

## TRANSITORY AQUATIC TAXOCENOSIS IN TWO NEOTROPICAL LIMESTONE CAVES

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**Resumo:** Nos ambientes cársticos, áreas de recarga hídrica são caracterizadas por feições externas, que capturam e veiculam as águas superficiais para compartimentos subterrâneos. O presente estudo teve como objetivo avaliar os efeitos das flutuações naturais de curto prazo no nível da água na composição e abundância das taxocenoses zooplanctônicas e ictiofauna no interior de duas cavernas calcárias. A água da chuva é filtrada através do exocarste acima das cavernas e goteja pelos espeleotemas. Além disso, pode ser também canalizada por meio de enxurradas, inundando parcialmente o piso das cavernas e misturando-se à água do lençol freático. Nas enxurradas são transportadas argila, troncos, folhas e fezes bovinas que se acumulam no piso das cavernas. Foram encontrados 3 grupos de invertebrados zooplanctônicos (Copepoda, Protozoa e Rotifera) e 3 táxons bentônicos/nectônicos (Annelida, Culicidae e *Hyaella* sp.), além de onze espécies de peixes (Characiformes e Siluriformes). Os eventos de inundação permitem que espécies de peixes e do zooplâncton oriundas do ambiente epígeo, tenham acesso e explorem locais inacessíveis da caverna durante a estação seca do ano. Ao final das estações chuvosas, aqueles organismos aprisionados em corpos de água temporários morrem e podem servir de recurso alimentar para outras espécies terrestres.

**Palavras chave:** Riqueza, composição de espécies, taxocenoses, espécies transitórias, cavernas.

**Abstract:** In karstic environments, water recharge areas are characterized by external features that capture and convey the surface water to subterranean compartments. The objective of the present study was to evaluate the effects of the short term natural water level fluctuations on the composition and abundance of the zooplankton and ichthyofauna inside two limestone caves. Zooplankton was sampled with filterings nets (120µm) and fish, with trawl nets, gillnets, wicker trap and hand nets. In both caves the rainwater is filtered through the exokarst directly above the caves and drips from speleothems. It can also come in flash floods, be filtered by the soil, or come through dolines, partially flooding the floors of the caves on its way to supply the water table. Through flooding clay, trunks, leaves and bovine feces are transported that accumulate on the floor of the caves. Three groups of invertebrate zooplankton were found in the two caves (Copepoda, Protozoa and Rotifera) and 3 benthic/nektonic taxa (Annelida, Culicidae and *Hyaella* sp.). Eleven species of fish were captured in Characiforms (*Astyanax* sp, *Astyanax bimaculatus*, *Astyanax fasciatus*, *Serrapinus heterodon*,

*Cheirodon piaba*, *Cyphocharax gilberi*, *Hyphessobrycon santae*, *Characidium lagosantense*, *Hoplias malabaricus*) and Siluriforms (*Rhandia quelen* and *Hoplosternum littorale*). The flood events in the two caves, caused by the water table level increase and flash floods during the rainy period allow fish and zooplankton species, originating from the epigeal environment, to have access and explore areas in the cave that are inaccessible during the dry season. At the end of the rainy season those organisms trapped in temporary bodies of water die and can serve as a food resource for other terrestrial species.

**Keywords:** Richness, species composition, taxocenosis, transitional species, caves.

## 1-INTRODUCTION

Natural subterranean cavities are elements that make up rocky reliefs known as karst. The karstic systems are comprised of large volumes of carbonate rock to which hydric recharge and discharge areas, usually allocated in distinct altimetric levels, are associated (Ford & Williams 2007).

Recharge areas are characterized by external features that capture and transmit the surface waters to subterranean compartments, and are indispensable in the movement, transport and deposition of sediment with the consequent formation of subterranean cavities (caves) (Ford & Williams 2007).

Caves are environments characterized by the permanent absence of light and consequently non-occurrence of photosynthetic producers. A large part of the organic resources present in cave ecosystem has allochthonous origins (Culver, 1982; Simon *et al.*, 2007; Souza-Silva *et al.*, 2007, Schneider *et al.* 2011).

Different openings and water courses, such as skylights, horizontal entrances, rivers and runoffs and waters that percolate the rock through pores or fractures, represent the particulate or dissolved organic matter form of transport to the caves (Simon *et al.* 2007, Souza-Silva *et al.*, 2007). Furthermore, carcasses and mainly the feces of animals that use the caves as shelters can also accumulate in the subterranean environment becoming important resources for the cave fauna, especially in permanently dry caves (Poulson, 1972, Culver, 1982, Ferrreira & Martins, 1999). Such imported organic materials frequently form deposits that make resources available for the subterranean fauna; however, the constancy of the supply of organic matter can determine the structure and dynamics of the communities of terrestrial invertebrate detritivores (Souza-Silva *et al.*, 2007, Schneider *et al.* 2011).

Countless aquatic invertebrates are also transported to the caves by running water that accesses its interior (Water 1981, Danielopol *et al.* 2003).

Cave environments present a wide variety of aquatic microhabitats (exokarst/epikarst waters, speleothems, rivers, temporary pools, permanent pools, tables, etc.). Some of these habitats can present a wide variety of micro and meso organisms (Johnson *et al.*, 2001).

Therefore, the subterranean waters can be inhabited by several organisms that range from microscopic to macroscopic size (Danielopol *et al.* 2003, Botosaneanu 1986, Wilkens *et al.* 2000, Chapelle 2001, Souza-Gomes 2008). For example, fish, amphibians, worms, arthropods, bacteria, protozoa and fungi can inhabit the sediment or be suspended in the subterranean or interstitial bodies of water (Griebler *et al.* 2002). Such organisms play important roles in the recycling of the organic matter transported by the water and in the redistribution of energy and matter, in cavities that vary in size from a few millimeters to meters (Ward *et al.* 1998, Simon & Benfield 2001, Boulton *et al.* 2002).

The secondary producer trophic level dominates the subterranean aquatic ecosystems as a consequence of the lack of radiant light energy to perform

photosynthesis (Gibert *et al.* 1994). The main representatives of the primary trophic level, when they occur, are represented by chemoautotrophic microorganisms, such as Bacteria and Archaeobacteria (Steven, 1997; Pedersen 2001).

Usually oligotrophy characterizes the natural subterranean aquatic habitats and plays an important role in community structuring (Gibert *et al.* 1994). As a consequence, in many subterranean aquatic systems the population densities are low, the food chains are simple, the trophic levels are few and the feeding behaviors are non-specialized (Ferreira & Martins 1999, Gibert 2001).

As a result, the imported organic resource availability rate can usually impose variations on the structure and dynamics of the terrestrial invertebrate detritivore communities (Souza-Silva *et al.*, 2007, Schneider *et al* 2011).

Grassle and Sanders (1973) proposed that short term fluctuations in the community structure are characterized by a "transitory diversity", as opposed to a permanent diversity in equilibrium. Transitory structure can be the product of biological factors such as predation, competition, parasitism or the result of physical or chemical environmental forces.

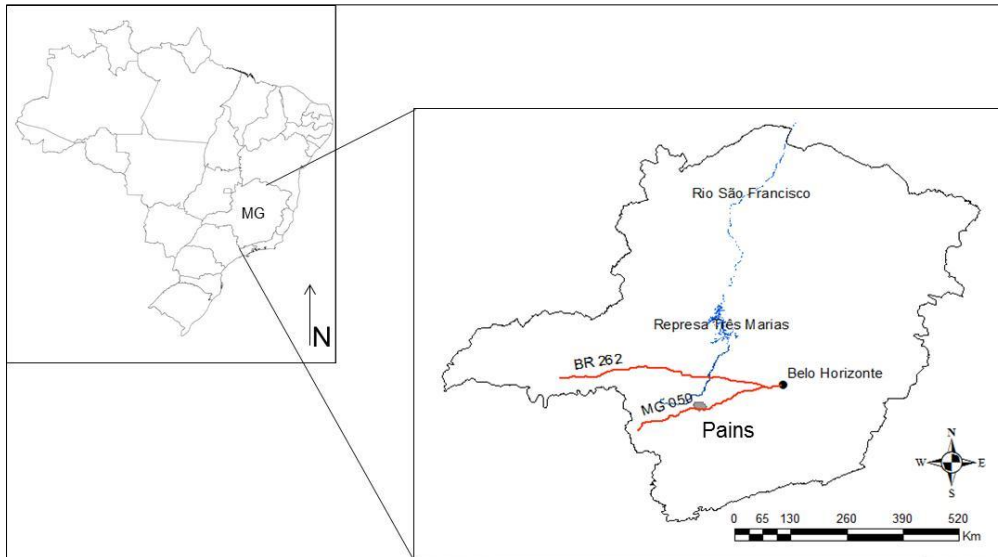
A large part of recent attention has been given to the role of anthropic alteration stress as being responsible for the transitory characteristic of some communities, but natural trophic and physical conditions are also important for the determination of transitory community structures. In the oceans, the origin could be the circulation of nutrients, seasonal variability, salinity, oxygen etc. (Nichols-Driscoll 1975). In cave environments it could be the humidity, brightness, food resource availability, water flow, etc. (Forbes 1998, Di-Russian *et al* 1999, Pentecost & Zhaohui 2001, Souza-Silva 2003, Schneider *et al* 2011).

The objective of the present study was to evaluate the effects of the natural short term water level fluctuations on the composition and abundance of the zooplanktonic and ichthyofauna taxocenosis in two limestone caves.

## 2-METHODOLOGY

### *Study site*

The study was conducted from April, 2009 to May, 2009 in permanent (groundwater outcroppings) and temporary (pools and temporary streams) aquatic environments of the Brega and Santuário caves (20°24'59.7"S - 45°46'20.6"WO), both located in the municipal district of Pains, Minas Gerais (Figure 1).



**Figure 1.** Map of the location of the city of Pains in Minas Gerais, Brazil.

Both caves are inserted in limestone outcrops, possess two opposing entrances and present bodies of water that undergo level increases, and also receive epigean water originating from dripping, temporary ponds and runoffs during the rainy season (October to March).

The Brega and Santuário caves are inserted in the sub-basin of the Patos Creek (530 km<sup>2</sup>). The basin is typically sparsely dense favored by direct infiltration in the limestone fissures and karstic absorption features such as sinkholes (Menegasse *et al* 2002).

The macroclimate of Pains region is characterized as mild temperate with hot, humid summers and dry winters. The average annual temperature is 20.7°C, July being the coldest month, with an average temperature of 16.3°C, and January the hottest month, with an average of 23.3°C. The local annual average precipitation is 1344 mm<sup>3</sup> (Menegasse *et al* 2002).

The local climate is responsible for the seasonal variation in the hydric dynamics of the Patos Creek, significantly altering the surrounding landscapes with periodic floods, during the rainy season (October to March), that corresponds to 81% of the annual precipitation (Menegasse *et al* 2002).

The high water volume in the microregion of the caves, associated to the relief with collector features (dolines), enables the increase of the water table level inside the caves, leaving much of their floors flooded (Figure 2).

The existing vegetation in the cave surroundings is composed of forest outcroppings and pastures for extensive cattle raising (Figure 2).



**Figure 2.** Representation of karstic features that promote water dynamics in the surroundings and inside the Brega and Santuário caves. Brega entrance (Be), Santuário entrance (Se), Temporary epigeian pond (TeP), Permanent epigeian pond (PeP).

**Field procedures**

**Zooplankton sampling**

For the analysis of the composition and abundance of the zooplankton 3 collections were conducted in each cave at 15-days intervals.

In the Santuário cave the collections were carried out in a temporary upwelling of groundwater, in a temporary travertine with dripping water and in groundwater with permanent water (Table 1, Figure 3). In the Brega cave two areas of runoff water accumulation (temporary) and a permanent groundwater site (Table 1) were sampled. It was not possible to conduct more collections, because the temporary bodies of water dried at the end of the rainy season.

**Table 1.** Characteristics of collection sites in the Brega and Santuário caves.

Local		N
P1 (Brega)	Runoff with clay accumulation and clay bottom (Figure. 3 D).	3
P2 (Brega)	Transparent groundwater and clay bottom	3
P3 (Brega)	Runoff with clay accumulation and clay bottom	1
P1 (Santuário)	Transparent upwelling, but receives runoff water (Figure. 3 B)	3
P2 (Santuário)	Travertine with clear water from dripping	3
P3 (Santuário)	Ground water with cloudy water not receiving runoff water (Figure. 3 A).	3

N = number of collections





**Figure 3.** Groundwater of the Santuário cave (A); Point 1 Santuário Cave (B); Ichthyofauna collection with drag net in the Santuário cave (C), Collection of zooplankton with bucket and net in the Brega cave (D).

For the zooplankton collection, 100 liters of water were filtered per collection station during each collection. The collection was made with using a 5 liter bucket and filtering carried out in a 120 $\mu$ m mesh zooplankton net. After filtering, the material was stored in polyethylene flasks and properly labeled, stained with rose bengal dye and preserved with 4% formaldehyde after a period of 15 minutes, still *in situ*. The collected organisms were identified in the laboratory and counted under a microscope.

### ***Ichthyofauna sampling***

The fish collection was only conducted in the Santuário cave on April 25th and 26th, 2009 after intense rains in the cave areas (Figure 3 C). For the collection, gillnet was placed in the cave for 24 hours. Furthermore, fishes were also collected with drag nets and hand nets. Dragging was done in shallow temporary pools in the cave (lentic environment). The gillnet was setup in a deep permanent pool (approximately 3-4m). The traps were baited with liver in the temporary streams inside the cave (lotic environments). All of the captured specimens were fixed in 5% formalin and preserved in 70% alcohol. In the laboratory the collected organisms were identified by a specialist.

### *Physical characterization of the cave surroundings*

Through observations *in loco* and with the use of Google Earth images it was possible to characterize the external karstic features that can influence the water dynamics and the cave aquatic communities (Figure 3).

### 3-RESULTS

In both caves the rainwater enters the cave through the exokarst/epikarst, runoffs or even filtered by the soil, through sinkholes, supplying the groundwater. The runoff water accumulates temporarily in a long passage and is also directly mixed with the groundwater (Figures 2 and 4). In the runoffs clay, trunks, leaves and bovine feces are transported that accumulate on the cave floor.

Close to the entrance of Santuário cave the rainwater accumulates in temporary epigean ponds and flows through the cave in runoffs or filtered by the soil (Figure 4 A and C). In the runoff clay, trunks, leaves and bovine feces are transported in small amounts.



**Figure 4.** Exo and endokarstic features in two limestone caves in Brazil. Temporary epigean pond near the Santuário cave entrance (A and B), temporary hypogean pond in the Santuário cave (C), Water that percolates through exokarst/epikarst and drips from speleothems (D), groundwater (E) and travertines in the Brega cave (F).

Using Google Images it was possible to infer that in intense flood periods Brega cave can be accessed by the Patos Creek's waters, through its second entrance, thus flooding a large part of its floor (Figure 2).

Much of the cave floor remains flooded during the period of intense rains. After flooding subsides, part of the water is captured by irregularities in the floor, forming discreet pools and small subterranean streams. As a result, there is the formation of lotic sites connected to the groundwater and disconnected lentic sites.

Three zooplanktonic invertebrate groups were found in the two caves (Copepoda, Protozoa and Rotifera) and 3 benthic/nektonic and xenobiotic/accidental taxa (Annelida, Culicidae larvae and Amphipoda: *Hyaella* sp) (Table 2). The highest abundance was represented by Copepoda (2736 ind. adults and larvae), followed by Protozoa (278 ind.), Rotifera (53 ind.), Culicidae larvae (Diptera) (23 ind.) and Amphipoda (*Hyaella* sp.) (15 ind.) (Table 2 and Figure 5).

In Brega cave the highest abundance was represented by Copepoda (2166 ind. adult and larvae), followed by the Protozoa (184 ind.), Rotifera (53 ind.), Culicidae (16 ind.) and *Hyaella* sp. (15 ind.). Those values refer to the total of the three collection points. In this cave annelids, *Hyaella* sp. and Culicidae larvae were only found in the temporary pool originating from water runoff. Rotifera were only found at Collection Point 1, with water also originating from runoffs (Figure 3, Table 2). In relation to the three collection points in Brega cave, the one that presented the highest number of organisms was the groundwater (Point 2, with 1118 individuals), followed by Point 1 (1089 individuals) and later the temporary pool (P3) with (234 individuals). In the three sites, the highest abundance was of Copepoda (Table 2). In the temporary pool a single collection was conducted (4/18/2009) finding 207 copepods, 16 diptera larvae, 15 amphipods and 5 protozoans.

In Santuário cave zooplanktonic organisms were only found at Point 1, closer to the water runoff access (Figure 3). The highest abundance in the Santuário cave was represented by Copepoda (570 ind.) including larval stages and adult individuals, followed by the protozoa (94 ind.) and Annelida (8 ind.).

Eleven fish species were captured in the Santuário cave, belonging to two orders: Characiformes (*Astyanax* sp, *Astyanax bimaculatus*, *Astyanax fasciatus*, *Serrapinus heterodon*, *Cheirodon piaba*, *Cyphocharax gilberi*, *Hyphessobrycon santae*, *Characidium lagsantense*, *Hoplias malabaricus*) and Siluriformes (*Rhandia quelen* and *Hoplosternum littorale*).

In both caves, when the groundwater level lowers, the fish species are trapped, forming small groups of Characiformes, catfish and wolf fish at different sites. In the dry season, many specimens die, others are trapped in the groundwater, and their trajectory in the cave is unknown.

The dead fish in the temporary aquatic environments serve as a food resource for detritivorous fauna in the caves. In Santuário cave an individual of *Endecous* sp. (Ensifera: Phalangopsidae) was observed feeding on a characiform carcass (Figure 6).

In the surroundings of the two caves anthropic alterations like highways, deforestation, soil tillage, dikes, pasturing and livestock (Figure 2) were observed. Inside the caves, alterations such as graffiti were observed at the entrance of Brega cave and motorcycle use inside Brega cave, with silting in both.

#### 4-DISCUSSION

The tendency towards environmental stability in caves (temperature and air humidity), compared to external environments, is well known (Culver 1982, Ferreira



2004). However, differences in the stability values can occur in function of the degree of isolation of different subterranean compartments in relation to the epigeal environment (Ferreira 2004). The general characteristics of number, positioning, distribution and extension of the entrances and their relation to the extension of the caves, are factors that can act in a direct way on the maintenance of the fluctuating atmospheric conditions in the subterranean environments (Ferreira 2004).

However, the effects of the water level fluctuations on community compositions have still not been discussed. In this study special attention is given to the physical characteristics and the micro and mesoorganisms in subterranean lentic environments, places little studied throughout the world (Sket 1999).

The local tropical climate, responsible for the seasonal variation in the water dynamics of Patos Creek, significantly alters the surrounding landscapes. It is during the wet season (October to March) that, according to Menegasse *et al.* (2002), the high water volume in the cave surroundings enables the increase of the surface water level and the groundwater present inside the cave. The sites in the two caves where a highest number of taxa were collected and highest abundances were found were those that receive runoff water.

Many invertebrate groups of the meiofauna (Microturbellaria, Gastrotricha, Rotifera, Nematoda, Oligochaeta, Tardigrada and Microcrustaceans) are important colonizers of interstitial environments, due to size and compatible shape (Schmid-Araya 1997). Furthermore, in general, microarthropod populations can present a transitory behavior in function of the water volume present in different subterranean aquatic compartments (Brancelj 2002; Pipan & Culver 2005).

Culver (2003) emphasizes the importance of the epikarst for the cave colonizing aquatic microfauna. However, in the Santuário cave zooplanktonic organisms associated to the travertine that receives only water that drips from the stalactites were not found. Probably, the deforestation, pasture and livestock anthropic alterations in the surroundings have affected the exokarst microfauna reducing its distribution and consequently its transit to the cave.

During the groundwater elevation period not only micro and meso organisms transit in the temporary bodies of water, but also fish species, probably originating from Patos Creek.

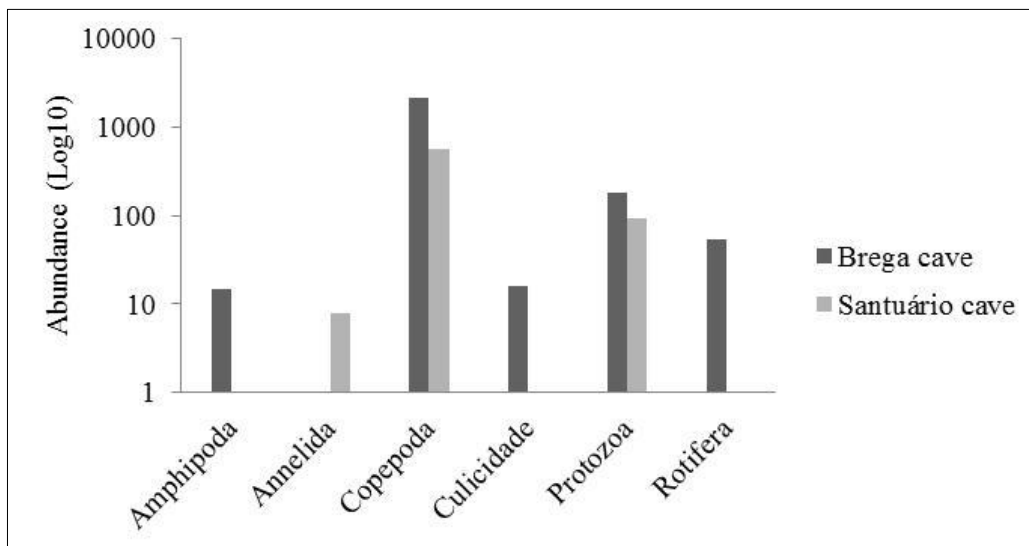
Among the fish species, it was verified that most of them are small in size, a fact that can favor their access to the cave interior. The dominance of Characiformes gives evidence of a transitory condition of such group, since for the world cave ichthyofauna, the predominance of Siluriformes and Cypriniformes is observed (Proudlove 2010). In this case, it evidences the importance of the piabas, as a food resource for the resident species like the wolf fish, as well as for the invertebrate fauna (Figure 6).

Therefore, the transport of macroscopic organic resources, the composition and abundance of the zooplankton and the ichthyofauna in the two caves seem to be strongly influenced by the rain dynamics in the area, allowing some environmental conditions to be highly fluctuating.

According to Souza-Silva and Ferreira (2009), who studied the fauna of intertidal caves, the high tide events and the force of the waves can periodically alter the availability and microhabitat structures in the caves, affecting the establishment of exclusively terrestrial or exclusively marine species. The resident species should be capable of tolerating different physiochemical conditions than those occurring during the high tide. High temperatures and desiccation stress have been considered important determinants for the distribution of organisms in rocky intertidal environments because these fluctuating conditions affect the physiology, competitive dominance and structure of invertebrate communities (Helmuth & Hofmann 2001).

**Table 2.** Abundance of invertebrates associated to different aquatic microhabitats of two limestone caves

	Brega Cave			Santuário Cave			Sum
	Upwelling	Groundwater	Pool	Upwelling	Travertine	Groundwater	
Amphipoda	0	0	15	0	0	0	15
Annelida	0	0	0	8	0	0	8
Copepoda	943	1016	207	570	0	0	2736
Culicidade	0	0	16	0	0	0	16
Protozoa	93	86	5	94	0	0	278
Rotifera	53	0	0	0	0	0	53



**Figure 5.** Total abundance of transitory invertebrates collected in different aquatic environments in the Brega and Santuário caves, Pains, MG, Brazil.



**Figure 6.** Terrestrial Phalangopsidae (*Endecous* sp.) feeding on Characiformes carcass (*Astyanax* sp.) in Santuário cave (Picture: Rodrigo Lopes Ferreira).

## 5-FINAL CONSIDERATIONS

In this study the influences of natural short term fluctuation conditions on the determination of a transitory taxocenosis in cave lentic environments are shown, even if in a preliminary way. The flood events in the two caves, caused by the increase of the groundwater level and runoffs during the rainy season allow fish and zooplankton species, originating from the epigeal environment, to have access and explore inaccessible places of the cave. During the dry seasons those organisms trapped in temporary bodies of water die and can serve as food resources for other terrestrial species.

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