Final Report “AbwasSARS-CoV-2”

Quantifying SARS-CoV-2 RNA in samples of six wastewater treatment plants and estimating the effective reproductive number R_e in Switzerland

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Sample collection at the wastewater treatment plant Zurich ARA Werdhölzli Photo Esther Michel

Executive Summary

Aim. To follow the dynamics of the Covid-19 pandemic independently of reported cases, the Federal Office of Public Health supported the development and application of wastewater-based epidemiology (WBE) through this research project *AbwasSARS-CoV-2*.

Monitoring period and locations. From 1 February 2021 to 30 April 2023, Eawag analyzed daily raw wastewater samples for SARS-CoV-2 RNA from six locations. These encompassed catchments of the six wastewater treatment plants Altenrhein, Chur, Geneva, Laupen, Lugano and Zurich, covering together approximately 14% of the Swiss population. Across all locations, SARS-CoV-2 RNA was detected in almost all samples[1]. The results were typically published once per week on a publically accessible online dashboard.

Comparison of wastewater and case data. During most of the monitoring period, Switzerland invested substantially in clinical case surveillance, allowing robust comparisons between wastewater and clinical data. The 7-day medians of wastewater data and reported positive cases showed similar trends until January 2023, when free clinical testing was abandoned. Consequently, the number of reported cases decreased substantially, while wastewater data was unaffected. The correlation between wastewater data and case data remained high, however, at a different scale. Although only every second infected person sheds SARS-CoV-2 RNA in feces, SARS-CoV-2 was detected almost all days except during a few days in summer 2021 when incidence was low. The findings highlight the potential of wastewater to provide objective insights into COVID-19 disease dynamics.

Effective reproductive number. Alignment between wastewater data and clinical case reporting was further assessed through comparison of the effective reproductive number R_e. R_e indicates the efficiency of SARS-CoV-2 spread in a given population. Throughout the study, the R_e estimated from clinical cases and wastewater largely agreed. Notably, during periods with low clinical surveillance – indicated by high test positivity rates or very low numbers of reported cases – the uncertainty of R_e based on case data increased substantially, while the uncertainty based on wastewater remained low.

Open challenges. Wastewater shows SARS-CoV-2 dynamics, but cannot yet be used to estimate an absolute number of infected individuals. This is due to uncertainty in: i) variation in the virus shedding rates among individuals, ii) fate of viral RNA during in-sewer transport, and iii) impacts of lab methodology such as efficiency of RNA extraction. Establishment of empirical relationships between RNA loads in wastewater and case numbers suffers from potential bias in case numbers due to the unknown fraction of unreported cases and varying testing regimes, which depended on regional capacity of testing facilities and willingness to test.

Wastewater as lead indicator. Whether or not wastewater data can serve as an early indicator of Covid-19 is highly dependent on the investments in clinical case surveillance. In Switzerland, clinical sample processing and reporting was sufficiently efficient such that wastewater data dynamics were coincident to clinical case dynamics. However, when there was limited or insufficient testing (high positivity rates), wastewater still provided timely information about the extent of circulation of SARS-CoV-2 in the population.

Variants of concern. The wastewater extracts obtained in this project were sequenced and the sequencing data was used to estimate the prevalence of (emerging) variants [sequencing and analysis was part of another contract]. Typically, new variants could be detected earlier – up to several weeks – in wastewater compared to sequencing clinical samples.

Outlook. In view of the suggested institutionalization of WBE by Swiss parliament[2] the experience from this project can inform future activities: to guarantee high quality data, it is recommended to take and analyze at least five wastewater samples per week. This will allow estimating reliably the effective reproductive number R_e and facilitate detecting the introduction and prevalence of emerging variants. Approximately 25% of the Swiss population could be covered when sampling ten large wastewater treatment plants. What has been shown successfully for SARS-CoV-2 in this project can be extended to other pathogens. When there is already investment in the infrastructure to collect, transport, and process samples, the surveillance can be extended to other pathogens at little additional cost, e.g. to respiratory viruses such as RSV or Influenza A and B. An additional beneficial potential is the analysis of wastewater for chemicals, e.g. pharmaceuticals with abuse potential, (il)licit drugs, antihistamines and other exogenous and endogenous health indicators.

[1] Detection of N-gene targeting the N1-region …
… on average in 88% of samples [Feb - Nov 2021, laboratory protocol based on centrifugal filter concentration]
… in over 99.4% of samples [Nov 2021 to Apr 2023, laboratory protocol based on direct total nucleic acid extraction]

[2] https://www.parlament.ch/de/ratsbetrieb/amtliches-bulletin/amtliches-bulletin-die-verhandlungen?SubjectId=60611

Background

Concept. Soon after the outbreak of the Covid-19 pandemic, researchers around the globe succeeded in measuring SARS-CoV-2 in wastewater samples. This is because SARS-CoV-2 viral RNA is shed in sufficient amounts in feces, RNA is sufficiently stable during in-sewer transport and the available detection methods are highly sensitive. Eawag and EPFL jointly developed a pipeline for Switzerland to collect, concentrate and analyze raw wastewater samples collected at the influent of wastewater treatment plants (WWTP). The advantages of observing the dynamics of a pandemic in wastewater compared to case data are: i) wastewater is independent of the test regime, i.e. capacity of testing facilities and willingness/ability of individuals to test and ii) wastewater requires much fewer samples, since the sewer system collects excreta from thousands of people. The limitations are that i) there is no information on an individual infections – nobody can be sent to quarantine based on a pooled wastewater sample – and ii) an individual that is not shedding sufficient amounts of RNA may not be detectable.

First data. With early samples from canton Ticino it was possible to retrospectively demonstrate that SARS-CoV-2 had already spread over catchments of nine WWTP by 29 February 2020[3]. These catchments cover >99% of the cantonal population and until that day, only a total of four individual cases had been clinically confirmed in this region.

For the first wave, wastewater data tracked the timing and shape of the peak better than case data[4]. This was concluded by comparing wastewater and case data against the results of a SEIR model[5] for the catchments of the two WWTP Lausanne and Lugano. Notably, the first wave was characterized by limited testing capacity and high test positivity. Surveillance was continued for the two WWTP Zurich and Lausanne revealing similar evidence in the second wave.

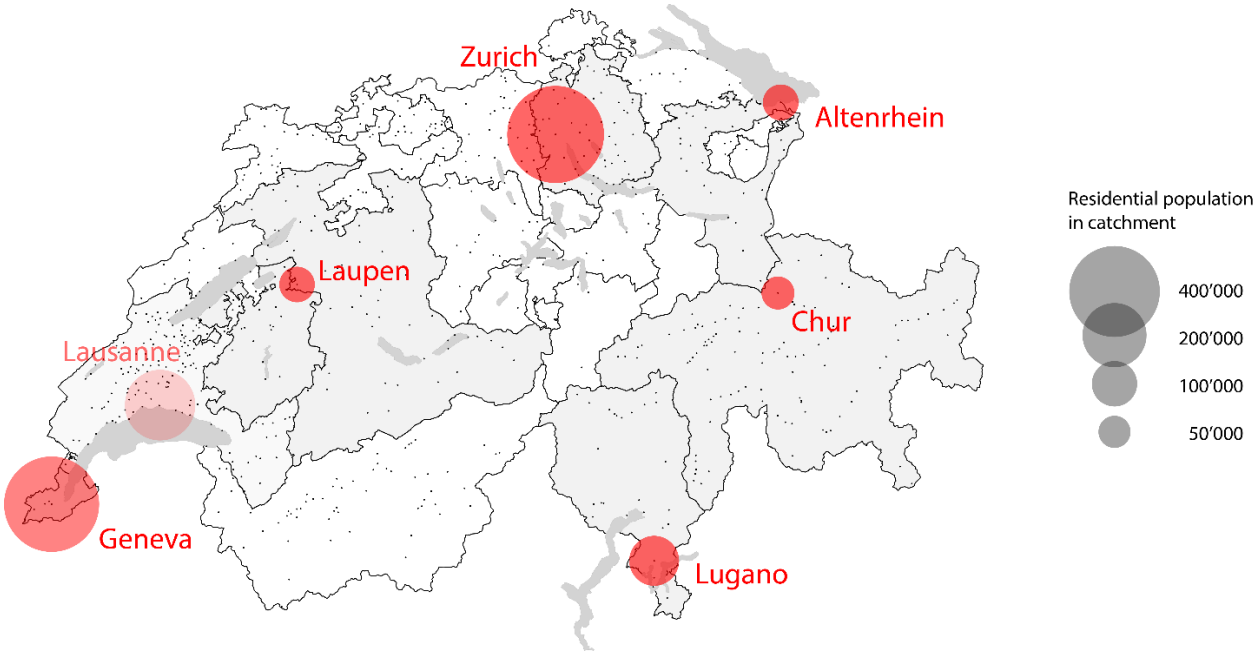

Figure 1. In Switzerland >97% of the population are connected to one of the >700 central wastewater treatment plants[6] (WWTP, black dots). Highlighted in red are the WWTP selected for the *AbwasSARS-CoV-2* project with the area of circles being proportional to the approximate residential population in the catchment. Lausanne was monitored until July 2021, Geneva from August 2021 onwards.

[3] Cariti et al. (2022) Wastewater Reveals the Spatiotemporal Spread of SARS-CoV-2 in the Canton of Ticino (Switzerland) during the Onset of the COVID-19 Pandemic https://doi.org/10.1021/acsestwater.2c00082
[4] Fernandez-Cassi et al. (2021) Wastewater monitoring outperforms case numbers as a tool to track COVID-19 incidence dynamics when test positivity rates are high. https://doi.org/10.1016/j.watres.2021.117252
[5] Susceptible-Exposed-Infected-Recovered; consistent with seroprevalence studies conducted in the region
[6] https://www.bafu.admin.ch/bafu/de/home/themen/wasser/dossiers/internationaler-tag-des-wassers-2017.html

Scope of the *AbwasSARS-CoV-2* project and link to other activities.

The *AbwasSARS-CoV-2* project, funded by the Federal Office of Public Health (FOPH), allowed an expansion of activities to daily sampling at six WWTP, covering approximately 14% of the Swiss population (Figure 1 and Table 1).

The project started on 1 February 2021, which was communicated at the Point de Presse on 9 March 2021[7]. The monitoring, initially planned until 31 December 2022, was extended cost-neutrally for four entire months until 30 April 2023. This was possible due to method improvements – implying lower consumable costs – achieved during the project.

In addition to quantifying SARS-CoV-2 RNA (N1 gene, see method section), the wastewater data was also used to estimate the effective reproductive number R_e[8]. This was done in the context of the *AbwasSARS-CoV-2* project in Prof. Tanja Stadler's group at ETHZ.

Furthermore, the RNA extracts were sent to the Functional Genomic Centre Zurich (FGCZ) for sequencing. The sequencing data was used by Prof. Niko Beerenwinkel's group at ETHZ to estimate the prevalence of variants of concern[9]. The sequencing was funded by FOPH through a different project. Therefore, no additional details are provided in this report. The results can be accessed on the new cov-spectrum dashboard[10], and a section is shown in Figure 2.

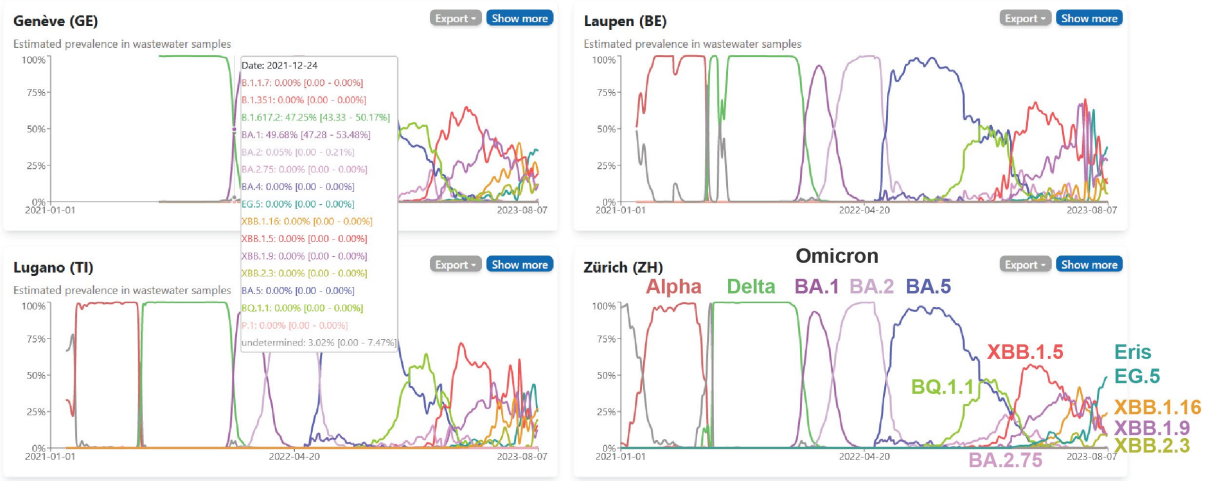

Figure 2. Annotated screenshot of a section of the cov-spectrum dashboard[10] (accessed on 27 August 2023) visualizing the prevalence of variants of concern as estimated from the sequenced wastewater extracts. RNA extracts were obtained within the *AbwasSARS-CoV-2* project, then sent to Functional Genomic Centre (FGCZ) for sequencing. The sequencing data was processed for estimating the prevalence of variants in the group of Prof. Niko Beerenwinkel at ETHZ.

[7] https://www.youtube.com/watch?app=desktop&v=PsJ49ZRbkcg&t=1623s
[8] Huisman et al. (2022) Wastewater-Based Estimation of the Effective Reproductive Number of SARS-CoV-2 https://doi.org/10.1289/EHP10050 and https://ibz-shiny.ethz.ch/wastewaterRe/
[9] Jahn et al. (2022) Early detection and surveillance of SARS-CoV-2 genomic variants in wastewater using COJAC https://doi.org/10.1038/s41564-022-01185-x
[10] https://cov-spectrum.org/stories/wastewater-in-switzerland

Methods

Selection of WWTP. The six WWTP were selected to cover i) a large fraction of the Swiss population, ii) different regions of Switzerland and i) not only metropolitan areas and commuter centers such as Lausanne and Zurich, but also smaller WWTP like Altenrhein and Laupen, both collecting wastewater from multiple municipalities with more countryside character. During the project, it was decided together with FOPH to change monitoring from Lausanne to Geneva due to Geneva being larger, closer to the border of France and having an international airport in the catchment (Zurich airport is not treated by Zurich ARA Werdhölzli, but by ARA Kloten-Opfikon).

Sampling and logistics. Raw wastewater samples were obtained from routinely collected 24-hour composite samples by WWTP staff, stored at 4°C and transported on ice to our laboratory at Eawag in Dübendorf twice per week. Typically, samples were processed and measured within one week after sample collection.

Analytical method. The methods are described in detail in Appendix A1, A2 and A3 and two peer-reviewed publications[11,12]. In brief, samples were processed following one of two general approaches. The first general method, referred to as ($v_{1\text{-}3}$; 1-3 representing minor protocol modifications) was conducted from February 2021 and November 2021. For $v_{1\text{-}3}$, the samples were processed as follows: 50 ml aliquots stirred were and clarified (either by sequential filtration through 2-µm and 0.22-µm filters, or by centrifugation) before being concentrated using centrifugal filter units (10kDa Centricon Plus-70; Millipore, at 3000xg for 30 minutes). The concentrate was extracted to 80 µl eluates using the QiaAmp Viral RNA Minikit (Qiagen) according to manufacturer's instructions. In the middle of November 2021, processing was switched to the method referred to as v_4, which used the Wizard® Enviro Total Nucleic Acid Kit (Promega Corporation, Madison, USA) according to manufacturer's instructions, also yielding an RNA extract of 80 µl. The method switch occurred after parallel testing over three weeks for all six WWTP. It showed that v_4 improved detectability of RNA in most locations. Additional benefits of v_4 were reduced cost and easier, faster protocols. Virus recovery was monitored in a subset of samples by spiking in Murine Hepatitis Virus (MHV; a mouse coronavirus) prior to sample clarification, with recovery efficiency estimated by comparing the concentrations of MHV added to the sample to the concentration of MHV recovered[11].

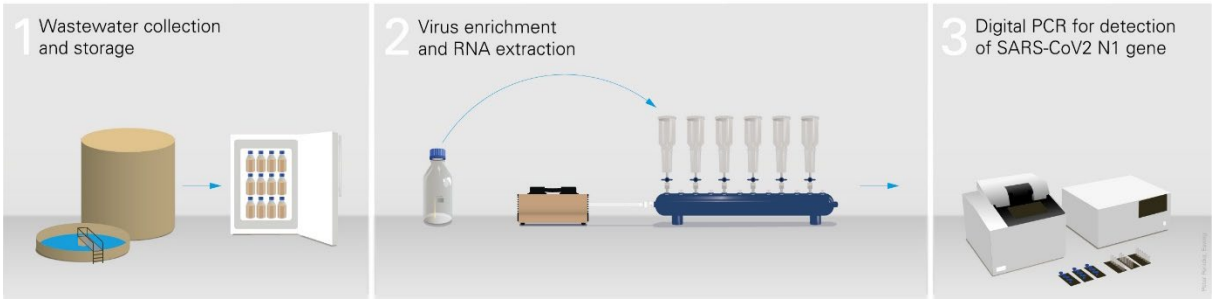

Figure 3. Conceptual model of the sample processing flow using the direct extraction protocol. Step 1: Samples are collected at the wastewater treatment plant where they are stored and transported to Eawag at 4C. Step 2: At Eawag, samples are concentrated and total nucleic acid is extracted, and then, Step 3 is the detection of SARS-CoV-2 RNA using a digital PCR.

Molecular targets were measured using the Naica System Crystal Digital PCR (Stilla Technologies, Villejuif, France) with the qScript XLT 1-Step RT-PCR Kit (QuantaBio). Reactions were prepared as 27 µL (using 5.4 µL template and 21.6 µL mastermix) pre-reactions, of which 25 µL was loaded into Sapphire Chips (Stilla Technologies) for an equivalent of 5 µL template per reaction. Primers and probes

[11] Huisman et al. (2022) Wastewater-Based Estimation of the Effective Reproductive Number of SARS-CoV-2 https://doi/10.1289/EHP10050 and https://ibz-shiny.ethz.ch/wastewaterRe/

[12] Fernandez-Cassi et al. (2021) Wastewater monitoring outperforms case numbers as a tool to track COVID-19 incidence dynamics when test positivity rates are high https://doi.org/10.1016/j.watres.2021.117252

were the 2019-nCov RUO Kit targeting N1 (Integrated DNA Technologies). Thermocycler conditions included partitioning droplets (40 °C for 12 min), reverse transcription (55 °C for 30 min) and polymerase activation (95 °C for 1 min), followed by 40 cycles of denaturation (95 °C for 10 s) and annealing/extension (55 °C for 30 s). All samples were tested for PCR inhibition using spike-in controls of synthetic SARS-CoV-2 RNA reference material[13].

Calculations. It is important to note that daily wastewater volumes can vary across WWTP catchments due to a number of factors. For example, during rain events, surface runoff enters sewers in combined sewer systems and dilutes the SARS-CoV-2 concentrations in the municipal wastewater. In the case of Zurich for example, this can be more than a factor of 4 (min 115'776 $m^3\,d^{-1}$ and max 493'753 $m^3\,d^{-1}$). Also, industrial inputs or extraneous water infiltrating sewer pipes below the groundwater table can dilute the SARS-CoV-2 concentrations. In Chur, the daily per-person wastewater volume was on average only 278 $l\,p^{-1}\,d^{-1}$, whereas it was 490 $l\,p^{-1}\,d^{-1}$ in Lausanne. Therefore, the measured SARS-CoV-2 concentrations were multiplied with daily wastewater volumes (provided by WWTP operators) and divided by population size (see Table 1). This results in population-normalized loads [units as number of SARS-CoV-2 RNA copies per 100'000 people per day] for objective comparison among WWTP catchments of different sizes and with different per capita wastewater volumes.

Effective reproductive number $R_{e,ww}$. Based on the measured viral loads in wastewater, the effective reproductive number were estimated for the different areas covered by the treatment plants through time. First, SARS-CoV-2 RNA measurements were transformed into a time series of infection incidence. This required deconvolving the wastewater data with a shedding load distribution, which describes the amount of virus an infected person sheds into the wastewater per day after infection. We parametrised this distribution as a combination of the incubation period distribution (the time from infection to symptom onset) and the gastrointestinal shedding load distribution for the time from symptom onset to shedding. The resulting time series of infection incidence was used to estimate $R_{e,ww}$ with a tool called EpiEstim[14] (for more details see[13]). When new data becomes available, the $R_{e,ww}$ values are updated.

[13] Huisman et al. (2022) Wastewater-Based Estimation of the Effective Reproductive Number of SARS-CoV-2 https://doi/10.1289/EHP10050 and https://ibz-shiny.ethz.ch/wastewaterRe/

[14] https://CRAN.R-project.org/package=EpiEstim

Results

Coverage and number of samples. The total population covered by the six WWTP is approximately 1.23 million people, which is around 14% of the Swiss population. In the period between 1 February 2021 and 30 April 2023, almost 5000 wastewater samples were shipped to Eawag and received in good conditions (cooled and in time). Results were published regularly, at least once per week, on our dashboard[15]. A total of 4658 samples passed all quality criteria (see Table 1). The main quality criterion was that the concentration estimates were not influenced by inhibition beyond an acceptable level.

Table 1. List of locations in the *AbwasSARS-CoV-2* project including the estimated residential population and the number of wastewater samples analyzed for each catchment (total of 4652 over the entire project period).

WWTP (ARA_ID)	Canton	**Residential population in WWTP catchment**	Description	Average wastewater volume over entire period [l/p/d]	**nr. of samples** with method v_x (nr. of samples >LOD [%])	
					v_1-v_3 1. Feb. 2021 to 30. Nov. 2021	v_4 10. Nov. 2021 to 30. Apr. 2023
Altenrhein (323700)	SG	**64'000**	8 municipalities in SG and 9 in AR	406	**291** (90.4)	**499** (99.8)
Chur (390101)	GR	**55'000**	city and 10 municipalities in GR	278	**274** (91.6)	**486** (99.4)
Geneva[a] STEP Aïre (664301)	GE	**454'000**	city incl.27 municipalities in CH and 4 in France	331	**113** (100.0)	**476** (100.0)
Laupen ARA Sensetal (66700)	BE/FR	**62'000**	13 municipalities in BE and 12 in FR	378	**298** (80.5)	**486** (100.0)
Lausanne[b] STEP Vidy (558600)	VD	**240'000**	city and 15 municipalities in VD	490	**180** (82.8)	-
Lugano (515100)	TI	**124'000**	city and 28 municipalities in TI	370	**254** (79.1)	**496** (100.0)
Zurich ARA Werdhölzli (26101)	ZH	**471'000**	city and 6 municipalities in ZH	407	**292** (95.9)	**513** (100.0)
Total 6 WWTP[c]		**1'230'000**			**1'702**	**2'956**

[a] studied within this project from 1 Aug 2021 to 30 Apr 2023 (see selection of WWTP on page 5)

[b] studied within this project from 1 Feb 2021 to 31 Jul 2021 (see selection of WWTP on page 5)

[c] without Lausanne

Case data. The catchment-specific case data was provided by FOPH. In a first step, the geographic WWTP catchment areas were intersected with the geographic areas of zip codes (PLZ). If a PLZ is only partly in the WWTP catchment, a case reported for that PLZ is counted with the percent of the area of the PLZ being within the WWTP catchment. Cases that were hospitalized are counted at their place of residency.

Over the entire period, in all WWTP catchments (excluding Lausanne due to the short period of measuring wastewater samples in this project), over 2.5 million individual tests were carried out and almost 500'000 positive cases were reported (see Table 2). The number of individual (clinical) tests per person carried out over the period of this project varies from 1.3 (Altenrhein) to 2.6 (Laupen). Also, the average positivity rate varies, from 17% (Geneva) to 26% (Altenrhein).

[15] https://sensors-eawag.ch/sars/overview.html

Table 2. Numbers of tests and reported positive cases per WWTP catchment during the project period.

WWTP	Residential population in WWTP catchment	Period from 1 Feb 2021 to 30 April 2023			
		Nr. of registered tests[a]	Average nr. of tests per person	**Reported positive cases**	Average positivity rate [%]
Altenrhein	64'000	**80'468**	1.3	**21'238**	26
Chur	55'000	**124'250**	2.3	**25'302**	20
Geneva	454'000	**935'391**	2.1	**159'036**	17
Laupen	62'000	**159'806**	2.6	**33'748**	21
Lugano	124'000	**237'812**	1.9	**51'333**	22
Zurich	471'000	**1'023'995**	2.2	**204'949**	20
Total 6 WWTPs	1'230'000	**2'561'722**	2.1	**495'605**	19

[a] PCR and antigen test

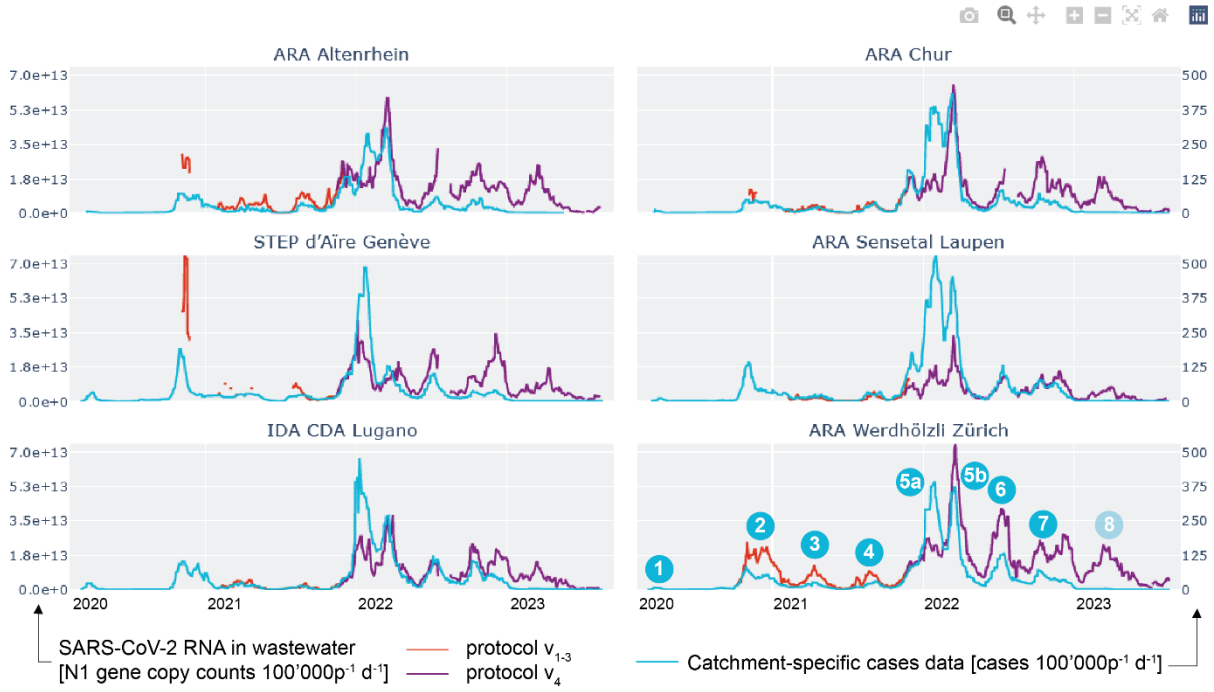

Figure 4. Screenshot of the overview dashboard[16] for all six wastewater treatment plants, i.e. 7-day medians of catchment-specific case data and wastewater measurements. The gap in the middle of July until middle of August 2022 is due to underestimation of loads caused by quality control issues with supplier-provided reagents. Samples during this period were measured again for Zurich only. The waves as seen in the case data are numbered consecutively in the panel for Zurich. Of note, when testing of individuals was reduced substantially and costs had to be carried by patients, the number of reported cases dropped substantially and wave 8 can be barely seen in the case data in the graphs above. In contrast, wave 8 can be clearly seen in wastewater to various extents in all six locations. Wave 8 is still visible in the positivity rate (see Figure 7E), to some extent in the R_e estimate based on cases (see Figure 8) and when re-scaling the axis of cases (see Figure 6).

[16] https://sensors-eawag.ch/sars/overview.html

General description of wastewater data. Besides the online dashboard[17] (see screenshot in Figures 4 and 5), more in-depth graphs summarize different aspects of the results (see Figure 7 and 8). All relevant details for Figures 4 and 5 can be found in the respective captions or online and are not further described here.

In general, the wastewater data and the case data show similar dynamics over the entire period at all locations. However, there are periods exhibiting differences when comparing numbers of cases and wastewater measurements. Most apparent are the discrepancies in the Omicron wave in January and February 2022. It appears as if wastewater underestimated the wave 5a to various extents at all locations (see Figures 4 and 5). The possible reasons remain speculative. One reason could be that during this period, mostly children and young adults were infected and tested positive. Children and young adults may shed SARS-CoV-2 virus at lower amounts than older adults and the elderly, resulting in lower amounts in wastewater per infection. This difference appears substantial on an absolute scale. However, it is important to note that although the magnitude of the waves differed, the dynamics (increases and subsequent decreases) were still the same during this period. As a result, the effective reproductive number – which mainly depends on relative changes – estimated from clinical cases remained similar to the one estimated from wastewater (see also description later in this report on page 12 onwards). Implications of differential shedding were investigated in more depth outside of this project[18]. Starting in July 2022, reported case data started to decouple from wastewater data. These observations hold true for all six locations. Particularly, the wave 8 is barely visible in the case data. One of the reasons is likely the reduced willingness to test and that the costs were not reimbursed anymore but had to be covered by the tested individuals since January 2023. When scaling the y-axis of the case data differently, it becomes evident that wastewater data and case data still correlate very (see Figure 6).

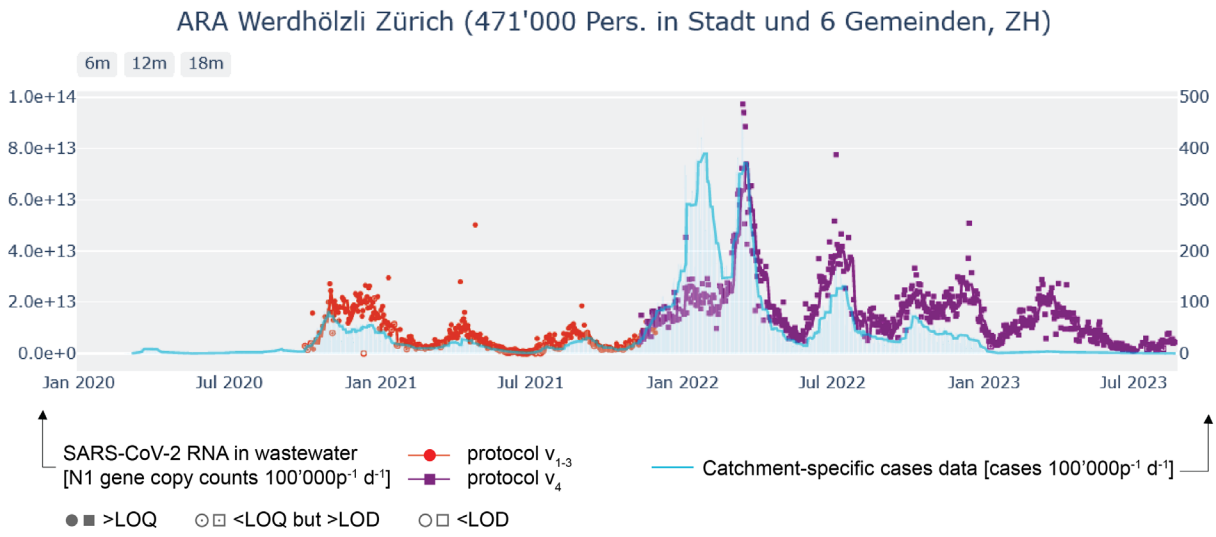

Figure 5. Screenshots of the online dashboard for Zurich as an example (best viewed online[19]). Lines are 7-day medians, dots (wastewater data) and thin bars (case data) are daily values. Please note that the two y-axes are not the same for the two locations. Scales of y-axes were selected such that the signals for wastewater and case data in the periods June and October 2021 (generally low wastewater concentrations and low positivity) visually overlapped and aligned well. LOQ = limit of quantification, LOD = limit of detection. For other locations, see online or Appendix A4. Note: on average, the protocol v_4 resulted in 2.5x higher concentrations than v_{1-3}, therefore the concentrations from the protocol v_{1-3} were multiplied with 2.5 for this graph.

[17] https://sensors-eawag.ch/sars/overview.html
[18] Dreifuss et al. (preprint 2023) Estimated transmission dynamics of SARS-CoV-2 variants from wastewater are robust to differential shedding https://doi.org/10.1101/2023.10.25.23297539
[19] https://sensors-eawag.ch/sars/zurich.html

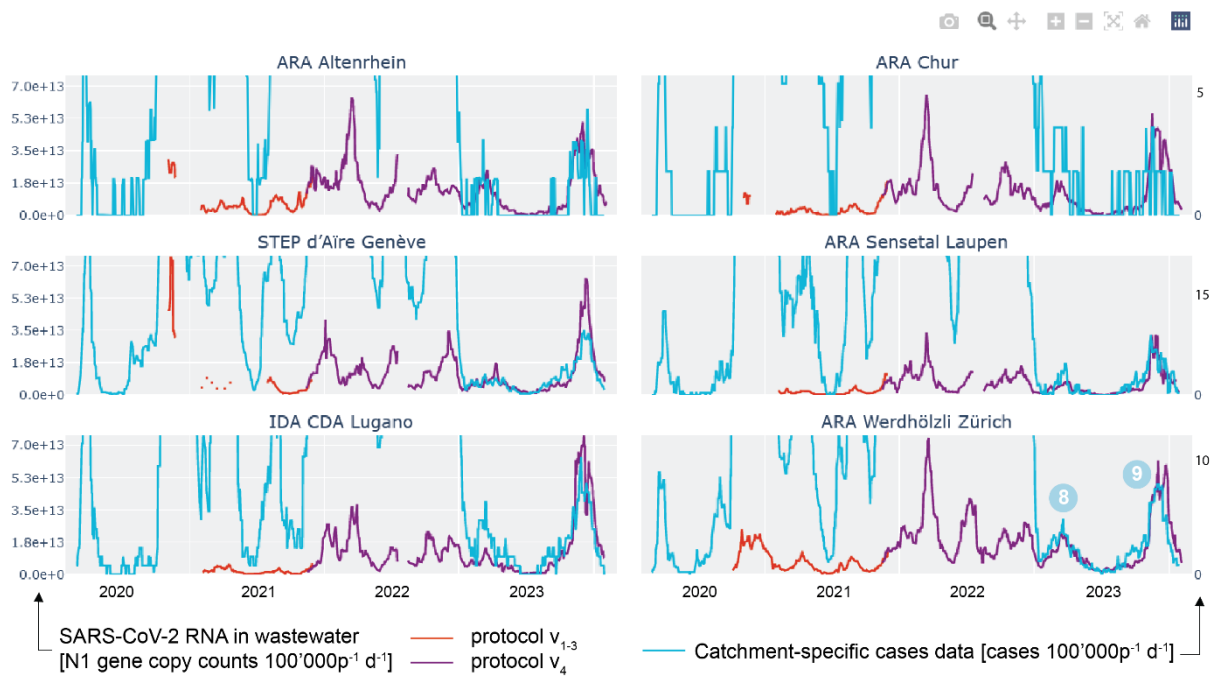

Figure 6. Screenshot of the overview dashboard[20] – same as Figure 4 – with y-axis for numbers of cases scaled differently. The secondary y-axes are stretched approximately a factor of 100 for Altenrhein and Chur (top row), a factor of 25 for Geneva and Laupen (middle row) and a factor of 50 for Lugano and Zurich (bottom row). For reference, wave 8 and 9 are numbered in the panel for Zurich (data after April 2023 is from the extension of this project). Note: on average, the protocol v_4 resulted in 2.5x higher concentrations than $v_{1\text{-}3}$, therefore the concentrations from the protocol $v_{1\text{-}3}$ were multiplied with 2.5 for this graph.

Quality control aspects. The project focused on measurement of SARS-CoV-2 RNA using a duplex digital PCR assay simultaneously targeting SARS-CoV-2 (N1 gene region) and the recovery control virus, MHV. Before the *AbwasSARS-CoV-2* project, Eawag had analyzed wastewater samples for both the N1 and the N2 gene regions of SARS-CoV-2. Due to the observed agreement in concentrations of N1 and N2 in Zurich (over seven months)[21] and Lausanne (over four months)[22], it was decided to focus quantification on N1 exclusively. Figure 7 shows additional measurements and allows a more in-depth analysis of data quality; detailed explanations are provided directly in the caption (see other locations in Appendix A5).

[20] https://sensors-eawag.ch/sars/overview.html
[21] https://sensors-eawag.ch/sarscov2/ARA_Werdhoelzli_ddPCR.html
[22] https://sensors-eawag.ch/sarscov2/STEP_Vidy_ddPCR.html

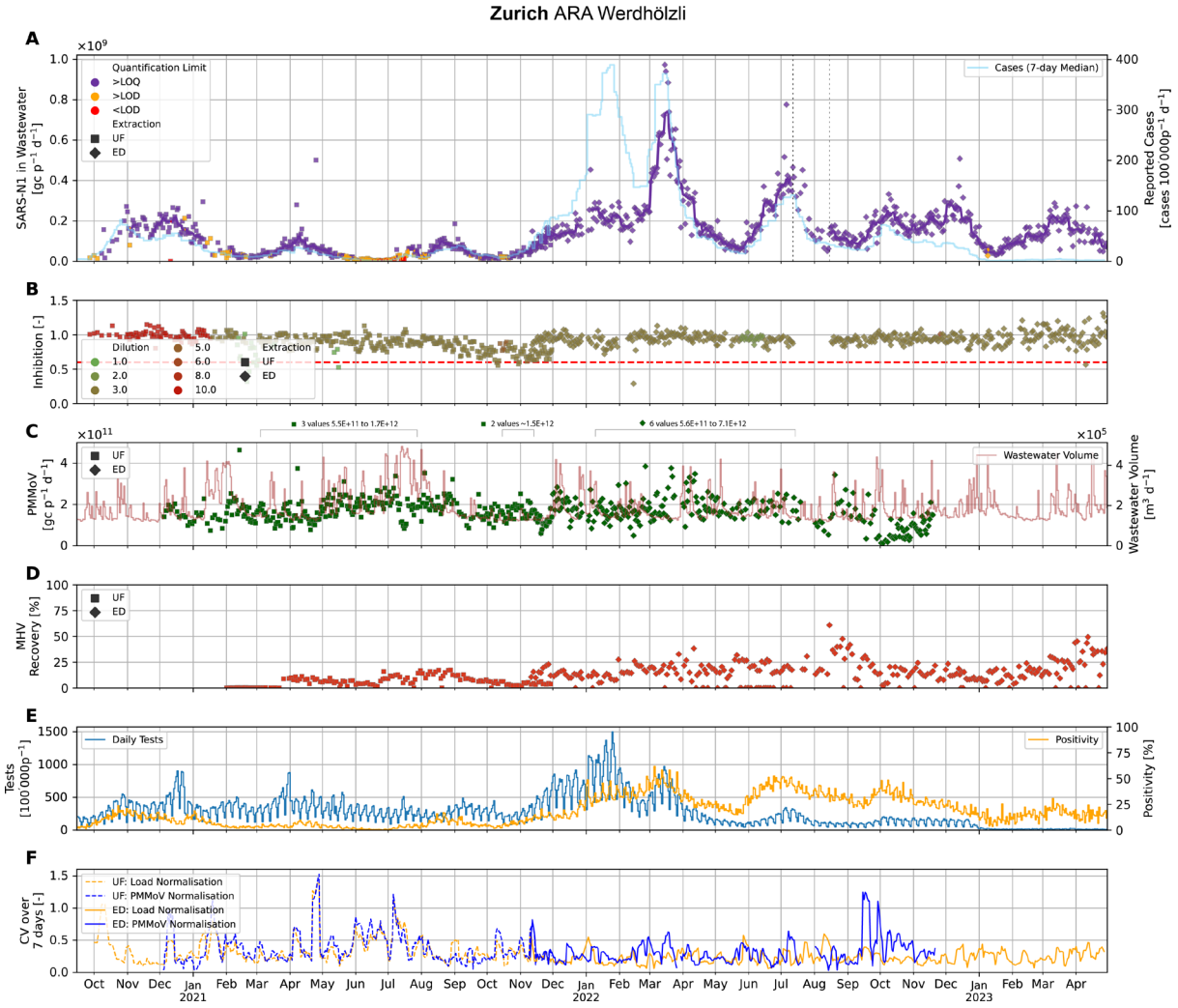

Figure 7. A: case and wastewater data (similar to Figures 4 and 5). Note: on average, the extraction method using enantiomeric digestion (ED) resulted in 2.5x higher concentrations than using ultrafiltration (UF), therefore the concentrations obtained with the UF protocol were multiplied by 2.5 for panel A in this figure. **B:** PCR inhibition controls: if more than 60% of spiked-in synthetic SARS-CoV-2 RNA reference material was recovered, it was deemed acceptable, i.e. the measurement was not be inhibited substantially. If this value was less than 60%, the sample was (further) diluted and re-measured. For samples with low concentrations this is a trade-off; on the one hand diluting a sample implies even lower concentrations that might fall below limit of quantification or limit of detection, on the other hand, inhibition might not be reduced to an acceptable level without dilution. Notably, from January 2023 onward, inhibition testing was reduced from averaging the results of duplicate samples to measuring only a single sample. This has the apparent impact of increasing variability of inhibition. **C:** daily wastewater volumes and pepper mild mottle virus (PMMoV) loads; PMMoV serves as quality control of the laboratory processing pipeline. PMMoV is present in wastewater because it is present in food products such as processed pepper products and is shed at an approximately consistent amount in a sufficiently large, healthy population. Samples with PMMoV loads outside of an acceptable (defined as mean +/- two standard deviations) range suggest a potential error in the sample processing. **D:** the method change $v_{1\text{-}3}$ to v_4 also implied higher recoveries of MHV; low values close to zero do not necessarily imply low recovery. **E:** catchment-specific number of individual clinical tests that were carried out and positivity rate. The weekend effect is clearly visible, with lower numbers of tests carried out on the weekend. Similar to positive cases, the positivity reflects the different waves to different degrees. **F:** variability of data for two possible ways of “normalizing” data: i) SARS-CoV-2 loads (i.e. SARS-CoV-2 RNA concentrations multiplied by daily wastewater volume) and ii) SARS-CoV-2 concentrations divided by PMMoV concentrations. In the latter case, the information about daily wastewater volumes is not needed and the uncertainty of (inaccurate) wastewater volumes cancels out. However, additional uncertainty about the consistent recovery of PMMoV adds to the observe variability. There is no substantial difference between the two approaches and our preferred way of presenting data was the load approach, since all WWTPs are equipped with a regularly checked flow meter and information on daily wastewater volumes are reliable. **Abbreviations: UF:** Ultrafiltration. **ED:** Enantiomeric Digestion. **LOQ:** Limit of quantification. **LOD:** Limit of detection.

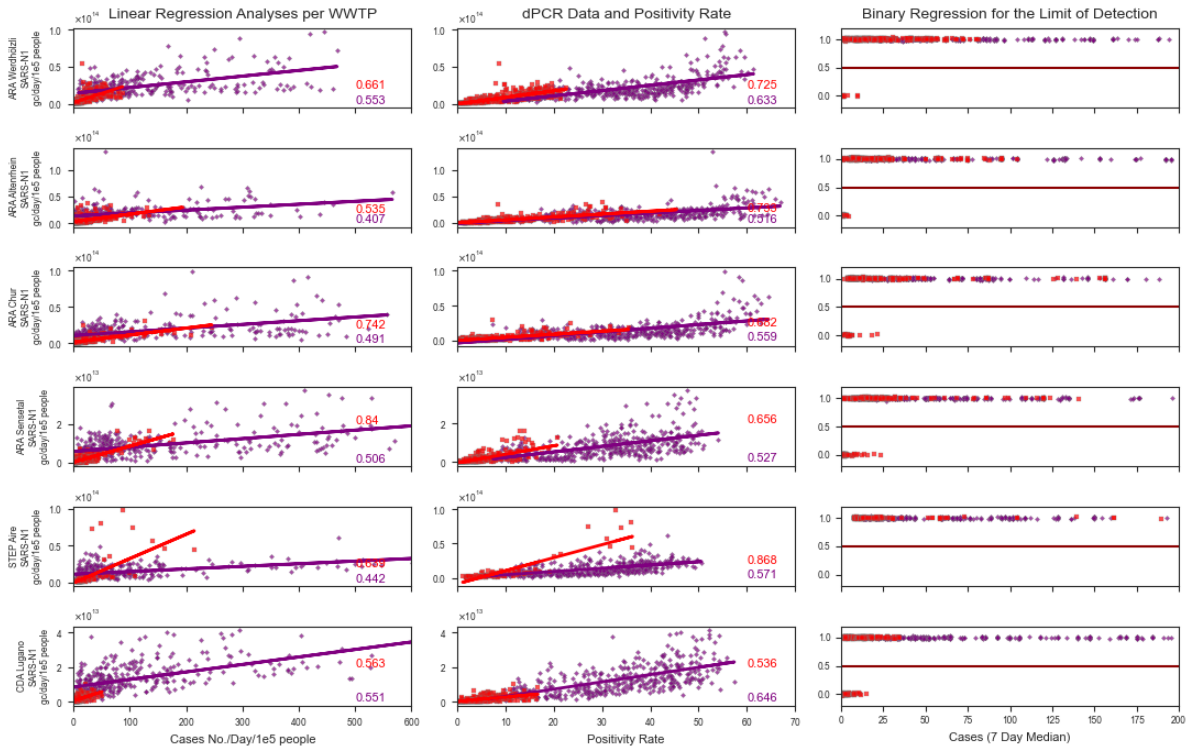

Figure 8. Linear regressions between wastewater data and case data (**left:** cases, **middle:** positivity) for all six locations for both protocols $v_{1\text{-}3}$ (red) and v_4 (purple). **Right:** binary regressions 0 = not detected in wastewater, 1 = detected in wastewater in dependence of reported cases.

Due to variable shedding rates of SARS-CoV-2 RNA, (changing) commuter patterns and analytical variability, population-normalized loads of N1 in wastewater are expected to vary from day to day. Case data shows a clear weekly pattern. Reduced testing on weekends implies lower reported numbers of positive cases (but higher positivity, see also Figure 7E). Therefore, to compare the relation between wastewater and cases beyond relative trends, linear regressions of 7-day medians were performed (see Figure 8). It is notable that R^2 values are typically slightly higher when comparing wastewater data with positivity (rather than cases). However, one can also note that the wastewater load per reported case varies from catchment to catchment. With the inhibition control – which might vary depending on general wastewater properties – systematic effects on the wastewater side seem to be unlikely. On the case data side, possible differences could originate from different testing behavior varying both geographically (see also Table 2) as well as over time (see Figure 7E). Focusing on the protocol $v_{1\text{-}3}$, used until November 2021 when testing was still relatively intensive also during times of low prevalence, SARS-CoV-2 was detected on most days in wastewater when the following numbers of new cases $100'000^{-1}$p d^{-1} were reported: 3 (Zurich) to 10 (Laupen) – evaluating the period June to August 2021 (the other cities are somewhere in between).

Effective reproductive number $R_{e,ww}$. Figure 9 shows a screenshot of the R_e dashboard[23] for the WWTP Sensetal/Laupen. The effective reproductive number $R_{e,ww}$ determined from viral load measurements in wastewater is shown together with the number $R_{e,cc}$, the R_e determined from confirmed cases in the catchment. During the period when clinical testing was extensive, we validated that the $R_{e,cc}$ and $R_{e,ww}$ largely agree[24]. We further analyzed how sensitive the $R_{e,ww}$ estimates were to model and parameter assumptions. Contrary to estimates of the total number of infected individuals, $R_{e,ww}$ estimation does not require exact knowledge of the total amount of viral shedding per person or the dilution factor from RNA shed to the wastewater. Instead, the estimates do depend on which shedding load distribution is assumed. Using the overlap between confirmed case- and wastewater-based R_e

[23] https://ibz-shiny.ethz.ch/wastewaterRe
[24] Huisman et al. (2022) Wastewater-Based Estimation of the Effective Reproductive Number of SARS-CoV-2 https://doi.org/10.1289/EHP10050

estimates as an indicator, we showed that our observations were compatible with parameters for the SARS-CoV-2 shedding load distribution estimated in clinical studies. Our wastewater-based $R_{e,ww}$ estimates were used widely in the reports of the COVID-19 Science Task Force (e.g., [25,26,27]), when sharing latest scientific information with the authorities.

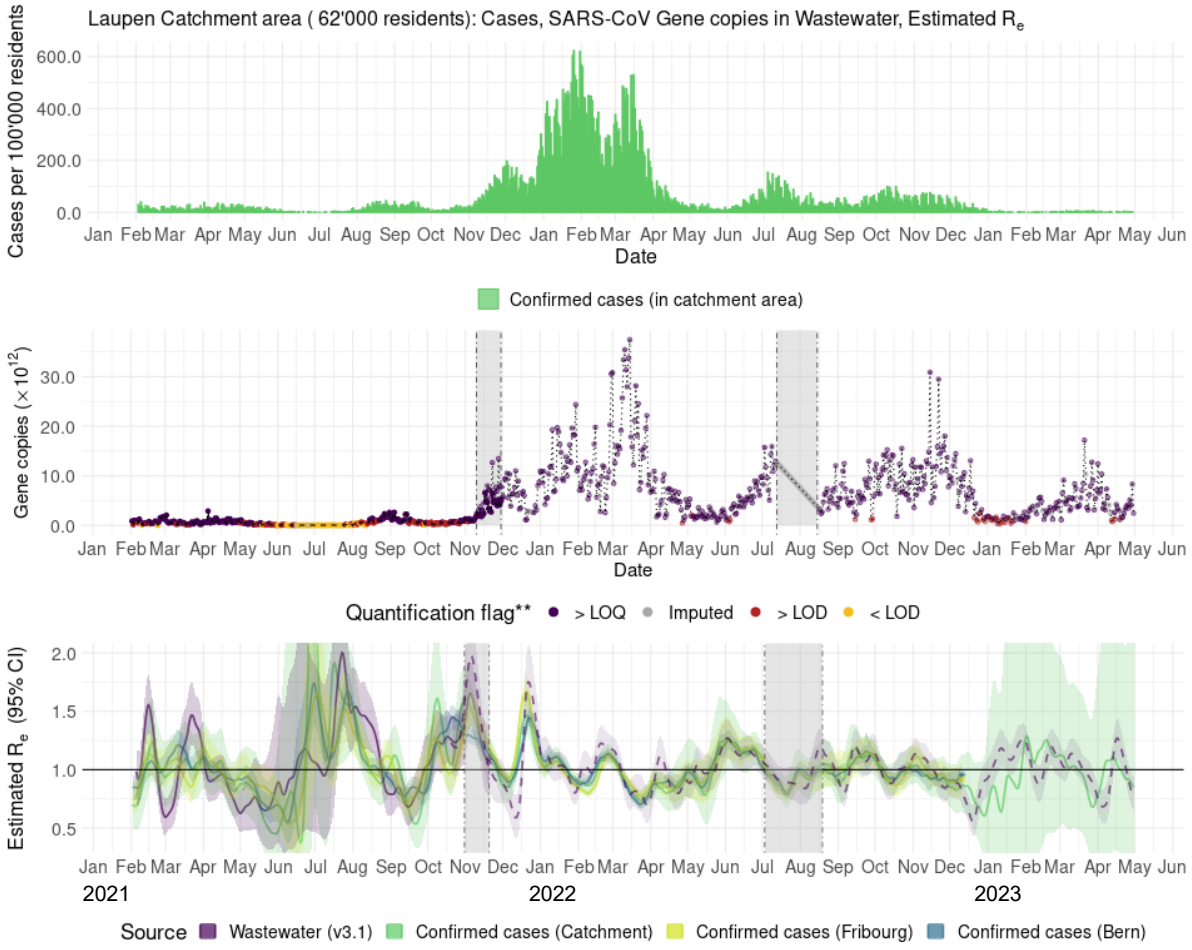

Figure 9. Screenshot of the R_e dashboard for the WWTP Sensetal (Laupen) receiving wastewater from 13 municipalities in canton Bern and 12 municipalities in canton Fribourg. Top: catchment-specific case data. Middle: population-normalized SARS-CoV-2 RNA in wastewater. Bottom: Re estimates made from i) confirmed cases in the catchment (green), ii) wastewater data (purple) and iii) cantonal case data (Bern in blue and Fribourg in yellowish). The shaded areas indicate the confidence interval of the estimates. The two gray areas indicate label periods with i) a method change in analyzing wastewater samples (November 2021) and ii) a period with missing wastewater data (July/August 2022; this gap is due to underestimation of loads caused by quality control issues with supplier-provided reagents – samples during this period were measured again for Zurich only).

Since January 2023, the Swiss government decided to halt the reimbursement of clinical testing for SARS-CoV-2 infections. Thus, the number of tests performed across Switzerland is currently so low that epidemiological trends cannot be accurately estimated from aggregated clinical case reporting. As shown during a period of substantial clinical testing, the wastewater R_e estimates are robust, allowing R_e estimates from wastewater to still provide a timely estimate of epidemiological trends in the absence of clinical data. The second indicator that is currently reliable is the hospital admission data, though this

[25] https://sciencetaskforce.ch/en/speech-by-tanja-stadler-at-the-point-de-presse-29-december-2021/
[26] https://sciencetaskforce.ch/en/scientific-update-of-25-january-2022/
[27] https://sciencetaskforce.ch/en/scientific-update-of-15-february-2022/

indicator lags the wastewater indicator. Going forward, we envision the wastewater indicator as a main indicator to obtain reliable and timely information on epidemic spread.

At times when new variants appeared, we combined the classic R_e estimation method based on wastewater data with information on variant frequencies based on sequencing data[28]. This allowed us to estimate variant specific effective reproductive numbers and the transmission advantage associated with newly appearing variants. This could help assess the risk of new pandemic waves triggered by variants of concern. We regularly shared this information with the authorities and plan writing a scientific manuscript on this topic.

Mpox. In addition to the SARS-CoV-2 measurements, the project team was also able to react on spontaneous requests. For example, in summer 2022, during the outbreak of mpox, the team was tasked with detecting and quantifying this virus in wastewater. Mpox DNA was always detected in wastewater when there were two or more reported cases per 100'000 people in Geneva and Zurich. In Lugano, concentrations were lower, as were the reported cases. From October onwards, the values measured in wastewater were below limit of detection confirming the low prevalence seen from case data. For more details see the related publication[29].

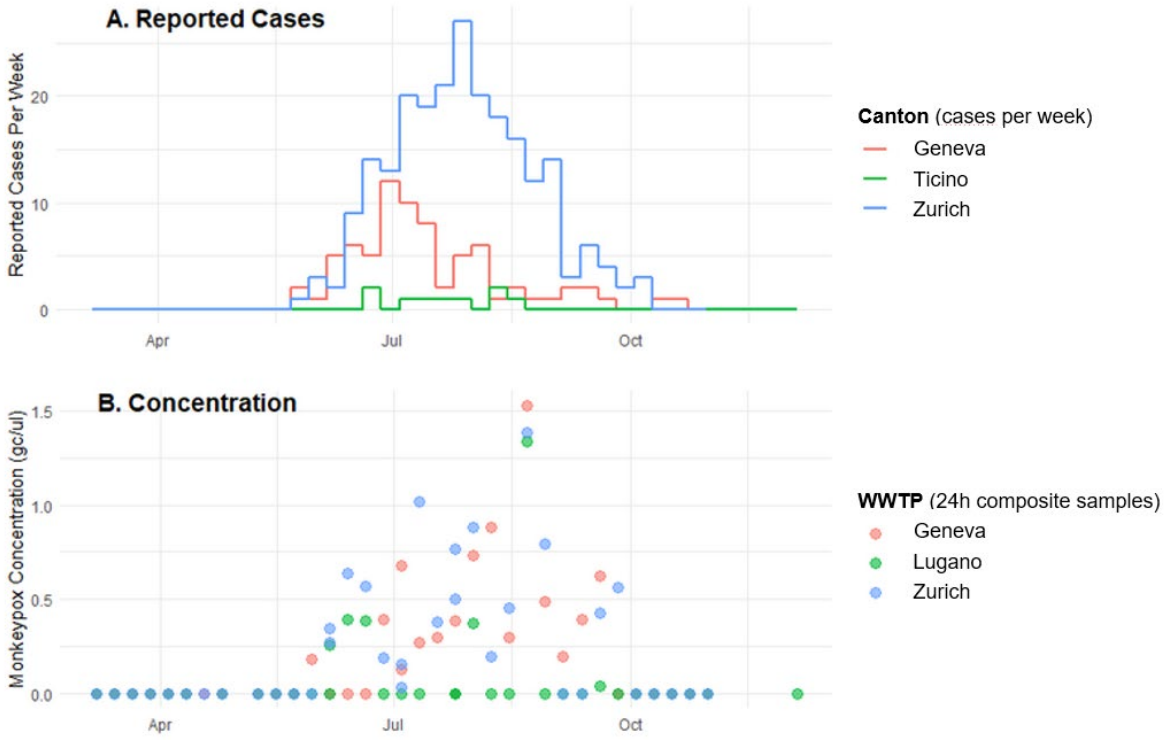

Figure 10. A: number of weekly cases reported in the cantons Geneva, Ticino and Zurich in summer 2022. **B:** concentrations in wastewater of the three large wastewater treatment plants covering substantial parts of the cantonal population they are located in (Geneva 90%, Lugano 35% and Zurich 31%).

[28] https://sciencetaskforce.ch/en/overview-and-evolution-of-the-situation-27-december-2021/
[29] Julian et al. (2024) Monitoring an Emergent Pathogen at Low Incidence in Wastewater Using qPCR: Mpox in Switzerland https://doi.org/10.1007/s12560-024-09603-5

Outlook and recommendations

A postulate to institutionalize wastewater-based epidemiology and sequencing of pathogens[30] was accepted by the parliament[31] in May 2023. With the experience from the project *AbwasSARS-CoV-2*, the following aspects should be considered when evaluating a possible future national implementation of such a surveillance for pathogens in wastewater:

Number of wastewater treatment plants: The six wastewater treatment plants considered in this study covered approximately 14% of the Swiss population. Including more wastewater treatment plants, would cover a higher portion of the population and would allow assessing disease transmission at higher spatial resolution. This was shown with the national monitoring program of the Federal Office of Public Health, which covered at its peak over 100 wastewater treatment plants representing over 70% of the population[32]. An analysis of this data set revealed that temporal dynamics can be clustered into approximately 10 to 20 groups showing similar dynamics. Drawing from this analysis, ***we advocate for the careful selection of around 15 major wastewater treatment plants***. This approach aims to encompass a significant portion of the population and to cover different regions with possibly different disease dynamics.

Number of samples: In this study, daily samples were collected over an extended period of over two years, which resulted in a unique data set of highest quality. While a general trend could be captured with fewer samples, the reliable real-time estimation of the effective reproductive number R_e from wastewater, requires at least three observations per week[33]. If the aim is to robustly estimate disease trends in **real time** using R_e estimates, ***we recommend analyzing at least five samples per week*** per wastewater treatment plant monitored. However, if the estimates do not have to be as timely, also **2-3 samples per week will give a very good longterm trend estimate**. This number of samples per week also supports other uses of the samples, such as sequencing for variants. Current estimates of variant proportions based on wastewater data relies on time series; five samples per week provide a sufficient frequency for robust variant proportion estimates.

Additional pathogens and chemicals: In the *AbwasSARS-CoV-2* project, wastewater-based epidemiology proved successfully that the dynamics of circulating pathogen causing a pandemic can be captured by analyzing wastewater samples. This was possible because wastewater data could be benchmarked against reliable, robust clinical testing, which required unprecedented efforts to set up testing facilities[34]. Wastewater samples can be similarly used to inform other diseases. ***This has already been shown to be feasible for the respiratory viruses such as RSV and Influenza A and B virus***[35], including the estimation of the effective reproductive number $R_{e,ww}$[36] for influenza. Using wastewater extracts with specifically tailored PCR assays – or detected with sequencing methods – enables ***tracking multiple additional pathogens at relatively little additional costs***. Investing in wastewater-based epidemiology allows for long-term, cost-effective and objective assessment of disease trajectories. Most importantly, it should be seen as complementary to, not a replacement of clinical testing. Wastewater samples can also be analyzed for an array of substances, e.g. alcohol and tobacco, pharmaceuticals with abuse potential, illicit drugs and health biomarkers (ongoing project with FOPH[37]). Example are the analysis of antihistamines[38], cough and antipyretic medication. Wastewater-based monitoring programs of these chemicals could assist in indirect syndromic surveillance as consumption could be tracked with high spatial-temporal resolution above and beyond what is feasible with existing pharmaceutical sales data. Therefore, ***we encourage complementing pathogen surveillance with chemical analysis of wastewater samples***.

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[30] https://www.parlament.ch/de/ratsbetrieb/suche-curia-vista/geschaeft?AffairId=20224271 submitted Nov 2022 by the Commission for Social Security and Health of the National Council; acceptance proposed by the Federal Council in Jan 2023

[31] https://www.parlament.ch/de/ratsbetrieb/amtliches-bulletin/amtliches-bulletin-die-verhandlungen?SubjectId=60611

[32] https://www.covid19.admin.ch/en/epidemiologic/waste-water?wasteWaterFacility=66700 see screenshot in Appendix 7

[33] Huisman et al. (2022) Wastewater-Based Estimation of the Effective Reproductive Number of SARS-CoV-2 https://doi.org/10.1289/EHP10050

[34] Over 24 million tests, PCR and rapid antigen in Switzerland and Liechtenstein, were carried out since the beginning of the pandemic, i.e. on average 2.7 tests per person https://www.covid19.admin.ch/en/epidemiologic/test?epiZoomDev=2020-02-09_2023-08-13&sum=cumulative&epiRelDev=abs

[35] https://www.eawag.ch/fileadmin/Domain1/Abteilungen/sww/projekte/SARS-CoV-2/WebsiteHTMLReport-EN.html

[36] https://wise.ethz.ch/influenza/

[37] https://www.eawag.ch/de/abteilung/sww/projekte/dromedario/

[38] Baumgartner et al. (preprint 2023) Wastewater-Based Analysis of Antihistamines to Estimate Pollinosis Disease Burden at Population-Scale https://doi.org/10.1101/2023.11.29.23299171

Dissemination

While our website[39] with the public online dashboard[40] was the main channel of communication, various news articles reported about this work. A selection of articles and TV broadcasts are listed on the website. In addition, members of the project team gave over 40 presentations at various occasions during the project period. They addressed mainly a scientific audience (including Swiss National Science Foundation, national and international conferences and FOPH events) but also outreach to the public and wastewater treatment plant operators, as well as a few presentations to industry (potential to optimize the detection/quantification methods) and internally (to establish new links to advance science in this inter- and transdisciplinary research field). The list can be found in Appendix A6.

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Without detailing everyone's individual contribution we would like to thank all who have contributed to the *AbwasSARS-CoV-2* project or its preparation in the time before, all listed alphabetically:

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[39] https://www.eawag.ch/covid19_sewage
[40] https://sensors-eawag.ch/sars/overview.html