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Surveillance of SARS-CoV-2 in sewage from buildings housing residents with different vulnerability levels

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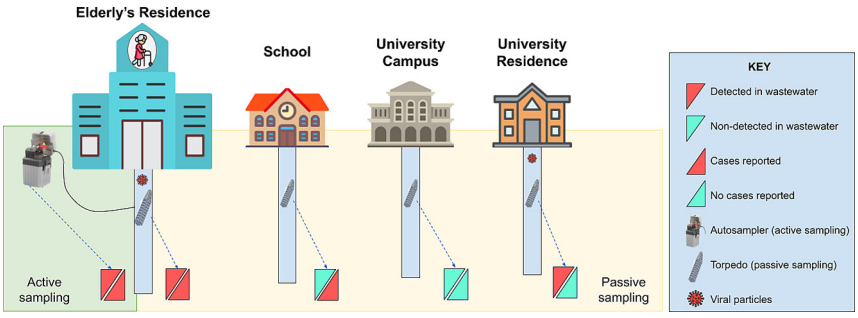
HIGHLIGHTS

- The COVID-19 prevalence detected *via* wastewater was 0.4 % using active sampling and 2.2 % using passive sampling.
- In non-residential buildings, infrequent toilet use causes a decoupling between cases and sewage viral load.
- Passive sampling is an affordable tool to monitor COVID-19 prevalence in buildings.
- Sewage surveillance using passive samplers is complementary to clinical testing.

GRAPHICAL ABSTRACT



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ABSTRACT

During the last three years, various restrictions have been set up to limit the transmission of the Coronavirus Disease (COVID-19). While these rules apply at a large scale (*e.g.*, country-wide level) human-to-human transmission of the virus that causes COVID-19, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), occurs at a small scale. Different preventive policies and testing protocols were implemented in buildings where COVID-19 poses a threat (*e.g.*, elderly residences) or constitutes a disruptive force (*e.g.*, schools). In this study, we sampled sewage from different buildings (a school, a university campus, a university residence, and an elderly residence) that host residents of different levels of vulnerability. Our main goal was to assess the agreement between the SARS-CoV-2 concentration in wastewater and the policies applied in these buildings. All buildings were sampled using passive samplers while 24 h composite samples were also collected from the elderly residence. Results showed that passive samplers performed comparably well to composite samples while being cost-effective to keep track of COVID-19 prevalence. In the elderly residence, the comparison of sampling protocols (passive *vs.* active) combined with the strict clinical testing allowed us to compare the sensitivities of the two methods. Active sampling was more sensitive than passive sampling, as the former was able to detect a COVID-19 prevalence of 0.4 %, compared to a prevalence of 2.2 % for passive sampling. The number of COVID-19-positive individuals was tracked clinically in all the monitored buildings. More frequent detection of SARS-CoV-2 in wastewater was observed in residential buildings than in non-residential buildings using passive samplers. In all buildings, sewage surveillance can be used to complement COVID-19 clinical testing regimes, as the detection of SARS-CoV-2 in wastewater remained positive even when no COVID-19-positive individuals were reported. Passive sampling is useful for building managers to adapt their COVID-19 mitigation policies.

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1. Introduction

The Coronavirus Disease (COVID-19) pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) lead to over 605 million confirmed cases and over 6,4 million deaths globally since its emergence in late 2019 (WHO,September 14, 2022). Apart from the cost in terms of human well-being, the restrictions imposed by governments as a response to the global spread of COVID-19 impaired the economic activity of many countries causing a worldwide economic crisis from which we are still recovering (Aktar et al., 2021). The main challenge for public and private institutions was to keep their activities running despite the emergence of local human-to-human SARS-CoV-2 transmission. In Spain, following the initial nationwide confinement (from March 12th to June 21st, 2020 in Spain, Viguria and Casamitjana, 2021), different measures were established at the building scale. Such measures involved the usage of protective equipment, limiting the number of people in common spaces, and the implementation of technologies to facilitate remote working. Furthermore, regular clinical testing of individuals was adopted in buildings other than hospitals and elderly residences to detect COVID-19 outbreaks as soon as possible and prevent further disruption of activity. Thus, it is in the interest of building managers to be proactive with their COVID-19 strategy. The progressive understanding of COVID-19 transmission routes, its treatment, and prophylaxis (*i.e.*, vaccination) allowed most countries to enter the "co-existing" scenario. Supplementary Table S1 describes the three strategies (named A, B, and C) applied in different buildings and refers to the increasing intensity of surveillance (A higher, C lower), which depends both on the vulnerability of the residents and on the legal responsibility of the institution concerning the reporting strategy imposed by Health authorities.

The measures employed to control COVID-19 varied in time, between countries, and between different institutions within the same country. The priority was testing essential workers and severe cases, first using PCR tests and, later, using rapid antigen tests. In this regard, massive random screening to detect mild and asymptomatic cases has been common in some vulnerable communities but not applied at large scale (*i.e.*, cities) because of its cost (Hassard et al., 2021).

Wastewater-based epidemiology (WBE) has been demonstrated to be a non-invasive, cost-effective tool to track the circulation of SARS-CoV-2 by sampling influent wastewater at Wastewater Treatment Plants (WWTPs). Since individuals infected with SARS-CoV-2 shed viruses in their feces (Wölfel et al., 2020), the viral genetic traces can be detected and quantified in wastewater (Medema et al., 2020). Several studies also demonstrated its effectiveness on a smaller scale, namely: in neighborhoods (Barrios et al., 2021; Spurbeck et al., 2021), in hospitals (Liu et al., 2022; Sharkey et al., 2021; Spurbeck et al., 2021; Wang et al., 2022), in universities (Anderson-Coughlin et al., 2022; Bivins et al., 2022; de Llanos et al., 2022; Fahrenfeld et al., 2022), in schools (*e.g.* Crowe et al., 2021; Kapoor et al., 2022), and households (*e.g.* Wong et al., 2021; Xu et al., 2021). One of the challenges of applying WBE at building scale is how to make the sampling cost-effective. At the entrance of WWTPs, samples are usually collected as a 24-h composite sample using costly autosamplers (between €5000 and €8000) that are not affordable for the collection of sewage in buildings. Grab sampling has been used as an alternative, but the uncertainties associated with sampling small communities are too large when compared to composite sampling (Ort et al., 2005). In the case of composite samples, the higher the sampling frequency (ideally continuous), the lower the associated uncertainty. An alternative is to use passive samplers, a method widely used to track chemicals in water (*i.e.*, Haas and Herrmann, 1998; Snow et al., 2012; Sultana et al., 2017) and recently adapted for monitoring SARS-CoV-2 (Schang et al., 2021). A passive sampler is a device that is exposed to the matrix of interest (*e.g.*, wastewater) over a known period of time (sampling period) and from which it entraps the analytes of interest (*e.g.* SARS-CoV-2). Some studies demonstrated that passive samplers are a cost-effective tool for the surveillance of SARS-CoV-2 in sewage, especially for small communities (Habtewold et al., 2022; Li et al., 2022; Liu et al., 2022; Schang et al., 2021; Mejías-Molina et al., 2023). While passive sampling is qualitative not quantitative as is active sampling, both sampling methods can be used to assess the circulation of the virus in terms of detection/non-detection.

Our main goal was to evaluate the efficacy and utility of sewage surveillance when implemented in buildings hosting communities of different ages, COVID-19 vulnerability, social habits, and case reporting. Although several papers have reported the application of wastewater surveillance on a small scale, only 2 of them have focused on schools and only one on an elderly residence (Davó et al., 2021). Besides, we have developed a sampling protocol that fits all these institutions. For that purpose, we monitored one outbreak in four buildings using passive sampling and, in the elderly residence, we also used active sampling to compare both protocols.

2. Materials and methods

2.1. Study sites

Sampling was performed in four different buildings located in two cities in Catalonia, Spain (Table 1). Buildings differed in the number of people gathering daily and in the age group of their residents. Two of the buildings (*ElderlyRes* and *UnivRes*) are residential and we thus assumed that their inhabitants used the toilets daily. In turn, inhabitants from non-residential buildings were less likely to use the premise's toilets to defecate. Thus, we expected that sewage surveillance would be more effective in the residential buildings since the collected samples would be more representative of the hosted community.

The studied buildings also differed on the COVID-19 reporting strategies and protective measures applied, namely:

- Elderly residence (*ElderlyRes*): was managed following a *surveillance strategy A*, consisting of a weekly rapid antigen test for all workers and residents, and extra tests when individuals showed COVID-like symptoms. Hence, data on the number of symptomatic and asymptomatic COVID-19 cases were available. If a community member tested positive, residents living in the same area of the building were tested and isolated in case of a

Table 1
Sampling points' characteristics. Due to ethical issues, the exact location of the buildings is not provided.

Type	ID	N° People	Age group	Living there?	Reporting strategy (A, B, C)
Elderly residence	ElderlyRes	500	Residents: >65 Staff: 16–65	Yes	A
School	School	500	Students: 3–12 Staff: 18–60	No	B*
University Campus residence	UnivRes	42 (rooms)	Residents: 17–30 Staff: 18–65	Yes	C
University Campus (Lecture building)	UniCamp	unknown	Residents 17–67	No	C

* The strategy changed from B to C on February 28th.

positive outcome until they tested negative. Visits from relatives were limited and entrance was only allowed after being negative in a rapid antigen test. This is the building having the most restrictive measures because it hosts the most vulnerable community. Accordingly, the community was very stable. Reporting new COVID-19 cases to health authorities was mandatory.

- Primary school (*School)*: was managed following *surveillance strategy B*. In this case, each student class was considered a 'bubble' (isolated group) and do not mix with other classes (separate bubbles). There was a protocol in place for quarantining new COVID-19 positives and their contacts. From the beginning of the sampling campaign (10th December 2021) until the 21st of February 2022, it was mandatory that all students that had been in contact with positive individuals underwent a rapid antigen test and, if positive, had to quarantine for 10 days. Additionally, if 5 students from the same class tested positive, all members of the class had to quarantine for 10 days. Accordingly, the number of positive cases was well-known during the study period due to the obligation to test and report positive cases to the health authority. On March 28th, 2022, the protocol changed, and only new positive cases had to undergo quarantine. As a result, schools lost track of the COVID-19 incidence and had no obligation to report cases. The community surveilled was dynamic (the individuals within this community changed frequently over time) because of the different isolation procedures.
- University Campus Residence (*UnivRes*): Wearing a mask was compulsory in shared spaces (*e.g.*, corridors). If an individual sharing an apartment tested positive, they were isolated into an individual apartment until testing negative. However, the administration of the building had no obligation to keep track of new COVID-19 cases. In this case, the responsibility lays on the individual, who had to directly report their situation to the health authority (*Strategy C*, Table 1). The health administration was then responsible to keep track of the individual close contacts.
- University Campus (*UniCamp*): The University had no obligation to report COVID-19 cases since the responsibility falls on the individual. The monitored community was also very dynamic and not very predictable since undergraduate students attend different classes at different locations and the reporting of attendance is not mandatory. Surveillance in this building was classified as *Strategy C* (Table 1).

The use of protective equipment such as face masks was compulsory even outdoors until the 8th of February 2022. After this date, the use of face masks was only mandatory indoors at all teaching buildings (*i.e.*, in the tests sites identified as *School*, *UnivRes*, and *UniCamp*).

2.2. Clinically confirmed cases

During the sampling campaign, all building managers agreed to provide the number of cases they recorded every day. For buildings with *Strategies B* and *C*, as positive individuals were isolated at home (and therefore they were not contributing to shedding within the studied building) we assumed that they were shedding the virus for 3 days before reporting. This assumption is a conservative approach taken from the range suggested by Miyama et al. (2022) between the symptoms onset and the reporting of cases (supplementary Fig. S1). For buildings under *Strategy A* (*ElderlyRes*), we were informed of the exact days that the case tested positive because they were repeatedly tested. This information was used to calculate the number of infected people per day.

2.3. Sampling frequency and protocols

The sampling period started on the 10th of December 2021 and ended on the 29th of March 2022 (the 6th COVID-19 wave in Catalonia). All buildings were sampled weekly except during two periods, namely: 1) between the 21st of December and the 10th of January no samples were collected from the school nor the University because these facilities were closed during the Christmas break; 2) between the 10th of January and the 10th of March 2022 we increased the sampling frequency to 2 samples per week after training technicians from the studied buildings and to detect the peak in COVID-19 positive cases.

Two sampling protocols were used, a passive sampling in all buildings and an active sampling only in the elderly residence. The active sampling was performed using a HACH-Bühler 2000 autosampler and the passive, using a torpedo-like 3D-printed device designed and published as open source by Schang et al. (2021). The latter contained 3 Mixed Cellulose Esters membranes (EZ-Pak® Membrane Filters, Millipore EZHAWG474). In both cases, the sampling lasted 24 h (from 9 a.m. to 9 a.m. of the following day). The main wastewater channel of each building was accessed *via* a manhole. The autosampler was programmed to collect an aliquot of wastewater every 15 min for 24 h. The autosampler had 24 separate bottles collecting samples from each hour allowing to manually compose a volume-proportional composite sample. We used a sample proportion of 3:2:1 for the morning peak:day:night periods.

Samples were stored refrigerated during collection and transport. Torpedoes were deployed for the same period. Sample processing in the laboratory took place within 24 h after collection. In this regard, 250 mL of the composite sample was used to analyze some physicochemical parameters such as total Kjeldahl nitrogen (TKN), total suspended solids (TSS), and chemical oxygen demand (COD) using standard methods.

2.4. Sample processing and molecular analyses

Passive sampler units were dismantled, and the inner membranes were used for the extraction of viral nucleic acids using the RNeasy Power Microbiome Kit (Qiagen) into a final extract volume of 50 μL.

For the wastewater samples collected through active sampling, 100 mL of wastewater was spiked with the bacteriophage MS2, at a final concentration of 1×10^5 gene copies per mL (GC·mL^{-1}), as a process control. Samples were then centrifuged to remove debris (4750 $\times g$ for 30 min) and 80 mL of the supernatant was ultrafiltered (150 KDa) using the automatic Concentration Pipette (CP-Select™, InnovaPrep, USA) into a final volume of 300 μL. Extraction of nucleic acids was performed using the QIAamp Viral RNA Mini kit using the QIAcube automatic system (Qiagen) into a final volume of 70 μL. A negative control of the extraction was included per batch of samples.

The presence of SARS-CoV-2 (N1 and N2 assays) and JC polyomavirus (positive control of human fecal pollution) were quantified in nucleic acids extracts using specific quantitative PCR (qPCR) assays as previously described (Moore et al., 2020; Pal et al., 2006). The primers, probes and qPCR programs used, and the list of Gblocks® designed are provided in supplementary material Tables S2 and S3. Commercially available Twist Synthetic SARS-CoV-2 RNA Control (Control 14, EPI_ISL_710528) was used to prepare standard curves for genome quantitation. JCPyV has been described as a useful human fecal indicator (Rusiñol et al., 2014). This virus is excreted by >60 % of the population, it is asymptomatic in most cases, and persistent. Besides, it has been described by Mayer et al. (2016) to be a better indicator than human adenovirus in small sewerage systems. We used the RNA Ultrasense™ One-Step RT-qPCR System (Invitrogen) and the TaqMan® Environmental Master Mix 2.0 (Thermofisher Scientific) for SARS-CoV-2 and JCPyV assays, respectively. All qPCR standards were prepared using either a synthetic SARS-CoV-2 control (Control 51 from Twist Biosciences) or a synthetic gBlock Gene fragment (IDT) for JCPyV (Rusiñol et al., 2020), which were previously quantified with a Qubit fluorometer (Thermofisher Scientific) 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 ± 0.2; $r^2 \geq 0.99$; and mean efficiency above 95 %. To evaluate enzymatic inhibition, undiluted and 10-fold dilutions of the sample extracts were analyzed. No replicates were performed. Recovery was assessed by quantifying phage MS2. The recovery mean was of 40 % (initial concentration of 1×10^5 GC·mL^{-1}) with an SD of 65 %. All the PCR assays included a non-template control (negative control). Quantification was performed in a QuantStudio™ Real-Time PCR System from ThermoFisher.

2.5. Data analysis

For samples collected using the autosampler, we calculated the geometric mean of the gene targets analyzed (N1 and N2, in GC·mL^{-1}). For the days where N1 or N2 were below the limit of quantification (LOQ), we used half of the LOQ to calculate the mean. The normality of the residuals was tested using a Shapiro-Wilk test and Homoscedasticity using a Breusch-Pagan test. Pearson correlation was performed between the geometric mean of N1 and N2 and the number of clinical cases reported at the *ElderlyRes*. Data from passive samples were interpreted as qualitative (*i.e.*, positive (detected) or negative (non-detected)). Detection of both or either N1 or N2 targets in a sample was considered positive, while a sample was considered negative when neither N1 nor N2 were detected above their limits of detection. When the control virus (JCPyV) was also not detected, the sample was discarded, see details in Supplementary Table S4. All data analysis was done using R Software (R Core Team, 2020) and visualized using the package *ggplot2* (Wickham, 2016).

3. Results

3.1. Elderly residence: cases vs. active sampling

At the elderly residence, 13 out of the 21 wastewater samples collected using active sampling (62 %) were positive for SARS-CoV-2 (Fig. 1). In the remaining 8 samples, both N1 and N2 were below the limit of detection of the assay. These negative samples were collected either at the early stages of the 6th wave (before cases rocketed) or during the end of the outbreak (after 21st February 2022). The mean concentration of N1 and N2 during the studied period ranged from 1.31 × 10^3 GC·L^{-1} to 2.72 × 10^5 GC·L^{-1}. The concentration of N1 ranged from 6.66 × 10^3 to 2.97 × 10^5 GC·L^{-1} and from 9.54 × 10^2 and 2.94 × 10^5 GC·L^{-1} for N2 (Suppl. Table S4). Wastewater characteristics from the *ElderlyRes* were typical for raw urban wastewater (Suppl. Table S5). The measured TSS was 223.33 ± 44.98 mg·L^{-1}, which was lower than the TSS threshold limit of 497 mg·L^{-1} suggested for passive sampling (Hayes et al., 2021).

On the 19th of December 2021, one worker tested positive and remained isolated off-site. However, the two composite sewage samples taken around that date were negative for both gene targets. The surge in cases linked to the outbreak of the 6th wave in Catalonia was reported in the *ElderyRes* on the 27th of December 2021. The first sample collected after Christmas holidays (11th January 2022) tested positive (4.37 × 10^3 GC·L^{-1}). Cases remained stable during most part of January but rose from 14 to 20 during the final week (24th of January 2022). This increase coincided with an increase in the concentration of SARS-CoV-2 gene targets in sewage (from 4.35 × 10^3 to 2.08 × 10^4 GC·L^{-1}). After peaking on the 1st of February, the number of cases went down to zero in 25 days. A concomitant decrease in the concentration of gene targets in wastewater was also observed, going from 2.72 × 10^5 GC·L^{-1} on the 8th of February to non-detectable levels on the 24th of the same month. Once cases were 0, viral RNA in wastewater were still detected in one sample (March 1st, 2022). High-frequency clinical testing allowed us to detect all the asymptomatic cases and we observed that they represent around 50 % of the total cases (15 asymptomatic out of 33 cases in the peak, Fig. 1).

A good and significant correlation (Pearson correlation coefficient R = 0.61, p-value 0.03426) was observed between cases and gene targets in wastewater (Fig. 2), indicating a linear relationship between the two variables (slope of 1.3, confidence interval of 0.3–2.3). The model of residuals displayed a normal distribution (p-value 0.142) and showed equal variance (p-value of 0.729).

3.2. Elderly residence: active vs. passive sampling

To assess the validity of passive sampling to detect SARS-CoV-2 in wastewater, we compared the results from torpedoes to those from composite samples. Since results from passive samplers were qualitative (detected/non-detected), quantitative results from composite samples were also converted into a binary outcome (values above the detection limit were considered "detected" and those below the limit of detection limit were considered "non-detected") (Table 2). Overall, we observed a good match between the detection of SARS-CoV-2 by both approaches since 14 out of 18 samples showed a perfect match (11 detected, 3 non-detected by both methods). The mismatch came for samples collected during dates of low COVID-19 prevalence, with SARS-CoV-2 results from composite samples close to the limit of detection. Three composite samples tested positive

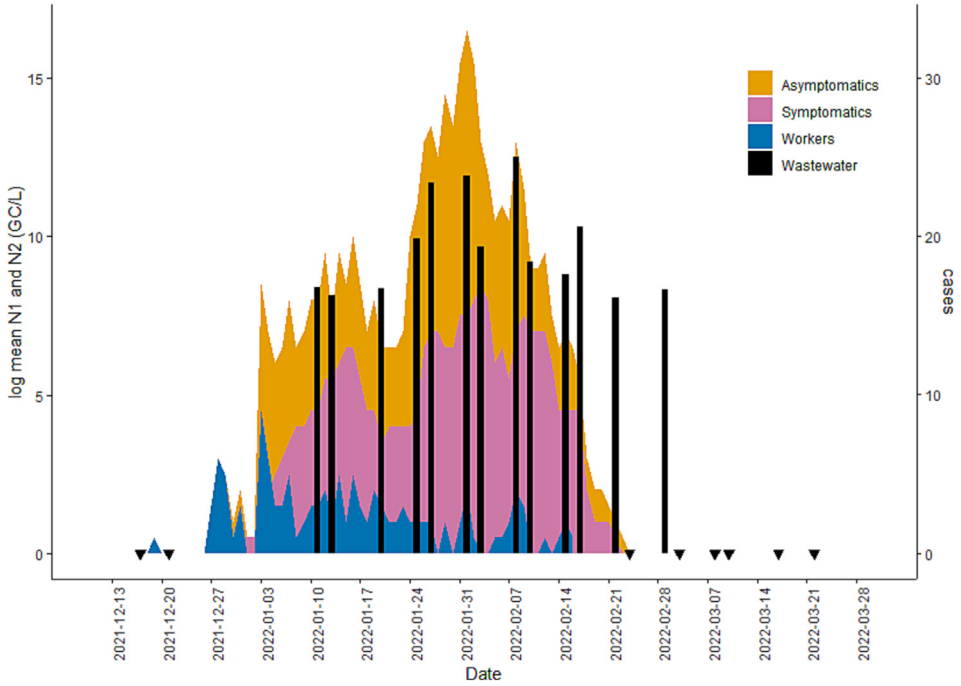

Fig. 1. Geometric Mean concentration of gene targets N1 and N2 (black bars, in log of GC·L^{-1}) and COVID-19 cases (shaded areas that are color-coded by subpopulation, see legend) in the elderly residence during the studied period (December 2021–March 2022). Inverted black triangles show the dates where both gene targets were below the limit of detection.

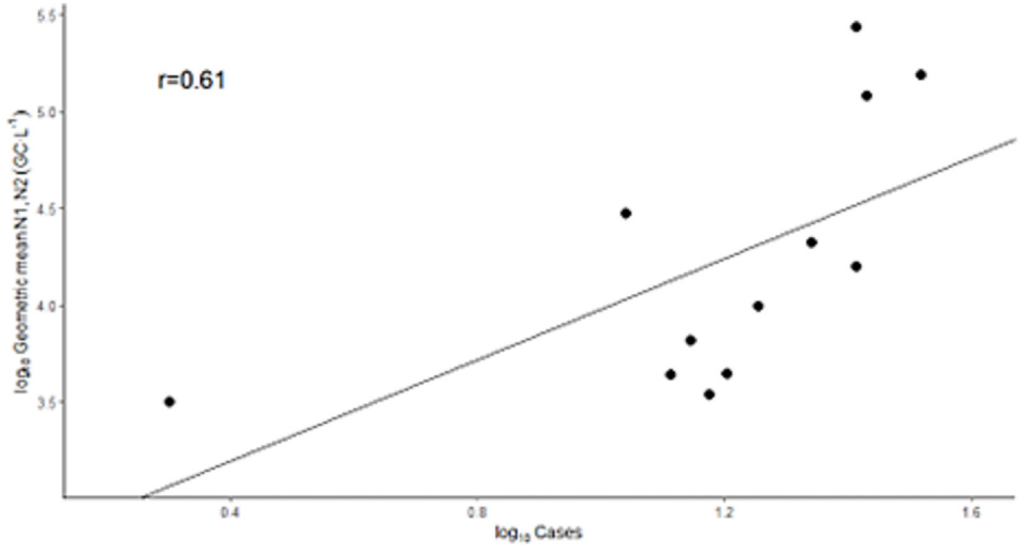

Fig. 2. Correlation between the reported cases and the geometric mean of the concentration of gene targets. Both variables were logarithmically transformed.

but negative in passive samplers, whereas 1 sample yielded the inverse outcome. With active sampling the reference, passive sampling had a sensitivity of 78 %, a specificity of 75 % and positive and negative predictive values of 91 % and 50 %, respectively. Nevertheless, the number of parallel samples was limited, and a larger sample size is needed to validate our preliminary results.

3.3. Evolution of pandemic through sewage in all buildings

Fig. 3 summarizes the evolution of the pandemic through sewage in all buildings monitored. In December 2021, most of the samples tested negative, except for the *School*, where a positive sample was detected and assigned to the positive clinical test reported on that date. In March 2022, most of the samples were also negative, coinciding with the end of the 6th wave in Catalonia. In between, we detected most of the positive samples. For the *UnivRes* and the *ElderlyRes,* we continuously observed positive detection during the 6th wave period. For the non-residential buildings (*UniCamp* and *School*), the detection was rather intermittent which probably reflects the fact that these buildings hosted a lot of temporary visitors.

The number of reported cases is also indicated in Fig. 3 (numbers in cells). It is worth pointing out that the correlation between wastewater gene copies and cases reported was high for the *ElderlyRes* (Fig. 2) where strict clinical testing was conducted on a weekly basis. In the case of the university residence (*UnivRes*), the sewage surveillance detected the circulation of SARS-CoV-2 but no cases were officially reported.

4. Discussion

The application of sewage surveillance to track the communal circulation of SARS-CoV-2 has proven useful at different scales, from large geographic areas (Rusiñol et al., 2021; Vincent-Hubert et al., 2022) to separate buildings of different typologies (de Llanos et al., 2022; Gutierrez et al., 2021; Spurbeck et al., 2021). Our study has revealed that sewage surveillance can also uncover blind spots in monitoring strategies implemented in different facilities to track and report COVID-19 cases among vulnerable populations. We observed differences between residential and non-residential buildings in relation to the intermittency of detection during COVID-19 outbreaks. The intermittencies detected might be explained by the different habits of toilet usage by residents since most (if not all) inhabitants in residential buildings use the toilet within the same building (thus shedding their viral load in place) in contrast to its seldom use in non-residential buildings.

Sewage surveillance is useful for buildings with clinical testing in place and implementing mandatory case reporting to Health authorities (*i.e.*, *strategy A*, Table S1). In this case, the frequency of clinical testing can be adjusted after sewage surveillance outcomes (*e.g.*, by increasing the testing frequency as soon as a positive detection in sewage is observed). Some studies at university campuses have demonstrated that sewage surveillance was able to anticipate COVID-19 outbreaks in comparison to clinical testing (*e.g.*, de Llanos et al., 2022; Wang et al., 2022; Welling et al., 2022). Our study did not provide evidence for an early warning of the outbreak in the elderly residence, since sample collection and analysis were discontinued during the Christmas holidays (21st of December 2021 – 10th of January 2022). Remarkably, however, we continued to detect SARS-CoV-2 RNA in sewage even when no cases were reported (1st, 3rd, and 10th of March), probably because viral shedding continued for some time after recovery (Zheng et al., 2020), especially in older people (Omori et al., 2021).

Sewage surveillance is also useful in buildings with no mandatory case reporting (*Strategies B* and *C*, Table 2) since it provides anonymous and cost-effective data on the communal circulation of SARS-CoV-2 in the target population, thus allowing the fast implementation of protective measures. The reporting strategy at the University residence and the University Campus facilities overlooked case counts and, therefore, protective measures were not further modified allowing the uncontrolled spread of infections.

Table 2
Comparison of passive and active sampling for the detection of SARS-CoV-2 in wastewater.

		Passive sampling (torpedoes)	
		Detected	Non-detected
Active sampling (composite samples)	Detected	11	3
	Non-detected	1	3

4.1. Defining the minimum number of cases for wastewater detection

The high frequency of clinical testing and case reporting at the elderly residence allowed us to obtain a reliable comparison between the number of cases and sewage signals (Fig. 2). This permitted an accurate estimation of the minimum number of cases that can be detected through wastewater monitoring. Samples collected using passive sampling did not test positive until there were 11 confirmed cases among a population of 500 residents (a prevalence detection limit (PDL) of 2.2 %). By using active sampling,

Fig. 3. Detection of N1 or N2 in the wastewater collected from the studied buildings using passive samplers for all cases except for the elderly residence where both active (*ElderlyRes-W*) and passive (*ElderlyRes-T*) sampling was used. Red cells indicate the dates where detection was positive and green cells when negative. The numbers inside the cells are the reported clinical cases in the corresponding building. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

we detected positive samples when only 2 cases were reported (PDL of 0.4 %). In a University residence hall, Corchis-Scott et al. (2021) detected SARS-CoV-2 in sewage when the COVID-19 prevalence in the community was 2.3 % (1 case among 86 individuals) using passive sampling. Using active sampling, Spurbeck et al. (2021) detected positive samples when the COVID-19 prevalence in the studied elderly residents was 1.5 %. At the small community scale, our PDL was within the ranges reported in the specialized literature. On a larger scale, such as WWTPs covering many thousands of inhabitants, the PDL tends to be much lower. Examples include detections with passive sampling when 0.02 – 0.03 % of the population was infected (Li et al., 2022; Liu et al., 2022; Schang et al., 2021). Using active sampling, wastewater detection was positive for PDL of 0.01 – 0.08 % (Rusiñol et al., 2021). The lower PDL in large catchment areas may respond to the fact that many cases (either asymptomatic or mild) within large communities remained unreported but the incidence is calculated from official case counts. In turn, the strict and periodical clinical testing carried out in small communities (*i.e.*, the elderly residence) allows the detection of all infected individuals, either symptomatic or not, thus providing a more reliable calculation of the actual prevalence. Accordingly, our results pointed out that the frequency of clinical testing (either by qPCR or rapid antigen tests) and not the type of sampling (passive *vs.* active) is a major driver explaining the mismatch between SARS-CoV-2 detection in sewage and reported cases. Also, the fact that in some buildings the new cases were immediately separated from the host community (*i.e.*, in the school) not only avoids the spread of infection but also removes its contribution to the communal waste. Under this scenario, detection in wastewater becomes unlikely despite the sampling protocol in use.

Another consideration to bear in mind, especially when comparing studies covering different pandemic waves, is the SARS-CoV-2 variant that prevails at the time of sampling. Our study was carried out amid the wave attributed to Omicron (lineage B.1.1.529) and recent studies reported that this variant produces lower fecal loads than previous ones (*e.g.*, alpha or delta, (Hay et al., 2022; Sentis et al., 2022). Thus, a larger number of shedders is necessary to reach the same RNA concentration in wastewater. Besides, the vaccination coverage among the population under study is also relevant. Levine-Tiefenbrun and coworkers reported that, after infection, vaccinated individuals shed fewer viruses in their feces than non-vaccinated ones (Levine-Tiefenbrun et al., 2021). During our study period, both residents and workers of the elderly residence were vaccinated, and the vaccination coverage of the university community was 90 %.

4.2. Effectiveness of passive samplers

Our study corroborates that passive sampling is a cost-effective alternative to active sampling, thus agreeing with previous studies on the matter (Habtewold et al., 2022; Li et al., 2022; Liu et al., 2022; Schang et al., 2021). While the appeal of passive samplers is clear mainly because of their cost-effectiveness and simple deployment, they also suffer from some limitations. Passive samplers have a more limited range of applicability since high COD and TSS in wastewater greatly affect their performance (Hayes et al., 2021). Also, there is no possibility either to collect proportional samples or to calculate the flow passing through, thus impeding quantitative measurements. In this regard, measurements are thus bound to inaccuracies since chemical and biological compounds, including SARS-CoV-2 particles, are known to fluctuate within a single day (Bivins et al., 2021). There is a common saying among photographers that states that “the best camera is the one you have with you”. Scientists applying sewage surveillance principles may thus consider these lines since the know-how and applicability of expensive and complex autosamplers is a limiting step when studying processes at a smaller scale.

5. Conclusions

Herein we show that passive sampling using torpedoes is a feasible, practical, and cost-effective method to monitor SARS-CoV-2 infections circulating in buildings housing different types of communities.

Sewage surveillance using passive samplers is complementary to clinical testing and we consider it especially useful as a tool to finely tune the intensity of epidemiological surveillance in buildings hosting vulnerable populations (*e.g.*, schools, elderly residences, among others). For buildings with less vulnerable communities, passive samplers can also be useful to rapidly detect outbreaks and avoid the massive spreading of infections. In comparison with active sampling, passive sampling performs similarly well since their detection pattern matches well, unless the prevalence is close to the limit of detection.

The minimum prevalence we were able to detect in the elderly residence was 0.4 % using active sampling and 2.2 % using passive sampling. These values are comparable to values previously reported for similar locations.

The knowledge of the daily habits of residents is key to properly interpret the results. In non-residential buildings, the detected intermittencies could be explained by the seldom use of toilets in these premises.

CRediT authorship contribution statement

Anna Pico-Tomàs: Formal analysis, Investigation, Data Curation, Writing - Original Draft; Visualization; **Cristina Mejías-Molina**: Investigation; **Ian Zammit**: Writing, Review & Editing; **Sílvia Bofill-Mas**: Funding acquisition, Project administration, supervision; **Marta Rusiñol**: Conceptualization, Review; **Carles M. Borrego**: Review & Editing, Supervision, Funding acquisition; **Lluís Corominas**: Conceptualization, Investigation, Formal analysis, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no competing financial or other interests.

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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.2023.162116.

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