Understanding and managing uncertainty and variability for wastewater monitoring beyond the pandemic: Lessons learned from the United Kingdom National COVID-19 Surveillance Programmes

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Abstract

The COVID-19 pandemic has put unprecedented pressure on public health resources around the world. From adversity opportunities have arisen to measure the state and dynamics of human disease at a scale not seen before. Early in the COVID-19 epidemic scientists and engineers demonstrated the use of wastewater as a medium by which the virus could be monitored both temporally and spatially. In the United Kingdom this evidence prompted the development of National wastewater surveillance programmes involving UK Government agencies academics and private companies. In terms of speed and scale the programmes have proven to be unique in its efforts to deliver measures of virus dynamics across a large proportion of the populations in all four regions of the country. This success has demonstrated that wastewater-based epidemiology (WBE) can be a critical component in public health protection at regional and national levels and looking beyond COVID-19 is likely to be a core tool in monitoring and informing on a range of biological and chemical markers of human health; some established (e.g. pharmaceutical

usage) and some emerging (e.g. metabolites of stress). We present here a discussion of uncertainty and variation associated with surveillance of wastewater focusing on lessons-learned from the UK programmes monitoring COVID-19 but addressing the areas that can broadly be applied to WBE more generally. Through discussion and the use of case studies we highlight that sources of uncertainty and variability that can impact measurement quality and importantly interpretation of data for public health decision-making are varied and complex. While some factors remain poorly understood and require dedicated research we present approaches taken by the UK programmes to manage and mitigate the more tractable components. This work provides a platform to integrate uncertainty management through data analysis quality assurance and modelling into the inevitable expansion of WBE activities as part of One Health initiatives.

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Abstract

The COVID-19 pandemic has put unprecedented pressure on public health resources around the world. From adversity, opportunities have arisen to measure the state and dynamics of human disease at a scale not seen before. Early in the COVID-19 epidemic, scientists and engineers demonstrated the use of wastewater as a medium by which the virus could be monitored, both temporally and spatially. In the United Kingdom, this evidence prompted the development of National wastewater surveillance programmes involving UK Government agencies, academics and private companies. In terms of speed and scale, the programmes have proven to be unique in its efforts to deliver measures of virus dynamics across a large proportion of the populations in all four regions of the country. This success has demonstrated that wastewater-based epidemiology (WBE) can be a critical component in public health protection at regional and national levels, and looking beyond COVID-19, is likely to be a core tool in monitoring and informing on a range of biological and chemical markers of human health; some established (e.g. pharmaceutical usage) and some emerging (e.g. metabolites of stress). We present here a discussion of uncertainty and variation associated with surveillance of wastewater, focusing on lessons-learned from the UK programmes monitoring COVID-19, but addressing the areas that can broadly be applied to WBE more generally. Through discussion and the use of case studies, we highlight that sources of uncertainty and variability that can impact measurement quality and, importantly, interpretation of data for public health decision-making, are varied and complex. While some factors remain poorly understood and require dedicated research, we present approaches taken by the UK programmes to manage and mitigate the more tractable components. This work provides a platform to integrate uncertainty management, through data analysis, quality assurance, and modelling, into the inevitable expansion of WBE activities as part of One Health initiatives.

Key words: COVID-19, Wastewater-based epidemiology, Measurement variability, Uncertainty

Introduction 1

Environmental surveillance for public health 2

The acquisition of data and extraction of information from environmental 3
samples to manage and improve public health has been a cornerstone of soci- 4
etal development for nearly 200 years [1]. However, in comparison with medical 5
and pharmaceutical innovation, much of the work in the field of environmen- 6
tal public health is largely unrecognised outside of the professional commu- 7
nities invested in its use. This is evident with wastewater, a conduit for an 8
array of bio- and chemical markers that can be analysed to provide information 9
on human activities, behaviours, and health status in populations [2, 3, 4, 5], 10
but which has remained a relatively untapped resource given its known poten- 11
tial [6, 7, 8, 9, 10]. However, the negative perception of wastewater as solely a 12
polluting substance, to be removed (from the human and natural environments) 13
and cleaned (often by energy intensive processes), has undergone re-evaluation 14
in recent years. The focus on sewage as a resource rather than a waste prod- 15
uct is driving innovation in the water industry. Accordingly, the diversity of 16
biotic and abiotic features within the sewage matrix presents an opportunity to 17
acquire actionable insights through routine monitoring and analysis of its com- 18
ponents. An increasing technological and computational capacity for deriving 19
knowledge from measurements and data has manifested in efforts to 'smarten' 20
the water industry [11], fusing data science with fundamental science and engi- 21
neering principles. This provides opportunities for greater utilisation of sewage 22
for the common good, be it in the production of resources such as energy and 23
high value chemicals [12], or as a proxy of human health and behaviours, which 24
will have transformative impacts for society. 25

Wastewater-based epidemiology in a time of crisis 26

The nature and extent of the COVID-19 pandemic has driven an unprece- 27
dented response from a diverse array of stakeholders, internationally. The efforts 28
to tackle both the spread of the disease and its impact on populations have high- 29
lighted the need for disparate communities of scientists, government agencies, 30
decision makers and the public to work together and collectively address the 31
multiplicity of public health, economic and social challenges that have emerged 32
over the course of the pandemic [13, 14]. This is also the case with the de- 33
velopment of wastewater-based epidemiology (WBE) as an important tool to 34
facilitate the detection and spatiotemporal monitoring of SARS-CoV-2 virus 35
dynamics in the environment being undertaken in many countries [15, 16, 17]. 36

Several studies have shown that the risk of infection by active SARS-CoV-2 37
virus in pre- or post-treated wastewater is low, particularly in modern sanitation 38
systems [18, 19, 20, 21]. Nevertheless, inactive fragments of the virus RNA have 39
been shown to persist longer in water than infectious virus [22]. Subsequently, 40
most reports on SARS-CoV-2 detection and quantification in wastewater have 41
focused on monitoring of the inactive virus, more specifically, the targeting of 42
small regions of the virus genome using an array of analytical methods, such as 43
reverse transcription-quantitative polymerase chain reaction (RT-qPCR) [23], 44

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genomic sequencing [24, 25, 26], and, more recently, mass spectrometry [27], to detect and identify the emergence and spread of novel variants in the population.

Beyond COVID-19

Although the COVID-19 pandemic has highlighted the benefits of *ex-situ* monitoring human-associated disease in the environment, WBE has also been successfully applied in other public health contexts, such as tracking pharmaceuticals, such as self-prescribed drug usage in cities [28, 29], antimicrobial resistance [30], and assessment of human exposure to environmental pollution [6, 31, 10]. The severity of the current pandemic is a strong motivation for increased and integrated public health and environmental surveillance at national and supra-national scales [32, 33]. Whether it is future-proofing for potential new pandemics [34] or *water fingerprinting* to determine factors impacting both physical and mental health in communities [35], wastewater surveillance will become a vital tool at the disposal of governments and public health authorities at the nexus of public and environmental health beyond COVID-19.

Wastewater and Public Health, an uncertain relationship

The manuscript is focused on the understanding and management of uncertainty in WBE, framed by, but not limited to, lessons-learned from wastewater surveillance during the COVID-19 pandemic. For broader discussion of WBE and its implementation as a tool for informing decision-making and policy, there are a plethora of excellent review articles that may be referred to [15, 36, 37]. The data rich, technologically diverse and computationally powerful resources available for WBE present an opportunity to deliver next-generation public health solutions in combination with targeted or passive environmental monitoring. Deriving an understanding of sources of uncertainty and implementing methods to estimate and account for measurement error is therefore critical for the design and implementation of wastewater surveillance to support public health decision-making. We posit that the insights presented here have wider consequences for WBE efforts beyond the pandemic.

Here we provide insights from our broad and extensive experiences gained while working with the United Kingdom (UK) WBE surveillance programmes and share this collective knowledge to support public health initiatives beyond the COVID-19 pandemic. The proceeding sections are then organised according to uncertainty and variability derived from source (population, shedding), in-network characteristics, and sampling and sample analysis. We finish by providing four case-studies related to separate aspects of applied WBE in which uncertainty and measurement variability are addressed and managed.

Uncertainty and its impact on wastewater surveillance

The perceived benefits of using measurements from wastewater samples for epidemiology include the relatively unbiased signal response to the determinant of focus (e.g. disease) and the provision of near real-time insights obtained from

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changes in the magnitude or direction of this response [38]. With respect to COVID-19, clinical sampling of individuals in the community, via nasopharyngeal swabs, saliva or serological tests, is subject to biases associated with factors common to sub-sampling of heterogeneous populations [39], and mass-testing to obtain a representative sample size is costly. Wastewater, on the other hand, provides an aggregated picture of community disease state through the measurement of virus RNA excreted by, theoretically, all viable shedders with the disease in the sewer catchment [40], and can be implemented at a relatively low-cost in comparison with clinical sampling [41]. In reality, however, the accuracy and representativeness of any measurement acquired from wastewater is subject to a number of influencing factors, which can be classed as observable (e.g. sample dilution by exogenous hydrological flows), or partially observable (e.g. in-network analyte decay/degradation). Two recent reviews of wastewater-based SARS-CoV-2 detection have focused on factors contributing to uncertainty in disease prevalence estimation [42] and, more specifically, errors associated with laboratory quantification using RT-qPCR [43].

It is well understood that environmental measurements are subject to extraneous factors that account for differing degrees of variability and uncertainty in the signal (see [44], for example), and surveillance for WBE is particularly impacted by the complexity of the media being sampled [35, 45, 42]. Figure 1 presents an overview of the known and potential sources of uncertainty in WBE for COVID-19, grouped into spatiotemporal classes (i.e., where and when the uncertainty is likely to impact the measurement). For COVID-19, variability manifests as a significant problem when different measured virus RNA concentrations are observed for, theoretically, the same proportion of infected individuals in the population. More precisely, it is the uncertainty arising between the target analyte (RNA) and its representation of the stressor of concern (disease prevalence or incidence). Unwanted variability can occur over time at a given sample site due, for example, to rainfall or snow melt entering into a combined sewer network during or after wet weather events, and diluting the analyte concentrations relative to a dry weather baseline. With target analytes such as virus particles, which can attach to solids in the network, the impact of increased flow in the sewer is likely non-linear due to the effects of turbulence and scouring on settled solids resuspension. Although, to our knowledge, no evidence of this currently exists for SARS-CoV-2.

Variation between sites is also a problem when using WBE measurements for comparison across geographies, or when aggregating to provide supra-catchment perspectives of target analyte dynamics. For example, a large catchment having a long residence time may systematically produce lower concentration measurements than a smaller site, even though the disease prevalence could be the same in both catchments. As shown in Figure 1, factors causing uncertainty or unwanted variability can range from large-scale processes, such as highly transient populations, to those at a smaller scale including laboratory specific methods [43]. In each case, strategies are needed to account for the variability in a way that is appropriate for the intended use of the data.

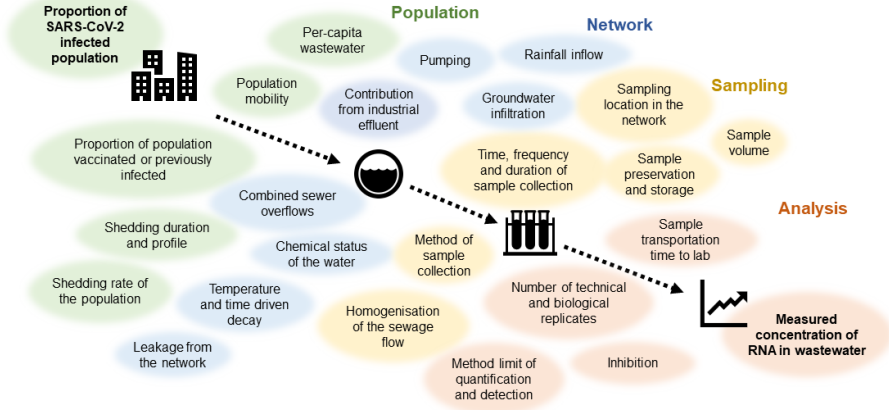Uncertainty is unexplained variability and, importantly, imposes a lower level

Figure 1: Summary of known and suspected sources of uncertainty for WBE; a perspective specific to UK wastewater surveillance of SARS-CoV-2

of confidence on a measurement than accountable and manageable (signal) variation [46]. Evaluation of uncertainty is necessary for WBE as quantifying the error bounds (and understanding the limits) of sample measurements is critical for capturing the inherent risk associated with public health decision-making processes. These risks are similar for likely all applications of WBE, i.e. incorrect estimation of target analyte(s); inability to compare measurements from different environments or under different conditions; loss of confidence in ability to detect or quantify the target analyte(s). The risks associated with uncertainty to wastewater surveillance of COVID-19 are wide-ranging and depend on its use-case. For example, recent attention focused on how using measurements from wastewater in epidemiological models [47, 48] could increase parametric uncertainty and error bounds on model estimates [49]. This, in turn, will affect the suitability of the model for tracking and predicting the dynamics of the disease [50]. Using raw wastewater measurements without accounting for factors that can bias interpretation, such as wastewater dilution or signal decay, may have a significant impact for decision-making when used to complement other sources of disease prevalence.

COVID-19 Wastewater Surveillance in the United Kingdom

England

Sampling of wastewater in England is being carried out by the Environment Agency, UK Water utilities, and the Environmental Monitoring for Health Protection team, part of the Joint Biosecurity Centre created to support government response to the COVID-19 pandemic. Sample collection started in June 2020 at the inlets to 44 sewage treatment works (STWs). The sites were selected to provide good population coverage and geographical representation across the

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country. In total, the original sites covered 17.7 million people (over 31% of the population of England). The sampling capacity was increased considerably at the start of 2021 and, as of July 2021, comprised 556 sites, including 263 STWs, 238 network sites (manholes or pumping stations in the sewer catchment), and 55 near-source sites (single or groups of buildings). The sites are distributed across the networks of the nine water utilities in England. By July 2021, wastewater sampling covered 39.4 million people (70% of the population of England). Recent sites were selected according to multiple criteria including demographic disease risk, population coverage and, for network sites, access points (e.g. manholes, pumping stations) that ensure safe access and well-mixed samples. STWs are sampled four times per week, using either autosamplers (composite) or by grab, or spot, sampling, post influent screening. The method of sampling is typically dependent on infrastructure at the STWs. An example of the potential for large variability in wastewater concentration between samples taken, either as grab or composite, is shown in Figure S3. In-network samples, collected upstream of the STWs, are used to constrain areas of concern in nine 'core' cities representing the largest conurbations in England, and three smaller strategic cities (based on historical COVID-19 trends). Samples from network sites are collected daily, mostly as grab samples, while near-source samples are largely taken using autosamplers at a fixed sub-sampling frequency (See Figure S1 for an overview, as of July 2021).

Scotland

Sampling and testing of wastewater across Scotland has been performed by Scottish Water and the Scottish Environmental Protection Agency (SEPA). In total there are over 1,800 sewage and wastewater treatment works in the Scottish Network, serving from fewer than 100 people to populations over 600,000. Initial testing of the premise that SARS-CoV-2 virus fragments were detectable in wastewater began in April 2020 with the development of a national monitoring programme operationalised by late May 2020. A network of 28 sites were initially prioritised, designed to maximise the coverage of population across Scotland's 14 National Health Service (NHS) Health Regions, while also ensuring that laboratory capacity was available at that time. The 28 sites covered a total of 2.6 million people (just over half of the five million sewered population in Scotland), with the goal of achieving a coverage of 40% in each of the 14 regions. As the need for wastewater monitoring has increased, so too has the monitoring network, which has expanded to 108 sites covering 4.2 million people. Autosamplers are used to obtain composite samples from the influent at each sewage works over a 24-hour period, which are then sent to SEPA for analysis. Results are then published via data visualisation dashboards. One dashboard, designed for the general public, holds the raw virus concentrations for each site (https://informatics.sepa.org.uk/RNAmonitoring), while a second dashboard, designed for public health officials, has additional metrics and comparisons to reported case numbers.

Additionally, Scottish Water, SEPA and a variety of NHS and Public Health professionals from across Scotland have been working together to collect and

analyse samples from within the sewer network itself (via manholes). These were sited at the request of health professionals in order to better understand the virus prevalence in areas of concern within larger sewer networks. These samples are taken by means of a grab sample. In total, 28 such sites have been monitored at one time with 14 still being active as of July 2021.

In total, over 5,000 samples have been tested and recorded as of July 2021. The sampling frequency varies between sites depending on several factors and has changed at different times as the needs of stakeholders has changed over time. Sampling at treatment work inlets has been variable, with the majority sampled at a frequency of once or twice a week, but some as much as 4 times per week, a trade-off between lab capacity and data density. All in-network samples are monitored five times per week in their initial week to establish a baseline before being sampled twice a week thereafter.

Wales

The Wales wastewater monitoring programme started as a pilot in March 2020 as the first wave of COVID-19 spread across the UK [51]. This early work highlighted the potential for tracking SARS-CoV-2 and also led to the development of robust methodologies for extracting and quantifying the virus in wastewater [52]. This pilot phase was then expanded in September 2020 to 20 sites across the country. These sites were initially sampled three times per week, increasing to five weekdays by June 2021, to try and reduce the variability in the wastewater SARS-CoV-2 RNA signal and, thus, improve its usability. One of the major challenges in Wales has been the lack of on-site infrastructure needed to take composite wastewater samples. Therefore, all samples are currently taken as grab samples, targeted at the early morning wastewater peak (between 08:00 and 11:00 h). However, it is now known from deploying the enveloped *Pseudomonas* virus, phi6, into the sewer network that this approach may miss the effluent peak, leading to an underestimation of viral abundance. Another challenge has been the poor geographical coverage in Wales. The country has two urban corridors centred around the northern and southern coasts in which 80% of the population resides. Consequently, wastewater surveillance has focused in these areas, leaving ca. 20% of the country, mainly in central and western Wales unmonitored, resulting in uncaptured localised outbreaks in small urban centres. Another major issue is that the capital city, Cardiff, is served by a very large centralised STW (930,000 people). Although this captures 30% of the Welsh population in one sample, the lack of granularity prevents the potential for using wastewater surveillance to target regions of the city to control localised COVID-19 outbreaks (e.g. implementation of surge testing and walk-in vaccination centres). The lack of sampling at weekends also prevents capturing of the large migration of tourists from North West England into North Wales. The wastewater samples taken in Wales were also used to pilot their potential to track other viruses of public health interest (e.g. influenza A and B, norovirus, respiratory syncytial virus, enterovirus D68). Analysis showed that wastewater contained all these viruses with the exception of Enterovirus D68. Looking forward, the Wales wastewater surveillance programme is now being expanded

to many more sites with the aim to capture 90% of the population on mains 247
sewerage and with analysis of a greater number of public health indicators. 248

Northern Ireland 249

Wastewater surveillance in Northern Ireland (NI) has several unique chal- 250
lenges compared to other parts of the UK, which are related to the urban and 251
rural distribution of population. NI has an extensive wastewater treatment net- 252
work operated by Northern Ireland Water (NI Water). In total, there are 1114 253
STWs in the NI Water network, serving just under 80% of the NI population. 254
Each STW serves a wastewater drainage catchment area of variable sizes. Up 255
to 68% of the NI Population is served by the 40 largest STWs. However, these 256
larger STWs serve predominantly urban, as opposed to rural communities, and 257
tend to be disproportionately located in eastern parts of NI. The integrated 258
wastewater testing and geographic surveillance programme for SARS-CoV-2 in 259
NI is led by Queen's University Belfast, funded by the Department of Agricul- 260
ture, Environment and Rural Affairs (DAERA) in collaboration with the Public 261
Health Agency NI (PHA-NI). 262

Currently there are SARS-CoV-2 wastewater samples being taken at 14 sam- 263
pling sites at STWs covering 35.3% of the NI population. The current sampling 264
strategy was based on several key factors, including population coverage, geo- 265
graphic distribution of wastewater surveillance and a close alignment and agile 266
response to PHA-NI test and trace results. Consideration is being given to sig- 267
nificantly expanding the sampling sites, allowing for the wastewater surveillance 268
of a significant portion of the NI population. 269

An important aspect of the approach in NI has been the use of Geographical 270
Information Systems (GIS) to develop spatial GIS-based wastewater monitor- 271
ing and reporting system integrating public health data to model population 272
geographies and align with wastewater drainage catchment areas. Modelling 273
population across NI using GIS provides an approach to estimate populations 274
covered by the wastewater network, the population within individual wastew- 275
ater drainage catchment areas, and an estimate of how to balance capturing 276
the maximum percentage of the population from a relatively limited number 277
of sample sites, while ensuring an adequate geographic spread across NI. This 278
has been achieved through the development of an interactive wastewater SARS- 279
CoV-2 Surveillance Dashboard. The Dashboard provides a display of the anal- 280
ysis results of sampling at various locations in NI and enables users to see and 281
understand population distribution modelling across NI wastewater network. 282
This offers the most efficient and informative sampling strategy for the pro- 283
gramme, and an approach to contextualise wastewater test results in terms of 284
socio-economic deprivation 285

An indication of the extent of wastewater surveillance across all four regions, 286
indicating the geospatial locations of sampling sites (as of July 2021), is shown 287
in Figure S2. 288

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Drivers of variability and sources of uncertainty

Population factors

Knowledge of the contributing population size upstream of the sampling location is important for calculation of per capita concentrations and to facilitate comparison between sample sites. It is important to have an accurate estimate of population size, a) to ensure that inter-site comparisons are made on an equivalent basis, and b) to account for the effects of intra-site population change on the loads of measured target(s) in the wastewater. Population size is, however, uncertain and variable. The mean population size may be estimated based on census data and additional demographic statistics, but such estimates cannot be easily updated to account for changes resulting from births, deaths and migration, and can quickly become outdated [53]. Fluctuations in population during the sampling period can contribute further uncertainty. These include, for example, weekly and seasons variations due to the flux of commuters and tourism or student populations, respectively. Dynamic population estimates may be obtained using water quality parameters such as ammonia and orthophosphate; however, this is subject to bias due to the contribution of additional sources such as industrial discharges [54]. The use of mobile device data [55] and biomarkers present in urine (e.g. caffeine, pharmaceuticals) [56], are alternative metrics that have been shown to significantly reduce measurement uncertainty when used to estimate population size for normalisation of target analyte concentrations.

To illustrate the potential effects of population variability, Figure 2 shows the impact of reporting per capita SARS-CoV-2 loads instead of SARS-CoV-2 concentrations on trends identified at a STW site in England. In this case, a site-specific mean daily ammonia discharge per capita ($\overline{x}$) is estimated using Equation 1, based on measured ammonia concentrations (X_d) and wastewater flow rates (Q_d) for the entire sampling period and the Office for National Statistics population estimate (P) for the catchment. SARS-CoV-2 gene copies per capita per day (L_d) are then calculated on a daily basis using Equation 2, based on this value and the measured SARS-CoV-2 (S_d) and ammonia concentrations for the current day.

$$\overline{x} = \frac{\overline{X_d Q_d}}{P} \tag{1}$$

$$L_d = \frac{S_d \overline{x}}{X_d} \tag{2}$$

Error bars are included in Figure 2 to indicate standard deviation resulting from variability in the site-specific ammoniacal nitrogen discharge per capita; these do not capture any other sources of uncertainty.

An important and poorly understood source of uncertainty related to proportion of contributing population is the quantity and rate of analyte released into the network through faecal or urinary shedding. Faecal shedding of SARS-CoV-2 RNA varies both between individuals and over the infection course of
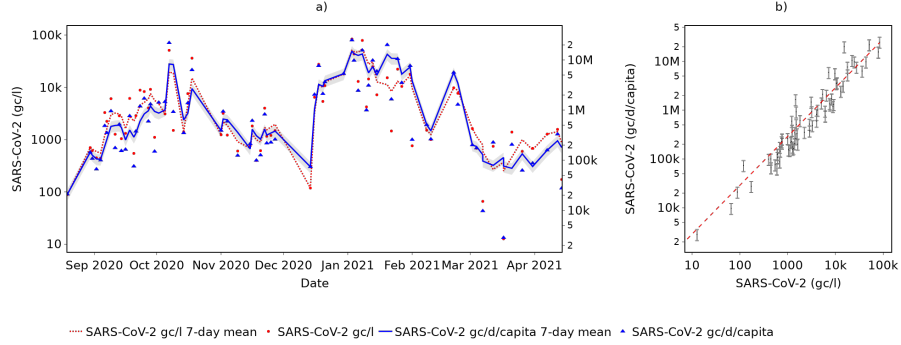
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Figure 2: Example comparison of wastewater SARS-CoV-2 concentrations and SARS-CoV-2 loads per capita at an English sewage treatment works.

any given individual [40]. Indeed, a recent study has indicated, from near- 328
source data, that faecal shedding peaks on average 6 days post-infection (95% 329
Uncertainty Interval 4 - 8 days) [57]. The impact of shedding variability be- 330
tween individuals is attenuated for large catchments and during high prevalence 331
periods because the sewerage system naturally averages the signal from many 332
people [58]. Due to the greater variability in the wastewater measurements 333
compared with clinical data sources, the power of WBE surveillance, at least 334
for COVID-19, is expected to be greatest when transmission (and prevalence) 335
or clinical testing is low; i.e. capturing (re)emergence of disease in a commu- 336
nity. However, quantitative estimates of the number of individuals infected are 337
likely to remain elusive when infection prevalence is low or the sampled popu- 338
lation is small, such as for near-source sampling. Temporal variability of viral 339
RNA shedding over the infection course implies that the concentration of SARS- 340
CoV-2 gene copies in wastewater is a convolution of disease incidence with the 341
shedding profile [59]. Consequently, techniques for relating epidemiological in- 342
dicators to wastewater-based signals need to consider multiple time lags, for 343
example by employing distributed lag models [60]. Studies to investigate viral 344
shedding prior to symptom onset are urgently required because existing data 345
have been collected from hospitalised patients [61, 62, 63]. Similarly, the im- 346
pact of vaccination on faecal shedding of viral RNA is unknown, although data 347
from nasal swabs suggest that viral loads are likely to be reduced [64]. Given 348
this, quantifying virus at near-source with any precision remains elusive, and 349
further work to understand faecal shedding distribution is critical for adoption 350
of wastewater measurements in epidemiological models for estimating transmis- 351
sion rates (i.e. effective reproduction number, R_{eff}) [65]. This information 352
can be applied broadly to other analytes routinely shed in the urine and faeces 353
that correlate to public health indicators, although shedding profiles could be 354
markedly different from those for viruses. 355

11

Characteristics of the sewage network (proportion of gravity-fed or pres- 357
surised pipes; size of the network; retention capacity; location and triggering of 358
combined sewer overflows (CSOs), and use of sustainable urban drainage, in- 359
frastructure to separate stormwater flow in the catchment) may impact both the 360
quantity of analytes of interest within the water and their distribution within 361
the sewage volumes. 362

The daily flow patterns in most wastewater systems are oscillatory, driven 363
by multiple factors such as sewer network design, industrial discharge events 364
and prevailing weather conditions. However, the flow signal, under dry weather 365
flow conditions, is governed by household water usage, which often presents as 366
morning and evening 'peak flow' pulses, especially in small catchments. These 367
daily oscillations are damped in catchments with a wide network or large storage 368
capacity where peak flow can be retained and processed later, leading to a 369
homogenisation of the signal [66]. Pumps across the network or at the inlet of 370
STWs can also homogenise analyte concentrations within the flow, with sumps 371
or wet wells acting as small retention tanks. Ingress of non-human derived flow, 372
e.g. from rainfall or snow melt, in combined sewers, or groundwater infiltration 373
in all sewers, can bias measurements by signal dilution. 374

Sewer network size, sewer gradient, pipe friction, and presence of retention 375
tanks can impact the time-of-travel of wastewater 'packets' (typically < 1 to 376
24-hours in the UK, dependent on catchment size), and may reduce target con- 377
centrations that are prone to degradation [67] (i.e. those with a short T90, 378
the time for one log unit reduction in concentration). Moreover, the type of 379
sewage system (gravity-fed or pressurised pipes) can directly impact the decay 380
rate of analytes of interests due to differences in biofilm composition within 381
these two environments [68] (fully anaerobic for rising mains, and mixed anaer- 382
obic/aerobic in gravity sewers). Further, the sheer stress created by cycling 383
between pressurised and unpressurised pipes might further hasten the decay 384
of labile analytes. Finally, retention tanks may also increase the binding of 385
hydrophobic targets, such as SARS-CoV-2 virions, with suspended solids to 386
form complex matrices [69], which may obfuscate their subsequent detection by 387
laboratory analysis, or result in settling-resuspension phenomena in the sewer 388
pipes [70], decoupling the temporal dynamics of the virus RNA from the dis- 389
charge event. Significant sewer pipe leakages may also influence the fate of 390
virus, and consequently its downstream detection and quantification, especially 391
in older networks. 392

Adjusting for the impact of network characteristics across a national pro- 393
gramme is challenging due to the need for quantitative, comparable information 394
for individual site networks. In England, this data is typically owned by private 395
water utilities and, in many cases, the precise configuration of the network is 396
not known, unless access is provided by the companies. However, the impact of 397
some site characteristics can be mitigated by taking into account co-dependent, 398
measurable parameters. For example, ammonia concentration can be used as a 399
proxy for the dilution effects in combined sewers, and catchment area is a rough 400

approximation for network size. While proxy variables are useful in the absence 401
of true measurements, their use in management of target measurement uncer- 402
tainty may be limited by how representative it is of the variable of interest. The 403
use of multi-biomarkers to better represent human wastewater contribution (See 404
Case Study 4), or GIS-based modelling and public health information to better 405
characterise catchment population are currently employed methods to mitigate 406
this limitation. 407

Sampling strategy 408

In the context of WBE and public health surveillance, acquiring a repre- 409
sentative sample that captures the analyte of interest is fundamental to sup- 410
port actions that have the potential to impact the well-being of individuals and 411
communities [66]. The source of the analyte(s) targeted, through urine (e.g. 412
metabolites of pharmaceuticals) or faeces (e.g. viruses), can impose additional 413
variability in measurements, and beyond the COVID-19 pandemic, wastewater 414
surveillance programmes will need to build in sampling flexibility to account for 415
this uncertainty [71]. 416

Broadly, there are two ways to take a sample: (i) a 'grab' or 'spot' sample 417
where a single sample of wastewater is taken using a small container, and (ii) a 418
'composite' sample where samples are taken regularly throughout the day using 419
an automated device (autosampler) and the samples mixed together in a sin- 420
gle container. Several autosampling modes may be used to create a composite 421
sample: *time-proportional*, where a constant sample volume is taken at regular 422
time intervals; *flow-proportional*, where the time interval is kept constant but 423
the sample volume adjusted to the flow in the sewers; and *volume-proportional* 424
sampling, where a constant volume sample is taken each time a fixed volume 425
passes through the sewer. Measurement uncertainties are heavily impacted by 426
the type, mode and timing of sampling depending on variability of flow and an- 427
alyte concentration over time [66]. The difference in the probability of detection 428
between sampling methods becomes greater as prevalence (of the target analyte) 429
decreases. Specifically, when concentration is low, detection likelihood via grab 430
sampling would be much lower than with composites, while as concentration 431
increases, the probability of detection using grabs becomes comparable. This 432
would suggest that composite sampling is preferable during periods of low tar- 433
get analyte concentration. However, if the daily signal is concentrated in time, 434
well-timed grab samples could capture higher concentrations than is possible 435
with composite samples (as shown in Figure 3). The nature of composite sam- 436
ples means that it dilutes a 'sharp' signal, which can be a disadvantage at low 437
prevalence times. Areas with a more temporally constant signal would be less 438
sensitive to the choice of sampling method. This risk can be mitigated somewhat 439
by the appropriate design and use of autosamplers. However, the autosampling 440
method can also impact measurement confidence. Time-proportional sampling 441
can lead to under- or over-weighting of sample during periods of high or low 442
flow, respectively, resulting in loss of representativeness. A flow-proportional 443
sampler extracts a fixed volume of sewage when a predetermined volume of flow 444
has accumulated. The resulting daily sample will be weighted by flow and could 445

13

be argued to be more representative of the conditions of that day, assuming that 446
the substance of interest is distributed uniformly through the day. However, on 447
low flow days the sample volume may be too low for effective analysis, while 448
on wet days the full volume may have been taken long before the end of the 449
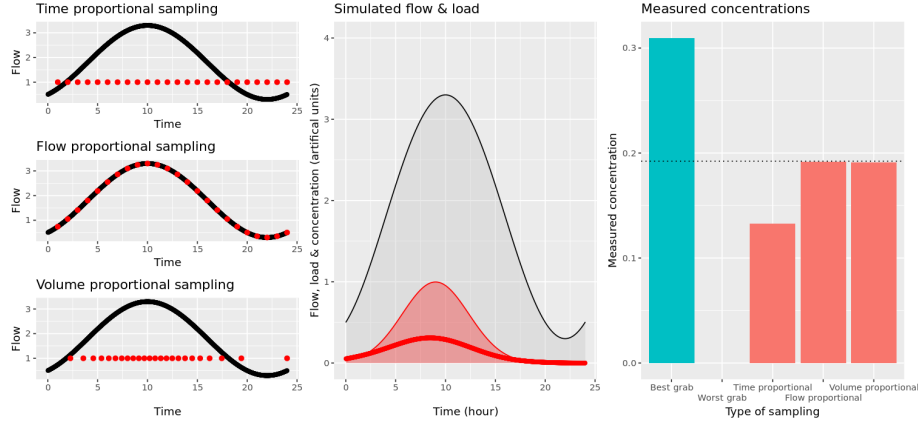sampling period. 450

Figure 3: Simulated sample concentrations using different sampling methods. Left: Representation of composite sampling methods; the flow is represented in black and sampling time and volume in red. Middle: Simulated flow (black), load (red), and resulting concentration (thick red line). Right: Resulting concentrations based on the sampling method. Highest and lowest values represent the maximal and minimal concentration that can be obtained with grab sampling from this simulated flow/load.

As samples are not always collected daily, sampling cadence must be con- 451
sidered when determining WBE sampling strategies. Aliasing effects may result 452
in incorrect interpretation of signal dynamics, or produce artefacts in mod- 453
els used for back-calculation of target stressors, for example [72]. A sampling 454
frequency as close to the daily cadence will reduce uncertainty arising from tem- 455
poral variability. This has been quantified through a data ablation experiment 456
for 186 network sites monitoring SARS-CoV-2 in England, for which a number 457
of samples were artificially removed to compute the relative bias introduced by 458
reducing the sampling cadence, as shown in Figure 4. Consideration of sampling 459
frequency in relation to sample location in the network is necessary. In small 460
catchments, or near-source applications (e.g. monitoring of critical infrastruc- 461
ture such as prisons, care homes and schools), high-rate composite sampling 462
may not be enough when all discharge events should be captured. Technolo- 463
gies that can provide continuous active sampling or passive samplers are more 464
suitable in this context [73, 74]. 465

Variability due to differences within and between site sampling deployment 466
have a potential be a significant influence on the measurement, particularly 467
when establishing a national surveillance system with a large number of sites 468
and different site personnel involved. Detailed and clear sampling protocols and 469
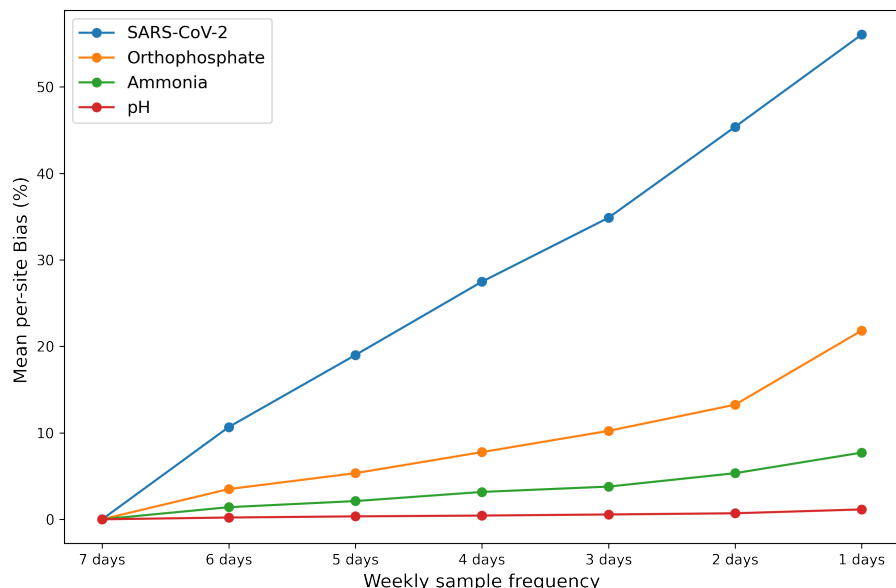ongoing training of staff are essential to minimise some of the sources of this 470

14

Figure 4: Per-site mean percent bias, compared to the 7-day baseline, in SARS-CoV-2 and other marker measurements when reducing the sampling frequency artificially for 186 network sites across England. Whilst the bias for ammonia, orthophosphate and pH is limited to $\sim 10\%$, a bias of up to $\sim 65\%$ can be introduced in the mean estimate of SARS-CoV-2 when decreasing the cadence frequency.

variation.

Sample analysis

Wastewater is a highly complex and variable media, containing compounds that can decrease detection sensitivity, which results in false-negative results, whilst also compromising the ability to quantify the analyte of interest, such as genetic fragments, accurately. As significant knowledge performing sample analysis has been gained while monitoring the COVID-19 pandemic, insights relating to the uncertainty arising from SARS-CoV-2 quantification have been addressed, leading to a consolidated application of wastewater lab-analysis for WBE.

Due to the low concentrations of SARS-CoV-2 in wastewater, methods are required to pre-concentrate the virus prior to analysis. The most commonly used methods include precipitation with salt or polyethene glycol (PEG [52]), electrostatically charged membrane filtration [75], ultrafiltration [76], or adsorption-precipitation with aluminium chloride or silica [77]. Due to the expense, poor availability and potential for blockages with ultrafiltration devices, the English wastewater surveillance programme initially adopted the PEG precipitation method. This was based on previous success at recovering viruses from

wastewater [78] and also that it does not require an extra step for pH measure- ₄₈₉ ment and correction. However, the overnight precipitation step in the method ₄₉₀ increased the time from sample collection to reporting. A decision was then ₄₉₁ made to switch from PEG to salt (ammonium sulphate, AS) precipitation as ₄₉₂ the latter only requires a 1-hour incubation step. Parallel studies with dupli- ₄₉₃ cate wastewater samples showed no significant differences in recovery between ₄₉₄ the two methods for SARS-CoV-2 RNA (data not shown). This AS workflow ₄₉₅ now allows viral RNA to be concentrated, extracted and quantified within a ₄₉₆ 24-hour window. ₄₉₇

Another key step in SARS-CoV-2 determination from wastewater is to pro- ₄₉₈ duce RNA extracts that ensure consistency in the quantity, quality, and purity ₄₉₉ of extracted nucleic acids for their applicability in downstream processes (e.g. ₅₀₀ detection, quantification, sequencing). SARS-CoV-2 determination is generally ₅₀₁ carried out with a nucleic acid-based PCR assay. However, given the wide-range ₅₀₂ of PCR inhibitors in wastewater and the options available for handling them, ₅₀₃ no single method serves all applications; a multifaceted approach being the best ₅₀₄ solution to avoid amplification failure. Therefore, efficient extraction methods ₅₀₅ are required to purify inhibitor-free RNA, together with the use of inhibitor- ₅₀₆ tolerant quantitative reverse transcription PCR (RT-qPCR) mixes containing ₅₀₇ enhancers/additives to help reduce inhibition (e.g. gp32 and BSA). On the other ₅₀₈ hand, the low levels of SARS-CoV-2 in wastewater means that sample dilution ₅₀₉ to alleviate inhibition is not recommended or should be limited [43]. Alter- ₅₁₀ natively, the samples can be analysed by one-step digital-PCR (dPCR) rather ₅₁₁ than RT-qPCR. To estimate the efficiency of viral RNA recovery, all samples ₅₁₂ in the English programme are spiked with phi6 virus. Typically, the recovery ₅₁₃ of phi6 ranges from 1 to 50%, indicating that the viral recovery methods still ₅₁₄ need to be optimised for some wastewater types. This is supported by studies ₅₁₅ from England where wastewater has been spiked with heat-treated SARS-CoV- ₅₁₆ 2 and where recovery is often incomplete (ca. 30 - 50% recovery; Kevill *et al.*, ₅₁₇ 2021, unpublished). Alongside SARS-CoV-2, a range of other faecal-marker ₅₁₈ viruses (e.g. crAssphage, pepper mild mottle virus) have been measured in ₅₁₉ wastewater [36]. In the English programme, crAssphage was initially used to ₅₂₀ help normalise the SARS-CoV-2 results to account for dilution by industrial ₅₂₁ wastewater and rainfall, however, this created extra workload and delayed the ₅₂₂ workflow, and was subsequently dropped in favour of other indicators of faecal ₅₂₃ load (e.g. ammonia). Case Study 3 presents some specific results from our ₅₂₄ management of laboratory analysis uncertainty and variability across the UK ₅₂₅ wastewater surveillance programmes. ₅₂₆

Management of variability and mitigation of uncertainty ₅₂₇

Population normalisation and measurement correction ₅₂₈

Measurement correction is key to addressing variation resulting from sam- ₅₂₉ pling, sample transport and storage, as well as possible errors linked with sample ₅₃₀

processing (including sample preparation: biomarker extraction from wastewater, concentration, and analysis). Normalisation of data is important to reduce uncertainties related to changing wastewater flows (resulting from diurnal changes and seasonal variability in rainfall patterns), movement of population, biomarker sources (e.g. intake vs. environmental occurrence) as well as biomarker stability and its transformation (e.g. human metabolism or metabolic degradation of sewer microorganisms). WBE in chemical exposure studies (e.g. illicit drugs, pesticides, industrial chemicals, pharmaceuticals) has been subject to comprehensive evaluation of uncertainties due to its application requiring a reliable quantitative measurement (e.g. per capita drug consumption). In chemistry-based WBE, 24-hour composite sampling is strongly advised, as well as having labelled internal standards (analogues of biomarkers that do not exist in nature, e.g. benzoylecgonine D8, which is used as an internal standard to benzoylecgonine) used to compensate for errors occurring throughout sample storage, processing and analysis. Flow measurements of wastewater are required, as well as an understanding of stability of biomarkers in wastewater and their extraction efficiency/matrix effects (e.g. interfering chemicals during analysis). However, with biology- or pathogen-based WBE, grab sampling is still the norm and there is an inherent lack of flow measurements in large national campaigns, as well as in near-source applications, which skew the results and make the studies more qualitative in nature. Biomarker selection in chemistry-based WBE requires its pre-use validation including the following requirements: (1) originating in human (with no other sources), (2) accounting for human metabolism, (3) stable in sewers, and (4) with excellent analytical performance in biomarker quantification (the latter is usually followed by inter-lab studies, or 'ring trials'). These factors are yet to be fully evaluated in biology-based WBE (and indeed in new chemistry WBE applications), where biomarkers are stressors themselves, and there is limited (albeit rapidly increasing) understanding of analytical method performance and stability of biomarkers. Most importantly, it is currently impossible to differentiate between different sources of stressor release to the sewerage systems.

Chemical analysis of certain biomarker groups, especially metabolites of high-usage, prescription only pharmaceuticals (e.g. antidepressants, antidiabetics, and antiepileptics) with well-defined consumption patterns, can provide important insights into diurnal changes in population size contributing to wastewater. Antidepressants are shown in Figure 5 as an example. Measurements were undertaken over seven consecutive days in five English towns/cities as discussed in Case Study 4. A significant positive relationship between the daily loads of antidepressants, their metabolites and the population size served by respective wastewater treatment plants was observed (Pearson coefficient, $r \geq 0.997$, $p < 0.0002$). As expected, metabolites showed the lowest spatiotemporal variability in the studied intercity catchment ($< 16\%$ for desmethylvenlafaxine and $<12\%$ for desmethylcitalopram), when compared to their respective parent antidepressants (venlafaxine and citalopram), which can be directly disposed-off into the drain. This indicates their suitability as population markers. Figure 5 also indicates the benefit of normalisation in trying to understand consumption patterns.

17

In the figure, double normalisation was applied to account for variable flows and population. As a result, per capita change in consumption patterns can be observed and conclusions drawn regarding the variable consumption patterns in cities with different socioeconomic status. Further discussion on how certain variables affect back-calculations of chemical intake can be found in Case Study 4.
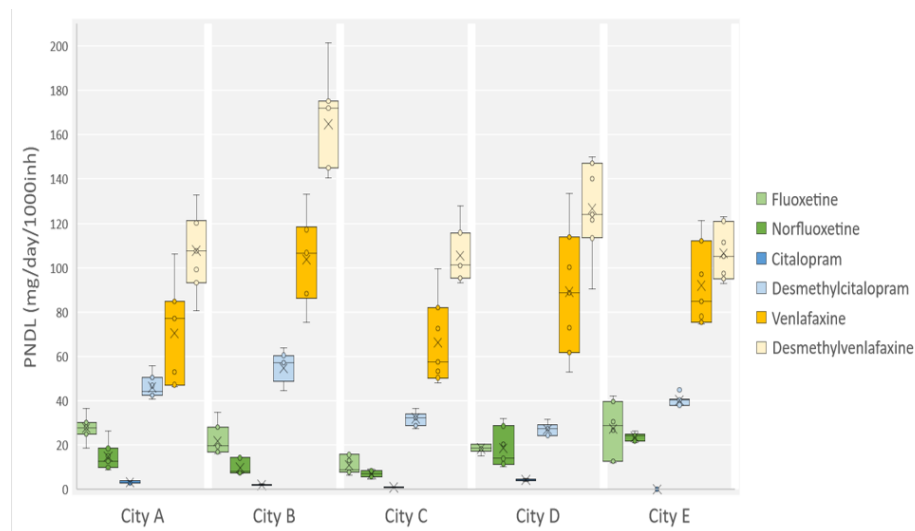

Figure 5: Population normalised daily loads (PNDL) of antidepressants (mg/day/1000 inhabitants) and their metabolites.

Design and implementation of sampling

The sampling strategies employed for SARS-CoV-2 surveillance across the UK have aimed primarily to address two key factors: percentage of the population covered and geographic representation, which includes both urban and rural area coverage. In addition, the sampling strategies have needed to allow for an agile sampling response to assist with surveillance of COVID-19 incidence clusters, as highlighted by governmental public health testing strategies. For sampling at STWs, these factors need to be facilitated by the regionally diverse privately and publicly owned sewage networks. Uncertainties arise in the actual population represented by the sampling strategies due to a mismatch between census administration geographies and population equivalents calculated for STWs. These may include estimates of the number of actual residents within a STW catchment, transient populations (i.e. those at workplaces, educational facilities, or communal gatherings such as sports or entertainment events), and the load placed upon each STW by industrial activity. Spatial data analysis approaches can be used to characterise the contribution of STW catchments to administrative geographies, which enable greater integration with public health case data [79].

For sampling at STWs, the use of composite samples can help mitigate uncertainty associated with diurnal flow variations (see Figure 3) but such samples may underestimate the magnitude of the peak concentration and, therefore, are more suited to understanding the average daily load within the sewer network catchment. Consideration of the ideal sampling site at each STW needs to account for the specific configuration of the inlet channels, equalisation storage, and mixing characteristics. In many cases, it is not possible to get a well-mixed sample with equal representation of all parts of the sewer catchment because of the design of the STW inlet piping. Several studies have sampled primary sewage sludge for SARS-CoV-2, with generally higher detection than from liquid influent samples [80, 81], although data on STW flows and process operation dynamics is required to fully characterise the period of time that each sample would represent. Sampling of solids has not been extensively performed in the UK. Mixed or combined sewerage systems (e.g. those receiving stormwater or industrial effluent) can also have a significant impact on sampling performance as dilution from additional flow and a more complex, or inhibitory, mix of wastewater constituents may obfuscate the ability to detect the signal (See Case Study 1 and Figure S4).

For network and near-source sampling, the large size and spatiotemporal complexity of urban water networks means that it is not economically or logistically feasible to collect a sufficient number of samples to ensure statistical significance of sampling results for estimating system wide average concentrations [82]. Consideration of the diurnal variation of flows from both domestic and industrial sources and impact of rainfall can help to select sampling locations that are less vulnerable to influence by these factors. Well-calibrated hydraulic models of the sewer networks can be a useful tool to understand dry weather and wet weather dynamics. For example, Figure 6 illustrates the modelled dry weather contribution to wastewater flow for one of the core cities sampled by the surveillance programme in England, showing that some locations are dominated by infiltration flows with less than 40% of total daily flow derived from domestic wastewater. Many of the network sites initially sampled in the UK consistently showed non-detectable levels of SARS-CoV-2 and ammonia, consistent with the model results, and these locations were subsequently removed from the sampling programme. Grab samples in sewer networks require precise timing to capture flows because many manholes are dry for large portions of the day, including near-source locations and upstream ends of the network. Many individual grab samples from network locations had non-detectable levels of SARS-CoV-2 in the core cities (see Figure S5). Sampling these locations daily, ideally with a slight variation in the time of sample collection, does not mitigate the underlying uncertainty associated with grab sampling but can assist with visualisation of trends and patterns despite the variability in individual sample results.
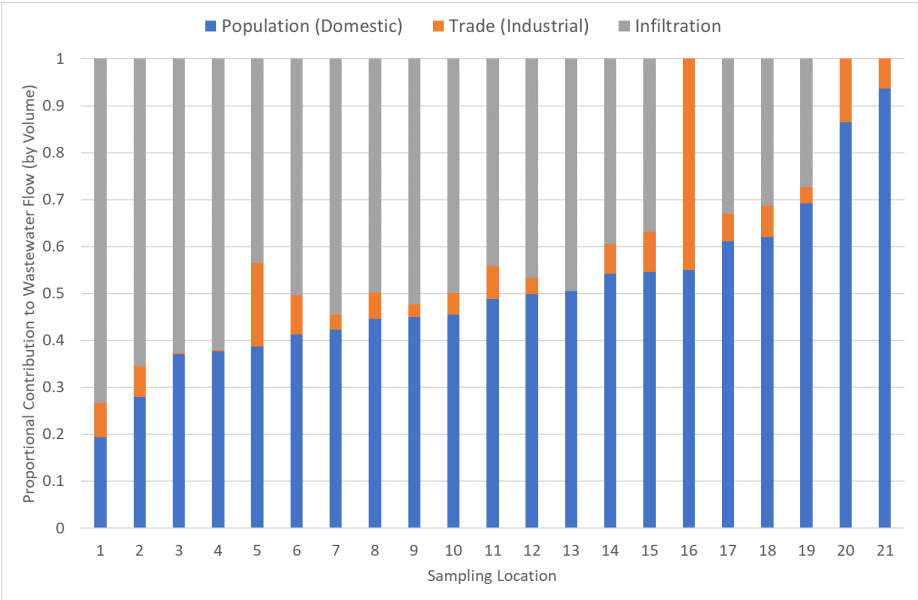
Figure 6: Contribution to wastewater flow by volume over a 24-hour dry weather day for sampling locations across one of the core cities, derived from the hydraulic model of the sewer network.

Case studies from the UK Wastewater Surveillance Programmes

Case study 1: Flow normalisation

Several approaches have been developed within the UK wastewater surveillance programmes to account for rainfall dilution of SARS-CoV-2 RNA measurements in wastewater. Given that flow data is only partially available across all the monitored sites, indirect normalisation techniques using other biochemical markers as a proxy for the real flow can be used, with an assumption that the majority of markers originated from a source with a constant load. Note that these corrections account for the temporal variability, but are not sufficient to estimate the average flow level, which can vary significantly between sites (as shown in the population normalisation section). Different techniques, based on a similar premise, are presented below for flow variability correction in (a) Scotland and (b) England. Dilution effects appear to only have a minor impact on SARS-CoV-2 concentrations in wastewater, with significant changes occurring only during heavy rainfall events or discharges from other sources. That is, variation due to dilution effects with sample-by-sample variability from other sources of noise (e.g. faecal shedding), appears to be minimal. However, from an epidemiological perspective, highly diluted measurements caused by storm events, for example, may result in the need to correct values by factors as much as 0.6 (results not shown), which would significantly skew interpretation of disease prevalence if ignored when interpreting the data.

20

a) Flow normalisation, as applied by the Scottish COVID-19 surveillance pro- 663
gramme 664

In Scotland, in addition to detecting and quantifying SARS-CoV-2, chemical 665
analytes, in particular ammonia, have been collected and processed from the 666
wastewater. These are available up to two weeks prior to flow measurements - 667
with flow, at some sites, not measured at all. As a result, a cross-site model 668
is used to relate ammonia concentrations with flow measurements, taking into 669
account population size as a proxy for faecal shedding in the catchments. 670

A linear mixed model (LMM), with flow related to ammonia and population 671
(on log10 scales), was developed and random intercepts and slopes were included 672
for each site. This model was shown to fit the Scottish data better than a simpler 673
linear regression model with the slopes for log10(ammonia concentration) and 674
for log10(population) fixed at -1 and +1, respectively. Model performances 675
were compared using Akaike Information Criterion (AIC) and a Kenward-Roger 676
approximation of the Wald test for LMMs. A review of the Scottish data at each 677
site, using a generalised additive model (GAM) with the Tweedie distribution, 678
showed that unnormalised data was equally or more noisy than normalised (but 679
scaled) data once trends were taken into account. A graph of example sites with 680
fitted ammonia/flow curves is shown in Figure S7. 681

Current practice in Scotland is to normalise by flow rate if available, then 682
ammonia concentration. If neither are available, then an estimate of flow based 683
on a spline function using recent ammonia trends is used (fitted on overall 684
national trends over time plus site specific effects). If 'capping' is an issue, where 685
CSOs prevent sewer overloading by discharging to natural water bodies, then 686
normalising against ammonia would be preferential as a more representative 687
measure of true flow. Anecdotally, it is not thought that capping is a major issue 688
in Scottish wastewater networks, based on communication with water sector 689
professionals. 690

b) Flow normalisation, as applied by the English COVID-19 surveillance pro- 691
gramme 692

The approach assumes that the flow F_t at time t is not directly observable. 693
Therefore, information about the flow can be obtained by observing the corre- 694
lation of concentrations ρ_{ti} of different markers i and that a dilution estimate 695
based on a single marker is not robust enough as it is not possible to distinguish 696
between a decrease in flow and an increase in marker load, e.g. due to a one-off 697
industrial or agricultural discharge. The model assumes 698

$$\log F_t \sim \text{Normal}\left(0, \lambda^2\right)$$
$$\log x_{ti} \sim \text{Normal}\left(\mu_i, \sigma_i^2\right)$$
$$\therefore \log \rho_{ti} = \log x_{ti} - \log F_t,$$

where λ^2 is the flow variance, μ_i and σ_i^2 are the mean and variance of the load 699
of marker i (all in log space). $\langle \log F_t \rangle$ is fixed at 0 to identify the model. 700

Accounting for variable dilution using multiple markers relies on the same basic premise as the approach presented for Scotland's case study, although with three key differences: first, using multiple markers (such as ammoniacal nitrogen and orthophosphate) jointly to estimate flow variability improves the accuracy of estimates. It also allows us to identify outliers (such as one-off discharges), and estimate flow variability as long as at least one marker is quantified (although with larger error bars). If no marker is quantified, the model predicts average flows with substantial error bars. Secondly, rather than assuming total marker loads are constant, they are assumed to be constant *in expectation*. In other words, natural variability of biomarker loads is accounted for. This allows to assign variable importance to different markers in a data-driven fashion. For example, crAssphage gene copy concentrations exhibit more natural variability than ammonia-nitrogen concentrations, and more importance should be assigned to the latter – although both can inform our dilution estimates. Finally, a generative modelling approach is used to test hypotheses *in silico*, and any inferences in the form of posterior distributions over parameter values include principled estimates of uncertainty. The model also handles missing data gracefully and can incorporate limits of detection where appropriate (not further considered here). Unfortunately, the model needs to be fit whenever new data become available, and it is more computationally expensive than other methods. Any combination of two or more markers can be used to estimate flows using the multi-marker method provided that their total loads are constant in expectation. An example of the correction for an English STW is presented on Figure 7.

Case study 2: Handling data anomalies
Identifying and reconciling anomalously low measurements in England

As discussed, the devolved administration programmes for COVID-19 wastewater surveillance have generated a large number of SARS-CoV-2 virus RNA measurements. As with all environmental measurements, the signal recovered will be subject to anomalies, or outliers, that diverge from the expected data trends, with some defined statistical significance. Measurements can vary by several orders of magnitude, with extrema possibly representing unaccountable occurrences such as ' 'super-spreader' events, single release of highly concentrated sewage (e.g. transported from non-networked sites, in-network holding tanks or wet wells), or due to sample capture of a highly aggregated, unmixed load. Alternatively, anomalies may represent measurement error or uncertainty due, for example, to inappropriate sampling frequency, miscalculation or unknown peak flow (for grab samples), or sample/laboratory contamination. Such data anomalies can cause many problems for further analysis or visualisation, and depending on context, different interventions are typically needed when they are detected.

In the English programme, post-laboratory analyses were conducted to attempt to identify measurements that may be anomalously low, by defining the likelihood that a measurement falls within some expectation criteria. In particular, a machine learning approach, using a Gradient Boosting for regression

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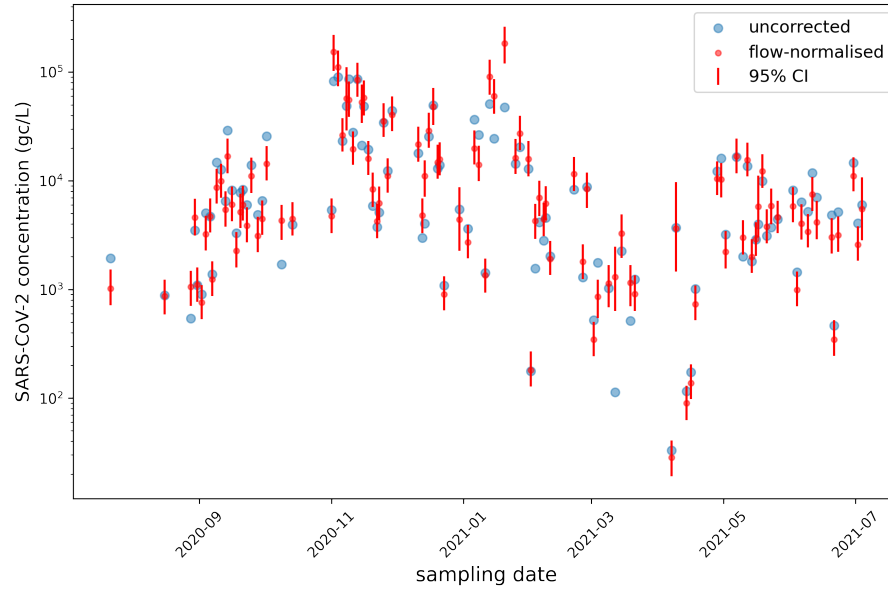
Figure 7: SARS-CoV-2 concentration (gc/L) over time with flow variability correction: example with Bolton sewage treatment works. Blue points represent the original uncorrected SARS-CoV-2 concentrations and red points and associated 95% confidence intervals are the estimates after accounting for flow variability.

model, was trained with a quantile loss function to predict 90% SARS-CoV-2 concentration intervals at the sampled sites. These predictions were used to explore unexpectedly low data points (below the 5th percentile prediction interval) where similar sites in terms of geography and collection method exhibited relatively high measurements.

The analysis identified 762 samples as anomalous out of 25,957 that did not report a quantified value. In particular, the model highlighted low measurements during January and February 2021 despite infection rates across the country were high. The analysis could be extended to explore any recorded values that do not fall within the predicted range, whether low or high. Figure 8 illustrates the frequency of anomalous data points when compared to ammonia concentrations, suggesting that lower concentrations of ammonia are associated with a higher proportion of unquantifiable samples. This suggests that flow dilution has the impact of reducing SARS-CoV-2 concentrations below the 5th percentile prediction interval.

Tracking measurement outliers in England

On a weekly basis, sites with rapid and sudden increase of SARS-CoV-2 are identified using parametric confidence bands around a linear regression model fit to the data. The model is used to predict the SARS-CoV-2 concentrations 7-days in advance and a 80% confidence band is calculated for this extended

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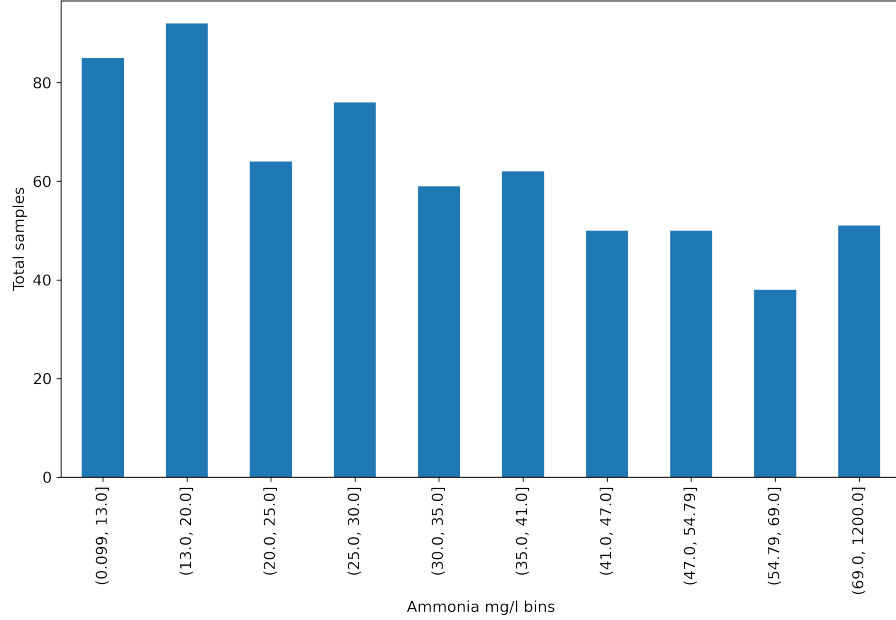
Figure 8: Frequency of anomalous SARS-CoV-2 RNA concentrations, identified using a gradient boosted regression and binned by the associated ammonia concentration. The data suggests that lower ammonia concentration and, by proxy, higher dilution, results in lower confidence in detection of viral RNA.

linear regression. This accounts for the uncertainty of the mean virus RNA ₇₆₆ concentrations over time. As new data is acquired, if the latest measurement ₇₆₇ falls outside the upper limit it indicates that the sample has exceeded the pre- ₇₆₈ dicted concentration and needs further investigation. Outliers identified with ₇₆₉ this method are visualised on a map of England and assessed alongside appro- ₇₇₀ priate meta-data, such as the inorganics (e.g., ammonia, orthophosphate), see ₇₇₁ Figure S8. Weekly maximums that lie above pre-defined threshold values are ₇₇₂ also flagged as outliers. After following this process, sites of concern are re- ₇₇₃ ported to the National Laboratory Service (NLS) who conducts further quality ₇₇₄ assurance. ₇₇₅

Identifying and reconciling anomalously high measurements in Scotland ₇₇₆

Under the Scottish programme, Biomathematics and Statistics Scotland ₇₇₇ (BioSS) conducted a similar procedure, though instead the focus was on anoma- ₇₇₈ lously high values (e.g. spikes), with the aim of flagging and potentially remov- ₇₇₉ ing anomalies as soon as they are recorded. A Generalised Additive Model ₇₈₀ (GAM) was used to identify when high amounts of wastewater COVID-19 (rel- ₇₈₁ ative to case rates, or relative to the previous variability of the site) is indicative ₇₈₂ of the wastewater measure not corresponding to future cases. With a suitable ₇₈₃ threshold, this was used to remove these measurements from aggregates, and/or ₇₈₄

24

trigger further investigation [83].   785

Case Study 3: Uncertainty arising from laboratory analysis of SARS-CoV-2,   786
and its mitigation within the UK wastewater surveillance programme   787

The analytical variability, in terms of both replicability and reproducibility,   788
for the estimation of SARS-CoV-2 in wastewater has been a major focus of   789
the UK wastewater surveillance programmes. In England, the use of two main   790
laboratories (required due to the need for high throughput analysis capacity)   791
provided significant challenges, but also opportunities to assess the reproducibil-   792
ity of sample analysis. Both laboratories employed the AS precipitation and,   793
despite some differences in the use of RT-qPCR reagents and quantification   794
standards, duplicate samples were analysed and found to be comparable (data   795
unpublished). In addition, an inter-laboratory ring trial was carried out involv-   796
ing five laboratories across the four nations, three using AS precipitation and   797
two using filtration (Walker *et al.*, 2021, unpublished). Significant differences   798
were found in the absolute SARS-CoV-2 concentrations measured by all labo-   799
ratories. However, these differences (less than one log between labs) were much   800
lower than reported in other ring trials [84]. Further, the variability between   801
the laboratories was similar to previous inter-laboratory trials for quantifying   802
viruses (e.g. Norovirus, Hepatitis A) in shellfish [85]. The differences in the   803
SARS-CoV-2 recovery between laboratories is likely due in part to the differ-   804
ences in the initial virus concentration method (e.g. ultrafiltration versus AS   805
precipitation) and the use of different RT-qPCR standards. The UK is now con-   806
tributing to discussions on the development of an ISO standard for quantifying   807
SARS-CoV-2 in wastewater. The development of an ISO standard will enable a   808
greater degree of international collaboration and provide the basis for external   809
proficiency testing schemes. The latter will give laboratories and accreditation   810
services a means to assess laboratory performance and flag potential quality   811
issues that require investigation.   812

The efficiency of downstream applications depends strongly on the purity of   813
the RNA sample used. In this regard, MIQE guidelines stipulate that a mea-   814
surement of a nucleic acid quantity is essential, while an assessment of purity   815
is desirable [86]. This is particularly important to avoid false negatives when   816
SARS-CoV-2 concentrations are too low to be quantified after dilution, requir-   817
ing the use of internal or external controls, such as RNA/DNA spikes, to detect   818
inhibitors and verify several other parameters of the workflow (See Figure S9).   819
Furthermore, the effect of wastewater properties has been assessed in a range   820
of mesocosm-based wastewater studies. These found that the presence of sus-   821
pended solids (turbidity range 10 - 400 NTU) or surfactants (0 - 100 mg/l)   822
had minimal impact on RNA recovery using PEG or AS precipitation methods   823
unless present at very high concentrations atypical of UK wastewater.   824

RT-qPCR can introduce additional variability at different steps during the   825
quantification of SARS-CoV-2. Firstly, the reverse transcription can vary with   826
the same samples by two to threefold depending on the amount and quality of   827
RNA [87]. On the other hand, sample variability increases when the target com-   828
plementary DNA (cDNA) is diluted, mainly when the quantification cycle (C_q)   829

values are greater than 30. This is due to stochastic amplification, measurement uncertainty, and subsampling error [88]. The RT-qPCR variability can easily range between 10% to 200% of the coefficient of variation (CV) and can only be minimised by interrogating a larger proportion of the sample using more technical replicates and applying the average C_q [88]. Figure S10 shows the variability of SARS-CoV 2 measurements in wastewater at different C_q values from an England pilot study.

Case Study 4: Population normalisation and measurement correction: lessons learned from WBE application in exposure studies beyond COVID

A study of multi-group chemical profiling in five contrasting urban populations, each served by a major STW contributing to one river catchment in South-West England and covering an area of approximately 2000 km^2 and a population of approximately 1.5 million (this constitutes >75% of the overall population in the catchment) was undertaken to understand measurement variability at an inter-city granularity (See Figure S6 for a map of the five study locations, and Table S1 for data on their network characteristics). A detailed discussion of multi-chemical fluxes in urban catchments has been provided by Proctor *et al.* [89] and the methodology used to measure chemicals and back-calculate mass loads and intake are found in recent literature [90, 10]. Key contributing factors to WBE uncertainties are carefully considered and included in the study to enable a fully quantitative measurement of city-wide intake for selected chemicals:

- Robust sampling and sample collection involving 24-hour flow proportional sampling in ice packed or refrigerated autosamplers maintaining biomarker stability;

- 7-day consecutive sampling to allow for temporal (weekday versus weekend) changes in biomarkers to be observed;

- Robust wastewater flow measurement and population size estimates;

- Fully validated analytical methods and the highest level of quality assurance (e.g. limits of detection and quantification, intra- and inter-day accuracy and precision, recovery from matrix);

- Characteristic biomarker selection for back-calculation of chemical exposure (e.g. metabolite versus parent compound to account for direct disposal of unused chemicals);

- Full biomarker mass balance in wastewater that accounts for biomarker presence in both solid and liquid phases with a full understanding of percentage biomarker recovered from the matrix.

The aim of the study is to understand and characterise key uncertainties to enable accurate back-calculation of city-wide exposure to chemicals. To validate the developed back-calculation protocol, high-resolution spatiotemporal NHS

pharmaceutical prescription databases are used for system calibration, in terms of biomarker selection and its correction factor, as well as for overall spatiotemporal system performance evaluation. A detailed discussion on multi-chemical exposure can be found in [10]. Here, focus is only given to carbamazepine and citalopram, two model chemicals, and two key variabilities for back-calculation of their usage at an inter-city level (that are not currently considered for UK SARS-CoV-2 monitoring): characteristic endogenous biomarker selection and establishment of correction factors accounting for human metabolism.

Carbamazepine intake (Figure 9: red line) is back-calculated using both parent compound (source carbamazepine) and its metabolite (carbamazepine-10,11-epoxide, CBZ10-11). While both biomarkers correlate well with NHS prescription data (Figure 9: blue line), using carbamazepine as a biomarker might lead to an overestimation of intake if direct disposal of unused carbamazepine takes place (see city A, Sunday, Figure 9). Interestingly, this is not the case if CBZ10-11 is used (no spike in city A during Sunday), which indicates its superiority over carbamazepine itself.

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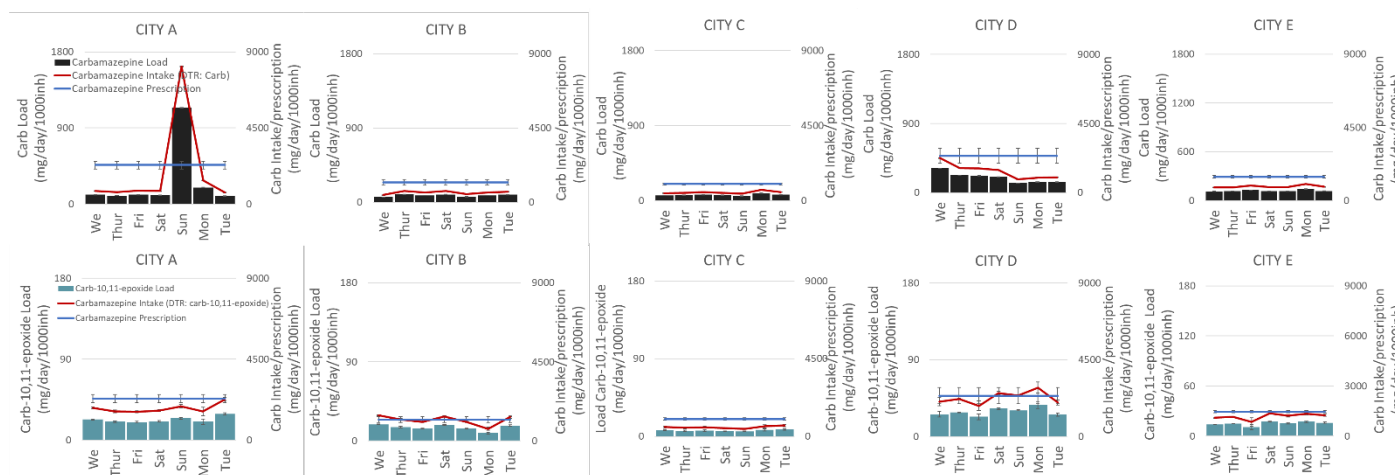
Figure 9: Comparison of carbamazepine daily loads, intake (calculated using both carbamazepine and carbamazepine-10,11-epoxide) and prescribed carbamazepine in five cities over a 7-day sampling week.

28

An understanding of the extent of metabolism of biomarkers or metabolic formation of biomarkers is key in quantitative back-calculation of chemical intake. Figure 10 shows an example of a significant overestimation of citalopram intake observed when using commonly applied weighted average correction factors based on the existing literature. This often include only phase I metabolism of chemical excreted in urine (desmethylcitalopram in this case), as opposed to the focused approach, where metabolism correction factors (mCFs) are calculated using only comprehensive datasets from studies combining phase I and II metabolites (glucuronides) excreted in both urine and faeces. Understanding biomarker excretion in faeces is of critical importance for compounds with a more hydrophobic nature, such as citalopram as it is, to a large extent, excreted in faeces. Additionally, citalopram and its metabolites undergo extensive glucuronide conjugation. Overlooking excretion in faeces and phase II metabolism will lead to incorrect CFs as seen in Figure 10. Having prescription data per 10 - 100 households/postcodes allows for the validation of the correction factors used. Prescription databases (if associated with well-defined regional units such as streets) can therefore serve as internal calibration systems.
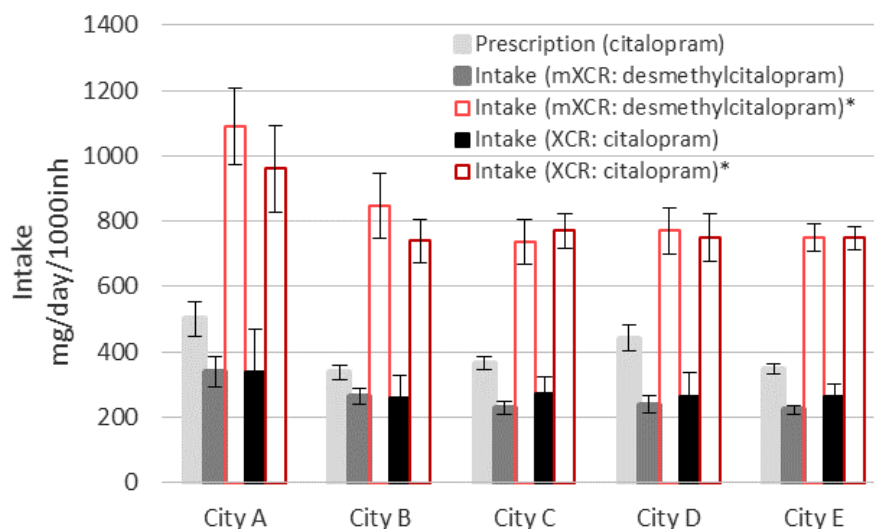
Figure 10: Citalopram intake calculated using citalopram and desmethyl-citalopram, with and without inclusion of phase II metabolites (Note: (*) indicates no inclusion of phase II metabolism, which leads to overestimation of intake).

This case study shows the importance of careful biomarker selection to enable highly accurate 'quantitative' calculation of per capita stressor intake. This is not currently performed with SARS-CoV-2 surveillance, where the stressor itself is used as a biomarker. As a result, various sources of the genetic material present in the wastewater sample are accounted for and, hence, calculation of

the per capita intake (or viral load) is not possible. Further work is required to establish a biomarker suite enabling quantitative measurement of SARS-CoV-2. In the interim, it is likely that WBE can only be used as an early warning system for SARS-CoV-2 surveillance and verification of disease prevalence trends at the community level, and not as a quantitative measure of community infection rates.

Conclusions

The scale of the COVID-19 pandemic has resulted in an unparalleled response from a diverse community of stakeholders, working collaboratively to control and reduce the transmission and impact of the disease. The early demonstration that wastewater was a viable medium for tracking the virus, led to academic and government initiatives to operationalise wastewater-based epidemiology for monitoring its dynamics at local, regional, and national scales. In the UK, COVID-19 surveillance programmes across the four nations (England, Wales, Scotland, and Northern Ireland), have demonstrated, perhaps uniquely, the opportunity for WBE to be used routinely and at unprecedented scale to combat a public health emergency. From their inception, the national wastewater surveillance programmes have delivered insights to support public health decision-making and to guide Government and key stakeholders in interpreting the measurements of SARS-CoV-2 in wastewater to provide a broader understanding of the disease in the populations.

This work has allowed for a broader appreciation of WBE as a tool for monitoring public health in populations at scale, with initiatives likely to focus on a 'beyond COVID' uplift of WBE as part of establishing One Health programmes across the world. However, their effectiveness requires that the data generated to support the function of WBE is meaningful and representative of the target(s) being monitored. Wastewater is a more complex environment than typical media used for monitoring of human health, with multiple factors potentially accounting for greater uncertainty or variability in the measured signal that in, for example, a clinical setting. Managing this uncertainty is one of the key challenges to ensure successful employment of WBE for public health protection.

Here perspectives are provided on the confidence in wastewater-derived measurements by those working across the national programmes, given work performed to understand, quantify and manage measurement uncertainty and variability. The work emphasises that while some sources of uncertainty may not be impactful, or can be adequately accounted for (e.g. extraneous flow dilution, sampling method), other sources are inconsistent or difficult to quantify directly (e.g. shedding distributions, in-network behaviour). While these intractable factors will, with consolidated research efforts, become less opaque, there is unlikely to be a general approach to manage measurement uncertainty for all applications of WBE beyond COVID. Making use of the greatly increased capacity for WBE in the UK, and more widely, will require new methods for

extracting actionable information from wastewater data, but also methods for determining the limits of its application.

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Disclaimer

References
984

[1] B. Choi, The past, present, and future of public health surveillance, Scientifica (Cairo) 2012 (2012) 875253. doi:10.6064/2012/875253. 985 986

[2] S. Castiglioni, L. Bijlsma, A. Covaci, et al., Evaluation of uncertainties associated with the determination of community drug use through the measurement of sewage drug biomarkers, Environ. Sci. Technol. 47 (3) (2013) 1452–1460. doi:10.1021/es302722f. 987 988 989 990

[3] J. O'Brien, Refining the uncertainties and expansion of wastewater-based epidemiology for assessing population exposure to chemicals, Ph.D. thesis, School of Medicine, The University of Queensland (2017). doi:10.14264/uql.2017.420. 991 992 993 994

[4] C. Daughton, Monitoring wastewater for assessing community health: Sewage Chemical-Information Mining (SCIM), Sci. Total Environ. 619-620 (2018) 748–764. doi:10.1016/j.scitotenv.2017.11.102. 995 996 997

[5] B. Subedi, Uncertainties associated with wastewater-based epidemiology for the estimation of community consumption of drugs, in: B. Subedi, D. Burgard, B. Loganathan (Eds.), Wastewater-Based Epidemiology: Estimation of Community Consumption of Drugs and Diets, ACS Symposium Series, 2019, pp. 79–98. doi:10.1021/bk-2019-1319.ch004. 998 999 1000 1001 1002

[6] A. Singer, J. Järhult, R. Grabic, et al., Compliance to oseltamivir among two populations in Oxfordshire, United Kingdom affected by influenza A(H1N1)pdm09, November 2009–a waste water epidemiology study, PLoS One 8 (4) (2013) e60221. doi:10.1371/journal.pone.0060221. 1003 1004 1005 1006

[7] A. Pruden, Balancing water sustainability and public health goals in the face of growing concerns about antibiotic resistance, Environ. Sci. Technol. 48 (1) (2014) 5–14. doi:10.1021/es403883p. 1007 1008 1009

[8] M. Lorenzo, Y. Picó, Wastewater-based epidemiology: current status and future prospects, Curr. Opin. Environ. Sci. Health 9 (2019) 77–84. doi:10.1016/j.coesh.2019.05.007. 1010 1011 1012

[9] I. González-Mariño, J. Baz-Lomba, N. Alygizakis, et al., Spatio-temporal assessment of illicit drug use at large scale: evidence from 7 years of international wastewater monitoring, Addiction 115 (2020) 109–120. doi:10.1111/add.14767. 1013 1014 1015 1016

[10] B. Kasprzyk-Hordern, K. Proctor, K. Jagadeesan, et al., Estimation of community-wide multi-chemical exposure via water-based chemical mining: Key research gaps drawn from a comprehensive multi-biomarker multi-city dataset, Environ. Int. 147 (2021) 106331. doi:10.1016/j.envint.2020.106331. 1017 1018 1019 1020 1021

32

[11] M. Wade, J.-P. Steyer, M. Ruano Garcia, Making Water Smart, Water Sci. Technol. 82 (12) (2020) v—-vii. doi:10.2166/wst.2020.581.

[12] P. Kehrein, M. van Loosdrecht, P. Osseweijer, M. Garfi, J. Dewulf, J. Posada, A critical review of resource recovery from municipal wastewater treatment plants – market supply potentials, technologies and bottlenecks, Environ. Sci.: Water Res. Technol. 6 (2020) 877–91. doi:10.1039/c9ew00905a.

[13] C. Kinsella, P. Santos, I. Postigo-Hidalgo, et al., Preparedness needs research: How fundamental science and international collaboration accelerated the response to COVID-19, PLoS Pathog. 16 (10) (2020) e1008902. doi:10.1371/journal.ppat.1008902.

[14] L. Lundy, D. Fatta-Kassinos, J. Slobodnik, et al., Making waves: Collaboration in the time of SARS-CoV-2 - rapid development of an international co-operation and wastewater surveillance database to support public health decision-making, Water Res. 199 (2021) 117167–117167. doi:10.1016/j.watres.2021.117167.

[15] A. Bivins, D. North, A. Ahmad, et al., Wastewater-Based Epidemiology: Global collaborative to maximize contributions in the fight against COVID-19, Environ. Sci. Technol. 54 (13) (2020) 7754–7757. doi:10.1021/acs.est.0c02388.

[16] M. Wade, D. Jones, A. Singer, et al., Wastewater COVID-19 monitoring in the UK: summary for SAGE, Tech. rep., Scientific Advisory Group for Emergencies (12 2020).

[17] C. Naughton, F. Roman, A. Alvarado, et al., Show us the Data: Global COVID-19 Wastewater Monitoring Efforts, Equity, and Gaps, medRxiv (2021). doi:10.1101/2021.03.14.21253564.

[18] A. Giacobbo, M. Rodrigues, J. Zoppas Ferreira, A. Bernardes, M. de Pinho, A critical review on SARS-CoV-2 infectivity in water and wastewater. What do we know? [published online ahead of print, 2021 Feb 9], Sci. Total Environ. 774 (2021) 145721. doi:10.1016/j.scitotenv.2021.145721.

[19] M. Kumar, M. Alamin, K. Kuroda, K. Dhangar, A. Hata, H. Yamaguchi, R. Honda, Potential discharge, attenuation and exposure risk of SARS-CoV-2 in natural water bodies receiving treated wastewater, npj Clean Water 4 (1) (2021). doi:10.1038/s41545-021-00098-2.

[20] B. Saawarn, S. Hait, Occurrence, fate and removal of SARS-CoV-2 in wastewater: Current knowledge and future perspectives, J. Environ. Chem. Eng. 9 (1) (2021) 104870. doi:10.1016/j.jece.2020.104870.

[21] H. Tran, S. Le, D. Nguyen, et al., SARS-CoV-2 coronavirus in water and wastewater: A critical review about presence and concern, Environ Res. 193 (2021) 110265. doi:10.1016/j.envres.2020.110265.

[22] A. Bivins, J. Greaves, R. Fischer, K. Yinda, W. Ahmed, M. Kitajima, V. Munster, K. Bibby, Persistence of SARS-CoV-2 in water and wastewater, Environ. Sci. Technol. Lett. acs.estlett.0c00730 (2020). doi:10.1021/acs.estlett.0c00730.

[23] N. Alygizakis, A. Markou, N. Rousis, et al., Analytical methodologies for the detection of SARS-CoV-2 in wastewater: Protocols and future perspectives, Trends Analyt. Chem. 134 (2021) 116125. doi:10.1016/j.trac.2020.116125.

[24] M. Brown, M. Wade, S. McIntyre-Nolan, et al., Wastewater Monitoring of SARS-CoV-2 Variants in England: Demonstration Case Study for Bristol (Dec 2020 - March 2021), Tech. rep., Scientific Advisory Group for Emergencies (04 2021).

[25] A. Crits-Christoph, R. Kantor, M. Olm, et al., Genome sequencing of sewage detects regionally prevalent SARS-CoV-2 variants, mBio 12 (1) (2021). arXiv:https://mbio.asm.org/content/12/1/e02703-20.full.pdf, doi:10.1128/mBio.02703-20.

[26] A. Pérez-Cataluña, Á. Chiner-Oms, E. Cuevas-Ferrando, et al., Detection of genomic variants of SARS-CoV-2 circulating in wastewater by high-throughput sequencing, medRxiv (2021). doi:10.1101/2021.02.08.21251355.

[27] L. Lara-Jacobo, G. Islam, J.-P. Desaulniers, A. Kirkwood, D. Simmons, First detection of SARS-CoV-2 proteins in wastewater samples by mass spectrometry, Research Square (2021). doi:10.21203/rs.3.rs-237512/v4.

[28] E. Zuccato, C. Chiabrando, S. Castiglioni, R. Bagnati, R. Fanelli, Estimating community drug abuse by wastewater analysis, Environ. Health Perspect. 116 (8) (2008) 1027–1032. doi:10.1289/ehp.11022.

[29] J. Baz-Lomba, C. Harman, M. Reid, K. Thomas, Comparison of pharmaceutical, illicit drug, alcohol, nicotine and caffeine levels in wastewater with sale, seizure and consumption data for 8 European cities, BMC Public Health 16 (1) (2016) 1035. doi:10.1016/j.watres.2017.05.041.

[30] R. Hendriksen, P. Munk, P. Njage, et al., Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage, Nat. Commun. 10 (2019) 1124. doi:10.1038/s41467-019-08853-3.

[31] E. Gracia-Lor, N. Rousis, F. Hernández, E. Zuccato, S. Castiglioni, Wastewater-based epidemiology as a novel biomonitoring tool to evaluate human exposure to pollutants, Environ. Sci. Technol. 52 (18) (2018) 10224–10226. doi:10.1021/acs.est.8b01403.

[32] D. Carroll, S. Morzaria, S. Briand, C. Johnson, D. Morens, K. Sumption, O. Tomori, S. Wacharphaueasadee, Preventing the next pandemic: the power of a global viral surveillance network, BMJ 372 (n485) (2021). doi:10.1136/bmj.n485.

[33] The European Commission, Commission Recommendation of 17.3.2021 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU, The European Commission, 2021.
URL https://ec.europa.eu/environment/pdf/water/recommendation_covid19_monitoring_wastewaters.pdf

[34] P. Daszak, C. das Neves, J. Amuasi, et al., Workshop Report on Biodiversity and Pandemics of the Intergovernmental Platform on Biodiversity and Ecosystem Services, Tech. rep., IPBES Secretariat, Bonn, Germany (2020). doi:10.5281/zenodo.4147317.

[35] N. Sims, B. Kasprzyk-Hordern, Future perspectives of wastewater-based epidemiology: Monitoring infectious disease spread and resistance to the community level, Environ. Int. 139 (2020) 105689. doi:10.1016/j.envint.2020.105689.

[36] K. Farkas, L. Hillary, S. Malham, J. McDonald, D. Jones, Wastewater and public health: the potential of wastewater surveillance for monitoring COVID-19, Curr. Opin. Environ. Sci. Health 17 (2020) 14–20. doi:10.1016/j.coesh.2020.06.001.

[37] D. Polo, M. Quintela Baluja, A. Corbishley, D. Jones, A. Singer, D. Graham, J. Romalde, Making waves: Wastewater-based epidemiology for COVID-19 – approaches and challenges for surveillance and prediction, Water Res. 186 (2020) 116404. doi:10.1016/j.watres.2020.116404.

[38] K. Mao, K. Zhang, W. Du, W. Ali, X. Feng, H. Zhang, The potential of wastewater-based epidemiology as surveillance and early warning of infectious disease outbreaks, Curr. Opin. Environ. Sci. Health 17 (2020) 1–7. doi:10.1016/j.coesh.2020.04.006.

[39] L. Hilborne, Z. Wagner, I. Cabreros, R. Brook, Linking statistics with testing policy to manage COVID-19 in the community, Am. J. Clin. Pathol. 154 (2) (2020) 142–148. doi:10.1093/ajcp/aqaa099.

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

[41] O. Hart, R. Halden, Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges, Sci. Total Env. 730 (2020) 138875. doi:10.1016/j.scitotenv.2020.138875.

[42] X. Li, S. Zhang, J. Shi, S. Luby, G. Jiang, Uncertainties in estimating SARS-CoV-2 prevalence by wastewater-based epidemiology, Chem. Eng. J. 415 (2021) 129039. doi:10.1016/j.cej.2021.129039.

[43] W. Ahmed, S. Simpson, P. Bertsch, et al., Minimizing Errors in RT-PCR Detection and Quantification of SARS-CoV-2 RNA for Wastewater Surveillance, Preprints (2021). doi:10.20944/preprints202104.0481.v1.

[44] National Research Council (US) Committee on Improving Risk Analysis Approaches Used by the U.S. EPA., Uncertainty and Variability: The Recurring and Recalcitrant Elements of Risk Assessment, National Academies Press (US), Washington (DC), 2009.

[45] R. Kantor, K. Nelson, H. Greenwald, L. Kennedy, Challenges in Measuring the Recovery of SARS-CoV-2 from Wastewater, Environ. Sci. Technol. 55 (6) (2021) 3514–3519. doi:10.1021/acs.est.0c08210.

[46] J. Lehmann, M. Rillig, Distinguishing variability from uncertainty, Nat. Clim. Change 4 (2014) 153. doi:10.1038/nclimate2133.

[47] C. McMahan, S. Self, L. Rennert, C. Kalbaugh, D. Kriebel, D. Graves, J. Deaver, S. Popat, T. Karanfil, D. Freedman, COVID-19 Wastewater Epidemiology: A Model to Estimate Infected Populations, medRxiv (2020). doi:10.1101/2020.11.05.20226738.

[48] C. Fuschi, H. Pu, M. Negri, R. Colwell, J. Chen, Wastewater-Based Epidemiology for Managing the COVID-19 Pandemic, Environ. Sci. Technol. Water 1 (6) (2021) 1352–1362. doi:10.3390/mps4010017.

[49] W. Edeling, H. Arabnejad, R. Sinclair, D. Suleimenova, K. Gopalakrishnan, B. Bosak, D. Groen, I. Mahmood, D. Crommelin, P. Coveney, The impact of uncertainty on predictions of the CovidSim epidemiological code, Nat. Comput. Sci. 1 (2) (2021) 128–135. doi:10.1038/s43588-021-00028-9.

[50] J. Saththasivam, S. El-Malah, T. Gomez, K. Jabbar, R. Remanan, A. Krishnankutty, O. Ogunbiyi, K. Rasool, S. Ashhab, S. Rashkeev, M. Bensaad, A. Ahmed, Y. Mohamoud, J. Malek, L. Abu Raddad, A. Jeremijenko, H. Abu Halaweh, J. Lawler, K. Mahmoud, COVID-19 (SARS-CoV-2) outbreak monitoring using wastewater-based epidemiology in Qatar, Sci. Total Environ. 774 (2021) 145608. doi:10.1016/j.scitotenv.2021.145608.

[51] L. Hillary, K. Farkas, K. Maher, A. Lucaci, J. Thorpe, M. Distaso, W. Gaze, S. Paterson, T. Burke, T. Connor, J. McDonald, S. Malham, D. Jones, Monitoring SARS-CoV-2 in municipal wastewater to evaluate the success of lockdown measures for controlling COVID-19 in the UK, Water Res. 200 (2020) 117214. doi:10.1016/j.watres.2021.117214.

[52] K. Farkas, L. Hillary, J. Thorpe, D. Walker, J. Lowther, J. McDonald, S. Malham, D. Jones, Concentration and Quantification of SARS-CoV-2

RNA in Wastewater Using Polyethylene Glycol-Based Concentration and qRT-PCR, Methods Protoc. 4 (1) (2021) 17. doi:10.3390/mps4010017.

[53] C. Daughton, Real-time estimation of small-area populations with human biomarkers in sewage, Sci. Total Environ. 414 (2012) 6–21. doi:10.1016/j.scitotenv.2011.11.015.

[54] F. Been, L. Rossi, C. Ort, S. Rudaz, O. Delémont, P. Esseiva, Population normalization with ammonium in wastewater-based epidemiology: application to illicit drug monitoring, Environ. Sci. Technol. 48 (14) (2014) 8162–8169. doi:10.1021/es5008388.

[55] K. Thomas, A. Amador, J. Baz-Lomba, M. Reid, Use of mobile device data to better estimate dynamic population size for wastewater-based epidemiology, Environ. Sci. Technol. 51 (19) (2017) 11363–11370. doi:10.1021/acs.est.7b02538.

[56] M. Rico, M. Andrés-Costa, Y. Picó, Estimating population size in wastewater-based epidemiology. Valencia metropolitan area as a case study, J. Hazard. Mater. 323 (Pt A) (2017) 156–165. doi:10.1016/j.jhazmat.2016.05.079.

[57] S. Cavany, A. Bivins, Z. Wu, D. North, K. Bibby, T. Perkins, Inferring SARS-CoV-2 rna shedding into wastewater relative to time of infection, medRxiv (2021). doi:10.1101/2021.06.03.212582383.

[58] H. Jones, M. Hickman, B. Kasprzyk-Hordern, N. Welton, D. Baker, A. Ades, Illicit and pharmaceutical drug consumption estimated via wastewater analysis. Part B: Placing back-calculations in a formal statistical framework, Sci. Total Environ. 487 (2014) 642–650. doi:10.1016/j.scitotenv.2014.02.101.

[59] F. Wu, A. Xiao, J. Zhang, K. Moniz, N. Endo, F. Armas, R. Bonneau, M. Brown, M. Bushman, P. Chai, C. Duvallet, T. Erickson, K. Foppe, N. Ghaeli, X. Gu, W. Hanage, K. Huang, W. Lee, M. Matus, K. McElroy, J. Nagler, S. Rhode, M. Santillana, J. Tucker, S. Wuertz, S. Zhao, J. Thompson, E. Alm, SARS-CoV-2 titers in wastewater foreshadow dynamics and clinical presentation of new COVID-19 cases, medRxiv (2020). doi:10.1101/2020.06.15.20117747.

[60] J. Peccia, A. Zulli, D. Brackney, N. Grubaugh, E. Kaplan, A. Casanovas-Massana, A. Ko, A. Malik, D. Wang, M. Wang, J. Warren, D. Weinberger, W. Arnold, S. Omer, Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics, Nat. Biotechnol. 38 (10) (2020) 1164–1167. doi:10.1038/s41587-020-0684-z.

[61] D. Jones, M. Quintela Baluja, D. Graham, A. Corbishley, J. McDonald, S. Malham, L. Hillary, T. Connor, W. Gaze, I. Moura, M. Wilcox, K. Farkas, 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, Sci. Total Environ. 749 (2020) 141364. doi:10.1016/j.scitotenv.2020.141364.

[62] R. Kantor, K. Nelson, H. Greenwald, L. Kennedy, Aligning SARS-CoV-2 indicators via an epidemic model: application to hospital admissions and rna detection in sewage sludge, Health Care Manag. Sci. 24 (2) (2021) 320–329. doi:10.1007/s10729-020-09525-1.

[63] F. Miura, M. Kitajima, R. Omori, 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, Sci. Total Environ. 769 (2021) 144549. doi:10.1016/j.scitotenv.2020.144549.

[64] M. Thompson, J. Burgess, A. Naleway, H. Tyner, S. Yoon, J. Meece, L. Olsho, A. Caban-Martinez, A. Fowlkes, K. Lutrick, H. Groom, K. Dunnigan, M. Odean, K. Hegmann, E. Stefanski, L. Edwards, N. Schaefer-Solle, L. Grant, K. Ellingson, J. Kuntz, T. Zunie, M. Thiese, L. Ivacic, M. Wesley, J. Mayo Lamberte, X. Sun, M. Smith, A. Phillips, K. Groover, Y. Yoo, J. Gerald, R. Brown, M. Herring, G. Joseph, S. Beitel, T. Morrill, J. Mak, P. Rivers, B. Poe, B. Lynch, Y. Zhou, J. Zhang, A. Kelleher, Y. Li, M. Dickerson, E. Hanson, K. Guenther, S. Tong, A. Bateman, E. Reisdorf, J. Barnes, E. Azziz-Baumgartner, D. Hunt, M. Arvay, P. Kutty, A. Fry, M. Gaglani, Prevention and attenuation of COVID-19 with the BNT162b2 and mRNA-1273 vaccines, N. Engl. J. Med. (2021). doi:10.1056/NEJMoa2107058.

[65] J. Huisman, J. Scire, L. Caduff, X. Fernandez-Cassi, P. Ganesanandamoorthy, A. Kull, A. Scheidegger, E. Stachler, A. Boehm, B. Hughes, A. Knudson, A. Topol, K. Wigginton, M. Wolfe, T. Kohn, C. Ort, T. Stadler, T. Julian, Wastewater-based estimation of the effective reproductive number of SARS-CoV-2, medRxiv (2021). doi:10.1101/2021.04.29.21255961.

[66] C. Ort, M. Lawrence, J. Rieckermann, J. A, Sampling for pharmaceuticals and personal care products (PPCPs) and illicit drugs in wastewater systems: are your conclusions valid? A critical review, Env. Sci. Technol. 44 (16) (2010) 6024–6035. doi:10.1021/es100779n.

[67] W. Ahmed, P. Bertsch, K. Bibby, E. Haramoto, J. Hewitt, F. Huygens, P. Gyawali, A. Korajkic, S. Riddell, S. Sherchan, S. Simpson, K. Sirikanchana, E. Symonds, R. Verhagen, S. Vasan, M. Kitajima, A. Bivins, Decay of SARS-CoV-2 and surrogate murine hepatitis virus RNA in untreated wastewater to inform application in wastewater-based epidemiolog, Environ. Res. 191 (2020) 110092. doi:10.1016/j.envres.2020.110092.

[68] A. Banks, F. Lai, J. Mueller, G. Jiang, S. Carter, P. Thai, Potential impact of the sewer system on the applicability of alcohol and tobacco biomarkers in wastewater-based epidemiology, Drug Test. Anal. 10 (3) (2018) 530–538. doi:10.1002/dta.2246.

[69] S. Balboa, M. Mauricio-Iglesias, S. Rodriguez, L. Martínez-Lamas, 1261
F. Vasallo, B. Regueiro, J. Lema, The fate of SARS-COV-2 in WWTPs 1262
points out the sludge line as a suitable spot for detection of COVID-19, Sci. 1263
Total Env. 772 (2021) 145268. doi:10.1016/j.scitotenv.2021.145268. 1264

[70] A. Solvi, Modelling the sewer-treatment-urban river system in view of the 1265
EU Water Framework Directive, Ph.D. thesis, Ghent University (2007). 1266

[71] C. Rose, A. Parker, B. Jefferson, E. Cartmell, The characterization of fe- 1267
ces and urine: A review of the literature to inform advanced treatment 1268
technology, Crit. Rev. Environ. Sci. Technol. 45 (17) (2015) 1827–1879. 1269
doi:10.1080/10643389.2014.1000761. 1270

[72] N. Chappell, T. Jones, W. Tych, Sampling frequency for water quality 1271
variables in streams: Systems analysis to quantify minimum monitoring 1272
rates, Water Res. 123 (2017) 49–57. doi:10.1016/j.watres.2017.06.047. 1273

[73] A. Coes, N. Paretti, W. Foreman, J. Iverson, A. DA, Sampling trace or- 1274
ganic compounds in water: a comparison of a continuous active sampler 1275
to continuous passive and discrete sampling methods, Sci. Total Environ. 1276
473–474 (2014) 731–741. doi:10.1016/j.scitotenv.2013.12.082. 1277

[74] J. Baz-Lomba, S. Salvatore, E. Gracia-Lor, et al., Passive sampling of 1278
wastewater as a tool for the long-term monitoring of community exposure: 1279
Illicit and prescription drug trends as a proof of concept, Water Res. 121 1280
(2017) 221–230. doi:10.1186/s12889-016-3686-5. 1281

[75] W. Ahmed, P. Bertsch, A. Bivins, K. Bibby, K. Farkas, A. Gather- 1282
cole, E. Haramoto, P. Gyawali, A. Korajkic, B. McMinn, J. Mueller, 1283
S. Simpson, W. Smith, E. Symonds, K. Thomas, R. Verhagen, M. Ki- 1284
tajima, Comparison of virus concentration methods for the RT-qPCR- 1285
based recovery of murine hepatitis virus, a surrogate for SARS-CoV-2 1286
from untreated wastewater, Sci. Total Environ. 739 (2020) 139960. doi: 1287
10.1016/j.scitotenv.2020.139960. 1288

[76] R. Izquierdo-Lara, G. Elsinga, L. Heijnen, B. Munnink, C. Schapendonk, 1289
D. Nieuwenhuijse, M. Kon, L. Lu, F. Aarestrup, S. Lycett, G. Medema, 1290
M. Koopmans, M. de Graaf, Monitoring SARS-CoV-2 circulation and diver- 1291
sity through community wastewater sequencing, the Netherlands and Bel- 1292
gium, Emerg. Infect. Dis. 27 (2021) 1405—-1415. doi:10.3201/eid2705. 1293
204410. 1294

[77] W. Randazzo, P. Truchado, E. Cuevas-Ferrando, P. Simón, A. Allende, 1295
G. Sánchez, SARS-CoV-2 RNA in wastewater anticipated COVID-19 oc- 1296
currence in a low prevalence area, Water Res. 181 (2020) 115942. doi: 1297
10.1016/j.watres.2020.115942. 1298

[78] K. Farkas, D. Cooper, J. McDonald, S. Malham, A. de Rougemont, D. Jones, Seasonal and spatial dynamics of enteric viruses in wastewater and in riverine and estuarine receiving waters, Sci. Total Environ. 634 (2018) 1174–1183. doi:10.1016/j.scitotenv.2018.04.038.

[79] J. McKinley, D. Cutting, N. Anderson, C. Graham, B. Johnston, U. Mueller, P. Atkinson, H. Van Woerden, D. Bradley, F. Kee, Association between community-based self-reported COVID-19 symptoms and social deprivation explored using symptom tracker apps: a repeated cross-sectional study in Northern Ireland, BMJ Open 11 (2021) e048333. doi:10.1136/bmjopen-2020-048333.

[80] P. D'Aoust, E. Mercier, D. Montpetit, J.-J. Jia, I. Alexandrov, N. Neault, A. Baig, J. Mayne, X. Zhang, T. Alain, M.-A. Langlois, M. Servos, M. MacKenzie, D. Figeys, A. MacKenzie, T. Graber, R. Delatolla, Quantitative analysis of SARS-CoV-2 RNA from wastewater solids communities with low COVID-19 incidence and prevalence, Water Res. 188 (2021) 116560. doi:10.1021/acs.est.0c06191.

[81] K. Graham, S. Loeb, M. Wolfe, D. Catoe, N. Sinott-Armstrong, S. Kim, K. Yamahara, L. Sassoubre, L. Mendoza Grijalva, L. Roldan-Hernandes, K. Langenfeld, K. Wigginton, A. Boehm, SARS-CoV-2 RNA in wastewater settled solids is associated with COVID-19 cases in a large urban sewershed, Env. Sci. Technol. 55 (1) (2021) 488–498. doi:10.1021/acs.est.0c06191.

[82] V. Speight, W. Kalsbeek, F. DiGiano, Randomized stratified sampling methodology for water quality in distribution systems, J. Water Res. Plan. Manag. 130 (4) (2004) 330–338. doi:10.1061/(ASCE)0733-9496(2004)130:4(330).

[83] Z. Fang, How outliers are identified in the wastewater data, in: Coronavirus (COVID-19): modelling the epidemic (Scottish Government Research Findings Report), Issue No. 60, The Scottish Government, Edinburgh, UK, 2021, pp. 25–27.

[84] B. Pecson, E. Darby, C. Haas, Y. Amha, M. Bartolo, R. Danielson, Y. Dearborn, G. Di Giovanni, C. Ferguson, S. Fevig, E. Gaddis, D. Gray, G. Lukasik, B. Mull, L. Olivas, A. Olivieri, Y. Qu, SARS-CoV-2 Interlaboratory Consortium, Reproducibility and sensitivity of 36 methods to quantify the SARS-CoV-2 genetic signal in raw wastewater: findings from an interlaboratory methods evaluation in the u.s., Environ. Sci. Water Res. Technol. 7 (2021) 504–520. doi:10.1039/d0ew00946f.

[85] J. Lowther, A. Bosch, S. Butot, J. Ollivier, D. Mäde, S. Rutjes, G. Hardouin, B. Lombard, P. In'T Veld, A. Leclercq, Validation of EN ISO method 15216 - Part 1 – Quantification of hepatitis A virus and norovirus in food matrices, Int. J. Food Microbiol. 288 (2019) 82–90. doi:10.1016/j.ijfoodmicro.2017.11.014.

[86] S. Bustin, V. Benes, J. Garson, J. Hellemans, J. Huggett, M. Kubista, R. Mueller, T. Nolan, M. Pfaffl, G. Shipley, J. Vandesompele, C. Wittwer, The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments, Clin. Chem. 55 (4) (2009) 611–622. doi:10.1373/clinchem.2008.112797.

[87] S. Bustin, H. Dhillon, S. Kirvell, C. Greenwood, M. Parker, G. Shipley, T. Nolan, Variability of the reverse transcription step: practical implications, Clin. Chem. 61 (1) (2015) 202–212. doi:10.1373/clinchem.2014.230615.

[88] S. Taylor, K. Nadeau, M. Abbasi, C. Lachance, M. Nguyen, J. Fenrich, The ultimate qPCR experiment: producing publication quality, reproducible data the first time, Trends Microbiol. 37 (7) (2019) 761–774. doi:10.1016/j.tibtech.2018.12.002.

[89] K. Proctor, B. Petrie, L. Lopardo, D. Muñoz, J. Rice, R. Barden, T. Arnot, B. Kasprzyk-Hordern, Micropollutant fluxes in urban environment - A catchment perspective, J. Hazard. Mater. 401 (2021) 123745. doi:10.1016/j.jhazmat.2020.123745.

[90] K. Proctor, B. Petrie, R. Barden, T. Arnot, B. Kasprzyk-Hordern, Multi-residue ultra-performance liquid chromatography coupled with tandem mass spectrometry method for comprehensive multi-class anthropogenic compounds of emerging concern analysis in a catchment-based exposure-driven study, Anal. Bioanal. Chem. 411 (2019) 7061–7086. doi:10.1007/s00216-019-02091-8.

Supporting Information

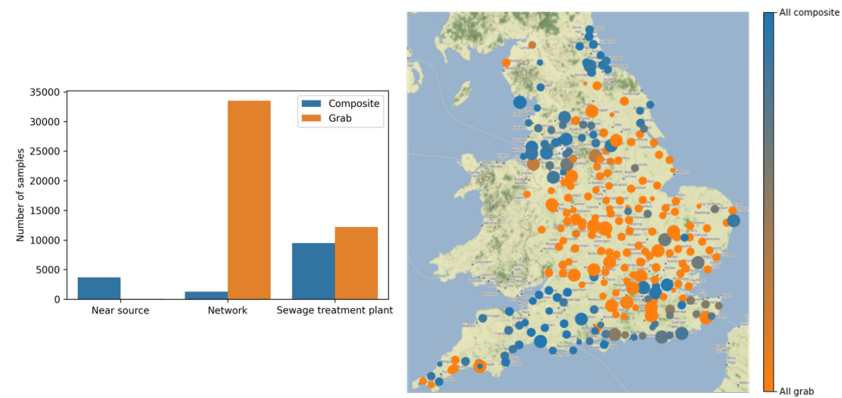*Views from the UK wastewater surveillance programmes*

Figure S1: A view of all wastewater sampling sites in England as of July 2021, indicating relative number of grab versus composite samples across the three site location types (near-source, in-network, and STW).
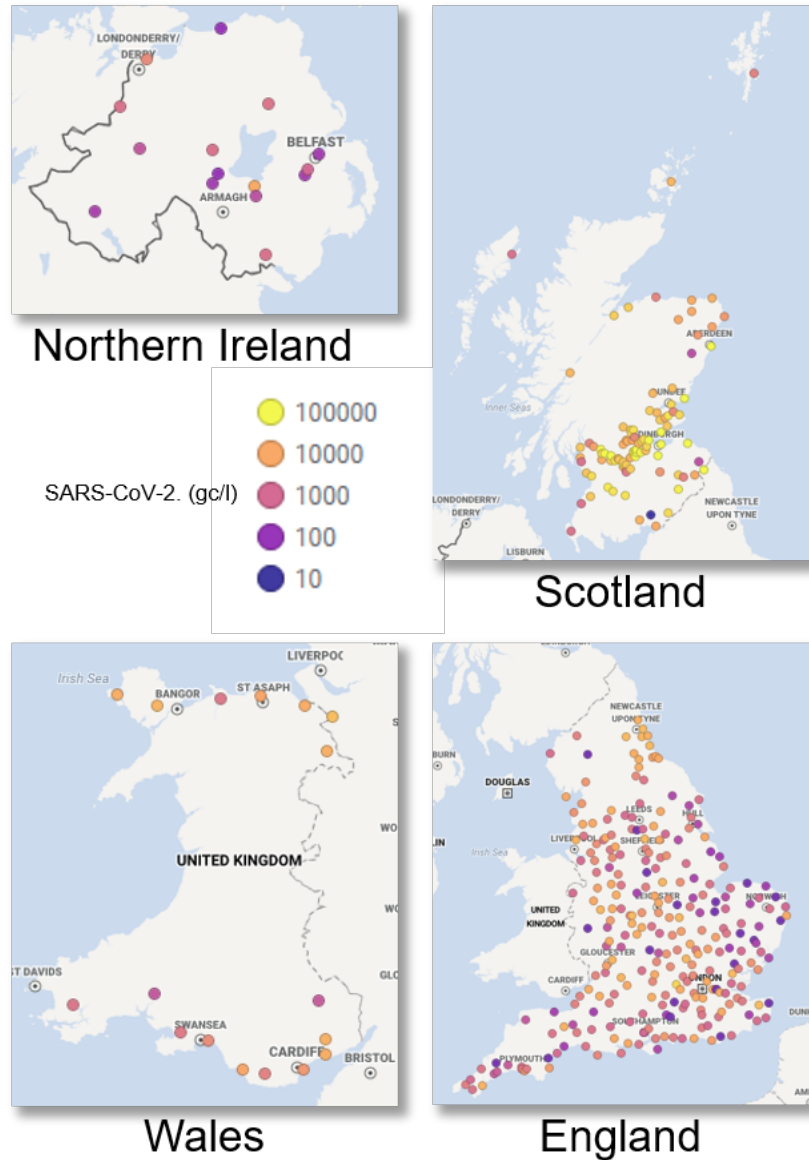
Figure S2: Maps of the four regions of the United Kingdom showing the wastewater sampling locations for the respective national COVID-19 surveillance programmes (as of July 2021). Markers represent centroids of the catchments serving the sample point and shading is the 7-day average SARS-CoV-2 RNA concentration (gene copy per litre) measurements at each site over the last week of June 2021. This is only an example of the spatial distribution of sampling in the UK and comparisons of concentrations between sites should not be made from these figures due to differences in sampling frequency and network characteristics across locations.
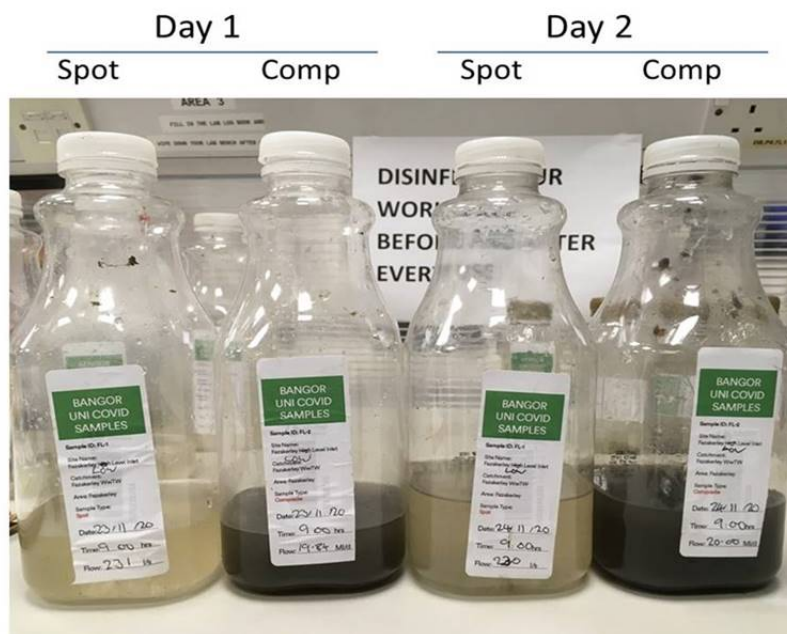
Figure S3: A visual representation of wastewater concentration variability between sampling methods (spot = grab, comp = composite) at a STW site in England. The samples were collected at the same time (09:00 h) on consecutive days for laboratory analysis at Bangor University. The darker the sample, the higher the likelihood of capturing a representative sample, while lighter samples suggest greater flow dilution, or that the sample has missed the peak discharge window.
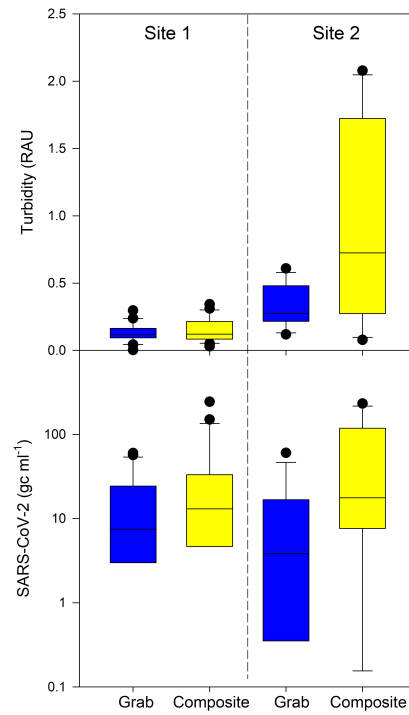
Figure S4: Comparison of grab and composite samples taken from two sites as part of the English wastewater surveillance programme: Site 1 - Domestic source; Site 2 - Domestic + Industrial sources. The boxplots suggest a greater degree of within sample and between-method variability for Site 2 than Site 1, suggesting that combined sewerage systems (i.e. those receiving stormwater or industrial inflow in addition to domestic flow) may impart greater signal variability . Additionally, the lower SARS-CoV-2 measurements for grabs at Site 2, implies that autosampling is more likely to capture the target analyte signal in complex or dilute media.
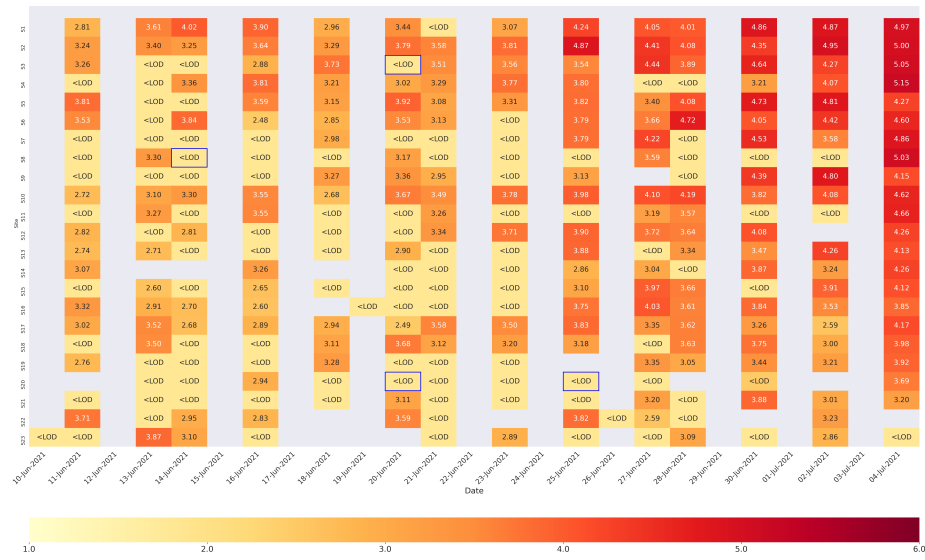
Figure S5: An anonymised heatmap view of 'core city' SARS-CoV-2 RNA concentrations measured in wastewater over a one month period from June - July 2021. Each row is an in-network sample location in the city and each column represents a sample day. Missing values represent a missing sample or no sample taken. Values are the log10 virus RNA concentrations (gene copies per litre). Cells with blue borders are flagged as likely being influenced by high dilution events, and < LOD are measurements below the laboratory limit of detection.
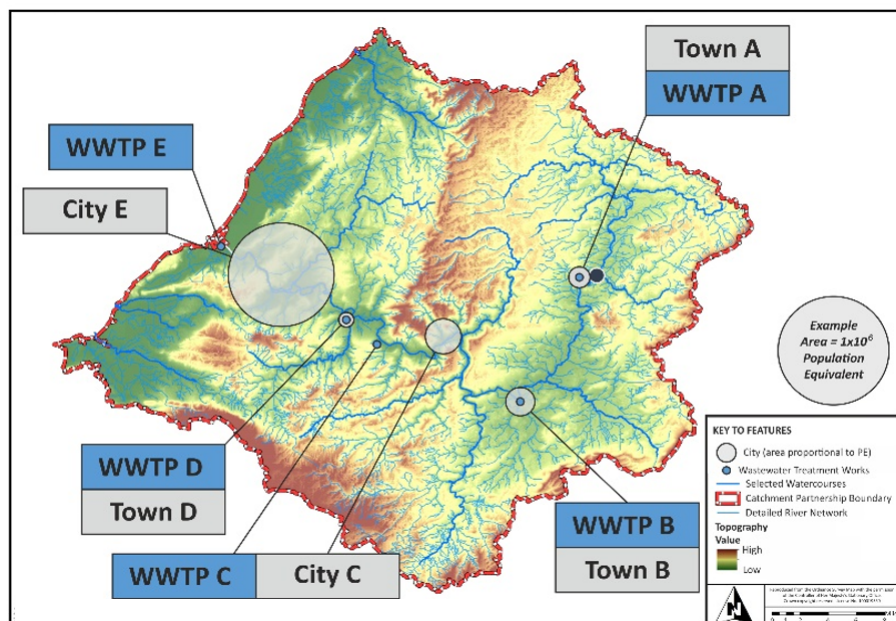
Figure S6: Site locations of studied STWs and corresponding cities and towns.

Table S1: Network characteristics for the studied sites. Residence times are given for typical summer dry-weather flows. P.E. = Population Equivalent.

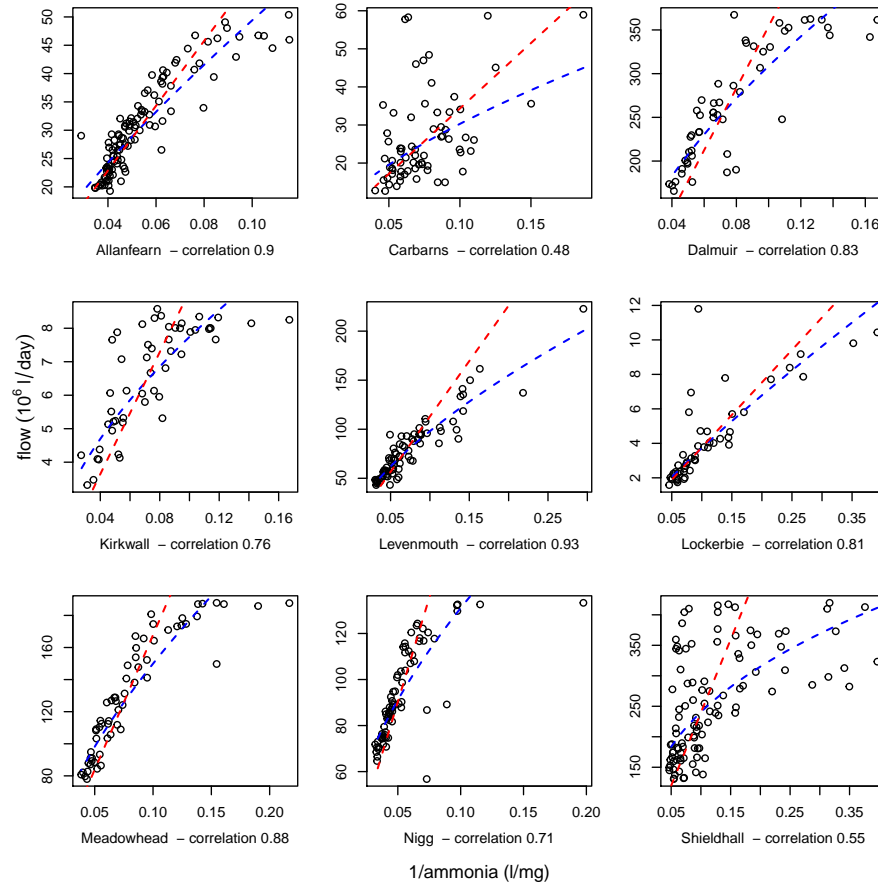| Site	Sewer residence time (h)	Popn. served (P.E.)	Ind. contrib. to P.E.	Mean flow rate (m^3 d^{-1})
A	<0.5 - 4	37,714	0.4 %	$8,242 \pm 3,085$
B	< 0.5 - 4	68.453	30.0 %	$11,202 \pm 3,202$
C	< 0.5 - 9	109,543	1.2 %	$24,875 \pm 2,167$
D	< 0.5 - 2	18,274	0.1 %	$2,924 \pm 199$
E	< 1 - 24	867,244	23.9 %	$153,061 \pm 12,245$

Figure S7: Relationship between flow rate and 1/ammonia concentration at Scottish wastewater sites with more than 40 coupled observations (up to 25 May 2021). The lines show the fitted regression estimates: blue is for the full random coefficient model and red is for the model with the slope for log ammonia fixed at -1. The strength of the relationship varies between sites, as shown by the correlations given. At some sites (e.g. Lockerbie), the fitted lines are quite close, and in other cases (e.g. Shieldhall), the difference is more marked.
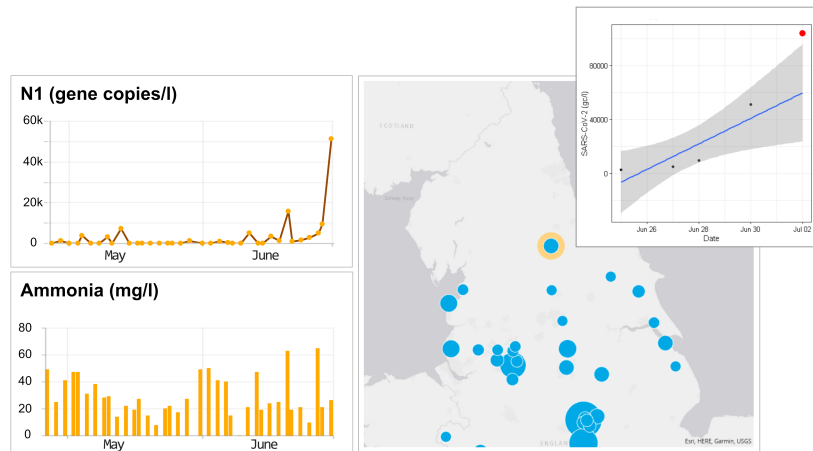
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Figure S8: An example output from a tool developed by the Environment Agency to visualise outliers in SARS-CoV-2 time-series measurements collected as part of the wastewater surveillance programme in England. Inset: Outlier detection using an 80% confidence interval around the linear regression; four weekly samples (black points) are used to generate a linear model and parametric confidence interval, a new sample (red point) is assessed against the confidence band and flagged as an outlier if it falls outside the band. This map was created using ArcGIS® software by Esri. ArcGIS® and ArcMap™ are the intellectual property of Esri and are used herein under license. Copyright © Esri and its licensors. All rights reserved.
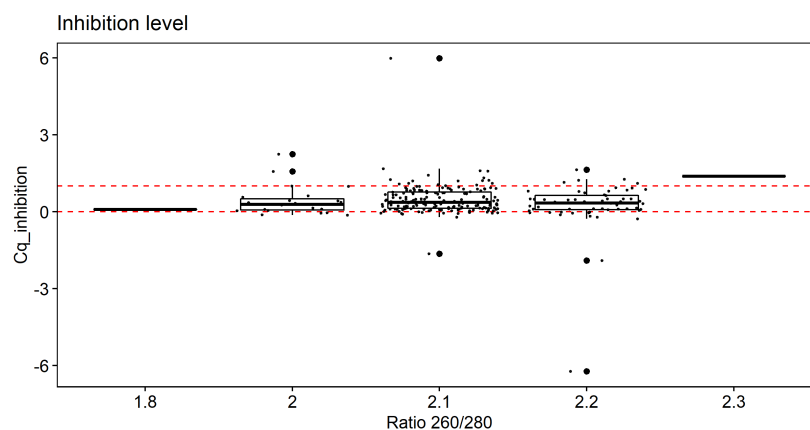
Figure S9: Inhibition level of clean samples spiked with synthetic single-stranded RNA (ss-RNA). The inhibition level was calculated by spiking ssRNA into wastewater extracts and comparing the measured C_q to RNA spiked into molecular negatives (no template controls). The modified PEG method keep the inhibition level below 1.0 C_q and the RNA quality between 2.0 and 2.2.
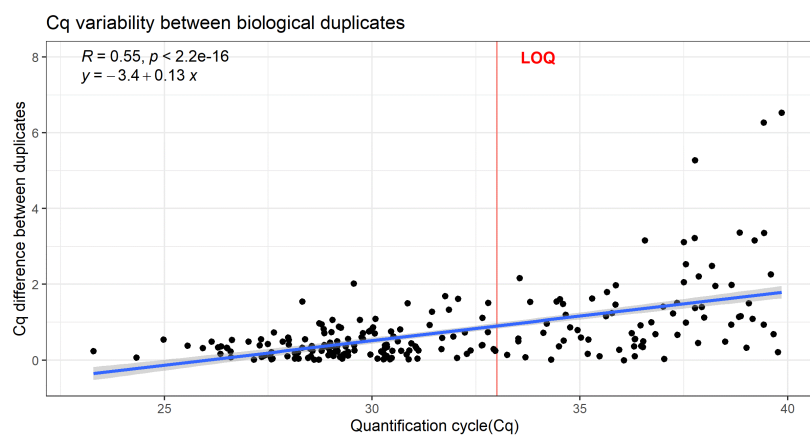

Figure S10: SARS-CoV-2 N1 gene variability between biological duplicates. The C_q variability increased with lower target concentrations (higher C_q). The CV was 1.0 ± 0.9 and 2.6 ± 2.3 for samples with C_q values below and above the LOQ, respectively.