Divergence of wastewater SARS-CoV-2 and reported laboratory-confirmed COVID-19 incident case data coincident with wide-spread availability of at-home COVID-19 antigen tests

Alexandria B. Boehm[1], Marlene K. Wolfe[2], Bradley White[3], Bridgette Hughes[3] and Dorothea Duong[3]

[1] Stanford University, Stanford, CA, United States of America
[2] Emory University, Atlanta, GA, United States of America
[3] Verily Life Sciences, South San Francisco, CA, United States of America

ABSTRACT

Concentrations of SARS-CoV-2 RNA in wastewater settled solids from publicly owned treatment works (POTWs) historically correlated strongly with laboratory confirmed incident COVID-19 case data. With the increased availability of at-home antigen tests since late 2021 and early 2022, laboratory test availability and test seeking behavior has decreased. In the United States, the results from at-home antigen tests are not typically reportable to public health agencies and thus are not counted in case reports. As a result, the number of reported laboratory-confirmed incident COVID-19 cases has decreased dramatically, even during times of increased test positivity rates and wastewater concentrations of SARS-CoV-2 RNA. Herein, we tested whether the correlative relationship between wastewater concentrations of SARS-CoV-2 RNA and reported laboratory-confirmed COVID-19 incidence rate has changed since 1 May 2022, a point in time immediately before the onset of the BA.2/BA.5 surge, the first surge to begin after at-home antigen test availability was high in the region. We used daily data from three POTWs in the Greater San Francisco Bay Area of California, USA for the analysis. We found that although there is a significant positive association between wastewater measurements and incident rate data collected after 1 May 2022, the parameters describing the relationship are different than those describing the relationship between the data collected prior to 1 May 2022. If laboratory test seeking or availability continues to change, the relationship between wastewater and reported case data will continue to change. Our results suggest, assuming SARS-CoV-2 RNA shedding remains relatively stable among those infected with the virus as different variants emerge, that wastewater concentrations of SARS-CoV-2 RNA can be used to estimate COVID-19 cases as they would have been during the time when laboratory testing availability and test seeking behavior were at a high (here, before 1 May 2022) using the historical relationship between SARS-CoV-2 RNA and COVID-19 case data.

Subjects Virology, Epidemiology, Environmental Contamination and Remediation, Environmental Health, COVID-19
Keywords COVID-19, Wastewater-based epidemiology, COVID-19 antigen tests, SARS-CoV-2, Viruses, Public health

INTRODUCTION

Concentrations of genetic material from various viruses in wastewater solids collected from publicly owned treatment works (POTWs) correlate well with clinical testing data on disease occurrence (*Wolfe et al., 2021a*; *Hughes et al., 2022*; *Wolfe et al., 2022*). SARS-CoV-2 RNA concentrations in wastewater solids have been shown to correlate strongly with laboratory-confirmed cases of COVID-19 (*Peccia et al., 2020*; *Graham et al., 2021*; *D'Aoust et al., 2021*). Similarly, concentrations of influenza A and B, RSV, metapneumovirus, parainfluenza, rhinovirus, seasonal coronaviruses, and mpox nucleic acids in POTW wastewater solids correlate well with clinical sample percent positivity rates and/or reported disease incidence in the contributing communities (*Hughes et al., 2022*; *Wolfe et al., 2022*; *Mercier et al., 2022*; *Boehm et al., 2022a*; *Wolfe et al., 2023*). As a result, wastewater monitoring for infectious targets has been used for public health decision making including decisions about vaccine and testing availability (*Wolfe et al., 2023*; *McClary-Gutierrez et al., 2021b*).

Wastewater can be a less biased approach than clinical testing for gaining insight to community health as it represents a composite biological sample containing contributions from the entire contributing population. Human excretions including feces, urine, sputum, saliva, and mucus are present in wastewater. Viruses and their genetic material adsorb to solids in the wastestream and as a result, their concentrations are enriched orders of magnitude in the solids compared to the liquid phase of wastewater (*Graham et al., 2021*; *Wolfe et al., 2023*; *Kim et al., 2022*; *Ye et al., 2016*; *Yin et al., 2018*). Therefore, solids represent a natural concentrator of virus in wastewater and an ideal matrix for measuring their concentrations for wastewater-based epidemiology. It should be noted that wastewater is not a viable option for community disease surveillance in locations where sewage is not treated at centralized wastewater treatment facilities.

Our and others' previous work showed strong correlations between SARS-CoV-2 RNA concentrations in wastewater and laboratory-confirmed COVID-19 case data (*Gonzalez et al., 2020*; *Wolfe et al., 2021b*; *Ahmed et al., 2020*; *Whitney et al., 2021*; *Duvallet et al., 2022*; *Hopkins et al., 2023*). This work was primarily completed before the emergence of the Omicron variant, and used COVID-19 case data compiled by public health departments, almost entirely reliant on testing of patient specimens performed in clinical laboratories. The emergence of Omicron occurred at the same time at-home antigen tests were first made available by the United States federal government free of charge on 16 January 2022 (at the height of the Omicron BA.1 surge) (*The White House, 2023*). The results of at-home antigen tests are not typically reportable to public health agencies so their positive results are not recorded in public health databases (*Radar et al., 2022*).

The goal of the present study is to compare the relationship between SARS-CoV-2 RNA concentrations measured in POTW wastewater solids to reported laboratory-confirmed COVID-19 incident cases before and after at-home antigen tests were widely available. Daily concentrations of SARS-CoV-2 from three large POTWs located in the Greater San Francisco Bay Area of California, USA are used for the analysis, along with publicly available data on incident laboratory-confirmed COVID-19 cases and laboratory test positivity rates.

METHODS

The study was reviewed by the IRB at Stanford University and the IRB has determined that this research does not involve human subjects as defined in 45 CFR 46.102(f) and therefore does not require IRB oversight.

Study sites

Daily samples between 1 December 2020 and 16 January 2023 of wastewater settled solids were obtained from three publicly owned treatment works (POTWs) San Jose (SJ) Oceanside (OS), and Sacramento (SAC) serving populations in Santa Clara County, San Francisco County, and Sacramento County, California USA, respectively. POTW leadership provided approval for study participation. The plants serve 1.5 million, 250,000, and 1.5 million people, respectively, representing 77%, 30%, and 93% of their county populations, respectively. Detailed methods for sample collection have been presented elsewhere (*Wolfe et al., 2021b*; *Topol et al., 2021a*; *Topol et al., 2021b*; *Topol et al., 2021c*; *Huisman et al., 2022*). In brief, POTW staff grab samples of solids were collected from the primary clarifier daily in sterile containers and placed at 4 °C and couriered to the laboratory where they were processed immediately. A total of 777, 737, and 769 samples were included in this study from SJ, OS, and SAC, respectively. Note that a subset of these data have already been published; in particular data from all three POTWs collected between 1 December 2020 and 31 March 2021 was previously published by *Wolfe et al. (2021b)* and data between 1 January 2022 and 13 April 2022 were published by *Boehm et al. (2022b)*. Otherwise, the data presented herein has not been previously published.

Laboratory analyses

Samples were processed the same day they were collected, with no storage, in the laboratory to measure concentrations of SARS-CoV-2 N gene and an internal endogenous control pepper mild mottle virus (PMMoV). The methods are detailed in other peer-reviewed publications (*Wolfe et al., 2021b*; *Topol et al., 2021a*) and available on protocols.io (*Topol et al., 2021a*; *Topol et al., 2021b*; *Topol et al., 2021c*). The N gene target is conserved across SARS-CoV-2 variants to date (Fig. S1). In brief, the samples were centrifuged to dewater the solids, resuspended in a buffer spiked with an exogenous viral control (bovine coronavirus, BCoV), homogenized, and then centrifuged. Nucleic-acids were extracted and purified from the supernatant in 10 replicates using a commercial kit and then the RNA extract was subjected to an inhibitor removal step. The resultant RNA from each of the 10 replicates was used immediately as template in reverse-transcription polymerase chain reactions (RT-PCR) in a digital droplet format to measure the SARS-CoV-2 N gene, PMMoV, and BCoV. Positive and negative extraction and RT-PCR controls were included in all runs, and we ensured that BCoV recovery was greater than 10% in all samples. Previous work showed that inhibition using this method was not present (*Huisman et al., 2022*). Results for SARS-CoV-2 N gene and PMMoV are reported as copies of target per dry weight of solids. Wastewater data are available through the Stanford Digital Repository (https://doi.org/10.25740/xy132dg9314).

Case data

Publicly available data on the laboratory-confirmed incidence rate, test positivity rate, and number of tests administered for Santa Clara, San Francisco, and Sacramento Counties are available online (https://data.chhs.ca.gov/dataset/covid-19-time-series-metrics-by-county-and-state). Incidence rates for populations served by specific POTWs (for each sewershed) were provided by California Department of Public Health. Incidence and positivity rate data are reported as a function of the "episode date" which is the earliest reported date associated with the specimen (symptom onset, collection, hospitalization, or death; but usually specimen collection date) and include results of nucleic-acid amplification tests (hereafter, "PCR tests"). Seven-day smoothed incidence and positivity rate data are used to represent incidence and positivity rate hereafter, because it is well understood that these data have substantial day-of-week effects due to reduced availability of testing on weekends (*Bergman et al., 2020*).

Statistical analysis

We used Pearson's r to test for linear relationships between sewershed-aggregated and county-aggregated incidence rate data for each POTW (three tests total). We used Kendall's tau to test for association between county-aggregated positivity and incidence rates for each POTW (three tests). We used Kendall's tau to test for association between wastewater and clinical case data sets. We tested for associations between measured N, N/PMMoV, 5-d trimmed average N, and 5-d trimmed N/PMMoV, and incidence rate and positivity rates for each POTW resulting in twenty-four Kendall's tau tests. We chose Kendall's tau as it does not require that data are normally distributed and therefore did not require data transformation; however, results are unchanged if other tests of association are used.

We tested whether the relationship between wastewater measurements of SARS-CoV-2 RNA and incidence rate differed for data collected before, and after 1 May 2022 using the following linear model:

$$Y = b + m*X + n*D + k*(D*X) \tag{1}$$

where Y is $\log_{10}$-transformed 7-day smoothed incidence rate data aggregated at the county where the POTW is located, X is the $\log_{10}$-transformed 5-d trimmed average N/PMMoV, D is a categorical, binary variable indicating whether the date is after 1 May 2022 ($D = 0$ if the date is before 1 May 2022, and 1 if it is on or after this date), b is the intercept, m is the coefficient on X, n is the coefficient on D, and k is the coefficient on the interaction term D*X. We chose 1 May 2022 as the date to bifurcate the data since this date occurs at the onset of the BA.2/BA.5 COVID-19 case surge in this region. This represents the first surge that began after at-home antigen tests became widely available in the region. We confirmed *via* visual examination that this date represented approximately when the relationship between incidence rate and wastewater concentrations appeared to change (see Results).

The model was applied to each sewershed (model was run three times). We chose to use the 5-d trimmed average wastewater concentration as it eliminates the influence of intrinsic variability associated with measurements from a complex, environmental matrix (*Wolfe et al., 2021b*); and we chose to use N/PMMoV as PMMoV normalization can control for

variation in RNA extraction efficiency and fecal strength of the solids (*Wolfe et al., 2021b*; *McClary-Gutierrez et al., 2021a*; *Simpson et al., 2021*). However, we explored using raw wastewater data (not 5-d trimmed averages) and unnormalized N gene concentrations as X in the model, and raw incidence rate as Y in the model, and results were generally unchanged.

We tested whether the relationship between positivity and incidence rate was different for data collected before, and after 1 May 2022 using Eq. (2):

$$Y = b + m*Z + n*D + k*(D*X) \qquad (2)$$

Here $Y = \log_{10}$-transformed incidence rate, and $Z = \log_{10}$-transformed positivity rate and the other variables have been defined. The model was applied to each sewershed (model was run 3 times).

We confirmed the Eq. (1) model residuals were approximately normal, and independent with equal variance with respect to the predictions. All statistical analyses were carried out in R studio (Version 1.4.1106; *RStudio Team, 2021*) using R (Version 4.0.5; *R Core Team, 2021*). We used alpha = 0.05 which corresponds to a p value of 0.0013 with a Bonferroni correction (0.05/36 tests).

RESULTS

Positive and negative RT-PCR and nucleic-acid extraction controls were positive and negative, respectively, indicating assays performed well and there was no contamination during sample pre-analytical and analytical processing. BCoV recovery was greater than 10% (median across samples from each POTW was 80%), and PMMoV concentrations were fairly consistent across samples (median ± interquartile range of $9.0 \times 10^8 \pm 4.3 \times 10^8$ cp/g (SAC), $5.2 \times 10^8 \pm 3.4 \times 10^8$ cp/g (OS), and $1.5 \times 10^9 \pm 6.0 \times 10^8$ cp/g (SJ)) indicating good RNA extraction efficiency and fairly consistent fecal strength of wastewater (Fig. S2). We previously published details related to the dMIQE (*The d MIQE Group & Huggett, 2020*) and EMMI (*Borchardt et al., 2021*) guidelines for these measurements (*Wolfe et al., 2021b*; *Boehm et al., 2022b*).

Sewershed-aggregated incidence rates correlate strongly with county-level incidence rates (Pearson's r of 1.00 for SJ, 0.99 for OS, and 0.93 for SAC, $p < 10^{-15}$ for all). We use county-aggregated incidence rate hereafter to represent sewershed-aggregated incidence rate given their high correlations, because the county-aggregated data are publicly available and can be shared readily. Positivity rate aggregated at the sewershed is not available to researchers, so we used county-aggregated positivity rate as a proxy for the sewershed positivity rate given the high degree of correlation between county and sewershed-aggregated incidence rate.

Across all three sewersheds, SARS-CoV-2 N gene concentrations, and those concentrations normalized by PMMoV (raw and 5-day trimmed averages) were positively and significantly correlated to county-aggregated COVID-19 incidence rate, and county-aggregated positivity rate (Kendall's tau between 0.51 and 0.62, $p < 10^{-15}$ for correlations with incidence rate, and Kendall's tau between 0.68 and 0.80, $p < 10^{-15}$ for correlations

with positivity rate), consistent with previously published work (*Peccia et al., 2020*; *D'Aoust et al., 2021*; *Duvallet et al., 2022*; *Feng et al., 2021*). The ranges reported in the previous sentence include tau calculated with the four considered wastewater variables for the three plants. It should be noted that the RNA targets measured in this study are highly persistent in wastewater matrixes over the time scales that wastewater is present in the wastewater collection system prior to sampling (< 1 day) (*Roldan-Hernandez et al., 2022*; *Burnet et al., 2023*) so differential decay of the markers in different systems is not expected.

County-aggregated incidence rate and positivity rate are well correlated (Kendall's tau 0.79 for SAC, 0.96 for SJ, 0.88 for OS, all $p < 10^{-15}$), but there is a change in their relationship starting approximately 1 May 2022, at the onset of the BA.2/BA.5 surge (Fig. S3). A multiple regression model was used to test whether the relationship between positivity rate and incidence rate was significantly different before and after 1 May 2022 at each of the considered counties, and those models indicated that the relationship was significantly different before and after 1 May 2022 with different slope and/or intercepts (Table S1). In general, slopes and y-intercepts after 1 May 2022 are larger.

When $\log_{10}$-transformed incidence rate and positivity rate are plotted against $\log_{10}$-transformed, 5-d trimmed average wastewater concentrations of SARS-CoV-2 N gene normalized by PMMoV at the three sewersheds, the data generally fall on a single line (Figs. 1–3). However, data collected starting approximately 1 May 2022 fall off the line represented by data collected before 1 May 2022 on the incidence rate—wastewater plot; the same pattern is not evident on the positivity rate—wastewater plot (Figs. 1–3). The relationship between $\log_{10}$-transformed incidence rate and $\log_{10}$-transformed N/PMMoV concentrations collected after 1 May 2022 appears approximately linear, but it is visually offset from earlier measurements.

Using multiple linear regression, we tested whether the linear relationship between $\log_{10}$-transformed incidence rate and $\log_{10}$-transformed 5-d trimmed average N/PMMoV is the same for data collected before and after 1 May 2022. Models for the three sites were statistically significant with adjusted R^2 values between 0.81 and 0.86 ($p < 10^{-15}$). Model residual errors appeared independent and normal with equal variance indicating model assumptions are not violated. For SAC, the slope of the relationship between the two variables is not different for data collected before and after 1 May 2022 (slope = 0.88) but the intercept is different (−0.26 compared to −0.87 for data collected before and after 1 May 2022, respectively). For SJ, the slope of the relationship between the two variables is not different for data collected before and after 1 May 2022 (slope = 0.96); but the intercept is different (0.29 compared to −0.66 before and after 1 May 2022, respectively). For OS, both the slope and the intercept of the relationship between incidence rate and wastewater are different for data collected before and after 1 May 2022 (slope = 0.65 and 0.35 for data before and after 1 May 2022, respectively; intercept = −1.2 compared to −2.73 before and after 1 May 2022, respectively). Results reported here are summarized in Table 1. We repeated the modeling using N (both raw and 5-d trimmed averaged data) and raw N/PMMoV as the dependent variables (X in Eq. (1)), as well as raw (unsmoothed) incidence rate data (Y in Eq. (1)), and results were similar. Model results for models using raw incidence rate and raw N/PMMoV data are provided in Table S2.

Boehm et al. (2023), *PeerJ*, DOI 10.7717/peerj.15631

7/18

Table 1 **Coefficients for the model $Y = b + m*X + n*D + k*(D*X)$ presented as Eq. (1) in the text where X is $\log_{10}$-transformed 5-d trimmed N/PMMoV and Y is $\log_{10}$-transformed incidence rate.** Adjusted R-square values and *p*-values for the model are provided, as well as the F statistic (and the degrees of freedom, DF). Coefficient values with standard errors are reported as well as *p*-values in parentheses. With Bonferroni corrections, *p* must be less than 0.0013 for alpha $= 0.05$. If *n* is significantly different from 0, then the intercept for the linear relationship between X and Y before and after 1 May 2022 is different and equal to $b+n$. If *k* is significantly different from 0, then the slope for the linear relationship between X and Y is different before and after 1 May 2022 and equal to $m+k$.

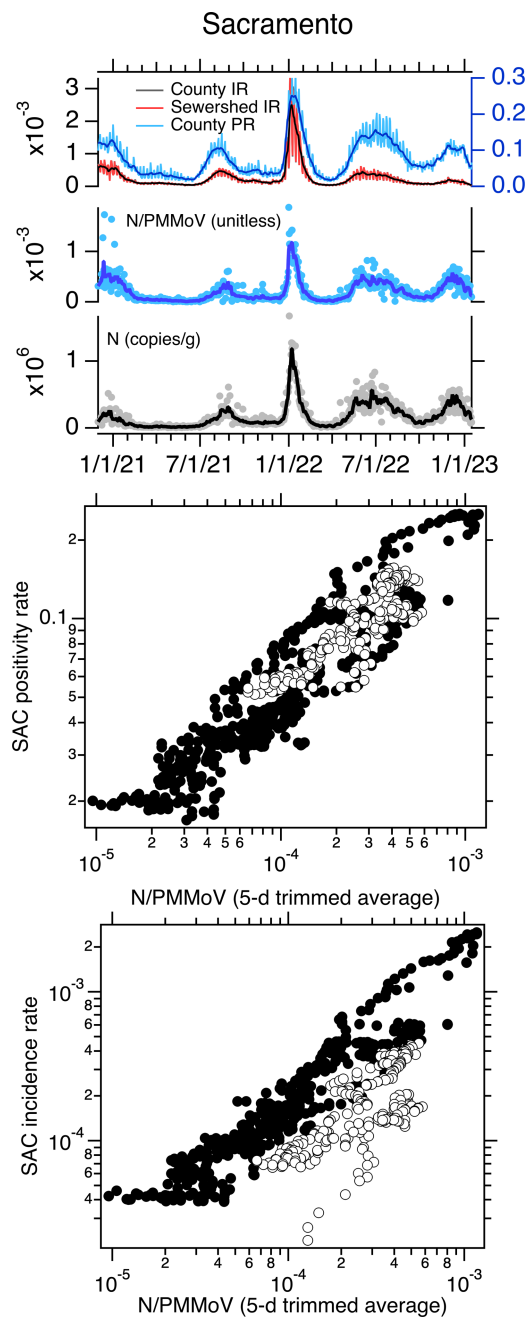| POTW	b	m	n	k	Adjusted R^2	*F*-statistic
SJ	0.29 ± 0.07 (1.3×10^{-5})	0.96 ± 0.02 $(<10^{-100})$	-0.96 ± 0.18 $(<10^{-7})$	-0.12 ± 0.05 (0.014)	0.86 $(<10^{-15})$	1545 on 3 and 773 DF
OS	-1.22 ± 0.05 $(<10^{-100})$	0.65 ± 0.01 $(<10^{-100})$	-1.51 ± 0.22 $(<10^{-11})$	-0.29 ± 0.06 $(<10^{-5})$	0.81 $(<10^{-15})$	1070 on 3 and 733 DF
SAC	-0.26 ± 0.06 (2.9×10^{-5})	0.88 ± 0.02 $(<10^{-100})$	-0.61 ± 0.15 (6.7×10^{-5})	-0.067 ± 0.04 (0.10)	0.83 $(<10^{-15})$	1270 on 3 and 765 DF

Figure 1 **Data for Sacramento POTW.** Top panel: Sacramento (SAC) time series of concentrations of N and N/PMMoV in wastewater, incidence rates (IRs), and positivity rates (PRs) between 1 December 2020 until 16 January 2023. IRs and PRs are unitless. The solid lines represent 5-d trimmed averages for the wastewater data, and 7-d moving averages for the incidence and positivity rates. The county-aggregated and sewershed-aggregated incidence rate data fall almost directly on top of each other, obscuring the view of both. The scales for incidence and positivity rates are on the left and right axes, respectively. Middle and bottom panels: County-aggregated (Sacramento County, SAC) positivity rate and incidence rate *versus* 5-d trimmed N/PMMoV; white symbols are data collected on and after 1 May 2022.
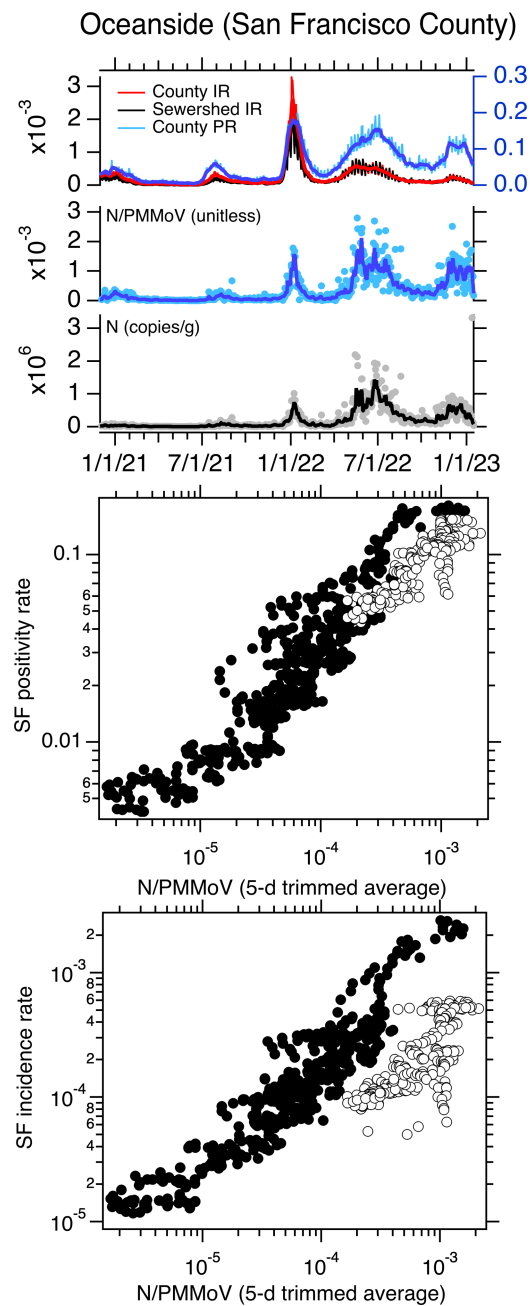
Full-size 🖼 DOI: 10.7717/peerj.15631/fig-1

Figure 2 **Data for Oceanside POTW.** Top panel: Oceanside (OS) time series of concentrations of N and N/PMMoV in wastewater, incidence rates (IRs), and positivity rates (PRs) between 1 December 2020 until 16 January 2023. IRs and PRs are unitless. The solid lines represent 5-d trimmed averages for the wastewater data, and 7-d moving averages for the incidence and positivity rates. The county-aggregated and sewershed-aggregated incidence rate data fall almost directly on top of each other, obscuring the view of both. The scales for incidence and positivity rates are on the left and right axes, respectively. Middle and bottom panels: County-aggregated (San Francisco County, SF) positivity rate and incidence rate *versus* 5-d trimmed N/PMMoV; white symbols are data collected on and after 1 May 2022.
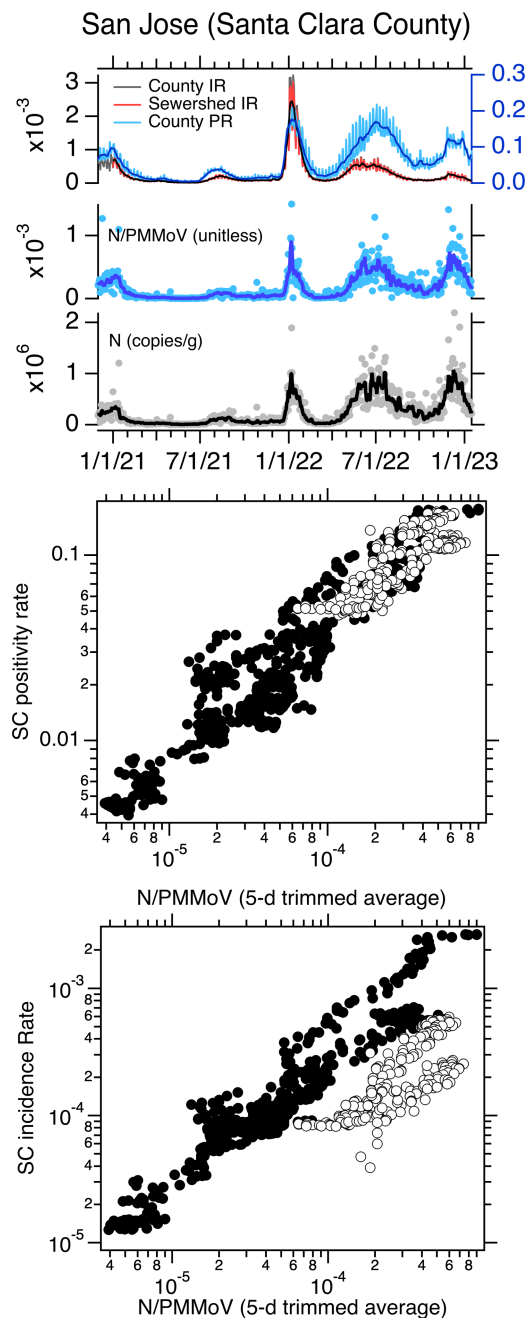
Full-size ⊡ DOI: 10.7717/peerj.15631/fig-2

Figure 3 **Data for San Jose POTW.** Top panel: San Jose (SJ) time series of concentrations of N and N/PMMoV in wastewater, incidence rates (IRs), and positivity rates (PRs) between 1 December 2020 until 16 January 2023. IRs and PRs are unitless. The solid lines represent 5-d trimmed averages for the wastewater data, and 7-d moving averages for the incidence and positivity rates. The county-aggregated and sewershed-aggregated incidence rate data fall almost directly on top of each other, obscuring the view of both. The scales for incidence and positivity rates are on the left and right axes, respectively. Middle and bottom panels: County-aggregated (Santa Clara County, SC) Positivity rate and incidence rate *versus* 5-d trimmed N/PMMoV; white symbols are data collected on and after 1 May 2022.

Full-size ◪ DOI: 10.7717/peerj.15631/fig-3

As Eq. (1) describes the relationship between $\log_{10}$-transformed incident rates and 5-d trimmed N/PMMoV well, it implies that incident rate and N/PMMoV are related by a power law relationship where incident rate $= 10^{intercept}$ (N/PMMoV)^{slope}.

DISCUSSION

In the Greater San Francisco Bay Area of California, USA, the BA.2/BA.5 COVID-19 case surge began around 1 May 2022 and represents the first surge in the region to begin after at-home antigen tests became widely available. The relationship between wastewater concentrations of SARS-CoV-2 RNA and laboratory-confirmed incidence rates of COVID-19 in the contributing community changed after 1 May 2022, likely a result of the change in regional PCR test seeking behaviors and testing availability. Because case data is likely underestimating the true cases in the community, this divergence indicates that wastewater may be particularly useful for obtaining a less biased estimate of community infection.

The relationship between SARS-CoV-2 RNA concentrations and laboratory-confirmed incident cases has previously been described as a power-law relationship (*i.e.,* it is linear on a log–log plot) (*Wolfe et al., 2021a*). We found that data collected both before and after 1 May 2022 at the three POTWs follow power-law relationships albeit with different parameter values. In particular, we found the slope of the log–log relationship to be similar for data collected before and after 1 May 2022 for two of the three POTWs, but the intercept to be different at all three. At all three POTWs, the intercept is smaller for data collected after 1 May 2022. This can be interpreted to mean that for data considered after 1 May 2022, that a one log-change in wastewater concentration indicates a log-change (between 0.35 and 0.96 log-change, depending on POTW) in incidence rates that is similar as to data presented before 1 May 2022 (between 0.65 and 0.96 log-change, depending on POTW), but a specific wastewater concentration corresponds to a lower observed incidence rate for data collected after 1 May 2022 than before 1 May 2022. This could be explained by fewer cases being reported through the public health case reporting system due to the increased use of at-home antigen tests, or decreased overall testing.

The change in the relationship between wastewater concentrations of SARS-CoV-2 RNA and reported laboratory-confirmed COVID-19 incidence rates is likely a result of decreased PCR test-seeking and availability, and increased availability of at-home COVID-19 antigen tests. It has been confirmed that PCR test seeking behavior has decreased as the availability of at-home antigen tests has increased (*Radar et al., 2022*). Reduced test seeking behavior will bias the number of laboratory-confirmed cases downward but would not necessarily change the PCR testing positivity rate, so long as reduced test-seeking behavior is similar across all individuals including those who ultimately are infected and not infected with SARS-CoV-2. The similar relationship between SARS-CoV-2 RNA wastewater concentrations and laboratory-confirmed test positivity rate for data collected before and after 1 May 2022 would be consistent with this explanation. The changed relationship between incidence rate and positivity rate after 1 May 2022 is also consistent with this explanation. It should be noted that positivity rate data may become unreliable if the number of tests administered is small. In the date range considered in this study, the

median number of weekly aggregated tests administered were 4,122, 4,702, and 2,478 tests per 100,000 people in San Francisco, Santa Clara, and Sacramento Counties (Fig. S4).

It is important to note that there are other potential reasons that the relationship between SARS-CoV-2 RNA wastewater concentrations and laboratory-confirmed COVID-19 incidence rates could change over time. The duration and magnitude of SARS-CoV-2 RNA shedding *via* human excretions that enter wastewater might change as different SARS-CoV-2 variants circulate (*Puhach, Meyer & Eckerle, 2022*). To date, there are limited quantitative, externally valid data (*i.e.,* measurements reported as concentrations with units of copies per mass or volume of excretion) on SARS-CoV-2 RNA in excretions among individuals infected with different variants, particularly with the newer variants that have emerged over the last year (*Daou et al., 2022*; *Anjos et al., 2022*). There is one study that inferred that the changing relationship between wastewater SARS-CoV-2 RNA concentration and case data between the Delta and Omicron surges in Arizona, USA was caused by changes in shedding (*Prasek et al., 2023*). Changes in severity of illness, or occurrence of asymptomatic infections may alter test seeking behavior, which would change case reporting, and yet is unrelated to the availability of at-home antigen tests.

It is highly likely that the relationship between wastewater concentrations and laboratory-confirmed COVID-19 incidence rates will continue to change as PCR test seeking and test availability change. For example, an official end of the COVID-19 public health emergency in the United States may make both clinical laboratory and at-home testing unaffordable or more difficult to obtain for some individuals. The work herein suggests that, assuming SARS-CoV-2 RNA shedding remains relatively stable among those infected with the virus as different variants emerge, that wastewater concentrations of SARS-CoV-2 RNA can be used estimate COVID-19 cases as they would have been during the time when PCR testing availability and test seeking behavior were at a high (here, before 1 May 2022) using the historical relationship between SARS-CoV-2 RNA and case data. This data may be useful for gaining insight into numbers of COVID-19 infections. In addition, research that enables estimation of true incidence rates of COVID-19 from wastewater monitoring data using coupled Susceptible-Exposed-Infectious-Recovered (SEIR) and environmental mass balance models could provide useful insights into true disease burdens from wastewater that are not dependent on test availability or individual test seeking behavior.

ACKNOWLEDGEMENTS

ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interests

Bradley J. White, Dorothea Duong, and Bridgette Hughes are employees of Verily Life Sciences, LLC. There are no other competing interests.

Author Contributions

- Alexandria B. Boehm conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Marlene K. Wolfe conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Bradley White conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Bridgette Hughes performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Dorothea Duong performed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

San Jose, Sacramento, and Oceanside wastewater treatment plants gave us permission to sample.

Data Availability

The following information was supplied regarding data availability:

The wastewater data is available at the Stanford Digital Repository: Boehm, A., Wolfe, M., White, B., Hughes, B., and Duong, D. (2023). Wastewater data for "Divergence of wastewater SARS-CoV-2 and reported laboratory-confirmed COVID-19 incident case data coincident with wide-spread availability of at-home COVID-19 antigen tests". Stanford Digital Repository. Available at https://purl.stanford.edu/xy132dg9314, https://doi.org/10.25740/xy132dg9314.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.15631#supplemental-information.

REFERENCES

Ahmed W, Bertsch PM, Angel N, Bibby K, Bivins A, Dierens L, Edson J, Ehret J, Gyawali P, Hamilton KA, Hosegood I, Hugenholtz P, Jiang G, Kitajima M, Sichani HT, Shi J, Shimko KM, Simpson SL, Smith WJM, Symonds EM, Thomas KV, Verhagen R, Zaugg J, Mueller JF. 2020. Detection of SARS-CoV-2 RNA in commercial passenger aircraft and cruise ship wastewater: a surveillance tool for assessing the presence of COVID-19 infected travellers. *Journal of Travel Medicine* **27**:taaa116 DOI 10.1093/jtm/taaa116.

Anjos D, Fiaccadori FS, do Servian PC, da Fonseca SG, Guilarde AO, Borges MASB, Franco FC, Ribeiro BM, Souza M. 2022. SARS-CoV-2 loads in urine, sera and stool specimens in association with clinical features of COVID-19 patients. *Journal of Clinical Virology Plus* **2(1)**:100059 DOI 10.1016/j.jcvp.2021.100059.

Bergman A, Sella Y, Agre P, Casadevall A. 2020. Oscillations in U.S. COVID-19 incidence and mortality data reflect diagnostic and reporting factors. *mSystems* **5(4)**:e00544-20 DOI 10.1128/mSystems.00544-20.

Boehm AB, Hughes B, Doung D, Chan-Herur V, Buchman A, Wolfe MK, White BJ. 2022a. Wastewater surveillance of human influenza, metapneumovirus, parainfluenza, respiratory syncytial virus (RSV), rhinovirus, and seasonal coronaviruses during the COVID-19 pandemic. *medRxiv*. ArXiv preprint. arXiv:2022.09.22.22280218 DOI 10.1101/2022.09.22.22280218.

Boehm AB, Hughes B, Wolfe MK, White BJ, Duong D, Chan-Herur V. 2022b. Regional replacement of SARS-CoV-2 variant Omicron BA.1 with BA.2 as observed through wastewater surveillance. *Environmental Science & Technology Letters* **9(6)**:575–580 DOI 10.1021/acs.estlett.2c00266.

Borchardt MA, Boehm AB, Salit M, Spencer SK, Wigginton KR, Noble RT. 2021. The environmental microbiology minimum information (EMMI) guidelines: QPCR and DPCR quality and reporting for environmental microbiology. *Environmental Science & Technology* **55(15)**:10210–10223 DOI 10.1021/acs.est.1c01767.

Burnet J-B, Cauchie H-M, Walczak C, Goeders N, Ogorzaly L. 2023. Persistence of endogenous RNA biomarkers of SARS-CoV-2 and PMMoV in raw wastewater: impact of temperature and implications for wastewater-based epidemiology. *Science of The Total Environment* **857**:159401 DOI 10.1016/j.scitotenv.2022.159401.

Daou M, Kannout H, Khalili M, Almarei M, Alhashami M, Alhalwachi Z, Alshamsi F, Tahseen Al Bataineh M, Azzam Kayasseh M, Al Khajeh A, Hasan SW, Tay GK, Feng SF, Ruta D, Yousef AF, Alsafar HS, on behalf of the UAE COVID-19 Collaborative Partnership. 2022. Analysis of SARS-CoV-2 viral loads in stool samples and nasopharyngeal swabs from COVID-19 patients in the United Arab Emirates. *PLOS ONE* **17(9)**:e0274961 DOI 10.1371/journal.pone.0274961.

D'Aoust PM, Mercier E, Montpetit D, Jia J-J, Alexandrov I, Neault N, Baig AT, Mayne J, Zhang X, Alain T, Langlois M-A, Servos MR, MacKenzie M, Figeys D, MacKenzie AE, Graber TE, Delatolla R. 2021. Quantitative analysis of SARS-CoV-2 RNA from wastewater solids in communities with low COVID-19 incidence and prevalence. *Water Research* **188**:116560 DOI 10.1016/j.watres.2020.116560.

Duvallet C, Wu F, McElroy KA, Imakaev M, Endo N, Xiao A, Zhang J, Floyd-O'Sullivan R, Powell MM, Mendola S, Wilson ST, Cruz F, Melman T, Sathyanarayana CL, Olesen SW, Erickson TB, Ghaeli N, Chai P, Alm EJ, Matus M. 2022. Nationwide trends in COVID-19 cases and SARS-CoV-2 RNA wastewater concentrations in the United States. *ACS ES&T Water* **2(11)**:1899–1909 DOI 10.1021/acsestwater.1c00434.

Feng S, Roguet A, McClary-Gutierrez JS, Newton RJ, Kloczko N, Meiman JG, McLellan SL. 2021. Evaluation of sampling, analysis, and normalization methods for SARS-CoV-2 concentrations in wastewater to assess COVID-19 burdens in wisconsin communities. *ACS ES & T Water* **1(8)**:1955–1965 DOI 10.1021/acsestwater.1c00160.

Gonzalez R, Curtis K, Bivins A, Bibby K, Weir MH, Yetka K, Thompson H, Keeling D, Mitchell J, Gonzalez D. 2020. COVID-19 surveillance in southeastern Virginia using wastewater-based epidemiology. *Water Research* **186**:116296 DOI 10.1016/j.watres.2020.116296.

Graham KE, Loeb SK, Wolfe MK, Catoe D, Sinnott-Armstrong N, Kim S, Yamahara KM, Sassoubre LM, Mendoza Grijalva LM, Roldan-Hernandez L, Langenfeld K, Wigginton KR, Boehm AB. 2021. SARS-CoV-2 RNA in wastewater settled solids is associated with COVID-19 cases in a large urban sewershed. *Environmental Science & Technology* **55(1)**:488–498 DOI 10.1021/acs.est.0c06191.

Hopkins L, Persse D, Caton K, Ensor K, Schneider R, McCall C, Stadler LB. 2023. Citywide wastewater SARS-CoV-2 levels strongly correlated with multiple disease surveillance indicators and outcomes over three COVID-19 waves. *Science of The Total Environment* **855**:158967 DOI 10.1016/j.scitotenv.2022.158967.

Hughes B, Duong D, White BJ, Wigginton KR, Chan EMG, Wolfe MK, Boehm AB. 2022. Respiratory syncytial virus (RSV) RNA in wastewater settled solids reflects RSV clinical positivity rates. *Environmental Science & Technology Letters* **9(2)**:173–178 DOI 10.1021/acs.estlett.1c00963.

Huisman JS, Scire J, Caduff L, Fernandez-Cassi X, Ganesanandamoorthy P, Kull A, Scheidegger A, Stachler E, Boehm AB, Hughes B, Knudson A, Topol A, Wigginton KR, Wolfe MK, Kohn T, Ort C, Stadler T, Julian TR. 2022. Wastewater-based estimation of the effective reproductive number of SARS-CoV-2. *Environmental Health Perspectives* **130(5)**:057011–1 DOI 10.1289/EHP10050.

Kim S, Kennedy LC, Wolfe MK, Criddle CS, Duong DH, Topol A, White BJ, Kantor RS, Nelson KL, Steele JA, Langlois K, Griffith JF, Zimmer-Faust AG, McLellan SL, Schussman MK, Ammerman M, Wigginton KR, Bakker KM, Boehm AB. 2022. SARS-CoV-2 RNA is enriched by orders of magnitude in primary settled solids relative to liquid wastewater at publicly owned treatment works. *Environmental Science: Water Research & Technology* **8**:757–770 DOI 10.1039/D1EW00826A.

McClary-Gutierrez JS, Aanderud ZT, Al-faliti M, Duvallet C, Gonzalez R, Guzman J, Holm RH, Jahne MA, Kantor RS, Katsivelis P, Kuhn KG, Langan LM, Mansfeldt C, McLellan SL, Mendoza Grijalva LM, Murnane KS, Naughton CC, Packman AI, Paraskevopoulos S, Radniecki TS, Roman FA, Shrestha A, Stadler LB, Steele JA, Swalla BM, Vikesl P, Wartell B, Wilusz CJ, Wong JCC, Boehm AB, Halden RU, Bibby K, Delgado Vela J. 2021a. Standardizing data reporting in the research community to enhance the utility of open data for SARS-CoV-2 wastewater surveillance. *Environmental Science: Water Research & Technology* **7**(**9**):1545–1551 DOI 10.1039/D1EW00235J.

McClary-Gutierrez JS, Mattioli MC, Marcenac P, Silverman AI, Boehm AB, Bibby K, Balliet M, de Los Reyes 3rd FL, Gerrity D, Griffith JF, Holden PA, Katehis D, Kester G, LaCross N, Lipp EK, Meiman J, Noble RT, Brossard D, McLellan SL. 2021b. SARS-CoV-2 wastewater surveillance for public health action. *Emerging Infectious Diseases* **27**(**9**):1–8 DOI 10.3201/eid2709.210753.

Mercier E, D'Aoust PM, Thakali O, Hegazy N, Jia J-J, Zhang Z, Eid W, Plaza-Diaz J, Kabir MP, Fang W, Cowan A, Stephenson SE, Pisharody L, MacKenzie AE, Graber TE, Wan S, Delatolla R. 2022. Municipal and neighbourhood level wastewater surveillance and subtyping of an influenza virus outbreak. *Scientific Reports* **12**(**1**):15777 DOI 10.1038/s41598-022-20076-z.

Peccia J, Zulli A, Brackney DE, Grubaugh ND, Kaplan EH, Casanovas-Massana A, Ko AI, Malik AA, Wang D, Wang M, Warren JL, Weinberger DM, Omer SB. 2020. SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics. *Nature Biotechnology* **38**:1164–1167 DOI 10.1038/s41587-020-0684-z.

Prasek SM, Pepper IL, Innes GK, Slinski S, Betancourt WQ, Foster AR, Yaglom HD, Porter WT, Engelthaler DM, Schmitz BW. 2023. Variant-specific SARS-CoV-2 shedding rates in wastewater. *Science of The Total Environment* **857**:159165 DOI 10.1016/j.scitotenv.2022.159165.

Puhach O, Meyer B, Eckerle I. 2022. SARS-CoV-2 viral load and shedding kinetics. *Nature Reviews Microbiology* **21**:147–161 DOI 10.1038/s41579-022-00822-w.

R Core Team. 2021. R: a language and environment for statistical computing. Version 4.0.5. Vienna: R Foundation for Statistical Computing. *Available at* https://www.r-project.org.

RStudio Team. 2021. RStudio: integrated development for R. Version 1.4.1106. Boston: RStudio, Inc. *Available at* http://www.rstudio.com/.

Radar B, Gertz A, Iuliano AD, Gilmer M, Wronski L, Astley CM, Sewalk K, Varrelman T, Cohen J, Parikh R, Reese HE, Reed C, Brownstein JS. 2022. Use of at-home COVID-19 tests —United States, August 23, 2021–March 12, 2022. *MMWR. Morbidity and Mortality Weekly Report* **71**:489–494 DOI 10.15585/mmwr.mm7113e1.

Roldan-Hernandez L, Graham KE, Duong D, Boehm AB. 2022. Persistence of endogenous SARS-CoV-2 and pepper mild mottle virus RNA in wastewater-settled solids. *ACS ES&T Water* **2**:1944–1952 DOI 10.1021/acsestwater.2c00003.

Simpson A, Topol A, White B, Wolfe MK, Wigginton K, Boehm AB. 2021. Effect of storage conditions on SARS-CoV-2 RNA quantification in wastewater solids. *PeerJ* **9**:e11933 DOI 10.7717/peerj.11933.

The dMIQE Group, Huggett JF. 2020. The digital MIQE guidelines update: minimum information for publication of quantitative digital pcr experiments for 2020. *Clinical Chemistry* **66(8)**:1012–1029 DOI 10.1093/clinchem/hvaa125.

The White House. Background press call on the rollout of 500 million free tests to American Homes. *Available at* https://www.whitehouse.gov/briefing-room/press-briefings/2022/01/14/background-press-call-on-the-rollout-of-500-million-free-tests-to-american-homes/ (accessed on 07 February 2023).

Topol A, Wolfe M, White B, Wigginton K, Boehm A. 2021a. High throughput pre-analytical processing of wastewater settled solids for SARS-CoV-2 RNA analyses. protocols.io.

Topol A, Wolfe M, White B, Wigginton K, Boehm A. 2021b. High throughput SARS-COV-2, PMMOV, and BCoV quantification in settled solids using digital RT-PCR. protocols.io.

Topol A, Wolfe M, Wigginton K, White B, Boehm A. 2021c. High throughput RNA extraction and PCR inhibitor removal of settled solids for wastewater surveillance of SARS-CoV-2 RNA. protocols.io.

Whitney ON, Kennedy LC, Fan VB, Hinkle A, Kantor R, Greenwald H, Crits-Christoph A, Al-Shayeb B, Chaplin M, Maurer AC, Tjian R, Nelson KL. 2021. Sewage, salt, silica, and SARS-CoV-2 (4S): an economical kit-free method for direct capture of SARS-CoV-2 RNA from wastewater. *Environmental Science & Technology* **55(8)**:4880–4888 DOI 10.1021/acs.est.0c08129.

Wolfe MK, Archana A, Catoe D, Coffman MM, Dorevich S, Graham KE, Kim S, Grijalva LM, Roldan-Hernandez L, Silverman AI, Sinnott-Armstrong N, Vugia DJ, Yu AT, Zambrana W, Wigginton KR, Boehm AB. 2021a. Scaling of SARS-CoV-2 RNA in settled solids from multiple wastewater treatment plants to compare incidence rates of laboratory-confirmed COVID-19 in their sewersheds. *Environmental Science & Technology* **8**:398–404 DOI 10.1021/acs.estlett.1c00184.

Wolfe MK, Duong D, Bakker KM, Ammerman M, Mortenson L, Hughes B, Arts P, Lauring AS, Fitzsimmons WJ, Bendall E, Hwang CE, Martin ET, White BJ, Boehm AB, Wigginton KR. 2022. Wastewater-based detection of two influenza outbreaks. *Environmental Science & Technology Letters* **9(8)**:687–692 DOI 10.1021/acs.estlett.2c00350.

Wolfe MK, Topol A, Knudson A, Simpson A, White B, Duc V, Yu A, Li L, Balliet M, Stoddard P, Han G, Wigginton KR, Boehm A. 2021b. High-frequency, high-throughput quantification of SARS-CoV-2 RNA in wastewater settled solids at eight publicly owned treatment works in Northern California shows strong association with COVID-19 incidence. *mSystems* **6(5)**:e00829-21 DOI 10.1128/mSystems.00829-21.

Wolfe MK, Yu AT, Duong D, Rane MS, Hughes B, Chan-Herur V, Donnelly M, Chai S, White BJ, Vugia DJ, Boehm AB. 2023. Use of wastewater for mpox outbreak

surveillance in California. *The New England Journal of Medicine* **388**:570–572 DOI 10.1056/NEJMc2213882.

Ye Y, Ellenberg RM, Graham KE, Wigginton KR. 2016. Survivability, partitioning and recovery of enveloped viruses in untreated municipal wastewater. *Environmental Science & Technology* **50(10)**:5077–5085 DOI 10.1021/acs.est.6b00876.

Yin Z, Voice T, Tarabara V, Xagoraraki I. 2018. Sorption of human adenovirus to wastewater solids. *Journal of Environmental Engineering* **144(11)**:06018008 DOI 10.1061/(ASCE)EE.1943-7870.0001463.