HAWAII STATE DEPARTMENT OF HEALTH

Wastewater Surveillance Report

09/05/2023

Background

Wastewater surveillance adds a useful layer of monitoring community levels of SARS-CoV-2. Due to the shift to self-testing and decreased clinical testing, reported COVID-19 case counts are lower than the *actual* COVID-19 case counts. Using wastewater surveillance data paired with the monitoring of COVID-19 case counts, hospitalizations, and fatalities, this allows for a more complete understanding of disease patterns. When trends are similar across these measures, confidence in the accuracy of those trends increases.

Advantages of monitoring wastewater concentrations of SARS-CoV-2:

- Wastewater based epidemiology:
  - Helpful when paired with clinical data since SARS-CoV-2 concentrations in sewage are positively correlated with COVID-19 case counts.
- Early warning for emerging outbreaks:
  - Infected people begin shedding 2 - 3 days before onset of symptoms.
  - SARS-CoV-2 is shed in feces by both individuals with asymptomatic and symptomatic COVID-19 infections.

Possible limitations of these data to consider:

- We cannot precisely predict case counts with the detection of concentrations of SARS-CoV-2 in wastewater.
- Wastewater surveillance might not capture low levels of infection in a community.
- Some communities and/or facilities are not connected to a Wastewater Treatment Plant (WWTP).
- Inhibitors could be present in wastewater that impacts the detection of SARS-CoV-2. Inhibition assessments are in place to ensure RNA quantification methods and viral recovery are performing as expected.

Interpretation of Trends

Not all peaks and surges in concentrations will correlate with a community-wide increase in cases. Wastewater is a highly variable mixture where concentrations of all pathogens like SARS-CoV-2 may vary based upon time of collection, or collection methods of sewage. For example, concentrations can vary on whether the sample was a grab sample (only captures at one point of time) or a 24-hour composite sample (more longitudinal variability over time). If an upward trend is observed in the data, this might represent an increase in cases that has yet to be confirmed through case-based surveillance. Additional data is required to confirm whether this trend will persist.

SARS-CoV-2 Variants in Sewage

SARS-CoV-2 is a constantly evolving virus. The detection of SARS-CoV-2 variants in wastewater is another useful layer of surveillance since some variants spread more rapidly than others. Information on dominant or new variants in a community assists in public health response. Additionally, in some cases, variants have been detected in wastewater prior to detection in clinical samples.

Wastewater Surveillance for the State of Hawaii

This report contains results for the SARS-CoV-2 surveillance of sewage in collaboration with the National Wastewater Surveillance System (NWSS). A total of 14 WWTP from the State of Hawaii are participating in this surveillance. Samples are collected weekly and analyzed by Biobot Analytics. Concentrations in this document are reported as SARS-CoV-2 copies per nanoliter of wastewater (unless otherwise stated).

For visualization and interpretation of trends, this report includes regression lines to help visualize possible changes in SARS-CoV-2 concentrations in sewage and COVID-19 case counts over time. For example, if the constant trend in SARS-CoV-2 concentrations is decreasing, we will likely observe a similar decrease in COVID-19 cases. Still, trends in concentrations do not always perfectly reflect community levels of SARS-CoV-2. Not all communities in Hawaii are sewered or are serviced by participating WWTP, which can disproportionately reflect more urban and often more heavily touristed areas. Tourism also frequently changes populations served by each WWTP and their travel may result in fluctuations in disease detection.

This report also includes information on the estimated composition of variants in wastewater through Next Generation Sequencing (NGS). NGS which is parallel to Whole Genome Sequencing (WGS) and allows for high-throughput and timely results. NGS is done with the use of Illumina instruments through Biobot's trusted sequencing partners. The estimated proportion of variants is calculated through the Freyja tool by measuring the frequency of variations at each position in the SARS-CoV-2 genome among mapped sequence fragments from a mixed SARS-CoV-2 sample. Data are reported in relative abundance. For example, if BA.5 (parent Omicron lineage) has the highest proportion, this means that BA.5 is the dominant variant relative to all other SARS-CoV-2 variants detected in the wastewater. These sequence data do not include information on the relative abundance of other pathogens present in wastewater. Variant composition estimates should be interpreted with caution, as substantial gaps in coverage across the reference genome and/or a lack of sequencing depth can produce inaccurate variant/lineage calls.

Due to the variability of wastewater and presence of inhibitors, concentrations reported have been normalized by flow and population served for each WWTP. Excessive rainfall and changes in water use can impact concentrations of SARS-CoV-2 in sewage over time. The use of a fecal indicator control (Pepper Mild Mottle Virus - PMMoV) helps account for any changes in human waste input in wastewater over time. For more information on how wastewater surveillance works, refer to the resources at the end of the report. At the Hawaii Department of Health's State Lab, we are in the process of validating our own protocols for processing wastewater on-site. This report includes SARS-CoV-2 concentrations from samples collected from 07/01/2023 to 08/29/2023, and relative variant proportions from 07/01/2022 to 08/25/2023. Report print date: 09/05/2023.

Metadata for participating WWTP by county

County	Number of WWTP	Total Population Served
Honolulu	5	906,000
Maui	3	160,000
Hawaii	3	21,507
Kauai	3	11,100

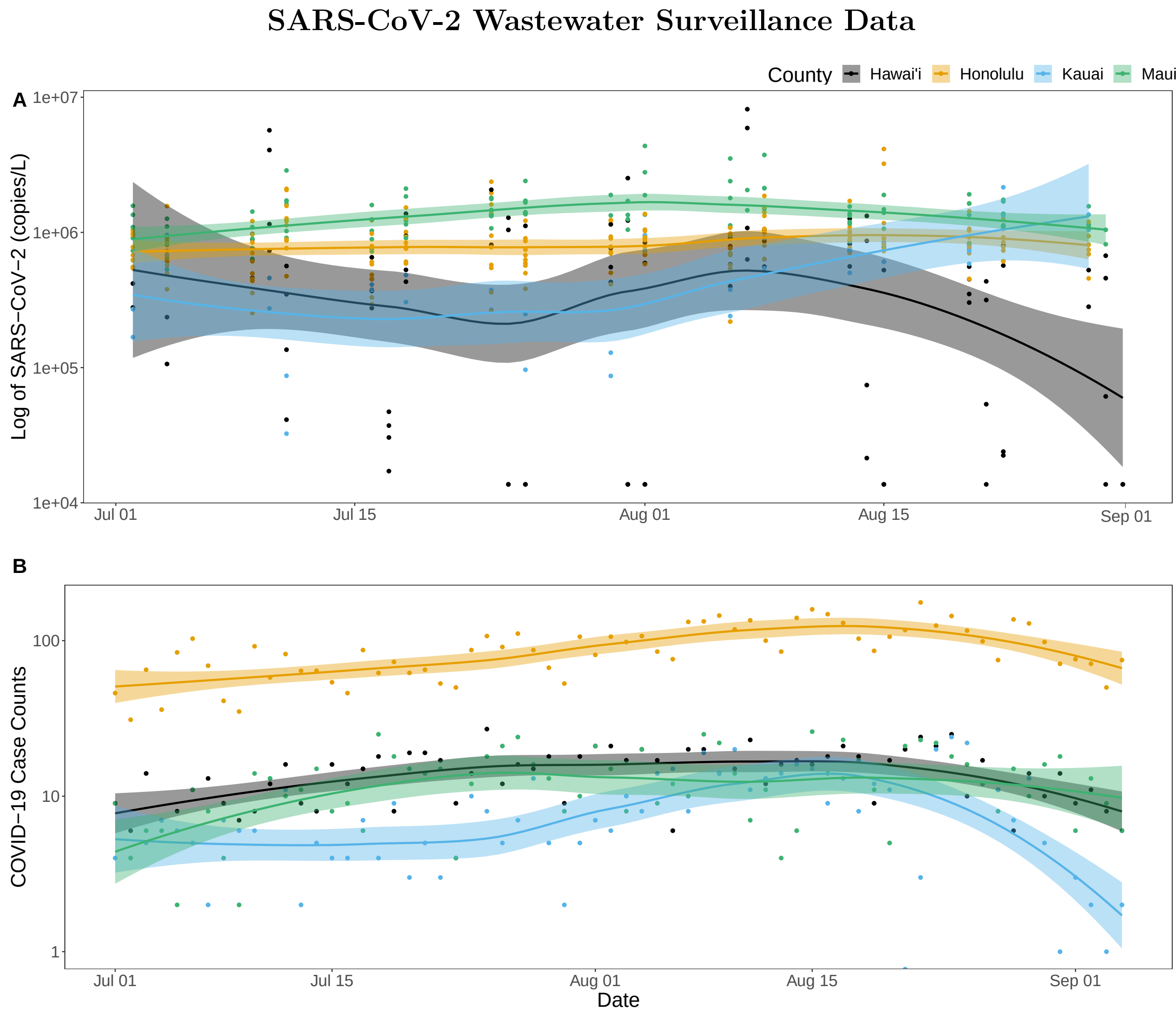

Figure 1. (A) Log transformed normalized concentrations of SARS-CoV-2 in sewage (copies/L) and (B) case counts for the state of Hawaii by each county. Regression line created with a prediction interval from a locally weighted (LOESS) regression. Shaded region represents 95% confidence interval.

Notes: Trends in raw sewage concentrations of SARS-CoV-2 are fluctuating in all counties.

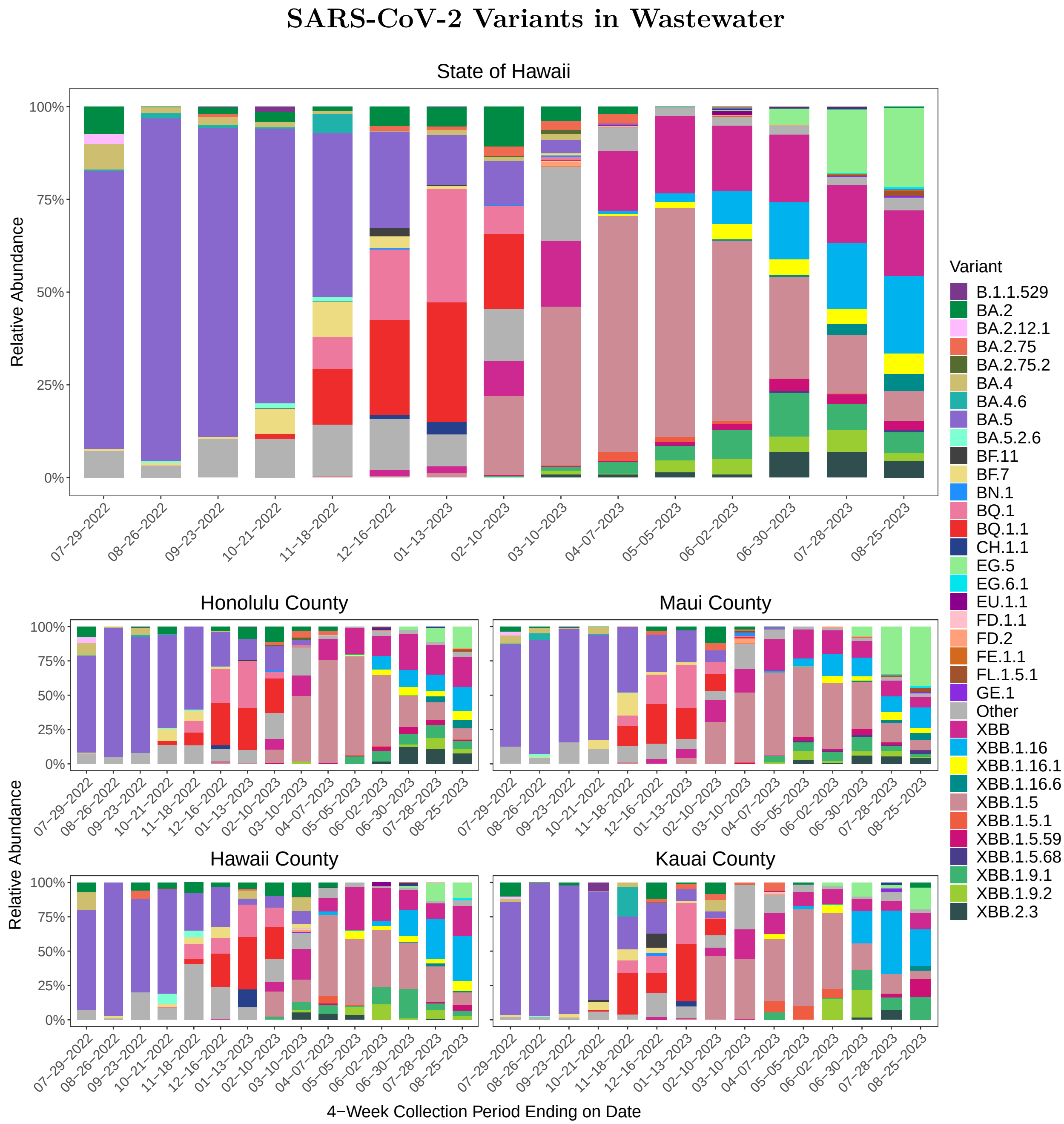

Figure 2. Stacked barplot demonstrating the relative abundance of SARS-CoV-2 aggregated lineages detected in wastewater across the State of Hawaii from 07/01/2022 to 08/25/2023.

Notes: Among the SARS-CoV-2 lineages detected, XBB* lineages have the highest relative abundance of estimated variants since February 2023 across the State of Hawaii. XBB* are all lineages and sublineages of Omicron. For a more detailed description of sequencing and information on clinical SARS-CoV-2 variants in the State of Hawaii, refer to the *State of Hawaii SARS-CoV-2 Sequencing and Variant Report* and the resources provided at the end of this report. These data are only a partial representation of the population from each county which might impact the abundance of certain lineages.

SARS-CoV-2 and Beyond: Additional Wastewater Surveillance Data

Wastewater based epidemiology has emerged as an effective method for monitoring various pathogens to obtain unbiased data on disease prevalence in a community. Other pathogens, excluding SARS-CoV-2, such as fungal, bacterial, or viral pathogens are suitable candidates for wastewater surveillance. To be monitored effectively, pathogens must be excreted or shed by humans, present in detectable concentrations during an active infection, and incapable of replication in the environment outside of a host (e.g., sewage systems).

Pathogens meeting these criteria, and of public health significance, can be surveyed similarly to SARS-CoV-2 concentrations in wastewater. Notable examples of pathogens that fulfill the requirements for wastewater surveillance include Norovirus (NoV), Influenza (commonly known as the "flu"), and MPOX (formerly known as monkeypox).

Noroviruses are single-stranded RNA viruses that cause millions of cases of acute gastroenteritis annually. Gastroenteritis is characterized by symptoms such as diarrhea, vomiting, nausea, and stomach pain and is commonly referred to as the "stomach flu." Inadequate handwashing before touching the mouth or consuming food, ingestion of contaminated food or liquids from infected individuals, and consumption of uncooked shellfish are common routes of transmission. NoV is excreted in the feces of infected individuals regardless of infection severity, and does not replicate in the environment without a zoological host, making NoV an ideal candidate for wastewater surveillance. Our wastewater detection focuses on the NoV GII strain, which causes 80% of norovirus infections worldwide.

Influenza viruses are highly contagious enveloped RNA viruses that infect the upper respiratory tract and are always accompanied by a fever. The two common subtypes, Flu A and Flu B, are responsibly for the majority of human infections during flu seasons. Infected individuals shed Influenza virus in bodily fluids like mucus and saliva for about 3 - 7 days in healthy adults, and up to 10 days or more in children depending on the viral titer. About 30% of infected individuals shed Influenza in their feces. Due to this viral shedding, Influenza A and B are deemed suitable for wastewater monitoring purposes.

MPOX is a double-stranded DNA virus closely related to the smallpox. Infected individuals experience a painful rash resembling pimples or blisters, which can appear on various body parts such as hands, feet, chest, face, mouth, and genitalia. The virus primarily spreads through direct contact with infected individuals with an active rash, contaminated objects (fomites), respiratory droplets, or vertical transmission from a parent to child. Infected individuals shed MPOX into wastewater through urine, semen, saliva, and feces. Wastewater-based epidemiology of MPOX began in 2022 and has been useful in assessing the prevalence of MPOX within communities.

The remainder of this report contains results for the SARS-CoV-2, Flu A, Flu B, MPOX, and NoV GII surveillance of sewage in collaboration with the National Wastewater Surveillance System (NWSS) and WastewaterSCAN. A total of 6 WWTP in two counties from the State of Hawaii are participating in this surveillance. Samples are collected three time a week and analyzed by WastewaterSCAN. WastewaterSCAN processes sludge, rather than the liquid fraction (which is what Biobot utilizes for processing). Concentrations in the following pages (unless otherwise stated) are reported as copies per g of dry sludge. This following includes samples collected from 07/01/2023 to 08/28/2023.

Metadata for participating WWTP by county

County	Number of WWTP	Total Population Served
Honolulu	5	906,000
Hawaii	1	16,257

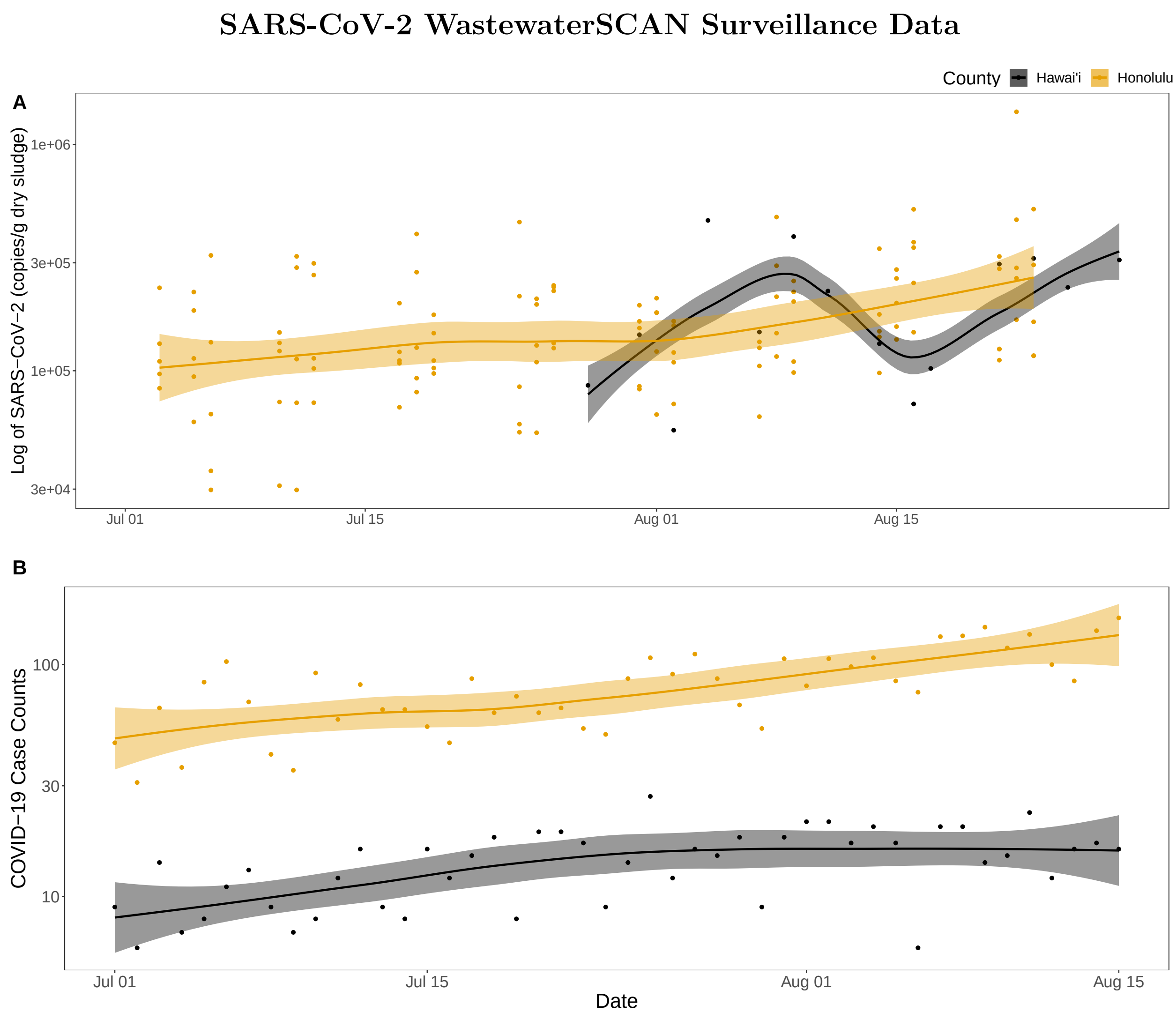

Figure 3. (A) Log transformed normalized concentrations of SARS-CoV-2 in sewage (copies/g dry sludge) quantified by WastewaterSCAN for one WWTP from Hawaii County and five from Honolulu County and (B) COVID-19 case counts for each county. These data are plotted separately since WastewaterSCAN utilizes sludge rather than the liquid fraction of raw sewage influent (which is used by Biobot). Regression line created with a prediction interval from a locally weighted (LOESS) regression. Shaded region represents 95% confidence interval for Honolulu County and 40% confidence interval for Hawaii County.

Notes: Trends in raw sewage concentrations of SARS-CoV-2 are fluctuating in these counties.

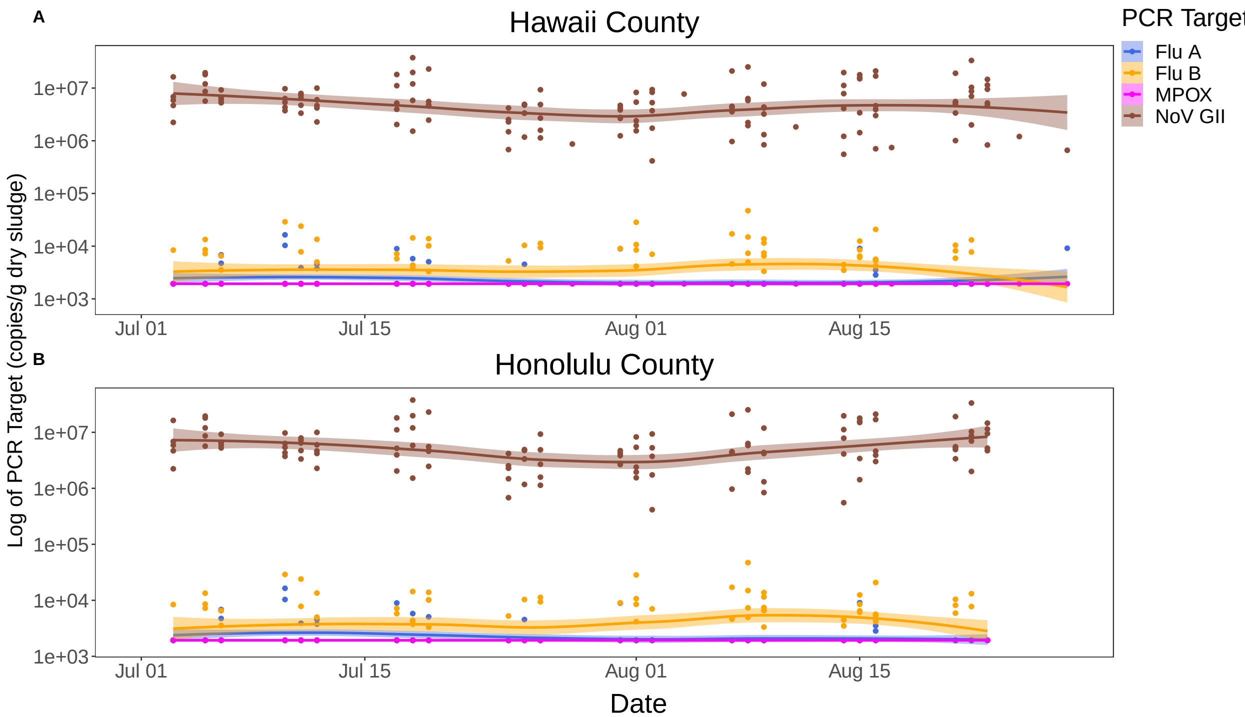

Figure 4. Log transformed normalized concentrations of Flu A, Flu B, MPOX, and NoV GII in sewage (copies/g dry sludge) quantified by WastewaterSCAN for one WWTP from (A) Hawaii County and five from (B) Honolulu County. Regression line created with a prediction interval from a locally weighted (LOESS) regression. Shaded region represents 95% confidence interval.

Notes: Concentrations of Flu A, Flu B, and MPOX are low and frequently not detected in samples. NoV GII is found at higher concentrations, likely since those infected primarily shed NoV GII in feces. Still, NoV GII trends are relatively stable across time.

Resources

- National Wastewater Surveillance System (NWSS): https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.html
  - How it works: https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/resources/how-wws-works.html
  - Data Reporting: https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/data-reporting-analytics.html
  - Data: https://covid.cdc.gov/covid-data-tracker/#wastewater-surveillance
  - Testing Method: https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/testing-methods.html
- Biobot Analytics: https://biobot.io/
  - Data: https://biobot.io/data/
  - Methodology and Protocol: https://biobot.io/covid19-community-plus-report-notes/
  - Sequencing: https://biobot.io/covid19-variants-report-notes/
- WastewaterSCAN: https://wastewaterscan.org/
  - Data: https://data.wastewaterscan.org/
  - Methodology and Protocol: https://data.wastewaterscan.org/about
  - https://www.protocols.io/view/high-throughput-pre-analytical-processing-of-waste-kxygxpod4l8j/v2
  - https://www.protocols.io/view/high-throughput-rna-extraction-and-pcr-inhibitor-r-81wgb72bovpk/v2
  - https://www.protocols.io/view/high-throughput-sars-cov-2-pmmov-and-bcov-quantifi-e6nvw5orwvmk/v5
  - https://www.protocols.io/view/quantification-of-various-sars-cov-2-variant-mutat-14egnzrrzg5d/v11
  - https://www.protocols.io/view/quantification-of-various-sars-cov-2-variant-mutat-14egnzrrzg5d/v11
- SARS-CoV-2 Variant Classifications:
  - https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html#:~:text=SARS%2DCoV%2D2%20has%20many,contain%20one%20or%20more%20mutations.
- Information on other pathogens surveyed by wastewater:
  - Norovirus: https://www.cdc.gov/hai/organisms/norovirus.html
  - https://www.cdc.gov/norovirus/lab/virus-classification.html
  - https://journals.asm.org/doi/10.1128/jvi.01364-10
  - Influenza: https://www.cdc.gov/flu/about/index.html
  - https://www.cdc.gov/flu/about/viruses/types.htm
  - MPOX: https://www.cdc.gov/poxvirus/mpox/symptoms/index.html
  - https://www.cdc.gov/poxvirus/mpox/index.html
- More about wastewater surveillance for SARS-CoV-2 and other pathogens:

– https://www.sciencedirect.com/science/article/pii/S2590049822000078
– https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8416286/
– https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7583624/
– https://www.nature.com/articles/s41586-022-04980-y
– https://www.nature.com/articles/s41586-022-05049-6
– https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10256456/
– https://pubmed.ncbi.nlm.nih.gov/34153546/
– https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9858235/
– https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8769679/