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MICROBIAL QUALITY AND RISK ASSESSMENT OF TYPE 2 NC RECLAIMED WATER FOR NON-POTABLE, AND POTABLE REUSE

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1 Acknowledgements (1pg limit)

WRRI (Water Resources Research Institute)

North Carolina Department of Environment and Natural Resources (NC DENR)

Participating Wastewater Utilities
- OWASA (Orange Water and Sewer Authority) Wastewater Treatment Plant
- Raleigh Neuse River Wastewater Treatment Plant
- North Durham Water Reclamation Facility
- Holly Springs Wastewater Treatment Plant
- North Cary Water Reclamation Facility

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2 Abstract

2.1 Title: Microbial Quality and Risk Assessment of Type 2 NC Reclaimed Water for Non-Potable, and Potable Reuse
2.2 Objectives: The goal of this research was to collect real world data on type 2 reclaimed water by conducting field studies on the performance of NCT2 like reclaimed water producing treatment facilities, as well as to evaluate the risk of exposure to this water in potable reuse scenarios by conducting microbiological water quality analyses and then quantitative microbial risk assessments (QMRAs). Field samples of wastewater and water were collected over a one-year period from 4 NCT2RW producing facilities, along with sewage impacted surface waters considered candidates for the 80/20 combination as sources for drinking water production. Water samples were examined for the microbial indicators specified in the NC legislation and for representative pathogens of public health interest.
2.3 Methods: Reclaimed water samples were collected from four water reclamation facilities producing NC Type 2-like reclaimed water and analyzed for indicator and pathogenic organisms by standard methods. Survival studies were conducted with both laboratory propagated indictor organisms and naturally occurring bacteria in surface waters to examine the effect of the 5 day storage period on microbial reduction. QMRA analyses were conducted in Analytica 4.6 (Lumina Decision Systems, Los Gatos, CA), with random variables sampled 10,000 times for each analysis.
2.4 Results: Based on microbial water quality analyses and QMRA analysis, there is evidence that the risks associated with consumption associated with potable reuse, are not reduced below the annual risk level of 1×10^{-4} set by US EPA for drinking water. Additionally, the 5 day storage period did not result in significant decreases in indicator organisms at either 4 or 20°C.
2.6 Conclusions: Based on the results presented here, it is apparent that: (1) the $\log_{10}$ reduction target for the virus indicator organisms (coliphages) are not documented as being met due to their low levels in the raw sewage, and 2) low but detectable levels of pathogens are present in the reclaimed water at levels that may pose human health risks from potable reuse exposures. A secondary issue is that little die-off occurs during the 5 day storage period, indicating that this may not be the most effective method of storage for pathogen reduction in reclaimed waters.
2.7: Recommendations: Additional research should be conducted on the pathogen content of NCT2RW, specifically on the concentrations of infectious viruses in this water. As adenoviruses were detected in high numbers, it is important to determine if any of these viruses are infectious and what risk they may pose to human health if this water is used for potable reuse purposes. Additional research should also be conducted using larger sample volumes for indicator virus analyses to determine if required $\log_{10}$ reductions are met during wastewater reclamation processes to achieve NCT2RW performance requirements, or if changes to this reduction standard or in methods to document compliance should be made.

3 Body of Report

3.1 Introduction

As of September 2014, the State of North Carolina has approved tertiary treated, dual disinfected (with UV radiation and free chlorine as defaults) wastewater (called type 2 reclaimed water, NCT2RW) for both non-potable agricultural use and potable reuse. This research evaluated both NCT2RW and riverine surface waters currently used as drinking water sources for their health-related microbial quality by quantifying fecal indicator organisms mandated for water quality testing by NC law and compared them to concentrations of culturable pathogenic bacteria (*Salmonella* spp.), human enteric viruses detected by cell culture (adenoviruses) and molecular (adenoviruses and noroviruses) methods, and protozoan parasites (Cryptosporidium and Giardia) detected by immunofluorescent microscopy methods in each type of water. Public health interest is generally focused on pathogen content in treated wastewater used as reclaimed water for both potable and non-potable purposes, but safety decisions and management systems on microbial quality are often based on concentrations of fecal indicator organisms. Therefore, a secondary focus of this research was to evaluate the relationships between microbial indicator and pathogenic microorganisms in NCT2RW and ambient surface waters.

The State of North Carolina defines potable reuse as a combination of up to 20% NCT2RW with at least 80% surface source water with a 5-day storage time under unspecified conditions. Performance targets are defined as allowable concentrations of fecal indicator bacteria (*Escherichia coli*), viruses (coliphages) and a protozoan parasite surrogate (*Clostridium perfringens*) in the reclaimed water, reductions in $\log_{10}$ concentrations of these fecal indicator microbes as well as monitored monthly geometric mean levels for each of these indicator microorganisms. Log_{10} reduction targets are 6 for bacteria, 5 for viruses, and 4 for protozoan parasite surrogates. The monthly geometric mean and daily maximum target concentrations for these fecal indicator bacteria, viruses, and the protozoan parasite surrogates in final effluent NCT2RW are summarized below.

- Monthly geometric mean *Escherichia coli* or fecal coliform levels of less than or equal to 3/100mL with a daily maximum of less than or equal to 25/100mL.
- Monthly geometric mean coliphage levels of less than or equal to 5/100mL with a daily maximum level of less than or equal to 25/100mL.
- Monthly geometric *C. perfringens* levels of less than or equal to 5/100mL with a daily maximum of less than or equal to 25/100mL.

After the 5-day storage time, the combined up to 20% NCT2RW and at least 80% surface source water blend is treated by traditional drinking water treatment processes. For conventional drinking water treatment processes, which typically involve the use of coagulation/flocculation followed by rapid granular media filtration and final disinfection, it is anticipated that this treatment will result in additional $\log_{10}$ reductions. Both the United States Environmental Protection Agency (US EPA) and the World Health Organization (WHO) have defined

performance targets for treated drinking water (US EPA, 2006;WHO, 2011); targets will be defined later in this report.

The microbial quality of reclaimed water produced by some reclaimed water treatment trains has been evaluated previously (Harwood et al., 2005; Rodriguez et al., 2009). However, the tertiary treated, dual disinfected reclaimed water proposed by the state of North Carolina for potable reuse and designated as type 2 has not been evaluated in full-scale production scenarios nor has it been studied when blended with surface source waters and stored for 5 days. A previous pilot scale study evaluating the dual UV and chlorine disinfection system for tertiary treated sewage as type 2 reclaimed water proposed in NC, concluded that dual disinfection is effective for reducing concentrations of fecal indicator bacteria, viruses and protozoan parasite surrogates in producing high quality reclaimed water (Sobsey et al., 2005). The goal of this research is to expand on this initial study by conducting field studies on the performance of NCT2-like reclaimed water producing treatment facilities, as well as to evaluate the risk of exposure to this water in various potable reuse scenarios by conducting quantitative microbial risk assessments (QMRAs).

3.2 Methods

3.2.1 Water samples

Raw sewage and reclaimed water samples were collected bi-monthly for 1 year, during and after storm events as grab samples using approved techniques (Standard Methods for the Examination of Water and Wastewater; SMEWW) from 4 different water reclamation facilities located in central North Carolina, resulting in 22 reclaimed water samples. Each of the water reclamation facilities produces NC Type 2 reclaimed water, which is characterized by both tertiary treatment and dual disinfection (typically treatment by UV radiation and chlorine disinfection).

Surface water samples were collected in 16L sample volumes and split into a 12L sample volume for pathogen analysis and a 4L volume for microbial indicator analysis. Samples were processed and concentrated according to the procedures described in section 2.2.2 with the addition of an initial centrifugation step applied to the enteric virus concentration method in order to remove sediment and other solids before hollow fiber ultrafiltration. Viruses in the centrifuged sediment pellet were recovered by elution from the sediment pellet and added back to the initial concentrated supernatant for further processing and analysis, as described in Sheih et al., 1997. Sample processing and concentration steps for surface water are summarized in Figure 3-1. Methods for the detection of pathogenic and indicator organisms are as described in section 2.2.3 – 2.2.8.

3.2.2 Sample Processing and Concentration Methods

Samples were split into two volumes upon arrival in the laboratory, one sample (larger volume) for pathogen analysis, and a second sample for microbial indicator analysis. Raw sewage samples were collected from wastewater treatment plants in 300mL volumes and split into a 200mL sample for pathogen analysis and a 100mL sample for microbial indicator by culture analyses. Reclaimed water samples were collected in 12L sample volumes and split into a 10L

sample volume for pathogen analysis and a 2L volume for microbial indicator analysis. Samples processed for indicator organisms were not further concentrated before analysis.

Primary concentration for reclaimed water samples was done by hollow fiber ultrafiltration using the protocol described in Hill et al., 2007 and Polaczyk et al., 2008, primary concentration for raw sewage samples was done by low speed centrifugation, involving an initial centrifugation at 1500 x *g* and sedimented pellet separation for IMS-FA to recover and detect protozoan parasites and pellet elution to recover sediment-asssociated viruses. Secondary concentration for viruses was done by polyethelyene glycol (PEG-8000) precipitation by the method described in Yamamoto et al., 1970, while secondary concentration and purification for protozoan parasites was done by immunomagnetic separation (IMS) and then protozoan parasite (oo)cyst staining with fluorescent antibodies followed by immunofluorescent microscopy for (oo)cyst examination and quantification. A diagram of surface water sample processing procedures is provided in Figure 2-1.

Briefly, 10L of reclaimed water was spiked with a commercially available positive internal control that is uniquely fluorescently labeled *Giardia* and *Cryptosporidium* (oo)cysts (BTF Precise Microbiology, Inc., Pittsburgh, PA), and then filtered through the Fresenius Optiflux F250NR hollowfiber ultrafilter using standard conditions of pressure and flow rate. Water samples were concentrated to produce a retentate liquid of approximately 100-200mL volume, and ultrafilters were backflushed with a solution containing 0.5% Tween 80, 0.01% Sodium polyphosphate (NAPP) (Sigma-Aldrich, cat# 305553-25G), and 0.001%Antifoam Y. The backflush liquid was added to the retentate liquid to produce a total microbe concentrate volume of approximately 200-250mLs. Next, reclaimed water samples were centrifuged at 1,500 x *g* for 30 minutes at 4°C to separate out protozoan parasites, the sedimented pellet from this centrifugation was eluted using 0.5M pH7.5 threonine for 1 hour at room temperature with a mixing speed of 60RPM to recover sediment-associated viruses. The eluted mixture was then re-centrifuged at 1,500 x *g*, and the supernatant was combined with the initial supernatant collected from the previous centrifuge step, while the sedimented pellet was processed for protozoan parasite (oo)cyst recovery by immunomagnetic separation (IMS), fluorescent antibody staining and then examination by fluorescence microscopy at a magnification of 200X using an oil emersion lens. Similarly, for raw sewage samples, a 200mL sample volume was centrifuged at 1,500 x *g* for 30 minutes and 4°C and the sedimented pellet from this centrifugation was eluted using 0.5M pH 7.5 threonine for 1 hour at room temperature with a mixing speed of 60RPM to recover viruses. After a second centrifugation at 1,500 x *g*, for 30 minutes at 4°C, the sedimented pellet was then processed by IMS to further concentrate and purify protozoan parasite (oo)cysts. IMS, fluorescent antibody staining and fluorescence microscopy analysis steps are described in section 2.2.9. Virus processing continued for both raw sewage and reclaimed water samples for their respective combined supernatant samples with an additional centrifugation step at 5,000 x *g* at 4°C for 30 minutes. The sedimented pellet from this centrifugation was also eluted with 0.5M pH 7.5 threonine for 1 hour at 60RPM, re-centrifuged at 5,000 x *g*, and then combined with the supernatant from the previous step for virus recovery. A secondary virus concentration step was then performed on the total supernatant using 10% PEG-8000 and 0.5M NaCl with an overnight incubation at 4°C. After incubation, the samples were then centrifuged at 5000 x *g* at 4°C for 30 minutes, the supernatant was discarded and the sedimented pellet was eluted with PBS-Tween (US EPA, 2012) to a volume of 4mLs for virus recovery.

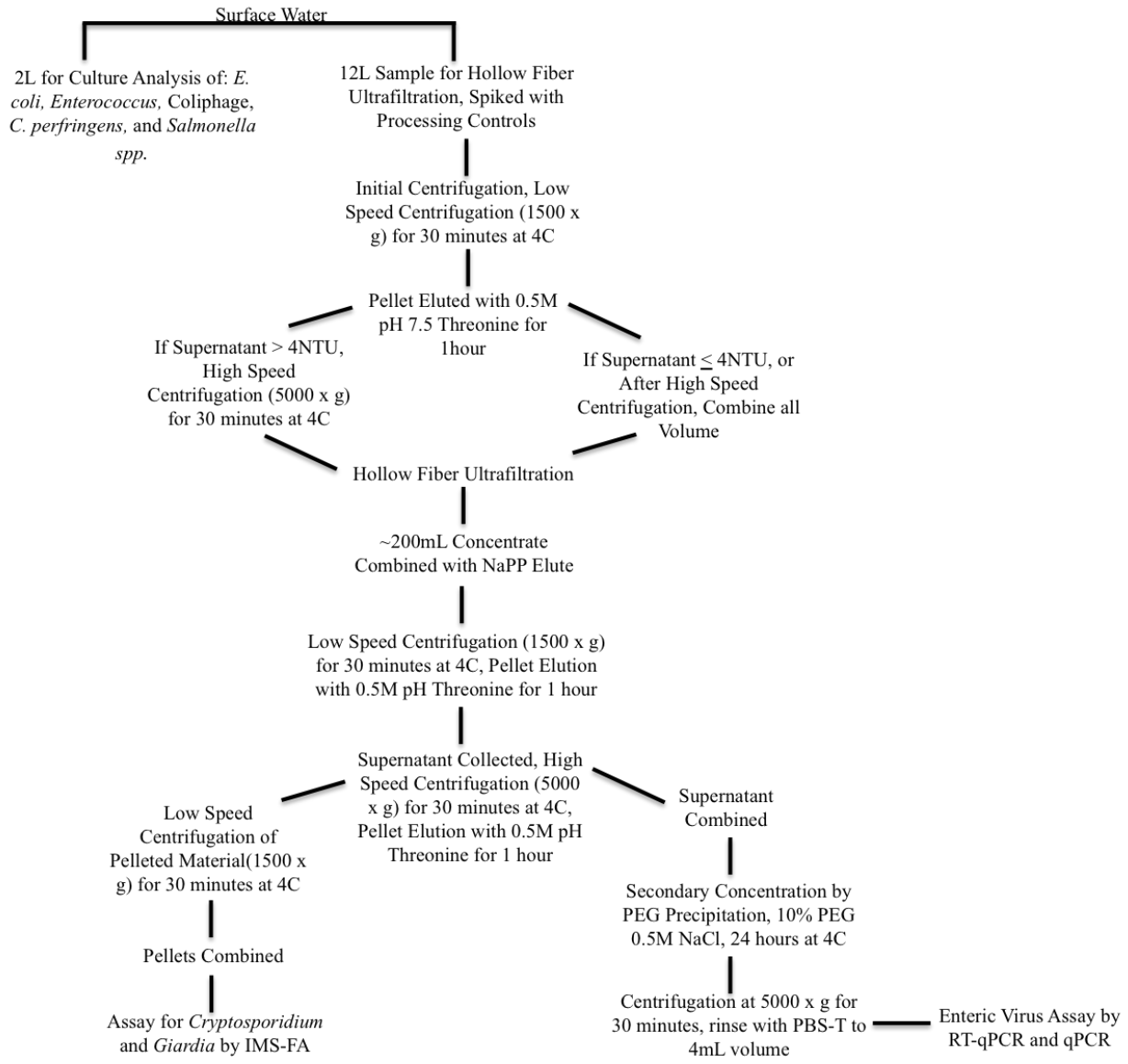

Figure 1: Diagram of reclaimed water sample processing for indicator and pathogenic microorganisms

3.2.3 Quantification of Bacterial Indicators

Concentrations of *E.coli* and *Enterococcus* spp. in 100 mL sample volumes were determined by a Most Probable Number (MPN) method with target bacteria growth on defined substrate media using the Quantitray 2000 multiwell system and Colilert and Enterolert media, respectively. Manufacturer instructions were followed and MPN values were determined using the manufacturer's MPN tables. Samples were processed in duplicate, diluted in phosphate buffered

saline (PBS) (US EPA, 2012) and incubated at 37°C for *E. coli* and 41.0°C for *Enterococcus* spp. for 24±4h; replicate MPN values were averaged to calculate the average MPN/100mL. The lower limits of detection for the Colilert and Enterolert methods are 1 MPN unit of *E. coli* or *Enterococcus* per 100mL sample volume.

3.2.4 Quantification of Coliphage Viruses

Somatic, F+/Male-specific and total coliphage concentrations were determined using US EPA Method 1602, the single agar layer method (US EPA, 2001). Samples of 100 mL were diluted using phosphate buffered saline (PBS) (US EPA, 2012). In this study, F+/Male-specific coliphage analysis was conducted using *E. coli* Famp as the *E. coli* host, with *E. coli* CN13 as the somatic coliphage host, and with *E. coli* CB390 as a total coliphage host. In work done by Guzmán et al, 2008, it has been proposed that *E. coli* CB390 can be used for the simultaneous detection of both F+/Male-specific and somatic coliphages. The limit of detection for the SAL method is 1 plaque forming unit (PFU) per 100mL.

3.2.5 Quantification of Protozoan Parasite Surrogates

Concentrations of *C. perfringens*, which are anaerobic, spore-forming enteric bacteria used as surrogate indicators for protozoan parasites, were determined using standard membrane filter (MF) methods modified from those originally developed for US EPA by Bisson and Cabelli (1980) using CP ChromoSelect Agar as the culture medium (Sigma-Aldrich, St. Louis, MO). Briefly, the agar base was prepared by adding 6.28 grams/100 mL deionized water, bringing to a boil on a hot plate and then removing to cool and keep molten at 55-60 degrees C. Once tempered to 55 degrees, 0.04 grams of D-Cycloserine were added per 100 mL of molten agar medium base. Supplemented medium was dispensed in 5-mL volumes in 60 mm diameter sterile, polystyrene petri dishes, which were then stored at 4°C until use. Samples were prepared by pasteurizing 100mL volumes of reclaimed water or 100mL of diluted raw sewage at 60°C for 30 minutes to detect only *C. perfringens* spores. Replicate samples were analyzed without pasteurizing to detect both spores and vegetative cells. Samples were diluted in phosphate buffered saline (PBS) (US EPA, 2012). After membrane filtration of samples by standard procedures and placing membranes on CP Chromoselect agar plates, membrane filtration plates were incubated in anaerobic jars at 45°C for 24±4h. After incubation, anaerobic jars were opened for at least 1 hour prior to counting to allow the characteristic color change to occur for *C. perfringens* colonies, which were then counted and recorded. The limit of detection for the MF method using CP ChromoSelect Agar is 1 colony forming unit (CFU) per 100mL.

3.2.6 Quantification of *Salmonella* Bacteria

Salmonella spp. bacteria concentrations were determined by a modification of the method described in both Hill & Sobsey 2001 and Krometis et al, 2010. Briefly, triplicate volumes of buffered peptone at pH 7.2 water were inoculated with three different sample volumes (for Reclaimed Water 300mL, 30mL, and 3mL; for Raw Sewage 1mL, 0.1mL, and 0.01mL) and incubated at 37°C for 24±4h as a pre enrichment step. After incubation, 10% of the enrichment culture volume was transferred into a volume of selective Rappaport-Vassilades (RV) broth and incubated at 41°C for 24±4h. After incubation, 10µL volumes of the RV broth enrichment were

then streaked on to Salmonella-Shigella (SS) agar plates and incubated at 37°C for 24±4h. Presumptive black positive colonies were identified and then confirmed as Salmonella-positive using the Triple Sugar Iron Agar slant biochemical test. A Salmonella-positive reaction was defined as a tube that fermented glucose and reduced sulfur. The presence of one or more *Salmonella* colonies on the SS agar was considered to be indicative of a positive broth culture enriched sample volume. *Salmonella* MPN concentrations were then determined using a 3 replicate, 3 dilution volume MPN table to calculate MPN volumes per 100mL.

3.2.7 Detection of Enteric Viruses

3.2.7.1 Virus Concentration and Nucleic Acid Extraction

Enteric viruses in reclaimed water and raw sewage were concentrated and processed as described above to obtain concentrated samples. Representative bacteriophages were used as positive controls for virus recovery in sample processing at each step in order to evaluate the efficiency of the virus recovery methods used. Specifically the Genogroup IV F+ RNA coliphage SP was used as a processing positive control for Norovirus and the Salmonella bacteriophage PRD1, a member of the *Tectiviridae* family, was used as a positive control for Adenovirus. PRD1 was provided by Dennis Bamford at the University of Helsinki, and SP was obtained from the American Type Culture Collection (ATCC, Rockville, Maryland). PRD1 was chosen because of it similarities to adenovirus, specifically its morphological characteristics (size and shape and double-stranded linear DNA genome); SP was chosen because it is a single stranded, non-enveloped RNA virus, much like Noroviruses. Positive control bacteriophages were added to 10L reclaimed water samples before hollow fiber ultrafiltration and to 200mL raw sewage samples before low speed centrifugation in order to follow as many processing steps as possible. A summary of processing control recovery efficiency is provided in Figure 2-1. Nucleic acids were extracted simultaneously by the method described in Rodríguez et al., 2012 from 100µL volumes of concentrated sample. Briefly, 100µL of Guanidinium thiocyanate (GuSCN) lysis buffer and concentrated sampled were vortex mixed together and incubated at room temperature for 10 minutes. A 200mL of 100% ethanol was then vortex mixed together with the sample and lysis buffer. The combined solution was then centrifuged for 1 minute at 14,000 x *g* at ? oC in a high bind RNA mini column (OMEGA BIOTEK, Norcross, GA). The waste effluent was discarded, and 500µL of 75% ethanol was added and the column was centrifuged at 14,000 x *g* for 1 minute at ? oC two more times. The mini column was then centrifuged an additional time at 14,000 x *g* and then placed in a new 1.5mL collection tube. A 50µL volume of RNAse free water (Sigma-Aldrich) was added to the column membrane and after 1 minute, the column was again spun at 14,000 x *g*. The purified nucleic acid solution were then collected and used for qPCR or RT-qPCR analysis.

3.2.7.2 Standard curve generation

Adenovirus standard curves were prepared using a stock of known concentration of Adenovirus 2 by the method described in Wu et al., 2011. Norovirus standard curves were generated using Quantitative Norovirus GII from the American Type Culture Collection (ATCC®, Manassas, VA, Product # VR-3235SD™). Samples were serially diluted in phosphate buffered saline (PBS, US EPA, 2012), and then dilutions were subjected to qPCR or RT-qPCR to generate standard curves corresponding to 10^0 to 10^8 copies

of adenovirus, and 10^0 to 10^5 copies of Norovirus. Positive control viruses were used for both adenovirus and Norovirus experiments. Adenovirus 2 (ATCC VR-846) was used as a positive control for qPCR experiments, and positive control Norovirus GII was provided by the lab of Dr. Ralph Baric (UNC Chapel Hill).

3.2.7.3 Prevention of PCR carryover contamination

Standard precautions were taken to prevent PCR contamination, including the use of dedicated laboratory spaces, pipettes, and barrier-filtered pipette tips. Two negative controls, containing no nucleic acid, were included in each run, and no indications of (RT-)PCR contamination were detected for any of the virus nucleic acids that were analyzed. Samples of positive control DNA and RNA were prepared in a separate room and never taken into the PCR set-up area for field samples.

Both the norovirus (RT-qPCR) and the adenovirus (qPCR) assays were performed using the QuantiTect Probe PCR Kit (Qiagen, CA) using a Bio-Rad CFX96 Touch Real Time PCR System in a 96 well format. Primers and probes used in Real-Time PCR are described in Table 2-2. Norovirus protocols were performed as described in Loisy et al., 2005. Briefly, the norovirus reaction mixture contained 2µL of extracted RNA, 200nM of GII primers and probe, 1.25U RNAse Inhibitor (Applied Biosystems, France), and 0.25µL Qiagen RT Enzyme (Qiagen, CA). Norovirus PCR conditions were as follows: reverse transcription for 30 minutes at 50°C, denaturation for 5 minutes at 95°C, and then 45 cycles of amplification with denaturation at 95°C for 15s and annealing and extension at 60°C for 1 minute. Protocols for SP, the Norovirus processing control were as described in Friedman et al., 2011. Briefly, the SP reaction mixture contained 2µL of extracted DNA, 10uM of the forward and reverse primers, 5uM of the probe, with a final volume of 25uL. The PCR conditions for SP required reverse transcription for 30 minutes at 50°C, denaturation for 15 minutes at 95°C, and then 45 cycles of amplification with denaturation at 95°C for 1s, annealing at 56°C for 30s and extension at 76°C for 1 30s. Adenovirus protocols were as described in Jothikumar et al., 2005. Briefly, the adenovirus reaction mixture contained 2µL of extracted DNA, 50uM of the forward and reverse primers, 5uM of the JJVXP probe, with a final volume of 25uL. The PCR conditions for adenovirus involved denaturation for 15 minutes at 95°C, followed by 45 cycles with denaturation for 10s at 95°C, annealing for 30s at 55°C, and elongation for 15s at 72°C. Protocols for PRD1, the adenovirus processing control, were as described in Dika et al., 2015. Briefly, the PRD1 reaction mixture contained 5µL of extracted DNA, 900nM of the forward and reverse primers, 225mM of the probe, with a final volume of 25uL. The PCR conditions for PRD1 involved denaturation for 3 minutes at 95°C, followed by 45 cycles with denaturation for 30s at 95°C, annealing for 30s at 55°C, and elongation for 1 minute at 72°C.

3.2.8 Adenovirus Integrated Cell Culture Polymerase Chain Reaction (ICC-PCR)

3.2.8.1 Cell Culture Infectivity Assay and mRNA Extraction

Cell culture infectivity assays were performed as described by Rodríguez et al., 2013 and Polston et al., 2014. Briefly, HEK 293 cells were grown in $25cm^2$ tissue culture flask with Eagle's Minimal Essential Medium (EMEM) (Gibco/Invitrogen, Carlsbad, California) and supplemented with 10% Fetal Bovine Serum (FBS) (Gibco/Invitrogen). Cells were incubated for 4-5 days at

37°C until at least 80% confluence was attained. After this level of confluence was reached, a 1.5mL inoculum was produced by diluting 350µL of concentrated adenovirus sample using 1050µL complete MEM medium without serum and containing 10µg kanamycin, 50µg gentamicin, and 20µg nystatin per mL. After 1 hour of incubation the inoculum was removed, 6mL of complete MEM medium with 2% bovine serum was added to each flask and cell cultures were incubated for 4-5 days at 37°C. After incubation, the cell culture medium and cells of each separate 25cm^2 tissue culture flask was disrupted and removed using 1mL pH 7.5 phosphate buffered saline (PBS) by vigorous pipetting up and down. Cells were transferred to a 1.5mL microfuge tube and centrifuged at full speed (16,000 x *g*) for two minutes at 4°C. The Qiagen RNeasy Mini Kit(Qiagen, Valencia, CA) was used for extracting nucleic acids from the recovered cells by the method described in Rodríguez et al., 2014. Briefly, cells were resuspended using RLT lysis buffer (provided in the Qiagen kit) and homogenized using QIAshredder minicolumns (Qiagen, Valencia, CA). Nucleic acid extraction was then performed using the RNeasy Kit and the final purified nucleic acids were collected in 50uL of nucleic acid free water.

3.2.8.2 Real-Time ICC-qRT-PCR

The adenovirus (qPCR) assays were performed using the QuantiTect Probe PCR Kit (Qiagen, CA) using a Bio-Rad CFX96 Touch Real Time PCR System in a 96 well format. Primers and probes used for the detection of mRNA are the same as those used for the detection of DNA described in Table 2-2. Protocols were performed as described in Rodríguez et al., 2013. Briefly, the adenovirus reaction mixture contained 2µL of extracted mRNA, 0.5µM of forward and reverse primers and probe, 1.25U RNAse Inhibitor (Applied Biosystems, France), and 0.25µL Qiagen RT Enzyme (Qiagen, CA). Adenovirus PCR conditions were as follows: reverse transcription for 30 minutes at 50°C, denaturation for 15 minutes at 94°C, and then 45 cycles of amplification with denaturation at 94°C for 15s and annealing for 30seconds at 58°C and extension at 72°C for 15 seconds.

3.2.8.3 Quality Assurance and Control

As described in section 2.2.7.4, standard precautions were taken to prevent PCR contamination. Positive control reference viruses (Adenovirus 2) were used for infectivity assays and a positive DNA control (the adenovirus viral hexon gene), as described by Rodríguez et al., 2013, were run parallel to each set of qPCR reactions for field samples. The titer of the adenovirus 2 viral stock as infectious units (MPNIU) was determined using end point dilution. Briefly, adenovirus stock was diluted serially ten-fold in PBS, with three replicates per dilution, in 6-well plates containing HEK 293 monolayers, with incubation in complete MEM medium at 37°C in a 5% CO_2 incubator. The viral hexon gene was detected after RNA extraction after up to 5 days post infection by the RT-PCR methods described above. Two negative phosphate buffered saline (PBS) control reactions were included in each ICC-qPCR run, and no indications of contamination were detected. The cycle threshold (C_t) is the cycle at which a significant increase in fluorescence occurs. A sample with a C_t value below 43, with no evidence of amplification in the negative controls (threshold not reached after 45 cycles) was considered positive.

Table 1: Summary of percent recovery data for processing control organisms

	Average Recovery Efficiencies (%)					
	Raw Sewage (n=22)			Reclaimed Water (n=22)		
Microbe	Average Percent Recovery	Standard Deviation	Range (%)	Average Percent Recovery	Standard Deviation	Range (%)
Adenovirus A-F (PRD1)	100.54%	7.63%	(86, 118)	109.36%	13.65%	(86, 139)
Norovirus GII (SP)	99.98%	5.14%	(92, 121)	104.64%	11.92%	(90, 127)
Cryptosporidium (ColorSeed)	84.40%	10.22%	(70, 101)	82.50%	6.47%	(71, 89)
Giardia (ColorSeed)	73.06%	18.07%	(40, 103)	63.50%	12.20%	(44, 85)

Table 2: Primers and Probes Used in Real-Time PCR

Assay	Oligonucleotide type	Oligonucleotide name	Sequence (5'- 3')	Position	Orientation
Norovirus GII					
	Forward Primer	QNIF2da[a]	ATGTTCAGRTGGATGAGRTTCTCWGA	5012–5037	+
	Reverse Primer	COG2Rb[b]	TCGACGCCATCTTCATTCACA	5080–5100	-
	Probe	QNIFSa[a]	FAM-AGCACGTGGGAGGGCGATCG-BHQ	5042–5061	+
SP					
	Forward Primer	IV Forward[d]	CGGYCAYCCGTCGTGGAAG	2941–2959	+
	Reverse Primer	IV Reverse[d]	AGT GAC TGC TTT ATT YGA AGT GCG	3082–3059	-
	Probe	IV Probe[d]	FAM-CCT GTC CGC AGG ATG TWA CCA AAC-BHQ	2964–2987	+
Adenovirus A-F					
	Forward Primer	JTVXFc[c]	GGACGCCTCGGAGTACCTGAG	18895–18915	+
	Reverse Primer	JTVXRc[c]	ACIGTGGGGTTTCTGAACTTGTT	18990–18968	-
	Probe	JTVXPc[c]	FAM-CTGGTGCAGTTCGCCCGTGCCA-BHQ	18923–18944	+
PRD1					
	Forward Primer	PRD1F[e]	AAACTTGACCCGAAAACGTG	9546-9565	+
	Reverse Primer	PRD1R[e]	CGGTACGGCTGGTGAAGTAT	9728-9747	-

Probe	PRD1P[e]	FAM-ATGGTAACGTGGGCTTTGTC-BHQ	9658-9677	+

[a] Norovirus GII forward primer and probe as described by Loisy et al., 2005

[b] Norovirus GII reverse primer as described by Kageyama et al., 2003

[c] Adenovirus groups A-F primers and probe as described by Jothikumar et al., 2005

[d] SP primers and probe described by Friedman et al., 2011

[e] PRD1 primers and probe described by Dika et al., 2015

3.2.9 Protozoan Parasite Detection and Quantification

Cryptosporidium spp. and *Giardia* spp. (oo)cysts were recovered and quantified in raw sewage and reclaimed water by modifications of EPA Method 1623. Primary concentration for reclaimed water samples was done using modifications of the hollow fiber ultrafiltration and elution protocol described in Hill et al., 2007 and Polaczyk et al., 2008 with Fresenius Optiflux F250NR hollow fiber ultrafilters (dialyzers). Briefly, 10L of reclaimed water was spiked with a commercially available positive internal control that is uniquely fluorescently labeled *Giardia* and *Cryptosporidium* (oo)cysts (BTF Precise Microbiology, Inc., Pittsburgh, PA), and filtered through the Fresenius Optiflux F250NR hollowfiber ultrafilter. Water samples were concentrated to produce a retentate liquid of approximately 100-200mL volumes, and ultrafilters were backflushed with a solution containing 0.5% Tween 80, 0.01% Sodium polyphosphate (NAPP) (Sigma-Aldrich, cat# 305553-25G), and 0.001%Antifoam Y. The backflush liquid was added to the retentate liquid to produce a total concentrate volume of approximately 200-250mLs. Next, reclaimed water samples were centrifuged at 1,500 x *g* for 30 minutes at 4°C to sediment out protozoan parasites, the sedimented pellet from this centrifugation was eluted using 0.5M pH7.5 threonine for 1 hour at room temperature with a mixing speed of 60RPM to elute and recover viruses. The eluted mixture was then re-centrifuged at 1,500 x *g*, and the supernatant was combined with the supernatant collected from the previous centrifuge step for further virus concentration. The sedimented pellet was processed for protozoan parasite recovery and purification by immunomagnetic separation (IMS) for subsequent fluorescent antibody staining and analysis by immunofluorescent microscopy . Similarly, for raw sewage samples, a 200mL sample was centrifuged at 1,500 x *g* for 30 minutes and 4°C and the sedimented pellet from this centrifugation was eluted using 0.5M pH7.5 threonine for 1 hour at room temperature with a mixing speed of 60RPM to recover viruses. After a second centrifugation at 1,500 x *g*, the sedimented pellet was then processed by IMS for protozoan parasite concentration and purification. Immunomagnetic separation was performed using the Dynabeads *Cryptosporidium/Giadia* combo kit (cat#:73012, Invitrogen, Carlsbad, CA) as per manufacturer's instructions. Briefly, a 0.5mL pellet volume was processed by combining 1mL of the provided 10X SL-buffer A and 10X SL-buffer B in a flat sided tube and then adding 100µL each of the Dynabeads *Cryptosporidium* and *Giardia* and incubating for 1 hour on a rotating mixer at 18RPM and 4°C. The tube was then sequentially placed in the Dynabeaads Magnetic Particle Concentrator (MPC-1) and concentrated to a 1.5mL sample volume. The sample was then placed in the MPC-M, the supernatant was eluted, and the pellet was rinsed with 50uL of 0.1N HCl after removing the MPC-M magnet. After a 10 minute incubation, the magnet was replaced, and the sample was transferred to a microscope slide containing 5uL of 1.0N NaOH. Microbe slides were stained and processed by fluorescent antibody methods using the Meriflour kit (Waterborne, Inc., New Orleans, LA), and slides were examined visually using a Leitz Orthoplan 2 fluorescent microscope at 200X magnification using an oil immersion lens. For raw

sewage samples primary concentration of protozoan parasite (oo)cysts was done by a simple centrifugation method, described previously in this report and in figure 1. For both reclaimed water and raw sewag samples primary concentration was followed by further concentration and purification by immunomagnetic separation (IMS) and then direct immunofluorescent microscopic (oo)cyst enumeration using the Merifluor kit (Waterborne, Inc., New Orleans, LA) along with a commercially available positive internal control that is uniquely fluorescently labeled *Giardia* and *Cryptosporidium* (oo)cysts (BTF Precise Microbiology, Inc., Pittsburgh, PA) (US EPA, 2012). The lower limit of detection for protozoan parasite recovery is 0.5 (oo)cysts per 100mL for raw sewage and 0.01 (oo)cysts per 100mL for reclaimed water. A summary of processing control recovery efficiency is provided in Table 4.

3.2.10 Statistical analysis for Reclaimed and Surface Waters

Microbial recovery efficiencies were determined by calculating the number of microbes recovered after an experiment (concentration multiplied by sample volume) and dividing that value by the number of each microbe present in the sample (concentration multiplied by sample volume) before the experiment and then multiplying that value by 100. Log_{10} reduction values were calculated by subtracting the log_{10} concentration of microorganism in NCT2RW from the log_{10} concentration in influent raw sewage.

Statistical analyses were conducted using GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA) or SPSS Version 24 (IBM Corporation, Armonk, NY). Data distributions were evaluated using the Shapiro-Wilk test, or relevant nonparametric tests for data that was not normally distributed, for log_{10} transformed data.

A binary logistic model was used to test the hypothesis that microbial indicator organism concentrations were predictive of the presence or absence of pathogens in surface water, as described in Harwood et al., 2005. Briefly, this method involved the use of continuous independent variables with non detect values being reported as a value of 0. The data for indicator microorganisms (total coliforms, *E. coli*, *C. perfringens*, and coliphages) were then be converted into a string of binary variables to represent the presence or absence of each indicator. The ability of the indicator data string to predict the presence of each pathogen (*Cryptosporidium, Giardia, Salmonella* spp., adenoviruses and noroviruses) was assessed separately and also for all viruses (Adenovirus groups A-F, Norovirus GII, and combined as an enteric viruses category). Results were then expressed as the percentage of samples correctly classified into the “pathogen present” and “pathogen absent” categories.

3.2.11 Survival Experiments

3.2.11.1 Survival Experiment Sample Collection

Grab samples of tertiary treated, dual disinfected (North Carolina “Type 2” like) reclaimed water, NCT2RW) samples were collected from the Orange County Wastewater Treatment plant in Chapel Hill, NC. Surface water was also collected as grab samples from the Cary/Apex Drinking Water Treatment Plant in Cary, NC. Samples were transported to the laboratory on ice

and stored for less than 1 week at 4°C until combined at the approved 80% surface water to 20% reclaimed water ratio.

3.2.11.2 Microorganisms for Survival Experiments

Five indicator organisms relevant to the NC legislation for reclaimed water were propagated from raw sewage. These organisms included *E. coli*, *Enterococcus* spp., F+/Male-Specific coliphages, somatic coliphages, and *Clostridium perfringens* spores. Each microorganism was propagated from a sample of raw sewage using selective media or selective hosts (for viruses). Briefly, media included, Bio-Rad Rapid *E. coli* 2 agar, mEnterococcus agar, *E. coli* Famp (for F+/male specific coliphages), *E. coli* CN13 (for somatic coliphages), and CP ChromoSelect Agar (for *C. perfringens*), each organism was grown in broth culture and stored at -80°C for future use.

3.2.11.3 Survival Experiment Protocol

Each of the propagated organisms was spiked into 100mL volumes of the 80/20 mix of surface water and reclaimed water at concentrations high enough to track a minimum 4-$\log_{10}$ reduction in each microorganism and incubated at both 4°C and 20°C, and at various mixing speeds (0,60, and 120 RPM), with samples analyzed at 0, 3, and 5 days. Organisms were quantified over the 5-day period using the spot-plate titer assay as described in Beck et al., 2009 using the selective agars or *E. coli* hosts (for coliphage viruses) as described above. Spot titer plates were incubated at 37°C for 24 ± 4h for *E. coli*, F+ and somatic coliphages and 48 hours for *Enterococcus* spp. CP Chromoselect spot plates for *C. perfringens* were incubated in anaerobic jars at 45°C for 24 ± 4h. Each experiment was conducted in the dark.

3.2.11.4 Sunlight Study

To evaluate the impact of sunlight on the survival of indicator organisms in the 80/20 mixture of surface and reclaimed water, the propagated organisms were also evaluated when exposed to natural sunlight. For these sunlight experiments, organisms were spiked into 100mL volumes of the 80/20 mix, placed in clear polyethylene bags and set in the sun for approximately 4 hours. These experiments were temperature controlled and samples were not allowed to reach temperatures greater than 20°C. Samples were collected at 0, 15, 30, 45, 90, 120, 180, and 240 minutes. Spot titer plate assays were done and incubated as described in section 2.2.11.3.

3.2.11.5 Natural Bacteria Survival

As propagated bacteria may not model the behavior of natural bacteria in the water environment, an experiment was also conducted with enteric bacteria naturally occurring in the surface waters. As not all of the indicator organisms were present at high enough concentrations to track their reductions, this experiment was conducted with total coliform bacteria, *E. coli* and *Enterococcus* spp. As with the other survival experiments, samples were evaluated at 20°C, and at various mixing speeds (0, 60, and 120 RPM), with samples analyed at 0, 3, and 5 days. Each experiment was conducted in the dark. Organisms were quantified over the 5-day period using a standard membrane filtration technique (Standard Methods for the Examination of Water and Wastewater,

SMEWW) with a selective agar for total coliforms and *E. coli*, Bio-Rad Rapid *E. coli* 2 agar, and the selective mEnterococcus agar for *Enterococcus* bacteria. Membrane filtration plates were incubated at 37°C for 24 ± 4h for total coliforms and *E. coli* and 48 hours for *Enterococcus* spp.

3.2.11.5 Statistical Analysis for Survival Experiments

The concentrations and changes in concentrations of regulated microbial indicators in samples of the 20% mixtures of NCT2RW plus 80% surface source waters at time = 0, 3 and after 5 days of storage at specified conditions of temperature and mixing were compared using Microsoft Excel (Microsoft Corporation, Redmond, WA) and GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA). Comparisons among the different microbial indicators were based on concentrations and $\log_{10}$ reductions. Log_{10} reduction values were calculated by subtracting the $\log_{10}$ concentration of microorganism in NCT2RW from the $\log_{10}$ concentration in influent raw sewage. For the sunlight study, the UV radiation was measured as Watts per meter squared per minute and summed to give a cumulative UV dose curve over time. The Chick-Watson (Log linear) kinetics model was then used to calculate the time to achieve a 4-$\log_{10}$ reduction with the observed cumulative UV dose.

3.2.12 Quantitative Microbial Risk Assessment

Data for key QMRA variables, including the exposure assessment parameter for numbers of pathogens constituting an exposure based on pathogen concentrations and exposure volumes, as well as recovery efficiencies for pathogens, pathogen infectivity, exposure routes and dose-response for infection and/or illness were entered into a Microsoft Excel (Microsoft Cooperation, Redmond, WA) spreadsheet. Calculations for risk and Monte Carlo simulations were performed using Analytica 4.6 (Lumina Decision Systems, Los Gatos, CA), with random variables sampled 10,000 times for each analysis. Details on the components of the risk assessment model, assumptions, recovery efficiencies, etc. are presented in detail in the sections below. Figure 5-3 presents a step-wise example calculation for the potential risks from potable reuse consumption.

3.2.12.1 Exposure Assessment

The focus of the exposure assessment is the estimation of the likelihood of an individual or a population to be exposed to the identified hazard as well as the estimation of the dose that is likely to be ingested. Based on the pathogen concentrations, recovery efficiencies, viability, and exposure scenarios, the average dose (N) of the pathogens constituting an exposure of interest was calculated using the following equation:

$$N = C \; x \; R^{-1} x \; I \; x \; V$$

In this equation, *N* is the dose or number of organisms (viruses, bacteria, or protozoan parasite (oo)cysts) ingested by a person through reclaimed water, surface water, or a combination of the two, C is the concentration of pathogens (organisms/L), R is the recovery efficiency of the detection method, I is the fraction of detected pathogens capable of causing infection, and V is the exposure scenario volume (L).

3.2.12.2 Concentrations of pathogens (C)

As risk estimates are best made based on unbiased estimates of the true mean pathogen concentration, the goal of this study was to model the behavior of pathogen concentrations in various water types. The concentration data from 4 NC type 2- like reclaimed water producing treatment plants were aggregated for analysis purposes. In order to model pathogen concentrations, previous studies (Ginneken and Oron, 2000; Hamilton et al., 2006) have fitted a normal distribution to log data. This method was used to model concentrations, C in this study for reclaimed, surface and blended waters for all pathogens, as concentration data were found to be lognormally distributed. The lognormal distribution for concentration was then multiplied by a correction factor in exposure analysis if pathogen die-off was considered (for exposures 4 and 5), and this is described in section 5.5. Distributions of microbial concentrations are summarized in Table 3. The potable reuse distribution was created by combining 0.8 parts of the surface water and 0.2 parts of the reclaimed water at their pathogen concentrations to create a potable reuse water that will be further 'treated' by 5 day storage and then conventional drinking water treatment. The concentrations of Norovirus GII were excluded from this analysis because no norovirus GII gene copies were detected in reclaimed or surface water samples.

Table 3: Lognormal distributions of microbial concentrations, C

Organism	Parameter	Reclaimed Water	Surface Water	Potable Reuse*
Adenovirus A-F (log_{10} GEC/L)	Mean	3.72	2.51	2.75
	Standard Deviation (+/-)	1.56	1.9	1.62
	Minimum, Maximum	1, 5.69	1, 5.56	1, 5.33
Salmonella spp. (log_{10} MPN/L)	Mean	0.13	0.76	0.63
	Standard Deviation	0.45	1.1	0.89
	Minimum, Maximum	0, 1.99	0, 4.9	0, 3.92
Cryptosporidium spp. (log_{10} oocysts/L)	Mean	0.22	0.73	0.63
	Standard Deviation	0.36	0.54	0.42
	Minimum, Maximum	-0.52, 0.79	-0.23, 1.85	-0.16, 1.64
Giardia spp. (log_{10} cysts/L)	Mean	-0.22	0.13	0.06
	Standard Deviation	0.38	0.5	0.4

Minimum, Maximum	-1, 0.38	-0.70, 1.18	-0.70, 1.01

*80/20Blend +5 day Storage + Conventional Drinking Water Treatment

3.2.12.3 Recovery efficiencies for pathogens (R)

The recovery efficiencies for Adenovirus groups A-F and *Cryptosporidium* spp. and *Giardia* spp in reclaimed and surface waters are summarized in Table 4. The recovery efficiency for Adenovirus group A-F was determined using the "adeno-like" salmonella phage PRD1 by PCR recovery methods, while the recovery efficiency for Cryptosporidium oocyts and Giardia cysts was determined using ColorSeed, a fluorescently labeled internal positive control by US EPA Method 1623 (BTF Precise Microbiology, Inc., Pittsburgh, PA) (US EPA, 2012). The recovery efficiency for the detection of *Salmonella* spp. was not determined empirically and is assumed to be 100% by the culture assay procedure used for the purposes of the QMRA modeling.

Table 4: Recovery efficiencies of processing control organisms in surface and reclaimed waters

	Reclaimed Water (n=26)		Surface Water (n=22)	
Microbe	Average Recovery (%)	Standard Deviation (%)	Average Recovery (%)	Standard Deviation (%)
Adenovirus A-F (PRD1)	109	13.7	109	9.11
Cryptosporidium spp. (ColorSeed)	82.5	6.47	95.8	8.11
Giardia spp. (ColorSeed)	63.5	12.2	78.3	14.2

2.2.12.4 Viability/Infectivity (I)

There are limited data available on the infectious fraction of pathogens in either reclaimed or surface waters. Data collected by ICC-qPCR on infectious adenovirus in reclaimed water samples, described in section 2.3.2 were used determine the fraction of infectious adenovirus of those that were detected by direct qPCR; this fraction was determined to be 38.5%. Similarly, a study conducted by Chapron et al., in fresh surface waters determined the fraction of viable infectious adenoviruses in this water type to be 37.9% (Chapron et al, 2000). For *Salmonella* spp., the estimated fraction of bacteria causing infection in humans from surface water exposures is 65% (Kapperud et al., 1998) Data collected on the fraction of viable (oo)cysts in surface water after chlorination was used to determine the infectious fraction of *Cryptosporidium* spp. and *Giardia* spp., these fractions were 25% and 13% respectively (M.W. LeChevallier, 1991; Gennaccaro et al., 2003).

3.2.12.5 Exposure Routes (V)

To assess the potential risks of exposure to pathogens associated with the use of reclaimed water for various purposes, five exposure scenarios were considered:

1. Scenario 1 (Accidental Exposure): A person is exposed to reclaimed water through a one time accidental ingestion of 10mL by consumption as drinking water. No pathogen die off is considered.
2. Scenario 2 (Recreational Exposure). A person swimming in recreational water is assumed to ingest 100mL of reclaimed water in a day (Haas, 1983). The person is assumed to swim for 2 hours per day on the weekends over a 5-month period, or 40 days per year. No pathogen die off is considered.
3. Scenario 3 (Reclaimed Water Exposure) It is assumed that a person ingests the 2L of water per day, as proposed by the US EPA Exposures Handbook (US EPA, 2011), of tertiary treated dual disinfected reclaimed water. As reclaimed water is currently piped to households in some North Carolina communities for non-potable reuse, there is a risk of ingestion exposure if pipes are mislabeled or water is inadvertently consumed. No pathogen die off is considered.
4. Scenario 4 (Potable Reuse Exposure). It is assumed that a person ingests 2L of blended reclaimed water after it has been combined at a 20% to 80% ratio with run-of-river intake raw source water, stored for 5 days and then treated by conventional drinking water treatment. Pathogen die-off is evaluated in the 5 day storage condition based on data presented in section Chapter 4. Pathogens are assumed to be reduced by conventional drinking water treatment by 1) the US EPA required $\log_{10}$ reductions and 2) the WHO risk based method of reducing pathogens in water and 3) a worst-case $\log_{10}$ reduction scenario based on real world data reviewed by Medema and Hijnen, 2007.

3.2.12.6 Dose-Response Modeling

For Adenovirus A-F, *Cryptosporidium* and *Giardia*, the exponential dose response model was used to determine the probability of infection from ingestion of various numbers of pathogens. The exponential model is below:

$$P(inf) = 1 - e^{-k*N}$$

In this equation, *P(inf)* is the probability of infection resulting form daily ingestion of the number of pathogens (N). *K* is the average dose, or number of microorganisms that must be ingested to initiate an infection. The best fit *K* values for Adenovirus, *C. parvum*, and *G. lamblia* are 6.07E-01 (Couch et al., 1966), 0.0042 (DuPont et al., 1995), and 0.0198 (Rose et al, 1991).

For *Salmonella* spp. the Beta-Poisson model was used, and this model is below:

$$P(inf) = 1 - [1 + N\frac{(2^{\frac{1}{\alpha}} - 1)}{N_{50}}]^{-\alpha}$$

Again, *P(inf)* is the probability of infection resulting from daily ingestion of the dose of pathogens (N), α is pathogen infectivity constant, and N_{50} is the LD_{50}, the dose that is lethal to 50% of individuals, divided by the ID_{50}, which is the median infective dose. The optimized

parameters for non-typhoid *Salmonella* are 2.1E-01 and 4.98E+01 for α and N_{50} respectively (Meynell and Meynell, 1958).
Estimates of daily risk may be extrapolated to the risk of infection over an extended period of time using the equation below (Haas, 1983). This equation was used to calculate yearly risks and surface water risks at the exposure scenario of 40 days of recreational water exposure.

$$P_t = 1 - (1 - P_d)^t$$

Here, P_t is the probability of infection after t days and P_d is the probability of infection after one day of exposure.

3.2.12.7 Potable Reuse Modeling

In order to evaluate the specific conditions proposed by the state of North Carolina, this QMRA model was designed to incorporate both the 5 day storage period and conventional drinking water treatment steps. The 5 day storage condition was modeled using data described in Chapter 4, where sewage propagated indicator microorganisms were subjected to 5 day storage conditions under various mixing speeds. The $\log_{10}$ reductions achieved on average by bacteria over a 5 day storage time were approximately -0.54 at 4°C and +2.0 at 20°C. A positive $\log_{10}$ reduction indicates an increase in concentration over the 5 day storage period. This observed increase in bacteria concentration is likely to be an artifact of using bacteria that were first propagated in culture in the laboratory and are not representative in physical and physiological state of the enteric bacteria present in fecally contaminated natural waters. Naturally occurring enteric bacteria in water modeled over this same period had an average $\log_{10}$ reduction of -0.61. Average viral indicator reductions were approximately -0.32 at 4°C and -1.22 at 20°C. For protozoan parasite surrogates, the average $\log_{10}$ reduction was -1.89 at 4°C and -1.64 at 20°C. Based on the statistical analysis presented in Chapter 4, there werea statistically significant differences in the extent of microbial reduction between the two temperatures evaluated for all organisms tested except *C. perfringens*, but not for the three mixing speeds. Therefore, storage conditions were modeled for both temperatures. The survival of organisms in reuse water was modeled as a normal distributions of the average $\log_{10}(N_t/N_o)$ values (presented in Chapter 4) at each temperature.

In addition to modeling the state mandated 5 day storage period at two temperatures, the effect of conventional drinking water treatment was also modeled to further evaluate the full scale production of NCT2RW and it use as potable drinking water. For this analysis, three drinking water treatment performance efficacy scenarios were evaluated: 1) the US EPA regulated $\log_{10}$ reductions for conventional drinking water treatment and disinfection (4 $\log_{10}$ for virus, 2 $\log_{10}$ *Cryptosporidium* and 3 $\log_{10}$ for *Giardia*), 2) the World Health Organization's (WHO) risk based reduction of pathogens based on Disability Adjusted Life Years (DALYs) and 3) 3) a worst-case $\log_{10}$ reduction scenario based on real world data reviewed by Medema and Hijnen, 2007. A more detailed description of these regulations is provided in Chapter 1; distributions were modeled as triangular distributions using the 95% confidence intervals of the required $\log_{10}$ reductions as the lower and upper bounds.

3.3 Results

3.3.1 Reclaimed Water Analysis

3.3.1.1 Bacterial Indicators

Table 5: *E. coli* and *Enterococcus* spp. Concentrations and Log_{10} Reductions from Raw Sewage and Reclaimed Water Samples

Date	Location	Sample	*E. coli* MPN Concentration (Lower 95% Confidence Interval, Upper 95% Confidence Limit)	*Enterococcus* MPN Concentration (Lower 95% Confidence Interval, Upper 95% Confidence Limit)
1.19.16	Cary	Raw Sewage	1.91E+07 (1.32E+07, 2.73E+07)	2.88E+05 (1.85E+05, 4.30E+05)
		Reclaimed Water	<1 (0.0, 3.7)	<1 (0.0, 3.7)
		Log_{10} Reduction	>7.28	>5.46
2.2.16	Raleigh	Raw Sewage	2.43E+06 (1.56E+06, 3.78E+06)	2.86E+05 (1.86E+05, 4.23E+05)
		Reclaimed Water	<1 (0.0, 3.7)	<1 (0.0, 3.7)
		Log_{10} Reduction	>6.40	>5.46
2.16.16	Holly Springs	Raw Sewage	1.61E+06 (9.40E+05, 2.67E+06)	3.08E+05 (2.00E+05, 4.52E+05)
		Reclaimed Water	<1 (0.0, 3.7)	<1 (0.0, 3.7)
		Log_{10} Reduction	>6.21	>5.49
2.23.16	OWASA	Raw Sewage	6.30E+05 (2.80E+05, 1.26E+06)	1.49E+05 (8.30E+04, 2.47E+05)
		Reclaimed Water	<1 (0.0, 3.7)	<1 (0.0, 3.7)
		Log_{10} Reduction	>5.80	>5.17
3.7.16	Durham	Raw Sewage	1.15E+06 (5.80E+05, 1.96E+06)	4.75E+05 (3.26E+05, 6.64E+05)
		Reclaimed Water	<1 (0.0, 3.7)	<1 (0.0, 3.7)
		Log_{10} Reduction	>6.06	>5.5
4.26.26	Holly Springs	Raw Sewage	2.22E+06 (1.35E+06, 3.44E+06)	4.07E+05 (2.73E+05, 5.78E+05)

		Reclaimed Water	<1 (0.0, 3.7)	<1 (0.0, 3.7)
		Log_{10} Reduction	>6.35	>5.61
5.10.16	Cary	Raw Sewage	1.46E+06 (8.10E+05, 2.40E+06)	5.94E+05 (4.18E+05, 8.24E+05)
		Reclaimed Water	<1 (0.0, 3.7)	<1 (0.0, 3.7)
		Log_{10} Reduction	>6.16	>5.77
5.23.16	Raleigh	Raw Sewage	2.86E+06 (1.86E+06, 4.23E+06)	2.69E+05 (1.73E+05, 4.02E+05)
		Reclaimed Water	<1 (0.0, 3.7)	<1 (0.0, 3.7)
		Log_{10} Reduction	>6.46	>5.43
6.6.16	OWASA	Raw Sewage	2.20E+06 (1.38E+06, 3.39E+06)	6.06E+05 (4.28E+05, 8.24E+05)
		Reclaimed Water	<1 (0.0, 3.7)	<1 (0.0, 3.7)
		Log_{10} Reduction	>6.34	>5.78
6.13.16	Durham	Raw Sewage	2.01E+06 (1.20E+06, 3.14E+06)	2.76E+05 (1.76E+05, 4.09E+05)
		Reclaimed Water	<1 (0.0, 3.7)	<1 (0.0, 3.7)
		Log_{10} Reduction	>6.30	>5.44
6.20.16	Holly Springs	Raw Sewage	4.98E+06 (3.51E+06, 6.92E+06)	1.23E+06 (8.93E+05, 1.66E+06)
		Reclaimed Water	<1 (0.0, 3.7)	<1 (0.0, 3.7)
		Log_{10} Reduction	>6.70	>6.09
6.27.16	OWASA	Raw Sewage	1.60E+06 (9.20E+05, 2.60E+06)	4.85E+05 (3.41E+05, 6.69E+05)
		Reclaimed Water	<1 (0.0, 3.7)	<1 (0.0, 3.7)
		Log_{10} Reduction	>6.20	>5.69
7.13.16	Cary	Raw Sewage	3.22E+06 (2.21E+06, 4.82E+06)	2.21E+06 (5.01E+05, 9.32E+05)
		Reclaimed Water	<1 (0.0, 3.7)	<1 (0.0, 3.7)
		Log_{10} Reduction	>6.52	>5.84

7.25.16	Raleigh	Raw Sewage	2.62E+06 (1.66E+06, 3.97E+06)	1.15E+05 (5.90E+04, 2.02E+05)
		Reclaimed Water	2.05 (0.4, 7.2)	<1 (0.0, 3.7)
		Log_{10} Reduction	>6.11	>5.06

Table 5 presents the log_{10} concentrations of fecal indicator bacteria in raw sewage and reclaimed water samples as well as the log_{10} reductions between these two samples. Based on this table, it is apparent that the concentrations of *Enterococcus* spp. in raw sewage are usually not high enough to track a 6 log_{10} reduction; however, for *E. coli*, the concentrations in raw sewage were sufficiently high most of the time to document a 6 log_{10} reduction or more.

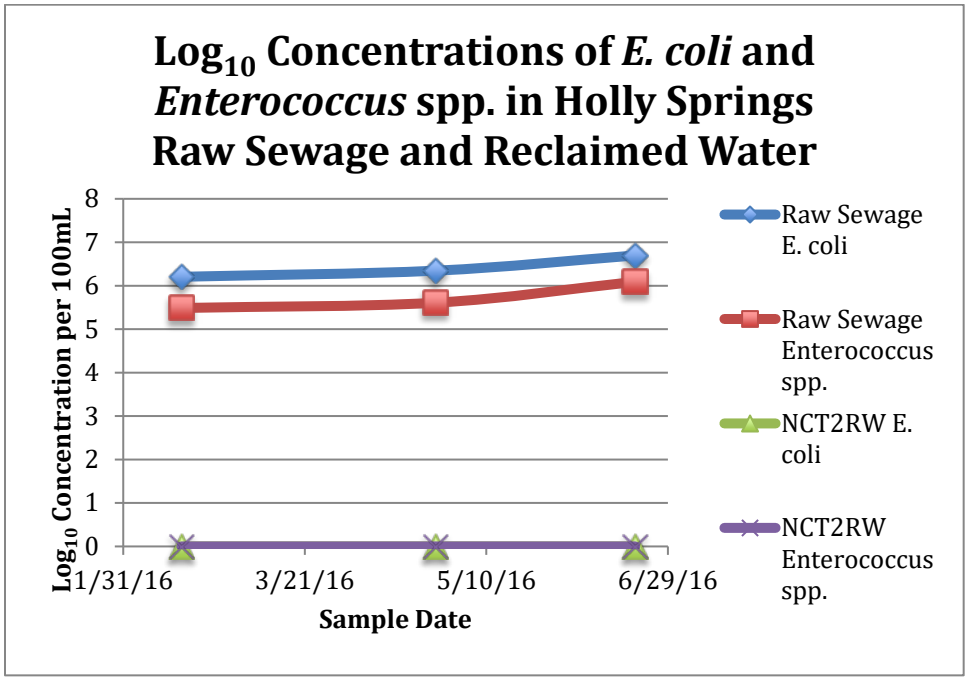

Figure 2: Log_{10} concentrations of *E. coli* in 3 sample pairs of Holly Springs raw sewage and tertiary treated, dual disinfected reclaimed water

Figure 2 displays the log_{10} concentrations of *E. coli* and *Enterococcus* spp. in 3 paired samples of raw sewage and tertiary treated dual disinfected reclaimed water from the Utley Creek Water Reclamation Facility (Holly Springs). From this figure and Table 5, it is apparent that for this type of wastewater treatment and disinfection scheme, the log_{10} reductions for *E. coli* and *Enterococcus* spp. were approximately 6 and 5.5 respectively. For theses samples, no *E. coli* or *Enterococcus* spp. were detected in the reclaimed water, so the lower detection limit (1MPN/100mL) value was used to calculate the log_{10} reduction as a greater than value.

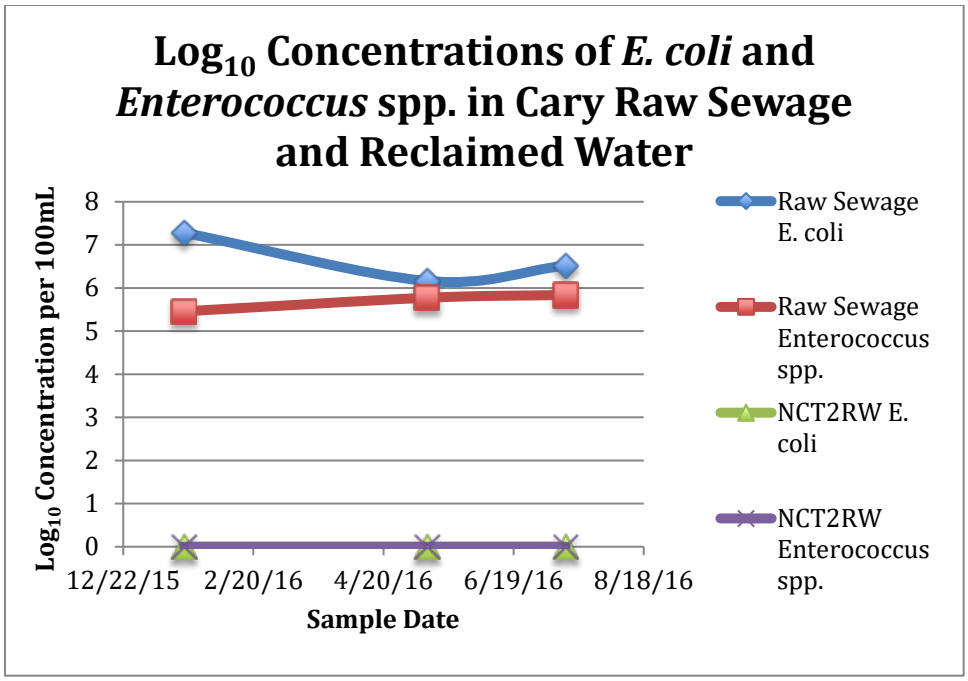

Figure 3: Log_{10} concentrations of *E. coli* in 3 sample pairs of Cary raw sewage and tertiary treated, dual disinfected reclaimed water

Figure 3 displays the log_{10} concentrations of *E. coli* in 3 paired samples of raw sewage and tertiary treated dual disinfected reclaimed water from the Cary Water Reclamation Facility. From this figure and Table 5, it is apparent that this type of wastewater treatment and disinfection scheme, achieves an approximate >6 to >6.5 log_{10} reduction for *E. coli* while for *Enterococcusthe reduction* is >5 to >6 log_{10}. For theses samples, no *E. coli* or *Enterococcus* spp. were detected in the reclaimed water, so the lower detection limit value (1MPN/100mL) was used to calculate the log_{10} reduction as a greater than value.

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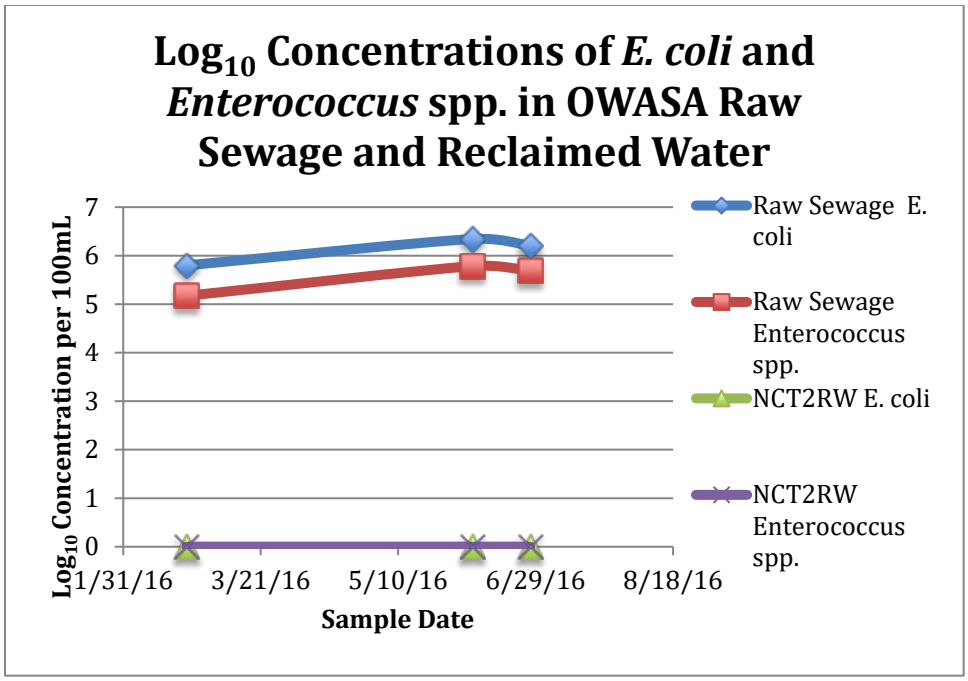

Figure 4: Log_{10} concentrations of *E. coli* in 3 sample pairs of OWASA raw sewage and tertiary treated dual disinfected reclaimed water

In Figure 4 the log_{10} concentrations of *E. coli* in 3 samples of Orange Water and Sewer Authority (OWASA) raw sewage and tertiary treated, dual disinfected reclaimed water are presented. From this figure and the log_{10} reduction data presented in Table 5, the log_{10} reductions achieved by the OWASA wastewater treatment plant for *E. coli* and *Enterococcus* spp. were approximately >6 and >5 to >5.5 respectively. For theses samples, no *E. coli* or *Enterococcus* spp. were detected in the reclaimed water, so the lower detection limit value (1MPN/100mL) was used to calculate the log_{10} reduction as a greater than value.

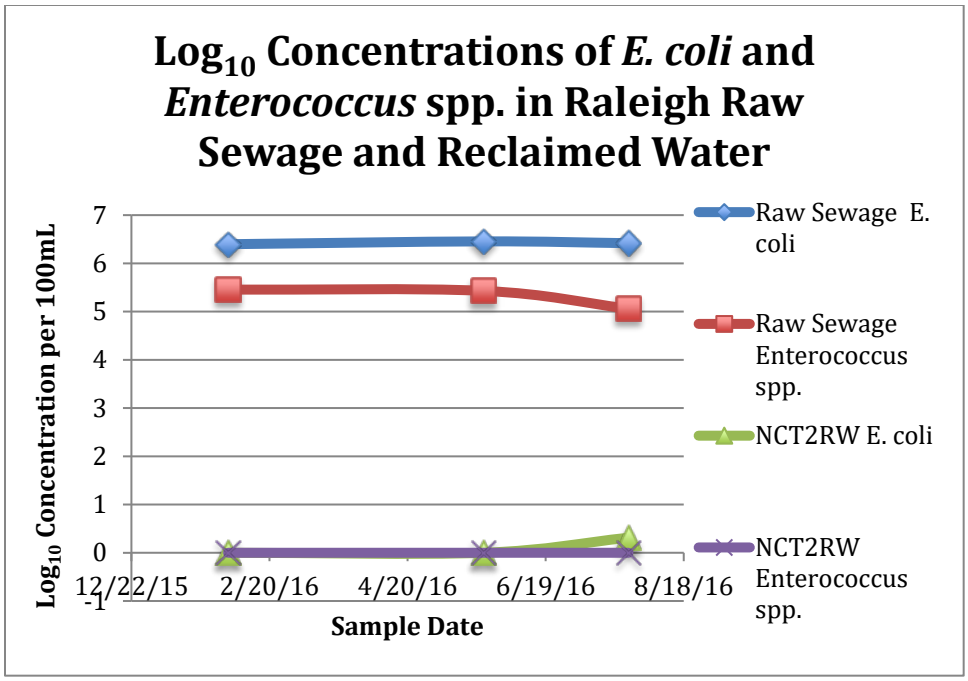

Figure 5: Log_{10} concentrations of *E. coli* in 3 sample pairs of Raleigh raw sewage and tertiary treated, dual disinfected reclaimed water

In Figure 5 the log_{10} concentrations of *E. coli* in 3 samples of raw sewage and tertiary treated, dual disinfected reclaimed water from the Neuse River Resource Recovery Facility (Raleigh) are presented. From this figure and the log_{10} reduction data presented in Table 5, the log_{10} reductions achieved by the Raleigh wastewater treatment plant are approximately >6.5 log_{10} for *E. coli* and >5 to >5.5 to *Enterococcus* spp.. With the exception of the NCT2RW sample analyzed for *E. coli* on 7/25/16, no *E. coli* or *Enterococcus* spp. were detected in the reclaimed water, so the lower detection limit value (1MPN/100mL) was used to calculate the log_{10} reduction as a greater than value.

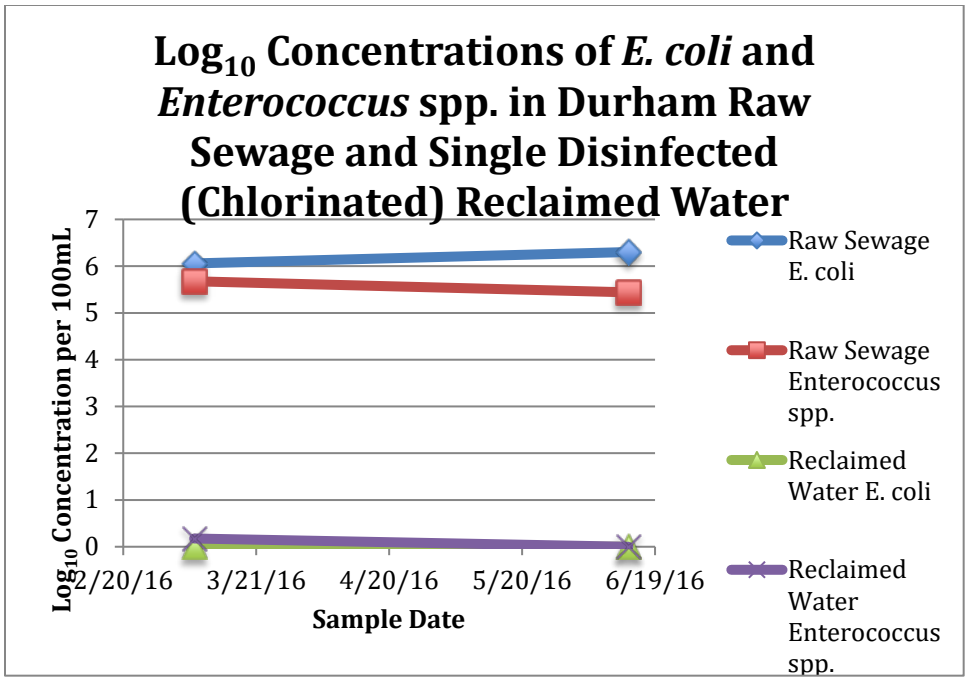

Figure 6: Log_{10} concentrations of *E. coli* in 2 sample pairs of Durham raw sewage and tertiary treated, chlorine disinfected reclaimed water

In Figure 6 the log_{10} concentrations of *E. coli* in 2 samples of raw sewage and tertiary treated, chlorine disinfected reclaimed water from the North Durham Water Reclamation Facility are presented. From this figure and the log_{10} reduction data presented in Table 5, the log_{10} reductions achieved by the Durham wastewater treatment plant are approximately >6 log_{10} for *E. coli* and approximately >5.5 for *Enterococcus* spp. With the exception of the NCT2RW sample analyzed for *Enterococcus* on 3/7/16, no *E. coli* or *Enterococcus* spp. were detected in the reclaimed water, so the lower detection limit value (1MPN/100mL) was used to calculate the log_{10} reduction as a greater then value.

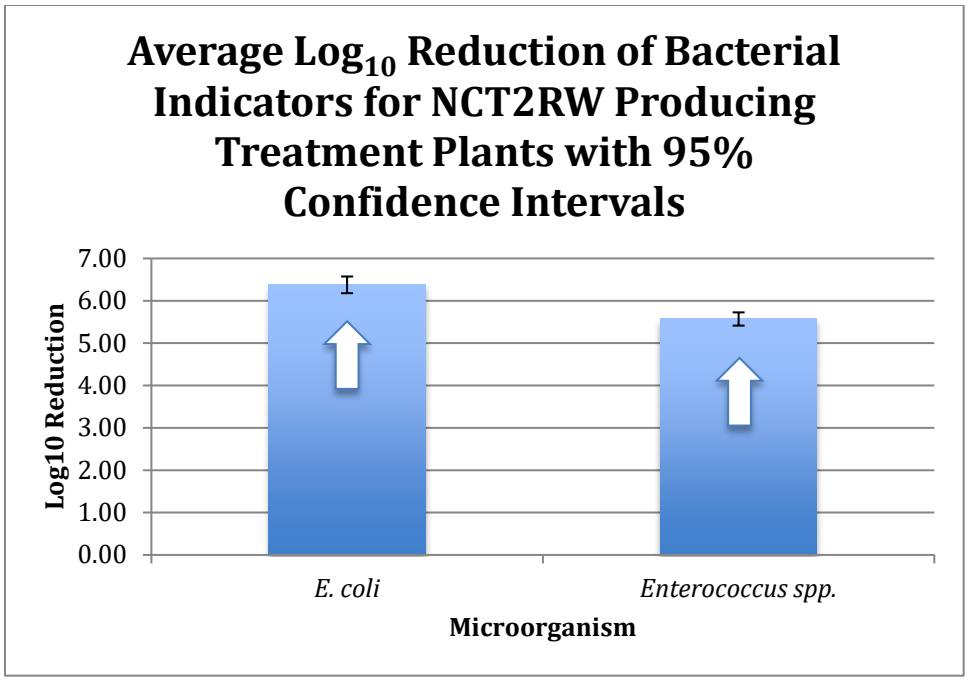

Figure 7: Average Log_{10} reductions for *E. coli* and *Enterococcus* spp. calculated from NCT2RW producing treatment plants, shown with 95% confidence intervals. Up arrows indicate greater than values.

In Figure 7 the average log_{10} reductions for *E. coli* and *Enterococcus* spp. for tertiary treated, dual disinfected NCT2RW samples are shown with 95% confidence intervals. Based on this figure, the average log_{10} reduction for *E. coli* in NCT2RW samples was approximately >6.4, wile the average log_{10} reduction for *Enterococcus* spp. was approximately >5.5. Based on Table 5, it appears that the reason for the lower log_{10} reduction in *Enterococcus* spp. is due to the lower concentrations of this microorganism in raw sewage, rather than its presence in the reclaimed water samples. Additionally, as *E. coli* and *Enterococcus* spp. were not detected in the final treated reclaimed water samples, these log_{10} reduction values represent upper limit detection values rather than the full magnitude of the treatment capabilities of each wastewater reclamation facility.

3.3.1.2 Coliphage Viruses

Coliphage data were collected using the single agar layer method (SAL, US EPA Method 1602) in 100mL samples. For the purpose of calculating log_{10} reducion values, non-detected values, were assumed to be the lower limit of detection, which for the SAL method is approximately 1 plauqe formining unit (PFU) per 100mL.

Table 6: Coliphage Concentrations and Log_{10} Reductions from Raw Sewage and Reclaimed Water Samples

Date	Location	Sample Type	Somatic Coliphage (PFU/100mL)	F+/Male Specific	Total Coliphage (PFU/100mL)

				Coliphage (PFU/100mL)	
1.19.16	Cary	Raw Sewage	2.40E+04	8.00E+03	5.40E+04
		Reclaimed Water	1.00E+00	1.00E+00	1.00E+00
		Log_{10} Reduction	>4.38	>3.90	>4.73
2.2.16	Raleigh	Raw Sewage	4.10E+04	8.00E+03	1.10E+04
		Reclaimed Water	1.00E+00	1.00E+00	7.00E+00
		Log_{10} Reduction	>4.61	>3.90	>3.20
2.16.16	Holly Springs	Raw Sewage	4.00E+03	8.00E+03	1.10E+04
		Reclaimed Water	1.00E+00	1.00E+00	1.00E+00
		Log_{10} Reduction	>3.60	>3.90	>4.04
2.23.16	OWASA	Raw Sewage	8.00E+03	3.00E+03	1.80E+04
		Reclaimed Water	3.00E+00	1.00E+00	1.50E+01
		Log_{10} Reduction	3.43	>3.48	3.08
3.7.16	Durham	Raw Sewage	5.00E+03	3.30E+04	5.00E+03
		Reclaimed Water	2.00E+00	2.00E+00	9.00E+00
		Log_{10} Reduction	3.40	4.22	2.74
4.26.26	Holly Springs	Raw Sewage	1.13E+04	5.67E+04	2.88E+04
		Reclaimed Water	1.00E+00	1.00E+00	1.00E+00
		Log_{10} Reduction	>4.05	>4.75	>4.46
5.10.16	Cary	Raw Sewage	1.53E+04	4.50E+03	4.20E+04
		Reclaimed Water	1.00E+00	1.00E+00	9.00E+00
		Log_{10} Reduction	>4.18	>3.65	3.67
5.23.16	Raleigh	Raw Sewage	7.40E+03	4.10E+03	1.30E+04
		Reclaimed Water	1.00E+00	1.00E+00	1.00E+00
		Log_{10} Reduction	>3.87	>3.61	>4.11
6.6.16	OWASA	Raw Sewage	7.65E+04	6.50E+03	7.40E+04
		Reclaimed Water	1.00E+00	1.50E+01	1.00E+00
		Log_{10} Reduction	>4.88	2.64	>4.87
6.13.16	Durham	Raw Sewage	3.13E+04	9.90E+03	2.40E+04

		Reclaimed Water	1.00E+00	1.00E+00	4.00E+00
		Log_{10} Reduction	>4.50	>4.00	3.78
6.20.16	Holly Springs	Raw Sewage	3.70E+04	1.71E+04	2.40E+04
		Reclaimed Water	3.00E+00	1.40E+01	5.00E+00
		Log_{10} Reduction	4.09	3.09	3.68
6.27.16	OWASA	Raw Sewage	5.50E+04	2.40E+03	1.30E+04
		Reclaimed Water	1.00E+00	1.00E+00	1.00E+00
		Log_{10} Reduction	>4.74	>3.38	>4.11
7.13.16	Cary	Raw Sewage	3.40E+04	1.96E+04	4.60E+04
		Reclaimed Water	1.00E+00	1.00E+00	1.00E+00
		Log_{10} Reduction	>4.53	>4.29	>4.66
7.25.16	Raleigh	Raw Sewage	1.80E+04	3.40E+03	2.40E+04
		Reclaimed Water	1.00E+00	1.00E+00	2.00E+00
		Log_{10} Reduction	>4.26	>3.53	4.08

Table 6 presents the log_{10} concentrations of somatic, F+/male specific, and total coliphage viruses in raw sewage and reclaimed water samples along with the calculated log_{10} reductions between these two samples. Based on this table, it is usually not possible to document that that the state mandated 5 log_{10} reduction target is met for all samples. In many samples this is because the lower detection limit coliphage concentration (none detected/100 mL) was reached in the reclaimed water and the initial concentration of coliphages in raw sewage was too low to document that the 5 log10 reduction was achieved. However, it is important to note all types of coliphage viruses were being eliminated by the treatment systems to their lower detection limit value (none detected) in many reclaimed water samples. treatment processes.

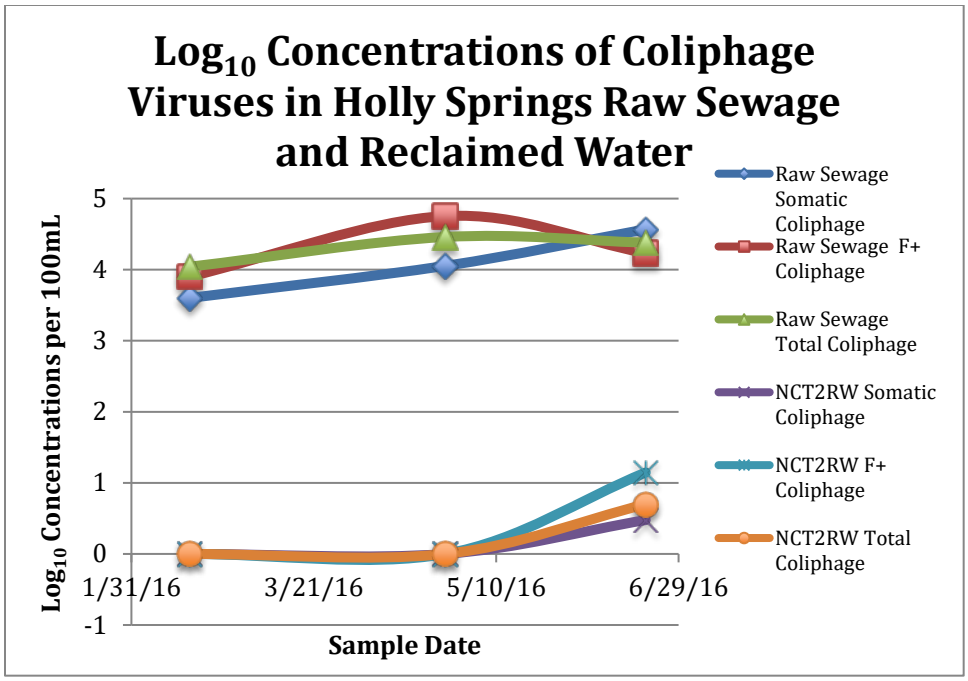

Figure 8: Log_{10} concentrations of coliphage viruses in Holly Springs raw sewage and tertiary treated dual disinfected reclaimed water reclaimed water for 3 sample pairs of

In Figure 8 the log_{10} concentrations of somatic, F+/male specific, and total coliphages are displayed for Holly Springs raw sewage and tertiary treated, dual disinfected reclaimed water. Based on this figure and the data presented in Table 6, the log_{10} reductions for somatic, F+ and total coliphages are approximately, >4 log_{10}, >3 to 4 log_{10}, and >3.5 to >4 log_{10} respectively. For these samples, coliphages were only detected in the reclaimed water sample tested on 6/20/16, for which the concentrations of somatic, F+, and total coliphages were 3.00E+00, 1.14E+01, and 5.00E+01 respectively.

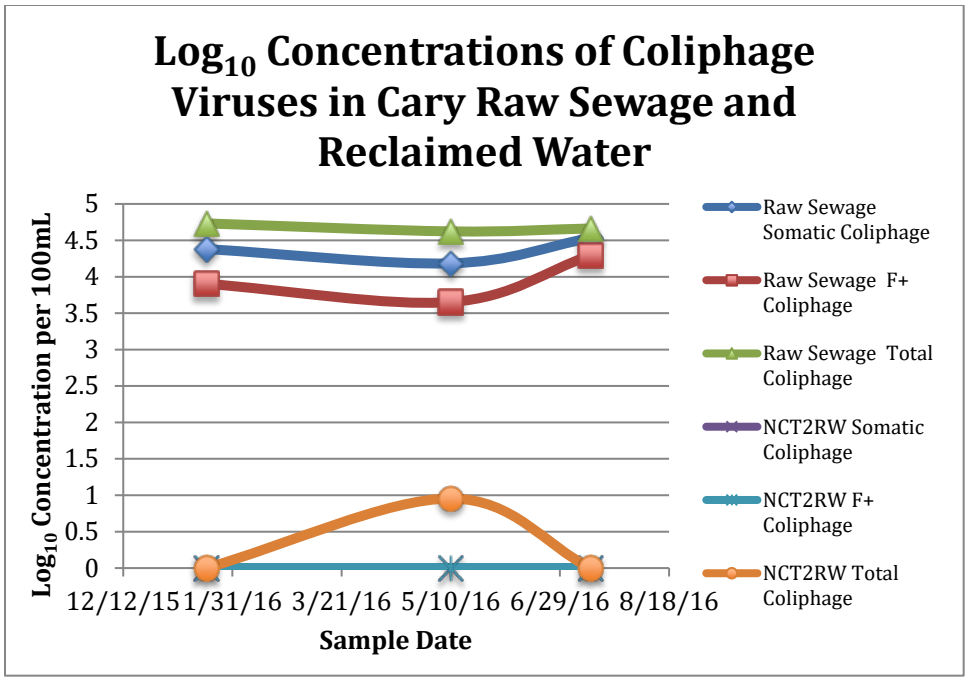

Figure 9: Log_{10} concentrations of coliphage viruses in Cary raw sewage and tertiary treated dual disinfected reclaimed water for 3 sample pairs

In Figure 9 the log_{10} concentrations of somatic, F+/male specific, and total coliphages are displayed for Cary raw sewage and tertiary treated, dual disinfected reclaimed water. Based on this figure and the data presented in Table 6, the log_{10} reductions for somatic, F+ and total coliphages, are approximately, >4 to >5 log_{10}, >4 log_{10}, and >4.5 log_{10} respectively. For these samples, total coliphages were detected in the reclaimed water sample analyzed on 5/10/16 at levels of 9.00E+00. For all other samples the lower detection limit value (1 PFU/ 100mL) was used to calculate the log_{10} reduction as a greater than value.

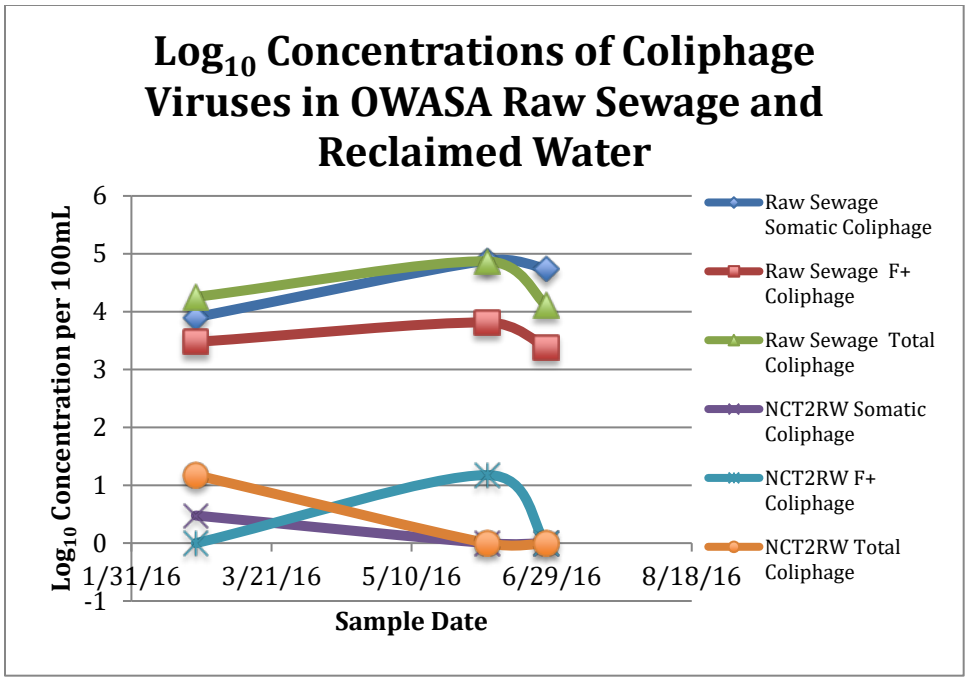

Figure 10: Log_{10} concentrations of coliphage viruses in OWASA raw sewage and tertiary treated dual disinfected reclaimed water for 3 sample pairs

In Figure 10 the log_{10} concentrations of somatic, F+/male specific, and total coliphages are displayed for OWASA raw sewage and tertiary treated, dual disinfected reclaimed water. Based on this figure and the data presented in Table 6, the log_{10} reductions for somatic, F+ and total coliphages, are approximately, .3.5 to >4.5 log_{10}, >3 log_{10}, and 3 to >4 log_{10} respectively. For these samples, coliphages were detected in many but not all samples of reclaimed water, as summarized in Table 4.

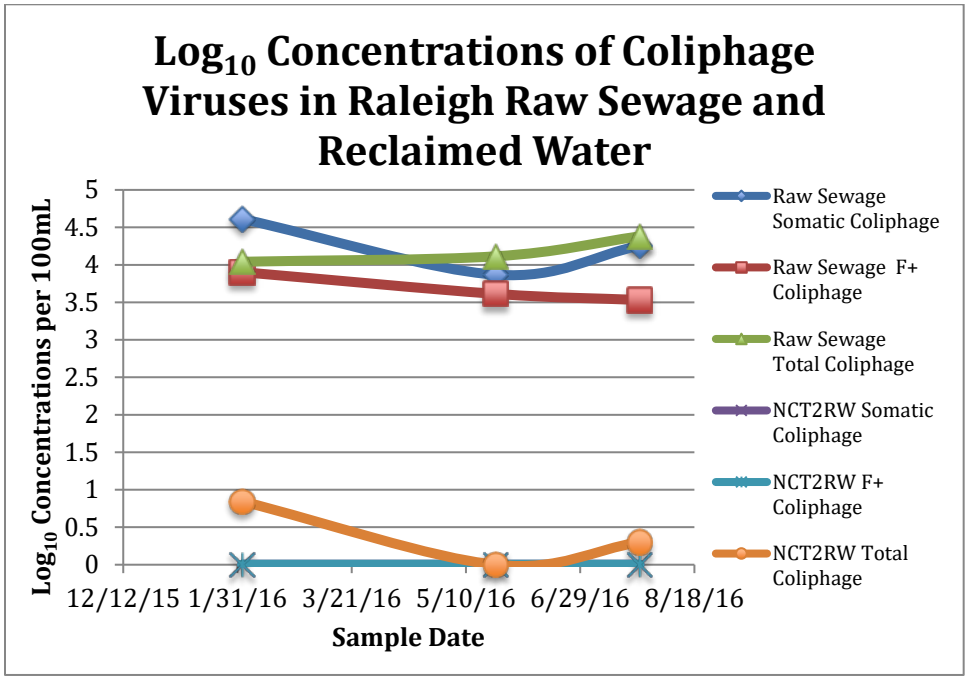

Figure 11: Log_{10} concentrations of coliphage viruses in Raleigh raw sewage and tertiary treated, dual disinfected reclaimed water for 3 sample pairs

In Figure 11 are displayed the log_{10} concentrations of somatic, F+/male specific, and total coliphages for Raleigh raw sewage and reclaimed water. Based on this figure and the data presented in Table 6, the log_{10} reductions for somatic, F+, and total coliphages, are approximately, >4 log_{10}, 3.5 to >4 log_{10}, and 4.5 to >5 log_{10} respectively. For these samples, no F+ coliphages or somatic coliphages were detected in the reclaimed water, so the detection limit (1 PFU/ 100mL) was used to calculate the log_{10} reduction as a greater value. Total coliphages were detected in the samples collected on 2/2/16 and 7/25/16 at the levels described in Table 4.

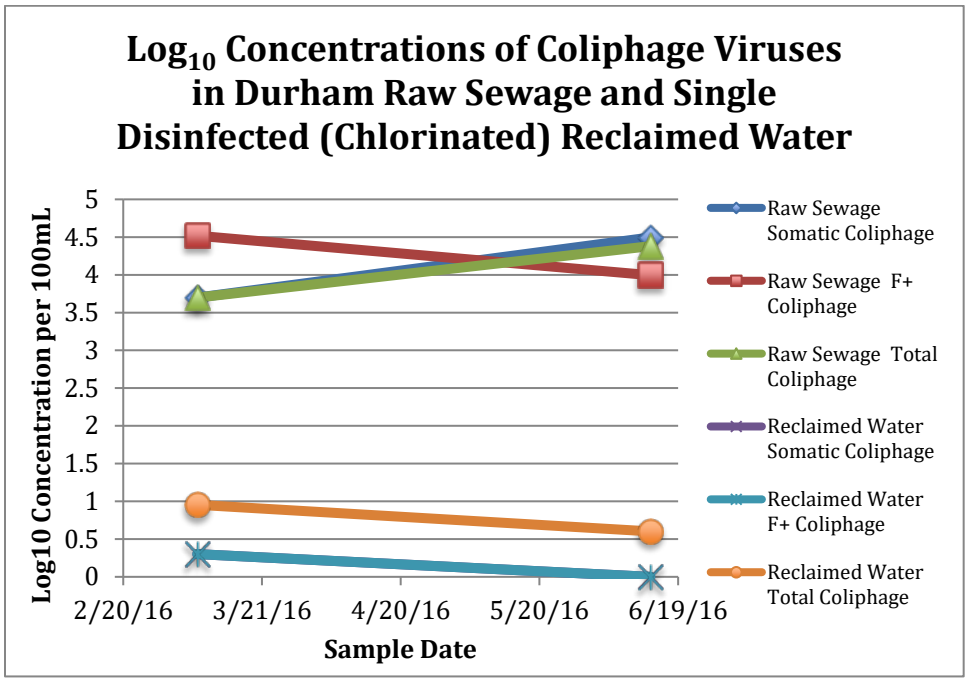

Figure 12: Log_{10} concentrations of coliphage viruses in Durham raw sewage and tertiary treated, chlorinated reclaimed water for 2 sample pairs

In Figure 12 log_{10} concentrations of somatic, F+/male specific, and total coliphages are displayed for Durham raw sewage and tertiary treated, chlorine disinfected reclaimed water. Based on this figure and the data presented in Table 6, the log_{10} reductions for somatic, F+, and total coliphages, are approximately, >3.5 to >4.5 log_{10}, 4 log_{10}, and 3 log_{10} respectively. For these samples, no F+ were detected in the reclaimed water, so the lower detection limit value (1 PFU/ 100mL) was used to calculate the log_{10} reduction as a greater than value. Somatic and total coliphages were detected at the levels summarized in Table 4.

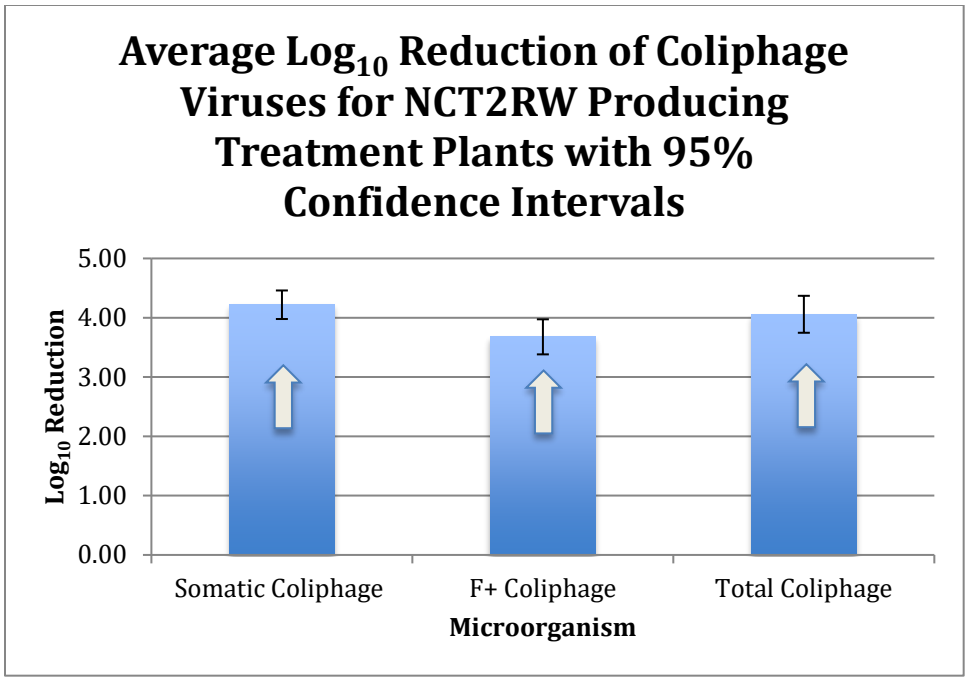

Figure 13: Average Log_{10} reductions for coliphage viruses from NCT2RW treatment plants, shown with 95% confidence intervals. Up arrow indicates that value is greater than that shown.

In Figure 13 the average log_{10} reductions for coliphage viruses for tertiary treated, dual disinfected NCT2RW samples are shown with 95% confidence intervals. Based on this figure, the average log_{10} reduction for somatic, F+ and total coliphages in NCT2RW samples were approximately >4.4, >4, and >4.7 respectively. Based on Table 6, it appears that the reason for the lower log_{10} reductions in somatic and F+ coliphages are due to the lower concentrations of this microorganisms in raw sewage, rather than their presence in the reclaimed water samples. Additionally, as these coliphages were generally not detected or detected at only low concentrations in the final treated reclaimed water samples, these values represent upper limit detection values rather than the fully measurable log_{10} reduction values achieved by the treatment system of each wastewater reclamation facility.

3.3.1.3 Protozoan Parasite Surrogates

Clostridium perfringens spores and vegetative bacteria as surrogates for protozoan parasites were detected using CP ChromoSelect agar using the membrane filtration method, and results are presented as colony forming units (CFU) per 100mL. For the purpose of calculating log_{10} reduction values, the lower limit of detection value (1 CFU per agar plate) was used in those cases where no *C. perfringens* were detected in the reclaimed water sample analyzed.

Table 7: *Clostridium perfringens* Concentrations and Log_{10} Reductions from Raw Sewage and Reclaimed Water Samples

Date	Location	Sample Type	*C. perfringens* Pasteurized	*C. perfringens* Unpasteurized

			Concentration (CFU/100mL)	Concentration (CFU/100mL)
1.19.16	Cary	Raw Sewage	4.67E+04	8.22E+04
		Reclaimed Water	1.00E+00	1.00E+00
		Log_{10} Reduction	>4.91	>4.67
2.2.16	Raleigh	Raw Sewage	6.67E+04	1.11E+03
		Reclaimed Water	1.00E+00	1.00E+00
		Log_{10} Reduction	>4.82	>3.05
2.16.16	Holly Springs	Raw Sewage	2.67E+04	4.33E+04
		Reclaimed Water	1.00E+00	1.00E+00
		Log_{10} Reduction	>4.43	>4.64
2.23.16	OWASA	Raw Sewage	3.22E+04	2.44E+04
		Reclaimed Water	1.00E+00	1.00E+00
		Log_{10} Reduction	>4.51	>4.39
3.7.16	Durham	Raw Sewage	3.44E+04	5.00E+04
		Reclaimed Water	3.97E+01	3.47E+01
		Log_{10} Reduction	>2.94	>3.16
4.26.26	Holly Springs	Raw Sewage	2.67E+04	3.11E+04
		Reclaimed Water	1.00E+00	1.00E+00
		Log_{10} Reduction	>4.43	>4.49
5.10.16	Cary	Raw Sewage	3.33E+04	3.89E+04
		Reclaimed Water	2.00E+00	6.67E-01
		Log_{10} Reduction	4.22	4.77
5.23.16	Raleigh	Raw Sewage	6.11E+04	4.67E+04
		Reclaimed Water	1.00E+00	1.00E+00
		Log_{10} Reduction	>4.79	>4.67
6.6.16	OWASA	Raw Sewage	1.04E+04	1.15E+04
		Reclaimed Water	1.00E+00	1.00E+00
		Log_{10} Reduction	>4.02	>4.06
6.13.16	Durham	Raw Sewage	3.41E+04	4.33E+04
		Reclaimed Water	5.33E+00	4.67E+00
		Log_{10} Reduction	3.81	3.97

6.20.16	Holly Springs	Raw Sewage	9.63E+03	2.56E+04
		Reclaimed Water	1.00E+00	1.00E+00
		Log_{10} Reduction	>3.98	>4.41
6.27.16	OWASA	Raw Sewage	1.44E+04	5.78E+04
		Reclaimed Water	1.00E+00	1.00E+00
		Log_{10} Reduction	>4.16	>4.76
7.13.16	Cary	Raw Sewage	1.07E+04	1.00E+04
		Reclaimed Water	1.00E+00	1.00E+00
		Log_{10} Reduction	>4.03	>4.00
7.25.16	Raleigh	Raw Sewage	5.00E+04	6.00E+04
		Reclaimed Water	2.67E+00	3.00E+00
		Log_{10} Reduction	4.27	4.30

In Table 7 are presented the log_{10} concentrations of pasteurized and unpasteurized *C. perfringens* in raw sewage and reclaimed water samples along with the calculated log_{10} reductions between these two samples. Based on this table, it is clear that the state mandated 4 log_{10} reduction target is met for most samples, with the exception of the Durham treatment plant employing only chlorine disinfection of reclaimed water. Furthermore, it is important to note that the raw sewage (influent) concentrations were at sufficiently high levels track a 4 log_{10} reduction in contrast to some of the other indicator organisms examined in this study.

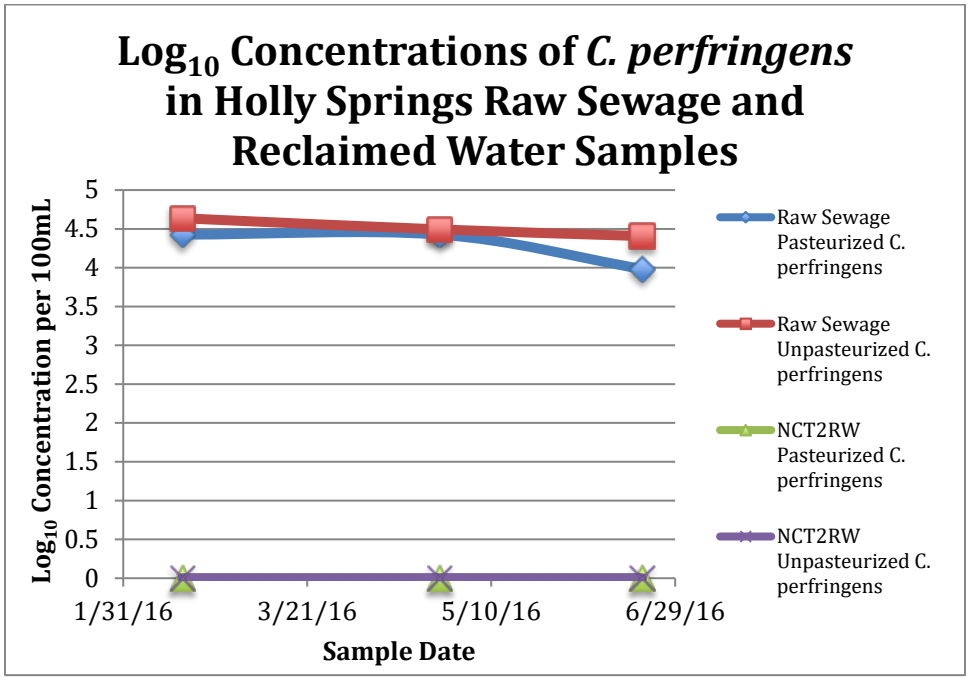

Figure 14: Log_{10} concentrations of *Clostridium perfringens* in Holly Springs raw sewage and tertiary treated dual disinfected reclaimed water for 3 sample pairs

In Figure 14 the log_{10} concentrations of unpasteurized and pasteurized *C. perfringens* are displayed for Holly Springs raw sewage and tertiary treated, dual disinfected reclaimed water. Based on the results shown this figure and the data presented in Table 7 the log_{10} reductions for pasteurized *C. perfringens* were approximately >4 log_{10}, while the log_{10} reduction of unpasteurized *C. perfringens* was approximately 4.5 log_{10}. For these samples, no *C. perfringens* of any type were detected in the reclaimed water, so the lower detection limit value (1 CFU/ 100mL) was used to calculate the log_{10} reduction as a greater than value.

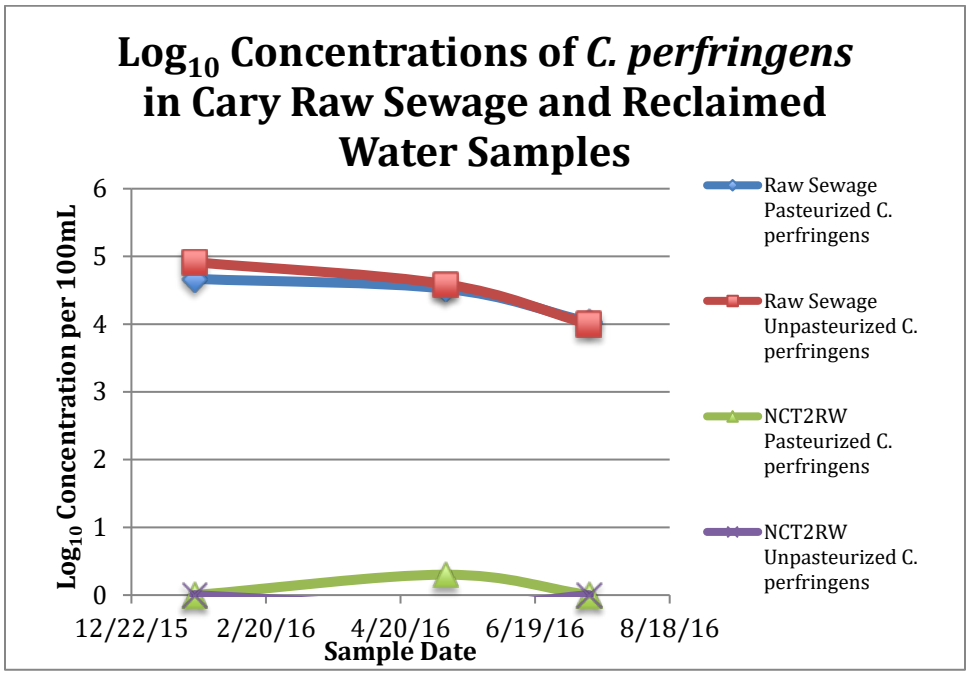

Figure 15: Log_{10} concentrations of *Clostridium perfringens* in Cary raw sewage and tertiary treated, dual disinfected reclaimed water for 3 sample pairs

In Figure 15 the log_{10} concentrations of unpasteurized and pasteurized *C. perfringens* are displayed for Cary raw sewage and tertiary treated dual disinfected reclaimed water. Based on the results in this figure and the data presented in Table 7, the log_{10} reductions for pasteurized *C. perfringens* were approximately >4 to >4.5 log_{10}, while the log_{10} reduction of unpasteurized *C. perfringens* was approximately >4.5 log_{10}. For these samples, *C. perfringens* were detected in reclaimed water only in the sample analyzed on 5/10/16; for other reclaimed water samples, the lower detection limit value (1 CFU/ 100mL) was used to calculate the log_{10} reduction as a greater than value.

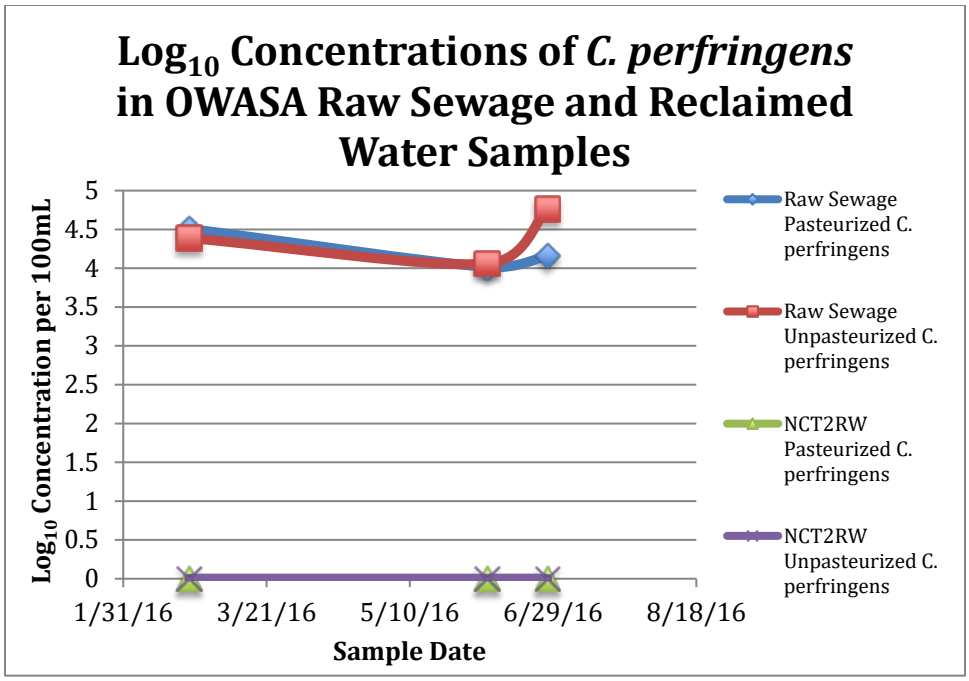

Figure 16: Log_{10} concentrations of *Clostridium perfringens* in OWASA raw sewage and tertiary treated dual disinfected reclaimed water for 3 sample pairs

In Figure 16 the log_{10} concentrations of unpasteurized and pasteurized *C. perfringens* are displayed for OWASA raw sewage and tertiary treated, dual disinfected reclaimed water. Based on the results in this figure and the data presented in Table 7, the log_{10} reductions for pasteurized *C. perfringens* were approximately >4 to >4.5 log_{10}, while the log_{10} reductions of unpasteurized *C. perfringens* were approximately >4.5 log_{10}. For these samples, no *C. perfringens* of any type were detected in the reclaimed water samples, so the lower detection limit values (1 CFU/ 100mL) were used to calculate the log_{10} reductions as greater than values.

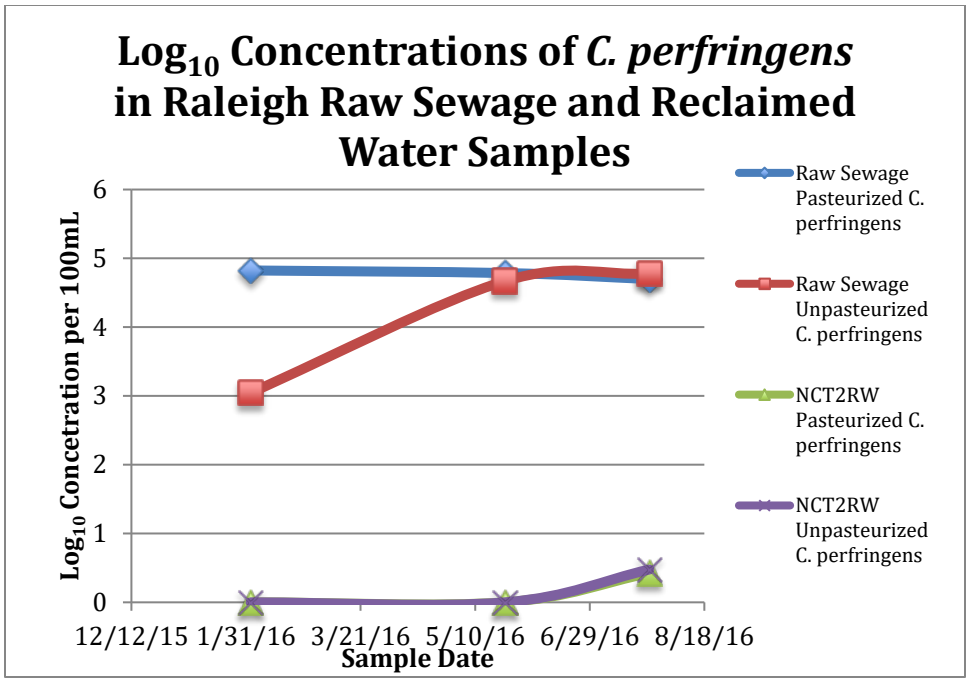

Figure 17: Log_{10} concentrations of *Clostridium perfringens* in Raleigh raw sewage and tertiary treated dual disinfected reclaimed water for 2 sample pairs

In Figure 17 the log_{10} concentrations of unpasteurized and pasteurized *C. perfringens* are displayed for Raleigh raw sewage and tertiary treated, dual disinfected reclaimed water. Based on the results this figure and the data presented in Table 7, the log_{10} reductions for pasteurized *C. perfringens* were approximately >4 to >4.5 log_{10}, while the log_{10} reductions of unpasteurized *C. perfringens* were approximately >3 to >4 log_{10}. For these samples, both pasteurized and unpasteurized *C. perfringens* were detected in the 7/25/16 sample at concentrations summarized in Table 5. For the other samples presented here, no *C. perfringens* of any type were detected in the reclaimed water, so the lower detection limit value (1 CFU/ 100mL) was used to calculate these log_{10} reductions and greater than values.

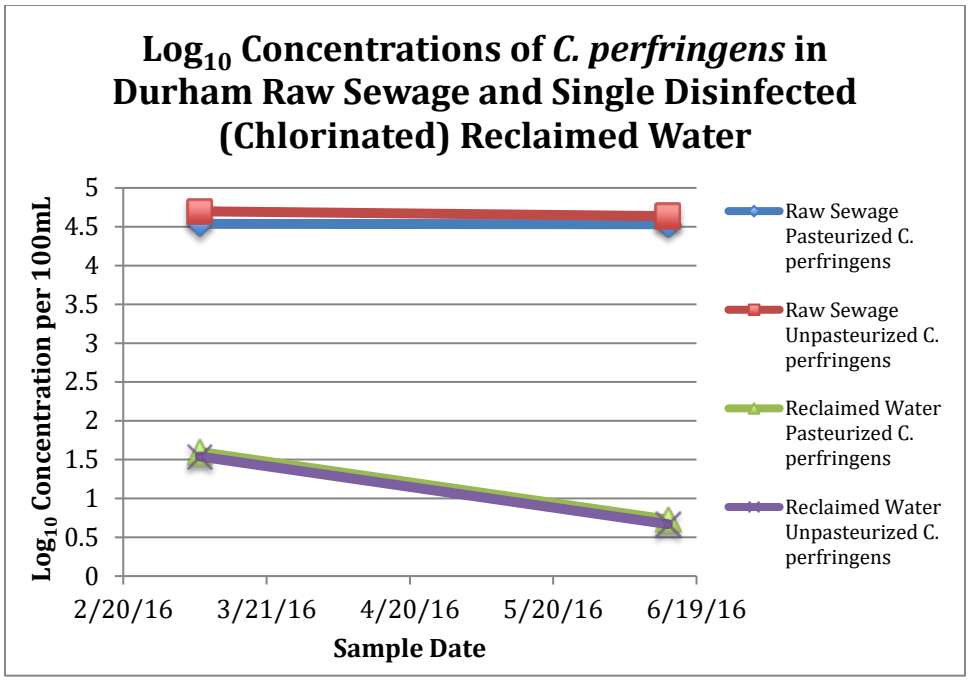

Figure 18: Log_{10} concentrations of *Clostridium perfringens* in Durham raw sewage and chlorinated reclaimed water for 2 sample pairs

In Figure 18 the log_{10} concentrations of unpasteurized and pasteurized *C. perfringens* are displayed for Durham raw sewage and chlorine disinfected reclaimed water. Based on the results this figure and the data presented in Table 7, the log_{10} reductions for pasteurized *C. perfringens* were approximately 3 to 3.8 log_{10}, while the log_{10} reductions of unpasteurized *C. perfringens* were approximately 3.5 log_{10}. For these samples, both pasteurized and unpasteurized *C. perfringens* were detected in both the 7/21/15 and 10/6/15 reclaimed water samples at concentrations described in Table 5.

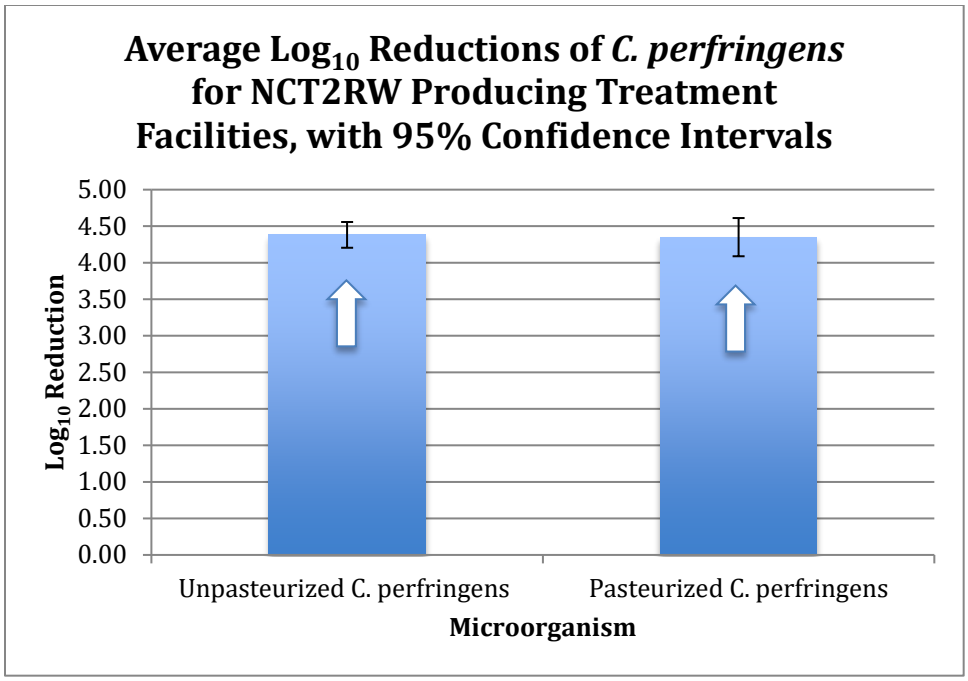

Figure 19: Average $\log_{10}$ reductions for pasteurized and unpasteurized *Clostridium perfringens* from NCT2RW treatment plants, shown with 95% confidence intervals

In Figure 19 the average $\log_{10}$ reductions for pasteurized and unpasteurized *C. perfringens* for tertiary treated, dual disinfected NCT2RW samples are shown with 95% confidence intervals. Based on this figure, the average $\log_{10}$ reductions for pasteurized and unpasteurized *C. perfringens* in NCT2RW samples were approximately >4.4 and >4.3 respectively. Based on the data in Table 7 and Figures 14-17, it is clear that almost no *C. perfringens* spores or vegetative cells were detected in the NCT2RW samples. As such, these values represent upper limit detection values rather than the true magnitude of microbial reduction achieved by the treatment systems of each wastewater reclamation facility.

3.3.1.4 Pathogenic Salmonella Bacteria

Salmonella spp. were detected in raw sewage and reclaimed water using the sequential methods of initial broth enrichment culture in multiple sample volumes in a non-selective medium, followed by further broth culture enrichment in a selective medium and then colony isolation of presumptive target bacteria on a differential-selective agar medium. Based on the distinctive appearance on the differential-selective agar of colonies from each enriched sample volume, a most probable number (MPN) concentration was estimated, with a subsequent biochemical confirmation plating step to confirm identification. Initially, three sample volumes were incubated in triplicate overnight (18-24 hours) in buffered peptone water at 37°C. The 9-volume MPN test volumes for the reclaimed water sample were 300 30, and 3 mLs; the test volumes of raw sewage samples were triplicate 1mL aliquots of serial 10-fold dilutions of the initial sewage sample. Next, a portion of the buffered peptone water was inoculated into a *Salmonella* selective broth, Rappaport Vassiliadis Broth, and incubated overnight at 37°C. Enriched samples were streak plated on Salmonella Shigella agar (BD) and incubated overnight at 37°C. Positive

presumptive Salmonella colonies were scored as colorless colonies with black centers, as indicated by the manufacturer. Presumptive positive colonies were then confirmed as positive using the Triple Sugar Iron Agar slant biochemical test. Confirmed positive results for analysis of the 9 sample sub-volumes of each sample were used to compute a most probable number concentration per 100 mL.

Table 8: *Salmonella* spp. Concentrations and Log_{10} Reductions from Raw Sewage and Tertiary Treated, Dual Disinfected Reclaimed Water Samples

Date	Location	Sample Type	*Salmonella* spp. MPN Concentration (Lower 95% Confidence Interval, Upper 95% Confidence Interval)
1.19.16	Cary	Raw Sewage	1.5E+03 (4.40E+02, 5.40E+03)
		Reclaimed Water	<1.00E-01 (1.20E-02, 8.20E-01)
		Log_{10} Reduction	>4.18
2.2.16	Raleigh	Raw Sewage	2.80E+03 (7.70+02, 9.90E+03)
		Reclaimed Water	<1.00E-01 (1.20E-02, 8.20E-01)
		Log_{10} Reduction	>4.45
2.16.16	Holly Springs	Raw Sewage	2.40E+03 (6.70E+02, 8.60E+03)
		Reclaimed Water	<1.00E-01 (1.20E-02, 8.20E-01)
		Log_{10} Reduction	>4.38
2.23.16	OWASA	Raw Sewage	2.40E+03 (6.70E+02, 8.60E+03)
		Reclaimed Water	<1.00E-01 (1.20E-02, 8.20E-01)
		Log_{10} Reduction	>4.38
3.7.16	Durham	Raw Sewage	7.50E+02 (1.80E+02, 3.20E+03)
		Reclaimed Water	<1.00E-01 (1.20E-02, 8.20E-01)
		Log_{10} Reduction	>3.88
4.26.26	Holly Springs	Raw Sewage	1.50E+03 (4.20E+02, 5.40E+03)
		Reclaimed Water	<1.00E-01 (1.20E-02, 8.20E-01)
		Log_{10} Reduction	>4.18
5.10.16	Cary	Raw Sewage	1.50E+03 (4.20E+02, 5.40E+03)
		Reclaimed Water	<1.00E-01 (1.20E-02, 8.20E-01)
		Log_{10} Reduction	>4.18
5.23.16	Raleigh	Raw Sewage	4.60E+03 (1.00E+03, 2.10E+04)

		Reclaimed Water	<1.00E-01 (1.20E-02, 8.20E-01)
		Log_{10} Reduction	>4.66
6.6.16	OWASA	Raw Sewage	1.10E+04 (2.60E+03, 4.70E+04)
		Reclaimed Water	<1.00E-01 (1.20E-02, 8.20E-01)
		Log_{10} Reduction	>5.04
6.13.16	Durham	Raw Sewage	1.50E+03 (4.20E+02, 5.40E+03)
		Reclaimed Water	1.20E-01 (1.70E-02, 8.40E-01)
		Log_{10} Reduction	4.10
6.20.16	Holly Springs	Raw Sewage	7.50E+02 (1.80E+02, 3.20E+03)
		Reclaimed Water	<1.00E-01 (1.20E-02, 8.20E-01)
		Log_{10} Reduction	>3.88
6.27.16	OWASA	Raw Sewage	9.50E+02 (2.40E+02, 3.80E+03)
		Reclaimed Water	<1.00E-01 (1.20E-02, 8.20E-01)
		Log_{10} Reduction	>3.98
7.13.16	Cary	Raw Sewage	1.10E+04 (2.60E+03, 4.70E+04)
		Reclaimed Water	<1.00E-01 (1.20E-02, 8.20E-01)
		Log_{10} Reduction	>5.04
7.25.16	Raleigh	Raw Sewage	2.40E+03 (6.70E+02, 8.60E+03)
		Reclaimed Water	<1.00E-01 (1.20E-02, 8.20E-01)
		Log_{10} Reduction	>3.38

Table 8 presents the log_{10} concentrations of *Salmonella* spp. bacteria in raw sewage and reclaimed water samples along with the calculated log_{10} reductions between these two samples. Based on this table, it appears that the log_{10} concentrations and corresponding log_{10} reductions for pathogenic bacteria are more variable than those of the indicator organisms displayed in Table 3 and that initial concentrations of *Salmonella* spp. were lower than *E. coli* concentrations, thus resulting in lower log_{10} reductions. Many reclaimed water samples were negative for *Salmonella* spp. bacteria in the sample volumes analyzed, and therefore, the estimated Salmonella concentrations in these samples were below the lower detection limit and were computed as less than values. *Salmonella* spp. concentrations in the raw sewage were not as high as those of the fecal indicator bacteria such as *E. coli*, and therefore, the estimated log_{10}

reductions were lower than the bacteria target reduction of 6 $\log_{10}$ and were computed as greater than values.

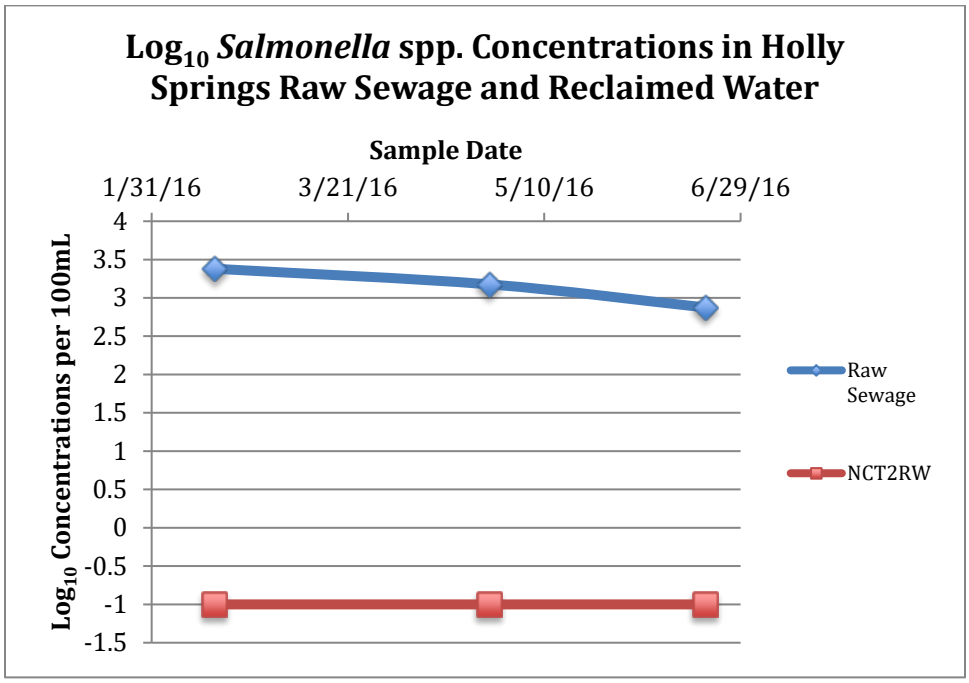

Figure 20: Log_{10} concentrations of *Salmonella* spp. in Holly Springs raw sewage and tertiary treated dual disinfected reclaimed water for 3 sample pairs

In Figure 20 the $\log_{10}$ concentrations of *Salmonella* spp. are displayed for Holly Springs raw sewage and tertiary treated dual disinfected reclaimed water. Based on the results in this figure and the data presented in Table 8, the $\log_{10}$ reductions for *Salmonella* spp. were approximately >4 $\log_{10}$. For these samples, no *Salmonella* spp. were detected in most of the reclaimed water samples analyzed, so the detection limit (0.1 MPN/ 100mL) was used to calculate the $\log_{10}$ reduction as a greater than value.

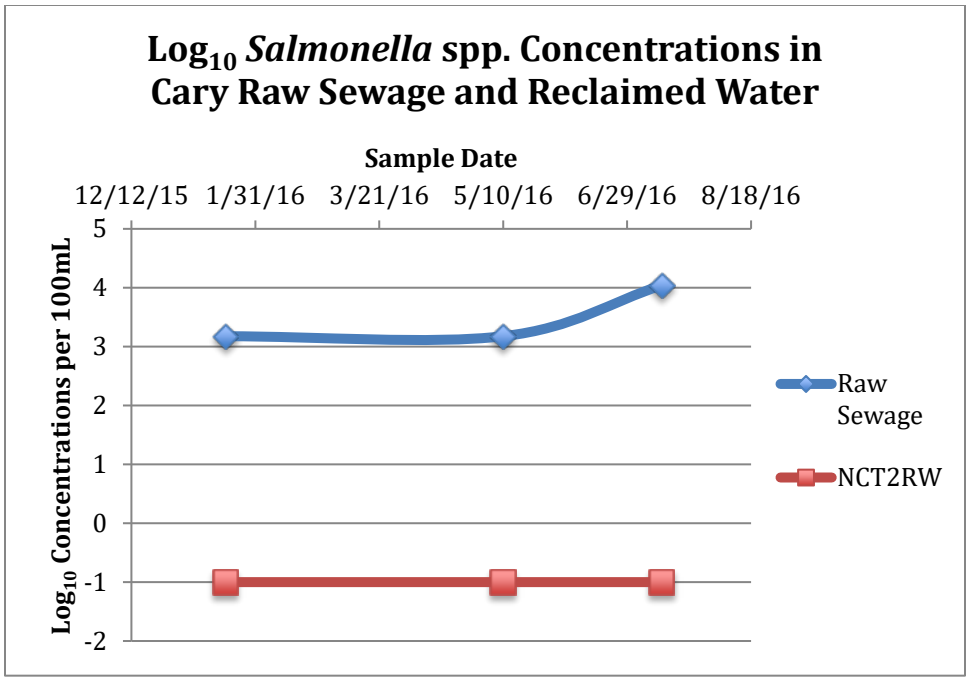

Figure 21: Log_{10} concentrations of *Salmonella* spp. in Cary raw sewage and tertiary treated dual disinfected reclaimed water for 3 sample pairs

In Figure 21 the log_{10} concentrations of *Salmonella* spp. are displayed for Cary raw sewage and tertiary treated, dual disinfected reclaimed water. Based on this figure and the data presented in Table 8, the log_{10} reductions for *Salmonella* spp. were approximately >4 to >5 log_{10}. For these samples, no *Salmonella* spp. were detected in the reclaimed water samples analyzed, so the lower detection limit value (0.1 MPN/ 100mL) was used to calculate the log_{10} reduction as a greater than value.

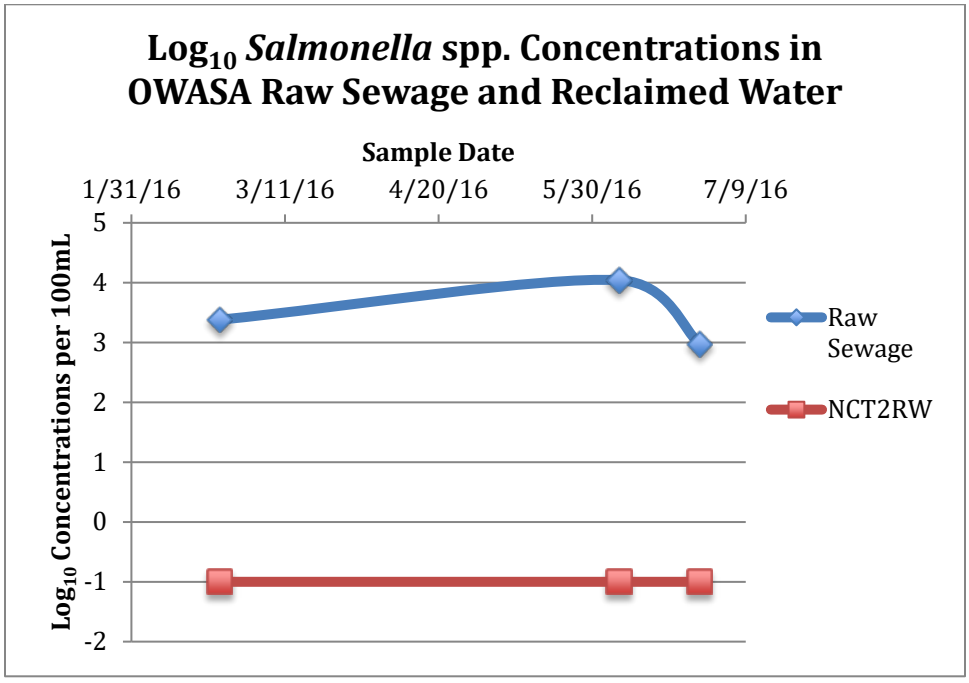

Figure 22: Log_{10} concentrations of *Salmonella* spp. in OWASA raw sewage and tertiary treated dual disinfected reclaimed water for 3 sample pairs

In Figure 22 the log_{10} concentrations of *Salmonella* spp. are displayed for OWASA raw sewage and tertiary treated, dual disinfected reclaimed water. Based on the results this figure and the data presented in Table 8, the log_{10} reductions for *Salmonella* spp. were approximately >4 to >5 log_{10}. For these samples, no *Salmonella* spp. were detected in the reclaimed water samples analyzed, so the lower detection limit value (0.1 MPN/ 100mL) was used to calculate the log_{10} reduction as a greater than value.

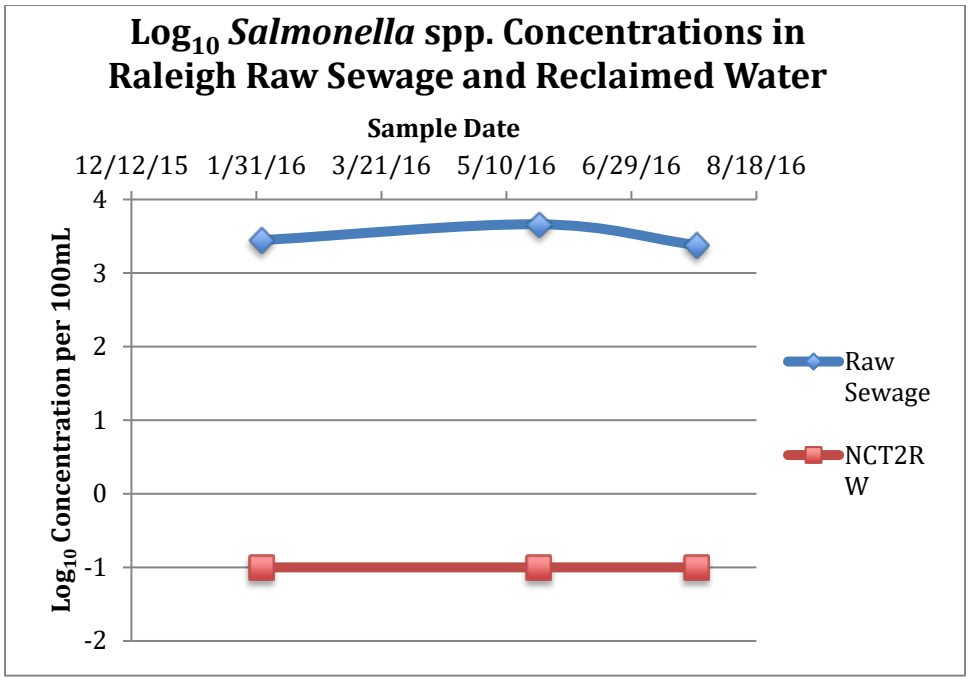

Figure 23: Log_{10} concentrations of *Salmonella* spp. in Raleigh raw sewage and tertiary treated dual disinfected reclaimed water for 2 sample pairs

In Figure 23 the log_{10} concentrations of *Salmonella* spp. are displayed for Raleigh raw sewage and tertiary treated, dual disinfected reclaimed water. Based on the results this figure and the data presented in Table 8, the log_{10} reductions for *Salmonella* spp. were approximately >3.5 to >4.5 log_{10}. For these samples, no *Salmonella* spp. were detected in the reclaimed water samples analyzed, so the lower detection limit value (0.1 MPN/ 100mL) was used to calculate the log_{10} reduction as a greater than value.

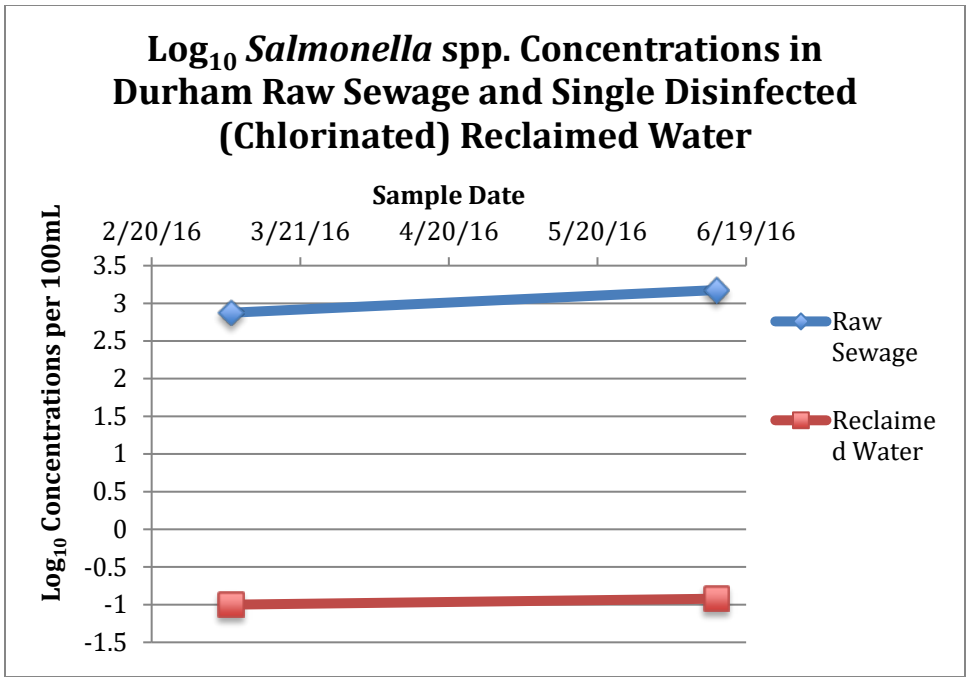

Figure 24: Log_{10} concentrations of *Salmonella* spp. in Durham raw sewage and tertiary treated, chlorinated reclaimed water for 2 sample pairs

In Figure 24 the log_{10} concentrations of *Salmonella* spp. are displayed for Durham raw sewage and tertiary treated, chlorine disinfected reclaimed water. Based on the results this figure and the data presented in Table 8, the log_{10} reductions for *Salmonella* spp. were approximately >4 log_{10}. For these samples, *Salmonella* spp. was detected only on in the reclaimed water sample collected on 6/13/16 at a concentration of 1.20E-01. For the reclaimed water sample collected on 3/7/16, no Salmonella spp. were detected in the sample volume analyzed, the lower detection limit value (0.1 MPN/ 100mL) was used to calculate the log_{10} reduction as a greater than value.

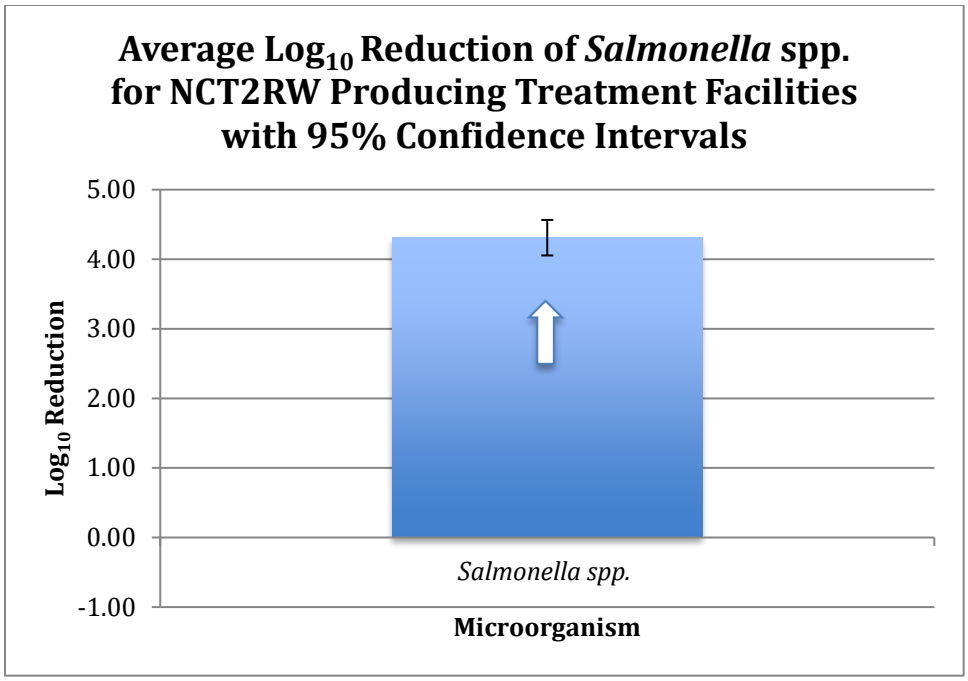

Figure 25: Average $\log_{10}$ reductions for *Salmonella* spp. from NCT2RW treatment plants, shown with 95% confidence intervals

In Figure 25 the average $\log_{10}$ reduction for *Salmonella* spp. for tertiary treated, dual disinfected NCT2RW samples is shown with a 95% confidence interval. Based on this figure, the average $\log_{10}$ reduction for *Salmonella* spp. in NCT2RW samples was approximately >4.3. Based on the data Table 8, and Figures 14-17 it is clear that almost no *Salmonella* spp. were detected in the NCT2RW samples volumes analyzed; as such, the$\log_{10}$ reduction values represent upper limit detection limit values rather than the full magnitude of the $\log_{10}$ reduction achieved by the treatment systems of each wastewater reclamation facility.

3.3.1.5 Enteric Viruses

Appropriate positive, negative, and specimen processing controls were prepared and evaluated in the calculation of the genome copy concentrations presented below.. The specimen processing control for Norovirus GII was the genogroup IV F+ RNA coliphage SP and the processing control for Adenovirus groups A-F was the adeno-like Salmonella phage PRD1. For the purpose of calculating $\log_{10}$ reduction values, non-detect qPCR values (ND) were assumed to be the lower limit of detection for each method, which is approximately 1 genome copy (GEC) per 100mL of sample for both Norovirus GII and Adenovirus groups A-F. Lower limit of detection information for the enteric virus analysis is provided in the methods section of this report (section 2).

Table 9: Enteric Virus Concentrations and Log_{10} Reductions from Raw Sewage and Reclaimed Water Samples

Date	Location	Sample Type	Norovirus (GEC per 100mL)	Adenovirus (GEC per 100mL)

1.19.16	Cary	Raw Sewage	4.33E+03	1.43E+05
		Reclaimed Water	ND	4.90E+04
		Log_{10} Reduction	>3.64	0.46
2.2.16	Raleigh	Raw Sewage	6.20E+02	6.79E+04
		Reclaimed Water	ND	ND
		Log_{10} Reduction	>2.79	4.83
2.16.16	Holly Springs	Raw Sewage	3.50E+03	2.54E+05
		Reclaimed Water	ND	1.88E+04
		Log_{10} Reduction	>3.54	1.13
2.23.16	OWASA	Raw Sewage	1.84E+03	1.43E+05
		Reclaimed Water	ND	1.92E+04
		Log_{10} Reduction	>3.26	0.87
3.7.16	Durham	Raw Sewage	1.17E+04	3.00E+03
		Reclaimed Water	ND	ND
		Log_{10} Reduction	>4.07	3.48
4.26.26	Holly Springs	Raw Sewage	6.34E+03	6.06E+04
		Reclaimed Water	ND	1.00E+00
		Log_{10} Reduction	>3.80	4.78
5.10.16	Cary	Raw Sewage	1.01E+03	6.22E+04
		Reclaimed Water	ND	7.45E+02
		Log_{10} Reduction	3.00	1.92
5.23.16	Raleigh	Raw Sewage	5.93E+03	4.41E+05
		Reclaimed Water	>ND	ND
		Log_{10} Reduction	3.77	5.64
6.6.16	OWASA	Raw Sewage	1.91E+03	2.79E+05
		Reclaimed Water	4.12E+02	3.39E+03
		Log_{10} Reduction	0.67	1.91
6.13.16	Durham	Raw Sewage	7.79E+03	4.31E+05
		Reclaimed Water	ND	2.48E+03

		Log_{10} Reduction	>3.89	2.24
6.20.16	Holly Springs	Raw Sewage	9.88E+05	9.64E+05
		Reclaimed Water	ND	3.23E+02
		Log_{10} Reduction	>5.99	3.48
6.27.16	OWASA	Raw Sewage	4.81E+03	3.19E+04
		Reclaimed Water	ND	1.24E+03
		Log_{10} Reduction	>3.68	1.41
7.13.16	Cary	Raw Sewage	1.11E+03	3.80E+04
		Reclaimed Water	ND	1.92E+03
		Log_{10} Reduction	>3.05	1.30
7.25.16	Raleigh	Raw Sewage	1.76E+03	1.27E+05
		Reclaimed Water	5.20E+02	1.33E+04
		Log_{10} Reduction	0.53	0.98

Table 9 presents the log_{10} concentrations of enteric viruses detected as genome copies per 100 mL in raw sewage and reclaimed water samples along with the calculated log_{10} reductions between these two samples. Based on this table, it appears that the log_{10} concentrations and corresponding log_{10} reductions for Norovirus GII and adenovirus groups A-F are lower and more variable than those of the coliphage viruses displayed in Table 6. Additionally, based on this table it is clear that concentrations of adenovirus (detected by qPCR) are not completely reduced after wastewater treatment when, in contrast, coliphage viruses and norovirus GII genome copies were completely eliminated when detected by culture infectivity methods and nucleic acid genome amplification methods, respectively.

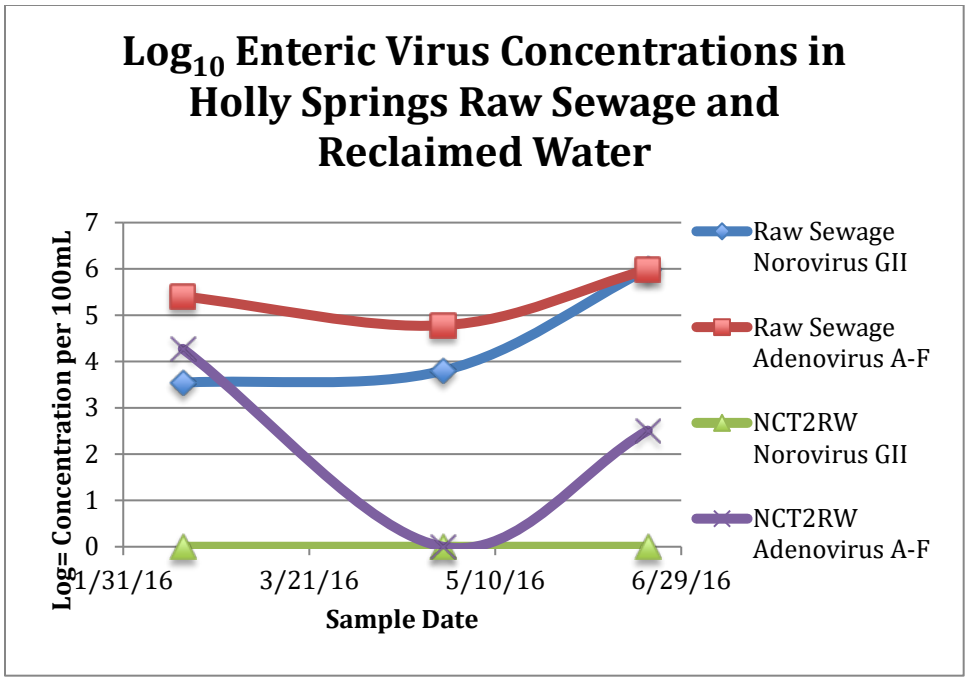

Figure 26: Log_{10} concentrations as genome copies of adenovirus groups A-F and norovirus GII in Holly Springs raw sewage and tertiary treated, dual disinfected reclaimed water for 3 sample pairs

In Figure 26 the log_{10} concentrations of both Norovirus GII and adenovirus groups A-F as genome copies are displayed for Holly Springs raw sewage and tertiary treated, dual disinfected reclaimed water. Based on the results I n this figure and the data presented in Table 9, the log_{10} reduction for Norovirus GII genome copies is approximately 3.5 to 6. The log_{10} reduction for adenovirus groups A-F is approximately >4.5 for the non-detect sample, and between 1 and 3.5 for the samples in which adenoviruses were detected in the reclaimed water. For these samples, no Norovirus GII genome copies were detected in the reclaimed water, so the lower detection detection limit (1 GEC/ 100mL) value was used to calculate the log_{10} reduction. In contrast to the Norovirus results, adenovirus gene copies were detected sometimes at high levels in the reclaimed water, particularly in the winter (January) and late summer months.

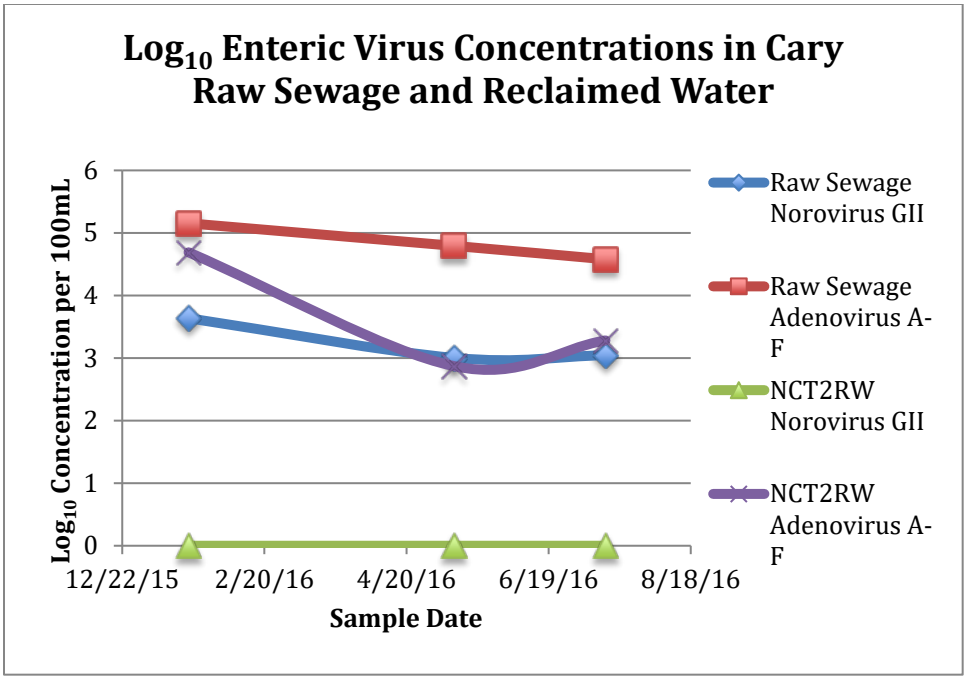

Figure 27: Log_{10} concentrations as genome copies of adenovirus groups A-F and norovirus GII in Cary raw sewage and tertiary treated dual disinfected reclaimed water for 3 sample pairs

In Figure 27 the log_{10} concentrations of both Norovirus GII and adenovirus groups A-F genome copies are displayed for Cary raw sewage and tertiary treated, dual disinfected reclaimed water. Based on the results in this figure and the data presented in Table 9, the log_{10} reduction for Norovirus GII gene copies is approximately >3 to >3.5. No Norovirus GII gene copies were detected in the reclaimed water samples analyzed, so the lower detection limit value (1 GEC/ 100mL) was used to calculate the log_{10} reduction as greater than values. Adenoviruses gene copies were detected at high levels in the reclaimed water samples. The log_{10} reduction for adenovirus groups A-F gene copies averages between nearly zero and 1.

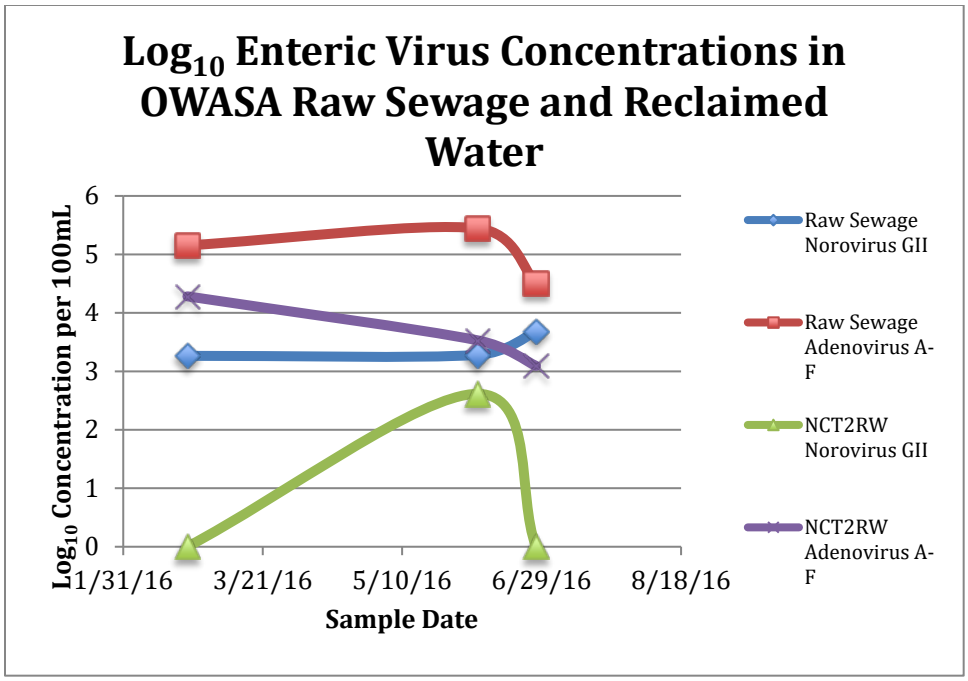

Figure 28: Log_{10} concentrations as genome copies of adenovirus groups A-F and norovirus GII in OWASA raw sewage and tertiary treated, dual disinfected reclaimed water for 3 sample pairs

In Figure 28 the log_{10} concentrations of genome copies of both Norovirus GII and adenovirus groups A-F are displayed for OWASA raw sewage and tertiary treated, dual disinfected reclaimed water samples. Based on the results this figure and the data presented in Table 9, the log_{10} reduction for Norovirus GII gene copies is approximately >3.5 for the non detect samples of reclaimed water, For these samples of reclaimed water, the lower detection limit value (1 GEC/ 100mL) was used to calculate the log_{10} reduction of Norovirus GII gene copies. For the sample where Norovirus GII gene copies were detected in the reclaimed water sample, the log_{10} reduction was approximately 1.. All of the reclaimed water samples contained gene copies of adenovirus groups A-F, and the log_{10} reductions averaged between 1 and 1.5.

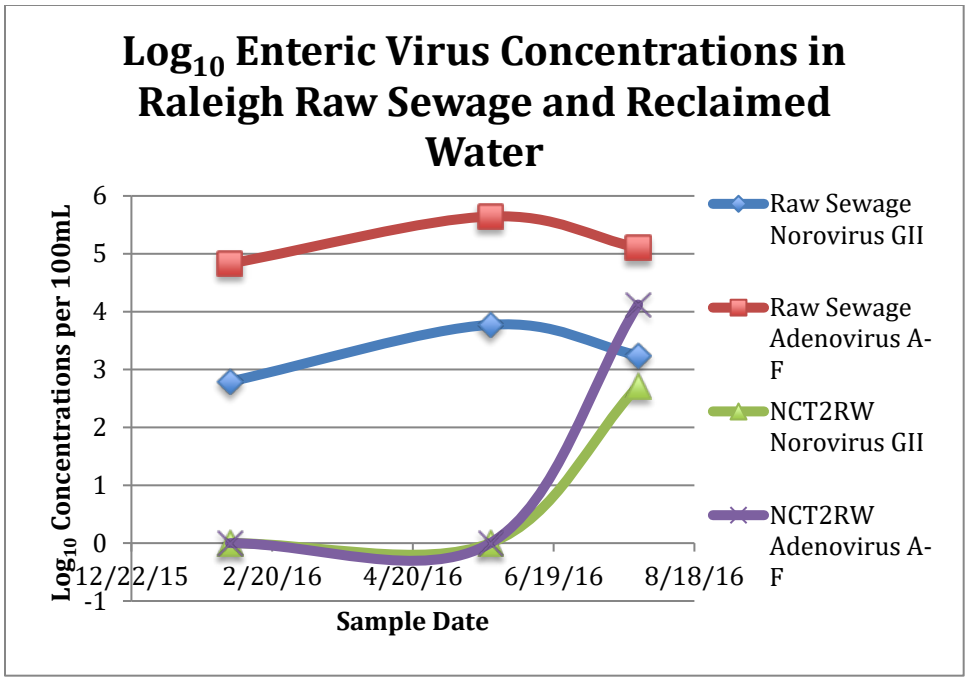

Figure 29: Log_{10} concentrations as genome copies of adenovirus groups A-F and norovirus GII in Raleigh raw sewage and tertiary treated, dual disinfected reclaimed water for 2 sample pairs

In Figure 29 the log_{10} concentrations of gene copies of both Norovirus GII and adenovirus groups A-F are displayed for Raleigh raw sewage and tertiary treated dual disinfected reclaimed water. Based on the results in this figure and the data presented in Table 9, the log_{10} reduction for the non-detect Norovirus GII samples of reclaimed water is approximately >3 log_{10}. For these samples of reclaimed water, the lower detection limit value (1 GEC/ 100mL) was used to calculate the log_{10} reduction of Norovirus GII gene copies. For the sample on 7/25/16 containing detectable Norovirus GII copies, the log_{10} reduction was approximately 0.5. For reclaimed water samples with non-detect levels of adenovirus groups A-F genome copies, the log_{10} reduction of genome copies was approximately >5 to >6_0, For the reclaimed water samples containing detectable concentrations of adenovirus groups A-F genome copies, the log_{10} reduction was approximately 1.

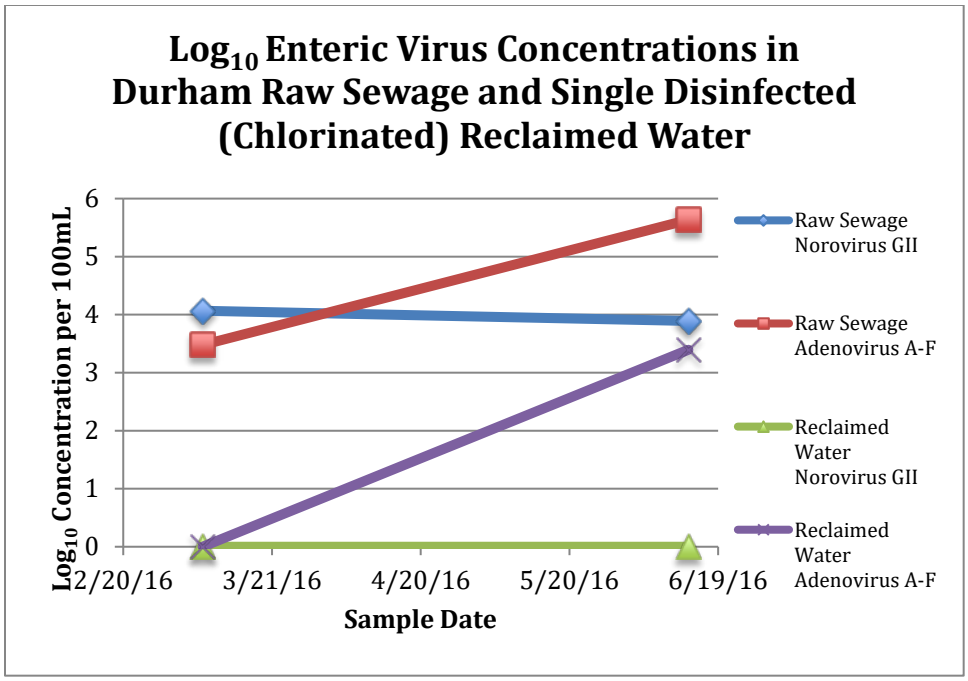

Figure 30: Figure 30: Log_{10} concentrations as genome copies of adenovirus groups A-F and norovirus GII in Durham raw sewage and tertiary treated, chlorinated reclaimed water for 2 sample pairs.

In Figure 30 the log_{10} concentrations of both Norovirus GII and adenovirus groups A-F genome copies are displayed for Durham raw sewage and chorine disinfected reclaimed water. Based on the results this figure and the data presented in Table 9, the log_{10} reduction for Norovirus GII genome copies is approximately >4 log_{10}. For these samples, no Norovirus GII genome copies were detected in the reclaimed water samples, so the lower detection limit value (1 GEC/ 100mL) was used to calculate the log_{10} reduction. For the adenovirus reclaimed water sample analyzed on 3/7/16, the log_{10} reduction was approximately >3.5, while the log_{10} reduction for the reclaimed water sample analyzed on 6/13/16 was 2.3..

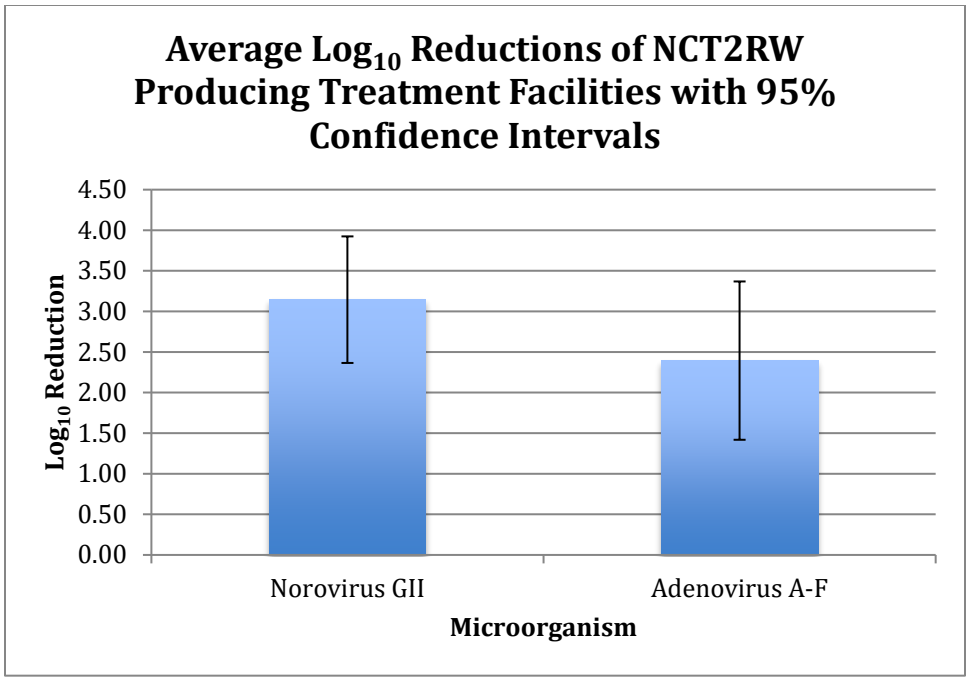

Figure 31: Average $\log_{10}$ reductions for adenovirus groups A-F and norovirus GII gene copies for NCT2RW treatment plants, shown with 95% confidence intervals

Shown in Figure 31 are the average $\log_{10}$ reductions with 95% confidence intervals for Norovirus GII and adenovirus groups A-F gene copies in tertiary treated, dual disinfected NCT2RW samplesbased on initial concentrations in raw sewage. Based on Table 9, and the summary figure above, the average $\log_{10}$ reduction for Norovirus GII gene copies in NCT2RW samples was approximately >3.1 from raw sewage. However, it is important to note that little to no Norovirus gene copies were detected in the reclaimed water samples. Therefore, Norovirus GII genome copy $\log_{10}$ reduction values were calculated using the lower limit detection value, The average $\log_{10}$ reduction for adenovirus groups A-F gene copies in reclaimed water produced from raw sewage is approximately 2.4 or more, with a 95% confidence interval indicating a wide range of $\log_{10}$ reductions that included some particularly low $\log_{10}$ reductions over the course of the sampling period.

3.3.1.6 Protozoan Parasites

Protozoan parasites were analyzed by US EPA Method 1623 and are presented as (oo)cysts per 100mL. Limit of detection information for protozoan parasite analysis is provided in section 2 of this report. It is important to note that the protozoan parasites detected by this method represent total numbers of cysts and oocysts and not infectious organisms. This is an important distinction as the number of infectious cysts and oocysts is potentially much lower than the total number detected in a given sample.

Table 10: Protozoan Parasite Concentrations and Log_{10} Reductions from Raw Sewage and Reclaimed Water Samples

Date	Location	Sample Type	*Cryptosporidium* spp. (oocysts per 100mL)	*Giardia* spp. (cysts per 100mL)
1.19.16	Cary	Raw Sewage	90	68
		Reclaimed Water	0.14	>0.01
		Log_{10} Reduction	2.81	>3.83
2.2.16	Raleigh	Raw Sewage	60	51
		Reclaimed Water	0.43	0.24
		Log_{10} Reduction	2.14	2.33
2.16.16	Holly Springs	Raw Sewage	18.5	23.5
		Reclaimed Water	0.17	0.04
		Log_{10} Reduction	2.04	2.77
2.23.16	OWASA	Raw Sewage	23	16.5
		Reclaimed Water	0.23	0.04
		Log_{10} Reduction	2.00	2.62
3.7.16	Durham	Raw Sewage	138	143
		Reclaimed Water	0.2	0.02
		Log_{10} Reduction	2.84	3.85
4.26.26	Holly Springs	Raw Sewage	34.5	34.5
		Reclaimed Water	0.22	0.21
		Log_{10} Reduction	2.20	2.22
5.10.16	Cary	Raw Sewage	94	69.5
		Reclaimed Water	0.25	0.1
		Log_{10} Reduction	2.58	2.84
5.23.16	Raleigh	Raw Sewage	56	42.5
		Reclaimed Water	0.42	0.1
		Log_{10} Reduction	2.12	2.63
6.6.16	OWASA	Raw Sewage	56	40.5
		Reclaimed Water	0.08	0.08

		Log_{10} Reduction	2.85	2.70
6.13.16	Durham	Raw Sewage	160	117
		Reclaimed Water	0.04	0.02
		Log_{10} Reduction	3.60	3.77
6.20.16	Holly Springs	Raw Sewage	96	111
		Reclaimed Water	0.14	0.02
		Log_{10} Reduction	2.84	3.74
6.27.16	OWASA	Raw Sewage	72.5	50.5
		Reclaimed Water	0.19	0.09
		Log_{10} Reduction	2.58	2.75
7.13.16	Cary	Raw Sewage	55	50
		Reclaimed Water	0.05	0.06
		Log_{10} Reduction	3.04	2.92
7.25.16	Raleigh	Raw Sewage	68	57
		Reclaimed Water	0.07	0.03
		Log_{10} Reduction	2.99	3.28

In Table 10 are shown the log_{10} concentrations of protozoan parasites detected by immunofluorescent microscopy in raw sewage and reclaimed water samples, along with the calculated log_{10} reductions between these two samples. Based on this table, it appears that the log_{10} concentrations and corresponding log_{10} reductions for *Cryptosporidium* spp. and *Giardia* spp. are lower and much more variable than those of pasteurized and unpasteurized *C. perfringens* protozoan parasite surrogates as displayed in Table 7. Additionally, based on the results in this table, it is apparent that concentrations of both *Cryptosporidium* and *Giardia* (detected by immunofluorescence microscopy) are not completely reduced after wastewater treatment processes. These log_{10} reductions for Cryptosporidium and Giardia are less than the target 4 log_{10} reduction for NCT2RW. In contrast, *C. perfringens* spores and vegetative cells were completely reduced to non-detectable levels to achieve > 4 log_{10} reductions when detected by culture-based plating methods of analysis.

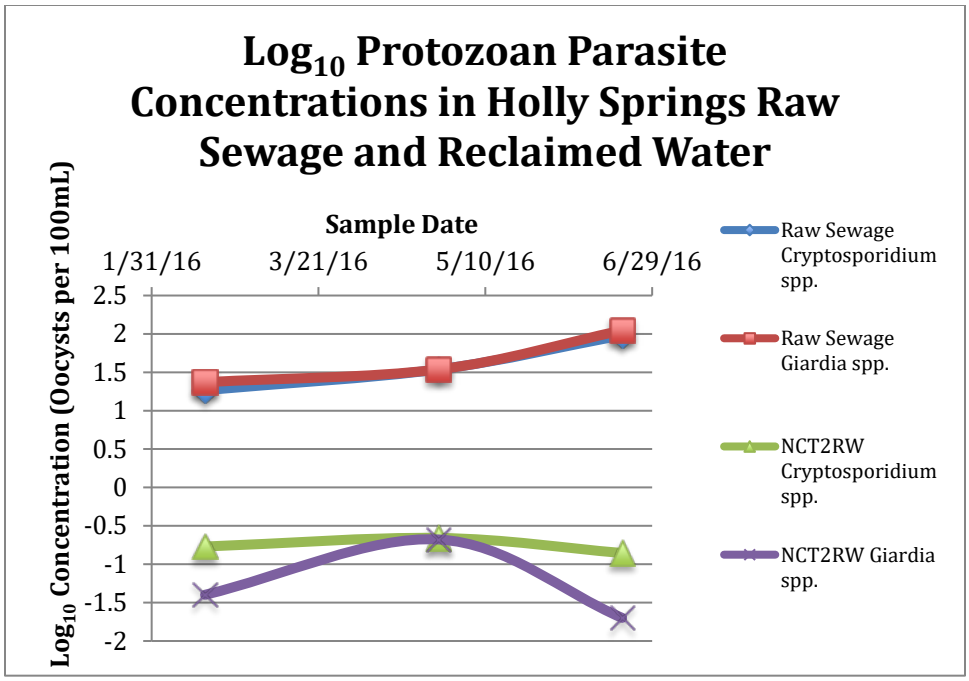

Figure 32: Log_{10} protozoan parasite concentrationss in Holly Springs raw sewage and tertiary treated, dual disinfected reclaimed water for 3 sample pairs

In Figure 32 the log_{10} concentrations of both *Cryptosporidium* spp. and *Giardia* spp. are displayed for Holly Springs raw sewage and tertiary treated, dual disinfected reclaimed water. Based on the results in this figure and the data presented in Table 10, the log_{10} reduction for *Cryptosporidium* is approximately 2.5 and the log_{10} reduction for *Giardia* is approximately 2.5 to 3.5.

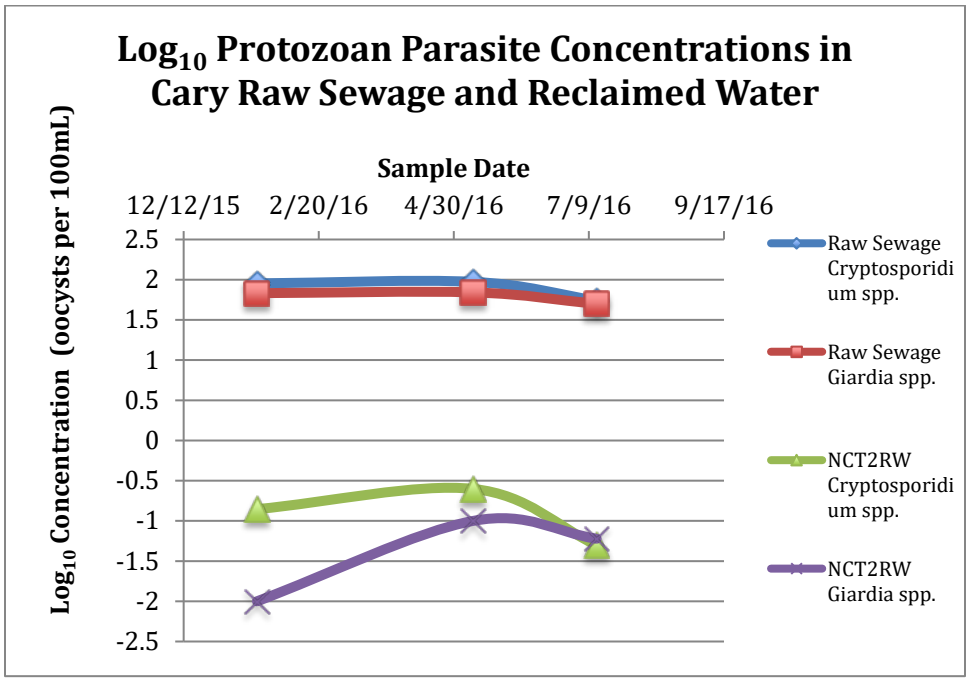

Figure 33: Log_{10} protozoan parasite concentrations in Cary raw sewage and tertiary treated, dual disinfected reclaimed water for 3 sample pairs

In Figure 33 the log_{10} concentrations of both *Cryptosporidium* spp. and *Giardia* spp. (oo)cysts are displayed for Cary raw sewage and tertiary treated, dual disinfected reclaimed water. Based on the results shown in this figure and the data presented in Table 10, the log_{10} reduction for *Cryptosporidium* is approximately 2.5 to 3 and the log_{10} reduction for *Giardia* is approximately 2.8 to 3.8. No *Giardia* spp. cysts were detected in the sample collected on 1/19/16, so the limit of detection (0.01 cysts/100ml) was used to calculate the log_{10} reduction for this sample. For the other reclaimed water samples, detectable levels of both *Cryptosporidium* and *Giardia* were detected.

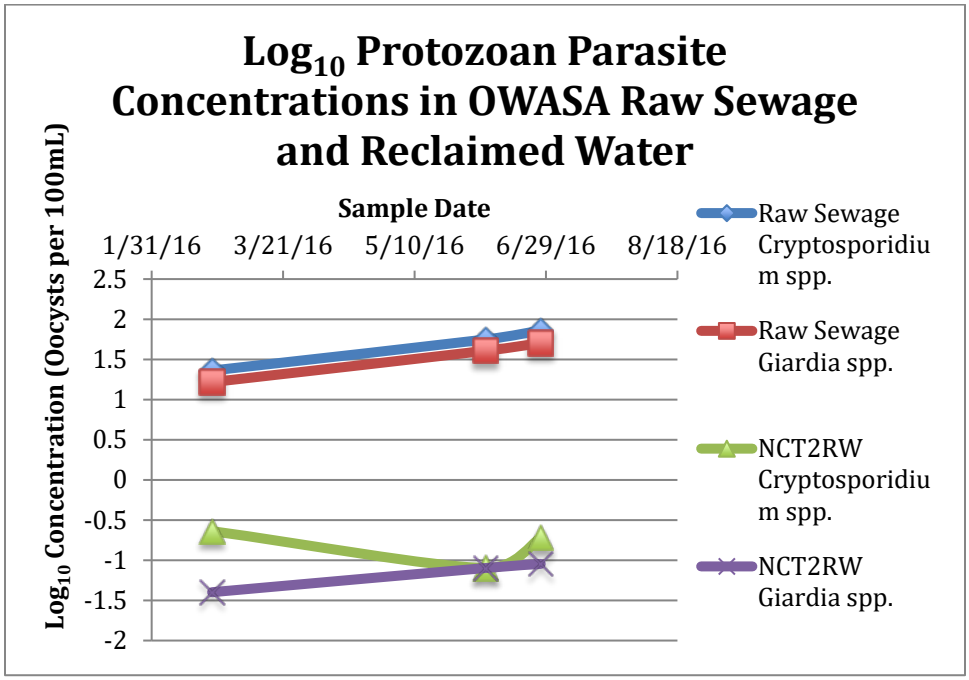

Figure 34: Log_{10} protozoan parasite concentrations in OWASA raw sewage and tertiary treated, dual disinfected reclaimed water for 3 sample pairs

In Figure 34 the log_{10} concentrations of both *Cryptosporidium* spp. and *Giardia* spp. are displayed for OWASA raw sewage and tertiary treated, dual disinfected reclaimed water. Based on the results in this figure and the data presented in Table 10, the log_{10} reduction for *Cryptosporidium* was approximately 2 to 2.8. The log_{10} reduction for *Giardia* was approximately 2.5 to 2.6. In all samples of OWASA reclaimed water, detectable levels of both *Cryptosporidium* and *Giardia* were detected.

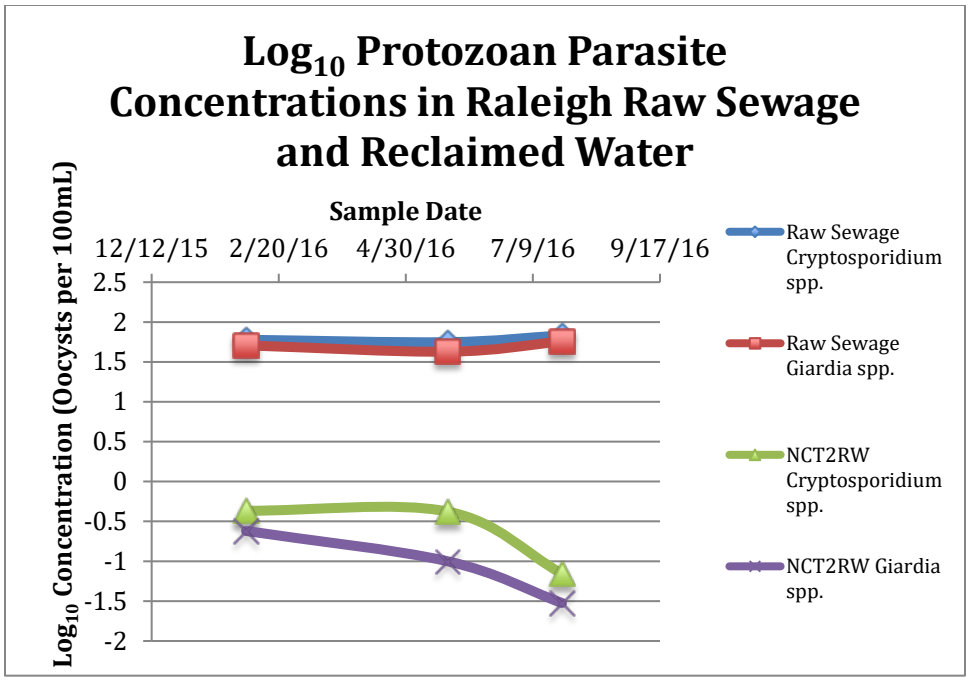

Figure 35: Log_{10} protozoan parasite concentrations in Raleigh raw sewage and dual disinfected, tertiary treated reclaimed water for 3 sample pairs

In Figure 35 the log_{10} concentrations of both *Cryptosporidium* spp. and *Giardia* spp. are displayed for Raleigh raw sewage and tertiary treated, dual disinfected reclaimed water. Based on the results in this figure and the data presented in Table 10, the log_{10} reductions for *Cryptosporidium* oocysts were approximately 2 to 3, while the log_{10} reductions for *Giardia* cysts were approximately 2.3 to 3.3. Detectable levels of both Cryptosporidium oocysts and Giardia cysts were still present in reclaimed water samples.

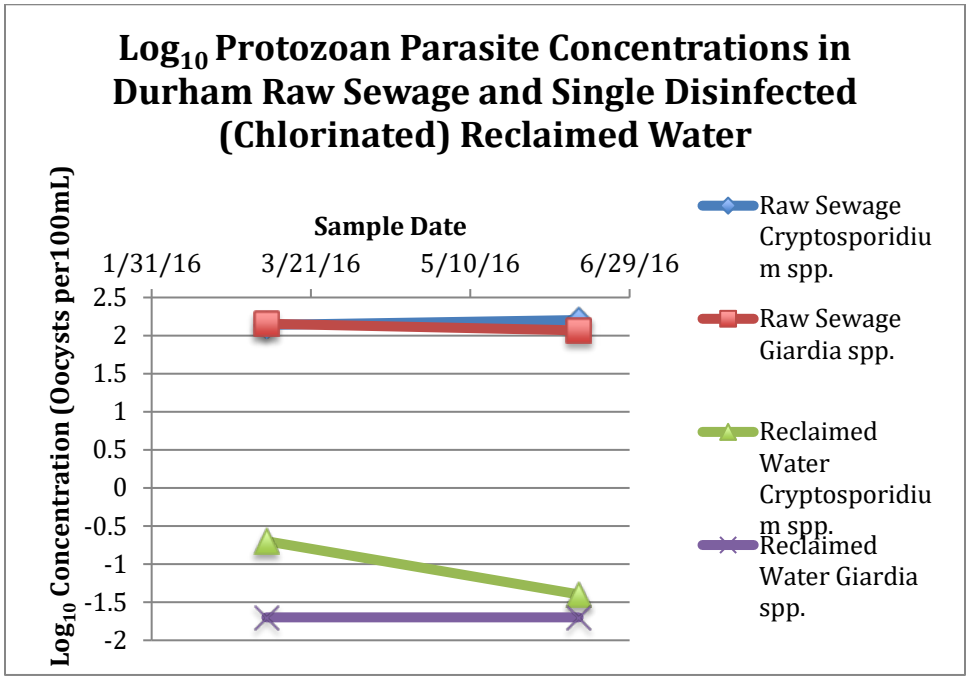

Figure 36: Log_{10} protozoan parasite concentrations s in Durham raw sewage and tertiary treated, chlorinated reclaimed water for 2 sample pairs

In Figure 36 the log_{10} concentrations of both *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts are displayed for Durham raw sewage and chlorine disinfected reclaimed water. Based on the results in this figure and the data presented in Table 10, the log_{10} reductions for *Cryptosporidium* were approximately 3 to 3.5, while the log_{10} reductions for *Giardia* were approximately 3.8. Low but detectable levels of both *Cryptosporidium* and *Giardia* were detected in all samples of Durham reclaimed water analyzed on these dates.

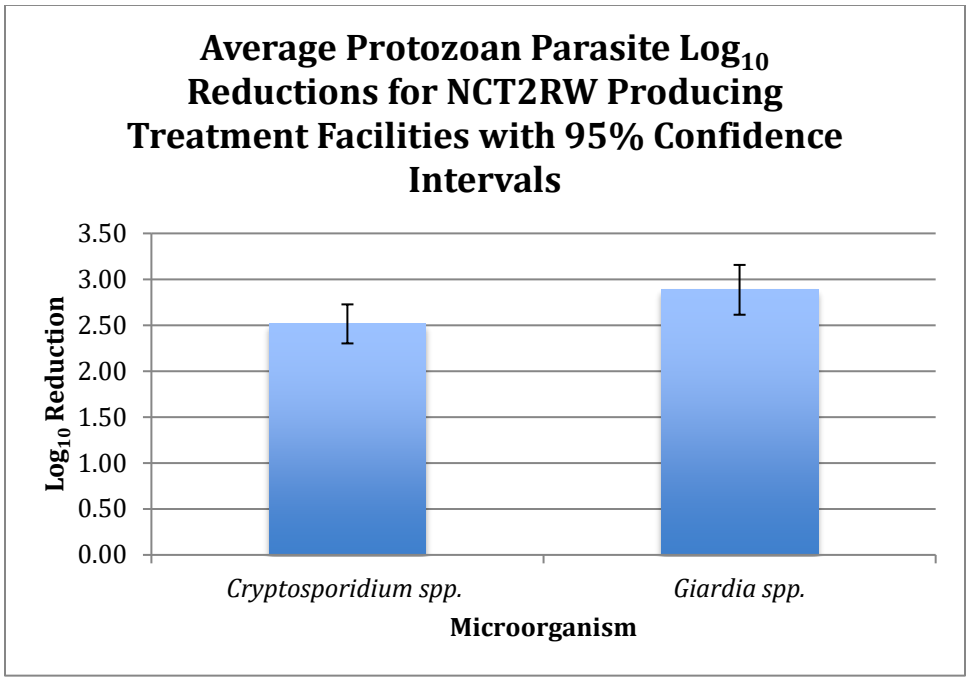

Figure 37: Average log_{10} reductions for protozoan parasites from NCT2RW treatment plants, shown with 95% confidence intervals

In Figure 37 the average log_{10} reductions for *Cryptosporidium* spp. oosysts and *Giardia* spp. cysts for tertiary treated and dual disinfected NCT2RW samples are shown with 95% confidence intervals. Based on the results this figure, the average log_{10} reduction for *Cryptosporidium* spp. was approximately 2.5, while the average log_{10} reduction for *Giardia* spp. was approximately 2.9. Low but detectable levels of *Cryptosporidium* oocysts and *Giardia* cysts were detected in nearly all reclaimed water samples. These results indicate the log_{10} performance of wastewater reclamation treatment facilities studied, which is lower than the target 4 log10 reduction for NCT2RW.

3.3.2 Surface Water Analysis

3.3.2.1 Bacterial Indicators

Table 11: *E. coli* and *Enterococcus* spp. Concentrations in Surface Water Samples

Date	Sample	*E. coli* MPN Concentration/100 mL (Lower 95% Confidence Interval, Upper 95% Confidence Limit)	*Enterococcus* MPN Concentration/100 mL(Lower 95% Confidence Interval, Upper 95% Confidence Limit)
7.13.15	Neuse River at Smithfield	4110 (2785, 5825)	117.8 (86.3, 158.2)
9.22.15	Falls Lake	<1 (0.0, 3.7)	<1 (0.0, 3.7)
9.22.15	Jordan Lake	1 (0.05, 4.6)	<1 (0.0, 3.7)
10.19.15	Eno River at Hillsborough	<1 (0.0, 3.7)	<1 (0.0, 3.7)
11.2.15	Smithfield Reservoir	10 (1, 55)	34.8 (23.5, 49.6)
11.2.15	Neuse River at Smithfield	4.1 (1.2, 9.1)	94 (64.2, 133.9)
11.8.15	Eno River at Hillsborough	139.1 (99.1, 190.8)	35.1 (23.3, 50.6)
11.16.15	Falls Lake	1 (0.1, 5.5)	<1 (0.0, 3.7)
11.17.15	Jordan Lake	1 (0.3, 6.5)	<1 (0.0, 3.7)
1.18.16	Eno River at Hillsborough	19 (11, 29.8)	4.1 (1.2, 9.8)
2.8.16	Jordan Lake	<1 (0.0, 3.7)	<1 (0.0, 3.7)
2.9.16	Falls Lake	33.9 (21.9, 49.7)	22.8 (39.1, 30.7)
2.23.16	Eno River at Hillsborough	66.5 (47.4, 91.2)	3.6 (1.3, 9.5)
2.29.16	Neuse River at Smithfield	24.4 (15.1, 37.0)	2.5 (0.5, 7.4)
5.29.16	Eno River at Hillsborough	5.8 (2.7, 12.2)	6.9 (3.1, 13.8)
5.31.16	Falls Lake	<1 (0.0, 3.7)	<1 (0.0, 3.7)
6.7.16	Jordan Lake	4.7 (1.8, 10.2)	1 (0.05, 4.6)
6.19.16	Eno River at Hillsborough	10.8 (5.2, 18.7)	1 (0.05, 4.6)
6.27.16	Jordan Lake	<1 (0.0, 3.7)	1 (0.05, 4.6)
7.15.16	Neuse River at Smithfield	7.4 (3.2, 14.4)	81.2 (58.6, 109.5)
7.15.16	Smithfield Reservoir	1 (0.1, 5.5)	1 (0.1, 5.5)
8.2.16	Falls Lake	2.6 (0.5, 8)	2 (0.4, 5.6)

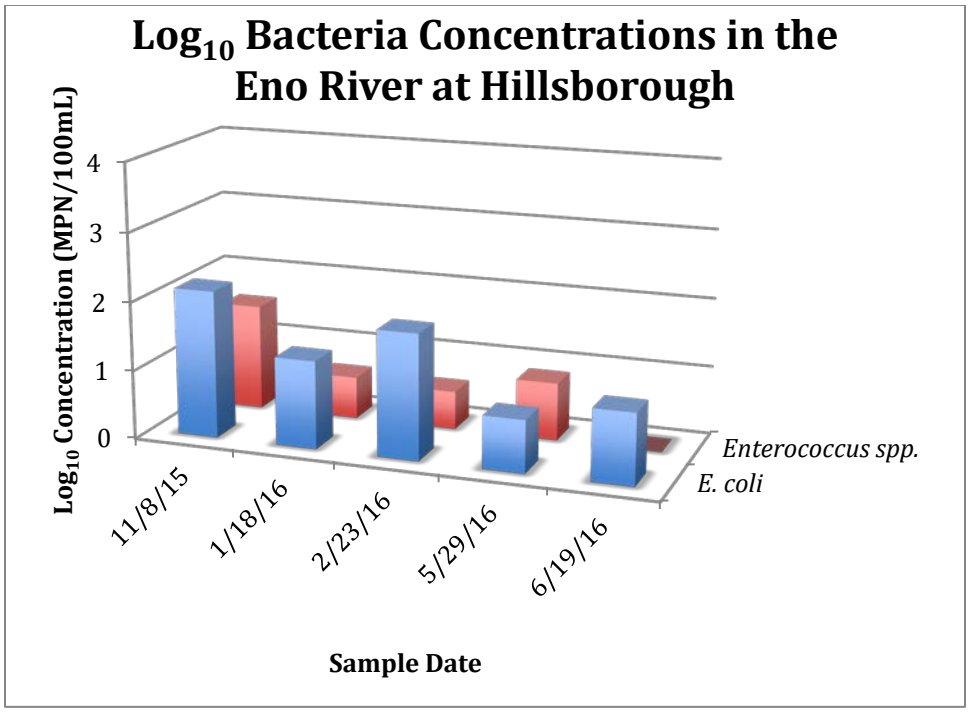

Figure 38: Log_{10} *E. coli* and *Enterococcus* spp. Concentrations in the Eno River at Hillsborough

In Figure 38 are shown the log_{10} concentrations of fecal indicator bacteria in the Eno River at Hillsborough. This water is used as the influent source water for the city of Hillsborough, NC water treatment plant. Based on the data presented in the figure above and in Table 11, the average log_{10} *E. coli* and *Enterococcus* spp. concentrations in the Eno River were 1.4 and 0.7 log_{10}/100 mL, respectively. For the sample analyzed on 6/19/16, no *Enterococcus* spp. was detected so the lower detection limit value (1MPN/100mL) is presented in Figure 38.

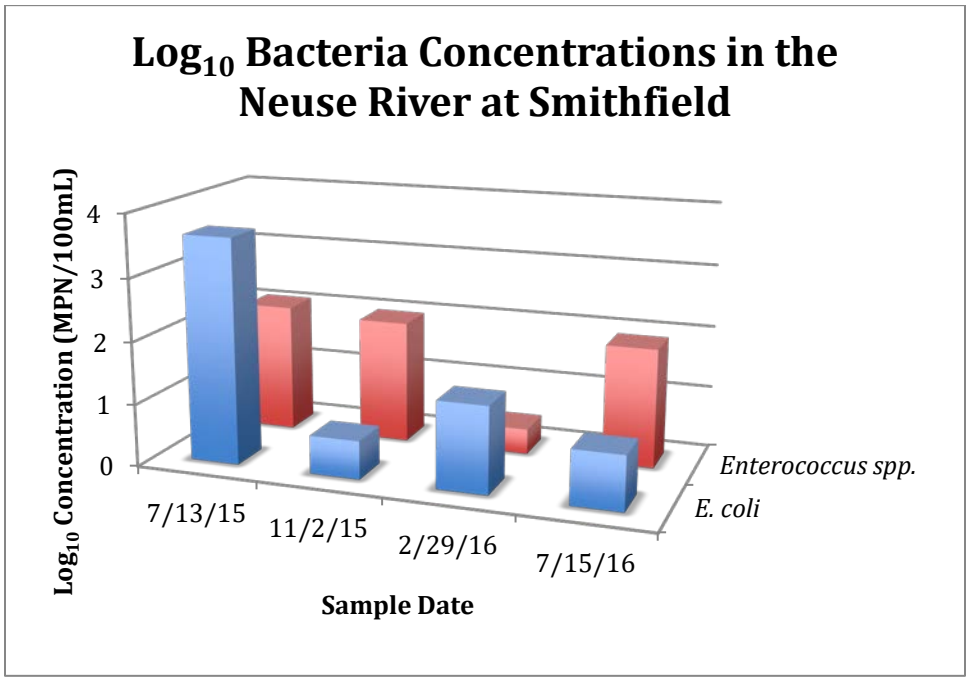

Figure 39: Log$_{10}$ *E. coli* and *Enterococcus* spp. Concentrations in the Neuse River at Smithfield

In Figure 39 are displayed the log$_{10}$ concentrations of fecal indicator bacteria in the Neuse River at Smithfield., This water was used as the influent source water for the city of Smithfield, NC water treatment plant on 7/13/15 and 2/29/15, when these samples were collected. Based on the data presented in the figure above and in Table 11, the average *E. coli* and *Enterococcus* spp. concentrations in the Neuse River were 1.62 and 1.59 log$_{10}$/100 mL, respectively.

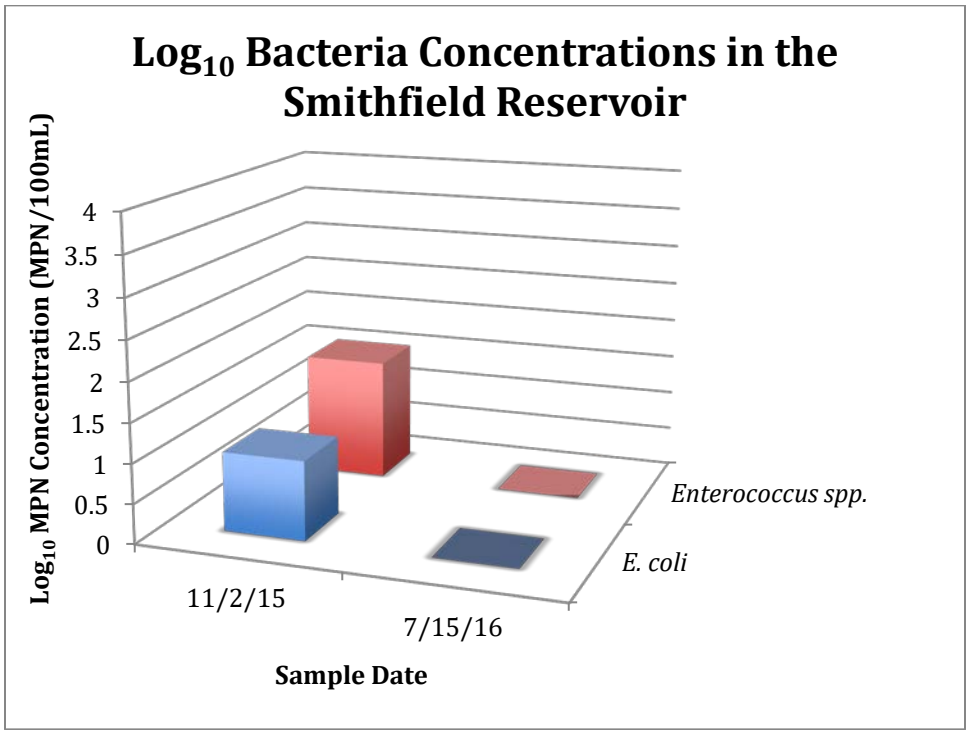

Figure 40: Log$_{10}$ *E. coli* and *Enterococcus* spp. Concentrations in the Smithfield Reservoir

In Figure 40 are shown the log$_{10}$ concentrations of fecal indicator bacteria in the Neuse River at Smithfield. , This water was used as the influent source water for the city of Smithfield, NC on the dates tested above. Based on the data presented in the figure above and in Table 11, the average *E. coli* and *Enterococcus* spp. concentrations in the Neuse River were 0.5 and 0.77 log$_{10}$/100 mL, respectively. In the samples analyzed on 7/15/16, no *E. coli* or *Enterococcus* spp. were detected, so the lower detection limit value (1 MPN/100mL) is presented in Figure 40.

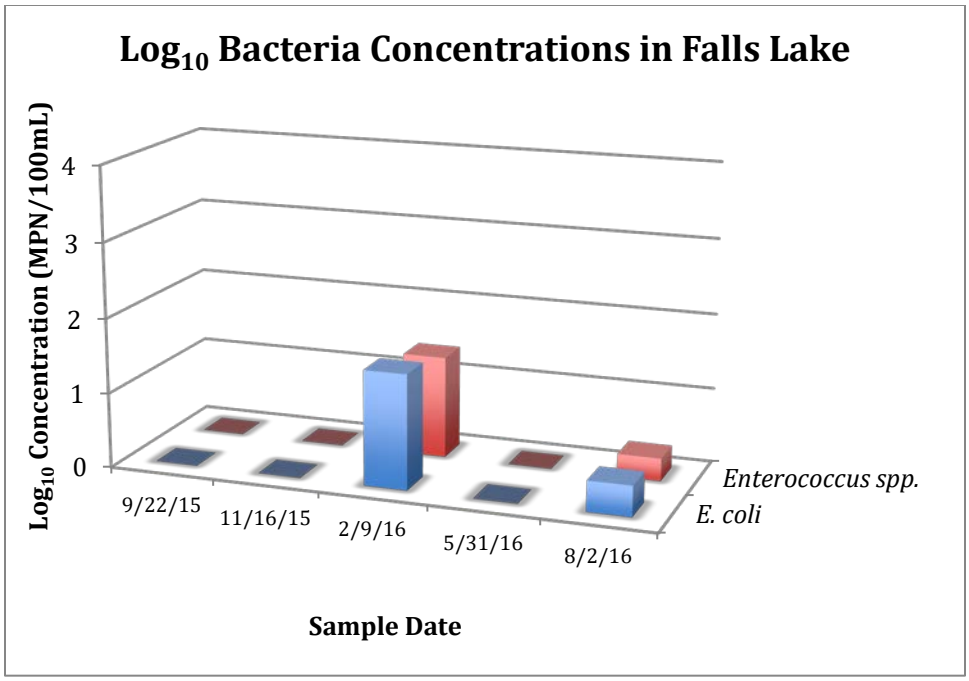

Figure 41: Log_{10} *E. coli* and *Enterococcus* spp. Concentrations in Falls Lake

In Figure 41 are shown the log_{10} concentrations of fecal indicator bacteria in Falls Lake. This water was used as the influent source water for the city of Raleigh, NC at the time of sample collection. Based on the data presented in the figure above and in Table 11, the average *E. coli* and *Enterococcus* spp. concentrations in the Neuse River were 0.39 and 0.33 log_{10}/100 mL,respectively. In many of the samples analyzed, no *E. coli* or *Enterococcus* spp. were detected, so the detection limit (1 MPN/100mL) is presented in Figure 41.

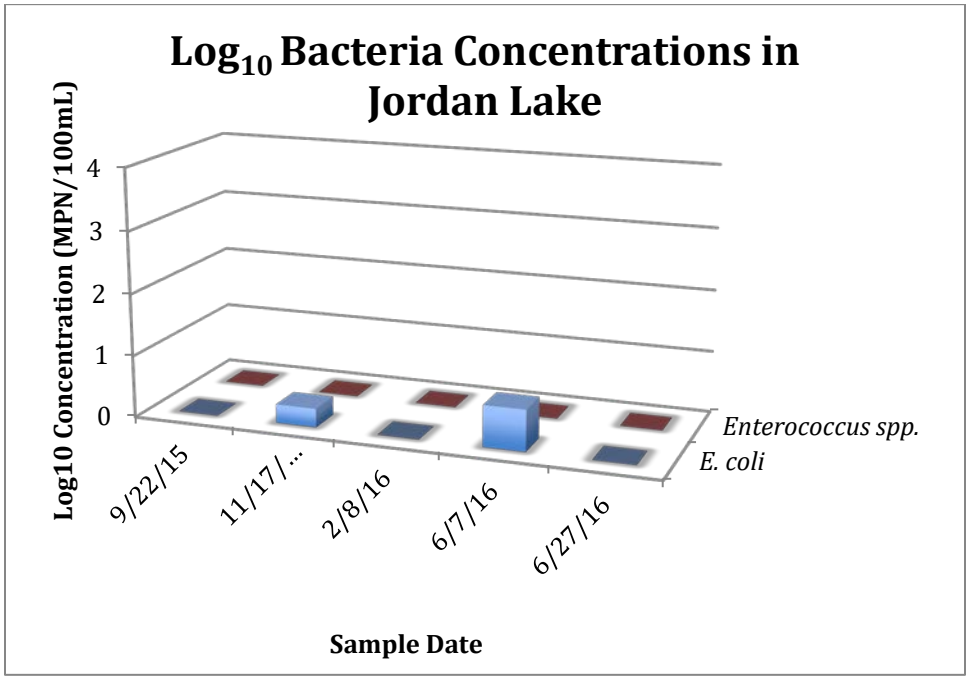

Figure 42: Log_{10} *E. coli* and *Enterococcus* spp. Concentrations in Jordan Lake

In Figure 42 are shown the $\log_{10}$ concentrations of fecal indicator bacteria in Jordan Lake. This water was used as the influent source water for the cities of Cary and Apex, NC when the samples were collected. Based on the data presented in the figure above and in Table 11, the average *E. coli* concentration was 0.19 $\log_{10}$/100 mL. No *Enterococcus* spp. was detected in these samples, and in many samples no *E. coli* was detected, so the lower detection limit vale (1 MPN/100mL) is presented in Figure 42.

3.3.2.2 Coliphage Viruses

Table 12: Coliphage Concentrations in Surface Water Samples

Date	Sample	Somatic Coliphage (PFU/100mL)	F+/Male Specific Coliphage (PFU/100mL)	Total Coliphage (PFU/100mL)
7.13.15	Neuse River at Smithfield	96	2	95
9.22.15	Falls Lake	<1	<1	<1
9.22.15	Jordan Lake	<1	<1	<1
10.19.15	Eno River at Hillsborough	<1	<1	<1
11.2.15	Smithfield Reservoir	17	3	16
11.2.15	Neuse River at Smithfield	179	3	175
11.8.15	Eno River at Hillsborough	16	<1	12
11.16.15	Falls Lake	<1	1	<1
11.17.15	Jordan Lake	1	<1	2
1.18.16	Eno River at Hillsborough	35	1	48
2.8.16	Jordan Lake	20	3	21
2.9.16	Falls Lake	53	5	55
2.23.16	Eno River at Hillsborough	27	<1	17
2.29.16	Neuse River at Smithfield	13	<1	19
5.29.16	Eno River at Hillsborough	18	<1	13
5.31.16	Falls Lake	13	<1	8
6.7.16	Jordan Lake	<1	<1	4
6.19.16	Eno River at Hillsborough	4	<1	2
6.27.16	Jordan Lake	2	<1	<1

7.15.16	Neuse River at Smithfield	27	<1	37
7.15.16	Smithfield Reservoir	9	<1	9
8.2.16	Falls Lake	2	<1	7

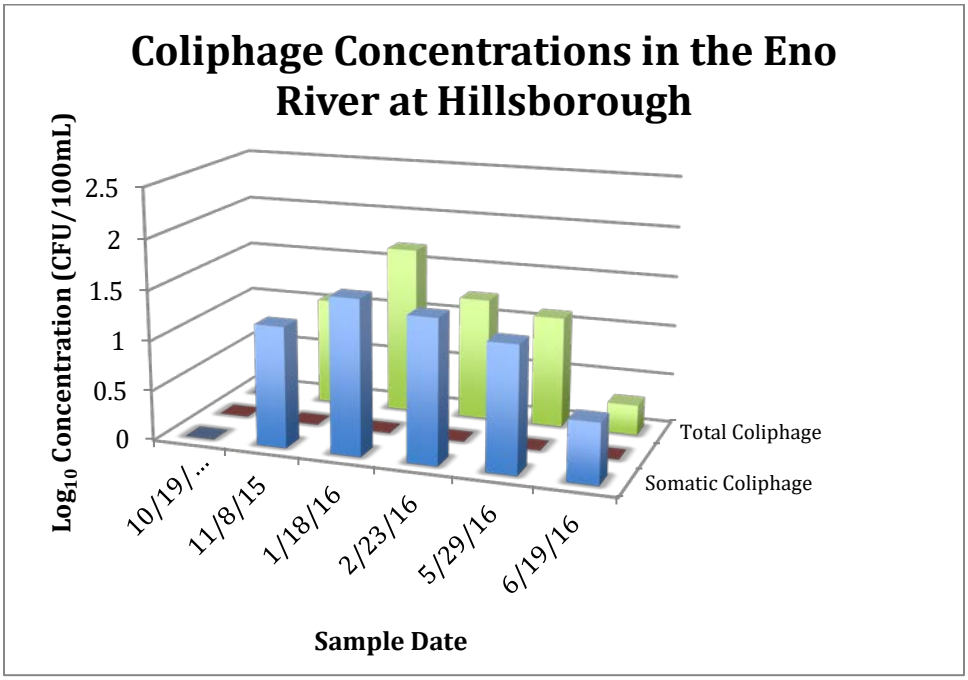

Figure 43: Log_{10} Coliphage Concentrations in the Eno River at Hillsborough

In Figure 43 are shown the log_{10} concentrations of coliphage viruses in the Eno River at Hillsborough. Based on the data presented in the figure above and in Table 12, the average log_{10} concentrations of somatic, and total coliphages were 1.01 and 0.90/100 mL, respectively. For the samples presented here, no F+ coliphage viruses were detected, so the lower detection limit value (1MPN/100mL) is presented in the figure above.

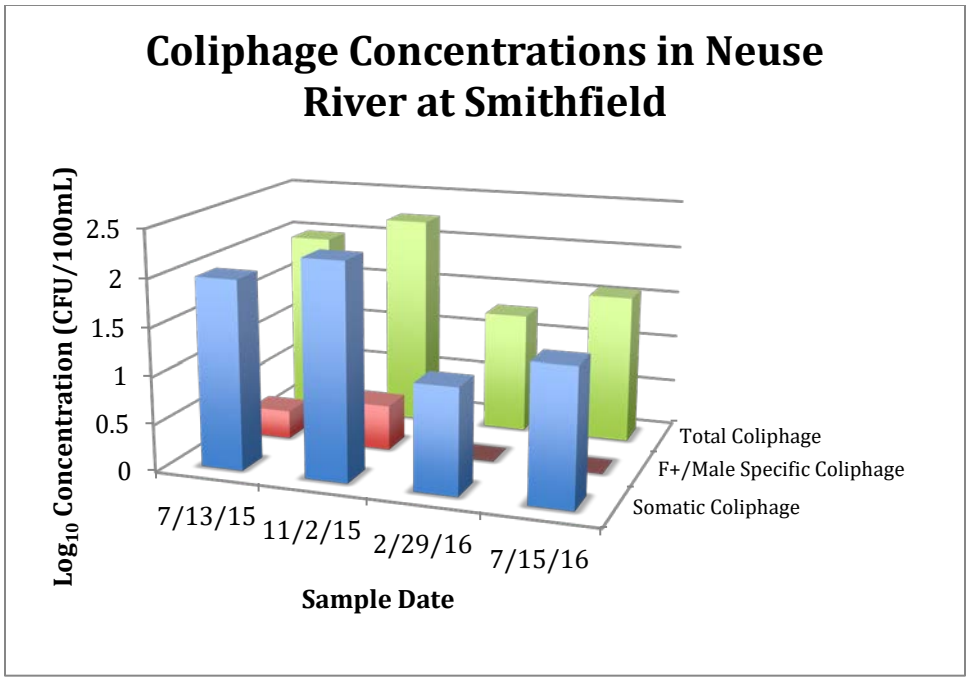

Figure 44: Log_{10} Coliphage Concentrations in the Neuse River at Smithfield

In Figure 44 are shown the log_{10} concentrations of coliphage viruses in the Neuse River at Smithfield. Based on the data presented in the figure above and in Table 12, the average log_{10} concentrations of somatic, F+ and total coliphages were 1.70, 0.19, and 1.77/100 ml, respectively. For the F+ coliphage samples analyzed on 2/29/16 and 7/15/16, no coliphages were detected, so the lower detection limit vlue (1MPN/100mL) is presented in the figure above.

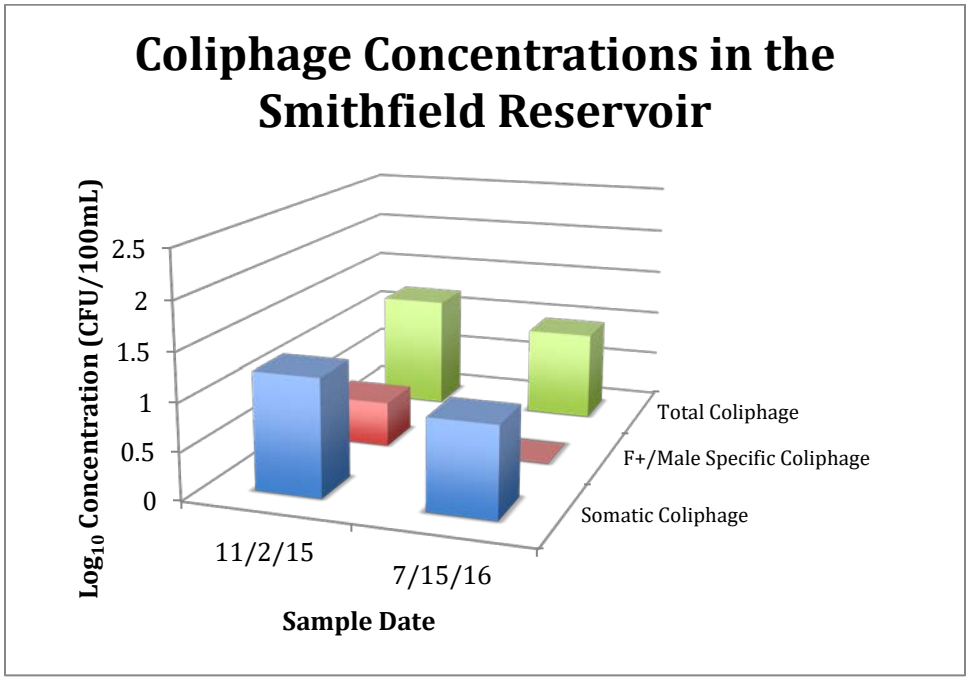

Figure 45: Log_{10} Coliphage Concentrations in the Smithfield Reservoir

In Figure 45 are shown the $\log_{10}$ concentrations of coliphage viruses in the Smithfield Reservoir. Based on the data presented in the figure above and in Table 12, the average $\log_{10}$ concentrations of somatic, F+ and total coliphages were 1.09, 0.24, and 1.08/100 mL, respectively. For the F+ coliphage samples analyzed on 7/15/16, no coliphages were detected, so the lower detection limit value (1MPN/100mL) is presented in the figure above.

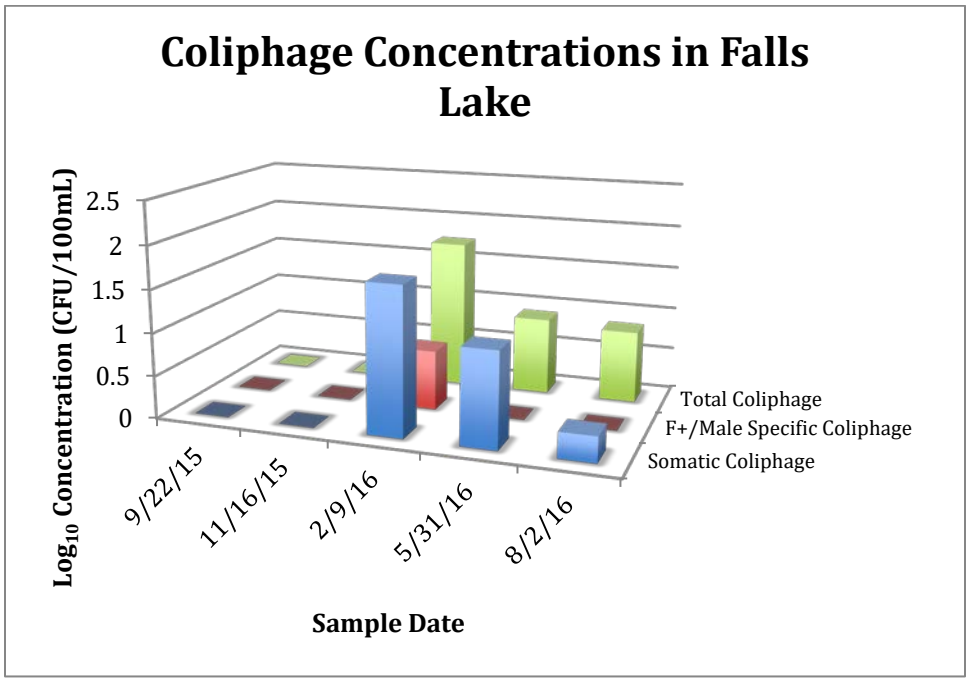

Figure 46: Log_{10} Coliphage Concentrations in Falls Lake

In Figure 46 are shown the $\log_{10}$ concentrations of coliphage viruses in Falls Lake. Based on the data presented in the figure above and in Table 12, the average $\log_{10}$ concentrations of somatic, F+ and total coliphages were 0.63, 0.14, and 0.70/100 mL, respectively. In many of the samples analyzed, no coliphage viruses were detected, so the lower detection limit value (1 MPN/100mL) is presented in Figure 46.

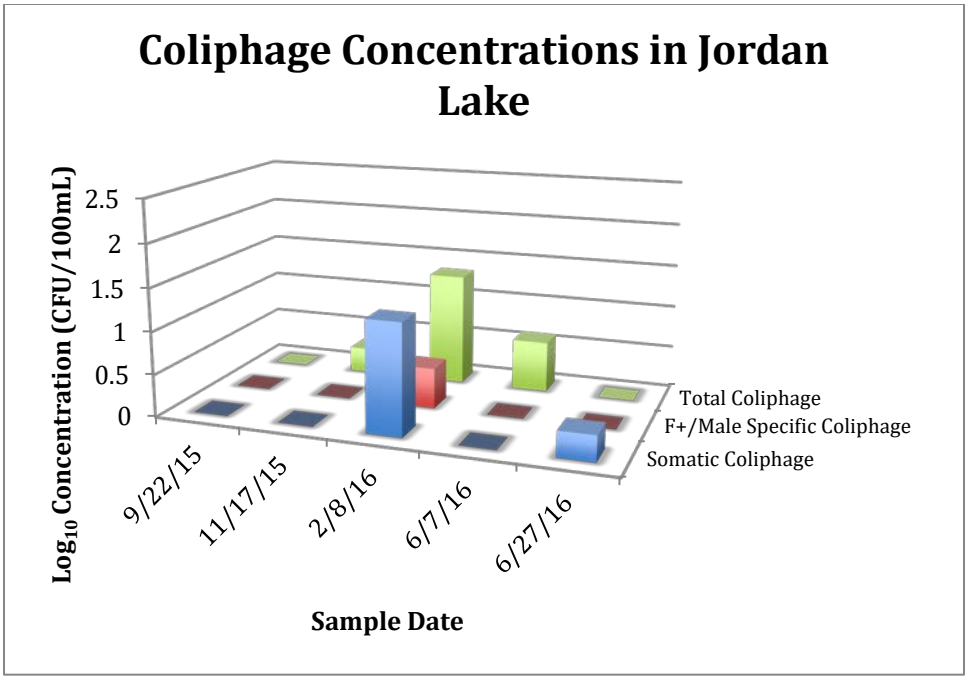

Figure 47: Log_{10} Coliphage Concentrations in Jordan Lake

In Figure 47 are shown the log_{10} concentrations of coliphage viruses in Jordan Lake. Based on the data presented in the figure above and in Table 12, the average log_{10} concentrations of somatic, F+ and total coliphages were 0.32, 0.10, and 0.45/100 mL, respectively. In many of the samples analyzed, no coliphage viruses were detected, so the lower detection limit value (1 MPN/100mL) is presented in the figure above.

3.3.2.3 Protozoan Parasite Surrogates

Table 13: *C. perfringens* Concentrations in Surface Water Samples

Date	Sample	Pasteurized *C. perfringens* Concentrations (CFU/100mL)	Unpasteurized *C. perfringens* Concentrations (CFU/100mL)
7.13.15	Neuse River at Smithfield	-	-
9.22.15	Falls Lake	1.11	1.11
9.22.15	Jordan Lake	4.44	3.33
10.19.15	Eno River at Hillsborough	31.11	22.22
11.2.15	Smithfield Reservoir	17.78	10.00
11.2.15	Neuse River at Smithfield	145.56	101.11
11.8.15	Eno River at Hillsborough	65.56	62.22
11.16.15	Falls Lake	12.22	16.67

11.17.15	Jordan Lake	12.22	16.67
1.18.16	Eno River at Hillsborough	27.78	22.22
2.8.16	Jordan Lake	108.33	84.44
2.9.16	Falls Lake	80.00	60.00
2.23.16	Eno River at Hillsborough	50.00	61.11
2.29.16	Neuse River at Smithfield	145.56	123.33
5.29.16	Eno River at Hillsborough	68.89	44.44
5.31.16	Falls Lake	10.00	7.78
6.7.16	Jordan Lake	71.11	66.67
6.19.16	Eno River at Hillsborough	112.22	77.78
6.27.16	Jordan Lake	41.11	43.33
7.15.16	Neuse River at Smithfield	300	444.44
7.15.16	Smithfield Reservoir	180.9	394.44
8.2.16	Falls Lake	20	15.56

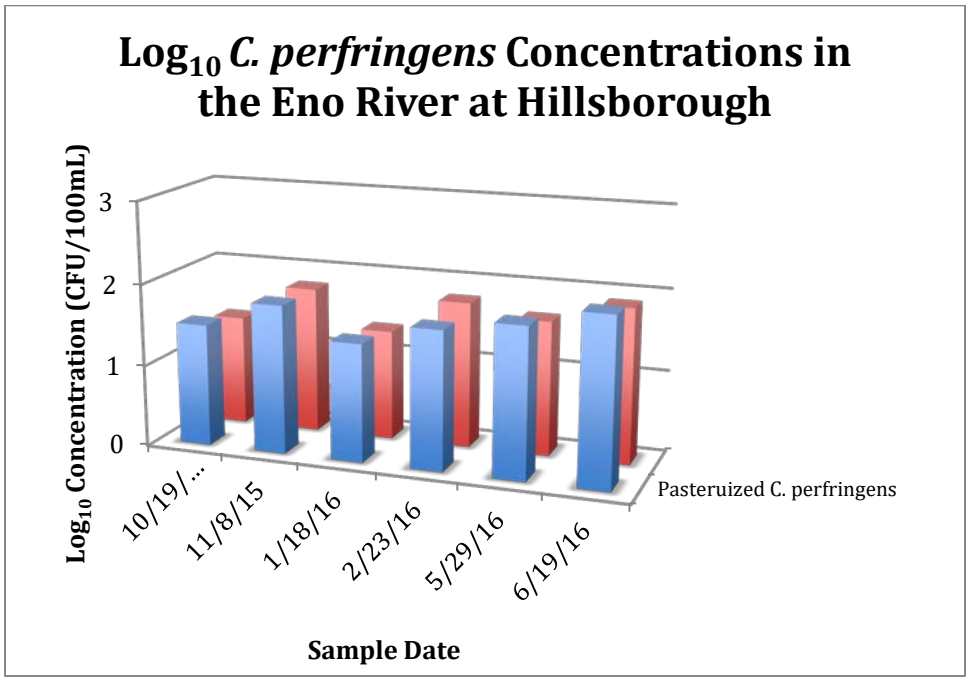

Figure 48: Log_{10} *C. perfringens* Concentrations in the Eno River at Hillsborough

In Figure 48 are shown the log_{10} concentrations of pasteurized and unpasteurized *C. perfringens* in the Eno River at Hillsborough. Based on the data presented in the figure above and in Table 13, the average log_{10} concentrations of pasteurized and unpasteurized *C. perfringens* were 1.72 and 1.64 log_{10}/100 mL, respectively.

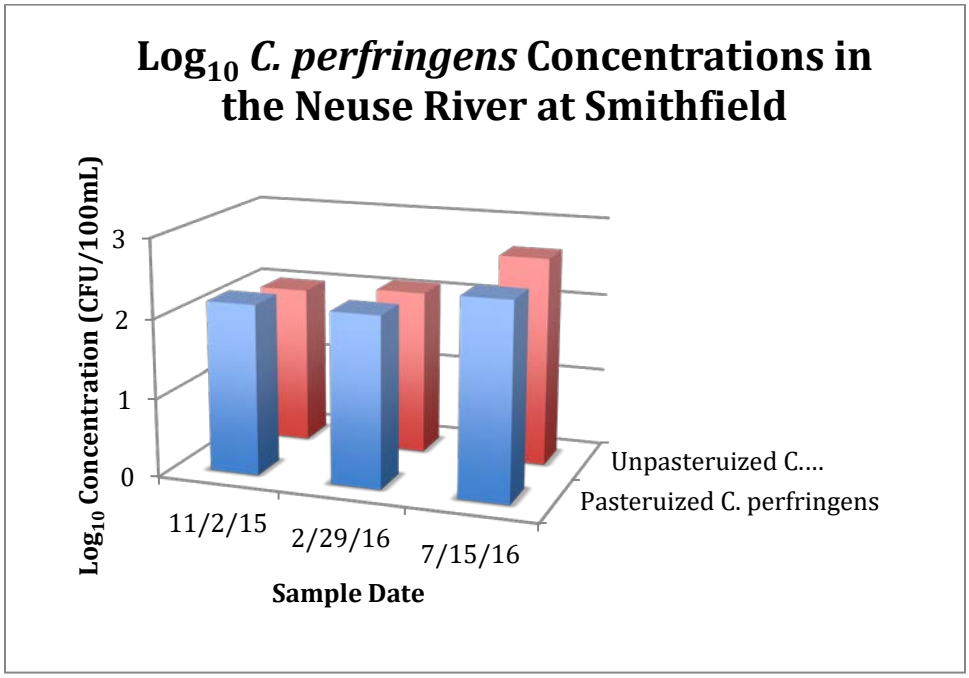

Figure 49: Log_{10} *C. perfringens* Concentrations in the Neuse River at Smithfield

In Figure 49 are shown the log_{10} concentrations of pasteurized and unpasteurized *C. perfringens* in the Neuse River at Smithfield. Based on the data presented in the figure above and in Table 13, the average log_{10} concentrations of pasteurized and unpasteurized *C. perfringens* were 2.27 and 2.25 log_{10}/100 mL, respectively.

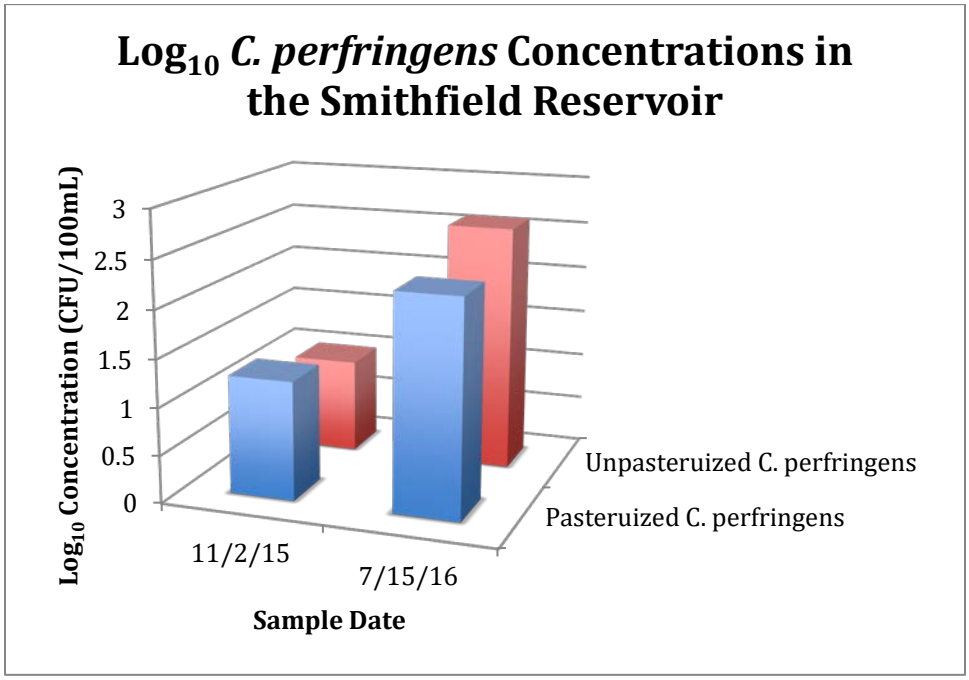

Figure 50: Log_{10} *C. perfringens* Concentrations in the Smithfield Reservoir

In Figure 50 are shown the $\log_{10}$ concentrations of pasteurized and unpasteurized *C. perfringens* in the Smithfield Reservoir. Based on the data presented in the figure above and in Table 13, the average $\log_{10}$ concentrations of pasteurized and unpasteurized *C. perfringens* were 1.75 and 1.80 $\log_{10}$/100 mL, respectively.

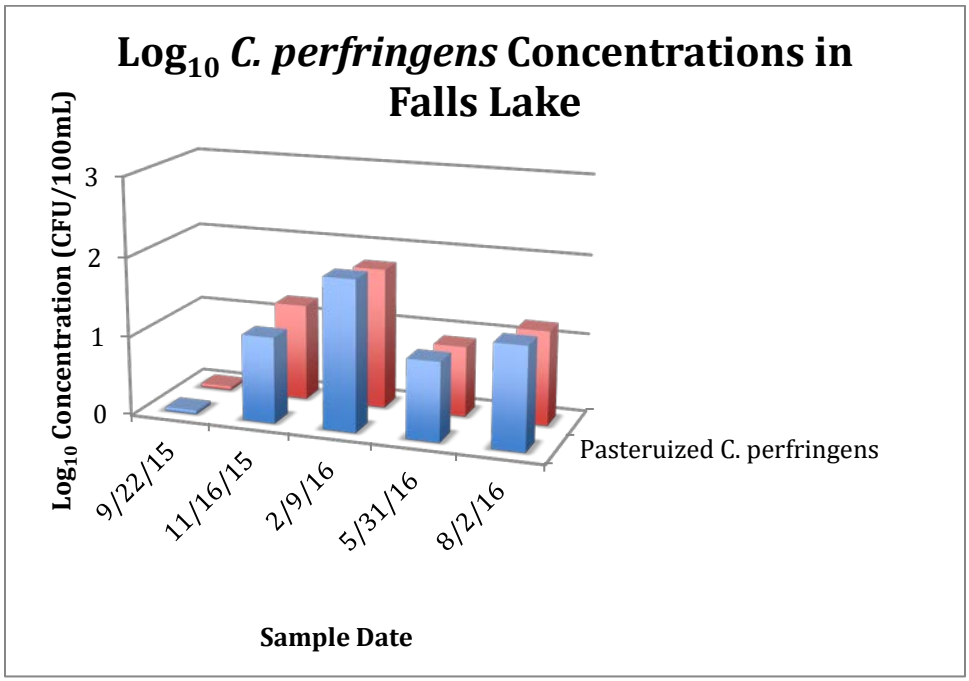

Figure 51: Log_{10} *C. perfringens* Concentrations in Falls Lake

In Figure 51 are shown the $\log_{10}$ concentrations of pasteurized and unpasteurized *C. perfringens* in Falls Lake. Based on the data presented in the figure above and in Table 13, the average $\log_{10}$ concentrations of pasteurized and unpasteurized *C. perfringens* were 1.07 and 1.03 $\log_{10}$/100 mL, respectively. In the sample analyzed on 9/22/15 no *C. perfringens* of any type was detected in Falls lake samples, so the lower detection limit value (1.11 CFU/100mL) is presented in the figure above.

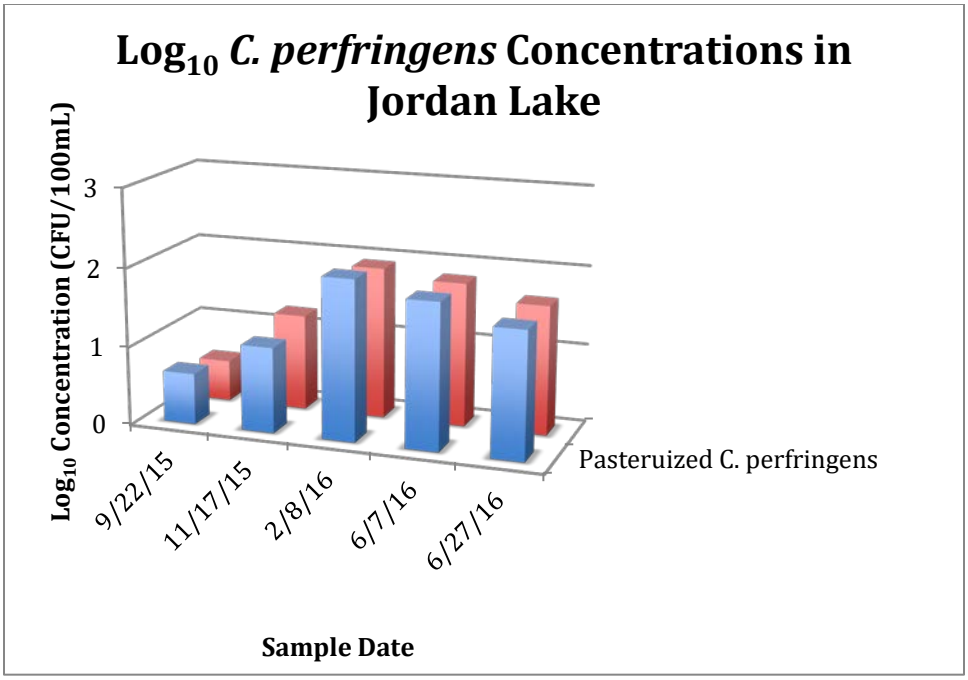

Figure 52: Log_{10} *C. perfringens* Concentrations in Jordan Lake

In Figure 52 are shown the log_{10} concentrations of pasteurized and unpasteurized *C. perfringens* in Jordan Lake. Based on the data presented in the figure above and in Table 13, the average log_{10} concentrations of pasteurized and unpasteurized *C. perfringens* were 1.45 and 1.43 log_{10}/100 mL, respectively.

3.3.2.4 Pathogenic Bacteria

Table 14: *Salmonella* spp. Concentrations in Surface Water Samples

Date	Sample	*Salmonella* spp. MPN Concentration/100mL Vol. (Lower 95% Confidence Interval, Upper 95% Confidence Interval)
7.13.15	Neuse River at Smithfield	8000 (2200, 29000)
9.22.15	Falls Lake	0.12 (0.017, 0.84)
9.22.15	Jordan Lake	0.95 (0.26, 3.4)
10.19.15	Eno River at Hillsborough	0.1 (0.013, 0.82)
11.2.15	Smithfield Reservoir	0.68 (0.2, 2.4)
11.2.15	Neuse River at Smithfield	89 (25, 320)
11.8.15	Eno River at Hillsborough	0.1 (0.013, 0.82)
11.16.15	Falls Lake	0.1 (0.013, 0.82)
11.17.15	Jordan Lake	0.77 (0.22, 2.7)

1.18.16	Eno River at Hillsborough	0.37 (0.099, 1.4)
2.8.16	Jordan Lake	0.31 (0.076, 1.2)
2.9.16	Falls Lake	0.12 (0.017, 0.84)
2.23.16	Eno River at Hillsborough	0.5 (0.14, 1.8)
2.29.16	Neuse River at Smithfield	0.12 (0.017, 0.84)
5.29.16	Eno River at Hillsborough	0.24 (0.054, 1.1)
5.31.16	Falls Lake	0.77 (0.22, 2.7)
6.7.16	Jordan Lake	0.24 (0.054, 1.1)
6.19.16	Eno River at Hillsborough	0.12 (0.017, 0.84)
6.27.16	Jordan Lake	0.12 (0.017, 0.84)
7.15.16	Neuse River at Smithfield	1.2 (0.31, 4.7)
7.15.16	Smithfield Reservoir	0.25 (0.055, 1.1)
8.2.16	Falls Lake	0.77 (0.22, 2.7)

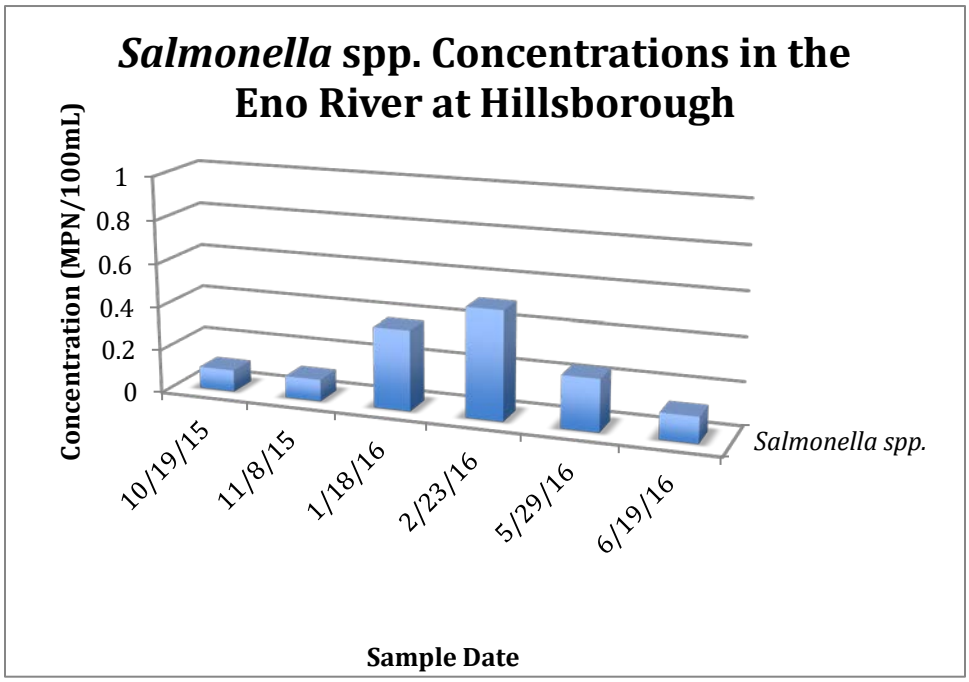

Figure 53: Concentrations of *Salmonella* spp. in the Eno River at Hillsborough

In Figure 53 are shown the concentrations of *Salmonella* spp. detected in the Eno River at Hillsborough between 10/19/15 and 6/19/16. Based on the data presented in the figure above and in Table 14, the average concentration of *Salmonella* spp. was 0.24 MPN/100mL. For the samples analyzed on 10/19/15 and 11/8/15, no *Salmonella* spp. was detected in surface water samples, so the lower detection limit value (0.1MPN/100mL is presented in the figure above.

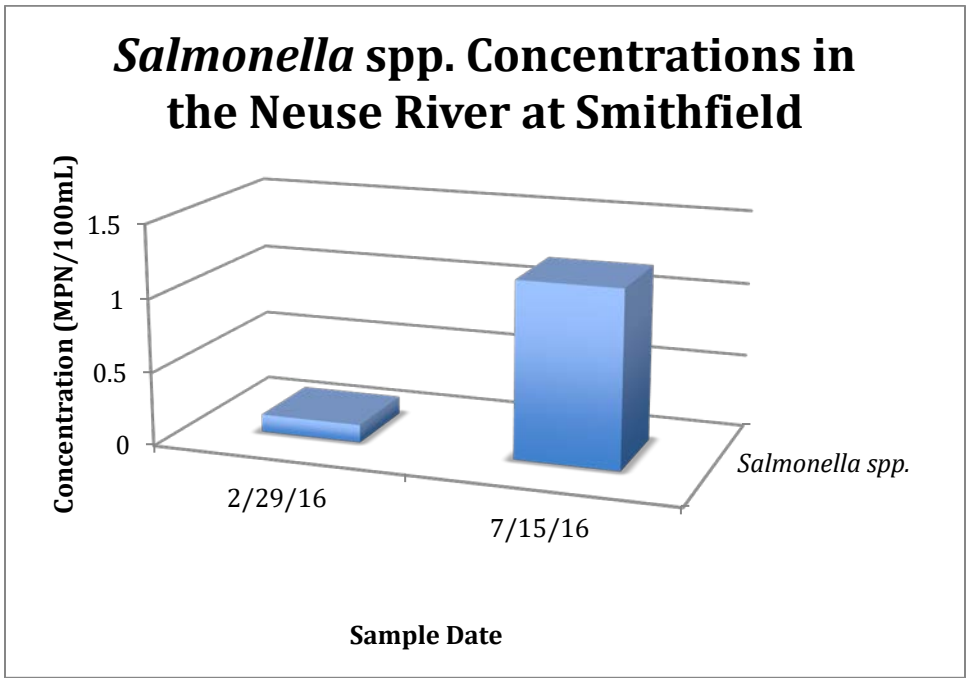

Figure 54: Concentrations of *Salmonella* spp. in the Neuse River at Smithfield

In Figure 54 are shown the concentrations of *Salmonella* spp. detected in the Neuse River at Smithfield on 2/29/16 and 7/15/16. Based on the data presented in the figure above and in Table 14, the average concentration of *Salmonella* spp. was 0.66 MPN/100mL.

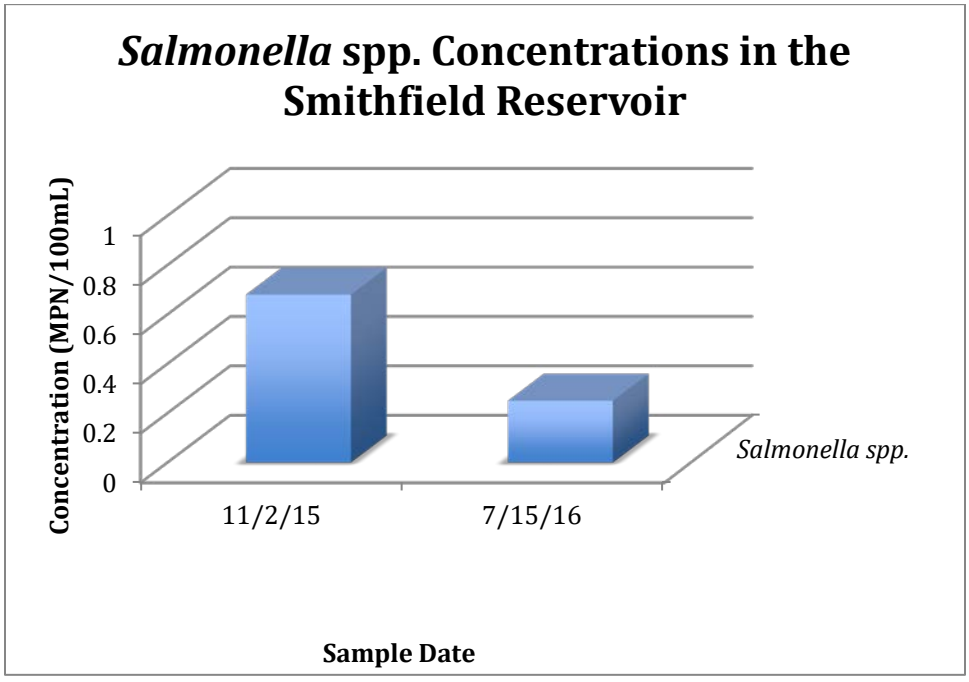

Figure 55: Concentrations of *Salmonella* spp. in the Smithfield Reservoir

In Figure 55 are shown the concentrations of *Salmonella* spp. detected in the Smithfield Reservoir on 11/2/15 and 7/15/16. Based on the data presented in the figure above and in Table 14, the average concentration of *Salmonella* spp. was 0.47 MPN/100mL.

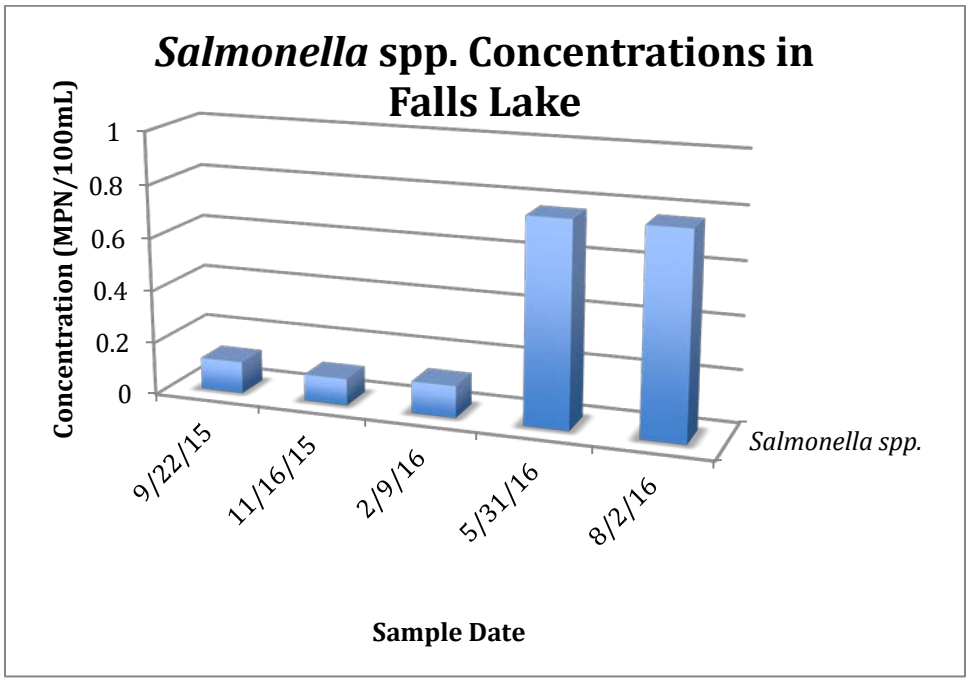

Figure 56: Concentrations of *Salmonella* spp. in Falls Lake

In Figure 56 are shown the concentrations of *Salmonella* spp. detected in Falls Lake between 9/22/15 and 8/2/16. Based on the data presented in the figure above and in Table 14, the average concentration of *Salmonella* spp. was 0.38 MPN/100mL. For the sample analyzed on11/16/15, no *Salmonella* spp. was detected, so the detection limit (0.1MPN/100mL is presented in the figure above.

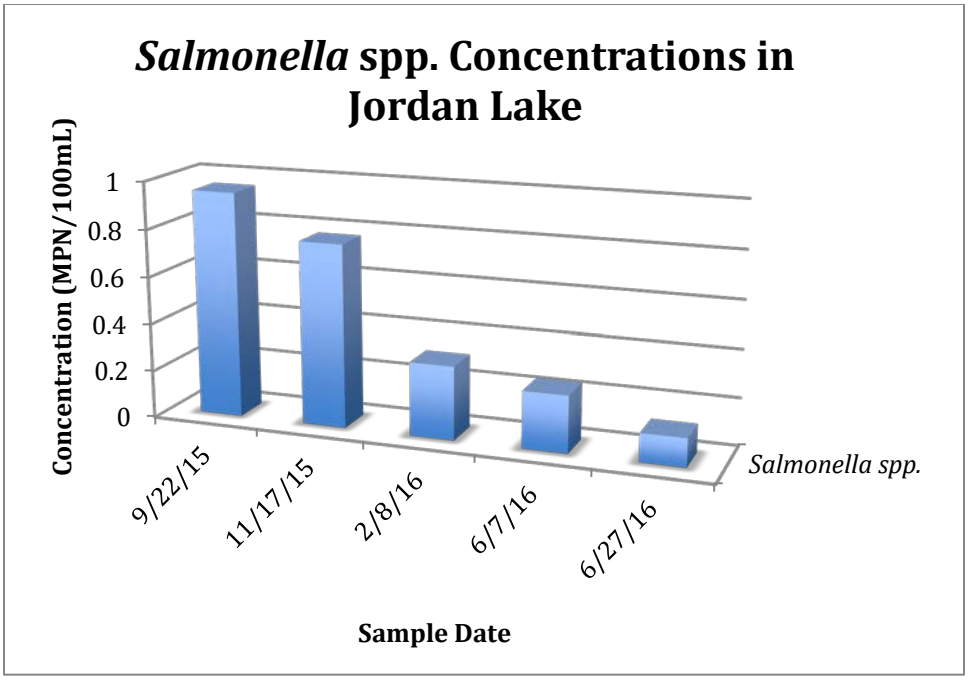

Figure 57: Concentrations of *Salmonella* spp. in Jordan Lake

In Figure 57 are shown the concentrations of *Salmonella* spp. detected in Jordan Lake between 9/22/15 and 6/27/16. Based on the data presented in the figure above and in Table 14, the average concentration of *Salmonella* spp. was 0.48 MPN/100mL.

3.3.2.5 Enteric Viruses

Table 15: Enteric Virus Concentrations in Surface Water Samples

Date	Sample	Norovirus (GEC/100mL)	Adenovirus (GEC/100mL)
7.13.15	Neuse River at Smithfield	ND	ND
9.22.15	Falls Lake	ND	ND
9.22.15	Jordan Lake	ND	ND
10.19.15	Eno River at Hillsborough	ND	1.28E+02
11.2.15	Smithfield Reservoir	ND	ND
11.2.15	Neuse River at Smithfield	ND	ND
11.8.15	Eno River at Hillsborough	ND	1.55E+02
11.16.15	Falls Lake	ND	ND
11.17.15	Jordan Lake	ND	ND
1.18.16	Eno River at Hillsborough	ND	ND

2.8.16	Jordan Lake	ND	ND
2.9.16	Falls Lake	ND	ND
2.23.16	Eno River at Hillsborough	ND	2.04E+04
2.29.16	Neuse River at Smithfield	ND	2.21E+04
5.29.16	Eno River at Hillsborough	ND	8.43E+03
5.31.16	Falls Lake	ND	ND
6.7.16	Jordan Lake	ND	ND
6.19.16	Eno River at Hillsborough	ND	2.68E+04
6.27.16	Jordan Lake	ND	1.36E+04
7.15.16	Neuse River at Smithfield	ND	ND
7.15.16	Smithfield Reservoir	ND	3.60E+04
8.2.16	Falls Lake	ND	1.70E+03

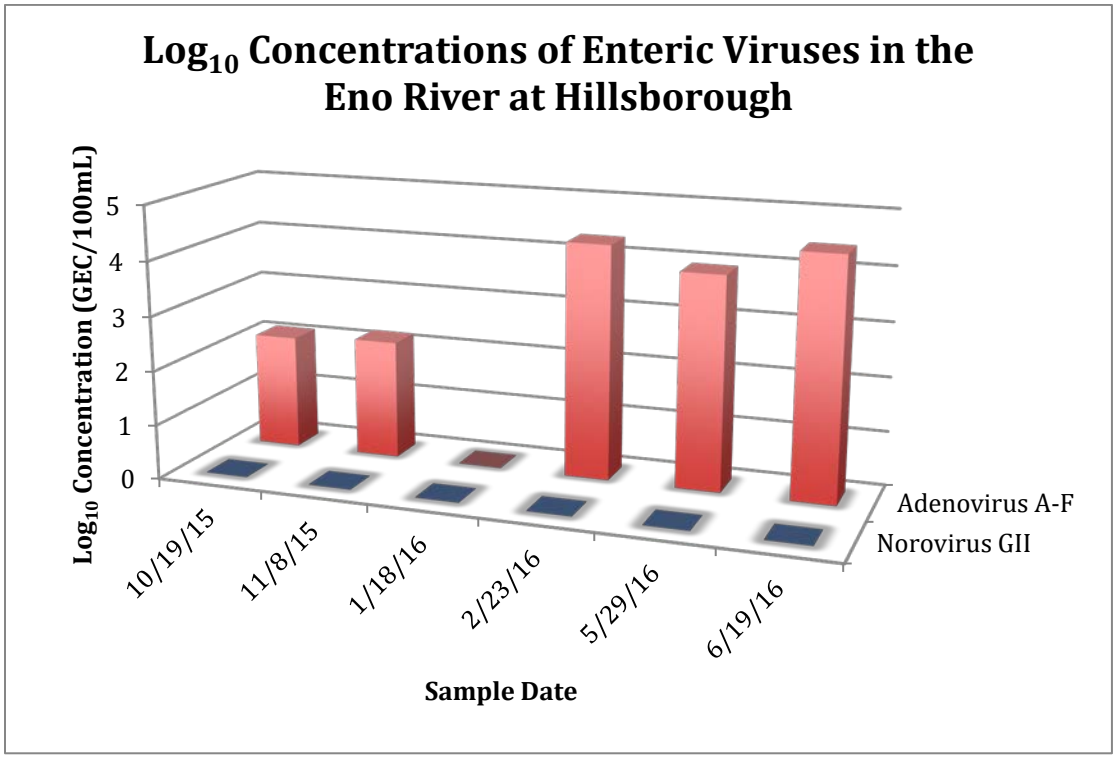

Figure 58: Log_{10} Concentrations of Enteric Virus Gene Copies/100 mL in the Eno River at Hillsborough

In Figure 58 are shown the concentrations of Norovirus GII and Adenovirus groups A-F gene copies/100 mL detected in the Eno River at Hillsborough between 10/19/15 and 6/19/16. Based on the data presented in the figure above and in Table 15, the average concentration of

adenovirus A-F gene copies detected was 2.83 GEC/100mL. No adenovirus was detected on 1/18/16, and no Norovirus was detected in any of the samples presented above. For samples where no enteric viruses were detected, the lower detection limit value (1 GEC/100mL) is presented in the figure above.

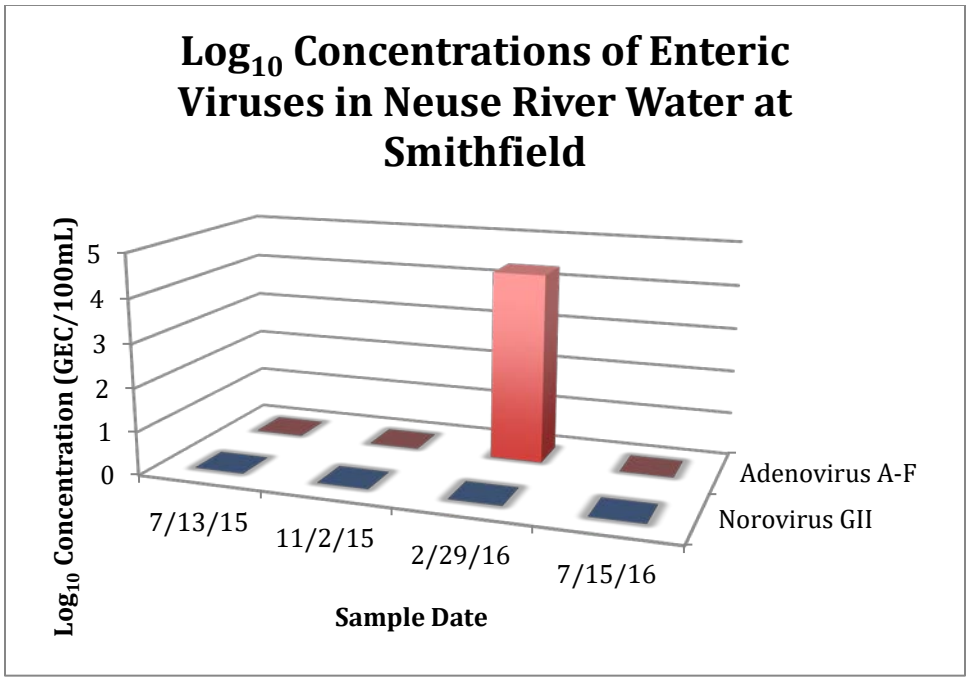

Figure 59: Log_{10} Concentrations of Enteric Virus Gene Copies/100 mLin the Neuse River at Smithfield

In Figure 59 are shown the concentrations of Norovirus GII and Adenovirus groups A-F gene copies per 100 mL detected in the Neuse River at Smithfield between 7/13/15 and 7/15/16. Based on the data presented in the figure above and in Table 15, the average concentration of adenovirus A-F gene copies detected was 1.09 GEC/100mL. Adenovirus was only detected on 2/29/16, and no Norovirus was detected in any of the samples presented above. For samples where no enteric viruses were detected, the lower detection limit value (1 GEC/100mL) is presented in the figure above.

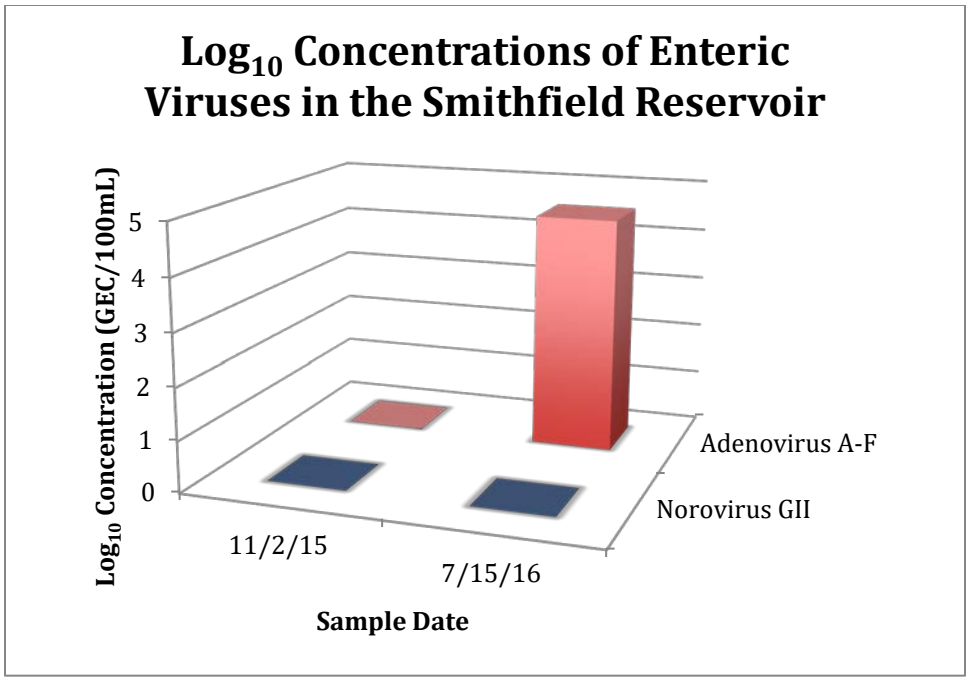

Figure 60: Log_{10} Concentrations of Enteric Virus Gene Copies/100 mLin the Smithfield Reservoir

In Figure 60 are shown the concentrations of Norovirus GII and Adenovirus groups A-F gene copies per 100 mL detected in the Smithfield Reservoir on 11/2/15 and 7/15/16. Based on the data presented in the figure above and in Table 15, the average concentration of adenovirus A-F detected was 2.28 GEC/100mL. Adenovirus was only detected on 7/15/16, and no Norovirus was detected in any of the samples presented above. For samples where no enteric viruses were detected, the lower detection limit value (1 GEC/100mL) is presented in the figure above.

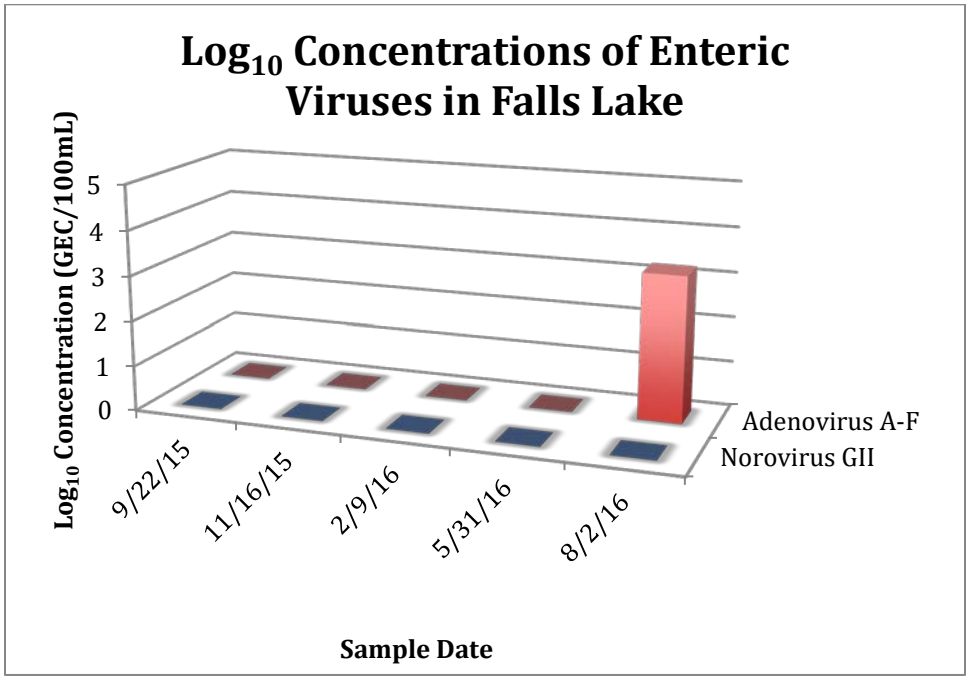

Figure 61: Log_{10} Concentrations of Enteric Virus Gene Copies/100 mL in Falls Lake

In Figure 61 are shown the concentrations of Norovirus GII and Adenovirus groups A-F gene copies per 100 mL detected in Falls Lake between 9/22/15 and 8/2/16. Based on the data presented in the figure above and in Table 15, the average concentration of adenovirus A-F detected was 0.65 GEC/100mL. Adenovirus was only detected on 8/2/16, and no Norovirus was detected in any of the samples presented above. For samples where no enteric viruses were detected, the lower detection limit value (1 GEC/100mL) is presented in the figure above.

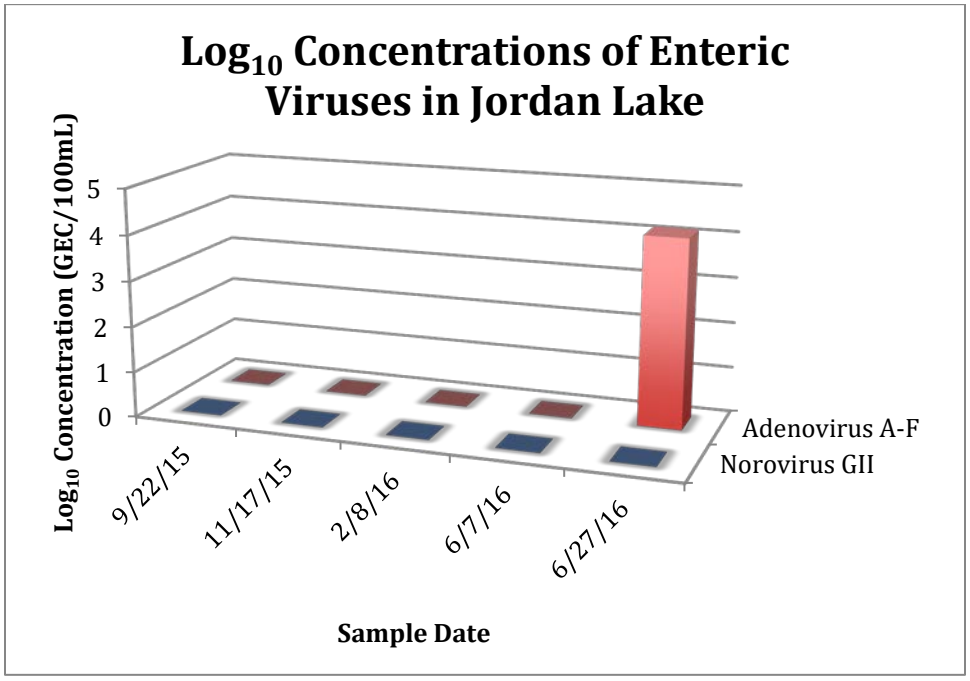

Figure 62: Log_{10} Concentrations of Enteric Virus Gene Copies/100 mL in Jordan Lake

In Figure 62are shown the concentrations of Norovirus GII and Adenovirus groups A-F gene copies/100 mL detected in Jordan Lake between 9/22/15 and 6/27/16. Based on the data presented in the figure above and in Table 15, the average concentration of adenovirus A-F detected was 0.83 GEC/100mL. Adenovirus was only detected on 6/27/16, and no Norovirus was detected in any of the samples presented above. For samples where no enteric viruses were detected, the lower detection limit value (1 GEC/100mL) is presented in the figure above.

3.3.2.6 Protozoan Parasites

Protozoan parasites were analyzed by US EPA Method 1623 and are presented as microscopically visible (oo)cysts per 100mL. Limit of detection information for protozoan parasite analysis is provided in section 2 of this report. It is important to note that the protozoan parasites detected by this method represent total numbers of cysts and oocysts and not infectious organisms. This is an important distinction as the number of infectious cysts and oocysts is expected to be lower than the total number of (oo)cysts detected microscopically detected in a given sample.

Table 16: Protozoan Parasite Concentrations in Surface Water Samples

Date	Sample	*Cryptosporidium* spp. Concentrations (oocysts per 100mL)	*Giardia* spp. Concentrations (cysts per 100mL)
7.13.15	Neuse River at Smithfield	4.05	0.70
9.22.15	Falls Lake	0.56	0.10
9.22.15	Jordan Lake	1.21	0.28
10.19.15	Eno River at Hillsborough	3.28	0.79
11.2.15	Smithfield Reservoir	0.24	0.09
11.2.15	Neuse River at Smithfield	7.02	1.52
11.8.15	Eno River at Hillsborough	2.11	0.51
11.16.15	Falls Lake	2.32	0.23
11.17.15	Jordan Lake	0.10	0.02
1.18.16	Eno River at Hillsborough	0.11	0.05
2.8.16	Jordan Lake	0.32	0.09
2.9.16	Falls Lake	0.17	0.03
2.23.16	Eno River at Hillsborough	0.23	0.08
2.29.16	Neuse River at Smithfield	0.75	0.23
5.29.16	Eno River at Hillsborough	0.32	0.06
5.31.16	Falls Lake	0.24	0.20
6.7.16	Jordan Lake	0.42	0.08
6.19.16	Eno River at Hillsborough	0.42	0.08
6.27.16	Jordan Lake	0.06	0.02
7.15.16	Neuse River at Smithfield	1.28	0.42
7.15.16	Smithfield Reservoir	0.28	0.10
8.2.16	Falls Lake	0.57	0.11

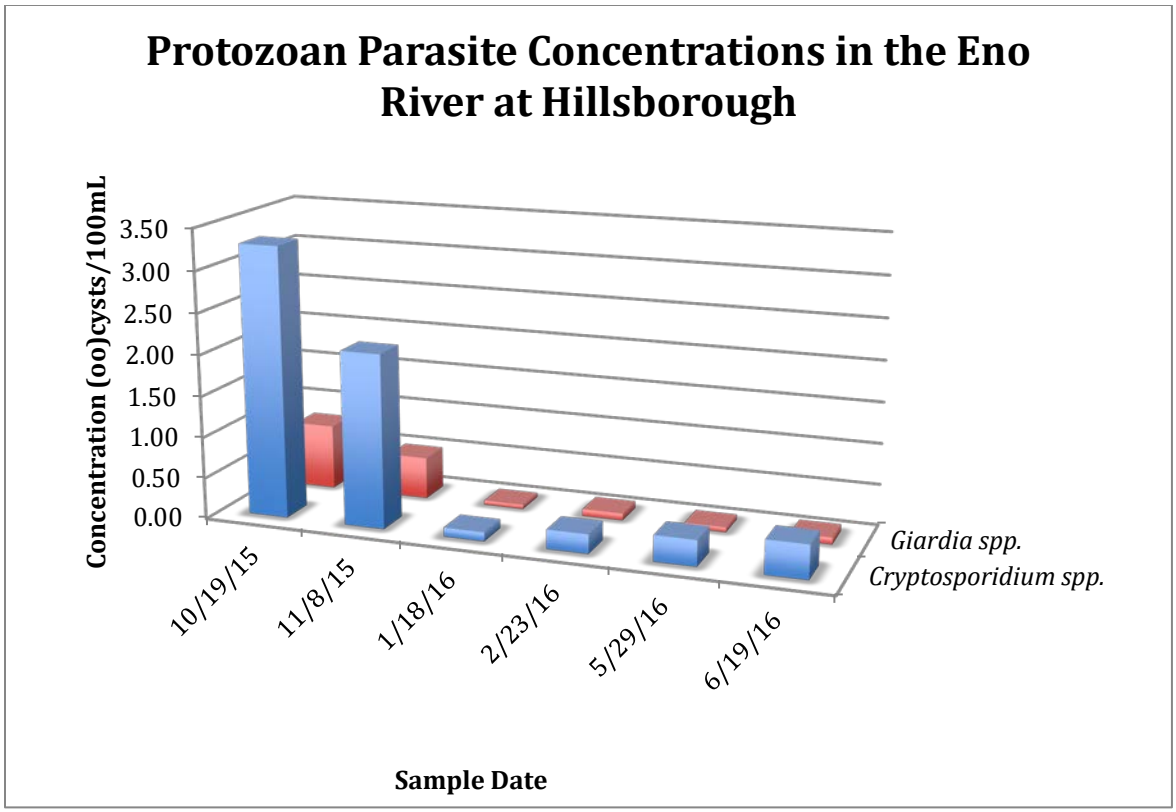

Figure 63: Concentrations of Protozoan Parasite (Oo)cysts in the Eno River at Hillsborough

In Figure 63 are shown the concentrations of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts detected in the Eno River at Hillsborough between 10/19/15 and 6/19/16. Based on the data presented in the figure above and in Table 16, the average concentration of *Cryptosporidium* was 1.08 oocysts per 100mL and the average concentration of *Giardia* was 0.26 cycsts per 100mL. Low but detectable levels of *Cryptosporidium* and *Giardia* were observed in all samples of Eno River water.

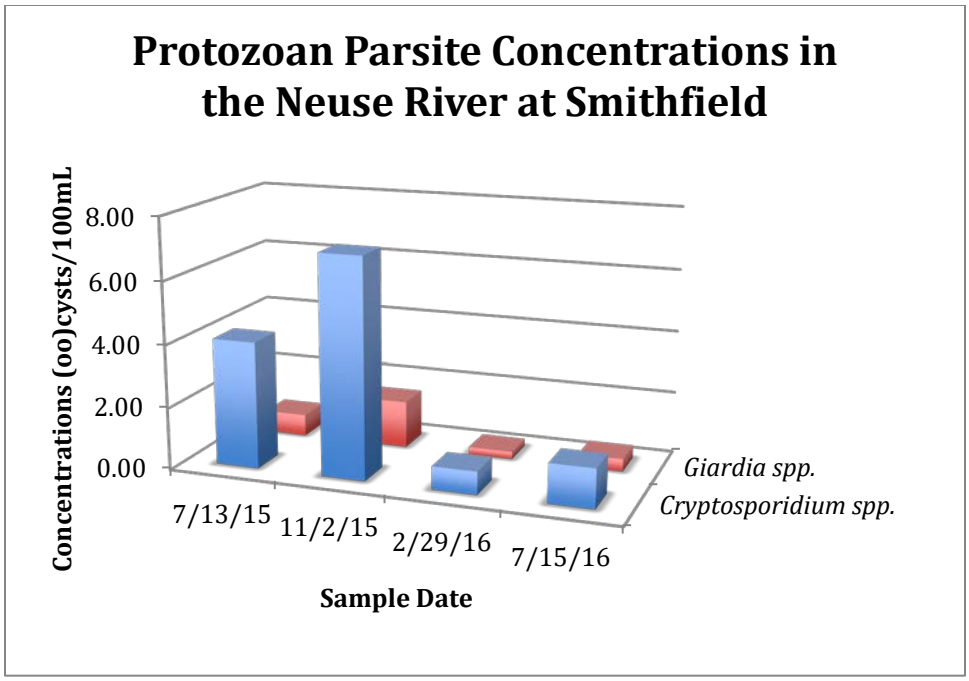

Figure 64: Concentrations of Protozoan Parasite (Oo)cysts s in the Neuse River at Hillsborough

In Figure 64 are shown the concentrations of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts detected in the Neuse River at Smithfield between 7/13/15 and 7/15/16. Based on the data presented in the figure above and in Table 16, the average concentration of *Cryptosporidium* was 3.28 oocysts per 100mL and the average concentration of *Giardia* was 0.72 cycsts per 100mL. Low but detectable levels of *Cryptosporidium* and *Giardia* were observed in all samples of Neuse River water.

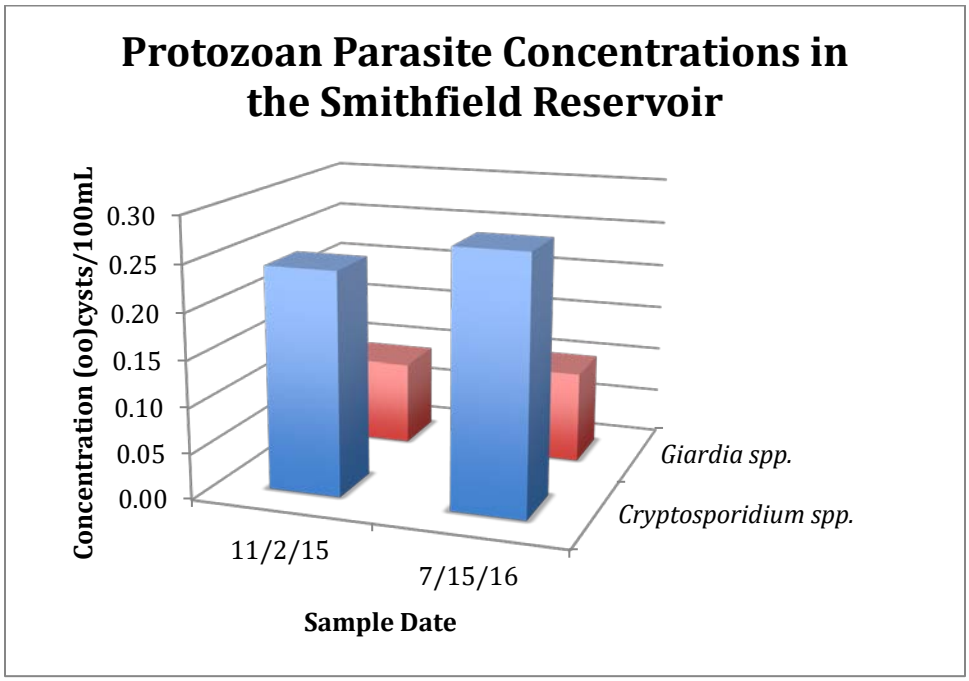

Figure 65: Concentrations of Protozoan Parasite (oo)cysts in the Smithfield Reservoir

In Figure 65 are shown the concentrations of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts detected in the Smithfield Reservoir on 11/2/15 and 7/15/16. Based on the data presented in the figure above and in Table 16, the average concentration of *Cryptosporidium* was 0.26 oocysts per 100mL and the average concentration of *Giardia* was 0.10 cycsts per 100mL. Low but detectable levels of *Cryptosporidium* and *Giardia* were observed in all samples of Smithfield Reservoir water.

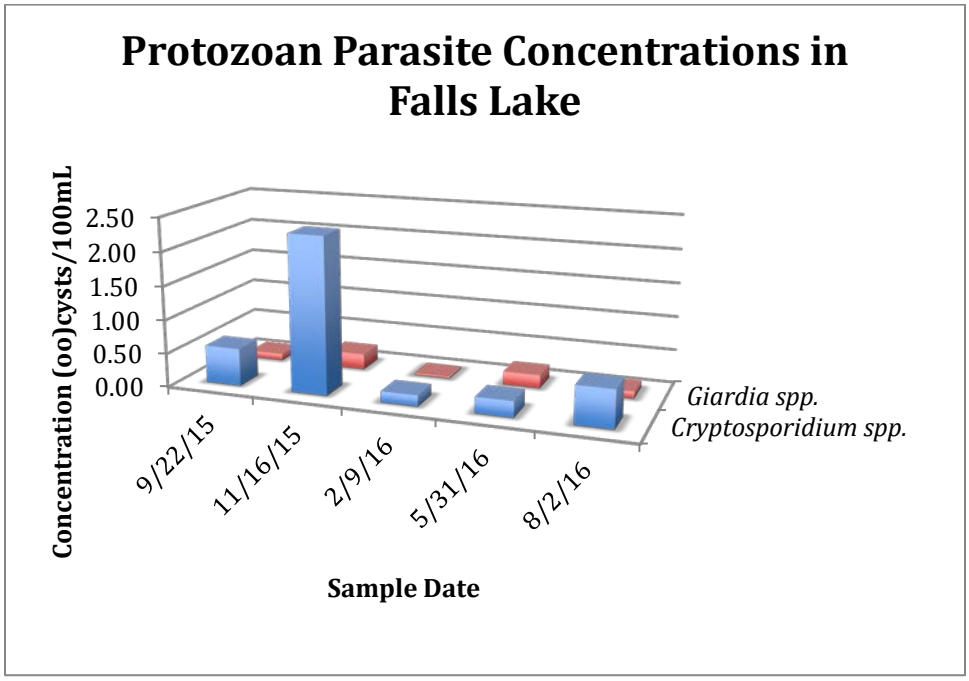

Figure 66: Concentrations of Protozoan Parasite (Oo)cysts s in Falls Lake

In Figure 66 are shown the concentrations of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts detected in Falls Lake between 9/22/15 and 8/2/16. Based on the data presented in the figure above and in Table 16, the average concentration of *Cryptosporidium* was 0.77 oocysts per 100mL and the average concentration of *Giardia* was 0.13 cycsts per 100mL. Low but detectable levels of *Cryptosporidium* and *Giardia* were observed in all samples of Falls Lake water.

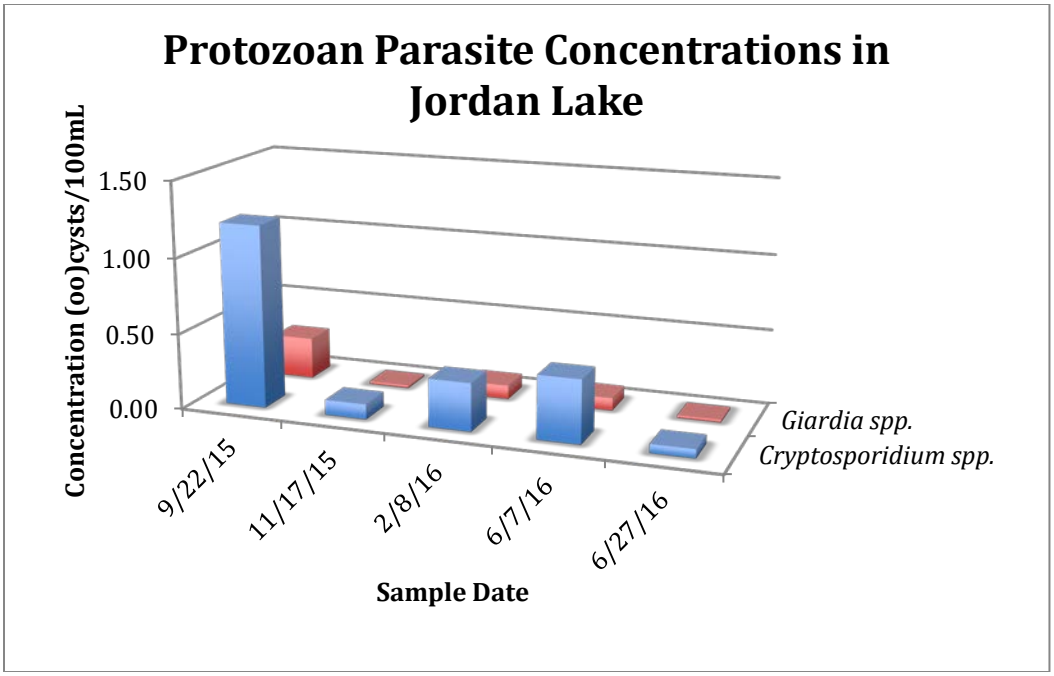

Figure 67: Concentrations of Protozoan Parasite (oo)cysts in Jordan Lake

In Figure 67 are shown the concentrations of *Cryptosporidium* spp. oocysts and *Giardia* spp. cysts detected in Jordan Lake between 9/22/15 and 6/27/16. Based on the data presented in the figure above and in Table 16, the average concentration of *Cryptosporidium* was 0.42 oocysts per 100mL and the average concentration of *Giardia* was 0.10 cycsts per 100mL. Low but detectable levels of *Cryptosporidium* and *Giardia* were observed in all samples of Jordan Lake water.

3.3.3 Survival Experiments

3.3.3.1 Sewage Propagated Organisms

Survival experiments were initially conducted with indicator organisms propagated from a single raw sewage sample collected from the Orange County Water and Sewer Authority (OWASA). The procedure for growing each organism is described in section 2.2.11. Conditions included mixing speeds of 0, 60 and 120 RPM, temperatures of 4 and 25°C, and sunlight exposure. As explained in the methods section above, propagated organisms were spiked into an 80/20 mix of 80% surface water to 20% reclaimed water. Experiments conducted at 4 and 25°C were conducted in triplicate with a PBS control over a period of 5 days. Sunlight experiments were also conducted in triplicate with a PBS control over a time period sufficient to track a 4 $\log_{10}$ reduction for most organisms. The figures below present the average $\log_{10}(N_t/N_0)$ survival of the three trials for each indicator organism at each temperature and mixing speed, as well as the results of the survival experiment.

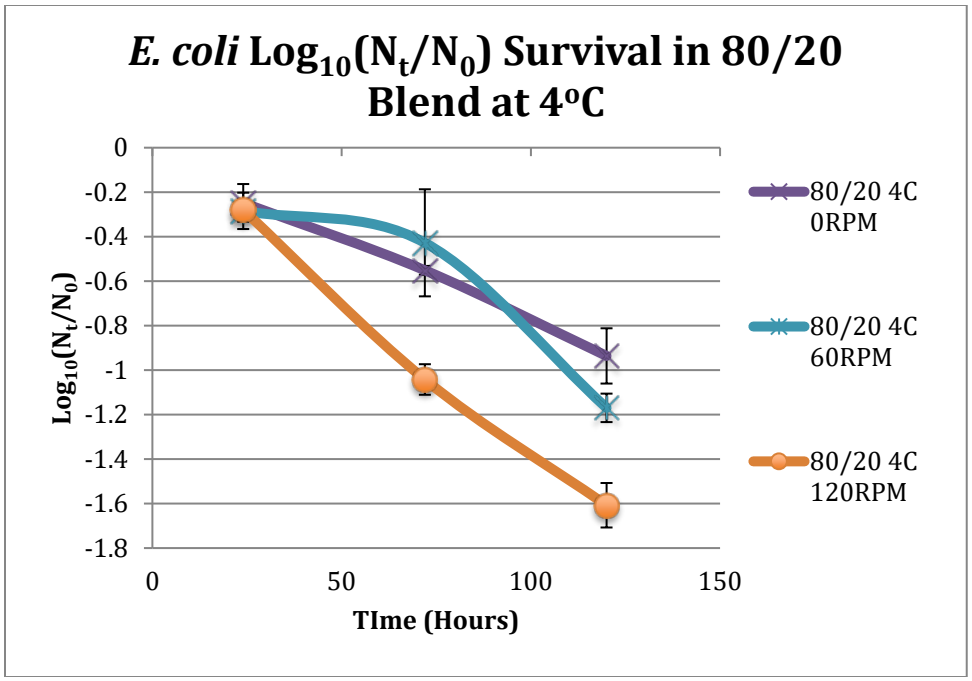

Figure 68: *E. coli* $\log_{10}(N_t/N_0)$ Survival in 80/20 Blend at 4°C with 95% Confidence Intervals

In Figure 68 are shown the survival ratios of *E. coli* at 4°C for mixing speeds of 0, 60, and 120RPM. Based on the results shwon, it appears that the 120 RPM mixing speed resulted in a slightly increased $\log_{10}$ reduction of *E. coli* over the 5 day time period, with a reduction of approximately 1.5, while the average reduction for 0 and 60 RPM was 0.8.

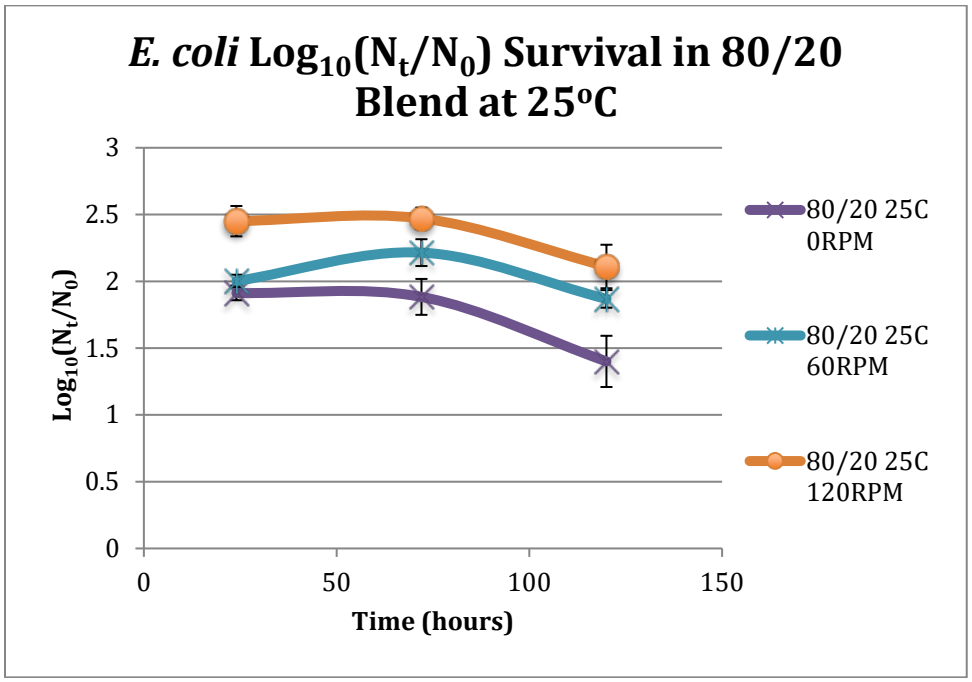

Figure 69: *E. coli* $\text{Log}_{10}(N_t/N_0)$ Survival in 80/20 Blend at 25°C with 95% Confidence Intervals

In Figure 69 are shown the $\log_{10}$ survivals of *E. coli* in the 80% to 20% reclaimed water blend over a 5 day storage period at 25°C. Based on the results presented in the figure above, there is an apparent increase in the concentration of *E. coli* after the initial 24 hour period of storage (~2$\log_{10}$ for all mixing conditions). After this initial increase, there is very little change in the concentrations of *E. coli* detected over the 5 day period. The samples mixed at 0 RPM decreased approximately 0.5 -1 $\log_{10}$, however the samples mixed 60 and 120 RPM decrease less than 0.5 $\log_{10}$. The significance and relevance of the apparent growth of the *E. coli* at 25°C will be further addressed in the experiments with natural bacteria in surface water.

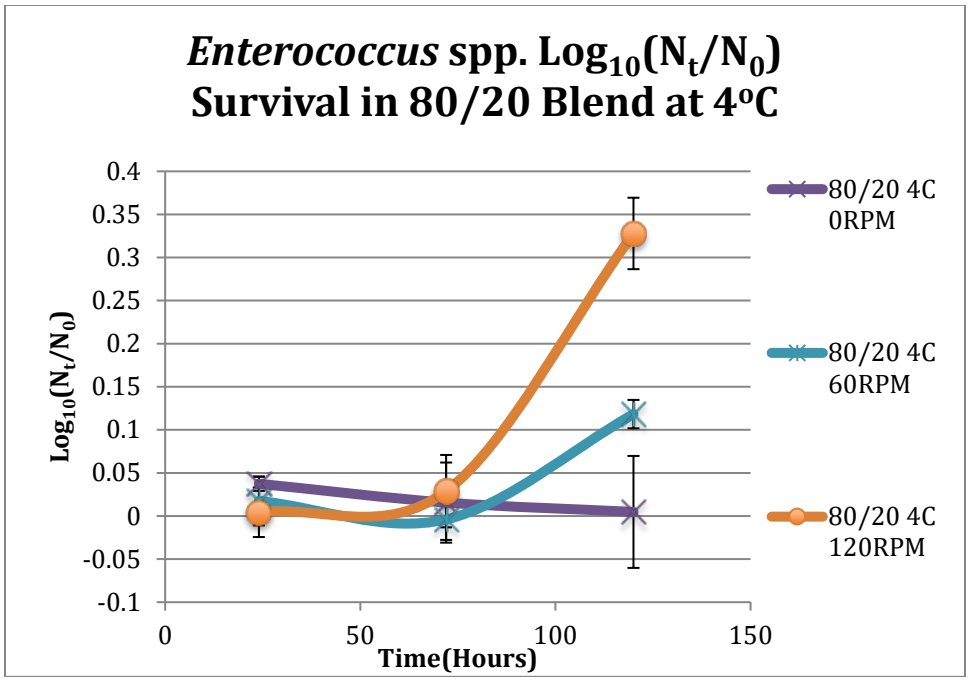

Figure 70: *Enterococcus* spp. $\log_{10}(N_t/N_0)$ Survival in 80/20 Blend at 4°C with 95% Confidence Intervals

In Figure 70 are shown the survivals of *Enterococcus* spp. in the 80/20 blend at 4°C at the various mixing speeds. Based on the results shown, it is apparent that very little change occurs in the survival of *Enterococcus* at 0 and 60 RPM mixing speeds, while an apparent increase of 0.3 $\log_{10}$ occurs at 120 RPM. In general, these changes are very small (<0.5 $\log_{10}$) indicating that there is little change in the concentration of this microorganism at this temperature under these conditions over a 5 day time period.

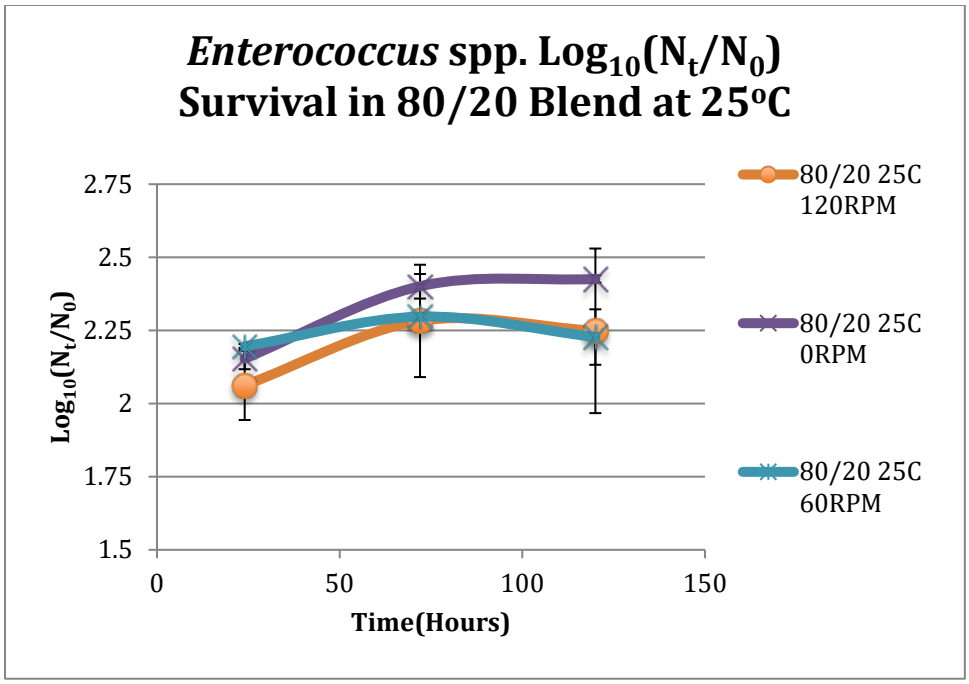

Figure 71: *Enterococcus* spp. Log_{10} (N_t/N_0) Survival in 80/20 Blend at 25°C with 95% Confidence Intervals

In Figure 71 are shown the log_{10} survivals of *Enterococcus* spp. over the 5 day storage period at 25°. As with the *E. coli* survival data presented in Figure 69, there is an apparent increase in the concentration of *Enterococcus* spp. after the initial 24 hour storage period (~2log_{10}) for all mixing speeds at this temperature. The significance and relevance of the apparent growth of the *Enterococcus* spp. at 25°C will be further addressed in the experiments with natural bacteria in surface water.

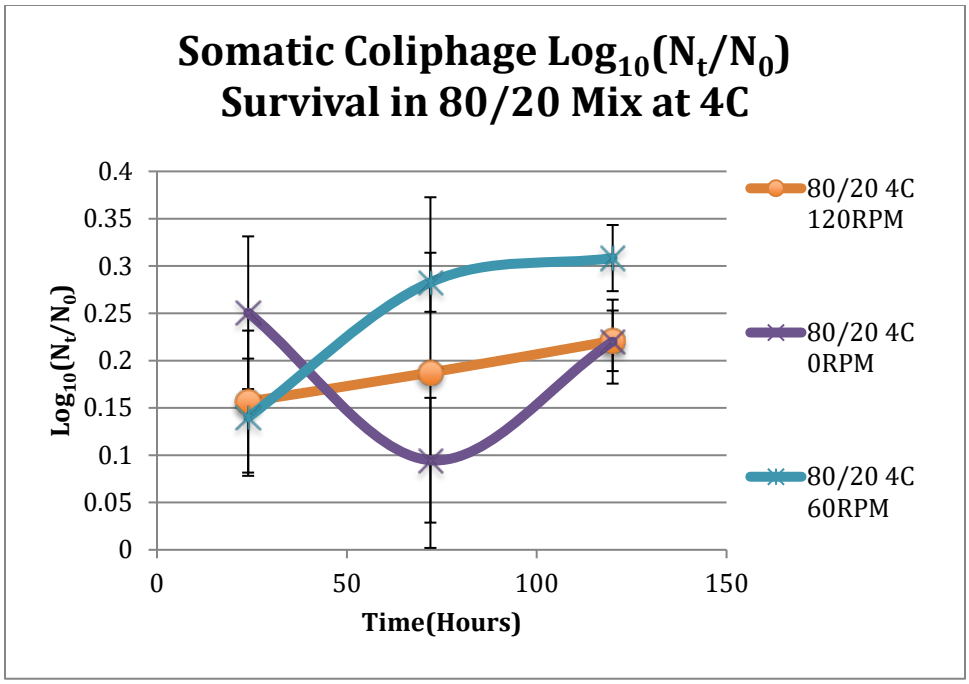

Figure 72: Somatic Coliphage Viruses $\log_{10}(N_t/N_0)$ Survival in 80/20 Mix at 4°C with 95% Confidence Intervals

Figure 72 displays the $\log_{10}(N_t/N_0)$ survival of somatic coliphage viruses at 0, 60, and 120 RPM at 4°C in the 80/20 surface water and reclaimed water mix. Based on the figure above, it is clear that there is very little change in the concentrations of somatic coliphages over a 5 day period at any mixing speed. For the samples mixed at 0 and 60 RPM, there was an average increase in concentration of approximately 0.1 $\log_{10}$. For the samples mixed at 120 RPM , there was an average increase in concentration of approximately 0.25 $\log_{10}$.

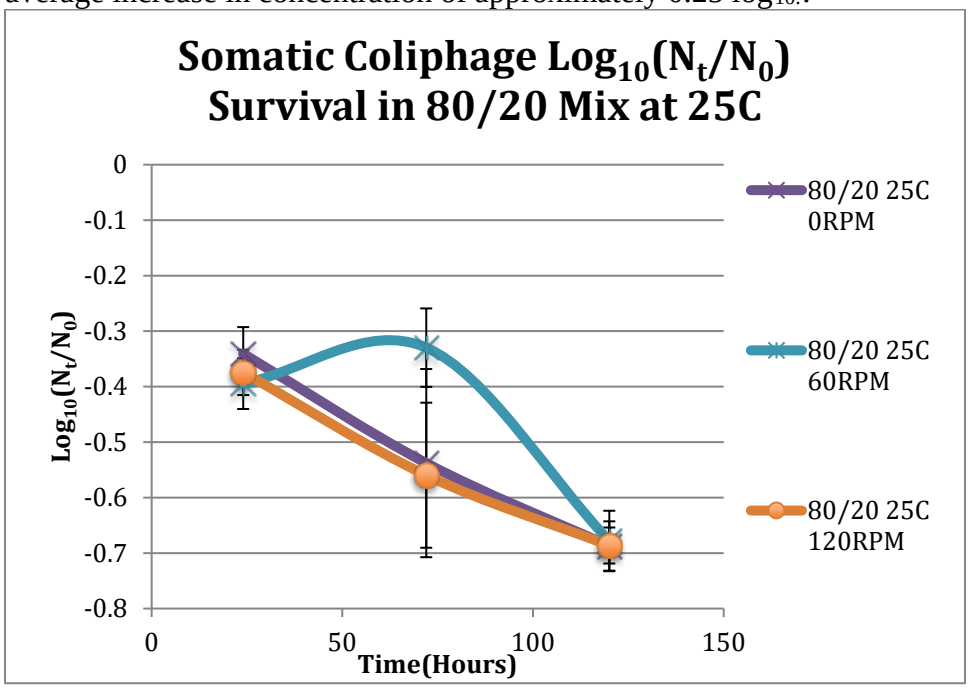

Figure 73:Somatic Coliphage Viruses Log_{10} (N_t/N_0) Survival in 80/20 Mix at 25°C with 95% Confidence Intervals

Figure 73 displays the log_{10} (N_t/N_0) survival of somatic coliphage viruses in the 80/20 mix stored over 5 days at 25°C and mixed at 0, 25, and 120 RPM. Based on the figure above, it is also clear that storage at 25°C has little impact on the survival of somatic coliphage viruses over a 5 day period. For the samples mixed at 0, 60 and 120 RPM, the average log_{10} reduction was approximately 0.3 log_{10} over the 5 day period. Additionally, nearly all mixing speeds were within the 95% confidence intervals of one another, indicating that there is no significant difference between mixing speed for somatic coliphage survival.

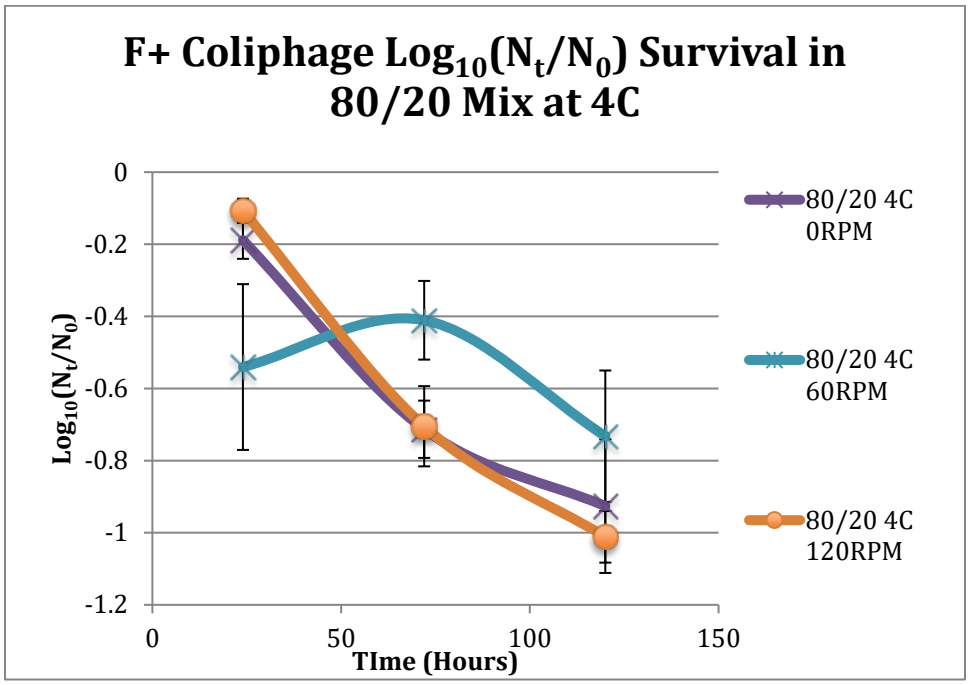

Figure 74: F+ Coliphage Viruses $log_{10}(N_t/N_0)$ Survival in 80/20 Mix at 4°C with 95% Confidence Intervals

Figure 74 displays the $log_{10}(N_t/N_0)$ survival of F+ coliphages in the 80/20 mix at 4°C at various mixing speeds. Based on the figure above, it is clear that F+ coliphages decline slightly over a 5 day period of storage at 4°C. At mixing speeds of 0 and 120 RPM, the log_{10} reduction was approximately 0.8 whereas the reduction for 60 RPM was approximately 0.2 log_{10}.

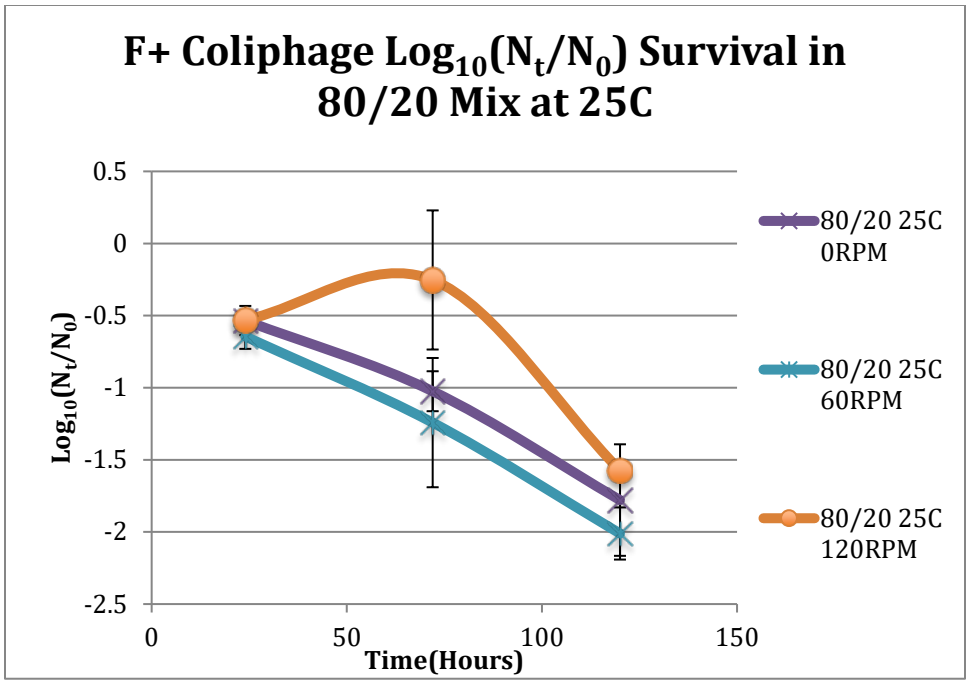

Figure 75: F+ Coliphage Viruses Log_{10} (N_t/N_0) Survival in 80/20 Mix at 25°C with 95% Confidence Intervals

Figure 75 displays the log_{10} (N_t/N_0) survival of F+ coliphages at 25°C over a 5 day period at various mixing speeds in the 80/20 mix of surface water and reclaimed water. As was the trend at 4°C, at 25°C, F+ coliphages decline slightly over a 5 day period. For mixing speeds of 0, 60, and 120 RPM, the average log_{10} reduction was approximately 1.5 log_{10}.

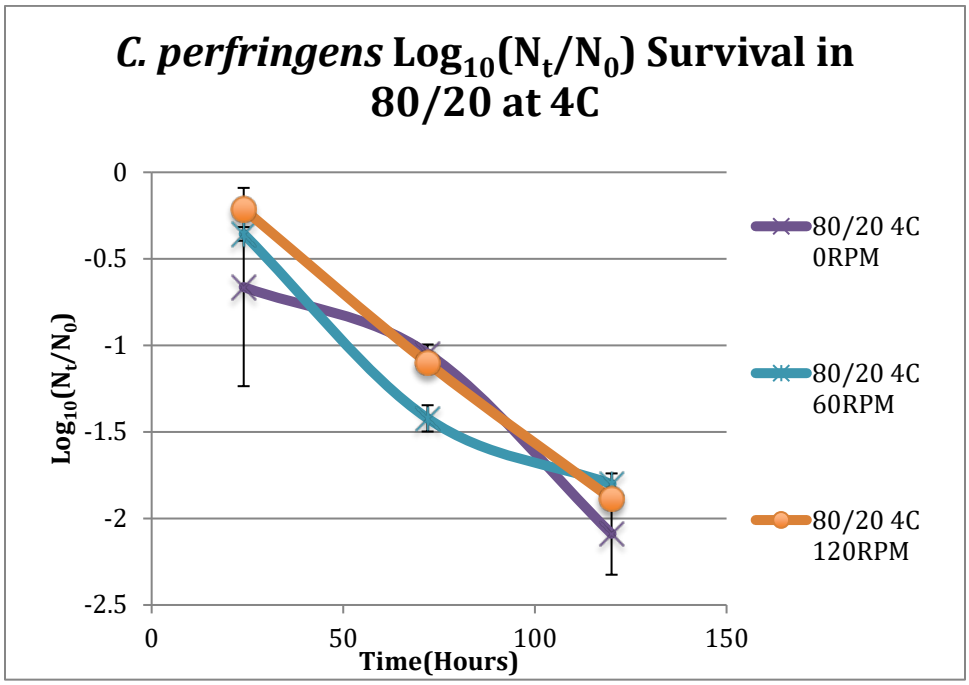

Figure 76: *C. perfringens* log_{10}(N_t/N_0) Survival in 80/20 Mix at 4°C with 95% Confidence Intervals

Figure 76 displays the $\log_{10}(N_t/N_0)$ survival of *C. perfringens* in the 80/20 mix at 4°C at various mixing speeds over the 5 day storage period. Based on the figure above, it appears that there is a slow initial reduction in *C. perfringens* after an initial storage period (of approximately 0.5 $\log_{10}$) followed by an additional 0.5 $\log_{10}$ on day 3 and another 0.5 to 1 $\log_{10}$ on day 5. This trend is consistent for all mixing speeds over the 5 day period, suggesting that sewage propagated *C. perfringens* does not survive for long periods at 4°C.

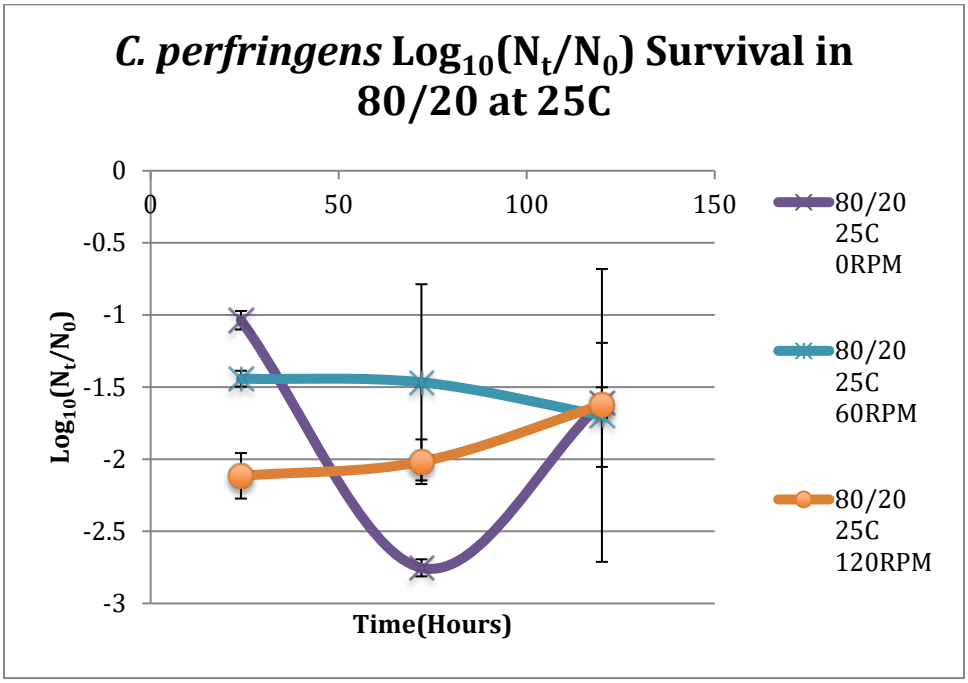

Figure 77: *C. perfringens* Log_{10} (N_t/N_0) Survival in 80/20 Mix at 25°C with 95% Confidence Intervals

Figure 77 displays the $\log_{10}$ (N_t/N_0) survival of *C. perfringens* at 25°C in the 80/20 mix at various mixing speeds over a 5 day storage period. Based on the figure above, it appears that for sewage propagated *C. perfringens*, there is an initial die-off of approximately 1-2 $\log_{10}$, for all mixing speeds. After this initial die-off, it appears that *C. perfringens* concentrations are generally constant for the rest of the 5 day experiment.

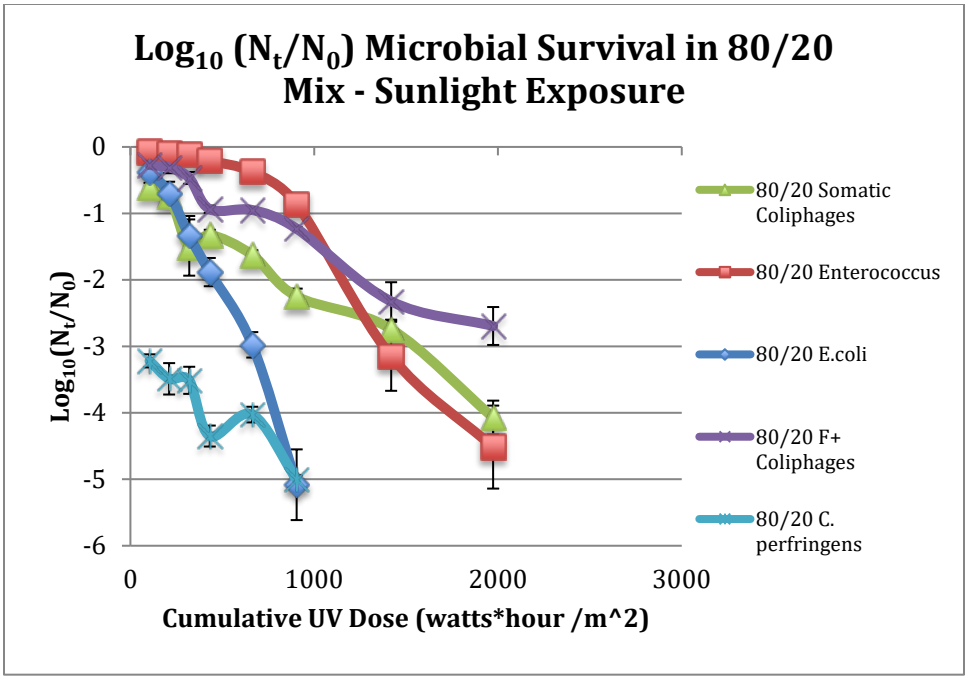

Figure 78: $Log_{10}(N_t/N_0)$ survival after sunlight exposure of sewage propagated microorganisms in 80/20 mix with 95% confidence intervals

Figure 78 displays the $log_{10}(N_t/N_0)$ survival of somatic and F+ coliphages, *Enterococcus* spp, *E. coli*, and *C. perfringens* after exposure to sunlight. Based on this figure, it is clear that sunlight exposure plays a key role in the inactivation of microorganisms in the 80/20 mix. As Figures 68-77 have displayed, it is not clear that significant changes occur in microorganism concentrations after water storage at 4 or 25°C over a 5 day period; however, in contrast after exposure to UV light, microorganisms begin to decrease after a relatively short amount of time. The cumulative UV doses required to achieve a 4 log_{10} reduction for *E. coli* and *Enterococcus* spp. are 692 and 1587 watts*hour/m^2 respectively. The doses required to achieve a 4 log_{10} reduction for somatic and F+ coliphage and 2327 and 2909 watts*hour/m^2 respectively, and the dose to achieve a 4 log_{10} reduction for *C. perfringens* is 4343 watts*hour/m^2.

3.3.3.2 Naturally Occurring Bacteria

Experiments with naturally occurring bacteria were conducted as described in section 2.2.11.5.The goal of these experiments was to further evaluate the survival of bacteria stored at 25°C, as the *E. coli* and *Enterococcus* spp. stored at this temperature grew by 2 to 2.5 log_{10} over the course of 5 days. The figures below present the survival of naturally occurring total coliforms, *E. coli* and *Enterococcus* spp. in surface water samples over a 5 day storage period at 25 $_oC$ and mixing speeds of 0, 60, and 120 RPM.

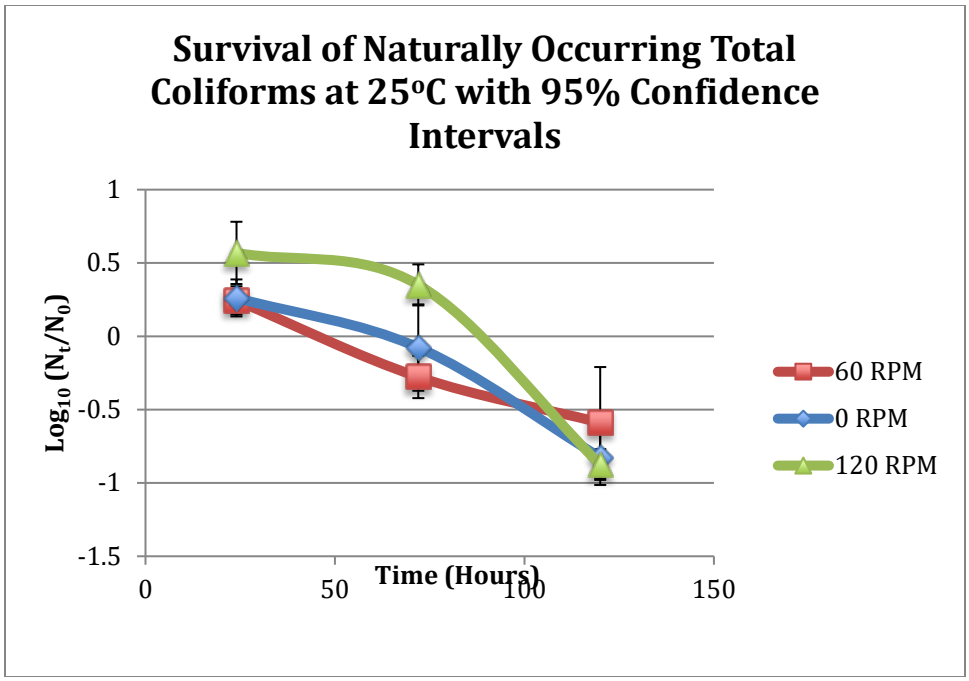

Figure 79: $Log_{10}(N_t/N_0)$ survival of naturally occurring total coliforms at 25°C with 95% confidence intervals

In Figure 79 are shown the log_{10} survivals of naturally occurring total coliform bacteria at 25°C at various mixing speeds. In contrast to the results for bacteria presented in the sewage propagated bacteria experiments, the results presented in this figure indicate that total coliform bacteria do not increase in surface water over a 5 day time period. For the mixing speeds evaluated here, bacteria concentrations decreased about 0.5-1 log_{10} over the 5 day storage period.

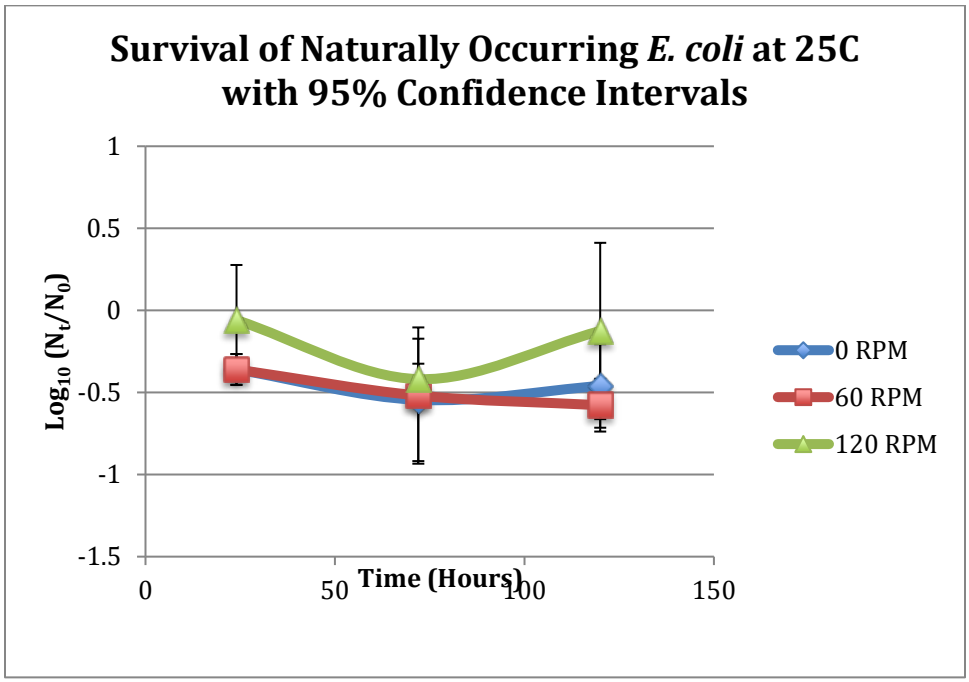

Figure 80: $Log_{10}(N_t/N_0)$ survival of naturally occurring *E. coli* at 25°C with 95% confidence intervals

In Figure 80 are shown the $\log_{10}$ survivals of naturally occurring *E. coli* at room temperature under various mixing conditions. As with the total coliform bacteria, *E. coli* appears to maintain approximately the same concentration throughout the 5 day storage experiment at this temperature at 0, 60, and 120 RPM mixing speeds.

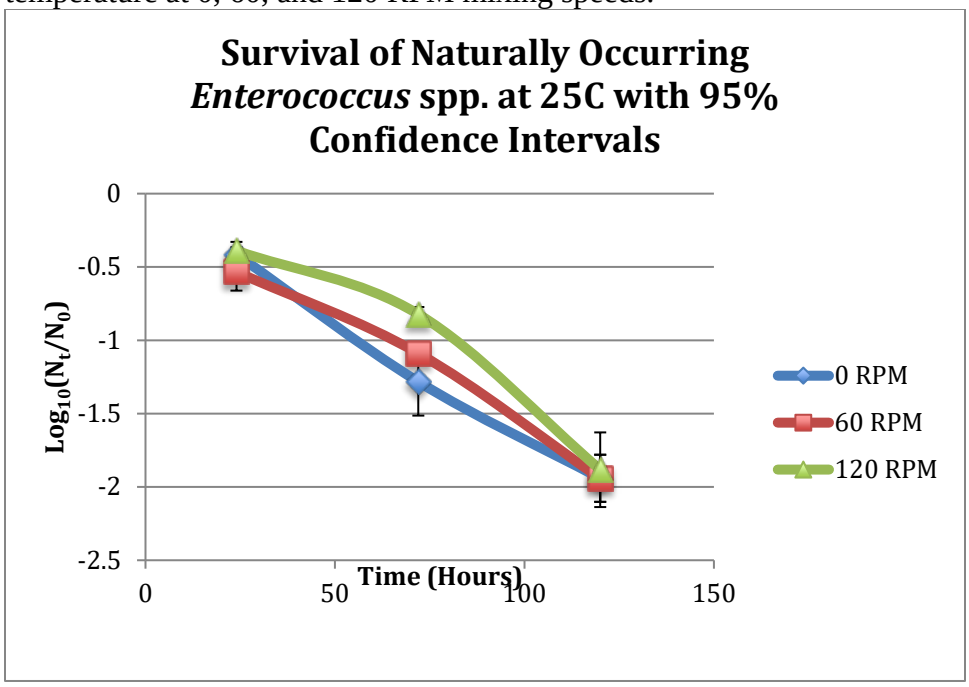

Figure 81: $\text{Log}_{10}(N_t/N_0)$ survival of naturally occurring *Enterococcus* spp. at 25°C with 95% confidence intervals

In Figure 81 are shown the $\log_{10}$ survivals of naturally occurring *Enterococcus* spp. bacteria at room temperature at 0, 60, and 120 RPM mixing speeds. Based on the figure above, it appears that for all three mixing speeds, the naturally occurring *Enterococcus* spp. decreased approximately 1.5 $\log_{10}$ over the 5 day storage period.

3.3.3.2 Survival of Lab Grown Fecal Indicator Microorganisms in 80/20 Water Blend over 5 Days

The average $\log_{10}$ survival data for the indicator organisms examined here (*E.* coli, *Enterococcus* spp., *C. perfringens*, F+ coliphage, and somatic coliphage) are summarized by temperature, either 4 or 20°C, and by mixing speed, either 0, 60 or 120 RPM, in Table 17. A positive $\log_{10}$ (N_t/N_0) value corresponds to an increase in microbe concentration over the 5 day storage period, while a negative $\log_{10}$ (N_t/N_0) value represents a decrease in microbe concentration. For the purposes of this analysis, the results for the 4°C temperature experiments at all mixing speeds will be presented first by microorganism, followed by the results of the 20°C experiments; additionally, mixing speed data will be presented in the order of 0, 60 and 120 RPM.

The starting concentration of *E. coli* was 6.8 x 10^6 CFU per 100mL. At 4°C and 0 RPM mixing speed, average $\log_{10}$ (N_t/N_0) at 5 days was -0.94. These results were statically significant from no change ($\log_{10}$ $(N_t/N_0)=0$, $p<0.0001$). For samples mixed at 60 RPM at a temperature of 4°C

the average $\log_{10}(N_t/N_0)$ was -1.17 at 5 days and was statistically significantly different from no change ($p<0.0001$), but not significantly different from the 0 RPM mixing speed ($p=0.0598$). For samples mixed at 120 RPM at 4°C, the average $\log_{10}(N_t/N_0)$ was -1.61 at 5 days. These results were statistically different from no change ($p<0.0001$) and from the 0 RM mixing speed ($p<0.0001$) and the 60 RPM mixing speed ($p=0.0019$).

For *E. coli* survival examined at temperatures of 20°C and a 0 RPM mixing speed, the average $\log_{10}(N_t/N_0)$ at 5 days was 1.40; this positive value indicates an increase in *E. coli* at this temperature and mixing speed. These results were statically different than no change ($p<0.0001$). At 60 RPM, the average $\log_{10}(N_t/N_0)$ at 5 days was 1.87, a value also statistically significantly different from no change ($p<0.0001$) and statically different from the 0 RPM mixing speed at this temperature ($p=0.0071$). At the 120 RPM mixing speed at 20°C, the average $\log_{10}(N_t/N_0)$ value was 2.11. This value was also statistically different than no change ($p<0.0001$), and 0 RPM ($p=0.0001$), but not statistically different than the $\log_{10}(N_t/N_0)$ 60 RPM mixing speed values ($p=0.2954$). For *E. coli*, all mixing speeds were statistically different ($p<0.0001$) for the two temperatures evaluated.

The starting concentration of *Enterococcus* spp. was 3.8×10^6 CFU per 100mL, and at 4°C and 0 RPM mixing speed, average $\log_{10}(N_t/N_0)$ value at 5 days was 0.00; this result was not statically different from no change ($p>0.99$). For samples mixed at 60 RPM, the average $\log_{10}(N_t/N_0)$ value at 5 days was 0.12, this value was also not statistically significantly different from zero ($p=0.9008$), or from the 0 RPM mixing speed ($p=0.9162$). At 120 RPM, the average $\log_{10}(N_t/N_0)$ value was 0.33, and there was not a statistically significant difference between this value and no change ($p=0.0739$), 0 RPM ($p=0.0800$) or 60 RPM mixing speeds ($p=0.4430$).

For *Enterococcus* spp. survival at 20°C, the average $\log_{10}(N_t/N_0)$ value at the 0 RPM mixing speed was 2.43, and there was a statically significant difference between this value and zero ($p<0.0001$). At the 60 RPM mixing speed, the average $\log_{10}(N_t/N_0)$ value was 2.23; there was also a statistically significant difference between this value and no change ($p<0.0001$), but not between this value and the 0 RPM mixing speed ($p=0.4926$). At 20°C and 120 RPM, the average $\log_{10}(N_t/N_0)$ value was 2.25; there was a statistically significant difference between this value and no change ($p<0.0001$), but not between this value and 0 RPM ($p=0.6120$), or between this value and 60 RPM ($p>0.9999$). In the comparison of mixing speed at the two temperatures, there was a statistically significant difference between each mixing speed (0, 60, and 120) for both temperatures evaluated ($p<0.0001$). The apparent increases in lab culture grown enterococcus at 20 ^oC will be discussed in a section of this report. These results are probably an artifact of the use of lab grown enterococci and several explanations for such results are possible.

The starting concentration of somatic coliphages was 7.6×10^7 PFU per 100mL. At 4°C and 0 RPM mixing speed, average $\log_{10}(N_t/N_0)$ at 5 days was 0.22. These results were statically significantly different from no change ($p=0.0002$). For samples mixed at 60 RPM at a temperature of 4°C the average $\log_{10}(N_t/N_0)$ was 0.31 at 5 days and was statistically significantly different from no change ($p<0.0001$), but not significantly different from the 0 RPM mixing speed ($p=0.1927$). For samples mixed at 120 RPM at 4°C, the average $\log_{10}(N_t/N_0)$ was 0.22 at 5 days. These results were statistically different from no change ($p=0.0002$) but not from the 0 RM mixing speed ($p>0.999$) and the 60 RPM mixing speed ($p=0.2006$).

For somatic coliphage survival examined at a temperature of 20°C and a 0 RPM mixing speed, the average $\log_{10}(N_t/N_0)$ at 5 days was -0.69. These results were statically different than no change ($p<0.0001$). At 60 RPM, the average $\log_{10}(N_t/N_0)$ at 5 days was -0.68, a value also statistically significantly different from no change ($p<0.0001$) but not statically different from the 0 RPM mixing speed at this temperature ($p>0.9999$). At the 120 RPM mixing speed at 20°C, the average $\log_{10}(N_t/N_0)$ value was -0.69. This value was also statistically different than no change ($p<0.0001$), but not statistically different than the $\log_{10}(N_t/N_0)$ 0 RPM or 60 RPM mixing speed values (p values >0.9999). For somatic coliphages, all mixing speeds were statistically different ($p<0.0001$) for the two temperatures evaluated.

The starting concentration of F+ coliphages was 4.20×10^6 PFU per 100mL, and at 4°C and 0 RM mixing speed, average $\log_{10}(N_t/N_0)$ value at 5 days was -0.93; this result was statically different from no change ($p=0.0013$). For samples mixed at 60 RPM, the average $\log_{10}(N_t/N_0)$ value at 5 days was -0.73, this value was also statistically significantly different from zero ($p=0.0099$), but not from the 0 RPM mixing speed ($p=0.9043$). At 120 RPM, the average $\log_{10}(N_t/N_0)$ value was -1.01, there was a statistically significant difference between this value and no change ($p=0.0005$), and 0 RPM ($p=0.0246$), but not the 60 RPM mixing speed ($p=0.6603$).

For F+ coliphage spp. survival at 20°C, the average $\log_{10}(N_t/N_0)$ value at the 0 RPM mixing speed was -1.78, there was a statically significant difference between this value and zero ($p<0.0001$). At the 60 RPM mixing speed, the average $\log_{10}(N_t/N_0)$ value was -2.01; there was also a statistically significant difference between this value and no change ($p<0.0001$), but not between this value and the 0 RPM mixing speed ($p=0.8112$). At 20°C and 120 RPM, the average $\log_{10}(N_t/N_0)$ value was -1.57; there was a statistically significant difference between this value and no change ($p<0.0001$), but not between this value and 0 RPM ($p=0.8791$), or between this value and 60 RPM ($p=0.2059$). In the comparison of mixing speed at the two temperatures, there was a statistically significant difference between each mixing speed (0, 60, and 120) for both temperatures evaluated ($p<0.0001$), except between the 4°C and 20°C 120RPM mixing speed, for these two conditions there was no significant difference ($p=0.0605$).

For *Clostridium perfringens,* the starting concentration was 4.03×10^7 CFU per 100mL. At 4°C and 0 RPM mixing speed, average $\log_{10}(N_t/N_0)$ at 5 days was -2.09. These results were statically significant from no change ($p=0.0012$). For samples mixed at 60 RPM at a temperature of 4°C the average $\log_{10}(N_t/N_0)$ was -1.80 at 5 days and was statistically significantly different from no change ($p=0.0044$), but not significantly different from the 0 RPM mixing speed ($p=0.9856$). For samples mixed at 120 RPM at 4°C, the average $\log_{10}(N_t/N_0)$ was -1.89 at 5 days. These results were statistically different from no change ($p=0.0030$) but not from the 0 RM mixing speed ($p=0.9977$) and the 60 RPM mixing speed ($p>0.9999$).

For *C. perfringens* survival examined at a temperature of 20°C and a 0 RPM mixing speed, the average $\log_{10}(N_t/N_0)$ at 5 days was -1.61. These results were statically different than no change ($p=0.0112$). At 60 RPM, the average $\log_{10}(N_t/N_0)$ at 5 days was -1.70, a value also statistically significantly different from no change ($p=0.0073$) but not statically different from the 0 RPM mixing speed at this temperature ($p>0.9999$). At the 120 RPM mixing speed at 20°C, the average $\log_{10}(N_t/N_0)$ value was -1.62. This value was also statistically different than no change

(p=0.0104), and the $\log_{10}(N_t/N_0)$ 0 RPM or 60 RPM mixing speed values (p values >0.9999). For *C. perfringens*, all mixing speeds were not statistically different (p>0.85) for the two temperatures evaluated.

Table 17: Survival data for lab grown indicator microorganisms spiked in to 80/20 blend of water stored for 5 days with mixing speeds of 0, 60 and 120 rpm and temperatures of 4 and 20 °C

	4°C						20°C					
	0 RPM		60 RPM		120 RPM		0 RPM		60 RPM		120 RPM	
	$\log_{10}(N_t/N_0)$*	SD+	$\log_{10}(N_t/N_0)$	SD	$\log_{10}(N_t/N_0)$	SD	$\log_{10}(N_t/N_0)$	SD	$\log_{10}(Nt/N0)$	SD	$\log_{10}(N_t/N_0)$	SD
E. coli	-0.94	0.11	-1.17	0.06	-1.61	0.09	1.40	0.17	1.87	0.06	2.11	0.14
Enterococcus spp.	0.00	0.06	0.12	0.01	0.33	0.04	2.43	0.01	2.23	0.08	2.25	0.25
C. perfringens	-2.09	0.21	-1.80	0.05	-1.89	0.02	-1.61	0.09	-1.70	0.90	-1.62	0.38
F+ Coliphage	-0.93	0.16	-0.73	0.16	-1.01	0.06	-1.78	0.34	-2.01	0.16	-1.57	0.06
Somatic Coliphage	0.22	0.04	0.31	0.03	0.22	0.03	-0.69	0.04	-0.68	0.05	-0.69	0.03

*$\log_{10}(N_t/N_0)$ is the average $\log_{10}$ reduction over the 5 day experiment
+SD is standard deviation

3.3.3.3 Survival of Indicator Organisms Exposed to Sunlight

Figure 82 displays the $\log_{10}$ survival of the 5 indicator organisms spiked into the 80/20 surface water/reclaimed water mixture versus the cumulative UV dose over time. Each point on the graph represents the average survival of 3 trials plated in triplicate versus UV dose at a specific time point (0, 15, 30, 45, 90, 120, 180, and 240 minutes). The temperature for these experiments was controlled, and samples did not reach temperatures above 20°C. Table 18 describes the best fit parameters for the log linear Chick-Watson model.

Based on these results it appears that the *E. coli* and *Enterococcus* spp. are the microorganisms declining most rapidly in the 80/20 blend, with decay constants of k = -0.0058 and k = -0.0025 respectively, the units of k are 1/ watts*h per m^2. There was not statistically significant difference between the rate of decay of *E. coli* and *Enterococcus* spp. (p=0.6185) and the estimated UV dose to achieve a 4-$\log_{10}$ reduction for *E. coli* and *Enterococcus* spp. are 692 Watts*h per m^2 and 1587 W/m^2 respectively.

As *C. perfringens* was not adequately modeled by the Chick-Watson model (R^2 value of 0.48), the decay of this organism was also modeled by the One-Hit Two Population Model. By this alternative model, which proposes a first phase of microorganism inactivation with a quicker rate of decay (*k*) followed by second, slower phase. This model was a better fit to the *C. perfringens* data, with an R^2 value of 0.63, and an initial *k* value of 0.003 and a secondary *k* value of 0.0029. The initial *k* value indicates that the initial phase of decay was slightly faster than the secondary phase. There was not a statistically significant difference between the UV dose required to inactivate *C. perfringens* and *E. coli* (p=0.2785), or between the dose required to inactivate *Enterococcus* spp. (p=0.9534).

As with the fecal indicator bacteria, the survival of the coliphage viruses was a good fit to the Chick-Watson model (R^2 values of 0.97). By this model, the decay rates for F+ coliphages and somatic coliphages were k = -0.0014 and k = -0.0017 respectively and the UV doses required to achieve a 4 $\log_{10}$ reduction was 2909 W/m^2 for F+ coliphage and 2327 W/m^2 for somatic coliphage. Compared to fecal indicator bacteria, there was not a statistically significant difference between the survival *E. coli* and somatic (p=0.1501) but there was a statistically significant difference between *E. coli* and F+ coliphages (p=0.0009) when exposed to sunlight. Similarly, there was also not a statistically significant difference between the survival of enterococci and somatic (p=0.7878) but was a statistically significant difference between F+ coliphages (p=0.0065). There was also a statically significant difference between the survival of the two types of coliphage viruses (p=0.0360).

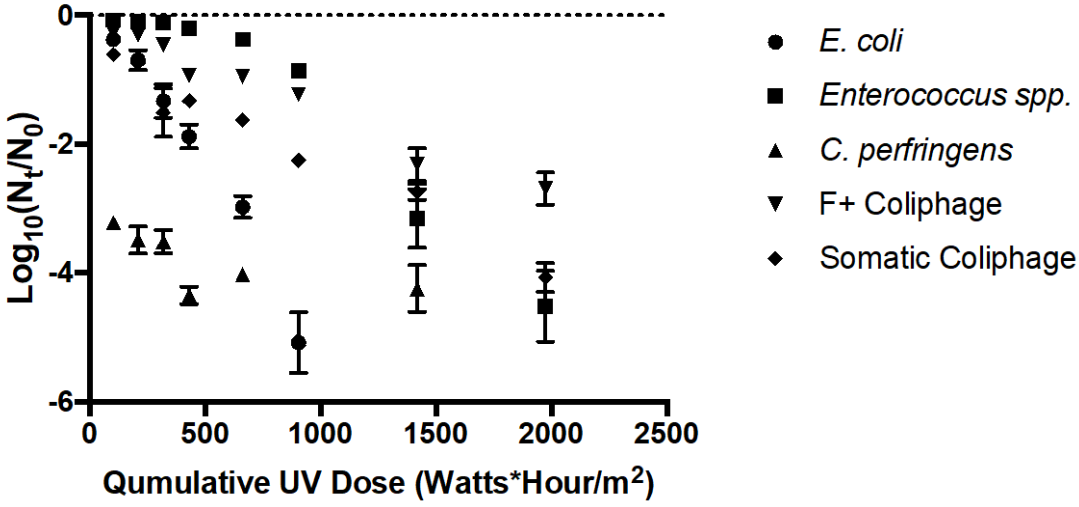

Figure 82: Survival of sewage propagated organisms exposed to sunlight $\log_{10}(N_t/N_o)$ vs. cumulative UV dose over time, 3 trials plotted per time point, shown with one standard deviation.

Table 18: Chick-Watson model parameters for sunlight survival data

Organism	Equation	*k*	R^2	UV Dose to Achieve 4 $\log_{10}$

				Reduction ($W*H/m^2$)
E. coli	Y = -0.005779x + 0.4789	-0.0058	0.9778	692
Enterococcus spp.	Y = -0.002519x + 0.7229	-0.0025	0.9323	1587
Cl. perfringens	Y = -0.000922x - 3.444	-0.0009	0.4676	4342
F+ Coliphage	Y = -0.001375x - 0.1117	-0.0014	0.9695	2909
Somatic Coliphage	Y = -0.001719x - 0.5647	-0.0017	0.9651	2327

3.3.3.4 Survival of Natural Bacteria in Surface Waters

Figures 79, 80, and 81 display the survival of indigenous populations of total coliforms, *E. coli* and *Enterococcus* spp. in surface waters at 20°C over a period of 5 days. Each data point represents the average of 3 trials plated in triplicate. The starting concentration of Total coliforms was 1.33×10^4 CFU per 100mL. At 20°C and 0 RPM mixing speed, average $\log_{10}(N_t/N_0)$ at 5 days was -0.83. There was a statistically significant difference between these results and no change ($\log_{10}(N_t/N_0)=0$, p=0.0114). For samples mixed at 60 RPM at a temperature of 20°C the average $\log_{10}(N_t/N_0)$ was -0.59 at 5 days and was not statistically significantly different from no change (p=0.0614), or from the 0 RPM mixing speed (p=0.6273). For samples mixed at 120 RPM at 20°C, the average $\log_{10}(N_t/N_0)$ was -0.87 at 5 days. These results were statistically different from no change (p=0.0082) but not statistically different from the 0 (p=0.9938) and the 60 RPM mixing speed (p=0.4891).

For *E. coli* survival examined at a temperature of 20°C and a 0 RPM mixing speed, starting concentrations were 6.67×10^0 CFU per 100mL and the average $\log_{10}(N_t/N_0)$ at 5 days was -0.46. These results were not statically different than no change (p=0.3870). At 60 RPM, the average $\log_{10}(N_t/N_0)$ at 5 days was -0.58, a value also not statistically significantly different from no change (p=0.2248) and also not statically different from the 0 RPM mixing speed at this temperature (p=0.9713). At the 120 RPM mixing speed at 20°C, the average $\log_{10}(N_t/N_0)$ value was -0.16. This value was not statistically different than no change (p=0.9656), and the $\log_{10}(N_t/N_0)$ 0 RPM (p=0.6266) or 60 RPM mixing speed values (p=0.4004). At all mixing speeds, the natural *E. coli* were significantly different than the sewage propagated *E. coli* also mixed at 0, 60 and 120 RPM at 20°C (p<0.0001). However, for sewage propagated organisms mixed at 4°C, there was no significant difference between the survival of natural *E. coli* mixed at 0 RPM and sewage propagated bacteria mixed at 0 or 60 RPM mixing speeds (p-values:0.3645; 0.0798). There was also no significant difference between the survival of naturally occurring *E. coli* mixed at 60 RPM and sewage propagated bacteria mixed at 0 RPM, 4°C (p=0.6390) or at 60 RPM (0.1791).

The starting concentration of *Enterococcus* spp. in natural waters was 3.67×10^1 CFU per 100mL. At 20°C and 0 RPM mixing speed, average $\log_{10}(N_t/N_0)$ at 5 days was -1.94. There was a statistically significant difference between these results and no change ($\log_{10}(N_t/N_0)=0$, p<0.0001). For samples mixed at 60 RPM at a temperature of 20°C the average $\log_{10}(N_t/N_0)$ was -1.94 at 5 days and was statistically significantly different from no change (p<0.0001) but not from the 0 RPM mixing speed (p>0.9999). For samples mixed at 120 RPM at 20°C, the

average $\log_{10}(N_t/N_0)$ was -1.88 at 5 days. These results were statistically different from no change ($p<0.0001$) but not statistically different from the 0 and the 60 RPM mixing speed (p values = 0.9790). Much like the comparison for *E. coli*, for *Enterococcus* spp., at all mixing speeds the natural *E. coli* were significantly different than the sewage propagated Enterococci also mixed at 0, 60 and 120 RPM at 20°C ($p<0.0001$). Similarly at 4°C, at all mixing speeds there was a statistically significant difference between the naturally occurring *Enterococcus* spp. mixed at 20°C and the sewage propagated organisms mixed at 20°C.

3.3.4 Quantitative Microbial Risk Assessment

3.3.4.1 Results and Risk Characterization

In this analysis the mean risk of infection was analyzed for reuse scenarios relevant to human health risk associated with exposure to treated and untreated reclaimed water. Table 19 presents a stepwise calculation of the potable reuse model for the US EPA mandated $\log_{10}$ reductions and storage at 20°C. Table 20 displays the mean risk of infection and upper and lower 95% confidence intervals for the 4 risk scenarios. It is important to note that the calculation of risks for *Cryptosporidium* and *Giardia* spp. are based on total counts (not on infectivity data) and despite accounting for infectivity in the QMRA model, risk may be overestimated. Additionally, US EPA Method 1623 does not differentiate between human infectious species and all species of *Cryptosporidium* and *Giardia*, which may also result in an overestimation of human health risks. For *Salmonella* spp., the risks of infection may also be overestimated as a result of the lack of data on the recovery efficiency, (R), and similar to the protozoan parasite data, a lack of differentiation between human infectious species and all *Salmonella* species. However, for adenovirus, the fraction of infectious viruses was determined by ICC-qPCR and likely does more closely estimate the risk of exposure in these exposure scenarios. In order to perform risk characterizations, an annual acceptable risk level for microbial infection of 1×10^{-4} was applied for waterborne exposure by potable water (Regli et al., 1991; Ryu et al.; 2007).

For exposure 1, which is a one time accidental exposure to 10mL of reclaimed water, the average risks for all pathogens were below the acceptable risk level with the exception of the mean risk of infection for adenovirus, which was 7.39×10^{-3} with a 95% confidence interval of 3.73×10^{-3} to 1.43×10^{-2}. Based on this analysis it is clear that there is a low risk of infection from the accidental exposure to 10mL of reclaimed water from the pathogens analyzed, with the exception of adenovirus, which still poses a risk at this level. For scenario 2, the risks of infection for 40 days of recreational exposure per year were compared to the US EPA acceptable risk level of 30 infections per 1000 people (3×10^{-4}). Based on this level the average annual risks were below the acceptable microbial risk level for *Giardia*, with average infection risks of 4.28×10^{-4} with a 95% confidence interval of 2.69×10^{-5} to 6.20×10^{-3}. The mean risk *Cryptosporidium* was slightly above the acceptable risk level, with a mean level of 2.58×10^{-3} and a confidence interval of 8.62×10^{-4} to 7.63×10^{-3}. The mean risk of exposure for both *Salmonella* spp. and adenovirus were above the US EPA acceptable risk level with levels of 1.18×10^{-1} and 8.23×10^{-1}, with highest levels of risk for adenovirus for the 40 day exposure period.

In scenario 3, the annual risks of infection after exposure to reclaimed water piped to a household as drinking water and consumed at a rate of 2L per day 365 days per year are

considered. In this scenario, the risks for adenovirus infection are >1, indicating that there is a very high risk of adenovirus risk under this scenario, which involve no additional treatment after initial wastewater treatment. The average risk of *Salmonella* spp. was 8.50×10^{-1} with a 95% confidence interval of 1.29×10^{-1} to 1.00×10^{0}, as with adenovirus these values are above the US EPA acceptable risk level for drinking water. Additionally, the acceptable risk levels for *Cryptosporidium* and *Giardia* are 1.00×10^{-1} and 2.75×10^{-1} respectively, and are also above the acceptable risk levels set by US EPA. As all of the average risks of infection for each pathogen for this exposure level are above the US EPA acceptable risk of infection, there is a high risk of infection by this exposure route.

The potable reuse scenarios analyzed in Scenario 4 include 1) the US EPA regulated $\log_{10}$ reductions for conventional drinking water treatment and disinfection (4 $\log_{10}$ for virus, 2 $\log_{10}$ *Cryptosporidium* and 3 $\log_{10}$ for *Giardia*), 2) the World Health Organization's (WHO) risk based reduction of pathogens based on Disability Adjusted Life Years (DALYs) and 3) 3) a worst-case $\log_{10}$ reduction scenario based on real world data reviewed by Medema and Hijnen, 2007. Each of these scenarios were evaluated for 5 day storage conditions at both 4 and 20°C.

Based on these $\log_{10}$ reduction scenarios, it is clear that the US EPA and WHO $\log_{10}$ reduction targets produce reclaimed water that complies with the 10^{-4} annual risk of infection target set by US EPA for *Cryptosporidium* and *Giardia* at both 4 and 20°C storage temperatures. In contrast, the risks of infection from *Salmonella* spp. and adenovirus are not reduced below the acceptable level of risk for either storage temperature. The US EPA $\log_{10}$ reduction targets are based on reducing viruses, and *Cryptosporidium* and *Giardia*, while the WHO targets are based on reducing risk of illness based on exposure to organisms in source water. For the worst-case scenario, which was based on a real world data set reviewed by Medema and Hijnen, 2007, the average annual risk of infection from all microorganism was higher at all temperatures, with a similar pattern of *Cryptosporidium* and *Giardia* reduction below the acceptable risk level and adenovirus and *Salmonella* risk continuing to be above this risk level. In general, the risks of adenovirus infection were the greatest for all exposure routes, this is partly due to the higher concentrations of adenovirus is reclaimed waters, surface water, and as a result the combined waters. Temperature does not play a large role in reducing average annual risk of infection based on this analysis, for all scenarios analyzed the risks are approximately the same for both temperatures.

Table 19: Step-wise potable reuse example calculation

	Units	Adenovirus	*Salmonella* spp.	Cryptosporidium spp.	*Giardia* spp.
Microbe Concentration, C	Organisms per liter	1	1	1	1
Average Reduction by 5 day storage (20°C), 5 day	% Reduction	0.94	0.91	0.98	0.98
Reduction by EPA Mandated conventional	% Reduction	0.9999	0.999	0.99	0.99

drinking water treatment, DWTP					
Potable Reuse Water, RW	Organisms per liter	6.00E-06	9.00E-05	2.00E-04	2.00E-04
Recovery Efficiency, R	%	109	100	82.5	63.5
Viability, I	%	38.5	65	25	13
Volume of Water Consumed, V	Liters per day	2	2	2	2
Exposure by drinking water, N	Organisms per day	4.62E-04	1.17E-02	1.00E-02	5.20E-03
Dose-response, r	Probability of infection per organism	6.07E-01	2.10E-01	4.20E-03	1.98E-02
Risk of infection, Pinf,d	Per day	2.80E-04	2.46E-03	4.20E-05	1.03E-04
Risk of infection, Pinf,y	Per year	9.73E-02	5.93E-01	1.52E-02	3.69E-02

Formulas:	RW = C*1-DWTP*1-5day
	N = RW*C*R^-1*I*V
	Pinf,d=N*r
	Pinf,y=1-(1-Pinf,d)^365

Table 20: Risks of infection for pathogens from five exposure scenarios to reclaimed water

Scenario 1 (One Time Accidental Exposure to 10mL Reclaimed Water)

Organism	Mean	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Salmonella spp.	2.63E-05	1.90E-06	3.60E-04
Adenovirus A-F	7.39E-03	3.73E-03	1.43E-02
Cryptosporidium spp.	1.45E-06	2.27E-07	9.68E-06
Giardia spp.	4.41E-06	6.81E-07	3.21E-05

Scenario 2 (Recreational Exposure to 100mL Surface Water, 40 days)

Organism	Mean	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Salmonella spp.	1.18E-01	2.25E-02	4.85E-01

Adenovirus A-F	8.23E-01	4.36E-01	9.94E-01
Cryptosporidium spp.	2.58E-03	8.62E-04	7.63E-03
Giardia spp.	4.28E-04	2.69E-05	6.20E-03

Scenario 3 (Reclaimed Water Exposure 2L/day, 365 days)

Organism	Mean	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Salmonella spp.	8.50E-01	1.29E-01	1.00E+00
Adenovirus A-F	1.00E+00	1.00E+00	1.00E+00
Cryptosporidium spp.	1.00E-01	1.64E-02	5.07E-01
Giardia spp.	2.75E-01	4.85E-02	9.04E-01

Scenario 4-1 (Potable Reuse Exposure - US EPA 2L/day 365 days)

	Storage at 4°C			Storage at 20°C		
Organism	Mean	Lower 95% Confidence Interval	Upper 95% Confidence Interval	Mean	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Salmonella spp.	4.62E-03	4.76E-04	3.69E-02	4.58E-03	4.51E-04	3.53E-02
Adenovirus A-F	3.63E-02	6.41E-03	1.43E-01	1.01E-02	1.44E-03	4.86E-02
Cryptosporidium spp.	1.57E-04	2.61E-05	6.81E-04	4.55E-04	6.32E-05	2.38E-03
Giardia spp.	7.63E-07	2.38E-08	2.43E-05	2.22E-06	6.03E-08	7.44E-05

Scenario 4-2 (Potable Reuse Exposure - WHO 2L/day 365 days)

	Storage at 4°C			Storage at 20°C		
Organism	Mean	Lower 95% Confidence Interval	Upper 95% Confidence Interval	Mean	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Salmonella spp.	4.69E-03	4.62E-04	3.88E-02	4.62E-03	4.49E-04	3.76E-02
Adenovirus A-F	3.59E-02	6.23E-03	1.42E-01	9.95E-03	1.42E-03	4.84E-02
Cryptosporidium spp.	1.60E-05	2.75E-06	7.06E-05	4.63E-05	6.56E-06	2.44E-04
Giardia spp.	7.64E-07	2.35E-08	2.42E-05	2.22E-06	6.17E-08	7.49E-05

Scenario 4-3 (Potable Reuse Exposure - Worst Case 2L/day 365 days)

	Storage at 4°C			Storage at 20°C		
Organism	Mean	Lower 95% Confidence Interval	Upper 95% Confidence Interval	Mean	Lower 95% Confidence Interval	Upper 95% Confidence Interval
Salmonella spp.	2.62E-02	3.01E-03	1.84E-01	2.54E-02	2.94E-03	1.78E-01

Adenovirus A-F	5.70E-01	9.79E-02	9.74E-01	2.06E-01	2.46E-02	7.02E-01
Cryptosporidium spp.	9.65E-06	1.50E-06	4.34E-05	2.79E-05	3.47E-06	1.55E-04
Giardia spp.	4.71E-07	1.42E-08	1.48E-05	1.37E-06	3.63E-08	4.60E-05

4 Discussion

4.1 Log_{10} Reduction Analysis for Indicator and Pathogenic Microorganisms

4.1.1 Indicator Organisms

As indicator organisms are typically detected in samples of water and wastewater as a model of the behavior of pathogenic microorganisms, one of the goals of the work presented here was to detect both the indicator organisms suggested by the state of NC for the evaluation of reclaimed water quality as well as to detect representative pathogens relevant to public health concern. In North Carolina, specific treatment recommendations were established for reclaimed water to be used for potable reuse purposes, specifically tertiary wastewater treatment followed by dual disinfection (typically UV and chlorine). In addition to treatment recommendations, log_{10} reductions and effluent quality requirements have been established to monitor the quality of this "Type 2" reclaimed water. Specific design criteria for wastewater treatment to meet NCT2RW requirements must achieve log_{10} reductions of 6 for *E. coli*, 5 for coliphages and 4 for *Clostridium perfringens*. Effluent quality for NCT2RW must also meet a geometric mean concentration of 3/100 mL for *E. coli* and 5/100 mL for both coliphages and *Clostridium perfringens*, with daily maxima of 25/100 mL for each of these three target microbes.

The results presented in this report represent a series of samples from 4 plants producing NC Type 2-like reclaimed water, characterized by tertiary treatment and dual disinfection (UV and chlorine treatment) as well as samples from 1 treatment plant producing reclaimed water treated by a single disinfection (chlorine only) step only. The log_{10} reductions are presented by microorganism and by treatment plant in the tables and figures above. As the tertiary treated, dual disinfected reclaimed water proposed for potable reuse applications has not been evaluated for microbiological quality and the log_{10} reduction performance has not been examined, one of the goals of this study was to address this need for data.

A summary of the fecal indictor bacteria detected in raw sewage and reclaimed water samples along with the calculated log_{10} reduction values calculated between the two types of samples are presented in table 5 and Figures 2 – 7. Based on the results presented here, the average log_{10} reduction was achieved by NCT2RW producing wastewater treatment facilities (presented in Figure 7) indicates that an average log_{10} reduction of 6.38 was achieved for *E. coli* while an average reduction of 5.57 was achieved for *Enterococcus* spp. Based on this analysis it appears that for *E. coli* the state mandated log_{10} reduction performance standard is met and that for *Enterococcus* spp. it is not met based on a 6 log_{10} reduction standard. It is important to note that the values used for the log_{10} reduction calculations incorporated the limit of detection (1MPN/100mL) for all samples of NCT2RW as no *E. coli* or *Enterococcus* spp. was detected in the reclaimed water. As such, these log_{10} reductions are upper limit detection values based on the concentration of *E. coli* and *Enterococcus* spp. present in the raw wastewater rather than the

potential treatment effect achievable by the wastewater treatment facilities. Another important consideration is that all of the reclaimed water samples were below the daily geometric mean of 3 *E. coli* per 100mL and a daily maximum of 25/ 100mL. In contrast to the dual disinfected samples, despite similar starting concentrations of bacteria, the chlorine only treated samples did not achieve complete removal or a 6-$\log_{10}$ reduction. These samples also did not meet the daily or monthly geometric mean limits.

Somatic, F+/male-specific, and total coliphage concentrations detected in raw sewage and reclaimed water samples along with calculated $\log_{10}$ reductions are presented in Table 7 and Figures 8 – 13. On average, the $\log_{10}$ reductions for somatic, F+/male specific, and total coliphages were 4.2, 3.7, and 4.1 respectively. Again, it is important to note that these values are based on an upper limit detection value as there were few to no coliphages detected in the reclaimed water samples after dual disinfection treatment. However, based on this analysis, it is not clear that the NCT2RW producing treatment plants have met the 5 $\log_{10}$ reduction standard established for indicator viruses. The monthly geometric mean and daily maxima of 5/100mL and 25/100mL respectively were not exceeded for any samples of NCT2RW. For the samples disinfected by chlorine only, the average $\log_{10}$ reduction was approximately the same as the NCT2RW treatment plats (between 4 and 4.5 per 100mL $\log_{10}$) while the daily maxima and monthly geometric mean values were not exceeded for any samples. In contrast to the bacterial indicator results, for the virus results, it is not clear that there was a significant difference between adding a secondary disinfection step to the NCT2RW treatment process.

Concentrations of pasteurized and unpasteurized *C. perfringens* detected in raw sewage and reclaimed water samples as well as calculated $\log_{10}$ reductions between the two sample types are presented in Table 8 and Figures 14 – 19. Average $\log_{10}$ reductions for pasteurized and unpasteurized *Clostridium perfringens* were 4.4 and 4.4 respectively. As with the other indicator organisms, with most samples of NCT2RW, little to no *C. perfringens* spores or vegetative cells were detected. As such, these values represent an upper limit detection value rather than the true performance of the wastewater treatment system. The monthly geometric mean and daily maxima of 5/100ml and 25/100ml respectively were not exceeded for any samples of NCT2RW. For chlorine only disinfected samples collected from the Durham treatment plant, the average $\log_{10}$ reduction for pasteurized and unpasteurized *C. perfringens* was 1.2 and 1.1 respectively. Much like the bacterial indicator results, there is a significant difference in the average $\log_{10}$ reductions achieved by chlorine only disinfection versus a dual disinfection system for the protozoan parasite surrogates.

Log_{10} reduction values are a common measure of treatment efficacy (Rose et al., 1996; 2001), but do not always give an accurate picture of treatment effect. For example, in the indicator organism data presented here, the data are limited by an upper limit detection value in that the $\log_{10}$ reduction is calculated based on value detected in raw sewage and detection limit value in reclaimed water. The actual $\log_{10}$ reduction based on treatment may be higher; however values are limited based on the values in the influent raw sewage and the detection limit values in the reclaimed water. As these values are not entirely representative of the treatment effect, the $\log_{10}$ concentrations are presented for both the influent and NCT2RW samples.

There are several important limitations to the data presented here, including the sample volume of NCT2RW analyzed for indicator organisms. As per standard methods, 100mL of sample was examined for indicator organism. This low volume of sample resulted in a non detect values for many of the NCT2RW samples. Other study limitations include the number of samples analyzed across and between wastewater treatment plants.

4.1.2 Pathogens

Although the North Carolina legislation on reclaimed water specifies indicator organisms to track the performance of wastewater utilities in the production of waters for potable reuse purposes, in order to evaluate the health effects of drinking these waters, it is necessary to evaluate the reduction performance for pathogenic microorganisms of human health concern. In selecting pathogens of interest, it is important to evaluate microorganisms that are present and persistent in wastewater and that can be tracked through wastewater treatment processes. Important considerations include the method of detection, and associated considerations such as time, ease, cost, etc. Another important factor is whether or not the method detects viability or infectivity of the given pathogen.

For this analysis, reference pathogens were selected for each class of microorganism and were detected in raw sewage and reclaimed water samples from 4 plants producing NC Type 2-like reclaimed water, characterized by tertiary treatment and dual disinfection (UV and chlorine treatment) as well as samples from 1 treatment plant producing reclaimed water treated by a single disinfection (chlorine only) step only. The $\log_{10}$ reductions are presented by microorganism and by treatment plant in the tables and figures above.

A summary of the concentrations of pathogenic *Salmonella* bacteria detected in raw sewage and reclaimed water samples as well as the calculated $\log_{10}$ reductions between these two types of samples are presented in Table 9 and Figures 20 – 25. The average $\log_{10}$ reduction for *Salmonella* spp. bacteria was 4.31. Much like the *E. coli* and *Enterococcus* spp. results, the majority of the samples of NCT2RW analyzed for pathogenic bacteria did not contain high concentrations of *Salmonella* spp. As such, these $\log_{10}$ reduction values are the result of upper limit detection values rather than a reflection of the $\log_{10}$ reduction performance of the wastewater treatment system. It is also important to note that there is a lower background concentration of *Salmonella* spp. in raw sewage when compared to that of *E. coli* and *Enterococcus* spp. (as expected) resulting in a lower $\log_{10}$. Additionally, the concentrations of *Salmonella* spp. were more variable than those of the indictor organisms, as the method for the detection of *Salmonella* bacteria is more complicated than that of the indicator organisms. If *Salmonella* spp. concentrations in the final reclaimed water are compared to state mandated daily maxima or monthly geometric mean values, samples from all NCT2RW treatment plants are below state mandated values.

Enteric virus concentrations detected in raw sewage and reclaimed water samples along with calculated $\log_{10}$ reductions are presented in Table 10 and Figures 26 – 31. The average $\log_{10}$ reduction for Norovirus GII and adenovirus groups A-F are 3.1 and 2.4 respectively. These values include non-detect values, assuming the limit of detection for the qPCR or RT-qPCR method (1 genome copy, GEC). As with the coliphage viruses, for Norovirus, the $\log_{10}$ reduction

value represents an upper limit detection value in that there was little to no Norovirus GII detected in the NCT2RW samples. The $\log_{10}$ reduction value in this case represents the concentration of Norovirus in the raw sewage, which is lower and more variable than the concentrations of coliphages in influent waters. Adenovirus, in contrast, was detected in NCT2RW at relatively high concentrations, resulting in low and variable $\log_{10}$ reduction values. Adenovirus concentrations in raw sewage samples ranged from approximately 10^2 to 10^5 where concentrations in the NCT2RW ranged from undetectable levels to approximately 10^4. The levels of Norovirus GII are approximately comparable to the levels of F+ coliphages present in raw sewage ($\sim10^3$) whereas the levels of adenoviruses are approximately comparable to the levels of somatic and total coliphages ($\sim10^4$-10^5); however, the $\log_{10}$ reduction level achieved for the pathogens is not comparable for adenoviruses based on the detection methods used here. It is important to note that the coliphage viruses detected here were detected by the single agar layer method (SAL), which detects infectious coliphages by a culture method, whereas adenoviruses (and noroviruses) were detected by (RT)qPCR which detects gene copies rather than infectious units of viruses. Based on this analysis the levels of Norovirus detected in NCT2RW would meet the daily maxima and monthly geometric mean levels mandated by the state of NC, but the levels of adenovirus do not meet these standards.

Concentrations of protozoan parasites detected in raw sewage and reclaimed water samples as well as calculated $\log_{10}$ reductions between the two sample types are presented in Table 11 and Figures 32 – 37. The average $\log_{10}$ reductions for *Cryptosporidium* and *Giardia* spp. were 2.5 and 2.9 respectively. As with the enteric viruses, these $\log_{10}$ reductions are lower than those for the indicator organisms, which were approximately 4 $\log_{10}$ per 100mL. This discrepancy is related to the lower concentrations of *Cryptosporidium* and *Giardia* in raw sewage as well as the low but detectable levels of these organisms in the NCT2RW samples. Important factors to consider in the comparison between the $\log_{10}$ reductions between the indicator and pathogenic microorganisms are the method of detection, and volume of concentrated sample. The indicator organisms were detected by a standard membrane filtration method, which resulted in culture (infectivity) data whereas protozoan parasites were detected by concentration by hollow fiber ultrafiltration followed by immunofluroescent microscopy, which does not provide any information on viability or infectivity. Additionally, *C. perfringnens* was not frequently detected in 300mL samples of NCT2RW, compared to the 10L concentrated by hollow fiber ultrafiltration for protozoan parasite analysis. Based on the $\log_{10}$ reductions presented here, the protozoan parasites would not meet the state mandated $\log_{10}$ reduction performance requirements, however, as explained previously, this relates to the low background concentrations of (oo)cysts in the raw sewage samples.

The goal of this $\log_{10}$ reduction analysis was to compare the $\log_{10}$ reductions performance for the indicator organisms to that of the pathogenic microorganisms. In general the $\log_{10}$ reductions for pathogenic microorganisms were lower and more variable when compared to indicator organisms. Much of this variation is related to the detection method for each pathogen, which are more complicated and difficult to preform when compared to the methods used for detecting indicator organisms. The lower $\log_{10}$ reductions are also related to the lower concentrations of the pathogenic organisms in raw sewage samples, with the exception of the adenovirus $\log_{10}$ reduction, which is low because of relatively high concentrations of adenovirus in NCT2RW samples. Despite not achieving the state mandated 6-5-4 $\log_{10}$ reduction targets for bacteria,

viruses, and protozoan parasite surrogates or pathogens, the monthly geometric mean and daily maxima levels were not exceeded except for adenovirus.

Important limitations of this analysis include the lack of infectivity data for pathogenic microorganisms, particularly for the enteric viruses. As adenoviruses were detected in NCT2RW samples at high levels (on average 10^3 GEC per 100mL) there is a need to determine if these PCR units are infectious after the dual disinfection treatment. The protozoan parasites detected in these samples were also detected by total count methods, specifically immunofluroscence microscopy, rather than infectivity methods, resulting in data that do not reflect the ability of these organisms to infect after dual disinfection treatment. Another important limitation includes the sample volume analyzed for indicator organisms, which although standard, was not large enough to detect indicator organisms in the majority of NCT2RW samples, resulting in the inability to calculate log10 reductions for the performance of the dual disinfection system. Finally, the number of samples analyzed across and within treatment plants is a limitation of the analysis presented here. Additional samples are necessary to document the performance of the dual disinfection treatment system as well as the pathogen concentration in the NCT2RW.

4.3 Surface Water Samples

The current surface water quality monitoring approach targets indicator organisms, specifically total or fecal coliforms in surface water in either a single daily grab sample or as a composite sample. In North Carolina, the suggested targets for drinking water intake sources are $\leq$300-50 fecal coliforms or *E. coli* per 100mL depending on off stream storage (0.5 to 4 hours) (NC DENR, 1996). Additionally, US EPA has specific requirements for the treatment of surface waters based on the reduction of *Cryptosporidium* and viruses in these sources. The most recent rule, the Long Term 2 Enhanced Surface Water Treatment Rule (US EPA, 2006) established a minimum 2 $\log_{10}$ reduction of *Cryptosporidium*, with a requirement of filter monitoring to minimize the effects of poor performance. The goal of this rule was to expand protection for high risk surface water sources, such as those that may store water in open reservoirs, by requiring water utilities to either cover open reservoirs or to achieve additional $\log_{10}$ reduction performance requirements (4 $\log_{10}$ for virus, 3 $\log_{10}$ for *Giardia lamblia*, and 2 $\log_{10}$ for *Cryptosporidium*) (US EPA, 2006).

Though indicator microorganisms are typically used as predictors of fecal contamination and therefore, considered indirectly representative of pathogen content in water, this relationship is imperfect (Havelaar et al., 1993; Simpson et al., 2003; Harwood et al., 2005). One of the goals of this research was to evaluate the relationship between indicator microorganisms and pathogens they are intended to represent in surface waters to determine any predictive relationship between the two categories, fecal indicators and pathogens, in this type of water. As the detection of indicator microorganisms is typically the only microbiological testing done by most drinking water treatment facilities, the link between and representativeness pathogens and their indicators is an important consideration for water supply system and water supply regulators including public health regulators of water supplies.

In this study it was found that pathogens were detectable in nearly all samples of sewage impacted surface water analyzed. *Salmonella* spp. was found in 91% of all samples at

concentrations ranging from 0.1 to 1.2 MPN/100 mL, Adenoviruses that were detected based on the presence and concentrations of their nucleic acids were found in 41% of all samples at concentrations ranging from 1 to 3.60 x 10^4 GECs/100 mL *Cryptosporidium* and *Giardia* as detected by immunofluorescent microscopy were found in 100% and 81% of all samples, respectively. Total coliforms, *E. coli*, and *Enterococcus* were detected in 95%, 64%, and 50% samples respectively, while somatic, F+ and total coliphage viruses were detected in 77%, 32% and 77% of samples respectively. *C. perfringens* spores and vegetative cells were detected in 91% of all samples. As pathogens and indicators were detected in different volumes of surface water, it is likely that this larger sample volume impacted the detectability of pathogens in surface waters. A larger sample volume and sample size is desirable and likely improved the detection of pathogens in this study.

As US EPA Method 1623 does not allow for the determination of (oo)cyst infectivity or the detection of human specific (oo)cysts, one important limitation to this study is the lack of infectivity data on the protozoan parasites (US EPA, 2012). In this study, *Cryptosporidium* and *Giardia* were found at low levels by immunoflorescent microscopy, in nearly all surface water samples, but infectivity was not assessed due to lack of time and additional resources to process these surface water samples. An important limitation of this work is the lack of infectivity data on human pathogens and the inability to predict human health risk based on the detection of these organisms in surface waters. Although the presence of pathogens in surface water is of concern, it is difficult to evaluate the human health risk of these microorganisms.

Indicator presence or absence was not consistently predictive of pathogen presence or absence by binary logistic regression, and the results of this study yielded a high number of false negative or false positive results for one of the indicator pathogen combinations, specifically the adenovirus/F+ coliphage relationship. The relationship of indicators with pathogens that were detected more frequently, such as adenovirus, showed a higher frequency of false positives (pathogens absent, indictors present). This result is not necessarily undesirable because the goal of an indicator is to alert for pathogen presence, rather than for pathogens to be present at equal or greater numbers than the indicator. The relationship of indicators with pathogens detected less frequently, such as *Salmonella* spp., showed a higher frequency of true positives (pathogens present, indicators present); however as the *Salmonella* was detected at concentrations on average 100-fold lower than the indicator organism, this represents an ideal characteristic of an indicator organism. FIB occurrence was not predictive of *Salmonella spp.* presence by binary logistic regression, but *Salmonella* spp. statistically significantly correlated with the concentrations of total coliform by Spearman's correlation analysis. This study suggests that there may not be one "ideal" indicator for the prediction of survival or presence of pathogens in surface water; however, based on correlation analysis, there is evidence that the $\log_{10}$ concentrations of indicator organisms are correlated with pathogen concentrations.

Though individual indicator organisms and pathogens were weakly correlated or uncorrelated by binary logistic regression, there is some evidence that $\log_{10}$ concentrations of indicator organisms are correlated with $\log_{10}$ concentrations of pathogens in surface water. The results presented here indicates that enteric pathogens, including Salmonella bacteria, human enteric viruses such as Adenoviruses and the protozoan parasites *Cryptosporidium* and *Giardia* are often present at detectable concentrations in surface waters that could be used as drinking water

sources. In the comparison of reclaimed water to surface waters presented here, it is clear that the quality of surface waters is not of the same microbiological quality as NC Type 2-like reclaimed water. For nearly every microorganism examined, concentrations in surface water were at minimum 10 fold greater in surface waters samples. Additional studies are needed to more thoroughly and rigorously evaluate the relationships between the fecal indicators and the enteric pathogens in these waters. Important consideration should be given to infectivity and culturability of protozoan parasites and enteric viruses in order to more accurately evaluate the human health risk from these pathogens. Additionally, this study only represents a small number of samples (n=22), from a limited number of sample sites (n=5), future work should expand on both the number of samples and the surface water sources to provide a more representative selection of the range of conditions that occurring in surface water sources used for drinking water.

4.4 Survival Experiments

Based on the results of this study, there was a statically significant difference between the $\log_{10}(N_t/N_0)$ value at 5 days and a $\log_{10}(N_t/N_0)$ of no change ($\log_{10}(N_t/N_0)=0$) for all organisms examined under all conditions except sewage propagated *Enterococcus* examined at 4°C and naturally occurring *E. coli* examined at 20°C. Additionally, there was no statically significant difference between mixing conditions at either temperature. This suggests that the concentrations of the organisms examined here change over the course of the 5 day storage period. For sewage propagated organisms stored at 4°C, concentrations of *E. coli*, *C. perfringens*, and F+ coliphage decreased over the 5 day period; however, there were slight increases of enterococci and somatic coliphages. Additionally, at the 20°C storage temperature for these organisms, there were much larger (2 $\log_{10}$) increases in concentration for *E. coli* and *Enterococcus* spp., while *C. perfringens*, F+ and somatic coliphages decreased in concentration. Potential reasons for this increase include the regrowth of bacteria due to the initial propagation, and the disaggregation of aggregated bacteria. Despite the regrowth of *E. coli* and enterococci in the sewage propagated bacteria experiments, there was no regrowth, or growth, of these organisms or of the total coliform bacteria in the natural bacteria experiments. This suggests that it is not the tendency of natural bacteria to grow at 20°C, but rather that that this was an artifact of the propagation of the bacteria or the disaggregation of these lab cultured bacteria used the spiking experiment. Although the regrowth of total coliform bacteria has been well documented (CT Butterfield, 1933; MW LeChevallier, 1990; Hammes et al., 2010), no increases in naturally occurring total coliform bacteria in the water tested were observed in these experiments. In the natural bacteria experiments, there was no significant difference between no change ($\log_{10}(N_t/N_0)=0$) and the survival of the natural total coliform bacteria or the *E. coli*, but there was a significant difference between the survival of *Enterococcus* spp. over the 5 day period. Much like the sewage propagated bacteria experiments; there was no significant difference between mixing speed.

Previous studies have documented the survival of indicator organisms in reclaimed water; however none have considered this mix of surface water to reclaimed water for potable reuse storage. Bitton et al., found that in groundwater *E. coli* and f2 coliphage declined at rates of 0.0066 and 0.059 hr^{-1} respectively. In other studies, it has been suggested that changes in the concentrations of bacteria in freshwater, including the potential for growth, may be a result of inhibitory substances (Klein and Alexander, 1986), or due to the activities of predatory and lytic

organisms (KP Flint, 1987; González et al., 1990). However, for the factors evaluated in this study, there was no statically significant effect related to the mixing speed, or temperature on the survival of sewage propagated or naturally occurring microorganisms over the 5 day period. Additionally, in comparing the naturally occurring microorganism to the sewage propagated microorganisms, there was no significant difference between sewage propagated *E. coli* stored at 4°C.

In the evaluation of sunlight inactivation, there was no significant difference between the $\log_{10}(N_t/N_0)$ values over time for many of the microorganisms examined here. Specifically, there was no difference between the inactivation of *E. coli* and *Enterococcus* over the 5 day period, or between *C. perfringens* and *E. coli* or enterococci. However, there was a significant difference between the inactivation of F+ and somatic coliphages. In this study F+ coliphages appear to be more resistant to disinfection by sunlight than somatic coliphages in the mix of reclaimed and surface water. This same pattern of F+ coliphage resistance to sunlight in freshwater was also found in a study done by Sinton et al. in 2002. Other studies have evaluated the survival of microorganisms exposed to UV light (Gutiérrez-Alfaro et al., 2015) and have found *C. perfringens* to be the most resistant to sunlight disinfection. Much like the results presented here, the work done by Gutiérrez-Alfaro et al. concluded that increased exposure times were required to achieved increased $\log_{10}$ reductions for protozoan parasite surrogates. Hijnen et al., 2006 also found that for environmental spores (such as the strains used here) have increased UV resistance and may required additional UV treatment to achieve $\log_{10}$ reductions necessary for wastewater treatment.

The results of this study suggest that the 5 day storage period proposed for the 80/20 blend of surface water and reclaimed water to be used as a component of potable reuse treatment, does have an impact on the survival of indicator microorganisms. Based on statistical analyses of survival concentrations, there was a significant difference between the concentrations of all or organisms stored over the 5 day period at all temperatures and at all mixing speeds with the exception of *E. coli* (natural and sewage propagated). At a temperature of 20°C, there was a $\log_{10}$ reduction of approximately 1 $\log_{10}$ for all microorganisms, with the exception of *E. coli* and *Enterococcus* spp. (a 1 $\log_{10}$ reduction was achieved for enterococci in the natural bacteria experiments). This suggests a 1 $\log_{10}$ reduction credit would be appropriate for a 5 day storage system based on the results presented here. Higher $\log_{10}$ reduction credits could be achieved with documented reductions using increased treatment during storage; treatment conditions could include, sunlight inactivation, increased temperature, etc. These results have practical implications for wastewater utilities considering potable reuse schemes, particularly those that require 5 day storage.

4.3 Quantitative Microbial Risk Assessment

In the evaluation of the North Carolina potable reuse scheme it appears that the proposed combination of 80% surface water with 20% reclaimed water, followed by 5 days of storage, and conventional drinking water treatment does not adequately decrease the risks of infection for the pathogens assessed by this study if $\log_{10}$ reduction targets set by US EPA and WHO are met by drinking water utilities. The goal of this study was to evaluate the health risk associated with exposure to pathogens in of reclaimed water after specific exposures related to potable reuse, and

based on the four exposure scenarios presented here, the risk of exposure to pathogens is not adequately reduced after complete treatment. Adenovirus and *Salmonella* were the two pathogens not reduced below the US EPA acceptable level of risk for drinking water exposures. For the US EPA $\log_{10}$ reduction targets, the average annual risk of infection for adenovirus was approximately 10^{-2} per person per year for storage at 4 and 20°C, while for the WHO $\log_{10}$ reduction targets the average annual risk was 10^{-2} at 4°C and 10^{-3} at 20°C. For *Salmonella*, under the US EPA and WHO $\log_{10}$ reduction targets, annual risks were on average 10^{-3} per person per year at both temperatures. The risks of infection from both *Cryptosporidium* and *Giardia* were reduced below the US EPA acceptable risk level by potable reuse treatment for both the US EPA and WHO $\log_{10}$ reduction targets at both temperatures.

A secondary goal of this study was to evaluate the risk of infection from recreational exposure to reclaimed water (Scenario 2). The risks calculated for this route of exposure also indicate that the average annual risks for adenovirus and *Salmonella* spp. were higher than the US EPA acceptable limit of 1×10^{-4} per person per year. As mentioned in section 5.6, the risk calculations presented here are conservative estimates and may be higher than what can be expected from potable reuse or drinking water treatment in practice. The numbers for recreational exposures do not consider sunlight exposure and the potential die-off as a result of sunlight UV exposure, a factor that is likely to play a role in microorganism survival.

Because high levels of adenovirus were detected by qPCR, additional methods were used (ICC-qPCR) to determine the infectivity of adenoviruses in reclaimed water samples. Rodríguez et al., 2013 and Polston et al., 2014, have previously evaluated the infectivity of adenovirus in wastewater and surface samples and in those studies approximately 50% of raw sewage and 44% of surface water samples positive for adenovirus based on the presence of genome copies were also positive on the basis of cell cuture infectivity. The present study found that approximately 38% of adenovirus positive reclaimed water samples for gene copies were also positive for infectious adenoviruses. With a high concentration of adenovirus in the reclaimed water (detected by qPCR), and a high percentage of infectious adenovirus (determined by ICC-RTqPCR), there results a high estimation of annual risk for this pathogen under scenarios considering incomplete drinking water treatment steps. However, when full drinking water treatment processes were evaluated, the risk of adenovirus infection decreased, but was still not reduced below the US EPA acceptable level of annual risk of infection. Despite this, additional study is needed to further evaluate the relationship between direct detection of viruses (DNA/RNA) and the infectivity of those viruses after treatment by disinfection and other processes in wastewater and surface water treatment applications.

Although conventional wastewater treatment is known to reduce numbers of *Cryptosporidium* and *Giardia* by 3 and 4 $\log_{10}$ respectively, numbers of (oo)cysts are often detected in tertiary treated effluents (Gennaccaro et al., 2003). The risk of infection related to exposure to *Cryptosporidium* and *Giardia* in reclaimed waters has been previously evaluated for non-potable uses (Ryu et al., 2007) as well as from surface water exposures (Jacob et al., 2015). These studies found low but detectable levels of protozoan parasites in these waters used for either agricultural use or drinking water purposes, but with health risks below($<10^{-4}$ per person per year, the US EPA acceptable risk level. In this study, both *Cryptosporidium* and *Giardia* were detected in nearly all reclaimed water samples at low concentrations. Despite this, and similar to the

previously cited studies, the average annual risks of infection were below the US EPA average annual acceptable risk of infection. Infectivity of protozoan parasites was not assessed in this study, and therefore, further study is also needed to evaluate the infectivity of human infectious protozoan parasites after UV and other reclaimed water and drinking water treatment processes and trains. Such infectivity studies are potentially possible for Cryptosporidium oocysts although not for Giardia cysts.

Based on the analyses and findings presented here, it is clear that potable reuse under conditions that provide for advanced drinking water treatment or storage options is possible and a potentially a viable option for communities with the capacity to first treat wastewater by tertiary treatment processes followed dual disinfection processes. As the risks of adenovirus and *Salmonella* infections for drinking water and recreational exposures are still above the US EPA acceptable annual risk limit, it may be advisable for wastewater treatment plants to increase or otherwise re-evaluate virus treatment methods. As a 5 day storage time is required for potable reuse in NC, open air tanks with some form of mixing and enhanced sunlight exposure may be effective in decreasing the microbial concentrations in reclaimed waters blended with raw surface waters for further treatment to reduce pathogens. Additional sunlight exposure and aeration, such as by aeration that sprays the water into the air may be an effective approach for enhancing virus inactivation in the stored water.

Reclaimed water has become more attractive with increases in population, patricianly around urban centers, and with appropriate use of available technology, it is possible to use waters currently produced by wastewater utilities and the treatment capabilities of drinking water treatment plants to provide high quality reclaimed water for potable reuse.

5 Summary

The goal of this study was to address the need for real world data on the quality of the North Carolina approved reclaimed water, designated as type 2 (NCT2RW). This goal was accomplished by conducting field studies on reclaimed water currently produced by four wastewater reclamation facilities producing North Carolina type 2-like water as well as on ambient surface waters used or potentially useable for drinking at 4 drinking water treatment facilities. These water samples were evaluated for both fecal indicator microorganisms of interest that are regulated by the state of North Carolina, and also enteric pathogens of public health interest. In addition, microorganisms in these waters were evaluated for their survival characteristics in a blended water consisting of 20% NCT2-like RW and 80% surface water over the state mandated 5-day storage period required for potable reuse in NC. Finally, quantitative microbial risk assessments (QMRAs) were conducted for various water exposure scenarios for both potable reuse and agricultural reuse applications.

This research provides further health-related microbiological and associated health risk assessment information on the type of reclaimed water proposed by North Carolina for potable reuse purposes. As potable reuse is becoming a topic of increased interest to areas experiencing either drought or population growth or both, the microbial quality of source waters proposed for the expansion of water resources for potable use is an important component of the design water reuse systems (NAS, 2012). Although chemical contaminants are also of concern when

evaluating reclaimed water schemes, microbial hazards can also pose immediate health risks if treatment is not designed to adequately remove or inactivate pathogens prior to consumption of reclaimed waters. In the North Carolina potable reuse scheme, potable reuse is designed as wastewater treatment involving full tertiary treatment by physical and biological processes, typically primary and secondary treatment followed by granular media filtration as tertiary treatment to produce well oxidized reclaimed water that is then subjected to dual disinfection, typically by UV irradiation and then chlorine disinfection. The resulting reclaimed water is then blended at up to 20% of the flow with at least 80% ambient surface water and stored for 5 days, followed by conventional drinking water treatment to produce potable water. This treatment scheme is designed to remove and inactivate microorganisms (by wastewater treatment and disinfection) and to further reduce microbial hazards by storage and combining with surface waters currently used as drinking water sources.

Conducted at a pilot scale, previous research done on the NC proposed tertiary treated, dual disinfected reclaimed water by Sobsey et al., indicated that the dual UV and chlorine disinfection system was effective in reducing numbers of fecal indicator bacteria, viruses and protozoan parasite surrogates for the production of high quality reclaimed water (Sobsey et al., 2005). When using a single disinfection step (either UV or chlorine), other studies have demonstrated that reclaimed waters may have detectable levels of pathogens, particularly by molecular detection of nucleic acid genome targets for viruses and by microscopic detection of protozoan parasites in contrast to the detection of infectious pathogens (Harwood et al., 2005). Based on the limited amount of available literature on the microbial quality type 2 reclaimed water, there was a need and motivation for additional data on both the microbial quality of this water and its associated health risks from potable reuse exposures as drinking water. This study sought to evaluate not only the microbial quality of the reclaimed water proposed for potable reuse, but also the microbial quality of surface waters currently used as sources for drinking water in North Carolina, and the microbial risks posed by these potable reuse-derived waters as determined by QMRA. Key research questions included:

1. Can NCT2-like RW-producing treatment plants that meet the indicator performance requirements, based on their fecal indicator microbe concentrations and $\log_{10}$ microbial reductions, also reduce enteric pathogens to the same or similar extent?
2. If pathogens are reduced to low levels in NCT2RW as documented by required $\log_{10}$ reductions, are they also reduced to sufficiently low levels based on their concentrations to achieve acceptably low human health risks, if used as drinking source water?
3. What is the microbial quality of run of river surface waters based on concentrations of microbial indicators and pathogens as potential source waters proposed for blending by at least 80% of combined flow with up to 20% NCT2RW as a combined source water for drinking water supply?
4. Does 5-day storage of this blended water have any effect on the concentrations of fecal indicator microorganisms?
5. Is the NCT2RW used to make drinking water by blending at a ratio of no more than 20:80 by volume with surface source water, then stored for 5 days and subjected to conventional water treatment likely to achieve the US EPA acceptable microbial drinking water risk level of 10^{-4} infections/person/year, based on the allowable

parameters and conditions for treatment, microbial quality, $\log_{10}$ microbial reductions, blending and storage?

5.1.1 Pathogens Detected in Reclaimed and Surface Waters

In this research, both indicators relevant to the NC reclaimed water regulations (NC DENR 2011; 2014) and pathogens of public health concern were detected in 22 North Carolina type 2-like reclaimed water samples and 22 sewage impacted surface water samples. The indicators examined in this research included total coliforms, *E. coli*, and *Enterococcus* spp. as the bacteria, F+/male-specific, somatic, and total coliphage as indicator viruses, and vegetative and spore forming *Clostridium perfringens* as the protozoan parasite surrogate. The pathogenic microorganisms detected by this research included, *Salmonella* spp. as the pathogenic bacteria, Norovirus GII and Adenovirus groups A-F as target human enteric viruses, and *Cryptosporidium* spp. and *Giardia* spp. as the protozoan parasites.

In the evaluation of pathogen concentrations in NC type 2-like reclaimed waters, nearly all samples of reclaimed water had low but detectable levels of pathogens after tertiary wastewater treatment and dual disinfection. This result is particularly important for the concentrations of adenoviruses, as the levels detected by real time qPCR (genome copies, not infectious units) were on average quite high at 5.24×10^2 GEC per 100mL. In addition, levels of infectious adenoviruses, detected by ICC-RTqPCR were also high, at average concentrations of 6.79×10^1 MPNIU per 100mL. Protozoan parasites were also detected by immunofluorescent microscopy in nearly all samples of reclaimed water, but these cysts and oocysts were not assayed for infectivity. In surface waters pathogens were also detected in many samples, with adenoviruses above the detection limits in 41% of samples, with an average concentration of 1.44×10^4 GEC per 100mL. For the protozoan parasites, *Cryptosporidium* oocysts were detected in 86% and *Giardia* cysts were detected in 81% of all surface water samples analyzed. Because the North Carolina potable reuse scheme proposes to combine type 2 reclaimed waters up to 20% flow with ambient surface waters at 80% flow or more, the quality of such surface waters becomes an important contributor to the potential microbial risks associated with potable reuse. In many of the samples evaluated, the concentrations of pathogens were equal to or higher in the surface waters than in the NC type 2 reclaimed water. Although infectivity was not evaluated for the protozoan parasites, there are a significantly higher number of both Giardia cysts and Cryptosporidium oocysts in surface waters at 5.37 and 1.35 per 100 mL, respectively, when compared to the tertiary treated reclaimed waters at 1.66 and 1.66/100 mL, respectively. For *Salmonella* spp., there were also significantly more in the surface water with concentrations of 5.75 per 100mL compared to 1.35 per 100mL in the reclaimed water. For adenovirus, concentrations were higher in the tertiary treated reclaimed waters with concentrations of 5.25×10^4 compared to concentrations in surface waters of 3.24×10^3 per 100mL.

Based on the results of the samples analyzed for microbial quality in this study, the reclaimed water samples produced by type 2-line water reclamation facilities are of higher or comparable microbial quality than the samples run of river or sewage impacted surface waters currently used by drinking water treatment facilities. Therefore, the addition of 20% of the volume of higher quality NCT2RW to the lower quality ambient surface water further treated as source water by these drinking water treatment facilities may reduce the microbial risk associated with their source waters. However, the high concentrations of adenoviruses in both of these water types

along with the occurrence of protozoan parasites in both water types is of health concern for both potable reuse applications and conventional drinking water supplies using surface water sources.

5.1.2 Log_{10} Microbial Reductions Relative to NCT2RW Performance Targets for Potable Reuse

The State of North Carolina specifies that source water for further drinking water treatment to produce potable water when there is potable reuse must be a combination of up to 20% NCT2RW with at least 80% surface source water, followed by a 5-day storage time under unspecified conditions. Performance targets for reclaimed water are defined as reductions in log_{10} concentrations of indicator bacteria, viruses, and a protozoan parasite surrogate as well as monitored monthly geometric mean and single sample maximum concentrations that must not be exceeded for each microorganism. Log_{10} reduction targets are 6-log_{10} for bacteria, a 5-log_{10} for viruses, and a 4-log_{10} for protozoan parasite surrogates. The monthly geometric mean and daily maximum targets for bacteria are a monthly geometric mean of less than or equal to 3 per 100mL (CFU) with a daily maximum of less than or equal to 25 per 100mL. For coliphage and *C. perfringens,* there is a monthly geometric mean concentration of no more than 5 per 100mL (PFU and CFU respectively), with a daily maximum concentration of less than or equal to 25 PFU or CFU per 100mL, respectively.

Based on these reduction targets, it is not clear that the log_{10} reductions achieved by the wastewater reclamation systems studied always meet the NC standards for indicator microorganisms. The log_{10} reduction targets for bacteria (*E. coli)* and the protozoan parasite surrogate (both pasteurized and unpasteurized *C. perfringens)* did meet the log_{10} reduction targets, with reductions of 6.36, 4.26, and 4.43 respectively. However, the quantifiable reductions for indicator viruses were below the 5-log_{10} reduction performance target for somatic, F+, and total coliphages. As several of these log_{10} reduction values, specifically the *E. coli,* and the coliphage values, are based on upper and lower limit detection values, it is unclear whether these log_{10} reductions are indicative of those actually achieved because they are censored values and could be greater than the log_{10} reductions reported. Even though the log_{10} reductions performance targets were not met for the viral indicators, the reclaimed water samples examined were below the state mandated monthly geometric mean and daily maximum concentration values for all indicator organisms (including viruses). In contrast to the low concentrations of fecal indicator microorganisms in the NCT2RW, there were high occurrences and concentrations of pathogenic bacteria (Salmonella spp.), enteric viruses (enteric adenoviruses) and protozoan parasites (Cryptosporidium and Giardia) in these reclaimed water samples. Of particular interest were the high (~10^2-10^3 GEC/100 mL) concentrations of adenovirus groups A-F detected by real time qPCR. Although these viruses were not detected by infectivity methods, this high concentration of adenovirus in tertiary treated, dual disinfected reclaimed waters is of potential health concern for potable reuse applications. Additionally, the concentrations of human enteric viruses detected in reclaimed waters (by qPCR) do not seem to correlate with coliphage virus levels detected by SAL, which were often not detectable at all the 100 mL sample volumes analyzed. The relationship between infectious and genome copies of adenoviruses detected in the NCT2RW samples analyzed is discussed in section 7.1.3 that follows.

5.1.3 Detection of Adenovirus Infectivity Based on ICC-RTqPCR

To address the North Carolina T2RW regulation performance for indicator viruses, somatic, F+, and total coliphage viruses were detected by US EPA method 1602, the Single Agar Layer method in 100mL reclaimed water samples. The NCT2RW samples analyzed met the concentration limits for these coliphages, with very low or non-detectable levels in 100 mL sample volumes. The average concentrations of somatic, F+ and total coliphages were 1.17, 1.28, and 1.17 per 100mL respectively, below the allowable limit of 5 per 100 mL. However, as stated previously, high levels of enteric adenoviruses were detected in reclaimed water samples. In order to evaluate the number of infectious adenoviruses in reclaimed water samples, adenovirus infectivity was evaluated based on a semi-quantitative integrated cell culture real time RT-qPCR assay. Out of the 22 reclaimed water samples examined, 19 were positive for adenoviruses by direct qPCR, with the average viral genome copies of the positive samples at 1.36×10^5 per 100mL. Out of the 19 positive samples, only 7 were positive for infectious adenoviruses, the average mRNA-IU was 6.79×10^1 per 100mL.

Rodríguez et al., 2014 and Polston et al., 2014, have previously evaluated the infectivity of adenovirus in wastewater and surface samples and in those studies approximately 50% of raw sewage and 44% of surface water samples positive for adenovirus were also infectious. This discrepancy between infectious adenovirus and genome copies detected by real time qPCR has also been examined by Rodríguez et al., 2013 and Polston et al., 2014, who found that approximately 50% of raw sewage and 44% of surface water samples respectively testing positive for adenovirus DNA were infectious. The results presented here indicate that approximately 32% of type 2 reclaimed water samples positive for adenovirus DNA were infectious. As the virus indicator specified by the State of NC, coliphages, were very low in the reclaimed water samples (~1 PFU/100mL), these results suggest that this indicator may not accurately reflect the concentrations of pathogenic viruses present in these water samples after treatment. The $\log_{10}$ reduction target as well as the daily and monthly maxima are designed as monitoring approach for wastewater utilities to examine the quality of type 2 reclaimed water before it is used for potable reuse. However, these results indicate that the tertiary treatment plus dual disinfection scheme for type 2 like reclaimed water may not be effective in reducing pathogenic viruses, and that the NC standards for reclaimed water (which include a coliphage reduction target) may not be adequate for monitoring the virological quality of this water.

5.1.4 NCT2RW Quality and US EPA Acceptable Risk Level for Potable Reuse

In order to assess the risks associated with potable reuse exposures, a quantitative microbial risk assessment (QMRA) model was built to evaluate multiple exposure scenarios. These scenarios included 1) accidental exposure, 2) recreational exposure, 3) reclaimed water exposure by drinking as piped water, 4) 80/20 blend + 5 day storage + conventional drinking water treatment, and 5) agricultural exposure through irrigation with reclaimed water. Based on this analysis, there were no potable reuse scenario for which the acceptable risk level was not exceeded by one or more class of pathogen; however, agriculture reuse using the DALY target of 10^{-6} per person per year was met using subsurface drip irrigation.

For the analysis of drinking water exposures, the risks of adenovirus infection were the greatest for all exposure routes, this is partly due to the higher concentrations of adenovirus is reclaimed waters, surface water, and as a result the combined waters. In the 4th scenario, in which pathogen die-off and treatment effect were considered, the risk of adenovirus infection still does not meet the acceptable annual risk level of 1 x 10^{-4} infections per person per year set by US EPA. However, in the analysis of bacteria and protozoan parasites in this analysis, the risks are lower than the US EPA acceptable risk level.

Based on these analyses, it is not clear that the water consumption risks associated with consumption of potable reuse water, are reduced consistently below the annual risk level of 1 x 10^{-4} set by US EPA. The results have implications for the practical use of this type of reclaimed water, which is currently only used for landscape irrigation.

6 Conclusions

A key step in assessing risk is collecting and evaluating microbial data on exposure and human health effects to inform quantitative microbial risk assessments that conservatively estimate the hazard associated with microbial exposure. From the results of this study, it is concluded that the risks of potable reuse, based on exposures to drinking water produced from raw sewage by tertiary treatment, dual disinfection, followed by 5 day storage, and then drinking water treatment, are potentially higher than the US EPA allowable annual 1 x 10^{-4} risk of infection per person per year. This result is largely based on high concentrations of infectious adenovirus in the treated reclaimed water and the detection of all classes of pathogens (bacteria, viruses, and protozoan parasites) in reclaimed and surface waters. Irrigation of raw food crops with type 2 like reclaimed water (expected to be eaten raw) also has a higher risk of infection than the level of risk for bacteria and protozoan parasite pathogens and also may be higher than a tolerable level of risk, depending the level of risk considered tolerable.

This research is consistent with the work of Harwood et al., 2005 in that fecal indicator microorganisms do not always, if at all, correlate with pathogens in wastewater or treated wastewater samples. This present research shows that in many cases, fecal indicator microorganisms were not detected in reclaimed waters samples of standard volume (100 mL), but that pathogens were detected in this water with detectable levels also in 100mL volumes. In the case of the coliphage viruses, very low or non-detectable levels of coliphages, below the specified allowable level, were detected in almost all samples, but high levels of adenoviruses DNA detected by direct qPCR and infectious adenoviruses detected as mRNA of adenovirus-infected mammalian host cells were detected. Additionally, in the analysis of the correlation between indicators and pathogens in surface waters, very few organisms were correlated. Specifically, $\log_{10}$ concentrations of *Salmonella* spp. were correlated with $\log_{10}$ concentrations total coliforms, and $\log_{10}$ concentrations of adenovirus were correlated with $\log_{10}$ concentrations of F+ coliphages.

In conclusion, at the present treatment level and disinfection efficacy, NC type 2 like reclaimed water may or may not be of high enough quality to be used for potable reuse applications when compared to the quality of surface water sources, based on the data and data analysis of this study. Further study is needed to evaluate the infectivity of the protozoan parasites detected in the reclaimed water, as well as the surface water currently used for drinking water sources to

better assess human health risks as the risk of virus infection based on the proposed potable reuse scheme and the concentrations of viruses detected in NCT2RW and ambient sources water is higher than the acceptable risk level of US EPA for drinking water exposure. Additional treatment steps would be necessary to further decrease the risk of virus infection from the levels of risk determined in this study. For agricultural use, the survival of bacteria and protozoan parasites on raw fruits and vegetables is of concern and additional treatment steps are necessary to either reduce the concentrations of these organisms by disinfection processes or physically remove them before distribution to consumers.

7 Recommendations

While this research has addressed some of the knowledge gaps on the quality of NC type 2 like reclaimed water, there are still many questions that remain about the risks associated with both potable reuse of NCT2RW. As much of this analysis relies on assumptions of pathogen infectivity, one of the main areas of additional research is the incorporation of infectivity analysis into protozoan parasite detection, specifically for *C. parvum.* In this study, *Cryptosporidium* and *Giardia* were detected by US EPA method 1623, which results in a total count of (oo)cysts rather than a determination of infectivity. Future research on the number of infectious oocysts in both treated reclaimed water and in surface waters used for drinking will expand and further inform the current risk assessment on potable reuse applications. Additionally, more research should be done to expand the information available on the infectivity of adenovirus, particularly as many GEC were detected in reclaimed water and surface water samples. Future research should include integrated cell culture RT-qPCR assays for mRNA for all samples analyzed.

Another research area in need further of study is the microbiological quality of the surface water, particularly on sewage impacted, or run of river waters that may be candidates for this type of combination of NCT2RW and surface water for potable reuse. There is little to no available data on the enteric pathogen content of such surface waters and this data could be used to inform future risk assessments, as this water is used for many purposes (drinking, irritation, recreation, etc.).

This research also addressed knowledge gaps in the area of the survival of indicator organisms in the NC approved 80% surface water 20% reclaimed water blend; however, little is known about the survival of pathogens in this matrix. Future research could determine the survival of enteric pathogens over the 5 day storage period in this matrix to determine if the die-off or survival relationship between fecal indicators and pathogens are similar, as wells as to determine the survival kinetics of pathogens in this type of water. Additionally, the survival of relevant pathogens has also not been examined after sunlight exposure. Because this was an important exposure factor for fecal indicators, it is possible that exposure to sunlight would also have a large impact on pathogens. The survival and persistence of fecal indicator and pathogenic microorganisms may help to inform design and operation characteristics of reclaimed water systems, particularly if a specific characteristic (sunlight or temperature) has a significant impact on survival.
Finally, much of the data presented in this report could be further reexamined and reanalyzed for various trends in estimating microbial concentrations, $\log_{10}$ reductions and human health risks.

In particular the relationship between indicators and pathogens in raw sewage and reclaimed water could be further characterized by using additional assumptions and alternative analytical methods such as Bayesian analysis. Although these aspects were not central questions in the current study, it may be relevant and of interest for future work.

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Appendix 1

List of Abbreviations

CFU	Colony forming unit
C_t	Cycle Threshold
DALY	Disability Adjusted Life Year
DNA	Deoxyribonucleic acid
GEC	Genetic equivalent copies
ICC-PCR	Integrated cell culture polymerase chain reaction
ICC-qPCR	Integrated cell culture quantitative polymerase chain reaction
ICC-RTqPCR	Integrated cell culture reverse transcriptase quantitative polymerase chain reaction
IMS-FA	Immunomagnetic separation – fluorescence assay microscopy
IU	Infectious unit
L	Liter
M	Meter
MF	Membrane filtration
mL	Milliliter

MPN	Most probable number
MPNIU	Most probable number of infectious units
mRNA	Messenger ribonucleic acid
NAS	National Academy of Science
NC	North Carolina
NC DENR	North Carolina Department of Environment and Natural Resources
NCT2RW	North Carolina Type 2 Reclaimed Water
OWASA	Orange Water and Sewer Authority
PFU	Plaque forming unit
QMRA	Quantitative microbial risk assessment
qPCR	Quantitative polymerase chain reaction
RT-qPCR	Reverse transcriptase quantitative polymerase chain reaction
RNA	Ribonucleic acid
RT	Reverse transcriptase
RV	Rappaport-Vassilades broth
SMEWW	Standard Methods for the Examination of Water and Wastewater
SS	Salmonella-Shigella agar
Spp.	Species
µL	Microliter
US	United States
US EPA	United States Environmental Protection Agency
UV	Ultraviolet
WHO	World Health Organization