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Bacterial Water Quality Monitoring as Citizen Science in Congaree National Park, South Carolina

Shea McCarthy

BACTERIAL WATER QUALITY MONITORING AS CITIZEN SCIENCE IN CONGAREE NATIONAL PARK, SOUTH CAROLINA

By

Shea McCarthy

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Accepted by:

Dwayne Porter, Director of Thesis

David Shelley, Reader

Geoff Scott, Reader

Cheryl L. Addy, Vice Provost and Dean of the Graduate School

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I owe all of my achievements to my loved ones, my family, and my friends. They have given me the opportunity to be successful and I am forever grateful for their daily support and encouragement.

ABSTRACT

Congaree National Park aims to preserve its natural and cultural resources while protecting these benefits for current and future enjoyment. Hydrologic features make up a large portion of the park and attract visitor recreation. There are several known sources of pollution that enter these waterways, mainly from upstream sources of waste discharge. The national park and its surrounding areas hold great ecological significance, however, there are many threats to the surface water quality in this area. Water quality degradation can impact the ecosystem, wildlife, and visitor experience. This project specifically considers exposure to fecal contamination in surface waters from upstream and local sources. In addition to its detriments to the environment, exposure to fecal contamination also poses a risk for human health in recreational waters. The overall goals of this project were to assess fecal contamination levels in the waters of the Congaree National Park and to design a monitoring program that incorporates citizen science to regularly test for bacteria levels in park waters. Through the course of this project, bacterial water quality sampling was conducted, and the analyses reviewed, and the sampling methodology was optimized and documented to be transferable to a citizen science program. Development of a citizen science approach allows for sustainable practices and civic engagement with results that can benefit both park and public information, while minimizing staff requirements from Congaree National Park.

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LIST OF ABBREVIATIONS

CFU ..... Colony Forming Units

CONG ..... Congaree National Park

CWA ..... Clean Water Act

DHEC ..... Department of Health and Environmental Control

DI ..... Deionized

E. coli ..... Escherichia coli

EDC ..... Endocrine Disrupting Chemicals

ENHS ..... Environmental Health Sciences

EPA ..... Environmental Protection Agency

GI ..... Gastrointestinal

MST ..... Molecular Source Tracking

MUG 4-Methylumbelliferyl-β-D-glucuronide

NIH ..... National Institute of Health

NOAA ..... National Oceanic and Atmospheric Administration

NPDES ..... National Pollutant Discharge Elimination System

NPS ..... National Park Service

NRW ..... National Resource Water

OGBFREC ..... Old-Growth Bottomland Forest Research and Education Center

ONPG ..... ortho-Nitrophenyl-β-galactoside

ONRW ..... Outstanding National Resource Water

UNESCO ..... United Nations Educational, Scientific, and Cultural Organization

USC ..... University of South Carolina

USGS ..... United States Geological Survey

UV ..... Ultraviolet

VIP ..... Volunteers in Parks

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

Congaree National Park (CONG) in Hopkins, South Carolina was the state's first national park. CONG preserves and protects approximately 27,000 acres including forest, floodplains, and surface water systems. Along with its unique ecosystem, national and state champion trees and biological diversity, CONG is also well-known for its old-growth bottomland hardwood forest, which is the largest remaining intact area of its kind in the southeastern United States. The floodplain forests of CONG regularly receive flood waters from the Congaree and Wateree Rivers, as well as additional tributary systems.[1]

According to the Foundation Document for CONG, *"The park is sustained by the rivers that bound it. Periodic floodwaters from the Congaree and Wateree rivers sweep through the bottomland forest, carrying nutrients and sediments that nourish and rejuvenate the rich floodplain ecosystem and its diverse assemblage of plants and animals."*[2]

Hydrologic features make up a great part of the resources and visitor appeal of CONG, as most of the park is either aquatic, wetland, or floodplain. CONG is bordered by the Congaree River and the Wateree River, as well as additional tributary systems in the park include Cedar Creek, Tom's Creek, Dry Branch, and others which feed the Congaree and Wateree Rivers. Cedar Creek is arguably the primary hydrologic feature

within park boundaries as it runs through the majority of the park, connects other water systems, and is frequently used for recreation by park visitors. CONG floods several times per year with overflowing waters from the Congaree and Wateree Rivers as well as tributary rivers, creeks, and lakes. Therefore, even surface waters external to the park contribute to the floodwaters that cover park land during floods, carrying nutrients and pollutants from the earth, soil, and impervious surfaces back into surface water systems.[2]

River and creek systems within the park, as well as those that contribute to the park's floodplain, provide for a diverse and bountiful habitat for a multitude of organisms. As well as being home to a variety of species, CONG also entertains more than 160,000 visitors per year. Visitors enjoy the park through canoe tours, kayaking, camping, fishing, bird watching, and hiking along trails and boardwalks. With such a majority of the park's area being either aquatic or wetland, a significant portion of visitor experiences at CONG are directly related to its water systems. Many activities put park visitors, volunteers, and staff in direct contact with river and creek waters.[1]

1.2 SIGNIFICANCE

Each national park provides a document that outlines the mission of the National Park Service (NPS) as well as the individual park's purpose, significance, fundamental resources and values, and management practices.[2] According to the Foundation Document for CONG, there are several natural resources of high value to CONG that are to be protected through management practices. These are considered fundamental resources and include the following: bottomland hardwood forest, big trees, floodplain, Cedar Creek, biodiversity, wilderness, and historic and prehistoric sites. The most

significant attributes of CONG which influence park planning and management due to their importance are outlined as follows:

- *"Congaree National Park protects the nation's largest remaining tract of Southern old-growth bottomland forest and a significant expanse of associated floodplain."*
- *"Congaree National Park preserves unique regional cultural history, archeological sites, and landscape features that document evolving agricultural, commercial, and social practices in the bottomlands and forests of the South Carolina Midlands."*
- *"The Congaree National Park Wilderness preserves the wilderness character of the largest expanse of old-growth bottomland forest in the National Wilderness Preservation System and provides opportunities to experience solitude, challenge, and adventure that are unique to this landscape"*[2]

Portions of Cedar Creek within CONG boundaries are designated as National Resource Waters (NRW) and Outstanding National Resource Waters (ONRW). The ONRW recognition is reserved for water bodies that have both remarkable water quality and great ecological significance. This designation is especially beneficial for the protection of water systems as it can be used as a management tool by discouraging development and promoting protection of water quality. Waters with such designations should maintain a high standard of water quality conditions and have strict restrictions regarding dumping or discharge of urban or agricultural waste.[3] Additionally, CONG is included in an area designated as an international Biosphere Reserve by the United

Nations Educational, Scientific and Cultural Organization (UNESCO). This international recognition defines and protects areas of environmental and cultural significance and also promotes sustainable development in such areas.[4]

1.3 PROBLEM STATEMENT

Good surface water quality is beneficial to various aspects of CONG including visitor experience, health of habitats, and wildlife success. Water quality degradation by fecal contamination is of particular concern in this area, especially considering potential bacterial pollutants from upstream sources such as faulty sewage systems and waste discharges which are further outlined in Chapter 3. Animal sources of fecal contamination also have high potential to contaminate CONG surface waters from upstream agricultural land and from animal populations, both native and non-native, that inhabit the area.[5] The various sources of fecal contamination and potential for high levels of such contamination is concerning due to potential water quality degradation and human health concerns. Exposure to fecal coliform bacteria, especially E. coli, can be a major risk to human health. Some strains of Escherichia coli (E. coli) are pathogenic and can cause very serious illnesses in humans including gastrointestinal (GI) illness and bacterial infection.[6]

There is local understanding and concern regarding fecal contamination in park waterways. Significant threats to contamination of surface waters include human waste discharge and agricultural runoff. Due to the significance of water quality to the park, improvement of water quality monitoring is in the top 20% of priorities for the CONG five-year strategic plan. It is of high priority in the CONG “data needs” planning to establish a water quality monitoring database to inform both park staff and visitors about

water quality trends.[2] Planning for management that protects this this resource includes collecting water quality monitoring data and expanding monitoring sites to monitor waters entering and exiting the park.[2] The specific need for bacterial water quality monitoring in recreational waters of Congaree National Park aims to protect both water quality and human health.

1.4 OBJECTIVES

The overall objective of this project was to develop and establish a water quality monitoring program in Congaree National Park to address monitor levels of bacterial contamination. Due to staffing and resource limitations, there is currently no regular bacterial water quality monitoring being conducted directly by the park. Recent water quality testing done by other groups in and around the park have usually excluded bacterial parameters. This proposed program would regularly test for total coliform and E. coli bacteria concentrations at multiple sites within CONG, mainly along Cedar Creek. Water quality monitoring would analyze changes in bacteria concentrations over time and how they are related to factors such as precipitation events or seasonal patterns.

In order to address the realities of staffing limitations and create a sustainable program that can be continued in the park, this water quality monitoring program was developed and proposed as a citizen science project. CONG has a large and enthusiastic base of volunteers that are regularly involved with park projects. Executing this monitoring program as citizen science and allowing for volunteer participations creates potential to sustain this project to continue into the future. To make this possible, project objectives also included creating and compiling documents to guide the continuation of bacterial water quality monitoring.

1.5 DOCUMENT OVERVIEW

There were various factors and steps that each played a significant part in the completion of this project, which are each laid out through this document. The document is organized as follows: literature reviews are detailed in Chapter 2, providing background for developing this program for CONG. Chapter 3 details preliminary bacterial water quality sampling, including methods and results. The methodology practices and results were taken further to adopt this project for citizen science and other types of transferability, which can be seen in the chapters that follow. Part of this adoptability was explored in Chapter 4, which analyzes laboratory methodology alternatives for scenarios of limited laboratory resources, funding, or time. Then, Chapter 5 outlines all of the steps taken to develop and implement a citizen science bacterial water quality monitoring program, including documentation for CONG to use for the continuation of this project. Continuing with the idea of adoptability, Chapter 6 provides professional recommendations, specific to CONG, on the benefits of continuing bacterial water quality monitoring and the feasibility of doing so. The overall findings and understandings as they were developed through this project are summed in Chapter 7, along with the significance of this work and final conclusions.

CHAPTER 2
LITERATURE REVIEW

2.1 OVERVIEW

The main purpose of conducting a thorough local literature review was to better understand the topics of this project and how they have been addressed in the past. Research projects, local partnerships, and CONG management efforts were each topics of interest. Research topics include fecal contamination in surface waters, bacterial water quality monitoring, the use of citizen science in national parks, and relevant research projects that have been conducted in CONG boundaries. Relevant research projects have included short-term water quality sampling in and around CONG that have been conducted by organizations or university students in partnership with CONG.

2.2 WATER QUALITY CONCERNS

There are several potential threats to surface water quality in the watersheds impacting CONG. CONG is approximately 20 miles downstream from the state capital of Columbia, South Carolina as seen in Figure 2.1. The city of Columbia has a 2018 population of 133,451,while the metropolitan statistical area of Columbia has a population of 832,666.[7] While CONG protects over 20,000 acres of undeveloped land, the large, metropolitan areas of developed land and their proximity to the park are potentially detrimental to surface water quality.

CONG is located at the downstream end of the Saluda and Broad River basins, both of which feed surface water to CONG. Figure 2.2 shows the major river basins of South Carolina and highlights the two river basins, Broad and Saluda, which feed CONG – represented by a dot in the center of the state. This figure and others was obtained from the South Carolina Department of Health and Environmental Control (DHEC) Watershed Atlas, which is an interactive online map with data available regarding various geographic data.[8]

The Saluda River Basin stretches from the Upstate to the Midlands of South Carolina. Among numerous over river and stream systems (totaling 5,609 stream miles) this basin includes the Saluda River, which joins with the Broad River to form the Congaree River at its confluence. The Saluda River Basin was comprised of mainly forested land making up 53.7% of land use, followed by 26.1% agricultural land, and 12.9% urban land as of 2011.[9] The other river basin contributing to CONG waters is the Broad River Basin which has the following land use breakdown: 60.6% forested, 23.8% agriculture, etc.[10] The Broad River spans all the way from North Carolina to the South Carolina midlands where it converges with Cedar Creek and multiple other creeks before meeting the Saluda River in Columbia.[10] The Congaree River watershed includes the river itself along with the tributaries from Cedar Creek. In this area, land use includes 35.8% forested land, 27% agricultural, and 24.4% forested wetland. Land use in these river basins is demonstrated in Figures 2.3, 2.4, and 2.5.[9]

The Clean Water Act (CWA) was established in 1972 by US Congress, and under this law, the United States Environmental Protection Agency (EPA) regulates surface water quality by controlling pollutant discharges. The CWA establishes water quality standards

for pollutants, sets regulations for wastewater discharges, and restricts the discharge of pollutants without a registered permit. These permits are National Pollutant Discharge Elimination System (NPDES) permits which are regulated by the EPA and authorized by the state government, such as South Carolina DHEC.[11]

NPDES permits are required for sites of point source discharges including wastewater treatment plant effluent, private or residential wastewater discharge, and stormwater discharges from industrial, municipal, or construction areas.[12] Upstream of CONG, there are a few dozen known waste discharge sites, permitted by DHEC as NPDES locations. Figure 2.6 shows each NPDES permit site and highlights the Broad and Saluda river basins.[8] Among these sites are two of the largest wastewater treatment facilities in the state, both of which are located directly on the Congaree River less than 20 miles upstream of CONG. Figure 2.7 shows the West Columbia wastewater treatment plant and Columbia sewage treatment plan as well as their proximities to the Congaree River.[13]

The presence of fecal contamination in recreational waters is associated with higher risk of GI illnesses due the potential that E. coli bacteria is present. The EPA considers E. coli to be a good predictor of GI illness risk in fresh recreational waters. The EPA water quality criteria previously recommended that fecal coliform bacteria be used as water quality indicators for recreational waters. However, beginning in 1983, studies showed that E. coli is the best predictor of illness risk in recreational fresh waters. EPA's 1986 water quality criteria identifies the standard of E. coli levels in recreational waters to be an average of 126 CFU/100ml.[14] Colony forming units (CFU) and Most Probable Number (MPN) are used interchangeably and both represent measurements of

bacteria in a sample.[15] The EPA recommends that individual states set water quality criteria that consider risk management.[14] In South Carolina, DHEC water standards indicate that E. coli concentrations in freshwaters should not exceed a monthly mean of 126 MPN/100ml or a daily maximum of 349 MPN/100ml.[16] DHEC quality criteria for freshwater are listed in Appendix A.

To understand the impact of land use on water quality, a recent article by Petersen et al. (2018) explores the relationship between local land use practices and water quality parameters.[17] Results show that among different types of land use (e.g. forest, agriculture, developed) agricultural land was most associated with degraded water quality parameters. Sample sites with high agricultural land use had very low dissolved oxygen rates as well as the highest concentrations of E. coli, while the best water quality parameter results were found at sample sites with forested or undeveloped land use. This study also showed that fluctuations in precipitation events and runoff were related to changes in E. coli concentrations in surface waters.[17] These findings are important for the sake of this study considering the significant and growing percentage of agricultural and developed land upstream of CONG. The Saluda and Broad river basins also contain dozens of swine, poultry, dairy, and cattle farms, including some in the immediate area of CONG as shown in Figure 2.8.[8] Additionally, the immediate surrounding area of CONG, including private residences, schools, business, and industries is almost entirely on septic systems, and reports show septic failure rates as high as 72%.[18]

Another potential source of fecal contamination in park waters is from animal populations within the park. There are significant mammalian populations such as deer and hogs regularly seen in park boundaries. Alligators may also cause concern for fecal

contamination while not overly abundant. Most importantly, American alligators show significantly higher concentrations of fecal coliform bacteria per weight of feces than any other population of concern.[19] Especially concerning is the large population of non-native feral hogs that live in and around CONG. Along with causing detriment to the local habitat by the destruction of forest floor and uprooting of native vegetation, they also contribute to bacterial contamination of water sources.[20] Fecal contamination is escalated from both large amounts of hog waste and increased soil erosion. Feral hogs pose a threat to natural resources in the park mainly due to the massive size of the hog population which is a result of the species being highly adaptive, reproducing quickly, and a lack of natural competitors.[20]

2.3 IMPAIRED WATERS

Water quality monitoring is conducted in the South Carolina by DHEC to assess trends and identify areas for improvement of water quality. DHEC regularly collects surface water samples from sites throughout the state to test for both chemical and physical water quality parameters which can inform management decisions.[21] Results are compared to state water quality standards listed in the South Carolina DHEC Regulation 61-68 Water Classifications and Standards. These regulations outline safe thresholds and limits for water quality parameters. These criteria from DHEC Regulation 61-68 are listed in Appendix A. By following these regulations, DHEC analyzes the data to identify areas of poor water quality, including the 303(d) list of impaired waters.[16]

There are several impaired water sites from the DHEC 2016 303(d) list within the Saluda and Broad river basins which can be seen in Figure 2.9 while Figure 2.10 shows a closer image of 303(d) sites in and around CONG.[8] Eight of these listed 303(d) sites are

within the Congaree River watershed and six sites are either within park boundaries or immediately adjacent (within 3 miles) of CONG. The six 303(d) listed sites directly impacting CONG are shown in Figure 2.11. Two of these sites, shown in red on Figure 2.11, are 303(d) listed sites that are within the ONRW designated area of Cedar Creek.[5] Additionally, three of these sites, which are within CONG boundaries, are listed as 303(d) impaired waters due to elevated levels of E. coli bacteria. Figure 2.12 shows the three sites, listed as impaired waters due to high E. coli concentrations, in relation to CONG.

2.4 CITIZEN SCIENCE

Several management and policy documents note the threat of fecal contamination in surface waters of CONG and the importance of protecting the waters of interest.[3–5] Citizen science can be a beneficial approach to implementing the collection and monitoring of bacterial water quality monitoring at CONG. Citizen science is a successful and growing concept that is especially popular in federal organizations, such as the NPS, because of their need for data collection as well as volunteer interest in working with such organizations. This approach engages active volunteers in scientific projects and data collection, even if they have little or no scientific experience. Citizen science aims to makes scientific processes understandable and approachable, and there is significant mutual benefit in that volunteers get hands on experiences as they contribute to projects in their local community or areas that they are passionate about, while organizations such as NPS are able to gain scientific data and results from the labor and assistance of citizen scientists volunteering their time.[22,23]

According to the guide, “Choosing and Using Citizen Science”, utilizing citizen science in data collection projects can be cost effective and it lessens the need for staff involvement. Studies regarding citizen science participations show extremely high interest in such opportunities and civic engagement in scientific projects.[24] Citizen science is especially beneficial in long-term monitoring projects as many volunteers show interest in long-term involvement in which they can see and understand the benefit of their work. Monitoring programs generally require short time commitment but repeated over long periods of time. The use of this approach in monitoring is also efficient because it allows for larger groups to collect data which can broaden the scope of sampling and increase the number of samples collected. Data from citizen science monitoring programs can be used to provide the organization at hand with monitoring results, and such data can inform them of environmental concerns or resource management priorities.[24]

Citizen science is also shown to increase engagement between organizations and local volunteers, creating stronger connections within communities. Some limitations of citizen science programs, however, include that volunteers need to be recruited, projects must be relatively simple, and several resources and materials are required. According to this guide, there are several factors that lead to the success of a citizen science approach, and they include: the establishment of a clear goal, the need for citizen engagement, access to the necessary resources, relatively simple protocol, and motivation for participant involvement. Citizens are generally highly motivated to be involved in projects that benefit their local community or organizations that they support. It is especially important for continued volunteer participation that there is a clear goal of the project that benefits

the local community, natural resources in the area, the leading organization, etc. as volunteer interest is driven by motivation to be helpful.[24]

Citizen science in the NPS has been especially popular due to volunteer interest in being involved with national parks and the access to funding or resources for such projects.[22,24] In 2012, for example, a citizen science project was developed within CONG with the goal to collect data on bird observations from park staff, volunteers, and visitors. This is a great example of citizen scientist involvement in a project with a simple protocol, as there is no prior experience or special skills required. Additionally, participation in this project may be motivated by the opportunity to drive, hike, and boat through CONG – an enjoyable experience that will also benefit understanding of bird populations in the area. Results were to be added to online databases which collect bird observations from all over the world where they are analyzed by scientists for monitoring trends in bird populations.[25] Other examples in CONG include a partnership project which recruits volunteers to collect dragonfly larvae to be tested for mercury concentrations. Additionally, volunteers are involved throughout the year for organized butterfly counts in CONG to monitor the amount and types of butterflies found.

An additional citizen science program that was proposed for CONG aimed to monitor surface water quality. This project, conducted in 2008, suggested regularly testing CONG surface waters for water quality parameters including pH, temperature, dissolved oxygen, turbidity, and specific conductance. Sample collection was to be conducted by trained citizen scientists, for the park to receive regular water quality data. Bacteria concentration testing as a parameter of water quality monitoring was added to this project, however, the project is no longer active.[26]

Citizen science is a familiar concept to groups that work in and around CONG, and the base of volunteers willing to participate in the area is relatively large. In the immediate area, citizen science-based research and other volunteer or non-profit efforts related to natural resource management are very common through a wide variety of groups in the area. These organizations can serve as a great source of communication regarding water quality efforts as well as potential citizen scientists. Some of these organizations include the following:

- The Congaree Land Trust conserves natural lands and waterways and works with the local community and volunteers to protect natural resources.[27]
- The Friends of Congaree Swamp advocates for CONG through public awareness and supports volunteer efforts that benefit the park's natural resources.[28]
- The COWASEE Basin is a group of local community members and landowners surrounding the Congaree, Wateree, and Santee Rivers who work to protect the forests and floodplains including CONG.[29]
- The Gills Creek Watershed Association regularly relies on volunteer help and donations to restore and advocate for the Gills Creek which has multiple water systems that flow into the Congaree River and to CONG.[30]

Specifically considering citizen science success in the area, there are programs designed specifically for civic engagement to improve water quality. For example, the Adopt-a-Stream program is run through Clemson University and is entirely citizen science based. Citizen scientists are trained to collect water quality monitoring data and

upload it to an online database for screening and monitoring purposes. Participants are motivated to be involved by "adopting" an area of a stream or creek in their local community or in an area they are passionate about. Following their training, Adopt-a-Stream participants are able to work individually and on their own time to collect water quality samples.[31] The Midlands Rivers Coalition works to protect and promote water quality of South Carolina rivers by collecting regular data on bacteria concentrations. This organization partners with various groups such as the Congaree Riverkeeper, DHEC, the University of South Carolina (USC), local property owners and other stakeholders. Through this partnership, regular bacterial monitoring is conducted along the Congaree, Saluda, and Broad Rivers. Bacteria results are collected and analyzed weekly and sample results are posted on a public website (HowsMySCRiver.org) to inform local populations of up to date water quality parameters – including warnings when parameters, such as bacteria, are at unsafe levels for recreation.[32,33] These examples of volunteer and non-profit efforts shows the success of citizen intervention in natural resource projects and supports the idea of citizen science as a feasible approach.

2.5 RELEVANT RESEARCH PROJECTS

Congaree National Park has partnered with several individuals and organizations for water quality testing and monitoring efforts in the past. Collaborations vary from USC faculty, staff, and students to federal organizations and such partnerships are mutually beneficial as it allows for researchers to utilize park resources and access protected areas for their work while the park benefits from the results and information collected. Patel (2010) explored the variability in fecal contamination throughout the Toms Creek watershed. Water quality samples were taken from sites both upstream of CONG as well

as within park boundaries. Patel (2010) also explored whether sources of fecal contamination at each site were from human or animal sources, to identify potential issues in waste discharge or animal population management. This project found that high concentrations of bacteria upstream of the park were mainly from human waste discharges. Bacteria concentrations internal to the park were very high and were attributed to both human waste discharge and animal waste. Animal populations contributing to high fecal bacteria contamination may be either native (e.g. deer) or non-native (e.g. feral hogs).[34] This project is very important for the topic of fecal contamination concerns in CONG because it demonstrated that there are high bacteria concentrations in park surface waters and also determined that both upstream wastewater discharges and excessive animal populations are significant sources of fecal contamination directly impacting the park.[34]

An additional project internal to CONG was executed by a student in 2007 to analyze bacterial concentrations (Enterococci and E. coli) in surface waters of CONG. This project included sampling water along Cedar Creek, Tom's Creek, Wise Lake, and Weston Lake and testing for bacteria concentrations. Bacteriological parameters were compared to EPA standards for recreational waters. It was determined that sample sites along Cedar Creek had the highest bacteria concentrations and results regularly exceeded EPA standards. Important takeaways from this project include the identification of high bacteria concentrations in CONG surface waters, the success of using the IDEXX Colilert system to test for E. coli concentrations, and the need to continue bacteriological testing in CONG.[35]

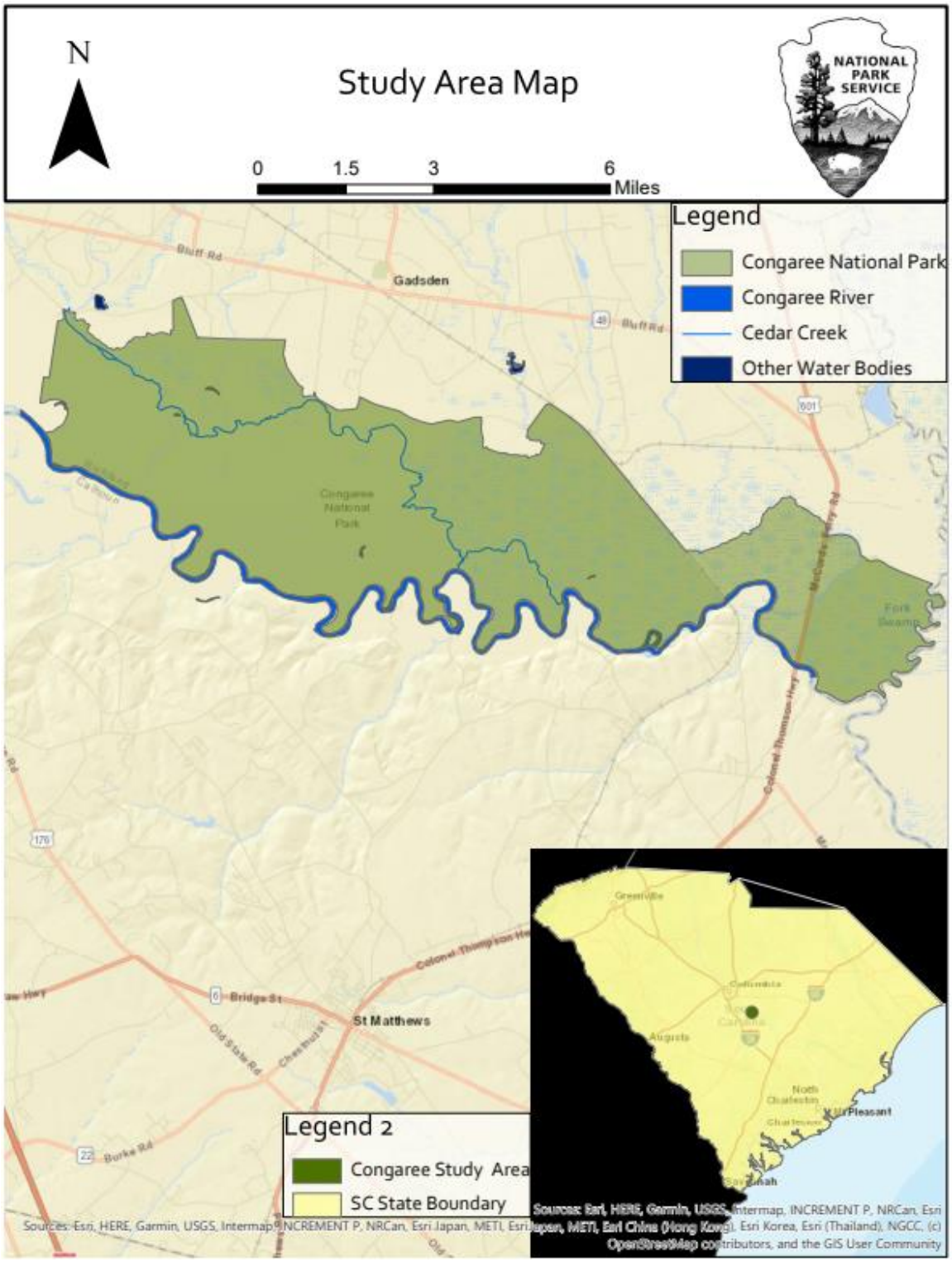


Figure 2.1 This map was created in ArcGIS to represent the study area and its most significant hydrological features.

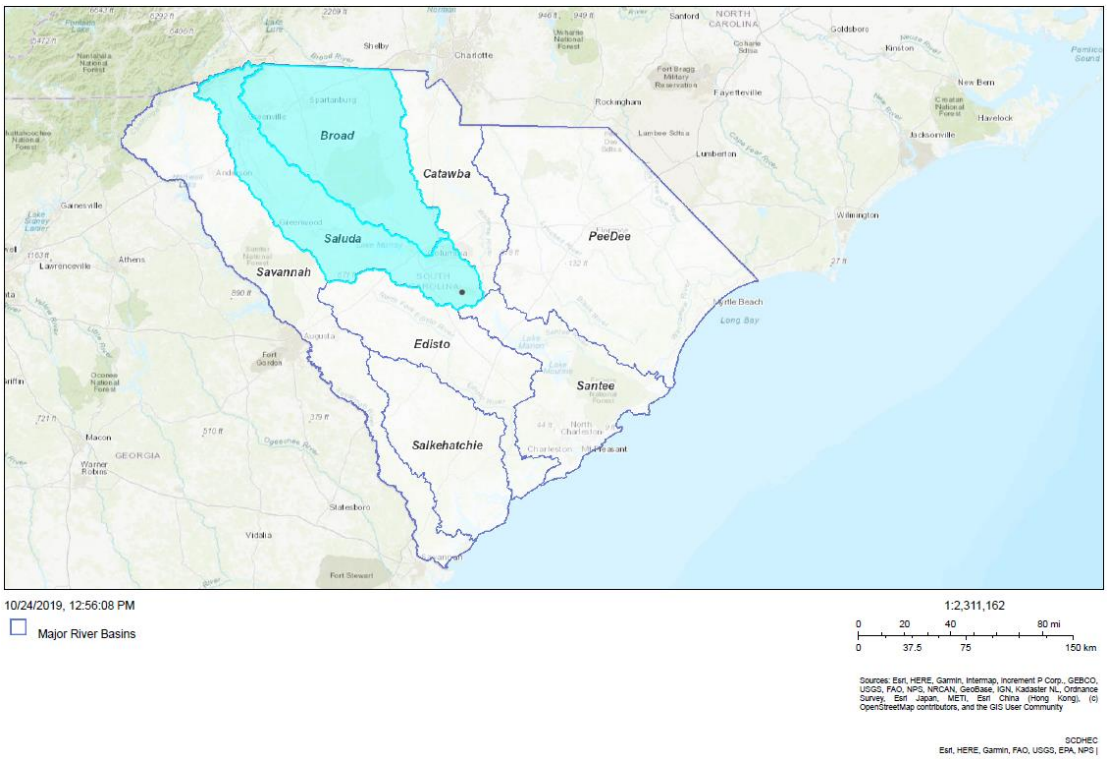


Figure 2.2 This map demonstrates the major river basins of South Carolina with the gray dot in the center of the state showing the location of CONG. This map was obtained from the DHEC Watershed Atlas.[8]

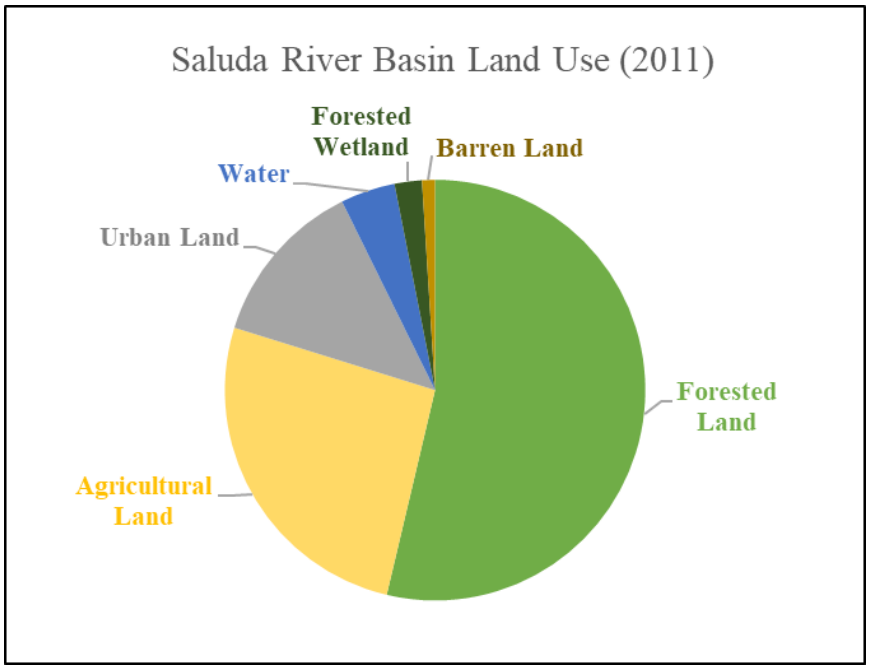


Figure 2.3 This pie chart represents the percentage of different land use types in the Saluda River Basin according to DHEC.[9]

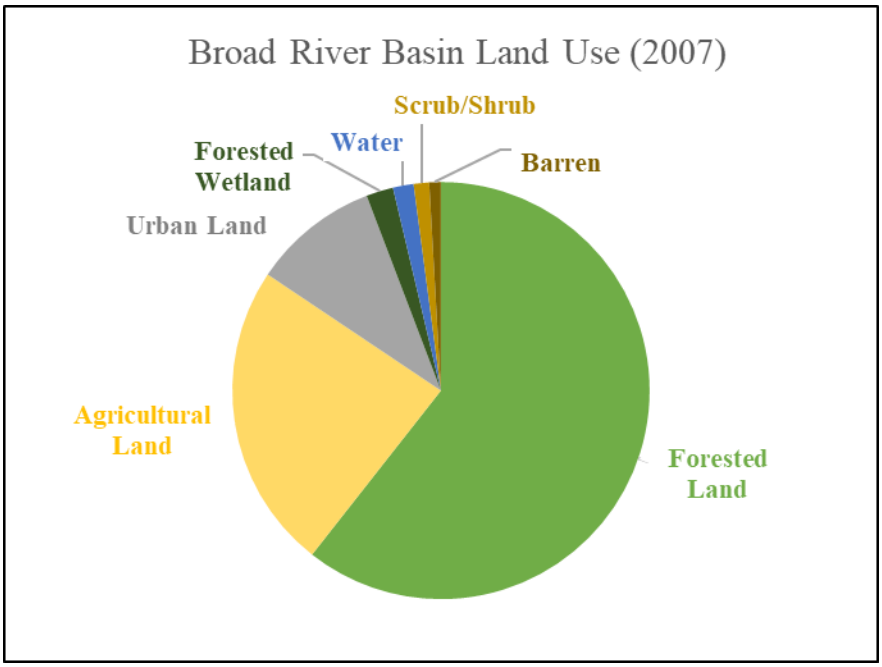


Figure 2.4 The division of land use in the Broad River Basin is demonstrated based on reports by DHEC.[10]

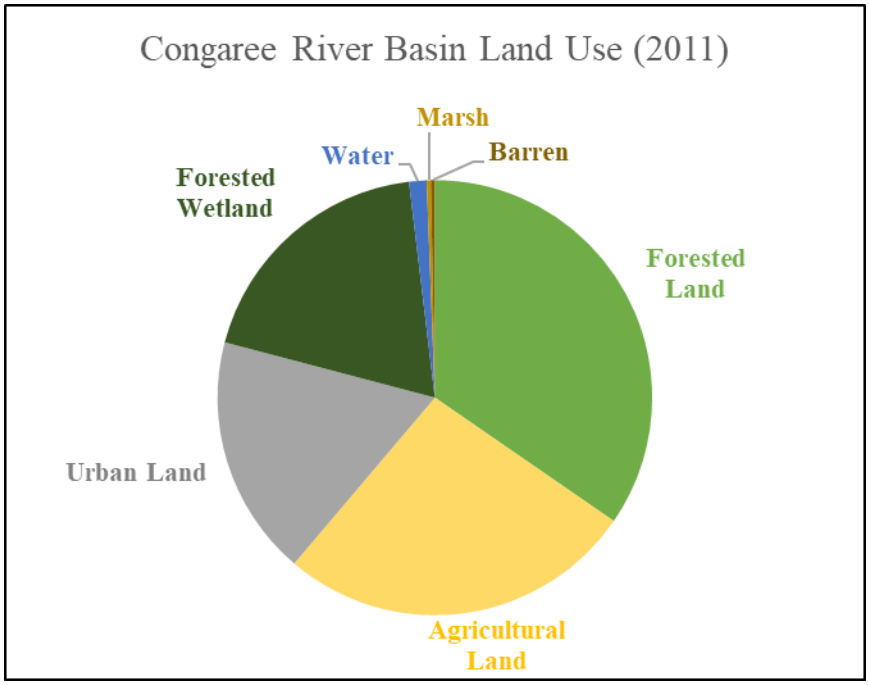


Figure 2.5 The percentages of land use types in the Congaree River Basin are demonstrated based on DHEC reports.[9]

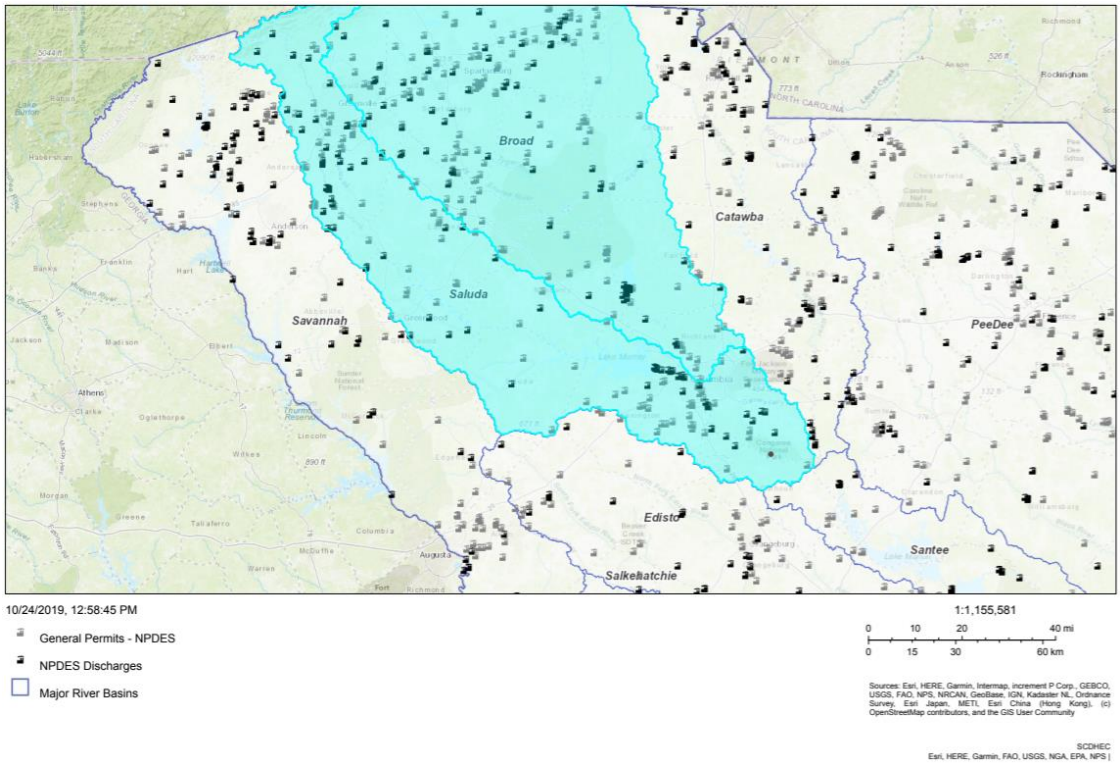


Figure 2.6 Obtained from the DHEC Watershed Atlas, this map of South Carolina shows NPDES discharge sites with the river basins that drain to CONG highlighted.[8]

Figure 2.7 This satellite image shows the location of two Columbia wastewater treatment plants in relation to the Congaree River.[13]

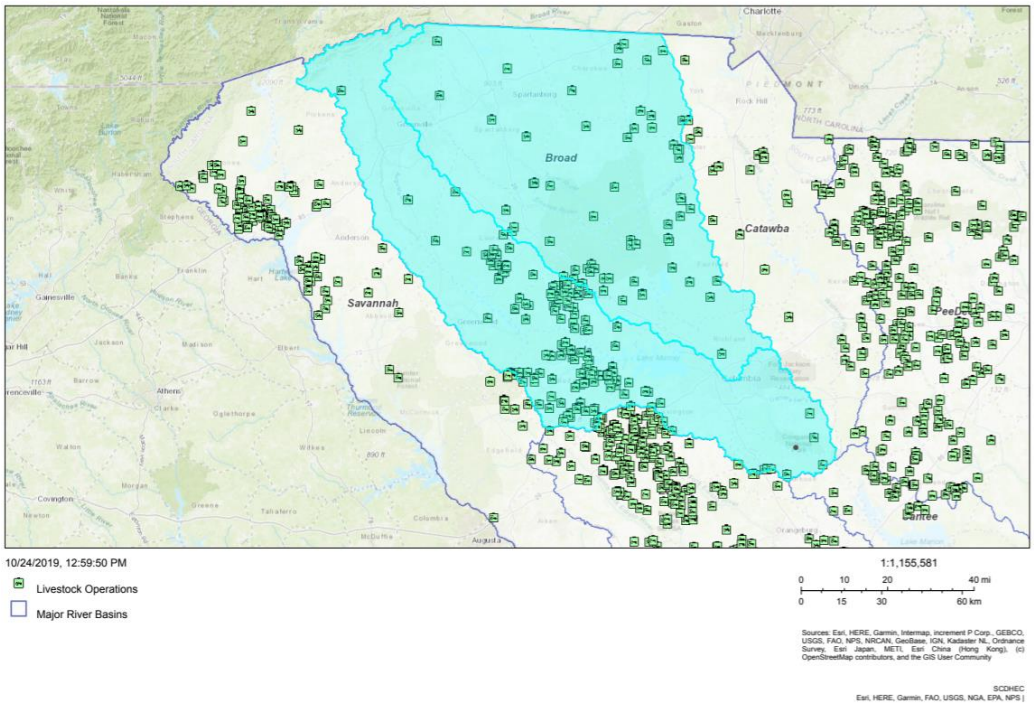


Figure 2.8 This map of South Carolina is also from the DHEC Watershed Atlas and represents livestock operations in the state with river basins that drain to CONG highlighted.[8]

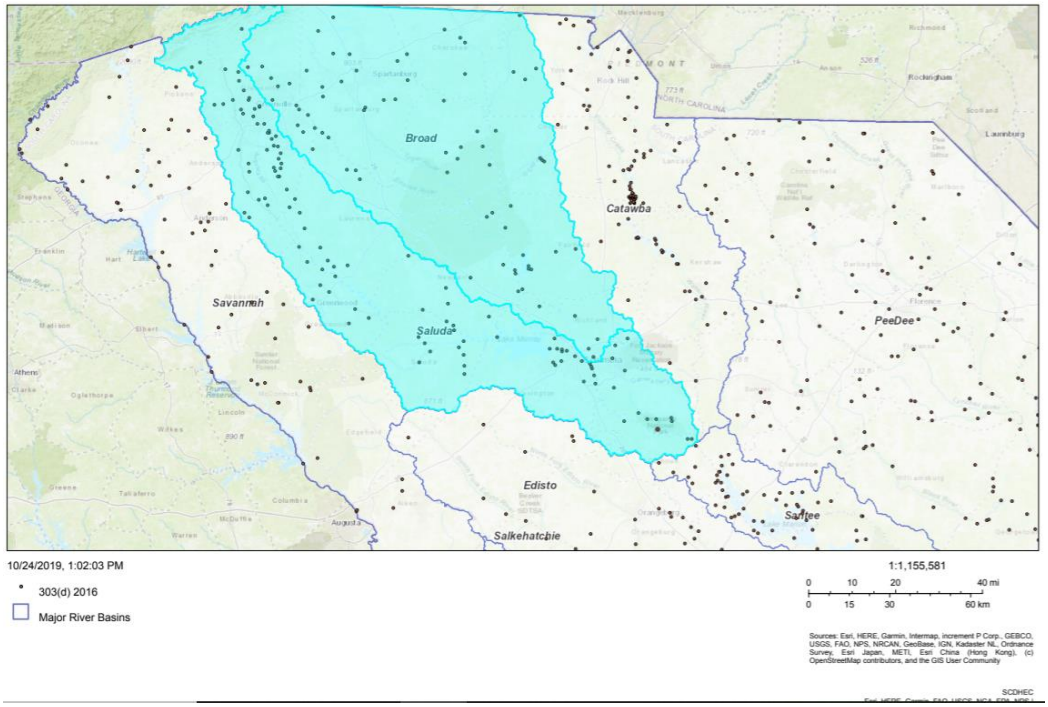


Figure 2.9 From the DHEC Watershed Atlas, this map represents sites on the 303(d) list of impaired waters for 2016.[8]

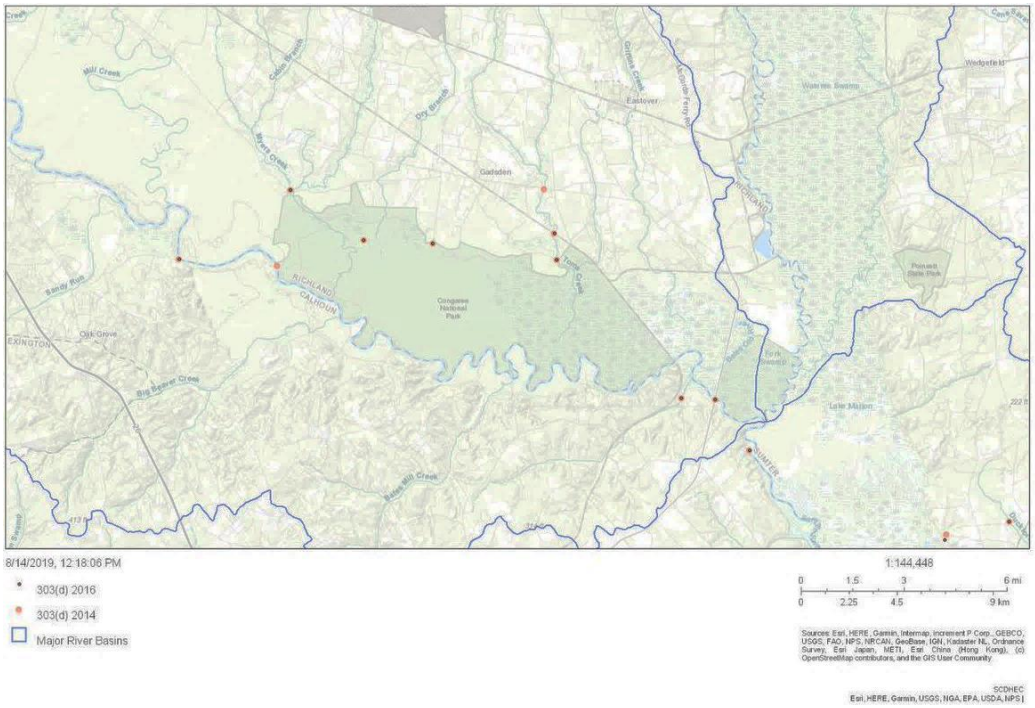


Figure 2.10 This map shows 303(d) impaired water sites near CONG.[8]

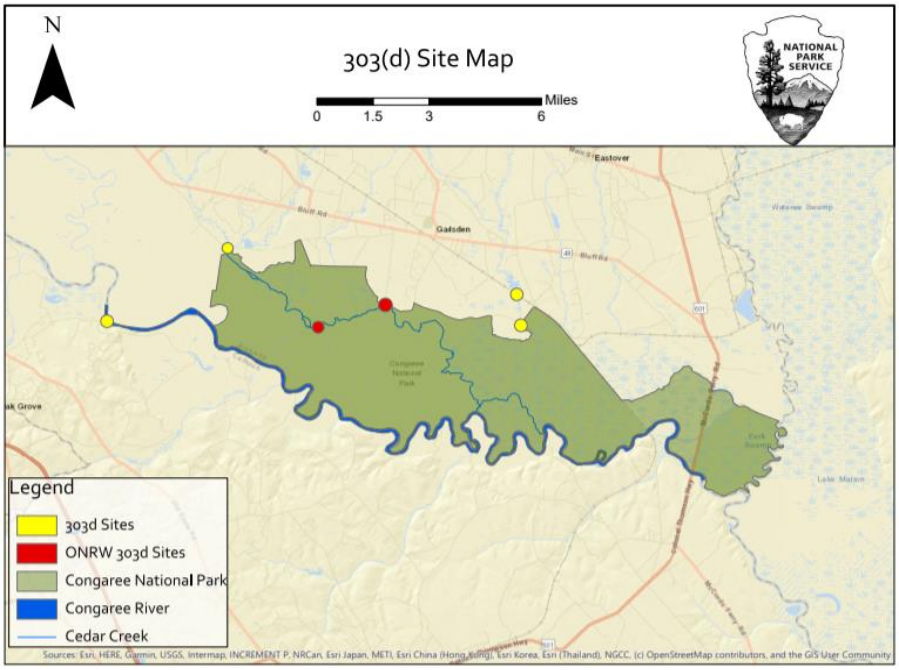


Figure 2.11 This map was created with ArcGIS to demonstrate impaired water sites in and around CONG with red sites representing sites on the ONRW portion of Cedar Creek.

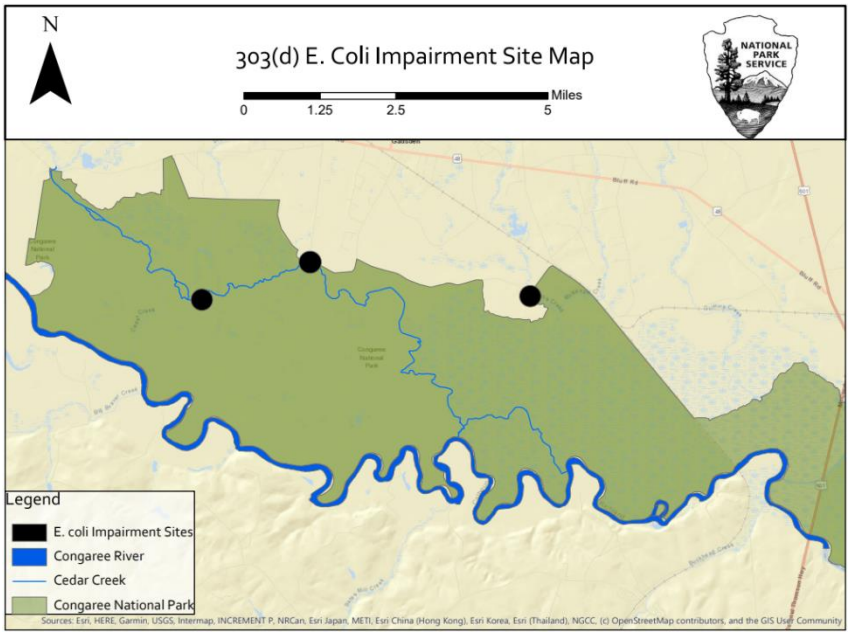


Figure 2.12 This map was created to demonstrate 303(d) sites in CONG that are impaired due to elevated E. coli levels.

CHAPTER 3
BACTERIAL SAMPLE ANALYSIS

3.1 BACKGROUND

Concerns for fecal contamination have been outlined as wastewater discharges, agricultural runoff, septic failure, and animal populations (native and non-native) in the local area.[18] It was also demonstrated that there are known point sources of waste discharge upstream from CONG and in close proximity to its receiving waters.[12] There is also potential for water quality impairment due to the large population of feral hogs in CONG boundaries because of their unmanageable population and habitat disturbance.[20] Fecal contamination in this area is concerning because of its threat to degrade water quality in protected areas and ecologically significant waters of CONG, but also for the threat of human illness from contact with contaminated waters. For these reasons, it is important for CONG to understand fecal contamination trends in its surface waters.

Following the same justification, the United States Geological Survey (USGS) is conducting a study at CONG which includes molecular source tracking (MST) of enteric bacteria in the Cedar Creek watershed. This project is being conducted mainly by staff of the South Atlantic Water Science Center of USGS.[36] Part of this bacterial sample analysis project was completed in partnership with the USGS MST project and with the advisement of the aforementioned USGS staff. The main objective of the USGS MST project is to sample water at sites upstream, within, and downstream of park boundaries

to identify specific sources of fecal contamination at various sites. The goal of this design is to determine if contamination within park boundaries is from sources internal to the park (e.g. feral hogs) or from upstream, external sources (e.g. wastewater discharge, agriculture) for the sake of informing management decisions.[36]

3.2 OBJECTIVE

Collecting water samples in CONG and testing for bacterial concentrations had a two-fold purpose. The first objective of this sample analysis was to compile and examine results from all bacterial testing conducted over the course of this project. Water samples were taken in CONG surface waters and tested for bacterial concentrations to inform park staff and visitors. Concentration results were observed in comparison to DHEC water quality standards to understand bacterial concentration trends in CONG waters and how they may differ based on factors such as precipitation. The second objective of executing sampling was to optimize the sampling methods and practice the procedures in order to transfer them to a citizen science training program.

3.3 METHODOLOGY

Water samples were collected periodically between September 2018 and October 2019 to test for bacteria concentrations. Throughout the process of this project, sampling methods were slightly changed and improved based on further research, experience, and advice with the goal of creating a procedure that is transferrable for citizen science. Beginning in October 2018 and ending in June 2019, USGS field staff would collect water samples for the MST project and would simultaneously collect water samples for the sake of this project for a total of five sampling events. Otherwise, sample collection was completed individually, or with the assistance of park staff or volunteers.

Sample sites were mainly along Cedar Creek, as it is the area of highest significance and concern, but other sample sites included the Congaree River, Tom's Creek, Dry Branch, and Myer's Creek. Figure 3.1 is a map of sample sites from the USGS MST project proposal which includes both temporal sites to be sampled six times per year and synoptic sample sites to be sampled once per year during the high-water season.[36] Shown on Figure 3.2 are all of the sample sites included in this bacterial sample analysis. The majority of sample sites are along Cedar Creek while the others (Tom's Creek, Dry Branch, and Myer's Creek above Cedar Creek) are tributary creeks that feed into Cedar Creek.

Samples were collected by submerging a sterile bottle into the flow of water. Water samples were collected either in a 1-liter Nalgene bottle sterilized by autoclaving or in a sterile 100 ml bottle. After collection, sample bottles were immediately sealed and placed in separate Ziploc bags. Samples were kept cool by ice packs during collection and were then kept in an iced cooler. After returning from sample collection, all samples were run in the convertible laboratory space of the CONG Old-Growth Bottomland Forest Research and Education Center (OGBFREC) building. Samples must be run for bacterial concentration testing within 6 hours of collection.[37]

Before testing water samples for bacterial concentrations, dilutions were made with sterile deionized (DI) water. There are two important justifications for diluting samples – the first being to get more accurate, quantifiable counts of bacterial concentrations. Without diluting environmental samples with high bacterial concentrations, it is very likely that concentrations will be underestimated. Secondly, the bacteria testing process being used requires detecting a color change to determine a positive result. A positive

result for fecal coliform bacteria is determined from a yellow color that can range from very pale yellow to a deep golden yellow. Environmental samples naturally have a brown, tan, or yellow color that can alter the reading of color change results. Therefore, dilutions are used both to reduce the color of the sample and the bacteria concentration. Dilutions were generally made in the factors of 1:1, 1:10, and 1:100 (sample: DI water), although dilutions were occasionally made at 1:0 and 1:1,000. Testing with three different dilutions also allowed for each sample site to be tested multiple times to also test for accuracy. All results were multiplied by a dilution factor in order to make results comparable.

Water samples were tested for both presence and concentration of bacteria using the EPA-approved IDEXX Colilert test. The procedure for this method is attached in Appendix B. This method simultaneously tests for total coliform bacteria and E. coli. Each dilution of a sample is made in a sterile 100 ml sample bottle combined with a pre-measured reagent which includes two carbon based nutrient indicators, ortho-Nitrophenyl-β-galactoside (ONPG) and 4-Methylumbelliferyl-β-D-glucuronide (MUG). The reagent is gently mixed into the sample water until homogenized and then the liquid is transferred into a Quanti-Tray 2000, a plastic tray containing 48 small wells, 48 large wells, and one overflow well. The tray is then held in a rubber mold for stability and run through a Quanti-tray sealer, the process of which evenly distributes the 100 ml of sample liquid into the wells of the tray. The sealed tray is then labelled and incubated at 35 degrees Celsius for 24 to 28 hours.[37,38]

The process of identifying both coliform bacteria and E. coli in the sample is identical but occurs with separate nutrient indicators. During incubation, coliforms that are present

in the sample will metabolize ONPG to create a color change which can appear in a range of pale yellow to a golden yellow. A yellow well indicates a positive result for total coliform bacteria in that well and this test can identify even a single viable coliform per sample.[38] In order to determine if a well is "yellow enough" to be considered positive, the IDEXX Comparator is used, which is a Quanti-tray 2000 filled with a pale-yellow liquid which matches the minimum color change that still indicates a positive result. In a similar process, the presence of E. coli in a sample will metabolize MUG during incubation and will make the liquid of a positive well fluorescent when viewed under a 365-nm ultraviolet (UV) light. If a well is both yellow and fluorescent under UV light, that well is positive for E. coli.[38]

In order to quantify bacteria concentrations, yellow wells (positive for coliform) are counted and recorded with a maximum of 48 small wells and 49 large wells - including the overflow well as one large well. The same counting process is used for fluorescent wells that are positive for E. coli. The counts of small wells and large wells for both total coliform and E. coli are compared on the IDEXX MPN table. This method gives results for bacteria densities with MPN per 100 ml sample.[37] The IDEXX MPN table is listed in Appendix C. As well as matching numbers on the MPN table by hand, MPN was also calculated using the IDEXX MPN Generator 1.4.4 computer application which allows the input of small and large well counts and outputs the corresponding MPN.[38] Coliform counts, E. coli counts, and MPN results were documented in an Excel spreadsheet and MPN results were multiplied by the appropriate dilution factor when necessary. For example, MPN would be multiplied by 100 for a 1:100 dilution, by 10 for a 1:10 dilution, and by 2 for a 1:1 dilution.

For quality assurance, blank tests were run to test for contamination throughout the sampling and laboratory process. During sampling, a sterile sample bottle was filled with sterile DI water, sealed in a Ziploc bag, and carried into the field. These field blanks were treated the same as environmental samples and tested for bacteria. Cooler blanks were also used with the same process, keeping the sample in the field cooler. These blanks were used to check for contamination throughout the sample collection process. Additionally, lab blanks were run between one and three times throughout the testing process which included filling a sample bottle with sterile DI and testing for bacteria through the IDEXX system in the same way as environmental samples. The purpose of running lab blanks was to test for contamination throughout the laboratory practices. Different types of blanks were run to check for potential contamination and to identify where in the process it may have occurred.

Total coliform bacteria are naturally occurring in the environment and are generally harmless as they do not always pose specific risk to human health. The presence of total coliform bacteria, however, indicates that there is a potential for fecal bacteria to be present. E. coli is a strain of fecal coliform bacteria that may be present in a coliform positive sample, and E. coli does have potential to cause human illness.[39] Following the state water quality standards, average monthly E. coli concentrations should not exceed 126 MPN/100ml and a single sample should not exceed 349 MPN/100ml. There is not a state standard for coliform bacteria in environmental samples.

3.4 RESULTS

Very early samples (September 2018) had the highest concentrations of coliform bacteria and E. coli compared to later sampling events. In event, environmental samples

were not yet being diluted, so it is possible that these results are not an accurate count which is justified with an extremely high standard deviation in comparison to other samples. For all following events, concentrations were variable, but are seen at more reasonable levels than the first event. Mean bacteria results for each sample site from every sampling event are listed in Appendix D. There were a handful of instances of sample site averages exceeding the state water quality criteria for E. coli of 349 MPN/100ml. This standard is set for a one time maximum, although the standard for monthly average is 126 MPN/100ml. Although this data does not include monthly means from multiple samples in each month, approximately half of the results for E. coli concentrations exceed 126 MPN/100ml. Mean concentrations for each sample site and event are shown in Figure 3.3 and compared to both the EPA and DHEC E. coli concentration standards for recreational waters.

Figure 3.4 shows E. coli concentration results for sampling events compared to the 24 hour and monthly precipitation readings for that time. Precipitation results were obtained from the National Oceanic and Atmospheric Administration (NOAA) National Weather Service Forecast.[40] Precipitation results must be considered with discretion because the weather station referenced is located in Columbia, SC approximately 15 miles from CONG so precipitation results are only suggestive, and not representative of the study area. However, this weather station is upstream of CONG so precipitation events could still impact CONG waters. In considering the association between precipitation events and bacteria concentrations, it is important to widen the scope of the time frame considered as earlier precipitation events could later impact runoff, water levels, and bacteria concentrations. For instance, the two highest E. coli readings were in September

2018 and October 2019. The 24-hour precipitation readings for these two sampling events were relatively high, and on both occasions, the highest 24-hour precipitation reading for the month was the day before the sampling event. In 2018, the highest daily precipitation was 1.62 inches on September 26th, and bacteria concentrations on September 27th were the highest readings throughout the project. Similarly, the highest daily precipitation for October 2019 was on the 19th, and on October 20th, E. coli concentrations more than doubled the state standard at 848.8 MPN/100 ml.

For each sampling event, the Cedar Creek water level as measured by the USGS static gage was noted in order to compare water level to bacteria concentrations. The USGS National Water Information System shares creek height levels from their permanent gage on the creek in CONG.[41] A higher creek height reading may suggest a precipitation event and higher bacteria concentration levels were generally associated with greater creek height. Figure 3.5 compares total coliform bacteria results from each sampling event with the gage height for that day and Figure 3.6 compares E. coli concentration results and gage height. Results also show that high concentrations of total coliform bacteria were not always indicative of higher E. coli concentrations and therefore cannot be predictive of E. coli results. Throughout each sampling event, no field blanks, cooler blanks, or lab blanks ever tested positive for any trace of bacteria. Each blank sample showed negative results for coliform bacteria and E. coli, indicating that there was no contamination through the sampling or testing process that could have altered sample results.

The USGS MST project has completed sampling and is currently in the analysis phase. MST testing is meant to identify primary sources of fecal contamination in park

waters. Results from this project must be considered with the following disclaimer, "this information is preliminary or provisional and is subject to revision. It is being provided to meet the need for timely best science. The information has not received final approval by the U.S. Geological Survey (USGS) and is provided on the condition that neither the USGS nor the U.S. Government shall be held liable for any damages resulting from the authorized or unauthorized use of the information." The statement of results from USGS is as follows: “Based on preliminary results, frequent detections of the pig microbial source tracking marker indicate that feral pigs are common contributors of fecal contamination to the sampled water bodies of Congaree National Park. While the human marker was occasionally detected within the park, the detections were not at the most proximal downstream sites from the Park's on-site septic system, suggesting sources of human contamination may be external or from sources other than the septic system. The lack of detection of the cow MST marker and infrequent detections of the ruminant marker relative to detections of the human and pig markers suggest that neither cows nor deer are primary sources of fecal contamination to the sampled water bodies of Congaree National Park; however, testing to ensure all assays are equally sensitive would be necessary to prove this. This information is preliminary or provisional and is subject to revision. It is being provided to meet the need for timely best science. The information has not received final approval by the U.S. Geological Survey (USGS) and is provided on the condition that neither the USGS nor the U.S. Government shall be held liable for any damages resulting from the authorized or unauthorized use of the information.”

3.5 DISCUSSION

The preliminary results from the USGS MST project indicate that the biggest sources of concern for fecal contamination in CONG are from hog and human waste. The high concentration of human markers along the Congaree River sample sites is likely due to the wastewater treatment plants situated upstream of the Congaree River. Cedar Creek runs through CONG and sample sites along it had very frequent and concentrated markers for pig, likely due to the large population of feral hogs in CONG and surrounding areas. These results not only show that there are high levels of fecal bacteria and E. coli in park waters, but also identifies their most significant sources. This is beneficial for park information because it can influence future management decisions for the park based on the identified sources of bacterial contamination. This project identifies the following suggestions for best management practices that may be beneficial based on the results of this project: improving park infrastructure, relocating animal or human waste sources from ecologically sensitive areas, improving human waste treatment, increasing feral hog population management.[36]

The IDEXX Colilert test has a maximum result of 2,419.6 MPN/100m. If samples are not diluted at a low enough concentration, it is possible to get inaccurate readings from this test that may show deceptively high concentration results. For example, a water sample from Cedar Creek with very high bacterial concentration run at a 1:0 dilution had 100% of wells (28 small wells and 49 large wells) read positive for coliform bacteria. This translates to an MPN of 2,419.6 which is the maximum detectable MPN for this test. However, the same sample run at a 1:1 dilution, which is diluted by half, had the same positive results of 100% yellow wells. Dilutions of 1:1, 1:10, and 1:100 were run and

their calculated MPN results (multiplied by the dilution factor) were 4,839.2, 5,475, and 6,630 MPN/100 ml. In this case, the undiluted sample (1:0) was removed from the calculated average MPN for that site to avoid a misleadingly estimation due to a procedural error.

Overall results of this bacterial testing analysis show that E. coli levels are relatively high in CONG waters with concentrations reaching levels that could be unsafe for recreation. E. coli levels that surpass the outlined state water quality criteria greatly increase the risk of human illness through contact with contaminated water.[16] Cedar Creek showed especially high measurements of bacteria and E. coli, which is concerning for recreational purposes such as boating and fishing. It is important to note, however, that CONG does not permit swimming. High bacteria concentrations in Cedar Creek also threaten the quality of this protected waterway. In comparison, bacteria measurements were lower in tributary creeks such as Dry Branch, Toms Creek than in Cedar Creek. Both coliform bacteria and E. coli can degrade water quality based on state and national standards and elevated bacteria contamination can decrease wildlife support in these areas.

The association between water level and bacteria levels is also informative because it can suggest precipitation events or seasonal trends. It is generally expected that precipitation events will lead to increased bacteria levels in surface waters from floodwaters and runoff that sweep contaminants into waterways. These results also suggest slight seasonal trends in bacteria concentrations, with generally higher concentrations during warmer seasons.

3.6 CONCLUSION

Bacteria concentration results were found to be variable, but relatively high as they approached or exceeded state water quality levels. E. coli concentrations are of particular concern and some results significantly surpassed the single sample criteria for E. coli concentration of 349 MPN/100ml. Half of the samples were above the monthly mean criteria of 126 MPN/100ml. All blank samples run through this procedure had negative results for bacteria concentrations. Cedar Creek samples generally had higher bacteria concentration results than other tributary creeks. The association between climate and bacteria concentrations can be seen in that high creek water levels were associated with spikes in bacteria concentrations on some occasions, especially during warmer months. Additionally, large precipitation events are shown to increase bacteria loading in that two of the days with greatest precipitation were followed by extremely high bacteria concentrations. These results can be used to inform park staff, volunteers, and visitors of known instances and risks of high bacteria levels. E. coli levels are especially concerning, and this data can be used to further identify concerns for human health risk and water quality degradation.

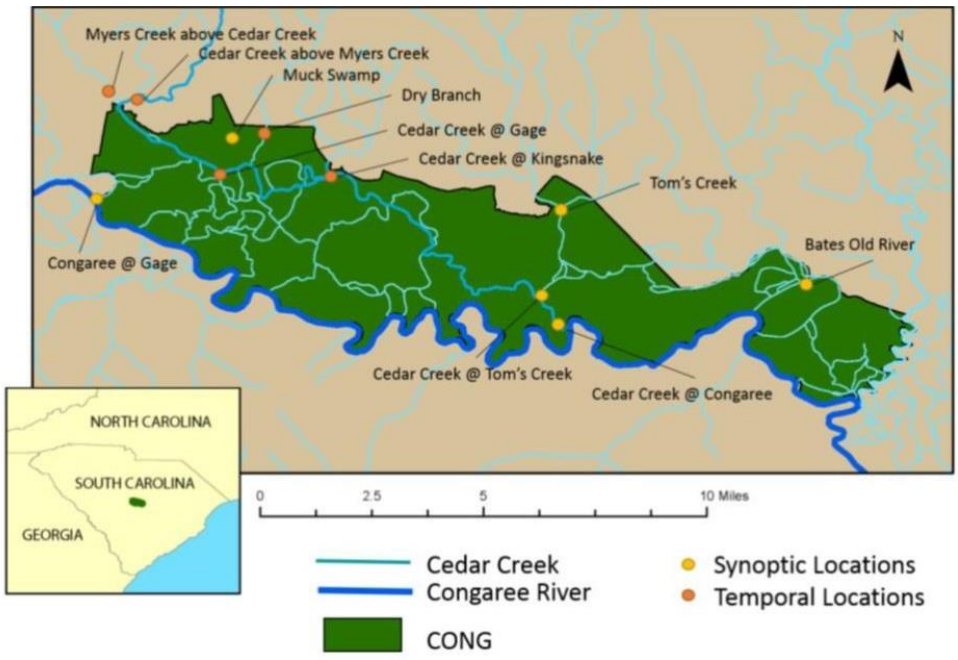


Figure 3.1 This map from the USGS MST project demonstrates the study area and CONG boundaries as well as the location of sample sites.[36]

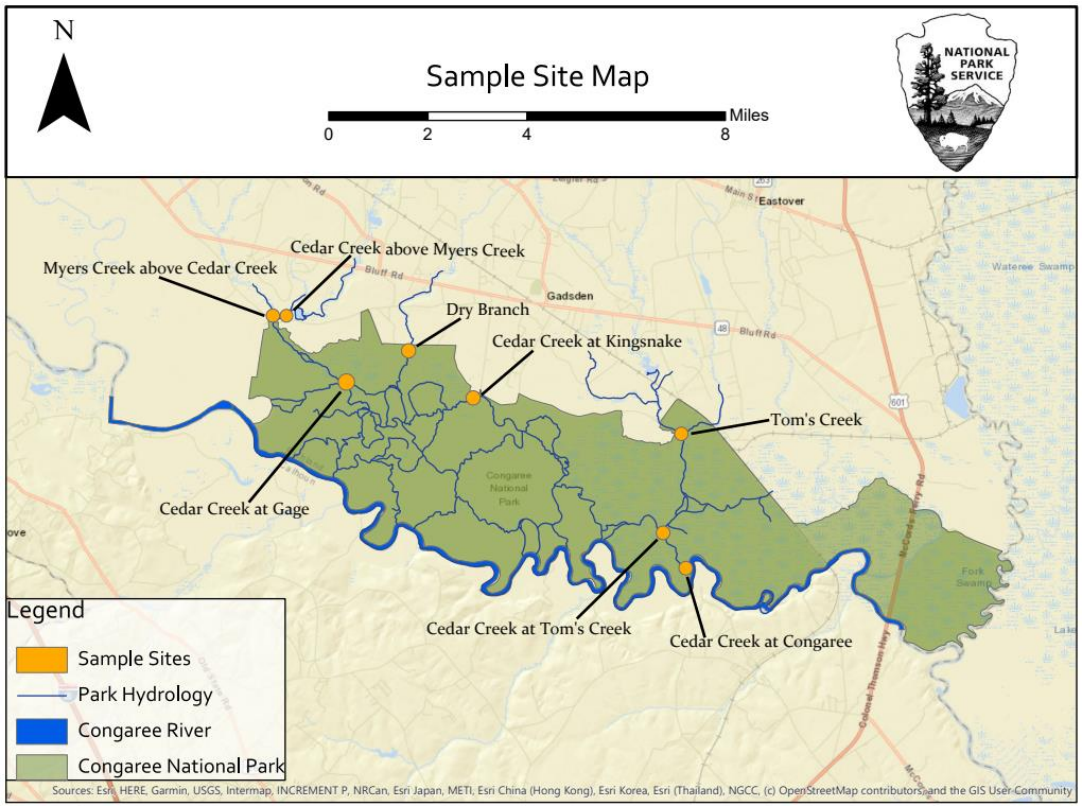


Figure 3.2 This map was created to show the sites for water sample collection.

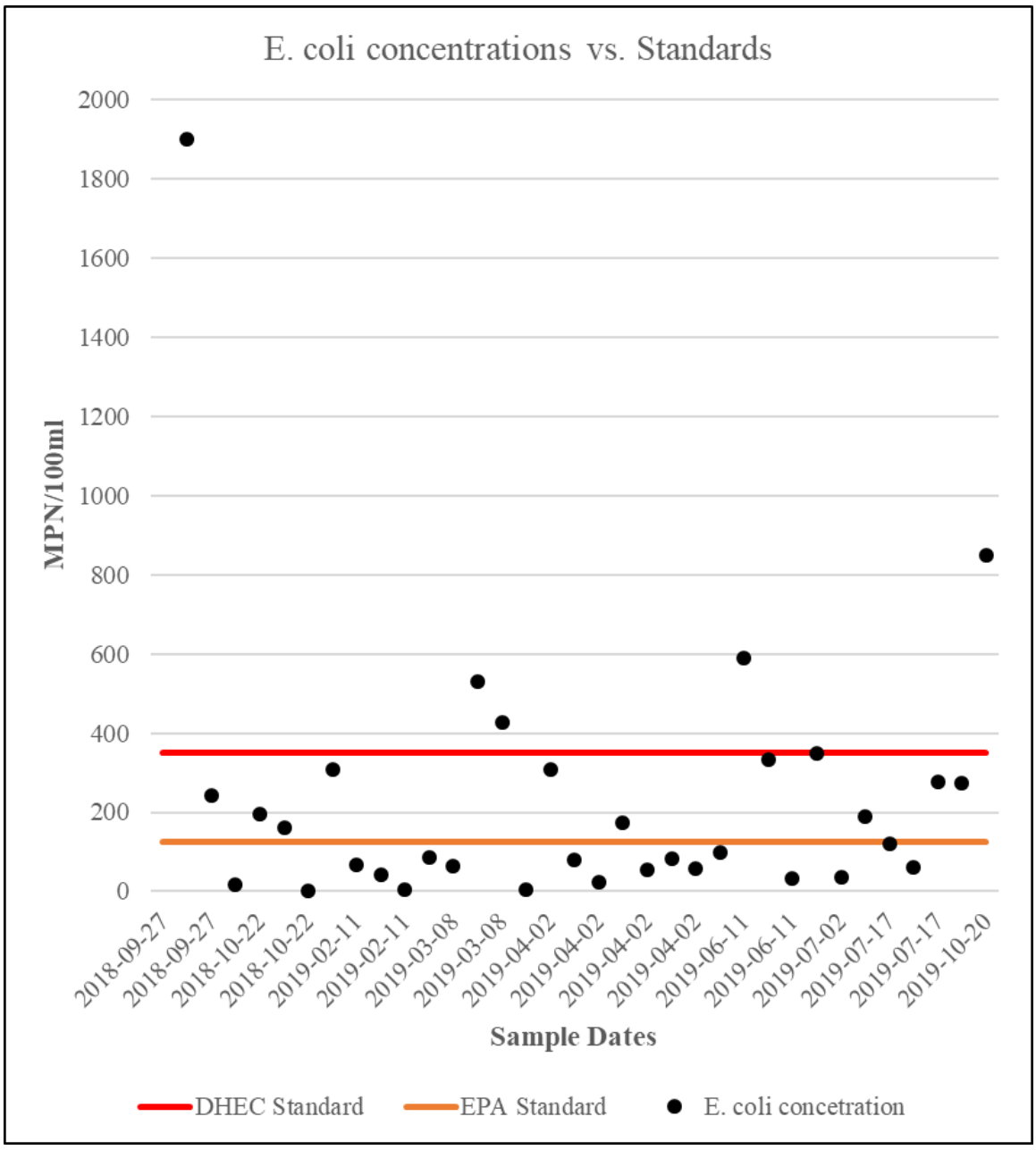


Figure 3.3 This graph shows the E. coli concentration results from each sampling event in comparison to the DHEC and EPA standards for recreational water.

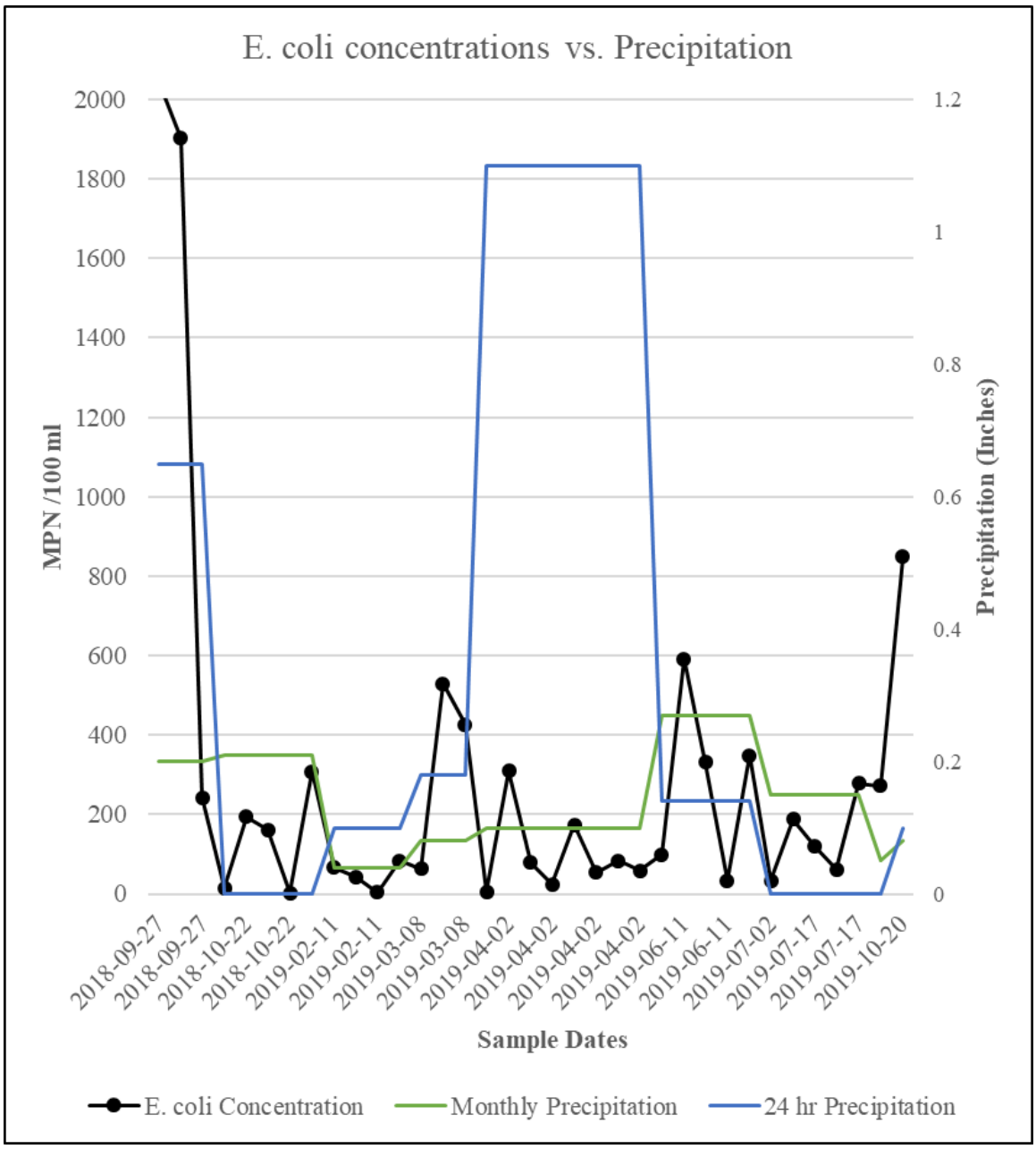


Figure 3.4 This graph shows E. coli concentrations compared to the precipitation averages for the day and month of the sampling event.

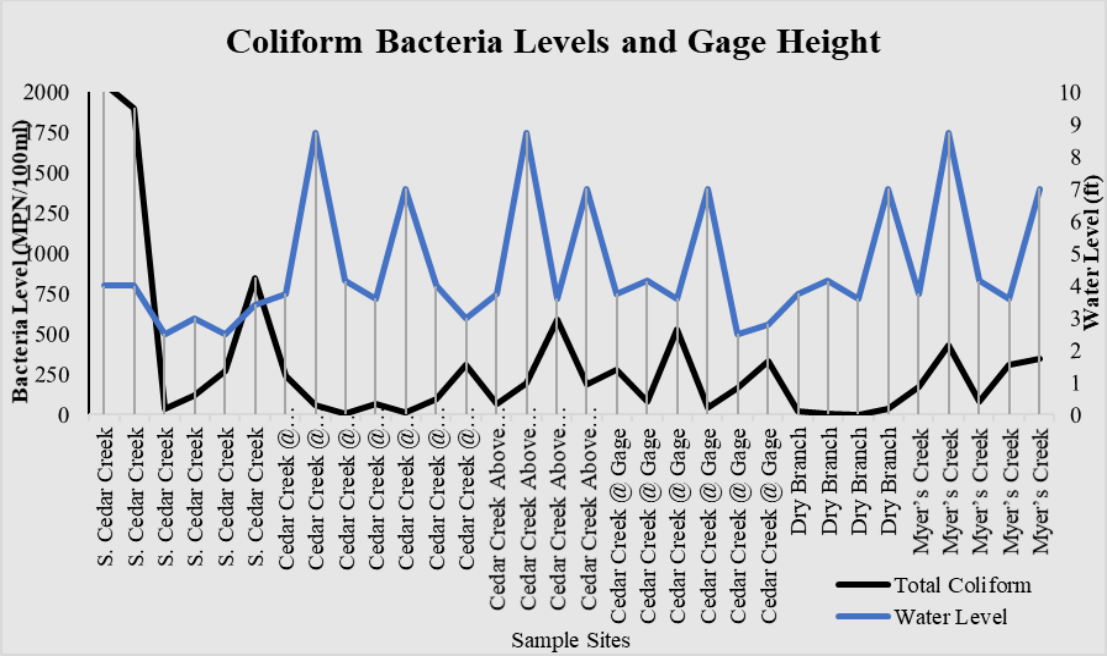


Figure 3.5 This graph shows Coliform bacteria concentrations for each sample site compared to the Cedar Creek water level at the time of sampling.

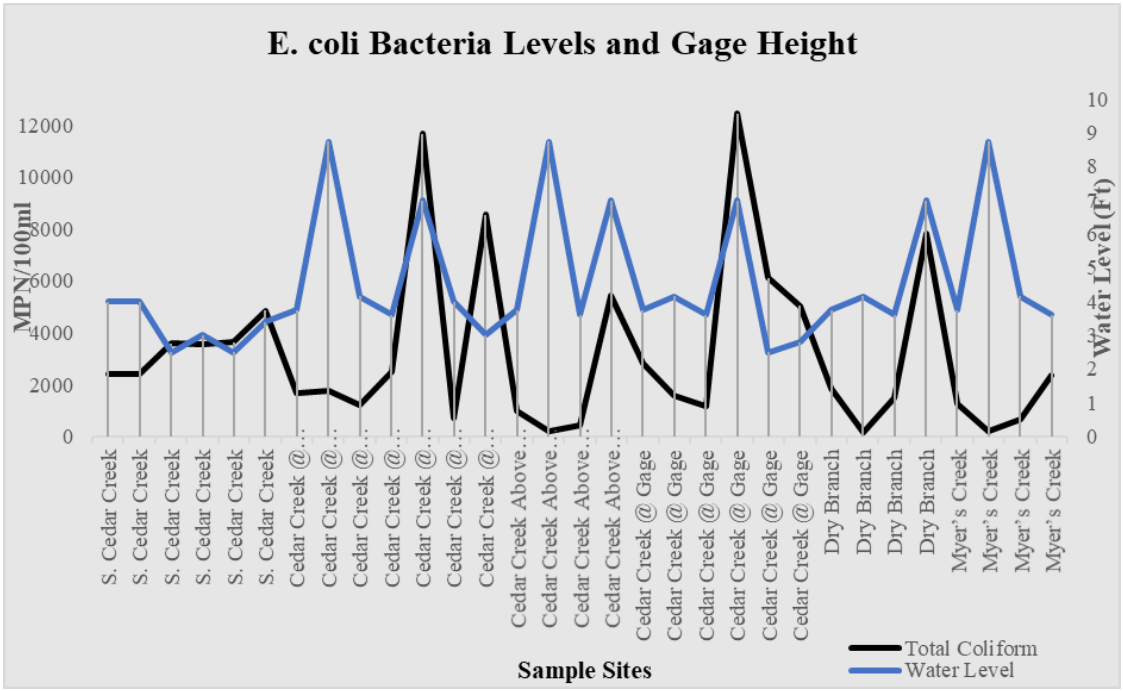


Figure 3.6 This graph shows E. coli concentration results for each sample site compared to the Cedar Creek water level at the time of the sampling event.

CHAPTER 4
ALTERNATIVES FOR WATER STERILIZATION METHODS

4.1 BACKGROUND

According to the National Institutes of Health (NIH) there are several standards of water quality that need to be met for laboratory purposes. Laboratory water should be clean and pure in order to eliminate risk of variability of alteration of laboratory results. The NIH states that "does not interfere with the specificity, accuracy, and precision of the procedure." While DI and reverse osmosis waters are purified to an extent, they are not considered sterile. Sterile water has been treated to remove traces of microorganisms and bacteria.[42]

For the sake of the bacterial concentration testing explain in Chapter 3, it is crucial that the laboratory water used be sterilized. Laboratory water is a significant part of this project's methodology because it is used as the base for sample dilutions as well as for blank samples. Using sterilized laboratory water is essential because it reduces the risk of altering bacterial concentration results.[42]

Methods for water sterilization can be time consuming and inconvenient. Some methods require extensive laboratory equipment and resources. For the sake of bacterial concentration testing at CONG, all laboratory practices are conducted in the park's learning center – a shared space for OGBFREC programs and educational opportunities (e.g. field trip and summer camp activities) as well as for staff use and storage. This

space is sufficient for this project's needs and provides the necessary space and equipment to complete bacteria testing experiments. There are limited laboratory resources in this space and a set budget for all OGBFREC programs, which including this bacterial water quality monitoring program.

4.2 OBJECTIVE

The purpose of this study is to explore various methods of water sterilization for laboratory use and their functionality. The main objective is to understand the efficiency, viability, and accessibility of each method for use in experiments such as bacterial concentration testing. Exploring these options will show differences in time requirements, cost, resources, and feasibility. The main goal is to present these options for use in scenarios of limited laboratory resources or funding. Considering this project of bacterial concentration testing at CONG and continuous monitoring, important factors for the sustainability of this project include that it is affordable, efficient, and can be completed entirely on-site.

4.3 METHODOLOGY

Multiple water sterilization methods were used through the completion of this project. Each option was influenced by the time and resources availability and they include: store bought distilled water, non-sterile DI water, DI water sterilized by autoclave, and water sterilized by a UV water purifier.

The two water sterilization methods used include using an autoclave and the Steripen Ultra – a handheld UV water purifier that is generally used for back country sterilization of drinking water.[43] The CONG learning center does not have equipment for DI water or an autoclave. In order to get DI water or to sterilize water using an autoclave, the student

transported equipment to the USC campus and used equipment in the Environmental Health Sciences (ENHS) department and laboratories.

The Steripen equipment was purchased by OGBFREC, is relatively affordable, and is handheld.[43] It can easily be used on-site at CONG and it is both user friendly and time efficient. In order to test the feasibility of this product as an alternative to water sterilization by autoclave, environmental samples were tested for bacteria concentrations before and after sterilizing water with the Steripen.

The three water sources tested for this experiment were DI water, tap water from the Learning Center, and an environmental water sample taken from Cedar Creek. Each water source was tested for coliform bacteria and E. coli concentrations using the IDEXX Colilert system with methodology outlined in Chapter 3.3. The procedures for this method can be found in Appendix This method includes adding a reagent to 100 ml of a water sample, homogenizing, and transferring the liquid to a tray. The tray is then incubated which creates a color change and fluorescence if there is a presence of coliform bacteria or E. coli. The DI and tap water were tested without being diluted, but the environmental sample was run without dilution (1:0) as well as at dilution factors of 1:1, 1:10, and 1:100 to ensure accurate bacterial concentration results since bacteria levels were expected to be high in this environmental sample. Approximately one liter of each water source was then treated with the Steripen following the product instructions. This basically involves turning the UV light on, choosing the amount of water being treated, submerging the light into the water, and stirring for 90 seconds.[43] After each water type had been treated, they were tested with the IDEXX procedure an additional time.

The IDEXX method suggests testing bacterial samples within 6 hours of sample collection, which was successfully completed in the first round of the experiment. However, in order to see how additional treatments of the Steripen would affect bacteria concentrations, supplementary rounds of treatment were tested on the same environmental sample the following day. The Steripen advises one use of the 90 second treatment, but this experiment was extended by treating the environmental sample a second and third time and testing for bacteria concentration after each additional treatment. At the time of the second and third treatments, the environmental sample had been collected approximately 24 hours prior, so it is expected that bacteria concentrations would be lower than when the sample was originally taken.

4.4 RESULTS

Table 4.1 shows that both DI water and OGBFREC tap water had entirely negative results for both coliform and E. coli both before and after UV sterilization, indicating that the original water source had no bacterial contamination. Additionally, all blank samples (explained in Chapter 3.3) had negative results for bacteria in each instance. This indicates that none of the bacterial concentrations results throughout this testing were altered by laboratory water.

Table 4.2 shows the total coliform bacteria results for the original environmental sample (before sterilization) and after each round of sterilization. Similarly, Table 4.3 shows E. coli concentrations before and after sterilization. In testing the feasibility of water sterilization with the Steripen, the environmental sample was tested as an extreme case because it was expected to have bacteria concentrations significantly higher than any water source that would be used for laboratory practices. Before UV sterilization, the

average concentration of total coliform bacteria in the environmental sample was extremely high at 5,684 MPN/100 ml and E. coli concentration was 848.8 MPN/100ml, more than double the state standard for water quality of 349 MPN/100ml.

Table 4.4 lists coliform and E. coli bacteria results after each round of sterilization and the percent reduction between each round. After just one round of UV sterilization, total coliform bacteria in the sample was reduced to 542.8 MPN/100ml (90.39% reduction) and E. coli concentration dropped to 89 MPN/100ml (89.51% reduction). For the additional testing of UV sterilization on the same sample, the environmental sample may have had a beginning concentration lower than the first sterilization results since these additional tests were done after the suggested time frame for bacterial testing of 6 hours after sample collection. However, the second round of UV sterilization reduced coliform bacteria concentrations by an addition 99.13% from the first round, dropping the concentration to a minute 4.7 MPN/100ml. Additionally, the second round of UV sterilization reduced the E. coli concentration to zero, a 100% reduction. By the third round of UV sterilization, coliform bacteria concentration was reduced to zero as well.

4.5 DISCUSSION

A UV light Steripen was tested for feasibility as an alternative to using an autoclave to sterilize laboratory water. The use of an autoclave for the bacterial water quality monitoring program required additional time commitments and is not feasible for citizen science. This process included packing laboratory equipment from the CONG learning center, transporting equipment to the USC campus, and receiving both access and permission to utilize a USC professor's laboratory equipment. The autoclave process for laboratory water takes approximately one hour, followed by an additional hour of waiting

for the water to cool before sealing the containers, packing the equipment, and transporting it back to CONG. While this process was extremely helpful through the course of this project and the ability to utilize USC lab equipment is greatly appreciated, this method is not feasible for the continuation of a water quality monitoring program.

The UV Steripen alternative was successful in creating sterile laboratory water from either DI water or tap water. The UV sterilization method, applied once, successfully eliminated approximately 90% of bacteria in a highly concentrated sample. After three rounds of the UV sterilization method, a sample with extremely high coliform bacteria and E. coli concentrations was successfully sterilized and tests were negative for both coliform and E coli. Each round of UV sterilization takes only 90 seconds per liter of water. The Steripen could be used to sterilize DI, tap, or store-bought distilled water for use as laboratory water. To be entirely thorough, the water could be run through three rounds of UV sterilization, since this experiment showed that three rounds can eliminate even extremely high concentrations of bacteria.

Autoclaves are extremely expensive pieces of laboratory equipment that require regular maintenance. While the OGBFREC has funding for research projects, the probability of allocating funds for an autoclave, utilizing space for it, and keeping up with maintenance is unlikely – especially considering it may not be used for many additional projects. The UV Steripen, however, is relatively affordable at about $100. It is also rechargeable and the bulb is estimated to work for up to 8,000 uses.[43] The Steripen is also very efficient because it is handheld, user friendly, and can sterilize one liter of water in under 5 minutes.

4.6 CONCLUSION

This experiment compared two alternatives of water sterilization methods, using an autoclave or a UV Steripen, for laboratory water in resource-limited laboratory scenarios. The UV Steripen is demonstrated as a viable alternative to autoclaving laboratory water. The two methods were compared by cost, time, accessibility, and efficiency, and the UV Steripen was successful in each category. Results show that the Steripen effectively removes approximately 90% of bacteria from water sources after one round of sterilization and shows 100% reduction after three rounds. Therefore, UV sterilization is a more practical investment for labs with limited resources.

Table 4.1 Tap and DI Water Sterilization Results

Sample	Dilution			Yellow Wells (Positive			Calculations		Fluorescent Wells			Calculations	
Long Name	Water (ml)	Sample (ml)	Dilution factor	Large	Small	Raw MPN	MPN	Mean	Large	Small	Raw MPN	MPN	Mean
DI - Before Sterilization	1	0	1	0	0	0	0.0	0.0	0.0	0.0	0.0	0	0.0
DI - After Sterilization	1	0	1	0	0	0	0.0	0.0	0.0	0.0	0.0	0	0.0
Tap - Before Sterilization	1	0	1	0	0	0	0.0	0.0	0.0	0.0	0.0	0	0.0
Tap - After Sterilization	1	0	1	0	0	0	0.0	0.0	0.0	0.0	0.0	0	0.0

Table 4.2 Water Sterilization Coliform Results

Sample	Dilution			Yellow Wells (Positive Coliform)			Calculations			
Long Name	Water (ml)	Sample (ml)	Dilution factor	Large	Small	Raw MPN	Calculated MPN	n	Mean	SD
Before Sterilization (1:100)	99	1	100	35	5	66.3	6,630.0	4.0	4,841.0	1,776.3
Before Sterilization (1:10)	90	10	10	49	28	547.5	5,475.0	4.0		
Before Sterilization (1:1)	50	50	2	49	48	2,419.6	4,839.2	4.0		
First Sterilization (1:100)	99	1	100	5	0	5.2	520.0	5.0	542.8	110.0
First Sterilization (1:10)	90	10	10	27	3	42.0	420.0	5.0		
First Sterilization (1:1)	50	50	2	48	20	272.3	544.6	5.0		
Second Sterilization (1:100)	99	1	100	0	0	0.0	0.0	4.0	4.7	5.0
Second Sterilization (1:10)	90	10	10	1	0	1.0	10.0	4.0		
Second Sterilization (1:1)	50	50	2	2	0	2.0	4.0	4.0		
Third Sterilization (1:100)	99	1	100	0	0	0.0	0.0	4.0	0.0	0.0
Third Sterilization (1:10)	90	10	10	0	0	0.0	0.0	4.0		
Third Sterilization (1:1)	50	50	2	0	0	0.0	0.0	4.0		

Table 4.3 Water Sterilization E. coli Results

Sample	Dilution			Fluorescent Wells			Calculations			
Long Name	Water (ml)	Sample (ml)	Dilution factor	Large	Small	Raw MPN	Calculated MPN	n	Mean	SD
Before Sterilization (1:100)	99	1	100	6	2	8.4	840.0	4.0	848.8	108.8
Before Sterilization (1:10)	90	10	10	37	4	71.2	712.0	4.0		
Before Sterilization (1:1)	50	50	2	49	26	488.4	976.8	4.0		
First Sterilization (1:100)	99	1	100	1	0	1.0	100.0	4.0	89.0	13.0
First Sterilization (1:10)	90	10	10	9	0	9.8	98.0	4.0		
First Sterilization (1:1)	50	50	2	24	3	35.9	71.8	4.0		
Second Sterilization (1:100)	99	1	100	0	0	0.0	0.0	4.0	0.0	0.0
Second Sterilization (1:10)	90	10	10	0	0	0.0	0.0	4.0		
Second Sterilization (1:1)	50	50	2	0	0	0.0	0.0	4.0		
Third Sterilization (1:100)	99	1	100	0	0	0.0	0.0	4.0	0.0	0.0
Third Sterilization (1:10)	90	10	10	0	0	0.0	0.0	4.0		
Third Sterilization (1:1)	50	50	2	0	0	0.0	0.0	4.0		

Table 4.4 Water Sterilization Percent Reduction

Sterilization	Coliform	% Reduction	E. coli	% Reduction
Before Sterilization	5684.0	--	848.8	--
First Sterilization	542.8	90.39%	89.0	89.51%
Second Sterilization	4.7	99.13%	0.0	100.00%
Third Sterilization	0.0	100.00%	0.0	--

CHAPTER 5
CITIZEN SCIENCE PROGRAM DEVELOPMENT

5.1 BACKGROUND

Citizen science is a successful concept that is gaining popularity and becoming more common in organizations such as the NPS. National parks utilize citizen science for data collection and analysis that is informative and beneficial to the park. While park staff oversee the planning and execution of a citizen science program, it is the volunteered time and effort of citizen science that implement such projects and get results. Citizen science allows civic engagement and involvement in a project, that can provide results with great quality and accuracy.[44] As described in Chapter 2.4, citizen science approaches are successful when the ideas and processes are transferrable. By creating approachable procedures through proper planning and volunteer trainings, citizen science can be executed by almost any interested volunteer. Therefore, scientific projects can be completed by citizen scientists with little or no scientific experience. This approach is mutually beneficial as it involves volunteers in efforts that they are enthusiastic about and provides the leading organization with project results or data collection.

Citizen science is especially advantageous for data collection and observation because it allows for a greater scope of sample collection. For example, the Adopt-a-Stream program, as mentioned in Chapter 2.4 relies entirely on trained volunteers to collect water quality samples from all over the state of South Carolina. With this program, staff

involvement is limited and project funds can be put towards providing resources. In this case, citizen science is successful in reducing time commitments of several employees while significantly increasing the amount of data collected. Additionally, observational studies, such as the aforementioned volunteer bird count successfully use citizen science by recruiting community members that are already interested and involved in the topic. In both scenarios, civic engagement is motivated by the idea of benefitting the area, population, or organization of interest. Much like the Adopt-a-Stream program, this program development encourages local community volunteers to be involved and informed in the state of their local environment. Citizen science is a beneficial approach, but it works best in specific scenarios that have resources or funding available and can reach a large audience of volunteers. If this is the case, however, citizen science has the potential to greatly expand the reach of a project by increasing data collection.

Citizen science is especially beneficial in areas where volunteer interest and participation is high. CONG has a large and enthusiastic base of volunteers in parks (VIPs) that are regularly involved in park projects. Many VIPs have expressed interest in hands on opportunities and volunteer research. The lack of water quality monitoring within CONG is mainly due to staffing limitations, so the use of citizen science bypasses that issue by giving the responsibility of data collection to citizen scientists. The overarching goal of this project is to develop and implement a sustainable, citizen science bacterial water quality monitoring program.

5.2 PROJECT DEVELOPMENT

The development of this program includes two parts: the creation of documentation that outlines water quality monitoring methods and practices for the completion of a

water quality monitoring event. Based on the experiences and improvements developed from the aforementioned sampling events and laboratory work in Chapter 2, the methodology for water sampling, bacteria testing, and results reporting is outlined in the training manual found in Appendix E. It also includes participant expectations, preparation for field sampling and laboratory work, safety considerations, sample collection methods, laboratory methods, and procedures for sample analysis. There is also a checklist for all of the required steps to complete a sampling event.

Studies on the success of citizen science, as previously outlined, suggest that citizen science programs have a clear goal and concise instructions, especially when some of the work may be independent.[24] This criteria were considered in creating thorough instructions that help to avoid inconsistencies in data collection by citizen scientists. The training manual also provides step by step instructions as well as a chronological checklist to make the design relatively simple and easy to follow. Successful citizen science programs also provide motivation for participation involvement by showing clear benefits of their work.[24] This program is designed to provide the park with water quality results which can be used for informing visitors, monitoring environmental changes, and influencing management decisions. These goals will benefit both CONG and the local community.

Program development also included designing, planning, and executing volunteer training sessions. Multiple training sessions were held with park staff and volunteers as well as members of the USC ENHS department. Volunteer training sessions include an overview of the training manual and program goals, field practice with site visits and sample collection, and hands-on laboratory practice. After the completion of a training

sessions, VIPs are trained and equipped to be involved in future sampling events and the continuation of the program. The results of sampling events have been recorded in a spreadsheet that can be shared with park staff and volunteers. This also includes formatting to calculate MPN results and average site concentrations. This is a running document that can be accessed by park staff and it serves as a base to collect all counts from sampling events.

5.3 RESULTS

Volunteer training sessions were continuously improved based on informal participant feedback. Trainings ranged from a few volunteers to a large group of 9 participants. It was found that volunteer trainings were most successful with fewer than 6 participants to have more one on one interaction. Early training events with colleagues and fellow students were used as trial training runs, but useful data was still collected. Through these trials, feedback and experiences helped shape changes and improvements that were made to the program design. Aspects such as scheduling and organization of the training session and the delivery of background information were improved. VIPs were generally very interested in getting into the field and hiking to sample sites. They were also generally enthusiastic about laboratory work and feedback indicated that the processes were relatively easy to learn. Feedback regarding improvements included taking less time to verbally explain guidelines and allowing VIPs to use condensed print outs of instructions to spend more time with hands on practice. There were several instances of training session participants expressing interest in further participation in the project. Additional VIPs and park visitors have also shown interest in joining future volunteer training sessions and sampling events.

5.4 TRANSFERRABILITY

The documents and instructions created for the implementation of this citizen science projects are thorough enough to guide the program but are also general enough to be applied to a different area or organization. This project has the potential to be continued at CONG, but the methods and execution are transferrable to other scenarios. In order to make this information publicly available, this project has been submitted to CitizenScience.gov which is a website that catalogs citizen science projects funded by federal organizations (e.g. NPS) for public use. This website includes ongoing projects that are actively recruited and completed citizen science projects. This resource allows for increased knowledge, popularity, and volunteer recruitment for a project and also provides descriptions of various citizen science projects for inspiration or information. An additional benefit of this website is the opportunity to report citizen science results for public access.

CHAPTER 6
RECOMMENDATIONS AND CONCLUSIONS

6.1 RECOMMENDATIONS FOR CONG

Based on the experiences and the results of this project, overall recommendations for CONG are to continue regular bacterial water quality monitoring. Findings from this project describe serious concerns of fecal contamination in park waters and relatively high levels of E. coli bacteria. The main sources of bacteria, including wastewater discharges, agricultural runoff, and animal wastes, are not expected to lessen. If anything, increases in population and development in the Columbia area, as well as upstream areas, will only continue to add to discharge of pollutants into surface waters. The CONG Foundation Document outlines trends that can impact ecological factors related to the park and its surrounding area into the future. Changes in climate trends that can impact water quality include increased precipitation frequency and intensity and higher winter temperatures which can impact ecological processes in the area and the wildlife in that habitat. Additionally, land use changes, water flow changes (e.g. dams), increased demand for groundwater, and upstream pollution can alter the floodplain and watershed.[2]

While most factors contributing to water quality degradation cannot be controlled, CONG and partners must consider management practices that can help protect surface waters. With the feral hog population in CONG causing such detriment and contributing to fecal contamination in park waters, CONG should prioritize feral hog population

management. This idea is addressed and outlined in the foundations document and it is recognized that hog management has not been able to successfully reduce the population to an acceptable number.[2] This is still the case at CONG and the impact of this population on water quality may make feral hog management even more of a priority.

For the protection of surface waters in CONG, it is highly recommended that CONG continue the use of the citizen science bacterial water quality monitoring program presented in Chapter 5. The training manual and supplemental documents provided allow for CONG to continue the outlined methodology for collecting bacterial concentration samples from park waters. Park staff or educated VIPs should conduct additional volunteer training sessions for interested volunteers and continue implementing regular sampling events to collect data on water quality trends in CONG. Results from this study as well as those from a similar water quality testing project in 2008 found that Cedar Creek and Toms Creek had relatively low water quality. Other surface waters in the park that had generally higher water quality include Dry Branch, Wise Lake, and Weston Lake.[35]

For convenience, the following list includes the short name for each sample site as well as any long name that each site has been referenced as throughout this document or on sample site maps.

- BABR – Bannister Bridge / Cedar Creek above Myer's Creek
- CECR – Cedar Creek at Tom's Creek
- CONG – Cedar Creek at Congaree River
- DRBR – Dry Branch
- GAGE – Cedar Creek at gage / Bridge B

- KING – Cedar Creek at Kingsnake / South Cedar Creek
- LAKE – Weston Lake
- MYCR – Myer's Creek above Cedar Creek
- TOMS – Tom's Creek

Future sampling events should consider multiple factors when choosing sample sites including site accessibility, potential for human contact, ecological significance, and areas of concern. For instance, Cedar Creek is the hydrologic feature of greatest concern because of its ecological significance and previous results showing high bacteria levels. Sample sites have each been given a four-letter reference name for the sake of discussing sample sites moving forward. In consideration of accessibility, sample sites including KING and BABR are both canoe launches which are regularly used for fishing and kayak or canoe inputs. Based on these considerations and to provide options for the extent of sampling events, three options for sampling events are proposed:

1. **KING, BABR, LAKE**

    a. Overview - This option for sample sites is the simplest and most accessible group of sample sites, therefore, it can be completely very quickly

    b. Accessibility – The first two sites are canoe launches which are only a short walk from a parking area. The third site is easily accessible by a short hike on a well-maintained trail.

    c. Significance – The first two sites are on Cedar Creek, which is an area of concern and all three sample sites have high potential for human contact.

d. Limitations – This option provides limited breadth of study results. LAKE is less of a concern for fecal contamination as it is less susceptible to significant pollution from upstream sources and has had relatively low bacteria levels in the past.

2. KING, BABR, GAGE, DRBR

a. Overview – This is the mid-range option for sampling events which balances the range of sample sites with adequate justification for each site.

b. Accessibility – Again, the first two sites are easily accessible canoe launches. The GAGE sample site intersects with a popular hiking trail in CONG which makes it accessible by a moderate hike. DRBR is not on Cedar Creek but is rather a tributary creek that feeds into it. This site can be accessed by a moderate hike with some off-trail hiking.

c. Significance – KING, BABR, and GAGE are all areas of high potential for human contact and sites on Cedar Creek. Additionally, Bridge B is in the ONRW portion of the creek. DRBR has previously had relatively low bacteria levels so it could be used as a comparison to understand the differences in levels along Cedar Creek. Results from this site would give insight on a water system separate other than Cedar Creek. Figure 6.1 from the CONG foundation document demonstrates that most visitor attractions (boardwalk trails, hiking trails, visitors center, canoe launches) are concentrated on the West end of the park while the East end has more wilderness area and is more difficult to access. For this reason, this option

concentrates sample sites on the West end of the park as well to represent areas of high visitation and accessibility. The proposed sample sites for this option are displayed in Figure 6.2.

d. Limitations – This option is still limited in breadth as it is concentrated on one end of the park. However, this is justified by the increased visitor activity in that area. This option does still exclude numerous other surface water sources in the park.

3. KING, BABR, GAGE, LAKE, Bates Old River, TOMS, CONG

a. Overview – This option proposed the highest number and most difficult to access sample sites. The purpose of a sampling event of this extent is not to do so regularly, but to be sampled approximately twice a year to get a synoptic sample of a wide range of sites.

b. Accessibility – There are challenges of accessibility to reach some of these sample sites as they are deeper in the park and require longer and more difficult hikes. This type of sampling would require multiple participants and could potentially benefit separating into small groups to collect samples at different sites. Figure 3.1 from the USGS MST project sample sites shows some of the sample sites listed above.[36]

c. Significance – A sampling event of this size would be very beneficial to the information of bacterial water quality monitoring as sample sites are spread throughout CONG land and are on several different hydrologic features. The sample sites for the USGS MST project were meant to

represent all areas and waterbodies of CONG to get a thorough understanding of bacteria concentrations.[36] For the same reason, it would be beneficial for park information to have occasional synoptic samplings of this wide range of sample sites.

d. Limitations – The major limitation of this option is the time, effort, resources, and participation required for its completion. Some sample sites should only be accessed by knowledgeable and capable participants and the time commitment required to collect samples from all seven sites is exponentially higher than other sampling options.

The three sampling options that are outlined can each be beneficial sources of information for CONG, but with varying degrees of time, effort, resources, and feasibility. All three options, however, include two sites, KING and BABR for the purpose of consistency in at least two sites even with the varying use of sample site options.

The ideal recommendation is for citizen science monitoring efforts to use sampling option #2 on a monthly or bi-monthly basis as the baseline. Additionally, if resources and participation allow, synoptic sampling events using option #3 can be conducted once or twice annually to get a broad understanding of bacteria levels throughout CONG waters. Option #1 is a more conservative, limited sampling event but can be useful for collecting regular data when participation, time, or resources are limited.

In choosing future sampling events, along with accessibility and importance of sample sites, we must also consider the resources required for each option. For the

number of sample sites being tested in one sampling event (between 1 and 7) the resources required were calculated and include sample bottles, IDEXX reagents, IDEXX trays, and DI water. The total number of samples run includes running three samples per sample site as well as the necessary lab blanks, cooler blanks, and field blanks required. For reference, Figure 6.3 outlines the amount of DI water and the additional cost of IDEXX resources required for each number of sample sites being tested. These are considered additional resource costs because they only include the cost of IDEXX bottles, reagents, and trays and do not consider the additional costs of overall testing such as the IDEXX tray sealer, extra sample bottles, pipettes, and other laboratory equipment. Cost estimates were derived from past orders of the IDEXX Colilert kit and prices may vary.

An additional recommendation to extend and improve water quality monitoring in CONG is to include the collection of other water quality parameters concurrently with bacteria concentrations. To accomplish this, the use of a handheld multiparameter water quality meter (e.g. YSI, ProDSS) could be purchased and used at sample sites. Meters of this type can collect water quality parameters including temperature, pH, dissolved oxygen, specific conductance, and turbidity. These data could be stored along with bacteria data to have a fuller understanding of water quality parameters in the park by providing a wide range of water quality parameters. Depending on the future of the program and the available budget, it is also suggested that OGBFREC invest in a small autoclave for the laboratory space. Waste management became an issue as it is suggested to sterilize IDEXX waste before disposal. A small, tabletop autoclave could be used in the convertible laboratory space for sterilization.

6.2 CONCLUSIONS

In summary, the main objective of this project was to develop a water quality monitoring program for CONG. Previous water quality monitoring efforts in the area and through partnerships have been relatively long term and most have not considered bacterial levels in water quality assessments. Fecal contamination and high bacterial levels are of a great concern for CONG water quality and environmental issues may escalate into the future. Such concerns are related to the public health risk of exposure to fecal contamination and its potential to cause human illness as well as the degradation of habitats. The water quality concerns outlined in Chapter 2 demonstrated the potential susceptibility of severe fecal contamination in CONG waters due to upstream wastewater discharges, faulty sewage systems, agricultural and industrial runoff, and pollution from animal populations. It is important to understand the sources of bacterial contamination and current management practices in order to make informed planning decisions for the future protection of ecologically significant waterways. Bacterial sample analysis, presented in Chapter 3, shows that there are high levels of E. coli in surface waters of CONG. Of specific concern are areas along Cedar Creek and sites where recreational is common and there is greater risk of exposure. Results also suggest that bacteria levels are higher following precipitation events, generally when creek levels are high. Elevated bacteria levels are also associated with increased precipitation events and warmer weather. This is especially concerning when considering management practices into the future, because climate trends are showing generally warmer temperatures as well as more frequent and more intense precipitation events.

In order to create a sustainable practice for bacterial water quality monitoring at CONG, Chapters 4 and 5 outline methodology and procedures for citizen science that includes collecting water samples and testing bacteria concentrations. The continuation of this project is justified thorough recommendations for implementing this program into the future. The maintenance of this program would provide regular data for water quality assessment in and around CONG, which greatly benefits park staff in understanding water quality dynamics and can be shared with park visitors for the sake of recreational safety.

Table 6.1 Resource Estimates for Sampling Events

# of Sample Sites	Total Samples Run	Cost of Resources	DI Water
1	7	$25.90	1.2
2	10	$37.00	1.7
3	13	$48.10	2.2
4	16	$59.20	2.6
5	19	$70.30	3.1
6	22	$81.40	3.6
7	25	$92.50	4.1

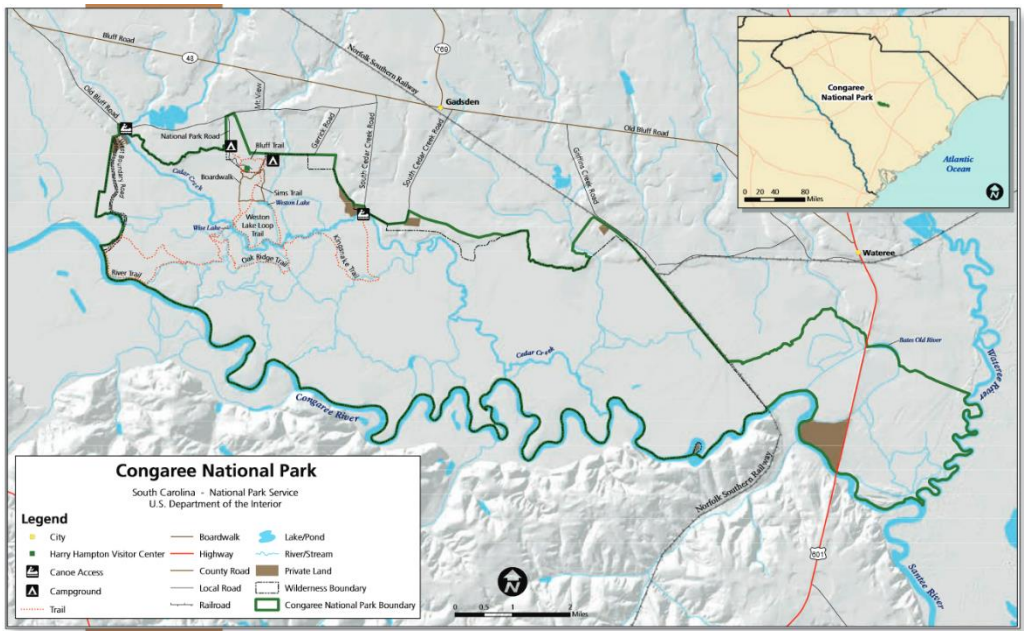


Figure 6.1 This map of CONG boundaries, hydrology, and other features is from the park's Foundation Document.[2]

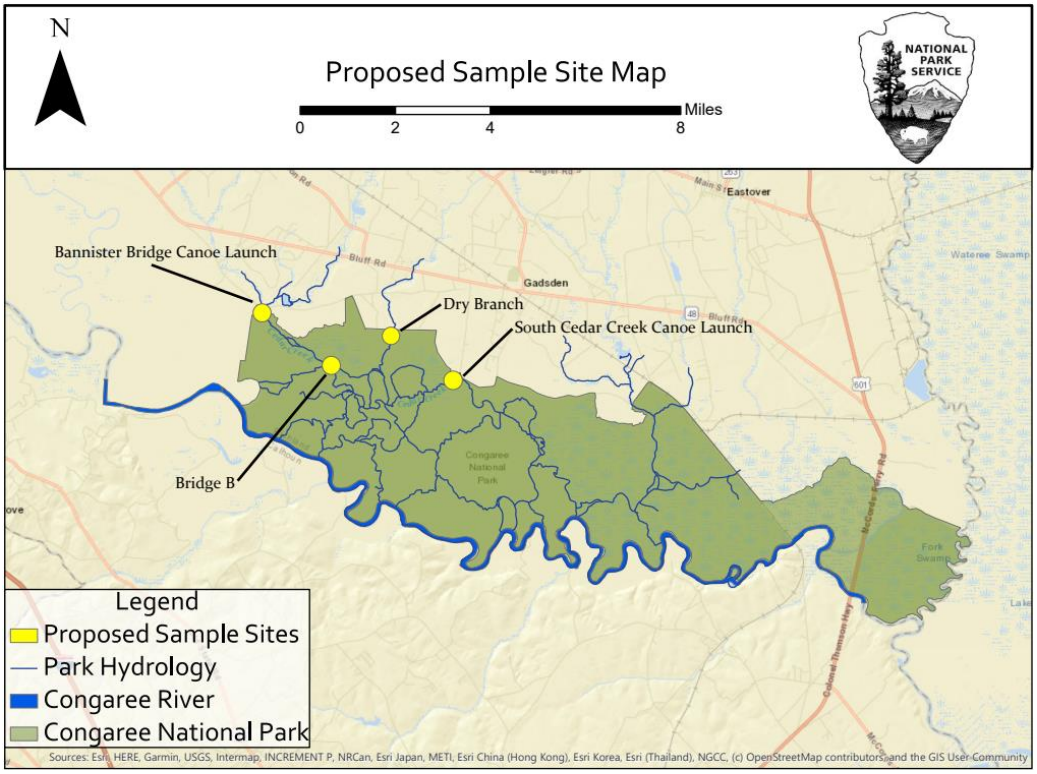


Figure 6.2 This map was created in ArcGIS to show the location of the proposed sample sites for bacteria testing.

REFERENCES

1. Hopkins MA 100 NPR, Us S 29061 P-4396 C. Congaree National Park (U.S. National Park Service). https://www.nps.gov/cong/index.htm. Accessed March 22, 2019.

2. Foundation Document - Congaree National Park South Carolina. October 2014.

3. Barbara West. Outstanding National Resource Waters: A Natural Resource Management Tool. http://npshistory.com/publications/water/onrw.pdf. Accessed August 2, 2019.

4. UNESCO. Ecological Sciences for Sustainable Development. United Nations Educational, Scientific and Cultural Organization & Man and the Biosphere Programme.

5. South Carolina Department of Health and Environmental Control. The State of South Carolina's 2016 Integrated Report Part I: Listing of Impaired Waters.

6. CDC. E. coli (Escherichia coli). https://www.cdc.gov/ecoli/general/index.html. Published January 11, 2019.

7. U.S. Census Bureau QuickFacts: Columbia city, South Carolina. https://www.census.gov/quickfacts/fact/table/saludacountysouthcarolina,lexingtoncountysouthcarolina,kershawcountysouthcarolina,fairfieldcountysouthcarolina,richlandcountysouthcarolina,calhouncountysouthcarolina/PST045218. Accessed October 23, 2019.

8. SC Watershed Atlas. https://gis.dhec.sc.gov/watersheds/. Accessed October 24, 2019.

9. DHEC. Watershed Water Quality Assessment. 2011.

10. DHEC. Watershed Water Quality Assessment - Broad River Basin. 2007.

11. US EPA. Summary of the Clean Water Act. US EPA. https://www.epa.gov/laws-regulations/summary-clean-water-act. Published March 11, 2019.

12. DHEC. National Pollutant Discharge Elimination System (NPDES) Overview.

13. Google Maps. Google Maps. https://www.google.com/maps/place/Columbia+Sewage+Treatment+Plant/@33.938

3748,-81.0183209,17z/data=!3m1!4b1!4m5!3m4!1s0x88f8ba409535bfb7:0xe9d403c9a86451c2!8m2!3d33.9383748!4d-81.0161322. Accessed October 24, 2019.

14. EPA. Office of Water. Recreational Water Quality Criteria. 2012.

15. IDEXX. What's the Difference between MPN and CFU? Water Testing Solutions Blog. https://www.idexx.com/en/blogs/idexx-water-testing-solutions/what-s-the-difference-between-mpn-and-cfu/.

16. SC Department of Health and Environmental Control. Water Regulations & Standards: Water Classification and Standards.

17. Petersen F, Hubert JA, Kellner E, Kutta E. Land-use-mediated Escherichia coli concentrations in a contemporary Appalachian watershed. *Environ Earth Sci*. 2018;22(2018). https://www.researchgate.net/publication/328962595_Land-use-mediated_Escherichia_coli_concentrations_in_a_contemporary_Appalachian_watershed.

18. Congaree River Keeper. Lower Richland Sewer Project. 2014.

19. Johnston MA, Porter DE, Scott GI, Rhodes WE, Webster LF. Isolation of faecal coliform bacteria from the American alligator (Alligator mississippiensis). *J Appl Microbiol*. 2010;108(3):965-973. doi:10.1111/j.1365-2672.2009.04498.x

20. Feral Hogs at Congaree National Park. Old-Growth Bottomland Forest RLC (U.S. National Park Service). https://www.nps.gov/rlc/ogbfrec/feral-hogs-at-congaree-national-park.htm. Published August 2018.

21. SC Department of Health and Environmental Control. How DHEC Measures Surface Water Quality.

22. Citizen Science - Research Learning Centers (U.S. National Park Service). https://www.nps.gov/rlc/citizenscience.htm.

23. CitizenScience.gov. https://www.citizenscience.gov/about/.

24. Pocock MJO, Chapman DS, Sheppard LJ, Roy HE. Choosing and Using Citizen Science: a guide to when and how to use citizen science to monitor biodiversity and the environment. Centre for Ecology & Hydrology.

25. Shelley, D. Citizen Science Birding Research. 2012.

26. Young, B. Citizen Science Based Aquatic Monitoring in Congaree National Park, South Carolina.

27. Congaree Land Trust. Congaree Land Trust. https://congareelt.org/. Published 2019.

28. Grego, J. Friends of Congaree Swamp. Friends of Congaree Swamp. https://friendsofcongaree.org/. Published 2019.

29. Department of Natural Resources. COWASEE Basin - Congaree, Wateree and Santee Rivers Basin. COWASEE Basin. http://cowasee.dnr.sc.gov/.

30. Gills Creek Watershed Association. Gills Creek Watershed Association. https://www.gillscreekwatershed.org/Index.asp. Published 2019.

31. The SC Adopt-a-Stream Program. Clemson Public Service and Agriculture. https://www.clemson.edu/public/water/watershed/scaas/index.html. Published 2019.

32. Midlands Rivers Coalition | SCDHEC. https://scdhec.gov/environment/your-water-coast/midlands-rivers-coalition.

33. Congaree Riverkeeper | Clean Water, Healthy Rivers. Congaree Riverkeeper. https://www.congareeriverkeeper.org/. Published 2019.

34. Patel, A. Locating Sources of Bacterial Contamination in the Toms Creek Watershed. 2010. https://scholarcommons.sc.edu/etd/235.

35. Montebello. An alaysis of the concentrations of Enterococci and Escherichia coli in Cedar Creek, Tom's Creek, Wise Lake and Weston Lake in Congaree National Park. *AWWAWEA S C*. 2008;38(3).

36. USGS. USGS/National Park Service Water Quality Partnership: Molecular Source Tracking of Enteric Bacteria in 303d-Listed Cedar Creek Watershed.

37. IDEXX. Colilert Test Procedures. 2019.

38. Colilert - IDEXX US. https://www.idexx.com/en/water/water-products-services/colilert/.

39. US EPA R 08. Addressing Total Coliform Positive or E.coli Positive Sample Results in EPA Region 8. US EPA.. Published February 22, 2013.

40. National Oceanic and Atmospheric Administration. National Weather Service Forecast Office. NOAA Online Weather Data. https://w2.weather.gov/climate/xmacis.php?wfo=cae. Accessed December 10, 2019.

41. USGS Current Conditions for USGS 02169672 CEDAR CREEK AT CONGAREE NP NEAR GADSDEN, SC. USGS National Water Information System. https://waterdata.usgs.gov/sc/nwis/uv?site_no=02169672. Published 2019. Accessed October 3, 2019.

42. NIH. Division of Technical Resources. Laboratory Water. March 2013.

43. Steripen Ultra™. https://www.katadyn.com/us/us/36833-ULT-MP-Ultra.

44. National Park Service. Citizen Science Resources. NPS: Common Learning Portal. Published 2019.

APPENDIX A
DHEC WATER QUALITY CRITERIA

10. **Freshwaters (FW)** are freshwaters suitable for primary and secondary contact recreation and as a source for drinking water supply after conventional treatment in accordance with the requirements of the Department. Suitable for fishing and the survival and propagation of a balanced indigenous aquatic community of fauna and flora. Suitable also for industrial and agricultural uses.

Quality Standards for Freshwaters	
ITEMS	**STANDARDS**
a. Garbage, cinders, ashes, oils, sludge, or other refuse	None allowed.
b. Treated wastes, toxic wastes, deleterious substances, colored or other wastes except those given in a. above.	None alone or in combination with other substances or wastes in sufficient amounts to make the waters unsafe or unsuitable for primary contact recreation or to impair the waters for any other best usage as determined for the specific waters which are assigned to this class.
c. Toxic pollutants listed in the appendix.	As prescribed in Section E of this regulation.
d. Stormwater, and other nonpoint source runoff, including that from agricultural uses, or permitted discharge from aquatic farms, concentrated aquatic animal production facilities, and uncontaminated groundwater from mining.	Allowed if water quality necessary for existing and classified uses shall be maintained and protected consistent with antidegradation rules.
e. Dissolved oxygen.	Daily average not less than 5.0 mg/l with a low of 4.0 mg/1.
f. *E. coli*	Not to exceed a geometric mean of 126/100 ml based on at least four samples collected from a given sampling site over a 30 day period, nor shall a single sample maximum exceed 349/100 ml.
g. pH.	Between 6.0 and 8.5.
h. Temperature.	As prescribed in E.12. of this regulation.

APPENDIX B

IDEXX COLILERT TEST PROCEDURE

Colilert® Test Kit

Introduction

Colilert® simultaneously detects total coliforms and *E. coli* in water. It is based on IDEXX's proprietary Defined Substrate Technology®. When total coliforms metabolize Colilert's DST® nutrient-indicator, ONPG, the sample turns yellow. When *E. coli* metabolize Colilert's DST® nutrient-indicator, MUG, the sample also fluoresces. Colilert can simultaneously detect these bacteria at 1 cfu/100 mL within 24 hours even with as many as 2 million heterotrophic bacteria per 100 mL present.

Storage

Store at 2–30°C away from light.

Presence/Absence (P/A) Procedure

1. Add contents of one pack to a 100 mL sample in a sterile, transparent, nonfluorescing vessel.
2. Cap vessel and shake.
3. Incubate at 35 ± 0.5°C for 24 hours.
4. Read results according to Result Interpretation table below.

Quanti-Tray® Enumeration Procedure

1. Add contents of one pack to a 100 mL water sample in a sterile vessel.
2. Cap vessel and shake until dissolved.
3. Pour sample/reagent mixture into a Quanti-Tray® or Quanti-Tray®/2000 and seal in an IDEXX Quanti-Tray® Sealer.
4. Place the sealed tray in a 35 ± 0.5°C incubator for 24 hours.
5. Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.

Result Interpretation

Appearance	Result
Less yellow than the comparator	Negative for total coliforms and *E. coli*
Yellow equal to or greater than the comparator	Positive for total coliforms
Yellow and fluorescence equal to or greater than the comparator	Positive for *E. coli*

- Look for fluorescence with a 6-watt, 365-nm UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample.
- Colilert results are to be read after 24 hours of incubation.
- However, if the results are ambiguous to the analyst based on the initial reading, incubate up to an additional four hours (but not to exceed 28 hours total) to allow the color and/or fluorescence to intensify.
- Positives for both total coliforms and *E. coli* observed before 24 hours and negatives observed after 28 hours are also valid.
- In addition, laboratories may incubate samples for additional time (up to 28 hours total) for their convenience.

Procedural Notes

- This insert may not reflect your local regulations. For compliance testing, be sure to follow appropriate regulatory procedures. For example, samples run in other countries are incubated at 36 ± 2°C for 24–28 hours.
- Colilert can be run in any multiple tube format. *Standard Methods for the Examination of Water and Wastewater*[2] MPN tables should be used to find Most Probable Numbers (MPNs).
- If a water sample has some background color, compare inoculated Colilert sample to a control blank of the same water sample.
- If sample dilutions are made, multiply the MPN value by the dilution factor to obtain the proper quantitative result.
- Use only sterile, nonbuffered, oxidant-free water for dilutions.
- Colilert is a primary water test. Colilert performance characteristics do not apply to samples altered by any pre-enrichment or concentration.
- In samples with excessive chlorine, a blue flash may be seen when adding Colilert. If this is seen, consider sample invalid and discontinue testing.
- Aseptic technique should always be followed when using Colilert. Dispose of in accordance with Good Laboratory Practices.

Quality Control Procedures

1. One of the following quality control procedures is recommended for each lot of Colilert:
   A. IDEXX-QC Coliform and *E.coli* [1]: *Escherichia coli*, *Klebsiella variicola*, and *Pseudomonas aeruginosa*
   B. Quanti-Cult®[1]: *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.
   C. Fill three sterile vessels with 100 mL of sterile nonbuffered oxidant-free water and inoculate with a sterile loop of ATCC® strains, *Escherichia coli* ATCC 25922/WDCM 00013 or ATCC 11775/WDCM 00090, *Klebsiella variicola* ATCC 31488/ WDCM 00206 and *Pseudomonas aeruginosa* ATCC 10145/WDCM 00024 or ATCC 27853.
2. Follow the P/A Procedure or Quanti-Tray Enumeration Procedure above.
3. Results should match the Result Interpretation table above.

NOTE: IDEXX internal quality control testing is performed in accordance with ISO 11133:2014. Quality Control Certificates are available at idexx.com/water.

Step 1

Add reagent to sample.

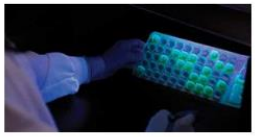
Step 2

Pour into Quanti-Tray (counts from 1–200) or Quanti-Tray/2000 (counts from 1–2,419).

Step 3

Seal in Quanti-Tray Sealer and place in 35°C ± 0.5°C incubator for 24 hours (in other countries, the temperature requirement may be different per regulatory requirements).

Step 4: Quanti-Tray



Quanti-Tray—Read results:

- Yellow wells = total coliforms
- Yellow/fluorescent wells = *E. coli*
- Count positive wells and refer to MPN table

Step 4: Quanti-Tray/2000

Quanti-Tray/2000—Read results:

- Yellow wells = total coliforms
- Yellow/fluorescent wells = *E. coli*
- Count positive wells and refer to MPN table

APPENDIX C

IDEXX MPN TABLE

IDEXX Quanti-Tray®/2000 MPN Table *(per 100ml)*

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.6	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	435.2

09-63235-01

IDEXX Quanti-Tray®/2000 MPN Table *(per 100ml)*

# Large Wells Positive	# Small Wells Positive																							
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1998.3	2419.6	>2419.6

09-63235-01

APPENDIX D
BACTERIAL SAMPLE ANALYSIS RESULTS

9/27/2019		Yellow (total coliform)				Blacklight (E. coli)			
		N	Average (95% C.I.)	St. Dev (n, 1s)	RSD (%)	N	Average (95% C.I.)	St. Dev	RSD (%)
Cedar Creek @ Bannister Bride	BABR	3	710.8	145.0	20.4%	3	241.9	70.0	29.0%
S. Cedar Creek Fast Water	FAST	3	2,419.6	0.0	0.0%	3	2,046.3	646.6	31.6%
S. Cedar Creek Slow Water	SLOW	3	2,419.6	0.0	0.0%	3	1,901.9	457.3	24.0%
Field Blank	FIBL	1	0.0	0.0	0.0%	1	0.0	0.0	0.0%
Lab Blank	LABL	3	0.0	0.0	0.0%	1	0.0	0.0	0.0%

10/22/2018		Yellow (total coliform)				Blacklight (E. coli)			
		N	Average (95% C.I.)	St. Dev (n, 1s)	RSD (%)	N	Average (95% C.I.)	St. Dev	RSD (%)
Cedar Cr. @ Gage (Bridge B)	GAGE	3	1,175.5	865.2	73.6%	3	194.3	32.0	16.4%
Cedar Cr. @ Kingsnake	KING	3	2,505.4	100.2	4.0%	3	161.3	56.7	35.2%
Cedar Cr. above Meyers Cr.	CECR	3	439.1	424.4	96.7%	3	15.6	4.5	28.9%
Dry Branch	DRBR	3	1,497.9	506.3	33.8%	3	1.7	3.0	173.2%
Field Blank	FIBL	3	0.0	0.0	0.0%	3	0.0	0.0	0.0%
Lab Blank	LABL	3	0.0	0.0	0.0%	3	0.0	0.0	0.0%
Meyers Cr. above Cedar Cr.	MYCR	3	2,374.2	1,183.2	49.8%	3	307.8	13.7	4.4%

2/11/2019		Yellow (total coliform)				Blacklight (E. coli)			
		N	Average (95% C.I.)	St. Dev (n, 1s)	RSD (%)	N	Average (95% C.I.)	St. Dev (1s)	RSD (%)
Cedar Cr. @ Gage (Bridge B)	GAGE	3	1,595.5	749.3	47.0%	3	66.6	58.4	87.7%
Cedar Cr. @ Kingsnake	KING	3	1,230.2	497.8	40.5%	3	42.6	38.8	91.0%
Meyers Cr.	MYCR	3	678.7	63.6	9.4%	3	84.3	89.9	106.6%
Dry Branch	DRBR	3	165.6	165.5	99.9%	3	4.4	5.1	117.2%
Field Blank	FIBL	2	0.0	0.0	0.0%	2	0.0	0.0	0.0%
Lab Blank	LABL	3	0.0	0.0	0.0%	3	0.0	0.0	0.0%
Cooler Blank	COBL	2	0.0	0.0	0.0%	2	0.0	0.0	0.0%

3/8/2019		Yellow (total coliform)				Blacklight (E. coli)			
		N	Average	St. Dev (n,	RSD (%)	N	Average	St. Dev	RSD (%)
Cedar Cr. above Meyers Cr.	CECR	3	227.6	137.4	60.3%	3	62.8	33.1	52.8%
Cedar Cr. @ Kingsnake	KING	3	1,744.2	589.5	33.8%	3	529.6	119.4	22.5%
Meyers Cr.	MYCR	3	205.9	125.7	61.0%	3	426.9	583.5	136.7%
Field Blank	FIBL	2	0.0	0.0	0.0%	2	0.0	0.0	0.0%
Lab Blank	LABL	2	0.0	0.0	0.0%	2	0.0	0.0	0.0%
Cooler Blank	COBL	2	0.0	0.0	0.0%	2	0.0	0.0	0.0%

4/2/2019		Yellow (total coliform)				Blacklight (E. coli)			
		N	Average (95% C.I.)	St. Dev (n, 1s)	RSD (%)	N	Average (95% C.I.)	St. Dev (1s)	RSD (%)
Cedar Cr. @ Gage	GAGE	3	2,835.5	874.2	30.8%	3	309.1	89.8	29.0%
Dry Branch	DRBR	3	1,862.1	118.6	6.4%	3	24.0	21.4	89.1%
Cedar Cr. above Meyers	CECR	3	997.1	140.1	14.0%	3	5.8	5.2	89.5%
Cedar Cr. @Kingsnake	KING	3	1,663.2	447.6	26.9%	3	79.4	18.8	23.7%
Meyers Cr.	MYCR	3	1,249.4	286.1	22.9%	3	174.1	32.7	18.8%
Stump Gut	STGT	3	1,401.9	387.3	27.6%	3	53.9	46.9	86.9%
Cedar Cr. Above Mazyck's	MAZY	3	1,750.1	478.0	27.3%	3	83.3	36.0	43.2%
Congaree River above Mazyck's	CONG	3	418.5	95.2	22.8%	3	59.1	35.5	60.0%
Lab Blank	LABL	3	0.0	0.0	0.0%	3	0.0	0.0	0.0%

6/11/2019		Yellow (total coliform)				Blacklight (E. coli)			
		N	Average (95% C.I.)	St. Dev (n, 1s)	RSD (%)	N	Average (95% C.I.)	St. Dev (1s)	RSD (%)
Myer's Creek Above Cedar Creek	MYCR	3	6,874.4	1,924.2	28.0%	3	347.9	149.2	42.9%
Cedar Creek Above Myer's Creek	CECR	3	5,429.1	919.1	16.9%	3	99.1	87.7	88.6%
Cedar Creek @ Gage	GAGE	3	12,439.4	6,671.5	53.6%	3	591.0	66.2	11.2%
Dry Branch	DRBR	3	7,828.7	3,568.9	45.6%	3	33.3	30.2	90.7%
Cedar Creek @ Kingsnake	KING	3	11,659.4	6,323.9	54.2%	3	331.9	95.5	28.8%
Lab Blank	LABL	3	0.0	0.0	0.0%	3	0.0	0.0	0.0%
Cooler Blank	COBL	1	0.0	0.0	0.0%	1	0.0	0.0	0.0%
Field Blank	FIBL	1	0.0	0.0	0.0%	1	0.0	0.0	0.0%

7/2/2019		Yellow (total coliform)				Blacklight (E. coli)			
		N	Average (95% C.I.)	St. Dev (n, 1s)	RSD (%)	N	Average (95% C.I.)	St. Dev (1s)	RSD (%)
Cedar Creek @ GAGE	GAGE	3	6,116.7	1,270.5	20.8%	3	188.6	100.2	53.1%
S. Cedar Creek	CECR	3	3,587.2	589.2	16.4%	3	34.4	31.9	92.7%
Field Blank	FIBL	1	0.0	0.0	0.0%	1	0.0	0.0	0.0%

7/17/2019		Yellow (total coliform)				Blacklight (E. coli)			
		N	Average (95% C.I.)	St. Dev (n, 1s)	RSD (%)	N	Average (95% C.I.)	St. Dev (1s)	RSD (%)
S. Cedar Creek Canoe Launch	CECR	3	3,535.9	677.8	19.2%	3	118.9	55.7	46.9%
Bannister Bridge Canoe Launch	BABR	3	8,555.7	3,437.1	40.2%	3	61.5	33.3	54.2%
Lab Blank	LABL	1	0.0	0.0	0.0%	1	0.0	0.0	0.0%
Cooler Blank	COBL	1	0.0	0.0	0.0%	1	0.0	0.0	0.0%

7/18/2019		Yellow (total coliform)				Blacklight (E. coli)			
		N	Average (95% C.I.)	St. Dev (n, 1s)	RSD (%)	N	Average (95% C.I.)	St. Dev (1s)	RSD (%)
Cedar Creek @ Gage - Group A	GAGE-A	3	4,895.5	1,881.2	38.4%	3	287.9	115.0	40.0%
Cedar Creek @ Gage - Group B	GAGE-B	3	4,317.7	810.5	18.8%	3	290.5	200.6	69.0%
Cedar Creek @ Gage - Group C	GAGE-C	3	5,856.4	1,263.0	21.6%	3	255.3	54.5	21.4%
Cooler Blank	COBL	1	0.0	0.0	0.0%	1	0.0	0.0	0.0%
Lab Blank	LABL	2	0.0	0.0	0.0%	2	0.0	0.0	0.0%

8/2/2019		Yellow (total coliform)				Blacklight (E. coli)			
		N	Average (95% C.I.)	St. Dev (n, 1s)	RSD (%)	N	Average (95% C.I.)	St. Dev (1s)	RSD (%)
S. Cedar Creek Canoe Launch	CECR	4	3,640.3	496.1	13.6%	4	272.4	50.3	18.5%
Lab Blank	LABL	1	0.0	0.0	0.0%	1	0.0	0.0	0.0%

10/20/2019		Yellow (total coliform)				Blacklight (E. coli)			
		N	Average (95% C.I.)	St. Dev (n, 1s)	RSD (%)	N	Average (95% C.I.)	St. Dev (1s)	RSD (%)
Before	**B4**	4	4,841.0	1,776.3	36.7%	4	848.8	108.8	12.8%
First	**UV1**	4	542.8	110.0	20.3%	5	89.0	13.0	14.6%
Second	**UV2**	4	4.7	5.0	107.9%	4	0.0	0.0	0.0%
Third	**UV3**	4	0.0	0.0	0.0%	4	0.0	0.0	0.0%
Lab Blank	**LABL**	1	0.0	0.0	0.0%	1	0.0	0.0	0.0%

APPENDIX E

BACTERIAL WATER QUALITY MONITORING PROGRAM DOCUMENTS

CongaReesearch:

Bacterial Water Quality Monitoring

Citizen-Science Training Program

Summer 2019

Contents

I. Background

This **emerging** program at Congaree National Park is focused on implementing water quality monitoring in various waters within the park. There are several known sites of impaired waters in park boundaries as well as areas of **high fecal coliform bacteria and E. coli concentrations**. The goals of this program include regularly sampling water at multiple sites, testing water samples for bacteria concentrations, and reporting results to park staff to keep on record for both staff and visitor information. This program will be citizen science based in hopes of the continuation of the program by trained VIPs (Volunteers in Parks). Through the completion of this training sessions, **VIPs will learn the methods and protocols for water quality monitoring** and will be invited to be involved in future sampling events on a regular schedule as they are interested and available. This is one of many volunteer opportunities that greatly benefits Congaree National Park and **could not be completed without the help of our VIPs**! Please feel free to direct any questions, comments, or suggestions to CONG intern and program lead, Shea McCarthy at *shea_mccarthy@partner.nps.gov*.

II. Trainee Expectations

1. **Prepare** for a sampling event as you would for a day hike – show up hydrated, fed, and **enthusiastic!**
2. **Wear** appropriate walking hiking boots or walking shoes
3. **Dress** comfortably for hiking in the given weather and bug condition (long pants and sleeves that are lightweight and breathable are great for sun and bug protection)
4. **Pack** sunscreen, bug spray, water, snacks, and any other necessities

Notes:

- **Training will be a shortened version of a sampling event**
- **A full sampling day will last approximately 6 hours and may include up to 4 hours in the field**

III.

IV. Sampling Day Protocol

a. Lab Preparation

1. Check incubator temperatures
2. Keep overhead fans off
3. Lysol wipe all work benches
4. Set up equipment and materials on work benches

b. Labelling

- We will run two types of samples

1. environmental samples – from our sample sites

2. blanks - sterile DI water treated as a sample to test for contamination

- Both types of samples will be given a four-letter short name. Use the following examples for reference:
  - Cedar Creek @ Gage – GAGE
  - Myers Creek – MYCR
  - Cedar Creek @ Kingsnake – KING
  - Lab Blank – LABL
  - Cooler Blank – COBL
  - Field Blank – FIBL

c. Field Prep

- Fill two sample bottles with sterile deionized (DI) water
- Label one bottle and its lid as “COBL” for cooler blank
- Label second bottle and its lid as “FIBL” for field blank
- Put each bottle in a separate Ziploc bag and label corresponding bags as “cooler blank” and “field blank” and include the date
- For each sample site, label one bottle with the site’s short name and “sample” on both the bottle and lid
- Keep the plastic seal on sample bottles until arrival at sample site
- Place each sample site bottle in a separate Ziploc bag and label bag with site short name and date
- Pack cooler
  - Trash bag of ice
  - Ziploc bag with ice pack or a small amount of ice

- Field blank, cooler blank, sample bottles

d. Safety

Before going into the field, it is crucial to discuss and understand safety precautions and potential hazards. Field hazards may include

- Heat exhaustion/heat stroke
- Dehydration
- Thunderstorms
- Uneven or slippery terrain
- Poison Ivy
- Potentially dangerous animals (snakes, spiders, feral hogs)
- Driving safety

e. Sample Collection

For each sample site:

1. Pack field blank and sample site bottles in the bag with the ice pack and carry in a backpack – leaving the cooler in a central location (Visitors Center, Learning Center, vehicle)

Note: ALWAYS keep sample bottles in their respective Ziploc bags until they are taken out to collect a water sample

2. Upon arrival to the sample site, make notes in field notebook of sample site location and description, sample site short name, general water level, weather, spot of sample grab (ex. Left side of bridge coming from the parking lot) and any other relevant observations
3. Remove plastic seal and put in Ziploc bag (leave no trace!)
4. Stand as close as possible to the bank in an area where flow is not blocked by logs or other interferences (or wade when necessary and safe)
5. Face the mouth of the bottle into the stream flow, ensuring that you, your hands, and any other possible interferences are downstream of the bottle
6. When prepared to take the sample, remove the lid directly above the water (spend as little time with the lid off of the bottle as possible)
7. Dip the bottle into the water facing the flow
8. Slightly tilt the bottle while submerged to remove any large air bubbles
9. Remove the sample from the water and immediately screw on lid

10. Return sample bottle to its Ziploc bag and put it back with the ice pack in a backpack
11. When possible, return collected sample to cooler at central location before going to the next site
12. Repeat all steps for each sample site

f. IDEXX Testing

Note: Samples must be kept on ice after collection and must be run for bacterial testing within 6 hours of collection

- We will run three sample tests for each sample site at three different dilutions (1:100, 1:10, 1:1)
- At least one field blank, cooler blank, and lab blank will be run to test for contamination throughout the process, additional blanks will be run if testing a larger amount of samples

Set up:

1. Make sure all benches are cleared off and organized
2. Ensure overhead fans are turned off
3. For each sample site (ex. GAGE) label three sample bottles and their lids with short name and dilution:
1. GAGE 1:100
2. GAGE 1:10
3. GAGE 1:1
4. For each blank, label a sample bottle and lid with short name (ex. LABL)
5. Label one quanti-tray for each sample being run (each bottle should have a corresponding tray)
- ONLY write on the silver side of trays – writing on the back/white side before sealing the trays can damage the sealer
- Label trays with small writing along the side of the wells
6. Set up work benches
1. Bench #1 – Sterile Deionized water, graduated cylinder, sample bottles
2. Bench #2 – cooler, autopipettor
3. Bench #3 – IDEXX reagents, trays, sealer
7. Plug in sealer, turn on, and allow to warm up (orange light will turn green when ready)

Notes:

- Wear gloves at all times
- Change gloves every time you begin handling a new sample site or blank
- Avoid contamination by keeping all lids closed when bottles are not in use

The following pages give step by step instructions for each work bench. Each of the three following pages can be left at each work bench for easy reference.

Bench #1

This bench should only hold DI water and sterile equipment

Fill DI water:

Sample Type	Amount of DI water
1:100 dilution	99 ml
1:10 dilution	90 ml
1:1 dilution	50 ml
Field Blank	None
Cooler Blank	None
Lab Blank	100 ml

1. **Fill graduated cylinder with DI water to the desired amount**
2. **Transfer water from graduated cylinder into appropriate sample**
3. **Put lid on sample bottle and set aside**

Best practice notes:

- Fill graduated cylinder until meniscus is at the line of desired amount
- One method is to unscrew the lid and pour water close to desired amount and then use squeeze bottle lid to slowly fill the rest of the way
- Avoid touching the graduated cylinder to the mouth of the bottle when pouring
- If you overfill the graduated cylinder, simply pour some of the water back into the DI water bottle

Lab Blank	**none**	**N/A**

Bench #2

Best practices notes:

- Between each sample, wipe down benchtop and change gloves
- Put on new pipette tip with clean gloves
- Always hold autopipettor upright/vertical

For each environmental sample site

1. Invert sample 10-15 times
2. Set autopipettor volume to 1 ml
3. Practice collecting 1 ml of sample and returning it 2-3 times
   - To collect, press the knob to the first stop
   - Submerge the tip into the sample
   - Release knob to collect water, wait until water stops rising, and remove tip from water
   - To empty, push knob to first stop, then push to second stop to empty
4. Add 1 ml of sample water to the 1:100 dilution
5. Set autopipettor volume to 10 ml
6. Practice collecting 10 ml of sample water and returning it 2-3 times
7. Add 10 ml of sample water to the 1:10 dilution
8. Add 10 ml of sample water 5 times (for a total of 50 ml) to the 1:1 dilution

Bench #3

1. **Shake reagent pack and tap on table to move powder downward**
2. **Pop tab of reagent pack open**
   - **Open away from your face and others as some powder is released when the reagent is popped open**
3. **Pour reagent into bottle**
4. **Dispose of empty reagent packs in the storage bin labelled for IDEXX waste**
5. **Put lid on sample bottle and invert 30 times**
   - **Invert gently to avoid creating bubbles**
   - **If reagent is not dissolved after 30 inversions, continue inverting 10-15 additional times until fully dissolved**
6. **Pour sample into corresponding tray**
7. **Place tray in rubber tray holder**
8. **Send tray through sealer with white paper side up**
9. **When the tray is released, label the white paper side with the date, time, short site name, and dilution factor (ex. 8/2 10:45 CECR 1:100)**
10. **Place tray in incubator**
11. **Repeat for all samples**

Best practices notes:

- Always wear safety goggles when handling reagents
- Between each sample, wipe down benchtop and change gloves

V. Reading Day Protocol

- Samples must be read within 24-28 hours of incubation
- Wear gloves when reading samples – skin must be protected from the black light – if sleeves do not cover wrists, be cautious to only put hand and not wrist in the reader
- Yellow wells are positive for coliform bacteria and wells that are both yellow and fluoresce under the black light are positive for E. coli
- Use the IDEXX comparator when marking positive wells – any well that is as yellow as or more yellow than the comparator is a positive well

1. Plug in black light reader and turn on
2. For each tray, fill in sample read form with sample name, dilution, date and time in incubator, and date and time being read
3. For each yellow well, use a Sharpie to make a hash mark (half of an X) on the positive well
4. Place the tray in the black light reader and look through the viewer
5. For each fluorescent well, use a Sharpie to make a hash mark the opposite way (completing the X) on the positive well
6. Remove the tray from the light and count each large and small well that is positive for coliform bacteria and each large and small well that is positive for E. coli
7. Note the positive counts on the sample read form
8. Using the MPN chart, match the number of positive large wells and the number of positive small wells to get the MPN count and write it on the sample read form
9. Have 1-2 additional participants count positive wells and check MPNs to avoid miscounting or misreading
10. Dispose of trays in the storage bin labelled IDEXX waste

VI. Task Checklist

WQ Monitoring Checklist		
One Week Before Sampling		
Inventory	Inventory	Need to Order
IDEXX bottle		50
IDEXX Quanti-trays		50
IDEXX reagents		50
IDEXX Comparator		1
Tasks	Complete (initial)	
Check IDEXX reagent expiration date		
Check IDEXX comparator expiration date		
Order IDEXX supplies as necessary		
Schedule use of USC lab		
2-3 Days Prior		
Pack equipment that needs to be autoclaved		
Fill DI water (3-4 Liters)		
Fill cooler blank and field blanks with DI		
Autoclave DI		
Autoclave equipment		
Pack sterile equipment in sealed Ziploc bags		
Label sample bottles and Ziploc bags with sample site		
Seal sample bottles in corresponding bag		
One business day prior		
Fill incubators with sample bottles of water		
Turn incubators on to 95 degrees F		
Check incubators at least 4 hours later		
Plug in black light and check bulb		
Organize learning center and clear all tables		
Check stock of gargabe bags, paper towels, surface cleaner		
Check stock of gloves, sharpies, safety goggles		
Clean out cooler		

Day of Sampling		
Keep notes in lab notebook including time started		
Check incubator temperatures		
Organize learning center and clear all tables		
Keep overhead fans off		
Lysol wipe all work benches		
Set up bench #1 sterile DI, graduated cylinders, sample bottles		
Set up bench #2 samples, autopipettor, pipette tips		
Set up bench #3 reagents, trays, sealer, rubber tray		
Plug in sealer and turn on		
Make water comparator/blank		
Label all sample bottles		
Label all Quanti-trays		
Make note of sample sites and short names in lab notebook		
Run samples within 6 hours of collection		
End of Sampling Day		
Dispose of reagent packets in labelled waste bin and close top		
Wash sample bottles		
Put equipment in Ziploc bag and label as used/needs autoclave		
Put away all additional equipment		
Unplug and store sealer		
Lysol wipe all surfaces		
Set up black light on bench #3		
Empty and clean cooler		
Make sure incubator doors are closed		
Note end time and notes in lab notebook		
Day of Sample Reading		
Read samples after 24-28 hours of incubation		
Print sample reading form		
Note start time and notes in lab notebook		
Plug in and turn on black light		
Read all samples and note results on sample read form		
Dispose of trays in labelled waste bin and close top		
Unplug and store black light		
Turn off incubators		
Note end time and additional notes		
Organize learning center		
Calculate MPN		
Note MPN results in document		
Note water level at time of sampling		