna is the temperature. The thermal stability observed along the stream in the hypogean environment provides certain stability for the benthic community and provides a more homogeneous habitat concerning thermal variations.

The high values of pH and electric conductivity registered may be related to the limestone dissolution. According to Gillieson (1996) and to Zhang and collaborators (2000) the dissolution process of limestone provides calcium ions to the water, increasing its pH and electric conductivity. The negative relation observed between the electric conductivity and the temperature may be a consequence of the dissolution process of limestone by the stream towards deeper zones of the cave. It is possible to observe an increasing variation of electric conductivity towards these zones (from H2 to H8).

However, the first sampling station (H1) presented a high value of electric conductivity, which could have been influenced by decomposition of organic matter occurring at this site. According to Tundisi (2008), there are regions in rivers where the nutrients accumulate and function as source of organic matter and ions to the water

system. The high value of EC registered at the first sampling station (H1) may be a consequence of a large deposit of hematophagous bat guano and accumulation of allochtonous vegetal debris in this station. The organic matter deposits could be adding ions to the system, causing this enhancement. The positive and significant relation between the electric conductivity and the percentage of organic matter corroborates with this hypothesis.

Rocha and colleagues (2009) mention a significant positive relation between pH and concentration of dissolved oxygen in aquatic environments (floodplain lakes) due to photosynthetic activity. We have also registered a positive relation; however, it is unlikely this result is consequence of photosynthetic activity, since the photosynthetic organisms are not adapted to the hypogean systems. In the case of caves, this relation is possibly related to other factors such as the accumulation of calcium ions towards deeper zones of the cave (Gillieson 1996; Zhang et al 2000). These deeper zones are also colder and consequently present higher levels of dissolved oxygen.

Similarity

Most stations presented a high similarity. This was probably caused by the similar environmental characteristics that could be providing similar conditions for the establishment of these invertebrates along the stream. The low similarity observed between the last station (H8) and all the other sampling stations may be a result of physical environmental differences not included in the present study, such as granulometry of substrate, type of organic matter, width or even depth of the stream (it is a very shallow stretch, not more than a few centimeters deep).

Physical-chemical parameters influencing the fauna

As previously mentioned, Esteves (1998) stated that temperature is one of the most important variables influencing the aquatic fauna. Regier and colleagues (1990) have shown in their study that the temperature affects different aspects and levels of a food web, and therefore, is an important variable affecting the aquatic fauna. It is also well known that the distribution of species is restricted to a certain temperature range. Water temperatures usually affect feeding activity, digestion rate, and respiratory rate (Allan 1995). *Melanoides tuberculata* is a warm water related species which feeds mainly on algae and vegetal debris present on sediment (Murray 1971). Although the temperature did not vary a lot, it was possible to observe a certain preference of *M. tuberculata* and Sphaeridae for warmer microhabitats. Both presented a positive significant relation with the temperature, which may be related to their behavior (g.e. feeding habits) or even physiological needs.

The negative relation of dissolved oxygen with *M. tuberculata* and Spaeridae sp1 is possibly consequence of their stronger positive relation with temperature. It is widely known that temperature normally has a negative influence on the rates of dissolved oxygen (Regier et al, 1990; Tundisi, 2008). It is plausible to think that organisms will interact with environmental variables in different ways and levels. Environmental variables have different levels of interaction strength according to intra and interspecific specifications. It is possible that the negative relation with the dissolved oxygen here observed is mainly a consequence of the strong relation between this taxa and the temperature, which is a variable that correlated negatively with the dissolved oxygen.

According to Tundisi (2008) the chemical characteristics of the water influence the aquatic fauna and flora. It is widely known that the electric conductivity may interfere in physiological functions, such as absorption of nutrients. The significant relation presented by *M. tuberculata* with electric conductivity could be a result of such influence. However, there could be other variables influencing this relation. More detailed studies should be performed to better understand the relation observed in this study.

7. CONCLUSION

The watersheds running through a karstic area are exposed to many different environmental variables and a diversity of landscape characteristics. The differences of environmental characteristics posed by different systems may affect the fauna inhabiting the caves.

The relations observed between certain species (*M. tuberculata* and Sphaeridae sp1) and the environmental variables show the influence these variables might have on the subterranean fauna. The strong relation observed between the temperature and dissolved oxygen on *M. tuberculata* and Sphaeridae sp1 highlights the importance of understanding how the ecosystem functions to be able to preserve these environments. Any strong environmental change on the watersheds (even caused by human activities) could have strong negative impact on the entire cave biodiversity. These impacts may be responsible for serious ecologic disturbance.

Aiming to preserve the subterranean biodiversity, more studies should be performed for a better understanding of the cave aquatic fauna and the elements influencing it. These are important tools concerning cave fauna conservation and management

8. AKNOWLODGEMENTS

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