A Compendium of U.S. Wastewater Surveillance to Support COVID-19 Public Health Response

EPA-830-R-21-004
September 2021

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- Top Left: Katie Watkins, Houston Public Media. The Houston Health Department collects a wastewater sample as part of the city's wastewater surveillance program supporting COVID-19 public health efforts (Watkins, 2021).
- Middle Left: Stock photo. Wastewater treatment plant aeration basin.
- Bottom Left: Ben Siegel, Ohio University. Engineering students prepare to collect a wastewater sample for SARS-CoV-2 monitoring to support Ohio University's COVID-19 public health efforts (OHIO News, 2021).
- Top Right: Stock photo. SARS-CoV-2 analyses of wastewater samples in a laboratory.
- Bottom Right: Stock photo. Aerial wastewater treatment plant photo.

Acknowledgements

EPA Office of Wastewater Management and Office of Research and Development would like to acknowledge the efforts of the following people in reviewing and providing feedback:

- Leonardo Angelone, National Institutes of Health
- Tracy Bone, EPA Office of Science and Technology
- Nicole Brinkman, EPA Office of Research and Development
- Jay Garland, EPA Office of Research and Development
- Amy Kirby, Centers for Disease Control and Prevention
- Lisa McFadden, Water Environment Federation
- Jeffrey Mercante, Centers for Disease Control and Prevention
- Kevin Oshima, EPA Office of Research and Development
- Jorge SantoDomingo, EPA Office of Research and Development

EPA would also like to acknowledge the participation of practitioners from the case study wastewater surveillance programs included in this report who provided information, participated in discussions, and reviewed their case study write-ups. Contributions of the practitioners are recognized below:

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Indiana	■ Jim McGoff, Indiana Finance Authority ■ Erica Walker, 120Water ■ Kyle Bibby and Alex Perkins, University of Notre Dame
Michigan	■ Rhiannon Bednar, Robert Orellana, Susan Peters, and Mary Grace Stobierski, Michigan Department of Health and Human Services ■ Shannon Briggs, Michigan Department of Environment, Great Lakes, and Energy ■ Kevin Bakker and Chuanwu Xi, University of Michigan ■ Erin Dreelin and Joan Rose, Michigan State University
New Mexico	■ Justin Garoutte, John Rhoderick, and Rebecca Roose, New Mexico Environment Department
Ohio	■ Zuzana Bohrerova, Ohio Water Resources Center
Wyoming	■ Ali Harrist, Franz Fuchs, Stefan Johansson, and Stephanie Pyle, Wyoming Department of Health ■ Wanda Manley and Cari Sloma, Wyoming Public Health Laboratory ■ Bledar Bisha and Sarah Collins, University of Wyoming
Hampton Roads Sanitation District, Virginia	■ Raul Gonzalez and Jim Pletl, Hampton Roads Sanitation District
Houston, Texas	■ Loren Hopkins, Houston Health Department ■ Lauren Stadler, Rice University
Tempe, Arizona	■ Wydale Holmes and Rosa Inchuasta, City of Tempe
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In addition, a formal technical review of the draft document was conducted by professionals with experience in wastewater surveillance in accordance with EPA Peer Review Guidelines. While every effort was made to accommodate all the Peer Review comments, the results and conclusions do not indicate consensus and may not represent the views of all the reviewers. The technical reviewers of this document included the following:

- Jerome Oliver, EPA Office of Wastewater Management
- Jason Turgeon, EPA Region 1
- Christobel Ferguson, Water Research Foundation
- Jonathan Yoder, Centers for Disease Control and Prevention

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Abbreviations

APHL	Association of Public Health Laboratories
ASTHO	Association of State and Territorial Health Officials
ASU	Arizona State University
CARES Act	2020 Coronavirus Aid, Relief, and Economic Security Act
CDC	Centers for Disease Control and Prevention
cDNA	complementary deoxyribonucleic acid
COVID-19	coronavirus disease 2019
CT	cycle threshold
DCIPHER	Data Collation and Integration for Public Health Event Response
DHS	United States Department of Homeland Security
EGLE	Michigan Department of Environment, Great Lakes, and Energy
ELC	Epidemiology and Laboratory Capacity for Prevention and Control of Emerging Infectious Disease (CDC funding)
EPA	United States Environmental Protection Agency
HHD	Houston Health Department
HHS	United States Department of Health and Human Services
HRSD	Hampton Roads Sanitation District
IFA	Indiana Finance Authority
L	liter
MDHHS	Michigan Department of Health and Human Services
mL	milliliter
N	nucleocapsid gene
NACCHO	National Association of County and City Health Officials
NACWA	National Association of Clean Water Agencies
NEHA	National Environmental Health Association
NIH	United States National Institutes of Health
NIST	United States National Institute of Standards and Technology
NSF	United States National Science Foundation
NSSIL	National Sewage Surveillance Interagency Leadership
NWSS	National Wastewater Surveillance System
ODH	Ohio Department of Health
Ohio EPA	Ohio Environmental Protection Agency
Ohio WRC	Ohio Water Resources Center
ORD	Office of Research and Development (within EPA)
PCR	polymerase chain reaction
RADx^{SM}	Rapid Acceleration of Diagnostics (NIH initiative)

RADx-rad	RADx^{SM} Radical (NIH initiative)
RAPID	Rapid Response Research (NSF funding mechanism)
RNA	ribonucleic acid
RT-ddPCR	reverse transcription digital droplet PCR
RT-PCR	reverse transcription PCR
RT-qPCR	reverse transcription-quantitative PCR
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
WBE	wastewater-based epidemiology
WDOH	Wyoming Department of Health
WEF	Water Environment Federation
WEST	Water and Energy Sustainable Technology (at the University of Arizona)
WHO	World Health Organization
WPHL	Wyoming Public Health Laboratory
WRF	Water Research Foundation
WWTP	wastewater treatment plant

Executive Summary

Wastewater surveillance is a community-level approach for monitoring disease or chemical biomarkers that are excreted in human urine and feces and collected in sewers. Since early 2020, with the start of the coronavirus disease 2019 (COVID-19) pandemic, scientists and public health practitioners across the globe have been developing methods and implementing programs to track severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, in wastewater. Even though SARS-CoV-2 is a respiratory virus, wastewater surveillance can be used to track its spread since it can be shed in the feces of individuals who are symptomatic and asymptomatic (including pre-symptomatic). Monitoring SARS-CoV-2 levels in untreated wastewater relies on approaches and technologies that have been and continue to be rapidly deployed and evaluated by federal agencies, non-governmental organizations, states, wastewater utilities, universities, and industry. Despite the rapidly evolving science in this field, these entities were able to establish wastewater surveillance programs while developing sampling and analytical methods. The results of these programs provide useful information to assist communities in their public health response to the COVID-19 pandemic—highlighting the potential for wastewater monitoring to serve as a complementary approach to current and future infectious disease surveillance systems.

The United States Environmental Protection Agency (EPA) created this document to provide information to those who are interested in implementing wastewater surveillance programs to monitor SARS-CoV-2 or other pathogenic disease agents and chemical exposures in the future. To support that goal, this compendium documents the efforts of federal, state, local, and tribal agencies—as well as associations, universities, and the private sector—throughout 2020 and into early 2021 to explore federal and other funding sources, develop and implement wastewater surveillance for SARS-CoV-2, and provide information on how programs were implemented through case studies.

The report describes funding mechanisms from federal agencies, non-governmental organizations, private foundations, or funding by other means, such as reprogramming of existing funding, that were used to establish wastewater surveillance programs. The report also discusses how stakeholder collaboration through workshops, trainings, and other mechanisms allowed the research community to build upon established protocols for waterborne enteric viruses to advance wastewater surveillance for SARS-CoV-2. This collaboration was also critical for developing analytical methods for SARS-CoV-2 in wastewater, refining sampling collection procedures, and interpreting the wastewater testing results. Tied to the last point, this report documents how some programs communicated the results through online dashboards and then translated those results into public health responses.

Through its research conducted in early 2021, EPA identified 14 states with large-scale SARS-CoV-2 wastewater surveillance programs, along with 160 local communities or academic institutions conducting wastewater surveillance. While these groups developed and relied on different methods, their efforts were ultimately successful in detecting SARS-CoV-2 and initiating action to prevent the continued spread of COVID-19. From these programs, EPA selected 10 case studies that highlight different approaches for implementing programs and analyzing wastewater data to track the presence of SARS-CoV-2. These examples can inform how to establish and implement wastewater surveillance throughout the COVID-19 pandemic or for future pandemics and public health crises. For example, the case study programs highlighted that much of their success was dependent on effective collaboration with multiple partners, flexibility to adapt their programs as the science evolved, support from within their organization, transparent communication with stakeholders on how wastewater testing results could be used to help their communities respond to the pandemic, and adequate funding. These programs highlighted how wastewater surveillance can be an important and effective tool for early detection of

SARS-CoV-2, especially among disadvantaged or vulnerable populations where clinical testing may not be widely available.

1 Introduction

Wastewater surveillance is a community-level approach for monitoring chemical metabolites, bacteria, and viral pathogens that are excreted in human urine and feces and collected in sewers. It has been successfully used to detect various agents of diseases (e.g., poliovirus, hepatitis B, norovirus) in populations for decades and more recently to understand community-level drug use (e.g., opioids). A major advantage of wastewater-based methods is that they are not subject to the same reporting and recall biases that can occur when epidemiologic data are collected from individual community members or health care providers. For example, wastewater samples can be used to identify the true spectrum of drugs being consumed by a population rather than relying on individual self-reported information. In addition, wastewater-based methods can produce near real-time data that represent an entire community or smaller subsets of a community (e.g., at the sub-county level, at the individual facility or building level).

Beginning in early 2020, wastewater surveillance received renewed attention in light of the COVID-19 pandemic. Because individuals with symptomatic or asymptomatic infection can shed the SARS-CoV-2 virus in their feces, quantitative measures of SARS-CoV-2 in wastewater can provide useful information on changes in total COVID-19 infection in the community contributing to the wastewater (CDC, 2021a). Scientists and public health practitioners across the globe therefore quickly mobilized to develop methods and programs to track SARS-CoV-2 in wastewater. For example, researchers in the Netherlands began testing sewage for SARS-CoV-2 from six cities and the Schiphol airport in February 2020, before the Netherlands reported their first COVID-19 case. A month after the wastewater program began, the Netherlands detected low levels of the virus in sewage from several sites. Subsequent increases in sewage viral concentrations correlated with increases in reported COVID-19 prevalence in the community (Medema et al., 2020). Italian researchers also began testing for SARS-CoV-2 in wastewater in areas of high (e.g., Milan) and low (e.g., Rome) COVID-19 prevalence in February 2020, with first detections of SARS-CoV-2 in wastewater observed in late February (La Rosa et al., 2020). Studies conducted in the United States in March and April 2020 had similar success detecting the presence of SARS-CoV-2 in wastewater (e.g., Massachusetts) (Wu et al., 2020). Early efforts such as these highlighted the potential for sewage surveillance to serve as a complementary measure for monitoring COVID-19 spread in a community.

Federal, state, public health, and environment departments throughout the United States, as well as academic institutions, tribes, utilities, and others have developed wastewater monitoring programs for SARS-CoV-2 to monitor trends within a sewershed or at a targeted site (e.g., a facility or building). According to the Centers for Disease Control and Prevention (CDC), the virus has in some cases been detected in wastewater prior to reported cases in the community and trends in virus concentrations in wastewater have preceded trends in newly reported cases by multiple days (CDC, 2021a). These findings suggest that wastewater surveillance can serve as an early warning system of increased COVID-19 spread. These programs can also provide data at the smaller sewershed or facility level and in communities where timely COVID-19 individual testing is underutilized or unavailable. There are numerous documented instances where SARS-CoV-2 wastewater surveillance data have been

Wastewater Analysis for SARS-CoV-2

Multiple testing methods are used to quantify SARS-CoV-2 in wastewater. These laboratory tests typically quantify ribonucleic acid (RNA) —or the genetic signature of SARS-CoV-2 in wastewater. This means that wastewater surveillance programs for SARS-CoV-2 are not measuring the infectious virus directly, but instead are measuring viral RNA as an indicator of virus concentrations. Note that where this report refers to measurements of the virus in wastewater, the SARS-CoV-2 genetic material is what is being measured.

used to prioritize individual testing resources, inform other community mitigation strategies, and monitor effectiveness of interventions over the past year. For example, a state or local public health department may increase individual testing or ramp up public health messaging and outreach following a rise in SARS-CoV-2 measurements in wastewater.

For most community-level wastewater surveillance programs, samples are typically collected at the influent of a wastewater treatment plant (WWTP). Other smaller scale programs sample at locations throughout the collection system, including at lift stations or from manholes that carry sewage from individual buildings. Wastewater samples are then analyzed for the presence of SARS-CoV-2 using a nucleic acid–based reverse transcription polymerase chain reaction (RT-PCR) assay for gene markers that are unique to the virus. Results provide insight on COVID-19 among the population served by the utility's collection system and/or subsections within a community.

It is important to note that wastewater surveillance for SARS-CoV-2 is a developing field and researchers are still learning about the dynamics of viral shedding in feces and viral persistence in wastewater. More data on the prevalence and concentrations of SARS-CoV-2 shed in the feces of infected individuals are needed to better understand the relationship between SARS-CoV-2 concentrations in wastewater and the number of individuals infected with COVID-19. Furthermore, low levels of infection in a community may not be captured by sewage surveillance (CDC, 2021a). Other complexities that arise when interpreting wastewater data include the mobility of the population contributing to the wastewater, industrial wastewater contribution, stormwater, other factors (e.g., cleaning, dilution), and variability in wastewater flow and fecal load. It is also important to note that community-level monitoring at a WWTP does not capture residences and businesses on a septic-based system or facilities that have their own system (e.g., correctional facilities); however, some separate wastewater surveillance efforts are also monitoring community septic tanks.

Because of these limitations, SARS-CoV-2 wastewater data are often considered in tandem with other COVID-19-related data to inform public health. Data from wastewater surveillance programs are not meant to replace other COVID-19 surveillance systems, but rather to offer complementary data.

2 Purpose

EPA compiled this report to capture some of the notable and unique efforts to develop and use wastewater surveillance to detect and monitor the SARS-CoV-2 virus genetic material in untreated wastewater throughout 2020 and into early 2021. As a result, this report does not include advancements in wastewater surveillance that may have occurred after February 2021. The report includes details on developing and establishing wastewater monitoring programs throughout the United States and summarizes details such as funding mechanisms, sampling approaches used, sample analysis methods development, data interpretation, and public health responses. The report also describes the robust collaboration between organizations to share knowledge and optimize the wastewater surveillance process that occurred during this period.

Through discussions with some key programs, EPA compiled lessons learned for the development and implementation of wastewater surveillance programs, in the face of rapidly evolving science and a global pandemic. These examples and the many success stories can inform wastewater surveillance efforts for future pandemics and public health crises.

This report documents SARS-CoV-2 wastewater surveillance efforts throughout the country by federal agencies, non-governmental stakeholders, state agencies, tribal agencies, local agencies, utilities, academic institutions, and private entities. The report begins by describing how EPA gathered information (Section 3) and then summarizes funding mechanisms that have provided financial support to researchers and other groups implementing wastewater surveillance (Section 4). Next, the document provides examples for how entities worked collaboratively to advance wastewater surveillance practices (Section 5.1), develop and research analytical methods (Section 5.2), provide ongoing support for wastewater surveillance strategies (Section 5.3), serve rural and underserved populations (Section 5.4), consider ethical and legal concerns (Section 5.5), and develop resources to protect wastewater utility workers (Section 5.6). The report also provides an overview of some of the SARS-CoV-2 wastewater surveillance programs that were established by various entities (Section 6.1) and presents 10 case studies, which offer unique examples of program leadership, collaboration, funding, sampling design, data presentation, and data interpretation (Section 6.2), with a summary of lessons learned from wastewater surveillance programs (Section 7). Appendix A includes two tables summarizing SARS-CoV-2 wastewater surveillance programs for states (Table A-1) and municipalities and universities (Table A-2), based on a review of publicly available information in January 2021.

This report is not intended to serve as a comprehensive summary of all wastewater surveillance efforts for SARS-CoV-2 in the U.S. or as a guidance/framework document. Rather, it documents and provides examples and perspective on the wide variety of activities conducted by diverse entities to develop and implement ways to use wastewater surveillance to complement existing public health measures for the COVID-19 pandemic.

It is important to note that this report and EPA do not endorse or make any judgement or provide guidance on a specific model for wastewater surveillance programs. This report is intended to provide information, lessons learned, and examples to assist communities in designing, developing, and implementing these programs as needed.

3 Report Development Approach

To summarize programs providing financial support and methods development research/guidance for wastewater surveillance of SARs-CoV-2 over the past year (see Sections 4 and 5), EPA searched for and reviewed federal agency, non-governmental organization, and other stakeholder (e.g., professional associations) websites with a focus on wastewater and public health. EPA also relied on information from press releases, publicly available grant/contracting documents, webinars/virtual conference presentations, research project updates from funding agencies, and news articles. Except for the 10 case studies presented in Section 6.2, EPA did not meet with the federal agencies, non-governmental organizations, or other stakeholders described throughout this report to collect additional information on their wastewater surveillance programs. Additionally, EPA did not conduct a comprehensive literature review in support of this report.

As part of this report, EPA also compiled details on existing wastewater surveillance programs in the United States using publicly available information (see Section 6 and Appendix A). EPA's goal in this effort was to identify and understand the breadth of state, local (e.g., city, county), tribal, and university wastewater surveillance programs. To gather this information, EPA conducted keyword searches in January 2021 using various combinations of wastewater, COVID-19, and program-specific search terms presented in Table 1 and an internet search engine. For example, a combination of "wastewater," "monitoring," "COVID-19," and "university" keywords used to identify surveillance efforts on college/university campuses.

Table 1. List of search terms used to identify wastewater surveillance programs.

Wastewater Search Terms	COVID-19 Search Terms	Program-Specific Terms
Wastewater	COVID-19	University
Sewage	COVID	College
Manhole	SARS-CoV-2	School
WWTP	Outbreak	City
Surveillance		County
Monitoring		Local
Dashboard		State
Wastewater Testing		State-wide
		Tribe
		Territories

EPA also reviewed websites of known collaborative initiatives (e.g., the COVID-19 Wastewater Based Epidemiology [WBE] Collaborative [COVID-19 WBE Collaborative, 2021]) to identify additional surveillance programs. EPA also reviewed the COVIDPoops19 website (UC Merced, 2021), which compiles wastewater surveillance programs for SARS-CoV-2 across the globe, and a Slack workspace website for informal communication regarding SARS-CoV-2 wastewater surveillance (see additional discussion in Section 5.1).

Once the wastewater surveillance programs were identified, EPA compiled information on the programs from program websites and dashboards, news articles and press releases, program summaries and reports, and more. EPA searched for and reviewed information across all state wastewater surveillance programs. However, EPA's ability to summarize information about all the local, tribal, and university programs identified was limited given the number of such programs identified (more than 150). For all of the state programs and a subset of the local and university programs, EPA recorded publicly available details, including:

- Program location (e.g., city, state).
- Organization leading the program.
- Partners or collaborating organizations.
- Wastewater surveillance program website link.
- Sampling start date and end date, if applicable.
- Summary of sampling locations (e.g., WWTP influent, manholes at correctional facilities or dormitories).
- Number of sampling locations.
- Analytical methods (e.g., reverse transcription-quantitative PCR [RT-qPCR]).
- Wastewater testing results presentation (e.g., tables, graphs, normalization, units).
- Public health actions/outcomes as available.

EPA then reviewed the identified state, local, and university-led programs to select a diverse subset with unique aspects to highlight as case studies. EPA selected programs that demonstrate the wide variety of ways that programs were financed (e.g., self-funded versus using Coronavirus Aid, Relief, and Economic Security [CARES] Act funding) and implemented (e.g., sampling locations, analytical methods), as well as how the programs interpreted the wastewater results (e.g., compared to individual cases, normalized by wastewater flow rate or population size) and the public health measures the programs took based on the wastewater results (e.g., individual testing, public education and outreach). Once EPA identified the programs for case studies, EPA met with the practitioners and researchers leading each program in order to gather additional information beyond what was found in the search of publicly available sources listed above. EPA used this information to develop the case study summaries presented in Section 6.2.

4 Financial Support

As more information became available on the ability to detect SARS-CoV-2 in untreated wastewater, federal agencies, non-governmental organizations, and various stakeholders quickly recognized the need to develop and support wastewater surveillance programs.

On March 27, 2020, the federal government enacted the CARES Act to address the health, economic, and societal impacts of the COVID-19 pandemic. Federal agencies distributed CARES Act funds through grants, contracts, and other mechanisms to interested researchers and other groups. The CARES Act funded many different types of programs (e.g., individual testing, unemployment relief), including funds to support wastewater surveillance programs. Some federal agencies, non-governmental organizations, and other stakeholders also provided financial support from their non-CARES Act annual funding to support wastewater surveillance programs.

Early in the COVID-19 response, numerous federal agencies and non-governmental stakeholders initiated independent programs tied to wastewater surveillance, including providing funding to support the COVID-19 response. To coordinate multiple initiatiatives across interested groups, the U.S. Department of Health and Human Services (HHS) and CDC created the National Sewage Surveillance Interagency Leadership (NSSIL) Committee in June 2020. Since late July of 2020, the NSSIL Committee has held monthly meetings "to exchange information and discuss federal agency-specific missions, roles, activities, and stakeholder engagement related to wastewater surveillance of SARS-CoV-2" (CDC, 2020a). The NSSIL Committee includes involvement from the following federal agencies:

- HHS
- CDC
- EPA
- Department of Homeland Security (DHS)
- Department of Defense
- United States Geological Survey
- National Institutes of Health (NIH)
- National Science Foundation (NSF)
- Department of Veterans Affairs

The NSSIL Committee also includes the following non-governmental stakeholders:

- Association of Public Health Laboratories (APHL)
- Association of State and Territorial Health Officials (ASTHO)
- Council of State and Territorial Epidemiologists
- National Association of County and City Health Officials (NACCHO)
- National Environmental Health Association (NEHA)
- Water Environment Federation (WEF)
- WRF

The NSSIL Committee originally included three federal interagency workgroups: Implementation and Planning Workgroup, Science and Technology Evaluation for Practice Research, and Wastewater Testing Surge Capacity (CDC, 2020a). The Implementation and Planning Workgroup develops and implements the National Wastewater Surveillance System (NWSS), sewage sampling and testing capacity, and guidance documents for sewage sampling, testing, and data interpretation for public health action. The NWSS links to a real-time, public health data platform known as DCIPHER (Data Collation and Integration for Public Health Event Response) developed by CDC and designed to store, analyze, and display public health data, including SARS-CoV-2 wastewater data collected throughout the country (CDC, 2021a). See Section 5.3 for more information on CDC's NWSS. The Science and Technology Evaluation for Practice Workgroup coordinates the exchange of information on wastewater sampling, testing, and data interpretation throughout federal agencies. This workgroup also coordinates forums to connect and inform the public and partner organizations (CDC, 2020a). The Wastewater Testing Surge Capacity workgroup was originally designed to utilize federal laboratories to provide timely as-needed wastewater analyses (CDC, 2020a). However, NSSIL decided this workgroup was no longer necessary because of the rapid increase in wastewater analytical support from commercial and state laboratories (CDC, 2021b).

Federal agencies and non-governmental stakeholders of the NSSIL prioritized making resources available for SARS-CoV-2 wastewater surveillance programs that would support public health efforts to decrease the spread of the virus. One identified resource was financial support for COVID-19-related wastewater surveillance research, development, and implementation.

This section summarizes some of the funding mechanisms provided by federal agencies, non-governmental organizations, and other stakeholders; it is not an exhaustive list of all funding provided to wastewater surveillance programs. There are many wastewater surveillance programs that used or are using funding from other sources (e.g., private foundations, reallocations of existing funding). See the case studies in Section 6.2 for several detailed examples of how specific entities funded their wastewater surveillance programs.

4.1 National Science Foundation

On April 3, 2020, NSF announced a request for proposals with a focus on "non-medical, non-clinical-care research" to better understand the spread of COVID-19, educate on the science of virus transmission and prevention, and develop processes to address the pandemic. NSF used their Rapid Response Research (RAPID) funding mechanism, which allows the agency to quickly review proposals and award grants for projects that have a critical urgency with regard to identified circumstances (NSF, 2020a). Traditional grant programs often take months between an agency's releases of a request for proposal and project funding, which can be ineffective during emergencies such as the COVID-19 pandemic (NSF, 2020b). NSF's RAPID grant proposal requirements are also more streamlined than traditional grant programs, requiring no more than five pages explaining why the proposed research is urgent and why RAPID is the most appropriate grant program (NSF, 2020c). NSF's announcement indicated up to $200,000 in funding for projects conducted over a period of up to one year. However, NSF noted that they would consider projects of a longer duration with sufficient justification (NSF, 2020a).

NSF received $76 million from the CARES Act, with $75 million allocated for grants and $1 million for the administration of those grants (NSF, 2020d). As of January 2021, NSF granted a total of 1,229 awards for COVID-19 projects using the CARES Act funding and $208 million from their fiscal year 2020 budget (NSF, 2020e). As of April 2021, 16 of these grants supported research on SARS-CoV-2 in wastewater, as shown in Table 2 (NSF, 2020f).

Table 2. NSF-funded research for wastewater surveillance.

Project Title	Award Number	Description
Collaborative Research: RAPID: Wastewater Informed Epidemiological Monitoring for SARS-CoV-2	2027752 and 2027758	University of Notre Dame and Georgia Tech to develop methods to monitor SARS-CoV-2 in wastewater and connect those measurements with epidemiological data to model outbreak dynamics and estimate the overall prevalence of the virus in communities over time. The issued project budget was $153,000 to Notre Dame and $45,503 to Georgia Tech (NSF, 2020g; NSF, 2020h).
Collaborative Research: RAPID: Coronavirus Persistence, Transmission, and Circulation in the Environment	2023057 and 2022877	University of Michigan and Stanford University to monitor SARS-CoV-2 in WWTPs in the San Francisco Bay Area to determine whether WWTP monitoring can be used to catch outbreaks early. This is part of a larger project funded with $68,591 issued to University of Michigan and $130,000 issued to Stanford University (NSF, 2020i; NSF, 2020j).
RAPID: Tracking the Coronavirus in Municipal Wastewater	2027679	Oregon State University to analyze SARS-CoV-2 from samples collected at WWTPs and sewer access points throughout Oregon to identify infected communities, determine virus levels within each community, and recognize possible underlying contributing factors of the continued spread of the virus. The total project budget was issued at $100,000 (NSF, 2020k).
RAPID: Tribal Capacity to Evaluate COVID-19 using Wastewater-based Epidemiology	2038372	Arizona State University to investigate the feasibility of using SARS-CoV-2 wastewater surveillance to monitor COVID-19 in tribal communities and to develop culturally appropriate research training and educational materials for wastewater utility operators, health professionals, and tribal leaders. The total project budget was issued as $200,000 (NSF, 2020l).
RAPID: COVID-19's Impact on the Urban Environment, Behavior, and Wellbeing	2028564	Arizona State University to assess how public health interventions in response to the COVID-19 pandemic impact the environment, human behavior, and wellbeing of the public. Researchers will analyze urban wastewater samples collected daily in Tempe, Arizona, in an effort to characterize various biomarkers of environmental health and wellbeing (e.g., air pollutants, medications, allergy suppressants, stimulants and depressants, drugs of abuse, dietary markers). The project budget is $199,998 (NSF, 2020m).
RAPID: Viral Structure-Function-activity in the Engineered Wastewater Cycle	2026599	Columbia University to research the fate of SARS-CoV-2 and other viruses during WWTP processes, with a total funding of $198,388 (NSF, 2020n).

Table 2. NSF-funded research for wastewater surveillance.

Project Title	Award Number	Description
RAPID: Monitoring for SARS-CoV-2 to Elucidate Infection Dynamics Across Major Metropolitan Areas of the U.S.	2029025	North Carolina State University, the University of Southern California, Rice University, and Howard University to monitor SARS-CoV-2 in wastewater in four cities, one each in California, North Carolina, Texas, and Washington, D.C. to address knowledge gaps in the use of wastewater surveillance as a public health monitoring tool for a variety of communities. The project budget was issued as $200,000 (NSF, 2020o). See additional details in the "Monitoring SARS-CoV-2 in Major Metropolitan Areas of the U.S." call-out box below.
RAPID: Determine Community Disease Burden of COVID-19 by Probing Wastewater Microbiome	2027059	University of Hawaii to develop a highly efficient concentration and detection method for enveloped viruses (like SARS-CoV-2) in wastewater and to collect time-sensitive wastewater samples from communities impacted by the disease to determine the abundance, diversity, and temporal dynamics of SARS-CoV-2 and other enveloped viruses. The total project budget was $151,956 (NSF, 2020p).
RAPID: Determination of Health Risks and Status from SARS-CoV-2 Presence in Urban Water Cycle	2029515	University of Utah to develop efficient techniques to extract and monitor SARS-CoV-2 in wastewater and to understand human health risks associated with the presence of SARS-CoV-2 in influent and treated effluent at WWTPs. Total funding issued for this project was $123,706 (NSF, 2020q).
Research Coordination Network for Wastewater Surveillance of SARS-CoV-2	2038087	University of Notre Dame, Howard University, Stanford University, and Arizona State University to create a Research Coordination Network to connect researchers from across the country for quick and efficient knowledge transfer on SARS-CoV-2 wastewater surveillance with a project budget of $299,995 (NSF, 2020r). See additional details in the "Creating the Research Coordination Network for SARS-CoV-2" call-out box below.
RAPID: Identifying Geographic and Demographic Drivers of Rural Disease Transmission for Improved Modeling and Decision Making	2029866	University of North Carolina to examine drivers of disease transmission in rural areas and explore differences with urban areas, with the ultimate goal of improving pandemic management in rural areas. Researchers will use data (e.g., health surveillance data, cellphone-based mobility data, land use features, commuting patterns) from three rural and three urban counties in North Carolina to develop a susceptible-exposed-infected-recovered model. They will also collect wastewater samples to quantify the prevalence of SARS-CoV-2 and to provide a complementary non-clinical metric to validate their model. Total funding issued for this project was $135,593 (NSF, 2020s).
SBIR Phase I: Automated In-Situ High-Resolution COVID-19 Wastewater-Based Epidemiology	2041400	FLUIDION US, Inc. to develop an optimized RT-qPCR approach for in-situ sampling and analysis of different viruses (including SARS-CoV-2, its variants, and other emerging viruses) and an instrumentation platform that is applicable to the early detection of viruses. As part of this work, researchers will explore the latest advances in molecular biology protocols, such as an extraction-free single step RT-qPCR. Total funding issued for this project was $256,000 (NSF, 2020t).

Table 2. NSF-funded research for wastewater surveillance.

Project Title	Award Number	Description
RAPID COVID-19 DCL response: Wastewater Pathogen Tracking Dashboard	2033137	Battelle Memorial Institute to evaluate wastewater data from four locations to determine the prevalence of SARS-CoV-2 and other viral pathogens, as well as to detect and quantify viral mutations through the use of gene sequencing. Additionally, researchers will develop a predictive risk model to identify neighborhoods to initiate contact tracing due to high SARS-CoV-2 in the wastewater relative to the number of confirmed COVID-19 cases. Total funding issued for this project was $197,375 (NSF, 2020u).
RAPID-REU Site: Mitigating the Impact of COVID-19 Pandemic on Undergraduate Research Training in the Biosciences	2034045	Montana State University to quantify SARS-CoV-2 in wastewater from a Montana WWTP using a CDC test kit protocol as an indicator of community spread of the virus for use by public health officials. This is just one of several projects covered by the $75,042 project budget, all of which will engage underrepresented minority students in research through projects that can be conducted remotely (NSF, 2020v).

Monitoring SARS-CoV-2 in Major Metropolitan Areas of the U.S.

In May 2020, NSF awarded a $200,000 RAPID grant (award number 2029025) to a collaboration between North Carolina State University, the University of Southern California, Rice University, and Howard University to create SARS-CoV-2 wastewater surveillance programs in four major metropolitan areas with varying sewershed sizes/populations, infection rates, and required COVID-19 prevention strategies (e.g., mask mandates). Each university received 25 percent of the NSF grant to purchase analytical equipment and materials and to collectively develop an analytical method for SARS-CoV-2 in wastewater. Each university was responsible for optimizing a portion of the analytical method (e.g., RNA extraction, sample storage, virus concentration) using samples taken from their respective cities (USC, 2021). The program collected samples from WWTPs in Los Angeles, California; the District of Columbia; Raleigh, North Carolina; and Houston, Texas, to address knowledge gaps in the use of wastewater surveillance as a public health monitoring tool. Researchers acknowledged that the largest hurdles with the project were deciding between analytical approaches, considering how much virus each method recovers, the associated expenses for the supplies, and the required duration of the methods. Additionally, the project team initially had to overcome supply and equipment shortages due to the pandemic, limiting their ability explore certain analytical methods (NSF, 2020o; Shah, 2020; USC, 2021).

Creating the Research Coordination Network for SARS-CoV-2

In July 2020, NSF provided nearly $300,000 to researchers collaborating from the University of Notre Dame, Howard University, Stanford University, and Arizona State University to create a Research Coordination Network to connect interested research groups studying SARS-CoV-2 in wastewater across the United States (award number 2038087). The Research Coordination Network addresses knowledge gaps in the development of wastewater analysis methods by initiating virtual activities such as conferences, workshops, training videos, and seminars. Additionally, the researchers support sharing knowledge globally by connecting with international wastewater surveillance networks and efforts. The goal of the Research Coordination Network is to exchange of knowledge in an effort to accelerate program optimization and facilitate the ongoing development of wastewater surveillance (NSF, 2020r).

4.2 Centers for Disease Control and Prevention

In place since 1995, CDC's Epidemiology and Laboratory Capacity for Prevention and Control of Emerging Infectious Diseases (ELC) Cooperative Agreement has provided funding to "all 50 states, several large health departments, and U.S. territories, and affiliates to detect, respond to, control, and prevent infectious diseases" (CDC, 2021b). On April 23, 2020, HHS announced that CDC[1] received $631 million in CARES Act funding to support state and local COVID-19 response efforts for the 64 health department recipients (i.e., 50 states, six large cities, and eight territories[2]) already supported in the current five-year funding period (CDC, 2020c). This funding was intended to support "key activities related to COVID-19 in the areas of epidemiology, laboratory, and informatics" (CDC, 2020c). None of this initial CARES Act funding was used to support wastewater surveillance programs (CDC, 2021c). On May 18, 2020, CDC announced that it would distribute an additional $10.25 billion in CARES Act

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[1] While CDC is an agency within HHS, CDC distributed the CARES Act funding independently through existing CDC financial mechanisms.

[2] The six large cities are: Chicago, IL; Houston, TX; Los Angeles County, CA; New York City, NY; Philadelphia, PA; and Washington, D.C. The eight territories are: American Samoa, Federated States of Micronesia, Guam, Mariana Islands, Marshall Islands, Palau, Puerto Rico, and U.S. Virgin Islands (CDC, 2021c).

funding to states, territories, and local jurisdictions through the ELC cooperative agreements (CDC, 2020d). Six states and two local jurisdictions allocated some of this funding to support wastewater surveillance efforts (CDC, 2021c). In September 2020, CDC distributed a supplemental ELC award totaling $2.5 million to eight states[3] to establish the early implementer network for NWSS. This initial funding was used to support public health department capacity to coordinate sample collection and testing, along with data submission to NWSS (CDC, 2021c).

In January 2021, ELC awarded an additional $19 billion in 2021 Coronavirus Response and Relief Supplemental Appropriations Act funding to support an expanded COVID-19 response, which could include further development, implementation, and expansion of wastewater surveillance programs (CDC, 2021d). Thirty states, one city, and two territories opted to allocate a total of $203 million to wastewater surveillance efforts (CDC, 2021c). This included support to Ohio and Houston, Texas (see case studies for Ohio in Section 6.2.4 and Houston, Texas, in Section 6.2.7). As of August 2021, 43 jurisdictions are using CDC-distributed funds to support wastewater surveillance, including 37 state health departments, four local health departments (Chicago, IL; Houston, TX; Philadelphia, PA; and Washington, D.C.), and health departments from two U.S. territories (see Figure 1) (CDC, 2021g).

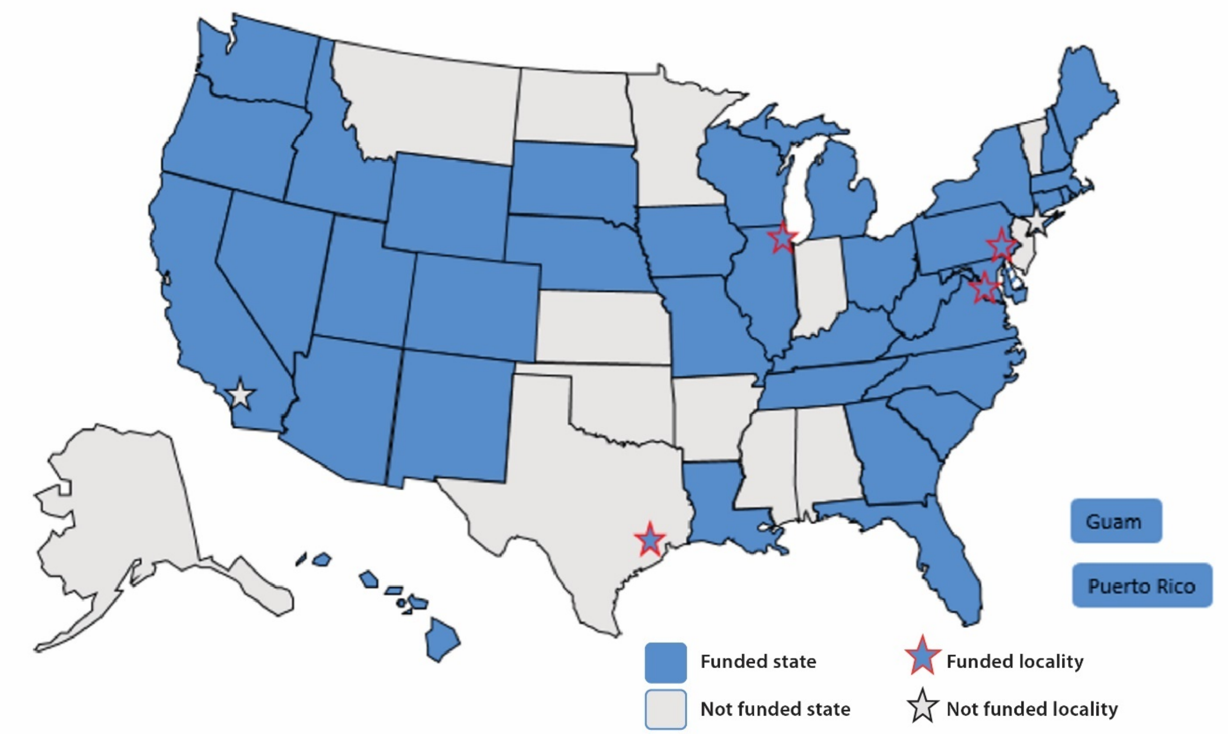

Figure 1. Jurisdictions using CDC funds to support wastewater surveillance for SARS-CoV-2 as of August 2021 (CDC, 2021g).

4.3 National Institutes of Health

NIH[4] launched the Rapid Acceleration of Diagnostics (RADx[SM]) initiative on April 29, 2020, to "speed innovation in the development, commercialization, and implementation of technologies for COVID-19

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[3] The eight states are: California, North Carolina, Ohio, South Carolina, Utah, Virginia, Washington, and Wisconsin (CDC, 2021c).

[4] While NIH is an agency within HHS, NIH established the RADx[SM] initiative independently from HHS.

testing" (NIH, 2021a). The initiative has four programs, each with a unique focus area and budget to fund projects (NIH, 2020a):

1. RADxSM Tech
2. RADxSM Advanced Technology Platforms
3. RADxSM Underserved Populations
4. RADx^{SM} Radical

The RADx^{SM} Radical (RADx-rad) supports new nontraditional approaches to address gaps in COVID-19 testing, including wastewater analysis to identify the virus and measure the spread of infection, particularly among high-risk populations (NIH, 2020a). RADx-rad has a total budget of $200 million, with grants and supplements supported by 11 NIH institutes and centers (NIH, 2020b; NIH, 2020c).

In August 2020, as part of the RADx-rad program, NIH issued a series of funding opportunities for wastewater surveillance through notices of special interest and emergency awards for researchers to apply for project funding. NIH issues notices of special interest to support high-priority and high-opportunity areas of science. NIH uses emergency awards to provide expedited funding in response to public health emergencies such as the one declared for COVID-19. NIH announced emergency awards to support wastewater detection of SARS-CoV-2, with $19 million available to fund five to ten wastewater surveillance initiatives (NIH, 2020d). In December 2020, NIH issued seven awards to support wastewater surveillance and one award to support data coordination across grantees (NIH, 2021b). All listed wastewater surveillance projects have a start date of January 2021 and are projected to end in December 2022; the data coordination effort is expected to continue through November 2024. A brief summary of the awards related to wastewater surveillance is provided in Table 3 and a summary of the data coordination center is presented in the following call-out box.

Table 3. NIH wastewater surveillance projects funded under RADx-rad.

Project Title	Project Number	Description
Development and Proof-of-Concept Implementation of the South Florida Miami RADx-rad SARS-CoV-2 Wastewater-Based Surveillance Infrastructure	1U01DA053941-01	The University of Miami (partnering with Weill Cornell Graduate School of Medical Sciences) to develop data standards and infrastructure for wastewater surveillance program data, optimize wastewater surveillance sampling and analytical methods, and integrate the results with public health data to develop predictive models for community spread utilizing total project funding of $2.7 million (NIH, 2021c).
Wastewater Analysis of SARS-CoV-2 in Tribal Communities	1U01DA053976-01	Arizona State University to develop a WBE Tribal Coordination Center and measure SARS-CoV-2 in wastewater across U.S. reservations, with a project budget of $1.5 million. Another goal of this project is to "show that WBE is a non-invasive, culturally appropriate biomonitoring strategy that can be adopted and implemented by tribal communities" (NIH, 2021d).
Improved Scalability, Sensitivity, and Interpretability of Pathogen Detection, Including SARS-CoV-2, in Wastewater Using High-throughput, Highly Multiplexed Digital Array PCR Technology	1U01DA053899-01	University of North Carolina Chapel Hill to explore limitations of molecular technologies like RT-qPCR to quantify SARS-CoV-2 (e.g., lack of streamlined pre-analytical processing steps) and to demonstrate other comprehensive and low-cost approaches that could be used to rapidly address novel pathogen threats in the future, with a project budget of $1 million (NIH, 2021e).
Wastewater Assessment for Coronavirus in Kentucky: Implementing Enhanced Surveillance Technology	1U01DA053903-01	University of Kentucky to develop analytical methods for rapid quantification in the field (i.e., "create a sensitive, robust, and field-friendly platform for testing wastewater for SARS-CoV-2 RNA" rather than a laboratory setting), to validate the approach with side-by-side comparisons to conventional wastewater surveillance, and then establish wastewater surveillance programs by training WWTP operators to use these methods in remote areas of Appalachian Kentucky. The issued project budget was $1.8 million (NIH, 2021f).
Wastewater Detection of COVID-19	1U01DA053893-01	Missouri State Department of Health and Senior Services to support wastewater surveillance at congregate facilities and to use these data to help estimate the per patient contribution and longevity of SARS-CoV-2 RNA in wastewater. This will be done by increasing the number of facilities sampled, adjusting the sampling frequency, and comparing the wastewater results to individual testing. Missouri is also using these funds to evaluate and define factors that contribute to SARS-CoV-2 signal suppression in wastewater, with a total funding of $2 million (NIH, 2021g).

Table 3. NIH wastewater surveillance projects funded under RADx-rad.

Project Title	Project Number	Description
Optimizing SARS-CoV-2 Wastewater-based Surveillance in Urban and University Campus Settings	1U01DA053949-01	Columbia University Health Sciences to optimize wastewater surveillance conducted at a diverse, urban university (including dorms, research buildings, and medical facilities) and the surrounding sewersheds and WWTPs. Researchers will also model case counts using normalized wastewater data. The team was awarded $2.5 million for this effort (NIH, 2021h).
Bioinformatics Framework for Wastewater-based Surveillance of Infectious Diseases	301LM013129-02S2	Arizona State University (Tempe campus) to develop and implement a near real-time wastewater surveillance bioinformatics framework for SARS-CoV-2 at the national, city, and intra-sewershed or neighborhood level, and to translate SARs-CoV-2 data from RT-qPCR analysis and high-throughput sequencing into information for monitoring population health. The goal of this work is to "lead to a better understanding of how WBE can support population-level monitoring of SARS-CoV-2 and similar infectious disease threats" (NIH, 2021i). As part of this work, researchers will also incorporate findings into and expand the online dashboard Arizona State University developed in collaboration with Tempe, Arizona (see case study in Section 6.2.8). The project was funded at $571,000 in funding (NIH, 2021i).

Establishing a RADx-rad Data Coordination Center

On August 6, 2020, NIH released an emergency award under RADx-rad to fund the development of a RADx-rad Data Coordination Center to provide support to RADx-rad awardees with administration operations and logistics; data collection, integration, and sharing; and data management and use. Beyond connecting RADx-rad programs, the Data Coordination Center also provides data to the NIH-based data center that supports other NIH initiatives (NIH, 2020e).

On December 21, 2020, NIH awarded the University of California San Diego and the University of Texas Health Science Center at Houston $5,954,423 in funding from the Paycheck Protection Program and Health Care Enhancement Act of 2020 for initial development of the Data Coordination Center in 2021 (project number 1U24LM013755-01) (NIH, 2020e; NIH 2021j). The project team designed the RADx-rad Discoveries and Data Coordination Center to include three main functions (NIH, 2021k):

1. Administration Core: To focus on logistics and communications for the Data Coordination Center and facilitate data sharing and data use agreements to collect and distribute data generated by the RADx-rad awardees.
2. Data Core: To develop tools and approaches to assist the RADx-rad awardees with data collection, harmonization, and sharing (e.g., common metadata requirements, mapping standards, data hosting).
3. Discovery and Diagnostic Core: To support RADx-rad awardees in ensuring the data they collect is usable for the Data Coordination Center by providing expert consultation, developing best practices, and more.

The Data Coordination Center includes information on all NIH RADx-rad awardees, a list of resources for the awardees that includes topics such as data flow and guidance for common data elements, and training and networking events for researchers (NIH, 2021k).

4.4 U.S. Department of Health and Human Services

In November 2020, HHS (with support from CDC) awarded a competitive contract of $1.55 million in CARES Act funding to AquaVitas, a commercial wastewater testing and analytics company, to complete a six-week wastewater testing pilot study of WWTPs serving about 10 percent of the U.S. population for a period of six weeks (CDC, 2020b; FPDS, 2021). The results from this pilot test were provided to state and local public health agencies and incorporated into CDC's NWSS (CDC, 2020b).

In February 2021, HHS issued a follow-on request for proposals to collect wastewater samples, analyze samples, and transfer results from at least 320 WWTP throughout the United States, including tribes and territories (the total representing approximately 30 percent of the U.S. population), into CDC's NWSS (CDC, 2020b; HHS, 2021). Biobot Analytics, a private company based out of Boston, Massachusetts, was awarded the contract on May 24, 2021, with sample collection scheduled anticipated to begin on June 14, 2021, and anticipated run for a period of nine weeks (Biobot, 2021a; Biobot, 2021b). Under this contract, Biobot Analytics will expand on the previous HHS-led wastewater epidemiology program, while incorporating genomic sequencing in an effort to track COVID-19 variants (Biobot, 2021a).

4.5 Water Research Foundation

WRF initiated and funded multiple research projects to advance wastewater surveillance for SARs-CoV-2 based on the research needs identified at their first International Water Research Summit in April 2020 (WRF, 2020a) (see additional details in Section 5.1). Three research areas were identified for additional funding and research: (1) interlaboratory and methods assessment, (2) stability of SARS-CoV-

2 genetic signal in wastewater, and (3) impact of storage and pre-treatment methods on signal strength (WRF, 2020b). Funds were awarded to address the first two topic areas to research teams following a competitive contract process.

In June 2020, WRF published a request for qualifications to identify a research team to evaluate existing laboratory methods for sensitivity and precision to detect the SARS-CoV-2 in untreated wastewater (WRF, 2020c). In July 2020, WRF named Trussell Technologies as the recipient of $200,000 to lead the "Interlaboratory and Methods Assessment of the SARS-CoV-2 Genetic Signal in Wastewater" study (WRF, 2020d). The work started in early Fall 2020 and involved collecting composite wastewater samples tested by the research team's laboratory and submitted to other participating laboratories for analysis using their own methods. WRF funded the project using internal funds as well as supplemental funding from the Bill and Melinda Gates Foundation (WRF, 2020e).

In August 2020, WRF announced another request for qualifications to find a research team that could identify ways to optimize SARS-CoV-2 sewage sampling designs in order to quickly detect COVID-19 spread through communities contributing to sewersheds of various sizes (WRF, 2020f). In October 2020, WRF named the University of California at Irvine as the recipient of $300,000 for a study titled "Understanding the Factors that Affect the Detection and Variability of SARS-CoV-2 in Wastewater" (WRF, 2020g). Using wastewater samples (sewage and septage) collected in Los Angeles County and analyzed for SARS-CoV-2, the research team is developing recommendations for optimal sample design at three different scales: large urban sewersheds, medium-sized regional sewersheds, and small regional systems (WRF, 2020h). Funding for this project was provided by WRF as well as the Bill and Melinda Gates Foundation, and work is anticipated to be completed in 2021 (WRF, 2020h).

WRF has also funded at least one other study related to wastewater monitoring for SARS-CoV-2. In September of 2019, WRF awarded $295,000 to the University of Notre Dame to evaluate the persistence and disinfection of Lassa virus in WWTPs (WRF, 2020i). The scope was later expanded to include SARS-CoV-2. The goal of this study is to develop a user-friendly model capable of estimating environmental releases and worker exposures to these two viruses to help prepare the wastewater industry for the next epidemic or pandemic. Work is anticipated to be completed in 2022 (WRF, 2020i).

4.6 Water Environment Federation

WEF has entered into a cooperative agreement with CDC to support CDC in providing information on SARS-CoV-2 guidance to the water and wastewater utility sector, which comprises the majority of WEF's membership. WEF is developing a network for information sharing within the sector. The Network for Wastewater-based Epidemiology will host the utility community of practice for wastewater surveillance (WEF, 2021b). In addition, WEF will provide training on wastewater surveillance to utilities and public health personnel (WEF, 2021a). WEF is also working with CDC to evaluate use of wastewater surveillance in correctional facilities as a supplement to case surveillance data and as a possible early warning for COVID-19 in these facilities. For this effort, WEF will be working with multiple states, the first of which is Oklahoma (WEF, 2021c).

4.7 Other Funding Opportunities

Beyond funding opportunities through federal agencies and non-governmental organizations, wastewater surveillance programs are utilizing funds from private foundations, existing program funding, and pro-bono resources. Two examples of private foundations funding wastewater surveillance programs or research on SARS-CoV-2 analytical methods include:

- The Bill and Melinda Gates Foundation, which provided almost $390,000 to the California Institute of Technology to develop a rapid quantification method for detecting SARS-CoV-2 in wastewater (BMGF, 2021).
- The Foundation for a Healthy Kentucky, which provided funding to pay for the equipment needed to analyze wastewater samples collected from the Mayfield WWTP for SARS-CoV-2, as part of a larger $60,000 project. The project team includes Graves County Health Department, Mayfield Electric and Water Systems, Murray State University, and the University of Louisville (Healthy KY, 2020).

Some wastewater surveillance programs were supported by funds from other existing programs. For example, Hampton Roads Sanitation District (HRSD) used available funds from ratepayers to conduct SARS-CoV-2 wastewater sampling and analysis (see case study in Section 6.2.6 for additional details) (HRSD, 2021b). Wyoming also reprogrammed existing funding they received from CDC's ELC to support their wastewater surveillance program (see case study in Section 6.2.5) (Wyoming, 2021).

An example of pro-bono wastewater surveillance support comes from BioBot Analytics. BioBot initiated a nationwide pro-bono wastewater sampling and analysis program in April 2020 to help establish their SARS-CoV-2 testing protocols and expand wastewater surveillance efforts. From March 25 through May 31, 2020, BioBot Analytics received over 1,800 wastewater samples from 360 WWTPs across 43 U.S. states and Canadian providences (Biobot, 2020). Results highlighted the utility of wastewater surveillance as an early warning of a potential COVID-19 outbreak. For example, wastewater data from New Castle County, Delaware, spiked between three and seven days before a spike in COVID-19 cases was identified through individual testing (BioBot, 2020). Biobot was able to quickly develop this program by leveraging some of their previous work monitoring wastewater for opioids.

5 Program Development

Numerous international efforts have demonstrated that wastewater surveillance can be used to detect outbreaks of other viruses (e.g., poliovirus, norovirus) earlier than through clinical surveys (Brouwer et al., 2018; Deshpande et al., 2003; Ivanova et al., 2019; Kazama et al., 2017; Lago et al., 2003). At the start of the COVID-19 pandemic, researchers and practitioners needed to quickly develop and/or adapt analytical methods, sample collection procedures, and data interpretation approaches specific to SARS-CoV-2. Much of this work began in early 2020 with collaborative efforts among international experts from utilities; universities; and local, state, and federal governments. Many of these efforts were held virtually and recorded for future reference. As the science rapidly evolved, teams leading surveillance programs throughout the United States and in other countries continually learned from one another via frequently revised online forums that documented research findings, lessons learned, best practices, guidelines, and recommendations.

As the field continued to evolve over the past year, new technologies emerged that improved the speed and accessibility of wastewater testing and analysis for implementation at various scales. Over time, multiple analytical methods and program frameworks were proven successful and were shared widely across the wastewater surveillance community. The field has matured by having reliable methods, programs, safety measures, and ethical considerations, many of which are being constantly refined and updated with the latest research and information. This section discusses some of the key areas of program development.

5.1 Peer-to-peer Communication and Resource Sharing

Early in the COVID-19 pandemic, there were few guidelines and recommendations available on sampling and analysis methods for SARS-CoV-2 in wastewater. The global research community worked in partnership to build upon established protocols for waterborne enteric viruses and further progress the field of wastewater surveillance. This international collaboration among experts was a crucial step in the success of wastewater surveillance programs for SARS-CoV-2 during the pandemic. Federal, state, tribal, and local agencies; utilities; universities; and other groups have all participated in and benefited from the continued collaborative spirit and partnership across the field. Some of these efforts are discussed below.

5.1.1 Workshops and Trainings

WRF released a 42-question survey via emails campaigns, LinkedIn, Instagram, Twitter, and Facebook from April 16–24, 2020, to gather information on sampling design (e.g., locations, frequency, composite vs. grab, shipping, storage), sample processing (e.g., preliminary treatment, concentration, purification), extraction, detection, sample volume requirements, and analytical methods validation and controls (Zhou et al., 2020). WRF used the results from the survey to inform the content for their virtual multi-day International Water Research Summit on COVID-19 held in late April 2020. WRF convened over 50 international experts from utilities, academia, consulting, and government to advance the work researchers were performing throughout the world (WRF, 2020a). The International Water Research Summit included discussions on the following topics in an effort to identify best practices and recommended approaches:

- Sample collection and preservation
- Analytical methods for identifying SARS-CoV-2 in wastewater
- SARS-CoV-2 concentrations in wastewater in comparison to community COVID-19 cases

- Communication of results

During the International Water Research Summit, participants also developed a "near-term research roadmap" that identified critical knowledge gaps requiring immediate research (WRF, 2020a). Participants categorized the necessary research into four themes: (1) analytical methods variability, reproducibility, and reliability; (2) viral shedding rate of SARS-CoV-2 in feces and the associated genetic signal; (3) interpretation of results to support public health efforts; and (4) risk assessment of potential exposure pathways for wastewater workers (WRF, 2020j). Participants also identified 12 research opportunities to advance wastewater surveillance for SARS-CoV-2 across these themes, as shown in Table 4 (WRF, 2020j). Based on these findings, WRF sought funding partners and began soliciting applications for qualified organizations to conduct research on these topics. See Section 4.5 for details on some of the projects WRF funded.

Table 4. Research opportunities identified by WRF to support wastewater surveillance of SARS-CoV-2 (WRF, 2020j).

Priority	Theme	Research Opportunity
High	Methods	Intra- and interlaboratory assessments on sampling regimes and molecular methods.
High	Shedding rate and genetic signal	Effect of wastewater pre-treatment on genetic signal.
High	Shedding rate and genetic signal	Dilution and persistence of the genetic signal in the sewer collection system. Targeted integrated study (in well-characterized systems that have good hydraulic models).
High	Risk	Evaluation of potential for infectious virus in wastewater and generation of aerosols.
High	Interpretation of results	Correlations to clinical data for the assessment of community prevalence. How to leverage wastewater surveillance to provide useful data to public health stakeholders.
High	Interpretation of results	Define partnership opportunities.
High	Shedding rate and genetic signal	Viral shedding rate, duration, and demographics in symptomatic and asymptomatic infections (RNA copies per gram of feces).
Medium	Methods	Impacts of sample collection method (composite vs. grab, duration of composite, time of day).
Medium	Methods	Distribution of virus (or RNA copies) in liquid and solid phase.
Medium	Interpretation of results	How to effectively translate COVID-19 research into pandemic preparedness and wastewater surveillance for future needs.
Low	Methods	Which spike organism to use for quality assurance/quality control purposes.
Low	Methods	Comparative methods review for enveloped viruses, focusing on the concentration from wastewater matrix.

WRF continues work on wastewater surveillance and most recently in April 2021 held a second International Symposium on COVID-19 Wastewater Surveillance (WRF, 2021a).

As another example, the National Institute of Standards and Technology (NIST) held a virtual workshop in June 2020 to discuss challenges with measuring and detecting SARS-CoV-2 in wastewater with five expert presenters.[5] The main goals for the workshop included discussing the merits of applying NIST's expertise in producing standards and reference materials to the most pressing monitoring issues (NIST, 2020).

Tailored to applying surveillance on a more local level, NACCHO and CDC hosted a webinar in June 2020 titled "Water, Sanitation, and Hygiene During the COVID-19 Pandemic" (NACCHO, 2020a; NACCHO, 2020b). The webinar featured three speakers, two from CDC and one from a local health department.[6] The topics included general information on proper cleaning and disinfection, minimizing risk of Legionella in drinking water before reopening unoccupied buildings, options for disaster shelters, along with wastewater surveillance for SARS-CoV-2. Panelists reviewed sampling and analysis techniques for wastewater testing of SARS-CoV-2, discussed the benefits of early detection of SARS-CoV-2, and raised concerns regarding common misconceptions about the field (e.g., the virus is always infectious in sewage) (NACCHO, 2020a; NACCHO, 2020b). NACCHO included wastewater surveillance for SARS-CoV-2 as one of the sessions in their Preparedness Summit annual conference in April 2021 (NACCHO, 2021).

5.1.2 Online Platforms

In addition to collaboration through workshops and webinars, the wastewater surveillance community has developed numerous interactive online resources. One example is the COVIDPoops19 dashboard, created and managed by the University of California at Merced. This dashboard provides a global map of reported SARS-CoV-2 wastewater surveillance efforts, including the number of public dashboards, universities, countries, and surveillance programs (referred to as testing sites), all of which is updated daily. For each identified program, the dashboard provides a link to the program website, relevant news articles, and/or associated publications for the surveillance program (UC Merced, 2021). The University of California at Merced published the methods for developing the COVIDPoops19 dashboard (Naughton et al., 2021).

As of early May 2021, the COVIDPoops19 dashboard includes 80 dashboards, 256 universities, 54 countries, and 2,216 surveillance programs, as shown in Figure 2 (UC Merced, 2021). This dashboard is part of the larger COVID-19 WBE Collaborative that encourages partnership and discussions among those in the wastewater surveillance field (COVID-19 WBE Collaborative, 2021). The COVID-19 WBE Collaborative website provides a list of resources, including a COVID-19 WBE Slack workspace that is an organized web-based channel for groups to collaborate and talk about their programs; a Protocols.io group that houses information on sample collection techniques, analytical methods, quality control, and results interpretation; and a link to the website for Research Coordination Network on wastewater surveillance. The Research Coordination Network website, funded by NSF and designed by researchers from the University of Notre Dame, Howard University, Stanford University, and Arizona State University, offers networking opportunities to increase collaboration among wastewater surveillance programs (University of Notre Dame, 2021). Information is provided through webinars, workshops,

[5] Bharat Ramakrishan, OpenBiome; Manoj Dadlani, CosmosID; Katerina Papp, Southern Nevada Water Authority; Dr. Kyle Bibby, University of Notre Dame; and Aparna Keshaviah, Mathematica.
[6] Amy E. Kirby, PhD, MPH, CDC; Jasen Kunz, MPH, CDC; Rob Blake, MPH, Transylvania County Department of Public Health.

training videos, and links to other resources (e.g., open data repositories) (see additional details on this NSF project in Section 4.1).

Figure 2. COVIDPoops19 dashboard (UC Merced, 2021).

Another example of an interactive online resource is the international Water Action Platform, created and managed by Isle Utilities and supported by various sponsors. While the platform was initiated in March of 2020 in response to the COVID-19 pandemic, it has since expanded to cover a wide range of wastewater-related topics (e.g., asset management, communications, sustainability). The Water Action Platform encourages peer-to-peer collaboration by hosting regular webinars to share lessons learned and best practices from utilities around the world, summarizing news updates and press releases, and supporting a series of "knowledge hubs" via WhatsApp. As of September 2020, more than 1,300 people from 758 organizations across 92 countries are participating in the Water Action Platform (Water Action Platform, 2021).

WEF is also compiling articles, research, news updates, and links to other sites for up-to-date information on best practices for wastewater surveillance on a weekly basis. WEF's coronavirus website breaks out resources into sections by topics such as vaccine resources, water sector–specific information, training and education resources, and water sector coronavirus assistance. The water sector–specific information includes recent articles, podcasts, webcasts, and fact sheets that were published by WEF or other sources (WEF, 2021d). WEF has also developed a Network of Wastewater-Based Epidemiology website focusing on U.S. utilities performing wastewater surveillance. As of May 13, 2021, WEF's dashboard included 517 utilities, 159 academic institutions, eight laboratories, and one other program performing or supporting wastewater surveillance programs, as shown in Figure 3 (WEF, 2021b).

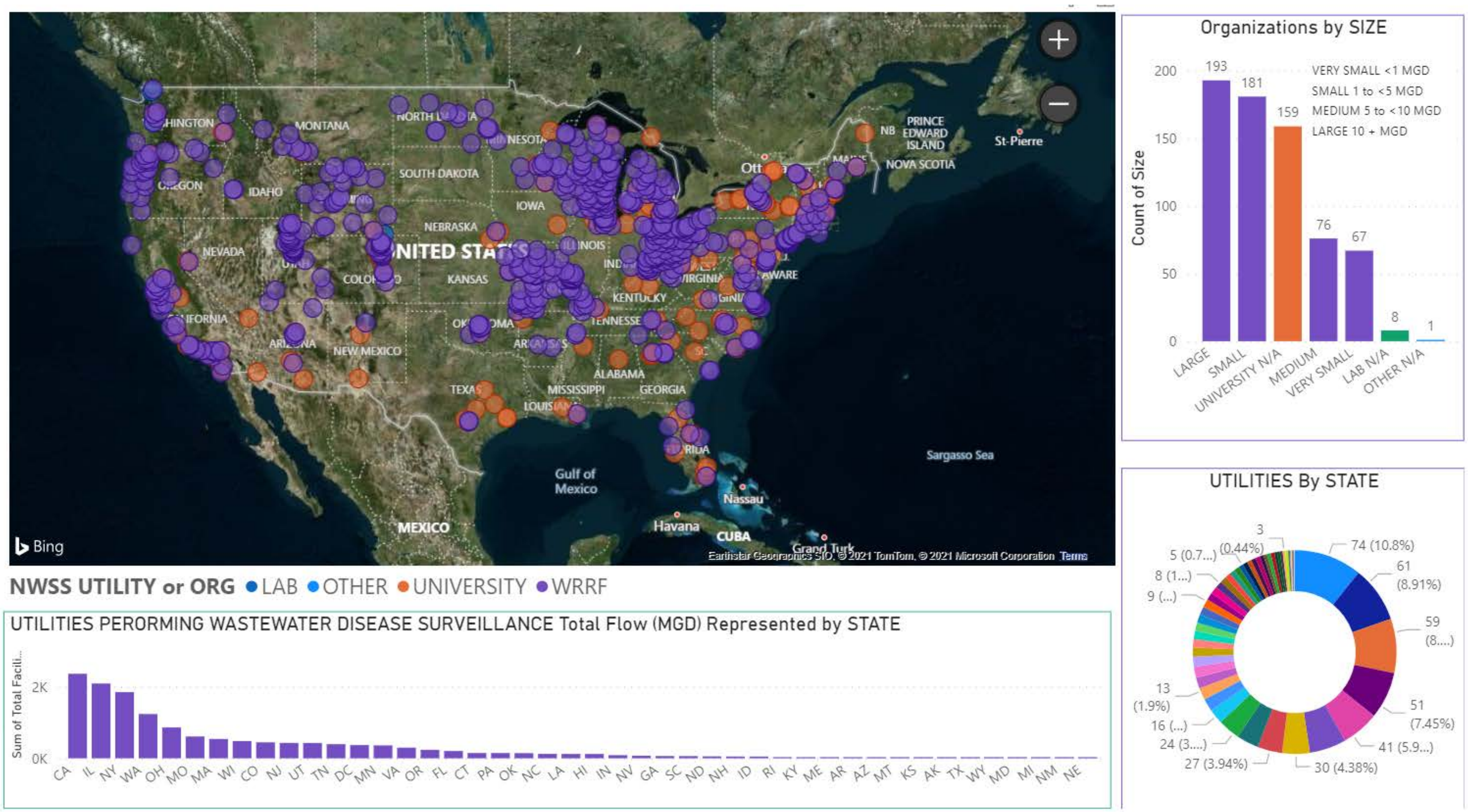

Figure 3. Utilities performing wastewater surveillance in the United States (WEF, 2021b).

5.2 Researching and Developing Analytical Methods

Beginning early in the COVID-19 pandemic, many federal agencies, state agencies, non-governmental stakeholders, and the academic research community worked both independently and together to quickly develop and refine methods to support wastewater surveillance for SARS-CoV-2. This section presents several examples of such efforts.

Laboratory analytical methods for the detection of SARS-CoV-2 RNA in wastewater have continually evolved based on new science and resources. The NWSS website (CDC, 2021a) outlines commonly used wastewater surveillance methods for:

- Sample preparation (e.g., storage, homogenization, sample clarification).
- Sample concentration (e.g., ultrafiltration, polyethylene glycol precipitation, ultracentrifugation).
- RNA extraction.
- SARS-CoV-2 RNA detection and quantification (e.g., RT-qPCR and reverse transcription digital droplet PCR [RT-ddPCR]).

SARS-CoV-2 RNA Measurement Methods

Once the SARS-CoV-2 RNA is extracted from the wastewater sample, laboratories quantify the amount of RNA using RT-qPCR, RT-ddPCR, or other less common forms of RT-PCR. The viral RNA is enzymatically transcribed to complementary deoxyribonucleic acid (cDNA) in a process called reverse transcription (RT). The cDNA is then used in a second enzymatic reaction (PCR) to make many copies of a small fragment of the transcribed viral RNA. RT-PCR (qPCR or ddPCR) can occur in two steps, where these enzymatic reactions take place sequentially in separate tubes, or as a one-step reaction where the two enzymatic processes occur in sequence in the same tube (CDC, 2021f).

CDC has also developed an online resource that lists reverse transcription PCR (RT-PCR) primers and probes for two N gene assays of SARS-CoV-2, last updated in June 2020 (CDC, 2020e). The APHL has compiled a list of selected COVID-19 molecular testing resources for individual testing and their key characteristics (e.g., test name, manufacturer, complexity, testing throughput capacity) (APHL, 2020). APHL also provides a downloadable Excel file summarizing a longer list of test methods for individual testing, with additional details on performance and limits of detection (APHL, 2021a), and a dashboard summarizing laboratory testing capacity and capabilities based on results of a weekly survey of 99 state, local, and territorial health laboratories, focusing on individual testing (APHL, 2021b). While the APHL resources are focused on individual testing, some of the information may be useful to consider for wastewater samples (e.g., buffer supply limitations). One example of a common laboratory analytical method is that developed by IDEXX Reference Laboratories. IDEXX developed a novel, highly sensitive RT-qPCR test to detect N1 and N2 gene targets of SARS-CoV-2 (IDEXX, 2021).

SARS-CoV-2 N Gene Targets

There are two distinct assays targeting the SARS-CoV-2 nucleocapsid gene, N1 and N2, that can be measured using RT-qPCR or RT-ddPCR (CDC, 2021f).

In the summer of 2020, NIST developed reference materials consisting of synthetic fragments of the SARS-CoV-2 virus RNA to aid in the analysis and development of RT-qPCR assays for SARS-CoV-2 (360dx, 2020; NIST, 2020). Researchers and laboratories can use the reference materials to assess detection limits for SARS-CoV-2 assays or calibrate other in-house or commercial SARS-CoV-2 controls. This allowed researchers and laboratories to further refine their analytical methods for SARS-CoV-2. NIST provided the reference material free of charge through funding from the CARES Act in exchange for user feedback to further improve and develop the materials (360dx, 2020; NIST, 2020). NIST is continuing to work with the DHS Science and Technology Directorate and the University of Louisville School of Medicine to further develop and refine reference materials and standards for quantifying SARS-CoV-2 with RT-qPCR assays, which will allow for additional development in the analytical methods (DHS, 2021). The new standards will allow SARS-CoV-2 wastewater surveillance results to be more readily shared and compared across surveillance programs, which will in turn inform more useful healthcare decisions (DHS, 2021).

As mentioned in Section 4.5, WRF funded several studies to further advance the wastewater surveillance field based on research needs identified at their International Water Research Summit (WRF, 2020h; WRF, 2020e). One such study was a methods assessment and interlaboratory comparison of wastewater results for SARS-CoV-2, in which WRF assessed the reliability of various SARS-CoV-2 testing methods to produce accurate and repeatable results (WRF, 2020c). For this, WRF invited laboratories to participate and analyze the same composite samples using their own analytical methods. The study ultimately compared distinct RNA extraction and quantification methods used at 32 U.S.-based laboratories, which included a combination of commercial; city, state, and federal government; academic research; and wastewater utility laboratories, as well as some commercial manufacturers. Study results provided critical information on the performance of various methods and their use when conducting laboratory analyses for wastewater surveillance studies and programs. The results are summarized in an executive summary available on WRF's website and a journal article (WRF, 2020k; Pecson et al., 2021).

In addition to WRF's interlaboratory comparison, in June 2020, EPA's Office of Research and Development (ORD) began researching and developing a method for concentrating and then quantifying the amount of SARS-CoV-2 virus in wastewater using samples from WWTPs in Ohio (see Section 6.2.4 for details on Ohio's wastewater surveillance program). Some of ORD's research also evaluated the ability to concentrate and detect a surrogate coronavirus called OC43 from larger sample volumes of wastewater (McMinn et al., 2021). EPA is continuing research on applying this approach to detect SARS-CoV-2 and developing culture-based methods to detect SARS-CoV-2 in wastewater, similar to the culture-based methods for detecting coliforms where bacteria grow on media. EPA has also collaborated with Australia's Commonwealth Scientific and Industrial Research Organization on several review and research activities on SARS-CoV-2 wastewater surveillance ranging from sampling design (Ahmed et al., 2021), stability of molecular targets (Ahmed et al., 2020a), and recoveries using surrogate coronavirus (Ahmed et al., 2020b). All these research activities further support the refinement of SARS-CoV-2 wastewater methods, with a long-term goal of developing "sensitive, standardized methods to detect and quantify SARS-CoV-2 in raw sewage, including infectious virus" (EPA, 2021).

Other laboratory quantification methods have since evolved to allow experts and nonexperts alike to quickly extract viral RNA from wastewater and quantify viral RNA in the field rather than in a laboratory setting, potentially offering same-day SARS-CoV-2 wastewater results. For example, LuminUltra—in collaboration with Halifax Water and Dalhousie University Halifax, Nova Scotia—created a portable and rapid SARS-CoV-2 wastewater testing solution. This new technology is described by the company as being able to run multiple samples within 90 minutes, without the need for extensive laboratory expertise (LuminUltra, 2020; LuminUltra, 2021). LuminUltra's quantification kit and others, such as those developed by BioRad, QIAGEN, and Zymo, have been developed to support cost-effective and rapid expansion of wastewater testing methods for SARS-CoV-2 (Bio-Rad, 2021; Qiagen, 2021; Zymo, 2021).

In early 2021, the research community also began exploring methods for detecting and quantifying SARS-CoV-2 variants (e.g., the United Kingdom variant, B.1.1.7 [Alpha]; the South African variant, B.1.351 [Beta]). This work is being done through genomic sequencing of wastewater samples and the development of primers and probes capable of detecting individual mutations that characterize specific variants via RT-qPCR. Some examples are Rice University sequencing the whole SARS-CoV-2 genome (see Houston Case Study in Section 6.2.7) and Clemson University quantifying the B.1.1.7 in the wastewater (see Clemson University Case Study in Section 6.2.9) (Houston, 2021; Clemson, 2021b).

5.3 Ongoing Wastewater Surveillance Support

As the field of SARS-CoV-2 wastewater surveillance continues to grow, organizations have developed and shared best practices and guidance with their stakeholders. The organizations include large international groups, such as the World Health Organization (WHO) that published recommendations on the ethics of wastewater surveillance, to smaller nonprofits such as WEF that published safety information for WWTP workers. This section describes the work of organizations like these that have or are providing ongoing support for wastewater surveillance.

Various federal agencies have contributed to the field of SARS-CoV-2 wastewater surveillance on a national level. For example, and as described in Section 4, CDC and HHS, in collaboration with other federal agencies, established the NWSS in September 2020 to, in part, provide support and coordination for wastewater surveillance of SARS-CoV-2. The NWSS website offers technical support for sampling methods (e.g., location and frequency), laboratory methods, and public health interpretation of wastewater testing data (CDC, 2021a). The NWSS DCIPHER platform is a national database of wastewater testing results for SARS-CoV-2 collected by state, tribal, local, and territorial health departments in coordination with wastewater utilities. Data are submitted to NWSS through the DCIPHER platform by state, tribal, local, and territorial health departments using a standard collection instrument that defines the minimum set of reporting guidelines (CDC, 2021a). CDC analyzes the data submitted to the system in real time and reports results to health departments via the DCIPHER data reporting analytics dashboard (CDC, 2021a).

The NWSS DCIPHER analytics system corrects wastewater testing results for laboratory method performance, as well as the composition of wastewater and the number of people living in the community under surveillance—ensuring comparability across labs, across communities, and through time (WRF, 2021a). The data reporting dashboard presents maps of WWTPs sewersheds under surveillance, time series plots of wastewater results, and information on SARS-CoV-2 trend status (e.g., sustained increase, increasing, plateaued, decreasing, sustained decrease) at each sampling location for use by public health departments (WRF, 2021a). A screen shot of part of the data reporting dashboard is shown in Figure 4 (CDC, 2021g). As of the end of August 2021, SARS-CoV-2 wastewater results for almost 20,000 samples have been submitted to NWSS (see Figure 5) (CDC, 2021g).

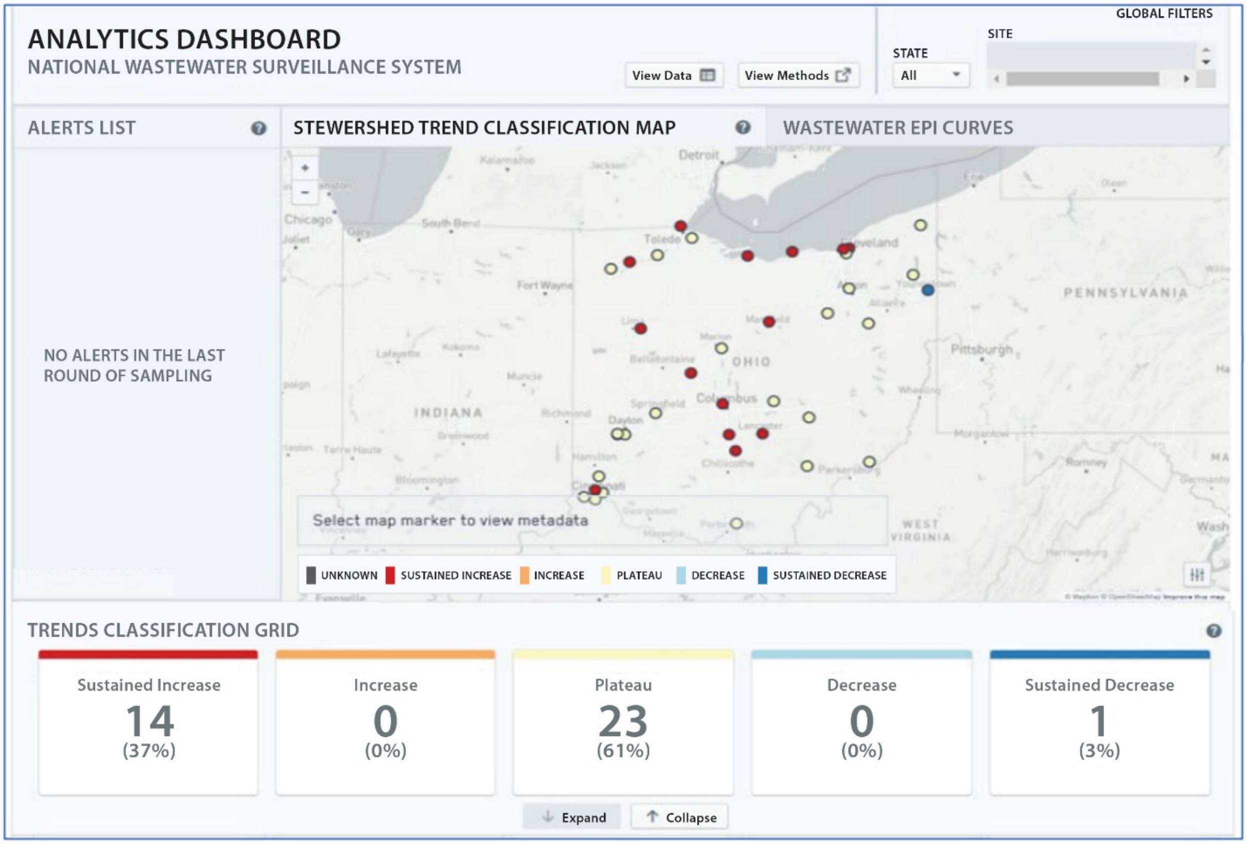

Figure 4. CDC's NWSS DCIPHER analytics dashboard for SARS-CoV-2 wastewater results (CDC, 2021g).

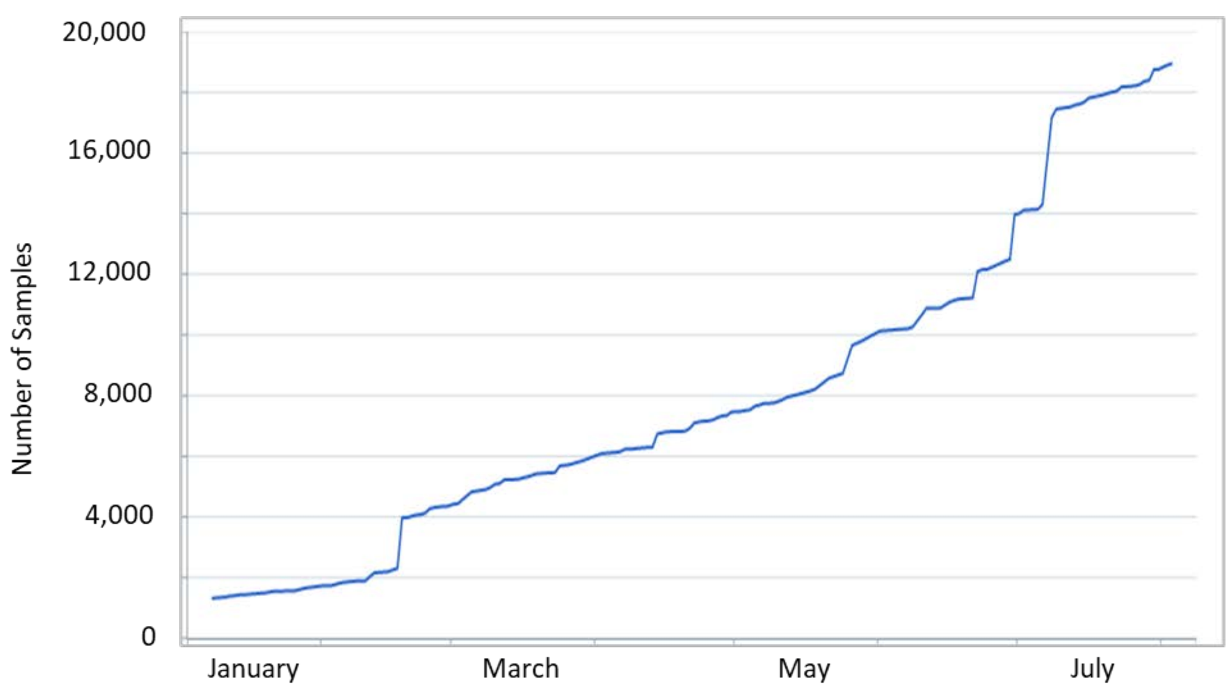

Figure 5. Cumulative samples in the NWSS DCIPHER system (CDC, 2021g).

As mentioned in Section 4.4, HHS (with support from CDC) funded wastewater testing from WWTPs serving 10 percent of the U.S. population (estimated at 100 WWTPs) in late 2020 for a period of six weeks. HHS is continuing to support this nationwide pilot study to test the scalability of an early warning system and public health response framework through a second phase that is anticipated to cover 30 percent of the U.S. population. The results of this work are especially relevant in smaller communities with the potential for underfunded individual testing programs that may be underreporting cases of COVID-19 (CNBC, 2020).

As mentioned in Section 4.5, WRF funded a research project to develop specific implementation and sample design recommendations at different types of sewersheds: large urban sewersheds, medium-sized regional sewersheds, and small regional systems (WRF, 2020h).

On a more local level, multiple organizations have and continue to provide general guidance about sampling and analysis methods to support programs. For example, in June of 2020, the National Association of Clean Water Agencies (NACWA) released a guidance document for utilities to aid in the development of wastewater surveillance programs for SARS-CoV-2. The document is based on lessons learned from NACWA member utilities who were already sampling for SARS-CoV-2 in wastewater. NACWA's document includes a recommendation for utilities to engage and work with their local and/or state health departments for the wastewater surveillance program. NACWA prepared this document in response to concerns that SARS-CoV-2 wastewater surveillance and communication of results are outside the purview of a wastewater utility; however, utilities are receiving requests to conduct surveillance from community members, private companies, and other organizations. Utilities were also concerned about assessing costs and data validation since there is no consistent analytical method. NACWA indicated that the resource is a "living document" and will be updated as guidance and information evolves (NACWA, 2020).

As another example, the Environmental Research Institute of the States and ASTHO, under a Memorandum of Agreement with EPA ORD, published an issue brief on the detection of SARS-CoV-2 in wastewater in November 2020. This brief summarizes early studies conducted by states, identifies research gaps, outlines common practices for collecting wastewater samples (e.g., sample location, type, and frequency; documentation; analytical methods; reporting), and provides recommendations to scientists and public health and environmental officials moving forward (ERIS and ASTHO, 2020).

Based on lessons learned at their April 2020 International Water Research Summit, WRF has shared multiple resources for surveillance programs. One such document is a detailed summary of recommendations from global experts that participated in the International Water Research Summit, covering "potential uses of wastewater surveillance for tracking COVID-19, sampling design, analytical tools, and communication of results to public health decision-makers, the public, and other key stakeholders" (WRF, 2020j). WRF also published an appendix on best practices for sample collection and storage and developed a field sample collection form that outlines the information that should be recorded during sample collection (e.g., wastewater flow rate, type of sewer system, air temperature, sample water characteristics such as temperature, total suspended solids, chlorine residual, pH) (WRF, 2020l). The field collection form also provides space to record information about sample storage and processing at the laboratory (e.g., temperature of sample upon receipt at the laboratory). WRF's website includes links to their press releases on current projects and funding opportunities (WRF, 2021b).

5.4 Inclusion of Rural and Underserved Populations

As wastewater surveillance efforts expanded across the country, federal, state, tribal, and local agencies and other non-governmental stakeholders have recognized the importance of selecting sites with consideration for potential vulnerabilities of the community (e.g., at-risk populations, rural areas, communities underserved by health care systems). Ohio's COVID-19 Populations Needs Assessment acknowledged that COVID-19 would likely disproportionally impact people of color, rural populations, and individuals with disabilities since these groups already deal with less access to healthcare and more negative social determinants of health than others (Nemeth et al., 2020). Wastewater surveillance can provide insight into COVID-19 spread without biases that may be associated with individual testing, such as race, economic status, age, testing site hours and locations, and many other factors.

For example, NEHA published an article outlining the benefits and best practices of wastewater surveillance in rural areas. The article, "Sewage Monitoring in Rural Communities: A Powerful Strategy for COVID-19 Surveillance," explains how wastewater surveillance is a strong supplement to individual testing programs in these areas because rural and underserved areas face challenges for detection and management of the outbreak. As an example, individual testing may not be feasible or cost-effective in these areas, and wastewater surveillance can provide greater coverage and broader detection in smaller towns. However, the article notes that wastewater surveillance in rural communities may be challenging due to the number of people (estimated at 20 percent) that live in areas without WWTPs and instead use septic systems. Additionally, rural areas may not have trained individuals to conduct the sampling and perform the laboratory analyses, so the article suggests rural wastewater surveillance will require coordination between WWTPs, laboratories (commercial and academic), and public health departments (NEHA, 2020).

One thing to consider when establishing wastewater surveillance programs is the number of people contributing to the wastewater. If the population is too small, robust individual testing and contact tracing programs may be more effective at evaluating the spread of COVID-19 than wastewater surveillance. However, if individual testing and contract tracing are not available, then wastewater surveillance may be useful for public health officials.

Multiple case study programs considered vulnerable and underserved communities when creating or executing the sampling program. The Ohio wastewater surveillance program prioritized sampling at WWTPs that service large and/or vulnerable populations. The previously mentioned vulnerability report that ODH and Ohio State University developed strongly guided Ohio's sampling site selection (Ohio, 2021; Nemeth et al., 2020). Other programs used community relations to institute public health measures more effectively within vulnerable populations. Tempe, Arizona partnered with Guadalupe, Arizona, to monitor the Guadalupe's wastewater, primarily comprised of low-income and minority families. The Mayor and City Council of Guadalupe used the wastewater results to enforce COVID-19 public health messaging and guide future decision-making. Tempe also created a multi-lingual door-to-door education campaign about COVID-19 and how to protect oneself while distributing masks and care packages for use for vulnerable populations within Tempe (Tempe, 2021d). The Houston Health Department (HHD) used the wastewater results to identify zip codes most in need of public health interventions and used leaders within the local communities to connect with residents. HHD'S community partnership was to conduct COVID-19 prevention outreach, such as scheduling appointments and organizing secure transportation to testing and vaccination sites more effectively to vulnerable and difficult to reach areas (Houston, 2021).

5.5 Ethical and Legal Considerations

Beyond guidance for sampling and methods, the wastewater surveillance community has considered and developed guidance on related ethical concerns. The Canadian Water Network (CWN) convened experts in a Public Health Advisory Group to discuss public health application and communication and the ethical issues posed by wastewater monitoring (CWN, 2020a). The Canadian Water Network also released ethics and communication guidance in September 2020, applying WHO's guidelines on ethical issues in public health surveillance (WHO, 2017) to wastewater-based SARS-CoV-2 data (CWN, 2020b). WHO released a document in August 2020 outlining the uses of wastewater surveillance programs and considerations when creating a program, including ethical and legal considerations. WHO specifically addressed concerns about potential stigmatization of the community but also noted that wastewater surveillance would be less likely than individual testing to be stigmatizing due to the wastewater characteristic as a pooled sample. WHO also acknowledged that these concerns may be heightened because wastewater surveillance is often done without community consent. WHO cautioned programs to avoid disproportionately targeting already-stigmatized communities with public health and social measures (WHO, 2020). Researchers from multiple universities also published an academic paper on the legal and ethical considerations with wastewater surveillance (Gable et al., 2020).

5.6 Worker Safety

Guidance has also been developed regarding utility worker safety when supporting wastewater surveillance efforts for SARS-CoV-2. For example, WEF compiled information about the viability of SARS-CoV-2 in wastewater and the related safety concerns for workers at the WWTP and throughout the collection system into a web-based "Water Professional's Guide to COVID-19" (WEF, 2020a). This provides resources for safety based on the hierarchy of controls, the most effective control solutions for the most employees, and guidance from the Occupational Safety and Health Administration (WEF, 2020a). WEF also published a document titled "Protecting Wastewater Professionals from COVID-19 and Other Biological Hazards" based on the findings of an expert panel of academics and practitioners (WEF, 2020b). The document summarizes the most current, evidence-based information on protecting worker health and safety with respect to exposure to biological hazards associated with wastewater from agencies and authorities external to WEF, as well as the peer-reviewed literature. Occupational Safety and Health Administration also has recommended guidance for solid waste and wastewater management workers and employers during the COVID-19 pandemic (OSHA, 2020).

6 Implementation of Surveillance Programs

As wastewater surveillance for SARS-CoV-2 gained traction in the United States, many state and local agencies, utilities, and universities, along with a few tribal agencies, developed and implemented monitoring programs. Section 6.1 briefly summarizes publicly available information on programs initiated by 14 states and 161 tribes, municipalities, and universities. Details are provided for each program in Appendix A, with Table A-1 summarizing state programs and Table A-2 summarizing municipality and university programs. Section 6.2 provides additional information for a subset of ten diverse programs, gathered through open-ended interviews with program leads.

This discussion is not meant to serve as a comprehensive summary of all wastewater surveillance efforts across the country. EPA identified the programs in this document in January and February of 2021 through publicly available resources, such as the COVIDPoops19 dashboard (see Section 5.1), and by searching the internet for program websites, data dashboards, research papers, reports, news articles, and press releases that provided general information on SARS-CoV-2 monitoring programs (UC Merced, 2021). EPA included programs based on the amount of publicly available information found at the time. EPA recognizes that there are many other ongoing wastewater surveillance programs for SARS-CoV-2 at both the state and local level, including tribes. Refer to Section 3 for additional information on data collection methods.

6.1 Overview of Surveillance Programs

Most early monitoring efforts for SARS-CoV-2 in wastewater focused on wastewater influent (i.e., untreated sewage that enters a WWTP). As wastewater monitoring gained traction throughout the pandemic, however, programs expanded to collect wastewater from sewer manholes, sewer lines, lift stations, and sewer cleanouts to target smaller isolated communities or specific facility populations (e.g., correctional facilities, long-term care facilities, K-12 schools). Universities also developed programs to monitor SARS-CoV-2 in sewage from student housing on campus, and in some cases, within the surrounding community. Universities were either able to quickly initiate programs by leveraging longstanding research programs that were already in place involving pathogens in wastewater (e.g., Michigan State University) or by pivoting from previous research and adapting their expertise to wastewater monitoring (e.g., Clemson University) (Michigan, 2021a; Clemson, 2021b). The Berkeley Water Center at the University of California Berkeley summarized the experience of 25 college and university wastewater surveillance programs in the United States from the Fall 2020 semester (Harris-Lovett et al., 2021). Many universities also provided laboratory support and technical expertise to local and state agencies (e.g., Rice University), as well as individual utilities (e.g., University of Arizona) (Houston, 2021; University of Arizona, 2021).

Wastewater surveillance programs have been implemented at the national, state, tribal, and local level using both commercial and academic research laboratories. At the national level, for example, Biobot Analytics initiated a nationwide pro bono sampling and analysis program in April 2020, and HHS started wastewater testing at 100 WWTPs across the country for a period of six weeks beginning in November 2020 (see Section 4) (BioBot, 2020; FPDS, 2021). HHS, with support from CDC, has also established a nationwide wastewater surveillance program evaluating SARS-CoV-2 in WWTP influent (see Section 4.4 for details). At the state level, many state health and environment departments have developed statewide surveillance programs (e.g., Ohio) or funded them on a more local level (e.g., Michigan) (Ohio DOH, 2020; Michigan, 2021b). Local agencies and individual utilities have initiated monitoring programs as well, typically with funds from local government or ratepayers. In some cases, states and local government have worked together to execute programs (e.g., Vermont) (Burlington, 2021). In other cases, states worked directly with utilities (e.g., Indiana) (Indiana, 2020). Some wastewater surveillance programs receive support from consulting firms. For example, the wastewater surveillance program in Bergen County, New Jersey, is supported by AECOM and Columbia University (AECOM, 2020), while CDM Smith is supporting the U.S. Department of Veterans Affairs with their wastewater surveillance pilot project (CDM Smith, 2020).

Methods employed in early programs vary greatly with respect to sample location, sample type, frequency and time of collection, laboratory processing and analysis, and data interpretation. Regarding sample collection, the number of sampling locations varies from small, localized programs with less than ten unique sampling locations (e.g., Boise, Idaho; Burlington, Vermont) to larger statewide programs with upwards of 300 locations (e.g., Michigan) (Boise, 2021; Burlington, 2021; Michigan, 2021a). Sampling frequency similarly varies across programs, with most programs collecting samples once or twice weekly and a few collecting samples daily.

Wastewater samples are collected as either composite samples or one-time grab samples. While composite samples are often considered more representative of community fecal contributions, grab samples are sometimes advantageous since they can be collected quickly and at a much lower cost (CDC, 2021e). Composite samples are most frequently collected at the influent of WWTPs, while grab samples are collected when access is limited (e.g., no electric hookup) or there are security concerns at a given sampling location. In general, grab samples are collected in the morning hours when a greater percentage of the population is assumed to use the restroom, as indicated by the daily peak in wastewater flow. During the early months of the pandemic, automatic composite samplers were in limited supply and many programs had no choice but to collect grab samples. Depending on the program, samples are collected by utility staff, private contractors, or students.

Sample Collection Types

Grab samples consist of a single discrete sample that is representative of the wastewater at the time of collection.

Composite samples are collected over time to represent the average wastewater over that time. A common composite sampling approach is to collect a set volume every 15 minutes for 24-hours using a programmed automatic composite sampler (EPA, 2013).

State and local programs typically send wastewater samples to commercial or university laboratories for analysis, while most universities analyze their program samples in on-campus laboratories. In some cases, programs send samples to multiple laboratories as part of an interlaboratory comparison to validate and improve analytical methods. Several programs started by using a commercial laboratory and then later switched to a local university or state laboratory to decrease costs and reduce turnaround time between sample collection and receiving the results (e.g., Boise, Idaho; Houston, Texas) (Boise, 2021; Houston, 2021). Most programs analyze their wastewater samples with RT-qPCR, while a smaller percentage rely on the RT-ddPCR method. A handful of programs use both technologies. Programs typically analyze samples for both the N1 and N2 gene targets of SARS-CoV-2, while several also analyze fecal indicator organisms such as the pepper mild mottle virus.

Many state, local, and university wastewater surveillance programs communicate their results to the public in real time through the use of online data dashboards. Several have also produced more detailed summary reports (e.g., Indiana) (IFA, 2020) or published their methods and preliminary results in peer-reviewed journals (e.g., HRSD in Virginia) (Indiana, 2020; Gonzalez et al., 2020).[7] Within these

[7] As of February 2021, many articles on this topic are only available in preprint version and have therefore not yet been certified by a formal peer review process.

resources, programs present the concentration of the virus measured in wastewater (e.g., "genome copies/liter [L]"), normalized virus concentrations (e.g., normalized by population size, wastewater flow), or both concentrations. Normalized concentrations are used to account for changes in wastewater dilution (e.g., due to water other than wastewater entering the sewer system) and differences in relative human waste input over time (CDC, 2021e).

One common design for public dashboards used by multiple jurisdictions is to have a map showing the location of the WWTPs that are sampled along with geographic boundaries of the collection system served by each WWTP. Users can then click a specific sampling location to view a line graph of virus concentrations measured in wastewater through time. Some dashboards also present individual COVID-19 testing results, either as a separate graph (e.g., Utah, Wisconsin, Colorado) or overlaid with the wastewater data (e.g., Ohio) (Utah DEQ, 2021; Wisconsin DOHS, 2020; Colorado DPH, 2021; Ohio DOH, 2020). This allows users to compare trends between SARS-CoV-2 wastewater results and COVID-19 case counts based on individual testing.

Boise, Idaho, is one example of a city with a public dashboard that displays wastewater testing results along with individual COVID-19 testing results. Boise has been sampling and testing influent to its two WWTPs since May 2020. Boise presents the average of the daily SARS-CoV-2 wastewater results from the two WWTPs (weighted based on the WWTP's flow) on their data dashboard, as presented in Figure 6. The top graph shows the wastewater results in virus copies/L and the bottom graph shows new cases of COVID-19 over the same timeframe for the entire county (Boise, 2021).

In some cases, dashboards indicate recent trends in wastewater results either with descriptive text or with a specific symbol or color on their sampling location maps. One example is the Utah SARS-CoV-2 Sewage Monitoring Program, which collects samples weekly at 42 WWTPs representing approximately 80 percent of the state's population. Utah's dashboard includes a map depicting each WWTP's service area with color-coded symbols to indicate the recent sewage trend based on the virus concentrations from the four most recent samples (see Figure 7). Utah combines wastewater flow and service area population data to estimate viral concentrations in units of SARS-CoV-2 gene copies per person, per day, for each WWTP, shown in the top graph. Utah's dashboard also shows the daily new cases per 100,000 residents for each WWTP sewershed, shown in the bottom graph (Utah DEQ, 2021).

Various other state, local, and university programs have started to define trends in wastewater data, albeit using different methods. The Wisconsin COVID-19 Wastewater Surveillance dashboard shows a map of the WWTP service areas along with wastewater SARS-CoV-2 results presented in gene copies per person, per day, along with the seven-day average COVID-19 case rate for the sewershed for each of the 65 participating WWTPs (as of April 2021). Wisconsin defines trends in SARS-CoV-2 concentrations and individual COVID-19 testing results using a linear regression model and the most recent two weeks of data. Increasing trends are when the change from the prior seven-day period to the most recent seven-day period is greater than or equal to 10 percent and statistically significant (Wisconsin DOHS, 2021). The Ohio Coronavirus Wastewater Monitoring Network dashboard compares the average of the most recent two samples with the average of the prior third and fourth sample to determine the percent change between those averages; less than 50 percent change is defined as stable, 50 to 100 percent change is defined as increasing or decreasing, and more than 100 percent change is defined as substantially increasing or decreasing (Ohio DOH, 2021). The Missouri Sewershed Surveillance Project's COVID-19 Tracking Tool uses a story map to summarize wastewater data from more than 50 participating WWTPs. Missouri presents trends in the SARS-CoV-2 wastewater results over the past two weeks using map symbology (see Figure 8) (Missouri DHSS, 2021).

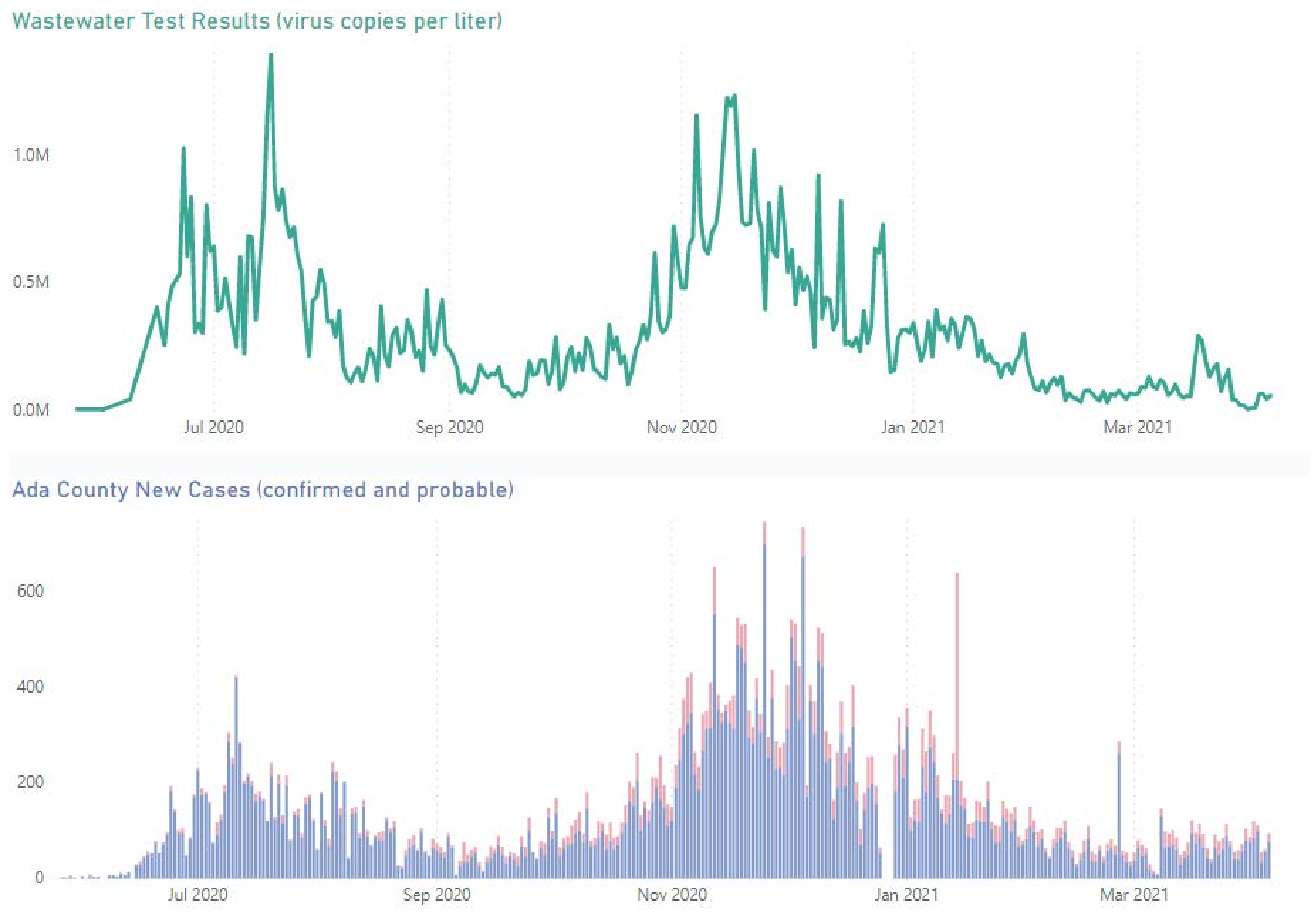

Figure 6. Boise Wastewater Surveillance Dashboard with wastewater SARS-CoV-2 virus copies per liter on the top graph and confirmed and probable COVID-19 cases on the bottom graph (Boise, 2021).

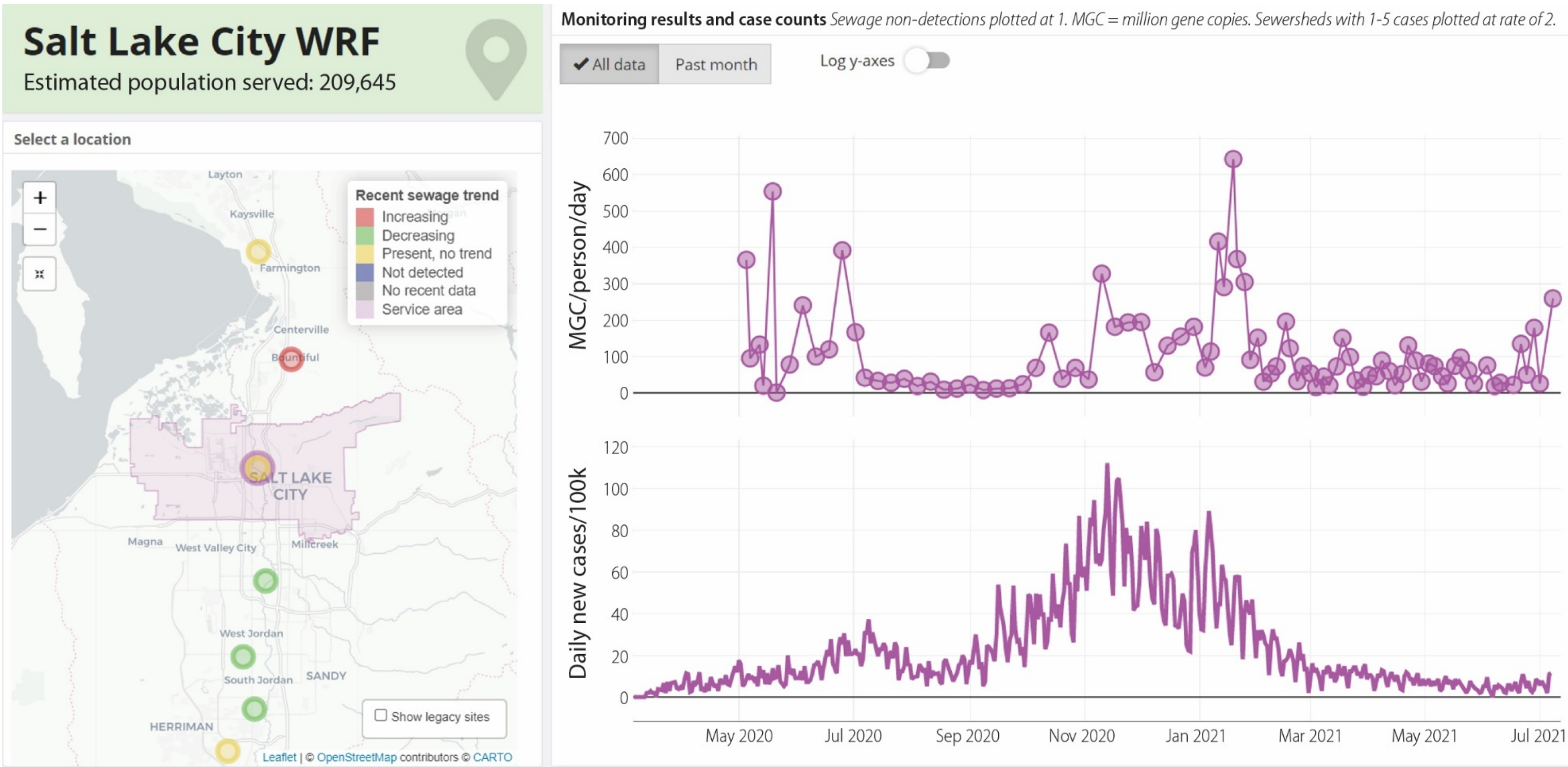

Figure 7. Utah SARS-CoV-Wastewater Surveillance Dashboard with the service are and recent wastewater trend on the left, SARS-CoV-2 million gene copies per person, per day on the top right graph, and daily new cases per 100,000 residents on the bottom right graph (Utah DEQ, 2021).

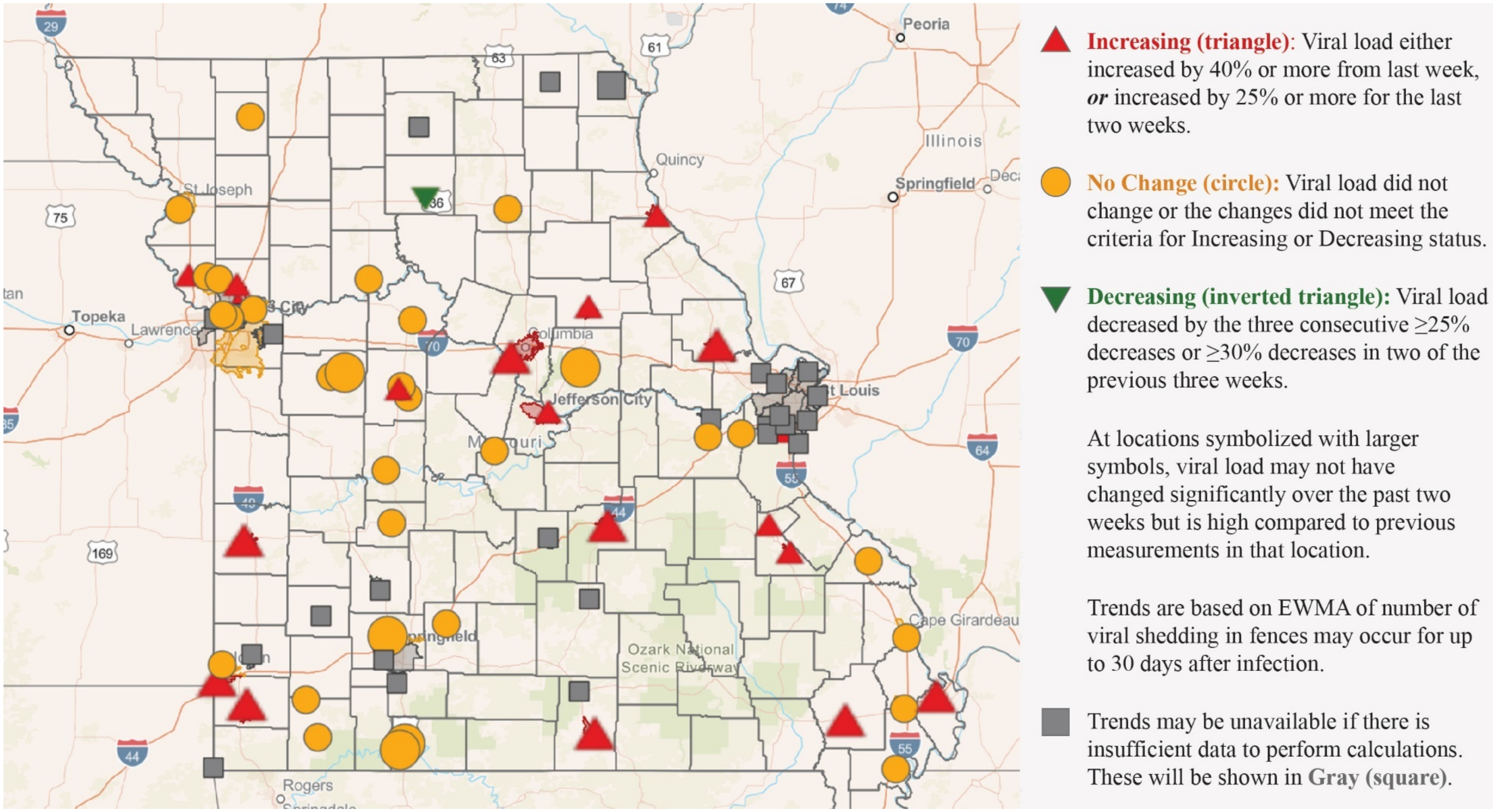

Figure 8. Missouri Department of Health and Human Services Wastewater Surveillance Dashboard with color coded symbols to indicate recent SARS-CoV-2 wastewater trends (Missouri DHSS, 2021).

Due to the recent emergence of SARS-CoV-2, there is no standardized way to predict the number of infected individuals reliably and accurately from wastewater sample results. However, some programs have used wastewater data to make predictions about the number of people with COVID-19 based on the SARS-CoV-2 wastewater results. For example, Yale University collects and analyzes daily wastewater samples for SARS-CoV-2 from seven WWTPs across Connecticut, covering nearly one million residents (Yale University, 2021b). For each participating WWTP, Yale graphs the wastewater SARS-CoV-2 results over time along with a seven-day moving average. Yale also graphs the available daily COVID-19 cases. For the most recent approximately two weeks Yale predicts the daily COVID-19 cases using the wastewater results. Yale replaces the predicted data with individual COVID-19 testing results as that information becomes available. Figure 9 presents an example of the graphs Yale generates for the Hartford South Meadows WWTP (Yale University, 2021a). Another example is summarized in the Wyoming case study in Section 6.2.5.

In some cases, wastewater surveillance programs have tied trends in wastewater data to events that occurred in the local communities where the wastewater samples are collected. For example, the University of Arizona consistently observed spikes in SARS-CoV-2 wastewater results collected from the WWTP a week after major events and holidays; when this occurred, they often saw similar spikes in individual COVID-19 cases the following week. The University of Arizona was also able to track the effectiveness of public health interventions through the wastewater sampling. For example, one week after Arizona issued the stay-at-home policy, the SARS-CoV-2 wastewater results decreased, with the individual COVID-19 cases decreasing the subsequent week (University of Arizona, 2021).

Some public-facing dashboards indicate events, like these, in an effort to help explain trends observed in SARS-CoV-2 measurements from wastewater. For example, the dashboard for Athens-Clarke County, Georgia, indicates significant events on the timeseries plot with vertical dashed lines. These dashed lines refer to events such as the day that Athens-Clarke County's mask mandate was put into place, the first day of the fall semester at the University of Georgia, and the day of Georgia's broad reopening (see Figure 10) (Lott et al., 2020).

In general, wastewater surveillance programs have been initiated to complement individual testing or to support more comprehensive surveillance for COVID-19 in areas with limited resources. Documented public health decisions triggered by or directly linked to wastewater monitoring results range from mask mandates to canvassing neighborhoods with educational materials. In some cases, wastewater data have served as an early warning of a potential COVID-19 outbreak, providing information ahead of individual testing data and allowing leadership to quickly intervene (e.g., increase individual testing to quarantine infected individuals, initiate targeted educational outreach efforts). This is most often seen at the local- or facility-level, where wastewater data represent smaller segments of the population (e.g., sub-sewershed monitoring) or a specific facility (e.g., correctional facility, university dorm). For example, in September 2020, the University of San Diego detected SARS-CoV-2 in wastewater from the Revelle College area. Within 14 hours of these results and out of an abundance of caution, the university sent targeted messages to members of the campus community to notify them of the results and to encourage them to get tested as soon as possible and monitor themselves for symptoms (UC San Diego, 2020). The university immediately expanded individual testing to enable campus employees and students to get tested over the following two days. The university tested more than 650 individuals and ultimately identified two positive cases. These individuals immediately self-isolated, potentially preventing a larger outbreak (Clark, 2020). Additional examples are provided in the case studies included in Section 6.2.

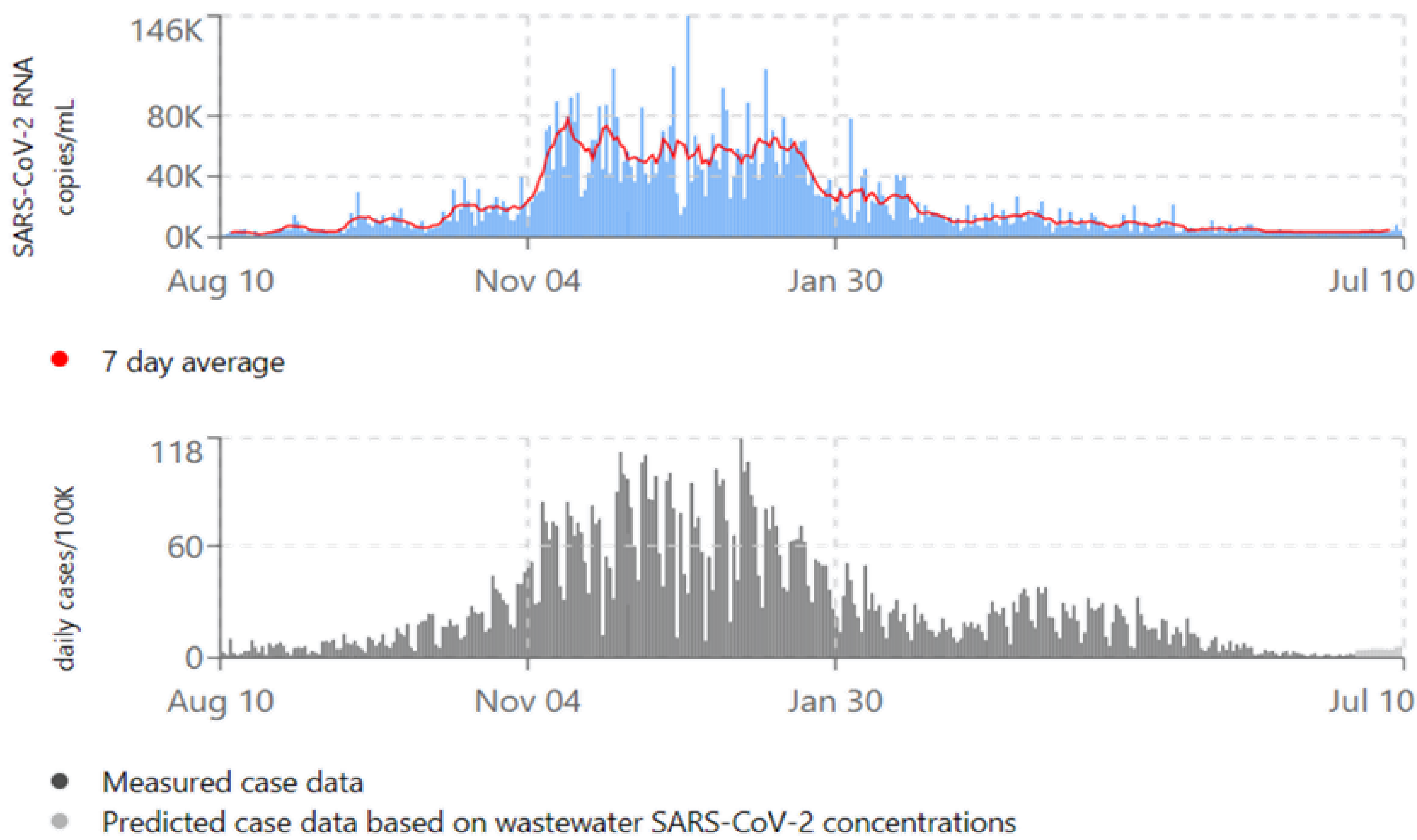

Figure 9. Yale University Wastewater Surveillance Dashboard – Hartford South Meadows WWTP serving Hartford, West Hartford, Newington, Bloomfield, and Wethersfield with SARS-CoV-2 wastewater results in copies per milliliter on the top graph and daily COVID-19 cases per 100,000 residents on the bottom graph (Yale University, 2021a).

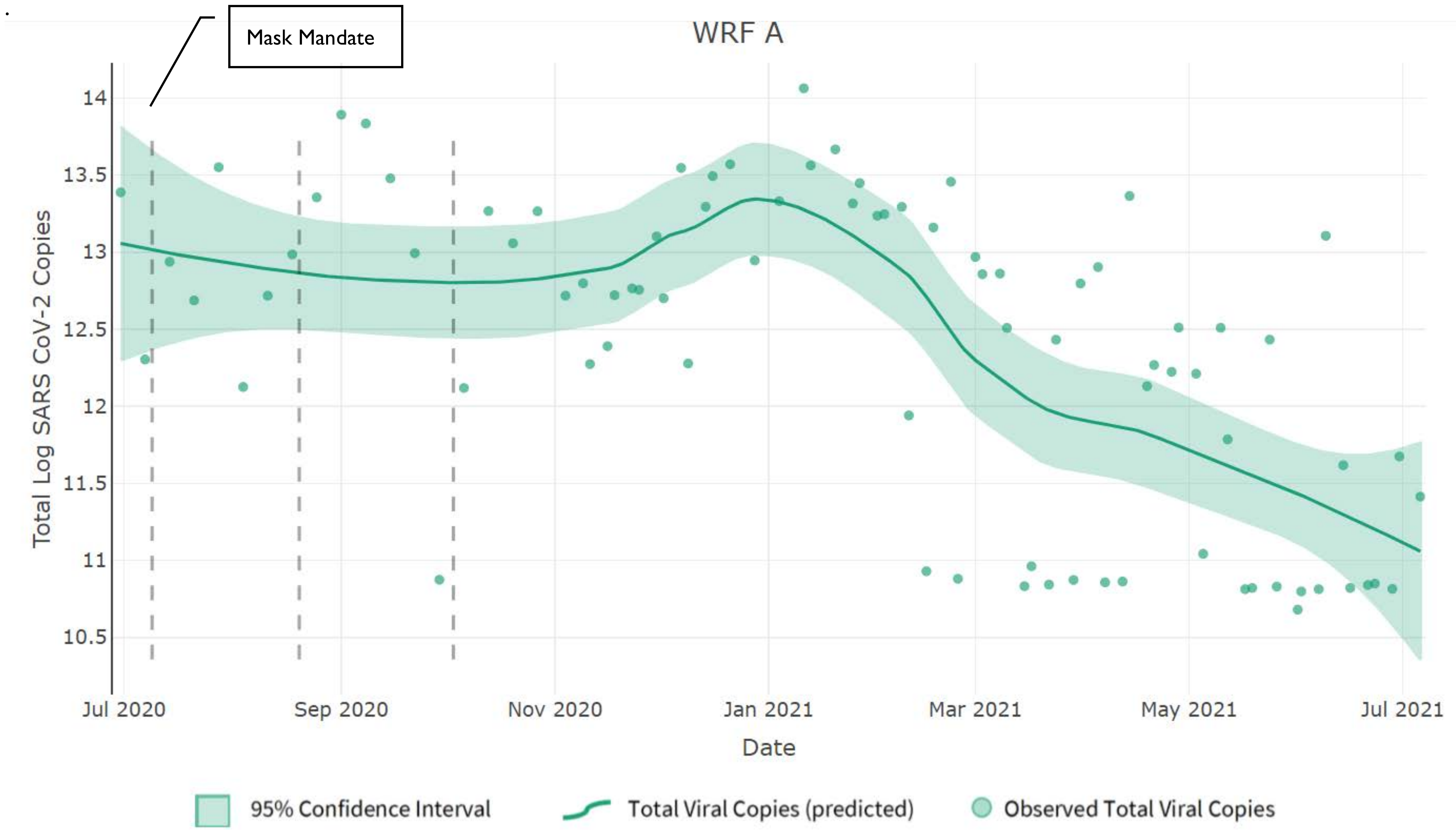

Figure 10. Athens-Clark County Wastewater Surveillance Dashboard for one of the WWTPs (Lott et al., 2020).

6.2 Wastewater Surveillance Case Studies

EPA conducted a series of interviews with a subset of practitioners and researchers leading wastewater monitoring efforts at the state and local level, as well as on university campuses. For this, and as described in Section 3, EPA focused on ten programs that demonstrate the wide variety of ways that programs were financed and implemented. The goal of this effort was to learn more about the diverse history and use of wastewater surveillance for SARS-CoV-2 among participants, including what prompted each initiative, initial and ongoing funding mechanisms, collaboration and organization with other entities, and sample collection and laboratory analytical methods. EPA also gathered insight on how wastewater data have been used to support decision-making and critical lessons learned along the way. EPA interviewed technical leads for the following wastewater surveillance programs listed in Table 5. These programs are also included in Appendix A summarizing the collaborations, sampling details, methods, and dashboards.

Table 5. Summary of case study wastewater surveillance programs.

Wastewater Surveillance Program		Unique Aspects of the Program Highlighted in the Case Study
State Programs	Indiana	The program was led by the Indiana Finance Authority (IFA). Participating utilities conducted sample collection at individual facilities and WWTPs. The program focused on communities with universities/colleges due to the transient nature of student populations and published a report documenting their decision-making process. See details in Section 6.2.1.
	Michigan	The statewide program included 20 different projects led by various universities and utilities. It was built off the existing state laboratory network and workflow used to support the beach water monitoring program. See details in Section 6.2.2.
	New Mexico	The program focuses sampling efforts entirely at individual facilities (e.g., correctional facilities, youth shelters). At some facilities, samples are collected at multiple locations that divide the facility into smaller resident populations. The state developed a public-facing dashboard summarizing results and published a press release highlighting a success story at a facility. See details in Section 6.2.3.
	Ohio	The program involves large-scale collaboration between multiple state agencies, U.S. EPA ORD, and numerous universities. WWTPs are sampled throughout the state. The program involves an analytical methods group—which includes eight laboratories—to foster SARS-CoV-2 methods development. The state developed a public-facing dashboard that depicts trends in wastewater results. See details in Section 6.2.4.
	Wyoming	The state provided financial incentives to participating utilities. A public-facing dashboard shows wastewater results as well as modeled predictions for the percent of the population infected with COVID-19, based on wastewater data. See details in Section 6.2.5.

Table 5. Summary of case study wastewater surveillance programs.

Wastewater Surveillance Program		Unique Aspects of the Program Highlighted in the Case Study
Local Programs	Hampton Roads Sanitation District in Hampton Roads, Virginia	The utility collects samples in the local community and analyzes them for SARS-CoV-2 at their own laboratory. The utility also analyzes wastewater samples from other utilities and organizations throughout the state and has documented their approach in a peer-reviewed journal article. See details in Section 6.2.6.
	Houston, Texas	The city collects wastewater samples from all WWTPs within the city, as well as at individual facilities and other locations within the sewershed. The city uses the wastewater data, along with other data sources such as COVID-19 individual testing results and vaccination rates, to identify "hot spots" for targeted public health intervention. Members of the local community support outreach efforts. See details in Section 6.2.7.
	Tempe, Arizona	The city quickly developed and implemented a wastewater surveillance program for SARS-CoV-2 by building off its existing opioid wastewater monitoring program. The city compares local events to the wastewater results. See details in Section 6.2.8.
University Programs	Clemson University in Clemson, South Carolina	The university categorizes wastewater results by "impact level" on a public-facing dashboard. This dashboard also includes results for variants. The university uses the wastewater data to support its COVID-19 response and the city of Clemson used the wastewater results to support its mask mandate. See details in Section 6.2.9.
	University of Arizona in Tucson, Arizona	The university first analyzed samples for SARS-CoV-2 from utilities across the country and then began analyzing samples collected on campus. The university has developed action levels for their campus wastewater surveillance program and used the wastewater data to prevent an outbreak in a dorm—a story that garnered national attention. See details in Section 6.2.10.

6.2.1 Indiana

The IFA recognized the possibility of accessing CARES Act funding to develop a wastewater surveillance program for SARS-CoV-2. IFA made a request to the state and was subsequently provided the CARES funding. IFA is a state agency that oversees Indiana's debt issuance and provides efficient and effective financing solutions for state, local, and business interests (IFA, 2021). In addition, the IFA manages the state's federally funded State Revolving Fund Loan Programs. As a result, IFA already had mechanisms in place to distribute the CARES Act funding in support of the wastewater surveillance program, as opposed to the Indiana State Department of Health or the Indiana Department of Environmental Management. Indiana established a wastewater surveillance program for SARS-CoV-2 from August to December of 2020 in 14 communities across the state. IFA chose to focus exclusively on communities with college and university campuses due to the transient nature of student populations and the potential increased spread of infectious diseases (Indiana, 2021).

IFA selected 120Water to manage the initiative and work directly with participating utilities (i.e., eligible utilities included those that served public or private universities with at least 1,000 enrolled students and student housing; all eligible utilities were invited to participate). 120Water hosted virtual training sessions on sample collection procedures, sent weekly sampling kits, coordinated between participating labs, leveraged technology for data collection and results management and collaboratively developed a final report. Utility partners collected their own samples and saw themselves as "stewards of the data," which many shared with public health decision-makers. Utilities identified sampling locations within the university collection systems (e.g., influent to WWTPs on-campus, manholes or lift stations that represent specific dormitory populations, Greek housing) and then collected the samples. Some utilities also chose to collect samples at other locations in the community (e.g., off-campus housing apartment complexes, correctional facilities, long-term care facilities). IFA and 120Water encouraged utilities to collect wastewater samples at additional locations that would provide useful information to their communities. In return for their participation, utilities received sample test kits, site selection guidance, and expert review and interpretation of the analytical results (Indiana, 2021).

For this program, utilities sent the wastewater samples to either Microbac Laboratories or the University of Notre Dame for SARS-CoV-2 analysis. Indiana included two laboratories to explore and better understand the precision of the novel analytical methods being used (Indiana, 2021). Utilities sent portions of the same wastewater sample to both laboratories in support of an interlaboratory comparison study. Microbac Laboratories analyzed samples via RT-PCR while the University of Notre Dame used RT-ddPCR. Staff from the two laboratories met weekly to discuss the results and identify ways to further refine and improve their methods, fostering a strong partnership. In general, wastewater results were comparable across laboratories (Indiana, 2020).

After sending samples to the laboratories, utilities received a weekly summary report that included graphs of wastewater results through time along with other relevant information (see Figure 11). In some cases, utilities communicated these results to and worked directly with local health departments (Indiana, 2021).

IFA released a public report on the program in late 2020 that summarized the purpose of the wastewater surveillance, the interlaboratory study, and trends in wastewater results (IFA, 2020). The report also included a detailed analysis of temporal trends in SARS-CoV-2 in wastewater compared to temporal trends in COVID-19 cases at both the community and campus level (see Figure 12). The report notes that temporal trends in wastewater mirrored trends in individual COVID-19 testing results for most communities, but that the trends appeared to occur at the same time (Indiana, 2020). In general, the wastewater data were not found to serve as a leading indicator of new cases for the Indiana program due to extended sample turnaround time and difficult-to-interpret fluctuations in the results. However, the wastewater results offered useful complementary information to other health data on COVID-19 (e.g., individual testing results) (Indiana, 2021).

The project's overall goal was to provide university communities with a tool for and knowledge about identifying COVID-19 outbreak risks, and to evaluate ways that communities could utilize wastewater surveillance in the future. Utilities were eager to contribute to this area of research, though they were unsure how to interpret results accurately (Indiana, 2021).

Although Indiana has used all the CARES Act funding allocated to this program, IFA sees wastewater surveillance as a valuable tool for the future. IFA is exploring other funding mechanisms for new surveillance opportunities (Indiana, 2021).

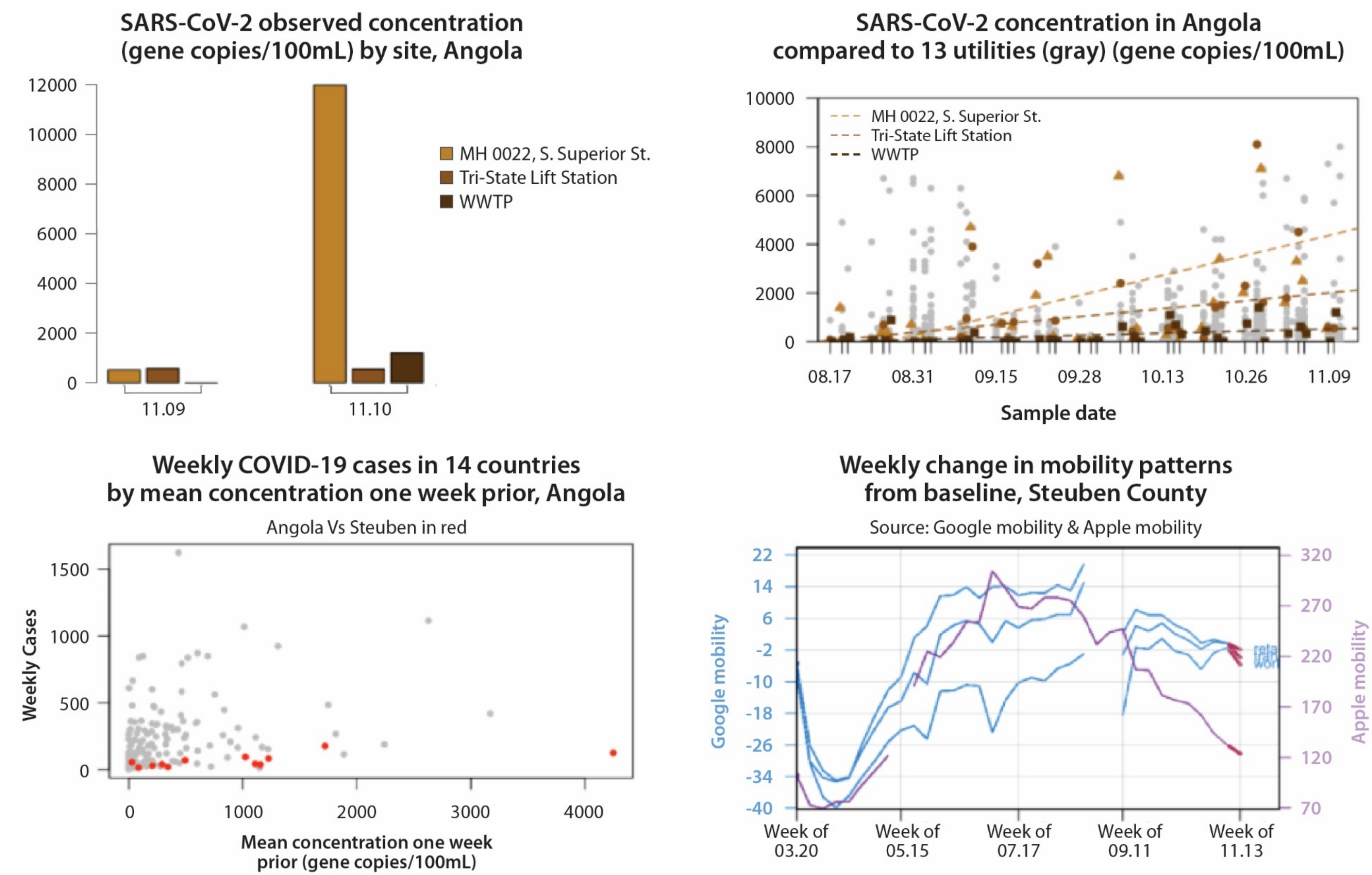

Figure 11. Example plots provided to the utilities including viral gene copies per 100 milliters (mL) for recent samples, concentrations over time as compared to the other utilities, weekly COVID-19 cases, and mobility metrics from Google and Apple (IFA, 2020)

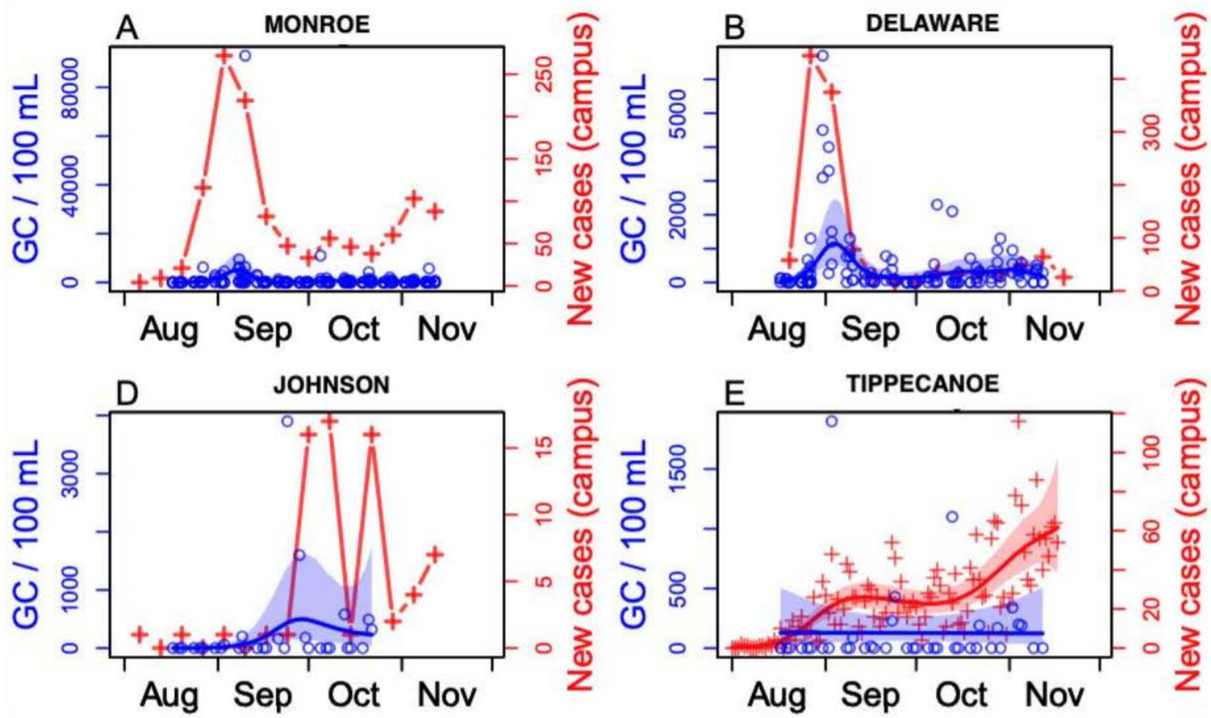

Figure 12. Plots of viral gene copies (GC) per mL in Indiana communities (Indiana, 2020).

6.2.2 Michigan

The Michigan wastewater surveillance program, which is led by the Michigan Department of Health and Human Services (MDHHS) and the Michigan Department of Environment, Great Lakes, and Energy (EGLE), began in September 2020 and ended February 2021. To quickly stand up a wastewater testing pilot project, EGLE relied on their established sampling program and laboratory network used to support the beach water monitoring program. As part of the beach program, EGLE had previously provided $500,000 to the laboratory network, led by Michigan State University, to procure RT-qPCR machines that the laboratories used to establish RT-qPCR analytical methods for *E. coli.* As a result, these laboratories were able to easily transition to measuring SARS-CoV-2 in wastewater (Michigan, 2021a).

Michigan funded the SARS-CoV-2 wastewater surveillance pilot project using CARES Act funding. MDHHS received CARES Act funding and provided these funds to EGLE for the statewide wastewater surveillance pilot project. EGLE used their existing grant process to distribute funding for local wastewater surveillance programs throughout the state. The grant application required a partnership with a wastewater utility and local health department, and ideally a laboratory to analyze the wastewater samples (Michigan, 2021b). EGLE distributed $10 million in grants to 20 projects that included over 270 sampling locations and community-scale samples from WWTPs and building samples from colleges and universities, assisted living and long-term care facilities, and K-12 schools with in-person learning (shown in Figure 13) (Michigan, 2021a).

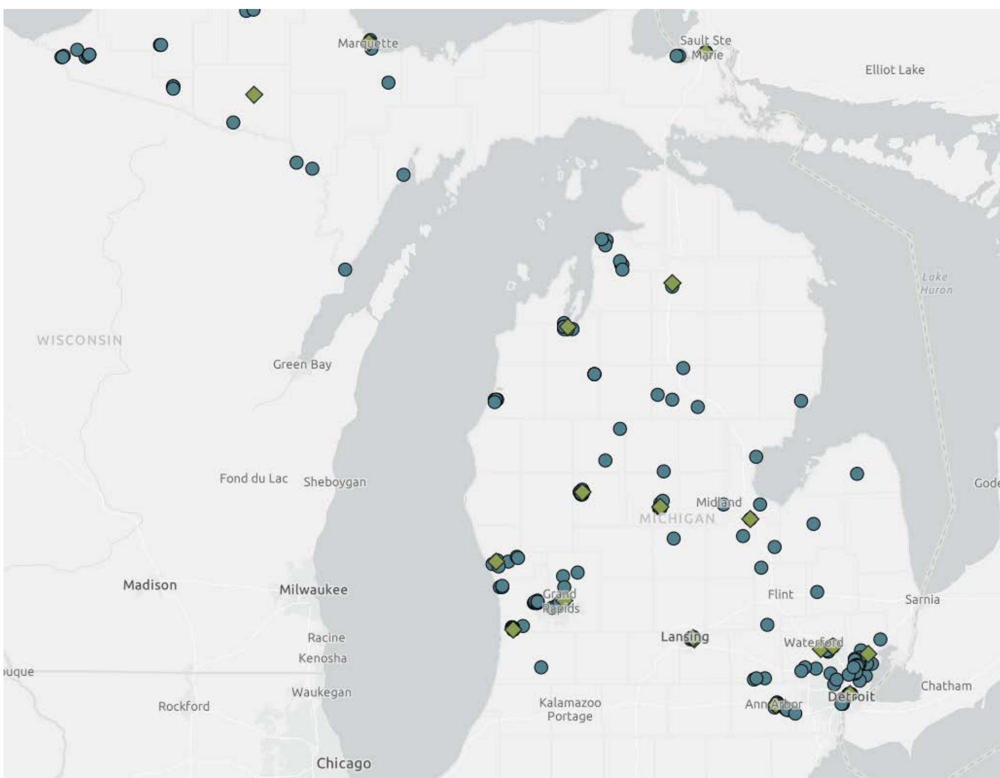

Figure 13. Michigan Wastewater Surveillance Program projects (green diamonds) and sampling locations (blue circles) (Michigan EGLE, 2021).

The 19 public, private, and academic laboratories participating in Michigan's program worked collectively to quickly develop a SARS-CoV-2 analytical method (Michigan, 2021a; Michigan, 2021b). All laboratories purchased the PCR primers and probes identified by CDC. Due to potential supply issues with RT-qPCR machines and supplies, most of the laboratories shifted to using RT-ddPCR. Michigan State University lead the RT-ddPCR analytical methods development. To ensure the SARS-CoV-2 wastewater results were consistent between laboratories, EGLE developed a Quality Assurance Project Plan that identified the control requirements and calculation methods, such as converting the cycle threshold (CT) score into virus copies/L (Michigan, 2021a). Michigan State University and Saginaw Valley State University participated in the WRF's interlaboratory methods evaluation study (WRF, 2020e).

Collaboration was a strength of Michigan's wastewater surveillance program. Michigan State University led weekly calls with the whole project team and hosted a Microsoft Teams page that included details on methods and quality assurance procedures. Michigan State University also hosted virtual office hours for all participants to answer questions and troubleshoot efforts (Michigan, 2021a).

Local projects provided the results to their local communities in real time to support public health actions. For example, the University of Michigan shared the SARS-CoV-2 wastewater results with the local health department and the campus COVID-19 response team. The campus COVID-19 response team conducted individual testing on students living in dormitories that had high levels of the virus in wastewater. In addition, the campus closed public locations, such as gyms, if high levels were detected from those buildings (Michigan, 2021a). As another example, one of the Michigan project teams was sampling wastewater from a Native American-owned casino, and the community decided to close the casino based on a combination of the wastewater surveillance data and community testing.

The Michigan wastewater surveillance pilot project ended in February 2021 after spending all the allocated CARES Act funding. Some of the participating sites continued wastewater surveillance using other funding sources. As of April 2021, MDHHS is reestablishing the statewide SARS-CoV-2 wastewater surveillance program using allocated funding from CDC's ELC Emerging Detection Enhancement award, with tentative plans to start in June 2021 (Peters, 2021).

6.2.3 New Mexico

The New Mexico wastewater surveillance program, led by the state's Environment Department, began in late 2020. Funded in part by the CARES Act, the program tracks the SARS-CoV-2 virus in wastewater from congregate facilities (e.g., youth shelters, detention centers). New Mexico chose to focus on these facilities to support direct public health action and because residents of congregate facilities are at higher risk of exposure to and illness from COVID-19. The wastewater results serve as an early indicator that—along with personal nasal or saliva testing and other public health interventions—is used to prevent or minimize a potential COVID-19 outbreak (NMED, 2021b).

As of early April 2021, New Mexico sampled 17 facilities in various regions of the state once or twice a week. Some facilities have multiple sampling points that divide the facility into smaller resident populations and/or designate separate COVID-19 quarantine areas (NMED, 2021a). This generates wastewater results that are more actionable for the facilities and public health professionals (NMED, 2021b).

New Mexico publishes the wastewater sampling results on a dashboard, categorizing the results by current level of concern and current trend, similar to other program dashboards (e.g., Clemson University) (NMED, 2021a). The current level of concern is defined as:

- less than 5,000 virus copies/L = no/minimal impact
- 5,000 to 10,000 virus copies/L = low
- 10,000 to 100,000 virus copes/L = moderate

- 100,000 to 1,000,000 virus copies/L = high
- greater than 1,000,000 virus copies/L = very high

The current trend is described as no trend, increasing, or decreasing based on the three most recent samples (NMED, 2021d).

New Mexico's wastewater surveillance program has helped multiple facilities successfully identify COVID-19 positive residents or staff through follow-up testing. One example is at J. Paul Taylor Center, a juvenile detention facility, where SARS-CoV-2 levels in the wastewater were consistently non-detectable. Then, on December 23, 2020, the surveillance program detected increased SARS-CoV-2 levels in the wastewater sample and notified the facility (see Figure 14) (NMED, 2021a). In response, the J. Paul Taylor Center conducted individual testing of all its residents and staff and found three positive staff members, one of whom was at the facility on the day that the sample was collected. The positive staff members quarantined off site, and the subsequent wastewater results returned to non-detectable levels (NMED, 2021c).

Another example is from Luna County Detention Center, which has two sampling points: one on the west side of the facility, which includes a COVID-19 quarantine area, and one on the east side of the facility, which should not have any residents with COVID-19. The wastewater sample from the east side was in the no/low impact level of concern until January 5, 2021, when the sample showed SARS-CoV-2 levels of over 1,000,000 virus copies/L (see Figure 15) (NMED, 2021a). Once again, the program notified the facility, which conducted 100 percent individual testing and identified 27 residents and 10 staff members with COVID-19. The inmates were transferred to the quarantine area on the west side of the facility and the staff quarantined off site, resulting in decreased SARS-CoV-2 levels in the wastewater (NMED, 2021b).

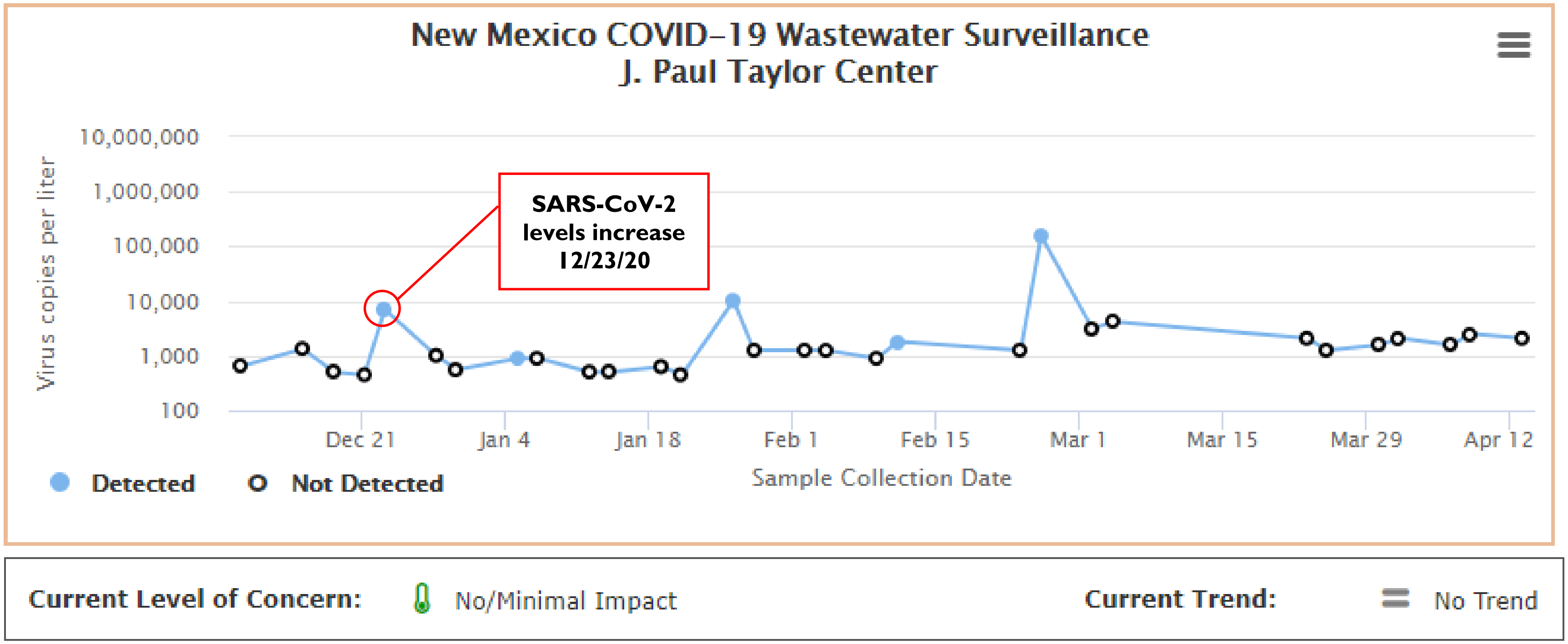

Figure 14. Wastewater surveillance data for the J. Paul Taylor Center (NMED, 2021a).

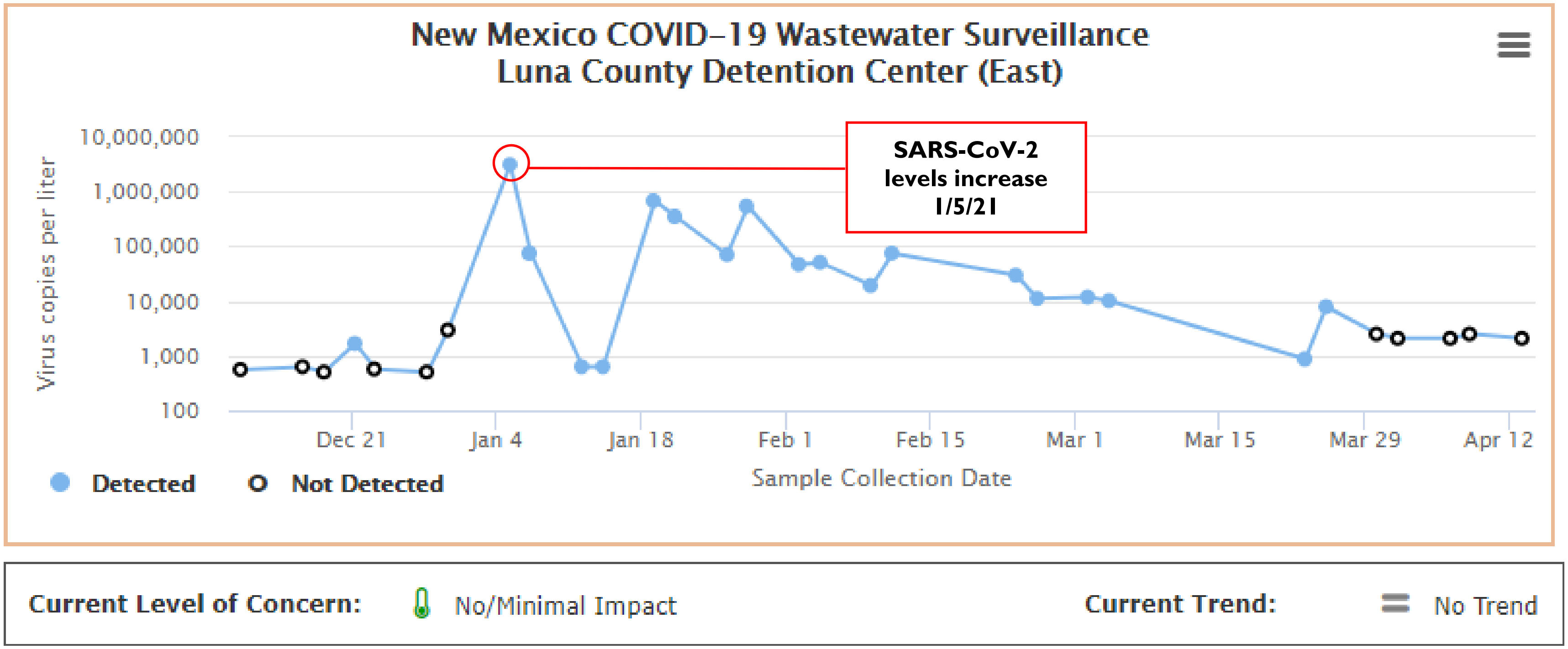

Figure 15. Wastewater surveillance data for Luna County Detention Center (east side) (NMED, 2021a).

6.2.4 Ohio

The Ohio wastewater surveillance program, led by the Ohio Department of Health (ODH) and Ohio Environmental Protection Agency (Ohio EPA), began in July 2020 after Ohio Governor DeWine became aware of the utility of wastewater surveillance and wanted to develop a program. ODH and Ohio EPA coordinated with the Ohio Water Resources Center (Ohio WRC) to implement the surveillance program using CARES Act funding. Ohio WRC had existing relationships with universities in Ohio, along with mechanisms to distribute funding to project teams. The U.S. EPA ORD in Cincinnati, Ohio also supported the program using internal funding (Ohio, 2021; Ohio DOH, 2020).

To begin the program, Ohio WRC invited researchers from about 10 universities and U.S. EPA to participate in a statewide monitoring program kickoff. The team established four subgroups to quickly develop every aspect of the program: a leadership group, site selection group, analytical methods group, and statistical modeling group. The site selection group had a goal to sample influent to WWTPs in as many counties to include as many people as possible throughout Ohio. The site selection group solicited partnerships with utilities directly and through state organizations, prioritizing WWTPs that served large or vulnerable populations and were willing to participate (Ohio, 2021). The site selection group used the vulnerability report that ODH and Ohio State University developed to support Ohio's COVID-19 efforts. The vulnerability report identified populations that are at risk for disproportionate burdens of illness and death from SARS-CoV-2 overlaid with social factors (e.g., median household income, occupation, education level) and healthcare resources (e.g., hospitals) (Nemeth et al., 2020). As of March 2021, Ohio's program included 65 WWTPs throughout the state, as presented in Figure 16 (Ohio DOH, 2020).

The analytical methods group included a network of university laboratories, ORD's laboratory, and out-of-state commercial laboratories. Each of the eight participating laboratories developed their own analytical method to foster SARS-CoV-2 methods development. Due to the pandemic creating supply chain issues for components of the analytical methods (e.g., buffers), some of the labs also had to adjust their method based on availability. Once a month, the program performs an interlaboratory validation whereby each lab receives a sample from the same WWTP for analysis. Once results are available, the participating laboratories meet to discuss the results and troubleshoot any items. For example, none of the laboratories pasteurize the samples due to lessons learned that pasteurization reduced the RNA PCR signal. Ohio WRC and U.S. EPA ORD also developed quality assurance and quality control requirements using positive spikes and human marker comparisons (Ohio, 2021). Additionally, U.S. EPA ORD and Ohio State University participated in the WRF's interlaboratory methods evaluation study (WRF, 2020e).

The statistical modeling group's focus was to evaluate whether the SARS-CoV-2 wastewater results could serve as an early warning system for potential COVID-19 outbreaks. The number of samples per week and analytical turnaround time were critical to the program's success. The program originally began with weekly sampling, but shifted to sampling twice per week to get more frequent results for the local health departments and enable more reliable trend analysis. The labs provide the results to the programs within three days of sample collection (Ohio, 2021). The statistical modeling group evaluated different trend analysis methods and modeling approaches to estimate the number of people shedding SARS-CoV-2 in sampled areas. The statistical modeling group decided to perform linear regression of the last ten wastewater results (normalized by flow). Ohio WRC said these linear regressions are not yet on the dashboard as of April 2021 (Bohrerova, 2021).

Ohio WRC compiles the results from all the labs, normalizes the results by WWTP influent flow rate to account for variations in flow rate due to rainfall, and uploads daily the results to ODH to be published on the program's dashboard (shown in Figure 17). ODH together with Ohio WRC and other stakeholders determined thresholds based on the results for the dashboard by comparing the average of the last two samples with the average of the prior third and fourth samples (Ohio, 2021; Ohio DOH, 2020). The thresholds are:

- Substantial increase in SARS-CoV-2 levels (purple) (greater than 100% increase)
- Increase in SARS-CoV-2 levels (red) (50% to 100% increase)
- Steady SARS-CoV-2 levels (gray) (49% decrease to 49% increase)
- Decrease in SARS-CoV-2 levels (blue) (greater than 50% decrease)

For utilities with significant increases in SARS-CoV-2 in the wastewater, ODH contacts the local health district and provides toolkits to assist with messaging about public health measures. ODH also coordinates with the local health districts to provide additional testing and contact tracing capabilities if necessary (Ohio, 2021).

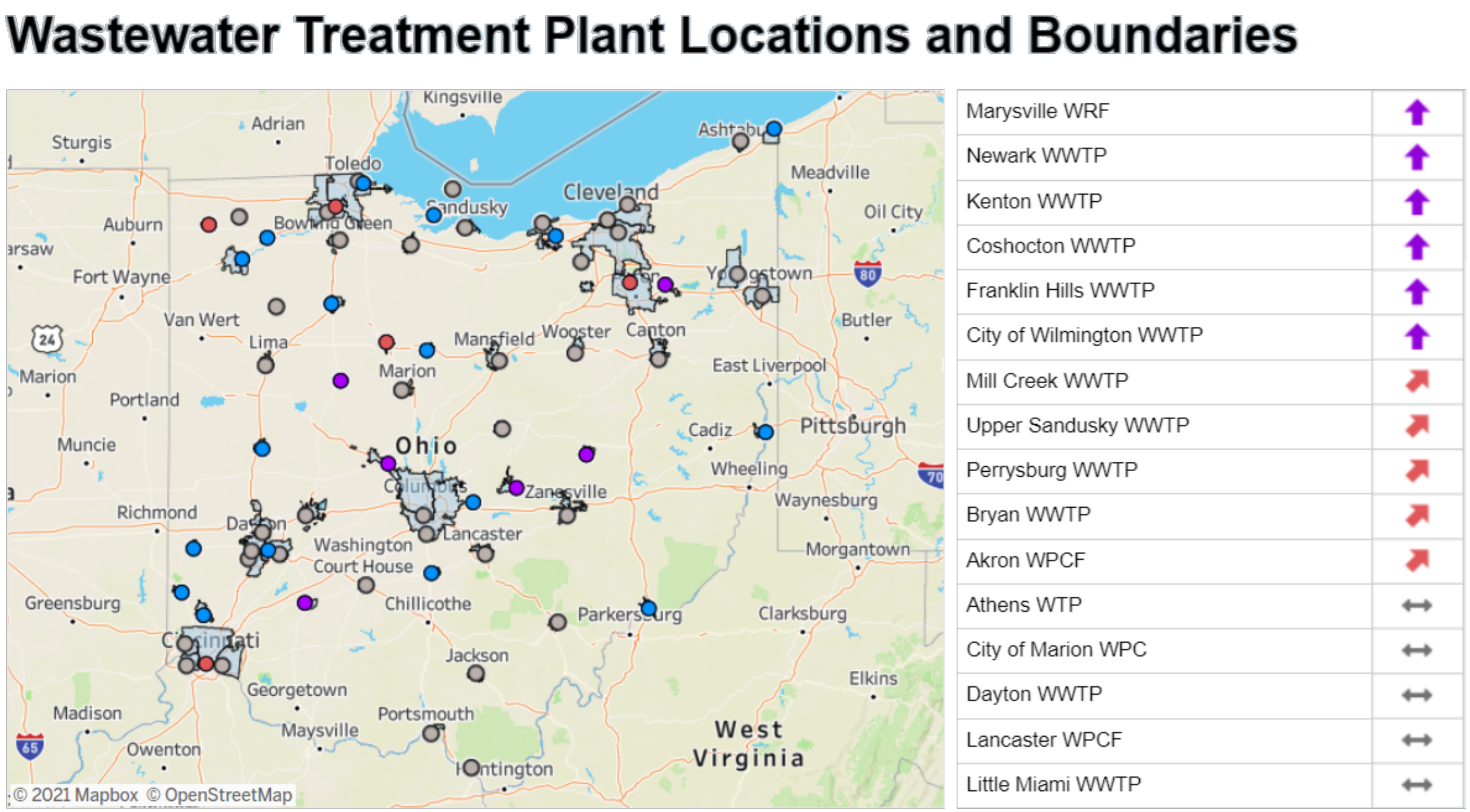

Figure 16. Ohio Coronavirus Wastewater Monitoring Network Dashboard showing all the participating utilities on the map and a list of utilities in order of the trend based on the most recent results (as of April 19, 2021) (Ohio DOH, 2020).

Figure 17. Ohio Coronavirus Wastewater Monitoring Network Dashboard showing city-specific results normalized by WWTP influent flow rate (top graph) and compared to the number of COVID-19 cases from individual testing (bottom graph) (Ohio DOH, 2020).

6.2.5 Wyoming

The Wyoming wastewater surveillance program, led by the Wyoming Public Health Laboratory (WPHL), Wyoming Department of Health (WDOH), and University of Wyoming, began in May 2020 using CARES Act funding and CDC's ELC funding. The program sampled both individual facility wastewater and community wastewater, including WWTP influent and lift stations within the communities' wastewater collection systems (Wyoming, 2021).

Wyoming incentivized community participation in the sampling efforts. First, Wyoming provided the wastewater sampling kits and coordinated sample shipping for each of the participating communities. In addition, Wyoming paid each community $300 per sample for their time and efforts and provided bonuses to communities that were consistently sampling. Most communities sample the WWTP influent twice a week. Next, since Wyoming required composite samples for participation in the program, Wyoming purchased composite samplers for the communities and reimbursed up to $4,000 for each composite sampler that communities purchased on their own. Wyoming also provided technical assistance to communities through the Wyoming Association of Rural Water Systems. Wyoming paid the Wyoming Association of Rural Water Systems for each community that participated in the program to further incentivize community participation (Wyoming, 2021). As a result, the Wyoming program currently includes 31 communities (Wyoming PHL, 2021). However, barriers to participation included concern from some communities that the detection of SARS-CoV-2 in the wastewater could lead to more restrictive public health measures, such as business shutdowns or gathering restrictions. Other communities were concerned a detection of SARS-CoV-2 in the wastewater would further stigmatize the community (Wyoming, 2021).

In addition to the WWTP influent or collection system sampling conducted by communities, the Wyoming program currently samples from 15 community living facilities with high-risk populations. These include Wyoming Department of Corrections facilities, Wyoming Department of Family Services housing, and dorms at the University of Wyoming and Laramie County Community College. Wyoming hired a contractor to collect composite samples from each facility to minimize the burden on the facilities (Wyoming, 2021).

Initially, WPHL analyzed all the wastewater samples, but as the program grew, WDOH began coordinating with the University of Wyoming to analyze some of the samples. The cooperation was enhanced because WPHL had previously established relationships with University of Wyoming faculty and had plans for other collaborative activities. WPHL and the University of Wyoming shared the same testing methods to ensure consistency through the testing process. Additionally, certain laboratory staff had been trained and worked at WPHL and then transferred to work at the university. WDOH then combines the data from both laboratories into one file for further statistical analysis (Wyoming, 2021). WDOH also models the estimated prevalence rates and uncertainty using the wastewater result as a proxy estimate for the percent of people infected with COVID-19 in each community (i.e., not for individual facilities) (Wyoming PHL, 2021). WDOH felt the prevalence rate was important to communicate the results to the participating communities and the general public, since CT and copies/mL are harder to understand (Wyoming, 2021). WDOH relied on information from a German study on fecal shedding rates for nine individuals and then ran data simulations to develop the estimation model (Wyoming PHL, 2021; Wölfel et al., 2020).

Wyoming's online wastewater surveillance dashboard includes a map of each community participating in the program. The color of the icon corresponds to the prevalence level of the virus (see Figure 18) with gray dots indicating communities without samples in the last 14 days. In addition, icons on the map outlined in red indicate an increasing prevalence level in the community. Once a user selects a community, Wyoming's dashboard adds three additional graphs of estimated prevalence rate, CT count (i.e., raw data from qPCR), and virus gene copies/mL all over time (see Figure 19). Wyoming's dashboard also includes graphs of virus gene copies/mL for each of the facility monitoring locations.

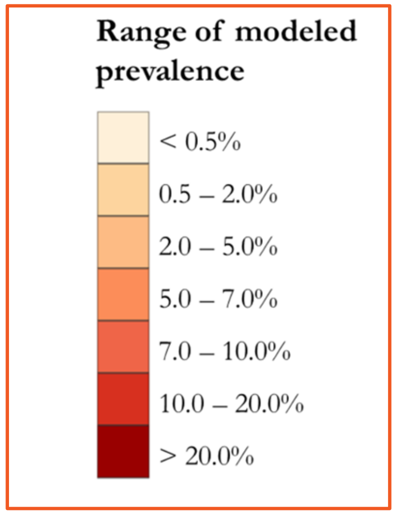

Figure 18. Wyoming State SARS-CoV-2 Wastewater Surveillance Dashboard prevalence ranges (Wyoming PHL, 2021).

Wyoming is comparing the community-level wastewater results to the state individual testing results to identify potential gaps in individual testing. As vaccines are available, Wyoming plans on using the SARS-CoV-2 wastewater program to monitor the spread of COVID-19 in communities with more limited access to vaccines. The facilities are also using the wastewater results to make public health decisions. For example, Honor Farm, a Wyoming Department of Corrections facility, conducted individual testing of all inmates and staff due to an increase in SARS-CoV-2 in the wastewater (Wyoming, 2021).

Beyond COVID-19, Wyoming is evaluating options for future wastewater surveillance use, such as in detection of other emerging diseases or antibiotic resistance (Wyoming, 2021).

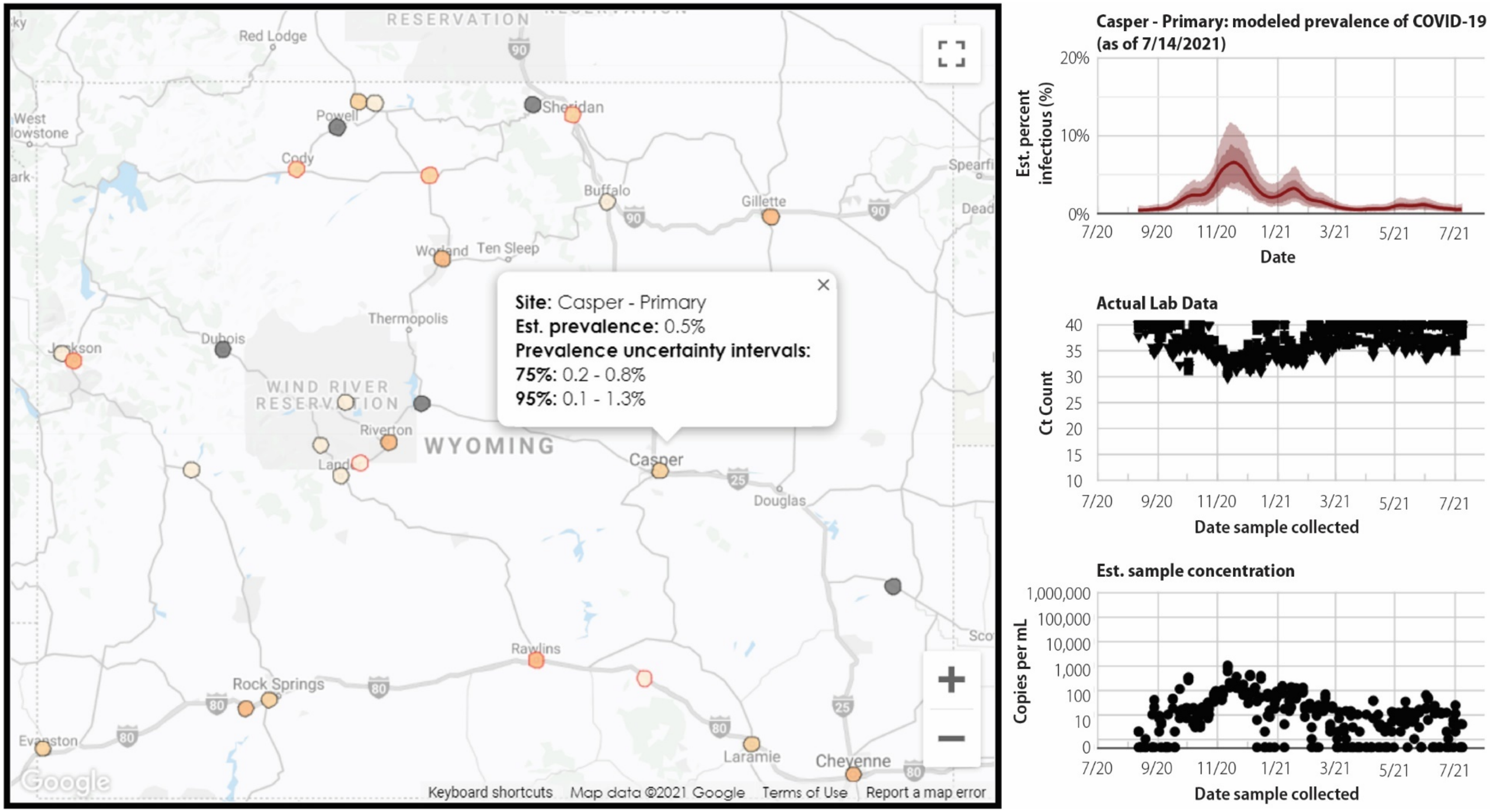

Figure 19. Wyoming State SARS-CoV-2 Wastewater Surveillance Dashboard (Wyoming PHL, 2021).

6.2.6 Hampton Roads Sanitation District in Hampton Roads, Virginia

The HRSD is a wastewater utility serving 20 cities and counties in southeastern Virginia. HRSD treats wastewater for more than 1.7 million people over a service area of nearly 5,000 square miles by operating 17 WWTPs of varying sizes. HRSD began a pilot microbial source tracking program to detect surface waters microbes in 2014. This provided HRSD with molecular experience, as well as the sampling and analytical equipment necessary when the COVID-19 pandemic started. As soon as CDC developed the clinical SARS-CoV-2 RT-qPCR diagnostic panel, HRSD shifted their focus to quantifying SARS-CoV-2 RNA in local raw influent wastewater. In early March 2020, HRSD began a SARS-CoV-2 wastewater surveillance pilot study using existing utility funding (HRSD, 2021b). In collaboration with experts from the University of Notre Dame and Ohio State University, HRSD then published a research paper on the results of their 21-week pilot program that analyzed WWTP influent from the nine large WWTPs in the region. The manuscript includes details on the program's analytical methods and data analyses (Gonzalez et al., 2020).

HRSD has continued weekly sampling of WWTP influent for SARS-CoV-2 beyond the pilot program for the large WWTPs in the region. In addition, HRSD participated in the WRF's interlaboratory methods evaluation (Pecson et al., 2021) and continues to refine their analytical methods over time as more information on SARS-CoV-2 becomes available (HRSD, 2021b).

HRSD displays up-to-date surveillance results on their HRSD COVID-19 Surveillance Dashboard as a graph of the total Hampton Roads viral SARS-CoV-2 load in local wastewater over time compared to clinical cases (see Figure 20). The figure includes the timing of state and local restrictions. HRSD's dashboard also presents the SARS-CoV-2 concentration spatially over time in a dynamic image. Figure 21 shows a snapshot in time of the spatial variation in normalized SARS-CoV-2 loading across the region. HRSD also provides wastewater results to the Virginia Department of Health and uploads the results to CDC's NWSS (HRSD, 2021b).

HRSD also analyzes wastewater samples for other Virginia government surveillance programs at-cost. As of April 2021, HRSD was analyzing wastewater samples from all 40 Virginia Department of Corrections facilities, as well as wastewater samples from some military barracks located in Virginia. In addition, HRSD has analyzed samples from the Western Virginia Water Authority in Roanoke, Virginia, since May 2020 (HRSD, 2021b).

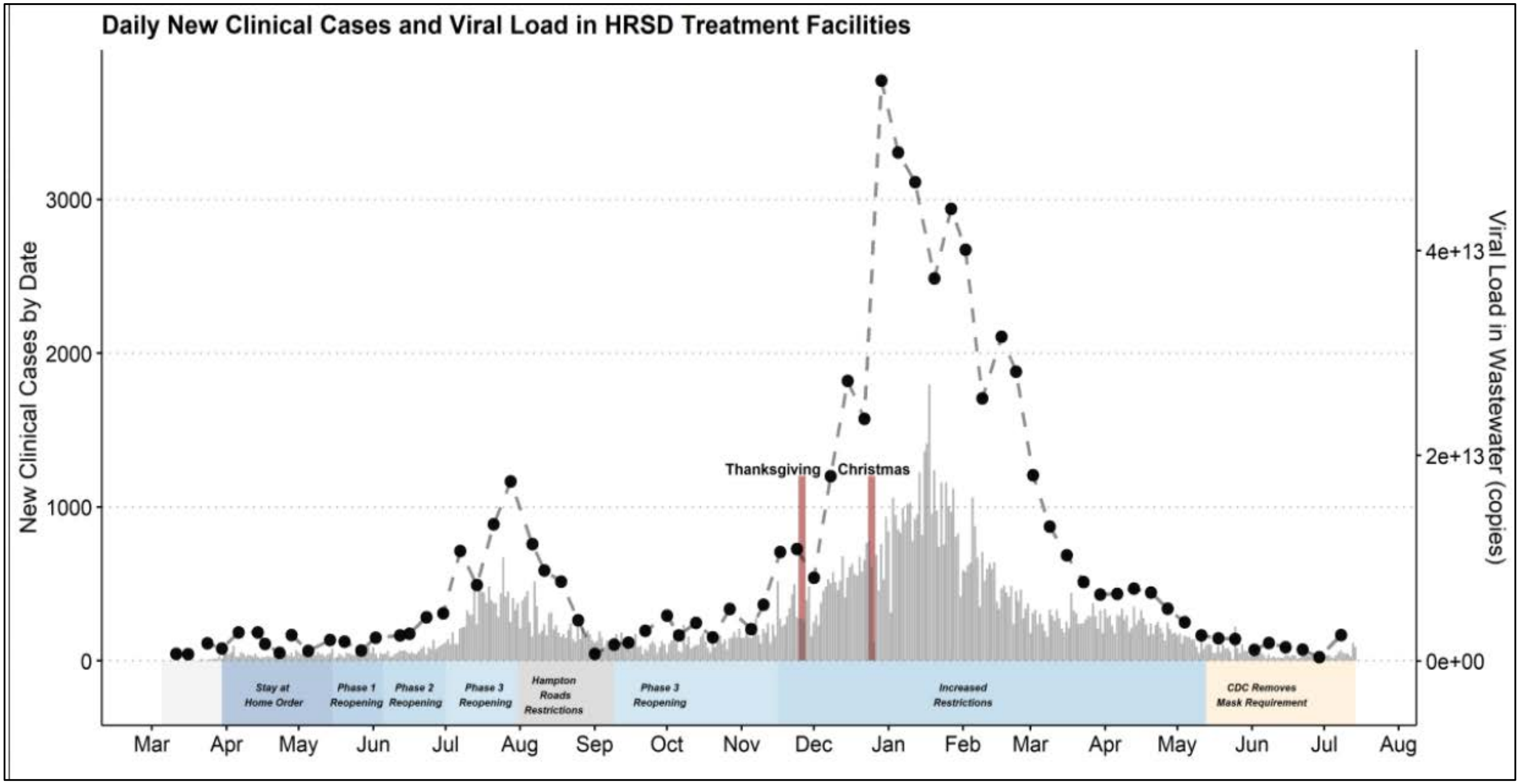

Figure 20. HRSD's dashboard presents the SARS-CoV-2 wastewater concentration and the new individual COVID-19 cases over time (HRSD, 2021a).

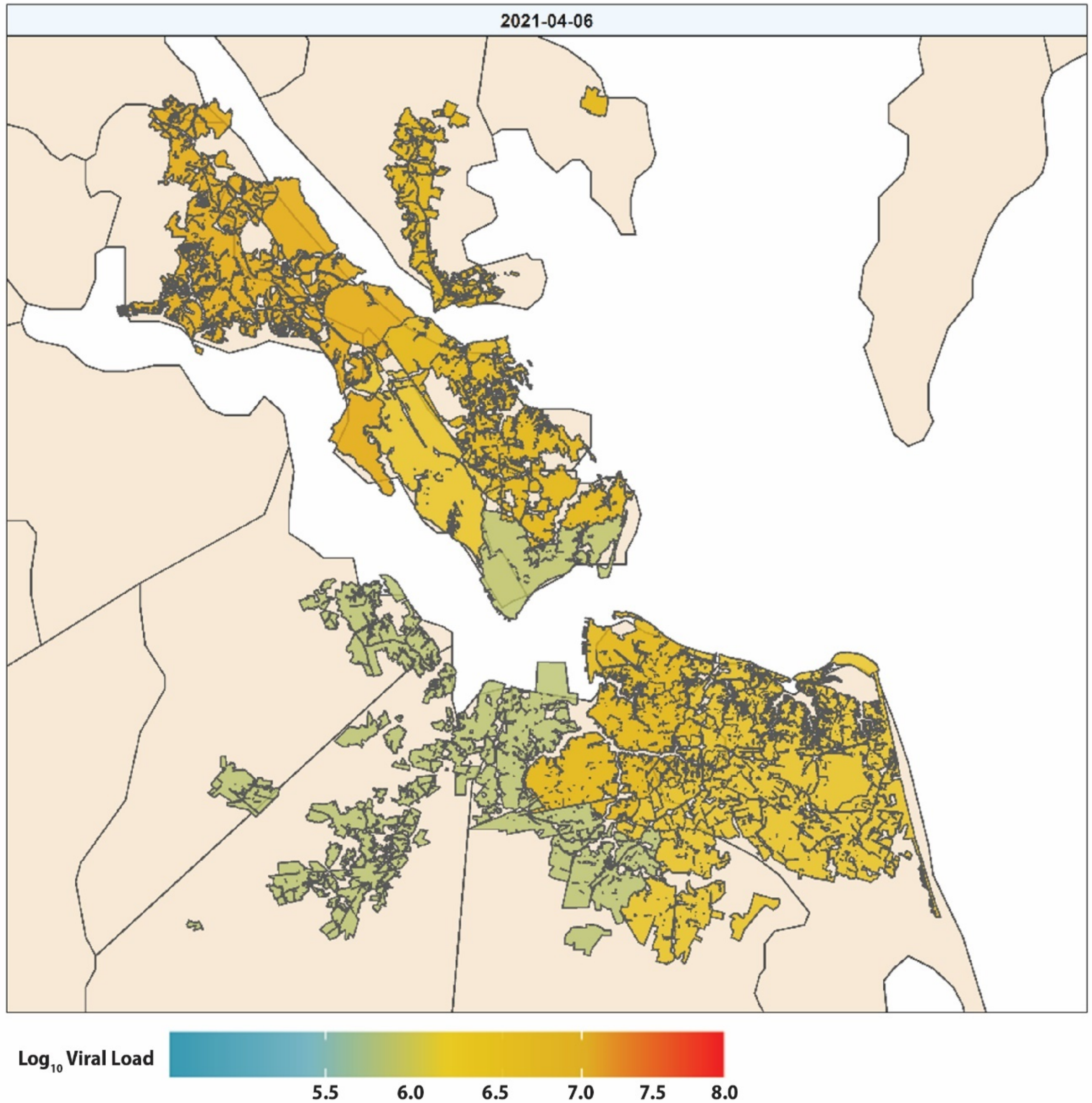

Figure 21. HRSD's dashboard presents the SARS-CoV-2 wastewater concentration spatially throughout the collection system over time (HRSD, 2021a)

6.2.7 Houston, Texas

The Houston wastewater surveillance program began in March 2020, led by HHD and in collaboration with Rice University for technical expertise and laboratory analyses. Baylor College of Medicine also provides laboratory support (HHD, 2020; Rice, 2020). The program was initially funded by Rice University an $200,000 ($35,000 subaward to Rice) NSF RAPID grant (Award #2029025), funding from CDC Foundation of $65,000, and received additional funding to expand through the CARES Act (Houston, 2021; NSF, 2020o). Houston also reprogrammed some of their funding received from CDC's ELC cooperative agreement to support the wastewater surveillance program (Houston, 2021).

Houston Water collects weekly wastewater samples at the influent of all 39 WWTPs in Houston, while HHD collects samples at other manhole and lift station locations throughout the city. Figure 22 presents a map of the areas the Houston surveillance program covers. As of March 2021, the program includes monitoring at 25 nursing homes, 51 K-12 schools, a correctional facility, and two homeless shelters, as well as at 30 lift stations to capture smaller segments of the community. Houston collects 24-hour composite samples at all locations, with the exception of schools, where the city collects six- to eight-hour composite samples to represent typical school hours (Houston, 2021).

HHD initially sent samples to a commercial laboratory but subsequently switched to local universities, Rice and Baylor, to reduce costs and minimize sampling result turnaround time. HHD worked closely with researchers at Rice University, who have provided technical expertise and laboratory analysis throughout the duration of the program, but also sent samples to Baylor for validation and standardization of the analytical methods (Houston, 2021). Although the universities used different methods (i.e., Rice used RT-ddPCR and Baylor used RT-qPCR), the universities worked together to optimize their approaches and analyzed their samples in triplicate to further verify results (Houston, 2021). For example, in order to choose the best method for standardization, the researchers compared five different RNA concentration and extraction methods. As the program grew, Rice also supported the transition of some of the wastewater sample analyses over to HHD's laboratory. HHD identified the flexible and adaptive nature of these academic partners as a key contributor to the success of their program, noting the rapid evolution in how data had to be analyzed, interpreted, and communicated to the public as more information became available (Houston, 2021).

HHD uses the wastewater data, along with other public health information, to identify "hot spot zones" for targeted public health interventions. HHD develops weekly reports that summarize available data for each of the 105 zip codes in Houston. HHD then identifies "hot spot zones" based on a detailed review of individual COVID-19 testing results, comparisons of wastewater results to the prior week and to peak levels observed in the summer of 2020 (see Figure 24), vaccination rates, and known presence of COVID-19 variants. HHD meets every other week to discuss the zip code summary reports, and in general, considers the wastewater data to be the most important metric (Houston, 2021).

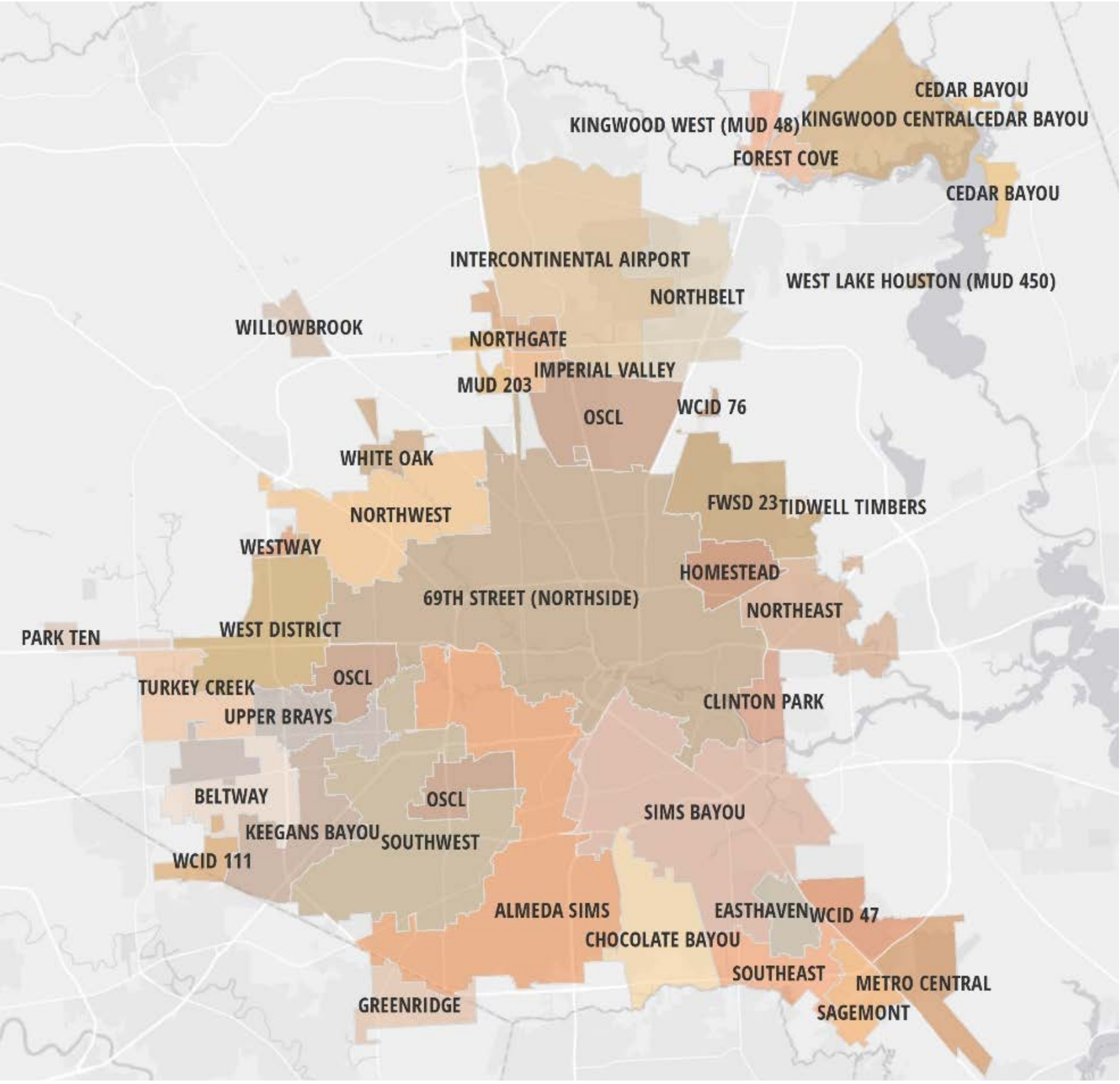

Figure 22. Areas of Houston covered by the wastewater surveillance program (Houston, 2021).

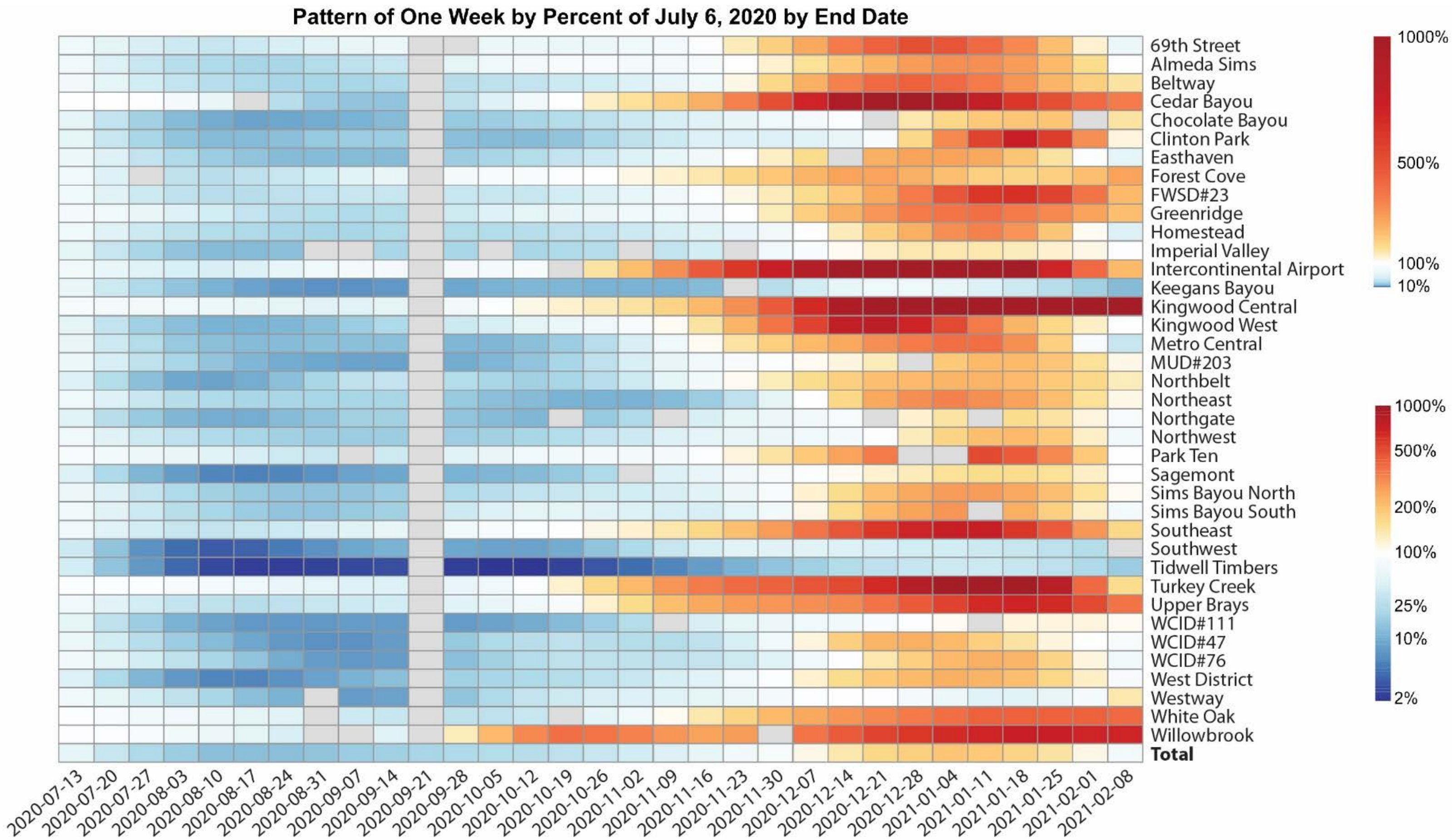

Figure 23. Percent change in wastewater results from July 6, 2020 (Peak) for areas of Houston covered by the wastewater surveillance program (Houston, 2021).

When HHD identifies new zip codes for public health intervention, they set up additional temporary vaccination and individual testing sites. HHD also identifies support in the local community (e.g., local churches or faith groups) to help connect with residents and determine barriers to testing and vaccination, as well as to schedule appointments and secure transportation to testing and vaccination sites. HHD provides funds to the local community groups for their involvement. Where wastewater samples are collected at individual facilities, HHD provides those facilities with the wastewater results weekly (Houston, 2021).

HHD attributes much of the success of their program to collaborations with researchers at Rice and Baylor, who supported the laboratory methods research while understanding the critical need for wastewater data to be actionable and to Rice for statistical interpretation and forecasting (Houston, 2021). Rice has recently begun quantifying two specific mutations in wastewater samples that are characteristic of the United Kingdom variant (B.1.1.7). Based on initial data, Rice is now working with HHD on sequencing the whole SARS-CoV-2 genome. While this approach does not definitively determine whether the variant is present, it provides information on the likelihood of variants being present among the population contributing to local wastewater. HHD uses the wastewater mutation sample data as a screening mechanism to identify communities where the mutations might be present and to help HHD determine where to allocate resources for genome sequencing of positive personal tests (Houston, 2021).

Beyond COVID-19, HHD and Rice are exploring future uses of wastewater surveillance (e.g., influenza and other viral pathogens) with hopes of developing a long-term robust monitoring program for the city (Houston, 2021).

6.2.8 Tempe, Arizona

In 2018, Tempe and Arizona State University's (ASU's) Biodesign Institute initiated a program to study opioids in wastewater with funding from the city council's Innovation Fund (Tempe, 2021d). For this effort, the city analyzed samples from five WWTPs for various parent drug compounds (e.g., fentanyl) and their metabolites (e.g., norfentanyl) (Tempe, 2021c). Tempe used the results to provide insight on illicit drug use in the city and to support public health decisions such as where to allocate resources and strategies for reducing opioid use. To foster community buy-in for this project, Tempe held a town hall meeting where a diverse panel of experts, including public health officials and the fire chief, described the project and then presented the data in the form of an online data dashboard. Tempe credits this meeting and the development of a publicly available dashboard as establishing the necessary foundation of transparency and trust with community members for the opioid wastewater surveillance program (Tempe, 2021d).

When the idea of testing wastewater for SARS-CoV-2 came about in February 2020, the city was able to quickly establish a program by leveraging community support and lessons learned from the city's existing opioids program, as well as systems like the partnership with ASU for laboratory analyses and the online data dashboard for disseminating results. Tempe began wastewater surveillance for SARS-CoV-2 in late March 2020 from one sewershed and expanded throughout the city shortly after (Tempe, 2021d). As of February 2021, Tempe collects 24-hour composite samples two to seven times per week in seven sewersheds (Tempe, 2021b). ASU then performs the laboratory analysis of SARS-CoV-2 via RT-qPCR on the wastewater samples using funding from NIH and NSF grants. Tempe presents the results in real time on an online data dashboard for use, along with other public health data, to drive decisions (e.g., when to close or reopen certain establishments) (Tempe, 2021d).

Tempe's success piqued the interest of, and partnership offer from the neighboring town of Guadalupe, which has a large population of essential workers and low-income and minority families. Guadalupe has since partnered with Tempe and ASU to monitor wastewater for SARS-CoV-2. Tempe posts the Guadalupe results on Tempe's online dashboard. Guadalupe's Mayor and Council use the results to support its public health messaging (e.g., the importance of social distancing) and decision-making (Tempe, 2021d).

Tempe's online data dashboard presents an interactive bar chart with average weekly SARS-CoV-2 wastewater results for each geographic collection area (see Figure 24) (Tempe, 2021a). The dashboard also includes an "event timeline" that indicates when the city implemented certain public health measures that may be tied to trends observed in the wastewater data (e.g., when dine-in restaurants were closed, when schools were closed, when the stay-at-home order was lifted) (Tempe, 2021a). Tempe also developed a timeline with user-friendly background information on local and state events related to COVID-19 that include events like (Tempe, 2021a):

- 3/14/2020 – Arizona closed schools statewide
- 3/29/2020 – Arizona issued a stay-at-home order
- 5/14/2020 – Arizona's stay at home order ended
- 6/10/2020 – COVID-19 testing became available in the Town of Guadalupe
- 6/19/2020 – Maricopa County required masks in public spaces
- 12/15/2020 – Tempe began phase 1A of the vaccine roll out
- 1/28/2021 – Arizona detected the first case of the U.K. COVID-19 variant
- 3/14/2021 – Tempe Elementary School District and Tempe Union High School District resumed in-person learning

Tempe's wastewater program for SARS-CoV-2 has offered an affordable way for the city to track COVID-19 in the community and has, in some cases, served as an early warning of increased COVID-19 spread (Tempe, 2021d). Tempe has also used the data to understand the distribution of COVID-19 cases throughout the city and to determine where to deploy additional services to educate the public and increase awareness on implemented public health measures. A key driver of this effort was to address equity issues within the community and determine if there were disproportionate concentrations of cases in certain locations. As an example, early in the program, Tempe observed elevated concentrations of SARS-CoV-2 in one area. Based on the wastewater data and the community demographics, Tempe recognized the need to provide additional resources and launched an educational campaign for the residents focused on reducing COVID-19 risk. City workers campaigned door to door throughout the community and distributed educational materials in Spanish and English, face masks, stickers, and care packages. After the campaign, the city observed slight decreases in SARS-CoV-2 wastewater measurements (Tempe, 2021d).

Beyond COVID-19, the city is evaluating options for future wastewater surveillance for other biomarkers that can indicate the presence of other viruses, drugs and alcohol, and other health concerns (e.g., asthma medicine) to aid in city planning (e.g., public education and outreach, allergy-friendly tree canopy selection) (Tempe, 2021d).

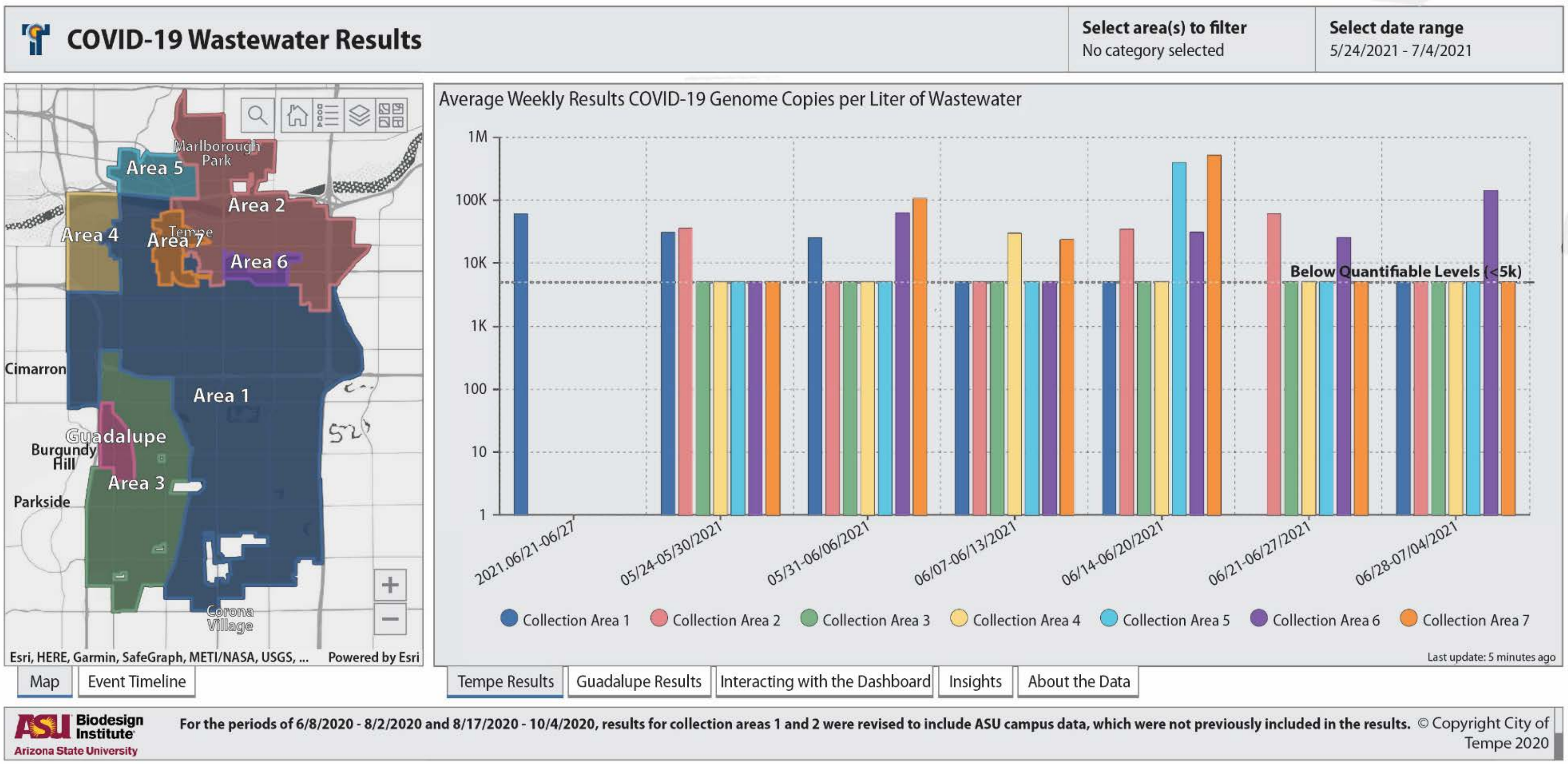

Figure 24. The City of Tempe's wastewater dashboard for SARS-CoV-2 with a map of the areas sampled (Tempe, 2021a).

6.2.9 Clemson University in Clemson, South Carolina

The Clemson University wastewater surveillance program, led by Dr. David Freedman, a professor in the Department of Environmental Engineering and Earth Sciences, began in late May 2020. Backed by university funding as part of Clemson's initiative to return students to campus amidst the COVID-19 pandemic (Clemson, 2021b), the program tracks both student transmission of the SARS-CoV-2 virus on campus, as well as student and public transmission of the virus in the city of Clemson and town of Pendleton, South Carolina (Clemson, 2021a).

Once or twice a week, 24-hour composite samples of influent wastewater are collected from an on-campus WWTP and two off-campus WWTPs (Clemson, 2021a; Clemson, 2021b). The samples are then sent to SiREM, a commercial lab in Knoxville, Tennessee, which analyzes for the virus using RT-qPCR for the N1 and N2 genes of SARS-CoV-2 (Clemson, 2021b; Clemson, 2020). Beginning in early March of 2021, Clemson expanded their analysis of the SARS-CoV-2 virus in wastewater to include the B.1.1.7 variant (i.e., the United Kingdom [U.K.] variant) and the B1.351 variant (i.e., the South African [S.A] variant). The laboratory uses the same molecular test used to quantify virus, except that the test is highly specific for the RNA that is uniquely associated with the variants.[8] Wastewater results for each variant are reported as percentages, where the variant RNA copies/L are divided by the total RNA virus copies/L (Clemson, 2021b). When none of the variant RNA is detected, the percentage is zero. When all of the total RNA virus detected consists of a variant, the percentage is 100.

Clemson's surveillance program reports each sampling location's SARS-CoV-2 results in copies/L on its online COVID-19 Wastewater Dashboard (see Figure 25) (Clemson, 2021a). The dashboard ranks the severity of the SARS-CoV-2 copies/L in wastewater by impact level. The impact levels allow the public to interpret the potential risk of COVID-19 from the wastewater more clearly. Dr. Freedman created the ranking system by comparing cases and surveillance results from European cities (e.g., Paris, Barcelona) to Clemson's results. The impact levels are as follows (Clemson, 2021b; Clemson, 2020):

- less than 4,000 virus copies/L = no impact
- 4,000 to 9,999 virus copies/L = minimal impact
- 10,000 to 99,999 virus copies/L = potential increasing impact
- 100,000 to 999,999 virus copies/L = potential moderate impact
- greater than 1,000,000 virus copies/L = potential significant impact

As of April 2021, the dashboard also includes results for the B.1.1.7 variant (see Figure 28) (Clemson, 2021a).

[8] SiREM quantifies variants in wastewater samples for Clemson University using the same RNA extracted to quantify the total SARS-CoV-2 virus. SiREM first uses the TaqPath COVD-19 PCR assay kit to quantify the total virus. This kit detects the S gene, N gene, and ORF1ab gene sequence. SiREM then uses a second PCR kit (GSD NovaType SARS-CoV-2 ID PCR) to detect the U.K. variant and the S.A. variant. Based on differences in Ct levels during the PCR reaction for RNA that is subjected to both kits, SiREM delineates what percentage of the total virus counts are attributable to the U.K. and S.A. variants. Differences in Ct levels are a result of mutations to the S gene that are present in the variants (Clemson, 2021b).

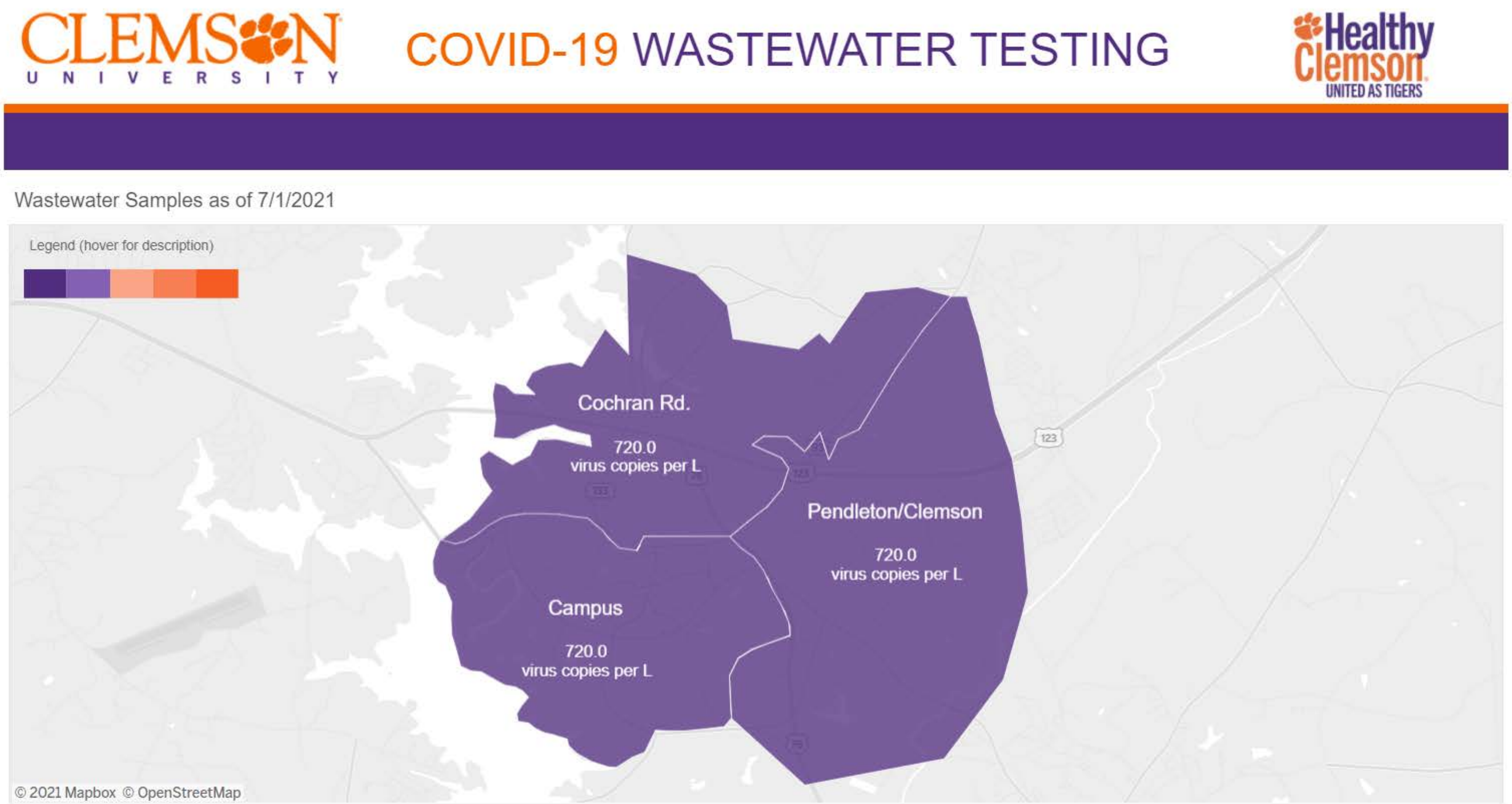

Figure 25. The Clemson University COVID-19 Wastewater Dashboard color codes the impact level on a map of each WWTP's collection system area (Clemson, 2021a).

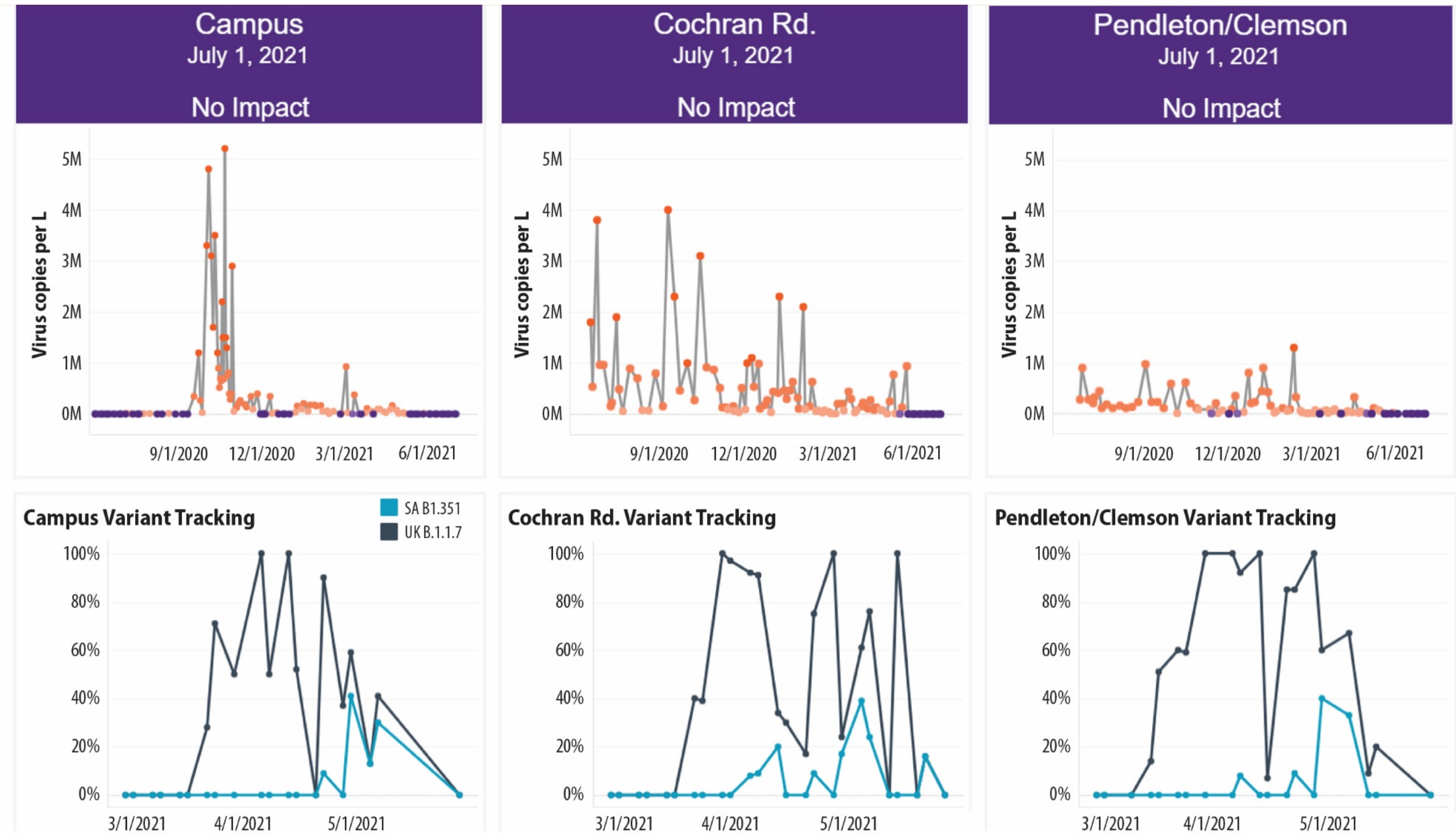

Figure 26. The Clemson University COVID-19 Wastewater Dashboard demonstrates the impact level and virus copies/L in each WWTP influent sampling point over time. The dashboard also includes variant tracking results (Clemson, 2021a).

Both the city of Clemson and Clemson University are using the wastewater surveillance program for their COVID-19 response (Clemson, 2021a; City of Clemson, 2021). In June 2020, when the city council began considering a mask ordinance, the city manager invited Dr. Freedman to present the elevated SARS-CoV-2 wastewater results from the city's Cochran Road WWTP (City of Clemson, 2020a). Shortly after hearing about the health concerns surrounding COVID-19 levels in the community, the city instituted a face mask ordinance citing the wastewater surveillance data and continues to do so with each subsequent extension of the ordinance. In October 2020, the city council extended the face mask ordinance until Christmas based on new elevated levels of SARS-CoV-2 in wastewater (City of Clemson, 2020b; Clemson, 2021b).

Clemson University increased the frequency of testing for SARS-CoV-2 in the wastewater after students returned to on-campus dormitories in Fall 2020. The wastewater results had detectable SARS-CoV-2, which did not align with the university's expectation that COVID-19 would be minimal on campus since students were required to have a negative COVID-19 test before and after arriving. Soon thereafter, Clemson University ramped up their individual testing plan for the fall semester to incorporate weekly testing for all students who live on campus. This was extended in the Spring 2021 semester to include weekly testing of any person who accessed a university building (Clemson, 2021b).

Clemson plans to continue monitoring the wastewater into the Summer of 2021, with declining concentrations anticipated in response to increasing levels of vaccination (Clemson, 2021b).

6.2.10 University of Arizona in Tucson, Arizona

The University of Arizona is leading and participating in multiple wastewater surveillance programs for SARS-CoV-2, much of which is spearheaded by Dr. Ian Pepper, Dr. Charles Gerba, and their team at the Water and Energy Sustainable Technology (WEST) Center (University of Arizona, 2021; University of Arizona, 2020b). The university's work in this field began in March 2020 when Dr. Pepper posted an advertisement on the WEST Center's website for SARS-CoV-2 analyses of the N1 and N2 gene variant in wastewater that utilities could receive for a fee. The WEST Center was able to quickly set up a laboratory for this work given their prior experience testing wastewater for other viruses, which also meant they already had the necessary equipment. Between March and the end of 2020, they analyzed approximately 350 samples from utilities throughout the U.S. and in Canada. In addition to providing SARS-CoV-2 wastewater results, Dr. Pepper and his team prepared summary reports with each data package that included a brief interpretation of the results and comparisons to the number of clinical cases in the local area at the time (University of Arizona, 2021; University of Arizona, 2020b).

In June 2020, the university created a task force to develop a plan and coordinate logistics for monitoring COVID-19 on campus with both personal testing and wastewater surveillance. The University President was supportive of the wastewater surveillance program and provided funding from the university (University of Arizona, 2021). Shortly after the wastewater program began and students returned to campus in late August, the university detected SARS-CoV-2 in the wastewater from one of the dormitories. Based on the wastewater results, the university tested all students in the dormitory, ultimately identifying two asymptomatic students who tested positive for COVID-19. The students were immediately isolated and SARS-CoV-2 was not detected in wastewater collected the next day—a success story that has garnered national attention (University of Arizona, 2021; University of Arizona, 2020a). The university has repeated this success and potentially prevented outbreaks nearly one hundred other times, where SARS-CoV-2 detected in wastewater triggered personal testing that identified students with COVID-19 for isolation. In general, wastewater testing was found to be a good predictor of COVID-19 cases. When SARS-CoV-2 was detected in wastewater, the university often found students who tested positive for COVID-19 in the same dormitory. Conversely, when SARS-

CoV-2 was not detected in wastewater, students often did not test positive for COVID-19 (Betancourt et al., 2021).

The university currently collects grab samples three times per week at 18 dormitories on campus and analyzes the samples on the same day they are collected (University of Arizona, 2021). To ensure that the wastewater data reflect a distinct population and are actionable, samples are collected at dormitories with a single sewer line. Tied to this, the university has developed action levels for their campus wastewater surveillance program (see Table 6). For example, non-detect wastewater results require no action, while concentrations between 1,000 and 10,000 copies/L trigger 20 percent random personal testing of dormitory residents. This program is a strong example for how rapid wastewater results and an efficient alert system can help minimize the spread of COVID-19 (Gerba, 2021).

Table 6. University of Arizona's levels of concern and associated actions for ranges of SARS-CoV-2 wastewater concentrations.

Level of Concern	Wastewater SARS-CoV-2 Concentration Order of Magnitude (gene copies/L)	Action Item
0	Non-detect	No action
1	10 to less than 1,000	Enhanced awareness and disinfection
2	1,000 to less than 100,000	20% random testing of residents
3	100,000 to less than 10,000,000	40% random testing of residents
4	10,000,000 or higher	All residents tested

Based on the success of the on-campus wastewater surveillance program, the university's Yuma Center of Excellence for Desert Agriculture received funding from the Arizona Department of Health in January of 2021 to monitor wastewater in Yuma County. Dr. Pepper's team helped colleagues establish an off-campus laboratory and develop a sampling plan. This plan involved dividing the county into 13 distinct regions and then identifying "high-risk" facilities (e.g., schools, nursing homes) for personal testing when community wastewater samples show increased levels of SARS-CoV-2 (University of Arizona, 2021; Ducey, 2021; Gerba, 2021).

In addition to these implementation programs, Dr. Pepper and the WEST Center team are actively researching methods for predicting numbers of infected individuals with wastewater data. For example, they are currently using personal testing and wastewater data collected on campus to predict the shedding rate of the virus in feces. The university is also testing the wastewater for mutations of the SARS-CoV-2 virus to evaluate the likelihood of variants present (University of Arizona, 2021).

7 Wastewater Surveillance Lessons Learned

EPA identified 14 states with large-scale SARS-CoV-2 wastewater surveillance programs, along with 160 local communities or academic institutions conducting wastewater surveillance. Each of these groups relied on different approaches for their program, from analytical methods to funding mechanisms to communication of the results. All the programs showed that wastewater surveillance can be an important and effective tool for early detection of SARS-CoV-2, giving the programs the ability to take action to prevent the continued spread of COVID-19. The programs also noted that adequate and sustained funding was critical to their success and that without it, a surveillance program would be hard to maintain.

EPA selected ten of these programs to highlight in the case studies presented in Section 6.2. These case studies present the different approaches for implementing programs and analyzing wastewater data to track the presence of SARS-CoV-2. The technical leads for the case study programs noted that much of their success was dependent on four key aspects of their wastewater surveillance programs: collaboration, flexibility, transparent communication, and adequate funding. In terms of collaboration, the wastewater surveillance programs leveraged resources through establishing partnerships and relationships with entities within their programs, as well as with external groups. For example, the Wyoming wastewater surveillance program initiated its own outreach efforts, but also relied on outreach support from the Wyoming Association of Rural Water Systems. These collaborative efforts helped educate utilities on the benefits of wastewater surveillance, ultimately leading to their voluntary participation in the state's program (Wyoming, 2021).

The success of these programs was also tied to their ability to be flexible and open to the rapidly developing science around wastewater surveillance for SARS-CoV-2. This flexibility included adapting to ongoing developments in analytical methods and data use by public health officials, as well as availability of necessary materials for sample collection and data analysis. For example, the Michigan wastewater surveillance program originally used RT-qPCR for laboratory analysis, but later shifted to RT-ddPCR due to supply chain issues with some of the materials needed for RT-qPCR methods (Michigan, 2021a). As another example, the Ohio wastewater surveillance program initially launched their program with weekly wastewater sampling, but then changed to twice per week as it provided more meaningful and actionable data for the local health departments (Ohio, 2021).

Program leads also highlighted the necessity of transparent communication within their organizations and with stakeholders in order to gain community support. For example, the Ohio wastewater surveillance program began after Ohio Governor DeWine became aware of wastewater surveillance. This led to a successful program because of the support from the highest levels within the state, including leadership at the ODH and Ohio EPA (Ohio, 2021). As another example, the opioid wastewater surveillance program in Tempe, Arizona, established strong community buy-in by having public health officials and the local fire chief share information during town hall meetings. Tempe was able to build on these relationships and the confidence established within the local community to quickly implement a wastewater surveillance program for SARS-CoV-2 (Tempe, 2021d).

The programs also identified that adequate and sustained funding was critical to their success in providing consistent support for public health. For example, Michigan's wastewater surveillance pilot project ended in February 2021 after spending all their CARES Act funding. Some of the participating sites continued wastewater surveillance using other funding sources, but some sites had to stop sampling. MDHHS was able to reestablish the wastewater surveillance program using funding from CDC's ELC Emerging Detection Enhancement award with tentative plans to start in June 2021 (Peters, 2021). On the other hand, Wyoming had sufficient funding to incentivize community participation by paying the communities $300 per sample, providing bonuses to communities that consistently sampled, and paying technical assistance providers for onboarding communities into the program. Wyoming used CARES Act and CDC ELC funding, originally budgeting for 50 to 100 communities participating. With the 30 communities that Wyoming included in early 2021, they estimated there were about 30 months of funding remaining (Wyoming, 2021).

Wastewater-based surveillance is a new and changing field. This report highlights the unique aspects of some of the wastewater surveillance implementation work and lessons learned. This rapidly evolving field can be an effective tool for early detection of SARS-CoV-2 and other pathogens in the future, especially among disadvantaged or vulnerable populations where clinical testing may not be widely available.

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Appendix A.
Summary of Wastewater Surveillance Programs

Table A-1. State wastewater surveillance programs.

State	Lead Agency	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard	Dashboard Description (Link Included in Reference)
Colorado	Colorado Department of Public Health and Environment	Colorado State University, Metropolitan State University, GT Molecular	August 2020	22 WWTPs	Unknown	Yes	Map of sewersheds with a timeseries of wastewater results in copies/L. Also includes a bar chart of personal testing data (Colorado DPH, 2021).
Connecticut	Connecticut Department of Health	Yale University, Connecticut Agricultural Experimental Station	August 2020	7 WWTPs	RT-qPCR	Yes	Map of WWTP locations, chart of wastewater N1 and N2 data in copies/L with a seven-day trendline, and a chart of personal testing data (Yale University, 2021a).
Indiana	Indiana Finance Authority	120Water, University of Notre Dame, Indiana University, Microbac	August 2020	15 WWTPs, 44 other locations	RT-ddPCR and RT-qPCR	No	Not applicable. Indiana published a report with the results (Indiana, 2020).
Kansas	Kansas Department of Health and Environment	University of Kansas	May 2020	12 WWTPs	Unknown	No	Not applicable. No centralized dashboard, but some cities have posted results (University of Kansas, 2020; Lawrence, 2021).
Maryland	Maryland Department of the Environment	Maryland Department of Health, St. Mary's College, MetCom	Pilot phase: Summer 2020 Follow-on phase: Winter 2021	2020 pilot phase: 27 low-income housing areas and 10 correctional facilities Follow-on phase: As of May 2021, 22 sites	RT-qPCR	Yes	Map of sampling locations with color-coded map markers based on SARS-CoV-2 trend with plots of copies/L over time (Maryland, 2021).

Table A-1. State wastewater surveillance programs.

State	Lead Agency	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard	Dashboard Description (Link Included in Reference)
Michigan	Michigan Department of Health and Human Services	Michigan Environment, Great Lakes, and Energy; universities	September 2020	270 WWTPs and facilities	RT-ddPCR	Yes	Map of testing sites and relevant detail such as lab name, grant award and amount, start date, sample type, and partner name (Michigan EGLE, 2021).
Missouri	Missouri Department of Health and Senior Services	Missouri Department of Natural Resources, University of Missouri	July 2020	50 WWTPs	Unknown	Yes	Map of WWTPs with color-coded points based on wastewater trend, and a timeseries plot of viral copies/day (Missouri DHSS, 2021).
New Mexico	New Mexico Environment Department	None	December 2020	22 congregate facilities	Unknown	Yes	Timeseries plots of copies/L sampling results over time for each facility, along with current level of concern and current trend (NMED, 2021a).
North Dakota	North Dakota Department of Environmental Quality	North Dakota State University	July 2020	21 WWTPs	Unknown	No	Not applicable. No public dashboard (Dura, 2020; Cooper, 2021).
Ohio	Ohio Department of Health	Ohio EPA, Ohio WRC, U.S. EPA, and other participating universities	July 2020	66 WWTPs	Unknown	Yes	A map of WWTPs color-coded to show the status of SARS-CoV-2 in wastewater (e.g., increasing). Also includes a timeseries plot for each WWTP with showing million gene copies/day and average N2 gene copies/L over time (Ohio DOH, 2020).

Table A-1. State wastewater surveillance programs.

State	Lead Agency	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard	Dashboard Description (Link Included in Reference)
Oregon	Oregon Health Authority	Oregon State University	August 2020	42 WWTPs	Unknown	Yes	Map of WWTPs that indicates whether SARS-CoV-2 was detected or not detected in the week of monitoring; can adjust the date to see over time (Oregon Health Authority, 2021).
Utah	Utah Department of Environmental Quality	Utah Department of Health. University of Utah, Utah State University, and Brigham Young University	April 2020	42 WWTPs	Unknown	Yes	Map of WWTP sewersheds with timeseries plot of wastewater data in million gene copies/person/day and daily new individual testing cases (Utah DEQ, 2021).
Wisconsin	Wisconsin Department of Health Services	Wisconsin State Lab of Hygiene, University of Wisconsin-Milwaukee	October 2020	65 WWTPs, including 4 tribal WWTPs	Unknown	Yes	Map of WWTP sewersheds with a timeseries plot of SARS-CoV-2 in million gene copes/person/day and the seven-day average individual case rate of COVID-19 (Wisconsin DOHS, 2020).
Wyoming	Wyoming Public Health Laboratory	Wyoming Department of Health, University of Wyoming, Wyoming Association of Rural Water Systems	July 2020	34 WWTPs and 13 facilities	RT-qPCR	Yes	Map of WWTPs color coded by percent of modeled prevalence of COVID-19 infections based on wastewater results. Also includes timeseries plots of modeled prevalence, Ct count, and copies/mL for WWTPs and facilities (Wyoming PHL, 2021)

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
University of Alaska–Anchorage	AK	Unknown—program details were not researched (see Section 3)					
University of Alaska–Fairbanks	AK	Unknown—program details were not researched (see Section 3)					
Birmingham Southern College	AL	Unknown—program details were not researched (see Section 3)					
University of Arkansas	AR	Unknown—program details were not researched (see Section 3)					
Gilbert	AZ	Arizona State University	May 2020	3 areas in collection system	RT-qPCR	Yes	Map of sewershed areas for each sample location with a timeseries bar chart with average weekly results in copies/L (Gilbert, 2021).
Tempe and Guadalupe	AZ	Arizona State University	March 2020	8 WWTPs and sewersheds	RT-qPCR	Yes	Map of sewershed areas for each sample location with a timeseries bar charts with average weekly results in copies/L (Tempe, 2021a).
University of Arizona	AZ	University of Nevada- Las Vegas	March 2020	18 dorms	RT-qPCR	No	Not Applicable. No public dashboard (University of Arizona, 2020a; University of Arizona, 2020b; University of Arizona, 2021).
University of Northern Arizona	AZ	Unknown—program details were not researched (see Section 3)					

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
Navajo Nation	AZ	Unknown—program details were not researched (see Section 3)					
Alameda County	CA	Unknown—program details were not researched (see Section 3)					
City of Davis	CA	University of California - Davis	November 2020	11 sewersheds	Unknown	Yes	Timeseries plot for each sewershed with virus concentration normalized to fecal strength, city-wide wastewater results, and detection limit (Healthy Davis Together, 2021).
City of Palm Springs	CA	GT Molecular	August 2020	1 WWTP	Unknown	Yes	Weekly reports with timeseries plots of copies/L and trendline; includes significant events. Also evaluating for variants (Palm Springs, 2021).
Contra Costa County	CA	Unknown—program details were not researched (see Section 3)					
Loma Linda University	CA	Unknown—program details were not researched (see Section 3)					
Los Angeles County Sanitation Districts	CA	University of Arizona, California State Water Resources Control Board	August 2020	2 WWTPs	Unknown	Yes	Timeseries plots with weekly average copies/L, seven-day average new COVID-19 cases, and three-day average LA County hospitalizations (LA County, 2021).
Marin County	CA	Unknown—program details were not researched (see Section 3)					

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
Mariposa County	CA	Biobot Analytics	May 2020	3 locations	Unknown	Yes	Timeseries plots with data from each of the three sampling locations as virus concentration/L (Mariposa County, 2021).
San Francisco County	CA	Unknown—program details were not researched (see Section 3)					
Santa Clara County	CA	Santa Clara County Department of Environmental Health, City of Palo Alto, City of San Jose, Sunnyvale, Gilroy, Stanford University	October 2020	4 WWTPs	Unknown	Yes	Timeseries plot for each WWTP with the S and N genes normalized to pepper mild mottle virus (Santa Clara County, 2021).
Stanford University	CA	Unknown—program details were not researched (see Section 3)					
University of California–Berkeley	CA	None	August 2020	5 locations on campus	RT-qPCR	Yes	Map of sampling locations with icons indicating detected or not detected SARS-CoV-2 or no sample collected (UC Berkeley, 2021).
University of California–Irvine	CA	Unknown—program details were not researched (see Section 3)					
University of California–Merced	CA	Unknown—program details were not researched (see Section 3)					

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
University of California–San Diego	CA	None	Summer 2020	640 campus buildings	RT-qPCR	Yes	A campus map that highlights buildings different colors based on if they are monitored/not monitored and if SARS-CoV-2 was detected/not detected in the wastewater (UC San Diego, 2021).
University of California–Santa Barbara	CA	Unknown—program details were not researched (see Section 3)					
University of Southern California	CA	None	October 2020	Dorms, athletic facilities, and office buildings	RT-ddPCR	No	Not applicable. No public dashboard (USC, 2021).
Colorado College	CO	Unknown—program details were not researched (see Section 3)					
Colorado State University	CO	None	Beginning of fall semester 2020	17 locations on campus, focused on dorms	RT-ddPCR	No	Not applicable. No public dashboard (CO State, 2020).
University of Colorado–Boulder	CO	None	August 2020	23 sites on-campus (residence halls, other buildings)	RT-qPCR	No	Not applicable. No public dashboard (CU Boulder, 2020).
University of Denver	CO	Unknown—program details were not researched (see Section 3)					
Metro State University	CO	Unknown—program details were not researched (see Section 3)					

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
University of Connecticut	CT	None	June 2020	1 WWTP and 14 buildings	RT-qPCR	Yes	Timeseries plot of the ratio of the E and N1 genes to a fecal indicator virus on collection dates for each of the sampling locations (UConn, 2021).
University of Hartford	CT	Unknown—program details were not researched (see Section 3)					
Howard University	DC	Unknown—program details were not researched (see Section 3)					
New Castle County	DE	Biobot Analytics, University of Delaware	April 2020	3 WWTPs and sewersheds	PCR	Yes	Timeseries plot of the aggregate sewer system viral copies/L and rolling seven-day average confirmed COVID-19 cases. Also includes latest viral copes/L for each sampling location and timeseries plots of viral copies/L with 95% confidence interval shading for each sampling result. Also includes sewershed maps for each sampling location (NCCo, 2021).
Broward County	FL	Unknown—program details were not researched (see Section 3)					
Florida Atlantic University	FL	None	March 2020	Campus lift stations	PCR	No	Not applicable. No public dashboard (Randall, 2020).

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
Loxahatchee River District	FL	Biobot Analytics, Florida Department of Health	May 2020	1 WWTP	qPCR	Yes	Timeseries plots with copies/L in log scale and linear scale are compared to new clinical cases and seven-day rolling average clinical cases (LRD, 2021).
Miami-Dade County	FL	Biobot Analytics	March 2020	3 WWTPs	Unknown	No	Not applicable. No public dashboard (WaterWorld, 2020).
Ringling College of Art and Design	FL	Unknown—program details were not researched (see Section 3)					
Rollins College	FL	Unknown—program details were not researched (see Section 3)					
University of Florida	FL	Unknown—program details were not researched (see Section 3)					
Athens-Clarke County	GA	University of Georgia	June 2020	3 WWTPs	RT-PCR	Yes	Timeseries plot of total viral copies with a 95% confidence interval, predicted total viral copies, along with daily and seven-day rolling average COVID-19 reported cases (UGA, 2021).
Emory University	GA	Unknown—program details were not researched (see Section 3)					
University of Hawaii–Manoa	HI	Unknown—program details were not researched (see Section 3)					
City of Boise	ID	University of Missouri, Boise State University	May 2020	2 WWTPs	Unknown	Yes	Timeseries plot with wastewater results in copies/L and a plot of confirmed and probable COVID-19 cases (Boise, 2021).

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
City of Moscow	ID	Biobot Analytics, University of Idaho	May 2020	1 WWTP	RT-PCR	No	Not applicable. No public dashboard (Argonaut, 2020).
Twin Falls	ID	Unknown—program details were not researched (see Section 3)					
University of Idaho	ID	None	May 2020	9 campus locations	RT-PCR	No	Not applicable. No public dashboard (Argonaut, 2020).
Chicago	IL	University of Illinois - Chicago, Northwestern University, Argonne National Laboratory, Metropolitan Water Reclamation District of Greater Chicago, Chicago Department of Public Health	Unknown	3 WWTPs and sewersheds	Unknown	No	Not applicable. No public dashboard available (UIC, 2020).
Kendell County	IL	Yorkville-Bristol Sanitary District, RJN Group, GT Molecular	November 2020	1 WWTP	Unknown	Yes	Map with WWTP sewershed, timeseries plot of virus concentration and number of positive COVID-19 tests and average COVID-19 positivity percent for Kendall County. Also includes wastewater result trend (YBSD, 2021).

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
Northern Illinois University	IL	Unknown—program details were not researched (see Section 3)					
University of Illinois at Chicago–School of Public Health	IL	Unknown—program details were not researched (see Section 3)					
City of Carmel	IN	Biobot Analytics, University of Notre Dame, Pace Analytical	May 2020	1 WWTP	Unknown	No	Not applicable. No public dashboard available (Carmel, 2020).
University of Notre Dame	IN	None	July 2020	Throughout campus	RT-ddPCR	No	Not applicable. No public dashboard available (Zacharias, 2020).
City of Lawrence	KS	University of Kansas	June 2020	2 WWTPs	Unknown	Yes	Map of the sewersheds for each WWTP along with interactive timeseries plots with the N1 and N2 copies/L and average in copies/day, along with the 14-day rolling average new COVID-19 cases (Lawrence, 2021).
University of Kentucky	KY	None	September 2020	Campus dorms	PCR	No	Not applicable. No public dashboard available (Chapin, 2020).
University of Louisville	KY	Unknown—program details were not researched (see Section 3)					
Murray State University	KY	Unknown—program details were not researched (see Section 3)					

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
Louisiana State University	LA	Baton Rouge	June 2020	Throughout campus and surrounding community	qPCR	No	Not applicable. No public dashboard available (Rddad, 2020).
Tulane University	LA	New Orleans	January 2020	Throughout campus and surrounding community	Unknown	No	Not applicable. No public dashboard available (Zobel, 2020).
Massachusetts Institute of Technology	MA	None	October 2020	7 campus buildings	RT-qPCR	No	Not applicable. No public dashboard available (Winn, 2020).
Northeastern University	MA	Unknown—program details were not researched (see Section 3)					
Town and County of Nantucket	MA	Biobot Analytics	April 2020	1 WWTP	RT-qPCR	Yes	PDF with weekly results that includes a timeseries plot of viral copies/L sewage with the daily and seven-day rolling average of new COVID-19 cases. Also includes comparisons of wastewater to samples nationwide. Includes Biobot's COVID-19 incidence estimate of new cases/day based on the wastewater results (Nantucket, 2021).
University of Massachusetts Lowell	MA	Unknown—program details were not researched (see Section 3)					

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
Williams College	MA	Unknown—program details were not researched (see Section 3)					
Frederick County	MD	CosmosID	May 2020	1 WWTP	RT-qPCR	No	Not applicable. No public dashboard available (Frederick County, 2020).
Mount St. Mary's University	MD	Unknown—program details were not researched (see Section 3)					
St. Mary's College of Maryland	MD	Unknown—program details were not researched (see Section 3)					
St. Mary's County	MD	St. Mary's County Health Department, St. Mary's College of Maryland, St. Mary's County Metropolitan Commission	July 2020	9 WWTPs	Unknown	Yes	Map of the WWTP service areas along with timeseries plots for each of the WWTPs with the virus copies/L (St. Mary's, 2021).
University of Maryland	MD	None	September 2020	Throughout campus	Unknown	No	Not applicable. No public dashboard available (Neugeboren, 2020).
Washington Suburban Sanitary Commission	MD	University of Maryland, Montgomery County, Prince George's County	Unknown	6 locations	Unknown	No	Not applicable. No public dashboard available (UMD, 2020).
Saint Joseph's College of Maine	ME	Unknown—program details were not researched (see Section 3)					

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
University of Maine–Orono, Fort Kent, and Presque Isle and University of Southern Maine–Gorham	ME	CES, Inc., Orono, Farmington	July 2020	9 campus locations and 2 WWTPs	Unknown	Yes	Tables for each sample location with positive/negative/ indeterminate categorization and viral equivalence/L for positive results (Maine, 2021).
University of Southern Maine	ME	Unknown—program details were not researched (see Section 3)					
Albion College	MI	Unknown—program details were not researched (see Section 3)					
Alma College	MI	Unknown—program details were not researched (see Section 3)					
Central Michigan University	MI	Unknown—program details were not researched (see Section 3)					
Eastern Michigan University	MI	Unknown—program details were not researched (see Section 3)					
Ferris State University	MI	Unknown—program details were not researched (see Section 3)					
Grand Valley State University–AWRI	MI	Unknown—program details were not researched (see Section 3)					
Hope College	MI	Trident Laboratories, Holland BPW	Unknown	9 campus locations and 1 WWTP	qPCR	No	Not applicable. No public dashboard available (Hope College, 2021)
Michigan State University	MI	None	May 2020	Campus manholes	Unknown	No	Not applicable. No public dashboard available (Lavery, 2020).
Oakland University	MI	Unknown—program details were not researched (see Section 3)					

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
Saginaw Valley State University	MI	Unknown—program details were not researched (see Section 3)					
University of Michigan	MI	None	Unknown	Throughout campus	Unknown	No	Not applicable. No public dashboard available (UMich, 2020).
University of Minnesota	MN	Unknown—program details were not researched (see Section 3)					
University of Minnesota Duluth	MN	Unknown—program details were not researched (see Section 3)					
University of Minnesota Rochester	MN	Unknown—program details were not researched (see Section 3)					
Missouri State University	MO	Unknown—program details were not researched (see Section 3)					
University of Mississippi	MS	Unknown—program details were not researched (see Section 3)					
Carroll College	MT	Unknown—program details were not researched (see Section 3)					
Montana State University	MT	Unknown—program details were not researched (see Section 3)					
Appalachian State University	NC	University of North Carolina	Fall 2020	8 dorms	RT-ddPCR	No	Not applicable. No public dashboard available (Buffy, 2021).

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
Jackson County	NC	Tuckaseigee Water and Sewer Authority, Jackson County Department of Public Health, University of Wisconsin-Milwaukee, Dogwood Health Trust, Mathematica	July 2020	1 WWTP	Unknown	No	Not applicable. No public dashboard available (Mathematica, 2020).
Raleigh	NC	North Carolina State University	March 2020	1 WWTP, 2 residential sewersheds, and hospital	Unknown	No	Not applicable. No public dashboard available (Sherman, 2020).
North Carolina State University	NC	Unknown—program details were not researched (see Section 3)					
University of North Carolina–Chapel Hill	NC	Unknown—program details were not researched (see Section 3)					
University of North Carolina–Charlotte	NC	None	Unknown	20 locations throughout campus	Unknown	No	Not applicable. No public dashboard available (UNC Charlotte, 2020).
University of North Carolina–Wilmington	NC	Unknown—program details were not researched (see Section 3)					

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
North Dakota State University	ND	Unknown—program details were not researched (see Section 3)					
University of Nebraska	NE	Unknown—program details were not researched (see Section 3)					
Darmouth-Hitchcock Medical Center	NH	Unknown—program details were not researched (see Section 3)					
Keene State College	NH	City of Keene, Cheshire Medical Center	August 2020	Campus and off-campus WWTPs	Unknown	Yes	Bar chart with sampling results for both sampling locations along with two trendlines in viral copies/L. Chart also includes significant events. Note that the dashboard does not include the entire timeframe (Keene, 2021).
University of New Hampshire	NH	None	August 2020	Throughout campus	RT-ddPCR	No	Not Applicable. No public dashboard available (UNH, 2020).
Bergen County	NJ	Columbia University, Bergen County Utilities Authority, AECOM	March 2020	1 WWTP and throughout sewershed	RT-qPCR	No	Not applicable. No public dashboard available (AECOM, 2020; Chen, 2020).
New Jersey Institute of Technology	NJ	None	September 2020	Each occupied dorm	PCR	No	Not applicable. No public dashboard available (NJIT, 2021).
New Mexico State University	NM	Unknown—program details were not researched (see Section 3)					

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
University of New Mexico	NM	Unknown—program details were not researched (see Section 3)					
University of Nevada–Reno	NV	Unknown—program details were not researched (see Section 3)					
University of Nevada–Las Vegas	NV	Unknown—program details were not researched (see Section 3)					
Clarkson University–Potsdam Hill Campus	NY	Unknown—program details were not researched (see Section 3)					
Colgate University	NY	Unknown—program details were not researched (see Section 3)					
Columbia University	NY	Unknown—program details were not researched (see Section 3)					
New York City	NY	New York City Department of Environmental Protection, New York City Department of Health and Mental Hygiene	April 2020	14 WWTPs	Unknown	No	Not applicable. No public dashboard available (Kilgannon, 2020).

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
Onondaga County	NY	Syracuse University, State University of New York College of Environmental Science and Forestry, State University of New York Upstate Medical University	August 2020	6 WWTPs	Unknown	No	Not applicable. No public dashboard available (Cox, 2020).
Queens College, City University of New York	NY	Unknown—program details were not researched (see Section 3)					
Rochester Institute of Technology	NY	None	August 2020	15 locations throughout campus	Unknown	No	Not applicable. No public dashboard available (Auburn, 2020).
Siena College	NY	None	August 2020	9 areas of campus	Unknown	No	Not applicable. No public dashboard available (Siena, 2020).
St. John Fisher College	NY	None	August 2020	All dorms	Unknown	No	Not applicable. No public dashboard available (St. John Fisher, 2020).
St. Lawrence University	NY	Unknown—program details were not researched (see Section 3)					
State University of New York–Albany	NY	Unknown—program details were not researched (see Section 3)					
State University of New York–Canton	NY	Unknown—program details were not researched (see Section 3)					

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
State University of New York–College of Environmental Science and Forestry	NY	Unknown—program details were not researched (see Section 3)					
State University of New York–Morrisville	NY	Unknown—program details were not researched (see Section 3)					
State University of New York–Oneonta	NY	Cornell, Syracuse University, Upstate Medical, Quadrant Biosciences	August 2020	3 points throughout campus	Unknown	No	Not applicable. No public dashboard available (Kelvin, 2020).
State University of New York–Oswego	NY	Unknown—program details were not researched (see Section 3)					
Stony Brook University	NY	Unknown—program details were not researched (see Section 3)					
Kent State University	OH	Unknown—program details were not researched (see Section 3)					
Kenyon College	OH	Unknown—program details were not researched (see Section 3)					
Ohio University	OH	Unknown—program details were not researched (see Section 3)					
University of Oklahoma	OK	Unknown—program details were not researched (see Section 3)					

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
City of Corvallis	OR	Oregon State University, Oregon Health Authority	July 2020	5 sewershed locations and 1 WWTP	RT-qPCR	Yes	Map of sampling locations with a timeseries plot of copies/L for each sampling location and for all sampling locations overlaid (Corvallis, 2021).
Borough of Indiana	PA	Biobot Analytics	April 2020	1 WWTP	Unknown	Yes	Timeseries plot of average daily flow, viral copies, cumulative COVID-19 cases, and weekly COVID-19 case change. Also includes a trend indicator with decreasing, no change, or increasing trends (Borough of Indiana, 2021).
Erie County	PA	Erie County Department of Health, Biobot Analytics	May 2020	1 WWTP	Unknown	No	Not applicable. No public dashboard available (Erie News Now, 2021).
Franklin and Marshall College	PA	Unknown—program details were not researched (see Section 3)					
Lehigh University	PA	Unknown—program details were not researched (see Section 3)					
Penn State University	PA	None	September 2020	Campus WWTP and sewersheds, off-campus WWTP	Unknown	No	Not applicable. No public dashboard available (LaJeunesse, 2020).
Susquehanna University	PA	Unknown—program details were not researched (see Section 3)					

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
Clemson University	SC	City of Clemson	May 2020	3 WWTPs	RT-qPCR	Yes	A map of collection areas color coded by the current impact based on the wastewater results. Also includes timeseries plots of results in viral copies/L for each WWTP and timeseries plots of variant ratios in percentages over the same time (Clemson, 2021a).
University of South Carolina	SC	Unknown—program details were not researched (see Section 3)					
City of Chattanooga	TN	Biobot Analytics	May 2020	1 WWTP	RT-qPCR	Yes	Bar chart with SARS-CoV-2 concentrations. Also includes Biobot weekly reports with normalized viral copies/L sewage compared to daily and seven-day rolling average COVID-19 cases; includes comparisons of wastewater to samples nationwide and Biobot's COVID-19 incidence estimate of new cases/day based on the wastewater results (Chattanooga, 2021).
Gallatin City–County Health Department	MT	Archer Biologicals, LLC; Montana State University	March 2020	5 WWTP	qPCR	Yes	Timeseries plot of N1 and N2 in genome copies/L compared to the number of COVID-19 cases for Bozeman. Remaining WWTPs present table with results (Healthy Gallatin, 2021).

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
University of Tennessee	TN	None	Unknown	At least 10 campus buildings	Unknown	No	Not applicable. No public dashboard available (Crawford, 2020).
Baylor College of Medicine	TX	Unknown—program details were not researched (see Section 3)					
Houston	TX	Houston Health Department, Rice University, Baylor College of Medicine	May 2020	39 WWTPs	RT-ddPCR and RT-qPCR	No	Not applicable. No public dashboard available (Houston, 2021).
Texas A&M University	TX	Unknown—program details were not researched (see Section 3)					
University of Texas	TX	Unknown—program details were not researched (see Section 3)					
University of Texas–San Antonio	TX	Unknown—program details were not researched (see Section 3)					
Utah State University	UT	Utah Department of Environmental Quality	July 2020	6 communities at Logan campus, 4 dorms at Eastern campus, and 2 dorms at Blanding campus	Unknown	No	Not applicable. No public dashboard available (USU, 2021).

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
Hampton Roads Sanitation District	VA	University of Notre Dame, Ohio State University	March 2020	9 WWTPs	RT-ddPCR	Yes	Timeseries plot of viral load for all WWTPs normalized by WWTP flow rate with new clinical cases. Also includes a mapping of results normalized to population over time for each of the service areas (HRSD, 2021a).
Stafford County	VA	Biobot Analytics, Harvard University, Massachusetts Institute of Technology, and Brigham and Women's Hospital	April 2020	2 WWTPs	Unknown	No	Not applicable. No public dashboard available (Stafford County, 2020).
University of Virginia	VA	None	September 2020	15 dorms and university hospital	Unknown	No	Not applicable. No public dashboard available (Whitman, 2020).
Virginia Tech	VA	None	September 2020	15 dorms	Unknown	No	Not applicable. No public dashboard available (Adams, 2020).

Table A-2. Municipality and university wastewater surveillance programs.

Locality Name or Academic Institution	State	Collaborators/ Partners	Sampling Start Date	Types and Number of Sampling Locations	Analytical Method	Dashboard?	Dashboard Description
City of Burlington	VT	Vermont Department of Health, Dartmouth Hitchcock Medical Center, University of Vermont, GoAigua	August 2020	3 WWTPs	Unknown	Yes	Timeseries plot of copies/L for each of the WWTPs; timeseries plot of percent of variant (Del6970 [B117], N501Y [B117], and E484K [Unknown]) for each WWTP starting February 2021 (Burlington, 2021).
Norwich University	VT	Unknown—program details were not researched (see Section 3)					
University of Vermont	VT	Unknown—program details were not researched (see Section 3)					
University of Washington	WA	Unknown—program details were not researched (see Section 3)					
University of Wisconsin–Oshkosh	WI	Unknown—program details were not researched (see Section 3)					
West Virginia University	WV	Unknown—program details were not researched (see Section 3)					
University of Wyoming	WY	Unknown—program details were not researched (see Section 3)					