

When case reporting becomes untenable: Can sewer networks tell us where COVID-19 transmission occurs?

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ABSTRACT

Monitoring SARS-CoV-2 in wastewater is a valuable approach to track COVID-19 transmission. Designing wastewater surveillance (WWS) with representative sampling sites and quantifiable results requires knowledge of the sewerage system and virus fate and transport. We developed a multi-level WWS system to track COVID-19 in Atlanta using an adaptive nested sampling strategy. From March 2021 to April 2022, 868 wastewater samples were collected from influent lines to wastewater treatment facilities and upstream community manholes. Variations in SARS-CoV-2 concentrations in influent line samples preceded similar variations in numbers of reported COVID-19 cases in the corresponding catchment areas. Community sites under nested sampling represented mutually-exclusive catchment areas. Community sites with high SARS-CoV-2 detection rates in wastewater covered high COVID-19 incidence areas, and adaptive sampling enabled identification and tracing of COVID-19 hotspots. This study demonstrates how a well-designed WWS provides actionable information including early warning of surges in cases and identification of disease hotspots.

1. Introduction

Since the early stages of the COVID-19 pandemic, wastewater surveillance (WWS) has been recognized as a valuable tool that can complement traditional epidemiological surveillance, to monitor the spread of COVID-19 and indicate trends in infection at different levels (Peccia et al., 2020; Izquierdo-Lara et al., 2021; Kirby et al., 2021; Karthikeyan et al., 2021). Ideally, traditional epidemiological surveillance through monitoring the number of people who tested positive for the SARS-CoV-2, would provide timely and accurate estimation of COVID-19 incidence. However, many factors like mild or asymptomatic infections, non-specific symptoms, diagnostic test sensitivity, limited diagnostic test reagents and testing centers, increased use of at-home test kits, inadequate data collection and overburdened reporting systems, and public health policies and human behavior could contribute to under-ascertainment, under-reporting, and delayed reporting of COVID-19 using standard disease surveillance approaches (Peixoto et al., 2020; Wang et al., 2021). With detection of SARS-CoV-2 RNA in

wastewater (Ahmed et al., 2020; Medema et al., 2020; Sherchan et al., 2020) and in stools from COVID-19 patients (Chen et al., 2020; Wu et al., 2020) during the early COVID-19 pandemic, WWS, previously utilized to monitor enteric diseases (Hovi et al., 2012; Hellmér et al., 2014; Andrews et al., 2020; Wang et al., 2020), was proposed as an inclusive, non-intrusive, inexpensive, sensitive, and scalable strategy to guide public health response to the COVID-19 pandemic (Daughton, 2020; Larsen and Wigginton, 2020). By September 2022, federal and local governments, health departments, municipalities, and universities in at least 70 countries had utilized WWS to monitor COVID-19 (Anon, 2022a). The results of WWS have been used to alert local jurisdictions, guide resource allocation, enable targeted communications, and forecast clinical resource needs (Kirby et al., 2021).

Most WWS studies have monitored and estimated the COVID-19 incidence at the city level (Peccia et al., 2020; Gonzalez et al., 2020; Wu et al., 2021) by collecting repeated grab or composite wastewater samples from downstream sites (e.g., inlets of wastewater treatment

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facilities), that serve a large population. When COVID-19 is spreading, wastewater samples from downstream sites consistently contain detectable levels of SARS-CoV-2 RNA. To monitor temporal trends of COVID-19 incidence in the catchment population, quantification of SARS-CoV-2 RNA concentration in wastewater is required. WWS has also been widely used at the institution level to monitor disease transmission and provide early warning of surges in cases or outbreaks (Karthikeyan et al., 2021; Gibas et al., 2021; Scott et al., 2021; Wang et al., 2022). Usually, WWS for early warning is considered when the incidence is low. Frequent sampling is necessary to enable a quick confirmation of an outbreak and a timely response. The catchments of sampling sites would preferably be small, which enables targeted control measures. Ethical concerns (e.g., risk of identifying individuals) could be greater when monitoring small populations and should be properly addressed (Hrudey et al., 2021; Wolfe, 2022). However, few WWS studies have focused on sampling upstream sites at the community/neighborhood level (Barrios et al., 2021; Haak et al., 2022). Sampling upstream has the potential to identify disease hotspots within a larger area, which enables monitoring localized disease transmission and targeted public health interventions (Haak et al., 2022; Li et al., 2022). Unlike sampling at wastewater treatment facilities, sample collection at community sites across the sewer network is more complicated and requires more strategic sampling design in order to optimize the value of information and guide interpretation of the results (Wang et al., 2020, 2012; McCall et al., 2017; Larson et al., 2020; Calle et al., 2021; Domokos et al., 2022).

In this study, we conducted COVID-19 WWS at the city and community level in the city of Atlanta. The objectives were to: (1) strategically design the WWS sampling by describing the sewer network in the city of Atlanta from a disease monitoring perspective, and developing and applying an algorithm to select and adapt community sampling sites across sewer networks; (2) examine the temporal correlation between WWS results and COVID-19 incidence at the city level; and (3) illustrate the capacity of WWS to detect and monitor spatial COVID-19 hotspots at the community level. The knowledge gained in this study demonstrates the value of strategic sampling design for WWS to provide actionable information to guide public health response to COVID-19.

2. Methods

2.1. Study area

The current study was conducted in the city of Atlanta, which is the capital and most populous city (498,715 in 2020) within the state of Georgia. The City of Atlanta Department of Watershed Management (DWM) manages the wastewater system in Atlanta and serves 1.2 million customers within the Atlanta Metropolitan Area. There are three water reclamation centers (WRCs) within the city limits of Atlanta, and each WRC has multiple influent lines (Fig. 1a). South River WRC has a permitted treatment capacity of 48 million gallons per day (MGD) and provides wastewater treatment for portions of Atlanta, East Point, Hapeville, College Park, and parts of DeKalb County and Clayton County. Utoy Creek WRC has a permitted treatment capacity of 40 MGD and provides wastewater treatment for portions of Southwest and Northwest Atlanta, East Point, and Fulton County. R.M. Clayton is the largest among the three WRCs, with a permitted treatment capacity of 100 MGD. R.M. Clayton provides wastewater treatment services for the City of Atlanta, primarily north of Interstate 20, a portion of Sandy Springs, and Northern DeKalb County. Detailed information on the WRCs and the sampled influent lines can be found in Supplementary Table 1. The community sampling sites in this study were selected mainly in the southern part of the city (south of Interstate 20), where underserved neighborhoods (e.g., Adamsville, Oakland City, Pittsburgh, Mechanicsville, and the West End) are located (Fig. 1b). The Georgia Department of Community Affairs defined underserved areas as areas with the highest unemployment rate, the lowest per-capita income

and the highest percentage of residents whose incomes are below the poverty level. People living in the underserved areas, when infected by SARS-CoV-2, are less likely to seek health care and get tested, leading to higher levels of COVID-19 under-reporting and under-ascertainment via traditional epidemiological surveillance.

2.2. Sewer network and catchment identification

Once the SARS-CoV-2 viruses in feces enter sewage, they are transported through the sewerage system consisting of sewers (gravity sewers and force mains), manholes, pumping stations, and wastewater treatment facilities. A sewerage system can be viewed as a flow network with manholes (potential sampling sites) as nodes, sewer segments as edges, and wastewater treatment facilities as sinks which only have incoming flows. The directions of edges represent the flow direction of sewage in sewer lines. If pumping stations are not involved, the sewage flow is only driven by gravity, and the flow direction is determined by the elevation.

A simplified flow network (e.g., rivers, sewerage system) could have a line, tree, or net topology. In a line topology network, the water flows from one upstream point (node) to one downstream point without merging or splitting (Fig. 2a). The indegree (the number of edges entering the node) and outdegree (the number edges leaving the node) both equal 1 for all the nodes in the network. A tree topology network has multiple upstream branches (edges) that merge as they flow downstream (Fig. 2b). The indegree could be larger than 1 for some nodes while the outdegree equals 1. The larger the average indegree of nodes, the more branches exist within the tree network. In a net topology network, the flow could split and merge multiple times as the water travels downstream (Fig. 2c). The indegree and outdegree both are larger than 1. The larger the indegree and outdegree, the higher the connectivity within the network. A sewer network has a hybrid topology integrating line, tree, and net topologies. Usually, sewage is collected from geographically widespread points and moves to the final destination of a wastewater treatment facility. In this type of network, the majority of nodes have outdegrees equal to 1, and the sewer lines (edges) are merging, developing mainly a tree topology. In this study, we examined the network statistics (indegree and outdegree) and network topology of the Atlanta sewer network using geospatial data for the Atlanta sewerage system (with flow direction), in shapefile format, provided by the DWM.

For WWS, it is critical to understand the geographic area and approximately how many people are represented in each wastewater sample. Based on the sewer network data, we identified all the upstream points (nodes) and sewer lines (edges) for any potential sampling site (manhole) in the sewer network using “lucy” (Ness, 2016), a wrapper for the igraph package in R. In the current study, such an upstream sub-network was defined as the catchment of a sampling point. The catchment area was estimated as the polygon area covering this sub-network using the “concaveman” package (Park and Oh, 2012) in R, and the catchment size was approximated by information on the number of upstream manholes (including the sampled manhole).

2.3. Sampling design and sample collection

One of the main purposes of this WWS study was to monitor COVID-19 incidence in the city of Atlanta at a city level. In this study, the incidence was defined as the number of new COVID-19 cases reported to the Georgia Department of Public Health (GDPH) over a specific period of time. During March 2021–April 2022, weekly 1 L grab samples of wastewater were collected on Monday mornings at six influent lines of two water reclamation centers (Utoy Creek and South River), identified by partners at the DWM. Since November 2021, samples were also collected from three additional influent lines at the R.M. Clayton WRC. The estimated catchment populations for influent lines are between 5427 and 178,077 (Supplementary Table 1).

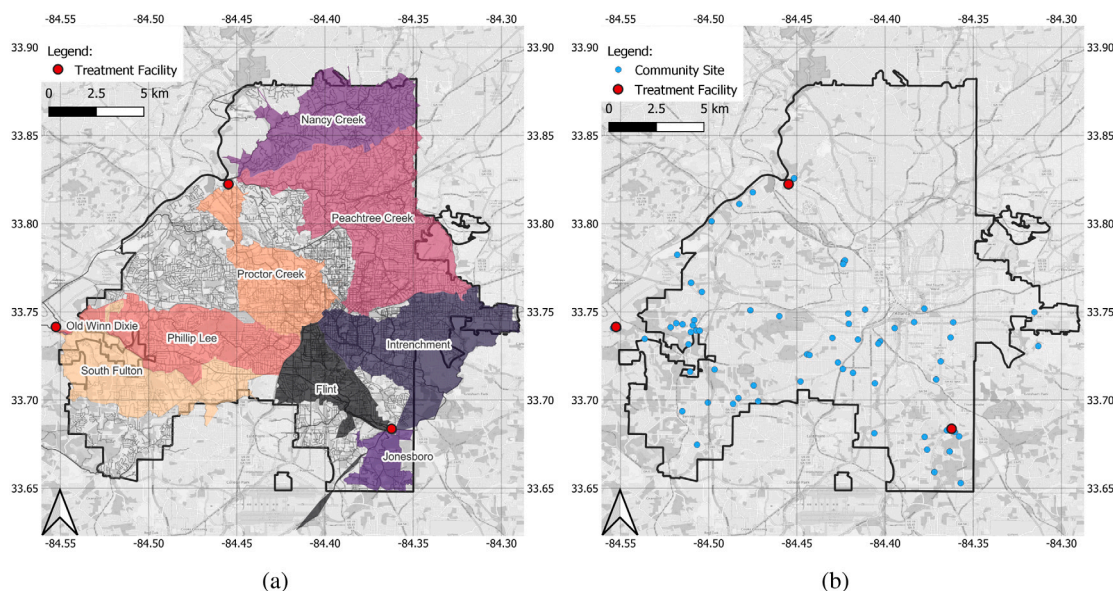


Fig. 1. (a) Estimated catchment areas of nine influent lines sites in the city of Atlanta; The catchment area of Old Winn Dixie, which is very small, may not be visible. (b) locations for community sampling sites and school sampling sites.

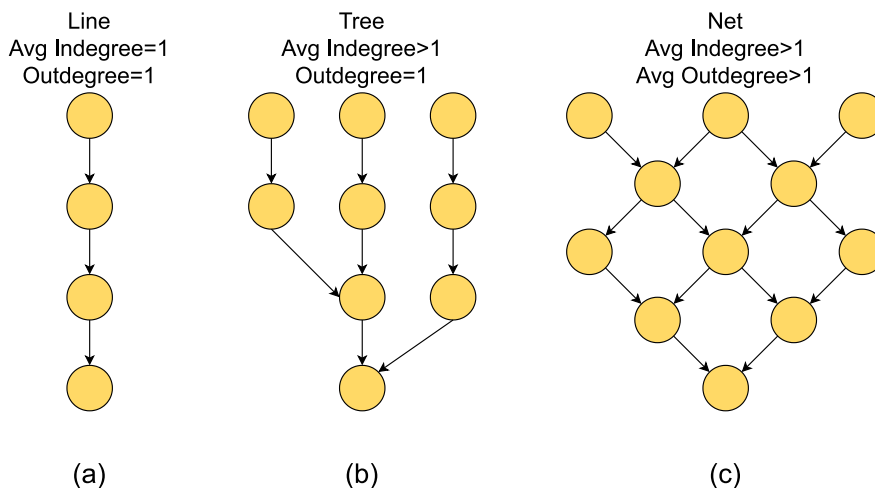


Fig. 2. Indegree and outdegree of different flow network topologies.

The samples from each influent line represent large areas of the city (Fig. 1a) and were intended to monitor temporal trends of incidence within each catchment area. To further assess COVID-19 incidence in smaller geographic areas, samples were collected from upstream community sites at manholes. Unlike wastewater from the influent lines, upstream wastewater is less likely to contain SARS-CoV-2 RNA. The Moore swabs method (Liu et al., 2022), a low-cost composite sampling approach, was used for community site sampling. During April 2021–April 2022, Moore swabs were placed weekly in manholes across the city of Atlanta (Fig. 1b) and retrieved after approximately 24 h. The details of site identification, sampling requirements, sampling schedule, and the mobile data collection process are described in Supplementary Material A.

We developed the Atlanta WWS sampling design for community sites in three phases. In the pilot phase 1 (April–May 2021), information related to the sewer network was not available. We conducted convenience sampling by selecting community sampling sites (manholes) in low-income neighborhoods in South Atlanta based on insights from our partners at DWM. The sampling sites were relocated to new sites every two weeks to explore sampling in different areas of the city and examine the variation in detection rates at different community

sites. In phase 2 (June–August 2021), we obtained the Atlanta sewer network shapefile from the DWM, which enabled us to identify the catchment areas and catchment sizes for all manholes. Based on the catchment sizes (measured as number of manholes upstream of the target manhole), we were able to select sampling sites with small (≤ 100 manholes), medium (100–500 manholes), and large (> 500 manholes) catchment sizes (Supplementary Table 2). Three community sites with low detection rates were relocated after four weeks. In phase 3 (September 2021–April 2022), we set up a nested sampling design by sampling one downstream influent line site along with multiple upstream *branch sites* (community manholes), which was defined as a sampling cluster. Using the Phillip Lee sampling cluster as an example (Supplementary Figure 1), we identified the *main trunk*, which was the longest path in the sewer network with the influent line site as the end point. Tributary *branch sites* were defined as the points (nodes) before merging into the *main trunk*. The *branch sites* were independent of each other (no upstream and downstream relationship), and the initial goal was to cover as many manholes as possible. Independence between different *branch sites* is critical to identify sub-areas with high incidence. In phase 3, we also applied the adaptive sampling process (Wang et al., 2020) to relocate community sites within the nested sampling design

in January 2022. Sites that were rarely positive for SARS-CoV-2 RNA were replaced with new *branch sites*, which were proposed following the descending order of their catchment sizes (largest to smallest).

2.4. Adaptive sampling process

Usually, the independent upstream sampling sites with small catchment areas could not cover the entire influent line site catchment area that we wanted to monitor. Some high incidence areas, if not covered, could be undetected by WWS. The adaptive sampling process relocated a small proportion of sites periodically based on the most recent WWS results collected. The adaptive sampling process has the following steps:

Initialization Assuming we are designing nested sampling on a sewer network and n sampling sites are planned, one sample site will be located at a downstream location of the sewer network (end point of sewer network), and $n - 1$ branching sites (before entering the *main trunk*) will be selected based on their cumulative weight. Each of the manholes (nodes) was given a weight as 1 at initialization. The cumulative weight for each manhole is calculated by adding the weights of all the manholes upstream of the target manhole. The *branch sites* are selected as independent (no upstream and downstream relationship between *branch sites*) sites with the largest cumulative weights.

Weighting Once the sampling sites have been initialized, wastewater is sampled and tested for a couple of rounds. When the presence of SARS-CoV-2 RNA (positive) is detected in samples from a specific site, the weights of manholes within the catchment of this site increase. The percent that the weight increases depends on the proportion of positive samples. On the other hand, if no positive result is detected in samples from a specific site, the weights of manholes within the catchment of this site decrease.

Updating After the weights of manholes have been adjusted, the *branch sites* are re-selected following the process same as initialization but based on updated cumulative weights. The weighting and updating steps are repeated periodically, and the WWS can be transformed into a dynamic system that updates itself based on the most recent results.

Fig. 3 illustrates the application of the adaptive sampling process in a simulation study. The hypothetical high-risk area is located at the left top corner of the sewer network. Here, a high risk of COVID-19 transmission means a large number of shedders contribute SARS-CoV-2 into wastewater. We initialized the sampling with one site at the end of the sewer network and five branching sites. The weighting and updating steps were conducted after every two rounds of weekly data collection. As the update process went on, one or two sampling sites were relocated and the weight of the manholes (represented as the size of nodes) on the left top corner increased. The source code of the adaptive sampling process can be found on Github (<https://github.com/YWAN446/COVID-WWS-ATL>).

2.5. Sample processing and lab testing

Between March–October 2021, three methods were used to concentrate SARS-CoV-2 from wastewater samples and extract viral RNA: (1) The Membrane Filtration method, which incorporates the use of a 0.45 μm membrane filter, was used to capture SARS-CoV-2 from wastewater grab samples. The Qiagen RNeasy Mini kit (Qiagen, Germany) was used for RNA extraction (Liu et al., 2022). (2) The Skim Milk method was used to concentrate of SARS-CoV-2 in Moore Swab samples and the same Qiagen RNeasy Mini kit was used as the Membrane Filtration method ([dx.doi.org/10.17504/protocols.io.b2uwqex6](https://doi.org/10.17504/protocols.io.b2uwqex6)). (3) The Manual Nanotrap[®] Concentration method, which involves CERES Nanotrap[®] magnetic hydrogel particles and an enhancement

reagent (Ceres Nanosciences Inc., USA), was used for both grab samples and Moore Swab samples. The Nanotrap[®] Magnetic Virus Particles capture and bind the SARS-CoV-2 viral particles in the wastewater so that they can be concentrated into a smaller volume. The Qiagen QIAamp Viral RNA Mini kit was used for RNA extraction ([dx.doi.org/10.17504/protocols.io.b2uzqex6](https://doi.org/10.17504/protocols.io.b2uzqex6)). The transition between these three methods to our final automated KingFisher Apex system occurred in October 2021 and the MagMax Viral/Pathogen Nucleic Acid Isolation kit (Thermo Fisher Scientific, USA) was incorporated in the KingFisher platform ([dx.doi.org/10.17504/protocols.io.b2nkqdcw](https://doi.org/10.17504/protocols.io.b2nkqdcw)). Each protocol mentioned above utilized a sample processing control of Bovine Respiratory Syncytial Virus (BRSV) (MWI Animal Health, USA), which was spiked directly into wastewater samples prior to sample processing. After concentration and RNA extraction, SARS-CoV-2 and BRSV were detected by a singleplex real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) ([dx.doi.org/10.17504/protocols.io.b2nkqdcw](https://doi.org/10.17504/protocols.io.b2nkqdcw)) between March–November 2021. After December 2021, a duplex RT-qPCR platform for simultaneous detection of SAS-CoV-2 and BRSV was used between December 2021–April 2022. The details for the RT-qPCR methods were described by Liu et al. (2022). Briefly, SARS-CoV-2 RNA was detected via RT-qPCR using the primers/probe developed by the U.S. CDC and the TaqPath[™] qPCR Master Mix (Thermo Fisher Scientific, USA) was used for RT-qPCR assay. We used Bland–Altman plot and Cohen's kappa coefficient to show that the results from different concentration methods were comparable. In addition, we conducted a sensitivity analysis using results only from the KingFisher method, which had similar delayed correlation patterns compared to using all the results from different concentration methods. The result was classified as positive when both CT values were present from the duplicate wells and at least one CT value was below 36. If both CT values were present and above 36, the sample was considered as weak positive. When any CT from duplicate wells was absent, the sample was considered negative. For grab samples, the concentrations of SARS-CoV-2 RNA in wastewater were estimated from CT values using standard curves.

2.6. Comparing wastewater surveillance results with reported case data

COVID-19 cases reported to the GDPH from March 2021 through April 2022 in Georgia were geocoded using Esri's ArcGIS Streetmap Premium location information for the North America region by GDPH. A set of data sources were used with resident address data from the case report form having the highest priority, followed by electronic laboratory records, and administrative data from other agencies serving Georgia's residents. Coordinates from geocoded resident addresses were used if they passed a series of quality score measures (e.g., resident address matched a single address with the highest score). Records not meeting these quality criteria were excluded. The final data file was the most complete and precise representation of resident address for COVID-19 cases in Georgia.

Wastewater results from any sampling site were matched with a subset of geocoded COVID-19 cases located within the catchment area of the sampling site. For each influent line site, we examined the temporal trends of SARS-CoV-2 RNA concentrations in wastewater and case numbers reported in the catchment area. The concentrations of SARS-CoV-2 RNA were smoothed over time using the LOESS (locally estimated scatterplot smoothing) method. The smoothed concentrations of SARS-CoV-2 RNA and the numbers of daily reported COVID-19 cases in Fulton County/catchment area were analyzed for correlation and temporally lagged correlation. For community sites, we examined the spatial agreement between catchment areas of wastewater sampling sites with high SARS-CoV-2 detection rates and high COVID-19 incidence areas from the geocoded case data. The wastewater detection rate of a site was calculated as the percentage of positive samples and heatmaps of COVID-19 cases were generated using 2D kernel density

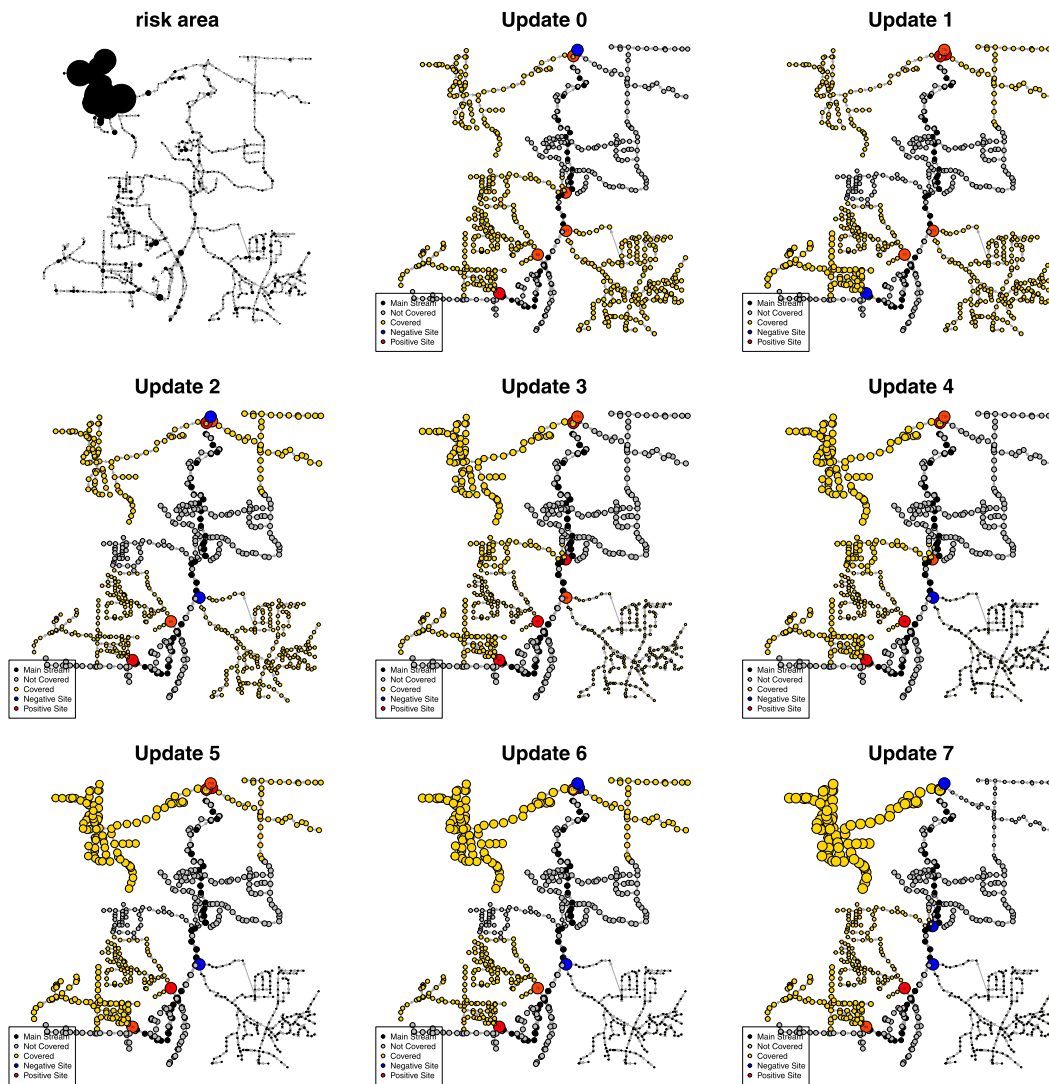


Fig. 3. Illustration of adaptive sampling process in a simulation study. Each node is a manhole in the sewer network. For the risk area subfigure, the size of nodes represents the number of shedders that contribute SARS-CoV-2 into wastewater, as an indicator of COVID-19 infection risk. For subfigures update 0–7, manholes shown in black are on the longest line in the sewer network, defined as the *main trunk* (main stream). Manholes shown in yellow are covered by the selected sampling sites, while manholes shown in gray are not covered. Red manholes represent sites with positive wastewater samples (detection of SARS-CoV-2). Blue manholes represent sites where the wastewater samples were negative. The sizes of nodes represent the weights of sites. The larger the weight, the higher estimated risk of COVID-19 transmission. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

estimation. Data analysis and data visualization in this study were conducted using R 4.0.1. The maps in Fig. 1 were generated by ArcGIS.

The GDPH Institutional Review Board determined that this analysis was exempt from the requirement for IRB review and approval and informed consent was not required.

3. Results

3.1. Atlanta sewer network

We examined the network statistics (indegree and outdegree) and network topology of the Atlanta sewerage system. The indegree and outdegree of a manhole in the sewer network are the numbers of sewer pipes going into and going out of the manhole, respectively. The city of Atlanta sewer network has a total of 52,787 sewer segments and 51,155 manholes. After excluding nodes with zero indegree or zero outdegree, the average indegree and outdegree were 1.40 and 1.04 respectively indicating it was mainly a tree topology network with some net topology structures. However, the sewer network topology varied in different areas. In newly developed areas with low population density,

the sewer network includes more line topology components. In densely populated inner city areas with older sewerage structures, there are more cross-connections and greater connectivity. Fig. 4 illustrates two sub-networks in our study: one near the center of the city (upstream of manhole 23370330601) and the other located more remotely (upstream of manhole 23330211001). The average indegree and outdegree were larger for the sewer network in the city center, which displays a more connected structure. Figs. 5a and 5b show the spatial distribution of in- and outdegree in the Atlanta sewer network. The nodes with large indegree and outdegree are located mainly at the center of the city where the sewers are mainly combined sewers (Fig. 5c).

3.2. Influent line wastewater surveillance results

A total of 362 samples were collected from nine influent lines entering three wastewater treatment facilities during March 20, 2021–April 11, 2022 (Fig. 1a). Fig. 6 shows the concentrations of SARS-CoV-2 RNA in the wastewater samples from influent lines increased between July and August 2021, matching the temporal trend of reported cases in Fulton County, Georgia. When the reported cases decreased in

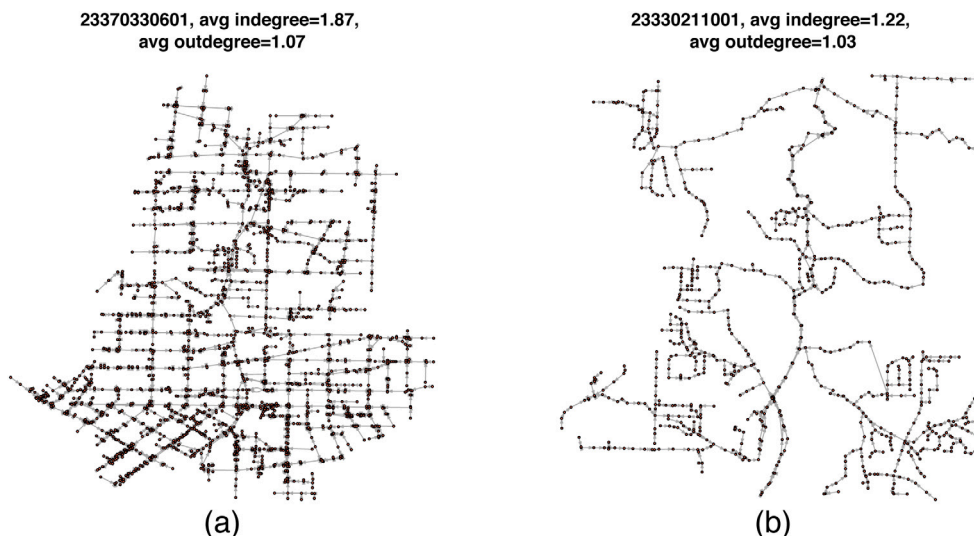


Fig. 4. Two sewer sub-networks (a) within the center of the city and (b) in the periphery of the city.

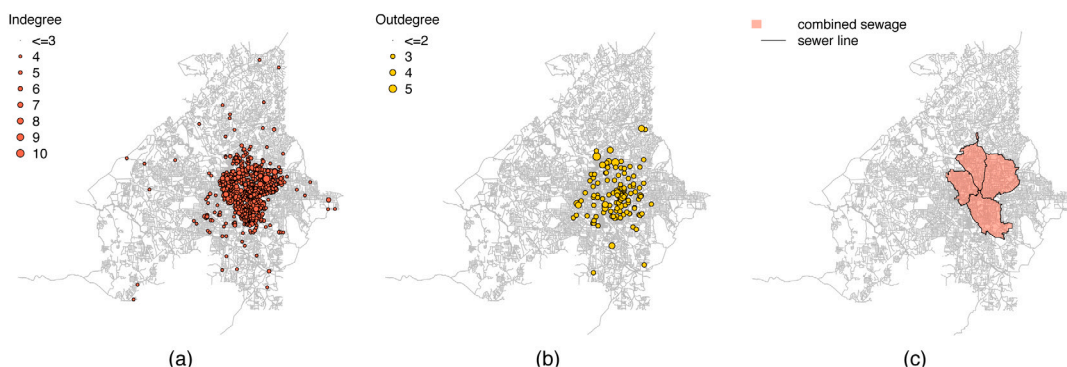


Fig. 5. (a) Geographical distribution of manholes with large indegree on the sewer network; (b) Geographical distribution of manholes with large outdegree on the sewer network; (c) Areas with combined sewers in the city of Atlanta.

October and November 2021, the SARS-CoV-2 RNA concentrations in wastewater stayed relatively high. In late December, the SARS-CoV-2 RNA concentration in wastewater and the reported case numbers surged rapidly and then decreased in January 2022.

Correlation analysis showed that the concentration of SARS-CoV-2 RNA in wastewater and the number of daily reported COVID-19 cases in Fulton County were moderately correlated (Spearman ρ between 0.54–0.71) except for the South Fulton site (Spearman $\rho = 0.34$). We also found strong correlations (Pearson’s r between 0.70–0.98 and Spearman ρ between 0.67–0.95) between the SARS-CoV-2 RNA concentrations in the wastewater samples from those six influent line sites sampled for the entire study period (Supplementary Figure 2). For most influent line sites, the correlations between the concentrations of SARS-CoV-2 RNA in wastewater and the reported cases (in Fulton County or in the catchment area) 7–12 days later were higher than the correlation between the concentrations of SARS-CoV-2 RNA in wastewater and the reported cases at the day of wastewater sampling (Supplementary Figures 3 and 4).

3.3. Community site wastewater surveillance results

Fig. 7 shows the overall WWS results from community sampling sites. A total of 506 Moore swab samples were collected from 56 manholes (Fig. 1b). In the pilot phase 1 (April–May 2021), we collected wastewater samples from 20 community sites, each for a time period of two weeks. We detected samples positive for SARS-CoV-2 RNA at 7 sites, while the other 13 sites only produced weak positive or negative

samples. The detection rates for SARS-CoV-2 RNA at community sites varied even between manholes in close proximity. In phase 2 with the targeted sampling (June–August 2021), the frequency of SARS-CoV-2 detection in the wastewater samples from community sites increased in July–September 2021 along with a surge in number of reported COVID-19 cases in Fulton County. In phase 3 with the nested sampling (September 2021–April 2022), samples from some community sites consistently had detectable SARS-CoV-2 RNA throughout the study period while other sites were only positive in December 2021, when the numbers of reported cases surged to an unprecedented high level. Four community sites (Ruby Harper Blvd, Southside Industrial Parkway, Village Dr, and Walmart), which had low percentages of positive wastewater samples, were relocated at the end of December 2021 to four new sites, which had more positive samples compared to those sites that were dropped. In March–April 2022, when the incidence decreased to a relatively low level, some community sites were still positive for SARS-CoV-2 RNA while their corresponding downstream influent line samples were negative (Fig. 8, Supplementary Figures 5, 6, and 7).

With the nested sampling design in phase 3, we observed spatial agreement between SARS-CoV-2 RNA detection in wastewater from a community site and the number of cases reported within the catchment area of the same site. Fig. 8 shows WWS results and cases reported side by side for the Phillip Lee sampling cluster (one downstream influent line site with multiple upstream community sites) as an example. Catchment areas of community sites with high SARS-CoV-2 detection rates in the wastewater (i.e., Chatham Ave and Plainville Trail) overlapped

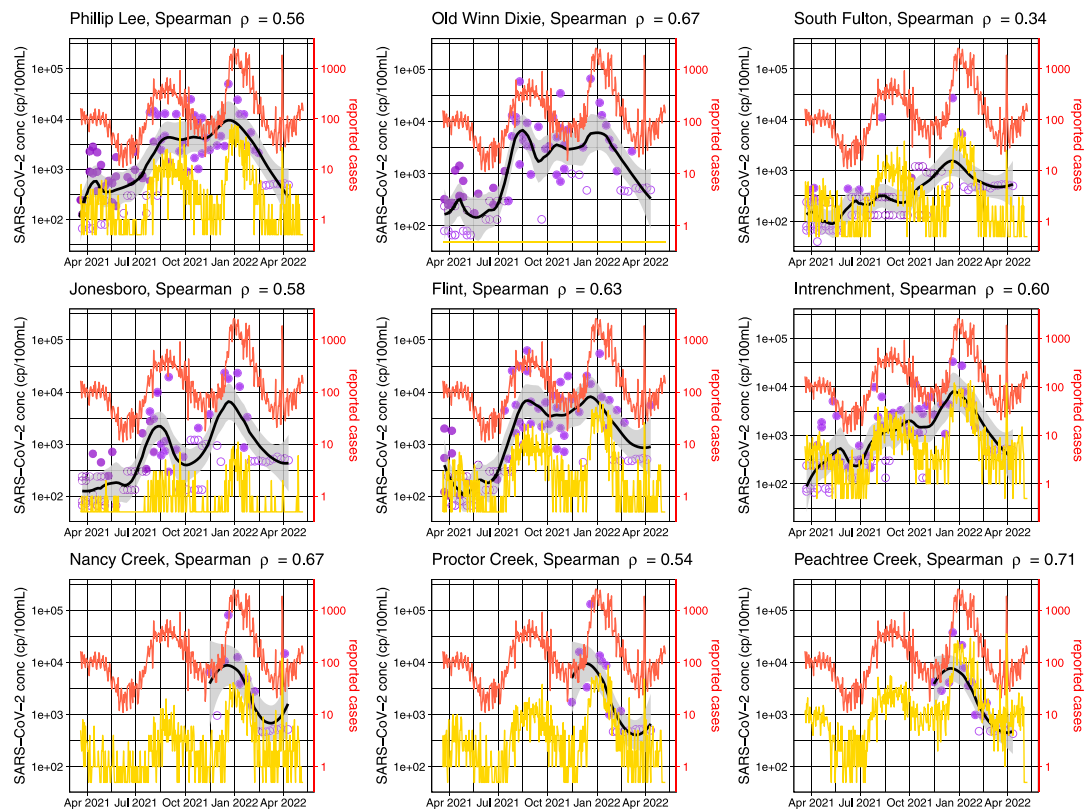


Fig. 6. Temporal trends of the concentrations of SARS-CoV-2 RNA in wastewater from nine influent line sites and the reported case numbers during March 20, 2021–May 8, 2022. Solid purple symbols represent positive samples and open purple symbols show negative samples at the detection limit. The black line is the LOESS line for concentration of SARS-CoV-2 RNA, and the gray band represent the 95% confidence interval. The red line is the number of reported cases in Fulton County, Georgia. The yellow line is the number of reported cases in the catchment area of the influent site. Throughout the study period, no case was reported in the very small catchment area of Old Winn Dixie. The graph caption shows the Spearman correlation between the smoothed concentrations of SARS-CoV-2 RNA in wastewater and the number of reported cases in Fulton County. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with areas with high numbers of reported COVID-19 cases. In contrast, samples from the Walmart site, which has a relatively large catchment area, were only positive for SARS-CoV-2 RNA twice out of 13 samples. In this area, few cases were reported during the surveillance period. The results from other sampling clusters are included in the Supplementary Figures 5, 6, and 7).

4. Discussion

As new variants emerge, SARS-CoV-2 continues to cause waves of infection across the globe (Karim and Karim, 2021). After more than two years of COVID-19 pandemic, adherence to COVID-19 rules and guidance has declined (Michie et al., 2020; Petherick et al., 2021), and there are signs of fatigue in performing PCR tests for individual nasal swabs and reporting daily confirmed COVID-19 case numbers accumulated among patients, caregivers, laboratories, and health departments. Such pandemic fatigue contributes to increasing and more heterogeneous underestimation of COVID-19 incidence all over the world. Meanwhile, it is unrealistic to sustain large scale epidemiological surveillance systems based on individual diagnostic testing given the economic burden especially in countries where the resources are limited. WWS, as an inexpensive, sensitive, and non-intrusive method, could provide complementary information about COVID-19 incidence at different geographic levels and is especially useful for populations where COVID-19 is under-ascertained and/or under-reported due to limited access to diagnostic testing or health behavior. With careful sampling designs, WWS can be utilized to examine temporal trends of COVID-19 incidence at the city level and to identify spatial COVID-19 hotspots at the community level. Such information differentiated by geographic level can directly inform and guide public health responses to COVID-19.

4.1. Spatially explicit sampling across sewer networks

Designing a sensitive and actionable WWS system requires a good understanding of what contributing population or catchment area is represented in a wastewater sample. With a well-characterized sewerage system, the catchment area (and population) can be determined for any sampling site within the sewer network. SARS-CoV-2 RNA concentrations in wastewater collected from such a sampling site reflect ongoing infections in the population who reside in its catchment area. However, sewerage systems usually develop along with the urbanization process. Combined sewers are common in some historical areas, and sanitary sewers are usually added as a city develops and expands. In the city of Atlanta, combined sewers are mainly located in the city center, which is also the most densely populated area of the sewer network. For WWS, sampling at sites from combined sewers poses challenges to interpretation of the results. First, because the combined sewer collects both sewage and stormwater, the concentration of SARS-CoV-2 RNA in the wastewater can be impacted by extreme variation in flow rate and velocity (caused by rainfall) and any chemicals in the stormwater, which may reduce the sensitivity or inhibit the lab assay. In the city of Atlanta, the Intrenchment, Proctor Creek, and Peachtree Creek influent line sites catch large proportions of combined sewers. Second, areas with combined sewers were observed to have many cross-connections (higher connectivity), which may have been designed to avoid blockage and overflow. Catchment areas and populations cannot be precisely determined for sampling sites in such sewer networks.

It is critical that catchments of different sampling sites are discrete areas so that the sources of human excreta entering the catchment can be considered independent. Usually, influent lines are separate and distinct before merging at a wastewater treatment facility. Each

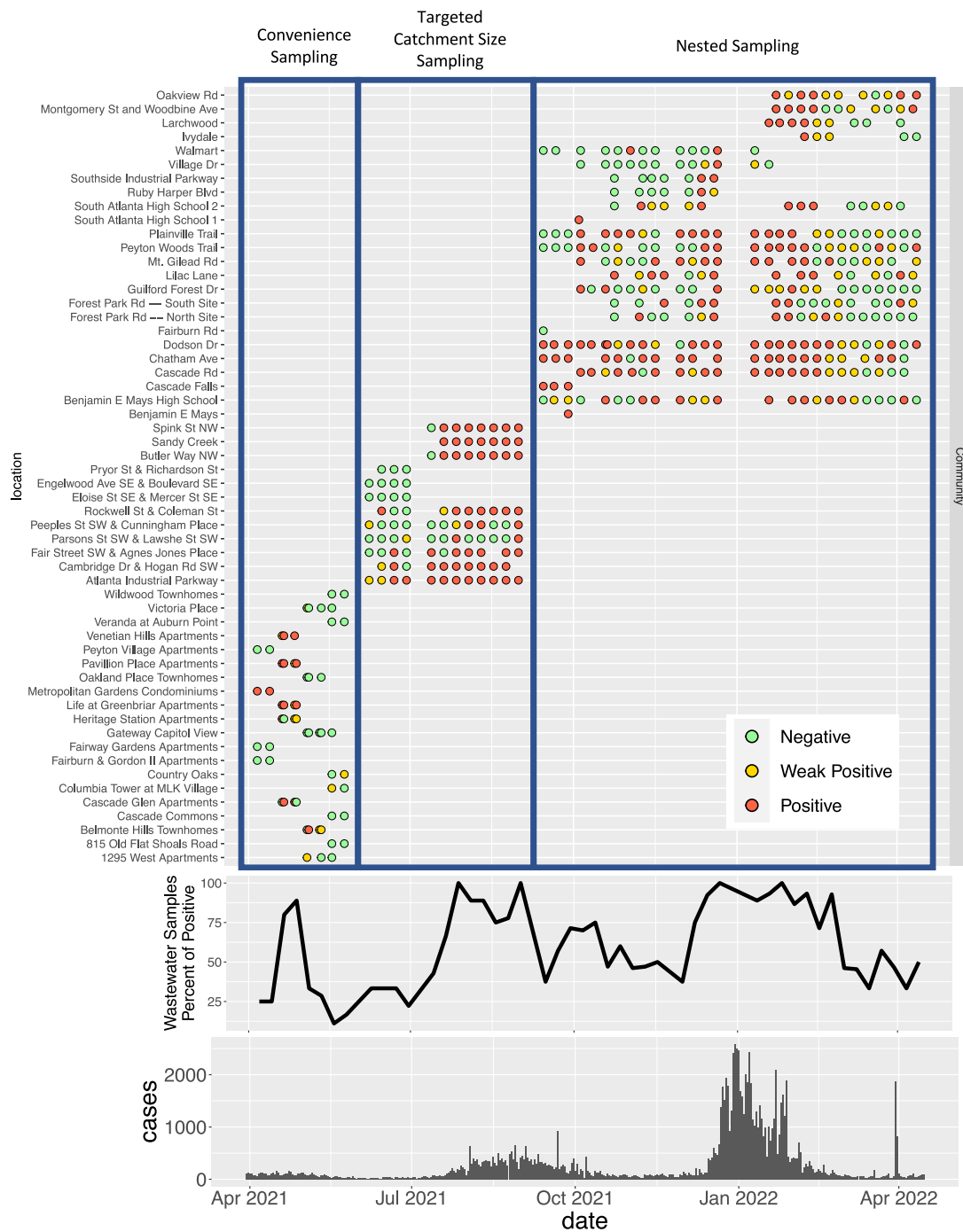


Fig. 7. SARS-CoV-2 wastewater surveillance results for community sites and reported case numbers in Fulton County between March 20, 2021–April 15, 2022. The high reported case numbers in April 2022 were caused by a data dump (i.e., delayed reporting), and did not represent a surge in cases during that month. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

line typically represents a large proportion of the city. For community manhole sites, any upstream and downstream connection creates challenges interpreting results regardless of whether they are quantified RNA estimates or presence/absence. For example, when upstream and downstream sites are both positive for SARS-CoV-2, we cannot determine whether there are COVID-19 infections located in areas covered by the downstream site but not by the upstream site. Independent catchment areas enable a more straightforward approach for linking WWS results with SARS-CoV-2 infections in specific catchment areas (and populations). The selection of independent locations within the network can be achieved with network partitioning methods (Larson et al., 2020; Calle et al., 2021), and in this study we developed

a “main trunk and branch sites” method to achieve this goal. Some limitations are introduced by forcing independence between sites. First, it is challenging to identify independent sites in a sewer network with many cross-connections. The sites selected could have extremely large or extremely small catchment areas. Second, in a tree topology sewer network, a small number of branch sampling sites may only be able to cover a proportion of the sewer network. A careful selection of branch sites (multiple sites as a system) should be located on a tree topology sanitary sewer network, have independent catchment areas, and together capture wastewater inputs from as large an area of interest as possible.

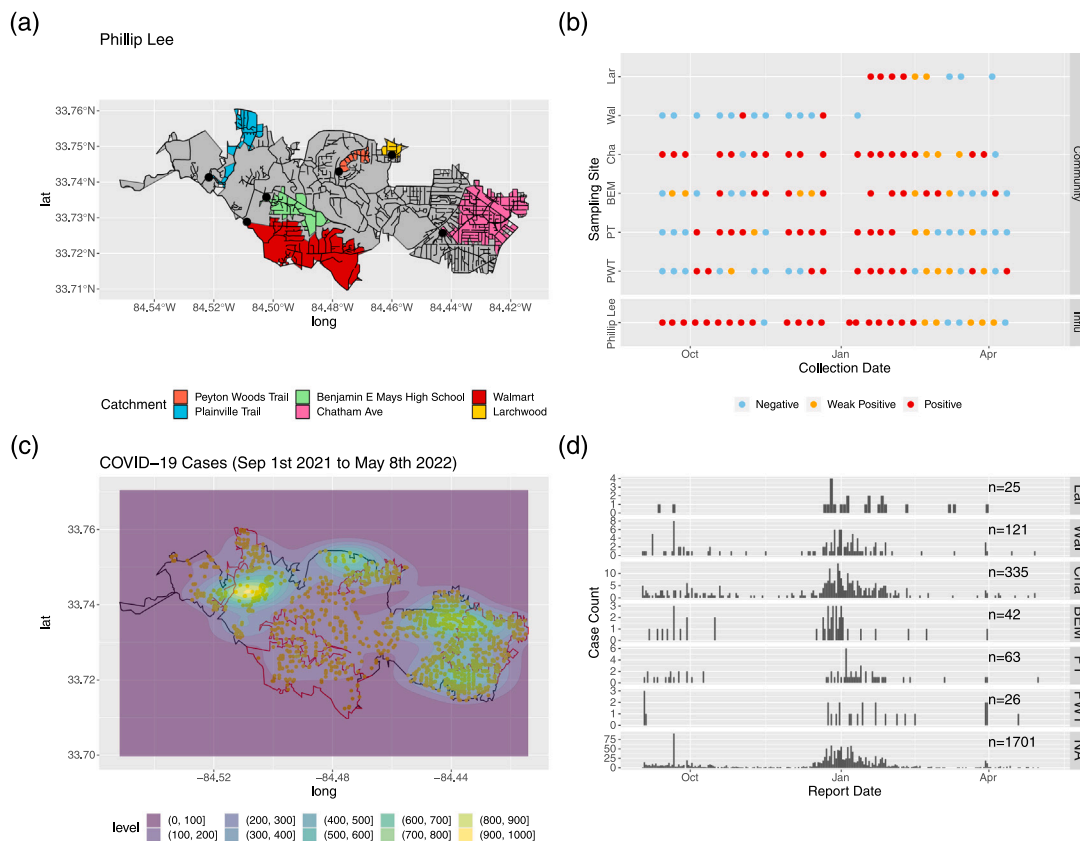


Fig. 8. SARS-CoV-2 wastewater surveillance results for community sites and reported COVID-19 case numbers in the catchment area for the Phillip Lee sampling cluster between September 2021–May 2022. Subfigure (a) shows the catchment areas of each community site nested within the overall catchment of the influent line site (in gray). The black lines represent the sewer network lines. Subfigure (b) shows the weekly wastewater surveillance results (RT-PCR detection of SARS-CoV-2 RNA). Subfigure (c) shows the heatmap of reported COVID-19 cases between September 1st 2021–May 8th 2022 within the influent catchment area. Subfigure (d) shows the epidemic curves of COVID-19 within each community site catchment area and NA represents all the cases in the catchment area of the influent line site that are not in the catchment area of a specific community site. PWT, PT, BEM, Cha, Wal, and Lar represent Peyton Woods Trail, Plainville Trail, Benjamin E Mays High School, Chatham Ave, Walmart, and Larchwood respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.2. Wastewater surveillance at the city level

At the city level, routine COVID-19 WWS, with one or more samples per week, could supplement or even replace epidemiological surveillance for obtaining information on the spread of COVID-19 in large populations (large catchment areas within a city). Translation of SARS-CoV-2 concentrations in wastewater into numbers of cases requires much information: virus concentrations in wastewater must be quantified reliably (and consistently); concentrations of human fecal matter in wastewater must be estimated using markers like pepper mild mottle virus (PMMoV); fecal shedding of SARS-CoV-2 in infected subjects must be quantified (fraction of infected subjects who shed the virus fecally, kinetics of fecal shedding); and information on mobility to establish locations for infected people detected via wastewater. Even though predicting the number of COVID-19 cases or SARS-CoV-2 infections (symptomatic and asymptomatic) from wastewater results is challenging and involves many factors (Wade et al., 2022), it is reasonable to assume that the time course of SARS-CoV-2 RNA levels in wastewater should reflect the time course of numbers of viral shedders in the population connected to the sewerage system. Similar to previous studies (Weidhaas et al., 2021; Ai et al., 2021; Feng et al., 2021; Wu et al., 2022), this study found temporal correlations between SARS-CoV-2 RNA concentrations in wastewater and numbers of daily COVID-19 cases reported. With weekly sample collection, we observed a 7–12 days lead time in wastewater signal trends in influent line samples compared to trends in reported cases in the catchment areas for these influent line sites. This finding demonstrates the potential of WWS to provide early warning of COVID-19 case surges in the city, which could

forecast needs for clinical and diagnostic testing resources. However, there was also some discordance between the trends of the SARS-CoV-2 RNA concentration in wastewater and the number of reported COVID-19 cases. Many wastewater samples from the South Fulton influent line were negative for SARS-CoV-2 RNA even when there was a large number of reported cases in the catchment area. We have been exploring the reasons for this finding by measuring pH values, suspended solids, and turbidity of all the wastewater samples from influent lines since July 2021. Higher pH values (>10) were frequently measured (68%) in the wastewater samples collected from the South Fulton influent line compared to pH values (around 7) in the wastewater samples from other influent lines. High pH may denature the virus and degrade the viral RNA, and it may also indicate the presence of other chemicals in wastewater that may cause PCR inhibition. We also observed sustained high SARS-CoV-2 RNA concentrations in some influent line samples after the reported COVID-19 case numbers declined, which could be caused by prolonged fecal shedding (Zhang et al., 2021) or may indicate continuing silent transmission in some communities. The SARS-CoV-2 RNA concentration in wastewater may be considered to represent the number of infected people currently shedding the virus in their feces, rather than COVID-19 incidence. Therefore, concentrations of SARS-CoV-2 RNA in wastewater can be considered a distorted reflection of the reported numbers of COVID-19 cases, convoluted by the fecal shedding curve which causes delay and averages over a certain time period. This distortion weakens the correlation between concentrations of SARS-CoV-2 RNA in wastewater and numbers of COVID-19 cases reported. Silent transmission occurs when an asymptomatic case passes the virus to someone else. As the pandemic continues, there will

be more people with some level of immunity, who may be infected with mild or no symptoms. Those infections, which may be not captured by epidemiological surveillance based on diagnostic testing, are still contributing to the disease transmission and are shedding SARS-CoV-2 in their feces. In such a scenario, WWS is valuable to guide public health response especially in settings where resources are limited or there is severe under-reporting/under-ascertainment.

4.3. Tracing COVID-19 hotspots in communities

Sampling wastewater from upstream community sites in addition to sampling from downstream influent sites (i.e., nested sampling) improves the sensitivity of wastewater-based surveillance system. SARS-CoV-2 RNA signals at downstream sites could be subject to large dilution, rapid degradation of virus and viral RNA, and substantial loss of signal while moving through the sewer lines (Wang et al., 2020). In this study, while the samples from South Fulton influent line site frequently failed to detect SARS-CoV-2 potentially due to high pH values, wastewater samples from its upstream community sites consistently tested positive for SARS-CoV-2 RNA. In this type of situation, sampling only from the downstream influent line site produced false negative results. And when the disease incidence is low, the concentration of SARS-CoV-2 RNA could be diluted to a level below the limit of detection whereas sampling at the upstream sites, that are closer to the shedders, could be more sensitive (Wang et al., 2020).

With an estimated over 413 million people infected with SARS-CoV-2 and 4.27 billion people fully vaccinated worldwide (Anon, 2022b), immunity has gradually increased in the population. However, acceptance and availability of COVID-19 vaccines are not uniformly distributed (Hughes et al., 2021) resulting in more heterogeneous and localized COVID-19 transmission (Andersen et al., 2021). Given these circumstances, more targeted prevention and control measures are needed to allocate the resources (testing capacity, vaccines, health communications, and healthcare resources) to those communities at risk. Reported COVID-19 cases can be geocoded with the assistance of other existing data systems (e.g., voting registration, vehicle registration, medical records etc.) to identify spatial clustering of cases. However, geocoding all reported cases is not a standard procedure in the epidemiological surveillance and requires tremendous effort. In this study, we evaluated the feasibility of using WWS at the community level to trace disease hotspots in a spatial area. With a nested sampling design, *branch sites* that cover high COVID-19 incidence areas tend to have high SARS-CoV-2 RNA detection rates. This study demonstrates how such spatial coincidence enables WWS to be deployed to locate infection clusters.

Community level WWS with a small number of independent sampling sites usually cannot cover the entire geographic area. The adaptive sampling method in the current study allows relocation of sites not detecting SARS-CoV-2 and searching for hotspots in the area. Furthermore, with immunity waxing and waning in the population, and the periodic introduction of new SARS-CoV-2 variants, disease transmission will continue to vary both temporally and spatially. For example, populations in COVID-19 hotspots during the latest Omicron wave (December 2021–February 2022) may gain some level of immunity and be less likely to contribute to COVID-19 transmission for several months afterwards. Then, the COVID-19 hotspots for the next wave of infection could move to areas where the population has a low level of immunity. An adaptive sampling approach can transform WWS into a dynamic system to identify and trace COVID-19 hotspots in communities.

5. Conclusions

This study highlights the importance of carefully designed sampling strategies for developing a sensitive and sustainable WWS. We have developed, applied, and validated a novel nested sampling strategy

with adaptive sampling process, which enables identification and tracing of COVID-19 hotspots. The strategic sampling design described here is critical for long-term sustainability of WWS for COVID-19 and other diseases, and provides maximum value of information from a minimum number of samples. The spatial granularity of this wastewater surveillance data provides actionable information to guide COVID-19 prevention and control measures at different geographic levels.

Code availability

The codes for performing the data analyses, data visualization, adaptive sampling process, and simulation study are available at <https://github.com/YWAN446/COVID-WWS-ATL>.

CRediT authorship contribution statement

Yuke Wang: Conceptualization, Methodology, Software, Formal analysis, Writing – original draft. **Pengbo Liu:** Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision. **Jamie VanTassel:** Investigation, Supervision, Writing – review & editing. **Stephen P. Hilton:** Software, Data Curation, Writing – review & editing. **Lizheng Guo:** Investigation. **Orlando Sablon:** Investigation, Writing – review & editing. **Marlene Wolfe:** Conceptualization. **Lorenzo Freeman:** Investigation, Writing – review & editing. **Wayne Rose:** Investigation. **Carl Holt:** Software, Data Curation, Writing – review & editing, Visualization. **Mikita Browning:** Investigation, Writing – review & editing, Supervision. **Michael Bryan:** Data Curation, Writing – review & editing. **Lance Waller:** Writing – review & editing. **Peter F.M. Teunis:** Conceptualization, Formal analysis, Writing – review & editing. **Christine L. Moe:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The dataset of WWS results is available at <https://github.com/YWAN446/COVID-WWS-ATL/tree/main/data>. The geocoded COVID-19 case data used in this study is available through the Public Health Information Portal data request process (<https://dph.georgia.gov/php-data-request>).

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.watres.2022.119516>.

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