

Supplementary Material

SARS-CoV-2 RNA abundance in wastewater as a function of distinct urban sewershed size

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Supplement A

Table A1. Inventory of samples used in this study by date and site. Samples collected from August 17, 2020 to April 5, 2021 of which N=727 were included in analysis.

Date	MFQTC	DRGWQTC	CCWQTC	FFWQTP	HCWQTP	Shawnee Park CSO189	34th Street PS	MH40870 Muddy Forks PS	MH32985 Wood Road & Terry Road	MH09837 Ashby Lane & Mill Creek	MH08915A CSO140	MH50495 CSO108	MH23290 W. Indian Trail	MH57769 Pineland Drive & Oakmont Dr.	MH57350 Preston & South Park	MH71910 CSO146	MH70101 15th & Wilson	Total Good Observations	Total Lab Quality Excluded Observations	Total Not in Study Design Observations
17-Aug-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
18-Aug-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-77	16	0	1
19-Aug-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
20-Aug-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
24-Aug-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
25-Aug-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-77	16	0	1
26-Aug-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
27-Aug-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
31-Aug-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
1-Sep-20	1	0	0	0	0	1	1	0	-77	-77	0	0	0	0	-77	0	-77	3	10	4
2-Sep-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
3-Sep-20	-77	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-77	15	0	2
8-Sep-20	1	1	1	1	0	1	1	-77	0	0	0	0	1	0	0	0	-77	7	8	2
9-Sep-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
10-Sep-20	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	-77	15	1	1
14-Sep-20	0	-77	-77	-77	-77	-77	0	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	0	2	15
15-Sep-20	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	16	1	0
16-Sep-20	0	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	0	1	16
17-Sep-20	1	1	1	1	1	1	1	1	0	1	0	0	1	1	0	1	0	12	5	0
21-Sep-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
22-Sep-20	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	16	1	0
23-Sep-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
24-Sep-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
28-Sep-20	0	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	0	1	16
29-Sep-20	0	1	1	0	0	0	0	0	0	1	-77	0	1	0	1	0	0	5	11	1
30-Sep-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
1-Oct-20	0	1	1	1	1	1	1	-77	1	1	0	-77	1	0	1	1	1	12	3	2
5-Oct-20	1	1	1	1	0	1	1	0	1	1	0	1	1	1	1	0	1	13	4	0
6-Oct-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
7-Oct-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16

Date	MFWQTC	DRGWQTC	CCWQTC	FFWQTP	HCWQTP	Shawnee Park CSO189	34th Street PS	MH40870 Muddy Forks PS	MH32985 Wood Road & Terry Road	MH09837 Ashby Lane & Mill Creek	MH08915A CSO140	MH50495 CSO108	MH23290 W. Indian Trail	MH57769 Pineland Drive & Oakmont Dr.	MH57350 Preston & South Park	MH71910 CSO146	MH70101 15th & Wilson	Total Good Observations	Total Lab Quality Excluded Observations	Total Not in Study Design Observations
8-Oct-20	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	15	2	0
12-Oct-20	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	0	1	14	3	0
13-Oct-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
14-Oct-20	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	16	1	0
15-Oct-20	0	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	0	1	16
19-Oct-20	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	16	1	0
20-Oct-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
21-Oct-20	0	1	1	1	1	1	0	0	1	1	-77	1	1	1	1	0	0	11	5	1
22-Oct-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
26-Oct-20	1	1	1	1	1	1	1	1	1	1	-77	1	1	1	1	1	1	16	0	1
27-Oct-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
28-Oct-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
29-Oct-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
2-Nov-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	16	1	0
3-Nov-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
4-Nov-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
5-Nov-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
9-Nov-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
10-Nov-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
11-Nov-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
12-Nov-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
16-Nov-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
17-Nov-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
18-Nov-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
19-Nov-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
23-Nov-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
24-Nov-20	1	1	1	1	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	5	0	12
30-Nov-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
1-Dec-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
2-Dec-20	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	16	1	0
3-Dec-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
7-Dec-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
8-Dec-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
9-Dec-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
10-Dec-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16

Date	MFWQTC	DRGWQTC	CCWQTC	FFWQTP	HCWQTP	Shawnee Park CSO189	34th Street PS	MH40870 Muddy Forks PS	MH32985 Wood Road & Terry Road	MH09837 Ashby Lane & Mill Creek	MH08915A CSO140	MH50495 CSO108	MH23290 W. Indian Trail	MH57769 Pineland Drive & Oakmont Dr.	MH57350 Preston & South Park	MH71910 CSO146	MH70101 15th & Wilson	Total Good Observations	Total Lab Quality Excluded Observations	Total Not in Study Design Observations
14-Dec-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
15-Dec-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
16-Dec-20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
17-Dec-20	1	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	-77	1	0	16
4-Jan-21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
11-Jan-21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-77	16	0	1
18-Jan-21	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	17	0	0
25-Jan-21	1	1	1	1	1	1	1	1	1	1	0	-77	1	1	1	1	-77	14	1	2
1-Feb-21	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	14	3	0
8-Feb-21	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	16	1	0
15-Feb-21	1	1	1	1	1	1	1	0	1	1	-77	0	-77	1	1	1	1	13	2	2
22-Feb-21	1	1	1	1	1	1	1	1	1	1	1	1	-77	1	1	1	1	16	0	1
1-Mar-21	1	1	0	1	1	1	1	1	1	1	-77	0	-77	1	1	-77	0	11	3	3
8-Mar-21	1	1	1	1	1	1	1	-77	1	1	0	-77	-77	1	1	1	1	13	1	3
15-Mar-21	1	1	1	1	1	1	1	0	1	1	-77	-77	-77	1	1	1	1	13	1	3
22-Mar-21	1	1	1	1	1	1	1	-77	1	1	1	1	-77	1	1	1	1	15	0	2
29-Mar-21	1	1	1	1	1	1	1	-77	1	1	1	1	-77	1	1	1	1	15	0	2
5-Apr-21	1	1	1	1	1	1	1	0	1	1	1	0	-77	1	1	1	1	14	2	1
Total	74	47	46	46	43	46	45	30	43	44	31	34	38	43	44	39	34	727	77	607

1 refers to good data

0 refers to data excluded due to bad lab quality

-77 refers to data excluded due to not in study design

Supplement B

Table B1. Primer and probe sequences used for RT-qPCR in study.

Target	Primer Name	Sequence (5'→3')	Probes
N1	2019-nCoV_N1-F	5'-GACCCCAAAATCAGCGAAAT-3'	None
	2019-nCoV_N1-R	5'-TCTGGTTACTGCCAGTTGAATCTG-3'	None
	2019-nCoV_N1-P	5'- FAM -ACCCCGCATTACGTTTGGTGGACC- QSY -3'	FAM, BHQ-1
RNase	RNase P-F	5'-AGATTTGGACCTGCGAGCG-3'	None
	RNase P-R	5'-GAGCGGCTGTCTCCACAAGT-3'	None
	RNase P-P	5'- JUN -TTCTGACCTGAAGGCTCTGCGCG- QSY -3'	JUN, BHQ-1
ORF	CoV_ORF1ab-F	5'-GTCGTAGTGGTGAGACACTTG-3'	None
	CoV_ORF1ab-R	5'-GGCCACCAGCTCCTTTATTA-3'	None
	CoV_ORF1ab-P	5'- FAM -ATACCAGTGGCTTACCGCAAGGTT- QSY -3'	FAM, BHQ-1
PMMoV	PMMoV-F	5'-GAGTGGTTTGACCTTAACGTTTGA-3'	None
	PMMoV-R	5'-TTGTCGGTTGCAATGCAAGT-3'	None
	PMMoV-P	5'- VIC -CCTACCGAAGCAAATG- QSY -3'	VIC, BHQ-1
CrAssphage	CrAssphage-F	5'- CAGAAGTACAACTCCTAAAAAACGTAGAG- 3'	None
	CrAssphage-R	5'-GATGACCAATAAACAAGCCATTAGC-3'	None
	CrAssphage-P	5'- JUN -AATAACGATTACGTGATGTAAC- QSY -3'	JUN, BHQ-1

Supplement C

Data Analysis

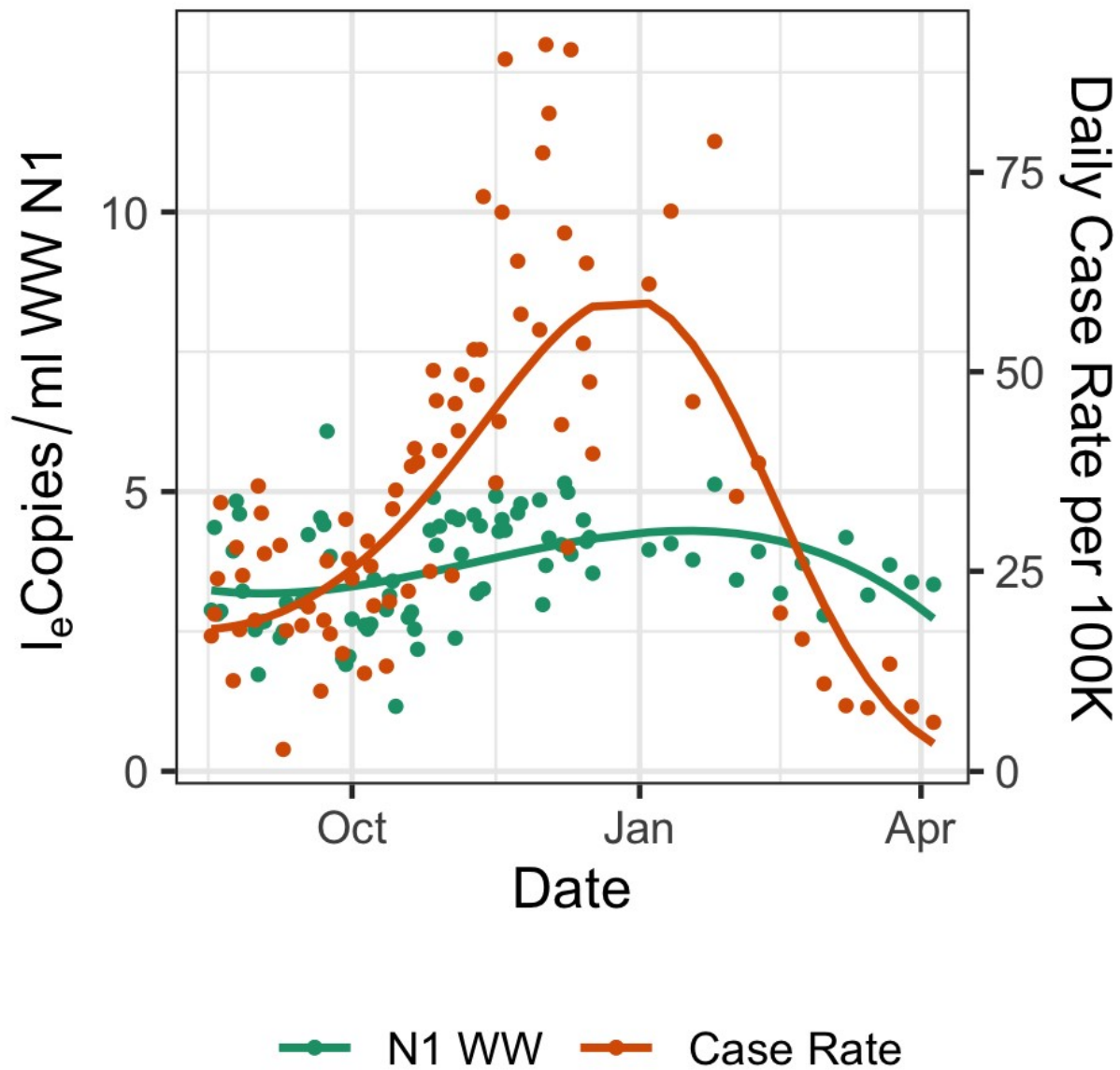


Figure C1. Mean $\log_e$ SARS-CoV-2 RNA (N1) wastewater concentration across Louisville/Jefferson County Metropolitan Sewer District (MSD) sample locations and number of new daily cases in Louisville/Jefferson County as reported by Louisville/Jefferson County Information Consortium and Louisville Metro Department of Public Health and Wellness, from August 2020 to April 2021. The lines represent the natural cubic spline fit as a function of time.

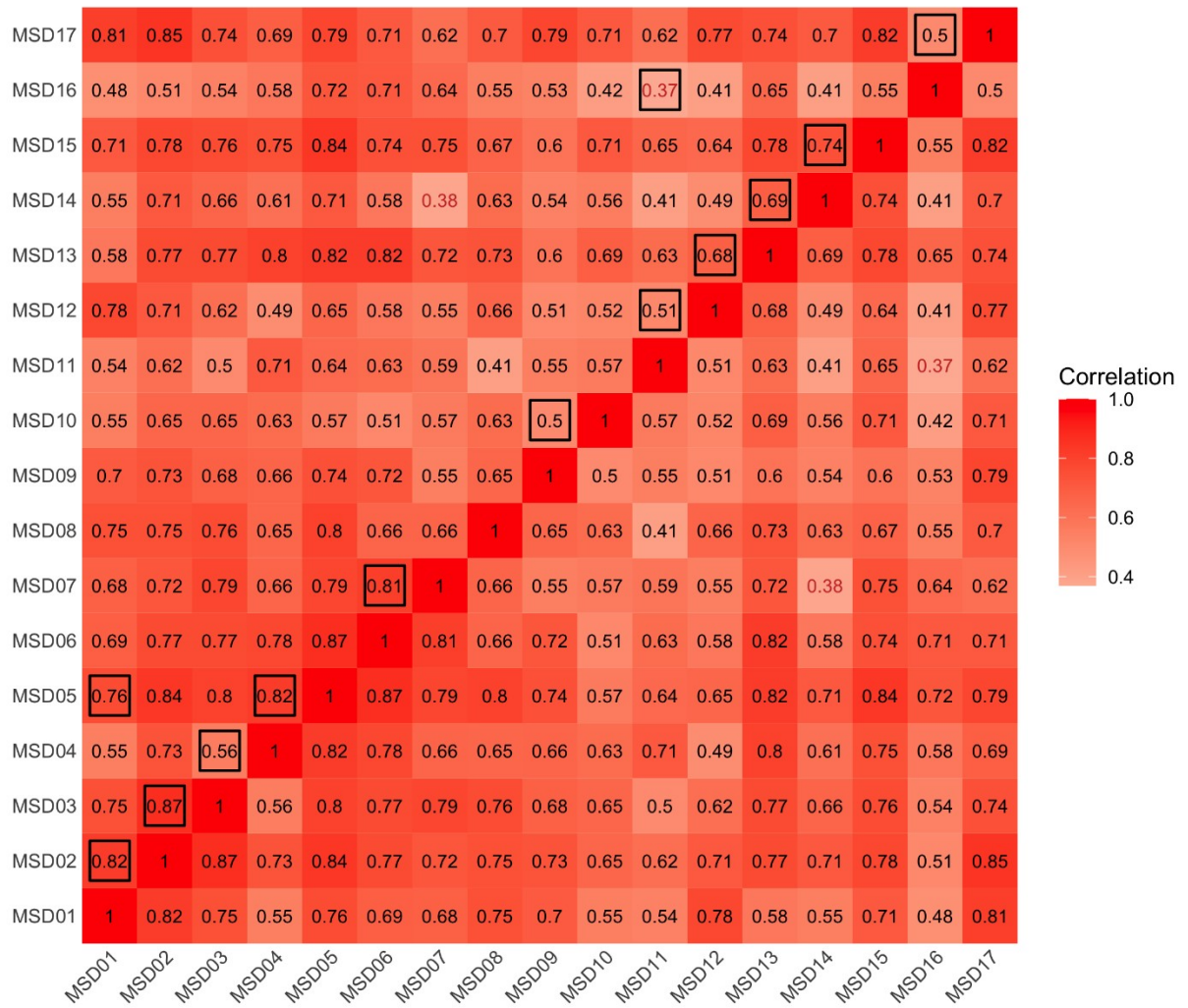


Figure C2. Correlations for date paired sites (N=25 paired sets for the 17 sites) between the $\log_e$ SARS-CoV-2 RNA (N1) normalized by $\log_e$ PMMoV. The correlation values in light colored fonts are not significant. Black boxes outline the correlation values corresponding to geographically adjacent sites.

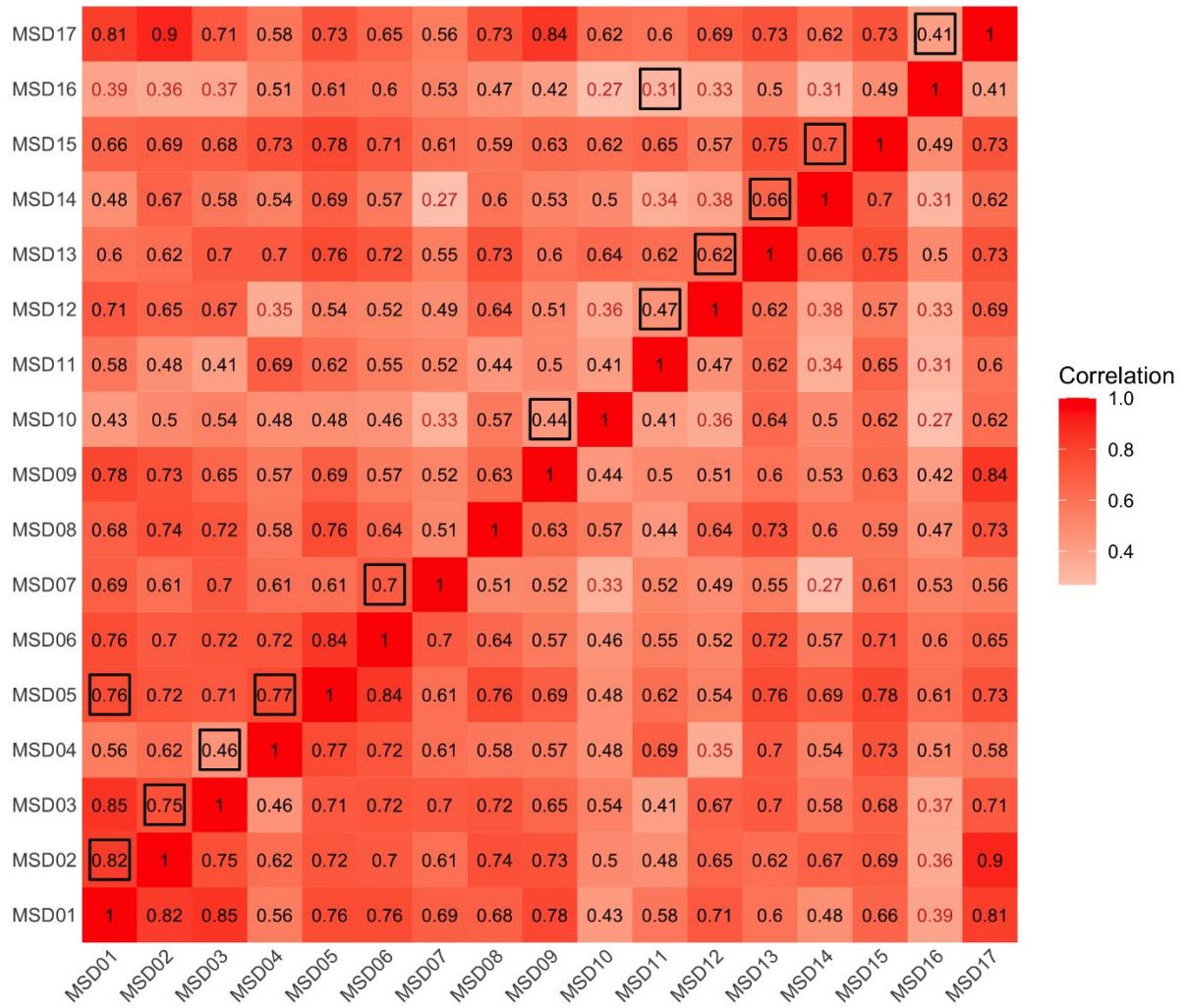


Figure C3. Correlations for date paired sites (N=25 paired sets for the 17 sites) between the $\log_e$ SARS-CoV-2 RNA (N1) normalized by $\log_e$ CrAssphage. The correlation values in light colored fonts are not significant. Black boxes outline the correlation values corresponding to geographically adjacent sites.

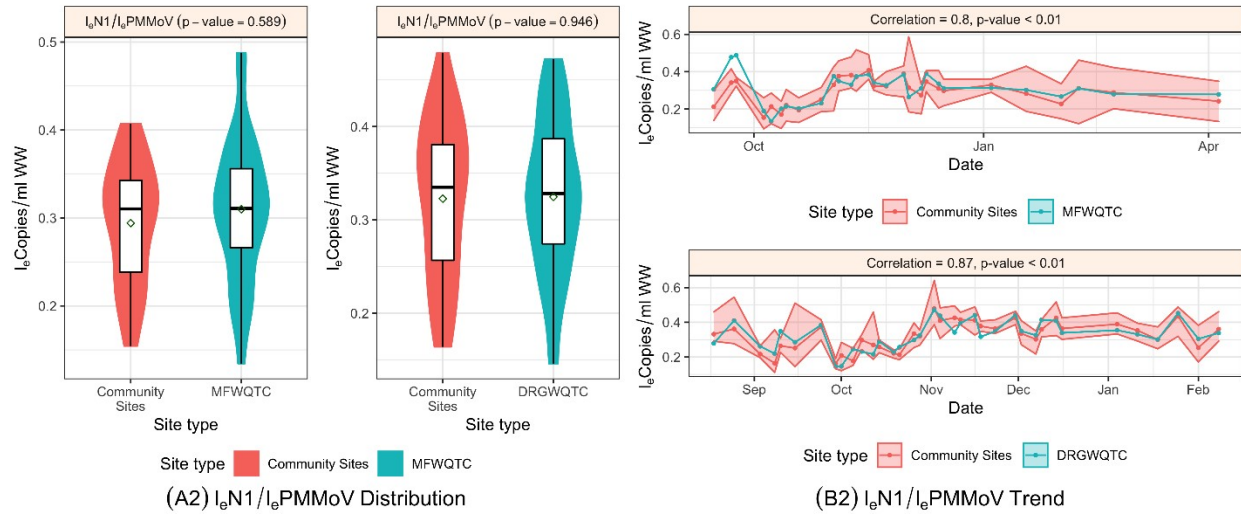


Figure C4. Aggregate sites (treatment centers) compared to contributing sites flowing from the upstream locations versus $\log_e$ SARS-CoV-2 RNA (N1) normalized by $\log_e$ PMMoV. Morris Forman Water Quality Treatment Center (MSD01, MFWQTC; N=7 contributing sewersheds) and Derek R. Guthrie Water Quality Treatment Center (MSD02, DRGWQTC; N=5 contributing sewersheds). The shaded regions represent the distributions of $\log_e$, the red dots represent the outliers. The p-value was based on the Kruskal-Wallis test (A). Temporal trends of $\log_e$ SARS-CoV-2 RNA (N1) normalized by $\log_e$ PMMoV at aggregate sites (treatment centers) compared to contributing sites. The shaded regions represent the minimum and maximum values of the data from the respective contributing sites. The p-value was based on the correlation test (B).

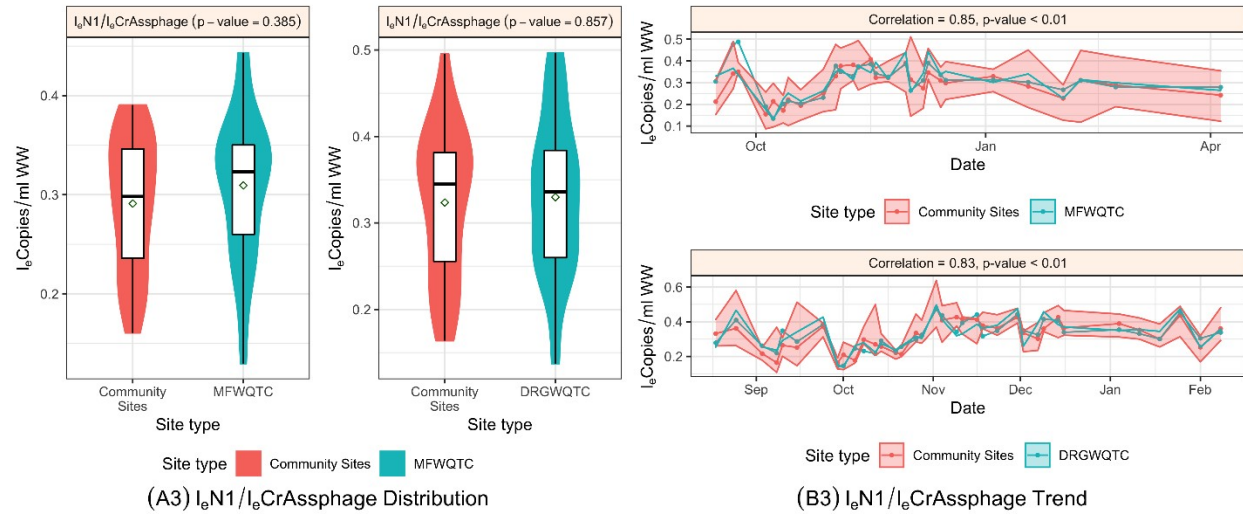


Figure C5. Aggregate sites (treatment centers) compared to contributing sites flowing from the upstream locations versus $\log_e$ SARS-CoV-2 RNA (N1) normalized by $\log_e$ CrAssphage. Morris Forman Water Quality Treatment Center (MSD01, MFWQTC; N=7 contributing sewersheds) and Derek R. Guthrie Water Quality Treatment Center (MSD02, DRGWQTC; N=5 contributing sewersheds). The shaded regions represent the distributions of $\log_e$, the red dots represent the outliers. The p-value was based on the Kruskal-Wallis test (A). Temporal trends of $\log_e$ SARS-CoV-2 RNA (N1) normalized by $\log_e$ CrAssphage at aggregate sites (treatment centers) compared to contributing sites. The shaded regions represent the minimum and maximum values of the data from the respective contributing sites. The p-value was based on the correlation test (B).

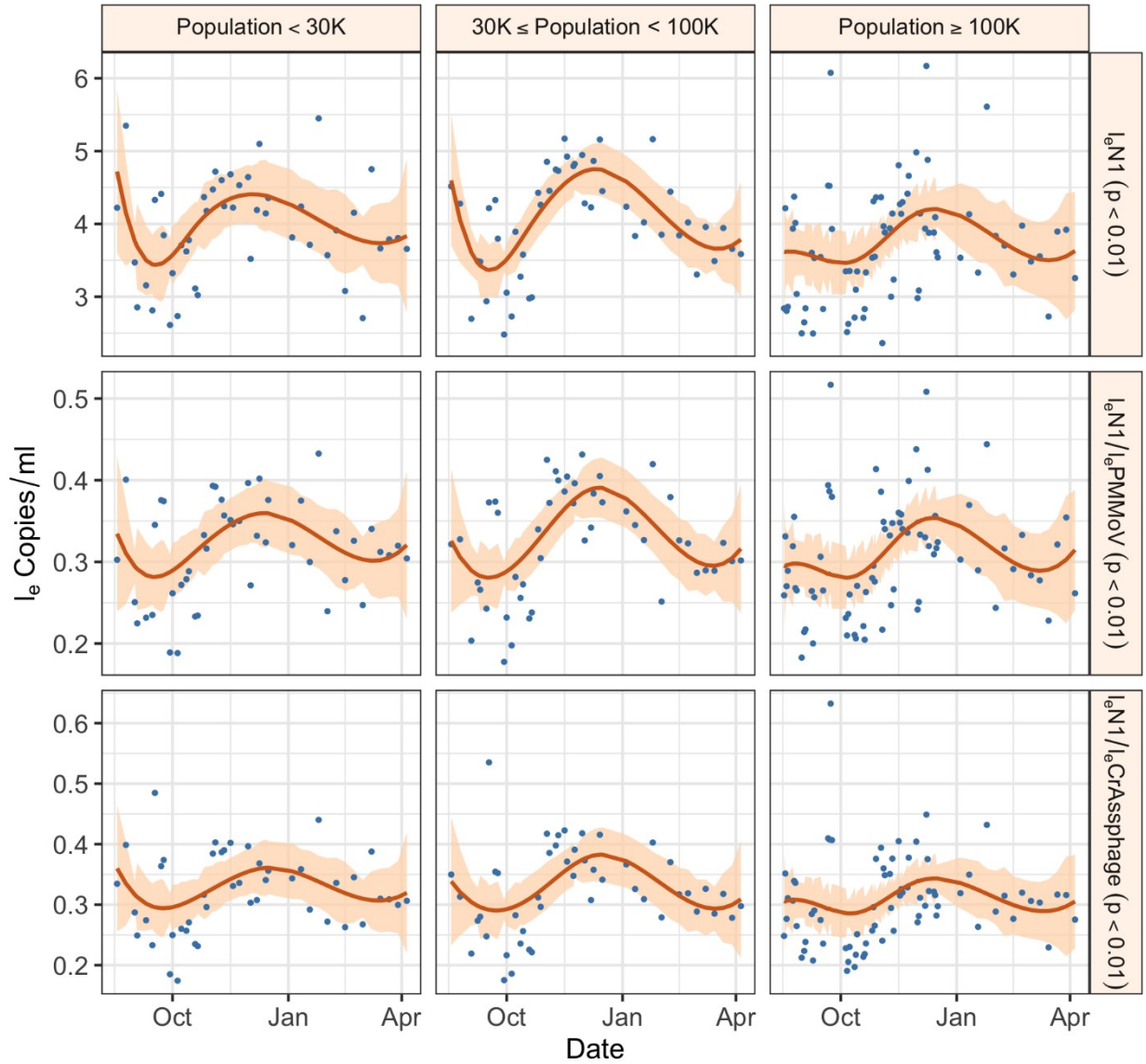


Figure C6. Temporal variability of $\log_e$ SARS-CoV-2 RNA (N1), SARS-CoV-2 RNA (N1) normalized by $\log_e$ PMMoV; and $\log_e$ SARS-CoV-2 RNA (N1) normalized by $\log_e$ CrAssphage across Louisville/Jefferson County Metropolitan Sewer District (MSD) sample locations, from August 2020 to April 2021 in the intervals $\leq 30,000$, $30,000$ to $100,000$ and $\geq 100,000$. The scatter plot represents the raw data, and the lines represent the natural cubic spline fit as a function of time in each of the population categories, the shaded areas is the 95% confidence band around the spline estimates.

Table C1. Summary statistics of triplicate untransformed wastewater data (i.e., viral gene copies/ml wastewater) of SARS-CoV-2 RNA, pepper mild mottle virus, and cross-assembly phage by sampling site and site location type for sample collection in Louisville/Jefferson County, from August 2020 to April 2021.

		N1 (copies/ml)						PMMoV (copies/ml)					CrAssphage (copies/ml)					
		N	mean	median	sd	min	max	mean	median	sd	min	max	mean	median	sd	min	max	
TOTAL		2,001	120	54	690	8	22,707	1,244,471	246,531	12,323,769	172	515,815,180	1,993,017	206,381	16,803,814	6,461	387,397,516	
Site	Map ID																	
	MFWQTC	1	213	73	39	275	8	3,956	496,729	172,141	1,332,544	3,873	10,896,886	3,376,673	110,334	27,986,729	17,719	377,366,734
	DRGWQTC	2	129	101	64	107	15	576	1,510,589	298,442	7,741,482	23,588	83,628,321	1,293,485	201,300	4,240,904	8,830	31,137,709
	CCWQTC	3	120	97	77	74	10	400	1,010,129	420,553	2,518,230	28,881	23,339,668	2,747,433	250,167	13,314,695	22,857	126,189,202
	FFWQTP	4	126	64	49	44	9	252	900,520	248,340	3,503,639	234	33,773,508	591,815	138,332	2,688,648	26,917	28,857,995
	HCWQTP	5	114	92	60	147	10	1,497	372,918	242,161	489,708	2,805	3,569,841	795,212	166,945	1,923,123	24,652	10,670,963
	Shawnee Park CSO189	6	123	289	91	1,521	9	16,915	1,216,089	333,774	2,468,706	19,902	15,194,145	3,379,796	411,281	23,117,228	15,036	256,254,775
	34th Street PS	7	126	75	41	83	10	467	4,642,365	193,998	45,921,802	12,401	515,815,180	7,625,425	290,905	47,159,501	6,461	387,397,516
	MH40870 Muddy Forks PS	8	84	58	43	52	8	328	1,406,842	181,670	5,442,684	1,572	41,831,342	871,844	290,145	2,122,839	28,240	14,678,998
	MH32985 Wood Road & Terry Road	9	129	173	117	179	12	1,212	2,671,999	438,822	10,135,184	19,771	91,204,093	2,421,106	387,317	6,973,055	17,719	56,083,351
	MH09837 Ashby Lane & Mill Creek	10	126	244	82	924	11	10,264	1,294,032	435,306	3,422,680	2,268	33,698,122	2,398,156	472,553	7,158,338	20,743	54,705,579
	MH08915A CSO140	11	90	88	53	123	9	701	1,344,428	269,346	3,765,133	26,016	29,921,108	1,278,457	258,932	7,700,764	16,660	73,360,757
	MH50495 CSO108	12	84	45	37	42	8	216	403,660	116,701	1,153,070	7,695	9,319,411	586,372	180,695	1,351,734	14,531	9,867,268
	MH23290 W. Indian Trail	13	108	285	54	2,179	8	22,707	772,037	304,347	1,343,266	2,438	10,852,851	753,656	218,720	2,463,353	13,006	22,080,088
	MH57769 Pineland Drive & Oakmont Dr.	14	126	108	46	266	8	2,720	382,591	145,427	632,396	172	4,390,820	407,125	167,637	692,253	13,265	5,459,626
	MH57350 Preston & South Park	15	123	90	41	227	8	2,414	820,791	161,053	5,407,571	5,525	59,948,300	981,919	165,640	5,341,263	14,324	53,043,461
	MH71910 CSO146	16	99	59	34	86	10	670	1,160,689	123,320	6,673,729	2,212	63,413,078	348,890	121,126	1,209,040	12,232	11,538,806
	MH70101 15th & Wilson	17	81	56	47	43	10	211	611,571	120,189	1,707,300	26,881	13,334,209	1,116,685	99,036	4,353,112	9,593	27,853,480
Site type																		
	Manhole	966	135	54	816	8	22,707	1,096,927	227,289	5,076,482	172	91,204,093	1,197,999	208,591	5,037,864	9,593	73,360,757	
	Pump Station	333	150	56	930	8	16,915	2,560,636	262,844	28,395,015	1,572	515,815,180	4,353,614	334,622	32,285,327	6,461	387,397,516	
	Treatment Center	702	84	54	173	8	3,956	823,166	260,202	3,865,691	234	83,628,321	1,967,245	174,094	16,537,696	8,830	377,366,734	

Table C2. Summary statistics of untransformed solid data (i.e., gc/g dry weight) of SARS-CoV-2 RNA and pepper mild mottle virus by treatment plant site and sample type in Louisville/Jefferson County Metropolitan Sewer District (MSD), from November 2020 to December 2020.

		N Gene (gc/g dry weight)						PMMoV (gc/g dry weight)				
		N	mean	median	sd	min	max	mean	median	sd	min	max
	Total	42	31,368	29,477	21,494	2,833	80,974	182,636,274	164,751,547	140,224,620	39,263,220	734,907,439
Site	Sample type											
MSD01	Anaerobic centrifuge cake	6	8,347	8,367	4,175	2,833	13,223	142,311,438	91,502,353	136,786,827	48,463,132	408,785,701
MSD01	Anaerobic primary sludge	6	48,162	46,950	16,514	32,356	72,071	104,175,906	98,712,570	34,396,344	71,115,600	167,826,701
MSD01	Anaerobic waste activated sludge	6	18,373	14,159	13,639	9,501	45,780	76,301,637	54,506,781	50,921,376	39,263,220	172,626,163
MSD02	Aerobic digesters	6	58,731	62,004	15,499	36,264	80,974	288,418,619	231,687,045	231,997,213	77,650,769	734,907,439
MSD03	Aerobic digesters	6	45,227	47,929	11,092	28,212	12,848	161,142,729	191,250,950	67,266,308	50,491,999	219,357,245
MSD04	Aerobic digesters	6	27,539	27,522	12,848	8,688	47,571	274,622,316	215,402,890	170,475,257	146,241,378	603,401,214
MSD05	Aerobic digesters	6	13,199	12,033	8,004	5,112	24,718	231,481,277	238,194,820	37,870,186	174,743,672	277,516,793

Table C3: Comparison of concentrations by composite or grab sample collection.

Variables	Total (N=727)	Composite (N=704)	Grab (N=23)	P Value
Log_e N1 (copies/ml)				<i>0.040</i>
N	727	704	23	
Mean ± SD	3.93 ± 1.01	3.94 ± 1.01	3.51 ± 0.84	
Median (min - max)	3.90 (2.02 - 8.16)	3.91 (2.02 - 8.16)	3.61 (2.18 - 5.42)	
Log_e PMMoV (copies/ml)				<i>0.175</i>
N	727	704	23	
Mean ± SD	12.55 ± 1.39	12.54 ± 1.40	12.94 ± 1.10	
Median (min - max)	12.46 (6.23 - 17.38)	12.44 (6.23 - 17.38)	12.53 (11.03 - 15.28)	
Log_e CrAssphage (copies/ml)				<i>0.234</i>
N	727	704	23	
Mean ± SD	12.68 ± 1.47	12.66 ± 1.46	13.03 ± 1.66	
Median (min - max)	12.36 (9.18 - 18.65)	12.36 (9.18 - 18.65)	12.67 (9.73 - 16.87)	
Log_e(N1 mean)/ Log_e(PMMoV mean) (copies/ml)				<i>0.014</i>
N	727	704	23	
Mean ± SD	0.32 ± 0.09	0.32 ± 0.09	0.27 ± 0.07	
Median (min - max)	0.31 (0.14 - 0.93)	0.32 (0.14 - 0.93)	0.26 (0.17 - 0.46)	
Log_e(N1 mean)/ Log_e(CrAss mean) (copies/ml)				<i>0.017</i>
N	727	704	23	
Mean ± SD	0.31 ± 0.09	0.32 ± 0.09	0.27 ± 0.07	

Variables	Total (N=727)	Composite (N=704)	Grab (N=23)	P Value
Median (min - max)	0.31 (0.13 - 0.64)	0.32 (0.13 - 0.64)	0.28 (0.17 - 0.47)	

P value refers GLM for unbalanced and balanced ANOVA. Log calculated with log base e.

Table C4: Comparison by sample collection types: street line manhole, pump station, and treatment center.

Variables	Total (N=727)	Manhole (N=350)	Pump Station (N=121)	Treatment Center (N=256)	P Value
Log_e N1 (copies/ml)					<i>0.609</i>
N	727	350	121	256	
Mean ± SD	3.93 ± 1.01	3.93 ± 1.09	4.00 ± 1.01	3.89 ± 0.89	
Median (min - max)	3.90 (2.02 - 8.16)	3.88 (2.02 - 8.16)	3.88 (2.16 - 6.53)	3.92 (2.05 - 6.08)	
Log_e PMMoV (copies/ml)					<i>0.930</i>
N	727	350	121	256	
Mean ± SD	12.55 ± 1.39	12.54 ± 1.45	12.60 ± 1.41	12.55 ± 1.30	
Median (min - max)	12.46 (6.23 - 17.38)	12.38 (7.79 - 17.38)	12.46 (8.23 - 16.05)	12.52 (6.23 - 16.53)	
Log_e CrAssphage (copies/ml)					<i>0.003</i>
N	727	350	121	256	
Mean ± SD	12.68 ± 1.47	12.64 ± 1.43	13.08 ± 1.48	12.54 ± 1.49	
Median (min - max)	12.36 (9.18 - 18.65)	12.31 (9.56 - 17.10)	12.90 (9.75 - 18.60)	12.26 (9.18 - 18.65)	
Log_e(N1 mean)/ Log_e(PMMoV mean) (copies/ml)					<i>0.720</i>
N	727	350	121	256	
Mean ± SD	0.32 ± 0.09	0.32 ± 0.10	0.32 ± 0.08	0.31 ± 0.08	
Median (min - max)	0.31 (0.14 - 0.93)	0.31 (0.14 - 0.93)	0.32 (0.15 - 0.52)	0.32 (0.14 - 0.68)	
Log_e(N1 mean)/ Log_e(CrAss mean) (copies/ml)					<i>0.847</i>
N	727	350	121	256	

Variables	Total (N=727)	Manhole (N=350)	Pump Station (N=121)	Treatment Center (N=256)	P Value
Mean $\pm$ SD	0.31 $\pm$ 0.09	0.31 $\pm$ 0.091	0.31 $\pm$ 0.084	0.31 $\pm$ 0.078	
Median (min - max)	0.31 (0.13 - 0.64)	0.31 (0.13 - 0.64)	0.31 (0.15 - 0.50)	0.32 (0.14 - 0.50)	

P value refers GLM for unbalanced and balanced ANOVA. Log calculated with log base e.

Table C5: Comparison of characteristics for Louisville/Jefferson County Metropolitan Sewer District (MSD) sites by contributing sites leading to DRGWQTC compared to aggregate results from DRGWQTC.

Variables	Total (N=259)	Leading to DRGWQTC (N=212)	DRGWQTC (N=47)	P Value
Log_e N1 (copies/ml)				<i>0.729</i>
N	259	212	47	
Mean ± SD	4.17 ± 1.09	4.19 ± 1.12	4.13 ± 0.93	
Median (min - max)	4.14 (2.02 - 8.16)	4.14 (2.02 - 8.16)	4.12 (2.06 - 5.90)	
Log_e PMMoV (copies/ml)				<i>0.668</i>
N	259	212	47	
Mean ± SD	12.74 ± 1.40	12.72 ± 1.46	12.82 ± 1.07	
Median (min - max)	12.62 (7.79 - 17.38)	12.62 (7.79 - 17.38)	12.59 (10.19 - 15.08)	
Log_e CrAssphage (copies/ml)				<i>0.407</i>
N	259	212	47	
Mean ± SD	12.81 ± 1.45	12.84 ± 1.43	12.65 ± 1.52	
Median (min - max)	12.51 (9.18 - 17.10)	12.53 (10.03 - 17.10)	12.45 (9.18 - 16.30)	
Log_e(N1 mean)/ Log_e(PMMoV mean) (copies/ml)				<i>0.537</i>
N	259	212	47	
Mean ± SD	0.33 ± 0.10	0.33 ± 0.10	0.32 ± 0.08	
Median (min - max)	0.33 (0.14 - 0.93)	0.33 (0.14 - 0.93)	0.33 (0.15 - 0.47)	
Log_e(N1 mean)/ Log_e(CrAss mean) (copies/ml)				<i>0.922</i>
N	259	212	47	

Variables	Total (N=259)	Leading to DRGWQTC (N=212)	DRGWQTC (N=47)	P Value
Mean $\pm$ SD	0.33 $\pm$ 0.09	0.33 $\pm$ 0.09	0.33 $\pm$ 0.08	0.310
Median (min - max)	0.33 (0.14 - 0.64)	0.32 (0.15 - 0.64)	0.34 (0.14 - 0.50)	

P value refers GLM for unbalanced and balanced ANOVA. Log calculated with log base e.

Table C6: Comparison of characteristics for Louisville/Jefferson County Metropolitan Sewer District (MSD) sites by contributing sites leading to MFWQTC compared to aggregate results from MFWQTC.

Variables	Total (N=333)	Leading to MFWQTC (N=259)	MFWQTC (N=74)	P Value
Log_e N1 (copies/ml)				<i>0.230</i>
N	333	259	74	
Mean ± SD	3.73 ± 0.95	3.76 ± 0.98	3.61 ± 0.84	
Median (min - max)	3.76 (2.05 - 6.53)	3.76 (2.05 - 6.53)	3.68 (2.05 - 6.08)	
Log_e PMMoV (copies/ml)				<i>0.153</i>
N	333	259	74	
Mean ± SD	12.36 ± 1.40	12.42 ± 1.41	12.16 ± 1.37	
Median (min - max)	12.26 (8.23 - 16.87)	12.27 (8.23 - 16.87)	12.24 (8.89 - 15.25)	
Log_e CrAssphage (copies/ml)				<i>0.094</i>
N	333	259	74	
Mean ± SD	12.60 ± 1.53	12.68 ± 1.47	12.34 ± 1.71	
Median (min - max)	12.36 (9.40 - 18.65)	12.42 (9.56 - 18.60)	12.14 (9.40 - 18.65)	
Log_e(N1 mean)/ Log_e(PMMoV mean) (copies/ml)				<i>0.633</i>
N	333	259	74	
Mean ± SD	0.30 ± 0.08	0.31 ± 0.08	0.30 ± 0.08	
Median (min – max)	0.31 (0.14 – 0.63)	0.31 (0.14 – 0.59)	0.30 (0.15 – 0.63)	
Log_e(N1 mean)/ Log_e(CrAss mean) (copies/ml)				<i>0.732</i>
N	333	259	74	

Variables	Total (N=333)	Leading to MFWQTC (N=259)	MFWQTC (N=74)	P Value
Mean $\pm$ SD	0.30 $\pm$ 0.08	0.30 $\pm$ 0.08	0.30 $\pm$ 0.07	
Median (min – max)	0.30 (0.13 – 0.51)	0.29 (0.13 – 0.51)	0.30 (0.16 – 0.49)	

P value refers GLM for unbalanced and balanced ANOVA. Log calculated with log base e.

Table C7: Comparison of wastewater mean $\log_e$ SARS-CoV-2 RNA, $\log_e$ pepper mild mottle virus, and $\log_e$ cross-assembly phage by temperature at time of sample collection in Louisville/Jefferson County Metropolitan Sewer District (MSD) sites, from August 2020 to April 2021.

Temperature at time of sample collection	Variables	Log _e N1 mean			Log _e PMMov mean			Log _e CrAssphage mean		Log _e (N1 mean) / Log _e (PMMov mean)		Log _e (N1 mean) / Log _e (CrAssphage mean)				
		N	Mean ± SD	Median (min - max)	N	Mean ± SD	Median (min - max)	N	Mean ± SD	Median (min - max)	N	Mean ± SD	Median (min - max)	N	Mean ± SD	Median (min - max)
	Total	650	3.96 ± 1.02	3.92 (2.02 - 8.16)	650	12.59 ± 1.39	12.46 (6.23 - 17.38)	650	12.71 ± 1.43	12.39 (9.18 - 18.60)	650	0.32 ± 0.09	0.32 (0.14 - 0.93)	650	0.32 ± 0.09	0.32 (0.13 - 0.64)
	32°F-34°F	75	4.52 ± 1.11	4.40 (2.21 - 6.51)	75	12.99 ± 1.36	12.84 (10.14 - 16.03)	75	12.92 ± 1.38	12.80 (9.75 - 16.63)	75	0.35 ± 0.08	0.35 (0.15 - 0.49)	75	0.35 ± 0.09	0.36 (0.14 - 0.50)
	35°F-37°F	204	4.27 ± 0.97	4.29 (2.07 - 8.16)	204	12.68 ± 1.30	12.68 (8.23 - 16.37)	204	12.93 ± 1.60	12.46 (9.18 - 18.60)	204	0.34 ± 0.09	0.35 (0.14 - 0.93)	204	0.34 ± 0.09	0.34 (0.13 - 0.63)
	38°F-40°F	245	3.81 ± 0.93	3.81 (2.05 - 7.01)	245	12.50 ± 1.35	12.36 (6.23 - 17.38)	245	12.58 ± 1.30	12.26 (9.71 - 17.07)	245	0.31 ± 0.08	0.31 (0.15 - 0.68)	245	0.31 ± 0.08	0.31 (0.15 - 0.58)
41°F-43°F	95	3.53 ± 0.92	3.48 (2.05 - 7.04)	95	12.27 ± 1.57	12.24 (7.79 - 16.87)	95	12.41 ± 1.36	12.15 (9.92 - 16.48)	95	0.29 ± 0.09	0.29 (0.14 - 0.70)	95	0.29 ± 0.08	0.28 (0.15 - 0.64)	
44°F+	31	3.16 ± 0.79	3.00 (2.02 - 4.77)	31	12.79 ± 1.49	12.51 (8.98 - 16.91)	31	12.68 ± 1.33	12.52 (9.73 - 16.00)	31	0.25 ± 0.07	0.24 (0.15 - 0.38)	31	0.25 ± 0.07	0.25 (0.16 - 0.41)	
	P Value		<.001			0.008			0.014			<.001			<.001	
P value A refers to GLM for unbalanced and balanced ANOVA.																

Supplement D

Table D1. Louisville/Jefferson County properties without sewer connections, by sewershed.

Map ID	Site	Sample location type	Number of properties without sewer connections, by sewershed ^a
1	MFWQTC ^b	Treatment plant	2258
2	DRGWQTC ^c	Treatment plant	2184
3	CCWQTC	Treatment plant	1557
4	FFWQTP	Treatment plant	1324
5	HCWQTP	Treatment plant	462
6	Shawnee Park CSO189	Community	50
7	34th Street PS	Community	61
8	MH40870 Muddy Forks PS	Community	40
9	MH32985 Wood Road & Terry Road	Community	121
10	MH09837 Ashby Lane & Mill Creek	Community	90
11	MH08915A CSO140	Community	97
12	MH50495 CSO108	Community	616
13	MH23290 W. Indian Trail	Community	3
14	MH57769 Pineland Drive & Oakmont Dr.	Community	476
15	MH57350 Preston & South Park	Community	324
16	MH71910 CSO146	Community	19
17	MH70101 15th & Wilson	Community	85
	Not within any boundary		2162
	TOTAL PROPERTIES		9947

^a Based on 2018 U.S Census Bureau American Community Survey (ACS)

^b The MFWQTC sewershed has a total of 2,184 households with no connection, this value also includes 1,014 households captured in other contributing sewershed sampling.

^c The DRGWQTC sewershed has a total of 2,258 households with no connection, this value also includes 968 households captured in other contributing sewershed sampling.