HAWAII STATE DEPARTMENT OF HEALTH

Wastewater Surveillance Report

04/30/2024

Contents

1 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 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.

2 National Wastewater Surveillance System (NWSS)

The following pages of this report contain results for the surveillance of sewage for SARS-CoV-2 in collaboration with the National Wastewater Surveillance System (NWSS). A total of 8 WWTP from the State of Hawaii are participating in this surveillance. Samples are collected weekly and analyzed by a contracted lab by NWSS (previously Biobot Analytics). Concentrations in this document are reported as log-transformed SARS-CoV-2 copies per liter 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 is parallel to Whole Genome Sequencing (WGS) and allows for high-throughput and timely results. NWSS previously contracted lab (Biobot Analytics) conducted NGS with Illumina instruments through their trusted sequencing partners. They calculated the estimated proportion of variants with 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. Please see previous reports for historical relative variant proportions from 07/01/2022 to 09/22/2023. Quantitative SARS-CoV-2 concentrations in wastewater for participating WWTP include data from 02/01/2024 to 04/29/2024. The relative abundance of SARS-CoV-2 variants in wastewater for participating WWTP include data from 02/14/2024 to 04/23/2024. Report print date: 04/30/2024.

Metadata for participating WWTP by county

County	Number of WWTP	Total Estimated Population Served
Honolulu	5	906,000
Maui	2	105,700
Hawaii	1	32,604

2.1 SARS-CoV-2 Wastewater Surveillance Data

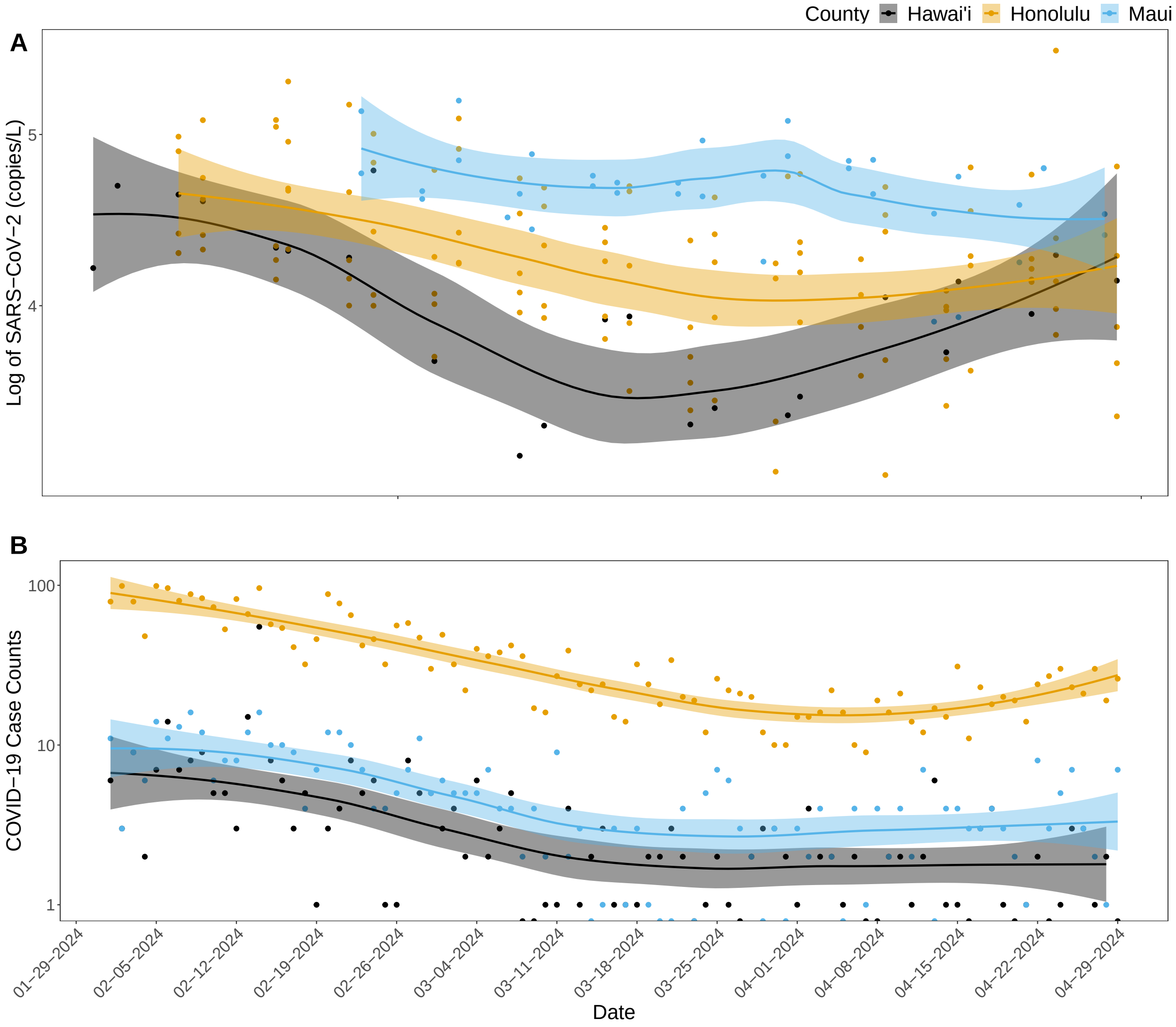

Figure 1. (A) Log transformed normalized concentrations of SARS-CoV-2 in sewage (copies/L) and (B) case counts for the State of Hawaii for Hawaii, Honolulu, and Maui counties. Regression line was created with a prediction interval from a locally weighted (LOESS) regression. Shaded region represents 95%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

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

2.2 SARS-CoV-2 Variants in Wastewater

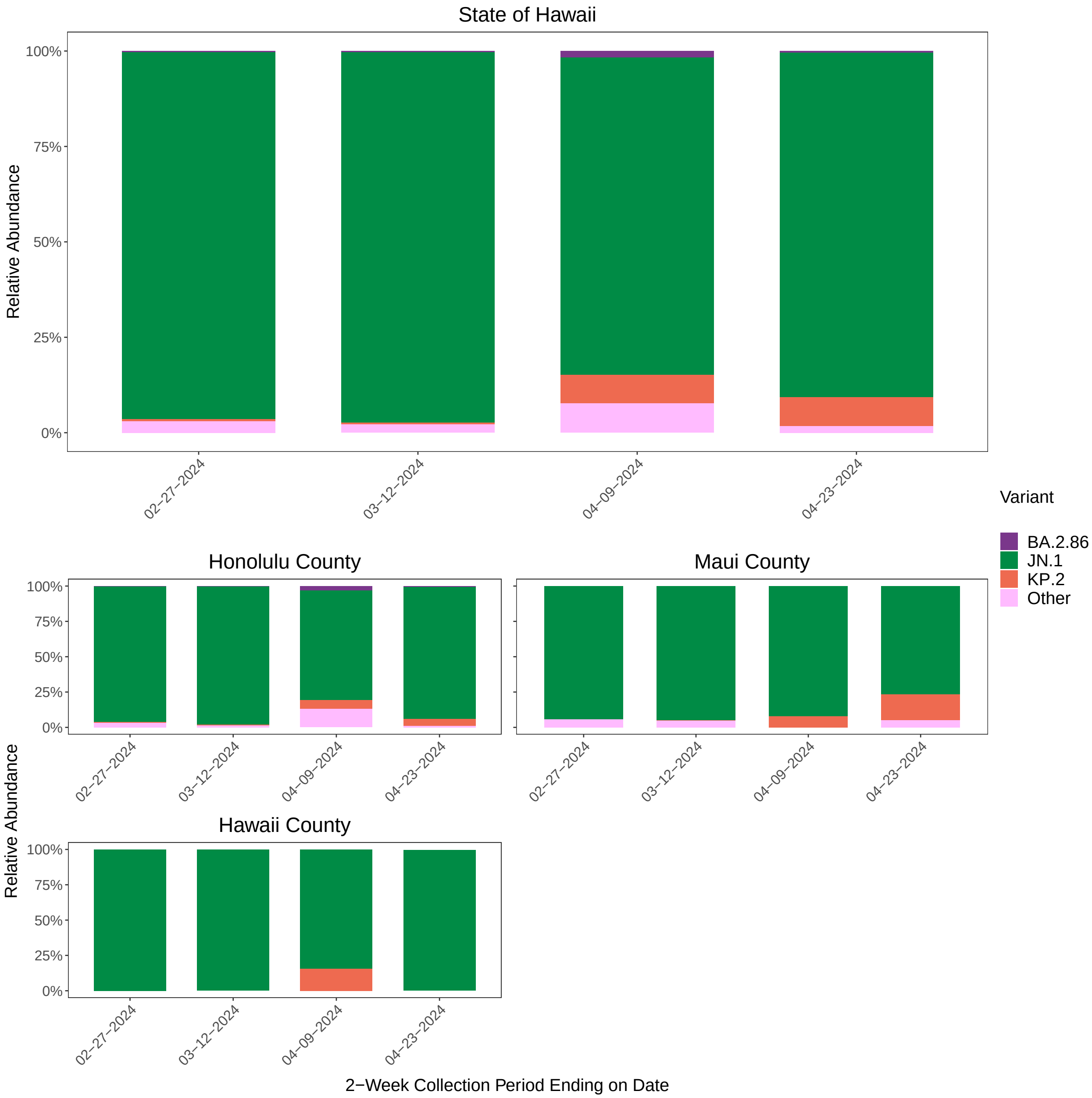

Figure 2. Stacked barplot demonstrating the relative abundance of SARS-CoV-2 aggregated lineages detected in wastewater across the State of Hawaii from 02/14/2024 to 04/23/2024.

Notes: Among the SARS-CoV-2 lineages detected in the most recent data included in this report, BN.1 lineages have the highest relative abundance of estimated variants since February 2024 across the State of Hawaii. 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.

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

Wastewater-based epidemiology (WBE) 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 Influenza (commonly known as the "flu"), Respiratory Syncytial Virus (RSV), Norovirus (NoV), MPOX (formerly known as monkeypox), and *Candida auris*.

Other Respiratory Pathogens

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 responsible 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. Infected individuals can also shed Influenza in their feces. Due to this viral shedding, Flu A and Flu B are deemed suitable for wastewater monitoring purposes.

RSV is a single-stranded enveloped RNA virus that infects the respiratory tract with symptoms ranging from congestion, dry cough, fever, to trouble breathing. While RSV typically induces mild symptoms in healthy individuals, it poses a higher risk for severe infections in infants, the immunocompromised, and the elderly. RSV primarily spreads through large droplets or through contact with contaminated objects. Notably, the virus can survive on surfaces for several hours, potentially contributing to its spread. RSV has been detected in the stool of infected individuals, and its concentrations in wastewater have shown a significant positive correlation with clinically confirmed cases of RSV, demonstrating the effectiveness to track community prevalence of RSV with wastewater surveillance.

Gastrointestinal Pathogens

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. WastewaterSCAN data focuses on the NoV GII strain, which causes 80% of norovirus infections worldwide.

Other Pathogens of Concern

MPOX is a double-stranded DNA virus closely related to 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. WBE of MPOX began in 2022 and has been useful in assessing the prevalence of MPOX within communities.

Candida auris (*C. auris*) is an opportunistic fungal pathogen known for its resistance to multiple antifungal agents. While *C. auris* generally poses minimal risk to healthy individuals, it significantly impacts individuals in long-term healthcare facilities, with underlying medical conditions, those with a history of exposure to antifungal agents, or patients requiring invasive medical devices. This pathogen primarily infects the skin through open wounds and the ears, often accompanied by fever and chills. However, *C. auris* may enter the body and infect the bloodstream, heart, or brain (similar to bacterial infections but do not improve with the use of antibiotics). Therefore, infections caused by *C. auris* can be severe and may lead to fatalities. While colonization with *C. auris* can occur without causing any noticeable symptoms, individuals can still transmit the pathogen to others through direct contact or contact with contaminated fomites. *C. auris* has been found in human excreta (feces and urine) as well as in wastewater. Utilizing routine wastewater surveillance to track new introductions of *C. auris* into specific jurisdiction's is vital for infection prevention and control efforts, especially in healthcare settings.

The remainder of this report contains results for wastewater surveillance of SARS-CoV-2, other respiratory pathogens (Flu A, Flu B, and RSV), gastrointestinal pathogens (NoV GII) and other pathogens of concern (MPOX and *C. auris*) in collaboration with the National Wastewater Surveillance System (NWSS) and WastewaterSCAN. **Note: a total of five WWTP from the State of Hawaii are actively participating in this surveillance with WastewaterSCAN.** Samples are collected three times a week and analyzed by WastewaterSCAN. WastewaterSCAN processes sludge from participating WWTP in Hawaii rather than the liquid fraction (which is what NWSS contracted lab Biobot Analytics previously utilized for processing). Concentrations of each target quantified by WastewaterSCAN are reported as log-transformed copies per g of dry sludge. The following section includes samples collected from 11/15/2023 to 04/23/2024.

Metadata for participating WWTP by county

County	Number of WWTP	Total Population Served
Honolulu	5	906,000

3.1 SARS-CoV-2 WastewaterSCAN Surveillance Data

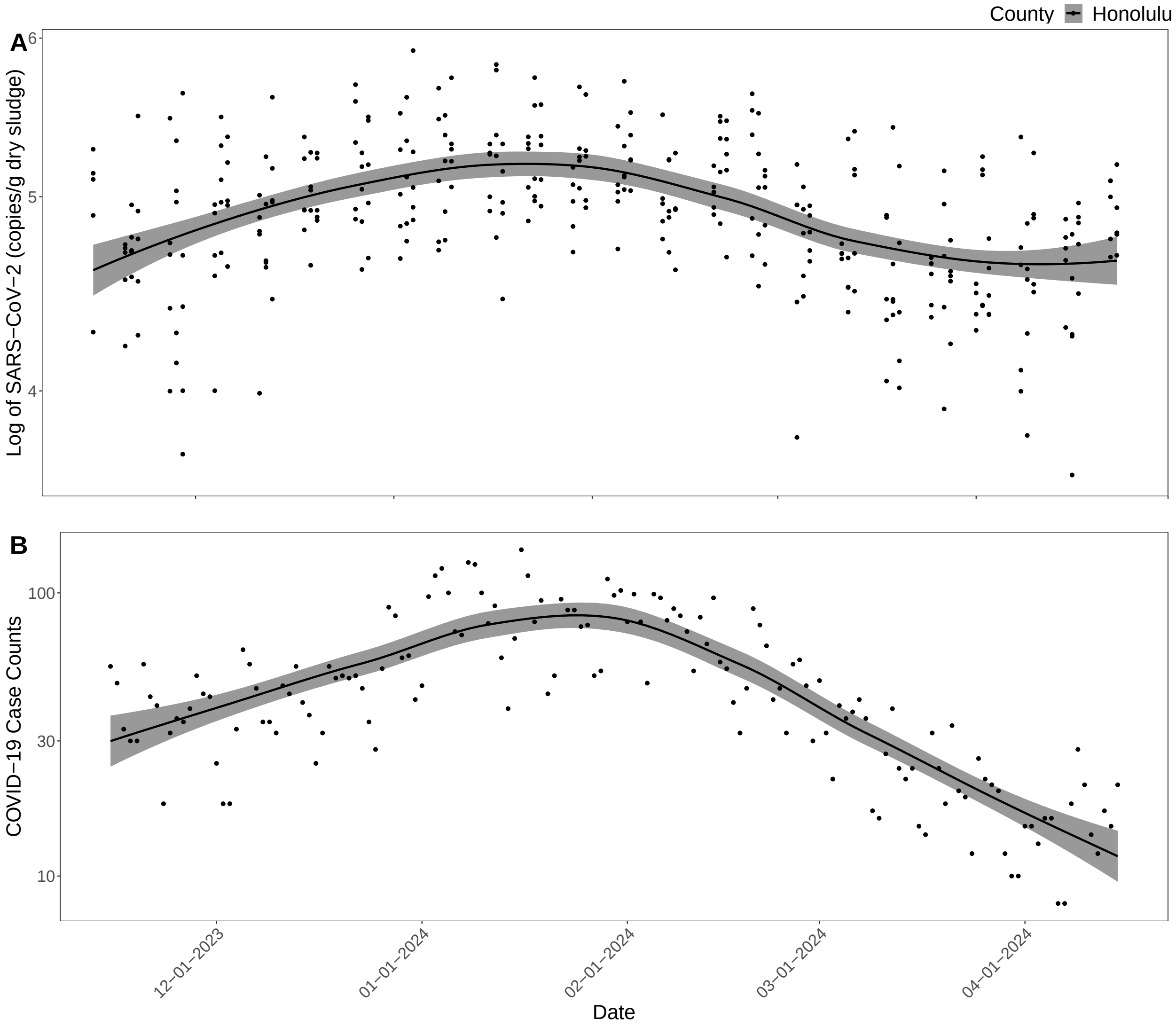

Figure 3. (A) Log transformed normalized concentrations of SARS-CoV-2 in sewage (copies/g dry sludge) quantified by WastewaterSCAN for five WWTP from Honolulu County and (B) Honolulu County COVID-19 case counts. Regression line was created with a prediction interval from a locally weighted (LOESS) regression. Shaded region represents 95% confidence interval.

Notes: WastewaterSCAN has estimated that the limit of detection for targets is ~1000 copies/g. Trends in raw sewage concentrations of SARS-CoV-2 are fluctuating in this county.

3.2 Wastewater Surveillance Data for Respiratory Pathogens

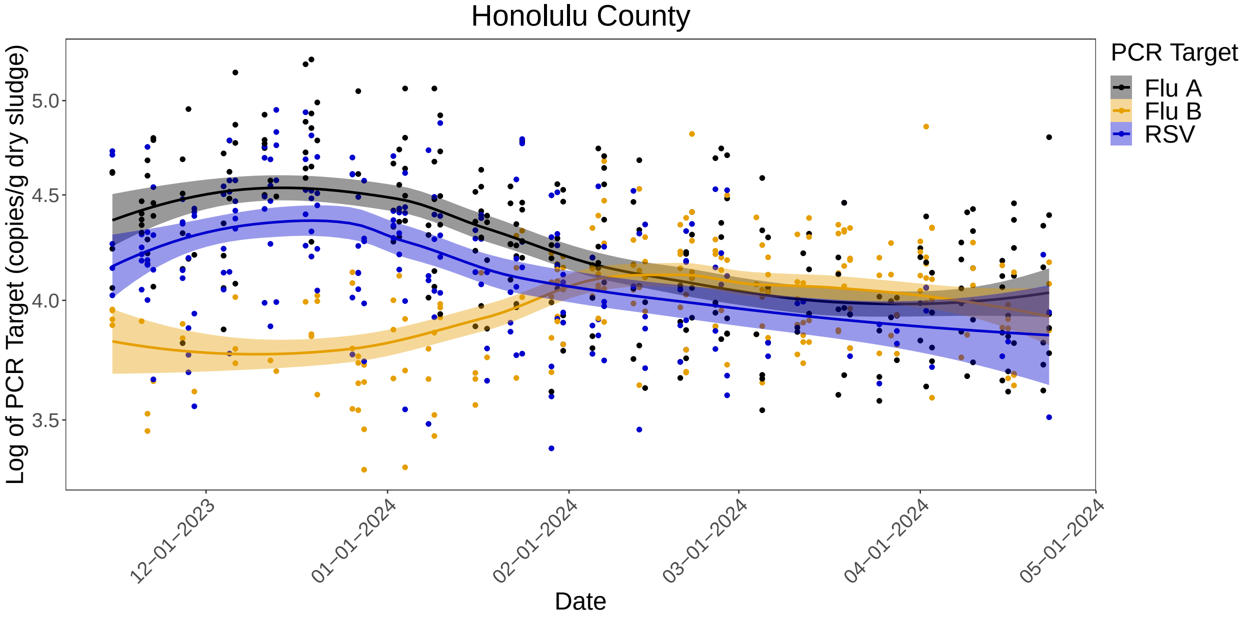

Figure 4. Log transformed normalized concentrations of Flu A, Flu B, and RSV in sewage (copies/g dry sludge) quantified by WastewaterSCAN for five WWTP from Honolulu County. Regression line created with a prediction interval from a locally weighted (LOESS) regression. Shaded region represents 95% confidence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

Notes: WastewaterSCAN has estimated that the limit of detection for targets is ~1000 copies/g. Concentrations of all respiratory pathogens are relatively stable in recent data.

3.3 Wastewater Surveillance Data for Gastrointestinal Pathogens

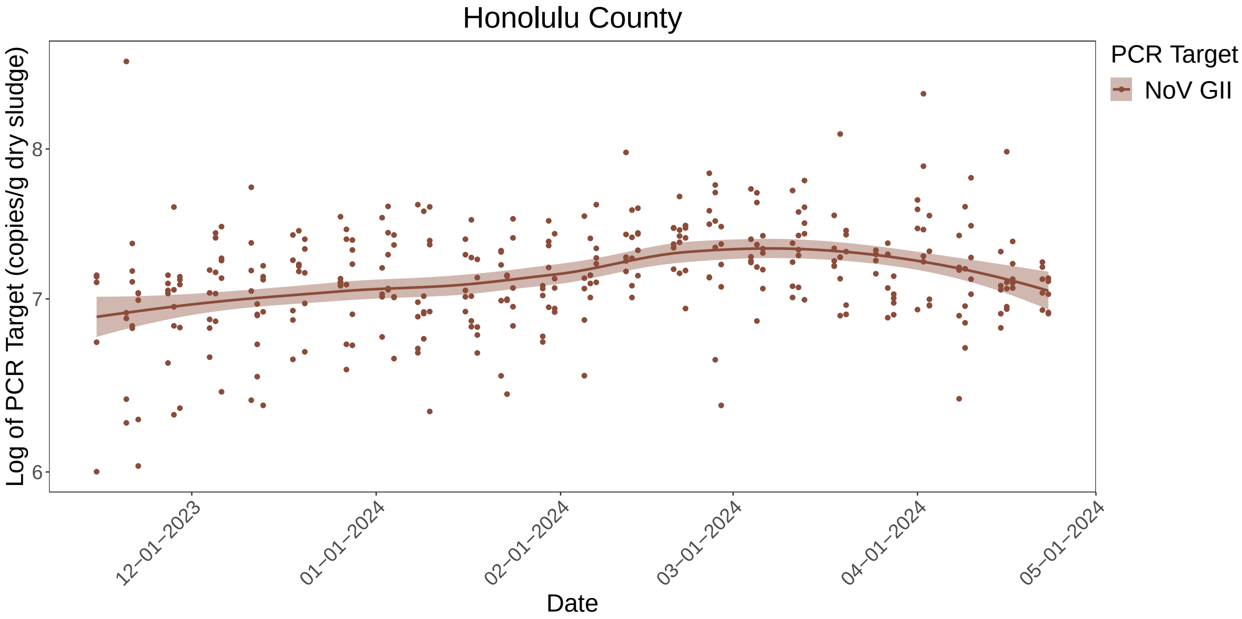

Figure 5. Log transformed normalized concentrations of NoV GII in sewage (copies/g dry sludge) quantified by WastewaterSCAN for five WWTP from Honolulu County. Regression line created with a prediction interval from a locally weighted (LOESS) regression. Shaded region represents 95% confidence interval.

Notes: WastewaterSCAN has estimated that the limit of detection for targets is ~1000 copies/g. NoV GII is found at consistently high concentrations compared to other pathogens likely since those infected primarily shed NoV GII in feces. Still, NoV GII trends are relatively stable across time in Honolulu County.

3.4 Wastewater Surveillance Data for Other Pathogens of Concern

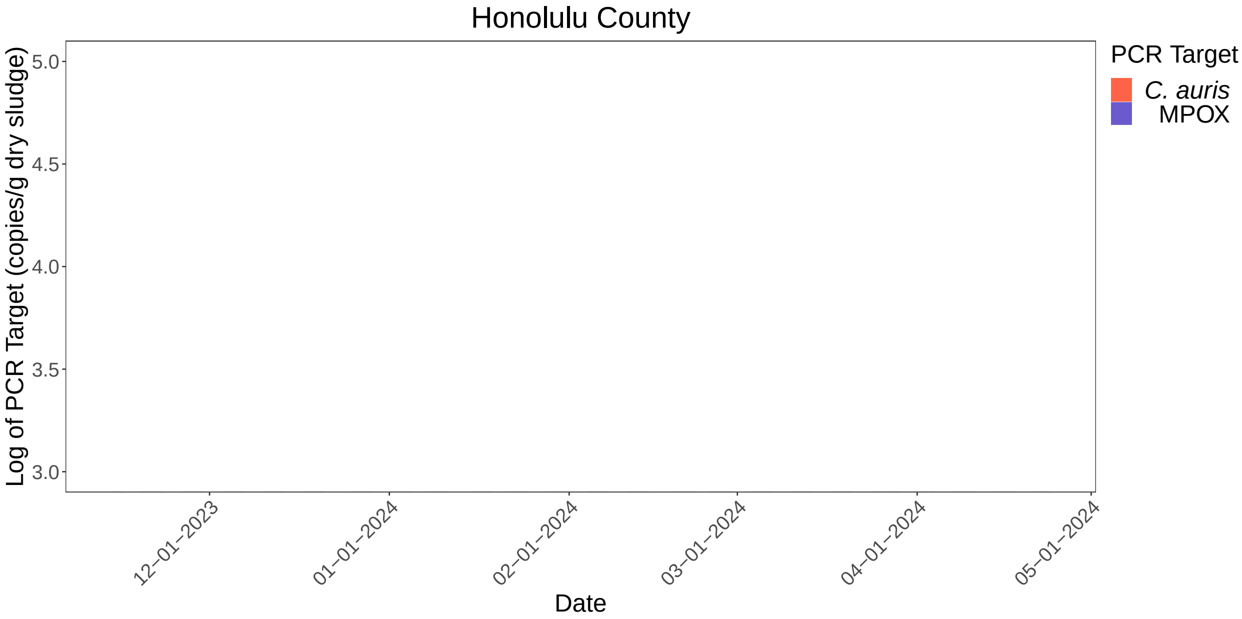

Figure 6. Bar chart of log transformed normalized concentrations of *C. auris* and MPOX in sewage (copies/g dry sludge) quantified by WastewaterSCAN for five WWTP from Honolulu County.

Notes: WastewaterSCAN has estimated that the limit of detection (LOD) for targets is ~1000 copies/g. Samples that fall below the LOD for *C. auris* and MPOX do not appear on the figure. Detection of MPOX and *C. auris* in samples is rare. No bars appear on the figure since there have been no recent detections above the LOD for MPOX and *C. auris* in Honolulu wastewater.

4 Hawaii State Laboratories Division: Wastewater Surveillance Data

The Hawaii State Laboratories Division (SLD) is developing local wastewater testing capabilities. All participating WWTP collect sewage samples once weekly which are sent to the state laboratory for processing. Methods for processing includes concentration, DNA/RNA extraction, and pathogen quantification with digital PCR (dPCR). These methodologies differ from contracted labs from NWSS (Biobot), and WastewaterSCAN. Additionally, the liquid fraction of raw influent is utilized for processing unlike WastewaterSCAN which uses sludge. Currently, dPCR assays are targeting respiratory pathogens specifically SARS-CoV-2, Flu A, Flu B, and RSV. Still, methods are in continual development such as improving the recovery of targeted pathogens and expanding dPCR assays. For this report, data is included from Kauai County and Hawaii County. Concentrations of each target quantified by the Hawaii State Laboratories Division are reported as log-transformed copies per L of raw sewage. The following section includes samples collected from 10/01/2023 to 04/22/2024.

Metadata for participating WWTP by county

County	Number of WWTP	Total Population Served
Kauai	1	10,000
Hawaii	1	32,604

4.1 Hawaii SLD Wastewater Data: Respiratory Pathogens

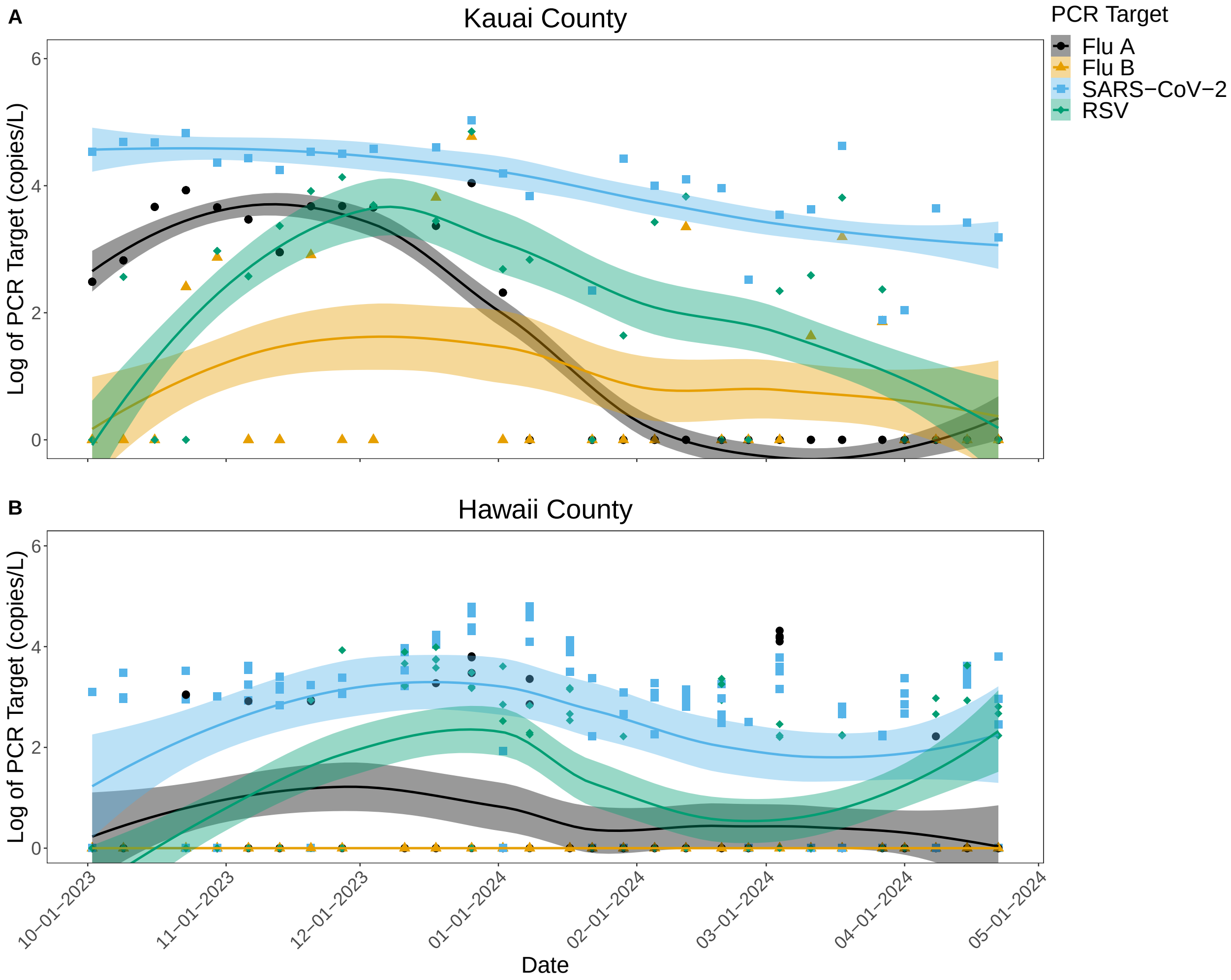

Figure 7. Log transformed average concentrations of Influenza A, Influenza B, SARS-CoV-2, and RSV in sewage from (A) Kauai County WWTP and (B) Hawaii County WWTP over time. Regression line created with a prediction interval from a locally weighted (LOESS) regression. Shaded region represents 95% confidence interval. Values below the limit of detection (LOD) are reported as 0 copies/L.

Notes: The SLD has recently switched wastewater concentration methods, which may reflect changes in concentrations of targets. Across both counties, SARS-CoV-2 is relatively stable while other respiratory pathogens are fluctuating. Flu B wastewater concentrations in both counties are frequently below the LOD.

5 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:
  - Influenza: https://www.cdc.gov/flu/about/index.html
  - https://www.cdc.gov/flu/about/viruses/types.htm
  - RSV: https://www.cdc.gov/rsv/index.html
  - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3461981/
  - 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>
  - MPOX: https://www.cdc.gov/poxvirus/mpox/symptoms/index.html
  - https://www.cdc.gov/poxvirus/mpox/index.html

  - *C. auris*: https://www.cdc.gov/fungal/candida-auris/index.html
  - https://www.cdc.gov/drugresistance/pdf/threats-report/candida-auris-508.pdf
  - https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7211321/
  - https://www.ncbi.nlm.nih.gov/books/NBK563297/

- 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/
  - https://pubs.acs.org/doi/10.1021/acs.estlett.1c00963
  - https://pubs.acs.org/doi/10.1021/acs.est.2c07763