COVID-19 Surveillance Project Update

Presentation to the SCCWRP Commission

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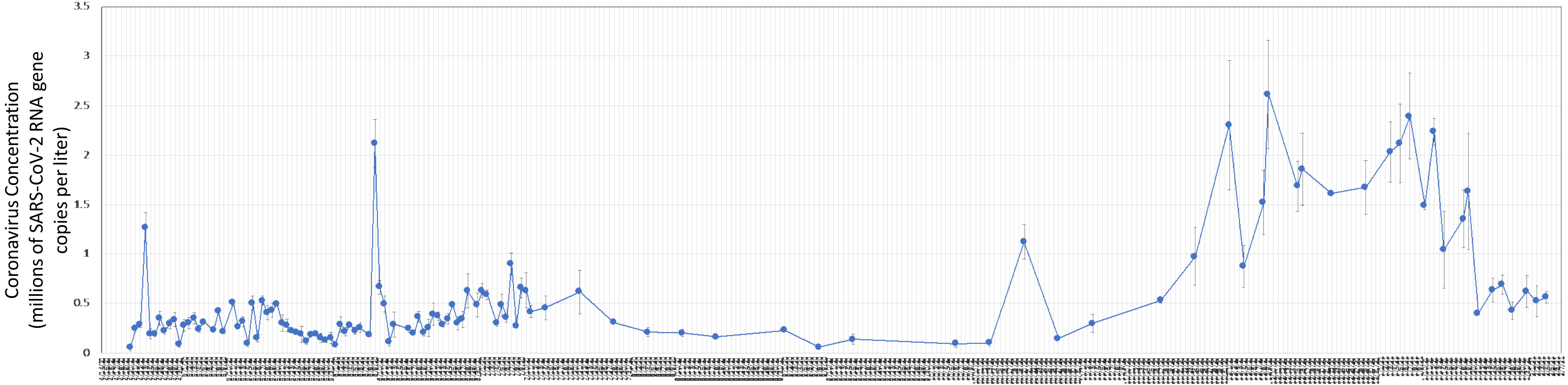
Background

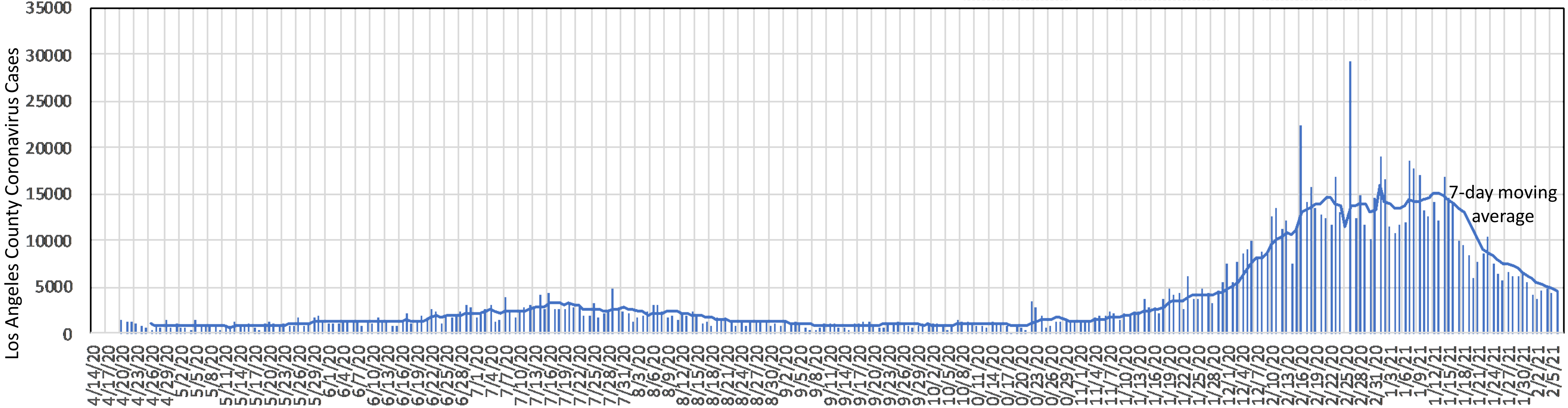
- Wastewater epidemiology is a great concept
  - Allows for tracking population levels of the SARS-CoV-2 virus
  - None of the drawbacks of individual testing

- Differences in methods lead to lack of compatibility of results across labs, plants, and regions
  - Comparison studies show wide variability across methods

- Our focus is on the relationship between variability and methodological choices

Coronavirus Concentration in HTP Influent and Case Counts 4/20-2/21





Potential Sources of Variability

Sample Type
Influent or
Settled
Solids

Sampling Strategy
Grab or
Composite

Sampling Frequency
Daily? Weekly?

Fresh Processing, Storage, or Preservation
Refrigerate
Freeze
Chemically preserve

Virus Inactivation
Heat
Chemical

Virus Capture
Direct processing,
Filter,
Precipitate,
Concentrate

Purification of Viral RNA
Commercial kit,
published protocol,
In-house protocol

Target Selection
Which gene target?

Virus Quantification
RT-qPCR or RT-ddPCR

- Large Effect (> 1 order magnitude)
- Moderate Effect (0.5 - 1 order magnitiude)
- Inconsequential Effect (0 – 0.5 order magnitude)

- In Progress

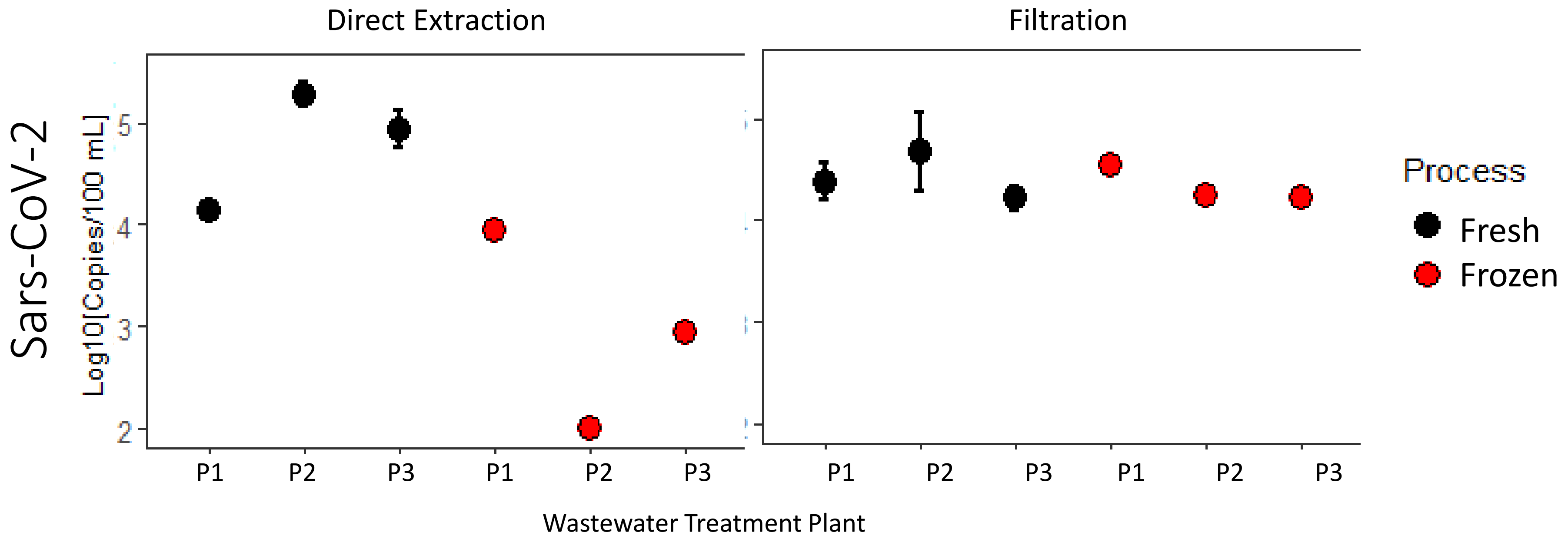
Biggest Sources of Variability

- Freezing and thawing
    - Reduces sensitivity by up to 4 orders of magnitude

- Heat Treatment (Pasteurization)
    - Reduces sensitivity by up to 3 orders of magnitude

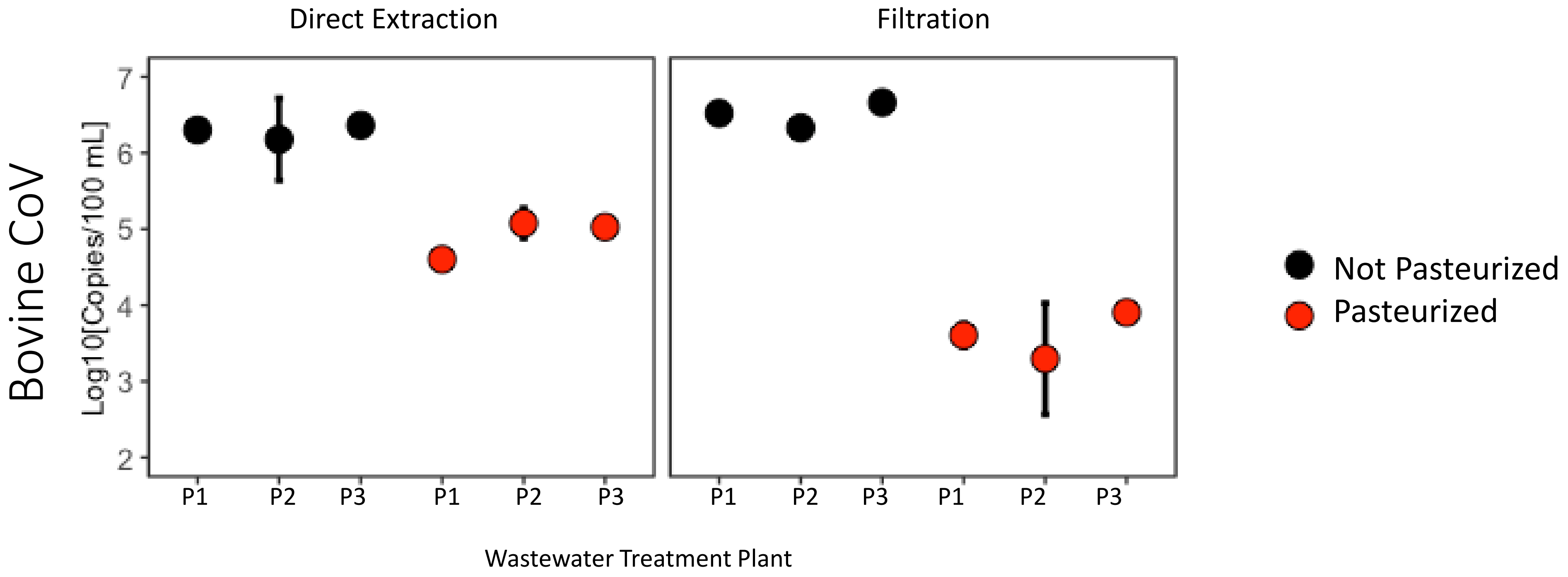
Effect of Freezing

- When you can - minimize freeze thaws



Heat Treatment

- ~1 to 3 log reduction in concentrations in pasteurized sample



Two other critical things we learned

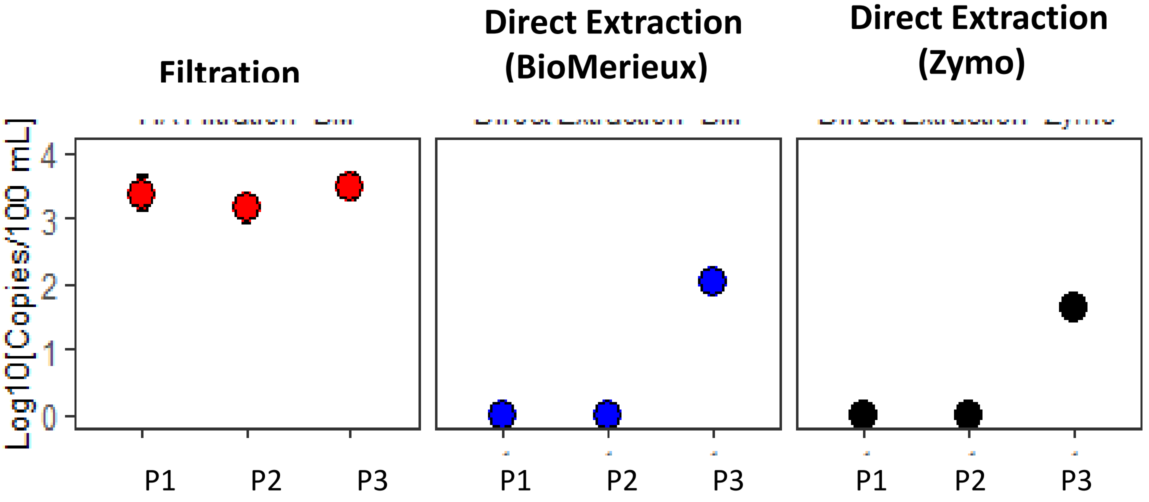
- Direct extraction vs. filtration
  - Trade-off between expediency and method sensitivity when virus titers are low
- Frequency matters
  - Sampling once per week is inadequate

Direct Extraction vs. Filtration

- Direct extraction more expedient

- BUT- trade off with limit of detection and method sensitivity

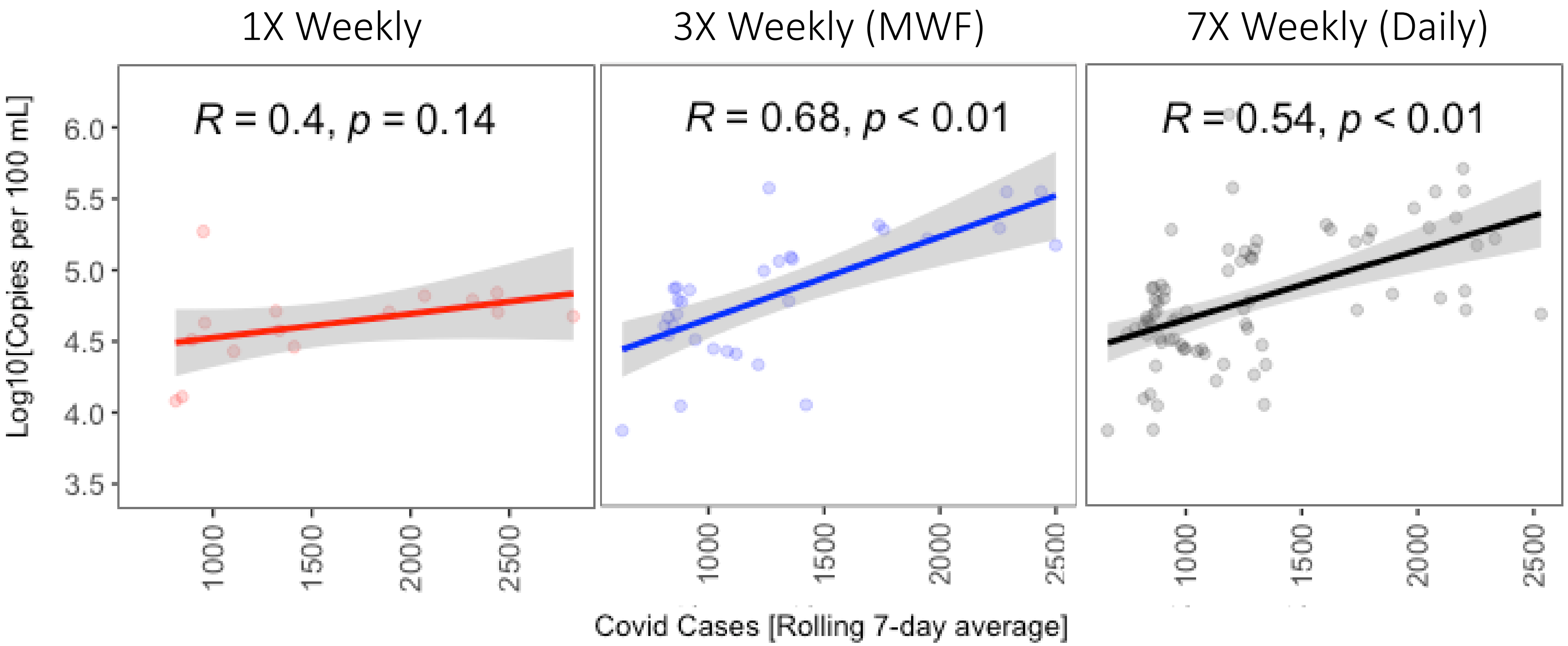
- Direct extraction may not be best option with lower SARS-CoV-2 concentrations



Sars-CoV-2

How frequently to sample?

- No significant relationship between Covid-19 case counts and **weekly** wastewater samples
- Significant relationship with **3X** or **daily** samples



Work in Progress

- Items remaining in Flow Chart
  - Comparison between paired influent and settled solids samples
  - Comparison of virus purification methods
  - Comparison of RTqPCR vs. RT-ddPCR

- Sequencing of SAR-CoV-2 in influent to track spread of variants
  - Collaboration with Stanford and UCI