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Comparison of residential dormitory COVID-19 monitoring via weekly saliva testing and sewage monitoring

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HIGHLIGHTS
- Wastewater COVID-19 detection from dorms compared to weekly individual testing.
- N1 and N2 were detected in weekly wastewater samples, peaking January or February.
- N1 detected on all 3 campuses studied, N2 only on one campus.
- N1 and N2 gene concentrations correlated with previous week’s individual testing.
- Weekly wastewater surveillance was a lagging indicator of COVID-19 cases.

GRAPHICAL ABSTRACT

ABSTRACT
Wastewater surveillance has been used as a tool for COVID-19 outbreak detection particularly where there was not capability in place for routine and robust individual testing. Given clinical reports that earlier detection is possible following infection from throat/nasal samples compared to fecal samples for COVID-19 patients, the utility of wastewater testing where robust individual testing is possible is less clear. The objective of this study was to compare the results of weekly required COVID-19 saliva tests to weekly wastewater monitoring for residential buildings (i.e., dormitories) located across three college campuses capturing wastewater from 80 to 441 occupants per sampling location. Sampling occurred during the spring semester of the 2021 academic year which captured the third wave of SARS-CoV-2 cases in the study region. Comparison of the saliva and wastewater testing results indicated that the wastewater SARS-CoV-2 concentrations had a strong linear correlation with the previous week’s percentage of positive saliva test results and a weak linear correlation with the saliva testing results surrounding the wastewater sampling (four days before and 3 days after). Given that no correlation was observed between the wastewater and the saliva testing from the following week, the weekly saliva testing captured spikes in COVID-19 cases earlier than the weekly wastewater sampling. Interestingly, the N1 gene was observed in buildings on all campuses, but N2 was observed in wastewater on only one of the campuses. N1 and N2 were also observed in sewer biofilm. The campus-specific challenges associated with implementation of wastewater surveillance are discussed. Overall, these results can help inform design of surveillance for early detection of SARS-CoV-2 in residential settings thereby informing mitigation strategies to slow or prevent the spread of the virus among residents in congregate living.

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1. Introduction

SARS-CoV-2 is shed in the feces of COVID-19 patients including asymptomatic individuals, making it a suitable target for wastewater-based monitoring. Reports of SARS-CoV-2 detection have been made in wastewater from around the world (Ahmed et al., 2020; Kitajima et al., 2020; Kumar et al., 2020; La Rosa et al., 2020; Medema et al., 2020) and build upon longer history of using wastewater monitoring for viral disease and vaccinations (Xagorarakis and O’Brien, 2020). Of particular interest during the COVID-19 pandemic is the design of monitoring programs for congregate living and university campuses, where transmission rates may be high due to shared living, bathing, and dining spaces and the highly social nature of the population. Universities have implemented a range of techniques for monitoring including daily symptom reporting, wellness checks, required individual testing prior to or upon campus move-in, movement tracking, and contact tracing, among other measures (Table 1). Many campuses implemented wastewater surveillance as a part of their monitoring programs and several campus representatives surveyed express unequivocally that the wastewater monitoring was “worth the efforts,” (Harris-Lovett et al., 2021).

Reports of implementation of building-level SARS-CoV-2 monitoring in university dorms range in length (38–178 d) often corresponding to the length of a semester with a variety of sampling designs from daily (Karthikeyan et al., 2021) to variable (Colosi et al., 2021). In particular, many were implemented on campuses with individual testing ranging from capturing only symptomatic and clinical cases (Betancourt et al., 2021; Colosi et al., 2021; Gibas et al., 2021) up to required asymptomatic testing weekly (Bivins and Bibby, 2021) to every two weeks (Scott et al., 2021). The outcome of a US nationwide survey effort noted the need remains to understand how to optimize wastewater monitoring with clinical testing to improve outcomes, including early detection and mitigation (Harris-Lovett et al., 2021).

Where wastewater surveillance was more frequent and individual testing less frequent, dormitory wastewater surveillance was reported as predictive of cases, including asymptomatic cases, and capturing outbreaks early (Betancourt et al., 2021; Gibas et al., 2021; Karthikeyan et al., 2021; Scott et al., 2021). Wastewater was a lagging indicator when individual testing was more frequent (Bivins and Bibby, 2021). Results from hospitalized patients indicate that viral shedding in fecal matter occurs ~3–7 days after a positive PCR test can be obtained from a nasal swab and that shedding in feces could continue for 6–10 days following a negative nasal swab (Chen et al., 2020; Foladori et al., 2020). Therefore, where regular individual testing of a population including asymptomatic individuals is implemented, one would expect wastewater surveillance to be a lagging indicator and where individual testing is not routinely implemented or testing compliance is insufficient, then wastewater surveillance would be a leading indicator.

### Table 1

| Reference | Location (state) | Frequency of WW sample collection | Individual testing | Length of study | Other surveillance techniques reported for institution | SARS-CoV-2 gene target(s) | Normalization |
|-----------|------------------|----------------------------------|--------------------|----------------|-------------------------------------------------------|--------------------------|--------------|
| Gibas et al., (2021) | NC | 3 per week | Symptomatic testing for all, scheduled testing of student athletes | 56 d | In-house contact tracing, daily symptom reporting | N1 |  |
| Scott et al., (2021) | LA | 1 per week | Every 2 weeks, more frequently during high prevalence | 104 d |  | N1, N2 | PMMoV |
| Colosi et al., (2021) | VA | Variable, 2 to 12 days | Clinical | 178 d |  | N1, N2 |  |
| Karthikeyan et al., (2021) | CA | Daily | Every 2 weeks | 38 d | Contact tracing | N1, N2, E | PMMoV, BRSV |
| Bivins and Bibby, (2021) | IN | Daily | Weekly | 42 d | Daily health checks | N1 | PMMoV |
| Betancourt et al., (2021) | AZ | Daily to 2 times per week | Symptomatic | 95 d | Testing upon move-in, contact tracing | N1, N2 |  |
| This study | NJ | 1 per week | Weekly | 203 d | Testing upon move-in, contact tracing, symptom checks | N1, N2 | PMMoV |

The objective of this study was to compare the results of COVID-19 saliva tests to wastewater monitoring for residential buildings on college campuses. To do this, weekly wastewater samples were collected from access sites within or outside of the buildings and the SARS-CoV-2 N1 and N2 gene copies were quantified with qPCR. Wastewater data were compared to the required weekly saliva test results reported by building residents to the college. Normalization of results to water quality and a control virus was tested. Results presented provide insight into the different monitoring approaches for capturing viral disease outbreaks.

### Table 2

| Campus | Sampling site | Residents (total number) | Buildings sampled (number) | Wastewater samples (number) | Grab samples (number, date month/day/year) |
|--------|---------------|--------------------------|----------------------------|-----------------------------|------------------------------------------|
| C1     | a             | 165                      | 1                          | 13                          | b, 80, 1, 12, 0                          |
|        | a-1           | 245                      | 1                          | 4                           | b-2, 245, 1, 10                          |
|        | a-2           | 245                      | 1                          | 10                          | c-2, 190, 2, 16                          |
| C2     | a             | 81                       | 3                          | 26                          | b, 414, 5, 20, 0                         |
|        | a-1           | 190                      | 2                          | 14                          | c-1, 190, 2, 14                          |
|        | a-2           | 190                      | 2                          | 16                          | c-2, 190, 2, 16                          |
| C3     | a             | 239                      | 1                          | 24                          | d, 239, 1, 24, 0                         |
covering in public spaces, diagnostic and surveillance testing (described further below), contact tracing, disinfection protocols, limitations on guests in the residence halls, and quarantine/isolation/separation requirements (including before or upon arrival to campus). Positive cases and their close contacts were relocated to buildings that were not part of the sewage monitoring program (with the rare exceptions for C1 and C2). Positive cases were relocated within 24 h of notification and saliva test results were returned generally within 24 to 72 h after testing, based upon laboratory volume.

All persons living in university housing and/or working on campus on average once or more a week were required to complete weekly COVID-19 saliva testing. Test kits could be submitted on weekdays (Monday–Friday) at least seven days after the prior test (i.e., tests were not necessarily submitted on the same day of the week by a given resident). The testing scheme was designed to capture cases of individuals who were not experiencing symptoms of COVID-19 (e.g., fever, cough, shortness of breath, sore throat, fatigue, muscle aches, loss of sense of smell or taste, or stomach upset). Persons experiencing symptoms were advised to quarantine and contact their medical provider. All students moving into on-campus housing or working on campus were required to undergo COVID-19 PCR testing one to three weeks prior to move-in day and a second test was performed for on-campus housing students at move-in. Test kits were available at multiple locations throughout the campus via in-person pick up, test kit vending machines, and drop boxes. Saliva tests were processed at a commercial lab using PCR (Accurate Diagnostics Lab). Results were reported to the individuals and the university.

The identities of individuals known to be infected with COVID-19 were not disclosed but contact tracing was pursued for persons in close contact with infected individuals per CDC and state guidelines. Persons entering campus buildings also completed online symptom check surveys (i.e., whether the person was experiencing fever or chills, cough, shortness of breath or difficulty breathing, new loss of taste or smell, sore throat, etc., within the past 24 h, whether there was close physical contact with a symptomatic or asymptomatic COVID-19 case, or whether the person was asked to self-quarantine due to travel advisories) before entry. After receiving a positive saliva test, re-testing would not occur until 90 days after the positive test. Vaccines became available to the community across the study period. Regardless of vaccine status, university housing residents were required to complete weekly testing (this policy changed after the study period). This study reviewed by the Rutgers Institutional Review Board as secondary research and determined to be exempt following review.

2.2. Building sewage monitoring and biofilm sampling

Wastewater sampling sites were selected on each campus based on the availability of sampling access locations that only captured residents’ wastewater (e.g., multiuse buildings with housing and classrooms, stores, or restaurants were not included). Sampling was performed at manholes or cleanouts, capturing one to five congregate living residential buildings. Sampling began at select locations in winter 2020 and continued on a weekly basis from January 2021 through May 2021, corresponding with the time students were in residence in the dormitories, details are provided in Table 2. All samples in 2021 were collected either on Tuesdays (N = 5/19) or Wednesdays (N = 13/19), with the exception of the February 4, 2021 sampling which was delayed until Thursday due to a major snow storm.

The 24-h composite samples (1 L) were collected using automatic portable samplers (Model WS700, Xylem, Rye Brook, NY, USA). At select sampling locations and dates, the automatic sampler did not collect large enough sample volumes for processing and in this situation, grab samples were collected (N = 5 grab / 140 samples total). After collection, the samples were stored at 4 °C and transported to the lab in a cooler with ice. Upon arrival at the lab, the wastewater samples were pasteurized in a water bath at 55 °C for 1 h or in an incubator at 55 °C for 90 min to inactivate viral particles (Wu et al., 2020). Immediately after pasteurization, the samples were processed for concentration and precipitation of viral particles, as described below. For quality assurance, field blanks consisting of deionized water were included each week during sampling, rotating between the campuses. A total of 18 field blanks were processed during the study period in parallel with the wastewater samples.

Grab biofilm samples were collected on April 27, 2021 in triplicate, from four of the locations where the wastewater samples were collected (C1a, C3a, C3b, C3c-1). Samples were collected by attaching a sterile cotton swab to a ~3 m long PVC pipe to reach and scrape the biofilm formed in the bottom of the manholes. Then, the tip of the cotton swab containing the sample was placed in a 2 mL plastic O-ring tube and stored in a cooler with ice for transportation. Upon arrival to the lab, the tubes were stored at ~80 °C.

2.3. Concentration and precipitation of viral particles in wastewater

Concentration and precipitation of viral particles from wastewater was done following a polyethylene glycol (PEG) precipitation method (Wu et al., 2020). Briefly, 200 mL of wastewater was filtered through a 0.22 μm mixed cellulose esters (MCE) membrane (Millipore Sigma, St. Louis, MO). The filtrate was then precipitated with polyethylene glycol 8000 (8% wt/wt) and NaCl (0.3 M). The samples were shaken by hand and incubated at room temperature for ~20 min, until the chemicals were dissolved. The sample solution (200 mL of filtrate with PEG and NaCl) was centrifuged for 2 h at 12000 × g immediately after holding overnight at 4 °C. Then, ~150 mL of supernatant of each centrifuge tube was discarded and the remaining 50 mL was vortexed and transferred to a 50 mL falcon tube. The 50 mL falcon tube was then centrifuged at 12000 × g for 45 min or until a pellet was visible. The supernatant was discarded and the pellet was used for total RNA extraction.

2.4. RNA extraction from wastewater pellets

Total RNA and DNA extraction of the wastewater pellets was performed using the RNeasy® PowerWater® kit (Qiagen, Germantown, MD). The wastewater pellet was resuspended with 990 μL of the PM1 solution from the kit and 10 μL 2-mercapto-ethanol, then transferred to the PowerWater DNA Bead tube. For the biofilm samples, the swab was transferred to the PowerWater DNA Bead tube. The rest of the protocol was completed following the manufacturer’s instructions for simultaneous total RNA and DNA extraction for both sample types. Total RNA and DNA suspension was stored at ~80 °C in plastic O-ring tubes.

2.5. Biomolecular analysis

Total RNA from the wastewater samples was analyzed by reverse transcription quantitative PCR (RT-qPCR) to detect and quantify SARS-CoV-2 N1 and N2 and pepper mottle virus (PMoV) (Haramoto et al., 2013) gene copies in the samples. The reverse transcription and PCR reactions were performed using the iTaq Universal Probes One-step kit (Bio-Rad, catalog no. 1725141) and the US CDC N1 and N2 primer-probe (Table A1, IDT, Coralville, Iowa). The PCR reaction mixture consisted of 10 μL of 2 × iTaq PCR reaction mix, 0.5 μM of forward, 0.7 μM and reverse primers, 0.2 μM of the FAM probe, 0.25 μL of iScript reverse transcriptase, 4 μL of RNA template (sample) and RNase free ddH2O to a final volume of 20 μL. The RT-qPCR was carried out on a Real Time Thermocycler (BioRad CFX96 Touch, Hercules, CA).

For the N1 and N2 genes, the one-step PCR conditions for both primer-probe sets were the following: a reverse transcription cycle of 10 min at 50 °C, a denaturing step of 10 min at 95 °C, followed by 45 cycles of 10 s at 95 °C and 30 s at 60 °C. A commercial plasmid containing the complete SARS-CoV-2 nucleocapsid gene was used as positive control for the construction of a five-point standard curve for both N1 and N2 (IDT, Coralville, IA). The range of the standard curve was 10^3 to 10^7 copies per reaction. All standards, samples and a no-template control (NTC) were analyzed in triplicate (technical replicates) on each 96-well qPCR plate. The average R² of the standard curves and efficiency in all reactions for N1 were 0.98 ± 0.01.
and 93.3 ± 5.10% and N2 were 0.98 ± 0.01 and 92.5 ± 4.8%, respectively. Matrix spike recoveries are reported in Table A1 for raw wastewater and RNA extracts.

The limit of quantitation (LOQ) for N1 and N2 in the wastewater samples was 1.25 gene copies/mL as determined based on the lowest qPCR standard. The limit of detection (LOD) was the same as the LOQ for N1 and N2, as positive control dilution lower than 10^2 copies/mL resulted in no detection. Agarose gel electrophoresis was used to confirm the presence of amplicons with the correct insert length and detection below the LOQ.

For the PMMoV primer-probe set, PCR conditions included a reverse transcription cycle of 10 min at 50 °C, a denaturing step of 10 min at 95 °C, followed by 45 cycles of 10 s at 95 °C and 1 min at 52 °C and 1 min at 72 °C. Lyophilized virus served as the positive control for PMMoV (agida, Elkhart, IN) which was resuspended in the PM1 solution from RNA extraction kit, as described above, and RNA was extracted following the manufacturer's instructions. The concentration of RNA in the extracts was measured via Nanodrop and the equation described by Rosario et al. (2009) was used to calculate gene copy concentration. A seven-point calibration curve was created with serial dilutions of the PMMoV extraction, the range of the standard curve was 5 × 10^8 to 5 × 10^2 for the PMMoV measurements. The average R^2 of the standard curves for all reactions were 0.99 ± 0.001 and efficiencies were 96.2 ± 4.8%. Matrix spike recoveries into RNA extracts were 90.7 ± 1.8% for PMMoV.

### 2.6 Water quality measurements

Conductivity was measured using a calibrated multimeter (Orion Star A329, Thermo Scientific) and pH was measured using a calibrated Oakton pH 700 (Oakton Instruments) in the laboratory. Of the 140 wastewater samples collected, four samples had insufficient volume for analysis.

### 2.7 Data analysis

Statistical tests were performed in R (www.r-project.org). Pearson correlations were tested between the percentage of positive saliva tests (number of positive tests/number of building residents) for the seven days prior, the days surrounding (i.e., 4 days before and 3 days after), or the seven days after the wastewater sample collection and the gene copy concentrations observed in the wastewater. Pearson correlations were tested also between PMMoV and water quality normalized N1 and N2 data and the percentage of positive saliva tests. To compare the observed concentrations of N1 and N2 gene fragments in wastewater, a paired Wilcoxon Rank Sum test was performed. Pearson correlations were also tested between the concentrations of the two gene fragments. Gene copy concentrations across time were compared between campuses using a paired Wilcoxon Rank Sum test. Average gene copy concentrations were compared using a Kruskal Wallis test followed by a post hoc pairwise test with a Bonferroni correction for multiple comparisons.

### 3. Results and discussion

#### 3.1 Observations of SARS-CoV-2 in university residential wastewater

SARS-CoV-2 N1 and N2 genes were each detected in 69.2–94.7% and 0–50%, respectively, of the wastewater collected at each sampling location. N1 concentrations, when detected and quantifiable, were 36 ± 204 gene copies/mL with a maximum of 1800 copies/mL. The concentrations detected were generally within the ranges we observed in municipal wastewater collected at the treatment plant intakes (data not shown). The maximum N1 concentration observed in university residential wastewater (3.25-log10 copies/mL) was an order of magnitude higher than the peak N1 concentration we observed in municipal wastewater (data not shown) and within the ranges reported by other dormitory studies that reported gene copy concentrations in wastewater (Betancourt et al., 2021; Scott et al., 2021).

The date of peak wastewater concentration for N1 varied by sampling location with maximums occurring on January 13, January 27, February 10, or February 24 (Fig. 1). These peak concentrations were all from composite samples (N1 was not detected in two of the grab samples, and ranged from <LOQ to 3.8 gene copies/mL in the other three grab samples). Differences were also observed between wastewater samples collected on the same day and campus from different locations, for example between C3a and C3b, both located on the same campus (paired Wilcoxon, p = 0.045). Across the sampling period, the N1 gene copy concentrations were not and the N2 gene copy concentrations were significantly different between the sampling sites (Kruskal Wallis test, p = 0.44 and 0.0027, respectively). Spring semester classes started January 19 and spring recess began March 13 and ended March 21, therefore these peak concentrations corresponded with the period following students return to campus and classes.

Comparing the results for the N1 and N2 genes, interestingly, the N2 gene was observed at select sampling dates across the Campus 3 sampling sites but was never above detection on the other two campuses (Fig. A1). The N1 gene was detected more frequently and at significantly higher concentrations than the N2 gene across the samples collected (paired Wilcoxon, p < 2.2 × 10^-16) and a strong linear correlation was observed between the concentrations of the two genes (Pearson, R^2 = 0.998, p < 2.2 × 10^-15) and a moderate linear correlation between the log10 normalized concentrations of the two genes (Pearson, R^2 = 0.50, p = 5.6 × 10^-9, Fig. 2). (Correlations were tested with all data.) Other researchers reported N1 and N2 observations generally followed similar trends (Betancourt et al., 2021; Scott et al., 2021) or were highly correlated (Colosi et al., 2021). One study was able to attribute differences in N1 and N2 observations to the type of bathroom in the dorm (i.e., suite style versus communal) (Scott et al., 2021). Other researchers redesigned N2 primers to improve the assay’s sensitivity (Feng et al., 2021). Matrix spikes were performed for an additional eight samples across the study period (1/27/2021–4/21/2021) where N1 was observed ranging from <LOQ to 35 N1 copies/mL and N2 not detected. These tests confirmed that matrix interferences did not explain why N2 was not observed in these samples given that matrix spike recoveries were comparable for the two genes (paired Wilcoxon Rank Sum, p = 0.87). In clinical testing, a single mutation in the N gene resulted in non-detection for the N2 gene while the E gene was still detected in case reports submitted in September 2020 from Germany (Ziegler et al., 2020) and January 2021 from New Zealand (Fox-Lewis et al., 2021). Sequencing of the wastewater samples would be needed to confirm if this was the cause of the low detection of the N2 gene in the present study.

#### 3.2 Water quality measurements

Water quality as measured by pH and conductivity was relatively consistent across the sampling locations (pairwise t-test, all p > 0.28) with the only site to site significant difference for conductivity between C2b and C1a (pairwise t-test, p = 0.047, Fig. A3). Collection of total suspended solids and chemical oxygen demand data were beyond the scope of this project; however, these data were collected prior to the study period at select sampling locations during a period where the N1 and N2 gene were below detection in all wastewater samples collected (Table A2).

#### 3.3 Biofilm observations

Field sewer biofilm samples were collected to screen for the potential of SARS-CoV-2 RNA to accumulate in sewer biofilms, which if sloughed and containing the target gene copies could impact wastewater concentrations. qPCR analysis of the N1 and N2 viral nucleocapsid gene fragments resulted in detection below the LOQ in all the biofilm samples. PMMoV concentrations in the biofilm ranged from 3.3 to 4.6-log_{10} gene copies per swab (Table A3). The low abundance of SARS-CoV-2 RNA in the biofilm samples was not surprising because when the samples were collected (April 27, 2021), the N1 gene qPCR signal in the wastewater samples collected from these locations was below the LOQ at two of the sites (C3a and C1a) and
ranged between 0.01 and 10 copies/mL at sites C3b and C3c-1 (Fig. 1). In addition, the signal obtained from the N2 gene in wastewater samples collected on April 28, 2021 was below LOQ in all the samples except for C3c-1 (Fig. A1). Previous studies in our lab showed that accumulation of SARS-CoV-2 RNA in simulated biofilm samples at peak concentrations observed had a ~1:1000 ratio for N2 to PMMoV gene copies (Morales Medina, 2020; Morales Medina et al., in review).

3.4. Comparison of wastewater and saliva testing

A total of 268 positive saliva tests were recorded across the study sites ranging from five (for a site with 80 residents) to 142 (for a site with 414 residents) total across the spring semester sampling campaign. The maximum reported positive tests in a day for a given sampling site ranged from one to 30 and the maximum percentage of building residents with a positive test on a given day ranged from 0.04 (mixed graduate and undergraduate population) to 7.2% (undergraduate students, only). The dates of the peak percentage of positive tests did not coincide across sites, but were observed during January and February (Fig. A2). While the testing of the residents across the study period was robust in comparison to many other campuses that required no asymptomatic to less frequent asymptomatic testing (Table 1), the testing strategy was designed to capture asymptomatic cases and residents experiencing COVID-19 symptoms were asked to quarantine rather than complete a test, therefore these results would be expected to represent an underestimate of total cases if those with asymptomatic cases followed these instructions.

The number of tests completed each week varied across the study period. For example, for all students submitting samples on Campus 3, the number of weekly tests completed by students ranged from ~1600 to >4900 (including those living in residence hall and those working on campus) with an average of ~3300 per week. Therefore, percent positive tests were calculated based on the number of residents for a given sampling site and used for comparison to the wastewater data, presented below. Data were collected by the university on negative and inconclusive tests and reported on a weekly basis. For Campus 3, an average of 1.51 ± 0.01% of tests were positive and 0.30 ± 0.13% of tests were inconclusive each week across the study period. For paired weekly totals, the inconclusive tests represented 34 ± 30% of the total positive cases reported (i.e., inconclusive/positives test results), and thus represented a potentially significant source of error.

Correlations were tested between the wastewater N1 or N2 gene copy concentrations and the saliva test percent positive test results (100 × number of positive samples / number of residents) for a given sampling site (Fig. 3). Moderate correlations were observed between the N1 gene and the positive saliva tests collected during the seven days before wastewater collection (Pearson cor = 0.48, p = 2.5 × 10⁻⁸). A weak positive correlation was observed between the N1 gene and the surrounding seven days positive saliva test results (Pearson cor = 0.21, p = 0.018) and no correlation was observed between the following seven days positive saliva test results (Pearson cor = 0.12, p > 0.20). N2 similarly had Pearson correlations with the positive saliva tests during the seven days prior to wastewater collection (p = 3.3 × 10⁻⁸), weak positive correlation with the surrounding seven days (p = 0.020), and no correlation with the following seven days saliva tests (p = 0.22). All correlation coefficients are listed in Table A3. Given the strongest correlation was observed with the wastewater N1 gene concentrations and the previous week’s saliva test results and no correlation was observed with the following week’s saliva test results, the wastewater data here represent a lagging indicator of COVID-19 infections.
in the buildings tested. The possibility of fecal shedding into the dorm sewers continued for 48 to 96 h after a positive saliva test (including both the time to receive results and be relocated). And the positive cases were not-recounted, as re-testing did not occur for 90 d following the initial positive saliva test. This is similar to results reported for a study performed during a campus vaccination campaign that included required weekly testing for asymptomatic persons and observed correlations between wastewater and rolling 7-day average cases (Bivins and Bibby, 2021). While this may seem in contrast to reports of wastewater serving as a leading indicator of SARS-CoV-2 infections (Betancourt et al., 2021; Gibas et al., 2021; Karthikeyan et al., 2021), less frequent individual testing (sampling less frequently than one sample per week) was implemented in the sewer catchment for those studies. Thus, when individual testing is not robust, weekly or more frequent wastewater sampling may still serve as a leading

![Fig. 2. Log10 transformed concentration of N2 gene copies versus N1 gene copies with colors corresponding to different sampling sites. Red line represents the linear regression and shaded area the 95% confidence interval. Non-detects are shown intersecting with the respective axes.](image)

![Fig. 3. Concentrations of N1 and N2 gene copies versus the percent positive tests (number of positives per sampling location / total residents per location) for the 7 days before the wastewater (WW) collection. Data points are color coded by site, shape corresponds to the campus. Error bars represent standard deviation of technical replicates (N = 3).](image)
indicator of outbreaks. The seven-day windows of COVID-19 cases were selected for these comparisons because residents submitted their saliva tests at least seven days apart, assuming testing compliance. Thus, seven-day windows would capture at most one sample per resident (unless involved in contact tracing). The stronger correlations observed for the previous week’s compared to surrounding week’s saliva tests may be due to the reported delay in clinical patients between a positive saliva test and the onset of fecal shedding (Chen et al., 2020; Foldatori et al., 2020). Other researchers have compared clinical testing results or hospitalizations on varying time scales to wastewater SARS-CoV-2 observations for in-sewer or sewershed-wide studies ranging from daily cases (Ai et al., 2021), 3 to 7 day average or cumulative cases (Agrawal et al., 2021; Galani et al., 2022; Giraud-Billoud et al., 2021; Koureas et al., 2021), and 10 to 20 day cumulative cases (Barrios et al., 2021).

The correlations when observed could have been improved if more data were available to help normalize the gene copy concentrations observed in the wastewater. Various normalization schemes have been suggested and tested to date for SARS-CoV-2 including correcting for flow, variation in water quality, and use of other biological markers to improve understanding of fecal loading in a given sample (Betancourt et al., 2021; Feng et al., 2021). Here, flow meters were not installed at the sampling locations, therefore wastewater flow during the composite sampling period is not known. Correlations were tested with the PMMoV normalized N1 and N2 gene copy observations. The PMMoV concentrations observed were on average 3.5 ± 1.1 log10 copies/mL, which is within the ranges reported for untreated wastewater (10−7–107 gene copies/mL) (Kitajima et al., 2018) (Fig. A4). Correlations were not observed for the PMMoV normalized N1 data compared to the saliva test results. The PMMoV normalized N2 data produced similar correlations to the saliva test results as the non-normalized N2 data (Table A5, Fig. A5). Thus, as reported by others at the municipal scale (Feng et al., 2021), normalizing SARS-CoV-2 to PMMoV did not improve the correlations observed with saliva testing results in this study. Likewise, normalizing the SARS-CoV-2 nucleocapsid gene concentrations to pH or conductivity did not improve the correlations between saliva test results (Tables A6, A7).

Of interest is the potential for false positives or negatives for wastewater sampling. Given the strongest correlation was observed between N1 gene copy concentrations and the previous week’s saliva test results and the wastewater samples, the data were also compared for when there was detection in both 41% of samples, detection in neither sample type (9% of samples), and detection in wastewater but not saliva testing data (42.6% of samples), and no detection in wastewater but detection in saliva data (9% of samples). The latter combined 51.6% of samples represent either false positive or negatives and may be due to a variety of factors including variable shedding rates and durations in feaces among individuals during infection, non-compliance with testing, use of other toilet facilities on campus from an individual residence, relocation of positive cases to other locations, among other possibilities (Harris-Lovett et al., 2021). Others have attributed false-negatives to low population in the catchment (Colosi et al., 2021). The challenges of interpreting wastewater results given the potential for false negatives and false positives was previously discussed (Harris-Lovett et al., 2021). The presumption that use patterns could vary on the two days without regular classes scheduled. It is possible that the correlations presented here would be different if more frequent wastewater sampling was performed, as several others have done (Table I).

4. Conclusions

Weekly saliva testing resulted in earlier detection of COVID-19 cases in dormitory populations as compared to weekly wastewater surveillance on the campuses studied. These results in combination with low case rates and vaccine mandates all contributed to decision among the administrators in the present study to pause the wastewater surveillance program on the campuses studied. When using these results for design of campus surveillance, it would be advisable to consider the testing compliance rates when determining if the results of our study would be repeated. Here, a small population was present on campus and the study was performed before and during the time vaccines became available to increase patient enrollment. Comparison of these results to other study designs as well as other considerations including cost and practicality (Harris-Lovett et al., 2021) will likely inform future monitoring study designs.

The results presented highlight the need for further study to understand whether the lack of N2 detection on C1 and C2 was due to different SARS-CoV-2 variants present; application of sequencing on preserved samples may provide insight. The observation of N1 and N2 in the C3 biofilm collected on April 27, 2021 was below LOD in all the samples except for C3c-1 (Fig. A1). Therefore, the potential for sloughing of biofilms containing viral gene copies to impact the observations here is not clear.

3.5. Challenges associated with implementing the wastewater surveillance program

Limitations of the wastewater sampling pilot implemented were noted including the sampling locations and frequency. The university housing for Campus 3 has over 100 buildings, and while not all were occupied during the study period, the labor and supplies including installation of sampling infrastructure was a limitation due to uncertainty with the methods and pandemic related budget constraints. Others have noted this challenge (Harris-Lovett et al., 2021) and solutions include implementing cascading sampling, sampling combined sites and moving upstream when there were positive detections to increase throughput (Karthikeyan et al., 2021). Some sampling sites selected for this study captured wastewater from multiple buildings. For our purposes, this provided pilot data for these buildings to compare with individual testing results with fewer wastewater sampling sites, thus saving time and funds. However, one can imagine scenarios where sampling all buildings separately would be desirable or the adoption of the cascading sampling. Inclusion of upstream sampling sites, even if only analyzed when the targets were detected, could provide more precise information needed for decision making (i.e., whole building quarantine or individual resident re-testing) which with this sampling scheme would impact potentially more students than needed.

For some housing units on the campuses in this study, it was not possible to sample residential wastewater separately from the other building uses given the sewage collection systems were not designed with this purpose in mind. These multi-use units were therefore excluded from this pilot study. Some other researchers sampled early morning in efforts capturing building residents rather than visitors or non-resident employees (Betancourt et al., 2021). Otherwise, a retrofit would be required for current buildings and future plumbing design may wish to take WBE considerations into account when designing collection systems in multi-use buildings.

The sampling design for this pilot study involved weekly sampling. The day of the week for sampling was selected for multiple reasons. Sampling Tuesday or Wednesday allowed for staff that work weekdays to set up and collect samples during regular work hours and for the lab to report data during regular business hours, as data were available by Friday afternoons for reporting. Sampling on the weekend was also avoided given the presumption that use patterns could vary on the two days without regular classes scheduled. It is possible that the correlations presented here would be different if more frequent wastewater sampling was performed, as several others have done (Table I).
samples also highlights the need for further understanding of this sewer matrix, for which a separate study was performed (Morales Medina, 2020; Morales Medina et al., in review) indicating SARS-CoV-2 can accumulate in sewer biofilms during periods of high COVID-19 cases.

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**CRediT authorship contribution statement**

N.L. Fahrenfeld: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft. William R. Morales Medina: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – review & editing. Stephanie D’Elia: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. Maureen Modica: Conceptualization, Data curation, Methodology, Project administration, Writing – review & editing. Alejandro Ruiz: Conceptualization, Data curation, Methodology, Project administration, Writing – review & editing. Mark McLane: Conceptualization, Data curation, Funding acquisition, Project administration, Writing – review & editing.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.151947.

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