Using Detrending to Assess SARS-CoV-2 Wastewater Loads as a Leading Indicator of Fluctuations in COVID-19 Cases at Fine Temporal Scales: Correlations Across Twenty Sewersheds in North Carolina

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Abstract

Wastewater surveillance emerged during the COVID-19 pandemic as a novel strategy for tracking the burden of illness in communities. Previous work has shown that trends in wastewater SARS-CoV-2 viral loads correlate well with reported COVID-19 case trends over longer time periods (i.e., months). We used detrending time series to reveal shorter sub-trend patterns (i.e., weeks) to identify leads or lags in the temporal alignment of the wastewater/case relationship. Daily incident COVID-19 cases and twice-weekly wastewater SARS-CoV-2 viral loads measured at 20 North Carolina sewersheds in 2021 were detrended using smoothing ranges of ∞, 16, 8, 4 and 2 weeks, to produce detrended cases and wastewater viral loads at progressively finer time scales. For each sewershed and smoothing range, we calculated the Spearman correlation between the cases and the wastewater viral loads with offsets of -7 to +7 days. We identified a conclusive lead/lag relationship at 15 of 20 sewersheds, with detrended wastewater loads temporally leading detrended COVID-19 cases at 11 of these sites. For the 11 leading sites, the correlation between wastewater loads and cases was greatest for wastewater loads sampled at a median lead time of 6 days before the cases were reported. Distinct lead/lag relationships were the most pronounced after detrending with smoothing ranges of 4–8 weeks, suggesting that SARS-CoV-2 wastewater viral loads can track fluctuations in COVID-19 case incidence rates at fine time scales and may serve as a leading indicator in many settings. These results could help public health officials identify, and deploy timely responses in, areas where cases are increasing faster than the overall pandemic trend.

Introduction

The first lab-confirmed COVID-19 case was reported in North Carolina (NC) on March 3, 2020, and over the next two and a half years, the number of reported positive cases statewide increased to more than three million [1,2]. However, the true burden of disease far exceeded this number due to underreporting, access to testing, unreported at-home tests, asymptomatic illness and other factors [3–5]. Testing was not uniformly distributed among populations due to unequal availability and pervasive mistrust of public health recommendations by historically marginalized persons [6–8]. As a result, there is need for non-clinical means of tracking COVID-19 trends to augment case-based reporting.

One promising approach is wastewater-based epidemiology (WBE), which measures substances shed in human feces and derived from a condition of interest, such as pathogen nucleic acids or pharmaceutical metabolites, by sampling sewage containing human fecal waste and byproducts of water usage [9]. WBE has been increasingly utilized to track COVID-19 infection trends at the community level by quantifying SARS-CoV-2 RNA in sewage. Twice-weekly testing of SARS-CoV-2 loads in wastewater can provide information on changes in COVID-19 burden in the sewershed population and can be used as a method to detect periods of increasing COVID-19 cases from far fewer samples than required for clinical case reporting since wastewater samples represent pooled samples of multiple individuals [10]. Unlike case-based surveillance, wastewater surveillance does not rely on individual healthcare-seeking behavior or access to testing, which are strongly impacted by well-documented societal inequities [11]. Additionally, SARS-CoV-2 is shed in the feces of both symptomatic and asymptomatic individuals, allowing the capture of data on a range of infected individuals [12–14] at varying stages of infection. Numerous studies have shown that when clinical testing coverage is high, wastewater SARS-CoV-2 loads and documented COVID-19 cases follow similar trends and are highly correlated [15–18]. Therefore, given the cost and human resource savings, WBE may provide an effective complement to case-based surveillance that addresses some of the limitations of traditional clinical surveillance approaches.

However, the values typically measured in wastewater, such as viral genome copies per liter, are not directly interpretable in terms of familiar population health metrics, like the prevalence or incidence rate of infection in a defined population. To effectively inform public health response and mitigation strategies using WBE, it is necessary to relate wastewater-based measurements to interpretable population-level metrics. One critical aspect is the temporal relationship between SARS-CoV-2 wastewater loads measured at a wastewater treatment plant (WWTP) and reported COVID-19 cases in the corresponding sewershed served by the plant [5,19]. Past work has demonstrated that increases in SARS-CoV-2 wastewater loads may occur prior to a rise in lab-confirmed sewershed COVID-19 cases in a sewershed, allowing for WBE to be used as an early warning system [4,20–22]. Such leading signals in wastewater were reported during the earlier phases of the pandemic in some North Carolina sewersheds [10,23] as well as during more recent pandemic phases [24].

As the pandemic becomes endemic, trends lasting several months have been widely reported to anticipate trends in COVID-19 infections, as later indicated by population surveillance metrics [21,22,25,26]. However, the time alignment between trends in wastewater load and trends in cases can be difficult to determine since its small temporal lead or lag may be eclipsed by the longer time scale of trends. In this situation, kernel detrending can be used to remove these longer pandemic trends and reveal shorter-term fluctuations that may help identify leads or lags in the temporal alignment of the detrended wastewater and detrended case relationship [27–31]. While the correlation between wastewater-based measurements of pathogens of concern and clinical cases over longer time periods (i.e., months) is useful for informing longer-term public health response, much less is known about short-term sub-trends (i.e., weekly or even daily), which may be more relevant for ongoing, day-to-day public health decision making. Therefore, there is a need for research to better understand and anticipate changes in COVID-19 incidence on shorter time scales to inform timely, targeted, and cost-effective public health action, particularly at the local level. *Detrending* the wastewater and case data is done by modeling these longer-term trends and removing them to obtain detrended wastewater loads and detrended cases, also referred to as wastewater load residuals and case residuals, respectively. If wastewater load residuals can predict a fine-scale fluctuation in case residuals, then public health measures can be taken *locally* and for *short* durations in sewersheds where cases are anticipated to rise at levels greater than that of the baseline trend. This methodology may also be applicable for other pathogens beyond SARS-CoV-2 as wastewater surveillance expands to new targets in the future.

Our work aims to contribute to previous studies by refining the time scale at which correlations between wastewater and cases are assessed. Accordingly, we investigate the temporal relationship (i.e., lead or lag) that maximizes correlation between detrended wastewater SARS-CoV-2 viral loads and detrended COVID-19 clinical cases at the finest time-scale possible for 20 sewersheds across North Carolina in 2021. Furthermore, to operationalize this approach, we propose and validate a set of reproducible criteria that can be easily deployed by public health agencies to support the application of WBE approaches beyond North Carolina.

Materials and Methods

Ongoing Wastewater-Based Epidemiology in North Carolina

In collaboration with University of North Carolina (UNC) system researchers, the North Carolina Department of Health and Human Services (NCDHHS) was one of eight state health departments initially funded by the Centers for Disease Control and Prevention (CDC) to participate in the National Wastewater Surveillance System (NWSS). The NCDHHS NC Wastewater Monitoring Network is a multi-disciplinary collaboration between epidemiologists, laboratory scientists, water reclamation managers, environmental engineers, and public health officials with promising applications for genomic, large-scale pathogen monitoring, as well as COVID-19. The development of this state surveillance network benefited from a collaboration funded by the North Carolina State Legislature among North Carolina universities at the start of the pandemic in 2020. This group of experts created the NC Wastewater Pathogen Research Network to develop sampling techniques, laboratory capabilities, and analysis of SARS-CoV-2 in wastewater [32]. The NC Wastewater Pathogen Research Network, in collaboration with NCDHHS, established a strong foundation for WBE, and founding contributors continue to be essential partners in the NC Wastewater Monitoring Network using a framework of innovative research to inform public health surveillance and action in North Carolina.

As part of the NC Wastewater Monitoring Network data collection in 2021, wastewater samples were collected twice per week by WWTP staff and shipped to the UNC-Chapel Hill Institute of Marine Sciences (IMS, Morehead City, NC) for laboratory analysis. Samples were analyzed for SARS-CoV-2 by reverse-transcription droplet digital polymerase chain reaction (RT-ddPCR) following a standardized protocol [33], for which additional details are provided in the Supplementary Material [34]. Sewer network spatial data (e.g., gravity mains, force mains, manholes, pump stations) obtained from North Carolina wastewater utilities and local geographic information systems departments were used to delineate a sewershed polygon using ArcGIS Pro 2.8 (ESRI, Redlands, CA). COVID-19 clinical cases reported to NCDHHS were geocoded in ArcMap 10.7.1 (ESRI) and matched to the sewershed within which they resided using a custom composite geocoder built from state and county address data. Lastly, wastewater sample results and recorded clinical cases in the sewershed were submitted to NCDHHS and uploaded weekly the CDC NWSS analytics platform for epidemiologic trend analysis. COVID-19 cases were given a date based on the following hierarchy: date of symptom onset, date of specimen collection, and date of result. Daily incidence rates per 100,000 estimated sewershed population were calculated. Wastewater sample results were normalized to flow within each municipal utility to represent a 24-hour viral load. These analyzed data are posted publicly on the CDC COVID-19 Data Tracker and the NCDHHS COVID Dashboard (https://covid19.ncdhhs.gov/dashboard/wastewater-monitoring).

Relating Wastewater Loads and COVID-19 Incidence

During a ten-month study period from January 2021 through October 2021, we compared SARS-CoV-2 viral loads in influent wastewater collected at the 20 WWTPs in the NC Wastewater Monitoring Network with COVID-19 incidence in the corresponding sewersheds. Nine sites were sampled for the entire duration of the study period, two sites were sampled beginning in January and ending before October 2021, and nine sites were added in the summer and sampled from June 2021 through October 2021 (Table 1). We retrieved calculated wastewater viral loads and clinical COVID-19 incidence rates in the sewershed for each of the 20 sites from the CDC NWSS analytics platform. Twice-weekly wastewater loads were provided as the sample-specific geometric mean of measured N1 and N2 target copy numbers per liter (L) of wastewater [35], normalized by multiplying by the average daily flow and dividing by the estimated sewershed population. Half the target-specific limit of detection (LOD) was substituted for the concentration when a target was not detected in the sample (see Supplemental Material). The resulting population-normalized viral loads, with units of SARS-CoV-2 N gene copies (GC) per person per day (pppd), were $\log_{10}$-transformed for all analyses, which were conducted in R version 4.1.2 [36].

Table 1. Characteristics of NC Wastewater Monitoring Network Sites

WWTP Name	Population (2019)	Area (km^2)	Capacity (ML/day)	First Sample	Last Sample	Number of Samples
Newport	3,731	6.1	5	1/12/2021	8/18/2021	64
Pittsboro	3,799	10.3	3	1/5/2021	5/25/2021	39
Beaufort	3,992	7.4	7	1/5/2021	10/20/2021	83
Marion	7,793	22.9	14	6/17/2021	10/14/2021	35
Laurinburg	15,407	37.4	18	6/17/2021	10/19/2021	36
Roanoke Rapids	19,335	43.9	38	6/19/2021	10/20/2021	33
Wilson	51,285	164.4	64	6/19/2021	10/19/2021	33
New Hanover Co.	51,401	81.4	48	1/22/2021	10/20/2021	78
Wilmington	65,081	62.5	25	1/5/2021	10/20/2021	79
Charlotte 1	77,278	126	55	1/5/2021	10/19/2021	76
Chapel Hill	84,729	89.8	66	1/6/2021	10/20/2021	81
Greenville	94,194	95.2	80	1/5/2021	10/20/2021	81
South Durham	98,068	100.7	91	1/6/2021	10/20/2021	81
Charlotte 3	122,063	122.2	55	6/3/2021	10/19/2021	38
Greensboro	144,539	143.6	82	6/18/2021	10/20/2021	36
Charlotte 2	154,519	105.3	127	1/4/2021	10/19/2021	80
Fayetteville	159,000	250.8	95	6/19/2021	10/20/2021	36
Winston Salem	177,520	319.6	70	6/19/2021	10/20/2021	33
MSD of Buncombe Co.	188,927	534.4	182	6/19/2021	10/20/2021	33
Raleigh	551,534	536.7	341	1/6/2021	10/20/2021	74

Exponential kernel smoothing is a technique used in space/time geostatistics to estimate spatial and temporal trends of environmental and health processes at a variety of spatial and temporal scales [27–31]. Here, we used exponential kernel smoothing to estimate trends in wastewater viral loads and COVID-19 incidence rates at different temporal scales. For each observed response, a smoothed estimate was obtained as the average of all observations weighted by an exponentially decaying function of the temporal distance from the estimation time point. The rate of exponential decay was determined by a smoothing range parameter, corresponding to the temporal duration below which variations in the response are smoothed out of the mean trend to retain only those variations of greater duration than the smoothing range. For a response $y(t)$ observed at time t, the smoothed estimate was obtained as the mean trend $m_y(t;T)$ with smoothing range of duration T:

$$m_y(t;T) = \sum_{j=1}^{N} k_j y(t_j) \tag{1}$$

where $y(t_j)$, j=1,…N, are the observations at observation times t_j and the exponential kernel smoothing weights k_j are given by

$$k_j = \frac{exp\left(\frac{-3\left|t_j - t\right|}{T}\right)}{\sum_{j=1}^{N} exp\left(\frac{-3\left|t_j - t\right|}{T}\right)} \tag{2}$$

Scaling the exponential decay function by -3 ensured that the influence of observations with temporal distance equal to the smoothing range T was diminished by ~95%, with the estimation point itself receiving the highest weight. As T increased, observations further away in time were allowed greater influence on the mean trend, increasing the extent of smoothing until converging to a constant value at the arithmetic mean of all the data for T of infinite duration.

As the mean trend $m_y(t;T)$ only retained variations in the response of greater duration than the smoothing range T, we detrended the observed responses by subtracting the mean trend estimated at time t to obtain the residual response:

$$\tilde{y}(t;T) = y(t) - m_y(t;T) \tag{3}$$

which captured the fluctuations around the trend at temporal scales shorter than the smoothing range T (including any measurement error). In short, we decomposed the signal $y_i(t)$ into a time trend $m_{y_i}(t;T)$ that captured variation of time scales greater than T and a detrended signal $\tilde{y}_i(t;T)$ that captured fluctuations of time scale shorter than T, corresponding to the shorter-term variations around pandemic trends that are of particular relevance to timely public health action.

To examine the time scales at which wastewater signals may lead (i.e., precede) or lag (i.e., follow) clinical cases at North Carolina Wastewater Monitoring Network sites, we evaluated the cross-correlation between *detrended* wastewater viral loads, denoted $\tilde{w}(t;T)$, and *detrended*

COVID-19 incidence rates $\tilde{y}(t;T)$ across various detrending kernel smoothing ranges for observations from January – October, 2021. The cross-correlation between two time series was determined as the set of correlations between pairs of observations for different temporal offsets τ, given by

$$r(\tau;T) = corr(\tilde{w}(t+\tau;T), \tilde{y}(t;T)) \tag{4}$$

for which $\tau < 0$ indicated the detrended wastewater load signal leads the detrended signal obtained from COVID-19 incidence rates; conversely, $\tau > 0$ indicated the signal from detrended wastewater loads lags that of detrended COVID-19 incidence.

We examined detrended wastewater loads and detrended COVID-19 incidence rates with detrending smoothing ranges of $T = \infty$, 16, 8, 4 and 2 weeks separately for each site. Because subtracting a constant does not affect correlation estimates, using the $T = \infty$ detrended residuals was equivalent to performing the analysis without detrending. As we anticipated nonlinear associations, we estimated Spearman rank correlations to assess the monotonic relationships between the two surveillance systems for temporal offsets ranging from $\tau = -7$ to $\tau = +7$ days. The optimal combination of detrending smoothing range and temporal offset to characterize the lead/lag relationship between wastewater and incidence over relevant time scales was identified for each site by applying a reproducible set of criteria. For each detrending smoothing range T, starting from $T = \infty$ down to $T = 2$, we:

1. Identified the span of consecutive lead/lag values τ for which $r(\tau;T)$ was a statistically significant positive correlation.
2. Accepted τ if (a) it was less than 7 days (identifiable), (b) it lasted at least 2 days (persistent), and (c) it contained the maximum $r(\tau;T)$ value (predictive). Otherwise, it was rejected and deemed inconclusive.

Finally, the optimal smoothing range was obtained by choosing the shortest detrending smoothing range *T* that successfully identified a conclusive lead or lag. Detecting fluctuations over a shorter duration is ideal because these can be addressed with more timely public health measures. We selected criteria that favor identifiability, persistence, and predictivity; however, this framework may easily be extended to additional or alternative criteria as required by the specific application.

This analysis did not involve human subjects in its research.

Results

Charlotte 1 Sewershed Case Study

In this case study, we demonstrate the use of kernel detrending in the cross-correlation analysis of SARS-CoV-2 wastewater loads and COVID-19 incidence in the Charlotte 1 sewershed. One of three WWTPs in the Charlotte metropolitan area monitored by the NC Wastewater Monitoring Network during the study period, the Charlotte 1 sewershed covers 126 km^2 in the northeast of the city and serves approximately 80,000 people. From January to October 2021, 76 wastewater samples were collected at Charlotte 1 with a SARS-CoV-2 RNA detection frequency of 98% and a mean daily load of 9.2×10^6 GC pppd. The maximum load was an order of magnitude higher at 4.7×10^7 GC pppd and the minimum load was 7.9×10^4 GC pppd. A total of 6,039 COVID-19 cases were reported in the Charlotte 1 sewershed over the 10-month study period, with a daily incidence rate of 30 cases/100,000 people on average and a maximum of 132 cases/100,000 people. There was only one day with zero COVID-19 cases reported (0.3%, n = 293 days).

Visual inspection of trends in the Charlotte 1 sewershed indicated the wastewater loads generally mirrored the COVID-19 incidence rates, with a peak in January, a gradual decline through July followed by a sharper increase in August and second peak around September (Figure 1a and 1b). The mean trend was estimated at each time point for smoothing ranges of

$T = \infty$, 16, 8, 4 and 2 weeks. Using $T = \infty$ resulted in a flat (i.e. constant) trend line. Then, as the kernel smoothing range became finer (i.e. $T =$ 16, 8, 4 and 2 weeks), the trend line captured more of the inflections in the wastewater and case trends.

Subtracting the various mean trends from the wastewater and case observations yielded residuals retaining the variation in the observations at time scales shorter than the corresponding smoothing range T. With an 8-week range, the detrended wastewater loads and detrended cases demonstrated lower temporal variability compared to the variability seen without detrending (Figures 1c and 1d). Scatterplots comparing the detrended wastewater loads and detrended cases on the same day (i.e., temporal offset $\tau = 0$) are presented in Figures 1e and 1f for detrending smoothing ranges $T = \infty$ weeks and $T =$ 8 weeks, respectively. As anticipated, we observed that the pairwise correspondence between detrended wastewater loads and detrended cases declined with decreasing detrending smoothing range (i.e., as $T = \infty$, 16, 8, 4 and 2 weeks) because more of the pandemic-scale trend was removed and only shorter-term fluctuations remained. However, detrended residuals were significantly positively correlated for all detrending smoothing ranges other than $T =$ 2 (the shortest range considered, Spearman's $\rho = 0.19$, $p = 0.11$).

We then calculated, for each detrending smoothing range T, not only the correlation for detrended wastewater observations on the same day as each case date ($\tau = 0$), but also for wastewater observations up to 7 days before ($\tau = -7$) and 7 days after ($\tau = +7$) each case date (Figure 1g). Based on our proposed criteria, we determined the shortest smoothing range T to conclusively identify a time offset τ for predicting detrended cases from detrended wastewater loads in the Charlotte 1 sewershed was $T =$ 8 weeks, which revealed positive correlations for wastewater measured 0 to 3 days before cases were reported. This set of contiguous positive correlations spanned more than 2 and fewer than 7 contiguous days and included the maximum correlation value, satisfying our proposed criteria for identifiable and predictive lead/lag relationships. Longer detrending smoothing ranges ($T = \infty$ and $T = 16$ weeks) demonstrated significant positive correlations at all temporal offsets, suggesting that the lead/lag relationships were not identifiable because they were dominated by overall pandemic trends that obscured the short-term fluctuations relevant to timely public health action. Conversely, the shorter 4- and 2-week detrending smoothing ranges removed so much of the trend that the residuals were not predictive at any contiguous sets of temporal offsets, rendering the lead/lag relationships inconclusive. We therefore concluded that the finest detrending time-scale at which wastewater loads predicted COVID-19 cases in the Charlotte 1 sewershed during our study period—based on our reproducible criteria for identifiability, persistency and predictivity—was 8-weeks, and that the correlation between detrended wastewater loads and detrended cases was greatest for wastewater loads sampled with a lead time of 0 to 3 days before the cases were reported.

Figure 1. Kernel smoothing of the (A) SARS-CoV-2 wastewater loads (log GC pppd) and (B) COVID-19 incidence (cases/100k) observed at Charlotte 1 sewershed from January to October 2021, using various range parameters indicated by the colored lines in the legend. The smoothed estimates were subtracted from the observations to yield the (C) detrended wastewater loads and (D) detrended cases, shown here for a detrending smoothing range of 8-weeks. The pairwise correspondence of the detrended wastewater and case residuals on the same day (i.e. temporal offset of zero) were compared in scatterplots with added spearman correlation lines prior to evaluating any temporal offsets for detrending smoothing ranges of (E) $T = \infty$ weeks and (F) $T = 8$ weeks. A cross-correlation plot (G) between the detrended wastewater and case residuals was created for each detrending smoothing range and temporal offset to be used with the criteria to assess the lead/lag relationship. Note: The temporal offset values on the x-axis are in relation to the case date, such that negative values indicate the correlation was performed when the wastewater preceded the cases and positive values indicate the correlation was performed when the wastewater lagged the cases. Statistically significant correlations are indicated with a filled-in circle and the intersecting line represents the 95% confidence interval.

Wastewater Loads and COVID-19 Incidence Across All Sites

The observed COVID-19 incidence rates and SARS-CoV-2 wastewater loads varied across the 20 North Carolina sewersheds participating in this study (Figure 2). The sites were distributed across North Carolina, covering approximately 20% of the population and about 2% of the land area. There was a wide range in sewershed size, with the largest sewershed, Raleigh, serving 551,534 people at a capacity of 341 ML/day and the smallest sewershed, Newport, serving 3,731 people at a capacity of 5 ML/day. During the study period, the number of samples collected per site ranged from 33 (Wilson, Buncombe, Roanoke Rapids, and Winston-Salem) to 83 (Beaufort). SARS-CoV-2 RNA was detectable in 74% of the 1,129 wastewater samples across all 20 sites. Sewersheds with larger populations tended to have higher detection frequencies, with 50% of all the non-detects occurring at the three smallest sites with populations under 5,000 people. The lowest mean daily load was 5.0 x 10^5 GC pppd, observed at Newport, while the highest mean daily load of 2.3 x 10^7 GC pppd was observed at Fayetteville. The median mean daily load was 7.8 x 10^6 GC pppd, observed at Buncombe County. There was a total of 122,444 COVID-19 cases reported across all 20 sites during the study period, with the average daily incidence rate ranging from 1 case/100,000 people (Pittsboro) to 148 cases/100,000 people (Raleigh). The median daily incidence rate was 16 cases/100,000 people, observed at South Durham. Comparable to the wastewater loads, the three smallest sewersheds accounted for almost 75% of the observed days with zero reported COVID-19 cases.

The maximum daily population normalized loads (henceforth referred to simply as loads) for each site ranged from 4.7×10^6 GC pppd to 4.3×10^8 GC pppd, with most of these values occurring in January or late August/early September, during which peaks in COVID-19 cases were also observed with daily incidence rates as high as 235 cases/100,000 people. For the 10 sites that were sampled for the entire 10-month period, there was also a noticeable lull during the period of May to July 2021 for both the wastewater loads and cases. All but one sewershed had significant positive correlations between the wastewater loads and cases observed on the same day, with the significant Spearman's coefficients ranging from $\rho = 0.38$ to $\rho = 0.85$, with a median of $\rho = 0.72$. The smallest sewershed (Newport) had a non-significant correlation with a coefficient of $\rho = 0.21$ and p-value of 0.09.

Figure 2. Time series of SARS-CoV-2 wastewater loads (log GC pppd) and COVID-19 incidence (cases/100k) for each of the 20 sites from January through October 2021. Note: COVID-19 incidence is shown as a 7-day rolling average with the blue line. SARS-CoV-2 wastewater loads are depicted with the orange dots and a LOESS curve was fitted to these values, depicted by the orange line (span=0.3).

Detrending Reveals Short-Term Associations

Applying each detrending smoothing range ($T = \infty$, 16, 8, 4 and 2 weeks) across temporal offsets ($\tau = -7$ to $\tau = +7$ days) allowed us to evaluate the lead/lag relationship between the detrended wastewater and case residuals at progressively finer time scales. Correlation plots similar to Figure 1e were generated for all 20 sewersheds, and the proposed criteria were used to identify the optimal detrending smoothing range for each site, which was defined as the shortest kernel smoothing range that revealed an identifiable lead or lag (Figure 3). Eighteen of the 20 sewersheds exhibited statistically significant correlation coefficients at all temporal offsets when $T = \infty$ weeks, indicating that additional detrending was needed to reveal the fine time scale fluctuations required for a lead/lag analysis. Beaufort and Pittsboro were the only sewersheds for which the $T = \infty$ weeks range was optimal for identifying the lead/lag relationship; the detrended wastewater and case residuals were no longer significantly correlated over any 2-day span of temporal offsets using shorter detrending smoothing ranges.

Of the remaining sewersheds, one site had an optimal detrending smoothing range of T = 16 weeks, eight sites had an optimal detrending smoothing range of T = 8 weeks, and four sites had an optimal detrending smoothing range of T = 4 weeks (Figure 3). As a general pattern, the detrending smoothing ranges greater than the identified optimal T either had significant positive correlations at all temporal offsets, such that no lead/lag pattern was identifiable, or additional detrending allowed us to detect fluctuations over a shorter duration while still meeting all the proposed criteria. Conversely, too much of the trend was removed when using values for T smaller than the optimal detrending smoothing range, such that the detrended residuals were no longer significantly correlated for any span of contiguous temporal offsets. Five of the 20 sewersheds were deemed inconclusive as none of the detrending smoothing ranges revealed an identifiable lead or lag between the detrended wastewater loads and cases, according to the proposed criteria. We identified two reasons for this: 1) the span of consecutive lead/lag values was longer than 7 days for larger T values (not identifiable) and shorter than 2 days at smaller T values (not persistent), or 2) the longest range of consecutive lead/lag values did not include the maximum correlation coefficient (not predictive). The inconclusive nature of the lead/lag relationship in these sewersheds may be linked to the short sampling duration or the small size of the sewershed as all five sites had data for only half of the study duration and all but Buncombe County were among the smallest sewersheds.

Figure 3. Plots of Spearman correlation coefficient versus temporal offset at the optimal detrending smoothing range for each of the 15 conclusive sites, ordered from longest lead to longest lag. The highest correlation value is colored in red, the identified lead or lag span is represented with brackets, and the optimal smoothing range is listed in the bottom right corner of each plot. Note: The lead/lag relationship was inconclusive for Wilson, Laurinburg, Marion, MSD of Buncombe County, and Roanoke Rapids, and these plots are therefore not presented.

Detrended wastewater loads were temporally leading detrended COVID-19 cases in 11 of the 15 sewersheds where we were able to identify optimal detrending smoothing ranges (Figure 4). For these sites, the highest correlation was observed for wastewater loads sampled at a median lead time of 6 days before the cases were reported, with a contiguous span of elevated correlations lasting a median of 3 days. At four sewersheds, the correlation between detrended wastewater loads and detrended cases was greatest when the detrended wastewater loads were *lagging*, with the highest correlation identified at a median of 3.5 days after the cases were reported and a median contiguous span of elevated correlations of 2 days. Although the smaller sewersheds were more likely to be inconclusive, size did not seem to influence the lead/lag relationship at the 15 conclusive sites, with about the same proportion of leading vs lagging between groups of the smallest and largest sewersheds. However, the optimal detrending smoothing range seemed to be related to the lead/lag relationship, as 64% (7/11) of the leading sewersheds had an optimal detrending smoothing range of T = 8 weeks and 75% (3/4) of the lagging sewersheds had an optimal detrending smoothing range of T = 4 weeks, suggesting that it may be easier to identify detrended wastewater loads lagging detrended COVID-19 cases at shorter detrending time scales. A summary of the optimal smoothing range, relationship, span, and temporal offset with the highest correlation identified for each sewershed is included in the Supplemental Material (Table S2).

Figure 4. Locations of NC Wastewater Monitoring Network sewersheds participating between January and October 2021. In 11 sewersheds, detrended wastewater leads cases (lead), in 4 sewersheds detrended wastewater lags cases (lag) and in 5 sewersheds results were inconclusive.

Discussion

Wastewater surveillance emerged during the pandemic as a potential leading indicator of COVID-19 infection trends in the community. Although previous research analyzed the overall correlation between SARS-CoV-2 wastewater loads and clinical cases, this analysis used kernel detrending to characterize short-term relationships and identify sub-trends. By detrending wastewater viral loads and cases in the sewershed using various kernel smoothing ranges, we were able to characterize lead/lag relationships at 15 of the 20 North Carolina sewersheds assessed using a set of reproducible criteria, reducing the proportion of inconclusive results from 90% without detrending to 25% using the optimal detrending smoothing range. Furthermore, we found that detrended wastewater loads temporally led detrended cases at almost three times as many sewersheds (N=11) as sewersheds where detrended wastewater loads lagged detrended cases (N=4), further highlighting the utility of wastewater as a leading indicator of COVID-19 cases in North Carolina. The optimal detrending kernel smoothing range that removed long-scale pandemic trends while retaining sufficient temporal correlation to identify lead/lag relationships was in the range of 4 to 8 weeks at 12 of the 15 sites with conclusive relationships. Because detrending with a given smoothing range retains only the variation in the observations at time scales shorter than the corresponding timeframe, this finding suggests that this approach is ideal for identifying the leading or lagging nature of wastewater and case trends in most sewersheds experiencing a sustained period of increasing SARS-CoV-2 infection rates lasting at least 4 to 8 weeks. A sustained 4 to 8 weeks increase in COVID-19 incidence corresponding to the emergence of the Delta variant (B.1.617.2*)* in late July 2021 was observed in wastewater loads at 19 of the 20 study sites, further supporting the wider relevance of this range during the study period. However, due to onboarding schedules, some sewersheds were only sampled for half of the study period, and the shorter sampling history appeared related to inconclusive results at these sites.

A strength of our study is that we performed a lead/lag analysis across a wide-range of WWTP systems, including both rural and urban municipal systems serving sewershed populations ranging from under 4,000 to 550,000 people [16,24,37–39]. Although we identified a leading relationship in the majority of North Carolina sewersheds, those within the same county or in adjacent counties did not always exhibit the same lead/lag relationship nor have the same optimal detrending smoothing range (Figure 4). For example, we found that detrended wastewater loads led detrended cases at Charlotte 1 and Charlotte 3 but lagged detrended cases at Charlotte 2 (Figure 4, Table S2). Wastewater led cases in both the Wilmington sewershed and the sewershed encompassing surrounding areas of New Hanover County, but the optimal detrending smoothing range was 8 weeks for the city and 16 weeks in the county, which covers a larger land area but serves fewer people (Table 1). Differences in the temporal relationship or optimal smoothing range at each sewershed could be due to conditions at a given site: virus loads measured in wastewater can be impacted by sewer network infrastructure age, sewer residence time, or weather [38,40,41], and clinical surveillance is subject to underreporting due to testing access, home test usage, or fluctuations in populations from tourists and commuters [42]. To minimize the potential impact of testing behavior on the evaluation of relationships between SARS-CoV-2 loads and COVID-19 cases presented in this work, we chose to perform the analysis for a period ending prior to November 2021, when clinical testing penetration was still relatively high and home testing was not yet widely used in North Carolina communities.

Given that site-specific conditions can influence wastewater results, public health agencies leading wastewater surveillance programs in their jurisdictions may want to validate their wastewater data against other foundational COVID-19 metrics to determine how wastewater surveillance fits into their larger surveillance strategies. For states or jurisdictions less familiar with wastewater data, a lead/lag analysis between wastewater loads and reported cases would be a useful method to help understand the temporal relationship between wastewater-based pathogen and other decision-making metrics. Our method can be employed by public health agencies participating in CDC NWSS across the United States by using an R Markdown document that applies set criteria to identify the leading or lagging relationships between wastewater and reported cases [43]. As counts of reported cases become less reliable over time due to an increase in non-reportable results from at-home-testing kits, as well as an overall reduction in PCR-based, reportable, COVID-19 clinical testing, this method can be adapted to utilize surveillance metrics besides cases, including hospitalizations, emergency department visits (syndromic surveillance data), or mortality [17].

Results from our analysis characterizing the shortest time ranges at which wastewater loads are associated with cases have been formative in elevating wastewater as a reliable metric for tracking trends in North Carolina, not only to anticipate the start of long-term cycles (such as the start of elevated rates in winter), but also for short duration fluctuations within any given long-term cycle. The leading nature of wastewater-based COVID-19 findings at most sites provides the foundation and rationale for including wastewater loads as an early warning metric alongside reported cases, emergency department visits, and hospitalizations, which are highlighted on statewide data surveillance dashboards such as the NCDHHS COVID-19 dashboard (https://covid19.ncdhhs.gov/dashboard/wastewater-monitoring).

In under two years, COVID-19 wastewater surveillance in the United States expanded from 8 pilot state health agencies participating in the CDC National Wastewater Surveillance System in 2020 to 46 states, 5 cities, 3 territories, and 7 tribes participating in 2022 [44]. Similarly, the global portal expanded to coverage of 70 countries, reporting for 3,807 sites, indicating widespread use of wastewater surveillance data [45]. With the explosive growth in both the academic literature on, and implementation of, wastewater surveillance programs globally, public health professionals developed a wide range of approaches to utilizing wastewater data for decision making. Our method shows how detrended wastewater loads can predict *fine* scale fluctuations in detrended cases, which can allow public health officials to respond more *locally* and *timely* when COVID-19 burden, or other disease burden as wastewater surveillance expands to new targets, is increasing at levels *greater* than the baseline trend. Examples of mitigation strategies that can be deployed at local levels and for short durations, while being complementary to long lasting statewide measures, may include the following: (a) officials could quickly alert local hospitals about a potential increase in cases above the statewide trend and provide recommendations to community leaders to implement short-duration restrictions, such as limiting indoor gatherings and reducing business capacity [46]; (b) jurisdictions could mobilize pop-up testing and take steps to increase vaccination in the community [47]; (c) increasing public health communications regarding masking, handwashing, vaccination, and social distancing to help contain the spread of the virus; and d) interacting with local public health officials and hospital administrators to indicate periods of higher ICU bed, PPE, and medical staffing needs. This has already been observed during a large sport fishing tournament that took place in a small coastal North Carolina sewershed where NCDHHS notified local health department and city officials of an increase in wastewater viral load. In response to this increase, local health department and city officials reinforced recommended mitigation strategies outlined in the Governor's Executive order to the event leadership, like additional hand-washing stations and frequent disinfection of high touch surfaces (Nina Oliver, Carteret County Health Director, personal communication, June 21-22 2021 & February 6, 2023). Local notices were also used to encourage the surrounding community to take precautions through vaccinations, masking, social distancing, and frequent handwashing [48]. Immediately following the event, county and city officials met routinely to review wastewater, as well as other COVID-19 metrics, and to ensure levels were decreasing (Nina Oliver, personal communication, February 6, 2023).

As public health officials and the scientific community continue to rely on wastewater surveillance both for large-scale pandemic decision-making and localized action as described here, there is a growing need for increasing equitable access to wastewater services, particularly in cases of municipal underbounding, and for investing in substantial infrastructure improvements. This is especially important in jurisdictions like North Carolina, where half of households rely on private septic and package treatment plants [49]. In some cases, racial disparities in access to and disproportionate exclusion from municipal water and sewer service have been documented [49–51]. In other areas, distance, lack of gradient, and groundwater height play a role in decisions to use centralized versus decentralized waste treatment systems. For wastewater to continue to be useful for disease tracking and public health decision-making beyond COVID-19, additional resources are needed to achieve equitable access to centralized wastewater treatment where it is desired and environmentally relevant. In other rural areas where this is not the case, we need to improve our technical capabilities to characterize decentralized waste systems.

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References

1. NCDHHS. North Carolina COVID-19 Dashboard. In: North Carolina Department of Health and Human Services [Internet]. 2023 [cited 12 Sep 2022]. Available: https://covid19.ncdhhs.gov/dashboard

2. NCDHHS. North Carolina Identifies First Case of COVID-19. North Carolina Department of Health and Human Services; 2020. Available: https://www.ncdhhs.gov/news/press-releases/2020/03/03/north-carolina-identifies-first-case-covid-19

3. Alwan NA. Surveillance is underestimating the burden of the COVID-19 pandemic. The Lancet. 2020;396: e24. doi:10.1016/S0140-6736(20)31823-7

4. Wu F, Xiao A, Zhang J, Moniz K, Endo N, Armas F, et al. SARS-CoV-2 RNA concentrations in wastewater foreshadow dynamics and clinical presentation of new COVID-19 cases. Science of The Total Environment. 2022;805: 150121. doi:10.1016/j.scitotenv.2021.150121

5. Xiao A, Wu F, Bushman M, Zhang J, Imakaev M, Chai PR, et al. Metrics to relate COVID-19 wastewater data to clinical testing dynamics. Water Res. 2022;212: 118070. doi:10.1016/j.watres.2022.118070

6. Brandt K, Goel V, Keeler C, Bell GJ, Aiello AE, Corbie-Smith G, et al. SARS-CoV-2 testing in North Carolina: Racial, ethnic, and geographic disparities. Health & Place. 2021;69: 102576. doi:10.1016/j.healthplace.2021.102576

7. Jacobson M, Chang TY, Shah M, Pramanik R, Shah SB. Racial and Ethnic Disparities in SARS-CoV-2 Testing and COVID-19 Outcomes in a Medicaid Managed Care Cohort. American Journal of Preventive Medicine. 2021;61: 644–651. doi:10.1016/j.amepre.2021.05.015

8. Lieberman-Cribbin W, Tuminello S, Flores RM, Taioli E. Disparities in COVID-19 Testing and Positivity in New York City. American Journal of Preventive Medicine. 2020;59: 326–332. doi:10.1016/j.amepre.2020.06.005

9. Bivins A, North D, Ahmad A, Ahmed W, Alm E, Been F, et al. Wastewater-Based Epidemiology: Global Collaborative to Maximize Contributions in the Fight Against COVID-19. Environmental Science & Technology. 2020; acs.est.0c02388. doi:10.1021/acs.est.0c02388

10. Barua VB, Juel MAI, Blackwood AD, Clerkin T, Ciesielski M, Sorinolu AJ, et al. Tracking the temporal variation of COVID-19 surges through wastewater-based epidemiology during the peak of the pandemic: A six-month long study in Charlotte, North Carolina. Science of The Total Environment. 2022;814: 152503. doi:10.1016/j.scitotenv.2021.152503

11. Murakami M, Hata A, Honda R, Watanabe T. Letter to the Editor: Wastewater-Based Epidemiology Can Overcome Representativeness and Stigma Issues Related to COVID-19. Environ Sci Technol. 2020; acs.est.0c02172. doi:10.1021/acs.est.0c02172

12. Crank K, Chen W, Bivins A, Lowry S, Bibby K. Contribution of SARS-CoV-2 RNA shedding routes to RNA loads in wastewater. Science of The Total Environment. 2021; 150376. doi:10.1016/j.scitotenv.2021.150376

13. Natarajan A, Zlitni S, Brooks EF, Vance SE, Dahlen A, Hedlin H, et al. Gastrointestinal symptoms and fecal shedding of SARS-CoV-2 RNA suggest prolonged gastrointestinal infection. Med. 2022;3: 371-387.e9. doi:10.1016/j.medj.2022.04.001

14. Schmitz BW, Innes GK, Prasek SM, Betancourt WQ, Stark ER, Foster AR, et al. Enumerating asymptomatic COVID-19 cases and estimating SARS-CoV-2 fecal shedding rates via wastewater-based epidemiology. Science of The Total Environment. 2021;801: 149794. doi:10.1016/j.scitotenv.2021.149794

15. Fernandez-Cassi X, Scheidegger A, Bänziger C, Cariti F, Tuñas Corzon A, Ganesanandamoorthy P, et al. Wastewater monitoring outperforms case numbers as a tool to track COVID-19 incidence dynamics when test positivity rates are high. Water Research. 2021;200: 117252. doi:10.1016/j.watres.2021.117252

16. Fitzgerald SF, Rossi G, Low AS, McAteer SP, O'Keefe B, Findlay D, et al. Site Specific Relationships between COVID-19 Cases and SARS-CoV-2 Viral Load in Wastewater Treatment Plant Influent. Environmental Science & Technology. 2021;55: 15276–15286. doi:10.1021/acs.est.1c05029

17. Kotlarz N, Holcomb D, Pasha T, Reckling S, Kays J, Lai Y, et al. Timing and trends for municipal wastewater, clinically confirmed case, and syndromic case surveillance of COVID-19 in Raleigh, North Carolina, USA. American Journal of Public Health. 2023;113: 79–88. doi:10.2105/AJPH.2022.307108

18. Weidhaas J, Aanderud ZT, Roper DK, VanDerslice J, Gaddis EB, Ostermiller J, et al. Correlation of SARS-CoV-2 RNA in wastewater with COVID-19 disease burden in sewersheds. Science of the Total Environment. 2021;775: 145790. doi:10.1016/j.scitotenv.2021.145790

19. Graham KE, Loeb SK, Wolfe MK, Catoe D, Sinnott-Armstrong N, Kim S, et al. SARS-CoV-2 RNA in Wastewater Settled Solids Is Associated with COVID-19 Cases in a Large Urban Sewershed. Environmental Science & Technology. 2020; acs.est.0c06191. doi:10.1021/acs.est.0c06191

20. Omori R, Miura F, Kitajima M. Age-dependent association between SARS-CoV-2 cases reported by passive surveillance and viral load in wastewater. Science of The Total Environment. 2021;792: 148442. doi:10.1016/j.scitotenv.2021.148442

21. Peccia J, Zulli A, Brackney DE, Grubaugh ND, Kaplan EH, Casanovas-Massana A, et al. Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics. Nature Biotechnology. 2020;38: 1164–1167. doi:10.1038/s41587-020-0684-z

22. Zhao L, Zou Y, Li Y, Miyani B, Spooner M, Gentry Z, et al. Five-week warning of COVID-19 peaks prior to the Omicron surge in Detroit, Michigan using wastewater surveillance. Science of The Total Environment. 2022;844: 157040. doi:10.1016/j.scitotenv.2022.157040

23. Grube A, Coleman C, LaMontagne C, Miller M, Kothegal N, Holcomb D, et al. Detection of SARS-CoV-2 RNA in wastewater and comparison to COVID-19 cases in two sewersheds, North Carolina, USA. Submitted. 2022.

24. Keshaviah A, Huff I, Hu XC, Guidry V, Christensen A, Berkowitz S, et al. Separating Signal from Noise in Wastewater Data: An Algorithm to Identify Community-Level COVID-19 Surges. medRxiv [preprint]. 2022. doi:10.1101/2022.09.19.22280095

25. Bibby K, Bivins A, Wu Z, North D. Making waves: Plausible lead time for wastewater based epidemiology as an early warning system for COVID-19. Water Research. 2021;202: 117438. doi:10.1016/j.watres.2021.117438

26. Olesen SW, Imakaev M, Duvallet C. Making waves: Defining the lead time of wastewater-based epidemiology for COVID-19. Water Research. 2021;202: 117433. doi:10.1016/j.watres.2021.117433

27. Akita Y, Carter G, Serre ML. Spatiotemporal Nonattainment Assessment of Surface Water Tetrachloroethylene in New Jersey. Journal of Environmental Quality. 2007;36: 508–520. doi:10.2134/jeq2005.0426

28. Cleland SE, West JJ, Jia Y, Reid S, Raffuse S, O'Neill S, et al. Estimating Wildfire Smoke Concentrations During the October 2017 California Fires Through BME Space/Time Data Fusion of Observed, Modeled, and Satellite-Derived PM2.5. Environmental science & technology. 2020;54: 13439. doi:10.1021/acs.est.0c03761

29. de Nazelle A, Arunachalam S, Serre ML. Bayesian Maximum Entropy Integration of Ozone Observations and Model Predictions: An Application for Attainment Demonstration in North Carolina. Environmental science & technology. 2010;44: 5707–5713. doi:10.1021/es100228w

30. Jat P, Serre ML. Bayesian Maximum Entropy Space/time Estimation of Surface Water Chloride in Maryland Using River Distances. Environmental Pollution. 2016;219: 1148–1155. doi:10.1016/j.envpol.2016.09.020

31. Rudolph JE, Cole SR, Edwards JK, Whitsel EA, Serre ML, Richardson DB. Estimating Associations Between Annual Concentrations of Particulate Matter and Mortality in the United States, Using Data Linkage and Bayesian Maximum Entropy. Epidemiology. 2022;33: 157–166. doi:10.1097/EDE.0000000000001447

32. NC Wastewater Pathogen Research Network. Tracking SARS-CoV-2 in the Wastewater Across a Range of North Carolina Municipalities: Final Report. North Carolina Policy Collaboratory; 2021 Feb. Available: https://collaboratory.unc.edu/wp-content/uploads/sites/476/2021/02/tracking-sars-cov-2-in-the-wastewater-across-a-range-of-north-carolina-municipalities-report.pdf

33. Beattie RE, Blackwood AD, Clerkin T, Dinga C, Noble RT. Evaluating the impact of sample storage, handling, and technical ability on the decay and recovery of SARS-CoV-2 in wastewater. PLOS ONE. 2022;17: 1–16. doi:10.1371/journal.pone.0270659

34. Borchardt MA, Boehm AB, Salit M, Spencer SK, Wigginton KR, Noble RT. The Environmental Microbiology Minimum Information (EMMI) Guidelines: qPCR and dPCR

Quality and Reporting for Environmental Microbiology. Environmental Science & Technology. 2021. doi:10.1021/acs.est.1c01767

35. Lu X, Wang L, Sakthivel SK, Whitaker B, Murray J, Kamili S, et al. US CDC Real-Time Reverse Transcription PCR Panel for Detection of Severe Acute Respiratory Syndrome Coronavirus 2. Emerging Infectious Diseases. 2020;26: 1654–1665. doi:10.3201/eid2608.201246

36. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. 2021. Available: https://www.R-project.org/

37. Feng S, Roguet A, McClary-Gutierrez JS, Newton RJ, Kloczko N, Meiman JG, et al. Evaluation of Sampling, Analysis, and Normalization Methods for SARS-CoV-2 Concentrations in Wastewater to Assess COVID-19 Burdens in Wisconsin Communities. ACS ES&T Water. 2021;1: 1955–1965. doi:10.1021/acsestwater.1c00160

38. Hoar C, Chauvin F, Clare A, McGibbon H, Castro E, Patinella S, et al. Monitoring SARS-CoV-2 in wastewater during New York City's second wave of COVID-19: sewershed-level trends and relationships to publicly available clinical testing data. Environmental Science: Water Research & Technology. 2022;8: 1021–1035. doi:10.1039/D1EW00747E

39. Holm RH, Mukherjee A, Rai JP, Yeager RA, Talley D, Rai SN, et al. SARS-CoV-2 RNA abundance in wastewater as a function of distinct urban sewershed size. Environmental Science: Water Research & Technology. 2022; 10.1039.D1EW00672J. doi:10.1039/D1EW00672J

40. Guo Y, Sivakumar M, Jiang G. Decay of four enteric pathogens and implications to wastewater-based epidemiology: Effects of temperature and wastewater dilutions. Science of The Total Environment. 2022;819: 152000. doi:10.1016/j.scitotenv.2021.152000

41. Zhu Y, Oishi W, Maruo C, Saito M, Chen R, Kitajima M, et al. Early warning of COVID-19 via wastewater-based epidemiology: potential and bottlenecks. Science of The Total Environment. 2021;767: 145124. doi:10.1016/j.scitotenv.2021.145124

42. Sharara N, Endo N, Duvallet C, Ghaeli N, Matus M, Heussner J, et al. Wastewater network infrastructure in public health: Applications and learnings from the COVID-19 pandemic. PLOS Global Public Health. 2021;1: e0000061. doi:10.1371/journal.pgph.0000061

43. rmarkdown: Dynamic Documents for R. RStudio; 2023. Available: https://github.com/rstudio/rmarkdown

44. CDC. Wastewater Surveillance: A New Frontier for Public Health. In: Centers for Disease Control and Prevention [Internet]. 19 Jul 2022 [cited 12 Sep 2022]. Available: https://www.cdc.gov/amd/whats-new/wastewater-surveillance.html

45. Naughton CC, Roman, Fernando A J, Alvarado AGF, Tariqi AQ, Deeming MA, Kadonsky KF, et al. Show us the data: global COVID-19 wastewater monitoring efforts, equity, and gaps. FEMS Microbes. 2023;4. doi:10.1093/femsmc/xtad003

46. Garcia K, Christensen A, Reckling S, Berkowitz S, Guidry V. Local Partnership Building – NC Wastewater Monitoring Program for SARS-CoV-2. Council of State and Territorial Epidemiologists (CSTE) Annual Conference; 2022 Jun; Louisville, KY.

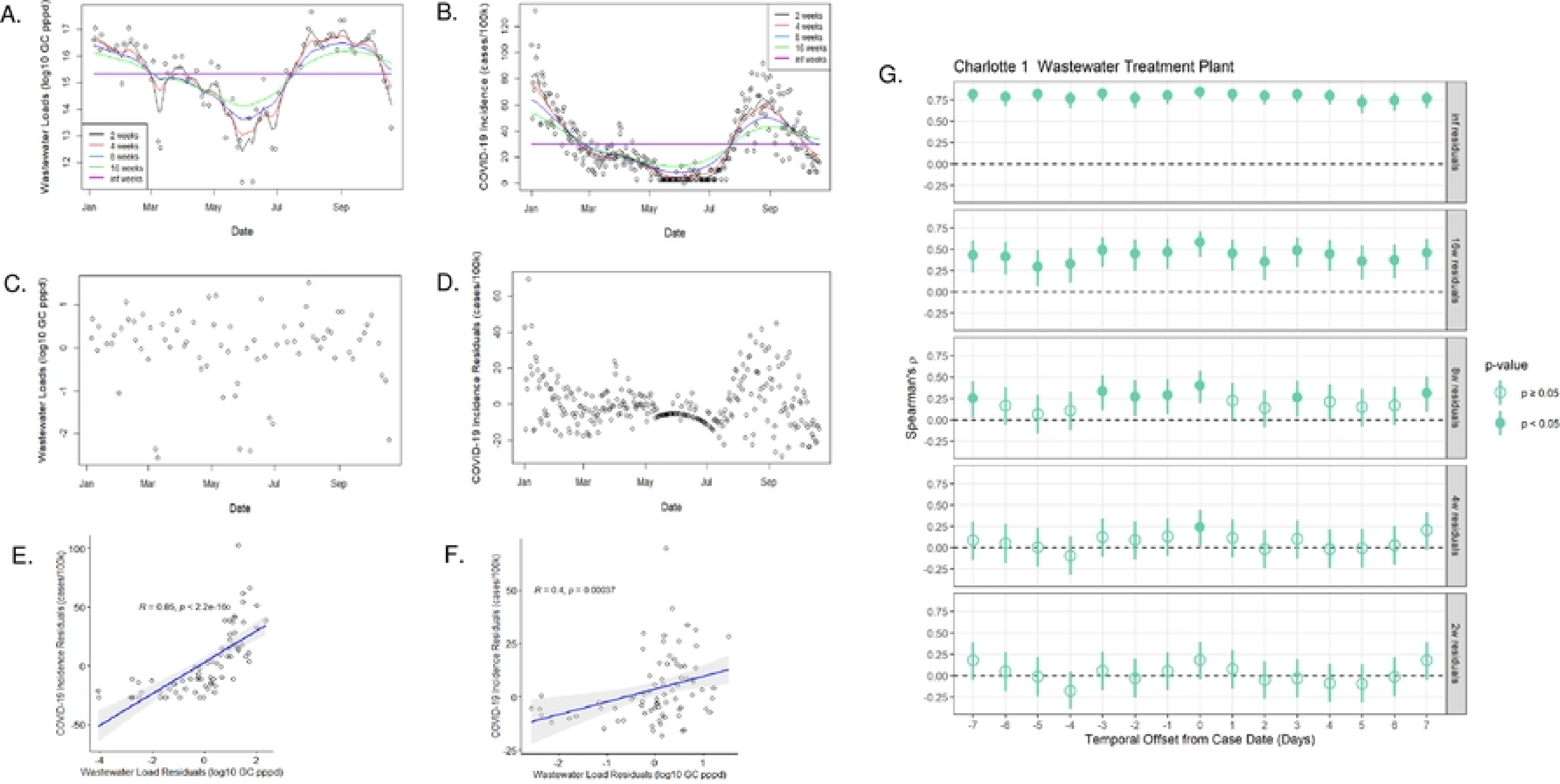
47. Stadler LB, Ensor KB, Clark JR, Kalvapalle P, LaTurner ZW, Mojica L, et al. Wastewater Analysis of SARS-CoV-2 as a Predictive Metric of Positivity Rate for a Major Metropolis. medRxiv [preprint]. 2020. doi:10.1101/2020.11.04.20226191

48. Johnson R, Oliver N. COVID-19 Monitoring Detects Elevated Levels of COVID-19 in Wastewater. Town of Beaufort, Carteret County Health Department, North Carolina Department of Health and Human Services; 2021. Available: https://www.beaufortnc.org/sites/default/files/fileattachments/public_utilities/page/10091/wastewater_joint_release_.pdf

49. Leker HG, Gibson JM. Relationship between race and community water and sewer service in North Carolina, USA. PLOS ONE. 2018;13: e0193225. doi:10.1371/journal.pone.0193225

50. MacDonald Gibson J, DeFelice N, Sebastian D, Leker H. Racial Disparities in Access to Community Water Supply Service in Wake County, North Carolina. American Journal of Public Health. 2014;104: e45. doi:10.2105/AJPH.2014.10412e45.1

51. Marsh B, Parnell A, Joyner A. Institutionalization of Racial Inequality in Local Political Geographies. Urban Geography. 2010;31: 691–709. doi:10.2747/0272-3638.31.5.691



A.
B.
C.
D.
E.
F.
G.
Charlotte 1 Wastewater Treatment Plant
Date
Temporal Offset from Case Date (Days)
Spearman's ρ
p-value
p ≥ 0.05
p < 0.05
inf residuals
10w residuals
6w residuals
4w residuals
2w residuals

Fig1

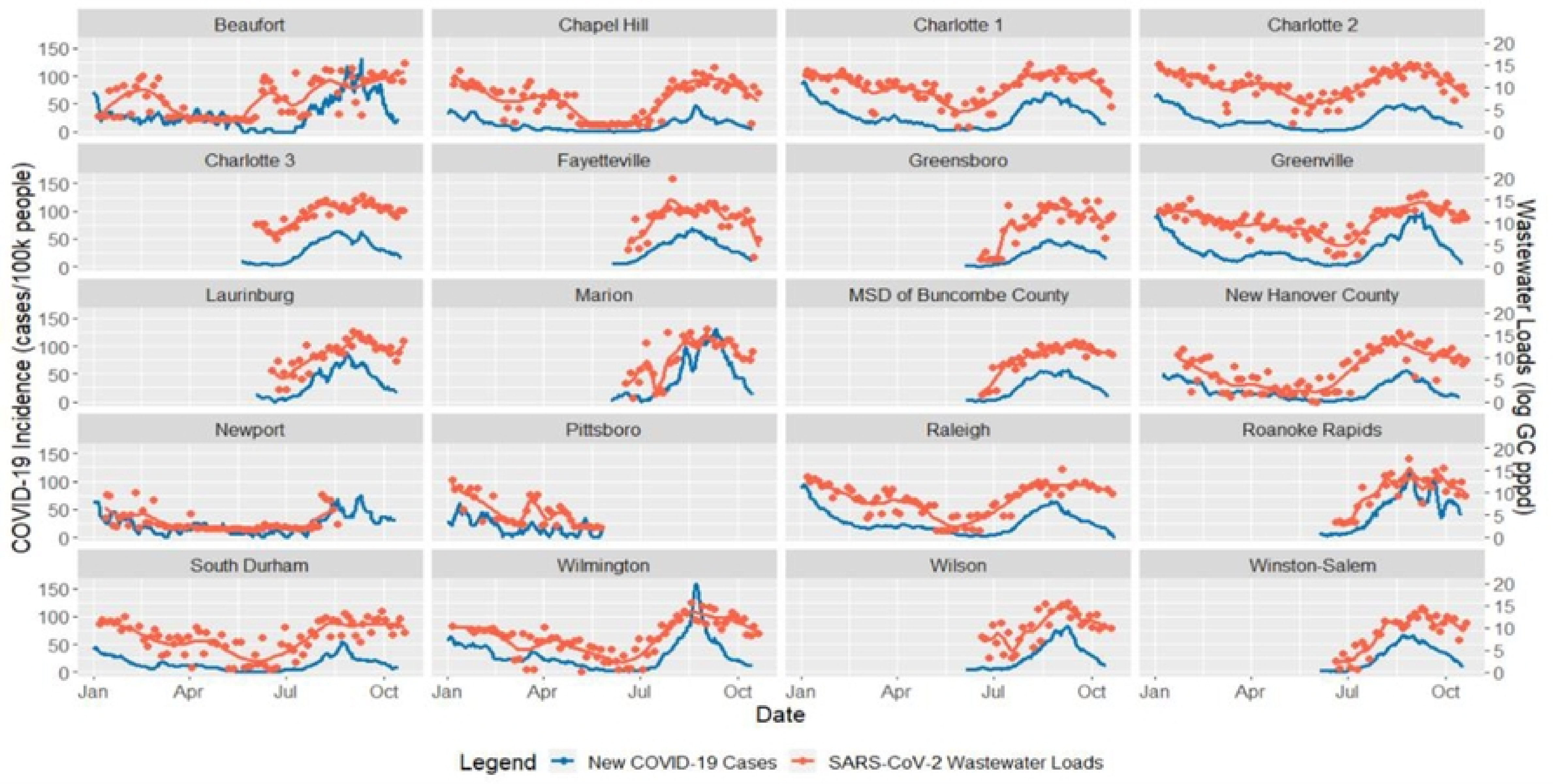

Beaufort
Chapel Hill
Charlotte 1
Charlotte 2
Charlotte 3
Fayetteville
Greensboro
Greenville
Laurinburg
Marion
MSD of Buncombe County
New Hanover County
Newport
Pittsboro
Raleigh
Roanoke Rapids
South Durham
Wilmington
Wilson
Winston-Salem
COVID-19 Incidence (cases/100k people)
Wastewater Loads (log GC pppd)
0
50
100
150
0
5
10
15
20
Jan
Apr
Jul
Oct
Date
Legend
New COVID-19 Cases
SARS-CoV-2 Wastewater Loads

Fig2

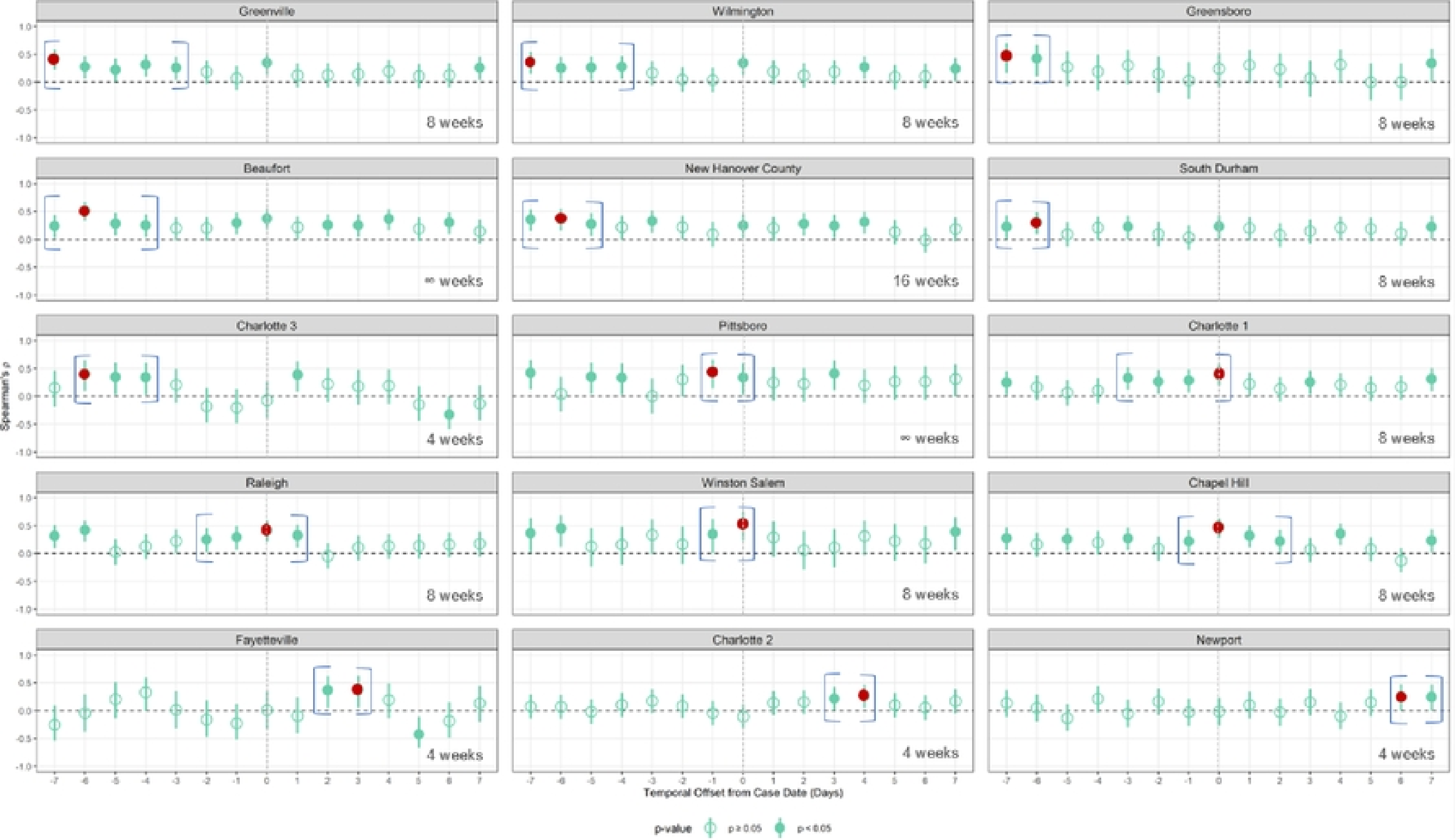
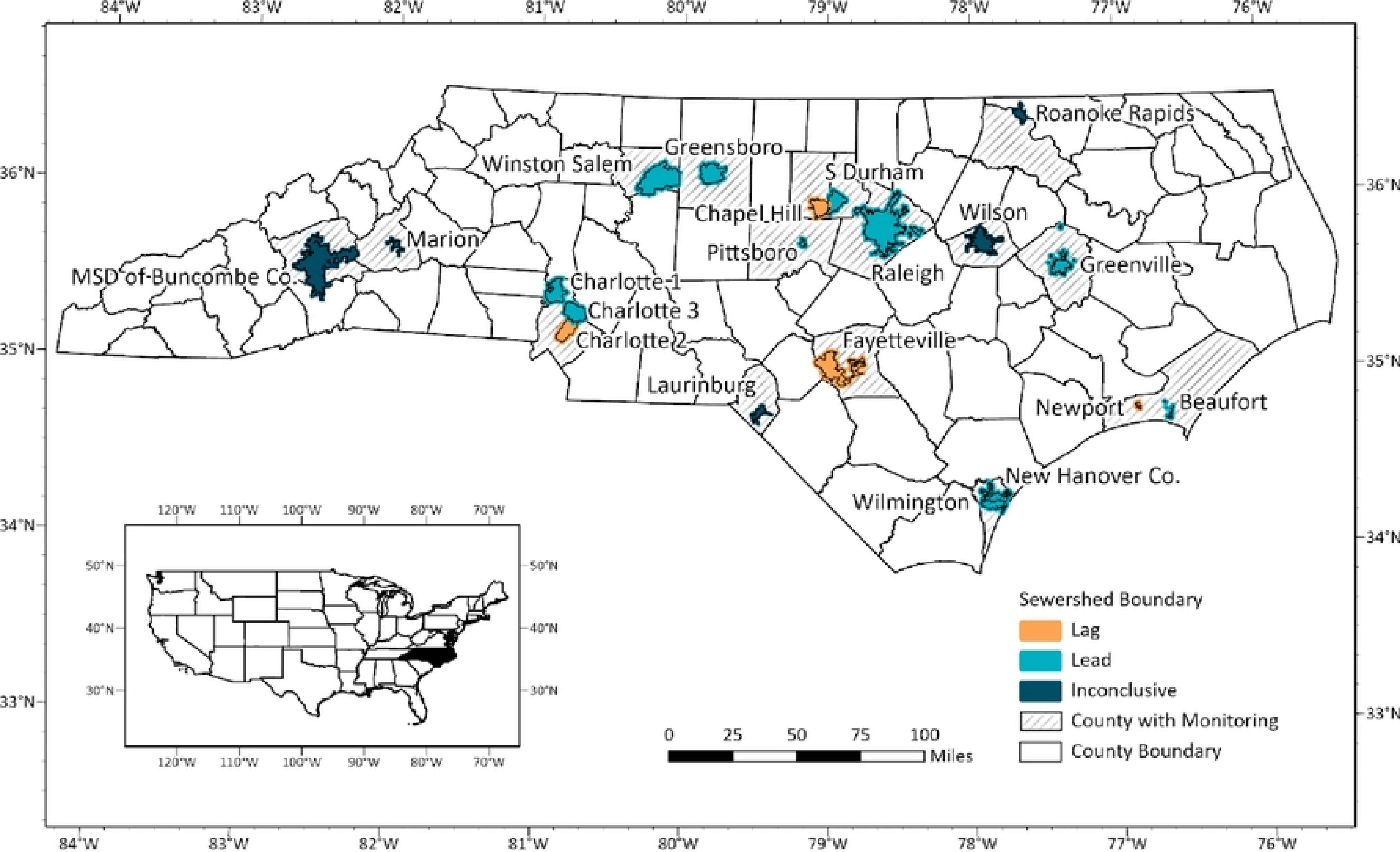

Greenville
8 weeks
Wilmington
8 weeks
Greensboro
8 weeks
Beaufort
∞ weeks
New Hanover County
16 weeks
South Durham
8 weeks
Charlotte 3
4 weeks
Pittsboro
∞ weeks
Charlotte 1
8 weeks
Raleigh
8 weeks
Winston Salem
8 weeks
Chapel Hill
8 weeks
Fayetteville
4 weeks
Charlotte 2
4 weeks
Newport
4 weeks
Spearman's ρ
Temporal Offset from Case Date (Days)
p-value
p ≥ 0.05
p < 0.05

Fig3



Roanoke Rapids
Greensboro
Winston Salem
S Durham
Chapel Hill
Wilson
Marion
Pittsboro
MSD of Buncombe Co.
Raleigh
Greenville
Charlotte 1
Charlotte 3
Charlotte 2
Fayetteville
Laurinburg
Newport
Beaufort
New Hanover Co.
Wilmington
Sewershed Boundary
Lag
Lead
Inconclusive
County with Monitoring
County Boundary
0 25 50 75 100
Miles
84°W 83°W 82°W 81°W 80°W 79°W 78°W 77°W 76°W
36°N 35°N 34°N 33°N
120°W 110°W 100°W 90°W 80°W 70°W
50°N 40°N 30°N

Fig4