Biospeleology Session

Session chair: Dr. Sarah Keenan
Thursday, June 15, 2022 9:00am-5:om
Location: Bridger-Steel

The Biospeleology Session includes presentations that highlight the latest research in Biospeleology. These presentations cover a variety of topics ranging from cave microbiology, biogeochemistry, species inventories and diversity and cave ecosystem ecology.

Biospeleology Session Schedule

Time	Presenter	Title
9:00-9:10	Introduction and Welcome	
9:10-9:40	Hazel A. Barton	The Weird Microbiology of the Mulu Caves, Borneo
9:40-10:10	Kathleen Lavoie	UVC to control WNS: limitations and effects on microbial communities in caves
10:10-10:40	Reilly Blackwell	More than Speleothems: Measuring and Maximizing Microbial Calcium Carbonate Precipitation
10:40-11:00	BREAK	
11:00-11:30	Annette S. Engel	Hawaiian Lava Tube Biology along the Space-Time Continuum, the Next Generation
11:30-12:00	Diana E. Northup	The Effects of Surface Wildfires on Microbial Communities in Lava Caves
12:00 - 2:20	LUNCH BREAK	
2:20-2:50	Robert Weck	Life history patterns of the Enigmatic Cavesnail, *Fontigens antroecetes*, revealed through captive breeding under simulated cave conditions
2:50-3:20	Hannah R. Rigoni	Microbes of the Barton Springs Segment of the Edwards Aquifer, Texas
3:20-3:50	Benjamin Schwartz	Hyporheic stygobiont diversity and distribution in Texas, USA
3:50-4:20	Hazel A. Barton	The Microbiology of the Grottedal Caves of Greenland

Biospeleology Session Abstracts
(listed in alphabetical order by main presenter)

The Weird Microbiology of the Mulu Caves, Borneo

Hazel A. Barton and George Breley
bartonh@uakron.edu

During the 2019 Mulu Caves Expedition in Borneo, we carried out a preliminary assessment of a number of unusual features common to these caves to determine whether microbial activity was involved in their formation. This initial work was geared at examining four formations:

phytokarst and phytokarren (the curved stalactites at cave entrances), white encrustations, and a unique fluted erosion. Of these features, only the phytokarst and phytokarren had a demonstrably significant biogenic component, while the white encrustations were either calcite or nitrate deposits. The most interesting feature unique to the Mulu caves were the fluted surfaces, which appear to form through the microbial decomposition of swiftlet guano. This decomposition appears to produce a strong acid, possibly nitric, that leads to highly aggressive dissolution of the host rock. Such acid production may also explain the rapid degradation of nylon ropes rigged in the caves. These preliminary field observations suggest that there is a significant, but undescribed microbial ecosystem within the caves, driven by the temperatures, humidity and uniquely high levels of organic carbon input by swiftlets.

The Microbiology of the Grottedal Caves of Greenland

Hazel A. Barton, George J. Breley, M. Paul Smith and Gina E. Moseley
bartonh@uakron.edu

During the Northeast Greenland Caves expedition to Grottedal, Kronprins Christian Land, at 80°N, over 20 caves and dissolutional features were identified. Despite the sub-freezing temperatures, desiccating conditions, and poor organic content of these caves, we examined them for evidence of microbial activity and found significant evidence of microbial activity, dominated by photosynthetic species in near-entrance zones. This included the presence of extremophilic green algae (chlorophytes), along with cyanobacteria that formed photokarren. Other microbial activity was observed indirectly, including microbialites and iron-oxide deposits, which may indicate microbial contributions to speleogenesis when the region was warmer and cave development was occurring. The Grottedal area and caves have several environmental features in common with Mars: a polar desert under desiccating, low-light conditions and could provide an important testbed for future astrobiological investigations and instrument development.

More than Speleothems: Measuring and Maximizing Microbial Calcium Carbonate Precipitation

Reilly Blackwell, George Breley, Hazel A. Barton
reilly.s.blackwell@gmail.com

Pure calcium carbonate is a valuable material for a wide variety of industrial uses and is primarily sourced by mining or quarrying, which is costly and environmentally destructive, and it threatens karst all over the world. There is an alternative: microbially influenced calcium carbonate precipitation (MICP) produces very pure calcium carbonate ($CaCO_3$). Bacteria in caves use MICP to deal with the environmental stress of high Ca^{2+} ions when they grow, using atmospheric CO_2 to create the carbonate. We have been able to create conditions in the lab where we can make the model bacteria *Escherichia coli* precipitate $CaCO_3$. Using the information we know about the cave environment, we have investigated ways to increase microbial carbonate precipitation, create new methods to determine the efficacy of precipitation, and investigate the effects of other factors such as buffers, carbon dioxide concentration, and temperature on MICP. These efforts inform the design and construction of a bioreactor (a closed system with bacteria continuously producing $CaCO_3$) to produce microbial $CaCO_3$ precipitation at an industrial scale.

Hawaiian Lava Tube Biology along the Space-Time Continuum, the Next Generation

Annette Summers Engel[1], Megan L. Porter[2], Rebecca A. Chong[2], Alan G. Hudson[2], Michael E. Slay[3],
Christy M. Slay[4], Scott A. Engel[5], Veda Hackell[6], Tomislav Gracanin[6], Peter Bosted[6]
1 Department of Earth and Planetary Sciences, University of Tennessee–Knoxville, TN
2 Department of Biology, University of Hawai'i at Mānoa, Honolulu, HI
3 The Nature Conservancy, Little Rock, AR
4 The Sustainability Consortium, University of Arkansas, Fayetteville, AR
5 Jacobs, Knoxville, TN
6 Cave Conservancy of Hawai'i, Ocean View HI

Hawai'i island has the longest lava tubes in the world and diverse cave-adapted invertebrate species, although most subterranean habitats remain unexplored and many species have not been formally described. New biological surveys from 2015 through 2022 (not including 2020) in lava tubes on Kīlauea, Hualālai, and Mauna Loa volcanoes uncovered new species and expanded the known geographic distributions for some taxa. Of the >1100 person-hours of search effort from 65 different lava tubes sections of different geologic ages, geographic locations, and elevations, the most common cave-adapted fauna encountered included cambalid millipedes, erebid moths, trigonidiid crickets, cixiid planthoppers, lycosid spiders, philosciid isopods, talitrid amphipods, reduviid threadlegged bugs, mesovellid cave treaders, and carabid beetles. We also documented non-cave fauna and invasive species, which is critical for assessing potential ecological threats to the ecosystems. Exposed roots of native ʻōhiʻa lehua trees support the cave ecosystems, from which at least four trophic levels exist based on stable isotope ratio analyses. Of >1500 lots of specimens collected to date, at least six new species have been identified from lava tubes on Mauna Loa. Abundance surveys in root and non-root areas in 10 different sections revealed repeated spatial patterns where cave taxa were more abundant in the deep cave compared to near entrances, regardless of root occurrence. Some taxa (e.g., *Oliarus* spp.) dominated within-root communities, with nearly 20 individuals per patch. These findings reinforce that these subterranean ecosystems are complex and develop similarly across the space-time continuum. Proper identification and description of new species and population distributions are essential to discovering high-priority areas for conservation and protection.

UVC to control WNS: limitations and effects on microbial communities in caves

Kathleen Lavoie, Anna Pittis, Christopher Gallegos, Alice MacComb-Coubrey, Eric Dinger, Rick Toomey, and Diana Northup
Lavoiekh@plattsburgh.edu

The *P. destructans* fungus that causes WNS in bats is very sensitive to the effects of UVC radiation. Using UVC has several limitations; it is physically shadowed by any objects (tan lines), and is shielded by cells absorbing the irradiation. As part of a larger study of testing the potential of UVC to control *P. destructans* in caves, we also looked at the UVC sensitivity of native microbes cultured on low nutrient media from the study caves to evaluate possible collateral damage to the microbes at the base of the food chain in caves. We cultured microbes from three NPS caves: Oregon Caves National Monument & Preserve (ORCA), Mammoth Cave (MACA), and Lava Beds National Monument (LABE). We selected unique isolates from each cave (ORCA n =83; MACA n = 84; LABE n = 89) and tested their sensitivity to UVC at doses of 0, 0.62, 1.24, and 2.48 mJ/cm^2. We found that the majority of isolates was very resistant or resistant to UVC, with ORCA having more sensitives and MACA the most resistant isolates. While several bacterial genera overlapped across the three parks to differing degrees, there were 25 genera that only occurred in one park's caves. Future plans include work at three cave sites in MACA in July to field test the utility of UVC at reducing *P. destructans* in the environment while minimizing collateral damage to native microbes. Our results will shed light on the advisability of using UVC to eliminate *P. destructans* in caves.

The Effects of Surface Wildfires on Microbial Communities in Lava Caves?

Northup, Diana E.[1], Medley, Joseph J.[1], Hathaway, J.J.M. [1], Kulkarni, Harshad V.[2], Datta, S. [2]
[1]Biology, University of New Mexico; [2] Department of Earth and Planetary Sciences, University of Texas at San Antonio

Wildfires can cause devastating impacts on surface vegetation and soil microbial communities, but do they impact microbial communities in shallow lava caves? Lava Beds National Monument (LABE), home to over 800 hundred lava caves, experienced devastating wildfires in 2020 (Caldwell Fire) and 2021 (Antelope Fire) that burned more than 90% of the surface environment. Previous studies of the impact of wildfires on surface soil communities demonstrated dramatic shifts in the bacterial soil community composition. To test whether wildfires would impact microbial communities in lava caves that are a few meters below the surface, we sampled in four LABE lava caves in burned areas. We had previously sampled these four caves in 2017-2019 in a NASA-funded project to study analog sites for the search for extraterrestrial life. Sample sites included more microbial features (microbial mats and ooze) and more mineral features (mineral crusts, coralloids, polyps), as well as a surface soil sample above each cave. To test changes in the microbial communities, we extracted DNA and conducted Illumina next gen sequencing that targeted bacterial and archaeal inhabitants of these different sample sites, to compare them to sites sampled in December 2021. The results of our study will elucidate the impact of surface wildfire on subsurface lava cave microbial diversity.

Microbes of the Barton Springs Segment of the Edwards Aquifer, Texas

Hannah R. Rigoni[1*], Audrey T. Paterson[1], Annette Summers Engel[1], Benjamin Hutchins[2], Benjamin Schwartz[2]
[1]Department of Earth and Planetary Science, University of Tennessee, 1621 Cumberland Ave., Knoxville TN 37996
[2] Edwards Aquifer Research and Data Center, Texas State University, 601 University Dr., San Marcos TX 78666
*hrigoni@vols.utk.edu

Understanding the microbial diversity and functional roles of aquifer ecosystems can lead to better conservation, protection, and management efforts by maintaining water quality and stygobiont biodiversity. The Barton Springs Segment of the Edwards Aquifer, in south-central Texas, has one of the most biodiverse stygobiont communities in the US, but the microbiology has not been described. The Edwards Aquifer is comprised of three hydrologically separated segments with geochemically diverse zones. In the San Antonio Segment of the Edwards Aquifer, chemolithoautotrophic microbial communities support stygobiont communities and we hypothesized that comparable chemolithoautotrophs provide energy and food for the Barton Springs stygobionts. To quantify microbial diversity, we analyzed water geochemistry, 16S and 18S rRNA gene sequences from 19 wells, four springs, and three creeks near Austin. We used metagenomics from five representative wells and springs to assess metabolic potential. Freshwater wells and springs ranged from 300 to 600 mg/L total dissolved solids (TDS) and 3.6 to 7.5 mg/L dissolved oxygen (DO), but saline wells ranged from 1,200 to 9,000 mg/L TDS and had 0 to 1.9 mg/L DO. Although the phyla Bacteroidota, Firmicutes, and Proteobacteria were present in all samples, diversity among site types (i.e., spring, creek, freshwater well, saline well) was significantly different and correlated with DO, ammonium, and sulfate concentrations. Spring microbial communities had the greatest variability. In Old Mill Springs, saline water contributes to the flow, and the microbial communities were more similar to saline well communities, in which Firmicutes, Nitrospirota, and Desulfobacterota dominated. Based on metagenomes, chemolithoautotrophic microbes were relatively more abundant in springs, freshwater, and saline wells than in creeks. Specifically, freshwater wells were comprised of nitrate-reducers and ammonia-oxidizers, but saline wells were comprised of sulfate-reducers, nitrate-reducers, and chemolithoautotrophs. Our results indicate that chemolithoautotrophy is present across the aquifer, but microbial diversity and metabolic potential vary with geochemistry.

Hyporheic stygobiont diversity and distribution in Texas, USA.

Benjamin Schwartz (bs37@txstate.edu)[1,2] Benjamin Hutchins[2], Ashley Cottrell[2], Kathryn Perez[3], Annette Engel[4], Ethan Sweet[4], Audrey Paterson[4], Peter H. Diaz[5], Aaron Swink[6]

[1]Department of Biology, Texas State University, 601 University Dr., San Marcos TX 78666
[2] Edwards Aquifer Research and Data Center, Texas State University, 601 University Dr., San Marcos TX 78666
[3]Department of Biology, The University of Texas Rio Grande Valley, 1201 W. University Avenue, Edinburg TX 78562

[4]Department of Earth and Planetary Sciences, University of Tennessee, 713 Strong Hall, 1621 Cumberland Ave, Knoxville, TN 37996
[5]Texas Fish and Wildlife Conservation Office, United States Fish and Wildlife Service, 500 East McCarty Lane, San Marcos, Texas, 78666, USA.
[6]State of Hawaiʻi, Division of Aquatic Resources, 3060 ʻEiwa Street, Room 306, Līhuʻe, HI 96766-1875

Previous stygobiont diversity and distribution work has primarily focused on karst aquifer habitats. Seven years of sampling in diverse hyporheic habitats across the state of Texas, USA supports the notion that stygobiont diversity and abundance are generally related to proximity to karst and sites with 'karst-like' physicochemical properties. However, stygobionts have also found in diverse non-karst groundwater-dependent and hyporheic habitats.
Hundreds of samples at 130 sites span hydrologic gradients from isolated desert and mountain-top seep springs, to large, coastal-plain rivers. These sites also represent ranges and gradients in substrate permeability, physicochemical properties, geologic substrates, hydrologic disturbance, connectivity to karst, discharge, latitude, temperature, precipitation, and anthropogenic disturbances. Current and future work includes nested reach-scale, watershed-scale, and state-wide studies, as well as microbiological characterization to identify relationships with nutrient cycling and redox conditions
We conclude that many stygobionts have larger ranges than previously documented, can be exceptionally abundant in some hyporheic samples (relative to wells and caves), and occupy diverse sites and habitats previously deemed unlikely for stygobionts. Ranges for described taxa are expanding, and new taxa are being discovered at a rate that exceeds our ability to describe them. Results of this work are needed by resource managers, for species status assessments, and for species and habitat conservation, and can be used to guide future investigations at non-karst sites.

Life history patterns of the Enigmatic Cavesnail, *Fontigens antroecetes*, revealed through captive breeding under simulated cave conditions

Robert Weck
Southwestern Illinois College, 2500 Carlyle Avenue, Belleville, IL 62221
Email address: bob.weck@swic.edu

The Enigmatic Cavesnail, *Fontigens antroecetes*, is a cave adapted Hydrobiid snail listed as state endangered in Illinois. It is known from only one cave in Illinois, Stemler Cave, and from several caves in the eastern Ozark ecoregion of Missouri. Little is known about the snail's reproductive habits, embryological development, or growth rates. I attempted to gain basic life history information by breeding Enigmatic Cavesnails under simulated cave conditions in the laboratory. Six adult snails were collected from Stemler Cave and held in aerated containers of cave water with one or two rocks from the cave stream. Containers of snails were housed in incubators set at the average cave water temperature of 13° C. The snails produced 49 embryos in captivity over the course of 34 weeks. Eggs were deposited singly, attached to the underside of rocks within small pits or crevices. Nearly 82% of embryos developed to hatching. Mean estimated development time of embryos was 70.7 days. Survival of hatchling snails was poor.

Limited data available from surviving hatchling snails suggests slow growth rates. The process was replicated with nine Enigmatic Cavesnails collected from Cliff Cave in St. Louis County MO. Captive Cliff Cave snails produced 32 embryos over 46 weeks and varied from the Stemler population in their oviposition behavior, with a majority of eggs deposited on the top surface of rocks. Cliff Cave snail embryos also had longer mean estimated development times (82.8 days).