Supplementary Information (SI) for Environmental Science: Water Research & Technology.

Supporting Information

1 Supplemental Methods

1.1 University Policies Before and After Fall Vaccination Mandate

The policies in place for Spring 2021 semester (pre-vaccine period), when undergraduate students returned to on-campus housing, were in place to ensure a low-incidence of disease. Students were housed at reduced capacity (50% pre-pandemic density) to allow for social distancing and the use of single occupancy rooms. Prior to attending any in-person classes or activities, all students were required to receive two negative PCR test results spaced two days apart through the JHU testing program. Other residence hall restrictions to prevent viral transmission included assigned sinks and showers for shared bathrooms and prohibition of visitation for non-residents. Campus-wide precautions included mandatory indoor masking, daily health checks via smartphone app, no congregate dining, and location-based contact tracing.

In the Fall 2021 semester (post-vaccine period), several modifications were made to on-campus mitigation policies. These included a return to primarily in-classroom classes, allowing medium-sized student gatherings, and full on-campus housing occupancy. Testing and quarantine of students upon move-in was no longer required. All undergraduate students were required to be fully vaccinated and regularly tested once per week for asymptomatic disease. Unvaccinated students (with an approved exception) made up less than 5% of the students in residence halls and were required to test twice per week. Students who had obtained a vaccine that was not FDA-authorized, were required to obtain an FDA-authorized vaccine prior to return to campus. Masks were required on campus and all other COVID mitigation efforts remained in place. Notably, the fall semester coincided with the circulation of the B.1.617.2 (Delta) variant of SARS-CoV-2.

1.2 Wastewater Sampling and Collection

Samples were collected from each dorm using an ISCO 6712 portable autosampler (Teledyne ISCO; Lincoln, NE) using varying access points inside and outside buildings Figure S1). For buildings without manhole access, pipe cleanouts were selected to enable access to horizontal building mains. Segments of rigid polyethylene (PE) tubing (1/2" OD; 3/8" ID) between 10-20 ft (3-6 m) length attached to low-flow stainless steel strainers (Teledyne ISCO) were used as suction lines into the autosamplers. The hard plastic tubing allowed the strainer end to be guided around sharp bends from access pipes into the desired sample locations in the main. Strainers were positioned horizontally in the bottom of building mains in the direction of flow. Access to building mains and strainer placement were determined using a combination of building schematics and pipe inspection cameras. Suction lines were run through cleanout caps tapped with compression fittings, allowing for sampling for cleanouts in a sealed environment. For sewer manholes, segments of flexible vinyl tubing (½" OD; 3/8" ID) were used to allow strainers to be placed down the vertical manholes under low tension. Ultra low-flow stainless steel strainers (Teledyne ISCO) containing flat weighted plates and suction from the bottom side only were used to facilitate easy placement in open-channel troughs found in manholes. Manhole samples were collected outside in enclosed shelters.

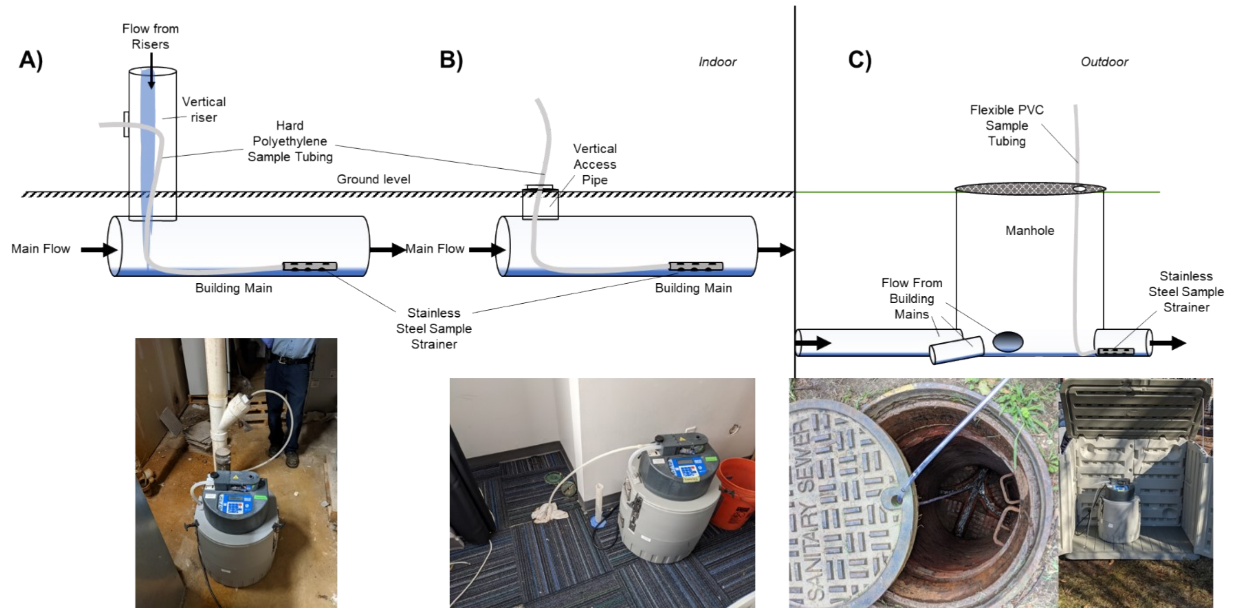

Figure S 1. Building-level wastewater sample collection methods employed during the study. Samples collected from the indoors were drawn from building sewer mains below residence halls accessed through either (A) vertical risers or (B) vertical cleanout access points. Collection from outside of buildings were accessed through (C) sewer manholes.

Collected samples were dispensed and stored in 10 L polypropylene (PP) carboys lined with clean, disposable PE liner bags. The following morning after collection, composite samples were mixed and dispensed into autoclaved 1 L PP screw-cap bottles and delivered to the laboratory for same day processing. Samplers were cleaned and reset for subsequent collection the afternoon of sample drop-off. Samples were collected on 32 different dates in the Spring and 28 in the Fall, totaling 60 for this study.

1.3 Processing, Extraction, and Detection of SARS-CoV-2 RNA

Samples were received in the laboratory within 12 hours of the end of the composite sampling window. Sealed sample bottles were immediately placed in a temperature-controlled water bath at 75°C for 2 hours to inactivate wastewater pathogens before further handling. Samples were processed following a Biosafety Level 2 protocol pre-approved by the JHU Department of Health, Safety, and Environment. During extraction, DNase I was applied to remove genomic DNA from the extract. The extraction resulted in a 100 µL nucleic acid eluent available for analysis. Primers and probes used in RT-qPCR assays are listed below in Table S 1.

Table S 1. Primers and probes utilized in RT-qPCR assay

	2019-nCoV_N1	*BCoV*
F Primer	5’-GAC CCC AAA ATC AGC GAA AT-3’	5'- CTGGAAGTTGGTGGAGTT -3'
R Primer	5’-TCT GGT TAC TGC CAG TTG AAT CTG-3’	5'- ATTATCGGCCTAACATACATC -3'
Probe	5’-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3’	5' - FAM CCT TCA TAT – Nova – CTA TAC ACA TCA AGT TGT T - BHQ1-3'

2 Additional Results

2.1 Wastewater optical density measurements

Optical density (OD_{600}; λ=600 nm; l= 1 cm) was measured for each undilute sample at the time of filtration and used as a simple indicator of turbidity among samples. Measurements were conducted using a Hach DR6000 UV Vis Spectrophotometer (Hach, Loveland, CO) using polystyrene cuvettes (Brand GMBH Wertheim, Germany). Measured OD_{600} values ranged from 0.057 to 2.84, with median and mean values of 0.202 and 0.293 ± 0.307 (1 standard deviation) respectively. Samples from unique sites also showed considerable variability, with median values from 0.144 to 0.414 (Figure S 2).

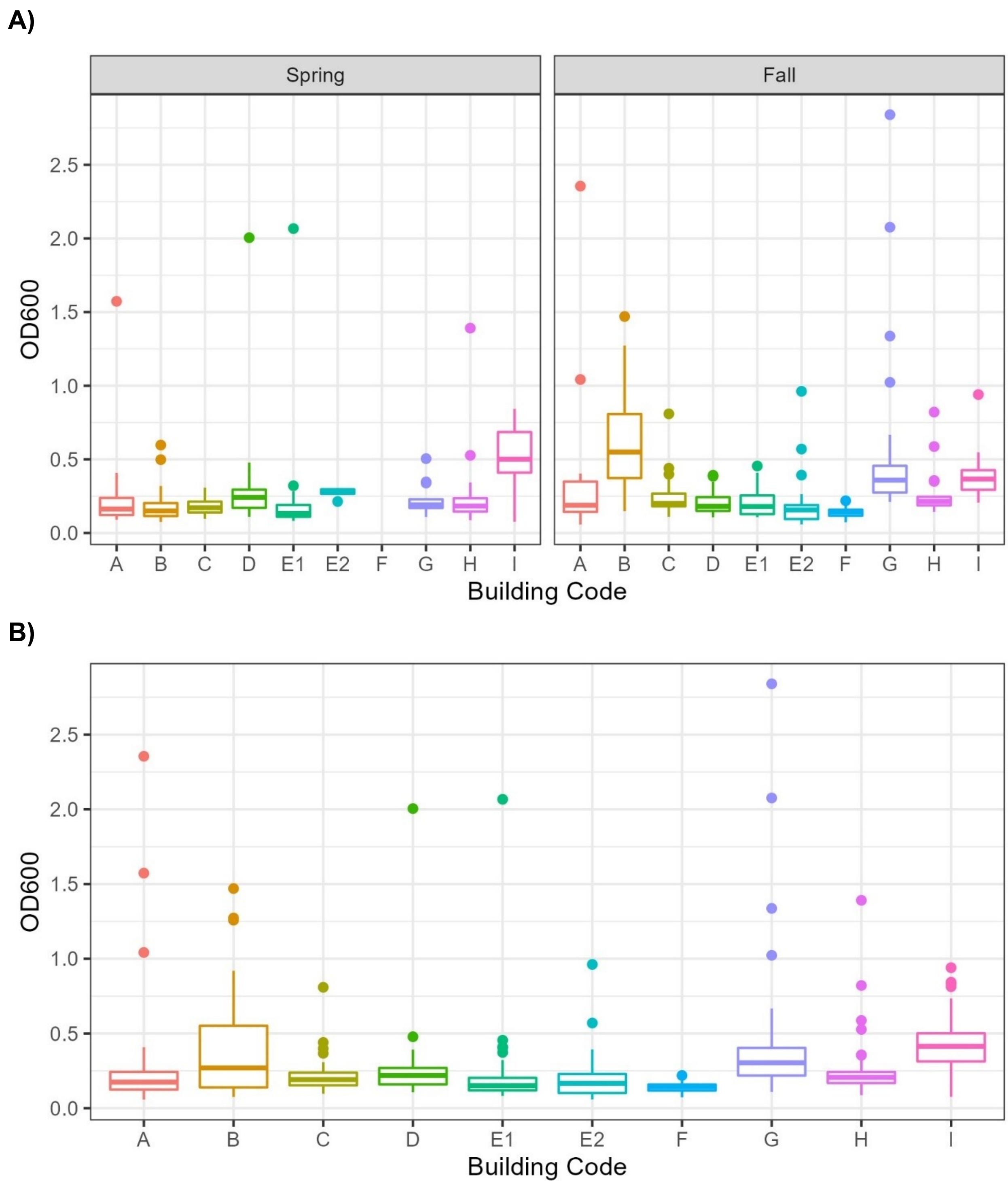

Figure S 2. Boxplot comparisons of OD_{600} measurements for samples collected at each wastewater surveillance site. (A) Measurements separated by semester. (B) Measurements for entire study period. Boxes contain 25th percentile, median, and 75th percentile values; whiskers represent 1.5× of the interquartile range; and individual data points are outliers.

Table S 2. RT-qPCR results for all positive detected wastewater samples in the study

Semester	Sample Date	Sample Location	Sars-CoV-2, Ct		BOV, Ct		BOV	Note
			Undil [a]	*10^{-1}* [b]	*Undil* [a]	*10^{-1}* [b]	*Δ Ct*	
Spring	01/27/2021	B	n.d.	37.0 *	30.7	32.8	-2.2	* Results from direct extraction
Spring	01/27/2021	D	36.3 *	n.d.	30.6	33.2	-2.6	* Results from direct extraction
Spring	01/31/2021	B	37.4	37.4	35.7	34.1	1.5	
Spring	01/31/2021	E1	35.9	n.d.	32.4	33.4	-1.0	
Spring	01/31/2021	D	34.4	n.d.	31.1	37.5	-6.4	
Spring	02/02/2021	A	37.4	n.d.	30.1	31.3	-1.3	
Spring	02/02/2021	E1	32.0 *	32.7	31.8	32.8	-1.1	* Averaged from 2 filters
Spring	02/02/2021	D	32.5	35.5	30.7	33.5	-2.8	
Spring	02/02/2021	C	36.3	n.d.	30.8	34.2	-3.5	
Spring	02/09/2021	C	38.6	n.d.	30.6	34.2	-3.6	
Spring	02/14/2021	D	37.1	n.d.	30.3	34.2	-3.9	
Spring	02/23/2021	D	36.8	38.1	37.3	35.6	1.8	
Spring	03/07/2021	H	31.9	34.6	30.7	33.4	-2.7	
Spring	03/07/2021	D	35.2	n.d.	32.4	36.4	-4.0	
Spring	03/09/2021	G	32.6	35.5	29.1	33.2	-4.1	
Spring	03/09/2021	H	33.9	n.d.	31.6	36.0	-4.4	
Spring	03/16/2021	C	34.1	n.d.	29.7	33.8	-4.1	
Spring	03/23/2021	G	36.0	n.d.	33.9	37.2	-3.4	
Spring	04/04/2021	C	35.7	n.d.	33.7	35.6	-1.9	
Fall	09/14/2021	C	37.9	n.d.	29.4	32.3	-2.8	
Fall	10/10/2021	F	35.6	n.d.	29.6	33.1	-3.5	
Fall	11/02/2021	B	n.d.	39.0	29.3	33.9	-4.6	
Fall	11/30/2021	E2	38.1	n.d.	28.2	32.5	-4.3	
Fall	11/30/2021	C	38.1	n.d.	29.9	34.4	-4.5	
Fall	12/05/2021	E2	38.9	n.d.	35.1	35.7	-0.5	
Fall	12/12/2021	D	37.6	n.d.	27.9	31.3	-3.4	

a. Extraction of undilute wastewater sample

b. Extraction of 10^{-1} diluted wastewater

n.d. – no signal crossing C_t threshold of 40

Table S3 Synthetic Wastewater

Composition of Synthetic wastewater per liter

Compounds	Concentration
CH_3COONa	1000 mg/L (varied based on target COD conc.)
NH_4Cl	60 mg/L
$NaH_2PO_4 \cdot 2H_2O$	15 mg/L
$NaHCO_3$	200 mg/L
$MgSO_4$	12.5 mg/L
Nutrient Solution*	0.5 mL/L
KCl	5 mg/L
$NaNO_3$	33 mg/L

*Nutrient Solution

compounds	g/L
$FeCl_3$	0.9
H_3BO_3	0.15
$CuSO_4 \cdot 5H_2O$	0.03
KI	0.18
$MnSO_4 \cdot$ H2O	0.12
$NaMoO_4 \cdot 2H_2O$	0.06
$ZnSO_4 \cdot 7H_2O$	0.12
$Co(NO_3)_2 \cdot 6H_2O$	0.15

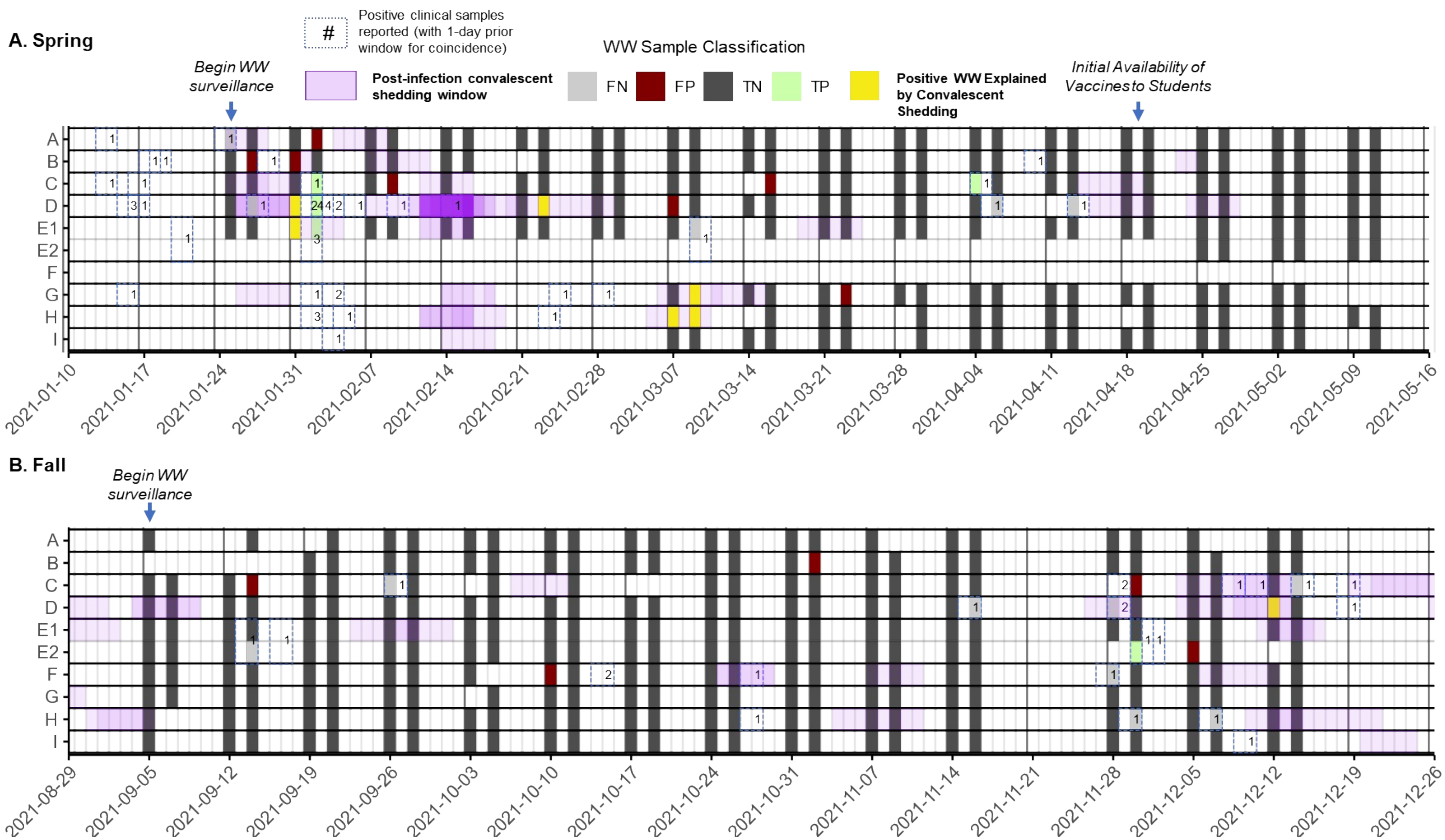

Figure S 3. Coincidence of WW surveillance sample data with windows of convalescent shedding from students returning from isolation. Windows of convalescent shedding were determined as the days between a student's return from isolation housing and 14 days after the initial positive clinical test. Shedding windows are highlighted purple with greater color intensity indicative of a higher density of returned students potentially shedding. WW positives previously classified as FPs but fall within the convalescent shedding window are highlighted in yellow. A) Spring and B) Fall semesters of 2021.