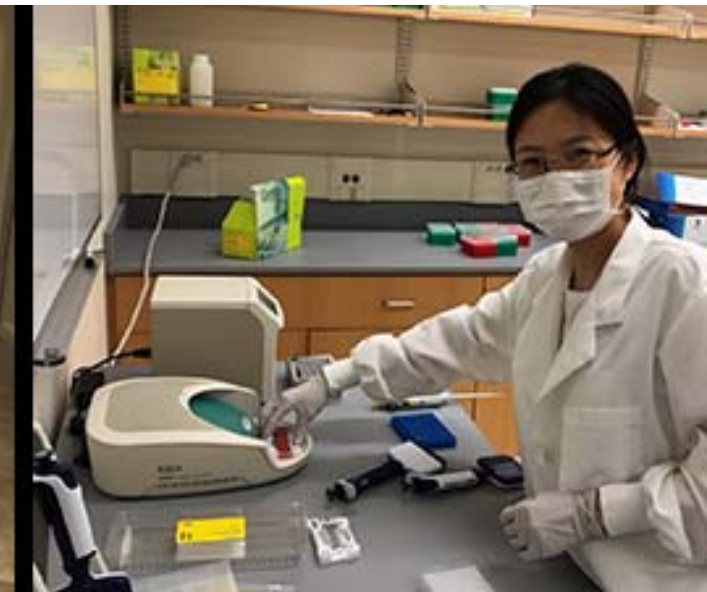
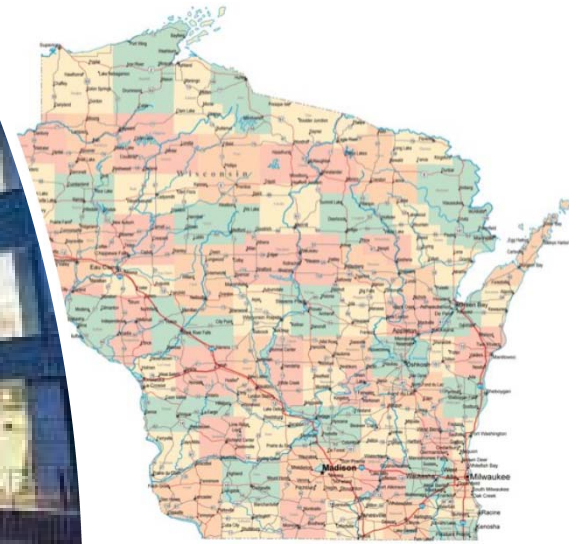


Wastewater surveillance  
for SARS-CoV-2 to assess  
citywide trends:  
variability, sensitivity, and  
correlation to case data

Sandra McLellan, Professor

School of Freshwater Sciences,  
University of Wisconsin-Milwaukee



# Wisconsin SARS-CoV-2 Wastewater Surveillance

## SARS-CoV-2 surveillance team

Adelaide Rouget, Post Doc

Deb Dila, Research Associate

Melinda Bootsma, Technician

Lexi Passante, Grad Student

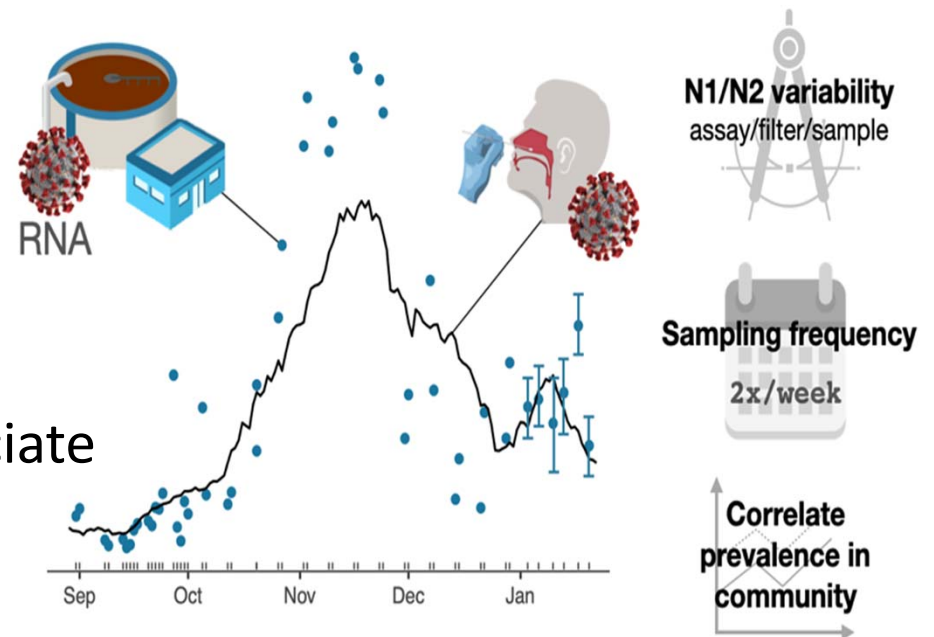
Melissa Schussmann, Grad Student

Angela Schmoldt, GLGC Research Associate

Shuchen Feng, Post Doc

Jill McClary, Post Doc

Ryan Newton, Assistant Professor



# McLellan Lab Research

New indicators of fecal pollution  
*Microbiome of animals and sewage*

sewage population structure



water analysis



What are the organisms in sewage?  
What is the health risk and viral load?

Should the beach be  
closed?

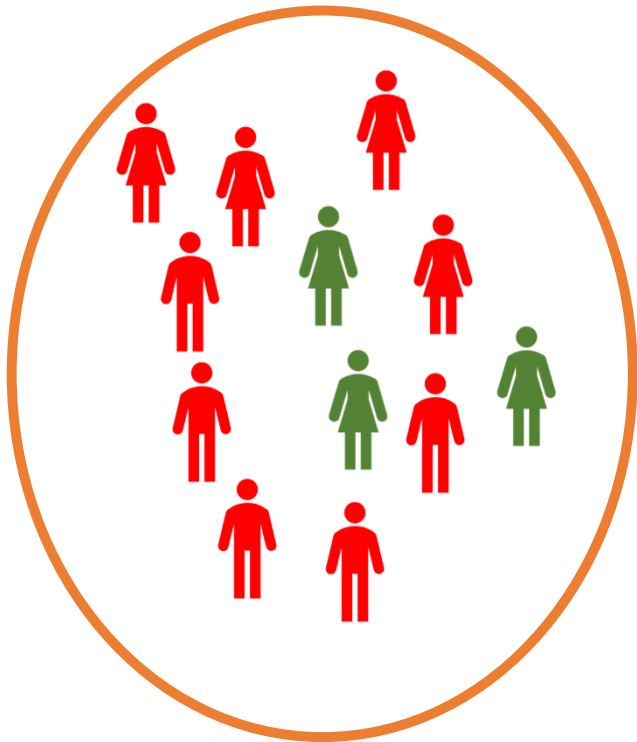
# How can we test the whole community at once?

Sewage is a representative sample of humans



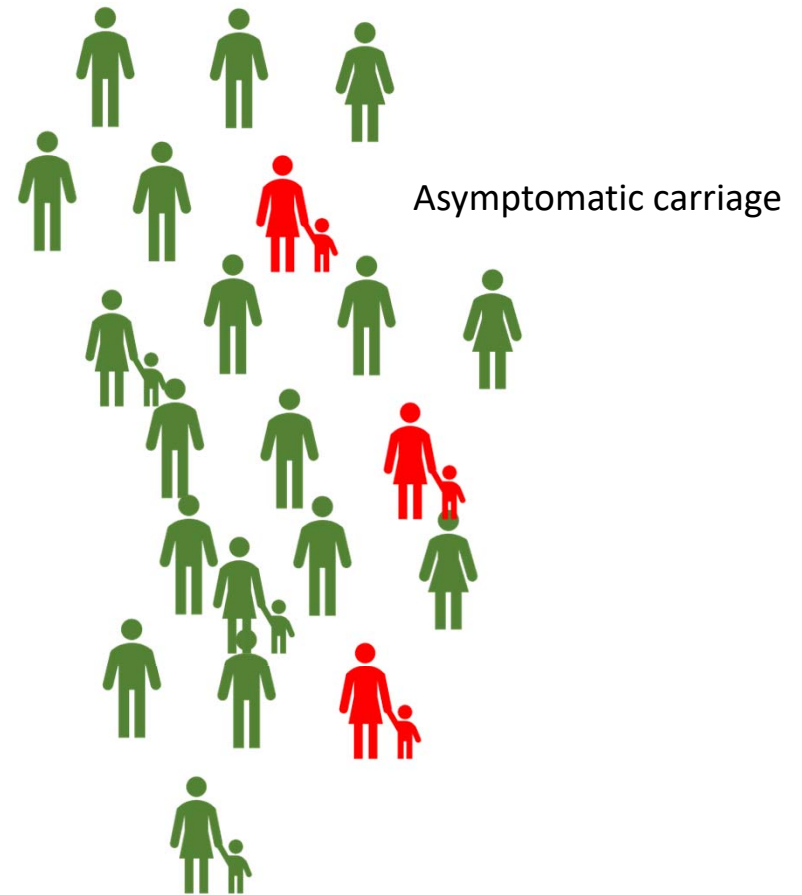
SARS-CoV-2 (viruses that causes Covid-19) is shed in feces, also enters sewer system through kleenex, saliva

How many people have Covid-19?  
This was the early testing scenario

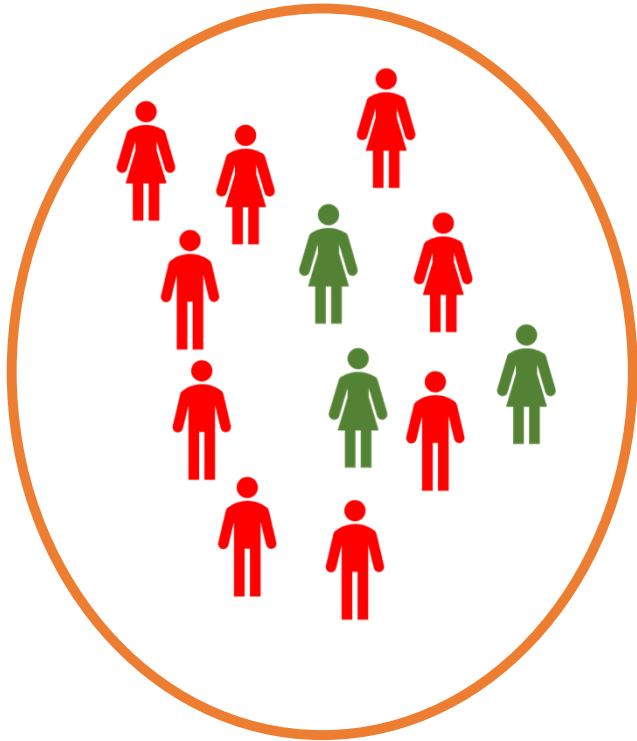


Test people who have symptoms

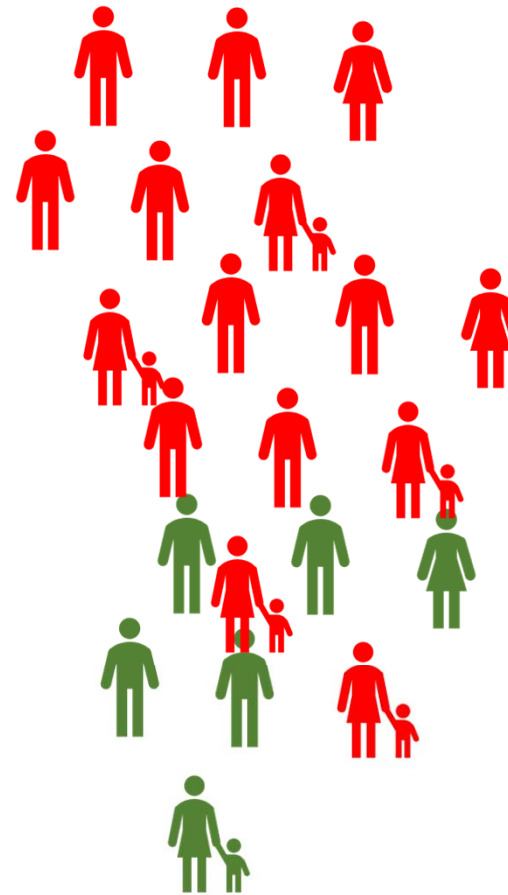
?



How many people have Covid-19?



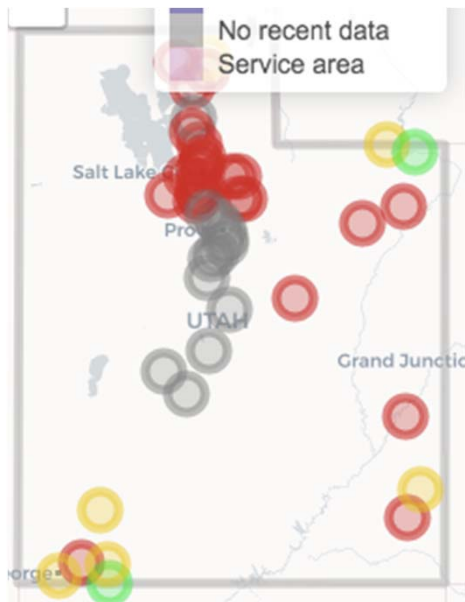
or?





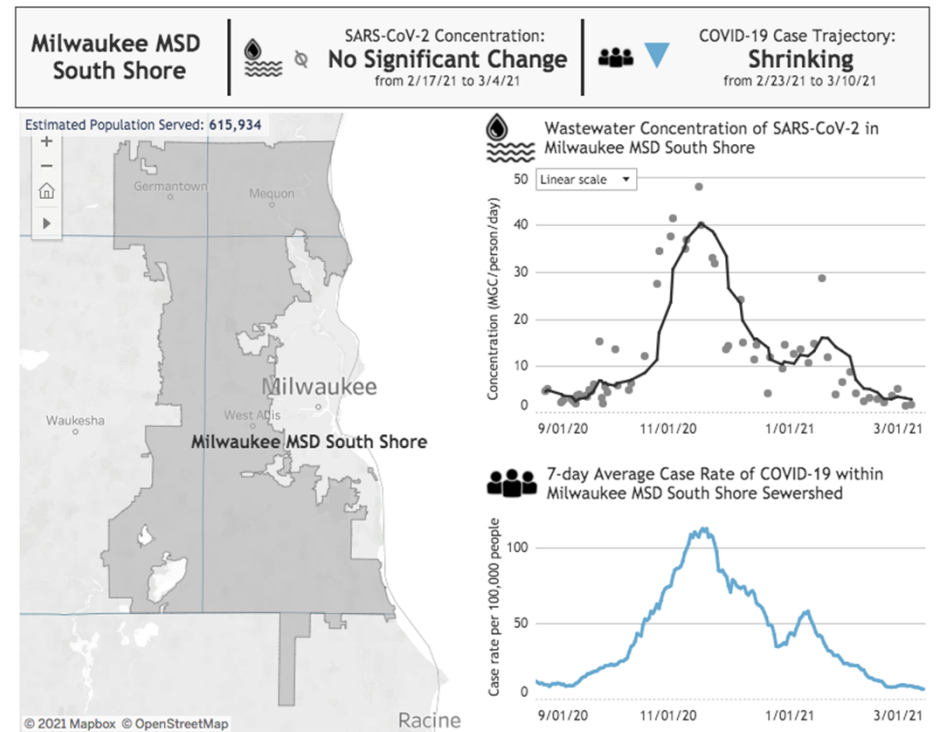
**Barrier 2.** Public health agencies want to see SARS-CoV-2 wastewater data in their own communities to gain confidence in its application and utility.

Growing examples in many areas



Wisconsin

Utah



Broad insights  
Experts Panel  
Public health insights

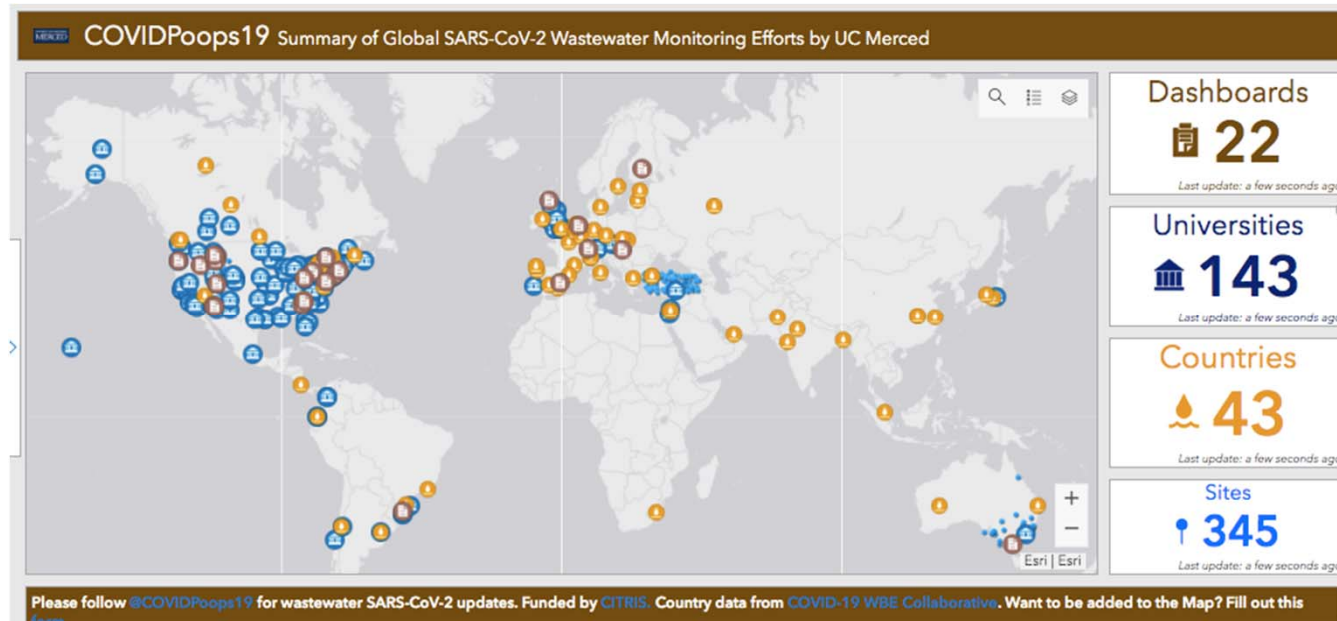
Data is not self-standing

Data needs to be  
simplified and easy to use

Proof of concept

Explanations on variability

Timely



<https://www.covid19wbec.org/>



# Wisconsin SARS-CoV-2 Wastewater Surveillance

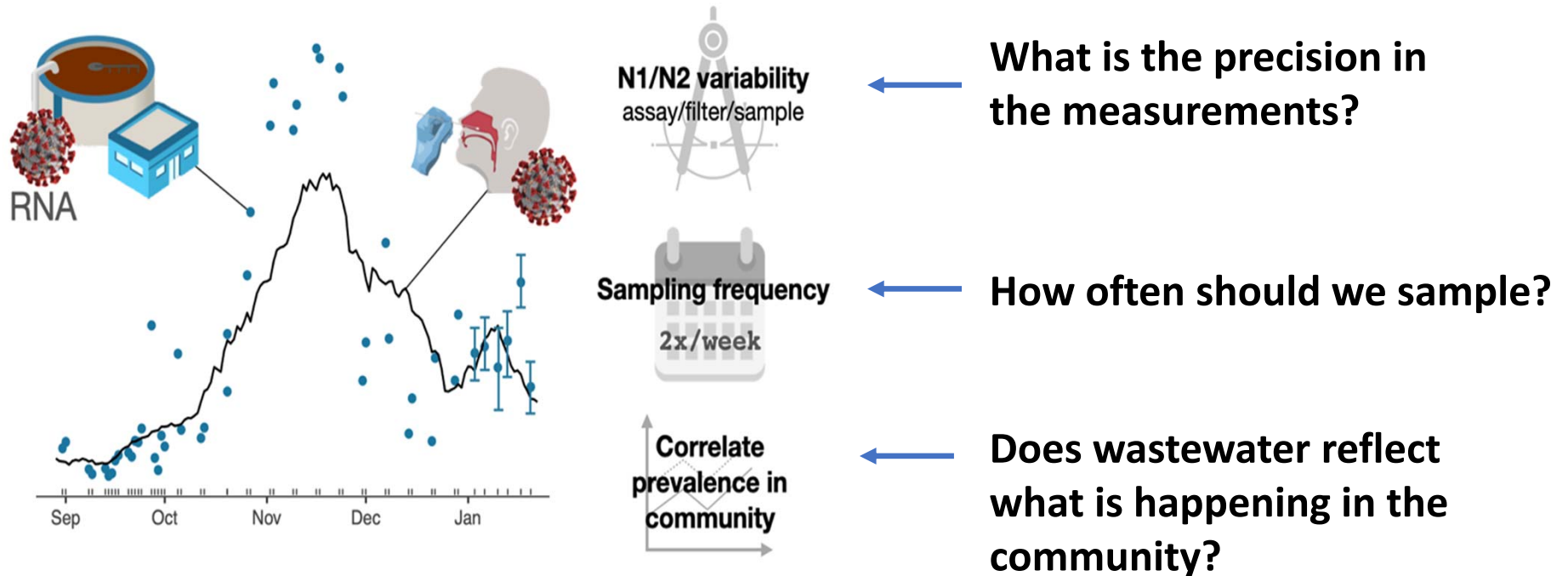
## McLellan Lab Activities

- Initiated monitoring for Racine, Milwaukee, and Green Bay as part of a Statewide surveillance effort with the Wisconsin State Laboratory of Hygiene at UW-Madison, funded by Department of Health Services, now sampling **12 WWTPs**
- Developing methods for tracking variants in sewage

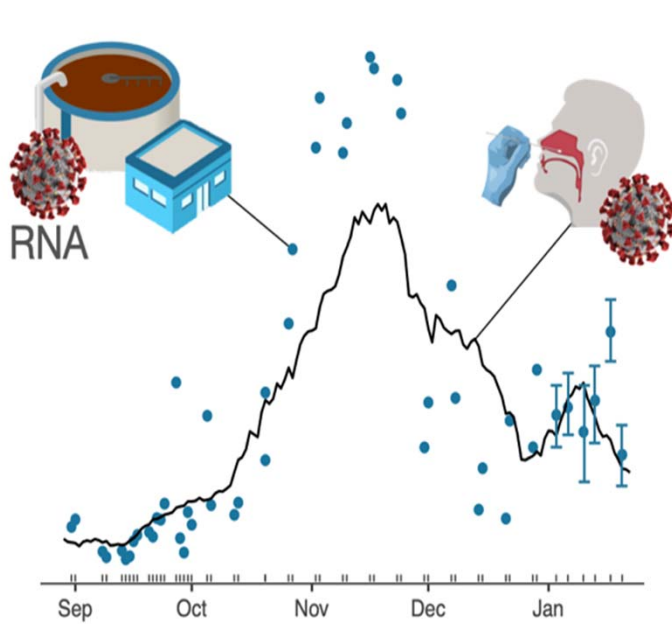


12 service areas  
~~1.8 M people

# Paper submitted to Environmental Science and Technology



# Paper submitted to Environmental Science and Technology



**N1/N2 variability**  
assay/filter/sample



**What is the precision in  
the measurements?**

**Sampling frequency**



**How often should we sample?**

**Correlate  
prevalence in  
community**



**Does wastewater reflect  
what is happening in the  
community?**

# Sampling and method overview

24 hour flow weighted sample at the WWTP



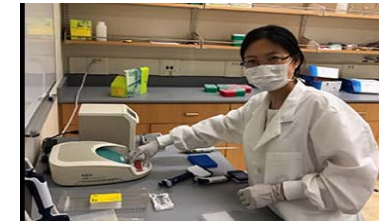
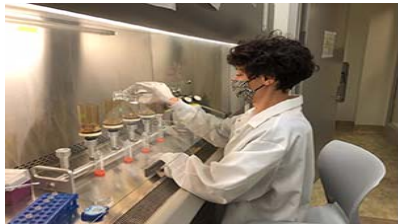
Sample processing  
(RNA concentration)



RNA extraction



ddPCR quantification



# Sampling and method overview

24 hour flow weighted sample at the WWTP



Standard



Tricky



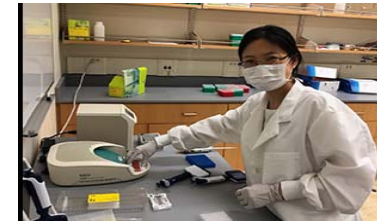
Just like the clinical lab



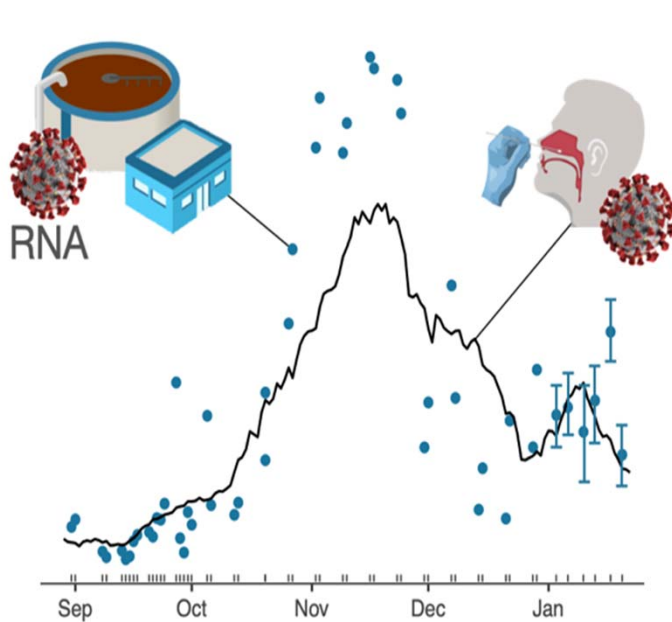
Sample processing  
(RNA concentration)

RNA extraction

ddPCR quantification



# Paper submitted to Environmental Science and Technology



**N1/N2 variability**  
assay/filter/sample



← What is the precision in the measurements?

**Sampling frequency**



← **How often should we sample?**

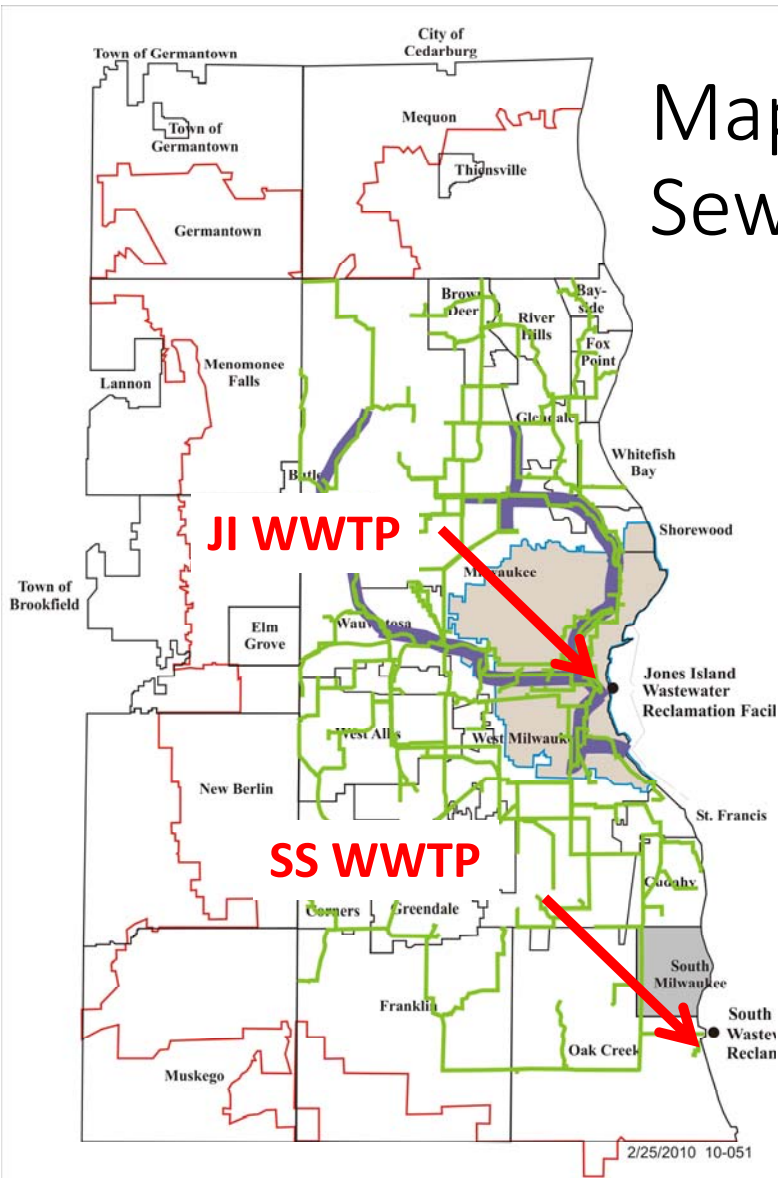
**Correlate prevalence in community**



← Does wastewater reflect what is happening in the community?



# Map of Milwaukee, WI Sewer Service Area



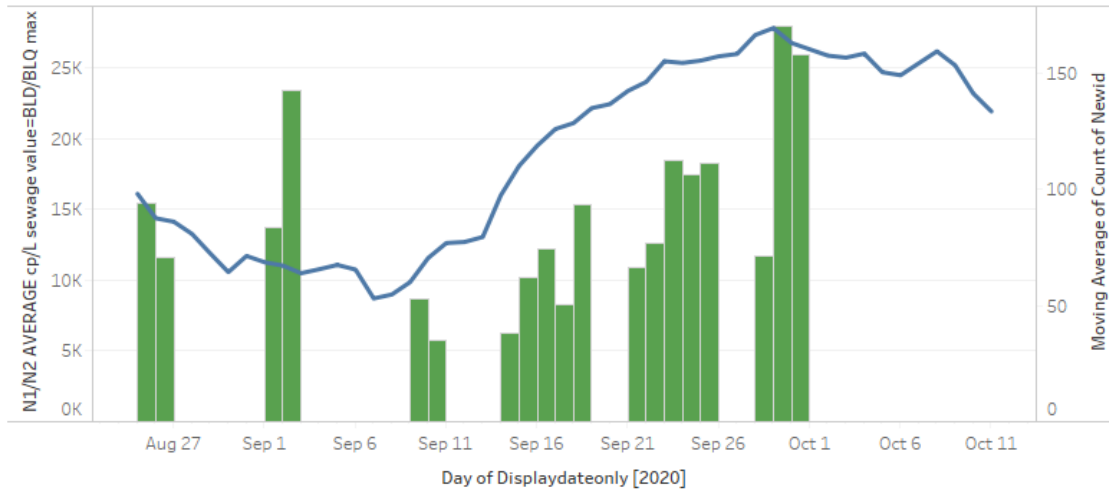
400 square miles  
6,000 miles of pipes

1M people

Two treatment plants

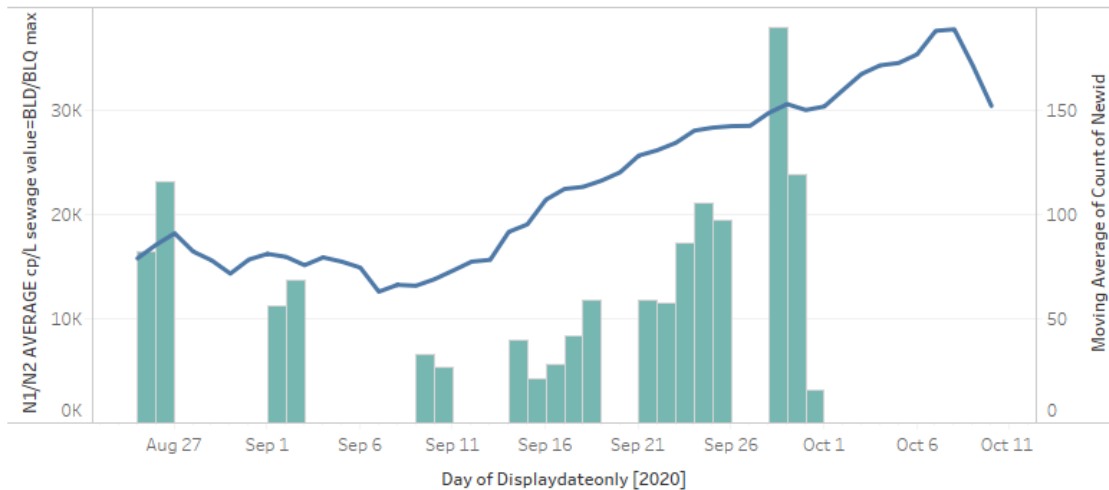
Separated sewer system (88%)  
Combined sewer system (12%)

MKE\_Jones Island



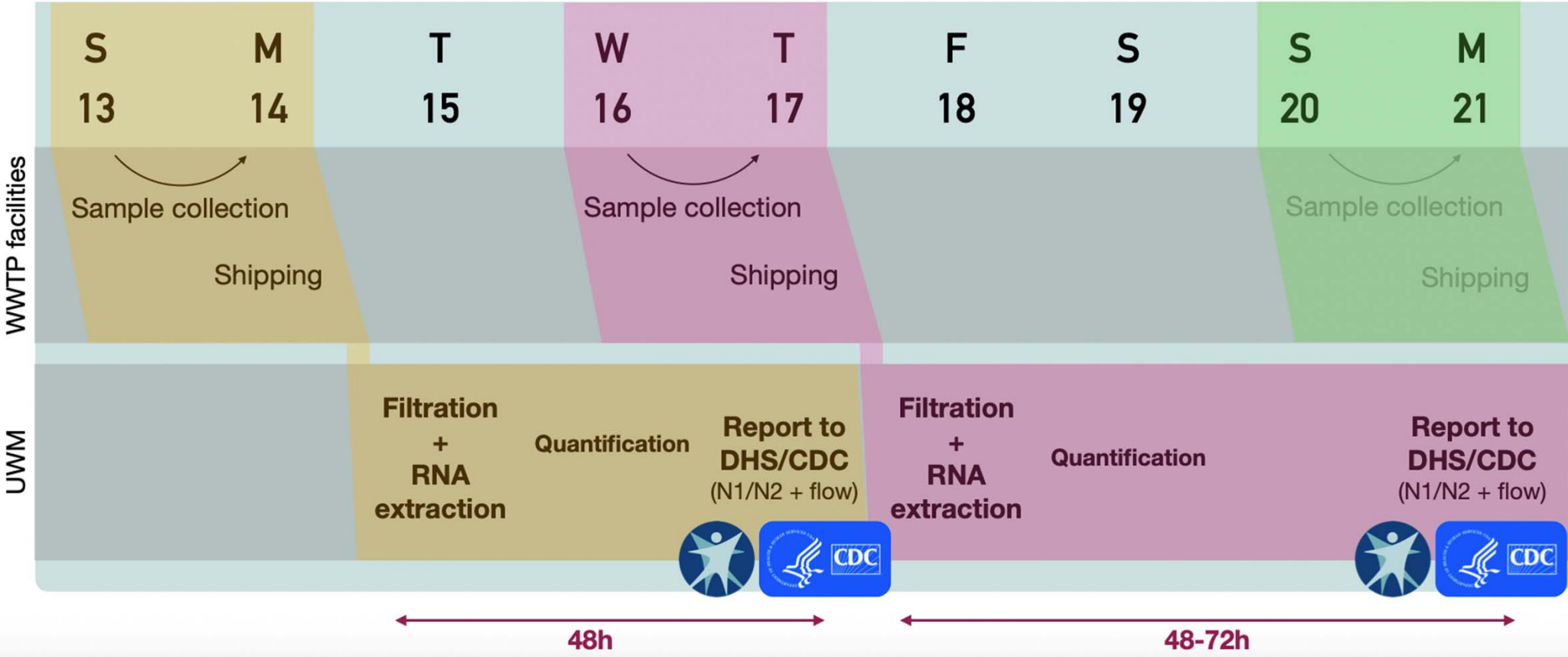
Two days a week could capture the trends

MKE\_South Shore

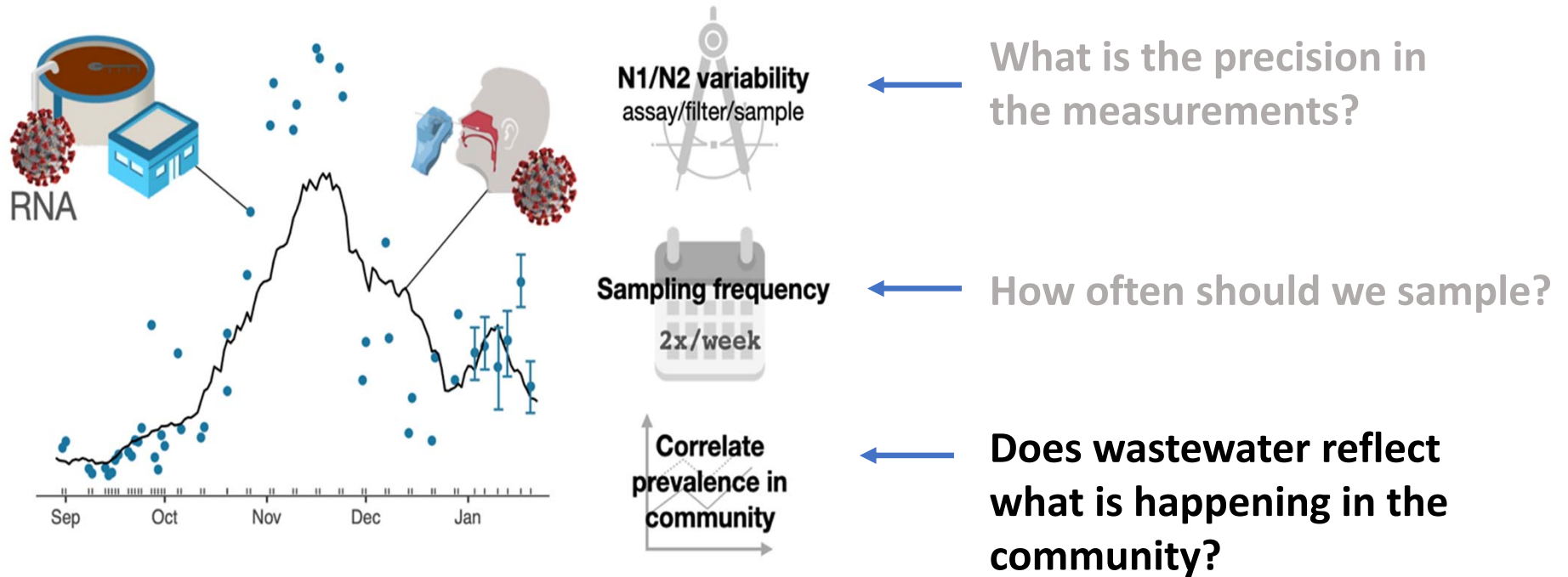


Each sample can be \$350-\$450  
Need to be cost effective while providing good data

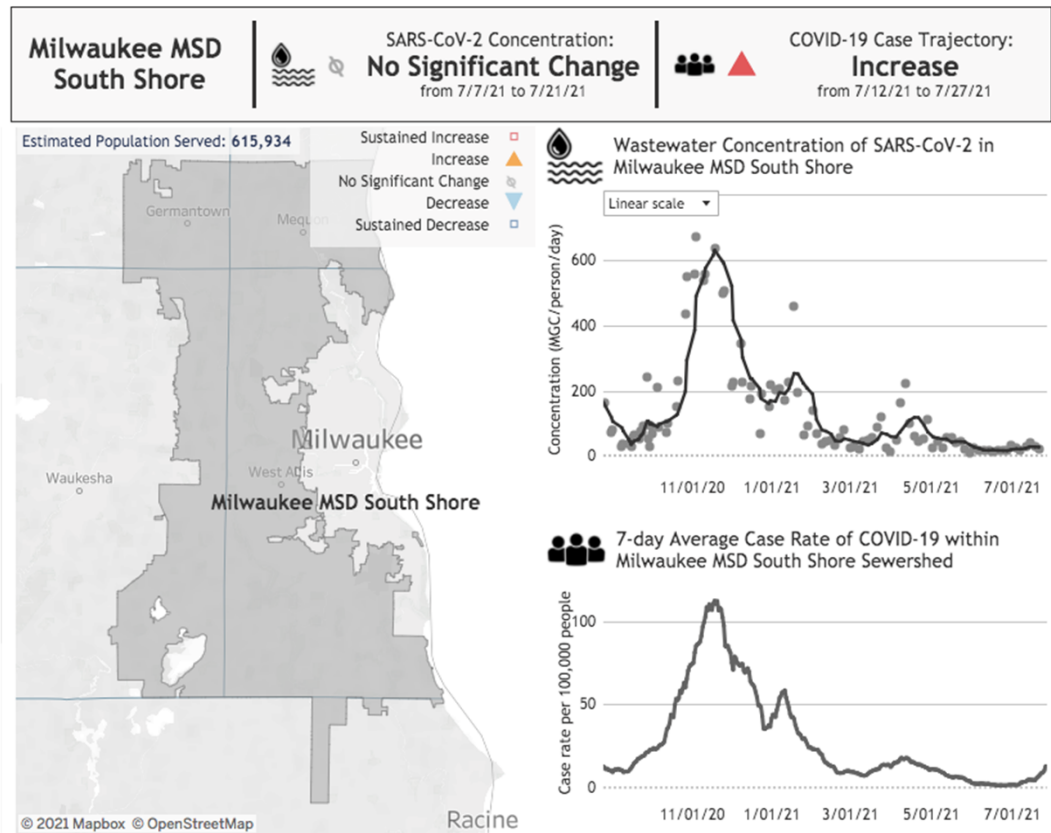
# December 2020



# Paper submitted to Environmental Science and Technology



# Wastewater dashboard



# Milwaukee MSD South Shore



SARS-CoV-2 Concentration:  
**No Significant Change**  
from 7/7/21 to 7/21/21



COVID-19 Case Trajectory:  
**Increase**  
from 7/12/21 to 7/27/21

Estimated Population Served: 615,934

- Sustained Increase
- Increase
- No Significant Change
- Decrease
- Sustained Decrease

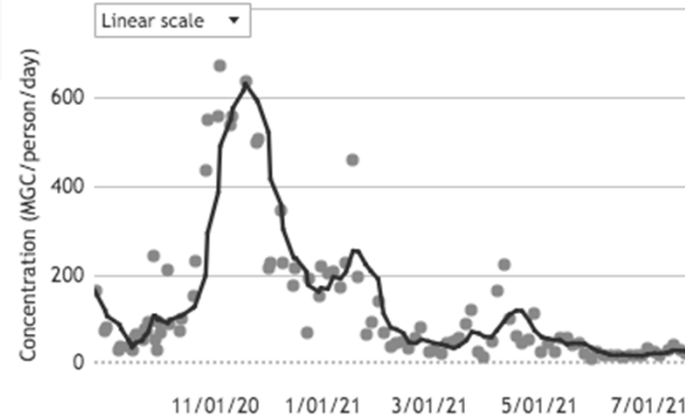


© 2021 Mapbox © OpenStreetMap

Racine



## Wastewater Concentration of SARS-CoV-2 in Milwaukee MSD South Shore



## 7-day Average Case Rate of COVID-19 within Milwaukee MSD South Shore Sewershed





# Milwaukee MSD Jones Island



SARS-CoV-2 Concentration:  
**No Significant Change**  
from 7/7/21 to 7/21/21

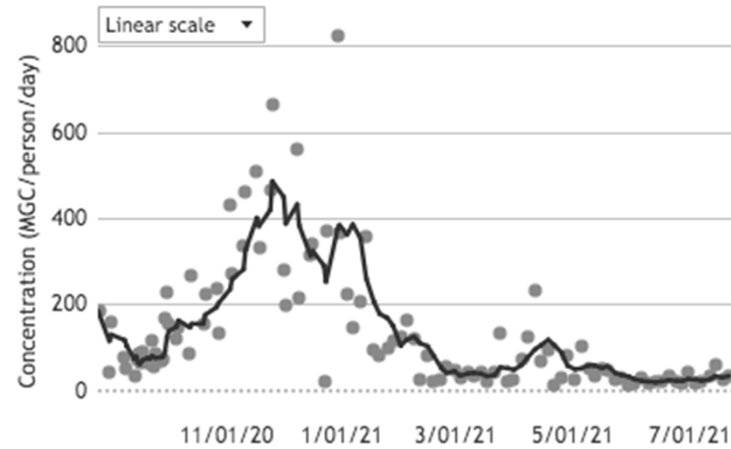


COVID-19 Case Trajectory:  
**Increase**  
from 7/12/21 to 7/27/21

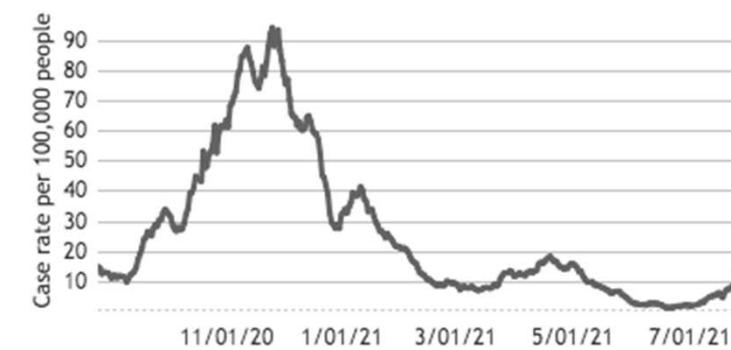
Estimated Population Served: 470,007



### Wastewater Concentration of SARS-CoV-2 in Milwaukee MSD Jones Island



### 7-day Average Case Rate of COVID-19 within Milwaukee MSD Jones Island Sewershed



# Racine Wastewater Utility

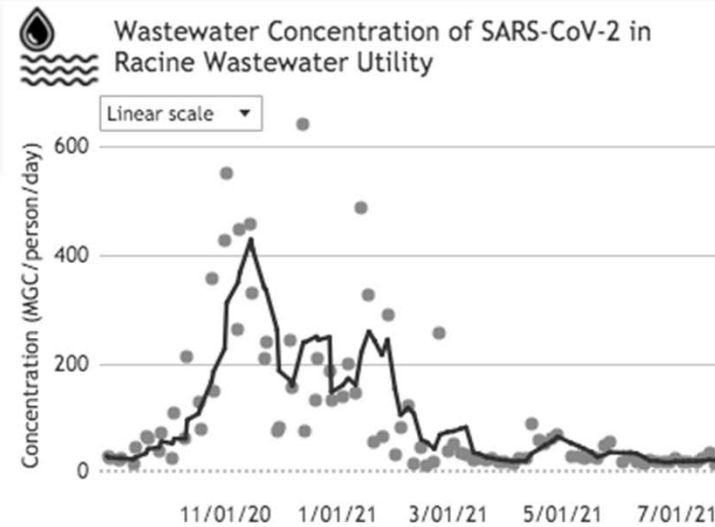
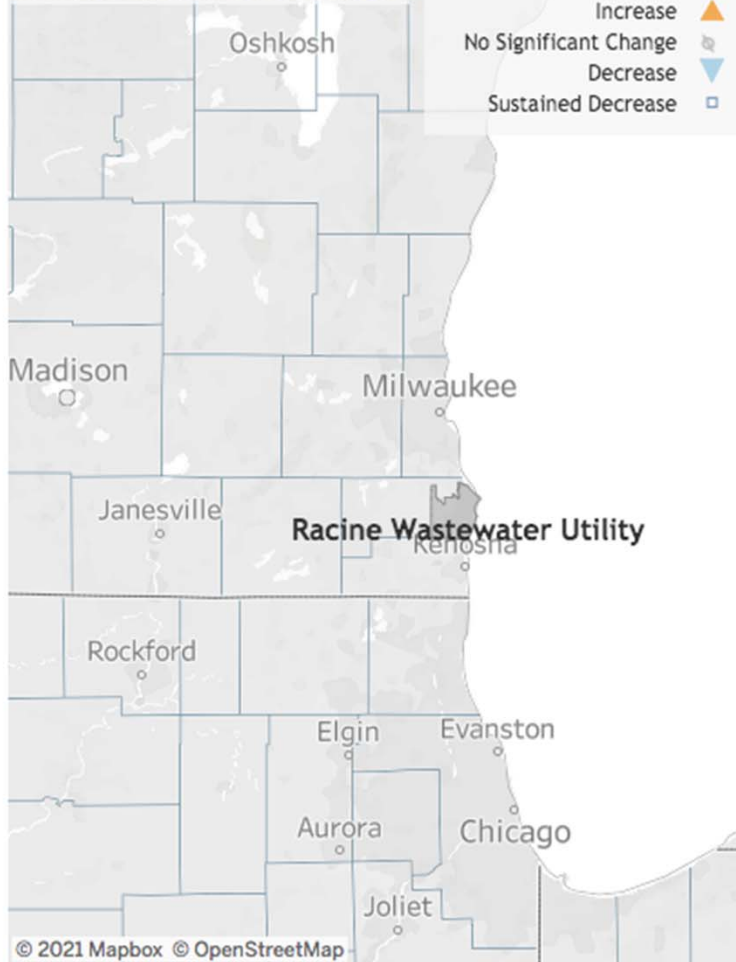


SARS-CoV-2 Concentration:  
**No Significant Change**  
from 7/7/21 to 7/21/21

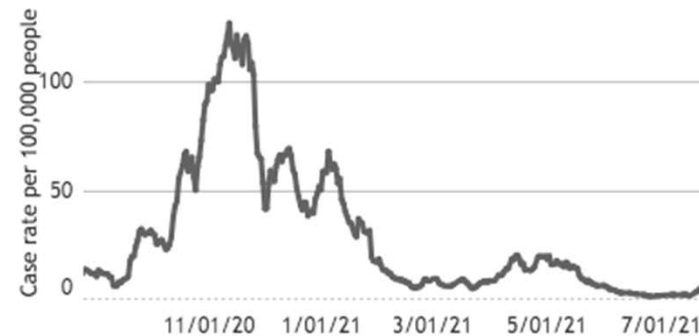


COVID-19 Case Trajectory:  
**Increase**  
from 7/12/21 to 7/27/21

Estimated Population Served: 139,000



### 7-day Average Case Rate of COVID-19 within Racine Wastewater Utility Sewershed



# Green Bay MSD



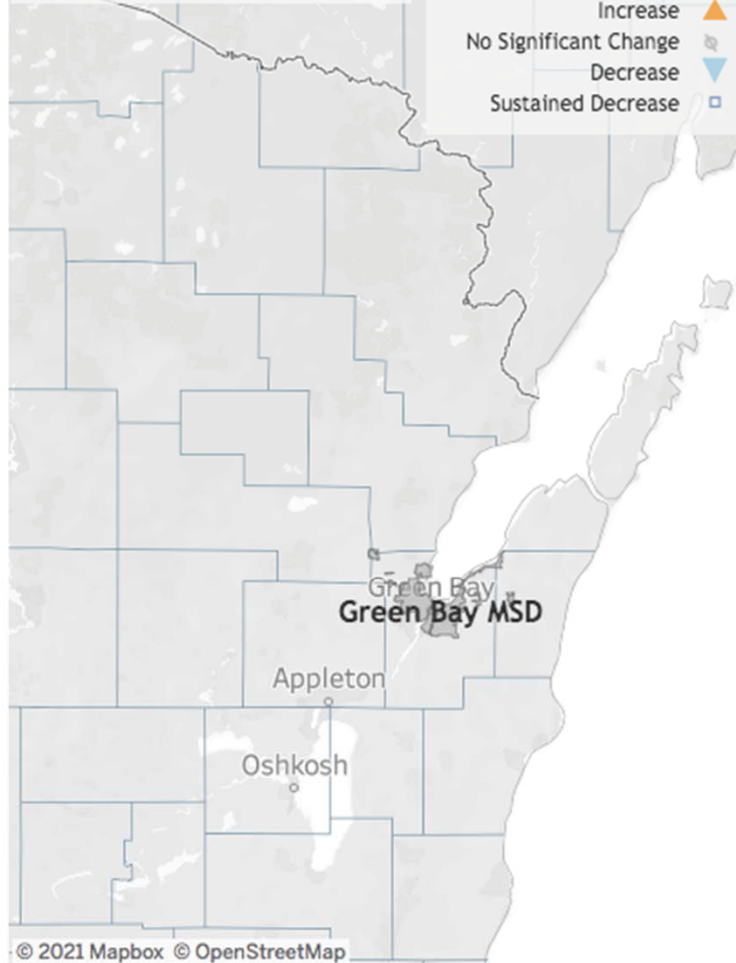
SARS-CoV-2 Concentration:  
**No Significant Change**  
from 7/5/21 to 7/21/21



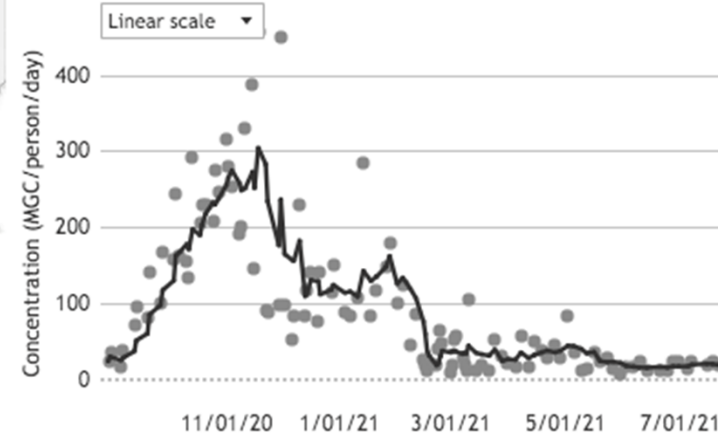
COVID-19 Case Trajectory:  
**Increase**  
from 7/12/21 to 7/27/21

Estimated Population Served: 189,000

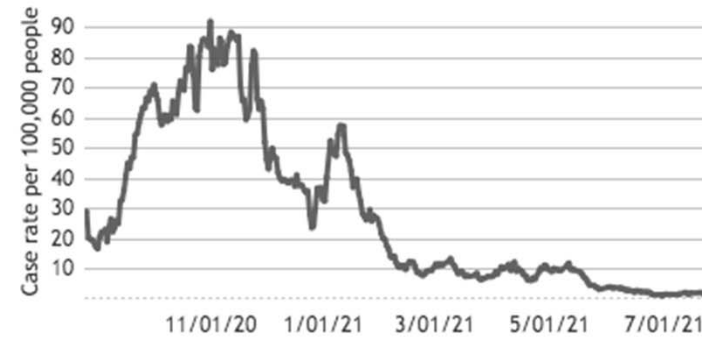
- Sustained Increase
- Increase
- No Significant Change
- Decrease
- Sustained Decrease



## Wastewater Concentration of SARS-CoV-2 in Green Bay MSD



## 7-day Average Case Rate of COVID-19 within Green Bay MSD Sewershed





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[COVID-19 activity level](#)

## COVID-19: Wisconsin Coronavirus Wastewater Monitoring Network

Jump to specific COVID-19 chart on this page:

- [Wisconsin wastewater surveillance](#)
- [Learn how to download our data](#)

### COVID-19: Data Pages

[Activity Level by Region and County](#) >

[Alternate Care Facility](#) >

<https://www.dhs.wisconsin.gov/covid-19/wastewater.htm>

## Evaluation of Sampling, Analysis, and Normalization Methods for SARS-CoV-2 Concentrations in Wastewater to Assess COVID-19 Burdens in Wisconsin Communities

Shuchen Feng, Adelaide Roguet, Jill S. McClary-Gutierrez, Ryan J. Newton, Nathan Kloczko, Jonathan G. Meiman, and Sandra L. McLellan\*



Cite This: <https://doi.org/10.1021/acsestwater.1c00160>



Read Online

Recently accepted by Emerging Infectious Disease



*Disclaimer: Early release articles are not considered as final versions. Any changes will be reflected in the online version in the month the article is officially released.*

Volume 27, Number 9—September 2021

*Online Report*

## SARS-CoV-2 Wastewater Surveillance for Public Health Action

Jill S. McClary-Gutierrez<sup>1</sup>, Mia C. Mattioli, Perrine Marcenac, Andrea I. Silverman, Alexandria B. Boehm, Kyle Bibby, Michael Balliet, Francis L. de los Reyes, Daniel Gerrity, John F. Griffith, Patricia A. Holden, Dimitrios Katehis, Greg Kester, Nathan LaCross, Erin K. Lipp, Jonathan Meiman, Rachel T. Noble, Dominique Brossard<sup>✉</sup>, and Sandra L. McLellan<sup>✉</sup>

### On This Page

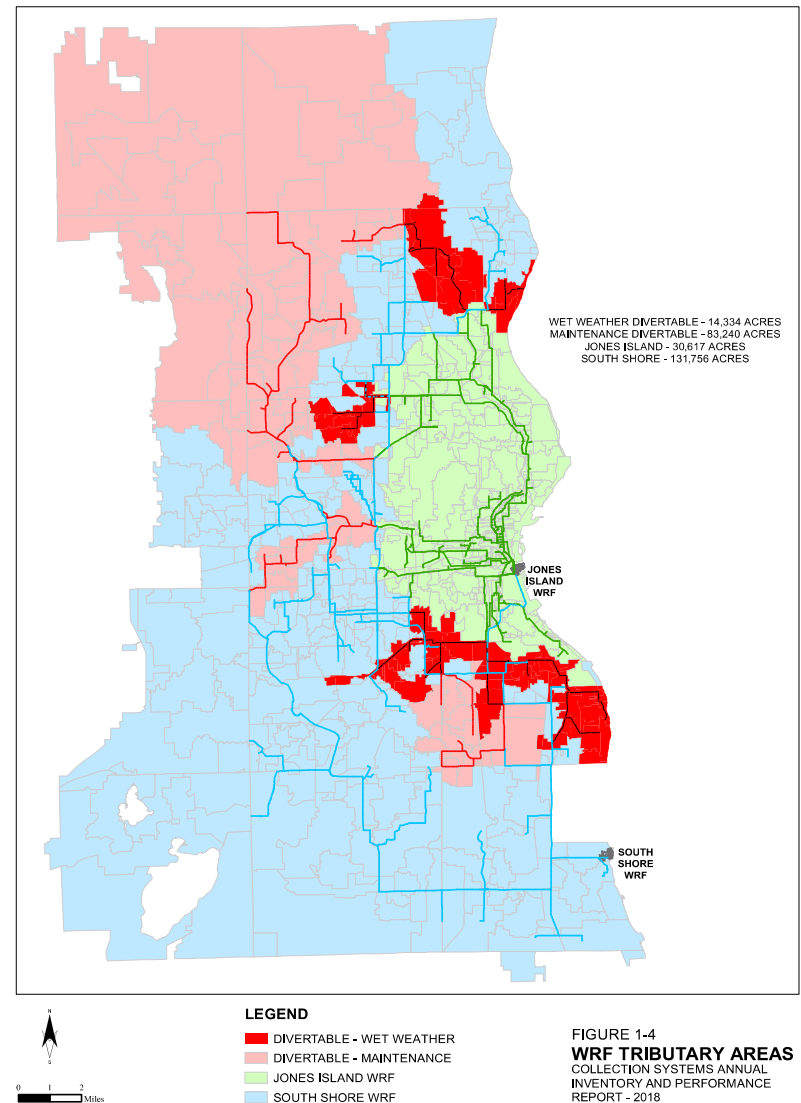
[Interdisciplinary Focus Group Discussions](#)



Many basic science questions remain

What is the effect of travel time?

What is the effect of temperature?

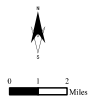
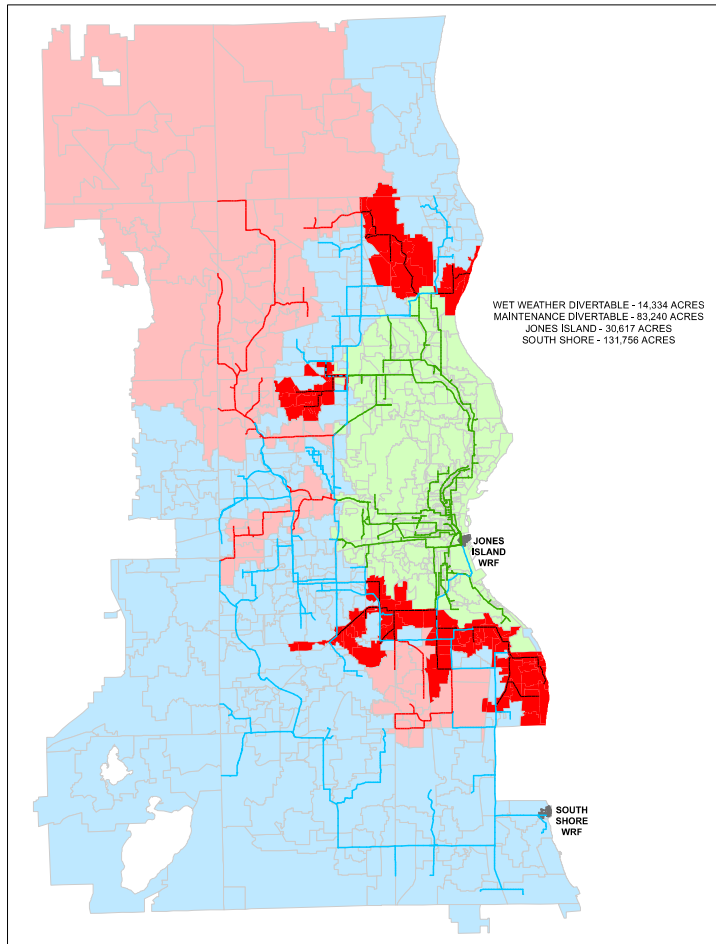


Many basic science questions remain

What is the effect of travel time?

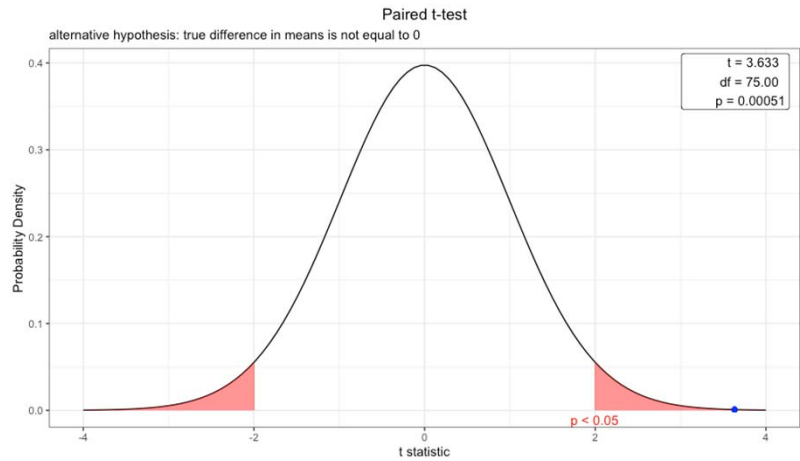
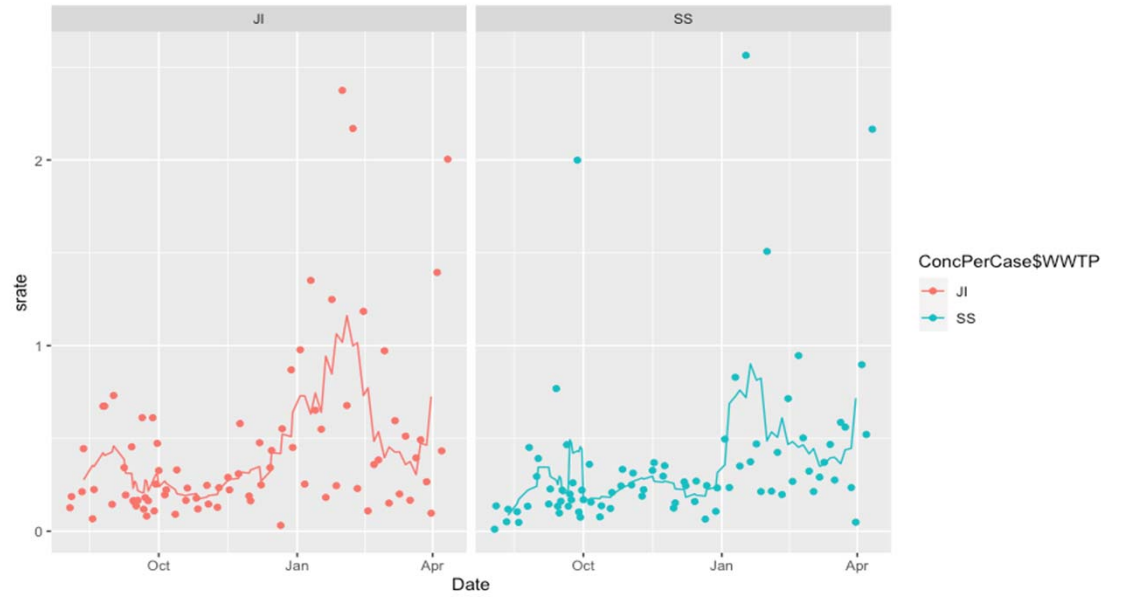
What is the effect of temperature?



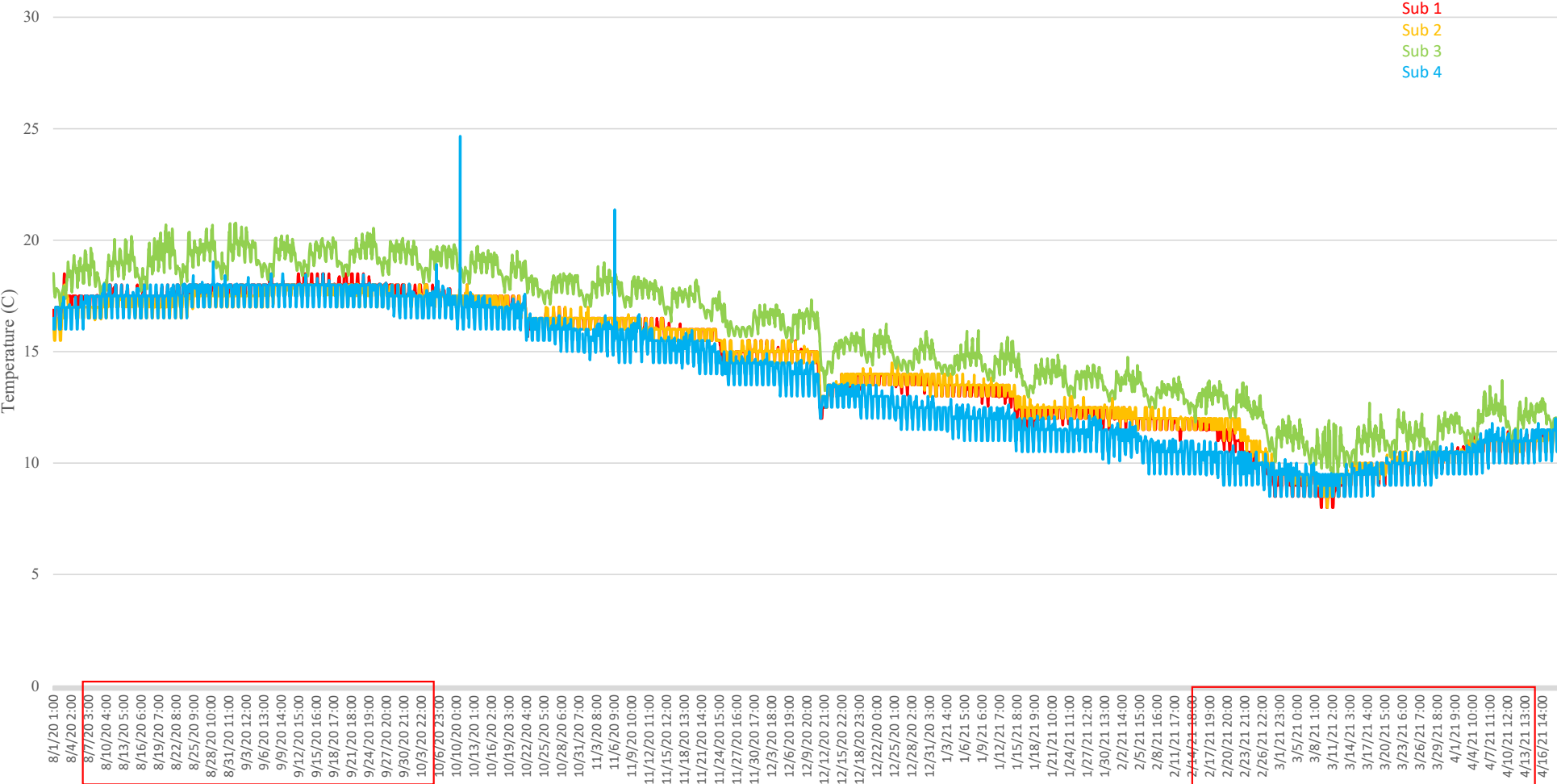


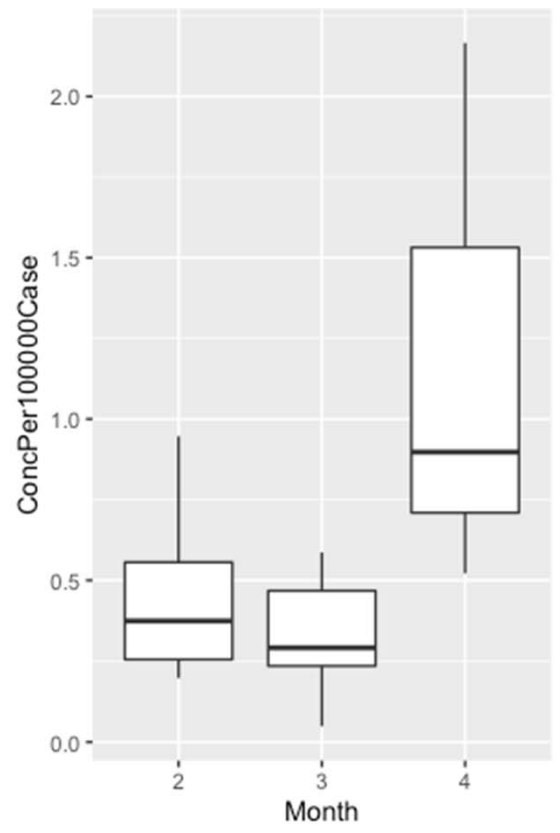
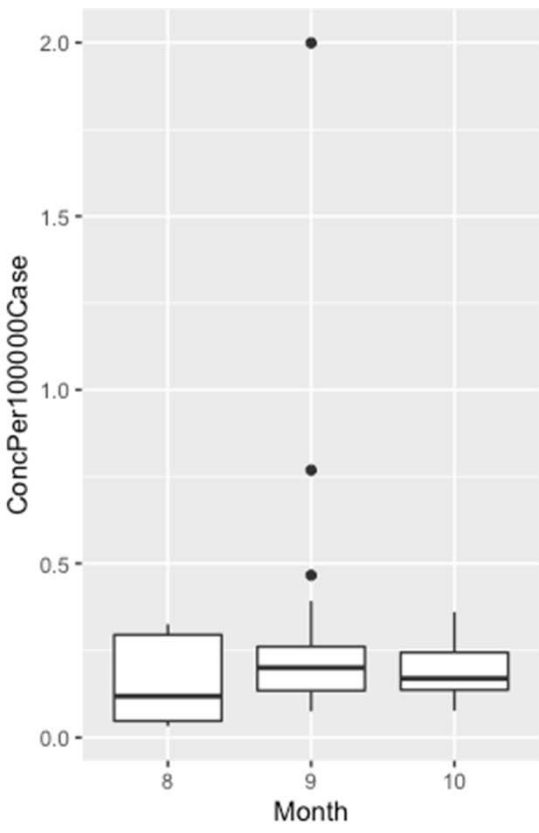
- LEGEND**
- DIVERTABLE - WET WEATHER
  - DIVERTABLE - MAINTENANCE
  - JONES ISLAND WRF
  - SOUTH SHORE WRF

**FIGURE 1-4**  
**WRF TRIBUTARY AREAS**  
 COLLECTION SYSTEMS ANNUAL  
 INVENTORY AND PERFORMANCE  
 REPORT - 2018



# August 2020 - April 2021 Temperatures by Subsystem





```

Response AveTemp :
                Df Sum Sq Mean Sq F value Pr(>F)
ConcPer100000Case  1  58.53   58.532   6.7993 0.01075 *
Residuals        86  740.33    8.608

Response Month :
                Df Sum Sq Mean Sq F value Pr(>F)
ConcPer100000Case  1  44.15   44.154   6.8276 0.01059 *
Residuals        86  556.16    6.467
  
```



# Variants are evolving

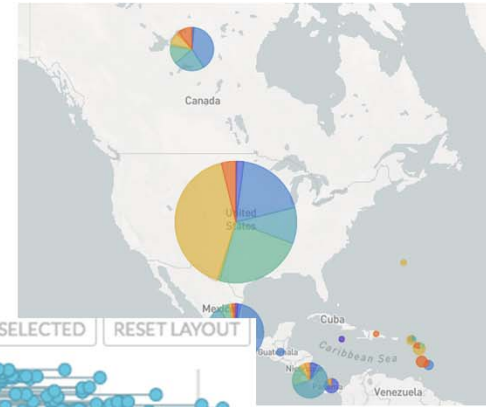
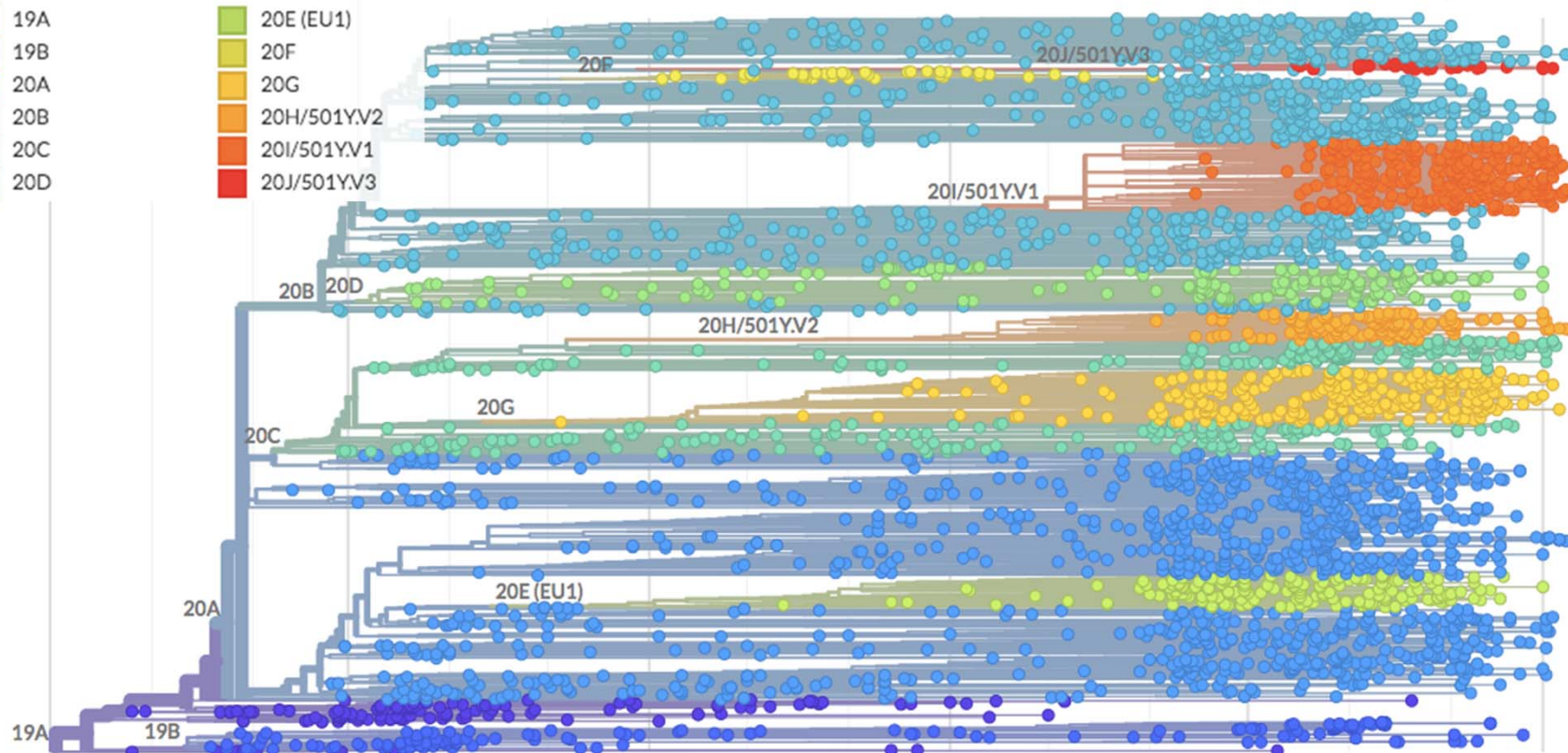
nextstrain.org

## Phylogeny

Clade ^

- 19A
- 19B
- 20A
- 20B
- 20C
- 20D

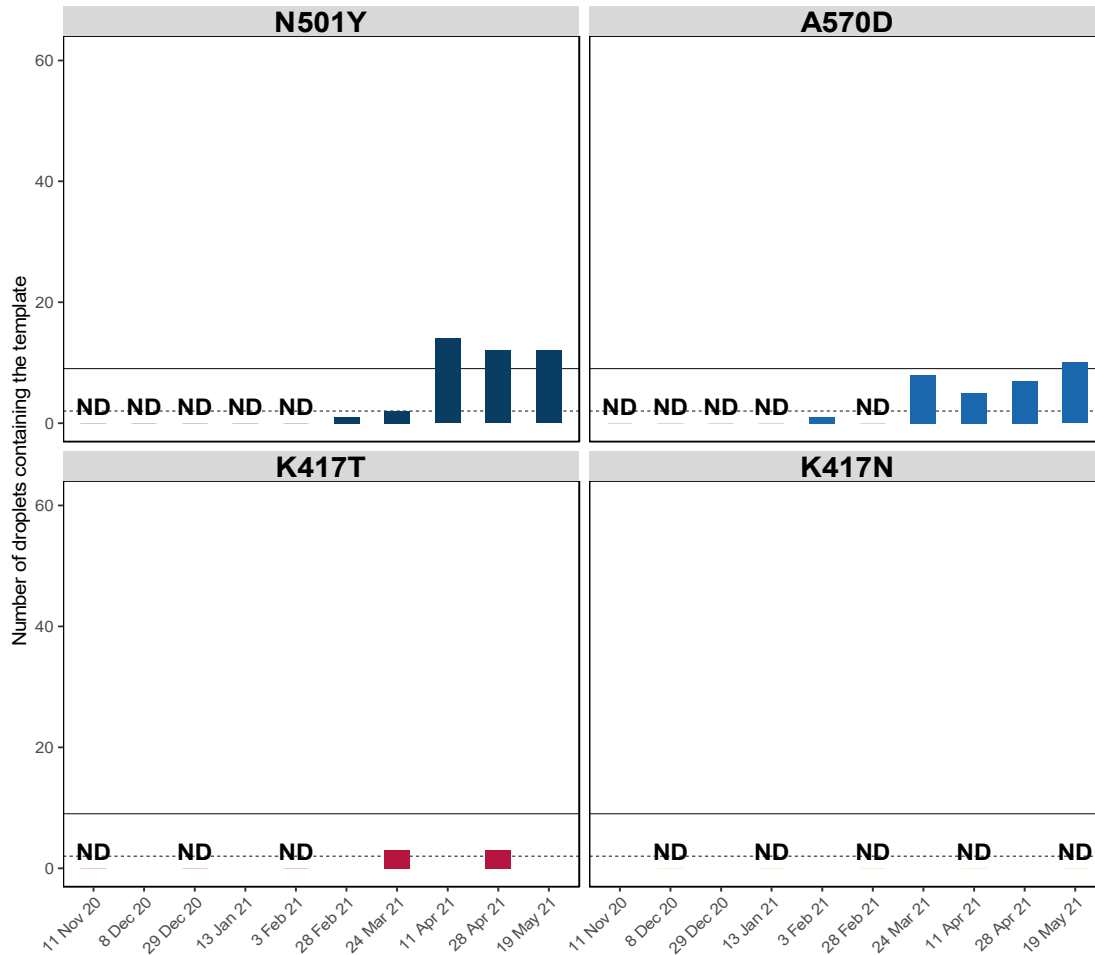
- 20E (EU1)
- 20F
- 20G
- 20H/501Y.V2
- 20I/501Y.V1
- 20J/501Y.V3



ZOOM TO SELECTED

RESET LAYOUT

## Detection level of SARS-CoV-2 mutations (dark colors) and wild-type (light colors)



N501Y targets Alpha, Gamma, and Beta variants

A570D targets Alpha  
K417T targets Gamma  
K417N targets Beta

High levels of the wild-type SARS-CoV-2 were detected in November to February, followed by a drop in overall levels, and increasing detection of the Alpha and Gamma variants.



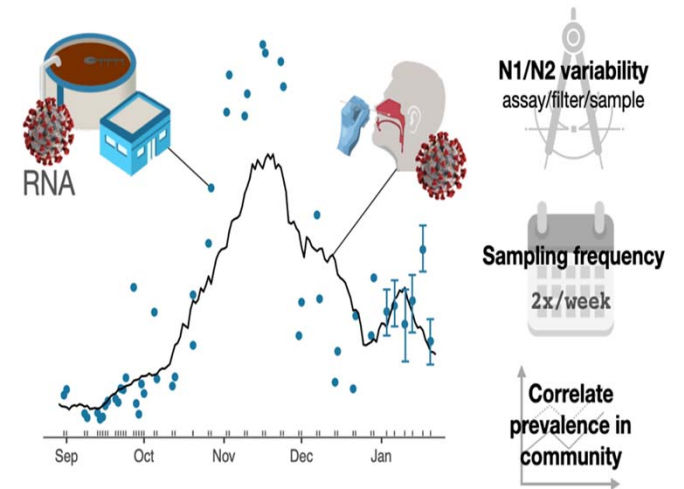
# Conclusions

Wastewater monitoring for SARS-CoV-2 captures the community burden of COVID-19

Low sampling and technical variability makes this method sensitive to changes in COVID-19 burdens: 9 cases /100,000

Ongoing research is needed to understand fate and transport of the virus to aid in result interpretation

**Detection and sequencing of variants in sewage samples is useful to benchmark spread and success of different variants**



# Acknowledgments

SCHOOL OF  
**Freshwater Sciences**



Ryan Newton, Assistant Professor  
Adelaide Roguet, Post Doc  
Melissa Schussman  
Elexius Passante  
Deb Dila, Research Specialist  
Angela Schmoltdt, GLGC

Shuchen Feng, Post Doc\*  
Jill McClary, Post Doc\*

Angela Schmoltdt, GLGC



Dagmara Antkiewicz, Scientist  
Jocelyn Hemming, Senior Microbiologist  
Martin Shafer, Senior Scientist  
Martin Collins, Senior Microbiologist  
Dawn Perkins, Environmental Toxicologist



WISCONSIN DEPARTMENT  
*of* HEALTH SERVICES

Jon Meiman, Chief Medical Officer  
Nathan Kloczko, Applied Epidemiology Fellow

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*UWM Office of the Chancellor  
School of Freshwater Sciences  
Department of Health Services, State of Wisconsin  
Alfred P. Sloan Foundation  
Milwaukee Metropolitan Sewerage District student fellowships **and in kind sampling***

Questions?