

Supplementary material

Evaluating the Use of Alternative Normalization Approaches on SARS-CoV-2 Concentrations in Wastewater: Experiences from Two Catchments in Northern Sweden

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Table S1. Content of total nitrogen (TN) in domestic wastewater (expressed as g/p/d) based on literature values of excretion in faeces and urine as well as contribution from greywater.

TN1 [1]	TN2 [2]	TN3 [3]	TN4 [4]
14.0	13.9	12.5	11.0

Table S2. Content of total phosphorous (TP) in domestic wastewater (expressed as g/p/d) based on literature values of excretion in faeces and urine as well as contribution from greywater.

TP1 [1]	TP2 [2]	TP3 [3]	TP4 [4]
2.1	2.0	1.7	1.2

Table S3. Weekend and weekday average TP and TN population estimations and corresponding relative standard deviation (RSD) at UDD WWTP.

	Population Estimation			
	TN1	TN2	TN3	TN4
Weekday average	77957.6	78518.5	87312.6	99218.8
RSD (%)			5.7	
Weekend average	75802.0	76347.3	84898.2	96475.2
RSD (%)			4.1	
	TP1	TP2	TP3	TP4
Weekday average	55828.3	58619.8	68964.4	97699.6
RSD (%)			10.6	
Weekend average	56821.2	59662.3	70190.9	99437.2
RSD (%)			6.1	

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Table S4. Weekend and weekday average TP and TN population estimations and corresponding relative standard deviation (RSD) at RAN WWTP.

Population estimation				
	TN1	TN2	TN3	TN4
Weekday average	1956.9	1971.0	2191.8	2490.6
RSD (%)			24.5	
Weekend average	1923.6	1937.5	2154.5	2448.3
RSD (%)			8.3	
	TP1	TP2	TP3	TP4
Weekday average	1395.9	1465.7	1724.4	2442.9
RSD (%)			25.4	
Weekend average	1725.3	1811.5	2131.2	3019.2
RSD (%)			29.1	

Table S5. UDD WWTP Ct values and calculated Ct difference between replicates as well as calculated concentrations in each sample. Each replicate is based on an average value from RT-qPCR triplicates targeting the N1 gene of SARS-CoV-2.

Sample ID	Sample date	Ct (#)			Concentration (copies/mL)	
		Rep 1	Rep 2	Ct difference	Rep 1	Rep 2
UDD 1	2021-01-13	32.14	32.58	-0.44	50.71	37.22
UDD 2	2021-01-19	32.56	32.44	0.12	37.82	41.26
UDD 3	2021-01-22	33.20	33.45	-0.25	24.26	20.33
UDD 4	2021-01-25	32.77	33.11	-0.34	32.63	25.86
UDD 5	2021-01-27	33.16	32.20	0.96*	24.86	48.56
UDD 6	2021-01-29	33.26	33.48	-0.22	23.30	19.99
UDD 7	2021-02-01	34.13	32.80	1.3*	12.61	32.01
UDD 8	2021-02-03	31.72	32.34	-0.62	68.29	44.20
UDD 9	2021-02-05	30.22	30.43	-0.21	193.66	167.65
UDD 10	2021-02-08	30.89	31.09	-0.20	121.51	105.91
UDD 11	2021-02-10	33.34	34.45	-1.1*	21.94	10.11
UDD 12	2021-02-12	32.26	32.02	0.24	46.68	55.11
UDD 13	2021-02-15	31.13	30.19	0.94*	102.76	197.84
UDD 14	2021-02-17	30.20	31.13	-0.93*	196.80	103.04
UDD 15	2021-02-19	30.69	30.68	0.010	140.00	140.26
UDD 16	2021-02-22	29.95	30.06	-0.11	234.00	217.16
UDD 17	2021-02-24	30.05	29.76	0.29	218.97	267.67
UDD 18	2021-02-26	30.88	30.57	0.31	122.55	151.47
UDD 19	2021-03-01	29.87	29.21	0.66	246.70	391.67

Key: *=Ct difference >0.90.

Table S6. RAN WWTP Ct values and calculated Ct difference between replicates as well as calculated concentrations in each sample. Each replicate is based on an average value from RT-qPCR triplicates targeting the N1 gene of SARS-CoV-2.

Sample ID	Sample date	Ct (#)			Concentration (copies/mL)	
		Rep 1	Rep 2	Ct difference	Rep 1	Rep 2
RAN 1	2021-02-01	34.76	34.06	0.70	8.15	13.33

RAN 2	2021-02-03	nd	nd	-	nd	nd
RAN 3	2021-02-05	nd	nd	-	nd	nd
RAN 4	2021-02-08	nd	nd	-	nd	nd
RAN 5	2021-02-10	33.17	32.96	0.21	24.68	28.57
RAN 6	2021-02-12	31.83	31.34	0.49	63.16	88.70
RAN 7	2021-02-15	30.50	30.13	0.37	159.37	206.04
RAN 8	2021-02-17	30.64	30.99	-0.35	144.83	112.58
RAN 9	2021-02-19	30.59	30.63	-0.040	149.64	145.20
RAN 10	2021-02-22	29.86	31.61	-1.8*	248.61	73.55
RAN 11	2021-02-24	30.78	31.22	-0.44	131.43	96.27
RAN 12	2021-02-26	30.29	30.51	-0.22	184.61	158.24
RAN 13	2021-03-01	31.91	31.75	0.16	59.78	66.75

Key: nd=not detected, *=Ct difference >0.90.

Table S7. UDD WWTP Ct values from RT-qPCR of PMMoV and calculated Ct difference as well as calculated concentrations in each sample replicate. Each replicate is based on an average value from RT-qPCR duplicates.

Sample ID	Sample date	Ct (#)			Concentration (copies/mL)	
		Rep 1	Rep 2	Ct difference	Rep 1	Rep 2
UDD 1	2021-01-13	23.93	24.25	-0.32	49292.4	49828.8
UDD 2	2021-01-19	24.10	23.79	0.31	43952.6	46150.2
UDD 3	2021-01-22	-	23.88	-	-	51906.8
UDD 4	2021-01-25	23.09**	23.46	-0.37	87786.8	61943.4
UDD 5	2021-01-27	23.52	24.26	-0.74	65509.1	35006.0
UDD 6	2021-01-29	24.38	23.59**	0.79	36119.6	73821.5
UDD 7	2021-02-01	24.01**	24.83	-0.82	46762.8	31153.6
UDD 8	2021-02-03	23.51	23.56	-0.050	65629.7	68902.8
UDD 9	2021-02-05	23.07	23.77	-0.70	88902.4	59830.4
UDD 10	2021-02-08	24.00	23.77	0.23	47044.9	40832.8
UDD 11	2021-02-10	22.86**	23.44**	-0.58	103016.5	79077.0
UDD 12	2021-02-12	22.44**	22.75**	-0.31	136938.6	106412.9
UDD 13	2021-02-15	22.88**	23.86	-0.98*	101552.8	63789.7
UDD 14	2021-02-17	23.74	23.79	-0.020	55969.9	59227.8
UDD 15	2021-02-19	23.47	23.60	-0.13	67682.8	64280.8
UDD 16	2021-02-22	24.02	23.09**	0.93*	46258.5	102083.3
UDD 17	2021-02-24	22.65**	22.27**	0.38	119080.6	154443.5
UDD 18	2021-02-26	22.82**	24.99	-2.2*	106038.6	24190.0
UDD 19	2021-03-01	23.48	23.70	-0.22	67129.1	56721.1

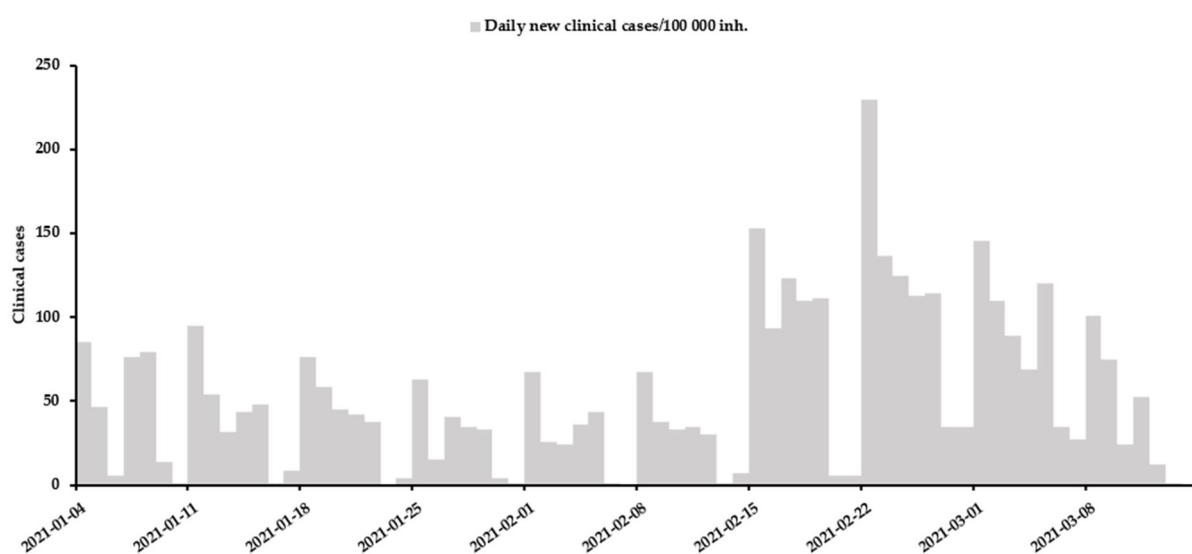
Key: *=Ct difference >0.90, **= reanalysed samples.

Table S8. RAN WWTP Ct values from RT-qPCR of PMMoV and calculated Ct difference as well as calculated concentrations in each sample replicate. Each replicate is based on an average value from RT-qPCR duplicates.

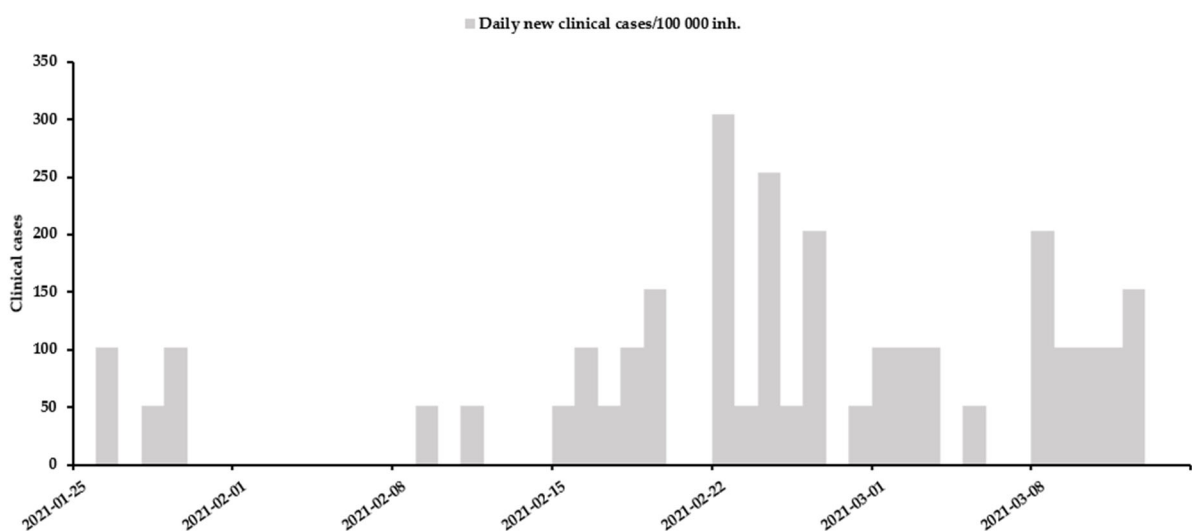
Sample ID	Sample date	Ct (#)			Concentration (copies/mL)	
		Rep 1	Rep 2	Ct difference	Rep 1	Rep 2
RAN 1	2021-02-01	24.41	23.78	0.63	35487.1	54753.8
RAN 2	2021-02-03	23.92	24.16	-0.24	49614.0	41914.9
RAN 3	2021-02-05	23.02	21.99**	1.0*	91920.2	186888.9

RAN 4	2021-02-08	23.13**	24.59	-1.5*	85470.0	31276.2
RAN 5	2021-02-10	23.41	23.24	0.17	70394.7	79329.7
RAN 6	2021-02-12	23.96	23.46	0.50	48366.2	68067.7
RAN 7	2021-02-15	22.21	21.89	0.32	160840.7	200011.2
RAN 8	2021-02-17	23.22	23.53**	-0.31	80258.6	64954.7
RAN 9	2021-02-19	24.31**	24.59**	-0.28	37976.3	31313.3
RAN 10	2021-02-22	23.42	23.13**	0.29	70018.5	85170.7
RAN 11	2021-02-24	24.56**	23.43	1.1*	31936.9	69656.6
RAN 12	2021-02-26	23.66	23.51**	0.15	59123.9	65547.6
RAN 13	2021-03-01	23.16	22.42	0.74	83866.1	139092.2

Key: *=Ct difference >0.90, **= reanalysed samples.



(a)



(b)

Figure S1. Daily new clinical cases per 100 000 inh. over the sampling period in the catchment area of: (a) UDD WWTP and (b) RAN WWTP. The datemark display is held on the first Monday of every new week.

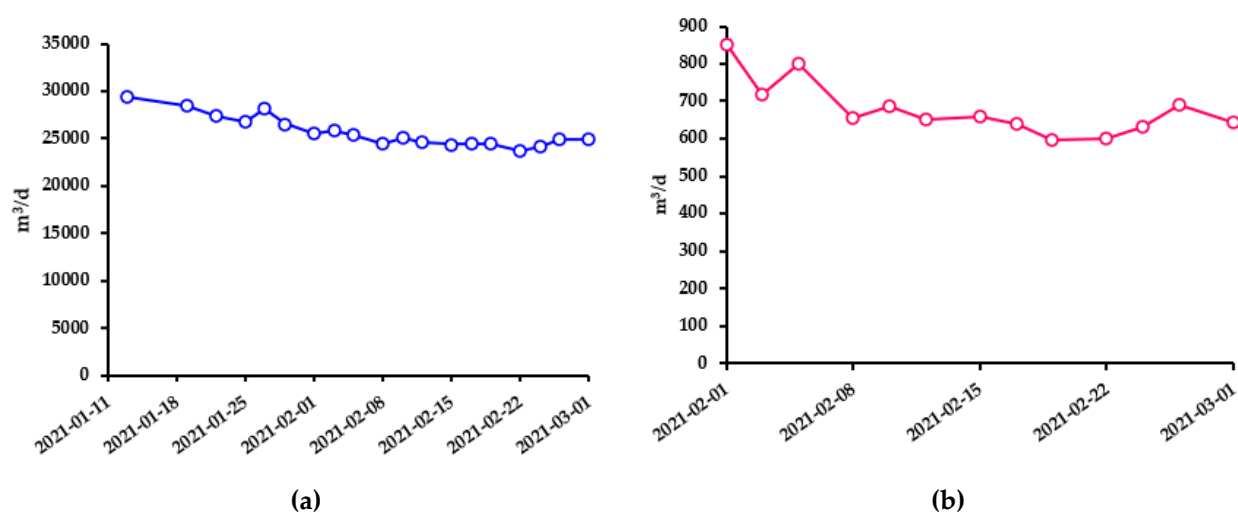
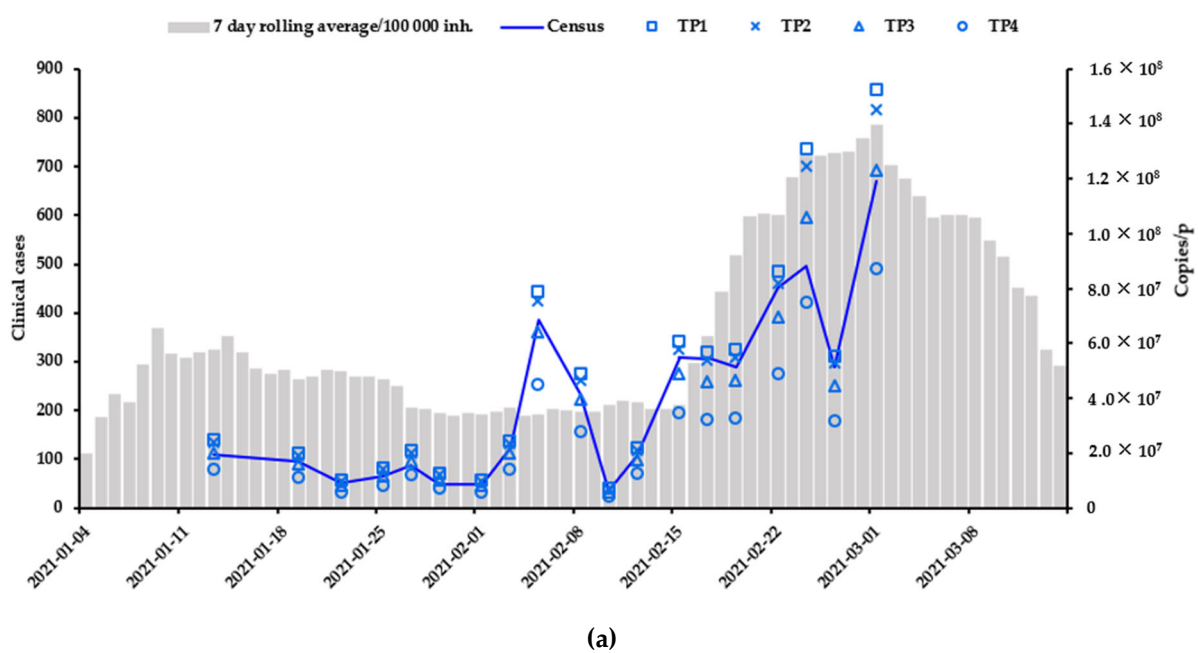


Figure S2. Average daily WWTP flow over the sampling period at: (a) UDD WWTP and (b) RAN WWTP.



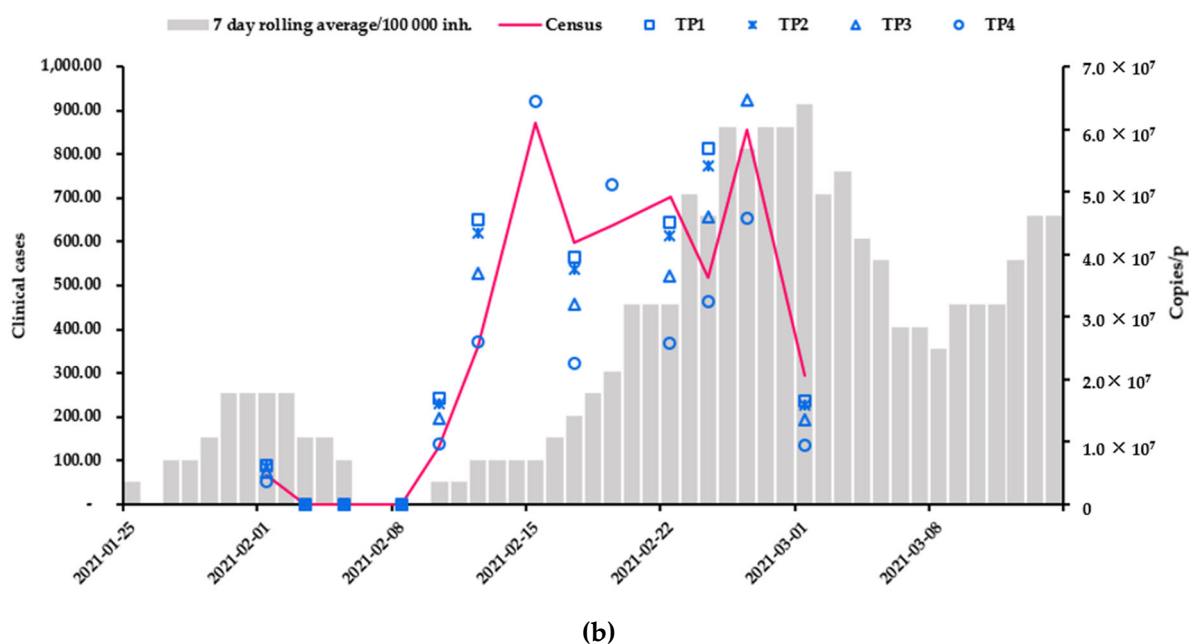


Figure S3. Longitudinal data of SARS-CoV-2 viral loads normalized to census population (census) and TP estimated population size (TP1-TP5) benchmarked with daily new clinical cases per 100 000 inh. based on day of testing at: (a) UDD WWTP and (b) RAN WWTP. The datemark display is held on the first Monday of every new week.

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