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Front cover: The River Styx Spring, Mammoth Cave, Kentucky. See Trimboli and Toomey in this issue.

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HALOTOLERANT AND CHAOTOLERANT MICROFUNGI FROM LITTORAL ANCHIALINE CAVES GOLUBINKA AND MEDOVA BUŽA (CROATIA)

Andrzej Chlebicki[1, C] and Natalia Jakus[2]

Abstract

Rock-inhabiting fungi were isolated for rock walls in Golubinka and Medova Buža littoral anchialine caves in Croatia and tested for their halotolerance. Isolates were identified as *Cladosporium psychrotolerans*, *C. delicatulum*, *Mucor circinelloides*, *Rhizopus stolonifer*, *Aureobasidium pullulans* var. *pullulans*, and *Talaromyces diversus*. Of them, *T. diversus* appears most resistant to cosmotropic (NaCl) and chaotropic (KCl) salts. It was also the most common species isolated from rock walls. Despite the negative influence of salinity on acid production, we propose that some fungal strains can be recognized as active agents in carbonate dissolution and as good competitors on rocks in saline environments. Anchialine caves can be a refugium for unique halotolerant fungi.

Introduction

The coastal sea area including islands of the Republic of Croatia is about 33,200 km^2. In comparison with the area of the eastern Adriatic territorial waters, the groups of islands covers a large area, being divided into several groups (Duplančić et al. 2004). The Adriatic Carbonate Platform (AdCP), is covered by Palaeozoic–Triassic deposits, Late Cretaceous–Palaeogene flysch, or Neogene and Quaternary deposits (Vlahović et al. 2005). The major types of rocks that build AdCP are freshwater or brackish and foraminiferal limestones; mudstones; marles; and silicoclastics. The region was changed by several tectonic events during the Palaeogene, Oligocene, and Miocene (Vlahović et al. 2005). Palaeogene compressional tectonics strongly influenced the area by faults oriented south-west to north-east. This direction is typical for this part of the karst terrain in the region, and it is often called Dinaric strike or even Dinaric karst. Rocks are eroded among zones of weakness by the hydraulic power of the waves and the abrasive force of suspended particles in water. Corrosion and bioerosion also have a significant influence on coastal shape in the mixing zone of fresh and sea water (Pikelj and Juračić 2013), and as a result, sea caves are formed. Among other karst features on islands are panholes, channels and furrows, uvalas and others. More than 235 submerged caves and pits have been noted along the Croatian coast and islands (Surić et al. 2010).

Anchialine cave, are the cave systems occurring in coastlines that are flooded with seawater. Pohlman et al. (1997) presents the first extensive description of the ecological and biogeochemical relationships of the anchialine cave ecosystem. They hypothesized that organic matter supporting the anchialine ecosystem would be: algal detritus transported by groundwater flow from the cenotes; soil particulates percolating from the tropical forest soil through the cracks and fissures of the limestone bedrock into the caves; coastal-borne particulate organic matter transported into the cave systems with tidally-exchanged seawater; and the potential for chemoautotrophic production of organic matter by bacteria. Only soil particulates from the forest did not occur in our caves due to lack of forest cover in the rock massive. Instead of forest particulates, small and scattered deposits of bat guano were found in both investigated caves.

There are many papers dealing with fungi in caves and mines (Nováková 2009; Nováková et al. 2012, Ogórek et al. 2013). A worldwide review of Fungi and Slime molds in caves was provided by Vanderwolf et al. (2013). Fungi and Slime molds are heterotrophic, using organic carbon for carbon and energy. Mineral pores and cavities, cracks and fissures are inhabited by cryptoendoliths (Ehrlich 1981; Sterflinger 2000; Burford et al. 2003). Rock-inhabiting fungi (RIF) dissolve minerals by excretion of enzymes, metal-binding ligands, siderophores, and acids. They transform minerals by dissolution and secondary mineral formation (Gadd 1993, 1999; Verrecchia 2000; Burford et al. 2003; Barton 2006; Barton and Northup 2007). Fungi are biosorbents for metal cations, such as Cd, Cu, Zn, Au, Ni, Fe, Ag and others (Tobin 2001). The presence of melanin and chitin in fungal cells increases the rate of biosorption (Gadd and Mowll 1985). Investigations of fungi in marine sediments and organisms (sponges) have also been performed (Sponga et al. 1999).

The salinity of Mediterranean sea water ranges from 38–39 ‰. There are solids such as cosmotropic NaCl (86 % of total solids), as well as Mg, S, Ca, K, Br, C, Sr and B (Masuma et al. 2001). Cosmotropic salts are stabilizing salts, such as NaCl and KCl, that stabilize proteins, whereas chaotropic salts are recognized as destabilizing salts like $MgCl_2$ that unfold proteins and increase solubility of hydrophobic chemicals.

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The stimulation of CO_2 production by low salt concentrations (1 % NaCl) has been confirmed by numerous experiments with yeast (Speakman et al. 1928). Marine fungi can grow abundantly in a medium with 4 % NaCl, whereas the same NaCl concentration suppressed growth of terrestrial fungi (Masuma et al. 2001).

We were interested in the occurrence of rock-inhabiting fungi (RIF) that can decompose natural rocks in a cave. We tested acidification of media by isolating fungi that can indicate the possibility of dissolution of rock minerals. We also tested the ability of isolated fungi for their ability to grow in dissolved cosmotropic salt (NaCl) and chaotropic salt ($MgCl_2$).

Methods

Study Sites

Two anchialine caves were chosen for investigation: Medova Buža from Rab Island and Golubinka from Dugi Otok. Dugi Otok (Long Island in English) and Rab Island belong to the megageomorphologic region of the Dinaric Mountain Belt, specifically to the macrogeomorphologic region of central Dalmatia with archipelago (Bognar 2001).

Dugi Otok is the largest and easternmost of the Zadarian Islands and derives its name from its distinctive shape. The semi-dark Golubinka Cave (Fig. 1) located on this island is one of 57 speleological features observed on Dugi Otok Island (Dzaja 2003). This 100 m long cave with an entrance accessible from the sea is situated on the steep southern slopes near Brbinjšćica Bay. Its main development extends to the north. The rock composition of the cave is dominated by Mesozoic carbonates. The Golubinka Cave is inhabited by many bats; among them are approximately 2000 *Myotis emarginatus* and approximately 1000 *Rhinolophus ferrumequinum* as well as *Tadarida teniotis* and *Plecotus austriacus* (Pavlinič et al. 2010). Fifty species of Porifera occur on the submerged rock walls. The bottom of the cave corridor is submerged; moreover, the lower parts of the cave walls are seasonally submerged or splashed by sea water such that bat guano and other organic detritus cannot be deposited in these places. Only small deposits can be attached to the upper part of cave walls.

Medova Buža Cave (107 m long) is located near Lopar on Rab Island (Fig. 1). It is composed of four rooms; only the first was investigated by us. This cave stretches approximately 60 m into Rab Island and is connected to the sea. The

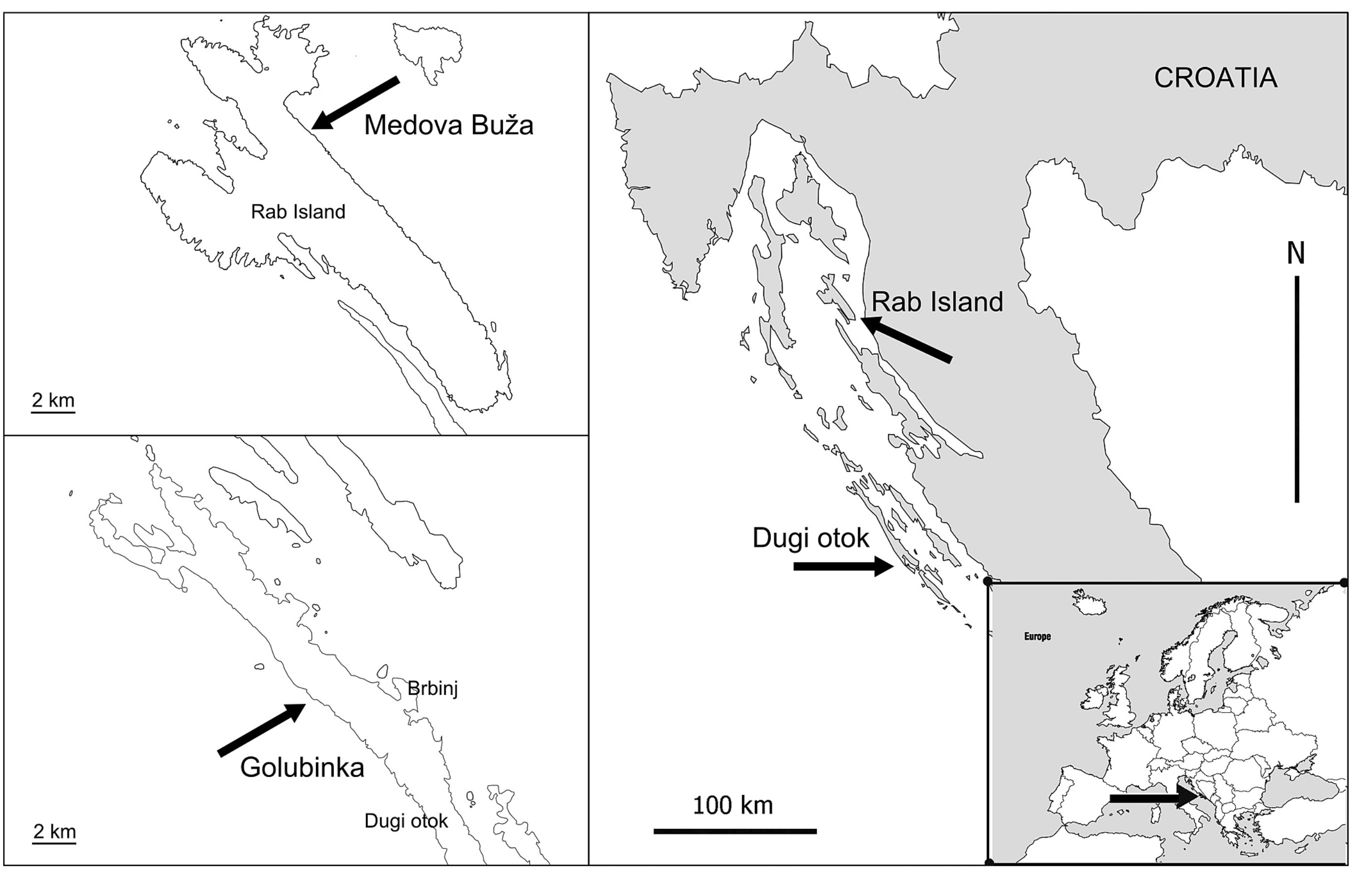

Figure 1. Anchialine caves in Croatia: A, B – Golubinka Cave on Dugi Otok Island; C, D – Medova Buža Cave on Rab Island.

entrance is 4 m below the surface. The bottom of this cave corridor is also submerged. The cave is inhabited by 4270 bats (Inf.Eurobats.Ac.19 2004) as *Miniopterus schreiberii*, *M. emarginatus* and *Rhinolophus euryale*.

Dugi Otok Island is made of carbonates of the Cretaceous age that have been deposited in deeper environments compared to carbonates from most of the other localities in coastal Croatia. These are commonly micritic-type limestones, occasionally with evidence of deposition from mass-flow and slope instabilities (Perica et al. 2004).

The oldest exposed rocks on Rab Island are Cretaceous carbonates, represented mainly by foraminiferal limestones, which are overlain by Eocene carbonates and clastics (limestones, marls and sandstones). The contact between Cretaceous and Eocene deposits is characterised by unconformity and occurrences of bauxite (Marjanac and Marjanac 2007). Important influences also include abrasion, corrosion in the mixing zone of water and bioerosion (Pikelj and Juračić 2013), especially in weak zones of cliffs.

Material Collection

Medova Buža: material was collected May 25, 2015 from its entrance; Golubinka Cave: material was collected June 2, 2015 from rock walls inside the cave. We collected chips of rocks approximately 150 cm above sea level (Fig. 2) because the lower parts of the cave walls were seasonally submerged or splashed by sea water. We used an ethanol flame-sterilized metal hammer to chip off cave rock walls. All collected materials were placed in sterile plastic boxes and refrigerated. After six days material was transported to Poland and placed in an incubator at 10 °C. In the laboratory the samples were mechanically crushed into small rock fragments.

Strain isolation Strains were isolated on RBC (Rose-Bengal Chloramphenicol, Merck KgaA) medium. Afterward, PDA (Potato Dextrose Agar, Merck KgaA) and MEA (Malt Extract Agar, Merck KgaA) media were used. At the beginning, several additions of NaCl to the MEA media were used: 1.7 %, 3.4 % and 6.8 % NaCl. Strains put in RBC were incubated at 10 °C; other material was stored at room temperature.

Morphology

The morphological characters of the living fungi were examined in water and in lactophenol cotton blue using light microscopy (Nikon SMZ 1500, Nikon Labophot 2 and Nikon Eclipse 800) equipped with a digital camera. For identification of fungi we used taxonomic guides and articles (Bensch et al., 2010, 2012; Owczarek-Kościelniak et al. 2016; Schipper 1976, 1984; Yilmaz et al., 2014; Zalar et al. 2007). Colony diameters were measured with a ruler.

Halotolerance

Halotolerance (ionic NaCl) and chaotolerance (ionic $MgCl_2$) were identified from YMA (Yeast Salt Agar, Sigma-Aldrich) (Jančič et al. 2015). Conidia were suspended in 0.9 % NaCl and 0.05 % agar and were point-inoculated in triplicate on each plate onto prepared media in 9 cm Petri dishes incubated at room temperature for 7, 14, and 28 days. Ionic $MgCl_2$ at 4, 9, 15, 16 and 17 % and ionic NaCl at 10, 12, 14, 16, 18, 20, 24 and 28 % were used in six replicates in each combination. Colony diameters were read after 14 days. We used the halotolerant strain of *Wallemia mellicola* Jancic, Nguyen, Seifert & Gunde-Cimerman (Jančič et al. 2015) for comparison of its halotolerance with those of isolated species.

Acidity

For experiments with pH indicators, we used YMA medium with 20 drops dibromothymolsulphonephthalein and 10 drops of 10 % KOH. Such media were green at the beginning (pH = 7) and turned yellow with acidity. A change in medium color from green to yellow indicates production and secretion of acids by these fungi.

Results

Fungi Isolated from Rocks in the Caves

Cladosporium delicatulum

According to Bensch et al. (2012), this species has been isolated from indoor air, building materials, dead leaves, fruits and tubers. It is a common and widely distributed hyphomycete, clustered as a sister to *Cladosporium cladosporioides* (Bensch et al. 2010).

Cladosporium psychrotolerans

This species was isolated from hypersaline water in Slovenia (Zalar et al. 2007). It was also reported from indoor environments and on plant leaves (Bensch, et al., 2012). Typical strains have a maximum salt concentration tolerance of 17%. We have viable cultures growing on PDA with 16 % salt concentration (Fig. 3).

Mucor circinelloides

This species was present in all plates and often overgrowing other fungal species. The fungus occurs on fruits, as well as in soil; compost; guts and dung of animals; pellets; river water and hypersaline water of saltpans; air; decaying plant material; and living animals, such as *Troglophilus neglectus* (Gunde-Cimerman et al. 1998), *Ornithorhynchus*

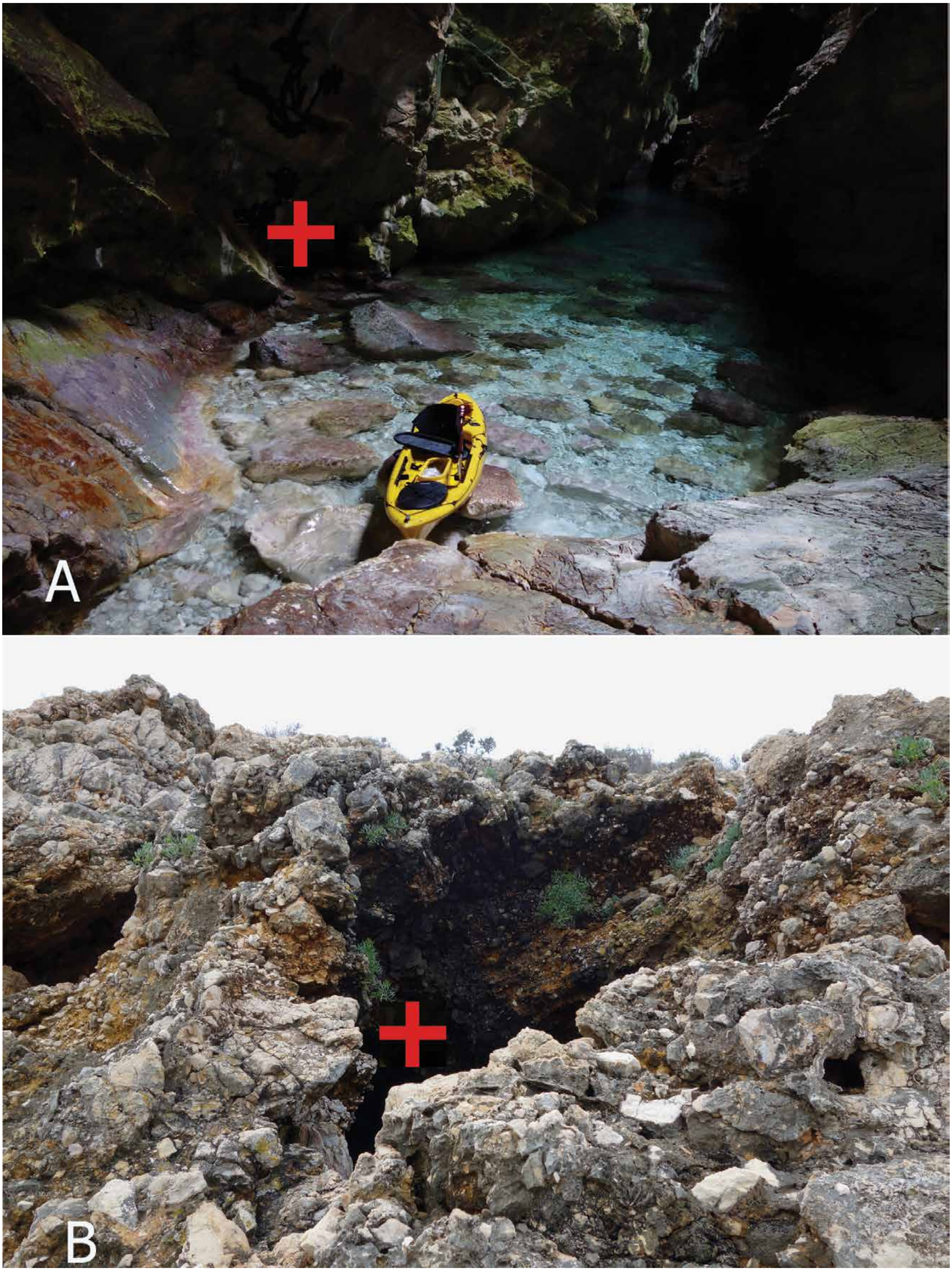

Figure 2. Anchialine caves in Croatia: A – central part of Golubinka Cave under window of rock vault, inside Provler Trident 11 kayak (80 cm wide); B – entrance of Medova Buža Cave. Sample collection sites marked.

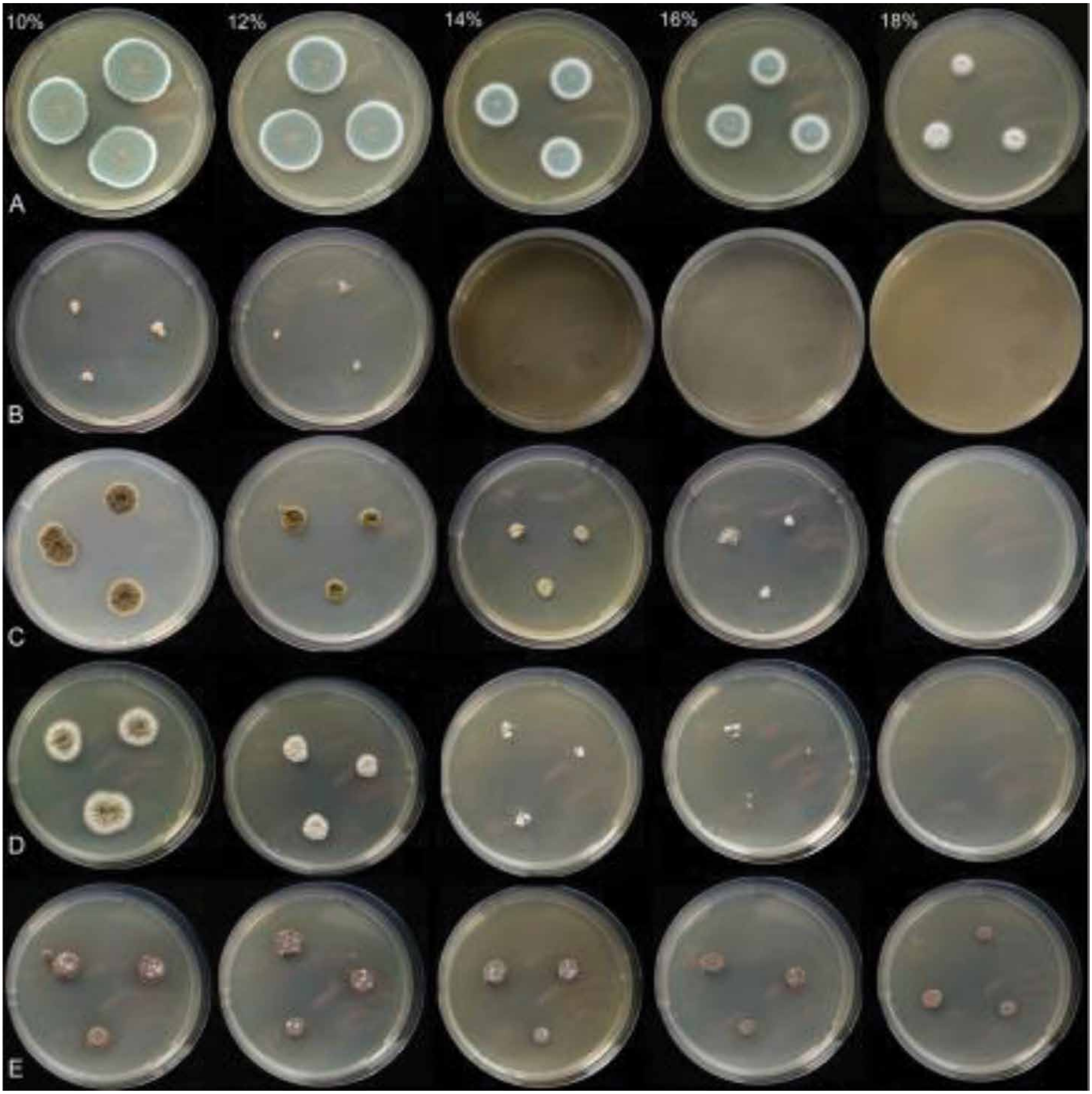

Figure 3. Halotolerance: A – Talaromyces diversus; B – Aureobasidium pullulans; C – Cladosporium delicatulum; D – Cladosporium psychrotolerans; E – Wallemia mellicola. Concentration of NaCl from left to right: 10 %, 12 %, 14 %, 16 %, and 18 %.

anatinus (Stewart et al. 1999) and *Pelteobagrus fulvidraco* (Ke et al. 2009). The species is reported to infect other animals (cattle, swine, and fowl) in anaerobic condition (Lübbehüsen et al. 2003). The fungus has a growth optimum of approximately 30 °C (Michailides 1991), and its upper temperature limit is 38 °C. It has a yeast-like growth phase in an anaerobic atmosphere and in the presence of 30 % CO_2 (Lübbehüsen et al. 2003). It is a carotene producer.

Rhizopus stolonifer

A single colony was isolated from an RBC plate. It is very common species, normally found at the entrance and interior of caves (Ogórek et al. 2014).

Aureobasidium pullulans var. *pullulans*

Colonies are mucoid and pink, hyphae septate and spores one-celled. Our strain was isolated from a single plate. The species appeared sensitive to high salt concentrations, however, other strains of this species are able to grow at relatively high concentrations of NaCl (Zajc et al. 2014). Our strain (unpublished data) has identical ITS sequence as a fungus isolated from roots of *Juncus trifidus* (see Owczarek-Kościelniak et al. 2016).

Talaromyces diversus

This species belongs to the section *Trachyspermi* Yaguchi & Udagawa (Yilmaz et al. 2014). Novakova (2009) isolated similar species – *Talaromyces flavus* (Klöcker) Stolk & Samson (syn. *T. vermiculatus*) from earthworm casts in the Dominica cave system in Slovakia. Nováková et al. (2012) noted it from timber in the abandoned Jeroným uranium mine at Jáchymov. Moreover, the fungus can synthesise colloidal silver nanoparticles (Ganachari et al. 2012) and produce mitorubrinic acid (Yilmaz et al. 2014).

Halotolerance

It is evident that some isolates are well accommodated to saline environments. *Talaromyces diversus* is the most resistant halotolerant species (Table 2). Also, both species of *Cladosporium* are well adapted to saline environments. At the same time, *T. diversus* is also the most resistant strain to chaotropic salt (Table 3).

Table 1. Fungi isolated from two littoral anchialine caves in Croatia.

Fungus Name	Golubinka Cave	Medova Buža Cave
Aureobasidium pullulans var. pullulans (de Bary & Löwenthal) G. Arnaud	−	+
Cladosporium psychrotolerans Zalar, de Hoog & Gunde-Cimerman	+	−
Cladosporium delicatulum Cooke	−	+
Mucor circinelloides Tiegh.	+	+
Rhizopus stolonifer (Ehrenb.) Vuill.	+	−
Talaromyces diversus (Raper & Fennell) Samson, Yilmaz & Frisvad	+	−

Note: + indicates presence of fungus in the cave.
− indicates absence of fungus in the cave.

Table 2. Halotolerance of fungi isolated from two anchialine caves in Croatia. (*Wallemia mellicola* added for comparison.)

Species	10 % NaCl	12 % NaCl	14 % NaCl	16 % NaCl	18 % NaCl	20 % NaCl	24 % NaCl
Aureobasidium pullulans	+	+	±	−	−	−	−
Cladosporium delicatulum	+	+	+	±	−	−	−
Cladosporium psychrotolerans	+	+	+	±	−	−	−
Mucor circinelloides	+	−	−	−	−	−	−
Talaromyces diversus	+	+	+	+	+	+	±
Wallemia mellicola	+	+	+	+	+	+	−

Note: + indicates that fungus can grow in these media with salt solution.
− indicates fungus growth inhibition by salt solution.

Table 3. Chaotolerance of fungi isolated from two anchialine caves in Croatia. (*Wallemia mellicola* added for comparison.)

Species	9 % $MgCl_2$	15 % $MgCl_2$	16 % $MgCl_2$	17 % $MgCl_2$
Aureobasidium pullulans	+	−	−	−
Cladosporioum delicatulum	+	−	−	−
Cladosporium psychrotolerans	+	−	−	−
Mucor circinelloides	±	−	−	−
Talaromyces diversus	+	+	+	−
Wallemia mellicola	+	+	±	−

Note: + indicates presence of fungus in the cave.
− indicates absence of fungus in the cave.

Table 4. Acid production by fungi isolated from two anchialine caves in Croatia. (+ indicates extent of colour change on PDA medium with dibromothymolsulphonephthalein indicator.)

Fungus	0 % NaCl	3.4 % NaCl	5 % NaCl	10 % NaCl
Aureobasidium pullulans	+	+	+	−
Cladosporium delicatulum	++	+	+	−
Cladosporium psychrotolerans	++	+	+	+
Mucor circinelloides	−	−	−	−
Talaromyces diversus	++++	+++	++	+
Wallemia mellicola	++	+	−	−

Note: ++++ indicates bigger size of color change.
+++ indicates medium size of color change.
++ indicates small size of color change.
+ indicates very small size of color change.
− indicates lack of acid production.

Chaotolerance

Aureobasidium pullulans, both species of *Cladosporium*, and *Mucor circinelloides* were inhibited by 15 % $MgCl_2$. Whereas *Talaromyces diversus* and *Wallemia mellicola* were the most resistant strains to chaotropic salt (Table 3).

Acid Production

We tested isolated strains for acid production in media with dibromothymolsulphonephthalein, (pH 7). Species such as *Cladosporium delicatulum*, *T. diversus,* and *Aureobasidium pullulans* var. *pullulans* alter pH in PDA plates (Table 4). However, acid production and secretion in *Mucor circinelloides* was absent. Colony shape of *A. pullulans* var. *pullulans* changes with increasing salinity of YMA medium. Margins of colonies in the absence of NaCl were fimbriate, whereas other colonies influenced by NaCl contained slightly undulate to entire margins (10 % NaCl).

Discussion

Both caves investigated are littoral anchialine caves. Typical anchialine caves connect sweet water from the sea and form a distinct boundary layer between them. Soil particulates mentioned by Pohlman et al. (1997) percolating from the tropical forest soil into the caves did not occur in our caves due to a lack of forest cover on rock massifs. Instead of forest particulates, bat guano was found in both caves.

Bat guano contains many phosphates and nitrates that can dissolve sandstone (Hosono et al. 2006). However, the lower parts of cave walls are seasonally submerged or splashed by sea water. Also, drops of the marine water can reach higher-located rocks so surfaces will be covered by various concentrations of diluted NaCl and small amounts of other solids. Among fungi isolated on rocks in our caves were coprophilous species from the genera *Aspergillus*, *Mucor* and *Rhizopus*. These fungi did not grow on media with highly concentrated NaCl and $MgCl_2$. However, species of *Cladosporium*, *A. pullulans* and *T. diversus* grown in media with 14 % concentrations of NaCl (Table 2) can be recognized as halotolerant fungi (see Cantrell et al. 2006). Some species of fungi belonging to the genus *Cladosporium* have been described from hypersaline environments (Zalar et al. 2007), among them *C. psychrotolerans*, noted by us in Golubinka Cave. Fungal deterioration of rocks can be made by acid excretion. *Talaromyces diversus* produces mitorubrinic acid (Yilmaz et al. 2014), which can dissolve limestone rocks. However, salinity agents appear to decrease acid production and excretion (Table 4). According to Harrison et al. (2015), differences in salinity influence microbial responses to mineralization. NaCl is an influencing factor that can decrease the amount of secondary metabolites (Huang et al. 2011). *Mucor circinelloides* did not produce acid, and its growth was diminished distinctly by NaCl. The species most resistant to NaCl and $MgCl_2$ appears to be *T. diversus*. This species was the most common fungal strain isolated from the cave rocks.

Wallemia ichthyophaga is the most halotolerant known fungus (Zajc et al. 2014). Used by us, *W. mellicola* has a halotolerance of 4–24 % NaCl (Jančič et al. 2015). The colony diameter of *W. mellicola* changed minimally (Fig. 3). *W. mellicola* is an advanced halotolerant species. Also, *A. pullulans* var. *pullulans* only slightly changed its colony diameter. The species is also known for its halotolerance. However, our strains appeared sensitive to high concentrations of NaCl. Despite the negative influence of salinity on acid production, we suppose that some fungal strains (i.e., *Talaromyces diversus*) can be recognized as active agents in carbonte dissolution. These halotolerant fungi can be good competitors on rocks in saline environments. Fungi identified here play a role in transforming the mineral environment of the cave.

Conclusion

Anchialine caves have reduced food resources and low microbial diversity (see Culver and Sket, 2000). Ours is the first report of fungal diversity in anchialine caves. These caves can be a kind of 'refugium' for preservation of these unique halotolerant microorganisms.

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THE CONCEPT OF REGULATED USE OF NATURAL HERITAGE SITES INCLUDED IN THE TOURIST CLUSTER: A RUSSIAN CAVES CASE STUDY

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Abstract

The rationale for our research arises from non-regulated use of caves for commercial and sightseeing purposes, which can significantly damage objects of cultural heritage or result in their complete destruction. In this respect, our paper aims to define the factors necessary for the development of nature preserves and tourist centers that provide for the preservation of caves that are properly maintained and used by the public. The paper employs a multidisciplinary approach based on inter-connections among geological, social, and economic research methods. Geological methods provide complex monitoring of cave environments to ensure that they are not being adversely impacted and remain popular tourist destinations. Social and economic methods allow development of a business model to ensure that their operation is economically sustainable. The authors apply a model of step-by-step formation and development of a cave-related nature preserve and tourist center, exemplified with the case study of the Sablinsky tourist center (Leningrad District), including complex research of the geology, establishment of a nature preserve and tourist unit, development of a self-financing operating plan, cave zoning that allots areas for natural and cultural interpretation, but restricts tourists to special routes, and touristic infrastructure. This paper is intended as a practical guide for promoting the development of domestic tourism in Russian regions and creating new tourist clusters around objects of natural and cultural heritage, while implementing an effective protection of the natural geology and limiting anthropogenic impacts.

Introduction

Since the turn of the 21st century, people have become increasingly interested in nature. This is especially true in the highly industrialized, developed countries. Artificial, urbanized landscapes can no longer provide a satisfying life style for people who need to feel a part of nature now more than ever. This trend has resulted in the global development of tourism focused on outdoor recreation and adventure sport. As a result, there is a growing number of tourists who prefer to spend their vacation in the mountains or woods, thus increasing the anthropogenic pressure on the remaining wilderness areas. Therefore, there is a necessity to extend nature conservation work by increasing the number of nature reserves, wildlife reserves, national parks, and state nature sanctuaries. In the Russian Federation, these processes are hindered by economic challenges. There are cases when specially protected areas are illegally used as commercially profitable tourist attractions. While using nature sanctuaries for tourism seems to have negligible consequences, many sites are under pressure because of the absence of systematic monitoring and many businesses do not realize their role or take responsibility for the preservation of natural and cultural heritage sites.

There are many facts demonstrating illegal use of such specially protected natural areas such as caves. Because they evoke great tourist interest, there is a temptation for caves to be used for commercial purposes. Easy access and short distance from urban centers aggravate the problem. Caves become meeting places for so-called adherents of nonconformist, youth subculture, and radical religious groups. Unfortunately, caves are extremely vulnerable to inadvertent or malicious destruction. Caves are an important element of ecosystems, especially the karst hydrology, and as sources of drinking water. Unique deposits, dating to ancient epochs, have accumulated in caves. They often preserve distinctive remnants of our human ancestors and their tools. Most notable are the priceless discoveries of Paleolithic cave paintings, which are primary masterpieces of early human art. Caves may also preserve paleontological remains of rare or extinct animals that disappeared from the surface of our Earth millions of years ago. In the publics' mind, caves are most commonly associated as a refuge for bats. Among mammals, the order Chiroptera is second only to rodents in the number of species worldwide. For all of these reasons, caves are especially popular tourist destinations.

In England alone, there are over 500 caves used for commercial recreation, while in Russia they are too few. It is not because they are not popular; it is mainly because they are used and facilitated without the proper legal grounds agreed upon with nature preserve authorities and, even worse, without an operating plan and regulations for use. Meanwhile, there are many caves suitable for recreation purposes in the Russian Federation.

Commercialization of specially protected natural areas has a devastating effect on both the environment and social sphere, i.e., local people and their way of life. By now, there has been quite extensive experience of both positive and

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negative impacts of tourism on the social and cultural sphere. The negative impact from intense tourist use of specially protected natural areas is manifested as follows: (1) A shift from the traditional types of economic activity to tourism, due to the fact that tourist industry might bring better income; (2) When dealing with international tourists (mainly the wealthy), locals feel underprivileged, due to their low incomes and unsatisfactory way of life; (3) Local people might experience negative emotions when the tourists visit religious sites without permission granted by the local religious leaders. This is the problem for a number of Russian regions, including Mari El Republic; (4) Locals are driven away from the villages, which are turned into summer residences for city dwellers, due to their intense tourist attraction.

To create a positive impact from tourism, especially in the rural areas of Russia with an extended tourist capacity, like specially-protected natural areas, attention should be paid to the establishment of a tourism cluster. This is also defined by the Federal Target Program "Domestic and inbound tourism development in the Russian Federation (2011–2018)". The tourism cluster was based on so-called core business, i.e., creating a tourist product. We believe that the core tourist product in this case may be the tourist potential of specially-protected natural areas. However, the use of the natural heritage should be implemented extremely carefully. In the remainder of our paper, we will concentrate on the concept of regulated use of natural heritage sites.

Background

The methodology of the concept of regulated use of national heritage sites is under- developed. The Russian scientist Yu. S. Lyakhnitsky first gave name to the concept, although the history of studies and the practical use of caves has been the focus of scientific attention for more than 100 years. It took decades to research into caves with high scientific value.

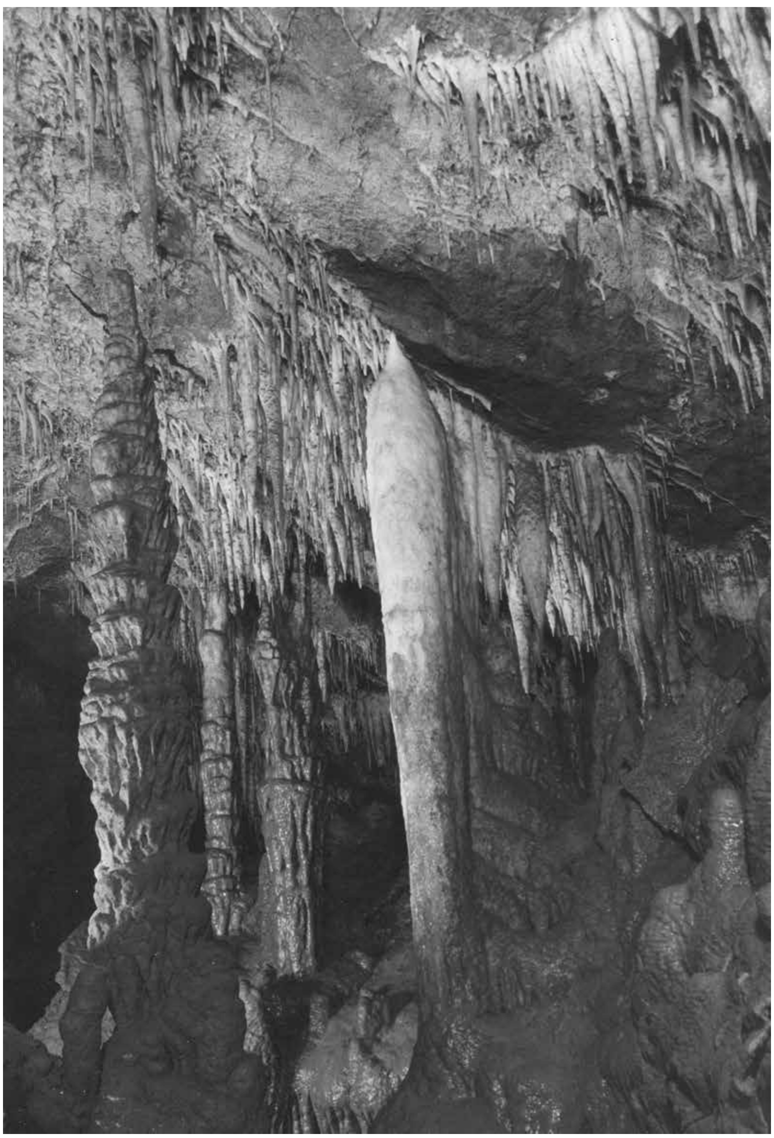

Figure 1. Sinter deposits in the Egyptian Hall of the Labirynthnaya Cave being part of Vorontsovskaya Cave Complex (stalagmites, stalagnates, stalactite).

In our research, we focus on the negative anthropogenic impact on caves and the counter actions taken to preserve their uniqueness.

Caves represent complex, environmental systems with historically established tunnels. As examples of the negative impact to caves, we can refer to tunneling in Kungur Cave (Lukin and Dorofeev, 1993, Mavlyudov, 2008) and the construction of a solid concrete wall in Karaulnaya-2 and Borodinskaya caves in the 1970s that resulted in degradation caused by perennial icing (Tsykin, 1978, Mavlyudov, 2008).

Extensive tourist visits increase the inflow of carbon dioxide into the cave environment, thus influencing the processes of corrosion and mineral formation in the cave. Among others, this problem was characteristic of Novo-Afonskaya Cave (Dublyansky, 1978) and Mramornaya Cave (Vakhrushev et al., 1999). The cumulative effect of the temperature increase, artificial light, spores, seeds and other biological material, brought in by the visitors, may result in so called "lamp flora" – initiation of chlorophyll formation and beginning the growth of moss, fungus, and ferns (Bartoli, 2003).

Lamp flora was found in many caves equipped with artificial lightning. Plant and bacterial communities inhabiting the cave surfaces cause degradation processes, which are especially damaging in caves with Paleolithic paintings. In addition, many types of bacterial flora are potentially pathogenic for humans and may cause allergies (Mazina, 2007).

Unfortunately, many caves with artificial lightning use incandescent-filament lamps as a light source. The artificial light flora is a problem in Kungur Cave (Naumkin et al., 2004), Vorontsovskaya Cave (Mazina, 2007), and other caves. Some tour guides, bringing visitors to Kungur Cave, initiated the tradition of throwing coins into underground lakes (Rapp, 1999). During early use of Shulgan-Tash Cave for tourism and recreation (prior to more stringent regulations on tour guides and cave security in the 1990s), the graffiti left by visitors covered more than 90 percent of accessible surfaces, significantly damaging the original Paleolithic paintings.

The issues of safe and secure use of caves are not sufficiently discussed in the literature (Maksimovich, 1963, Dublyansky, 1990, Dublyansky and Dublyanskaya, 2004). Legal aspects of cave-based tourist and excursion complexes are formally considered in the works of V.D. Rezvan (Rezvan, 2004). However, not all the cave trails are licensed (Lyakhnitsky, 2002).

The notion of cluster was initially mentioned and analyzed by Michael Porter (2000). According to his definition, a cluster is a "geographic concentration of competing and cooperating companies, service providers and associated institutions in a particular field, linked by commonalities and externalities mutually reinforcing each other" (Porter, 2000, p. 16). Porter stressed that successful cluster development requires strategic development. The cluster formation is mainly aimed at the economic development of the local businesses, based on a long-term (5–10 years) development strategy. The Russian scientist A. Yu. Aleksandrova devised the concept of cluster theory for international tourism development. Based on the existing aspects of tourism industry (i.e., range of inter-industry relations, its fragmented structure, prevalence of small and medium-sized businesses, non-material nature of the tourist product, and others), the author stresses the applicability of the cluster approach for tourism development (Aleksandrova, 2007).

Today, the idea of cluster approach as the model for regional tourism development is extensively discussed in Russian science. This issue is addressed in the works of N.V. Shabalina (2007), A.E. Illarionov (2008), and Yu.P. Kovalev (2011). Particular attention to the formation and development of the strategy for regional tourism development (ethnic, rural) is discussed in the works of A.N. Polukhina (2010, 2012, Polukhina and Talalaev, 2014). The theory of clusters used for the concept of regulated use of national heritage sites in tourism is understudied and requires further elaboration.

Study Area

The number of caves used for recreational purposes in Russia is 14. Unfortunately, they are often exploited and without any legal protections or operating agreements obtained from state authorities. An even more serious violation is the absence of research-based development projects with a detailed and regulated visiting plan.

Planning an underground tourist trail should be based on detailed and well-grounded research results: passage morphology, cave roof structure, hydrology, hydrochemistry, mineralogy, microclimatology, microbiology, background radiation, radon dangers, etc. It is evident that speleological engineering is the basis for scientific speleology. No commercial tourist company or speleologist specializing in tourist caves can solve this task on their own.

In the Caucasus, the Urals, and eastern Russia, there are areas with well-developed karst landscape. Also, in the Pinego-Kuloisky district of the Arkhangelsk Region there are a few gypsum caves. In the Caucasus, in Sochi National Park and Logonaki Plateau, there are Vorontsovskaya and Krestik-Turist caves, which are over 10 kms long. In the eastern Sayan mountains there is the largest-in-area cave in the world, known as Oreshnaya Cave, which is about 60 kms long. On the tributary of the Oredezh River in the Leningrad Region, close to V. Nabokov's residence, there is a unique erosion "Svyataya" (Sacred) cave. It is a picturesque grotto with a small stream. By its size and aesthetic characteristics, it is equal to some karst caves in the Caucasus or the Urals. There are no organized excursions or proper security, even though this pseudo-karst cave is worthy of tourist attention.

Kungur Cave is located in the suburb of Kungur city in the Perm region. It is formed of gypsum and anhydrite of Permian age in the massive Ledyanaya Mountain. This cave is the most popular and the most visited cave in Russia. It has been extensively studied. The first mention of the cave dates to 1703, and it has been the focus of on-site research since 1948. In 1972–1973, a 109-m-long tunnel was excavated into the grotto Vyshka. The gallery leading from Bolshoe (meaning Large), an underground lake, was equipped with electric lighting (Lukin and Dorofeev, 1993). The cave was studied for the purpose of learning more about the microclimatic, hydrological, and hydro-chemical factors of the cave (Kungur Cave, 2005).

The cave and the adjacent territory are included within the historical and nature preserve complex known as "Ledyanaya Mountain and Kungur Ledyanaya Cave" that was established by the edict of the Governor of Perm region No 163, dated 26 June, 2001 (Naumkin et al, 2004).

The Levoberezhnaya Artificial Cave (Left Bank Artificial Cave) is located in Leningrad region, at a distance of 40 km from St. Petersburg on the territory of the Sablinsky state complex natural monument. The cave represents an ancient excavation of 5.5 km, where the mining of quartzose sandstone began about 150 years ago for the glass industry. Inside the cave there are three lakes, a stream, and numerous landslide halls. Sablinsky State Complex was established in 1976, although it was not properly protected, which inevitably lead to its degradation. In 1992, driven by a public initiative among speleologists, geologists, and ecologists, the authorities of the Leningradskaya Region created

Figure 2. “Svyataya” Cave in Rozhdestveno, located on the tributary of River Oredezh. A rare case of pseudo karst grotto developed as a result of Devonian sandstone erosion and suffusion.

the Sablinsky Nature Preservation Excursion and Tourist Center. The work was initiated and supervised by Yu. S. Lyakhnitsky. All tour guides working in the cave use flashlights; however, this weak-working light compromises tourist’s safety. To preserve the cave and guarantee safety of the visitors, the hydrologic and microclimatic environment of the cave is carefully monitored and supported.

There are Paleolithic painting exhibits from Kapovaya Cave, model site of the Stone Age man sculpture composition, demonstrating a process of sandstone mining and mineralogical mining. In October 2005, one of the cave rooms was turned into an underground chapel of St. Nicholas the Wonderworker. On religious holidays the church priests hold religious services in this chapel. There was also a primary reconstruction of the surface trail, especially limestone staircases constructed in the steep, canyon paths, which caused ecological excursion trails to be broken. At the entrance to the cave next to the gatehouse, there is a display of ancient animals: trilobite, or thoceratites, ammonite, guard fish, dinosaurs, and mammoth. Large concrete sculptures highlight minor details of the anatomy of these ancient animals to create the picture of fauna evolution on our planet. The natural monument annually accommodates more than 40,000 people, mainly school children. Thus, this self-supporting economic plan allows the people involved both to educate the public and protect the nature complex, preserving the resident bat population.

Pyatigorsky Proval mine is located within the boundaries of Pyatigorsk city (Stavropol Territory), on the southeast shoulder of Mashuk Mountain (Dublyansky, 2008). The mine was mentioned in the novel *Knyazhna Mary* by the famous Russian writer and poet M. Yu. Lermontov. People used to believe in the healing properties of underground waters and descended to the underground lake, which contains hydrogen sulfide. Mr. Lermontov was one of the first who volunteered to experiment with this theory. In 1858, a horizontal 43.8 m long tunnel was built into the cave, which made the underground lake accessible to visitors (Maksimovich, 1980).

Another cave, called Bolshaya Azishkaya, is located in Krasnodarsky Territory in the southern part of the Azish-Tau Mountain chain (next to Lagonagsky highland) at a height of 1,600 m above sea level (Samoilenko, 2001). According to B.R. Mavlyudov (2002), the foot traffic varies from 5,000–7,000 people per year. The cave is equipped with electric lighting, metal passageways, and staircases. Initially, the cave was lit by filament lamps that are now being replaced by light-emitting diodes (LEDs). The trail has been functioning since 1989. S. P. Lozovoj in his book, *Lagonar Upland,* describes the terrible condition of the cave, resulting from its uncontrolled visitation before security provisions and regulated tourist application. “The cave was littered with broken glass, trash, bottles, with damaged stalactites and stalagmites. Over 1,000 dripstones were destroyed.” The situation is currently improving. New passageways and staircases have been constructed. Unfortunately, the choice of construction material (metal) was not properly selected. In the course of time, the metal passageways will become unfit for use. They should be replaced with corrosion resistant materials.

As an example of a positive geological heritage preservation story, we may refer to the project aimed at preserving the unique East European Paleolithic paintings in Karpovaya Cave (Shulgan-Tash) in Bashkortostan. It is located on the Belaya (White) River, in the National Park Shulgan-Tash (Lyakhnitsky and Chuiko, 1999). The site is a complex natural monument of world value, the only cave in the Russian Federation that still contains various ancient paintings in relatively good condition. The scientific estimate for the age of these paintings is greater than 17,000 years. In the near past, the cave was visited by tourists, who chose their own unequipped trails to see the paintings. Shulgan-Tash Cave is one of the oldest excursion caves in Russia. The first tourist visits date to the end of 19th century (Kudryashov, 1977, Kichaeva, 2004).

In 1959, the Natural Park zoologist, A.V. Ryumin, discovered the Late Paleolithic-age cave paintings. As a result of his discovery, the cave instantly became the focus of tourist and scientist's attention on a national and international level. Soon after the discovery and after the spread of the legend on the healing qualities of underground waters and clay, a large number of sick people from all over the former Soviet Union began coming to Shulgan-Tash. In 1967, a new tourist itinerary, No. 57, was launched on the Belaya River that included a visit to the cave. As a result, the anthropogenic impact on the site has significantly increased. I.K Kudryashov provides a description of the unsanitary condition of the cave at the time it was first inspected during the summer of 1977: "Here and there you can see different items left by the sick people: glass jars, bottles, wood pallets, paper, left-over food. In some places it is hard to breathe due to unpleasant odor."

Today, due to the efforts of the Ministry of Culture and National Policy of Bashkortostan and with participation of experts from A. P. Karpinsky All-Russian Institute of Geological Research and the Russian Geographical Society, it was possible to develop a local tour that included visiting a small part of the cave close to the entrance, thus limiting access to authentic paintings located deep inside the cave. Tourists enjoy numerous copies of ancient paintings, a huge Main Gallery, and an entrance grotto called "Portal." Currently there is a precisely careful and scientifically well-grounded design plan for a second tourist itinerary that involves a vertical aspect in addition to the horizontal tour. Visitors will be able to come up to the intermediary terrace of the gallery and enjoy a view of the entrance to the cave from above, which is meant to enhance the emotional impact of the visit. The concept of the first regulated itinerary was approved by French experts who visited the cave in 2004. Unfortunately, a few paintings were destroyed by the incoming water. Consequently, new measures have been taken to correct the hydrological regime and microclimate of the cave. It is also planned to restore and provide better security for the paintings. Beside the actions already carried out, there is a plan to create a contemporary Historical and Archaeological Landscape and a Speleological, Cultural and Educational Center dedicated to this unique monument of natural and cultural heritage.

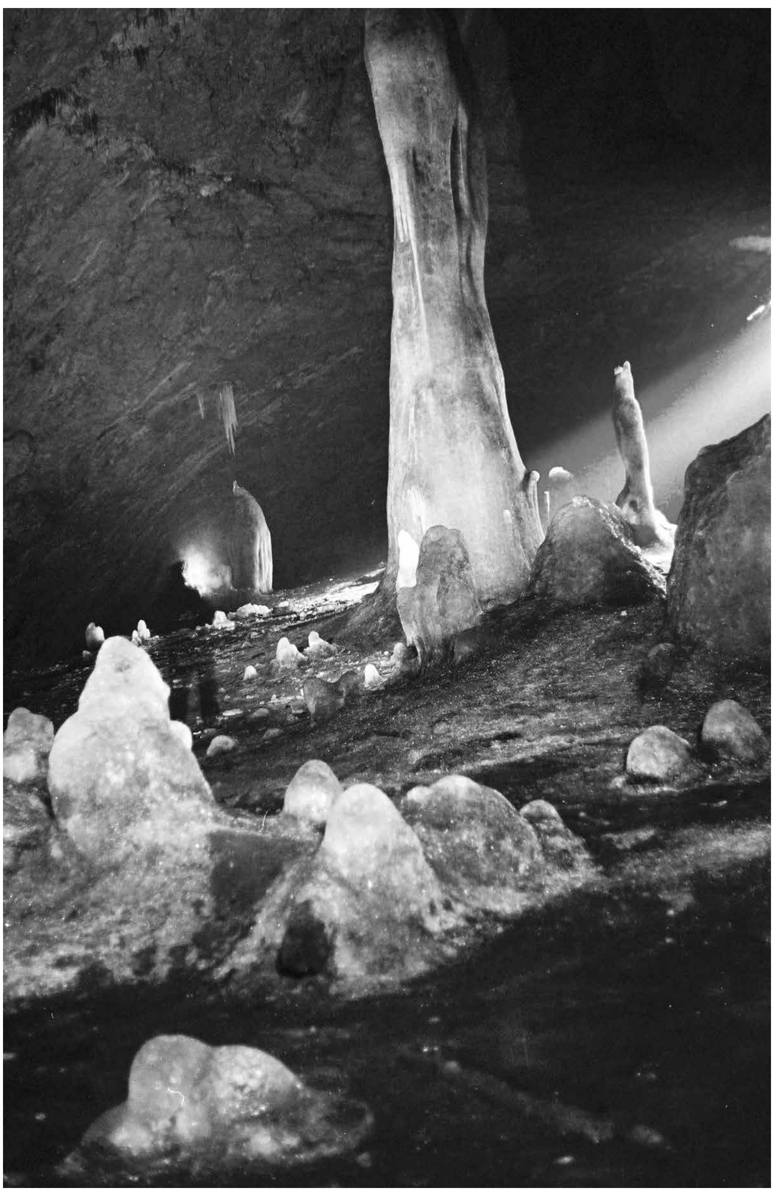

Figure 3. Askynskaya Ice Cave in Bashkortostan. Longstanding ice stalagmites and stalagnates are preserved due to microclimate of the cave.

We are justified to state that implementing this project will become a very valuable contribution to protecting natural monuments in the Russian Federation. Thus, using systematic research, regular monitoring, and careful implementation of a short tourist trail, it was possible to save the ancient paintings and Karpovaya Cave itself.

Generally speaking, it is important to reiterate that even sites that are specified as nature preserves are not properly guarded, usually understaffed, and lack proper management. The status of specially protected natural areas and the implementing documents alone cannot replace actual protection and security on site. In this respect, we have to admit that mass violations of the security regime are done by both general public and state authorities. In practice, there is a huge number of examples of natural monument degradation that was the result of such an irresponsible attitude. Even with all these negative tendencies occurring, it is still possible to improve the situation. Back in the 1990s, Yu. S. Lyakhnitsky suggested creating a nature preserve and tourist center, a natural museum based on a self-supporting financial plan (Lyakhnitsky, 2002). However, there is a great number of organizational, legal, and financial challenges to establishing such a center. The initial stage should be supported by the state. The Sablinsky

Figure 4. Long-eared bat wintering in Levoberezhnaya Cave. The adopted cave protection and excursion practice does not interfere with the natural wintering of chiropterous animals.

natural monument was selected as the first such experimental site.

Sablinsky Nature Preserve and Tourist Center was created within the existing legislation and has been successfully functioning for more than 20 years. During this period, it was visited by a 100,000 school students and families. For most visitors it was a unique experience that allowed individuals to discover the beauty of nature, geology, ecology, and speleology. The educational value of a site visit is considerably more effective than the museum and classroom experience alone.

Methods

The methods applied can be classified into geological and social-economic. The first group of methods is aimed at doing baseline research of the future tourist destination. Thus, special attention should be paid to measures for preserving the Paleolithic paintings under conditions of increased, regulated excursion and tourist activity that would occur with an improved and updated tourist itinerary.

To achieve these goals, it is necessary to conduct complex monitoring procedures using hydrologic, hydro-chemical, gas, geo-ecological and microclimatic studies (temperature, humidity, airflow, condensation, heat and mass transfer) to develop and implement measures with the purpose of creating favorable, microclimatic conditions.

The second group of methods is concerned with research into operational conditions of the future tourist cluster, created for the specially, protected natural area (i.e., the cave). Systematic methods and comparative analysis, supported by statistical methods, are needed to identify the contemporary conditions and predict the impact to the local economy by development of the area under study. Methods of evaluation and cluster analyses by experts make it possible to design a business model for development of the future tourist cluster. Application of in-depth interviews and other social and psychological examination methods are necessary to monitor public opinion and constructively communicate with the local community, which is an essential element of any tourist cluster.

Results

The experience gained and the material collected makes it possible to address a very important challenge: to design nature preserve excursion and tourist centers taking into consideration the development of infrastructure to support visitation of complex natural sites. We would suggest using the concept of regulated use of natural heritage sites developed by Yu. S. Lyakhnitsky when establishing a regional tourist cluster. Taking into consideration the special importance of a natural site should be the foundation of the tourist product at the initial stage of development with the express purpose of preserving the natural site.

Yu. S. Lyakhnitsky suggests establishing a monitored and regulated nature preserve and tourist center on the territory of a specially protected natural area or cave complex. The main operational principals of this center are as follows:

1. Design a research-based professional plan that would rely on procedural rules. Research should include the following:
    - Cartographic work;
    - Detailed geological mapping of the cave and surrounding locality (maps, plans and profiles);
    - Mining and engineering research of the cave structure, to identify trouble areas for the possibility of rock slides; develop measures to strengthen those areas and select safe trail routes;
    - Hydro-chemical research to check natural water levels aggressively; pollution, contamination, etc.
    - Radiation and radon research, necessary to provide tourist and staff safety;
    - Gas monitoring for the contents of CO^2, CO, methane, and other gases in the cave.

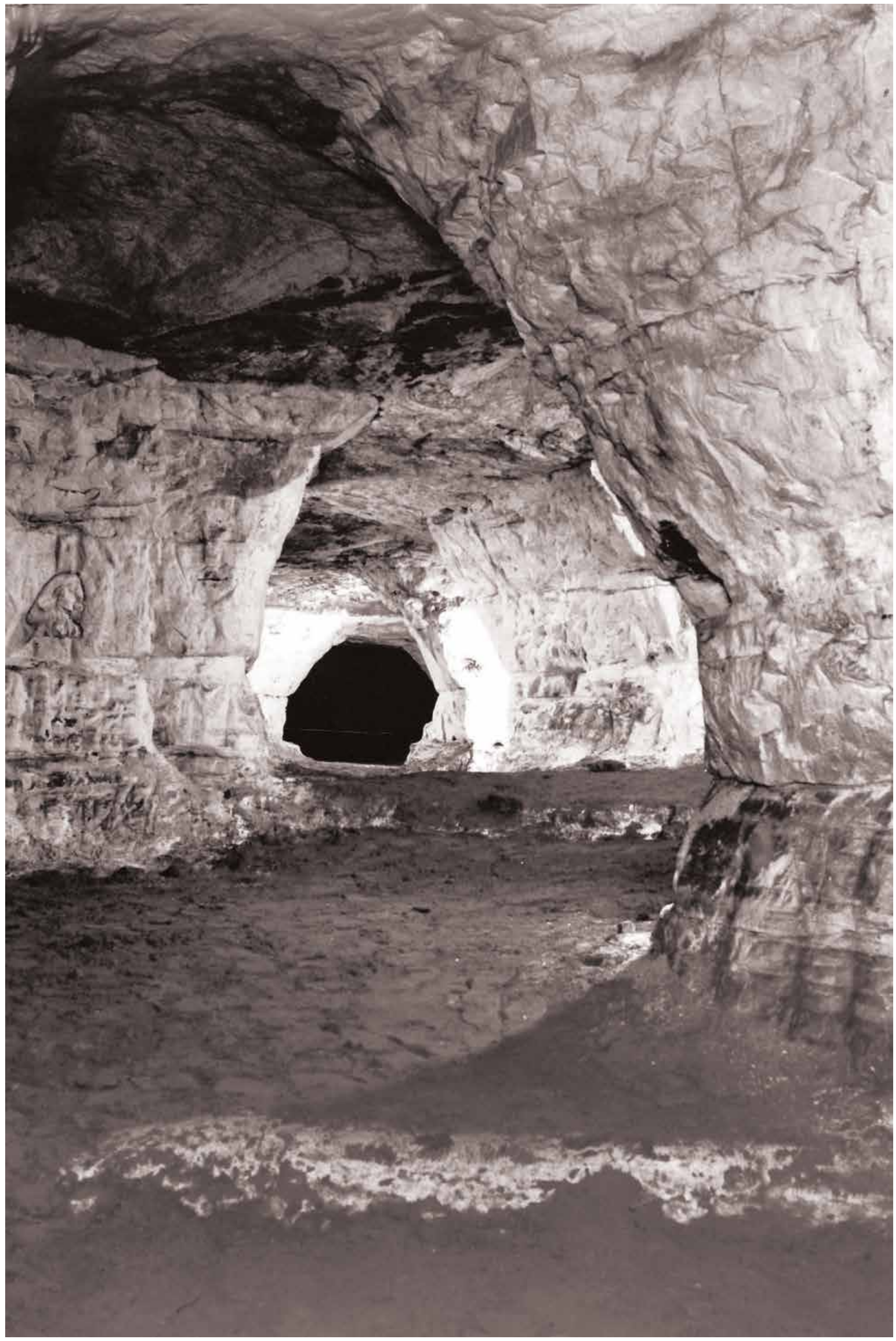

Figure 5. Labyrinth of Levberezhnaya Cave. The cave walls consist of the white, Cambrian "glass" sandstones of Sablinskaya series; the cave roofs consist of Ordovician ferruginous, cross-stratified sandstone.

2. Nature preserve excursion and tourist organization should be functioning in agreement with the subject of the Russian Federation (territory or the republic). In this respect, the expertise of staff involved is very important: paleontologists should be responsible for the fossil fauna and speleologists should be responsible for the caves. Should a commercial company be involved in tourist use of the cave, it should be obliged to make agreements with professional scientists to develop reasonable projects and monitor the environmental conditions of the site.
3. Financial self-support of the site or minimization of costs. The efforts of companies to develop the cave as a tourist attraction should be based on the principle of self-funding.
4. Proper zoning of the cave, allotting areas for different use and degree of protection, should include the following: reserved, unattended, tourist and recreational areas. To be properly preserved and protected, tourist and

Figure 6. The first gallery of the Kapovaya Cave as an example of large, underground canyon in carbonic limestone. Figures of people can hardly be seen in the huge cavern.

recreational areas should be smaller in size in comparison to the total size of the cave. However, this can be achieved only in large cave complexes. Small caves (in terms of volume and chambers) can be visited by small, guided groups or individual tourists without establishing tourist complexes around them. Nevertheless, small caves also require regulated tourist use.

5. Distribution of tourist impacts on a specially equipped excursion, environmental, or recreational itineraries (i.e., environmental excursion trails).
6. Creating properly managed tourist areas with displays. Limitation of excursions in accordance with the standards of anthropogenic impacts and continuous monitoring.
7. Application of nature preserve's geo-ecological and educational principles when carrying out research activities in caves. Complex program development aimed at protection and use of variable tourist itineraries. "Horror" caves might improperly educate the youth, and restaurants located inside caves will pollute the environment, causing harm to the natural cave processes. This kind of use inevitably leads to degradation of the cave environment and deprives the public of a natural experience.
8. Tourist infrastructure and service industry facilities should be located in adjacent or urbanized areas.
9. Minimized administrative staff.
10. Organization and coordination of associated advertising, educational activities, and souvenir and printed material emphasizing selling points.

The points mentioned above suggests the necessity of establishing a special unit that would monitor natural, geological monuments with caves. This state unit would take responsibility for regulating protection and use of natural, geological monuments (caves) with Research and Development Centers of the Ministry of Culture located in the subjects of the Russian Federation.

The authors suggest establishing a tourist cave cluster of natural geological monuments. Nature preserve and tourist centers described by Yu. V. Lyakhnitsky (2002, 2006) can be taken as the basis of M. Porter's "root" organization. Right now, a contemporary nature preserve and tourist center is being established based on Kapovaya Cave. The status of this object is the Federal Museum Reserve. In order to monitor the condition of the natural monument and its proper protection, it is necessary to have continuous speleological, geological, and ecological monitoring.

When establishing a tourist cluster, where the nature preserve and tourist center act as the tourist attracting component, other commercial and non-commercial organizations are interconnected. We refer to commercial organizations as those providing accommodation and catering services. However, it should be taken into consideration that their location should not degrade the cave complex, i.e., tourist infrastructure should be located at a short, but reasonable, distance from the site (this does not apply to bio-toilets, that should be at a walking distance from the tourist itinerary or trail, but not cause pollution to the immediate environment). Souvenir and other types of shops (jewelry, arts and crafts, local products) connected with the complex should be properly located. This allows more stakeholders to be included in the social and economic process, and gain their loyalty to the growing number of tourists, which will keep their interest in the end result. On the other hand, this aspect significantly increases the net profit margin of the project, and provides a positive feedback effect.

The role of non-commercial entities is also very important. These include various non-profit organizations, such as educational, or social and rehabilitation services. The impact of these organizations

Figure 7. Paleolithic composition "Horses of the Chaos Hall" as an example of the Paleolithic art unique for eastern Europe. In the picture, we can see two, semi-abstract horses and an abstract symbol typical for the Karpovaya Cave–a kind of trapeze.

is hard to overestimate, especially in the low, populated regions, as they exert a strong, positive (or negative) influence on the public opinion.

Tourist clusters in the Russian Federation cannot exist without the governmental support, which first requires adoption of legislative and normative acts, that would regulate the operation of nature preserve and tourist centers, or the whole tourist cluster of a region.

In this respect, we will refer to the Sablinsky nature monument case study. At the beginning of the 1990s, when the western countries were still discussing the "geopark" concept, a group of St. Petersburg scientists, supervised by Yu. S. Lyakhnitsky, took some practical actions to save the Sablinsky nature monument. The area received status as a Nature Monument in 1976, but it was not properly managed or protected at the time, which led to its degradation. The area surrounding the specially protected, natural area was built-up; it contained a lot of waste deposits, the caves collapsed, and others were occupied by trouble-makers—religious fanatics and drug addicts; making it was dangerous to organize guided tours there. The situation is quite different today. Sablinsky Complex Nature Monument is one of the most valuable monuments in the North-West of Russia. It is located 40 kms from St. Petersburg. There are 14 artificial caves (former mine openings significantly altered by natural processes), two waterfalls, the Sablinka and Tosna river valleys, paleontological and mineralogical objects, mineral sources and other tourist attractions connected with the history and culture of the Russian Federation. In 1992, the work of creating Sablinsky Nature Protection Tourist Center was initiated by the public, and the relevant decision was made by the authorities of the Leningradskaya region. The key concept of the decision is organization of the actual monitoring and protection of the specially-protected, natural area using the resources from regulated, tourist excursion activity. The project was approved and received the funding for implementation. Thus, the process of regulated monument development began.

In Levoberezhnaya Cave, there was an equipped underground tourist trail that included strengthening unstable areas, concrete casing of entrance headwalls, regulation of hydrological and microclimatic regimes, removing a tourist trail, and mounting protective screens in the most valuable parts of the monument.

To control and protect the monument, a public, non-commercial organization was established. It included specialists from the fields of speleology, geology, ecology, tourism, and cultural professionals. Nowadays, Levoberezhnaya Cave is properly supervised and guarded around-the-clock; the adjoining area is regularly patrolled; there are around-the-season tour itineraries (a few options). Tourists can choose bus or walking tours, speleological hiking trips that include boating on the underground lakes, etc. In addition to the cave, visitors have the opportunity to enjoy two canyon-shaped river valleys, waterfalls, picturesque rocks, ferriferous minerals, and sulfurated sources. The area is complemented by unique tourist attractions, i.e. the standing post of Tsar Alexander Nevsky before his famous battle with the Swedes. Premise security is carried out by preserving the existing level of anthropogenic pressure on urbanized areas, without increasing the number of visitors. Specially protected areas are kept free from excessive tourist visits.

In general, the experience gained from establishing Sablinsky Nature Preserve and Tourist Center demonstrates that the selected direction first, improved the condition of the object, and serves both educational and aesthetic purposes, and second, improves the general environmental and social situation in the region. In fact, Sablinsky represents the first "geological park" that is much spoken about, but very little is being done to put these ideas into practice. Thus, we have to stress that the center was established within the current legislation and keeps functioning despite the currently emerging challenges. For the past year it has become a very popular visiting site for school students, families, and people of different ages and professions. It is especially valuable that the largest visiting cohort are young people who realize the beauty of the nature, geology, ecology, and speleology after coming to the place.

Discussion

It is evident that the problems discussed in this paper, even though the reasons are apparent on the surface, do not have easy solutions. We identified the major obstacles hindering the implementation of simple and efficient measures, leading to establishment of nature preserve tourist centers and tourist clusters around cave complexes, and their successful functioning and reliable protection. These are as follows:

1. Lack or absence of initial financing from the state budget or nature protection authorities.
2. Absence of real state nature protection policy in the issues of establishment of nature preserve tourist centers and tourist clusters.
3. Legal obstacles impeding establishment of new, specially-protected nature areas. Position of local authorities that prioritizes immediate profit to environmental processes by selling forest resources, and lending parts of specially-protected areas by breaking the specific character of their use.
4. Passive position of scientists, lack of self-confidence, that they can independently organize nature preserve tourist centers, and corruption on different levels of power from municipal to the federal levels.
5. Absence of generally-accepted federal, scientific and methodological norms of use and protection of the objects of natural and, especially, geological heritage. The municipal and regional committees of nature manage-

ment experience lack of the legal framework; the civil servants have nothing to rely on in their everyday work with specially-protected nature areas.

6. Insufficient local public awareness efforts on the value of nature preserve tourist center and tourist cluster development. No opportunities for local initiatives to manifest on the levels of legislation and finance.

Nevertheless, certain positive examples make us believe that the solution to this problem is still possible.

Conclusion

There are noticeable, positive tendencies in protection and use of natural monuments. However, for successful development of this process, and to save the geological heritage of Russia, it is necessary to activate state support. The strategy of preserving Russia's natural heritage is aimed at implementing the concept of regulated use of natural sites, in developing research and methodological directives on the state level, and at least minimum funding of the process of nature preserve tourist centers and tourism clusters establishment, while the tactics are to activate practical work on a regional level. The driving force behind the process are scientists, on the one hand, and nature preserve state authorities, on the other. It is also necessary to keep in mind that natural monuments, representing aesthetic and intellectually important elements of the landscape, exert an important emotional and educational influence on children and young people, and therefore, are ethnically formative and supportive factors. The concept of providing a reliable security of natural geological monuments, using the funds obtained from regulated tourist activity, on condition of professional approach and localization of anthropogenic pressure, is justified. It can act as the basis for saving the natural objects of the Russian Federation. Establishing a tourism complex in the area on the basis of caves raises local tourist appeal. Additionally, it can help with overcoming bureaucratic obstacles in development of the tourist potential of caves.

Acknowledgements

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TEMPERATURE AND REVERSE-FLOW PATTERNS OF THE RIVER STYX, MAMMOTH CAVE, KENTUCKY

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Abstract

River Styx and Echo River are two, closely-associated underground rivers located in the Mammoth Cave System. Both rivers are home to a variety of aquatic cave life, including the endangered Kentucky cave shrimp (*Palaemonias ganteri*). Typically, both underground rivers emerge at their respective springs and flow into the Green River. During flooding conditions, the Green River can back up into its subterranean tributaries, including the River Styx and Echo River. In addition, the hydraulic gradient in the River Styx can reverse under non-flood conditions of the Green River and create a stable, reverse-flow pattern. This phenomenon was identified at least as early as the 1920s, when a dye trace study noted that, after a rain event, Green River water was flowing into River Styx Spring and coming out at Echo River Spring. However, detailed studies on the River Styx's stable, reverse-flow patterns were not conducted until the 1950s, and little additional research has been conducted. Water temperature data were collected between October 2009 and October 2012 on the River Styx, Echo River, and Green River. During the study period, the Green River had a mean water temperature of 15.5 ± 7.2 °C, while River Styx and Echo River had cooler and more stable mean water temperatures of 13.5 ± 2.7 °C and 13.4 ± 0.6 °C, respectively. Water temperature was used as a proxy for determining whether the River Styx was flowing forward (out of the cave) or backward (into the cave). Periods of time when the River Styx was flowing into the cave were classified as being due to back-flooding or a stable reverse-flow. During the times when data were available for all three rivers, the River Styx flowed out of the cave 77 % of the time, was in a stable reverse-flow 17 % of the time, and it was back-flooding 3 % of the time. These results differ from the original studies' results that identified the River Styx's stable, reverse-flow pattern. The different results could be due to anthropogenic influences on the Green River and/or due to differences in precipitation patterns, possibly as a result of climate change.

Introduction

Karst topography can be found throughout most of south-central Kentucky. As in other karst landscapes, much of the precipitation that falls in south-central Kentucky quickly infiltrates into caves and smaller subterranean passages. The meteoric waters then join cave streams and rivers that flow underground until they emerge at springs located on, or near, one of a relatively small number of perennial streams or rivers. Each spring is the outlet of a karst basin, encompassing the karst catchment area that feeds the associated cave streams during times of normal, low karst flow. During periods of high karst flow, water from one karst basin can spill over into a neighboring karst basin if upper-level connecting passages are available.

Mammoth Cave National Park (Fig. 1) is located in south-central Kentucky and is home to the longest known cave in the world, the Mammoth Cave System. The park is bisected by the Green River (Fig. 2), which is the master stream for south-central Kentucky and is approximately 618 km long. Six modern underground rivers flow through the Mammoth Cave System and are subterranean tributaries of the Green River. Two of Mammoth Cave's underground rivers are the River Styx and Echo River. Both underground rivers are home to many cave-adapted, aquatic organisms including: cave crayfish (*Orconectes pellucidus*), two species of eyeless cavefish (*Typhlichthys subterraneus* and *Amblyopsis spelaea*), and the endangered Kentucky cave shrimp (*Palaemonias ganteri*), which is endemic to the Mammoth Cave region.

Under base-flow conditions, the Echo River karst basin (21.7 km^2) and the River Styx karst basin (2.2 km^2) are almost completely within the park's boundaries (Mammoth Cave data, 2017). However, during periods of high karst flow, waters from the Turnhole Bend karst basin (254.4 km^2) can overflow into the neighboring Echo River karst basin (Meiman, 2006). When this occurs, water from the primarily agricultural lands surrounding the park enters the Echo River karst basin and brings with it potential contaminants from outside of the park. Under flood conditions, water from the Green River backs up into all of its tributaries, including Echo River and River Styx. These back-flooding events can bring contaminants that entered the Green River upstream of the park into the cave system.

Greensburg, Ky. (Fig. 2) is more than 100 river-kilometers upstream on the Green River from Mammoth Cave National Park. In 1958, oil-drilling operations increased near Greensburg. The drilling operations caused a significant increase in the chloride concentration of the Green River (Brown, 1966). These concentrations could still be detected when

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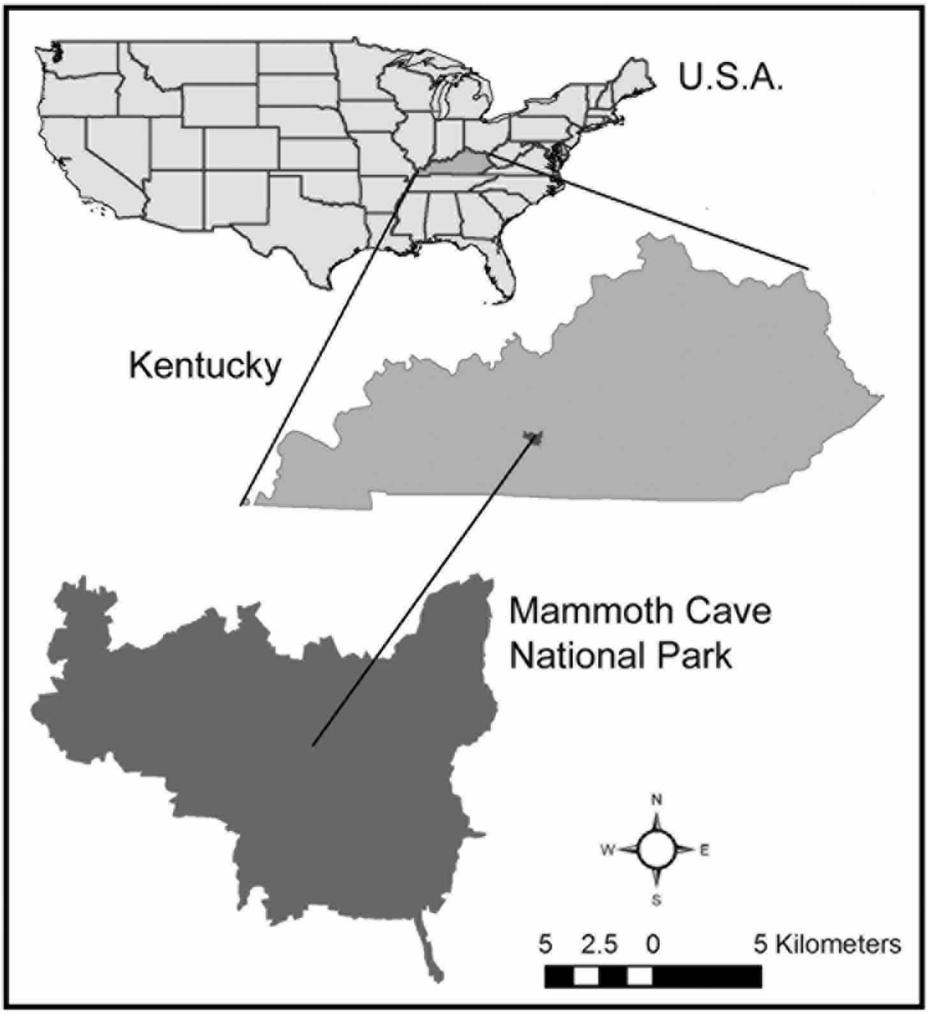

Figure 1. Location of Mammoth Cave National Park within Kentucky and the United States of America.

the Green River reached Mammoth Cave. Between 1958 and 1959, the USGS conducted an extensive hydrological study of the Mammoth Cave area, using the increased chloride content of the Green River as a tracer (Hendrickson, 1961; Brown, 1966). The USGS study found that the River Styx frequently flows backward, even when the Green River is not flooding (Hendrickson, 1961; Brown, 1966). When the River Styx flows backward, Green River surface water flows into the cave through the River Styx Spring. The backward flow of the River Styx forces it to reverse the direction of its flow, cross into the Echo River karst basin, and flow out of Echo River Spring (Figs. 3 and 4).

The River Styx's reverse-flow events can be due to Green River flooding or they can be a stable, relatively long-term, divergent-flow pattern that occurs even when the Green River is not under flood conditions. For the purposes of this paper, reverse-flow events, due to Green River flooding, are referred to as back-flooding events, while reverse-flow events that occur when the Green River is not flooding, are referred to as stable, reverse-flow events. The more general phrase, reverse flow, is used when no distinction is being made between back-flooding events and stable, reverse-flow events.

Stable, reverse-flow events typically take place when the Green River is at a higher stage than the River Styx and when the karst basin is at low flow (Hendrickson, 1961; Brown, 1966; Meiman, 2006). These conditions are more common in the winter. Thus, stable, reverse-flow events are more common during the winter. However, stable, reverse-flow events also occur in the summer when the necessary hydrological conditions are met.

Understanding the River Styx's reverse flow patterns is important because they can affect the biological, geological, cultural, and archeological resources in the cave. Meiman (2006) reported distinct changes in the water quality of the River Styx and Echo River during River Styx reverse flow events. During the River Styx's reverse-flow events, any chemical contaminants, found within the Green River, when it reaches the River Styx Spring, are circulated through the River Styx and the affected portion of Echo River. These contaminants have the potential to impact the aquatic ecosystems found within the two underground rivers; however, those potential impacts have not been quantified.

When Green River water enters the cave, it also carries non-troglobitic fish (Ruhl, 2005) and nutrients from the surface into the low-energy, cave aquatic ecosystem. In moderation, increased nutrients can be a boon to the ecosystem; however, too many nutrients can be a detriment to such a low-energy system. The overall impact of the non-troglobitic fish is unknown because they have the potential to provide additional nutrients when they die, or to consume or compete with troglobitic species, depending on how long the non-troglobitic species survive in the cave.

During the winter, reverse-flow events bring cold water from the surface into the cave. The cold surface water cools the River Styx and can create condensation or fog in the nearby passages. In the summer, reverse-flow events bring warm, surface water into the cave and can have impacts similar to those seen during winter reverse-flow conditions. However, the seasonal differences in the cave airflow patterns cause condensation to occur in different locations during summer and winter reversals. Anecdotal observations by park staff and partners suggest these micro-climate changes could be fairly significant and extend quite a distance away from the immediate River Styx area.

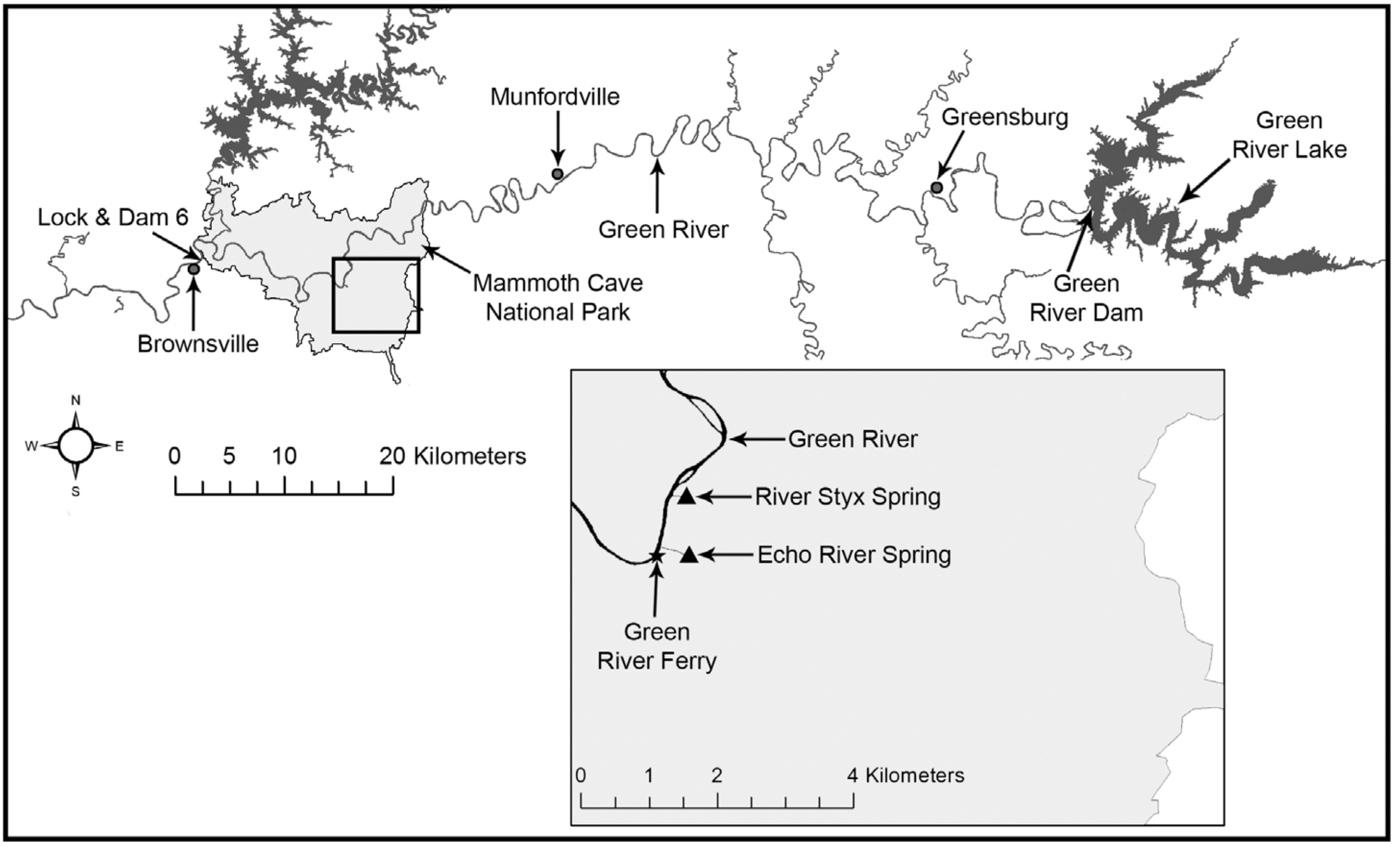

Figure 2. Regional overview with inset showing locations of River Styx Spring, Echo River Spring, and Green River Ferry in Mammoth Cave National Park.

Condensation due to seasonal weather patterns and anthropogenic changes to the Historic Entrance have resulted in increased fungal growth on archeological artifacts and cultural resources in Mammoth Cave's upper passages (Olson, 1996). We speculate that condensation and fog, resulting from reverse-flow events in the River Styx, may have similar effects on archeological and cultural resources in the lower passages of the cave. Research to quantify the micro-climatic changes in the lower passages, due to River Styx reverse-flow events, was recently begun (French and Trimboli, 2017).

The colder or warmer surface water, depending on the season, coming into the cave could also influence the behaviors of cave aquatic organisms that live in the affected portion of the underground rivers. For example, cave aquatic organisms could temporarily or permanently avoid areas of the underground rivers, where the temperature was too cold, too warm, or too variable. In fact, Edwards (2009) found temperature changes to be the most pronounced indication of reverse-flow events, when compared to turbidity, specific conductivity, and pH.

With the exception of the USGS (2015) study, there has been little research published on the River Styx's reverse-flow patterns. In 2008, a seventh-grade science teacher from T. K. Stone Middle School contacted the Mammoth Cave International Center for Science and Learning. She was interested in opportunities for her students to conduct research at Mammoth Cave National Park. In the fall of 2009, T. K. Stone Middle School and the Mammoth Cave International Center for Science and Learning partnered to study River Styx's reverse-flow patterns. Trimboli et al. (2011) provided details about the development of the project and lessons learned from conducting research with students. The current paper provides an in-depth analysis of the data collected through this research partnership.

Study Area

The Green River (Figs. 2, 3, and 5A) is the master stream for most of south-central Kentucky. It is heavily influenced by anthropogenic controls over much of its length. Green River Dam, located approximately 169 river kilometers upstream of the Green River Ferry (Fig. 2), is the primary anthropogenic control on the Green River. The U.S. Army Corps of Engineers completed the dam in 1969. Though the dam was built primarily for flood control, it also created the Green River Lake Reservoir, which has become a popular recreational spot. The timing of water releases and the amount of water released from the dam heavily influences the downstream level of the Green River, irrespective of local precipitation patterns.

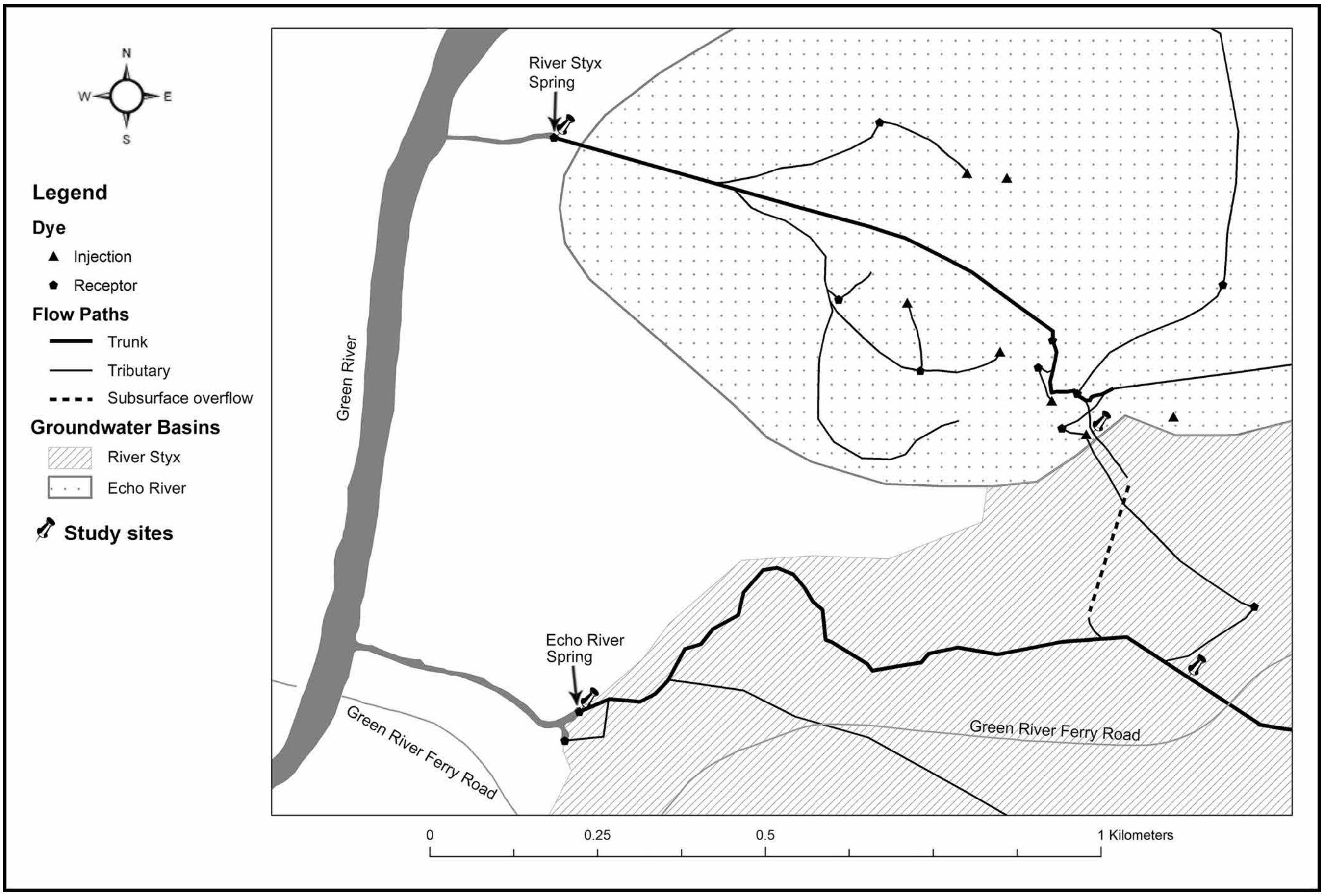

Figure 3. Close-up of the study area, showing a portion of the River Styx and Echo River drainage basins and the flow paths of each river, as determined by previous dye trace studies. When the River Styx flows backward, it flows into Echo River along the dashed subsurface overflow shown on the map. Pushpins show the study sites at River Styx, Echo River, and the two springs.

Another anthropogenic control of the Green River is a series of locks, built in the late 1800s and early 1900s to allow commercial river navigation. Lock and Dam 6 was completed in 1906, approximately 24 river kilometers downstream of Mammoth Cave National Park's Green River Ferry (Fig. 2). Behind Lock and Dam 6, the pool extended upriver beyond Echo River Spring and River Styx Spring. It artificially raised the level of the Green River and its tributaries located within the pooled area, including Echo River and the River Styx.

The River Styx and Echo River are two underground rivers located in Mammoth Cave, and they are subterranean tributaries to the Green River (Figs. 3 and 4). In cave, the Echo River and River Styx karst basins are separated by what is essentially a low, wide sandbar (Fig. 6). By a combination of walking and swimming, one could follow the same large cave passage to get from the River Styx site to the Echo River site (Figs. 4 and 6). However, because of the need to swim through a cave river, that is not the preferred route between sites. Echo River has several large tributaries (e.g. Roaring River, Mystic River, and Hanson's Lost River), while there are fewer River Styx tributaries, which tend to be much smaller. On the surface, the River Styx Spring (Figs. 2, 3 and 5B) is located approximately 1.6 river kilometers upstream from the Echo River Spring on the Green River (Figs. 2, 3 and 5C).

Study sites were located in River Styx, Echo River, the Green River, at River Styx Spring, and at Echo River Spring (Fig. 4). The River Styx sites were located in the areas of the river known as the Dead Sea and Lake Lethe. No differences were seen between the data at these two sites. Thus, the data were analyzed collectively as the River Styx data. The Echo River site was located in Echo River between Hanson's Lost River and the point where Roaring River enters Echo River. This location is not affected by the River Styx's stable, reverse-flow events.

The original Green River site was located at the Green River Ferry, immediately downstream of Echo River Spring. However, multiple early losses of the data loggers, due to flooding and vandalism, required the research team to abandon this location. Scouting from the ferry to shortly upstream of River Styx revealed no locations that: 1) the students could safely access, and 2) would not have the same potential for flooding and vandalism issues as the original location.

The USGS maintains a river station (#03308500) approximately 47-river kilometers upstream of the Green River Ferry at Munfordville, Ky. (Fig. 2). Relatively, little difference was seen between the original data collected at the Green

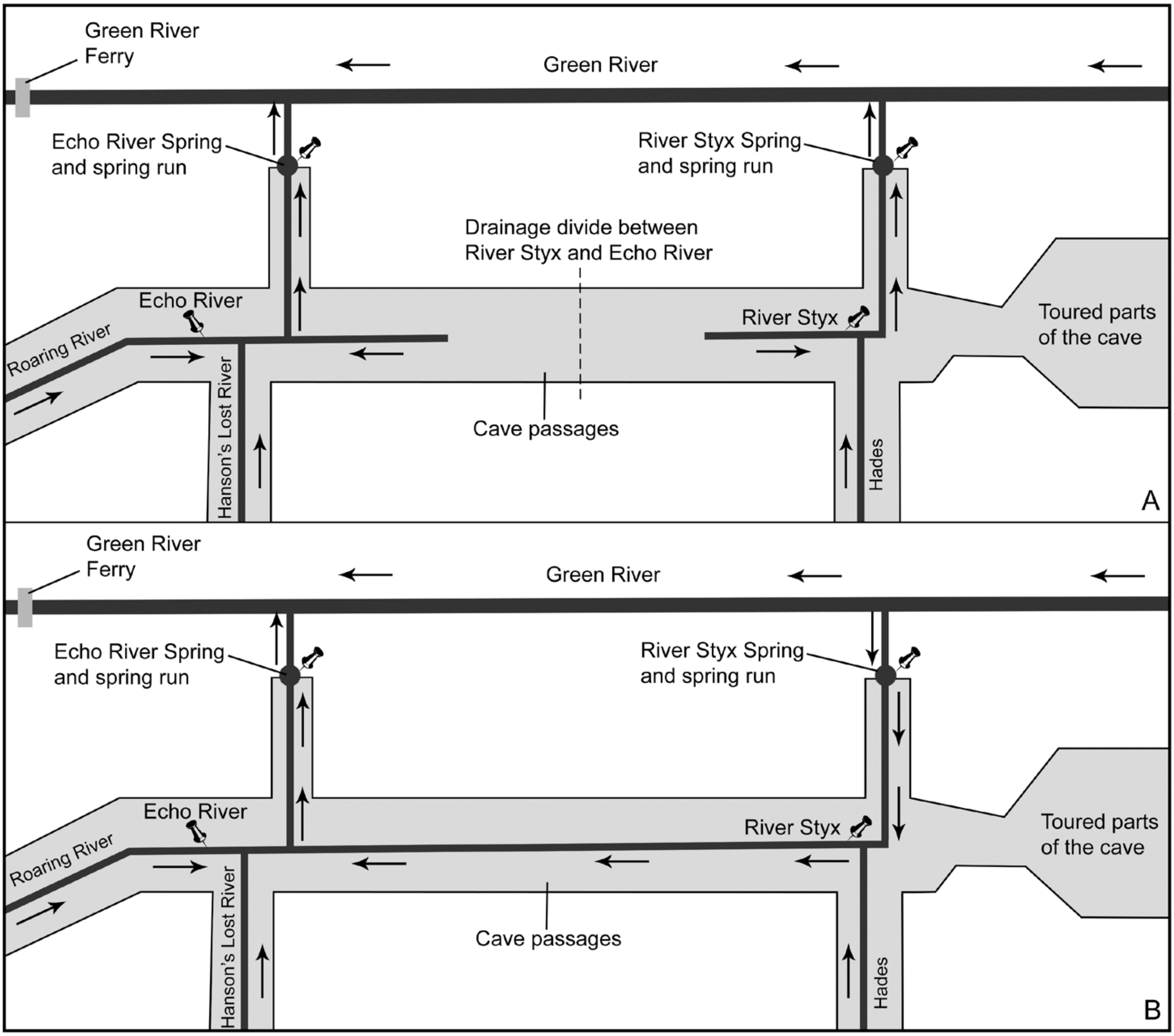

Figure 4. Schematic diagrams illustrating a) the normal flow pattern of River Styx out of the cave, and b) the reverse-flow pattern of River Styx into the cave. In diagram A, River Styx and Echo River are separated by a low, wide sandbar, which serves as a divide between the two karst drainage basins. When the River Styx reverses direction, it flows over the sandbar and into Echo River, as illustrated in diagram B. Pushpins show the study sites at River Styx, Echo River, and the two springs. Diagrams are highly simplified and not drawn to scale.

River Ferry and data collected by the USGS station in Munfordville for the same time period. The data trends for the two sites showed even less variation than the actual data points and, for this study, the patterns created by the data trends on the Green River were more important than the actual data points. Therefore, the decision was made to use data downloaded from the USGS station (USGS, 2015) as the Green River data during the remainder of the project. Earlier student analyses used a combination of data from the original site and the Munfordville station; however, only Green River data from the USGS station in Munfordville were used in this analysis.

Materials and Methods

Onset HOBO Pendant temperature/light data loggers (UA-002-64) were installed in late October 2009. The data loggers have an accuracy of ± 0.53 °C from 0 °C to 50 °C (Onset, 2017). The data loggers were programmed to record water temperature every two hours.

Two data loggers were initially installed to provide backup and quality assurance of the data in River Styx, in Echo River, and at River Styx Spring. During periods of high karst flow, Echo River Spring can issue forth large amounts

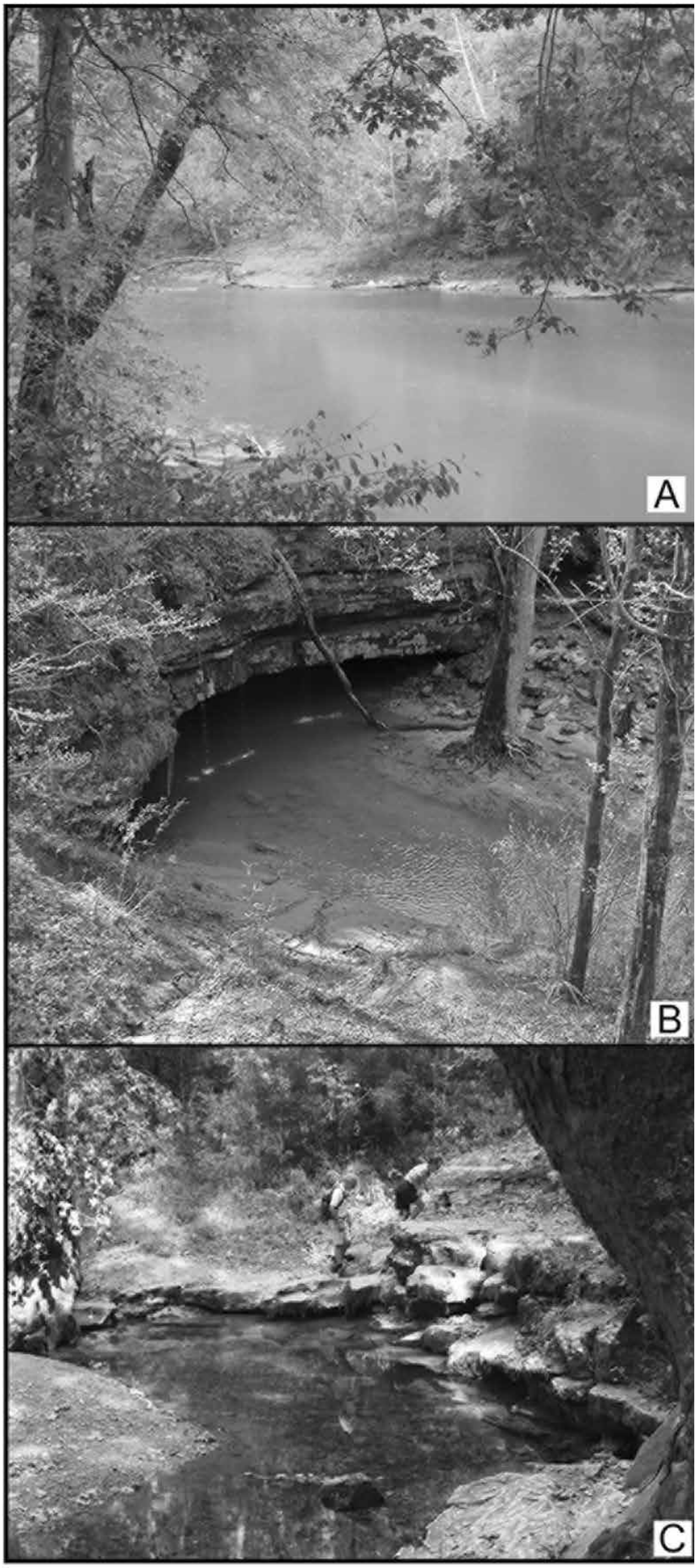

Figure 5. a) The Green River near the outflow of River Styx Spring, after the removal of Lock and Dam 6, looking downstream toward the River Styx Spring, b) River Styx Spring, and c) Echo River Spring.

of water at a rapid rate. This limited the locations where data loggers would: 1) remain in the water during low karst flow, 2) not be swept away during high karst flow events, 3) be easily accessible to the students, and 4) not be highly visible to the public. The best location that met all four requirements was a crevice that only had space for one data logger, thus only one data logger was installed at Echo River Spring. Even with backup data loggers in place, flooding and equipment failure resulted in occasional data gaps for all of the study sites. Lost data loggers were replaced as resources allowed.

This study analyzes the data collected between October 2009 and October 2012. Whenever a site had two data loggers simultaneously recording data, the mean temperature recorded for both data loggers was used. Overall, there was relatively little variance between the temperatures recorded by the two data loggers at a given site. When the data loggers differed by more than 1 °C, the data were inspected for quality control purposes. If it appeared one of the data loggers was out of the water, and thus, recording air temperature, or the data logger appeared to be malfunctioning, then those data points were removed from analysis.

Water temperature was graphed over time for each of the rivers for each week, where corresponding data were available from River Styx, Echo River, and the Green River. The graphs represented 103 weeks and more than 8,600 data points per river. Water temperature in the underground rivers is relatively constant year-round unless a reverse-flow event (from either back-flooding or a stable, reverse-flow) is occurring.

Temperature was used as a proxy for identifying the flow direction of the River Styx. Data were analyzed by comparing the River Styx temperatures to those of Echo River and the Green River. When the River Styx temperature was more similar to the Echo River temperature than to the Green River temperature, the River Styx was assumed to be flowing in its normal direction (out of the cave). If the River Styx temperature was more similar to the Green River temperature than to the Echo River temperature, then it was assumed to be in a stable, reverse-flow. The Green River was assumed to be back-flooding into both underground rivers if the temperatures for all three rivers were similar.

Occasionally, the river direction could not be determined because the Green River temperature was too close to the mean temperature of Echo River and River Styx (such as in the spring or fall), or because there was no clear pattern when comparing the graphs of the three rivers. The lack of a clear pattern occurred most often for short events, such as a possible, brief return to forward flow (out of the

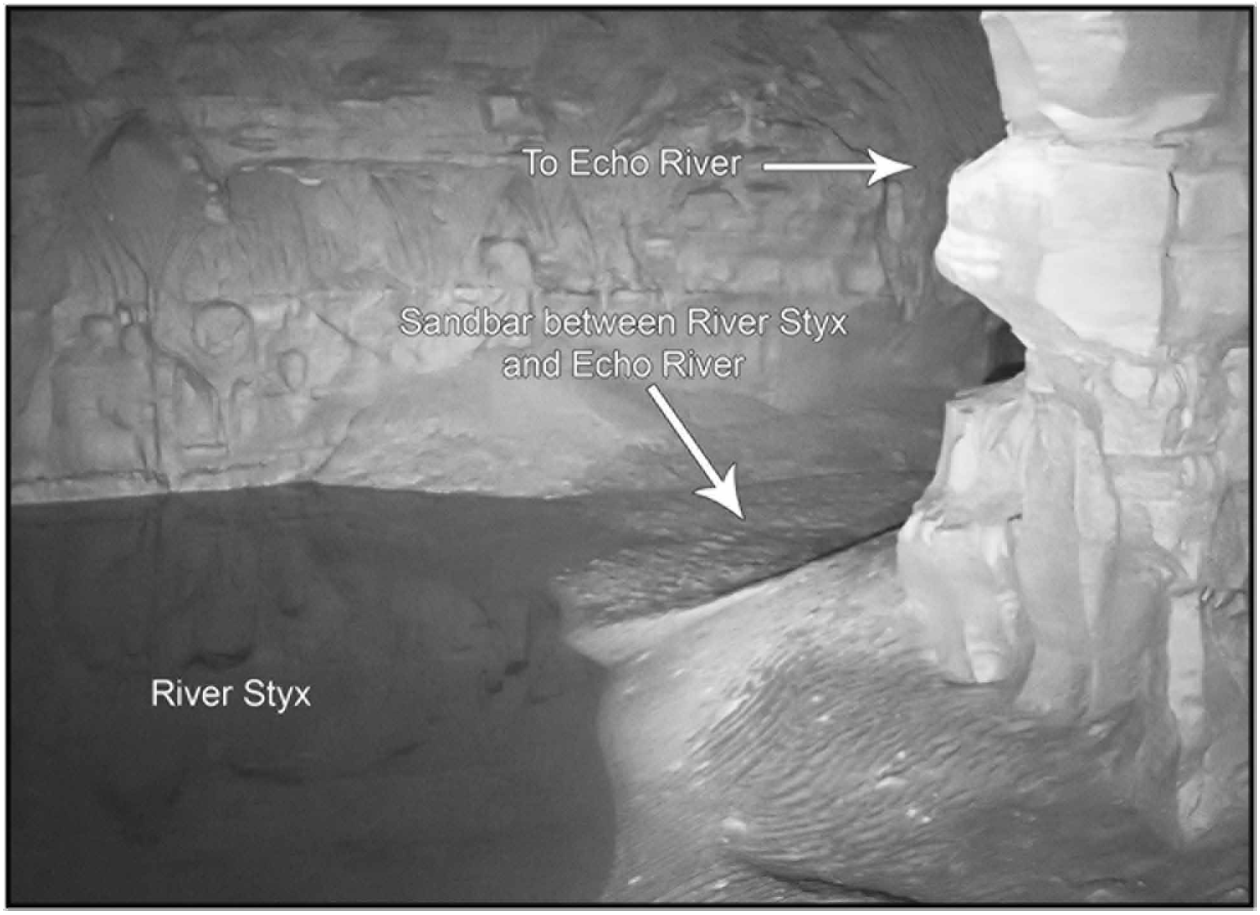

Figure 6. Under normal flow conditions, River Styx and Echo River are separated by the low, wide sandbar seen in this photo. River Styx is visible in the foreground. Continuing down the passage by walking across the sandbar would lead to Echo River. One could then wade or swim through Echo River to the Echo River study site. When the River Styx reverses, this sandbar is submerged, and River Styx flows freely into Echo River.

cave) between two reverse-flow events. There were also a few instances where it was obvious that the River Styx was reversed, but a determination could not be made as to whether it was a back-flooding event or a stable, reverse-flow event. This occurred most often during transition periods, as from back-flooding to stable, reverse-flow events.

Mammoth Cave National Park staff record the level of the Green River at the Green River Ferry twice a day, when the ferry is operating. River levels for each day of the study period were obtained from park records. The two data points for each day were averaged to give a mean, daily level of the Green River. Mean, daily precipitation from the three park weather stations was also obtained from park records. To analyze the influence of precipitation upstream of the park, daily precipitation totals from the NOAA climate station at Greensburg were downloaded from NOAA's website (NOAA, 2016). The station at Greensburg was chosen because it was located on the Green River, upstream of the park, and was well outside of the park boundary.

Each day of the study was coded as to whether River Styx was predominantly flowing forward (out of the cave) or was predominantly reversed (flowing into the cave). The reverse flows were also coded as either back-flooding or stable, reverse-flow events. Logistic regression, using XLStat 2017 software, was used to compare the flow direction of the River Styx (forward or reversed) to the level of the Green River, the local daily precipitation amounts, and the daily amount of precipitation at Greensburg, Ky. Logistic regressions allow the user to model the relationship between a binary, dependent variable (e.g., presence of a reverse flow) with one or more independent variables (e.g., river levels or daily precipitation) (XLSTAT, 2017).

Results

Water temperature

Between October 2009 and October 2012, the temperature in Echo River remained relatively constant with a mean temperature of 13.4 °C and a standard deviation of ± 0.6 °C (Table 1). The mean temperature in River Styx was similar

Table 1. Minimum, maximum, and mean temperatures recorded for each site between October 2009 and October 2012.

Temperature Range	Temperature, °C				
	River Styx (underground) *n* = 13,004	Echo River (underground) *n* = 8,602	Green River (surface) *n* = 12,581	River Styx Spring *n* = 13,002	Echo River Spring *n* = 7,571
Minimum	3.6	9.2	0.7	2.2	4.2
Maximum	23.8	14.4	29.5	26.3	18.4
Mean	13.5 ± 2.7	13.4 ± 0.6	15.5 ± 7.2	13.0 ± 3.5	13.0 ± 1.6

Table 2. Water temperature in each river and at both springs, when the lowest water temperature was recorded at each site between October 2009 and October 2012. The * indicates the lowest-recorded temperature at that site.

Date	Time	Temperature, ºC					Condition of River Styx Based on Graphs
		River Styx	Echo River	Green River	River Styx Spring	Echo River Spring	
02/01/2010	1600	3.6 *	13.1	3.5	3.5	6.4	Stable, reverse flow
12/08/2010	0200	8.2	9.2 *	7.9	7.8	8.5	Back-flooded
01/08/2010	0800	11.1	13.3	0.7 *	6.4	12.6	Normal flow direction
12/16/2010	0200	11.8	12.4	2.9	2.2 *	11.4	Normal flow direction
12/14/2010	0600	11.8	12.3	2.9	7.9	4.2 *	Normal flow direction

to that of Echo River, but it had a higher degree of variability (13.5 ± 2.7 °C, Table 1). The Green River had the highest mean temperature and the greatest degree of variability (15.5 ± 7.2 °C, Table 1).

The minimum temperature recorded at River Styx was 3.6 °C on February 1, 2010 (Table 2). River Styx remained at 3.6 °C for approximately four hours and was within 0.5 °C of the recorded low for approximately one day. Temperature at Echo River Spring at that time was 6.4 °C (Table 2). The temperature in Echo River at that time was 13.1 °C, indicating the River Styx was in a stable, reverse-flow pattern, and contributing a large percentage of the flow out of Echo River Spring. Analysis of the graph (Fig. 7) for this time period confirmed this interpretation.

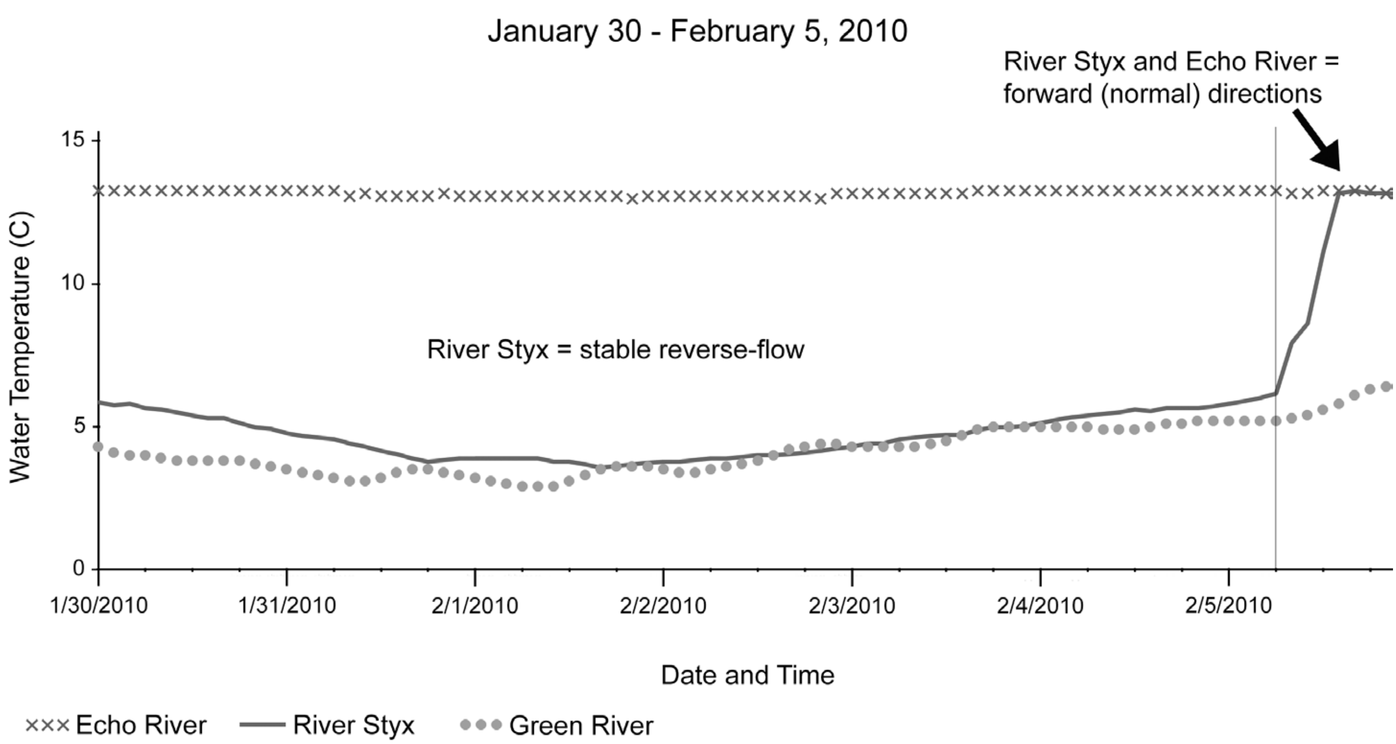

Figure 7. Time-series graph of water temperatures in River Styx, Echo River, and the Green River for the week of January 30 to February 5, 2010. Comparing the pattern of the River Styx water temperatures with those of the Green River and Echo River, confirms that River Styx was in a stable, reverse-flow pattern, when it reached its lowest recorded temperature of 3.6 ºC on February 1, 2010.

Minimum temperature recorded in Echo River was 9.2 °C on December 8, 2010 (Table 2). Echo River remained at 9.2 °C for approximately eight hours, and was within 0.5 °C of the recorded low for approximately 0.75 days. At the same time, River Styx was 8.2 °C and the Green River was 7.9 °C. Both springs also had similar temperatures. The similar water temperatures at all five sites indicate that the Green River was flooding and forcing water back into its tributaries. Based on the associated graph, shortly after the minimum temperature was recorded at Echo River, the back-flooding stopped and Echo River returned to a normal flow direction, while River Styx entered a stable, reverse-flow pattern (Fig. 8).

The maximum temperature recorded at Echo River was 14.4 °C on November 1, 2009 (Table 3), and the temperature remained at 14.4 °C for approximately 2.25 days. River Styx, Green River, and River Styx Spring all had similar temperatures (Table 3). Although the temperatures were similar and above Echo River's mean, the associated graph indicates that all three rivers were flowing in their normal directions during this time (Fig. 9). This interpretation makes sense, given the time of year, and illustrates why the patterns represented in the graphs were important to the analysis.

Maximum temperature recorded for River Styx was 23.8 °C on September 3, 2010 (Table 3), and lasted approximately two hours. The temperature was within 0.5 °C of 23.8 °C for approximately eight hours. Similar temperatures were recorded at the same time for Green River and River Styx Spring (Table 3). Whether the maximum temperature in River Styx was due to a stable, reverse-flow event or a back-flooding event cannot be determined because no data from the corresponding times are available from Echo River.

Reverse flows

The periods when data were recorded for all three rivers were October 23, 2009 to April 2, 2010, October 25, 2010 to May 15, 2011, and November 3, 2011 to October 24, 2012. Analysis of the graphs from these periods identified 34 times when the River Styx was in a stable, reverse-flow condition, five back-flooding events, when Green River water backed up into both cave rivers, and 10 times when the River Styx could not be classified as flowing forward (out of the cave), back-flooding, or in a stable, reverse-flow. Throughout the study, the River Styx flowed in its normal direction (out of the cave) approximately 77 % of the time and was either back-flooding or in a stable, reverse-flow approximately 20 % of the time. In each year, at least one stable, reverse-flow event was recorded every month from December through February. Two of the three years also showed stable, reverse-flow events in November and March.

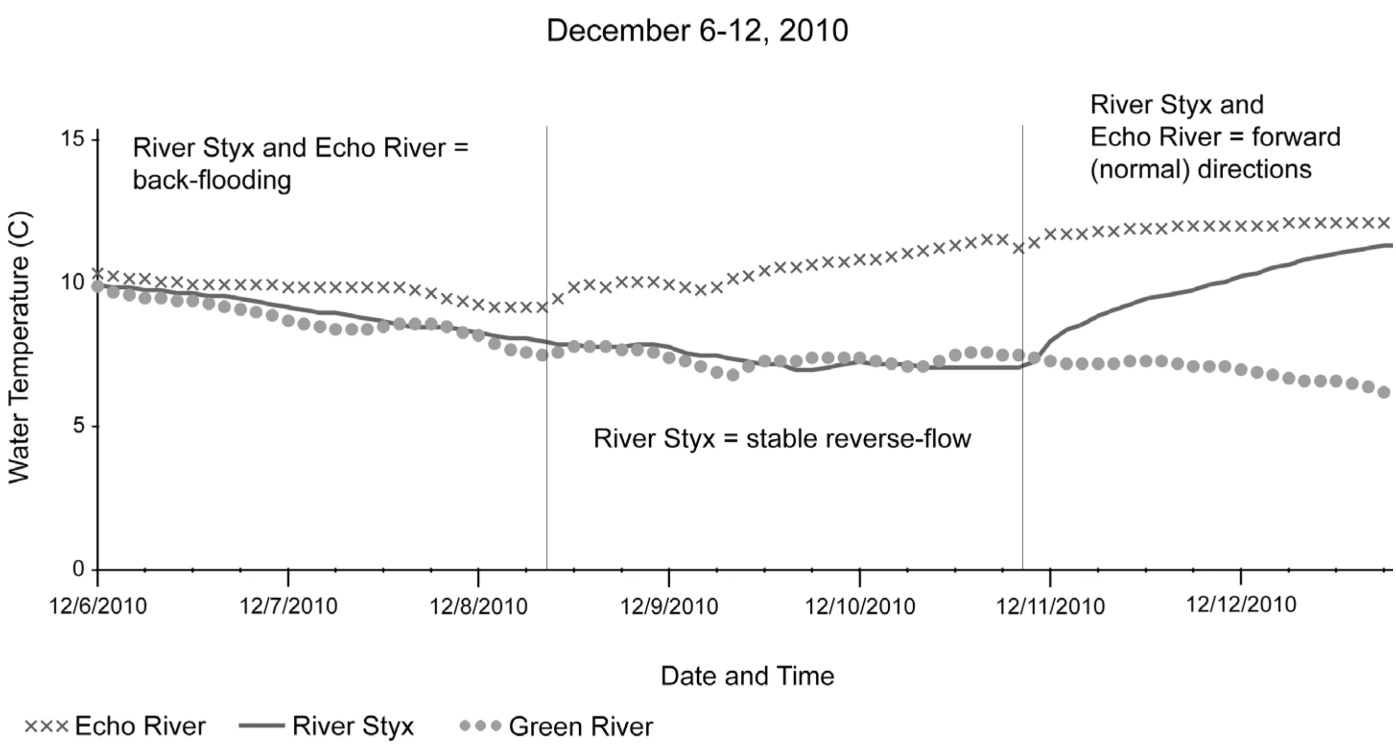

Figure 8. Time-series graph of water temperatures in River Styx, Echo River, and Green River for the week of December 6−12, 2010. Comparing the pattern of River Styx and Echo River's water temperatures with those of the Green River, confirms that River Styx and Echo River were back-flooding when Echo River reached its lowest-recorded temperature on December 8, 2010. The graph also shows that Echo River stopped back-flooding shortly after reaching its lowest-recorded temperature, while River Styx entered a stable, reverse-flow pattern.

Table 3. Water temperature in each river and at both springs, when the highest water temperature was recorded at each site between October 2009 and October 2012. The * indicates the highest-recorded temperature at that site. No graphs could be created for periods when there were no Echo River temperature data.

Date	Time	Temperature, ºC					Condition of River Styx Based on Graphs
		River Styx	Echo River	Green River	River Styx Spring	Echo River Spring	
09/03/2010	0400	23.8 *	•••	22.1	22.5	•••	Unknown—no graph
11/01/2009	0400	14.3	14.4 *	14.3	14.3	•••	Normal flow direction
07/27/2012	1800	13.6	13.8	29.5 *	13.8	13.8	Normal flow direction
07/13/2011	1600	17.1	•••	24.6	26.3 *	14.2	Unknown—no graph
06/23/2011	0600	20.8	•••	19.6	20.2	18.4 *	Unknown—no graph

Duration of the stable, reverse-flow events varied greatly. Using temperature as a proxy for determining flow direction, some events appeared to last only a few hours, while others lasted for more than a week. Often, River Styx shifted flow direction multiple times in a week. The frequent switching of directions was most pronounced from mid-March 2011 through, at least mid-May 2011, when the data logger at Echo River stopped recording. During these two months, the River Styx switched 13 times between stable, reverse-flow events, periods of indeterminate flow direction, and normal flow patterns. Two approximately eight-day periods were the longest continuous periods when the River Styx was flowing forward (out of the cave) during this time. River depth at the Green River Ferry during this time ranged from a minimum of 1.6 m to a maximum of 10.2 m, with a mean of 4.7 m, and a standard deviation of 2.1 m.

Back-flooding events during the study period were rare compared to stable, reverse-flow events. However, there appears to be a relationship between back-flooding and stable, reverse-flow events. All five back-flooding events identified during this study were immediately preceded or followed by a stable, reverse-flow event. Two of the back-flooding events were sandwiched between two stable, reverse-flow events.

Logistic Regression

No significant relationship was found between local daily precipitation (P = 0.66) or daily precipitation at Greensburg (P = 0.43) and the flow direction of the River Styx. Logistic regression showed a strong relationship between the level of the Green River and the direction in which River Styx was flowing (p < 0.0001).

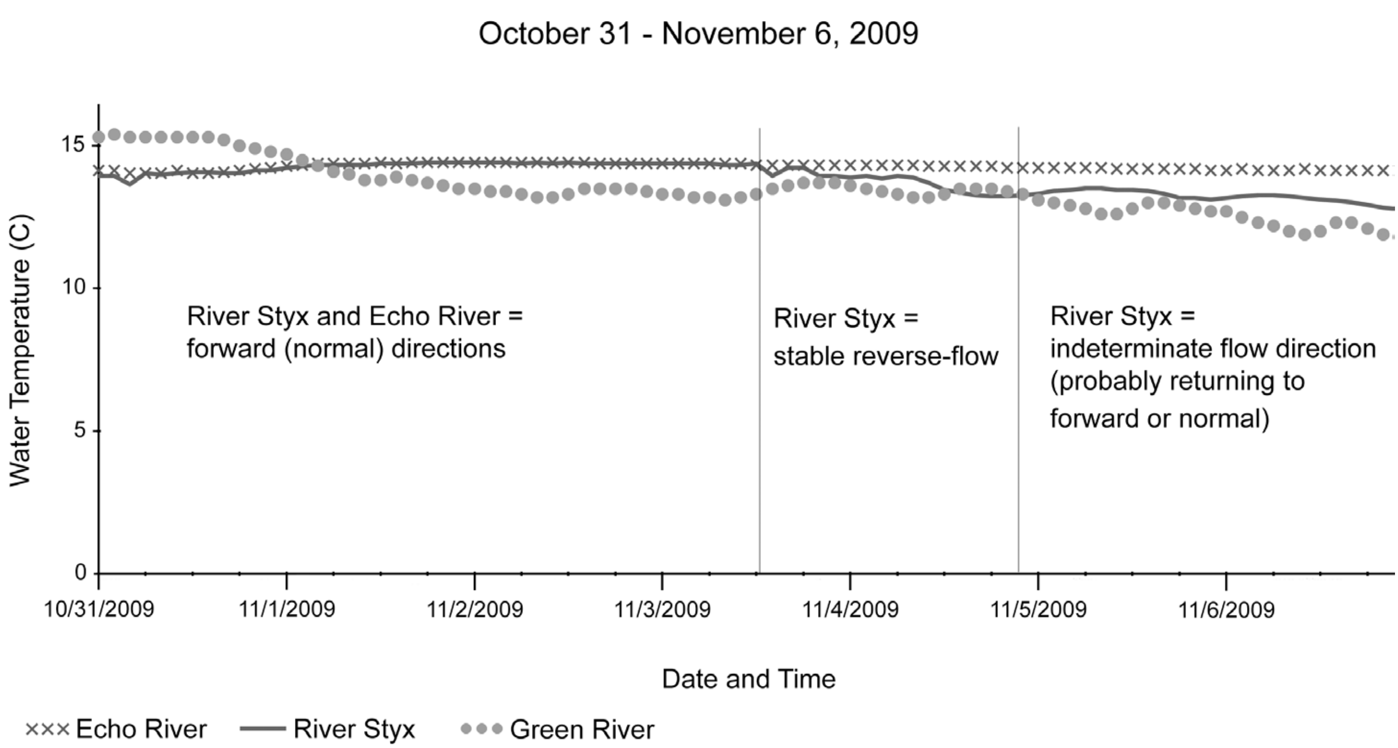

Figure 9. Time-series graph of water temperatures in River Styx, Echo River, and Green River for the week of October 31 to November 6, 2009. Comparing the pattern of River Styx and Echo River's water temperatures with those of the Green River, confirms that Echo River and River Styx were flowing forward (out of the cave), when the maximum temperature was recorded in Echo River on November 1, 2009. Both underground rivers remained relatively constant during this time, while the Green River was decreasing in temperature.

Table 4. Number of days and percentage of days when the River Styx was primarily flowing forward (out of the cave), backwards (into the cave, subdivided into stable reverse-flow or back-flooding), or the direction could not be determined.

River Styx	Periods of Examination and Days and Percent Days of Flow											
	Oct. 2009 to April 2010		Oct. 2010 to May 2011		Nov. 2011 to May 2012		June 2012 to Oct. 2012		Oct. to May 2009, 2010, 2011		All Four Seasons	
	Days	Percent	Days	Percent	Days	Percent	Days	Percent	Days	Percent	Days	Percent
Forward (out of the cave)	106	65	145	71	158	75	146	100	409	71	555	77
Backward (into the cave)	46	29	54	27	48	23	0	0	148	26	148	20
Stable, Reverse-Flow	45	28.4	48	24	36	17	0	0	129	23	129	17
Back-Flooding	1	0.6	6	3	12	6	0	0	19	3	19	3
Unknown	10	6	4	2	5	2	0	0	19	3	19	3

Discussion

Using Proxies

Edwards (2009) compared several proxies and found temperature changes to be the best indicator of reverse flow events in River Styx. However, relying on a proxy, such as water temperature to determine flow direction, presents inherent challenges to any study. Obviously, the temperature of the underground rivers does not instantaneously change when the river direction changes. There is a lag time between when a reverse flow event begins and when the change in water temperature is detected by the data loggers, or is evident in a graph. Another lag time occurs at the end of a reverse-flow event and may be a different duration than the beginning lag time. The original USGS studies (Hendrickson, 1961; Brown, 1966) would have suffered from similar challenges, but in terms of salinity instead of temperature. Despite the challenges associated with using proxies to determine flow direction, the patterns identified are supported by the data.

Timing and Duration

Based on data from April 30 to May 16, 1954, Brown (1966) concluded the River Styx stays in a stable, reverse-flow condition for most of the winter and early spring. He also concluded that the stable, reverse-flow conditions would be interrupted only when locally heavy rainfall resulted in a temporary rise in the level of the River Styx. The data from this study failed to support Brown's conclusions.

During the three winters and early springs of this study, the number of days when the River Styx primarily flowed backward, either due to stable, reverse-flow events or back-flooding, was only 148 days or approximately 26 % of the time (Table 4). The highest percentage of time when the River Styx was reversed during any of the three winters and early springs was 29 % of the time between October 23, 2009 and April 2, 2010. No reverse flows were detected in November 2010 or April 2011. Overall, the River Styx primarily flowed forward and exited the cave at River Styx Spring for approximately two-thirds to three-quarters of the time during each of the 2009–2010, 2010–2011, and 2011–2012 winters.

One potential explanation to the differing results of the two studies could be a matter of scale. Brown (1966) indicated that the River Styx Spring could potentially alternate between in-flow and out-flow several times within a half-hour period. If this is the case, then the current study could have missed a substantial number of rapid, reverse-flow events because the loggers only recorded data once every two hours. Also, visual analysis of the data was conducted from graphs, representing one week of data. If the data were re-analyzed by graphing the data on a daily basis, it may be possible to detect smaller-magnitude or shorter-duration, reverse-flow events that were undetectable on a weekly basis. This conclusion is supported by comparing the current analysis with the original analysis done by the students.

The students originally graphed the data on a monthly basis and identified 15 stable, reverse-flow events. By graphing the data on a weekly basis, this study identified more than twice as many stable, reverse-flow events. However, increasing the number of stable, reverse-flow events detected does not necessarily increase the amount of time in which the River Styx was detected as flowing backward. Many shorter events could add up to approximately the same length of time, or possibly even less time, than a single event that encompassed all of the shorter events. Therefore, it seems unlikely that the different rate of data recording and scale of analysis could fully explain the high degree of difference observed between Brown's study and the current study. To fully explain the difference, physical explanations, such as the influence of the Green River stage, the influence of precipitation patterns, and anthropogenic influences on the Green River, need to be explored.

Influence of Green River Stage

Hendrickson (1961) indicated that reverse-flow conditions were possible whenever the Green River is above a gage height of 129.1 m. This would be equivalent to a reading of 1.1 m on the NPS staff gage at the Green River Ferry, before Lock and Dam 6 failed. (A level of 1 m on the Green River Ferry staff gage prior to Lock and Dam 6 failing was equal to approximately 128 m in the older USGS studies, which were reported as height above mean sea level.) Brown (1966) concluded the stable, reverse-flow conditions would occur whenever the Green River stage is high and the River Styx stage is low. According to Brown (1966), only a 0.06 m difference in stage heights between the River Styx and the Green River could change the direction that the River Styx was flowing.

Logistic regression confirmed a strong relationship between the level of the Green River and River Styx reversals. However, it is not a simple relationship because there were numerous times during the study period, when the Green River was high but the River Styx was flowing out of the cave. One potential explanation could be that the karst basin was at high flow during those times, thus preventing the Green River from entering River Styx Spring. This explanation fits with the conclusions of Hendrickson (1961), Brown (1966), and Meiman (2006), that stable, reverse-flow events typically take place when the Green River is at a higher stage than the River Styx, and the karst basin is at low flow.

Influence of Precipitation

Brown (1966) stated that locally heavy rainfall could result in interruptions of stable, reverse-flow events. Precipitation is also one of the factors that can affect the level of the Green River. Local precipitation and/or precipitation upriver from the park, therefore, could be important factors contributing to the reverse-flow events. However, logistic regression failed to detect a significant relationship between daily precipitation and the direction of the River Styx for either local precipitation or precipitation upriver from the park at Greensburg.

Despite the lack of a direct relationship between the River Styx reverse-flow events and daily precipitation, an indirect relationship with precipitation still exists. Climate change in the southeastern U.S. is predicted to affect the timing, duration, and intensity of precipitation events (Melillo et al., 2014). These changes will have obvious impacts on the timing and duration of any regional or local flood or drought conditions, and thus, on the level of the Green River and the amount of karst flow. As discussed earlier, there is a strong relationship between the level of the Green River and the River Styx's flow direction. Therefore, it stands to reason that any changes in the timing, duration, and frequency of droughts or floods in the Green River, Echo River, and River Styx drainage basins will also affect the timing, duration, and frequency of the River Styx reverse-flow events.

Anthropogenic Influences

The potential role of precipitation in stable, reverse-flow or back-flooding events is complicated by the fact that the level of the Green River is only partially controlled by precipitation. A number of artificial structures also greatly affect the level of the Green River.

Green River Dam is the largest anthropogenic influence on the Green River. The dam did not exist when Hendrickson and Brown conducted their studies on the River Styx and its stable, reverse-flow events. At that time, precipitation was the primary factor influencing the level of the Green River. However, that is no longer the case. In the spring and summer, water is held behind the dam for flood control and recreational purposes. Each fall, the U.S. Army Corps of Engineers releases 2.1 m of water from the Green River Dam to drop the lake to winter pool level. Those releases temporarily raise the level of the Green River through Mammoth Cave National Park, and can cause the River Styx to enter a stable, reverse-flow or back-flooding event.

The dam releases do not necessarily correspond to daily precipitation events, and can complicate any conclusions based solely on recorded precipitation. Over the last few years, the timing of the annual winter drawdown has shifted from earlier in the fall to later in the fall. This later timeframe is closer to the time when winter rains in the area would naturally begin causing the River Styx to flood, or set up a stable, reverse-flow pattern. However, while the general timing of the releases may be closer to a more natural, precipitation-driven pattern for higher Green River levels, that still does not mean there is a simple correlation between daily precipitation patterns and river level.

Until recently, Lock and Dam 6 also influenced the level of the Green River within Mammoth Cave National Park. The pool created by Lock and Dam 6 artificially raised the level of the Green River, Echo River and River Styx. The U.S. Army Corp of Engineers stopped operating Lock and Dam 6 in 1951. In a report to the National Park Service Advisory Board, Dunn (1951) recommended the U.S. Army Corps of Engineers remove Lock and Dam 6 to allow the Green River, and its underground tributaries within Mammoth Cave, to return to their more natural 19th-century levels. From 1951 through 2016, Lock and Dam 6 continued to deteriorate, while the U.S. Army Corp of Engineers conducted numerous studies on the Green River locks and dams.

On November 25, 2016, Lock and Dam 6 failed, causing the level of the Green River at the Green River Ferry to drop approximately 0.34 m in the first eight hours after the failure. At the Green River Ferry, the level of the Green River continued to drop at a much slower rate until the afternoon of November 28, 2016, when a rain event moved into the area. The level of the Green River is still adjusting to the new conditions, and research on the impacts of Lock and Dam 6 on the levels of the Green River and its underground tributaries is ongoing. It is hypothesized that the breach of Lock and

Dam 6 will result in fewer River Styx back-flooding and stable, reverse-flow events. Fewer stable, reverse-flow events are hypothesized because the lower Green River base-flow elevation will require an additional rise before reaching the stage where it begins to flow into the River Styx Spring. This will likely be a more natural condition than what has been experienced over the last 110 years. Data collected in this study will provide important additional data for testing this hypothesis.

Conclusions

Proxies can provide important information on stable, reverse-flow and back-flooding events in River Styx. To gain a more detailed understanding of these events, especially during transition periods, future research is needed that directly correlates proxies with a direct measurement of flow direction.

Over the last 50 years, the frequency and duration of reverse-flow events in River Styx has changed. The primary cause of this change is likely due to the anthropogenic influences of the Green River Dam on the level of the Green River.

This study provides a baseline, multi-year dataset of temperature variation in Mammoth Cave's underground rivers before the removal of Lock and Dam 6. As such, the data are now irreplaceable.

With Lock and Dam 6 in place, back-flooding and stable reverse-flow events could affect the temperature, nutrient availability, and potential contaminants in River Styx for approximately 20 % of the time each year. These changes could directly affect the aquatic ecosystems of River Styx, including habitat for the endangered Kentucky cave shrimp. By driving micro-climatic changes in the lower passages, the reverse flow events also had the potential to indirectly affect archeological and geological resources.

Now that Lock and Dam 6 has been removed, this study needs to be repeated to better understand the effect of Lock and Dam 6 on the River Styx and its stable, reverse-flow events. If, as hypothesized, the removal of Lock and Dam 6 results in fewer stable, reverse-flow events, new studies may be needed to determine how the aquatic ecosystem in River Styx responds to the more stable water temperatures resulting from fewer stable, reverse-flow events.

Future studies should include collecting water level data in the River Styx, at the River Styx Spring, and in the Green River near where the spring run for the River Styx Spring enters the Green River. The data on water levels will provide a better understanding of how the level of the Green River and the amount of karst flow interact, especially during stable, reverse-flow events and back-flooding events.

Additional studies are needed on the interactions of cave sediments and karst flow in River Styx and Echo River. During back-flooding and high, karst flow events, large amounts of sediment can be rearranged in the vicinities of the underground rivers. This includes creating and removing natural sediment dams in parts of the underground rivers. The patterns of movement related to these sediments could change as a result of the lower Green River, River Styx, and Echo River levels since the removal of Lock and Dam 6.

Better understanding the causes and impacts of the stable, reverse-flow events in River Styx could provide additional information for making science-informed, management decisions about anthropogenic controls of the Green River, such as releasing water from the Green River Dam. In recent years, the U.S. Army Corps of Engineers changed the timing and pattern of its water releases in the fall to minimize the impacts on freshwater mussels living in the Green River. Currently, little to nothing is known about the impact of the water releases on the endangered Kentucky cave shrimp or other cave aquatic organisms.

New research is needed to monitor the cave meteorology in the lower passages of Mammoth Cave. The cave meteorology data will help determine how the stable, reverse-flow events and back-flooding events in River Styx influence the micro-climates of the lower cave passages, and how far those effects extend away from the underground river passages. Understanding the micro-climatic changes related to the River Styx stable, reverse-flow and back-flooding events is important because those changes can affect the biological, geological, archeological, and cultural resources found in the cave's terrestrial environments. Long-term collection of water temperature and river levels in River Styx, Echo River, and the Green River could help document and better understand the direct effects of climate change on Mammoth Cave's aquatic environments and the indirect effects on resources found in the cave's terrestrial passages.

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MULTI-YEAR CAVE DRIPWATER FREQUENCY AND HYDROCHEMICAL MONITORING OF THREE CAVES IN EASTERN NORTH AMERICA: IMPLICATIONS FOR SPELEOTHEM PALEOCLIMATOLOGY

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Abstract

A cave monitoring program of three caves in southeastern West Virginia, USA, was undertaken from September 2011 to December 2013. Culverson Creek Cave, Buckeye Creek Cave, and Lost World Caverns were continuously monitored for temperature and relative humidity, revealing a highly-stable environment year-round. The caves were visited approximately every three months during the study period, when discrete CO_2 measurements were taken, revealing a seasonal ventilation cycle characteristic of temperate-region caves. Dripwaters from 12 sampling stations were collected throughout the first year, from which the isotopic results show the relationship between cave dripwaters and meteoric precipitation. Two sampling periods, those of March 2012 and October 2012, were distinctly different than most of the other isotope values that fell on, or very near, the Global Meteoric Water Line (GMWL). The March 2012 dripwater isotopes were very negative, resulting from several days of heavy meteoric precipitation preceding the collection time that likely pushed water through the vadose zone that had accumulated in the previous winter months. The October 2012 samples displayed a positive linear trend, falling to the right of the GMWL, indicating that those samples were comprised of waters with evaporative loss. Drip frequency loggers placed above the cave allow a direct comparison between surface precipitation and six cave drip-frequency loggers, placed strategically throughout the study caves. These frequency data help to characterize the drips, where one was shown to be highly responsive and underwent flow-switching. Two are shown to have a seasonal-response and three demonstrated no response, characteristic of slow seepage flow. Stalagmites formed as a result of the latter are generally regarded as the most suitable for long-term paleoclimate studies. Monitoring programs performed prior to stalagmite collection for paleoclimate reconstructions could aid in the selection of suitable samples, thereby preserving priceless cave formations, as well as aiding in the interpretation of geochemical proxy variations in speleothem calcite.

Introduction

Speleothems as paleoclimate archives have been increasingly utilized over the past few decades (Baker et al., 1997; Dorale et al., 1998; Roberts et al., 1998; Hellstrom and McCulloch, 2000; Wang et al., 2001; Poore et al., 2003; Dykoski et al., 2005; Cheng et al., 2006; Spötl et al., 2006; Vollweiler et al., 2006; Borsato et al., 2007; Cruz et al., 2007; Mattey et al., 2008; Lambert and Aharon, 2010; Stríkis et al., 2011). Stalagmites are the product of cave dripwaters, which have complex surface-to-cave hydrologic pathways, seasonal distributions, and unique hydrochemical histories. Isotopic and trace element variation between coeval speleothems underscores the importance of an understanding of dripwater variability, as speleothem records may represent different aspects of the climate system. The complexity of hydrologic evolution from the soil zone through the vadose and phreatic zones in the epikarst has prohibited a complete understanding of the hydrologic histories of individual cave drips. However, the dripwater frequency and modern hydrochemistry of dripwaters can give clues as to the hydrologic pathway of the individual drips and may aid in the interpretation of stalagmite records.

Speleothems form due to the CO_2 degassing of supersaturated waters, which is governed by soil gas CO_2 and the cave air CO_2 concentrations (Fairchild et al., 2006). Upon entering the cave atmosphere, the dripwaters with high CO_2 encounter the lower-CO_2 cave air, thereby driving the precipitation and deposition of calcite as the CO_2 degasses. Density-driven seasonal ventilation exerts a significant control on the annual growth distribution of calcite laminae, as the concentration of cave air CO_2 is generally highest in the warmer months, because the relatively colder cave air remains in the topographic lows of the cave. In contrast, the cold, dense air of the colder months sinks into the cave, thereby increasing ventilation and lowering cave air CO_2 (Fairchild and Baker, 2012).

Previous work on karst vadose hydrology and speleothem geochemistry has focused on three major themes: drip variability and flow regimes (Smart and Friederich, 1986; Baker et al., 1997; Baker and Brunsdon, 2003), hydrochemical studies of dripwaters (Huang et al., 2001; Tooth and Fairchild, 2003; McDonald et al., 2004, 2007; Karmann et al., 2007; Lambert and Aharon, 2010, 2011), and experimental/quantitative models of calcite deposition and/or isotopic evolution (Buhmann and Dreybrodt, 1985; Dreybrodt, 1996; Wackerbarth et al., 2010). Many recent studies have included

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aspects of the three themes (Baldini et al., 2006, 2008; Verheyden et al., 2008; Sherwin and Baldini, 2011). In a pioneering study of discharge from a karst aquifer in Mendip Hills, UK, Smart and Friederich (1986) determined that for cave drips with low recharge rates, flow within the aquifer is predominantly vertical and slow through the matrix (seepage). However, with increasingly higher drip rates, flow switches recharge to vertical shafts (fracture) and overflow behavior was observed as rapid responses to rainfall events. An understanding of the variation of flow routes of speleothem-forming dripwaters would aid in the interpretation of paleoclimate records.

Setting

Culverson Creek Cave (CCC), Buckeye Creek Cave (BCC) and Lost World Caverns (LWC), in Greenbrier County, southeastern West Virginia (Fig. 1), were selected for a monitoring study for several reasons: 1) stalagmites from CCC and BCC have been used in numerous paleoclimate studies (Springer et al., 2008, 2009, 2014; Hardt et al., 2010; Buckles and Rowe, 2016), 2) the caves are located near one another with relative ease of access, and 3) the caves exhibit a wide range of morphologies and drip behaviors. These caves are formed within the Union Limestone Member (massive limestone with abundant chert and marine fossils) of the Greenbrier Group, which is Mississippian in age and is composed of interbedded limestone, shale, and sandstone (Dasher and Balfour, 1994). The Union Limestone ranges from 100 to 325 feet below the top of the Greenbrier Series and is notable due to its purity, containing 62–91 % of calcium carbonate with low amounts of magnesium carbonate (Reger and Price, 1926).

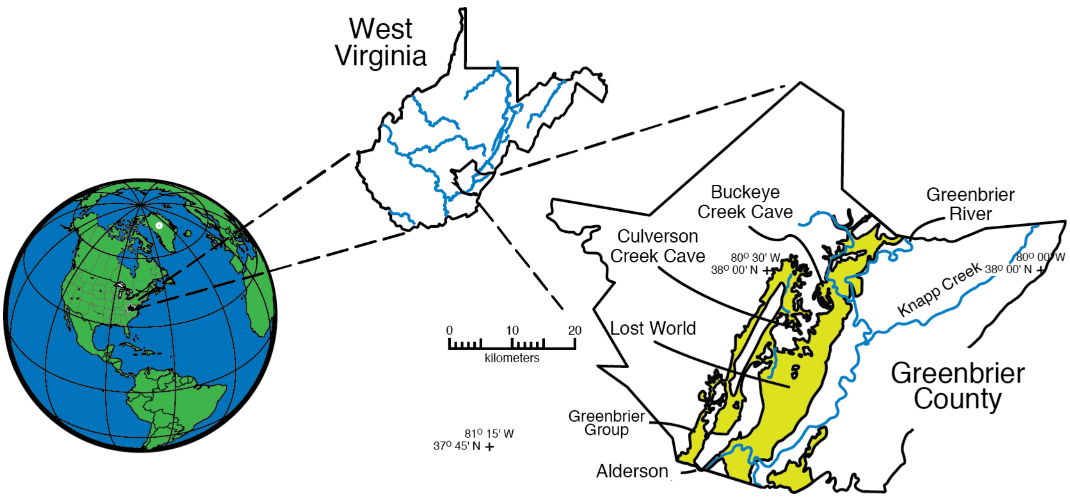

Figure 1. Regional map of Greenbrier County, West Virginia, showing the locations of Buckeye Creek Cave (37°58'33.37" N, 80°23'58.66" W), Culverson Creek Cave (37°56'11.15" N, 80°25'23.51" W), and Lost World Caverns (37°49'56.94" N, 80°26'47.53" W) in the Greenbrier Group.

CCC is a large, shallow cave system with multiple entrances, though only the portion of the cave used in this study (Peterbilt Passage) is shown (Fig. 2). The nearest entrance to the monitoring station is called the SSS entrance, which is a small, stream passage that is prone to flooding during periods of extended precipitation or snowmelt.

BCC is a large cave system with multiple, horizontal networks above the basal Buckeye Creek stream passage, and is accessed through the Buckeye Creek entrance (Fig. 3). This cave system is also prone to flooding, with the narrow canyon (located approximately 250 m from the entrance) and the near-siphon, Watergate Sump, being especially sensitive to rises in water level. Two monitoring stations were established, with Station 1 located in the basal stream passage to the east of the canyon, and Station 2 further back in the cave on the secondary level. Periodic flooding of the Watergate Sump proved to be a significant barrier to accessing Station 2.

LWC, formerly Grapevine Cave, is a heavily decorated cave located approximately 16 km from the entrance to BCC (Fig. 4). LWC has two main sections: the commercial section and the wild section, which are separated by a narrow passage of breakdown rubble. LWC receives tourists year-round, and the commercial section is frequented by visitors daily. The wild section is used for cave tours, though on a less frequent basis. LWC was ideal for an examination of the intra-cave variability in dripwater frequency and hydrochemistry for this monitoring study because it is accessible year-round, not prone to flooding, and has a wide variety of speleothem formations and drips. However, it was less ideal for CO_2 measurements due to the unquantified, anthropogenic contribution of year-round visitors and artificial ventilation at the entrance to the commercial section.

Methods

In August 2011, surficial and cave monitoring stations were established at the three study caves to record meteoric precipitation frequency, cave microclimate, and the drip frequency and isotopic characteristics of dripwaters. Dripwater station locations were chosen to represent the variability of drip behaviors present in each cave. Trips to the study

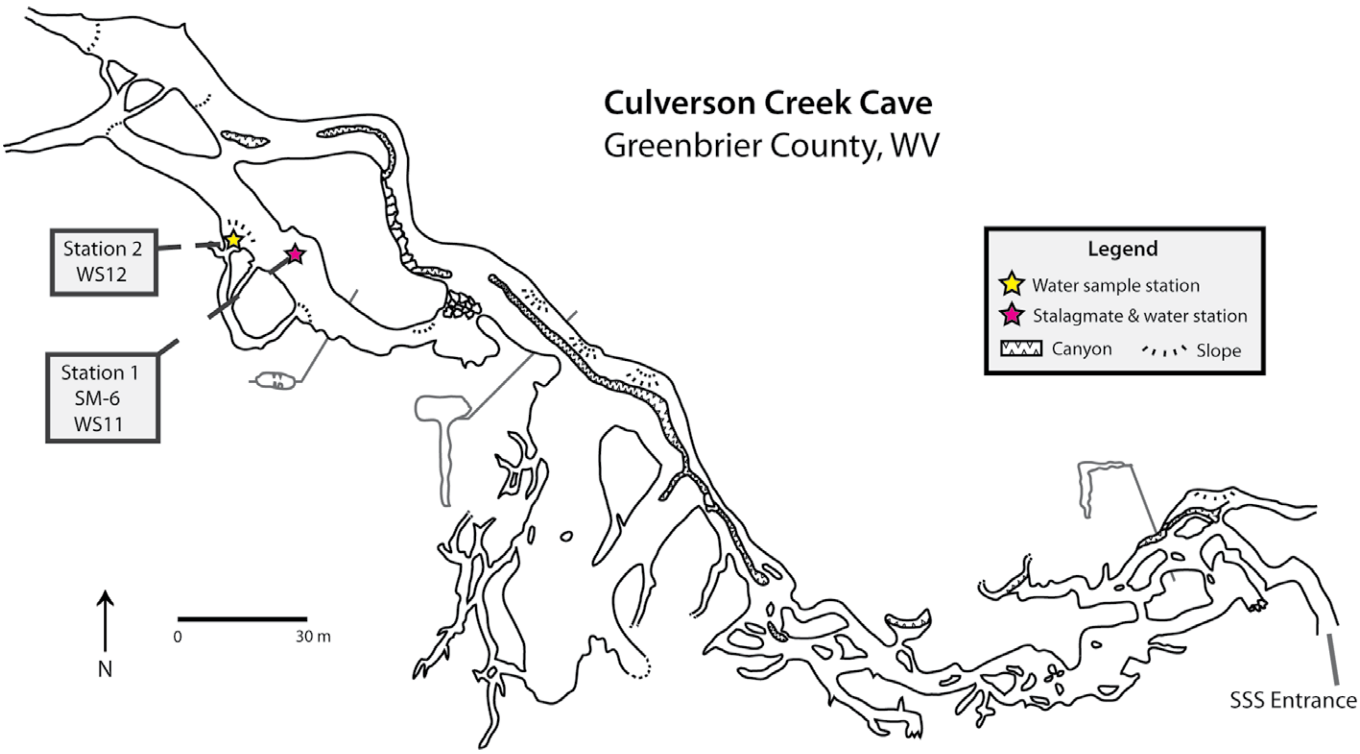

Figure 2. Map of Culverson Creek Cave, Greenbrier County, W.Va., with monitoring stations labeled (SM = Stalagmite; WS = Water Sample). Modified from map published in Dasher and Balfour, 1994.

caves were undertaken approximately every three months during the 2.5-year period (August 2011to December 2013) to retrieve data from the continuous data loggers, obtain discrete CO_2 measurements, and obtain water samples for isotopic analysis.

Cave temperature and relative humidity were measured continuously in each of the caves over the study period at 30-minute intervals with EasyLogger USB RH/Temp loggers, manufactured by Lascar Electronics, UK that recorded

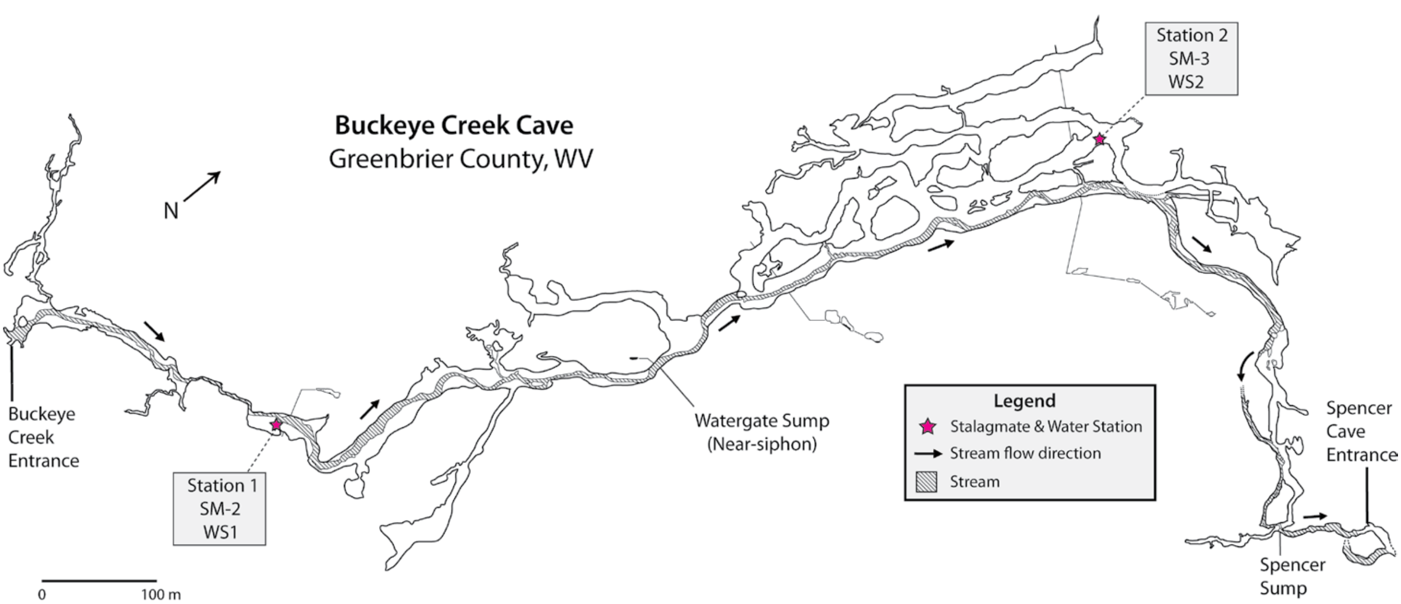

Figure 3. Map of Buckeye Creek Cave, Greenbrier County, W.Va. Modified from original survey sketches (West Virginia Association for Cave Studies), with monitoring stations labeled (SM = Stalagmite; WS = Water Sample).

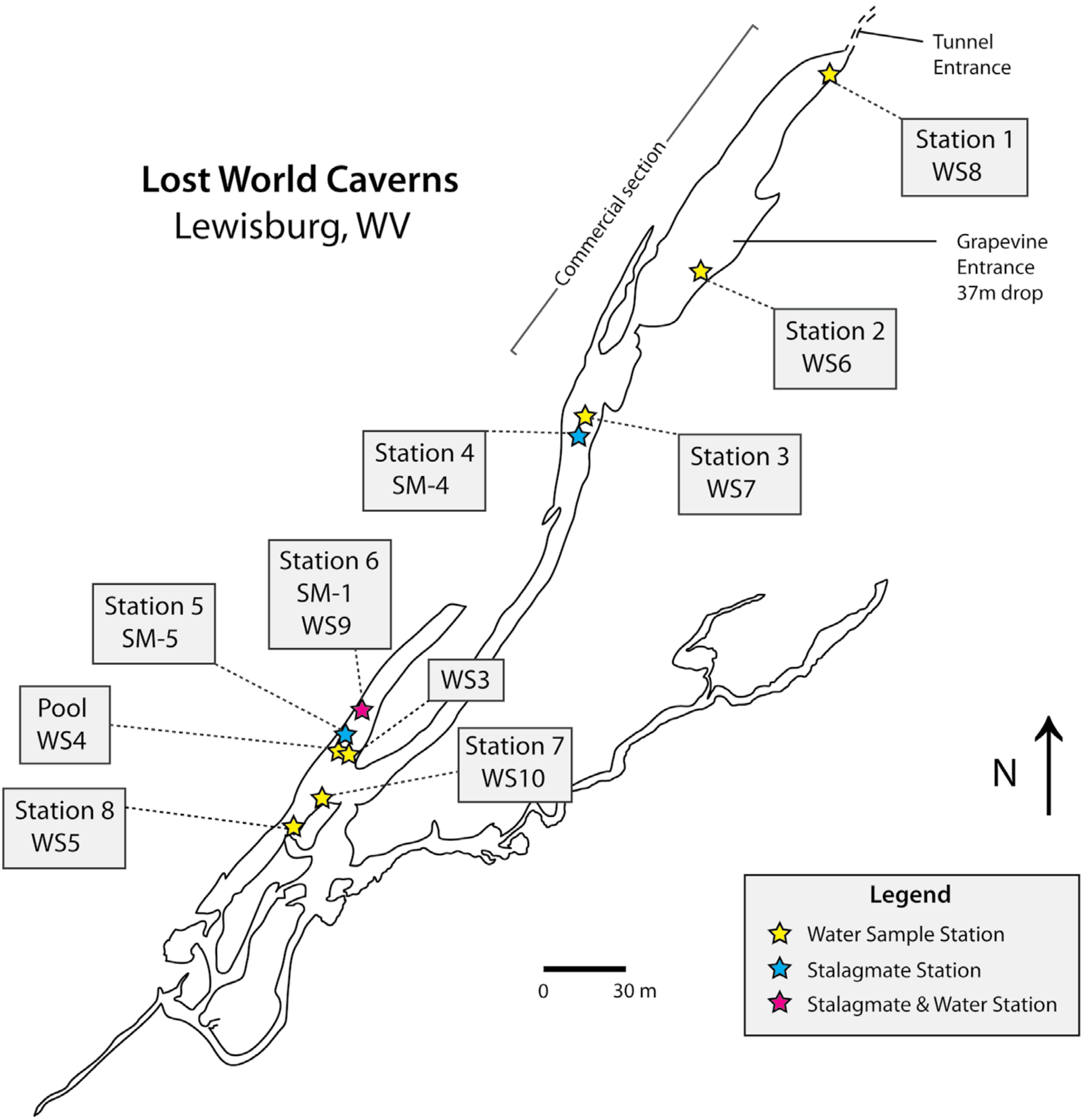

Figure 4. Map of Lost World Caverns, Lewisburg, W.Va., with monitoring stations labeled (SM = Stalagmite; WS = Water Sample). West Virginia Association for Cave Studies.

values at 1.0 degree and 0.5 % increments. Cave air CO_2 concentrations were measured during each of the sampling trips using a 0 to 10,000 ppm K33-ELG data logger, manufactured and calibrated by Dataq Instruments.

During each of the sampling trips, two visits to each of the study caves were to obtain enough dripwater for multiple analyses. Throughout the first set of cave trips, water catchment cups were deployed under drip sites (designated Water Sampling Stations, Table 1) and allowed to accumulate between 24 to 48 hours, after which dripwater samples were then collected. The relative humidity values remained high (99.7–99.99 %) throughout the entire study interval for all three caves. While potential, in-cave evaporation of dripwater is possible, simply due to the time necessary to collect enough dripwater, headspace was minimized in water sampling containers to reduce the possibility of evaporation of waters in transit.

Table 1. Dripwater source characteristics for WS1 – 12 (water sample) from Buckeye Creek Cave (BCC), Culverson Creek Cave (CCC), and Lost World Caverns (LWC) in southeastern West Virginia, USA.

Cave	Sample	Description	Notes
BCC	WS1	Stalactite-fed	Shared with Station 1 (SM-2)
	WS2	Stalactite-fed	Shared with Station 2 (SM-3)
LWC	WS3	Stalactite-fed	High-rate drip on side of large flowstone
	WS4	Pool water	
	WS5	Stalactite-fed	Periodically inactive/very slow drip
	WS6	Stalactite-fed	
	WS7	Stalactite-fed	
	WS8	Stalactite-fed	High-rate drip on side of large stalagmite
	WS9	Fed by soda straw	Soda straw approx. 30 cm in length
	WS10	Stalactite-fed	
CCC	WS11	Fed by collection of stalactites	
	WS12	Fed by collection of soda straws	

Upon collection, dripwater samples were stored in VWR high-density, polyethylene bottles. On several occasions some of the samples did not accumulate enough water to allow complete analyses to be performed. Isotopic analyses were performed at Texas State University. $\delta^{18}O$ and δD were measured using a Los Gatos Research Liquid Water Stable Isotope Analyzer (Model 908-0008), and are reported relative to the V-SMOW (Vienna Standard Mean Ocean Water) laboratory standard, with accuracies of ±0.3 ‰ for δD and ±0.1 ‰ for $\delta^{18}O$.

Figure 5. Set-up of Stalagmite monitoring station SM-4; temporary platforms for Stalagmite drip loggers were built with aluminum foil onto actively-growing stalagmites for the study duration.

Meteoric precipitation and cave dripwater frequency were monitored using Driptych Pluvimate and Stalagmite drip-frequency acoustic loggers with a 10-minute interval. One Pluvimate was placed at the surface of LWC and one at CCC (BCC is within 5 km, so the logger provides surface precipitation data relevant to both CCC and BCC). Six stalagmites were installed on top of actively-growing stalagmites (two in BCC, three in LWC, and one in CCC), under drips that exhibited different behaviors, in an effort to document a variety of dripwater responses to surface precipitation events.

Estimating the amount of meteoric water infiltrating the cave is important, as the effects of evapotranspiration can be substantial. This is accomplished through the calculation of Water Excess (*WE*), which serves as an indicator for the amount of infiltration. *WE* was calculated for Lewisburg, W.Va. (Genty and Deflandre, 1998) $WE = R - ETP$ [mm/month] where R is the total rainfall per month (mm) and ETP is the estimated monthly evapotranspiration, which was calculated using the Thornthwaite method (Thornthwaite, 1954) $ETP = 16(10\theta/I)^{a}F(\lambda)$ [mm/month] where θ = monthly temperature in degrees C; $a = (6.75 \times 10^{-7})I^{3} - (7.71 \times 10^{-5})I^{2} + (1.79 \times 10^{-2})I + 0.49239$; I = annual thermal index, which is the sum of the 12 monthly i indices; $i = (\theta/5)^{1.514}$; $F(\lambda)$ = latitude index (for 38 °N).

Table 2. Cave temperature, relative humidity, and CO2 measurements during sampling trips for the study period. Sampling sites were the commercial and wild sections of Lost World Caverns (LWC), Culverson Creek Cave (CCC), and Buckeye Creek Cave (BCC).

Location	Date	Temp., °C	RH, %	CO_2, ppm
LWC (Commercial)	8/23/2011	11.67	100	3654
	10/22/2011	11.67	100	2492
	12/29/2011	11.67	100	955
	3/12/2012	11.67	100	941
	6/18/2012	11.67	99.5	1635
	10/2/2012	11.67	99.5	1147
	1/15/2013	11.67	99.5	1424
	8/10/2013	11.67	100	3011
	12/7/2013	11.67	99.5	1380
	Average	11.67[a]	99.9[a]	1848.78
	Std. Dev.	0[a]	0.24[a]	973.69
LWC (Wild)	8/23/2011	11.67	100	1746
	10/22/2011	11.67	100	2673
	12/28/2011	11.67	100	2010
	3/12/2012	11.67	100	1461
	6/18/2012	11.67	99.5	2169
	10/2/2012	11.67	99.5	2500
	1/15/2013	11.67	99.5	1614
	8/10/2013	11.67	100	4745
	12/7/2013	11.67	99.5	1379
	Average	11.67[a]	99.9	2255.22
	Std. Dev.	0[a]	0.24	1035.01
CCC	8/22/2011	11.67	100	640
	10/22/2011	11.67	100	746
	1/4/2012	11.67	100	999
	3/14/2012	11.67	100	1180
	6/22/2012	11.67	99.5	826
	9/29/2012	11.67	100	557
	1/4/2013	12.23	99.5	693
	8/11/2013	12.23	99.5	1341
	12/7/2013	...	...	...
	Average	11.83[a]	99.97[a]	872.75
	Std. Dev.	1.09[a]	0.13[a]	276.21
BCC	8/21/2011	11.11	100	634
	10/22/2011	11.11	100	785
	1/4/2012	10.56	100	1002
	3/14/2012	10.56	100	1293
	6/22/2012	10.56	100	1077
	9/30/2012	10.00	100	1158
	1/4/2013	10.00	100	1280
	8/11/2013	9.44	100	1857
	12/7/2013	...	...	...
	Average	10.44[a]	100[a]	1135.75
	Std. Dev.	1.0[5]a	0[a]	370.78

[a] Indicates values calculated from larger dataset.

Results

The microclimate monitoring results for the studied caves confirm very humid, temperature-stable environments. The average surface temperature for the region (Lewisburg, W.Va.) over the study interval was 10.6 °C, with a seasonal range of 10 °C (Menne et al., 2012; NOAA National Climatic Data Center), which falls within the temperature range of the studied caves (9.44 to 11.67 °C). While it is well established that temperatures in the interior of caves generally represent the annual surface temperature of the region (Poulson and White, 1969), this is an important aspect in speleothem paleoclimatology as the $\delta^{18}O$ of speleothem calcite is governed by the isotopic composition of the dripwater and the cave temperature (Lachniet, 2009). The average temperature and relative humidity measurements of the three caves were highly consistent year-round (Table 2).

Temperatures in BCC were slightly lower and relative humidity slightly higher than the other two caves, both likely due to the streamflow of Buckeye Creek through BCC. The low sensitivity of these instruments (recording only whole temperature unit increments (1.0 °C) and 0.5 % steps for relative humidity values) made them less than ideal for cave monitoring studies, as slight seasonal variations would not be recorded.

Carbon dioxide concentrations were obtained as discrete measurements during the water sampling trips (Fig. 6). Minor variability for CCC and BCC was observed, with LWC exhibiting the widest range of values. The CO_2 values range from 941 to 4,745 ppm for LWC, 557 to 1,341 ppm for CCC, and 634 to 1,857 ppm for BCC. The

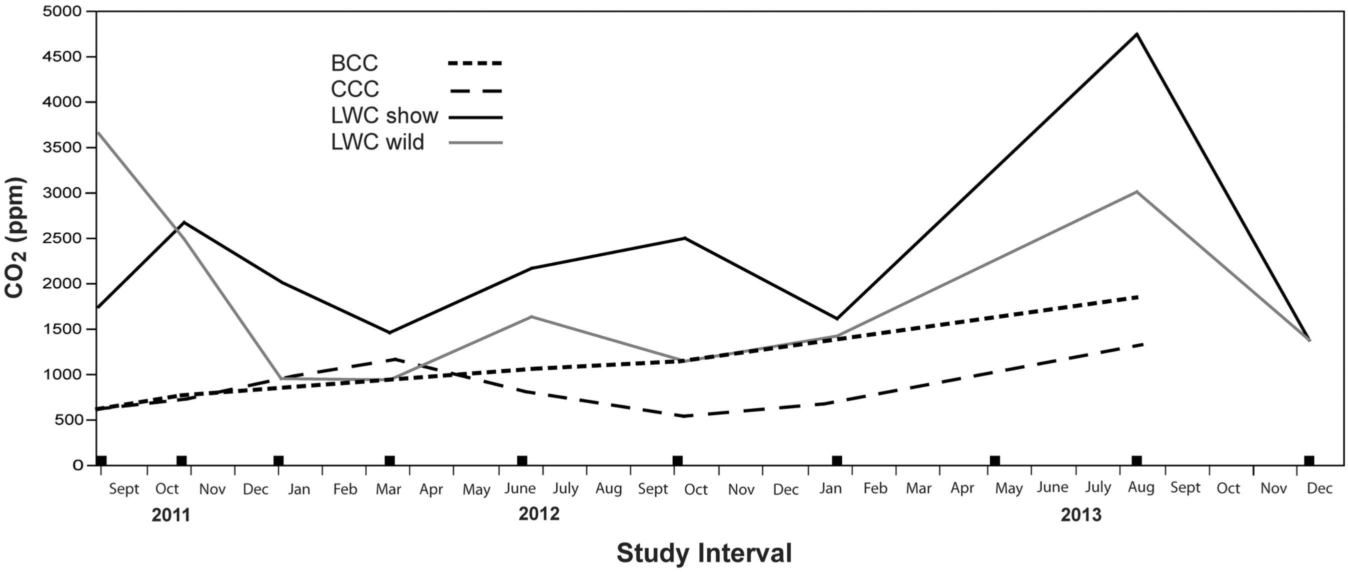

Figure 6. Periodic CO2 concentration values (ppm) for Buckeye Creek Cave (BCC), Culverson Creek Cave (CCC), and the show and wild sections of Lost World Caverns (LWC). Measurements were obtained during sampling trips (black boxes) which occurred approximately every three months during the study interval from August 2011 to December 2013. Note: values for BCC and CCC were not obtained during the December 2013 trip due to flooding.

discrete CO_2 measurements taken at each of the caves during each sampling trip permitted brief glimpses into the seasonal ventilation cycle.

CCC had the highest values in March 2012 and August 2013, but was the lowest overall, with a maximum of 1,341 ppm. Surprisingly, BCC displayed a linear trend over the study interval and also experienced the maximum of 1,857 ppm in August 2013. LWC, a show cave with year-round visitors, had the highest CO_2 concentrations with a maximum of 4,745 ppm, though it is impossible in this study to separate the natural and anthropogenic components.

Pluvimate and Stalagmite drip logger results are shown for the monitoring interval for CCC (Fig. 7), BCC (Fig. 8), and LWC (Fig. 9), as compared to monthly instrumental climate data from Lewisburg, W.Va. (ncdc.noaa.gov). The drip-logger frequency data can be found in supplemental data. The objective for the drip-frequency loggers was to compare high-resolution surface, meteoric precipitation frequency to several cave drips in an attempt to characterize the drip behavior and flow paths. This objective was met with varying degrees of success as several difficulties were experienced, including software/hardware failure, inaccessible instrumentation (i.e., flooding and sumped passages), and harsh-climate effects on the surface loggers, which resulted in gaps to monitoring data (dashed lines, Figs. 7–9). Additionally, the funnels covering the Pluvimate loggers were occasionally clogged with debris (e.g., leaf litter, wasp nests), as well as ice in some winter months. Because of these problems, a qualitative comparison is more appropriate for the drip frequency results.

For CCC, surface temperature and rainfall records for the region (Fig. 7a, b) show a wet spring and dry summer for 2012, and both show a wet spring and summer for 2013, as illustrated by the several intense rainfall events (>5k counts) recorded by PM-2, which occurred from June 2013 to August 2013 (Fig. 7c). *WE* was calculated for this region (Fig. 7d; note inverted axis to align with Fig. 7c) (Genty and Deflandre, 1998). During months when ETP (mm) exceeded precipitation (mm), it is assumed that no infiltration occurred. June 2012 to August, 2012, as well as July 2013 and October, 2013 were such months, when *WE* calculations produced negative results (exhibited as zero on Fig. 7d for ease of display) and can be considered zero as water excess.

WE is an important consideration in understanding the cave drip frequency and response to precipitation events. For example, the surface precipitation (Fig. 7c) during the months of June 2013 to August 2013 show many substantial precipitation events. However, the increase in evapotranspiration during these summer months meant that *WE* was minimal during this period, as opposed to the winter months during the study interval. When the highest *WE* values were calculated SM-6, the Stalagmite in CCC exhibited only a small response to surface precipitation events, as drip counts increased from two to three drips per 10-minute recording intervals, corresponding to a discharge of 0.014 to 0.028 mL/min, based on the typical stalactite water-drop volume of 0.14 mL, as reported by Genty and Deflandre, 1998 (Fig. 7e). The relationship between water-drop volume and the opening from which it drops was established by Collister and Mattey (2008). However, measurements of stalactite and soda straw apertures were not possible in this study due to inaccessibility.

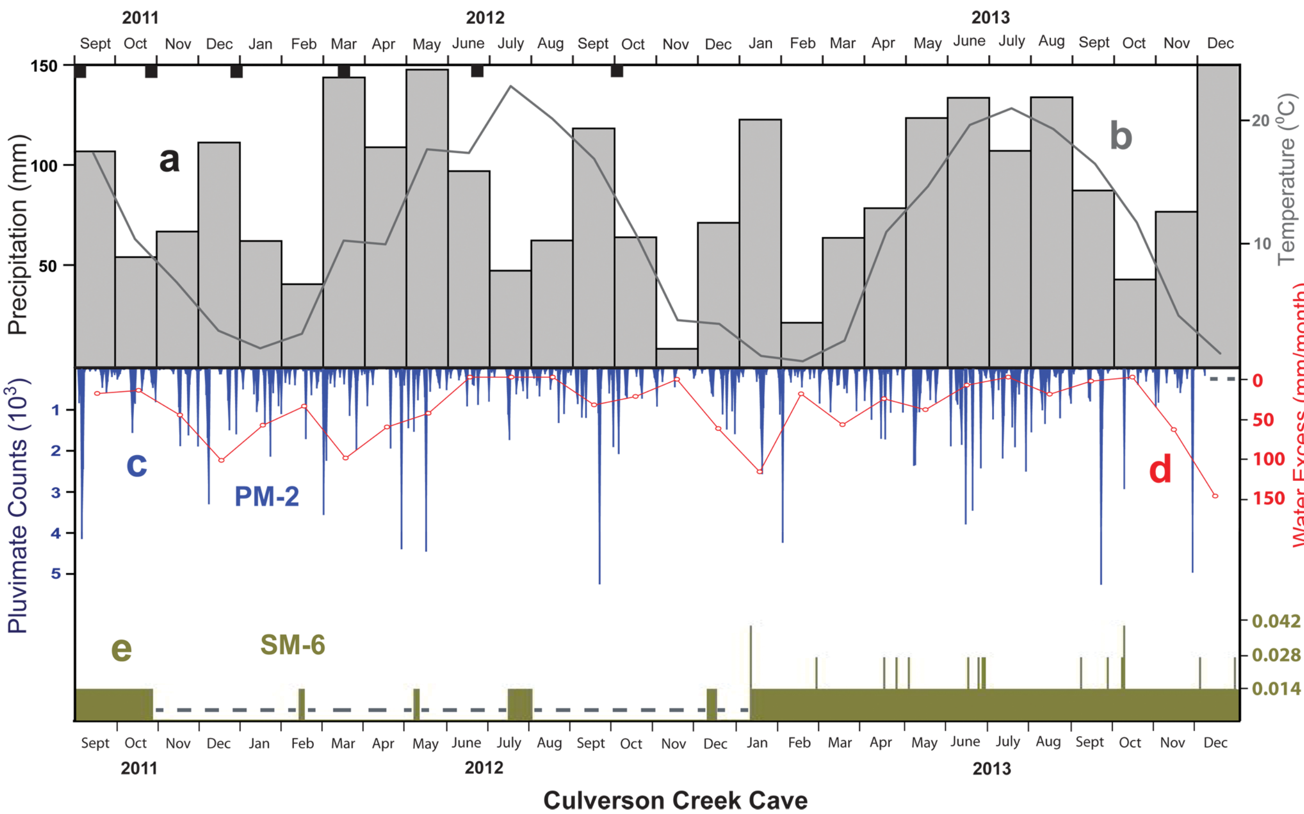

Figure 7. Drip frequency monitoring results for Culverson Creek Cave, where a) monthly precipitation totals (mm) (bar graph), and b) monthly temperature averages (°C) for Lewisburg, W.Va. (ncdc.noaa.gov); c) surface meteoric, precipitation drip-frequency logger data (PM-2) are shown as daily drip counts; d) water excess (mm/month) for Lewisburg, W.Va; and e) cave dripwater discharge (mL/min) of drip-frequency logger (SM-6). Black boxes on the top time axis denote cave dripwater sampling periods. Dashed lines indicate gaps in data.

The two Stalagmite loggers in BCC (SM-2 and SM-3) displayed very different responses to the surface precipitation (PM-2) events (Fig. 8). SM-2 (Fig. 8e) was especially responsive to surface precipitation events during the months of December to June of both 2012 and 2013. From June to November of the same years, however, the drip rate decreased to a baseline value and became largely unresponsive to precipitation events. The quiescence of SM-2 cave drips during the early summer is likely due to both a decrease in rainfall and an increase in evapotranspiration, which results in minimal infiltration of water into the epikarst. SM-3 (Fig. 8f) displays seepage flow behavior and responded to drips most similarly to SM-6 (Fig. 7e) and never exceeds three drips per interval. The data gaps for SM-3 are due to site inaccessibility of the station during sampling trips for data retrieval.

The cave drip frequency results for LWC exhibit a wide range of behaviors. The surface precipitation logger (PM-3) recorded quite similar results (Fig. 9c) as PM-2, with the main difference being the presence of two, brief data-gap intervals in 2013. SM-1 was highly responsive to surface precipitation events (Fig. 9e), with a high drip volume until late November 2012. Then, the drip suddenly changed behavior to a low-volume drip with a baseline of approximately 16 drips per sampling interval, corresponding to a discharge of 0.224 mL/min (Fig. 9f). This sudden change is not believed to be an instrumental or placement error as SM-1 was tested for functionality and correct placement. These test results demonstrated that the equipment was functioning properly and recording drips in the correct location. These data are, therefore, considered to reflect the changing behavior of the drip throughout the study interval. The drip data for SM-1 continued at this low-flow, relatively unresponsive state for the rest of the study.

Drip logger SM-4 displays seasonal behavior (Fig. 9g) similar to that of SM-2 (Fig. 8e), where the drip is most active between the winter to early summer months and decreases to a baseline flow of two to three drops per sampling interval, corresponding to a discharge of 0.028 mL/min to 0.042 mL/min during the rest of the year. SM-4 experienced the maximum drips during the March 2012 to May 2012-period, coincident with the period of highest rainfall of that year. Interestingly, the large rain event, which occurred in late September 2012, did not bring SM-4 above the baseline flow, likely due to the relatively dry state of the epikarst from low infiltration during the summer months (low rainfall and high

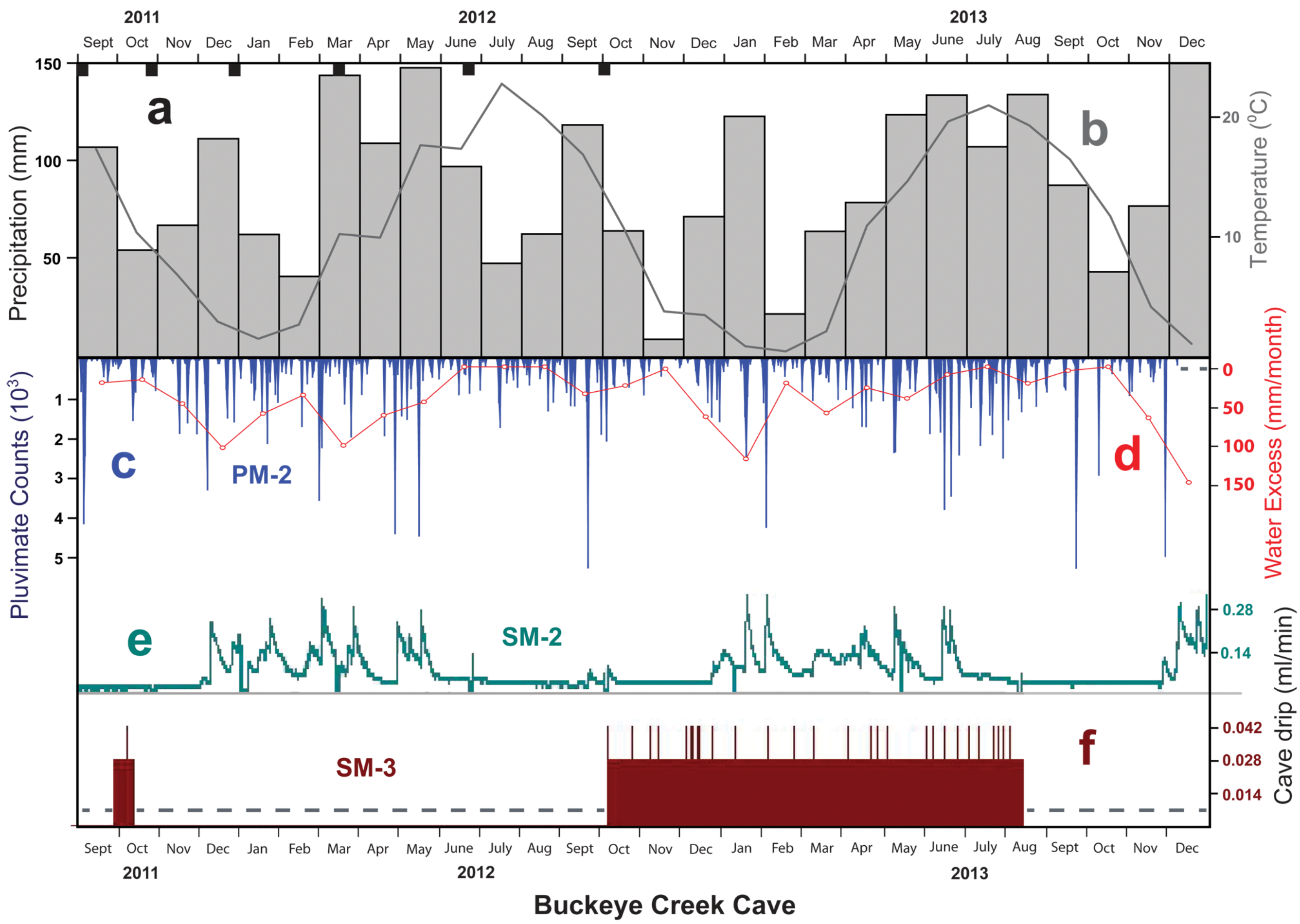

Figure 8. Drip-frequency monitoring results for Buckeye Creek Cave, where a) monthly precipitation totals (mm) (bar graph) and b) monthly temperature averages (° C) for Lewisburg, W.Va. (ncdc.noaa.gov); c) surface meteoric precipitation drip frequency logger data (PM-2) are displayed as daily counts; d) water excess (mm/month) for Lewisburg, W.Va.; and e) cave dripwater discharge (mL/min) of drip-frequency loggers (SM-2); and f) SM-3. Black boxes on the top time axis denote cave dripwater sampling periods. Dashed lines indicate gaps in data.

evapotranspiration). The final drip logger, SM-5 (Fig. 9h) displayed seepage-flow behavior similar to both SM-6 (Fig. 7e) and SM-3 (Fig. 8f). There were unknown instrumental and/or possible user error problems with SM-5 and SM-6, which resulted in gaps of the recorded data at random periods (however, the gaps in SM-3 are due to site inaccessibility).

Isotope ($\delta^{18}O$ and δD) values of water samples were obtained for each sampling trip (Fig. 10, see supplemental data) and are compared to the Global Meteoric Water Line (GMWL) (Craig, 1961) and Local Meteoric Water Line of the closest Global Network of Isotopes in Precipitation (GNIP) station in Coshocton, Ohio (IAEA/WMO, 2016). If dripwaters retain the isotopic signature of their parent meteoric waters, then it follows that these values should plot near those of the LMWL (see Discussion).

The comparison of cave dripwaters to the GMWL (Fig. 10) allows for the identification of possible fractionation processes from surface to cave dripwaters, as the values would presumably be identical under equilibrium conditions. This is the case with most of the dripwater samples, which fall within the range of the meteoric waters used to plot the Local Mean Water Line (LMWL, Coshocton, Ohio GNIP station, ncdc.noaa.gov). The two dripwater batches that deviate from this line are March 2012 and October 2012 (a few samples overlap with October 2011 as well). The October 2012 and October 2013 isotope values are the most positive, falling off the LWML to the side, typical of waters that have experienced evaporative loss. The dripwaters sampled in both October 2012 and October 2013 likely comprised waters that had accumulated in the epikarst during the summer months, which had experienced significant evaporation in the soil zone.

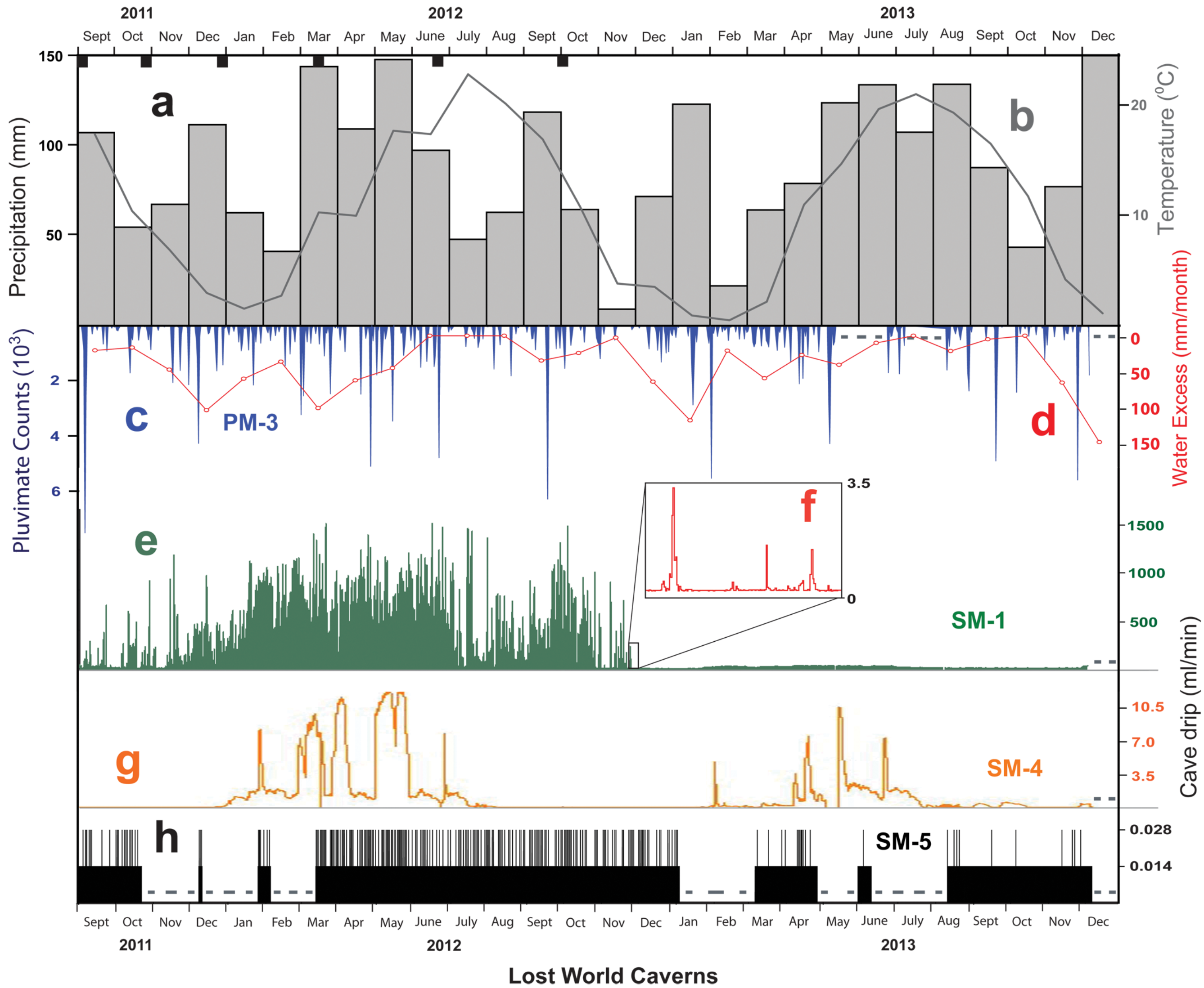

Figure 9. Drip frequency monitoring results for Lost World Caverns, where a) monthly precipitation totals (mm) (bar graph) and b) monthly temperature averages (° C) for Lewisburg, W.Va. (ncdc.noaa.gov); c) surface meteoric, precipitation drip-frequency logger data (PM-3) are displayed as daily counts; d) water excess (mm/month) for Lewisburg, W.Va; e) cave dripwater discharge (mL/min) of drip-frequency loggers (SM-1); f) inset for SM-1 (Note: scale); g) SM-4; and h) SM-5. Black boxes on the top time axis denote cave dripwater sampling periods. Dashed lines indicate gaps in data.

Discussion

Cave Drip Characterization

The stalagmite drip frequency data over the 28-month study interval recorded three primary drip behaviors in response to meteoric precipitation: no response, where the drip frequency is generally slow and unchanging throughout the year (SM-3, SM-5, and SM -6); seasonal response, where cave drips are only active (or more active) for part of the year (SM-2, SM-4); and highly responsive, where a strong relationship exists between meteoric, precipitation frequency and cave drip frequency (SM-1). The decrease in drip frequency of SM-1 that began in December 2012, and persisted until the end of the study, may represent flow-switching, where a very high-frequency drip becomes blocked and entirely or partially reroutes through a different outlet (Baldini et al., 2006).

The important role of *WE* in cave infiltration is emphasized in the results that display a seasonal response (SM-2, SM-4, Figs. 8 and 9), as the drips are most frequent during the winter to early summer months, when WE values are at their highest for the year. The large meteoric precipitation events that occurred during non-recharge months (Fig. 8c) do not produce a subsequent increase in cave drip frequency (e.g., the large rain event which occurred in late September 2013), like those which occur when *WE* values are high.

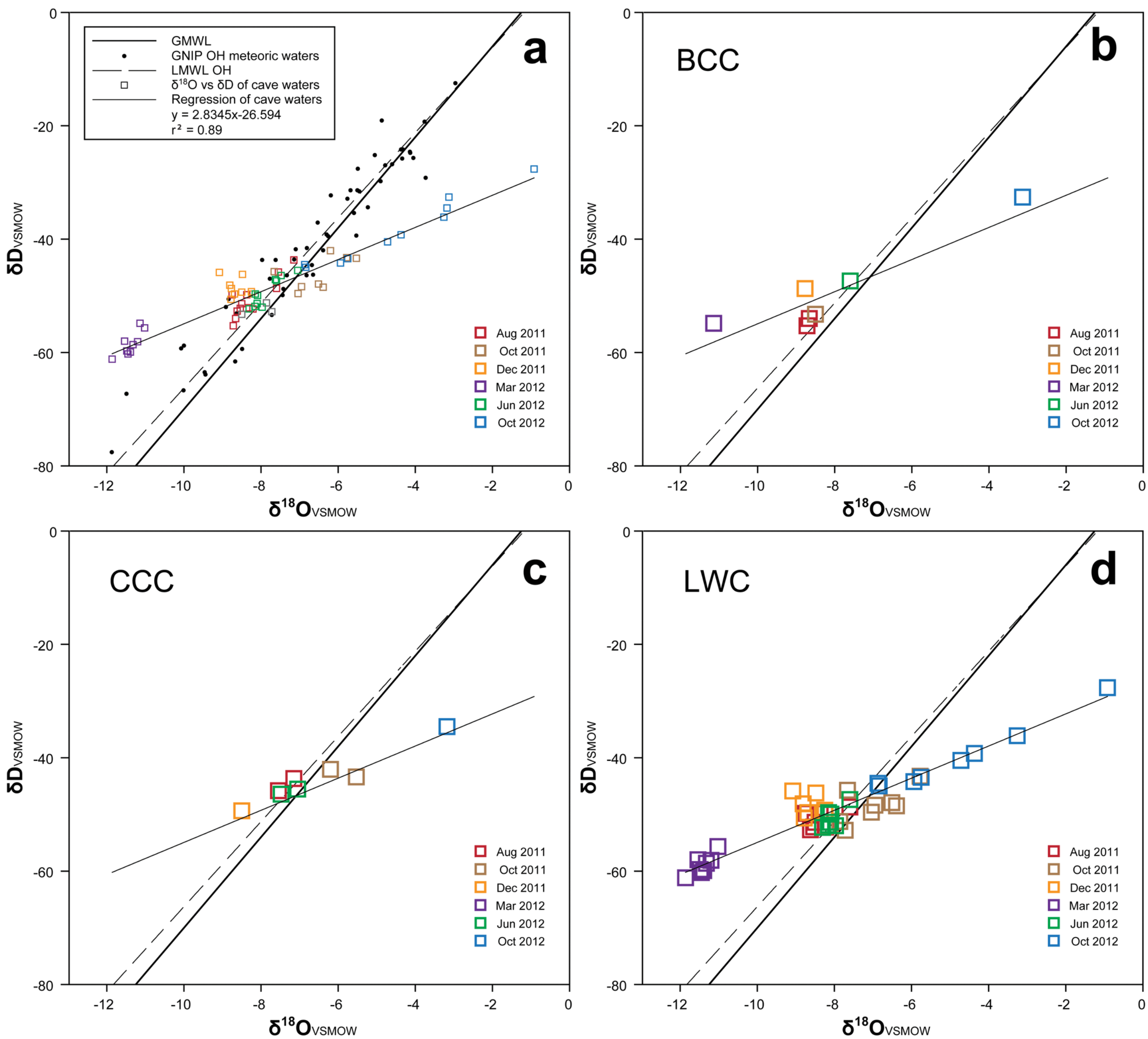

Figure 10. a) Cross-plot of cave dripwater sample δD and δ18O values standardized to VSMOW (squares; this study). Global Meteoric Water Line (GMWL) (thick black line) as δD = 8 × δ18O + 10 ‰ (Craig, 1961). Global Network of Isotopes in Precipitation (GNIP) of meteoric precipitation from closest station in Coshocton, Ohio (black dots), whose regression line provides the Local Meteoric Water Line (LMWL, dashed line) (y = 7.5096x + 8.8099; R2 = 0.9729), combined cave dripwater samples (this study) (y = 2.8345x − 26.594; R2 = 0.8900), b) cave dripwater samples from Buckeye Creek Cave; c) cave dripwater samples from Culverson Creek Cave; and d) cave dripwater samples from Lost World Caverns.

Cause of Low Dripwater Isotope Values for March 2012 Samples

The low δD and $\delta^{18}O$ isotope values of the March 2012 water samples are enigmatic, but are likely related to the anomalously-high rainfall in March 2012 (nearly twice the amount compared to March 2013). The cave dripwater samples were collected on March 12, 2012, 10 to 12 days after several substantial rain events that occurred from February 29, 2012 to March 2, 2012, as identified through pluvimate data. There are four scenarios that may account for these isotope values: the source of the moist air masses, which resulted in said rain events, was of polar or high altitude origin; the waters in the vadose zone had experienced significant evaporation resulting in kinetic, disequilibrium effects of the water vapor that could re-condense to dripwaters (Dansgaard, 1964); cave dripwater samples experienced substantial evaporation during the collection process; and the influx of the substantial amount of rain flushed out the wa-

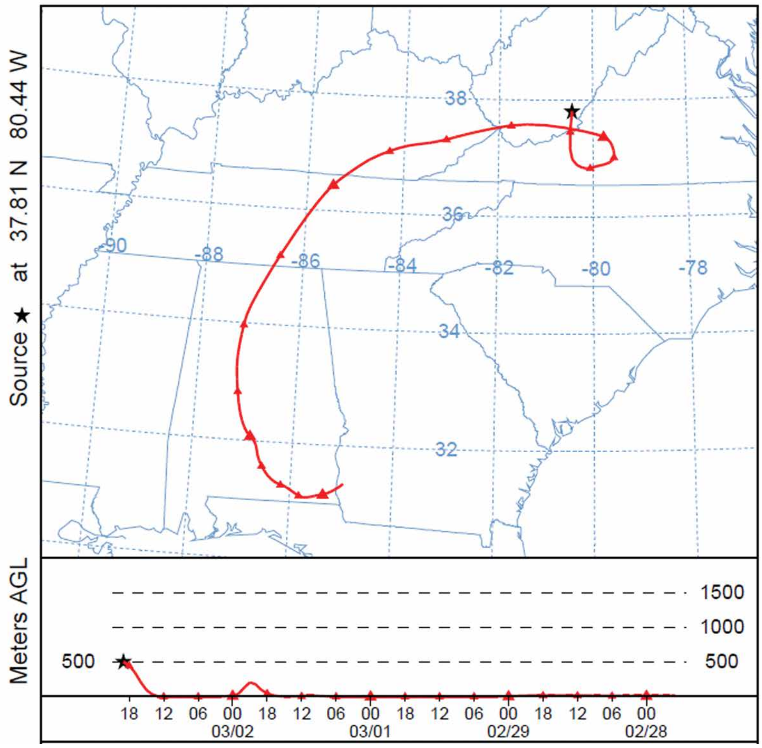

Figure 11. Hybrid Single Particle Lagrangian Integrated Trajectory Model (HYSPLIT), backward trajectory analysis for the study area during the interval of February 28 2012 to March 2, 2012 (Stein et al., 2015).

ters from the vadose zone that were comprised of meteoric precipitation, which had occurred during the cold winter months (December to February) of the preceding winter.

Scenario 1 can be tested using the back-trajectory analysis feature of the National Oceanic and Atmospheric Administration's (NOAA) Hybrid Single Particle Lagrangian Integrated Trajectory Model (HYSPLIT), which is used to calculate the trajectories of air parcels, utilizing archived weather data (Stein et al., 2015). A backward trajectory analysis was performed for the study area during the interval of February 28, 2012 to March 2, 2012, and this revealed that the air masses originated to the south of the study area at low altitudes (Fig. 11), which eliminates this scenario as a possible cause of the negative-dripwater isotope values.

Scenario 2 recognizes the possibility for kinetic, disequilibrium effects resulting from the evaporation of waters in the vadose zone. If re-condensed, the isotopic values of the resulting waters would be more negative. This scenario is unlikely to be the cause of the negative values of the March 2012 dripwater samples for two reasons. First, the water vapors, resulting from evaporation in the vadose zone, cannot be expected to remain in place and re-condense in-situ as the vadose zone is not a closed system. Second, the likelihood of this scenario is lessened by the fact that all dripwater samples from all three study caves exhibited similar negative isotopic values despite different vadose zone thicknesses and morphologies, and different drip behaviors. While evaporation and disequilibrium effects of waters in the vadose zone may occur under specific circumstances, it is highly unlikely that vadose zone evaporation during the winter months affected all three caves and all resulting dripwater samples to the same magnitude.

Scenario 3 probes the possibility that cave dripwater samples experienced substantial evaporation during the collection process. While this occurrence cannot be definitively ruled out, it is unlikely. All stalagmite loggers that were actively logging during the February 2012 to March 2012 interval (SM-1, SM-2, and SM-4) recorded relatively high drip rates, which minimized time for dripwater sample collection. The CO_2 (ppm) values measured during the March 2012 sampling period were 941 to 1,461 ppm. While CO_2 concentrations are typically lower in caves during the cold winter months due to seasonal ventilation effects, these values are likely not low enough to drive the evaporation of samples to such a degree for all three study caves.

Scenario 4 states that the influx of meteoric precipitation during the late February 2012 to early March 2012 period to the study region effectively flushed out the waters that had accumulated in the vadose zone during the preceding winter months. The meteoric precipitation falling in the cold winter months would be more negative and fall to the left of the GMWL (Dansgaard, 1964). Because all of the dripwater samples from the three caves had similar negative isotope values, this scenario is the most likely. While Scenarios 2 and 3 cannot be completely ruled out, it is unlikely that such specific circumstances would be active in all three study caves, affecting all of the dripwater samples to the same degree.

Implications for Using Speleothems for Paleoclimate Studies

This study highlights the wide range of concurrent drip behaviors that can be found in caves. From the frequency monitoring of six cave drips, one was shown to be highly responsive, two demonstrated a seasonal response, and the remaining three exhibited slow, exceedingly stable, year-round drip behavior. If these stalagmites were collected and sampled geochemically ($\delta^{18}O$, $\delta^{13}C$, trace metals, etc.) for the purpose of paleoclimate reconstructions, they would likely record very different geochemical records of the same time period simply because they are recording different aspects of the hydrologic system. For paleoclimate studies of long-term climate changes, the ideal stalagmites would be the result of slow, steady drip behaviors. While it is not feasible to perform multi-year cave monitoring research programs for every stalagmite utilized in paleoclimate studies, such a monitoring program would aid in the identification and collection of only the most appropriate and promising stalagmite samples. This conservative approach would be beneficial not only for the preservation of priceless cave formations, but it would also aid in the interpretation of the relationship between proxy values and meteoric precipitation.

Conclusions

The wide range of cave drip behaviors and isotopic values over this 28-month study highlight the dynamic nature of these systems, as well as the importance in understanding the geochemical implications of resulting speleothem calcite to paleoclimate reconstructions. The drip frequency results of the six Stalagmite loggers used in this study revealed one high-frequency drip that underwent flow-switching during the study (SM-1), two seasonal drips that were most active during the winter to early summer months (SM-2 and SM-4), and three seepage flow drips that were very slow and constant throughout the year (SM-6, SM-3, and SM-5). If stalagmites forming under these drips were used in paleoclimate reconstructions, they would likely preserve very different climate signals. SM-1 would be unsuitable as the high-frequency drip would likely dissolve more calcite than it deposited, as a small dissolution pit is present at the top of the stalagmite. The calcite laminae precipitating from the seasonal drips would likely be biased to the winter to early summer months, when most of the drips were recorded. The seepage drips, however, would most likely preserve a long-term climate signal with minimal seasonal bias and would be the most suitable for long-term paleoclimate reconstructions.

Most of the dripwater isotope values fall within the range of meteoric waters, as would be expected for mixed karst waters. However, two important sets of outliers are present in these data. March 2012 dripwaters present, as an isotopically-distinct group, that is likely the result of heavy rainfall infiltrating the vadose zone, and flushing out the isotopically-negative waters that had accumulated during the previous cold winter months. Dripwaters from October 2012 (and to a lesser extent, October 2011) likely represent heavily-evaporated soil waters from the summer months previous. These dripwater data allow for a greater understanding of the seasonal variations of isotope values, and aid in the identification of dripwaters that have undergone disequilibrium processes in the soil and epikarst zones.

While cave monitoring programs have inherent difficulties (time/financial commitment, instrument failure, etc.), they are essential in understanding the climatological implications of speleothem geochemistry. The prior monitoring and selection of stalagmites for paleoclimate reconstructions based on its drip characteristics would not only allow for a better understanding of what the geochemical proxies represent in the climate system but would also help to conserve stalagmites if only the most appropriate were collected.

Acknowledgments

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COMBINED USE OF HYDROGEOLOGICAL, HYDROGEOCHEMICAL AND ISOTOPIC TECHNIQUES TO IDENTIFY THE IMPACT OF A SALT DIAPIR ON SURROUNDING AQUIFERS, SOUTHERN IRAN

Zohreh Alemansour and Mehdi Zarei[1]

Abstract

More than 120 exposed salt diapirs in southern Iran are connected to the adjacent aquifers and likely constitute the main sources of groundwater salinization in the region. Located in southern Iran, the Korsia salt diapir is surrounded by alluvial and karst groundwater aquifers. To investigate the impact of the salt body of Korsia on the groundwater quality of surrounding aquifers, electrical conductivity, total dissolved solids and dissolved calcium, magnesium, sodium, potassium, chloride, bromide, and sulfate concentrations were measured at 41 sampling points, including 32 exploitation wells, 7 springs and 2 surface water stations. Additionally, oxygen-18 and deuterium isotopes were analyzed at 7 sampling points to investigate the source of the salinity in the area. Our hydrogeological, hydrogeochemical, and isotopic evaluations show that the Korsia diapir deteriorates groundwater quality of the eastern karst and southern alluvial aquifers through infiltration of a spring's brine into limestone, and flow of the surface brine originated from the diapir, respectively. A karst aquifer west of the diapir is not influenced by the diapir brine because its hydraulic connectivity is interrupted by an impermeable geological formation. Construction of salt basins or diversion of brine is suggested to increase water quality of the surrounding aquifers. These procedures can be applied not only in the Korsia diapir, but also in tens of diapirs of southern Iran as remediation methods to improve water quality of their adjacent aquifers in this arid region.

Introduction

There are more than 120 emerged salt diapirs in the Zagros Mountain Ranges of southern Iran (Talbot and Alavi, 1996; Bosák et al., 1999; FDA, 2016; Zarei, 2016). Intrusion of brines from these salt diapirs into their surrounding water resources contributes to degrade the quality of surface and underground waters of southern Iran (Zarei, 2010; Zarei et al., 2014). Salt diapirs are also reported in the United States (the Gulf Coast region and southeast Utah), the Dead Sea coasts, the northern German Plain, and northeast Spain (Frumkin, 1994; Bosák, 1999; Kloppmann et al., 2001; Hamlin, 2006; Lucha et al., 2008). Investigations dealing with exposed salt diapirs in Spain and the Dead Sea coasts have been mainly the subject of salt speleogenetic studies, whereas their impact on water quality on the surrounding water resources have not been considered so far. The salt diapirs in the United States (Hamlin, 2006) and Northern Germany (Kloppmann et al., 2001) have no exposure at the surface and their effect on water quality has been evaluated in detail.

Geomorphological and hydrogeological aspects of salt diapirs of southern Iran have been studied in detail by several investigators over the last two decades. Bruthans et al. (2000) characterized the factors affecting morphogenesis of salt karst in southern Iran, pointing to thickness of caprock as a major factor that influences superficial and underground karst forms. The most important factors affected by caprock thickness are, in turn, the density of recharge points, the amounts of concentrated recharge, the rate of ground surface lowering, the dissolution capacity of water, and the size and amount of load transported by underground flood-streams into cave systems. Bruthans et al. (2006) estimated the age, depositional history and uplift rates of marine terraces of the Hormoz and Namakdan salt diapir in the Persian Gulf, based on radiocarbon dating. The erosion rates of residuum and rock salt exposures on several salt diapirs with different climatic settings were measured in southern Iran for a period of five years by Bruthans et al. (2008). They found that denudation of rock salt exposures is much faster than the diapirs covered by weathering residuum. Bruthans et al. (2009) studied surficial deposits of 11 Iranian salt diapirs, and characterized that the source material, diapir relief, climatic conditions, and vegetation cover were the main factors affecting the development and erosion of surficial deposits. Evolution of salt diapir and karst morphology of the coastal salt diapir of Namakdan, southern Iran, were evaluated based on known sea-level oscillations, radiometric dating, and geological evidence (Bruthans et al., 2010).

Comparing the evolution of Namakdan diapir with the Hormoz and Larak diapirs, they showed that the evolution of diapir morphology is strongly affected by the differences in uplift rates and geological settings. Bruthans et al. (2017) studied soil, drip, stream, and flood waters from different environments at several diapirs of southern Iran, and they found that the soil water chemistry depends on both the climate and cap soil thickness. Abirifard et al. (2018) studied model of flow direction and hydrochemical effects of the Jahani salt diapir on the adjacent water. They also characterized the major factors controlling the morphological aspects of salt karst at the Jahani diapir.

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A variety of chemical constituents and ratios have been suggested to distinguish halite-solution brine from other potential sources of salinization, such as evaporation of groundwater (Richter et al., 1991; Kloppmann et al., 2001; Davidson and Mace, 2006; Jirsa et al., 2013; Ying et al., 2013; Kumar, 2014; Salameh et al., 2014; Lichun Ma et al., 2016; Ebrahimi et al., 2016;). Intrusion of halite-solution brines typically produces significant changes in groundwater chemistry from more Ca-HCO_3 to Na-Cl type (Kreitler and Richter, 1986). The molar ratio of Na/Cl has been also suggested by Leonard and Ward (1962) to characterize halite-solution brines. Sodium (Na) and chloride (Cl) are present in halite at equal molar concentrations, and therefore, the Na/Cl molar ratio is close to one in brines that originate from salt diapirs (Richter et al., 1990). Bromide is extensively used in combination with chloride as a useful tracer in the study of saline waters (Richter et al., 1991; Cartwright et al., 2006; Zarei, 2010; Charef, et al., 2012). Both constituents (Br and Cl) are conservative and they are not easily removed by processes such as ion exchange or precipitation)Kreitler and Richter, 1986). Their ratio (Br/Cl) can be used as a tracer of salinization sources. The Br/Cl ratio in brines related to halite dissolution (Br/Cl $<4 \times 10^{-4}$) is typically one order of magnitude smaller than in other water sources. Many authors have applied Br/Cl ratio in their studies to identify sources of salinization, including Whittemore and Pollock (1979), Kreitler and Richter (1986), Morton (1986), Kreitler (1993) and Kharroubi et al. (2012).

The water quality of alluvial and karst aquifers surrounding the salt diapirs of southern Iran are typically deteriorated by intrusion of diapir-derived brine, and this represents one of the main hydrogeological problems affecting karst aquifers in Iran (Karimi and Taheri, 2010; Taheri et al., 2016, 2017). Zarei (2016) evaluated 62 salt diapirs in southern Iran to identify factors governing the impact of salt diapirs on the surrounding water resources. The author concluded that the main controlling factors are: i) the evolutionary stage of the diapirs, ii) the geology, iii) the hydrogeological setting, and iv) the anthropogenic activities. Moreover, Mehdizadeh et al. (2015) studied how the 62 salt diapirs of southern Iran influence water quality in the surrounding aquifers, reporting that the main mechanisms of the adjacent aquifers' deterioration are related to: a) diapir-brine intrusion in the subsurface, b) re-infiltration of brine emerging from springs, and c) infiltration of saline runoff originating from the surface of the diapirs. The effects of several, individual salt diapirs on adjacent aquifers in southern Iran have been also evaluated at the Konarsiah diapir (Sharafi et al., 1996; Zarei and Raeisi, 2010; Zarei et al., 2013), Gaztavileh diapir (Sharafi et al., 2002), Bastak diapir (Zarei et al., 2014), and Karmustadje diapir (Nekouei and Zarei, 2016). For instance, Sharafi et al. (2002) studied the impact of the Gaztavileh salt diapir on the adjacent karst aquifer and reported that the water quality is degraded by the diapir brine. Nekouei et al. (2016) evaluated the influence of Karmustadj salt diapir on aquifers using hydrogeochemical and isotopic techniques. They found that diapir-derived brines intrude the surrounding alluvial aquifers but have no degrading impact on the adjoining karst aquifer.

Salt has a particularly low-yield strength, and consequently, is subject to plastic deformation under differential pressure (Anderson and Brown, 1992). The role of halokinesis in salt karst hydrology has been evaluated by some researchers. Frumkin (2000) studied how halokinesis inhibit deep development of salt caves in salt diapirs of the Dead Sea area. Chiesi et al. (2010) related the sudden increase in salinity of springing waters in Poiano area, Italy, to active halokinesis causing new bodies of rock salt to reach the water table (De Waele et al., 2017).

The Korsia salt diapir of southern Iran is in direct contact with adjacent karst and alluvial aquifers, and in addition, shows several brine springs that emerge from the diapir. The surrounding wells and springs are characterized by fresh to brackish waters. The overall purposes of this study are to: i) evaluate the salinity distribution of waters in the Korsia area, ii) demonstrate that the salt diapir is the main source of aquifer salinization, and iii) describe how this diapir influences water quality.

Geological, Geomorphological and Hydrogeological Setting

The salt diapir of Korsia is located in south-central Iran, 15 km west from the city of Darab (Fig. 1). The diapir occurs on the southern limb of the Shahneshin Anticline, situated in the Zagros Mountains. The Shahneshin Anticline follows the general NW-SE trend of the Zagros. James and Wynd (1965) and Falcon (1974) described the stratigraphical and structural characteristics of the Zagros sedimentary sequence. The exposed geological formations of the study area, from the oldest to the youngest, include Hormuz salts (Precambrian-Middle Cambrian), Sarvak limestones (Cretaceous), Radiolarite Unit (Cretaceous), Tarbur limestones (Upper Cretaceous), Sachun marlstones (Upper Cretaceous- Paleocene), Jahrum limestones and dolostones (Oligocene), Razak shales and marlstones (Oligo-Miocene), and Gachsaran marls and evaporites (Tertiary). The bedrock is unconformably overlain by Quaternary alluvium that characterizes the alluvial plain (Falcon, 1967; Berberian and King, 1981; Alavi, 2004). The salt rocks of Korsia diapir belong to the Hormuz Formation with an approximate original thickness of 1 km (Stöcklin, 1968; Kent, 1979).

The Korsia salt diapir has an elliptical shape with an area of 2.64 km^2 (Fig. 2a). Mehdizadeh et al. (2015) characterized it as an active diapir in terms of the evolutionary stages of salt diapirs. An active salt diapir has a positive relief with significant area of salt rock exposures. Table 1 summarizes the morphological characteristics of the Korsia diaper, the maximum length and width of the diapir being 2.40 and 1.48 km, respectively. The maximum elevation of the diapir is

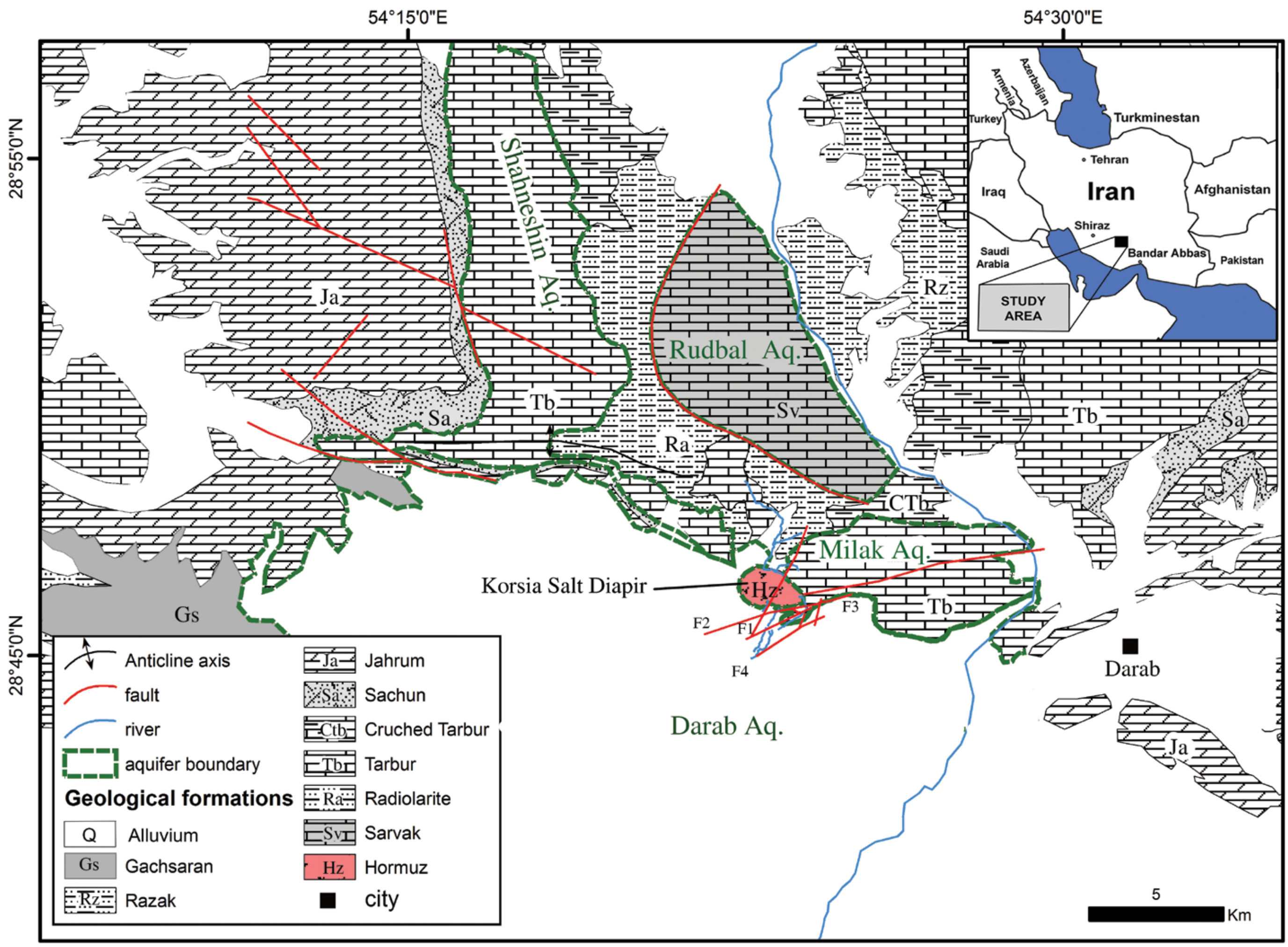

Figure 1. Geological map of the study area.

1300 meters above sea level (m.a.s.l.) and 200 m above the surrounding plain, which is located in the western section.

The diapir can be separated into two different morphological portions: i) summit and ii) low land zones (Fig. 2). The western portion, with high relief, comprises the top of the diapir (summit zone), whereas the eastern one has a low and hummocky morphology (low land zone) and is highly dissected by several faults. In the summit zone, salt is covered by residual soil, while in the low-land, it is mostly exposed.

The study area is located in a semi-arid region with a mean annual precipitation of 360 mm (Alemansour, 2015) that takes place mainly during late fall and winter. The salt diapir of Korsia is surrounded by four aquifers (Fig. 1):

Milak karst aquifer: it is located east of the diapir (Fig. 1). The aquifer is composed of well-karstified limestones of the Tarbur Formation and is in direct contact with the Korsia diapir. Milak karst aquifer discharges about 800 L/s of groundwater through two springs (S8: Korsia, and S9: Shahijan) with electrical conductivities of 2.25 and 0.44 mS/cm, respectively. They have become dry during recent years (Fig. 3a). The northern and eastern boundaries of Milak aquifer are mainly bordered by the Rudbal River.

Shahneshin karst aquifer: as for the Milak karst aquifer, it is associated with the Tarbur limestones in the northwest side of the Korsia diapir (Fig.1). Shahneshin Aquifer is drained by Golabi spring (S7). The emergence point of the spring is only 1 km away from the Korsia diapir (Fig. 3). However, the spring is of bicarbonate water type with electrical conductivity of 0.60 mS/cm.

Rudbal karst aquifer: it develops in the limestone rocks of Sarvak Formation, north of the Korsia diapir (Fig. 1). The NW boundary of the Rudbal aquifer is in contact with Rudbal River. Hydraulic connectivity of the Korsia diapir with Rudbal aquifer is interrupted by the impermeable Radiolarite Unit. No evident springs discharge the Rudbal aquifer, which likely feeds the Rudbal River. Electrical conductivity in the two sampling sites of Rudbal River (R1 to R2) decreases from 0.69 mS/cm (R1) to 0.45 mS/cm (R2) along this section, which is thought to be related to input of high-quality water from Sarvak aquifer.

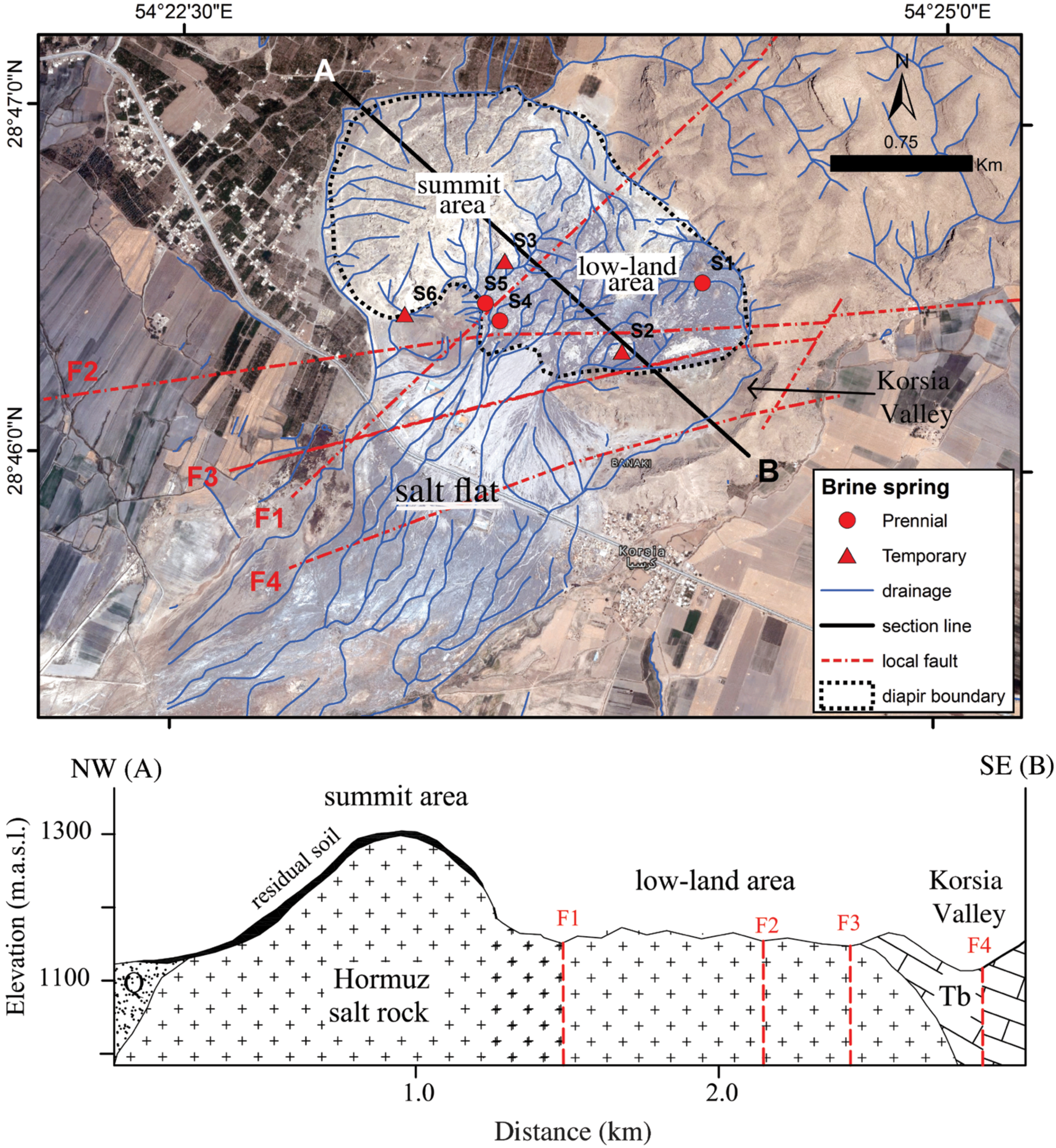

Figure 2. a) Satellite image of the study area; b) NW-SE cross-section of the Korsia salt diapir along the section line of AB.

Darab alluvial aquifer: it borders the southern and western portions of the Korsia salt diapir. In addition, the area between the Darab plain and Korsia diapir is characterized by a salt flat (Fig. 3). The electrical conductivity of groundwater in Darab plain, near the Korsia diapir, ranges from 0.40 to 5.05 mS/cm.

The Korsia salt diapir is drained by three permanent and three temporary brine springs (Fig.2a), characterized by flow rates lower than 2 L/s (Table 2). The surface runoff of the summit zone presents a radial drainage network and flows toward the surrounding Darab plain. Part of the diapir runoff and spring S1 pass through the Korsia valley,

Table 1. Morphological characteristics of the Korsia diapir[a].

Measurement	Value
Area	2.64 km^2
Perimeter	6.27 km
Maximum length	2.40 km
Maximum width	1.48 km
Maximum Elevation	1300 m.a.s.l.
Minimum Elevation	1180 m.a.s.l.

[a] Korsia diapir shape = elliptical.

which is partly in contact with limestones of the Milak karst aquifer (Figs. 2a and 2b). The spring-derived brines and saline-surface runoff from the diapir finally flow southward to the Darab plain, and this results in the development of a salt flat south of the diapir (Figs. 2 and 3).

Sampling and Analytical Methods

Electrical conductivity (EC), temperature, and pH of the 41 sampling points (Table 3) were measured in April 2013, using a portable instrument (Hach Company, model Hq40d). The sampling points include 32 exploitation wells,

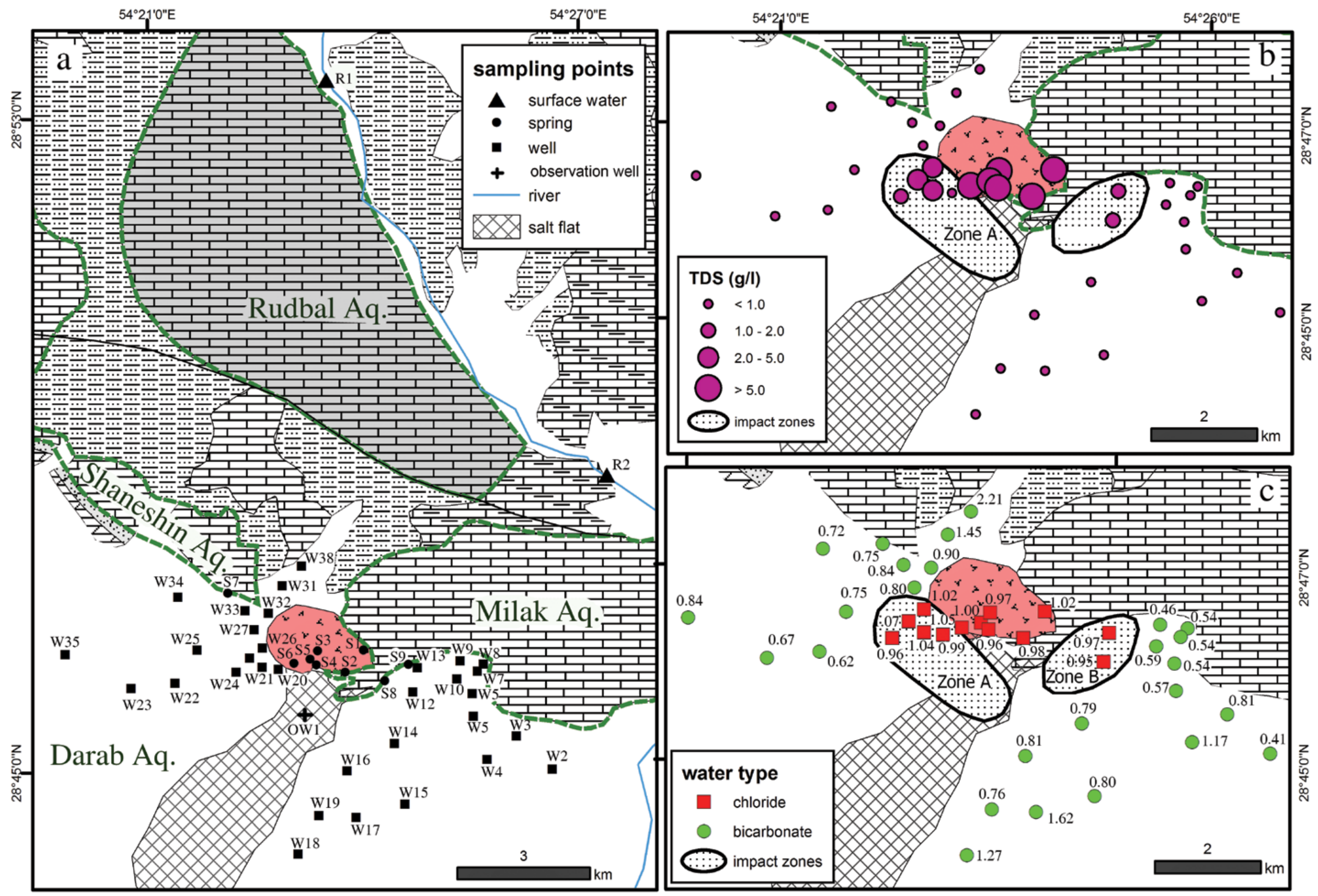

Figure 3. a) Location map of sampling points of the study area; b. distribution map of groundwater salinity (TDS: total dissolved solids); c) water type and molar ratio of Na/Cl in the study area.

Table 2. Measured parameters of the springs of the study area.

Spring Code	Spring Location	Flow Rate (L/s)	Elec. Cond. (mS/cm)	Current Status	Date of Measurement
S1	Korsia diapir	0.5	160	active	2013
S2	Korsia diapir	0.2	150	active	2013
S3	Korsia diapir	0.3	165	active	2013
S4	Korsia diapir	0.2	155	active	2013
S5	Korsia diapir	0.5	145	active	2013
S6	Korsia diapir	0.2	170	active	2013
S7 (Golabi)	Shahneshin Aq.	395	0.60	active	2013
S8 (Korsia)	Milak Aq.	430	2.25	dry	2002
S9 (Shahijan)	Milak Aq.	360	0.44	dry	2002

Table 3. List and location of sampling points.

		UTM-Zone 40N		
Sampling Location	**Source Type**	**Easting**	**Northing**	**Aquifer**
S1	Brine spring	246526	3185870	Korsia diapir
S2	Brine spring	246114	3185368	Korsia diapir
S3	Brine spring	245547	3186047	Korsia diapir
S4	Brine spring	245380	3185713	Korsia diapir
S5	Brine spring	245368	3185762	Korsia diapir
S6	Brine spring	244939	3185703	Korsia diapir
S7 (Golabi spring)	Karst spring	243437	3187289	Shahneshin aquifer
S8 (Korsia spring)	Karst spring	246982	3185317	Milak aquifer
S9 (Shahijan spring)	Karst spring	247504	3185668	Milak aquifer
W2	well	250560	3183879	East side of Darab aquifer
W3	well	250074	3183820	East side of Darab aquifer
W4	well	249603	3183760	East side of Darab aquifer
W5	well	248999	3184505	East side of Darab aquifer
W7	well	249119	3185406	East side of Darab aquifer
W8	well	249088	3185525	East side of Darab aquifer
W9	well	249222	3185688	East side of Darab aquifer
W10	well	248793	3186025	East side of Darab aquifer
W11	well	248764	3185953	East side of Darab aquifer
W12	well	247996	3185466	East side of Darab aquifer
W13	well	247712	3185715	East side of Darab aquifer
W14	well	246652	3184187	East side of Darab aquifer
W15	well	246317	3183144	East side of Darab aquifer
W16	well	246373	3182878	East side of Darab aquifer
W17	well	246197	3182452	East side of Darab aquifer
W18	well	244902	3182558	East side of Darab aquifer
W19	well	244817	3181912	East side of Darab aquifer
W20	well	244563	3185415	West side of Darab aquifer
W21	well	244411	3185748	West side of Darab aquifer
W22	well	244094	3185699	West side of Darab aquifer
W23	well	244030	3185767	West side of Darab aquifer
W2	well	243950	3186118	West side of Darab aquifer
W25	well	243836	3186132	West side of Darab aquifer
W26	well	243780	3186173	West side of Darab aquifer
W27	well	243975	3186373	West side of Darab aquifer
W28	well	244138	3186330	West side of Darab aquifer
W31	well	244669	3187460	West side of Darab aquifer
W32	well	244325	3187177	West side of Darab aquifer
W33	well	243944	3187005	West side of Darab aquifer
W34	well	242303	3187197	West side of Darab aquifer
W35	well	239751	3185896	West side of Darab aquifer
W38	well	244746	3188289	West side of Darab aquifer
R1	river	245469	3198796	Rudbal River
R2	river	246469	3196203	Rudbal River
S2	Brine spring	246114	3185368	Korsia diapir
S3	Brine spring	245547	3186047	Korsia diapir
S4	Brine spring	245380	3185713	Korsia diapir
S5	Brine spring	245368	3185762	Korsia diapir
S6	Brine spring	244939	3185703	Korsia diapir

seven springs, and two surface water stations (Fig. 3a). Water samples were taken in new, pre-rinsed polyethylene bottles to measure major- and minor-dissolved constituents.

Chemical analyses of the water samples were performed in the laboratories of the Geological Department of TU Bergakademie, Freiberg, Germany. The concentrations of major and minor ions, including calcium, magnesium, sodium, potassium, chloride, bromide, and sulfate, were determined by ion chromatography (Metrohm Compact IC Pro 881 for anions and Metrohm Professional IC 850 for cations). Bicarbonate was determined by titration with HCl, using methyl orange as indicator. Table 4 reports the results of the chemical analysis of water samples. The ion balance error did not exceed 5 % in any of the samples analyzed.

In addition, seven of the 41 samples were analyzed for stable isotopes of oxygen-18 and deuterium in the laboratories of TU Bergakademie, Freiberg, Germany. The isotopic composition ($\delta^{18}O$ and $\delta^{2}H$) of the water samples were measured with a laser-based device, Picarro L1102-I. Since all the brine samples are of Na-Cl type, the laboratory results of isotopes measurements are not expected to be affected by salinity effect (Sofer and Gat, 1972). Therefore, no specific correction was applied.

Results and Discussion

The Influence of the Diapir on the Surrounding Aquifers

The Korsia salt diapir is surrounded by three karst aquifers: Rudbal, Milak, Shahneshin, and an alluvial aquifer, Darab. We do not have sufficient data from the groundwaters of the Rudbal aquifer because there are no observable emerging springs or observation/exploitation wells in the aquifer. Therefore, the flow regime of the Rudbal aquifer has

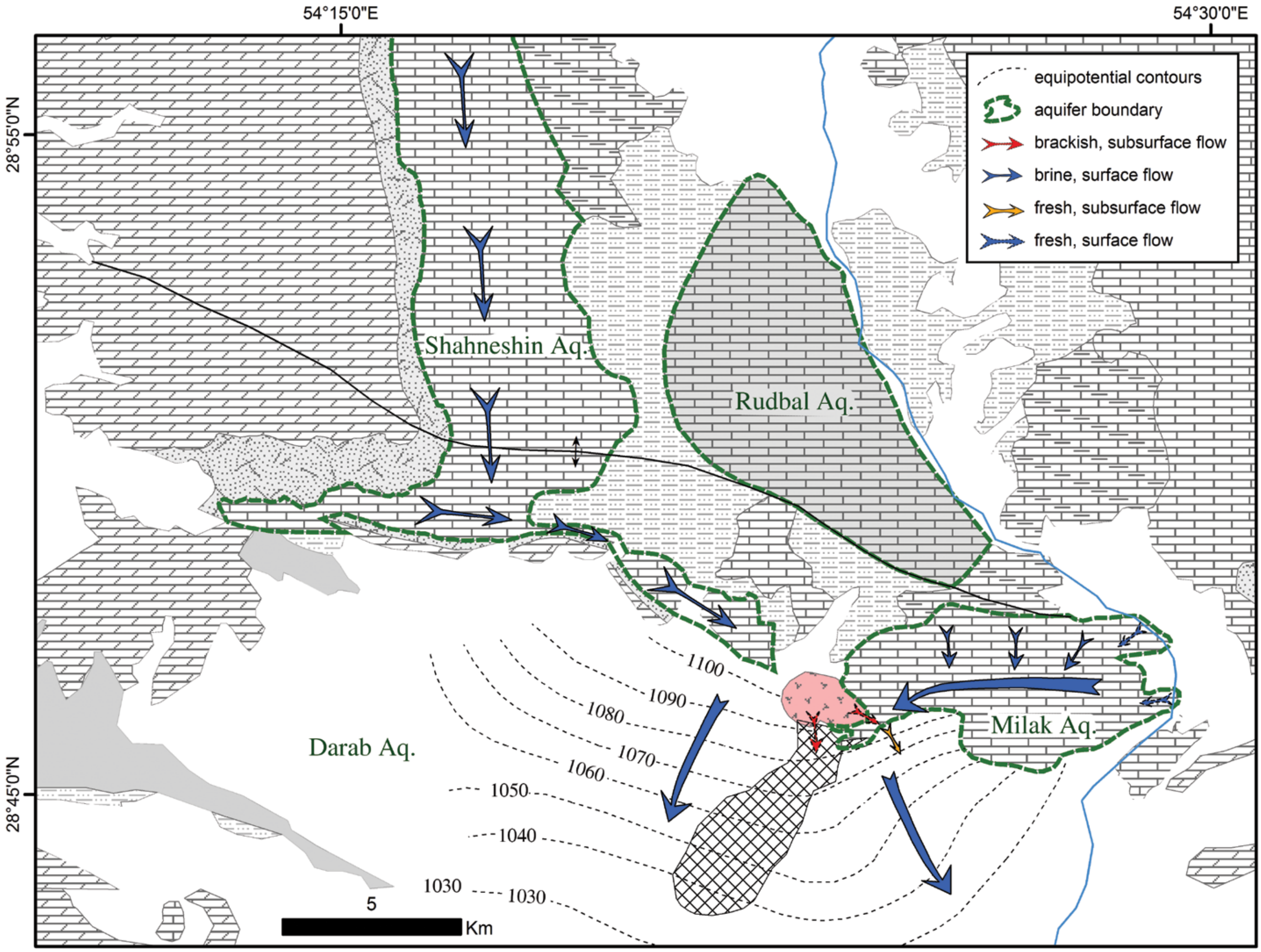

Figure 4. Conceptual groundwater flow model of the study area.

not been evaluated in the current work. However, the impact of the diapir on the karst Rudbal aquifer is unlikely, in particular, because its hydraulic connectivity is interrupted by the impermeable Radiolarite Unit.

Milak Karst Aquifer

Our observations allowed us to understand the general groundwater circulation in the Milak aquifer (Fig. 4). The main components of water recharge to the aquifer include: i) meteoric precipitation, and ii) infiltration of waters from Rudbal River. Part of the meteoric waters falling on the surface of Milak area recharge the aquifer and flow southward, following the dip direction of the strata, and then westward, parallel to the strike of limestones. In addition, the Milak aquifer receives a significant volume of water from the Rudbal River, where it flows in direct contact with the aquifer. These waters used to emerge at springs S8 and S9, located in the southwest portion of the aquifer, where the limestone crops out at the lowest elevation. The brine intrusion occurs in the western part of the aquifer, and the EC of the waters emerging at spring S8 increases from an expected value for karst waters (<0.5 to 2.25 mS/cm) (Table 2). As mentioned earlier, the springs have dried up in the last 10 years because of a severe drought in the region, and the construction of a dam in the Rudbal River, located upstream of the studied area. Perhaps, after drying out of the springs, karst waters from Milak aquifer discharge through flow to the alluvial Darab plain.

The following reasons justify the proposed water circulation model for the Milak karst aquifer:

1. The result of water balance estimation for the Milak aquifer indicates that the total outcrop area of the aquifer is not sufficient to provide flow rates of 430 and 360 L/s from the springs S8 (Korsia) and S9 (Shahijan), respectively. The annual volume of precipitation recharging the Milak aquifer is estimated using Equation (1) and information provided in Table 5.

$$V = PIA \tag{1}$$

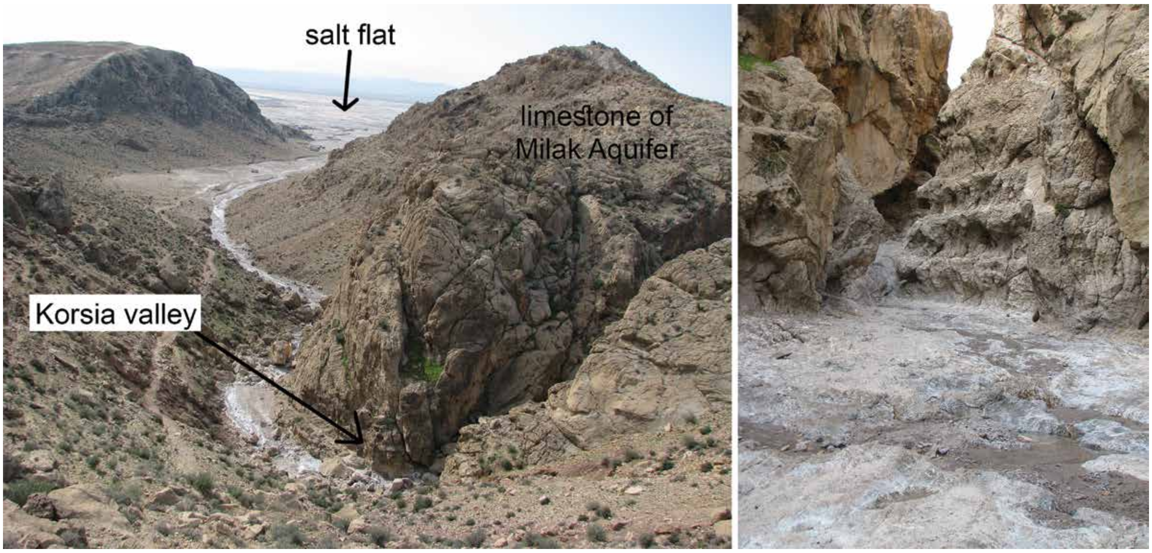

Figure 5. a) A photograph looking south showing Korsia valley located in the east of the diapir; b) flow of brine originating from spring S1, infiltrating into the limestone of Milak Aquifer on the bed of Korsia Valley.

where V is the total annual volume of recharge to the aquifer, P is the annual precipitation on the aquifer outcrop (0.340 m), I is the recharge coefficient (0.40 m), and A is the Milak aquifer outcrop area (28.7 km^2). The calculated value for the recharge volume V to the aquifer is 110 L/s, which is significantly lower than the discharge volume observed at springs S8 and S9. This suggests that there is another recharge source for the aquifer, in addition to the direct precipitation on the aquifer. Regarding the direct contact of the aquifer with the Rudbal River, seepage from the river is the most likely source of extra recharge for the Milak aquifer, especially through the Shahijan Fault (F2 on Fig. 1). A significant decrease in flow rate of the river, after dam construction, resulted in a quick lowering of water table in the Milak karst aquifer. Therefore, springs S8 and S9 dried up and karst water of the Milak aquifer discharges to the Darab alluvial plain as a subsurface flow, pointed out by the equipotential map of the Darab alluvial Plain in this area (Fig. 4).

b. Comparing the water quality of springs S8 and S9, we observed that spring S8 presents EC of 2.25 mS/cm and Na-Cl waters, mainly influenced by diapir brines, which have, on the other hand, no impact on S9 water, showing EC of 0.44 mS/cm and Ca-HCO_3 waters. A portion of the brine flows into the Milak aquifer somewhere between the emerging points of these two springs. Therefore, the part of karst water flowing westward and discharging through spring S8 receives some brine infiltrated into the aquifer. In addition, our field observations indicate that the brine infiltration into the limestone of the Milak aquifer occurs from the eastern side of the diapir. Part of the diapir-derived brine flows eastward on limestone outcrops in Korsia Valley (Fig. 2 and Fig. 5). Flowing through the valley, salt water of the brine stream infiltrates into the limestones at the valley bottom.

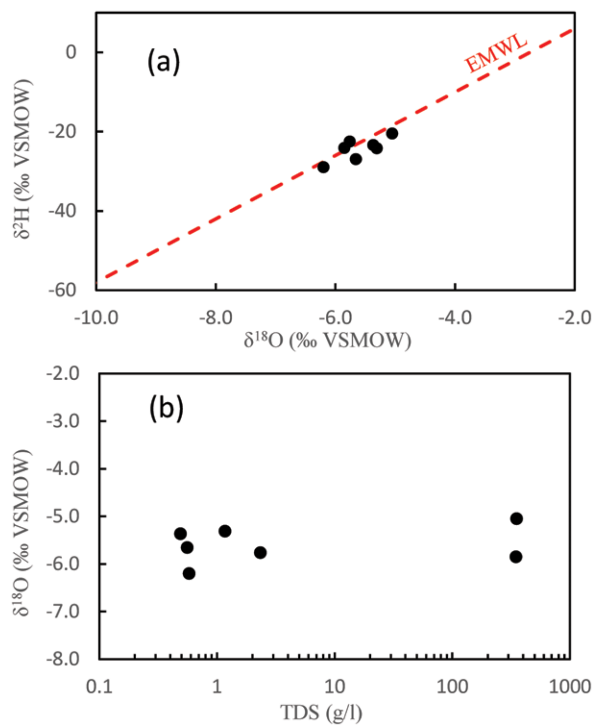

Figure 6. a) δ2H vs. δ18O diagram for water samples, EMWL: eastern Mediterranean meteoric water line; b) variation of δ18O vs. TDS (total dissolved solids) of groundwater samples.

c. Spatial distribution of the groundwater quality in the alluvial Darab aquifer shows an increase in the salinity of the exploitation wells located in a zone, where the Milak aquifer discharges into the Darab Alluvium. The front of brine intrusion has propagated to the east during recent years. Consequently, well W13, located close to the former emerging point of spring S9, with EC of 0.44 mS/cm, has EC of 1.71 mS/cm, with a chloride water type at the present time.

d. The molar ratio of Na/Cl of groundwater samples has been plotted in Figure 3c. Investigation of ion ratios indicates that karst water discharging to Darab aquifer is influenced by diapir brine in the western sector of the discharge zone. The Na/Cl molar ratio of wells W12 and W13, located close to the diapir, are 0.95 and

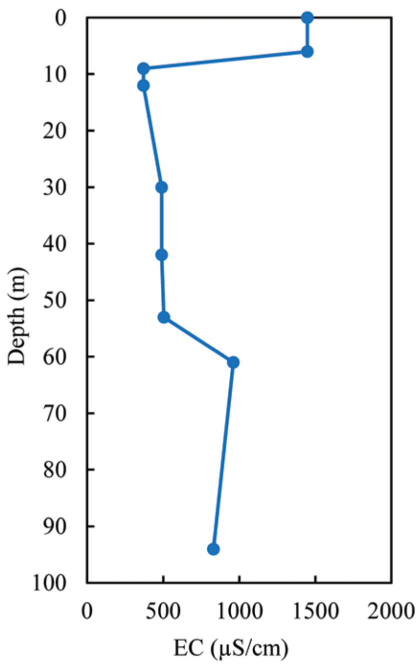

Figure 7. Variations of electrical conductivity (EC) with depth in observation well of OW1.

0.97, respectively, which are especially close to the average Na/Cl ratio of brine springs of the diapir (0.99). The ratio is lower than 0.8 in wells located further east, including W7, W8, W9, W10 and W11, which indicates no impact of diapir brine in that area (Fig. 3c). In addition, the weight ratios of Br/Cl (Table 4) are less than 4×10^{-4} in groundwater samples from W12 and W13, falling in the proposed range of Br/Cl ratio for saline water resulting from halite dissolution (Richter et al., 1991), and is also close to the ratio calculated for the brine springs of Korsia diapir (2.5×10^{-4}).

Shahneshin Karst Aquifer

Shahneshin karst aquifer is located on the west side of the Korsia salt diapir (Fig. 1). Part of the meteoric precipitation falling on the limestone outcrops recharges to the aquifer. The general flow direction in the aquifer is mainly N-S, across the axis of an anticline. Then karst water flows toward the southeast, following the strike of the limestone strata, finally emerging at Golabi Spring (S7) with a flow rate of 395 L/s, as the only discharging point of the aquifer.

The limestone of Tarbur is sandwiched between two impermeable layers. It is underlain by the Radiolarite Unit and overlain by the Sachun Formation. Therefore, discharge of karst water is only likely in the far southeast of the aquifer, where the limestone outcrops lay at the lowest elevation, and where spring S7 is located. The presence of the impermeable Sachun Formation, in the southern boundary of the aquifer, prevents subsurface discharge of karst water into the Darab alluvial aquifer.

Water budget calculations indicate that meteoric precipitation recharging to the outcrop area of the Shahneshin aquifer is sufficient to provide the karst water discharging via spring S7, with an annual volume discharge of 12.5 MCM or 395 L/s (Table 5). The annual recharge volume to the Shahneshin Aquifer is estimated at 12.6 MCM (equivalent to 400 L/s) using Equation (1), given the outcrop area of 78.05 km^2, an annual precipitation of 405 mm and a recharge coefficient of 0.40.

Golabi spring (S7) is located in the eastern sector of the Shahneshin aquifer and 1000 m away from the salt diapir. However, electrical conductivity of the spring water is 0.60 mS/cm, which is characteristic of typical fresh waters dominated by Ca-HCO_3 water type. These confirm the lack of brine intrusion from Korsia diapir into the Shahneshin aquifer.

Darab Alluvial Aquifer

Darab alluvial aquifer is located in the south side of the Korsia diapir (Fig. 1), whereas its northern part is in direct contact with Milak karst aquifer. Generally, groundwater flows from the northern toward the southern sector (Fig. 4). Therefore, recharge in the northern mountains, as surface runoff and subsurface flow, represents the main water source for the alluvial aquifer. Figure 3b indicates the spatial distribution of salinity in Darab aquifer around the Korsia Diapir and suggests two zones of high-salinity groundwater: Zone A (adjacent to the Korsia diapir) and Zone B (adjacent to the Milak aquifer). Figure 4 illustrates the general flow direction in the Darab Aquifer. The following reasons justify the proposed model:

Zone A (south of the diapir (is mainly influenced by brines emerging from springs. In addition, flow of saline surface runoff, originated from the surface of the diapir, causes additional salinization of Zone A. Infiltration of surface saline water in Zone A causes an increase in the salinity of groundwater in this zone.

Subsurface inflow of brackish water from Milak Aquifer causes an increase of groundwater salinity in Zone B (adjacent to Milak Aquifer). As mentioned earlier, part of the diapir brine flows into the western section of Milak aquifer. Then brackish karst water of Milak Aquifer flows into the alluvium of Darab Aquifer, which increases salinity of exploitation wells located in Zone B.

Generally, Darab Aquifer presents bicarbonate (HCO_3) waters, which in Zones A and B have become chloride waters because of the intrusion of diapir-derived brine.

The molar ratio of Na/Cl of groundwater samples from Zones A and B varies between 0.95 and 1.07 (Fig. 3b), which are markedly close to the average Na/Cl ratio of brine springs of the Korsia diapir (0.99). Furthermore, the ratio of Br/Cl is lower than 5×10^{-4} (Table 4) in groundwater samples of wells located in Zones A and B, which suggests that the source of salinity is salt diapir (Richter et al., 1991).

The isotopic composition of all groundwater samples was plotted on the $\delta^{18}O$-δ^2H diagram of Figure 6a. Since there is no local meteoric line in the study area, and meteoric precipitation mainly originated from Mediterranean air masses, the Eastern Mediterranean Meteoric Water Line (EMWL) is considered as the meteoric water line of the area. All samples plot very close to the EMWL, which shows that evaporation does not play any significant role on groundwater salin-

Table 4 Results of the chemical analysis of water sample in the study area.

Sampling Location	Water Type	Elec. Cond. (mS/cm)	TDS (g/L)	Ca (mg/L)	Mg (mg/L)	Na (mg/L)	K (mg/L)	SO_4 (mg/L)	Cl (mg/L)	HCO_3 (mg/L)	Br (mg/L)	Na/Cl (molar)	Br/Cl [$\times 10^{-4}$] (molar)
S1	Cl	160	352	1550	349.4	138400	496.2	2260	209200	42.7	39.7	1.02	1.9
S2	Cl	150	341	1680	273.4	130900	471.1	2430	205600	48.8	···	0.98	···
S3	Cl	165	378	950.0	1220	141900	1610	5260	226900	61.0	···	0.97	···
S4	Cl	155	335	1380	683.6	127900	581.4	3130	205600	30.5	···	0.96	···
S5	Cl	145	349	2510	106.3	133900	691.6	4250	207400	30.5	70.5	1.00	3.4
S6	Cl	170	362	1300	607.6	144900	671.3	2080	212700	30.5	48.9	1.05	2.3
S7	HCO_3	0.60	0.558	76.2	23.1	25.8	1.56	64.8	53.18	305.1	0.069	0.75	13.0
W2	HCO_3	0.80	0.652	78.2	41.3	47.4	1.96	92.7	177.30	213.6	···	0.41	···
W3	HCO_3	0.72	0.709	64.1	46.2	65.1	2.35	169.6	124.10	238.0	0.434	0.81	35.0
W4	HCO_3	0.55	0.532	60.1	48.6	26.9	1.56	127.3	35.45	231.9	···	1.17	···
W5	HCO_3	0.81	0.721	82.2	43.8	67.8	1.56	103.3	184.40	238.0	···	0.57	···
W7	HCO_3	0.50	0.543	60.1	30.4	24.6	1.56	92.7	70.91	262.4	0.121	0.54	17.1
W8	HCO_3	0.54	0.518	62.1	48.6	14.9	1.17	43.7	42.54	305.1	···	0.54	···
W9	HCO_3	0.42	0.504	78.2	29.2	13.8	1.17	78.8	28.36	274.6	···	0.75	···
W10	HCO_3	0.43	0.492	74.1	26.7	12.2	1.17	101.3	31.91	244.1	···	0.59	···
W11	HCO_3	0.40	0.511	92.2	15.8	17.9	1.17	85.5	60.27	238.0	0.072	0.46	11.9
W12	Cl	1.40	1.016	134.3	64.4	214	2.35	162.3	354.50	213.6	0.106	0.95	3.0
W13	Cl	1.71	1.174	100.2	52.3	280	1.56	78.8	453.80	238.0	0.095	0.97	2.1
W14	HCO_3	0.70	0.661	90.2	45.0	67.6	2.35	96.1	131.20	268.5	0.223	0.79	17.0
W15	HCO_3	0.50	0.555	58.1	52.3	25.8	1.96	92.7	49.63	274.6	···	0.80	···
W16	HCO_3	0.72	0.670	90.2	41.3	28.1	1.96	101.3	53.18	353.9	···	0.81	···
W17	HCO_3	0.40	0.474	64.1	28.0	26.0	1.56	54.8	24.82	274.6	···	1.62	···
W18	HCO_3	0.52	0.522	40.1	42.5	46.7	1.17	47.6	56.72	286.8	0.091	1.27	16.0
W19	HCO_3	0.60	0.576	66.1	42.5	43.5	1.56	76.9	88.63	256.3	0.152	0.76	17.1
W20	Cl	5.05	0.850	100.2	48.6	1190.0	1.56	827.8	1860.00	384.4	0.279	0.99	1.5
W21	Cl	3.02	2.337	30.1	30.4	513.3	2.74	447.2	759.70	366.1	0.137	1.04	1.8
W22	HCO_3	0.80	0.694	110.2	24.3	45.3	1.96	75.4	113.4	323.4	0.261	0.62	23.0
W23	HCO_3	0.80	0.811	108.2	43.8	41.6	1.96	153.7	95.72	366.1	···	0.67	···
W24	Cl	1.90	1.275	220.4	30.4	286.3	3.13	231.1	460.90	292.9	0.23	0.96	5.0
W25	HCO_3	0.70	0.653	90.2	32.8	36.3	1.56	131.1	74.45	286.8	···	0.75	···
W26	Cl	3.10	2.381	116.2	51.0	579.6	1.96	482.2	833.10	317.3	···	1.07	···
W27	HCO_3	0.60	0.595	84.2	42.5	31.3	1.17	101.3	60.27	274.6	116	0.80	19.2
W28	Cl	4.40	2.643	78.2	28.0	820	4.30	172.9	1241.00	299.0	0.397	1.02	3.2
W31	HCO_3	1.03	0.955	90.2	48.6	133.3	2.35	148.4	141.80	390.5	···	1.45	···
W32	HCO_3	0.90	0.791	96.2	45.0	64.4	1.96	138.3	109.90	335.6	0.165	0.90	15.0
W33	HCO_3	0.62	0.638	70.1	41.3	36.8	3.91	101.3	67.36	317.3	···	0.84	···
W34	HCO_3	0.40	0.508	66.1	35.2	19.8	1.56	92.7	42.54	250.2	···	0.72	···
W35	HCO_3	0.90	0.777	110.2	42.5	53.8	2.35	113.8	99.63	366.1	···	0.84	···
W38	HCO_3	0.90	0.571	44.1	28.0	86.2	1.96	75.4	60.27	274.6	···	2.22	···
R1	HCO_3	0.69	0.875	125.1	50.7	25.3	2.30	145.1	49.36	336.8	0.095	0.79	19.2
R2	HCO_3	0.45	0.530	84.2	29.2	17.0	1.17	83.1	28.36	286.8	···	0.93	···

Table 5. Water balance parameters of Milak and Shahneshin Aquifers.

Parameter	Milak	Shahneshin
Outcrop area (km^2)	78.05	28.70
Annual precipitation (mm)	405	340
Recharge coefficient (%)	40	40
Calculated recharge (Mm^3)	12.6	3.5
Aquifer discharge through springs (Mm^3)	12.5	24.9

Table 6. Results of analysis of stable isotope in selected water samples.

Sample	VSMOW		Elec. Cond. (mS/cm)
	δ^{2}H, %	δ^{18}O, %	
S1	−20.4	−5.1	352.3
S5	−24.1	−5.9	348.9
S7	−27.0	−5.7	0.56
W19	−28.9	−6.2	0.58
W21	−22.5	−5.8	2.34
W13	−24.2	−5.3	1.17
W10	−23.3	−5.4	0.49

ity of the water samples. Additionally, this is confirmed by Figure 6b, which shows no enrichment of δ^{18}O with increasing salinity.

A salt flat has been developed south of the diapir, in Zone A. Field observations show (Figs. 2 and 5) flow of direct runoff and discharge of brine springs toward the surface of Darab Aquifer in Zone A. In addition, the satellite image in Figure 2 indicates that there is no cultivation south of the Korsia diapir, which justifies low quality of groundwater and soil in Zone A, while agricultural activities in the west of the diapir indicates no impact of the diapir on this area.

A monitoring well was drilled in the south of the diapir by Water Authority of Fars Province (Well OW1 in Fig. 3a). Figure 7 illustrates the results of measurement of electrical conductivity with depth in this well. A general declining trend of salinity with depth is observed, which suggests that Zone A is influenced by brine mainly flowing in the upper portion of the Darab aquifer.

Conclusion

Fragility of the karst environment determines high possibility for pollution's rapid transport within karst conduits (Allshorn et al., 2007; Ford and Williams, 2007), with serious problems in recovering a contaminated aquifer to its pre-contamination state (Milanovic, 1981; Kacaroglu, 1999; Stevanovic et al., 2010; Parise et al. 2015, 2018). In Iran, more than 50 % of salt diapirs in the southern part of the country deteriorate water quality of the surrounding aquifers (Zarei, 2016). Located in the south-central Iran, Korsia salt diapir is surrounded by alluvial and karst aquifers. Our investigations indicate that the Korsia diapir influences the eastern karst aquifer (Milak Aquifer) and the southern alluvial aquifer (Darab Aquifer), whereas it has no impact on the western karst aquifer of Shahneshin. Infiltration of brine emerging from a spring in the east of the Korsia diapir leads to an increase of up to 2.25 mS/cm in the salinity of karst waters in the western part of Milak aquifer. The westward flow direction of karst water in the Milak Aquifer prevents flow of saline water to the eastern section of the aquifer. However, a decline in the recharge rate of the aquifer, subsequent to the construction of a dam on the Rudbal River, caused an eastward advance of the salinity front in recent years. Thereafter, salt karst water in the west of the Milak Aquifer discharges as subsurface flow toward the adjoining alluvium of Darab Aquifer, deteriorating the quality of the aquifer in this zone. Conveying brine from perennial spring S1 to salt-evaporation basins would improve water quality of the karst aquifer and would result in economic benefits by producing salt for local and industrial uses after the required treatments. The Darab Aquifer is also influenced by infiltration of saline surface runoff from the diapir and brine emerging via springs around the diaper that drain southward to the alluvium of the Darab Aquifer. Construction of salt evaporation basins could be used to collect surface saline waters originating from the diapir. This would prevent infiltration of brine into the ground and would increase the quality of the groundwater and soil of the alluvial aquifer. The karst waters coming from Shahneshin Aquifer and emerging in Golabi Spring (S7), located less than 1 km away from the west of Korsia salt diapir, show high quality. The salt diapir has no impact on water quality of the Shahneshin Aquifer due to the presence of the impermeable Radiolarite Unit that interrupts the hydraulic connectivity of the diapir with the Shahneshin karst aquifer.

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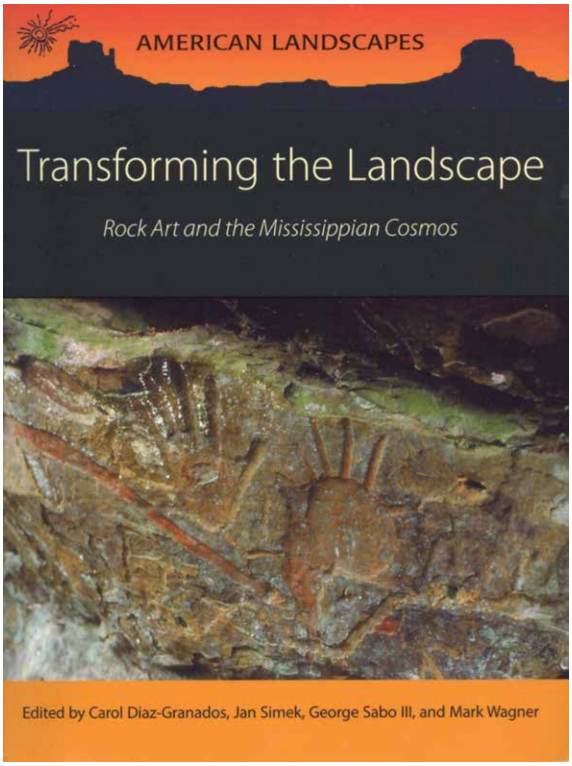

Transforming the Landscape: Rock Art and the Mississippian Cosmos

Diaz-Granados, C., Simek, J., Sabo III, G., and Wagner, M. (eds.), 2018, 1950 Lawrence Rd., Havertown, PA 19083, Oxbow Books, 240 p., 7.1 x 9.7 inches, ISBN 9781785706288, paperback, $34.95.

Although much of the prehistoric Indian rock art in North America is above ground and is not restricted to caves, symbols found in caves play a significant role. This book is well laid out with colorful graphics and location maps. It includes well reproduced drawings and photographs of the rock art, much of which is no longer available or cannot be seen by the Public. It deals with the general understanding of what the rock art means, especially as it relates to the prehistoric Indian's understanding of the spirit world. It is of interest to a general audience, although it includes detail and some wording best suited to anthropologists. The book can be easily read by the average reader, and the emphasis on spiritual beliefs gleaned from the rock art makes for interesting reading. Because of the growing amount of research on rock art in North America, the book focuses on interpreting rock art created during the Mississippian period of eastern North American prehistory (post-AD 900), emphasizing the connection of rock art with its physical location and with the Indian's concept of the universe. Did rock art have a spiritual meaning, and was its placement of special importance? In order to try to understand the meaning of the symbols used in the rock art, the authors went to the oral traditions passed down to the descendants of the prehistoric Indians. The concepts are intriguing.

The book has seven chapters. The first is an overview of how Native American peoples tried to connect their world and the spirit world. Examples indicate Native Americans from the Great Plains and Eastern Woodlands to the Maya in Mesoamerica all felt a relationship between the visible and the spirit world. They attached significance to specific places and generally held a belief that the universe has several levels with different occupants and powers. Humans are in a central place and they must keep the order in balance. Portals, or specific places on Earth, could be used to cross between levels to ask for favors, such as a good harvest. These spots could be marked, for example with petroglyphs. Chapter 2 concentrates on the big five petroglyph sites in Missouri and uses interviews with indigenous Osage Indians to interpret the rock art. They feel the north-south orientation of the sites was placed to align with the local Indians symbolism of the cosmos. Chapter 3 discusses the role of Old or First Woman in the Osage spiritual beliefs. Fertility is a common theme with graphic depictions. Chapter 4 is a study of central Arkansas rock art along the Arkansas River. Imagery on the north side of the river reflected the spirit world while on the south the imagery was worldly. This corresponds with the beliefs of living Indians. Chapter 5 describes Mississippian rock art and mounds in Illinois. An important site, Millstone Bluff was a late Mississippian settlement on a natural mound in southeastern Illinois whose shape resembles a burial mound. There are three rock art panels on the site thought to represent the belief in a three-tiered universe commonly held by the Indians of the pre-European Southeast. Chapter 6 deals with rock art on the Cumberland Plateau. This covers Kentucky, Tennessee and Alabama. Rock art along the Cumberland Plateau is associated with bedrock mortars, animals and animal tracks. The authors suggest the Kentucky rock art defined borders apparently between different cultures. Tennessee rock art was primarily placed at high points along the Cumberland Escarpment, while rock art in Alabama may have had a spiritual significance with pictographs (symbols) dominant in northern sites (representing the higher Mississippian spirit world) and petroglyphs to the south (human, middle world). Chapter 7 describes petroglyphs in the mountains of northern Georgia and western North Carolina which were selectively placed along trails. They connected inhabited bottom land settlements with sacred mountain tops or mound sites. The petroglyphs pointed the way to the place where the spirit world could be entered.

The book could be used as a road log for anyone interested in exploring rock art sites, although it might be of most interest to someone already interested in rock art. However, anyone who has seen North American rock art would find the interpretations in this book intriguing, with the goal of interpreting what prehistoric people believed about an after life. Utilizing the traditional beliefs of living descendants, the symbols take on new meaning with less speculation involved.

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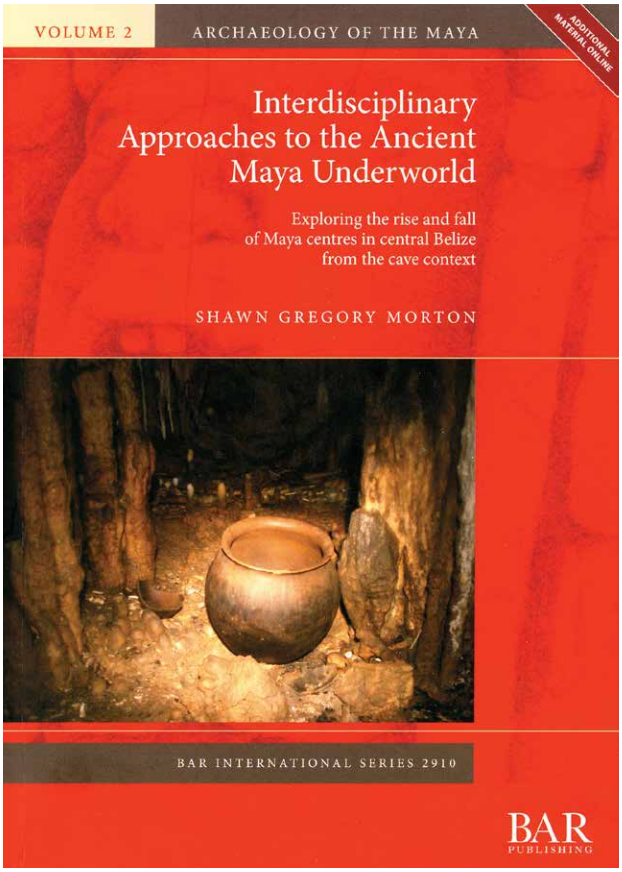

Interdisciplinary Approaches to the Ancient Maya Underworld: Exploring the Rise and Fall of Maya Centres in Central Belize from the Cave Context

Shawn Gregory Morton, 2018. Oxford, UK, Vol. 2 of the series Archaeology of the Maya, Elizabeth Graham et al., eds., BAR Publishing (British Archaeological Reports, Ltd.), International Series 2910, www.barpublishing.com, 187 p., 8.2 x 11.6 inches, softbound, ISBN 978-1-4073-1666-6. £37 (approx. $74 retail, USA).

The Maya inhabited much of Mesoamerica, with cultures dating from before the years 300 CE, blossoming during what are considered the Classic (300 to 900 CE) and post-Classic (900 to ~1300 CE) periods, and followed by their rapid decline. This book focuses on their colonization of the Maya Mountains of central Belize, which are located along the southeastern margin of the Yucatán Peninsula, in the drainage basins of the Roaring Creek and Caves Branch River along the Hummingbird Highway. The area consists of a rugged upland located on karst-rich Cretaceous limestone. The major civic and ceremonial center of Tipan Chen Uitz has recently been discovered there and is presently under investigation by the Central Belize Archaeological Survey (CBAS). Neighboring areas have received far less study.

Dr. Morton has written many scholarly articles on the Maya culture and is currently on the faculty of the Anthropology Department at Northern Arizona University. This book is based mainly on his doctoral research in central Belize for the University of Calgary (Alberta) and several additional years of work as a senior staff member, and now co-Director, of CBAS. His primary goal is to clarify the origin of the local pre-Columbian people, to document their cave use from artifacts and related clues, and to show how they interacted with their neighbors. Throughout most of the region, little prior attention had been given to the history and culture of the Maya. Archeological sites in Belize are protected by law from unauthorized disturbance. This study was properly sanctioned and involved minimal disturbance. Abundant credit is given to previous investigators with a 27-page bibliography, including publications by at least half a dozen prominent members of the National Speleological Society.

This book is nicely printed and securely bound on semi-gloss paper. The text is scholarly, clear, and shows great care in preparation, with many detailed maps and diagrams. It is well illustrated with monochrome and color photos. Some photos have distinctly low-key lighting, but most important features are highlighted, and they portray well the dark and somewhat gloomy settings of the cave interiors.

The central theme of the book is: How did Mayan use of caves reflect on the complex history, social life, and economic status of the region during the development and ultimate decay of their culture? Discoveries over the past decade have greatly changed our understanding of the topic, and there is much more to be learned. This book focuses on ritual as the human expression of culture – i.e., rites of exchange, communion, affliction, feasting, fasting, politics, prayer, etc. This is not simply a catalogue of artifacts. Instead, it attempts to reach into the minds and thoughts of the local inhabitants. Abundant references and footnotes tie ever-greater parts of the subject together in a scholarly manner. There are still many unanswered questions, and these are summoned as an aid to future investigators. The extensive reference list includes works of interest to anyone involved in archeological field work, especially those who wish to learn about the Mayan culture in depth, and not solely in reference to caves.

The book opens in a rather academic style that reflects its origin as a dissertation, but it quickly becomes more relaxed as the interpretation unfolds. It consists of two sections divided into nine chapters. The first part summarizes the archeology of the Maya, with a focus on the use of caves in daily life, ritual, and religion. The second part gives specific details of the author's field research, including many maps of caves and interpretation of their contents. It is much more than a simple description of local sites and archeological relics. Caves are treated in a holistic way, with descriptions of how they influenced the Maya culture. It is an enjoyable read, interspersed with thought-provoking quotes drawn from authors ranging from Aristotle and Plato to fantasy novelist Terry Pratchett.

Section 1 introduces the caves in terms of ritual, iconography, and sense of community. The author treats the subject as an adventure in time, space, and evolving culture. It includes a broad discussion of Mayan life and how caves were used for living space, water supply, religious rites, art galleries, and burial sites, as well as how caves influenced their ways of thinking. Topics include modern investigations highlighted by the production of cave maps that portray archeological content, glyphs and paintings, and interpretation of the culture. Included are myths and aspects of religious worship, some of which continue today. The concept of caves as homes and shelters is treated at some length, including how they served as the sources or representation of fertility, wealth, communication with ancestors, and abodes of supernatural beings. As sources of drinking water, caves and cenotes probably had the most important control of population density. Caves were also used as burial grounds, crematoria, and ossuaries. There is some evidence of sacrifice.

Present evidence indicates that nucleated habitation came late to this area. All of the datable artifacts in caves reveal some use during the final cultural phases late in the Maya history (approximately 1000 to 1400 CE). Similarity of pottery types suggests that the Caves Branch River Valley was linked to a broad network of exchange and consumption, while marine shells give evidence that there was also contact with population centers along the Caribbean coast. The primary remaining question is how the changing patterns of pre-Columbian use of caves relates to the complex historical, social, political, and economic conditions during the occupation, expansion, and eventual collapse of Tipan Chen Uitz and other nearby cultural centers.

Section 2 includes many cave maps based on careful surveys by the author and his team, including the identification and spatial arrangement of artifacts. Each site is described with location, description, geomorphic context, excavation details, and archeological summary. Portrayal of artifacts is aided by histograms of their frequency (some with eye-popping effects where wide frequency ranges are displayed at a uniform scale). Exploration and mapping of the caves are described, including carefully detailed surveys made either with Brunton compass and tape, or with Leica Disto.

Caves are described in terms of their multiple purposes: as homes, shelters, entry-and-exit points to and from the underworld, sites of ritual acts, water sources, etc. Caves serve as a concept, rather than simply physical spaces – as sources of sustenance, even human life itself. Not all events took place specifically in caves, but the caves serve as a back-drop for most of the inhabitants' lives. Caves were also recognized as the abodes of witches, ghouls, and bizarre phenomena. The location of a cave also affected how it was used.

The book also represents caves in a broader perspective than the voids themselves – for example, the views from their entrances. Peering out of a cave entrance reveals what the ancient Maya saw, and it gives a broader perspective of the cave's meaning. A cave also serves as a point of contact with ancestors.

The final chapter summarizes the topic with two different perspectives. Does a cave mean more when you look into it, or when you look out at the rest of the world? What do you see in each case? Such philosophical points of view may seem off the subject, but they delve into the minds of the ancient Maya and set the reader to thinking. The book ends with a forecast of future directions in the field. It is highly recommended for anyone interested in the Mayan culture and the field of archeology in general.

Four appendices are available as free downloads with the link available in the book: (1) Ceramics: summary and catalog; (2) Lithics (worked rock chips, carving, etc.); (3) Faunal catalog; and (4) Radiocarbon dating.

Reviewed by Margaret V. Palmer and Arthur N. Palmer, 619 Winney Hill Rd., Oneonta, NY 13820, April 25, 2019.

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