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 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.

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

Table 1. NC Pathogen Research Network Collaborative Partners and MunicipalStakeholders.

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.

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

Table 2. NC Pathogen Research Network Industry Partners

	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.

References cited:

Pecson, B., Darby, E., Haas, C.N. et al., 2020. Reproducibility and sensitivity of 36 methods to

quantify the SARS-CoV-2 genetic signal in raw wastewater: Findings from an interlaboratory methods evaluation in the U.S. SARS-CoV-2 Interlaboratory Consortium medRxiv 2020.11.02.20221622; doi: <u>https://doi.org/10.1101/2020.11.02.20221622</u>

Bivins, A., North, D., Ahmad, A. et al., 2020. Wastewater-Based Epidemiology: Global Collaborative to Maximize Contributions in the Fight Against COVID-19. Environmental Science & Technology 2020 *54* (13), 7754-7757 DOI: 10.1021/acs.est.0c02388

Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., and A. Brouwer. 2020. Presence of SARS-Coronavirus-2 in sewage. Environmental Science & Technology Letters. DOI: 10.1021/acs.estlett.0c00357

Visva Bharati Barua, Md Ariful Islam Juel, Adeola Sorinolu, Isaiah Young, David Holcomb, Mark Ciesielski, Thomas Clerkin, Denene Blackwood, Rachel Noble, and Mariya Munir. Quantitative analysis of SARS-CoV-2 in wastewater and tracking the temporal variation of COVID-19 surges/cases in Charlotte, North Carolina using wastewater based epidemiology. February 2021. In preparation. Science of the Total Environment

Md Ariful Islam Juel, Visva Bharati Barua, Blackwood, Denene, Noble, Rachel, and Mariya Munir. Comparative evaluation of virus concentration methods for detecting SARS-CoV-2 virus in wastewater. February 2021. In preparation. Environment Science and Pollution Research

Mark Ciesielski, Denene Blackwood, Thomas Clerkin, Raul Gonzalez, Hannah Thompson, Allison Larson, Rachel Noble*. Assessing Sensitivity and Reproducibility of Two Molecular Workflows for the Detection of SARS-CoV-2 in Wastewater. February 2021. In preparation. Journal of Virological Methods. Table 1: Personnel supported over the duration of NC Collaboratory-funded projectentitled "Tracking SARS-CoV-2 in the Wastewater Across a Range of North CarolinaMunicipalities".

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

			methods specialist, technical
			support, advanced molecular
			training and troubleshooting,
			industry partnerships
Jayne Boyer	ESE	Lab manager	I 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	ministration &
		coordinator	accounting support
Megan Miller	ESE	GRA, MS student	
Misty Parrish	IMS	Administrative	urces support
		support specialist	
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	t
		support specialist	
Tom Clerkin	IMS	Research	
		technician	industry partnerships, technical
			support
Wayne	IMS	Business manager	ministration and
Johannessen			accounting support
Carly Dinga	IMS	Undergraduate	data analysis
		Student	and reporting
Unber Ahmad	Carolina	Data analyst	epidemiological data
	Population		analysis
	Center		
University rolin	a at Charlotte		
Mariya Munir		Co-PI,	author, methods
		professor	development, community
			prevalence studies, Charlotte
			Water stakeholder interaction,
			industry partnerships, campus
			wastewater surveillance project
Adeola	CEE	PhD student	
Sorinolu			technical support for filtration and
			RNA extraction
Isaiah Young	CEE	URA, technical	
		support	supporting sample collection

Kim Wilson	CEE	Business services	Finance and accounting support
		and	
		grant coordinator	
Md Ariful Islam	CEE	PhD student	Lead author on virus concentration
Juel			method comparison paper, co-
			author on other papers;
			Dissertation Chapter, qPCR
			method development and
			optimization, qPCR analysis
Visva Bharati	CEE	Post Doctoral	Lead author on Charlotte area
Barua		Research Associate	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 rolin	a at Wilmington		
Art Frampton	BMB	Co-PI, Associate	author, methods
		professor	development, Wilmington
			stakeholder interaction, campus
			wastewater surveillance
Larry Cahoon	BMB	Co-PI, Professor	author, stakeholder
			interaction, WWTP industry
			partnerships, inflow and infiltration
			modeling
Jacob	BMB	Masters student	ntributions
Kazenelson			
North Carol			
Francis L. de	CCEE	Co-PI, Professor	author, methods
los Reyes III			development, WWTP industry
			partnerships, stakeholder
			interaction
Angela Harris	CCEE	Co-PI, Assistant	author, methods
		Professor	development, community
			prevalence studies and
			stakeholder interaction
Jane Hoppin	Biological	Professor, Co-PI	author, industry
	Sciences		partnerships, stakeholder
			interaction and collaborative
			specialist
Helena	CGA, MEAS	Professor, Co-PI	author, sewershed
Mitasova			analysis software development,
			sewershed data requests and data

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

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

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.

















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, 24hour 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.

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

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.



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 HIII Michael Ramsey (lead) and Rachel Noble (co-PI)

Appendix G: Predicted outcomes resulting from collaboratory framework

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

				1
			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	Francis de los Reves.	Stakeholder	
,	Kotlarz, Iulia	Angela Harris, Helena	engagement: how	
	Kaplan	Mitasova	hest to engage	
	inapiun	interso va	stakeholders	
			(utilities DHHS	
			communities): what	
			do they need to	
			make policy	
			decisions how to	
			look at uncortainty	
			in the date	
Angele Henrie	Tanuin Dacha	Nadina Katlara Caan	In the data	
Angela Harris;	Tanvir Pasna	Nadine Kotlarz, Sean	How do trends of	
Francis de los		Daly, Steven	SARS-COV-2 In	article
Reyes		Berkowitz	wastewater	
			compare between	
			samples collected at	
			the nead of the	
			treatment plant and	
			samples from within	
			the sewershed?	
Noble	Ciesielski	Blackwood, Clerkin	DDPCR v QPCR	
			comparison	article, in
				preparation
				Journal
				Virological
				Methods
Noble	Ciesielski	Munir, Barua, Clerkin,	DDPCR v	
		Blackwood, Steele,	QPCR/DDPCR	article, in
		Griffith, Stewart	COMPARISON	preparation
Noble	Blackwood,	Stewart, Munir,	DDPCR based	
	Ciesielski,	Frampton, De Los	assessment of cross	article
	Clerkin	Reyes, Harris	laboratory,	
		-	extraction	
			efficiency.	
			sensitivity,	

			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,	Larry Engel	David Holcomb,	What is the change	antiala
Millasova, Gulul y		Kellily Chell, Kelly	that can be detected	article
		Noble L others in the	that can be detected	
			MUNTE SARS CoV2	
		enid and spatial	massured	
		groun	concentration when	
		Broup	accounting for lab	
			measurement	
			errors, lab control.	
			uncertainty in I&I	
			flow, persistence of	
			virus + shape and	
			size of sewer system	
Stewart	uncertain	Alyssa Grube/Knox	Reporting treatment	
		Coleman/Connor	plant data (vs	article
		LaMontagne/Megan	time?); include	
		Miller/David	crude case counts;	
		Holcomb/Nikhil	methods	
		Kothegal/Larry Engel	assessment	
			(opportunities for	
			short notes/letters	
			publications)	
Mitasova, Serre,		Reckling, Petras	Match COVID-19	Data and
Engel			case to sewershed	Data and
				soltware
Sarra Mitacova	Corinne	Stacov Rockling	Model shedding of	Sheet
Fngel	Wiesner	Vaclay Petras	SARS-CoV-2 along	Data and
Linger	Wieshei	Richard Strott? Kelly	the sewer network	Software
		Hoffman? Nadine	the sewer network	sheet
		Kotlarz?		Sheet
Berkowitz, Serre	Claire Dust		calculate the	
			fraction of	report
			sewershed resident	_
			who have septic	
			systems	
Larry Cahoon,	TBD	Richard Strott, Noble,	Calculate inflow and	Product
Serre, Mitasova		Clerkin	infiltration daily %.	
Art Frampton	Art Frampton	Larry Cahoon	Compare direct	
			extracts versus HA	article
			filter methods.	
			Evaluate persistence	
			of SAKS-CoV-2	
			signal. Identify	
			detected nathogon	
Berkowitz, Serre Larry Cahoon, Serre, Mitasova Art Frampton	Claire Dust TBD Art Frampton	Richard Strott?, Kelly Hoffman?, Nadine Kotlarz? Richard Strott, Noble, Clerkin Larry Cahoon	the sewer network calculate the fraction of sewershed resident who have septic systems Calculate inflow and infiltration daily %. Compare direct extracts versus HA filter methods. Evaluate persistence of SARS-CoV-2 signal. Identify physical nature of detected pathogen	Software sheet report Product article

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