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# A case study of a community-organized wastewater surveillance in a small community: correlating weekly reported COVID-19 cases with SARS-CoV-2 RNA concentrations during fall 2020 to summer 2021 in Yarmouth, ME

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## ABSTRACT

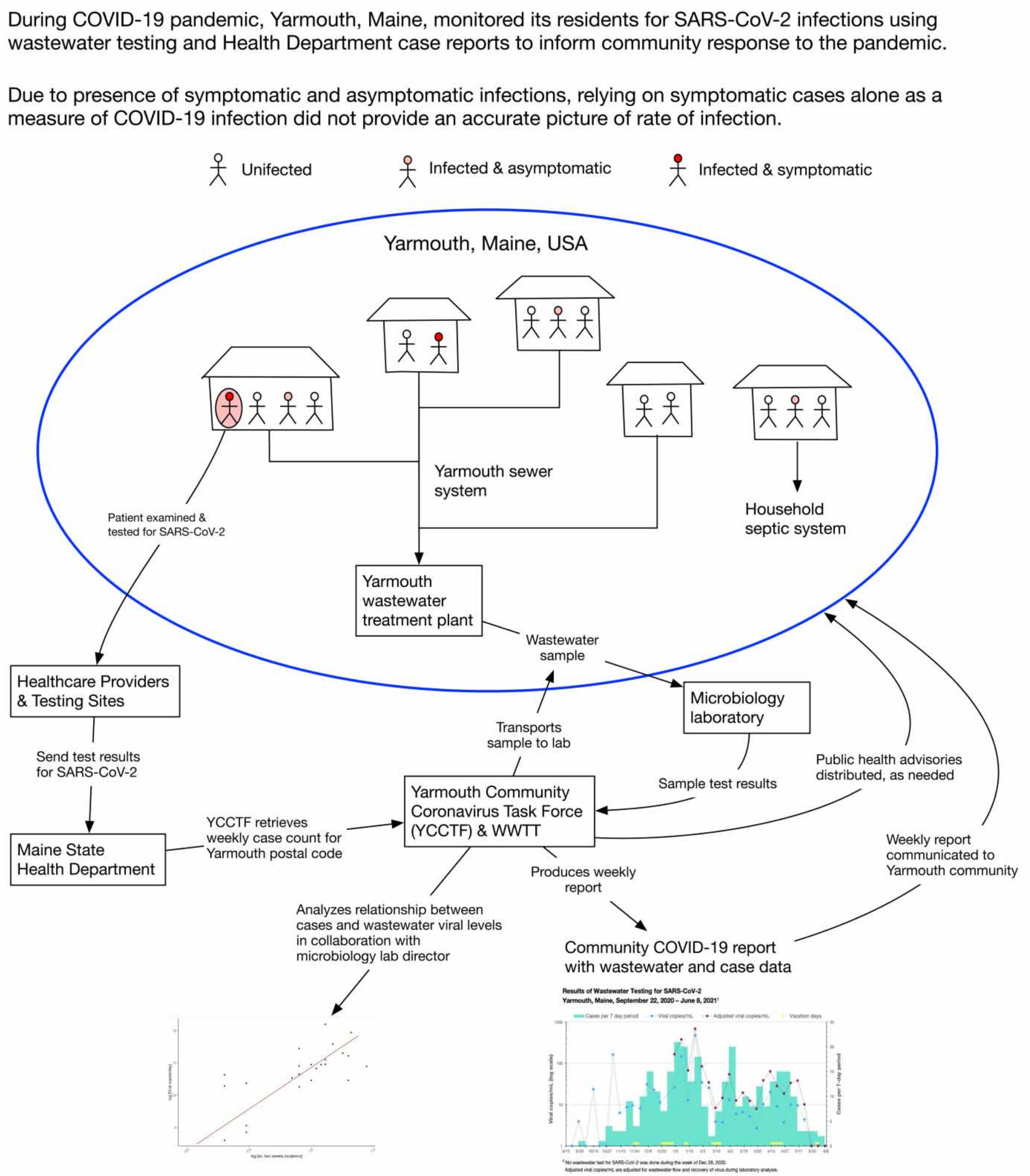
Wastewater surveillance offers a rapid evaluation of SARS-CoV-2 transmission in a community. We describe how a community group, the Yarmouth Wastewater Testing Team (YWTT), in Yarmouth, Maine, (population 8,990) utilized an asset-based community design framework to organize and manage a program to monitor SARS-CoV-2 RNA concentrations. From September 22, 2020 through June 8, 2021, the YWTT disseminated weekly reports of the wastewater results and reported COVID-19 cases within the Yarmouth postal code. After high and increasing SARS-CoV-2 RNA concentrations, the YWTT issued two community advisories to encourage extra care to reduce exposure. Correlations between SARS-CoV-2 RNA concentrations and COVID-19 cases were stronger the week after sampling, and the average of the COVID-19 cases during the week of sampling and the following week, indicating that surveillance provided advance notice of cases. A 10% increase in SARS-CoV-2 RNA concentrations was associated with a 13.29% increase in the average number of weekly reported cases of COVID-19 during the week of sampling and the following week ($R2 = 0.42$; $p < 0.001$). Adjusting for viral recovery (December 21, 2020 through June 8, 2021), improved R2 from 0.60 to 0.68. Wastewater surveillance was an effective tool for the YWTT to quickly respond to viral transmission.

**Key words:** community partnerships, COVID-19, RT-PCR, SARS-CoV-2, wastewater surveillance

## HIGHLIGHTS

- Community organizations can successfully design wastewater surveillance programs.
- Weekly wastewater surveillance correlates to weekly reported COVID-19 cases.
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA concentrations are best correlated to cases the same week and the following week.
- Adjusting for viral recovery increased the fit of linear regression by 8%.
- Community-based initiatives can effectively respond to an increased viral transmission.

## GRAPHICAL ABSTRACT




## BACKGROUND

Wastewater-based epidemiology has been used as a public health tool to evaluate trends in the transmission of various infectious viruses such as hepatitis A, polio, adenovirus, norovirus GII, sapovirus, human herpesvirus 6, human herpesvirus 8, and influenza A (Lago *et al.* 2003; McCall *et al.* 2020; Bi *et al.* 2021). Trends in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA concentrations have been tracked in wastewater treatment plant (WWTP) influent in many communities across the world (Ahmed *et al.* 2020; Kumar *et al.* 2020; Medema *et al.* 2020; Sherchan *et al.* 2020; Prado *et al.* 2021; Street *et al.* 2021). Many of these studies have investigated wastewater surveillance in urban communities with populations $\geq$380,000. These systems are a mixture of industrial and residential wastewater and can have less microbial variability than municipal wastewater systems in small communities (Gudra *et al.* 2022).

Few studies have investigated the potential of wastewater surveillance in smaller municipal wastewater systems to detect and track trends in SARS-CoV-2 transmission. In a rural community of <5,000 people in Eastern Ontario, SARS-CoV-2 N1 and N2 markers were more readily detected from 24-h composite samples ($n = 5$ sampling dates) from pumping stations than those in the downstream lagoon (D'Aoust *et al.* 2021). Significant correlations between reported coronavirus disease (COVID-19) cases and concentrations of SARS-CoV-2 N2 markers ($n = 20$ or 21) were found in only one of the four WWTPs that served <25,000 people in Finland (Tiwari *et al.* 2022). Additionally, in a WWTP serving approximately 17,000 connections in Bexar County, Texas, trends in SARS-CoV-2 RNA concentrations from composite samples of wastewater influent ($n = 13$) were significantly correlated with weekly COVID-19 infection rates (Al-Duroobi *et al.* 2021). These studies demonstrate that wastewater-based surveillance can be used in smaller communities to detect SARS-CoV-2 RNA and correlate the concentrations to reported COVID-19 cases over short periods. More data are needed to demonstrate how wastewater-based surveillance can be used as a public health tool over longer periods in smaller communities and how local communities can meaningfully utilize these data to respond to the pandemic.

Traditional approaches to solving social problems, such as a public health crisis, view communities as having deficits and lacking resources. In contrast, asset-based community development (ABCD) begins with the assumption that all communities regardless of their privilege have people who can, and do, serve as 'connectors' to work collaboratively when strengths, interests, and passions are made visible (Kretzmann & McKnight 1993). ABCD includes individuals who identify their skills and interests and collaborate with existing institutions such as schools and public agencies (Hopes *et al.* 2015). During the COVID-19 pandemic, citizens in many large and small localities came together, illustrating the principles of ABCD to do the following activities: provide housing and home-based resources, run screenings, offer public health information for immigrant groups and other marginalized communities, prepare and deliver food for isolated individuals, and coordinate early childhood education (Hogan 2020; Ishisaka 2020; Michener *et al.* 2021). There are few studies, however, that have documented community-organized wastewater surveillance programs.

Many municipal wastewater surveillance programs are collaborations between local health departments, municipalities, utility companies, and testing laboratories; most of these programs have been housed in health departments, corporations, or local universities. Few wastewater surveillance programs have integrated ABCD into their approach. In this study, we describe a unique community, public utility, and collegiate partnership to monitor SARS-CoV-2 transmission in Yarmouth, ME, a small coastal community (population ~8,990), and to inform public health recommendations. The specific objectives of this study were to:

1. monitor SARS-CoV-2 transmission using wastewater influent from the Yarmouth municipal WWTP independent of weekly reported COVID-19 cases by the Maine Center for Disease Control (ME CDC);
2. qualitatively describe the ABCD initiatives that combat the transmission of SARS-CoV-2 in the Yarmouth community; and
3. mathematically evaluate the relationship between the concentrations of SARS-CoV-2 RNA in wastewater influent and weekly reported COVID-19 cases in a small community, Yarmouth, ME with linear regression analyses and Spearman's correlation coefficients. We also evaluate how lags and leads in SARS-CoV-2 RNA concentrations as well as viral recovery affect the correlation of SARS-CoV-2 RNA concentrations to weekly reported COVID-19 cases.

## METHODS

### Study area and the establishment of a community-based initiative to curb SARS-CoV-2 transmission in Yarmouth, ME

The Town of Yarmouth is a coastal Maine community with a population of 8,990 (U.S. Census Bureau 2020), which is principally a residential community approximately 15 min from Maine's largest municipality, Portland. The Town covers the same geographic area as the Yarmouth zip code, and about 70% (~6,300) of its residents are served by the Yarmouth WWTP. The remaining residents (~2,700) use residential septic systems. The wastewater system contains 30 pump stations and does not have any permitted industrial discharges. The Yarmouth WWTP is a sanitary sewage system with a capacity of 5,910 $m^3$/day of influent, and the mean flow during sample collection was 2,815 $m^3$/day (s.d. = 814 $m^3$/day).

In February 2020, Yarmouth community members became concerned about the arrival of COVID-19 in the United States and, after discussions with Town officials, formed the Yarmouth Community Coronavirus Task Force (YCCTF). This group was comprised of over 50 core individuals from a range of backgrounds that first gathered in March 2020 for a presentation by a local epidemiologist on the science of pandemics. Citizens volunteered to serve on various subcommittees that performed a wide range of duties. For example, while some sewed and promoted face masks, others served as 'neighborhood communicators' whose job it was to connect community members with updates and important information regarding COVID-19 prevention.

### First-hand accounts of establishing a wastewater surveillance program from members of the YCCTF

In July 2020, several YCCTF members learned that some communities were measuring SARS-CoV2 RNA concentrations in wastewater to evaluate viral transmission (Biobot Analytics 2020; Gallatin City-County Health Department 2020). They also learned of the possible establishment of a national wastewater testing program for COVID-19 (Mehrotra *et al.* 2020). After discussions with Town of Yarmouth officials and staff at the Yarmouth WWTP, the YCCTF established the Yarmouth Wastewater Testing Team (YWTT) consisting of six Yarmouth residents with various backgrounds.

The YWTT worked with the Yarmouth Town Manager to identify initial funding for a wastewater surveillance program for 6 months (mid-September 2020–mid-March 2021) through the CARES Act's *Keep Maine Healthy Grant – Waste Water*. An additional 3 months of testing from mid-March 2021 to early June 2021 was funded jointly by the Town of Yarmouth and individual contributions. The YWTT volunteers managed the Yarmouth wastewater surveillance program, including locating a local laboratory, organizing the logistics of coordinating sample collection and transportation, and communicating results to the public.

### Wastewater collection, the concentration of viral nucleic acids, and the quantification of SARS-CoV-2 N1 and N2

From September 22, 2020 to June 8, 2021, 24-h composite samples of untreated influent were aseptically collected once a week from the Yarmouth WWTP, typically starting on Monday at 7:00 am and finishing on Tuesday at 7:00 am; 300 ml of influent was collected between every 15,000 and 30,000 gal using a Hach AS 950 (Hach Company, Loveland, CO) with a refrigeration unit that stored the composite sample at 4 °C. The total volume of each composite sample was ~2 L. The average pH of the composite was 7.6 (s.d. = 0.1). The average concentrations of suspended solids and BOD were 231 mg/L (s.d. = 55 mg/L) and 276 mg/L (s.d. = 336 mg/L), respectively, and were consistently measured in samples collected 1 day after the composite samples. No sample was collected and tested during the week of December 28, 2020. The samples were placed in a cooler with ice and transported to the laboratory. At the laboratory, samples were stored at 4 °C and analyzed within 72 h after the sampling event. For the samples collected from December 21, 2020, to June 8, 2021, within 72 h of sample collection, 131.4 μl of 1:10 dilution of bovine respiratory syncytial virus (BRSV, Zoetis, Parsipanny, NJ) was added to 105 ml of the wastewater influent sample prior to the concentration step. Using the Water SARS-CoV-2 reverse transcription polymerase chain (RT-PCR) test (IDEXX Laboratories, Westbrook, ME), the biological material was concentrated with two centrifugation steps: 4,700 × *g* for 30 min followed by the addition of 10.5 g of polyethylene glycol 8000 and 2.364 g of NaCl per sample and a centrifugation step of 12,000 × *g* for 2 h. Prior to the extraction of nucleic acids, the IDEXX Water Internal Control (IDEXX Laboratories, Westbrook, ME) was added to each sample, positive control, and negative extraction control (sterile $dH_2O$). The nucleic acids were extracted using the IDEXX Water DNA/RNA Magnetic Bead kit. The quantitative PCRs (qPCRs) were measured using absolute concentrations of the N1 and N2 markers in a single reaction using the QuantStudio 3 (Applied BioSytems, Waltham, MA). Sample volumes of 5 μl were added to the RT-qPCR run for all samples, non-template controls, positive controls, and all steps of the standard curve. Each RT-qPCR run contained duplicates of the following: each sample, non-template control, one step of the standard curve (2,000 or 20,000 copies/5 μl), negative extraction control (nuclease-free $H_2O$), and positive control of undiluted BRSV (starting on December 21, 2021) and 20 μl of 1:100 dilution of SARS-CoV-2 culture fluid (Isolate: USA-WA1/2020; ZeptoMetrix, Buffalo, NY). The 2019-nCoV_N_Positive Control plasmid (Integrated DNA Technologies Inc., Coralville, IA) was the quantitative standard, and the standard curve included five steps. A new standard curve was made every four qPCR runs, and each step was run in triplicate in the qPCR along with a negative control. The standard curves had a limit of quantification at either 2 copies or 20 copies per reaction (average $C_t$ values = 39.36 and 36.19, respectively) combined for both the N1 and N2 markers. Samples with detectable but not quantifiable concentrations of SARS-Cov-2 N1/N2 markers were reported at the experimental limit of quantification, either 2 copies/rxn or 20 copies/rxn. All samples that contained undetectable SARS-CoV-2 N1/N2 markers were reported at half of the theoretical limit of detection, either 1 copy/rxn or 10 copies/rxn, as previously recommended (CDC n.d.).

### Collection of reported cases, data analyses, and statistical analyses

In all samples, concentrations of SARS-CoV-2 RNA were adjusted for the 24 h flow of the wastewater; and from December 21, 2020, to June 8, 2021, the SARS-CoV-2 RNA concentrations were also adjusted for viral recovery using BRSV spiked into each sample.

The number of COVID-19 cases each week for the Yarmouth, Maine, zip code consisted of cases reported to and compiled by the Maine Center for Disease Control & Prevention (Maine CDC). The weekly reported cases spanned from Monday to Sunday each week and were publicly available on the subsequent Wednesday (Supplementary Material, Figure S1).

All statistical analyses were performed in R Version 4.1.3 using the base and stats package (R Core Team 2022). The alpha value for all statistical tests was 0.05. All graphical plots were created using the ggplot2, ggpubr, and ggpmisc packages. Linear regression was used to analyze associations between the number of reported COVID-19 cases and concentrations of SARS-CoV-2 RNA, as this type of regression analysis provided a good fit in wastewater surveillance in medium and larger communities (Anneser *et al.* 2022). The following equation was used:

$$\log(S_n) = m_n * \log(I_n) + b \quad (1)$$

$S_n$ was the concentration of SARS-CoV-2 RNA per day normalized with the flow rate where:
$S_1$ included samples collected from September 22, 2020, to June 8, 2021 but did not account for the viral recovery;
$S_2$ included samples collected from December 21, 2020 to June 8, 2021 but did not account for viral recovery; and
$S_3$ included samples collected from December 21, 2020 to June 8, 2021 and accounted for viral recovery with BRSV.

Within each variation of $S_n$, five variations of the independent variable, $I_n$, were used to evaluate the various lags and leads in the number of weekly reported COVID-19 cases for individuals residing in the Yarmouth zip code (Supplementary Material, Figure S1).

$I_1$ was the reported cases for the week before wastewater sampling (1–7 days prior to wastewater sampling);
$I_2$ was the average reported cases for the same week as wastewater sampling and the week prior to wastewater sampling;
$I_3$ was the reported cases for the week of sampling (0–6 days after wastewater sampling);
$I_4$ was the average of the reported cases for the same week as wastewater sampling and the week after wastewater sampling; and
$I_5$ was the reported cases for the week after sampling (7–13 days after sampling).

Within each variation of $S_n$, the best-fit equation from the variations of $I_n$ was the equation with the smallest Akaike Information Criterion (AIC) value. Comparisons of best-fitted equations among $S_{1-3}$ were analyzed using their $R^2$ values.

Non-parametric Spearman's rho correlation coefficients also evaluated the relationships between SARS-CoV-2 RNA concentrations ($S_n$) to the various lags and leads in reported COVID-19 cases ($I_n$). The non-parametric test was chosen as the Shapiro–Wilk test demonstrated that the data were not normally distributed.

## RESULTS

### Summary of wastewater samples and SARS-CoV-2 RNA concentrations

In total, 37 weekly samples from 24-h composites of wastewater influent were collected from the Yarmouth WWTP from September 22, 2020 to June 8, 2020. No sampling occurred on December 28, 2020. A summary of the concentrations of SARS-CoV-2 RNA adjusted for the flow rate and recovery, as well as the weekly reported cases of COVID-19 in Yarmouth, are available in the Supplementary Material, Example report to stakeholders. The detection limit (and the limit of quantification) of the concentration of SARS-CoV-2 RNA was generally <log 9.1 copies/day (and between log 9.9 and 10.6 copies/day; Figure 1(a)) and was dependent on the flow rate (and the viral recovery for samples collected from December 21 to June 8, 2021). Seven samples (19%) had undetectable concentrations of SARS-CoV-2 RNA: September 22, 2020; October 6, 2020; October 20, 2020; October 27, 2020; May 25, 2021; June 1, 2021; and June 8, 2021 (Figure 1(a)). Two samples (5%) had detectable but not quantifiable concentrations of SARS-CoV-2 RNA (November 10, 2020 and February 22, 2021). The average concentration of SARS-CoV-2 RNA adjusted for the flow rate only (and recovery) was log 10.16 copies/day (log 10.68 copies/day) and ranged from <log 8.51 to log 12.01 copies/day (<log 8.60 to log 12.19 copies/day; Figure 1(a)). For the recovery-adjusted samples, the recovery of BRSV averaged 40%, with a range of 18–96%, which was >10% recovery, which was the

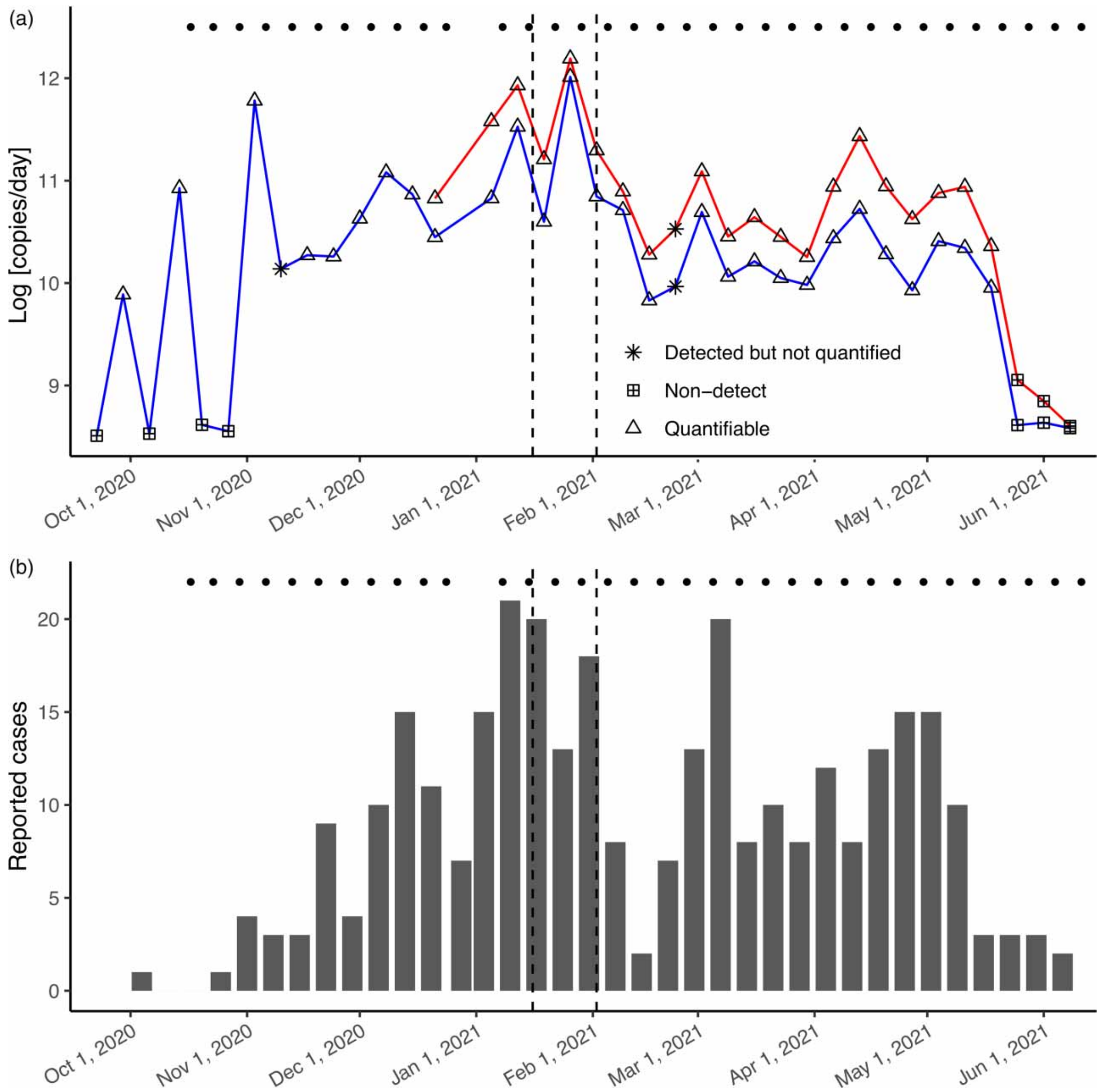



**Figure 1** | (a) Log concentrations of SARS-CoV-2 RNA (copies/day) in the wastewater influent from the Yarmouth WWTP from September 22, 2020 to June 8, 2021. Values were adjusted for the flow rate only (blue) and for the flow rate and recovery (red). No wastewater sample was analyzed during the week of December 28, 2020. Samples with undetected concentrations of SARS-CoV-2 RNA are represented as the log of ½ value of the detection limit and adjusted for the flow rate (and recovery if applicable). Concentrations of SARS-CoV-2 RNA that were detected but not quantifiable are represented as the log-transformed limit of quantification and were adjusted for the flow rate (and recovery if applicable). (b) Number of cases of COVID-19 within the zip code for Yarmouth, ME as reported weekly by the Maine CDC. The dashed line represents the date of public advisories of high SARS-CoV-2 RNA concentrations in RNA. The black dots represent the dates that weekly reports of SARS-CoV-2 RNA were announced to the public. Please refer to the online version of this paper to see this figure in colour: https://dx.doi.org/10.2166/wh.2023.238.

threshold to re-run samples in a study of SARS-CoV-2 RNA measured from settled solids in untreated wastewater (Yu *et al.* 2021).

### Description of the trends in SARS-CoV-2 RNA concentrations in wastewater and weekly reported COVID-19 cases in Yarmouth, ME between September 22, 2020 and June 8, 2021

From September 22 to October 27, 2020, SARS-CoV-2 RNA concentrations fluctuated each week between non-detected and $\geq$1 log above the detection limit (Figure 1(a)). Concentrations of SARS-CoV-2 RNA were detected from November 3, 2020 to May 18, 2021. Many peaks in SARS-CoV-2 RNA concentrations, as defined by $>$1 log change in detectable concentrations adjusted for the flow rate, occurred within 2 weeks after holidays and Yarmouth public school vacations, such as October

14, 2020 (2 days after Indigenous People's Day/Columbus Day); January 12, 2021 (8 days after the return from Christmas/New Year's break); and January 26, 2021 (8 days after Martin Luther King Day) (Figure 1(a)).

There were <5 weekly reported COVID-19 cases from residents in Yarmouth during the weeks starting on September 22, 2020 to November 15, 2020, the week of February 8, 2021, and May 10, 2021 to June 6, 2021 (Figure 1(b)). As shown in Figure 1(b), weekly cases rose during fall 2020 and peaked in early January 2021. Additional peaks occurred in early March and late April 2021.

## Comparing the SARS-CoV-2 RNA concentrations and reported COVID-19 cases in Yarmouth, ME

Overall, linear regression analyses demonstrated a significant temporal correlation between the log concentrations of SARS-CoV-2 RNA and the log number of weekly reported COVID-19 cases even when including a lag or a lead up to 1 week ($p < 0.05$; Figure 2(a)–2(o)). The best-fit regressions for each dependent variable of $S_{1-3}$ and the log concentration of SARS-CoV-2 RNA vs. the log of the number of reported COVID-19 cases were with $I_4$, which represents the average of the reported COVID-19 cases for the same week and the week after the wastewater sample was collected (Figure 2(j)–2(l)). From September 22, 2020 to June 8, 2021, a 13.3% increase in $I_4$ was associated with a 10% increase in the concentration of SARS-CoV-2 RNA, $S_1$ (Figure 2(j); AIC = 77.82; $N = 35$). For the period from December 21, 2020 to June 8, 2021, the log concentrations of SARS-CoV-2 RNA were adjusted for the flow rate only, $S_2$, or the flow rate and viral recovery, $S_3$. Adjusting for viral recovery in addition to flow rate resulted in a 2.7% increase of COVID-19 cases associated with a 10% increase in SARS-CoV-2 RNA concentrations. Specifically, the best-fit equation for $S_2$, indicated an 18.9% increase in $I_4$ was associated with a 10% increase in $S_2$ (Figure 2(k); AIC = 40.85; $N = 24$), while the best-fit equation for S3 indicated a 21.6% increase in $I_4$ was associated with a 10% increase in $S_3$ (Figure 2(l); AIC = 38.89; $N = 24$).

We compared the $R^2$ values of the three best-fit equations from $S_{1-3}$ to evaluate which linear equation best represented the relationship between the SARS-CoV-2 RNA concentrations and the number of reported COVID-19 cases, $I_4$. The smallest $R^2$ value, 0.4 (Figure 2(a)–2(o)), was from the $S_1$ equation, which explained 42% of the variation in $I_4$. The largest $R^2$ was from $S_3$, 0.68, which explained 68% of the variation in $I_4$. The $R^2$ value of the $S_2$ equation was 0.60. Thus, adjusting for viral recovery in addtion to flow rate improved the fit of the linear regression model by 8%.

Overall, Spearman's rho correlation coefficients of the concentrations of SARS-CoV-2 RNA, $S_n$, and the reported cases of COVID-19, $I_n$, were positively and significantly correlated with all leads and lags in the reported clinical cases ($p < 0.05$) except for three instances: $S_2$ vs. $I_1$, $S_2$ vs. $I_2$ and $S_3$ vs. $I_1$ (Table 1). The largest Spearman's rho correlation coefficients for the concentrations of SARS-CoV-2 RNA adjusted for flow rates only ($S_1$and $S_2$) were with the number of reported COVID-19 cases 1 week after the wastewater sample, $I_5$ ($\text{rho}_{S_1-I_5} = 0.744$ and $\text{rho}_{S_2-I_5} = 0.613$, respectively; Table 1). When viral recovery was considered along with flow rate ($S_3$) the largest Spearman's rho correlation coefficient of the concentrations of SARS-CoV-2 RNA was with average of the number of COVID-19 cases reported for the week of, and 1 week after, wastewater sampling, $I_4$ ($\text{rho}_{S_3-I_4} = 0.706$). Reported COVID-19 cases measured 1 week after sampling had a similar rho of 0.700 (Table 1).

## Qualitative description of the formation of the YCCTF, a community-based organization

Qualitative feedback from members of the YCCTF illustrated that the ABCD model can be an effective approach for understanding local organizing. As one team member explained, getting 'well-known' residents involved in ABCD from the beginning was critical in establishing buy-in among members of the public. As volunteers identified duties and roles that often fit with their professional line of work or personal experiences, the Yarmouth community focused its efforts on the needs of seniors, K-12 students, and business owners. A member of the YWTT who has lived in the community for 36 years and has been involved in both social service management and political campaigns explained: 'I know how to organize people to do good stuff.' Echoing other members' sentiments, she explained that she and community members 'began to create capacity to meet the needs of citizens through ordinary channels.' The Task Force was established as a grassroots, loose collective that was non-hierarchical. In addition, she emphasized that the response to the pandemic was a collective effort, separate from the Town's government. She added that we were 'doing what we think will benefit the Yarmouth people.' Another member, an environmental scientist who has lived in Yarmouth for 30 years described the process to identify the strengths of community members as 'organic.' His introduction to the YCCTF's work occurred through the Rotary Club. He first helped with grocery shopping and later joined the YWTT, given his experience with wastewater treatment. He explained: 'I figured out what it would take to do this work that I understood.' While he identified his specific skills

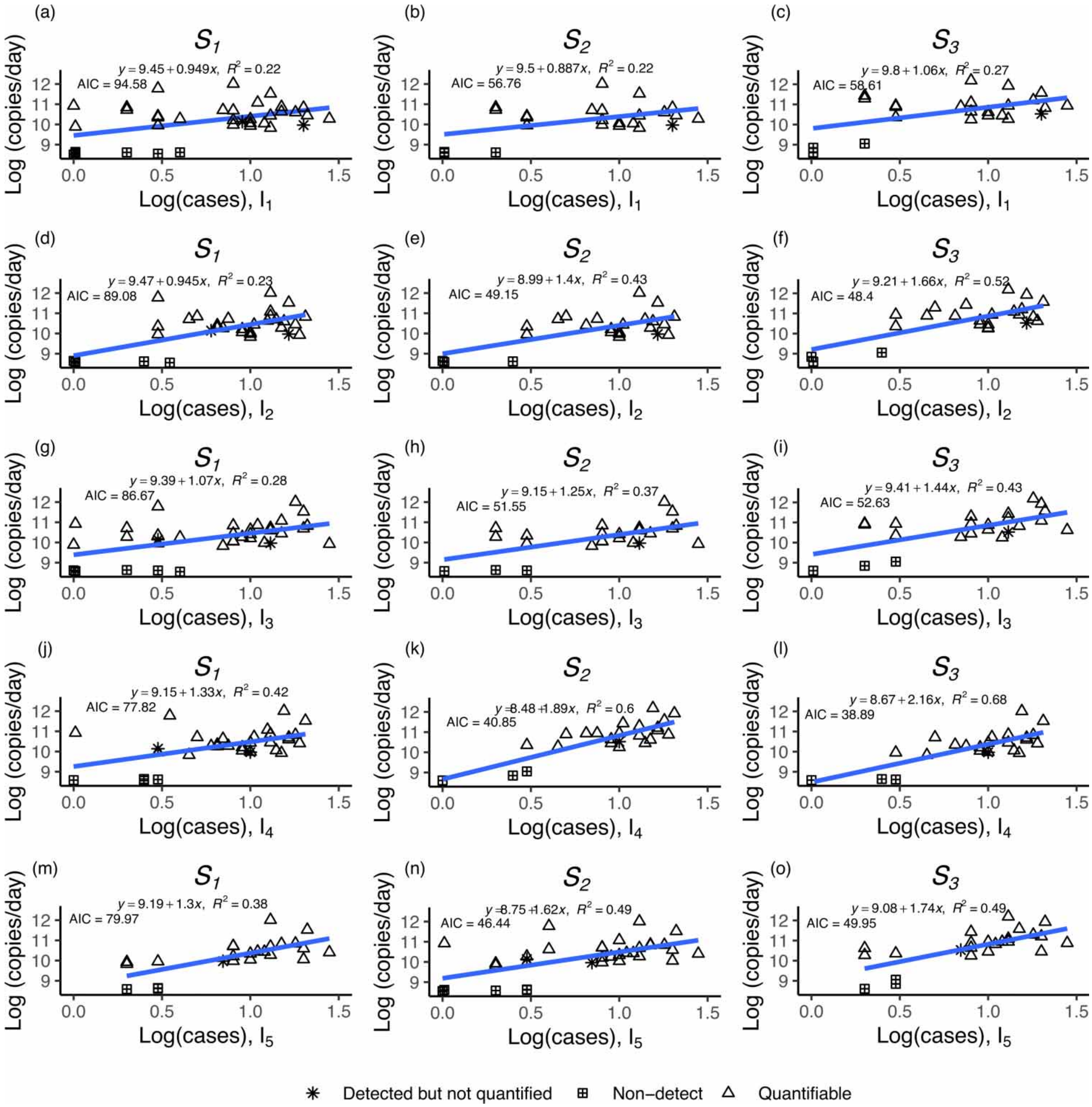



**Figure 2** | (a–o) Evaluation of the association of the log-transformed of the number of weekly reported COVID-19 cases within the zip code for Yarmouth with leads and lags of up to 1 week from the sample collection, $I_{1-5}$, to the log-transformed concentrations of SARS-CoV-2 RNA (copies/day) adjusted for the flow rate, $S_1$ (a, d, g, j, m), during September 22, 2020 to June 8, 2021; $S_2$ (b, e, h, k, n), during December 21, 2020 to June 8, 2021; and $S_3$ (c, f, i, l, o), adjusted for the flow rate and viral recovery during December 21, 2020 to June 8, 2021. $I_1$ was the number of weekly reported cases 1 week prior to sample collection. $I_2$ was the average of the weekly reported cases for 1 week before and the same week as the wastewater was collected. $I_3$ was the number of weekly reported cases for the same week as the wastewater was collected. $I_4$ was the average of the weekly reported cases for the same week and 1 week after the wastewater was collected. $I_5$ was the number of weekly reported cases 1 week after the wastewater was collected. The blue lines represent the linear regression of each dataset with the corresponding parameters of each equation detailed in each graph. Samples with non-detected concentrations of SARS-CoV-2 are represented at the log-transformed ½ value of the detection limit and adjusted for the flow rate. Concentrations of SARS-CoV-2 that were detected but not quantifiable are represented at the log-transformed limit of quantification and were adjusted for the flow rate. Please refer to the online version of this paper to see this figure in colour: https://dx.doi.org/10.2166/wh.2023.238.

**Table 1** | Spearman rho correlation coefficients of the log concentrations of SARS-CoV-2 RNA adjusted for flow rates, $S_1$ and $S_2$, during September 22, 2020 to June 8, 2021 and December 21, 2020 to June 8, 2021, respectively, and $S_3$, which evaluated viral recovery and flow rate during December 21, 2020 to June 8, 2021 to the log-transformed number of weekly reported COVID-19 cases (with variations in lead and lag times up to 1 week from the wastewater sample collection, $I_{1-5}$) in the Town of Yarmouth, ME, $I_n$

| Weekly reported cases of COVID-19, log $I_n$ | N | Spearman's rho |
|---|---|---|
| $S_1$ Dates of samples – September 22, 2020–June 8, 2021, viral recovery not measured | | |
| $I_1$ (1 week before the wastewater sample) | 37 | 0.331* |
| $I_2$ (average of the same week as sampling and the week before) | 36 | 0.406* |
| $I_3$ (same week as the wastewater sample) | 36 | 0.472** |
| $I_4$ (average of the same week as sampling and the week after) | 35 | 0.561*** |
| $I_5$ (1 week after the wastewater sample) | 35 | 0.613*** |
| $S_2$ Dates of samples – December 21, 2020–June 8, 2021, viral recovery not measured | | |
| $I_1$ (1 week before the wastewater sample) | 24 | 0.219 |
| $I_2$ (average of the same week as sampling and the week before) | 24 | 0.383 |
| $I_3$ (same week as the wastewater sample) | 24 | 0.490* |
| $I_4$ (average of the same week as sampling and the week after) | 24 | 0.692*** |
| $I_5$ (1 week after wastewater sample) | 24 | 0.744*** |
| $S_3$ Dates of samples – December 21, 2020–June 8, 2021, viral recovery measured | | |
| $I_1$ (1 week before the wastewater sample) | 24 | 0.302 |
| $I_2$ (average of the same week as sampling and the week before) | 24 | 0.485* |
| $I_3$ (same week as the wastewater sample) | 24 | 0.532** |
| $I_4$ (average of the same week as sampling and the week after) | 24 | 0.706*** |
| $I_5$ (1 week after the wastewater sample) | 24 | 0.700*** |

*p*-values: * $<0.05$; ** $<0.01$; *** $<0.001$.

and expertise, he also was quick to highlight the egalitarian way others on the YCCTF functioned, adding that 'nobody cared about who got credit for [the] work.' Merging personal interests and professional expertise, the YWTT members collaborated with the community's water management facility to obtain wastewater samples, identified a college to conduct laboratory analyses, and continuously reassessed their goals and the implications of their work to the community. Members of the YWTT team spoke about the challenge of justifying the expenses associated with testing the wastewater. Members did not want the testing to be simply a 'scientific exercise,' but rather something that could be translated into action should the testing yield unfavorable results. As one member explained, the YWTT saw testing as 'another way to view information [about the pandemic] and as a tool to inform neighbors.' Additionally, a member emphasized that 'Too often these projects are undertaken by people/organizations removed from the local community and there is not the support or use of the data by the community contributing the samples or meant to act on it.' Similar to challenges identified by others studying ABCD, the YWTT continuously engaged in discussion about the purpose, duties, and costs of wastewater surveillance.

## Dissemination of wastewater surveillance data to the community and the YCCTF's response to changing concentrations of SARS-CoV-2 RNA and reported COVID-19 cases

The YWTT waited until October 17, 2020, after 4 initial weeks of wastewater testing, to begin preparing and disseminating weekly wastewater testing reports to the Yarmouth Town Manager, the Superintendent of the Yarmouth WWTP, Yarmouth Community Services, and Yarmouth residents (Figure 1(a) and 1(b)). The YWTT issued subsequent reports for every week that testing was performed. An example of the reports is provided in the Supplementary Material. The first communications began as emails with a report that included a brief overview of wastewater testing for SARS-CoV-2 RNA, a graph and table with historical and current wastewater data, a note on the wastewater sampling methodology, and frequently asked questions. Weekly COVID-19 cases reported by the Maine CDC for the Yarmouth zip code were added in early November 2020. Eventually, concentrations of SARS-CoV-2 RNA that were adjusted for the flow rate and viral recovery were added. The reports were posted each week on 'Be Well Yarmouth,' the Town of Yarmouth's COVID-19 website (Yarmouth Community

Coronavirus Task Force 2022). Beginning on December 13, 2020, the report was also sent to the Maine Department of Health and Human Services and a larger local audience; an email summary and interpretation of each report's findings were added to facilitate communication. Beginning with the first report, neighborhood communicators informed their neighbors of the recent wastewater results and weekly reported COVID-19 cases in Yarmouth. Additionally, they relayed pertinent information regarding the COVID-19 response in the community, such as community testing events, vaccination events, and other resources available to community members. On January 16, 2021 and February 2, 2021, when concentrations of SARS-CoV-2 RNA were higher and increasing from the previous weeks, the YWTT prepared and the YCCTF issued community advisories alongside the wastewater reports to encourage extra care to reduce exposure in the coming weeks (Figure 1(a) and 1(b)). The extra care emphasized in the reports included actions such as wearing masks and following US CDC and Maine CDC recommendations regarding when to get tested for COVID-19, quarantining, isolation, and locations of testing facilities. The advisories were sent based on the recommendations of two or three members of the YCCTF who regularly reviewed the clinical and wastewater data to look for increasing trends in cases in the previous 4 weeks. At the time of the dissemination of the advisories, the existing literature did not recommend specific quantitative thresholds for genetic markers measured from wastewater or clinical cases to trigger public health action to mitigate the transmission of SARS-CoV-2.

Retrospective analysis of the clinical and wastewater data revealed that the advisories were disseminated when (1) significant increases in unadjusted wastewater SARS-CoV-2 levels (at least 3$\times$ the concentrations in the previous 2–3 weeks and concentrations $>$100,000 copies/L, as compared to an unadjusted 'baseline' of about 5,000–10,000 copies/L) and (2) concurrent increases or high numbers of COVID cases in the Yarmouth zip code area, as reported by Maine CDC, or in Yarmouth schools over a 2–4-week period. (We did not have SARS-CoV-2 RNA concentrations adjusted for viral recovery for the test results on which the first advisory was based.) There was no other period from September 2020 to June 2021 when these conditions were met. There were increases in SARS-CoV-2 RNA concentrations and reported cases from February 22 to March 7 and during April 2021, but the RNA concentrations never reached the levels seen in January 2021.

## DISCUSSION

Wastewater surveillance is a popular public health tool that can help monitor the transmission of SARS-CoV-2 and other pathogens. The purpose of this study was to demonstrate the performance of a wastewater surveillance program using an ABCD framework in a small community, Yarmouth, ME. Specifically, the YCCTF and the Town of Yarmouth, ME, established and funded a community-based wastewater surveillance program, which monitored the presence and concentrations of SARS-CoV-2 RNA in the majority of the Yarmouth community for ~\$400 per sample for 37 weeks. To our knowledge, this is one of the few documented examples of community-based wastewater surveillance in a small community.

We described the results of longitudinal wastewater surveillance and concurrent weekly reported COVID-19 cases in the Town of Yarmouth. We also evaluated the relationship between the concentrations of SARS-CoV-2 RNA and various lags and leads in weekly reported COVID-19 cases. Temporal trends in Yarmouth's wastewater and clinical data followed general trends reported in the Northeast United States. Specifically, in Yarmouth and metropolitan Boston, MA, clinical cases increased from September 23, 2020 until mid-January 2021, followed by another spike in mid-April 2021 (Xiao *et al.* 2022). Overall, our analyses demonstrated that there was a significant and positive temporal correlation between the number of weekly reported COVID-19 cases in Yarmouth ($I_n$) and the concentrations of SARS-CoV-2 RNA ($S_{1-3}$) in the municipal wastewater with both linear regression and Spearman's rho correlations (Table 1 and Figure 2(a)–2(o)). Similarly, a wastewater surveillance study of a WWTP in metropolitan Charlotte, NC, USA (population = 68,895) demonstrated that linear regressions and Spearman's correlations of RT-qPCR measurements of SARS-CoV-2 N1 and N2 markers with reported COVID-19 cases on the same day and up to 14 days later were significantly and positively correlated (Barua *et al.* 2022). Additionally, Spearman's rho and linear regression analyses provided a positive and significant correlation between the concentrations of N2 markers measured from grab samples in WWTPs in metropolitan Reno-Sparks, NV with reported COVID-19 cases on the same day as sampling as well as a 7-day lead in cases ($p < 0.001$; Li *et al.* 2022). The significant association between the SARS-CoV-2 RNA concentrations and lags in reported cases could be the result of viral shedding by infected individuals in wastewater prior to the onset of symptoms, delays in seeking diagnostic testing, or lags in reporting positive COVID-19 cases to health officials.

Specifically, we compared the linear relationship between SARS-CoV-2 RNA concentrations, $S_{1-3}$, in Yarmouth wastewater to various leads and lags in the reported COVID-19 cases ($I_{1-5}$). Overall, the best-fit of the equations, as evaluated by the lowest AIC value, was from the average number of COVID-19 cases reported from the week of sampling and the following week ($I_4$; Table 1). Similarly, a study of municipal wastewater surveillance in metropolitan Boston, MA found better correlations between daily measurements of SARS-CoV-2 RNA concentrations 4–5 days prior to the daily COVID-19 case counts (Wu *et al.* 2022). Wastewater surveillance in three municipal WWTPs in the Charlotte, NC metropolitan area demonstrated the best correlation of daily measurements of SARS-CoV-2 N1 and N2 markers in 24 h composite samples to the 7-day rolling average of reported COVID-19 cases with a wastewater sample lead of 5–11 days (Barua *et al.* 2022). Our results provide further evidence that reported cases of COVID-19 in sewersheds servicing small and large communities were best correlated with retrospective concentrations of SARS-CoV-2 RNA measured with daily and weekly sampling schedules. Therefore, wastewater surveillance can be used as an early warning system in both large and small communities.

Spearman's correlations also demonstrated that the strongest correlations of SARS-CoV-2 RNA concentrations, $S_{1-3}$, were with reported COVID-19 cases averaged from the same week and the week after sampling, $I_4$, or the week after sampling, $I_5$. Spearman's correlation and linear regressions in SARS-CoV-2 RNA concentrations measured between December 21, 2020 and June 8, 2021 adjusted for the flow rate only, $S_2$ differed in which the type of lag in reported COVID-19 cases had the strongest relationship, $I_4$ for linear regression and $I_5$ for Spearman's correlation. Specifically, Spearman's correlation (and $R^2$ values) with $I_4$ had a rho value of 0.052 larger (or 0.105 smaller) than $I_5$. This difference in the ranks of the strongest relationship of the variables measured with Spearman's correlation coefficients and linear regression analyses could be attributed to the increased variability of an evaluation of a subset of our entire dataset. Although a previous study demonstrated that linear regression analyses can adequately explain the relationship between SARS-CoV-2 RNA concentrations and reported COVID-19 cases (Anneser *et al.* 2022), such an analysis assumes a constant slope between the variables, which is not required to calculate Spearman's correlation coefficients.

In our study, there were 10 instances (26% of samples) in which the concentration of SARS-CoV-2 RNA was either below detection or detected but not quantifiable. In the best-fit model for $S_1$, 60% of the undetected ($n = 4$) and detected but not quantifiable samples ($n = 6$) occurred when $I_4 \leq 3$ cases. This indicates that the correlation between $S_1$ and $I_4$was still meaningful when $S_1$ was nondetectable.

The adjustment of the SARS-CoV-2 RNA concentrations for viral recovery from December 21, 2020 to June 8, 2021 improved the goodness-of-fit measure, $R^2$, from 0.60 to 0.68 when plotted against the average reported COVID-19 cases during the week of and the week after sampling, $I_4$ (Figure 2(k) and 2(l)). The significance of the correlation of $I_4$ and $S_{2-3}$ did not change. Although previous studies demonstrated that normalizing viral RNA concentrations for viral recovery improved correlations to case data in the samples gathered from municipal WWTPs servicing smaller communities, the correlations were still statistically significant without the normalization (Feng *et al.* 2021; Nagarkar *et al.* 2022; Vadde *et al.* 2022). Thus, our data, and data from previous studies, indicate that the added time and cost required to assess viral recovery in smaller municipal systems may not be necessary to measure SARS-CoV-2 RNA concentrations from wastewater influent.

On November 3, 2020, the SARS-CoV-2 RNA concentration increased to log 11.78 copies/day, but during the week of sampling and the following week, there were 5 reported COVID-19 cases (Figure 1(a) and 1(b)). The sampling occurred the week that Yarmouth's high school switched to remote learning due to an outbreak at the school. It is possible that the COVID-19 infections were underreported among high-school students due to asymptomatic infections and symptomatic students who did not have sought care. Similarly, college students living in dormitories were less likely to have symptomatic infections (Poletti *et al.* 2021; Schmitz *et al.* 2021) and may not have sought testing. Wastewater surveillance of SARS-CoV-2 in a southern Nevada community (population of 16,399) estimated 3.3 times more COVID-19 infections than the number of confirmed cases from March 2020 to April 2021 (Vo *et al.* 2022). Additionally, wastewater surveillance of SARS-CoV-2 in metropolitan Boston, MA estimated at least 3.8 times more infections than reported cases during March 18–23, 2020 (Wu *et al.* 2020). These studies indicate that reported cases underrepresent the total COVID-19 cases in a sewershed, and wastewater-based epidemiology can provide a better estimate of the total caseload.

Our study supports the theory that community-based organizations, such as the YCCTF, can quickly engage community members and provide them with information and local resources to mitigate COVID-19 transmission (Korfmacher & Harris-Lovett 2022). Alongside the weekly wastewater testing reports, the YCCTF also provided approachable explanations of the testing results to the local community. Neighborhood leaders sought out and interacted with members of harder-to-reach populations. After viral peaks in the wastewater, neighborhood leaders encouraged residents to be more careful, which may have helped reduce SARS-CoV-2 RNA concentrations in the subsequent weeks. Additionally, the wastewater surveillance program provided reassurance to the Yarmouth community that COVID-19 was being monitored and addressed at the local level. The wastewater program's timely information about SARS-CoV-2 transmission in the community was viewed as being beneficial enough to the community's COVID-19 monitoring and response activities that the Town of Yarmouth and individual residents contributed funds to extend the initial 6-month pilot program for 3 months and, after a 3-month break, to resume the program in September 2021 for another 6 months.

The wastewater surveillance program in Yarmouth had some limitations. Although we were able to establish significant correlations between weekly measurements of SARS-CoV-2 RNA and reported COVID-19 cases, previous studies demonstrated that once weekly wastewater sampling did not identify significant trends in SARS-CoV-2 transmission in the sewersheds of larger communities (population >200,000) where COVID-19 cases are reported daily (Feng *et al.* 2021; Xie *et al.* 2021). Another limitation was ~4 days turnaround time from sample collection to receiving results. Quicker availability of testing results would allow timelier assessment and reporting of SARS-CoV-2 levels in the community and faster mobilization of community support in response to increased viral levels. Because the Yarmouth sewershed only covers about 70% of Yarmouth residents, the wastewater surveillance program provided an incomplete picture of viral transmission in Yarmouth, including the presence of non-resident workers and the absence of some residents during part of the weekly 24-h sampling period, which may have also contributed to a possibly unrepresentative picture of viral levels in the community. Lastly, the correlation of SARS-CoV-2 RNA concentrations with reported COIVD-19 cases is dependent on the availability and use of individual testing in the community.

Overall, this study demonstrates the feasibility and usefulness of weekly wastewater testing in a small community. Our study also documents a community-based initiative to respond to the COVID-19 pandemic. The study of the Yarmouth wastewater surveillance program can be used as a framework to help other small communities establish wastewater surveillance, interpret surveillance data, and disseminate testing results to the community. Future studies could evaluate the cost–benefit analysis of a wastewater surveillance program in a small community.

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## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

## CONFLICT OF INTEREST

Y.M.B. received in-kind materials from IDEXX Laboratories, Inc.

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