



## Zooming in to the neighborhood level: A year-long wastewater-based epidemiology monitoring campaign for COVID-19 in small intraurban catchments

Ian Zammit<sup>a,b,1</sup>, Sergi Badia<sup>a,b,1</sup>, Cristina Mejías-Molina<sup>c,d</sup>, Marta Rusiñol<sup>c,d</sup>, Sílvia Bofill-Mas<sup>c,d</sup>, Carles M. Borrego<sup>a,e</sup>, Lluís Corominas<sup>a,b,\*</sup>

<sup>a</sup> Catalan Institute for Water Research (ICRA-CERCA), Emili Grahit 101, 17003 Girona, Spain

<sup>b</sup> University of Girona, Plaça de Sant Domènec 3, 17004 Girona, Spain

<sup>c</sup> Laboratory of Viruses Contaminants of Water and Food, Genetics, Microbiology & Statistics Dept., Universitat de Barcelona, Barcelona, Catalonia, Spain

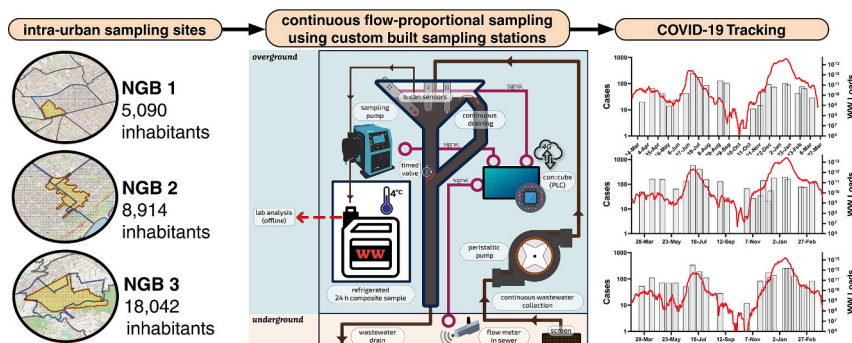
<sup>d</sup> The Water Research Institute (IdRA), Universitat de Barcelona, Barcelona, Catalonia, Spain

<sup>e</sup> Group of Molecular Microbial Ecology, Institute of Aquatic Ecology, University of Girona, Girona, Catalonia, Spain

### HIGHLIGHTS

- SARS-CoV-2 surveillance in sewage at the neighborhood level was compared to the WWTP level.
- Correlations were higher at the neighborhood level as compared to the WWTP.
- Lower loads per clinical case were found during the Omicron wave.
- No differences were found across neighborhoods differing in socioeconomic status.

### GRAPHICAL ABSTRACT



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### ABSTRACT

In recent years, wastewater-based epidemiology (WBE) has emerged as a valuable and cost-effective tool for monitoring the prevalence of COVID-19. Large-scale monitoring efforts have been implemented in numerous countries, primarily focusing on sampling at the entrance of wastewater treatment plants (WWTPs) to cover a large population. However, sampling at a finer spatial scale, such as at the neighborhood level (NGBs), pose new challenges, including the absence of composite sampling infrastructure and increased uncertainty due to the dynamics of small catchments. This study aims to investigate the feasibility and accuracy of WBE when deployed at the neighborhood level (sampling in sewers) compared to the city level (sampling at the entrance of a WWTP). To achieve this, we deployed specific WBE sampling stations at the intraurban scale within three NGBs in Barcelona, Spain. The study period covers the 5th and the 6th waves of COVID-19 in Spain, spanning from March 2021 to March 2022, along with the WWTP downstream from the NGBs. The results showed a strong correlation

\* Corresponding author at: Catalan Institute for Water Research (ICRA-CERCA), Emili Grahit 101, 17003 Girona, Spain.

E-mail address: [lcorominas@icra.cat](mailto:lcorominas@icra.cat) (L. Corominas).

<sup>1</sup> Shared first authorship.

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between the dynamics of COVID-19 clinical cases and wastewater SARS-CoV-2 loads at both the NGB and city levels. Notably, during the 5th wave, which was dominated by the Delta SARS-CoV-2 variant, wastewater loads were higher than during the 6th wave (Omicron variant), despite a lower number of clinical cases recorded during the 5th wave. The correlations between wastewater loads and clinical cases at the NGB level were stronger than at the WWTP level. However, the early warning potential varied across neighborhoods and waves, with some cases showing a one-week early warning and others lacking any significant early warning signal. Interestingly, the prevalence of COVID-19 did not exhibit major differences among NGBs with different socioeconomic statuses.

## 1. Introduction

After the initial outbreak of COVID-19, wastewater-based epidemiology (WBE) emerged as a valuable strategy for monitoring the communal prevalence of infections (Lodder and de Roda Husman, 2020) by taking advantage of the fact that individuals infected with SARS-CoV-2 shed the virus in their feces (Wölfel et al., 2020). Proof-of-concept studies quickly demonstrated the successful quantification of SARS-CoV-2 RNA in wastewater (Ahmed et al., 2020; Medema et al., 2020), leading to the rapid implementation of nationwide wastewater surveillance networks in various countries, including Australia, the United Kingdom, the United States, Spain, and Switzerland.

Traditionally, these large-scale monitoring efforts have relied on the sampling of influent wastewater in wastewater treatment plants (WWTPs) as it allows for tracking the circulation of SARS-CoV-2 among the served populations and estimating communal COVID-19 prevalence. WWTPs are convenient sampling points as they collect wastewater from a significant number of inhabitants (from a few thousand to more than a million), and the sampling infrastructure is already available. However, surveillance at smaller scales, such as neighborhoods or buildings, could provide valuable insights for better disease management by identifying infection hotspots within a city and enabling the implementation of control measures at the source (e.g., local containment or quarantines). In this regard, wastewater from airplanes and cruise ships (Ahmed et al., 2021b; Albastaki et al., 2021), university campuses (Betancourt et al., 2021; Gibas et al., 2021), schools (Crowe et al., 2021), and elderly residence homes (Pico-Tomás et al., 2023) have also been successfully monitored for SARS-CoV-2.

Although 24-hour flow-proportional composite sampling is recommended by the European Union (European Commission, 2021) and widely used at WWTPs in COVID-19 WBE studies (e.g., Pardo-Figueroa et al., 2022; Rusiñol et al., 2021; Tiwari et al., 2022) and surveillance networks (e.g., Guerrero-Latorre et al., 2022), this sampling methodology is not commonly employed when sampling directly from sewers. This is because expensive sampling equipment would be vulnerable to vandalism or theft, leaving grab sampling (e.g., Betancourt et al., 2021; Zdenkova et al., 2022) or composite sampling lasting <24 h (Barrios et al., 2021; Rubio-Acero et al., 2021; Saingam et al., 2023) as the only feasible options. However, grab sampling not only fails to account for diurnal variations in SARS-CoV-2 concentrations (Ahmed et al., 2021a; Bivins et al., 2021; Gerrity et al., 2021; Li et al., 2021; Mendoza Grijalva et al., 2022) but also introduce large uncertainty. The latter is particularly pronounced in small catchments with fewer served inhabitants, as observed for chemical markers (Ort et al., 2005) since the analyte of interest is released in discrete pulses that are harder to capture as they occur less frequently. Given that SARS-CoV-2 is predominantly shed in feces, resulting in less frequent pulses compared to analytes excreted in urine, continuous sampling can help reduce such uncertainties. Alternatively, passive samplers offer an advantageous option that combines many of the benefits of grab sampling and composite sampling and have been demonstrated to be a cost-effective alternative when qualitative results are sufficient (Li et al., 2022; Mejías-Molina et al., 2023; Schang et al., 2021).

Considering the challenges associated with tracking SARS-CoV-2 in sewers, we aim to address the following questions: 1) Is wastewater-

based epidemiology (WBE) feasible and accurate when deployed at the neighborhood level compared to the city level through a wastewater treatment plant (WWTP)? 2) Are there differences in the dynamics of COVID-19 outbreaks across neighborhoods within the same city? To answer these questions, we conducted a year-long WBE monitoring campaign to track COVID-19 prevalence within three neighborhoods (NGBs) in Barcelona (Spain). Sampling was performed in well-defined urban areas using dedicated sampling stations equipped with sensors and flow meters specifically designed for neighborhood-level wastewater sampling. Continuous, flow-proportional composite samples were collected over a 24-hour period at these stations, and SARS-CoV-2 viral loads were quantified. The viral load data were compared to clinically reported cases, spatially filtered by corresponding healthcare precincts as reported by the regional health authority. Also, a large WWTP downstream from all three neighborhoods was sampled on a weekly or fortnightly basis to assess the communal COVID-19 prevalence at the city level. Additionally, the wastewater from a hospital serving as a reference point for many residents in the eastern part of Barcelona was also monitored. The results presented in this study provide a comprehensive overview of the COVID-19 pandemic in Barcelona during the 5th (Delta variant) and 6th (Omicron variant) waves. Importantly, this is the first study to directly compare WW SARS-CoV-2 levels in neighborhoods, the city, and a hospital within the same area and time frame using highly accurate 24-hour continuous composite samples.

## 2. Material and methods

### 2.1. Description of sampled locations

#### 2.1.1. Neighborhoods

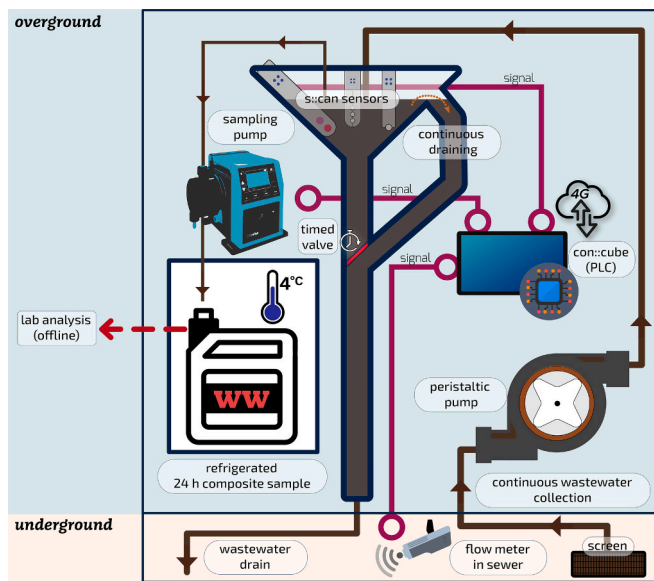
Three sampling stations were constructed in three distinct NGBs of Barcelona, representing different socioeconomic levels including high, mid, and low-income areas. Table 1 provides details on the sampled population, household income, and catchment area associated with each NGB.

Fig. 1 illustrates the configuration of the sampling stations utilized for wastewater collection from the three NGBs (a picture of a station is presented in the supplementary material Fig. S1). The sampling procedure involved the use of a large peristaltic pump to continuously extract wastewater from the sewer and transfer it to a reservoir equipped with a top drain. Within this reservoir, online sensors were positioned to monitor wastewater parameters, and an inlet silicone tube connected to a secondary sampling pump (Watson Marlow Qdos 30) was employed for sample collection. The sampling pump was linked to a Scan Concube unit (s::can GmbH) via a 4–20 mA analogue connection to regulate the flow rate. Each composite sample spanned a 24-hour period and was

**Table 1**

Number of inhabitants, area of monitored site and income of the sampled neighborhoods.

Neighborhood	Inhabitants	Monitored area size (m <sup>2</sup> )	Average household income (€/yr)
1	5090	183,617	78,476
2	8914	252,195	38,430
3	18,042	529,096	25,849



**Fig. 1.** Simplified illustration of the sampling stations built at neighborhood level.

obtained in a continuous, flow-proportional manner. Proportional sampling was divided into three stages (peak, day, and night), with each stage corresponding to different flow rates of the sampling pump. These flow rates were determined based on field observations conducted at the same stations, utilizing the installed flow meters within the sewer pipe. In terms of volume, each composite sample consisted of 32 % collected between 07:00 and 12:00 (peak flow rate), 54 % collected between 12:01 and 22:00 (day flow rate), and 14 % collected between 22:01 and 06:59 of the following day (night flow rate).

Between March 2021 and September 2021, the sampling frequency was established as one sample every two weeks. Subsequently, from October 2021 to March 2022, sampling occurred once per week. Throughout the entire year-long period from March 2021 to March 2022, a total of 77 composite samples were collected from the three NGBs.

### 2.1.2. Hospital

The chosen hospital for monitoring purposes was used as reference hospital for a significant portion of the Barcelona population, including the three NGBs and a large portion of the sewershed connected to the WWTP. The hospital has a staff of 4100 individuals and a total of 516 beds. Strict protocols were in place for patients in the hospital, including thorough testing and the requirement of a negative test result before discharge. The hospital administration maintained a daily record of active cases, representing the number of infected individuals within the hospital, and shared this data with our research team. Wastewater samples from the hospital were collected between July 2021 and March 2022. To collect these samples, a HACH Bühler 2000 autosampler was employed. This autosampler collected 26 time-proportional composite samples over a 24-hour period, with a sampling frequency of every 15 min and a constant volume of wastewater collected. The aim of this sampling was to investigate any potential time lag between the peak of COVID-19 in the community and the peak of hospitalizations within the hospital setting both using clinical data and wastewater data.

### 2.1.3. Wastewater treatment plant

To assess the overall dynamics of SARS-CoV-2 levels at the city level and compare them with the neighborhood level, we sampled the largest wastewater treatment plant (WWTP) in Barcelona, which is located downstream from all three monitored neighborhoods. This WWTP serves a population of 1.4 million inhabitants in the Barcelona

Metropolitan area. For this purpose, we collected 56 weekly influent wastewater (WW) samples from March 2021 to March 2022. An auto-sampler was utilized to collect high-frequency point samples, which were stored separately. These samples were later combined to create flow-proportional composite daily samples. This sampling approach allowed us to quantify SARS-CoV-2 levels in wastewater at the city level and compare them with the levels observed at the neighborhood level.

## 2.2. Viral analyses

Upon reception, wastewater samples were spiked with bacteriophage MS2 as control process at a final concentration of  $10^5$  gene copies (GC)/mL. Samples (100 mL) were then centrifuged to remove large debris ( $4750 \times g$  for 30 min) and 80 mL of the supernatant was collected and ultrafiltered (150 KDa) using an automatic concentration pipette with 150KDa tips (CP-Select™, InnovaPrep; MO, USA) into a final volume of 300  $\mu$ L. Nucleic acids were then extracted using the QIAamp Viral RNA Mini kit using the QIAcube automatic system (Qiagen; Hilden, Germany) into a final volume of 70  $\mu$ L. Undiluted and 10-fold dilutions of the nucleic acid extracts were analyzed in duplicate and used to assess the potential inhibition of the amplification reaction. Each batch of 12 samples included a negative control of the extraction process.

The abundances of SARS-CoV-2 (N1 and N2 assays) and JC polyomavirus (JCPyV), which was used as human fecal indicator (Rusiñol et al., 2014), were determined in the extracts using quantitative PCR (qPCR) assays as previously described (Moore et al., 2020; Pal et al., 2006). RNA Ultrasense™ One-Step RT-qPCR System (Invitrogen; MA, USA) and the TaqMan® Environmental Master Mix 2.0 (ThermoFisher Scientific; MA, USA) were used for SARS-CoV-2 and JCPyV assays, respectively. qPCR standards were prepared using a synthetic SARS-CoV-2 control (Control 51 from Twist Biosciences; CA, USA) or a synthetic gBlock Gene fragment (IDT, IA, USA) for JCPyV as already explained in (Rusiñol et al., 2020). These standards were previously quantified with a Qubit fluorometer (ThermoFisher Scientific; MA, USA) and serially diluted from  $10^6$  to  $10^1$  copies per reaction. Standard qPCR curves were accepted under the following parameters: mean slope  $-3.4 \pm 0.2$ ;  $r^2 \geq 0.99$ ; and mean efficiency above 95 %. To evaluate RT-qPCR inhibition, undiluted and 10-fold dilutions of sample extracts were quantified. Recovery was assessed by quantifying phage MS2 (Pecson et al., 2009). The mean recovery was of 40 % (initial concentration of  $1 \times 10^5$  GC/mL with a standard deviation of 65 % of the recovery mean). The total amount of WW sample analyzed corresponds to 5.86 mL for SARS-CoV-2 (N1 and N2 assays) and 11.73 mL for JCPyV. All PCR assays included a non-template control (negative control). Quantification was performed in a QuantStudio™ Real-Time PCR System from ThermoFisher Scientific.

## 2.3. Clinical data and data analysis

The estimation of active COVID-19 cases in each catchment area, including the three neighborhoods and the WWTP sewershed, was based on clinical data obtained from PCR tests conducted on individuals displaying COVID-19 symptoms. These tests were carried out under the supervision of the Catalan Health Authority (GENCAT, 2022). The clinical datasets were updated daily throughout the monitoring period and linked to a health territorial unit known as the *Àrea Bàsica de Salut* (ABS), which represents a geographical unit. To access and filter this data by location, the Catalan Institute for Water Research (ICRA) developed a web platform called <https://legir-api-covid19.icradev.cat/>. This platform allowed us to match the clinical cases with the sewersheds of each sampled area. It should be noted that while the sewersheds of the NGBs and ABSs overlapped to some extent, they were not entirely identical. The overlap between these areas can be visualized in S.I. Fig. 2, provided in the supplementary information. The total number of cases, both in terms of time and space, was used to estimate the number of active cases in each location. Specifically, one estimation



method referred to as “cases f7d” involved summing the number of daily PCR-positive individuals over the 7-day period following each sampling event. This estimation was utilized in Fig. 1 of the results section. To examine the time lag or anticipation of cases detected in wastewater compared to clinical cases, the sum of cases over multiple 7-day windows was calculated. These time windows ranged from “C–3” to “C+7,” where “C” represents the center of the window (*i.e.*, the sampling day), and the number after the “C” indicates the direction and number of days the center is shifted. For example, “C+3” indicates a shift of 3 days into the future relative to “C.” The concept of these 7-day windows is visually represented in S.I. Fig. 3, included in the supplementary information. These estimations of cases were used in heatmap Fig. 4 of the results section.

The number of COVID-19 cases at the hospital was directly reported by the hospital administration and did not involve estimation. The administration recorded and updated the data daily, capturing new admissions and discharges, which allowed for the determination of the number of active cases within the hospital premises on any given day. At the three sampling stations, daily wastewater flow was measured using installed flow sensors, specifically the Hach Flo-Dar (in NGB2), Nivus PCM 4 (in NGB1), and Nivus NFM 550 (in NGB3). The flow data for the WWTP was provided by the site management. To facilitate a better comparison among the sampled sites, as these sites had varying water discharge levels *per capita*, viral concentrations (expressed in gene copies per liter, GC/L) were multiplied by the daily wastewater flow to calculate the daily viral load (GC/day). It is important to note that at the hospital, where a flowmeter could not be installed, the data is presented as a concentration (GC/L). In cases where the N1 gene was not detected, a value equivalent to half of the limit of quantification (LOQ) for the N1 gene (LOQ N1 = 3623 GC/L) was considered.

### 3. Results

#### 3.1. Time series

The study period encompassed the 5th and 6th waves of the COVID-19 pandemic that affected Catalonia during the summer of 2021 and winter of 2022, respectively. The 5th wave was attributed to the Delta variant (PANGO lineage B.1.617.2), while the 6th wave was driven by the rapid surge and spread of the Omicron variant (PANGO lineages BA.1, BA.2, BA.4, BA.5) in both Catalonia and other parts of Europe (Ciruela-Navas et al., 2021; ECDC, 2023; Ort et al., 2021; O’Toole et al., 2021; SIVIC COVID-19, 2021). Fig. 1 illustrates the dynamics of COVID-19 cases reported and SARS-CoV-2 load in wastewater at the four monitored sites.

Viral loads at the WWTP ranged from  $4.1 \times 10^{11}$  GC/d to  $5.8 \times 10^{14}$  GC/d. The highest loads were observed on July 6th 2021, during the 5th wave, and on January 25th 2022, during the 6th wave. In the sampled neighborhoods, N1 loads ranged from  $3.3 \times 10^9$  GC/d to  $1.4 \times 10^{12}$  GC/d, with the highest loads also observed on July 6th 2021, during the 5th wave, and in mid to late January 2022 during the 6th wave. Notably, the maximum viral loads in wastewater occurred during the same week in all locations, and the peaks in the number of reported clinical cases aligned with the timing of the wastewater peaks across all locations (Fig. 2). The lowest loads recorded (seen in September 2021 for all study sites in Fig. 2) in wastewater also coincided with the lowest clinically recorded cases. Detailed information on N1 loads and reported cases by ABS data per sampling day for the WWTP and NGBs can be found in the supplementary Tables S1 and S2.

N1 concentrations in the wastewater collected at the hospital ranged from  $1.8 \times 10^3$  to  $8.4 \times 10^5$  GC/L (Fig. 3). However, on four sampling dates (Jan. 10th, Jan. 18th, Jan. 20th, and Feb. 15th 2022, shown as inverted triangles in Fig. 3), we observed inconsistent results between the number of active cases and viral load in wastewater. These dates coincided with the presence of significant foam in the sewer channel where the autosampler was installed (Fig. S4). It is suspected that

intensive cleaning activities were carried out at the hospital on these days, as the foam was accompanied by a noticeable chlorine smell. It is noteworthy that while the peak in reported clinical cases and wastewater viral load during the 5th wave occurred in the same week in the three neighborhoods and the hospital, this was not the case during the 6th wave. In the Omicron wave, the wastewater peak at the hospital occurred four weeks after the wastewater peaks observed in the NGBs (August 3rd 2021). Detailed information on N1 concentrations and active reported cases per sampled day can be found in Table S3.

#### 3.2. Correlations between WW loads and cases

The correlations between N1 loads and concentrations with reported cases are shown in Fig. 4.

The Pearson's correlation coefficient ( $r$ ) was calculated for each location and each wave using a 7-day window of summed daily cases, ranging from C–3 (6 days before sampling and the day of sampling) to C+7 (3 days after sampling to 10 days after sampling, corresponding to the longest lead time of early warning). The window is visually explained in S.I. Fig. S4. The neighborhoods showed higher correlations than the WWTP, as the correlations ranged from 0.437 to 0.762 at the WWTP, from 0.465 to 0.936 at the NGB1, from 0.730 to 0.977 at the NGB2, and from 0.671 to 0.939 at the NGB3. A gradient of correlations was obtained for each of the sites, yielding the highest correlations using C+3 at the NGB2 ( $r = 0.98$ ) and the WWTP ( $r = 0.76$ ), C–1 at the NGB1 ( $r = 0.94$ ), C–2 at the NGB3 ( $r = 0.94$ ). Lead times of early warning were different across waves and sites. In the 5th wave, the longest lead times of early warning were for the NGB3 (C+5), followed by the NGB2 (C+3) and the WWTP (C+3). In the 6th wave, the longest lead times of early warning were for the NGB1 (C+7) and the NGB2 (C+6).

### 4. Discussion

#### 4.1. Efficacy of WBE at neighborhood-level

This study provides valuable insights at the neighborhood level over a 1-year monitoring period, utilizing accurate and continuous 24-hour flow-proportional composite sampling with specialized infrastructure. Our findings suggest that SARS-CoV-2 surveillance at the neighborhood level is both feasible and accurate, as evidenced by the strong congruence observed between N1 loads and concentrations with clinical cases. Interestingly, the correlations at the neighborhood level were found to be better than those at the city level. This contradicts the findings by Acosta et al. (2022) in which data at WWTP level correlated better with clinical cases than that at neighborhood-level in Calgary. While their justification relies on their wastewater sewersheds separation from the stormwater system, we think that the sampling approach they employed might also played a role. Whereas in our study we employed continuous flow-based composite sampling, they employed time-weighted discrete composite sampling in 8 out of the 9 sampling sites. In small catchments like the NGBs monitored in this study, the number of COVID-19 cases typically ranged between 10 and 100 individuals (Fig. 2). Accumulated grab sampling, commonly used in composite sampling, is more susceptible to stochastic events in such scenarios. It can easily miss relevant pulses or overestimate viral loads by oversampling during periods of high viral load. Given the low number of relevant pulses (in the order of 10–100 a day, assuming an average of one defecation per day per individual), the uncertainties of a random process cannot be sufficiently smoothed out due to the limited number of pulses involved. Additionally, the residence time of wastewater at the NGBs was much shorter (a couple of hours) compared to the WWTP (over 12 h, as indicated in Table S4). Consequently, in-sewer degradation of the virus is expected to be less significant at the NGBs compared to the WWTP (Li et al., 2023). The correlation coefficients obtained in our study are at the upper range of values reported in the literature for WWTP and sewers with connected populations below 20,000 (average  $r$  of 0.63 in sewer studies, see Excel

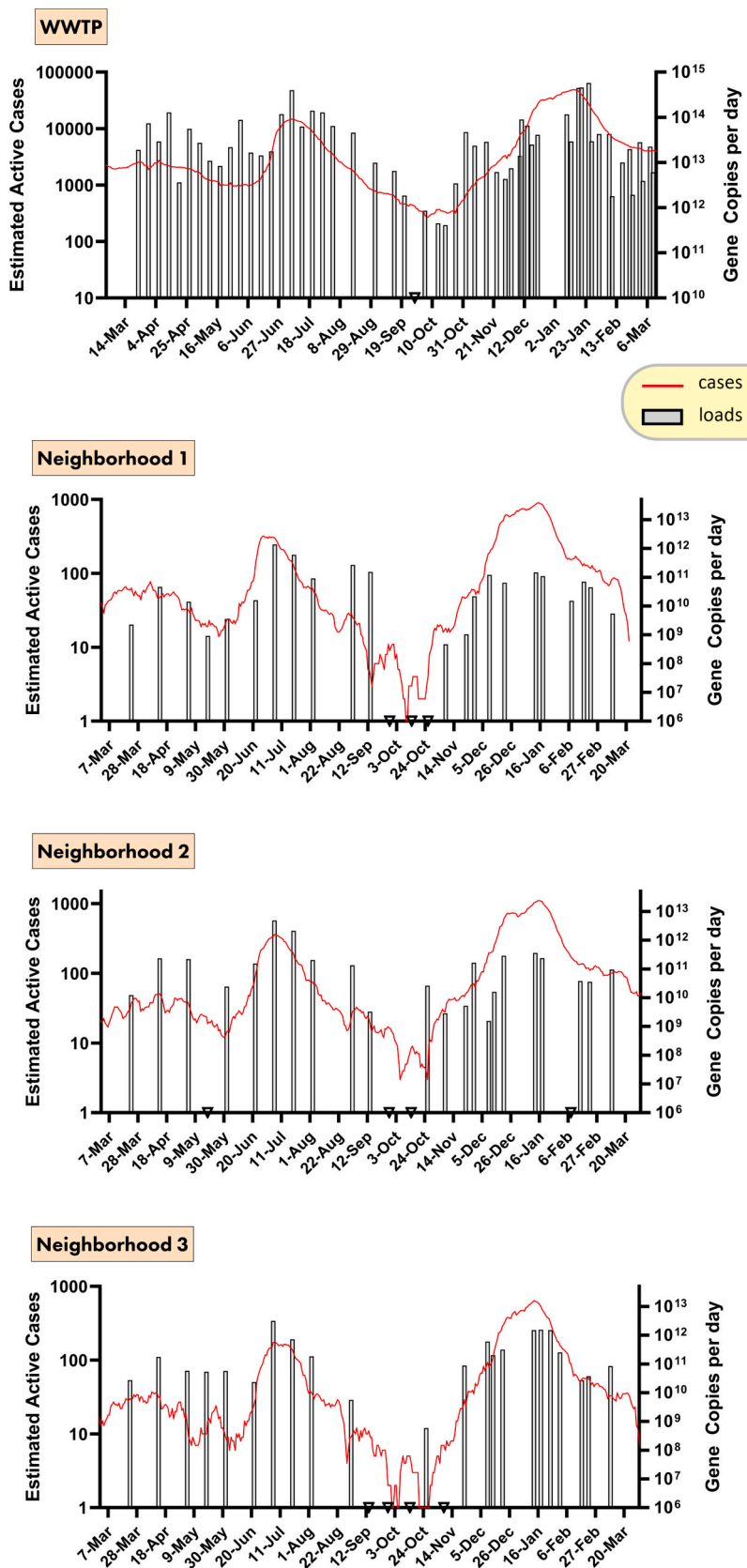


Fig. 2. Time series of reported clinical COVID-19 cases as estimated by summing the daily new cases from the day of sampling to 7-days after (red line – left y-axis) and SARS-CoV-2 loads (bars - right y-axis) at the four studied sites. Inverse triangles indicate the sampling dates where WW concentrations were below the detection limit of the assay.

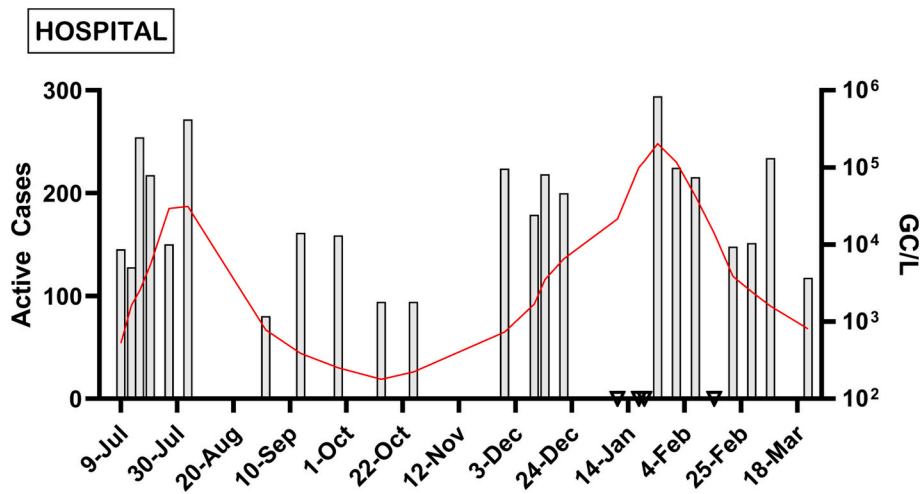


Fig. 3. Time series reported active clinical COVID-19 cases in the sampled hospital (red line – left y-axis) and gene copies per liter (bars – right y-axis). Inverted triangles indicate the dates where wastewater concentrations were below the detection limit of the assay.

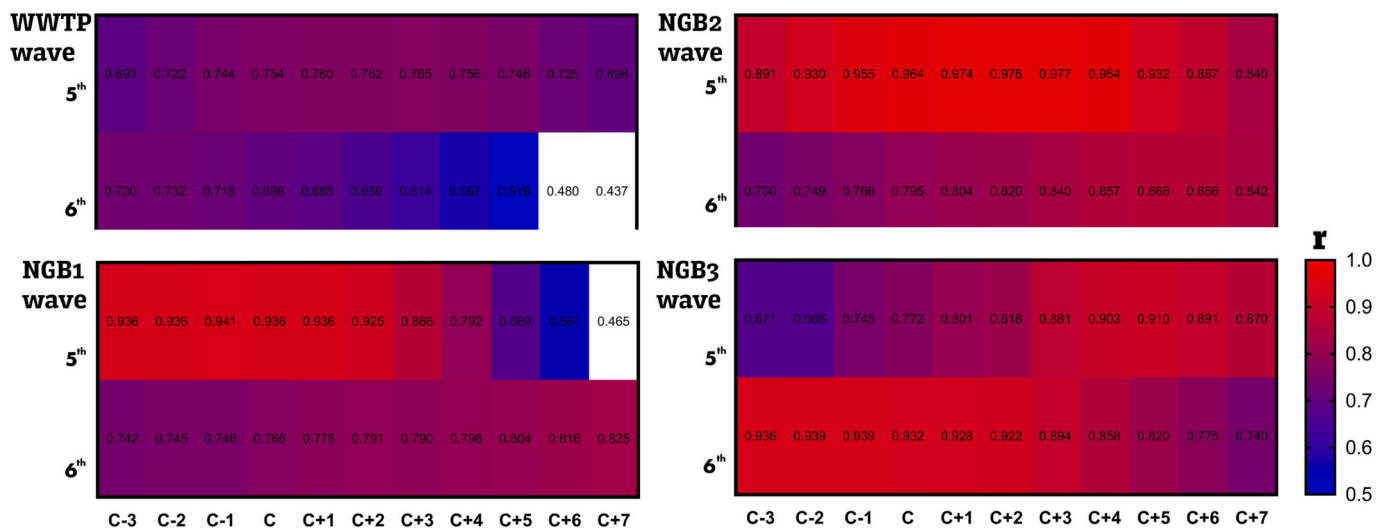


Fig. 4. Heatmap showing the values of correlation coefficients ( $r$ ) between wastewater N1 loads and reported COVID-19 cases as estimated by daily new detections during the two monitored COVID-19 waves (rows in each panel). In all sites, active cases were estimated with a 7-day time sliding window of summed daily cases and taking the day of sampling as reference. Thus, “C” is centered on the day of sampling and sums up the cases occurring 3 days before and after the sampling day as well as the day of sampling itself. C–3 corresponds to the sum of daily cases from six days before and including the sampling day. Each value from C–2 to C+7 is shifting the 7-day sliding window by 1 day in the future.

spreadsheet in Supporting Information). Notably, NGB 3 consistently exhibited  $r$ -values above 0.9 during both waves, although the time window for clinical case estimation differed between the waves (see Fig. 4, C–1 and C+5). An explanation for such a high correlation is that NGB3 has the largest population (18,042 residents) and features a steep sewer network gradient (0.020 m/m), resulting in higher flow velocities and shorter retention times. The other two NGBs have lower slopes (0.05 and 0.012 m/m), resulting in lower velocities and longer retention times. These hydraulic factors influence the contact time between SARS-CoV-2 and the sewage system components. Longer retention times can enhance the potential for viral removal through both physical and biological mechanisms, affecting sedimentation and biofilm growth, which contribute to the removal of SARS-CoV-2 (Maere et al., 2021; Morales Medina et al., 2022).

The N1 load normalized by clinical cases varied between the two waves. On a *per capita* basis, a higher load was recorded during the 5th wave when the Delta variant was dominant, compared to the 6th wave when the Omicron variant was prevalent (see Fig. 2). This WBE data

aligns with existing literature indicating higher shedding rates of patients during the Delta wave compared to the Omicron wave (Prasek et al., 2023; Puhach et al., 2022). It is attributed to differences in viral variant pathology and the higher vaccination rates in high-income countries during the Omicron wave. Studies by Acosta et al. (2023) observed higher N1 concentrations and N1 normalized by Pepper mild mottle virus (PMMoV) during the Omicron wave relative to the Delta wave in hospital settings, although the number of recorded patients during the Omicron wave was often several times higher, thus matching our *per capita* observations.

#### 4.2. Hospital WBE monitoring

The sampled hospital in this study serves a significant portion of the same population as the monitored WWTP and NGBs. The objective was not to assess the prevalence of infection within the hospital, as this information is already well documented. Instead, the aim was to assess hospitalizations resulting from community infections.

One interesting observation from the hospital monitoring was the discrepancy in timing between the WW peaks during the 5th and 6th waves compared to the peaks of clinical cases in the general population. During the 5th wave, the WWTP, NGBs, and the hospital all experienced peak viral loads simultaneously. However, this was not the case during the 6th wave, where the peak in WW viral load and hospitalizations occurred approximately a month later at the hospital compared to the general population (as shown in Fig. 2 relative to Fig. 3). The reasons behind this discrepancy are unclear. One possibility is that the 6th wave, dominated by the Omicron variant, resulted in fewer hospitalizations and/or shorter hospital stays due to milder symptoms (Wise, 2022). This reduced the immediate need for hospitalization. A similar time lag between community cases and hospitalizations was observed during the first COVID-19 wave in Sweden by Saguti et al. (2021) and in Austria during the Delta and Omicron waves by Schenk et al. (2023). This lag was consistently observed across multiple WWTPs and hospitals within the same areas, with longer lag times observed during the Delta wave compared to the Omicron wave. Hospital WBE monitoring has also been utilized to track nosocomial SARS-CoV-2 infections and correlate spikes in hospital wastewater with patients and healthcare workers within the hospital premises (Acosta et al., 2021).

Despite the accurate recording and control of the number of COVID-19 patients within the hospital, the correlations between cases and concentrations at the hospitals were relatively poor. This could be attributed to potential variations in viral shedding rates among different hospitalized individuals. For instance, a few “superspreaders” among a patient population of only a few tens could significantly impact the viral load measurements. Achieving strong correlations between cases and concentrations at hospitals has proven challenging, as observed by de Araújo et al. (2023) in their study tracking three hospitals.

#### 4.3. Lead times of early warning

Our study shows that lead times of early warning were different across waves and sites. Numerous studies in literature demonstrated the ability of SARS-CoV-2 surveillance in sewage to anticipate the number of diagnosed COVID-19 cases several days in advance relative to clinical monitoring and reporting. Examples of such studies include Peccia et al. (2020), Rusiñol et al. (2021), Saguti et al. (2021). The range of lead times varies as well across published studies, meaning that factors related to both SARS-CoV-2 virus shedding (i.e., vaccination rates, predominant variants) and clinical monitoring can influence the early warning potential. The efficiency of clinically detecting cases can vary. Factors such as the intensity of clinical testing and resource availability can affect the early detection of symptomatic individuals, thereby influencing the correlations between wastewater viral load and clinical cases. Higher testing intensity and better resource availability would result in the earlier diagnosis of individuals with symptoms, leading to stronger correlations with less anticipation (towards the left side of Fig. 4). The challenge associated with utilizing WBE as an early warning system lies in its inherent retrospective nature. Its level of relevance becomes apparent only when compared to clinical data. For instance, during the 5th wave, the wastewater analysis from NGB3 exhibited a stronger correlation with future COVID-19 cases than that of NGB1. However, at the time of analysis, this predictive capability was unknown. Given this retrospective aspect, we propose that WBE serves a more valuable role as an independent monitoring method for tracking prevalence. This approach remains consistent over time and is not contingent on the population's willingness to undergo testing. Moreover, it provides a real-time snapshot of the situation on the ground at the time of sampling.

#### 4.4. Socioeconomic status and risk of infection

While some studies have reported that individuals from economically disadvantaged backgrounds are more likely to be infected with

COVID-19 (Capasso et al., 2022; Oh et al., 2021), our study did not reveal a clear association between socioeconomic status and SARS-CoV-2 circulation. Interestingly, we observed a higher number of infections in the middle-income neighborhood (NGB2), which also happens to be a popular tourist destination and has a relatively young average resident age. Lancaster et al. (2022) collected data from eight cities in Ohio and concluded that wastewater viral concentrations were negatively associated with poverty, and positively associated with median income, community health centers, and onsite rapid testing locations. It's important to note that the distances between the three neighborhoods in our study were approximately 3 km, whereas in Lancaster's study, the distances among the eight cities spanned around 40 km. Additionally, variations in healthcare systems, with Europe having socialized systems and the USA having privatized systems, could contribute to these differences.

In general, the peaks of the two waves studied occurred simultaneously in all three neighborhoods, suggesting a concurrent pattern of viral circulation. Additionally, the maximum and median gene copies per day and per inhabitant were comparable across the neighborhoods (Table 2).

During the period from March 2021 to March 2022, the maximum estimated number of COVID-19 cases in the overlapping areas of the ABSs with NGB 1, 2, and 3 were 903, 1179, and 638 cases, respectively. It is worth noting that each ABS covers approximately the same population. The observed pattern of similar case numbers across neighborhoods can be attributed to the general reopening of activities in the later stages of the pandemic and high mobility in Barcelona. In the early stages of the pandemic, essential workers who were unable to work remotely were more likely to come from lower income households (Capasso et al., 2022). However, as the pandemic spread and most workers returned to their workplaces, the risk of infection became similar across income levels. This likely contributed to the convergence of infection rates among different income groups in the studied neighborhoods.

All three NGBs included in the study are located within the inner perimeter of the city of Barcelona. Public transportation within the city is accessible to most of the population, regardless of their neighborhood of residence. In 2017, the city's public transportation system recorded an average of over 6 million trips per day, with 68 % of those trips made by city residents (Ajuntament de Barcelona, 2017). The high mobility and intermingling of people within the city would contribute to lower differences in COVID-19 infection rates between individuals residing in different neighborhoods.

#### 4.5. WBE at neighborhood level: next steps

Although our study demonstrates the feasibility of monitoring SARS-CoV-2 at the neighborhood level and the stronger correlation with clinical cases compared to the WWTP-level, we did not find any essential advantage that neighborhood-level monitoring offers over sampling at the WWTP level, especially in highly interconnected areas. The peaks of N1 loads at all sites occurred in the same weeks, indicating a consistent pattern of viral shedding in the studied neighborhoods. While there may be a small benefit in terms of early warning of infections, implementing restrictions at a neighborhood scale would be challenging for a widely prevalent pathogen like SARS-CoV-2, as it has been throughout most of the past years. The widespread nature of the virus would require more comprehensive and coordinated measures at the city or regional level

**Table 2**

Max and median GC values per inhabitant recorded in the 3 neighborhoods of different socioeconomic status.

Measure	NGB1	NGB2	NGB3
Max GC/(day × inhabitant)	$2.69 \times 10^8$	$5.45 \times 10^8$	$1.73 \times 10^8$
Median GC/(day × inhabitant)	$4.30 \times 10^6$	$4.44 \times 10^6$	$3.95 \times 10^6$



rather than focusing solely on individual neighborhoods. A neighborhood-level monitoring effort would be most valuable at the early stages of a pathogen outbreak, before it evolves into an epidemic, assuming that the sampling frequency is sufficiently high. Early detection and localized interventions may be more effective in containing the spread of a newly emerging pathogen within a specific neighborhood. However, as the virus becomes more widespread, broader surveillance and intervention strategies are typically needed to address the larger-scale transmission dynamics.

## 5. Conclusions

Using WBE methods, this study successfully monitored the prevalence of COVID-19 in three small catchments as well as a reference hospital and the WWTP downstream to these catchments. The specialized infrastructure and continuous, flow-proportional, 24-hour composite sampling approach employed in this study yielded a strong correlation between SARS-CoV-2 RNA levels in wastewater and clinically reported cases. The correlation coefficient ( $r$ ) values obtained in this study were among the highest reported in the literature, indicating the robustness of the findings. Notably, the correlations between NI loads and clinical cases were stronger at the neighborhood level compared to the wastewater treatment plant (WWTP) level. Interestingly, higher viral loads were observed during the 5th wave of the pandemic compared to the 6th wave, despite a higher number of clinical cases being recorded in the latter. This suggests that other factors, such as viral shedding rates or testing and reporting practices, may have influenced the observed differences. Furthermore, the study did not find any apparent association between socioeconomic status and susceptibility to COVID-19 among the monitored populations. This indicates that the prevalence of infection was not significantly influenced by socioeconomic factors in these specific neighborhoods. Hospital WBE monitoring was not useful in benchmarking community infections but differences in hospitalizations and community infection rates were recorded through the clinical data provided by the hospital.

## CRedit authorship contribution statement

**I. Zammit:** Investigation, Writing - Original Draft, Review & Editing, Formal analysis, Visualization; **S. Badia:** Investigation, Writing - Original Draft, Visualization; **C. Mejías-Molina:** Investigation; **M. Rusiñol:** Writing - Review; **S. Bofill-Mas:** Resources, Supervision Writing - Review; **C. Borrego:** Writing - Review & Editing; **LL. Corominas:** Supervision, Conceptualization, Resources, Project administration, Funding acquisition, Writing - Review & Editing

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.167811>.

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