SARS-CoV-2 Wastewater Surveillance NSF Research Coordination Network

Kickoff Meeting

advancing the science of water®

COLLEGE OF ENGINEERING

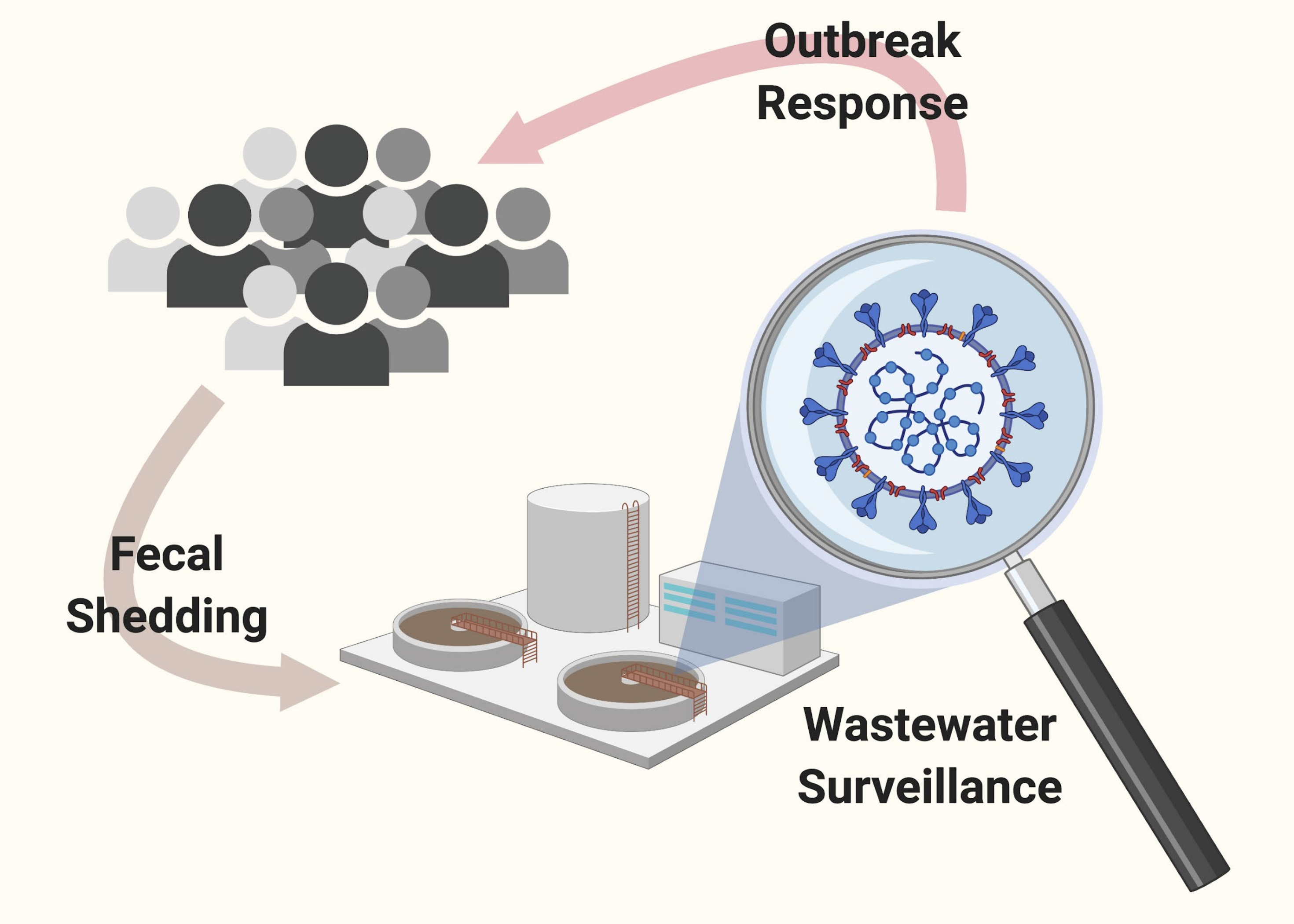
RCN: Wastewater Surveillance of SARS-CoV-2

Kyle Bibby PhD, PE
Associate Professor and Wanzek Collegiate Chair
Civil and Environmental Engineering and Earth Sciences
University of Notre Dame
KBibby@nd.edu **@kylejbibby**

Project CBET 2038087 (#1440funded)

CoPIs – Boehm (Stanford), Delgado-Vela (Howard), Halden (Arizona State)





Aaron Bivins et al. Wastewater-Based Epidemiology: Global Collaborative to Maximize Contributions in the Fight Against COVID-19 *Environmental Science & Technology* 2020 *54* (13), 7754-7757

Is It Safe to Come Out of Lockdown? Check the Sewer

Wastewater could provide early, painless and localized data about the rise or fall of coronavirus levels.

As some states reopen, studying sewage could help stop the coronavirus pandemic

Dinah Voyles Pulver USA TODAY NETWORK

Published 11:01 a.m. ET May 19, 2020 | **Updated 1:47 p.m. ET May 19, 2020**

nature

Subscribe

NEWS · 03 APRIL 2020 · CORRECTION 03 APRIL 2020

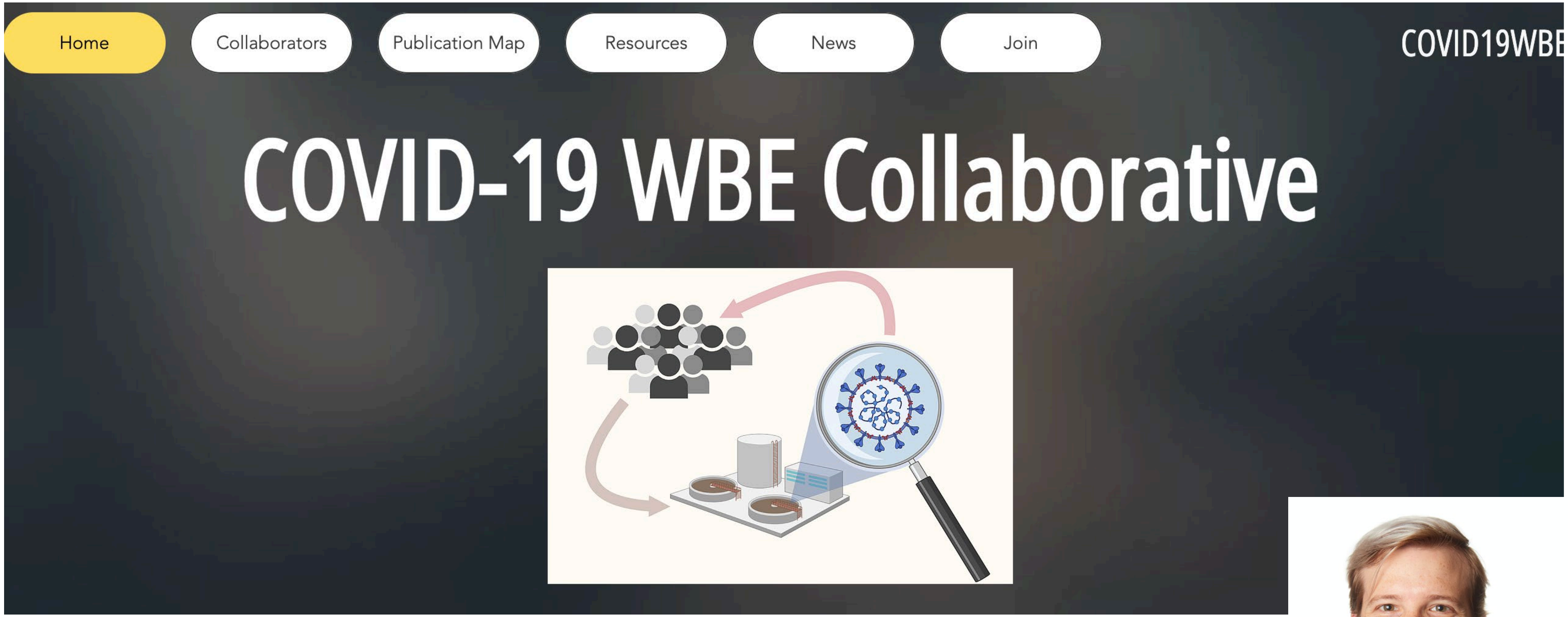
How sewage could reveal true scale of coronavirus outbreak

The CDC wants state and local sewage systems tested for coronavirus

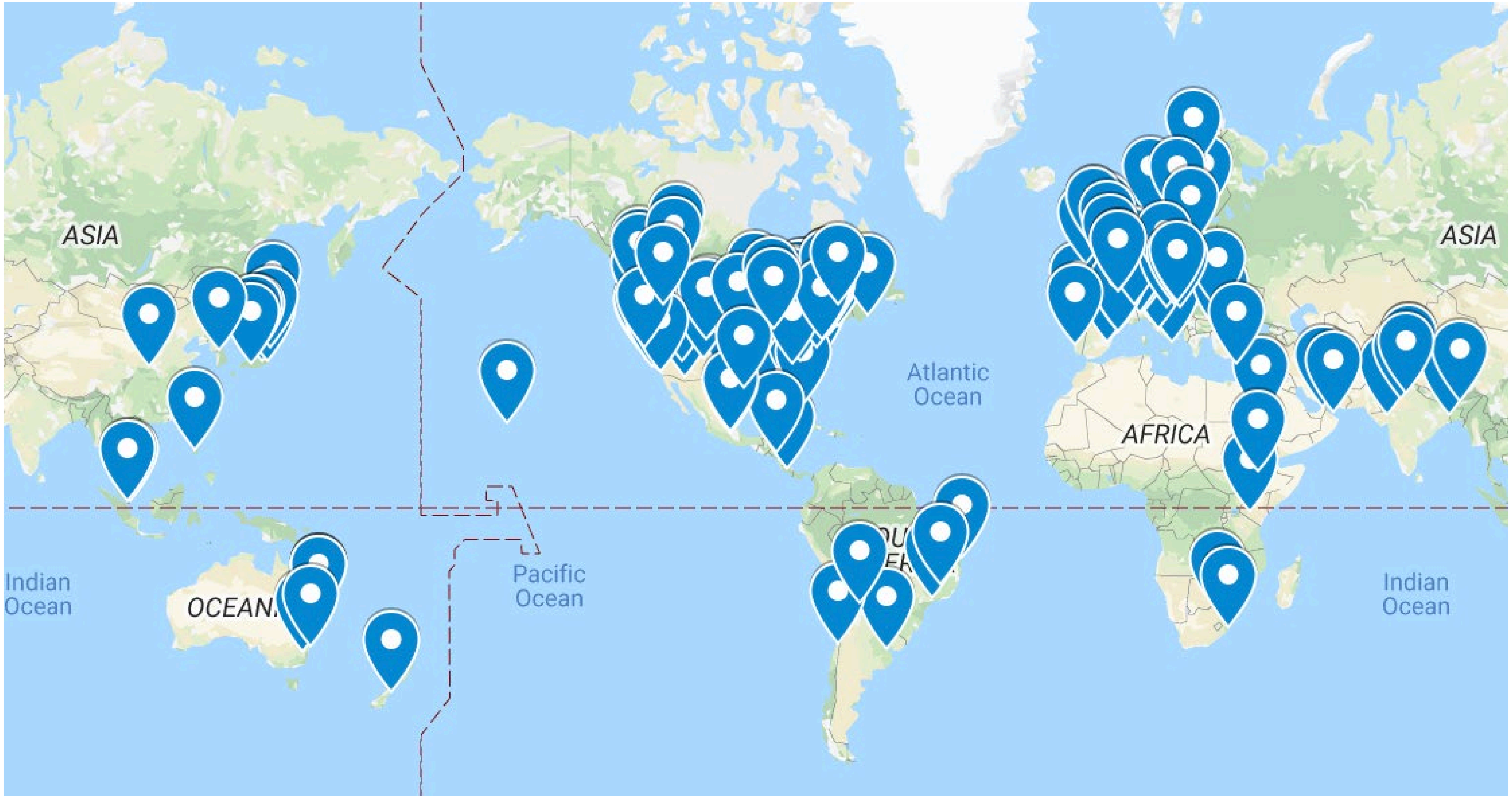
PUBLISHED WED, AUG 19 2020•9:32 AM EDT

Significant technical challenges remain!

Collaboration is Key!



www.COVID19wbec.org


ASIA
ASIA
Atlantic
Ocean
AFRICA
Pacific
Ocean
Indian
Ocean
Indian
Ocean

COLLEGE OF ENGINEERING

Research Coordination Network Goals

1. advance a field…by supporting groups of investigators to communicate and coordinate their research
2. training and educational activities across disciplinary, organizational, geographic and international boundaries

RCN Activities

Open door participation!

Seminars

Quarterly workshops

Student Training Videos

Data Sharing

Sample Sharing

Seeking a Postdoc

COLLEGE OF ENGINEERING

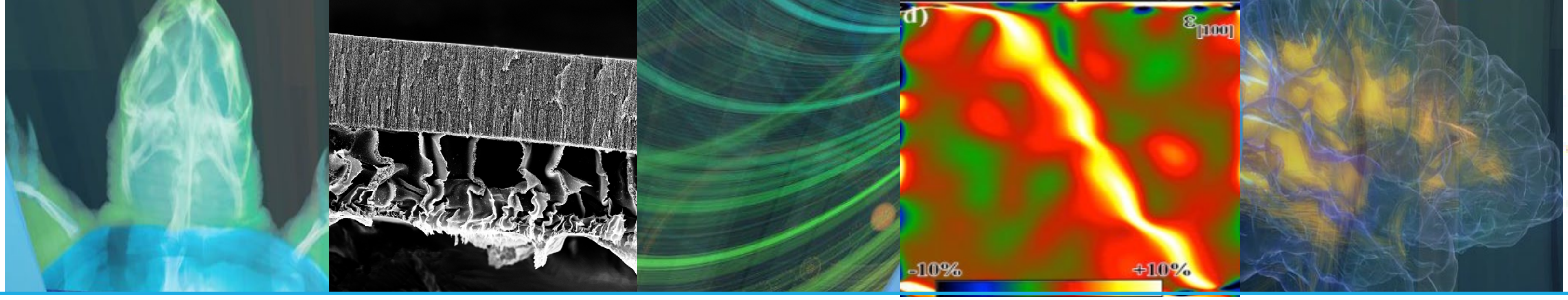
Help coordinate RCN activities.

Can be entirely remote.

Please send a CV and information for at least two references to kbibby@nd.edu

KBibby@nd.edu @kylejbibby

www.COVID19wbec.org



SARS CoV-2 Wastewater Surveillance Research Coordination Network

SARS CoV-2 Research Support

11:15-11:20 AM

September 2, 2020

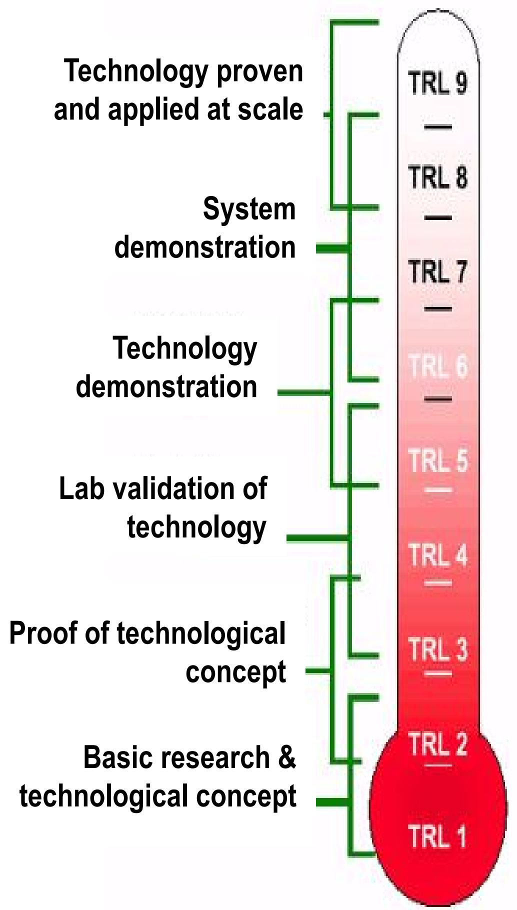
Virtually

Karl Rockne, PhD, PE, BCEE: 1440 Environmental Engineering Program Director

Chemical, Bioengineering, Environmental and Transport Processes

National Science Foundation

NSF Support of Engineering Research



Translational, Commercialization

ENG/IIP Small Business Innovation Research (SBIR), and Small Business Technology Transfer (STTR)

Fundamental, Precompetitive

ENG Core Programs (CBET, CMMI, ECCS), Centers (EEC), and Emerging Frontiers in Research and Innovations (EFMA)

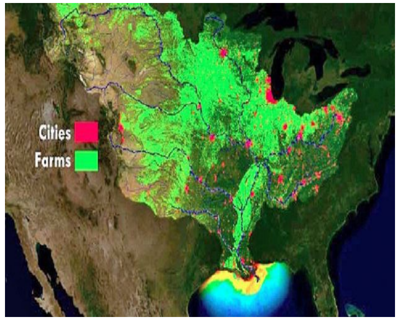
NSF Funding Mechanisms

- **Core/Unsolicited:** 2-4 years; Individual or multi-institutional collaborative teams
- **Solicitations/DCLs:** Variable funding; multiple divisions/directorates involved
  - COVID RAPID DCL
  - URoL: Microbiome Theory & Mechanisms…
- **RAPID:** Ephemeral data (COVID-19), Disasters: Fires, Hurricanes/extreme weather
- **Research Coordination Networks (RCNs):** Submitted through the core program
- **Workshops/Conferences:** Where can NSF funding make a difference?
  - Product/outcomes leading to new **knowledge**, **directions**, and/or **identification of needs**

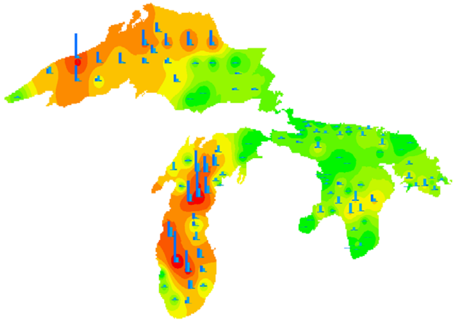
CBET Environmental Engineering:
Program Emphasis Areas (1440)

Program Goal: Fund **transformational** and high risk/high reward research to:

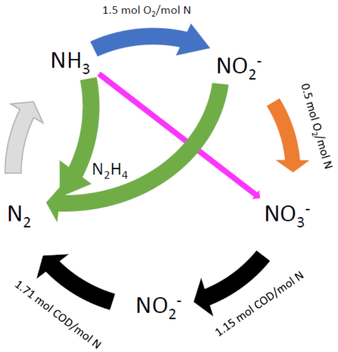
- **Prevent/minimize release** of pollution to **soil**, **water,** and **air**
- **Mitigate:** Ecological and human health impacts of such releases by smart/adaptive manipulation of the environment
- **Remediate** polluted environments through engineered **chemical**, **biological,** and **geo/physical** processes
- Integral to achieving these goals is the **fundamental understanding of pollutant transport** in the environment and **how to harness and control their biological, chemical, and geo/physical reactions**



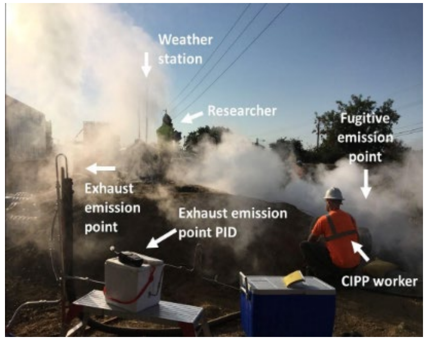
USGS (2007)



Guo et al. (2017)

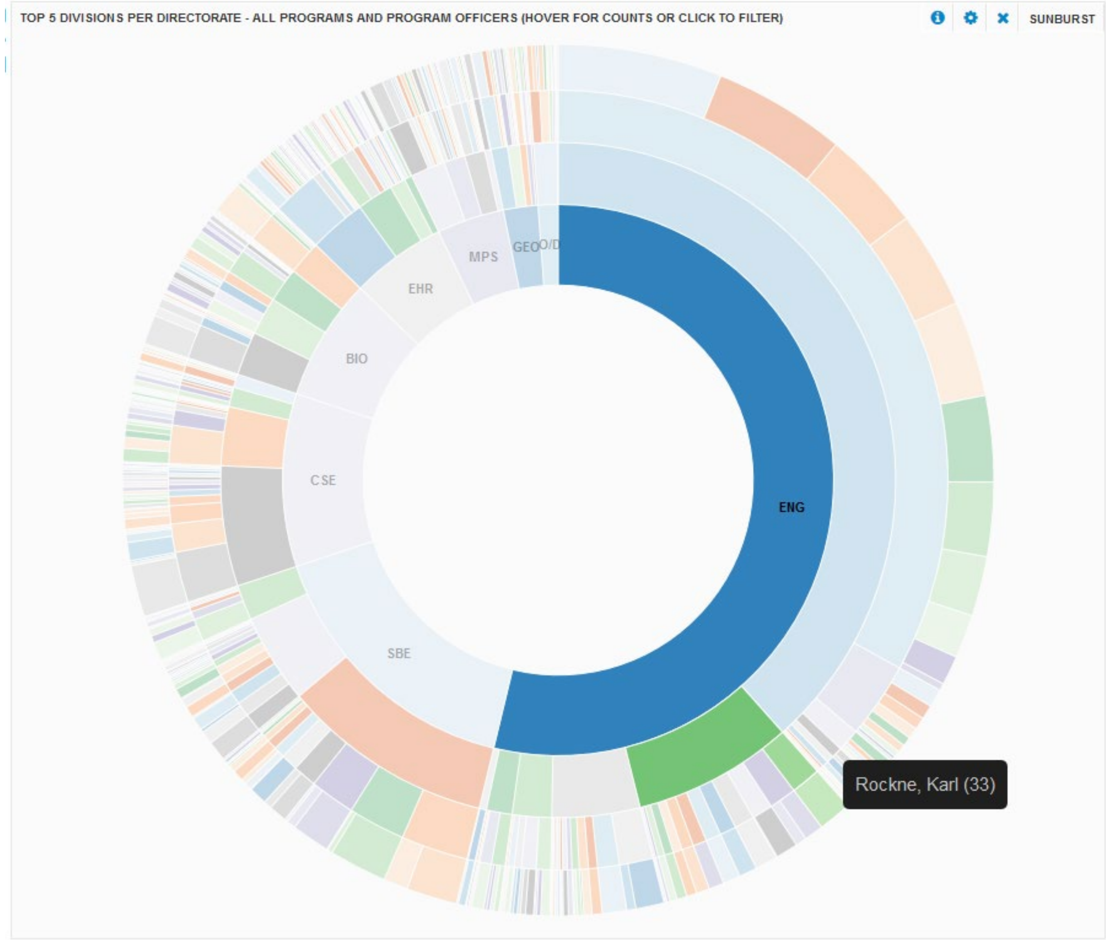


Chandran et al. (2017)

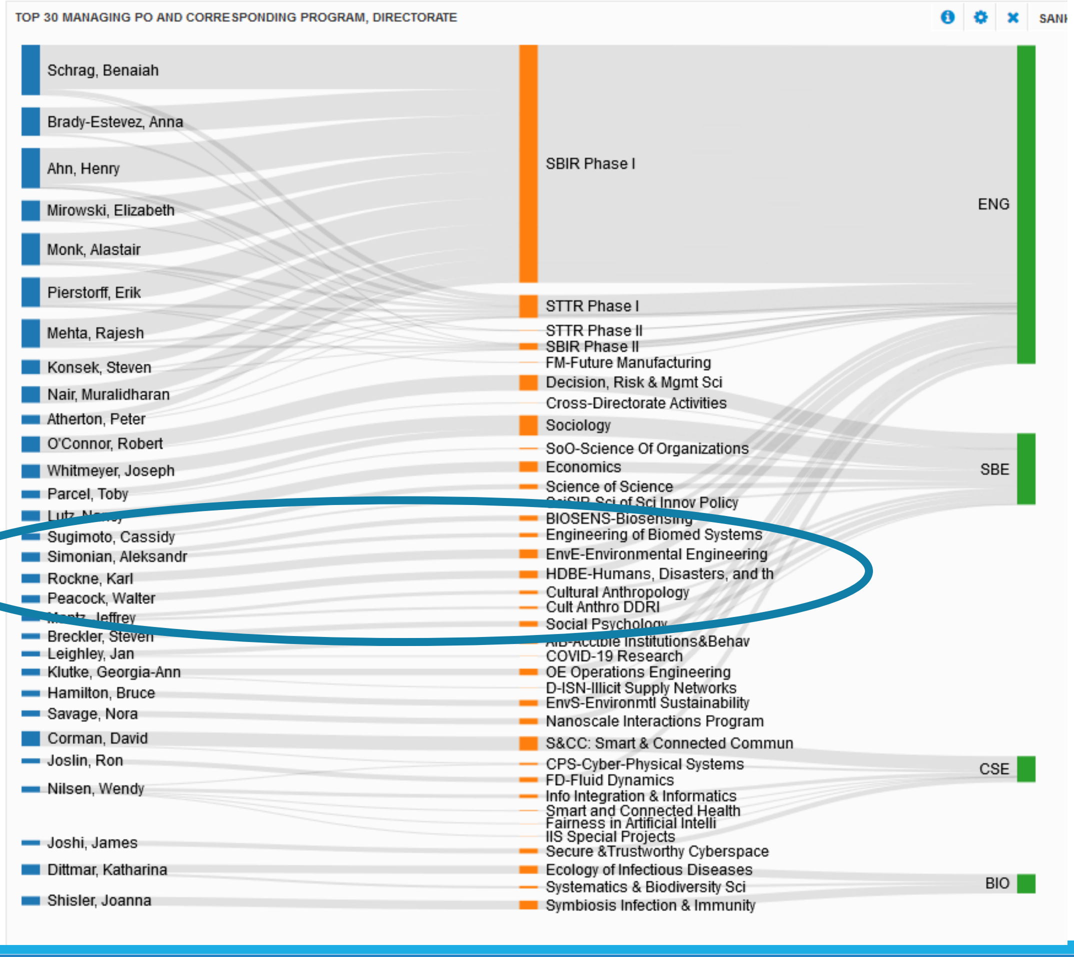


Whelton, Purdue Univ (*2017)*

CARES Act: COVID RAPID DCL



Top programs: SBIR DRMS ENG



Selected WBE grants in 1440

Wastewater Surveillance			Public Abstract
Krista Wigginton, Boehm, Ali	University of Michigan/ Stanford	Collaborative Research: RAPID: Coronavirus persistence, transmission, and circulation in the environment	https://nsf.gov/awardsearch/showAward?AWD_ID=2023057&HistoricalAwards=false
Bibby, Kyle/ Brown, Joe	Notre Dame/ Georgia Tech	Collaborative Research: RAPID: Wastewater Informed Epidemiological Monitoring of SARS-CoV-2	https://nsf.gov/awardsearch/showAward?AWD_ID=2027752&HistoricalAwards=false
Chandran, Kartik	Columbia University	RAPID: Viral structure-function-activity in the engineered wastewater cycle	https://nsf.gov/awardsearch/showAward?AWD_ID=2026599&HistoricalAwards=false
Yan, Tao	University of Hawaii	RAPID: Determine Community Disease Burden of COVID-19 by Probing Wastewater Microbiome	https://nsf.gov/awardsearch/showAward?AWD_ID=2027059&HistoricalAwards=false
Radniecki, Tyler S	Oregon State University	RAPID: Tracking the Coronovirus in municipal wastewater	https://nsf.gov/awardsearch/showAward?AWD_ID=2027679&HistoricalAwards=false
Halden, Rolf U	Arizona State University	RAPID: COVID-19's Impact on the Urban Environment, Behavior, and Wellbeing	https://nsf.gov/awardsearch/showAward?AWD_ID=2028564&HistoricalAwards=false
De los Reyes III, Francis L	North Carolina State University	RAPID: Monitoring for SARS-CoV-2 in municipal wastewater and sewage to elucidate infection dynamics across major metropolitan areas of the United States	https://nsf.gov/awardsearch/showAward?AWD_ID=2029025&HistoricalAwards=false
Goel, Ramesh	University of Utah	RAPID: Determination of health risks and Status from SARS-CoV-2 Presence in Urban Water cycle	https://nsf.gov/awardsearch/showAward?AWD_ID=2029515&HistoricalAwards=false
Conroy-Ben, Otakuye	Arizona State University	RAPID: Tribal capacity to evaluate COVID-19 using wastewater-based epidemiology	https://nsf.gov/awardsearch/showAward?AWD_ID=2038372&HistoricalAwards=false

Results and Press

Research News

Water quality could be altered in buildings closed during COVID-19 pandemic

Quality of water left sitting in pipes could change

Purdue engineer Andrew Whelton holds up a water sample from a building faucet.

Credit and Larger Version

April 21, 2020

While restaurants, gyms, schools and other buildings are closed indefinitely to prevent the spread of COVID-19, the quality of water left sitting in pipes could change.

In buildings nationwide, water left sitting for long periods of time could contain excessive amounts of heavy metals and pathogens that are concentrated in pipes, say researchers who have begun a field study on the impact of the pandemic shutdown on buildings.

ASU researchers believe sewage gives them a way to track the coronavirus

By Bailey Miller | Published April 28 | Coronavirus in Arizona | FOX 10 Phoenix

ASU researchers believe sewage gives them a way to track the coronavirus

Researchers at Arizona State University think they have found a way to track coronavirus, and keep economies going in places where the virus is not prevalent.

TEMPE, Ariz. - Researchers at Arizona State University think they have found a way to track coronavirus, and keep economies going in places where the virus is not prevalent.

It's an interesting concept, and one that could focus resources and restrictions to where they're needed most.

The Arizona cities of Tempe, Guadalupe and Gilbert are benefitting from this technology. Researchers argue they can find where hotspots of coronavirus are, and that will help determine what locations to put on a stay at home order, rather than a one-size-fits-all.

The answer, finding where the coronavirus is spreading, is all in the flush of that toilet.

"Whether you like it or not, the information we need is in the sewer, and the sewer doesn't lie," said Rolf Halden, an ASU professor with the Biodesign Institute.

Halden and other researchers created an early warning system to detect the seasonal flu, by analyzing stool in the sewage systems. Little did they know how important their work would become during the ongoing COVID-19 pandemic.

"What we have in mind is to have a proactive system, where we see an increase in viruses and can react before people that are asymptomatic present at the health care facilities," said Halden.

Halden argues by collecting sewage samples, he can produce data for an entire city within 24 hours, and 80 % of the U.S. population within a week, adding that his method is much cheaper and faster than the clinical testing

Coronavirus Updates View More

- LIVE Blog: Coronavirus in Arizona - Latest case numbers
- MAP: Arizona coronavirus cases by zip code
- LIST: Arizona districts announce return to school plans
- LIST: Arizona cities with face mask requirements
- LIST: Coronavirus testing locations in Arizona

The New York Times

'A Smoking Gun': Infectious Coronavirus Retrieved From Hospital Air

Airborne virus plays a significant role in community transmission, many experts believe. A new study fills in the missing piece: Floating virus can infect cells.

Doctors in Johannesburg demonstrated how to place a device called an intubox over a patient, to help curb the spread of viral droplets during intubation. Michele Spatari/Agence France-Presse — Getty Images

By Apoorva Mandavilli

Aug. 11, 2020

UM, Stanford study early warning system for coronavirus through sewage

Kim Kozlowski, The Detroit News Published 12:01 a.m. ET March 30, 2020 | Updated 12:11 a.m. ET March 30, 2020

As the world works to stem the tide of the coronavirus, researchers are racing to better understand the virus, its transmission, and how to stop it.

One potential answer could be in the water — wastewater, to be precise.

Though the COVID-19 is a respiratory disease, researchers at the University of Michigan and Stanford University say that people who have contracted COVID-19 shed the genetic material of the virus through their waste and into a community's sewage system.

That's why they are looking for early signs of the novel coronavirus in the wastewater of some California and Midwest communities to develop a tool that might inform a community of the virus' presence before a widespread outbreak.

If successful, it would be an early-warning system that detects when a virus is circulating before sick people appear in hospitals. That could prompt quicker and shorter calls for social isolation without crippling the economy.

"It would be like a real-time assessment of what is happening in the community," said Krista Wigginton, a UM civil and environmental engineering associate professor who is currently a visiting professor at Stanford.

More than a third of U.S. counties don't have a single reported case of the COVID-19, the disease caused by the virus, according to a surveillance system set up by Johns Hopkins University. But because symptoms can take 2-14 days to present, an absence of positive tests does not mean the virus isn't already circulating.

Share your feedback to help improve our site experience!

MORE STORIES

- State health chief: No decision yet on gym reopenings — Sept. 1, 2020, 6:13 p.m.
- Porn star Ron Jeremy charged with 20 more counts of sex abuse — Aug. 31, 2020, 7:56 p.m.
- Feds accuse Novi man of $3M COVID-19 scheme — Sept. 1, 2020, 4:27 p.m.
- Fireworks light up pandemic-weary hearts remotely — Aug. 31, 2020, 10:38 p.m.
- Whitmer task force urges big COVID-19 nursing home policy shifts — Aug. 31, 2020, 6:44 p.m.
- Officials: Deputies fatally shoot Black man who dropped gun — Sept. 1, 2020, 3:13 p.m.

The New York Times

After Coronavirus, Office Workers Might Face Unexpected Health Threats

Stagnant plumbing systems in emptied commercial buildings could put returning employees at risk of Legionnaires' and other illnesses.

Office buildings on Park Avenue in Manhattan earlier this month. Chang W. Lee/The New York Times

By Max Horberry

Published May 20, 2020 Updated Aug. 27, 2020

GREGORY BARBER SCIENCE 04.07.2020 08:00 AM

One Way to Potentially Track Covid-19? Sewage Surveillance

How many people have been infected with the new coronavirus? A group of Bay Area researchers aims to find out—by tracking what's in the local wastewater.

An early warning system for coronavirus infections could be found in your toilet

From the U.S. to Europe to Australia, scientists have detected the virus in wastewater ahead of spikes in local cases

Researchers from Brazil's National Institute of Science and Technology and Minas Gerais sanitation employees collect sewage samples to try to detect the coronavirus on April 16 in Belo Horizonte, Brazil. (Douglas Magno/AFP/Getty Images)

By Brady Dennis

May 1

David Hirschberg has a simple explanation for why a growing number of scientists are looking to sewage to help track the spread of the novel coronavirus in communities around the world.

"S--- is a great source of information," said Hirschberg, founder of a nonprofit biotech firm and professor

ARTICLE IN PRESS

Aerosol and Air Quality Research, x: 1–5, xxxx
ISSN: 1680-8584 print / 2071-1409 online
https://doi.org/10.4209/aaqr.2020.05.0202

Collection of SARS-CoV-2 Virus from the Air of a Clinic Within a University Student Health Care Center and Analyses of the Viral Genomic Sequence

John A. Lednicky[1,2], Sripriya N. Shankar[3], Maha A. Elbadry[1,2], Julia C. Gibson[1,2], Md. Mahbubul Alam[1,2], Caroline J. Stephenson[1,2], Arantzazu Eiguren-Fernandez[4], J. Glenn Morris[2,5], Carla N. Mavian[2,6], Marco Salemi[2,6], James R. Clugston[7,8], Chang-Yu Wu[3*]

[1] Department of Environmental and Global Health, University of Florida, Gainesville, FL 32611, USA
[2] Emerging Pathogens Institute, University of Florida, Gainesville, FL 32611, USA
[3] Department of Environmental Engineering Sciences, University of Florida, Gainesville, FL 32611, USA
[4] Aerosol Dynamics Inc., Berkeley, CA 94710, USA
[5] College of Medicine, University of Florida, Gainesville, FL 32611, USA
[6] Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida, Gainesville, FL 32611, USA
[7] Student Health Care Center, University of Florida, Gainesville, FL 32611, USA
[8] Department of Community Health and Family Medicine, University of Florida, Gainesville, FL 32611, USA

ABSTRACT

The progression of COVID-19 worldwide can be tracked by identifying mutations within the genomic sequence of SARS-

Selected Federal Efforts

- **NIH RADx-rad:** *Wastewater detection of SARS CoV-2* ($19M)
  - https://grants.nih.gov/grants/guide/rfa-files/RFA-OD-20-015.html
- **CDC/HHS:** *A new public health tool to understand COVID-19 community spread*
- **NIST:** *Measuring SARS CoV-2 in wastewater and fecal material: A call for standards*
- **HHS/USPHS et al:** *National Sewage Surveillance Interagency Leadership Committee*

1440 support/liaison:

- **NASEM WSTB:**
  - *Wastewater surveillance of SARS CoV-2* (1440-supported PI Wigginton presenting)
- **NASEM EHMI:**
  - *Airborne Transport of SARS CoV-2* (1440-supported PI Marr, Haas, Miller presenting)

Important Questions

- **QA/QC:** Methods for scientifically defensible results
- **Privacy:** How to ensure citizen privacy (e.g. HIPPA)
  - Sampling in the sewershed vs plant
- **Interpretation:** How to interpret concentration, loading, …
  - System hydraulics, usage
- **Health Outcomes Linkage:** How do we link data with health outcomes?
  - Finer resolution vs larger capture zone
  - Concomitant community health markers (metabolites, pharmaceuticals…)

Thoughts on the future…

- **Think beyond traditional boundaries:** Collaboration is key
  - Public health, epidemiology, medicine
  - Environmental Health
- **Is the pendulum swinging back? "**Sanitary Engineering" makes a comeback
  - "Urban penalty": Sanitary/human health focused
  - CWA/EPA…: Environmental health focused
  - Remember: The first Director of the CDC was an engineer!

Get More Information/ Chat

www.nsf.gov

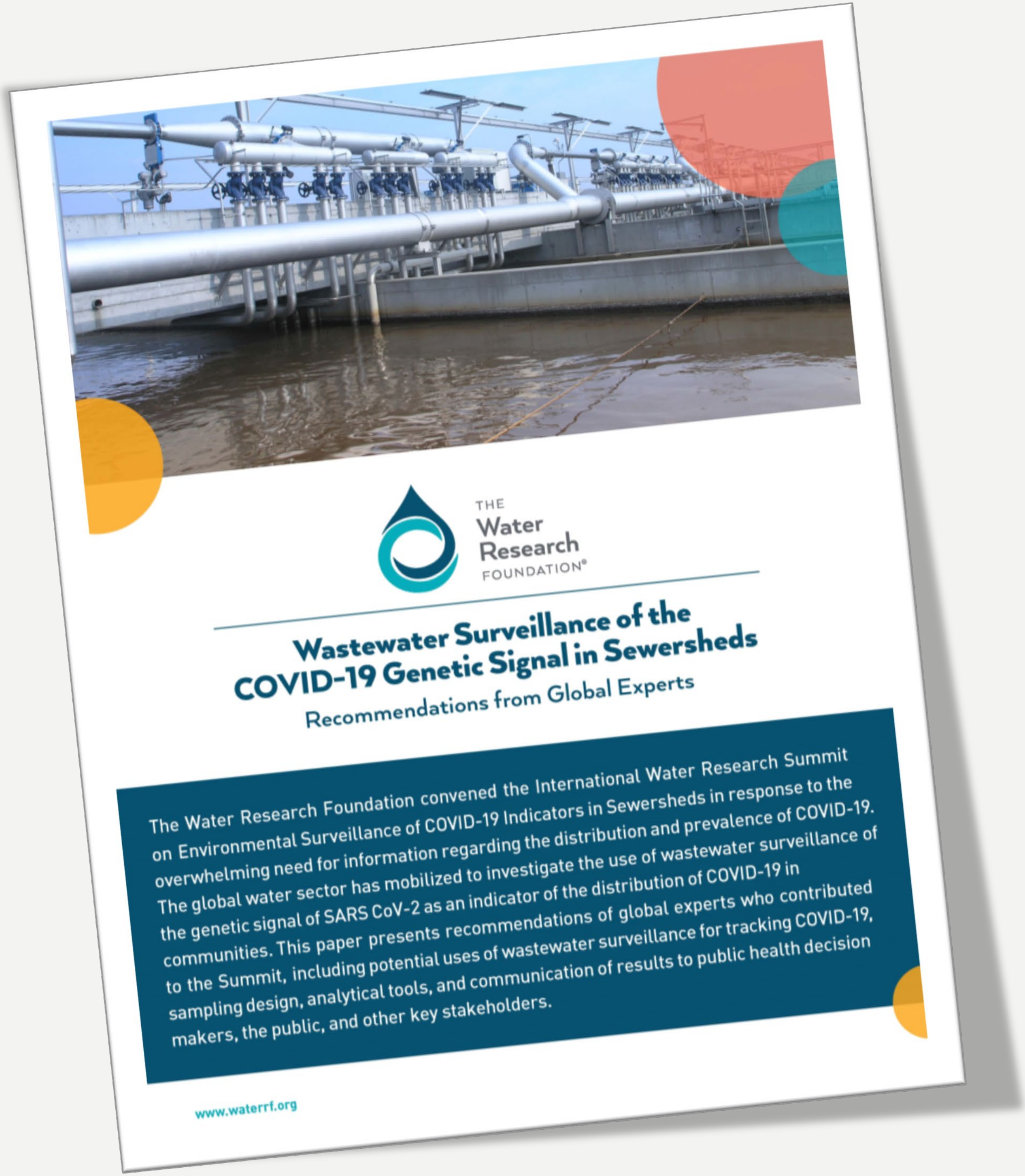
Karl Rockne
KROCKNE@nsf.gov

@DOCROCKORG

SARS-CoV-2 Monitoring

Peter Grevatt, PhD, Chief Executive Officer
John Albert, Chief Research Officer
Christobel Ferguson, PhD, Chief Innovation Officer

advancing the science of water®



Virtual International Water Research Summit on COVID-19
www.waterrf.org/event/virtual-international-water-research-summit-covid-19

Recommendations from Global Experts Whitepaper (following Summit)

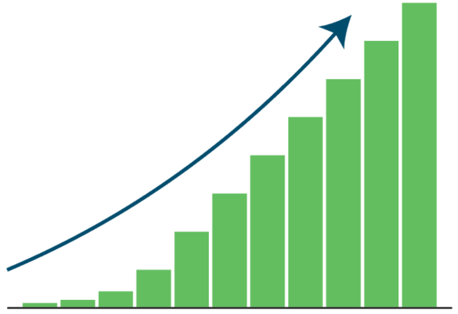
www.waterrf.org/sites/default/files/file/2020-06/COVID-19_SummitHandout-v3b.pdf

Virtual Congressional Briefing on Environmental Surveillance of the Genetic Fingerprint of COVID-19 in Sewersheds: www.waterrf.org/event/virtual-congressional-briefing-environmental-surveillance-genetic-fingerprint-covid-19

What Can You Use Sewershed Surveillance Data For ?

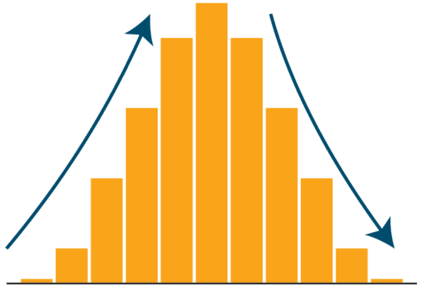
General Use Cases	Can Inform	Current Feasibility
Trends/Changes in Occurrence	Early detection of Occurrence. Tracking the impact of medical and social interventions: A) curve increasing; B) curves decreasing	A) ++ B) +
Assessment of Community Infection	Tracking disease prevalence in the community Identification of areas of concern	+/-
Risk Assessment	Risk to utility workers and those exposed to raw sewage	+/-
Viral Evolution	Source tracking of the virus	-

Trend Occurrence



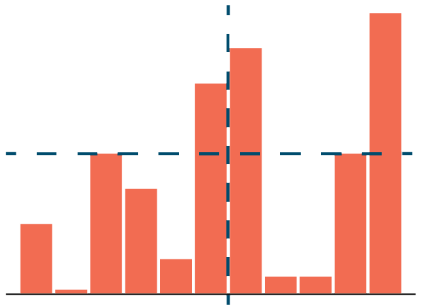
1 variable, 1 direction

Changes in Trends



1 variable, 2 directions

Community Prevalence



multiple variables, need to establish trigger levels

4 Priority Areas to Accelerate the Pace of Progress

1. Standardized procedures for the collection and storage of wastewater samples
2. Use of tools to identify the genetic signal of SARS-CoV-2 in wastewater samples
3. Recommended approaches for the use of data on the genetic signal of SARS-CoV-2 to inform trends and estimates of community prevalence
4. Strategies to communicate the implications of wastewater surveillance results with the public health community, elected officials, wastewater workers, and the public

COVID-19 RESEARCH PROJECTS

- Interlaboratory and Methods Assessment of the SARS-CoV-2 Genetic Signal in Wastewater (#5089)
- Understanding the Factors that Affect the Detection and Variability of SARS-CoV-2 in Wastewater (#5093)
- Environmental Persistence and Disinfection of Lassa Virus and SARS-CoV-2 to Protect Worker and Public Safety (#5029)
- Impact of Storage and Pre-Treatment Methods on Signal Strength of SARS-CoV-2 Genetic Signal in Wastewater *(under development)*
- NSF Research Coordination Network on SARS-CoV-2 wastewater surveillance (*recently awarded*)

ADD-ONS TO PROJECTS ALREADY UNDERWAY THROUGH A GRANT FROM CALIFORNIA STATE WATER BOARD

Measuring Pathogens in Wastewater (#4989 & #4952)

SARS-CoV-2 added to list of organisms of concern; research team is investigating the feasibility of analyzing SARS-CoV-2 in samples archived since Nov. 2019 and going forward

Collecting Pathogens in Wastewater During Outbreaks (#4990)

Added coronavirus to the list of organisms of concern

Interlaboratory and Methods Assessment of the SARS-CoV-2 Genetic Signal (#5089)

Lab Selection (63 --> 33 US Labs)

Experimental Plan and QAPP – liaising with CWN to compare approaches

Wastewater Sampling and Interlaboratory Analysis (Aug 17th – Aug 28th)

Evaluate Results and Prepare Report (>50% of results submitted – delays due to reagent shortages)

Participants virtual workshop end of September 2020

Publication early October 2020

Understanding the Factors that Affect the Detection and Variability of SARS-CoV-2 in Wastewater (#5093)

- Scale
- Study goals / Use cases
- Grab vs. Composites, study-specific
- Timing within an event
- Sensitivity vs. Quantitation in monitoring
- Complexity of wastewater infrastructure
- Frequency & Duration
- Representativeness
- Comparability

2-Minute Lightening Talks

Wastewater-Based Epidemiology at the University of Arizona WEST Center

The Likins Dorm Case Study - Timeline

Date	Time	Event
August 25	8:30am	Likins Dorm wastewater sampled
	8:30am – 5:00pm	Sample analyzed
	6:00pm	positive for SARS-CV-2 and announced to UA Task Force
	11:00pm	Emergency meeting, Dr. Pepper with Task Force including President Robbins
August 26	8:00am	Emergency meeting – decision made to test students in Likins Hall
	8:30am	5 samples, one every 5 minutes, collected from Likins Dorm sewage
	11:00am	Antigen and PCR tests of students in Likins Dorm
	5:30pm	All 5 samples positive for SARS-CoV-2. Concentrations virtually identical in all 5 samples. Two students positive for antigen test (asymtomatic) and removed from Likins Dorm
August 27	8:30am	Wastewater sample collected from Likins Dorm
	12:30pm	Additional sample collected from Likins Dorm
	5:00pm	All samples found to be negative
August 28	8:30am	Likins Hall sample collected
	5:00pm	Sample found to be negative
August 30		Plans made to double WBE testing capacity - Originally 10 locations twice weekly - New projection 24 locations 3x a week

A comparison of SARS-CoV-2 wastewater concentration methods in use for environmental surveillance

Sarah E. Philo, Erika Keim, Rachael Swanstrom, Angelo Q.W. Ong, Elisabeth Burnor, Alexandra Kossik, Joanna C. Harrison, Bethel A. Demeke, Nicolette A. Zhou, Nicola K. Beck, Jeffry H. Shirai, **J. Scott Meschke**
Contact info: jmeschke@uw.edu; +1 206-221-5470

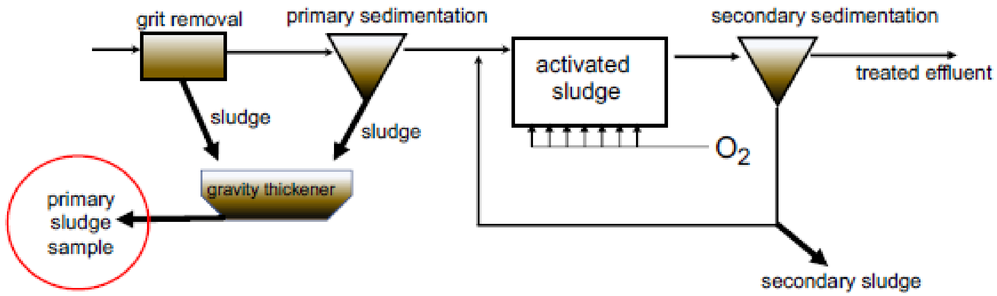
- Conducted seeded recovery control experiments with OC43 in wastewater from 3 Seattle WWTPs

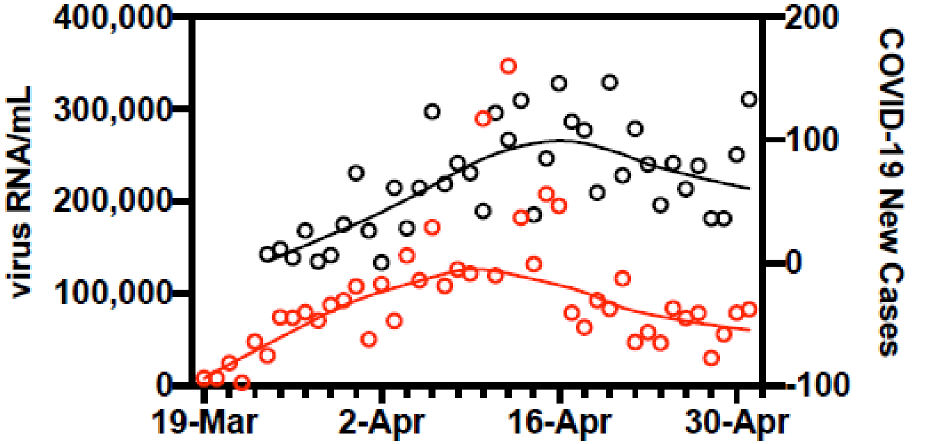
Method	Mean OC43 Cq (95% CI)	Mean percent OC43 recovery (95% CI)	Mean effective volume assayed (mL) (95% CI)	Detection of SARS-CoV-2 (%)	Sample Number
Skimmed milk flocculation	24.3 (23.8-24.9)	9.1 (6.2-11.9)	1.1 (1.0-1.2)	60.0	45
Skimmed milk flocculation with Vertrel Extraction	25.0 (24.4-25.7)	6.5 (2.9-10.1)	1.4 (1.3-1.5)	60.0	45
BMFS	25.5 (24.5-26.5)	0.69 (0.35-1.0)	11.9 (10.7-13.1)	48.9	45
BMFS with Vertrel Extraction	28.8 (27.3-30.4)	0.038 (0.0097-0.066)	10.4 (8.4-12.4)	66.7	9
PEG precipitation	25.2 (24.5-26.0)	3.2 (1.3-5.1)	1.1 (0.92-1.2)	25.0	4
Ultrafiltration	26.0 (24.7-27.3)	1.0 (0.50-1.6)	5.3 (4.8-5.8)	68.2	22

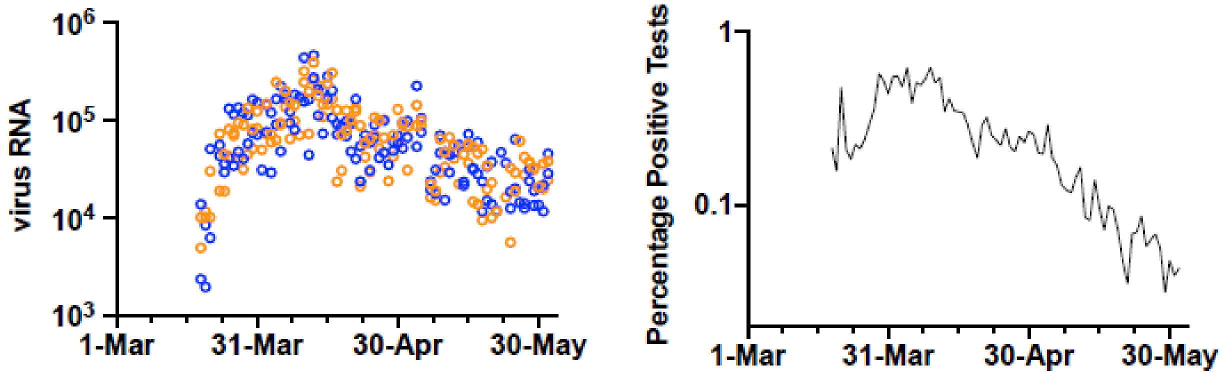
- Skimmed milk flocculation performed the most consistently, and is feasible in low-resource settings
- Additional work needed to optimize SARS-CoV-2 RT-qPCR assays for detection in wastewater
    - Variability in N1, N2, and N3 standard curves between runs and in different matrices

SARS-CoV-2 RNA concentrations in municipal wastewater primary sludge as an early indicator of COVID-19 outbreak dynamics

Alessandro Zulli, Yale University







- Lead times were found using a distributed lag measurement error time series model
- Lead times of 6-8 days on reported test data
- Lead times shortened to 0-2 days for specimen collection

Yale SCHOOL OF ENGINEERING & APPLIED SCIENCE

J. Peccia, A. Zulli, et al., Nature Biotech. Accepted, 2020

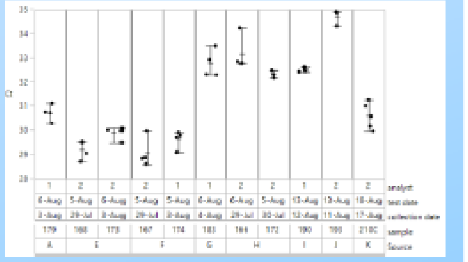
What do WBE methods require to be valuable for **large scale public health efforts?**

1) Full method validation

2) Consistent results

3) Availability & robust quality assurance

IDEXX RT-qPCR and Extraction Kits Study Findings



- ✓ Validated nationwide with diverse sewage samples
- ✓ Full method virus/test coef. of variation: 6.6 – 37.5%
- ✓ Simple one-step reaction minimizes variation
- ✓ Efficient (95-105%), linear (R^2>0.997) standard curves
- ✓ Demonstrated inhibitory substance removal by magnetic beads & PEG

- ✓ IDEXX has maintained >99.9% key product availability
- ✓ >10 years of manufacturing for PCR and wastewater in ISO/GMP facility
- ✓ Training videos and live technical support

IDEXX would like to partner with researchers to support WBE at scale

R&D Contact: Brian Swalla, PhD, Brian-Swalla@IDEXX.com

IDEXX

ddPCR Quantification of SARS-CoV-2 in Wastewater Treatment Facilities across New Hampshire

University of New Hampshire
College of Engineering and Physical Sciences
Civil and Environmental Engineering

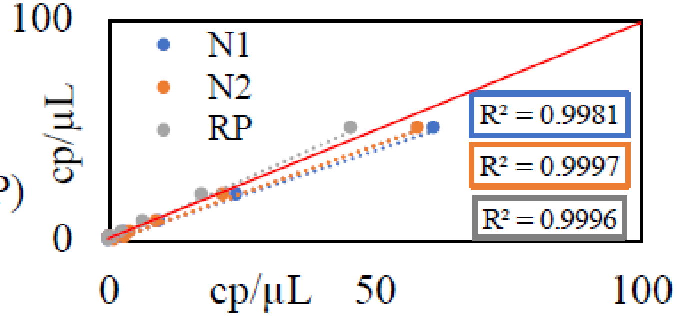
Objectives:

1. Geographic assessment of SARS-CoV-2 biomarkers in New Hampshire WWTFs
2. Temporal study of SARS-CoV-2 presence in selected WWTFs (ongoing)
3. Estimation of SARS-CoV-2 removal through conventional treatment processes (ongoing)

Methods:

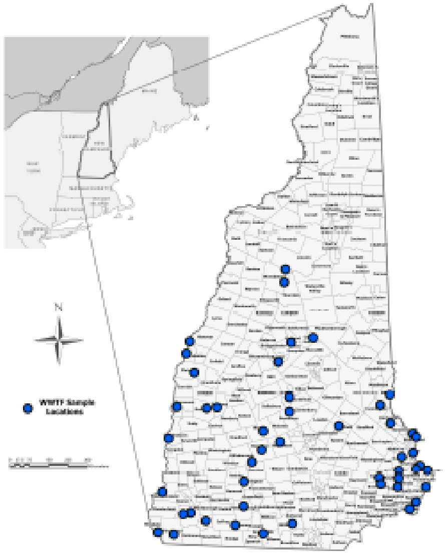
1. Solids removal
2. PEG precipitation
3. RNA extraction
4. ddPCR (N1, N2 and RP)

Linearity of Standard in WW



Detection Limits, Extraction Efficiency

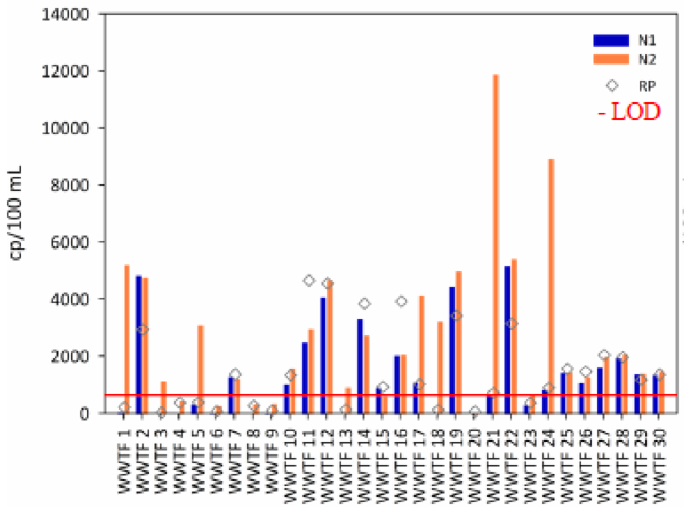
Parameter	Result
RNA Recovery	48.4 ± 3.9%
Limit of Detection	619.5 cp/µL



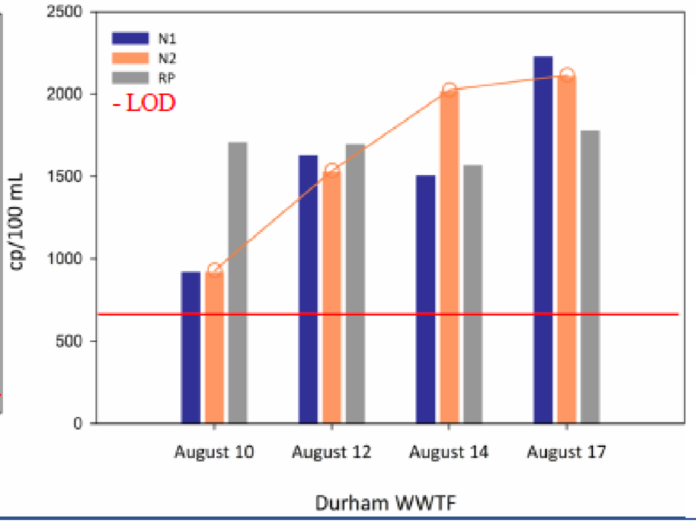
45 Sampling locations

Results:

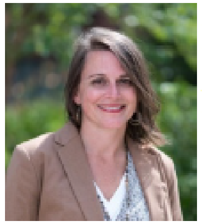
Objective 1



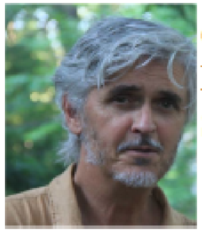
Objective 2



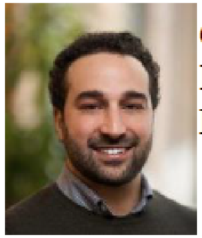
Project Team



PI: Dr. Paula Mouser
Associate Prof. CEE
Paula.Mouser@unh.edu

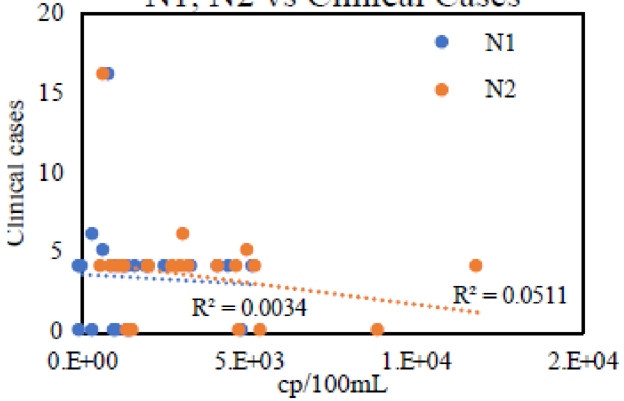


Co-PI: Dr Stephen Jones
Research Assoc. Prof. NRE
Stephen.Jones@unh.edu



Co-PI: Dr Fabrizio Colosimo
Post-Doc Researcher, CEE
Fabrizio.Colosimo@unh.edu

N1, N2 vs Clinical Cases



Practical Points:

1. Sample poles wedged in as guides
2. Toilet paper will always be a nightmare
3. Flow out from straight channel through manholes easiest to sample

University of Colorado Boulder

Cresten Mansfeldt cresten.mansfeldt@colorado.edu

COVID-19 Risk in Rural NC

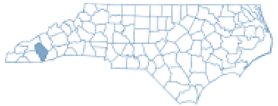
Results from a wastewater pilot study in a rural Western North Carolina town that is home to a major regional university.

Authors:

Aparna Keshaviah, Sc.M.
Cindy Hu, Sc.D.
Marisa Henry, M.S.

Jackson County, NC

- Population: ~43,000 residents + ~12,000 Western Carolina University (WCU) students
- Low but rising case load (~1% infected), few deaths



Wastewater as a Leading Indicator

- **Wastewater**: High viral loads on 7/28, then linear decrease
- **Confirmed cases**: Spike on 8/6, then sustained decrease
- **Proxy symptoms**: Spike on 8/5, then linear decrease

Wastewater Sampling

- 500 mL samples collected at one of three WWTPs (serving ~14,000 residents + WCU)
- 24-hour timed composites (once/hour) collected Mondays & Tuesdays (7/28 – 8/18)
- Samples very clear, with minimal solids – impacted control recovery?

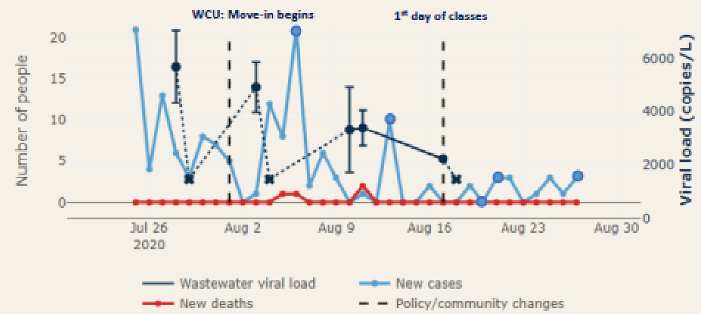
Wastewater Testing

- qPCR with LOQ = 2200 CN/L at McLellan lab (U of WI-Milwaukee);
- 0.10% - 2.19% recovery of internal control (BCoV)
- High correlation (Pearson r=0.81) between N1 and N2 values
- Mon vs. Tue levels varied by ~50% (3/4 samples >LOQ on Mon vs. 0/4 on Tue)

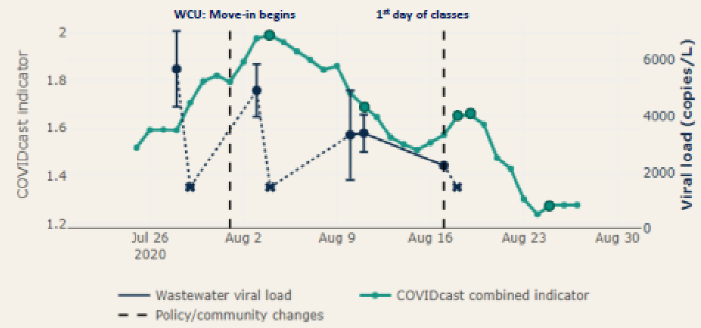
Other Data: NYT, U of WA, Delphi Research Group, COVID Tracking Project, NIEHS, CDC, RWJF, Google, Census

Results: Wastewater data could **provide a lead time of 8 to 9 days** for changes in SARS-CoV-2 levels.

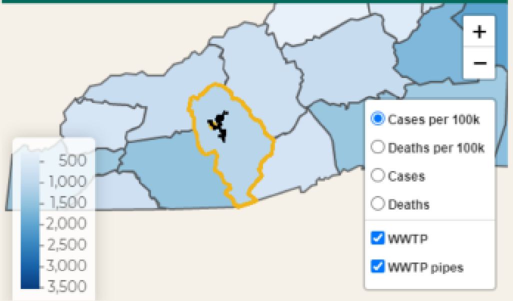
WASTEWATER VIRAL LOAD, CASES & DEATHS



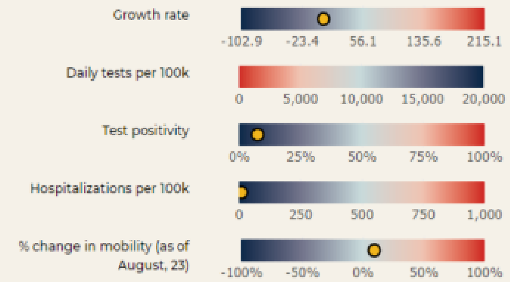
WASTEWATER VIRAL LOAD & COVIDCAST INDICATOR



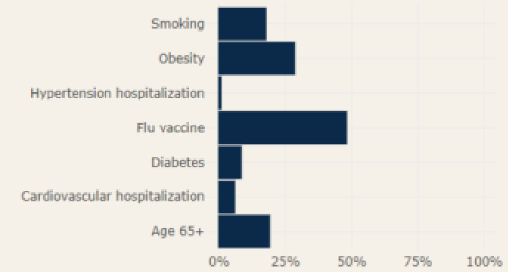
CASES & DEATHS



KEY INDICATORS



UNDERLYING CONDITIONS



10,532
Total tests (county-level)

476
Total cases (county-level)

7
Total deaths (county-level)

1.1
Estimated case underreporting factor (county-level)

0.91 - 1.35
Estimated R(t) (state-level)

0.51
Pandemic vulnerability index (county-level)

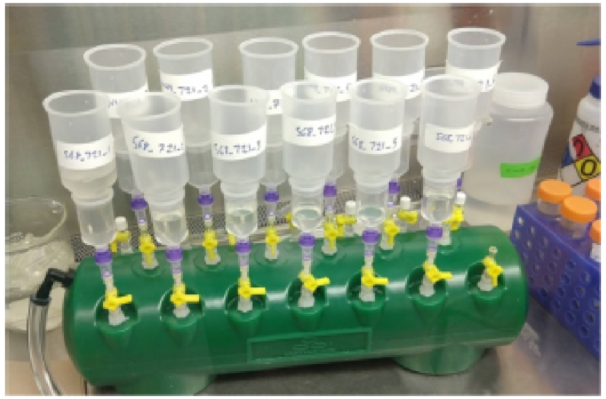
Mathematica

In collaboration with:
- Tuckaseigee Water and Sewer Authority
- Jackson County Department of Public Health
- University of Wisconsin-Milwaukee

WBE at the University of California, Berkeley

WBE Research:

- Direct extraction method
  - Salt lysis, heat pasteurization, and silica column concentration
  - WRF interlab comparison study
- Sequencing SARS-CoV-2 from wastewater
- Pilot projects: sampling upstream in sewersheds



The 4S method: dx.doi.org/10.17504/protocols.io.bi3dkgi6

Operationalizing WBE:

- COVID-WEB: SF Bay Area Regional Monitoring Program
  - Guided by working group
  - Data sharing platform
- High-throughput, pop-up testing lab underway
- UC Berkeley campus testing program

Covid-WEB
Wastewater Epidemiology for the Bay Area

Team: Prof. Kara Nelson (karanelson@berkeley.edu), Dr. Rose Kantor, Dr. Sasha Harris-Lovett, Prof. Jill Banfield, Matthew Metzger, Alex Crits-Christoph, Hannah Greenwald, Lauren Kennedy, Oscar Whitney, Adrian Hinkle, Vinson Fan, Melissa Thornton, Lauren Liao, Basem Al-Shayeb, Avi Flamholz, Anmol Seth, Joaquin Bradley Silva. **Funding:** Innovative Genomics Institute, Center for Information Technology Research in the Interest of Society, Private Foundation

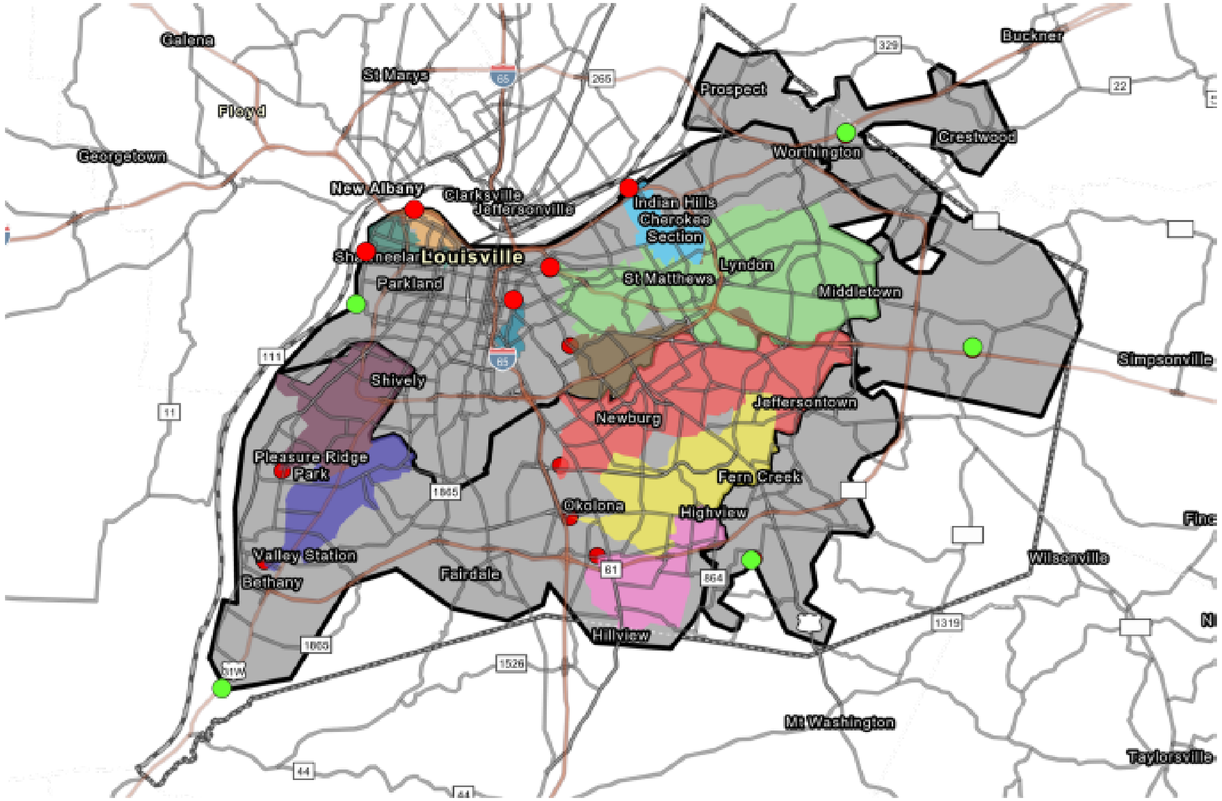
UNIVERSITY OF LOUISVILLE

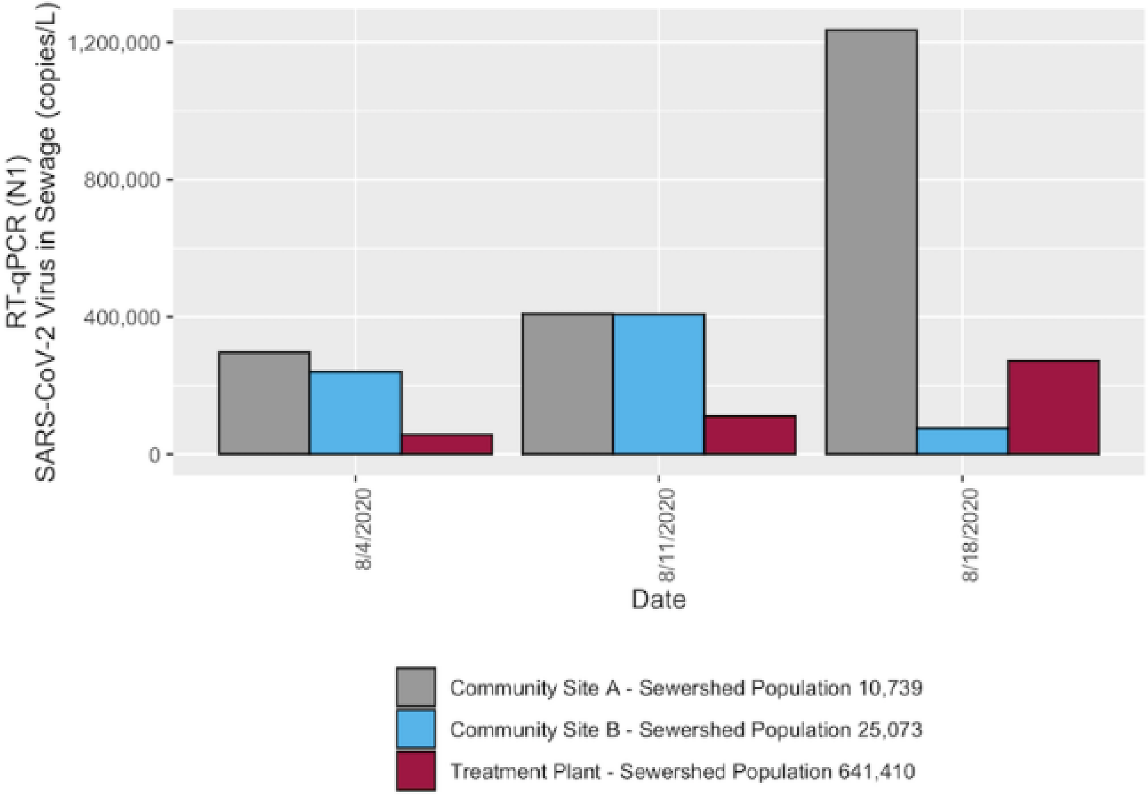
CHRISTINA LEE BROWN ENVIROME INSTITUTE

Community-level wastewater sample site selection for SARS-CoV-2

Ray A. Yeager, Rochelle H. Holm, Kumar Saurabh, Joshua L. Fuqua, Daymond Talley, Aruni Bhatnagar, Ted Smith*

*ted.smith@louisville.edu



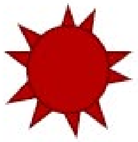


- Treatment plants lack the spatial resolution to reconstruct neighborhood-level clusters of infection
- Sampling sites along influent sewer lines from geographically and demographically distinct areas corresponding with participant locations in a concurrent nasal diagnostic clinical study.

Field collection for safe sampling and defensible data

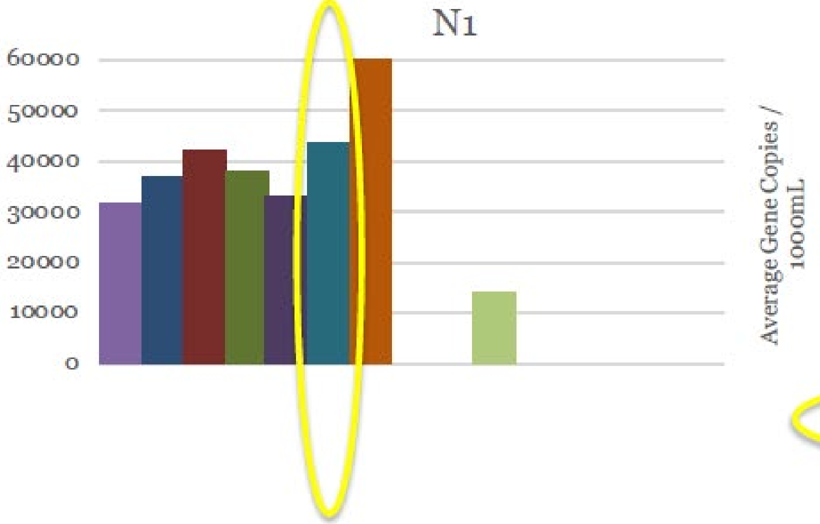
- Neighborhood scale epidemiological modeling and monitoring of SARS-CoV-2 requires consistent collection protocols that protect the field staff.
- SARS-CoV-2 requires greater attention to quality control samples.

Development of a Concentration Method for High Throughput of Samples to Detect SARS-CoV-2

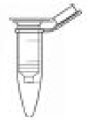


Filter comparisons

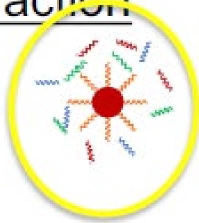
0.45μm vs 0.8μm
25mL vs 40mL
HA vs cellulose nitrate
no treatment
pH (3.5-4)
25mM $MgCl_2$
pH + $MgCl_2$
soaked in 25mM $MgCl_2$
PEI treated
PEI beads



RNA extraction



vs



qPCR

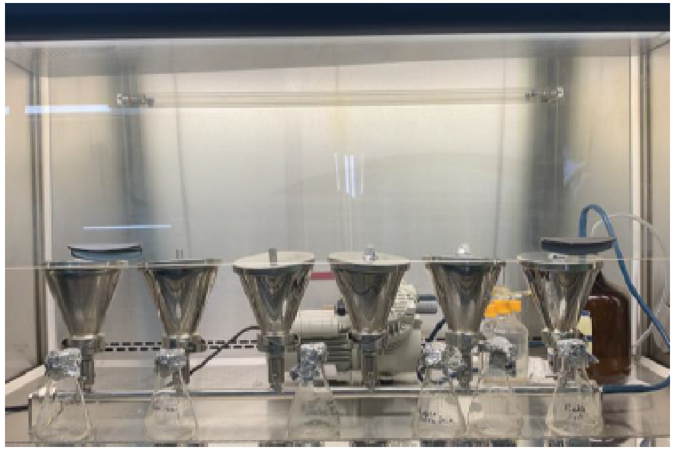
Multiplex homemade kit
vs
Manufactured kit

Kayley H. Janssen, Dagmara S. Antkiewicz, Paige Mullins, Rebecca Fahney, and Martin M. Shafer
Wisconsin State Laboratory of Hygiene

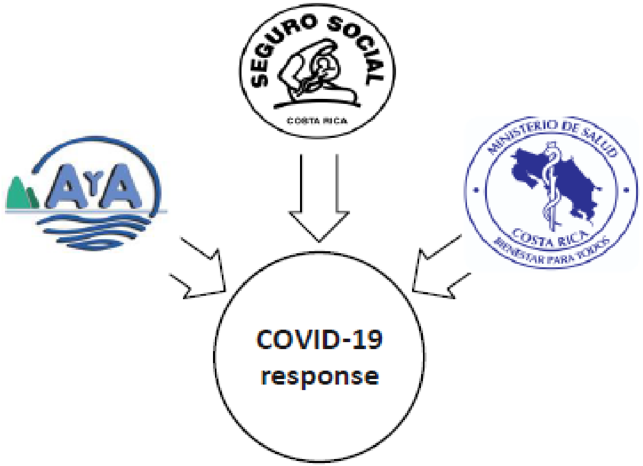
A case study of SARS-CoV-2 wastewater surveillance in areas with minimal centralized sanitation infrastructure from a government laboratory's perspective in Costa Rica

A. Badilla A., P. Rivera N., E.M. Symonds., E. Alfaro A., I. Vega G., J. Orozco G., D. Mora A.

Lack of centralized sanitation systems → **creative** sampling solutions



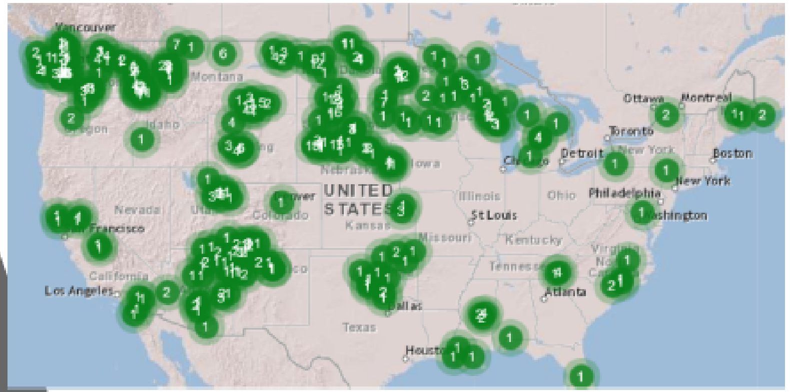
WW samples, Moore swabs, adsorption-elution & RT-qPCR (N1, N2, N3 assays)



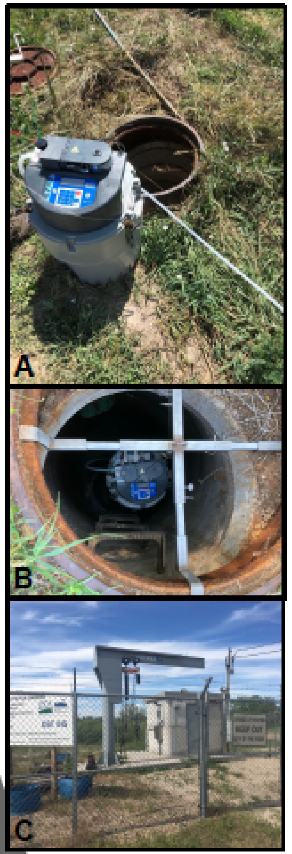
Inter-institutional collaboration is key

Coronavirus in Tribal Communities

Otakuye Conroy-Ben, Erin Driver, Rebecca Muenich, Marcus Denetdale, Rolf Halden
Sustainable Engineering and the Built Environment
The Biodesign Institute
Arizona State University, Tempe, AZ



- Tribal wastewater infrastructure is unique
  - 574 Federally recognized Tribes
  - > 300 with a land base
  - 95 Tribes with NPDES permits (FRS, ICIS)
  - 225 – 250 MGD of sewer flow in Indian Country
  - ~ 2.8×10^6 individuals



- Objectives
  - Determine Tribal communities that can utilize WBE
  - Quantify SARS-CoV-2 in Tribal wastewater
    - Influent and effluent
  - Develop training materials for Tribal wastewater operators and health administrators

- Tribal Partnerships
  - Approvals: IHS, Tribal Research Board, Tribal Water Division, ASU Tribal Research Review

Collaborators: InterTribal Council of Arizona
Funding: NSF Award #2038372 ,The Catena Foundation
Contact: Otakuye.Conroy@asu.edu

IRA A. FULTON SCHOOLS OF engineering
ARIZONA STATE UNIVERSITY

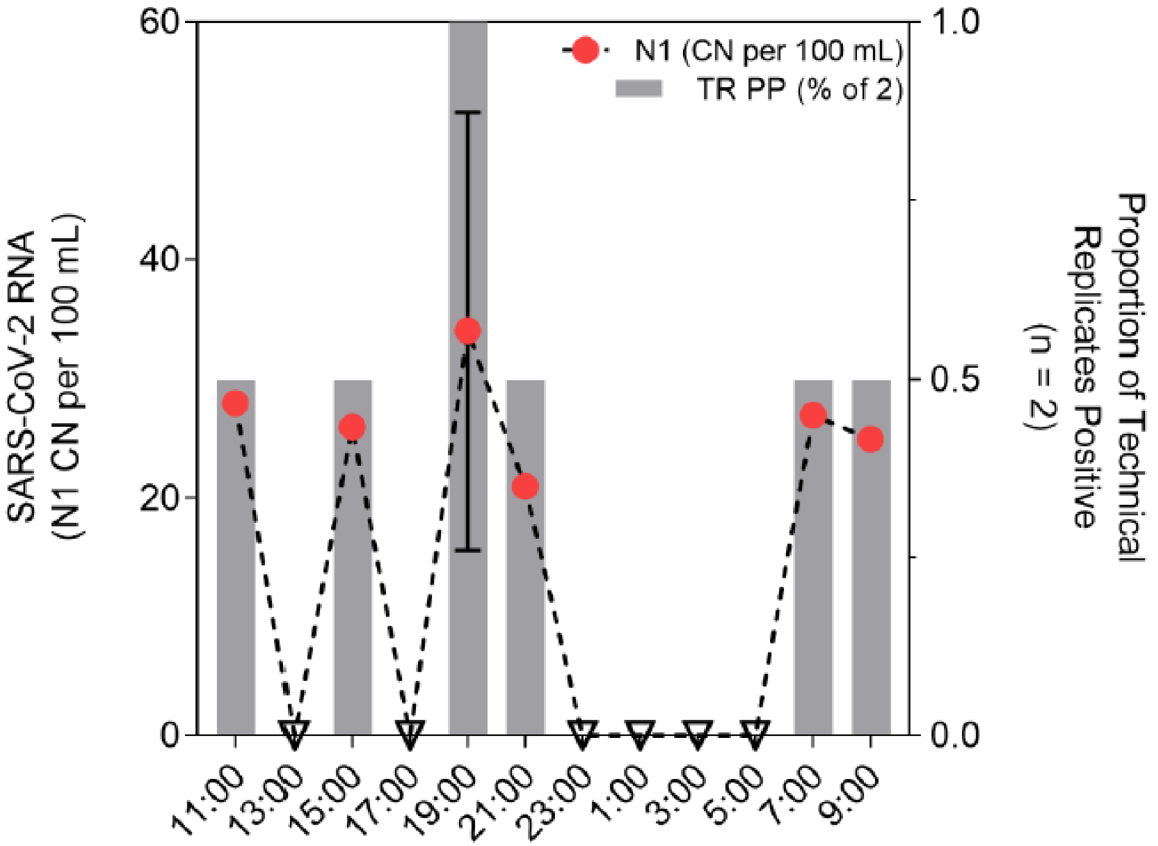
UNIVERSITY OF NOTRE DAME | COLLEGE OF ENGINEERING

Aaron Bivins, PhD, PE
Postdoctoral Research Fellow
GERM Lab

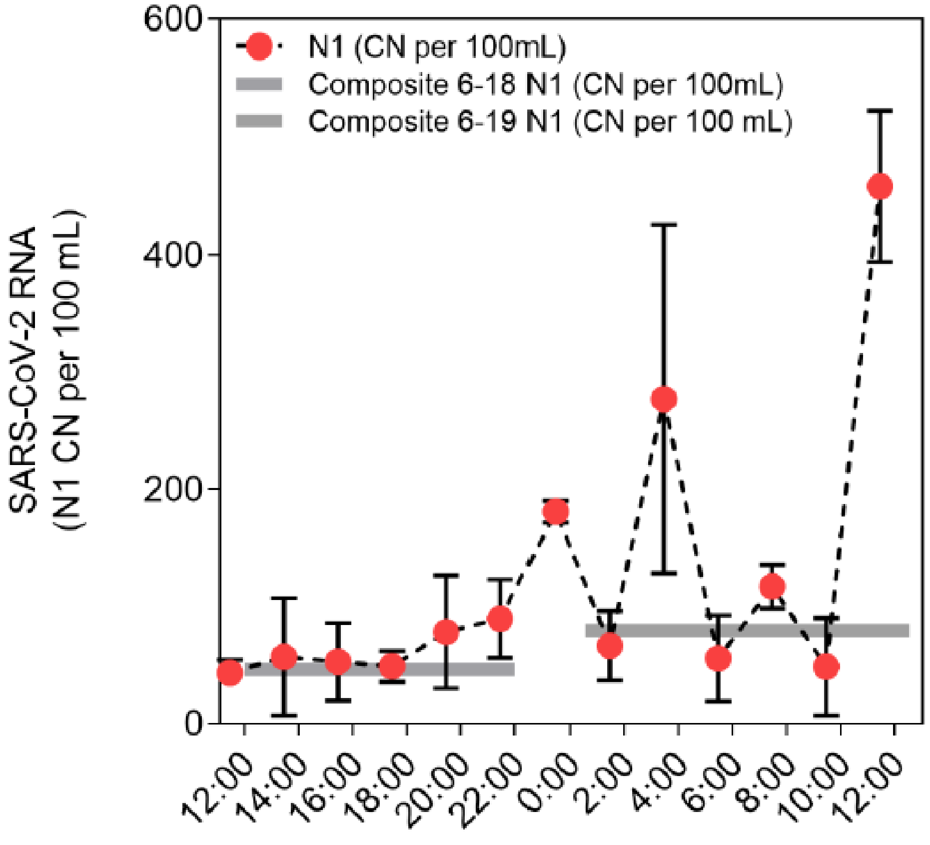
Primary Influent 2-hour Serial Sampling

Funding by NSF CBNET 2027752

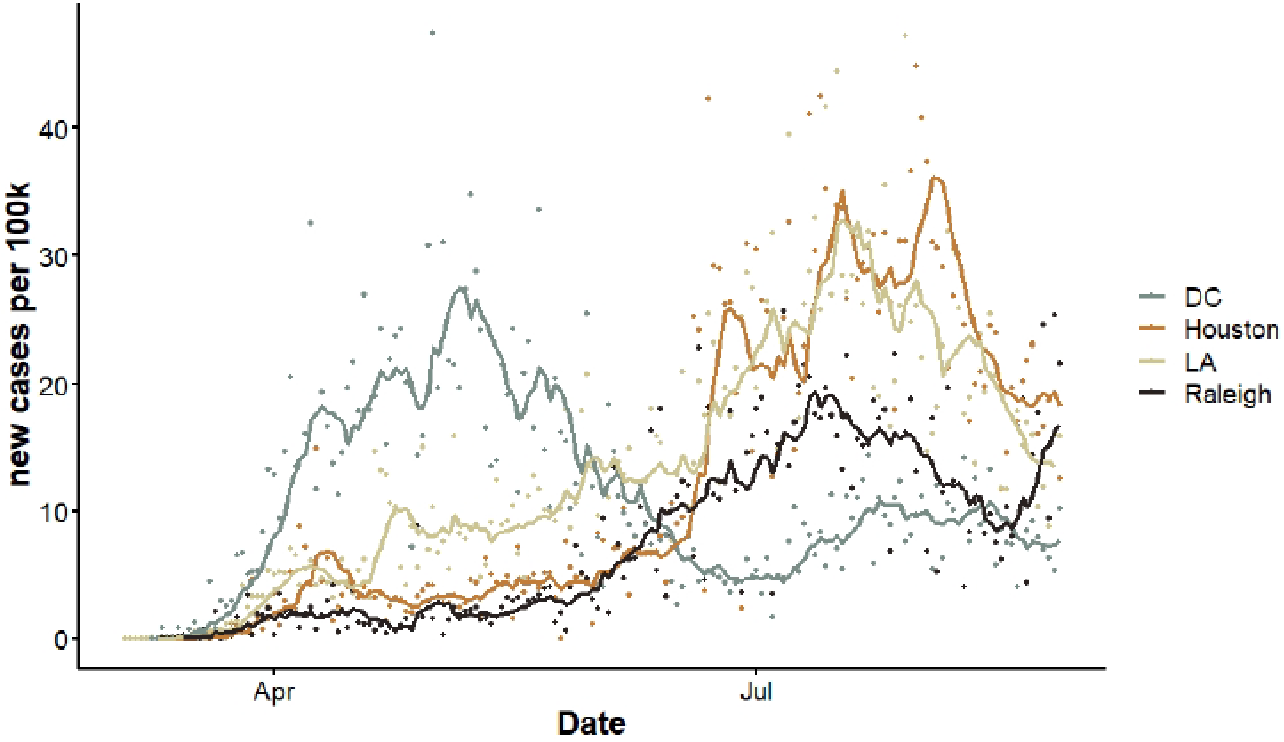
Community A
7-day moving average: 18 – 21 cases



Community B
7-day moving average: 50 – 78 cases



We are collaborating nationally to develop relationships between wastewater and public health data.



DC, Raleigh, Houston, and Los Angeles
- Varying types of sewage systems, degree of centralization, and public health data

Surveying over 45 treatment plants in these metropolitan areas ranging in populations served between 2700 residents to 4,000,000 residents.

Total population we are surveying is over 15M

Jeseth Delgado Vela
Jeseth.delgadovela@howard.edu

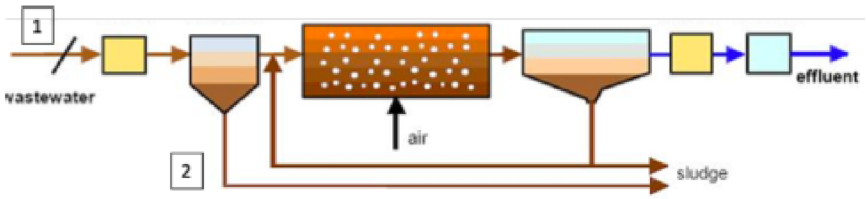
Adam Smith

Nadine Kotlarz
Angela Harris
Francis de los Reyes

Lauren Stadler

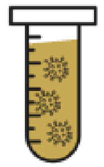
SCAN: Sewer Coronavirus Alert Network

Sample Collection

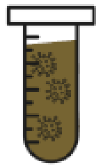


- 49 treatment plants
- Paired influent and primary settled solids
- Focus on 2 plants for methods

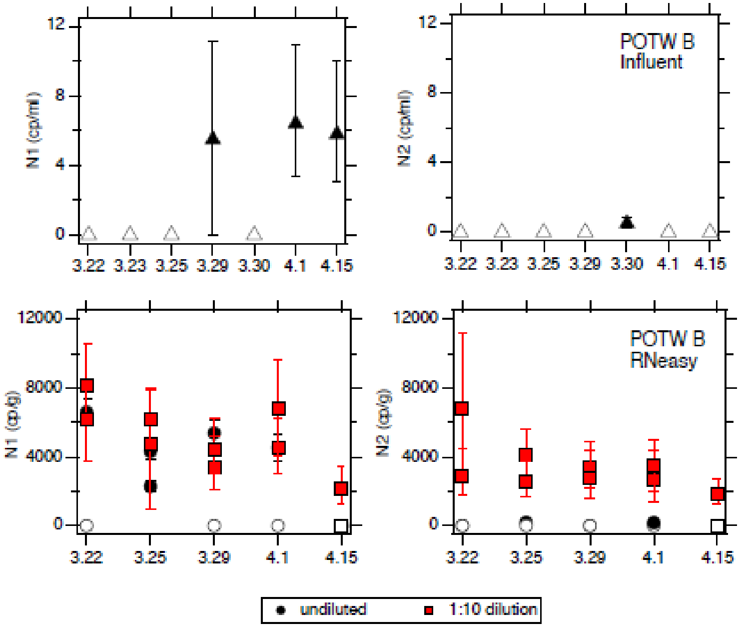
Methods Development



Influent:
PEG precipitation

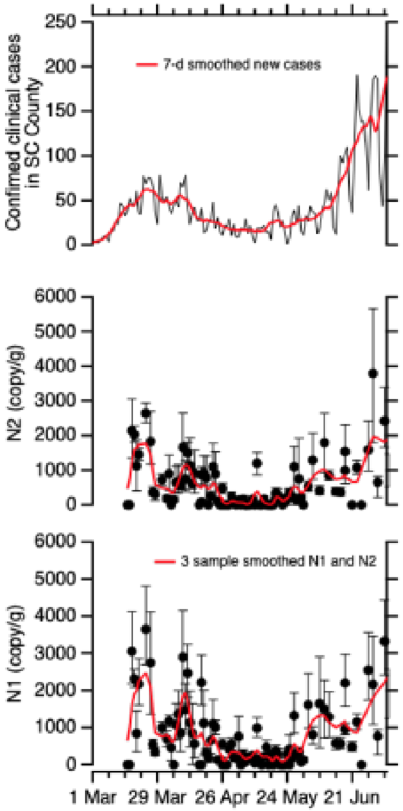


Solids:
Direct extraction



- Many viruses have affinity for solids
- More consistent **detection** in solids
- **Higher concentration** in solids
- **ddPCR** outperformed qPCR

Longitudinal Analysis



- Longitudinal analysis of San Jose solids
- SARS-CoV-2 N1 and N2 genes track clinical cases
- **Cases correlated with viral RNA** with 3-4 day lead time

Next Step: Modeling

Stanford University

University of Michigan

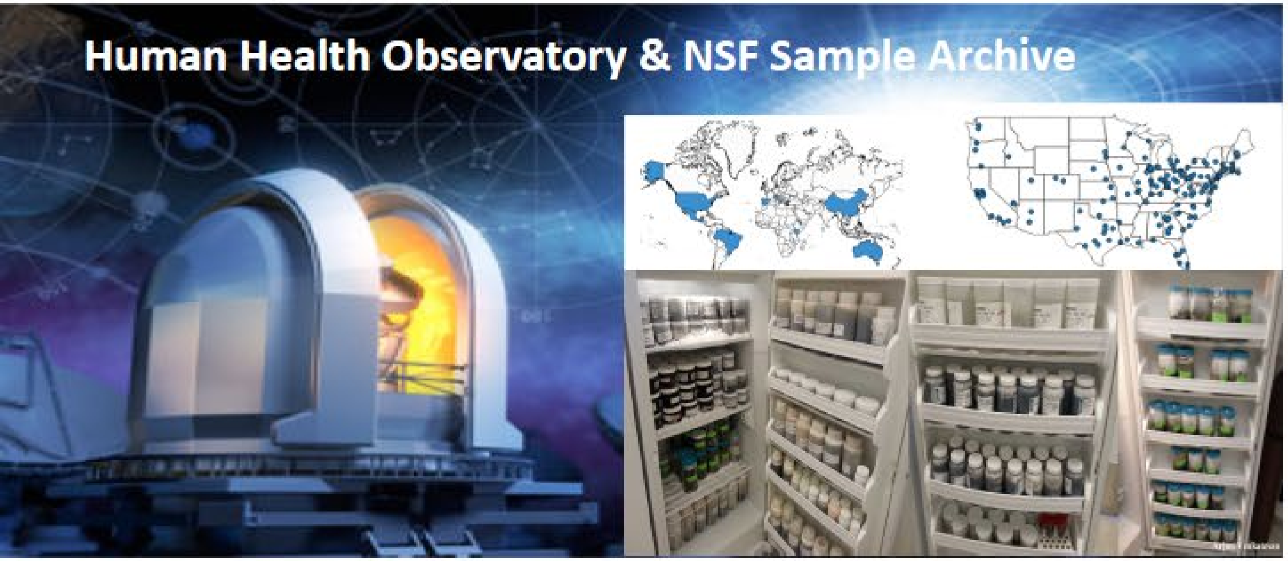
PIs: Alexandria Boehm, Krista Wigginton
Study Team: Katy Graham, Stephanie Loeb, Marlene Wolfe, Nasa Sinnot-Armstrong, Lorelay Mendoza, Laura Roldan, Suzy Kim, Kevan Yamahara, Lauren Sassoubre, David Catoe

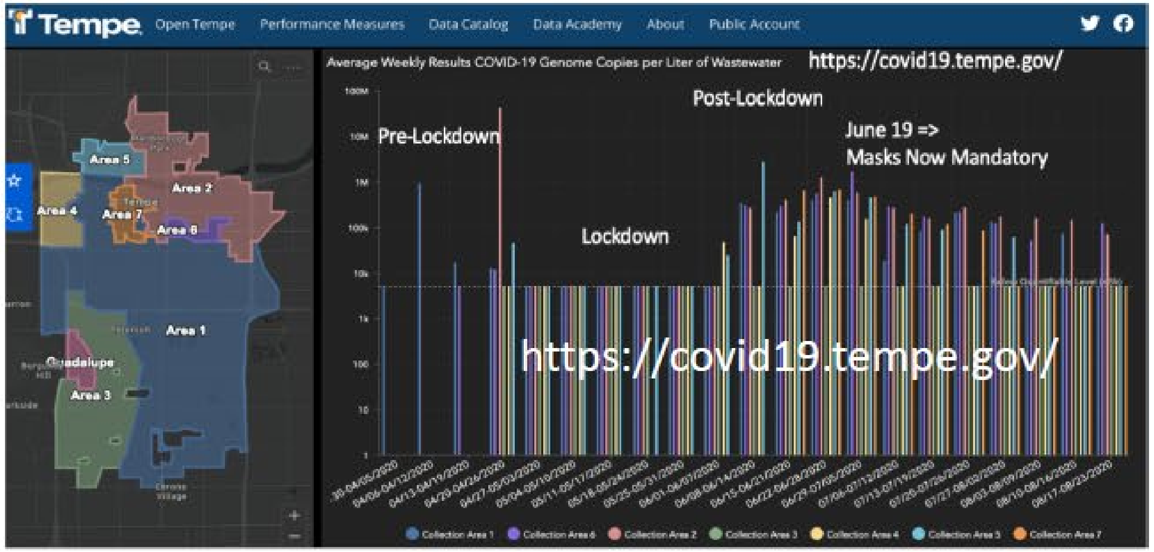
Contact: marlene.wolfe@stanford.edu
aboehm@stanford.edu
kwigg@umich.edu

RCN Resources

Rolf Halden, Co-PI - ASU

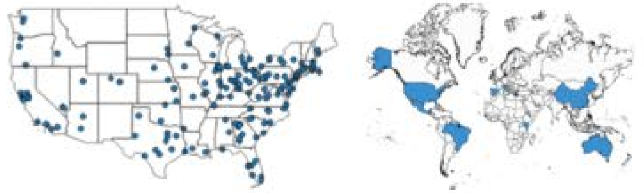
NSF 2038087, 2028564; NIH 1R01LM013129





Tracking Infectious Diseases with ASU's HHO

RH (Presenter) | Matthew Scotch | Arvind Varsani



Human Health Observatory (HHO) at Arizona State University's Biodesign Institute

2019: US Nationwide Infectious Disease Monitoring Network

Surveillance of known disease agents & discovery of 1000s of new DNA & RNA viruses

NATURE MEDICINE | VOL 24 | OCTOBER 2018 | 1484–1490 | www.nature.com/naturemedicine

The Importance of Concentration Factor in SARS-CoV-2 Wastewater Epidemiology Methods

A. Page, D. Alburty, D. Goad, InnovaPrep, Drexel Missouri

Concentration Factor is a single factor that provides a direct comparison of sample preparation/concentration methods and their impact on method detection limit – taking into account:

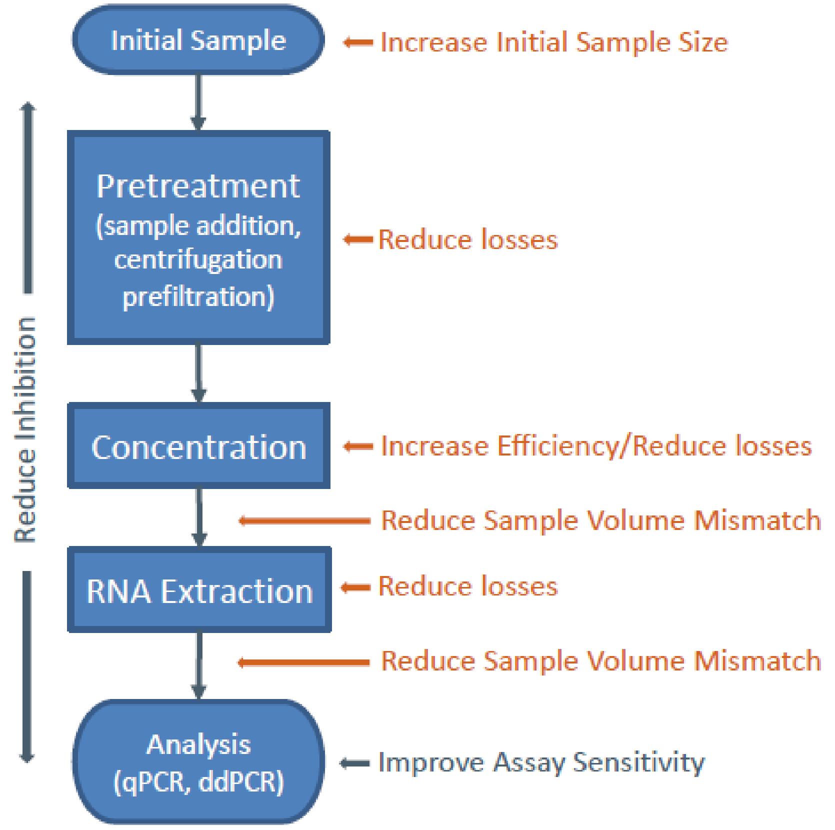
- Initial Sample Volume
- Efficiency of each step
- Volume mismatch between steps

CF = Concentration Factor
$V_{Initial}$ = Volume of Initial Sample (mL)
V_{Final} = Volume of Final Sample (mL)
E_{Method} = Combined Efficiency of Entire Method (%)*
**includes losses due to volume mismatch between steps*

$$CF = \frac{V_{Initial}}{V_{Final}} x E_{Method}$$

CF = Concentration Factor
T_{Final} = Titer of the Final Sample (CFU/mL)
$T_{Initial}$ = Titer of the Initial Sample (CFU/mL)

$$CF = \frac{T_{Final}}{T_{Initial}}$$



Takeaway: A 50% reduction in initial volume, 50% efficiency or a loss of 50% of sample due to volume mismatch between steps all impact CF and ultimately the method detection limit equally.

INNOVAPREP

Andy Page, apage@innovaprep.com, InnovaPrep LLC, www.innovaprep.com

Communicating Sewage Surveillance (CoSeS)

Interactive group of researchers working with public health and communication scholars to build capacity and communication networks for sewage surveillance

- Funded by the Alfred P. Sloan Foundation

Objective 1: Work with cites to implement wastewater surveillance

Objective 2: Draw upon national expertise to create a communication strategy that is responsive to public health

Investigators: McLellan, Silverman, Boehm, Bibby, Brossard
Research Expertise: Lipp, Noble, Gerrity, Griffith, de los Reyes, Holden, Wigginton

Public Health and Wastewater Expertise
Wisconsin Dept Health Services, Utah Depart of Health, California Association of Sanitation Agencies, NYC DEP, CDC

CoSeS
Communicating Sewage Surveillance

home / about / panel recommendations / network and resources / news and upcoming events

HOME

Communicating Sewage Surveillance (CoSeS)

5-Minute Lightening Talks

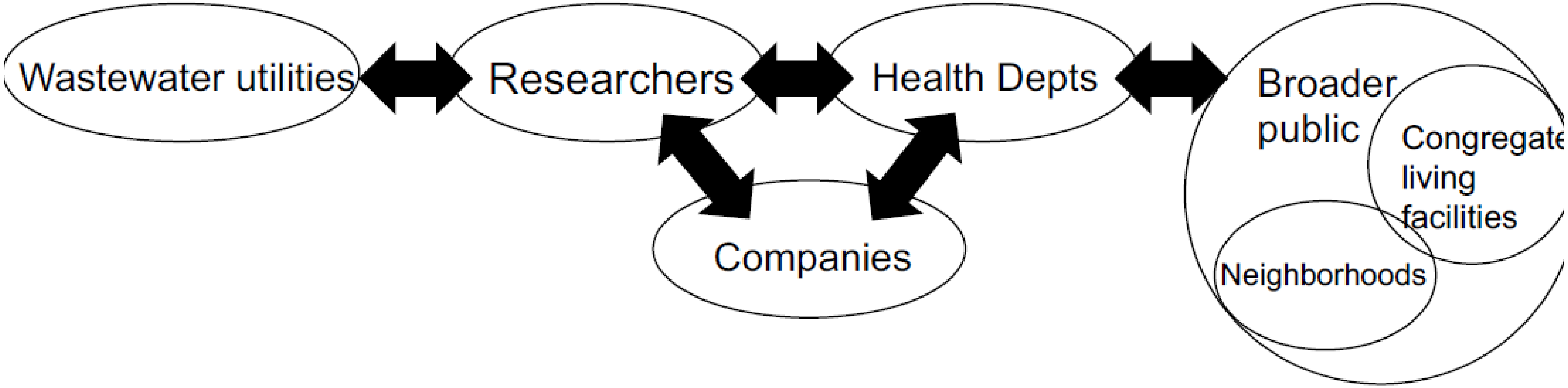
NC STATE UNIVERSITY
Center for Human Health and the Environment

Engaging stakeholders in SARS-CoV-2 wastewater surveillance research to maximize public health benefit

Nadine Kotlarz, Angela Harris, Jane Hoppin,
Helena Mitasova, Francis de los Reyes

RAPID # 2029025

Stakeholder engagement matters



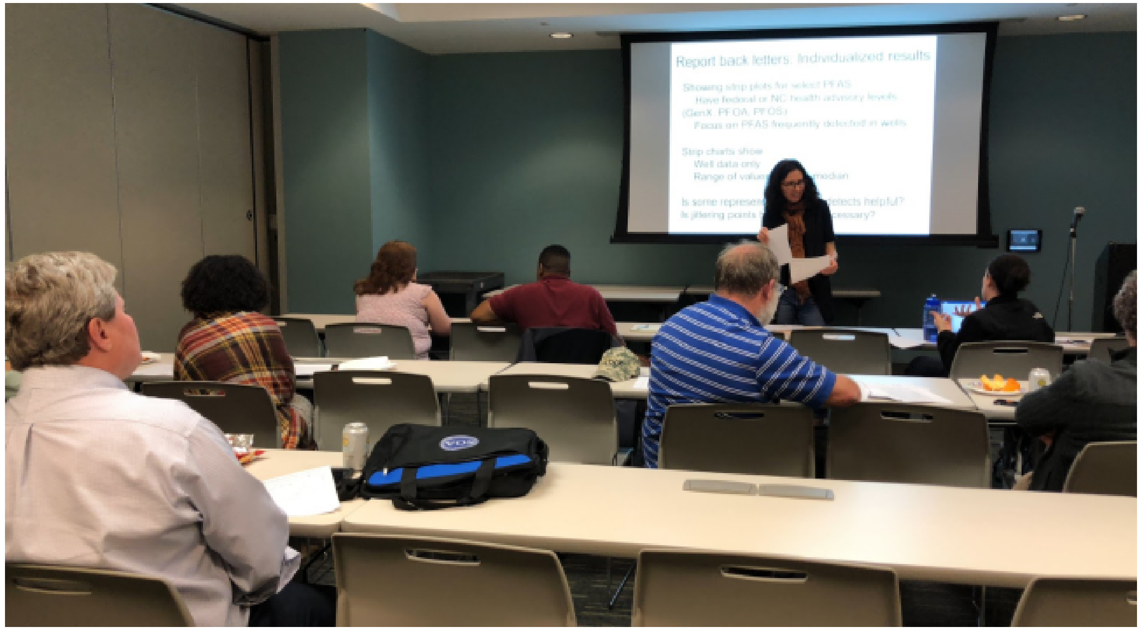
What is Community (or Stakeholder) Engaged Research?

Embraced by Environmental Health researchers but slower to be adopted by Environmental Engineers

Without an engaged approach, we risk:

1) not realizing full public health benefit
2) repeating mistakes

Lessons learned from our GenX Exposure Study



GenX Exposure Study

https://genxstudy.ncsu.edu/

1. Build a community science advisory panel
2. Discuss uncertainty
3. Return results to stakeholders first (before publication)

Takeaways so far

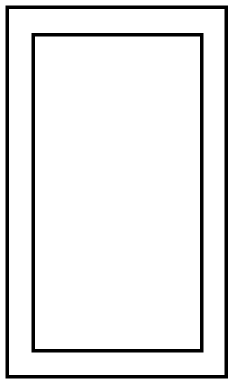
Common questions

Is this scientific research or will there be application?

If everyone is doing this, why are the methods still being worked out?

How much increase/decrease warrants action?

Framing is key



Wastewater is a complementary tool

Relating to healthcare surveillance approaches (e.g., cases, hospitalizations) critical for adoption

Engage stakeholders to *integrate* wastewater surveillance with healthcare surveillance efforts to meet local needs

Spatio-temporal monitoring of SARS-CoV-2 in Houston's Wastewater

LaTurner, Zachary W. [a]**;** Raja, Pavan M. V. [a]; Kalvapalle, Prashant[a]; Crosby, Tessa[a]; Ali, Priyanka[a]; Hollstein, Marielle[a]; Zong, David[a]; Lou, Esther[a]; Senehi, Naomi[a]; Bedient, Phil; Fang, Nick; Hopkins, Loren; Ensor, Kathy; Maresso, Anthony; **Stadler, Lauren B.** [a]

[a]Department of Civil and Environmental Engineering University, Rice University
Stadler Group

zach.laturner@gmail.com, lauren.stadler@rice.edu

HOUSTON HEALTH DEPARTMENT

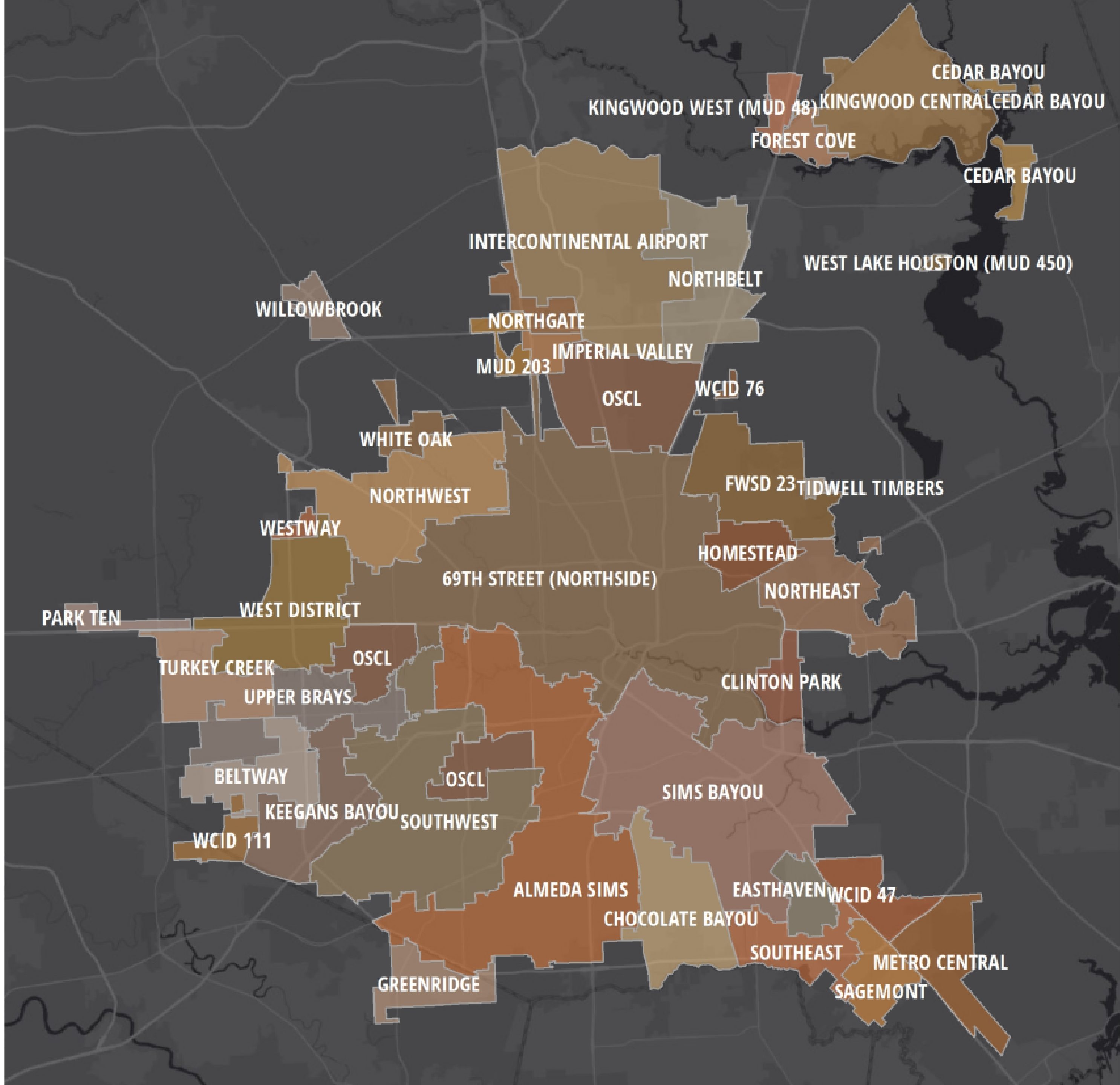
HOUSTON PUBLIC WORKS

tailφr Tailored Antibacterials and Innovative Laboratories for phage (Φ) Research

Baylor College of Medicine

RICE Unconventional Wisdom

NSF RAPID #2029015



Surveillance Results

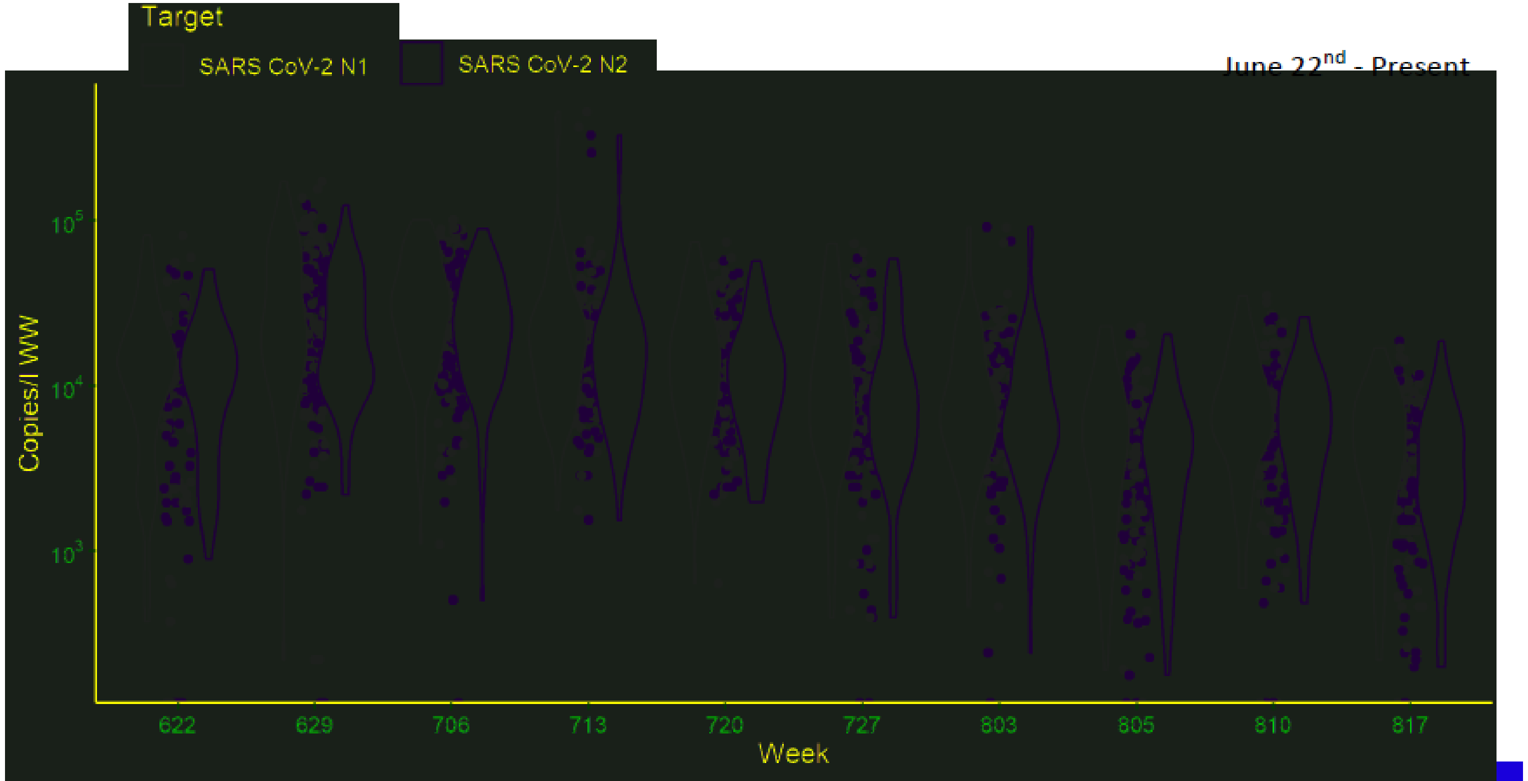
Processing Methods	
Sampling:	Weekly, 24 Hour Composite
Concentration:	Electronegative Adsorption
Extraction:	AllPrep PowerViral DNA/RNA
Quantification:	N1/N2 – ddPCR, BCoV - qPCR

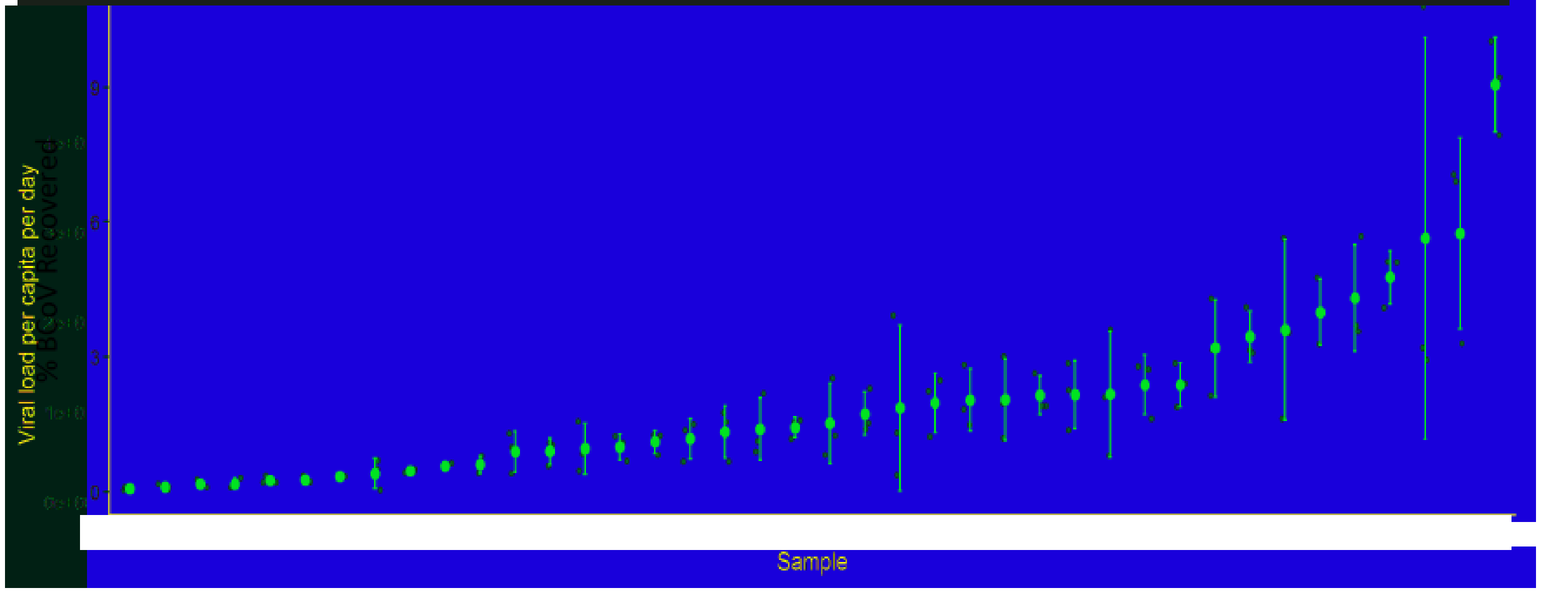
City of Houston uses data for:

- Trend tracking
- Identification of hotspots
- Supplement clinical testing data

Measuring recovery with BCoV:

- Variable recovery across WWTP
- Variable recovery across weeks at same WWTP
- Identifying wastewater parameters that effect recovery





Decay in Wastewater

Conditions for preliminary decay rate results:

- Aerobic, room temperature, single WWTP

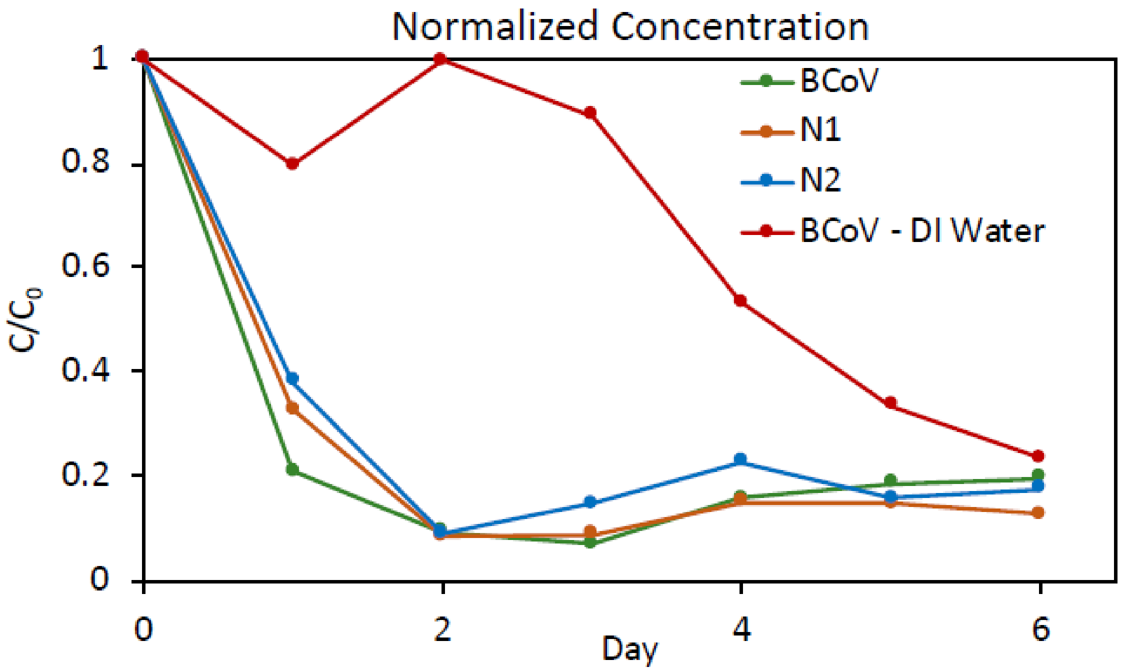
In progress:

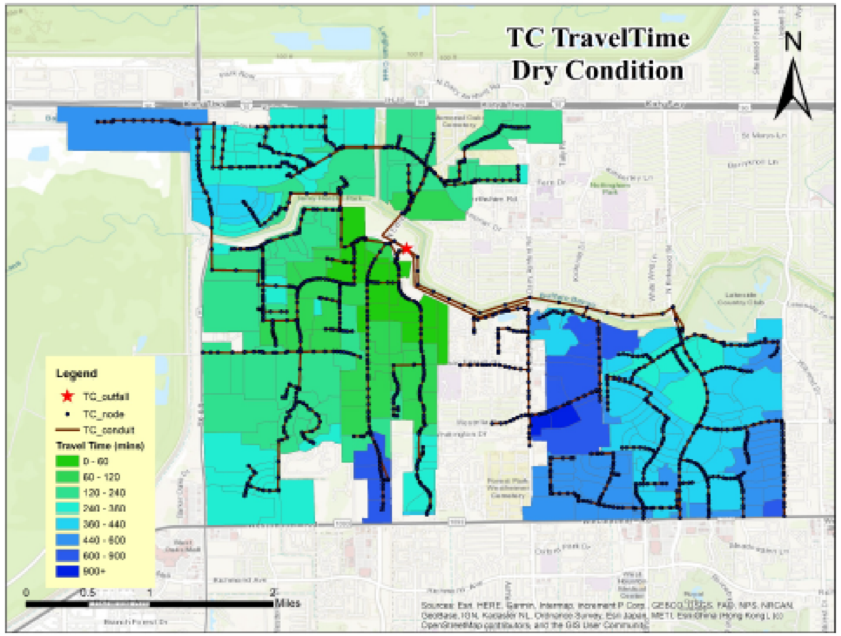
- Anaerobic
- 4 °C (Storage), 37 °C
- Multiple WWTP

Half Life		
BCoV	12	hrs
N1	16	hrs
N2	20	hrs

Travel time is not constant throughout single catchment system

- ~0 – 15 hrs
- Wet weather conditions change travel time
- Locations closer to WWTP give a stronger signal than locations further away





Acknowledgements

HOUSTON HEALTH DEPARTMENT

Loren Hopkins, Ph.D.
Lilian Mojica
Braulio Garcia

HOUSTON PUBLIC WORKS

Paul Zappi, P.E.
Carol LaBreche, P.E.
Rae Mills, P.E.

tailΦr
Tailored Antibacterials and Innovative Laboratories for phage (Φ) Research

Baylor College of Medicine

Anthony Maresso, Ph.D.
Austen Terwilliger, Ph.D.
Justin Clark, Ph.D.
Haroldo de Santos

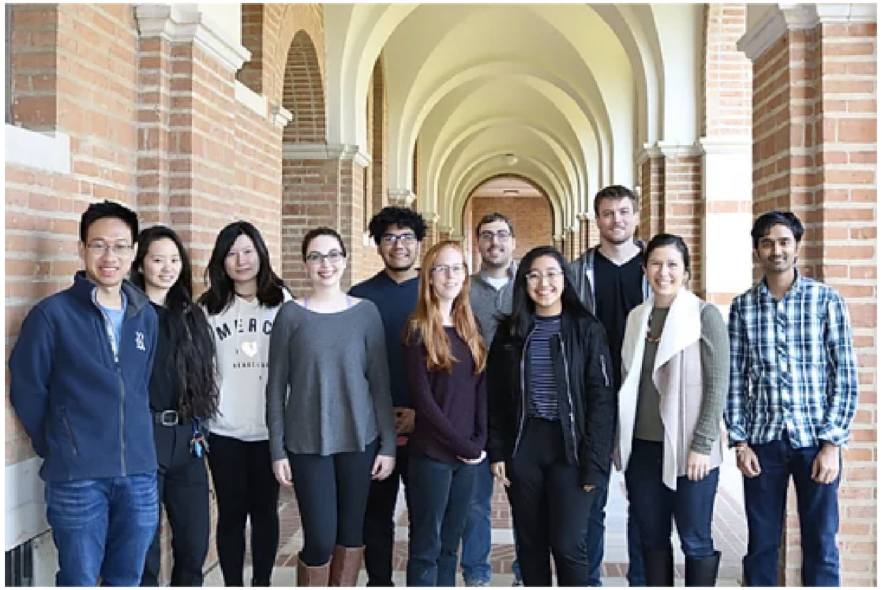
RICE
Unconventional Wisdom

Kathy Ensor, Ph.D.
Phil Bedient, Ph.D.
Nick Fang, Ph.D.

NSF RAPID
#2029025

Francis de los Reyes, Ph.D.
Nadine Kotlarz, Ph.D.
Jeseth Delgado Vela, Ph.D.
Adam Smith, Ph.D.
Angela Harris, Ph.D.

Stadler Group
Lauren Stadler Ph.D. (Advisor)
Priyanka Ali
Tessa Crosby
Kiara Reyes Gamas (Graphics)
Marielle Hollstein
Prashant Kalvapalle
Zach LaTurner
Esther Lou
Pavan Raja, Ph.D.
Naomi Senehi
David Zong, Ph.D.



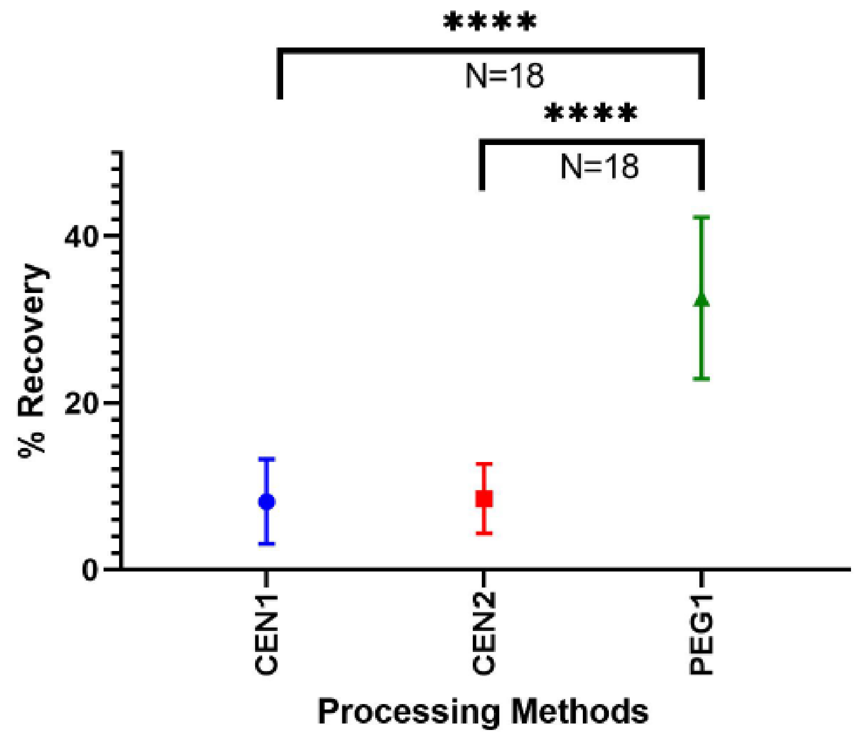
Wastewater-based Surveillance of COVID-19:Addressing Community Health in Michigan

Michigan State University Team: Matthew Flood, Nishita Dsouza, Rebecca Ives, Joan B. Rose, Amber Pearson, MSU's Infrastructure Planning and Facilities (IPF)

Our Goals:

1. *To assess and monitor the prevalence and transmission of the SARS-CoV-2 virus in Michigan using wastewater-based epidemiology as a tool to provide "early warnings" for communities.*
2. *To determine which processing/concentration method is most effective for wastewater samples from Michigan*

Figure 1. Phi6 Percent Recovery based on ddPCR



Michigan Communities being Served:

- Pontiac, Oakland County (2 WWTPs) – April 6, 2020
  - Population 59,515
- Genesee County (1 WWTP) – April 15, 2020
  - Population 425,790
- Marquette (1 WWTP) – April 29, 2020
  - Population 21,355
- MSU Campus (Sanitary Sewers) – May 11, 2020 (including E. Lansing and Meridian Townships populations 47,988 and 43,318)
- Warren (1 WWTP) – July 27, 2020
  - Population 136,961
- Manistee, MI (Sanitary Sewers) – August 8, 2020
  - Little River Band of Ottawa Indians
  - Population 8,156
- Macomb County, MI (Sanitary Sewers) – August 8, 2020
  - Population 100,800

Figure 1. Marquette SARS-CoV-2 N1, N2, and E Gene Results by Date

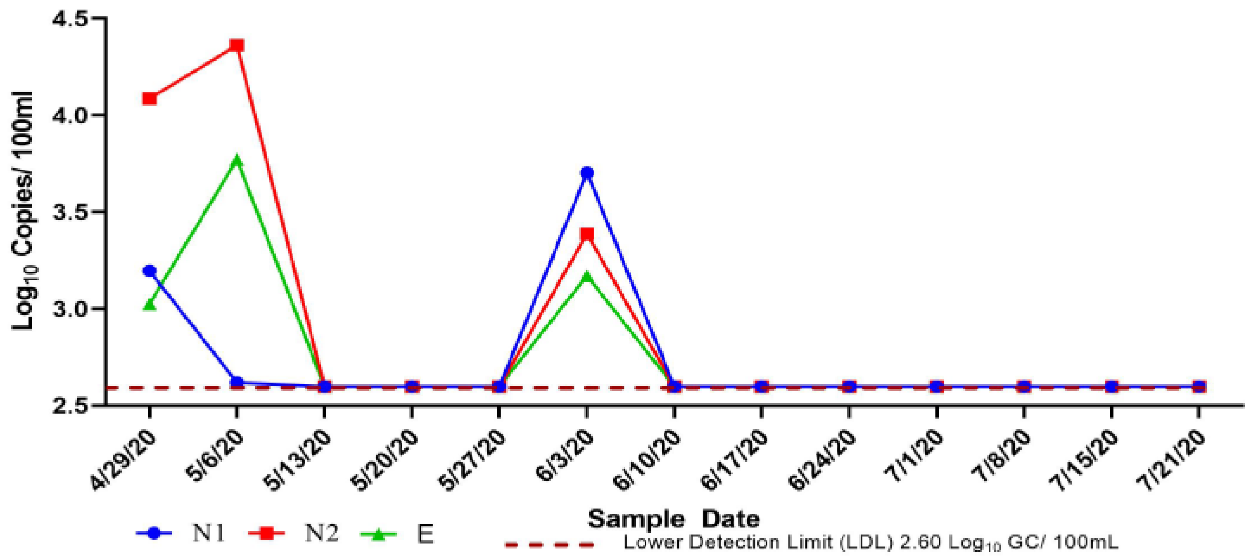
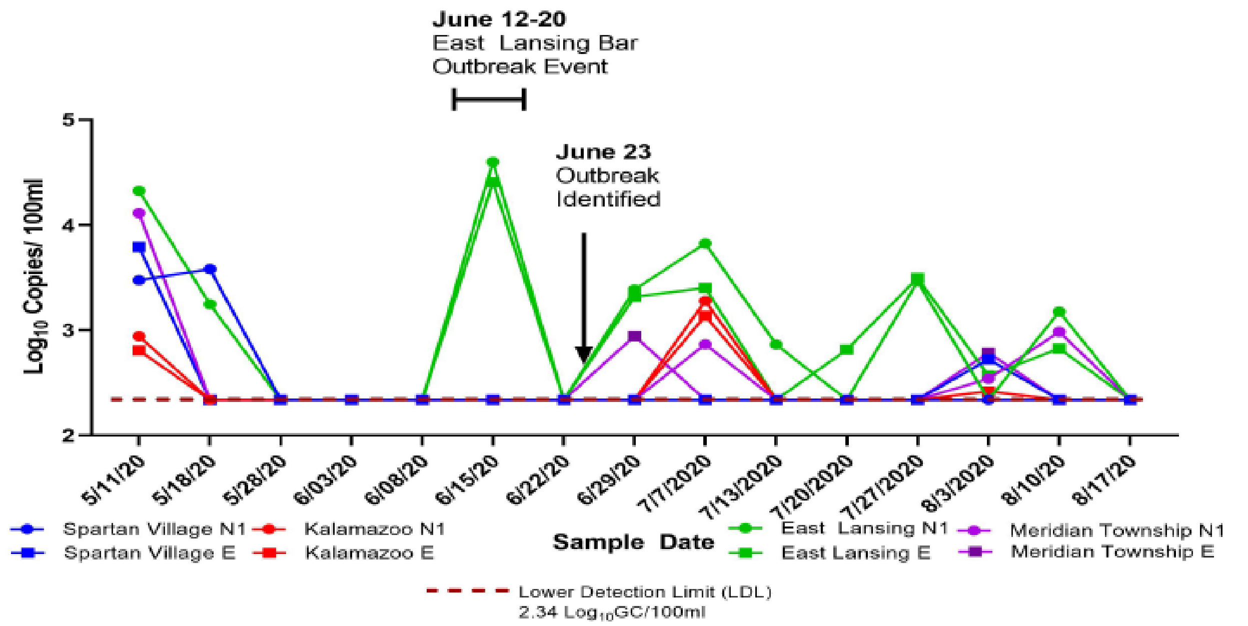


Figure 1. MSU Sanitary Sewer SARS-CoV-2 N1 and E Gene Results



Ongoing Efforts and Next Steps

- Expanding on Campus (MSU) testing to include dormitories (September 2020)
- Working with the state (EGLE) to set up a Pilot study and train a statewide consortium of labs to test for SARS-CoV-2 in wastewater (Academic, Health, and Utilities collaborating) (October 1, 2020)
- Helping to create a state dashboard for SARS-CoV-2 reporting (September 2020)

Initial Prototype Results Dashboard for State

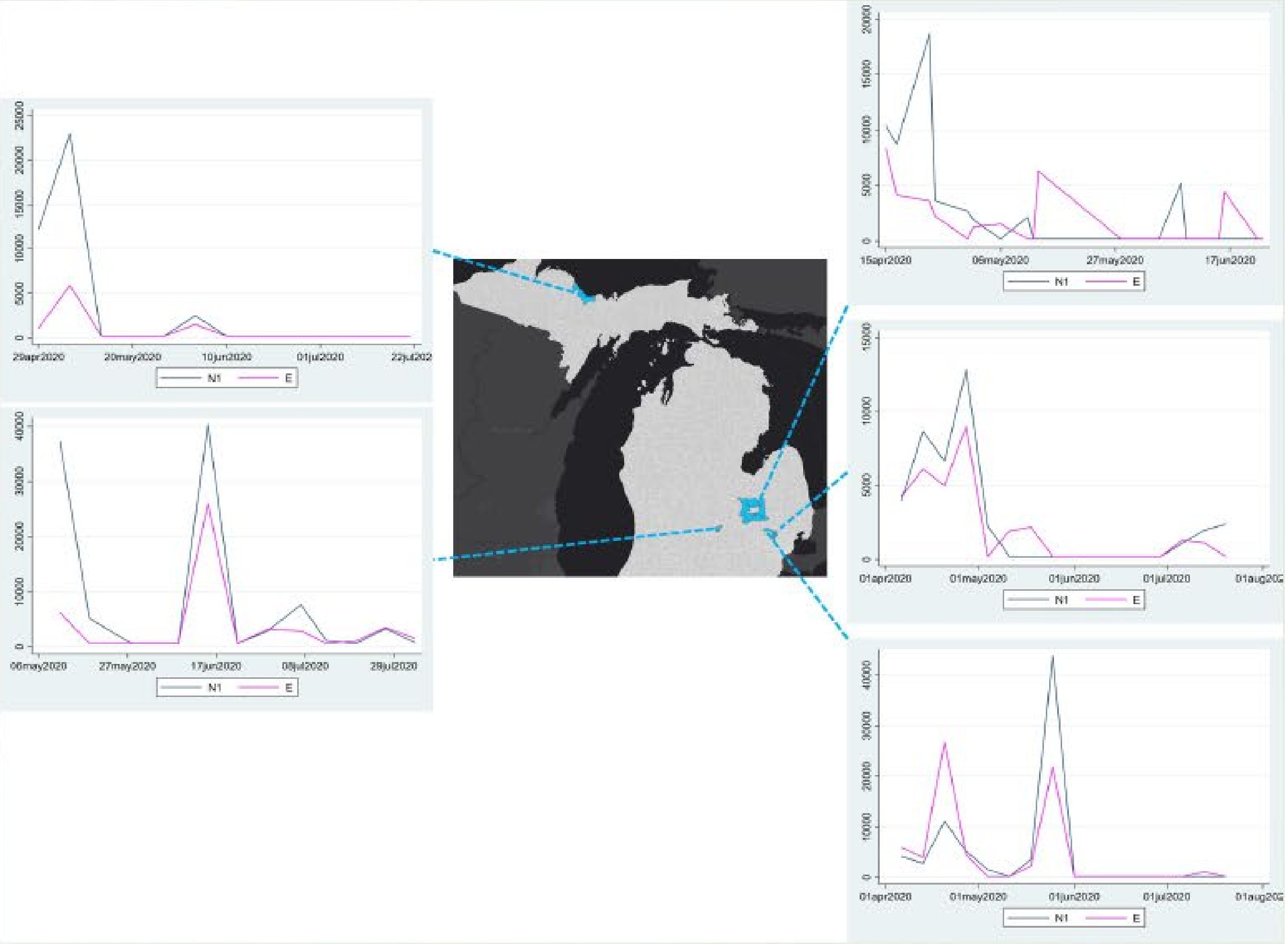


Image courtesy of: Amber Pearson

Micro-sewershed surveillance paired with random household sampling during a COVID-19 outbreak in Newport, Oregon

NSF RCN Kick-off Meeting

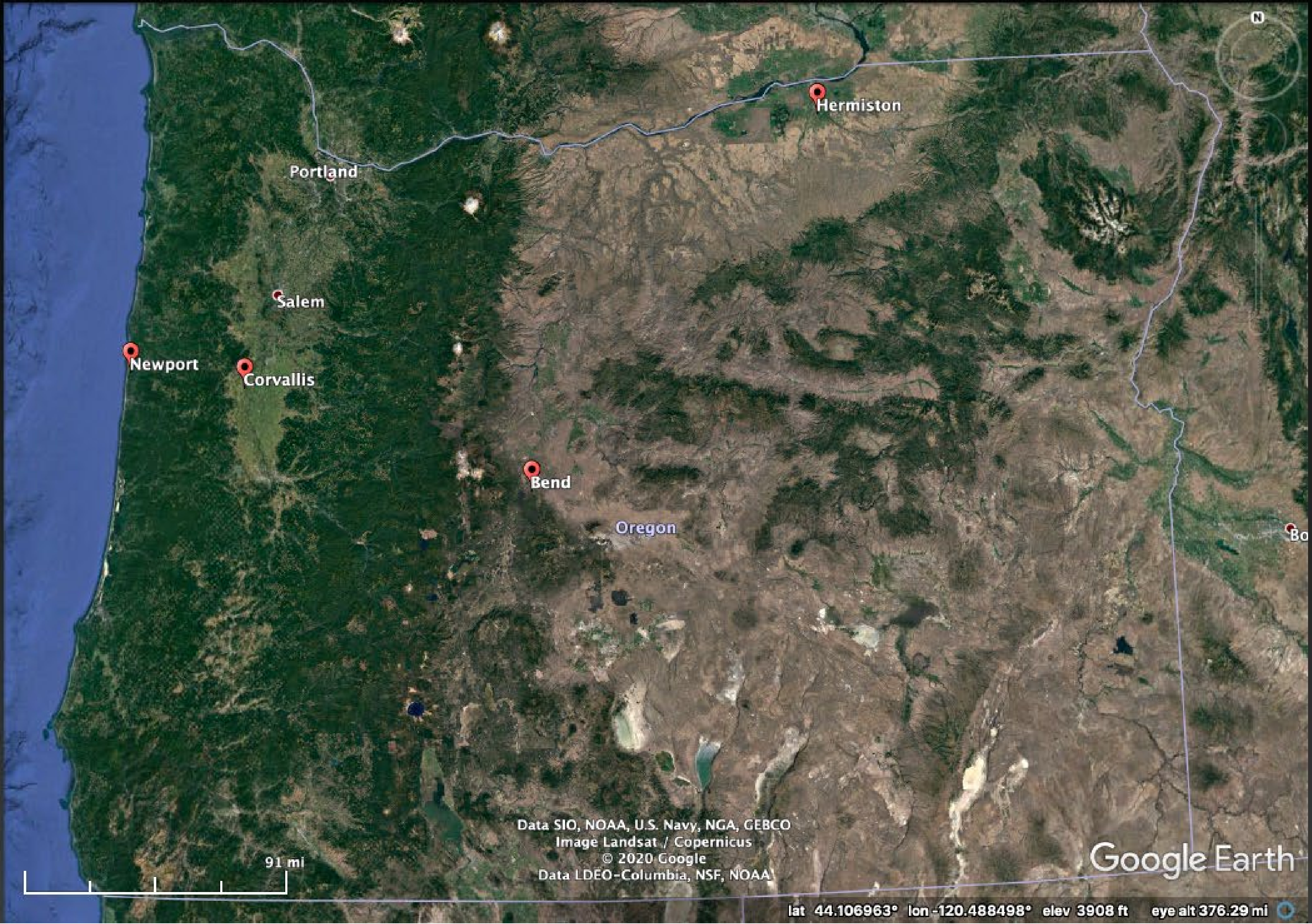
September 2, 2020

Oregon State University

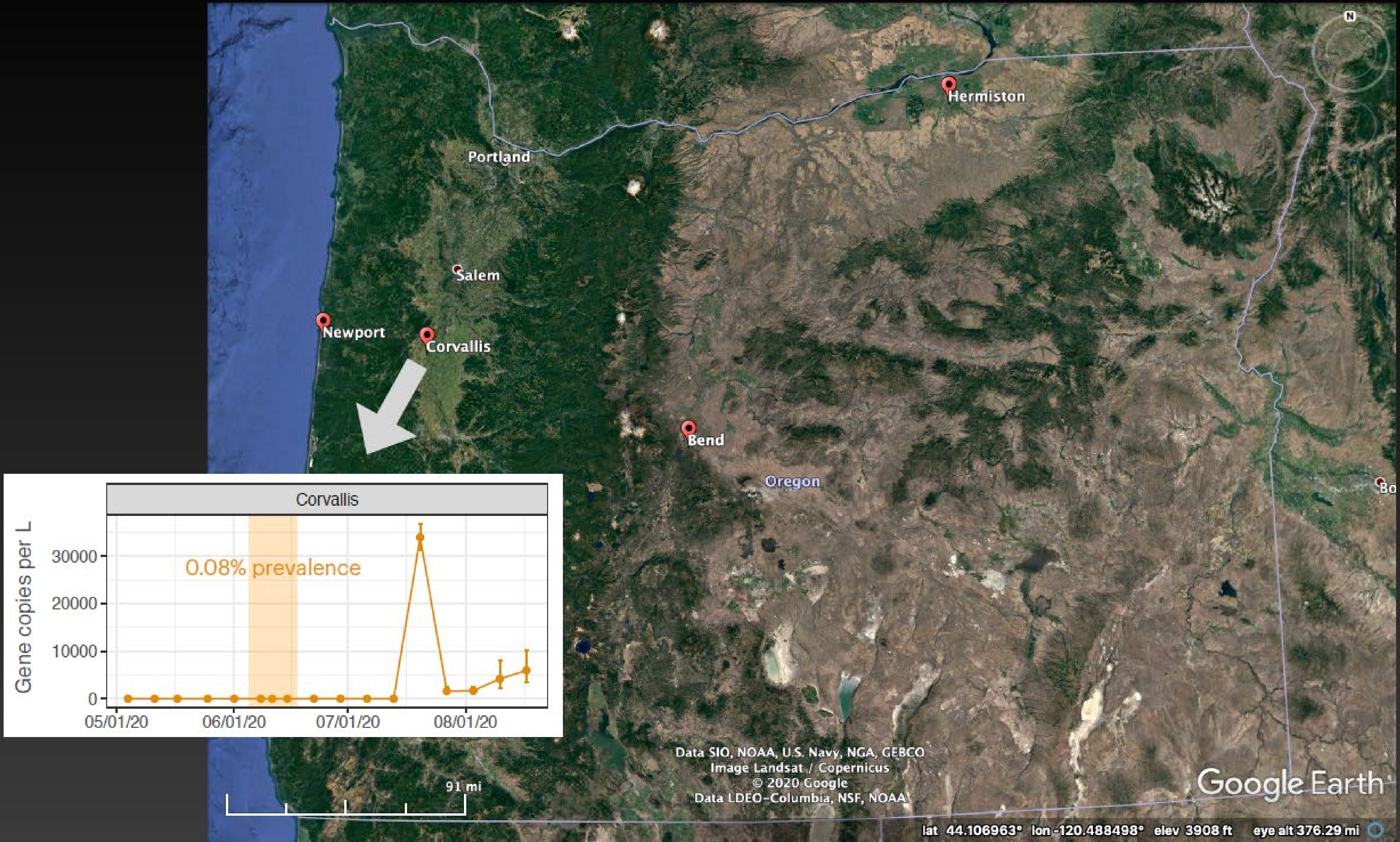
Clean Water Services

Blythe Layton, Christine Kelly, Ken Williamson, Jeff Bethel, Benjamin Dalziel, Dana Gibbon, Roy Haggerty, Kathryn Higley, Denise Hynes, Jane Lubchenco, Katherine McLaughlin, F. Javier Nieto, Aslan Noakes, Justin Sanders, Brett Tyler, and Tyler Radniecki

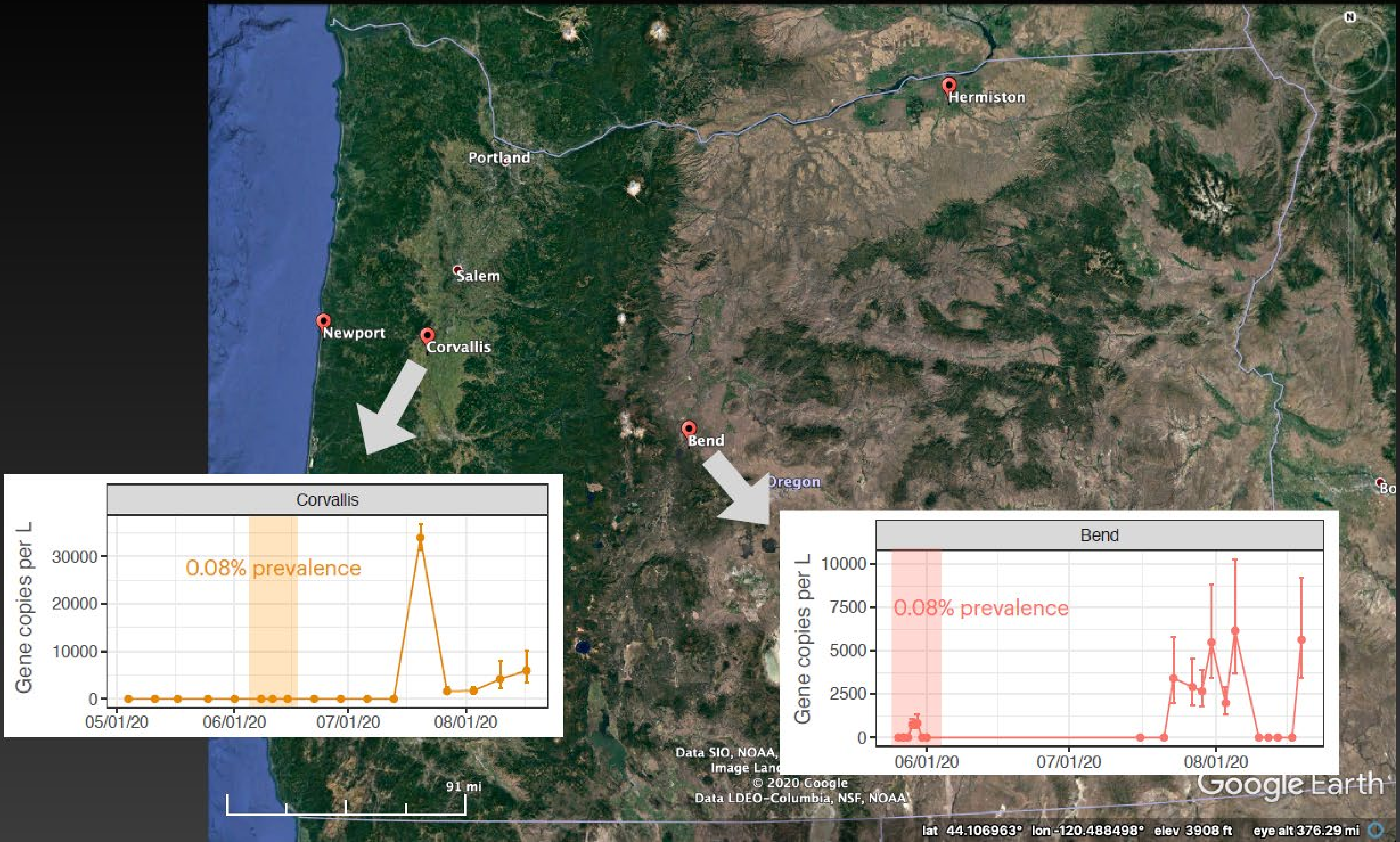
TRACE: Influent + Randomized Nasal Swabs



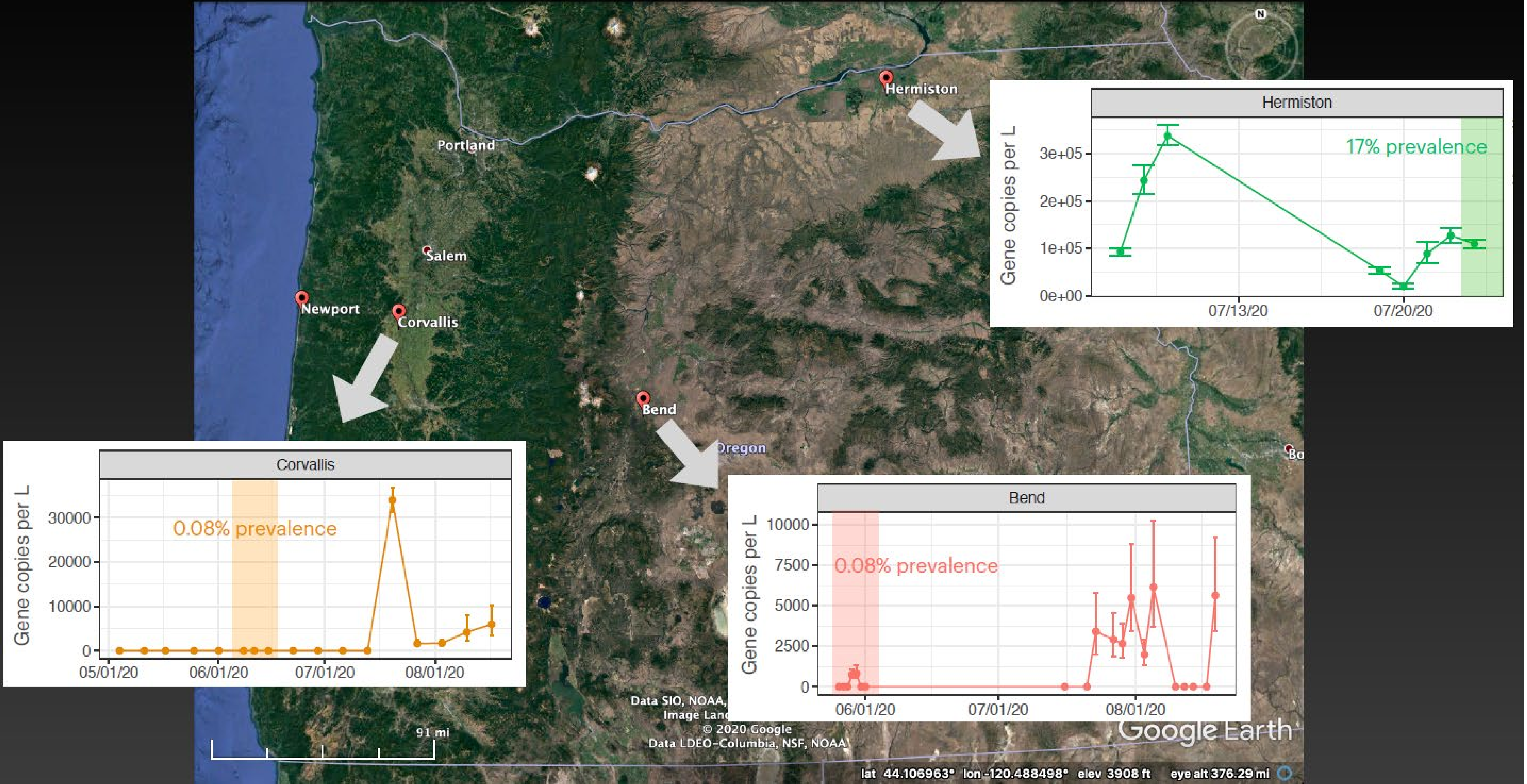
TRACE: Influent + Randomized Nasal Swabs



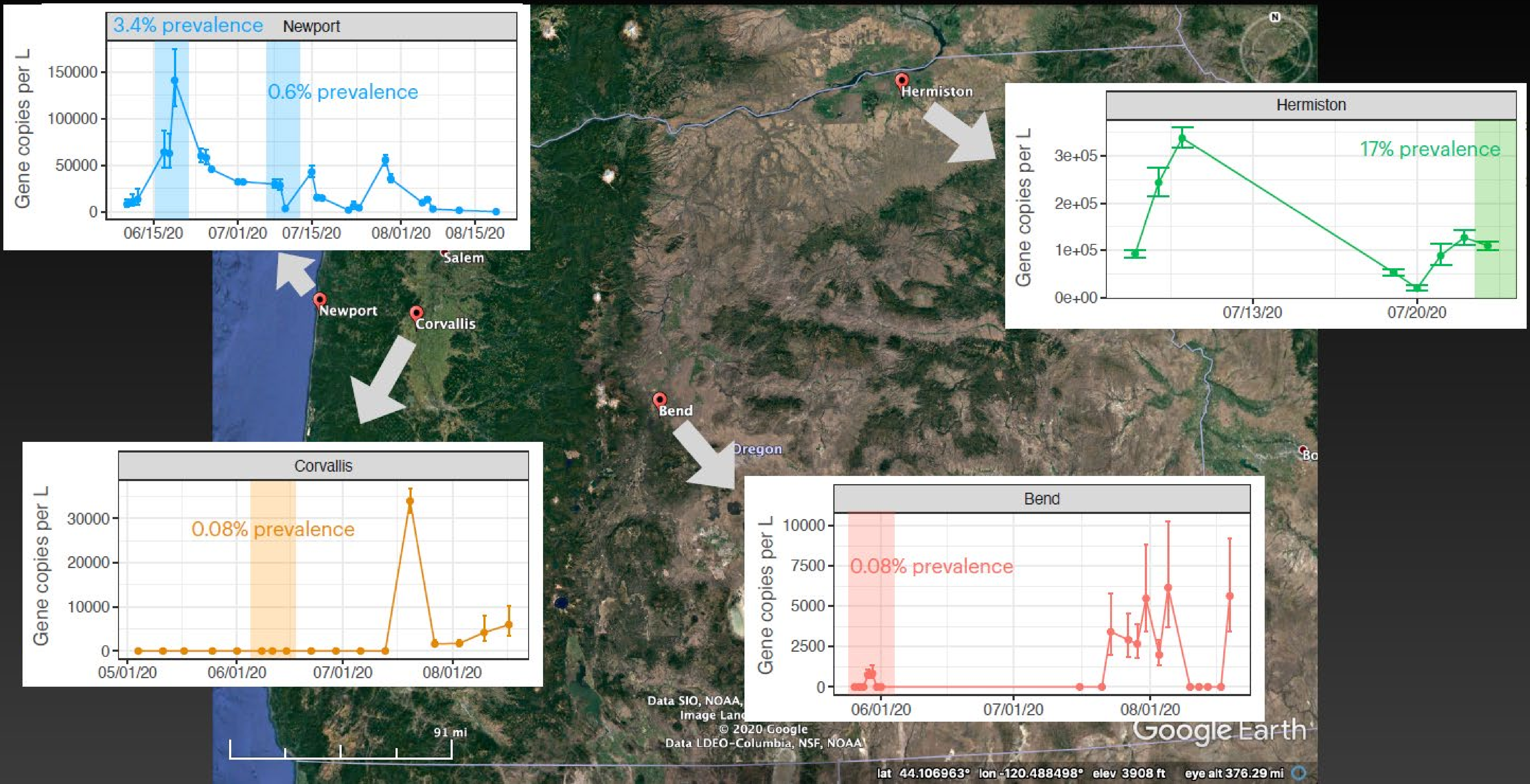
TRACE: Influent + Randomized Nasal Swabs

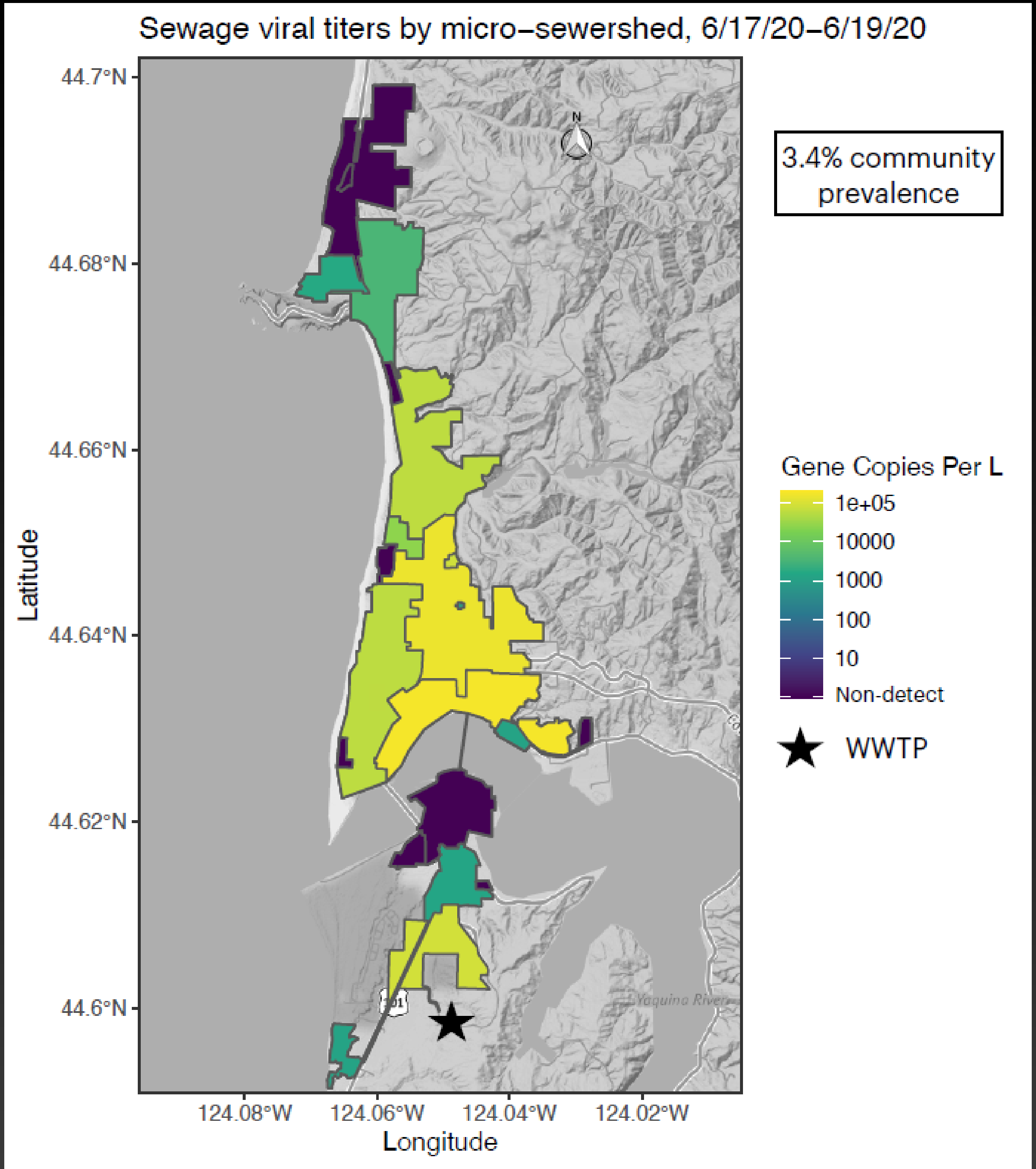


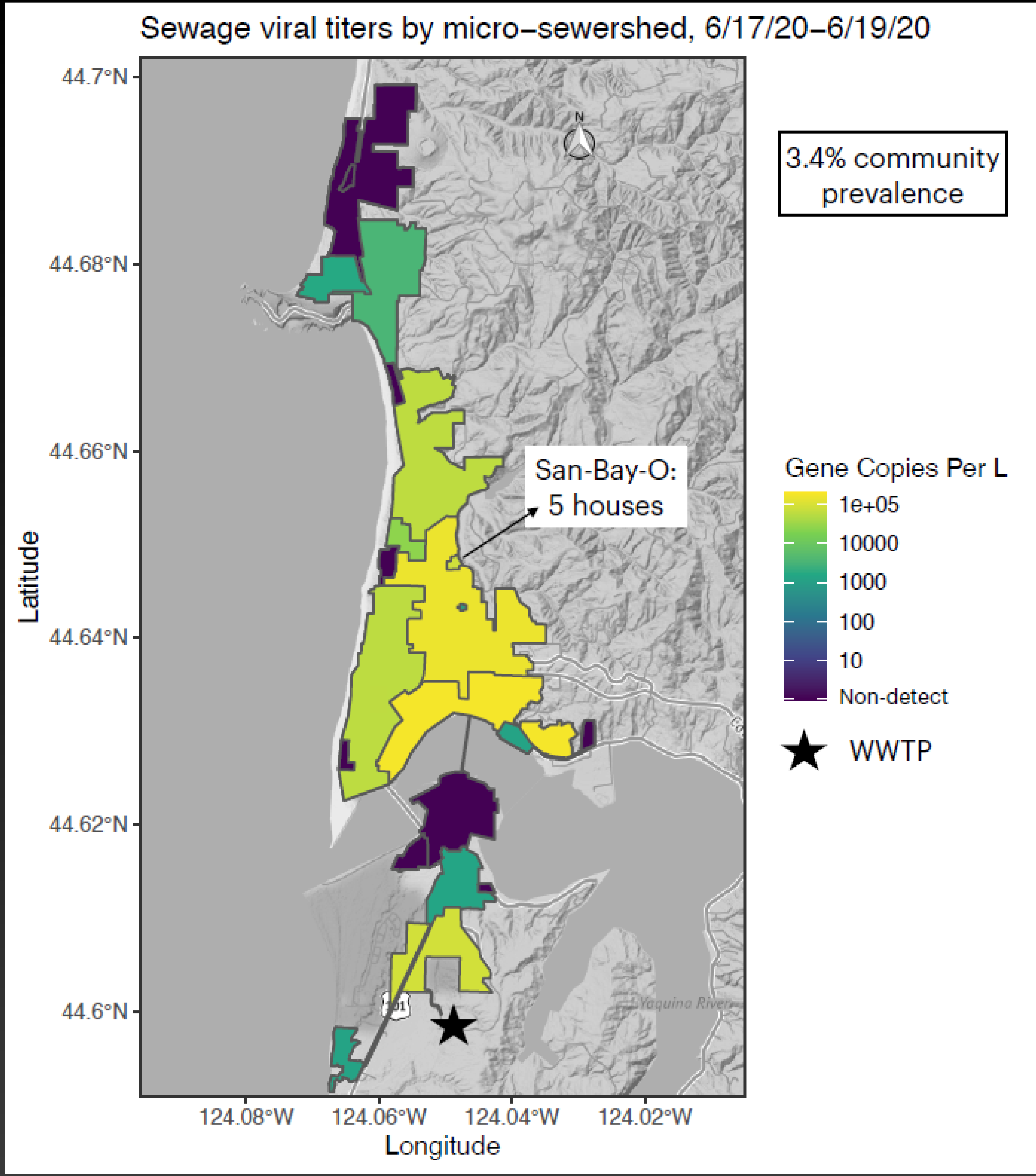
TRACE: Influent + Randomized Nasal Swabs

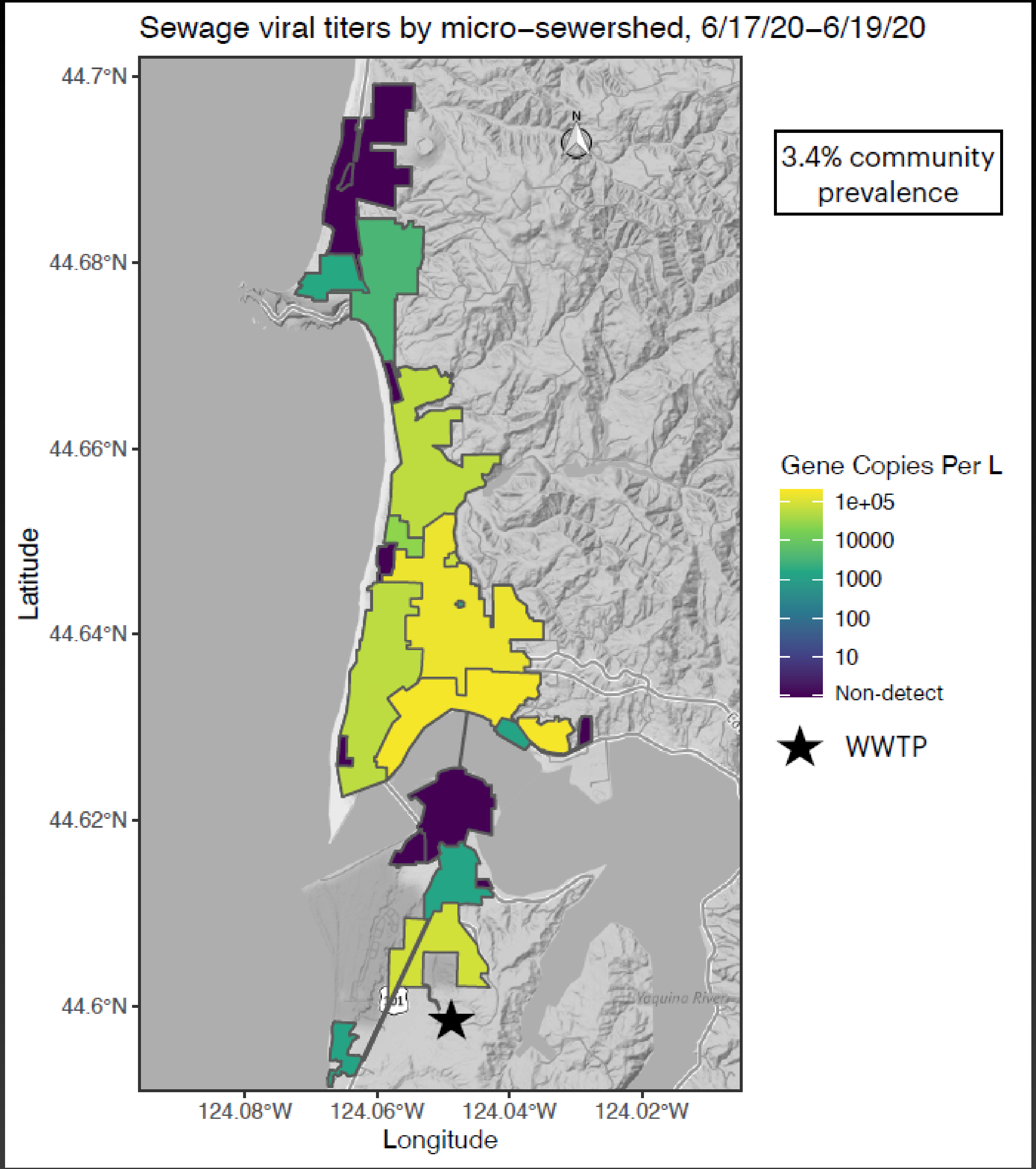


TRACE: Influent + Randomized Nasal Swabs

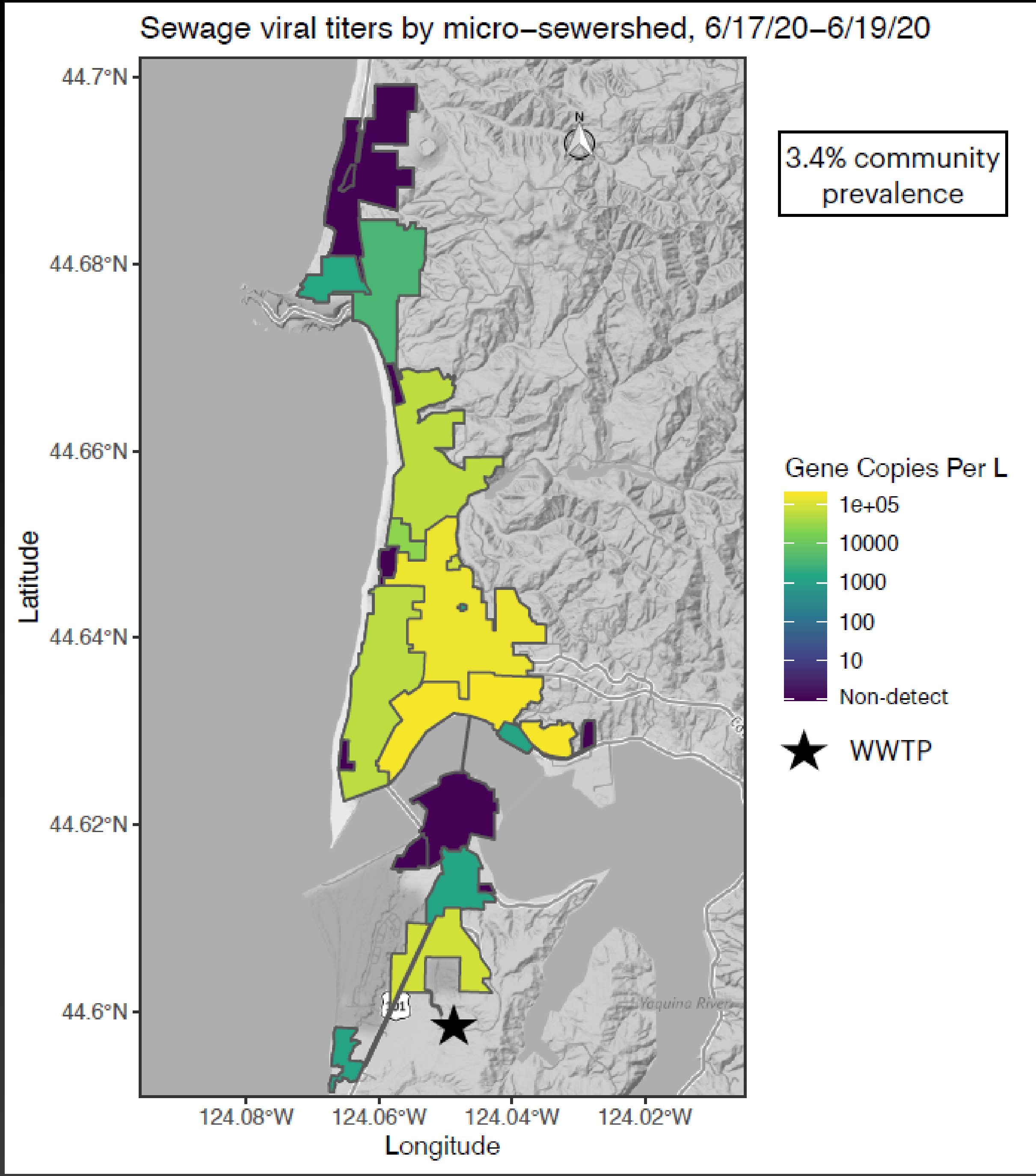


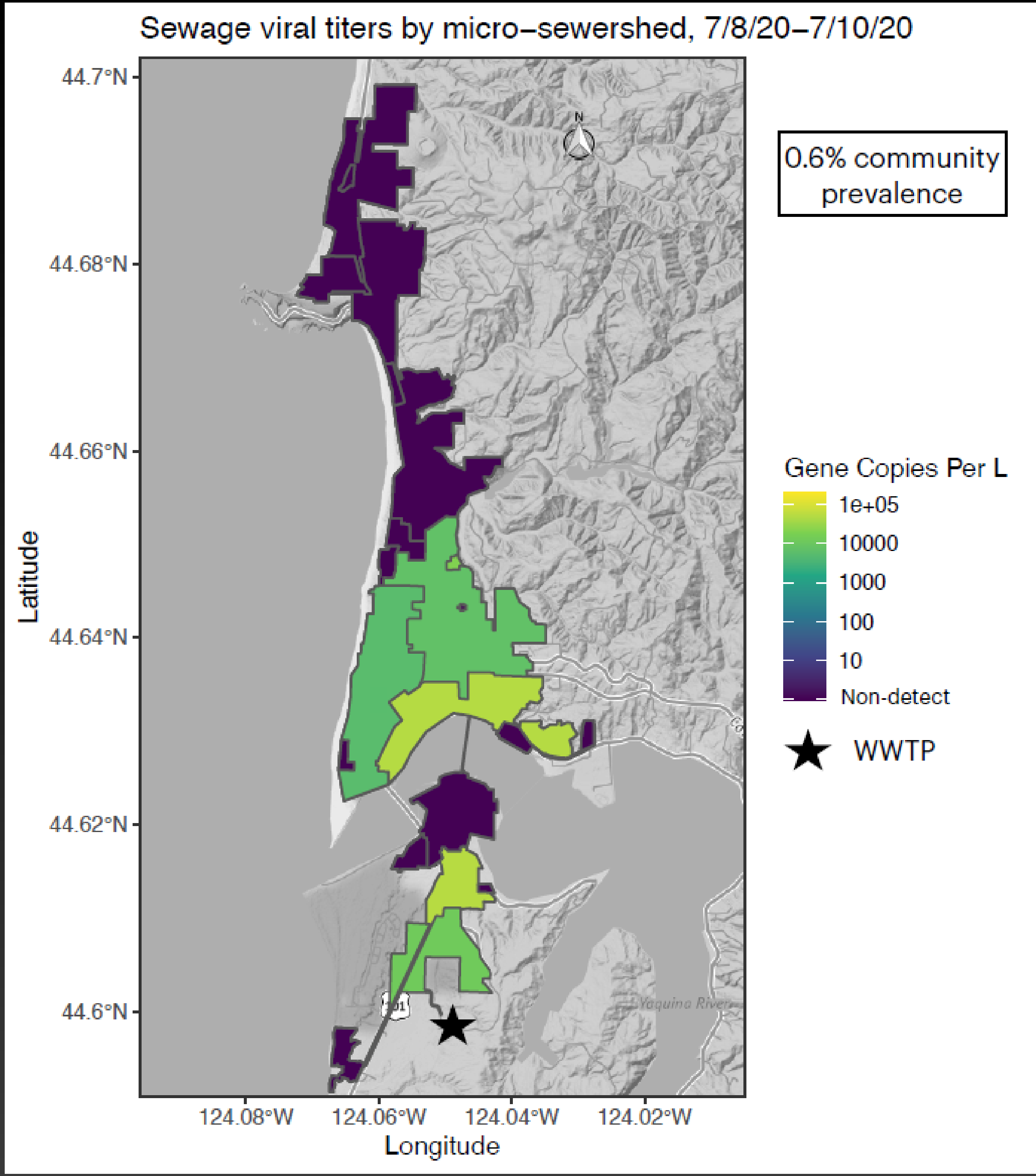

Sewage viral titers by micro−sewershed, 6/17/20−6/19/20
3.4% community prevalence
Gene Copies Per L
1e+05
10000
1000
100
10
Non-detect
WWTP
Latitude
44.7°N
44.68°N
44.66°N
44.64°N
44.62°N
44.6°N
Longitude
124.08°W
124.06°W
124.04°W
124.02°W
Yaquina River

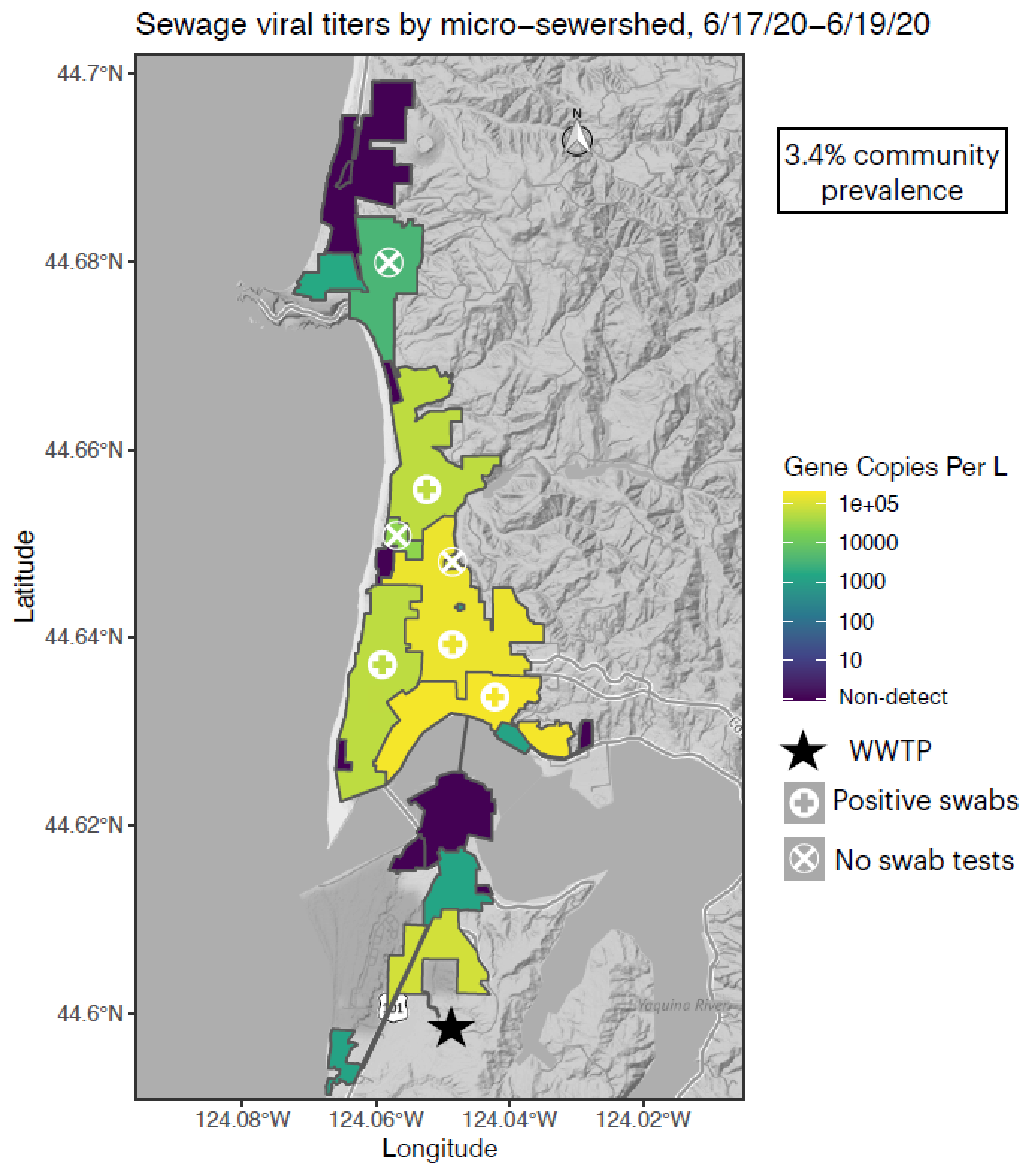

Sewage viral titers by micro–sewershed, 6/17/20–6/19/20
3.4% community prevalence
San-Bay-O: 5 houses
Gene Copies Per L
1e+05
10000
1000
100
10
Non-detect
WWTP
Latitude
44.7°N
44.68°N
44.66°N
44.64°N
44.62°N
44.6°N
Longitude
124.08°W
124.06°W
124.04°W
124.02°W
Yaquina River
101
N



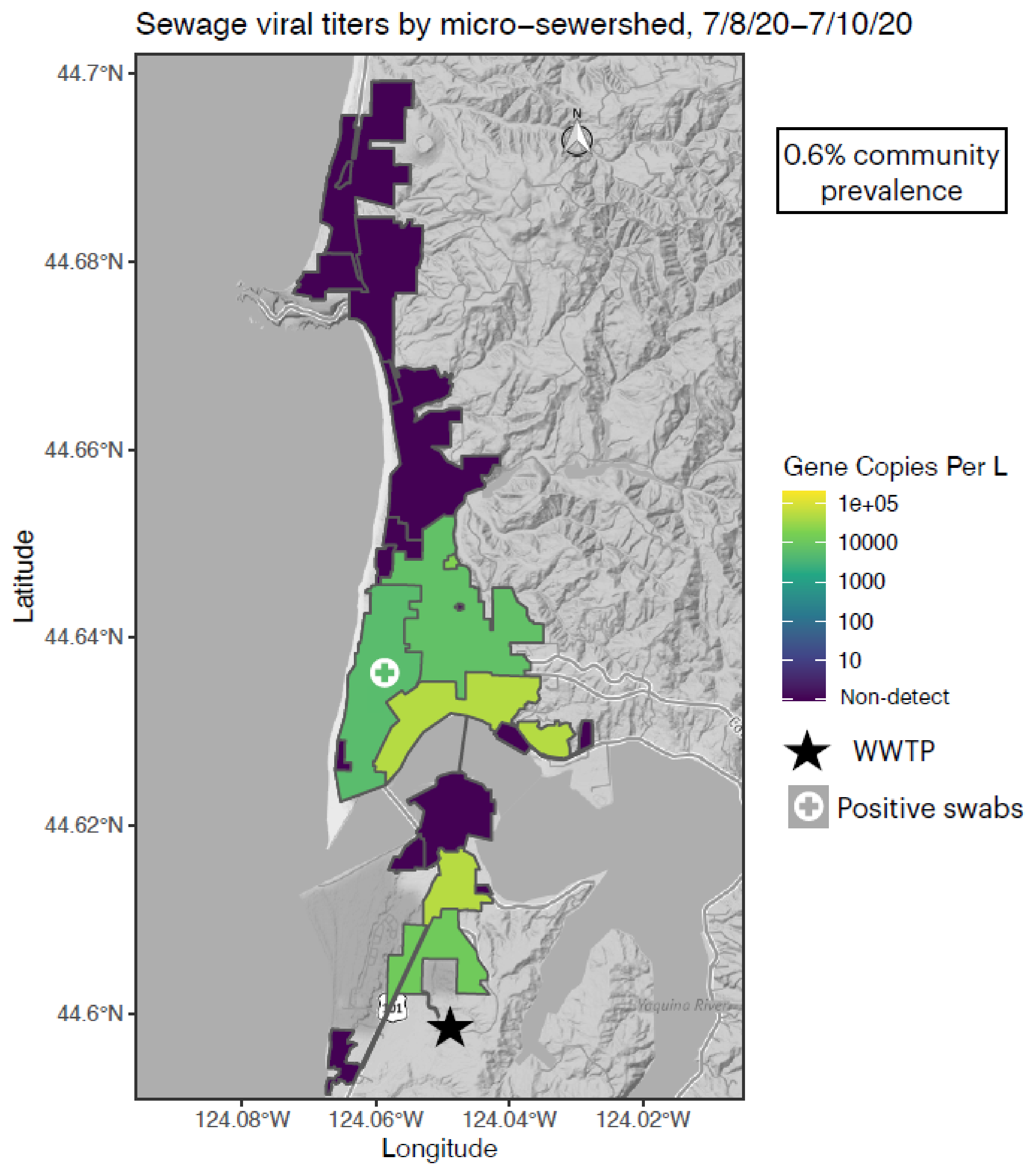
Sewage viral titers by micro−sewershed, 6/17/20−6/19/20
3.4% community prevalence
Gene Copies Per L
1e+05
10000
1000
100
10
Non-detect
WWTP
44.7°N
44.68°N
44.66°N
44.64°N
44.62°N
44.6°N
Latitude
124.08°W
124.06°W
124.04°W
124.02°W
Longitude
N
Yaquina River
101


Sewage viral titers by micro–sewershed, 6/17/20–6/19/20
3.4% community prevalence
Gene Copies Per L
1e+05
10000
1000
100
10
Non-detect
WWTP
Latitude
44.7°N
44.68°N
44.66°N
44.64°N
44.62°N
44.6°N
Longitude
124.08°W
124.06°W
124.04°W
124.02°W
Yaquina River

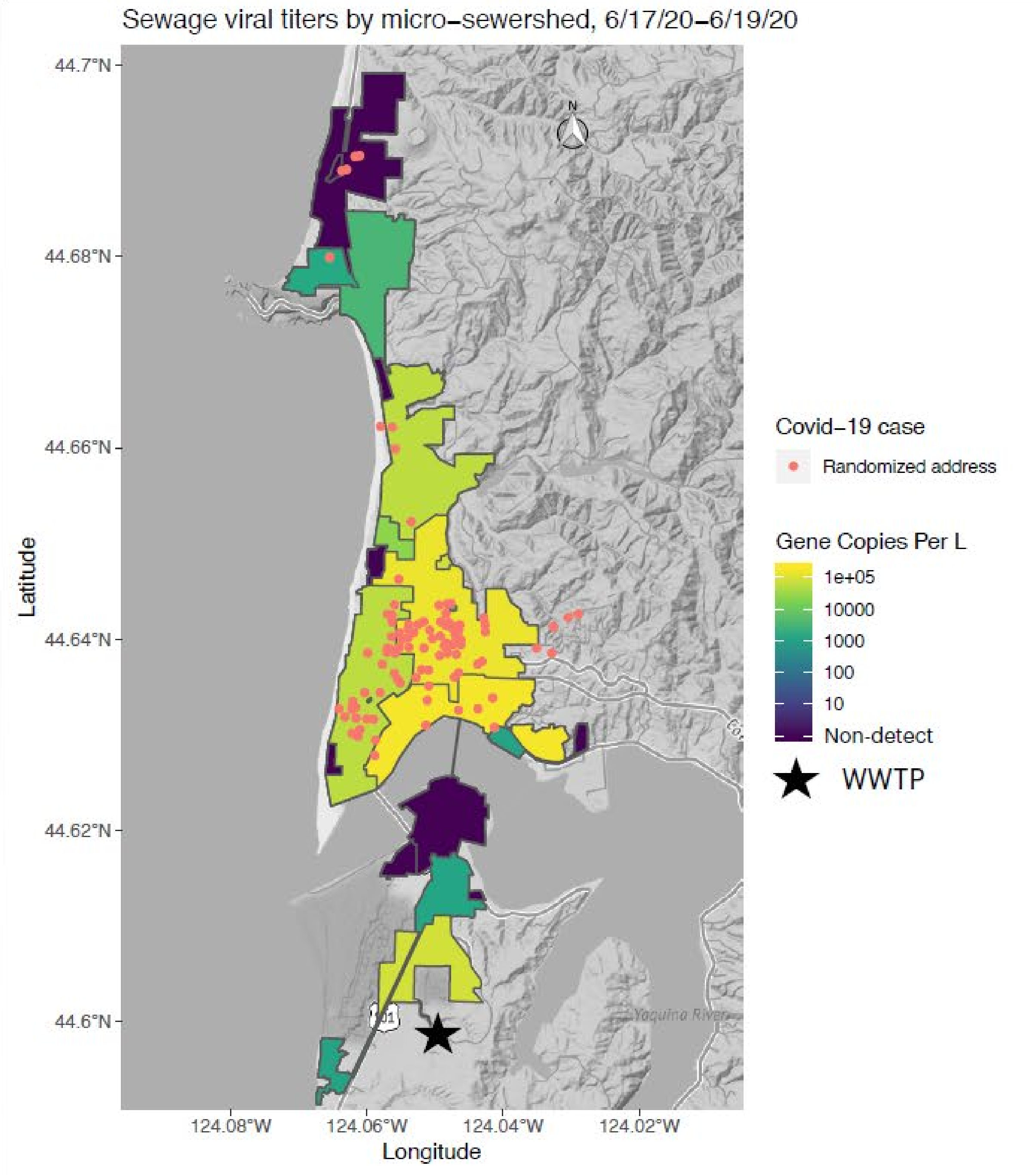

Sewage viral titers by micro–sewershed, 7/8/20–7/10/20
0.6% community prevalence
Gene Copies Per L
1e+05
10000
1000
100
10
Non-detect
WWTP
Latitude
44.7°N
44.68°N
44.66°N
44.64°N
44.62°N
44.6°N
Longitude
124.08°W
124.06°W
124.04°W
124.02°W
Yaquina River

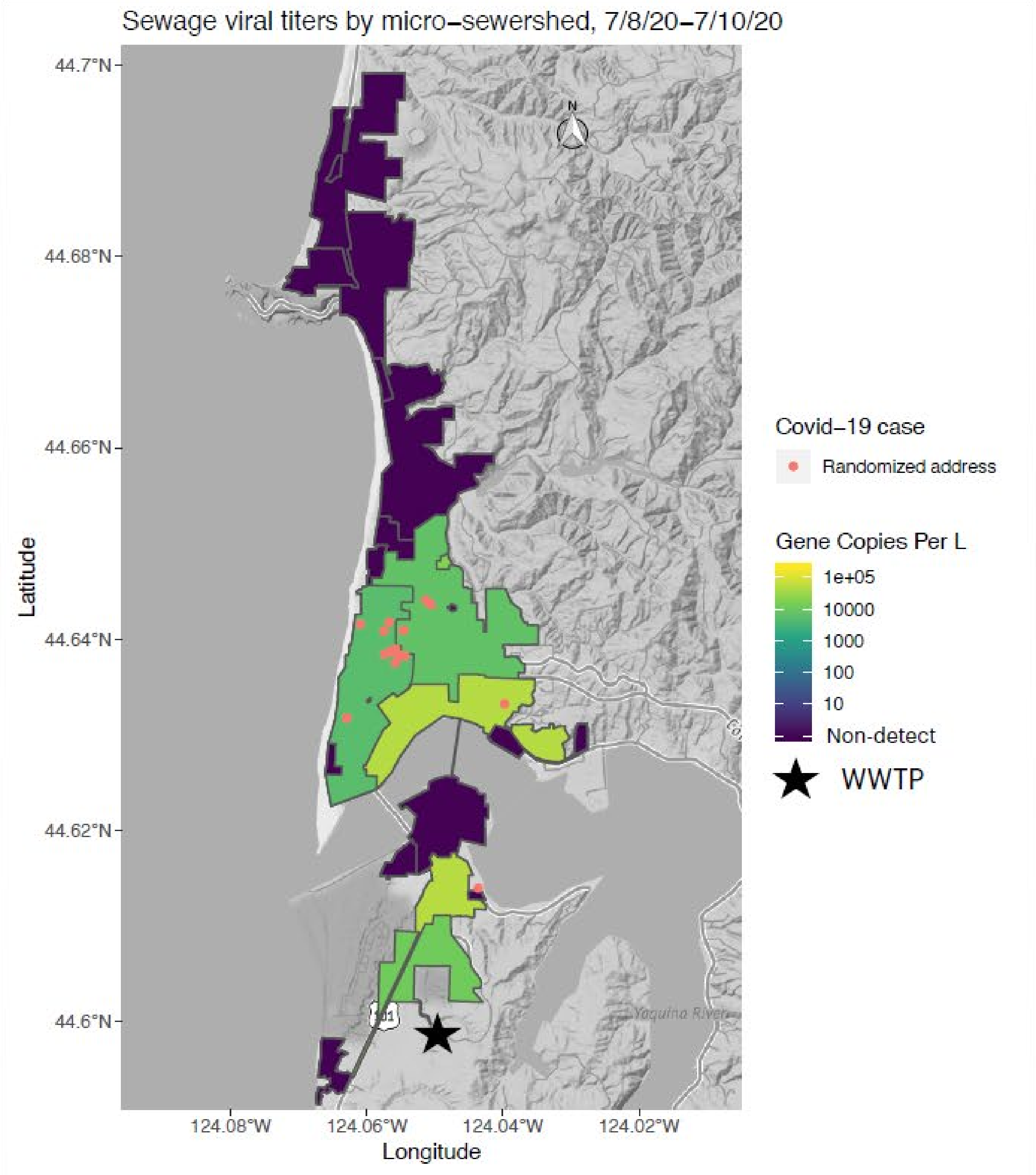


Sewage viral titers by micro-sewershed, 6/17/20–6/19/20
3.4% community prevalence
Gene Copies Per L
1e+05
10000
1000
100
10
Non-detect
WWTP
Positive swabs
No swab tests
Latitude
44.7°N
44.68°N
44.66°N
44.64°N
44.62°N
44.6°N
Longitude
124.08°W
124.06°W
124.04°W
124.02°W



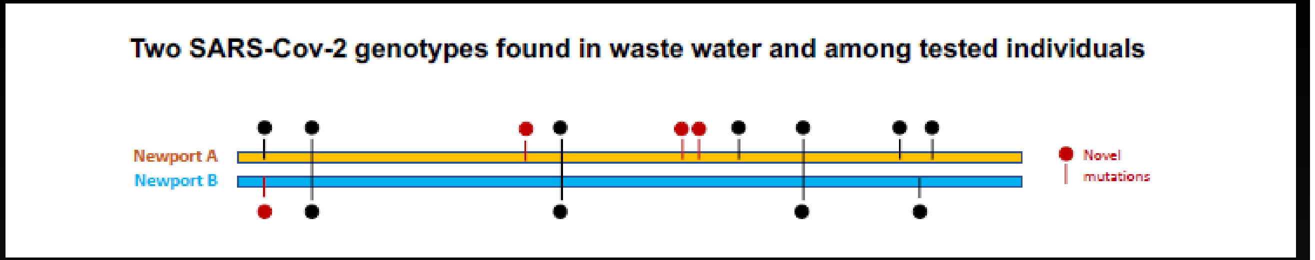
Sewage viral titers by micro-sewershed, 7/8/20–7/10/20
0.6% community prevalence
Gene Copies Per L
1e+05
10000
1000
100
10
Non-detect
WWTP
Positive swabs
Latitude
44.7°N
44.68°N
44.66°N
44.64°N
44.62°N
44.6°N
Longitude
124.08°W
124.06°W
124.04°W
124.02°W


Sewage viral titers by micro−sewershed, 6/17/20−6/19/20
44.7°N
44.68°N
44.66°N
44.64°N
44.62°N
44.6°N
Latitude
124.08°W
124.06°W
124.04°W
124.02°W
Longitude
Yaquina River
101
Covid−19 case
Randomized address
Gene Copies Per L
1e+05
10000
1000
100
10
Non-detect
WWTP

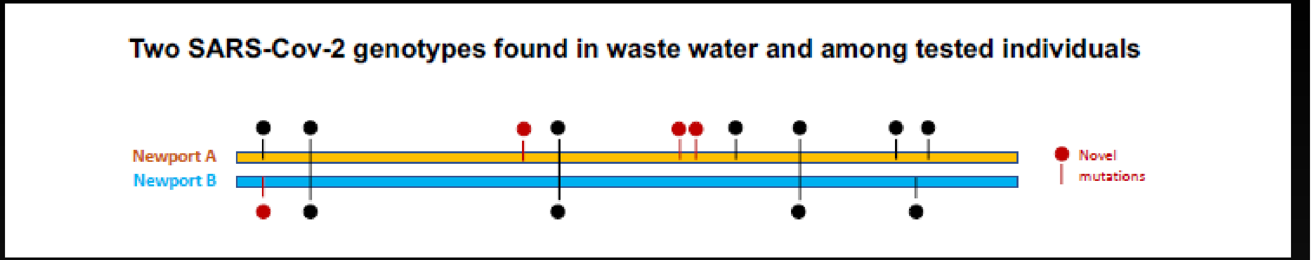

Sewage viral titers by micro−sewershed, 7/8/20−7/10/20
44.7°N
44.68°N
44.66°N
44.64°N
44.62°N
44.6°N
Latitude
124.08°W
124.06°W
124.04°W
124.02°W
Longitude
Yaquina River
101
Covid−19 case
Randomized address
Gene Copies Per L
1e+05
10000
1000
100
10
Non-detect
WWTP

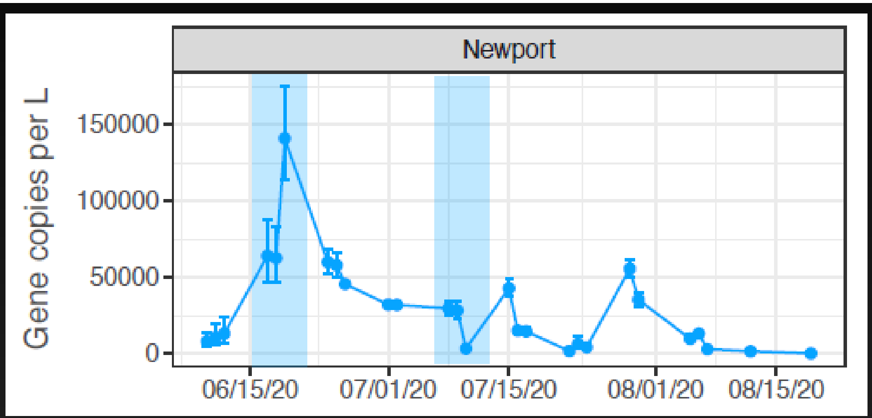
Sequencing Revealed
Two Novel Genotypes

Sequencing Revealed Two Novel Genotypes

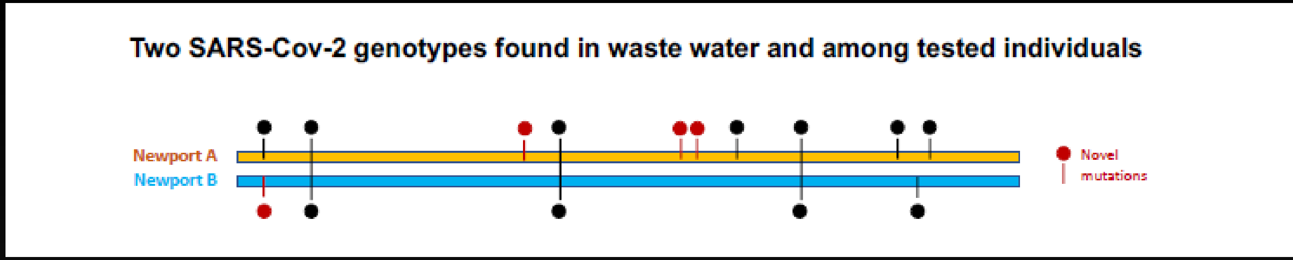


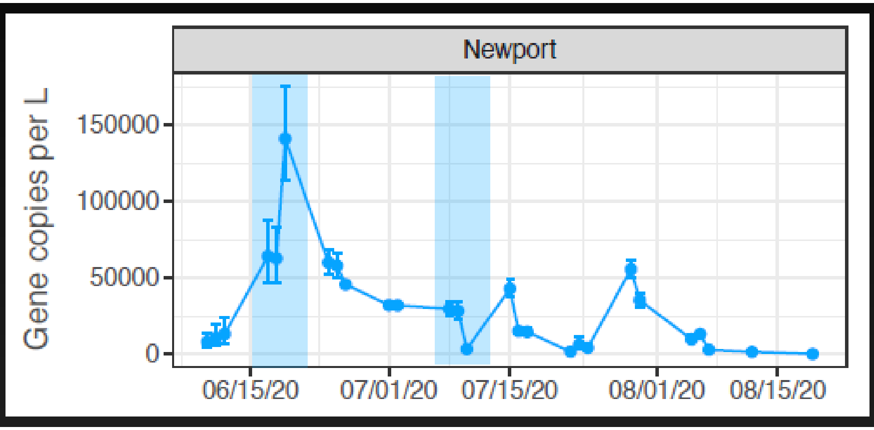
Sequencing Revealed Two Novel Genotypes

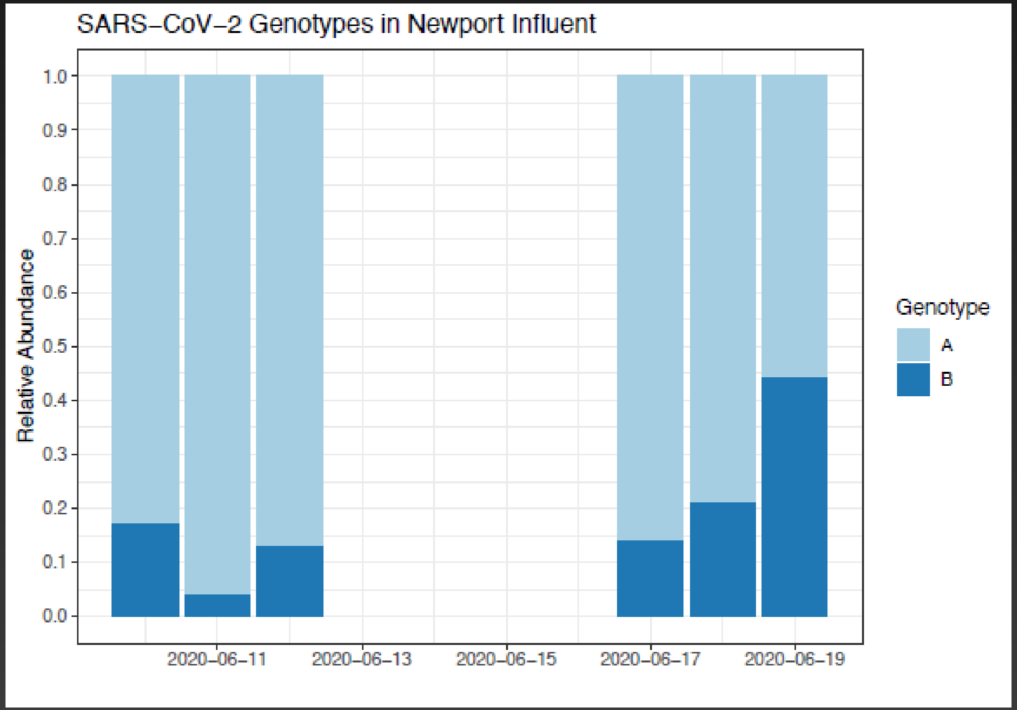




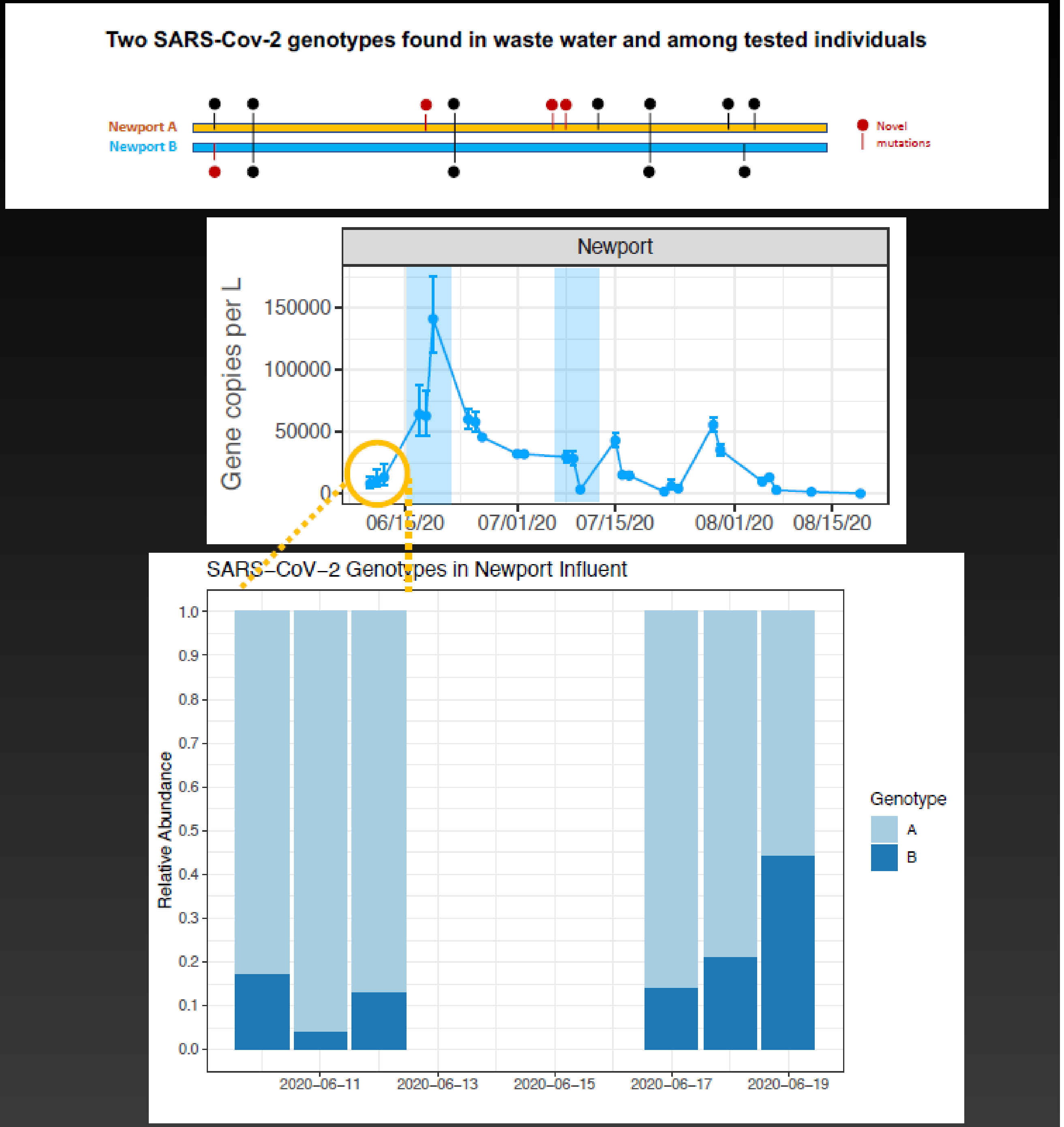
Sequencing Revealed Two Novel Genotypes



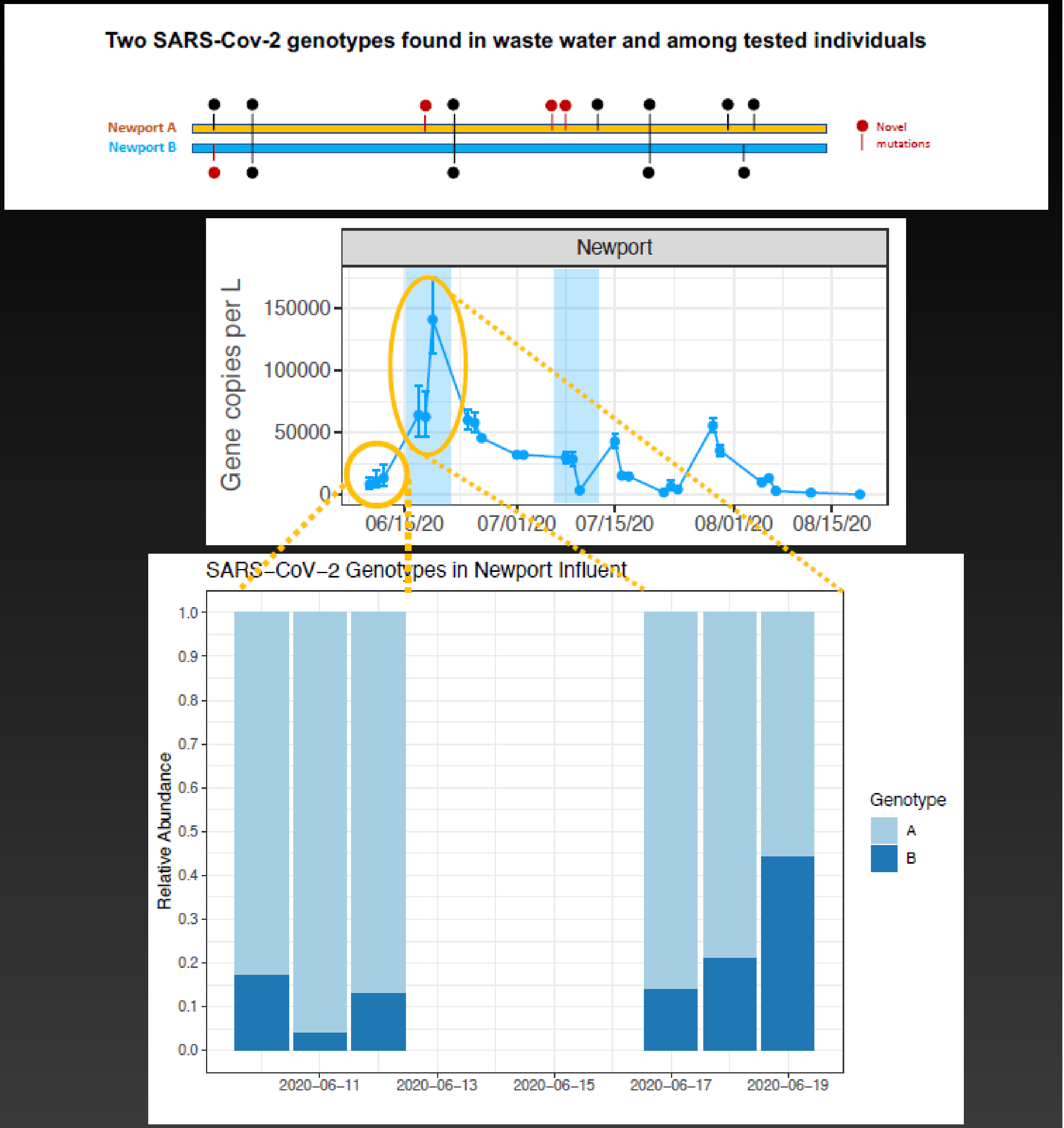




Sequencing Revealed Two Novel Genotypes

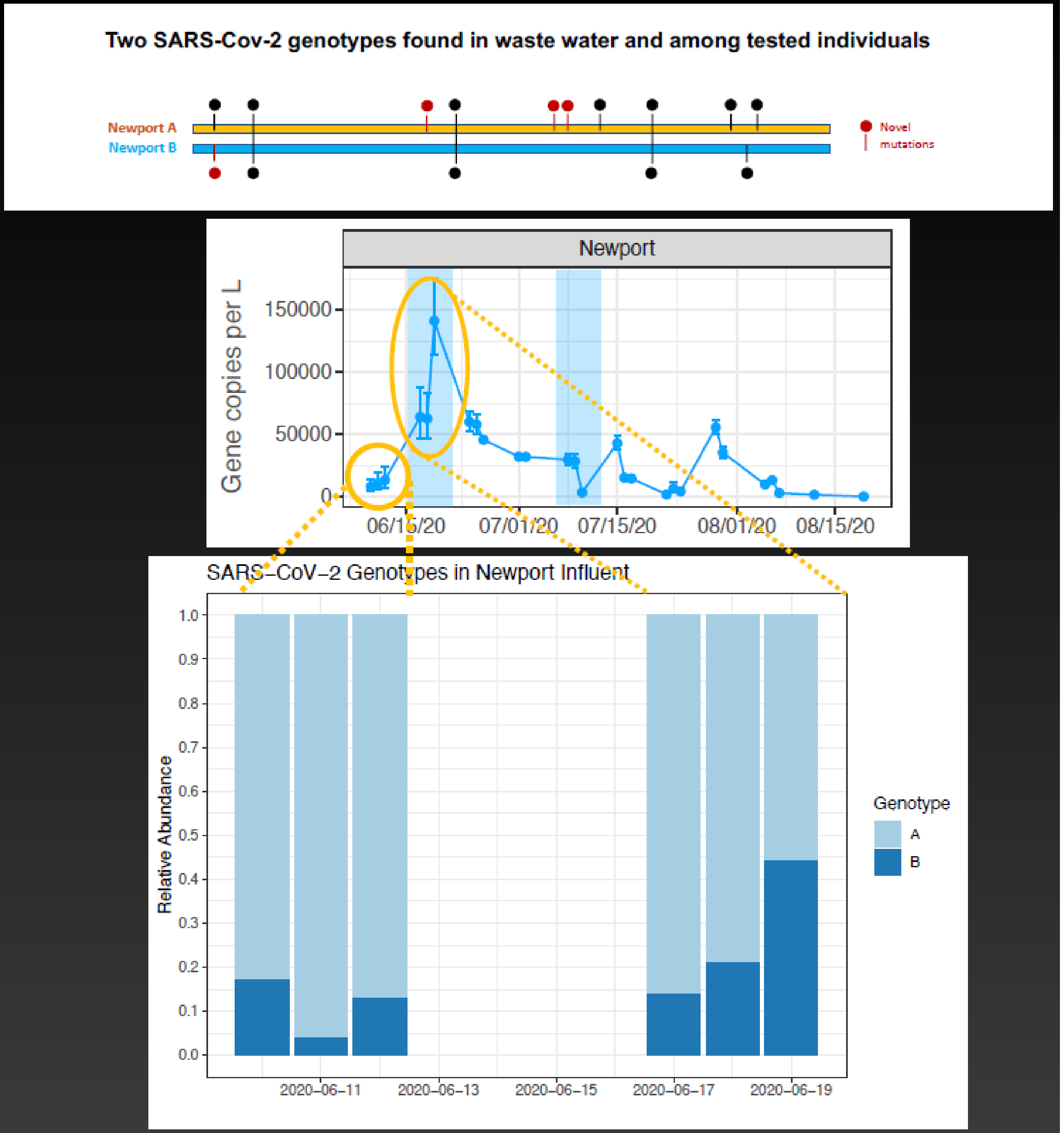


Sequencing Revealed Two Novel Genotypes



Sequencing Revealed Two Novel Genotypes

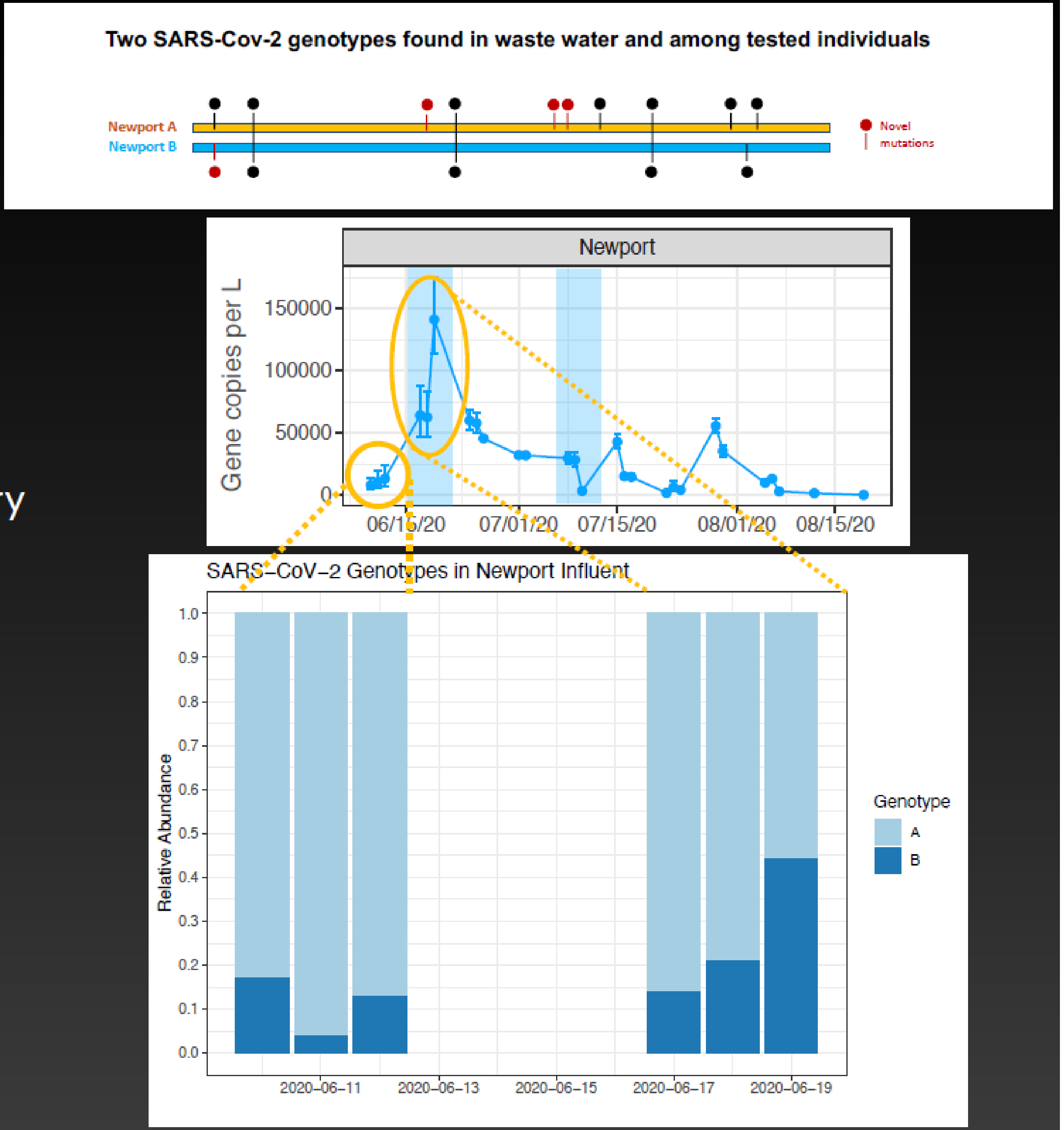
N2: High sensitivity, but potential non-specificity



Sequencing Revealed Two Novel Genotypes

N2: High sensitivity, but potential non-specificity

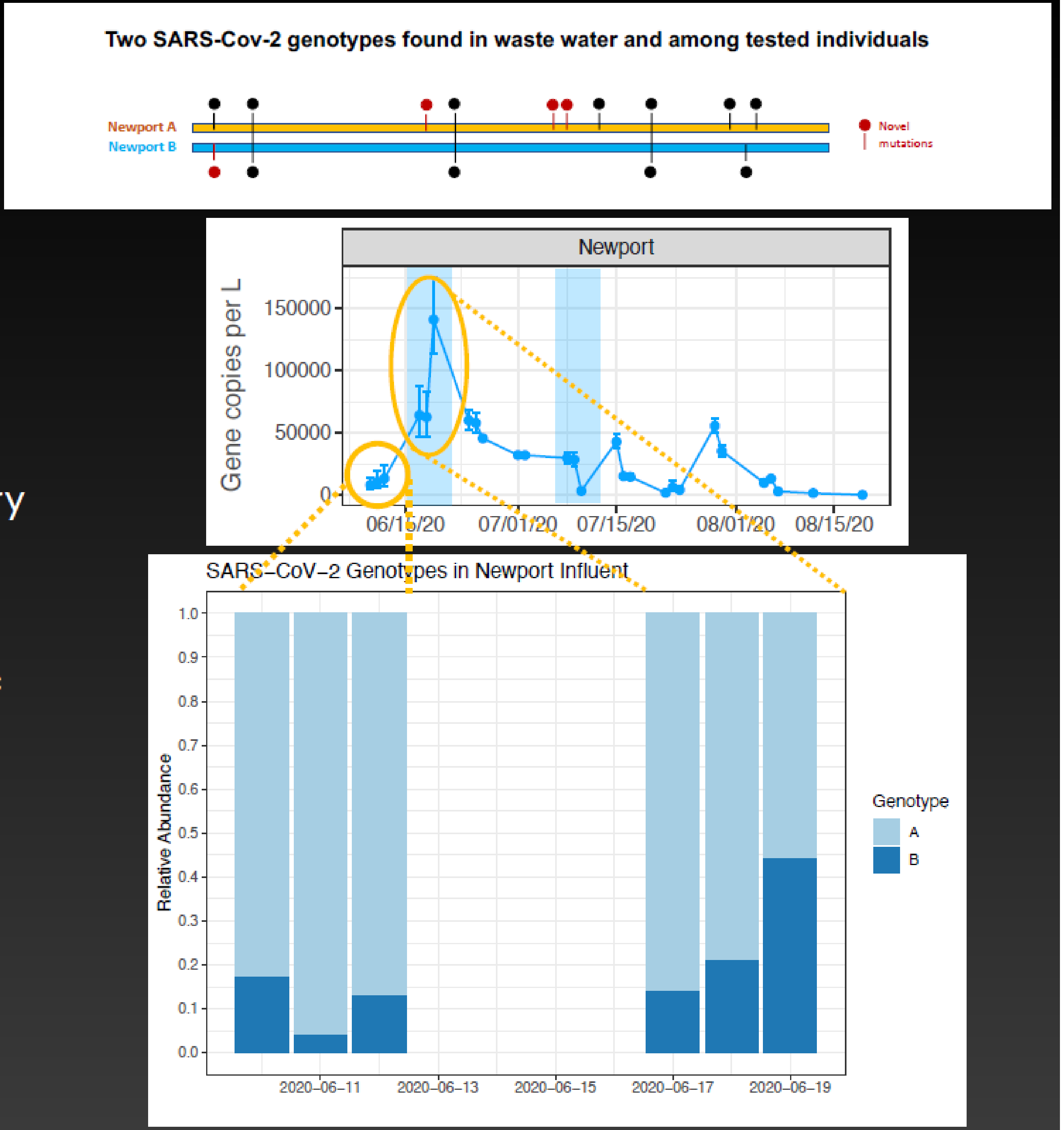
- Samples positive for N2 (only) by ddPCR had very few aligned reads - but appear to be true hits



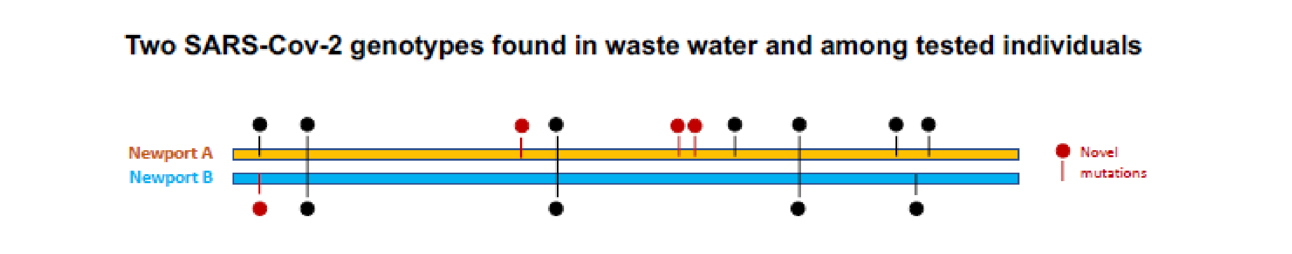
Sequencing Revealed Two Novel Genotypes

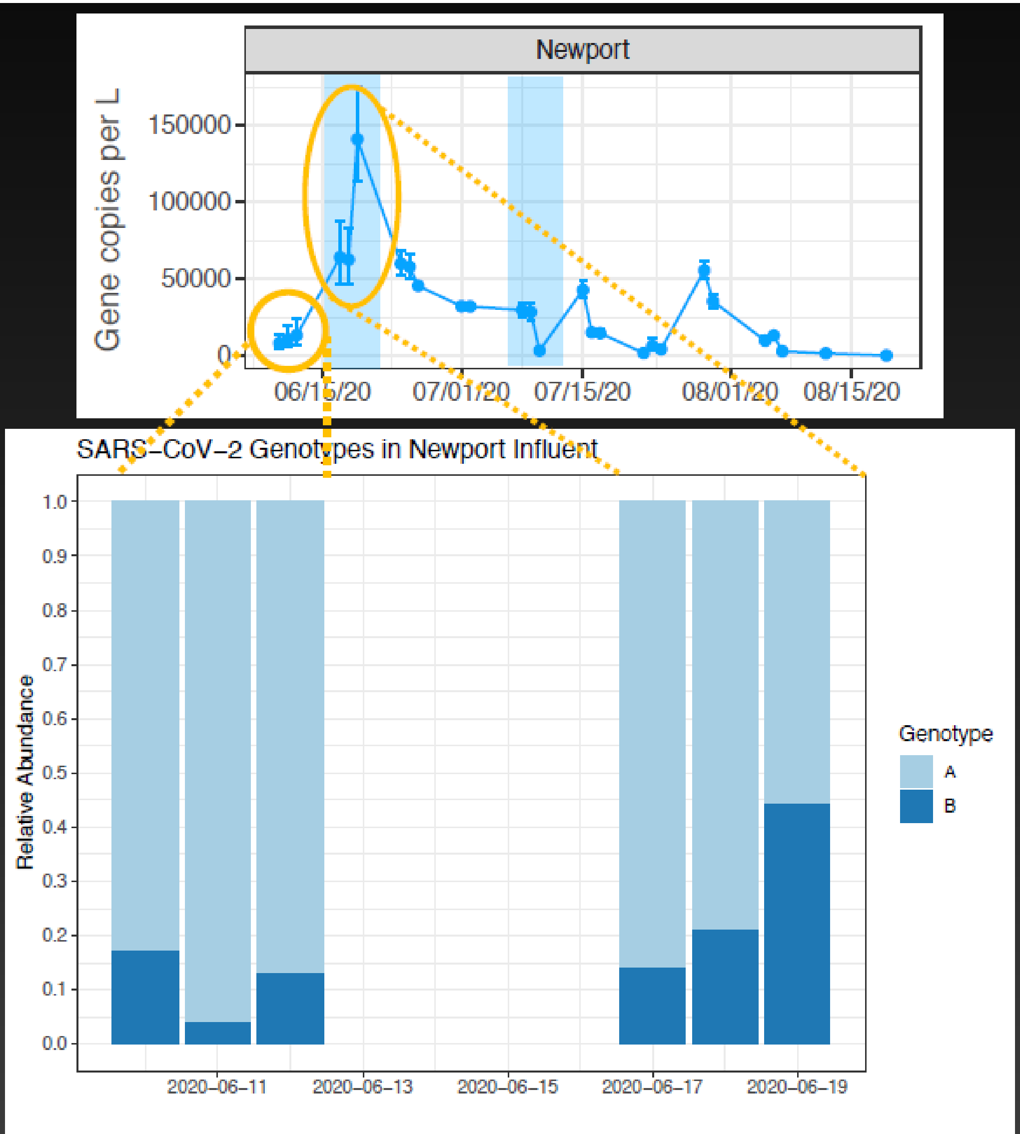
N2: High sensitivity, but potential non-specificity

- Samples positive for N2 (only) by ddPCR had very few aligned reads - but appear to be true hits
- Limit of Blank was 2x higher for N2 than N1, suggesting greater mis-priming and nonspecific amplification



Sequencing Revealed Two Novel Genotypes





N2: High sensitivity, but potential non-specificity

- Samples positive for N2 (only) by ddPCR had very few aligned reads - but appear to be true hits
- Limit of Blank was 2x higher for N2 than N1, suggesting greater mis-priming and nonspecific amplification
- *in silico* amplification of triplex assay revealed 15 possible primer dimers, 5 involving the N2 probe

Sequencing Revealed Two Novel Genotypes

N2: High sensitivity, but potential non-specificity

- Samples positive for N2 (only) by ddPCR had very few aligned reads - but appear to be true hits
- Limit of Blank was 2x higher for N2 than N1, suggesting greater mis-priming and nonspecific amplification
- *in silico* amplification of triplex assay revealed 15 possible primer dimers, 5 involving the N2 probe

Potential primer-dimer for primers: N2-P and N2-F

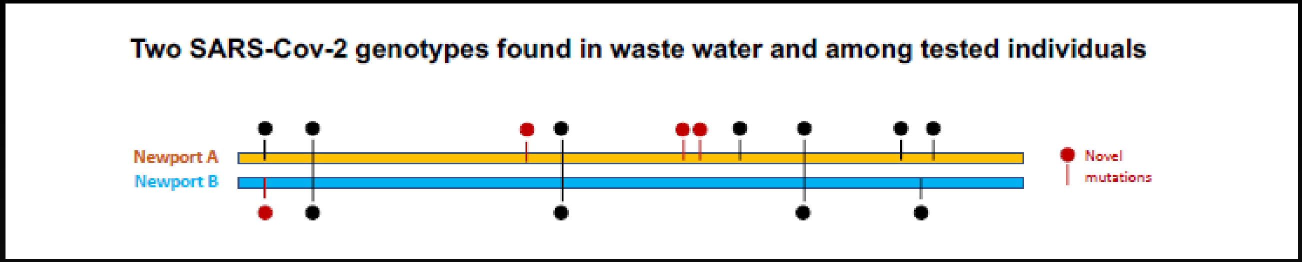
Overlap = 19, Quality = 110.0

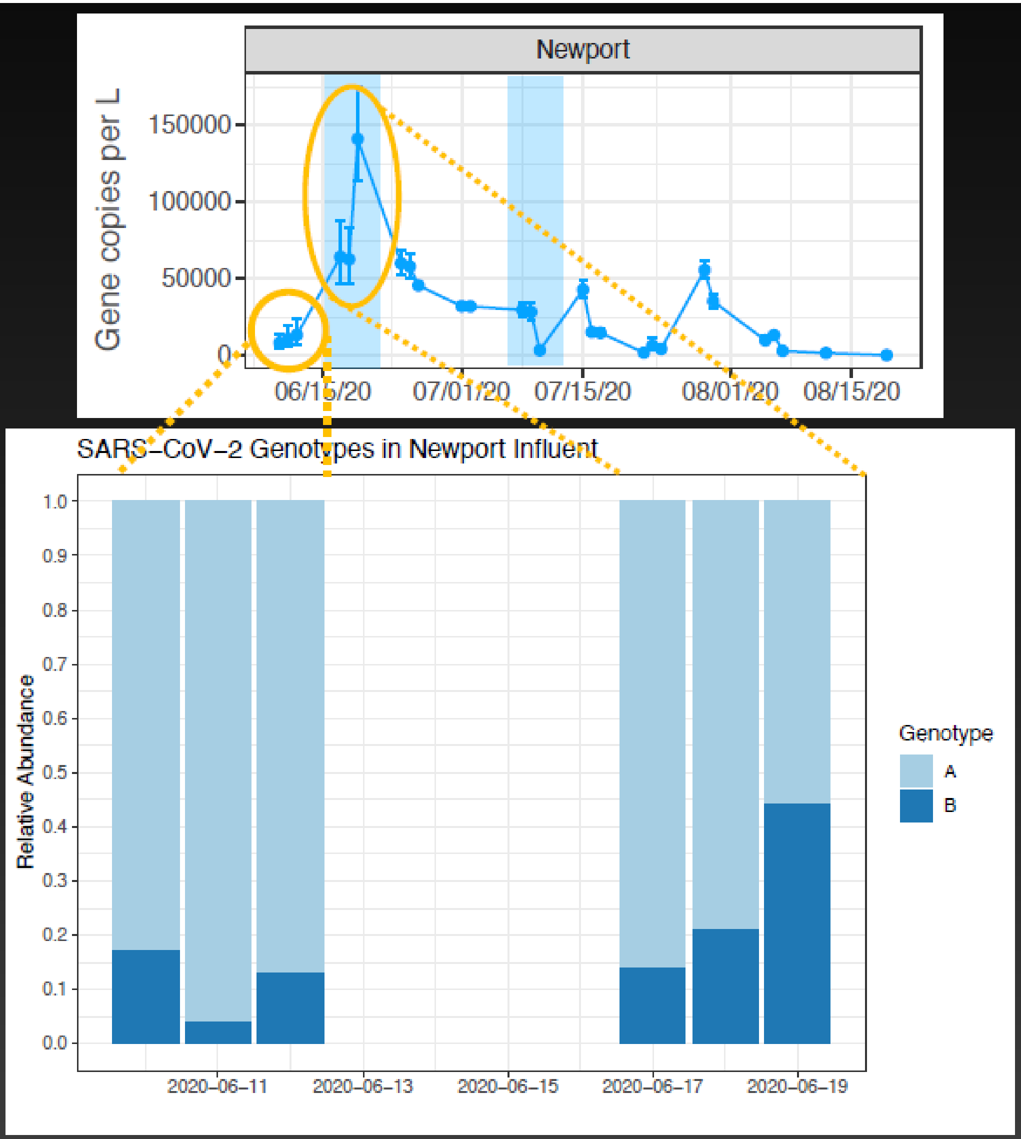
```
N2-P    5′ ACAATTTGCCCCCAGCGCTTCAG 3′
N2-F          3′ AAACGCCGGTTACAAACATT 5′
```

Potential primer-dimer for primers: N2-P and N2-P

Overlap = 14, Quality = 100.0

```
N2-P    5′ ACAATTTGCCCCCAGCGCTTCAG 3′
N2-P               3′ GACTTCGCGACCCCCGTTTAACA 5′
```





Oregon Health Authority

the David & Lucile Packard Foundation

PacificSource Health Plans

NSF

RAPID #2027679

Thank you!

Special thanks to the entire TRACE team and the CGRB:

Jeff Bethel, Katherine Carter, **Benjamin Dalziel**, Dana Gibbon, Anne-Marie Girard-Pohjanpelto, Roy Haggerty, Kathryn Higley, Denise Hynes, Jane Lubchenco, Katherine McLaughlin, F. Javier Nieto, Aslan Noakes, Justin Sanders, Brett Tyler

Oregon State University Foundation

Contact: tyler.radniecki@oregonstate.edu

Oregon State University

Detection of SARS-CoV-2 in Wastewater

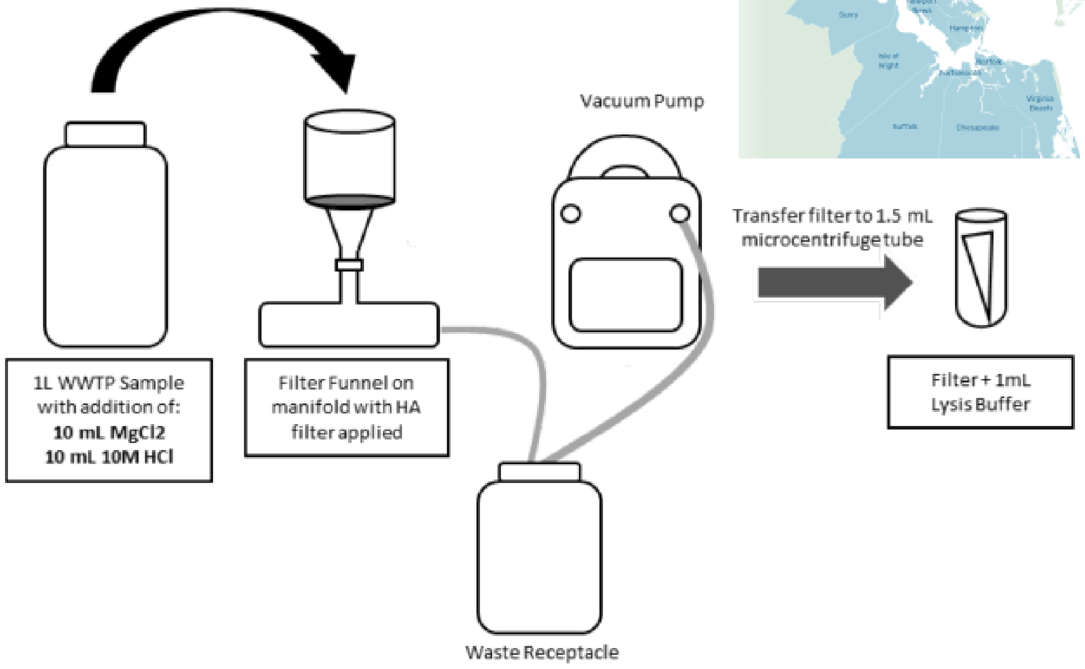
Assessing Sensitivity of Two Molecular Workflows

Mark Ciesielski, Thomas Clerkin, Denene Blackwood, Hannah Thompson, Allison Larson, Raul Gonzalez & Rachel Noble

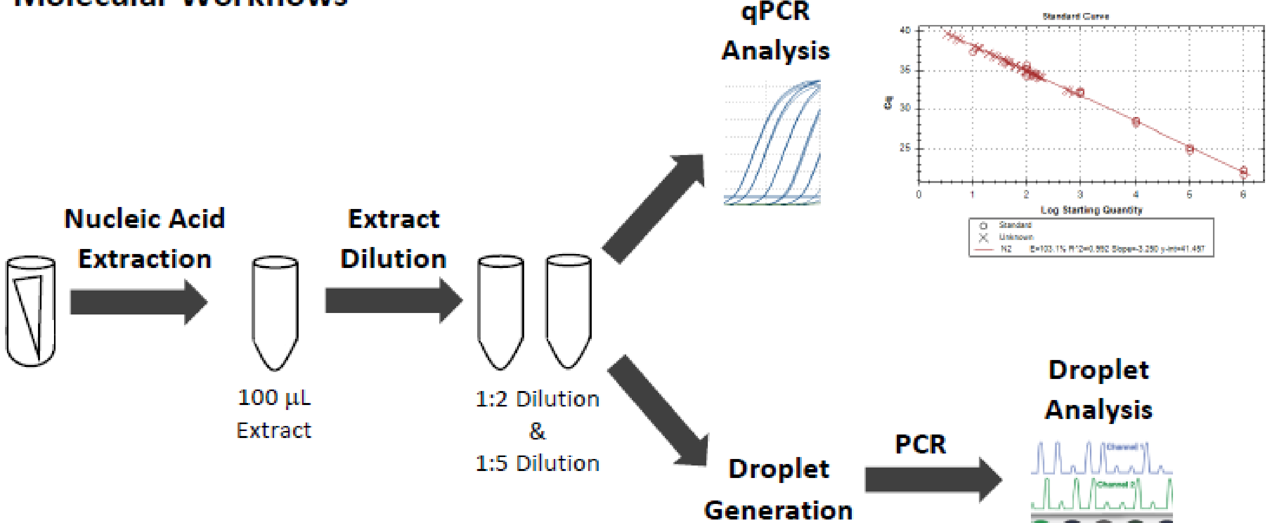
Wastewater Surveillance Workflows

Sample Processing Setup

- 60 samples derived from multi-month collection period
- Collected from 9 Facilities



Molecular Workflows

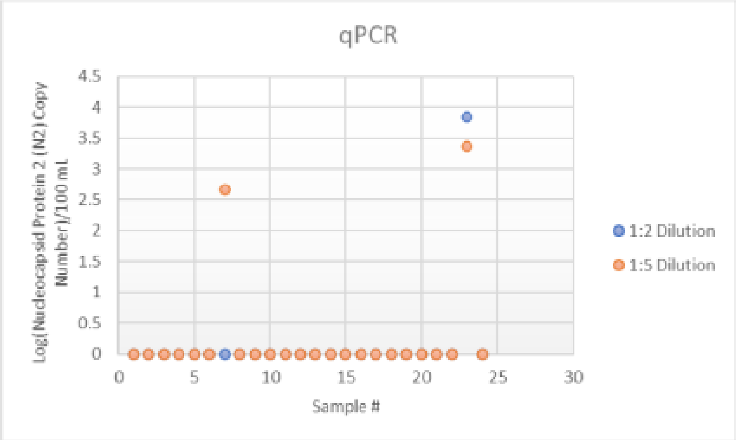


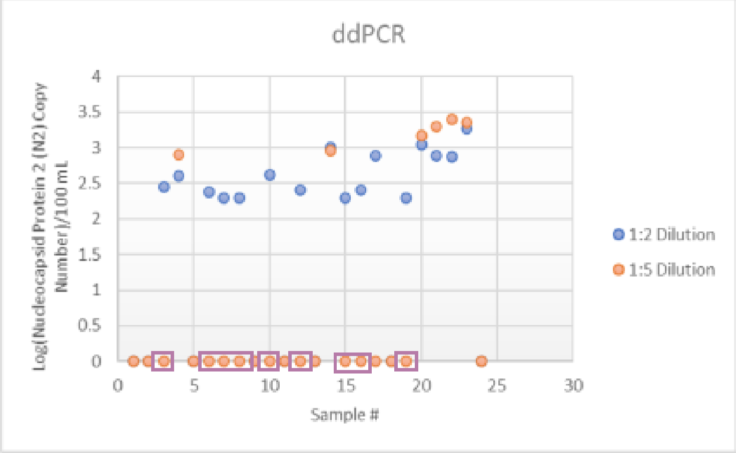
Adherence to MIQE Guidelines

- Negative Extraction Control
- Negative Reverse Transcription Control
- Negative Template Control
- Processing Control

Differences in Quantification across Target Concentrations

Lower Target Concentration

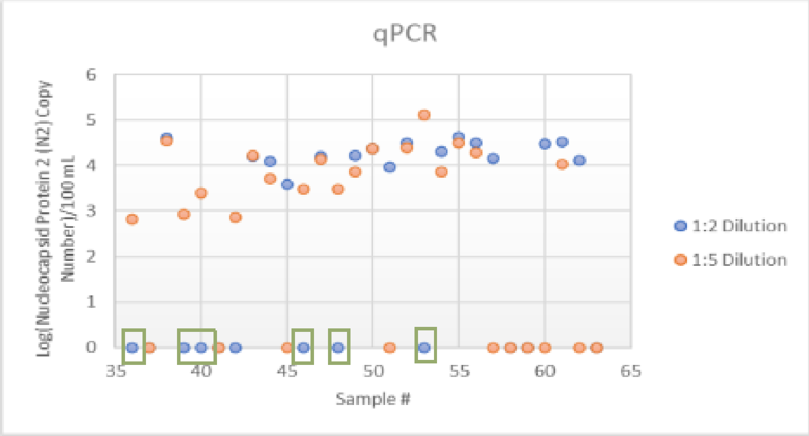


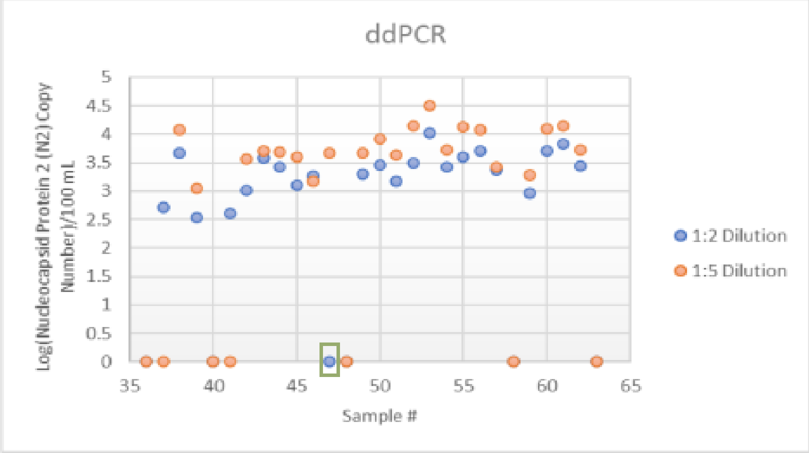


Spearman Rank Correlation qPCR vs ddPCR

1:2 Dilution: **0.36**
1:5 Dilution: **-0.13**

Higher Target Concentration





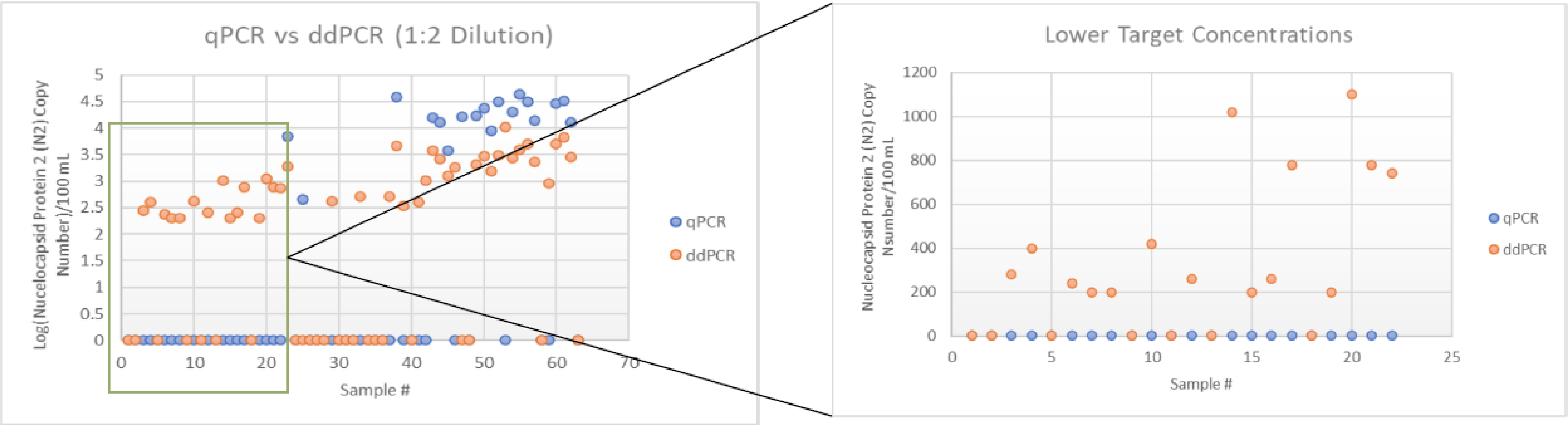
Spearman Rank Correlation qPCR vs ddPCR

1:2 Dilution: **0.53**
1:5 Dilution: **0.66**

Sample is positive at a 1:2 Dilution and is non-detectable at a 1:5 Dilution

Sample is non-detectable at a 1:2 Dilution and is positive at a 1:5 Dilution

Sensitivity of ddPCR versus qPCR



Test Method	qPCR +	qPCR -
ddPCR+	18	22
ddPCR -	2	22

Positive Percent Agreement: 90%
Negative Percent Agreement: 50%
Overall Percent Agreement: **62.5%**

Contact Information: Mark Ciesielski (mciesi3@email.unc.edu) & Rachel Noble (rtnoble@email.unc.edu)

Thank you!

advancing the science of water®