Starting Guide

Getting Started

Overview

This package is intended to make the process of analyzing epidemiological wastewater data easier and more insightful. It's intended primarily for researchers and epidemiologists but could potentially be used by anyone with an interest in the topic who has a working knowledge of R programming, epidemiology, and statistics.

Requirements: - Knowledge of R programming - Familiarity with epidemiology - A base understanding of statistics

This package provides a set of core utilities for preparing your data, analyzing your data, and visualizing the results.

Features: - Outlier detection - Various data smoothing techniques - Normalization techniques - Time series analysis - Wastewater/case offset analysis - Visualization / graphing tools - Sample data set courtesy of the Wisconsin Department of Health Services

Note that the package also includes a set of example data. This was done in order to provide a set of real-world instructive examples which make the code easier to understand and apply to your own data sets.

What is Wastewater Based Epidemiology?

Epidemiology is the process of investigating and monitoring the prevalence of disease agents within a population or an environment. This has been most commonly performed by collecting case data from hospitals and other public health agencies. Wastewater-based epidemiology takes a different approach by looking for disease agents in the population's wastewater as it is collected in sewage treatment plants.

Problems with Traditional Case-Based Epidemiology

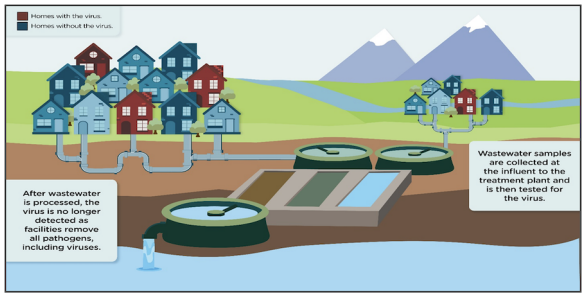
During the Covid-19 pandemic, many problems associated with the traditional case-based epidemiology approach became evident.

- Variations in testing
- Variations in reporting
- Privacy concerns associated with the collection of individual health data
- Disease can take time before onset and presentation in a doctor's office, so the technique is not very timely.

The Process of Wastewater-Based Epidemiology

To address these problems, attention has shifted to an alternate/complementary approach - wastewater-based epidemiology. Wastewater epidemiology is conducted using the following process:

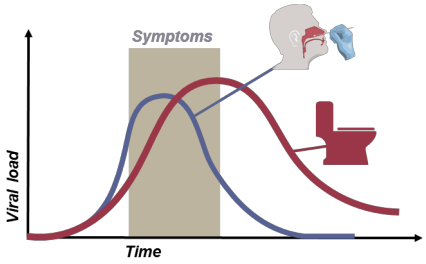
- Wastewater samples are periodically collected from sewer sheds
- Samples are sent to state laboratories for analysis
- Analysis results are communicated to the state health services agencies, where they are compared with reported case rates



Benefits of Wastewater-Based Epidemiology

The advantages of wastewater-based epidemiology compared with case-based epidemiology are as follows:

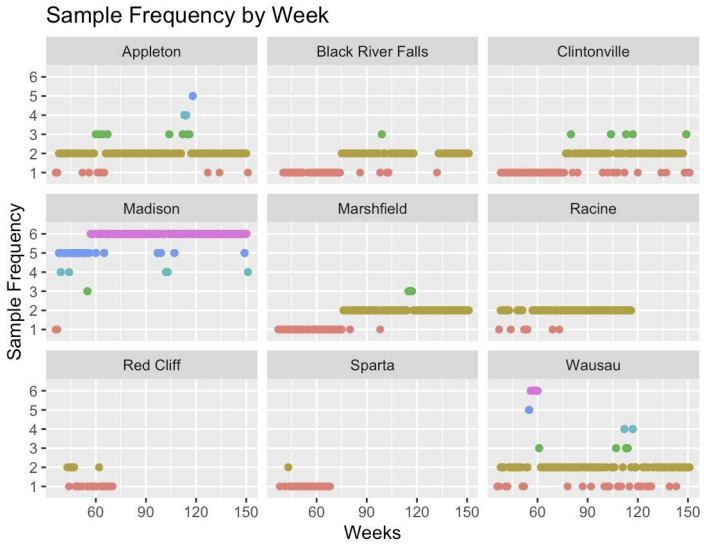
- Can provide more timely, almost "real-time" information
- Is well suited for an epidemiological early warning system
- Maintains the anonymity of individuals
- Does not rely upon voluntary testing by individuals so it has the potential to be more consistent.



Challenges Associated with Wastewater-Based Epidemiology

Despite the very attractive characteristics and promising results of wastewater-based testing, there are also a number of potential challenges associated with this approach which can make it difficult to implement:

- Data is inherently noisy
- There are often significant sampling differences between communities (once per day versus once per week, for example)
- There are often differences in methodology (qpcr versus dpcr etc.)
- There are many cofactors related to wastewater collection and testing which can make interpretation of results difficult.



The Role of This Software

Because of these various complicating factors and the difficulty in performing wastewater-based analysis and interpreting results, software such as this can serve as a valuable aid in making the analysis and interpretation of this data easier and more reliable.

Loading and Viewing Data

The data in this package is a combination of data provided to us from the following sources: - Wisconsin Department of Health Services (DHS) - Wisconsin State Lab of Hygiene (SLH) - Open-source data

All data can be found in the /data directory as .RData objects. Alternatively, when our package is installed, these data sets can be loaded by using the command:

```
data(<name here>, package = "Covid19Wastewater")
```

where <name here> is replaced with one of the following:

Data List

- Aux_info_data Extra data that can be merged with WasteWater_data
- Case_data Case information for all of Wisconsin from 2020-01-22 to 2022-12-08
- Covariants_data Statewide variant proportions
- Example_data A merged and shortened version of Case_data and WasteWater_data from 3 sites
- HFGCase_data High-frequency data from 6 weeks involving ten sites
- HFGWaste_data High-frequency data from 6 weeks involving ten sites
- InterceptorCase_data Madison specific data
- Pop_data Population data along with region, county, and lab submitter

- WasteWater_data Wastewater epidemiological data from across Wisconsin can be merged with Aux_info_data

Here is the key to all the column names in the data: https://github.com/UW-Madison-DSI/Covid19Wastewater/blob/main/docs/data/data_columns_discription.md

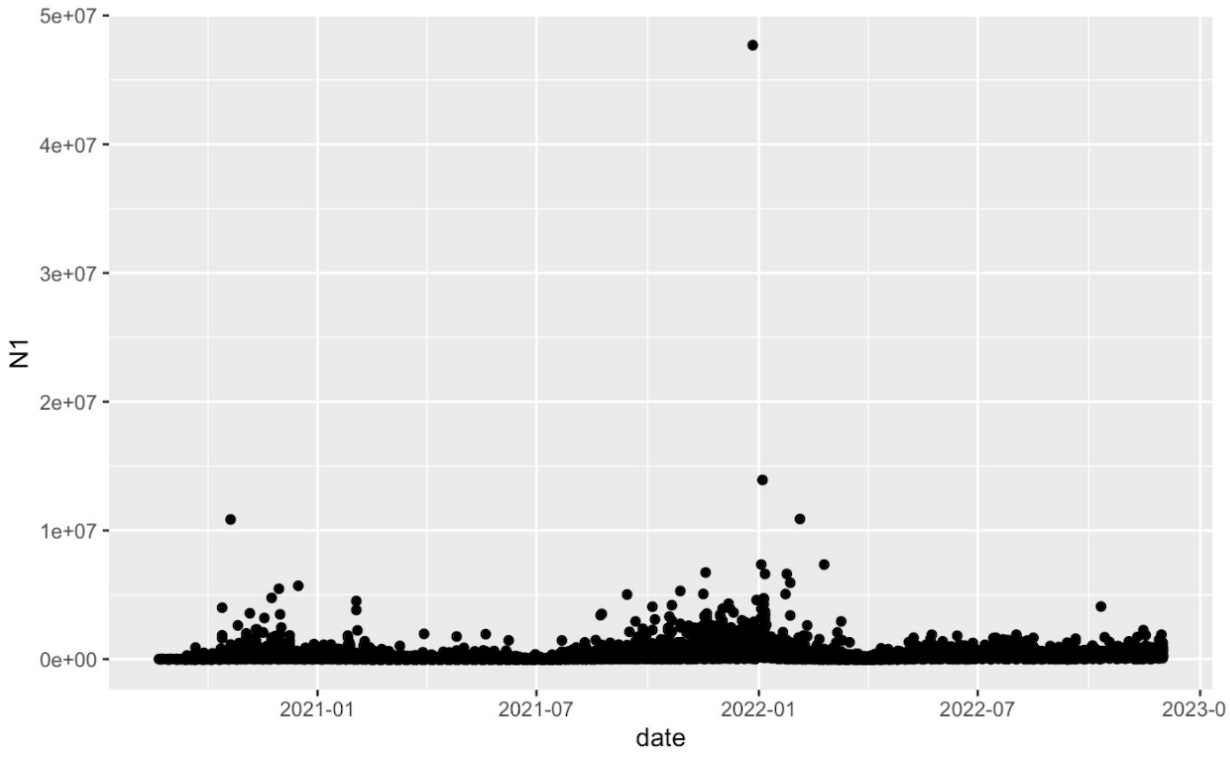
Examples

Viewing Gene Markers Over Time

The prevalence of covid is determined using the genome markers called "N1" and "N2". A simple starting point is to load in the data and then graph N1 or N2 over time.

```
data("WasteWater_data", package = "Covid19Wastewater")

WasteWater_data %>% ggplot(aes(x=date,y=N1)) +
  geom_point()
```

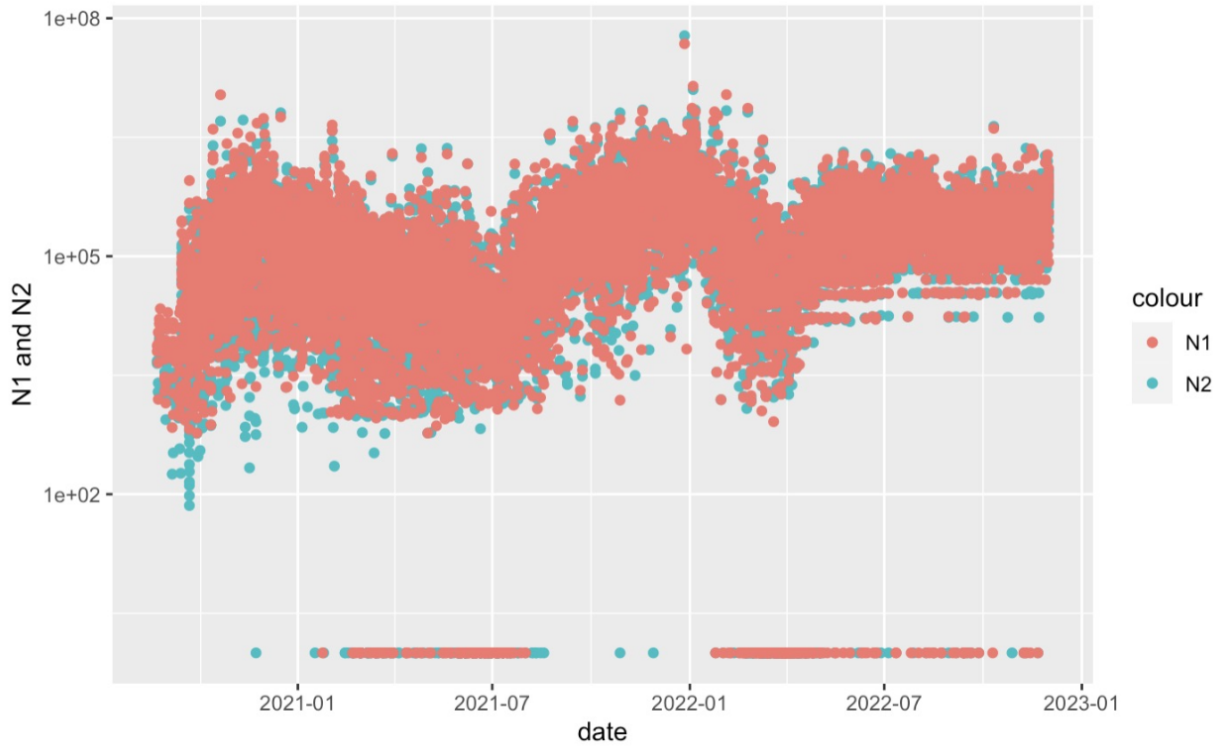


Adding Color

With a few extra lines of code, we can add some color coding in order to display N1 and N2 in a more visually appealing way.

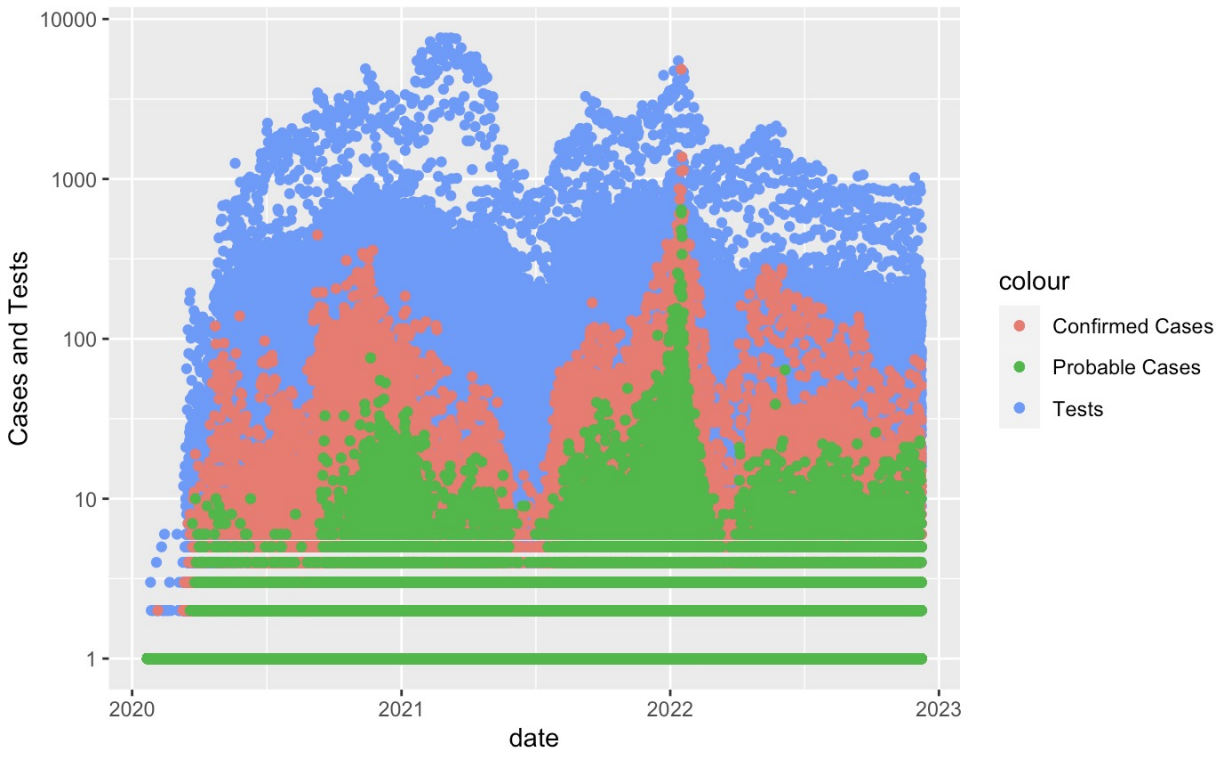
```
data("WasteWater_data", package = "Covid19Wastewater")

WasteWater_data %>% ggplot() +
  geom_point(aes(x=date,y=N2+1, color = "N2")) + #plus 1 to have a nice log
  geom_point(aes(x=date,y=N1+1, color = "N1")) +
  scale_y_log10() +
  ylab("N1 and N2")
```



Taking a similar approach to case data we can make a similar graph for comparing probable cases, confirmed cases, and tests

```
data("Case_data", package = "Covid19Wastewater")
Case_data %>%
  ggplot() +
  geom_point(aes(x=date,y=tests+1, color = "Tests")) +
#plus 1 to have a nice log
  geom_point(aes(x=date,y=conf_case+1, color = "Confirmed Cases")) +
  geom_point(aes(x=date,y=prob_case+1, color = "Probable Cases")) +
  scale_y_log10() +
  ylab("Cases and Tests")
```



Merging Datasets

Below, we show a set of examples of merging different datasets.

1. Merging Wastewater and Case Data

When merging wastewater and case data, it is best to merge by site and data to identify each entry uniquely.

```
data("WasteWater_data", package = "Covid19Wastewater")
data("Case_data", package = "Covid19Wastewater")

WasteAndCaseMerged_data <- merge(Case_data, WasteWater_data, by = c("site","date"))
head(WasteAndCaseMerged_data)
```

site	date	tests	prob_case	conf_case	prob_death	conf_death	sample_id	N1	N2	PMMoV	flow	ph	pcr_type	n1_sars_cov2_lod	n2_sars_cov2_lod	n1_lod	n1_loq	n2_lod	n2_loq
Algoma	2020-10-06	46	0	7	0	0	529795001	1068	NA	146797007	0.498	7.7	qPCR	TRUE	TRUE	20000	64000	33000	68000
Algoma	2020-10-13	60	0	6	0	0	530920001	52846	55955	77956087	0.499	7.9	qPCR	FALSE	FALSE	20000	64000	33000	68000
Algoma	2020-10-20	47	0	8	0	0	532236001	40090	22193	11431157	0.402	7.8	qPCR	FALSE	TRUE	20000	64000	33000	68000
Algoma	2020-10-27	30	0	4	0	0	533319001	183437	165726	6958094	0.670	8.1	qPCR	FALSE	FALSE	20000	64000	33000	68000
Algoma	2020-11-03	38	1	5	0	0	534556001	1574735	1896871	4132435	0.489	7.9	qPCR	FALSE	FALSE	20000	64000	33000	68000
Algoma	2020-11-10	67	1	9	0	0	535601001	267387	232507	11649229	0.463	7.9	qPCR	FALSE	FALSE	20000	64000	33000	68000

2. Merging High-Frequency Wastewater and Case Data

We include high-frequency datasets for both wastewater and case data which can be merged as follows:

```
data("HFGWaste_data", package = "Covid19Wastewater")
data("HFGCase_data", package = "Covid19Wastewater")

HFGWasteAndCaseMerged_data <- merge(HFGCase_data,HFGWaste_data, by = c("site","date"))
head(HFGWasteAndCaseMerged_data)
```

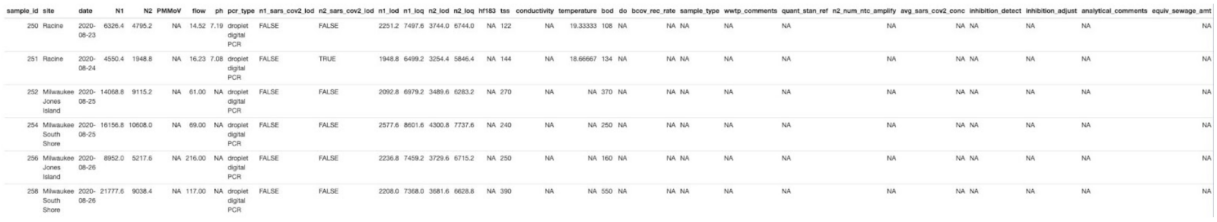
site	date	ReportedCases	EpisodeCases	CollectedCases	ConfirmedCases	Filter	Well	N1	N1LOD	N2	N2LOD	PMMOV	BCoV	HF183	CrP
Hudson	2021-01-25	-999	-999	6	5	2	3	98634	FALSE	67862	FALSE	7103485	6.10	296482397	97912594
Hudson	2021-01-25	-999	-999	6	5	3	1	123509	FALSE	88829	FALSE	6643187	2.43	164425133	78106846
Hudson	2021-01-25	-999	-999	6	5	3	2	107217	FALSE	65828	FALSE	8215284	2.69	170701740	74252234
Hudson	2021-01-25	-999	-999	6	5	2	2	168132	FALSE	107241	FALSE	6917604	4.76	203828374	91220128
Hudson	2021-01-25	-999	-999	6	5	2	1	138137	FALSE	110372	FALSE	6945237	4.15	265156508	98203103
Hudson	2021-01-25	-999	-999	6	5	3	3	93576	FALSE	93760	FALSE	6655620	2.58	161122042	58830450

3. Merging Wastewater and Aux Data

When merging the auxiliary information, it can only be done with sample_id (Aux_info_data can only be merged with WateWater_data)

```
data("WasteWater_data", package = "Covid19Wastewater")
data("Aux_info_data", package = "Covid19Wastewater")

WastewaterAndAuxInfo_data <- merge(WasteWater_data,Aux_info_data, by = "sample_id")
head(WastewaterAndAuxInfo_data)
```



4. Merging Wastewater and Population Data

Population data can be merged with any dataframe that contains site data.

```
data("WasteWater_data", package = "CovidWastewater")
data("pop_data", package = "Covid19Wastewater")
data("Case_data", package = "Covid19Wastewater")
```

```
WastewaterAndPop_data <- merge(WasteWater_data, pop_data, by = "site")
head(WastewaterAndPop_data)

CaseAndPop_data <- merge(Case_data, pop_data, by = "site")
head(CaseAndPop_data)
```

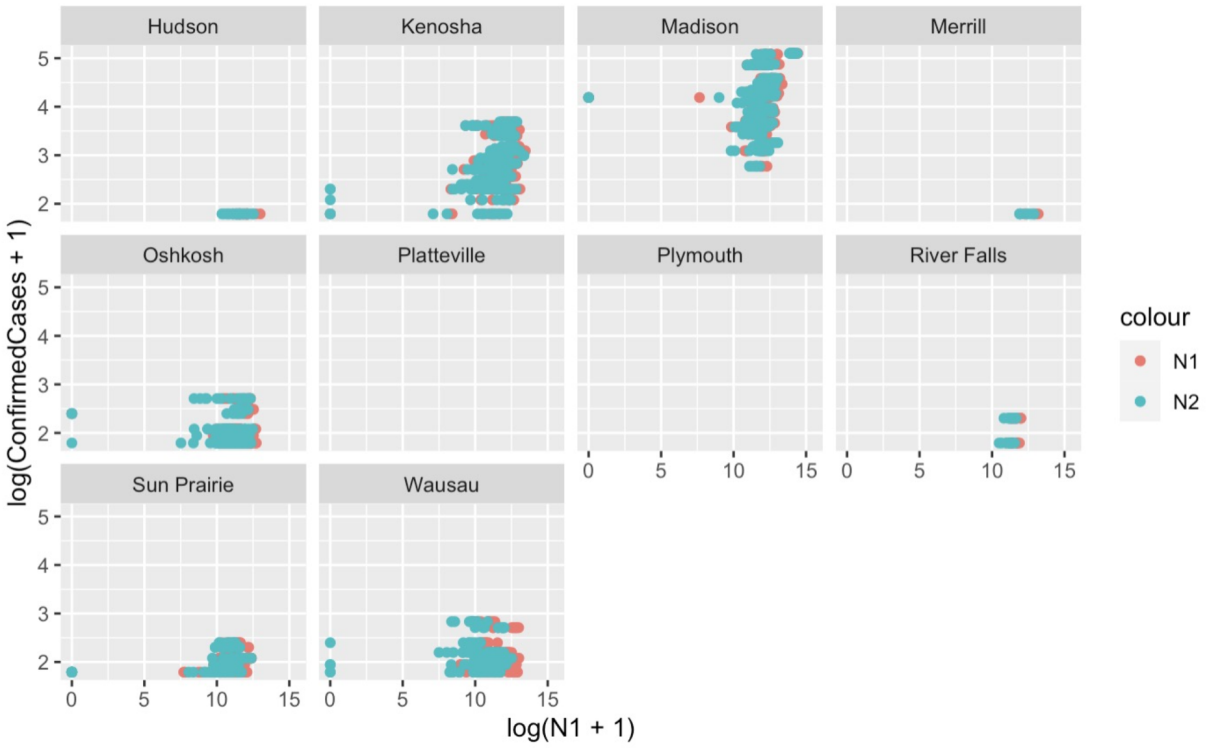
site	sample_id	date	N1	N2	PMMoV	flow	ph	pcr_type	n1_sars_cov2_lod	n2_sars_cov2_lod	n1_lod	n1_loq	n2_lod	n2_loq	regions	county	lab_submitter	pop
Algoma	555990001	2021-04-13	11300	NA	8245987	0.810	7.9	qPCR	TRUE	TRUE	40000	130000	66000	140000	Northeastern	Algoma	SLH	3171
Algoma	540468001	2020-12-15	109432	143498	20942986	0.474	7.9	qPCR	FALSE	FALSE	40000	130000	66000	140000	Northeastern	Algoma	SLH	3171
Algoma	535601001	2020-11-10	267387	232507	11649229	0.463	7.9	qPCR	FALSE	FALSE	20000	64000	33000	68000	Northeastern	Algoma	SLH	3171
Algoma	539283001	2020-12-08	264854	277608	13964405	0.471	7.9	qPCR	FALSE	FALSE	20000	64000	33000	68000	Northeastern	Algoma	SLH	3171
Algoma	546044001	2021-02-02	4515894	3840368	11244204	0.437	7.9	qPCR	FALSE	FALSE	40000	130000	66000	140000	Northeastern	Algoma	SLH	3171
Algoma	532236001	2020-10-20	40090	22193	11431157	0.402	7.8	qPCR	FALSE	TRUE	20000	64000	33000	68000	Northeastern	Algoma	SLH	3171

site	date	tests	prob_case	conf_case	prob_death	conf_death	regions	county	lab_submitter	pop
Algoma	2020-01-22	0	0	0	0	0	Northeastern	Algoma	SLH	3171
Algoma	2020-01-23	0	0	0	0	0	Northeastern	Algoma	SLH	3171
Algoma	2020-01-24	0	0	0	0	0	Northeastern	Algoma	SLH	3171
Algoma	2020-01-25	0	0	0	0	0	Northeastern	Algoma	SLH	3171
Algoma	2020-01-26	0	0	0	0	0	Northeastern	Algoma	SLH	3171
Algoma	2020-01-27	0	0	0	0	0	Northeastern	Algoma	SLH	3171

4. Merging Wastewater and Confirmed Cases Data

With the data now merged, we can perform many more analyses. In the analysis below, we show the number of confirmed cases.

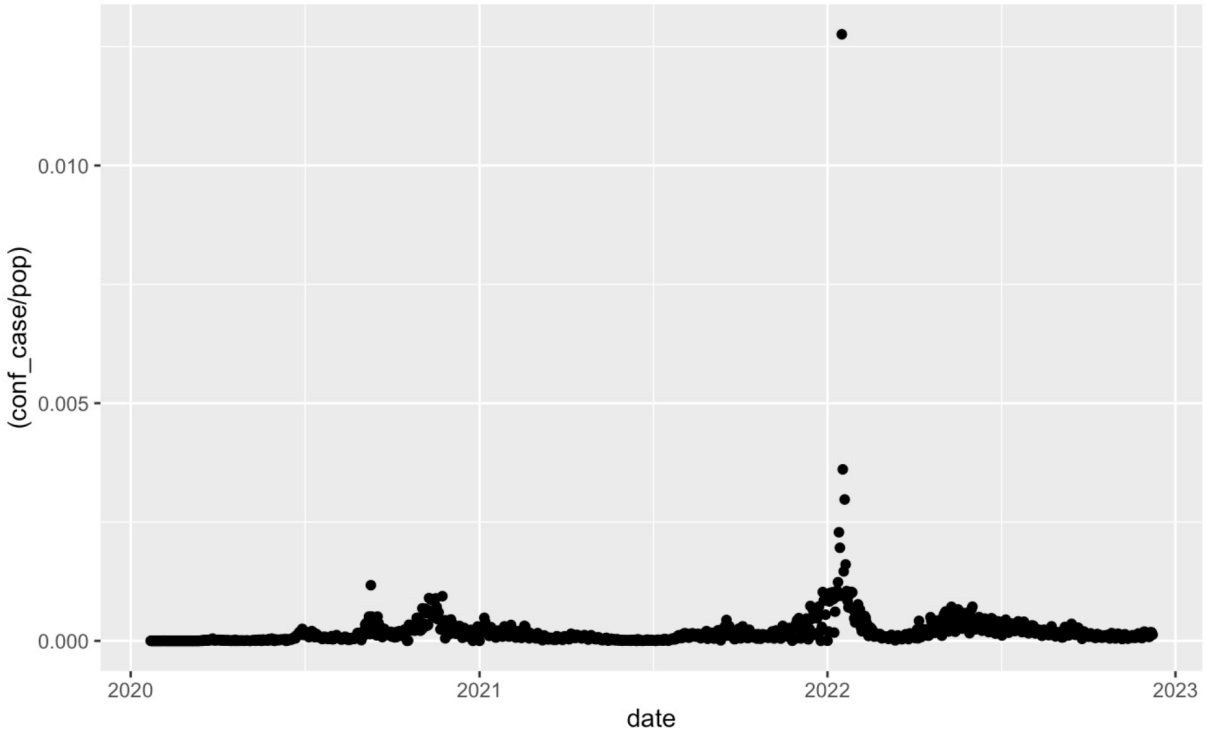
```
HFGWasteAndCaseMerged_data %>%
  ggplot() +
  geom_point(aes(x=log(N1+1),y=log(ConfirmedCases+1),color="N1")) +
  geom_point(aes(x=log(N2+1),y=log(ConfirmedCases+1),color="N2")) +
  facet_wrap("site")
```



4. Normalizing by Population

Below, we display the number of confirmed cases normalized by population as a function of time.

```
CaseAndPop_data %>%
    filter(site == "Madison") %>%
    ggplot(aes(x=date,y=(conf_case/pop)))+
    geom_point()
```



Tips

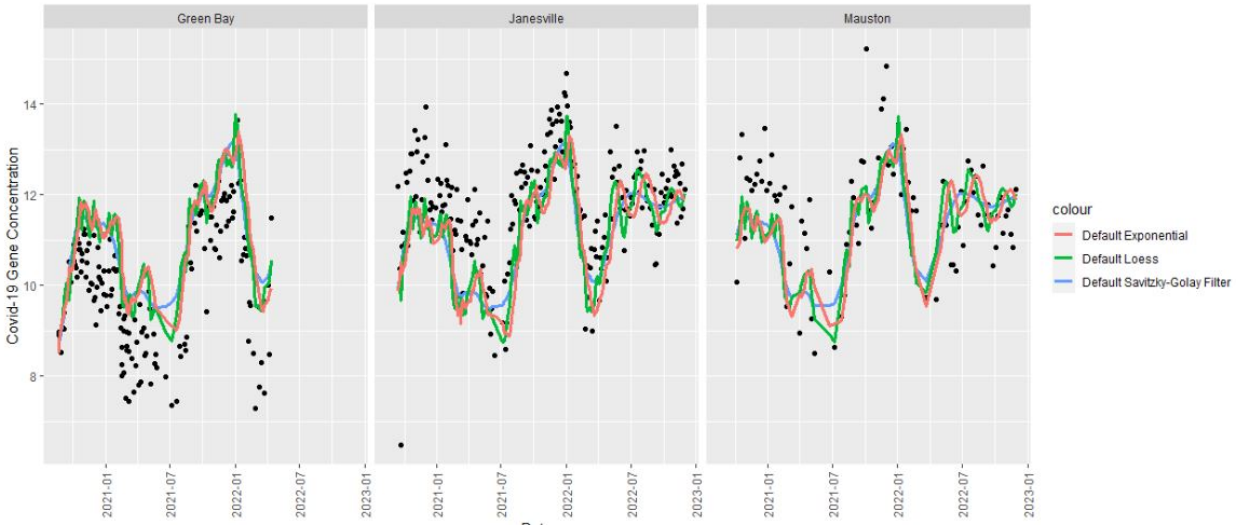
Always make sure that when merging, the "by =" should always be able to identify the information you are merging uniquely. (i.e. don't merge waste and case data by date alone)

Data Preparation

The data preparation takes two main forms: - Outlier detection and Removal - Smoothing methods

Smoothing methods

There are three smoothing methods available to get a more stable Wastewater measurement. - loessSmoothMod - expSmoothMod - sgolaySmoothMod Each one can generate a consistent signal from weekly data. A comprehensive Guide on the methods is available here. Below are the smoothing methods applied with their default values to three Citys in the Wastewater dataset.



Outlier detection

There are two main ways to detect outliers in this package. - Deviance from the trend - Unusual spikes from adjacent values Below is a quick description of these methods but a more comprehensive document can be found here.

Deviance from the trend

This process has two steps. First, you need a trend. This can normally be done with the smoothing in the previous section. Then the trend can be used to find points sufficiently greater than it. This is normally set to 2.5 standard deviations. This example of this method with all the default values applied to Janesville. This method has a ton of flexibility and offers the most accuracy when the trend is accurate. The main issue with this method is that it normally will not work on recent data. Most trend methods do not capture the true trend on the edges of the data effects.

```
WasteWater_flag  <- WasteWater_data%>%
    filter(site == "Janesville")%>%
    mutate(N1 = log(N1 + 1))%>%
    select(site, date, N1)%>%
    loessSmoothMod("N1", "N1_loess")%>%
    Flag_From_Trend(N1, N1_loess)

WasteWater_flag %>%
  ggplot(aes(x = date))+
  geom_point(aes(y = N1, color = flagged_outlier))+
geom_line(aes(y = N1_loess, color = "N1 Loess"))+
  theme(plot.title = element_text(hjust = 0.5),
        axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1))+
  labs(y = "Covid-19 Gene Concentration",
       x = "Date",
       color = "Flagged Outlier"
       )
```

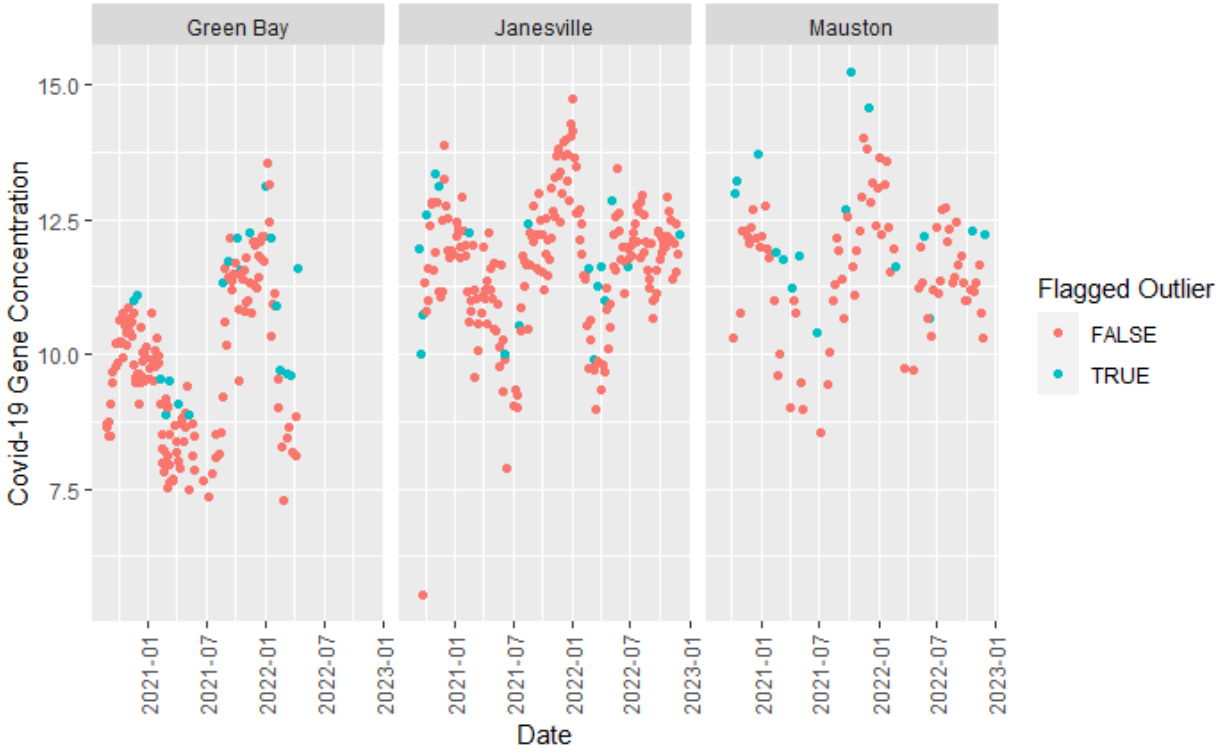
Unusual spikes from adjacent values

This method involves calculating the difference between two adjacent data points and subsequently assessing the extent to which this difference deviates from the distribution of all other such differences. Focusing on the relative size of the jumps, it requires very little prediction of the trend and future movement. This means it can function with only one extra data point after the measurement. This leads to an answer that can be less precise but captures the worst outliers and is more applicable to long-term trends.

```
WasteWater_data <- WasteWater_data%>%
  select(site, date, N1, N2)%>%
  filter(N1 != 0, N2 != 0)%>%
  mutate(N1 = log(N1), N2 = log(N2),
    N12_avg = (N1 + N2) / 2)
df_data <- computeJumps(WasteWater_data)
ranked_data <- rankJumps(df_data)
classied_data <- flagOutliers(ranked_quantile_data, 9, MessureRank)%>%
  select(site, date, N12_avg, MessureRank, FlaggedOutlier)

classied_data%>%
  ggplot(aes(x = date))+
  geom_point(aes(y = N12_avg, color = FlaggedOutlier))+
  facet_wrap(~site)+
  theme(plot.title = element_text(hjust = 0.5),
        axis.text.x = element_text(angle = 90, vjust = 0.5, hjust=1))+
  labs(y = "Covid-19 Gene Concentration",
       x = "Date",
       color = "Flagged Outlier"
```

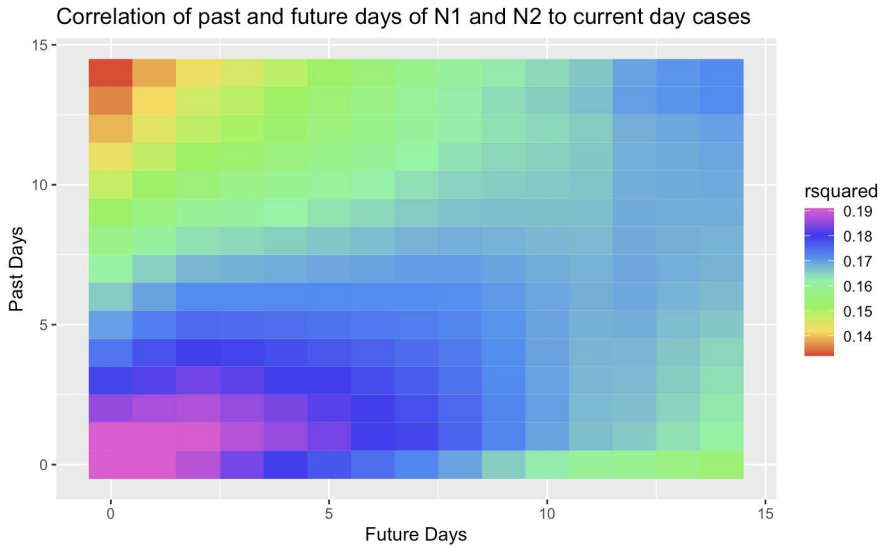
```
)
```



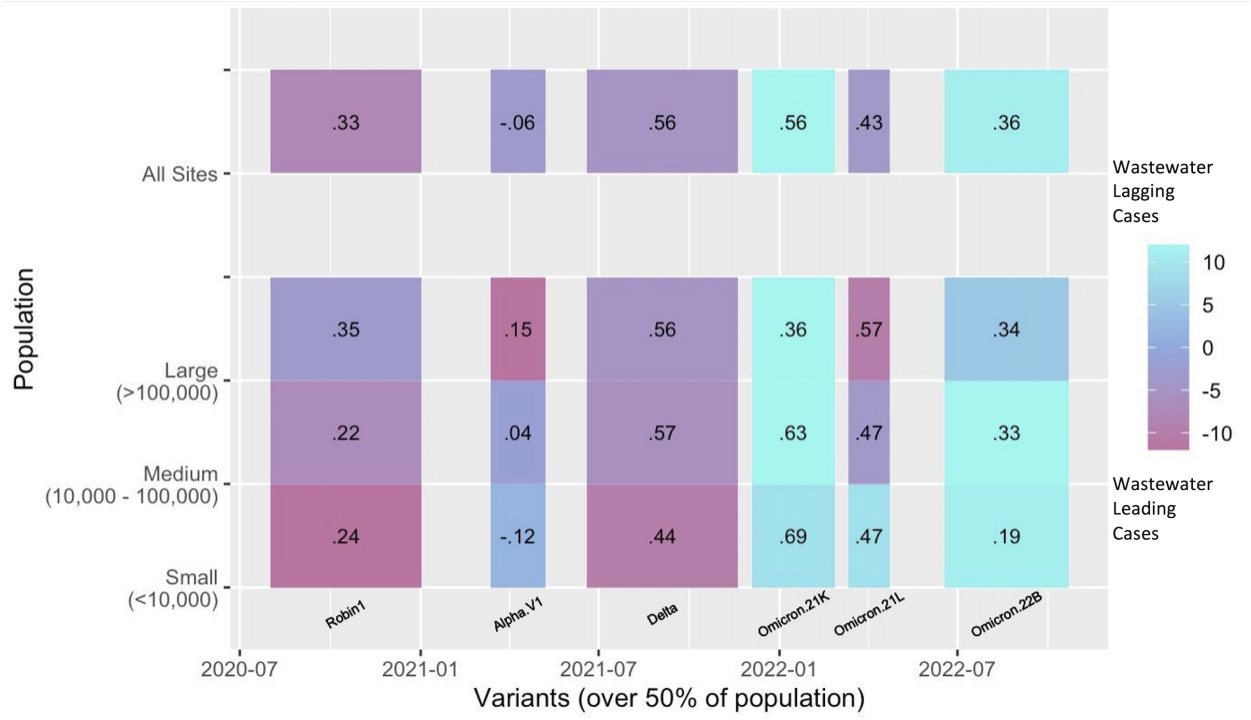
Data Analysis

Time Series Analysis

Shedding is an ongoing process from the first day of infection to days or even weeks after symptoms subside. Thus it is hard to know exactly how many individuals in the community are infected at any given time just using wastewater data. Since we have mostly reliable case data, if we can find the offset that best correlates with the 2 data sets, we can work backward from only wastewater data in the future.



This analysis was done by finding the R-squared correlation between the wastewater data of the current day Z and the combined case data from past Y many days and X number of future days. Thus we can find which moving window of days best represents the wastewater data for the next analysis.



Using the window of case data days that best correlates to the wastewater data, we can find the offset that best corresponds to the time between shedding at its peak and when the individual got tested.

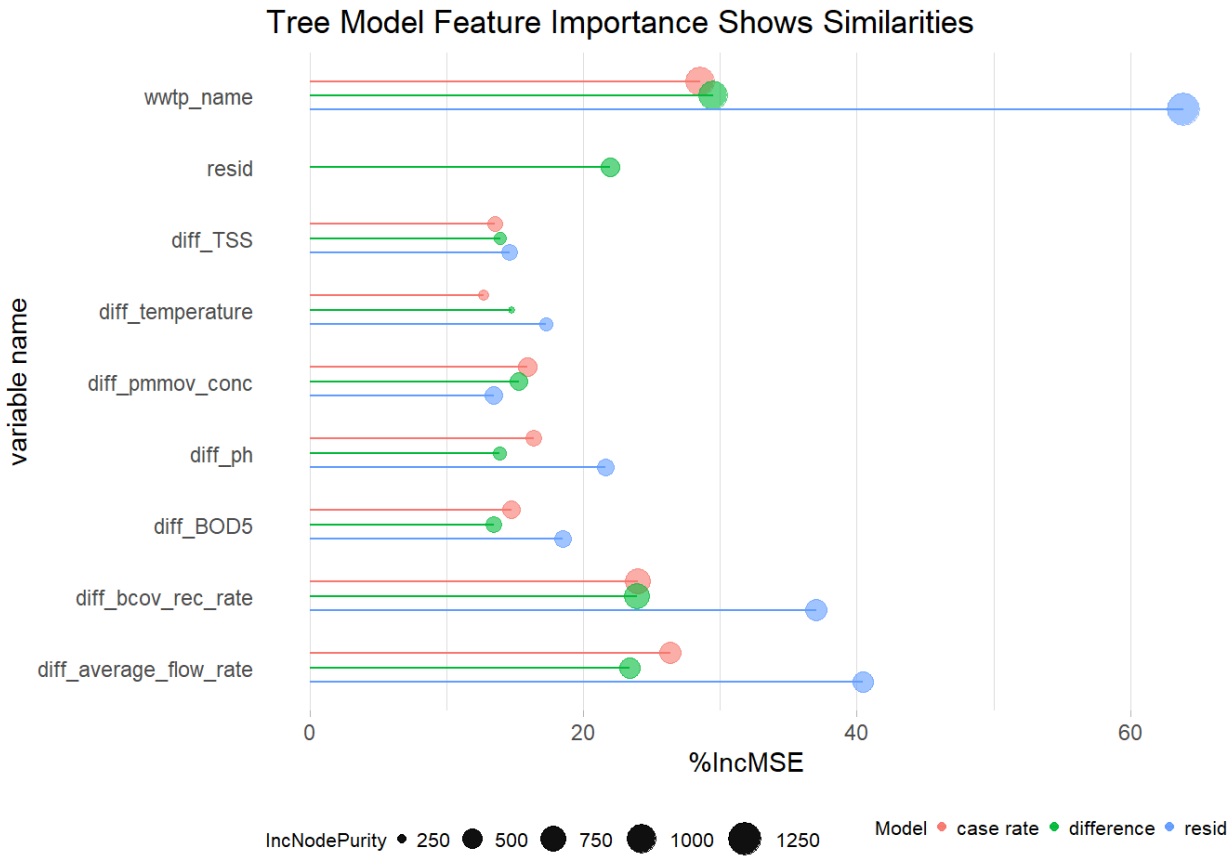
View the vignette to learn more here: Time Series Offset

Or view the vignette in your R interpreter:

```
vignette("time_series_offset", package = "Covid19Wastewater")
```

Random Linear Forests

We introduce a powerful tool called "random_linear_forest" that offers a unique approach to generating linear relationships between two variables while accounting for the influence of covariates. This innovative method combines the principles of random forests with linear tree modeling, resulting in a versatile and robust framework for understanding the intricate interplay between variables and their covariates. Below is the result of using this model on the Longitudinal data done here.



Conclusion

We hope you have had a successful and enjoyable experience using this software package. If you would like to share your results and/or feedback with the package authors, contact information is listed below:

- Marlin Lee - (mailto:mrlee6@wisc.edu)
- Kyllan Wunder - (mailto:kwunder@wisc.edu)
- Abe Megahed - (mailto:amegahed@wisc.edu) You may also submit comments, feedback, feature requests, and bug reports through the GitHub repository at: https://github.com/UW-Madison-DSI/Covid19Wastewater

Acknowledgements

This package was made possible through support from the University of Wisconsin Data Science Institute in collaboration with the Wisconsin Department of Health Services (DHS) and the State Lab of Hygiene (SLH).