Environmental Science Water Research & Technology

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Environ. Sci.: Water Res. Technol.*, 2025, **11**, 114

Beyond campus borders: wastewater surveillance sheds light on university COVID-19 interventions and their community impact†

David Lisboa, [a] Devrim Kaya,[ab] Michael Harry,[a] Casey Kanalos,[a] Gabriel Davis,[a] Oumaima Hachimi,[a] Shana Jaaf,[a] David Mickle,[a] Dana Alegre,[c] Katherine Carter,[c] Steven Carrell,[c] Mark Dasenko,[c] Nathan Davidson,[c] Justin Elser,[c] Matthew Geniza,[c] Anne-Marie Girard,[c] Brent Kronmiller,[c] Matthew Peterson, [c] Elizabeth Zepeda,[c] Christine Kelly[a] and Tyler S. Radniecki [*a]

Received 29th February 2024,
Accepted 28th June 2024

DOI: 10.1039/d4ew00168k

rsc.li/es-water

The evaluation of COVID-19 policy effectiveness on university campuses, particularly in mitigating spread to neighboring cities (*i.e.*, "campus spill-over"), is challenging due to asymptomatic transmission, biases in case reporting, and spatial case reporting limitations. Wastewater surveillance offers a less biased and more spatially precise alternative to conventional clinical surveillance, thus providing reliable data for university COVID-19 policy evaluation. Wastewater surveillance data spanning the academic terms from Fall 2020 through Spring 2022 was used to evaluate the impact of university COVID-19 policies. During the campus closure to external visitors (09/21/2020–9/15/2021), campus viral concentrations and variant compositions were dissimilar from those of the host and neighboring cities (MAPE = 0.25 ± 0.14; Bray–Curtis = 0.68 ± 0.1, respectively), indicating relative isolation of the campus from its surroundings. Upon the campus reopening to visitors (9/15/2021–2/27/2022), the viral concentrations and variant compositions matched more closely with the host and neighboring cities (MAPE = 0.21 ± 0.1; Bray–Curtis = 0.14 ± 0.08, respectively). Furthermore, post-lifting of campus and state mask mandates (2/27/2022–6/12/2022), the campus, host and neighboring city viral concentrations and variant compositions became indistinguishable (MAPE = 0.06 ± 0.02; Bray–Curtis = 0.07 ± 0.05, respectively). This data suggests that university COVID-19 policies effectively prevented campus-spill over, with no significant contribution to COVID-19 spread into the surrounding communities. Conversely, it was the surrounding communities that led to the spread of COVID-19 onto the campus. Therefore, wastewater surveillance proves instrumental in monitoring COVID-19 trends in surrounding areas, aiding in predicting the impact of easing campus restrictions on campus health.

Water impact

This study utilized wastewater surveillance to assess the efficacy of university COVID-19 policies to protect campus and community health. The findings demonstrated that university COVID-19 policies were effective at preventing campus spill-over to neighboring communities. This research highlights wastewater surveillance's potential as a vital tool in evaluating the effectiveness of university public health policies.

1. Introduction

The implementation of university COVID-19 restrictions and policies has been heavily influenced by clinical testing as well as recommendations from local, city, and federal health officials. These policies, designed to mitigate the suspected and feared spread of COVID-19 into its surrounding communities (*i.e.* campus spill-over), included shifting to online-only instruction, enforcing social distancing, and mandating the use of masks on campus.[1] While the effects of these COVID-19 policies in universities have been proven to reduce transmission,[2–4] there has been limited effort in understanding how effective these policies were at preventing campus spill-over nor at how easing these restrictions affects the campus and surrounding communities.

[a] *School of Chemical, Biological and Environmental Engineering, Oregon State University, Corvallis, OR, USA. E-mail: tyler.radniecki@oregonstate.edu*
[b] *School of Public Health and SDSU-IV, San Diego State University, San Diego, CA, USA*
[c] *Center for Quantitative Life Sciences, Oregon State University, Corvallis, OR, USA*

† Electronic supplementary information (ESI) available. See DOI: **https://doi.org/10.1039/d4ew00168k**

Traditional clinical surveillance methods, such as PCR testing, contact tracing, and isolating, have been essential in both identifying and isolating cases to prevent COVID-19 spread.[5] However, there are significant limitations within this. These include asymptomatic transmission, underreporting, test availability, and time delays due to infection-to-symptom onset.[6] Additionally, early COVID-19 testing were shown to disproportionately represent both Black and Hispanic populations due to geographic disparities of testing sites.[7–9] Although clinical surveillance is useful in monitoring county and state-wide data, it often fails to capture the whole picture of COVID-19 viral transmission, especially within dynamic populations such as university campuses.

In contrast, wastewater surveillance offers a less biased and more spatially precise alternative.[10–12] This method uses PCR-based approaches to quantify pathogens, like the SARS-CoV-2 virus, that are shed in an infected individual's stool and enters the waste stream after flushing. Additionally, pathogen variant composition can be identified *via* amplicon sequencing.[13] This tool has been used to provide an accurate representation of the pathogenic activity within a neighborhood or community, providing an additional source of data to assist public health officials in making informed decisions on how to respond to the pandemic dynamics.[14–18] Wastewater surveillance as a whole is cost-effective, non-invasive, and can be performed in areas where clinical testing is constrained.[19,20]

Despite its advantages, wastewater surveillance is also linked to potential biases and constraints such as variations in water use, rainwater infiltration and inundation (I and I), and sampling frequency. This study addresses these challenges through regular maintenance of autosamplers and excluding data from significant rainfall events. Additionally, SARS-CoV-2 quantification underwent rigorous quality control measures to mitigate the risk of inaccurate data.

Wastewater surveillance has been utilized to identify COVID-19 hotspots at the community scale[12] and to provide initial screening efforts at the building scale.[21] At the university level, wastewater surveillance has been successful in monitoring the viral burden of a campus community.[20,22] Furthermore, wastewater surveillance has been effective as a tool to identify locations of positive individuals and provide intervention to contain outbreaks.[21,23–25] However, to date, wastewater surveillance has not been used to evaluate the effectiveness of university COVID-19 policies.

A common concern of many living in university host cities was the increased risk of COVID-19 spreading through the community and neighboring communities due to the influx of out-of-area students.[26–29] Universities responded to this concern, in addition to concern for the well-being of their students, faculty and staff, with a variety of COVID-19 policies. These policies included campus closures, isolation policies in residential halls for infected students, and mask mandates. However, quantifying the impact of college students entering into a community, as well as the effectiveness of university COVID-19 policies to reduce SARS-CoV-2 transmission, can be challenging using traditional clinical surveillance metrics due to the small-geographic scale of a university campus,[26,30] as well as the clinical testing biases mentioned above.

This study utilized wastewater surveillance to investigate the effectiveness of university COVID-19 policies at Oregon State University (OSU) in reducing the spread of COVID-19 on campus. Additionally, it evaluated the effects of rainwater infiltration and inundation (I and I) as well as autosampler maintenance times to make recommendations towards more accurate data collection. Furthermore, wastewater surveillance data of the university host city (Corvallis, OR) and that of a neighboring city (Albany, OR) were compared to the campus community to help determine the effectiveness of university COVID-19 policies in preventing the spread of COVID-19 from the campus into its surrounding communities, (*i.e.* campus spill-over).

2. Materials and methods

2.1 Wastewater sampling sites and collection

Untreated influent wastewater was collected from the Corvallis and Albany wastewater reclamation plants (WWRPs) located in Benton and Linn County, Oregon, respectively. Samples consisted of 24 h time-weighted composites following Layton, *et al.*[16] and were collected 4 times and 2 times per week from the Corvallis and Albany WWRP, respectively. Additionally, 24 hour time-weighted composite samples, with a 15 minute sampling interval, were collected from manhole locations at 15 on-campus sites (Fig. S1†)

Table 1 Sampling locations for the campus and surrounding communities

Group	Location	Number of samples	Sampling period
Main line	East Campus	127	09/21/20–06/04/22
	International Living-Learning Center (ILLC)	133	09/27/20–06/18/22
	Arnold Dining	142	10/04/20–06/11/22
Building clusters	Poling/NW Weatherford	114	09/27/20–06/11/22
	Goss Stadium	139	10/04/20–06/04/22
	Halsell	109	09/27/20–06/11/22
	Beth Ray Center	135	10/07/20–06/04/22
Isolated buildings	Sackett	115	09/21/20–06/11/22
	West	112	09/30/20–06/08/22
	Finley	149	09/21/20–06/18/22
	Callahan	123	09/21/20–06/08/22
	Gem	118	09/27/20–06/11/22
	Hawley/Buxton	118	09/21/20–06/08/22
	Wilson/McNary/Tebeau	118	09/21/20–06/11/22
	SE Weatherford	112	09/30/20–06/11/22
WW treatment plants	Corvallis	374	04/21/20–06/05/22
	Albany	151	09/23/20–08/12/22

twice per week (Table 1). All samples were collected between September 2020 and June 2022.

Campus sampling sites were divided into three categories: main line, building clusters, and isolated buildings. Main line locations were those in which most of the wastewater effluents from buildings across campus intersected and flowed. Building clusters contained wastewater effluent from at least ten campus buildings which included a mix of residential halls and academic buildings. Finally, isolated buildings were those in which wastewater effluent contributing to the sample was only from residential halls. Isolated buildings were primarily a single residential hall but could also include residential hall groups (*e.g.* Hawley/Buxton and Wilson/McNary/Tebeau). The International Living-Learning Center (ILLC) and East Campus locations were used to represent the entire campus' wastewater data as the majority of the university's effluent flowed through these points before exiting to the city. The East Campus location was used as the primary source for representing the entire campus while the ILLC location was used when sampling failures occurred at the East Campus location.

2.2 Wastewater sample collection and SARS-CoV-2 quantification

All wastewater samples were processed as described in Layton *et al.*[16] In brief, during collection, all composite wastewater samples were kept on ice. Within 8 hours of collection, 30 mL of each wastewater composite was vacuum filtered through a 0.45 μm electronegative HA membrane filter. The filter was placed into 2 mL tubes containing 0.7 mm garnet beads and 1 mL of DNA/RNA Shield (Zymo Research, Irvine, CA) and were bead beaten for 2 min. The samples were extracted from 200–400 μL of lysate using the MagMAX Viral/Pathogen kit on a KingFisher Flex automated instrument per manufacturer's instructions (catalog #A48310 ThermoFisher Scientific).

SARS-CoV-2 targets (N1 and N2) and Human RNaseP (internal control, RP) were quantified *via* RT-ddPCR using BioRad's 2019-nCoV CDC ddPCR Triplex Probe Assay and the One-Step RT-ddPCR Advanced Kit for Probes (Bio-Rad Laboratories, Hercules, CA) on a QX-200 ddPCR system with an automated droplet generator and droplet reader, per manufacturer's instructions (Bio-Rad Laboratories). All assay conditions were performed as specified in the Bio-Rad assay protocol with a template concentration of 5.5 μL of RNA per reaction.[31] One-step thermal cycling conditions were as follows: reverse transcription at 50 °C for 60 min, enzyme activation at 95 °C for 10 min; 40 cycles of denaturation at 94 °C for 30s and annealing/extension at 55 °C for 60 s; enzyme inactivation at 98 °C for 10 min; droplet stabilization at 4 °C for 30 min to a maximum of overnight.

A minimum of three positive droplets was required for a sample to be identified as positive for SARS-CoV-2. Samples negative for SARS-CoV-2 detection were assigned a concentration of $\frac{1}{2}$ the limit of detection. All samples were run in duplicate and the N1 and N2 concentrations were averaged together for a final concentration per sample. As determined by Layton *et al.*, the limit of blank for N1 and N2 were 2.0 and 4.2 copies per reaction and the estimated limit of detection for N1 and N2 were 4 and 12 copies per reaction, respectively.[16] Additionally, the process recovery efficiency (using bovine coronavirus as a surrogate) was determined to be 57%.

Each ddPCR plate contains the following controls in duplicate; field blanks (FBs), extraction blanks (EBs), negative control reactions (containing human genomic DNA), positive control reactions (containing synthetic RNA of N1 and N2 and synthetic DNA of RP), and no template controls (NTCs). For a ddPCR run to be considered valid, a minimum of 6000 droplets per reaction must have been produced, all negative controls (FBs, EBs, NTCs and negative control reactions) must have less than 3 positive droplets, and all positive controls (synthetic RNA of N1 and N2 and synthetic DNA of RP) must have passed the three-positive droplet threshold. Per manufacturer's instructions, for an individual ddPCR reaction to be considered valid, at least one of the targets (N1, N2 or RP) must have passed the three-positive droplet threshold. Reactions that did not pass this threshold were excluded from further analyses.

2.3 Sequencing of wastewater SARS-CoV-2

All wastewater samples containing SARS-CoV-2 at a concentration of $4.0 \log_{10}$ gc L^{-1} or greater were sequenced to identify their SARS-CoV-2 variant composition, as previously described.[32] A minimum sequencing template concentration of $4.0 \log_{10}$ gc L^{-1} was required to achieve sufficient sequencing depth. In brief, an amplicon-based sequencing approach was taken to amplify the entire SARS-CoV-2 genome using the Swift Amplicon SARS-CoV-2 Panel and Amplicon Combinatorial Dual indexed adapters (Swift Biosciences, Ann Arbor, MI), per manufacturer's instructions. The average amplicon length was 150 bp which were sequenced on either a HiSeq 3000 or NextSeq 2000 sequencer (Illumina, San Diego, CA) to an average depth of 4–5 million sequence reads per sample.

Sequence reads were demultiplexed, trimmed to the reference sequence (Wuhan-Hu1, GenBank accession no. NC_045512.2), and coordinate-sorted with SAMtools version 1.10 (Genome Research Limited, **http://www.sanger.ac.uk**). The primer sequences were trimmed and GATK version 4.2.0.0 (Broad Institute, **https://www.broadinstitute.org**) was used to identify mutations compared to the reference genome sequence.

A multilocus sequence typing approach was then used to identify SARS-CoV-2 variants by matching amplicon mutations to known SARS-CoV-2 variant mutation sequences. For a positive identification of a variant, a lower limit of 5% of sequence reads (with a minimum of six total reads) was required to span the mutation site. Additionally, a minimum of two different mutations unique to each variant was required.

2.4 Rainfall and wastewater conductivity data

Rainfall totals were obtained for Corvallis from the Hyslop Weather Station. Wastewater collection occurred over 24 hours that spanned over two days. Therefore, 48 h rainfall totals, that included the days the wastewater collection started and ended for each sample, were used for correlation analyses (section 2.6.2). Wastewater conductivity of the 24 h composite samples was measured using a VWR conductivity electrode (catalog #89231-618) and symphony B40PCID benchtop meter (VWR SympHony B40PCID) within 4 hours of sample collection.

To determine the effects of rainwater inundation and infiltration (I and I), spearman correlations were conducted between 48 h rainfall totals and wastewater conductivity. Additionally, regression analyses were performed comparing East Campus wastewater SARS-CoV-2 concentrations to reported on-campus COVID-19 cases under two conditions: 1) using all samples regardless of rainfall and 2) removing samples if the 48 h rainfall total was greater than 15 mm. This cutoff was chosen using the IQR method of outlier detection.

2.5 Campus policies

During Spring Term of the 2020–2021 academic year, OSU closed its campus to outside visitors, held remote classes and only allowed essential staff and researchers onto campus. Additionally, the residential halls were open to students at 50% capacity, housing ∼2200 students in 13 residential halls. For students living in the OSU residential halls at this time, the following policies were enforced: residents and visitors were required to wear face coverings at all times in shared spaces, except for their room as long as no visitors were present; residents were required to maintain a distance of 6 feet at all times; residents could only host visitors in their room if the guest is a resident of the same building; and residents could not attend social gatherings of more than 10 people at any location within Oregon. Additionally, residents who showed COVID-19 symptoms, had a positive COVID-19 diagnosis, or had been exposed to someone who tested positive for COVID-19 were required to isolate until ten days had passed since the onset of symptoms.[33]

During the 2021–2022 academic year, the OSU Corvallis Campus was opened to outside visitors, non-essential employees, and non-resident hall students, and policies followed guidelines provided by the CDC. These requirements included: receiving the first set of vaccinations before arriving on campus, weekly testing for those exempt from vaccination, wearing face coverings at all times in shared spaces, and maintaining a distance of 6 feet at all times.[34] During Spring 2022, the mask mandate was lifted, and all other policies remained in place. During the 2021–2022 academic year, the OSU Corvallis Campus was opened to outside visitors, non-essential employees, and non-resident hall students. Classes were held in person and the resident hall population increased to ∼4700 students across the 13 residential halls. A state-wide mask mandate remained in place until March 12, 2022, requiring masks to be worn inside.

2.6 Transmission and vaccination rates

Community transmission levels for Linn and Benton County were received from historical data provided by the CDC.[35] Transmission was given a category of Low, Moderate, Substantial, and High based on two criteria; total new cases per 100 000 persons in the last 7 days, and percentage of NAATs (nucleic acid amplification tests) that are positive during the past 7 days.[36] Community vaccination rates for Linn and Benton county were received from historical data provided by the CDC.[37] An individual was considered vaccinated if they had received at least their first dose of their vaccination series.

2.7 Statistical analysis

2.7.1 Mean sample event failure rate. The recommended period between autosampler inspection and maintenance services was determined using the mean time before failure (MTBF). This metric quantified the average period of time between autosampler failures (eqn (1)).[38]

$$\mathrm{MTBF} = \frac{\text{Operation uptime}}{\#\text{ of failures}} \tag{1}$$

2.7.2 Correlation analysis. Spearman's correlation coefficients (r_s) were calculated for the following regression analyses: rainfall amounts *versus* wastewater conductivity values; reported COVID-19 cases per 10 000 persons *vs.* wastewater SARS-CoV-2 concentrations; and viral concentrations across three communities, Corvallis, Albany, and OSU.[39] The following categories were assigned to indicate the strength of the correlation: r_s values greater than 0.7 were considered "strong", r_s values from 0.5–0.7 were "moderate", r_s values from 0.3 to 0.5 were "weak" and r_s <0.3 were "none" or "very weak".

In the comparison of rainfall and wastewater conductivity, the interquartile range (IQR) method of outlier detection was used to identify and filter out outliers that would skew the data. This method defines an outlier as a data point in which its value is 1.5 times greater than or 1.5 times less than the IQR.[40]

Additionally, the Fisher's Z-transformation was utilized when comparing between different correlations. This test transforms the samples to become normally distributed and then returns a "z-score" that allows for the testing of the significance of the difference between two correlation coefficients.[41] The Fisher's Z-transformation was used to assess whether rainfall events resulted in significant variations in the wastewater data.

2.7.3 Similarity amongst groups. Similarity analyses were performed on the data using the mean absolute percent error[42] (MAPE) and the Bray–Curtis dissimilarity test.[43] The MAPE (eqn (2)) was used to quantify the average difference between viral concentrations in Corvallis, Albany, and the campus, with a range of 0–∞.

$$\text{MAPE} = \frac{\sum_{i=1}^{n} \left|\hat{y}_i - y_i\right|}{n} \tag{2}$$

where $\hat{y}_i$ = log-transformed SARS-CoV-2 concentration of one community

y_i = log-transformed SARS-CoV-2 concentration of another community

n = number of samples collected

The Bray–Curtis dissimilarity was used to quantify the differences between communities based on the variant composition of the wastewater samples (eqn (3)). Bray–Curtis returns a value in the range of 0–1 where 1 indicates the populations have complete dissimilarity from each other.

$$\text{BC}_{ij} = 1 - \frac{2C_{ij}}{S_i + S_j} \tag{3}$$

where i & j = the two communities

S_i = sum of total variant percentage counted in the community i

S_j = sum of total variance percentage counted in community j

C_{ij} = the sum of only the lesser counts for each variant in both sites

2.7.4 Coefficient of variation. The coefficient of variation (CV) measures the dispersion of a standard deviation relative to the mean (eqn (4)).[44] The CV was used to compare the variability of wastewater viral concentrations during different time points of the study.

$$\text{CV} = \frac{\sigma}{\bar{x}} \tag{4}$$

where CV = coefficient of variation

σ = standard deviation

$\bar{x}$ = sample mean

3. Results and discussion

3.1 Wastewater surveillance reliability metrics

3.1.1 Autosampler failure events. As composite wastewater samples were collected throughout the study period, several events (*e.g.*, worn tubing, clogging, low battery) prevented the autosamplers from properly functioning. These were denoted as "failure events" and were one cause of data disruption upon analysis. A total of 217 failure events occurred, accounting for approximately 11% of the total sampling events, with a mean time before failure (MTBF) of 40 ± 6 days, n = 2081 (Fig. S2, Table S1†).

These failure events impacted the data collected by creating gaps in the data, potentially leading to an underrepresentation of the viral load during those times. To mitigate this impact, data from the days with failure events

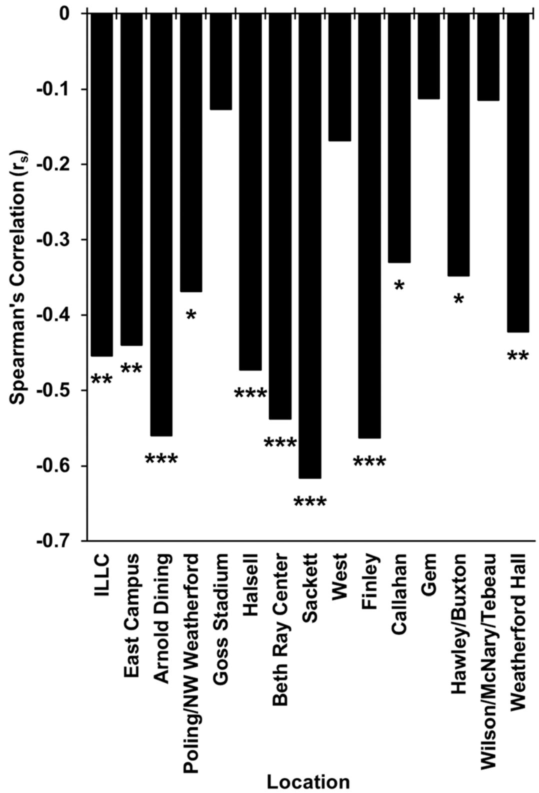

Fig. 1 Correlation between conductivity and rainfall at different sampling points across campus. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

(7 out of 144 samples) were excluded from this study to ensure that the analysis accounted for these missing points. Thus, autosamplers used in campus wastewater surveillance efforts should undergo preventative maintenance approximately every 4–5 weeks to minimize risk of these disruptions and biases.

3.1.2 Effect of rainwater dilution on sampling. A low-to-moderate negative correlation (r_s = −0.12 to −0.62), and median (IQR) of −0.42 (0.75), was found between 48 h rainfall totals and wastewater conductivity across all campus sampling locations (Fig. 1). Correlations were strongest (r_s = −0.13 to −0.56), with a median (IQR) of −0.45 (0.10), and generally statistically significant ($p < 0.05$) in areas that collected the largest volumes of wastewater. This included the main line and building clusters, which had the greatest opportunities for rainwater inundation and infiltration (I and I). Likewise, correlations were weakest (r_s = −0.12 to −0.62), with a median (IQR) of −0.34 (0.30) and generally not statistically significant ($p > 0.05$) in areas that collected the smallest volumes of wastewater. This was primarily the isolated buildings, which had the smallest opportunities for rainwater I and I. Thus, rainfall was not a big factor in interpreting results from individual buildings but may introduce variability when considering SARS-CoV-2 concentrations at locations that drain the entire campus (*e.g.*, East Campus and the ILLC). These results are in line with

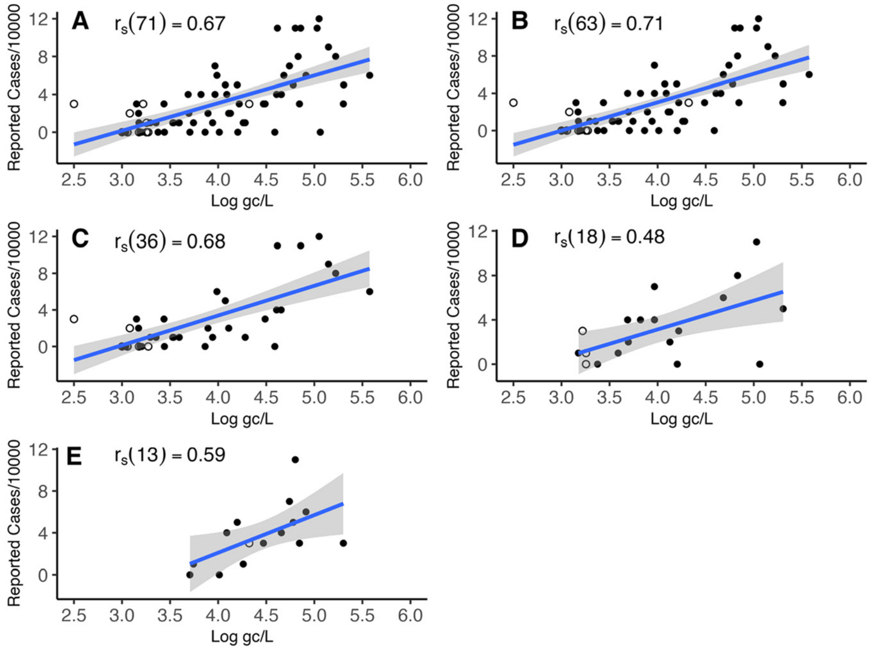

Fig. 2 Linear regression analysis of weekly reported cases and campus log gc L^{-1} during three major university policy decisions: (A) all sampling dates – all data, (B) all sampling dates – rainfall influenced data removed, (C) campus closure, (D) campus opening, and (E) the mask mandate removal. Open-faced points represent samples below the limit of detection. The shaded region represents the 95% confidence interval.

previous studies which found that wastewater viral concentrations were influenced by rainwater I and I.[45–47]

The removal of samples with 48 h rainfall totals greater than 15 mm increased the correlation from $r_s(71) = 0.67$ to $r_s(63) = 0.71$ (Fig. 2A and B). However, a Fisher's r to z transformation identified that these values were not significantly different from each other ($p > 0.05$). Additionally, 71% of sampling events occurred during periods of little-to-no precipitation, and more than 90% of the rainfalls were less than 15 mm over 48 hours. Thus, it was concluded that rainfall events were not of significant consequence to this study, and no further transformation of the data was necessary.

It should also be noted that while the influence of I and I on wastewater surveillance results is often ignored, it is also highly site specific. For instance, in new housing developments with new separated sewer systems, the influence of I and I on wastewater surveillance would be negligible. However, when conducting wastewater surveillance in established communities with combined sewer systems or older conveyance lines, the influence of I and I can be substantial. Especially if a sizable area drains to that location. Thus, we recommend that the influence of I and I be investigated at all sampling locations through identifying potential sources of I and I as well as through measuring wastewater parameters, such as conductivity, that may indicate if I and I is occurring during the sampling period.

3.2 Exploring the campus spill-over hypothesis

3.2.1 Evaluation of the campus closure policy. During the campus closure of the 2020–2021 academic year, when residential students were the vast majority of people on campus, a strong positive correlation ($r_s(36) = 0.68$, $p < 0.001$) was observed between the reported campus COVID-19 cases and the East Campus wastewater SARS-CoV-2 concentrations (Fig. 2C). These results follow similar trends found in other COVID-19 surveillance studies,[16,48,49] showing correlations between 0.67 to 0.71. Likewise, Betancourt *et al.*[24] compared wastewater viral concentrations to mandatory clinical testing among students in the University of Arizona's dormitories, finding that 91 out of 111 (82%) positive wastewater samples corresponded with at least one resident testing positive for COVID-19. These results validate the use of wastewater surveillance as an effective early-warning system for outbreak detection at the building scale, bolstering confidence in this method's ability in capturing the campus's disease burden accurately.

Additionally, during the campus closure, the viral wastewater concentrations at the East Campus location were weakly correlated with those in Corvallis and Albany, showing r values of 0.31 and −0.18 (Fig. 3A and D) and MAPE values of 0.87 and 1.16, respectively (Fig. 4B). Notably, except for the period from January 17, 2021, to March 14, 2021, the East Campus wastewater viral concentrations were generally lower than those observed in Corvallis or Albany. Meanwhile, during the campus closure, the correlation of wastewater viral concentrations between Corvallis and Albany remained moderate, with a r_s value of 0.50 and a MAPE of 0.39 (Fig. 3G and 4B), indicating some interaction between these communities. Finally, during the campus closure, the wastewater SARS-CoV-2 variant composition remained distinct for the East Campus location compared with the

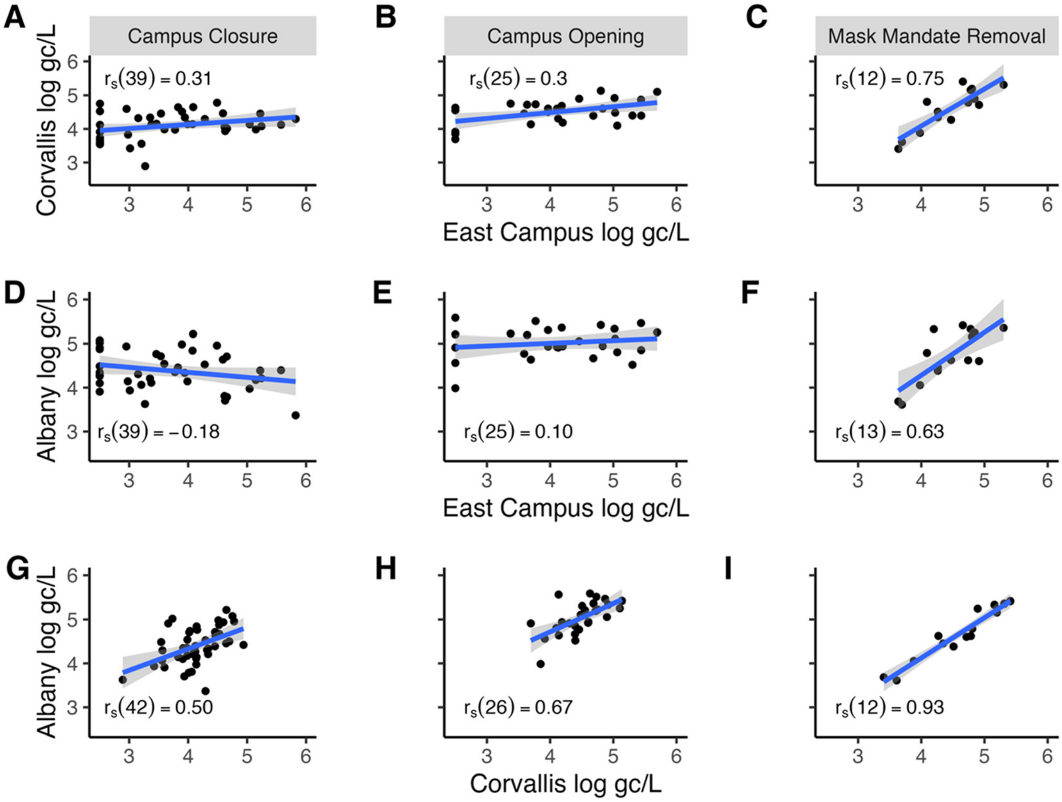

Fig. 3 Linear regression analysis of Albany, Corvallis, and East Campus during three major university policy decisions: (A–C) East Campus and Corvallis, (D–F) East Campus and Albany, (G–I) Corvallis and Albany. The shaded regions represent the 95% confidence interval.

Corvallis and Albany wastewater SARS-CoV-2 variant compositions with a Bray Curtis dissimilarity index of 0.63 and 0.73, respectively (Fig. 5C).

The high correlation between East Campus SARS-CoV-2 wastewater concentrations and the reported campus COVID-19 cases suggests that the wastewater accurately captured the campus disease burden. Additionally, the moderate correlation between the campus and Corvallis wastewater SARS-CoV-2 concentrations in combination with the low correlation between the wastewater SARS-CoV-2 concentrations and variant composition with the cities of Corvallis and Albany suggests that the campus was insular from the outside communities and did not strongly influence the adjacent communities. This is unlike Corvallis and Albany themselves, which have moderate correlations between wastewater SARS-CoV-2 concentrations and variant compositions suggesting that there was an influence of these two communities on one another.

These results are consistent with other non-wastewater studies that estimated the effects of opening the campus to the surrounding community. Arnold *et al.*,[26] conducted a clinical study that quantified the presence of anti-SARS-CoV-2 antibodies of Pennsylvania State University residents and those residing in the surrounding neighborhoods during the time students were allowed to return to campus. There was little evidence of a significant increase in COVID-19 cases in the surrounding community following the return of students to campus. Similarly, Valesano *et al.*,[28] conducted a genomic analysis of SARS-CoV-2 in positive individuals both on-campus and within the community. This study found that most of the variants present in the outside community were not linked to variants within the campus population. Thus, these findings collectively demonstrate minimal evidence of a campus spillover into the surrounding communities.

3.2.2 Evaluation of the campus opening policy. When the campus opened for the 2021–2022 academic year, with restrictions in place, the correlation between the reported COVID-19 cases and East Campus wastewater SARS-CoV-2 concentrations weakened with a moderate correlation of $r_s(18) = 0.48$ ($p < 0.05$) observed (Fig. 2D). Additionally, the correlation between the wastewater SARS-CoV-2 concentrations at East Campus with the city of Corvallis increased slightly with a r_s value of 0.30 and a MAPE of 0.79 while the correlations were still weak with Albany with a r_s values of 0.10 and a MAPE of 1.15 (Fig. 3B and E and 4B). Meanwhile, the correlation between Corvallis and Albany increased to strong with a r_s value of 0.67 and a MAPE of 0.54. Finally, the wastewater SARS-CoV-2 variant composition for the East Campus became more similar to the Corvallis and Albany wastewater SARS-CoV-2 variant compositions with a Bray Curtis Dissimilarity index of 0.14 and 0.14, respectively (Fig. 5C).

The weakened correlation between reported campus COVID-19 cases and East Campus wastewater SARS-CoV-2

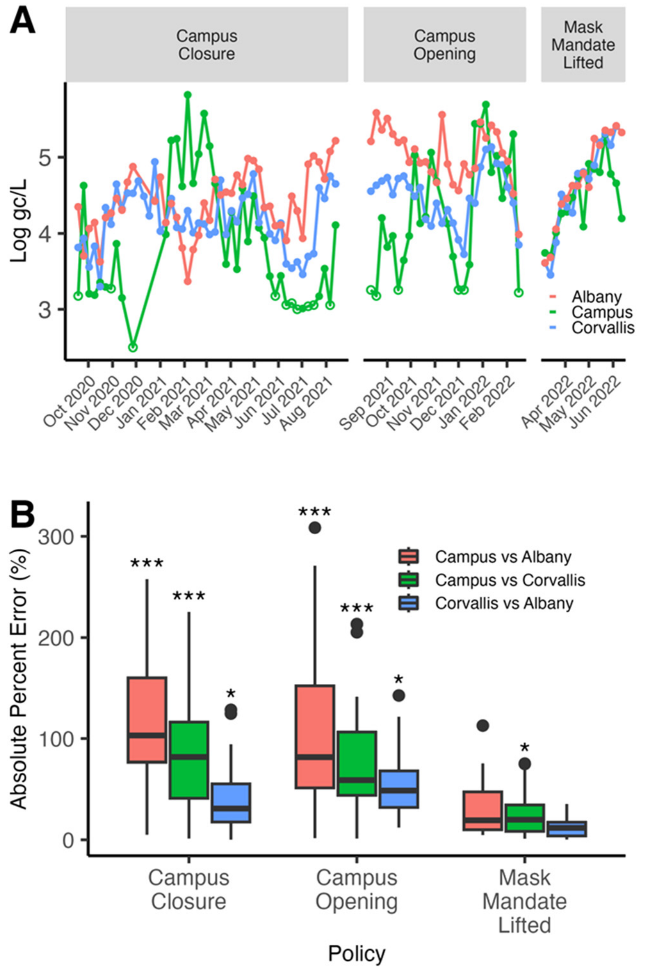

Fig. 4 (A) Time series analysis of East Campus, Corvallis, and Albany logged viral concentrations during the three major policy decisions, (B) absolute percent error between viral concentrations of the three locations at difference policy changes. * = $p < 0.05$, *** = $p < 0.001$.

concentrations was indicative of the campus community becoming less insular from the surrounding communities. In particular, the shallower slope between wastewater SARS-CoV-2 concentrations and reported campus COVID-19 cases (*i.e.* fewer reported COVID-19 cases per wastewater SARS-CoV-2 concentration) indicated that infected individuals arrived on campus, contributed to SARS-CoV-2 signals in the wastewater but were not reported as campus-based COVID-19 infections, since their residences were located off-campus.

Similarly, the increased correlation between East Campus and Corvallis wastewater SARS-CoV-2 concentrations suggests a greater interaction between these two communities while the improved, but still weak, correlation between East Campus and Albany SARS-CoV-2 wastewater concentrations suggests that these two communities are still largely insular from one another. Additionally, the increased similarity of the wastewater SARS-CoV-2 variant composition also indicates increased interactions between the three communities (Fig. 5). However, the rapid rise of the Delta variant being dominant across the state at this time weakens the usefulness of this comparison.[16] Nonetheless, various Omicron variants were detected in the Corvallis wastewater before the East Campus wastewater, indicating that these variants did not enter the city communities through the campus community members (Fig. 5B).

Taken as a whole, the increased correlation between East Campus and Corvallis indicates that either East Campus is influencing Corvallis or *vice versa*. However, the increased correlation between Corvallis and Albany combined with the weakened correlation between East Campus and Albany suggests that there was not a campus spill-over effect into either Corvallis or Albany. Instead, this data indicates the exact opposite with Corvallis spilling over onto campus. These findings coincide with a study performed by Platt *et al.*,[4] where in-class instruction with a mask mandate did not increase viral transmission on campus, but students who had become infected had potential exposure points from interactions with the outer community.

This theory of the surrounding communities increasing the COVID-19 burden on campus is also supported by the transmission rates and vaccination coverage reported in the Corvallis (Benton County) and Albany (Linn County) communities during this time. The non-detections of SARS-CoV-2 in the East Campus wastewater at the start of the campus opening period indicated a low level of COVID-19 transmission on campus (Fig. 4A). This was likely due to the near 100% vaccination level of university students, faculty, and staff at that time, as required by university policy. In contrast, at this time the COVID-19 transmission rates in Corvallis (Benton County) and Albany (Linn County) were classified as "high" to "substantial" and the single dose vaccination rates in Corvallis and Albany, were much lower at rates of 68% and 49%, respectively (Fig. S3†).[35,37] Thus, the introduction of unvaccinated individuals from communities with relatively high transmission rates onto campus may have likely contributed to the onto-campus spill-over effect.

3.2.3 Evaluation of lifting the indoor mask policy. On March 12, 2022, the state of Oregon lifted the indoor mask policy for schools and indoor public spaces, including the OSU Corvallis campus. After the mask mandate was lifted, the correlation between reported campus COVID-19 cases and the East Campus wastewater SARS-CoV-2 concentrations remained at a moderately-strong level, $r_s(13) = 0.59$, $p < 0.05$ (Fig. 2E). Additionally, the correlation between the wastewater SARS-CoV-2 concentrations at East Campus and cities of Corvallis and Albany increased substantially to be very strong and moderately strong with r_s values of 0.75 and 0.63 (Fig. 3C and F) and MAPE values of 0.25 and 34 (Fig. 4B), respectively. Finally, the correlation between Corvallis and Albany also increased with a r_s value of 0.93 and a MAPE of 0.14 (Fig. 3I and 4B).

The further increase in correlation between East Campus wastewater SARS-CoV-2 concentrations with those from both Corvallis and Albany suggests an even greater interaction

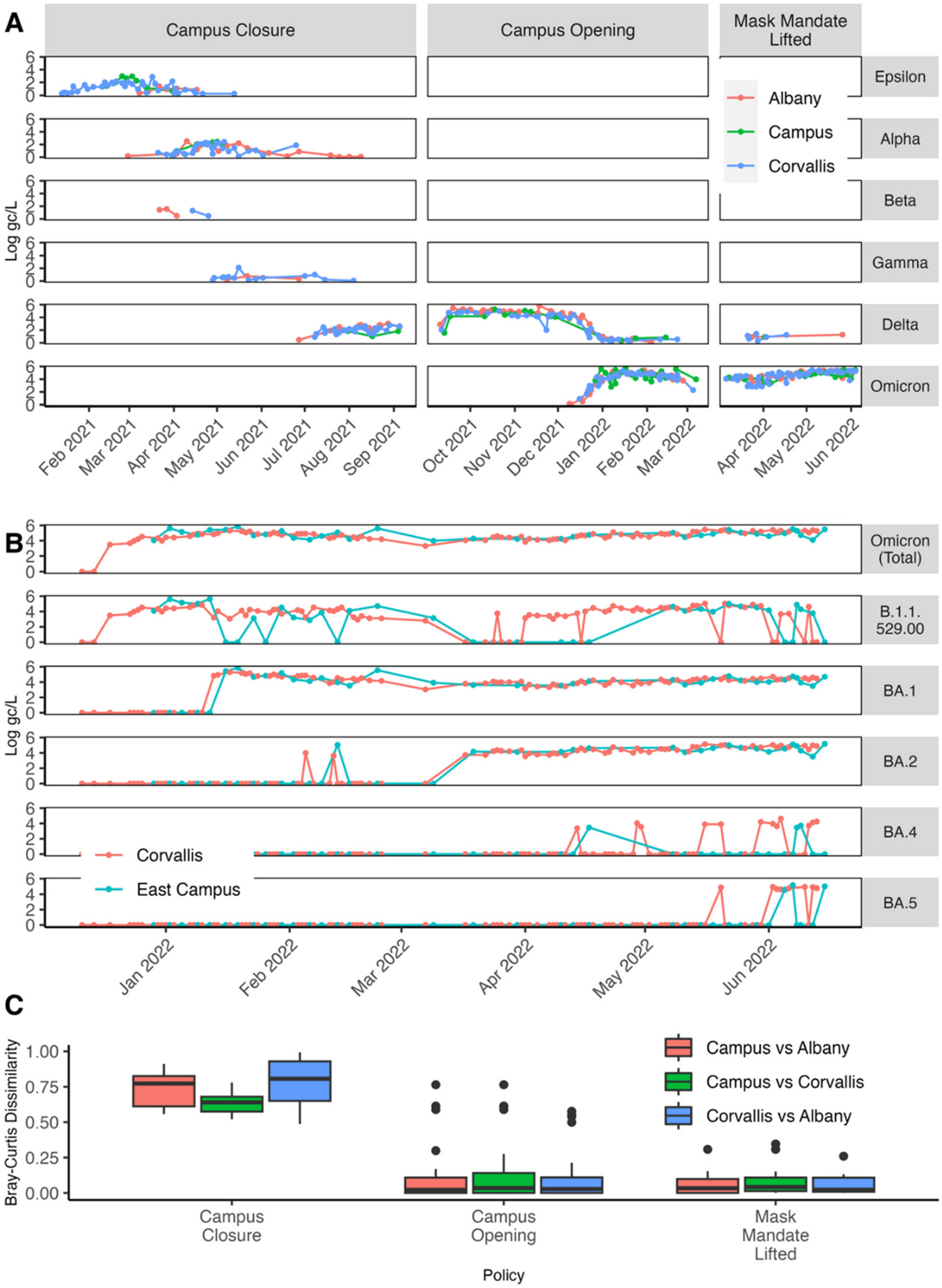

Fig. 5 (A) Variant concentrations in wastewater from the campus, Corvallis, and Albany, over the duration of the three major policy decisions. (B) Omicron variant concentrations in wastewater. (C) Bray–Curtis dissimilarity of variant compositions between the three communities.

between the three communities after the mask mandate was lifted (Fig. 4). At this time, transmission rates in both communities remained classified as "high" to "substantial" and the single dose vaccination rates in Corvallis and Albany were still lower than the university campus at 80% and 60%, respectively (Fig. S3†). Similar to before the mask mandate was lifted, the stronger correlation between Corvallis and Albany compared to East Campus indicates that campus spillover was not occurring but rather the surrounding communities were spilling over into the campus. Finally, the wastewater SARS-CoV-2 variant composition of East Campus, Corvallis, and Albany also increased in similarity after the mask mandate was lifted (Fig. 5). However, just as with Delta, a single variant, Omicron, was dominant across the state at this time weakening the usefulness of this comparison.[16]

4. Conclusion

This study used wastewater surveillance to evaluate how university COVID-19 policies influenced SARS-CoV-2 viral burdens on campus in the surrounding communities. The findings suggest that regular autosampler maintenance every 4–6 weeks can minimize the risk of missed sampling due to equipment failure. Additionally, data normalization due to rainwater infiltration and inundation (I and I) effects, was not necessary for this study as there were no significant differences in data when samples collected during large rainfall events were removed.

This study also demonstrated that the enactment of campus closures and mask mandates were highly effective in reducing SARS-CoV-2 viral burdens and dampened the influence of campus residents on the surrounding communities and *vice versa*. Additionally, the findings of this study indicate that the campus spill-over hypothesis can be dispelled, showing that the increase in the SARS-CoV-2 viral load on the campus primarily originated from the surrounding community rather than from the campus spreading the virus to the community.

As the campus COVID-19 restrictions on campus were adjusted, the viral dynamics on the campus began to mirror those of the host city. This suggests that university policymakers can use the viral activity patterns of the host city as a benchmark to estimate the consequences of lifting COVID-19 restrictions on campus. Additionally, through wastewater surveillance's ability to track viral trends down to the neighborhood and building scale, this method could be utilized to create targeted interventions that alleviate transmission potential from building to building. On a university-scale, this could include enforcing testing and isolation of that area, without having to spend resources to test the entire campus. Overall, wastewater surveillance in conjunction with traditional clinical surveillance methods, can serve as a valuable tool towards refining policy decisions based on accurate, real-time data.

Data availability

Numerical data for Campus Reported Cases, Rainfall, Wastewater Conductivity, and the SARS-CoV-2 $\log_{10}$ gc L^{-1} for East Campus, Corvallis and Albany is provided in the ESI† section (Numerical_Data_Archive.xlsx). All wastewater sequences were deposited in the National Center for Biotechnology Information's short read archive, under BioProject ID # PRJNA1121307.

Author contributions

David Lisboa – formal analyses, data curation, writing – original draft, review and editing, visualization; Devrim Kaya – methodology, validation, formal analyses, investigation, data curation, writing – review and editing; Michael Harry – methodology, validation, investigation; Casey Kanalos – investigation; Gabriel Davis – methodology, validation, investigation; Oumaima Hachimi – methodology, validation, investigation, writing – review and editing; Shana Jaaf – methodology, validation, investigation; David Mickle – methodology, validation, investigation, writing – review and editing; Dana Alegre –methodology, validation, investigation; Katherine Carter – methodology, validation, investigation, writing – review and editing; Steven Carrell – investigation, writing – review and editing; Mark Dasenko – methodology, validation, investigation, writing – review and editing; Nathan Davidson – investigation; Justin Elser – investigation, writing – review and editing; Matthew Geniza – methodology, validation, investigation; Anne-Marie Girard – methodology, validation, investigation, writing – review and editing; Brent Kronmiller – methodology, validation, investigation, writing – review and editing; Matthew Peterson – methodology, validation, investigation; Elizabeth Zepeda – methodology, validation, investigation, writing – review and editing; Christine Kelly – conceptualization, investigation, supervision, project administration, funding acquisition, writing – review and editing; Tyler S. Radniecki – conceptualization, investigation, supervision, project administration, funding acquisition, writing – original draft, writing – review and editing.

Conflicts of interest

None to report.

Acknowledgements

This work was supported by Oregon State University. We are grateful to the Corvallis Wastewater Treatment Facility, especially Gloria Zeller, John Hoppner, and Max Hildebrand for their participation in our wastewater surveillance program. We would also like to thank David Gilby and the rest of the team at the Albany Wastewater Treatment Facility for their participation. Additionally, we would like to thank our OSU wastewater collection undergraduate team, especially Jim Gallagher, and the TRACE OSU COVID-19 team, especially Jeff Bethel, Ben Dalziel, Roy Haggerty, Kathy Higley, Jane Lubchenco, Katie McLaughlin, Allison Meyers, Javier Nieto and Justin Sanders, for their support and advice for field collection, sample preparation and data analysis activities, and Leslie Dietz for proofreading.

References

1 A. Hatibie, S. Majumdar, M. Pyarali, R. Koch, A. Wood and T. Hale, *Variation in US states' responses to COVID-19*.
2 J. M. Brauner, S. Mindermann, M. Sharma, D. Johnston, J. Salvatier, T. Gavenčiak, A. B. Stephenson, G. Leech, G. Altman, V. Mikulik, A. J. Norman, J. T. Monrad, T. Besiroglu, H. Ge, M. A. Hartwick, Y. W. Teh, L. Chindelevitch, Y. Gal and J. Kulveit, Inferring the effectiveness of government interventions against COVID-19, *Science*, 2021, **371**, eabd9338.
3 A. Mendez-Brito, C. El Bcheraoui and F. Pozo-Martin, Systematic review of empirical studies comparing the

effectiveness of non-pharmaceutical interventions against COVID-19, *J. Infect.*, 2021, **83**, 281–293.

4 K. Kuhfeldt, J. Turcinovic, M. Sullivan, L. Landaverde, L. Doucette-Stamm, D. H. Hamer, J. T. Platt, C. Klapperich, H. E. Landsberg and J. H. Connor, Examination of SARS-CoV-2 In-Class Transmission at a Large Urban University With Public Health Mandates Using Epidemiological and Genomic Methodology, *JAMA Netw. Open*, 2022, **5**, e2225430.

5 H. Littlecott, C. Herd, J. O'Rourke, L. T. Chaparro, M. Keeling, G. James Rubin and E. Fearon, Effectiveness of testing, contact tracing and isolation interventions among the general population on reducing transmission of SARS-CoV-2: a systematic review, *Philos. Trans. R. Soc., A*, 2023, **381**, 20230131.

6 K. Sherratt, S. Abbott, S. R. Meakin, J. Hellewell, J. D. Munday, N. Bosse, M. Jit and S. Funk, Exploring surveillance data biases when estimating the reproduction number: with insights into subpopulation transmission of COVID-19 in England, *Philos. Trans. R. Soc., B*, 2021, **376**, 20200283.

7 D. S. Grigsby-Toussaint, J. C. Shin and A. Jones, Disparities in the distribution of COVID-19 testing sites in black and Latino areas in new York City, *Prev. Med.*, 2021, **147**, 106463.

8 E. N. Pond, L. Rutkow, B. Blauer, A. Aliseda Alonso, S. Bertran de Lis and J. B. Nuzzo, Disparities in SARS-CoV-2 Testing for Hispanic/Latino Populations: An Analysis of State-Published Demographic Data, *J. Public Health Manag. Pract.*, 2022, **28**, 330.

9 N. P. Dalva-Baird, W. M. Alobuia, E. Bendavid and J. Bhattacharya, Racial and ethnic inequities in the early distribution of U.S. COVID-19 testing sites and mortality, *Eur. J. Clin. Invest.*, 2021, **51**, e13669.

10 D. A. Larsen and K. R. Wigginton, Tracking COVID-19 with wastewater, *Nat. Biotechnol.*, 2020, **38**, 1151–1153.

11 C. Hoar, F. Chauvin, A. Clare, H. McGibbon, E. Castro, S. Patinella, D. Katehis, J. J. Dennehy, M. Trujillo, D. S. Smyth and A. I. Silverman, Monitoring SARS-CoV-2 in wastewater during New York City's second wave of COVID-19: sewershed-level trends and relationships to publicly available clinical testing data, *Environ. Sci.: Water Res. Technol.*, 2022, **8**, 1021–1035.

12 B. J. Paterson and D. N. Durrheim, Wastewater surveillance: an effective and adaptable surveillance tool in settings with a low prevalence of COVID-19, *Lancet Planet. Health*, 2022, **6**, e87–e88.

13 D. Kaya, R. Falender, T. Radniecki, M. Geniza, P. Cieslak, C. Kelly, N. Lininger and M. Sutton, Correlation between Clinical and Wastewater SARS-CoV-2 Genomic Surveillance, Oregon, USA - Volume 28, Number 9—September 2022, *Emerg. Infect. Dis.*, 2022, **28**(9), 1906–1908.

14 CDC, National Wastewater Surveillance System, **https://www.cdc.gov/nwss/wastewater-surveillance/index.html**, (accessed 28 February 2023).

15 M. B. Diamond, A. Keshaviah, A. I. Bento, O. Conroy-Ben, E. M. Driver, K. B. Ensor, R. U. Halden, L. P. Hopkins, K. G. Kuhn, C. L. Moe, E. C. Rouchka, T. Smith, B. S. Stevenson, Z. Susswein, J. R. Vogel, M. K. Wolfe, L. B. Stadler and S. V. Scarpino, Wastewater surveillance of pathogens can inform public health responses, *Nat. Med.*, 2022, **28**, 1992–1995.

16 B. A. Layton, D. Kaya, C. Kelly, K. J. Williamson, D. Alegre, S. M. Bachhuber, P. G. Banwarth, J. W. Bethel, K. Carter, B. D. Dalziel, M. Dasenko, M. Geniza, A. George, A.-M. Girard, R. Haggerty, K. A. Higley, D. M. Hynes, J. Lubchenco, K. R. McLaughlin, F. J. Nieto, A. Noakes, M. Peterson, A. D. Piemonti, J. L. Sanders, B. M. Tyler and T. S. Radniecki, Evaluation of a Wastewater-Based Epidemiological Approach to Estimate the Prevalence of SARS-CoV-2 Infections and the Detection of Viral Variants in Disparate Oregon Communities at City and Neighborhood Scales, *Environ. Health Perspect.*, 2022, **130**(6), 067010.

17 A. E. Kirby, M. S. Walters, W. C. Jennings, R. Fugitt, N. LaCross, M. Mattioli, Z. A. Marsh, V. A. Roberts, J. W. Mercante, J. Yoder and V. R. Hill, Using Wastewater Surveillance Data to Support the COVID-19 Response — United States, 2020–2021, *MMWR Morb. Mortal. Wkly. Rep.*, 2021, **70**, 1242–1244.

18 T. Prado, T. M. Fumian, C. F. Mannarino, P. C. Resende, F. C. Motta, A. L. F. Eppinghaus, V. H. Chagas do Vale, R. M. S. Braz, J. da S. R. de Andrade, A. G. Maranhão and M. P. Miagostovich, Wastewater-based epidemiology as a useful tool to track SARS-CoV-2 and support public health policies at municipal level in Brazil, *Water Res.*, 2021, **191**, 116810.

19 S. Shrestha, E. Yoshinaga, S. K. Chapagain, G. Mohan, A. Gasparatos and K. Fukushi, Wastewater-Based Epidemiology for Cost-Effective Mass Surveillance of COVID-19 in Low- and Middle-Income Countries: Challenges and Opportunities, *Water*, 2021, **13**, 2897.

20 P. Liu, M. Ibaraki, J. VanTassell, K. Geith, M. Cavallo, R. Kann, L. Guo and C. L. Moe, A sensitive, simple, and low-cost method for COVID-19 wastewater surveillance at an institutional level, *Sci. Total Environ.*, 2022, **807**, 151047.

21 A. L. Rainey, K. Buschang, A. O'Connor, D. Love, A. M. Wormington, R. L. Messcher, J. C. Loeb, S. E. Robinson, H. Ponder, S. Waldo, R. Williams, J. Shapiro, E. B. McAlister, M. Lauzardo, J. A. Lednicky, A. T. Maurelli, T. Sabo-Attwood and J. H. Bisesi, Retrospective Analysis of Wastewater-Based Epidemiology of SARS-CoV-2 in Residences on a Large College Campus: Relationships between Wastewater Outcomes and COVID-19 Cases across Two Semesters with Different COVID-19 Mitigation Policies, *ACS EST Water*, 2023, **3**(1), 16–29.

22 S. Harris-Lovett, K. L. Nelson, P. Beamer, H. N. Bischel, A. Bivins, A. Bruder, C. Butler, T. D. Camenisch, S. K. De Long, S. Karthikeyan, D. A. Larsen, K. Meierdiercks, P. J. Mouser, S. Pagsuyoin, S. M. Prasek, T. S. Radniecki, J. L. Ram, D. K. Roper, H. Safford, S. P. Sherchan, W. Shuster, T. Stalder, R. T. Wheeler and K. S. Korfmacher, Wastewater Surveillance for SARS-CoV-2 on College Campuses: Initial Efforts, Lessons Learned, and Research Needs, *Int. J. Environ. Res. Public Health*, 2021, **18**, 4455.

23 C. M. Welling, D. R. Singleton, S. B. Haase, C. H. Browning, B. R. Stoner, C. K. Gunsch and S. Grego, Predictive values of

time-dense SARS-CoV-2 wastewater analysis in university campus buildings, *Sci. Total Environ.*, 2022, **835**, 155401.

24 W. Q. Betancourt, B. W. Schmitz, G. K. Innes, S. M. Prasek, K. M. Pogreba Brown, E. R. Stark, A. R. Foster, R. S. Sprissler, D. T. Harris, S. P. Sherchan, C. P. Gerba and I. L. Pepper, COVID-19 containment on a college campus via wastewater-based epidemiology, targeted clinical testing and an intervention, *Sci. Total Environ.*, 2021, **779**, 146408.

25 L. C. Scott, A. Aubee, L. Babahaji, K. Vigil, S. Tims and T. G. Aw, Targeted wastewater surveillance of SARS-CoV-2 on a university campus for COVID-19 outbreak detection and mitigation, *Environ. Res.*, 2021, **200**, 111374.

26 C. R. K. Arnold, S. Srinivasan, S. Rodriguez, N. Rydzak, C. M. Herzog, A. Gontu, N. Bharti, M. Small, C. J. Rogers, M. M. Schade, S. V. Kuchipudi, V. Kapur, A. F. Read and M. J. Ferrari, A longitudinal study of the impact of university student return to campus on the SARS-CoV-2 seroprevalence among the community members, *Sci. Rep.*, 2022, **12**, 8586.

27 L. E. Cipriano, W. M. R. Haddara, G. S. Zaric and E. A. Enns, Impact of university re-opening on total community COVID-19 burden, *PLoS One*, 2021, **16**, e0255782.

28 A. L. Valesano, W. J. Fitzsimmons, C. N. Blair, R. J. Woods, J. Gilbert, D. Rudnik, L. Mortenson, T. C. Friedrich, D. H. O'Connor, D. R. MacCannell, J. G. Petrie, E. T. Martin and A. S. Lauring, SARS-CoV-2 Genomic Surveillance Reveals Little Spread From a Large University Campus to the Surrounding Community, *Open Forum Infect. Dis.*, 2021, **8**, ofab518.

29 N. Bharti, B. Lambert, C. Exten, C. Faust, M. Ferrari and A. Robinson, Large university with high COVID-19 incidence is not associated with excess cases in non-student population, *Sci. Rep.*, 2022, **12**, 3313.

30 C. J. Courtemanche, A. H. Le, A. Yelowitz and R. Zimmer, 2021.

31 Bio-Rad, Instructions for Use: SARS-CoV-2 ddPCR Kit - Part Number 12013743, https://www.bio-rad.com/webroot/web/pdf/lsr/literature/10000130776.pdf, (accessed 1 May 2020).

32 M. Sutton, T. S. Radniecki, D. Kaya, D. Alegre, M. Geniza, A.-M. Girard, K. Carter, M. Dasenko, J. L. Sanders, P. R. Cieslak, C. Kelly and B. M. Tyler, Detection of SARS-CoV-2 B.1.351 (Beta) Variant through Wastewater Surveillance before Case Detection in a Community, Oregon, USA, *Emerg. Infect. Dis.*, 2022, **28**, 1101–1109.

33 UHDS, The Student Policy and Information Guide, https://uhds.oregonstate.edu/sites/uhds.oregonstate.edu/files/uhds_policy_guide_sept_2020.pdf, (accessed 15 January 2023).

34 COVID-19 Information, https://studenthealth.oregonstate.edu/covid-19-info, (accessed 23 May 2024).

35 United States COVID-19 County Level of Community Transmission Historical Changes - ARCHIVED|Data|Centers for Disease Control and Prevention, https://data.cdc.gov/Public-Health-Surveillance/United-States-COVID-19-County-Level-of-Community-T/nra9-vzzn, (accessed 28 May 2024).

36 A. Christie, Guidance for Implementing COVID-19 Prevention Strategies in the Context of Varying Community Transmission Levels and Vaccination Coverage, *MMWR Morb. Mortal. Wkly. Rep.*, 2021, **70**(30), 1044–1047.

37 COVID-19 Vaccinations in the United States, County|Data|Centers for Disease Control and Prevention, https://data.cdc.gov/Vaccinations/COVID-19-Vaccinations-in-the-United-States-County/8xkx-amqh/explore/, (accessed 28 May 2024).

38 What is Mean Time Between Failure MTBF?, https://www.upkeep.com/learning/mean-time-between-failure/, (accessed 22 May 2023).

39 D. Freedman, R. Pisani and R. Purves, *Statistics*, W.W. Norton & Co, New York, 4th edn, 2007.

40 Zach, How to Find Outliers Using the Interquartile Range, https://www.statology.org/find-outliers-with-iqr/, (accessed 18 July 2023).

41 Fisher Z-Transformation - Statistics How To, https://www.statisticshowto.com/fisher-z/, (accessed 22 May 2023).

42 A. D. George, D. Kaya, B. A. Layton, K. Bailey, S. Mansell, C. Kelly, K. J. Williamson and T. S. Radniecki, Impact of Sampling Type, Frequency, and Scale of the Collection System on SARS-CoV-2 Quantification Fidelity, *Environ. Sci. Technol. Lett.*, 2022, **9**, 160–165.

43 Stephanie, Bray Curtis Dissimilarity, https://www.statisticshowto.com/bray-curtis-dissimilarity/, (accessed 22 May 2023).

44 How to Find a Coefficient of Variation, https://www.statisticshowto.com/probability-and-statistics/how-to-find-a-coefficient-of-variation/, (accessed 5 June 2023).

45 B. Nguyen Quoc, P. Saingam, R. RedCorn, J. A. Carter, T. Jain, P. Candry, M. Gattuso, M.-L. W. Huang, A. L. Greninger, J. S. Meschke, A. Bryan and M. K. H. Winkler, Case Study: Impact of Diurnal Variations and Stormwater Dilution on SARS-CoV-2 RNA Signal Intensity at Neighborhood Scale Wastewater Pumping Stations, *ACS EST Water*, 2022, **2**, 1964–1975.

46 Neighbourhood-scale wastewater-based epidemiology for covid-19: Opportunities and challenges|Journal of Hydrology (New Zealand), https://search.informit.org/doi/abs/10.3316/informit.548064549025013, (accessed 28 February 2023).

47 A. Lastra, J. Botello, A. Pinilla, J. I. Urrutia, J. Canora, J. Sánchez, P. Fernández, F. J. Candel, A. Zapatero, M. Ortega and J. Flores, SARS-CoV-2 detection in wastewater as an early warning indicator for COVID-19 pandemic. Madrid region case study, *Environ. Res.*, 2022, **203**, 111852.

48 S. Arora, A. Nag, A. Kalra, V. Sinha, E. Meena, S. Saxena, D. Sutaria, M. Kaur, T. Pamnani, K. Sharma, S. Saxena, S. K. Shrivastava, A. B. Gupta, X. Li and G. Jiang, Successful application of wastewater-based epidemiology in prediction and monitoring of the second wave of COVID-19 with fragmented sewerage systems–a case study of Jaipur (India), *Environ. Monit. Assess.*, 2022, **194**, 342.

49 B. Layton, D. Kaya, C. Kelly, K. Williamson, S. Bachhuber, P. Banwarth, J. Bethel, K. Carter, B. Dalziel, M. Dasenko, M. Geniza, D. Gibbon, A.-M. Girard, R. Haggerty, K. Higley, D. Hynes, J. Lubchenco, K. McLaughlin, F. J. Nieto, A. Noakes, M. Peterson, A. Piemonti, J. Sanders, B. Tyler and T. Radniecki, *Wastewater-based epidemiology predicts COVID-19 community prevalence*, In Review, 2021.