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# Wastewater surveillance of SARS-CoV-2 and influenza in preK-12 schools shows school, community, and citywide infections

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A R T I C L E I N F O

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A B S T R A C T

Wastewater surveillance is a passive and efficient way to monitor the spread of infectious diseases in large populations and high transmission areas such as preK-12 schools. Infections caused by respiratory viruses in school-aged children are likely underreported, particularly because many children may be asymptomatic or mildly symptomatic. Wastewater monitoring of SARS-CoV-2 has been studied extensively and primarily by sampling at centralized wastewater treatment plants, and there are limited studies on SARS-CoV-2 in preK-12 school wastewater. Similarly, wastewater detections of influenza have only been reported in wastewater treatment plant and university manhole samples. Here, we present the results of a 17-month wastewater monitoring program for SARS-CoV-2 (n = 2176 samples) and influenza A and B (n = 1217 samples) in 51 preK-12 schools. We show that school wastewater concentrations of SARS-CoV-2 RNA were strongly associated with COVID-19 cases in schools and community positivity rates, and that influenza detections in school wastewater were significantly associated with citywide influenza diagnosis rates. Results were communicated back to schools and local communities to enable mitigation strategies to stop the spread, and direct resources such as testing and vaccination clinics. This study demonstrates that school wastewater surveillance is reflective of local infections at several population levels and plays a crucial role in the detection and mitigation of outbreaks.

## 1. Introduction

Schools are a known source of respiratory viral outbreaks in communities (Wang et al., 2014). The large number of students and staff in close proximity to one another makes schools a high-risk area and enables the transmission of infectious diseases (Murray et al., 2021). Consequently, the onset of school sessions are correlated with acute respiratory infections, especially among students ages 5–17 (Temte et al., 2019). This correlation is also seen among other age groups, suggesting secondary transmission within student households (Temte et al., 2019). COVID-19 outbreaks in schools created increases in secondary transmission and, in turn, school closures that may have led to negative effects on student learning and increased strain on families (Falk et al., 2021; National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases, 2020; Tomasik et al., 2021). Influenza outbreaks are also common in schools, with preK-12 children facing the highest age-based influenza attack rate of approximately 30% (Heymann et al., 2009; Wang et al., 2014). School transmission of influenza and SARS-CoV-2 not only affects school-aged children and their families, but is also associated with community-wide outbreaks (Chao et al., 2010; Chernozhukov et al., 2021). Rapid detection of these viruses in schools can provide the time needed to initiate measures to mitigate transmission. Precautions such as contact tracing and isolation of close contacts are associated with reductions in

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transmission of SARS-CoV-2 and influenza, but require ample time for implementation (Sinha et al., 2021; Wang et al., 2014). The ability to quickly recognize the onset of potential outbreaks in schools is crucial in preventing further spread into the community.

Clinical testing has traditionally been used to monitor respiratory viral outbreaks such as influenza and SARS-CoV-2. However, compiling testing data from various health care facilities such as clinics, hospitals, and emergency rooms to obtain an accurate incidence estimate is time-consuming and can allow the virus more time to proliferate throughout the community (Lu et al., 2018). Additionally, clinical surveillance metrics only capture a fraction of infections. This issue is exacerbated by infected individuals with asymptomatic or mild cases that do not seek medical care (under-ascertainment) as well as underreporting due to non-reportable at-home tests and atypical presentations that lead to misdiagnoses (Bivins et al., 2020; McCarthy et al., 2020; Rader et al., 2022; Wolfe et al., 2022). Under-ascertainment is a relevant problem in schools because children with COVID-19 may be more likely than adults to be asymptomatic or to have a mild case, making it less likely that their infection will be detected using clinical methods (Falk et al., 2021; Ludvigsson, 2020; National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases, 2020).

In contrast, wastewater surveillance is a passive, efficient, and relatively fast method that overcomes many of the challenges faced by clinical diagnostic testing. Wastewater information is not biased by differences in access to testing resources or by health-seeking behavior (Olesen et al., 2021). Wastewater surveillance's pooled sample strategy allows for the simultaneous and rapid sampling of large groups of people (Bibby et al., 2021; D'Aoust et al., 2021; Larsen and Wigginton, 2020; Sutton et al., 2022). Wastewater viral concentrations for both influenza and SARS-CoV-2 have been shown to correlate with community-wide clinical diagnostic trends (Al-Duroobi et al., 2021; Bethel et al., 2021; Kaya et al., 2022; Layton et al., 2022; Mercier et al., 2022; Peccia et al., 2020). Using wastewater surveillance can enable the detection of infection outbreaks where clinical data is not able to rapidly detect (Mao et al., 2020; Wu et al., 2022).

In addition to monitoring large populations by sampling at centralized wastewater treatment plants, wastewater monitoring has also been applied to monitor individual facilities and buildings. Previous studies detected SARS-CoV-2 in the wastewater of facilities such as nursing homes, universities, and hospitals (Gibas et al., 2021; Scott et al., 2021; Spurbeck et al., 2021). In a study monitoring nursing home wastewater, a positive wastewater detection of SARS-CoV-2 occurred when only one out of sixty residents were positive for COVID-19 (Spurbeck et al., 2021). Additionally, a university-based study detected asymptomatic cases that were not otherwise identified by the campus monitoring program (Gibas et al., 2021). School wastewater surveillance of SARS-CoV-2 has shown a correlation between wastewater viral concentrations of SARS-CoV-2 in schools and community cases of COVID-19 (Castro-Gutierrez et al., 2022). Therefore, school wastewater monitoring could serve as building-level surveillance with the ability to identify infections within the facility and reflect the infection burden of the larger community that the school serves (Crowe et al., 2021).

While there are a limited number of studies on wastewater monitoring of SARS-CoV-2 in preK-12 school settings (Castro-Gutierrez et al., 2022; Crowe et al., 2021; Fielding-Miller et al., 2021), no previous studies have expanded school monitoring to include other respiratory viruses such as influenza. Influenza and respiratory syncytial virus (RSV) are also shed in stool and are detectable in wastewater (Akbari et al., 2017; Al Khatib et al., 2021; Chan et al., 2011; Heijnen and Medema, 2011; Hirose et al., 2016; Hughes et al., 2022; To et al., 2010; von Linstow et al., 2006). Only two studies to date have applied wastewater surveillance for influenza and they demonstrated its ability to detect community outbreaks by monitoring wastewater treatment plants or manholes that serve >10,000 people (Mercier et al., 2022; Wolfe et al., 2022).

Here, we expand on wastewater disease surveillance for respiratory viruses in preK-12 school settings. We quantified SARS-CoV-2 and influenza A in the wastewater of 51 preK-12 schools across Houston over a 17-month period. We compared wastewater detections to available diagnostic PCR testing data performed at the schools, as well as community (zip code level) positivity rates for COVID-19, and citywide syndromic surveillance data on influenza. We show that wastewater monitoring is an effective pooled-sample monitoring strategy that reflects the prevalence of infection in the school as well as the surrounding community. We describe how the information was communicated to the schools and used by the Houston Health Department for targeted vaccination campaigns.

## 2. Methods

### 2.1. Selection of schools

Samples were obtained from 51 preK-12 schools across Houston, Texas from December 7th, 2020 to May 9th, 2022. The schools included 39 elementary schools and early childhood centers, 5 middle schools, 5 high schools, and 2 combined grade-level schools. Enrollment information for each school is provided in Table S1. These schools were categorized as "high risk" schools based on zip code level COVID positivity rates, vaccination rates, and COVID-19 Community Vulnerability Index (CCVI). The location of the schools within Houston is shown in Fig. 1 and they were clustered in three groups: North, Southwest, and Southeast based on driving routes for sample pickup.

### 2.2. Sample collection

Wastewater samples were collected once a week using refrigerated, time-weighted composite autosamplers located in or adjacent to the school manholes. The composite samplers drew from manholes that only received wastewater from the selected schools with no contributions from other sources. On sample collection days, the composite samplers were programmed to collect 250 mL every 15 min between the hours of 6am and 12pm. This ensured that samples were fresh and not stored in an autosampler for more than a few hours. Once a week, team members from the Houston Health Department collected and delivered the school wastewater samples to Rice University for processing and analysis. Samples were kept on ice during transport and stored at 4 °C in the laboratory. The wastewater samples were aliquoted into 50 ml centrifuge tubes and were concentrated within 24 h of collection. The 17-month sampling campaign for SARS-CoV-2 spanned from December 7th, 2020 to May 9th, 2022 and we added influenza A and B quantification on September 27th, 2021, for a total of 7 months of influenza analysis. Over the entire study period, 2176 samples were analyzed for SARS-CoV-2 and 1217 samples were analyzed for influenza.

### 2.3. Concentration, extraction, and quantification of SARS-CoV-2 and influenza A and B

Wastewater samples were concentrated using an HA filtration and bead beating method as previously described (LaTurner et al., 2021; Lou et al., 2022). Between December 7th, 2020 and January 25, 2021, RNA extraction was performed using the Maxwell 48 RSC automated platform (AS8500, Promega) and the modified protocol for the Maxwell RSC PureFood GMO and Authentication Kit (AS1600, Promega). Beginning February 1st, 2021 and for the remainder of the study period, the Chemagic™ Prime Viral DNA/RNA 300 Kit H96 (Chemagic, CMG-1433, PerkinElmer) was used for RNA extraction as described by Lou et al. (2022). We performed a head-to-head comparison of SARS-CoV-2 concentrations using Maxwell and Chemagic extraction methods to determine an adjustment factor for converting Maxwell concentration levels to Chemagic levels (detailed in Supplemental Material Sections S1.4 and S2.3).

The concentrations of SARS-CoV-2 N1 and N2 genes and influenza A

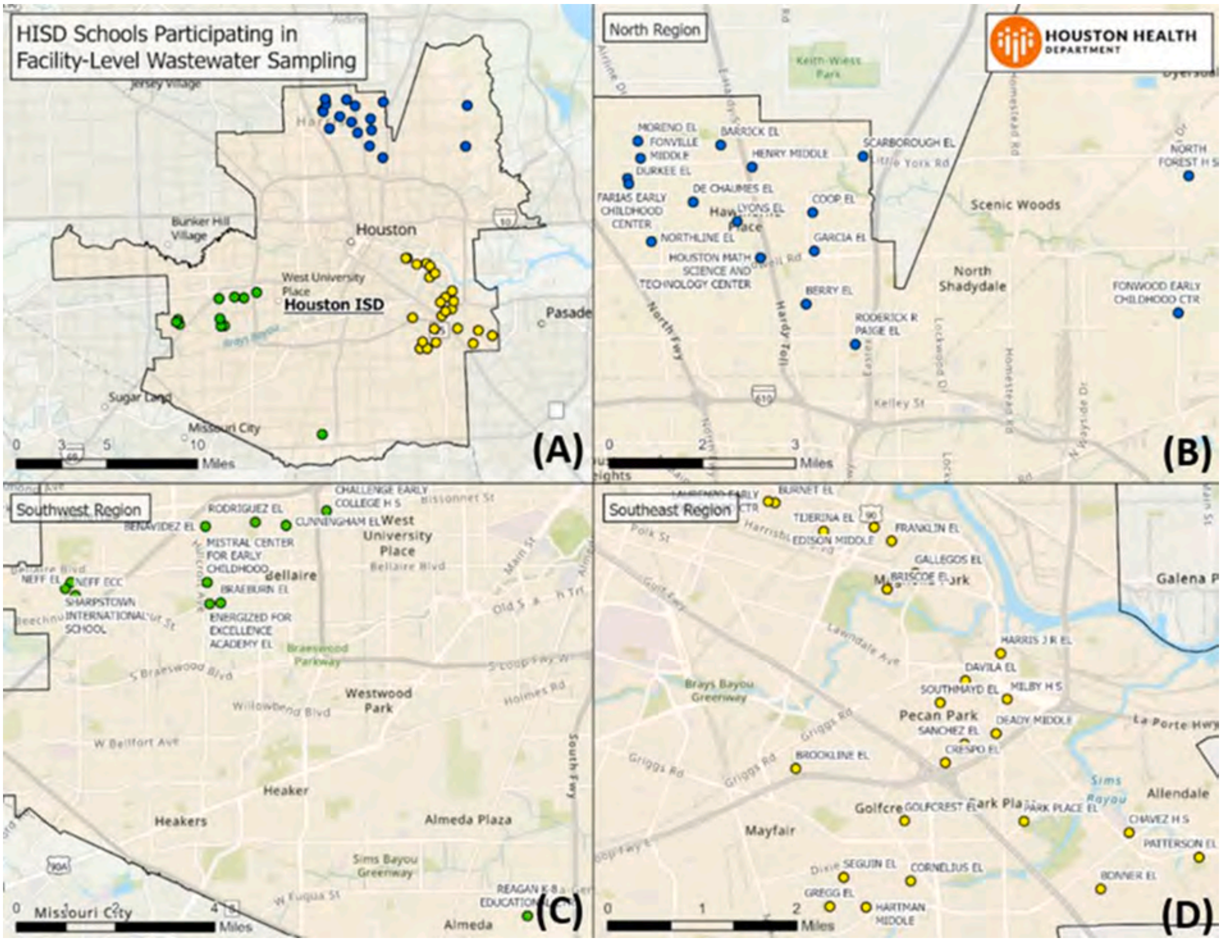


**Fig. 1.** Locations of the 51 schools monitored for SARS-CoV-2 and influenza during this study (A). Zoomed-in map of the schools located in the north (B), southwest (C), and southeast (D) regions of Houston, Texas.

and B were quantified using a one-step RT-ddPCR assay. RT-ddPCR was completed using the QX200 AutoDG Droplet Digital PCR System (Bio-Rad) and a C1000 Thermal Cycler (Bio-Rad). SARS-CoV-2 and influenza quantifications require identical thermal cycler conditions, so RT-ddPCR was often performed for SARS-CoV-2 and influenza simultaneously. Additional details on Maxwell RNA extraction, primers and probes, assay setup, and thermal cycling conditions are also available in the Supplemental Material. Detailed protocols for concentration factor calculations, RT-ddPCR, thermal cycler conditions, limit of detection (LOD), RT-ddPCR reaction compositions, and quality control measures are as described previously (Lou et al., 2022).

### 2.4. Classification of results as positive, negative, or inconclusive

Each wastewater sample was processed as two concentration replicates, and we classified the sample as either positive, negative, or inconclusive for SARS-CoV-2 and influenza A and B using the following logic: For SARS-CoV-2 there were 4 SARS-CoV-2 measurements for each sample (N1 and N2 for each concentration replicate). When $>3$ measurements were above LOD, samples were called positive for SARS-CoV-2. When no measurements were greater than the LOD, samples were called negative for SARS-CoV-2. In cases with only 1 or 2 measurements greater than the LOD, the samples were called inconclusive. For influenza, results were based on a single target measurement for 2 concentration replicates for each sample for both influenza A and B. When the 2 measurements for influenza A were greater than the LOD, the samples were determined to be positive for influenza A. When neither measurement was greater than the LOD, the samples were identified as negative for influenza A. In cases with 1 measurement greater than the LOD and 1 measurement below, the samples were deemed inconclusive. This logic was also applied for influenza B analysis.

### 2.5. Clinical testing of individuals for SARS-CoV-2 at schools

The Houston Health Department carried out a SARS-CoV-2 PCR testing program for consenting students and staff at 259 preK-12 schools, 46 of which were part of this study's school wastewater monitoring program. Consenting participants ($n = 3213$ participants) were offered free PCR testing weekly but could choose to opt out. Up to 12 weeks of diagnostic testing data was obtained from the schools, and an average of 9.3 weeks of data per school was gathered across all 46 sites. Over the course of the testing program, the weekly positivity rates (positive tests divided by total tests performed) for each school were provided by the Houston Health Department. The specimen collection dates were used to aggregate the clinical testing results by school week (Monday-Friday). The PCR diagnostic testing data for each school was compared to the measured concentrations of SARS-CoV-2 in its wastewater samples collected the same week. All procedures involving human participants were approved by the Institutional Review Board (IRB-FY2020-300-Modeling of Covid19 lab test results in space and times) and were performed in accordance with applicable guidelines.

### 2.6. Aggregated zip code school wastewater and PCR data

We used imputation to replace wastewater measurements below the limit of detection. The imputed values were set as the maximum between the actual observed value and half the LOD. Here, the LOD was assumed to be 2556 copies/L, which corresponds to the approximate concentration of 3 positive droplets per reaction well. The wastewater concentrations of SARS-CoV-2 of schools within the same zip code were aggregated by averaging the N1 and N2 measurements of each school after imputation and taking the average of those measurements across schools in the same zip code.

COVID-19 diagnostic (PCR) testing results were extracted from the Houston Electronic Disease Surveillance System (HEDSS) and included persons located within one of the 105 Houston-area zip codes. Test records were deduplicated by person by specimen date such that if a person was tested multiple times in a single day, they were counted only once for that day, and if a person tested across multiple days, they were counted once per each of those days. Results were aggregated by specimen date and the daily positivity rate was calculated as the number of persons with a positive test divided by the number of persons PCR tested. The daily positivity rates of all schools in the same zip code collected within the same week were averaged to obtain weekly zip code positivity rates.

### 2.7. Citywide influenza rates

Syndromic surveillance was used to determine a citywide percentage of discharge diagnosis influenza from reporting healthcare facilities, including hospitals, emergency departments, community and school-based clinics and surgery centers. To obtain the percentage of visits with a discharge diagnosis of influenza, the Houston Health Department's Electronic Syndromic Surveillance System for the Early Notification of Community-Based Epidemics (ESSENCE) was searched for the number of visits with an influenza-related code in the discharge diagnosis, regardless of influenza strain. This number was then divided by the number of total visits in ESSENCE and multiplied by 100. All visits were filtered by zip code and/or county to only include patients from the Houston/Harris County area. We chose to compare discharge diagnosis influenza rates as opposed to influenza-like illness (ILIs) rates because of the abundance of similar respiratory viruses, such as SARS-CoV-2 and RSV, that were likely contributing to ILI rates. Counts were aggregated by week (Sunday to Saturday).

### 2.8. Statistical analysis

We used R for all data analyses. Our code for these analyses is available at https://github.com/hou-wastewater-epi-org/school-wastewater-surveillance. Since the SARS-CoV-2 positivity rates at the schools were all very low or zero, we transformed the positivity rate measurement into a binary (0 for zero positivity rate, 1 for positive positivity rate) variable. We used this binary variable as our dependent variable in a logistic regression model with the log wastewater concentration on the associated date as the independent variable. Using logistic regression, we estimated the effect of the wastewater concentration on the probability of a nonzero number of positive cases that week.

We used linear regression to model the effect of wastewater concentration on the positivity rate at the zip code level for SARS-CoV-2. We explored various regression models including addition of random effects components for each replicate but ultimately chose the simple linear model for its combination of good model fit and interpretability. For influenza, we used linear regression to model the relationship between the citywide rate of influenza and the school influenza wastewater detection rates. We used the proportion of the 51 schools with a positive detection of influenza in a given week as our independent variable with the citywide rate of visits diagnosed with influenza as the dependent variable.

## 3. Results

### 3.1. School wastewater concentrations of SARS-CoV-2 RNA were representative of COVID-19 cases in schools

We collected 2176 school wastewater samples over a 17-month period. We assayed 2176 samples for SARS-CoV-2 and 1217 samples for influenza A and B. Of the 2176 samples assayed for SARS-CoV-2, 22.3% (486) were positive, 9.1% (197) were inconclusive, and 68.6% (1493) were below the limit of detection. Of the positive samples, wastewater concentrations averaged 141,248 (10th percentile: 3293; 90th percentile: 226,617) copies/L. There was no relationship between wastewater concentration and school enrollment numbers (Fig. 2B). Fig. 3 shows the distribution of weekly SARS-CoV-2 detections in 51 preK-12 schools over the study period.

PCR diagnostic testing was completed at selected sites to examine the relationship between wastewater concentrations of SARS-CoV-2 in schools and school COVID-19 cases. Only 13 weeks of diagnostic PCR testing data from the schools was collected and the majority of the school PCR positivity rates were zero (71%). Further, the PCR testing program was not mandatory, and thus the testing results were not comprehensive. Despite these limitations, we observed a significant positive relationship between school wastewater concentrations for SARS-CoV-2 and the probability of a non-zero SARS-CoV-2 positivity rate at the school (Fig. 4; $\beta_1 \approx 1.14$; $p < 0.0001$). In other words, schools with a 10 times higher SARS-CoV-2 wastewater concentration have an estimated 3.1 times higher odds of a positive test result, based on the results of the logistic regression model.

### 3.2. School wastewater concentrations of SARS-CoV-2 RNA were representative of community positivity rates for SARS-CoV-2

We compared school wastewater concentrations of SARS-CoV-2 to the positivity rate of the zip code that they are located in. This was performed by averaging the wastewater SARS-CoV-2 concentrations of the schools located in the same zip code, and using linear regression to model the effect of the averaged school wastewater concentration on the zip code positivity rate at the zip code level (Fig. 5). The wastewater concentrations were significant predictors of zip code positivity rates (Fig. 5; $\beta_1 \approx 0.05$; $p < 0.0001$). The regression model estimates an expected increase of 0.05 in the zip code positivity rate for every 1 unit increase in log SARS-CoV-2 copies per liter. This positive association indicates that school wastewater concentrations of SARS-CoV-2 RNA were representative of community positivity rates of SARS-CoV-2.

### 3.3. Influenza A detections in school wastewater were representative of citywide influenza rates

Detections of influenza A RNA in school wastewater were compared to the citywide rates of discharge diagnosed influenza. Of the 1217 wastewater samples assayed for influenza A and B, none were positive for influenza B. For influenza A, 2.6% (32) of samples were positive, 1.6% (19) were inconclusive, and 95.8% (1166) were below the limit of detection. Fig. 6 shows detections of influenza A in school wastewater over the course of the study period. Of the positive samples for influenza A, wastewater concentrations averaged 31,570 (10th percentile: 1929; 90th percentile: 58,300) copies/L. The wastewater concentrations of influenza A were generally lower than the positive SARS-CoV-2 concentrations (Fig. S1). This pattern remained consistent when concentrations of SARS-CoV–2 and influenza A were compared across grade levels (Fig. S2).

Citywide rates of visits with a discharge diagnosis of influenza over the study period are shown in Fig. 7A. A linear regression model revealed a significant positive relationship between the proportion of schools with a positive wastewater detection and the citywide rate of visits with a discharge diagnosis of influenza (Fig. 7B; $\beta_1 \approx 0.128$; $p <$

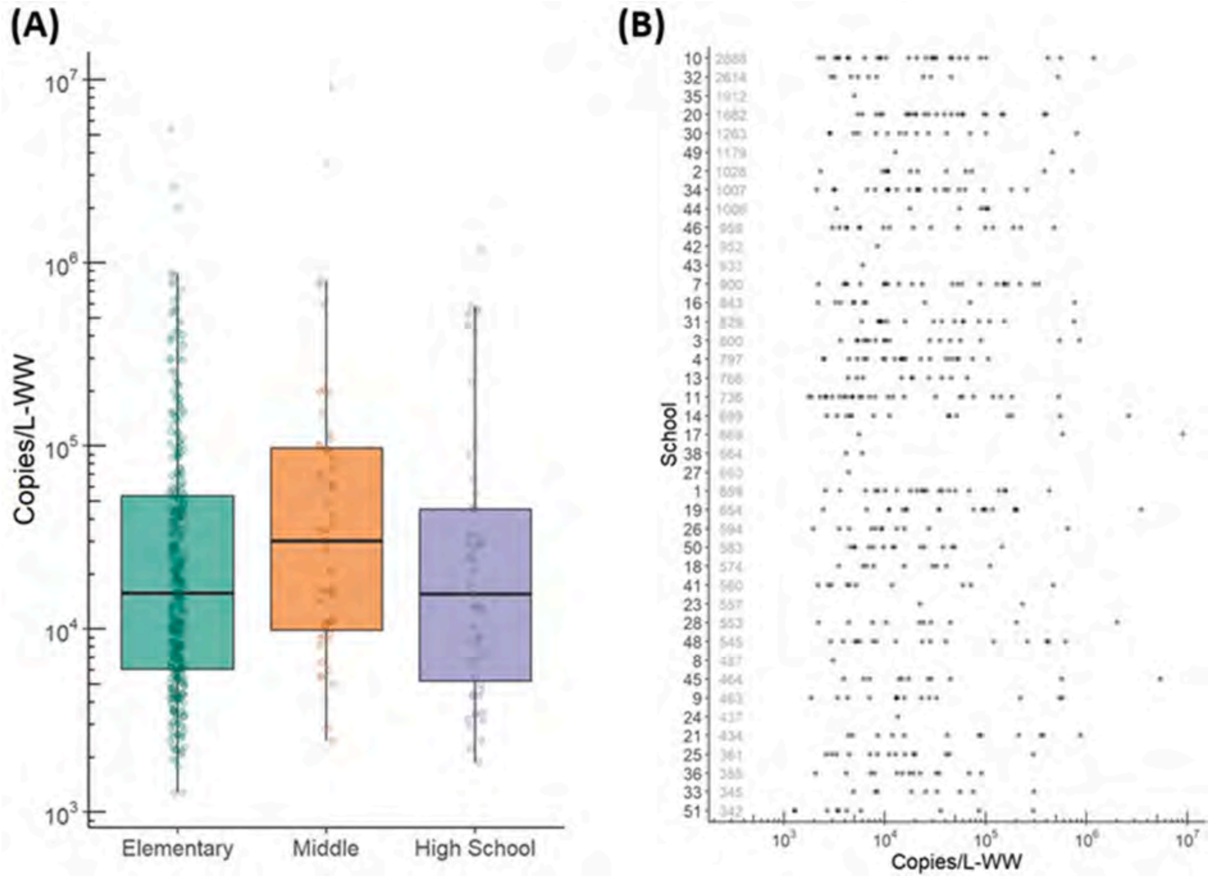


**Fig. 2.** (A) Positive SARS-CoV-2 wastewater concentrations by grade level in copies/L. Each point represents the averaged N1 and N2 concentrations for that sample. (B) Positive SARS-CoV-2 wastewater concentrations by school in copies/L. Numbers to the right of the school numbers represent each school's student enrollment as of October 2019.

0.0001). These results indicate that influenza detections in school wastewater were representative of citywide influenza diagnosis rates.

## 4. Discussion

Our findings indicate that wastewater can be used to passively monitor respiratory viruses in schools without requiring participants to engage in health-seeking behaviors, unlike other passive surveillance systems based on clinical testing. We show that wastewater detections of SARS-CoV-2 were consistent with reported cases within the school and community positivity rates, and influenza detections were consistent with citywide influenza diagnosis rates. Wastewater monitoring can offer valuable insight into school outbreaks and enable timely mitigation strategies that decrease the likelihood of secondary transmission into the students' families and communities. Targeted mitigation strategies include identifying positive individuals through diagnostic testing, vaccinations, and masking in schools and local communities. Wastewater surveillance of SARS-CoV-2 and influenza is especially crucial in preK-12 schools as many infected children have asymptomatic or mild cases and do not seek medical attention or diagnostic testing, likely leading to an underreporting of infections (Zimmermann and Curtis, 2021). The ability to identify these cases is crucial in preventing the spread of outbreaks as preK-12 schools are often areas of high transmission for respiratory viruses and other pathogens (Ghani et al., 2010; Glatman-Freedman et al., 2012; Murray et al., 2021). This wastewater monitoring process is flexible and can be modified to detect various pathogens that are shed in stool and urine with relative ease. School wastewater monitoring's ability to passively reflect community infections is critical in detecting and preventing the spread of future outbreaks.

Here, we expand on the findings of the limited number of studies that have investigated SARS-CoV-2 in preK-12 school wastewater by performing a 17-month school wastewater surveillance program that spans three different COVID-19 waves in Houston (driven by the Alpha, Delta, and Omicron variants) at 51 preK-12 schools, resulting in 2176 samples. Crowe et al. (2021) performed a 5-week pilot investigation of three K-12 schools that supplemented saliva-based PCR testing with environmental samples from wastewater, surfaces, and indoor air (Crowe et al., 2021). SARS-CoV-2 was detected in school wastewater for 12 of the 14 (85.7%) individual school weeks that also experienced positive PCR test results. This generally consistent relationship between SARS-CoV-2 wastewater detections and clinical diagnostic testing is similar to the association found in our study. Our study found a significant, positive relationship between school wastewater SARS-CoV-2 concentration and the probability of a school's nonzero positivity rate based on nasal-based PCR testing (Fig. 4; $\beta_1 \approx 1.14$; $p < 0.0001$). Fielding-Miller et al. (2021) identified associations between positive school wastewater detections of SARS-CoV-2 and positive school cases of COVID-19 (Fielding-Miller et al., 2021). They sampled nine K-12 schools over the course of 12 weeks, totaling 447 samples. Weekly diagnostic testing was offered to students and staff, and 67% of positive cases identified by this testing were associated with a positive wastewater detection of SARS-CoV-2. Castro-Gutierrez et al. (2022) analyzed 296 samples for SARS-CoV-2 over a 9-week wastewater monitoring study of 16 schools (Castro-Gutierrez et al., 2022). They detected SARS-CoV-2 in 47.3% of wastewater samples. They reported a significant positive correlation between school wastewater weekly positivity rates and community COVID-19 cases (Pearson's correlation coefficient = 0.33; $p < 0.01$)

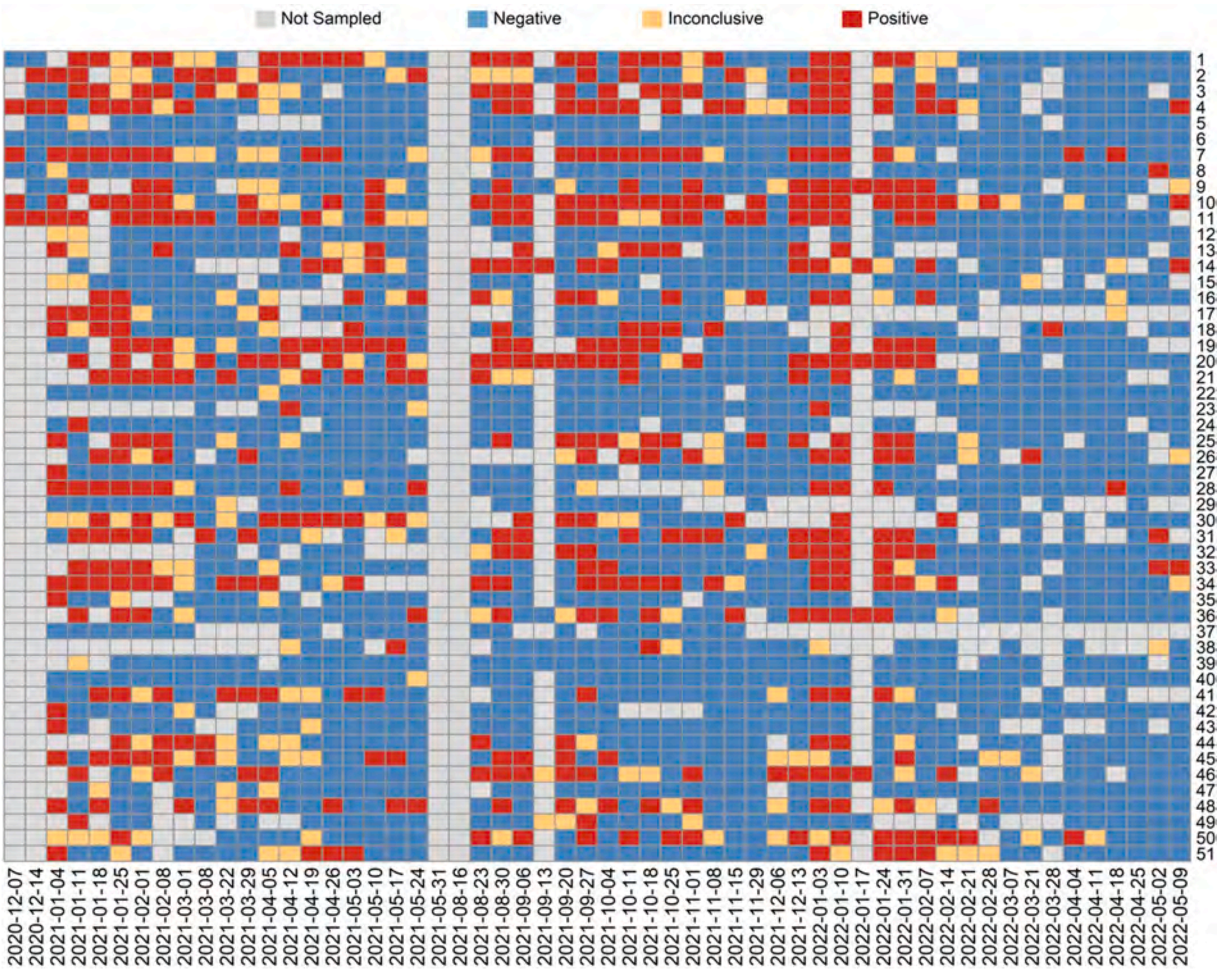


**Fig. 3.** Heat map of SARS-CoV-2 wastewater detections for 51 schools (n = 2176 samples) collected between December 7, 2020 and May 9th, 2022. Red indicates a positive detection of SARS-CoV-2, blue indicates a sample below the limit of detection, yellow indicates an inconclusive result, and gray denotes schools not sampled that week.

when school wastewater rates led community cases by two weeks.

We also extended the wastewater surveillance of influenza into preK-12 schools for the first time. We monitored influenza A and B concentrations in the wastewater of 51 preK-12 schools over the course of 7 months (n = 1217 samples). While no other studies to date have implemented influenza wastewater surveillance in preK-12 schools, a limited number of previous studies have demonstrated the ability to detect community influenza outbreaks using wastewater (Mercier et al., 2022; Wolfe et al., 2022). One study investigated two separate influenza A outbreaks in universities (University of Michigan and Stanford University) by comparing the universities' influenza A wastewater concentrations to clinical data gathered from university health services and student athlete surveillance, respectively (Wolfe et al., 2022). The concentrations of influenza A in the University of Michigan's wastewater ranged from nondetect to $2.63\times10^4$ copies/g with a median of $1.03\times10^4$ copies/g. Stanford University wastewater concentrations of influenza ranged from nondetect to $1.27\times10^5$ copies/g with nondetect as the median. The average influenza A concentration in our study on a per mass basis ($3.16\times10^1$ copies/mL, where mass of 1 mL = 1 g) was comparable to the levels reported in their study. Even though wastewater concentrations are a function of the number of infected individuals in the community, we observed concentration levels at similar orders of magnitude to results from previous studies. Wolfe et al.'s results indicated that wastewater concentrations of influenza A were strongly associated with the 5-day smoothed incidence rate of influenza A at both universities (University of Michigan: $\tau = 0.58$; $p < 10^{-7}$; n = 45 and Stanford University: $\tau = 0.67$; $p < 10^{-14}$; n = 43). Similarly, our study examined the relationship between the proportion of schools with a positive influenza wastewater detection and the citywide percentage of visits with discharge diagnosed influenza (Fig. 7B; $\beta_1 \approx 0.128$; $p < 0.0001$). Mercier et al., 2022 also compared influenza A and B concentrations in neighborhood wastewater and citywide primary clarified sludge with PCR test rates (Mercier et al., 2022). They reported a strong, positive association when the citywide primary sludge influenza concentrations led the PCR rates by 17-days ($r = 0.97$, $p < 0.05$, n = 14). They also detected influenza A in 60% (n = 79) of all samples- a much higher detection rate than observed in this study (2.6%). This difference in detection rates could be due to differences in community influenza rates, influenza RNA concentrations in primary clarified sludge versus raw wastewater, and/or sampling at centralized wastewater treatment plant versus at manholes that capture wastewater from individual schools. Additionally, these studies measured influenza A in solid fractions as opposed to liquid fractions, providing evidence that influenza A partitions to solids (Mercier et al., 2022; Wolfe et al., 2022). This could lead to variability in detection rates as inhibition may differentially impact the recoveries of influenza from the liquid and solid fractions. Consistent across all of these studies and our work is that wastewater detections of influenza reflected clinical infection rates. Future research should investigate factors that impact influenza recovery from both the liquid and solid fractions of wastewater samples as well as optimization of sampling schemes for public health interventions.

The findings of our wastewater monitoring program were reported

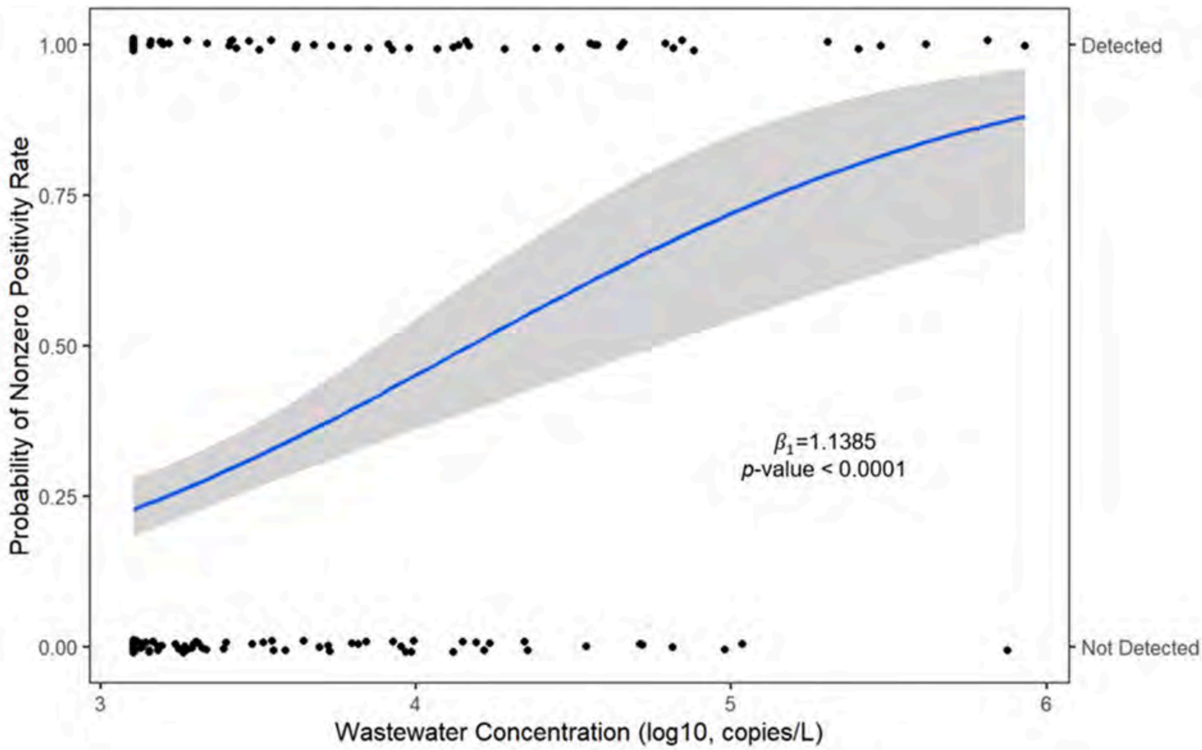


**Fig. 4.** Logistic regression of the probability of a non-zero PCR positivity rate at a school estimated using the school wastewater concentration of SARS-CoV-2 (log scale) in log10 copies/L. Gray shaded area represents the 95% confidence interval of the line.

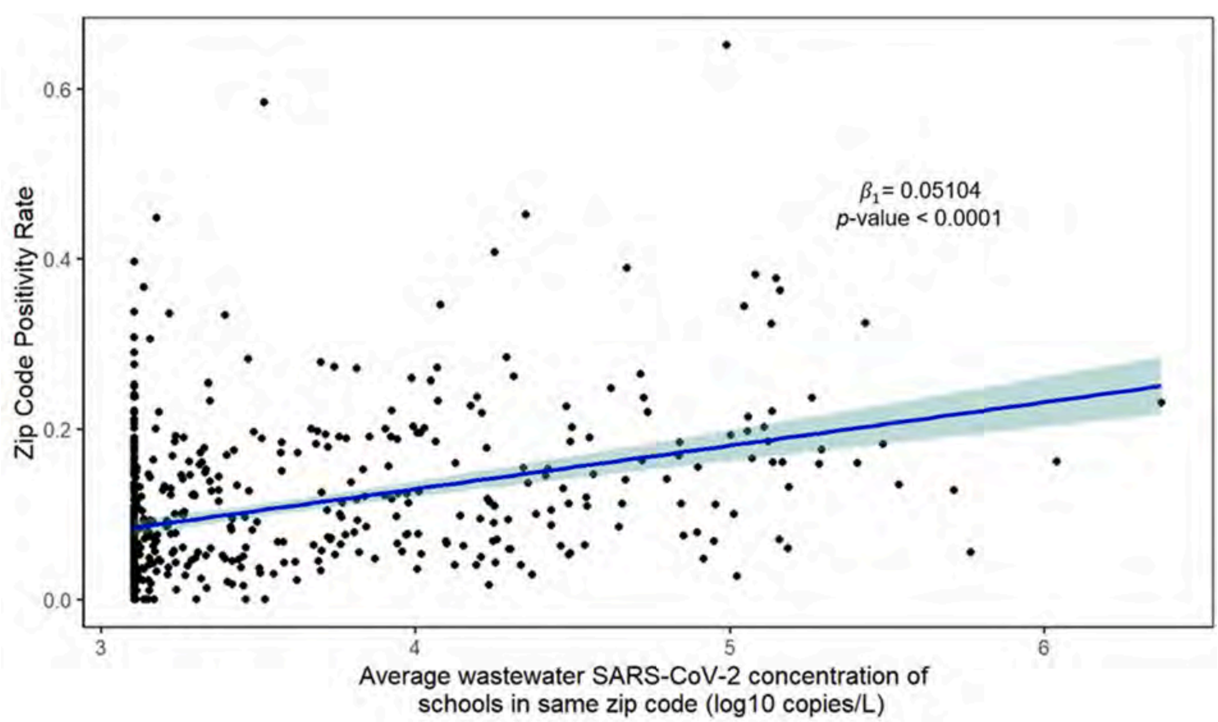


**Fig. 5.** Linear regression of zip code positivity rate estimated using the average wastewater SARS-CoV-2 concentration for schools located in the same zip code in log10 copies/L. Green shaded area represents 95% confidence interval of the line.

back to the schools and used by the Houston Health Department to target interventions in the local communities. Every week, the SARS-CoV-2 and influenza school wastewater results were communicated to each school's point of contact (typically the nurse and/or principal). This line of communication assisted with identifying positive COVID-19 and influenza cases and enabled the timely implementation of mitigation strategies. In the case of a positive school wastewater detection of SARS-CoV-2, the Houston Health Department assessed the school's COVID-19 history and its current COVID-19 status. They prioritized any cases in need of contact tracing that had not yet been reached. In the case of a

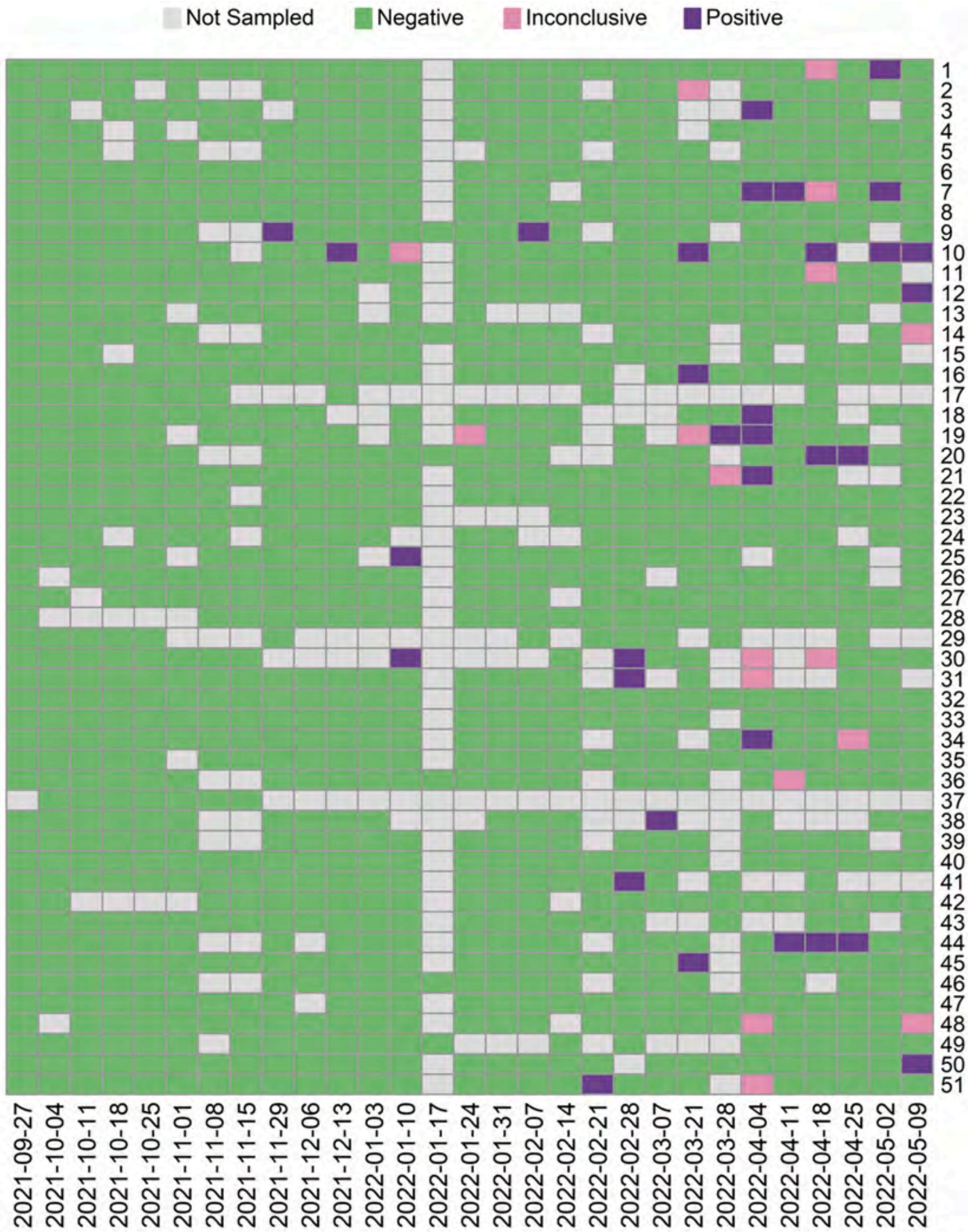


**Fig. 6.** Heat map of influenza A wastewater detections for 51 schools (n= 1217 samples) collected between September 27, 2021 and May 9th, 2022. Purple indicates a positive detection of influenza A or B, green indicates a sample below the limit of detection, pink indicates an inconclusive result, and gray denotes schools not sampled that week.

COVID-19 outbreak in a school, the health department planned an intervention and scheduled an enhanced site visit within 24–72 h. Houston Health Department maintained active outreach with the school's point of contact to ensure daily reporting and distributed isolation and quarantine letters to cases and contacts as needed. The Houston Health Department (HHD) also used data from the wastewater monitoring program to create the HHD Weekly Influenza Reports. These reports were shared internally with Houston Health Department leadership, the Texas Department of State Health Services (DSHS), local and nearby counties and jurisdictions, and Rice University leadership. Additionally, the Houston Health Department published the reports on their website for public viewing.

Weekly wastewater monitoring results were also shared with the community through SARS-CoV-2 zip code fact sheets and the City of Houston SARS-CoV-2 Wastewater Monitoring Dashboard (covidwwtp.spatialstudieslab.org). The fact sheets provided the SARS-CoV-2 wastewater detection status for every school in the given zip code, as well as information on community COVID-19 vaccination rates. These fact sheets were part of a Houston Health Department initiative to use schools as a springboard into the community to encourage vaccinations. The health department hosted free influenza and COVID-19 vaccine clinics for communities based on the results of the school wastewater monitoring program.

While there are many advantages to school wastewater monitoring, there are still limitations. Wastewater is a complex matrix that is affected by environmental factors that are not always identified, leading

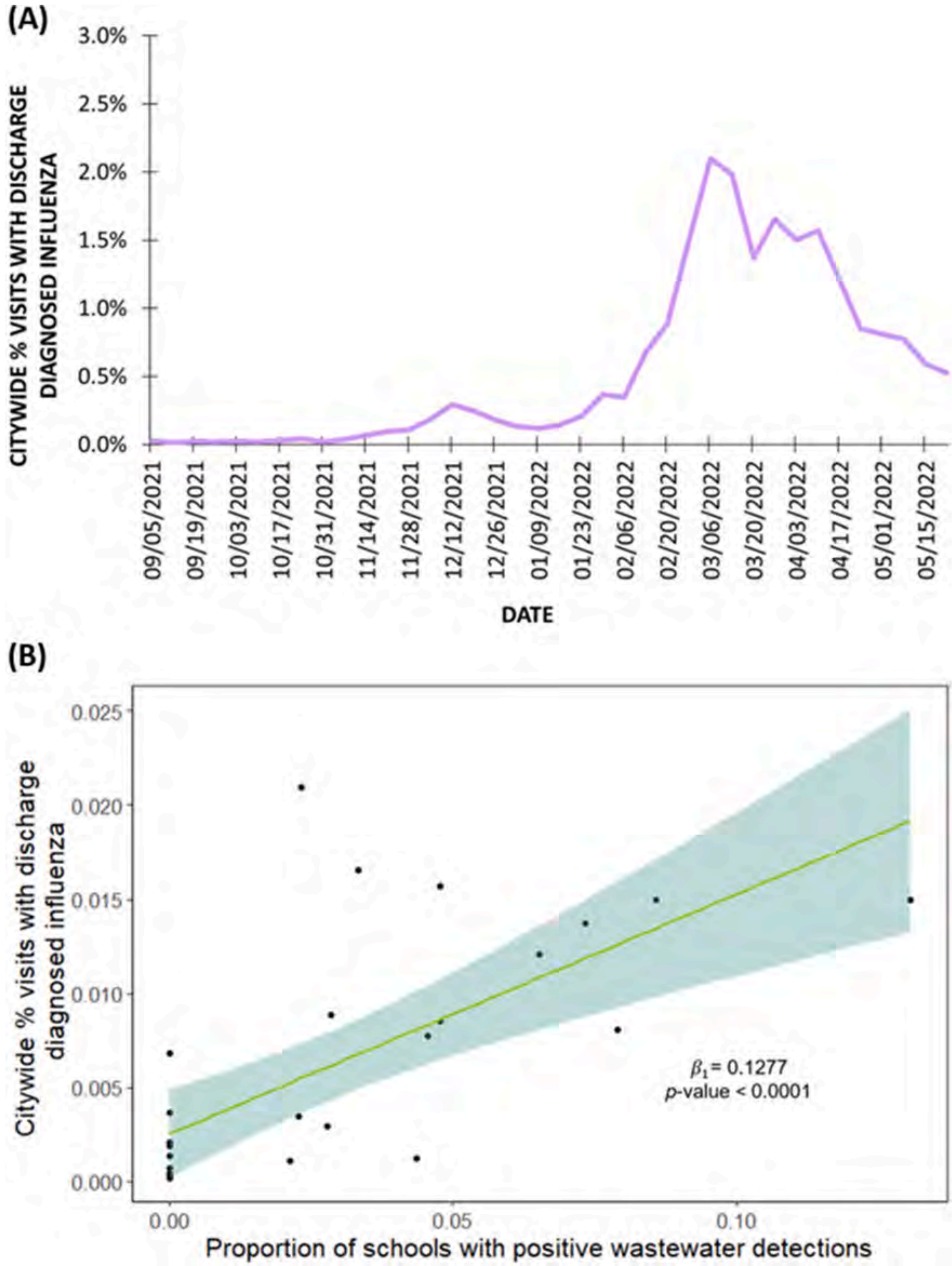


**Fig. 7.** (A) Citywide percentage of visits with a discharge diagnosis of influenza by week from September 5, 2021 to May 15th, 2022. (B) Linear regression of the citywide percentage of visits with discharge diagnosed influenza estimated using the proportion of schools with positive wastewater detections of influenza. Green shaded area represents the 95% confidence interval of the line.

to inherent variability and uncertainties (Pulicharla et al., 2021). This can make it difficult to determine how directly wastewater concentrations reflect the number of infected individuals. Some infections are likely missed due to low viral loads in the wastewater that are either not obtained by sampling or are lower than the limit of detection (CDC, 2022). Toilet use behavior in schools is also a source of variability that impacts the likelihood of detecting infected individuals at school via wastewater monitoring. Additionally, the aggregation of school positivity rates from PCR testing, which was performed over several days, could lead to discrepancies when compared to wastewater data which was analyzed one day per week. Most of the clinical testing was completed the same day that the wastewater was sampled. However, some testing was performed on other days of the week to accommodate the schools and testing teams. The relative timing of PCR and wastewater testing data trends is an area that requires further investigation and could be informed by fecal shedding distributions for each virus (and their variants).

Fecal shedding of influenza, and specifically whether influenza infects intestinal cells and is consequently shed in the feces is not well established. However, some studies suggest that swallowed nasopharyngeal secretions that contain influenza or the dissemination of influenza through the blood (Chan et al., 2011) result in influenza in the intestinal tract. Influenza may bind to colonic epithelial cells via an influenza virus receptor, sialic acid α 2,3-Gal, and thus explain the process by which influenza is shed in the feces (Pinsky et al., 2010). Previous studies investigated the proportion of influenza-infected individuals that shed the virus fecally (Chan et al., 2011; Hirose et al., 2016). Hirose et al. (2016) found influenza A or influenza B RNA in the feces of 36.4% of influenza A- or influenza B-infected individuals, respectively (Hirose et al., 2016). A similar study detected influenza A in the feces of 47.1% of influenza A-infected individuals (Chan et al., 2011). These results suggest that while not all individuals infected with influenza shed the virus fecally, a substantial proportion of infected individuals do, thus enabling the use of wastewater surveillance. Additionally, SARS-CoV-2 and influenza can be shed through other bodily secretions such as sputum and mucus and can in turn enter the wastewater system through this route. Future research is needed to investigate these limitations, as well as other factors such as how fecal shedding rates for SARS-CoV-2 and influenza differ between school-aged children and the general population and could in turn affect the comparability of results between the groups. Further investigations could also examine how vaccination rates affect school wastewater detection results and could determine if schools with higher student vaccination rates experience lower concentrations of SARS-CoV-2 and influenza in their wastewater.

## 5. Conclusions

The results of this study show that preK-12 school wastewater levels of SARS-CoV-2 and influenza A were reflective of school, community, and citywide infections. School wastewater measurements can complement clinical disease surveillance by providing a pooled sample that is not dependent on symptoms or health-seeking behavior. PreK-12 schools are especially critical surveillance locations as they are sources of high transmission, and their outbreaks can lead to further spread into local communities. Monitoring of other diseases common to school-aged children via wastewater surveillance could be implemented to reduce the burden of disease on communities and the number of schooldays missed due to illness.

## 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.

## Data availability

The authors do not have permission to share data.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2023.119648.

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