CRANFIELD UNIVERSITY

MILES GEORGE TERENCE FOLKES

Source to Sink Wastewater Surveillance of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) for COVID-19 monitoring.

SWEE
MSc by Research in Water

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Academic Year: 2021 - 2022

Supervisor: Dr Francis Hassard
Associate Supervisor: Professor Ana Soares
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This thesis is submitted in fulfilment of the requirements for the degree of MSc. by Research in Water

ABSTRACT

Wastewater-based surveillance (WBS) complements individual testing to assess disease burden within geographically defined communities. Here, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) RNA fragments of N1 and E genes were monitored intermittently over ~16-month period (19th March 2020 – 21st July 2021) from large buildings on a university campus (near-source), in-sewer, raw wastewater and treated effluents to monitor infection burden within a small University in England, United Kingdom. SARS-CoV-2 abundance positively correlated with ammonia at near-source (Spearman's Rank; $\rho(14) = 0.82$, $p < 0.01$) and at the in-sewer (Spearman's Rank; $\rho(26) = 0.54$, $p < 0.01$) spatial scales but not within the onsite wastewater treatment works (WWTW) inlet or treatment process interstage samples. Campus infections and detection of SARS-CoV-2 RNA in wastewater occurred consistently through the survey and increasing trends lagged local area infection data and community cases of emerging / dominant variants of concern. Sequencing of the SARS-CoV-2 genomes from wastewater suggested detection of Alpha (B.1.1.7) Variant of Concern from wastewater samples. The University secondary WWTW (roughing and nitrifying trickling filters) did not removal substantial quantities of SARS-CoV-2 and the virus was regularly detected in permitted discharges, despite complete compliance to conventional wastewater consents during the survey. Although the virus was detected, there is very strong confidence in it not being active and thus it is not infectious. Remote and rural WWTW may not be effective at breaking down the RNA of enveloped viruses such as SARS-CoV-2 prior to discharge. In conclusion SARS-CoV-2 WBS can be used to proactively manage the health of campus-based communities as a complimentary measure of health status. Testing WBS at near-source, in-sewer and interstage WWTW provides the first single source to sink surveillance program to support broader roll out of WBS as a surveillance method.

Keywords: wastewater surveillance; wastewater-based epidemiology (WBE); surveillance COVID-19; SARS-CoV-2; coronavirus; Pepper mild mottle virus (PMMoV).

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Φ6	*Pseudomonas bacteriophage Φ6*
ACE2	Angiotensin-Converting Enzyme 2
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
COVID-19	Coronavirus Disease 2019
FS	Faecal Shedding
GP	General Practitioner
HDPE	High Density Polyethylene
IT	Information Technology
LOD	Limit of Detection
LOQ	Limit of Quantification
LTLA	Lower Tier Local Authority
N1	Nucleocapsid gene 1
PE	Population Equivalent
PMMoV	Pepper Mild Mottle Virus
R_0	Basic Reproduction Number
RNA	Ribonucleic Acid
RT-qPCR	Reverse Transcription Quantitative Polymerase Chain Reaction
SARS-CoV-1	Severe Acute Respiratory Syndrome Coronavirus 1
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
TSS	Total Suspended Solids
VoC	Variant of Concern
WBS	Wastewater-Based Surveillance
WWTW	Wastewater Treatment Works

1 Introduction

The Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) is a highly infectious zoonotic virus, thought to originate from Wuhan, China in late 2019. At the time of writing (February 2022) there have been at least 426 million confirmed cases and over 5.8 million deaths attributed to the COVID-19 disease worldwide (World Health Organization, 2021).

SARS-CoV-2 is an enveloped virus, which has a host cell membrane derived lipid bilayer which encapsulates its helical nucleocapsid that contains the linear, positive-sense single-stranded RNA genome (Kumar et al., 2020; V'kovski et al., 2020). The SARS-CoV-2 genome encodes, amongst other accessory proteins, three major structural proteins: the spike surface glycoprotein (S), membrane (M), nucleocapsid (N) and the envelope protein (E). SARS-CoV-2 binds to the angiotensin converting enzyme 2 receptor on host cell membranes (e.g., human epithelial cells in the respiratory tract and gut of humans and some animals) using the S protein. After binding, confirmational S protein shape change facilitates the fusion of the viral membrane with the host cell leading to nucleocapsid entry into the host cell (Kumar et al., 2020; Letko et al., 2020) The nucleocapsid, which contains the viral genome, interacts with host cell organelles leading to the release of the viral genome into the cytoplasm. The viral genome is then replicated and translated creating multiple SARS-CoV-2 viruses, and in susceptible individuals COVID-19 develops. Studies using human epithelial cells in culture conditions have reported the 10^5-10^7 virions per infected cell, typically resulting in 10-100 infectious units per cellular infection cycle (Sender et al., 2021). Once replication is complete the viruses are released from the cell in vesicles. This process triggers the innate immune system to release pro-inflammatory cytokines (Hosseini et al., 2020). If levels of pro-inflammatory cytokines are sufficiently high, as is often the case in a severe infection, this can result in the mobilization of large numbers of various immune cells (e.g., neutrophils, macrophages, and dendritic cells) to the affected area (Hosseini et al., 2020). Immune cells can then attack healthy cells and/or tissues, resulting in damage to the infected person. Lung damage, capillary damage and/or

multiorgan damage/failure have all been reported for hospitalised COVID-19 patients, with a mortality rate of 5% (Hosseini et al., 2020; Li et al., 2020). However, mortality has shown considerable variation amongst different cohorts and has been shown to be influenced by, *inter alia*, age, disease severity and existing health conditions such as cardiovascular disease (Wu and McGoogan, 2020). Alongside COVID-19's high mortality, over 50 long-term health impacts (e.g., pulmonary fibrosis) have been identified (Lopez-Leon et al., 2021). SARS-CoV-2 remains a significant global public health concern despite a range of pharmaceutical interventions e.g., anti-viral therapy and vaccinations. SARS-CoV-2 infections continue to be driven by, *inter alia*, new, and emerging Variants of Concern (VoC), vaccine hesitancy, slow rollout of vaccination and slow availability and rollout of vaccines in numerous countries (David Ainslie et al., 2021; Sah et al., 2021).

SARS-CoV-2 has proven particularly challenging to manage for a number of reasons. Firstly, it binds strongly to the ACE2 cell-surface receptor, with 10-20 times more affinity than SARS-CoV-1 (Hwang et al., 2020). This is thought to contribute to the increased infectivity of SARS-CoV-2 compared to other identified coronaviruses (Kumar et al., 2020; Laurini et al., 2021). Transmissibility of diseases can be measured in terms of their Reproduction Number - R_0. Whilst there are several flaws regarding the use of R_0 in managing disease, this metric is the most commonly used in the literature (Smith et al., 2011). R_0 is an indication of how many secondary infections could, when averaged across a population, be infected by a single infector within a defined and susceptible population (Wang et al., 2021). R_0 is affected by numerous factors and can change throughout the course of a disease being present in a community (Delamater et al., 2019). Multiple factors affect the R_0, such as biology (e.g., virus binding to cell surface receptors), sociobehavioral (e.g., people wearing masks and keeping physical distance between themselves) and environmental factors (e.g., adequate ventilation in buildings) (Delamater et al., 2019). In short, the higher the R_0 the more transmissible the disease is.

Secondly, SARS-CoV-2 appears to present a particularly high degree of mutagenicity (specifically the S protein coding sections of the SARS-CoV-2 genome) which can help its transmissibility and potential evasion of vaccines or immunity (Harvey et al., 2021). For example, the VoC B.1.1.7 – the Alpha variant first identified in Kent, United Kingdom - is estimated to have a mean R_0 29% higher than SARS-CoV-2 lineage A, all other factors being the same. The Alpha variant was responsible for an outbreak in England during periods of lockdown in winter 2020 (Campbell et al., 2021). The VoC B.1.617.2 (Delta)– has been estimated as having an R_0 97% greater than the wild-type SARS-CoV-2 lineage (variant A) (Campbell et al., 2021), and is regarded as being responsible for the majority of SARS-CoV-2 infections post-15th May 2021. In many countries, a recently emerged VoC so called Omicron (B.1.1.529) has displaced Delta as the dominant strain with an estimated R_0 as high as 10 (Burki, 2022) but mooted lower mortality rates possible due to changes to the biology of the virus and success of vaccination in some countries.

Furthermore, for COVID-19 disease, a significant proportion of infectious individuals are asymptomatic, which means individuals present mild or absent symptoms associated with SARS-CoV-2 infection. A study by Al-Qahtani et al. (2021) found that 67.6% of people who tested positive for SARS-CoV-2 were asymptomatic, and that most people who are asymptomatic remain asymptomatic throughout their infection cycle. Pre-symptomatic, asymptomatic, and symptomatic individuals are capable of infecting others and shed virus in various bodily fluids including stools (Furukawa, Brooks, and Sobel, 2020; Rothe *et al*., 2020; Yu *et al*., 2020). Asymptomatic individuals contribute to the spread of SARS-CoV-2 infections (Karthikeyan et al., 2021b). Therefore, identifying all people infected with SARS-CoV-2, not just those who are symptomatic, and controlling the spread of infections is of great importance to understanding and managing SARS-CoV-2 infections.

Several methods exist for tracking the spread of infectious diseases within communities. Sims & Kasprzyk-Hordern (2020), reviewed techniques and their respective advantages and disadvantages which has been updated here and

presented within Table 1. It was suggested that current methods which are employed for assessing infection burden (e.g., clinical testing) are sometimes unsuitable for controlling the spread of highly infectious and novel diseases. For example, for clinical testing to be effective large numbers of people must take regular tests, and this testing needs to be repeated frequently (i.e., longitudinal) during the course of the disease progression through communities. For SARS-CoV-2, these tests typically utilise nasopharyngeal swabs to collect sputum and saliva samples for analysis. Test swabs are inserted through the nostril until the upper part of the throat (nasopharynx) is reached, the swab is then rotated against the nasopharynx to obtain the sample. This method of collecting a sample is highly invasive, uncomfortable, and difficult for vulnerable populations (elderly, infirm, infants, and people with disabilities) (Blaschke et al., 2011). In addition, certain groups have lower rates of testing due to perceptions, historical injustice, and cultural reasons for not participating in clinical testing (Bruns et al., 2020). Whilst it can be effective for identifying asymptomatic individuals, clinical testing is reliant on adherence to and acceptance of a testing programme, that self-administered tests are conducted properly, and that people are correctly reporting the results (European Centre for Disease Prevention and Control, 2021). This can limit the ability for healthcare professionals to identify and isolate infected individuals, and those who may have been exposed to them. Furthermore, clinical testing is expensive and difficult to scale for testing large populations of people rapidly enough to control outbreaks via public health interventions (Hassard et al., 2021). Thus, clinical testing alone may not be enough to control the spread of infection with highly infectious disease agents including SARS-CoV-2 (Bivins et al., 2020; Hart and Halden, 2020). An emerging approach for monitoring public health status of communities is wastewater based surveillance (Table 1).

Table 1: Methods of infectious disease surveillance, adapted from Sims & Kasprzyk-Hordern (2020).

Technique	Example	Advantages	Disadvantages	References
Sentinel Surveillance	General practitioner's (GPs) reporting cases of influenza	Utilises existing systems Increased communication in communities	Rare and novel pathogens likely to be missed Limited scope of disease focus	(Lee et al., 2010)
Clinical-based Surveillance	UK Health Security Agency Antimicrobial Resistant Pathogen monitoring	Increased transfer of knowledge between epidemiologists and clinical laboratories Detailed genomic information of studied organisms available	Requires significant facilities, resources, trained staff, and good communication links. Selection bias on which samples are sent to the laboratory	(Choi, 2012; HM Government, 2019)
Hospital admission data	The Emerging Infectious Disease Surveillance Network	Can provide data on severity of infections and their incidences Potential to identify new diseases	Significant skilled human resource requirements in often busy environments Potential confidentiality issues when sharing data with public health agencies	(Hirshon, 2000)
Wastewater-based Surveillance	Assess exposure to chemicals or infectious disease agents at the community level	Capable of spatial and temporal trends Data in near-real time Anonymous contribution to samples (spatial scale dependent)	Selection of biomarkers can be challenging Biomarker stability in wastewater Uncertainties related to contributing population and wastewater flows	(Been et al., 2017; Choi et al., 2019; Lopardo et al., 2018; Rousis et al., 2017a)

1.1 Wastewater-based surveillance of disease-causing agents.

One complimentary approach to clinical testing for disease surveillance is WBS, also called wastewater-based epidemiology (Karthikeyan et al., 2021b). WBS is defined as the retrieval of human health information from wastewater through the analysis of specific chemicals or human metabolites, excreta or disease linked products (Castiglioni et al., 2014; Rousis et al., 2017b) and Figure 1 demonstrates the potential scale and sample locations of WBS. Wastewater in this context refers to the wastewater (e.g., combined sewage, greywater, blackwater) effluents produced by people through everyday practices such as defecation, urination, showering and the laundering of clothes. WBS has historically been used for a variety of purposes, including but not limited to identification of people infected with poliovirus (Lago et al., 2003), monitoring for illicit drug consumption (Rousis et al., 2017b), and monitoring for biomarkers for incidence of lifestyle diseases such as

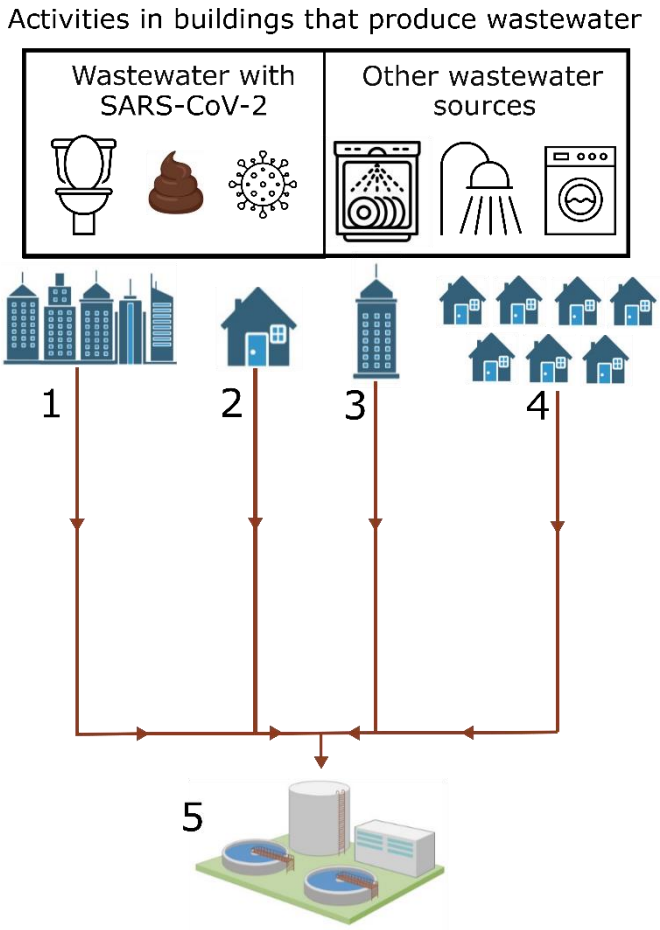

Figure 1: Conceptual model of WBS at various spatial scales including: 1 – groups of large buildings, 2 – Individual houses, 3 – Individual large buildings, 4 – groups of houses and 5 – WWTW. Wastewater sample can be taken at these scales. Note that WBS does not include human tissue samples (including faeces, throat / nose, and anal swabs) which require elevated ethical approval.

alcoholism (Thomas and Reid, 2011). WBS operates on the assumption that the sample taken is representative of the population that reside within the geographically defined wastewater catchment to which the population contributes (Wade et al., 2022). It is argued that WBS is ethically acceptable as the samples taken for WBS are anonymous (Kwiatkowska et al., 2021). Another proposed benefit of WBS is that it provides a snapshot of the overall community disease burden for a fraction of the cost of clinical sampling. One study from a University in the USA suggested that the costs of clinical sampling were $17.5 per person compared to an equivalent WBS of $0.31 per person tested (Wright et al., 2022). An overwhelming body of evidence suggests that WBS can be used to support public health monitoring campaigns as complimentary datasets to traditional methods e.g., clinical testing (Fielding-Miller et al., 2021; Hart and Halden, 2020; Kaplan et al., 2021; Karthikeyan et al., 2021b; Wright et al., 2022). However, sampling WWTW in isolation can, in some circumstances, result in poor spatial resolution if the sewer catchment is very large (Figure 1).

Sampling at different locations within the wastewater collection and conveyance network permits spatial trends in disease burden to be identified and directly linked to the contributing population (Kitajima et al., 2020; Wade et al., 2022). Repeated measurements from these locations provides the temporal dimension to WBS datasets; greater frequency sampling events are required at sample points nearer to the source. This is to establish the difference between real trends from natural variability in human populations (e.g., occupancy), missing flush events and differences in defaecation events. For example, near-source sampling obtains samples close to the source of wastewater effluents, such as from sewer inspection chambers associated with large buildings and could be used to identify individuals within a building infected with a disease. Samples from WWTW would be representative of everyone residing within the WWTW catchment providing a more holistic appreciation of disease burden in that area. Although concerns about sample storage (Ahmed et al., 2020b), contamination with RT-qPCR inhibitors from the wastewater (Ahmed et al., 2022) and dilution from stormwater in combined municipal waste and stormwater systems (Sims and Kasprzyk-Hordern, 2020) have been suggested to limit the utility of WBS, it

has been previously used to track and predict hospitalisations prior to clinical tests for diseases (Berchenko et al., 2017; Lago et al., 2003). Thus, it is hypothesized that WBS can provide a leading indicator of infection burden within a geographically defined populace, allowing clusters of potentially infected individuals to be identified. These identified groups can then be encouraged to quarantine and be targeted for clinical testing, with the aim of breaking chains of infection. Communicating this data with public health officials can allow for resources to be distributed to areas needed, allowing for a coordinated and more effective public health response (Karthikeyan et al., 2021b).

1.2 Uncertainty in WBS for SARS-CoV-2 monitoring

The use of WBS has been subject to debate. Wastewater is an inherently complex matrix, presenting a challenging medium in which to operate. Often an aggregate of many different effluent streams, wastewater will contain a complex mix of compounds (e.g., pharmaceuticals, detergents, and fire retardants) and organisms (e.g., bacteria, fungi, and viral communities) (Rose et al., 2015). Whilst this potentially provides a reservoir of sources of data and despite technical advancements of WBS analytes in recent years, extracting useful information from wastewater streams can be challenging, due to low concentrations or stability of the target or analyte or inhibition concerns (Hart & Halden, 2020). Furthermore, network factors can limit the utility of the WBS approach. For example, the flow patterns of combined sewers, drains, and foul-lines are not always established, and sampling regimes can further complicate obtaining reliable samples which are representative of the population (Wade et al., 2022). Another challenge with WBS is the difficulty in estimating the size of the contributing population to a wastewater catchment, especially when the target individuals are highly dynamic e.g., with large cities that have significant daily/weekly in and outfluxes of commuters and/or tourists (Rosselló et al., 2017; Wade et al., 2022).

There are specific uncertainties associated with the use of WBS for SARS-CoV-2 infection monitoring. Of these, one of the most significant is the uncertainty surrounding the shedding of SARS-CoV-2 genetic material in faeces between

individuals (Jones et al., 2020). The shedding dynamics at different stages of COVID-19 disease pathogenesis is variable and knowledge of them limited due to only a few studies having been conducted which have focused primarily on hospitalised individuals with more severe disease than the general population (Table 2). The presence of detectable SARS-CoV-2 in stools from symptomatic individuals is highly variable, ranging from 15.3-100%. The shedding rate is between 2.4-7.2 log SARS-CoV-2 GC g^{-1} wet faeces and normally peaks 5 days after the onset of symptoms in symptomatic hospitalised individuals (Hoffmann and Alsing, 2021). However significant uncertainty exists in faecal shedding estimates, as shedding has been shown to vary from person to person and throughout the course of infection (Hoffmann and Alsing, 2021). The impact of population demographics, the variant of infection and/or vaccination status has not been resolved at a population level. However, some clinical studies have tried to resolve these aspects through clinical data. For example, Singanayagam et al. (2021) showed that peak viral abundance within the respiratory tract was similar regardless of variant type (Wild-type, Alpha and Delta VoCs) and vaccination status. However, age was associated with a 'moderate' increase in the SARS-CoV-2 viral load in nasopharyngeal samples. Other studies e.g., Adhikari et al. (2021) and Milliere et al. (2021) did not show a significant relationship between SARS-CoV-2 abundance between nasopharyngeal and stool samples. Finally, the shedding dynamics of pre- and asymptomatic people infected with SARS-CoV-2 are not well established.

Despite these limitations, WBS has shown promise as a complimentary approach for surveillance of SARS-CoV-2 infections alongside clinical testing. Studies such as Kaplan et al. (2021), Li et al. (2022) and Fielding-Miller et al. (2021) have shown that the presence of SARS-CoV-2 genetic material in wastewater can represent a leading indicator of clinical cases and hospital admissions, whilst Colosi et al. (2021) and Nemudryi et al. (2020) demonstrated that wastewater was a lagging indicator of disease prevalence within a community when assessing disease burden from the point of onset of symptoms. A review of the literature by Shah et al. (2022) found that in most reported cases, detection of SARS-CoV-2 in wastewater was a leading indicator of COVID-19 disease in the

community. However, there remains significant uncertainty in i) estimated cases numbers from wastewater SARS-CoV-2 gene copies and ii) utilising wastewater in a predictive/preventative capacity (i.e., when to initiate a public health intervention based on WBS data). This uncertainty can partly be countered through normalisation of wastewater data, which will be discussed in Section 1.3. Overall, more research is needed to evaluate the appropriateness of WBS in SARS-CoV-2 management.

Table 2: Studies investigating shedding of SARS-CoV-2 RNA in faeces

Total Patients	SARS-CoV-2 RNA positive in stool samples	Mean N1 Gene copies in sample gc/ml (range)	Median FS duration since onset of symptoms (IQR)	FS % positive for SARS-CoV-2 after negative nasopharyngeal test, and median duration in days after negative nasopharyngeal result (IQR)	Reference
42	66% (28/42)	-	Overall: 11 (7-11) 9 days in 2 'uncomplicated' cases 8 (4.5-14) days in 29 'mild' cases 14 (9.5-18) days in 11 'severe' cases	64.29% (18/28) of patients 6.5 days 'uncomplicated' cases 8 (6-10) days in 'mild' cases 7 (6.5-9.5) days in 'severe' cases	(Chen et al., 2020)
95	47.7% (31/65)	-	-	-	(Lin et al., 2020)
66	100% (66/66)	-	Overall: no data > 21 days in 11 cases 11 (9.0-16.0) in 55 cases	78.2% (43/55) of patients 2.0 (1.0-4.0) days	(Ling et al., 2020)
84	33% (28/84)	-	-	26% (20/76)	(Wei et al., 2020)
9	89% (8/9)	N/A (Log 3 – 7)	-	-	(Wölfel et al., 2020)
74	55% (41/74)	-	Mean – 27.9	72.5% (29/40) Mean – 11.2 days	(Y. Wu et al., 2020)
73	53.42% (39/73)	-	-	43.59% (17/39) of patients	(Xiao et al., 2020b)
23	83.30% (10/12)	5623	22.0 (15.5-23.5)	-	(N. Zhang et al., 2020)
96	59.13% (55/93)	-	22 (17-31)	-	(Zheng et al., 2020)

1.3 Methods for normalising data from WBS

Due to the uncertainties in flow and other sources of wastewater (e.g., industrial inputs), there is interest in normalising the SARS-CoV-2 abundance to indicators of municipal wastewater such as ammonia or indicators of human population equivalent during a sampling period. Wastewater biomarkers can estimate the contributing population to a sewershed, which is especially useful where there is a variable sized/mobile population e.g., during monitoring of near-source and at sewer level where population dynamics are averaged over an area significantly smaller than at the WWTW level (Sweetapple et al., 2022). Biomarkers can help to normalise municipal wastewaters receiving industrial inputs and runoff in combined sewer systems. Biomarkers are specific compounds that are secreted by humans. This can be general (i.e., non-specific) compounds present from faeces and urine, such as ammonium (NH_4^+), phosphate (PO_4^{2-}) ions (Rose et al., 2015). Other wastewater quality parameters have also been used to estimate contributing populations, such as the chemical oxygen demand (COD) and the biological oxygen demand (BOD) to varying degrees of success (Sims and Kasprzyk-Hordern, 2020). van Nuijs et al. (2011) reported that each individual person excretes 1.7 g d^{-1} of Phosphate, 12.5 g d^{-1} of Nitrogen, 59 g d^{-1} of BOD and 128 g d^{-1} of COD on average in their combined urine and faeces. Ammonium is one commonly applied metric of contributing populations (Been et al., 2014). Unlike other wastewater quality parameters, ammonium is thought to be less affected by non-human sources that can increase values these values. Detergents from laundry can increase the phosphate concentration in samples in countries where it is permitted to include phosphates in detergents. However, in combined sewage and stormwater systems these conventional values will be reduced by the impact of rainfall events diluting the signal from the wastewater. In short, the concentrations of chemical biomarkers such as ammonium and phosphate in wastewater are not always influenced by the presence of faeces and urine in wastewater. Using them to normalise the concentration of SARS-CoV-2 may not be appropriate, as SARS-CoV-2 concentration in wastewater is hypothesised to be related to the number of people contributing to the wastewater

who are shedding SARS-CoV-2 RNA in their faeces. Thus, a robust biomarker that is shed in faeces is required for normalising viruses that are shed in faeces.

Enteric viruses have been widely applied as indicators of human faecal pollution (McMinn et al., 2017). Unlike chemical indicators such as ammonium and phosphate, their presence in the wastewater is directly related to the faeces in wastewater. In recent studies the plant virus Pepper Mild Mottle Virus (PMMoV) and a bacteriophage crAssphage have been widely applied in WBS (Wilder et al., 2021; F. Wu et al., 2020). Of these, PMMoV shows greater promise as PMMoV has a similar composition to SARS-CoV-2; it is a single-stranded positive sense RNA virus but unlike SARS-CoV-2 is non-enveloped (Fauquet et al., 2005), is stable in the wastewater matrix and is present in high viral titres. In a study by Rachmadi et al. (2016) wastewater samples containing PMMoV did not experience a reduction in their concentration of PMMoV, even after being incubated for 21 days at 37°C. A review by Symonds et al. (2018) reported concentrations of 10^6 to 10^{10} PMMoV gene copies L^{-1} in wastewater. These factors, combined with being detected in most wastewater samples (Rosario et al., 2009) suggest PMMoV could be a highly suitable biomarker for normalisation. Whilst highly specific and reliable, a significant drawback is the reliance on RT-qPCR for PMMoV identification and quantification (Symonds et al., 2018), thus making it potentially unsuitable where rapid results are required. However, this point is moot when the target requiring normalisation itself requires nucleic acid amplification or similarly complex and/or time consuming techniques. Another potential issue is that the shedding of PMMoV and CrAssphage differ from SARS-CoV-2. For example, the shedding rate of PMMoV can be influenced by dietary factors and infection profiles within vegetable matter and processed foods, with reported concentrations in dry faeces of 10^6-10^9 virions g^{-1} dry faeces (Zhang et al., 2005). The CrAssphage bacteriophage faecal shedding profiles have been shown to vary between individuals and with time due to infection and changes in the gut microbiome which could preclude its wider utility as a biomarker. Typical concentrations in faeces are $10^{3.5}$ and at $10^{8.5}$ GC ml^{-1} faeces (Langeveld et al., 2021). However, it is considered that these viral indicators could be more suitable for normalising samples than chemical markers because they are i) biogenic, ii)

have similar size and structure to SARS-CoV-2 and iii) could partition in a similar way within the wastewater matrix, unlike dissolved constituents such as ammonia.

1.4 Rationale

As of yet, few studies have attempted to monitor wastewater at the near-source spatial scale, and few have tracked the SARS-CoV-2 concentration through a sewer system to a contiguous wastewater treatment works (WWTW) for WBS. The source-to-sink approach without significant contributions from other sources reported below, represents the first study of its kind to my knowledge. The Cranfield campus has a large static geographically isolated residential population in student halls of residence and houses. The campus has significant daily movements of people onto campus (comprising visitors, staff, and students), and a range of business activities that occur within the campus and business park. In addition, Cranfield University WWTW does not receive wastewater from off-campus areas and the residential areas do not receive industrial effluents. The WWTW does receive industrial effluents due to research activities that take place on the campus. It is therefore argued that Cranfield is useful as a model wastewater catchment.

Good occupancy data for residential buildings is available during the period of the pandemic (19th March 2020-21st July 2021). As such, the Cranfield campus represents an idealised real scale test site for WBE studies. For example, population estimates would have stronger confidence than for other settlements where daily, weekly, or monthly population estimates are less precise and subject to significant variability. Further research is needed to contribute to the rapidly evolving field of WBS for management of SARS-CoV-2, and potentially for future disease management. More research is also needed to establish the removal of viruses (especially enveloped viruses) in wastewater treatment streams to protect the environment and human health.

1.5 Aims

The aim of this project was to evaluate the role of wastewater-based surveillance for tracking SARS-CoV-2 infections and assessing contributions from its source e.g., infected individuals on campus, investigate removal processes within the WWTW and determine loading to its sink (a small river tributary to the Great Ouse catchment in England).

1.6 Objectives

Table 3: Brief Objectives and links to specific hypotheses

Objective	Hypothesis
Quantify SARS-CoV-2 gene copies from wastewater at three scales on campus samples with assumed increasingly large daily wastewater flows.	WBS can identify magnitude of SARS-CoV-2 infections on the University Campus
Establish SARS-CoV-2 gene copy abundance correlation to wastewater constituents.	SARS-CoV-2 with wastewater constituents positive correlates at near-source level not WTWW
Establish SARS-CoV-2 gene copy concordance with COVID-19 infections on campus	SARS-CoV-2 wastewater samples will be positively related with on-campus infections
Isolate SARS-CoV-2 whole genomes from wastewater	Wastewater SARS-CoV-2 WBS can identify variants of concern on campus
Verify removal of SARS-CoV-2 RNA fragments from a small Trickling Filter WWTW treating water exclusively from campus population	Small linked WWTW removes SARS-CoV-2 RNA fragments from wastewater

Five key objectives were identified as follows:

1. Quantify SARS-CoV-2 gene copies obtained from large buildings, in-line sewer and WWTW level samples.
2. Investigate the relationship between SARS-CoV-2 gene copy abundance with wastewater characteristics.
3. Investigate if SARS-CoV-2 gene copy concentration in wastewater data correlates with confirmed and estimated infections.
4. Sequence SARS-CoV-2 genome data for identification of variants of concern responsible for on campus infections.
5. Establish ability of on campus WWTW to remove the SARS-CoV-2 RNA fragments from wastewater prior to discharge to the environment.

1.7 Hypotheses

1. WBS can be used to identify magnitude of SARS-CoV-2 infections on the University Campus
2. SARS-CoV-2 N1 gene copies will be positively correlated with a) total suspended solids, b) tCOD, c) pH and d) ammonia and e) orthophosphate concentrations at the "near-source" level due to the coupling between these parameters and faecal shedding near-source
3. Wastewater SARS-CoV-2 WBS can identify variants of concern on campus
4. The on campus WWTW will remove detectable fragments of SARS-CoV-2 RNA from wastewater.
5. SARS-CoV-2 gene copy concentration in wastewater samples will be positively correlated with on-campus infections

2 Source to Sink Wastewater Surveillance of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) for COVID-19 monitoring.

2.1 Introduction

Previous studies to monitor SARS-CoV-2 in wastewater have taken place on a variety of spatial scales and contexts. Schools (Castro Gutierrez et al., 2021; Fielding-Miller et al., 2021; Hassard et al., 2021), hospitals (Liu et al., 2022), University buildings and campuses (Corchis-Scott et al., 2021; Karthikeyan et al., 2021b; Scott et al., 2021) wastewater catchments through in sewer or pumping station monitoring and WWTW (Ai et al., 2021; Gonzalez et al., 2020; Kaplan et al., 2021; Wurtzer et al., 2020) have all been studied previously. The removal of SARS-CoV-2 within WWTW has also been investigated (Randazzo et al., 2020; Kumar et al., 2021), although no studies thus far could be found where monitoring programs have been conducted within a highly controlled catchment. An extensive discussion was provided by Safford et al. (2022) who suggested the value of WBS as a leading indicator of disease presence, but only in low prevalence scenarios in highly controlled environments. They highlighted that one major challenge of WBS is to know when to act on pooled wastewater results. This is because tracing individual infections to pooled wastewater samples requires testing large numbers of people and enforcing isolation. This has the potential to create ethical issues (e.g., through stigma), which is counterintuitive to the purported purpose of WBS. With this in mind, an indirect approach to WBS was deemed to be more appropriate for use in this study. Instead of targeted testing, it was proposed that students and staff would be alerted to increased levels of SARS-CoV-2 in a wastewater drainage basin and they would be encouraged to get a test and if possible, to isolate. Regulations regarding mask wearing and social distancing in England were, for much of the study, controlled and enforced by the UK Government. As such, we could not encourage people to start wearing masks and/or socially distance should the concentration of SARS-CoV-2 in the wastewater increase. However, it was possible to encourage

people to take a test for SARS-CoV-2 infection should there have been a need to.

2.1.1 State of the art on campus WBS

Several studies thus far have investigated the use of WBS on University Campuses, though the purposes of the studies have varied considerably. Several studies have used near-source sampling to identify buildings in which one or more infected people were located to target for mandatory testing (Barich and Slonczewski, 2021; Betancourt et al., 2021; Bivins and Bibby, 2021; Colosi et al., 2021; Corchis-Scott et al., 2021; Fahrenfeld et al., 2022; Gibas et al., 2021; Scott et al., 2021; Vo et al., 2022; Wang et al., 2022; Wright et al., 2022), others have used WBS and voluntary testing to monitor cases on campuses and inform health and safety policy (Karthikeyan et al., 2021b; Reeves et al., 2021; Travis et al., 2021), whilst others have only investigated how campus dynamics (e.g. population and SARS-CoV-2 positive individuals on the campus) influence the presence or abundance of SARS-CoV-2 in wastewater (Bivins et al., 2021; Liu et al., 2022; Sweetapple et al., 2022).

These studies have found, *inter alia*, that SARS-CoV-2 GC concentration correlates with clinically confirmed cases on university campuses (Bivins and Bibby, 2021; Fahrenfeld et al., 2022; Gibas et al., 2021; Scott et al., 2021; Wang et al., 2022; Wright et al., 2022), that wastewaters need to be sampled frequently (Gibas et al., 2021; Karthikeyan et al., 2021b; Reeves et al., 2021) and that variants can be identified from wastewater (Vo et al., 2022). However, thus far no studies have monitored SARS-CoV-2 in wastewater through a sewage system to a linked WWTW in a highly controlled sewershed on a longitudinal basis and monitored its removal at the linked WWTW.

Accordingly, the purpose of this study was to i) monitor wastewater effluent streams at the Near-Source, In-Sewer and WWTW level in a geographically isolated sewer catchment across a 16-month period for the presence and abundance of SARS-CoV-2 genetic material, ii) evaluate if there is a relationship between SARS-CoV-2 gene copies in the wastewater and wastewater characteristics, iii) investigate if SARS-CoV-2 gene copy data is correlated with

the number of cases on the campus/in the local community and iv) to assess the efficacy of the on-campus WWTW at removing detectable SARS-CoV-2 gene copies from the wastewater. In general terms it was hoped that I could ascertain the impact of national and local lockdown restrictions and VoC emergence on the abundance of SARS-CoV-2 gene copies in wastewater. To the best of my knowledge, this is the first study to combine Near-Source Tracking (NST) and In-Sewer WBS with a linked WWTW.

2.2 Materials and Methods

2.2.1 Sampling Site

Cranfield University is a postgraduate only university located in the county of Bedfordshire, England, United Kingdom. Cranfield University has 59 large buildings, a median resident population of 1200 and a transient population of 3800 staff and students on weekdays (2018-2019 data). On weekends the population on campus is estimated to be ~1300 to account for campus resident, and essential staff who work on campus at the weekend. During the COVID-19 pandemic, numerous national (England) and Cranfield University campus public health measures were implemented which together impact the population on campus. Cranfield University benefits from having its own contained sewer system which receives primarily wastewater for the university technical buildings, halls of residence and residential properties designed for campus based academics, students, and their families. A proportion of Cranfield University's wastewater is combined with rainfall and surface water drainage. Similar to many wastewater conveyance schemes, the proportion of the sewer network which is municipal only versus combined is not known. This is typical for USA and UK towns and cities and many other countries. The wastewater is stored prior to pumping up a gradient (1%) at Mitchell Hall pumping station to the onsite wastewater treatment works (Population equivalent [P.E] of 3278±914 in 2018) which currently treats 100% of its daily wastewater flow. The daily average wastewater flow was 465.50±129.82 m^3d^{-1} in 2018 and was 459.35±137.88 m^3d^{-1} in 2019 and in 2021 449.04±163.74 m^3d^{-1}, which positively correlates to the population on campus (Spearmans Rank; $\rho(186) = 0.673$, $p < 0.01$).

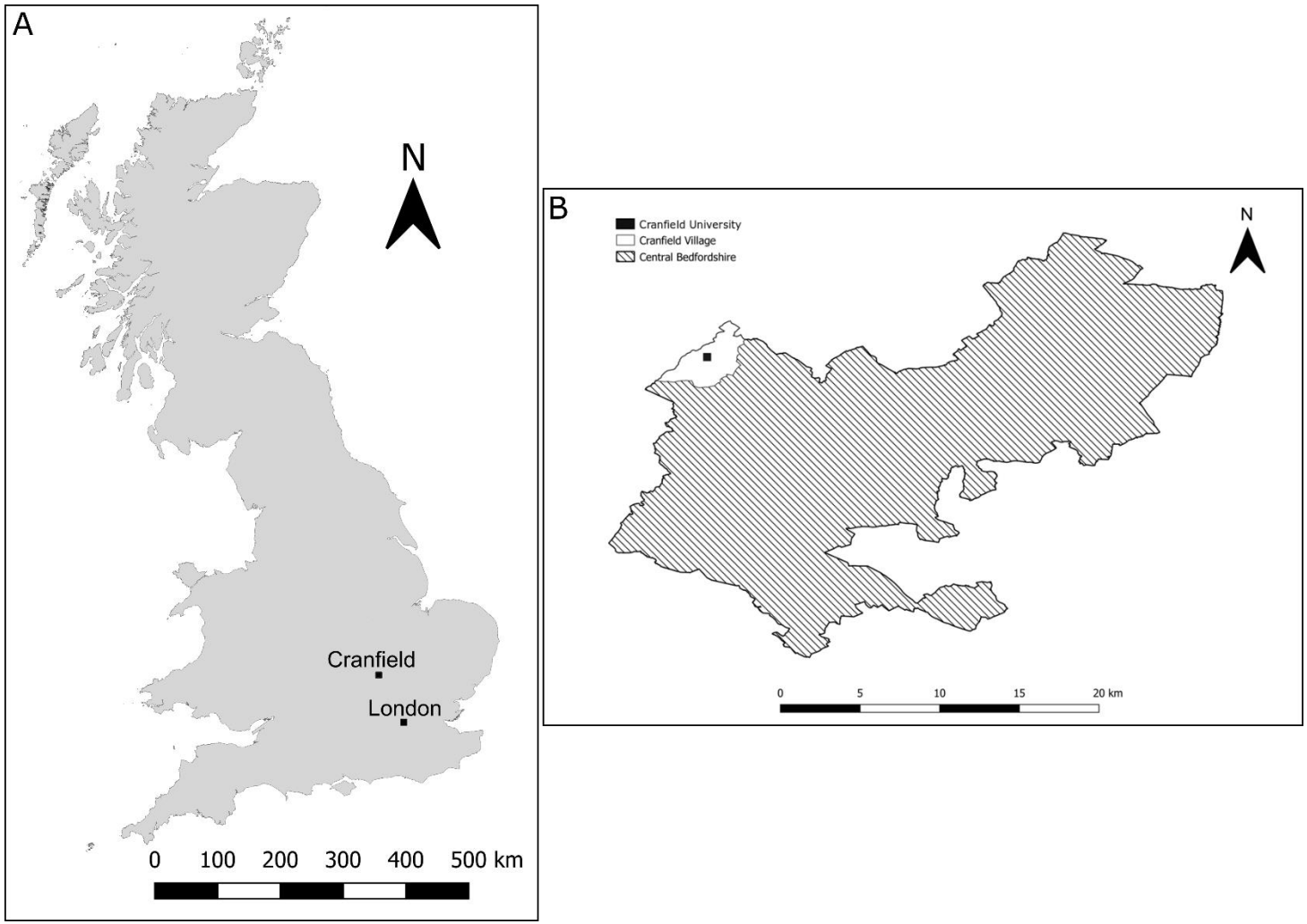

Figure 2: A - Location of Cranfield in the UK, and B - Location of Cranfield University in the Central Bedforshire local authority area in County of Central Bedforshire, England.

2.2.2 Sampling Site Selection

Sites that best represented the population of the campus resident population were selected. It was determined that three spatial scales (“Near-Source”, “In-Sewer” and WWTW) would provide the best overall population sampling which could be achieved and help determine possible sources of COVID-19 infection on campus. These are shown in Figure 3, below. A contractor (Aqua Jet, UK) with significant experience of the university wastewater network was consulted to identify four suitable wastewater nodes and their associated sewer inspection chambers from which samples could be obtained. A limited number of sample locations in this case were considered to enable detection within and between different groups of on-campus populations. This is because, during most of the study duration, teaching (i.e., lectures) were online and so students were mostly contained within halls of residence, whereas other sample locations helped isolate staff populations working in other large buildings and families living within campus based residential properties.

The sewer-catchment basins identified were:

- “Hall of Residence A” and “Hall of Residence B” – “Near-Source” sampling points in sewer access points that drained two of the student halls of residence comprising 57% of the student halls of residence population.
- “Residential Houses In-Sewer” – A Sewer-Scale sampling point located in a sewer access point downstream of the node of several sewer foul lines the drained multiple shared student and individual family houses
- “University Technical Buildings In-Sewer” – A Sewer-Scale sampling point in a sewer access point located downstream of the node of several sewer foul lines that drain the University Technical buildings

Figure 3: Cranfield Campus drainage areas which were sampled. 1- Halls of Residence A, 2 – University Technical Buildings In-Sewer, 3 – Residential Houses In-Sewer and 4 – Halls of Residence B. Stars indicate location of sampling point. Map not to scale.

The Cranfield Campus wastewater treatment works (WWTW) were also sampled, as shown in Figure 4 below. The post-primary lamella (settlement), the post-roughing trickling filter, post-secondary trickling filter outlets and final effluent was sampled at least weekly over a 6-month period (22nd January 2021-27th July 2021), and the post-balance tank settled influent (the “Influent") to the WWTW was sampled at least weekly over a 16-month period (19th March 2020-27th July 2021). The balance tank was assumed to be a representative sample of the entire campus population for the 24-hour period prior to sample collection. The post-

inlet sample points were sampled to monitor the removal of SARS-CoV-2 through the WWTW and prior to discharge into the environment.

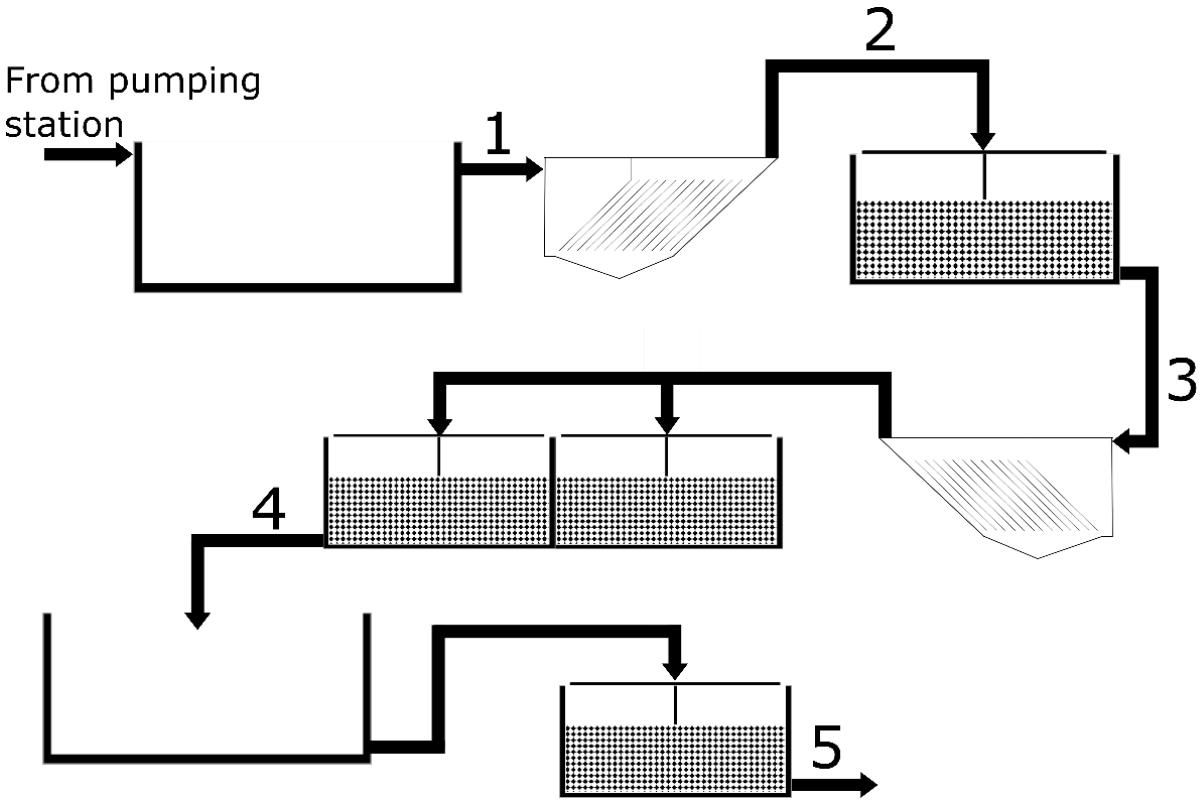

Figure 4: Wastewater Treatment Works Sample Points. 1: Settled Raw Wastewater at WWTW Inlet, 2: Post-Primary Lamella, 3: Post-Roughing Trickling Filter, 4: Post-Secondary Trickling Filter and 5: Final Effluent (prior to environmental discharge)

A brief description of the processes shown in Figure 4 that treat the wastewater at the campus WWTW, adapted from (Droste and Gehr, 2019), follows. The balance tank settles solids from water by decreasing the velocity of particles, allowing gravity-assisted sedimentation to occur within the tank. The lamella clarifier pumps water from the base of the unit and up through tubes contained in the clarifier. The up flow velocity rate is set so that it is lower than the settling velocity of many of the particles in the wastewater, encouraging the settling of particles. These then fall to the bottom of the clarifier and can be pumped out. The roughing filters consist of a bed of plastic matrix media, on which a biologically active biofilm forms. Wastewater is trickled over the filter media, and solids contained therein are passed through the matrix media. Solids are retained by the biofilms, which are then broken down by the microorganisms of the biofilm.

2.2.3 Sample collection and analysis

A total of 488 samples were analysed for SARS-CoV-2, comprising 389 separate samples. 377 individual samples were also analysed for wastewater characteristics. 112 samples (39.7%) tested positive for N1. The abundance of PMMoV was also quantified in 108 samples that had tested positive for SARS-CoV-2. A detailed breakdown of when and from where the samples were taken, the sampling methodology used to collect the sample, whether or not they were frozen prior to extraction and RNA extraction Protocol is provided in Table 12 in Appendix 1,

During the first phase of sampling (19/03/2020-15/01/2021), a grab sample of at least 250ml was taken weekly from the WWTW Inlet. This was then frozen at -80°C pending analysis when the University labs were re-opened for research. Sampling of the campus infrastructure began on the 22nd of January 2021. Aquacell P2-COMPACT (Aquamatic, UK) autosamplers were installed in the sewer access chambers. Samples were taken from each site at least once a week to obtain the highest resolution data that was obtainable considering time, budget, and site access restrictions. At each site 16.6 ml of the wastewater stream was taken every five min ~200 ml/hr over each 24hr period (7 am - 7 am). This formed a 4.8 L composite sample in the integrated 5 L HDPE collection vessel. Sample sites were visited between 07:00 am – 08:00 am on the days of sample collection. After thorough mixing, a 1 L sample was aliquoted for subsequent analysis into a 1 L polypropylene bottle. From the 22/01/2021, 1 L grab samples were taken from the inlet flow, post-primary lamella, the post-roughing trickling filter and post-secondary trickling filter outlets, and the final effluent of the WWTW at least once a week (Figure 4). For the final 3 months of the study, the inlet was sampled 4 times a week to better track overall disease burden on campus. Samples were transported on melting ice, manually transferred back to the laboratory, and immediately stored in a refrigerator at a temperature of 2.5 - 4°C. Three 200 ml aliquots were taken i) for immediate RNA extraction, ii) for cryogenic storage (-80°C) and iii) analysis of wastewater constituents with i) and ii) occurring same day and iii) within 48 hours.

Each wastewater sample was analysed in the laboratory for total suspended solids (TSS), ammonium (NH_4-N) orthophosphate (PO_4-P), total chemical oxygen demand (tCOD), pH, conductivity, and oxidation reduction potential (ORP) according to

standard methods for the examination of wastewater (American Public Health Association, 1994). Two Protocols were used for SARS-CoV-2 RNA extraction and purification: Protocol 1 and Protocol 2. During this project, significant method development was undertaken, and Protocol 2 was shown to have better RNA yield, therefore this protocol was used for subsequent samples. Sample numbers 86-175 (internal reference F001-F099) were extracted using Protocol 1. In detail, the falcon tubes were centrifuged for 30 minutes at 3,000 xg at 4°C, and the supernatant transferred to 250 ml PCCO centrifuge bottles (Thermo Fisher, UK). Supernatants were spiked with a surrogate enveloped virus (murine norovirus) for extraction control before concentration with polyethylene glycol (PEG) precipitation, with incubation in a refrigerator at 4°C for at least 12 hours. The samples were then centrifuged at 10,000 xg for 30 minutes at 4°C, excess PEG was discarded by pouring, followed by further centrifugation at 10,000 xg for 10 minutes at 4°C to form a final concentrated pellet. Excess supernatant was once again discarded. This pellet was then re-suspended in 0.5 mL of molecular biology grade phosphate buffered saline (PBS), pH 7.4. RNA extractions from the pellets were then conducted using the NUCLEISENS® RNA extraction kit on a MINIMAG® (BioMérieux, France). For samples F100-F303 and the defrosted samples, Protocol 2 was used. Protocol 2 was adapted from Amirouche et al. (2021). Samples were extracted in duplicate where possible. The falcon tubes were centrifuged for 30 minutes at 3000 xg at 4°C, and the supernatant transferred to 250 ml PCCO centrifuge bottles (Thermo Fisher, UK). Supernatants were spiked with a surrogate enveloped virus (Φ6) for extraction control before concentration with polyethylene glycol (PEG) precipitation. Samples were shaken at 200 rpm on an orbital shaker for 15 minutes at room temperature, prior to incubation in a refrigerator at 4°C for at least 2 hours. A pilot study on 3 wastewater samples extracted in triplicate revealed that between 2-24 hours of incubation did not impact the RNA yield or RNA quality. No significant difference was found between samples incubated for 2 - 24 hours. The samples were then centrifuged at 10,000 xg for 30 minutes at 4°C, excess PEG was discarded by pouring, followed by further centrifugation at 10,000 xg for 10 minutes at 4°C to form a final concentrated pellet. Excess supernatant was discarded, with 1 mL retained. This was used to resuspend the pellet prior to transfer to a 1.5 mL microcentrifuge tube. The samples were then centrifuged at 12,500 xg for 5 minutes, re-forming the pellet. The supernatant was then removed by pipetting.

The pellet was resuspended in 800 µL of TRIzol™ (Fisher Scientific, UK) by pipetting and vortexing to lyse the sample. Samples could then be stored at -20°C for later analysis should Project workload demand. This did not influence RNA recovery, and frequently enabled for optimised workflow increasing sample throughput. 200 µL of Chloroform (Fisher Scientific, UK) was then added, and mixed by vortex. The samples were incubated at room temperature for 3 minutes. The sample was then centrifuged at 12,000 xg for 15 minutes at 4°C. The aqueous layer containing the RNA was then transferred to sterile, RNAase free Lo-Bind Eppendorf tubes (Fisher Scientific, UK). RNA was then extracted and purified using a Macherey Nagel Nucleospin RNA kit (Fisher Scientific, UK).

Following extraction, RNA concentration was measured using Qubit™ (Fisher Scientific, UK). On occasions where the sample RNA concentration exceeded 100 ng/mL, these samples were subsequently diluted in RNAase free sterile water to at least 1:10 dilution. This was done as the acceptable range for the analysis required less than 100 ng/mL of RNA in samples, else the process efficiency was inhibited. SARS-CoV-2 RNA detection was performed by RT-qPCR using the RNA UltraSense™ One-Step Quantitative RT-PCR System (Thermo Fisher, UK) targeting the nucleoprotein (N1) gene fragment, and the envelope (E) protein gene fragment using a QuantStudio™ 7 Pro Real-Time PCR System (ThermoFisher, UK). These target gene sequences were chosen due the high degree of specificity and low mutagenicity, thereby reducing the risk of misidentification of SARS-CoV-2 or false negatives, as recommended by Centre for Disease Control and Prevention (2021), Corman et al. (2020) and Ahmed et al. (2022).

RNA samples were analysed in duplicate alongside negative (nuclease-free water) controls after Castro Gutierrez et al. (2021). The RNA extracts were quantified by plotting the quantification cycles (CT) to an external standard curve constructed from commercially available synthesised plasmids containing the target sequence. Samples where the N1 gene copy data exceeded the empirical limit of detection (LOD) for their respective extraction and purification protocol were considered to positive for SARS-CoV-2.

The LOD for Protocols 1 and 2 were calculated using the methodology outlined in Farkas et al. (2020). For Protocol 1 the LOD for N1 and E was 1,268 GC L^{-1} and 2,968

GC L^{-1}, respectively, as confirmed in Castro Gutierrez et al. (2021). For Protocol 2 the LOD for N1 and E was 956 GC L^{-1} and 2,401 GC L^{-1}, respectively. The limit of quantification for Protocols 1 and 2 were also calculated using the method outlined in Castro Gutierrez et al. (2021). The LOQ was determined through spiking SARS-CoV-2 negative RNA extracts from school wastewater with a range of defined quantities of Armored RNA standard (Asuragen Quant SARS-CoV-2 Panel - 52036, VH Bio Ltd., UK) with the LOQ being the lowest concentration which achieved a CV value not exceeding 25%. The LOQ for Protocol 1 was for N1 and E was 9,196 GC L^{-1} and 21,300 GC L^{-1}. The LOQ for Protocol 2 for N1 and E was 7,859 GC L^{-1} and 18,138 GC L^{-1}, respectively. A positive detection for SARS-CoV-2 occurred when the sample had concentration of SARS-CoV-2 N1 GC greater than the LOD. All samples that were negative were recorded as having an N1 GC L^{-1} of half of the LOD.

Protocol 1 was initially used due to its simplicity, low-cost and its utilisation of readily available materials (which was critical owing to the disruption to supply chains due to the pandemic) whilst still being reliable and robust enough to produce valid data. This was validated as part of this project through empirical measurement of limits of quantification and detection and quality of RNA which it yielded. However, a maximum of 12 samples could be extracted at once often forming a significant bottleneck in analysis, hence the transition to Protocol 2. When required, sample turnaround could be achieved in under 24hrs when using Protocol 2 and 24 samples could be extracted simultaneously. Similar concentrations of SARS-CoV-2 were found in linked samples (coefficient of variation <20%) between extraction methods. Thus, both protocols produced data that could be compared to one another.

108 previously extracted samples were tested for PMMoV. PMMoV was quantified using a methodology adapted from Jafferali et al. (2021) to optimise performance with existing equipment. Forward and reverse primer sequences recommended by Jafferali et al. (2021) were used. Custom TaqMan probes (ThermoFisher, UK) were used to quantify the target PMMoV genes. 5 µL of each sample tested was analysed. Thermal cycling (55 °C 60 mins, 95 °C 5 mins, followed by 45 cycles of 95 °C 15 s, 60 °C for 1 min, 65 °C for 1 min) was performed on a QuantStudio™ 7 Pro Real-Time PCR System (ThermoFisher, UK). Negative controls were included in each run. The RNA extracts were quantified by plotting the quantification cycles (CT) to an external standard curve constructed from commercially available synthesised plasmids

containing the target sequence. Samples were considered positive if the Ct value was below 45 cycles.

2.2.4 Normalising to wastewater biomarkers

Normalising wastewater to biomarkers is critical when flow information is not available, as it allows for more accurate estimation of population shedding. This is particularly useful where there is a highly variable population, such as Near-Source WBS. The following equation was used to normalise the gene copy data to a biomarker, as used by Sweetapple et al. (2022):

$$GC_n = \frac{GC}{X}$$

Where:

GC_n = GC normalised

GC = SARS-CoV-2 GC L^{-1}

X = Wastewater biomarker L^{-1} (e.g., ammonium or PMMoV)

Equation 1: Wastewater parameter normalised gene copies

2.2.5 Estimating campus infection burden

Campus residence occupancy data was obtained directly from the Cranfield Campus Residence Management Team. This consisted of a spreadsheet that listed the weekly occupancy figures for the different residences on the campus (e.g., Halls of Residence A and B, and the student and family houses). The sewer-catchment maps were combined with this data to estimate the population within the sewer catchment.

The campus infection rate, the % of people on campus who if tested with a PCR test would return a positive result, was assumed to be equal to that of the Lower Tier Local Authority (LTLA) PCR positive test rate. The LTLA PCR positive test rate was also assumed to be an accurate measure of the disease burden within the Central Bedfordshire administrative council region. The Cranfield campus is rural and geographically isolated and is thus unlikely to be equal to that of the rest of the LTLA. However, the LTLA positive PCR rate data was the highest resolution data that was

publicly available that was sufficiently accurately and reliable for used for comparison to wastewater / local infection data generated directly by this study.

The equation below demonstrates how the infected population was estimated using the LTLA data:

$$Ei = Li * P$$

Where:

Ei = Estimated infected

Li = LTLA positive PCR rate

P = Population within sewer-catchment

Equation 2: Estimating infection burden within sewershed using PCR positive rate data

Campus infection burden was also estimated from the wastewater data using an equation from Ahmed et al. (2020) :

$$People\ Infected = \frac{(N1) * (Q)}{\left(\frac{g\ faeces}{person\ d^{-1}}\right) * \left(\frac{N1}{g\ faeces}\right)}$$

Equation 3 – Estimating infection burden from faecal shedding of SARS-CoV-2

Where: Q = wastewater flow rate (L d^{-1}), SARS-CoV-2 N1 GC L^{-1} were quantified by RT-qPCR, litres of wastewater were estimated by multiplying the known sewer-catchment population by the typical per person wastewater production volume of 140 L d^{-1} (Brockett, 2019). Daily stool mass was assumed to be 128 g of wet faeces (Rose et al., 2015), and faecal shedding was based on viral shedding of $1x10^5$ N1 GC g^{-1} wet faeces which represents the median shedding of the wild-type SARS-CoV-2 in the faeces 10 days after symptom onset (Miura et al., 2021).

Cases of SARS-CoV-2 confirmed by clinical nasopharyngeal PCR test, where people were on campus 2 days prior to either displaying symptoms or testing positive by PCR, were supplied by the Health and Safety Team of Cranfield University. This data was a weekly aggregate of individuals who tested positive for SARS-CoV-2 within two days of being present on campus.

2.2.6 SARS-CoV-2 Variant of Concern Identification

SARS-CoV-2 VoCs were analysed using the Arctic multiplex PCR method for SARS-CoV-2 genome sequencing (Tyson et al., 2020) through the commercial partner Eurofins (Germany). In brief, this methodology identifies lineage defining mutations caused by single nucleotide polymorphisms, such as insertions and deletions, that significantly alter factors (e.g., virus transmissibility) that are characteristics of VOCs. In total, 4% of SARS-CoV-2 positive wastewater samples were sequenced, of which two had correct coverage to be interpreted in detail, with 13 whole genomes not having sufficient coverage in key genes to warrant further investigation. Once sequenced, complete SARS-CoV-2 genomes were extracted from the NCBI database and aligned using MUSCLE (Edgar, 2004). The evolutionary history was inferred using the Neighbour-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.007 was constructed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (values over 25 shown) (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes Cantor method (Jukes and Canthor, 1969) and are in the units of the number of base substitutions per site. The analysis involved 11 whole SARS-CoV-2 sequences. Evolutionary analyses were conducted in MEGA v11 (Tamura et al., 2021).

2.2.7 Statistics

The statistical analysis of the dataset was performed on SPSS (Version 28, IBM 2021) which contains the requisite statistical tests for the analysis in this study. Microsoft Excel (Version 2108, Microsoft 2021) was sued to maintain a database for all of the sample data.

The N1, E GC L^{-1} and wastewater characteristic data was tested for normality with the Shapiro-Wilk test. It was found that none of the data was normally distributed as, in all cases, $p < 0.05$. Spearman's rank-order correlation was used to test the statistical significance of the correlations between SARS-CoV-2 N1 and E GC L^{-1} wastewater and the wastewater data. Spearman's rank-order correlation is a non-parametric test for correlation between two variables and is thus appropriate for non-normally

distributed data. P values of less than 0.05 were considered as statistically significant. Other similar studies have also used Spearman's rank-order correlation to test for the power, direction, and statistical significance of correlations between wastewater characteristic data and SARS-CoV-2 gene copy concentrations such as (Bivins and Bibby, 2021).

SARS-CoV-2 removal was assessed by conducting a Kruskall-Wallis 1-way ANOVA on the N1 and E gene copy concentration in the effluent at each sampled stage of the WWTW. This was conducted to assess if there was a statistically significant difference between the median influent and final effluent SARS-CoV-2 gene copy concentrations, and to test the interstage removal of SARS-CoV-2 genetic material.

Microsoft Excel (Version 2108, Microsoft 2021) was used to identify instances of correspondence between positive detections of SARS-CoV-2 in pre- and proceeding positive cases of SARS-CoV-2 in the wastewater. Bootstrapping was performed in SPSS (Version 28, IBM 2021) to calculate bias corrected confidence intervals set at 95% confidence level.

3 Results and discussion

3.1 Study results

3.1.1 Wastewater SARS-CoV-2 correlates to on Campus Infections

Figure 5 and the metadata summarised in Table 4 demonstrate how changes in England's and campus restrictions impacted the concentration of SARS-CoV-2 N1 gene copies in the wastewater samples. The numbers 1-3, 5-7 and 9 on Table 4 represent changes to national and/or local restrictions, whilst numbers 4 and 8 represent periods of increased concentration of N1 GC in the wastewater on campus. At points 4 and 8, the site health and safety teams were notified of increasing trends in wastewater and likely increasing number of cases on campus, despite no obvious increase in clinical testing case numbers. Table 4 explains the changes to national and local restrictions, VOC, campus occupancy and LTLA level of vaccination as it was assumed that these were the dominant factors driving changes in the wastewater N1 GC concentration.

This study did not find a statistically significant correlation between SARS-CoV-2 N1 GC L^{-1} and the number of campus infections when using non-normalised data (Spearman's Rank; $\rho(22) = 0.23$, $p = 0.21$). Normalisation of the SARS-CoV-2 data was undertaken, but this did not yield a positive correlation between campus infections and N1 GC / ammonia (Spearman's Rank, $\rho(28) = 0.169$, $p = 0.389$). This is in contrast to other university campus based WBS studies that also noted a positive relationship, such as Fahrenfeld et al. (2022) (Pearson Correlation, $r = 0.21$, $p = 0.018$) and (Scott et al., 2021) (Pearson Correlation, $r = 0.181$, $p < 0.01$), Bivins and Bibby (2021) (Spearman's Rank, $\rho = 0.51$, $p = 0.039$), Gibas et al. (2021) (Pearson Correlation, $r = 0.769$), Wang et al. (2022) (Pearson Correlation, $r = 0.835$) and Wright et al. (2022) (Pearson Correlation, $r(13) = 0.71$, $p < 0.01$). Other researchers (Ai et al., 2021; Bhattarai et al., 2021; D'Aoust et al., 2021b, 2021a; Li et al., 2022; Medema et al., 2020; Nemudryi et al., 2020a; Scott et al., 2021; Weidhaas et al., 2021), who normalised their data either using flow or biomarkers (chemical and/or biological) to account for variable flows in wastewater streams have reported statistically significant correlations between SARS-CoV-2 GC L^{-1} and SARS-CoV-2 cases in non-University Campus studies.

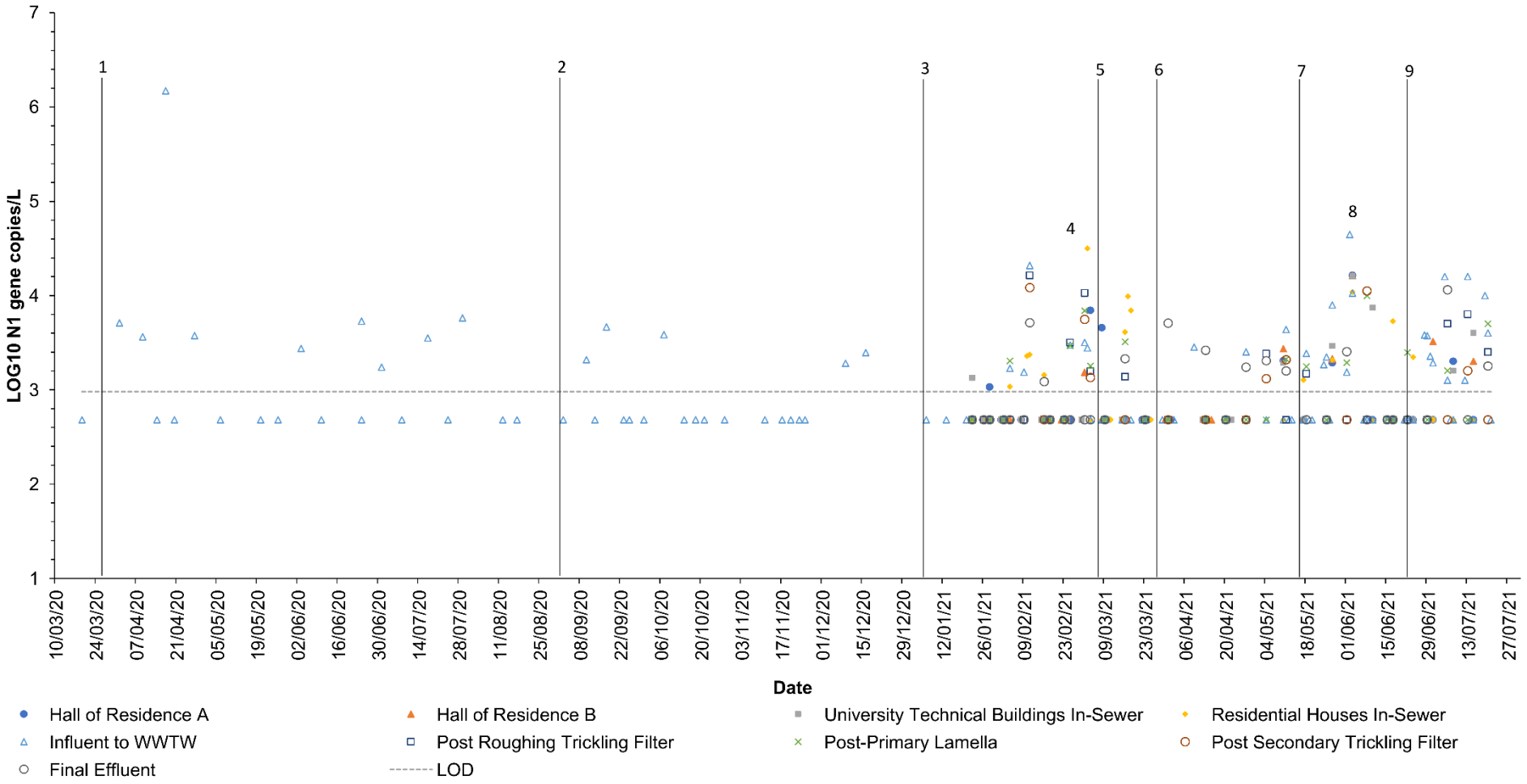

Figure 5: Wastewater N1 GC concentration at study sampling points.

Table 4: Summary of changes to England's national and university restrictions, VOC, campus population and LTLA vaccination rate. * (Public Health England, 2020)**, †** (Public Health England, 2021a)**, ‡** (Public Health England, 2021b)**, §** (Public Health England, 2021c)

Number	Date	National restrictions	Campus restrictions	Dominant Variant of Concern (VOC)	Median people living on campus	LTLA Level of 1st vaccination (%)	LTLA Level of 2nd vaccination (%)
1	25/03/2020	Full national lockdown.	Access to campus restricted.	Undetermined*	977	0	0
2	01/09/2020	Indoor and outdoor.	Face-to-face teaching permitted.	Undetermined*	572	0	0
3	06/01/2021	National lockdown: essential shops open.	Access to campus restricted.	Alpha-variant†	1175	1.73	0.36
4		No change.	No change.	Alpha-variant‡	1160	20.71	0.49
5	08/03/2021	No change.	Partial return to face-to-face teaching.	Alpha-variant§	1147	37.28	1.41
6	29/03/2021	Outdoor socialisation permitted in groups of 6.	No change.	Alpha-variant§	1147	56.21	3.85
7	17/05/2021	Most businesses re-open.	Full return to face-to-face teaching.	Delta-variant§	1081	64.75	32.92
8		No change.	No change.	Delta-variant§			
9	21/06/2021	Removal of all restrictions.	Campus access restrictions removed.	Delta-variant§	1013	82.61	64.15

On the 25th of March 2020 (1 on Table 4), the Coronavirus Act 2020 was given Royal Assent, and 'lockdown' was implemented across England the next day. The Government mandated that, unless they have a "reasonable excuse", people should not leave their homes. Permitted activities included but were not limited to: taking one form of exercise per day, shopping for essential items, travelling to and from work should they be unable to work from home and travelling for medical needs. Gatherings of 2 or more people were not permitted, with some exceptions to which education was not exempted. From the 25th of March 2020, Cranfield University transitioned to online lecturing and access to all non-essential buildings was restricted. During this period, monitoring at the WWTW Influent was undertaken, and a concentration of 5,108 N1 GC L^{-1} was detected in the wastewater on 1st April 2020. Campus clinical testing data was not recorded at this point. Prior to this on the 19th of March 2020, the concentration in the wastewater was below the LOD.

On the 1st of September 2020 (2 on Table 4), changes to the Cranfield campus restrictions were made to permit face-to-face teaching. At the WWTW Influent, the concentration of N1 GC was below the LOD on the 2nd of September 2020, increasing to 2,080 N1 GC L^{-1} on the 10th of September 2020, and increasing once more on the 17th of September 2020 to a concentration of 4,635 N1 GC L^{-1} before reducing to below the LOD on the 23rd of September 2020. Campus clinical testing data was still not being reported, and the campus population was a mean of 572 residents during this period.

On the 6th of January 2021 (3 on Table 4), a third National lockdown was announced for England due to the identification of the Alpha VoC which had become the dominant VoC in England, and then the rest of the UK. Data at the time suggested it was more infectious than the wild-type virus, with later studies reporting the R_0 for the Alpha VoC as 29% greater than the wild-type SARS-CoV-2 virus (Campbell et al., 2021). On the same day, 14 news cases of people were reported to be infected with SARS-CoV-2 on the campus. WWTW Influent N1 GC concentration was below the LOD on this day. The WWTW Influent N1 GC concentration data did not increase above the LOD until the 4th of February 2021, at which point a concentration of 1,678 N1 GC L^{-1} was reached. This suggests either i) infected individuals were self-isolating off campus or ii) that the wastewater logged campus infections not identified by clinical tests in this case. This would suggest a lag time of 30 days from clinically confirmed testing to

detection in the wastewater. A peak concentration in the WWTW Influent of 13,740 N1 GC L^{-1} on the 11th of February 2021, which was preceded by 8 clinically confirmed cases on the 13$^{th\ of}$ January 2021 suggesting another potential lag of 30 days between cases and detection in the wastewater. This could be associated with the arrival of the Alpha VoC on the campus, which was confirmed by genome sequencing of samples taken during this time. Due to the increased infectivity of the Alpha SARS-CoV-2 VoC, it can be assumed that its introduction to a new area will result in an increase in infections. This has previously been reported by (Corchis-Scott et al., 2021), who reported that the introduction of the Alpha variant to their study area led to a significant increase in infections. This 1.16 log increase above the LOD could also be attributed to the low level of vaccination in the resident campus population at this time. In the UK, prior to the 8th of June 2021, people below the age of 30 were unable to receive a first vaccine dose unless they were deemed being at “high risk” of harm from a SARS-CoV-2 infection (Roberts, 2021). Vaccination has been shown to reduce the rate of transmission of SARS-CoV-2 amongst populations (Sah et al., 2021; Thompson et al., 2021), and Bivins and Bibby (2021) have demonstrated a significant reduction in the concentration of SARS-CoV-2 genetic material on a University Campus following a vaccination campaign that led to 90% of students being vaccinated. Thus, it was assumed that very few of the resident population - most of whom were assumed to be younger than 30 years of age – would have been vaccinated against SARS-CoV-2. In this study the vaccination status of the Campus population was not known. A more infectious variant combined with a low percentage of vaccinated residents on the campus possibly contributed to the observed increased detection of SARS-CoV-2 in the wastewater during this period.

From the 16th of February 2021 until 22nd of March 2021 there was an increase in the concentration of N1 GC in the wastewater samples, especially in Residential In-Sewer wastewater samples. During this time period, there were 7 clinically confirmed cases on the campus. A peak concentration of 31,623 N1 GC L^{-1} was recorded from a sample taken from the Residential In-Sewer Sampling Point on the 3rd of March 2021. On the same day, a concentration of 2,754 N1 GC L^{-1} was recorded at the WWTW inlet. These peaks were reached 14 days after 4 clinically confirmed cases of SARS-CoV-2 infections were recorded on the 16th of February, suggesting a lag of 14 days from clinical testing to detection in the wastewater. From the peak, the N1 GC concentration

in the wastewater continued to decline. This trend of reducing SARS-CoV-2 GC continued until the 20th of March 2021.

On the 17th of May 2021, changes to National restrictions were lifted to allow business to re-open, unless they were deemed to be at 'high risk' of allowing SARS-CoV-2 transmission (e.g., nightclubs), and changes to Cranfield University campus were made that permitted a full return to face-to-face teaching (7 in Table 4). The concentration in the WWTW Influent peaked at 44,157 N1 GC L^{-1} on the 2nd of June 2021 and was preceded by 2 clinically confirmed SARS-CoV-2 infections on the 25th of May 2021 suggesting that detection in the wastewater lags 8 days behind positive cases.

On the 21st of June 2021, all National and Cranfield University Campus restrictions were lifted. From this date, wastewater concentration of N1 GC increased before reaching a peak concentration in the WWTW Influent of 15,849 N1 GC L^{-1} on the 5th of July 2021 from a previous value of 3,802 GC L^{-1} on the 28th of June 2021. Following this increase in the wastewater, there was an increase in the number of clinically confirmed SARS-CoV-2 infections from a previous weekly average of 2 cases for the period 22/06/2021-18/07/2021 to 20 cases. This would suggest that, in this instance, the wastewater data is a leading indicator of infection by 14 days. This increase in infections could be the result of the removal of restrictions that help to limit the spread of infection (e.g., social distancing and wearing masks), and the arrival of the Delta VoC in the UK. The Delta VoC is more 97% more transmissible than the wild-type SARS-CoV-2 virus (Campbell et al., 2021). Thus, its arrival is likely to be associated with an increase in infections.

Figure 6A and 6B show that estimating the number of infected people on the Cranfield University campus by use of the LTLA PCR positive test rate may not be appropriate. Despite the LTLA estimations that there would be 79 infected individuals on the campus on the 11th of February 2021 (Figure 6B), the wastewater data estimated that there would be 27 whilst the clinical testing data suggested that there were 2 infected individuals (Figure 6A). Applying disease burden trend data from large geographical regions with varying characteristics may not be appropriate for estimating disease burden in smaller, more isolated, and self-contained geographical regions or even at the localised level of individual buildings. Studies such as Karthikeyan et al., (2021),

(Scott et al., 2021), Goyal et al. (2021) and Nagarkar et al. (2021) have all recognised the importance of identifying clusters of outbreaks in controlling the spread of disease, and the differing relationships between wastewater data and SARS-CoV-2 infections at different spatial scales and study site characteristics. In this instance, using LTLA estimated infection data appeared to significantly overestimate the Campus and sewershed infections, whilst clinical data appeared to underestimate infections – possibly due to improper compliance with testing regimes. Thus, the findings of this study would appear to agree with the findings of others, that WBS is appropriate and useful when used to support clinical testing for estimating disease burden at the building and community level, and managing the spread of disease, especially in well-controlled environments such as on University Campuses and where disease burden is low but infections can still be substantial (Corchis-Scott et al., 2021; Goyal et al., 2021; Karthikeyan et al., 2021b; Scott et al., 2021; Sweetapple et al., 2022).

This shows that people infected with SARS-CoV-2 were on the campus and shedding virus particles in their faeces, even when there were no weekly reported cases throughout the course of the study. It is possible that these people were pre-symptomatic, asymptomatic, symptomatic and did not know/report that they were infected, or they had recovered and were continuing to shed SARS-CoV-2 in their faeces. SARS-CoV-2 N1 GC L^{-1} wastewater data was provided at twice monthly intervals as requested by the Site Access Group, however data could have been provided as often as 24hrs had it been required. Interventions (e.g., encouraging testing of individuals and isolating identified infected people) were not possible in this study. These have shown to be effective at limiting the spread of and even preventing outbreaks of SARS-CoV-2 in other studies (Betancourt et al., 2021; Corchis-Scott et al., 2021; Fielding-Miller et al., 2021; Karthikeyan et al., 2021b; Scott et al., 2021). The results of this study therefore appear to support the findings that England's and Cranfield Campus measures such as isolating infected individuals, wearing of facemasks, physical distancing, closing all but essential shops, online teaching, limiting access to campus buildings (e.g. laboratories and offices) were effective at limiting the spread of SARS-CoV-2 on the Cranfield Campus owing to the low-level of infected people in comparison to the rest of the LTLA (Goyal et al., 2021; Karthikeyan et al., 2021b; Scott et al., 2021; Wilder-Smith and Freedman, 2020).

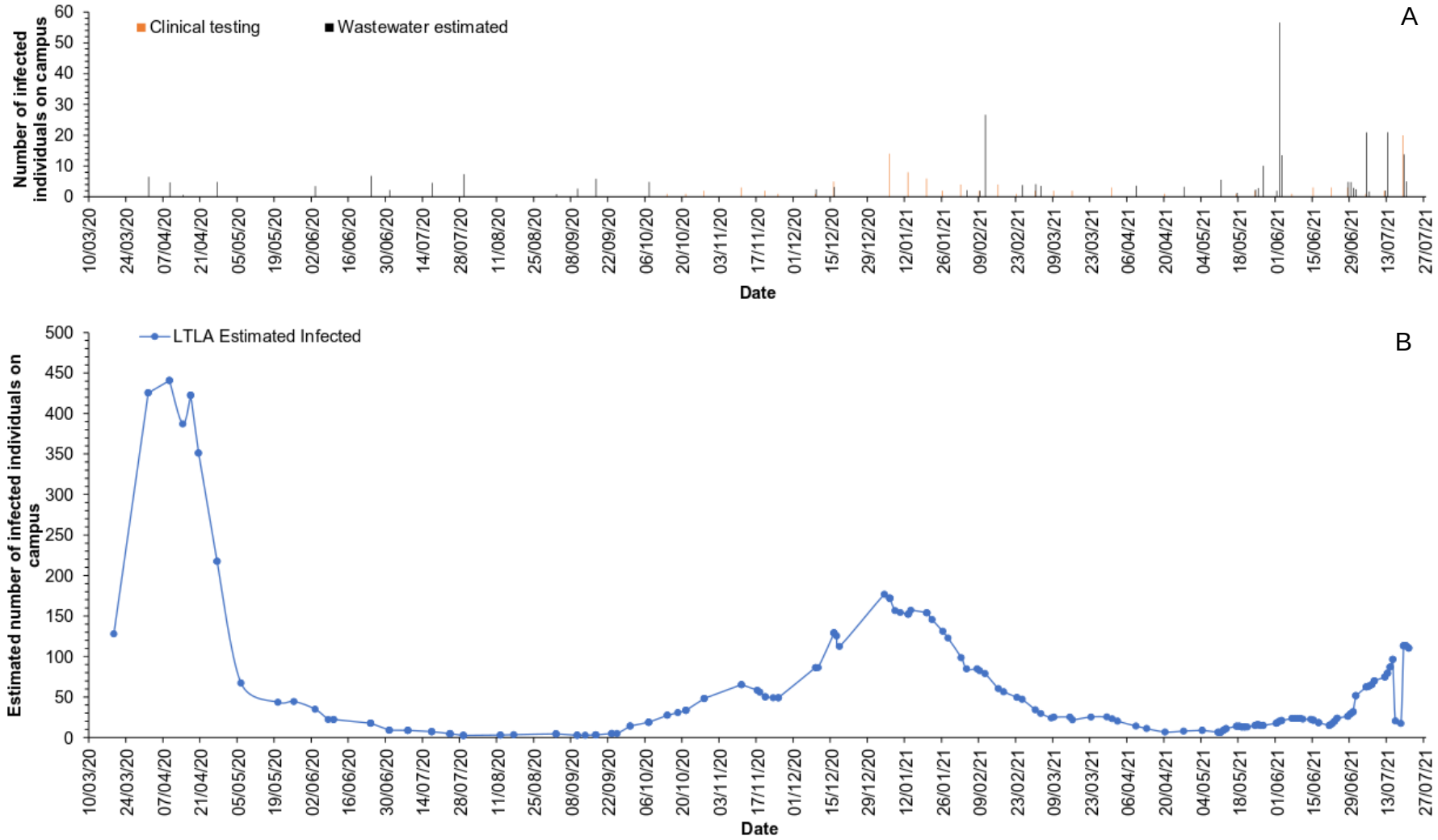

Figure 6: (A) Actual and Estimated (Wastewater), and LTLA Estimated (B) Cranfield campus SARS-CoV-2 Infections.

3.1.2 Identifying variants of concern within campus populations

Of the 15 samples sent off for genome sequencing, only 3 were positive for SARS-CoV-2 by genome sequencing and only 2 samples (internal reference F58 and F93) were sequenced (Table 5, below). Figure 7 below, shows the genome coverage of the sequenced samples (A – F58, B - F93) and the locations of amino acid mutations, and C shows that the samples that were successfully sequenced were closely aligned to the Alpha VoC. Other researchers have identified SARS-CoV-2 variants at the Near-Source level (Karthikeyan et al., 2021a; Vo et al., 2022), In-Sewer (Rios et al., 2021) and at the WWTW level (Nemudryi et al., 2020; Bar-Or et al., 2021; Crits-Christoph et al., 2021; Heijnen et al., 2021; Izquierdo-Lara et al., 2021; Jahn et al., 2021; la Rosa et al., 2021; Rios et al., 2021). This research still shows that WBS could be used to track the spread of SARS-CoV-2 VoCs and variants of interest at different spatial scales, but for it to be effective RNA extraction protocols must be optimised for preventing RNA fragmentation. It has been hypothesized that Protocol 2 may be responsible for the lack of sample genome sequencing, as the Trizol used in RNA extraction appears to have degraded and/or fragmented the RNA. This can be seen in Figure 8. The lanes that contain samples extracted using Protocol 2 show a distinct lack of banding, unlike the lanes that contain samples extracted using Protocol 1 show much stronger banding. Whilst fragmented RNA allows RT-qPCR to be effective, it makes sequencing less effective. Despite this variability in sequencing, it was hypothesised that WBS can indeed be used to identify variants responsible for on campus infections has Protocol 1 been feasible.

Table 5: Samples sequenced by Eurofins by Arctic Multiplex PCR for SARS-CoV-2 genome sequencing

Internal Reference	Sample location	Date collected	SARS-CoV-2 detected via sequencing	Sequenced	Variant identified
H10	Influent	17/04/2020	No	No	N/A
H29	Influent	24/06/2020	No	No	N/A
H47	Influent	29/07/2020	No	No	N/A
H63	Influent	17/09/2021	No	No	N/A
F20	Hall of Residence A	28/01/2021	No	No	N/A
F44	Influent	04/02/2021	No	No	N/A
F58	Influent	11/02/2021	Yes	Yes	B.1.1.7
F93	Residential In-Sewer	02/03/2021	Yes	Yes	B.1.1.7
F106	Hall of Residence A	08/03/2021	No	No	N/A
F129	Hall of Residence A	18/03/2021	Yes	No	N/A
F143	Influent	31/03/2021	No	No	N/A
F167	Technical In-Sewer	22/04/2021	No	No	N/A
F213A	Influent	27/05/2021	No	No	N/A
F233A	Hall of Residence B	10/06/2021	No	No	N/A
F271A	Hall of Residence B	01/07/2021	No	No	N/A

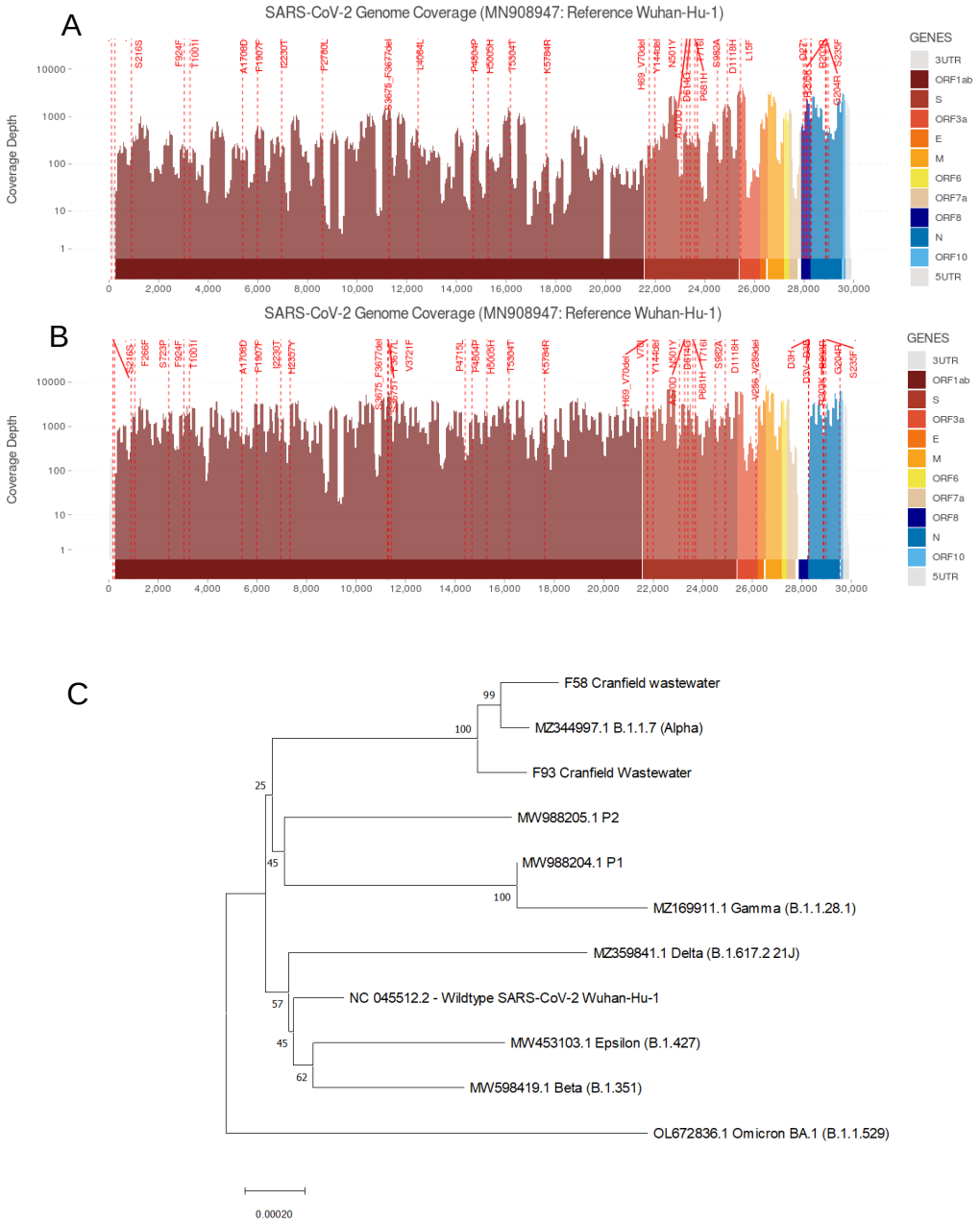

Figure 7: Genome coverage of sequenced samples A – F58 and B - F93. Coverage depth relates to the average number of unique reads of a targeted genes of the SARS-CoV-2 genes which were sequenced (Sims et al., 2014)**, and the red lines represent the genome positions of the amino acid mutations. C - Phylogenetic tree constructed from whole genome sequencies of SARS-CoV-2 obtained from campus wastewater and comparative VoCs and Variant Under investigation (VUI). Scale bar denotes number of base substitutions per site. Reference strains were downloaded from GenBank and labelled with their accession number, Pango lineage (e.g., B.1.1.7) and WHO label (e.g., Alpha)**

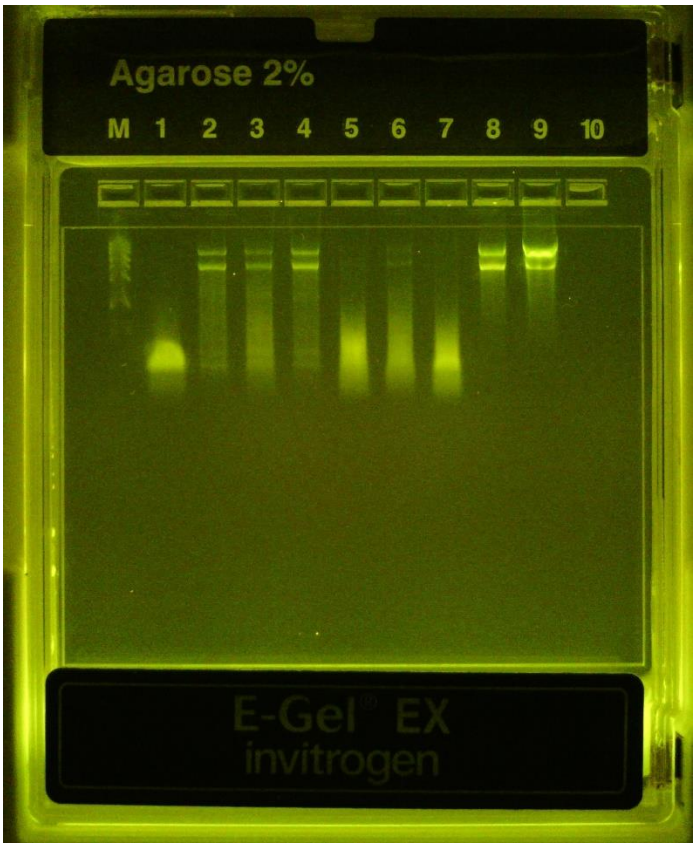

Figure 8: Gel electrophoresis of samples extracted by different methods. Lane M is the control SARS-CoV-2 genome. Lanes 1, and 5 to 7 were extracted with Protocol 2, lanes 2-4, 8 and 9 were extracted with Protocol 1.

3.1.3 SARS-CoV-2 N1 is coupled to wastewater constituents near-source

Table 6 and Table 7 (below) summarise the correlations between the wastewater N1 and E gene copy concentrations and wastewater parameters at the Near-Source and In-Sewer sampling points, respectively. Ammonium and phosphate are present in residential wastewater owing to the presence of urine and faeces (Rose et al., 2015), whilst N1 gene copies are present as SARS-CoV-2 virus particles are shed in the faeces (Foladori et al., 2020; Miura et al., 2021; T. Zhang et al., 2020). TSS consists of the non-dissolved solid matter that exists in the wastewater, and tCOD is the amount of oxygen equivalents consumed in the chemical oxidation of organic matter by a strong oxidant (Hu & Grasso, 2005 p.

325). This study found a strong positive correlation between the SARS-CoV-2 N1 GC concentration and the concentration of ammonium in the wastewater at the Near-Source level (Spearman's Rank; $\rho(14) = 0.823$, $p < 0.01$), supporting the findings of Sweetapple et al. (2022). The SARS-CoV-2 N1 GC concentration was also positively correlated with TSS (Spearman's Rank; $\rho(14) = 0.572$, $p < 0.01$) and tCOD (Spearman's Rank; $\rho(14) = 0.765$, $p < 0.01$). The SARS-CoV-2 E GC concentration was also positively correlated with ammonium (Spearman's Rank; $\rho(17) = 0.537$, $p < 0.01$). This was the only relationship for the SARS-CoV-2 E gene.

At the In-Sewer level, the SARS-CoV-2 N1 GC concentration was positively correlated with ammonia (Spearman's Rank; $\rho(26) = 0.543$, $p < 0.01$), TSS (Spearman's Rank; $\rho(27) = 0.408$, $p = 0.03$) and tCOD (Spearman's Rank; $\rho(25) = 0.528$, $p < 0.01$). The SARS-CoV-2 N1 GC concentration was positively correlated with phosphate, but this relationship was not significant (Spearman's Rank; $\rho(23) = 0.342$, $p = 0.09$). There were no statistically significant correlations between the SARS-CoV-2 E GC concentrations at the In-Sewer Level. There were no statistically significant relationships between any of the tested wastewater parameters and unnormalized N1 or E gene copies at the WWTW.

In this study there was no statistically significant relationship between phosphate concentration and N1 gene copy concentration in the wastewater at the Near-Source level. Whilst this could be due to the small number of samples that tested positive for SARS-CoV-2 at this spatial scale, other factors could also be affecting the relationship. Surfactants have been shown to influence the recovery of SARS-CoV-2 from wastewater. Kevill et al. (2022) found that there was a statistically significant negative correlation between increasing surfactant concentration in wastewater and decreasing recovery of SARS-CoV-2 genetic material with the PEG virus precipitation method used in both Protocols 1 and 2. This may explain the N1 SARS-CoV-2 GC in the wastewater and phosphate concentration, but it does not correlate as PO_4-P can be decoupled from viral shedding. Other factors, such as variable flow and sampling regime, may also have impacted the strength and significance of this relationship. Ingress of ground and/or soil water into the

sewer, in-network characteristics and sampling strategy can all influence results (Wade et al., 2022).

The data was reanalysed with SARS-CoV-2 data that had been normalised to ammonia, phosphate or PMMoV concentration in the sample, with the results shown in Tables 7-9. After normalisation, at the Near-Source level there was a negative correlation between ammonia normalised SARS-CoV-2 N1 GC concentration and phosphate (Spearman's Rank; $\rho(16) = -0.622$, $p = 0.01$). No other statistically significant correlations were observed at the Near-Source level. At the In-Sewer level, there was a negative correlation between the PMMoV normalised SARS-CoV-2 N1 GC concentration and tCOD (Spearman's Rank; $\rho(16) = -0.649$, $p < 0.01$). No other statistically significant correlations were found at the level. At the WWTW level, a positive correlation was found between the PMMoV normalised SARS-CoV-2 N1 GC concentration and the ammonia (Spearman's Rank; $\rho(29) = 0.400$ $p = 0.03$). This finding is interesting as it suggests that i) ammonia might be useful as a normalisation parameter in WWTW not impacted by industrial inputs and ii) that two readily applied markets of population i.e., viral (PMMoV) and chemical (ammonia) show the same trend. It is recommended that PMMoV is used in diluted municipal feeds, but that ammonia is quicker and cheaper for in sewer/normal municipal WWTW.

Table 6: Spearman rank-order correlations between SARS-CoV-2 N1 and E gene copies and wastewater constituents for Halls of Residence A and B. N.B: * significant to the 0.05 level, ** significant to the 0.01 level.

Wastewater Constituent (mg/L)	**Target Gene (GC L^{-1})**	
	Nucleocapsid Region 1 (N1)	Envelope Protein (E)
NH_4^+	0.823** N = 16	0.537** N = 19
PO_4^{2-}	0.466 N = 16	0.372 N = 18
TSS	0.572** N = 16	0.376 N = 18
tCOD	0.765** N = 16	0.315 N = 15

Table 7: Spearman rank-order correlations between SARS-CoV-2 N1 and E gene copies and wastewater constituents for Technical and Residential In-Sewer sample points. N.B: * significant to the 0.05 level, ** significant to the 0.01 level.

Wastewater Constituent	Target Gene (GC L^{-1})	
	Nucleocapsid Region 1 (N1)	Envelope Protein (E)
NH_4^+	0.543** N = 28	0.293 N = 19
PO_4^{2-}	0.342 N = 25	-0.130 N = 18
TSS	0.408* N = 29	0.381 N = 22
tCOD	0.528** N = 27	0.407 N = 21

Table 8: Spearman rank-order correlations between SARS-CoV-2 normalised N1 gene copy ratios and wastewater constituents for Halls of Residence A and B. N.B: * significant to the 0.05 level, ** significant to the 0.01 level.

Wastewater Constituent (mg/L)	N1 normalisation technique		
	N1/NH4	N1/PO4	N1/PMMoV
NH_4^+	-	-0.139 N = 14	0.651 N = 8
PO_4^{2-}	-0.622* N = 16	-	0.356 N = 8
TSS	-0.061 N = 14	-0.319 N = 14	0.506 N = 8
tCOD	0.337 N = 12	-0.415 N = 14	-0.800 N = 8

Table 9: Spearman rank-order correlations between SARS-CoV-2 normalised N1 gene copy ratios and wastewater constituents for Technical and Residential In-Sewer sample points. N.B: * significant to the 0.05 level, ** significant to the 0.01 level.

Wastewater Constituent (mg/L)	N1 normalisation technique		
	$N1/NH_4$-N	$N1/PO_4$-P	N1/PMMoV
NH_4^+	-	-0.296 N = 18	-0.452 N = 16
PO_4^{2-}	0.179 N = 21	-	-0.416 N = 13
TSS	0.132 N = 25	0.187 N = 19	-0.409 N = 17
tCOD	0.148 N = 23	-0.106 N = 18	-0.649* N = 16

Table 10: Spearman rank-order correlations between SARS-CoV-2 normalised N1 gene copy ratios and wastewater constituents for WWTW Influent. N.B: * significant to the 0.05 level, ** significant to the 0.01 level. $N1/PO_4$ data not available for the WWTW.

Wastewater Constituent (mg/L)	N1 normalisation technique		
	N1/NH4	N1/PO4	N1/PMMoV
NH_4^+	-	-	0.317 N = 31
PO_4^{2-}	-	-	-
TSS	-0.129 N = 45	-	-0.207 N = 35
BOD	0.020 N = 43	-	-0.261 N = 33

Samples testing positive for SARS-CoV-2 were also tested for PMMoV concentration. All of the 108 samples tested were positive for PMMoV. No significant relationships were identified between PMMoV GC L^{-1} at the Near-Source Level for ammonia (Spearman's Rank; $\rho(14) = -0.053$ $p = 0.857$), phosphate (Spearman's Rank; $\rho(14) = 0.143$ $p = 0.626$), TSS (Spearman's Rank; $\rho(14) = -0.451$ $p = 0.106$), tCOD (Spearman's Rank; $\rho(10) = -0.379$ $p = 0.280$) nor SARS-CoV-2 N1 GC L^{-1} (Spearman's Rank; $\rho(9) = -0.117$ $p = 0.765$). Positive correlations were found at the In-Sewer level between PMMoV L^{-1} and the wastewater concentrations of ammonia (Spearman's Rank; $\rho(19) = 0.535$ $p = 0.18$), and tCOD (Spearman's Rank; $\rho(24) = 0.490$ $p = 0.046$). No correlation was found between PMMoV GC L^{-1} and SARS-CoV-2 N1 GC L^{-1} at this level (Spearman's Rank; $\rho(11) = 0.427$ $p = 0.190$). At the WWTW Influent, PMMoV GC L^{-1} was found to negatively correlated with TSS (Spearman's Rank; $\rho(97) = -0.245$ $p = 0.016$), no other statistically significant relationships were found. The lack of relationship at the Near-Source level could be due to the small number of samples. Had more samples been tested for PMMoV a different relationship may have been established. The positive correlations between PMMoV and the wastewater characteristics at the In-Sewer level suggest that it is strongly associated with faeces. The lack of a statistically significant relationship at the WWTW could be due to the effects of external influences as the wastewater is conveyed through the wastewater network to the WWTW (Wade et al., 2022).

Analysis of the concentration of PMMoV at the WWTW Influent found that concentration of PMMoV L^{-1} was similar to the ranges reported by Symonds et al. (2018). The lower quartile concentration of PMMoV was 7.56×10^6 GC L^{-1}, the median value was 3.97×10^7 GC L^{-1}, the upper quartile value was 8.97×10^7 GC L^{-1} with an interquartile range of 8.21×10^7 GC L^{-1}. There was a positive correlation between PMMoV and sewershed population, but it was not significant (Spearman's Rank; $\rho(138) = 0.132$ $p = 0.14$) (Figure 9). After adjusting PMMoV concentration with the WWTW Inlet flow data to account for daily loading, was still not a statistically significant positive relationship between PMMoV and the population (Spearman's Rank; $\rho(99) = 0.027$ $p < 0.79$). There were no statistically

significant relationships between PMMoV GC and SARS-CoV-2 N1 GC concentrations in the wastewater at any level. This is the first study to my knowledge linking PMMoV a tightly defined population with a defined wastewater conveyance and treatment works. This study found that PMMoV was consistently detected in wastewater, and that concentrations in wastewaters remain within previously established ranges even at different spatial scales, and that increasing population leads to increasing daily loading. Thus, this study has found that PMMoV is a robust biomarker that can be used to normalise data when flow data is not available, supporting the findings of previous researchers (Ahmed et al., 2022; Schmitz et al., 2021; Symonds et al., 2018).

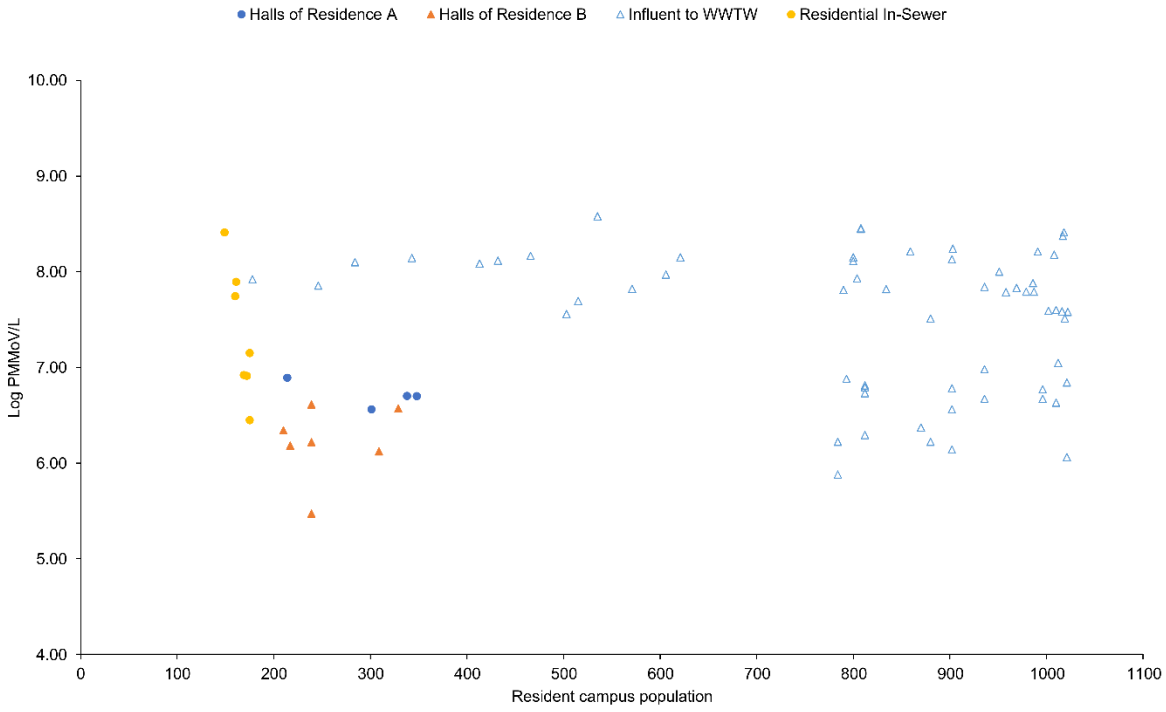

Figure 9: Correlation between PMMoV and people who live on campus at different study sample points.

As noted by Ort et al. (2010), the sampling strategy of a WBS study is likely to have a strong impact on the representativeness of the sample obtained. It was assumed that a grab sample from the balance tank effluent would be representative of the 24hr period prior to which it was taken, as the purpose of a balance tank is to 'balance' the packets of water that arrive at a WWTW to allow

it to operate at optimum efficiency. However, this assumption may not be correct, and thus the grab sample obtained from the balance tank may not be representative of the 24hr period of sampling and thus stools containing SARS-CoV-2 genetic material of people with COVID-19 may not be present in the sample.

3.1.4 Lead / Lag analysis of Wastewater Based Surveillance for SARS-CoV-2 monitoring.

A retrospective analysis of the concordance between positive detection of SARS-CoV-2 in the wastewater and cases on the campus found that of the 82 days that there was at least one positive case on the campus, 56.1% corresponded with a positive detection of SARS-CoV-2 in the wastewater at the WWTW Inlet in the preceding 7 days (95% CI: 45.2-67.1), 69.5% corresponded with a positive detection of SARS-CoV-2 in the wastewater at the WWTW Inlet in the preceding 14 previous days (CI: 59.3-79.3) and 92.7% corresponded with a positive detected at the WWTW Inlet in the preceding 30 days (CI: 86.8-97.6). This supports the findings of Fielding-Miller et al. (2021) who reported that 76% of positive cases in a school corresponded with positive detection of SARS-CoV-2 in their wastewater samples (95% CI: 68% - 75%), and Karthikeyan et al. (2021) who found that 84.5% of positive cases on the University of California San Diego campus corresponded with positive detection of SARS-CoV-2 in their wastewater samples one week prior to sample collection. This would appear to support the hypothesis that the wastewater SARS-CoV-2 data is a leading indicator of SARS-CoV-2 infections, as reported by Fielding-Miller et al. (2021), Kaplan et al. (2021), Li et al. (2022) and Peccia et al. (2020) amongst others. The concordance between cases and detection in the wastewater were much lower in this study than in Fielding-Miller et al. (2021) and Karthikeyan et al. (2021). As this study did not influence the SARS-CoV-2 testing regime on campus, instead relying on self-administered unmonitored routine testing, it is likely that SARS-CoV-2 infected individuals were contributing to the wastewater, but the Cranfield Campus Health and Safety team were not aware of their status. This could potentially have led to an underestimation of the number of cases on campus, affecting the relationships between campus cases and SARS-CoV-2 gene copies

in the wastewater. These factors together contributed to the lower rate of concordance between people testing positive for SARS-CoV-2 on the campus and positive detections for SARS-CoV-2 in the wastewater in this study in comparison to others, as opposed to any uncertainty in wastewater enumeration. Other studies, such as Fielding-Miller et al. (2021) and Karthikeyan et al. (2021), were able to encourage people in the wastewater catchments from which samples were obtained to take tests if there was a positive detection for SARS-CoV-2 in the wastewater helping to break chains of infection.

3.1.5 Removal of SARS-CoV-2 at on Campus WWTW

The wild-type SARS-CoV-2 was shown to be 87.2% genetically similar to SARS-CoV-1 in the binding region of the S protein. It was hypothesised that it could be spread by inhalation or ingestion of faecal matter, which is reported to have occurred with SARS-CoV-1 (Yu et al., 2004). A study by (Xiao et al., 2020a) reported recovering infectious SARS-CoV-2 virions from the stool of a patient infected with SARS-CoV-2. Several studies have reported that SRAS-CoV-2 is capable of replicating in the epithelial cells of the intestines (Giobbe et al., 2021; Qian et al., 2021; Xiao et al., 2020b). With this in mind, it is not unsurprising that there was concern that SARS-CoV-2 could spread by the faecal-oral route, such as by faecal aerosols or through discharge into the environment post-wastewater treatment (Amirian, 2020; Amoah et al., 2020; Foladori et al., 2020; Guo et al., 2021; Heller et al., 2020). To date, there have not been many studies that have specifically investigated SARS-CoV-2 removal in WWTW (Mohapatra et al., 2020). Studies that have reported the behaviour of SARS-CoV-2 in wastewater have based on investigations of the behaviour of coronavirus surrogates such as *Pseudomonas virus phi6* (Φ6), and often only in laboratory settings. Ye et al. (2016) reported 22% of Φ6 absorbed to the solids of untreated domestic wastewater, suggesting that enveloped viruses at least partially bind to the solids fraction of wastewater. Thus, it has been assumed that SARS-CoV-2 would be readily removed by primary and secondary treatment processes. A review by Foladori et al. (2020) identified only one peer-reviewed study reporting the removal of SARS-CoV-2 at WWTW. Randazzo et al. (2020) reported a detection

rate of 11% (2 of 18 samples) for SARS-CoV-2 after secondary treatment out of 18 samples taken, despite detection in the influent in 83% of samples (35 out of 42). Thus, further study of interstage removal of SARS-CoV-2 and other enveloped viruses is required, as there is a lack of studies investigating this.

As SARS-CoV-2 is hypothesized to bind to particulates in wastewater, an analysis of the interstage removal of TSS (kg) and SARS-CoV-2 (LOG GC) genetic material at the Cranfield Campus WWTW was conducted. Figure 10A and 10B show the results of this analysis for the TSS and N1 gene copy data, respectively. The results of the Kruskall-Wallis 1-way ANOVA test indicate that there is a statistically significant difference between the daily loading of the TSS across the WWTW ($p < 0.01$). The Post-hoc Dunn's test for pairwise comparisons revealed that the only statistically significant difference between the daily loading at the interstage level was between the Post-Secondary Trickling Filter and the Final Effluent ($p < 0.01$). The results of the Kruskall-Wallis 1-way ANOVA test indicate that there is not a statistically significant difference between the daily loadings of N1 gene copies at the interstage level of the on Campus WWTW ($p > 0.05$). This suggests that removal of SARS-CoV-2 does not occur at the Cranfield Campus WWTW. This contradicts what is reported in Randazzo et al., (2020). However, the Cranfield Campus WWTW does not use an activated sludge process as part of the treatment train unlike the WWTW sampled from in Randazzo et al. (2020). There is currently a significant lack of data regarding the removal of SARS-CoV-2 and other enveloped viruses in processes such as trickling filters and remote, rural, and remotely operated WWTW. This may explain why removal of SARS-CoV-2 did not occur in this study, as the biological processes of the activated sludge process likely enhanced the removal of SARS-CoV-2 genetic material in Randazzo et al. (2020). Whilst this may be concerning, studies have reported that SARS-CoV-2 is rapidly inactivated in wastewater matrices (Ahmed et al., 2020b). Thus, as there have yet to be confirmed cases of infection spread via the faecal-oral route, it is assumed that the risk of infection from SARS-CoV-2 from wastewater is minimal (Mohapatra et al., 2020; Rimoldi et al., 2020; WHO, 2020).

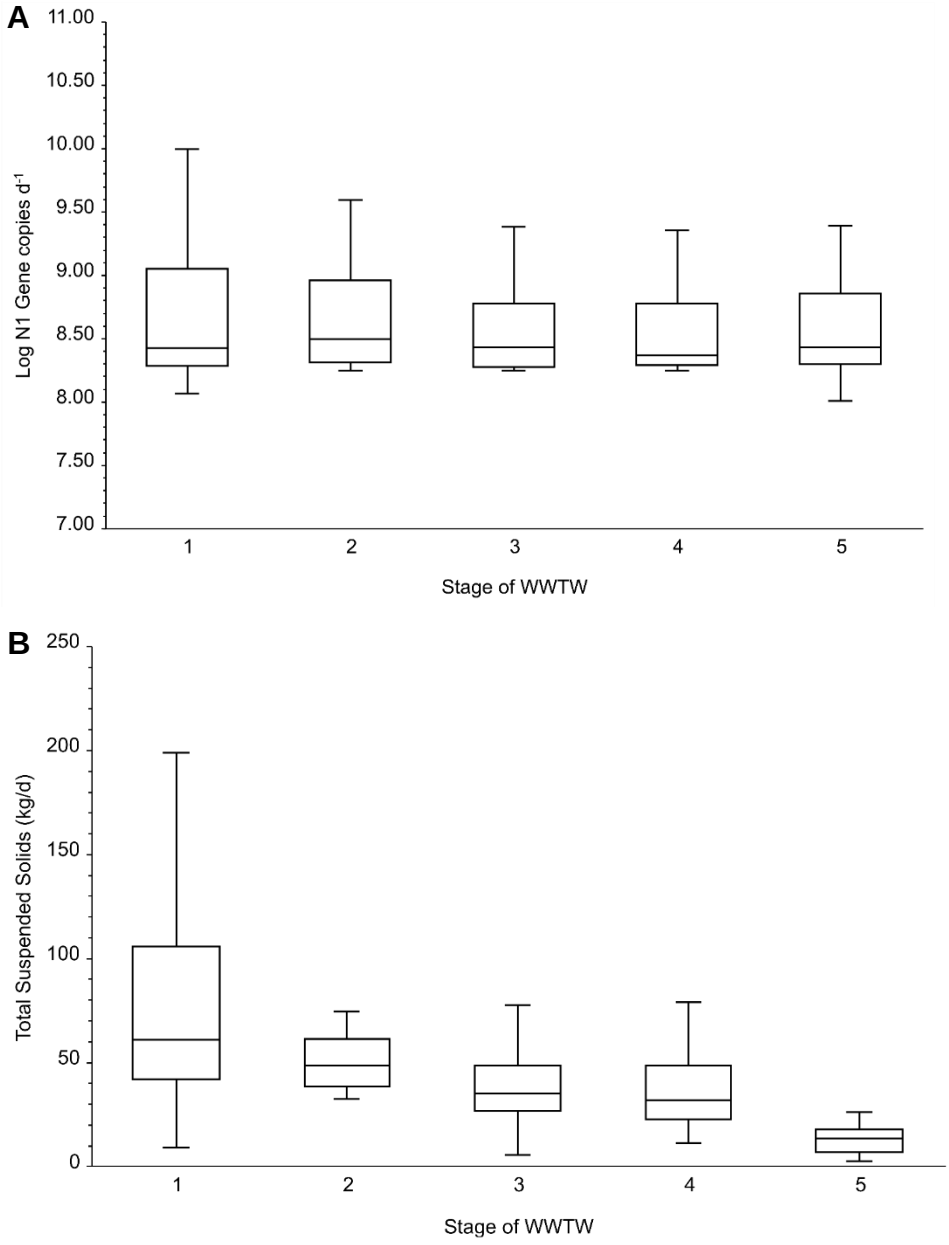

Figure 10: A - TSS (kg/d) at different stages of the WWTW; B - LOG N1 gene copies d^{-1} at different stages of the WWTW. Outliers removed. Stages are 1: Inlet, 2: Post-Primary Lamella, 3: Post-Roughing Trickling Filter, Post-Secondary Trickling Filter and 5: Final Effluent.

3.2 Uncertainties and assumptions during WBS studies

This study also encountered several areas of uncertainty that other researchers have previously highlighted in WBS. Different uncertainties and their magnitudes exist depending on the spatial scale at which WBS is conducted. What makes WBS so challenging to implement effectively is the inherent uncertainty of wastewater. However, the benefits of utilising WBS as a method of estimating and managing SARS-CoV-2 infections (and potentially in future pandemics) make for a strong case of understanding, minimising and/or overcoming uncertainty to develop effective WBS programs.

According to Wade et al. (2022), uncertainties can be broadly defined as “observable” and “partially-observable”. Observable uncertainties are those that are readily measurable or can be estimated with moderate to high confidence, and consist of factors such as: population, sampling, and virus quantification. Partially observable uncertainties are those that cannot be readily measurable and/or confidence in estimates is less than moderate, but can be confidently assumed or modelled, and consist of factors such as: sewerage characteristics, the behaviour of SARS-CoV-2 in wastewaters and faecal shedding of SARS-CoV-2.

3.2.1 Observable sources of uncertainty

As discussed previously, population is a source of uncertainty in WBS. For example, this study was unable to confidently calculate the sensitivity and specificity of the WBS system owing to uncertainties regarding the actual number of people contributing to the wastewater stream. Without tracking populations and monitoring toilet flushes (a potentially problematic ethical situation), it is not possible to know the exact number of people and their respective contributions to the wastewater stream. It is possible to estimate the contributing population by using flow data, but this is often only available at the WWTW level as in the case of this study. It is often challenging to measure flow in sewers, particularly at the Near-Source level. Though the challenges presented typically depend on the device used to measure the flow, the following presents a brief summary of the most common issues faced reported by Sun et al. (2021): difficulty in installing

monitoring equipment, difficulty in retrieving data from monitoring equipment, variable properties of domestic waters between different sites, flushed debris clogging equipment, clogging of pipes disrupting flow, corrosivity of wastewater and the difficulty caused by highly variable flows. To combat these issues, Sun et al. (2021) recommends the use of a “portable, multi-functional flowmeter”. This study intended to use the portable SQ-R Non-Contact Flowmeter (Sommer Messtechnik, Austria). This flowmeter is highly specialist and designed to operate in a variety of sampling environments, even in confined sewers, and overcomes the challenges listed above. Sadly, due to the short nature of this study the expense could not be justified. Thus, flow could not be calculated for the Near-Source and In-Sewer locations.

Where flow data cannot be obtained, biomarkers can instead be used to normalise the gene copies. Different biomarkers have advantages and disadvantages. For example, whilst ammonia and phosphate tests can be conducted rapidly, their concentrations in the wastewater can be influenced by the introduction of other non-biological sources e.g., detergents from cleaning. Viral biomarkers, such as PMMoV and crAssphage also confer significant advantages e.g., highly specificity and resistance to outside influences, their longer processing times in comparison to standard wastewater parameter tests may make them less useful (Symonds et al., 2018). For RT-qPCR to be sufficiently accurate for use in public health interventions, quality control and assurances must be built into the methods used to quantify SARS-CoV-2 abundance in wastewater. Ahmed et al. (2022) provide an extensive review of how to minimise errors in SARS-CoV-2 quantification. In short, for WBS for SARS-CoV-2 to be effective, robust, and streamlined laboratory practices must be implemented. A specific problem is created by the need to quantify several viruses, which can occur when using viral surrogates as extraction controls. For optimum turn-around time, these viruses would need to be quantified simultaneously. This could be achieved by parallel multiplex RT-qPCR reactions (either in different machines, or on the same plate should different programmable zones exist). Multiplexing typically allows 2-6 gene targets to be quantified simultaneously in the same tube (Hirschhorn et al., 2022). Multiplex RT-qPCR

reactions would need to be designed to quantify several viruses that are accurate and rapid enough to be of use in interventions. This would be challenging due to the nature of multiplex RT-qPCR reactions, such as the need to prevent the formation of primer-dimers and allow for optimum melting curves (Hirschhorn et al., 2022). These factors show that, whilst nucleic acid based biomarkers have significant advantages over using standard wastewater parameters for gene copy normalisation, the significant capital costs, skilled individuals, and time involved may not be appropriate in all scenarios, especially in low-income countries where the low-cost of WBS is especially attractive and useful (Liu et al., 2022). In these situations, less technically challenging nucleic acid amplifications, such as Loop-Mediated Isothermal Amplification, may be appropriate (Bivins et al., 2021). Thus, it can clearly be seen that estimating contributing population is an area fraught with uncertainty, and the different methods to reduce uncertainty in population themselves present areas for uncertainty and error.

Sampling regime is also a source of uncertainty in WBS. According to Ort et al. (2010), sampling regime strongly influences the representativeness and usefulness of a sample taken. At the near-source level well-timed grab samples can coincide with flush events, resulting in highly concentrated but unrepresentative samples. In contrast, composite samples are assumed to be more representative than grab samples but, depending on how the composites are collected, can be significantly more diluted than grab samples. Composite sampling regime is dependent on the site conditions (e.g., availability of power) and the equipment (e.g., volume of composite container). Ahmed, Bivins, et al. (2020) recommend that, where possible, a flow-weighted composite should be collected. Flow-weighted composite sampling regimes are more capable of dealing with the fluctuations of flow at different spatial scales. However, this is only possible in contexts where it is possible to monitor flow which, as previously stated, is very challenging as sewerage infrastructure may not enable it. Where a flow-weighted composite sampling regime is not possible, a more intensive sampling regime is an appropriate strategy. Ahmed, Bivins, et al. (2020) recommend collecting a sample every 10-15 minutes, especially when attempting to detect the shedding of SARS-CoV-2 in areas where the case rate is low. This

highlights the critical role of infrastructure and technology in WBS studies. Sampling is limited by being able to access the sewer, and how one can sample. It is therefore essential to develop robust and flexible sampling strategies that can reliably obtain representative samples in multiple scenarios.

The above can largely be measured for or confidently estimated and accounted in studies, and thus the influence on uncertainty can be estimated with confidence when interpreting results.

3.2.2 Partially observable sources of uncertainty

There is still debate regarding the influence of faecal shedding dynamics and the subsequent impact on detection of SARS-CoV-2 in the wastewater, due in part to the inconsistencies of investigation in medical literature. As shown in Table 2, there exists significant variation amongst reported shedding dynamics in studies that have investigated it, and a lack of studies investigating the gene copies shed in the faces. Reported incidences of detection of SARS-CoV-2 genetic material in patients range from as little as 15.3% to as high as 100%. The duration of faecal shedding has also shown considerable variation, with duration appearing to be influenced by severity of infection (Chen et al., 2020). Faecal shedding has been reported in patients that have recovered from SARS-CoV-2 infections and in those who are no longer considered infectious. However, much of these studies were conducted in the early stages of the pandemic, prior to the emergence of VoCs such as Delta and Omicron, and almost exclusively on hospital patients in small cohorts. Thus, there is little data available regarding faecal shedding of SARS-CoV-2 for pre- and asymptomatic infections. Whilst respiratory tract viral loads have been shown to be similar between pre-Alpha, Alpha and Delta SARS-CoV-2 variants, and people of different vaccination status (Singanayagam et al., 2021), it is currently unknown how faecal shedding varies between VoC infection and vaccination status but is has been hypothesised that viral loads in the faeces of vaccinated individuals will be lower (Thompson et al., 2021).

It is currently not known how SARS-CoV-2 behaves in wastewater matrices. A study by Shi et al. (2021) in model sewers reported that surrogates of coronaviruses rapidly decayed in wastewater, achieving 99% decay in 30 hours.

Kevill et al. (2022) investigated, amongst other factors, the influence of turbidity and surfactant concentration on the recovery of SARS-CoV-2 from wastewater samples and reported a statistically significant decrease in virus recovery from samples with increasing turbidity and/or surfactant concentration. Finally, different sewers show considerable differences in their in-network characteristics and can have multiple characteristics throughout the network (Wade et al., 2022). To date, no studies have investigated the behaviour nor recovery of SARS-CoV-2 or surrogates in sewers under different conditions.

All of this considered, it is challenging to relate SARS-CoV-2 gene copies in wastewater with on Campus COVID-19 infections when the combined impact of faecal shedding dynamics, sewer conditions and characteristics, wastewater matrix composition, population size and dynamics, sampling regime and SARS-CoV-2 extraction and quantification techniques, resident population and stakeholder involvement and communication is as of yet uncertain (Wade et al., 2022). This is, however, beyond the scope of this study. The usefulness of WBS can only be as good as the strength of the assumptions that are made in the design of the study, and the decisions that are made during interpretation of the data generated. Improperly designed WBS projects can potentially lead to unhelpful public health interventions. Thus, further research is needed to address these areas of uncertainty to improve the efficacy of WBS for SARS-CoV-2 management and for potential future pandemics.

4 Conclusions

The severity of the SARS-CoV-2 pandemic has catalysed significant interest in the use of WBS in the monitoring of disease burden, both in the current potentially in future pandemics. Clinical tests have proven to be unsuitable for managing the spread of such an infectious disease. They are not appropriate for all to use , are only effective when sufficient numbers of people adhere to a testing programme, and are expensive, challenging to scale, may not reliably identify asymptomatic individuals and not rapid enough to test large populations to control outbreaks of disease. The successful isolation and quantification of SARS-CoV-2 from wastewater led to the investigation of wastewater-based surveillance for use in disease management. Studies have since proven its usefulness to test large numbers of people in an ethically acceptable manner. Previous studies have investigated WBS's usefulness in different contexts and different spatial scales: schools, hospitals, University buildings and campuses and WWTW catchments have all been studied. To the best knowledge of the authors of this paper, this is the first study to investigate the use of WBS at different scales within a single connected WWTW drainage basin for this length of time. Table 11, below, summarises the objectives, related hypotheses, and findings in this study.

Table 11: Summary of study objectives, hypotheses, and results

Objective	Hypothesis	Results
Quantify SARS-CoV-2 gene copies from wastewater at three scales on campus samples.	WBS can identify magnitude of SARS-CoV-2 infections on the University Campus.	Study did not find a correlation between cases and concentration in the wastewater, but it was possible to identify which sewersheds had people shedding SARS-CoV-2 in their faeces
Establish SARS-CoV-2 gene copy abundance correlation to wastewater constituents.	SARS-CoV-2 with wastewater constituents positive correlates at near-source level not WTWW	Statistically significant correlations were found between N1 GC, and ammonium, TSS and tCOD at the Near-Source and In-Sewer level
Establish SARS-CoV-2 gene copy concordance with COVID-19 infections on campus	SARS-CoV-2 wastewater samples will be positively related with on-campus infections	This study found that the wastewater SARS-CoV-2 GC data was a leading indicator of infections
Isolate SARS-CoV-2 whole genomes from wastewater	Wastewater SARS-CoV-2 WBS can identify variants of concern on campus	2 samples were successfully identified as containing Alpha-variant SARS-CoV-2
Verify removal of SARS-CoV-2 RNA fragments from a small Trickling Filter WWTW treating water exclusively from campus population	Small linked WWTW removes SARS-CoV-2 RNA fragments from wastewater	On campus WWTW did not remove SARS-CoV-2 RNA fragments. SARS-CoV-2 RNA was frequently detected in the WWTW effluent.

This study found that it was feasible to identify in which sewershed infected individuals were on the campus. In contrast to other studies, there was not statistically significant positive correlation between normalised N1 gene copies in the wastewater at the WWTW and clinically confirmed campus SARS-CoV-2 infections. Other studies have highlighted the utility of WBS in identifying cases within a defined geographic area and potential for estimating the number of infected individuals contributing to the sample. This study has shown that it is critical to have real-time estimates of the numbers of infected people in a sewershed to ensure that data is accurate and reliable, thereby allowing relationships between cases and SARS-CoV-2 abundance in wastewaters to be firmly established. There were statistically significant positive correlations between N1 SARS-CoV-2 gene copies and wastewater TSS, tCOD and ammonia at the near-source spatial scale, but there was not a statistically significant correlation between N1 SARS-CoV-2 GC and phosphate in the wastewater at the same spatial scale. Campus cases appeared to be much lower than estimated using LTLA PCR positive rate if based solely on clinical testing, but the wastewater data suggests that SARS-CoV-2 was present and circulating the campus for much of the study period. Cases also appeared to lag behind the LTLA rate, likely due to the impacts of lockdown and the isolated nature of the campus. However, when national and local restrictions relaxed, cases rose in line with the LTLA rate. Current treatments at the on campus do not appear to be removing SARS-CoV-2 RNA from the wastewater, despite removing solids. This study has shown that, when combined with clinical testing, WBS can be a highly effective means of identifying infected individuals in a low-cost and rapid way.

This study has also identified several other factors that are essential for effective WBS:

- Research is urgently needed to assess how faecal shedding of SARS-CoV-2 in stools varies between pre-symptomatic, asymptomatic, vaccination status, variant infection, and demographic factors
- Stakeholders must be positively engaged throughout the process to allow the most accurate data to be collected, and to allow for effective coordination to facilitate useful interventions.
- Optimisation of sample processing is critical to ensure sufficiently rapid throughput of samples and minimisation of errors to enable effective public health interventions
- Further research is needed to assess the behaviour of SARS-CoV-2 in different wastewater matrices to elucidate how this impacts the relationship between gene copies in wastewater and cases within the sewershed

Overall, the use of WBS shows promise as a method to compliment clinical testing as a method to rapidly and inexpensively testing large numbers of people in an ethically acceptable manner. It warrants further research to address areas of uncertainty, and so develop a tool to help direct public health officials in designing effective public health interventions to limit the spread of disease Finally, the infrastructure and skills created as a result of the many WBS conducted during the pandemic can be utilised for future health and disease monitoring, and more research should be conducted to establish the limits of the usefulness of WBS for public health purposes.

5 Further research

This work has shown that WBS shows promise in supporting clinical testing by rapidly testing large numbers of potentially infected individuals. This allows for targeted clinical testing and provides public health officials with early warnings for potential new outbreaks, assisting them in their decision-making processes for public health interventions.

For WBS for SARS-CoV-2 to be fully effective further research is needed to assess, minimise and in some cases remove areas of uncertainty. Of these, the most critical is faecal shedding dynamics. There is still a lack of robust data that analyses faecal shedding across and between large cohorts of different characteristics. It is not yet known how, or if, faecal shedding varies between different SARS-CoV-2 variants, how vaccination status influences shedding, how shedding dynamics vary based on pre or asymptomatic infections and disease severity. Furthermore, another challenge exists in establishing if the concentration of SARS-CoV-2 in a wastewater sample is due to a few individuals who are shedding a very high amount of SARS-CoV-2 in their stools (e.g., 1 $x10^6$ N1 GC g^{-1} wet faeces), many individuals that are shedding low amounts of SARS-CoV-2 (e.g. 1 $x10^2$ N1 GC g^{-1} wet faeces) or mixtures of these combinations. There is currently a lack of understanding how SARS-CoV-2, and other enveloped viruses, behave in different wastewaters (e.g., what is their rate of inactivation?, how do they partition in the liquid and solids fractions? etc.). These factors, amongst others, affect the abundance of SARS-CoV-2 in samples. Fortunately, robust protocols to calculate the abundance of SARS-CoV-2 gene copies in samples are well established in the literature already, with strategies for optimising and streamlining laboratory analysis of samples beginning to be established. However, it is critical to develop a robust standardised protocol with built in process controls throughout the analysis procedure for SARS-CoV-2 analysis from wastewater and ensure consistency in reporting results in the literature. Without robust understanding of shedding dynamics of SARS-CoV-2, its behaviour in wastewater, estimations of the number of infected people contributing to a wastewater sample derived from gene copies contained therein

will not be sufficiently accurate. This has the potential of over or underestimating the number of infected people contributing to a wastewater sample, potentially leading to ineffective, unnecessary, or deleterious public health interventions. Finally, more research should also be conducted to evaluate the utility of WBS for monitoring health and disease prevalence, and also for preparedness for future potential pandemics.

REFERENCES

Adhikari, U. das, Eng, G., Farcasanu, M., Avena, L.E., Choudhary, M.C., Triant, V.A., Flagg, M., Schiff, A.E., Gomez, I., Froehle, L.M., Diefenbach, T.J., Ronsard, L., Lingwood, D., Lee, G.C., Rabi, S.A., Erstad, D., Velmahos, G., Li, J.Z., Hodin, R., Stone, J.R., Honko, A.N., Griffiths, A., Yilmaz, Ö.H., Kwon, D.S., 2021. Fecal Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-Cov-2) RNA Is Associated With Decreased Coronavirus Disease 2019 (COVID-19) Survival. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America. https://doi.org/10.1093/CID/CIAB623

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., Choi, P.M., Kitajima, M., Simpson, S.L., Li, J., Tscharke, B., Verhagen, R., Smith, W.J.M., Zaugg, J., Dierens, L., Hugenholtz, P., Thomas, K. v., Mueller, J.F., 2020a. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. Science of The Total Environment 728, 138764. https://doi.org/10.1016/J.SCITOTENV.2020.138764

Ahmed, W., Bertsch, P.M., Bibby, K., Haramoto, E., Hewitt, J., Huygens, F., Gyawali, P., Korajkic, A., Riddell, S., Sherchan, S.P., Simpson, S.L., Sirikanchana, K., Symonds, E.M., Verhagen, R., Vasan, S.S., Kitajima, M., Bivins, A., 2020b. Decay of SARS-CoV-2 and surrogate murine hepatitis virus RNA in untreated wastewater to inform application in wastewater-based epidemiology. Environmental Research 191. https://doi.org/10.1016/j.envres.2020.110092

Ahmed, W., Bivins, A., Bertsch, P.M., Bibby, K., Choi, P.M., Farkas, K., Gyawali, P., Hamilton, K.A., Haramoto, E., Kitajima, M., Simpson, S.L., Tandukar, S., Thomas, K. v., Mueller, J.F., 2020c. Surveillance of SARS-CoV-2 RNA in wastewater: Methods optimization and quality control are crucial for generating reliable public health information. Current Opinion in Environmental Science and Health. https://doi.org/10.1016/j.coesh.2020.09.003

Ahmed, W., Simpson, S.L., Bertsch, P.M., Bibby, K., Bivins, A., Blackall, L.L., Bofill-Mas, S., Bosch, A., Brandão, J., Choi, P.M., Ciesielski, M., Donner, E., D'Souza, N., Farnleitner, A.H., Gerrity, D., Gonzalez, R., Griffith, J.F., Gyawali, P., Haas, C.N., Hamilton, K.A., Hapuarachchi, H.C., Harwood, V.J., Haque, R., Jackson, G., Khan, S.J., Khan, W., Kitajima, M., Korajkic, A., la Rosa, G., Layton, B.A., Lipp, E., McLellan, S.L., McMinn, B., Medema, G., Metcalfe, S., Meijer, W.G., Mueller, J.F., Murphy, H., Naughton, C.C., Noble, R.T., Payyappat, S., Petterson, S., Pitkänen, T., Rajal, V.B., Reyneke, B., Roman, F.A., Rose, J.B., Rusiñol, M., Sadowsky, M.J., Sala-Comorera, L., Setoh, Y.X., Sherchan, S.P., Sirikanchana, K., Smith, W., Steele, J.A., Subburg, R., Symonds, E.M., Thai, P., Thomas, K. v., Tynan, J., Toze, S., Thompson, J., Whiteley,

A.S., Wong, J.C.C., Sano, D., Wuertz, S., Xagoraraki, I., Zhang, Q., Zimmer-Faust, A.G., Shanks, O.C., 2022. Minimizing errors in RT-PCR detection and quantification of SARS-CoV-2 RNA for wastewater surveillance. Science of The Total Environment 805, 149877. https://doi.org/10.1016/J.SCITOTENV.2021.149877

Ai, Y., Davis, A., Jones, D., Lemeshow, S., Tu, H., He, F., Ru, P., Pan, X., Bohrerova, Z., Lee, J., 2021. Wastewater SARS-CoV-2 monitoring as a community-level COVID-19 trend tracker and variants in Ohio, United States. The Science of the Total Environment 801, 149757. https://doi.org/10.1016/J.SCITOTENV.2021.149757

Al-Qahtani, M., AlAli, S., AbdulRahman, A.K., Salman Alsayyad, A., Otoom, S., Atkin, S.L., 2021. The prevalence of asymptomatic and symptomatic COVID-19 in a cohort of quarantined subjects. International Journal of Infectious Diseases 102, 285–288. https://doi.org/10.1016/j.ijid.2020.10.091

Amirian, E.S., 2020. Potential fecal transmission of SARS-CoV-2: Current evidence and implications for public health. International Journal of Infectious Diseases 95, 363. https://doi.org/10.1016/J.IJID.2020.04.057

Amoah, I.D., Kumari, S., Bux, F., 2020. Coronaviruses in wastewater processes: Source, fate and potential risks. Environment International 143, 105962. https://doi.org/10.1016/J.ENVINT.2020.105962

Barich, D., Slonczewski, J.L., 2021. Wastewater Virus Detection Complements Clinical COVID-19 Testing to Limit Spread of Infection at Kenyon College. medRxiv 2021.01.09.21249505. https://doi.org/10.1101/2021.01.09.21249505

Bar-Or, I., Weil, M., Indenbaum, V., Bucris, E., Bar-Ilan, D., Elul, M., Levi, N., Aguvaev, I., Cohen, Z., Shirazi, R., Erster, O., Sela-Brown, A., Sofer, D., Mor, O., Mendelson, E., Zuckerman, N.S., 2021. Detection of SARS-CoV-2 variants by genomic analysis of wastewater samples in Israel. Science of The Total Environment 789, 148002. https://doi.org/10.1016/J.SCITOTENV.2021.148002

Been, F., Bastiaensen, M., Lai, F.Y., van Nuijs, A.L.N., Covaci, A., 2017. Liquid Chromatography-Tandem Mass Spectrometry Analysis of Biomarkers of Exposure to Phosphorus Flame Retardants in Wastewater to Monitor Community-Wide Exposure. Analytical Chemistry 89, 10045–10053. https://doi.org/10.1021/ACS.ANALCHEM.7B02705/SUPPL_FILE/AC7B02705_SI_001.PDF

Been, F., Rossi, L., Ort, C., Rudaz, S., Delémont, O., Esseiva, P., 2014. Population normalization with ammonium in wastewater-based epidemiology: Application to illicit drug monitoring. Environmental Science and Technology 48, 8162–8169.

https://doi.org/10.1021/ES5008388/SUPPL_FILE/ES5008388_SI_001.PDF

Berchenko, Y., Manor, Y., Freedman, L.S., Kaliner, E., Grotto, I., Mendelson, E., Huppert, A., 2017. Estimation of polio infection prevalence from environmental surveillance data. Science Translational Medicine 9. https://doi.org/10.1126/scitranslmed.aaf6786

Betancourt, W.Q., Schmitz, B.W., Innes, G.K., Prasek, S.M., Pogreba Brown, K.M., Stark, E.R., Foster, A.R., Sprissler, R.S., Harris, D.T., Sherchan, S.P., Gerba, C.P., Pepper, I.L., 2021. COVID-19 containment on a college campus via wastewater-based epidemiology, targeted clinical testing and an intervention. The Science of the Total Environment 779, 146408. https://doi.org/10.1016/J.SCITOTENV.2021.146408

Bhattarai, B., Sahulka, S.Q., Podder, A., Hong, S., Li, H., Gilcrease, E., Beams, A., Steed, R., Goel, R., 2021. Prevalence of SARS-CoV-2 genes in water reclamation facilities: From influent to anaerobic digester. Science of The Total Environment 796, 148905. https://doi.org/10.1016/J.SCITOTENV.2021.148905

Bivins, A., Bibby, K., 2021. Wastewater Surveillance during Mass COVID-19 Vaccination on a College Campus. Environmental Science and Technology Letters 8, 792–798. https://doi.org/10.1021/ACS.ESTLETT.1C00519/SUPPL_FILE/EZ1C00519_SI_001.PDF

Bivins, A., Lott, M., Shaffer, M., Wu, Z., North, D., Lipp, E.K., Bibby, K., 2021. Building-level wastewater surveillance using tampon swabs and RT-LAMP for rapid SARS-CoV-2 RNA detection. Environmental Science: Water Research & Technology 8, 173–183. https://doi.org/10.1039/D1EW00496D

Brockett, J., 2019. Water Consumption: PR19 Challenge Report #5. London.

Bruns, D.P., Kraguljac, N.V., Bruns, T.R., 2020. COVID-19: Facts, Cultural Considerations, and Risk of Stigmatization. Journal of Transcultural Nursing 31, 326–332. https://doi.org/10.1177/1043659620917724

Burki, T.K., 2022. Omicron variant and booster COVID-19 vaccines. The Lancet Respiratory Medicine 10, e17. https://doi.org/10.1016/S2213-2600(21)00559-2

Campbell, F., Archer, B., Laurenson-Schafer, H., Jinnai, Y., Konings, F., Batra, N., Pavlin, B., Vandemaele, K., van Kerkhove, M.D., Jombart, T., Morgan, O., de Waroux, O.L.P., 2021. Increased transmissibility and global spread of SARSCoV- 2 variants of concern as at June 2021. Eurosurveillance 26, 1–6. https://doi.org/10.2807/1560-7917.ES.2021.26.24.2100509/CITE/PLAINTEXT

Castiglioni, S., Thomas, K. v., Kasprzyk-Hordern, B., Vandam, L., Griffiths, P., 2014. Testing wastewater to detect illicit drugs: State of the art, potential and research needs. Science of The Total Environment 487, 613–620. https://doi.org/10.1016/J.SCITOTENV.2013.10.034

Castro Gutierrez, V., Hassard, F., Vu, M., Leitao, R., Burczynska, B., Wildeboer, D., Stanton, I., Rahimzadeh, S., Baio, G., Garelick, H., Hofman, J., Kasprzyk-Hordern, B., Kwiatkowska, R., Majeed, A., Priest, S., Grimsley, J., Lundy, L., Singer, A.C., di Cesare, M., 2021. Monitoring occurrence of SARS-CoV-2 in school populations: a wastewater-based approach. medRxiv. https://doi.org/10.1101/2021.03.25.21254231

Chen, Y., Chen, L., Deng, Q., Zhang, G., Wu, K., Ni, L., Yang, Y., Liu, B., Wang, W., Wei, C., Yang, J., Ye, G., Cheng, Z., 2020. The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. Journal of Medical Virology 92, 833–840. https://doi.org/10.1002/JMV.25825

Choi, B.C.K., 2012. The past, present, and future of public health surveillance. Scientifica 2012, 1–26. https://doi.org/10.6064/2012/875253

Choi, P.M., Tscharke, B., Samanipour, S., Hall, W.D., Gartner, C.E., Mueller, J.F., Thomas, K. v., O'Brien, J.W., 2019. Social, demographic, and economic correlates of food and chemical consumption measured by wastewater-based epidemiology. Proceedings of the National Academy of Sciences of the United States of America 116, 21864–21873. https://doi.org/10.1073/PNAS.1910242116/-/DCSUPPLEMENTAL

Colosi, L.M., Barry, K.E., Kotay, S.M., Porter, M.D., Poulter, M.D., Ratliff, C., Simmons, W., Steinberg, L.I., Wilson, D.D., Morse, R., Zmick, P., Mathers, A.J., 2021. Development of Wastewater Pooled Surveillance of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) from Congregate Living Settings. Applied and Environmental Microbiology 87, 1–15. https://doi.org/10.1128/AEM.00433-21/ASSET/91DC28D9-AD14-4866-B743-CC4CDC4FF29E/ASSETS/IMAGES/LARGE/AEM.00433-21-F001.JPG

Corchis-Scott, R., Geng, Q., Seth, R., Ray, R., Beg, M., Biswas, N., Charron, L., Drouillard, K.D., D'Souza, R., Heath, D.D., Houser, C., Lawal, F., McGinlay, J., Menard, S.L., Porter, L.A., Rawlings, D., Scholl, M.L., Siu, K.W.M., Tong, Y., Weisener, C.G., Wilhelm, S.W., McKay, R.M.L., 2021. Averting an Outbreak of SARS-CoV-2 in a University Residence Hall through Wastewater Surveillance. Microbiology Spectrum 9. https://doi.org/10.1128/SPECTRUM.00792-21

Crits-Christoph, A., Kantor, R.S., Olm, M.R., Whitney, O.N., Al-Shayeb, B., Lou, Y.C., Flamholz, A., Kennedy, L.C., Greenwald, H., Hinkle, A., Hetzel, J., Spitzer, S., Koble, J., Tan, A., Hyde, F., Schroth, G., Kuersten, S., Banfield, J.F., Nelson, K.L., 2021. Genome sequencing of sewage detects regionally prevalent SARS-CoV-2 variants. mBio 12, 1–

9. https://doi.org/10.1128/MBIO.02703-20/ASSET/017A9265-9EFF-470B-8933-56CEB70D705C/ASSETS/GRAPHIC/MBIO.02703-20-F0003.JPEG

D'Aoust, P.M., Graber, T.E., Mercier, E., Montpetit, D., Alexandrov, I., Neault, N., Baig, A.T., Mayne, J., Zhang, X., Alain, T., Servos, M.R., Srikanthan, N., MacKenzie, M., Figeys, D., Manuel, D., Jüni, P., MacKenzie, A.E., Delatolla, R., 2021a. Catching a resurgence: Increase in SARS-CoV-2 viral RNA identified in wastewater 48 h before COVID-19 clinical tests and 96 h before hospitalizations. Science of The Total Environment 770, 145319. https://doi.org/10.1016/J.SCITOTENV.2021.145319

D'Aoust, P.M., Mercier, E., Montpetit, D., Jia, J.J., Alexandrov, I., Neault, N., Baig, A.T., Mayne, J., Zhang, X., Alain, T., Langlois, M.A., Servos, M.R., MacKenzie, M., Figeys, D., MacKenzie, A.E., Graber, T.E., Delatolla, R., 2021b. Quantitative analysis of SARS-CoV-2 RNA from wastewater solids in communities with low COVID-19 incidence and prevalence. Water Research 188, 116560. https://doi.org/10.1016/J.WATRES.2020.116560

David Ainslie, Caleb Ogwuru, Rebecca Sinclair, 2021. Coronavirus and vaccine hesitancy, Great Britain - Office for National Statistics. London.

Delamater, P.L., Street, E.J., Leslie, T.F., Yang, Y.T., Jacobsen, K.H., 2019. Complexity of the Basic Reproduction Number (R0) - Volume 25, Number 1—January 2019 - Emerging Infectious Diseases journal - CDC. Emerging Infectious Diseases 25, 1–4. https://doi.org/10.3201/EID2501.171901

Droste, R.L., Gehr, R.L., 2019. Theory and practice of water and wastewater treatment, 2nd ed. Wiley, Hoboken.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32, 1792–1797. https://doi.org/10.1093/NAR/GKH340

European Centre for Disease Prevention and Control, 2021. Considerations on the use of self-tests for COVID-19 in the EU/EEA Key messages Scope of this document, ECDC T. Solna.

Fahrenfeld, N.L., Morales Medina, W.R., D'Elia, S., Modica, M., Ruiz, A., McLane, M., 2022. Comparison of residential dormitory COVID-19 monitoring via weekly saliva testing and sewage monitoring. The Science of the Total Environment 814, 151947. https://doi.org/10.1016/J.SCITOTENV.2021.151947

Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball, L.A. (Eds.), 2005. Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses, Virus Taxonomy. Academic Press, San Diego.

Felsenstein, J., 1985. CONFIDENCE LIMITS ON PHYLOGENIES: AN APPROACH USING THE BOOTSTRAP. Evolution 39, 783–791. https://doi.org/10.1111/J.1558-5646.1985.TB00420.X

Fielding-Miller, R., Karthikeyan, S., Gaines, T., Garfein, R.S., Salido, R., Cantu, V., Kohn, L., Martin, N.K., Wijaya, C., Flores, M., Omaleki, V., Majnoonian, A., Gonzalez-Zuniga, P., Nguyen, M., Vo, A. v, Le, T., Duong, D., Hassani, A., Dahl, A., Tweeten, S., Jepsen, K., Henson, B., Hakim, A., Birmingham, A., Mark, A.M., Nasamran, C.A., Rosenthal, S.B., Moshiri, N., Fisch, K.M., Humphrey, G., Farmer, S., Tubb, H.M., Valles, T., Morris, J., Kang, J., Khaleghi, B., Young, C., Akel, A.D., Eilert, S., Eno, J., Curewitz, K., Laurent, L.C., Rosing, T., SEARCH, Knight, R., 2021. Wastewater and surface monitoring to detect COVID-19 in elementary school settings: The Safer at School Early Alert project. medRxiv 2021.10.19.21265226. https://doi.org/10.1101/2021.10.19.21265226

Foladori, P., Cutrupi, F., Segata, N., Manara, S., Pinto, F., Malpei, F., Bruni, L., la Rosa, G., 2020. SARS-CoV-2 from faeces to wastewater treatment: What do we know? A review. Science of the Total Environment 743. https://doi.org/10.1016/j.scitotenv.2020.140444

Gibas, C., Lambirth, K., Mittal, N., Juel, M.A.I., Barua, V.B., Roppolo Brazell, L., Hinton, K., Lontai, J., Stark, N., Young, I., Quach, C., Russ, M., Kauer, J., Nicolosi, B., Chen, D., Akella, S., Tang, W., Schlueter, J., Munir, M., 2021. Implementing building-level SARS-CoV-2 wastewater surveillance on a university campus. Science of The Total Environment 782, 146749. https://doi.org/10.1016/J.SCITOTENV.2021.146749

Giobbe, G.G., Bonfante, F., Jones, B.C., Gagliano, O., Luni, C., Zambaiti, E., Perin, S., Laterza, C., Busslinger, G., Stuart, H., Pagliari, M., Bortolami, A., Mazzetto, E., Manfredi, A., Colantuono, C., di Filippo, L., Pellegata, A.F., Panzarin, V., Thapar, N., Li, V.S.W., Eaton, S., Cacchiarelli, D., Clevers, H., Elvassore, N., de Coppi, P., 2021. SARS-CoV-2 infection and replication in human gastric organoids. Nature Communications 2021 12:1 12, 1–14. https://doi.org/10.1038/s41467-021-26762-2

Gonzalez, R., Curtis, K., Bivins, A., Bibby, K., Weir, M.H., Yetka, K., Thompson, H., Keeling, D., Mitchell, J., Gonzalez, D., 2020. COVID-19 surveillance in Southeastern Virginia using wastewater-based epidemiology. Water Research 186. https://doi.org/10.1016/j.watres.2020.116296

Goyal, R., Hotchkiss, J., Schooley, R.T., de Gruttola, V., Martin, N.K., 2021. Evaluation of SARS-CoV-2 transmission mitigation strategies on a university campus using an agent-based network model. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America 73, 1735–1741. https://doi.org/10.1093/CID/CIAB037

Guo, M., Tao, W., Flavell, R.A., Zhu, S., 2021. Potential intestinal infection and faecal–oral transmission of SARS-CoV-2. Nature Reviews Gastroenterology & Hepatology 2021 18:4 18, 269–283. https://doi.org/10.1038/s41575-021-00416-6

Hart, O.E., Halden, R.U., 2020. Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges. Science of the Total Environment 730. https://doi.org/10.1016/j.scitotenv.2020.138875

Harvey, W.T., Carabelli, A.M., Jackson, B., Gupta, R.K., Thomson, E.C., Harrison, E.M., Ludden, C., Reeve, R., Rambaut, A., Peacock, S.J., Robertson, D.L., 2021. SARS-CoV-2 variants, spike mutations and immune escape. Nature Reviews Microbiology 2021 19:7 19, 409–424. https://doi.org/10.1038/s41579-021-00573-0

Hassard, F., Lundy, L., Singer, A.C., Grimsley, J., di Cesare, M., 2021. Innovation in wastewater near-source tracking for rapid identification of COVID-19 in schools. The Lancet Microbe 2, e4–e5. https://doi.org/10.1016/S2666-5247(20)30193-2

Heijnen, L., Elsinga, G., de Graaf, M., Molenkamp, R., Koopmans, M.P.G., Medema, G., 2021. Droplet digital RT-PCR to detect SARS-CoV-2 signature mutations of variants of concern in wastewater. Science of The Total Environment 799, 149456. https://doi.org/10.1016/J.SCITOTENV.2021.149456

Heller, L., Mota, C.R., Greco, D.B., 2020. COVID-19 faecal-oral transmission: Are we asking the right questions? Science of The Total Environment 729, 138919. https://doi.org/10.1016/J.SCITOTENV.2020.138919

Hirschhorn, J., Schandl, C.A., Nolte, F.S., 2022. Polymerase Chain Reaction And Other Nucleic Acid Amplification Technology - ClinicalKey, in: McPherson, R.A., Pincus, M.R. (Eds.), Henry's Clinical Diagnosis and Management by Laboratory Methods. pp. 1387–1400.

Hirshon, J.M., 2000. The rationale for developing public health surveillance systems based on emergency department data. Academic emergency medicine : official journal of the Society for Academic Emergency Medicine 7, 1428–1432. https://doi.org/10.1111/J.1553-2712.2000.TB00503.X

HM Government, 2019. Contained and controlled: the UK's 20-year vision for antimicrobial resistance. London.

Hoffmann, T., Alsing, J., 2021. Faecal shedding models for SARS-CoV-2 RNA amongst hospitalised patients and implications for wastewater-based epidemiology. medRxiv 2021.03.16.21253603. https://doi.org/10.1101/2021.03.16.21253603

Hosseini, A., Hashemi, V., Shomali, N., Asghari, F., Gharibi, T., Akbari, M., Gholizadeh, S., Jafari, A., 2020. Innate and adaptive immune responses against coronavirus. Biomedicine & Pharmacotherapy 132, 110859. https://doi.org/10.1016/J.BIOPHA.2020.110859

Hu, Z., Grasso, D., 2005. WATER ANALYSIS | Chemical Oxygen Demand. Encyclopedia of Analytical Science: Second Edition 325–330. https://doi.org/10.1016/B0-12-369397-7/00663-4

Hwang, S.S., Lim, J., Yu, Z., Kong, P., Sefik, E., Xu, H., Harman, C.C.D., Kim, L.K., Lee, G.R., Li, H.B., Flavell, R.A., 2020. MRNA destabilization by BTG1 and BTG2 maintains T cell quiescence. Science 367, 1255–1260. https://doi.org/10.1126/SCIENCE.ABB2507/SUPPL_FILE/PAPV2.PDF

Izquierdo-Lara, R., Elsinga, G., Heijnen, L., Oude Munnink, B.B., Schapendonk, C.M.E., Nieuwenhuijse, D., Kon, M., Lu, L., Aarestrup, F.M., Lycett, S., Medema, G., Koopmans, M.P.G., de Graaf, M., 2021. Monitoring SARS-CoV-2 Circulation and Diversity through Community Wastewater Sequencing, the Netherlands and Belgium - Volume 27, Number 5—May 2021 - Emerging Infectious Diseases journal - CDC. Emerging Infectious Diseases 27, 1405–1415. https://doi.org/10.3201/EID2705.204410

Jafferali, M.H., Khatami, K., Atasoy, M., Birgersson, M., Williams, C., Cetecioglu, Z., 2021. Benchmarking virus concentration methods for quantification of SARS-CoV-2 in raw wastewater. Science of The Total Environment 755, 142939. https://doi.org/10.1016/J.SCITOTENV.2020.142939

Jahn, K., Dreifuss, D., Topolsky, I., Kull, A., Ganesanandamoorthy, P., Fernandez-Cassi, X., Bänziger, C., Devaux, A.J., Stachler, E., Caduff, L., Cariti, F., Corzón, A.T., Fuhrmann, L., Chen, C., Jablonski, K.P., Nadeau, S., Feldkamp, M., Beisel, C., Aquino, C., Stadler, T., Ort, C., Kohn, T., Julian, T.R., Beerenwinkel, N., 2021. Detection and surveillance of SARS-CoV-2 genomic variants in wastewater. medRxiv 2021.01.08.21249379. https://doi.org/10.1101/2021.01.08.21249379

Jones, D.L., Baluja, M.Q., Graham, D.W., Corbishley, A., McDonald, J.E., Malham, S.K., Hillary, L.S., Connor, T.R., Gaze, W.H., Moura, I.B., Wilcox, M.H., Farkas, K., 2020. Shedding of SARS-CoV-2 in feces and urine and its potential role in person-to-person transmission and the environment-based spread of COVID-19. Science of the Total Environment 749. https://doi.org/10.1016/j.scitotenv.2020.141364

Jukes, T.H., Canthor, C.R., 1969. Evolution of Protein Molecules. World journal of microbiology & biotechnology 30, 21–132. https://doi.org/10.1016/B978-1-4832-3211-9.50009-7

Kaplan, E.H., Wang, D., Wang, M., Malik, A.A., Zulli, A., Peccia, J., 2021. Aligning SARS-CoV-2 indicators via an epidemic model: application to

hospital admissions and RNA detection in sewage sludge. Health Care Management Science 24, 1. https://doi.org/10.1007/S10729-020-09525-1

Karthikeyan, S., Levy, J.I., Hoff, P. de, Humphrey, G., Birmingham, A., Jepsen, K., Farmer, S., Tubb, H.M., Valles, T., Tribelhorn, C.E., Tsai, R., Aigner, S., Sathe, S., Moshiri, N., Henson, B., Hakim, A., Baer, N.A., Barber, T., Belda-Ferre, P., Chacón, M., Cheung, W., Cresini, E.S., Eisner, E.R., Lastrella, A.L., Lawrence, E.S., Marotz, C.A., Ngo, T.T., Ostrander, T., Plascencia, A., Salido, R.A., Seaver, P., Smoot, E.W., McDonald, D., Neuhard, R.M., Scioscia, A.L., Satterlund, A.M., Simmons, E.H., Aceves, C.M., Anderson, Catelyn, Gangavarapu, K., Hufbauer, E., Kurzban, E., Lee, J., Matteson, N.L., Parker, E., Perkins, S.A., Ramesh, K.S., Robles-Sikisaka, R., Schwab, M.A., Spencer, E., Wohl, S., Nicholson, L., Mchardy, I.H., Dimmock, D.P., Hobbs, C.A., Bakhtar, O., Harding, A., Mendoza, A., Bolze, A., Becker, D., Cirulli, E.T., Isaksson, M., Barrett, K.M.S., Washington, N.L., Malone, J.D., Schafer, A.M., Gurfield, N., Stous, S., Fielding-Miller, R., Garfein, R., Gaines, T., Anderson, Cheryl, Martin, N.K., Schooley, R., Austin, B., Kingsmore, S.F., Lee, W., Shah, S., McDonald, E., Zeller, M., Fisch, K.M., Laurent, L., Yeo, G.W., Andersen, K.G., Knight, R., 2021a. Wastewater sequencing uncovers early, cryptic SARS-CoV-2 variant transmission. medRxiv 2021.12.21.21268143. https://doi.org/10.1101/2021.12.21.21268143

Karthikeyan, S., Nguyen, A., McDonald, D., Zong, Y., Ronquillo, N., Ren, J., Zou, J., Farmer, S., Humphrey, G., Henderson, D., Javidi, T., Messer, K., Anderson, C., Schooley, R., Martin, N.K., Knight, R., 2021b. Rapid, Large-Scale Wastewater Surveillance and Automated Reporting System Enable Early Detection of Nearly 85% of COVID-19 Cases on a University Campus. mSystems 6, 793–814. https://doi.org/10.1128/MSYSTEMS.00793-21/ASSET/031E9DD5-E2DF-43B4-A28A-692BC82488D9/ASSETS/IMAGES/MEDIUM/MSYSTEMS.00793-21-F003.GIF

Kevill, J.L., Pellett, C., Farkas, K., Brown, M.R., Bassano, I., Denise, H., McDonald, J.E., Malham, S.K., Porter, J., Warren, J., Evens, N.P., Paterson, S., Singer, A.C., Jones, D.L., 2022. A comparison of precipitation and filtration-based SARS-CoV-2 recovery methods and the influence of temperature, turbidity, and surfactant load in urban wastewater. Science of The Total Environment 808, 151916. https://doi.org/10.1016/J.SCITOTENV.2021.151916

Kitajima, M., Ahmed, W., Bibby, K., Carducci, A., Gerba, C.P., Hamilton, K.A., Haramoto, E., Rose, J.B., 2020. SARS-CoV-2 in wastewater: State of the knowledge and research needs. Science of The Total Environment 739, 139076. https://doi.org/10.1016/J.SCITOTENV.2020.139076

Kumar, S., Nyodu, R., Maurya, V.K., Saxena, S.K., 2020. Morphology, Genome Organization, Replication, and Pathogenesis of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Coronavirus Disease 2019 (COVID-19) 23. https://doi.org/10.1007/978-981-15-4814-7_3

Kwiatkowska, R., Ruhaak, A., Kasprzyk-Hordern, B., Hassard, F., Lundy, L., Cesare, M. di, Hickman, M., Singer, A., 2021. Wastewater-based epidemiology and group privacy: the elephant in the sewer? Environmental Science & Technology. https://doi.org/10.31219/OSF.IO/Q8RCD

la Rosa, G., Mancini, P., Bonanno Ferraro, G., Veneri, C., Iaconelli, M., Lucentini, L., Bonadonna, L., Brusaferro, S., Brandtner, D., Fasanella, A., Pace, L., Parisi, A., Galante, D., Suffredini, E., 2021. Rapid screening for SARS-CoV-2 variants of concern in clinical and environmental samples using nested RT-PCR assays targeting key mutations of the spike protein. Water Research 197, 117104. https://doi.org/10.1016/J.WATRES.2021.117104

Lago, P.M., Gary, H.E., Pérez, L.S., Cáceres, V., Olivera, J.B., Puentes, R.P., Corredor, M.B., Jímenez, P., Pallansch, M.A., Cruz, R.G., 2003. Poliovirus detection in wastewater and stools following an immunization campaign in Havana, Cuba. International journal of epidemiology 32, 772–777. https://doi.org/10.1093/IJE/DYG185

Langeveld, J., Schilperoort, R., Heijnen, L., Elsinga, G., Schapendonk, C.E.M., Fanoy, E., Schepper, E.I.T. de, Koopmans, M.P.G., Graaf, M. de, Medema, G., 2021. Normalisation of SARS-CoV-2 concentrations in wastewater: the use of flow, conductivity and CrAssphage. medRxiv 2021.11.30.21266889. https://doi.org/10.1101/2021.11.30.21266889

Laurini, E., Marson, D., Aulic, S., Fermeglia, A., Pricl, S., 2021. Computational Mutagenesis at the SARS-CoV-2 Spike Protein/Angiotensin-Converting Enzyme 2 Binding Interface: Comparison with Experimental Evidence. ACS Nano 15, 6929–6948. https://doi.org/10.1021/ACSNANO.0C10833/SUPPL_FILE/NN0C10833_SI_003.PDF

Lee, L.M., Teutsch, S.M., Thacker, S.B., Louis, M.E.S., 2010. Principles & Practice of Public Health Surveillance, Principles & Practice of Public Health Surveillance. Oxford University Press. https://doi.org/10.1093/ACPROF:OSO/9780195372922.001.0001

Letko, M., Marzi, A., Munster, V., 2020. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nature Microbiology 5, 1. https://doi.org/10.1038/S41564-020-0688-Y

Li, L., Mazurowski, L., Dewan, A., Carine, M., Haak, L., Guarin, T.C., Dastjerdi, N.G., Gerrity, D., Mentzer, C., Pagilla, K.R., 2022.

Longitudinal monitoring of SARS-CoV-2 in wastewater using viral genetic markers and the estimation of unconfirmed COVID-19 cases. The Science of the Total Environment 817, 152958. https://doi.org/10.1016/J.SCITOTENV.2022.152958

Li, L. quan, Huang, Tian, Wang, Yong qing, Wang, Z. ping, Liang, Y., Huang, Tao bi, Zhang, H. yun, Sun, W., Wang, Yuping, 2020. COVID-19 patients' clinical characteristics, discharge rate, and fatality rate of meta-analysis. Journal of Medical Virology 92, 577–583. https://doi.org/10.1002/JMV.25757

Lin, L., Jiang, X., Zhang, Zhenling, Huang, S., Zhang, Zhenyi, Fang, Z., Gu, Z., Gao, L., Shi, H., Mai, L., Liu, Y., Lin, X., Lai, R., Yan, Z., Li, X., Shan, H., 2020. Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. Gut 69, 997–1001. https://doi.org/10.1136/GUTJNL-2020-321013

Ling, Y., Xu, S.B., Lin, Y.X., Tian, D., Zhu, Z.Q., Dai, F.H., Wu, F., Song, Z.G., Huang, W., Chen, J., Hu, B.J., Wang, S., Mao, E.Q., Zhu, L., Zhang, W.H., Lu, H.Z., 2020. Persistence and clearance of viral RNA in 2019 novel coronavirus disease rehabilitation patients. Chinese medical journal 133, 1039–1043. https://doi.org/10.1097/CM9.0000000000000774

Liu, P., Ibaraki, M., VanTassell, J., Geith, K., Cavallo, M., Kann, R., Guo, L., Moe, C.L., 2022. A sensitive, simple, and low-cost method for COVID-19 wastewater surveillance at an institutional level. The Science of the Total Environment 807, 151047. https://doi.org/10.1016/J.SCITOTENV.2021.151047

Lopardo, L., Adams, D., Cummins, A., Kasprzyk-Hordern, B., 2018. Verifying community-wide exposure to endocrine disruptors in personal care products – In quest for metabolic biomarkers of exposure via in vitro studies and wastewater-based epidemiology. Water Research 143, 117–126. https://doi.org/10.1016/J.WATRES.2018.06.028

Lopez-Leon, S., Wegman-Ostrosky, T., Perelman, C., Sepulveda, R., Rebolledo, P.A., Cuapio, A., Villapol, S., 2021. More than 50 long-term effects of COVID-19: a systematic review and meta-analysis. Scientific Reports 2021 11:1 11, 1–12. https://doi.org/10.1038/s41598-021-95565-8

McMinn, B.R., Ashbolt, N.J., Korajkic, A., 2017. Bacteriophages as indicators of fecal pollution and enteric virus removal. Letters in applied microbiology 65, 11. https://doi.org/10.1111/LAM.12736

Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., Brouwer, A., 2020. Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands. Cite This: Environ. Sci. Technol. Lett 7, 511–516. https://doi.org/10.1021/acs.estlett.0c00357

Milliere, L., Engelmann, I., Tinez, C., Bouarouro, Y., Ouafi, M., Lazrek, M., Prevost, B., Hober, D., Bocket, L., Alidjinou, E.K., 2021. Stool samples versus nasopharyngeal specimens for the initial diagnosis of SARS-CoV-2 infection. Journal of Medical Virology 93, 4145–4146. https://doi.org/10.1002/JMV.26923

Milliere, L., Engelmann, I., Tinez, C., Bouarouro, Y., Ouafi, M., Lazrek, M., Prevost, B., Hober, D., Bocket, L., Kazali ALIDJINOU, E., Kazali Alidjinou, E., n.d. Stool samples versus nasopharyngeal specimens for the initial diagnosis of SARS-CoV-2 infection. Journal of Medical Virology. https://doi.org/10.1002/jmv.26923

Miura, F., Kitajima, M., Omori, R., 2021. Duration of SARS-CoV-2 viral shedding in faeces as a parameter for wastewater-based epidemiology: Re-analysis of patient data using a shedding dynamics model. Science of the Total Environment 769, 144549. https://doi.org/10.1016/j.scitotenv.2020.144549

Mohapatra, S., Menon, N. Gayathri, Mohapatra, G., Pisharody, L., Pattnaik, A., Menon, N. Gowri, Bhukya, P.L., Srivastava, M., Singh, M., Barman, M.K., Gin, K.Y.H., Mukherji, S., 2020. The novel SARS-CoV-2 pandemic: Possible environmental transmission, detection, persistence and fate during wastewater and water treatment. Science of the Total Environment 142746. https://doi.org/10.1016/j.scitotenv.2020.142746

Nagarkar, M., Keely, S.P., Jahne, M., Wheaton, E., Hart, C., Smith, B., Garland, J., Varughese, E.A., Braam, A., Wiechman, B., Morris, B., Brinkman, N.E., 2021. SARS-CoV-2 monitoring at three sewersheds of different scales and complexity demonstrates distinctive relationships between wastewater measurements and COVID-19 case data. Science of The Total Environment 151534. https://doi.org/10.1016/J.SCITOTENV.2021.151534

Nemudryi, A., Nemudraia, A., Wiegand, T., Surya, K., Buyukyoruk, M., Vanderwood, K.K., Wilkinson, R., Wiedenheft, B., 2020a. Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater. medRxiv. https://doi.org/10.1101/2020.04.15.20066746

Nemudryi, A., Nemudraia, A., Wiegand, T., Vanderwood, K.K., Wilkinson, R., Correspondence, B.W., Surya, K., Buyukyoruk, M., Cicha, C., Wiedenheft, B., 2020b. Temporal Detection and Phylogenetic Assessment of SARS-CoV-2 in Municipal Wastewater ll Temporal Detection and Phylogenetic Assessment of SARS-CoV-2 in Municipal Wastewater. https://doi.org/10.1016/j.xcrm.2020.100098

Ort, C., Lawrence, M.G., Rieckermann, J., Joss, A., 2010. Sampling for Pharmaceuticals and Personal Care Products (PPCPs) and Illicit Drugs in Wastewater Systems: Are Your Conclusions Valid? A Critical Review. Environmental Science and Technology 44, 6024–6035. https://doi.org/10.1021/ES100779N

Peccia, J., Zulli, A., Brackney, D.E., Grubaugh, N.D., Kaplan, E.H., Casanovas-Massana, A., Ko, A.I., Malik, A.A., Wang, D., Wang, M., Warren, J.L., Weinberger, D.M., Omer, S.B., 2020. SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics. medRxiv. https://doi.org/10.1101/2020.05.19.20105999

Public Health England, 2021a. Investigation of novel SARS-CoV-2 variant Variant of Concern 202012/01 Technical briefing 4. London.

Public Health England, 2021b. SARS-CoV-2 variants of concern and variants under investigation in England: Technical Briefing 7.

Public Health England, 2021c. SARS-CoV-2 variants of concern and variants under investigation: Technical Briefing 19. London.

Public Health England, 2020. Investigation of novel SARS-COV-2 variant Variant of Concern 202012/01. London.

Qian, Q., Fan, L., Liu, W., Li, J., Yue, J., Wang, M., Ke, X., Yin, Y., Chen, Q., Jiang, C., 2021. Direct Evidence of Active SARS-CoV-2 Replication in the Intestine. Clinical Infectious Diseases 73, 361–366. https://doi.org/10.1093/CID/CIAA925

Rachmadi, A.T., Kitajima, M., Pepper, I.L., Gerba, C.P., 2016. Enteric and indicator virus removal by surface flow wetlands. Science of The Total Environment 542, 976–982. https://doi.org/10.1016/J.SCITOTENV.2015.11.001

Randazzo, W., Truchado, P., Cuevas-Ferrando, E., Simón, P., Allende, A., Sánchez, G., 2020. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water Research 181. https://doi.org/10.1016/J.WATRES.2020.115942

Reeves, K., Liebig, J., Feula, A., Saldi, T., Lasda, E., Johnson, W., Lilienfeld, J., Maggi, J., Pulley, K., Wilkerson, P.J., Real, B., Zak, G., Davis, J., Fink, M., Gonzales, P., Hager, C., Ozeroff, C., Tat, K., Alkire, M., Butler, C., Coe, E., Darby, J., Freeman, N., Heuer, H., Jones, J.R., Karr, M., Key, S., Maxwell, K., Nelson, L., Saldana, E., Shea, R., Salveson, L., Tomlinson, K., Vargas-Barriga, J., Vigil, B., Brisson, G., Parker, R., Leinwand, L.A., Bjorkman, K., Mansfeldt, C., 2021. High-resolution within-sewer SARS-CoV-2 surveillance facilitates informed intervention. Water Research 204, 117613. https://doi.org/10.1016/J.WATRES.2021.117613

Rimoldi, S.G., Stefani, F., Gigantiello, A., Polesello, S., Comandatore, F., Mileto, D., Maresca, M., Longobardi, C., Mancon, A., Romeri, F., Pagani, C., Cappelli, F., Roscioli, C., Moja, L., Gismondo, M.R., Salerno, F., 2020. Presence and infectivity of SARS-CoV-2 virus in wastewaters and rivers. Science of The Total Environment 744, 140911. https://doi.org/10.1016/J.SCITOTENV.2020.140911

Rios, G., Lacoux, C., Leclercq, V., Diamant, A., Lebrigand, K., Lazuka, A., Soyeux, E., Lacroix, S., Fassy, J., Couesnon, A., Thiery, R., Mari, B., Pradier, C., Waldmann, R., Barbry, P., 2021. Monitoring SARS-CoV-2 variants alterations in Nice neighborhoods by wastewater nanopore sequencing. The Lancet Regional Health - Europe 10, 100202. https://doi.org/10.1016/J.LANEPE.2021.100202/ATTACHMENT/3A1A4178-BE29-430B-A765-E9CAB8619F8B/MMC4.DOCX

Roberts, M., 2021. Covid vaccine: 25 to 29-year-olds in England invited - BBC News [WWW Document]. BBC News. URL https://www.bbc.co.uk/news/health-57384099 (accessed 1.24.22).

Rosario, K., Symonds, E.M., Sinigalliano, C., Stewart, J., Breitbart, M., Petersburg, S., 2009. Pepper Mild Mottle Virus as an Indicator of Fecal Pollution. APPLIED AND ENVIRONMENTAL MICROBIOLOGY 75, 7261–7267. https://doi.org/10.1128/AEM.00410-09

Rose, C., Parker, A., Jefferson, B., Cartmell, E., 2015. The Characterization of Feces and Urine: A Review of the Literature to Inform Advanced Treatment Technology. Critical Reviews in Environmental Science and Technology 45, 1827. https://doi.org/10.1080/10643389.2014.1000761

Rosselló, J., Santana-Gallego, M., Awan, W., 2017. Infectious disease risk and international tourism demand. Health Policy and Planning 32, 538–548. https://doi.org/10.1093/HEAPOL/CZW177

Rousis, N.I., Gracia-Lor, E., Zuccato, E., Bade, R., Baz-Lomba, J.A., Castrignanò, E., Causanilles, A., Covaci, A., de Voogt, P., Hernàndez, F., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.K., Plósz, B.G., Ramin, P., Ryu, Y., Thomas, K. v., van Nuijs, A., Yang, Z., Castiglioni, S., 2017a. Wastewater-based epidemiology to assess pan-European pesticide exposure. Water research 121, 270–279. https://doi.org/10.1016/J.WATRES.2017.05.044

Rousis, N.I., Gracia-Lor, E., Zuccato, E., Bade, R., Baz-Lomba, J.A., Castrignanò, E., Causanilles, A., Covaci, A., de Voogt, P., Hernàndez, F., Kasprzyk-Hordern, B., Kinyua, J., McCall, A.K., Plósz, B.G., Ramin, P., Ryu, Y., Thomas, K. v., van Nuijs, A., Yang, Z., Castiglioni, S., 2017b. Wastewater-based epidemiology to assess pan-European pesticide exposure. Water Research 121, 270–279. https://doi.org/10.1016/J.WATRES.2017.05.044

Sah, P., Vilches, T.N., Moghadas, S.M., Fitzpatrick, M.C., Singer, B.H., Hotez, P.J., Galvani, A.P., 2021. Accelerated vaccine rollout is imperative to mitigate highly transmissible COVID-19 variants. EClinicalMedicine 35, 100865. https://doi.org/10.1016/J.ECLINM.2021.100865/ATTACHMENT/789F6C8A-ED4E-4A42-B7E3-BF7EF29BCF7E/MMC1.DOCX

Saitou, N., Nei, M., 1987. The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees'. Mol. Biol. Evol. 4, 406–425.

Schmitz, B.W., Innes, G.K., Prasek, S.M., Betancourt, W.Q., Stark, E.R., Foster, A.R., Abraham, A.G., Gerba, C.P., Pepper, I.L., 2021. Enumerating asymptomatic COVID-19 cases and estimating SARS-CoV-2 fecal shedding rates via wastewater-based epidemiology. https://doi.org/10.1016/j.scitotenv.2021.149794

Scott, L.C., Aubee, A., Babahaji, L., Vigil, K., Tims, S., Gim Aw, T., 2021. Targeted wastewater surveillance of SARS-CoV-2 on a university campus for COVID-19 outbreak detection and mitigation. Environmental Research 200, 111374. https://doi.org/10.1016/j.envres.2021.111374

Sender, R., Bar-On, Y.M., Gleizer, S., Bernshtein, B., Flamholz, A., Phillips, R., Milo, R., 2021. The total number and mass of SARS-CoV-2 virions. Proceedings of the National Academy of Sciences of the United States of America 118. https://doi.org/10.1073/PNAS.2024815118/-/DCSUPPLEMENTAL

Shah, S., Gwee, S.X.W., Ng, J.Q.X., Lau, N., Koh, J., Pang, J., 2022. Wastewater surveillance to infer COVID-19 transmission: A systematic review. Science of The Total Environment 804, 150060. https://doi.org/10.1016/J.SCITOTENV.2021.150060

Shi, J., Li, X., Zhang, S., Sharma, E., Sivakumar, M., Sherchan, S.P., Jiang, G., 2021. Enhanced decay of coronaviruses in sewers with domestic wastewater. Science of The Total Environment 151919. https://doi.org/10.1016/J.SCITOTENV.2021.151919

Sims, D., Sudbery, I., Ilott, N.E., Heger, A., Ponting, C.P., 2014. Sequencing depth and coverage: key considerations in genomic analyses. Nature Reviews Genetics 2014 15:2 15, 121–132. https://doi.org/10.1038/nrg3642

Sims, N., Kasprzyk-Hordern, B., 2020. Future perspectives of wastewater-based epidemiology: Monitoring infectious disease spread and resistance to the community level. Environment International 139, 105689. https://doi.org/10.1016/J.ENVINT.2020.105689

Singanayagam, A., Hakki, S., Dunning, J., Madon, K.J., Crone, M.A., Koycheva, A., Derqui-Fernandez, N., Barnett, J.L., Whitfield, M.G., Varro, R., Charlett, A., Kundu, R., Fenn, J., Cutajar, J., Quinn, V., Conibear, E., Barclay, W., Freemont, P.S., Taylor, G.P., Ahmad, S., Zambon, M., Ferguson, N.M., Lalvani, A., Badhan, A., Dustan, S., Tejpal, C., Ketkar, A. v., Narean, J.S., Hammett, S., McDermott, E., Pillay, T., Houston, H., Luca, C., Samuel, J., Bremang, S., Evetts, S., Poh, J., Anderson, C., Jackson, D., Miah, S., Ellis, J., Lackenby, A., 2021. Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study. The Lancet Infectious Diseases 0. https://doi.org/10.1016/S1473-3099(21)00648-4/ATTACHMENT/902121DE-37A1-4F39-BD8B-239C656F6218/MMC1.PDF

Smith, R.J., Li, J., Blakeley, D., 2011. The Failure of R 0. Computational and Mathematical Methods in Medicine 2011, 17. https://doi.org/10.1155/2011/527610

Sweetapple, C., Melville-Shreeve, P., Chen, A.S., Grimsley, J.M.S., Bunce, J.T., Gaze, W., Fielding, S., Wade, M.J., 2022. Building knowledge of university campus population dynamics to enhance near-to-source sewage surveillance for SARS-CoV-2 detection. Science of The Total Environment 806, 150406. https://doi.org/10.1016/J.SCITOTENV.2021.150406

Symonds, E.M., Nguyen, K.H., Harwood, V.J., Breitbart, M., 2018. Pepper mild mottle virus: A plant pathogen with a greater purpose in (waste)water treatment development and public health management. Water Research 144, 1–12. https://doi.org/10.1016/J.WATRES.2018.06.066

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution 38, 3022–3027. https://doi.org/10.1093/MOLBEV/MSAB120

Thomas, K. v, Reid, M.J., 2011. What Else Can the Analysis of Sewage for Urinary Biomarkers Reveal About Communities? Environ. Sci. Technol 45, 7611–7612. https://doi.org/10.1021/es202522d

Thompson, M.G., Burgess, J.L., Naleway, A.L., Tyner, H., Yoon, S.K., Meece, J., Olsho, L.E.W., Caban-Martinez, A.J., Fowlkes, A.L., Lutrick, K., Groom, H.C., Dunnigan, K., Odean, M.J., Hegmann, K., Stefanski, E., Edwards, L.J., Schaefer-Solle, N., Grant, L., Ellingson, K., Kuntz, J.L., Zunie, T., Thiese, M.S., Ivacic, L., Wesley, M.G., Mayo Lamberte, J., Sun, X., Smith, M.E., Phillips, A.L., Groover, K.D., Yoo, Y.M., Gerald, J., Brown, R.T., Herring, M.K., Joseph, G., Beitel, S., Morrill, T.C., Mak, J., Rivers, P., Poe, B.P., Lynch, B., Zhou, Y., Zhang, J., Kelleher, A., Li, Y., Dickerson, M., Hanson, E., Guenther, K., Tong, S., Bateman, A., Reisdorf, E., Barnes, J., Azziz-Baumgartner, E., Hunt, D.R., Arvay, M.L., Kutty, P., Fry, A.M., Gaglani, M., 2021. Prevention and Attenuation of Covid-19 with the BNT162b2 and mRNA-1273 Vaccines. New England Journal of Medicine 385, 320–329. https://doi.org/10.1056/NEJMOA2107058/SUPPL_FILE/NEJMOA2107058_DATA-SHARING.PDF

Travis, S.A., Best, A.A., Bochniak, K.S., Dunteman, N.D., Fellinger, J., Folkert, P.D., Koberna, T., Kopek, B.G., Krueger, B.P., Pestun, J., Pikaart, M.J., Sabo, C., Schuitema, A.J., 2021. Providing a Safe, In-Person, Residential College Experience During the COVID-19 Pandemic. Frontiers in Public Health 9, 786. https://doi.org/10.3389/FPUBH.2021.672344/BIBTEX

Tyson, J.R., James, P., Stoddart, D., Sparks, N., Wickenhagen, A., Hall, G., Choi, J.H., Lapointe, H., Kamelian, K., Smith, A.D., Prystajecky, N., Goodfellow, I., Wilson, S.J., Harrigan, R., Snutch, T.P., Loman, N.J.,

Quick, J., 2020. Improvements to the ARTIC multiplex PCR method for SARS-CoV-2 genome sequencing using nanopore. bioRxiv 3, 1. https://doi.org/10.1101/2020.09.04.283077

van Nuijs, A.L.N., Mougel, J.F., Tarcomnicu, I., Bervoets, L., Blust, R., Jorens, P.G., Neels, H., Covaci, A., 2011. Sewage epidemiology — A real-time approach to estimate the consumption of illicit drugs in Brussels, Belgium. Environment International 37, 612–621. https://doi.org/10.1016/J.ENVINT.2010.12.006

V'kovski, P., Kratzel, A., Steiner, S., Stalder, H., Thiel, V., 2020. Coronavirus biology and replication: implications for SARS-CoV-2. Nature Reviews Microbiology 2020 19:3 19, 155–170. https://doi.org/10.1038/s41579-020-00468-6

Vo, V., Tillett, R.L., Chang, C.L., Gerrity, D., Betancourt, W.Q., Oh, E.C., 2022. SARS-CoV-2 variant detection at a university dormitory using wastewater genomic tools. Science of The Total Environment 805, 149930. https://doi.org/10.1016/J.SCITOTENV.2021.149930

Wade, M.J., lo Jacomo, A., Armenise, E., Brown, M.R., Bunce, J.T., Cameron, G.J., Fang, Z., Farkas, K., Gilpin, D.F., Graham, D.W., Grimsley, J.M.S., Hart, A., Hoffmann, T., Jackson, K.J., Jones, D.L., Lilley, C.J., McGrath, J.W., McKinley, J.M., McSparron, C., Nejad, B.F., Morvan, M., Quintela-Baluja, M., Roberts, A.M.I., Singer, A.C., Souque, C., Speight, V.L., Sweetapple, C., Walker, D., Watts, G., Weightman, A., Kasprzyk-Hordern, B., 2022. Understanding and managing uncertainty and variability for wastewater monitoring beyond the pandemic: Lessons learned from the United Kingdom national COVID-19 surveillance programmes. Journal of Hazardous Materials 424, 127456. https://doi.org/10.1016/J.JHAZMAT.2021.127456

Wang, L., Didelot, X., Bi, Y., Gao, G.F., 2021. Comparative evaluation of the transmissibility of SARS-CoV-2 variants of concern. medRxiv 2021.06.25.21259565. https://doi.org/10.1101/2021.06.25.21259565

Wang, Y., Liu, P., Zhang, H., Ibaraki, M., VanTassell, J., Geith, K., Cavallo, M., Kann, R., Saber, L., Kraft, C.S., Lane, M., Shartar, S., Moe, C., 2022. Early warning of a COVID-19 surge on a university campus based on wastewater surveillance for SARS-CoV-2 at residence halls. The Science of the Total Environment 821, 153291. https://doi.org/10.1016/J.SCITOTENV.2022.153291

Wei, X.S., Wang, X., Niu, Y.R., Ye, L.L., Peng, W.B., Wang, Z.H., Yang, W.B., Yang, B.H., Zhang, J.C., Ma, W.L., Wang, X.R., Zhou, Q., 2020. Diarrhea Is Associated With Prolonged Symptoms and Viral Carriage in Corona Virus Disease 2019. Clinical Gastroenterology and Hepatology 18, 1753. https://doi.org/10.1016/J.CGH.2020.04.030

Weidhaas, J., Aanderud, Z.T., Roper, D.K., VanDerslice, J., Gaddis, E.B., Ostermiller, J., Hoffman, K., Jamal, R., Heck, P., Zhang, Y., Torgersen,

K., Laan, J. vander, LaCross, N., 2021. Correlation of SARS-CoV-2 RNA in wastewater with COVID-19 disease burden in sewersheds. The Science of the total environment 775. https://doi.org/10.1016/J.SCITOTENV.2021.145790

WHO, 2020. Water, sanitation, hygiene, and waste management for the COVID-19 virus. Geneva.

Wilder, M.L., Middleton, F., Larsen, D.A., Du, Q., Fenty, A., Zeng, T., Insaf, T., Kilaru, P., Collins, M., Kmush, B., Green, H.C., 2021. Co-quantification of crAssphage increases confidence in wastewater-based epidemiology for SARS-CoV-2 in low prevalence areas. Water Research X 11, 100100. https://doi.org/10.1016/J.WROA.2021.100100

Wilder-Smith, A., Freedman, D.O., 2020. Isolation, quarantine, social distancing and community containment: Pivotal role for old-style public health measures in the novel coronavirus (2019-nCoV) outbreak. Journal of Travel Medicine 27. https://doi.org/10.1093/JTM/TAAA020

Wölfel, R., Corman, V.M., Guggemos, W., Seilmaier, M., Zange, S., Müller, M.A., Niemeyer, D., Jones, T.C., Vollmar, P., Rothe, C., Hoelscher, M., Bleicker, T., Brünink, S., Schneider, J., Ehmann, R., Zwirglmaier, K., Drosten, C., Wendtner, C., 2020. Virological assessment of hospitalized patients with COVID-2019. Nature 2020 581:7809 581, 465–469. https://doi.org/10.1038/s41586-020-2196-x

World Health Organization, 2022. WHO Coronavirus (COVID-19) Dashboard [WWW Document]. URL https://covid19.who.int/ (accessed 2.24.22).

Wright, J., Driver, E.M., Bowes, D.A., Johnston, B., Halden, R.U., 2022. Comparison of high-frequency in-pipe SARS-CoV-2 wastewater-based surveillance to concurrent COVID-19 random clinical testing on a public U.S. university campus. Science of The Total Environment 820, 152877. https://doi.org/10.1016/J.SCITOTENV.2021.152877

Wu, F., Zhang, J., Xiao, A., Gu, X., Lee, W.L., Armas, F., Kauffman, K., Hanage, W., Matus, M., Ghaeli, N., Endo, N., Duvallet, C., Poyet, M., Moniz, K., Washburne, A.D., Erickson, T.B., Chai, P.R., Thompson, J., Alm, E.J., 2020. SARS-CoV-2 Titers in Wastewater Are Higher than Expected from Clinically Confirmed Cases. mSystems 5. https://doi.org/10.1128/MSYSTEMS.00614-20/ASSET/3176BDF3-0F21-4633-A5D6-65F5F9E59D15/ASSETS/GRAPHIC/MSYSTEMS.00614-20-F0004.JPEG

Wu, Y., Guo, C., Tang, L., Hong, Z., Zhou, J., Dong, X., Yin, H., Xiao, Q., Tang, Y., Qu, X., Kuang, L., Fang, X., Mishra, N., Lu, J., Shan, H., Jiang, G., Huang, X., 2020. Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. The Lancet Gastroenterology and Hepatology 5, 434–435. https://doi.org/10.1016/S2468-1253(20)30083-

2/ATTACHMENT/85B0900C-C533-4644-AF07-4AE386B6EA83/MMC2.PDF

Wu, Z., McGoogan, J.M., 2020. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. JAMA 323, 1239–1242. https://doi.org/10.1001/JAMA.2020.2648

Wurtzer, S., Marechal, V., Mouchel, J.M., Maday, Y., Teyssou, R., Richard, E., Almayrac, J.L., Moulin, L., 2020. Evaluation of lockdown impact on SARS-CoV-2 dynamics through viral genome quantification in Paris wastewaters. medRxiv. https://doi.org/10.1101/2020.04.12.20062679

Xiao, F., Sun, J., Xu, Y., Li, F., Huang, X., Li, H., Zhao, Jingxian, Huang, J., Zhao, Jincun, 2020a. Infectious SARS-CoV-2 in Feces of Patient with Severe COVID-19. Emerging Infectious Diseases 26, 1920. https://doi.org/10.3201/EID2608.200681

Xiao, F., Tang, M., Zheng, X., Liu, Y., Li, X., Shan, H., 2020b. Evidence for Gastrointestinal Infection of SARS-CoV-2. Gastroenterology 158, 1831. https://doi.org/10.1053/J.GASTRO.2020.02.055

Ye, Y., Ellenberg, R.M., Graham, K.E., Wigginton, K.R., 2016. Survivability, Partitioning, and Recovery of Enveloped Viruses in Untreated Municipal Wastewater. Environmental Science and Technology 50, 5077–5085. https://doi.org/10.1021/ACS.EST.6B00876/SUPPL_FILE/ES6B00876_SI_001.PDF

Yu, I.T.S., Li, Y., Wong, T.W., Tam, W., Chan, A.T., Lee, J.H.W., Leung, D.Y.C., Ho, T., 2004. Evidence of Airborne Transmission of the Severe Acute Respiratory Syndrome Virus. New England Journal of Medicine 350, 1731–1739. https://doi.org/10.1056/NEJMoa032867

Zhang, N., Gong, Y., Meng, F., Bi, Y., Yang, P., Wang, F., 2020. Virus shedding patterns in nasopharyngeal and fecal specimens of COVID-19 patients. medRxiv 2020.03.28.20043059. https://doi.org/10.1101/2020.03.28.20043059

Zhang, T., Breitbart, M., Lee, W.H., Run, J.Q., Wei, C.L., Soh, S.W.L., Hibberd, M.L., Liu, E.T., Rohwer, F., Ruan, Y., 2005. RNA Viral Community in Human Feces: Prevalence of Plant Pathogenic Viruses. PLOS Biology 4, e3. https://doi.org/10.1371/JOURNAL.PBIO.0040003

Zhang, T., Cui, X., Zhao, X., Wang, J., Zheng, J., Zheng, G., Guo, W., Cai, C., He, S., Xu, Y., 2020. Detectable SARS-CoV-2 viral RNA in feces of three children during recovery period of COVID-19 pneumonia. Journal of Medical Virology 92, 909–914. https://doi.org/10.1002/jmv.25795

Zheng, S., Fan, J., Yu, F., Feng, B., Lou, B., Zou, Q., Xie, G., Lin, S., Wang, R., Yang, X., Chen, W., Wang, Q., Zhang, D., Liu, Y., Gong, R., Ma, Z., Lu, S., Xiao, Y., Gu, Y., Zhang, J., Yao, H., Xu, K., Lu, X., Wei, G., Zhou,

J., Fang, Q., Cai, H., Qiu, Y., Sheng, J., Chen, Y., Liang, T., 2020. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ 369. https://doi.org/10.1136/BMJ.M1443

6 Appendix 1: Summary data of samples collected during study

Table 12: Summary of samples collected during study

*Location is as follows: 1 – Halls of Residence A; 2 – Halls of Residence B; 3 – Technical In-Sewer; 4 – Residential In-Sewer; 5 – Influent; 6 – Post-Primary Lamella; 7 – Post-Roughing Trickling Filter; 8 – Post-Secondary Trickling Filter and 9 – Final Effluent

Date collected	Internal Reference	Sample number	Frozen or Fresh	Location*	Sampling method	Extraction Protocol
19.3.20	H1	1	Frozen	5	Grab	2
1.4.20	H3a	2	Frozen	5	Grab	2
1.4.20	H3	3	Frozen	5	Grab	2
9.4.20	H4	4	Frozen	5	Grab	2
9.4.20	H6	5	Frozen	5	Grab	2
14.4.20	H8	6	Frozen	5	Grab	2
17.4.20	H10	7	Frozen	5	Grab	2
17.4.20	H12A	8	Frozen	5	Grab	2
17.4.20	H12B	9	Frozen	5	Grab	2
20.4.20	H13	10	Frozen	5	Grab	2
20.4.20	H14a	11	Frozen	5	Grab	2
27.4.20	H15	12	Frozen	5	Grab	2
27.4.20	H16	13	Frozen	5	Grab	2
6.5.20	H19	14	Frozen	5	Grab	2
20.5.20	H20	15	Frozen	5	Grab	2
26.5.20	H22	16	Frozen	5	Grab	2
3.6.20	H23	17	Frozen	5	Grab	2
3.6.20	H25	18	Frozen	5	Grab	2
10.6.20	H26	19	Frozen	5	Grab	2
10.6.20	H120	20	Frozen	5	Grab	2
24.6.20	H27	21	Frozen	5	Grab	2
24.6.20	H28	22	Frozen	5	Grab	2
24.6.20	H29	23	Frozen	5	Grab	2
1.7.20	H31A	24	Frozen	5	Grab	2
1.7.20	H31B	25	Frozen	5	Grab	2
8.7.20	H35	26	Frozen	5	Grab	2
8.7.20	H36	27	Frozen	5	Grab	2
8.7.20	H37	28	Frozen	5	Grab	2
17.7.20	H39a	29	Frozen	5	Grab	2
17.7.20	H40	30	Frozen	5	Grab	2
17.7.20	H39b	31	Frozen	5	Grab	2
24.7.20	H43	32	Frozen	5	Grab	2

24.7.20	H43	33	Frozen	5	Grab	2
24.7.20	H44	34	Frozen	5	Grab	2
24.7.20	H45	35	Frozen	5	Grab	2
29.7.20	H47	36	Frozen	5	Grab	2
29.7.20	H48A	37	Frozen	5	Grab	2
29.7.20	H49	38	Frozen	5	Grab	2
12.8.20	H50	39	Frozen	5	Grab	2
12.8.20	H111	40	Frozen	5	Grab	2
17.8.20	H53	41	Frozen	5	Grab	2
2.9.20	H54	42	Frozen	5	Grab	2
2.9.20	H55	43	Frozen	5	Grab	2
2.9.20	H56	44	Frozen	5	Grab	2
10.9.20	H59A	45	Frozen	5	Grab	2
10.9.20	H59B	46	Frozen	5	Grab	2
10.9.20	H60	47	Frozen	5	Grab	2
17.9.20	H63	48	Frozen	5	Grab	2
17.9.20	H64	49	Frozen	5	Grab	2
17.9.20	H65	50	Frozen	5	Grab	2
23.9.20	H67	51	Frozen	5	Grab	2
23.9.20	H69	52	Frozen	5	Grab	2
30.9.20	H70A	53	Frozen	5	Grab	2
30.9.20	H72A	54	Frozen	5	Grab	2
7.10.20	H75	55	Frozen	5	Grab	2
7.10.20	H76	56	Frozen	5	Grab	2
7.10.20	H77A	57	Frozen	5	Grab	2
14.10.20	H78	58	Frozen	5	Grab	2
14.10.20	H80	59	Frozen	5	Grab	2
21.10.20	H104	60	Frozen	5	Grab	2
21.10.20	H112A	61	Frozen	5	Grab	2
21.10.20	H112B	62	Frozen	5	Grab	2
28.10.20	H113	63	Frozen	5	Grab	2
28.10.20	H122	64	Frozen	5	Grab	2
11.11.20	H114	65	Frozen	5	Grab	2
11.11.20	H116	66	Frozen	5	Grab	2
20.11.20	H115	67	Frozen	5	Grab	2
20.11.20	H118	68	Frozen	5	Grab	2
25.11.20	H105	69	Frozen	5	Grab	2
25.11.20	H117	70	Frozen	5	Grab	2
9.12.20	H83A	71	Frozen	5	Grab	2
9.12.20	H85A	72	Frozen	5	Grab	2
16.12.20	H87	73	Frozen	5	Grab	2
16.12.20	H88A	74	Frozen	5	Grab	2
16.12.20	H88B	75	Frozen	5	Grab	2
6.1.21	H90	76	Frozen	5	Grab	2
6.1.21	H92	77	Frozen	5	Grab	2
6.1.21	H119	78	Frozen	5	Grab	2

6.1.21	H110	79	Frozen	5	Grab	2
13.1.21	H95	80	Frozen	5	Grab	2
13.1.21	H96	81	Frozen	5	Grab	2
13.1.21	H121	82	Frozen	5	Grab	2
20.1.21	H100	83	Frozen	5	Grab	2
20.1.21	H98	84	Frozen	5	Grab	2
20.1.21	H101	85	Frozen	5	Grab	2
22.1.21	F1	86	Fresh	1	Composite	1
22.1.21	F2	87	Fresh	2	Composite	1
22.1.21	F3	88	Fresh	3	Composite	1
22.1.21	F4	89	Fresh	4	Composite	1
22.1.21	F5	90	Fresh	5	Grab	1
22.1.21	F9	94	Fresh	6	Grab	1
22.1.21	F7	92	Fresh	7	Grab	1
22.1.21	F8	93	Fresh	8	Grab	1
22.1.21	F6	91	Fresh	9	Grab	1
26.1.21	F14	95	Fresh	1	Composite	1
26.1.21	F15	96	Fresh	3	Composite	1
26.1.21	F10	97	Fresh	5	Grab	1
26.1.21	F12	99	Fresh	7	Grab	1
26.1.21	F13	100	Fresh	8	Grab	1
26.1.21	F11	98	Fresh	9	Grab	1
27.1.21	F18	101	Fresh	2	Composite	1
27.1.21	F19	102	Fresh	4	Composite	1
28.1.21	F20	103	Fresh	1	Composite	1
28.1.21	F21	104	Fresh	3	Composite	1
28.1.21	F22	105	Fresh	5	Grab	1
28.1.21	F26	109	Fresh	6	Grab	1
28.1.21	F24	107	Fresh	7	Grab	1
28.1.21	F25	108	Fresh	8	Grab	1
28.1.21	F23	106	Fresh	9	Grab	1
1.2.21	F30	110	Fresh	1	Composite	1
1.2.21	F31	111	Fresh	3	Composite	1
2.2.21	F33	112	Fresh	3	Composite	1
2.2.21	F34	113	Fresh	5	Grab	1
2.2.21	F38	117	Fresh	6	Grab	1
2.2.21	F36	115	Fresh	7	Grab	1
2.2.21	F37	116	Fresh	8	Grab	1
2.2.21	F35	114	Fresh	9	Grab	1
3.2.21	F40	118	Fresh	2	Composite	1
3.2.21	F41	119	Fresh	4	Composite	1
4.2.21	F42	120	Fresh	2	Composite	1
4.2.21	F43	121	Fresh	4	Composite	1
4.2.21	F44	122	Fresh	5	Grab	1
4.2.21	F48	126	Fresh	6	Grab	1
4.2.21	F46	124	Fresh	7	Grab	1

4.2.21	F47	125	Fresh	8	Grab	1
4.2.21	F45	123	Fresh	9	Grab	1
8.2.21	F50	127	Fresh	1	Composite	1
8.2.21	F51	128	Fresh	3	Composite	1
9.2.21	F52	129	Fresh	5	Grab	1
9.2.21	F54	131	Fresh	7	Grab	1
9.2.21	F55	132	Fresh	8	Grab	1
9.2.21	F53	130	Fresh	9	Grab	1
10.2.21	F56	133	Fresh	4	Composite	1
11.2.21	F57	134	Fresh	4	Composite	1
11.2.21	F58	135	Fresh	5	Grab	1
11.2.21	F60	137	Fresh	7	Grab	1
11.2.21	F61	138	Fresh	8	Grab	1
11.2.21	F59	136	Fresh	9	Grab	1
15.2.21	F62	139	Fresh	3	Composite	1
16.2.21	F63	140	Fresh	4	Composite	1
16.2.21	F64	141	Fresh	5	Grab	1
16.2.21	F68	145	Fresh	6	Grab	1
16.2.21	F66	143	Fresh	7	Grab	1
16.2.21	F67	144	Fresh	8	Grab	1
16.2.21	F65	142	Fresh	9	Grab	1
17.2.21	F69	146	Fresh	1	Composite	1
18.2.21	F70	147	Fresh	2	Composite	1
18.2.21	F71	148	Fresh	5	Grab	1
18.2.21	F76	152	Fresh	6	Grab	1
18.2.21	F74	150	Fresh	7	Grab	1
18.2.21	F75	151	Fresh	8	Grab	1
18.2.21	F73	149	Fresh	9	Grab	1
22.2.21	F77	153	Fresh	2	Composite	1
23.2.21	F78	154	Fresh	4	Composite	1
23.2.21	F79	155	Fresh	5	Grab	1
23.2.21	F83	159	Fresh	6	Grab	1
23.2.21	F81	157	Fresh	7	Grab	1
23.2.21	F82	158	Fresh	8	Grab	1
23.2.21	F80	156	Fresh	9	Grab	1
24.2.21	F84	160	Fresh	3	Composite	1
25.2.21	F85	161	Fresh	1	Composite	1
25.2.21	F86	162	Fresh	5	Grab	1
25.2.21	F90	166	Fresh	6	Grab	1
25.2.21	F88	164	Fresh	7	Grab	1
25.2.21	F89	165	Fresh	8	Grab	1
25.2.21	F87	163	Fresh	9	Grab	1
1.3.21	F91	167	Fresh	3	Composite	1
2.3.21	F92	168	Fresh	2	Composite	1
2.3.21	F93	169	Fresh	4	Composite	1
2.3.21	F94	170	Fresh	5	Grab	1

2.3.21	F98	174	Fresh	6	Grab	1
2.3.21	F96	172	Fresh	7	Grab	1
2.3.21	F97	173	Fresh	8	Grab	1
2.3.21	F95	171	Fresh	9	Grab	1
3.3.21	F99	175	Fresh	4	Composite	1
4.3.21	F100	176	Fresh	1	Composite	2
4.3.21	F101	177	Fresh	5	Grab	2
4.3.21	F105	181	Fresh	6	Grab	2
4.3.21	F103	179	Fresh	7	Grab	2
4.3.21	F104	180	Fresh	8	Grab	2
4.3.21	F102	178	Fresh	9	Grab	2
8.3.21	F106	182	Fresh	1	Composite	2
8.3.21	F107	183	Fresh	2	Composite	2
9.3.21	F108	184	Fresh	4	Composite	2
9.3.21	F109	185	Fresh	5	Grab	2
9.3.21	F113	189	Fresh	6	Grab	2
9.3.21	F111	187	Fresh	7	Grab	2
9.3.21	F112	188	Fresh	8	Grab	2
9.3.21	F110	186	Fresh	9	Grab	2
10.3.21	F115	190	Fresh	3	Composite	2
10.3.21	F114	191	Fresh	4	Composite	2
11.3.21	F116	192	Fresh	4	Composite	2
15.3.21	F117	193	Fresh	3	Composite	2
15.3.21	F118	194	Fresh	4	Composite	2
16.3.21	F119	195	Fresh	1	Composite	2
16.3.21	F120	196	Fresh	4	Composite	2
16.3.21	F121	197	Fresh	5	Grab	2
16.3.21	F125	201	Fresh	6	Grab	2
16.3.21	F123	199	Fresh	7	Grab	2
16.3.21	F124	200	Fresh	8	Grab	2
16.3.21	F122	198	Fresh	9	Grab	2
17.3.21	F126	202	Fresh	2	Composite	2
17.3.21	F127	203	Fresh	4	Composite	2
18.3.21	F128	204	Fresh	4	Composite	2
18.3.21	F129	205	Fresh	4	Composite	2
22.3.21	F130	206	Fresh	1	Composite	2
22.3.21	F131	207	Fresh	4	Composite	2
23.3.21	F132	208	Fresh	2	Composite	2
23.3.21	F133	209	Fresh	3	Composite	2
23.3.21	F134	210	Fresh	5	Grab	2
23.3.21	F138	214	Fresh	6	Grab	2
23.3.21	F136	212	Fresh	7	Grab	2
23.3.21	F137	213	Fresh	8	Grab	2
23.3.21	F135	211	Fresh	9	Grab	2
24.3.21	F139	215	Fresh	1	Composite	2
25.3.21	F140	216	Fresh	4	Composite	2

30.3.21	F141	217	Fresh	2	Composite	2
31.3.21	F142	218	Fresh	4	Composite	2
31.3.21	F143	219	Fresh	5	Grab	2
31.3.21	F145	221	Fresh	7	Grab	2
31.3.21	F146	222	Fresh	8	Grab	2
31.3.21	F144	220	Fresh	9	Grab	2
1.4.21	F148	223	Fresh	1	Composite	2
9.4.21	F149	224	Fresh	5	Grab	2
12.4.21	F150	225	Fresh	3	Composite	2
13.4.21	F151	226	Fresh	1	Composite	2
13.4.21	F152	227	Fresh	5	Grab	2
13.4.21	F156	231	Fresh	6	Grab	2
13.4.21	F154	229	Fresh	7	Grab	2
13.4.21	F155	230	Fresh	8	Grab	2
13.4.21	F153	228	Fresh	9	Grab	2
14.4.21	F157	232	Fresh	4	Composite	2
15.4.21	F158	233	Fresh	2	Composite	2
20.4.21	F160	234	Fresh	4	Composite	2
20.4.21	F161	235	Fresh	5	Grab	2
20.4.21	F165	239	Fresh	6	Grab	2
20.4.21	F163	237	Fresh	7	Grab	2
20.4.21	F164	238	Fresh	8	Grab	2
20.4.21	F162	236	Fresh	9	Grab	2
21.4.21	F166	240	Fresh	1	Composite	2
22.4.21	F167	241	Fresh	3	Composite	2
27.4.21	F168	242	Fresh	5	Grab	2
27.4.21	F172	246	Fresh	6	Grab	2
27.4.21	F170	244	Fresh	7	Grab	2
27.4.21	F171	245	Fresh	8	Grab	2
27.4.21	F169	243	Fresh	9	Grab	2
4.5.21	F173A	247	Fresh	5	Grab	2
4.5.21	F173B	248	Fresh	5	Grab	2
4.5.21	177A	255	Fresh	6	Grab	2
4.5.21	177B	256	Fresh	6	Grab	2
4.5.21	175A	251	Fresh	7	Grab	2
4.5.21	175B	252	Fresh	7	Grab	2
4.5.21	176A	253	Fresh	8	Grab	2
4.5.21	176B	254	Fresh	8	Grab	2
4.5.21	174A	249	Fresh	9	Grab	2
4.5.21	174B	250	Fresh	9	Grab	2
10.5.21	179A	257	Fresh	1	Composite	2
10.5.21	179B	258	Fresh	1	Composite	2
10.5.21	178A	259	Fresh	2	Composite	2
10.5.21	178B	260	Fresh	2	Composite	2
10.5.21	180A	261	Fresh	3	Composite	2
10.5.21	180B	262	Fresh	3	Composite	2

10.5.21	181A	263	Fresh	4	Composite	2
10.5.21	181B	264	Fresh	4	Composite	2
10.5.21	182A	265	Fresh	5	Grab	2
10.5.21	182B	266	Fresh	5	Grab	2
11.5.21	183A	267	Fresh	5	Grab	2
11.5.21	183B	268	Fresh	5	Grab	2
11.5.21	187	272	Fresh	6	Grab	2
11.5.21	185	270	Fresh	7	Grab	2
11.5.21	186	271	Fresh	8	Grab	2
11.5.21	184	269	Fresh	9	Grab	2
12.5.21	188A	273	Fresh	5	Grab	2
12.5.21	188B	274	Fresh	5	Grab	2
13.5.21	189A	275	Fresh	5	Grab	2
13.5.21	189B	276	Fresh	5	Grab	2
17.5.21	190A	277	Fresh	1	Composite	2
17.5.21	190B	278	Fresh	1	Composite	2
17.5.21	191A	279	Fresh	2	Composite	2
17.5.21	191B	280	Fresh	2	Composite	2
17.5.21	192A	281	Fresh	3	Composite	2
17.5.21	192B	282	Fresh	3	Composite	2
17.5.21	193A	283	Fresh	4	Composite	2
17.5.21	193B	284	Fresh	4	Composite	2
17.5.21	194A	285	Fresh	5	Grab	2
17.5.21	194B	286	Fresh	5	Grab	2
18.5.21	195A	287	Fresh	5	Grab	2
18.5.21	195B	288	Fresh	5	Grab	2
18.5.21	199	292	Fresh	6	Grab	2
18.5.21	197	290	Fresh	7	Grab	2
18.5.21	198	291	Fresh	8	Grab	2
18.5.21	196	289	Fresh	9	Grab	2
19.5.21	200A	293	Fresh	5	Grab	2
19.5.21	200B	294	Fresh	5	Grab	2
20.5.21	201A	295	Fresh	5	Grab	2
20.5.21	201B	296	Fresh	5	Grab	2
24.5.21	202A	297	Fresh	5	Grab	2
24.5.21	202B	298	Fresh	5	Grab	2
25.5.21	203A	299	Fresh	5	Grab	2
25.5.21	203B	300	Fresh	5	Grab	2
25.5.21	207A	305	Fresh	6	Grab	2
25.5.21	207B	306	Fresh	6	Grab	2
25.5.21	205	303	Fresh	7	Grab	2
25.5.21	206	304	Fresh	8	Grab	2
25.5.21	204A	301	Fresh	9	Grab	2
25.5.21	204B	302	Fresh	9	Grab	2
26.5.21	F208A	307	Fresh	5	Grab	2
26.5.21	F208B	308	Fresh	5	Grab	2

27.5.21	F210A	309	Fresh	1	Composite	2
27.5.21	F210B	310	Fresh	1	Composite	2
27.5.21	F209A	311	Fresh	2	Composite	2
27.5.21	F209B	312	Fresh	2	Composite	2
27.5.21	F211A	313	Fresh	3	Composite	2
27.5.21	F211B	314	Fresh	3	Composite	2
27.5.21	F212A	315	Fresh	4	Composite	2
27.5.21	F212B	316	Fresh	4	Composite	2
27.5.21	F213A	317	Fresh	5	Grab	2
27.5.21	F213B	318	Fresh	5	Grab	2
1.6.21	F214A	319	Fresh	5	Grab	2
1.6.21	F214B	320	Fresh	5	Grab	2
1.6.21	F218A	325	Fresh	6	Grab	2
1.6.21	F218B	326	Fresh	6	Grab	2
1.6.21	F216	323	Fresh	7	Grab	2
1.6.21	F217	324	Fresh	8	Grab	2
1.6.21	F215A	321	Fresh	9	Grab	2
1.6.21	F215B	322	Fresh	9	Grab	2
2.6.21	F219A	327	Fresh	5	Grab	2
2.6.21	F219B	328	Fresh	5	Grab	2
3.6.21	F220A	329	Fresh	1	Composite	2
3.6.21	F220B	330	Fresh	1	Composite	2
3.6.21	F221A	331	Fresh	2	Composite	2
3.6.21	F221B	332	Fresh	2	Composite	2
3.6.21	F222A	333	Fresh	3	Composite	2
3.6.21	F222B	334	Fresh	3	Composite	2
3.6.21	F223A	335	Fresh	4	Composite	2
3.6.21	F223B	336	Fresh	4	Composite	2
3.6.21	F224A	337	Fresh	5	Grab	2
3.6.21	F224B	338	Fresh	5	Grab	2
7.6.21	F225A	339	Fresh	5	Grab	2
7.6.21	F225B	340	Fresh	5	Grab	2
8.6.21	F226A	341	Fresh	5	Grab	2
8.6.21	F226B	342	Fresh	5	Grab	2
8.6.21	F230A	348	Fresh	6	Grab	2
8.6.21	F230B	349	Fresh	6	Grab	2
8.6.21	F228A	345	Fresh	7	Grab	2
8.6.21	F229A	346	Fresh	8	Grab	2
8.6.21	F229B	347	Fresh	8	Grab	2
8.6.21	F227A	343	Fresh	9	Grab	2
8.6.21	F227B	344	Fresh	9	Grab	2
9.6.21	F231A	350	Fresh	5	Grab	2
9.6.21	F231B	351	Fresh	5	Grab	2
10.6.21	F232A	352	Fresh	1	Composite	2
10.6.21	F232B	353	Fresh	1	Composite	2
10.6.21	F233A	354	Fresh	2	Composite	2

10.6.21	F233B	355	Fresh	2	Composite	2
10.6.21	F234A	356	Fresh	3	Composite	2
10.6.21	F234B	357	Fresh	3	Composite	2
10.6.21	F235A	358	Fresh	4	Composite	2
10.6.21	F235B	359	Fresh	4	Composite	2
10.6.21	F236A	360	Fresh	5	Grab	2
10.6.21	F236B	361	Fresh	5	Grab	2
15.6.21	F237A	362	Fresh	5	Grab	2
15.6.21	F237B	363	Fresh	5	Grab	2
15.6.21	F241A	370	Fresh	6	Grab	2
15.6.21	F241B	371	Fresh	6	Grab	2
15.6.21	F239A	366	Fresh	7	Grab	2
15.6.21	F239B	367	Fresh	7	Grab	2
15.6.21	F240A	368	Fresh	8	Grab	2
15.6.21	F240B	369	Fresh	8	Grab	2
15.6.21	F238A	364	Fresh	9	Grab	2
15.6.21	F238B	365	Fresh	9	Grab	2
17.6.21	F243A	372	Fresh	2	Composite	2
17.6.21	F243B	373	Fresh	2	Composite	2
17.6.21	F244A	374	Fresh	3	Composite	2
17.6.21	F244B	375	Fresh	3	Composite	2
17.6.21	F245A	376	Fresh	4	Composite	2
17.6.21	F245B	377	Fresh	4	Composite	2
17.6.21	F246A	378	Fresh	5	Grab	2
17.6.21	F246B	379	Fresh	5	Grab	2
17.6.21	F250A	386	Fresh	6	Grab	2
17.6.21	F250B	387	Fresh	6	Grab	2
17.6.21	F248A	382	Fresh	7	Grab	2
17.6.21	F248B	383	Fresh	7	Grab	2
17.6.21	F249A	384	Fresh	8	Grab	2
17.6.21	F249B	385	Fresh	8	Grab	2
17.6.21	F247A	380	Fresh	9	Grab	2
17.6.21	F247B	381	Fresh	9	Grab	2
21.6.21	F251A	388	Fresh	5	Grab	2
21.6.21	F251B	389	Fresh	5	Grab	2
22.6.21	F252A	390	Fresh	5	Grab	2
22.6.21	F252B	391	Fresh	5	Grab	2
22.6.21	F252	395	Fresh	6	Grab	2
22.6.21	F254	393	Fresh	7	Grab	2
22.6.21	F255	394	Fresh	8	Grab	2
22.6.21	F253A	392	Fresh	9	Grab	2
23.6.21	F257A	396	Fresh	5	Grab	2
23.6.21	F257B	397	Fresh	5	Grab	2
24.6.21	F258A	398	Fresh	1	Composite	2
24.6.21	F258B	399	Fresh	1	Composite	2
24.6.21	F259A	400	Fresh	2	Composite	2

24.6.21	F259B	401	Fresh	2	Composite	2
24.6.21	F260A	402	Fresh	3	Composite	2
24.6.21	F260B	403	Fresh	3	Composite	2
24.6.21	F261A	404	Fresh	4	Grab	2
24.6.21	F261B	405	Fresh	4	Grab	2
24.6.21	F262A	406	Fresh	5	Grab	2
24.6.21	F262B	407	Fresh	5	Grab	2
28.6.21	F263A	408	Fresh	5	Grab	2
28.6.21	F263B	409	Fresh	5	Grab	2
29.6.21	F264A	410	Fresh	5	Grab	2
29.6.21	F264B	411	Fresh	5	Grab	2
29.6.21	F268A	416	Fresh	6	Grab	2
29.6.21	F268B	417	Fresh	6	Grab	2
29.6.21	F266A	414	Fresh	7	Grab	2
29.6.21	F267A	415	Fresh	8	Grab	2
29.6.21	F265A	412	Fresh	9	Grab	2
29.6.21	F265B	413	Fresh	9	Grab	2
30.6.21	F269A	418	Fresh	5	Grab	2
30.6.21	F269B	419	Fresh	5	Grab	2
1.7.21	F270A	420	Fresh	1	Composite	2
1.7.21	F270B	421	Fresh	1	Composite	2
1.7.21	F271A	422	Fresh	2	Composite	2
1.7.21	F271B	423	Fresh	2	Composite	2
1.7.21	F272A	424	Fresh	3	Composite	2
1.7.21	F272B	425	Fresh	3	Composite	2
1.7.21	F273A	426	Fresh	4	Composite	2
1.7.21	F273B	427	Fresh	4	Composite	2
1.7.21	F274A	428	Fresh	5	Grab	2
1.7.21	F274B	429	Fresh	5	Grab	2
5.7.21	F275A	430	Fresh	5	Grab	2
5.7.21	F275B	431	Fresh	5	Grab	2
6.7.21	F276A	432	Fresh	5	Grab	2
6.7.21	F276B	433	Fresh	5	Grab	2
6.7.21	F280A	438	Fresh	6	Grab	2
6.7.21	F280B	439	Fresh	6	Grab	2
6.7.21	F278	436	Fresh	7	Grab	2
6.7.21	F279	437	Fresh	8	Grab	2
6.7.21	F277A	434	Fresh	9	Grab	2
6.7.21	F277B	435	Fresh	9	Grab	2
7.7.21	F281A	440	Fresh	5	Grab	2
7.7.21	F281B	441	Fresh	5	Grab	2
8.7.21	F282A	442	Fresh	1	Composite	2
8.7.21	F282B	443	Fresh	1	Composite	2
8.7.21	F283A	444	Fresh	3	Composite	2
8.7.21	F283B	445	Fresh	3	Composite	2
8.7.21	F284A	446	Fresh	4	Composite	2

8.7.21	F284B	447	Fresh	4	Composite	2
8.7.21	F285A	448	Fresh	5	Grab	2
8.7.21	F285B	449	Fresh	5	Grab	2
12.7.21	F286A	450	Fresh	5	Grab	2
12.7.21	F286B	451	Fresh	5	Grab	2
13.7.21	F287A	452	Fresh	5	Grab	2
13.7.21	F287B	453	Fresh	5	Grab	2
13.7.21	F291A	460	Fresh	6	Grab	2
13.7.21	F291B	461	Fresh	6	Grab	2
13.7.21	F289A	456	Fresh	7	Grab	2
13.7.21	F289B	457	Fresh	7	Grab	2
13.7.21	F290A	458	Fresh	8	Grab	2
13.7.21	F290B	459	Fresh	8	Grab	2
13.7.21	F288A	454	Fresh	9	Grab	2
13.7.21	F288B	455	Fresh	9	Grab	2
14.7.21	F292A	462	Fresh	5	Grab	2
14.7.21	F292B	463	Fresh	5	Grab	2
15.7.21	F293A	464	Fresh	1	Composite	2
15.7.21	F293B	465	Fresh	1	Composite	2
15.7.21	F294A	466	Fresh	2	Composite	2
15.7.21	F294B	467	Fresh	2	Composite	2
15.7.21	F295A	468	Fresh	3	Composite	2
15.7.21	F295B	469	Fresh	3	Composite	2
15.7.21	F296A	470	Fresh	4	Composite	2
15.7.21	F296B	471	Fresh	4	Composite	2
15.7.21	F297A	472	Fresh	5	Grab	2
15.7.21	F297B	473	Fresh	5	Grab	2
19.7.21	F298A	474	Fresh	5	Grab	2
19.7.21	F298B	475	Fresh	5	Grab	2
20.7.21	F299A	476	Fresh	5	Grab	2
20.7.21	F299B	477	Fresh	5	Grab	2
20.7.21	F303A	482	Fresh	6	Grab	2
20.7.21	F303B	483	Fresh	6	Grab	2
20.7.21	F301	480	Fresh	7	Grab	2
20.7.21	F302	481	Fresh	8	Grab	2
20.7.21	F300A	478	Fresh	9	Grab	2
20.7.21	F300B	479	Fresh	9	Grab	2
21.7.21	F304A	484	Fresh	5	Grab	2
21.7.21	F304B	485	Fresh	5	Grab	2