

# WET WEATHER SEPTIC SYSTEM IMPACT TO WATER QUALITY STUDY

University of Georgia

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## Background

### The Metropolitan North Georgia Water Planning District

The Georgia General Assembly established the Metropolitan North Georgia Water Planning District (the District) in 2001 to oversee water resource planning in the metropolitan Atlanta area. Encompassing 15 counties, 96 cities, and over 50 water and wastewater providers, the District has played a crucial role in producing comprehensive water resources planning documents since its inception.

Over the years, the District Governing Board, alongside its Technical Coordinating Committee and Basin Advisory Councils, has initiated discussions regarding management policies for on-site sewage management systems, commonly known as septic systems. Addressed in the Water Resources Management Plan, these discussions cover various facets of septic management, such as land use planning, coordination among jurisdictional departments, collaboration with local Boards of Health, and strategies for proper septage disposal. Looking ahead, the District Governing Board is contemplating additional actions to enhance surface water quality in the region.

In pursuit of this goal, the Board directed the District to conduct a [study](#) on the impact of septic systems on water quality. An initial study was conducted by Geosyntec under dry weather conditions, and the results from that study are summarized below. This study aims to evaluate the contribution of septic systems to surface water quality, considering factors like fecal coliform and nutrients under wet weather conditions.

### Summary from Dry Weather Study Conducted in 2019

To assess the contribution of human waste from septic systems to surface waters in the Atlanta region, the study used microbial source tracking (MST), an advanced DNA-based tool, in conjunction with conventional monitoring of fecal indicator bacteria (FIB) and nutrients. The District, with support from Geosyntec Consultants, developed a Monitoring Plan to guide the sampling and analysis. The Plan incorporated a review of existing data and studies, detailed the sampling location selection process, specified parameters for monitoring, and provided information on sampling locations and frequencies.

The [findings](#) from the human waste marker, HF183, in different subwatersheds indicated generally low impacts of human waste on surface waters during dry weather, except for one site that may have been affected by a leaking sanitary sewer. Across other subwatersheds, there were no notable connections found between HF183 and various factors such as septic density, distance to the stream, fecal coliform, nitrate+nitrite, or dissolved phosphorus. The report concluded that septic systems were not identified as the primary source of fecal coliform nor were they significantly contributing to dissolved phosphorus concentrations during dry weather. However, they may contribute to increased nitrate+nitrite levels in streams under dry conditions. In light of these findings, the study concluded management actions targeting septic systems are unlikely to have a significant impact on fecal coliform or phosphorous concentrations in these streams during dry weather. As a result, no regional changes to septic policies were recommended. However, they did suggest conducting an investigation into the impact of septic systems during wet weather conditions.

In response to the findings from the dry weather study, the District sought to assess the impacts of septic systems during wet weather on bacterial and nutrient loading to surface waters in the metropolitan Atlanta region. This investigation would build on the findings of the aforementioned [2019 Septic System Impacts to Surface Water Quality Study](#).

## Project Description and Research Questions

The purpose of this study is to support the District in evaluating the impact of septic infrastructure on surface water quality in streams within their jurisdiction. Specifically, this study will employ time-sensitive sampling techniques to assess the effects of rain events on interactions between septic system characteristics (e.g., system age and density) on surface water conditions that compromise human health and the environment.

### Research Questions:

Our work was designed to address four primary research questions. They include:

- 1. Can we attribute declines in surface water quality with rainfall events in watersheds dominated by septic infrastructure and is there evidence that such declines are derived from human wastewater (i.e., increases in fecal coliform bacteria and the HF138 marker)?*
- 2. If increases in fecal coliform bacteria and the HF138 marker are detected, are they related to corresponding changes in conductivity, nitrogen, and phosphorus concentrations?*
- 3. If additional system specific data (e.g., exact location, detailed soil information, age, maintenance records, etc.) are integrated into the data collected from the 2019 study and analyzed using our proposed methods, what else can we learn about the relationships between surface water quality parameters and septic infrastructure from the data collected in that study?*
- 4. If changes in water quality due to rainfall events are documented in the watershed, are they maintained long enough to forgo the need for immediate sample collection in response to changing discharge (i.e., ISCO samplers)?*

Questions 1-3 address key points detailed in the project solicitation. The primary motive of this project is to relate wet weather to septic-derived surface water pollution. This is addressed through questions 1 and 2. The District also hopes to relate data collected in the new project to data collected in the 2019 study. We proposed to enhance the power of the data collected in 2019 by adding additional information to the dataset and then applying additional statistical analysis to the data. In addressing question 4, we hoped to inform future management and monitoring programs of the District. We proposed to separate the effects of the “first flush” of pollution moving through the system during a rain event from baseflow conditions.



## Study Design

### Study Sites and Number of Sampling Events

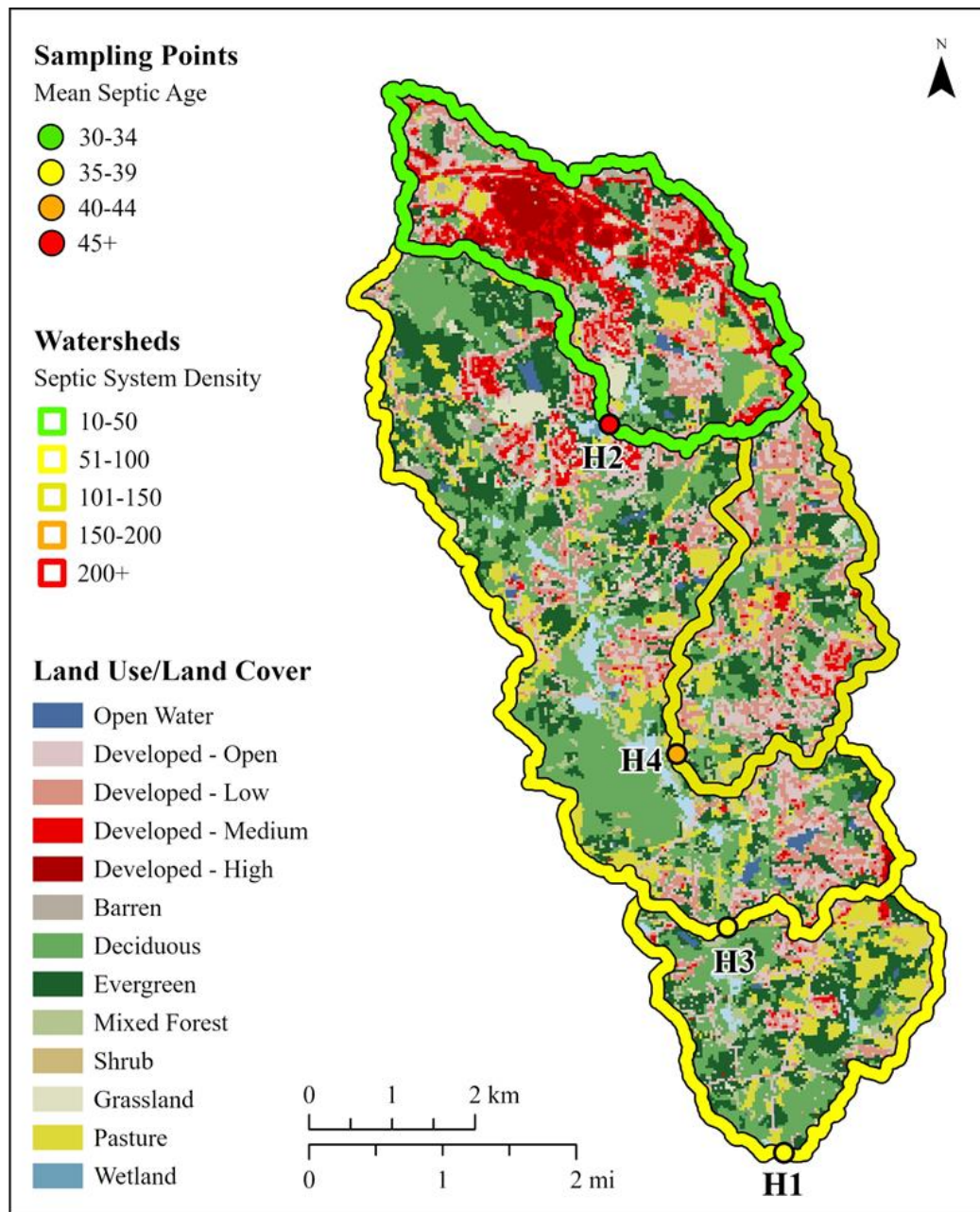
We collected data in four of the watersheds that were sampled in the 2019 Dry Weather Study (Figures 1-4; Appendix A). We sampled in Honey Creek (3 dry weather samples; 8 wet weather samples), Little Stone Mountain (3 dry weather samples; 8 wet weather samples), Pond Fork (3 dry weather samples; 5 wet weather samples and West Fork Little River (3 dry weather samples; 3 wet weather samples) between 28 September 2022 and 01 May 2023 (Table 1). Dry weather samples were collected under baseflow conditions.

Wet weather events were defined using the StreamStats tool (<https://www.usgs.gov/streamstats>) that we employed to calculate flow for floods of varying return periods. *Return periods* tell us how often we can expect a specific event, like a flood, to happen. For example, a 10-year return period flood has about a 1 in 10 chance of occurring each year. It helps us understand the likelihood of certain events over time based on historical data. The StreamStats tool uses regional data collected from streams with stream gauges and to predict flow for streams that do not have stream gauges using regression methods. We used these regression data and field surveys with an Acoustic Doppler Velocimeter (ADV) to estimate flow for lower return periods and determined the corresponding water depth for each of our four sample sites using a rating curve derived from the data from the ADV data. A *lower return period* refers to a shorter average time between occurrences of a specific event, such as a flood or other natural phenomena. In the context of hydrology and risk assessment, events with lower return periods are more frequent and have a higher likelihood of happening within a given timeframe.

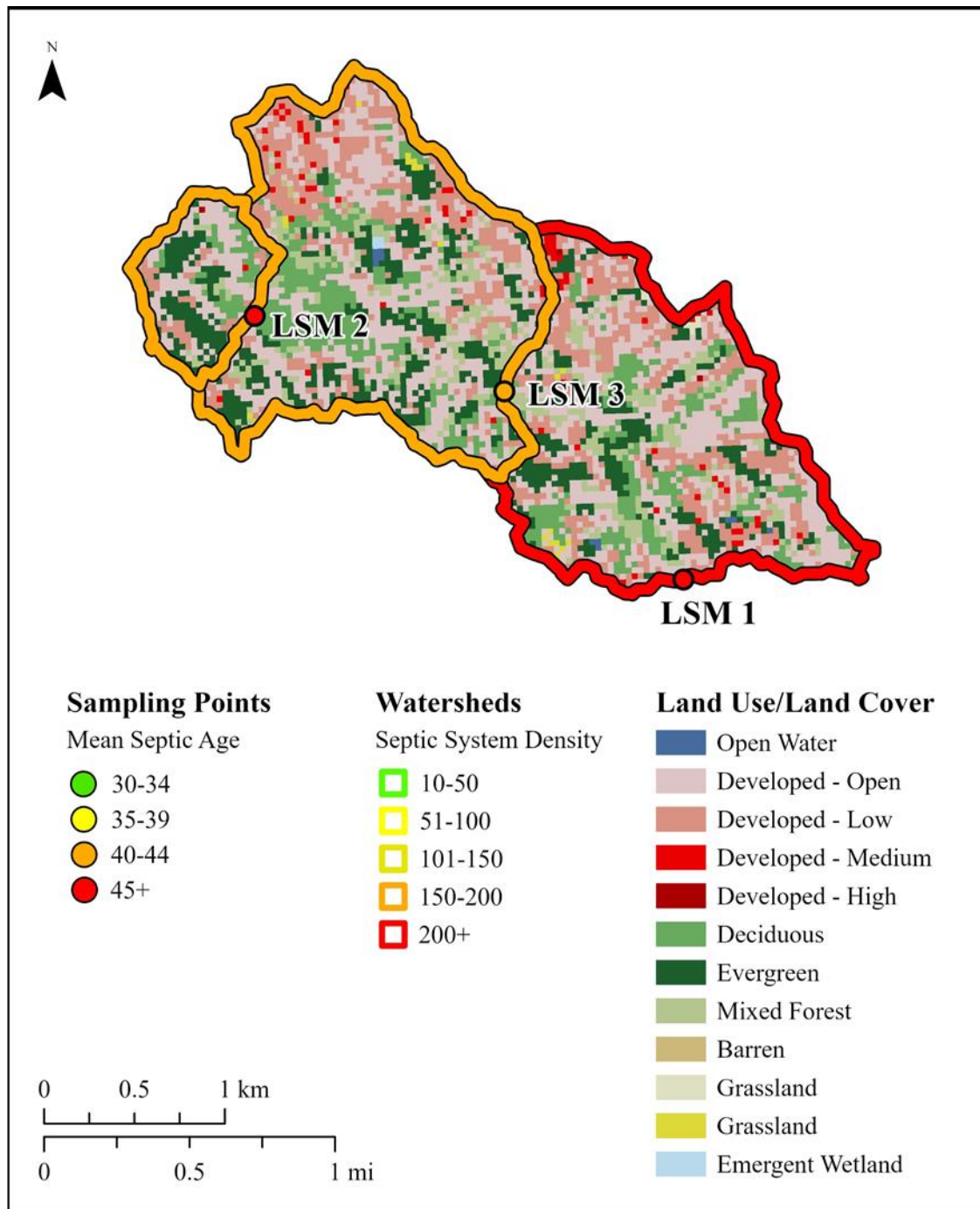
Automated sample collection was initiated when a specific water level indicative of a flood event in the watershed occurred and triggered automatic sample with our automatic sampler with refrigeration capabilities. We programmed the sensors (ISCOs) to sample as the water was rising (the rising limb of the hydrograph) and when the water levels were declining (the falling limb of the hydrograph). A *hydrograph* is a graph depicting the variation in water discharge over time at a specific location in a river or stream. When we visited the sites to remove the samples from our sampler, we also took a grab sample after the rain event. Sample collection during baseflow allowed us to assess the influence of rain events on surface water quality and to support data comparison between the two studies (Table 1). We also conducted additional trips to the sample locations to evaluate site characteristics using the Rapid Bioassessment Protocol developed by the US EPA, update site-specific land use conditions, and maintain in-situ sampling equipment (e.g., conductivity sensors, pressure transducers, ISCOs).

### Field and Laboratory Methods

Our research approach was designed to investigate relationships between septic systems and surface water quality, including, but not limited to, conductivity, nitrogen and phosphorus concentrations, and fecal coliform bacteria in surface water. We also conducted microbial source tracking (MST) in the study watersheds to evaluate the contribution of human waste to fecal coliform concentrations. We employed the same analytical methods to analyze surface water nutrients, fecal coliform concentrations, and microbial surface tracking detailed in the Final Report for the Septic System Impact on Surface Water Quality Study in Metropolitan Atlanta that was prepared by Geosyntec in 2019 to support direct comparisons of data collected in both studies. Specifically, we measured nitrate+nitrite as N using EPA Method

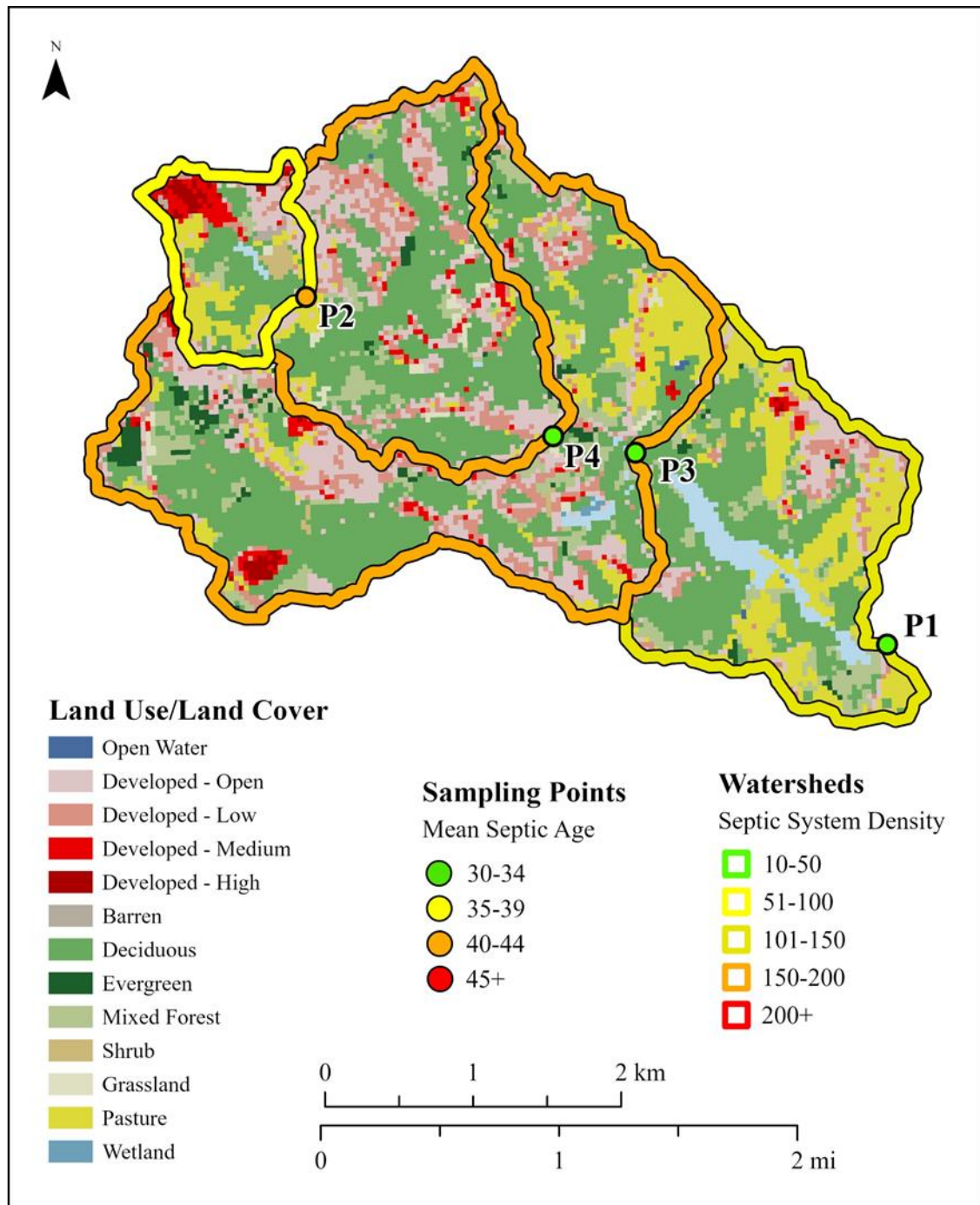


**Figure 1:** Honey Creek. Sampling sites are detailed by points. Sampling numbers reflect the site numbers in the 2019 study. The color of the point indicates the mean sample age of septic systems in the area drained by that point. The line color denotes the septic system density in the subwatershed. Land use/land cover data are denoted by the shaded areas within each watershed.

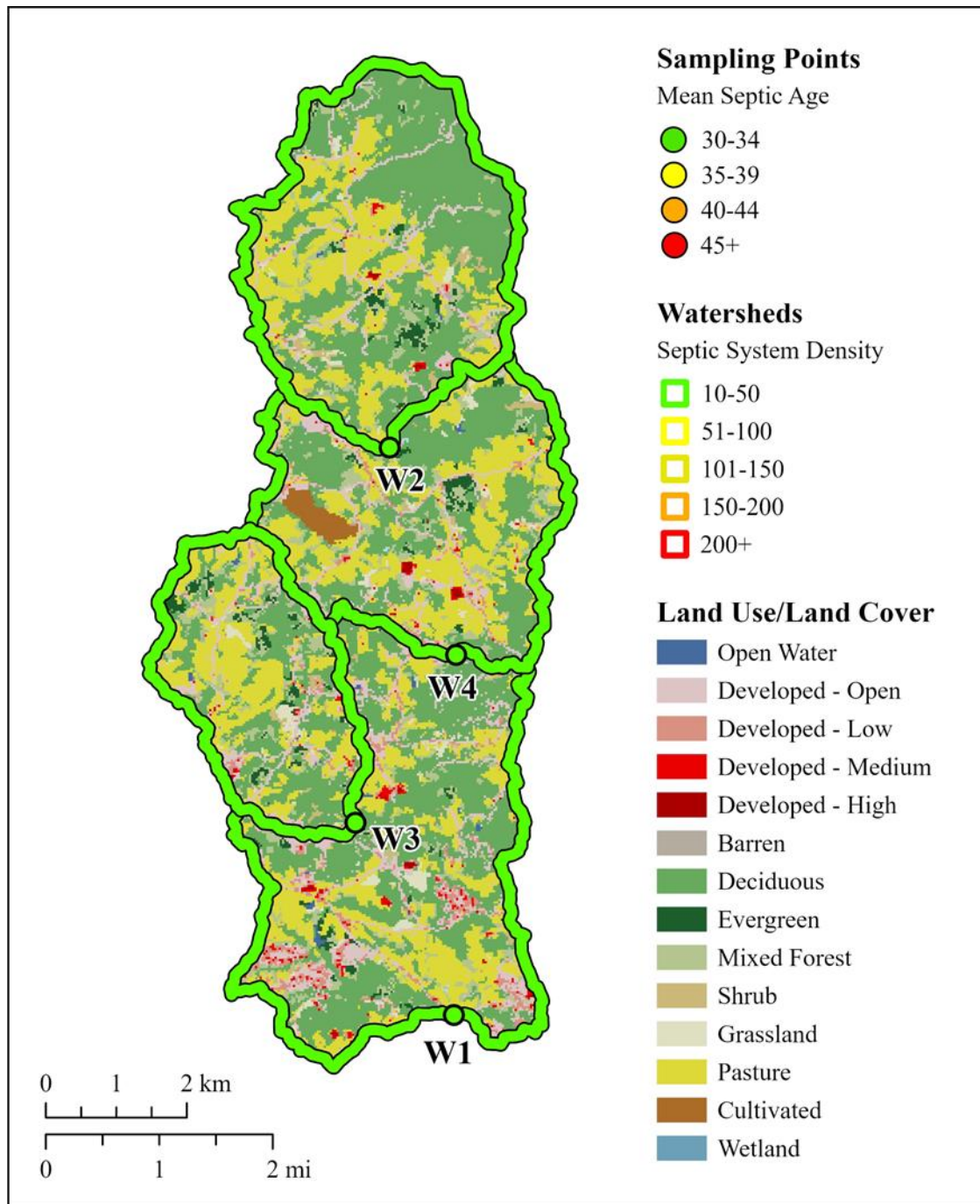


**Figure 2:** Little Stone Mountain. Sampling sites are detailed by points. Sampling numbers reflect the site numbers in the 2019 study. The color of the point indicates the mean sample age of septic systems in the area drained by that point. The line color denotes the septic system density in the subwatershed. Land use/land cover data are denoted by the shaded areas within each watershed.





**Figure 3:** Pond Fork. Sampling sites are detailed by points. Sampling numbers reflect the site numbers in the 2019 study. The color of the point indicates the mean sample age of septic systems in the area drained by that point. The line color denotes the septic system density in the subwatershed. Land use/land cover data are denoted by the shaded areas within each watershed.



**Figure 4:** West Fork Little River. Sampling sites are detailed by points. Sampling numbers reflect the site numbers in the 2019 study. The color of the point indicates the mean sample age of septic systems in the area drained by that point. The line color denotes the septic system density in the subwatershed. Land use/land cover data are denoted by the shaded areas within each watershed.

353.2 and dissolved phosphorus using EPA Method 365.1. Samples were also analyzed for ammonium concentrations, **but this was not paid with project funds, as it was outside of the scope of the project. This was conducted as an add-on activity to our work to benefit the project.** We estimated fecal coliform concentrations using Standard Method 9222D and quantified human fecal marker (HF183) using ddPCR. Handheld multiparameter probes were used to measure pH, temperature, dissolved oxygen, turbidity, and conductance using standard methods. We also measured discharge using a flow meter at each site during each sampling event. Additionally, we supplied and deployed continuous weather sensors and conductivity sensors in each of the study watersheds. Though limited by budget, we deployed four ISCO samplers and pressure transducers to estimate changes in discharge in the study watersheds. In each watershed, one ISCO sampler was deployed at the furthest downstream sampling point.

To comprehensively evaluate the impact of wet weather on stream water quality and identify the causes of poor stream water quality, we performed additional sampling to separate the effects of runoff and baseflow. First, we collected rainfall data in each watershed using a rain gauge, and the data were recorded on a cloud to enable near real-time monitoring of rainfall. Continuous stream gauge data were collected using a pressure transducer and we ran hydrograph analysis on the data for each storm to identify the start and end times of runoff to streams and the amount of runoff to streams. This was done using the Web-Based Hydrograph Analysis Tool. We collected streamwater samples during and after a storm event using an automated ISCO sampler. This enabled us to estimate the concentration of analytes carried in runoff and baseflow. **Analytes** are the specific substances or things that scientists or researchers are studying or measuring in a sample.

During each rainfall event, time-based samples were collected for the duration of the storm. We followed the procedure described in the dry weather sampling report to determine discharge areas for each sampling location and retained the number of sampling locations identified in the dry weather report for comparing between the two sampling periods.

Water samples were filtered within 24 hours of collection. Samples were diluted to 1:10 and 1:100 for Fecal Coliform Bacteria (FCB) analysis to ensure countable plates. Both dilutions were filtered, in duplicate, onto sterile 47 mm 0.45 µm pore size mixed cellulose ester membrane and incubated at 44.5°C for at least 16 hours in a cell foam box (USFDA Center for Food Safety and Applied Nutrition, 2020). Dark blue colonies were counted for each plate and recorded. Counts were reported as CFU (colony forming units) per 100 ml using one set of replicate plates for a single dilution (generally this is the lowest dilution plate with countable colonies, unless there are issues with replication or other noted problems).

An additional 50 mL of sampled water was filtered onto 47 mm 0.2 µm pore size polycarbonate filters for DNA extraction. Filters were processed using Zymo DNA Extraction kits and DNA eluted into a final volume of 100 µL. Extracted DNA was used for quantification of the human fecal marker HF183 using ddPCR (as described by Cao et al., 2015) at the GGBC. An additional aliquot was used for quantification of the fecal marker crAssphage using qPCR (Malla et al. 2019). **CrAssphage**, is a relatively recently identified bacteriophage associated with the human gut that shows promise for detecting human fecal contamination in the environment. It is predominantly found in human feces and sewage, and it often correlates

with the presence of other fecal indicators, making it a potential tool for monitoring water pollution from human sources (Park et al. 2020). A *bacteriophage* is a type of virus that infects and replicates within bacteria. **The analysis of crAssphage was not paid with project funds, as it was outside of the scope of the project. This was conducted as an add-on activity to our work to benefit the project.** We measured crAssphage concentrations to assess relationships between the bacteriophage and the other human fecal markers we measured in this study. Detailed field and laboratory standard operating procedures are included in Appendix B.

**Table 1:** Sample dates, weather conditions, number of sites within each watershed, and the number of samples collected for nutrient and fecal marker analysis at each of the four sample sites

Site Name	Sample Date	Weather Conditions	Number of Sites	Number of Samples Analyzed for Nutrients	Number of Samples Analyzed for Fecal Markers
Little Stone Mtn.	9/28/2022	Dry	3	3	3
Honey	9/28/2022	Dry	4	4	4
West Fork	9/29/2022	Dry	4	4	4
Pond	9/29/2022	Dry	4	4	4
West Fork	11/9/2022	Dry	4	4	4
Pond	11/9/2022	Dry	4	4	4
Little Stone Mtn.	11/10/2022	Dry	3	3	3
Honey	11/10/2022	Dry	4	4	4
Little Stone Mtn.	11/28/2022	Wet	1	2	2
Pond	11/30/2022	Wet	1	1	2
Little Stone Mtn.	12/1/2022	Wet	1	5	6
Honey	12/1/2022	Wet	1	7	7
West Fork	12/7/2022	Wet	1	2	2
Pond	12/7/2022	Wet	1	2	2
Little Stone Mtn.	12/10/2022	Wet	1	3	3
Little Stone Mtn.	12/15/2022	Wet	1	4	4
Honey	12/15/2022	Wet	1	5	5
Pond	12/15/2022	Wet	1	1	1
Honey	12/19/2022	Dry	4	4	4
Little Stone Mtn.	12/19/2022	Dry	3	3	3
West Fork	12/20/2022	Dry	4	4	4
Honey	1/5/2023	Wet	1	5	5
Little Stone Mtn.	1/5/2023	Wet	1	1	2
West Fork	1/5/2023	Wet	1	1	1
Pond	1/5/2023	Wet	1	1	1
Honey	1/23/2023	Wet	1	13	13
Little Stone Mtn.	1/23/2023	Wet	1	7	8
West Fork	1/23/2023	Wet	1	1	1
Pond	1/23/2023	Wet	1	1	1
Honey	1/26/2023	Wet	1	3	3
Little Stone Mtn.	1/26/2023	Wet	1	2	3
Pond	2/6/2023	Dry	4	4	4
Little Stone Mtn.	3/27/2023	Wet	1	3	4
Honey	3/27/2023	Wet	1	8	8
Honey	4/28/2023	Wet	1	1	1
Honey	5/1/2023	Wet	1	3	3

## Data Visualization and Statistical Methods

We created box plots of the concentrations of nutrients and fecal markers for each watershed during wet weather events (Figures 6-11). We also created plots of raw data collected by sample site in wet and dry weather events during this study. These plots are located in Appendix C. We also created visual comparisons of automated samples collected during all sampling events that can be viewed in Appendix E.

To examine differences in nutrient concentrations and fecal markers between wet and dry weather events within each watershed, we used *Wilcoxon signed-rank tests* which is an approach that can be used when you have pairs of related data and want to see if there's a significant difference between them. Instead of focusing on the actual values in a data set, it ranks the differences between paired observations, looks at the sum of positive and negative ranks, and determines if there's a consistent direction of difference. If the p-value is low (typically less than 0.05, you can conclude that there's a significant difference between the paired observations. The results from these analyses can be viewed in Tables 2 & 3.

We performed *Pearson correlation analyses* for different subsets of the data to examine relationships between the nutrient and human fecal markers within watersheds and between wet and dry sampling events. The analysis is a statistical method used to quantify the strength and direction of a linear relationship between two continuous variables. A *continuous variable* is a type of number that can take on any value within a range and can be measured with great precision. Examples of continuous variables include height, weight, temperature, and time. It can have infinite possibilities between any two points, making it different from variables that can only have specific, separate values. Pearson correlation assumes that there is a linear relationship between variables. A *linear relationship* between two variables means that when you graph their values, they form a straight line. If one variable goes up, the other goes up by a constant amount (positive slope), or if one goes up, the other goes down by a constant amount (negative slope). Pearson correlation coefficients are produced through the correlation analysis and they only range from values of -1 to +1. Values closer to +1 indicate that there is a positive relationship, meaning that as one variable increases, the other tends to increase as well. Values closer to -1 indicate a negative linear relationship, suggesting that as one variable increases, the other tends to decrease. The results from these analyses can be viewed in Figures 12-16.

We also attempted to perform a generalized linear mixed model (GLMM) using land use/land cover data from the four study watersheds. A *GLMM* is a statistical tool that handles diverse types of data. It can examine average relationships between variables and can be applied to many kinds of data. *Repeated measures* refer to gathering data from the same sites several times. This helps researchers see how things change within the same subjects across various time points, or in our case, wet and dry weather conditions. Unfortunately, because we were only able to outfit each of the watersheds with one automatic sampler (we paid for three of these machines with funding outside of this grant), the number of samples we were able to collect through this project were not sufficient to statistically model any of the measured variables (nutrients, human fecal markers) with the large number of other variables that we collected in each subwatershed. Therefore, we were unable to examine relationships between variables using this approach.



## Roles and Responsibilities

### **Dr. Krista Capps (Project Manager)**

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University of Georgia  
140 East Green St.  
Athens, GA 30602

**Education and Experience:** Dr. Capps has an undergraduate degree in biology and political science from Hope College, a MS degree in environmental science from Indiana University, and a PhD in Ecology and Evolutionary Biology with an emphasis in biogeochemistry from Cornell University. She is a freshwater ecologist who is interested in understanding how wastewater infrastructure influences water quality, biodiversity, and nutrient dynamics in freshwater systems. She has been funded by the Georgia Environmental Protection Division, the Georgia Water Resources Institute, and the National Science Foundation (NSF) to investigate the ways in which wastewater infrastructure influences stream water quality. Two of her NSF grants were led and/or co-authored by Drs. Abney and Gaur to study the ways in which septic system infrastructure may influence watershed nutrient dynamics. Dr. Capps has collaborated and published septic-related research with other academic colleagues and with water professionals from the local, regional and state-levels, including colleagues from Athens-Clarke County, the Georgia Department of Health, the Douglasville-Douglas County Water and Sewer Authority, and the Metropolitan North Georgia Water Planning District (Metro District). Dr. Capps has led several large collaborative research teams through her experience as a US Peace Corps Volunteer, through her graduate training, and through her work as a faculty member of UGA. She will apply these skills to the management of this interdisciplinary research team (Figure 1).

### **Dr. Rebecca Abney**

Forest Resources Building 4 room 303  
University of Georgia  
180 E Green St  
Athens, GA 30602

**Education and Experience:** Dr. Abney graduated with a BSc in Environmental Biology and Physical Geography from the University of St Andrews in 2012, and her PhD in Environmental Systems in 2017. Her PhD work focused on the combined impacts of wildfire and erosion on soil organic matter composition and fate in temperate forest systems. She then completed a postdoctoral scholar position at Indiana University until 2019, when she started at UGA. Dr. Abney is a soil biogeochemist, and broadly her work focuses on how disturbances impact soil organic matter and nutrient cycling. These disturbances include urban septic systems, which are widely understudied as potential nutrient hotspots. Dr. Abney has organized sessions at national conferences on urban soils, and she has presented her work at meetings of this scale.

### **Dr. Nandita Gaur**

3105 Miller Plant Sciences  
University of Georgia  
120 Carlton St  
Athens, GA, 30602

**Education and Experience:** Dr. Nandita Gaur is a faculty in the Department of Crop and Soil Sciences at the University of Georgia (UGA). She received her BS in Civil Engineering in 2008

from Punjab Engineering College, India and a Ph.D. in 2015 from the Biological and Agricultural Engineering department at Texas A & M University. Dr. Gaur subsequently worked as post-doctoral candidate at Texas A&M University where she worked in a large interdisciplinary team to design and install a watershed scale hydrological observatory. Thereafter, she joined UGA in 2018 as an Assistant Professor of Soil Physics. She is affiliated with the River Basin Center and the Institute of Resilient Infrastructure Systems. Dr. Gaur's current research focuses on applications that include 1) understanding of water movement and contaminant transport under different land uses and land covers, and 2) enabling the use of remote sensing data for environmental applications. Specific to this project, Dr. Gaur is currently an investigator on interdisciplinary grants (RAPID and SCC-PG) from NSF where she is using geophysics and physical measurements to study septic systems and their impact on water quality and soil biogeochemistry. She is also the lead PI on a grant funded by the Georgia Environmental Protection Division to map the distribution of septic systems in Jackson County, Georgia using remote sensing imagery and GIS. She is also an instructor for a short course on sub-surface hydrology (offered up to twice a year) for county extension agents at the Georgia Department of Public Health where she teaches about various hydrological tests related to septic systems' siting.

**Dr. Erin Lipp**

Environmental Health Science, Room 141  
University of Georgia  
150 East Green St  
Athens GA 30602

**Education and Experience:** Dr. Lipp received her bachelors degree in marine biology from New College of Florida and went on to earn her PhD in marine science from the University of South Florida where she specialized in environmental microbiology in coastal systems. She continued her work on microbiology of aquatic pathogens at the University of Maryland Center of Marine Biotechnology. Her work focuses on the ecology of aquatic pathogens and the role of water in the distribution and transmission of disease associated with humans and other animals. Her work has been funded by the EPA, NOAA, USGS, CDC, NIH, and NSF. Specific to this project, she has work on water quality issues associated with septic system placement and density in both coastal and inland areas. This has included chemical and microbiological tracer studies to track movement between drainfield, groundwater and surface water and to assess impacts from contaminated groundwater discharge on adjacent surface waters. Her lab also works on understanding pathogen introduction, distribution, and transmission associated with water. In Georgia, she has collaborated with state (GA DNR) and local agencies (Athens Clarke County Public Utilities).

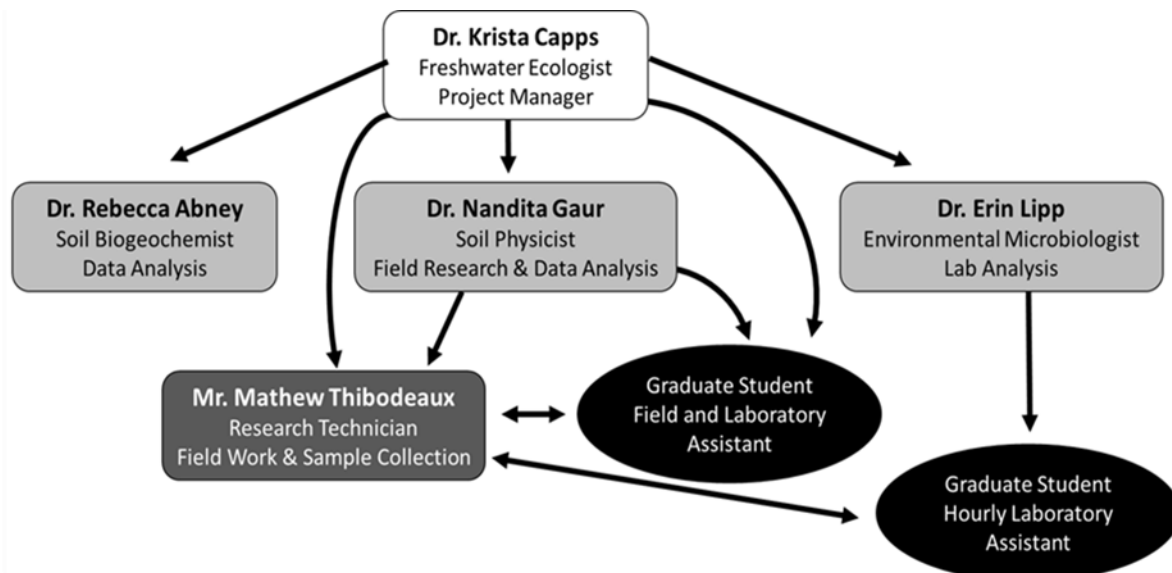
**Mr. Mathew Thibodeaux**

3403 Miller Plant Sciences  
University of Georgia  
120 Carlton St.  
Athens, GA, 30602

**Education and Experience:** Mr. Thibodeaux received his Bachelor of Science in Forest Science with a concentration in Hydrology in 2016 from the Warnell School of Forestry and Natural Resources from University of Georgia. He then worked as a Research Assistant in the Department of Crop and Soil Sciences in until 2017 before being promoted to Research

Technician and Research Professional. He is trained in wet lab work, stream water, and soil sampling and ArcGIS based analysis. Specific to this project, he has worked on two septic system related projects. The first was on the Lake Lanier Study (funded by Gwinnett Department of Water Resources) where he developed, installed, and monitored a network of 100+ groundwater observation wells inside of a septic tank effluent plume and collected samples for analyzing pH, DO, conductivity, nitrogen, phosphorus and e-coli following established standard protocols, took soil samples at depths of up to 6m, managed 6 runoff sites and collected runoff samples using ISCO's for measuring the various analytes mentioned above, opened and sampled directly from septic tanks and performed routine measurements of soil conductivity using an ERT instrument. He has also worked on a *Septic Sitter project (funded by the National Science Foundation)* where he coordinated with installers on installation of a septic tank riser, located septic tank drain lines, installed ultrasound sensors in drain lines of multiple sites, installed ultrasound sensors in septic tank, connected all sensors to a dedicated, cloud-capable monitoring platform.

**Graduate Student Researchers:** Graduate researchers will be selected from within the Odum School of Ecology and the School of Public Health. The students will be selected based on their previous field and lab experience and their willingness to learn geospatial statistics to analyze the data collected from this project and the previous dry-weather sampling effort. If it is of interest to the Metro District, the data from this project could be incorporated into a thesis or dissertation. However, this is not our expectation. The graduate student workers will support field and lab work associated with the project. They will be directly supervised by Drs Capps and Gaur or Dr. Lipp. Their time will be managed by Mr. Thibodeaux (ovals; Figure 2).



**Figure 5 FLOW CHART OF TEAM ORGANIZATIONAL STRUCTURE.** The project manager will oversee all aspects of the project and be responsible for preparing final reports. Mr. Thibodeaux's work will be overseen by Drs. Capps and Gaur. The unnamed graduate students will be based in the Odum School of Ecology or the Department of Environmental Health Science. The graduate students will be managed by Drs. Capps and Gaur or Dr. Lipp and will coordinate research activities with Mr. Thibodeaux.

## Analytical Facilities at the University of Georgia

**The Center for Applied Isotope Studies (CAIS)** was originally funded through a grant through the National Science Foundation and was designed to handle large sample sets (100s of samples per day) of water, soil, and plant and animal tissues with high degree of precision and accuracy. This facility caters to active research in ecology and has processed thousands of samples from various research centers. The CAIS has increased its capacity by adding equipment and a staff of dedicated chemists for the services offered. The lab is fully equipped to prepare, process, and analyze stable isotope, nutrient, and organic carbon samples. It maintains two Shimadzu TOC-5000A for DOC analysis, multiple Carlo-Erba NA1500 mass-spectrophotometers for stable isotope analysis (C, N, and S capabilities), Costech C and N elemental combustion analyzers, a Shimadzu AA-6800 atomic absorption spectrophotometer, and multiple Alpkems for water nutrient analysis. The nitrogen and phosphorus samples collected for this project was analyzed through CAIS and overseen by Dr. Krista Capps.

**The Georgia Genomics and Bioinformatics Core (GGBC)** is the University of Georgia (UGA) core laboratory for nucleic acid sequencing and bioinformatics. The mission includes research support, education, and training. The services of the GGBC services encompass the range of genomic techniques and applications, sequencing technologies, and bioinformatics analyses. GGBC operates multiple platforms for short-, long-, and single-molecule sequencing reads (i.e., Illumina MiSeq and NextSeq, PacBio Sequel, and Oxford Nanopore Minlon). They also have the ability to analyze samples using the droplet digital polymerase chain reaction (ddPCR) provides high-precision, absolute quantification of nucleic acid target sequences. With extensive applications in both research and clinical diagnostics, ddPCR is an ultrasensitive approach while simultaneously removing PCR bias, and the need for standard curves. The ddPCR samples collected for this project were analyzed through GGBC and overseen by Dr. Erin Lipp.

## Results

### Overview of Data Products

#### RAW DATA ([Link](#)):

Please note, sample sizes are different among the four watersheds. We ended up shifting focus to the higher-density watersheds in 2023, so Honey and Little Stone Mountain have more sampling events than the other two watersheds.

- Honey Creek
- Little Stone Mountain
- Pond Creek
- West Fork Little River

#### GEOSPATIAL ANALYSIS

- New geospatial analysis of land use/land cover in the subwatersheds ([Link](#))
- Revision of initial geospatial analysis completed in first study to address issues ([Link](#))

#### DATA VISUALIZATION AND STATISTICAL OUTPUT ([Link](#))

- Box plots of all wet weather events plotted per watershed.
- Dot plots of all dry and wet weather events plotted per event and watershed.
- Hydrographs showing grab vs automated sample comparisons
- Tables with outputs from the Wilcoxon signed-rank tests
- Person correlation plots for the different variables

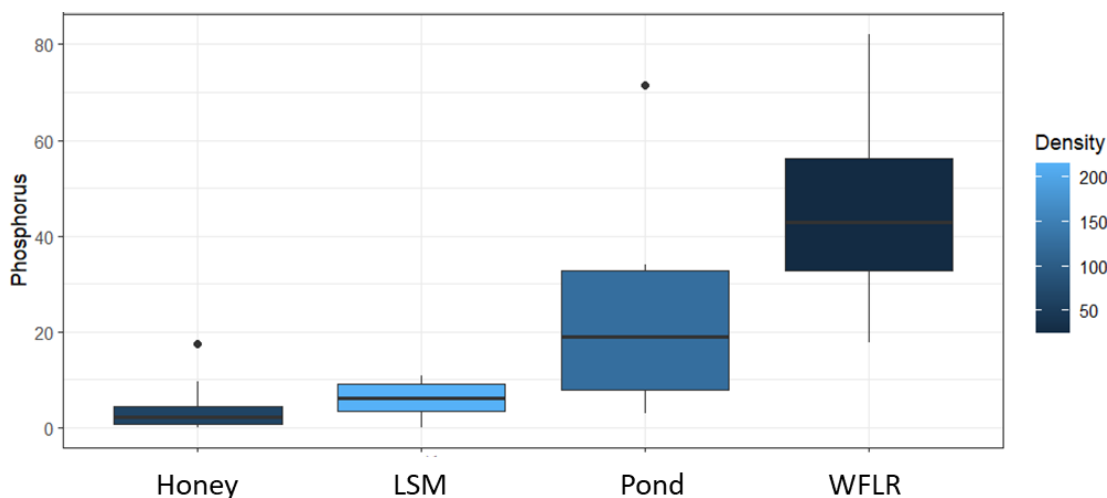
## Results: Nutrient Data

The Wilcoxon tests, which compare median values within sites, revealed distinct patterns in phosphorus, ammonium, and nitrate concentrations during wet and dry weather events within watersheds (Table 2).

**Table 2:** Wilcoxon signed-rank tests to assess differences in wet weather versus dry weather conditions for nutrients. If the p-value is low (typically less than 0.05), you can conclude that there's a significant difference between the paired observations. Relationships that are statistically significant are highlighted in yellow. These differences signal that within a watershed, there was a significant difference between conditions in wet vs. dry weather.

<i>T-Test: Wet vs. Dry (<math>\alpha = 0.05</math>)</i>			
<i>Watershed</i>	<i>Phosphorus</i>	<i>Ammonium</i>	<i>Nitrate</i>
Little Stone Mountain	<0.05	<0.05	<0.05
Honey	<0.05	<0.05	0.16
Pond	<0.05	<0.05	0.08
West Fork	<0.05	<0.05	0.17

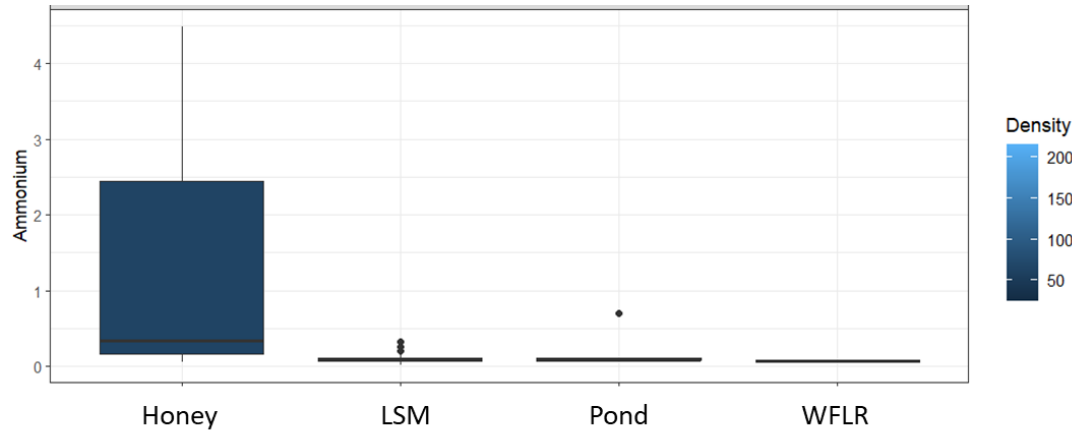
Collectively, wet weather events consistently exhibited higher levels of phosphorus and ammonium. Additionally, in the specific context of Little Stone Mountain, wet weather samples exhibited significantly elevated nitrate concentrations. These results emphasize the influence of weather conditions on nutrient concentrations in different watersheds, with notable variations observed in phosphorus, ammonium, and nitrate levels during wet weather events (Figures 2-4). However, there were no obvious effects of septic density on patterns in nutrient concentrations.



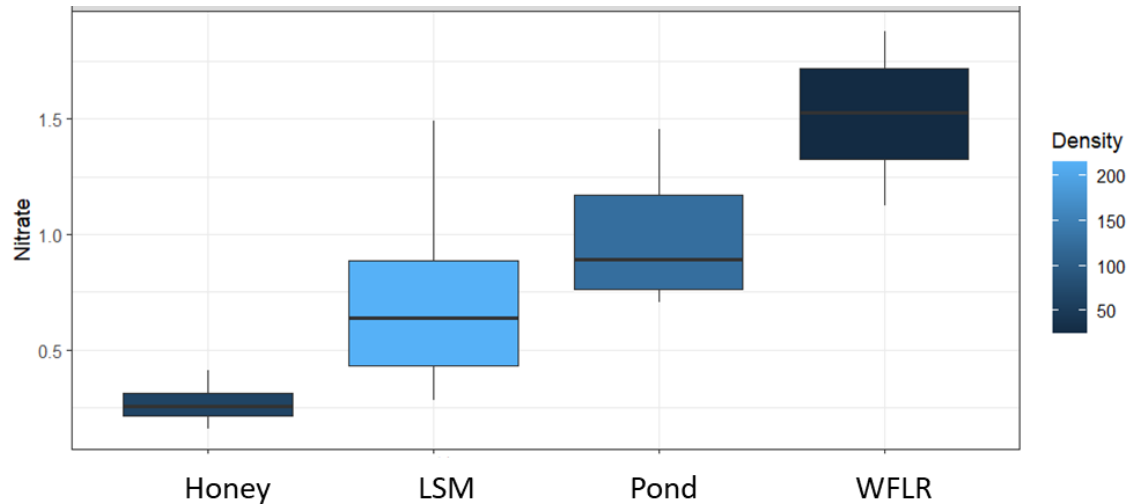
**Figure 6:** Box plots of phosphorus concentrations (ug/L) during wet weather events. Septic density in the watershed is emphasized with color, with lighter colors indicating greater septic density.

## Results: Fecal Markers

In the study, analyses of wet and dry weather events revealed a consistent trend of elevated fecal coliform concentrations during wet weather events. Specifically, wet weather samples exhibited higher concentrations of crAssphage in Honey ( $p = 0.02834$ ) and Pond ( $p = 0.03963$ ).



**Figure 7:** Box plots of ammonium concentrations (mg/L) during wet weather events. Septic density in the watershed is emphasized with color, with lighter colors indicating greater septic density.

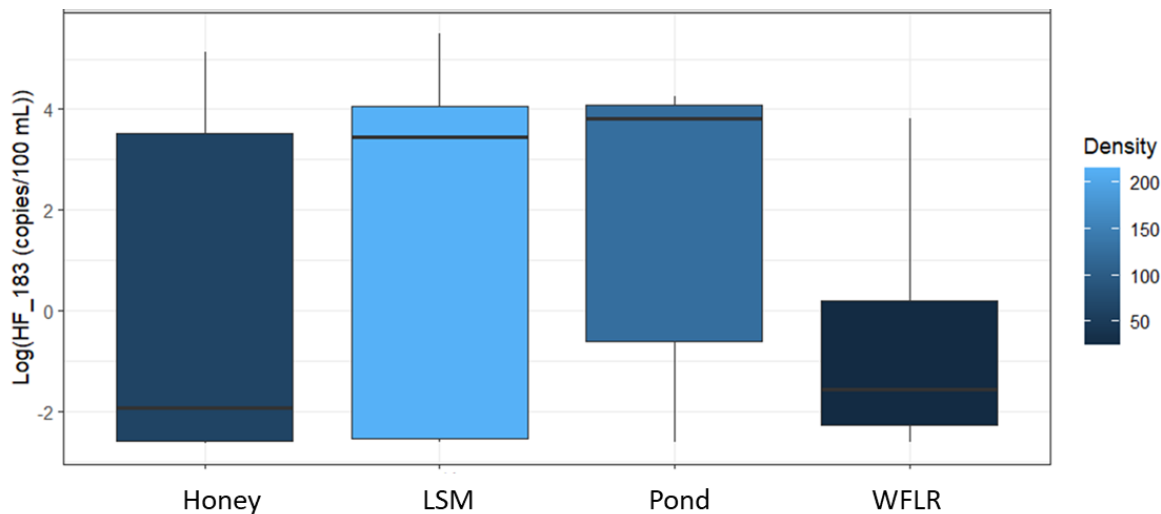


**Figure 8:** Box plots of nitrate concentrations (mg/L) during wet weather events. Septic density in the watershed is emphasized with color, with lighter colors indicating greater septic density.

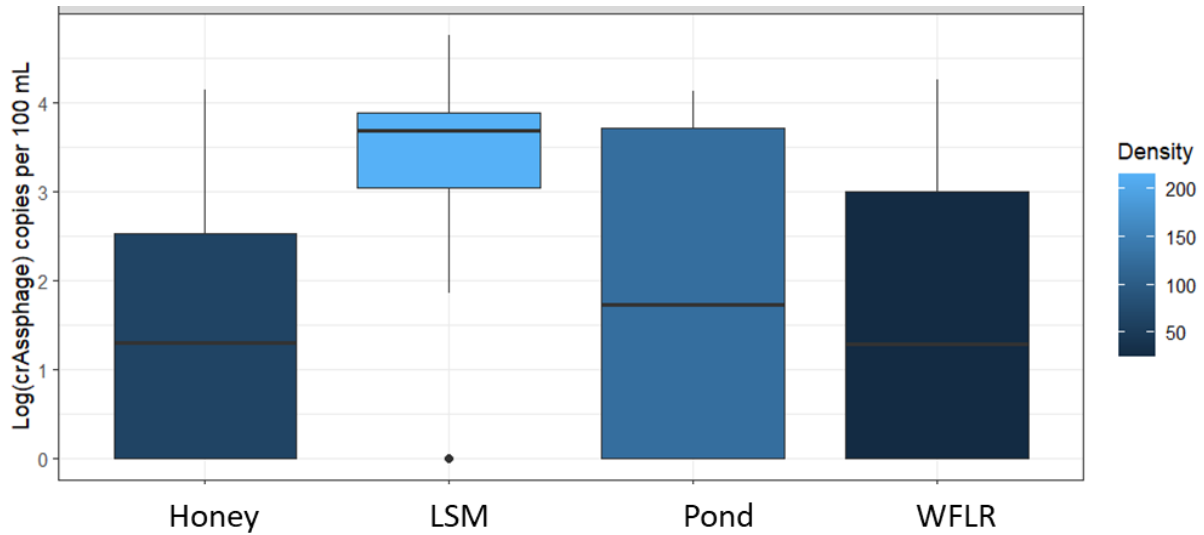
**Table 3:** Wilcoxon signed-rank tests to assess differences in wet weather versus dry weather conditions for fecal markers. If the p-value is low (typically less than 0.05), you can conclude that there's a significant difference between the paired observations. Relationships that are statistically significant are highlighted in yellow. These differences signal that within a watershed, there was a significant difference between conditions in wet vs. dry weather.

<i>T-Test: Wet vs. Dry (<math>\alpha = 0.05</math>)</i>			
<i>Watershed</i>	<i>Hf183</i>	<i>crAssphage</i>	<i>Fecal Coliform</i>
Little Stone Mountain	0.1075	0.1148	0.0003602
Honey	0.5877	0.02834	0.00007868
Pond	0.032	0.03963	0.0001077
West Fork	0.5989	0.1544	0.03077

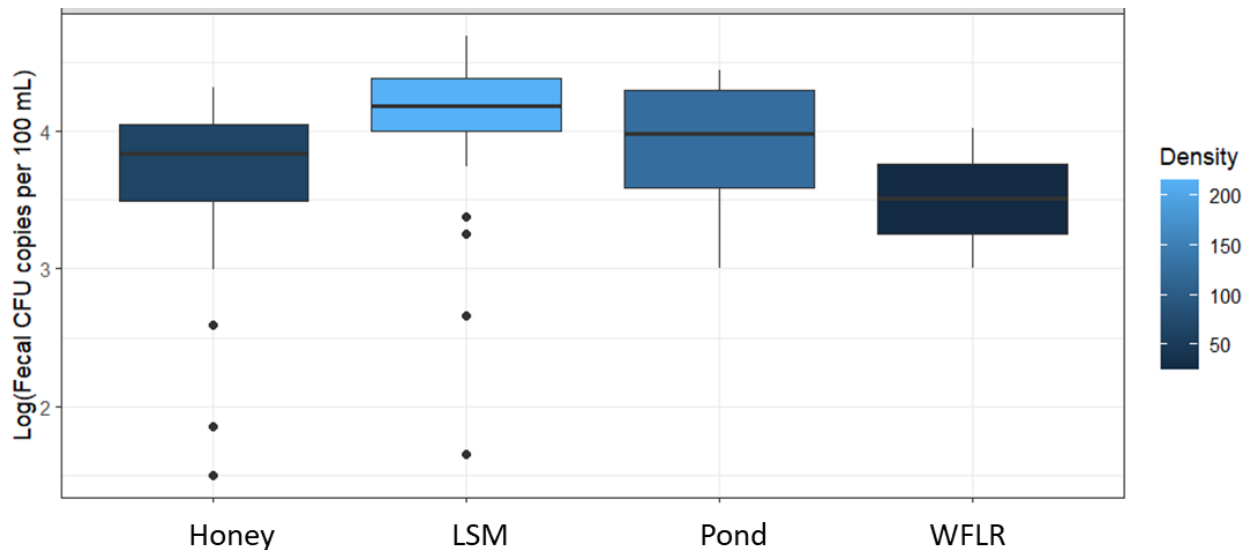
Furthermore, Pond wet weather samples demonstrated increased concentrations of HF183 ( $p = 0.032$ ). These findings underscore the impact of precipitation on fecal coliform levels, with notable variations observed in specific locations such as Honey and Pond during wet weather events (Figures 5-7).



**Figure 9:** Box plots of the human fecal marker (HF183) during wet weather events. Septic density in the watershed is emphasized with color, with lighter colors indicating greater septic density. The sample sizes vary substantially among watersheds (Table 1). Therefore, any perceived differences should be interpreted with caution.



**Figure 10:** Box plots of the human fecal marker, crAssphage, during wet weather events. Septic density in the watershed is emphasized with color, with lighter colors indicating greater septic density. The sample sizes vary substantially among watersheds (Table 1). Therefore, any perceived differences should be interpreted with caution.

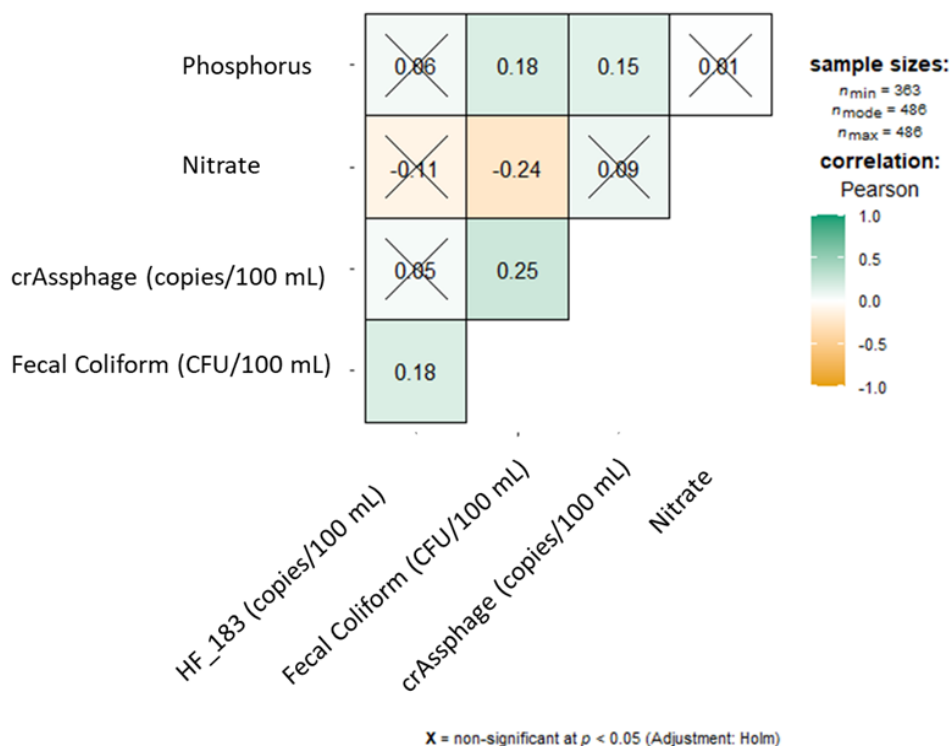


**Figure 11:** Box plots of the concentration of fecal coliform units, during wet weather events. Septic density in the watershed is emphasized with color, with lighter colors indicating greater septic density. The sample sizes vary substantially among watersheds (Table 1). Therefore, any perceived differences should be interpreted with caution.



## Results: Correlations among Variables

In the analysis that considered all of the data collected in wet and dry weather in both studies, we identified modest yet statistically significant correlations between some of the analytes. Notably, a negative correlation was observed between fecal coliform and nitrate. There was a weak but positive correlation noted between crAssphage and phosphorus. However, it's important to highlight that the correlations between bacterial and viral markers were not statistically significant. These findings highlight the complex relationships among the studied parameters (Figure 12).



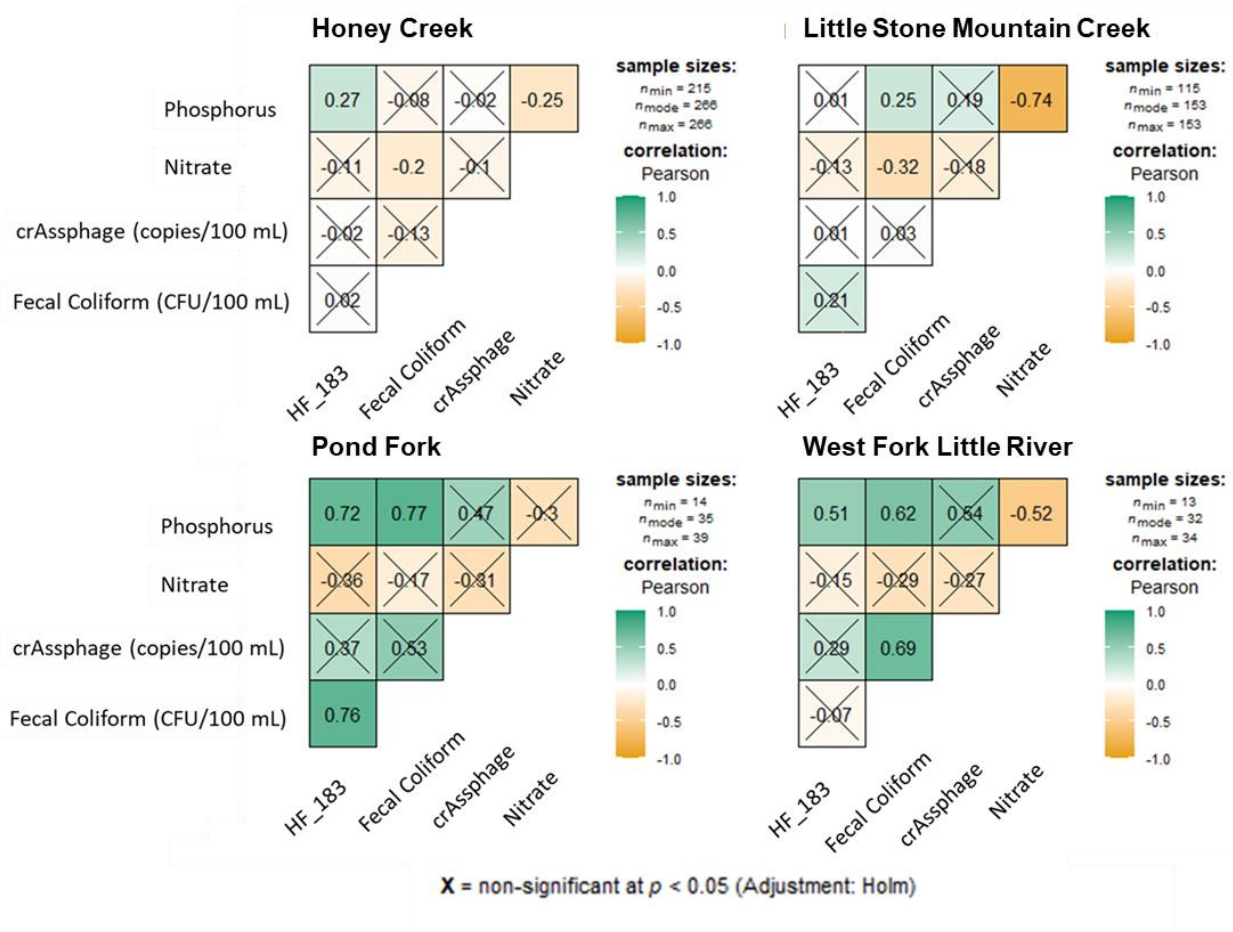
**Figure 12:** Pearson correlation analysis examining relationships between variables for all of the data collected in the 2019 and 2022/23 studies. Values closer to +1 indicate that there is a positive relationship, meaning that as one variable increases, the other tends to increase as well. Values closer to -1 indicate a negative linear relationship, suggesting that as one variable increases, the other tends to decrease.

The analysis produced several noteworthy correlations for within-watershed relationships between variables (Figure 13). Firstly, a negative correlation between nitrate and phosphorus was observed in the watersheds with the lowest and highest density of septic systems, suggesting that this relationship may not be density-driven. Secondly, in the lowest density watershed, fecal coliform displayed a strong positive correlation with the human fecal marker crAssphage, while in a relatively high-density watershed, it exhibited a similar correlation with HF183, underscoring the absence of clear relationships between analytes with septic system

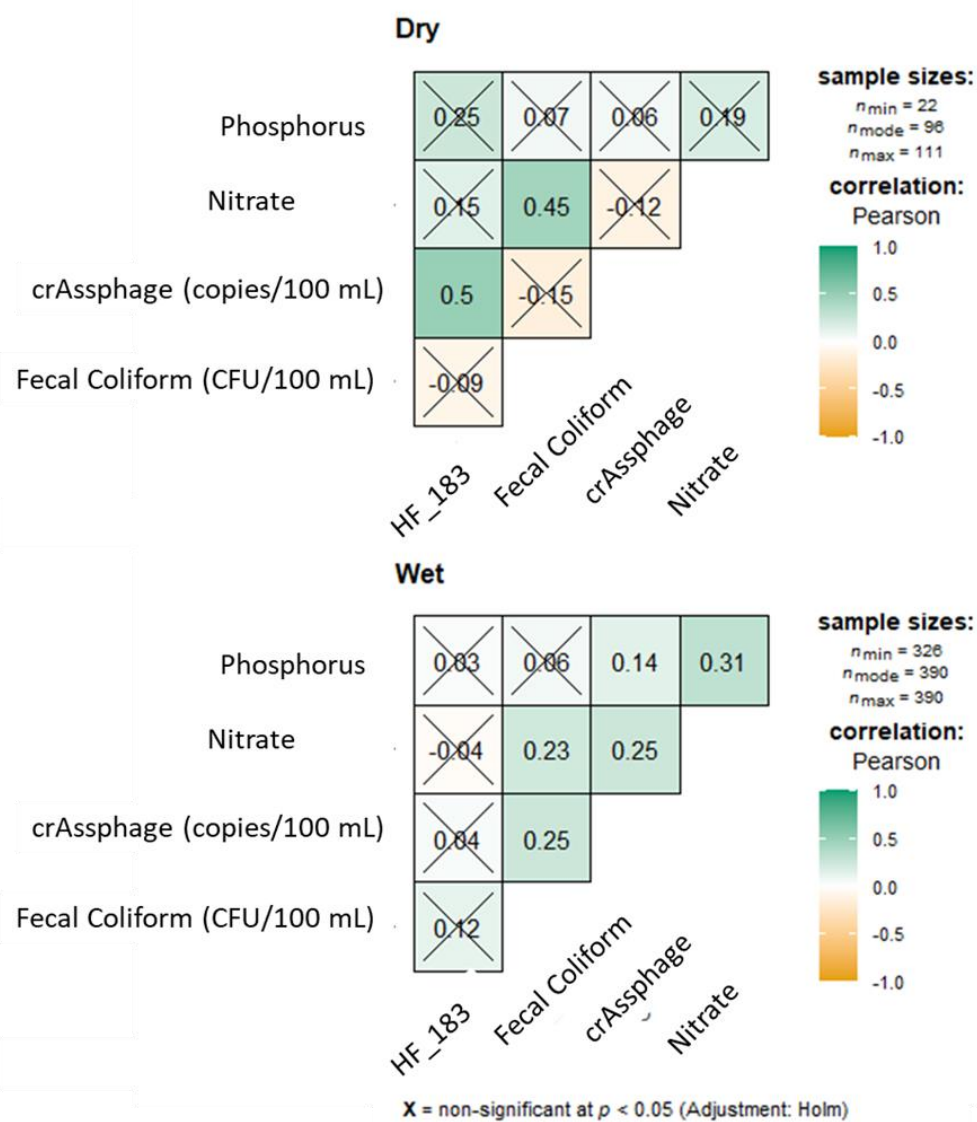
density in the watershed. Lastly, the correlations between human and viral markers of fecal contamination were generally weak and not statistically significant.

Because rain events influence the transport of pollutants overland and underground in different ways, we predicted there would be differences in correlations between variables between dry and wet weather events. During dry weather events, we expected there would be strong correlations between variables that are transported through groundwater, whereas we expected that variables that are often associated with soil or that dissolve in runoff would be strongly influenced by wet weather conditions. Our results partially supported these results (Figure 14). Unexpectedly, HF183 was not correlated with any other variable in wet weather. Conversely, fecal coliform and crAssphage were positively related in wet weather events, suggesting potential transport through runoff. In dry weather conditions, the viral and bacterial fecal indicators displayed statistically significant and positive correlations, a pattern not observed in wet weather. These findings highlight the dynamic and event-specific nature of correlations, emphasizing the importance of considering distinct weather conditions and local factors in understanding transport processes.

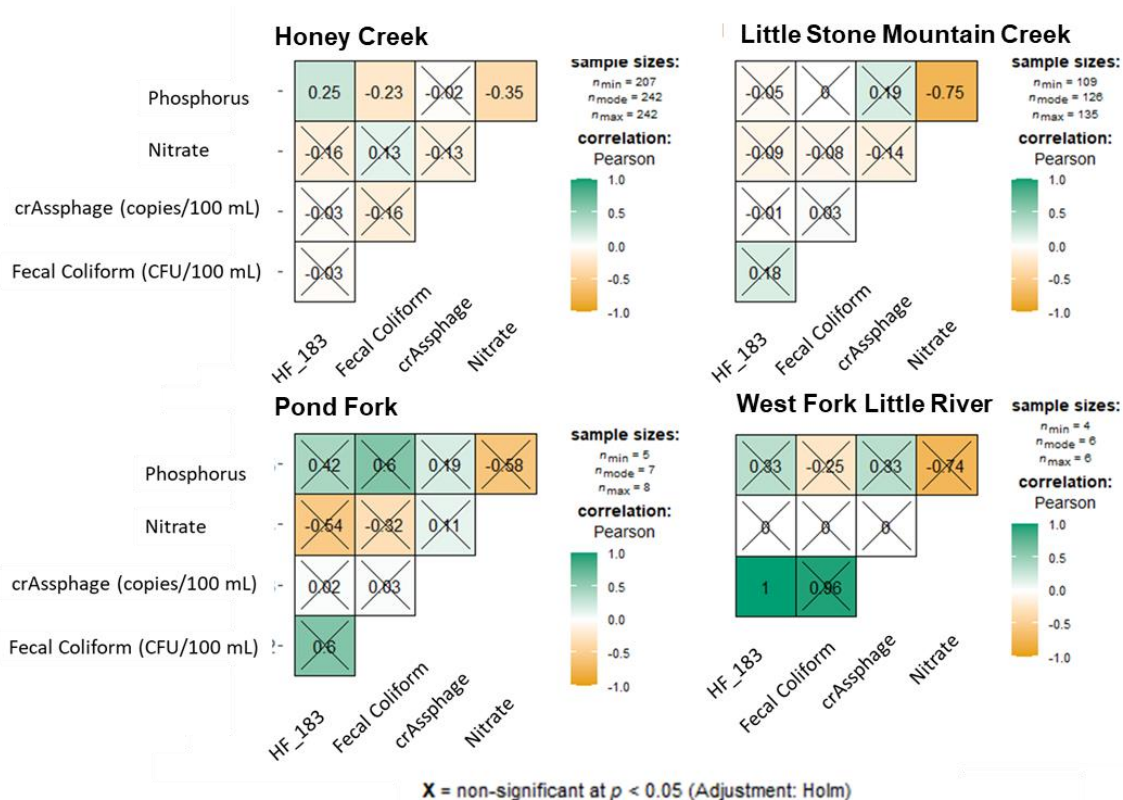
Relationships between variables was not only influenced by weather conditions, it was also site specific and relationships between variables changed among variables, highlighting the complex relationships between environmental conditions and septic infrastructure (Figures 15 & 16).



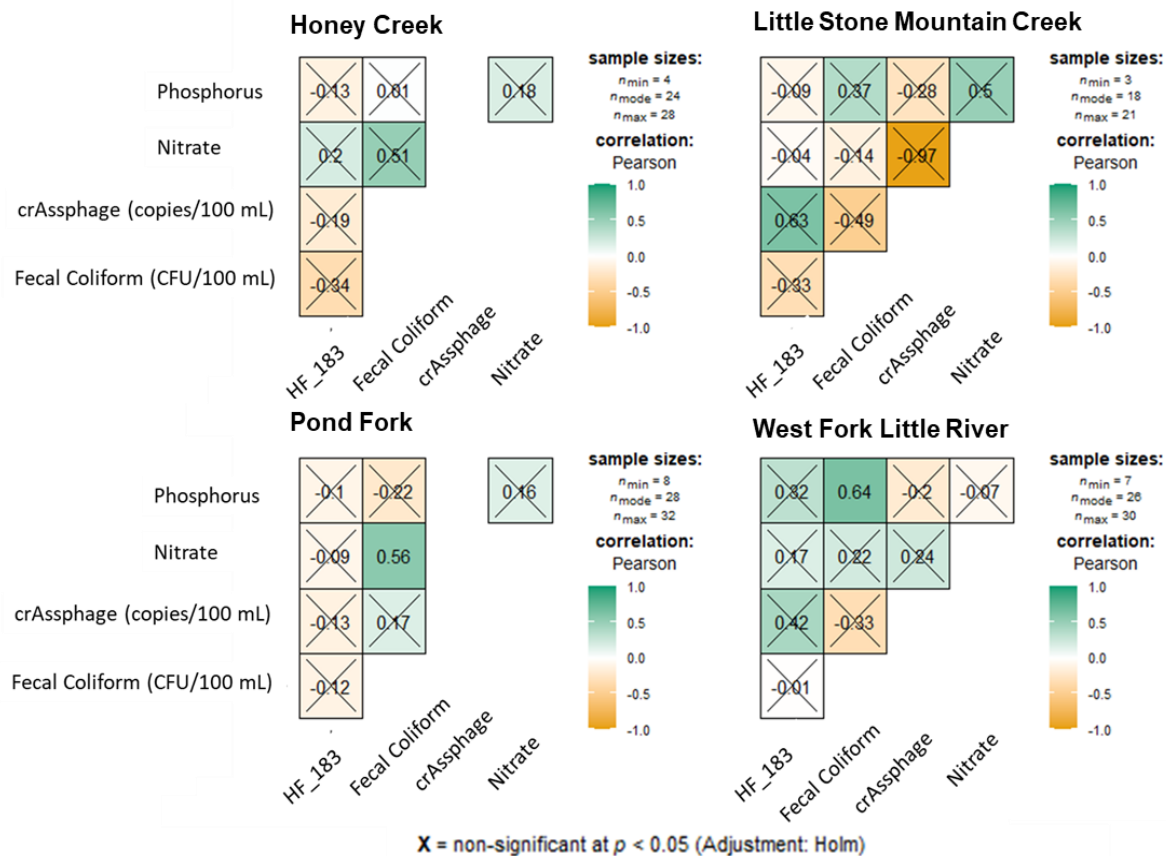
**Figure 13:** Pearson correlation analysis examining relationships between variables for all of the data collected in the 2019 and 2022/23 studies by watershed. Values closer to +1 indicate that there is a positive relationship, meaning that as one variable increases, the other tends to increase as well. Values closer to -1 indicate a negative linear relationship, suggesting that as one variable increases, the other tends to decrease.



**Figure 14:** Pearson correlation analysis examining relationships between variables for all of the data collected in the 2019 and 2022/23 studies by weather conditions. Values closer to +1 indicate that there is a positive relationship, meaning that as one variable increases, the other tends to increase as well. Values closer to -1 indicate a negative linear relationship, suggesting that as one variable increases, the other tends to decrease.



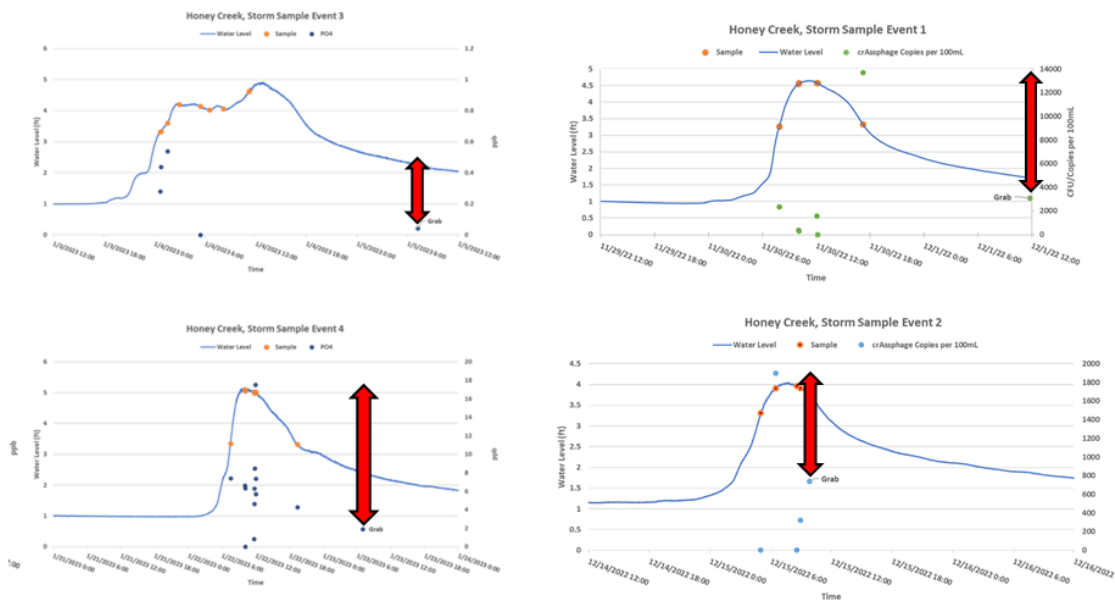
**Figure 15:** Pearson correlation analysis examining relationships between variables for all of the data collected in the 2019 and 2022/23 studies by watershed during wet weather. Values closer to +1 indicate that there is a positive relationship, meaning that as one variable increases, the other tends to increase as well. Values closer to -1 indicate a negative linear relationship, suggesting that as one variable increases, the other tends to decrease. Where values of the relationships could not be modeled using this approach, boxes remained empty.



**Figure 16:** Pearson correlation analysis examining relationships between variables for all of the data collected in the 2019 and 2022/23 studies by watershed during dry weather. Values closer to +1 indicate that there is a positive relationship, meaning that as one variable increases, the other tends to increase as well. Values closer to -1 indicate a negative linear relationship, suggesting that as one variable increases, the other tends to decrease. Where values of the relationships could not be modeled using this approach, boxes remained empty.

## Additional Observations

Though small sample sizes created a situation where we could not test the question statistically, we wanted to include some additional observations about Research Question 3 (If changes in water quality due to rainfall events are documented in the watershed, are they maintained long enough to forgo the need for immediate sample collection in response to changing discharge (i.e., ISCO samplers)?). We asked this question, in part, to support future decision-making and sample design for water quality monitoring programs. Our results suggest that monitoring efforts that do collect samples for chemistry and fecal markers during rain events as the hydrograph is rising and falling do generate different results than grab samples taken after the event. For example, there were large differences between the grab sample concentrations of analytes and the maximum value of each analyte collected along the hydrograph (Figure 17; Appendix E). Again, limited sample sizes precluded us from testing the statistical significance of the observed differences. However, if the observed difference is significant, it suggests that if environmental managers are concerned with spikes in concentrations of analytes, they may need to invest the relatively considerable resources needed to sample streams during rain events. During this study, water levels changed substantially (Figure 18; Appendix D and E) and during one of the rain events, one of the ISCO samplers was dragged downstream and up the bank. The sampling devices were buried multiple times and components of the sampling devices (e.g., batteries, solar panels) were stolen and lost. This was a valuable, but time intensive and resource intensive project.



**Figure 17:** Visual comparison of automated and grab samples during each sampling event. Information included in the figures includes the water level, the point in the hydrograph where the sample or samples were collected by the ISCO samplers, and the concentrations of the nutrients or human fecal markers collected during the storm event or in the grab sample collected after the storm event occurred. Red arrows indicate the differences between grab sample concentrations and the highest concentrations of an analyte during a particular rain event.





**Figure 18:** West Fork Little River during baseflow (top) and flooding (bottom). The red arrows indicate the same location in each photo. Dates and times of the images are denoted in white text.



## Conclusions and Recommendations

**Research Question 1:** Can we attribute declines in surface water quality with rainfall events in watersheds dominated by septic infrastructure and is there evidence that such declines are derived from human wastewater [i.e., increases in fecal coliform bacteria, the HF138 marker, and crAssphage (human fecal marker – viral)] using data from the 2019 study and this study?

- **Conclusions:**
  - Rain events were associated with declines in water quality across multiple variables, indicating potential issues with water contamination. The prevalence of fecal matter poses a widespread problem, although the data on human fecal contamination is not robust. Within watersheds, Wilcox results emphasize that wet weather exacerbates the deterioration of water quality. Despite the prevailing concerns about fecal contamination, there is insufficient evidence to support a density effect. It was only possible to analyze data at the watershed level, not the sub-watershed level, due to limitations in the number of ISCOs, highlighting a constraint in the granularity of the analysis.
- **Recommendations:**
  - The need for collecting additional data is underscored by the limitation of small sample sizes at the sub-watershed level. The current dataset lacks the granularity required for a more detailed analysis, emphasizing the importance of obtaining a larger and more diverse set of samples, especially from single sites with higher resolution. Increasing the volume of data at a finer scale can provide a more comprehensive understanding of the variables and their interrelationships, contributing to a more robust and nuanced interpretation of water quality dynamics.

**Research Question 2:** If increases in fecal coliform bacteria and the HF138 marker are detected, are they related to corresponding changes in nitrate-nitrite, ammonium, and phosphorus concentrations using data from the 2019 study and this study?

- **Conclusions:**
  - The correlation analyses revealed dynamic patterns that varied significantly by watershed, suggesting the influence of other governing processes. These variations were observed not only across different watersheds but also in response to different weather conditions. The findings emphasize the uniqueness of each watershed's dynamics and the impact of weather events, highlighting that there are no universal substitutes in understanding and interpreting the complex relationships within the studied processes.
- **Recommendations:**
  - Diverse and sometimes opposing relationships existed among variables in different watersheds and under various weather conditions in this study. Effectively monitoring and mitigating the impacts of failing septic infrastructure on surface water quality will likely require a site-specific approach until more data are generated to support effective modeling efforts. If understanding the impact of failing septic systems remains a concern to the District, we recommend investing in a longer-term (multi-year, continuous) monitoring program at multiple sites within one or two watersheds. If this effort is supported, managers should measure multiple water quality parameters across different weather conditions.

This initiative should involve automated samplers to gain a comprehensive understanding of interactions between long-term weather patterns and septic system behavior under different weather conditions and flood conditions. Concurrent efforts should focus on acquiring detailed information about septic infrastructure and maintenance records in the watershed. Collecting extensive data could facilitate more nuanced modeling efforts and a deeper comprehension of the relationships between septic infrastructure and environmental variables. This comprehensive approach may clarify some of the conflicting relationships identified in this study and yield results applicable to other watersheds.

If this recommendation is supported, we suggest the District should target watersheds that include stream reaches on the 303d list--reaches that violate Total Maximum Daily Load (TMDL) regulations. Even if the findings are not universally applicable to other watersheds, supporting improved surface water quality in these reaches may be worthwhile for District efforts to improve water quality.

**Research Question 3: If changes in water quality due to rainfall events are documented in the watershed, are they maintained long enough to forgo the need for immediate sample collection in response to changing discharge (i.e., ISCO samplers)?**

- **Conclusions:**
  - Changes in water quality resulting from rainfall events exhibit heterogeneity along the hydrograph, varying as water levels rise and fall in response to precipitation. However, these changes are not sustained for an extended period, emphasizing the importance of immediate sample collection in response to changing discharge. This underscores the necessity of sampling during the storm to accurately estimate the impacts of decentralized infrastructure on water quality, capturing the transient and dynamic nature of these effects.
- **Recommendations:**
  - Our findings indicate that if a particular water quality parameter is anticipated to exert significant acute effects on aquatic biota, such as elevated concentrations of salt or metals, or potentially pose substantial risks to human health, as in the case of untreated wastewater, it is advisable for managers to conduct water quality sampling in response to rising water flow triggered by precipitation. Nevertheless, the challenges faced by our team in consistently maintaining sampling equipment after minor precipitation events, coupled with equipment failure and losses during severe floods, underscore the exceptional cost associated with this type of sampling.
  - Natural resource managers seeking guidance on monitoring water quality for decentralized wastewater or other forms of non-point source pollution should consider contacting local resources such as county extension agents or university centers like the River Basin Center at the University of Georgia. While not guaranteed, researchers may be able to use their expertise to assist in planning. If time or expertise is lacking, they may be able to help identify suitable experts for support. As the land-grant institution for the state, the University of Georgia has a formal responsibility to use its personnel and resources to enhance life in Georgia, extending its tradition of service into the 21st century and beyond.

**Research Question 4:** If additional system specific data (e.g., exact location, age, maintenance records, etc.) are integrated into the data collected from the 2019 study and analyzed using our proposed methods, what else can we learn about the relationships between surface water quality parameters and septic infrastructure from the data collected in that study?

- ***Conclusions:***

- Even with the incorporation of additional system-specific data into the datasets collected through both septic projects and their analysis using our proposed methods, consistent relationships between surface water quality parameters and septic infrastructure proved elusive. This limitation primarily stemmed from the constraint of having only one automatic sampler in each of the sampled watersheds due to equipment cost, preventing the examination of environmental variables (both water quality and land cover variables) at the subwatershed level. Our inquiries were consequently restricted to the watershed level and limited the power of our analyses. Although we allocated project funds to acquire one additional ISCO sampler following the destruction of our original sampler in a flood, the other three ISCOs used were pre-existing equipment in our labs, highlighting the financial challenges associated with sub-watershed level research. It is crucial to acknowledge that, despite the inability to discern patterns due to limited data, the results from our study suggest that septic density did not consistently account for changes in water quality observed in both wet and dry weather conditions.

- ***Recommendations:***

- The complex and varied relationships among environmental variables in different weather conditions and across diverse watersheds suggest if the District hopes to ascertain general patterns in how septic density influences water quality, it should consider supporting projects that gather water quality samples with increased spatial and temporal resolution using automatic samplers in multiple locations within various watersheds during storm events. Our research underscores the considerable expenses and time commitments associated with such endeavors. An alternative strategy might involve evaluating the impact of septic infrastructure on water quality issues as they arise, focusing specifically on watersheds with high densities of older systems exhibiting poor water quality characterized by elevated concentrations of human fecal markers.

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# Appendices

# **Appendix A:**

## **Geospatial Data**

Sub-Watershed	z_m	slope_deg	slope_per	d2_m	hd2_m	ha_m	hha_m	YRBUILT	wsl_per	hwsl_per	Age
H1	253.68	3.78	6.61	298.85	462.33	13.94	18.09	1987	0.0566	0.0511	35
H2	262.23	3.42	5.99	325.53	509.67	11.86	1684.27	1982	0.0441	0.0411	40
H3	255.83	3.76	6.58	304.95	465.43	13.72	17.87	1986	0.0537	0.0488	36
H4	265.57	3.24	5.66	243.96	544.18	9.74	18.37	1977	0.0471	0.0440	45
LSM 1	307.38	5.09	8.96	411.28	598.62	20.78	24.63	1977	0.0607	0.0488	45
LSM 2	319.21	4.37	7.80	327.95	421.65	17.44	17.03	1969	0.0554	0.0429	53
LSM 3	313.15	4.71	8.30	383.33	524.47	18.83	20.37	1978	0.0587	0.0470	44
P1	304.74	4.76	8.35	201.07	374.85	14.71	20.91	1991	0.0821	0.0694	31
P2	329.41	5.13	8.86	279.02	451.39	18.82	24.28	1980	0.0683	0.0580	42
P3	307.08	4.89	8.57	196.67	366.16	14.77	21.36	1991	0.0836	0.0712	31
P4	312.32	4.93	8.65	197.17	396.99	13.76	22.54	1989	0.0787	0.0685	33
W1	396.66	5.79	10.20	256.17	437.15	20.01	25.35	1988	0.0973	0.0777	34
W2	430.88	6.10	10.73	237.51	451.05	22.57	29.18	1988	0.1040	0.0772	34
W3	401.82	4.85	8.54	269.18	439.08	18.10	23.64	1987	0.0764	0.0624	35
W4	415.39	5.34	9.40	285.82	492.96	22.40	26.72	1989	0.0904	0.0667	33

Sub-Watershed	DA	n	nO25	nO35	nO45	Density	Den_O25	Den_O35	Den_O45	TSL	TRL
H1	48.00	3102	2115	1367	931	64.77	44.16	28.54	19.44	51.7	282.4
H2	7.00	985	832	595	442	134.99	114.02	81.54	60.57	8.2	35.7
H3	40.00	2625	1848	1194	846	64.85	45.65	29.50	20.90	37.8	253.8
H4	10.00	220	168	154	135	21.02	16.05	14.71	12.90	6.2	131.4
LSM 1	5.66	1219	1106	992	725	215.45	195.47	175.33	128.14	3.90	34.95
LSM 2	0.53	100	99	87	86	189.22	187.33	164.62	162.73	0.24	3.91
LSM 3	3.18	631	541	463	406	198.29	170.01	145.50	127.59	2.31	19.23
P1	13.76	1743	899	587	406	126.69	65.35	42.67	29.51	22.74	46.28
P2	1.06	86	77	69	37	80.82	72.36	64.84	34.77	1.03	2.57
P3	10.34	1592	817	535	369	153.92	78.99	51.73	35.68	17.17	40.19
P4	4.55	872	492	353	277	191.75	108.19	77.62	60.91	6.67	18.10
W1	53.92	1389	814	562	356	25.76	15.10	10.42	6.60	77.97	143.74
W2	15.02	210	125	76	52	13.99	8.32	5.06	3.46	26.64	28.60
W3	8.48	206	135	85	55	24.29	15.92	10.02	6.49	9.82	22.17
W4	28.23	509	272	185	139	18.03	9.64	6.55	4.92	42.04	68.30



Sub-Watershed	Open Water	Perennial	Dev_Open	Dev_Low	Dev_Med	Dev_High	Barren	Deciduous	Evergreen	Mixed Forest	Shrub
H1	0.91	0.00	13.61	12.88	4.23	2.35	1.07	24.46	21.59	3.86	0.31
H2	0.47	0.00	17.30	21.70	2.38	0.52	0.06	20.71	18.39	4.03	0.27
H3	0.95	0.00	14.24	14.12	4.87	2.78	1.26	23.66	20.26	3.54	0.35
H4	0.57	0.00	16.20	18.57	14.21	9.82	2.88	12.99	13.99	1.31	0.11
LSM 1	0.19	0.00	33.38	17.48	0.54	0.03	0.06	17.75	19.40	10.38	0.13
LSM 2	0.00	0.00	31.12	8.33	0.17	0.00	0.00	19.22	33.84	6.97	0.00
LSM 3	0.14	0.00	31.79	16.91	0.34	0.03	0.11	18.44	21.65	9.88	0.23
P1	0.07	0.00	16.45	10.29	2.03	0.54	0.18	41.73	1.90	7.70	0.59
P2	0.00	0.00	14.33	10.90	9.97	3.10	1.84	29.92	0.17	3.77	3.86
P3	0.07	0.00	18.84	11.90	2.51	0.60	0.23	43.25	1.99	7.12	0.72
P4	0.02	0.00	20.41	12.16	3.22	0.73	0.45	46.89	0.79	5.18	0.91
W1	0.21	0.00	10.01	3.05	0.62	0.14	0.06	42.39	1.92	8.10	0.53
W2	0.10	0.00	6.47	0.76	0.28	0.05	0.07	51.33	2.09	7.02	0.13
W3	0.24	0.00	11.00	2.60	0.25	0.03	0.03	33.60	3.62	10.11	1.05
W4	0.09	0.00	7.90	1.65	0.36	0.13	0.09	44.09	1.83	8.11	0.39

Sub-Watershed	Grassla0	Pasture	Cultivated	Woody We	Herbaceous Wetla0
H1	1.72	11.21	0.00	1.73	0.05
H2	0.47	13.33	0.00	0.37	0.00
H3	1.94	10.20	0.00	1.78	0.06
H4	1.76	6.40	0.00	1.06	0.15
LSM 1	0.21	0.41	0.00	0.03	0.00
LSM 2	0.00	0.34	0.00	0.00	0.00
LSM 3	0.17	0.28	0.00	0.03	0.00
P1	0.86	15.57	0.00	1.97	0.11
P2	0.75	19.95	0.00	1.42	0.00
P3	1.10	11.09	0.00	0.44	0.12
P4	0.79	8.09	0.00	0.34	0.00
W1	2.10	30.15	0.69	0.03	0.00
W2	2.41	29.25	0.00	0.04	0.00
W3	2.13	35.32	0.00	0.00	0.00
W4	2.57	31.40	1.33	0.05	0.00

WS	WSn	z_m	slope_deg	slope_per	d2_m	hd2_m	ha_m	hha_m	YRBUILT	wsl_per	hwsl_per	Age
<i>Fourmile</i>	1	370.15	5.05	8.86	276.63	507.39	16.73	21.47	1994	0.0812	0.0614	28
<i>Fourmile</i>	2	395.65	5.54	9.75	215.68	440.85	21.06	27.70	1989	0.1017	0.0716	33
<i>Fourmile</i>	3	389.83	5.66	9.94	218.77	374.36	17.86	22.48	1991	0.0955	0.0722	31
<i>Fourmile</i>	4	379.79	5.12	8.96	244.14	399.21	16.59	20.55	1992	0.0867	0.0675	30
<i>Fourmile</i>	5	382.22	4.16	7.27	311.32	490.91	15.80	20.48	1988	0.0675	0.0527	34
<i>Panther</i>	1	259.49	4.90	8.59	203.71	332.07	12.62	17.22	1990	0.0760	0.0694	32
<i>Panther</i>	2	266.87	4.64	8.08	254.08	438.67	13.67	19.01	1996	0.0647	0.0572	26
<i>Panther</i>	3	260.18	5.11	8.97	198.99	344.29	12.52	17.29	1996	0.0800	0.0721	26
<i>Stamp</i>	1	322.50	4.82	8.48	247.73	467.65	14.63	21.53	1980	0.0882	0.0769	42
<i>Stamp</i>	2	320.65	4.08	7.20	309.29	558.16	12.20	18.10	1985	0.0413	0.0337	37
<i>WhiteOak</i>	1	259.27	4.28	7.50	400.65	828.30	15.48	21.33	1975	0.0488	0.0359	47
<i>WhiteOak</i>	2	264.72	4.36	7.69	324.58	585.50	15.12	19.05	1972	0.0548	0.0412	50
<i>WhiteOak</i>	3	255.52	4.12	7.20	460.65	987.24	15.88	23.30	1979	0.0418	0.0301	43
<i>WhiteOak</i>	4	269.49	4.42	7.80	335.97	614.50	14.76	18.89	1974	0.0526	0.0392	48
<i>Byrd</i>	1	386.19	5.96	10.54	232.27	375.99	19.57	26.46	1985	0.1013	0.0861	37
<i>Byrd</i>	2	398.71	6.06	10.69	229.50	331.73	21.79	26.81	1988	0.1137	0.0967	34

*The numbers represent summaries of the entire area within the watershed that is upstream of that location.*

Note: There was a discrepancy between the point on the map (which showed this sampling point on a tributary) and the coordinate provided on the report. The geospatial analysis for this location has been done based on the coordinates provided in the report and hence consider this point on the main channel. The numbers reflect the same.

WS	WSn	DA	n	n025	n035	n045	Density	Den_025	Den_035	Den_045	TSL	TRL
<i>Fourmile</i>	1	20.58	757	333	160	84	36.78	16.18	7.77	4.08	28.52	60.84
<i>Fourmile</i>	2	3.57	35	22	15	7	9.82	6.17	4.21	1.96	5.40	7.03
<i>Fourmile</i>	3	11.07	202	107	69	40	18.26	9.67	6.24	3.61	16.19	32.90
<i>Fourmile</i>	4	17.46	407	212	118	63	23.32	12.14	6.76	3.61	24.50	46.25
<i>Fourmile</i>	5	3.91	118	76	46	23	30.15	19.42	11.75	5.88	3.89	10.18
<i>Panther</i>	1	11.48	263	134	72	53	22.91	11.67	6.27	4.62	17.15	17.52
<i>Panther</i>	2	1.26	55	26	8	5	43.49	20.56	6.33	3.95	1.65	3.71
<i>Panther</i>	3	2.73	174	67	16	12	63.62	24.50	5.85	4.39	3.79	8.48
<i>Stamp</i>	1	46.26	74	51	35	22	1.60	1.10	0.76	0.48	71.49	66.95
<i>Stamp</i>	2	29.04	24	8	6	6	0.83	0.28	0.21	0.21	40.63	30.63
<i>WhiteOak</i>	1	41.66	326	263	206	121	7.82	6.31	4.94	2.90	41.26	74.34
<i>WhiteOak</i>	2	23.75	188	157	133	76	7.92	6.61	5.60	3.20	25.20	38.64
<i>WhiteOak</i>	3	9.38	89	70	52	36	9.49	7.46	5.55	3.84	9.28	17.63
<i>WhiteOak</i>	4	15.53	134	109	90	56	8.63	7.02	5.79	3.60	16.22	26.05
<i>Byrd</i>	1	8.15	136	78	58	34	16.70	9.58	7.12	4.17	14.19	17.09
<i>Byrd</i>	2	4.33	70	32	25	13	16.17	7.39	5.77	3.00	8.03	7.73

WS	WSn	Open Water	Perennial Snow/Ice	Dev-Open	Dev-Low	Dev-Med	Dev-High	Barren	Deciduous	Evergreen	Mixed Forest	Shrub
<i>Fourmile</i>	1	0.10	0.00	14.12	4.97	0.70	0.11	0.05	38.96	1.04	5.94	0.30
<i>Fourmile</i>	2	0.15	0.00	20.19	3.41	0.63	0.08	0.00	27.39	0.71	3.28	0.08
<i>Fourmile</i>	3	0.10	0.00	14.03	4.59	0.85	0.07	0.07	38.08	0.54	5.29	0.31
<i>Fourmile</i>	4	0.10	0.00	12.36	3.69	0.65	0.06	0.06	40.34	1.01	5.95	0.25
<i>Fourmile</i>	5	0.16	0.00	10.34	1.84	0.39	0.09	0.05	31.68	2.21	6.38	0.14
<i>Panther</i>	1	0.92	0.00	3.11	1.76	0.03	0.02	0.01	30.87	34.75	5.70	1.94
<i>Panther</i>	2	0.85	0.00	2.49	4.98	0.14	0.14	0.00	22.67	24.95	3.13	12.94
<i>Panther</i>	3	1.02	0.00	4.74	4.61	0.07	0.07	0.00	27.11	28.43	4.58	7.94
<i>Stamp</i>	1	0.02	0.00	1.15	0.10	0.00	0.00	0.00	37.43	24.53	30.71	1.99
<i>Stamp</i>	2	0.00	0.00	0.19	0.02	0.00	0.00	0.00	37.72	22.70	33.58	2.39
<i>WhiteOak</i>	1	1.00	0.00	4.09	0.78	0.01	0.00	0.03	43.75	18.65	7.44	1.43
<i>WhiteOak</i>	2	0.98	0.00	4.44	1.00	0.01	0.00	0.05	42.87	19.24	7.52	1.31
<i>WhiteOak</i>	3	1.84	0.00	3.26	0.50	0.01	0.00	0.01	45.25	20.39	8.88	0.90
<i>WhiteOak</i>	4	1.20	0.00	4.87	0.89	0.01	0.00	0.04	38.29	22.24	7.95	0.77
<i>Byrd</i>	1	0.19	0.00	5.67	1.28	0.10	0.03	0.08	43.12	13.98	16.35	0.94
<i>Byrd</i>	2	0.31	0.00	5.11	1.70	0.15	0.06	0.00	43.16	14.54	18.23	0.54

WS	WSn	Grassland	Pasture	Cultivated	Woody Wetland	Herbaceous Wetland
<i>Fourmile</i>	1	1.18	32.04	0.00	0.45	0.05
<i>Fourmile</i>	2	1.72	42.37	0.00	0.00	0.00
<i>Fourmile</i>	3	1.15	34.93	0.00	0.00	0.00
<i>Fourmile</i>	4	0.86	34.49	0.00	0.17	0.00
<i>Fourmile</i>	5	0.32	46.41	0.00	0.00	0.00
<i>Panther</i>	1	4.46	11.99	0.00	4.26	0.19
<i>Panther</i>	2	6.89	20.82	0.00	0.00	0.00
<i>Panther</i>	3	3.79	16.80	0.00	0.86	0.00
<i>Stamp</i>	1	3.24	0.71	0.00	0.14	0.00
<i>Stamp</i>	2	3.12	0.21	0.00	0.06	0.00
<i>WhiteOak</i>	1	2.32	15.18	0.00	5.05	0.25
<i>WhiteOak</i>	2	2.24	18.85	0.00	1.44	0.05
<i>WhiteOak</i>	3	1.82	10.63	0.00	6.51	0.00
<i>WhiteOak</i>	4	2.29	20.46	0.00	0.93	0.07
<i>Byrd</i>	1	1.85	16.41	0.00	0.00	0.00
<i>Byrd</i>	2	2.95	13.25	0.00	0.00	0.00

## **Appendix B:**

# **Methods and Standard Operating Procedures**

# **Wet Weather Septic System Impact to Water Quality Study**

## **Standard Operating Procedure**





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## Introduction

The purpose of this operating procedure is to standardize the field sampling and data collection methods for the Wet Weather Septic System Impact Study. The methods used in this project follow standard sample and data collection protocols. Sample and data analysis will be integrated to answer research questions outlined in the scope of the Wet Weather Septic System Impact Study.

## Sampling Overview

The watersheds to be sampled in this study were selected to compliment an accompanying 2019 study done by Geosyntec Consultants. Geosyntec's primary focus was sampling for stream water quality during "dry" weather and base flow conditions with little to no precipitation in a predetermined timeframe. This study will focus on wet weather conditions, in which samples and data will be collected during or within a 24-hour window following a major precipitation event. Field data and samples to be measured and collected will include automated stream flow samples, stream grab samples, in-situ water level and physical water quality parameters, photographic records, and site observations unique to each sampling event.

Automated stream flow samples will be collected using an ISCO Avalanche Portable Refrigerated Sampler. This sampler will be programmed to monitor, collect, and chill samples until the sampling team is on site to collect them within 24 to 48 hours after initial sample collection. The sampler will be programmed using the same parameters for each site, but parameter values will be unique to each site and based on measured and historic hydrologic behaviors. Stream grab samples will be collected anytime the sampling team is on site to collect samples from the ISCO Avalanche. In-situ water level data will be collected using an ONSET Hobo U20 Water level data logger and an ONSET Hobo Water Conductivity Data logger. Both of these loggers will remain in the streams for the duration of the study and data downloaded periodically. In-situ physical water quality parameters will be collected during a sampling event using a calibrated YSI Pro Plus Multiparameter Instrument and Hach Turbidimeter. These instruments will measure water temperature, pH, specific conductivity, dissolved oxygen, and turbidity. Photographic records will be collected using a mounted trail camera, which will remain at a fixed point for the duration of the study and will be programmed to take a photo at the same time each day. All data, with the exception of photographs, will be recorded onto a [Field Datasheet](#) designed to remind, confirm, and clearly display any field, sample, or site observation data unique to each site and sampling event.

## Sampling Set Up

### ISCO Avalanche

The ISCO Avalanche should be set up and positioned near the stream in order for it to collect a sample. It should also be positioned in a way that it will be safe from high water levels or falling debris, and stabilized so that it is not in danger of falling into the stream. The sampler is designed to be robust and uncovered so that it will not be affected by weather or temperature changes within an acceptable operating range. However, it is our recommendation that the sampler be placed inside a weather proof enclosure to ensure it remains operational and minimally effected by weather events.

### Materials Needed

- ISCO Avalanche
- Weather Proof Enclosure (if applicable)
- Sample Bottles
- ISCO Water Level Logger
- ISCO Sampling Hose
- ISCO Sampling Hose Strainer
- ISCO Sampling Accessories (if Applicable)
- 500 ml plastic graduated cylinder
- Shovel
- Solar Panel Post (if Applicable)
- Drill and appropriate screws
- Wooden block approximately 2x4x18 inches
- Compass
- 14 Gauge Wire and Wire Couplers
- 2 Deep Cycle Batteries
- Deep Cycle Battery Connecting Terminals
- Solar Panel (if Applicable)
- Solar Charging Controller (if Applicable)
- Hand Tools (Voltmeter, wire strippers, screw driver, crescent wrench, bubble level)
- Padlock

### Select a location to set up the sampler

Select a location within the stream that is representative of the stream environmental characteristics. Sampling should be done within well mixed stream intervals, such as in a riffle or immediately downstream of a waterfall. The sampling hose should also be positioned approximately 4 inches above the stream bed to reduce sediment intake. Also keep in mind the recommended sampling hose height limitations - the sampler cannot draw water up more than 23 ft.

Set up the sampler, enclosure, power source, and any other sampler accessories

1. Once a location has been selected, place the sampler on level ground. Or, if using an enclosure, place the enclosure on level ground and then place the sampler inside the enclosure. It may be necessary to dig, adjust, or level both the enclosure and the sampler.
2. Then set up a power source. This study will use a 90-amp solar panel. Position the post in a way that the mounted solar panel will receive adequate sunlight to recharge and maintain the sampler batteries. It is recommended that the solar panel is facing South (if in the Northern Hemisphere), but predicted site specific sunlight conditions will override solar panel North/South orientation. Dig a hole approximately 10 inches deep and the width of the solar panel post. Set the post inside the hole and pack excavated soil around the post to create a strong setting. Screw the solar panel bracket to the top of the post and attach the solar panel to the bracket. It's also recommended to use a small length of mounted 2x4 wood block to support the bottom of the solar panel to the post. Mount the solar panel charge controller inside the weatherproof enclosure using a drill and appropriate screws. The charge controller should be mounted on the side of the enclosure, out of the way but still accessible. Wire the solar panel to the sunlight charge controller using adequate length 14-gauge wires, making sure to wire them into the port labeled "solar". If unfamiliar with how to attach wires to a solar panel, review the manual that accompanied the solar panel.
3. Then wire the batteries in parallel and to the solar charger. This study will use 2 12-volt, deep cycle batteries per sampler. To wire the batteries in parallel - attach a red wire to the positive terminal of each battery using a wire coupler and battery post bolt. Then attach a third wire to both of the red battery wires using a wire coupler. You should now have one red wire attached to each of the positive battery terminals, then connected to a third wire using a wire coupler. You'll then wire the third wire into the solar charger positive battery terminal. Repeat this process for the negative battery posts using black wire and wire it into the solar charger negative battery terminal.
4. Finally, cut a small, 4-inch section of both red and black wires. Wire these into the "load" portion of the solar charger, with the red wire going to the positive terminal and the black going to the negative terminal. Then clamp the ISCO battery clamps to the load wires, with the red battery clamp attached to the red "load" wire and the black battery clamp being attached to the black "load" wire. The sampler should immediately power on. Then attach the sampling hose to the sampler, and place the other end in the stream where you will be sampling. The hose stream end should have a strainer attached to it to block any large debris from being sucked into the sampler. It should also be secured to the stream bed using T-posts, rope, blocks, etc.
5. Attach any accessories to the ISCO, including water level pressure transducers, area and velocity modules, or rain gauges. If using a water level pressure transducer, place the sensor in the stream next to the sampling hose strainer and secure it in the same way. If using a

rain gauge, it is recommended that the rain gauge be placed nearby, but free from any overhead obstructions, such as trees or other structures. One location could be the top of the solar panel post, or its own dedicated rain gauge post. Then bury all lines to limit trip hazards and animal interference.

### Program the sampler

1. Program the sampler in such a way that makes sense to your sampling application. We will be programming the sampler to take samples during a rain event according to calculated water level base flows and predicted hydrograph rate of change. It's also recommended to program the sampler in an office or lab before deploying to the field site. Refer to [Appendix A](#) for this project's sampler program
2. Once programmed, the sampler should now return to the run screen. Navigate to *Other Function, Manual Functions, Calibrate Volume*. Select the appropriate sample volume with regards to the 500 ml plastic graduated cylinder. Make sure the sample hose is in the water and highlight *Calibrate Volume* and press Enter. The sampler will then deposit the selected sample volume into one of the internal sample bottles. Afterwards, pour this sample into the graduated cylinder, note the sample volume, and enter it on the sampler screen when prompted. The sampler will use this as a calibration for future sampling events. This step should be done at the beginning of sampler deployment and at 6-month intervals.
3. Make sure the program has been started, close the sampler enclosure, and lock it using the padlock to prevent sampler tampering.

### Downloading Data

Sampler data should be downloaded each time the sampling team visits the site. The sampler memory is limited and downloading frequently will inhibit data loss. This project will use an ISCO Rapid Transfer Device (RTD). The RTD will act as a memory stick of sorts. The RTD plugs directly into the ISCO data transfer port and immediately begins downloading data, as indicated by the yellow blinking transfer light. When transfer is complete, the green complete light will be solid.

## METER Rain Gauge and Pressure Transducer

### Materials Needed

- METER rain gauge
- METER pressure transducer
- METER ZL6 data logger
- 6 AA rechargeable batteries
- T Post
- T Post driver or hammer
- 11" Zip ties
- Bluetooth capable smartphone or field laptop/tablet

### Site Selection

1. Select a location for both the rain gauge and pressure transducer. An ideal location should be free of overhead structures that could interfere with the rain gauge measurements, such as a tree or wall. It should also be close enough to the stream that the pressure transducer can be placed directly alongside or attached to the anchor point of the ISCO pressure transducer and sample hose. Drive the T Post in to the ground using the hammer or T Post Driver. Attach the rain gauge to the top of the T Post using zip ties
2. Mount the ZL6 data logger below the rain gauge by using zip ties. If using a ZL6 with a solar panel mounted into it, point the data logger and solar panel in the direction that it is most likely to get the fullest amount of sunlight.
3. Place the pressure transducer into the stream alongside the ISCO pressure transducer and, if possible, attach to the same anchor point using zip ties. If not possible, place the METER pressure transducer as close as possible to the ISCO pressure transducer, making sure to simulate the stream location as much as possible.
4. Insert 6 rechargeable batteries into the data logger. Both the green *OK* and *Error* lights should flash. This indicates that the logger is on and is ready to be set up. Plug the METER rain gauge into port 1 and the METER pressure transducer into port 2.
5. Using a Bluetooth enabled smartphone with the Zentra Utility app or a field laptop/tablet with the Zentra Utility Program installed, communicate with the data logger and confirm its location, data logging interval, data cloud upload time interval, and sensor options. This project will record data every 5 minutes and upload data every 15 minutes.
6. Then, using an internet browser, navigate to [zentracloud.com](https://zentracloud.com), create an account, and follow the prompts to claim the logger and confirm it's uploading data.

## ONSET Hobo Data Loggers

### Materials Needed

- ONSET Hobo Water Level U20 logger
  - ONSET Hobo Water Conductivity logger
  - ONSET Hobo U-DTW-1 Shuttle
  - Zip Tie
  - 3" diameter PVC Pipe, 6" long
  - 6" Stake, 1/2" diameter pipe, or T post
  - Hammer
  - Brick or Concrete Block
  - Rope
1. Set up both the data loggers with the appropriate names, parameters, and sampling time interval using the ONSET Hobo data logger computer program and the manual that came with each logger.
  2. Drill a series of holes into the 3" PVC pipe to allow water to pass through in all directions.
  3. Place the loggers inside and attach them to the end of the 3" PVC pipe using zip ties or string. These should be secure enough to prevent them from detaching and being lost downstream, but will need to be accessible for data download.
  4. Attach the 3" PVC pipe to the concrete block or brick using rope or any other non-rusting attachment device. This should be very secure in order to prevent loss of data loggers.
  5. Place loggers, 3" PVC pipe, and block into the stream at the sampling location. The location should have the highest probability of constant water flow and be nearest to the sampling point. This project will place these directly in front of the culverts under the road crossings.
  6. Dig, hammer in, or otherwise secure the 6" stake, 1/2" diameter pipe, or T post into the stream bank nearest the sampling location. Then tie an appropriate length of rope from the stream bank post to the block to secure the whole data logger unit to the stream bank. This will limit how far the unit can move and will deter it from being swept downstream during heavy current.

### Downloading Data

Download data from each data logger using the ONSET Hobo Shuttle. This should be done whenever collecting samples or approximately every 3 weeks, depending on the measurement interval and logger memory. This is a waterproof unit that uses a logger specific coupler to attach to and download data wirelessly. It is recommended to set up and test in lab to become familiar with the unit before using in the field. Refer to the operating manual and HOB0 Data logger program for function, battery life, and maintenance.



## Trail Cameras

### Materials Needed

- Trail Camera
  - 6 AA batteries
1. Set up the trail camera prior to use in the field. Refer to the operation manual for specific options and how to download data from the camera. This project will take a photo of the stream culverts at the same time every day (12, noon) since the camera motion sensor will be pointed at road crossings and likely be triggered unnecessarily by passing cars.
  2. Mount the camera in the field by attaching it using the cinch strap or mount that came with it. This should be securely attached to a tree, T post, or any structure that is unlikely to move and provide the camera with a constant vantage point. Then take a series of test photos or videos to confirm the point of interest is within frame of the camera and adjust accordingly. This project will download photos from the cameras during sampling or approximately every 3 weeks - the same download interval as the Hobo data loggers.

## Detailed Sampling Procedures

### Decision to Sample

The sampling and lab teams will be notified up to one week in advance before a predicted sampling time frame. The decision to sample will be made based on forecasted weather conditions, actual precipitation amounts, and previous weather patterns. The sampling team will confirm sampling the day before and the morning of a predicted sample event and within 24 hours of initial collection by the automated sampler. The sample team will remain in contact with the lab team for the duration of the sampling day and inform them of general sample amounts, any potential sample loss, and expected sample arrival in lab.

Wet weather conditions will be met when a 6-month flood event occurs within the watershed at the automated sampler site. The probable occurrence of such an event will be determined by threshold stream stage as recommended by the hydrologist. We will consider sampling from lower return periods if the 6-month flood event proves difficult to achieve within the first 3 months of the project start date. Dry weather conditions will be met when no rain event or increase in stream discharge has occurred in the watershed within a 72 hour period. Both wet and dry weather conditions will be monitored by the cloud capable rainfall gauge and pressure transducer mounted at each automated sampler site. These will also be checked the morning of a scheduled sampling event to confirm if a rain event has occurred at a sample site and to estimate if the ISCO automated sampler has logged a sample event.

### Sample Labeling Convention

This project will have four sample watersheds, each with an automated stream sampler and grab bottle sampling. These watersheds are West Fork Little River, Pond Fork, Little Stone Mountain Creek, and Honey Creek. The naming convention will be to use the first initial of each watershed, followed by the sampling point, then a hyphen and either the letter G or S to denote a grab or automated storm sample, and then the sample number. At the bottom of the sample bottle will also be the date of sample collection and the initials of the sampling team.

Watershed	Sample Point	Sample Type	Sample #
<b>WFL</b>	<b>1</b>	<b>G</b>	<b>1</b>
<b>WFL</b> - West Fork Little River <b>P</b> - Pond <b>LSM</b> - Little Stone Mountain <b>H</b> - Honey	<b>1</b> - Sample Point 1 <b>2</b> - Sample Point 2 ...	<b>G</b> - Grab <b>S</b> - Storm	<b>1</b> - Sample 1 <b>2</b> - Sample 2 ...

### *Examples:*

H3 - G1; 9/04/2022 MT → Honey Creek Point 3 - Grab Sample 1; September 4, 2022 Matthew Thibodeaux

LSM1 - S4; 10/26/2022 MT → Little Stone Mountain Point 1 - Storm Sample 4; October 26, 2022 Matthew Thibodeaux

### Pre-Sampling

The morning of a sample event, perform calibration checks on both the YSI multiparameter probe and the Hach turbidimeter. Log values on the [YSI Calibration Spreadsheet/Hach Turbidimeter Calibration Spreadsheet](#) and recalibrate if necessary. See [Appendix B](#) for calibration procedures and spreadsheets.

### Materials Needed for all sampling events

- Calibrated YSI multiparameter probe
- Calibrated Hach turbidimeter
- Field capable laptop/tablet
- Sharpie and Pen
- Cooler
- 1000 ml autoclaved sample bottle
- Ice or ice packs
- Nitrile gloves
- ONSET Hobo U-DTW-1 Shuttle
- Chest waders or rubber boots
- Field sample sheet (Appendix B)
- 20 60 ml syringes
- 20 50 ml acid washed bottles
- 25 25mm .45 um syringe filter

Wet Weather Sampling Only

- Automated sampler Rapid Transfer device (RTD)
- 25 acid washed automated sampler bottles and lids
- Both internal automated sampler tubes

### Wet Weather Sampling

1. Approach the site, unlock the automated sampler enclosure, and slowly open the lid. Be careful in case there are any insects or rodents that have found a way inside the enclosure.
2. Attach the RTD to the appropriate port of the sampler. Data download should begin automatically as noted by the blinking yellow transfer light. When finished downloading, as noted by the solid green complete light, remove the RTD from the sampler. Indicate completing the data download on the [Field Datasheet](#).
  - a. IF the sampler has turned off due to insufficient power supply, attach the battery clamps directly to the deep cycle battery and turn the sampler on. Then begin the data transfer
3. Open the sampler and remove any sample bottles that have sample in them. Label the bottles according to the labeling convention, along with the date and sample team initials. Record the number of bottles on the field sample sheet along with the labels and corresponding sampler bottle rack number (1-14). Place sample bottles in cooler with ice packs. Navigate to the sampler report and record any information on the [Field Datasheet](#)., including time of trigger water level, time of sampler sample collection, and any errors that occurred during the program run.
4. Replace used sampler bottles with clean acid washed bottles, replace internal tubing with acid washed tubing, restart sampler program, close the lid, and lock the sampler enclosure.
5. Continue on to step 3 of the [Dry Weather Sampling Protocol](#)

### Dry Weather Sampling

1. Approach the site, unlock the automated sampler enclosure, and slowly open the lid.
2. Attach the RTD to the appropriate port of the sampler. Data download should begin automatically as noted by the blinking yellow transfer light. When finished downloading, as noted by the solid green complete light, remove the RTD from the sampler. Indicate completing the data download on the [Field Datasheet](#). IF the sampler has turned off due to insufficient power supply, attach the battery clamps directly to the deep cycle battery and turn the sampler on. Then begin the data transfer
3. Put on waders or rubber boots, and enter the stream if it is safe enough to do so. Approach the HOBO data loggers with the waterproof shuttle and couplers. Attach the appropriate coupler to the shuttle, insert the logger into the coupler, and press down on the coupler lever to begin the data transfer. The yellow transfer light will blink as data is transferred. When data transfer is complete, the green complete light will blink. Remove the logger from

the coupler and place back into the stream stilling well. Repeat the process with the other logger. Indicate completing the data download on the [Field Datasheet](#).

4. Approach the automated sampler hose and pressure transducer in the stream. Take note of the unit orientation, clear any debris from the hose and transducer, and place in the same location and orientation. The unit should be facing upstream, with the transducer on one side and the sampler hose on the other. Record state of sampler intake unit on the [Field Datasheet](#).
5. While standing close to the sampler intake unit and wearing nitrile gloves, take a stream bacteria sample using the 1000 ml autoclaved sample bottle by lowering the bottle opening until it is below the water surface. Be sure to sample upstream of your immediate location as to not contaminate or bias the sample with sediment or other analytes stirred up from moving within the stream. Label the bottles according to the labeling convention, along with the date and sample team initials. Record the bottle on the [Field Datasheet](#) and place in cooler with ice packs.
6. While standing close to the sampler intake unit and wearing nitrile gloves, take 2 stream analyte samples using a 60 ml syringe. Place the syringe opening below the water surface and pull back on the plunger until the syringe is full. Be sure to sample upstream of your immediate location as to not contaminate or bias the sample with sediment or other analytes stirred up from moving within the stream. Attach a syringe filter to the end of the syringe, place the opening over a 60 ml acid washed sample bottle, and slowly depress the syringe, allowing the sample to pass through the filter and into the bottle. Fill the bottle with approximately 50 ml of sample. Repeat sample intake and filter process into the other acid washed 60 ml sample bottle using the same 60 ml syringe. If filter becomes significantly clogged or slow, it may be necessary to replace it with a new filter and continue to filter the sample into the sample bottle. Label the bottles according to the labeling convention, along with the date and sample team initials. Record the bottle on the [Field Datasheet](#) sheet and place in cooler with ice packs. Discard used filters and save used syringes to be acid washed and reused.
7. Turn on the YSI multiparameter probe and wait approximately 5 minutes for the probe to warm up. Unscrew the probe from the transfer cup and screw the probe guard on. While standing close to the sampler intake unit, slowly lower the probe until it is approximately midway in the water column. Be sure to sample upstream of your immediate location to not contaminate or bias the probe reading. Wait until the readings have stabilized and record the in-situ hydrologic data (e.g., temperature, DO, pH, specific conductivity) on the [Field Datasheet](#). Turn the probe off, screw the transfer cup back on the return the probe to the travel case
8. Turn on the Hach turbidimeter and remove the sample vial from the instrument. While standing close to the sampler intake unit, slowly lower the vial into the water until the opening is below the surface and fill the vial with sample. Lift vial out of the water and

discard. Then lower the vial into the water again and fill with sample. Be sure to sample upstream of your immediate location as to not contaminate or bias the sample with sediment or other analytes stirred up from moving within the stream. Screw the lid onto the vial and use the lint free cloth to dry the outside of the vial and clean off any smudges. Insert the vial into the instrument, close the lid, and press *read*. Record the value on the [Field Datasheet](#) and discard the sample. Turn the instrument off and return to case.

9. Exit the stream and record time spent on site along with any pertinent information including site observations, weather, and confirm all data has been recorded.

## In Lab Sample Processing

### In Lab Processing After Wet Weather Sampling

#### Materials Needed

- 47 mm, 0.45 um filter paper
  - 4 vacuum filtration units
  - 4 500 ml vacuum port Erlenmeyer flasks
  - Calibrated YSI multimeter
  - Calibrated Hach Turbidimeter
  - 4 150 ml acid washed beakers
  - Bench top vacuum and hose
  - Twice as many 60 ml acid washed bottles as you have automated sampler bottles
  - As many 1000 ml autoclaved sample bottles as you have automated sampler bottles
1. Remove all samples from the cooler and place in refrigerator. Samples should remain here at a constant temperature until filtered.
  2. Set up the vacuum filtration unit. The unit should have an open glass cup clamped to a porous glass funnel, which is then inserted into an Erlenmeyer flask with a vacuum port. Place one 47mm, 0.45 um filter In between the funnel and the cup.
  3. Remove the first automated sampler bottle from the refrigerator. This should be the first sample that the sampler took per site (XXX1 - S1). Shake by inversion for 10 seconds and pour approximately 500 ml of sample into a labeled, autoclaved 500 ml sample bottle. Place the autoclaved sample bottle in the refrigerator. There should be approximately 450 ml of sample still in the automated sampler bottle.
  4. Turn on the bench top vacuum and attach the hose to the vacuum filtration unit. Pour approximately 100 ml of sample into the vacuum filtration unit and allow this to filter into the Erlenmeyer flask. When done filtering, turn vacuum off, remove the vacuum filtration

unit, and discard the filter paper. Pour approximately 50 ml of sample into 2 labeled, acid washed 60 ml sample bottles. Place bottles into freezer.

5. Pour the remaining 350 ml of sample in the automated sampler bottle into a labeled, acid washed 150 ml beaker. Place the YSI multimeter into the beaker and allow readings to stabilize. Record specific conductivity, pH, and dissolved oxygen readings onto the [Field Datasheet](#). **DO NOT** record temperature.
6. After taking the YSI readings, take a small subsample out of the 400 ml beaker using the Hach turbidimeter vial and place into the Hach turbidimeter instrument. Record the reading onto the [Field Datasheet](#). Remove the turbidimeter vial from the instrument and discard the sample along with the remainder of the active sample.
7. In between filtering automated sampler bottle, rinse all equipment that came in contact with sample 5 times with deionized water. Also, be sure that each set of glassware is only used for 1 site. (e.g. use one set of glassware for all Honey Creek samples, use another set for all Little Stone Mountain samples, etc.). After rinsing, repeat filtration process with next sample in the queue (XXX1-S2). The entire filtration process can be done simultaneously with other sites.
8. Once all samples have been filtered, refrigerated or frozen, notify the lab team so the bacteria analysis can begin.

### [In Lab Processing After Dry Weather Sampling](#)

1. Upon return from dry weather sampling, place 60 ml sample bottles into the freezer and 1000 ml autoclaved sample bottles into the refrigerator. Notify the lab team so bacterial analysis can begin. There is no other processing needed.

### [Bacterial Methods Analysis](#)

#### **Water Sample Processing and DNA extraction for ddPCR and CrAssphage qPCR:**

1. Water Sample Filtration:
  - a. Set up a filtration manifold with two Erlenmeyer flasks with appropriately sized rubber stoppers and effluent tubing. They should be connected linearly to the vacuum pump in the following order:
    - i. Manifold -> Large Erlenmeyer flask -> Smaller Erlenmeyer flask -> vacuum pump nozzle.
  - b. Attach sterile filter towers in on the outer two filter tower ports.
  - c. Using flame sterilized forceps, remove polycarbonate filters from packaging and place **shiny side up** between filter tower magnetic housing and filter platform.
  - d. Rinse filter with enough autoclave sterilized 1X PBS to wet the entire filter surface.



- e. Remove water sample bottle from 4C storage and shake vigorously (~25 times) to ensure uniform bacterial distribution.
  - f. **With filter tower vacuum in the off position**, aliquot 100 mL of shaken water sample to the filter.
  - g. Turn filter tower vacuum to the on position and pull the entire water sample through the filter.
  - h. Once all the water sample has passed through the filter, rinse the side of the filter funnel with 20-30 mL of sterile 1X PBS to wash residual sample off the sides of the filter tower.
  - i. With flame sterilized forceps, place sample filter into a ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm).
  - j. Remove filter funnel tower and soak in 70% EtOH and rinse in DI water.
  - k. Repeat steps b-j for each replicate of each site and event.
2. Sample DNA Extraction: *Note: DNA extraction protocol from ZYMO DNA Extraction Mini Kit.*
- a. Add 750 uL of ZymoBIOMICS Lysis Solution to the tube with sample DNA and cap tightly.
  - b. Secure in BeadBeater with 2 mL tube holder assembly and process for the following parameters depending on number of tubes:
    - i. Biospec Mini-BeadBeater-96 with 2 ml BashingBead Tubes:
      1. 5 minutes at max RPM
      2. 5 min rest.
      3. Repeat cycle 4 times for a total of 20 min of bead beating.
    - ii. Biospec Mini-BeadBeater-96 with 96-well BashingBead lysis rack:
      1. 5 minutes at max RPM
      2. 5 min rest.
      3. Repeat cycle 4 times for a total of 40 min of bead beating.
  - c. Centrifuge Lysis tubes in micro centrifuge at 10,000 x g for 1 minute.
  - d. Transfer **at most** 400 uL supernatant to the Zymo-Spin III-F Filter in a Collection Tube and centrifuge at 8,000 x g for 1 minute. **Discard Zymo-Spin III-F Filter.**

#### **Droplet Digital PCR for HF183 (Cao et al., 2019 and BioRad Expert Design Assay [Appendix 1])**

- Method Development and Testing:
  - Purpose: Using a Gblock (synthetic gene fragment standard), create two standard curves in parallel to find ddPCR observed method limit of detection and discover possible PCR inhibition in stream water extracted samples.
- Resuspend HF183 G-block:
  - a. Before opening the tube, centrifuge at a minimum of 3000 x g for 2-3 seconds to ensure all materials are at the bottom of the tube.
  - b. Add TE buffer to reach final concentration of 10 ng/uL.
    - i. G-block we received was 500 ng, dried.
    - ii. Add 50 uL of TE buffer to achieve 50 ng/L of HF183 Gblock.

- c. Vortex briefly.
  - d. Incubate at 50C for 20 min.
  - e. Briefly vortex and centrifuge (minimum of 3000 x g for 2-3 seconds).
2. Standard curve preparation:
  - a. Calculate number of copies in per dry mass of Gblock:
    - i. 
$$\text{Number of copies} = \frac{! (\# . \% \& \& ' * \% )}{* + * \# \# \% \# \# ( ' * \% ( \# ) } \frac{\$ \% \&}{\#}$$
    - ii. X = Gblock dry mass (ng); N = Number of base pairs in Gblock
    - iii. Our Gblock was calculated to contain  $4.959 \times 10^{12}$  copies.
    - iv. That yields  $9.918 \times 10^{10}$  copies per uL.
  - b. Using suspended Gblock, make a serial dilution going from a  $10^8$  order of magnitude dilution down to a  $10^1$  using molecular grade water as the dilution solvent.
  - c. Repeat part b using extracted stream water DNA as the dilution solvent.
  - d. Aliquot 11 uL of standard curve samples into separate sets of strip tubes and send to University of Georgia Genomics core for ddPCR analysis.
3. ddPCR Sample analysis
  - a. Following DNA extraction, aliquot 11 uL of DNA extract into labeled strip tubes and deliver to University of Georgia Genomics Core for ddPCR with automated droplet generator assay.
    - i. ddPCR assay recipe and conditions adapted from Cao et al. (2019) and BioRad Expert Assay Design (Appendix 1).
    - ii. All results were back calculated and reported as copies per 100 mL.

#### **CrAssphage qPCR Assay (Malla et al., 2019):**

4. Master mix for CrAssphage qPCR assay was prepared by combining the following reagents in biosafety hoods to reduce risk of contamination in a pre-PCR designated room. Quantities of reagents are for a single reaction. Master Mixes were made in batches and calculated based on total number of samples and replicates needed. Reagents were added in the order indicated below. Master mix was vortexed after adding each reagent with exception of the addition of TaqPath. TaqPath denatures when vortexed which required us to pipette mix the master mix after adding TaqPath.
  - a. RNase-Free water: 9 uL
  - b. Forward Primer (20 uM): 0.4 uL
  - c. Reverse Primer (20 uM): 0.4 uL
  - d. Probe (FAM reporter; 20 uM): 0.2 uL
  - e. TaqPath: 5 uL
5. 54 uL of prepared master mix was aliquoted into individual strip tube wells.
6. 18 uL of each sample DNA extract were added to individual wells of 54 uL of master mix and pipette mixed.
7. 20 uL of each sample master mix solution was aliquoted in triplicate into a 384 well plate and sealed with optically active adhesive film for analysis using a BioRad CFX384

Touch Real-Time PCR Detection System under the below cycling conditions.

1. 95°C	30 seconds	Repeat for a total of 40 cycles
2. 95°C	5 seconds	
3. 56°C	30 seconds	

8. All results were converted to copies per 100 mL using a standard curve.

**Fecal Coliform Culturing (Center for Food Safety and Applied Nutrition, 2020):**

1. Water Sample Filtration for Fecal Coliform Bacteria:
  - a. Set up a filtration manifold with two Erlenmeyer flasks with appropriately sized rubber stoppers and effluent tubing. They should be connected linearly to the vacuum pump in the following order:
    - i. Manifold -> Large Erlenmeyer flask -> Smaller Erlenmeyer flask -> vacuum pump nozzle.
  - b. Attach sterile filter towers in on the outer two filter tower ports.
  - c. Using flame sterilized forceps, remove mixed cellulose ester filters from packaging and place **grided side up** between filter tower magnetic housing and filter platform.
  - d. Rinse filter with enough autoclave sterilized 1X PBS to wet the entire filter surface.
  - e. Remove water sample bottle from 4C storage and shake vigorously (~25 times) to ensure uniform bacterial distribution.
  - f. **With filter tower vacuum in the off position**, aliquot 10 mL of shaken water sample to the filter.
    - i. Due to observed variation in fecal coliform bacteria colony forming unit growth during method development, we diluted sample water 1:10 and 1:100 via serial dilution and plated both dilution levels.
    - ii. Diluting samples increased countability of plates, therefore reducing counting bias.
  - g. Turn filter tower vacuum to the on position and pull the entire water sample through the filter.
  - h. Once all the water sample has passed through the filter, rinse the side of the filter funnel with 20-30 mL of sterile 1X PBS to wash residual sample off the sides of the filter tower.
  - i. With flame sterilized forceps, we place filters directly onto 60x15mm mFC agar plates.
  - j. Remove filter funnel tower and soak in 70% EtOH and rinse in DI water.
  - k. Place sample plates with filters in a foam box in an incubator at 37°C for 2 hours. Afterwards, increase incubation temperature to 44.5°C for 16 hours. Total incubation time is 18 hours.
  - l. Repeat steps b-k for each replicate (2) of each dilution, site, and event.

2. Plate counting

- a. Fecal coliform bacteria colony forming units grow with a royal blue color on mFC plates.
- b. Pick dilution level for each sample based on countability of colony forming units. Countable range is between 20 and 80 colony forming units.
- c. Count colony forming units for each replicate at the determined countable dilution level.
- d. Average the two replicate counts together for final colony forming unit count for that sample.
  - i. All results were back calculated and reported as copies per 100 mL.

#### References for Bacterial Methods

1. Cao, Y., Raith, M. R., & Griffith, J. F. (2015). Droplet digital PCR for simultaneous quantification of general and human-associated fecal indicators for water quality assessment. *Water Research*, 70, 337–349. <https://doi.org/10.1016/j.watres.2014.12.008>
2. Center for Food Safety and Applied Nutrition. (2020). *Bam Chapter 4*. U.S. Food and Drug Administration. <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria>
3. Malla, B., Ghaju Shrestha, R., Tandukar, S., Sherchand, J. B., & Haramoto, E. (2019). Performance Evaluation of Human-Specific Viral Markers and Application of Pepper Mild Mottle Virus and CrAssphage to Environmental Water Samples as Fecal Pollution Markers in the Kathmandu Valley, Nepal. *Food and Environmental Virology*, 11(3), 274–287. <https://doi.org/10.1007/s12560-019-09389-x>

## Appendix A

### ISCO Automated Sampler Program Outline

1. Turn the sampler on if not already, navigate to the Program option, and press Enter. Options in each window hereafter will be selectable and should be decided with this project's goals in mind.
2. Highlight both the *Program Name* and *Site Description* and label each according to the program and site details
3. *Units Selected*: Ft; Temperature: F
4. *Units Selected*: Flow Rate - cfs; Flow Volume - cf
5. *Submerged Probe*: Level Only, this is to indicate we will be using the pressure transducer to only measure flow instead of estimating discharge
6. *Current Level*: 0, this is to calibrate the pressure transducer - take the probe out of the water, select and adjust the current level to 0

7. *Data Storage Interval*: 5 Minute Data Interval, this is the rate at which data will be stored in the memory partition.
8. *Number of bottles & Bottle Volume*: 14 bottles, 900 ml volume; *Suction Head*: XX feet, this is the sample hose length and will vary per site; *Auto Suction Head*: Enabled, the program will automatically determine the height of the sampler above the stream; *Rinses and Retries*: 1 Rinse, 0 Retries, this is the number of times the sampler will rinse the sample hose between samples and the number of times it will retry to take a sample if it doesn't detect any the first time
9. *One Part Program*: Enabled, this is the option to indicate if there will be one or two programs running simultaneously on the sampler.
10. *Pacing*: Event paced, Event 01: Level, Level Condition: Rate of Change, True when: Rises, Level Rises: XX ft in XX hours and XX minutes, Event 02: Level, Level Condition: Rate of Change, True when: Falls, Level Falls: XX ft in XX hours and XX minutes, Done; this is option set to indicate when to take samples when certain conditions are met. This project will rely on a water level rate of change to take samples during and after a rain event.
11. *Distribution*: Sequential, 1 bottle per sample event (14), Switch Bottles On: Number of Samples, Switch Bottles Every: 1 Samples, Run Continuously: No; this is to make sure the sampler samples once into each bottle and does not composite samples.
12. *Volume*: 900 ml samples, this is how much sample volume the sampler will deposit per sample
13. *Enable*: Level, Done, Level Condition: Set Point, Level Set Point: XX ft, Enabled When: Above Set Point; this is to indicate when the sampler should start the pacing portion of the program
14. *Enable: Repeatable Enable*: No, *Sample at Disable*: No, *Sample at Enable*: Yes, this is to indicate if the entire program should continue indefinitely once enabled or not.
15. *Enable: Sample Interval Reset at Enable*: Yes; this is to indicate to the sampler that it should restart the program every time the program is enabled (triggered)
16. *Enable: 0 Pause & Resumes*; this is to indicate if the sampler should be dormant during user defined times
17. *No Delay to Start*: Yes; this is to indicate if the program should start immediately after being selected to run
18. *Programming is Complete...*: Yes; this is to start the program after all other options have been decided. The instrument will then test the distributor arm and report errors, if any.

## Appendix B

### YSI Calibration Procedure

This procedure was summarized by the Soil Physics lab at UGA by reading the YSI operational manual. Refer to the manual as needed for any questions or concerns.

#### Supplies Needed:

- 4 60 ml bottles with lid
- Ring stand with clamp
- Conductivity standard 2060 uS/cm
- pH 4, 7, 10 standards
- 500 ml beaker
- Deionized water
- Non-Hazardous Waste Container
- Dedicated Non-Hazardous waste funnel
- Nitrile Gloves

#### Calibrating Specific Conductivity

1. Turn the instrument on and put on Nitrile gloves
2. Unscrew calibration cup from sensors and dispose of storage water. Rinse sensors and calibration cup with DI water before placing sensors with the calibration cup standing upside down in ring stand. After rinsing with DI, pour a small amount (approximately 10ml) of the 2060 Conductivity Standard into the cup and replace the cap. Take calibration cup off ring stand, and swirl the standard onto all of the sensors - this is to remove any DI remnants still left in the cup
3. Pour the rinse conductivity standard into the 500 ml beaker. This will be used as a waste beaker for the calibration procedure.
4. Put calibration cup back on to the ring stand upside down and unscrew top cap
5. Pour enough conductivity standard into calibration cup to cover all of the sensors
6. Hit Cal button on the YSI. This will take you to the Calibration Screen. Highlight Conductivity. Press ENTER on YSI
7. Highlight Sp. Conductance, then SPC-micro siemens per cm (uS/cm), press ENTER
8. Check to see that Calibration Value [2060] is the correct standard being used. If not, highlight it, press ENTER, and the backspace to clear. Next, enter 2060 and press ENTER
9. Let it sit 5 minutes to wait and see what the pre-cal value is. Then, record the Actual Reading in the Pre-Cal Cond. Std column on the [YSI Calibration Spreadsheet](#)

10. If the reading is within  $\pm 12$  for 2060 specific conductance (2048 -2072), press *ESC*. This is to prevent over calibrating. If the reading for SPC is off by  $\pm 12$  (2047 or below or 2073 or above), then you will need to calibrate the sensor. If calibration is needed - confirm the calibration value is correct, Highlight the Accept Calibration option and press ENTER
11. Once you press ENTER, it will take you to the run screen. Record it in the equipment calibration log as After Cal in the SPC column.
12. Pour used conductivity standard into a labeled 60 ml bottle. This standard can be disposed of in a waste beaker, used to calibrate in the future, or used to rinse the probe during the next conductivity calibration. Rinse probe and calibration cup with DI water

### Calibrating pH

It is essential to check pH 7 first using the pH 7 buffer solution to prevent over calibration. If the pH sensor needs to be calibrated, then calibrate with the other standards in order starting with pH 4 buffer solution, then pH 7 buffer solution, then pH 10 buffer solution.

1. Place sensors with the calibration cup standing upside down in ring stand. After rinsing with DI water and disposing in the waste beaker, pour approximately 10ml of the pH 7 standard into the cup and replace the cap. Take calibration cup off ring stand, and swirl the standard onto all of the sensors - this is to remove any DI remnants still left in the cup
2. Pour the rinse pH 7 standard into the waste beaker and put calibration cup back on to the ring stand upside down
3. Pour enough pH 7 standard into calibration cup to cover the pH probe
4. When checking pH with the pH 7 buffer solution, allow it to stabilize for 10 minutes. Record the value as Pre Cal-pH 7 on the [YSI Calibration Spreadsheet](#). If you check it and the pH is reading + or - .12 units from 7.00, (7.12- 6.88), then you will need to calibrate the sensor using all three standards. If you need to calibrate, pour out the pH 7 buffer solution into a 60 ml bottle. This can be used again to check the instrument at the very end of the calibration.



### *To calibrate pH*

1. Rinse the calibration cup with DI water. Discard into Non -hazardous waste container. Next, pour approximately 10ml of pH 4 buffer into transportation cup, screw cap on, and swirl. Discard this pH 4 rinse solution into the waste beaker. Put calibration cup back on to the ring stand upside down and unscrew top cap. Pour new pH 4 buffer solution into the calibration cup.
2. Press the Cal button and scroll down to ISE1 (pH), press ENTER. There will be a message at the bottom of the screen that says Ready for Point 1.
3. Make sure the calibration value is reading [4.00]. If not, highlight calibration value and change it.
4. Wait for 10 minutes to ensure the reading has stabilized before you accept it. Record this value under Pre Cal-pH 4 on the [YSI Calibration Spreadsheet](#). Highlight Accept Calibration and press ENTER
5. At the bottom of the screen, it will read Ready for point 2. The YSI is now ready for pH 7 buffer standard. Pour out the pH 4 buffer solution into a 60 ml bottle. This can be re-used when rinsing with pH4 standard during the next pH 4 calibration.
6. Rinse sensors with DI water. Discard into waste beaker. Then, rinse sensors with pH 7 buffer solution and discard into waste beaker. Put calibration cup back on to the ring stand upside down and unscrew top cap. Then fill calibration cup with enough pH 7 buffer solution to cover pH probe.
7. Make sure the calibration value is reading [7.00]. If not, highlight calibration value and change it. Wait for 10 minutes to ensure the reading has stabilized before you accept it. Record this value under Pre Cal pH7 in the Calibration Log Sheet. Highlight Accept Calibration and press ENTER
8. At the bottom of the screen, it will read Ready for point 3. The YSI is now ready for pH 10 buffer standard. Pour out the pH 7 buffer solution into a 60 ml bottle. This can be re-used when rinsing with pH 7 standard during the next pH 7 calibration.
9. Rinse sensors with DI water. Discard into waste beaker. Then, rinse sensors with pH 10 buffer solution and discard into waste beaker. Put calibration cup back on to the ring stand upside down and unscrew top cap. Then fill calibration cup with enough pH 10 buffer solution to cover pH probe.

10. Make sure the calibration value is reading [10.00]. If not, highlight calibration value and change it. Wait for 10 minutes to ensure the reading has stabilized before you accept it. Record this value under Pre Cal-pH 10 on the [YSI Calibration Spreadsheet](#). Highlight Accept Calibration and press ENTER. Then press the Cal button to finish calibrating the pH probe. You will then be taken back to the run screen.

#### *Check pH Calibration for Calibration Accuracy*

1. Rinse sensors with DI water and discard into waste beaker. Then, rinse sensors with pH 7 buffer solution and discard into waste beaker. Fill calibration cup with enough calibration check pH 7 buffer solution to cover pH probe.
2. Monitor the pH reading on the run screen and wait for it to stabilize. The pH should read closer to 7.00 while still in the calibration cup. If still off by +/-12 units after waiting 5 minutes, repeat the pH calibration procedure. If within +/- 12, record pH 7 reading under the After Cal pH 7 column on the [YSI Calibration Spreadsheet](#). Pour this pH 7 buffer solution into the waste beaker.

#### Calibrating DO %:

1. Screw the calibration cup off the probe (use grey sleeve if needed to grip the transportation cup if needed). Screw the black sensor guard onto the YSI probe
2. Using DI water, wet the small yellow sponge that came with the YSI instrument. This should be wet throughout but not dripping in order to create a moist environment. Drop the moistened sponge into the grey probe sleeve and place grey rubber tube over black sensor guard. Put calibration cup on to the ring stand right side up.
3. To calibrate, press Cal on YSI to take you to the Calibration Screen. Then select DO and DO %.
4. The actual reading should be within +/-12 of the 98 %. (86 - 110 %) Record under Pre Cal-column on equipment calibration log. If the DO% is within this range, the probe does not need to be calibrated. If it outside of this range then it needs to be calibrated
5. Highlight Accept Calibration and press Enter. You will then be returned to the run screen. Record the DO% reading under After Cal on the [YSI Calibration Spreadsheet](#).
6. Remove the probes from the black sleeve and unscrew the probe guard. Fill the calibration cup halfway with **tap water** and screw onto the probe housing. The unit has now been calibrated and can be turned off before placing back into case. Pour waste solution from waste beaker into a non-hazardous waste container.

## Hach Turbidimeter Calibration Procedure

This procedure was summarized by the Soil Physics lab at UGA by reading the Hach Turbidimeter operational manual. Full calibration should be done once every three months, and secondary calibrations done at the beginning of the sampling day.

1. This procedure will use StablCal standardized samples, as recommended by the manufacturer, in order to eliminate error likely to occur from making standards in lab.
2. Thoroughly clean the StablCal <0.1 NTU (S0) vial using a Kim Wipe or lint free cloth. This will allow the instrument to measure the standard and not be impacted by fingerprints or smudges. Insert the vial into the instrument, making sure to align the diamond on the vial to the line guide on the instrument, and close the lid. Turn the instrument on, and then press Signal Average, and then Cal, and then Read. The instrument will count down from 67 while it calibrates using the first standard. Once the countdown reaches 0, the instrument will display S1. Remove the <0.1 NTU (S0) and proceed to the next standard.
3. Clean the next standard, 20 NTU (S1) by using a Kim Wipe or lint free cloth, shaking by inversion 2-3 times, and letting the standard sit for a minute. Place it into the instrument, making sure to correctly align the vial. Close the lid and press Read. The instrument will countdown from 67 and will automatically indicate when it is ready for the next standard.
4. Repeat step 3 for the remaining 2 standards. S2 will be the 100 NTU sample, and S3 will be the 800 NTU sample. When the S3 standard has been read, the instrument will display S0 again. Remove the S3 standard, and press Cal. The instrument will calibrate and return to the home measurement screen.

## *Secondary Calibration*

1. Turn the instrument on, and set the Range mode to automatic. Clean each of the 3 secondary calibration vials using a Kim Wipe or lint free cloth. Place the first standard, 0-10 NTU, into the instrument making sure to align it properly. Close the lid and press Read. After a moment, the reading will be displayed. Record this value in the [Hach Turbidimeter Calibration Spreadsheet](#).
2. Repeat the reading with the other 2 secondary calibration standards. These should be standardized to 0-100 NTU and 0-1000 NTU. Record the readings in the [Hach Turbidimeter Calibration Spreadsheet](#). If the values have drifted more than 5% from the previous calibration check, or if they are not within the specified range, this is an indicator that the instrument needs to be fully calibrated before being used.

Project:				Sample Event: <input type="checkbox"/> Dry <input type="checkbox"/> Wet		Day:	
Date:				<div> <div>Data Download</div> <div> <div>Trailcam (photos)</div> <div>Y N</div> <div>Y N</div> </div> <div> <div>HOBO</div> <div>Y N</div> <div>Y N</div> </div> <div> <div>ISCO</div> <div>Y N</div> <div>Y N</div> </div> </div>			
Collectors:				<div> <div>Please note any potential sources of bacteria/nutrients under Site Notes</div> <div>Site Notes</div> </div>			

Site ID							
Time started (24 hr)		Time ended (24 hr)					
Weather Conditions	<input type="checkbox"/> Clear <input type="checkbox"/> Partly cloudy <input type="checkbox"/> Overcast <input type="checkbox"/> Fog						
Water Surface	<input type="checkbox"/> None <input type="checkbox"/> Sheen <input type="checkbox"/> Scum <input type="checkbox"/> Solids						
	<input type="checkbox"/> Trash <input type="checkbox"/> Algae <input type="checkbox"/> Foam <input type="checkbox"/> Other:						
Water Color	<input type="checkbox"/> Colorless <input type="checkbox"/> Green <input type="checkbox"/> Yellow <input type="checkbox"/> Brown						
Water Clarity	<input type="checkbox"/> Clear <input type="checkbox"/> Cloudy (>4" visibility) <input type="checkbox"/> Murky (<4" visibility)						
Water odor	<input type="checkbox"/> None <input type="checkbox"/> Musty <input type="checkbox"/> Decay <input type="checkbox"/> Other:						
	<input type="checkbox"/> Chlorine <input type="checkbox"/> Chemical <input type="checkbox"/> Sewage						
Trash	<input type="checkbox"/> None <input type="checkbox"/> Styrofoam <input type="checkbox"/> Plastic <input type="checkbox"/> Other:						
Water Temperature (°C)		pH					
DO (%)		DO (mg/L)					
Sp. Conductivity (µS/cm)		Turbidity (NTU)					
Grab Samples Collected							
Bottle 1-60 ml clear, 0.45-µm filtered		Nut (G1)	Y N				
Bottle 2-60 ml clear, 0.45-µm filtered		Nut (G2)	Y N				
Bottle 3-500 ml clear, unfiltered		Bac (G3)	Y N				
Automated Samples Collected							
Site ID:	Date/Time	Temp (°C)	pH	DO (%)	DO (mg/L)	Sp. Con (µS/cm)	Turbidity (NTU)

Site ID							
Time started (24 hr)		Time ended (24 hr)					
Weather	<input type="checkbox"/> Clear <input type="checkbox"/> Partly cloudy <input type="checkbox"/> Overcast <input type="checkbox"/> Fog						
Water Surface	<input type="checkbox"/> None <input type="checkbox"/> Sheen <input type="checkbox"/> Scum <input type="checkbox"/> Solids						
	<input type="checkbox"/> Trash <input type="checkbox"/> Algae <input type="checkbox"/> Foam <input type="checkbox"/> Other:						
Water Color	<input type="checkbox"/> Colorless <input type="checkbox"/> Green <input type="checkbox"/> Yellow <input type="checkbox"/> Brown						
Water Clarity	<input type="checkbox"/> Clear <input type="checkbox"/> Cloudy (>4" visibility) <input type="checkbox"/> Murky (<4" visibility)						
Water odor	<input type="checkbox"/> None <input type="checkbox"/> Musty <input type="checkbox"/> Decay <input type="checkbox"/> Other:						
	<input type="checkbox"/> Chlorine <input type="checkbox"/> Chemical <input type="checkbox"/> Sewage						
Trash	<input type="checkbox"/> None <input type="checkbox"/> Styrofoam <input type="checkbox"/> Plastic <input type="checkbox"/> Other:						
Water Temperature (°C)		pH					
DO (%)		DO (mg/L)					
Sp. Conductivity (µS/cm)		Turbidity (NTU)					
Grab Samples Collected							
Bottle 1-60 ml clear, 0.2-µm filtered		Nutrients	Y N				
Bottle 2-60 ml clear, 0.2-µm filtered		Nutrients	Y N				
Bottle 3-500 ml clear, unfiltered		Bacteria	Y N				
Automated Samples Collected							
Site ID:	Date/Time	Temp (°C)	pH	DO (%)	DO (mg/L)	Sp. Con (µS/cm)	Turbidity (NTU)

YSI Calibration Spreadsheet

Pre-Calibration: YSI Probe								Post-Calibration: YSI Probe				
Date	Calibrated by	YSI #	Sp. Conductivity (uS/cm)	pH4	pH7	pH10	Dissolved Oxygen %	Sp. Conductivity (uS/cm)	pH4	pH7	pH10	Dissolved Oxygen %
												27

Hach Turbidimeter Calibration Spreadsheet

Pre-Calibration: Hach Turbidimeter								Post-Calibration: Hach Turbidimeter				
Date	Calibrated by	Meter #	0-10 NTU	0-100 NTU		0-1000 NTU		0-10 NTU	0-100 NTU		0-1000 NTU	
												28

**Water Sample Processing and DNA extraction for ddPCR and CrAssphage qPCR:**

1. Water Sample Filtration:
  - a. Set up a filtration manifold with two Erlenmeyer flasks with appropriately sized rubber stoppers and effluent tubing. They should be connected linearly to the vacuum pump in the following order:
    - i. Manifold -> Large Erlenmeyer flask -> Smaller Erlenmeyer flask -> vacuum pump nozzle.
  - b. Attach sterile filter towers in on the outer two filter tower ports.
  - c. Using flame sterilized forceps, remove polycarbonate filters from packaging and place **shiny side up** between filter tower magnetic housing and filter platform.
  - d. Rinse filter with enough autoclave sterilized 1X PBS to wet the entire filter surface.
  - e. Remove water sample bottle from 4C storage and shake vigorously (~25 times) to ensure uniform bacterial distribution.
  - f. **With filter tower vacuum in the off position**, aliquot 100 mL of shaken water sample to the filter.
  - g. Turn filter tower vacuum to the on position and pull the entire water sample through the filter.
  - h. Once all the water sample has passed through the filter, rinse the side of the filter funnel with 20-30 mL of sterile 1X PBS to wash residual sample off the sides of the filter tower.
  - i. With flame sterilized forceps, place sample filter into a ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm).
  - j. Remove filter funnel tower and soak in 70% EtOH and rinse in DI water.
  - k. Repeat steps b-j for each replicate of each site and event.
2. Sample DNA Extraction: *Note: DNA extraction protocol from ZYMO DNA Extraction Mini Kit.*
  - a. Add 750 uL of ZymoBIOMICS Lysis Solution to the tube with sample DNA and cap tightly.
  - b. Secure in BeadBeater with 2 mL tube holder assembly and process for the following parameters depending on number of tubes:
    - i. Biospec Mini-BeadBeater-96 with 2 ml BashingBead Tubes:
      1. 5 minutes at max RPM
      2. 5 min rest.
      3. Repeat cycle 4 times for a total of 20 min of bead beating.
    - ii. Biospec Mini-BeadBeater-96 with 96-well BashingBead lysis rack:
      1. 5 minutes at max RPM
      2. 5 min rest.
      3. Repeat cycle 4 times for a total of 40 min of bead beating.
  - c. Centrifuge Lysis tubes in micro centrifuge at 10,000 x g for 1 minute.
  - d. Transfer **at most** 400 uL supernatant to the Zymo-Spin III-F Filter in a Collection Tube and centrifuge at 8,000 x g for 1 minute. **Discard Zymo-Spin III-F Filter.**

**Droplet Digital PCR for HF183 (Cao et al., 2019 and BioRad Expert Design Assay [Appendix 1])**

- Method Development and Testing:
    - Purpose: Using a Gblock (synthetic gene fragment standard), create two standard curves in parallel to find ddPCR observed method limit of detection and discover possible PCR inhibition in stream water extracted samples.
1. Resuspend HF183 G-block:
    - a. Before opening the tube, centrifuge at a minimum of 3000 x g for 2-3 seconds to ensure all materials are at the bottom of the tube.
    - b. Add TE buffer to reach final concentration of 10 ng/uL.
      - i. G-block we received was 500 ng, dried.
      - ii. Add 50 uL of TE buffer to achieve 50 ng/L of HF183 Gblock.
    - c. Vortex briefly.
    - d. Incubate at 50C for 20 min.
    - e. Briefly vortex and centrifuge (minimum of 3000 x g for 2-3 seconds).
  2. Standard curve preparation:
    - a. Calculate number of copies in per dry mass of Gblock:
      - i. 
$$\text{Number of copies} = \frac{X(6.0221 \times 10^{23})}{(N \times 660 \frac{g}{mol})(1 \times 10^9 \frac{ng}{g})}$$
      - ii. X = Gblock dry mass (ng); N = Number of base pairs in Gblock
      - iii. Our Gblock was calculated to contain  $4.959 \times 10^{12}$  copies.
      - iv. That yields  $9.918 \times 10^{10}$  copies per uL.
    - b. Using suspended Gblock, make a serial dilution going from a  $10^8$  order of magnitude dilution down to a  $10^1$  using molecular grade water as the dilution solvent.
    - c. Repeat part b using extracted stream water DNA as the dilution solvent.
    - d. Aliquot 11 uL of standard curve samples into separate sets of strip tubes and send to University of Georgia Genomics core for ddPCR analysis.
  3. ddPCR Sample analysis
    - a. Following DNA extraction, aliquot 11 uL of DNA extract into labeled strip tubes and deliver to University of Georgia Genomics Core for ddPCR with automated droplet generator assay.
      - i. ddPCR assay recipe and conditions adapted from Cao et al. (2019) and BioRad Expert Assay Design (Appendix 1).
      - ii. All results were back calculated and reported as copies per 100 mL.



**CrAssphage qPCR Assay (Malla et al., 2019):**

4. Master mix for CrAssphage qPCR assay was prepared by combining the following reagents in biosafety hoods to reduce risk of contamination in a pre-PCR designated room. Quantities of reagents are for a single reaction. Master Mixes were made in batches and calculated based on total number of samples and replicates needed. Reagents were added in the order indicated below. Master mix was vortexed after adding each reagent with exception of the addition of TaqPath. TaqPath denatures when vortexed which required us to pipette mix the master mix after adding TaqPath.
  - a. RNase-Free water: 9 uL
  - b. Forward Primer (20 uM): 0.4 uL
  - c. Reverse Primer (20 uM): 0.4 uL
  - d. Probe (FAM reporter; 20 uM): 0.2 uL
  - e. TaqPath: 5 uL
5. 54 uL of prepared master mix was aliquoted into individual strip tube wells.
6. 18 uL of each sample DNA extract were added to individual wells of 54 uL of master mix and pipette mixed.
7. 20 uL of each sample master mix solution was aliquoted in triplicate into a 384 well plate and sealed with optically active adhesive film for analysis using a BioRad CFX384 Touch Real-Time PCR Detection System under the below cycling conditions.

1. 95°C	30 seconds	Repeat for a total of 40 cycles
2. 95°C	5 seconds	
3. 56°C	30 seconds	

8. All results were converted to copies per 100 mL using a standard curve.

**Fecal Coliform Culturing (Center for Food Safety and Applied Nutrition, 2020):**

1. Water Sample Filtration for Fecal Coliform Bacteria:
  - a. Set up a filtration manifold with two Erlenmeyer flasks with appropriately sized rubber stoppers and effluent tubing. They should be connected linearly to the vacuum pump in the following order:
    - i. Manifold -> Large Erlenmeyer flask -> Smaller Erlenmeyer flask -> vacuum pump nozzle.
  - b. Attach sterile filter towers in on the outer two filter tower ports.
  - c. Using flame sterilized forceps, remove mixed cellulose ester filters from packaging and place **grided side up** between filter tower magnetic housing and filter platform.
  - d. Rinse filter with enough autoclave sterilized 1X PBS to wet the entire filter surface.
  - e. Remove water sample bottle from 4C storage and shake vigorously (~25 times) to ensure uniform bacterial distribution.
  - f. **With filter tower vacuum in the off position**, aliquot 10 mL of shaken water sample to the filter.
    - i. Due to observed variation in fecal coliform bacteria colony forming unit growth during method development, we diluted sample water 1:10 and 1:100 via serial dilution and plated both dilution levels.
    - ii. Diluting samples increased countability of plates, therefore reducing counting bias.
  - g. Turn filter tower vacuum to the on position and pull the entire water sample through the filter.
  - h. Once all the water sample has passed through the filter, rinse the side of the filter funnel with 20-30 mL of sterile 1X PBS to wash residual sample off the sides of the filter tower.
  - i. With flame sterilized forceps, we place filters directly onto 60x15mm mFC agar plates.
  - j. Remove filter funnel tower and soak in 70% EtOH and rinse in DI water.
  - k. Place sample plates with filters in a foam box in an incubator at 37°C for 2 hours. Afterwards, increase incubation temperature to 44.5°C for 16 hours. Total incubation time is 18 hours.
  - l. Repeat steps b-k for each replicate (2) of each dilution, site, and event.
2. Plate counting
  - a. Fecal coliform bacteria colony forming units grow with a royal blue color on mFC plates.
  - b. Pick dilution level for each sample based on countability of colony forming units. Countable range is between 20 and 80 colony forming units.
  - c. Count colony forming units for each replicate at the determined countable dilution level.

- d. Average the two replicate counts together for final colony forming unit count for that sample.
  - i. All results were back calculated and reported as copies per 100 mL.

#### Works Cited

1. Cao, Y., Raith, M. R., & Griffith, J. F. (2015). Droplet digital PCR for simultaneous quantification of general and human-associated fecal indicators for water quality assessment. *Water Research*, 70, 337–349. <https://doi.org/10.1016/j.watres.2014.12.008>
2. Center for Food Safety and Applied Nutrition. (2020). *Bam Chapter 4*. U.S. Food and Drug Administration. <https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria>
3. Malla, B., Ghaju Shrestha, R., Tandukar, S., Sherchand, J. B., & Haramoto, E. (2019). Performance Evaluation of Human-Specific Viral Markers and Application of Pepper Mild Mottle Virus and CrAssphage to Environmental Water Samples as Fecal Pollution Markers in the Kathmandu Valley, Nepal. *Food and Environmental Virology*, 11(3), 274–287. <https://doi.org/10.1007/s12560-019-09389-x>

## **Appendix 1:**

### **Expert Design Assay: Unique Assay ID dEXD79194188**

#### **Description**

The HF183 ddPCR assay is a 20X assay designed to quantify HF183 of *Bacteroides* in samples such as wastewater for epidemiology investigations using Droplet Digital PCR. This assay should be validated prior to use by the end-user.

#### **Targets Detected by the Assay**

Name	Target	Fluorophore	Amplicon length
HF183	DNA	FAM	92

#### **Synthetic DNA Sequence for Designing Positive Controls**

Gblock:

CCCATGAGACATACAAAAAGGTAATGCCGCCTCGCTAGGTGAGCTACAGCTCGATT  
GTCACGTTAAGCTGGCCAGTTCAAATCCCTGTTGCATTAATTTACCAGTAATGAGT  
CTTTTTCATCATGAGTTCACATGTCCGCATGATTAAAGGTATTTTCCGGTAGACGAT  
GGGGATGCGTTCCATTAGCTCGAGATAGTAGGCGGGGTAACGTGACCTGGCTGTA  
GCTTAGGAGTAGCATGTTCTTTACGATCATAGTTCATTCATGAAACTATTTTATTCAT  
CTCTCGGTGAAGCTTCAGAGAACTTTATTAGGTATGTTTACTTAACAAAAGAGTGCA  
TTGGGGGTGATGAAGCCTAGTCAAATTCACAGAAAGCTAAGGAGGTCTCGACTATA  
CGCCCGTTTTTCGGATC

#### **Host**

Human

#### **Other Reagents required**

ddPCR Supermix for Probes (No dUTP), 2 × 1 ml – 1863023

#### **ddPCR Reaction Setup**

Component	Volume per Reaction, $\mu$ l	Final Concentration
2x ddPCR Supermix for Probes (No dUTP)	10	1x
20x ddPCR Assay	1	1x
Target sample and/or DNase-free water	up to 9 $\mu$ l	variable
Total Volume	<b>20**</b>	–

\*\* For the Automated Droplet Generator, prepare 22  $\mu$ l per well.

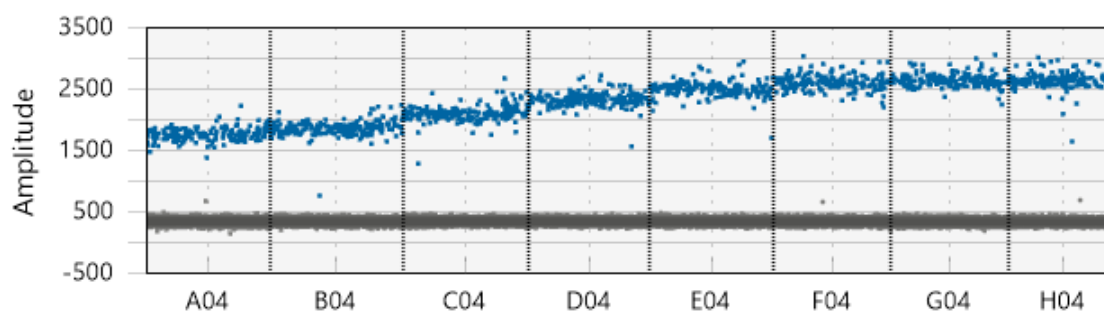


### **Recommended Thermal Cycling Conditions for Bio-Rad's C1000 Touch Thermal Cycler\***

Cycling Step	Temperature, °C	Time	Ramp Rate	Number of Cycles
Enzyme activation	95	10 min	2°C/sec	1
Denaturation	94	30 sec		40
Annealing/extension	55	1 min		1
Enzyme deactivation	98	10 min	1°C/sec	1
Hold (optional)	4	Infinite		1

\* Use a heated lid set to 105°C and set the sample volume to 40 µl. Further optimization of thermal cycling conditions may be performed if necessary.

### **Example Data**

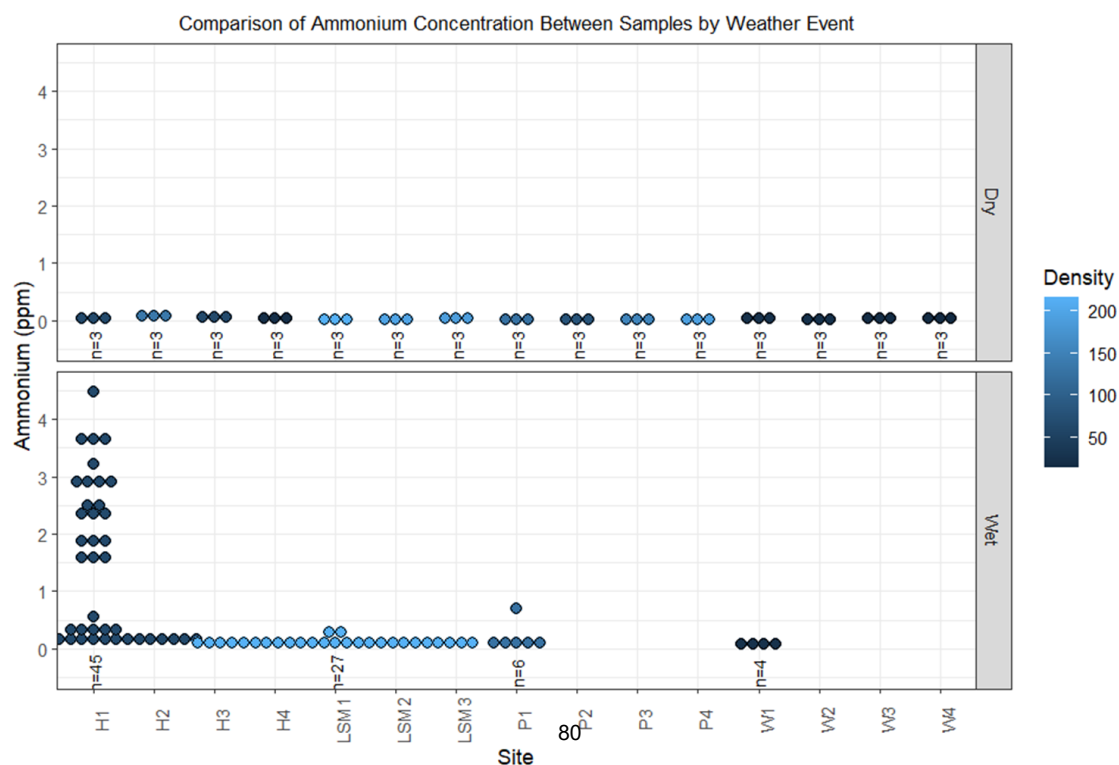
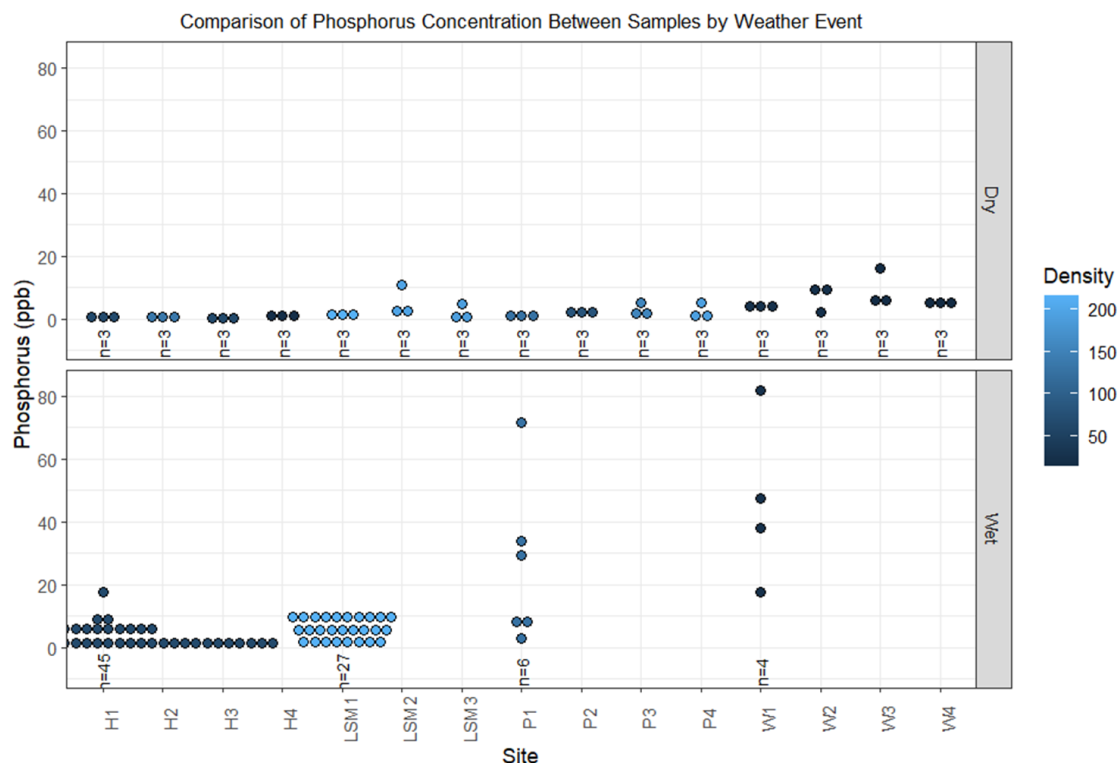


A 1-D droplet plot of an annealing/extension temperature gradient 62–52°C. Wells A04–H04 correspond to the temperature gradient 62–52°C.

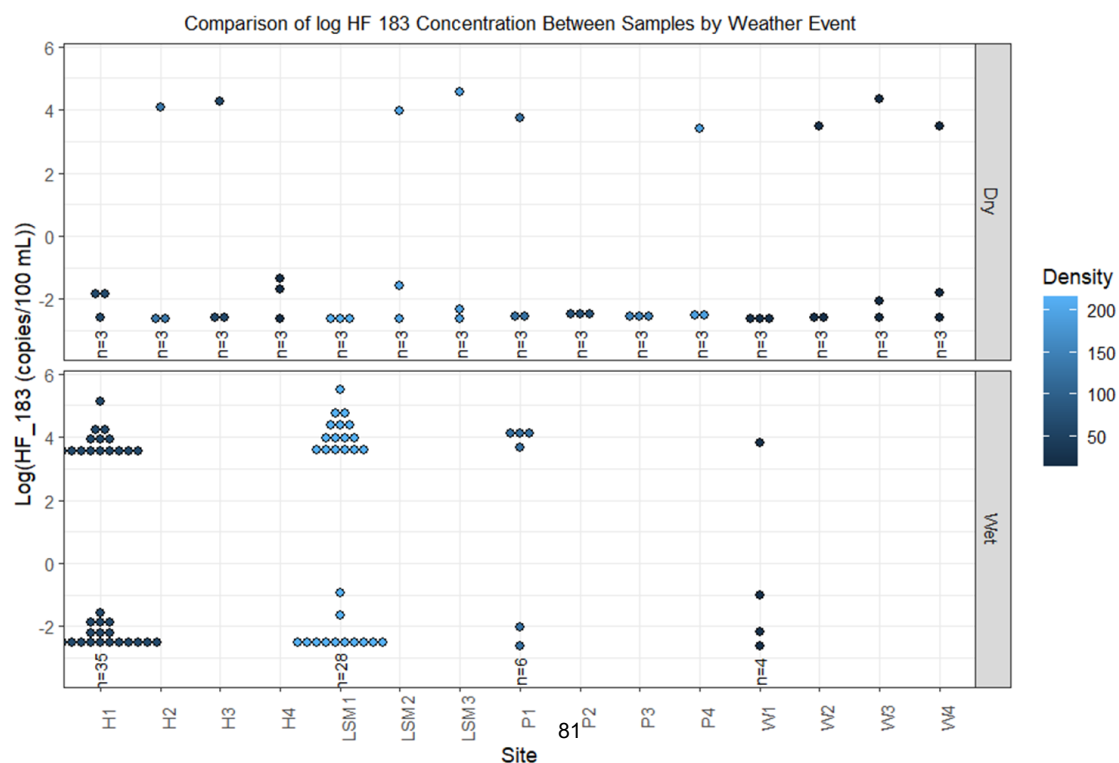
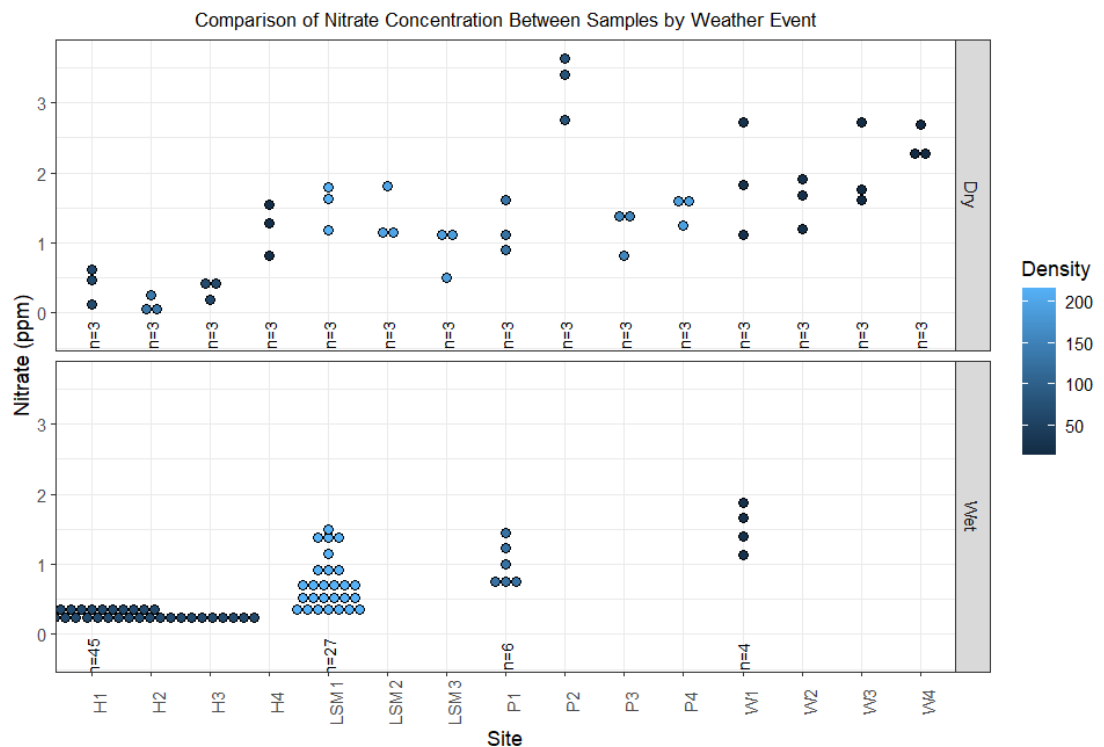
## **Appendix C:**

**Dry and wet weather data by watershed**

**Dry and wet weather data by watershed.** Letters indicate different watersheds (H: Honey Creek; LSM: Little Stone Mountain; P: Pond Fork; W: West Fork Little Creek) and numbers indicate different sites within each watershed. Only one site was outfitted with an ISCO sampler in each watershed; therefore, they were the only sites sampled during wet weather events. Numbers of samples collected at each site (n) in each weather condition (dry/wet) are indicated above each sample site.

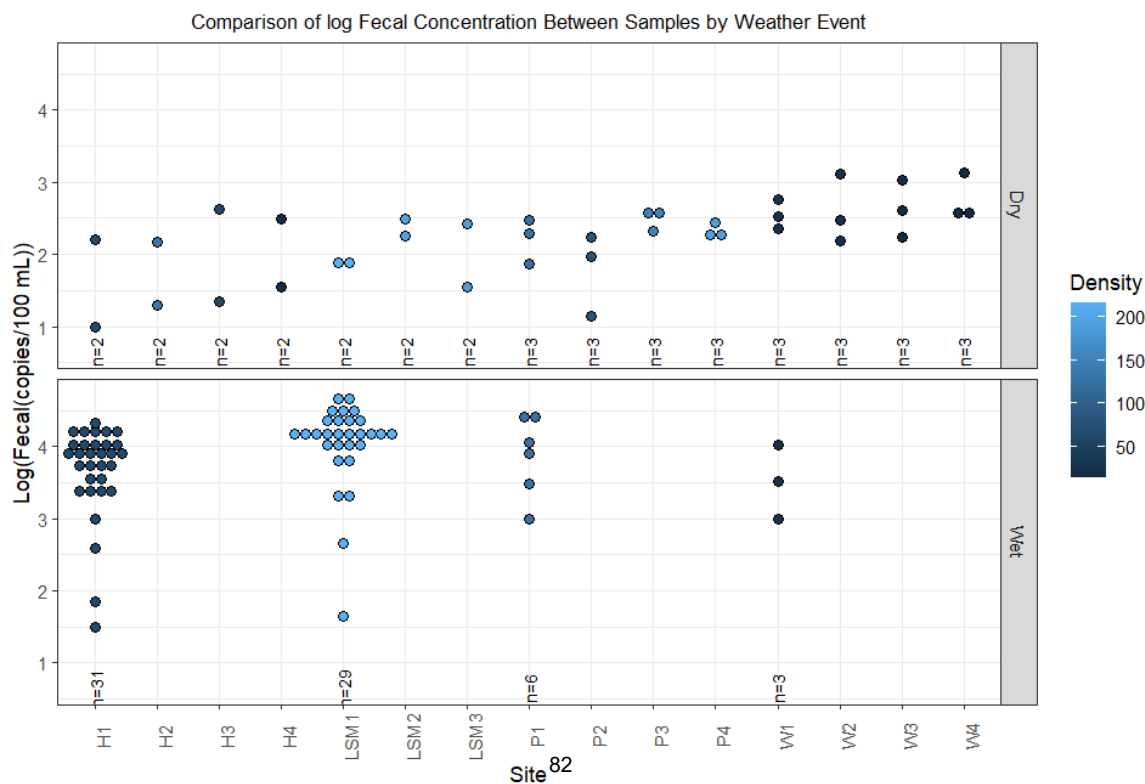
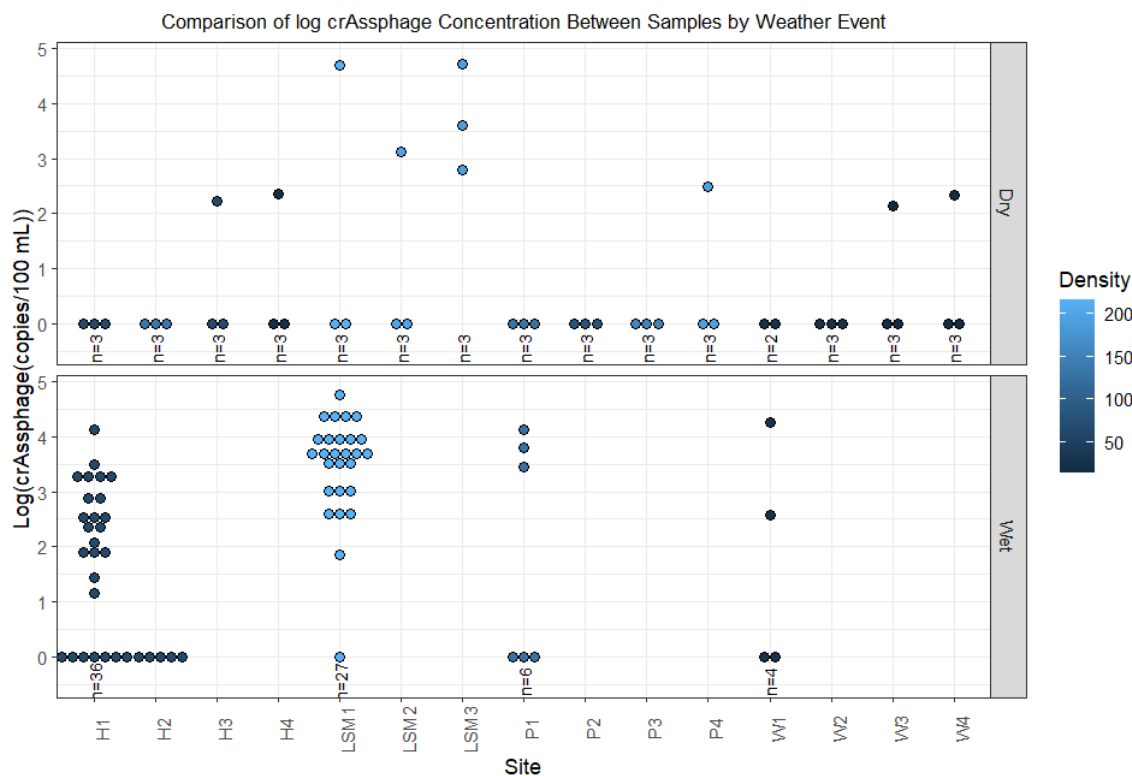


**Dry and wet weather data by watershed.** Letters indicate different watersheds (H: Honey Creek; LSM: Little Stone Mountain; P: Pond Fork; W: West Fork Little Creek) and numbers indicate different sites within each watershed. Only one site was outfitted with an ISCO sampler in each watershed; therefore, they were the only sites sampled during wet weather events. Numbers of samples collected at each site (n) in each weather condition (dry/wet) are indicated above each sample site.



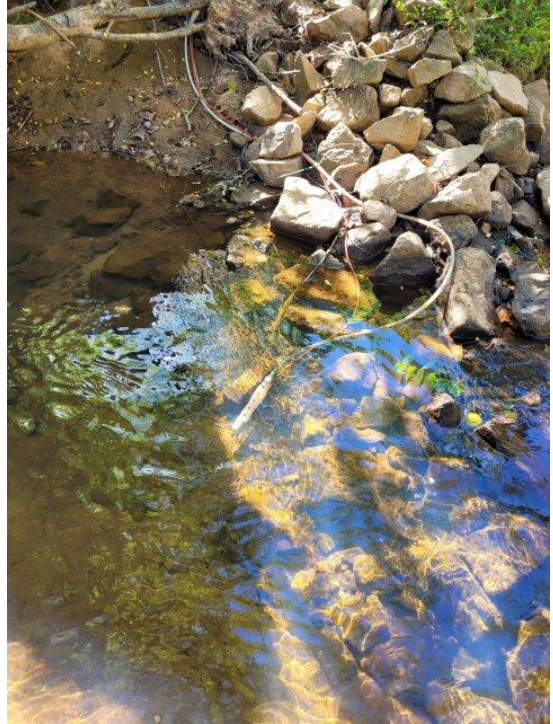


**Dry and wet weather data by watershed.** Letters indicate different watersheds (H: Honey Creek; LSM: Little Stone Mountain; P: Pond Fork; W: West Fork Little Creek) and numbers indicate different sites within each watershed. Only one site was outfitted with an ISCO sampler in each watershed; therefore, they were the only sites sampled during wet weather events. Numbers of samples collected at each site (n) in each weather condition (dry/wet) are indicated above each sample site.



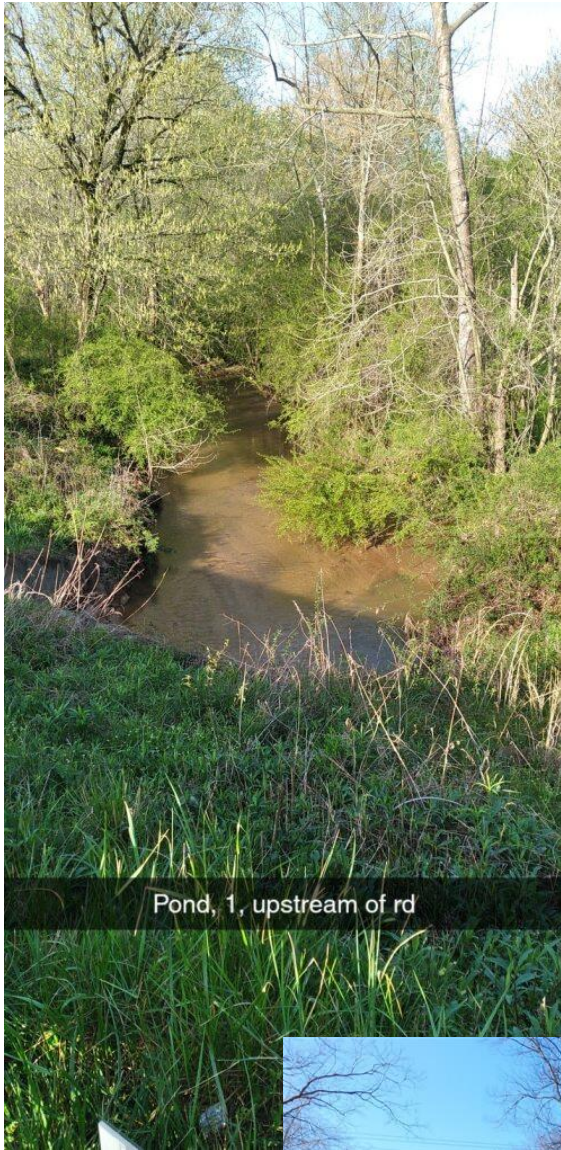
## **Appendix D:**

### **Images from the study**



ISCO set up





Pond before and after  
flood.

Note the equipment  
knocked over and  
downstream.







West Fork base flow and flooding.  
Images from trail cameras.



Little Stone Mountain base flow and flooding.  
Images from trail cameras.





Honey Creek base flow and flooding.  
Images from trail cameras.



West Fork, buried intake after storm moved sediment.



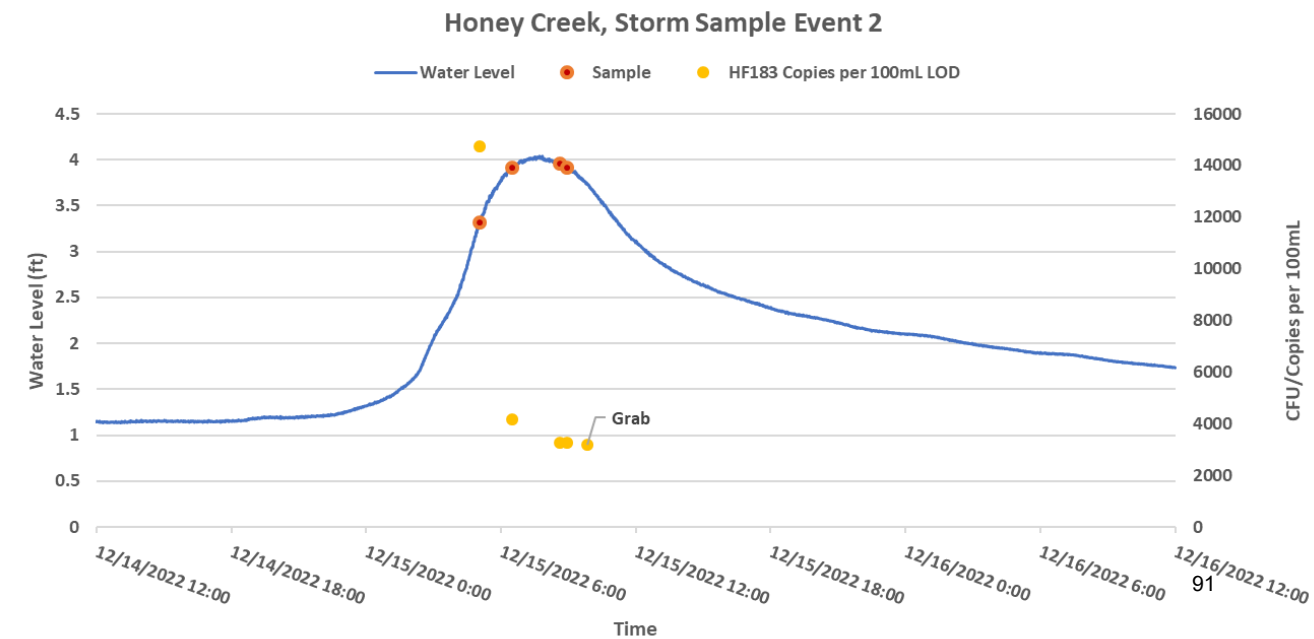
## **Appendix E:**

### **Visual comparison of automated and grab samples during sampling events**

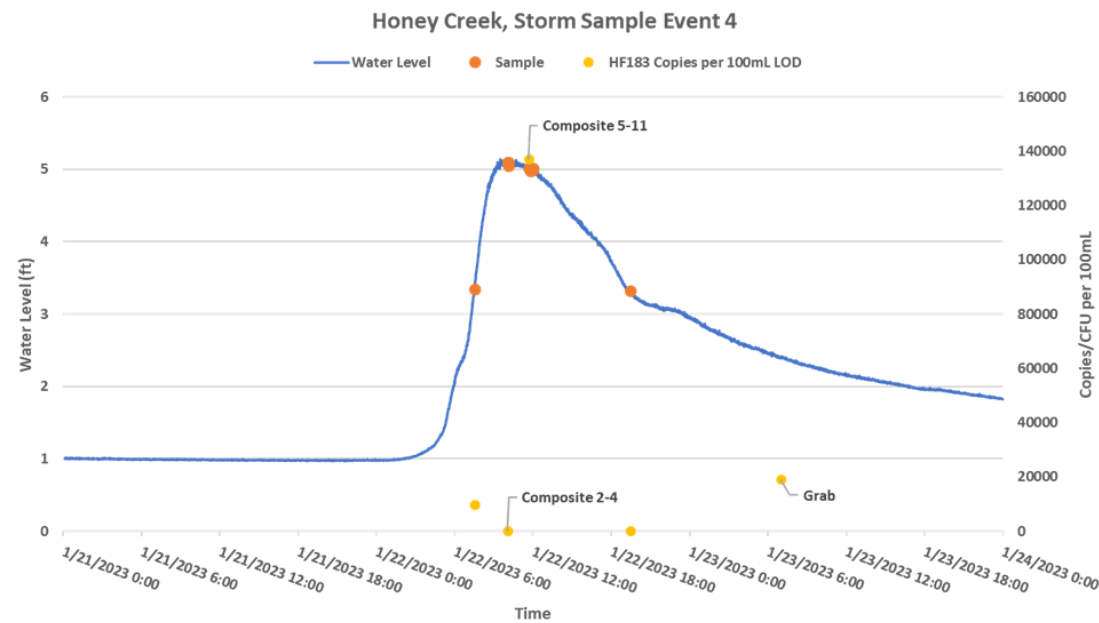
Please note, the Information included in the figures in this appendix includes the water level, the point in the hydrograph where the sample or samples were collected by the ISCO samplers, and the concentrations of the nutrients or human fecal markers collected during the storm event or in the grab sample collected after the storm event occurred. All images and raw data can be accessed here:

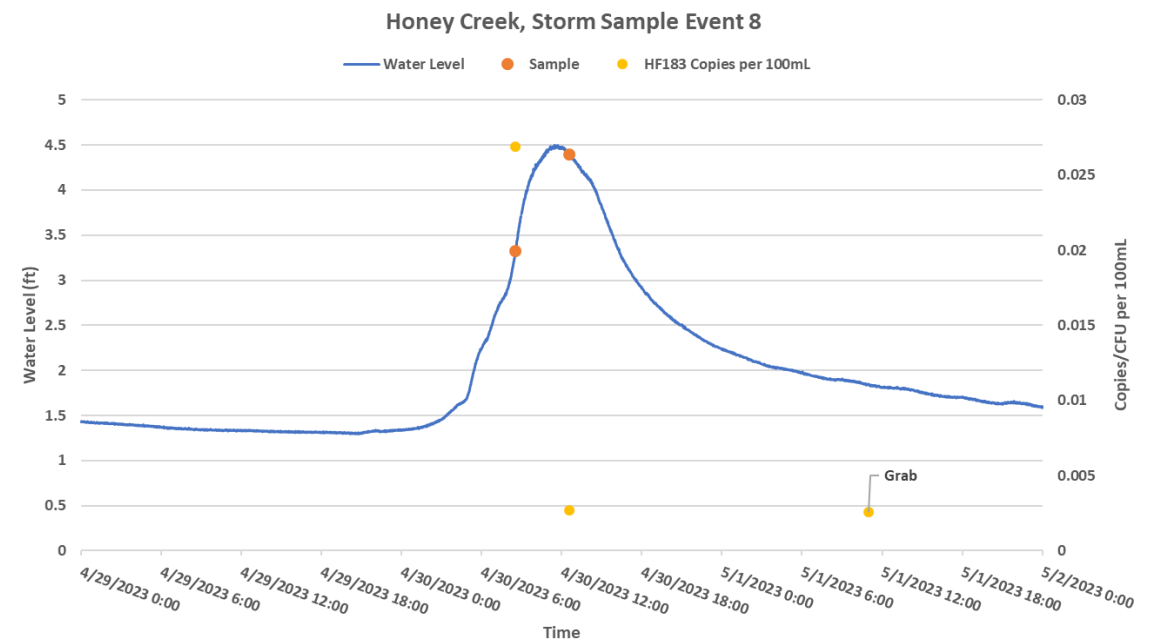
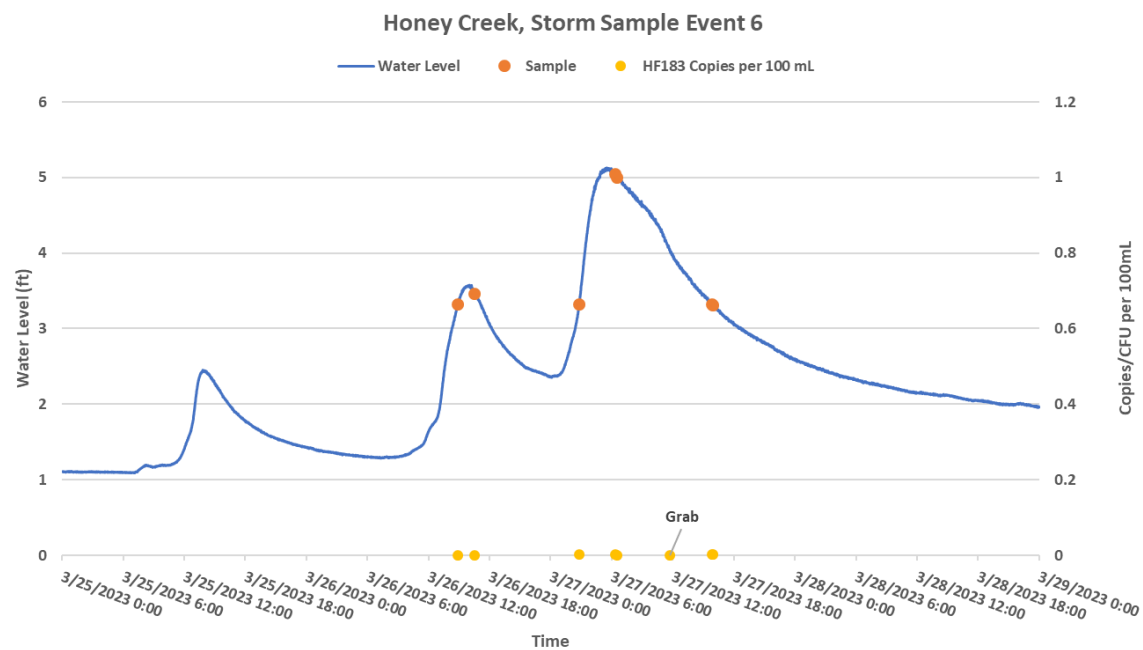
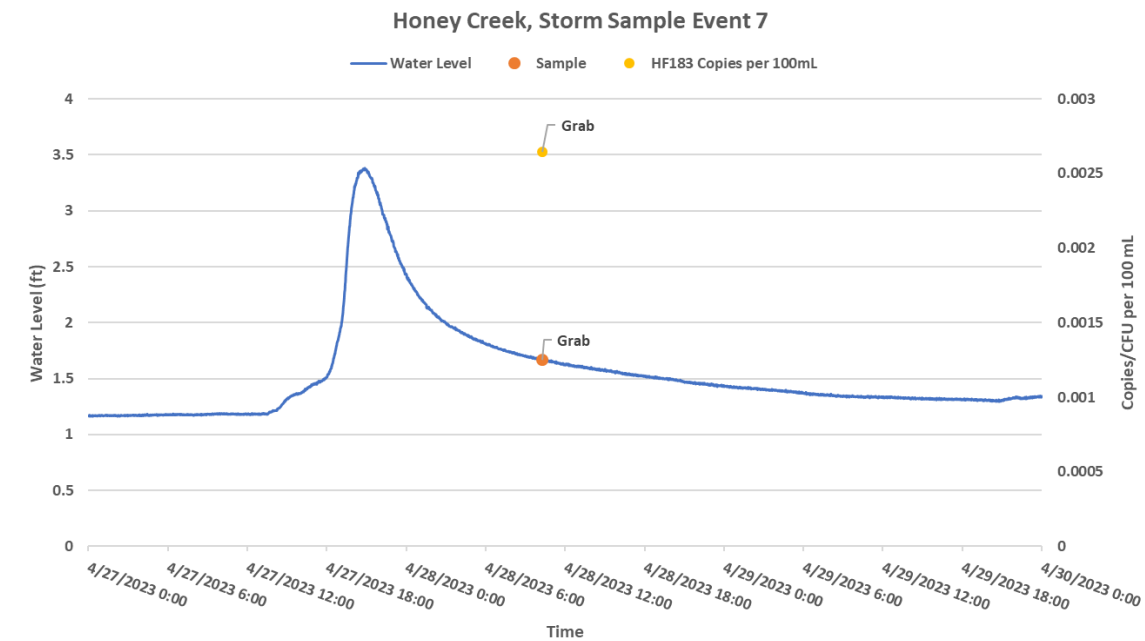
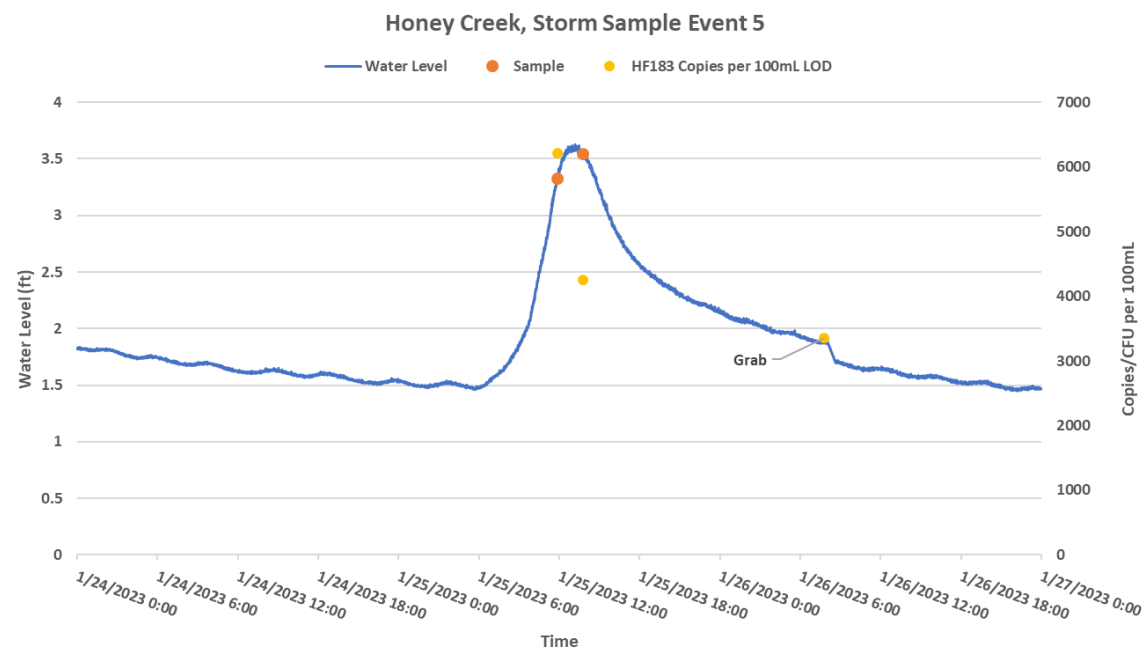
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● Sample      — Water Level      ● HF183 Copies per 100mL LOD



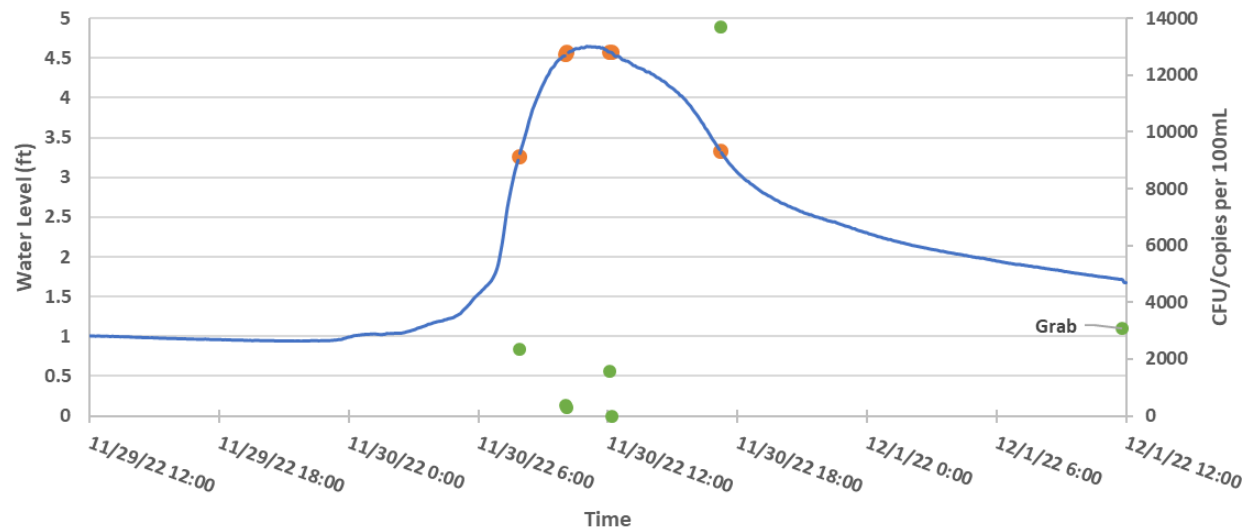
— Water Level      ● Sample      ● HF183 Copies per 100mL LOD





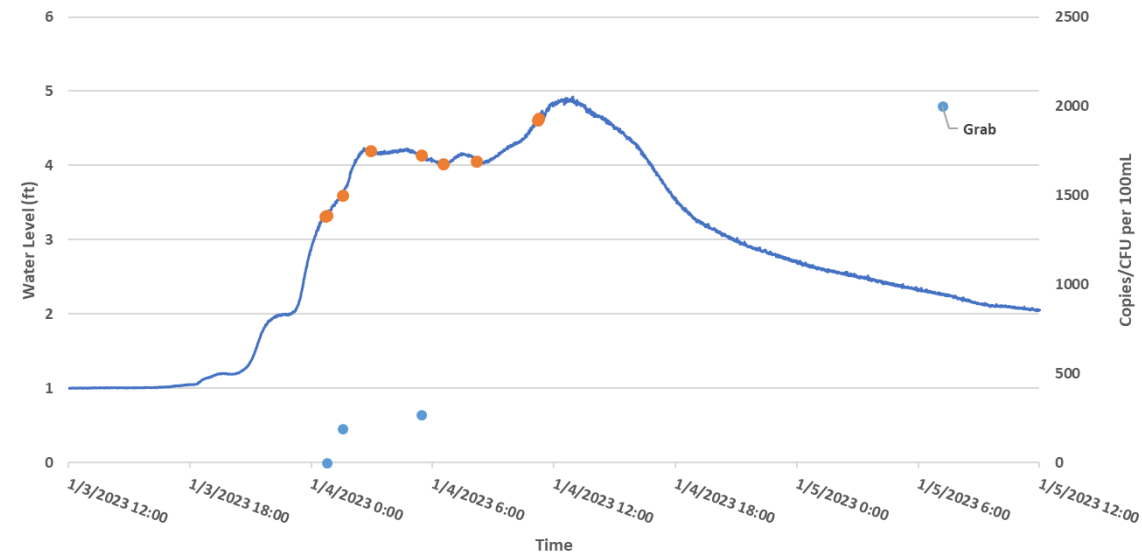
### Honey Creek, Storm Sample Event 1

● Sample — Water Level ● crAssphage Copies per 100mL



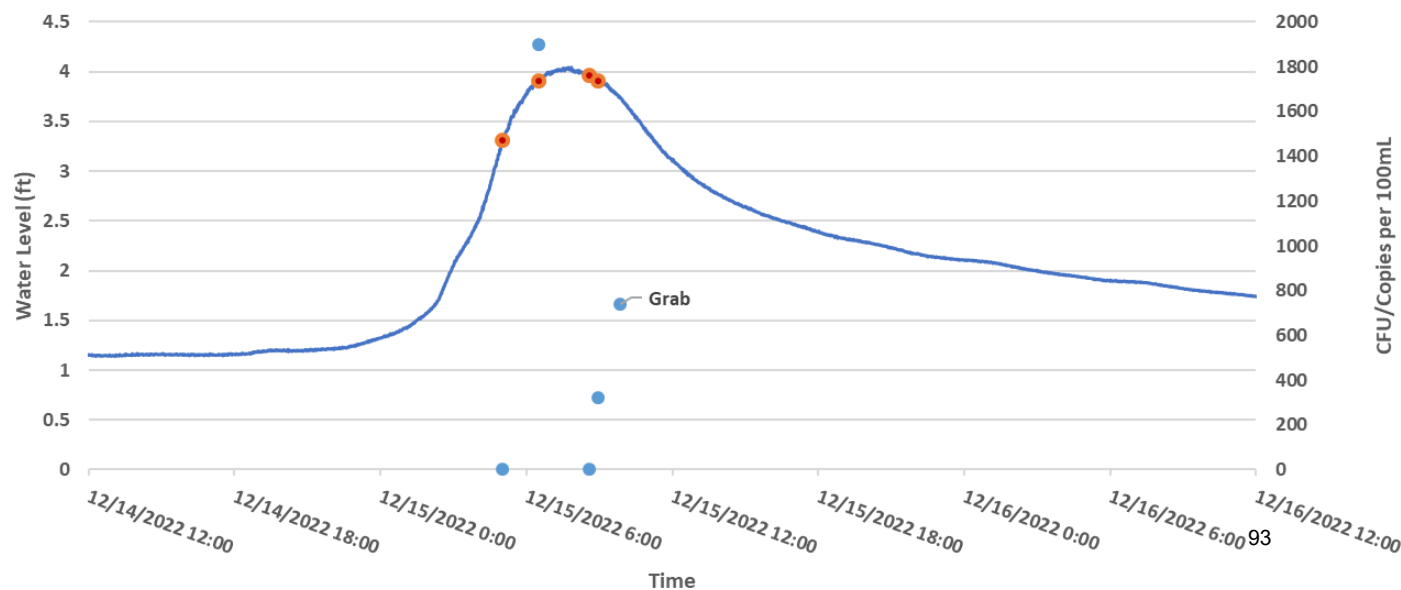
### Honey Creek, Storm Sample Event 3

— Water Level ● Sample ● crAssphage Copies per 100mL



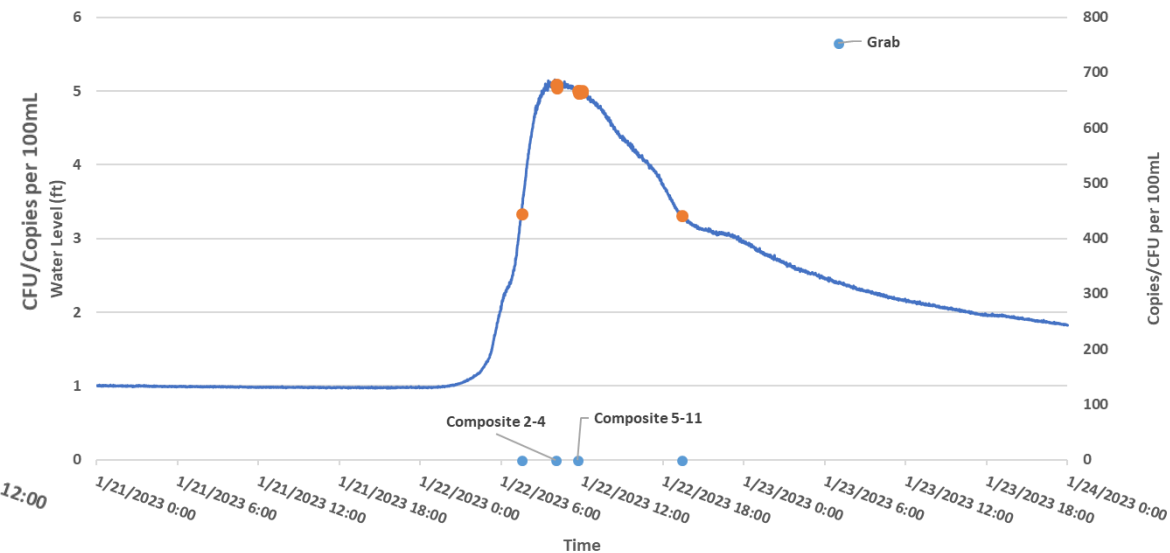
### Honey Creek, Storm Sample Event 2

— Water Level ● Sample ● crAssphage Copies per 100mL

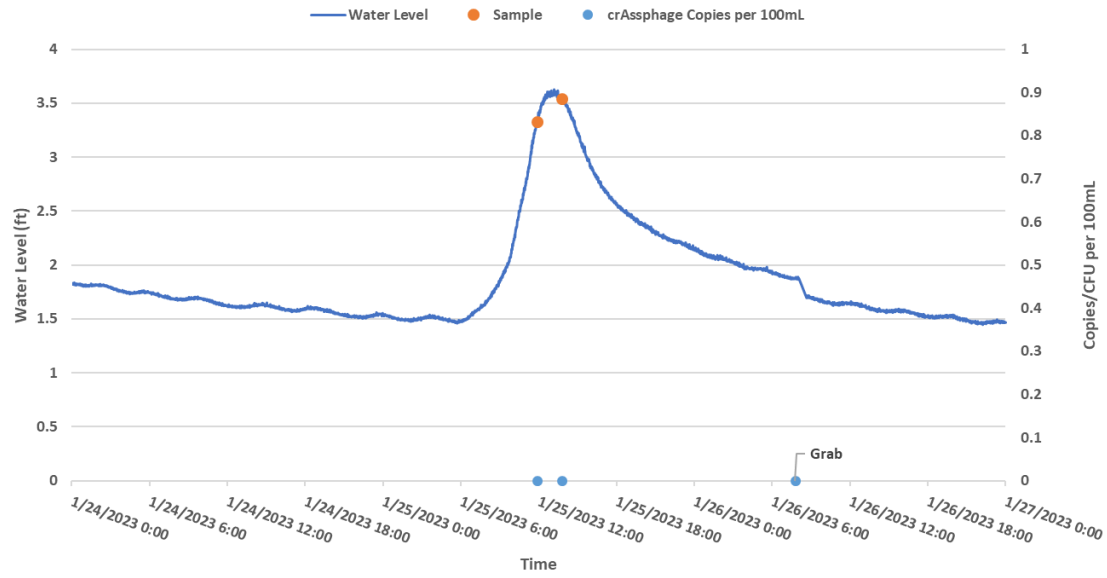


### Honey Creek, Storm Sample Event 4

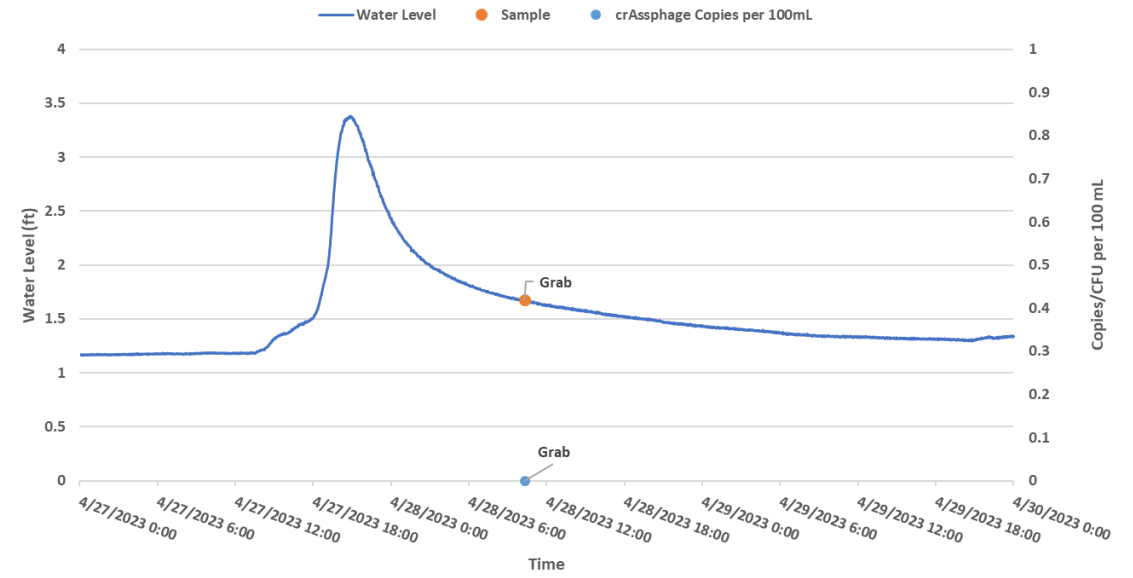
— Water Level ● Sample ● crAssphage Copies per 100mL



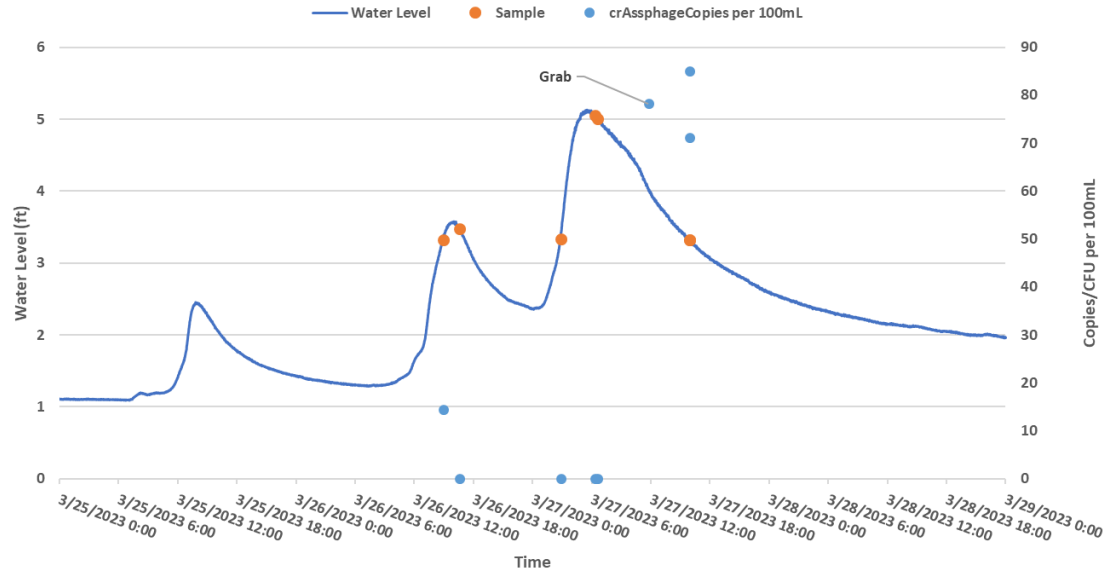
### Honey Creek, Storm Sample Event 5



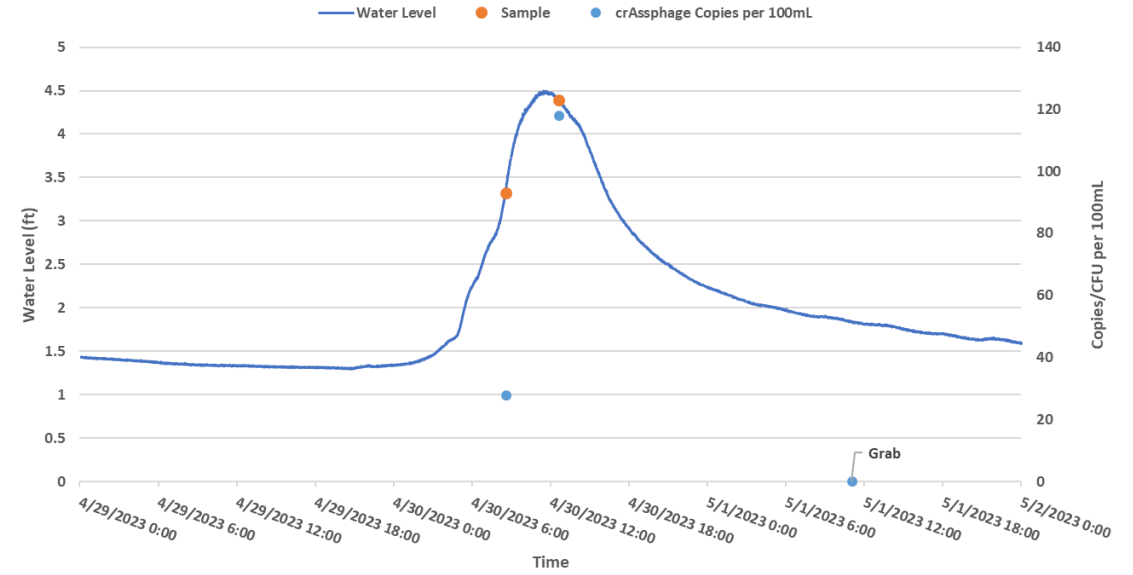
### Honey Creek, Storm Sample Event 7



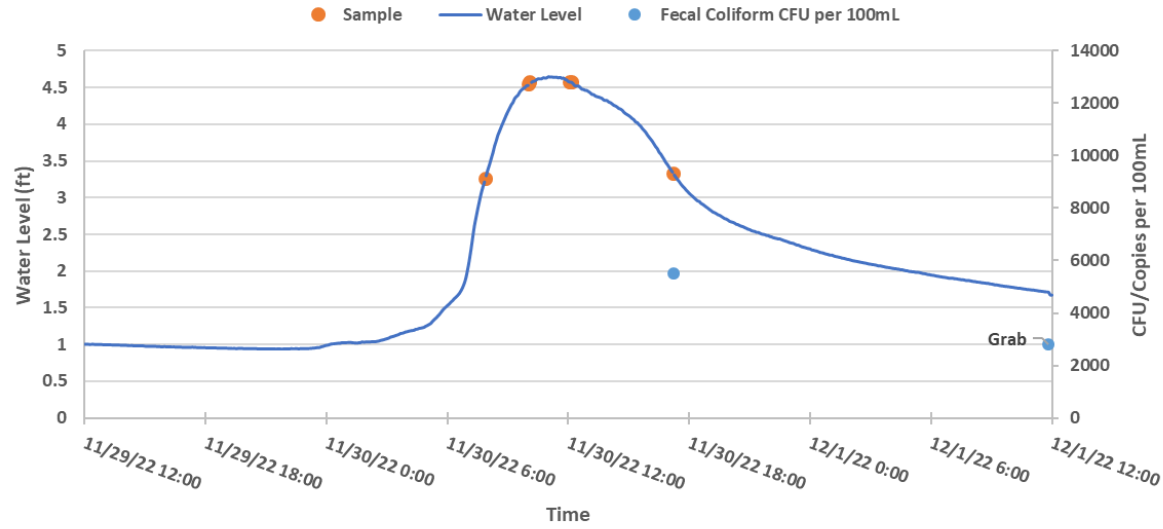
### Honey Creek, Storm Sample Event 6



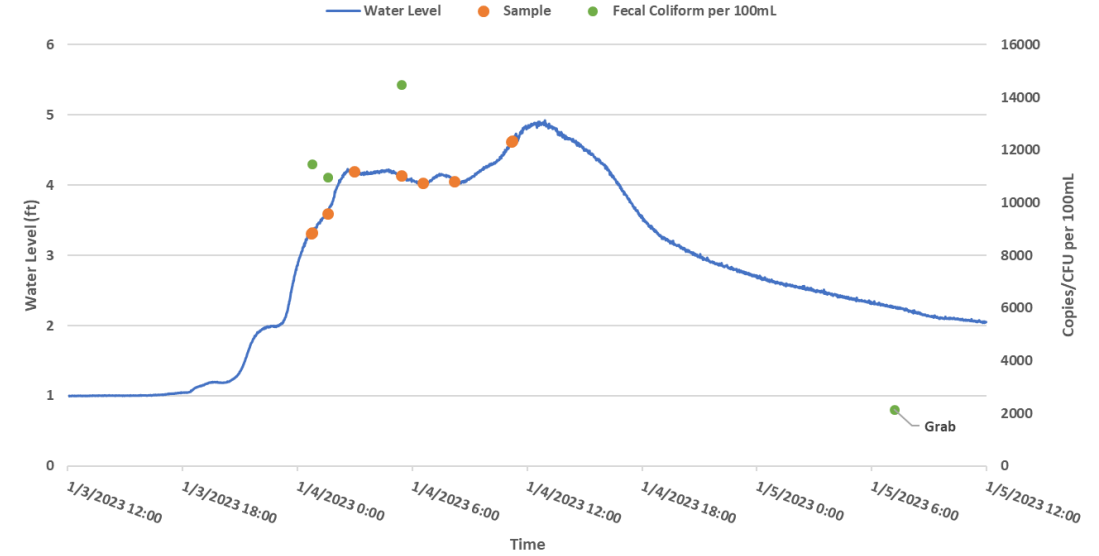
### Honey Creek, Storm Sample Event 8



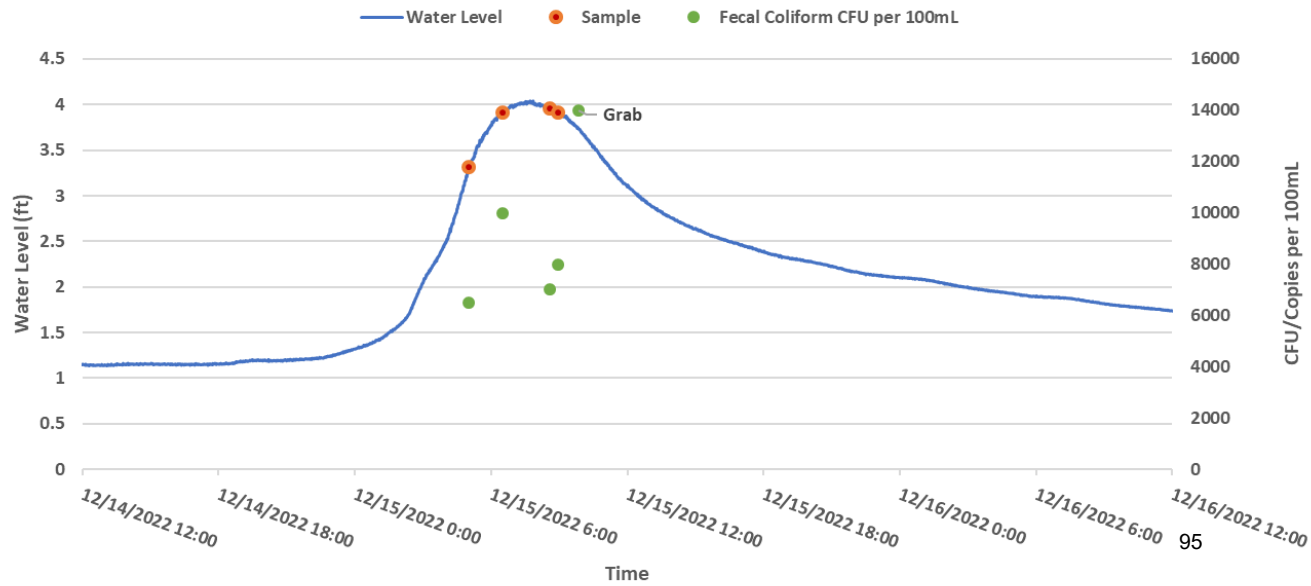
### Honey Creek, Storm Sample Event 1



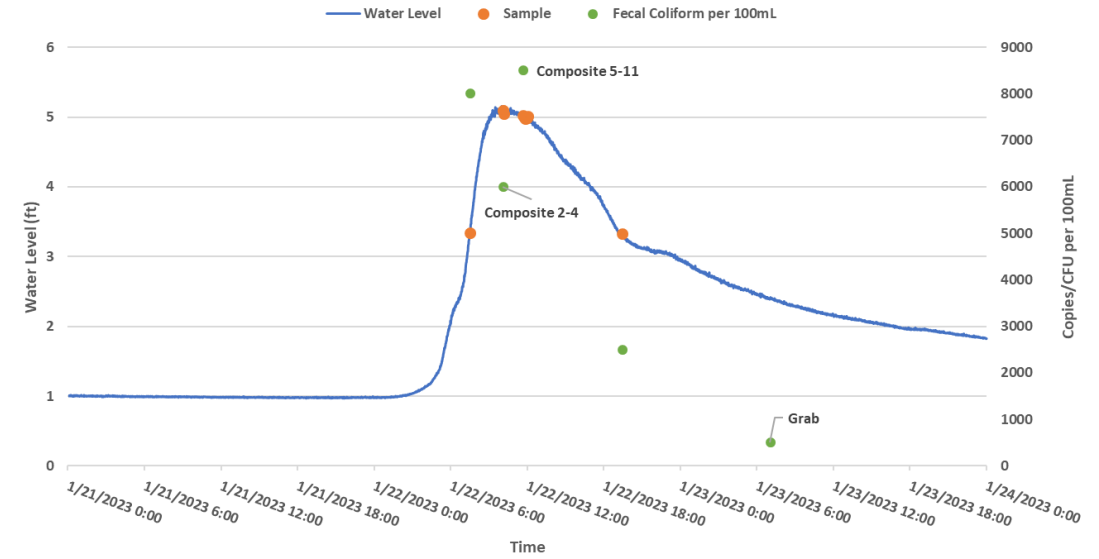
### Honey Creek, Storm Sample Event 3



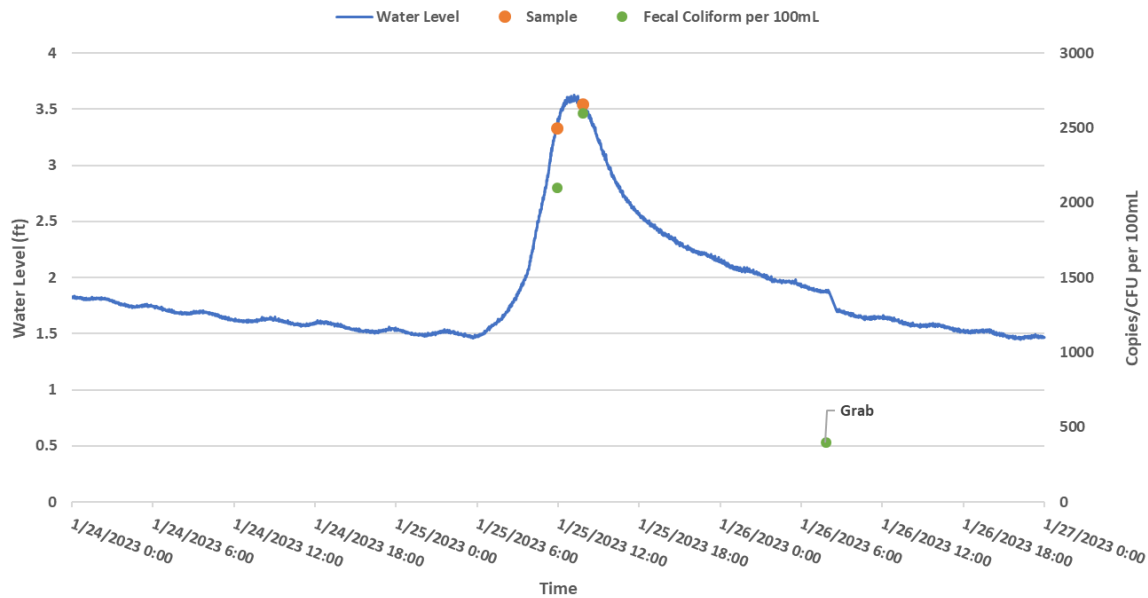
### Honey Creek, Storm Sample Event 2



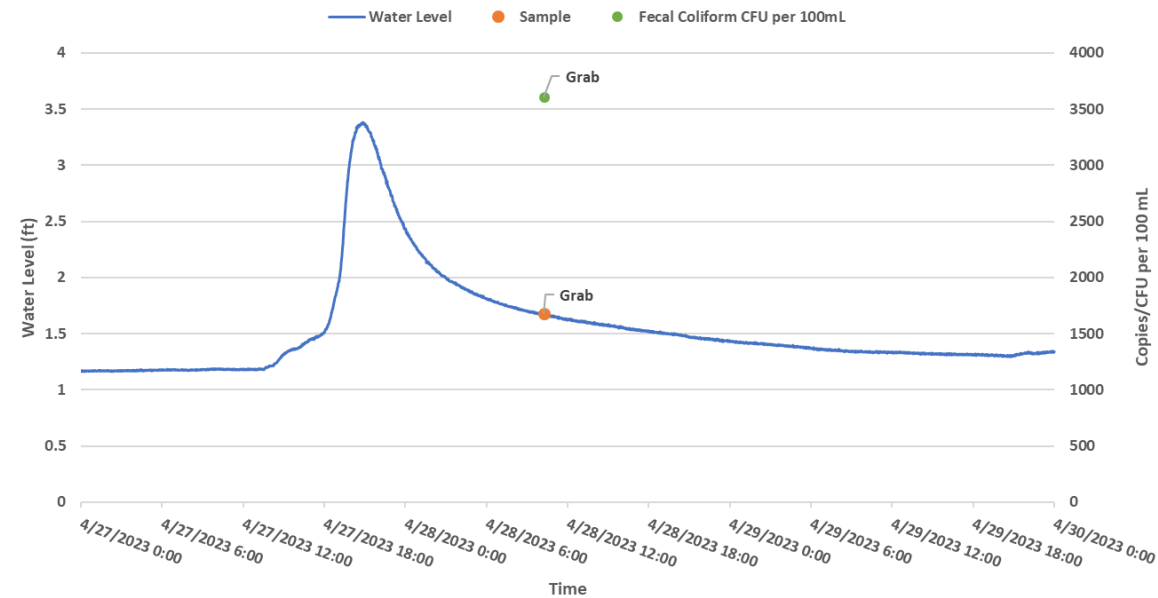
### Honey Creek, Storm Sample Event 4



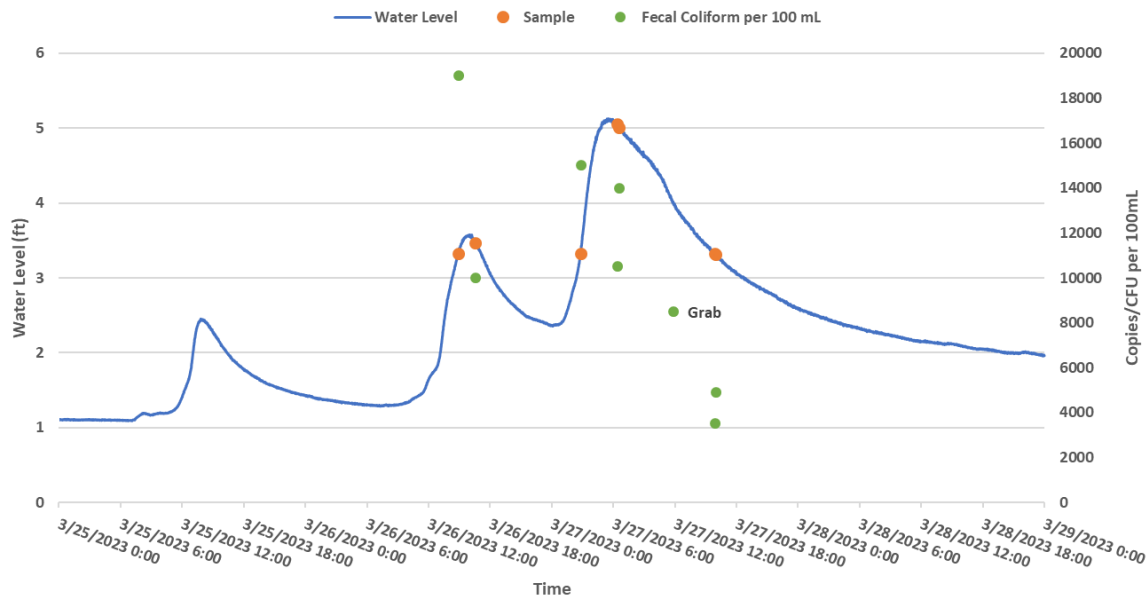
Honey Creek, Storm Sample Event 5



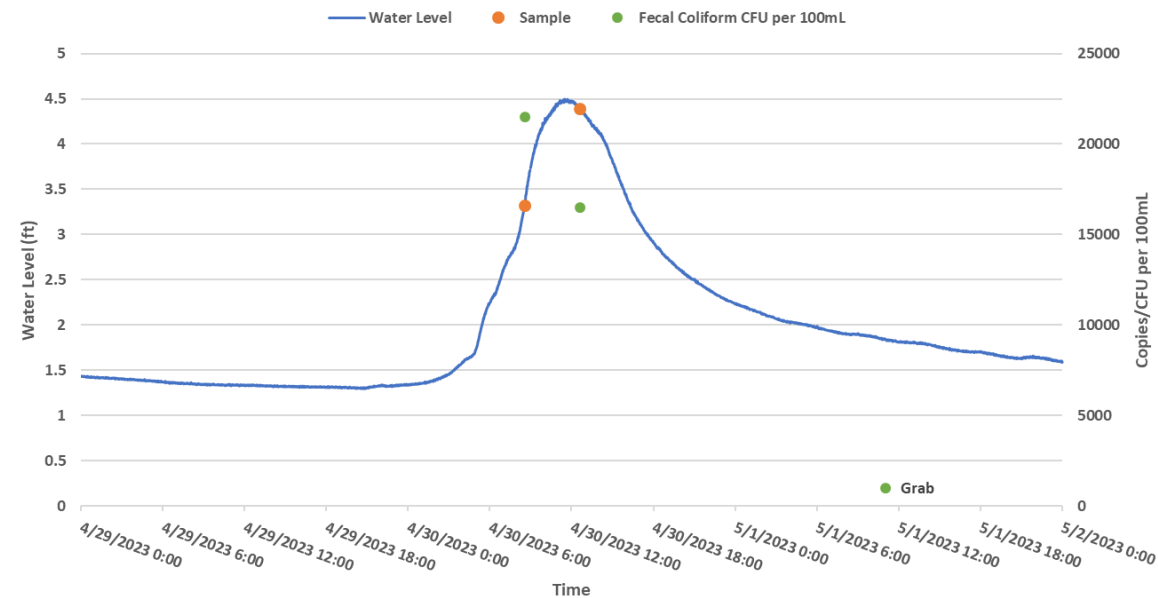
Honey Creek, Storm Sample Event 7



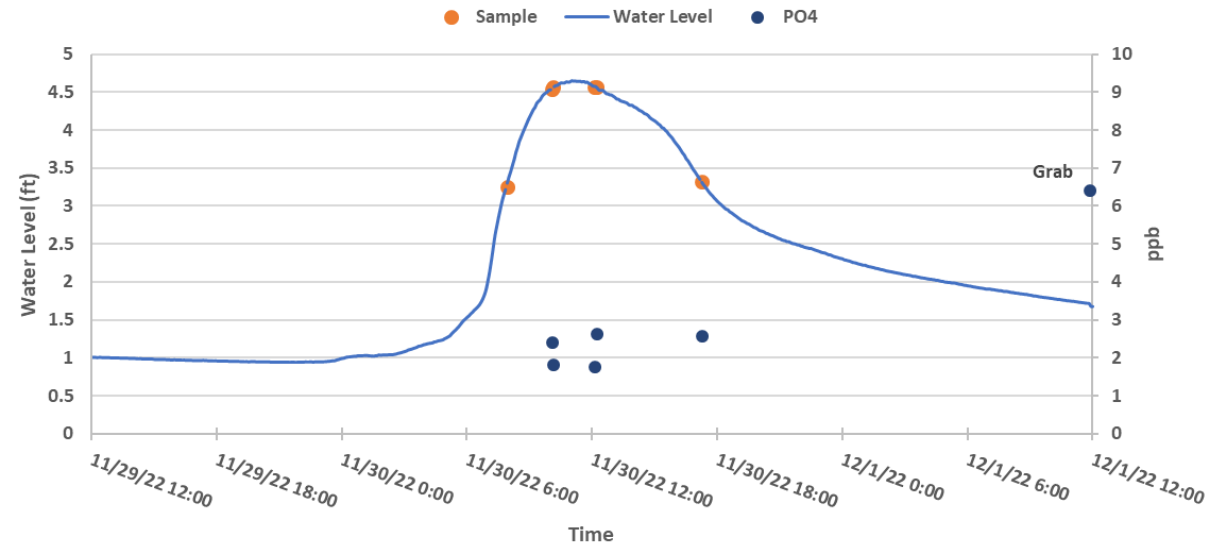
Honey Creek, Storm Sample Event 6



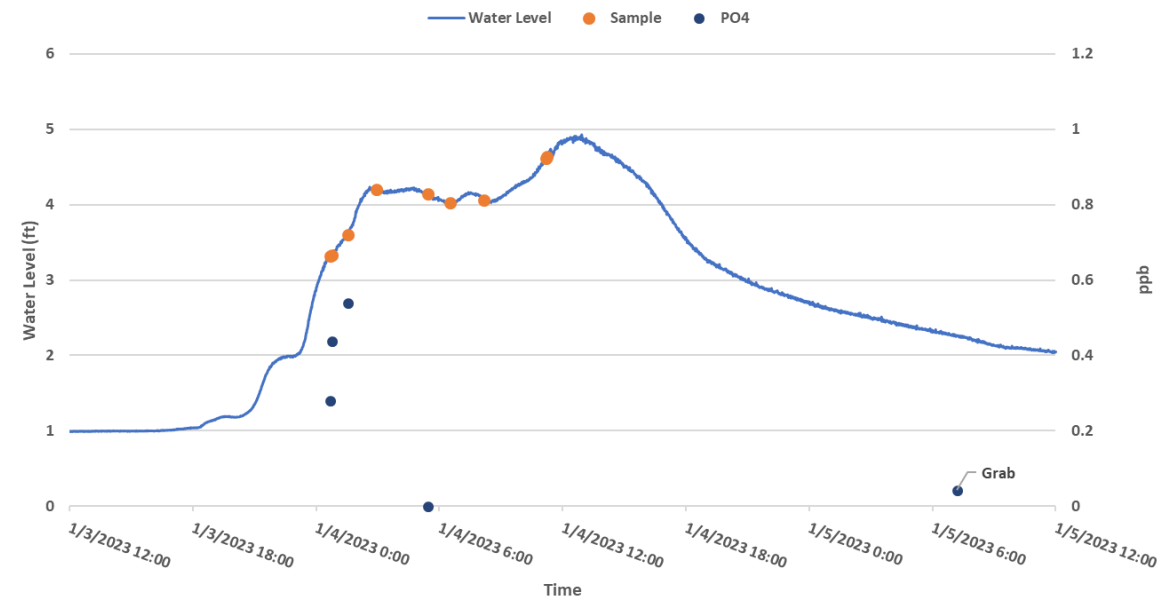
Honey Creek, Storm Sample Event 8



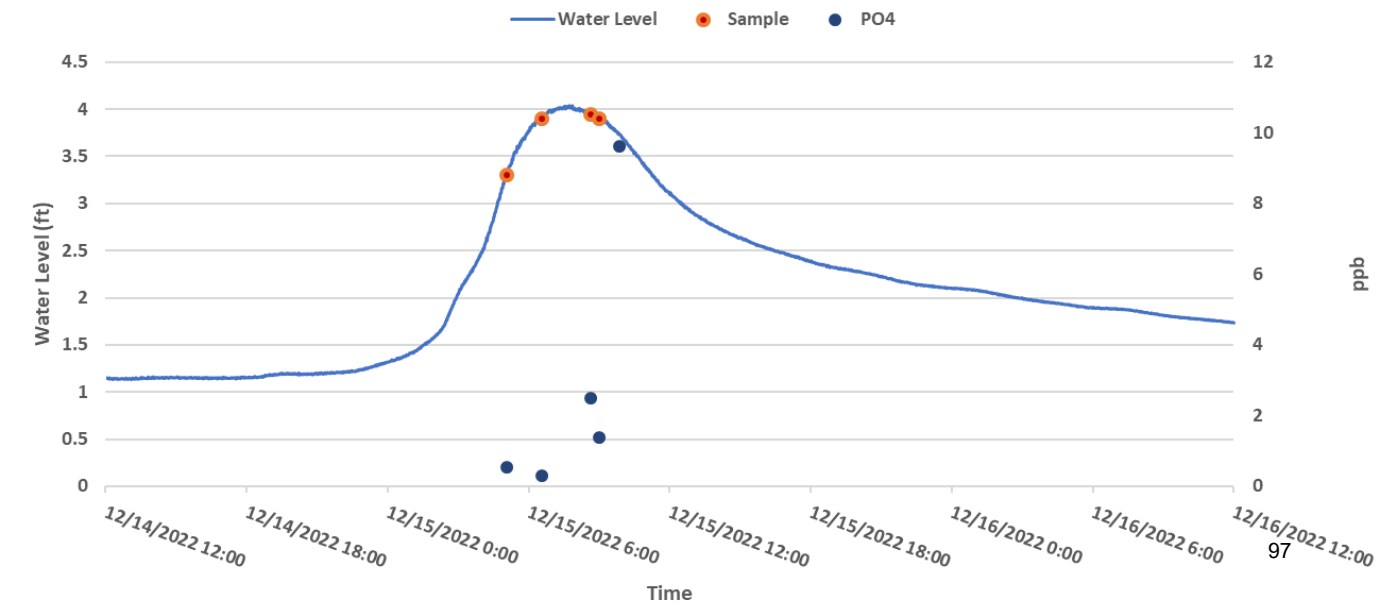
### Honey Creek, Storm Sample Event 1



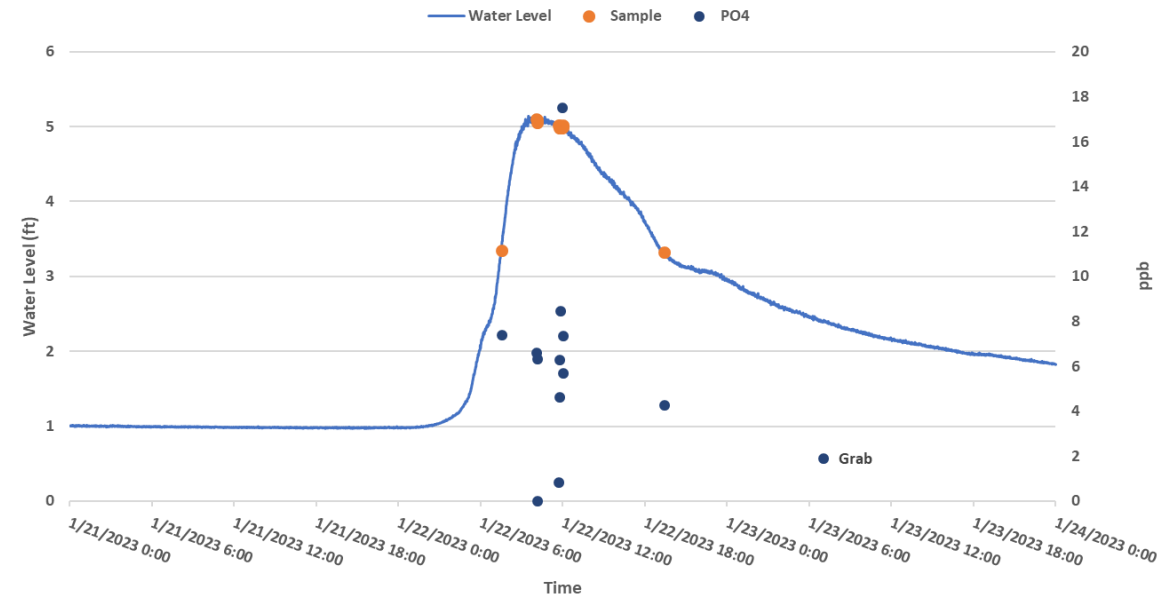
### Honey Creek, Storm Sample Event 3



### Honey Creek, Storm Sample Event 2

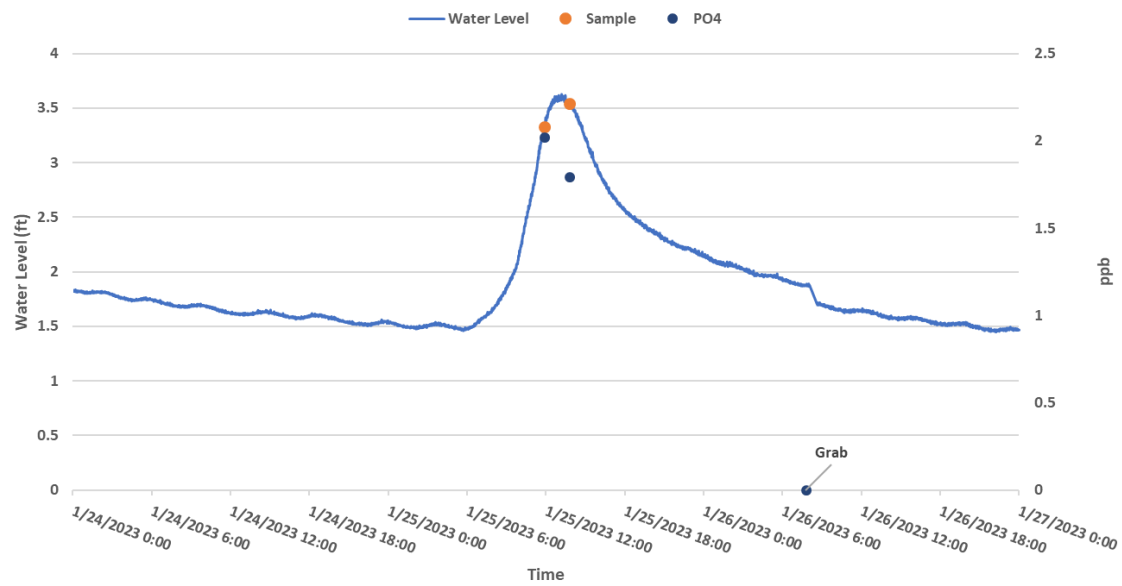


### Honey Creek, Storm Sample Event 4

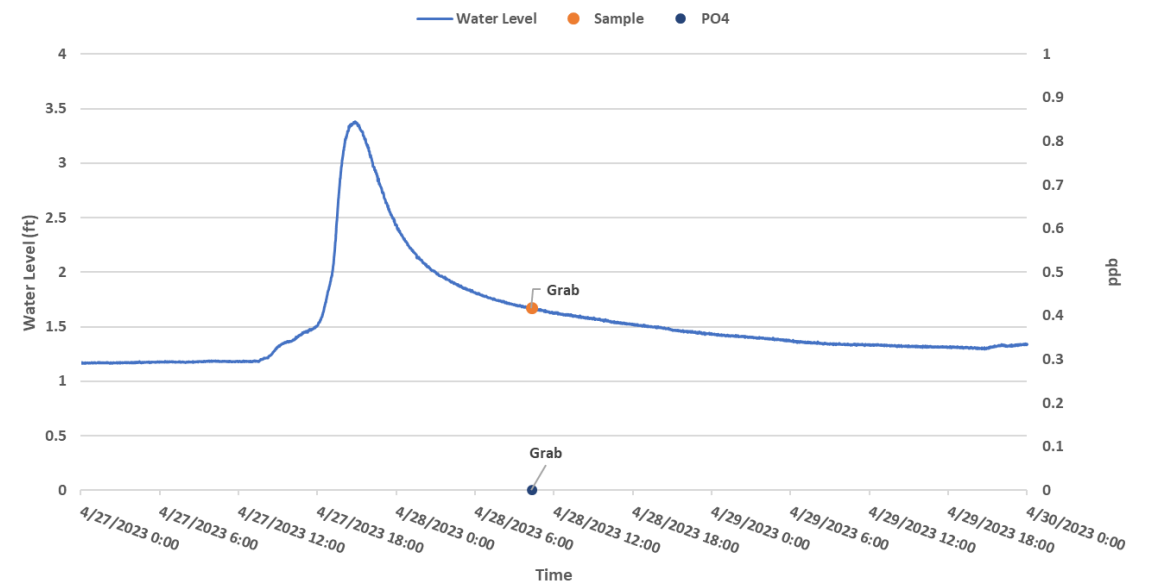




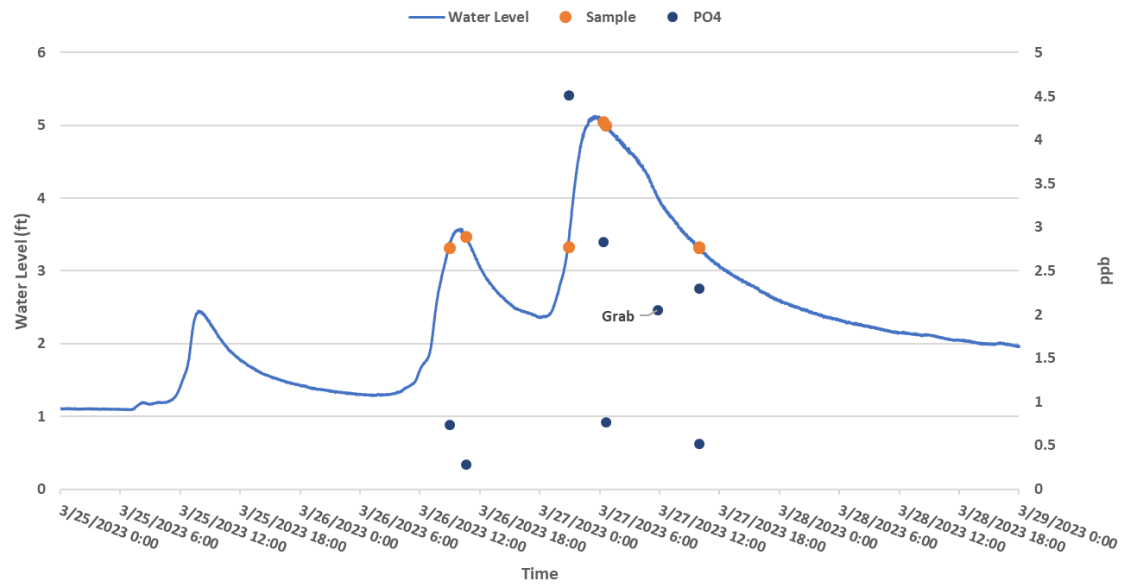
### Honey Creek, Storm Sample Event 5



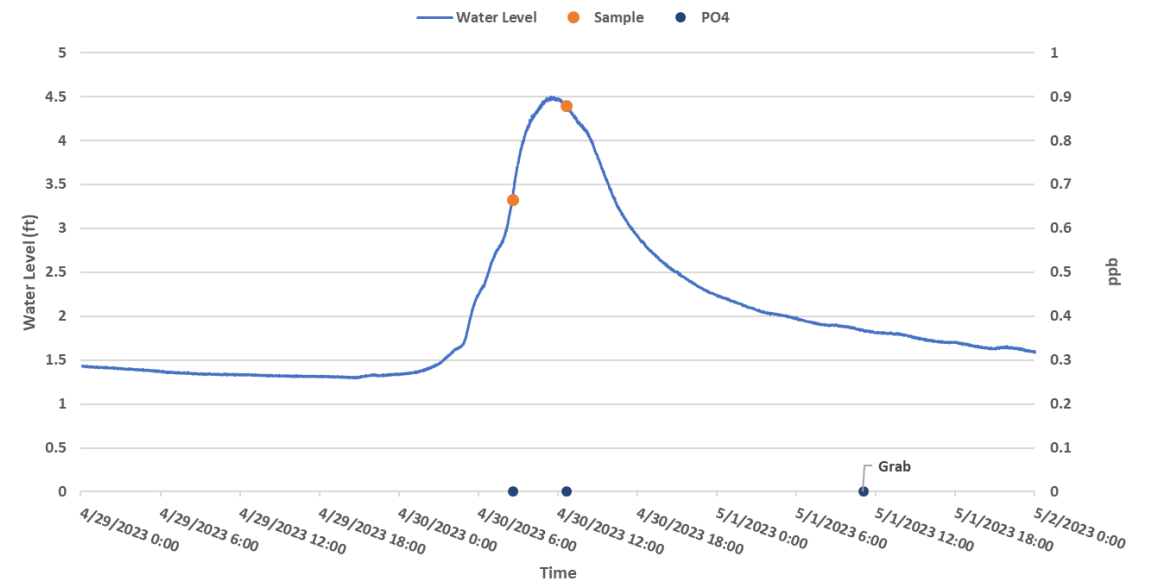
### Honey Creek, Storm Sample Event 7



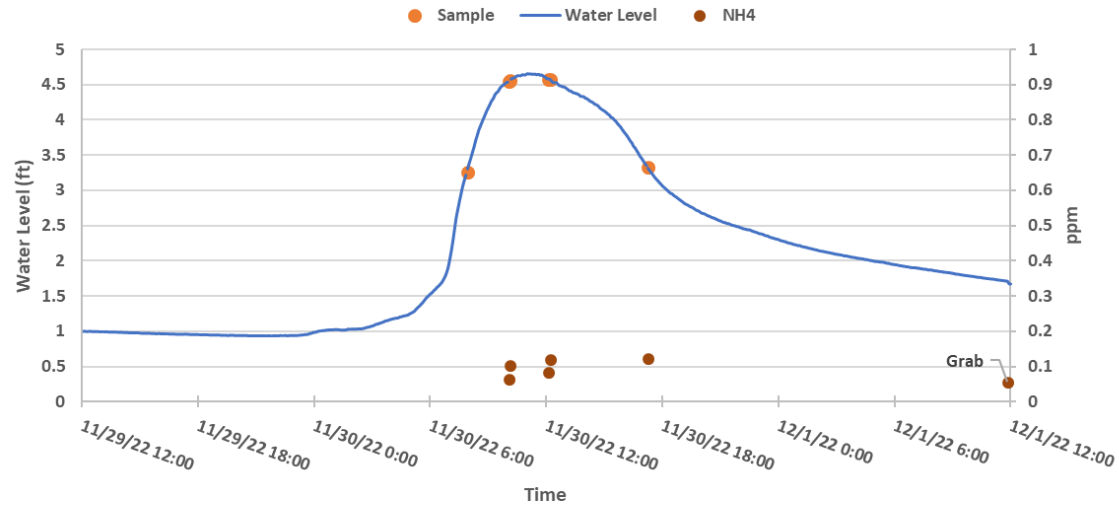
### Honey Creek, Storm Sample Event 6



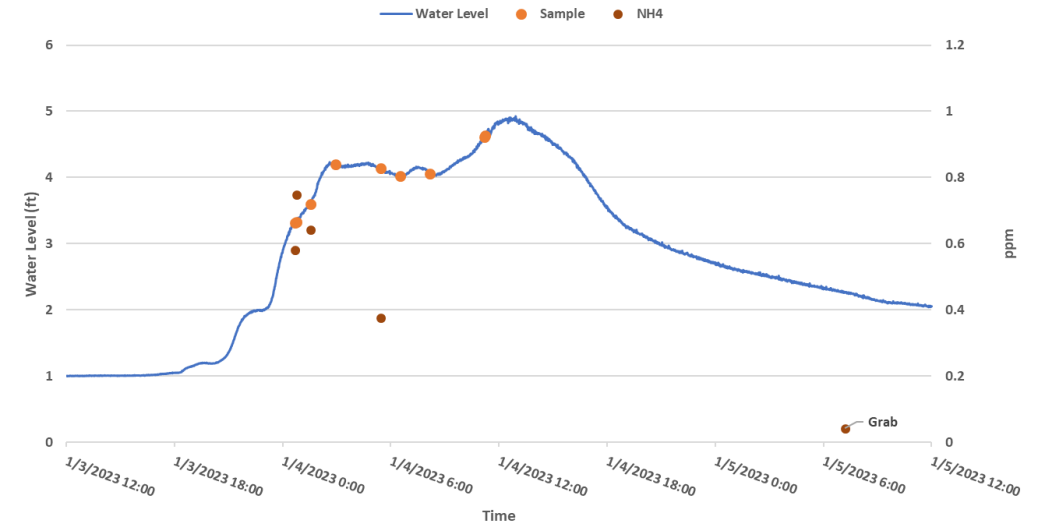
### Honey Creek, Storm Sample Event 8



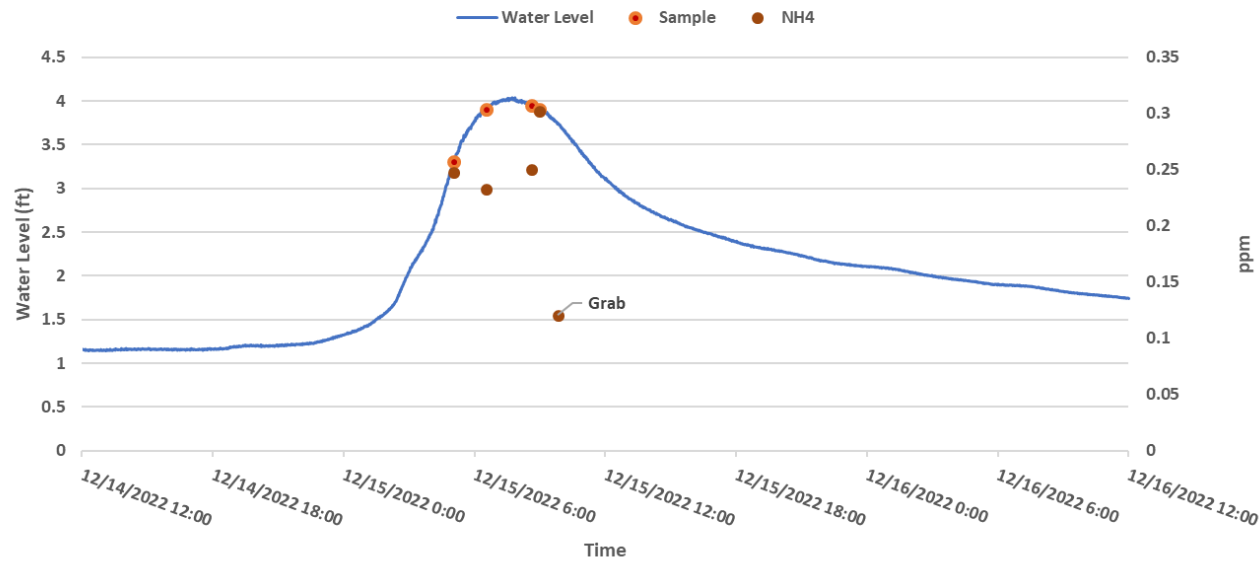
### Honey Creek, Storm Sample Event 1



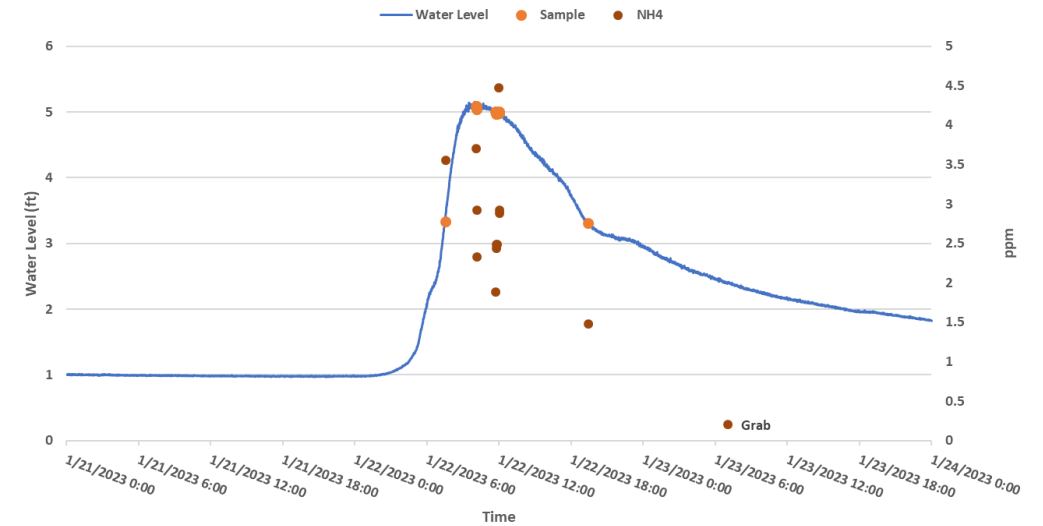
### Honey Creek, Storm Sample Event 3



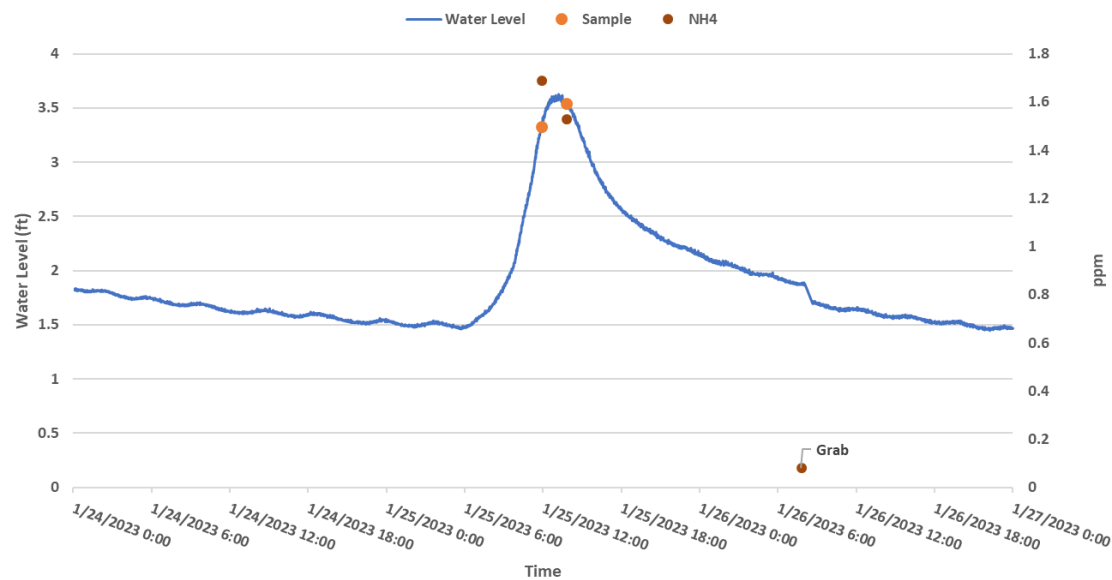
### Honey Creek, Storm Sample Event 2



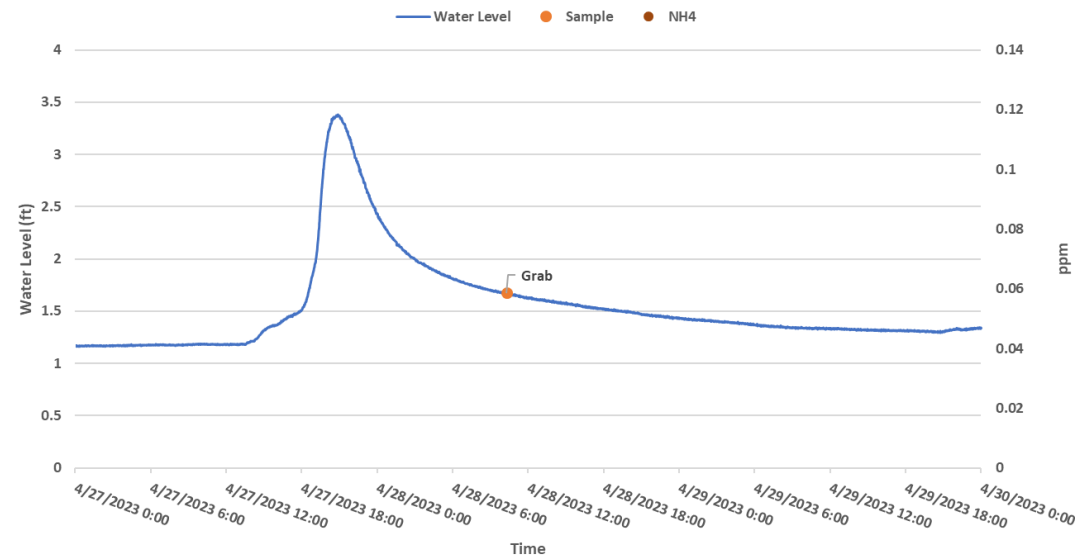
### Honey Creek, Storm Sample Event 4



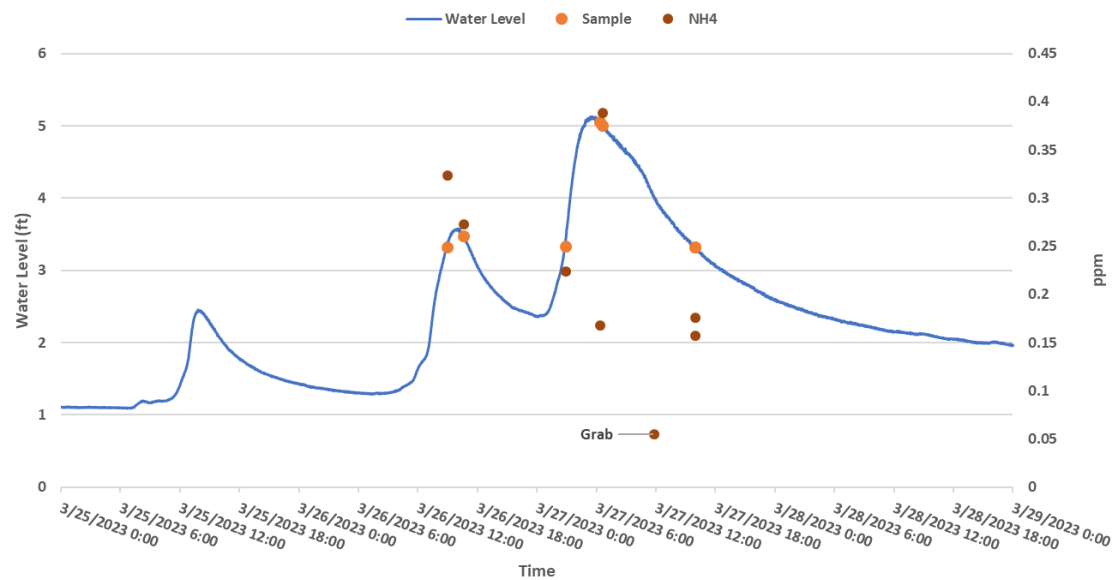
### Honey Creek, Storm Sample Event 5



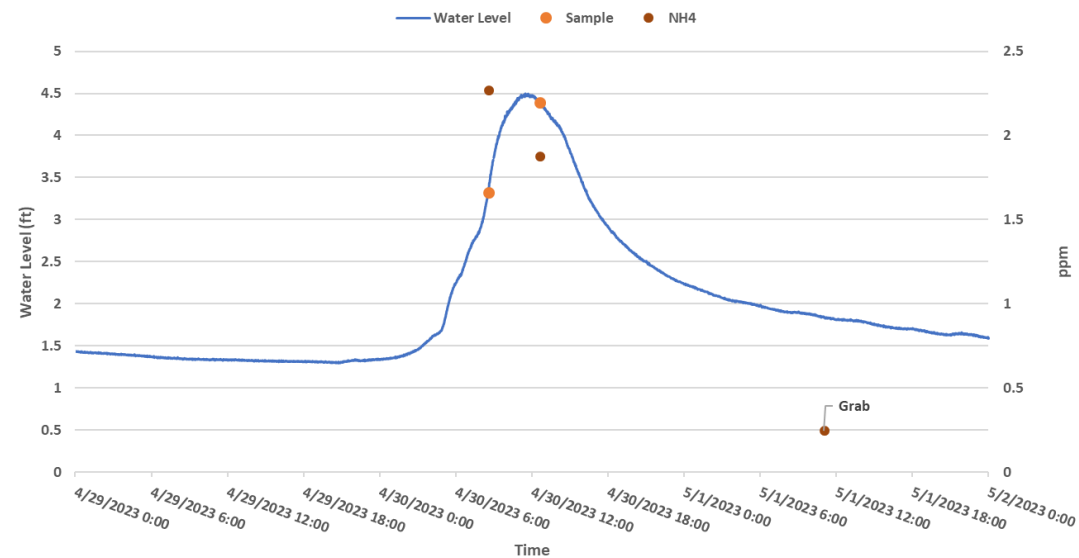
### Honey Creek, Storm Sample Event 7



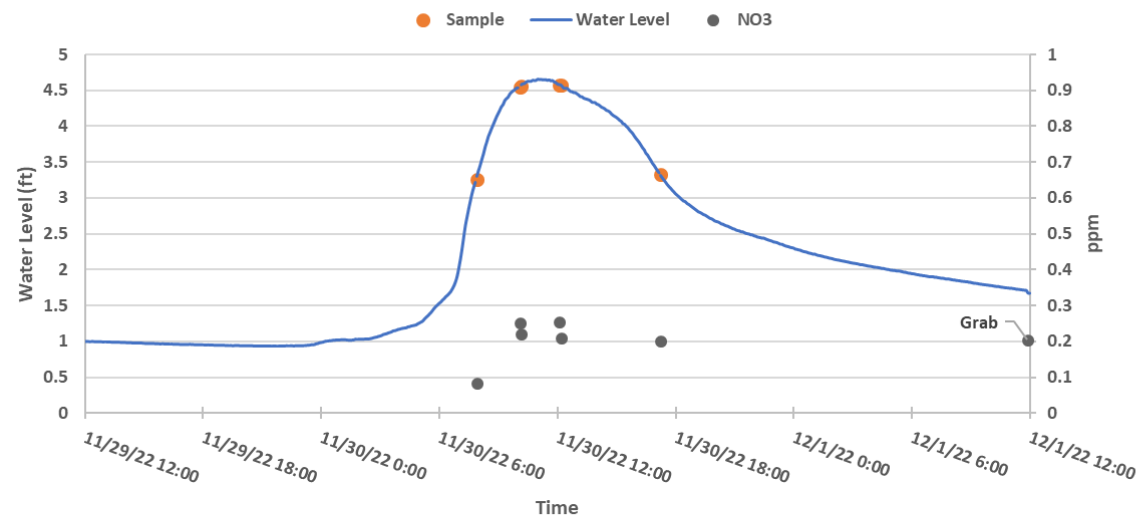
### Honey Creek, Storm Sample Event 6



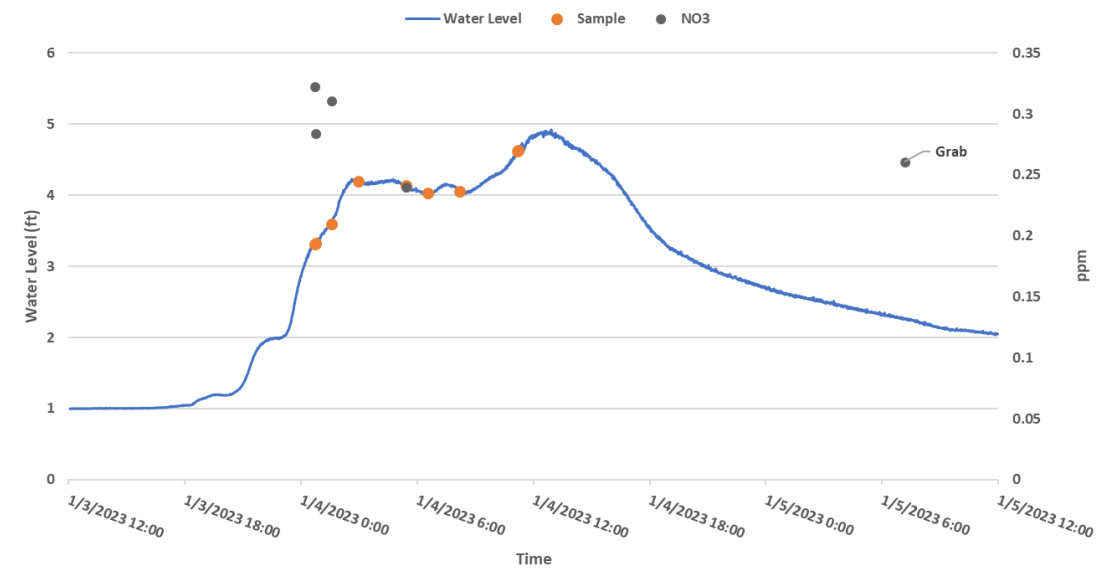
### Honey Creek, Storm Sample Event 8



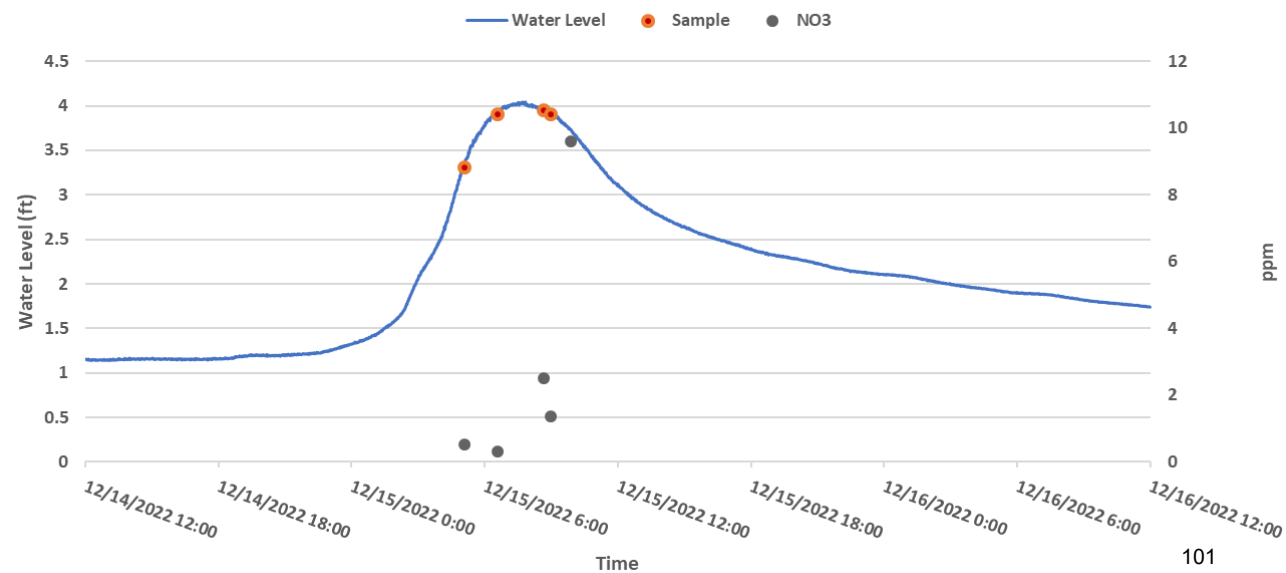
### Honey Creek, Storm Sample Event 1



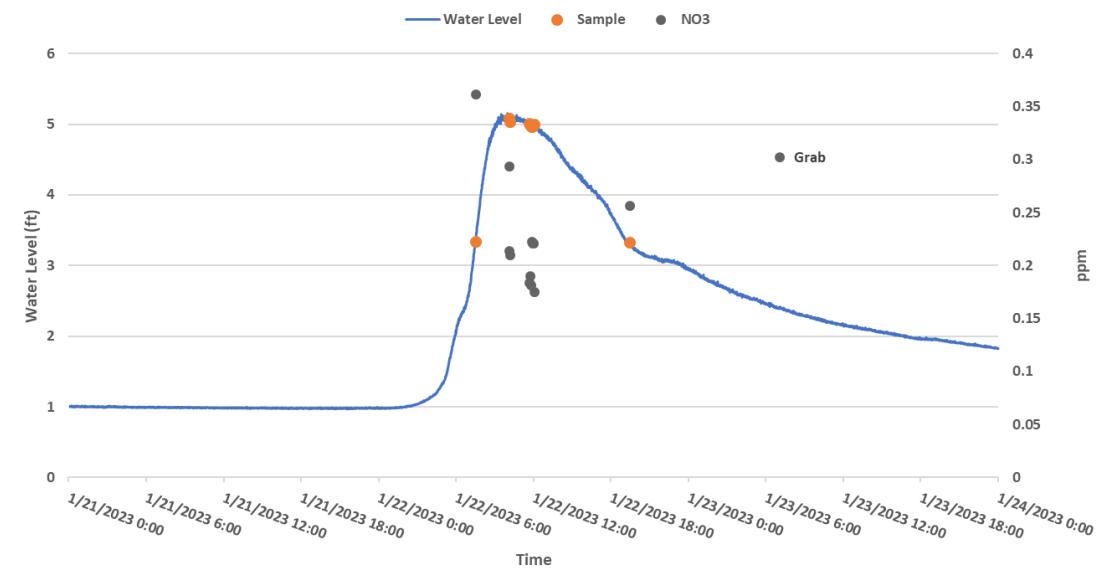
### Honey Creek, Storm Sample Event 3



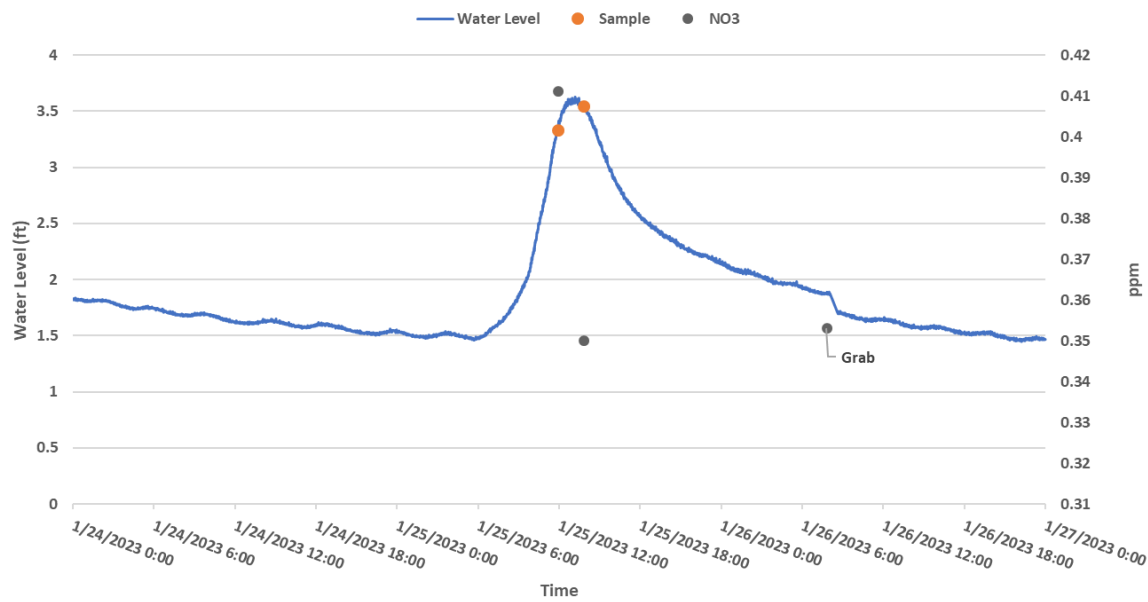
### Honey Creek, Storm Sample Event 2



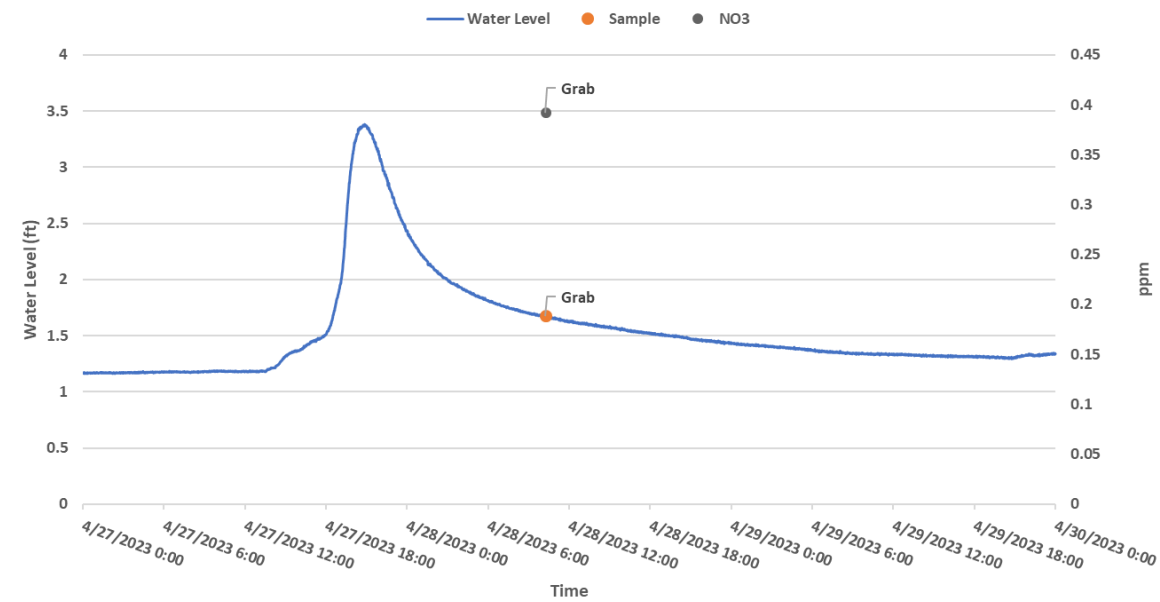
### Honey Creek, Storm Sample Event 4



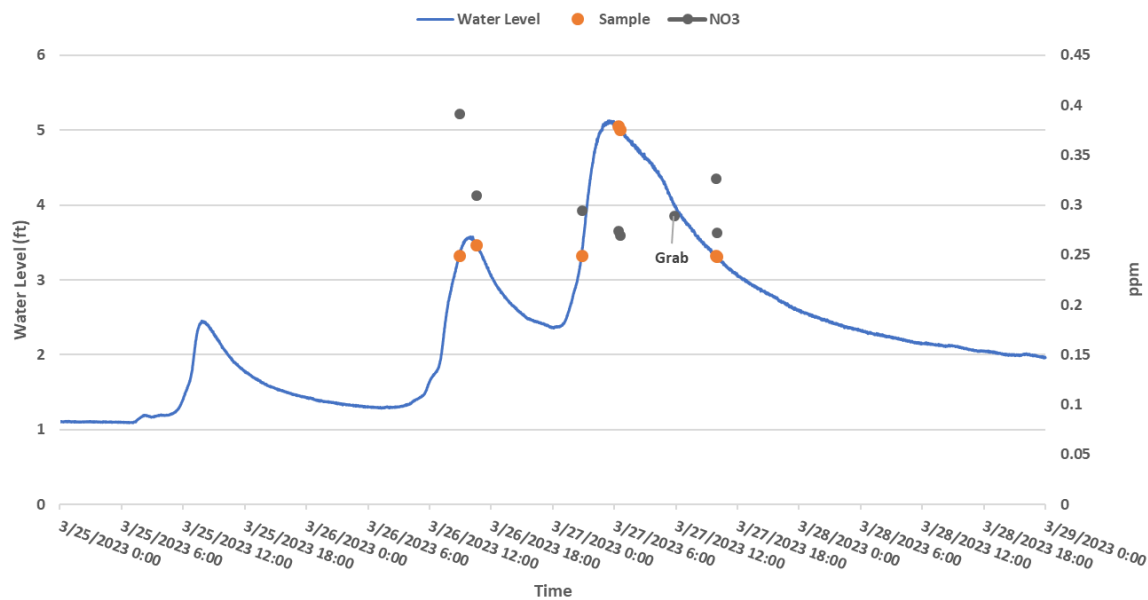
### Honey Creek, Storm Sample Event 5



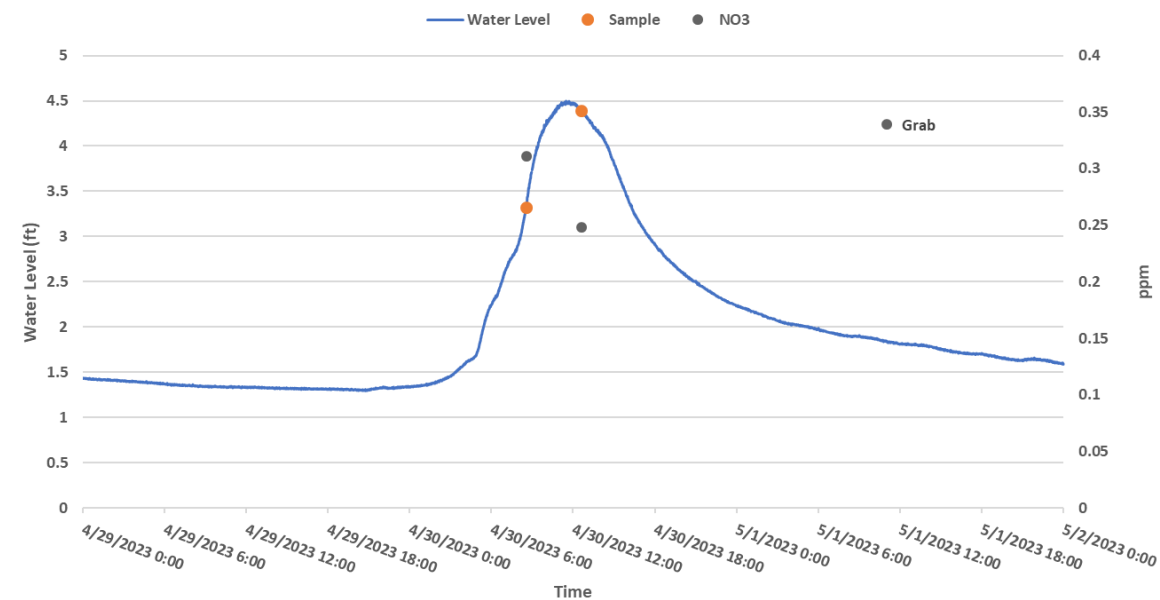
### Honey Creek, Storm Sample Event 7



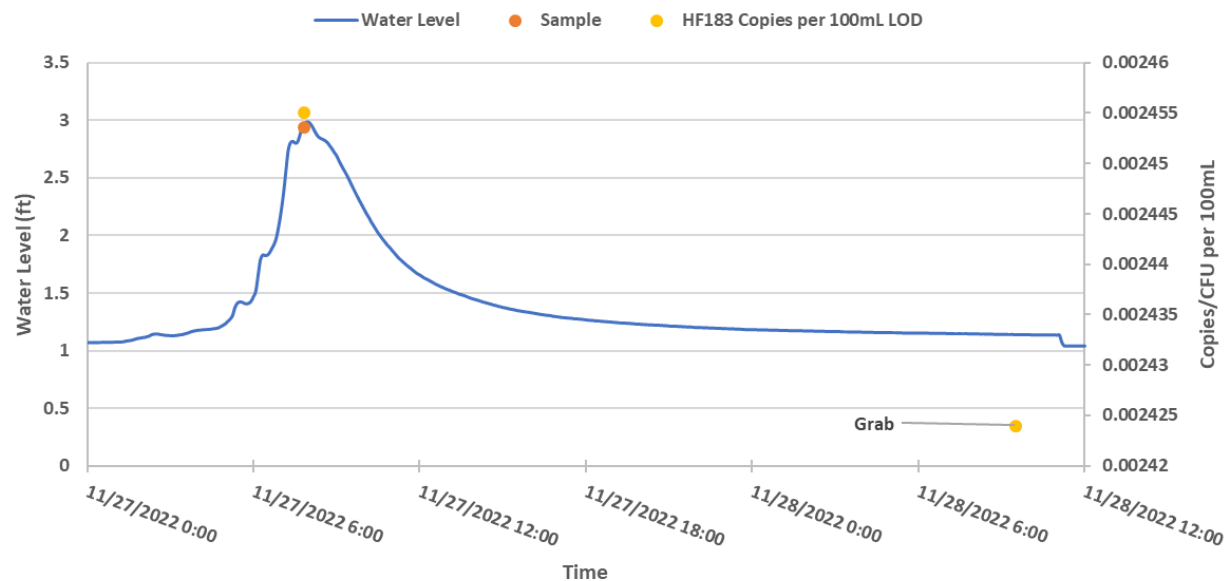
### Honey Creek, Storm Sample Event 6



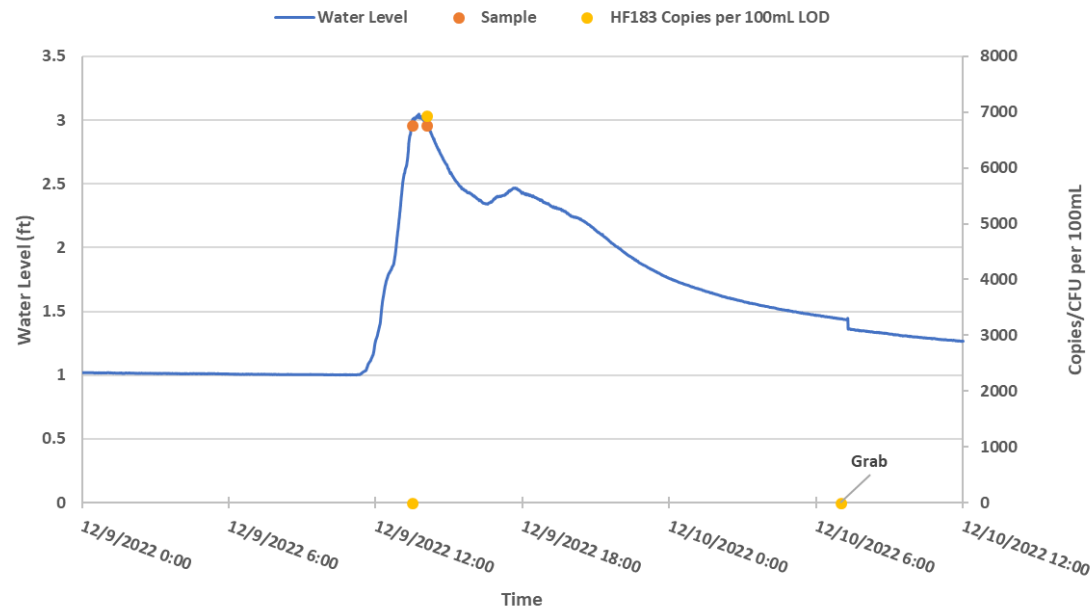
### Honey Creek, Storm Sample Event 8



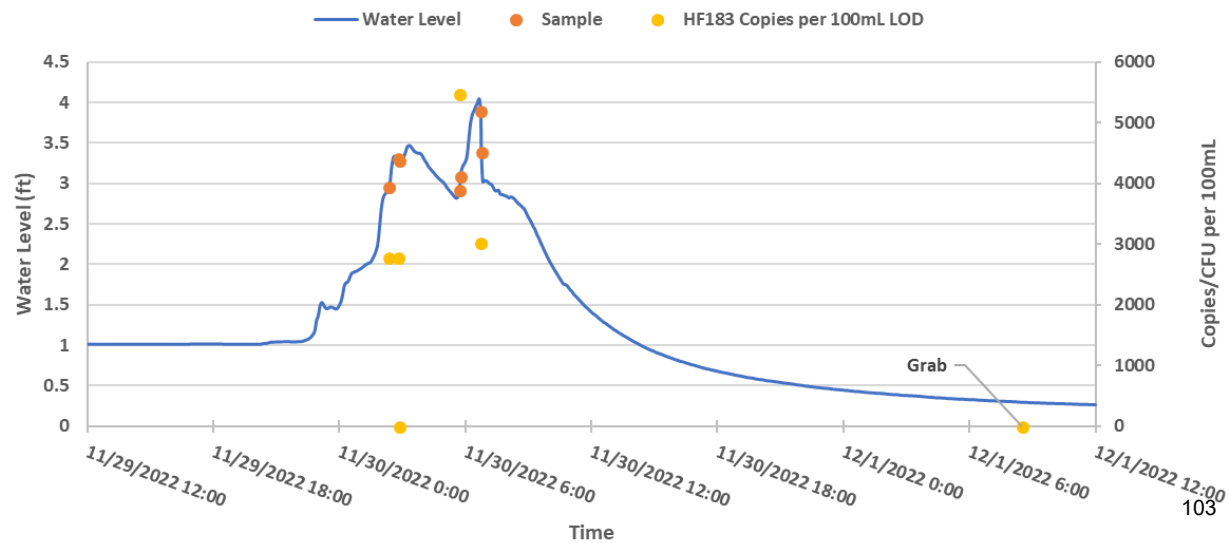
### Little Stone Mountain, Storm Sample Event 1



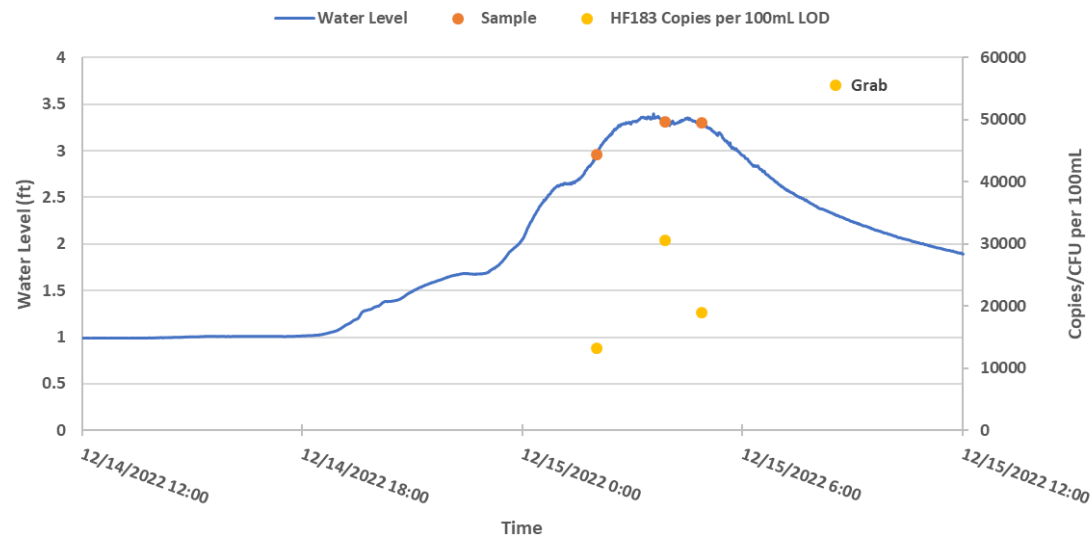
### Little Stone Mountain, Storm Sample Event 3



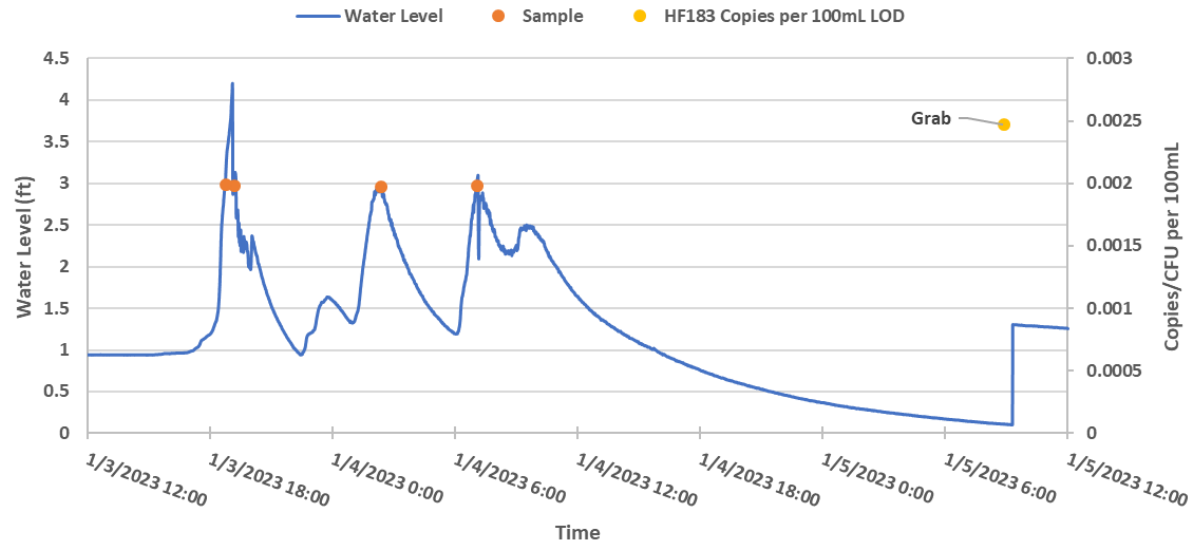
### Little Stone Mountain, Storm Sample Event 2



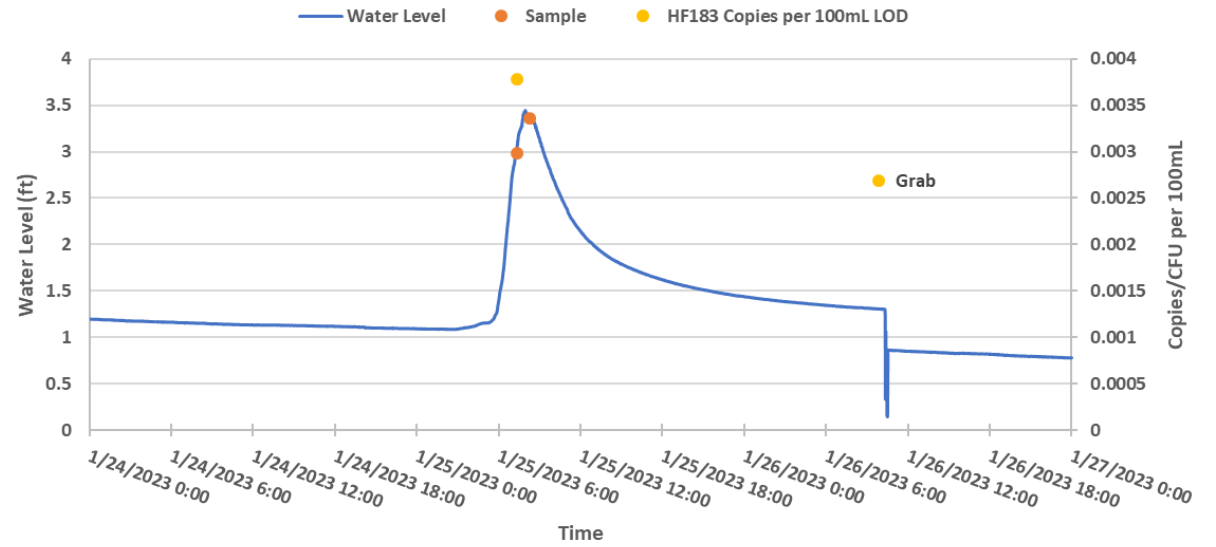
### Little Stone Mountain, Storm Sample Event 4



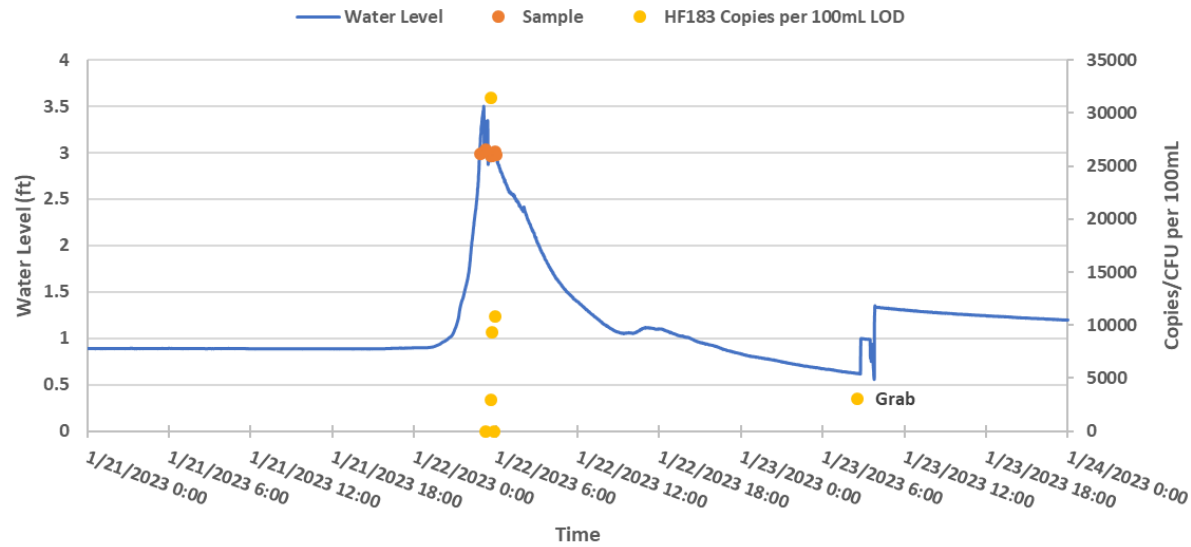
### Little Stone Mountain, Storm Sample Event 5



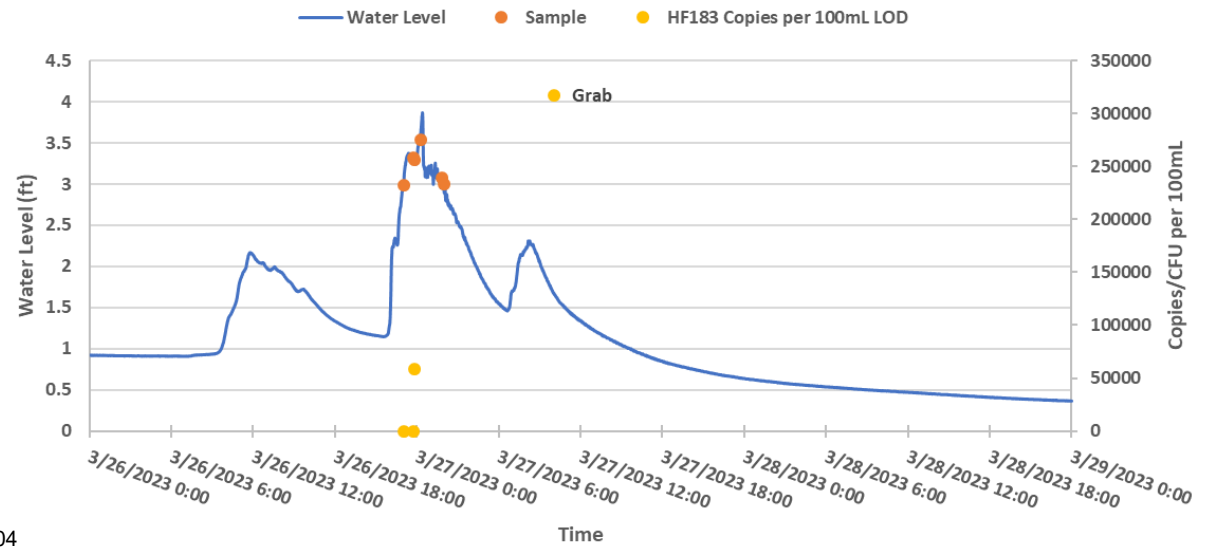
### Little Stone Mountain, Storm Sample Event 7



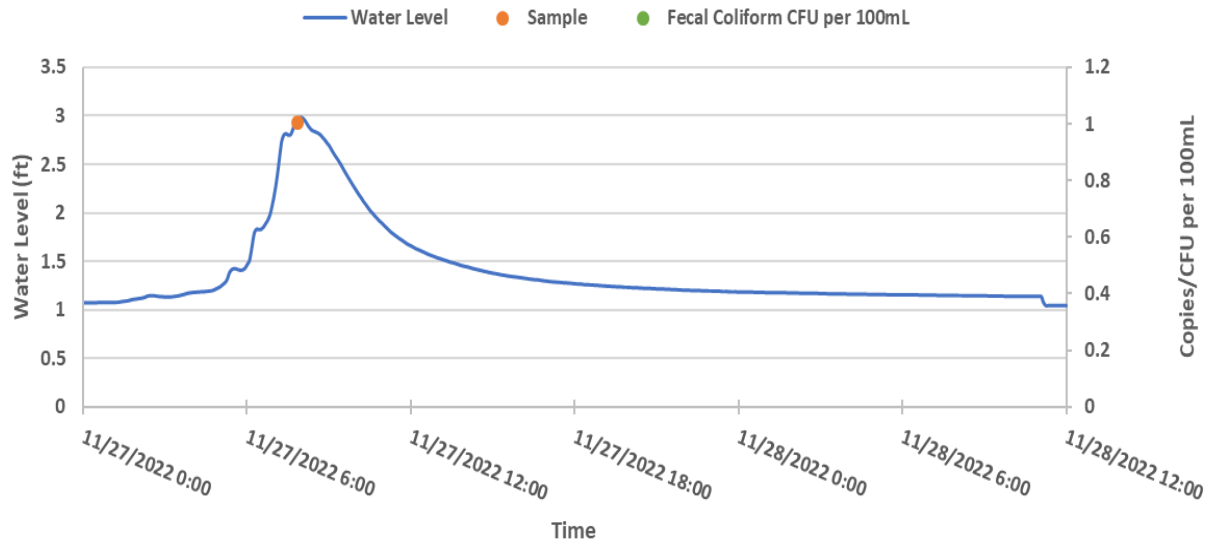
### Little Stone Mountain, Storm Sample Event 6



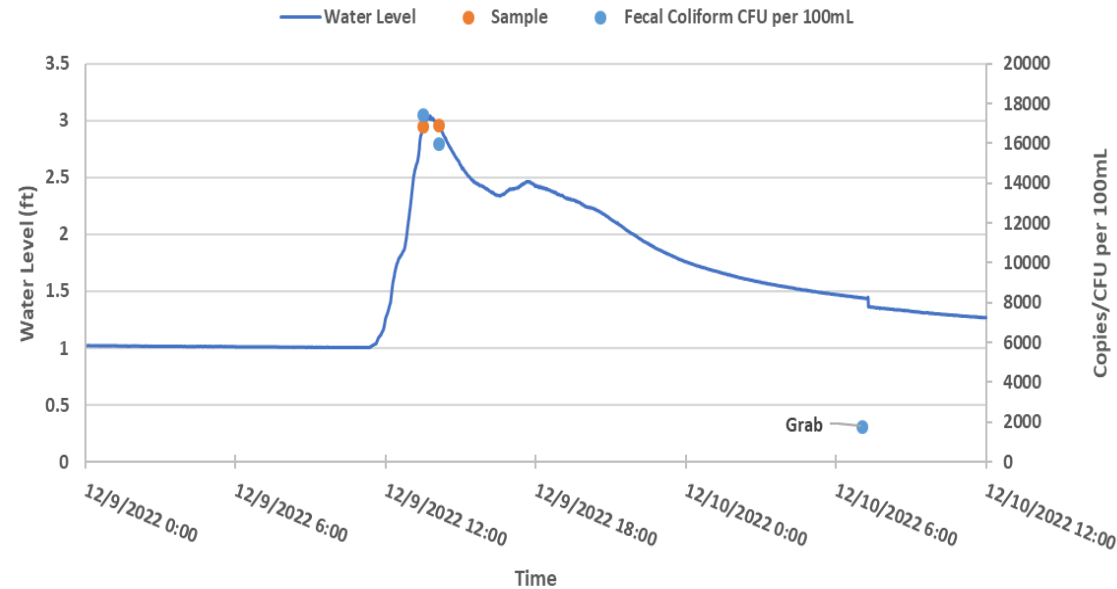
### Little Stone Mountain, Storm Sample Event 8



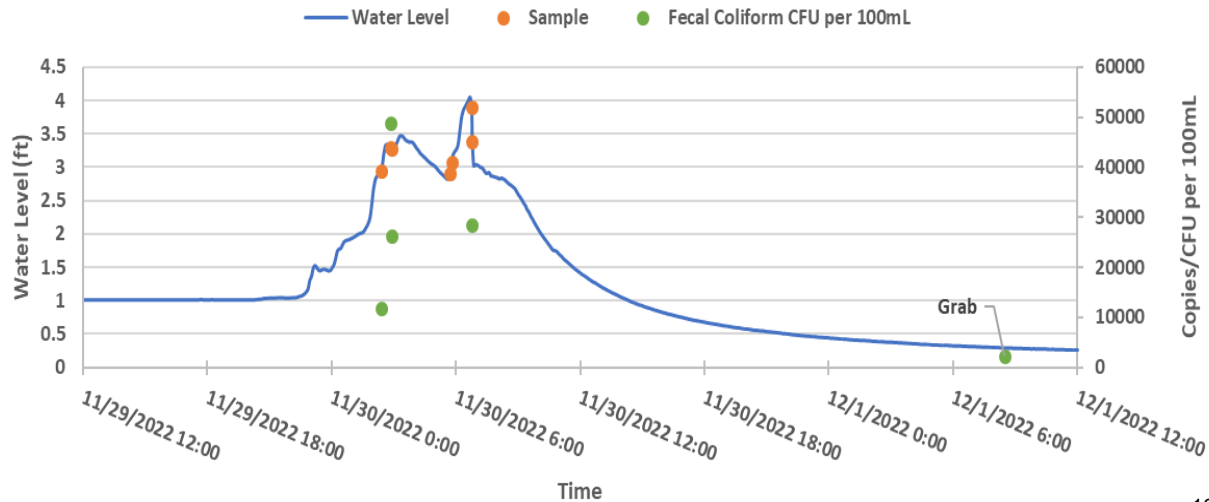
Little Stone Mountain, Storm Sample Event 1



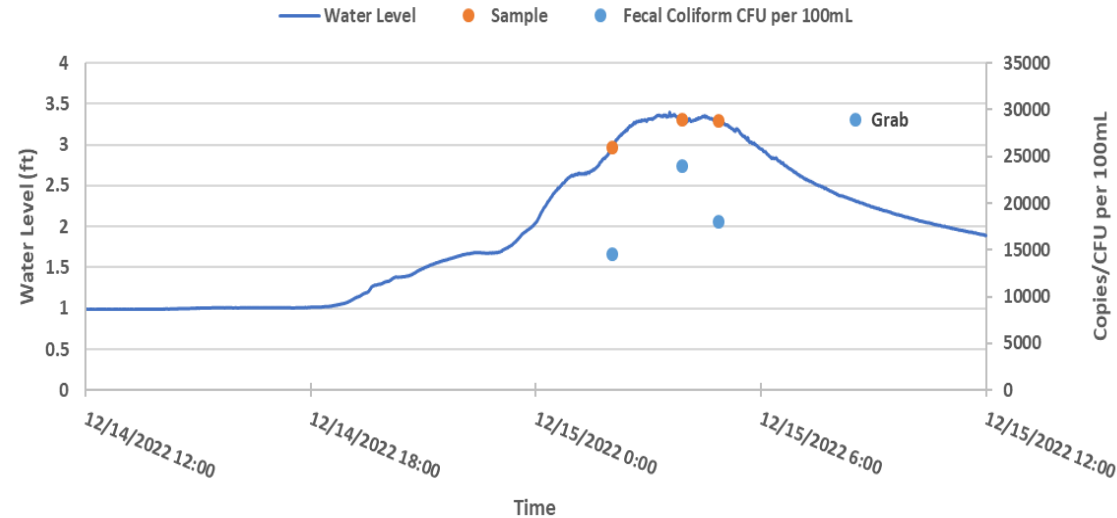
Little Stone Mountain, Storm Sample Event 3



Little Stone Mountain, Storm Sample Event 2

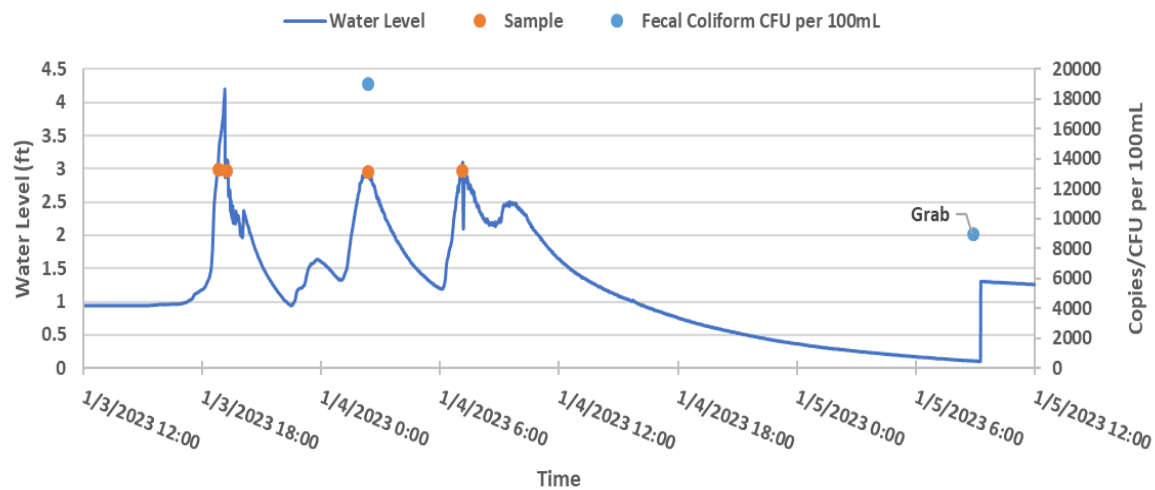


Little Stone Mountain, Storm Sample Event 4

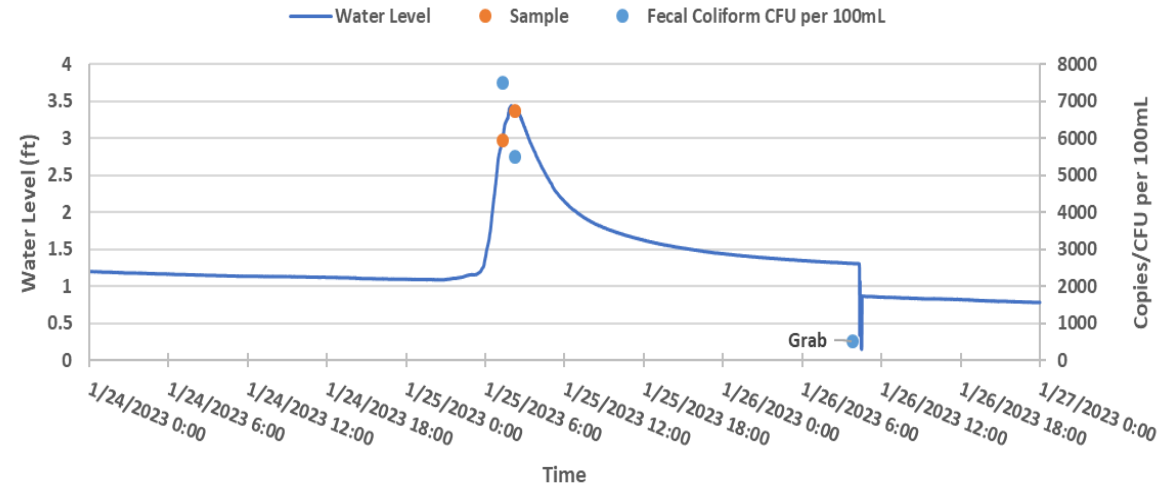




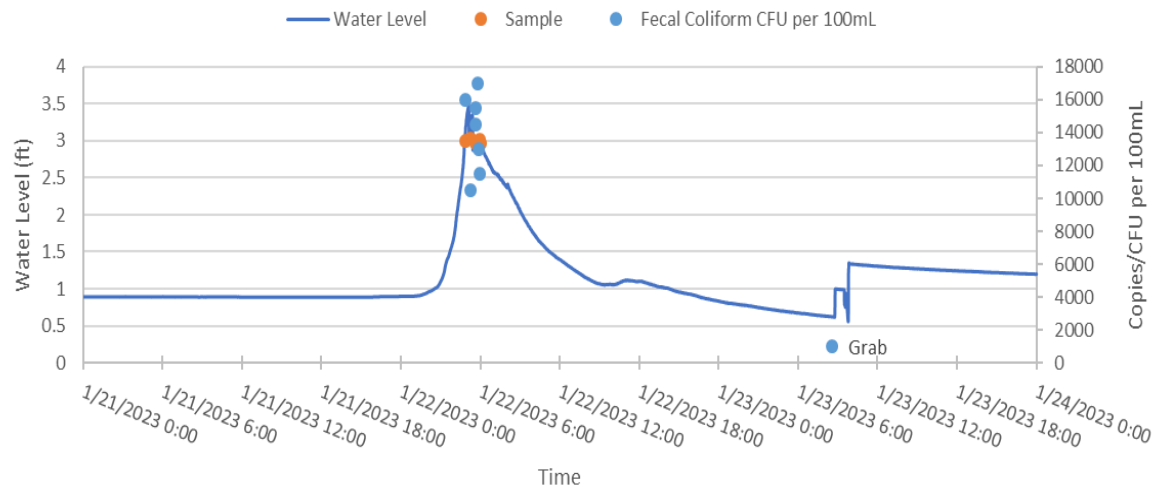
### Little Stone Mountain, Storm Sample Event 5



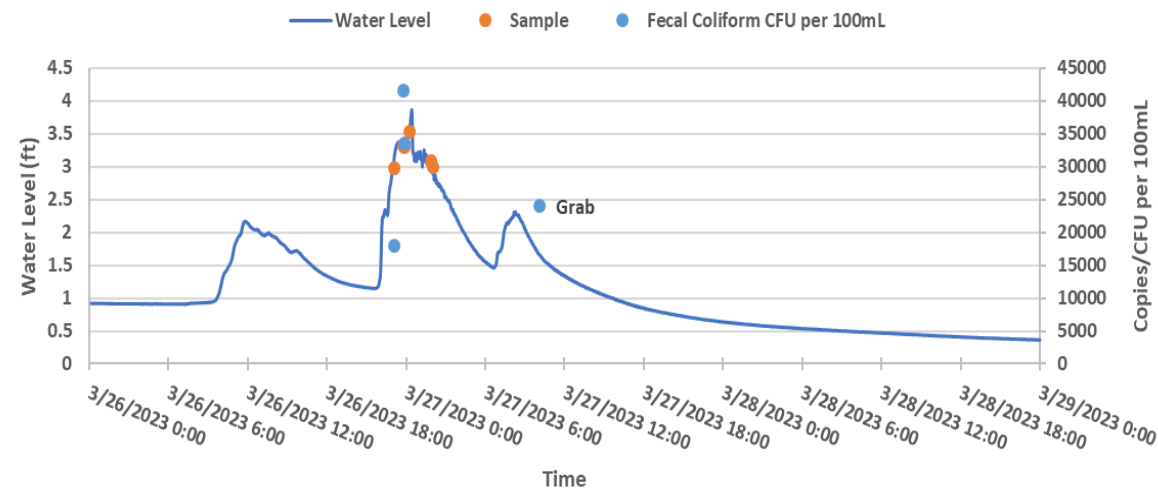
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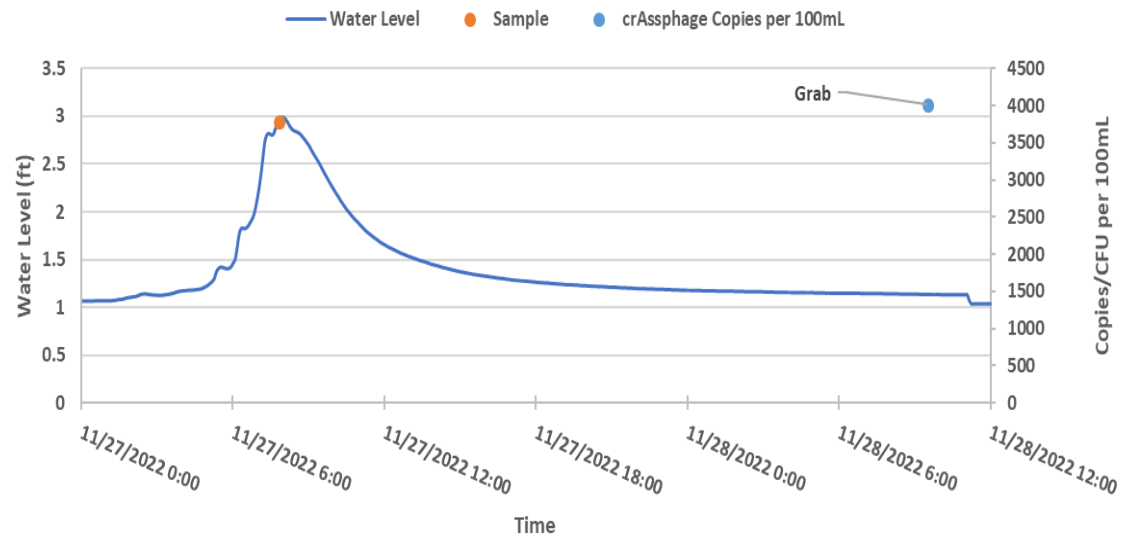
### Little Stone Mountain, Storm Sample Event 6



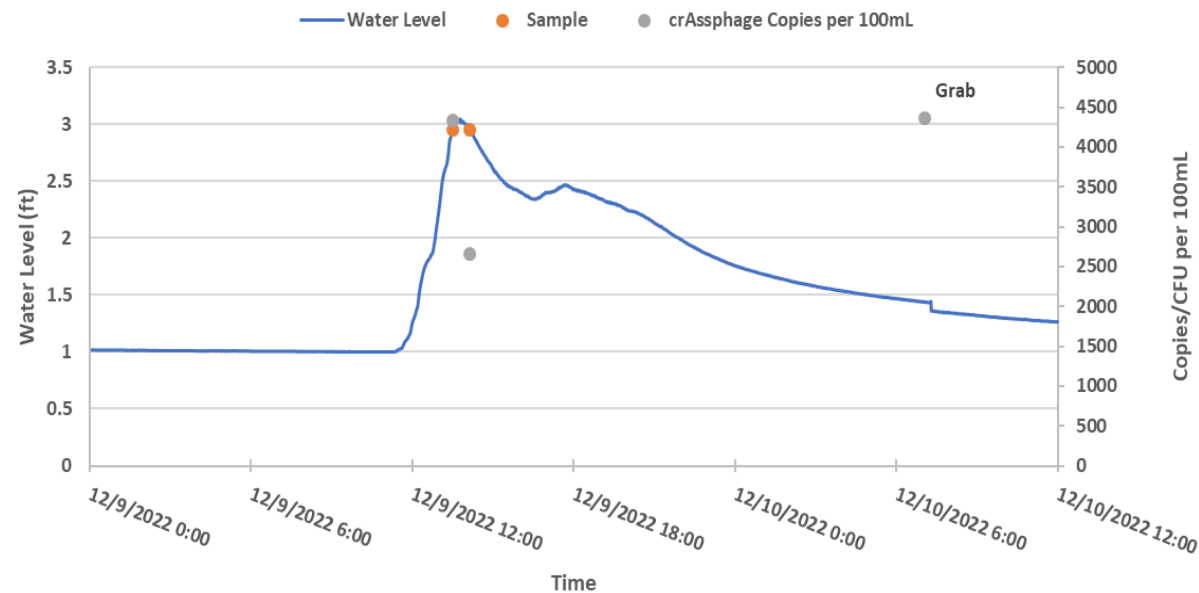
### Little Stone Mountain, Storm Sample Event 8



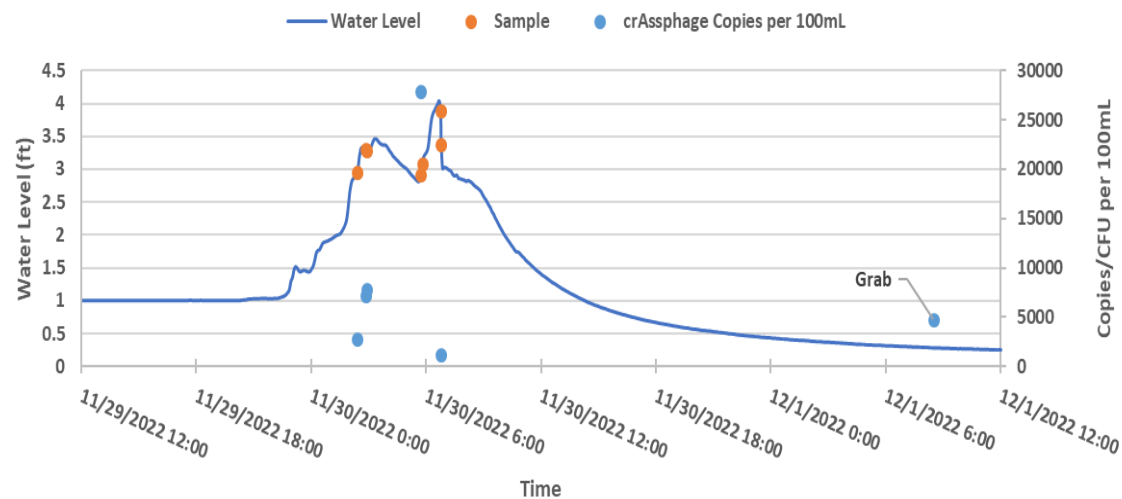
### Little Stone Mountain, Storm Sample Event 1



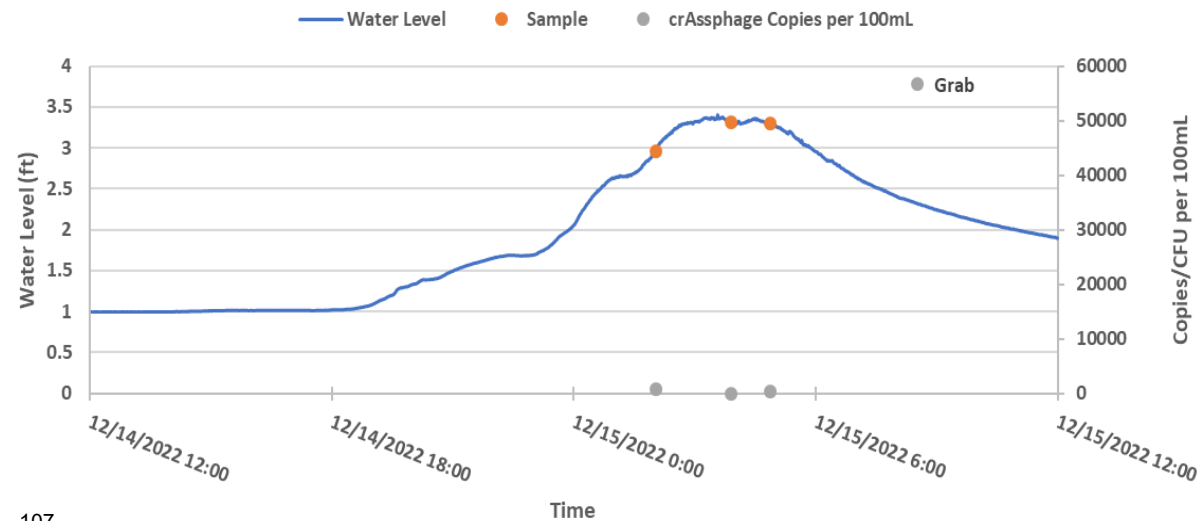
### Little Stone Mountain, Storm Sample Event 3



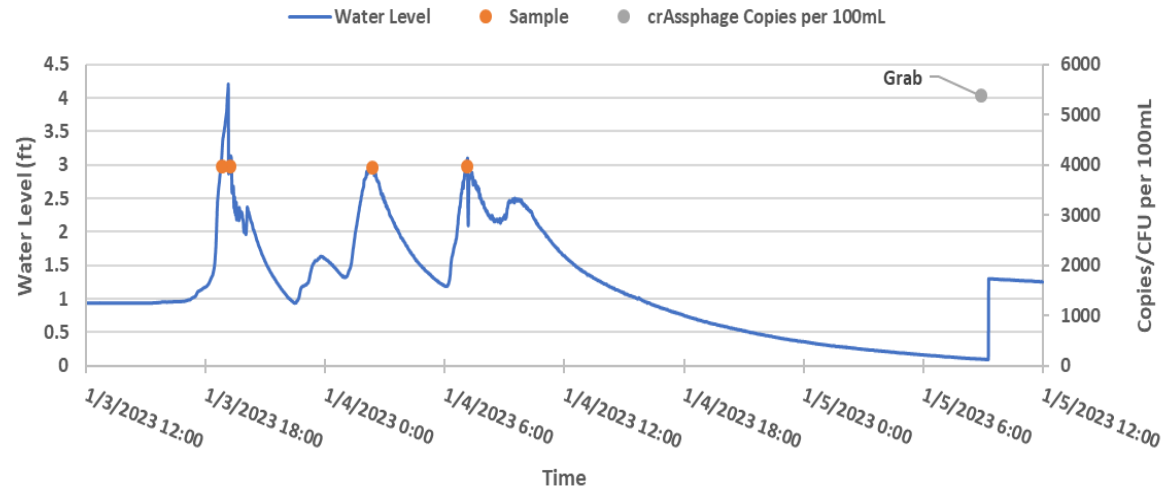
### Little Stone Mountain, Storm Sample Event 2



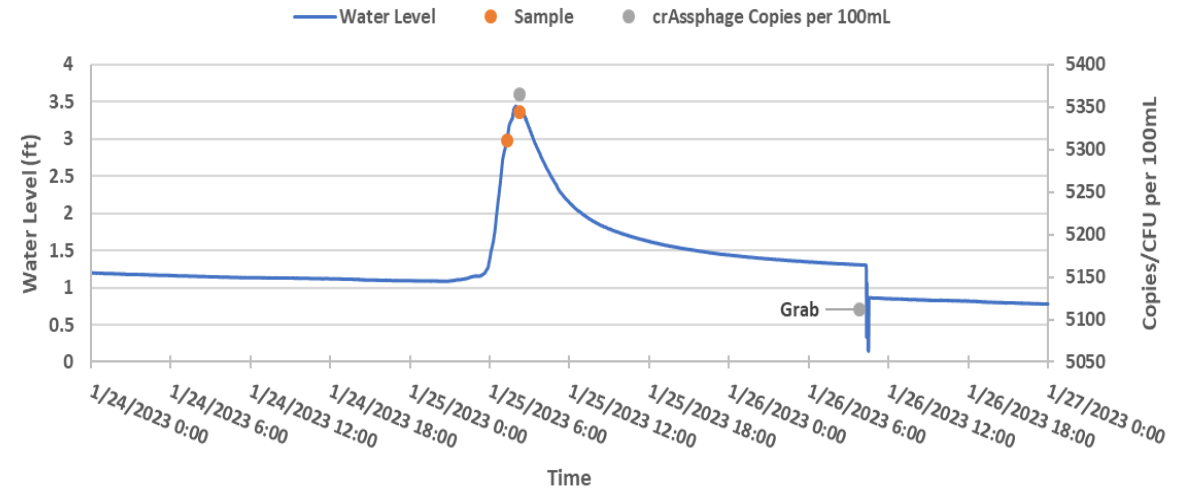
### Little Stone Mountain, Storm Sample Event 4



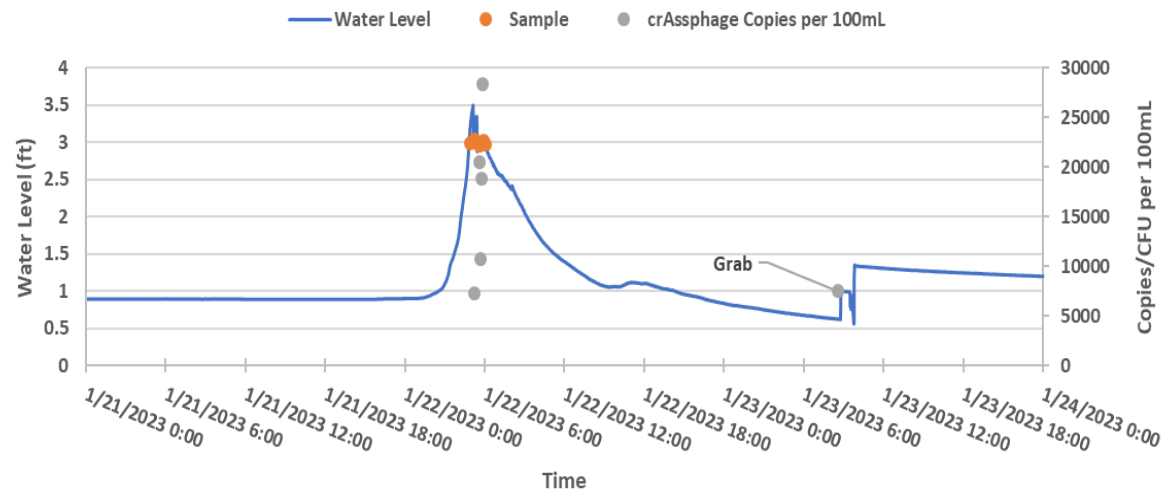
### Little Stone Mountain, Storm Sample Event 5



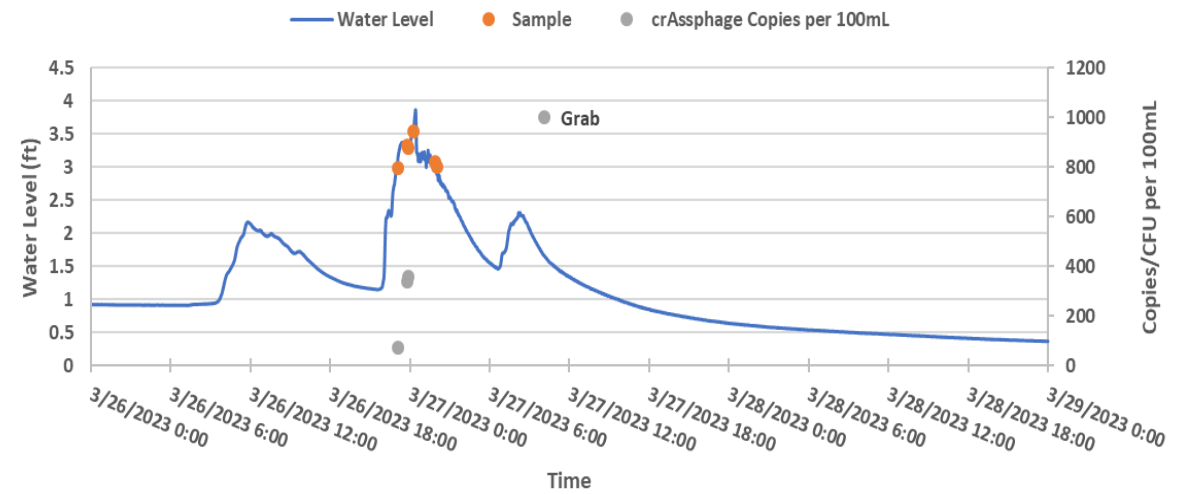
### Little Stone Mountain, Storm Sample Event 7



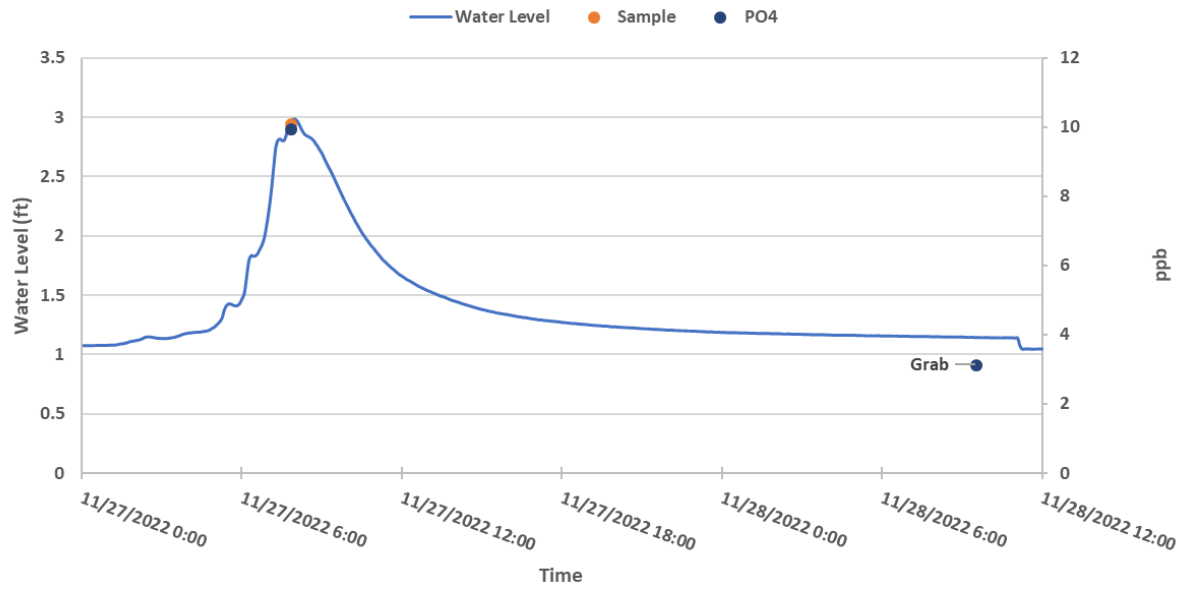
### Little Stone Mountain, Storm Sample Event 6



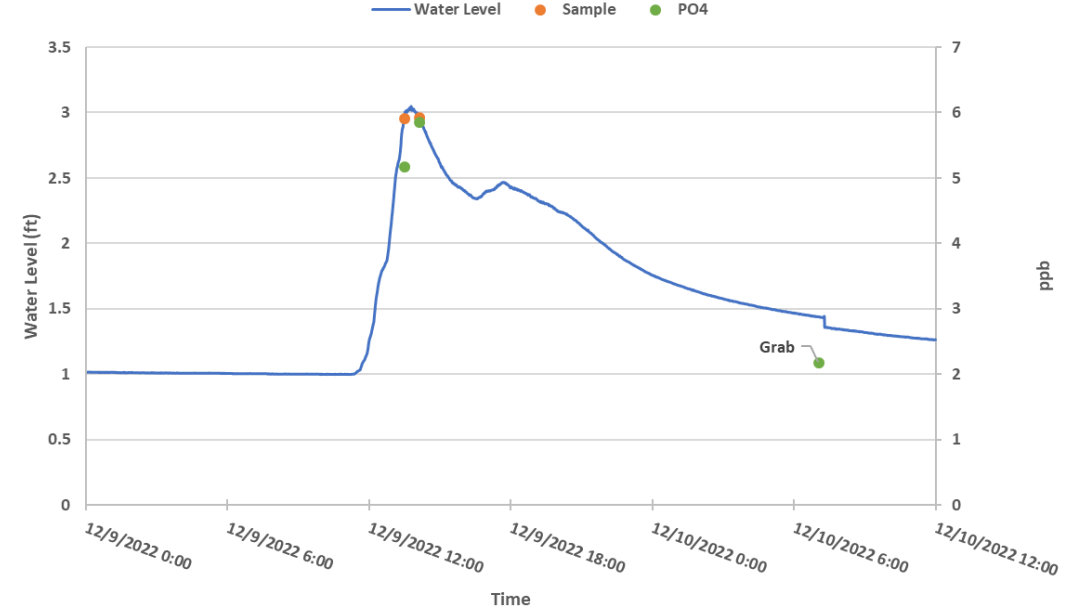
### Little Stone Mountain, Storm Sample Event 8



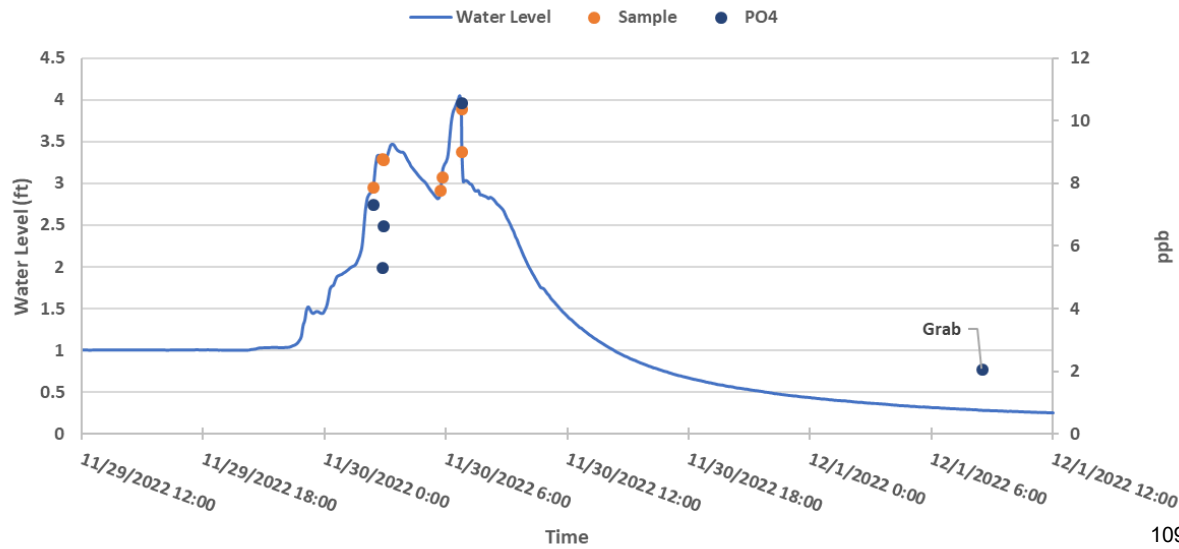
### Little Stone Mountain, Storm Sample Event 1



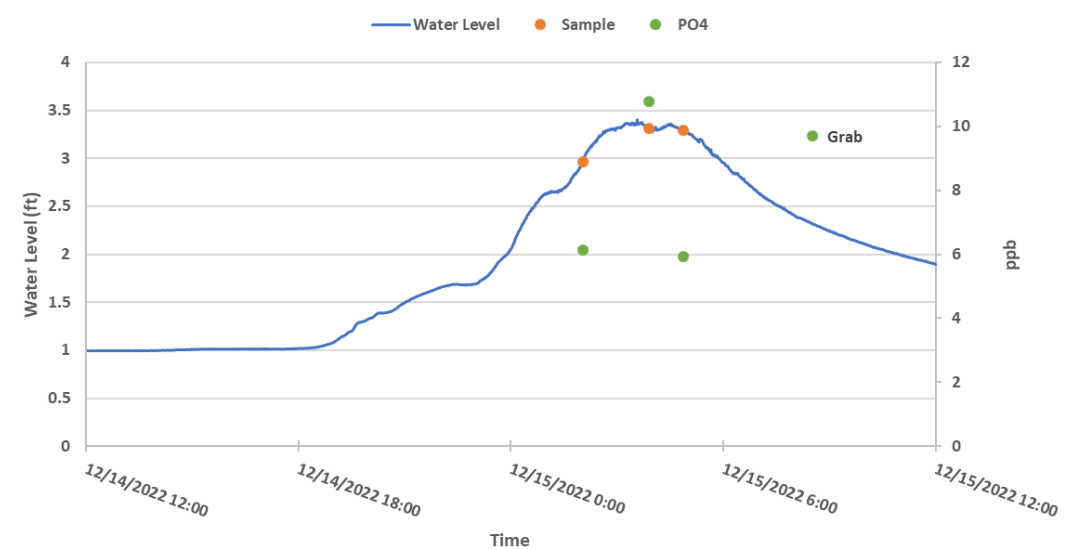
### Little Stone Mountain, Storm Sample Event 3



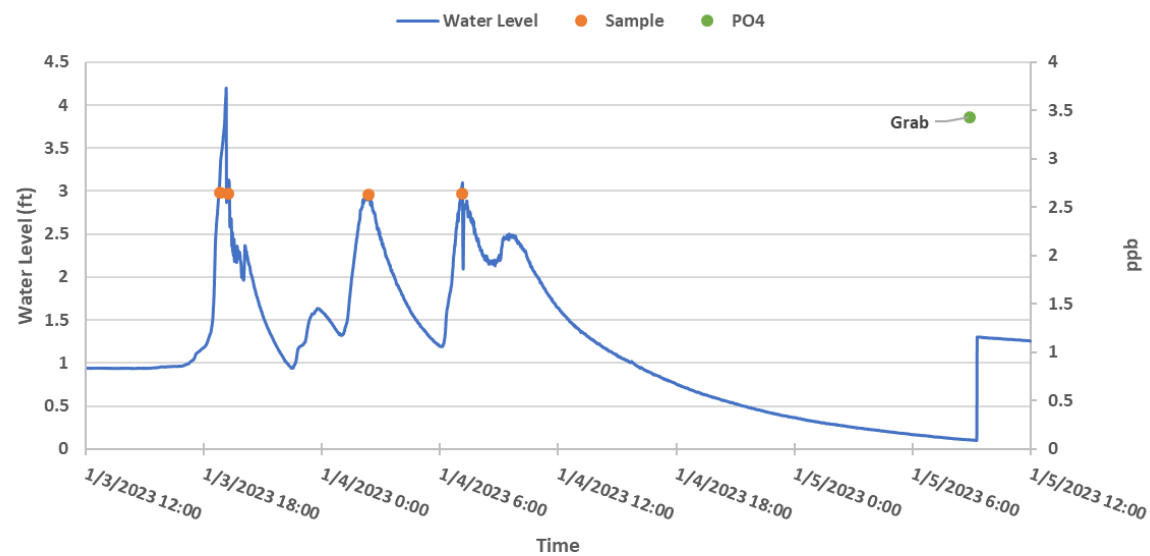
### Little Stone Mountain, Storm Sample Event 2



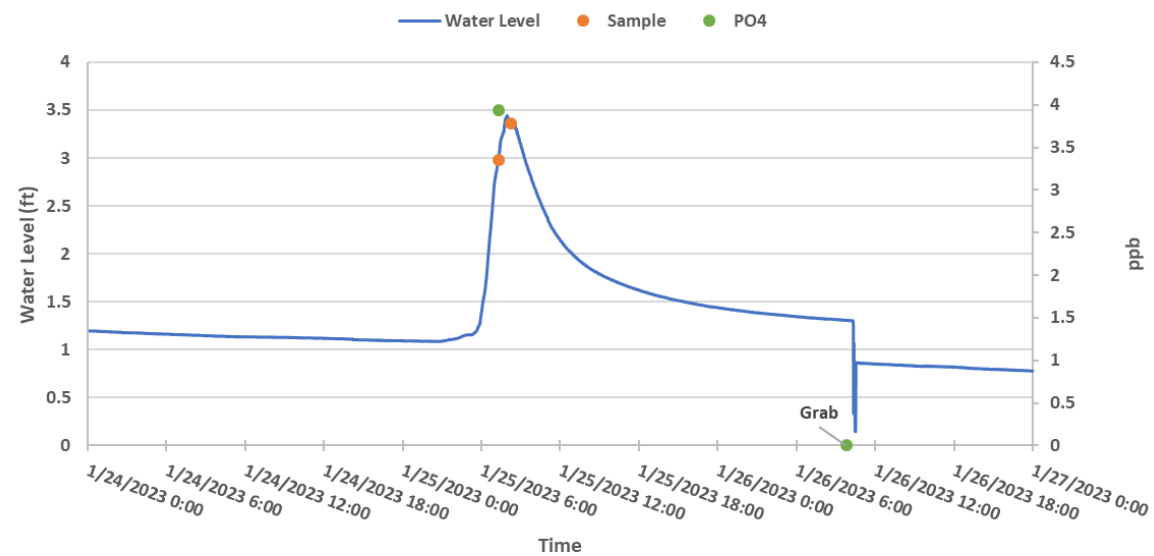
### Little Stone Mountain, Storm Sample Event 4



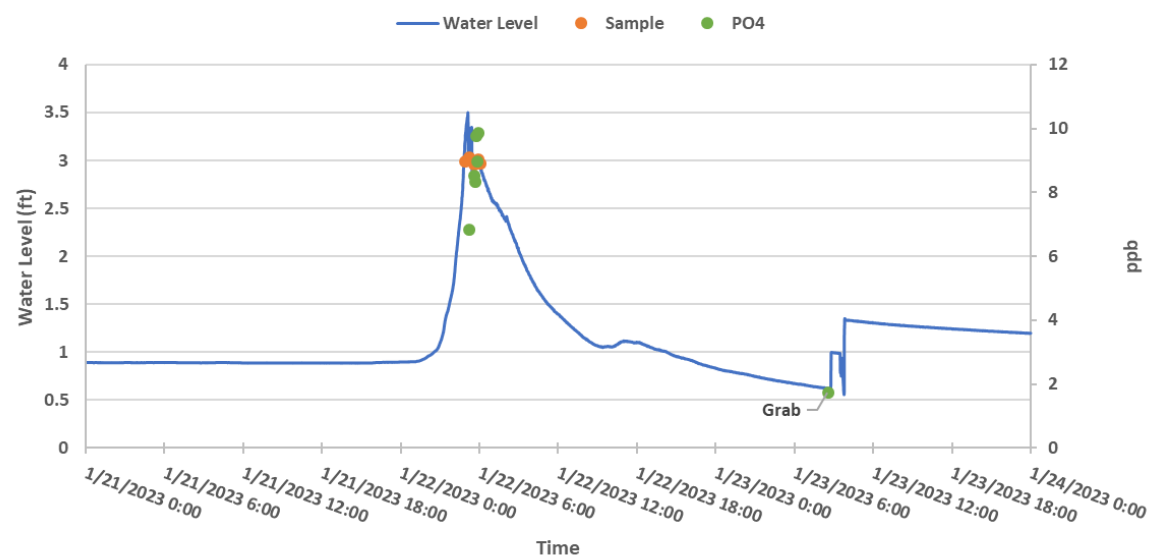
### Little Stone Mountain, Storm Sample Event 5



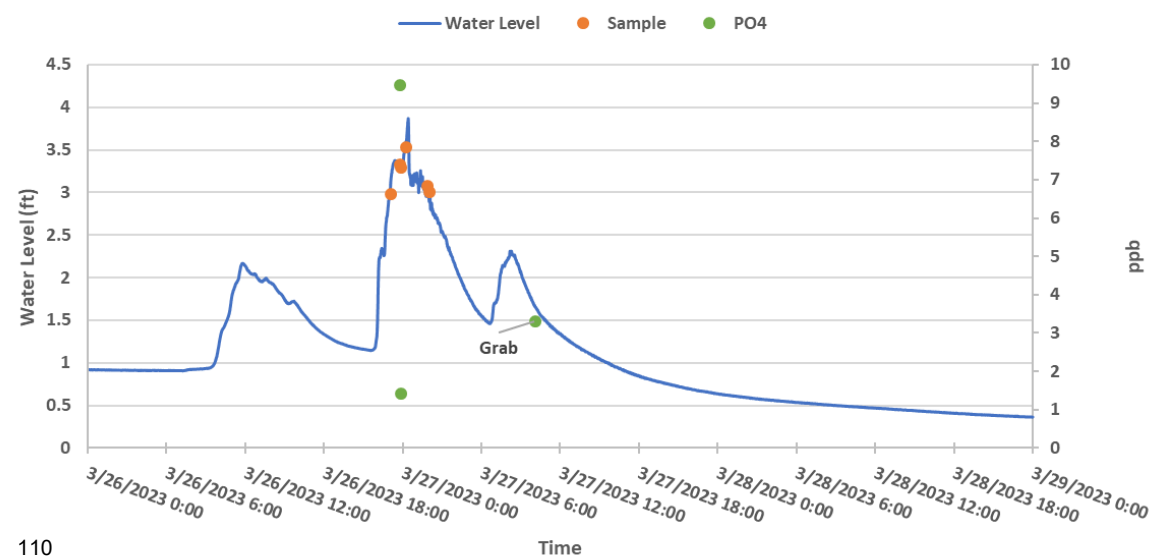
### Little Stone Mountain, Storm Sample Event 7



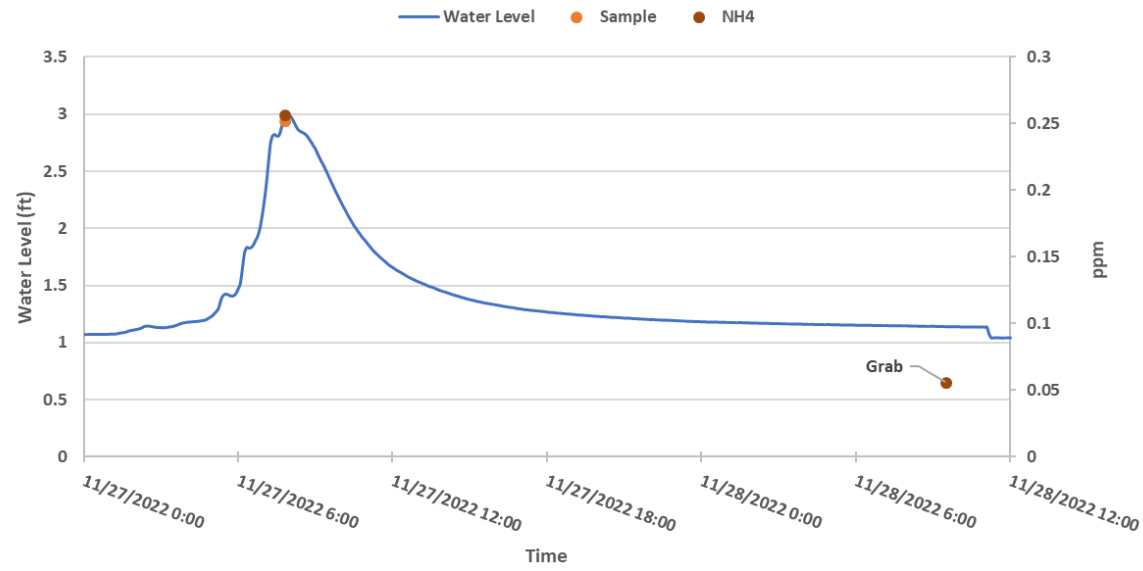
### Little Stone Mountain, Storm Sample Event 6



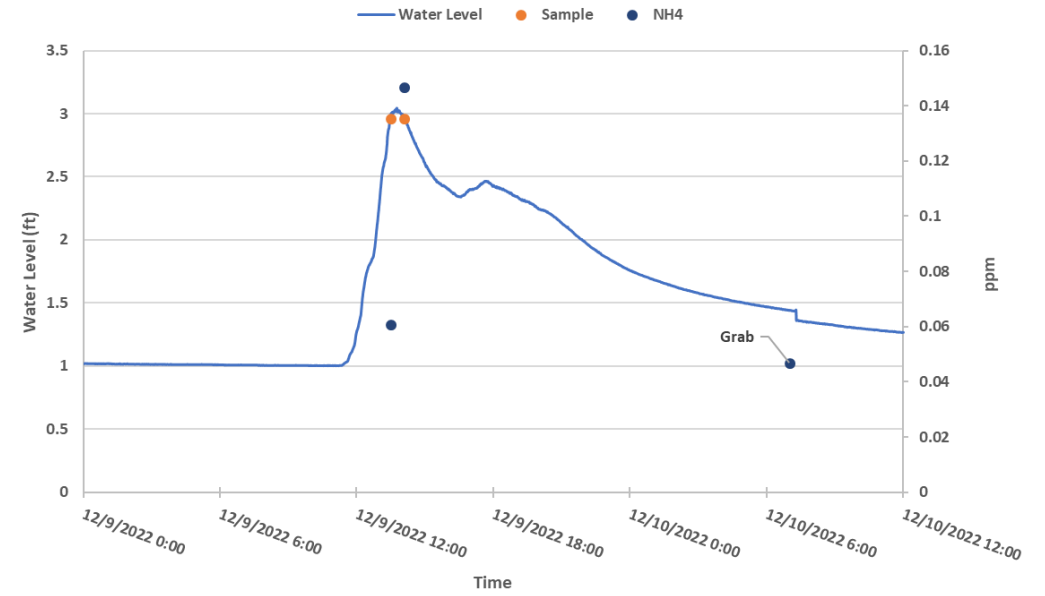
### Little Stone Mountain, Storm Sample Event 8



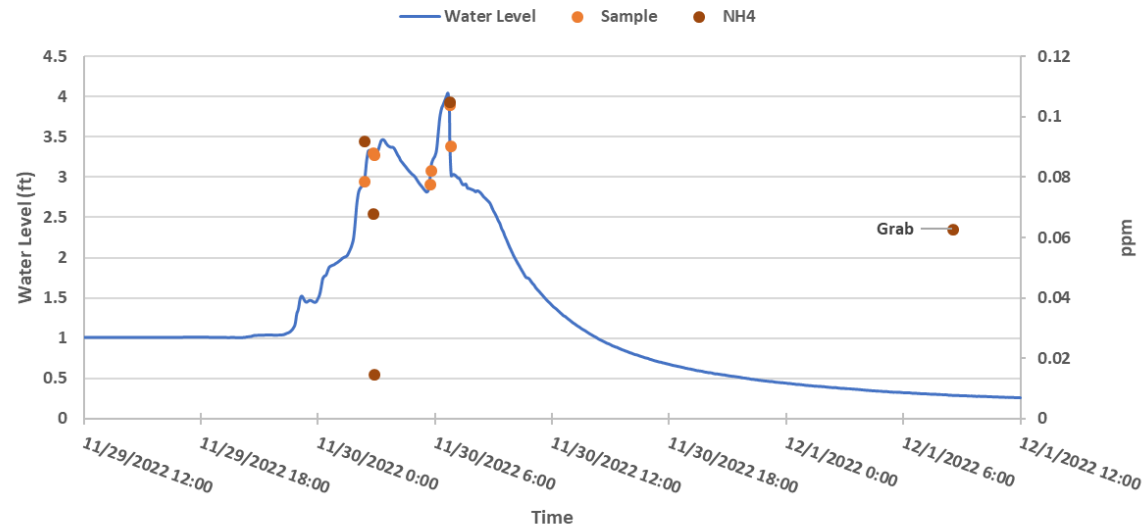
Little Stone Mountain, Storm Sample Event 1



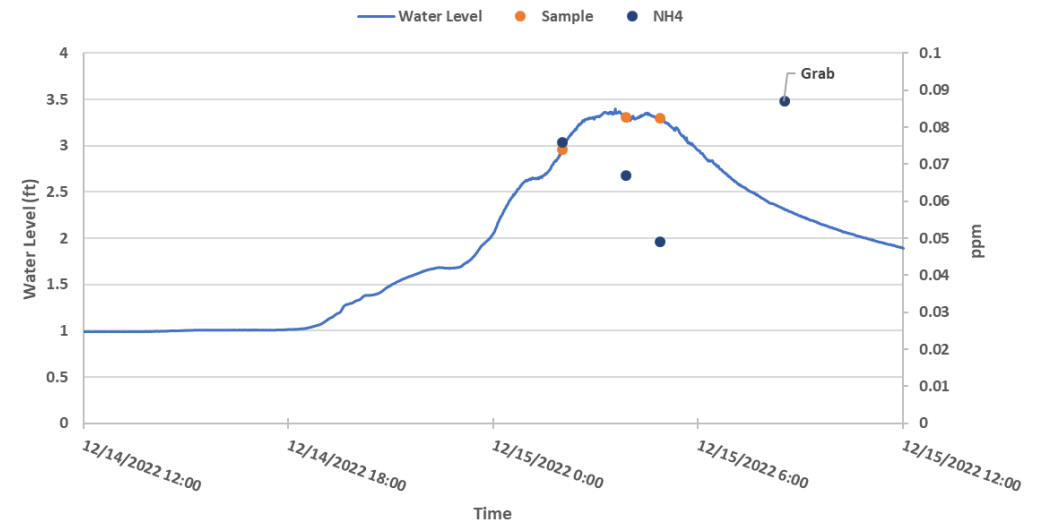
Little Stone Mountain, Storm Sample Event 3



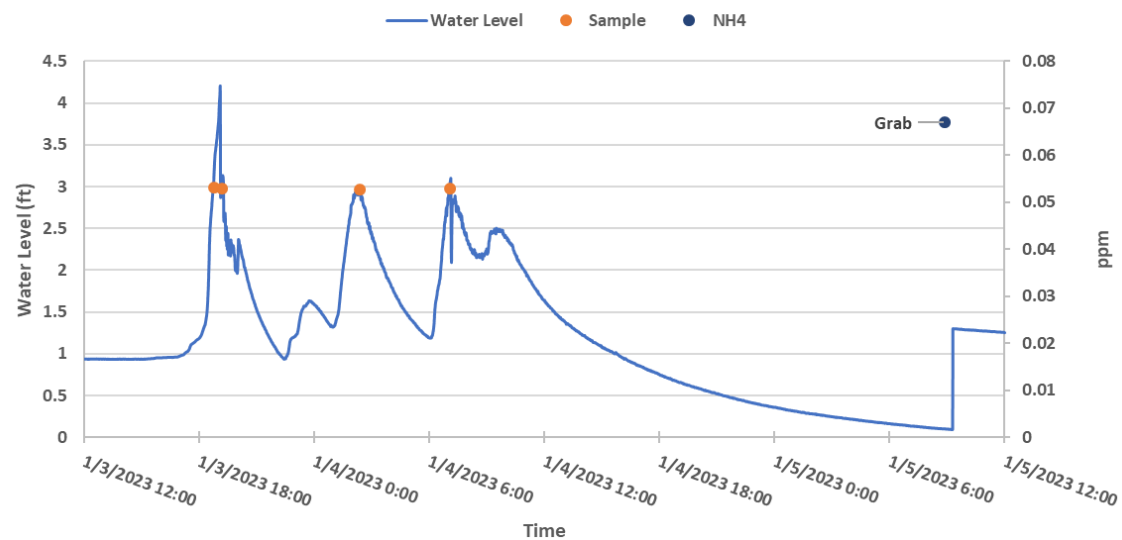
Little Stone Mountain, Storm Sample Event 2



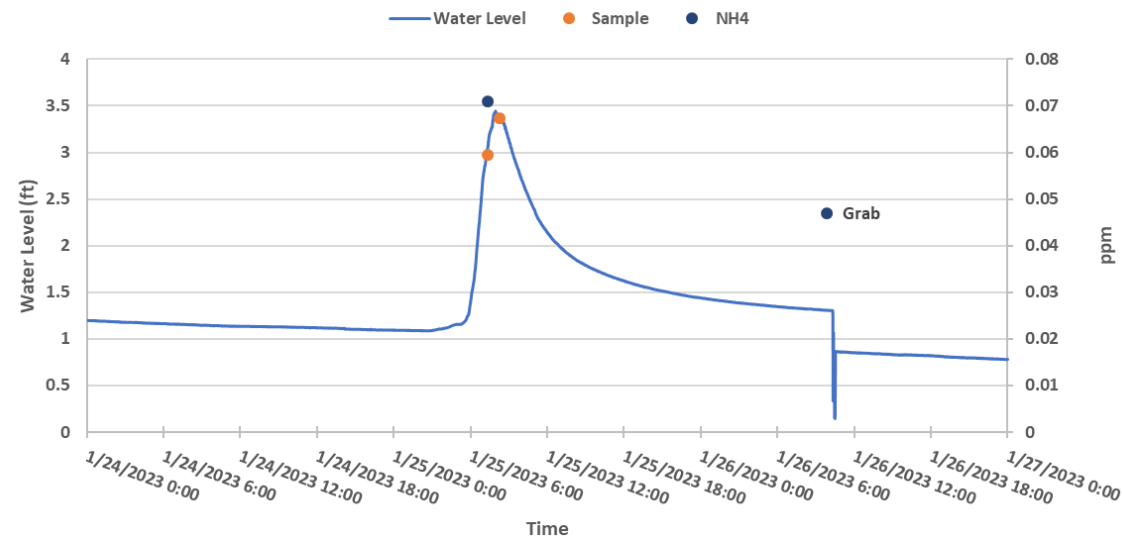
Little Stone Mountain, Storm Sample Event 4



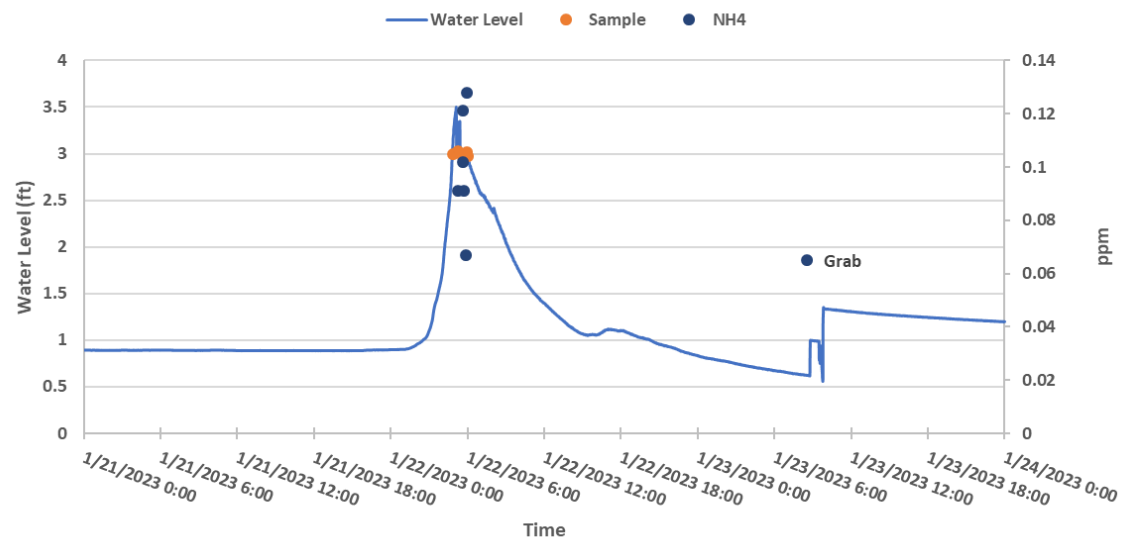
Little Stone Mountain, Storm Sample Event 5



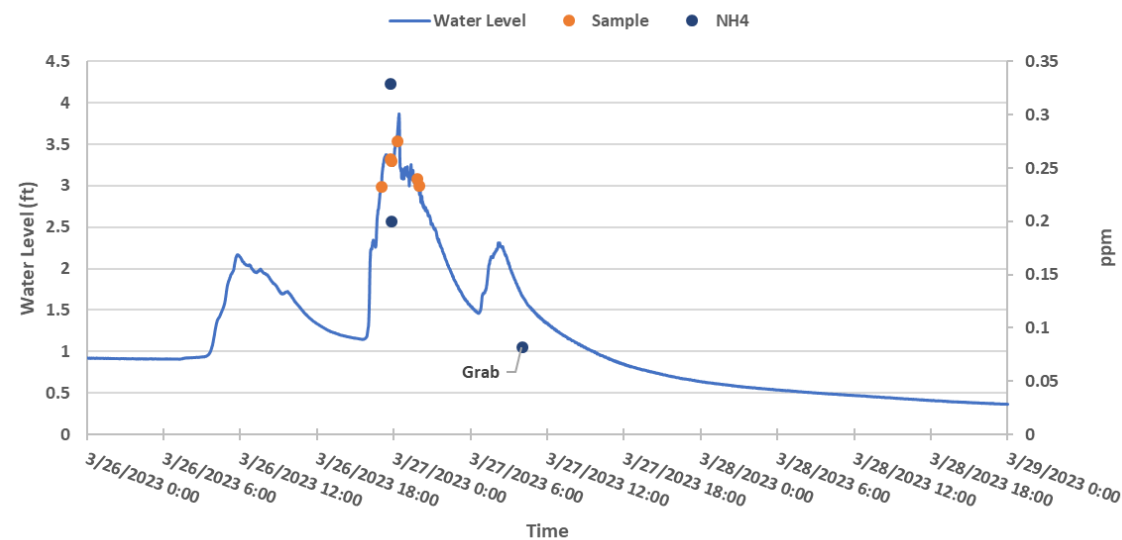
Little Stone Mountain, Storm Sample Event 7



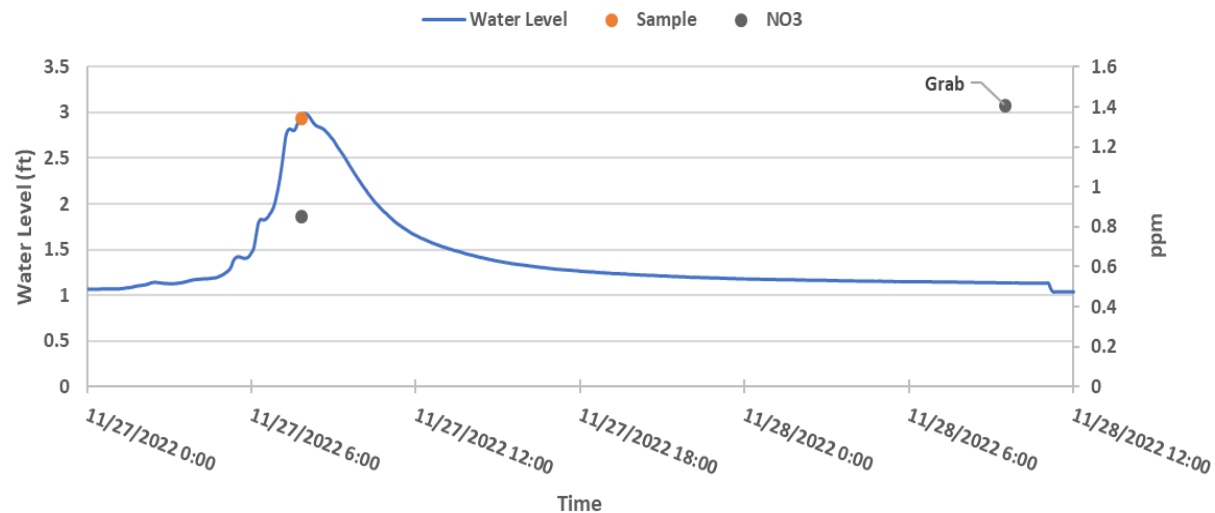
Little Stone Mountain, Storm Sample Event 6



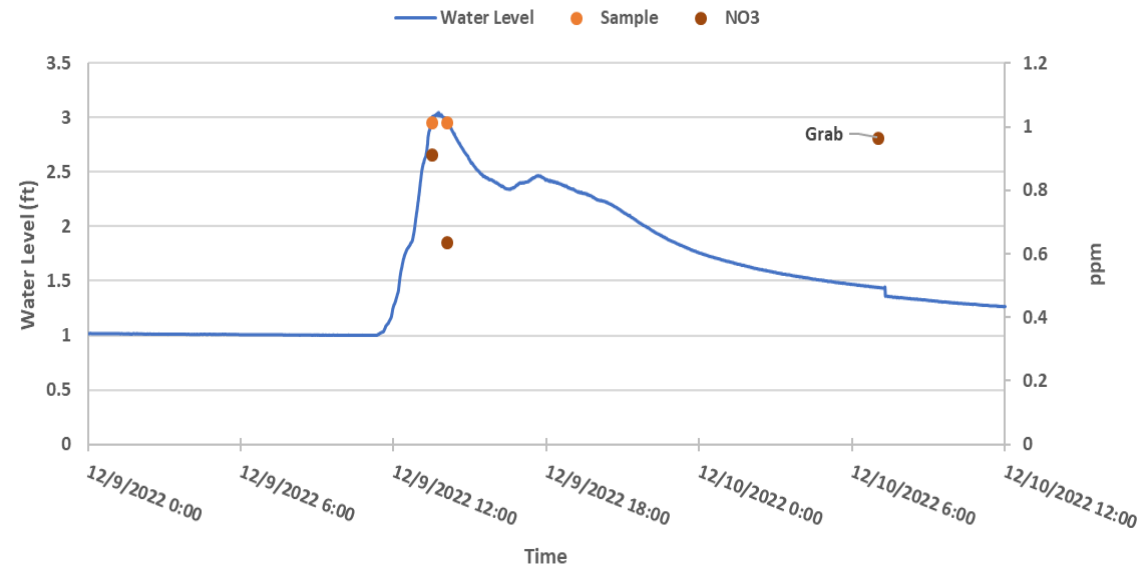
Little Stone Mountain, Storm Sample Event 8



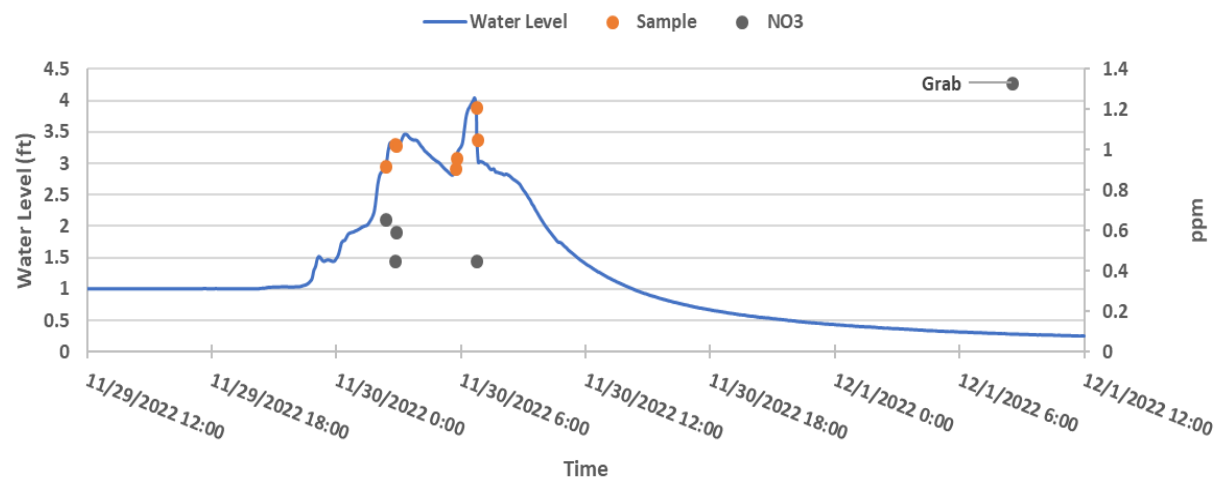
### Little Stone Mountain, Storm Sample Event 1



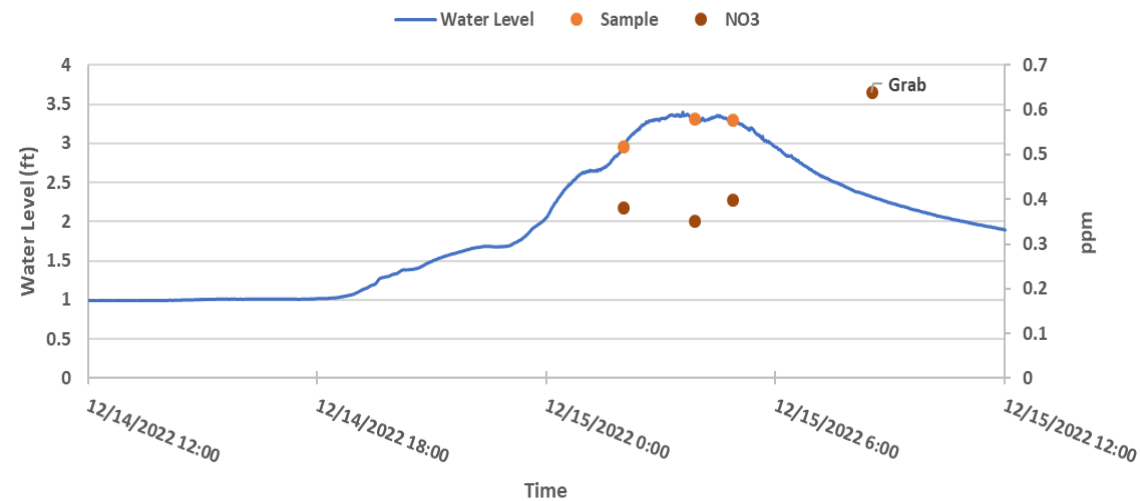
### Little Stone Mountain, Storm Sample Event 3



### Little Stone Mountain, Storm Sample Event 2

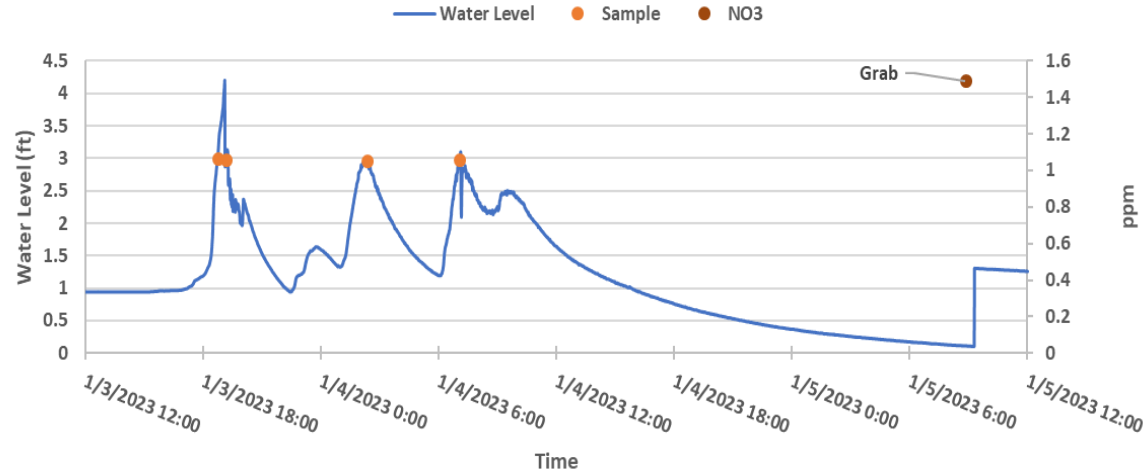


### Little Stone Mountain, Storm Sample Event 4

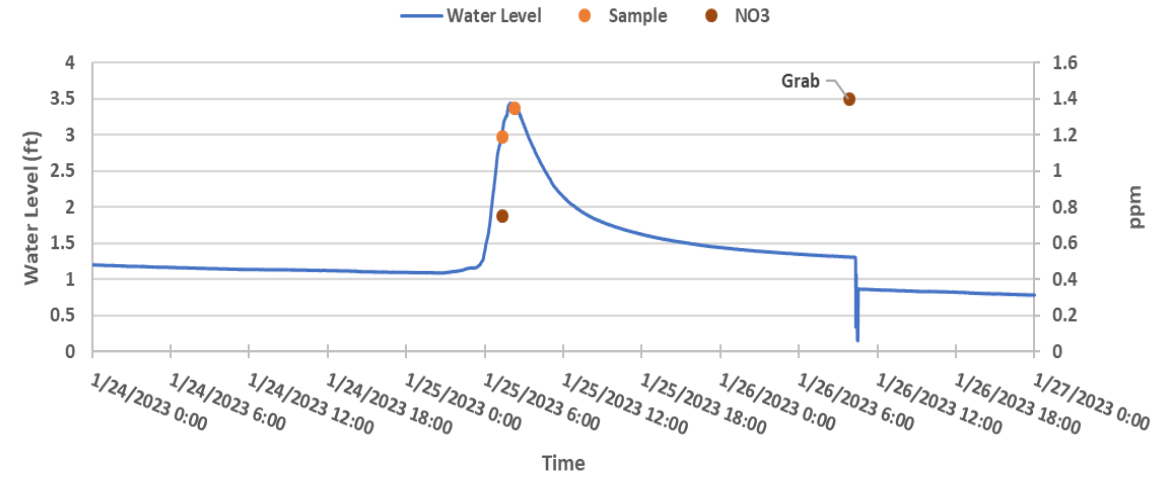




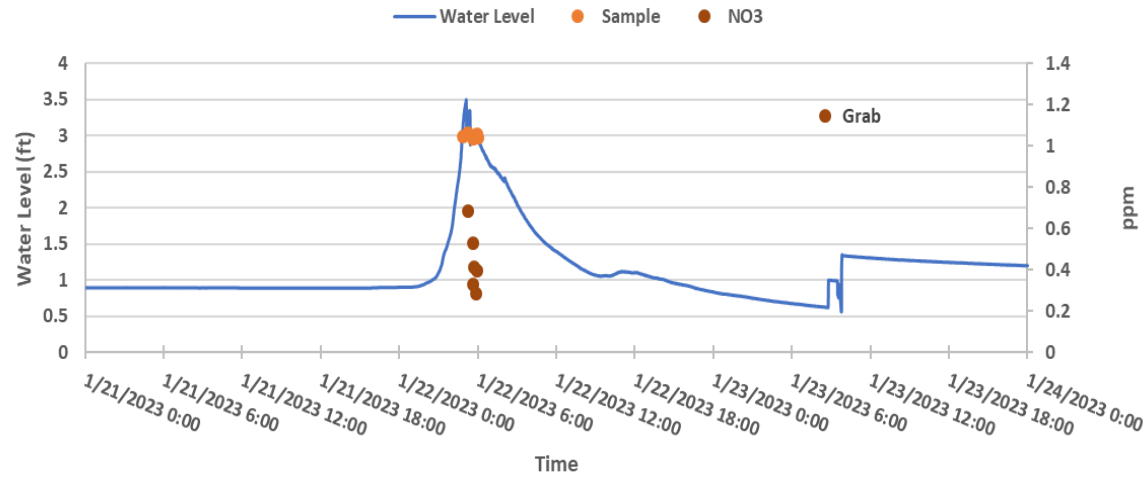
Little Stone Mountain, Storm Sample Event 5



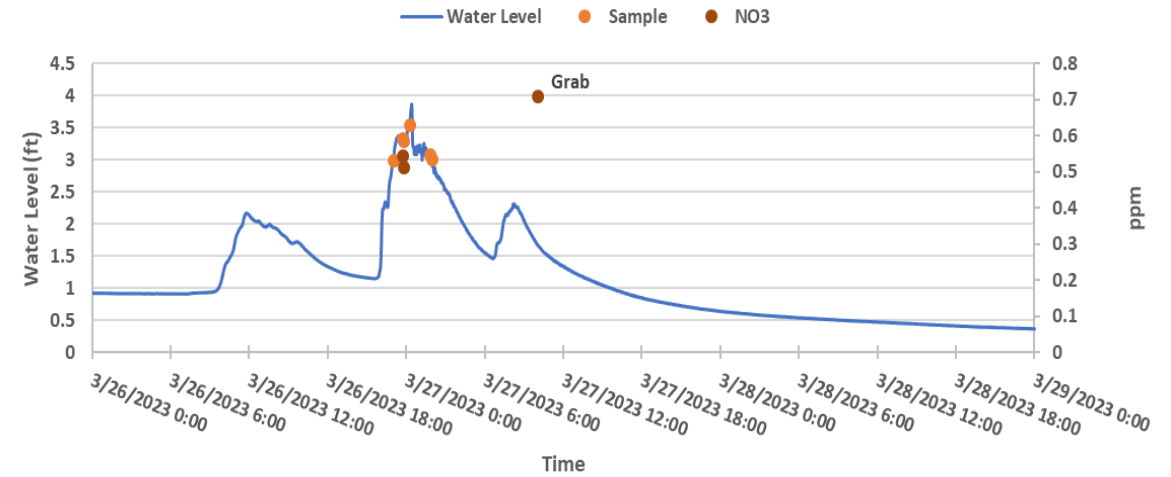
Little Stone Mountain, Storm Sample Event 7



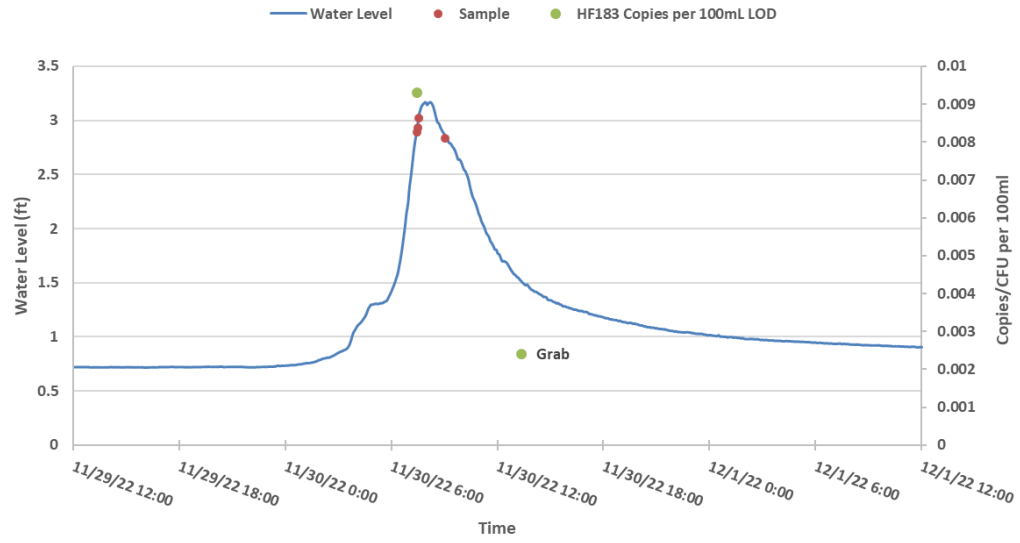
Little Stone Mountain, Storm Sample Event 6



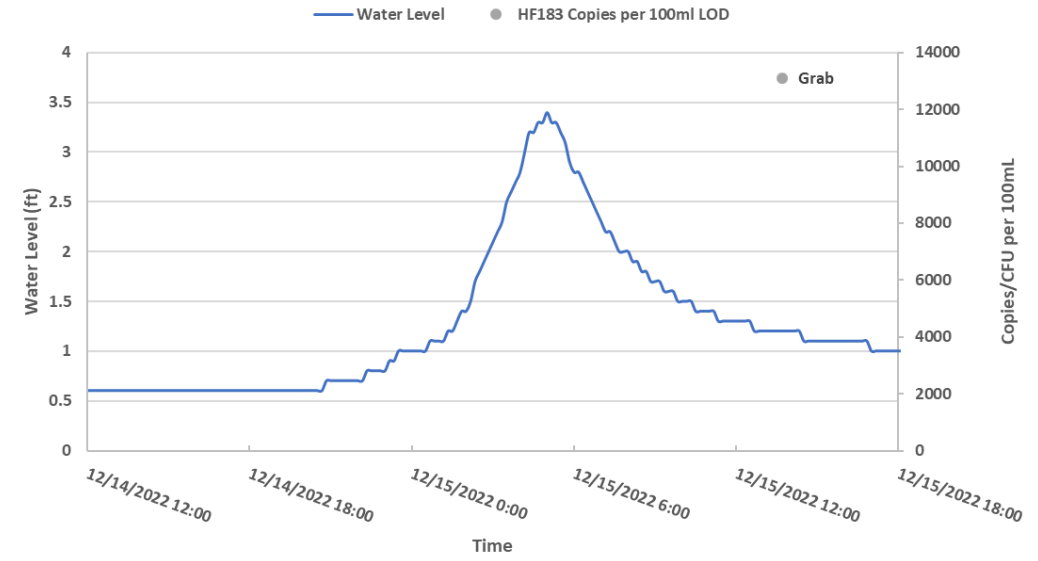
Little Stone Mountain, Storm Sample Event 8



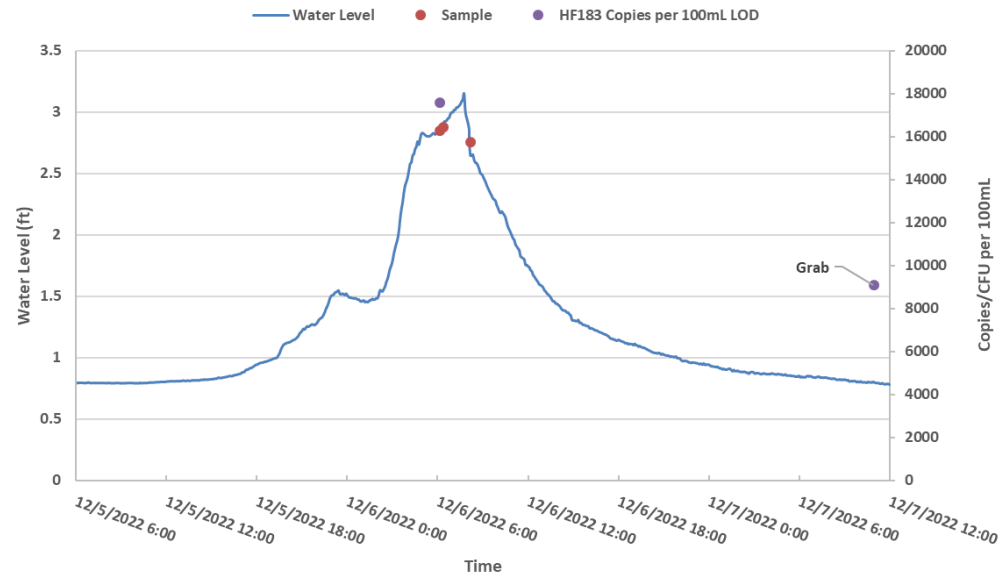
Pond Fork, Storm Sample Event 1



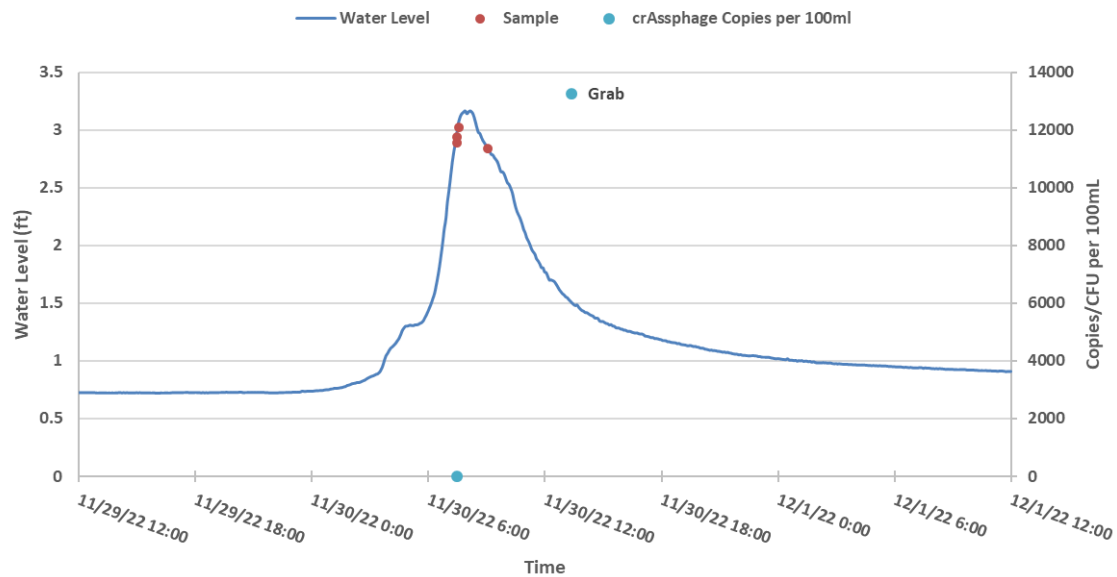
Pond Fork, Storm Sample Event 3



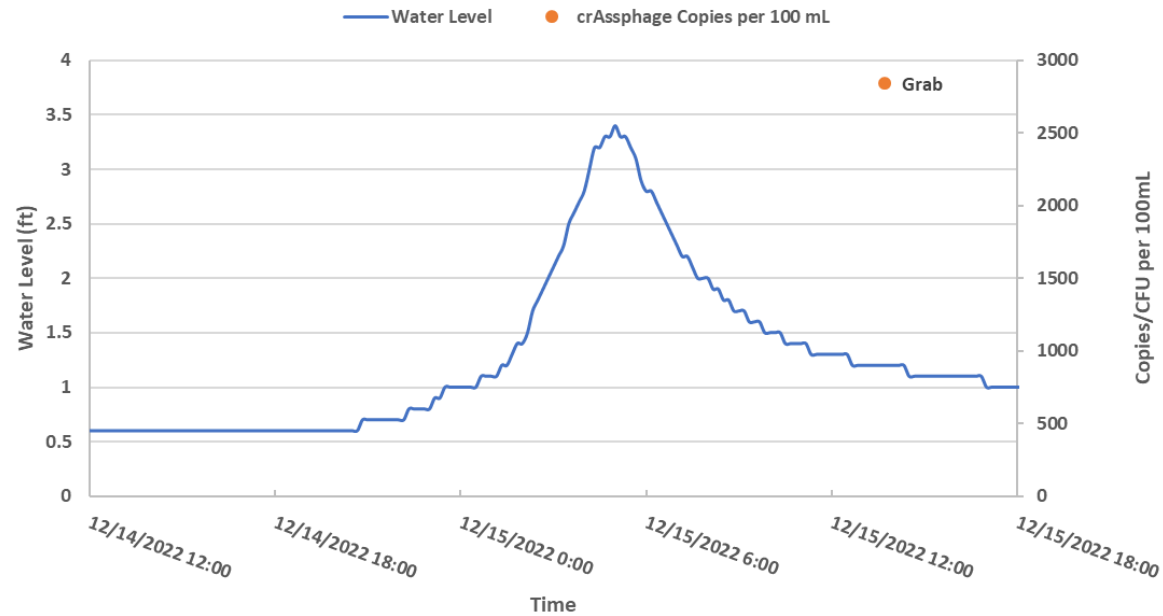
Pond Fork, Storm Sample Event 2



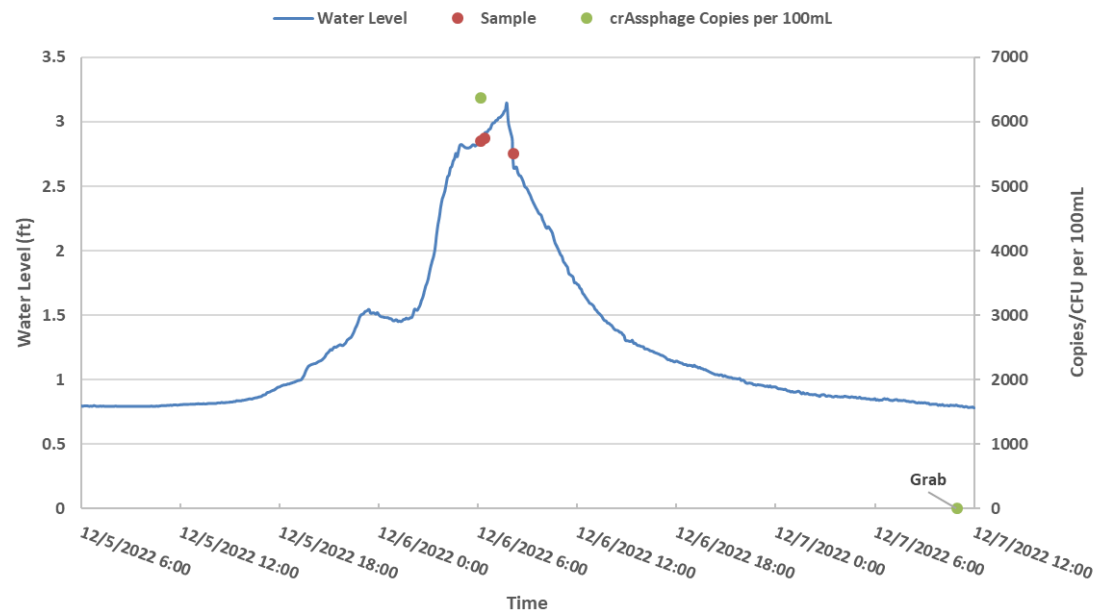
Pond Fork, Storm Sample Event 1



Pond Fork, Storm Sample Event 3

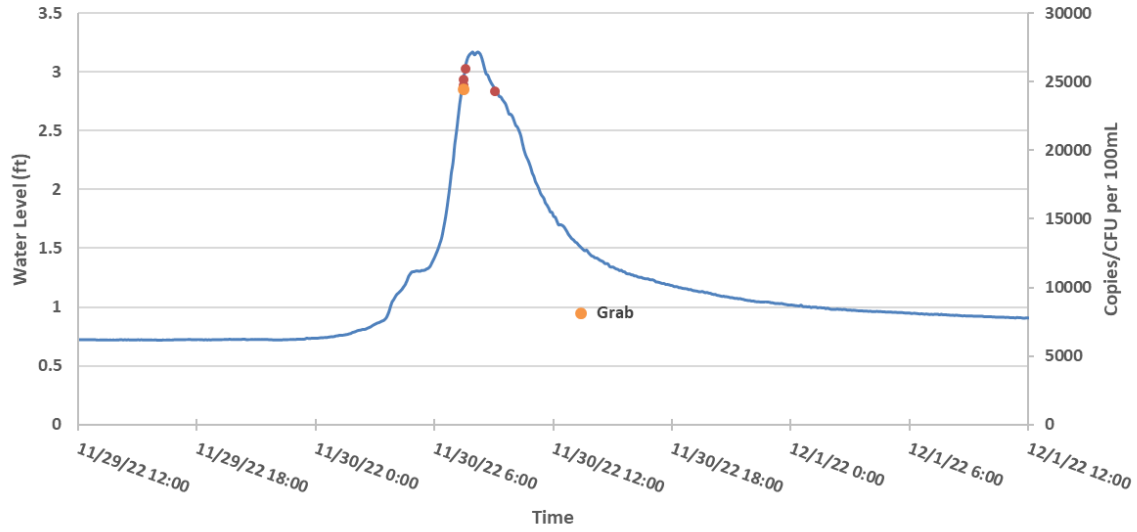


Pond Fork, Storm Sample Event 2



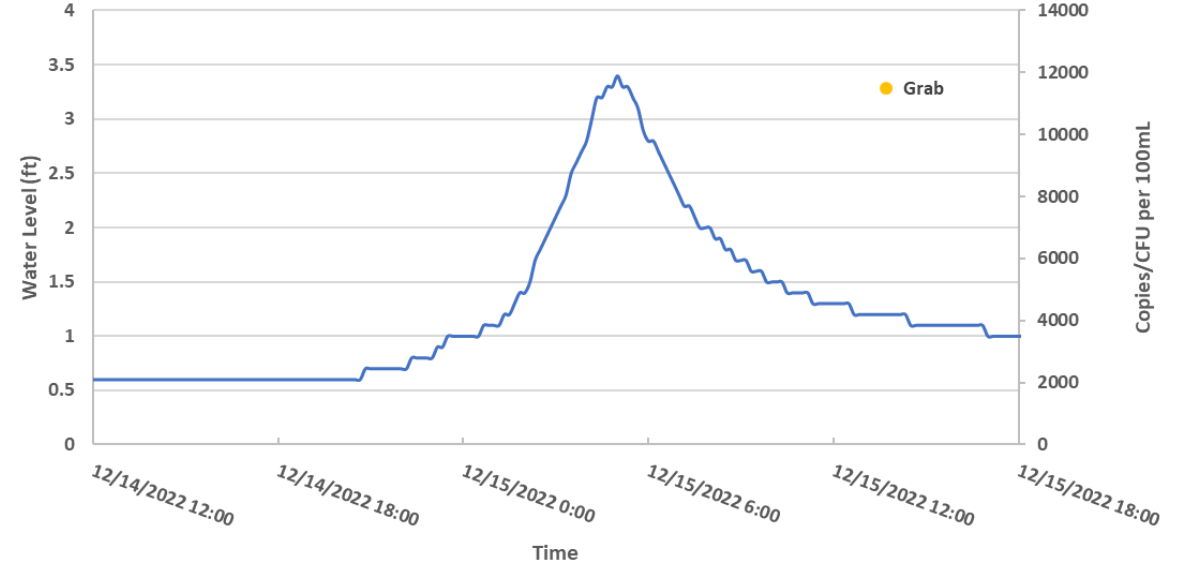
### Pond Fork, Storm Sample Event 1

Water Level Sample Fecal Coliform CFU per 100mL



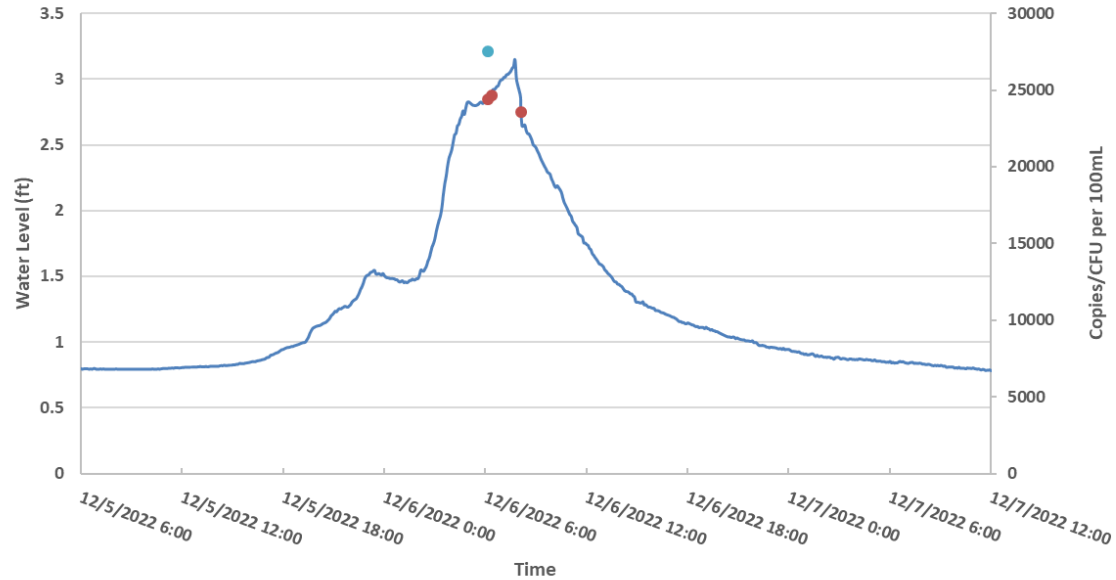
### Pond Fork, Storm Sample Event 3

Water Level Fecal Coliform CFU per 100 mL

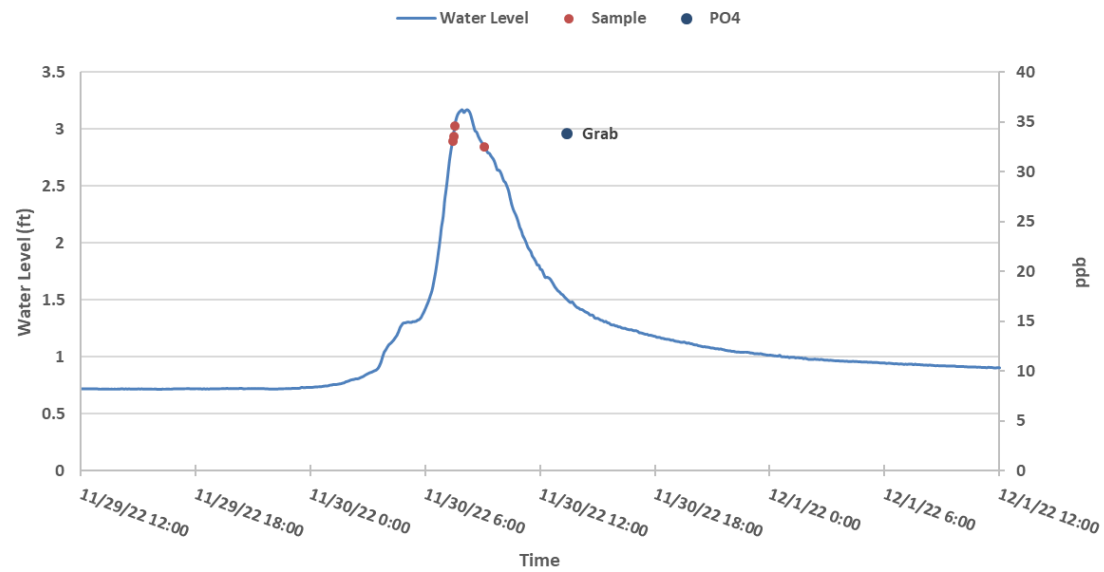


### Pond Fork, Storm Sample Event 2

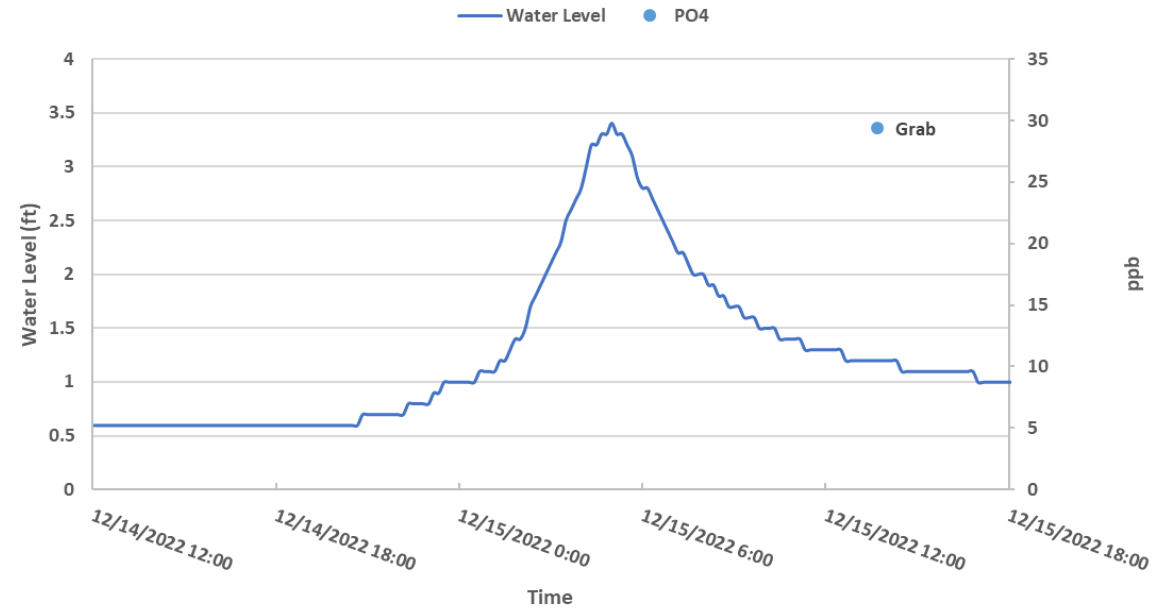
Water Level Sample Fecal Coliform CFU per 100mL



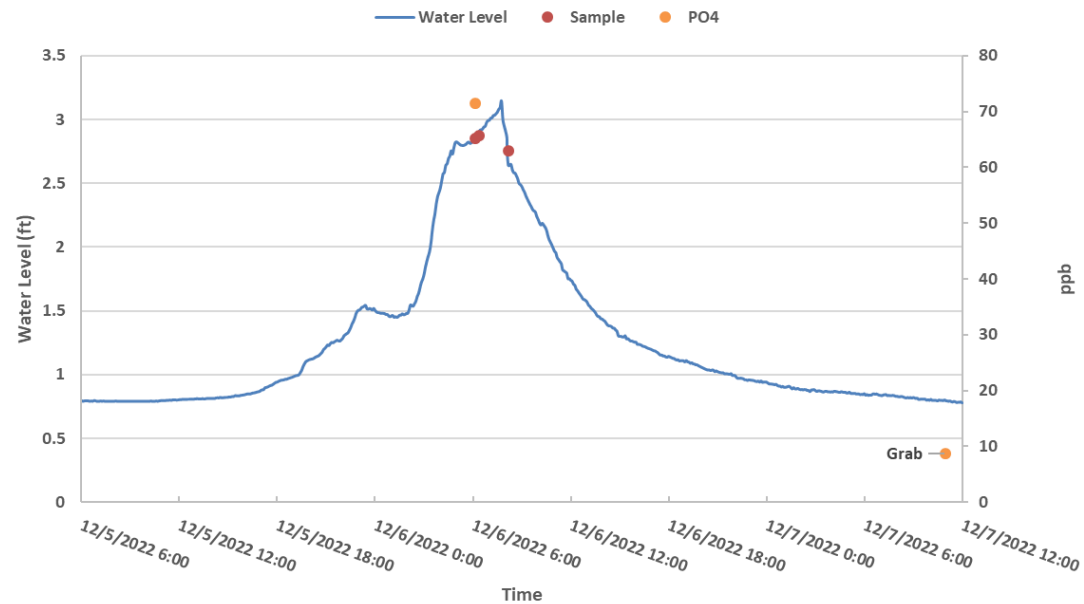
Pond Fork, Storm Sample Event 1



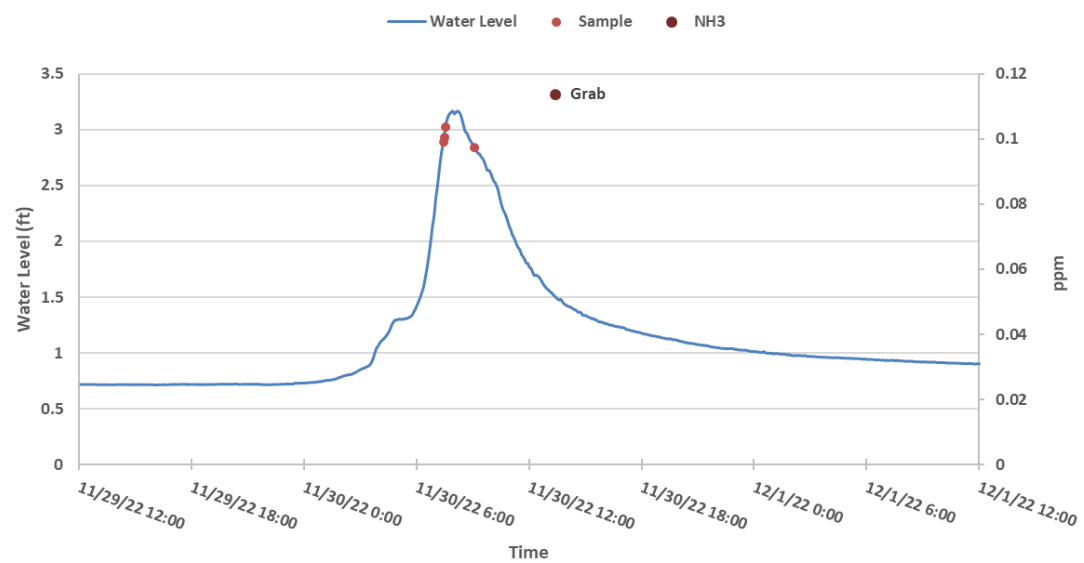
Pond Fork, Storm Sample Event 3



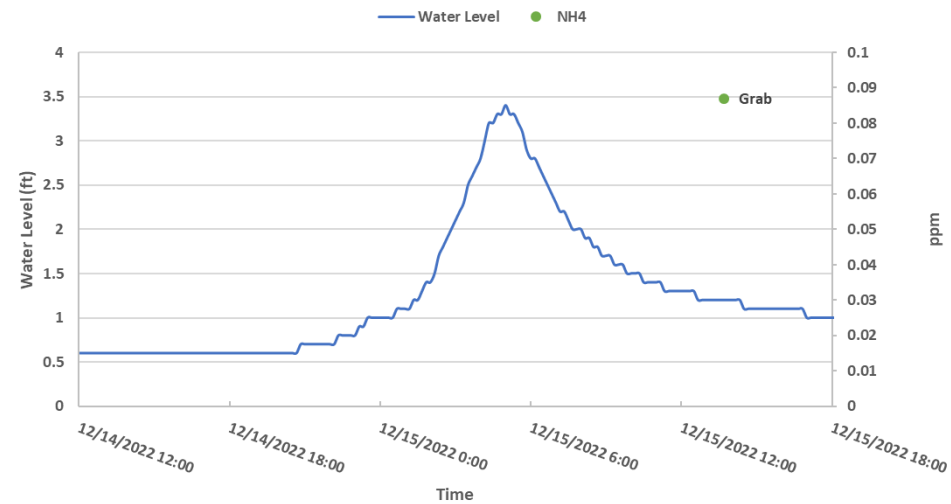
Pond Fork, Storm Sample Event 2



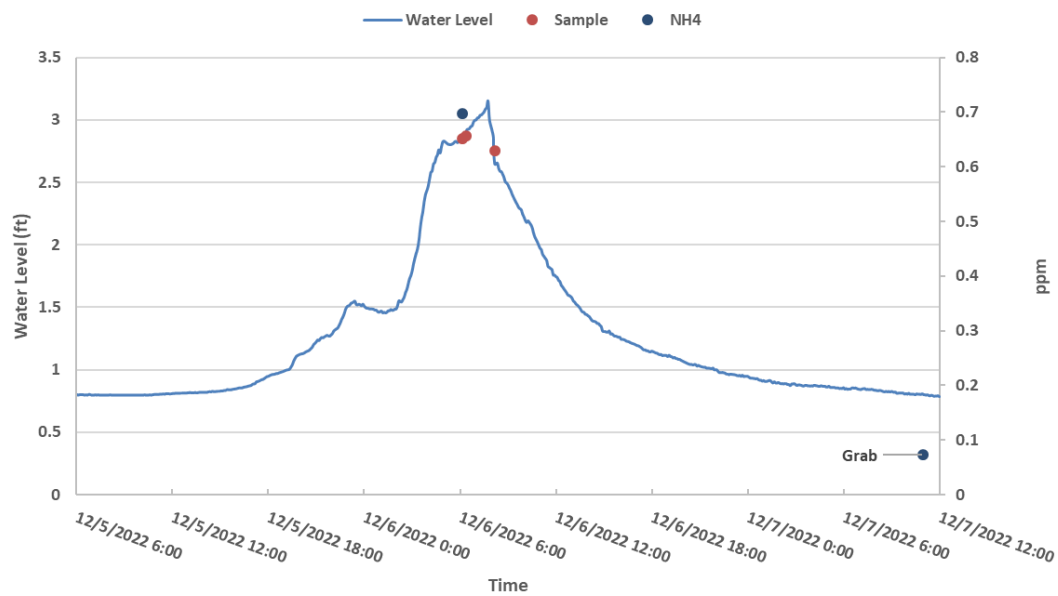
### Pond Fork, Storm Sample Event 1



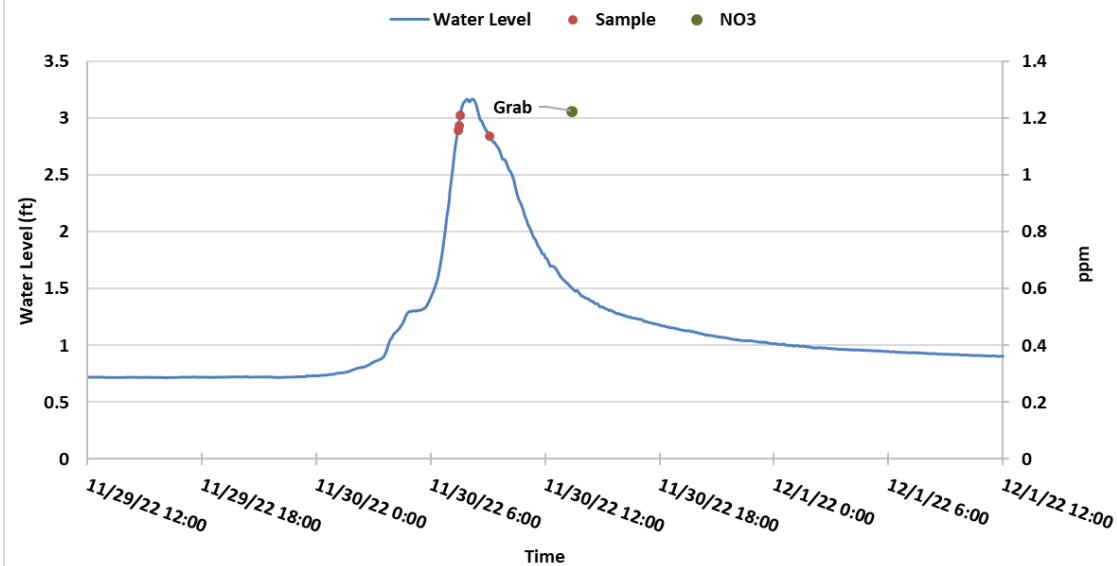
### Pond Fork, Storm Sample Event 3



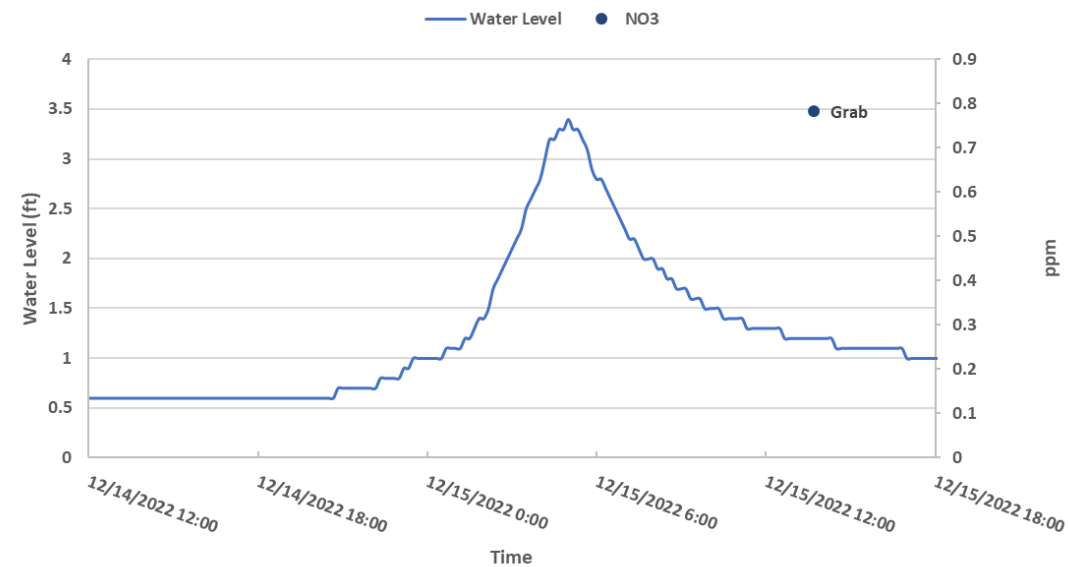
### Pond Fork, Storm Sample Event 2



Pond Fork, Storm Sample Event 1



Pond Fork, Storm Sample Event 3



Pond Fork, Storm Sample Event 2

