

Nevada Water News

April 2023

Photo by Sean McKenna

Written and compiled by
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Strain-specific Monitoring of SARS-CoV-2 in Rural Wastewater Systems

This project is funded through the National Institutes for Water Resources (NIWR) 104(b) grant.

Although wastewater monitoring is a reliable and accurate way to track pathogens, ranging from norovirus and hepatitis A to bacteria such as salmonella, almost all of these studies have focused on urban rather than rural areas. The COVID-19 pandemic studies have been no different. Most of the SARS-CoV-2 wastewater monitoring in the United States likewise focused on population centers, which meant that the degree and timing of infection in rural areas was much less understood. To bridge this knowledge gap, the focus of the project “Strain-specific Monitoring

of SARS-CoV-2 in Rural Wastewater Systems” was to determine the effectiveness of using wastewater monitoring to better understand and track viral spread in rural areas, as well as improve sampling and testing practices to cover surface waters that aren’t usually included in wastewater monitoring programs.

In the pilot phase of the study, DRI sampled several rural wastewater systems, developed techniques for sample concentration and RNA purification, and used reverse transcription-quantitative

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***If you have questions
about submitting
a NWRRRI proposal,
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***Visit the NWRRRI
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for current RFP information.***



(Project Spotlight continued)

polymerase chain reaction (RT-qPCR) tools to determine SARS-CoV-2 loadings in environmental samples. Concurrently, UNLV sequenced 200 SARS-CoV-2-positive specimens collected from nasal swabs collected in Nevada for variant analyses and further epidemiological studies, identifying several unique strains of the virus in the process. The teams also successfully collected, prepared, and sequenced a set of Las Vegas wastewater samples. The original scope of the project was to combine the capabilities of both research

groups to conduct a statewide survey of the rural SARS-CoV-2 strain distribution by sampling rural wastewater influents. “It’s a little hard to believe now, but there were some initial concerns that the pandemic might be over before we were able to collect our first sample,” says Dr. Duane Moser (DRI), the principal investigator of the project that also included co-investigator Dr. Edwin Oh (UNLV/National Institute for Personalized Medicine [NIPM]). “However, considering the U.S. and worldwide vaccination patterns at the time and the propensity of

particularly infectious Omicron variant forced the team to shift their plan. “Recognizing that we were the only team organized at a critical time to document a historic epidemiological event across a large, undermonitored region of the country, Dr. Oh and I held an emergency meeting on December 18th to plan a way forward,” Moser explains. “At this meeting, we decided to rescope and expand the project, marshalling new resources available through Dr. Oh’s lab to support sequence analysis from a much larger number of samples than was originally budgeted. Given how this virus seemed to move across huge swaths of country in waves, we thought it would be more informative to sacrifice some of the originally planned geographic coverage and reinvest in repeat samplings of a smaller number of sites from outlying areas surrounding the urban core of the greater Las Vegas and Henderson area in hopes of documenting the Omicron wave as it propagated outward.”

this virus to mutate toward immune evasion and increasing infectivity, we made the case in the summer of 2021 that additional infection waves were likely.”

The project started in September 2021 and the researchers spent the first couple months organizing a planned statewide wastewater survey of rural Nevada. However, by late November 2021, the new wave of the

The rescoping request was approved and with the help of runners from the Clark County Water Reclamation District, the research team was able to sample influent water from five



Dr. Duane Moser collecting the first project samples from Beatty, Nevada, on December 26, 2021 (photo courtesy of Christiana Manville).

(Project Spotlight continued)

rural wastewater plants (Indian Springs, Moapa Valley, Laughlin, Searchlight, and Blue Diamond) on a weekly basis. This sampling program was supplemented by a second weekly collection program focusing on rural Nye County with the assistance of the Great Basin Water Company and Beatty Water District. Samples were collected from five wastewater treatment plants or lift stations in Pahrump, Nevada, and one in Beatty, Nevada. To facilitate this ambitious plan, Dr. Moser converted his own 2008 Honda Fit into a mobile field laboratory in late December 2021 and hit the road. “For each sampling event, two one-liter bottles were collected. One bottle was then sent to UNLV for qPCR and full genomic analysis, and the other was archived in freezers at DRI, where a time capsule of the pandemic remains to this day,” Moser says. “At the time, we had no idea how resilient Omicron would turn out to be and we expected to have completed our study within a month or so. However, seemingly endless waves of subvariants occurred across the

region month after month, ultimately requiring 35 weekly rounds of sampling by the end of the contract period on August 31, 2022.”

In addition to the originally planned wastewater focus, urban runoff grab samples were collected over the same sampling interval from the Flamingo and Tropicana Washes in Las Vegas with the aim of gaining insights into the effects of the pandemic on unhoused populations known to occupy underground flood channels. This study of opportunity tracked both SARS-CoV-2 and a second universal human fecal indicator, the pepper mild mottle virus (PPMoV), finding PPMoV in almost all the wash samples (which confirmed fecal contamination) and SARS-CoV-2

in 28 percent to 44 percent of the samples. Full genomic sequencing resulted in the recognition of a progression of subvariants that roughly matched circulating clinical subvariants and three novel strains of the virus, including two that have yet to



Dr. Edwin Oh, of the UNLV National Institute for Personalized Medicine, giving an interview about the COVID-19 wastewater monitoring project for the PBS program *Nevada Week* in May of 2022 (photo courtesy of Duane Moser).

This project was featured on the PBS *Nevada Week* segment, which shows both PIs collecting samples and discussing the work: www.vegaspbs.org/blogs/nevada-week-season-4/covid19-snapshot-funds-community-good/.

(Project Spotlight continued)

DRI/UNLV School of Life Sciences graduate students, (left) Tejinder Singh and (right) Victoria Wuest, collecting water samples from the Tropicana Wash adjacent to DRI in Las Vegas, Nevada. Later samplings, which corresponded to high sunlight exposure periods from this site and Flamingo Wash, were collected at the tunnel outlets to prevent potential UV-light inactivation of the virus (photos courtesy of Duane Moser).

be seen anywhere else in the world. This work is now under peer review.

The strength of the sampling approach used in this study is that by sampling untreated water flowing into the treatment plants, the team was able to capture a combined viral signal for an entire community with just one sample collection. The results from the study were somewhat what the investigators expected based on the previous study and what they were witnessing in the community. They found progressive waves of variants and subvariants that would first appear in the population centers (Las Vegas and Henderson), and

then spike in the outlying areas approximately 10 to 20 days later. For example, after the first reported detection of Omicron BA.1 in Las Vegas followed by a maximum viral load around January 1, 2022, eight of the 10 rural sites showed high viral concentrations by January 20, 2022. “One surprise has been the extreme degree of variation in viral concentrations across our rural wastewater treatment plant network,” Moser says. “For example, the Indian Springs and Searchlight plants completely bypassed the initial Omicron BA.1 wave, perhaps reflecting the relative isolation of these two communities. Although most

sites did contribute to the initial Omicron BA.1 wave, subsequent variants were much more unpredictable in timing and intensity. In fact, four sites recorded six distinct spikes in viral abundance over the course of the study.”

The results of the project also highlighted some important lessons learned. The researchers found that viral waves could potentially come and go so quickly that weekly sampling and testing might not catch every wave. Another trend across the dataset was that rural wastewater plants tended to have higher viral loadings than urban sites. “For perspective,

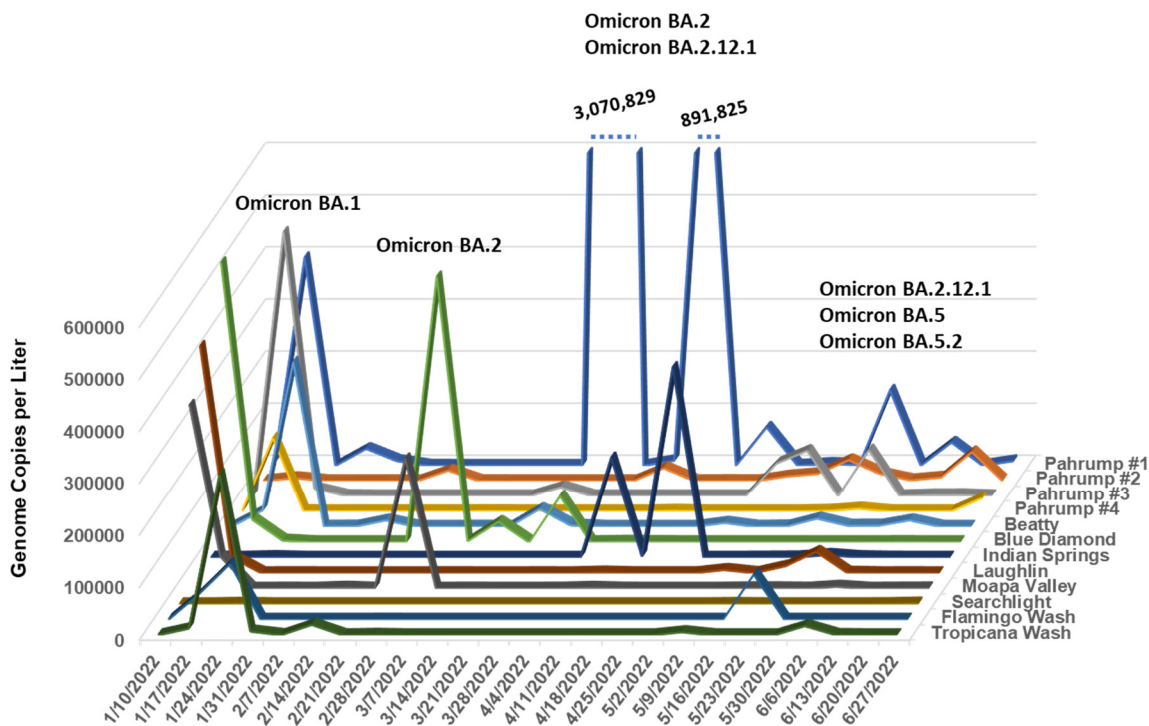
(Project Spotlight continued)

the highest viral concentrations reported since the beginning of the pandemic from the greater Las Vegas area were approximately 30,000 genome copies per milliliter,” Moser says. “Swings in viral loadings at the rural plants were much more pronounced, varying from undetectable to over 3,000,000 genome copies per milliliter in one case. The reason for this discrepancy is not entirely clear, but we suspect it is at least in part due to higher dilution rates in the much larger urban

wastewater systems.” One effect of higher viral concentrations in the rural systems might be increased assay sensitivity for otherwise hard-to-detect viruses. Therefore, finding ways for monitoring to be more equitably conducted in rural areas is an area of research that deserves further study.

This project also provided unique opportunities for the participating students to gain experience in leading-edge research techniques. Students were involved in all stages of the

project, from sample collection to extraction and analysis to viral RNA and data processing. Students affiliated with DRI participated in some of the field collections, whereas those affiliated with Dr. Oh’s lab handled much of the weekly sample processing, RNA sequencing, and analytics. In addition, the students had opportunities to interact with wastewater plant operators and local media, as well as contribute to developing the public-facing project dashboard, which can be



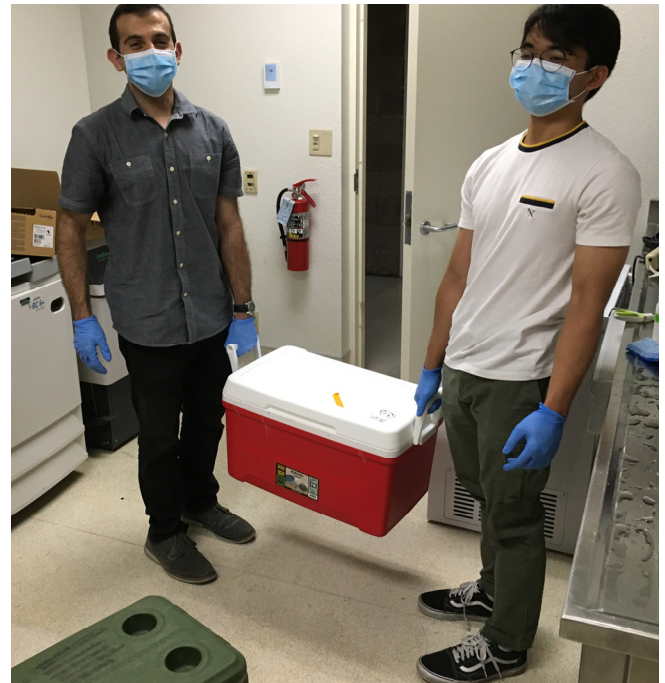
Graphical representation showing SARS-CoV-2 concentrations (qPCR data) from 10 rural wastewater plants and two urban storm drains from January through June of 2022. Note that most of the sites had high viral loadings at the beginning of the study, corresponding with the initial Omicron BA.1 wave that had appeared several weeks earlier in Las Vegas and Henderson, Nevada. Subsequent spikes in viral concentrations were more sporadic and generally corresponded to subvariants that had circulated in the nearby urban cores shortly prior to these detections. These data are currently under review and should be regarded as preliminary. Pahrump wastewater plant designations are randomized for this figure and do not correspond to formal plant names (figure courtesy of Duane Moser).

(Project Spotlight continued)

viewed on the Southern Nevada Health District website: empower.unlv.edu/.

A key success of this project is that it was a true teamwork effort, building and strengthening new relationships among DRI, UNLV/ NIPM, the Southern Nevada Health District, the Clark County Water Reclamation District, Great Basin Water Company, and Beatty Water and Sanitation District, as well as regional stakeholders and wastewater managers. This teamwork also helped advance the methods used in the pilot project. The original method required 10 L wastewater samples, which were then concentrated using a blood dialysis filter and other approaches to achieve a 2,000-fold concentration factor. Dr. Oh's lab infrastructure and sample analysis methods allowed the process to be scaled down, first from 10 L samples to 1 L samples, and then

to 50 mL samples. Being able to use such small samples while still achieving accurate results is expected to open up interesting possibilities for further tech innovations. "Wastewater monitoring is a perfect technique for tracking disease spread in almost real time and it can be scaled as quickly as needed for most needs, with the exception of rural septic systems," Moser explains. "This project was one of the best in the country to cover rural SARS-CoV-2 wastewater monitoring and assess the dynamics of viral



UNLV students (left) Michael Moshi and (right) Jose Yani Itorralba picking up the weekly sample submission from DRI prior to processing and analysis at UNLV (photo courtesy of Duane Moser).

spread from urban areas into rural areas. It also documented a historic event that caught us off guard and could catch us off guard again." ■

Events List

Please keep an eye on the event websites for changes in conference schedules.

19th Annual Truckee River Field Study Course
May 4 & 5, 2023
Reno, NV
www.nvwra.org/2023-truckee-river-tour

"Water Issues in Southern Nevada and the Colorado River Basin" by Kyle Roerink
May 9, 2023
Reno, NV
www.aegsnv.org/meetings

Chapman Conference: Hydrothermal Circulation and Seawater Chemistry
May 15-19, 2023
Agros, Cyprus
www.agu.org/Chapman-Hydrothermal-Circulation-Seawater-Chemistry

Nevada National Security Site Tour
May 16, 2023
Las Vegas, NV
www.nvwra.org/2023-may-nevada-national-security-test-site-tour

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Student Interview: Molly Devlin

We asked Molly Devlin, who is a fourth-year PhD student in the School of Life Sciences at UNLV and worked on the NWRRI COVID-19 wastewater monitoring project, about her current studies and plans for the future. Here's what she had to say:

1) What field are you currently studying and what sparked your interest in that field?

I study the microbial life of lava caves and deep aquifers. My interest in the subsurface began when I went caving for the first time and since then, I've been hooked!

2) You worked on the NWRRI project "Strain-specific Monitoring of SARS-CoV-2 in Rural Wastewater Systems." What did you learn from your experience on that project?

This was a great opportunity to work with and communicate to scientists in a wide range of disciplines. I learned how to better tailor presentations to my audience.

3) Was there anything that you learned while working on this project that you found particularly interesting or surprising?

It was interesting to see the network of people needed to obtain wastewater samples from multiple communities.

4) What are you currently studying/researching and what do you hope to learn more about?

My research currently focuses on life in a deep aquifer. I am interested in learning more about the abundance and composition of bacterial endospores in the subsurface and the roles they may play in evolutionary stability over time, as well as the metabolic diversity in these environments.



Photo courtesy of Molly Devlin

5) What are your goals for the next steps in your studies/career?

My next step after finishing my PhD is a postdoctoral position.

6) Coke or Pepsi?

Coke.

7) Cake or Pie?

Cake. ■

"This [project] was a great opportunity to work with and communicate to scientists in a wide range of disciplines. I learned how to better tailor presentations to my audience."

– Molly Devlin

Events List Continued

Please keep an eye on the event websites for changes in conference schedules.

119th Annual Meeting of the Cordilleran Section
May 17-19, 2023
Reno, NV
www.geosociety.org/GSA/Events/Section_Meetings/GSA/Sections/cd/2023mtg/home.aspx

World Environmental & Water Resources Congress
May 21-25, 2023
Henderson, NV
www.ewricongress.org/

Chapman Conference: Climate and Health for Africa
June 12-15, 2023
Washington DC
www.agu.org/Chapmans-Climate-and-Health

2023 Annual Water Resources Conference
June 13-15, 2023
Fort Collins, CO
ucowr.org/

Well Drilling Regulations Workshop
June 14, 2023
Reno, NV
www.nvwra.org/2023-june-well-regulations-workshop

Well Design, Construction, and Rehab Workshop
June 15, 2023
Reno, NV
www.nvwra.org/2023-june-well-design

2023 Sustainable Agronomy Conference
July 12, 19, 26, & August 2, 2023
Online
www.agronomy.org/meetings/sustainable-agronomy/

AWRA 2023 Summer Conference: Connecting Land & Water for Healthy Communities
July 17-19, 2023
Denver, CO
www.awra.org/Members/Events_and_Education/Events/2023_Summer_Conference/2023_Summer_Conference.aspx

Marlette Lake Tour
September 12, 2023
Carson City, NV
www.nvwra.org/2023-marlette-lake-tour



Photo by Jesse Juchter

2023 NWRA Fall Symposium
September 13 & 14, 2023
Reno, NV
www.nvwra.org/2023-fall-symposium

2023 Tahoe Science Conference
October 1-3, 2023
South Lake Tahoe, CA
www.nvwra.org/2023-tahoe-science-conference

GSA Connects 2023
October 15-18, 2023
Pittsburgh, PA
community.geosociety.org/gsa2023/home

2023 ASA, CSSA, SSSA International Annual Meeting
October 29-November 1, 2023
St. Louis, MO
www.acsmeetings.org/

2023 UNLV/NWRA Dinner Forum
November 1, 2023
Las Vegas, NV
www.nvwra.org/unlvnwra-dinner-forum

AGU23
December 11-15, 2023
San Francisco, CA
www.agu.org/Fall-Meeting

NWRRRI - Nevada Water Resources Research Institute

Success and the dedication to quality research have established the Division of Hydrologic Sciences (DHS) as the Nevada Water Resources Research Institute (NWRRRI) under the Water Resources Research Act of 1984 (as amended). As the NWRRRI, the continuing goals of DHS are to develop the water sciences knowledge and expertise that support Nevada's water needs, encourage our nation to manage water more responsibly, and train students to become productive professionals. The work conducted through the NWRRRI program is supported by the U.S. Geological Survey under Grant/Cooperative Agreement No. G21AP10578. The Desert Research Institute (DRI) administratively houses and logistically supports the operations of the NWRRRI.

About DRI

The Desert Research Institute (DRI) is a recognized world leader in basic and applied environmental research. Committed to scientific excellence and integrity, DRI faculty, students who work alongside them, and staff have developed scientific knowledge and innovative technologies in research projects around the globe. Since 1959, DRI's research has advanced scientific knowledge on topics ranging from humans' impact on the environment to the environment's impact on humans. DRI's impactful science and inspiring solutions support Nevada's diverse economy, provide science-based educational opportunities, and inform policymakers, business leaders, and community members. With campuses in Las Vegas and Reno, DRI serves as the nonprofit research arm of the Nevada System of Higher Education. For more information, please visit www.dri.edu.

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Banner photo: Rain over a small pond in Surprise Valley, California, which is an active hydrothermal area with many hot springs. Photo by Sean McKenna.

Pages 2-6: Project photos courtesy of Duane Moser and Christiana Manville; figure courtesy of Duane Moser.

Page 7: Photo courtesy of Molly Devlin.

Events list, page 8: The edible spring fruits of the native Joshua tree located on the eastern foothills of the Spring Mountain Range. Photo by Jesse Juchtzer.