Rapid Implementation of High-Frequency Wastewater Surveillance of SARS-CoV-2

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ABSTRACT: There have been over 507 million cases of COVID-19, the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), resulting in 6 million deaths globally. Wastewater surveillance has emerged as a valuable tool in understanding SARS-CoV-2 burden in communities. The National Wastewater Surveillance System (NWSS) partnered with the United States Geological Survey (USGS) to implement a high-frequency sampling program. This report describes basic surveillance and sampling statistics as well as a comparison of SARS-CoV-2 trends between high-frequency sampling 3–5 times per week, referred to as USGS samples, and routine sampling 1–2 times per week, referred to as NWSS samples. USGS samples provided a more nuanced impression of the changes in wastewater trends, which could be important in emergency response situations. Despite the rapid implementation time frame, USGS samples had similar data quality and testing turnaround times as NWSS samples. Ensuring there is a reliable sample collection and testing plan before an emergency arises will aid in the rapid implementation of a high-frequency sampling approach. High-frequency sampling requires a constant flow of information and supplies throughout sample collection, testing, analysis, and data sharing. High-frequency sampling may be a useful approach for increased resolution of disease trends in emergency response.

KEYWORDS: wastewater surveillance, SARS-CoV-2, wastewater treatment plant, community trends, wastewater-based epidemiology, wastewater sampling

INTRODUCTION

COVID-19, the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was declared a pandemic by the World Health Organization (WHO) in March 2020 and has resulted in over 507 million cases and 6 million deaths globally.¹ COVID-19 symptoms include fever, cough, and shortness of breath, but almost 30% of infected individuals are asymptomatic.²,³ Asymptomatic infections pose a challenge for public health control because the affected individuals often do not seek care or testing. As a result, the presence of asymptomatic infections leads to under-ascertainment of SARS-CoV-2 in a community. Other challenges to accurate ascertainment of cases include a lack of capacity and funding for large-scale clinical testing and healthcare. Public health agencies continue to face many challenges identifying and tracking the burden of COVID-19 in a community, but new methods to monitor the outbreak, such as wastewater surveillance, are emerging.⁴

SARS-CoV-2 is a respiratory pathogen; however, viral ribonucleic acid (RNA) may be detected in stool of infected individuals.⁵–⁶ A meta-analysis from Zhang et. al found that the pooled prevalence of SARS-CoV-2 RNA in stool samples from COVID-19 cases was 43%.⁷,⁸ Some research indicates that viral RNA can be detected in stools before symptom onset; however, it has also been noted that positive nasal swabs precede the detection of viral RNA in stool samples.⁵,⁶,⁷,⁹ Research continues to develop to refine the timeline of respiratory and fecal positivity in infected individuals.⁵ Because SARS-CoV-2 can be detected in stool, wastewater surveillance is a valuable tool in understanding the presence of SARS-CoV-2 in communities.⁴,⁵ With a single sample, wastewater surveillance can provide information on SARS-CoV-2 infections in communities. Importantly, both symptomatic and asymptomatic cases are reflected in wastewater data. Wastewater surveillance also enables public health agencies to monitor SARS-CoV-2 community trends, independent of...
clinical testing access and availability, and healthcare-seeking behaviors.\textsuperscript{10}

Given the benefits of wastewater surveillance, countries around the world have implemented wastewater surveillance to detect SARS-CoV-2 in communities.\textsuperscript{11−13} Programs for SARS-CoV-2 wastewater surveillance have shown great success globally.\textsuperscript{14−17} Wastewater surveillance for SARS-CoV-2 can help local health departments target mitigation efforts to control the spread of the virus.\textsuperscript{18}

The Centers for Disease Control and Prevention (CDC) established the National Wastewater Surveillance System (NWSS) in September 2020 to better understand trends in SARS-CoV-2 infections and provide an early warning of increasing infections in communities throughout the United States.\textsuperscript{18} State, tribal, local, and territorial health departments report wastewater testing data to NWSS 1−2 times per week for summarizing and interpreting data for public health action. These sampling efforts are considered routine wastewater surveillance because they are embedded into existing health department programmatic activities and focus on the longevity of data collection and use as a disease surveillance tool.

In August 2021, NWSS partnered with the United States Geological Survey (USGS) Environmental Health Program, which has long studied wastewater contaminants and impacts, to assess the feasibility and value of rapid scale-up of routine wastewater surveillance sampling to provide localized high-frequency testing of SARS-CoV-2 in wastewater.\textsuperscript{19,20} High-frequency sampling may provide more value than routine sampling by more accurately monitoring outbreak onset, estimating more timely disease trends, and understanding disease re-emergence. In particular, the ability to rapidly scale-up wastewater surveillance may be an effective surveillance tool in emergency response situations. USGS high-frequency sampling occurred 3−5 times per week at each wastewater treatment plant (WWTP). The purposes of this study were to understand the logistical challenges and solutions for rapid scale-up of wastewater sampling and to assess the added value of high-frequency data compared with routine samples by evaluating surveillance and sampling metrics as well as SARS-CoV-2 concentration trends.

\section{METHODS}

\textbf{Data Set.} Between September 7 and 29, 2021, the USGS collected 354 wastewater samples (referred to as USGS samples), conducted quantitative analysis for the presence of SARS-CoV-2, and reported the data to the CDC. The USGS sampling sites included 25 WWTPs in Colorado, Missouri, North Carolina, Ohio, Utah, and Wisconsin. The USGS samples were collected 3−5 times per week for high-frequency sampling, and the NWSS wastewater samples (referred to as NWSS samples) were collected 1−2 times per week for routine sampling.

Between September 7 and 29th, 2021, 10 state and local health departments collected 1758 wastewater samples at 368 WWTPs as part of routine NWSS surveillance. The NWSS samples and sampling locations that were not part of the USGS sampling efforts were excluded from the analysis (excluded number of samples = 1602; excluded number of WWTPs = 345). One WWTP included in USGS sampling was not part of the NWSS samples and was excluded from the analysis (excluded number of samples = 10). The NWSS samples were collected from 24 WWTPs also sampled by the USGS. The comprehensive data set consisted of 500 samples combined from the USGS and NWSS (344 and 156, respectively).

Quality control checks were applied to the NWSS and USGS data sets in the CDC DCIPHER data platform, which included required variables that were either missing or improperly formatted. Data reporters were able to view their quality control report at any time. Quality control issues were categorized as required or attention. Attention fields are not as critical but may indicate a problem with the quality of the data. Examples of attention issues include indicating a microbial human fecal marker was measured but not providing a concentration for this marker or improper formatting of the solids separation method.

Required issues denote problems with fields that must be included in the data file; otherwise, the sample might be excluded from analysis. Data fields required for inclusion of sample results include sample collection date, testing methods, flow rate, and population served. Some samples from the USGS comprehensive data set were excluded from the final data sets due to missing or invalid required variables. The final data set consisted of 452 samples combined from the USGS and NWSS (296 and 156, respectively).\textsuperscript{21}

Surveillance metrics included the WWTP state, population served (estimated number of persons whose sewage was captured by the sampling site as reported by the WWTP), and days from sample collection to sample testing (to assess timeliness). Sampling metrics included sample location (centralized WWTP or upstream) and sample type (grab [i.e., collecting one sample at a time] or composite [i.e., collecting many subsamples at regular intervals over a period of time or amount of flow]). The volumetric flow at the sampling location was reported as million gallons per day (MGD). Also, the PCR type (reverse transcriptase quantitative polymerase chain reaction [RT-qPCR] or reverse transcriptase droplet digital polymerase chain reaction [RT-ddPCR]) and gene target (N1, N2, or N1 and N2 combined) used to detect the presence of SARS-CoV-2 were reported. The SARS-CoV-2 concentration was reported as copies per liter (copies/L) of wastewater and was also dichotomized to identify whether SARS-CoV-2 was detected in the sample. Detection is defined as the SARS-CoV-2 concentration being above the assay and instrumentation limit of detection (LOD). All surveillance and sampling metrics were required to accompany each sample and were flagged by a quality control check if they were not present upon data submission.

\textbf{USGS Sample Collection and Testing.} Untreated influent wastewater samples were collected by USGS and WWTP personnel. Initial planning was conducted by the USGS in collaboration with each participating WWTP to establish facility-specific logistics, including safety and sampling protocols, access limitations, and personnel points of contact. Facility-specific sampling protocols based on modifications of the USGS national field manual methods for the collection and processing of water samples were employed along with personal protective equipment (PPE) and use protocols based on CDC guidance for protection against dermal and inhalation exposures to infectious diseases.\textsuperscript{19,22−24}

For high-frequency sampling, 24 h flow-based composite samples (250 mL) were collected by a refrigerated autosampler at grate or spigot access points. Composite samples and a tap water-filled temperature control (250 mL amber glass) were double bagged, bubble wrapped, encased in frozen gel packs (4 °C), and shipped after collection overnight for analysis. At the
USGS Eastern Ecology Science Center Biosafety Level 3 laboratory, samples were extracted using the kit-free “Sewage, Salt, Silica and SARS-CoV-2” (4S) method of the recent interlaboratory SARS-CoV-2 wastewater monitoring assessment. The 4S extraction method employs affordable and readily available sodium chloride (NaCl), ethanol, and silica RNA-capture matrices, thus minimizing supply chain constraints that may arise during pandemic and other emergency-response conditions. Mouse Hepatitis Virus (MHV) and the N2 and E genes of SARS-CoV-2 were quantified by RT-ddPCR using a multiplex assay kit. A single-plex RT-ddPCR quantification assay for pepper mild mottle virus (PMMoV) was conducted for normalization of human fecal load. See the Supporting Information for further details (Table S1).

NWSS Sample Collection and Testing. As part of the NWSS routine wastewater surveillance, WWTPs collected raw wastewater samples approximately twice a week at WWTPs or upstream in the wastewater network. WWTPs collected 22–25 h time-based or flow-based composite samples according to facility capacity and available resources. Samples were stored and transported at 4 °C and, where possible, refrigerated during the collection process. If samples could not be processed within 24 h, a matrix recovery control was spiked into the sample before refrigerating at 4 °C or freezing at −20 or −70 °C.

State health departments identified testing laboratories, which included state- and federal-owned, public health, academic, or commercial laboratories, to quantify SARS-CoV-2 in wastewater from the NWSS samples. Samples were concentrated using concentrating pipet ultrafiltration, membrane filtration, or polyethylene glycol (PEG) precipitation. RNA or total nucleic acid extraction kits were used to extract and isolate SARS-CoV-2 RNA from the samples. SARS-CoV-2 RNA concentrations were quantified using RT-qPCR or RT-ddPCR with N1 and/or N2 gene targets with a standard curve (if using RT-qPCR). To enable normalization of wastewater concentrations and to ensure the quality of each result, testing laboratories included recovery efficiency controls and endogenous human fecal markers including pepper mild mottle virus (PMMoV) and crAssphage (Table S1).

Statistical Analysis. Descriptive statistics were calculated for all surveillance and sampling metrics. Flow-population normalized SARS-CoV-2 concentrations were plotted by state, which provide an estimate of the total amount of SARS-CoV-2 RNA in the wastewater sample relative to a static population estimate for the sewershed. This normalization approach indicates whether the total number of individuals in the sewershed who are shedding SARS-CoV-2 RNA has changed. The flow-population normalization concentration calculation is log10(SARS-CoV-2 viral RNA concentration × flow rate)/population served. The average SARS-CoV-2 flow-population normalized concentrations were then calculated per day. Figures were constructed using the Microsoft Office Excel software.

Statistical trends were calculated using SparkR and PySpark in the DCIPHER platform (DCIPHER Foundry, Palantir Inc., Denver, CO, United States) using every third and fifth sample per sampling site. Trends were classified into five categories (sustained increase, increase, plateau, decrease, or sustained decrease). A trend was classified as “increasing” if the slope was statistically significantly positive or was classified as “decreasing” if the slope was statistically significantly negative over three samples (p < 0.05, two-tailed test). If the slope was statistically significantly positive or negative over five samples, it was considered a “sustained increase” or “sustained decrease”, respectively (p < 0.05, two-tailed test). If no statistically significant change at the α = 0.05 level was found over either number of samples, the trend is classified as a “plateau”. If fewer than three samples were available to calculate a trend, it is classified as “unknown”.

Statistical trends were compared by WWTP and sample collection date to identify trends that agreed. For example, if a USGS and NWSS sample both reported a plateau for the same WWTP and sample collection date, the trend agreed. If a USGS and NWSS sample reported a different trend for the same WWTP and sample collection date, the trend did not agree. The percent of time the trends agreed between the USGS and the NWSS is provided as support to the SARS-CoV-2 flow-population normalized concentrations.

RESULTS

Quality Control Review. Most quality control issues for the USGS and NWSS samples were for required fields (66.1%, n = 773 and 83.7%, n = 166, respectively) compared to attention fields (33.9%, n = 396 and 16.3%, n = 27, respectively). The most common required field issues for USGS samples included recovery-control spike concentration, PCR target, or WWTP name information. For NWSS sample variables, the most common required field issues included concentration method, extraction method, or sample-type information. The most common attention field issues for the USGS and NWSS samples were microbial target information and SARS-CoV-2 concentration 95% confidence intervals.

Surveillance Metrics. The final data set consisted of 452 samples collected from 24 WWTPs. The USGS collected an average of 13.1 (10.0–15.0) (mean (range)) samples per week, and the 6 NWSS states collected an average of 6.7 (6.0–7.0) (mean (range)) samples per week. The average population served by the WWTPs was 138 947 (11 883–488 000) (mean (range)) persons for USGS sites and 161 747 (11 883–488 000) (mean (range)) persons for NWSS sites. On average, there were 3.4 (2.0–7.0) (mean (range)) days between the beginning of sample collection and the completion of sample testing for USGS samples and 3.8 (0.0–15.0) (mean (range)) days for NWSS samples. Other surveillance metric characteristics of the wastewater samples are described in Table 1 and Table S1.

Sampling and Testing Metrics. In general, all USGS samples were collected as 24 h flow-based composites, except for four samples (22, 23, 25, and 26 h composites) at one location. The NWSS samples were collected as 22–25 h flow-based composite samples (90.4%, n = 141) and time-based composite samples (9.6%, n = 15). All USGS samples were tested using RT-ddPCR, and NWSS samples were tested by RT-qPCR (57.0%, n = 89) or RT-ddPCR (43.0%, n = 67). N2 gene targets were used in USGS testing, while N1 and N2 combined (70.5%, n = 110), N2 (18.6%, n = 29) and N1 (10.9%, n = 17) gene targets were used in NWSS testing (Table 1).

Virus in most NWSS samples was preconcentrated before extraction of nucleic acids by a range of methods, including membrane filtration (67.3%, n = 105), ultrafiltration (14.7%, n = 23), or PEG precipitation (5.2%, n = 8). Some NWSS samples, however, were extracted directly without viral preconcentration (12.8%, n = 20; see Table S1). Commercial extraction methods used for NWSS samples included Promega.
This study evaluated the rapid scale-up of wastewater sampling frequency and the utility of such efforts for community SARS-CoV-2 monitoring but also compared respective surveillance and sampling metrics and SARS-CoV-2 flow-population normalized concentrations and trends between the USGS and the NWSS. Sampling and testing methods for the USGS and NWSS varied; however, both programs successfully benefited from high-frequency sampling and the NWSS. Sampling and testing methods for the USGS showed similar trajectories of average SARS-CoV-2 concentration changes in each state. Colorado, Missouri, Ohio, Utah, and Wisconsin showed similar trajectories of average SARS-CoV-2 concentration changes compared to routine sampling. The USGS sampling in North Carolina demonstrated decreasing average SARS-CoV-2 flow-population normalized concentrations between USGS and NWSS sampling. However, the USGS results may more accurately capture fluctuations in average SARS-CoV-2 flow-population normalized concentrations because the USGS results. North Carolina was the only state with a slightly different trajectory between the USGS and the NWSS sampling. The USGS sampling in North Carolina demonstrated decreasing average SARS-CoV-2 flow-population normalized concentrations, whereas the NWSS sampling showed a slight increase, which could be due to the large number of nondetects.

## DISCUSSION

The microbial target concentration (in log10 copies/L) was 7.2 (5.7–9.0) (mean (range)) for the USGS samples and 7.7 (5.7–9.0) (mean (range)) for the NWSS samples (Table 1).

**SARS-CoV-2 Flow-Population Normalized Concentrations.** We compared the average SARS-CoV-2 flow-population normalized concentrations from USGS high-frequency sampling and NWSS routine sampling to understand the differences between the two data sources (Figure 1). We also compared statistical trends for USGS and NWSS samples by sample collection date and WWTP. Matched trends between the USGS and the NWSS samples agreed 58% (193/330) of the time (Table S2). Overall, high-frequency sampling provided a more nuanced indication of average SARS-CoV-2 flow-population normalized concentration changes in each state. Colorado, Missouri, Ohio, Utah, and Wisconsin showed similar trajectories of average SARS-CoV-2 flow-population normalized concentrations between USGS and NWSS sampling. However, the USGS results may more accurately capture fluctuations in average SARS-CoV-2 flow-population normalized concentrations better than the NWSS results. North Carolina was the only state with a slightly different trajectory between the USGS and the NWSS sampling. The USGS sampling in North Carolina demonstrated decreasing average SARS-CoV-2 flow-population normalized concentrations, whereas the NWSS sampling showed a slight increase, which could be due to the large number of nondetects.

### Table 1. Characteristics of the National Wastewater Surveillance System (NWSS) and the U.S. Geological Survey (USGS) Wastewater Sampling Data, September 7–29, 2021 (n = 452) ^{44}

| characteristic | USGS samples (n = 296) | NWSS samples (n = 156) |
|----------------|------------------------|------------------------|
| wastewater treatment plant state | | |
| Colorado | 47 (15.9) | 17 (10.9) |
| Missouri | 32 (10.8) | 8 (5.1) |
| North Carolina | 54 (18.2) | 34 (21.8) |
| Ohio | 65 (22.0) | 29 (18.6) |
| Utah | 43 (14.5) | 20 (12.8) |
| Wisconsin | 55 (18.6) | 48 (30.8) |
| composite sample type | | |
| flow based | 296 (100.0) | 141 (90.4) |
| time based | 0 (0.0) | 15 (9.6) |
| PCR type | | |
| RT-ddPCR | 296 (100.0) | 67 (43.0) |
| RT-qPCR | 0 (0.0) | 89 (57.0) |
| PCR target | | |
| N1 | 0 (0.0) | 17 (10.9) |
| N2 | 296 (100.0) | 29 (18.6) |
| N1 and N2 | 0 (0.0) | 110 (70.5) |
| SARS-CoV-2 detected | yes | 258 (87.2) |
| | no | 38 (12.8) |
| mean (range) | | |
| average limit of detection (log10 copies/L) | 3.5 (3.5–3.8) | 3.6 (2.1–4.9) |
| average number of samples per week | 13.1 (10.0–15.0) | 6.7 (6.0–7.0) |
| population served | 138 947 (11 883–488 000) | 161 747 (11 883–488 000) |
| days from sample collection to sample testing | 3.4 (2.0–7.0) | 3.8 (0.0–15.0) |
| flow rate (million gallons/day) | 19.2 (0.8–253.9) | 20.7 (0.7–220.4) |
| SARS-CoV-2 concentration | 7.0 (5.7–8.1) | 7.7 (5.7–9.0) |

^44 Abbreviations: RT-ddPCR, reverse transcriptase droplet digital polymerase chain reaction; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction; PEG, polyethylene glycol; PMMoV, pepper mild mottle virus. *The first and last day of the sampling time period were removed to allow for the calculation of averages per week. **Measured in copies per person per day, this measurement shows a ow-population normalized concentration, which provides an estimate of the total amount of SARS-CoV-2 RNA in the wastewater sample. Calculated by log10[(SARS-CoV-2 viral RNA concentration \times flow rate)/population served]. (HT and manual TNA kits; 30.8%, n = 48), QIAGEN QIAamp and AllPrep PowerViral DNA/RNA kits; 24.4%, n = 38), NucliSENS (magnetic bead kit; 21.8%, n = 34), and TRIzol (10.2%, n = 16). No virus was preconcentrated from the USGS samples. Instead, nucleic acids from the USGS samples (40 mL) were extracted directly by the 4S method. PMMoV was used as a human fecal marker for the USGS samples. PMMoV (63.5%, n = 99) and CPPs (18.6%, n = 29) were used as human fecal markers for most NWSS samples; however, some NWSS samples did not use a human fecal marker (17.9%, n = 28). All USGS samples used a murine coronavirus recovery efficiency control, while NWSS samples used a bovine coronavirus vaccine (71.2%, n = 111), OC43 (19.2%, n = 30), puro (5.1%, n = 8), and murine coronavirus (4.5%, n = 7). The average recovery efficiency control percent was 22.5% (<0.01–95.8%) (mean (range)) for the USGS samples and 27.3% (0.10–120.5%) (mean (range)) for the NWSS samples. The microbial target concentration (in log10 [copies per liter of wastewater]) was 6.5 (3.9–7.6) (mean (range)) for the USGS samples and 7.2 (5.7–9.2) (mean (range)) for the NWSS samples.

SARS-CoV-2 was detected in 87.2% (n = 258) of the USGS samples and 99.4% (n = 155) of the NWSS samples. The average limit of detection (in log10 copies/L) was 3.5 (3.5–3.8) (mean (range)) for the USGS samples and 3.6 (2.1–4.9) (mean (range)) for the NWSS samples. The average SARS-CoV-2 flow-population normalized concentration (in log10 [copies per person per day]) was 7.0 (5.7–8.1) (mean (range)) for the USGS samples and 7.7 (5.7–9.0) (mean (range)) for the NWSS samples.

**SARS-CoV-2 Flow-Population Normalized Concentrations.** We compared the average SARS-CoV-2 flow-population normalized concentrations from USGS high-frequency sampling and NWSS routine sampling to understand the differences between the two data sources (Figure 1). We also compared statistical trends for USGS and NWSS samples by sample collection date and WWTP. Matched trends between the USGS and the NWSS samples agreed 58% (193/330) of the time (Table S2). Overall, high-frequency sampling provided a more nuanced indication of average SARS-CoV-2 flow-population normalized concentration changes in each state. Colorado, Missouri, Ohio, Utah, and Wisconsin showed similar trajectories of average SARS-CoV-2 flow-population normalized concentrations between USGS and NWSS sampling. However, the USGS results may more accurately capture fluctuations in average SARS-CoV-2 flow-population normalized concentrations better than the NWSS results. North Carolina was the only state with a slightly different trajectory between the USGS and the NWSS sampling. The USGS sampling in North Carolina demonstrated decreasing average SARS-CoV-2 flow-population normalized concentrations, whereas the NWSS sampling showed a slight increase, which could be due to the large number of nondetects.

**DISCUSSION**

This study evaluated the rapid scale-up of wastewater sampling frequency and the utility of such efforts for community SARS-CoV-2 monitoring but also compared respective surveillance and sampling metrics and SARS-CoV-2 flow-population normalized concentrations and trends between the USGS and the NWSS. Sampling and testing methods for the USGS and NWSS varied; however, both programs successfully estimated SARS-CoV-2 concentrations in the same communities. Our data suggest that high-frequency sampling may benefit communities using wastewater surveillance for SARS-CoV-2 emergency response and provide more accurate fluctuations in SARS-CoV-2 flow-population normalized concentrations compared to routine sampling. Being able to understand subtle differences in SARS-CoV-2 flow-population normalized concentrations over time could be important in identifying emerging issues, particularly in emergency response settings where wastewater sampling can be used. High-frequency sampling can be supported by a variety of sampling and testing methods but requires careful planning and prepared staff to rapidly implement.
Figure 1. continued
Sampling methods differed between the USGS and the NWSS. Both sampling approaches produced quality data and successfully estimated SARS-CoV-2 flow-population normalized concentrations in communities. Despite the rapid scale-up of sampling by the USGS, data quality issues were minimal and similar to those for routine sampling. Most quality control issues for the USGS and NWSS were missing required fields. The attention fields for the USGS and NWSS were related to Figure 1. Average log10 SARS-CoV-2 flow-population normalized concentrations by the U.S. Geological Survey (USGS) and the National Wastewater Surveillance System (NWSS) Reporting States, September 7–29, 2021. Measured in copies per person per day; this measurement shows a flow-population normalization concentration, which provides an estimate of the total amount of SARS-CoV-2 RNA in the wastewater sample. Calculated by log10[(SARS-CoV-2 viral RNA concentration × flow rate)/population served]. Averages were then calculated per day.
the microbial fecal indicator target and the SARS-CoV-2 concentration 95% confidence interval. The high-quality data produced by both approaches are in part due to the standardization of data collection, which defines variables, allowed values, and proper formats before sample collection to ensure comparable results. Likewise, the delivery of high-frequency sampling data with minimal data quality issues, despite the increased logistical challenges, was attributable in large part to leveraging the existing network of well-trained USGS personnel around the country to quickly support sample collection and testing.

In addition to high-quality data, the time from sample collection to sample testing was comparable for USGS high-frequency and routine NWSS sampling approaches, differing by less than 1 day. This further exemplifies that rapid scaling of wastewater testing can also produce data equally as timely as routine surveillance. It has been shown that compared to clinical SARS-CoV-2 testing of individuals suspected to have COVID-19, wastewater surveillance can provide a beneficial lead time to identify an increase in SARS-CoV-2 in a community.\textsuperscript{36,37} Clinical testing for SARS-CoV-2 produces results around 3–9 days after symptom onset and is dependent on the availability of resources; wastewater sample results can be delivered on average 2–4 days quicker than clinical testing results.\textsuperscript{37} The USGS and NWSS tested samples within 4 days of collection, on average, which may provide results days before clinical testing results. Because of the potentially expedited results, wastewater surveillance may provide an earlier indication of increases in cases than clinical testing. In addition, there are barriers to clinical testing including the availability and funding of testing resources and the need for individuals to seek out testing. Despite different sample collection and testing methods, the sample turnaround time between the USGS and the NWSS was roughly the same, illustrating that high-frequency sampling approaches can be implemented with no reduction in timeliness of results.

High-frequency sampling provided a more nuanced impression of the trajectory of average SARS-CoV-2 flow-population normalized concentrations, which could be helpful in emergency response situations. Having an earlier indication of changing trends could be useful in emergency response settings to be able to take quick action on public health measures and better understand changes in disease occurrence.\textsuperscript{35} Pandemic influenza plans from the WHO and CDC include plans for disease surveillance, which often rely on clinical testing and data from hospitals, and emphasize reducing expenses and increasing effectiveness.\textsuperscript{38,39} Wastewater surveillance can be a valuable tool in natural disaster by providing an estimate of disease in a population based on a single wastewater sample. A natural disaster may disrupt travel, electrical, and communications systems, whereas sewage systems are underground and are less likely to incur damage. Residents in a community affected by a natural disaster will continue to use their sewage system but may be unable or less likely to travel to clinical testing facilities. In addition, a natural disaster may force residents in emergency shelters which provides an environment for diseases to spread. Although the focus of this study was on community-level wastewater sampling, institution- or building-level wastewater surveillance is possible. As a result, a single sample could represent the entire population housed in the shelters. The ability to identify a pathogen or observe an increase in pathogen levels is important to avoid an outbreak in these shelters. Our data suggest that a high-frequency sampling approach would be beneficial in emergency response and natural disaster settings to understand disease trends and enable quick public health action. High-frequency sampling requires a strategic approach while maximizing the utilization of resources to understand disease trends in communities.
A high-frequency sampling approach requires a constant flow of information and supplies throughout sample collection, testing, analysis, and data sharing. The goal of high-frequency sampling was to report results within 3 days of sample collection. Developing and piloting a high-frequency sampling approach before an emergency can help execute the sampling campaign under emergency conditions. As part of the emergency preparedness plan, it is important to establish a list of WWTPs in the area, along with plant operator contact information, to quickly contact the facility to communicate the sample collection and shipping plan. The plan for collecting and shipping samples may include how to distribute resources (e.g., PPE and sampling kits), identification and allocation of staffing to collect samples, and coordination of overnight shipping logistics (Figure 2). As with any emergency or disaster response, advanced preparation is critical for success. Therefore, a sample collection and testing plan will ensure the success and rapid implementation of a high-frequency sampling plan in emergency situations.

Challenges of wastewater surveillance include the lack of a standard method for testing samples in the United States, which makes direct comparisons of SARS-CoV-2 wastewater concentrations difficult and produces barriers to analysis between different WWTPs and wastewater surveillance programs. Therefore, much of our analysis is descriptive and not inferential in nature. However, research continues to support the variety of sampling methods used to detect SARS-CoV-2 in wastewater. It is important when analyzing and interpreting data to directly compare only concentration data obtained using the same laboratory methods due to confounding differences in sensitivity between methods. Differences in sample testing methods should be accounted for when constructing a surveillance plan, and the same laboratory methods and testing laboratory should be used when feasible. It is worth noting that wastewater surveillance is not a practicable outbreak monitoring approach for septic-system-dependent communities, which comprise approximately 25% of all U.S. households. Finally, wastewater surveillance might not be feasible in disaster settings if the disaster disrupts the wastewater network or WWTP.

### CONCLUSIONS

Nationwide programs for SARS-CoV-2 wastewater surveillance have proven to be a cost-effective approach to monitor trends of SARS-CoV-2 in communities. Wastewater surveillance can be used to allocate resources, assess the spread of emerging threats such as SARS-CoV-2, and understand disease trends in communities. This study demonstrates that high-frequency sampling can be conducted in emergency response and natural disaster settings to deliver more nuanced SARS-CoV-2 community assessment. Rapidly scaling up high-frequency wastewater surveillance requires a coordination and logistics preparedness plan paired with available capacity in trained staff and established testing laboratories. High-frequency sampling may be a useful approach for increased resolution of disease trends and enable emergency responders to act more quickly to avert disease spread.

### ASSOCIATED CONTENT

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.2c00094.

**Additional USGS methods for collecting, shipping, and testing wastewater samples (PDF)**

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Maximize Contributions in the Fight Against COVID-19.

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