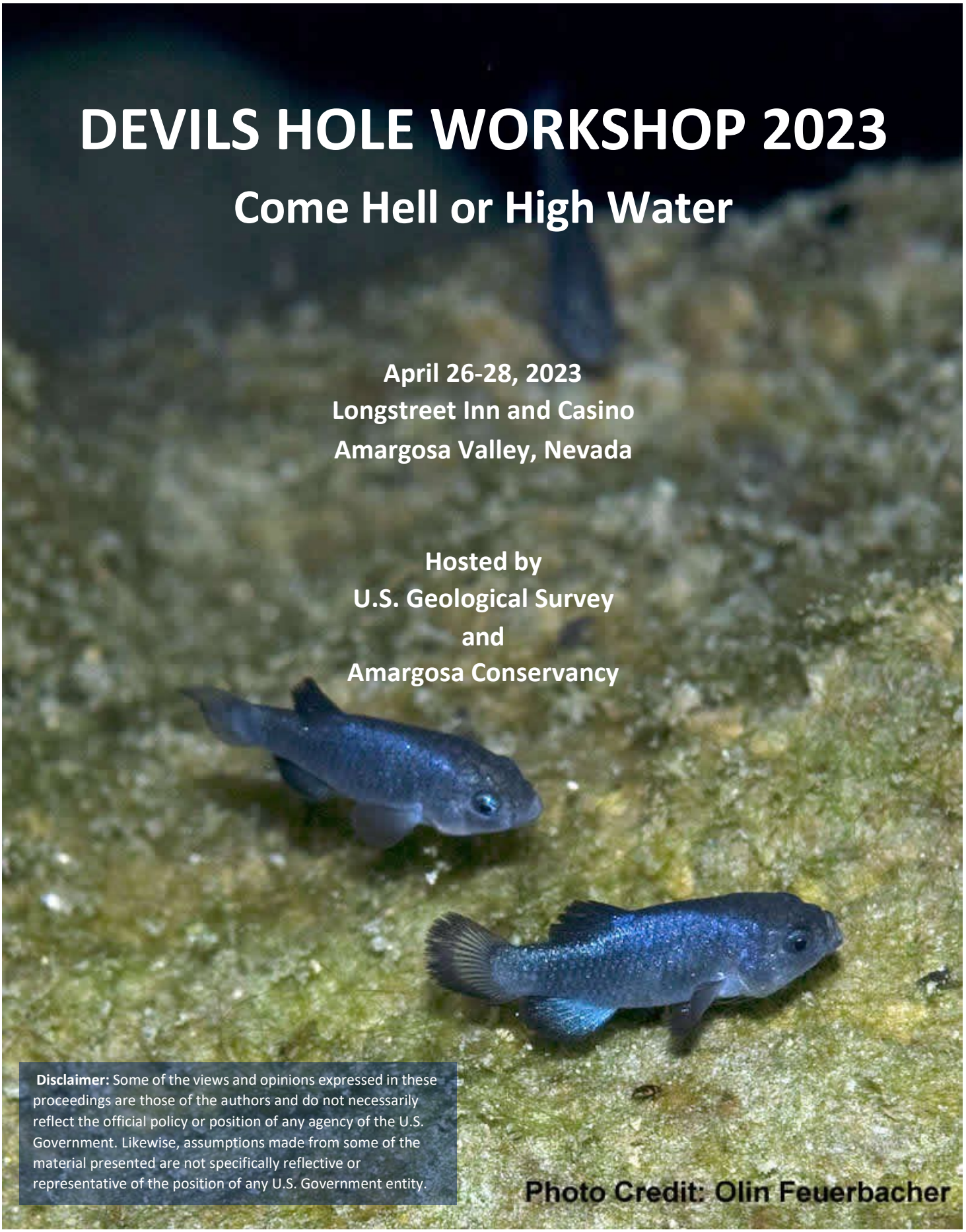
# DEVILS HOLE WORKSHOP 2023

## Come Hell or High Water

**April 26-28, 2023**
**Longstreet Inn and Casino**
**Amargosa Valley, Nevada**

**Hosted by**
**U.S. Geological Survey**
**and**
**Amargosa Conservancy**



**Disclaimer:** Some of the views and opinions expressed in these proceedings are those of the authors and do not necessarily reflect the official policy or position of any agency of the U.S. Government. Likewise, assumptions made from some of the material presented are not specifically reflective or representative of the position of any U.S. Government entity.

Photo Credit: Olin Feuerbacher

| | | |
|---|---|---|
| | **Devils Hole Workshop** | |
| | **"Come Hell or High Water"** | |
| | **April 26-28, 2023** | |
| | **Longstreet Inn and Casino** | |
| | **Amargosa Valley, Nevada** | |
| | | |
| **April 26 Wednesday** | | |
| | | |
| **Registration** | **Noon - 1 PM** | |
| | | |
| ***Session 1*** | ***Welcome & Pupfish Biology*** | Moderator: Wayne Belcher (USGS) |
| 1:00 PM | Welcome & Logistics | Wayne Belcher (USGS) |
| 1:10 PM | Introductory Remarks | Mike Reynolds (Superintendent Death Valley National Park) |
| 1:20 PM | Recent developments of the Devils Hole program and encouraging pupfish count numbers | Kevin Wilson (NPS) |
| 1:40 PM | Assessing Ecosystem and Population Dynamics to Determine the Primary Drivers of Devils Hole Pupfish Population Trends | John Umek (DRI) |
| 2:00 PM | Advances in captive rearing of Devils Hole pupfish at the Ash Meadows Fish Conservation Facility | Michael Schwemm (FWS) |
| | | |
| 2:20 PM | **BREAK** | |
| | | |
| ***Session 2*** | ***DNA in the Desert*** | Moderator: Geoff Moret (USGS) |
| 3:00 PM | DNA Tools for Desert Ecology and Hydrogeology: Updates from DRI's Environmental Microbiology Lab | Duane Moser (DRI) |
| 3:20 PM | A Decade of Evidence for Local Ecological Succession and Evolutionary Stasis in Continental Deep Subsurface Microbial Lineages | Alireza Saidi-Mehrabad (DRI) |
| 3:40 PM | Environmental DNA (eDNA) for Early Detection of Invasive Aquatic Species and Endemic Fish Surveillance in Desert Spring Systems | Victoria Wuest (DRI) |
| 4:00 PM | The Impact of Sampling Methodology on Obtaining Quality Microbiological Samples from Deep Subsurface | Molly Devlin (DRI) |
| 4:20 PM | ADJOURN; Announcements | Wayne Belcher (USGS) |
| | | |
| ***Posters*** | | |
| ***5:00 - 6:30 PM*** | | |
| | Quantification of Candidatus "Desulforudis audaxviator" Bacterial Endospores in the Deep Subsurface | Chelsea Black (DRI) |
| | The National Water Information System, U.S. Geological Survey – U.S. Department of Energy Cooperative Studies in Nevada, and Mercury Core Library and Data Center websites: What they are and how to use them | Seth Gainey, Steve Reiner (USGS) |
| | Tale of Two Wells: Comparing Microbial Communities from Recharge and Discharge Zones of the Death Valley Regional Flow System | Miriam Robertson (DRI) |
| | Environmental DNA (eDNA) for the tracking of endangered and invasive species in the Muddy River, NV, a desert warm spring system | Victoria Wuest (DRI) |
| | | |
| **April 27 Thursday** | | |
| | | |
| ***Session 3*** | ***Hydrology I*** | Moderator: Nancy Damar (USGS) |
| 9:00 AM | Announcements | |
| 9:10 AM | Historical Evolution of Conceptual Models of Furnace Creek Discharge | Keith Halford (Halford Hydrology) |
| 9:30 AM | Pervasive Preferential Flow through Thick Unsaturated Zones in the Death Valley Region | Joseph Fenelon (USGS, retired) |
| 9:50 AM | Water Level Trends in Southern Nye County – 2023 Update | John Klenke (Nye County) |
| | | |
| ***10:10 AM*** | ***BREAK*** | |
| | | |
| ***Session 4*** | ***Hydrology II*** | Moderator: Jeff Sanders (USGS) |
| 10:40 AM | Filling in the Blanks: Controlled Source Audio Magneto-Telluric (CSAMT) Geophysical Data as an aid to monitoring well placement | Kevin Day (Navarro) |
| 11:00 AM | Paleo-elevation constraints from a complexly deformed pre-Colorado River geomorphic surface: Significance of carnotite occurrences in the Southern Nevada region | Cady Johnson (Geologic VR LLC) |
| | | |
| ***11:20 AM*** | ***LUNCH*** | |
| | | |
| ***Session 5*** | ***Pupfish Award, Keynote Address, & Geology*** | Moderator: Wayne Belcher (USGS) |
| 1:30 PM | Tribute to John Bredehoeft | Mike King (Hydrodynamics Group) |
| 1:50 PM | Presentation of Pupfish Award | Sarah Peterson (USGS) |
| 2:00 PM | KEYNOTE ADDRESS: Three Things to Remember About Devils Hole | Peter Fahmy (NPS) |
| 2:30 PM | Enhancing Collaboration in the Amargosa Basin | Mason Voehl (Amargosa Conservancy) |
| 2:50 PM | Guided Discussion on Scientific Collaboration | Geoff Moret & Phil Gardner (USGS) |
| 3:10 PM | Field Trip Instructions | Keith Halford (Halford Hydrology) |
| 3:20 PM | Selection of 2024 Host | Jeff Sanders (USGS) |
| | | |
| ***3:30 PM*** | ***ADJOURN & BREAK*** | |
| | | |
| **April 28 Friday** | | |
| 8:00 AM | Field Trip | Keith Halford (Halford Hydrology) & Joseph Fenelon (USGS emeritus) |

**Wednesday April 26, 2023**

**Session 1 (1:00 – 2:30 PM): Pupfish Biology**

## Recent developments of the Devils Hole program and encouraging pupfish count numbers

Kevin Wilson[1], Ambre Chaudoin[1], Jeff Goldstein[1], John Wullschleger[1]; Michael Bower[2], Olin Feuerbacher[2], Jennifer Gumm[2], Michael Schwemm[2]; Brandon Senger[3]; John Umek[4]; and Mark Hausner[4]

[1]National Park Service
[2]US Fish and Wildlife Service
[3]Nevada Department of Wildlife
[4]Desert Research Institute

The Devils Hole pupfish (*Cyprinodon diabolis*) population has seen numerous highs and lows over the years. Climbing back from the brink of extinction and a record low of 35 fish in 2013, the 2022 spring and autumn counts produced the highest population estimates in nearly two decades. Visual surveys, using standard methodology (SCUBA and surface counters), recorded an estimated 175 fish in spring 2022 and spring 2023, the largest spring counts since 2000 when the population was an estimated 190 fish. Autumn 2022 surveys recorded 263 fish, an increase of 51% over the autumn 2021 count which recorded 174 fish. This represents the largest autumn count since 2003 when the population was an estimated 297 fish, and the first time in 19 years that the count has been greater than 200. Collaborative research and management currently focus on research gaps and implementation of the recently completed Devils Hole Pupfish Strategic Plan, including the development of an emergency evacuation plan, and other specific protocols. Additionally, the ICT is working with the Desert Research Institute on two separate projects. The first is a data synthesis project encompassing nearly 10 years of monitoring data (2011-2019) from the Devils Hole long-term ecosystem monitoring plan, plus other abiotic and biotic datasets going back to the 1960s; the second is a food web study to investigate trophic dynamics within the ecosystem, using stable isotope analysis techniques for comparison with previous food web studies in Devils Hole, yielding insights into the incorporation of natural and supplemental feeding.

## Assessing Ecosystem and Population Dynamics to Determine the Primary Drivers of Devils Hole Pupfish Population Trends

John Umek[1], Mark Hausner[1] and Kevin Wilson[2]

[1]Division of Hydrologic Sciences, Desert Research Institute, 2215 Raggio Pkwy,Reno, NV 89512.

[2]Death Valley National Park 1321 S. Hwy 160 Suite #1 Pahrump, NV 89048.

The critically endangered Devils Hole pupfish (DHP), *Cyprinodon diabolis*, found only in Devils Hole, was in a steady decline in population size beginning in the mid 1990s. The population reached an all-time low of 35 observable fish is the spring of 2013. Since this all-time low the population has increased to 100-180 observable fish. However, the population remains well below historical levels of 250-540 fish. Reasons for the steady decline and subsequent increase are not fully understood. A Long-Term Ecosystem Monitoring Program (LTEMP) was established in 2011 to collect ecosystem level information to better understand ecosystem processes and population dynamics of Devils Hole and the DHP. Prior to implementation of the LTEMP, however, data were collected from Devils Hole in a less systematic manner. In 2020, the Desert Research Institute (DRI) began a project to compile, organize, and document these data to make them accessible to researchers and resource managers. This project has two goals: (1) to compile the existing LTEMP data into a digitally ingestible format that can be easily accessed by researchers seeking to analyze the data; and (2) to perform data analysis to examine and quantify relationships between different drivers of habitat quality and metrics of the Devils Hole pupfish population. To accomplish goal 2, we examine ecosystem and population dynamics through an extensive statistical modeling project using Multivariate Autoregressive State-Space (MARSS) models to fit time-series data. Modeling outcomes will inform the adaptive management plans. Specifically, we use the

statistical methods to quantify the short and long-term trends of the Devils Hole pupfish population and the ecosystem processes driving these population trends to examine the interaction effects among biotic and abiotic parameters as they relate to aquatic community composition, trophic interactions, and pupfish habitat quality.

**Advances in captive rearing of Devils Hole pupfish at the Ash Meadows Fish Conservation Facility**

Olin Feuerbacher, Jennifer Gumm, Kevin Wilson, Ambre Chaudoin, Jeffrey Goldstein, Alex Jones, Michael Schwemm, John Wullschleger, Corey Lee, & Brandon Senger

The Ash Meadows Fish Conservation Facility began operation in 2013, primarily intended to develop a lifeboat population of Devils Hole Pupfish, *Cyprinodon diabolis*. Since the initial stocking of the 100,000-gallon refuge tank with 29 individuals in 2014, the population has grown to over 300 fish. Monthly collection of eggs from Devils Hole augments the population and provides gene flow into the captive population. Egg collections from the refuge tank have allowed for laboratory experimentation and breeding trials to refine captive rearing methods. This experimentation has provided insights into disease burden, fecundity, and egg viability, as well as hatch, survival, and growth rates. Comparison of data from fish reared from eggs produced in Devils Hole, the refuge tank, and aquaria have shown differences between the populations, particularly in egg viability and subsequent hatch and survival rates. Lower egg viability and increased rates of embryo malformations from fish in aquaria led to the development of new rearing methods and customized foods for broodstock.

## Session 2 (3:00 – 4:30 PM): DNA in the Desert

### DNA Tools for Desert Ecology and Hydrogeology: Updates from DRI's Environmental Microbiology Lab

[1]Duane Moser, [1]Alireza Saidi-Mehrabad, [1,2]Molly Devlin, [1,2]Victoria Wuest, [1,2]Miriam Robertson, [1,2]Chelsea Black, [1]Ron Hershey, [3]Eric Becraft, [4]Tim D'Angleo, [4]Melody Lindsay, [4]Julia McGonigle, [4]Beth Orcutt, [4]Ramunas Stepanauskas, [2]Brian Hedlund, [2]Cale Seymour, [5]Nancy Merino, [5]Annie Kersting, [5]Mavrik Zavarin; [6]Jenny Gumm, [6]Olin Feuerbacher, [6]Michael Schwemm, [7]Edwin Oh, [8]Tracie Jackson

[1]Desert Research Institute, Division of Hydrologic Sciences
[2]University of Nevada Las Vegas, School of Life Sciences
[3]University of Northern Alabama, Florence, AL
[4]Bigelow Lab for Ocean Sciences, Booth Bay, ME
[5]Livermore National Lab, Livermore, CA
[6]USFWS, Las Vegas, NV
[7]UNLV National Institute of Personalized Medicine;
[8]Unaffiliated

DRI's Environmental Microbiology Lab employs molecular tools to study life in the environment (viruses-to-humans). Our group was diverted during the pandemic for SARS-CoV-2 surveillance at 10 rural Nevada wastewater plants, establishing a pattern whereby new subvariants appeared first in urban centers and then outlying areas ~10 days later. Rural viral loadings tended to be much higher than urban and novel subvariants were identified on three occasions. The Lab's ongoing work with deep microbial ecosystems of the Death Valley Regional Flow System (DVRFS) continued. Microbial populations were used to confirm model-defined groundwater basins: Pahute Mesa–Oasis Valley (PMOV), Ash Meadows (AM), and Alkali Flat–Furnace Creek Ranch (AFFCR). Microbial diversity across the DVRFS corresponds to physicochemical drivers; with aerobes dominating in the recharge zone and known deep life (e.g. *C.* "Hadarchaea" and *Methanobacter* spp.) in hot, anoxic portions of the discharge zone. Wells, ER-EC-15 in the recharge zone and Inyo-BLM 1 (BLM-1) and Nevares Deep Well 2 in the discharge zone, were studied in detail. New evidence suggests that subsurface microbial populations are controlled by viruses and that hard pumping triggers a rolling progression of successional blooms that persists for years. Groundwater accessed by BLM-1 has become progressively enriched in *C. "*Desulforudis audaxviator" (CDA) in the 15 years since it was drilled and experienced a dramatic but transient bloom in *Methanobacterium* ~ 2011). Comparative genomics of CDA from BLM-1, Siberia, and South Africa revealed nearly identical genomes, a pattern that extends to other lineages such as *Methanobacterium*. "Omics" tools are revealing evolutionary history and ecological function of Candidate phyla, including Rokubacteria, Kiritimateallae, Nitrospirota and Nitrospinota, and Omnitrophota. New work is focusing on environmental DNA (eDNA) for surveillance of endemic desert fishes and invasive species. The lab is developing a suite of qPCR and metabarcoding assays targeting, for example, Moapa- and Ash Meadows speckled dace and Warm Springs pupfish and invasives such as red swamp crayfish. The exquisite sensitivity and specificity of these methods offer promise for tracking species composition over time and enabling early/actionable detection of invasions. We are performing all-life surveys from Devils Hole and the Ash Meadows Fish Conservation Facility to gain insights into the comparative lower trophic function of both. The lab is also characterizing the microbiome of the Devils Hole pupfish to better understand the effects of antimicrobial treatments used in husbandry of refuge populations.

### A Decade of Evidence for Local Ecological Succession and Evolutionary Stasis in Continental Deep Subsurface Microbial Lineages

Alireza Saidi-Mehrabad[1], Miriam Robertson[1], Melody Lindsay[2], Eric Becraft[2,3], Julia Brown[2], Molly Devlin[1], Victoria Wuest[1], Brian Hedlund[4,5], Tullis Onstott[6], Ramunas Stepanauskas[2], Duane Moser[1].

[1]Desert Research Institute, Las Vegas, NV, USA

[2]Bigelow Laboratory for Ocean Sciences, East Boothbay, ME, USA
[3]Department of Biology, University of North Alabama, Florence, AL, USA
[4]School of Life Sciences, University of Nevada, Las Vegas, NV, USA
[5]Nevada Institute of Personalized Medicine, Las Vegas, NV, USA
[6]Department of Geosciences, Princeton University, Princeton, NJ, USA

Current estimates suggest that ~$10^{29}$ microbial cells inhabit Earth's subsurface, an amount equal or greater than all life in the world's oceans and surface ecosystems. Most of this "deep life" is new to science, with physiologies, evolutionary relationships, metabolic potential, and contribution to Earth's biochemical cycles remaining largely unknown. Lacking direct energetic linkages to the photosphere, deep fractured rock aquifers are known for in situ biomass turnover times on the order of centuries-to-millennia. Here we share recent findings concerning life accessible via boreholes that intercept the Death Valley Regional Flow System (DVRFS), which special reference to Inyo-BLM 1 (BLM-1), a 750-m-deep well in the discharge zone of the DVRFS, which harbors rarely encountered signature deep subsurface lineages only found in Earth deepest habitats from around the world. Microbial community structure and activity were assessed via amplicon sequencing and DNA Stable Isotope probing (SIP), and evolutionary stasis, taxonomy, and potential shifts in metabolic activity via metagenomics, and single-cell genomics from pumped groundwater samples collected in 2007, 2011 and 2021. Omics tools demonstrated a dramatic shift in the community structure of bacteria and archaea from 2011 to 2021, whereby the community shifted from an archaeal (methanogen)-dominated system (64% *Methanothermobacter* spp) in 2011 to bacterial-domination (80% *Candidatus* "Desulforudis audaxviator" in 2021.

As with other life, microorganisms can be infected by viruses (e.g., bacteriophage), which in some cases can function as top predators, driving population dynamics. At BLM-1, a shift in viral abundance in metagenomic datasets, from 25% of total DNA in 2011 to 0.05% in 2021, appears to correspond to the concurrent shift from archaeal to bacterial dominance. Viral communities were dominated by lysogenic prophages of methanogens (Phages psiM2 and psiM100 related to double stranded DNA family of Siphoviridae), which initiate the host cell lysis when hydrogen, the main energy source for these methanogens, declines. An exhaustive analysis of single and metagenome-assembled genomesrevealed extensive overlap with microbes from South African gold mines or novel organisms lacking from genomic databases. Our calculations of the index of replication demonstrated that cells were alive but not actively replicating in both 2011 and 2021, consistent with ultra-oligotrophy as suggested by very low cell densities always encountered at this site. SIP samples, incubated under $^{13}CO$ atmosphere demonstrated the dominance of uncharacterized *Carboxydocella* sp., an obligate chemolithoautotrophic genus known to grow exclusively by hydrogenogenic CO oxidation, revealing a potential mechanism for hydrogen production for methanogens and other life of the deep biosphere. Future work focuses on statistical modeling, alpha and beta diversity analyses, SIP analysis of 2011 samples, and finally comparative metabolic analysis.

**Environmental DNA (eDNA) for Early Detection of Invasive Aquatic Species and Endemic Fish Surveillance in Desert Spring Systems**

[1,2]Victoria Wuest, [1]Alireza Saidi-Mehrabad, [1,2]Molly Devlin, [1,2]Chelsea Black, [3]Michael Schwemm, [1]Duane Moser

[1]Desert Research Institute, Las Vegas, NV
[2]School of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV
[3]US Fish and Wildlife Service, Southern Nevada Fish and Wildlife Office

Aquatic organisms continuously shed DNA into the environment (eDNA); producing a signal that is detectable with exquisite sensitivity and specificity through quantitative Polymerase Chain Reaction (qPCR) and/or metabarcoding. These methods are routinely being used for the tracking of coldwater fish

(i.e. salmonids) and Asian carp, for example. Much less is known concerning the efficacy of eDNA for surveillance of warm-water species. Spring-fed systems of the US Great Basin host an exceptional diversity of endemic and/or threatened aquatic organisms, whose fortunes are tied to hydrologic variables and competition/predation from invasive species. We posit that eDNA tools are ideal for surveillance of endemic desert aquatic organisms since many are difficult to monitor with traditional methods in complex habitats. In parallel, the work aims to validate rapidly deployable methods for detection of invasive species, either in response to new/suspected invasions or verifying extirpation status in restored habitats. Here we present a proof-of-concept study focused on the optimization of eDNA approaches in desert aquatic environments. This work examined a proposed workflow, emphasizing sampling (e.g. high-volume filtration), clean technique, DNA extraction protocols, and evaluation or development of effective molecular probes (qPCR primer sets). We are evaluating published and custom-designed primers targeting mitogenome targets to verify that endemic fishes (e.g. Moapa dace (*Moapa coriacea*) and Warm Springs pupfish (*Cyprinodon pectoralis nevadensis*)) and invasive species (red swamp crayfish (*Procambarus clarkii*), Australian redclaw crayfish (*Cherax quadricarinatus*), blue tilapia (*Oreochromis aureus*) and red shiner (*Cyprinella lutrensis*)) can be quantitatively monitored in several priority ecosystems (i.e. the Muddy River and Ash Meadows in Southern NV). Calibrations using tissue (fin clips), timed microcosm tests, and environmental sampling support a high level of sensitivity (~ 1 individual in 100,000 L of water for Moapa dace). Detection of several species (Moapa dace and red swamp crayfish was dramatically improved through development of custom PCR primers; whereas, published primers were effective for detection of mosquitofish (*Gambusia affinis*) and Australian redclaw crayfish. Results of a watershed-scale eDNA survey at Warm Springs Natural Area for Moapa dace, conducted in parallel with a snorkel survey, showed that eDNA is both sensitive and quantitative for this species. The results from this preliminary study support eDNA-based monitoring as a cost-effective and sensitive approach for the routine surveillance of endemic fishes and potential invaders in desert aquatic ecosystems.

**The Impact of Sampling Methodology on Obtaining Quality Microbiological Samples from Deep Subsurface**

Molly Devlin[1,2], Alireza Saidi-Mehrabad[1], Ronald L Hershey[1], Jamieson Walker[3], Julia McGonigle[4], Ramunas Stepanauskas[4], Duane P Moser[1]

[1]Division of Hydrological Sciences, Desert Research Institute, Las Vegas, NV
[2]School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV
[3]Jamieson Geological Inc affiliation, Pahrump, NV
[4] Bigelow Laboratory of Ocean Sciences, East Boothbay, ME

The fracture-rock networks and abundance of wells in the Death Valley Regional Flow System provide a world-class opportunity to study life in the deep subsurface. Microbial taxa recovered from this region, including *Candidatus* Desulforudis audaxviator, *Ca*. D8A-2, and *Ca.* Hadarcheota have been found in some of the deepest samples ever collected on multiple continents, highlighting the relevance of these sites to the global deep biosphere. One of the main challenges in studying this poorly understood biome is simply obtaining an uncontaminated sample from deeply sourced water. Although methods for obtaining physical and chemical measurements from wells and boreholes are well established (typically purging 3-5 well volumes and measuring physical and chemical parameters), no standard approach exists for microbiological sampling. Microbial life is particularly sensitive to artifacts created by reactive metal casings and static water columns, which highlights the importance of adequately purging a well prior to sampling. In this work, we track changes in chemistry (major ions, nutrients, etc.) and microbial community structure (16S rRNA gene amplicon libraries) from bailed samples of the static water column samples obtained by high-volume pumping of Inyo-BLM 1, a continuously cased well from an inferred fracture in Paleozoic carbonates at about 755 m depth in the discharge zone of the Death Valley Regional Flow System. During pumping, a distinct breakthrough of formation water was reflected in the pH, sulfate, and presence of the Candidate phylum Hadarcheota at one well volume of purging. Over the next

two days of pumping at an average of 200 GPM, a stable core microbiome of shared taxa was established, but the relative abundances of these core taxa shifted over time, The bailed sample, collected prior to pumping and from below the lower casing terminus at 750 m, had a nearly identical chemical composition to the pumped formation water but contained very little of the core microbiome, which suggests that chemistry is an imperfect marker for a sample indicative of pristine formation water. These data will be useful in informing the sampling design of future subsurface studies and better interpreting existing data.

**POSTERS (5:00 – 6:30 PM)**

**Quantification of *Candidatus* "Desulforudis audaxviator" Bacterial Endospores in the Deep Subsurface**

Chelsea Black[1,2], Molly Devlin[1,2], Duane Moser[1]

[1]Division of Hydrological Sciences, Desert Research Institute, Las Vegas, NV

[2]School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV

Terrestrial deep subsurface presents microbes with unique challenges from high temperatures or lack of surface-derived nutrients. Spore-forming bacteria are abundant in this challenging environment because they sense nutrient limitation or other environmental stressors and undergo sporulation. Sporulation involves the cessation of metabolism and formation of a protective spore coat allowing the organism to go dormant until favorable conditions return. *Candidatus* "Desulforudis audaxviator" (CDA) is a spore-forming, deep-living Firmicute that exists in the deepest known habitats on three continents, including Inyo-BLM 1 (BLM-1), a well in fractured Paleozoic carbonate rocks of the Death Valley Regional Flow System. Recently published evidence suggests that CDA populations from around the world have been in a state of evolutionary stasis for at least 200 million years. We hypothesize that this is due to the organism being in the spore form most of the time over geologic timescales. If no, or very few spores are present in this environment, spores are not likely to be important despite the abundance of predicted spore formers. If a large spore bank is present, the taxa it is composed of may shed light on the ecological role of spores in this environment. To test this hypothesis, we performed a spore-specific DNA extraction method on archived filters from pumped water samples collected from BLM-1 in 2021. A quantitative PCR (qPCR) assay specific to CDA was developed and applied to spore-specific and total microbial preparations. For this assay, we designed custom PCR primers targeting a hypervariable region of the 16S rRNA gene and confirmed their specificity *in silico*. The predicted optimal annealing temperature was validated using a gradient cycler. We tested for sensitivity and specificity by completing a melt curve and determined that we can detect as low as 4 gene copies with these precise primers. The primers will be able to quantify CDA in samples from BLM-1 or any environment, thus allowing us to determine the absolute proportions of vegetive CDA cells vs. those in spore form. A high proportion of spores would support a bias towards spore the form as a mechanism for maintaining extreme evolutionary stability.

**The National Water Information System, U.S. Geological Survey – U.S. Department of Energy Cooperative Studies in Nevada, and Mercury Core Library and Data Center websites: What they are and how to use them**

Seth R. Gainey[1], Peggy E. Elliott[1], and Steven R. Reiner[1]

[1]U.S. Geological Survey, Nevada Water Science Center, 500 Date Street, Boulder City, NV 89005

The U.S. Geological Survey (USGS) National Water Information System (NWIS) is a publicly available, national database that contains groundwater, surface-water, water-quality, and water-use data collected from about 1.9 million sites. These hydrologic data can be accessed through the interactive web interface, Water Data for the Nation (https://waterdata.usgs.gov/nwis), using navigation features to search for, find, retrieve, and compare data.

In addition, geologic, hydrologic, and geophysical data specific to the Nevada National Security Site (NNSS) can be accessed through the USGS-U.S. Department of Energy (DOE) Cooperative Studies in Nevada (https://nevada.usgs.gov/doe_nv/) and Mercury Core Library and Data Center websites (https://www.sciencebase.gov/mercury/#/). These websites provide project-specific data collected in support of various DOE programs.

We will provide an interactive overview of the Mercury Core Library and Data Center website focusing primarily on rock-sample images and information. Secondly, we will deliver an overview of the Water Data for the Nation website by highlighting common workflows to search for hydrologic data stored in

NWIS. Thirdly, we will provide a synopsis of the USGS-DOE Cooperative Studies in Nevada website, its features, and how to retrieve NNSS-specific data.

## A Tale of Two Wells: Comparing Microbial Communities from Recharge and Discharge Zones of the Death Valley Regional Flow System

Miriam Robertson[1], Alireza Saidi-Mehrabad[2], and Duane Moser[2]

[1]School of Life Sciences, University of Nevada Las Vegas, Las Vegas, NV
[2]Division of Hydrological Sciences, Desert Research Institute, Las Vegas, NV

The 100,000 $km^3$ Death Valley Regional Flow System (DVRFS) is a vast fractured rock aquifer that connects volcanic highlands in central Nevada to low-elevation discharge zones in eastern California. Numerous wells across the DVRFS are periodically sampled for hydrology and long-term surveillance of potential radionuclide contamination from underground Cold War nuclear weapons testing, creating a unique regional-scale deep biosphere observatory. Here we describe the microbiomes and associated physical and chemical environment from ER-EC-15, a well that intersects three discrete fractures in Tertiary volcanic rocks of the recharge zone and Nevares Deep Well 2 (Nevares-DW2), which samples the Lower Carbonate Aquifer of the discharge zone. Flowing groundwaters were subsampled from manifolds at the surface and planktonic microorganisms collected on 0.2-micron membrane filters. Total DNA was extracted and amplicon libraries of the 16S rRNA gene produced on the Illumina platform. Amplicon Sequence Variants (ASVs) were generated using the denoising method DADA2. Community clustering methods were used to compare samples from each well and depth. All samples were dominated by bacteria, with archaea making up less than 1% of the populations. The upper aquifer of ER-EC-15 was dominated by predicted aerobes, for example the bacterial Chloroflexi phylum, which accounts for 37.45% of the microbial community. ER-EC-15 Upper was the only zone with any significant archeal abundance (0.55% of the total community). Crenarchaeota (e.g. ammonia oxidizers such as the Thaumarchaeota) and Euryarchaeota accounted for 100% of archaea detected. ER-EC-15 Middle was dominated by the Proteobacteria (51.00%). ER-EC-15 Lower was dominated by the bacterial phylum Deinococcota (48.10%, i.e thermophiles such as *Thermus* spp.) 29.33% of the Nevares-DW2 microbial community was dominated by the bacterial phylum Nitrospirota (e.g. sulfate reducers, Thermodesulfovibrionia). Nevares-DW2 had the highest relative abundance of archaea, at 0.65% of the community. Nanoarchaeota (member of the DPANN superphylum) and Crenarchaeota collectively made up 65% of the archaeal community in Nevares DW-2, but 8 other phyla were also identified. Non-Metric Multidimensional Scaling plots further verified the distinctness of each sample based on a dissimilarity distance matrix. ER-EC-15 Upper and Middle plotted closest to each other, implying similarity between microbial communities, whereas ER-EC-15 Lower and Nevares-DW2 were highly dissimilar from each other and other samples. Temperature, redox status, and rock type appears to drive community composition. Future metagenomic analysis will interrogate the physiological potential of these microbial communities in great detail, provide more accurate taxonomy, and reveal rare community members.

**Environmental DNA (eDNA) for the tracking of endangered and invasive species in the Muddy River, NV, a desert warm spring system.**

[1,2]Victoria Wuest, [1]Duane Moser, [1]Alireza Saidi-Mehrabad, [3]Michael Schwemm

[1]Desert Research Institute, Las Vegas, NV
[2]School of Life Sciences, University of Nevada, Las Vegas, Las Vegas, NV
[3]US Fish and Wildlife Service, Southern Nevada Fish and Wildlife Office, 4701 N. Torrey Pines Dr., Las Vegas, NV 89130-2301

The Muddy River and thermal springs that form its headwaters near Moapa, Clark County, NV are home to the endemic Moapa dace. The most recent, dramatic declines of the Moapa dace were a direct result of the invasion of blue tilapia. Within the past few years (2012-2019) a focused restoration effort for the dace and other native species has been implemented, which included removal of all fishes (via the piscicide rotenone) in the Muddy River upstream of a barrier near I-15. Thus, a major goal of this work in the future will be to document the presumed removal of tilapia and concurrently, reestablishment of dace in the mainstem Muddy River. For the early detection of invasives, environmental DNA (eDNA) has proven to be a successful method. eDNA is found throughout the environment, as organisms shed genetic material into the water. This method enables the determination of the number of copies of DNA fragments specific to a taxonomic group in any sample. Thus, environmental DNA abundance/ concentration has the potential to be used as a direct proxy for fish biomass. Alternatively, sequencing general regions of a taxonomic group in an environmental sample, or eDNA metabarcoding, can determine biodiversity.

To date, eDNA assays have been little utilized for the monitoring of desert fishes. In the design of this project, we proposed that the method is particularly well-suited to spring habitats of Southern Nevada, where a small number of tiny fish may be almost impossible to find distributed across complex habitats such as marshes. We evaluated two eDNA survey methods side-by-side with a traditional visual survey method along the entire watershed of the Muddy River, Moapa, NV to 1) determine the best method for eDNA detection, 2) compare eDNA metabarcoding yielded the same detected species as visual survey data and 3) quantitatively compare eDNA qPCR results to visual survey data.

Calibrations using tissue (fin clips), timed microcosm tests, and environmental sampling support a high level of sensitivity (~ 1 individual in 100,000 L of water for Moapa dace and an unprotected surrogate, the Western mosquitofish (*Gambusia affinis*)) for the optimized approach. Results of a watershed-scale eDNA survey for Moapa dace, conducted in parallel with a traditional snorkel survey, will be presented. The results from this preliminary study support qPCR-based eDNA monitoring as a cost-effective and high-sensitivity approach for the routine surveillance of both endemic fishes and potential invaders in desert aquatic ecosystems.

**Thursday May 2, 2019**

**Session 3 (9:00 – 10:30 AM): Hydrology I**

### Historical Evolution of Conceptual Models of Furnace Creek Discharge

Keith J. Halford[1]

[1]Halford Hydrology LLC, khalford@halfordhydrology.com, Carson City, NV

Groundwater discharge from springs and phreatophytes in Furnace Creek has been recognized as anomalous for sixty years because discharge volumes from Furnace Creek springs far exceed local recharge. External sources have been postulated by successive investigators, where contemporaneous interpretations of regional geology, groundwater discharges, and water chemistry greatly influence conceptual models. Flow along faults in deep carbonate rocks from Pahrump Valley originally was postulated by Hunt and Robinson (1960) as the principal source of regional groundwater in Furnace Creek. Winograd and Thordarson (1975) rejected this conceptualization and used additional hydrologic and geochemical data to develop an alternative conceptual model of regional groundwater flow from basin fill in the Central Amargosa Desert. A source of regional flow to the basin fill was identified by Walker and Eakin (1963) as upwelling of water from carbonate rocks in Ash Meadows groundwater basin to overlying basin fill in the Central Amargosa Desert. While upwelling is certain, flow-rate estimates ranged from negligible to 3,000 acre-ft/yr. Plausibility of a deep-carbonate or basin-fill flow-path conceptualization has depended on (1) regional estimates of groundwater discharge from the Alkali Flat-Furnace Creek Ranch groundwater basin, which have ranged between 7,500 and 40,800 acre-ft/yr; and (2) hydrologic interpretations of the Funeral Range as an impermeable barrier or a transmissive feature. Consistent interpretation of available hydrologic and geochemical data suggests that the basin-fill flow path between Ash Meadows groundwater basin and Furnace Creek is more plausible.

### Pervasive Preferential Flow through Thick Unsaturated Zones in the Death Valley Region

Joseph M. Fenelon[1]

Tracie R. Jackson[2]

[1]U.S. Geological Survey (emeritus), jfenelon@usgs.gov
[2]U.S Geological Survey (former)

Recharge from preferential flow through thick (100–1000 m) unsaturated zones is a pervasive phenomenon in the Death Valley region. Wet and dry winters control water-level trends, with water levels rising within a few months to a year following a wet-winter recharge event and declining during sustained dry periods. Groundwater is composed of a mixture of diffuse-flow (paleo recharge) and preferential-flow (modern recharge). First-order approximations and simple mixing models of modern and late Pleistocene recharge water indicate that 10% to 40% of recharge is preferential flow and that modern recharge may play a larger role in the water budget than previously thought.

### Water Level Trends in Southern Nye County – 2023 Update

John Klenke[1]

[1]Nye County

Utilizing well data obtained from the Nye County Water District's Water Level Measurement Program (WLMP) and incorporating data from the USGS and NDWR websites, a map was created showing water level tends across southern Nye County from September 2004 to September 2022. Additionally, 2-year change maps were generated to depict short term changes across the area of study.

## Session 4 (10:40 – 11:20 AM): Hydrology II

### Filling in the Blanks: Controlled Source Audio Magneto-Telluric (CSAMT) Geophysical Data as an aid to monitoring well placement

Kevin Day[1]

[1]Navarro, Inc.

The U.S. Department of Energy (DOE) Environmental Management (EM) Nevada Program and its Environmental Program Services (EPS) contractor, Navarro Research and Engineering, Inc., have embraced innovation to safely, securely, and successfully execute several complex environmental restoration missions on the Nevada National Security Site (NNSS). As part of an ongoing effort to characterize and forecast the extent of impacted groundwater in support of closure of the Central and Western Pahute Mesa (PM) Corrective Action Units (CAUs) 101 & 102, the services of Zonge International, Inc. (Zonge) were procured to conduct a Controlled Source Audio Magneto-telluric (CSAMT) survey in an area targeted for groundwater observation well drilling in the coming field seasons. The results of the geophysical survey have provided insight into the subsurface and is an important component of the well site planning process.

The PM CAUs are areas of the NNSS located in south-central Nevada that were historically used for underground nuclear testing. Eighty-five underground nuclear tests were conducted at the PM CAUs between 1965 and 1992 (NNSA/NFO, 2015b). The detonation of Underground nuclear tests at or below the water table has resulted in test related radionuclides in the groundwater system in the vicinity of test locations and transport of these radionuclides to down gradient locations. Because of the existence of this underground groundwater contamination, the DOE, EM Nevada Program's Underground Test Area (UGTA) Activity is conducting a corrective action investigation (CAI) of the PM CAUs.

Borehole data collected over the course of testing activities and subsequent EM efforts, along with extensive geologic mapping and previous geophysical work has been assembled into a Hydrostratigraphic Framework Model (HFM) using EarthVision software (DGI, 2020) to provide both a decision-making tool and a foundation for groundwater flow and transport modeling. As can be expected, the HFM provides a more reliable depiction of subsurface hydrogeology and fault structures in areas where the borehole data is more concentrated and where previous geophysical surveys have been conducted. Outcropping formations also provide geologists with a better understanding of the subsurface in surrounding areas.

The CSAMT profiles that were surveyed and interpreted have provided important information about subsurface structures and the extent of volcanic aquifers and confining units. Analysis of the CSAMT profiles has included comparison with the Pahute Mesa-Oasis Valley HFM structures and deposits, correlation with nearby borehole geophysical data and evaluation of groundwater flow modeling results in the area of concern. These data comparisons suggest that the subsurface resistivity contrasts revealed by the geophysical investigation appear to align with features that influence or control groundwater flow as it leaves the testing area. It is also possible that the resistivity contrasts depicted by the CSAMT profiles are indicative of unknown structural features or alternative orientations of known structural features. These findings have provided project geologists with an important planning tool as locations are evaluated for proposed monitoring wells between the radionuclide sources and the discharge area in the vicinity of Beatty, NV. The project continues to evaluate the benefits of CSAMT surveys in subsurface investigations.

### Paleo-elevation constraints from a complexly deformed pre-Colorado River geomorphic surface: Significance of carnotite occurrences in the Southern Nevada region

Cady Johnson[1]

[1]GeoLogic VR LLC

It's been 100 years since D. Foster Hewett described sparse occurrences of carnotite, $K_2(UO_2)_2(VO_4)_2 \cdot 3H_2O$, near Goodsprings in southern Nevada. Near-surface carnotite occurrences near Goodsprings and several additional discoveries support the inference of an extensive pre- Colorado-River lowland in the Lake Mead region that can be quantified for modeling and visualization as a very flat parabolic cylinder that has been deformed by recently clarified

dynamic processes in Earth's mantle. Shallow groundwater associated with the paleovalley would precipitate carnotite given modest evaporation assuming the starting compositions resembled those in Recent samples from eastern Las Vegas Valley.

It turns out that there is carnotite associated with the "fossil water table" of Anderson (1969) streetside in Boulder City. Carnotite was also discovered in association with a radiometric anomaly near Mormon Mesa, part of a geomorphic surface equivalent to the Ivanpah Upland that developed unconformably on the Muddy Creek Formation. The Mormon Mesa caliche was long thought to be pedogenic but the carnotite occurrences investigated for this study prove otherwise based on field evidence, natural analogues, and reaction-path modeling of evaporative solution chemistry and sequential mineral precipitation. These carnotite occurrences give us a powerful tool to constrain the Mio-Pliocene paleo-elevation history of the Lake Mead region, but only if the role of groundwater in calcrete development is interpreted correctly.

A coupled finite-element model of regional groundwater and heat flow describes the present-day southeastern Nevada flow system and provides scaling relations for the larger, late Miocene system that was destroyed by crustal movements in the 6-4.4 Ma time frame. Models based on mantle dynamics indicate monotonic subsidence of ~1.5 km in the central-eastern Basin and Range since the middle Miocene, which would have eliminated a huge recharge area in northwestern Utah that is now in its collapsed form the West Desert. Uplift of 550 m of the former discharge environment area in the Lake Mead – Lower Colorado region before 4.4 Ma caused ponding and eventual spillage south as the lower Colorado River as we know it was born. This result begs the question: Can the deformed Ivanpah Upland be recognized in the Ash Meadows / Amargosa Flat area and beyond to reveal differing tectonic modifications of the same Mio-Pliocene land-surface datum?

The Mg-clay beds and surrounding calcite veins at Amargosa Flat are here interpreted to be associated with a remnant of the Ivanpah Upland, clearly owe their origins in large part to groundwater discharge, and given the low solubility of aluminosilicates suggest that either colloids played a role in moving the observed mineral mass to Amargosa Flats in the very short Pliocene (?) time frame or an extraordinarily long time with stable hydrology was required to precipitate these chemical sediments. Based on the maximum ages of ash beds in Lake Tecopa and occurrence of sepiolite low in the Tecopa lake beds, precipitation of Mg-clays in Amargosa Flat appears to have ended at about the time the spring-fed Amargosa River was born.

Extension east of the Spring Mountains had largely ceased by 6 Ma (Wernicke, 1988) so characterization of the transition from the Pliocene to the modern case where regional groundwater has been discharging to the collapsing East California Shear Zone for some 2.5 million years should be top priority in the context of the EPA-revised million-year regulatory time frame for a Yucca Mountain repository. We are not even close to achieving this.

**Session 5 (1:30 – 3:10 PM): Closing Session**

**A Tribute to Our Colleague Dr. John D. Bredehoeft, N.A.E.**

(presented by Michael King, Hydrodynamics Group)

DR. JOHN D. BREDEHOEFT, a consummate geo-scientist and educator, passed away on January 1, 2023 at the age of 89. John was born in St. Louis, Missouri on February 23, 1933. He showed an early interest and aptitude for engineering that he pursued throughout his life.

John earned a Bachelor of Science degree in Geological Engineering from Princeton University in 1955. In 1957 he earned a Master's degree in Geology from the University of Illinois at Urbana-Champaign. Following a brief position working with Humble Oil and the Desert Research Institute of the University of Nevada, John earned a Ph.D. in Geology at the University of Illinois. His educational research set the stage for his distinguished geological engineering career with the U.S. Geological Survey (USGS) and with *The Hydrodynamics Group*, LLC.

John contributed 32 years of public service at the USGS. He served for many years as Chief of the National Research Program of the Water Resources Division (WRD) of the USGS, which at that time employed close to 300 scientists and engineers. In this position, John substantially increased the relevance and visibility of their hydrologic research program. He later served for several years as Regional Hydrologist for the operational program in the eight-state Western Region of WRD. His successful efforts to create a 'research-in-the-District' program led to a better balance between scientific investigations and data collection throughout WRD. Perhaps the most amazing aspect of his time with the USGS is that John remained a productive scientist and researcher while also serving as a division manager. While at the USGS, John testified before Congress on a national policy regarding the geologic disposal and management of nuclear waste in the western United States.

John collaborated with Michael King to form The *Hydrodynamics* Group, LLC in 1997 with the vision that a team of nationally recognized experts in hydrogeology, geology, and geological engineering could provide principal-level consulting expertise for a wide spectrum of groundwater resource development and energy storage problems. John's 25 years of experience with *Hydrodynamics* includes oversight research on the Yucca Mountain High Level Nuclear Waste Repository for Inyo County, California; and on energy storage in deep geological structures for numerous projects around the world.

As an educator, John was as a visiting professor at the University of Illinois. He was the academic advisor for George Pinder at the University of Illinois in 1967-68. Together John and George developed and published the first widely utilized numerical groundwater flow model. They received the Horton Award of the American Geophysical Union for the groundbreaking work. They also developed the first widely used contaminant transport model, for which they received the Meinzer Award of the Geological Society of America. John was a consulting professor at Stanford for 8 years, and at the University of California, Santa Cruz, and San Francisco State University for many years. He served on numerous national advisory committees for the National Research Council, the National Science Foundation, and the Department of Energy. John was known for his ability to explain complex theories on the study of mass and energy transport in the earth to the U.S. Congress, the Nuclear Regulatory Agency, and other colleagues.

Most scientists get sufficient satisfaction from seeing their research results published and recognized by their peers. His contributions to hydrogeology, the storage of nuclear waste, and the development of groundwater resources are preserved in his numerous professional publications. John strived for more, and he recognized the need for society to benefit from the federally-funded research. He was a member of the National Academy of Sciences/National Research Council (NAS/NRC) Committee on the Waste Isolation Pilot Plant, and a member of the NAS/NRC Panel that reviewed groundwater concerns for the Yucca Mountain Nuclear Repository. John was also a member of the Russian Academy of Natural Sciences.

John's distinguished career has been acknowledged by receiving the Pemrose and Horton Medals. John also received the M.K. Hubbert, O.E. Meinzer, and Boggess Awards. Additionally, John received the Meritorious Service and Distinguished Service Awards from the Department of the Interior. Other honors included the Langbein and Halbourt Distinguished Lectures honors, and Life Time Member status with the National Groundwater Association.

John enjoyed his mini-vineyard in Sonoma where he grew several kinds of grapes. He enjoyed learning about and making wine and riding his tractor. John and his wife Beth spent summers in a remote cabin they built in Wyoming, where one year he encountered a cougar at the home. John truly enjoyed classical music. He is survived by his wife Beth Garbutt and four children John, Paul, and Chris Bredehoeft and Martha Clemensen.

John was a brilliant geoscientist who was at the forefront of many of the most important developments in the field of hydrogeology. He was also a generous friend and colleague who touched the lives of many. He loved his work at the U.S. Geological Survey and was deeply honored to be a member of the National Academy of Science.

## KEYNOTE ADDRESS

### Three Things You Must Remember About Devils Hole

Peter Fahmy, JD[1]

[1]Policy Analyst, NPS Water Rights Branch

The amount of information associated with Devils Hole is vast in scope and depth. However, there are three things that anyone who works on water rights and water resource issues associated with Devils Hole must absolutely remember. This presentation will succinctly describe these items and explain why knowledge of them is essential.

### Enhancing Collaboration in the Amargosa Basin

Mason Voehl[1]

[1]Executive Director, Amargosa Conservancy

The purpose of this presentation is to speak to recent events and efforts that have resulted in a renewed commitment to collaborative conservation in the Amargosa Basin ecoregion. The presentation will provide a brief overview of past conservation successes made possible by multi-partner collaborations and to speak to the need for enhancing communication and collaboration in light of the opportunities and challenges facing the watershed. Participants will gain a clearer understanding of the purpose and promise of watershed collaboratives as well as the role the Amargosa Conservancy has and will continue to play in acting as the convening organization driving collaboration forward. Opportunities for questions and information regarding how participants may become involved in the burgeoning collaborative will be provided.

The presentation will be delivered by Mason Voehl, the executive director of the Amargosa Conservancy and acting point of contact for the Amargosa Basin Coalition collaborative group.

## GUIDED DISCUSSION ON COLLABORATION IN THE DEATH VALLEY REGION (Geoff Moret and Phil Gardner, USGS)

**Friday, April 28, 2023**

**FIELD TRIP (8 AM – Noon) Keith Halford and Joseph Fenelon**

The field trip will focus on regional flow to Ash Meadows and to the Furnace Creek area. Ash Meadows and the downgradient Alkali Flat-Furnace Creek Ranch (AFFCR) groundwater basin, where Furnace Creek is located, are hydraulically connected. About 40 percent of discharge from the Furnace Creek area is recharged in the Ash Meadows groundwater basin and is conceptualized to flow into basin fill in the central Amargosa Desert via the megachannel. The megachannel is a large, transmissive, carbonate, groundwater pool that can be drained easily but fills up slowly. An alternative conceptualization of flow from Ash Meadows to the Furnace Creek area assumes that groundwater from the Ash Meadows groundwater basin flows through deep carbonate rocks that extend between Ash Meadows discharge area and the southeastern Funeral Mountains. Lithology, hydraulic properties, chemical evidence, and numerical simulation results demonstrate that the basin-fill flow path is more likely than the deep-carbonate flow path.

Significant hydrologic features will be visited and discussed during the field trip. These features define flow from the Ash Meadows groundwater basin to the Furnace Creek area. Basin-fill and deep-carbonate flow path conceptualizations will be discussed at each stop. The field trip will begin at Furnace Creek Ranch and end near Crystal Springs at the Ash Meadows visitor center.