NC Collaboratory Final Report

February 8, 2021

Title: Tracking SARS-CoV-2 in the Wastewater Across a Range of North Carolina Municipalities

Short title: NC Wastewater Pathogen Research Network (NC WW Path)

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BACKGROUND: The novel coronavirus referred to as SARS-CoV-2 causes severe respiratory distress, therefore its label as a SARS-like virus (Severe Acute Respiratory Syndrome). The disease that SARS-CoV-2 causes is referred to as COVID-19 (Coronavirus Infectious Disease 19). COVID-19 is caused by a group of viruses within the family "Coronaviridae" within the genus *Betacoronavirus*. Coronaviruses are positive-sense single-stranded RNA viruses. SARS-CoV-2 is contagious in humans and is the causative agent of a major pandemic outbreak that initiated in the Wuhan Region of China. At the current time, millions of cases have been counted, with case fatality rates (CFR) that range from less than 1% to as high at 15% in specific populations. Over the course of a pandemic that has seen millions of deaths, clinical testing is now well-entrenched in most countries. At the writing of this document (16 January 2021), over 195 universities, 983 sites, with 45 countries represented (https://www.covid19wbec.org/covidpoops19) globally were participating in wastewater-based epidemiological studies around the world. Furthermore, over the course of 2020, a collaborative team outlined the needs for wastewater-based epidemiology to provide important information on aggregate levels of community disease in a peer-reviewed publication (e.g. Bivins et al., 2020). It was noted early in the pandemic that quantification of the SARS-CoV-2 RNA in wastewater was a key, but often complex, step in being able to use wastewater-based epidemiology for public health management (WBE, e.g. Medema et al., 2020). It has already been shown that the tools for quantification, if conducted at a high level of technical molecular proficiency, can be accurate for understanding the dynamics of the pandemic in a way that is non-invasive and aggregate. Furthermore, cross-laboratory studies have shown that even if teams of researchers use slightly different approaches and materials, they tend to generate similar SARS-CoV-2 data for wastewater-based analyses (Pecson et al., 2020).

At the start of this funded project, research efforts to conduct wastewater-based epidemiology existed at few NC academic institutions. Importantly, over the course of this funded project, we successfully developed a collaborative team comprising academic (UNC System), State agencies (e.g. NC Department of Health and Human Services (NC DHHS), NC Department of Environmental Quality (NC DEQ)), and water utility stakeholders. This collaborative effort has successfully built the foundation and capacity for the State of NC to not only conduct, but to lead the development of, wastewater-based surveillance in the broadest sense (Figure 1).

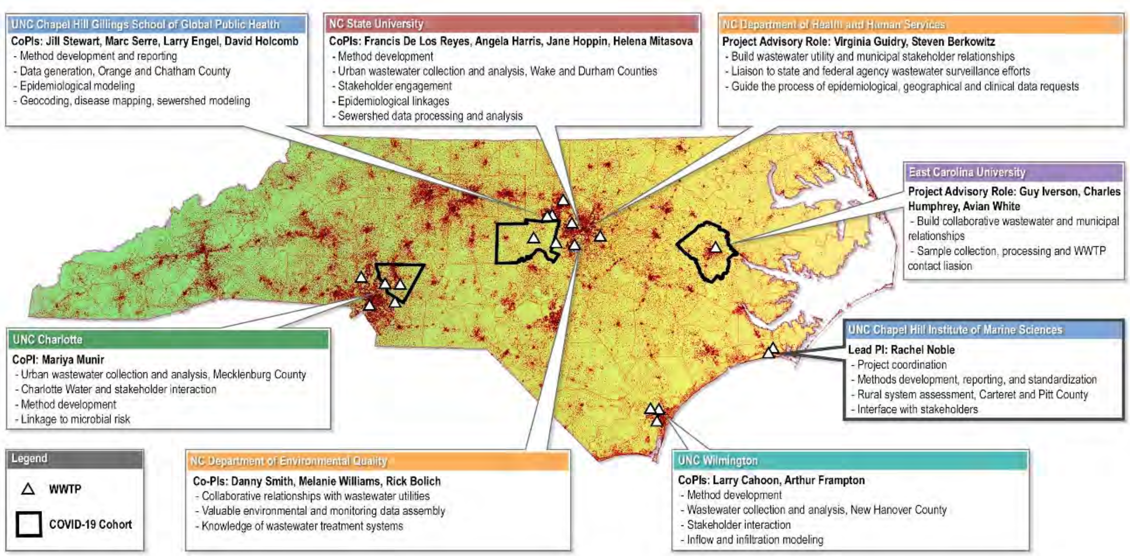

Figure 1: Map of North Carolina-based project and advisory team institutions, participants, and roles.

Now that a framework of collaborative teamwork, stakeholder interaction, epidemiological data access, and hardware and professional technical skills are established in North Carolina, this surveillance program is coordinating at the federal agency level (CDC National Wastewater Surveillance System) to develop an early warning system that is not dependent on clinical testing of COVID-19 infected individuals. *This will provide critical information to public health officials for COVID-19 crisis management, but should be envisioned as the start of a larger effort to create a pandemic preparedness system in the State of North Carolina for future outbreaks.*

The overall goal of the 2020-funded research project was to develop capacity and contribute valuable information to the State of NC on the presence and persistence of SARS-CoV-2 like viruses in complex wastewater infrastructure and environmental systems as a metric of community COVID-19 prevalence.

Key accomplishments:

- **Built laboratory, analytical and epidemiological research capacity for the State of North Carolina and beyond.**
- **Responded rapidly to the COVID-19 pandemic through coverage of over twenty municipal wastewater treatment plants over the duration of 2020.**
- **Built relationships with industry and agency partners, state (NC DHHS, NC DEQ), federal agency (US Centers for Disease Control and Prevention), and national and international academic partners (University of Wisconsin, Notre Dame, Stanford).**
- **Established new relationships with wastewater utilities, municipal stakeholders, sanitation districts, joint powers agencies, and others interested in developing improved surveillance systems using wastewater.**
- **Fostered collaboration with NC researchers to examine the use of WWTP-based COVID-19 data for improved management of clinical and medical resources.**
- **Garnered additional funding for technical, statistical, epidemiological, clinical, and molecular applications to improve research infrastructure in the State of North Carolina.**
- **Requested, compiled, analyzed and reported linkages between wastewater, sewershed, epidemiological and environmental data.**
- **Established relationships with other public health research teams studying COVID-19 infection dynamics in NC.**

Accomplishments over the course of the project are summarized and presented here according to the original objectives stated in the original scope of work.

OBJECTIVE 1: Develop cross-state collaborative project team to conduct WWTP collection, sampling and analysis relying on a framework of existing collaborative academic-state-industry relationships.

The collaborative team includes UNC Chapel Hill, UNC Charlotte, UNC Wilmington, and North Carolina State University, as well as the NC DHHS, and NC DEQ (see Appendix A for a personnel support summary for this project). We engaged with 20 municipal wastewater treatment plants (WWTP) in the study, with active sampling commencing at all of these, plus additional locations. The coverage of participating WWTPs can be seen in the map presented in Figure 1 by the open triangles. The municipal stakeholders who participated in this project are also highlighted in Table 1 below.

There were three initial steps to project team development. First, the group simultaneously developed and shared expertise, molecular protocols, and hardware/software applications knowledge. Each laboratory developed a sampling collection, processing and analysis program adhering to all quality controls and laboratory safety requirements (Appendix B). Sample and data collection from participating WWTPs was streamlined using shared files and approaches, and incorporated metrics from required monthly reporting including wastewater-specific data collection attributes such as flow, infiltration, inflow, precipitation, temperature, total suspended solids, and confounding factors. Each individual academic laboratory established relationships with their respective WWTP stakeholders, and collected and analyzed samples at frequencies of either one, two or three times per week depending on the location and the university role on the project.

Additional empirical investigations of overall processing efficiency, extraction efficiency and PCR efficiency were conducted by individual laboratories, often with side by side sampling and analysis conducted at the UNC Chapel Hill Institute of Marine Sciences. The project team optimized approaches to analyze SARS-CoV-2 in both liquid influent and solids samples collected at the primary clarifier of the WWTP. We generated data over the course of the entire project at each WWTP sampled, and paired that data with the 7-day rolling average of new cases reported (see Appendix C1 and C2 Figures).

SARS-CoV-2 concentration data generated from the efforts noted in Objective 1 were then utilized across the other objectives of the project. The capacity built over the course of the project has translated into funding for at least two additional projects at the time of this writing, 1) supported by CDC through the NC DHHS to support NC participating in the National Wastewater Surveillance System (total funding amount $160,000 over 6 months) and 2) support to Noble and collaborators through NIH to develop a highplex surveillance system for a wide range of bacterial and viral pathogens, and antibiotic resistant bacteria, for application not only in the state of NC, but also across the USA (total funding amount $1.3 M, Appendix F). A wide range of project presentations, outcomes, and stakeholder interaction examples are provided on the Sharepointe site, and can be requested for further presentation.

OBJECTIVE 2: Ensure that collaborating laboratories are making progress toward fully quantitative, validated wastewater analyses for SARS-CoV-2 and variants.

Over the course of this project, we developed and shared protocols (Appendix B) across five laboratories attending to quality assurance and quality control measures. Fully quantitative SARS-CoV-2 concentration data can be visualized from the example plots shared in the figures in Appendix C as part of this study. To accomplish this, we conducted extensive cross-project correspondence (weekly project team and methods-focused meetings), materials and protocol sharing, sharing of reagents, standards and controls, and other materials. We shared calculation spreadsheets and provided access to theory and application information to ensure that our quantification of SARS-CoV-2 and related metrics were accompanied by appropriate quality assurance and quality control measures to permit publication in peer-reviewed journals (e.g. Ciesielski et al. 2021, in preparation, Kotlarz et al. 2021 in preparation, and Munir et al. 2021, in preparation). This was a stepwise, vital, and necessary process, and the lead laboratories for this project have combined decades of experience with training for the use of these tools. The final workflow common to the laboratories conducted qRTPCR and qRTddPCR analyses is shown in Appendix B.

In addition to internal, project team driven collaboration, we collaborated with outside industry partners (Table 2) to improve the outcome of Objective 2, including Biogx (respiratory panel), Bio-Rad (validation of materials and reagents), Zymo (validation of extraction and concentration approaches), QIAGEN (validation and application of the QIAcube automated extraction system), and ThermoFisher (validation and application of the high-throughput KingFisher Magnetic Particle Analyzer for SARS-CoV-2 analyses and high recovery efficiencies). All of these commercial assessment activities will proceed through 2021, and serve to improve the overall quality of data generated on behalf of wastewater-based surveillance. In addition, three cross-state collaborative relationships have been built as part of this project, 1) Southern California Coastal Water Research Project (Steele, Griffith, Zimmer-Faust), 2) Hampton Roads Sanitation District (Gonzalez), and 3) State of Wisconsin (McLellan, Sloan Foundation).

OBJECTIVE 3: Initiate collaborative framework of municipal, county, and stakeholder interaction, through collaboration with the NC DEQ, UNC system researchers, NC DHHS, and County Health Departments, utilizing existing GIS mapping, epidemiological and modeling expertise.

Collaborative partnerships that include not just the UNC system research labs, but also municipal, county, and state health and environmental agencies across NC were developed. This collaborative network made it possible to establish the logistics of wastewater sampling, obtain and analyze state-wide public health (COVID19 testing) data, and potentially use the data to guide policy decisions (Table 1). Each partner contributed in different ways to the collaboration, resulting in a synergistic network that allowed integration of different data sources and expert/stakeholder inputs.

Table 1. NC Pathogen Research Network Collaborative Partners and Municipal Stakeholders.

Partner	**Type of agency**	**Role**	**Specific research outcomes/comments**
NC Department of Health and Human Services (DHHS)	State	Advise surveillance system development, coordinate CDC project, facilitate access to testing data, stakeholder engagement	Critical to obtaining data, liaising with county health officials
NC Disease Event Tracking and Epidemiologic Collection Tool (NC DETECT)	State	Access to testing, emergency room, and hospital admissions data	Extended existing relationships for data requests and collaboration
NC Department of Environmental Quality	State	Sample collection, access to wastewater data, wastewater monitoring reports	Connected municipalities to academic team, including access to monitoring data
Town of Beaufort WWTP	Municipal utility	Provide wastewater influent samples, access to monitoring data	Rural location, rural and coastal tourism service population
Town of Morehead City WWTP	Municipal utility	Provide wastewater influent samples, access to monitoring data	Rural location, rural and coastal tourism service population
Town of Newport WWTP	Municipal utility	Provide wastewater samples	Rural location
City of Wilmington (Southside, Northside-county, Northside-city)	Municipal utilities	Provide wastewater samples	Periurban location

City of Raleigh	Municipal utility	Wastewater sample collection logistics, wastewater data	Urban service population
Town of Cary (North, South, Western Wake)	Municipal utilities	Wastewater sample collection logistics, wastewater data	Urban and suburban service population
City of Durham (North, South)	Municipal utilities	Wastewater sample collection logistics, wastewater data	Urban and collegiate service population
Town of Brunswick (Northeast)	Municipal utilities	Provide wastewater samples	Suburban service population, inflow and infiltration studies
City of Charlotte (McDowell Creek, Mallard Creek, Sugar Creek)	Municipal utilities	Provide wastewater samples	Urban and suburban population, includes collegiate population
Orange Water and Sewerage Authority	Municipal utility	Provide wastewater samples	Urban and suburban service population
Town of Pittsboro	Municipal utility	Provide wastewater samples	Sparse suburban and collegiate population
Durham County Public Health	County	Workshop participants	Stakeholder participation and feedback on value of wastewater-based epidemiology
Wake County Public Health	County	Workshop participants	Stakeholder participation and feedback on value of wastewater-based epidemiology
WakeMed Hospitals	Hospital	Workshop participant	Stakeholder participation and feedback on value of wastewater-based epidemiology

A major challenge in the public health interpretation of SARS-CoV-2 wastewater surveillance data is that, currently, it is not recommended to convert wastewater concentrations into infection prevalence estimates. Therefore, once SARS-CoV-2 wastewater data is in hand, it is not clear how best to communicate it to public health officials. On Dec 2, 2020, NC State University hosted a virtual stakeholder workshop using Zoom with representatives from NC DHHS, local health departments, wastewater utilities and other organizations, to discuss communication strategies for SARS-CoV-2 wastewater surveillance data. Specifically, we discussed the relative importance of presenting 1) uncertainty and detail in wastewater surveillance data; 2) relationships between wastewater surveillance data and case data; and 3) specific formats aimed at public health decision making. There were 29 workshop attendees at the time of peak attendance. We sent an anonymous, electronic pre-survey before the workshop, and an anonymous, electronic post-survey after the workshop. During the workshop, we administered Zoom polls. We are working on compiling the survey and poll results, and reviewing the transcript from the discussion. The NC State IRB reviewed our protocol and determined this work to be IRB exempt. We are writing a commentary on the importance of effective data visualizations for SARS-CoV-2 wastewater surveillance.

Our project team has also worked collaboratively with PI Dr. Greg Characklis (UNC Chapel Hill) to provide wastewater sampling and concentration data that has been incorporated into a susceptible-exposed-infected-recovered (SEIR) epidemiological model making short-term ensemble projections of new hospital admissions in the Raleigh/Durham/Chapel Hill and Charlotte regions. The ensemble projections are being used to trigger hospital management decisions, including elective procedure cancellations and admission triage, under a range of COVID-19 outbreak scenarios. The project team running the model is running simulations to test if more resolved forecasts using wastewater sampling data can improve tradeoffs between capacity utilization, cost, and standard-of-care objectives during the potential outbreak periods. We have benefitted from this collaborative relationship, and view this research as a specific example of economic benefit potentially tied to wastewater-based pathogen concentration data.

OBJECTIVE 4: Continue to develop and optimize molecular diagnostic tools to build surveillance capabilities for the next decade, and for future pandemics.

Our team has worked over the course of 2020 to build and expand our collaborative relationships with industry partners that are playing a prominent role in the expansion of COVID-19 related molecular applications. During the project, Mark Ciesielski (Ph. D. Marine Sciences) and Rachel T. Noble presented a webinar on ddPCR and qPCR workflows as related to clinical microbiologists around the country as part of an invited webinar series led by Bio-Rad.

Table 2. NC Pathogen Research Network Industry Partners

Collaborative Partner	**Component**	**Outcome**	**Status**
BioGx, Inc.	Multiplex for pan-	Wastewater/clinical	In process

	respiratory panel	generated	
ThermoFisher	Streamlining speed of sample processing and throughput	Save cost, improve throughput and QA/QC	
QIAGEN	Streamlining speed of sample processing and throughput	Save cost, improve throughput and QA/QC	
Bio-Rad, Inc.	Streamlining, speed of reactions	Save cost, improve throughput	plete
Zymo Research, Inc.	Inhibitor removal, streamline sample processing	Reduce volumes necessary for processing	

OBJECTIVE 5: Interact with a team of epidemiologists (Dr. Larry Engel, Dr. David Holcomb, and Dr. Jane Hoppin), spatial and GIS modeling researchers (Dr. Marc Serre and Dr. Helena Mitasova) to understand the impact of social distancing and mitigation measures to place the data into a useful context for future surveillance system development.

There were several research areas that were advanced over the course of this project including geospatial analysis, epidemiological analysis, and disease mapping. Each of these are highlighted below with a short summary.

Geospatial analysis (NCSU): In order to acquire accurate, comprehensive digital sewer networks for each of the 19 monitored WWTPs, we requested sewer network data from local water utilities and GIS departments. Then we developed a GIS-based workflow to delineate sewershed boundaries for each of the individual WWTPs, based on their sewer networks. Relevant components of each sewer system's data, including, but not limited to, sewersheds, sewer pipes and their properties, and manholes, were integrated into a GIS database and converted to a shareable geospatial format for use in further analyses. Wastewater sampling was completed for a variety of sewersheds in North Carolina, including inland and coastal regions, as well as large, metropolitan and small, rural sewersheds. The area of the sewersheds varied from 2 to 207 square miles, with estimated service populations ranging from 3,000 to 550,000 people.

We generated several GIS-based methods which were automated and stored in a code-sharing repository so that they can be utilized for this and future wastewater-based monitoring projects. These efforts have been conducted across NC, even though the examples that we highlight here are from Raleigh. Of particular interest to current reporting of clinical data, we found that sewershed boundaries often didn't align with zip codes or county boundaries (Figure 2). Therefore, we developed a technique which could modify publicly available case data to more accurately reflect the number of COVID-19-positive cases that were contributing to SARS-CoV-2 detected in the wastewater samples. Specifically, this method calculates the proportion of people living inside the sewershed based on census block geography and the 2010 census population and weights the zip code cases according to the estimated population. We plan to compare the results generated from this method to other COVID case datasets, including county data, zip code cases weighted by area, and geocoded, address level case data aggregated to the sewersheds in order to better understand if publicly available data can be used if more granular data is not available.

In order to interpret the signal of viral pathogens stemming from areas contributing to a single wastewater influent source, it is necessary to understand sewerage transport time. This is because as sewage is moving from the source (home or business) to the wastewater treatment plant, the sewage is undergoing change and sometimes dilution along the way, as well as degradation of the viral pathogen nucleic acid. This travel time is likely to be an important factor impacting the integrated SARS CoV-2 signal that is observed in the municipal wastewater influent. During this project, we created a method that can estimate the time it takes for sewage to travel from any location in the sewershed to the WWTP based on the attributes of the sewer pipes. (Figure 3). We plan to incorporate these sewage travel times into a SARS-CoV-2 decay model in order to better understand how the level of SARS-CoV-2 detected in the WWTP influent is affected by the time it takes for the sewage to reach the plant. In particular, our efforts have demonstrated that even though degradation will likely contribute to changes in the overall concentrations of viral pathogen nucleic acid in wastewater influent, it is unlikely to be a major source of loss given the short time estimates for sewage transport.

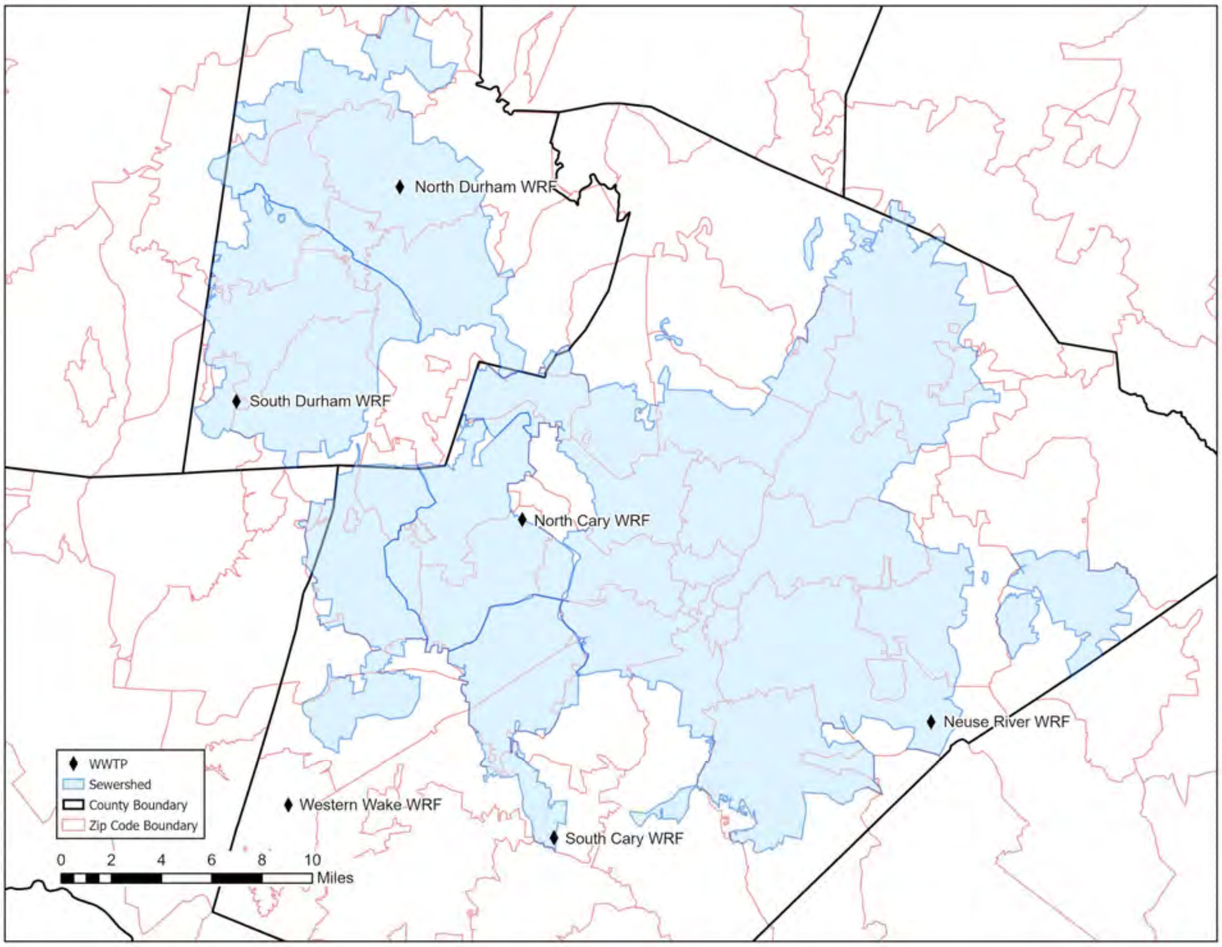

Figure 2. Sewershed boundary alignment with zip codes in Wake and Durham counties.

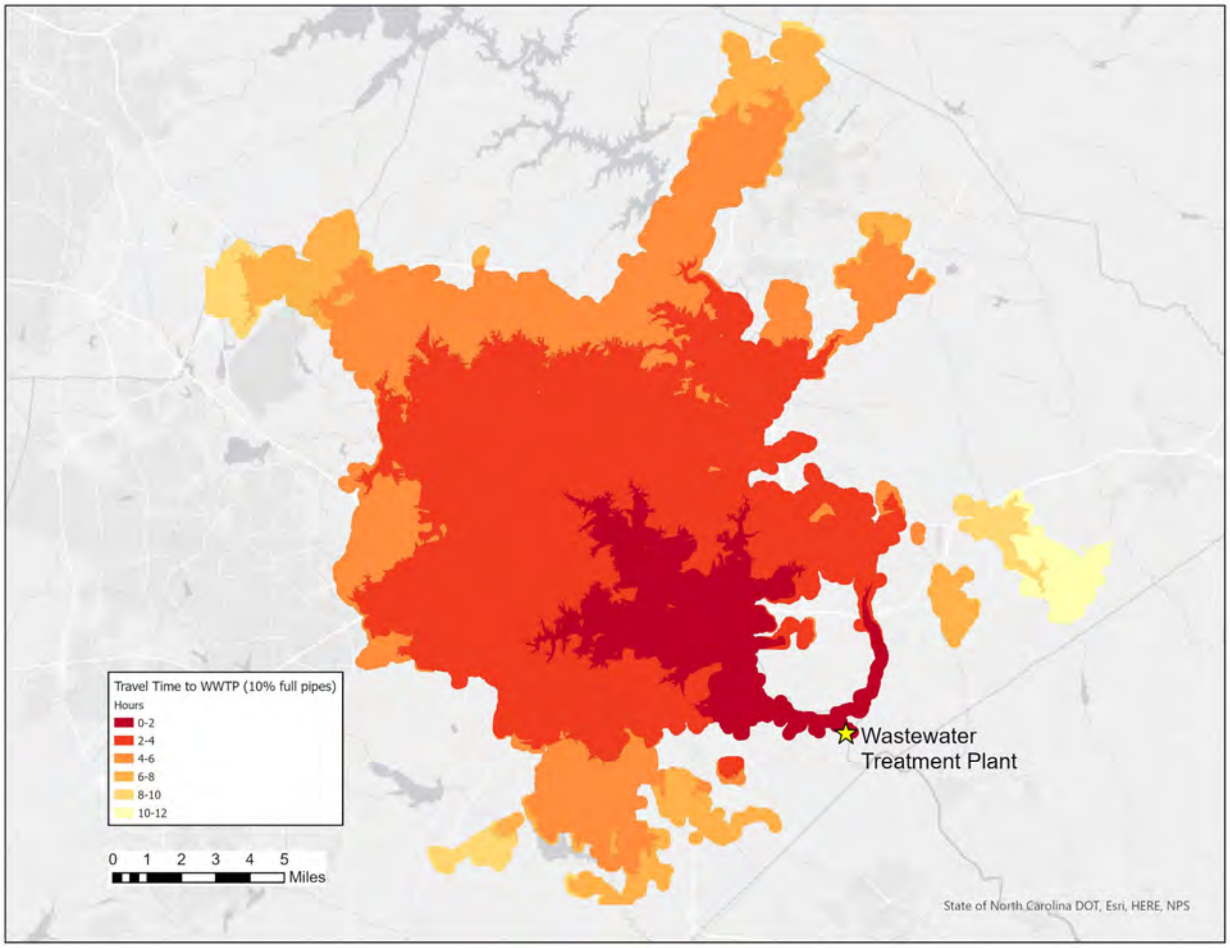

Figure 3. Sewer travel times to the WWTP within the Raleigh sewershed assuming 10% full gravity main pipes and variable sewage velocities.

A major limitation of this study is that the focus has been on analysis of WWTP influent in locations where university programs exist, and in areas where laboratory capacity could be built. Residences on septic systems do not contribute waste to WWTPs and, therefore, do not directly affect levels of SARS-CoV-2 in a WWTP's influent. The large proportion of homes on septic systems in NC is, consequently, likely to distort the relationship between population prevalence of COVID-19 infection and measured levels of SARS-CoV-2 in wastewater at WWTPs if not accounted for. To begin to address the balance of septic and sewershed dynamics in the State of NC, we acquired septic data (where available) and a number of parcels on septic within specific sewersheds was estimated and mapped. We used this approach to exclude parcels with septic systems, but clearly an improved outcome would be to consider random stratified sampling and analysis of septic system waste.

A major issue with a focus on WWTP and municipal waste is highlighted by the fact that NC is highly rural (38% of population resides in a rural county) when compared to other states (national average 21%). Critical social components to rural disease transmission are human behaviors, social mobility, demographics, landscape characteristics, and waste infrastructure. Rural systems are being thought of as the test-bed for initial “return-to-normal” measures nationally, but we have little understanding of disease transmission in these settings. In this project, we made progress with analysis of municipal WWTP that were in rural areas, but as a team, we will have to think carefully about applications to package treatment and septic systems to provide a more complete picture. Achieving the objectives outlined in this project were a vital first step in improving decision-making for COVID-19 in rural systems for this and future pandemics.

Epidemiologic analysis (UNC-CH)

All data sources available to estimate the incidence and prevalence of COVID-19 infection in North Carolina have important limitations, including likely substantial underascertainment of asymptomatic cases, non representativeness of tested populations, or small target populations. Syndromic surveillance systems can identify cases of COVID-like illness, which is especially important in regions/periods where testing is unavailable or inadequate, but they lack the confirmation of COVID-19 disease status from testing. In addition, publicly available data from the state dashboard are at a geographic resolution that generally aligns poorly with sewersheds, introducing additional error when estimating case counts.

To overcome these limitations, we obtained complementary, mostly high geographic resolution, data, with regular updates, from multiple sources. We are in the process of combining the data from these sources to better estimate the true prevalence of COVID-19 infection in each of our study sewersheds. These data include 1) results of all COVID-19 tests administered in North Carolina since the disease was first identified in the state and provided to the NC Department of Health and Human Services as part of the mandatory communicable disease reporting system; 2) the NC Disease Event Tracking and Epidemiologic Collection Tool (NC DETECT), which is North Carolina’s statewide syndromic surveillance system and allows identification of individuals presenting at Emergency Departments with COVID-like illness; 3) serial COVID-19 viral test results and limited demographics of participants in the prospective Chatham County COVID-19 Cohort (C4); and 4) serial COVID-19 viral test results and limited demographics of a representative sample of participants in the prospective MURDOCK Study Community Registry and Biorepository, which covers Cabarrus County and some surrounding areas. Of note, both the C4 and MURDOCK cohorts are located in, or overlapping with, sewersheds included in the current project. The geographic resolution of these data allow us to place individuals testing positive for COVID-19 or presenting at Emergency Departments with COVID-like illness within a given sewershed with certainty or near certainty, with the exception of the NC DETECT data, which are at the zip code level.

Due to the need to first address all legal, IRB, and data security concerns related to these sensitive data, we only recently obtained and began analyzing these data. To date, we have geocoded all individuals in the state testing database who tested positive for COVID-19 since 2/1/2020 and, based only on these data, estimated the number of individuals testing positive each day within each of our study sewersheds. Our plans, currently underway, are to estimate not only how many individuals within a sewershed test positive each day, but to estimate how many individuals within a sewershed are likely to be shedding SARS-CoV-2 in their feces each day, based on symptom reporting data in some of the data sources, together with viral shedding data from published studies. We will use the serial test results from the two prospective cohorts, together with census data, to estimate and account for asymptomatic cases not routinely captured in the state mandatory reporting system, with a particular focus on the two sewersheds in which these cohorts reside. In a further refinement of our models, we will account for viral transit time, which has been addressed above, and viral degradation in the sewer lines, based on the distance between each case's residence and their respective WWTP, as well as the ambient temperature. Because precipitation can, through inflow and infiltration, dilute wastewater and, consequently, distort the measured viral concentrations in that wastewater, we will use weather data, together with validated models, to account for the impact of the estimated inflow and infiltration. Precipitation from all NOAA stations across the states were used to estimate precipitation at each of the WWTPs in our study, and compared with the WWTP flow. Plots of the WWTP flow and estimated precipitation at the WWTPs in our study show that WWTP flow is significantly associated with precipitation, which indicates significant inflow and infiltration into the sewer lines (Appendix E).

Disease Mapping (UNC-CH)

Counts of confirmed cases of COVID-19 were obtained for all counties and zip codes across the state of North Carolina. These data were used as input for the Bayesian Maximum Entropy (BME) method of geostatistics to estimate the COVID-19 incidence across North Carolina for different time aggregates between March 12th, 2020 and January 5th, 2021 (See maps in Appendix D). The BME method was used because it accounts for the space/time autocorrelation in COVID-19 incidence rates and treats observations as interval or Gaussian soft data to smooth out outliers observed in counties with small populations.

The BME estimation of COVID-19 cases serves two purposes. The first purpose is to map the incidence rate of COVID-19 across sewersheds, which can then be used to model SARS-CoV-2 shedding into the sewers and improve the model used to predict COVID-19 cases from SARS-CoV-2 measured at WWTPs. The second purpose is to develop an early warning system that will use SARS-CoV-2 measured at WWTPs together with reported COVID-19 cases to create maps of COVID-19 that can anticipate outbreaks and allow public health measures to be taken as soon as levels are increasing.

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Appendix A

Table 1: Personnel supported over the duration of NC Collaboratory-funded project entitled “Tracking SARS-CoV-2 in the Wastewater Across a Range of North Carolina Municipalities”.

Personnel	Affiliations	Role	Outcomes/End products
University rolina at Chapel Hill			
Rachel Noble	IMS, MASC, ESE, IE	Lead PI, professor	Lead or co-stry collaboratio developmenchian State dorm project
Lawrence Engel	EPID	Co-PI, associate professor	Lead or co-emiological analysis andl case and epl data requests, im shedding estimations
David Holcomb	EPID	Co-PI, post-doc	Lead or co-emiological analysis, coy prevalence studies, sewershed-based analysis
Marc Serre	ESE	Co-PI, associate professor	Lead or co- geospatial aocoding, disease ma inflow and inpping of septic syms
Jill Stewart	E SE	Co-PI, professor	Lead or co- developmen prevalence collaborator, stakeholder interactions
Alyssa Grube	ESE	GRA, PhD student	support
Claire Dust	ESE	GRA, MSPH student	ping, mapping of septic system, practicum report
Collin Coleman	ESE	GRA, PhD student	support
Connor LaMontagne	ESE	GRA, PhD student	chapter
Corinne Wiesner	ESE	Unpaid, PhD student	
Da Zhe “Kenny” Chen	EPID	GRA, PhD	epidemiological analysis
Deanna Good	IMS	Administrative associate	Finance ad& accounting support
Denene Blackwood	IMS	Research specialist	advancemeeam

			methods specialist, technical support, advanced molecular training and troubleshooting, industry partnerships
Jayne Boyer	ESE	Lab manager	l support
Kelly Hoffman	ESE	Unpaid, MSPH student	mapping
Mark Ciesielski	MASC	GRA, PhD student	nd ddPCR comparison paper, co-author on other papers; dissertation chapter
Megan Hunter	IMS	Business services coordinator	ministration & accounting support
Megan Miller	ESE	GRA, MS student	
Misty Parrish	IMS	Administrative support specialist	urces support
Nikhil Kothegal	ESE	GRA, PhD student	support
Richard Strott	ESE	GRA, MSEE student	mapping, modeling of inflow and infiltration
Ryan Neve	IMS	IT and engineering support specialist	t
Tom Clerkin	IMS	Research technician	industry partnerships, technical support
Wayne Johannessen	IMS	Business manager	ministration and accounting support
Carly Dinga	IMS	Undergraduate Student	data analysis and reporting
Unber Ahmad	Carolina Population Center	Data analyst	epidemiological data analysis
University rolina at Charlotte			
Mariya Munir		Co-PI, professor	author, methods development, community prevalence studies, Charlotte Water stakeholder interaction, industry partnerships, campus wastewater surveillance project
Adeola Sorinolu	CEE	PhD student	technical support for filtration and RNA extraction
Isaiah Young	CEE	URA, technical support	supporting sample collection

Kim Wilson	CEE	Business services and grant coordinator	Finance and accounting support
Md Ariful Islam Juel	CEE	PhD student	Lead author on virus concentration method comparison paper, co-author on other papers; Dissertation Chapter, qPCR method development and optimization, qPCR analysis
Visva Bharati Barua	CEE	Post Doctoral Research Associate	Lead author on Charlotte area paper, co-author on others; overall project coordination including sample collection, filtration RNA extraction, qPCR analysis, provisioning, and report writing
Sol Park	CEE	Ph. D. Student	Technical research related to sample processing
University rolina at Wilmington			
Art Frampton	BMB	Co-PI, Associate professor	author, methods development, Wilmington stakeholder interaction, campus wastewater surveillance
Larry Cahoon	BMB	Co-PI, Professor	author, stakeholder interaction, WWTP industry partnerships, inflow and infiltration modeling
Jacob Kazenelson	BMB	Masters student	ntributions
North Carol			
Francis L. de los Reyes III	CCEE	Co-PI, Professor	author, methods development, WWTP industry partnerships, stakeholder interaction
Angela Harris	CCEE	Co-PI, Assistant Professor	author, methods development, community prevalence studies and stakeholder interaction
Jane Hoppin	Biological Sciences	Professor, Co-PI	author, industry partnerships, stakeholder interaction and collaborative specialist
Helena Mitasova	CGA, MEAS	Professor, Co-PI	author, sewershed analysis software development, sewershed data requests and data

			management, methods for public cases data processing
Daniel Cockson	CCEE	Undergraduate research assistant	SE
Emma Bolden	CCEE	Undergraduate research assistant	Diver, BS ENE
Gracie Hornsby	CCEE	Research assistant	support, provisioning
Jeremy Lowe	CCEE	Undergraduate research assistant	Diver, BS ENE
Judith Kays	CCEE	Research assistant	support for protocol development and RNA extractions
Julia Kaplan	Biological Sciences	Data Manager	ms paper, data download of publicly available data, data sources collaborator
Justyna Jeziorska	CGA	Research Associate	derived sewershed data, precipitation analysis data lead
Laura Gomez	CCEE	URA	
Marisa Incremona	Biological Sciences	Science Communicator	author, facilitator for stakeholder engagement project
Nadine Kotlarz	Biological Sciences	Post-doctoral research associate	solids paper and Stakeholder engagement paper, co-author on other papers
Sean Daly	CCEE	GRA	chapter
Sivaranjani Palani	Microbiology	GRA	analysis
Stacie Reckling	CGA	Research Associate	wershed data lead; sewershed analysis techniques and software
Tanvir Pasha	CCEE	GRA	ershed paper, Co-author on other papers; dissertation chapter
Vaclav Petras	CGA	Geospatial Software Engineer	software modules for sewershed and public cases data processing and visualization, sewershed and derived cases data co-lead
Victoria Ponthier	CCEE	URA	NE

Yi-Chun Lai	CCEE	GRA	Co-author; sample processing and analysis
Zach Bennett	CCEE	URA	Driver, BS CE
NCDEQ Dives			
Danny Smith	NCDEQ Division of Water Resources	Director	support for wastewater collection and treatment systems
Melanie Williams	NCDEQ Division of Water Resources	Environmental Program Consultant	wastewater collection and treatment systems
Rick Bolich	NCDEQ Division of Water Resources	Section Chief	support for wastewater collection and treatment systems

List of abbreviations:
BAE-Biological and Agricultural Engineering
BMB-Biology & Marine Biology
CCE- Civil and Environmental Engineering
CCEE -Civil, Construction, and Environmental Engineering
CE-Civil Engineering
CGA-Center for Geospatial Analytics
ENE-Environmental Engineering
EPID-Department of Epidemiology
ESE-Environmental Science & Engineering
GRA- graduate research assistant
IE-Institute for the Environment
IMS-Institute of Marine Sciences
ISE-Industrial and Systems Engineering
MASC-Marine Sciences
MEAS- Department of Marine, Earth and Atmospheric Sciences
RA-research assistant
URA-Undergraduate Research Assistant

Appendix B: The project team developed a consolidate workflow (Figure B-1) for liquid influent sample processing from municipal wastewater treatment plants. Generalized application of conditions, controls, and laboratory safety approaches are shown. This generalized consolidated sample processing approach is common to the five laboratories that are conducting wastewater treatment plant influent analyses across the State of North Carolina. In particular, progress has been made highlighting improved RNA recovery, streamlining of methods, time to results, and resource/cost conservation with this workflow.

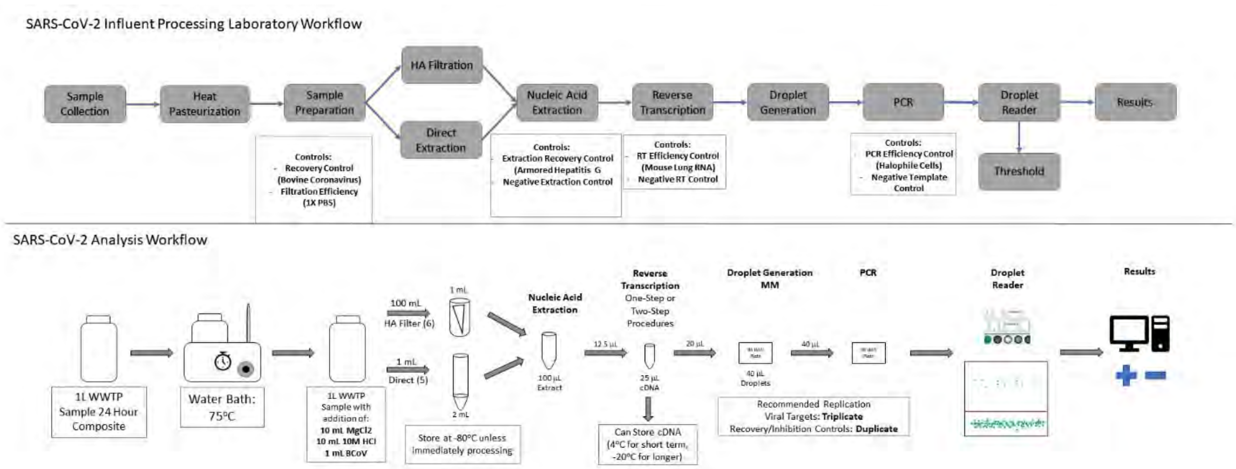
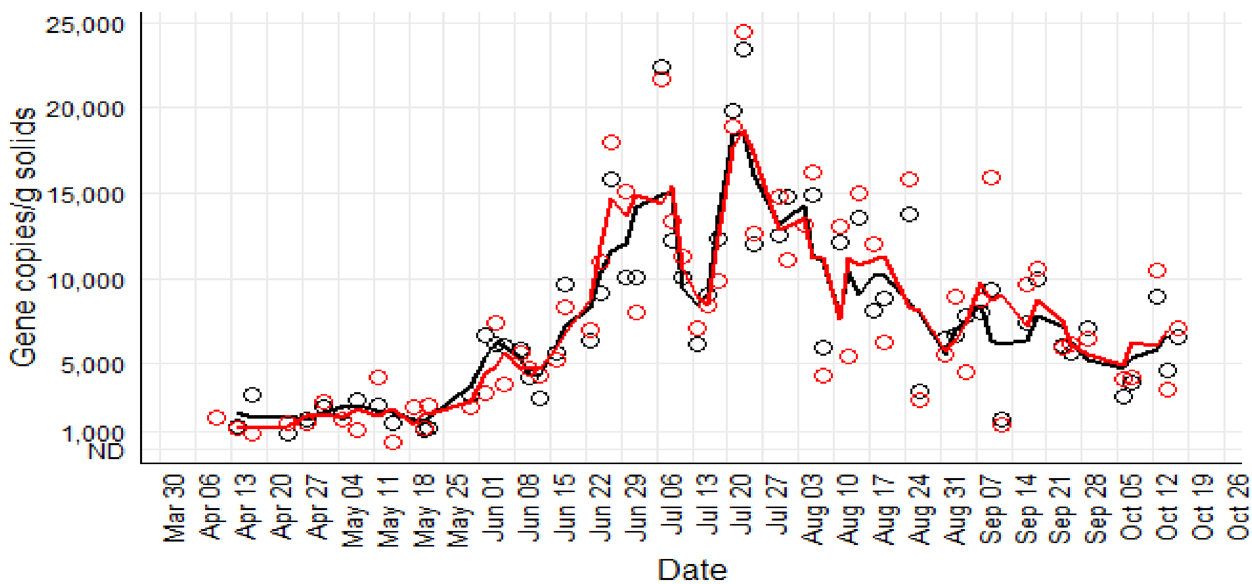
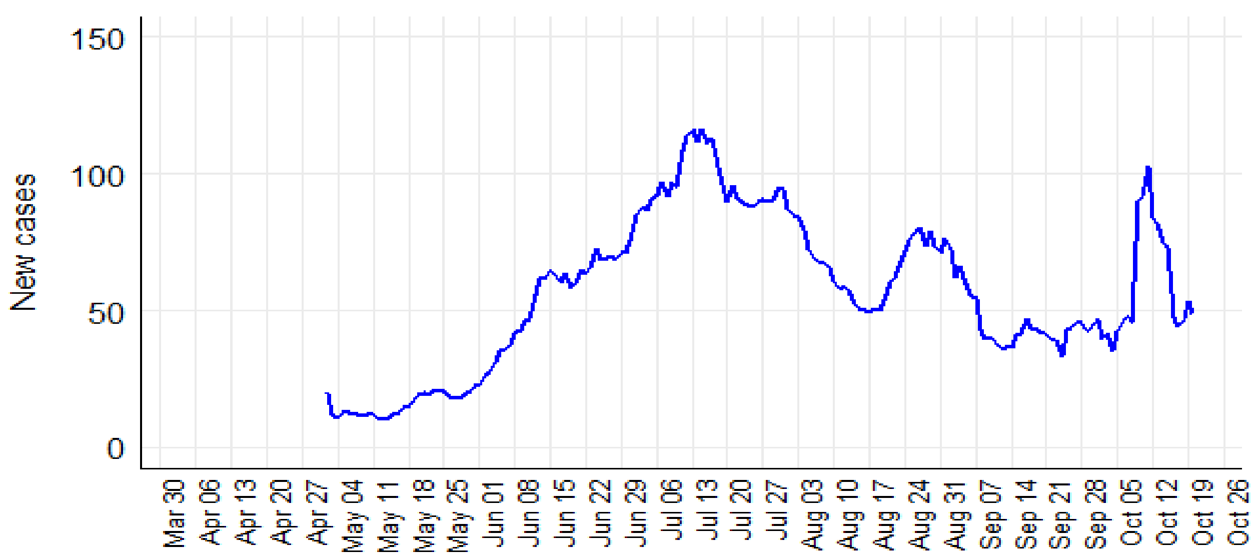


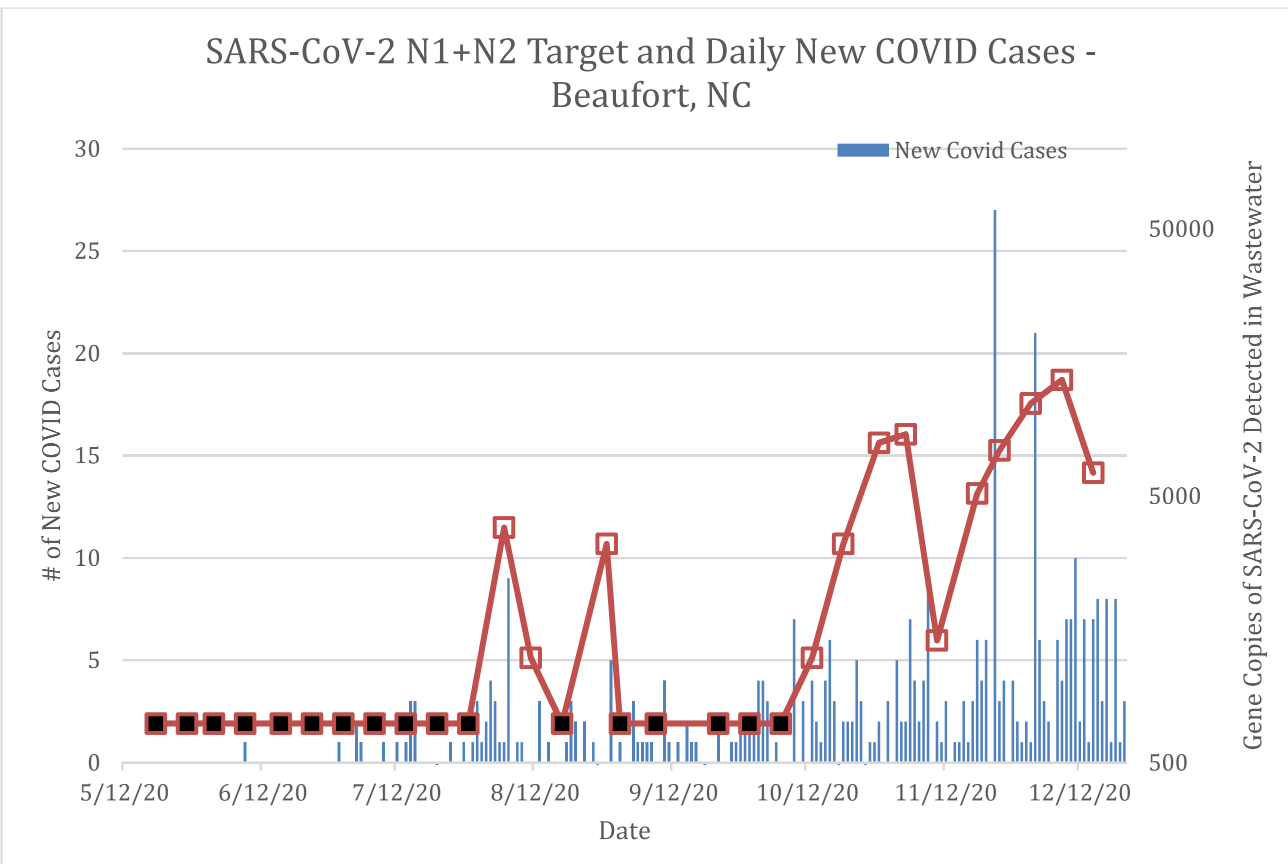
Figure B-1: Summarized workflow for liquid influent wastewater sample processing for treatment plants across the State of North Carolina

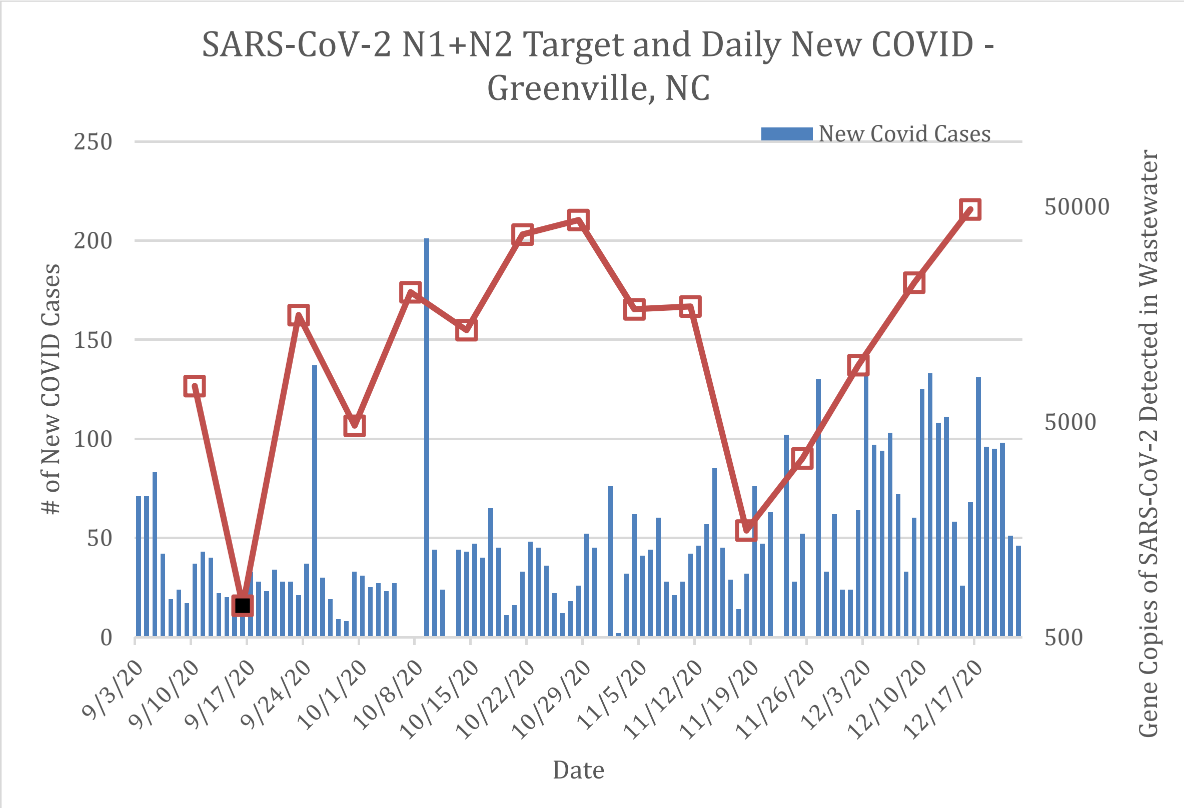
Appendix C1: SARS-CoV-2 RNA results for primary solids samples collected from Neuse River Resource Recovery Facility (Raleigh, NC) on 64 days between April 10, 2020 and Oct 17, 2020. SARS-CoV-2 N1 and N2 gene copy concentrations are normalized by total solids concentration. SARS-CoV-2 N1 concentrations ranged from 854 copies/g total solids (April 24, 2020) to 23,463 copies/g total solids (Jul 23, 2020); SARS-CoV-2 N2 concentrations ranged from 939 copies/g total solids (April 17, 2020) to 24,422 copies/g total solids (Jul 23, 2020). The Neuse River Resource Recovery Facility serves approximately 600,000 people. The blue line in the bottom figure is the 7-day rolling average of new COVID-19 cases in the Raleigh sewershed. The number of cases in the sewershed was approximated by summing the daily cases from every zipcode that intersects the Raleigh sewershed boundary, proportional to the overlapping area.

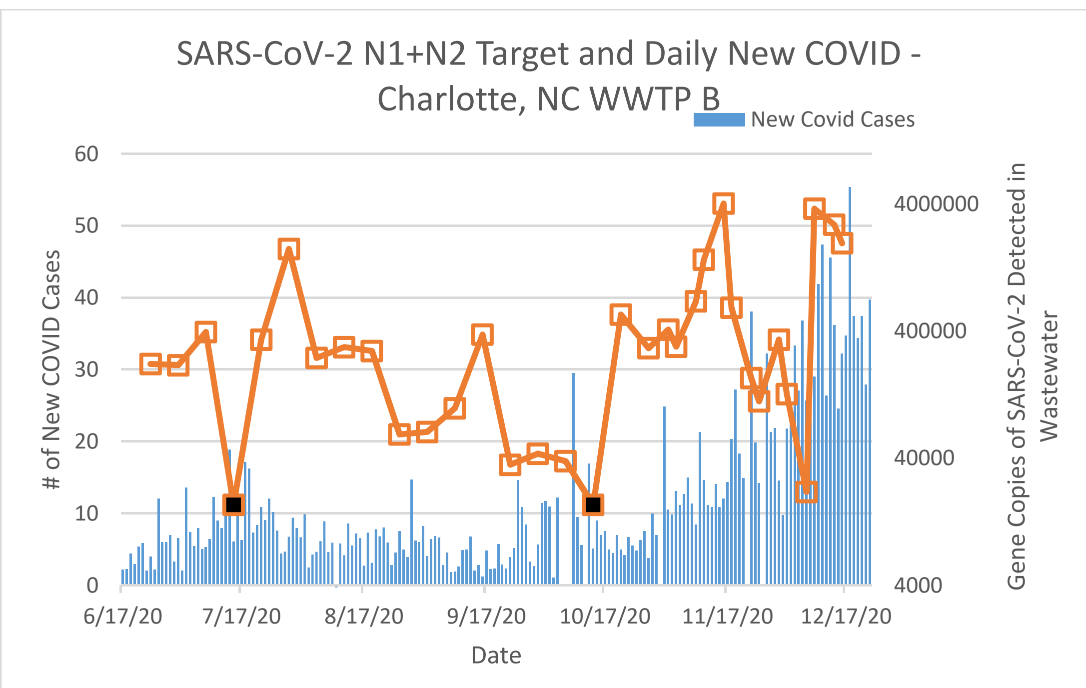


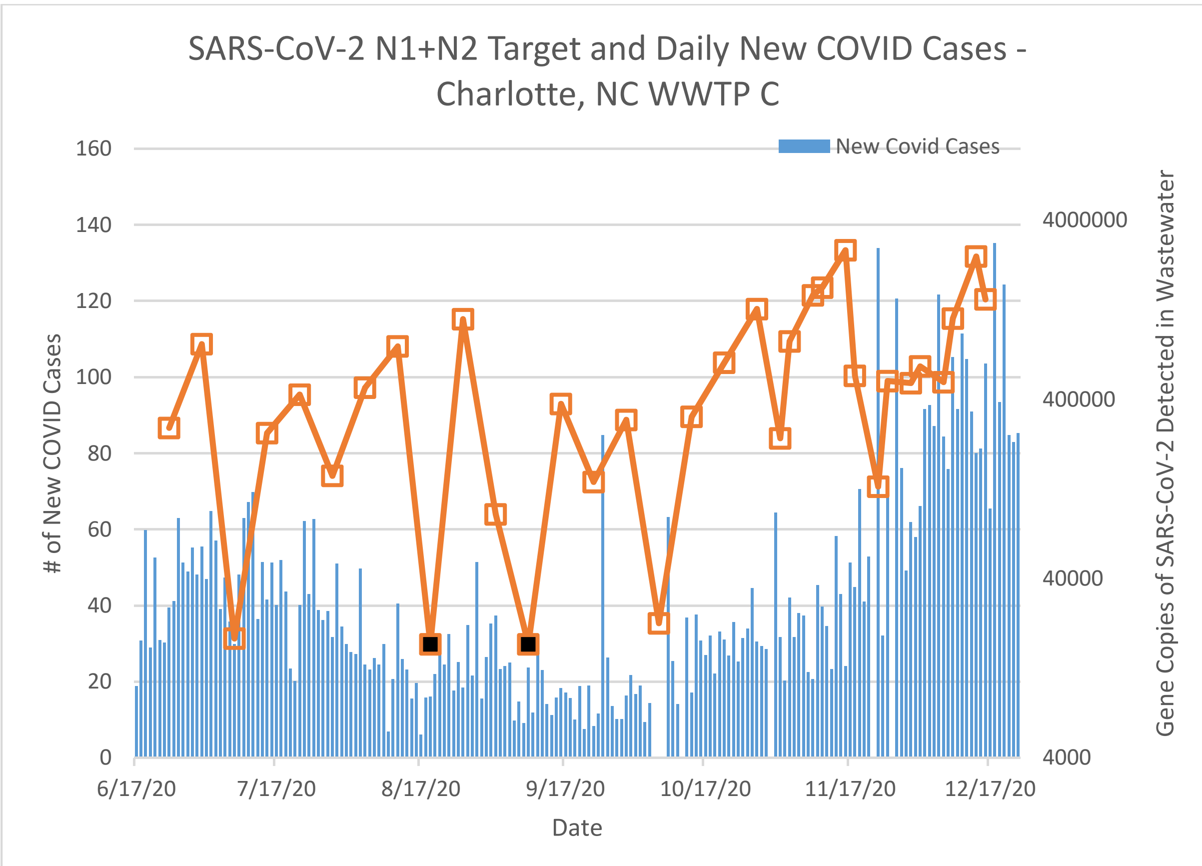


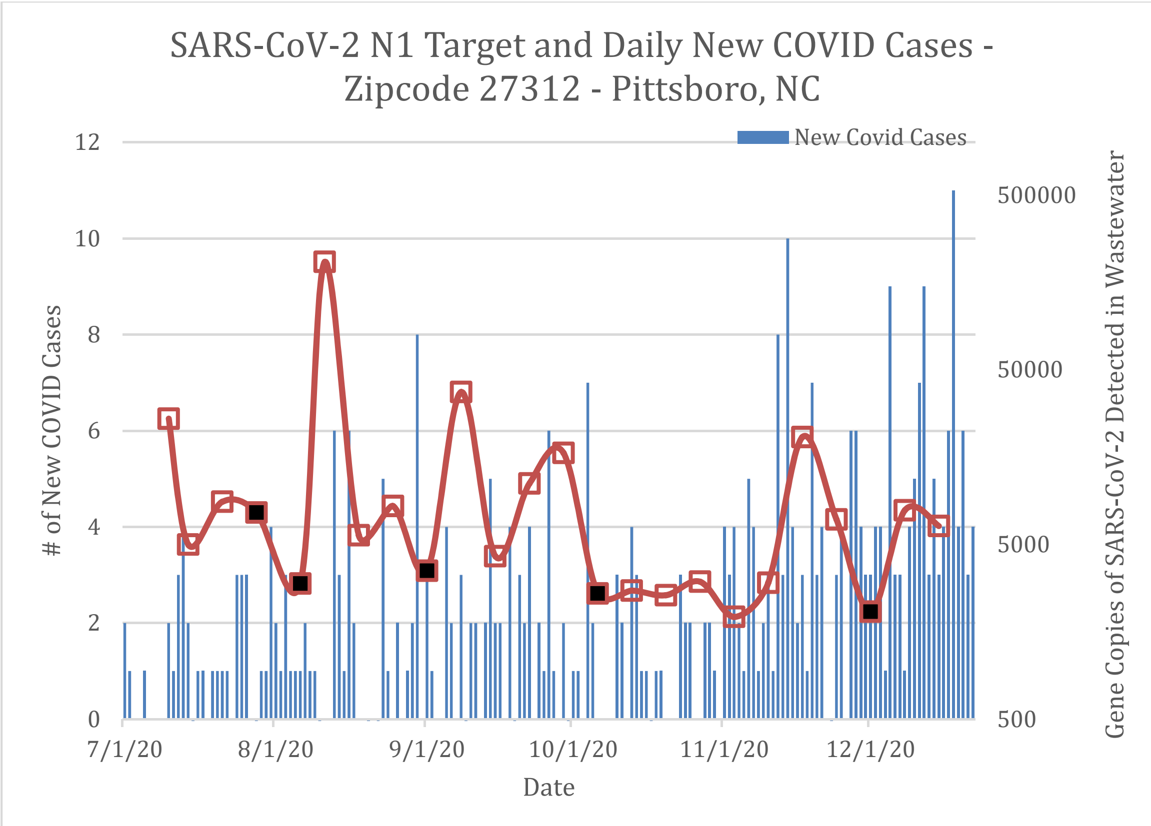
Appendix C2: Over the duration of the study over fifteen WWTP plants were sampled weekly at intervals of one, two, or three times per week and liquid influent samples were processed according to the liquid influent workflow presented in Appendix B. For the purposes of this report, we have selected 8 treatment plants that will be graphically represented for data from liquid influent sample collection plotted in relationship to new and cumulative cases for the service area (sewerage treatment municipal zipcode areas) for each of the WWTP. We have chosen to not show plots from every single location analyzed for the project but can provide those data for review and to municipal stakeholders upon request. Similar to clinical diagnostic tests conducted globally, SARS-CoV-2 gene targets for this liquid influent analysis are N1 (nucleocapside protein-1) and N2 (nucleocapsid protein-2) and are represented as a concentration reported for gene copies per liter of sewage influent. For each figure, the histogram shows the 7-day rolling average of new COVID-19 cases. The number of cases for each sewershed was approximated by summing the daily cases from every zipcode that intersects the WWTP sewershed boundary, proportional to the overlapping area of the service population. The symbols represent concentrations of the N1 and N2 gene targets per liter of wastewater analyzed from the respective WWTP.

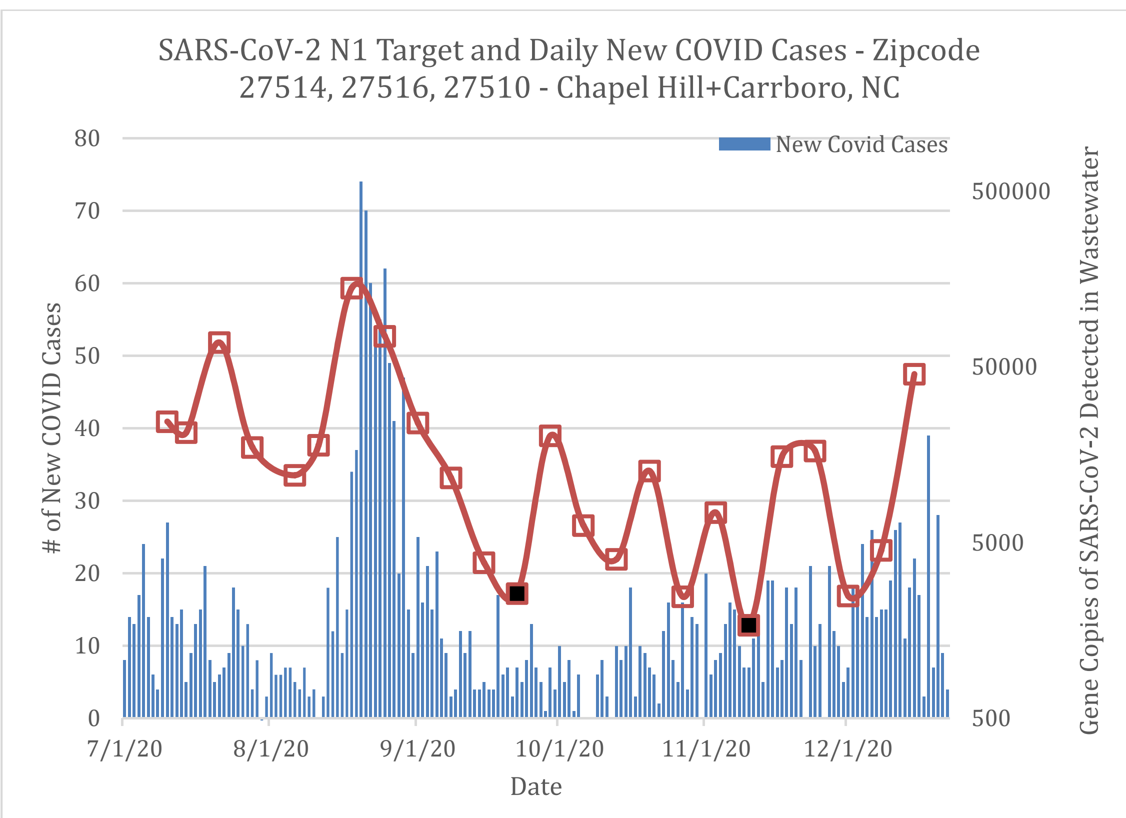


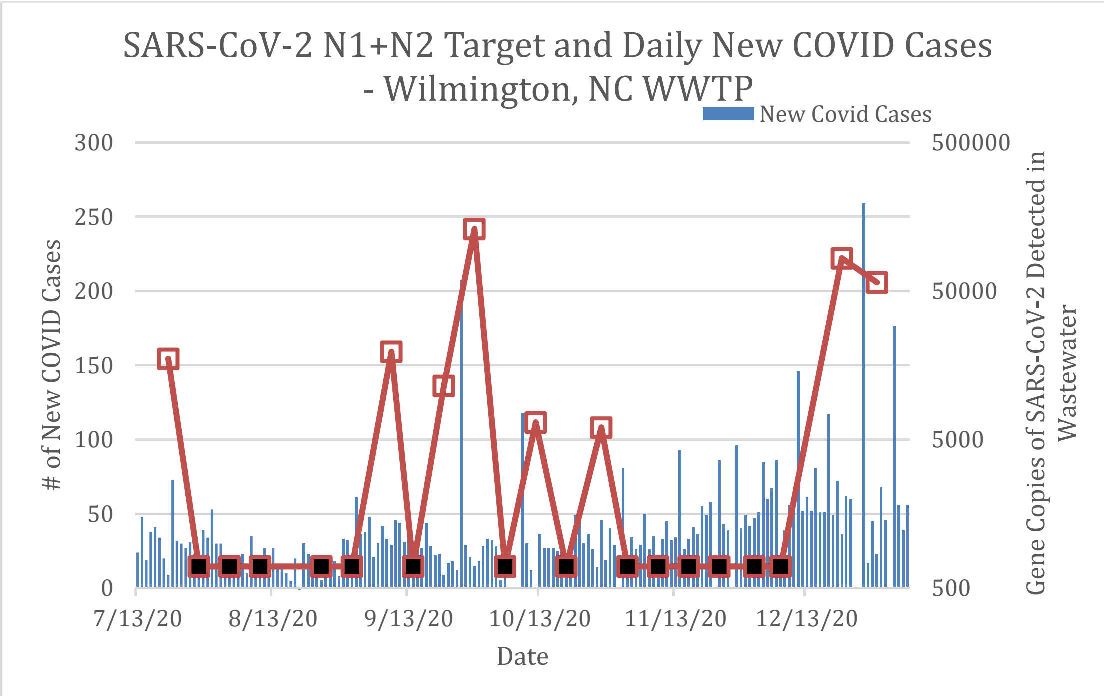

SARS-CoV-2 N1+N2 Target and Daily New COVID - Greenville, NC
New Covid Cases
250
200
150
100
50
0
50000
5000
500
# of New COVID Cases
Gene Copies of SARS-CoV-2 Detected in Wastewater
9/3/20
9/10/20
9/17/20
9/24/20
10/1/20
10/8/20
10/15/20
10/22/20
10/29/20
11/5/20
11/12/20
11/19/20
11/26/20
12/3/20
12/10/20
12/17/20
Date

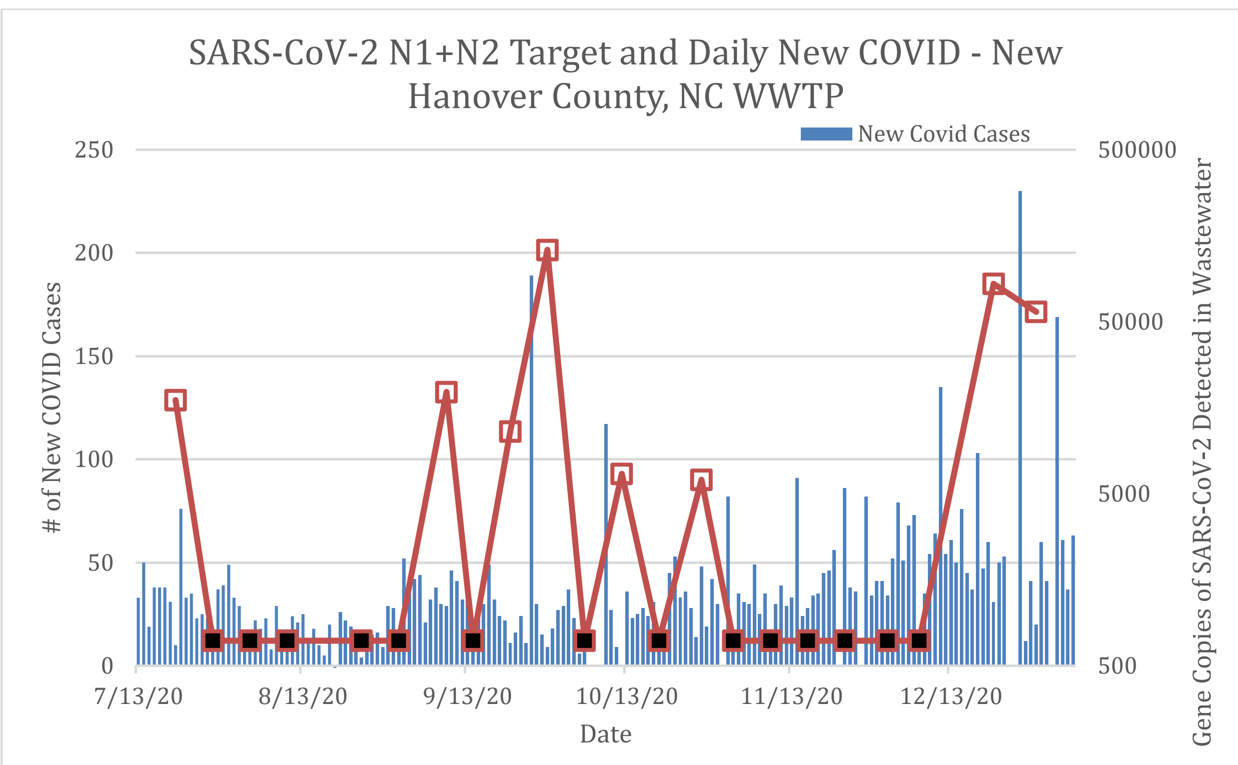

SARS-CoV-2 N1+N2 Target and Daily New COVID - Charlotte, NC WWTP B
New Covid Cases
60
50
40
30
20
10
0
4000000
400000
40000
4000
# of New COVID Cases
Gene Copies of SARS-CoV-2 Detected in Wastewater
6/17/20
7/17/20
8/17/20
9/17/20
10/17/20
11/17/20
12/17/20
Date


SARS-CoV-2 N1+N2 Target and Daily New COVID Cases - Charlotte, NC WWTP C
New Covid Cases
# of New COVID Cases
Gene Copies of SARS-CoV-2 Detected in Wastewater
Date
0
20
40
60
80
100
120
140
160
4000
40000
400000
4000000
6/17/20
7/17/20
8/17/20
9/17/20
10/17/20
11/17/20
12/17/20


SARS-CoV-2 N1 Target and Daily New COVID Cases - Zipcode 27312 - Pittsboro, NC
New Covid Cases
# of New COVID Cases
Gene Copies of SARS-CoV-2 Detected in Wastewater
12
10
8
6
4
2
0
500000
50000
5000
500
7/1/20
8/1/20
9/1/20
10/1/20
11/1/20
12/1/20
Date


SARS-CoV-2 N1 Target and Daily New COVID Cases - Zipcode 27514, 27516, 27510 - Chapel Hill+Carrboro, NC
New Covid Cases
# of New COVID Cases
Gene Copies of SARS-CoV-2 Detected in Wastewater
80
70
60
50
40
30
20
10
0
500000
50000
5000
500
7/1/20
8/1/20
9/1/20
10/1/20
11/1/20
12/1/20
Date





Detection of SARS-CoV-2 in wastewater and new COVID-19 cases for zip code serviced by the Town of Beaufort in Carteret County. Bars represent daily new COVID-19 cases for zip code 28516 obtained from the N.C. Department of Health and Human Services' zip code-level map as compiled by WRAL News*. Boxes connected by the line graph represent SARS-CoV-2 gene copies/L wastewater. With the assistance of staff at the Town of Beaufort Wastewater Treatment Facilities, 24-hour composite wastewater samples were collected weekly from 5/12/2020 to 12/16/2020, corresponding to 31 sampling dates with merged triplicate qPCR reactions for the quantification of both nucleocapsid gene (N1 and N2). Black boxes represent a SARS-CoV-2 detection event below the limit of quantification.

Detection of SARS-CoV-2 in wastewater and new COVID-19 cases for zip code serviced by Greenville in Pitt County. Bars represent daily new COVID-19 cases for zip codes 27812, 27834, 27858, and 28590 obtained from the City of Greenville treatment works staff. Boxes connected by the line graph represent SARS-CoV-2 gene copies/L wastewater. With the assistance of staff at the Greenville Wastewater Treatment Facilities, 24-hour composite wastewater samples were collected weekly from September through to December, corresponding to 15 sampling dates with merged triplicate qPCR reactions for the quantification of both nucleocapsid gene (N1 and N2). Black boxes represent a SARS-CoV-2 detection event below the limit of quantification.

Detection of SARS-CoV-2 in wastewater and new COVID-19 cases for zip code serviced by WWTP B (Mecklenburg County). Bars represent daily new COVID-19 cases for zip code 28078, 28213, 28262, 28269, 28027, 28075, and 28215 obtained from the N.C. Department of Health and Human Services' zip code-level map as compiled by WRAL News*. Boxes connected by the line graph represent SARS-CoV-2 gene copies/L wastewater. With the assistance of staff at the Wastewater Treatment Facilities, 24-hour composite wastewater samples were collected weekly from 6/24/2020 to 12/16/2020, corresponding to 33 sampling dates. Until October 2020 samples were collected once a week then from November to December 2020 bi-weekly samples were collected. SARS-CoV-2 detection data reflects merged triplicate qPCR reactions for the nucleocapsid gene (N1). Black boxes represent a SARS-CoV-2 detection event below the limit of quantification.

Detection of SARS-CoV-2 in wastewater and new COVID-19 cases for zip code serviced by WWTP C (Mecklenburg County). Bars represent daily new COVID-19 cases for zip code 28202, 28203, 28204, 28205, 28206, 28207, 28209, 28210, 28211, 28212, 28213, 28217, 28262, 28269, 28215 obtained from the N.C. Department of Health and Human Services' zip code-level map as compiled by WRAL News*. Boxes connected by the line graph represent SARS-CoV-2 gene copies/L wastewater. With the assistance of staff at the Wastewater Treatment Facilities, 24-hour composite wastewater samples were collected weekly from 6/24/2020 to 12/16/2020, corresponding to 33 sampling dates. Until October 30, 2020 samples were collected once a week then from November 2 to December 16, 2020, bi-weekly samples were collected. SARS-CoV-2 detection data reflects merged triplicate qPCR reactions for the nucleocapsid gene (N1). Black boxes represent a SARS-CoV-2 detection event below the limit of quantification.

Detection of SARS-CoV-2 in wastewater and new COVID-19 cases for zip code serviced by the Pittsboro Wastewater Treatment Facility. Bars represent daily new COVID-19 cases for zip code 27312 obtained from the N.C. Department of Health and Human Services' zip code-level map as compiled by WRAL News*. Boxes connected by the line graph represent SARS-CoV-2

gene copies/L wastewater. With the assistance of staff at the Pittsboro Wastewater Treatment Facility, 24-hour composite wastewater samples were collected weekly from 7/10/2020 to 12/15/2020, corresponding to 24 sampling dates. SARS-CoV-2 detection data reflects merged duplicate ddPCR reactions for the nucleocapsid gene (N1). Black boxes represent a SARS-CoV-2 detection event below the limit of quantification.
*https://github.com/wraldata/nc-covid-data/tree/master/zip_level_data/time_series_data/csv

Detection of SARS-CoV-2 in wastewater and new COVID-19 cases for zip codes serviced by the Orange County Water and Sewer Authority (OWASA) treatment facility. Bars represent daily new COVID-19 cases for zip codes 27510, 27514, and 27516 obtained from the N.C. Department of Health and Human Services' zip code-level map as compiled by WRAL News*. Boxes connected by the line graph represent SARS-CoV-2 gene copies/L wastewater. With the assistance of staff at the OWASA treatment facility, 24-hour composite wastewater samples were collected weekly from 7/10/2020 to 12/15/2020, corresponding to 24 sampling dates. SARS-CoV-2 detection data reflects merged duplicate ddPCR reactions for the nucleocapsid gene (N1). Black boxes represent a SARS-CoV-2 detection event below the limit of quantification.

Detection of SARS-CoV-2 in Wilmington, NC (Northside) wastewater and new COVID-19 cases by zip code. Bars represent daily new COVID-19 cases by zip codes. Boxes connected by the line graph represent SARS-CoV-2 gene copies/L wastewater. With the assistance of staff at the Cape Fear Public Utility Authority, 6-hour composite wastewater samples were collected weekly from June 29 to December 29 corresponding to 27 sampling dates with merged duplicate qPCR reactions for the quantification of both nucleocapsid gene (N1 and N2). Black boxes represent a SARS-CoV-2 detection event below the limit of quantification.

Detection of SARS-CoV-2 in Wilmington, NC (Southside) wastewater and new COVID-19 cases by zip code. Bars represent daily new COVID-19 cases by zip code. Boxes connected by the line graph represent SARS-CoV-2 gene copies/L wastewater. With the assistance of staff at the Cape Fear Public Utility Authority, 6-hour composite wastewater samples were collected weekly from June 29 to December 29 corresponding to 27 sampling dates with merged duplicate qPCR reactions for the quantification of both nucleocapsid gene (N1 and N2). Black boxes represent a SARS-CoV-2 detection event below the limit of quantification.

*https://github.com/wraldata/nc-covid-data/tree/master/zip_level_data/time_series_data/csv

Appendix D: Mapping COVID-19 incidence rate

The incidence rate of COVID-19 was mapped using the Bayesian Maximum Entropy (BME) method of geostatistics developed at UNC-CH. This method accounts for the spatio-temporal covariance of incidence rates, which exhibits autocorrelation over long distances and time ranges (Figure D-1). These long spatial and temporal autocorrelation ranges allow to interpolate observed county incidence rate to create maps describing the spatial variability of incidence across the State. Maps of the BME incidence rate on July 20, 21, 22 and 23 of 2020 (Figure D-2) show two localized outbreaks emerging in two counties at the beginning of the COVID pandemic. Maps of the BME incidence rate on 11/22, 11/26, 11/30 and 12/4 of 2020 show the emergence of two regional outbreaks at a later state of the pandemic (Figure D-3).

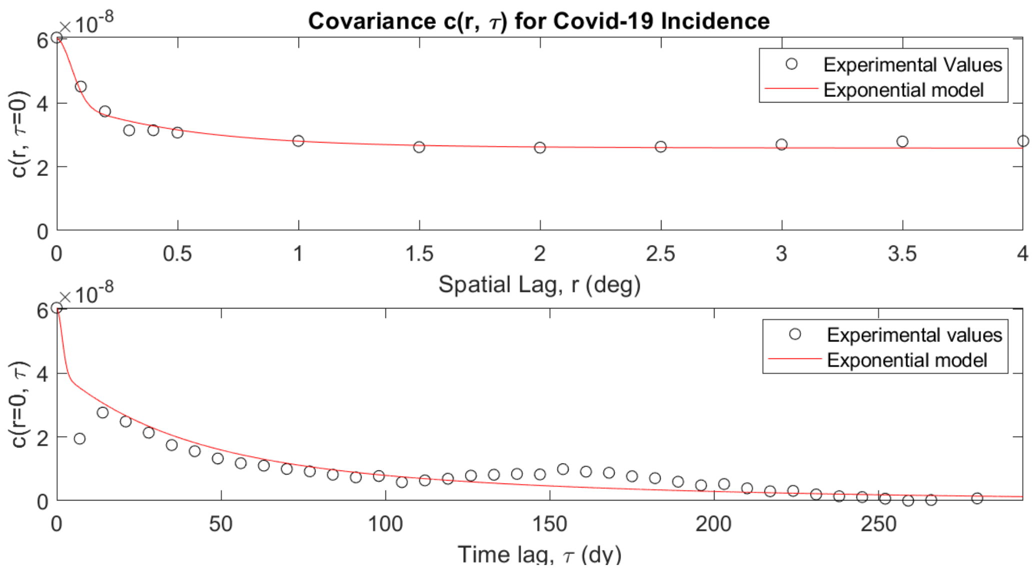


Figure D-1: Covariance of COVID-19 incidence rate

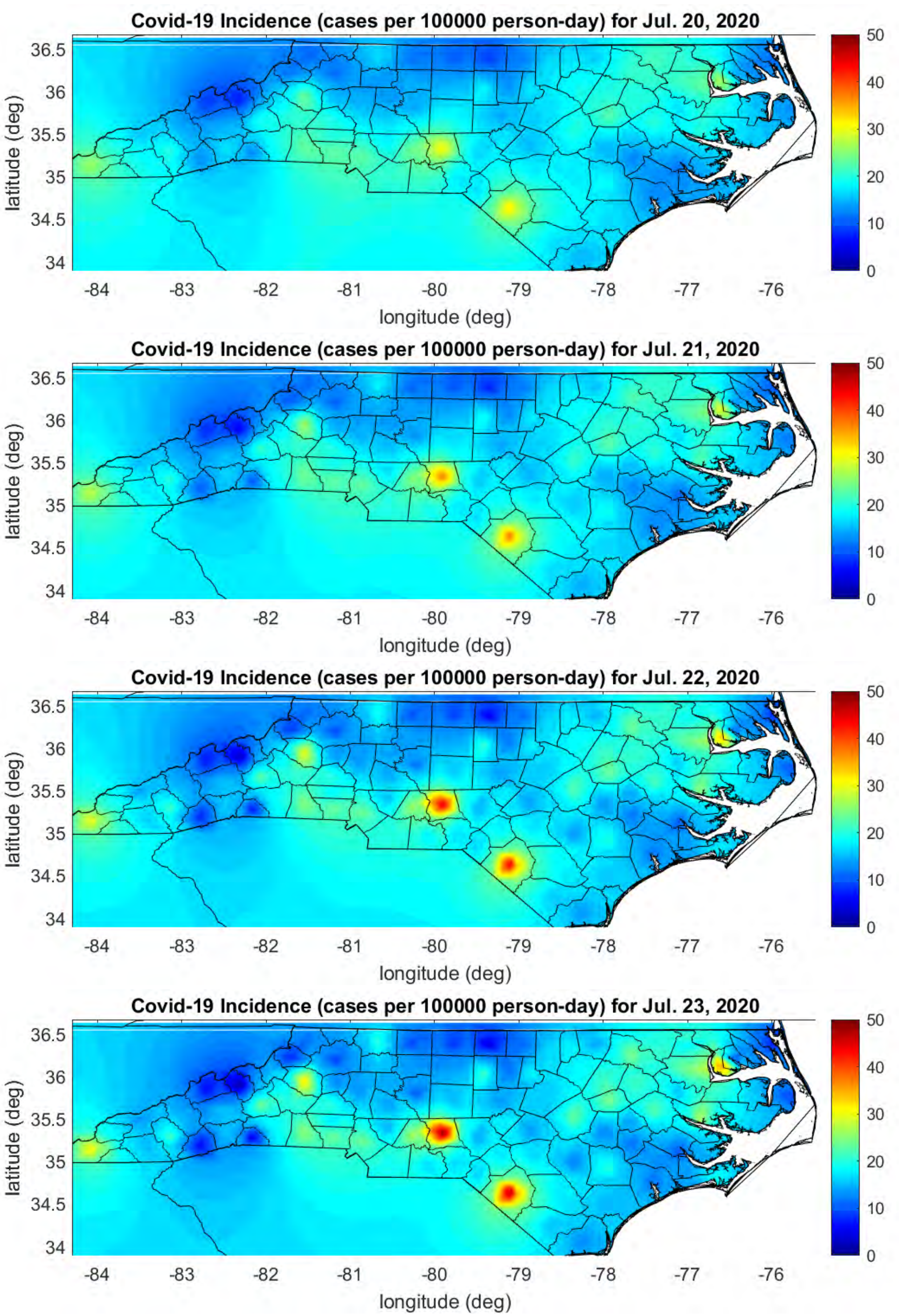


Figure D-2: Maps of the BME estimate of COVID-19 incidence rate on July 20, 21, 22 and 23 of 2020. These maps show two localized outbreaks emerging at the beginning of the COVID pandemic.

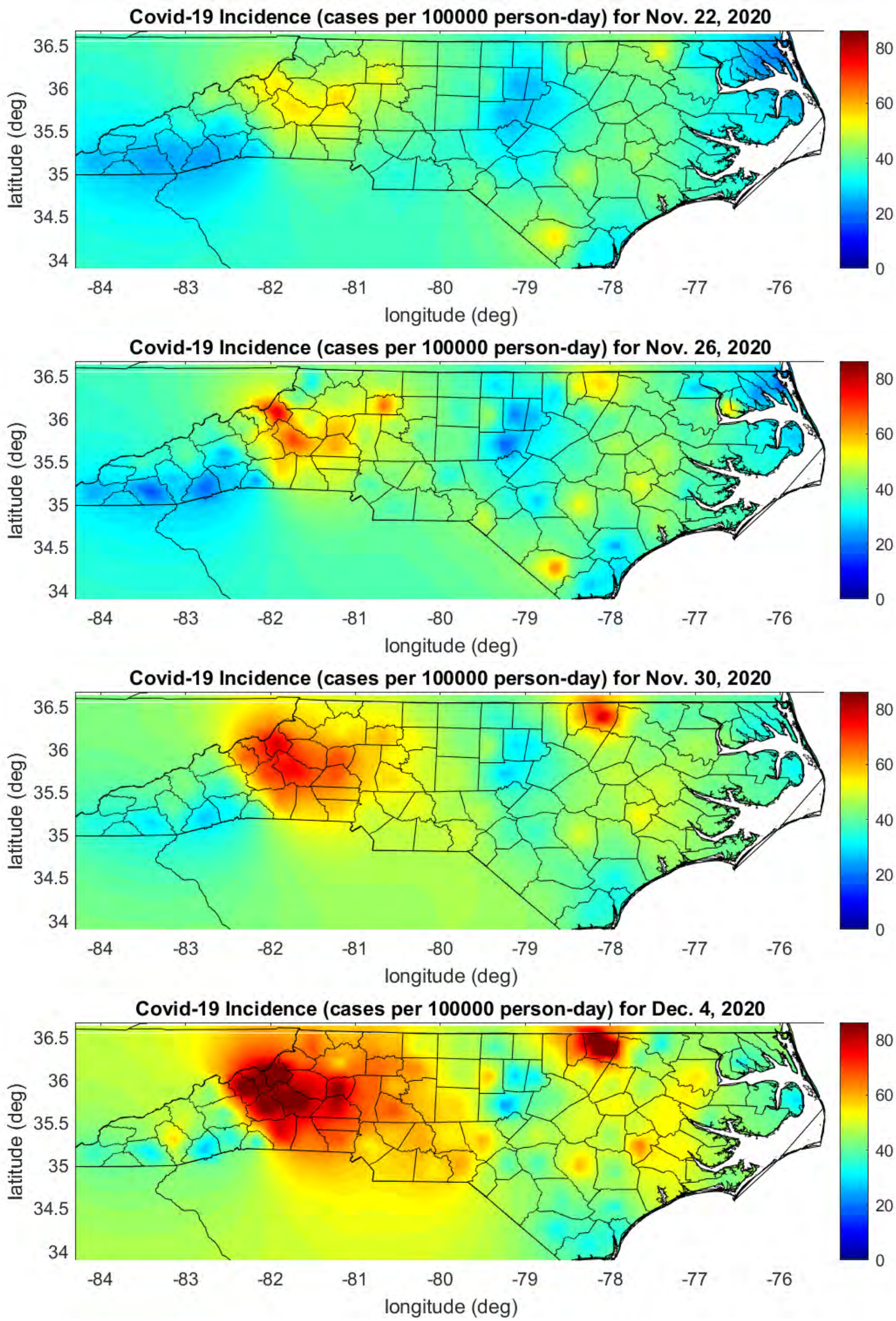


Figure D-3: Maps of the BME estimate of COVID-19 incidence rate on 11/22, 11/26, 11/30 and 12/4 2020. These maps show the emergence of two regional outbreaks at a later state of the pandemic.

Appendix E: Precipitation and WWTP flow

WWTP flows are correlated with precipitation because precipitation contributes to inflow and infiltration into the sewer lines. The covariance of daily precipitation exhibits autocorrelation over long distance ranges but short time range (Figure E-1). The long spatial range can be seen in the maps displayed in Figure E-2, which show the precipitation at all NOAA weather stations across the State on a day with no precipitation, on a day with low precipitation, and two days with high precipitation. The WWTP flow and the BME estimate of precipitation are shown in Figure E-3 at four plants in our study for the duration of our study. Similar plots were obtained for the other plants in our study. The plots show that precipitation is significantly correlated with WWTP flow, indicating a high amount of inflow and infiltration into sewer lines.

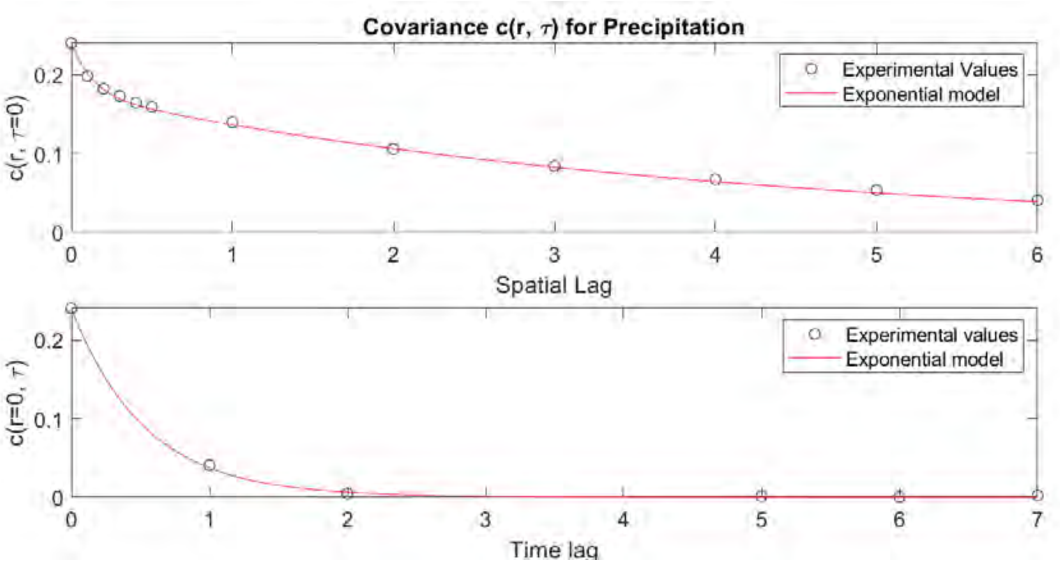


Figure E-1:

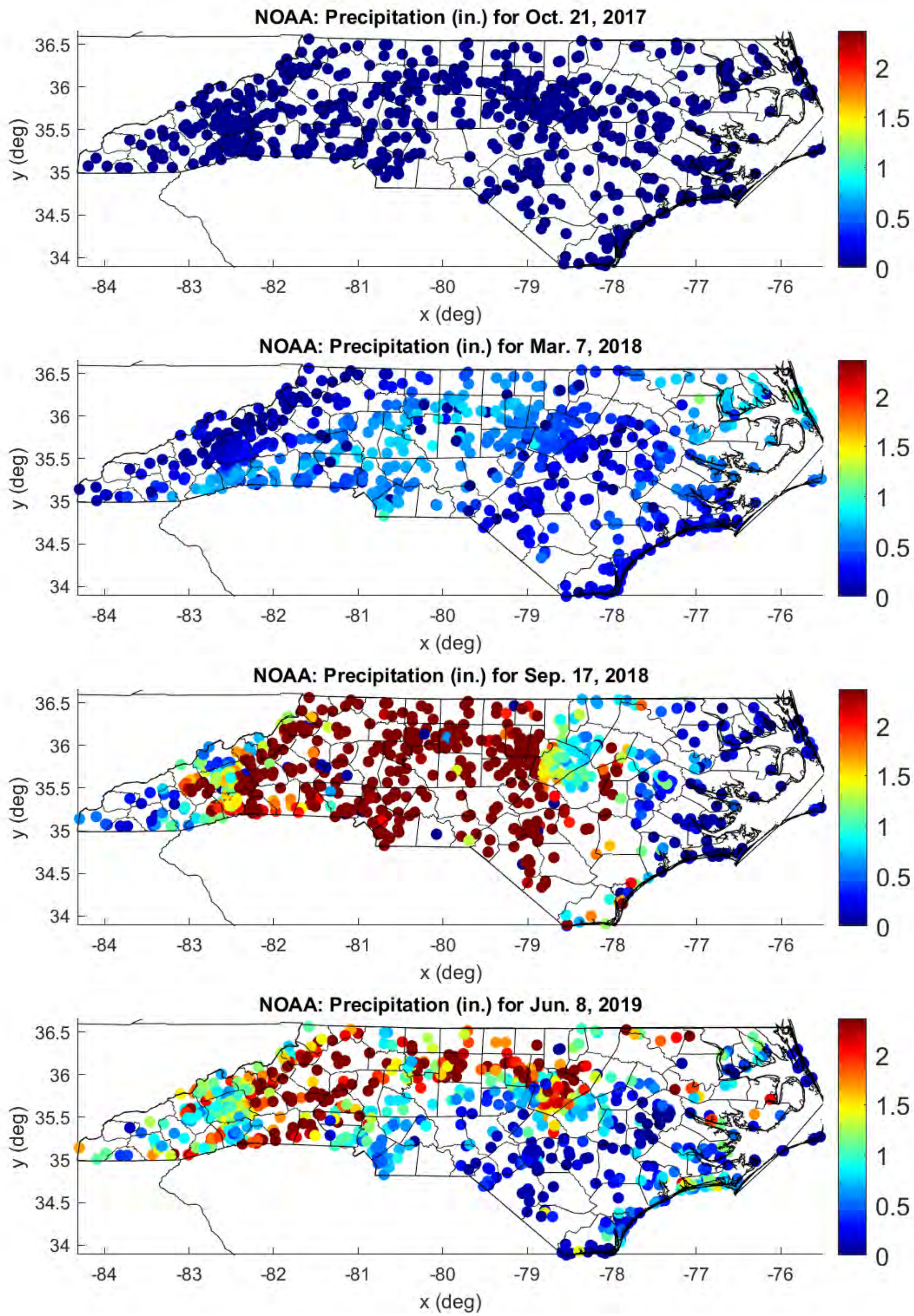
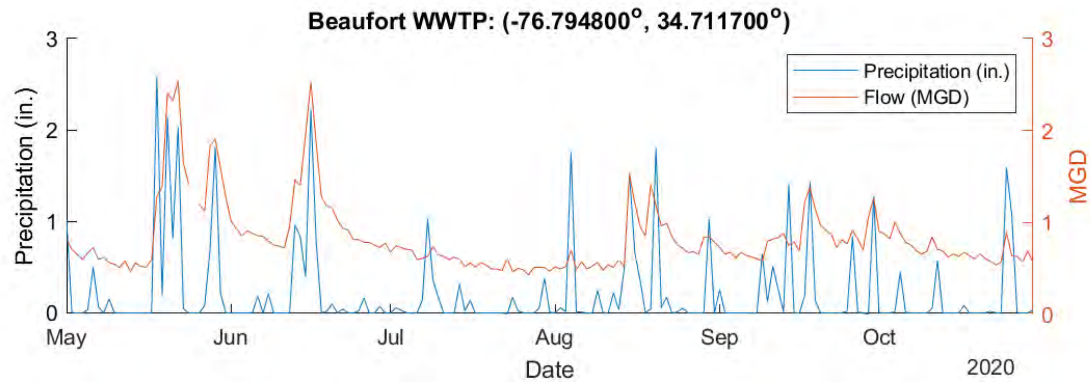
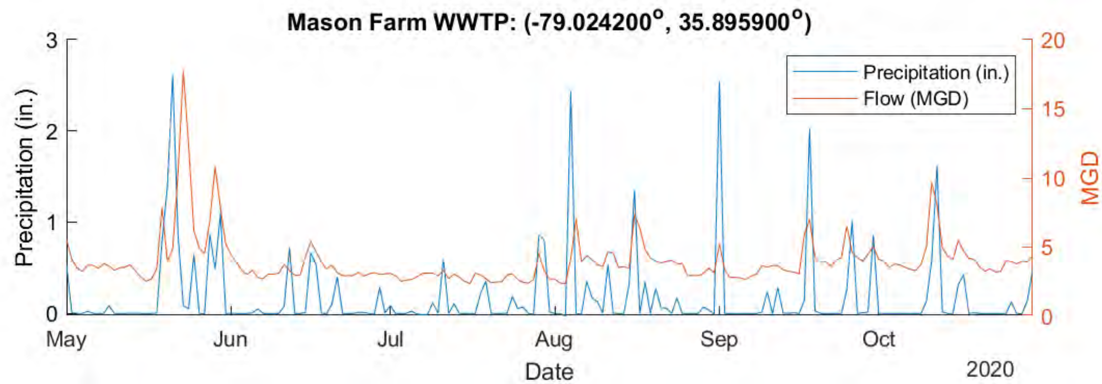


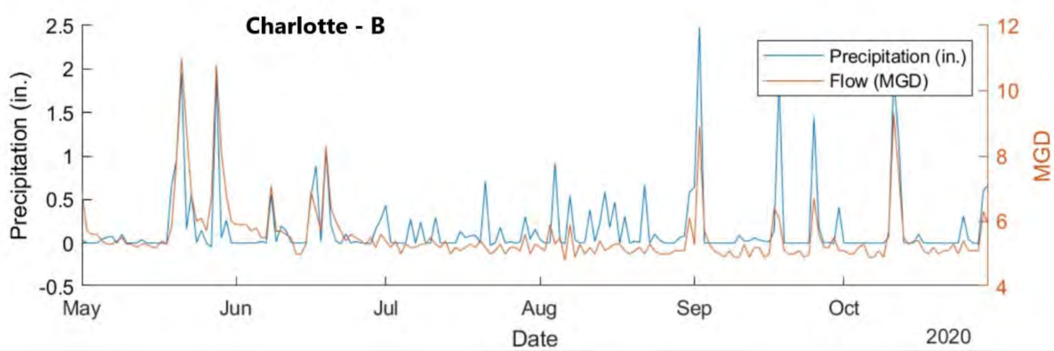
Figure E-2: Precipitation at NOAA stations across the State on 10/21/2017, 3/7/2018, 9/17/2018 and 6/8/2019. There was no precipitations on 10/21/2017, while precipitations were low on 3/7/2018, and high on 9/17/2018 and 6/8/2019.



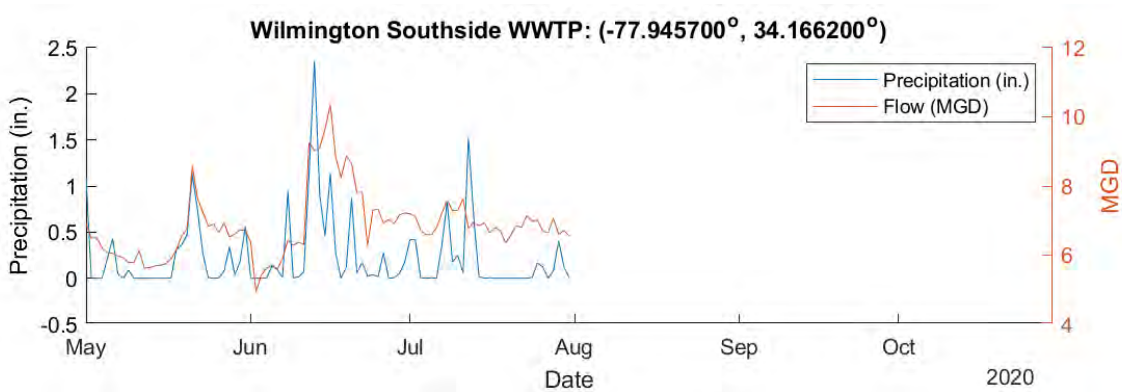
Beaufort WWTP: (-76.794800°, 34.711700°)
Precipitation (in.)
Flow (MGD)
Precipitation (in.)
MGD
Date
2020



Mason Farm WWTP: (-79.024200°, 35.895900°)
Precipitation (in.)
Flow (MGD)
Precipitation (in.)
MGD
Date
2020



Charlotte - B
Precipitation (in.)
Flow (MGD)
Precipitation (in.)
MGD
Date
2020



Wilmington Southside WWTP: (-77.945700°, 34.166200°)
Precipitation (in.)
Flow (MGD)
Precipitation (in.)
MGD
Date
2020

Figure E-3: WWTP flow and BME estimation of precipitation at four WWTPs for the duration of our study

Appendix F: New funding opportunities created by the funding received to this project team as part of the NC Collaboratory COVID-19 Package.

Funding Agency	Title	Project Duration	Project Team
CDC/NC DHHS	North Carolina DHHS/CDC Collaboration for National Wastewater Surveillance System	January 2021-July 2021	NC DHHS, UNC Chapel Hill, North Carolina State University, UNC Wilmington, UNC Charlotte North Carolina has been selected as one of eight pilot states in the Centers for Disease Control and Prevention (CDC) National Wastewater Surveillance System (NWSS). This system will provide information on the presence and persistence of SARS-CoV-2-like viruses in wastewater systems as a metric of community COVID-19 prevalence.
NIH RADx	Improved scalability, sensitivity, and interpretability of pathogen detection, including SARS-CoV-2, in wastewater using high-throughput, highly multiplexed digital array PCR technology	January 2021-January 2023	UNC Chapel HIll Michael Ramsey (lead) and Rachel Noble (co-PI)

Appendix G: Predicted outcomes resulting from collaboratory framework

PI	**Lead Investigator**	**Co-investigators**	**Research Objectives**	**Intended product**
Francis de los Reyes, Angela Harris	Nadine Kotlarz	Larry Engel, Tanvir Pasha, others in NCSU lab, DHHS	Wastewater solids - methods and correlation to public health data	Journal article
Francis de los Reyes; Helena Mitasova	Julia Kaplan, Stacie Reckling	Nadine Kotlarz, Jane Hoppin, Angela Harris	Defining the model: Which scale of health data	Journal article, model

			correlates best with COVID RNA measured in wastewater; Using the detailed model for Raleigh, evaluate different data sources to identify the minimal model that could be applied in settings without complete data to get the same answers.	
Jane Hoppin	Nadine Kotlarz, Julia Kaplan	Francis de los Reyes, Angela Harris, Helena Mitasova	Stakeholder engagement; how best to engage stakeholders (utilities, DHHS, communities); what do they need to make policy decisions, how to look at uncertainty in the data	
Angela Harris; Francis de los Reyes	Tanvir Pasha	Nadine Kotlarz, Sean Daly, Steven Berkowitz	How do trends of SARS-CoV-2 in wastewater compare between samples collected at the head of the treatment plant and samples from within the sewershed?	article
Noble	Ciesielski	Blackwood, Clerkin	DDPCR v QPCR comparison	article, in preparation Journal Virological Methods
Noble	Ciesielski	Munir, Barua, Clerkin, Blackwood, Steele, Griffith, Stewart	DDPCR v QPCR/DDPCR COMPARISON	article, in preparation
Noble	Blackwood, Ciesielski, Clerkin	Stewart, Munir, Frampton, De Los Reyes, Harris	DDPCR based assessment of cross laboratory, extraction efficiency, sensitivity,	article

			performance and protocol variations	
Noble/Harris/De Los Reyes	Noble or other	Combined relevant co authors from Noble lab and NCSU labs	Comparison of solids analysis analyzed by DDPCR two ways	article, would follow any initial submission led by Nadine on solids analysis
Noble	Noble or other	Blackwood, Clerkin, Ciesielski	Rural vs metropolitan/urban assessments of wastewater surveillance	article
Munir	Barua	co-authors from Munir's Lab, Noble or other Lab groups	Detection and persistence of SARS-CoV-2 in the municipal wastewater of Charlotte, North Carolina	study/journal
Munir	Juel	co-authors from Munir's Lab, and others	Comparison of virus concentration methods	article
Munir	Barua	co-authors from Munir's Lab, Noble or others	Co-relating SARS-CoV-2 with other indicator organism available in wastewater, looking at variations over time using metagenomic analysis	article
Serre, Engel, Mitasova, Guidry	TBD (possibilites include Richard Strott, Claire Dust, Marc Serre or a new student)	Kelly Hoffman?, Kenny Chen, David Holcomb, Corinne Wiesner? + others in the NCSU/UNC/DHHS epid and spatial group, Noble	Near real time spatiotemporal mapping of COVID-19 outbreaks across NC from March to December 2020	article
Engel , Serre , Mitasova, DHHS	David Holcomb	David Holcomb, Kenny Chen, Noble, Reckling, Petras,others in the NCSU/UNC/DHHS epid and spatial group	Effect of data sources and parameter models on the association between COVID-19 cases and WWTP	article

			SARS-CoV2 concentrations	
Engel, Serre, Mitasova, Guidry	Larry Engel	David Holcomb, Kenny Chen, Kelly Hoffman, Wiesner Noble + others in the NCSU/UNC/DHHS epid and spatial group	What is the change in incidence rate that can be detected by changes in WWTP SARS-CoV2 measured concentration when accounting for lab measurement errors, lab control, uncertainty in I&I flow, persistence of virus + shape and size of sewer system	article
Stewart	uncertain	Alyssa Grube/Knox Coleman/Connor LaMontagne/Megan Miller/David Holcomb/Nikhil Kothegal/Larry Engel	Reporting treatment plant data (vs time?); include crude case counts; methods assessment (opportunities for short notes/letters publications)	article
Mitasova, Serre, Engel		Reckling, Petras	Match COVID-19 case to sewershed	Data and Software sheet
Serre, Mitasova, Engel	Corinne Wiesner	Stacey Reckling, Vaclav Petras, Richard Strott?, Kelly Hoffman?, Nadine Kotlarz?	Model shedding of SARS-CoV-2 along the sewer network	Data and Software sheet
Berkowitz, Serre	Claire Dust		calculate the fraction of sewershed resident who have septic systems	report
Larry Cahoon, Serre, Mitasova	TBD	Richard Strott, Noble, Clerkin	Calculate inflow and infiltration daily %.	Product
Art Frampton	Art Frampton	Larry Cahoon	Compare direct extracts versus HA filter methods. Evaluate persistence of SARS-CoV-2 signal. Identify physical nature of detected pathogen	article

			using immunogold electron microscopy. Detection of variant strains in collected samples by genomic sequencing	