

## CORONASTEP Report 39 (Week 01) SARS-CoV-2 Sewage Surveillance in Luxembourg

### Summary

This report 39 presents the results of SARS-CoV-2 contamination of wastewater at the entrance of the 13 wastewater treatment plants (WWTPs) during the first week of 2021. Two sampling dates were performed during this week, excepted for Hespérange, Boevange-sur-Attert and Echternach, where only one sample was collected.

At the beginning of January 2021, the SARS-CoV-2 RNA fluxes in the treatment plants remain high and comparable to those observed during the last two weeks of 2020, indicating a still important prevalence of the virus in wastewater at national and regional level. The slow downward trend observed so far seems to have stabilised on a plateau.

For all the WWTPs studied individually, SARS-CoV-2 RNA fluxes still show irregular variations on a weekly basis. A downward trend can also be observed from the maximum peak of the current wave, followed by a stabilization of the signal for about two to three weeks. Hespérange seems to be behaving a little differently with a possible increase in the presence of the virus. However, this needs to be confirmed in the coming weeks.

Table 1 – National level of SARS-CoV-2 contamination of wastewaters in Luxembourg.



*Dark green: negative samples for SARS-CoV-2 gene E (-), Green to red: positive samples for SARS-CoV-2 gene E. The intensity of the color is related to the national SARS-CoV-2 flux (RNA copies / day / 100 000 equivalent inhabitants).*

Week	National Contamination Level
Week 3	Dark Green
Week 7	Dark Green
Week 9	Dark Green
Week 11	Dark Green
Week 14	Orange
Week 15	Yellow-Orange
Week 16	Yellow
Week 17	Yellow
Week 18	Yellow
Week 19	Yellow
Week 20	Yellow
Week 21	Yellow
Week 22	Yellow
Week 23	Yellow
Week 24	Yellow
Week 25	Yellow
Week 26	Yellow
Week 27	Yellow
Week 28	Yellow
Week 29	Yellow
Week 30	Yellow
Week 31	Yellow
Week 32	Yellow
Week 33	Yellow
Week 34	Yellow
Week 35	Yellow
Week 36	Yellow

Week	National Contamination Level
Week 37	Yellow
Week 38	Yellow
Week 39	Yellow
Week 40	Yellow
Week 41	Yellow
Week 42	Orange
Week 43	Red
Week 44-1	Red
Week 44-2	Red
Week 45-1	Red
Week 45-2	Red
Week 45-3	Red
Week 46-1	Red
Week 46-2	Red
Week 46-3	Red
Week 47-1	Red
Week 47-2	Red
Week 48-1	Red
Week 48-2	Red
Week 48-3	Red
Week 49-1	Red
Week 49-2	Red
Week 50-1	Yellow-Orange
Week 50-2	Yellow-Orange
Week 51-1	Yellow-Orange
Week 51-2	Yellow-Orange
Week 51-2	Yellow-Orange
Week 52	Yellow-Orange
Week 53	Yellow-Orange
Week 01-1	Yellow-Orange
Week 01-2	Yellow-Orange

Figure 1a – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in Luxembourgish wastewater samples from December 2019 to January 2021. Grey squares: daily-confirmed cases for Luxembourgish residents (<https://data.public.lu/fr/datasets/donnees-covid19/>), Blue dots: cumulative SARS-CoV-2 flux (RNA copies / day / 100 000 equivalent inhabitants).

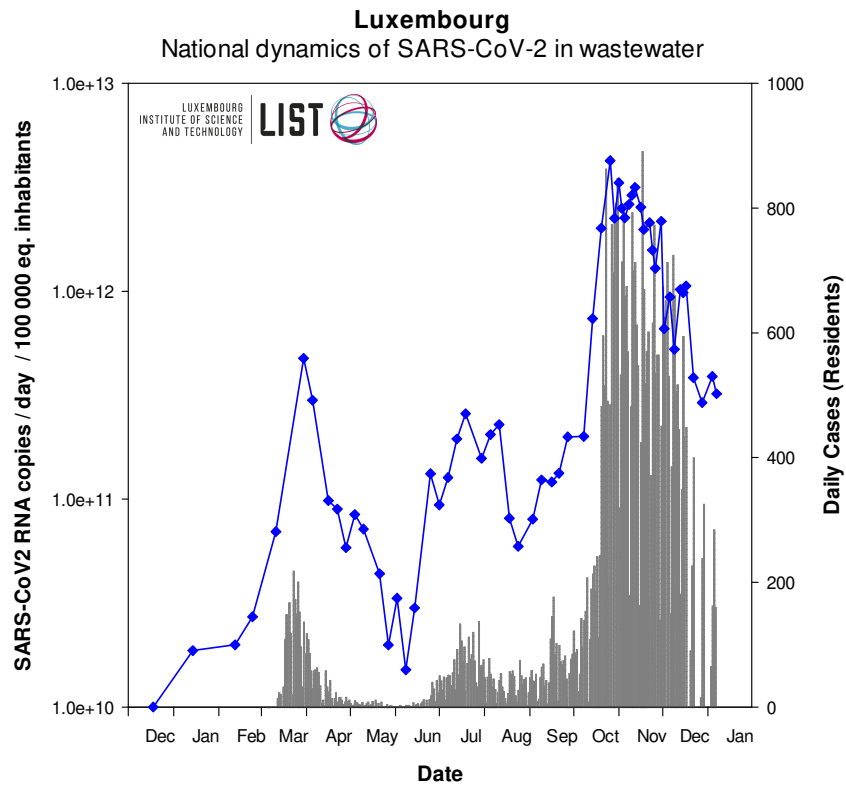


Figure 1b – Close-up of Figure 1a showing results from September 1<sup>st</sup> on.

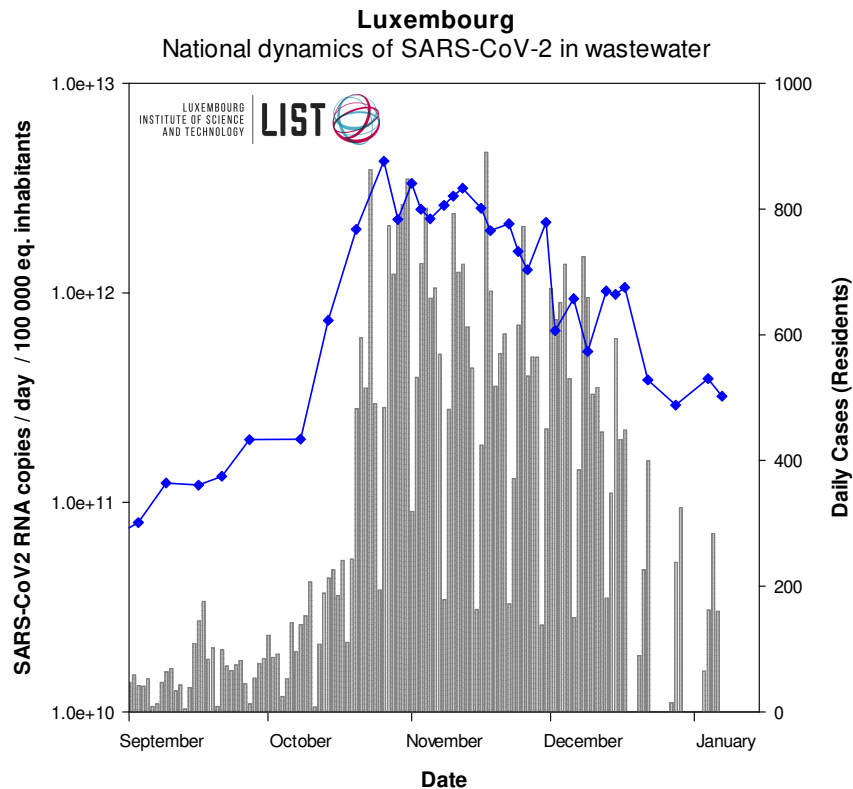




Figure 2a – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in the four most impacted wastewater treatment plants from March 2020 to January 2021. Grey squares: daily-confirmed cases for the contributory area of each wastewater treatment plant, dots: SARS-CoV-2 flux (RNA copies / day / 10 000 equivalent inhabitants).

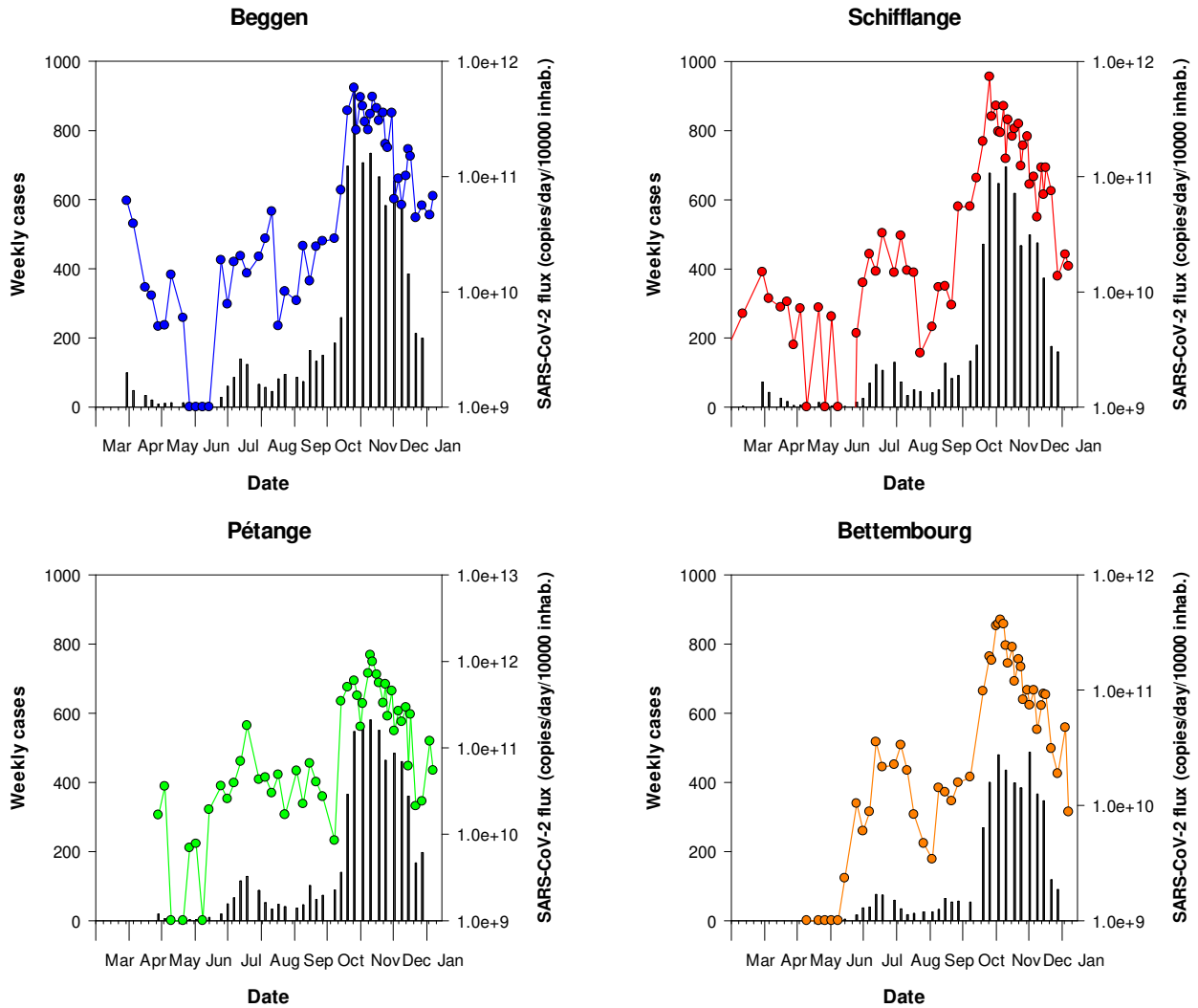


Figure 2b – Close-up of Figure 2a showing results from September 1<sup>st</sup> on.

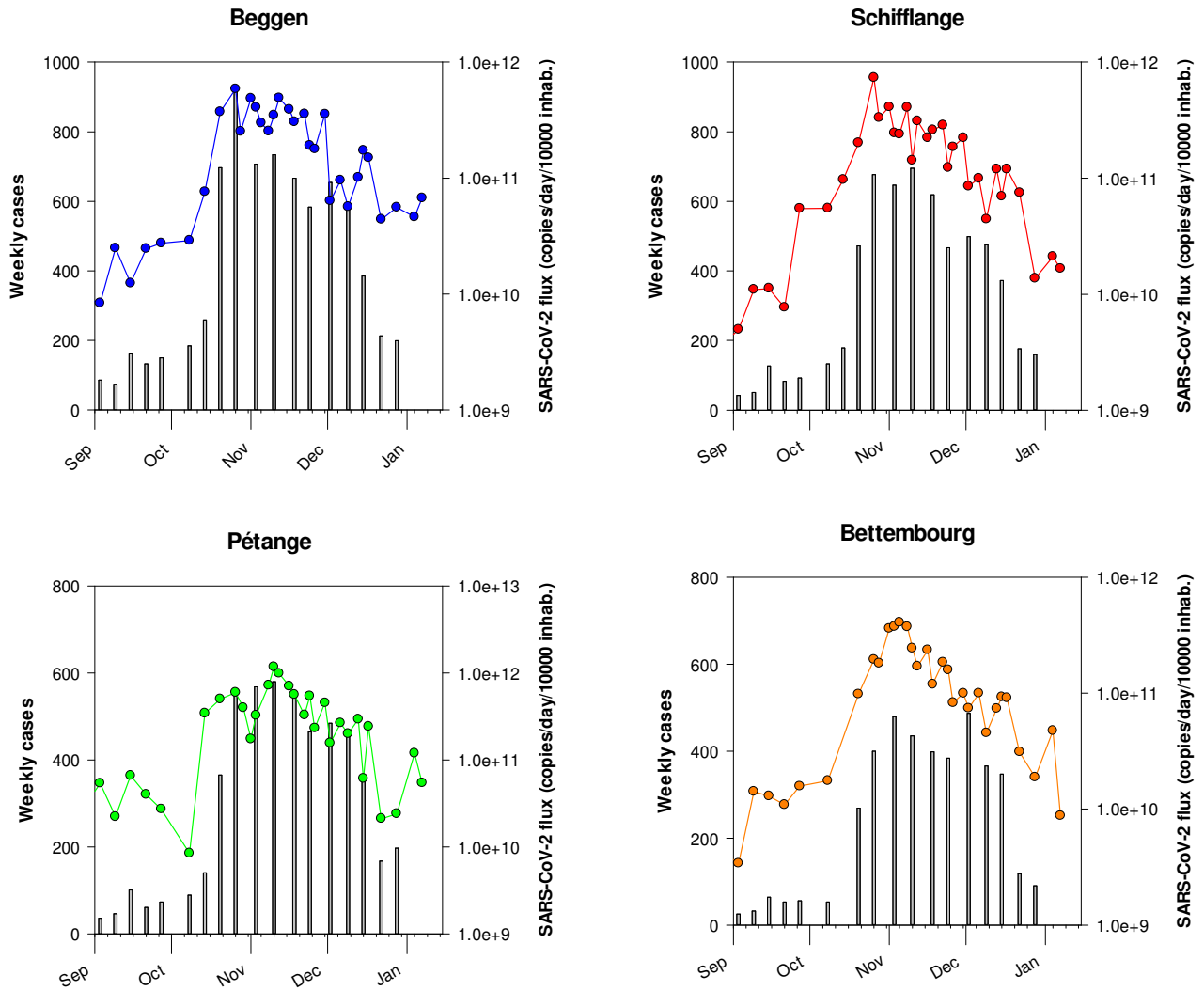


Figure 3a – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in Hespérange, Mersch and Boevange-sur-Attert wastewater treatment plants from March 2020 to January 2021. Grey squares: daily-confirmed cases for the contributory area of each wastewater treatment plant, dots: SARS-CoV-2 flux (RNA copies / day / 10 000 equivalent inhabitants).

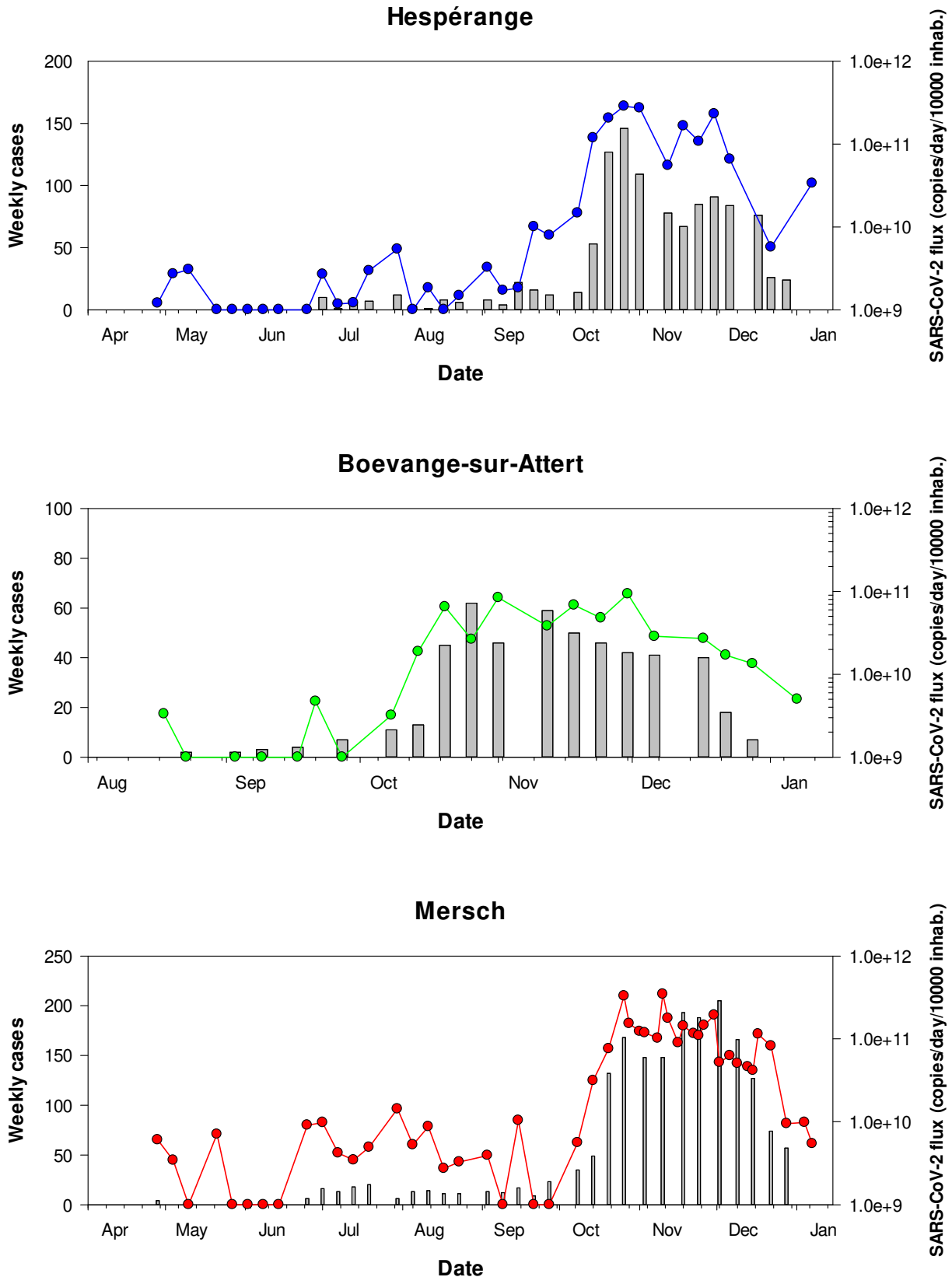


Figure 3b – Close-up of Figure 3a showing results from September 1<sup>st</sup> on.

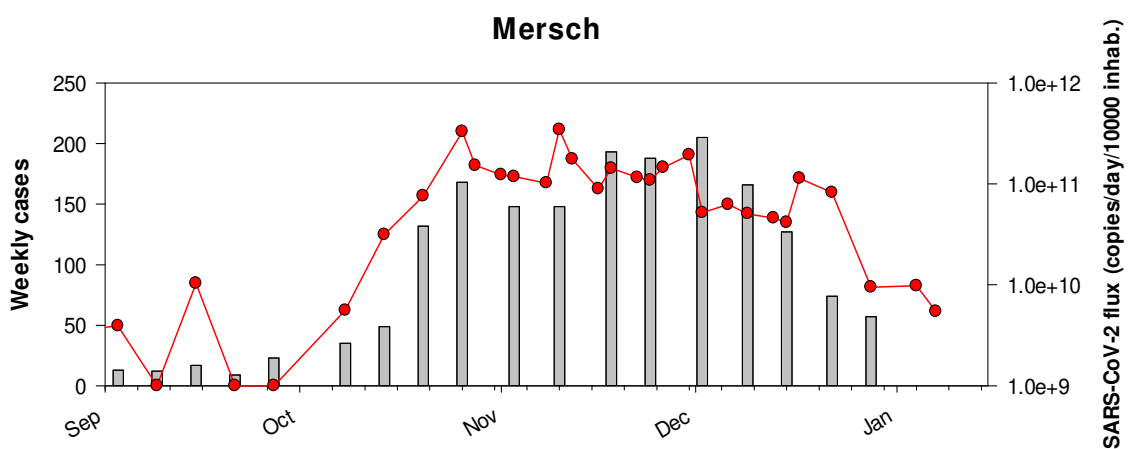
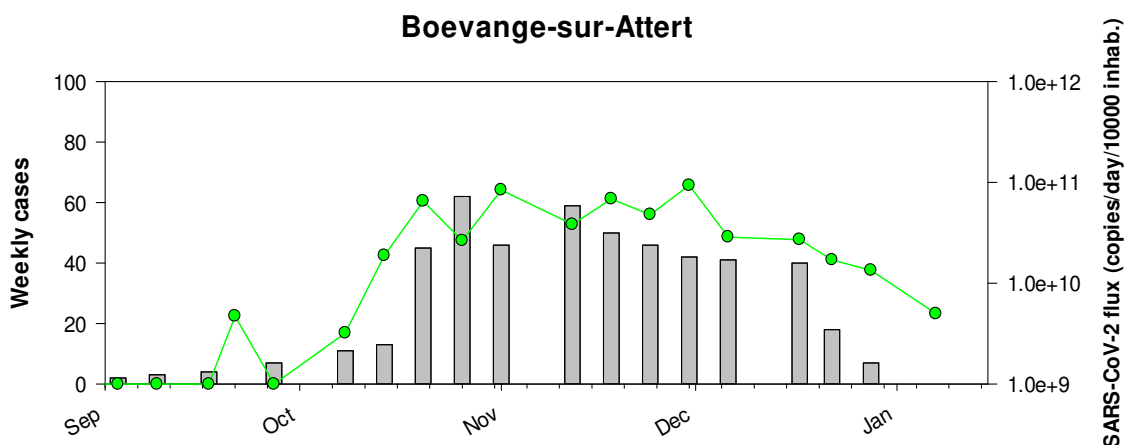
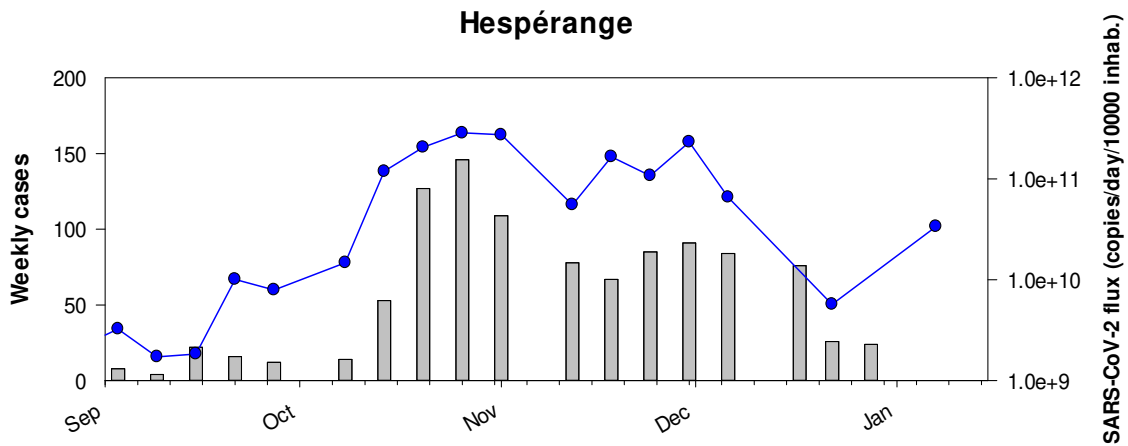


Figure 4a – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in SIDEST wastewater treatment plants from March 2020 to January 2021. Grey squares: daily-confirmed cases for the contributory area of each wastewater treatment plant, dots: SARS-CoV-2 flux (RNA copies / day / 10 000 equivalent inhabitants).

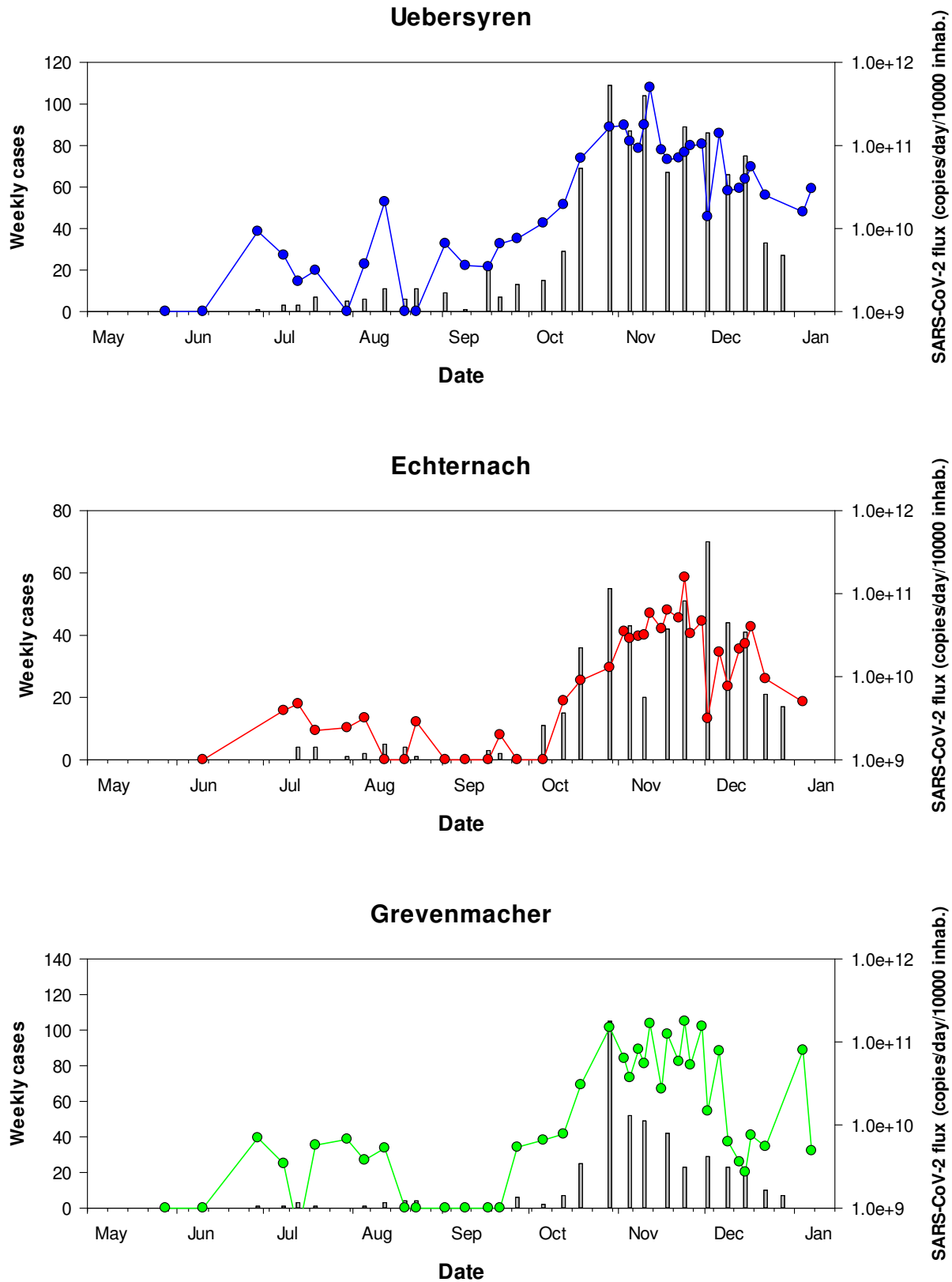




Figure 4b – Close-up of Figure 4a showing results from September 1st on.

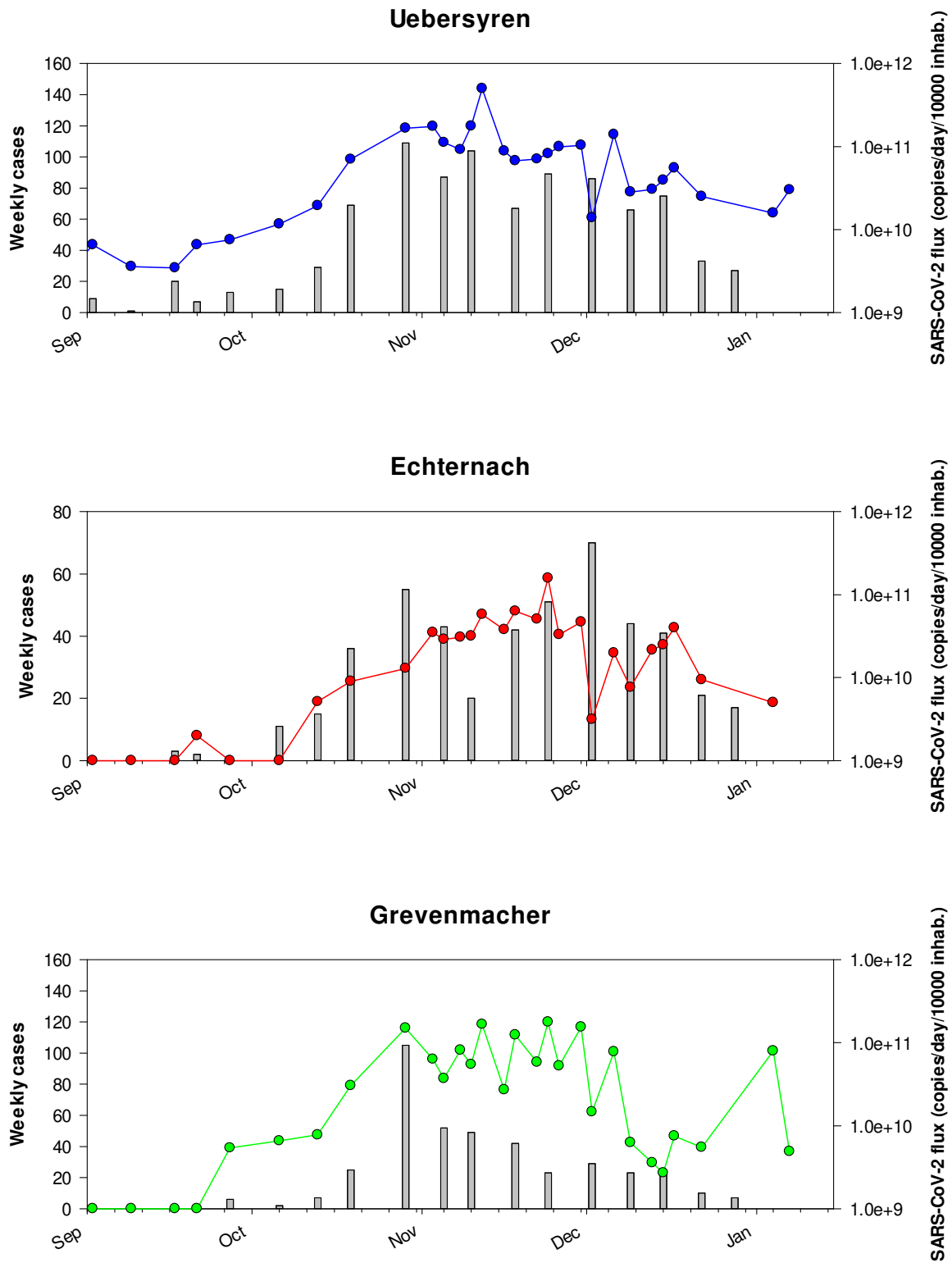


Figure 5a – RT-qPCR quantification time-course monitoring of SARS-CoV-2 (E gene) in SIDEN wastewater treatment plants from March 2020 to January 2021. Grey squares: daily-confirmed cases for the contributory area of each wastewater treatment plant, dots: SARS-CoV-2 flux (RNA copies / day / 10 000 equivalent inhabitants).

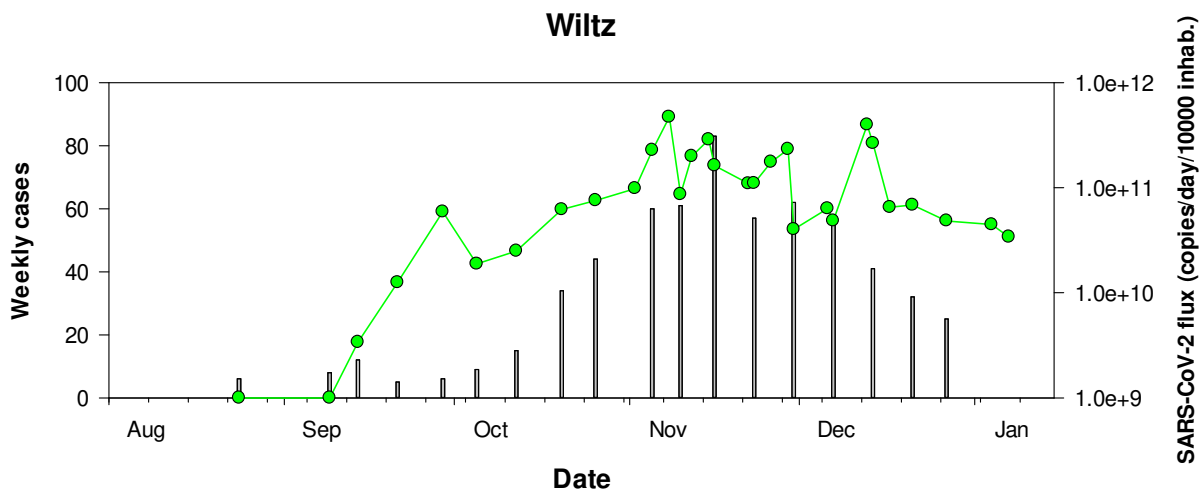
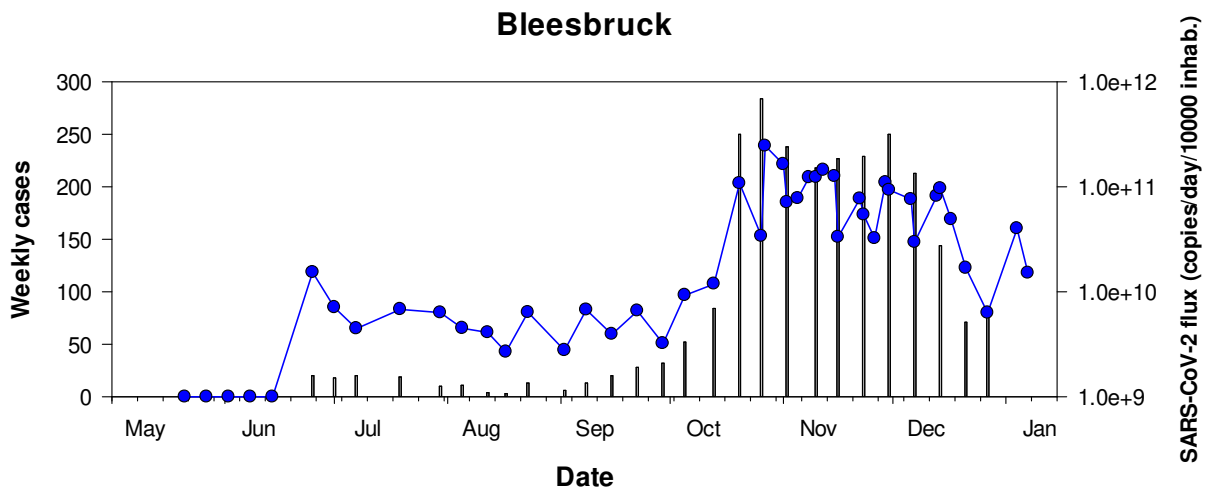
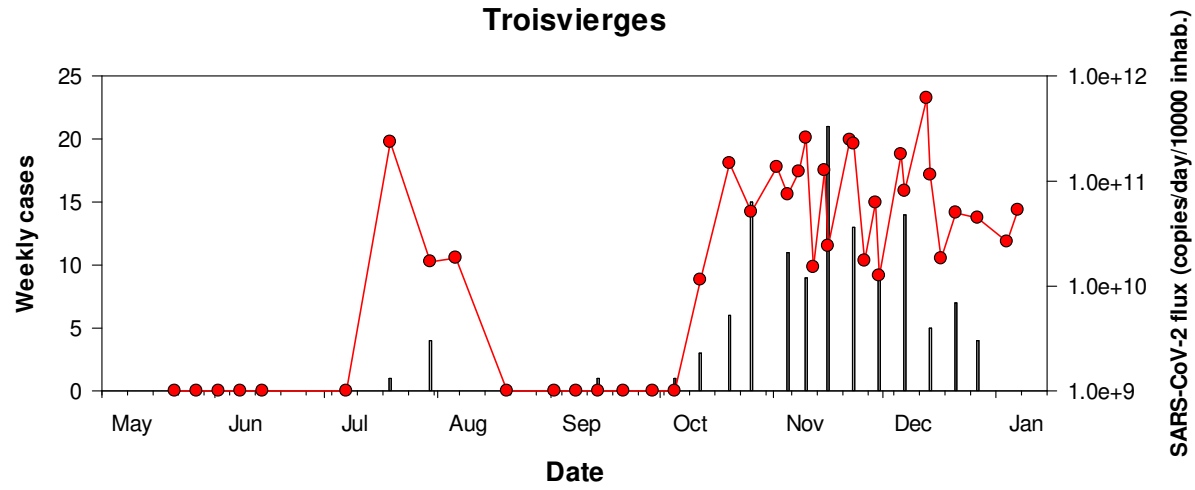
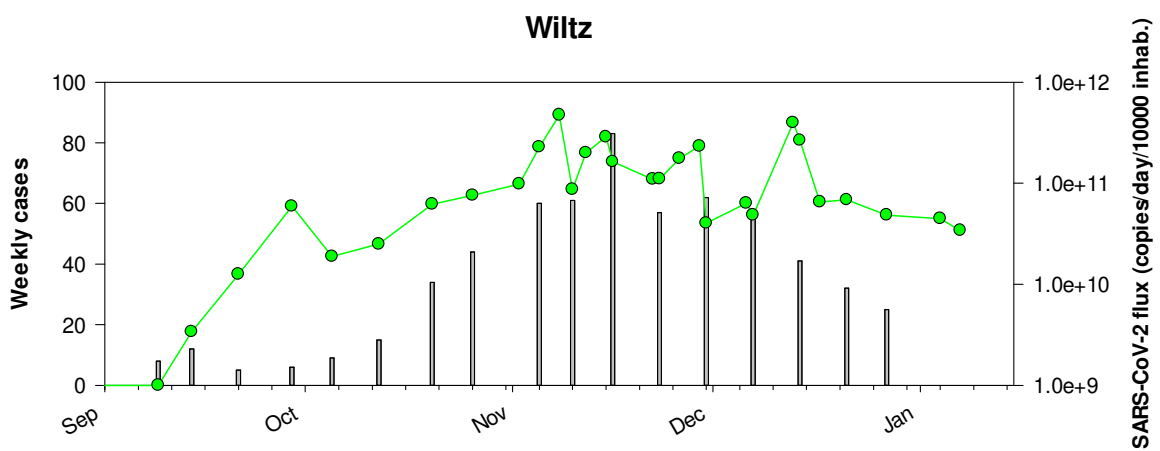
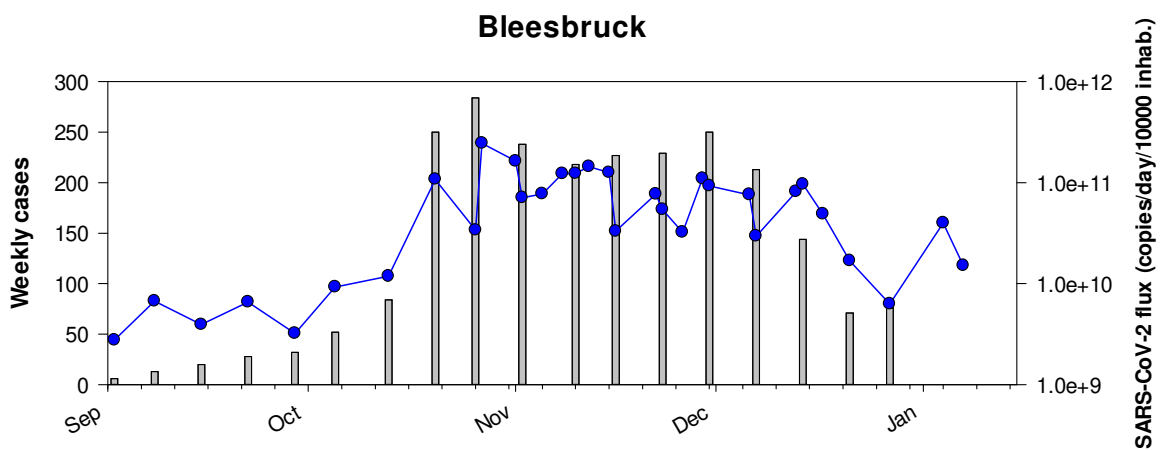
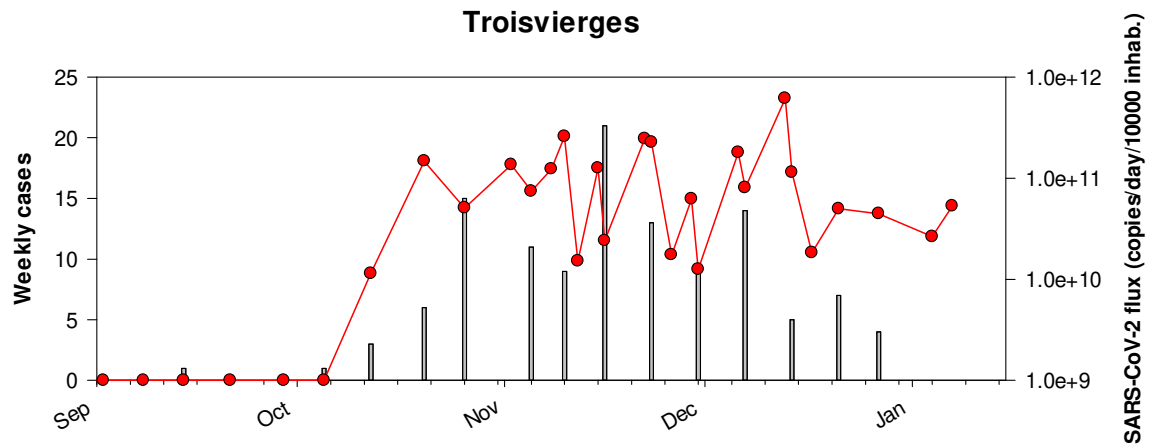


Figure 5b – Close-up of Figure 5a showing results from September 1st on.





## Materials and Methods

### Sewage samples

From March 2020 to January 2021, up to thirteen WWTPs were sampled at the inlet of the plant according to the planning presented in Table 3. The operators of the WWTPs sampled a 24-h composite sample of 96 samples according to your own sampling procedure. Composite sample was stored at 4°C until sample processing.

### Sample processing

The samples were transported to the laboratory at 4°C and viral RNA was isolated on the day of sampling. Larger particles (debris, bacteria) were removed from the samples by pelleting using centrifugation at 2,400 x g for 20 min at 4°C. A volume of 120 mL of supernatant was filtered through Amicon® Plus-15 centrifugal ultrafilter with a cut-off of 10 kDa (Millipore) by centrifugation at 3,220 x g for 25 min at 4°C. The resulting concentrate was collected and 140 µL of each concentrate was then processed to extract viral RNA using the QIAamp Viral RNA mini kit (Qiagen) according to the manufacturer's protocol. Elution of RNA was done in 60 µL of elution buffer.

### Real-time One-Step RT-PCR

Samples are screened for the presence of *Sarbecovirus* (*Coronaviridae*, *Betacoronaviruses*) and/or SARS-CoV-2 virus RNA by two distinct real-time one-step RT-PCR, one on the E gene (Envelope small membrane protein) and the second on the N gene (nucleoprotein). The E gene real-time RT-PCR can detect *Sarbecoviruses*, i.e. SARS-CoV, SARS-CoV-2 and closely related bat viruses. In the context of the COVID19 pandemic, it can be assumed that only SARS-CoV-2 strains will be detected by this assay given that SARS-CoV virus has been eradicated and other bat viruses do not commonly circulate in the human population. The E gene assay is adapted from Corman et al. [17]. The N gene real-time RT-PCR assay (N1 assay) specifically detects SARS-CoV-2 virus. It is adapted from the CDC protocol<sup>1</sup>. The two primers/probe sets are presented in Table 3. The RT-qPCR protocols and reagents were all provided by the LIH.

Table 4 – RT-qPCR primer-probe sets

Target	Primer name	Primer sequence (5' to 3')	References
E gene	E_Sarbeco_F1	5-ACAGGTACGTTAATAGTTAATAGCGT-3	Corman et al., 2020
	E_Sarbeco_R2	5-ATATTGCAGCAGTACGCACACA-3	
	E_Sarbeco_P1	5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1	
N gene	2019-nCoV_N1_Fw	5'-GAC CCC AAA ATC AGC GAA AT-3'	CDC
	2019-nCoV_N1_Rv	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	
	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'	

Each reaction contained 5 µL of RNA template, 5 µL of TaqPath 1-step RT-qPCR MasterMix (A15299, Life Technologies), 0.5 µL of each primer (20 µM) and probe (5 µM) and the reaction volume was adjusted to a final volume of 20 µL with molecular biology grade water. Thermal cycling reactions were carried out at 50 °C for 15 min, followed by 95 °C for 2 min and 45 cycles of 95 °C for 3 sec and 58°C (E gene) or 55°C (N gene) for 30 sec using a Viia7 Real-Time PCR Detection System (Life Technologies). Reactions were considered positive (limit of detection – LOD) if the cycle threshold (Ct value) was below 40 cycles.

<sup>1</sup> <https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf>

## Controls

A non-target RNA fragment commercially available (VetMAX™ Xeno™ IPC and VetMAX™ Xeno™ IPC Assay, ThermoFischer Scientific) was added to the viral RNA extract from sewage concentrates as an internal positive control (IPC). This IPC-RNA is used to control the performance of the RT-qPCR (E gene) and to detect the presence of RT-qPCR inhibitors.

Viral RNA copies quantification of both targeting genes in wastewater samples was performed using RT-qPCR standard curves generated using EDX SARS-CoV-2 Standard (Biorad). This standard is manufactured with synthetic RNA transcripts containing 5 targets (E, N, S, ORF1a, and RdRP genes of SARS-CoV-2, 200,000 copies/mL each). Using such a standard, the limits of quantification (LOQ) of both RT-qPCR assays were estimated to 1 RNA copy per reaction (Figure 6).

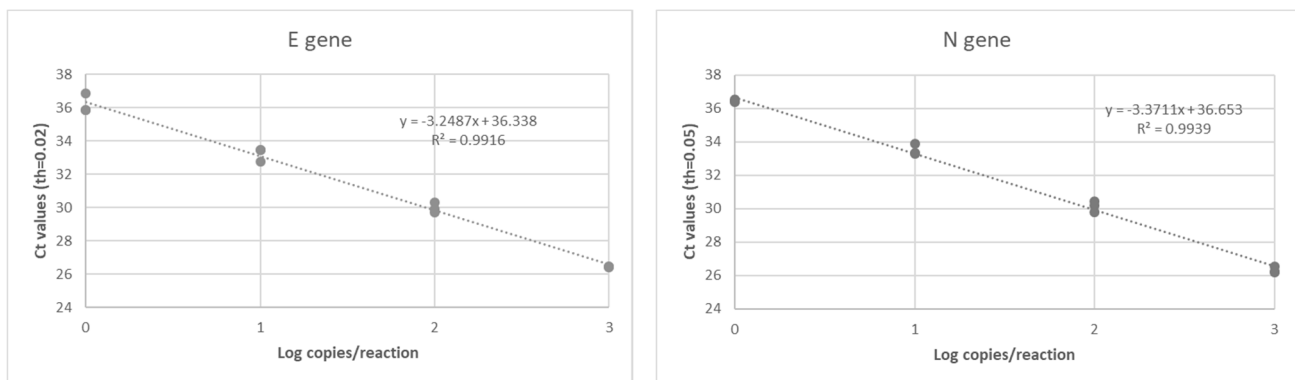


Figure 6 – RT-qPCR standard curves established for both targeting genes (E gene and N gene) of SARS-CoV-2 using a commercially available standard (Biorad).

## Data interpretation

A sample is declared positive for the presence of SARS-CoV-2 if both targets (E and N gene) are detected with Ct values less than or equal to the LOQ. If only one target is detected or if target genes are detected with Ct values between the LOD and the LOQ, samples are reported as presumptive positive (+/-). A sample is declared negative when no target genes are detected (Ct values superior to the LOD).

In case of presumptive positive, sample is tested again using another RT-qPCR detection assay (Allplex 2019-nCoV Assay, Seegene). This commercially available detection kit is a multiplex real-time RT-PCR assay for simultaneous detection of three target genes of SARS-CoV-2 in a single tube. The assay is designed to detect RdRP and N genes specific for SARS-CoV-2, and E gene specific for all *Sarbecovirus* including SARS-CoV-2.

As shown in Figure 7, a highly significant correlation (Pearson Correlation,  $R^2=0.964$ ,  $p = 5.979 \cdot 10^{-24}$ ) was obtained between the SARS-CoV-2 RNA concentrations estimated using the E gene and the N gene, respectively. Therefore, only the E gene results were presented in this report.

Figure 7 - Relationship between the SARS-CoV-2 RNA concentration (RNA copies / L of wastewater) estimated by the both distinct RT-qPCR systems targeting the E and N gene, respectively (n=415),

