PEDRO RATTON ALVES DE SOUSA

DA SUPERFÍCIE AO SUBTERRÂNEO: UM ESTUDO SOBRE AS INFLUÊNCIAS DO HABITAT FÍSICO NA ESTRUTURA DA COMUNIDADE EM UM HOSTPOT DE BIODIVERSIDADE SUBTERRÂNEA

LAVRAS – MG
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Dissertação apresentada a Universidade Federal de Lavras, como parte das exigências do curso de Pós-graduação em Ecologia Aplicada, área de conhecimento em Ecologia e Conservação de Paisagens Fragmentadas e Agroecossistemas, para obtenção do título de mestre.

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"Hoje entendo bem meu pai. Um homem precisa viajar. Precisa viajar por si, com seus olhos e pés, para entender o que é seu. Para um dia plantar as suas próprias árvores e dar-lhes valor. Conhecer o frio para desfrutar do calor. E o oposto. Sentir a distância e o desabrigo para estar bem sob o próprio teto. Um homem precisa viajar para lugares que não conhece para quebrar essa arrogância que nos faz ver o mundo como imaginamos, e não simplesmente como é ou pode ser; que nos faz professores e doutores do que não vimos, quando deveríamos ser alunos, e simplesmente ir ver".

Amyr Klink

RESUMO

O Brasil é o país com a maior concentração de rochas carbonáticas da América do Sul, estudos estimam que as áreas cársticas brasileiras abranjam aproximadamente 200 mil Km². Apesar de o país apresentar mais de 11 mil cavidades naturais cadastradas, estudos mais aprofundados sobre a fauna subterrânea são extremamente incipientes e fragmentados. Dessa forma, com essa dissertação, objetivou-se amostrar a fauna da Gruta do Éden bem como discutir sua importância em uma região considerada como um *Hospot* de biodiversidade subterrânea. Além disso, amostramos a ictiofauna e testamos as influências de características do habitat físico na estrutura da comunidade de peixes ao longo de um gradiente longitudinal (superfície-subterrâneo-superfície) em um riacho situado em uma micro bacia cárstica. Finalmente, foi avaliado o efeito de espaços subterrâneos como filtros ambientais. A amostragem foi realizada nas proximidades da cidade de Pains (Minas Gerais) durante as estações de seca e chuva do ano de 2012. Foram registradas 98 espécies de invertebrados, oito espécies de peixe, duas espécies de anfíbio e uma espécie de morcego no interior da Gruta do Éden. Dentre os invertebrados coletados, 13 compreendem espécies adaptadas ao ambiente cavernícola (troglóbios), esse valor corresponde a 15% do total de troglóbios para a região. A fauna de invertebrados se distribui de maneira desigual no interior da caverna. O conduto inferior possui valores de abundância e riqueza mais elevados quando comparado ao conduto superior. A amostragem de peixes e caracterização do habitat físico, ao longo do gradiente longitudinal do riacho (cinco trechos amostrais), ocorreu durante o período de seca (Julho) de 2012. Foram coletadas 28 espécies de peixe distribuídas em três ordens e nove famílias. Characiformes é o grupo mais diversificado (57% da fauna total) no riacho amostrado, com 16 espécies coletadas. Foram observadas variações significativas na estrutura da comunidade de peixes ao longo do gradiente longitudinal. Dentre as características do habitat físico, profundidade (25.75%) e cobertura de dossel (15.42%), foram as que mais influenciaram na estrutura da comunidade. Sugere-se que o número, tamanho e disposição de espaços subterrâneos ao longo de uma microbacia cárstica possuam influências adicionais na estruturação da comunidade de peixes. Portanto, acreditamos que o ambiente cavernícola, nesse contexto, esteja atuando como um filtro ambiental, selecionando espécies aptas a atravessá-lo e/ou colonizá-lo. A Gruta do Éden representa um desafio para conservação, pois se encontra em situação de extrema vulnerabilidade sob a ação de diversos impactos antrópicos que ameaçam a persistência de sua biodiversidade. Medidas emergenciais devem ser tomadas para que esse patrimônio natural possa continuar a existir.

Palavras-chave: Paisagens cársticas. Hostpots. Ecologia de riachos. Espécies endêmicas. Conservação.

ABSTRACT

Studies indicate that Karst areas cover about 200.000 km^2 of the Brazilian territory and that about 5% of the country's surface area show suitable conditions for cave formation. Besides the elevated number of registered natural cavities (approximately 11.000), subterranean biodiversity studies in Brazil are still quite fragmented and incomplete. Therefore, the purpose of this paper is to characterize the subterranean fauna of the Éden cave and to discuss the role of a single representative cavity in a broader area considered as a tropical *hotspot* of subterranean biodiversity. We also sampled the ichthyofauna and examined the effect of physical habitat characteristics on the composition of the fish assemblages on a longitudinal gradient in a karst stream. We finally analyzed if subterranean habitats have additional effects responsible for shaping the fish community composition and if it is acting as a filter selecting fish groups with particular traits. The field trips occurred during the dry and rainy periods of the year 2012, the samplings took place close to Pains city, Minas Gerais state. We observed 98 invertebrate species, eight fish species, two amphibians and one bat species within the cave habitat. Among these, 13 cave adapted invertebrate species were found. Such numbers represent 15% of the total amount of troglobiotic species known for the entire province. The invertebrate fauna showed distinct richness and abundance values according to cave levels. The lower level features a significantly higher number of organisms when compared to the upper level. The composition of the fish fauna and habitat attributes were investigated in five stream reaches along a longitudinal gradient of a stream during the dry period of the year 2012. We collected 28 fish species distributed in three orders and nine families within the five sampling sites. Characiforms represents the most diverse group in the studied area with 16 registered species (57 % of the sampled fauna). Depth (25.75%) and percentage of channel canopy cover (15.42%) were respectively the variables that account for most of the variation observed in the fish fauna composition. We believe that number, size and position of subterranean spaces within a karstic watershed have additional influences in the fish community composition through its selective pressures upon species traits. In this context we believe, that subterranean spaces may act as environmental filters, selecting species that are able to cross or colonize it. The Éden cave represents a hotspot inserted in a hotspot of subterranean biodiversity. The cave is in a very vulnerable condition and represents a challenge for conservation since it is located in an extremely altered watershed subject to major impacts. This Hotspot of Subterranean diversity is highly endangered and needs immediate attention.

Keywords: Karst landscape. Hostpots. Stream ecology. Endemic species. Conservation

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PRIMEIRA PARTE

REFERENCIAL TEÓRICO

Paisagens Cársticas

O termo “carste” é utilizado para se descrever um tipo singular de paisagem, caracterizada pela presença de afloramenos rochosos, cavernas, sumidouros, surgências, dolinas, vales cegos, abismos e um extenso sistema hidrológico subterrâneo que se desenvolve especialmente sobre rochas de elevada solubilidade. Rochas carbonáticas representam uma das principais litologias suscetíveis à formação de cavernas devido à sua porosidade e alta solubilidade. Os processos hidrológicos e químicos associados ao carste podem ser compreendidos de maneira mais clara sob um ponto de vista sistêmico (FORD & WILLIAMS, 2007).

O carste se comporta como um sistema aberto composto de dois “sub-sistemas” intimamente integrados: o hidrológico e o geoquímico, que atuam em conjunto transformado o relevo cárstico. As características superficiais e subterrâneas do carste são o produto de processos integrados desses dois “sub-sistemas”. Os ciclos hidrológicos determinam a evolução da hidrologia local nas paisagens cársticas. As características marcantes superficiais e subterrâneas dessas paisagens são resultado da ação da água que percorre passagens e condutos, esculpindo com o passar do tempo sua estrutura geológica (FORD & WILLIAMS, 2007). As cavernas são partes ou subunidades desta ampla unidade que apresenta diversos canais de escoamento hídrico (GIBERT *et. al.,* 1994).

Relevos cársticos podem favorecer à formação de aquíferos, formações rochosas com a capacidade de armazenar, conduzir e fornecer água em quantidade significante (FREEZE & CHERRY, 1979; DOMENICO & SCHWARTZ, 1998). Os aquíferos cársticos proporcionam aproximadamente 25% de toda a água utilizada para o abastecimento público no mundo sendo, portanto, de grande importância econômica (GILLIESON, 1996).

Características gerais dos sistemas subterrâneos

As cavernas, quando comparadas ao ambiente externo, apresentam uma marcante restrição na disponibilidade de recursos, em muitos casos sendo classificadas como ambientes oligotróficos (FERREIRA, 2004; CULVER & PIPAN, 2009). A principal causa desta condição é a ausência permanente de luz, uma das características mais marcantes observada nas cavernas. Na zona afótica (permanentemente escura) das cavidades, exclui-se a possibilidade da existência de produtores primários fotossintetizantes (salvo algumas raras exceções), sendo estes, encontrados apenas nas proximidades das entradas ou claraboias, locais que recebem luminosidade direta ou indireta. Portanto, grande parte da produção de energia no ambiente cavernícola é de origem secundária tendo como base uma cadeia de organismos detritívoros, fortemente dependente de recursos importados do ambiente externo. Em determinados casos, 100% da matéria orgânica presente no ambiente subterrâneo, provém do meio externo (HOWARTH, 1983).

O ambiente cavernícola é altamente conectado ao ambiente de superfície por meio da alta permeabilidade do relevo cárstico (HOWARTH, 1983; BOLSTAD & SWANK, 1997; GUNN *et al.*, 2000). Recursos orgânicos adentram as cavernas continuamente ou sazonalmente, transportados por distintos agentes físicos e biológicos (SOUZA-SILVA, 2003; SIMON *et. al.*, 2007). A água, além de ter um papel central na formação e desenvolvimento de cavidades naturais, é considerada importante agente físico de aporte de recursos orgânicos para o meio subterrâneo. A matéria orgânica pode ser carreada pelas águas de rios, riachos, enxurradas e de outros tipos de curso d´água que percolam no teto e paredes fluindo por meio de fendas e fraturas eventualmente formadas no interior de cavidades (GIBERT *et al.*, 1994).

Classificação dos organismos cavernícolas

Grande parte dos organismos que habitam o ambiente cavernícola possui pré- adaptações à vida nesses sistemas. Desta forma, grupos regularmente encontrados em hábitats úmidos e sombreados e que possuem uma dieta generalista são potencialmente mais aptos a colonizarem ambientes subterrâneos. Segundo Holsinger & Culver (1988), a partir de modificações no sistema de classificação de Schiner-Racovitza, os organismos cavernícolas podem ser classificados em três categorias:

1. **Troglóxenos:** representam organismos regularmente encontrados no ambiente subterrâneo, mas que periodicamente se deslocam para o ambiente externo geralmente em busca de alimento e/ou para completar seu ciclo de vida. Em geral, ocorrem nas porções mais próximas às entradas, mas populações, eventualmente, também podem ocorrer em zonas mais profundas. Muitos desses organismos são responsáveis pela importação de recursos alimentares provenientes da superfície, sendo muitas vezes os principais responsáveis pelo fluxo energético em cavernas, com importância acentuada em cavernas permanentemente secas.

2. Troglófilos: representam organismos facultativos no ambiente subterrâneo, capazes de completar todo o seu ciclo de vida no meio subterrâneo de forma independente do meio epígeo (superfície). Na superfície, tanto os troglóxenos quanto os troglófilos geralmente ocorrem em ambientes úmidos e sombreados.

3. Troglóbios: organismos restritos ao ambiente subterrâneo, que geralmente apresentam especializações morfológicas, fisiológicas e comportamentais que evoluíram em resposta às pressões seletivas do meio cavernícola e/ou à ausência de pressões seletivas típicas do ambiente externo. Tais organismos normalmente apresentam redução de estruturas oculares, despigmentação e alongamento de apêndices. Além disso, comumente apresentam distribuição restrita, baixa densidade populacional, baixa tolerância às flutuações ambientais e estratégia reprodutiva do tipo K. Essas características os tornam extremamente vulneráveis às alterações e impactos no seu ambiente (CULVER, 1982). Desta maneira, espécies troglóbias são consideradas como vulneráveis à extinção pela International Union for Conservation of Nature (IUCN).

Embora o sistema "Shinner-Racovitza" compreenda apenas três categorias, é importante ressaltar a existência de uma quarta categoria.. Tais organismos são denominados acidentais sendo representados por espécies que normalmente não são encontradas em cavernas. Os organismos acidentais, em geral, adentram o meio subterrâneo involuntariamente por quedas em entradas verticais e transportados pela água ou vento. Embora esses organismos não sejam considerados como efetivamente cavernícolas, eles possuem grande importância ecológica visto que, suas fezes e cadáveres contribuem de maneira significativa para o aporte energético no meio subterrâneo.

O cenário espeleológico do Brasil e de Minas Gerais

Atualmente, estima-se que o potencial espeleológico brasileiro possa ultrapassar 100.000 cavernas (AULER *et al.*, 2001). Contudo, pouco mais de 11.000 cavernas constam no Cadastro Nacional de Cavidades da Sociedade Brasileira de Espeleologia (SBE) e na base de dados do Centro de Estudos Proteção e Manejo de Cavidades (IBAMA/CECAV). Os principais grupos carbonáticos do país são os grupos Açungui, Una e Bambuí, este representando a maior área cárstica Brasileira. O estado de Minas Gerais possui o número mais elevado de cavernas dentro do território nacional, sendo que a província espeleológica de

Arcos/Pains/Doresópolis, parte do grupo geológico Bambuí, representa atualmente a maior concentração de cavernas do país, com mais de 1.300 cavidades naturais cadastradas (IBAMA/CECAV).

Embora a Província Espeleológica de Arcos, Pains, Doresópolis apresente uma grande concentração de cavidades, grande parte destas possuem dimensões reduzidas (menor que 100 metros). Uma exceção ao padrão local é a gruta do Éden, cadastrada desde 1989, que apresenta desenvolvimento horizontal de aproximadamente 1.931 metros, espeleotemas raros e o maior número de espécies troglomórficas dentre as cavernas amostradas na região sendo que a maior parte destas espécies ainda não foi descrita (ZAMPAULO, 2010).

Impactos ambientais em áreas cársticas

Cavidades inseridas em relevos com predominância de rochas carbonáticas são alvo de intensa exploração econômica de empreendimentos diversos. No município de Pains, empresas de mineração exercem forte pressão sobre esse tipo de litologia em função da retirada e utilização do carbonato de cálcio para a fabricação de cimento e cal (PILÓ, 1999). O calcário possui múltiplas utilidades econômicas, o Centro de Estudos Proteção e Manejo de Cavidades (IBAMA/CECAV) lista 44 aplicações produtivas para o carbonato de cálcio. Além disso, diversas outras atividades antrópicas como monocultura, criação de gado, criação de suínos, expansão urbana, dentre outros, tem transformado a paisagem local, representando impactos adicionais ao ecossistema. Os ecossistemas aquáticos também têm sofrido alterações significativas devido a múltiplos impactos ambientais decorrentes de atividades antrópicas. Diversos rios, riachos, lagos e até mesmo aquíferos têm sido fortemente impactados em diversas partes do globo devido ao crescimento desordenado de atividades humanas (MCALLISTER *et al*., 1997).

A relação intrínseca entre as feições cársticas superficiais e subterrâneas propicia uma maior suscetibilidade a impactos ambientais. Alterações antrópicas em paisagens cársticas podem atingir com grande velocidade áreas distantes como bacias hidrográficas adjacentes. A perda de cobertura vegetal pode acarretar alterações no fluxo hídrico, na evapotranspiração, na sedimentação, na morfologia do canal, na radiação solar, na temperatura e no "input" de matéria orgânica, gerando mudanças na estrutura de comunidades que habitam tanto as cavernas quanto a superfície (BURTON & LIKENS, 1973; POFF *et. al.*, 1997; RUTHERFORD *et al.*, 1997; SPONSELLER *et al.*, 2001; WOOD *et al.*, 2008).

O uso e ocupação do solo pelo homem tem uma relação direta com a estruturação da fauna cavernícola (aquática e terrestre) tendo em vista que esses organismos dependem primariamente de recursos provenientes do meio externo (SPONSELLER *et al.*, 2001). A água, nesse contexto, pode agir não só como agente importador de recursos para as cavernas, mas também como fator que influencia negativamente esses ambientes, sendo disseminadora de contaminantes. Esses efeitos negativos podem causar alterações nas características físico-químicas da água bem como impactar a biodiversidade associada ao ecossistema cavernícola.

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SEGUNDA PARTE

Artigo 1

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The Éden cave: a biodiversity hotspot within a highly threatened speleological province, southeastern Brazil

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ABSTRACT

The carbonatic speleological province of Arcos-Pains-Doresópolis (Minas Gerais state, Brazil) is considered a hotspot of subterranean biodiversity. It comprises more than 1300 registered caves, from which approximately 80 troglobitic species are known. The Éden cave is the largest in the province with unique geological and biological conditions compared to other caves in the area. The aim of this study was to make a complete assessment of the cave biodiversity at the dry and rainy periods of 2012 and highlight the urgency for conservation actions. We recorded 98 invertebrate species in the cave belonging to at least 44 families found in aquatic, terrestrial and epikarst cave compartments. Among these, 13 troglomorphic invertebrate species were recorded. This number represents 15% of the total amount of cave adapted species known for the entire province. The invertebrate fauna showed distinct richness and abundance values according to cave levels. The lower level features a significantly higher number of species when compared to the upper level. The Éden cave represents a biodiversity hotspot inserted in a highly threatened karst area. The cave is in a very vulnerable condition and represents a challenge for conservation since it is located in an extremely altered watershed subject to major impacts. Emergency actions must be taken to ensure the cave´s protection along with its surroundings.

KEY WORDS: Karst; Tropical habitat; Endemic species; Troglobites; Conservation.

INTRODUCTION

Biodiversity loss and species extinction far exceed conservation resources around the globe and the perspective is getting increasingly worse (Wilson 1992; Ehrlich 1994; Myers *et al.* 2000). It has been imperative to identify priorities to conservation in order to protect endangered ecosystems with a significant number of unique species. The delimitation of biodiversity *Hotspots,* which includes areas with outstanding concentration of endemic species subject to exceptional habitat loss, has shown to be an important strategy (Myers *et al.* 2000). Karst systems are notably vulnerable and fragile to anthropogenic impacts compared to most other natural ecosystems. The efficient surface drainage down to an array of subterranean conduits transmits the surface pollution in an incredible speed (Ford & Williams 2007). Human activities undertaken upstream of karst terrains are liable to lead to major environmental degradation (Ford & Williams 2007; Van-Beynen 2012). The rising concern with global biodiversity contributed to produce relevant information concerning species diversity patterns including also, different groups of cave organisms (Culver & Sket 2000).

Extensive studies on subterranean biodiversity are unevenly distributed with most part of the data from northern temperate karst areas. Culver and Sket (2000) working with patterns of subterranean biodiversity introduced the concept of *Hotspots of subterranean biodiversity*. They indentified 20 subterranean systems (18 caves and two wells) that matched their criteria (20 or more obligate subterranean species per site). These systems are mainly distributed in temperate regions (five in Slovenia, five in France, three in North

America, one in Australia, one in South East Asia and one in an Atlantic island) (Culver & Sket 2000).

Tropical subterranean habitats are not as extensively studied as temperate ones. During the last three decades, however, biodiversity data from caves located in tropical regions started to become available (Chapman 1980; Humphreys 1993; Gnaspini & Trajano 1994; Deharveng & Bedos 2000; Sharratt *et al.* 2000; Trajano 2000; Deharveng 2005; Prous & Ferreira 2009; Zampaulo 2010; Souza-Silva *et al.* 2011). Initially, most studies on the Brazilian cave fauna generated species lists which are particularly difficult to be compared, as most taxa were not identified to species level (Dessen *et al*. 1980; Chaimowicz 1984, 1986; Godoy 1986; Pinto-Da-rocha 1995; Ferreira & Horta 2001). Ferreira (2005) suggested that inventories of Brazilian cave fauna have been seriously incomplete. Recent research in previously unstudied areas are revealing dozens of new subterranean taxa, some of them described only in the last three years (Volkmer-Ribeiro *et al.* 2010; Souza & Ferreira 2010, 2011a, b, 2012; Cardoso *et al.* 2011; Hernandes *et al.* 2011; Machado *et al.* 2011; Pellegrini & Ferreira 2011a, b; Rizzato *et al.* 2011; Bernardi *et al.* 2012; Brescovit *et al.* 2012; Dantas-Torres *et al.* 2012; Prevorcnik *et al.* 2012; Iniesta *et al.* 2012; Rodrigues *et al.* 2012; Hoch & Ferreira 2012, 2013; Ázara & Ferreira 2013; Iniesta & Ferreira, 2013a, b; Fiser *et al.* 2013).

Studies on tropical patterns of subterranean biodiversity have revealed the existence of a rich troglobiotic community and therefore many tropical hotspots of subterranean biodiversity could also be delimited (Deharveng 2005). At this date there are approximately 12.000 officially registered caves in Brazil (Cecav 2014), although some estimates reveal that the real number is probably close to

100.000 (Auler *et al.* 2001). Studies indicate that karst areas cover about 200.000 km^2 of the national territory and that about 5% of the country's surface area show suitable conditions for cave formation (Auler *et al.* 2001). Subterranean biodiversity studies in Brazil are still quite fragmented with great part of the sampling effort concentrated in particular regions of the country. These areas deserve special attention due to their biodiversity and number of cave adapted species: Alto do Ribeira State Park, Intervales State Park, Peruaçu National Park, Serra da Bodoquena National Park, Arcos-Pains-Doresópolis speleological province and scattered karst areas located at the northeast part of the country (Pinto-da-Rocha 1993; Trajano 2000; Ferreira & Horta 2001; Ferreira 2003; Ferreira 2006; Trajano 2007; Medeiros 2008; Zampaulo 2010; Bento 2011; Souza 2012).

Subterranean habitats are notably detached with distinct species occurring in caves separated only by a few kilometers. In many cases the number of troglobites listed in a single cave is not incredibly high (less than 20 species) but when the entire region is considered, this value can increase significantly (Culver & Sket 2000). In fact, great part of the subterranean biodiversity described takes into account the sum of different species from adjacent sites. On the other hand, it is important not to neglect individual sites (e.g. single cave systems) as some of them show outstanding biodiversity and singularities. The protection of subterranean habitats and karst landscapes may start with the protection of a single cave, which can constitute an important step for preserving larger karst areas (Culver & Sket 2000).

The identification of key areas as well as very important caves regarding subterranean biodiversity in Brazil is essential to assure the protection of a variety of endemic and threatened species. Therewith, the purpose of this paper is to characterize the subterranean fauna of the Éden cave and to discuss the role of a single representative cavity in a broader area considered as a tropical *hotspot* of subterranean biodiversity.

MATERIAL AND METHODS

Study site

The field work was performed in the Éden cave (UTM 2023083/45 40018). The cave is inserted in limestone outcrops of the Bambui geological group and is located near the city of Pains, Minas Gerais state, Brazil (Fig. 1A). This area is surrounded by an important speleological province (Arcos-Pains-Doresópolis) with more than 1300 catalogued caves. This number represents approximately 32% of all known caves in the state (Cecav 2014). The region is characterized by hot and humid summers and dry winters with temperatures ranging from 23.3ºC in January (hottest) to 16.3ºC in July (coldest) with an annual average temperature of 20.7ºC and local annual average precipitation of 1344 mm³ (Menegasse *et al.* 2002). Cerrado vegetation predominates and features a natural gradient: from grasslands to arboreal vegetation. Over the karst landforms, the vegetation is characterized as “Montana Deciduous Forests” or “Dry forest” (Velloso *et al.* 1991).

The Éden cave is the largest known cave in this region with approximately 1.931 meters of linear length (Fig. 1C). The cave comprises a wide dissolution gallery, conditioned by North/South lineaments with linear morphology. Its evolution consists of vadose levels: an upper dry level, a lower level with a stream running through it; and a phreatic level where groundwater emerges in all its extension (Cadamuro 2007). The cave has only one natural entrance, a small skylight which gives access to the lower level and an artificial opening resulted from past mining activities which is now closed by an iron gate.

The cave was classified as a “pecilotrophic” environment due to its considerable size and the noticeable shifts in the amount of organic resources available in different cave compartments. However, great part of the cavity is considered “oligotrophic” (upper level) showing a striking absence of organic matter on both seasons (dry-rainy). At the upper level, during the rainy period, we observed a significant amount of water dripping from the ceiling and speleothems. We believe this might be the most important means of organic matter input in this cave compartment. The cave’s lower level, receives a considerable amount of organic matter carried by the stream such as leaves, wood debris and carcasses. The skylight located at a small separate chamber in this level also contributes to the organic matter input with the falling of leaves and branches, although this contribution is restricted to the area near this entrance.

The cavity´s surroundings are highly modified by anthropogenic activities such as agriculture, pastures, urban expansion and past mining activities. The cave is located within a mining area adjacent to the city of Pains (Fig. 1B). A stream reaches the cave’s lower level through an upwelling and flows for approximately 700 meters until it reaches a sinkhole. Before entering the cave, the stream runs through an impacted watershed subject to different types of human activities. The watershed’s natural Cerrado vegetation has been long removed. Pastures, agriculture and domestic sewage are among some of the most damaging activities affecting this water course. At the same time, the karst drainages that intersect the area are considered strategic since all the water utilized by the population of the city of Pains is collected from a single spring interconnected to the local karst system including the Éden cave (Cadamuro 2007; Freitas 2009).

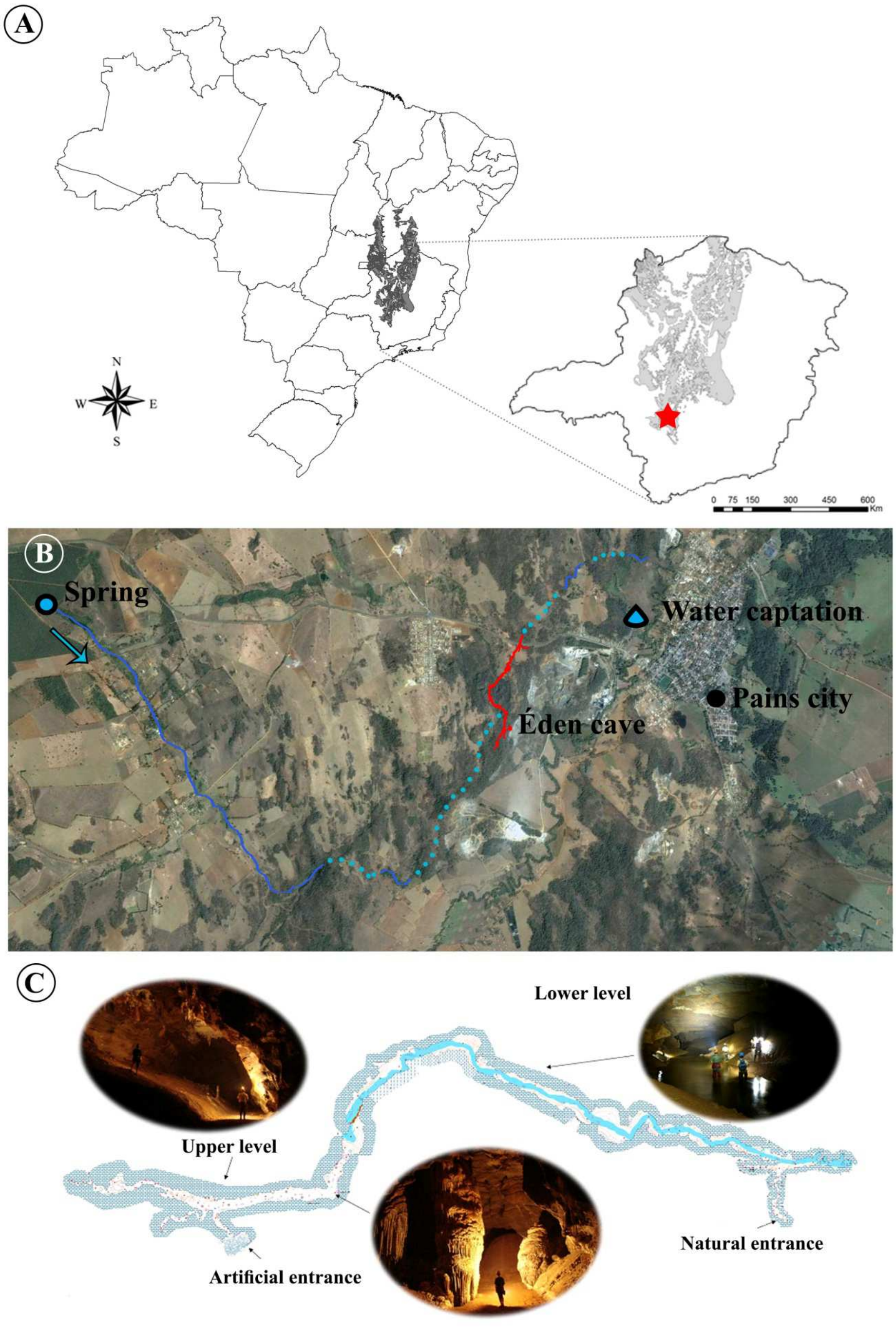

Fig. 1: (A) Brazilian map with the Bambui geological group in gray (Zoom: Minas Gerais State, red star indicates the city of Pains. (B) The sattelite image shows the Éden cave´s watershed. The river is highlighted in blue (subterranean stretches are represented by the dotted line). (C) Éden cave´s floor plan.

SAMPLING

Terrestrial invertebrates

We surveyed the cave’s invertebrate fauna in the rainy (January) and dry (July) periods of 2012. A rigorous search was carried out at the cave floor and walls with special attention to potential biotopes. The invertebrates were captured with the aid of fine brushes and tweezers and kept in 70% ethanol vials (Sharrat *et al.* 2000; Ferreira 2004; Souza-Silva *et al.* 2011). Previously known species had their abundance estimated by visual counts in field, avoiding unnecessary sampling and their position plotted in the cave´s floor plan (according to the methods proposed by Ferreira 2004). It is important to highlight that pitfall traps were not installed in the Éden cave, this method is considered to have low efficiency when applied to most tropical caves and can represent a threat to fragile troglobiotic populations (Weinstein & Slaney 1995; Sharrat *et al.* 2000).

All collected specimens were later identified under a stereo microscope and separated into “morphospecies” with the aid of taxonomic keys and were lodged in the collection of invertebrates of the Subterranean Biology Study Center, Biology department, Zoology Section, Lavras Federal University (ISLA) , Minas Gerais State, Brazil.

Epikarstic invertebrates

We installed “epikarstic” traps underneath ceiling and speleothem drips in order to sample the invertebrates that inhabit the cave “epikarst”. The traps consisted of 500ml plastic vials and one bucket in which we opened horizontal slits and attached a fine mash in order to drain the water and retain the organisms. The traps remained in the cave (upper level only) for a period of three months. The water retained inside the traps, was filtered with a zooplankton net, stained with rose bengal and stored in 70% ethanol plastic vials. We also surveyed all water filled pools and travertines for aquatic fauna.

Aquatic invertebrates

We sampled the benthic fauna during the dry period of 2012 in triplicates using surber nets (bottom area = 300mm x 300mm; 250µm mesh size). The cave´s lower conduit becomes flooded during strong rains, very common in the rainy period. Thus, working on this area during the rainy period can be a dangerous task, and this is why we opted to sample the benthic fauna only during the dry period. We selected three sampling transects equally distributed along the cave stream (upstream-midstream-downstream). Three samples were taken of each transect. Samples were preserved in plastic vials containing 70% ethanol and taken to the laboratory for identification. The invertebrate fauna was sorted in the laboratory under a stereomicroscope and identified to the lowest possible taxonomic level.

Ichthyofauna sampling

We sampled the cave´s fish fauna during the dry period only. We utilized kicking nets (4x2m 5mm mash size) and seines (80cm diameter; 1mm mash size) to capture the fish in an upstream direction. All specimens were fixed in 10% formalin and latter preserved in 70% ethanol. The specimens were identified and lodged at the Laboratory of fish Ecology, Ecology Department of Lavras Federal University, Minas Gerais State, Brazil.

RESULTS

We sampled a total of 98 invertebrate species in the Éden cave belonging to 44 families (Appendix. 1; Fig. 2-3). We also recorded two species of Anura: Hylidae (*Scinax fuscovarius*), Leptodactylidae: (*Leptodactylus* sp) and one bat species: Phyllostomidae (*Carollia perspicillata*).

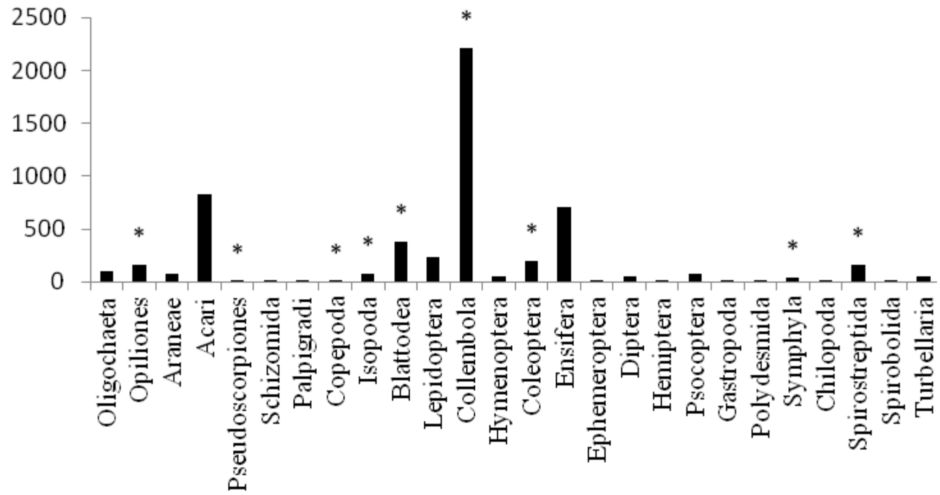

Fig. 2: The Éden cave´s total invertebrate abundance. The (*) represents taxons with troglobiotic species.

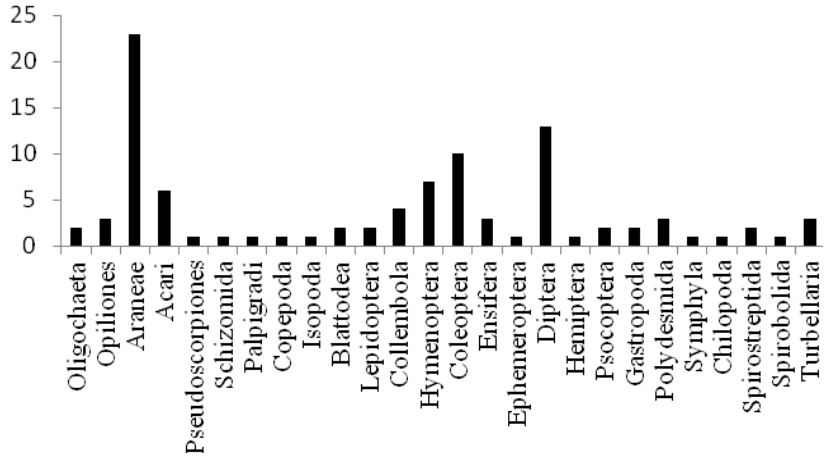

Fig. 3: The Éden Cave´s total invertebrate richness.

During the rainy period, we found a total of 66 invertebrate species, 50 species at the inferior level and 30 at the superior level. During the dry period, a total of 77 species were found, 64 at the inferior level and 25 at the superior level (Appendix. 1). The invertebrate fauna showed distinct richness and abundance values according to cave levels with the lower level featuring a higher number of species when compared to the upper level (Fig. 4).

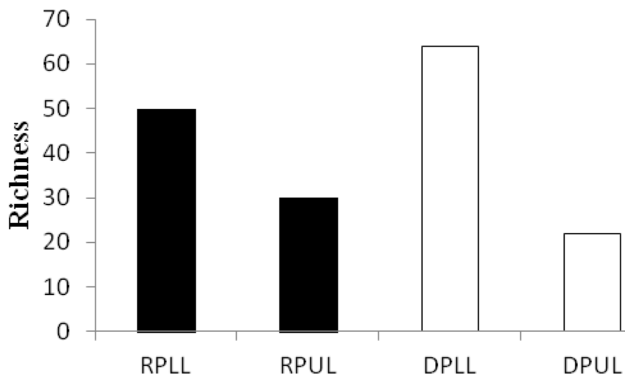

Fig. 4: Species richness according to season and cave levels: Rainy period lower level (RPLL), Rainy period upper level (RPUL), Dry period lower level (DPLL) and Dry period upper level (DPUL).

Among all sampled species, we found 13 invertebrate species with troglomorphic traits (Fig. 5): One species of Opiliones (Gonileptidae *Liops* sp.), one Pseudoscorpiones (Chthoniidae sp.), two Isopods (Styloniscidae sp.), one Blattodea, one Collembola (Arrhopalitidae *Arrhopalites* sp.), three Coleoptera (three species of Carabidae), one Symphyla, one Spirostreptida (Pseudonannolenidae *Pseudonannolene ambuatinga*, Iniesta and Ferreira 2013), one Sphaerodesmidae (Polydesmida) and one Copepoda (Harpacticoida) collected from epikarstic waters. Furthermore, it shelters a variety of phylogenetically distant obligate subterranean species that, in some cases, can present very large populations: *Arrhopalites* sp02 (1,306 ind); Blattodea sp (373 ind); *Pseudonannolene ambuatinga* (159 ind); *Lyops* sp (110 ind) (Fig. 2).

The subterranean stream features a very low aquatic invertebrate diversity. We collected a total of 10 species of aquatic invertebrates in the cave stream: four species of Gastropoda, two species of Diptera (Chironomidae and Stratiomyidae), one species of Trichoptera (Hydropsychidae), one species of Oligochaeta, one species of Odonata (Ghomphidae) and one species of Coleoptera (Scirtidae). The following fish species were also recorded in the cave: *Astyanax lacustris*, *Astyanax fasciatus*, *Hemigrammus marginatus, Imparfinis minutus, Pimelodella lateristriga, Pseudopimelodus charus*, *Hoplias malabaricus* and *Hypostomus lima*.

Although we did not measure the environmental impacts in the Éden cave we could visually identify some damage caused by human activities. At the upper level´s floor, there are collapsed speleothems along with big rock plates due to the past use of explosives. The subterranean stream conditions are worrying; we noticed a great amount of silt indicating intense sedimentation through its course.

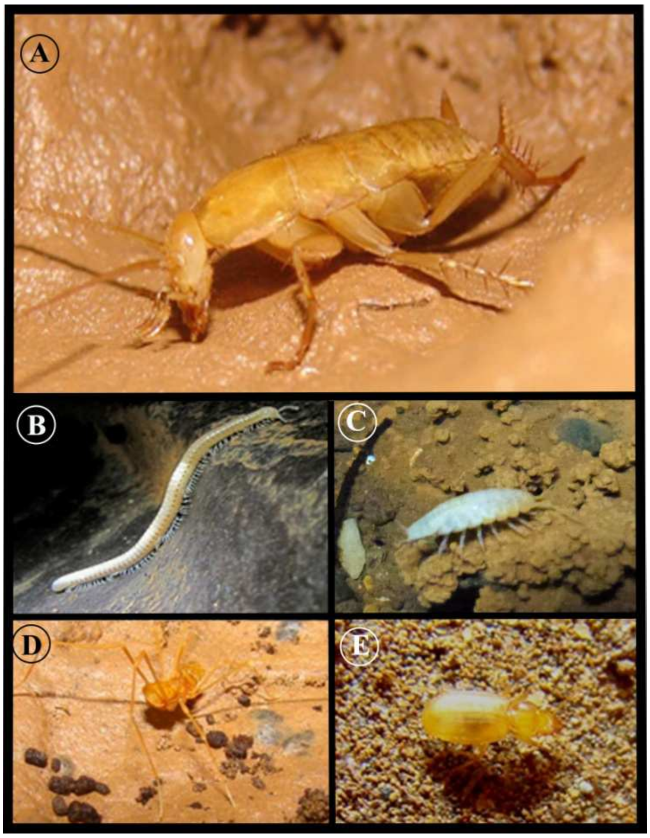

Fig. 5. Cave Troglobites: (A) Blattodea, (B) Spirostreptida (*Pseudonannolene ambuatinga*), (C) Isopoda (Styloniscidae), (D) Opiliones (*Lyops* sp) and (E) Coleoptera (Carabidae).

DISCUSSION

Besides the size, the Éden cave stands out from the entire karst area (Arcos-Pains-Doresópolis) in regard to the invertebrate richness and to the number and phylogenetic diversity of troglobites. From the 13 cave-dwelling species listed in this study, 12 are undescribed species and only one is formally described in the literature (*Pseudonannolene ambuatinga* – Iniesta & Ferreira 2013). Any information concerning the biology and ecology of these species is yet inexistent. This fact reflects the incipient knowledge on the tropical subterranean fauna and also the need for more studies and experts. The Brazilian territory possess large karts areas and great cave potential (Auler *et al.* 2001), however reduced governmental support and funding holds back speleological research and limits the volume of available information (Figueiredo 2009). Troglobites are considered to be very fragile due to its low density and its populations are commonly known for only a handful of specimens (Culver & Pipan 2009). The large number of individuals found for some of the obligate subterranean species in the Éden cave represents a rare event in nature (Culver & Pipan 2009). In this context, the Éden cave may be utilized as a natural laboratory, fostering a variety of future biological studies.

It is well established that food availability is an important selective force in cave ecosystems. The quality and distribution of resources within the cave habitat are important factors that influence the composition, abundance and spatial organization of the cave fauna (Decou & Decou 1964; Culver 1982; Culver & Pipan 2009; Ferreira & Martins 2009). Humphreys (1991) found that water and organic matter combined have a positive effect on the number of organisms in cave patches. Finally, seasonal events related to stochastic flow pulses are responsible to change the amount of available organic resources though time in the subterranean environment which may also have severe implications on the composition and distribution of the cave fauna (Souza-Silva *et al.* 2011).

The resource distribution within the Éden cave is patchy as great part of the upper level features very low to inexistent organic resources. The lower level shows the opposite scenario with a considerable amount of organic matter carried by the stream. It is not surprising that the cave invertebrate community would respond to this energetic availability difference. The distribution of cave invertebrates in the Éden limestone cave is highly influenced by this feature resulting in disproportionately greater richness and abundance values for the inferior level. It is interesting to highlight that this discrepancy between cave levels becomes even more pronounced during the dry period. At this time of the year the species richness decreases at the superior level and increases at the inferior. We believe that shifts in the amount of available organic matter within cave levels are causing the observed community variation. During the dry period the amount of dripping and percolating water was severely reduced at the upper level (personal observation). The reduction on water volume may have led to a decrease in the available dissolved organic matter in this cave compartment during part of the year which in turn possibly resulted in a reduction in the number of organisms. The increase in invertebrate richness in the inferior level may result from a higher detritus retention rate during the dry season thus providing larger amounts of organic resources for the community to explore. Further studies based on experimental approaches are needed to fully elucidate these questions.

Although the presence of a subterranean stream in the Éden cave has severe implications determining the structure of the cave community through its positive effects (organic matter input) on the terrestrial fauna, it is interesting to notice that its fish fauna and aquatic invertebrate community have extremely low diversity. This fact may be attributed to several reasons and the causes of the observed low diversity patterns remain unclear and demand further research. Information on the aquatic fauna is yet unavailable for the study region, past cave surveys were aimed at terrestrial invertebrates only (Zampaulo 2010) thus making it impossible for comparisons to be made. However, we noticed a high quantity of silt in the stream channel causing a sedimentation gradient in a downstream direction. The homogeneous muddy substrate found in the stream bed of the Éden cave might have negative effects on the aquatic community through the loss of habitat heterogeneity and complexity (Allan & Castillo 2007).

The Arcos-Pains-Doresópolis karst area represents the highest cave concentration in Brazil being characterized by the presence of small cavities (mean linear projection of 102.7 m) (Zampaulo 2010). It is also the most studied region within the country regarding the number of sampled caves (296 caves). Most part of this area has been subject to a severe fragmentation of its original vegetation due to decades of intense logging and agricultural exploitation. The remaining forest formations are restricted to the margins of São Miguel River and to the limestone rocky outcrops due to their particular morphological conditions which make these areas unsuitable for agricultural use. At the other hand, they receive considerable threat posed by the advance of mining activities (Teixeira & Dias 2003; Henriques-Junior 2006). During the 60´s several mining companies and calcination industries were settled in this region and became the main economical activities. The carbonate rocks have been overexploited since then, thereby causing irreversible damage to the karst landscapes as well as to natural cavities (Cherem & Magalhães-Junior 2007). At this date, this region represents one of the major conflicts between biological conservation and mining activities in Brazil.

This speleological province is remarkable for its invertebrate diversity (1.574 species) including approximately 80 species with troglomorphic traits and thus is considered a Hotspot of subterranean biodiversity (Zampaulo 2010). The total species richness (98) found in the Éden cave is very high when compared to the mean richness of the 296 surveyed caves in the region (35 ± 19.1 species/ cave) (Zampaulo 2010). This feature probably relates to the cave's dimension as it is the largest known cave for the region. The high richness values suggest an elevated ecological significance with a greater number of complex ecological interactions (Ferreira 2004). The Éden cave, when compared to the other 296 surveyed caves in the region, shows a singular and distinct invertebrate community featuring many rare species and the highest number of troglobites. In this sense, the Éden cave represents a Hotspot within a Hotspot of subterranean biodiversity (Arcos-Pains-Doresópolis) that needs immediate conservation actions.

The land use on the cave surroundings poses serious threats to the subterranean biodiversity as the Éden cave is situated in an extremely vulnerable area considered as a priority to conservation (Zampaulo 2010). The serious impacts posed on this fragile subterranean system have become a matter of public health care. The water pollution along with the removal of the native vegetation in the cave`s watershed, are liable to cause major water quality problems (Allan & Castillo 2007). This scenario can become very dangerous since studies utilizing fluorescent dye (Rhodamine Wt) revealed that the water utilized by the population of Pains is collected from a spring (S.A.A.E) that receives considerable amount of water from the karst system in which the Éden cave is located (Freitas 2009).

In the year 2009, an attempt to protect the cave and its surroundings was made through the proposition of a conservation unit: "Gardens of Eden Natural Monument" (Decreto Municipal 40/2009). At this time there was no information on the cave fauna yet, but its known singular dimensions and speleothems were enough to support the proposition. Unfortunately, this attempt failed and the conservation unit was never created. Thereby we strongly advise taking emergency actions in this situation, the Éden cave and its watershed must be urgently protected. Government authorities must reconsider creating a conservation unit to ensure the cave´s protection (as its surroundings).

Further biological and environmental information are needed to accurately delimitate the landscape area for the conservation unit to be created and to maximize the protection of natural resources. Therefore, the delimited area proposed in the 2009 official document must be reviewed. Studies on the aquatic habitat biotic integrity should be performed on the major streams that reach the Éden cave and provide water supply to the population of Pains. The ongoing activities responsible of causing impacts on river ecosystems must be identified and stopped, and habitat restoration measures must be taken.

The above proposed actions should represent the first step towards a broader environmental protection program focused on strategic karst areas in the Arcos-Pains-Doresópolis speleological province. This Hotspot of Subterranean diversity is highly endangered and needs immediate attention.

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Apêndice A - Species sampled in the Éden cave: LL/UL (lower/upper levels), S/A (Richness/Abundance).

Order	Taxons	Rainy Period				Dry Period			
		LL		UL		LL		L L	
		S	A	S	A	S	A	S	A
Oligochaeta	NI	2	39			2	56		
Opiliones	Gonyleptidae	3	52	1	1	3	103	1	8
	(Liops sp, M. taquara, E. astatus)								
Araneae	Ctenidae (*Ctenus*)	1	10	1	6	1		1	
	Ctenidae (*Isoctenus*)			1	1	1	2	1	4
	Ctenidae (*Enoploctenus*)							1	1
	NI	2	4	1	1	5	6		
	Theraphosidae					1	1		
	Clubionidae					1	1		
	Theridiidae	1	2	1	3	1	1	1	1
	Theridiidae (*Theridion*)			1	1				
	Uloboridae			1	1			1	1
	Symphytognathidae (*Anapistula*)					1	1		
	Oonopidae	1	2			2	2		
	Nemesiidae	1	2						
	Theridiosomatidae (*Plato*)	1	1						
	Pholcidae (*Mesabolivar*)	1	10			1	11		
Acari	Veigaiidae (*Veigaia*)	1	301			1	508		
	Eviphididae					1	3		
	Neotenogyniidae (*Neotenogynum*)	1	3			1	1		
	Podocinidae (*Podocinum*)	1	1						
	Rhagidiidae	2	2			1	4		
Pseudoscorpiones	Chthoniidae			1	2	1	3	1	2
Schizomida	NI			1	1				
Palpigradi	Eukoeneniidae	1	2	1	2	1	1		
	(*Eukoenenia florenciae*)								
Copepoda	Harpacticoida			1	4				
Isopoda	Styloniscidae			2	63			2	15
Blattodea	NI	1	184	2	8	1	176	1	9
Lepidoptera	Noctuidae (*Hypoena*)	1	117	1	104	1	1	1	8
	Tineidae			1	1				
Collembola	Entomobryidae	2	18	1	67	2	457	2	36
	Arrhopalitidae (*Arrhopalites*)	1	1195	1	90	2	337	1	10

Continuação

Hymenoptera	Eupelmidae							1	1
	NI			1	1				
	Formicidae	1	31	1	2	2	15		
	Formicidae (*Attini, Odontomachus*)	1	1			2	2		
Coleoptera	Pselaphidae	1	23			1	29		
	Staphylinidae					2	2		
	Carabidae	4	5	2	4	4	129		
	Larvae	1	2			2	2		
	Coleoptera larvae (*Coarazuphium*)			1	1				
Ensifera	Phalangopsidae (*Eidmanacris, Endecous*)	1	251	1	99	2	298	1	59
	Phalangopsidae	1	1	1	2	1	1		
Ephemeroptera	NI	1	2			1	2		
Diptera	Mycetophilidae					2	2	1	1
	Dolichopodidae			1	2			1	1
	Brachycera			1	1				
	Chloropidae			1	1				
	Sciaridae					2	2		
	Chironomidae					1	1		
	Chaoboridae	1	1			1	1		
	Phoridae	1	1						
	Psychodidae (*Lutzomia*)	1	3						
	Phoridae (*Conicera*)	1	1			1	31	1	6
Hemiptera	Cydnidae			1	3			1	8
Psocoptera	Psyllipsocidae	1	1	1	49			1	23
	Ptiloneuridae							1	4
Gastropoda	Valloniidae					1	18		
	NI	1	1						
Polydesmida	Sphaerodesmidae	1	1						
	Paradoxosomatidae					1	2		
	Chelodesmidae					1	1		
Symphyla	NI	1	12			1	31		
Chilopoda	Lithobiomorpha	1	1						
Spirostreptida	Pseudonannolenidae (*Pseudonannolene ambuatinga*)	1	43	1	4	1	106	1	6
	Pseudonannolenidae (*Pseudonannolene saguassu*)	1	2			1	1		
Spirobolida	NI	1	8			1	1		

Continuação

Turbellaria	Geoplanidae (*Geoplana*)			1	2
	Bipalidae	1	15	1	29
	NI	1	1	1	1

Artigo II

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Cave ecosystem as an ecological filter along a tropical stream

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Abstract

The composition of the fish fauna and habitat attributes were investigated in five sampling stations along a longitudinal gradient of a stream located in a karst landscape. We examined surface and subterranean sites in order to compare the fish fauna composition and physical habitat characteristics. We investigated the influence of each physical habitat characteristic on the fish community and analyzed if the subterranean habitats have additional effects responsible for shaping the fish community structure. During the sampling period, we collected 773 individuals representing 28 fish species distributed in three orders and nine families within the five sampling sites. Characiforms represent the most diverse group in the studied area with 16 registered species (57 % of the sampled fauna). We did not find cave adapted fish species during this study although four non-troglomorphic species were sampled exclusively in the Éden cave. Contrasting physical differences were identified between sampled sites and among superficial and subterranean sites. The species composition responded to these environmental differences showing distinct patterns between sites. Depth (25.75%) and percentage of channel canopy cover (15.42%) were respectively the variables that account for most of the variation observed in the fish fauna composition. In the studied karst watershed, Characiforms have higher probabilities of entering cave systems however, Siluriforms have greater chances of transposing and colonizing subterranean habitats. We believe that number, size and position of subterranean spaces

within a karst watershed have additional influences in the fish community composition through its selective pressures upon species traits.

Key words: Caves; karst landscape; stream ecology; community composition; aquatic habitat.

INTRODUCTION

Karst landscapes are unique settings of carbonate rocks subject to dissolution over very long periods of time. They are characterized by the presence of caves, sink holes, dry valleys and disappearing streams. These landscapes are known for its groundwater flow and efficient drainage of surface water through a wide network of subterranean conduits, fractures and caves. The water enters the subsurface through recharge zones and emerges at the surface through discharge zones in the form of springs and seeps (Ford & Williams, 2007).

The fish fauna is a very relevant element of aquatic subterranean habitats comprising top predators in several cave systems (Bichuette & Trajano, 2003). The subterranean fish fauna found in the Brazilian territory is remarkable for its elevated species richness and high evolutionary and ecological diversity. Approximately 25 fish species featuring morphological adaptations to the subterranean life (troglomorphisms) are currently known from different karst areas in Brazil. They occupy a variety of habitats, from epikarst zones to base level streams (Trajano et al., 2010). During the 90’s, a considerable time after the first Brazilian troglomorphic fish (*Pmelodella kronei* – Pavan, 1946) was described, a series of ecological studies focused on Brazilian troglomorphic fishes were conducted (e.g., Trajano, 1991; 1997a, b; Trajano & Bockmann, 1999; Trajano, 2001a,b; Trajano &

Bichuette, 2007; Sampaio, 2012a, b, Bastos et al., 2012). Much of the research effort concerning the subterranean ichytchyofauna aimed at cave adapted species, few studies have encompassed the non-troglomorphic fishes, very common in subterranean habitats (Poly & Boucher, 1996; Poly, 2000; Bichuette & Trajano, 2003; Mattox et al., 2008).

The composition of the subterranean ichythyofauna is highly influenced by historical and ecological factors that may determine the adoption of subterranean life by epigean (surface) species. (Poly & Boucher, 1996; Trajano, 2001). Different habitats (e.g. subterranean) may act as biological filters responsible to control the community composition by excluding unsuccessful strategists from a pool of potential colonists (Scarsbrook & Townsend, 1993). Permanent absence of light and food scarcity represent the most conspicuous ecological pressures posed on the cave fauna. These factors may also hamper the colonization of subterranean habitats by different type of fish (Bichuette & Trajano, 2003; Culver & Pipan, 2009).

The South American cave adapted ichythyofauna is dominated by Siluriforms. Their nocturnal habits and chemo-orientation constitute pre adaptations to the subterranean habitat, thus, making the life history traits of this group of fish preponderant in cave colonization events (Trajano et al., 2010). Neotropical fish can colonize a range of different subterranean habitats with distinct physical characteristics (Trajano et al., 2010). Habitat characteristics have been considered as the primary foundation in which biological communities are organized (Schoener, 1974), many researchers have sustained this generalization for fish communities as well (Gibbons & Gee, 1972; Werner et al., 1977; Schlosser & Toth, 1984). However, no study attempted to test the influence of physical habitat characteristics on the composition of the fish fauna in Brazilian streams draining karst landscapes.

We believe that physical habitat characteristics and the position of subterranean spaces within stream systems have major implications on the distribution and abundance of fish species. This paper examines the effect of physical habitat characteristics on the composition of the fish fauna within five sampling stations distributed on a longitudinal gradient in a karst stream. The spatial variability of the fish fauna was examined and the physical habitat characteristics that most influence this variation were determined. We also analyzed if subterranean habitats have additional effects responsible for shaping the fish community composition and if it is acting as a filter selecting fish groups with particular traits.

MEHTODS

Study Site

The field work was conducted in a third order stream draining a small karst watershed dominated by limestone outcrops and located at the municipality of Pains, Minas Gerais state, Brazil (Latitude: -20.371053; Longitude: -45.663369) (Fig. 1). The surveyed stream is part of the upper Rio São Francisco basin, one of the largest Neotropical drainages with approximately 630.000 km² (Fig. 1) (Sato & Godinho, 1999). The study site is located in an important speleological province (Arcos-Pains-Doresópolis) with the greatest cave density in the country and approximately 32% of all catalogued caves in the state (CECAV data base 2014). This karst area is inserted in the Bambui geological group formed by horizontally bedded limestone from the upper Proterozoic. The temperature ranges from 23.3 ºC in January to 16.3 ºC in July with annual average temperature of 20.7 ºC. The annual average precipitation is 1344 mm^3 (Menegasse et al., 2002). This location features a mild temperate microclimate (hot-humid summers and dry winters). The native vegetation is comprised by the Brazilian savannah like vegetation (Cerrado) depicting a natural gradient: from grasslands to arboreal vegetation. On the karst formations, the vegetation is referred as "Dry forest" or "Montana Deciduous Forests" (Veloso et al., 1991).

The surveyed stream is approximately 7.7 km long and its superficial course is interspersed by subterranean stretches. The stream reaches the Éden cave, the largest cave in the province (1.931 m), through an upwelling where it runs for 700 m in the cave's lower level until it disappears through a sinkhole (Fig. 2B). The stream is located in a permanently dark cave compartment.

This water course runs through an impacted watershed subject to different types of anthropogenic activities and the watershed's native vegetation has been severely altered. Agriculture, pastures, mining and urban expansion are some of the activities negatively affecting water courses in this area (Fig 2A). This scenario can become dangerous since a considerable amount of the fresh water that runs through this landscape and infiltrate in the limestone is withdrawn downstream from a spring interconnected to the local karst system and utilized by the population of Pains (Cadamuro, 2007; Freitas, 2009).

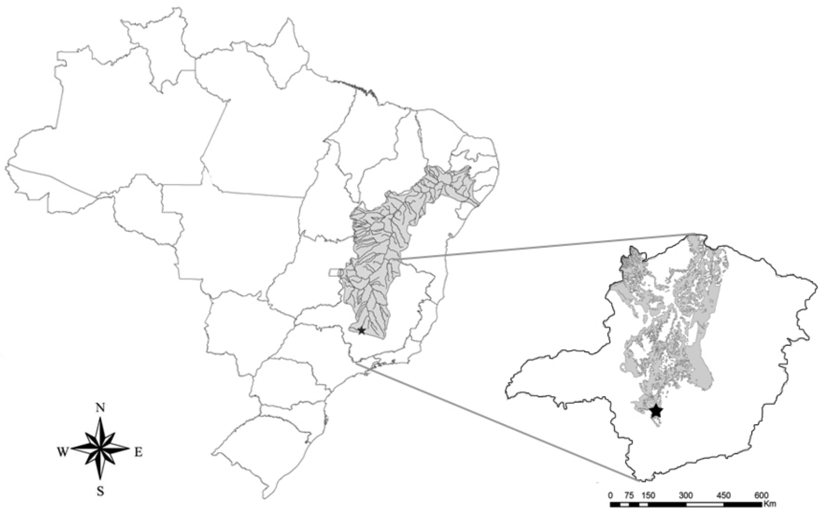

Fig. 1. Map of Brazil (Rio São Francisco basin in gray). Amplification of Minas Gerais state (Bambui geological group shown in gray) and the location of Pains city indicated by the star.

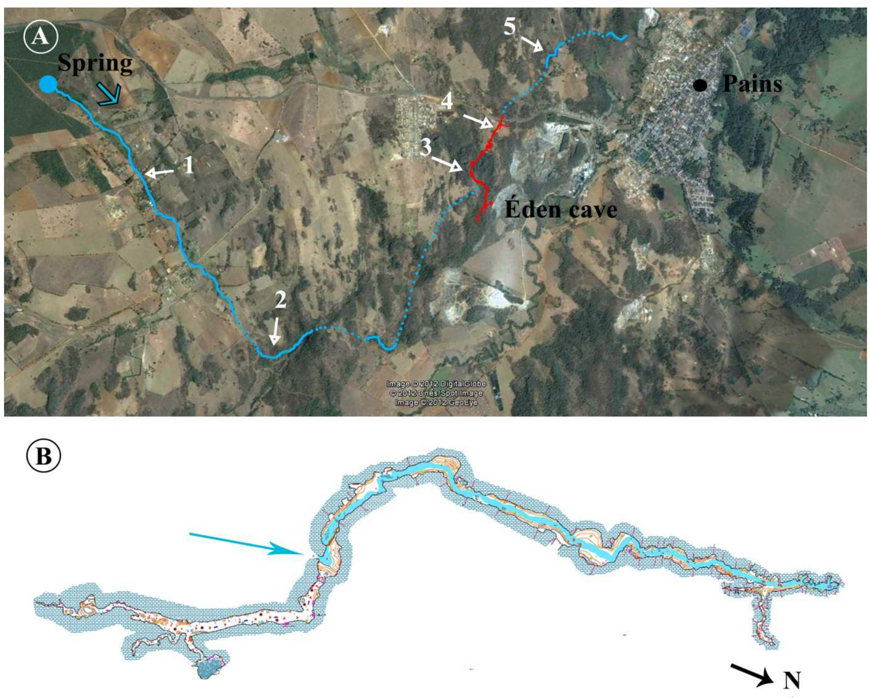

Fig. 2. (A) Satellite image showing the study site: The blue dot represents the spring (arrow indicates flow direction); the stream is marked in blue (continuous lines are surface stretches and dotted lines are subterranean). The white arrows indicate the sampled stream reaches, in red the Éden cave's floor plan. (B) Éden cave´s floor plan, blue arrow indicates the upwelling.

Sampling Design

A four-person field crew sampled five different wadeable stream reaches (1-5) distributed on a longitudinal gradient during the month of July (dry period) of 2012. The sites one, two and five comprehend surface reaches whereas the sites three and four are located inside the Éden cave and comprehend subterranean reaches (Fig. 2). The sampling design utilized in this study was based on the US-EMAP field procedures (Kaufmann et al., 1999). We established reach lengths of 40 times their low flow wetted width and a minimum length of 150m for each sampled reach in order incorporate habitat-scale variation (Kaufmann et al., 1999). After the downstream end of each reach was located, 11 transect positions were set, 1/10th at equal intervals along the sample reach (Kaufmann et al., 1999). Each transect was surveyed independently for the fish fauna and for the physical habitat attributes.

Fish data collection

The fish collections were conducted in an upstream direction with sieves (80cm diameter; 1mm mash size) and kicking nets (4x2m 5mm mash size) until depletion. All transects were independently sampled along the five studied stream reaches. The lower and upper ends of each transect were blocked with a seine during the collections. We determined a minimum sampling period of two hours for each stream reach or 12 minutes per transect (Junqueira, 2011). All fish were kept in separate bags identified by sampled reach and transect numbers and preserved in formalin 10%. The fish were sorted in the laboratory, washed in current water and transferred to 70% ethanol. The specimens were identified to species level when possible. All specimens were lodged at the Ichthyological Collection of The University of Lavras (CI-UFLA).

Habitat data collection

We conducted the physical habitat data collection based on the protocols developed by US-EMAP West Wadeable Streams (Olsen & Peck, 2008). A range of habitat parameters were measured at each sampled site: Depth; Width; Channel canopy cover; Total wood volume; Slope; Organic matter and Substrate (hard pan; fine gravel; sand; smooth rock; rough rock and silt). These information were collected on each transect located within the five sampled stream reaches. After filling the field protocols, the data was transferred to a digital worksheet for the calculation of the parameters (condensed observed values) (Kaufmann et al., 1999).

Data Analysis

We used a Non-metric Multidimensional-scaling (NMDS) model (Sorensen index) run in the software Primer E + Permanova v6 along with associated analysis of similarity (ANOSIM). We combined subterranean sites (3 and 4) for analysis. NMDS and ANOSIM were utilized to ordinate the fish fauna data and to compare the community composition of the sampled reaches. To verify the influence of the measured physical habitat characteristics on the fish fauna composition we performed a Distance based linear models (DistLM) analysis. To ordinate and visualize the DistLM models we conducted a Distance based redundancy analysis (dbRDA). We utilized the Kruskall Wallis test to check if there are significant differences in the physical habitat characteristics among surface and subterranean sites (software Statistica 7). We used a logistic regression and a G test to check if different fish groups (siluriforms and non siluriforms) have the same probabilities of entering and colonizing subterranean streams.

RESULTS

A total of 773 individuals representing 28 fish species distributed in three orders and nine families were collected during the survey period (Tab. 1). Characiforms represent the most diverse group in the studied area with 16 registered species (57 % of sampled fauna). The most abundant species were: *Hyphessobrycon santae* (194); *Hasemania nana* (154) and *Serrapinnus piaba* (82). Although troglomorphic species were not found among the sampled fish four non-troglomorphic species were sampled exclusively in the Éden cave (*Astyanax lacustris; Hemigrammus marginatus; Imparfinis minutus; Pseudopimelodus charus*). The subterranean ichythyofauna is equally divided between chariformes and siluriformes with four species distributed on each order (Tab. 1). The most common species found in the Éden cave are: *Astyanax fasciatus* (6) and *Pimelodella lateristriga* (5). It is interesting to observe that, although the stream's superficial course is interspersed by subterranean stretches, the species richness and abundance maintain a rising continuum in a downstream direction, although the Éden cave breaks the continuum featuring lower richness and especially abundance values (Fig 3-4).

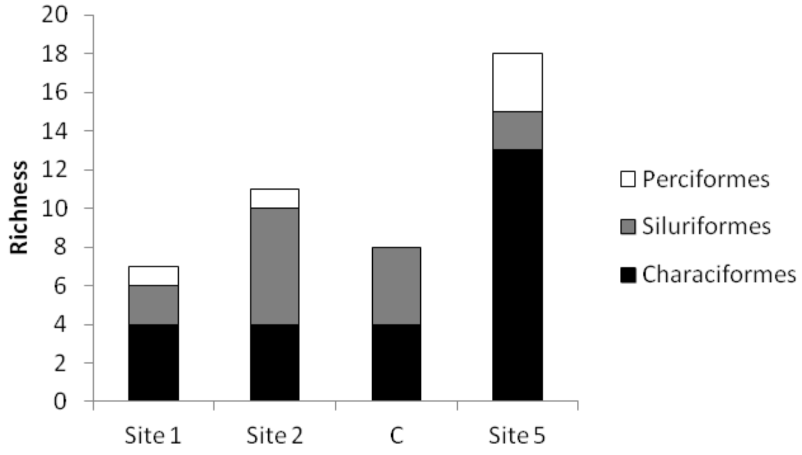

Fig. 3. Fish richness values according to sampled sites from upstream (1) to downstream (5), (c) represents combined cave sites (3 and 4).

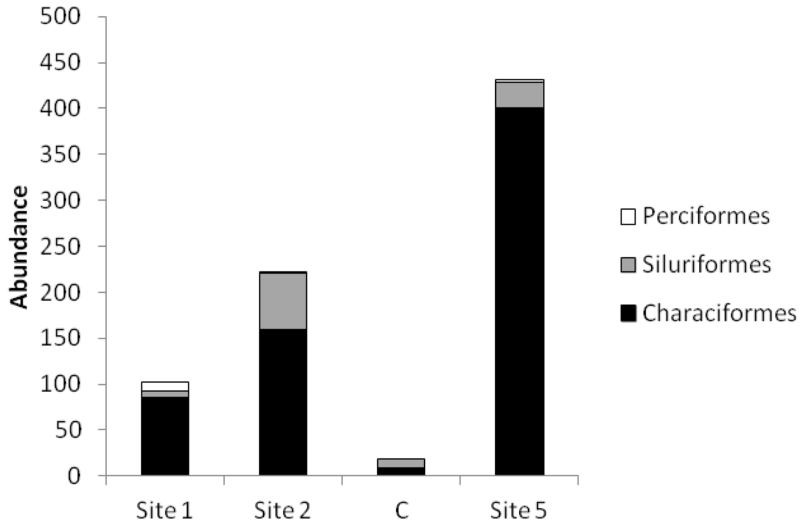

Fig. 4. Fish abundance values according to sampled sites from upstream (1) to downstream (5), (c) represents combined cave sites (3 and 4).

Table 1. Sampled fish species according to sampled sites (sites: 3 and 4 are subterranean sites).

	Site 1	Site 2	Site 3	Site 4	Site 5
Taxons					
CHARACIFORMES					
Characidae					
Astyanax fasciatus (Cuvier, 1819)			1	5	24
Astyanax lacustris (Linnaeus, 1758)			1		
Astyanax rivularis (Lütken, 1875)	3				7
Hasemania nana (Lütken, 1875)					154
Hemigrammus marginatus - Ellis, 1911			1		
Hyphessobrycon santae (Eigenmann, 1907)	77	88			29
Hysteronotus megalostomus - Eigenmann, 1922		2			8
Piabina argentea - Reinhardt, 1867		1			11
Serrapinnus heterodon (Eigenmann, 1915)					59
Serrapinnus piaba (Lütken, 1875)					82
Crenuchidae					
Characidium lagosantense - Lütken, 1875					3
Characidium sp		69			1
Characidium zebra - Eigenmann, 1909					21
Curimatidae					
Steindachnerina elegans (Steindachner, 1875)					1
Erythrinidae					
Hoplias intermedius (Günther, 1864)	4				1
Hoplias malabaricus (Bloch, 1794)	1			1	
PERCIFORMES					
Cichlidae					
Cichlasoma facetum (Jenyns, 1842)	10				1
Geophagus brasiliensis - Quoy & Gaimard, 1824		1			1
SILURIFORMES					
Heptapteridae					
Cetopsorhamdia iheringi - Schubart & Gomes, 1959		13			
Imparfinis minutus (Lütken, 1874)			1		
Rhamdiopsis sp		1			
Pimelodella lateristriga (Lichtenstein, 1823)		2	4	1	
Rhamdia quelen (Quoy & Gaimard, 1824)	6	2			
Loricariidae					
Hypoptopomatinae sp		33			26

Hypostomus lima (Lütken, 1874)		8		1	1
Pseudopimelodidae					
Microglanis sp					1
Pseudopimelodus charus (Humboldt 1821)			2		
Trichomycteridae					
Trichomycterus brasiliensis - Lütken, 1874	1	2			
Total	**102**	**222**	**10**	**8**	**431**

The NMDS analysis describes significant differences in species composition between distinct sampled sites. Epigean (surface) sites (1, 2 and 5) are grouped separately but describing a continuum in an upstream - downstream direction (1 to 5). The two combined subterranean sites (c) behave randomly (Fig. 5). The ANOSIM results based on the similarity matrix, indicate significant dissimilarities among the community composition found in distinct sampled sites (R = 0,304; p = 0.01).

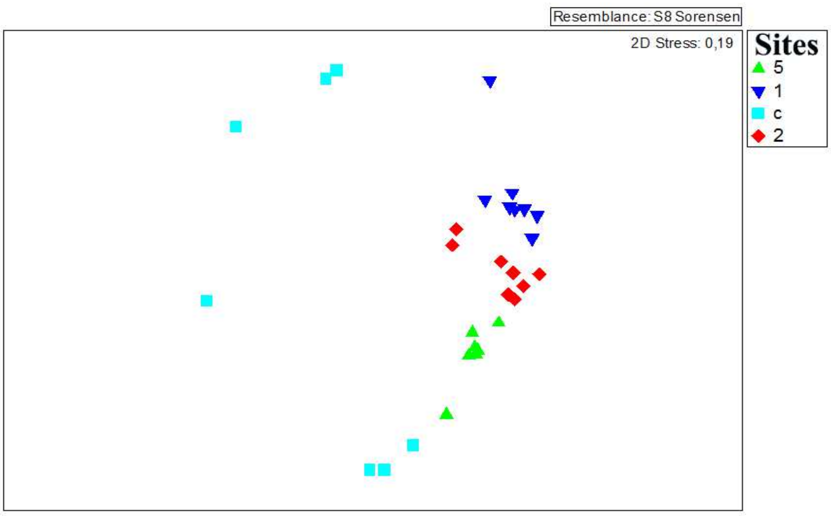

Fig. 5. NMDS ordination of species composition in the sampled sites from upstream (1) to downstream (5), (c) represents combined cave sites (3 and 4).

The Distance based linear models (DistLM) analysis revealed that among the measured physical habitat characteristics in the sampled sites, depth and percentage of channel canopy cover (25.75% and 15.42% out of total variation) represent respectively the variables that account for most of the variation in species composition between sampled sites (Adjusted R^2: 0.45). The graph (dbRDA) illustrates this characteristic with vectors indicating tendencies and strength of explanation (Fig. 6).

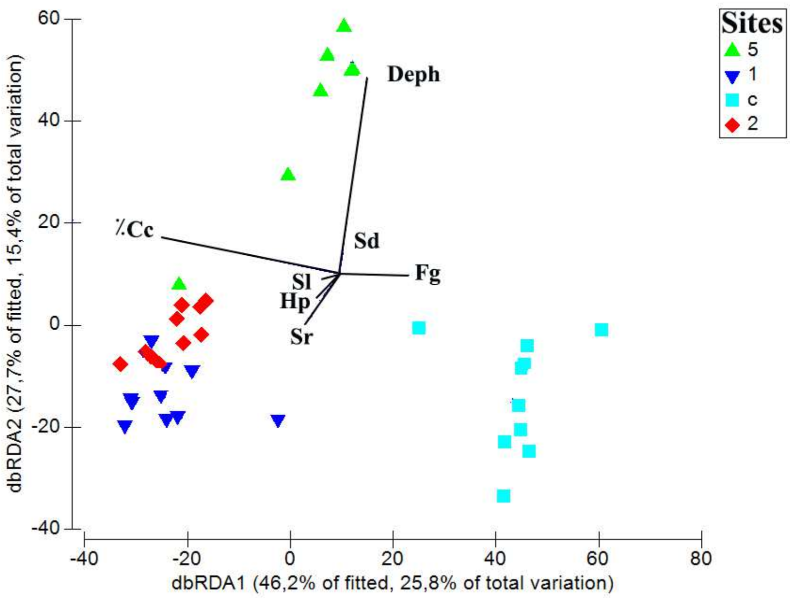

Fig. 6. The influence of physical habitat characteristics on the composition of the fish fauna. %Cc (channel canopy cover %); Sd (sand); Fg (fine gravel); Sr (Smooth rock); Hp (Hard pan); Sl (slope).

Significant differences in the following physical habitat characteristics between surface and subterranean sites were found. Channel width (KW – H (1;47) = 13.94; p= 0.0002); Channel Canopy cover (%) (KW – H (1;47) = 36.58; p= 0.00); slope (KW – H (1;47) = 11.99; p= 0.0005); % fine substrate (KW – H (1;47) = 12.84; p= 0.0003); wood total volume (KW – H (1;47) = 7.96; p= 0.0048); % organic substrate (KW – H (1;47) = 12.84; p= 0.0262) (Fig. 7).

Siluriforms do not have greater probabilities, compared to Characiforms, of entering the subterranean habitat (Éden cave) according to the logistic regression analysis (Z=0,986; p= 0,324). This result reflects the composition of the epigean (surface) fish fauna which is dominated by Characiforms. However, Siluriforms have greater chances of colonizing subterranean habitats since they are found in greater proportion inside the cave, when compared to surface areas (G= 14,5; p= 0,0001).

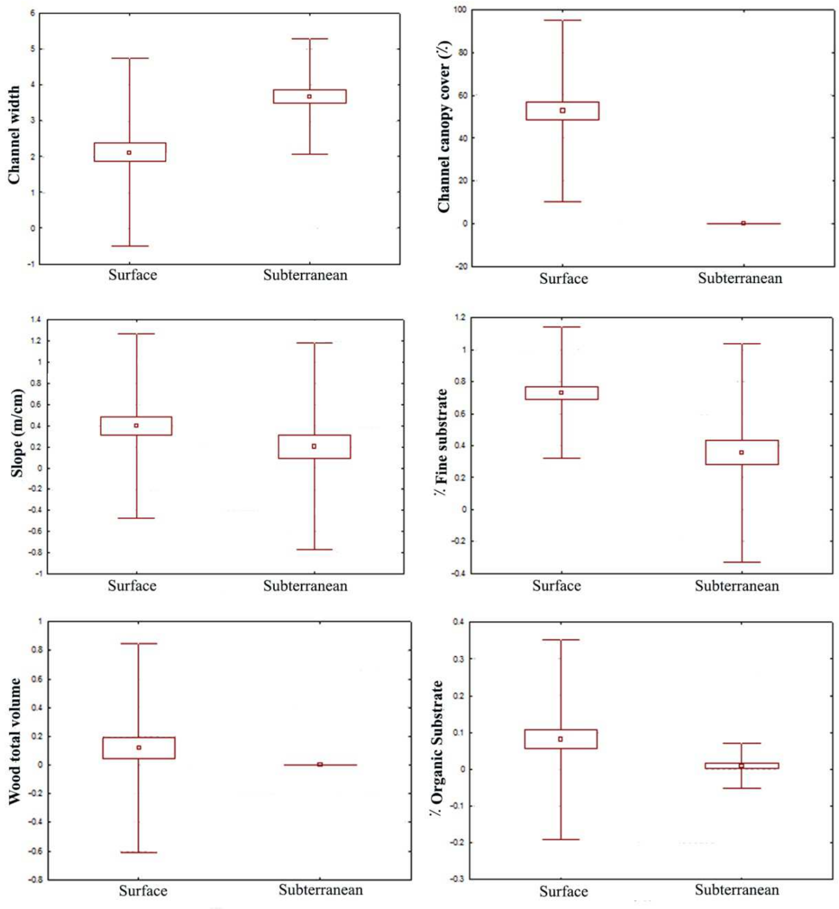

Fig. 7. Variation (Mean, Standard error and standard deviation) in physical habitat characteristics among combined Surface and Subterranean sites.

DISCUSSION

Although the speleological province (Arcos-Pains-Doresópolis) encompassing the study site is the most studied karst area in the country regarding number of sampled cave systems (Zampaulo, 2010), only one study assessed the subterranean fish fauna (Santuário cave) (Souza-silva et al., 2012). The Éden cave and Santuário cave share the following fish species: *Astyanax faciatus; Astyanax lacustris* and *Hoplias malabaricus*. The lack of information on the regional ichythyofauna makes further comparisons virtually impossible for the area, therefore more studies are needed in order to reveal any biological pattern. All fish species sampled during the present study are commonly found in Rio São Francisco basin. The number of sampled species (28) represents approximately 15.6% of the total fish fauna found in the basin (Britskiet et al., 1984; Sato & Godinho, 1999; Alves & Pompeu, 2001). The species collected exclusively in the Éden cave were all found deep into the aphotic zone at several hundred meters from the closest known cave entrance however, they probably occur in the surface as well since they are common in the upper São Francisco basin (Sato et al., 1987).

The fish community composition differs significantly between sampled sites. Despite several interruptions on the stream's superficial course by subterranean stretches, the number of species and the overall abundance in the sampled epigean sites increases continually as we move downstream. Even in karst landscapes, where stream continuity is naturally interrupted, the river continuum is still maintained, leading to serial alterations in the fish community composition coupled with habitat changes (Vanote et al., 1980). The subterranean site (c) breaks the river continuum punctually featuring a drastic decrease in species richness and abundance. These findings are attributed to differences in physical habitat characteristics within the karst watershed including the presence of subterranean passages.

Local habitat characteristics have long been pointed out as determinants of local community composition (Hilborn & Stearns, 1992; Poff, 1997). Among the measured physical habitat characteristics, depth (25.75%) and percentage of channel canopy cover (15.42%) are the ones that most influence the fish community composition. The depth vector (dbRDA) indicates gradual rising depths in a downstream direction from site one to five (deepest), and community composition seems to be intimately related to this habitat characteristic. Many studies have shown that depth has important influences on fish assemblages through its strong correlation with species richness and abundance (Sheldon, 1968; Evans & Noble, 1979; Taylor et al., 1993) and when combined with width, could be associated with habitat volume. Larger stream habitats should be able to accommodate a greater number of organisms reflecting on the local capacity to support fish biomass (Gerking, 1949). Schlosser (1987) postulated a natural gradient in fish community attributes from shallow to deeper habitats, leading to a gradual increase in species richness due to greater environmental stability and habitat heterogeneity. Deeper waters also allow vertical separation of fish species thus allowing more species to inhabit the same stream reach (Baker & Ross, 1981; Gorman, 1988a, b).

Percentage of channel canopy cover (%Cc) has also strong influences in the fish assemblages. Upstream sites (1 and 2) are characterized by greater density of riparian vegetation which affects the community composition. Matthews and Hill (1979) found that canopy cover increased shade in the stream channel therefore enhancing habitat use by certain group of fish. The reduction in sun light to stream reaches caused by the presence of canopy also reduces the stream's primary productivity thus lowering the density of algivorous species (Power, 1983). Vegas-Cendejas (2013), working on karst pools, revealed that arboreal vegetation, roots and aquatic vegetation positively influenced species diversity of fish assemblages. The cave site (c) is opposite to the %Cc vector (dbRDA) due to the lack of riparian vegetation. The permanently dark cave environment impedes photosynthetic production and prevents riparian vegetation growth, primary productivity is usually absent in aphotic subterranean environments with some rare exceptions (e.g. chemoautotrophic bacteria) (Sarbu et al., 1996; Chivian et al., 2008).

Cave communities are usually dependent upon allochthonous organic matter that may enter the subterranean environment carried by different agents (e.g. streams) (Simon et al., 2007; Souza-silva et al., 2011a, b). Although it was not tested, we believe that the Éden cave's architecture is another factor that greatly hampers the cave's organic matter input as the stream reaches the cave through an upwelling after running through subterranean water filled passages for a considerable distance (approximately 1km). Wood and organic substrates were significantly reduced in the subterranean habitat compared to surface sites. The presence of wood in rivers potentially affects biodiversity in aquatic ecosystems in many different ways. It represents a source of nutrients for aquatic insects having severe influences in the trophic structure and productivity in aquatic food webs (Wondzell & Bisson, 2003). Therefore, cave environments can represent "harsh" oligotrophic scenarios that may prevent the formation of viable populations (Culver & Pipan, 2009).

In the present study, we observed dominance of Chariforms (16 species), especially tetra characins, followed by Siluriforms (10 species) and Perciforms (2 species). The occurrence of Characiforms in Brazilian caves is quite common, in many cases they show signs of starvation, which probably indicates that they have been trapped in the cave interior unable to find food (Bichuette & Trajano, 2003). This condition is probably occurring in the Éden cave also. Although we did not analyze stomach contents, we observed that organic resources were scarce in the subterranean area. This feature can directly influence the distribution and abundance of aquatic invertebrates (Molles, 1982), leading to a depleted cave benthic community (Ratton, P. unpublished data) which in turn is negatively affecting the performance of visually oriented fish such as the tetra characins.

Among the cave adapted fish species recorded in Brazil, six occur in the Rio São Francisco basin in Minas Gerais (*Stygichtys typhlops; Trichomycterus itacarambiensis* and *Rhamdiopsis* sp.) and Bahia state (*Rhamdia enfurnada; Trichomycterus rubiolli* and *Rhamdiopsis krugi*.) (Mattox et al., 2008). Siluriforms are by far the commonest Brazilian fish group showing troglomorphic traits. They are considered pre-adapted to the subterranean habitat due to their nocturnal habits, chemo-orientation and omnivorous or generalist carnivorous diet (Bichuette & Trajano, 2003). Cave habitats usually show common selective forces (i.e. permanent darkness and few organic resources) (Culver & Pipan, 2009), therefore this environmental similarity should select species with similar traits (e.g. siluriforms) (Poff, 1997). In our study, we demonstrate that besides being outnumbered by Characiforms, considering the overall fish fauna, Siluriforms are able to colonize subterranean habitats more efficiently. This information indicates that the Éden cave, as well as other water filled subterranean spaces represent selective forces acting in a differential way towards different group of fish (e.g. Characiforms and Silurifoms). These permanent dark passages may be acting as "filters", selecting fish species with the necessary attributes to transpose or colonize them and therefore shaping the community composition within the karst watershed.

In conclusion, our results indicate that the fish community composition is influenced by local physical habitat characteristics. The observed dissimilarity on fish community composition between sampled sites is more pronounced than we expected, considering that stream length and distances between sites are both short. We believe that the number, size and position of subterranean spaces within a karst watershed have additional influences in the fish community composition through its "filtering" effects. More studies are needed in order to evaluate the strength of the influence of subterranean spaces on the fish community composition in water courses draining karst areas.

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