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SARS CoV-2 Wastewater Monitoring: Linking Research and Application To Meet Immediate Needs

Research Team

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- **Ohio Water Resource Center** Zuzana Bohrerova

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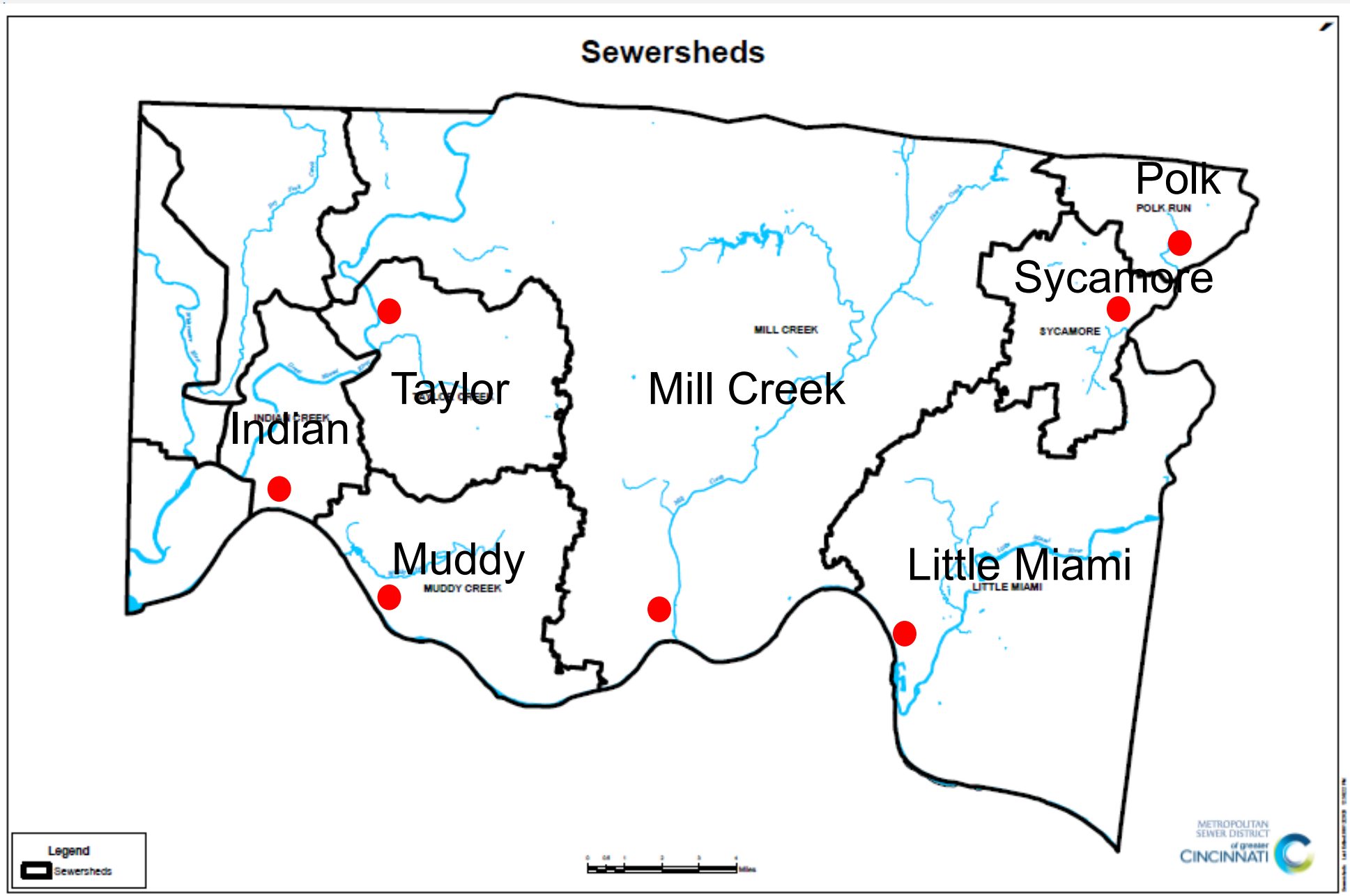
Into the *Sewer*

Characterizing and sampling a sewer district

Cincinnati Municipal Sewer District



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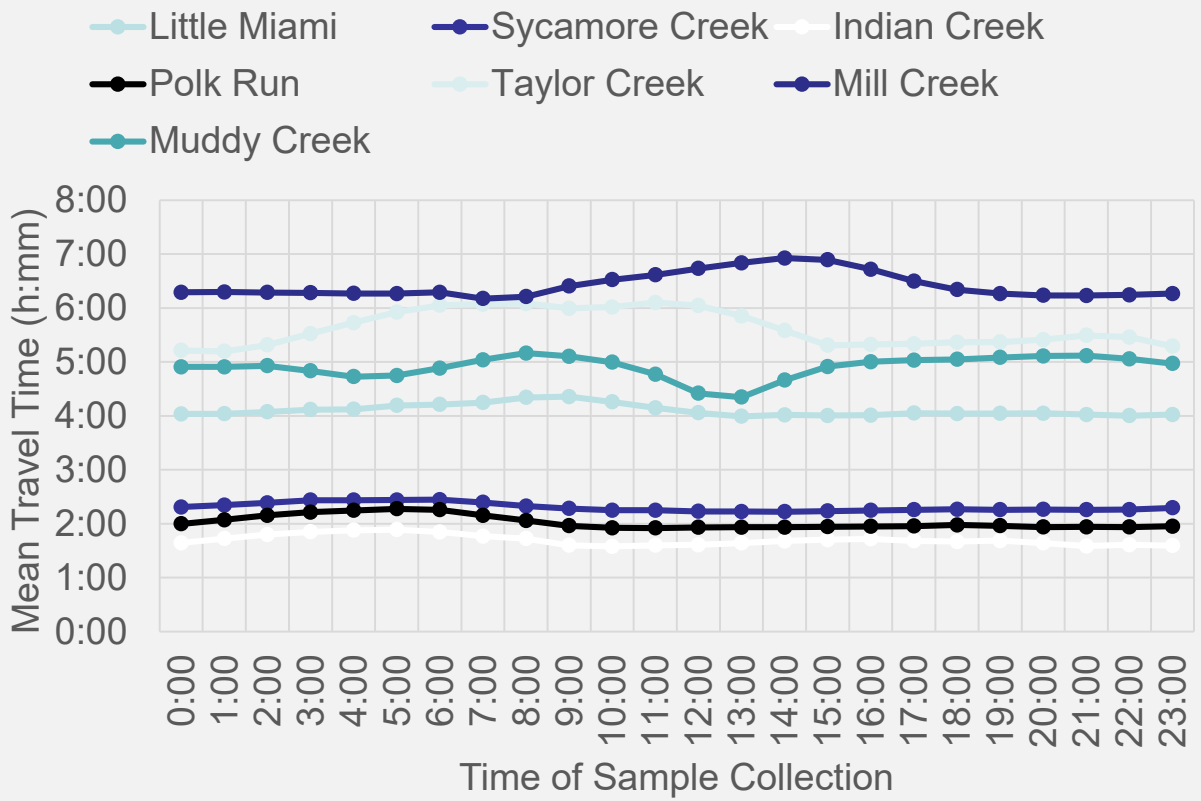
	% Industrial	% Combined	Dilution
Mill Creek *(118 MGD)*	5.0	40	0.5:1
Little Miami *(37 MGD)*	4.2	30	0.4:1
Muddy Creek *(14 MGD)*	<0.05	30	0.5:1
Sycamore Creek *(8 MGD)*	1.1	0	0.5:1
Polk Run *(5 MGD)*	<0.1	0	0.8:1
Indian Creek *(1 MGD)*	0	0	1:1
Taylor Creek *(3 MGD)*	0	0	1.8:1

Average Model Travel Times for Each WWTP



NOTE: “Mean Travel Time” includes 15 minutes of house-to-model time

Sub-Sewershed Sampling – Lick Run

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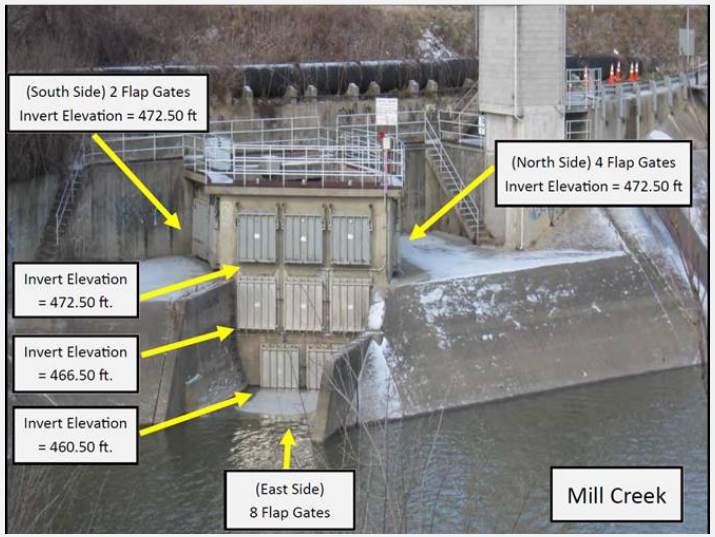
Combined Sewer Overflow



Remote Composite Sampler
~10L between 8-11 am
~500 ml every 15 min

Access to Sewer

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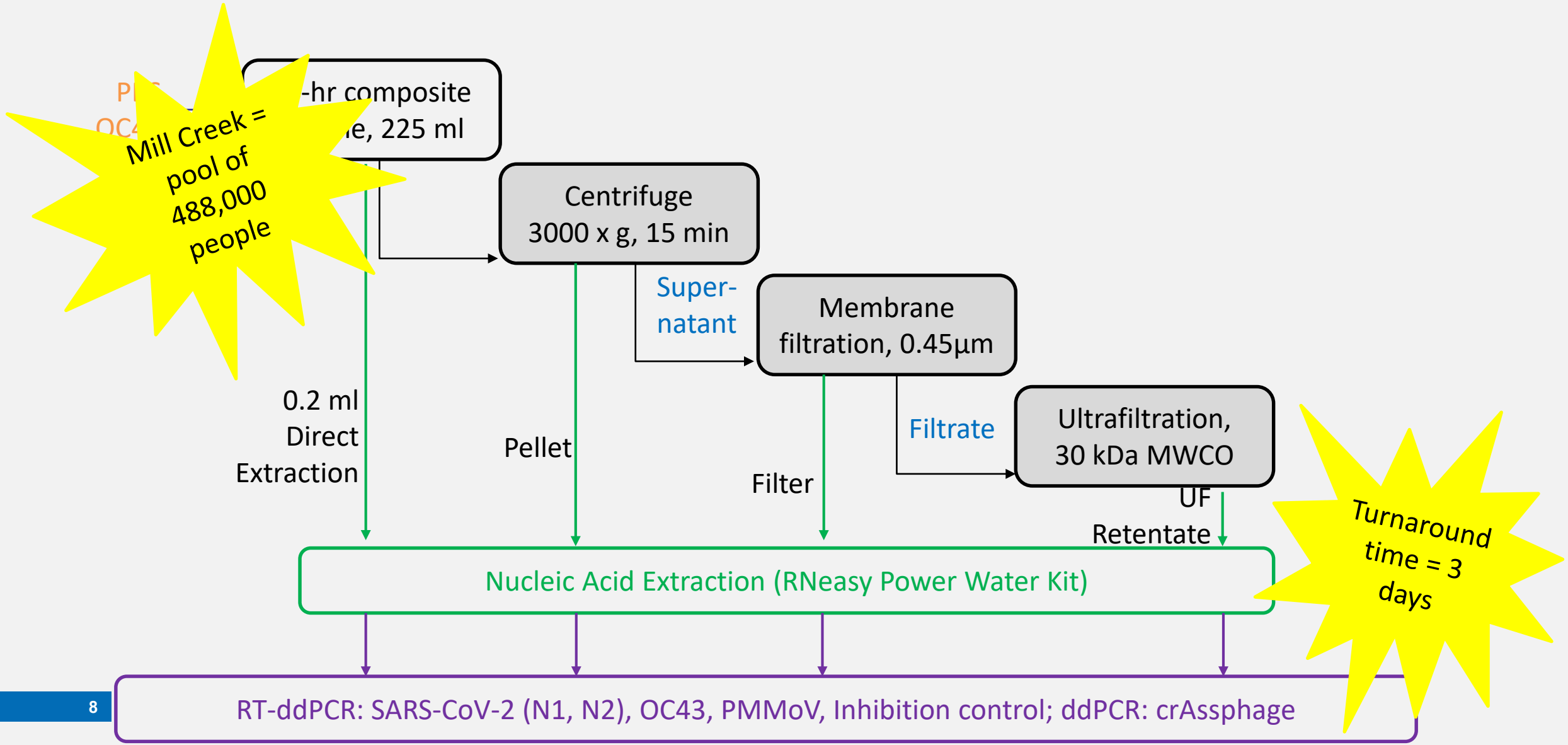
Back to the Lab

Analytical method development and application

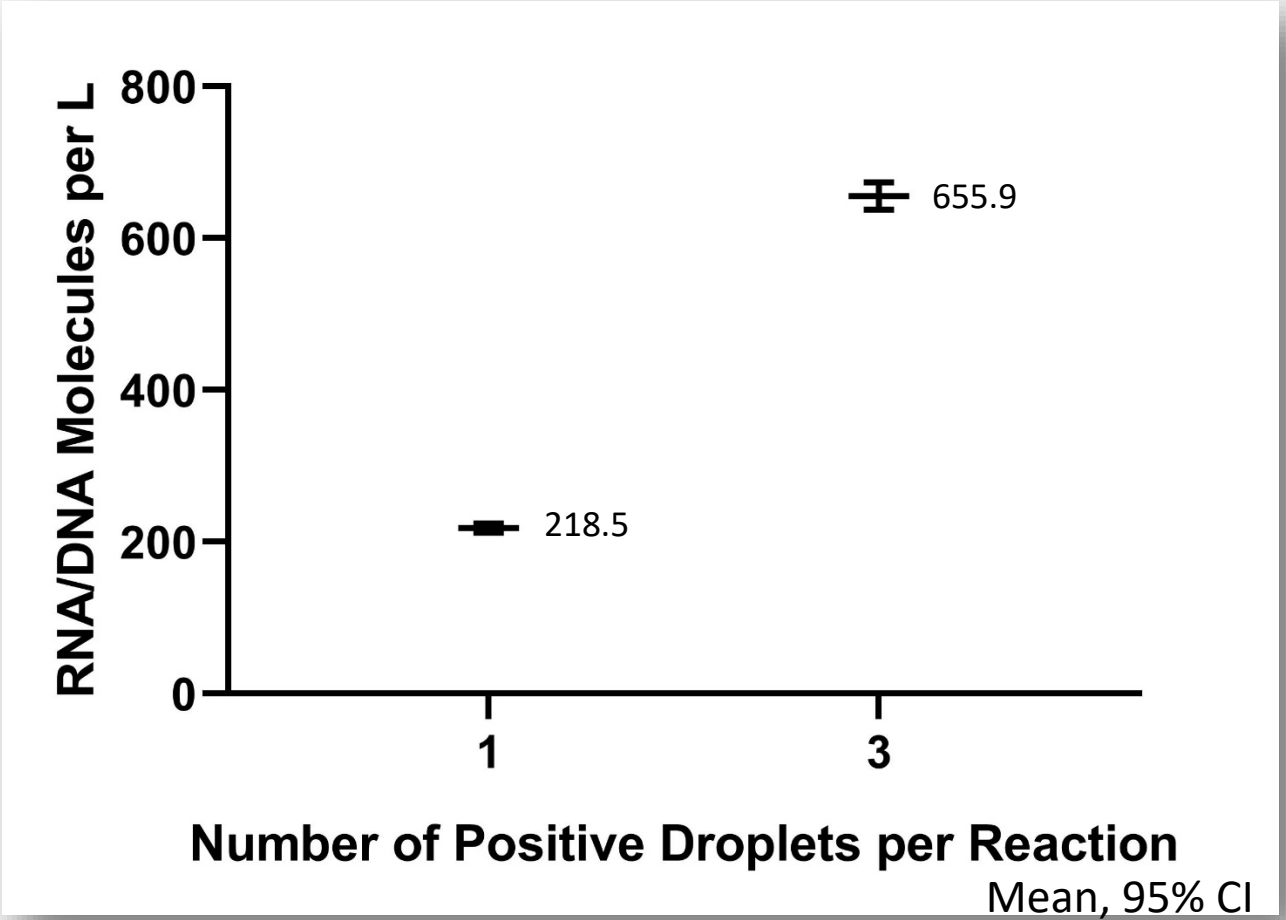
Sample Processing and Analysis



Limits of Detection/Quantification

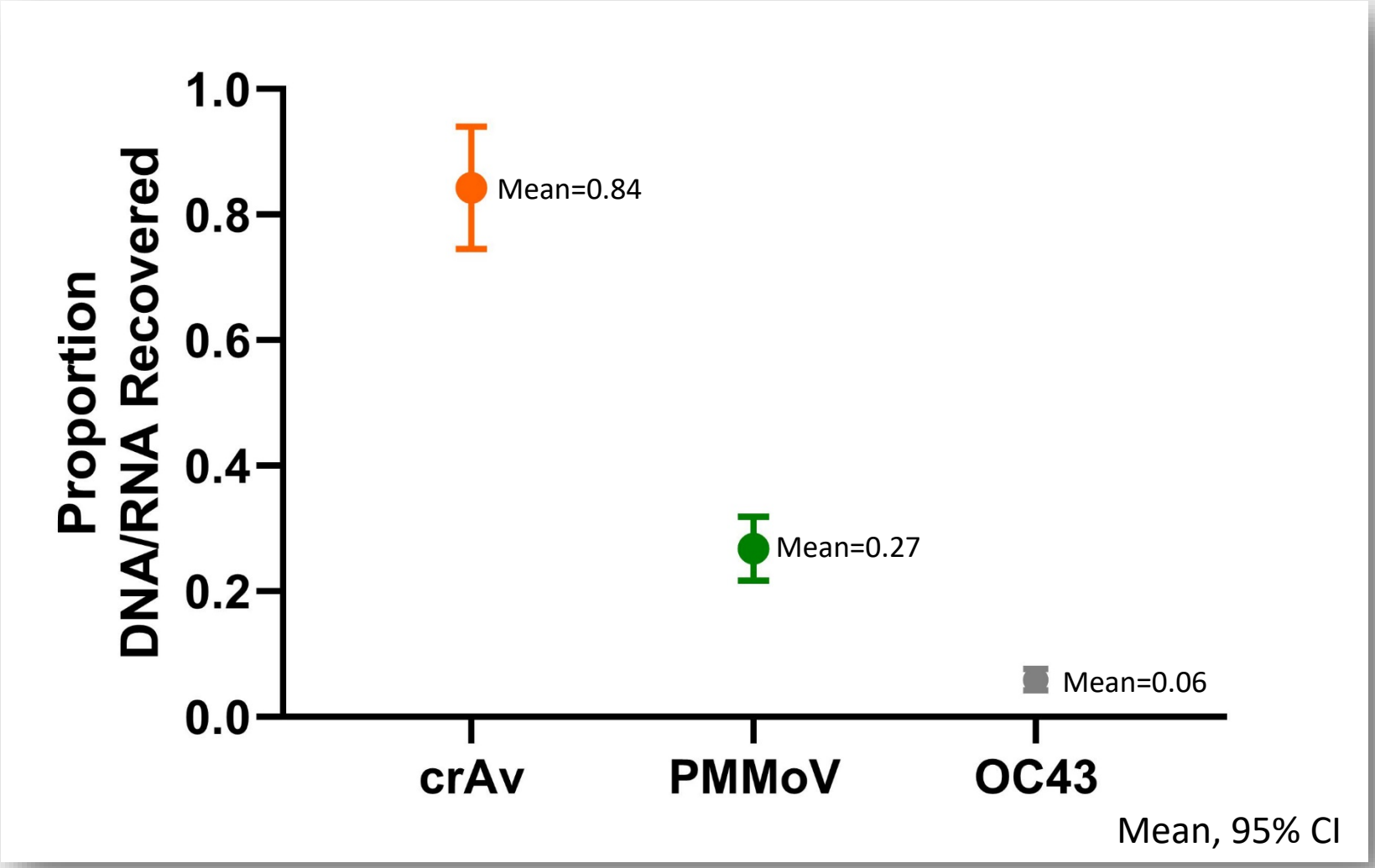
- Volume of sample processed
- Concentration factor
- Volume of processed sample analyzed
- Analytical sensitivity (i.e., minimum detectable concentration)

- Ideal conditions
- Practical limits likely higher due to losses during processing



Recovery Efficiency of Endogenous and Spiked Virus

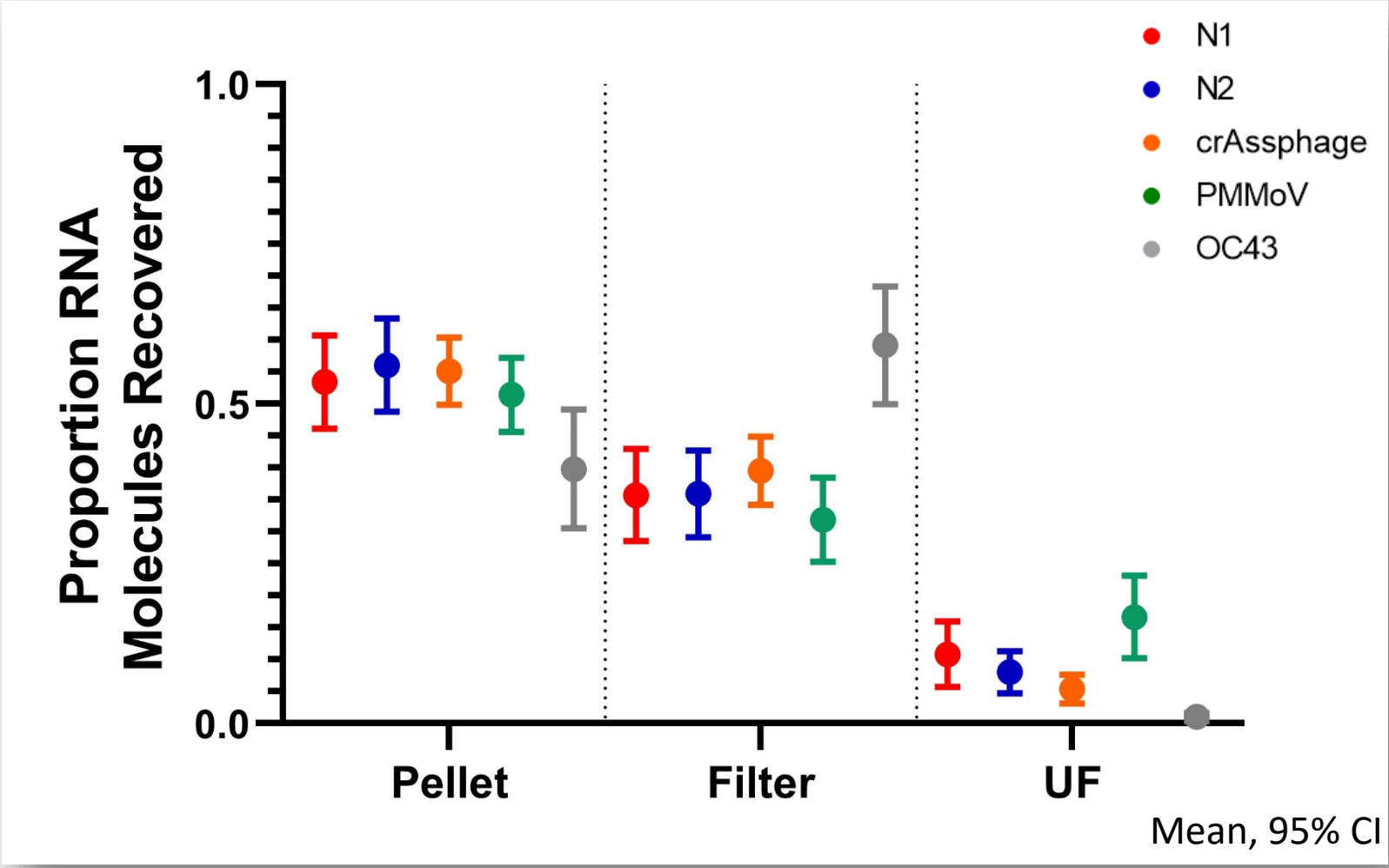
- Endogenous virus
  - crAssphage
  - Pepper Mild Mottle Virus
- Spiked virus
  - OC43
- Measure concentrations before and after sample processing



Partitioning of Virus in Sample Fractions

- Where are viruses recovered within samples?
- Proportion of total virus measured in each sample fraction

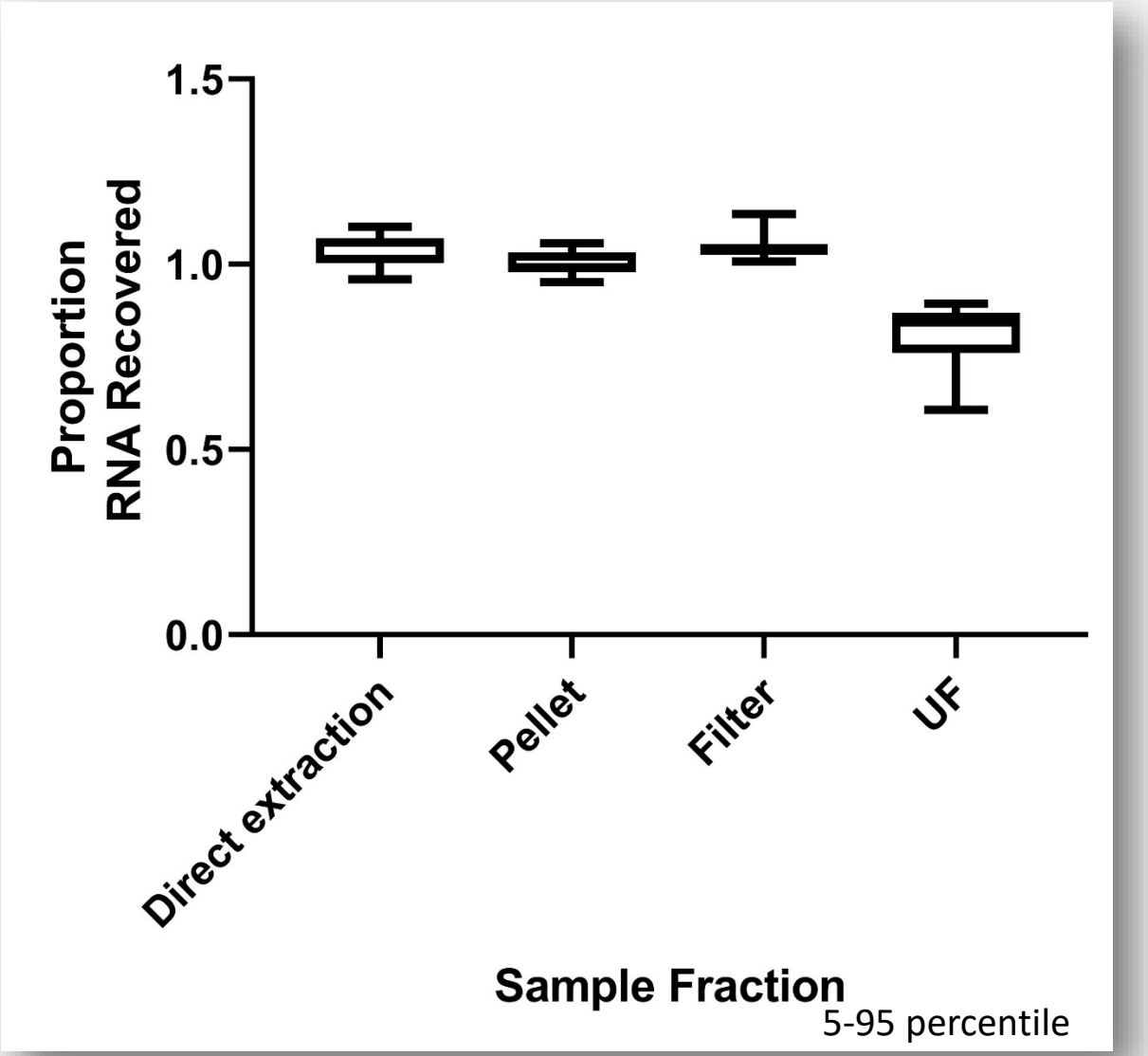
- ~ 90% measurable virus in pellet and filter fractions



RT-ddPCR Inhibition

- Add RNA before RT-ddPCR
- Compare RNA concentration in sewage sample extracts and matrix-free controls

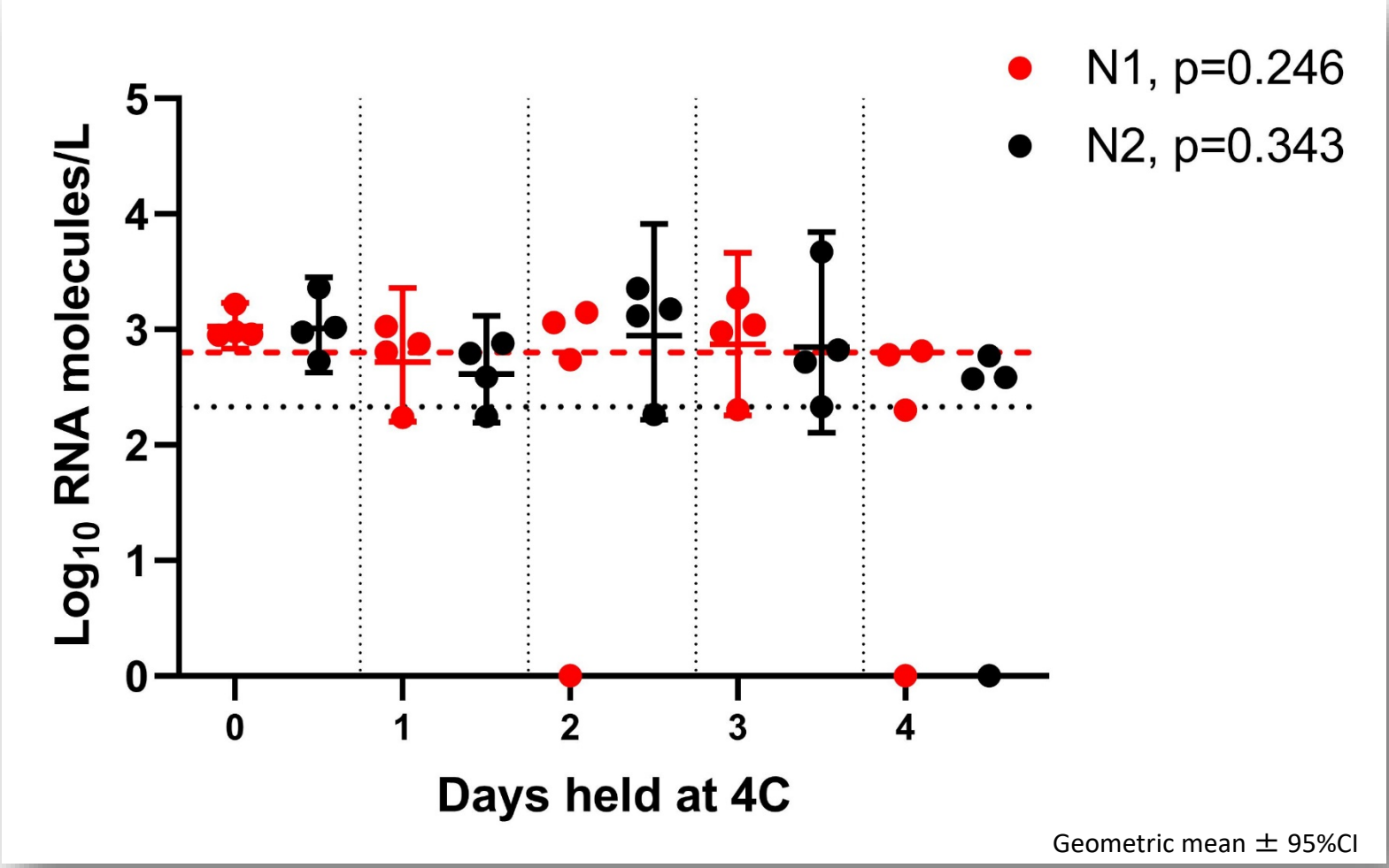
- Minimal RT-ddPCR inhibition observed



Sample Storage at 4° C

- 24- hour composite
- Shipped overnight
- How long can samples be stored before a significant decrease in viral RNA is observed?

- No significant difference in SARS-CoV-2 RNA up to 4 days at 4° C

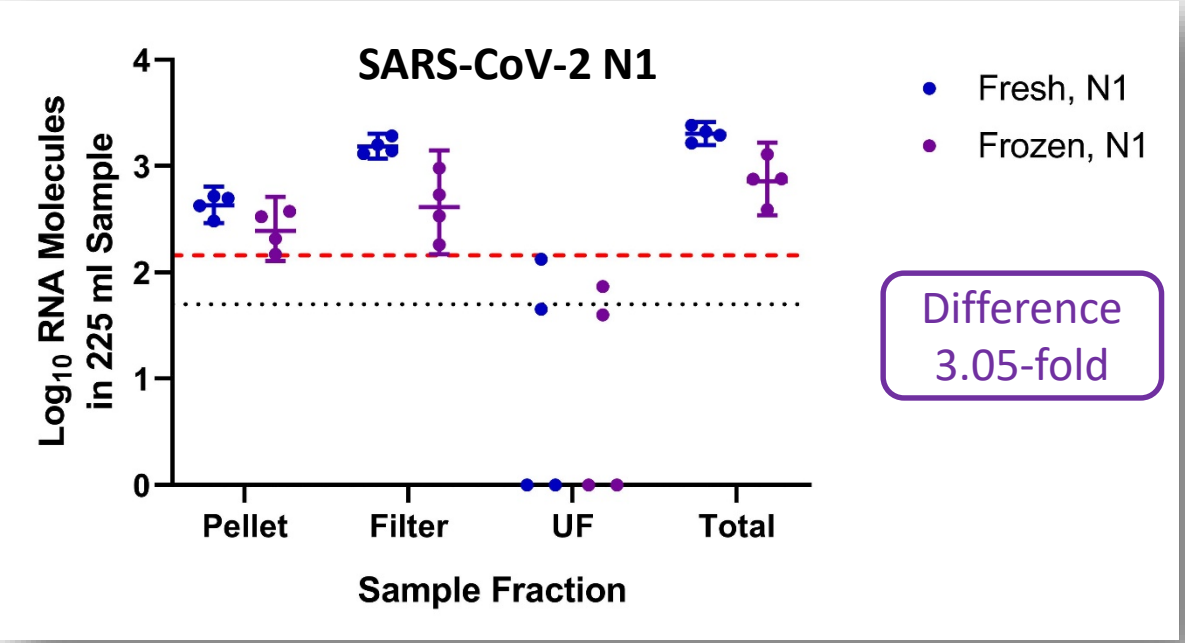


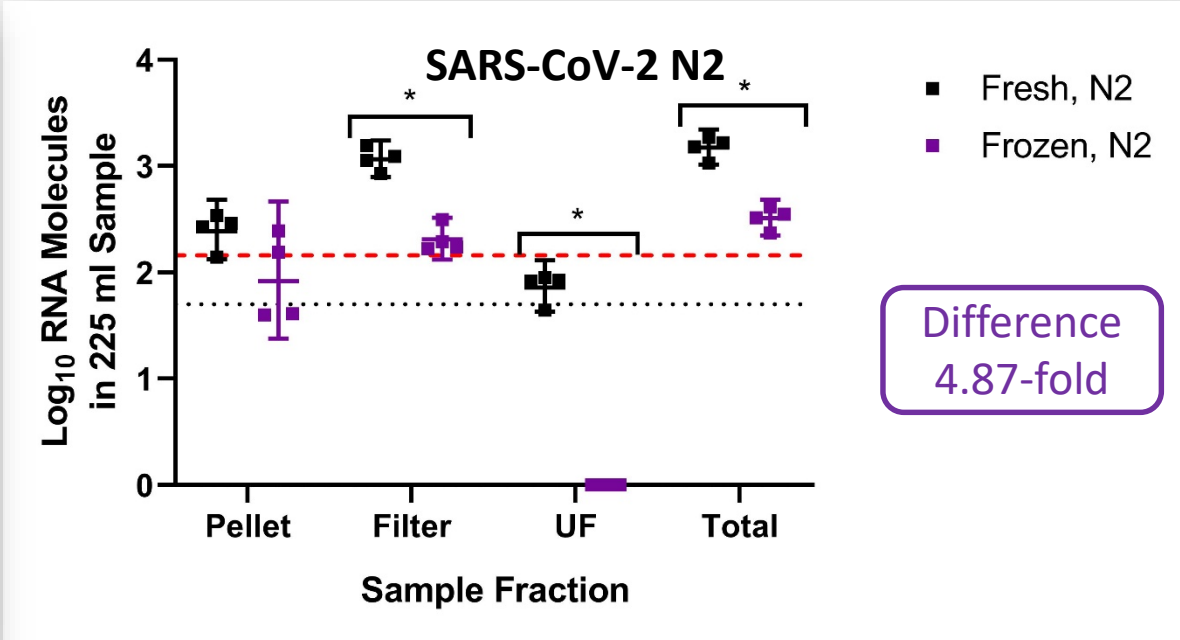
Geometric mean ± 95%CI

p-values for repeated measures ANOVA with time

Sample Storage at -70° C

- What is the effect of freeze-thaw cycle on detection of SARS-CoV-2 RNA?
- Sample collected and processed immediately
- Subsample frozen at -70° C, thawed at 37° C

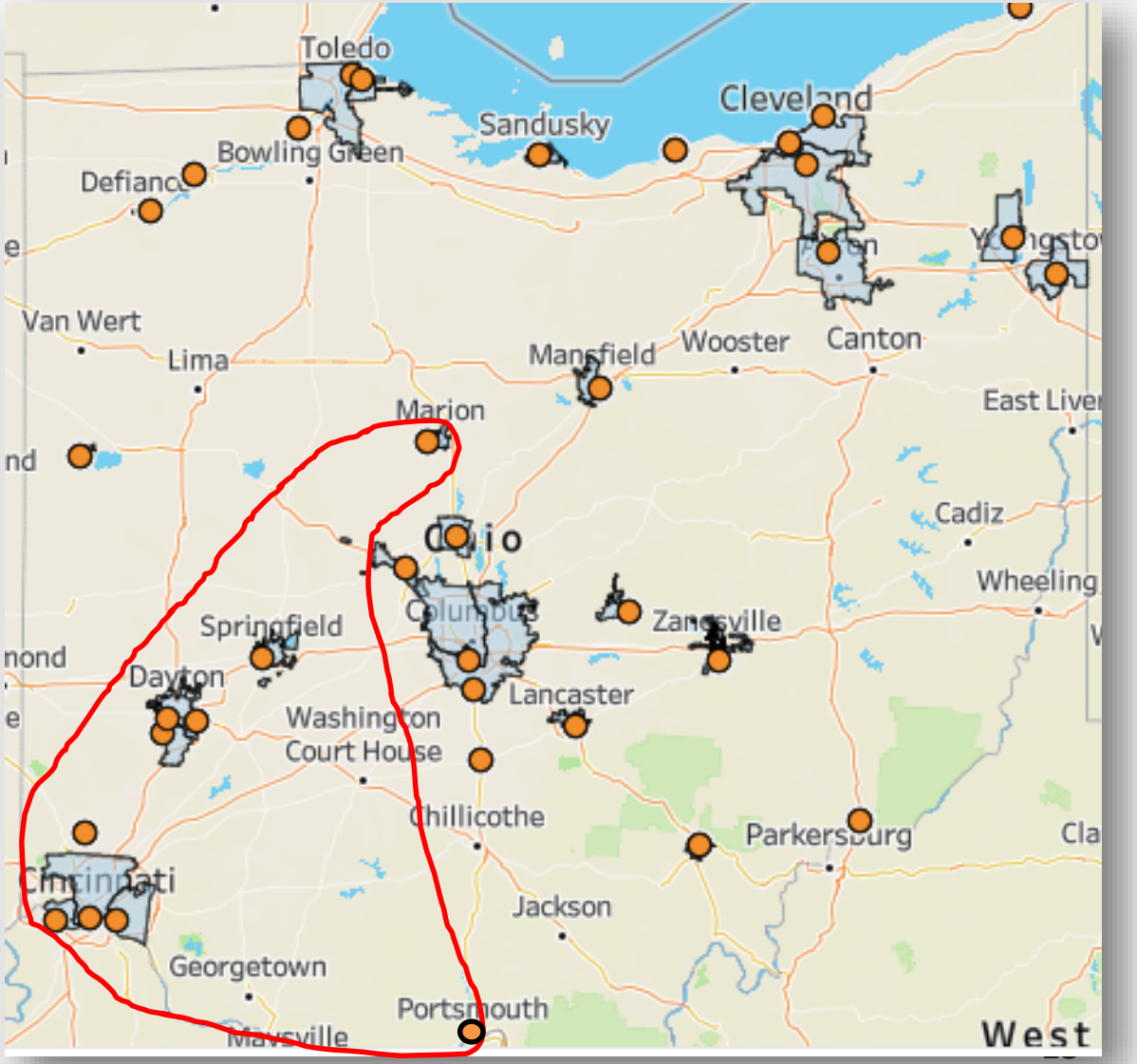




Weekly Wastewater Monitoring

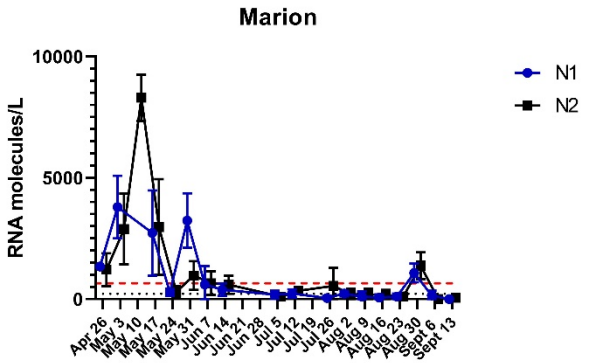
- OH Coronavirus Wastewater Monitoring Network
  - 36 sites, more will be added
  - Sample 1-2 times per week
  - ORD-Cincinnati = 10 sites

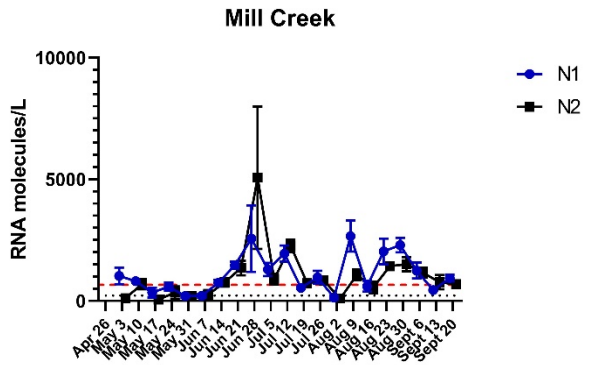
- Sewershed Scale
  - MSD
  - Mill Creek
    - Large flow, high dilution, high industrial input
  - Taylor Creek
    - Small flow, little dilution, little industrial input
  - Lick Run
    - Subsewershed of Mill Creek

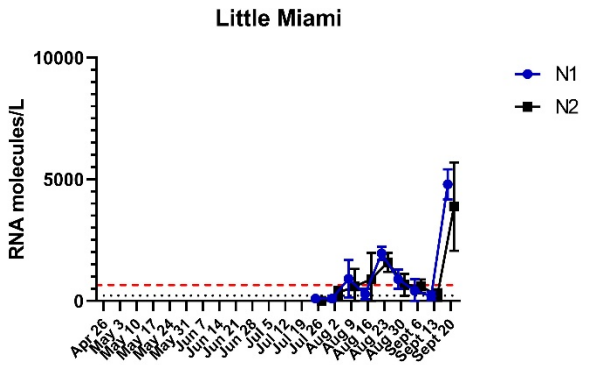


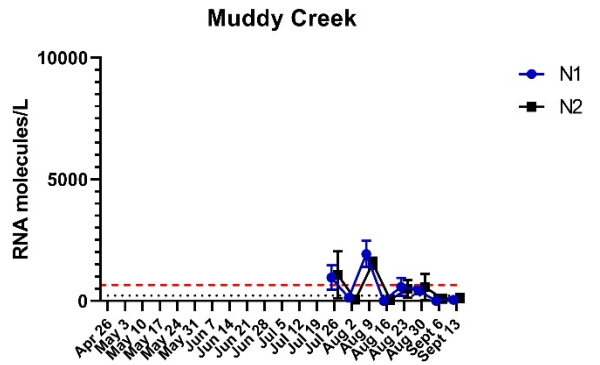
https://coronavirus.ohio.gov/wps/portal/gov/covid-19/dashboards/wastewater

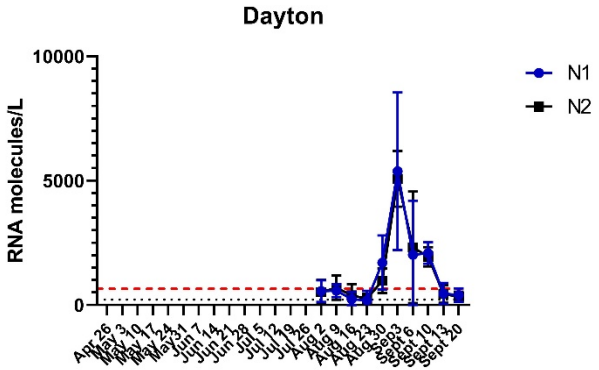
Temporal Trends of SARS-CoV-2 in Sewersheds

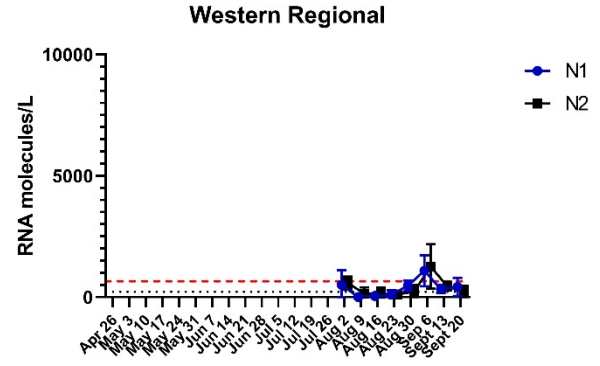


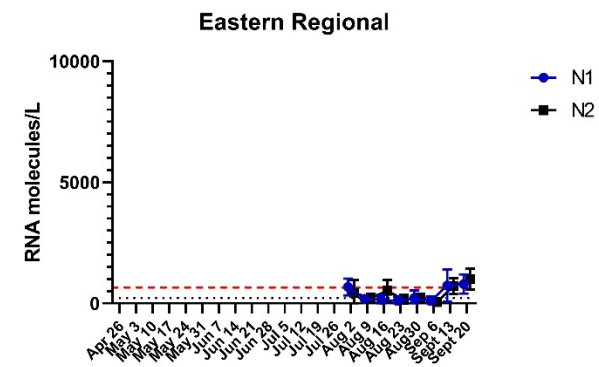


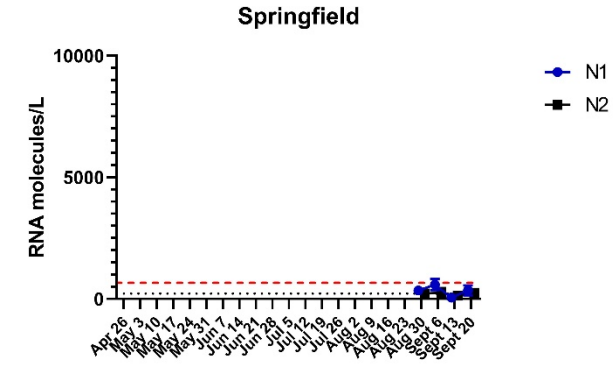


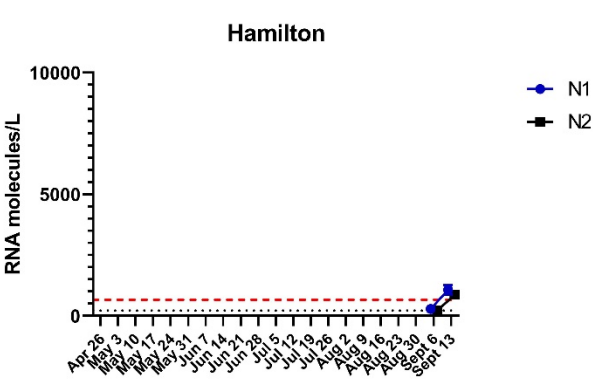




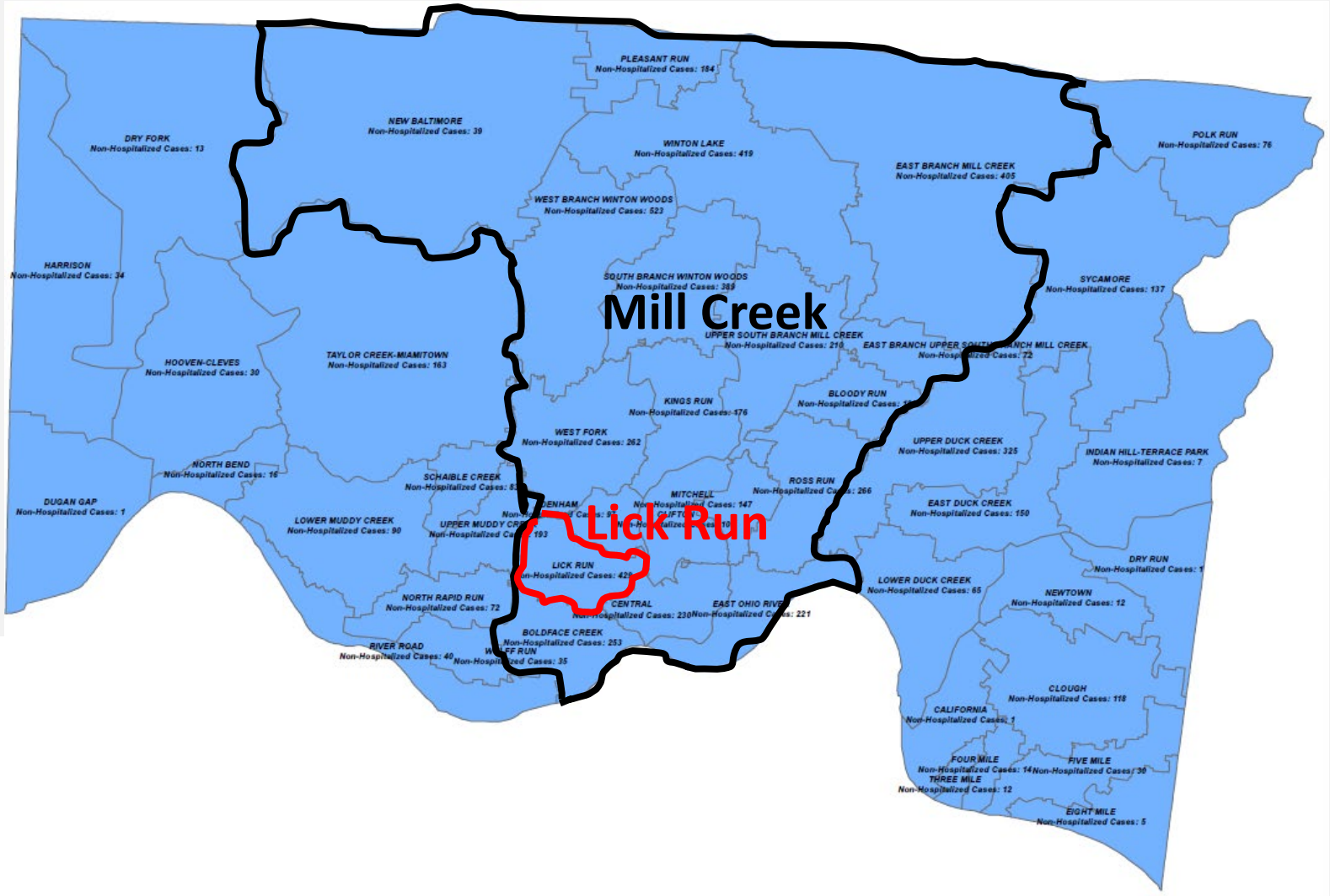


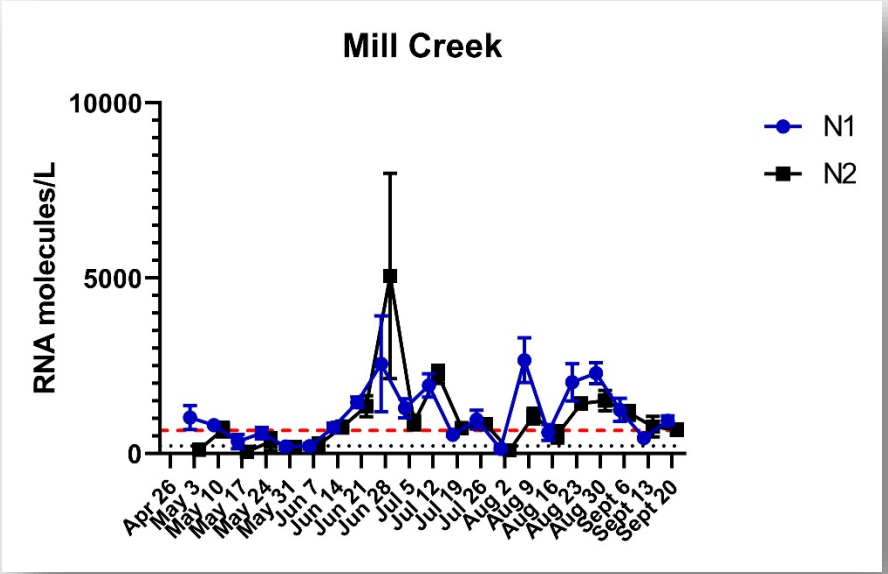


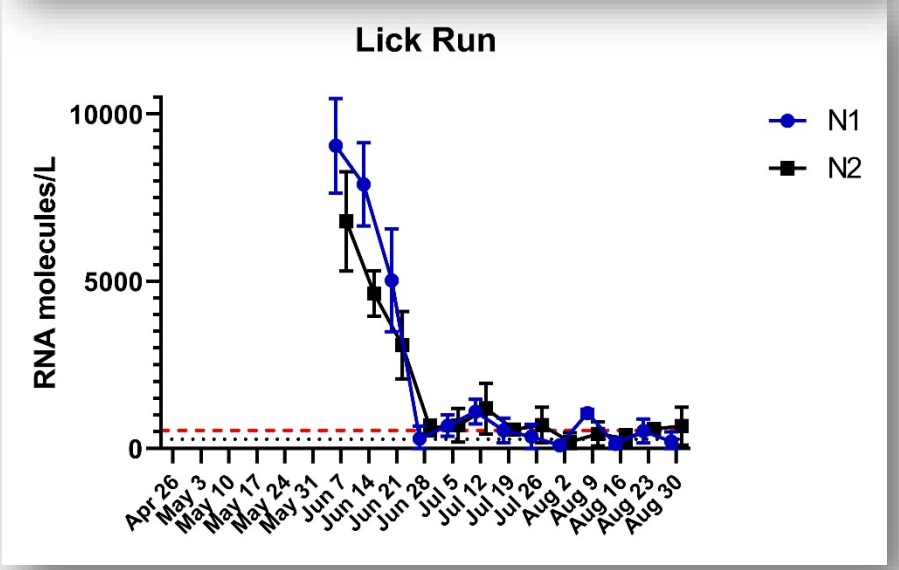




Lick Run Sub-Sewershed







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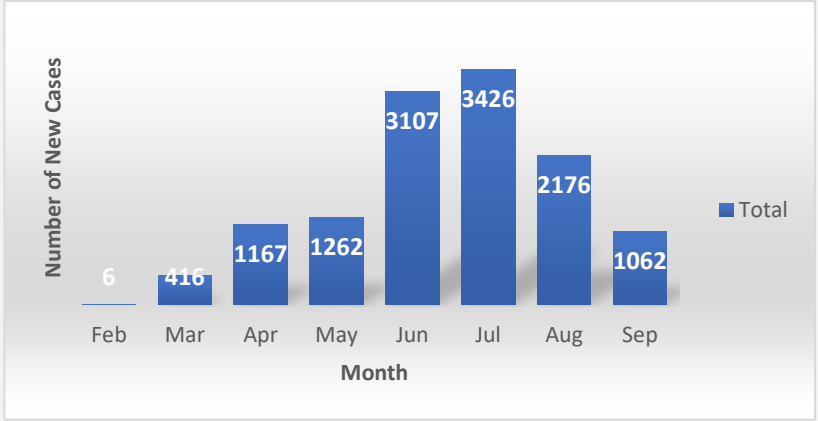
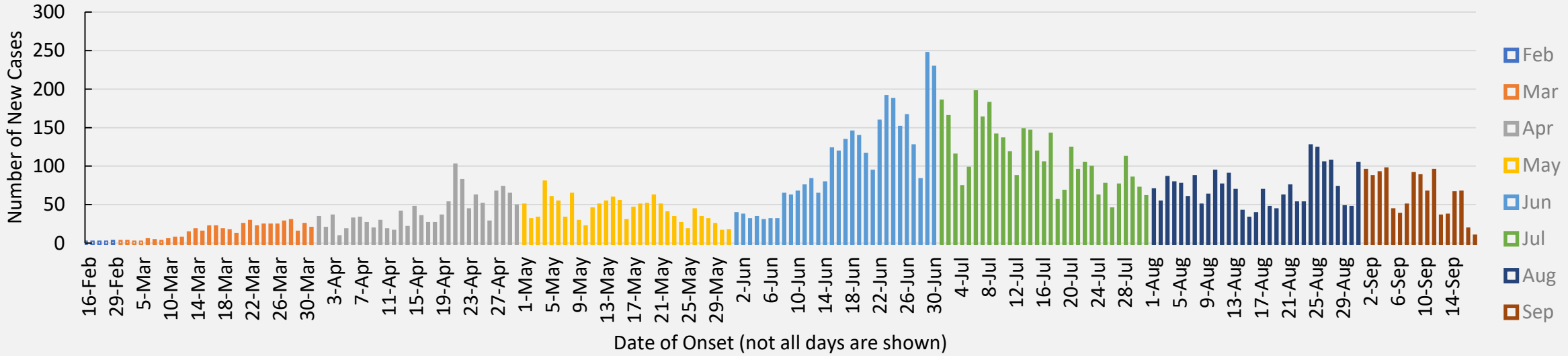
Looking in the Mirror

Relating the sewer signal to community case rates

New COVID-19 Cases (Hamilton County)





data download (Sept 18) from https://coronavirus.ohio.gov/wps/portal/gov/covid-19/dashboards/overview

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SARS-CoV-2 RNA and New COVID-19 cases

7-day case averages centered around the sample collection date

MC serves 488,000 individuals
118 MGD; MSD 186 MGD

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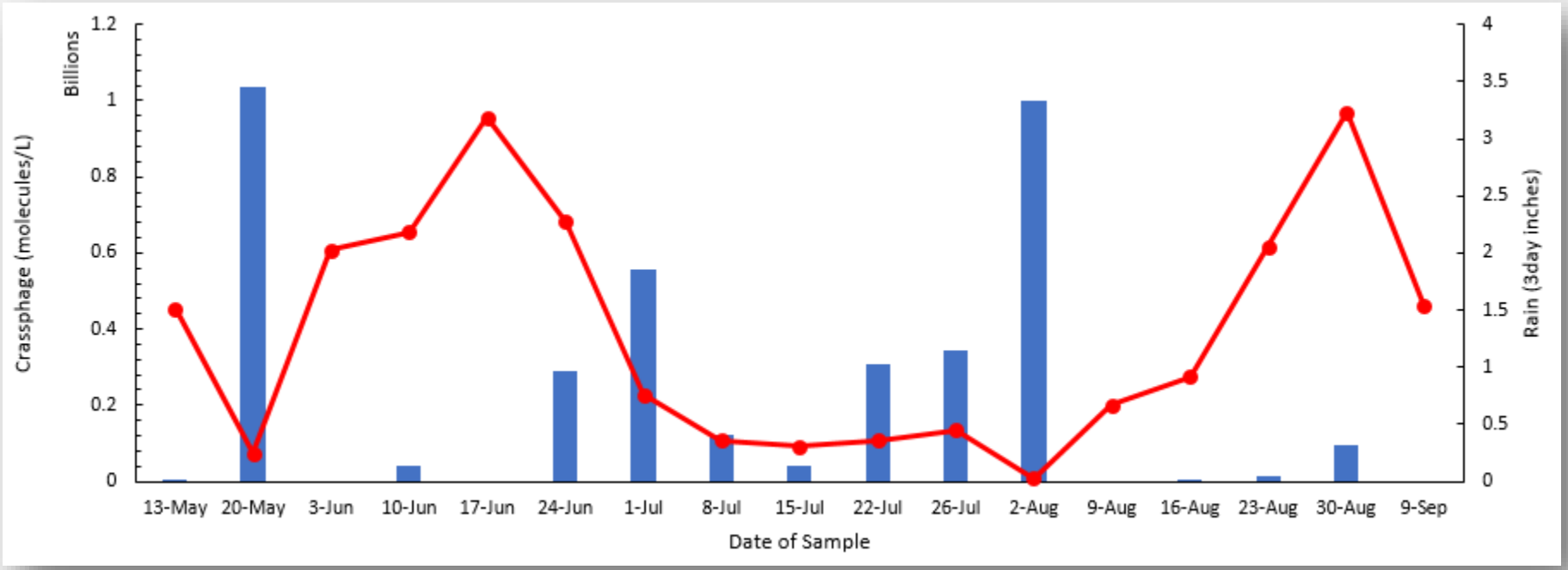
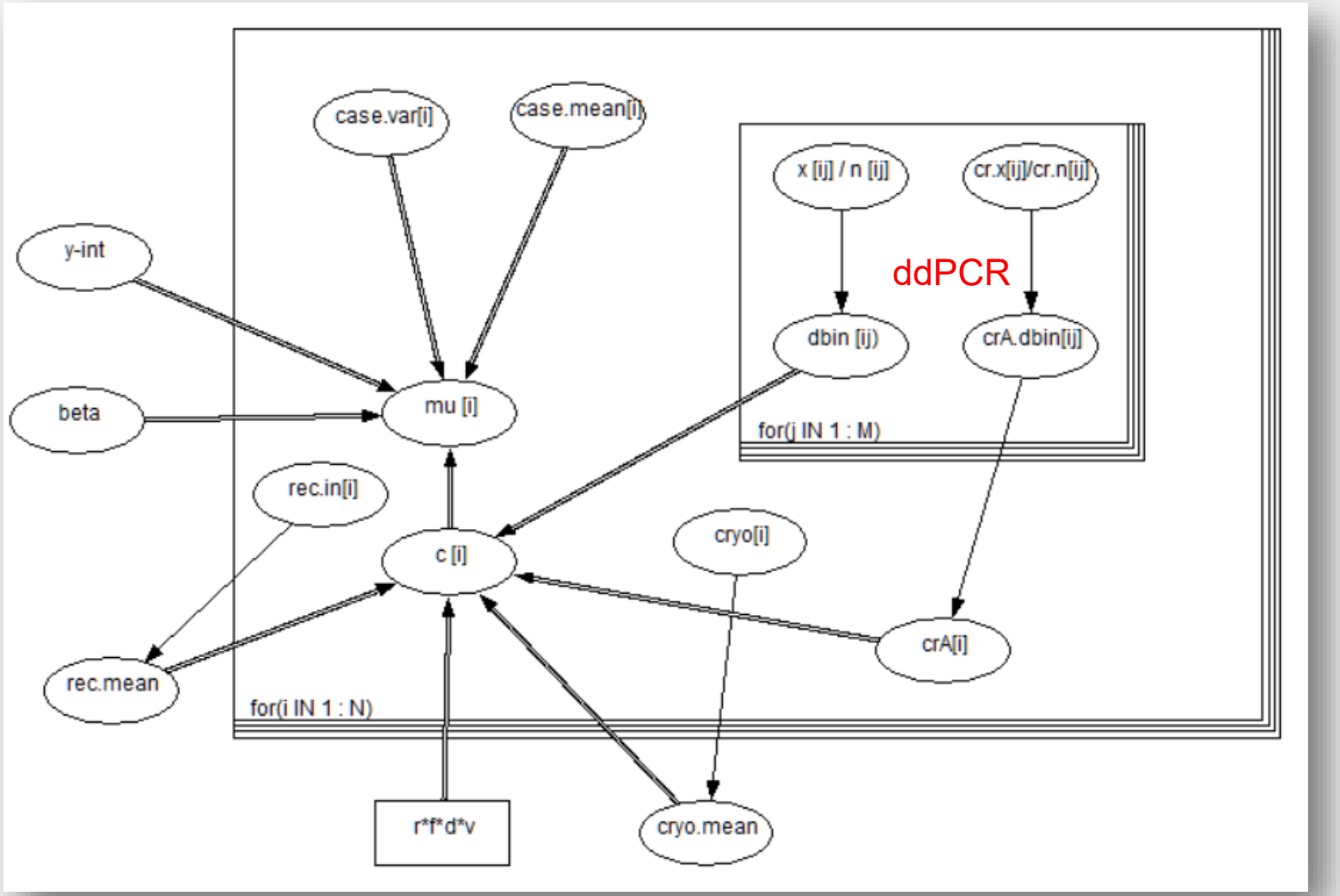
Association of Rain and Decline of CrAssphage

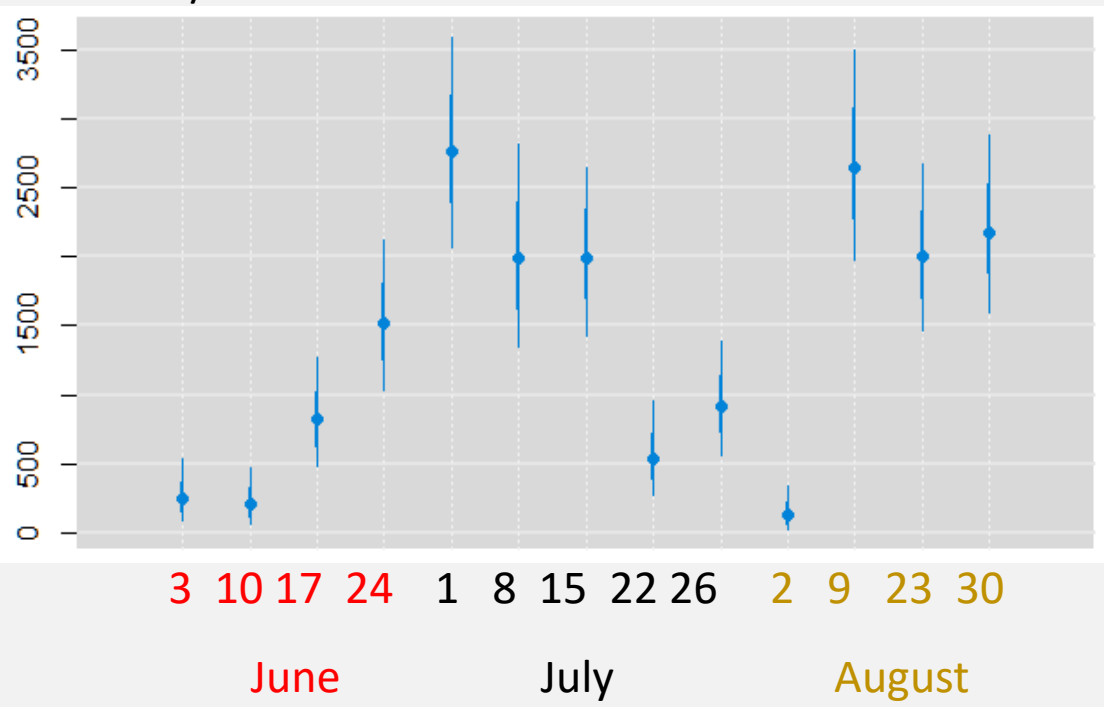


The Bayesian Framework

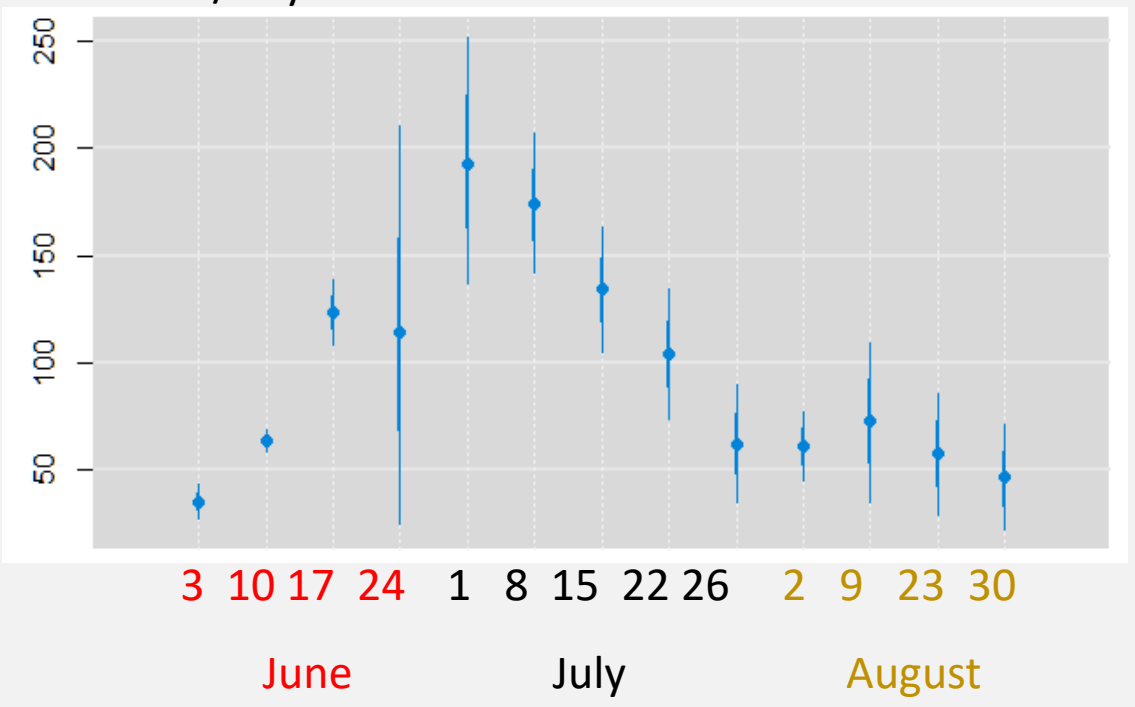


CoV-2 RNA vs New Cases

RNA/L

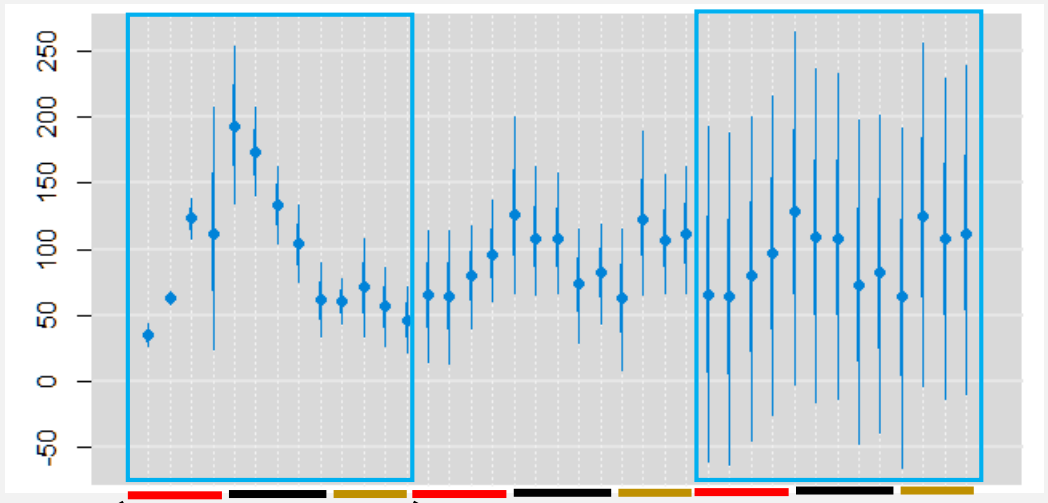


Cases/Day

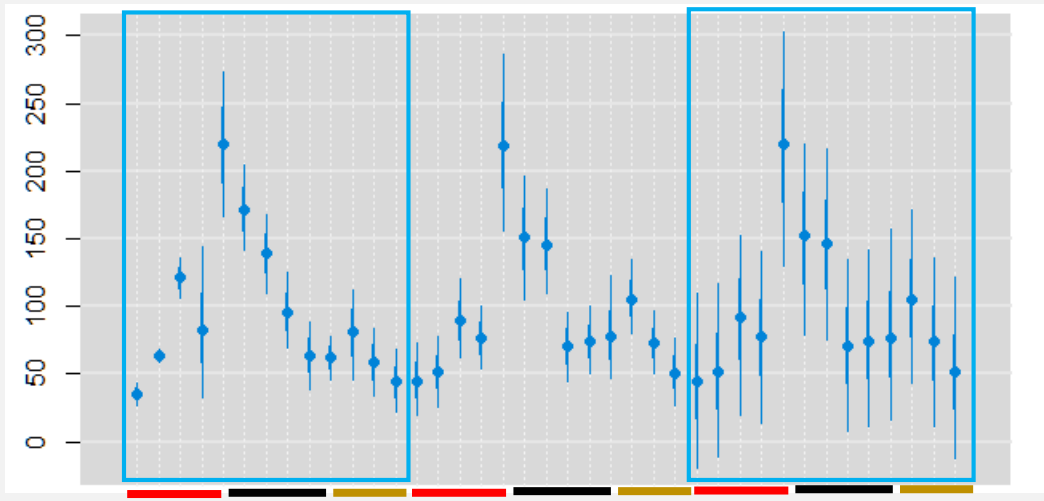


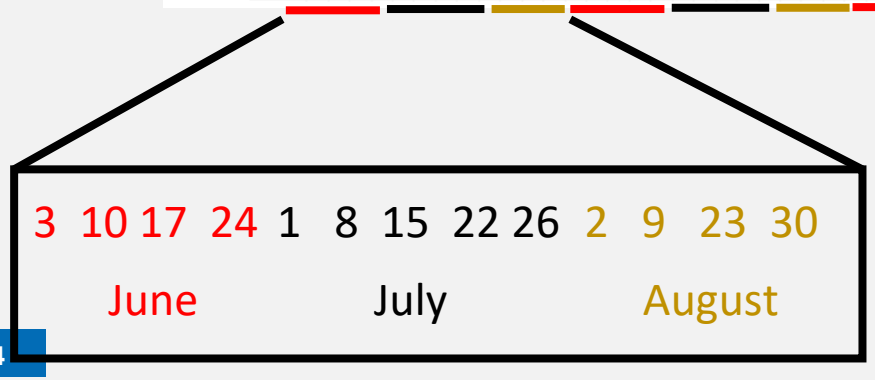
Model improved by accounting for OC43 recovery, freezing and CrAssphage normalization

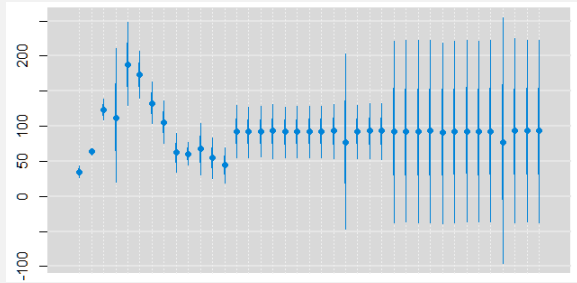
Before



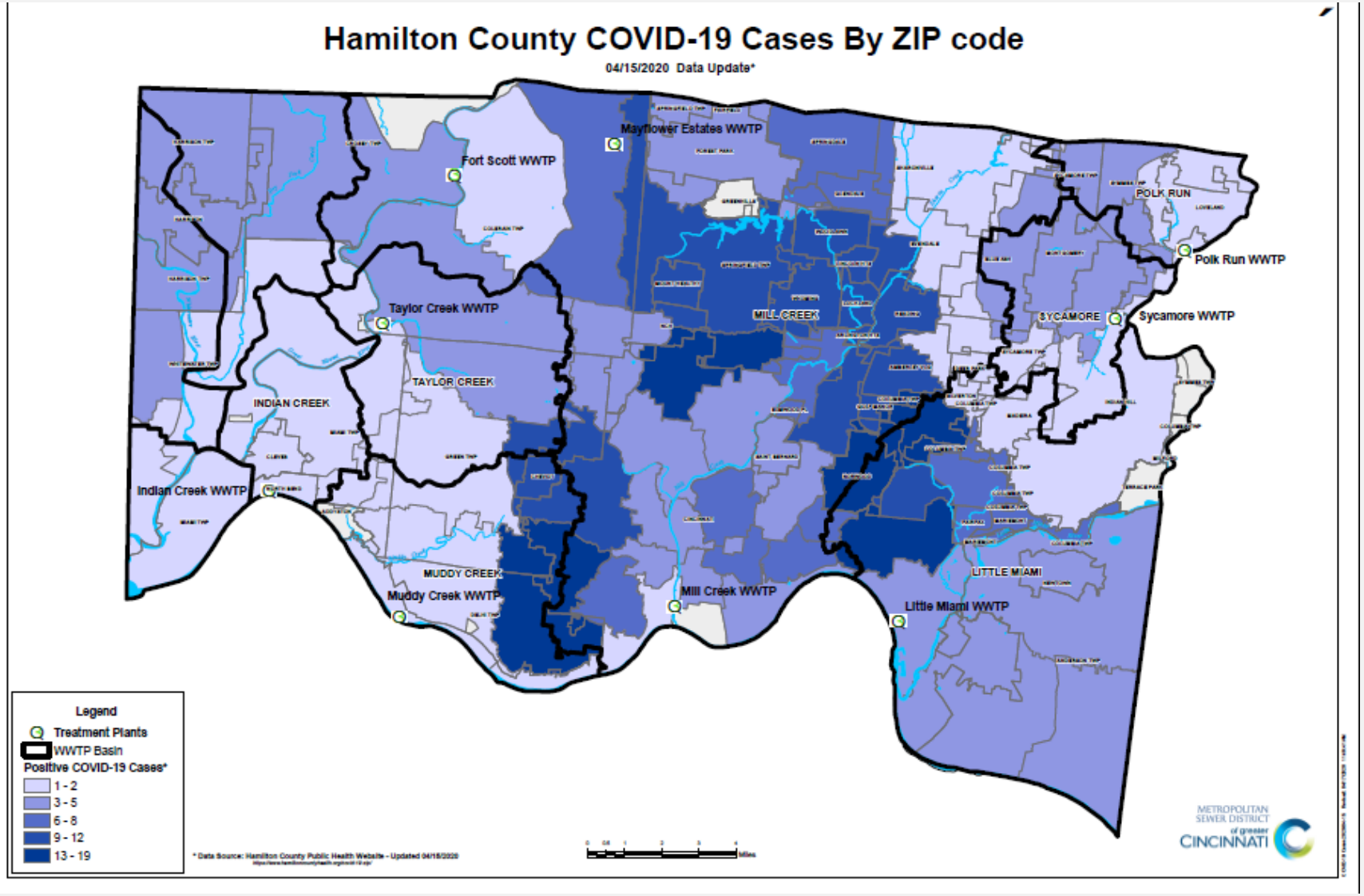
After







Randomized ddPCR Data



Next Steps – Relating Sewer Signal not to Zip codes or County Infections Rates

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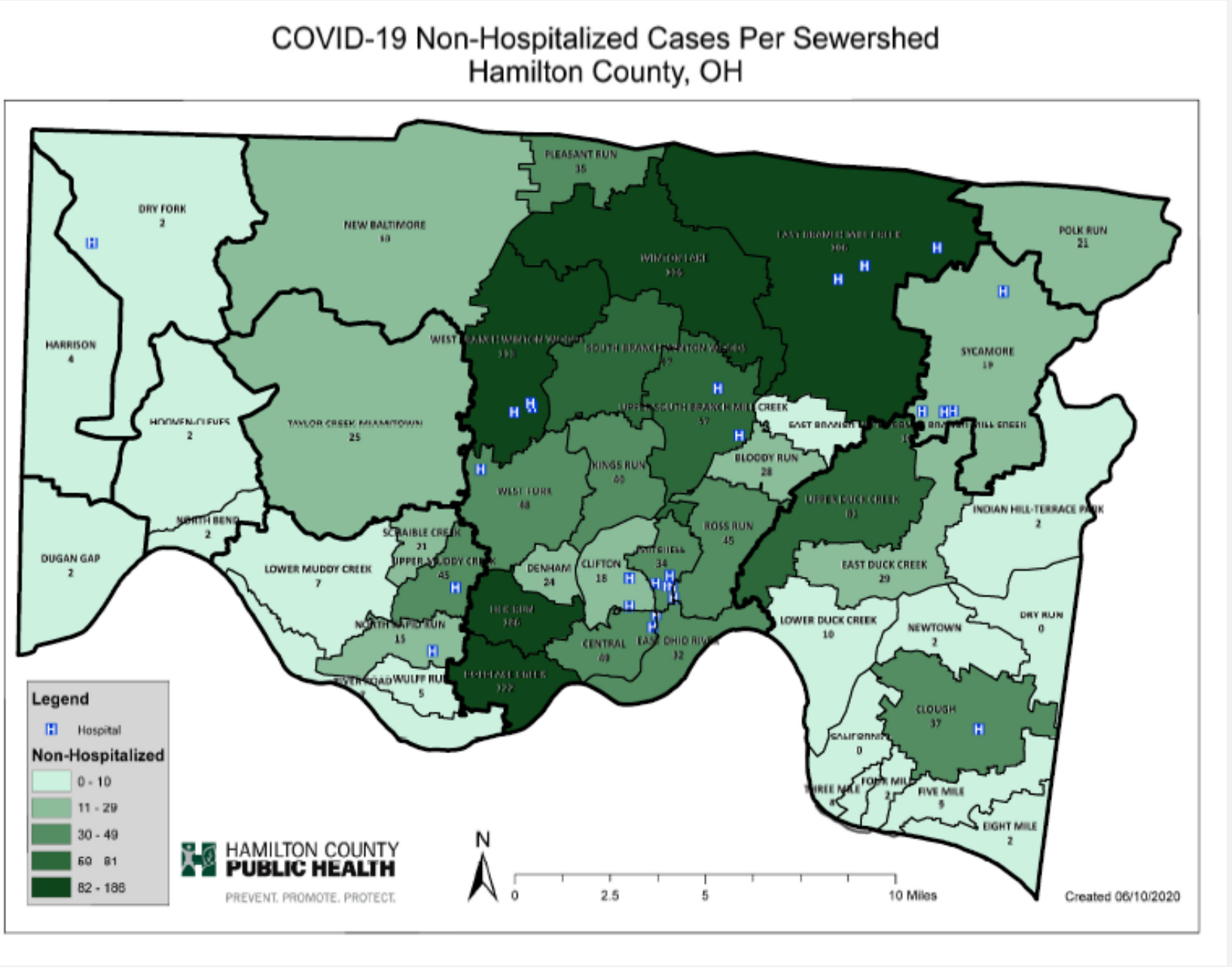

But to Sewersheds and Sub-sewersheds rates

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Connecting the Dots

Translating the information into public health decisions

Ohio Wastewater Monitoring Network

- Ohio received $2 million in CARES funding to **develop a statewide wastewater monitoring network in Ohio to monitor for coronavirus gene copies/fragments.**
- The purpose of the project is to determine the trends in the number of gene copies as a leading indicator of disease occurrence in a community, to help understand disease trends, prioritize resources and to inform community interventions to limit the spread of disease.
- At full capacity, the network will include 50 sites across the state monitored twice weekly.

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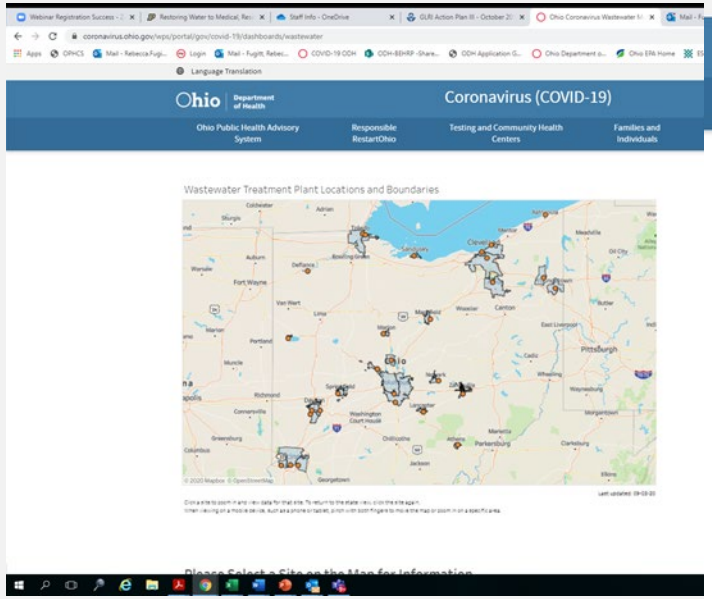

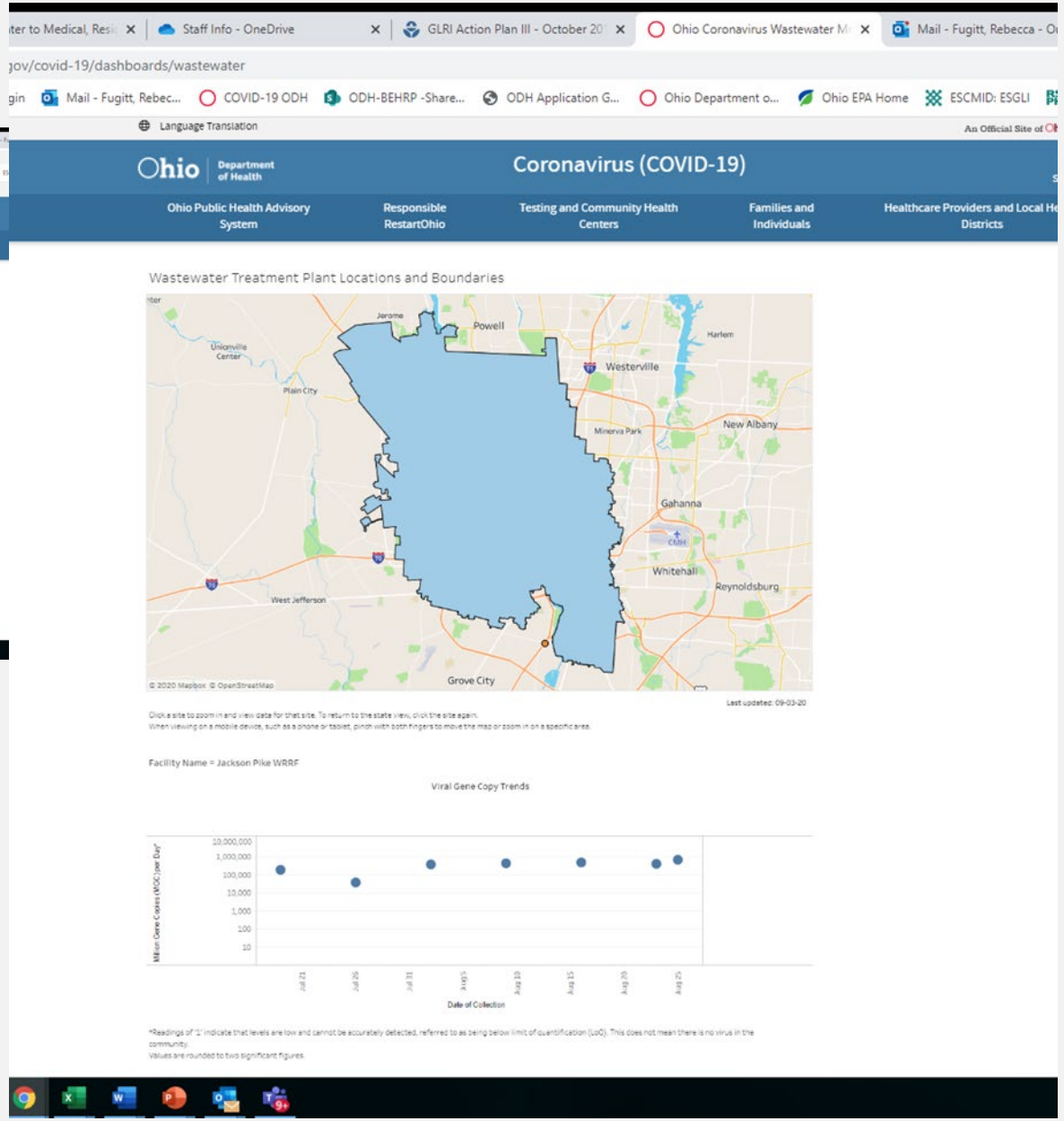

https://coronavirus.ohio.gov/wps/portal/gov/covid-19/dashboards/wastewater

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Public Health Applications

- The focus is on **trends or significant changes** in the number of viral gene copies detected.
- To serve as an early warning of infection in communities and possibly more targeted areas within communities.
- Additional messaging to public on best practices
- Mobilize additional testing or PPE
- Alert hospitals, physicians, other health care providers
- Closely monitor & evaluate data, (hot spots, contact tracing)
- Provide recommendations to local leaders to take direct actions

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Future Public Health Applications

- Develop methodologies/predictive models to translate viral loads detected for comparison to health surveillance data or percentage of infection in communities.
- Predict or compare results to the prevalence data study for specific communities to better understand factors affecting disease spread.
- Determine impacts on disproportionately affected communities (blue-collar, ethnic, race) where risk of infection is greater.
- Coordination with data used in the Ohio Public Health Advisory System

Final Summary

- Sewer signal can detect moderate levels of rising infections
  - Improved recovery efficiency is a priority
  - Further analysis underway to
    - Refine normalization approaches
    - Directly relate wastewater to sewershed populations
    - Define the degree of potential early warning

- On-going, collaborative evaluation of the value of the wastewater signal to inform public health
  - Threshold or trigger points for decision making?
  - Defining optimal “sentinel” sites appears complex
    - The right site, at the right time, adjusting the right knobs