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A Taxonomic Survey of Lamp Flora (Algae and Cyanobacteria) in Electrically Lit Passages within Mammoth Cave National Park, Kentucky

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Abstract:

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A taxonomic survey of the lamp flora from electrically lit passages in Mammoth Cave, Mammoth Cave National Park, identified 28 species. Overall, cyanobacteria were dominant represented by 14 species (50% of the total), green algae had eight species (29%), and six diatoms species (21%) were present. There was not a correlation between species diversity and temperature, but there is a general trend of increasing diversity with warmer temperatures. There were two algal or cyanobacterial species identified in this study that overlapped with previous studies. There is a lack of continuity between previous studies only having one species identified in more than one study. This suggests a high algal turnover and possible colonization rates.

Keywords: caves, Lamp flora, Mammoth cave, Kentucky, Algae, Cyanobacteria

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INTRODUCTION

Mammoth Cave National Park located in south-central Kentucky encompasses 21,380 hectares of primarily mixed mesophytic forest overlying most of the 580 km Mammoth Cave System. Mammoth Cave became a national park in 1941, and in subsequent decades lighting systems were installed. White fluorescent lamps and incandescent flood lamps were the primary sources of light. Presently, some of these lamps have been replaced with 500-Watt halogen bulbs or yellow LED lights. A common problem with electrical lighting systems in caves is the unwanted growth of lamp flora, which includes moss gametophytes, fern sporophytes, cyanobacteria, and algae (Fig. 1). Growth on cave formations and walls is a serious ecological distortion and also an aesthetic problem (Aley, 1972; Aley et al., 1985; Elliott, 1997; Olson, 2002).

Cyanobacteria and algae typically colonize lithic environments with an ample quantity of light for photosynthesis (Ariño & Saiz-Jimenez, 1996; Faimon et al., 2003; Grobbelaar, 2000; Marathe & Chaudhari, 1975). These pioneer species typically modify the rock surface they inhabit by producing carbonic acid during respiration (Shields & Durell, 1964). This weak acid is corrosive especially to cave formations, which are characteristically limestone (Marathe & Chaudhari, 1975). This dissolution of cave formations can have irreversible damage on speleothems (Grobbelaar, 2000).

Lamp flora or lampenflora has been studied throughout the world, with high levels of activity in Europe, North America and Israel (Abdelahad, 1989; Bahls, 1981; Claus, 1962; Couté, 1984, 1985; Couté & Chauveau, 1994; Dayner & Johansen, 1991; Davis & Rands, 1982; Dickson & Kirk, 1976; Friedmann, 1955, 1956, 1961, and 1962; St. Clair & Rushforth, 1976).

Previous studies at Mammoth Cave National Park, identified two major algal groups (green algae and diatoms) and cyanobacteria growing on cave formations, wet walls, and sediments inside the cave. These studies sampled the Frozen Niagara (Harris, 1981; Von Hoff, 1974), and Historic sections (Jones, 1965; Van Landingham, 1965a, 1965b, 1967) and Crystal Cave (Nagy, 1965).

The purpose of this study was to document the algal and cyanobacteria lamp flora species in selected areas of the Mammoth Cave system. The intent was to conduct a one-time collection of samples from tourist areas (Half-Day, Frozen Niagara, New Entrance, and Historic sections) and compare results with previous studies. This study may help in future managerial plans to determine appropriate actions for prevention of lamp flora growth. Mammoth Cave has one of the world's most diverse assemblages of cave adapted animals, and areas such as the Frozen Niagara section are biodiversity hotspots (Culver et al., 2003), which are put at risk by chemical cleaning of lamp flora

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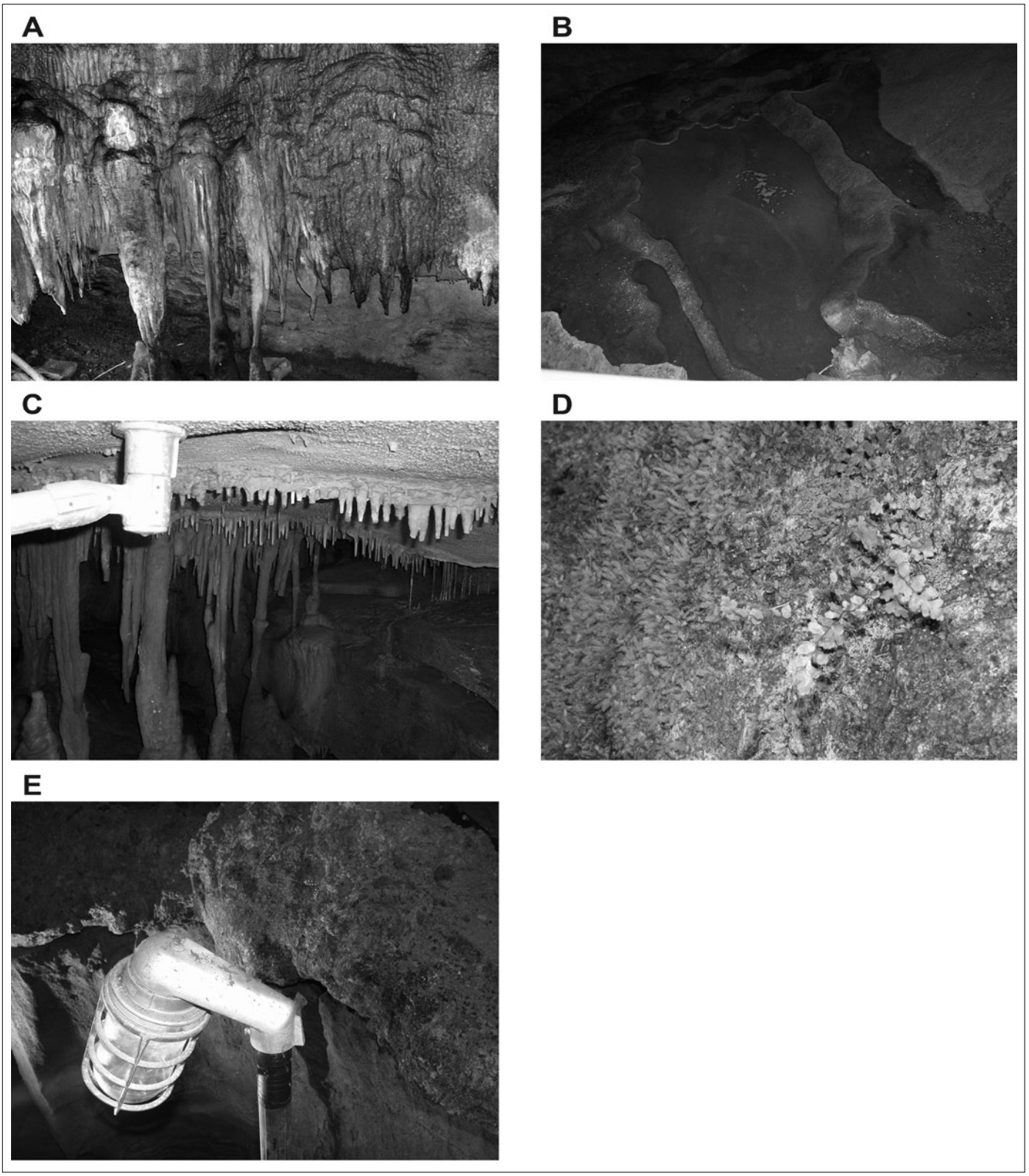

Fig.1. Lamp flora from Mammoth Cave National Park: A. Rainbow Dome (sample #32); B. Travertine dam at Rainbow dome (sample #30); C. Onyx Colonnade (sample #22 & #23); D. and E. Stairways in New Entrance to Mammoth Cave (sample #12 - 15)

(Boston, 2006; Elliot, 2006; Hildreth-Werker & Werker, 2006). By understanding which lamp flora species are colonizing artificially lighted areas of Mammoth Cave, we can acquire more detailed information on their specific biology, which may lead to alternative ways to interfere with their life cycle by non-toxic means. For clarification, algae will be defined; as any eukaryotic organism containing chlorophyll "a" in the Kingdom *Protista* and cyanobacteria is a prokaryotic organism containing chlorophyll "a"and phycobiliproteins in the Kingdom *Bacteria*.

SAMPLING SITES AND METHODS

Mammoth Cave Sampling Sites — Thirty-two sites were sampled on July, 2003. These sites were chosen because of lamp flora growth. There were 13 sites from the Frozen Niagara section, six sites from the New Entrance to Mammoth Cave, six sites from the Historic tour and

Mammoth Dome, four sites from Snowball Dining Room and Boone Avenue, and three sites from Doyle's Big Break at Central Station and Lover's Leap (Fig. 2, 3, & 4). All sites sampled were moist to dry except for sites in the Frozen Niagara's Drapery Room (sample #20 & 21), which had slow water flowing down the formation's face. Air temperature and light intensity were recorded at each sampling location (Table 1). Air temperatures were measured using a Kestrel 3000 Pocket Weather Station meter. Light intensities were measured with an Extech foot candle/lux meter Model # 401025 for comparative purposes between sites.

Lamp flora were removed using two sampling methods: First method, a soft toothbrush was used to lightly scrub in a circular motion a small area (20 cm^2) on cave walls and formations, then rinsed with distilled water. The toothbrush was cleaned with distilled water after each sample. This rinse water was collected in a sterile Whirl-pak® and preserved with M3 (American Public Health Association, 1992). The second method, the top layer of sediment with lamp flora were removed with a single edge razorblade, and placed in a sterile Whirl-pak® and preserved with M3. Semi-permanent slides were made with distilled water and sealed with epoxy (Smith, 2003). The algae and cyanobacteria were counted under a light microscope at 1000X until they enumerated 500 cells per slide. The species records from the previous studies on Mammoth cave lamp flora has been revised and updated to reflect current taxonomic understanding. Nomenclature, descriptions and keys follow Ettl & Gänter (1995), Dillard (1989a, 1989b, 1990), Komárek and Anagnostidis (1999, 2005), and Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b, 2000).

Microsoft EXCEL 2000 was used to calculate species diversity for each sampling site. A regression analysis was also conducted to search for any correlations; a p-value < 0.05 was used for significance. The Shannon-Wiener diversity index from Zar (1999) was used to determine species diversity from individual sites.

$$H' = -\sum_{i=1}^{s} p_i \log_e p_i$$

RESULTS AND DISCUSSION

Twenty-eight lamp flora species were identified from 32 sampling locations (Table 2). Cyanobacteria were the dominant group, represented by 14 species (50.0% of the reported species); green algae included 8 identified species (28.6%); and 6 species of diatoms (21.4%) were identified.

The dominant species in our study were represented by six cyanobacteria (*Gloeocapsa aeruginosa 9.7%*, *Leptolyngbya subtilissima 6.7%*, *Leptolyngbya tenuis 5.7%*, *Chroococcus minor 3.8%*, *Chroococcus helveticus 3.7%*, *Oscillatoria rupicola 2.2%*), three green algae (*Chlorella miniata 34.1%*, *Chlorella protothecoides 8.2%*, *Stigeoclonium terrestre 1.7%*) and two diatom species (*Diadesmis contenta 14.9%*, *Symploca cartilaginea 2.2%*). These 11 species comprised 93% of the community relative percentage.

Sample #	Light Intensity (foot candles)	Temp °C	Species Diversity
1	46.2	17.2	0.350
2	42.9	14.9	0.000
3	93.4	16.8	0.485
4	103.5	14.5	0.794
5	90.9	15.0	0.418
6	201	15.0	0.296
7	34.4	14.8	0.072
8	4.3	14.8	0.024
9	196	14.8	0.195
10	18.7	14.6	0.042
11	48.3	16.3	0.0113
12	6.3	19.3	0.524
13	29	19.5	0.454
14	27	19.5	0.382
15	107	17.9	0.355
16	45	17.2	0.145
17	70	16.7	0.057
18	31.8	16.3	0.329
19	8.1	16.3	0.440
20	114	15.2	0.834
21	5.7	15.2	0.476
22	109.3	16.2	0.042
23	4.5	16.5	0.484
24	2240	16.4	0.000
25	54.3	16.4	0.297
26	3780	15.0	0.000
27	2160	15.0	0.071
28	29.5	15.0	0.065
29	70.3	15.8	0.286
30	143	15.9	0.492
31	80.3	15.9	0.020
32	44.6	15.9	0.000

Table 1. Site light intensity (foot candles), temperature (°C) and species diversity (H') for each of the sampling locations.

Most species tended to be site specific with 17 species (60.7% of species identified) found at two or fewer sampling locations. Of these site specific species, six (*Aphanothece saxicola*, *Coconneis placentula*, *Nitzschia littoralis*, *Oscillatoria rupicola*, *Scytonema julianum*, and *Stephanodiscus hantzschii*) were found only in the Frozen Niagara section, five (*Cylindrocystis crassa*, *Cylindrocystis brebiissonii*, *Netrium oblongum*, *Nostoc muscorum*, and *Symploca cartilaginea*) were only found at the Lover's Leap site, and *Cyanothece aeruginosus* was only found on the New Entrance to Grand Central Station route. The Frozen Niagara section, Lover's Leap site, and the New Entrance to Grand Central Station route are all on the Frozen Niagara tour, which is 1.2 kilometers (0.75 miles) long. Therefore, these sites are all in reasonable proximity to one another. The Snowball Dining Room had two site specific species: *Chroococcus cohaeren* and *Gloeocystis vesiculosa*. *Euastrum sublobatum* and *Lyngbya truncicola* were both found in the Frozen Niagara Section but *E. sublobatum* was also found at Lover's Leap, while *L. truncicola* was found along the

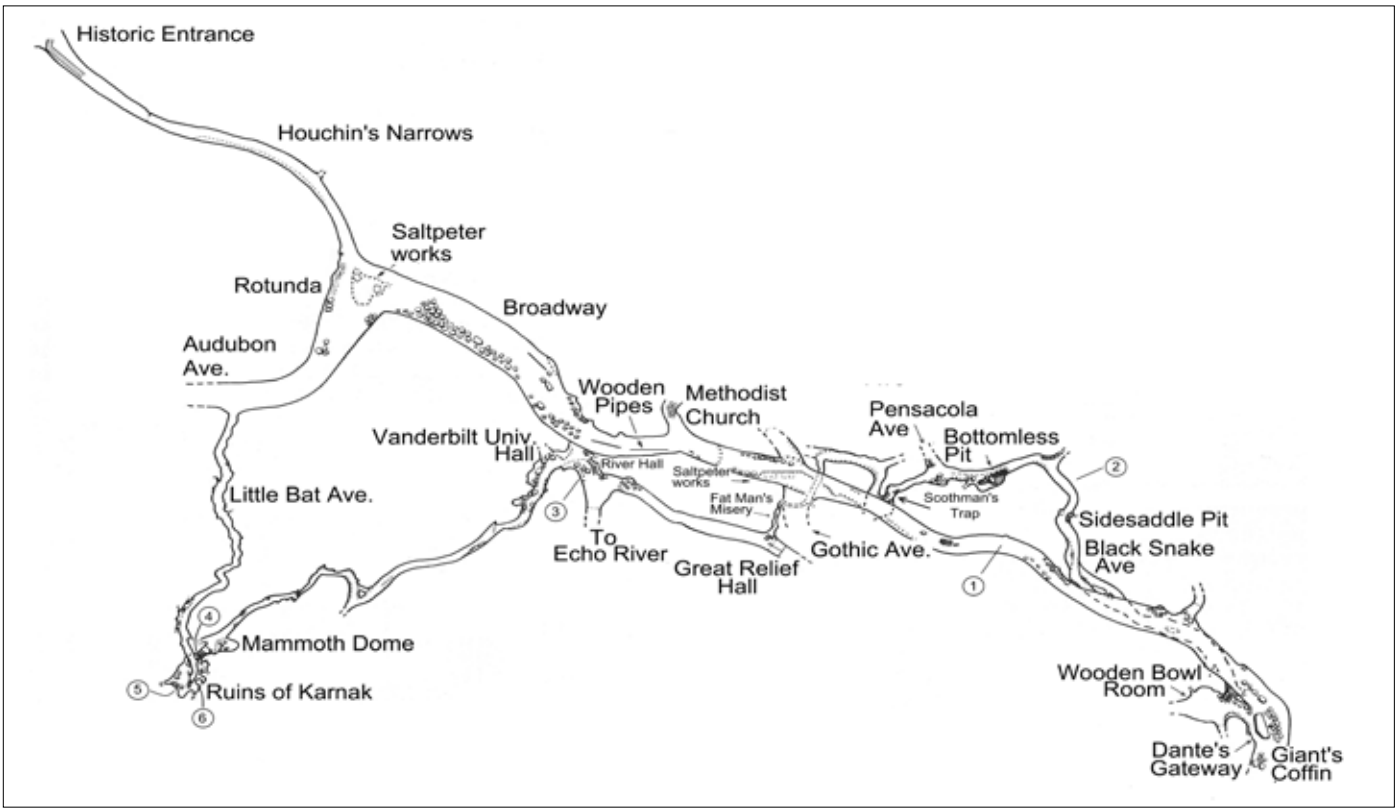

Fig.2. Map of the Historic tour with sample number and location. (Palmer, 1981)

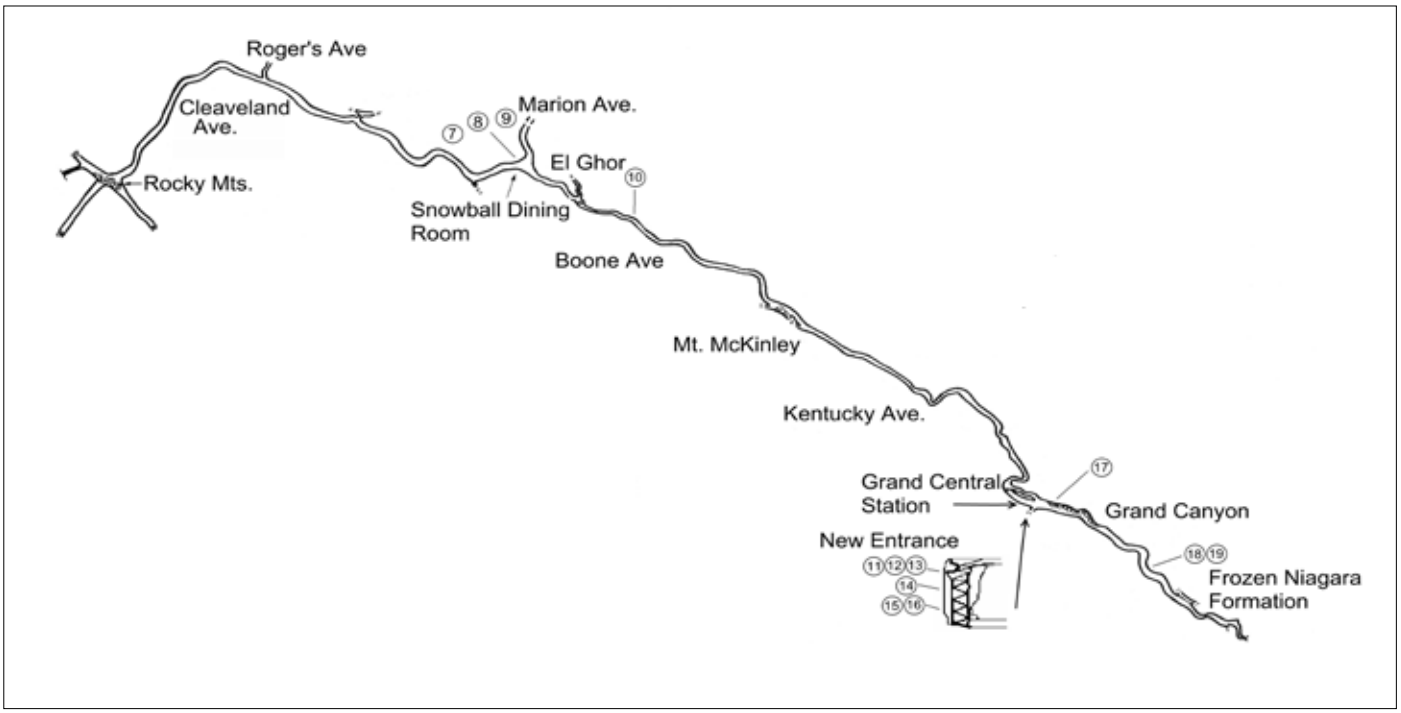

Fig.3. Map of the Half-Day and Frozen Niagara tour with sample number and location. (Palmer, 1981)

New Entrance route. *Porphyrosiphon martensiana* was only found in the Historic section at Mammoth Dome.

There was no clear conclusion in relationship to temperature and species diversity. When we ran a regression analysis, there was not a correlation ($p>0.05$) between temperature and species diversity (Table 3). In general, higher algal species diversities (H') tended to be found at warmer temperatures (Fig. 5). This may be due fluctuations in air flow and wind chill factor at or near cave entrances.

Dayner and Johansen's study (1991) identified lamp flora from Seneca Cave, Ohio. They found a similar low species richness (26 species identified), but found diatoms to be dominant (14 species). There were six species found in both studies (one cyanobacteria, two green algae, and three diatoms) with only *Chlorella miniata* and *Diadesmis contenta* being quite abundant and found in multiple sites.

During the previous algal studies in Mammoth Cave, using the lowest taxonomic resolution, investigators identified 54 species and 16 genera in Table 4, 5,

	SITE NUMBERS																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	**17**	**18**	**19**	20	21	22	23	24	25	26	27	28	29	30	31	32
Cyanobacteria																																
Chroococcus cohaeren (Brébisson) Nägeli									X																							
Chroococcus minor (Kützing) Nägeli	X		X	X												X				X												
Chroococcus helveticus Nägeli																			X	X	X											
Cyanothece aeruginosus (Nägeli) Komárek												X																				
Gloeocapsa aeruginosa Kützing												X		X	X	X														X		
Aphanocapsa muscicuola (Meneghini) Willie												X		X	X	X														X		
Aphanothece saxicola Nägeli																				X												
Leptolyngbya subtilissima (Kützing ex Hansgirg)							X	X	X											X	X											
Leptolyngbya tenuis (Gomont) Anagnostidis et Komárek								X	X			X	X	X	X	X				X			X		X		X	X				
Lyngbya truncicola Ghose														X						X												
Nostoc muscorum Kützing																		X														
Oscillatoria rupicola Hansgirg																								X								
Porphyrosiphon martensianus (Meneghini ex Gomont) Anagnostidis et Komárek					X	X																										
Scytonema julianum Meneghini																							X									
Symploca cartilaginea Gomont ex Gomont																		X														
Chlorophyta																																
Chlorella miniata (Nägeli) Oltmann	X	X	X		X		X		X	X	X	X	X				X										X	X	X	X	X	X
Chlorella protothecoides Kruegger	X		X	X	X													X	X			X	X		X	X						
Cylindrocystis brebiissonii Meneghini																			X													
Cylindrocystis crassa (DeBary) Wolle																			X													
Euastrum sublobatum Brebisson																			X	X												
Gloeocystis vesiculosa Nägeli							X																									
Netrium oblongum (DeBary) Luetkemueller																			X													
Stigeoclonium terrestre Iwanoff				X					X				X	X	X	X		X	X	X	X									X		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	**17**	**18**	**19**	20	21	22	23	24	25	26	27	28	29	30	31	32
Bacillariophyta																																
Achnanthes minutissima (Wm. Smith) Grunow					X	X																	X									
Diadesmis contenta Grunow in Van Heurck	X		X	X	X	X				X	X	X	X	X	X	X	X			X	X	X						X	X	X	X	
Nitzschia hantzschiana Rabenhorst							X								X	X	X															
Coconneis placentula Ehrenberg																													X			
Nitzschia littoralis (Agardh) W. Smith																							X									
Stephanodiscus hantzschii Grunow in Cleve & Grunow																													X			

Table 2. Lamp flora identified from Historic tour (1-6), Snow Ball Dining Room (7-9), Boone Avenue (10), New Entrance to Mammoth Cave stairs (11-16), Grand Central Station (17), Kentucky Avenue (18-19), and Frozen Niagara tour (20-32)

Variable	Results	Coefficient	Standard Error	P-Value
Temperature	Constant	-0.3717	0.4896	0.4536
	X-variable	0.03942	0.0303	0.2026

Table 3. Regression analysis results between temperature and species diversity.

and 6. Cyanobacteria were dominant with 20 species and six genera identified; green algae had 16 species and two genera; diatoms had 12 species and seven genera; red algae had two species and one genus; dinoflagellates had two species; and chrysophytes and xanthrophytes both had one species.

Results from our lamp flora species were not consistent with previous study results. There were two species (*Chlorella miniata* and *Achnanthes minutissima*) identified in our and from Van Hoff's (1974) study. There was one species (C*hroococcus minor*) similarity between Van Hoff and our study; only one other species (*Jaaginema neglectum*) was identified by two studies (Jones, 1965; Nagy, 1965). A difference in the taxonomists identifying the lamp flora may account for some of species disparity between these studies. They identified 54 species and only went to the genus taxonomic level for 16 individuals, which is 30% of the community identification.

Another possibility is a shift in the community structure between the different studies. The time span ranged from 8 to 23 years between studies. Jones and

	Study of Mammoth Cave National Park			
	Jones	**Nagy**	**Van Hoff**	**Harris**
Cyanobacteria				
Anacystis Meneghini			X	
Aphanothece gelatinosa (hennings) Lemmermann			X	
Beggiatoa alba (Vaucher) Trevisan	X			
Chamaesiphon confervicolus A. Braun in Rabenhorst		X		
Chroococcus minor (Kützing) Nägeli			X	
Cyanothece aeruginosus (Nägeli) Komárek			X	
Geitlerinema splendidum (Greville ex Gomont) Anagnostidis	X			
Gomontiella magyariana Claus	X			
Gleocapsa Kützing			X	
Heteroleibleinia pusilla (Hansgirg) Compére	X			
Hydrocoleus homeotrichus sensu Anagnostidis & al.				X
Jaaginema neglectum (Lemmermann) Anagnostidis et Komárek	X	X		
Jaaginema subtilissima (Kütz ex De Toni) Anagnostidis et Komárek	X			
Leptolyngbya cebennensis (Gomont) Umezaki et M. Watanabe	X			
Leptolyngbya subtruncata (Voronichin) Anagnostidis	X			
Lyngbya Agardh			X	
Microchaete tenera Thuret				X
Microcystis stagnalis Lemmermann	X			
Nostoc minitissimum Kütz emend. Claus	X			
Oscillatoria I, II, III Vaucher ex Gomont			X	
Oscillatoria clausiana Jones	X			
Phormidium automnale (Agardh) Trevisan ex Gomont				X
Planktolyngbya contorta (Lemmermann) Anagnostidis et Komárek				X
Tetrachloris inconstans Pascher	X			

Table 4. Cyanobacteria lamp flora identified from Jones (1965), Nagy (1965), Van Hoff (1974) and Harris (1981).

	Study of Mammoth Cave National Park			
	Jones	**Nagy**	**Van Hoff**	**Harris**
Chlorophyta				
Ankistrodesmus falcatus (Corda) Ralfs	X			
Astercoccus superbus (Cienk.) Schert.	X			
Chlorella ellipsoidea Gerneck				X
Chlorella vulgaris Beijer	X			
Chlorococcum humicola (Naegeli) Rabenhorst			X	
Chlorococcum sp. Meneghini				X
Chlorosarcina consociata (Klebs) GM Smith				X
Coccomyxa confluens (Kützing) Fott	X			
Desmodesmus brasiliensis (Lagerheim) E. Hegewald		X		
Diogenes sp. Naumann				X
Kirchneriella lunaris (Kirchn.) Mob	X			
Microthamnion kuetzingianum Naegeli				X
Nannochloris bacillaris Naumann				X
Oocystis lacustris Chod.	X			
Planophila laetevirens Gerneck	X			
Protococcus viriois Agardh				X
Scenedesmus abundans (Kirchn) Chod	X			
Ulothrix tenerriam Kützing	X			
Xanthophyta				
Xanthonema stichococcoides Pascher				X
Dinophyta				
Gymnodinium tenuissimum Lauterborn.		X		
Trachelomonas verrucosa Stokes	X			
Chrysophyta				
Chrysococcus klebsianus Pascher	X			
Rhodophyta				
Asterocytis smaragdiana (Reinsch) Forti		X		
Lemanea torulosa (Roth) Ag	X			
Rhodochorton sp. Nägeli				X

Table 5. Lamp flora identified from Jones (1965), Nagy (1965), Van Hoff (1974) and Harris (1981).

	Study of Mammoth Cave National Park		
	Van Hoff	**VanLandingham**	**Nagy**
Bacillariophyta			
Achnanthes minutissima Kützing		X	
Aulacoseira granulate var. angustissima (O. Müller) Simonsen		X	
Cocconeis Ehrenberg	X		
Cymbella brehmii Hustedt		X	
Cymbella delicatula Kützing		X	
Cymbella prostrate (Berkeley) Cleve		X	
Cymbella sp. Agardh		X	
Diatoma vulgaris Bory		X	
Fragilaria capunina Desmarzières		X	
Fragilaria l Lyngbye	X		
Fragilaria sp. Lyngbye		X	
Gophonema hotchkissii VanLandingham		X	
Gophonema parvulum (Kützing) Kützing		X	
Melosira Agardh	X		
Melosira varians Agardh		X	
Meridion circulare (Gréville) Agardh		X	
Navicula Bory	X		
Luticola nivalis (Ehrenberg) Mann			X
Stauroneis sp. Ehrenberg		X	

Table 6. *Bacillariophyta* lamp flora identified from Van Hoff (1974), VanLandingham (1965a, 1965b, and 1967), and Nagy (1965).

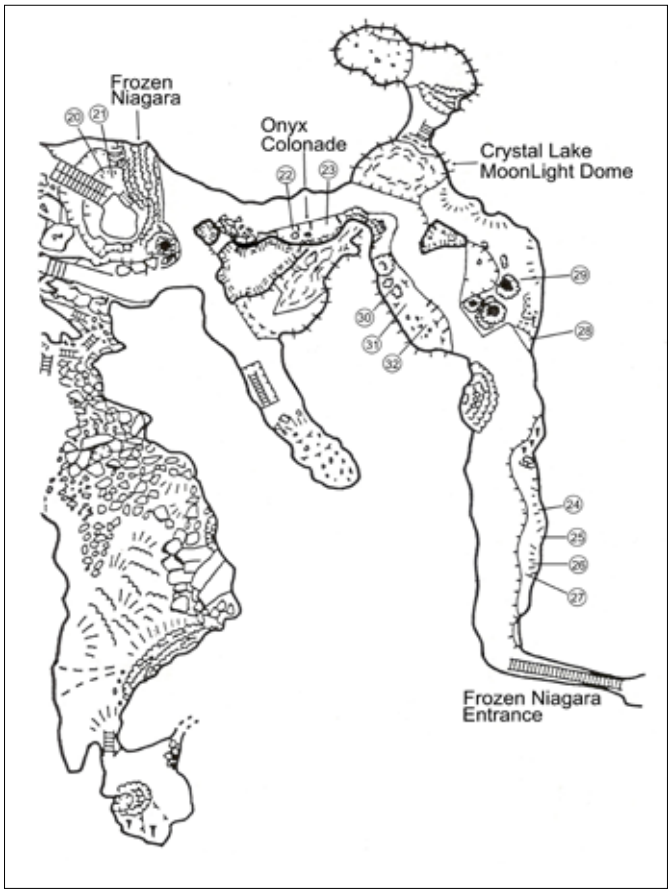

Fig.4. Map of the Half-Day and Frozen Niagara tour with sample number and location. (Palmer, 1981)

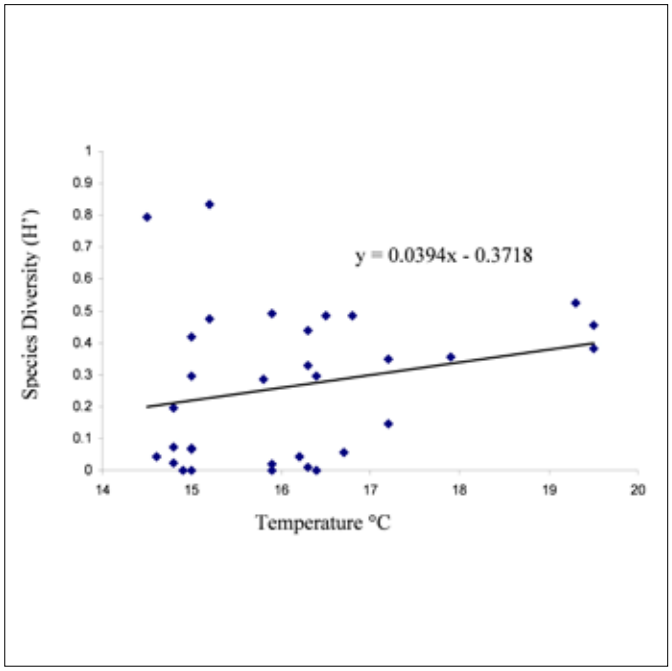

Fig.5. Map of the Frozen Niagara section with sample number and location.

Nagy's samples were collected in 1963 and Van Hoff sampled in 1974, an 11 year span. Harris sampled in 1981, a range of 7 years and we sampled in 2004, 23 years later.

Sites along tour trails with lights have wet surfaces of various types, which are ideal colonization sites. The National Park Service has cleaned the lamp flora off formations with diluted household bleach in the past. If park managers decide to continue this approach to lamp flora control, which is not recommended by cave geomicrobiologists (Boston et al., 2006), then such chemical cleaning will not only kill native microbes, it will also significantly alter algal species diversity in Mammoth Cave over time. Similar effects have been observed in algal recovery studies in aquatic systems (Peterson et al., 1994; Peterson & Stevenson, 1992). This would explain the few number of species found between studies suggesting a high turn over rate.

Iliopoulou-Georgoudaki et al. (1993) assessed the cleaning technique effectiveness on lamp flora growing on carbonate deposits in a cave in Greece. They found cleaning with sodium hypochlorite (bleach) was not completely effective. There were some cyanobacteria species surviving in carbonate microcavities. *Chroococcus minor* and *Scytonema julianum* are two

species that have been shown to survive this type of cleaning, and both were found during this study. *Chroococcus minor* was observed in four locations, three in the Historic Section and one in the Frozen Niagara Section's Drapery Room. *Scytonema julianum* was only seen on the Onyx Colonnade soil and formations. It is pale blue-green in color and capable of precipitating carbonate onto its mucilage sheath providing habitat for other algal species (Pietrini and Ricci, 1993). *S. julianum* species is known for its ability to mobilize calcium ions from calcareous substrata, as do the other cave-inhabiting cyanobacteria, including *Geitleria calcarea* and *Hepyzonema pulverulentum* (Ariño et al., 1997; Couté, 1985; Hernandéz-Mariné & Canals, 1994; Hernandéz-Mariné et al., 1999; Pietrini & Ricci, 1993). Park managers maybe concerned with the mobilization of calcium ions implying corrosion of the calcite surfaces cave formations that visitors come to see.

Olson (2006) installed narrow spectrum yellow LED's (595 λ) in 2005 in the Frozen Niagara section to control or eliminate lamp flora growth. When we sampled the Frozen Niagara section, no lamp flora growth was observed on the LED lighted formations. Previously, these formations contained a healthy lamp flora colony. Further observations of this area needs to be conducted because cyanobacteria can utilize wavelengths in the yellow band absorbed from the phycocyanin proteins. Cyanobacteria have been observed to adapt and respond to light composition by increasing specific phycobiliproteins concentrations that are able to absorb the precise available wavelengths (Albertano, 1991; Albertano & Caiola, 1988). They may grow back but at slower rate. Prevention of lamp flora growth through the use of yellow LEDs would eliminate the need to use chemicals such as bleach, or steam to remove growth as has been done previously in Mammoth Cave.

To help facilitate future managerial plans and prevent lamp flora growth, it is recommended that managers eliminate the cleaning of cave formations with sodium hypochlorite or bleach. Use of alternative light bulbs, especially LEDs, is in the best interest of the cave, its life, and visitors. Presently Mammoth Cave National Park personnel use selective wavelength lighting bulbs as an alternative lighting source that would emit wavelengths not usable for photosynthesis. This lighting would provide light for visitors' safety especially in the wet cave that can be slippery.

CONCLUSIONS

Mammoth Cave National Park is known worldwide for its outstanding subterranean resources. Unfortunately, lamp flora has flourished for decades since the introduction of lighting systems, which has resulted in some damage to the very resources visitors come to see. In some cases, live algae or cyanobacteria have become entombed within calcite, making it impossible to remove. In total, there were 28 species of lamp flora identified. Along tour routes in Mammoth Cave, cyanobacteria were dominant with 14 species, followed by green algae with eight species, and by diatoms with six species. Half of the species were site specific and higher species diversities were found at sites with high air temperatures, although the significance of this is unknown. Additional investigations need to be conducted to further understand immigration rates for different species and factors that influence them. It is only by understanding the biology of these opportunistic species that we can hope to devise alternative methods to eliminate them from an ecosystem where they do not occur naturally in such abundance. Their very presence, plus the chemicals used to control them, pose a serious risk to cave resources.

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