

HAWAII STATE DEPARTMENT OF HEALTH

Wastewater Surveillance Report

07/18/2023

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.

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 05/30/2023 to 07/11/2023, and relative variant proportions from 07/01/2022 to 07/04/2023. Report print date: 07/18/2023.

Metadata for participating WWTP by county

County	Number of WWTP	Total Population Served
Honolulu	5	906,000
Maui	3	160,000
Hawaii	3	37,850
Kauai	3	11,100

SARS-CoV-2 Wastewater Surveillance Data

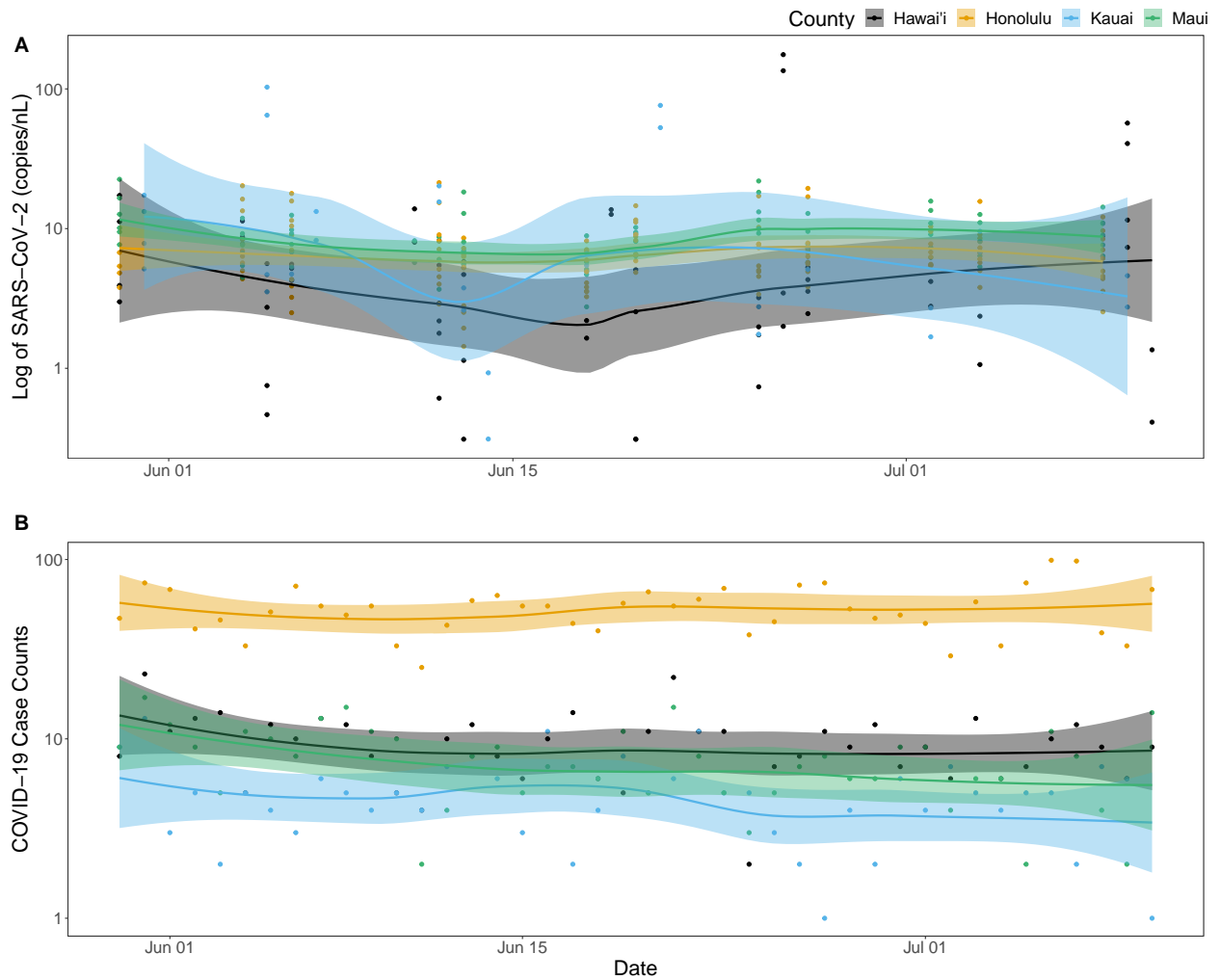


Figure 1. (A) Log transformed normalized concentrations of SARS-CoV-2 in sewage (copies/nL) and (B) case counts for the state of Hawaii by each county. Regression line created with a prediction interval from a locally weighted (LOESS) regression. Shaded region represents 95% confidence interval.

Notes: Trends in raw sewage concentrations of SARS-CoV-2 are fluctuating in all counties. Since the last report, no surges in COVID-19 cases were observed.

SARS-CoV-2 Variants in Wastewater

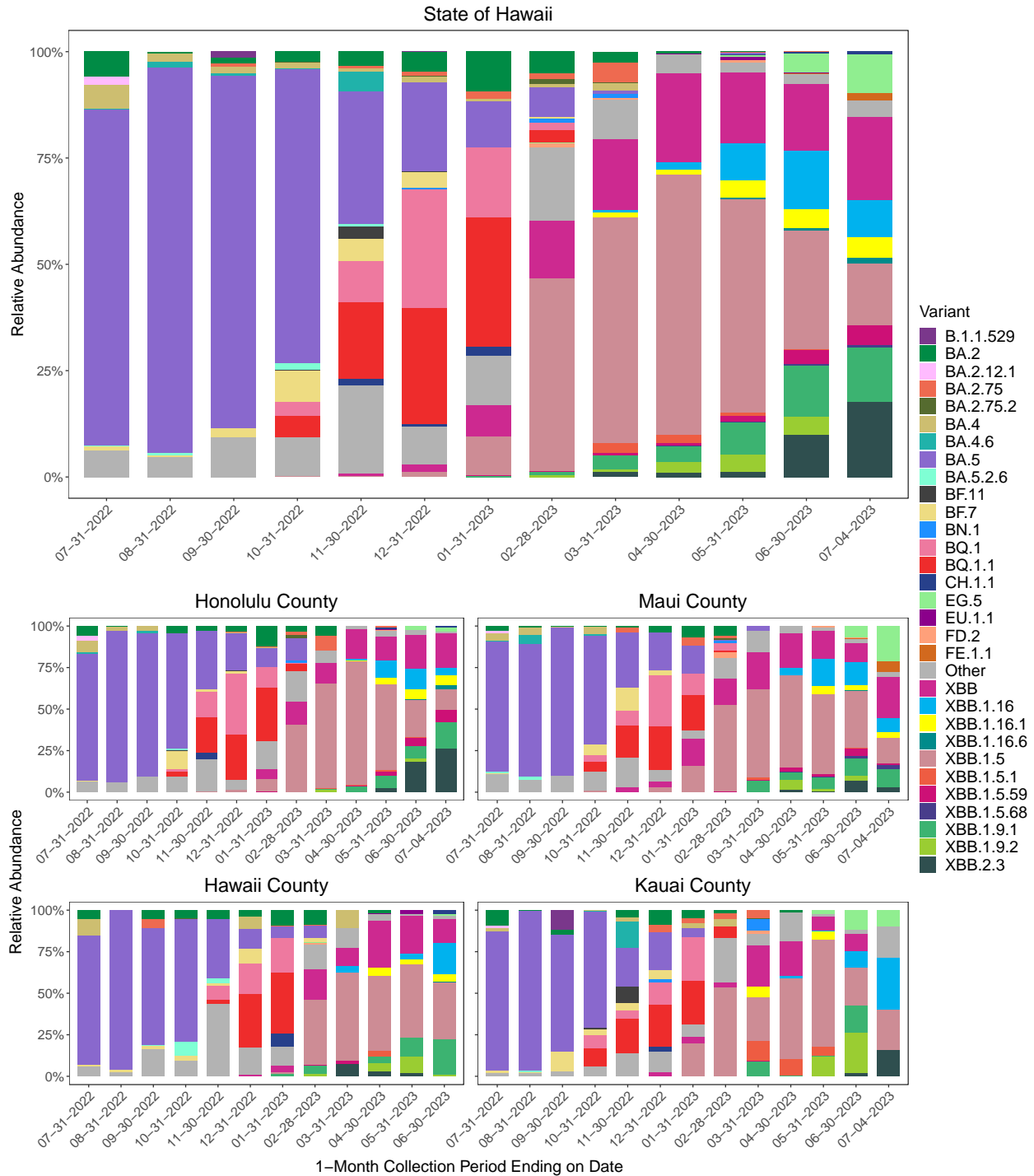


Figure 2. Stacked barplot demonstrating the relative abundance of SARS-CoV-2 aggregated lineages detected in wastewater across the State of Hawaii from samples collected from 07/01/2022 to 06/30/2023 for Hawaii County and 07/01/2022 to 7/04/2023 for Honolulu, Maui, and Kauai County.

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.

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/>
- 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.>
- More about wastewater surveillance for SARS-CoV-2:
 - <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>