INDIANA WASTEWATER MONITORING PROGRAM

Sampling Community Sewersheds For SARS-CoV-2

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EXECUTIVE SUMMARY

A community wastewater system can provide valuable information about public health conditions in an anonymous and rapidly accessible manner. One area where this is especially powerful is infectious diseases, which are shed into wastewater systems. In response to the global COVID-19 pandemic, the scientific community has rapidly mobilized to determine if wastewater monitoring for SARS-CoV-2, the virus that causes COVID-19, could be detected and quantified in wastewater streams and sludge. This approach is especially attractive, as it does not require symptoms or access to clinical testing to identify disease trends within a community. Many cities, states, and countries globally have been interested in this strategy because of its predictive power when paired with viral case data. Infected individuals excrete the viral RNA in their stool, which is then collected in the wastewater system. These viral detections can then be used to demonstrate the presence of the disease and disease prevalence trends in a manner that is not dependent on clinical testing or reporting lags.

The Indiana Finance Authority supported 14 communities in sampling wastewater for SARS-CoV-2 from August through December of 2020. Eligible wastewater utilities served public or private universities with student housing. Utilities sampled from wastewater treatment plants and from manholes or lift stations within the collection system. Additional collection system sites were selected because they represented a discrete portion of the population the community wanted to monitor (i.e., dormitories, prisons, low-income communities, etc.). In this report, we examined 13 weeks of wastewater sampling results for trends and relationships to reported county and university COVID-19 case rates. We also provide information about how participants viewed the study and utilized results.

KEY FINDINGS

- ➔ Utilities collected over 1900 samples from 15 wastewater treatment plants and 44 sites within collection systems
- ➔ An interlaboratory comparison indicated consistent and reliable results between labs for this type of analysis
- ➔ Temporal trends in wastewater were detectable in communities and college campuses and these trends mirrored trends in case data in most communities
- ➔ Trends in wastewater samples and case data appeared to occur at the same time, but it is possible that signals in wastewater could serve as leading indicators in real-time practice if a community is experiencing severe delays or shortages in testing
- ➔ Our results suggest wastewater monitoring is especially valuable for three purposes:
  - Locating hotspots at the building or neighborhood level
  - Identifying trends in conjunction with viral testing results
  - Monitoring for new outbreaks as vaccines are deployed and case rates decrease

INTRODUCTION

Wastewater-based epidemiology (WBE) or sewer surveillance can be used to observe community-level trends through analysis of various chemical or biological markers in wastewater entering wastewater treatment plants (WWTPs) or within the collection system.[1] Although many applications of WBE largely center around tracking drug use in communities, WBE has also been used to monitor the spread of infectious diseases such as the poliovirus [2] and has promising applications understanding human exposure to industrial chemicals and the emergence of antibiotic resistance in a community. Current research suggests WBE may be a useful tool in understanding outbreaks of the novel Coronavirus disease-19 (COVID-19) when paired with clinical viral testing datasets.[3]

WBE can be used as a system to monitor for the presence and infection trends of COVID-19 because it allows for the detection of mild, subclinical and asymptomatic infections present within a community. Both symptomatic and asymptomatic carriers of COVID-19 excrete the RNA signal of SARS-CoV-2, the virus that causes COVID-19, in their stools. International monitoring efforts successfully detected SARS-CoV-2 RNA in wastewater days to weeks before clinically confirmed cases.[4, 5] Beyond the potential for this system to monitor for increased infection rates, wastewater monitoring is also potentially useful to monitor for disease re-emergence following the availability of a vaccine and to confirm clinical case observations with a secondary data source. Notably, as WBE approaches monitor for SARS-CoV-2 RNA, these methods cannot identify infectious virus and are not indicative of infectious risk from wastewater sources.

To investigate the potential of this approach to inform Indiana's COVID-19 response, the Indiana Finance Authority provided 14 communities across the state with access to free wastewater analysis for SARS-CoV-2 in the Fall of 2020. The result of this effort was the first ever wastewater monitoring network in the state.

The primary goals of this initiative were to:

1. Provide cities with early information about COVID-19 outbreak risks in their communities
2. Understand which communities might benefit most from this testing strategy in the future

In this report, we will:

- Describe how the initiative was designed and implemented
- Detail findings from a comparison of 2 separate labs
- Share SARS-CoV-2 RNA results from all 14 communities over 13 weeks
- Compare wastewater results to county and local COVID-19 case rates
- Provide community testimonials about testing and data use

BACKGROUND

Communities with college and university campuses were an important focus area for the Indiana SARS-CoV-2 Monitoring Program. Colleges and Universities are national and international connectivity nodes in many communities and potential hot spots for the spread of infectious diseases. While there is no national tracking system for case rates, according to a recent survey conducted by the New York Times of over 1,600 institutions, over 252,000 positive cases of COVID-19 were reported as of early November 2020. These numbers are notable although mitigation and symptomatic testing rates are likely much higher at colleges and universities when compared with cities and even counties elsewhere in the US.

Eligible utilities included those that provide wastewater services to public or private colleges and universities in the state of Indiana with at least 1,000 students and on-campus housing. Fourteen communities opted to participate in phase I of the program (August 17- October 23) and 12 utilities opted to participate in phase II (October 26-January 1). The Indiana Finance Authority sponsored the program and selected 120Water to manage the logistics of the initiative including training sessions, water sampling kits, data management and lab coordination. Research partners at the University of Notre Dame provided technical guidance, conducted confirmatory analysis of wastewater treatment plant (WWTP) samples, and supplied weekly results briefs for each participant. Microbac Laboratories was the primary lab for this project and participated in the interlab comparison effort led by the University of Notre Dame.

Participants were located in 14 different counties and serve between 2,700 and 101,000 people, though this number fluctuates during active school semesters. According to an entry survey completed by all participants, most utilities (71%) experienced significant inflow and infiltration and most (78%) reported industrial and manufacturing sites as major inputs to their treatment facilities. Less than half (42%) receive waste from septic haulers or other WWTPs.

Sampling Methodology

All participants collected samples at the influent to the WWTP and between 1-9 different locations within the collection system three times a week for 13 weeks (Figure 1). Where possible, utilities collected at the primary influent after some screening or straining of biosolids (62% of utilities), or at the raw influent where autosamplers were already installed (30% of utilities). One utility selected an alternative sampling location at a manhole immediately upstream of the plant due to dilution concerns at the WTTP. All WWTP samples were composites collected hourly over 24 hours.

Wastewater utilities also selected a total of 44 sampling locations within the collection system (Figure 2). Sampling within the collection system may help to provide finer geographic resolution of potential COVID-19 'hotspots'. Participants were encouraged to collect samples from lift stations if possible (23% sampling locations) or manholes (77% of sampling locations) in gravity-fed systems or in instances where the lift station was not an ideal way to target a particular population (i.e. student housing). Lift stations are considered ideal sampling points because they provide a pseudo-composite sample without the need for additional sampling equipment. Over 50% of participants were able to deploy additional auto-composite samplers at collection system sites, leaving 15 manhole sites where true grab samples were collected.

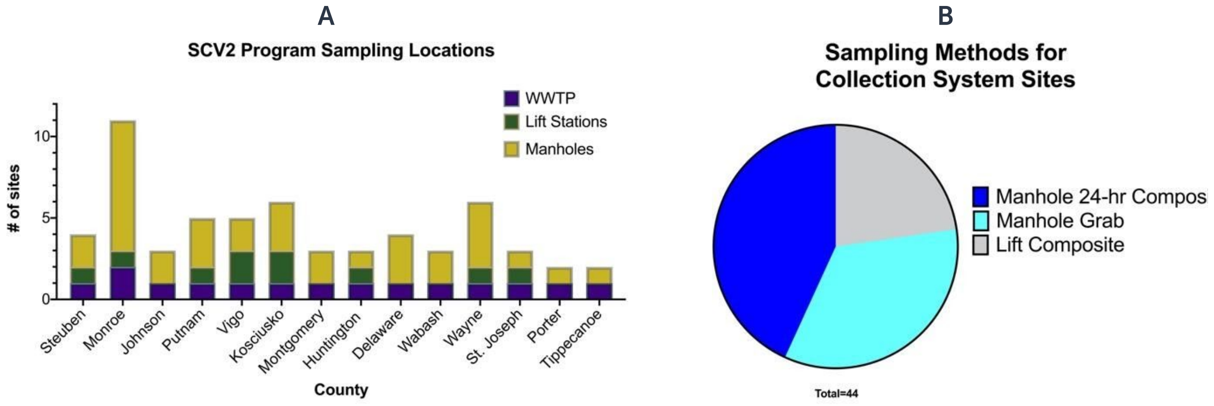

Figure 1. Sampling locations (A) selected by 14 communities and sampling methods at collection system sites (B).

Most utilities selected sampling sites representative of on-campus housing, the most downstream sampling point for the entire university, and off-campus student housing sites like large apartment complexes (Figure 2). Utilities interested in sampling non-university communities selected sites outside of low-income using units, nursing homes, prisons, and healthcare service centers.

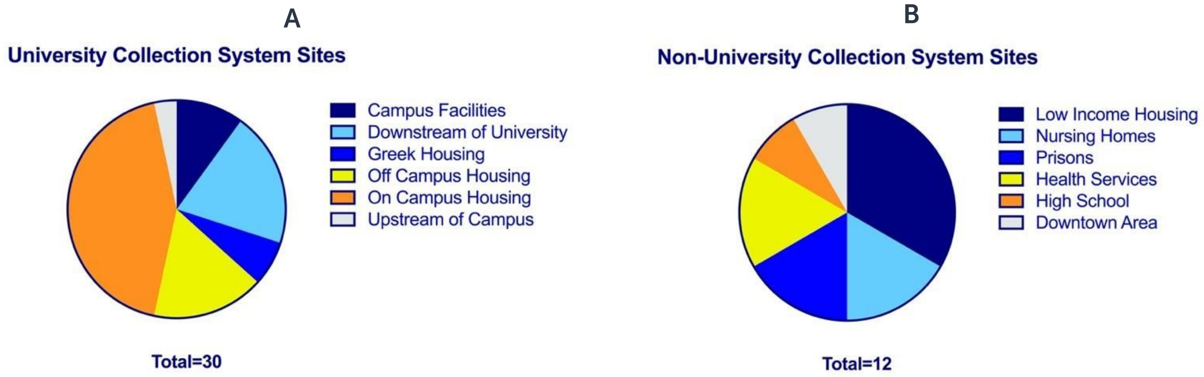

Figure 2. Populations targeted by university-based sampling locations (A) and populations represented by non-university-based sampling locations (B). Two sites were representative of more than one of these categories.

Analytical Methodology & Interlab Comparison

The overall sample processing approach used by Notre Dame is shown below (Figure 3). The Microbac processing approach was similar, with the exception of the differing sample extraction and quantification methods as described.

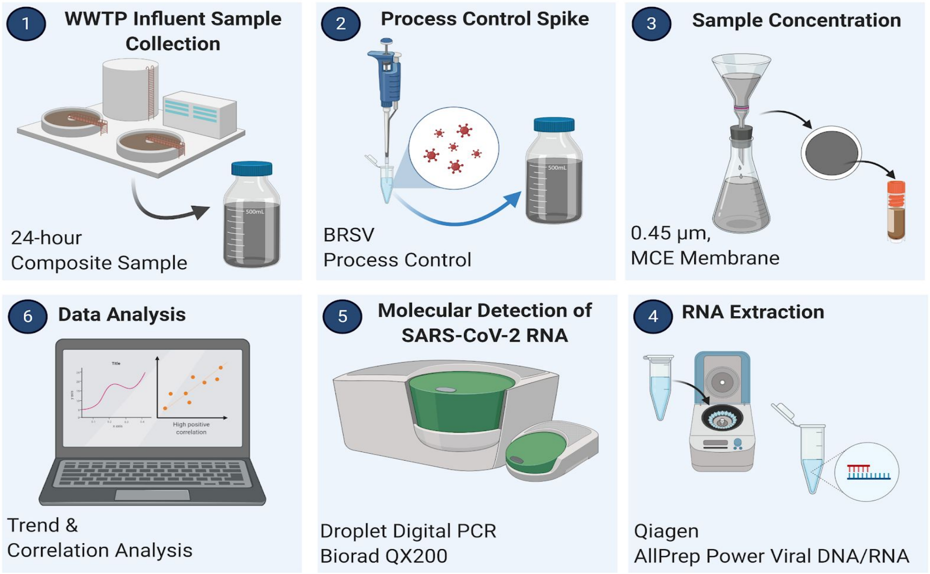

Figure 3. Representative sample collection and processing methodology. 1. Samples collected at the WWTP 2. Samples received and spiked with BRSV process control 3. Samples concentrated through a 0.45 µm filter 4.RNA extracted 5. Target quantification 6. Data analysis and reporting.

Upon sample receipt, live attenuated Bovine Respiratory Syncytial Virus (BRSV) is spiked to a target concentration of 10^3-10^5 copies per mL into the sample as a process control. This virus would not otherwise occur in the sample, and is an enveloped RNA virus similar to SARS-CoV-2 that can serve to represent the efficacy of sample processing.

The sample is then concentrated to improve our analytical ability to detect SARS-CoV-2. Both laboratories used a filter-based concentration method that was previously found to be the best performing method in a cross-comparison study.[6] Briefly, the sample pH is dropped to 3.5 to encourage virus particle association, and the sample is filtered through a 0.45 um hemicellulose acetate filter. This filter is then directly used for RNA extraction.

RNA extraction was performed using kit-based methodologies. The Notre Dame team used the Qiagen Power Viral kit. The Microbac team used the Idexx Viral RNA kit. Both kits are well-established and would be expected to perform adequately for this application.

Molecular targets were then quantified using PCR based methodologies. The Notre Dame team used digital droplet PCR (ddPCR) and the Microbac team used quantitative PCR (qPCR). In qPCR, the fluorescence is monitored during PCR amplification of the target genomic region, and this amplification profile is compared with a standard curve. Notre Dame used digital droplet PCR (ddPCR). In ddPCR, the PCR reaction is divided into >10,000 droplet sub-reactions. These sub-reactions are then thermal cycled to completion, and read as either positive or negative, enabling quantification of the target without a standard curve. While qPCR is more widely used in environmental microbiology applications, ddPCR has gained attention due to decreased inhibition and potentially greater sensitivity. PCR primer information is shown below in Table 1.

The primary monitoring target was the SARS-CoV-2 N1 assay as developed by the US CDC. This assay has been found to be the best performing SARS-CoV-2 assay in wastewater matrices. BRSV was also monitored as a process control as described above. Finally, Pepper Mild Mottle Virus (PMMoV) was measured as a potential normalization factor. PMMoV is a RNA plant virus that is found in sewage as a result of eating pepper products (e.g., hot sauce). In this application we are evaluating its potential use to normalize between sewer systems with differing inputs.

Table 1. PCR primers for representative wastewater surveillance monitoring targets.

Assay name	Primer	Primer Sequence (5'→3')
BRSV	BRSV-F	GCAATGCTGCAGGACTAGGTATAAT
	BRSV-R	ACACTGTAATTGATGACCCCATTCT
Pepper Mild Mottle Virus	PMMV-FP1	GAGTGGTTTGACCTTAACGTTTGA
	PMMV-RP1	TTGTCGGTTGCAATGCAAGT
SARS-CoV-2	N1-F	GACCCCAAAATCAGCGAAAT
	N1-R	TCTGGTTACTGCCAGTTGAATCTG

RESULTS

Interlab Comparison Findings

An interlab comparison study was included in the overall study design. Each week, approximately 15 sample splits were sent to both Microbac Laboratories and an academic laboratory at the University of Notre Dame. In total, 141 paired samples were included in this analysis (not all samples sites participated each study week). While there was technical coordination between the laboratories, results were blinded between participating laboratories. Overall, results demonstrated a moderate correlation between laboratories. Notably, qPCR quantifications demonstrated a wider quantitative range, and had a larger number of samples below the detection limit. Excluding data points below the detection limit for either laboratory and using Pearson's product-moment correlation analysis a correlation of 0.19 (p=0.095) was observed. If data below the detection limit was set as 0, a correlation of 0.19 (p=0.02) was observed.

Variability in observed correlation may be due to differences in sample handling by individuals, performance of the differing quantification approaches (qPCR vs. ddPCR), or other as yet undetermined factors. It should be noted that expected variability between biological replicates for molecular analyses is typically much higher than observed for chemical water quality measures. For example, a recent pre-print detailing a large lab comparison study for SARS-CoV-2 monitoring in wastewater reported a seven-log (ten million time) variation in reported recovery efficiency.[7] Considering the large variability inherent in these measures, the interlaboratory comparison suggests consistent and reliable results between the participating entities.

Trends in SARS-CoV-2 Concentrations in Wastewater

Across the 14 counties participating in this study, 1916 samples were collected and analyzed. Concentrations of SARS-CoV-2 varied from below the detection limit (10 gene copies per 100mL) to concentrations as high as 93,000 gene copies per 100mL (GC/100mL). During the first week of sampling, 35.8% of samples were above the minimum detection limit (Figure 4). The most recent week of sampling resulted in 65.1% of samples being above the minimum detection limit.

The first four weeks of sampling (day 0-24) occurred during the arrival of students to college campuses and corresponded to increases in the percent of samples above the detection limit (26.5% to 61.4%). Weeks 3-7 (day 28-45) resulted in a decrease in the percent of samples above the detection limit, with a minimum occurring on day 44, with only 11.1% of samples being above the limit of detection. Since week 7, the percentage of samples above detection limit have an increasing trend, with a maximum occurring on day 85, with 85.7% of samples above the detection limit (Figure 4).

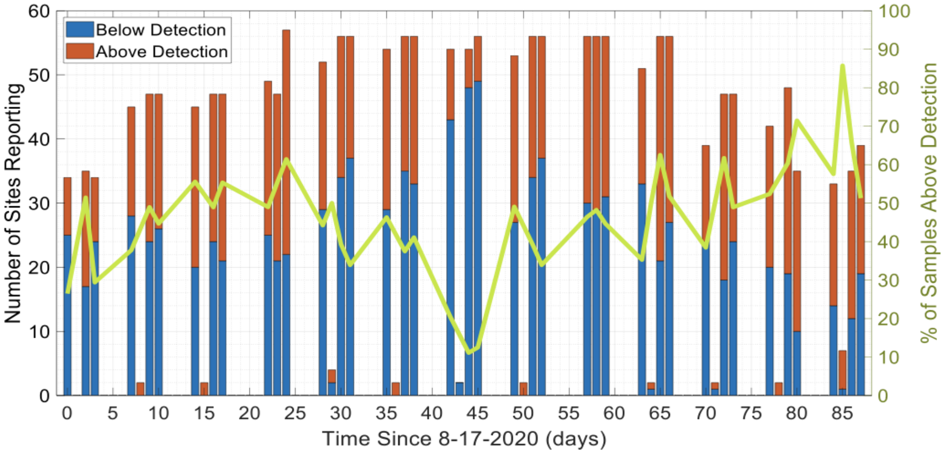

Figure 4. Number of samples collected each day and the percentage of samples above the limits of detection. Weekly average of samples above the detection limits have increased by 30% over the duration of this study.

Measured concentrations of SARS-CoV-2 spanned several orders of magnitude across sample sites and collection methodologies (<10-93,000 GC/100mL). Variability observed between sample locations resulted in the need for extended monitoring to identify trends within the data. Across the duration of monitoring, concentrations of SARS-CoV-2 have an increasing, positive trend, with a greater number of samples in phase II of sampling being above the minimum detection limit.

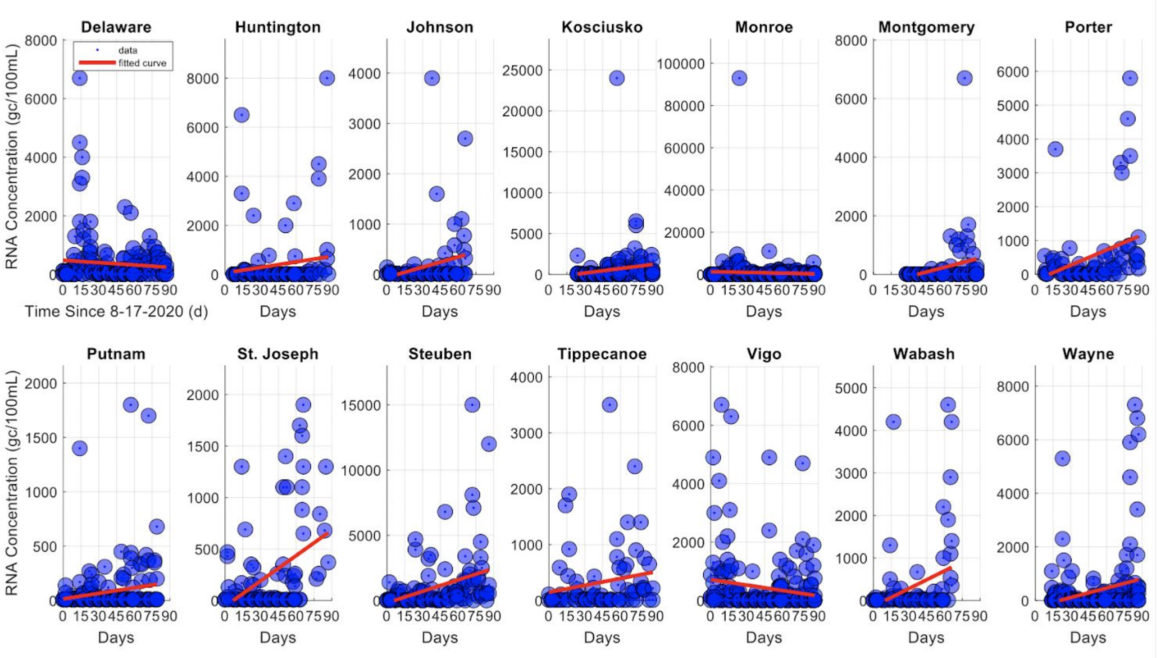

Figure 5. SARS-CoV-2 results by county for 13 weeks. Samples below the detection limit (10 gene copies per 100 mL) are included.

Trends in concentration across counties were also found to vary from decreasing (Figure 5 Delaware County), remaining constant (Figure 5 Monroe County), or increasing (Figure 5 St. Joseph County). Greek housing and Prison samples were the two target representation sites that showed a decreasing trend in of SARS-CoV-2 concentration, while campus facilities and off-campus student housing showed a minor increasing trend over the duration of sampling (Figure 6). The remaining five sites have significant increases in concentrations. Health services, low-income, and downstream-most campus locations recorded the most significant increases in concentrations.

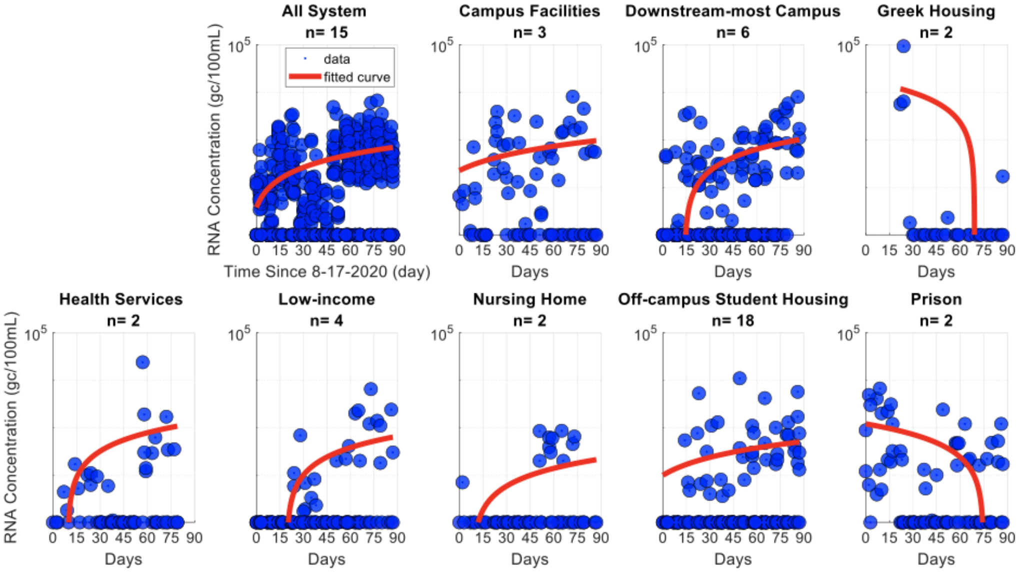

Figure 6. SARS-CoV-2 wastewater results for 13 weeks by population collection system sites represented. Samples below the detection limit (10 gene copies per 100 mL) are included.

Sampling Methodologies

Sample locations used in this study varied in their residence time, composition, and system representation. These traits have the potential to more accurately reflect/predict SARS-CoV-2 cases and may provide optimal monitoring locations. Specifically, samples were collected at manholes, lift stations, and wastewater treatment plants. Each sample location showed similar ranges in concentrations (Figure 7). Manhole locations recorded the greatest variability in concentrations, although concentrations from each location varied by up to two orders of magnitude. Trends across manhole and lift station locations were similar, while WWTP locations show a greater increasing trend. Ultimately, these results indicate that composite sampling within a manhole and lift station (i.e. within the collection system sites) provide comparable data, while samples collected at the WWTP may not be directly comparable to collection system samples.

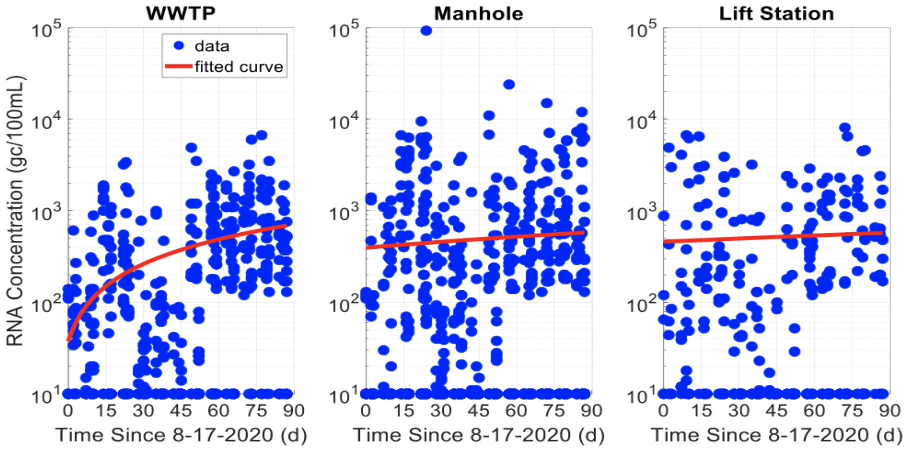

Figure 7. SARS-CoV-2 wastewater results by sampling location. Samples below the detection limit (10 gene copies per 100 mL) are included.

Wastewater Concentration & COVID-19 Cases

To understand how temporal trends of SARS-CoV-2 concentration in wastewater samples related to temporal trends of COVID-19 cases, we performed separate analyses at the community and college campus levels. At the community level, we used wastewater data from wastewater treatment plants only, as those were viewed as most representative of the community as a whole. Data on COVID-19 cases at the county level were obtained from a database maintained by the New York Times[8], which collates county-level data on daily cases and deaths attributable to COVID-19 as reported by local authorities across the United States. At the college campus level, we used wastewater data from sources most closely associated with campuses. Data on COVID-19 cases at the college campus level were obtained from COVID-19 dashboards from participating colleges, where available (9 of 14 communities). Seven of these dashboards reported data on new cases on a regular basis (ranging from daily to weekly), while one reported data on active cases and another reported data on students in isolation. For all data types, we detected trends in the data over time by fitting generalized additive models[10]. These models consider curves with a wide range of flexible shapes, select those that are best supported by the data, and safeguard against overfitting.

At the community level, temporal trends of SARS-CoV-2 concentration in wastewater samples were discernible in all 14 communities (Figure 8, blue), despite the noisy nature of the wastewater data (i.e., many very low values, relatively few very high values). Trends in some communities indicated a consistent upward trajectory in SARS-CoV-2 concentrations (Figure 8; B, D, E, G-N), which was consistent with trends in case data in all but one community (B). However, closer inspection of the wastewater data in that community suggests more consistency with the peaks than the statistical trend suggests (Figure 8; B, blue circles). In two communities (A, F), peaks were detected around the beginning of September that were consistent with peaks in case data at that time. In one other community (C), a small peak at the beginning of September was detected by the wastewater data that was not apparent in the case data, followed by increases in both wastewater data and case data later in the period of the analysis.

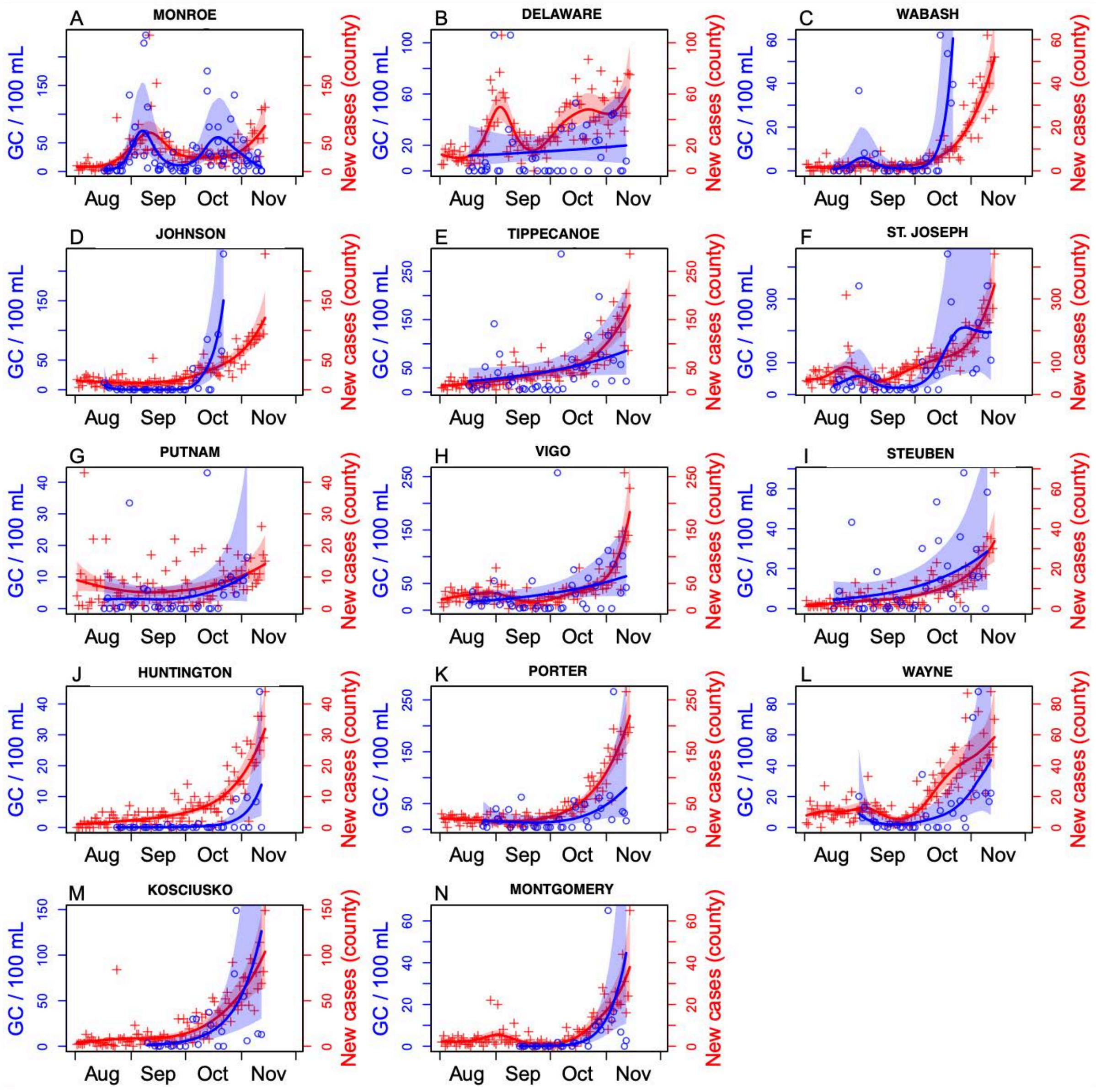

Figure 8. Temporal trends in SARS-CoV-2 wastewater data (blue) and COVID-19 case data (red) for 14 communities in Indiana from August through November, 2020. Points indicate raw data, lines indicate median trends, and bands indicate 95% confidence intervals.

Regarding the potential of wastewater data to serve as a leading indicator of cases, there was not much opportunity to evaluate this at the community level for two reasons. First, SARS-CoV-2 prevalence was generally high and mostly increasing across the time period of this study. Second, the temporal trends in wastewater data and case data were generally consistent with one another. Differences in the height or slope of the curves in Figure 8 should be interpreted cautiously, given that their appearance can be sensitive to the most extreme data points in each data set, which dictate the y-axis ranges. For example, whereas C and D may give the appearance of increases in wastewater preceding increases in cases, it should be noted that cases were also increasing at this time in early- to mid-October. In addition, had wastewater data been collected into November in C and D, further increases in wastewater data would have made the increases observed in early- to mid-October not seem as pronounced. To quantitatively assess the potential of wastewater data to serve as a leading indicator of cases, we performed a cross-correlation analysis similar to other published studies (e.g., Peccia et al. [11]) and found that correlations were strongest at a zero-day lag, meaning that temporal trends in wastewater data and case data were generally concurrent.

At the college campus level, temporal trends of SARS-CoV-2 concentration in wastewater samples were also discernible in all 14 communities (Figure 9, blue). Case data from college campuses was not available in five communities (I, J, L, M, N), but temporal trends based on wastewater data from those campuses, which were relatively small in terms of student population, generally mirrored those from the broader community. In the case of three communities (A, B, H), temporal trends from wastewater data matched temporal trends from case data extremely well, with both tracking either one (A, B) or two (H) peaks during the period of the study. Note that the relative magnitudes of the peaks are not necessarily meaningful, given the different scales of the data and the position of extreme outliers relative to the overall trend. In these communities, changes in temporal trends appeared relatively synchronous across the two data types.

In one community (F), the temporal trend from wastewater data mirrored an increase in cases beginning in late September but did not capture a peak in late August. Although the statistical trend did not capture that peak, there were data points in the wastewater samples in late August and early September that were consistent with such a peak (Figure 9, F; blue points). Had wastewater data collection begun earlier, the statistical trend would have likely captured this peak. In another community (E), no temporal trend was detected. If not for a single very high data point in late August (Figure 9, E; highest blue point), the temporal trend in the wastewater data may have more closely matched that in the case data. In three communities (C, D, G), there were some similarities between the temporal trends in wastewater data and case data, but these were difficult to discern given the small student populations involved and the very low numbers of cases overall.

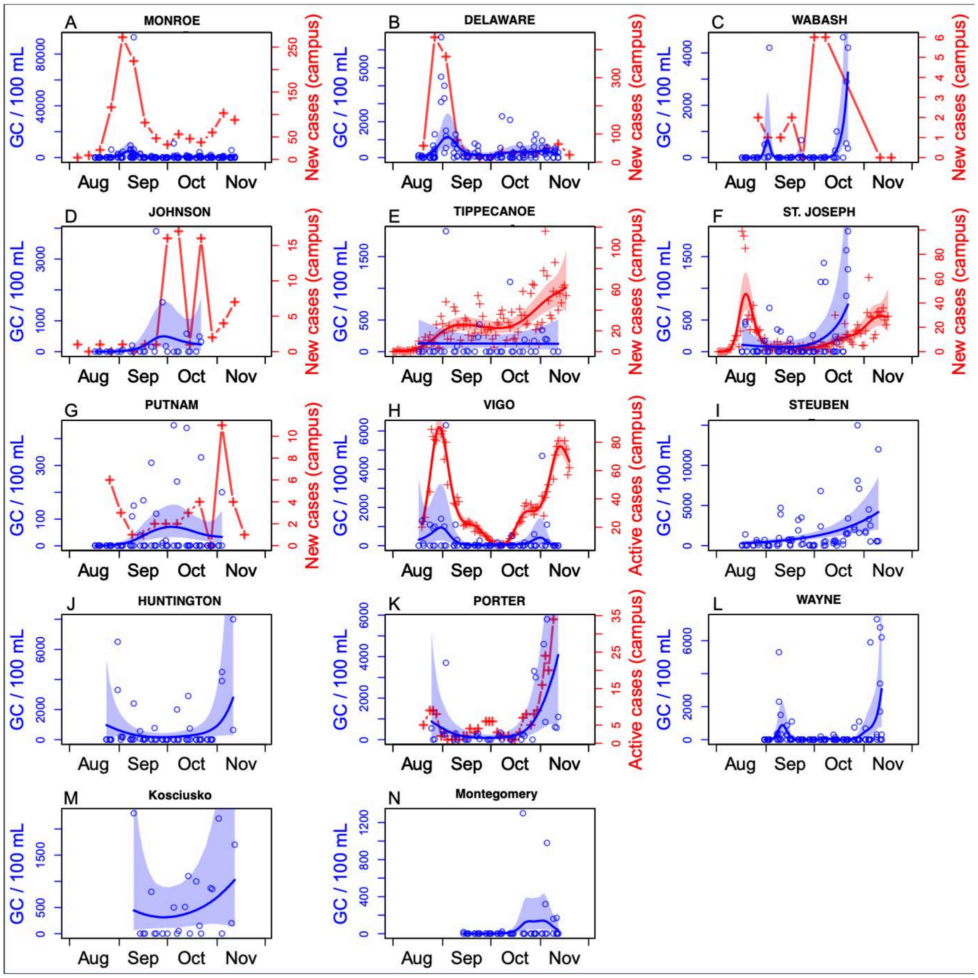

Figure 9. Temporal trends in SARS-CoV-2 wastewater data (blue) and COVID-19 case data (red) for 14 college campuses in Indiana from August through November 2020. Points indicate raw data, lines indicate median trends, and bands indicate 95% confidence intervals. In A-D, G, and K, data were insufficient to support detection of a statistical trend with a generalized additive model, so lines between successive raw data points are presented instead.

In summary, temporal trends in wastewater were detectable in communities and college campuses that participated in this study. These trends mirrored trends in case data in many of these settings. The few instances in which there was less clear concordance between wastewater and case data appear to be due to small sample sizes and noise in the data (e.g., on small college campuses with very low numbers of cases). Altogether, these results provide validation of wastewater samples for monitoring trends in SARS-CoV-2 activity. In broader community settings, changes in the trajectory of SARS-CoV-2 activity appear to occur on timescales of a month or more, given that these changes reflect trends in the overall epidemic in a community as a whole. In college campus settings, changes in the trajectory of SARS-CoV-2 activity were detectable on a timescale of one to two weeks, consistent with the more episodic nature of small outbreaks on some college campuses.

We did not find clear or consistent evidence of trends in wastewater samples preceding similar trends in case data; rather, they appeared to occur at similar times. There are a few possible reasons for this. First, excretion of SARS-CoV-2 in fecal matter and development of symptoms that would prompt testing both occur a few days after exposure to SARS-CoV-2 [9]. Data on the timing of cases used in this analysis reflect times at which testing took place, rather than times at which results from testing were available. Therefore, while wastewater samples may not appear to be a leading indicator of changes in the trajectory of cases in our results, it is possible that signals in wastewater could serve as leading indicators in real-time practice if a community is experiencing severe delays or shortages in testing. Second, most data on cases in this analysis reflect incidence (i.e., new cases), whereas wastewater data are more of a reflection of prevalence (i.e., active cases, which take time to clear after initial appearance). As a result, we would expect peaks and downturns in temporal trends to be somewhat delayed for wastewater data relative to case data.

In the event that this program is continued, the accrual of a longer time series of wastewater data would allow for additional clarity about the relationship between temporal trends in wastewater data and case data. The two-to-three-month period of this study was relatively short in terms of changes in SARS-CoV-2 activity in community settings, and not initiating the study prior to arrival of students prevented us from detecting leading indicators of initial outbreaks on college campuses in late August and early September. Continuation of this program would allow for clearer assessment of wastewater data to detect outbreaks on college campuses after students return from winter break. Building on the analysis from this study, continuation of this program would allow for better assessment of the utility of wastewater data as a leading indicator in real-time practice. Finally, one potentially very valuable extension of this program could be to measure concentrations of other respiratory viruses that could result in cases with similar symptom presentation as mild cases of COVID-19, such as influenza, respiratory syncytial virus, rhinoviruses, and seasonal coronaviruses. Simultaneous wastewater measurement of these viruses could help resolve differences in the trends of these viruses that are challenging to elucidate with case data alone.

USAGE BY PARTICIPANTS

Communities in the study expressed varying reasons for participating and likewise utilized results in unique ways. We summarize views shared in a qualitative survey and in personal conversations with participants throughout the course of the program.

1. Utilities are not public health officials and saw themselves as stewards of the data. Utilization of the information depends on having those decision-making networks in place and on identifying discrete actions public health partners can take as results change.

> We share results with University, Health Department, Local Healthcare & our Local COVID-19 Taskforce to try to provide an advanced warning of outbreak.

2. University collaborators and utilities said the wastewater testing results were a useful layer of information, especially where clinical testing rates were low.

> We feel we are benefitting from measuring the presence of SARS-CoV-2 at the operations facility in addition to the samples we are collecting associated with College Campuses.

3. Some participants expressed uncertainty about how to interpret or contextualize results for local public health decision makers. Some of this hesitation was adequately addressed through internal meetings and additional education, but some remains due to current knowledge gaps in the science around this strategy.

> We would like to have better understanding of the results & how to interpret them as it relates to public health decisions. What is a high viral concentration? How does that translate into a count of the people in the system who are infected?

4. Utilities in our study were eager to contribute to an ongoing area of research and some see wastewater monitoring more broadly as a way to enrich public health data and decision-making.

> The access to this science is important to our organization. We were already looking at the potential to engage with COVID-19 testing before contact with this study. The results from this opportunity will be used to develop engagement and possibly enhance the structural resilience of our communities health network.

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