Abstract

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Relationships Between the Occurrence and Concentrations of *Cryptosporidium*, *Giardia* and Fecal Indicator Microbes in Waters from Tributaries of the Kensico Reservoir
(Under the direction of Dr. Mark D. Sobsey)

As much as 96% of the surface waters in the US are contaminated by the protozoan parasites *Cryptosporidium parvum* and *Giardia lamblia*. Both of these organisms have low infectious doses and form (oo)cysts that are resistant to chlorine-based disinfection processes. Unfiltered drinking water systems, such as those of New York City, rely on chlorination processes as a primary method of ensuring consumer safety and are therefore especially concerned with *Cryptosporidium* and *Giardia*. This study was undertaken to determine the occurrence of *Cryptosporidium* and *Giardia* in New York City's Kensico Reservoir and the correlation of these protozoan parasites with fecal indicator microbes and various physicochemical water quality measures. During storm events, significant ($\alpha = 0.05$) Spearman rank correlations were found between *Giardia* concentrations and those of thermotolerant coliforms, enterococci, *Clostridium perfringens* spores, somatic coliphages, particles, turbidity, and total organic carbon. *Cryptosporidium* was significantly correlated with *Giardia* and temperature, but only during wet weather events. Within individual storm events, rising hydrographic limbs accounted for disproportionately high *E. coli* and TOC loadings when compared with peak and falling limbs on the basis of stream volume fraction, providing some evidence for a "first flush" for these variables. Water samples taken at the peak of each storm collectively represented 36% of total stream volume during this study, but accounted for approximately 60% of FIB and particle loading. These results indicate that wet weather events, and particularly the peak stages, should be priorities in reducing the water quality impacts of FIB and protozoan parasites. Enterococci may be a suitable microbial surrogate for *Giardia* in Kensico tributaries during both dry and wet weather periods.

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Table of Contents

List of Tables

List of Figures

BACKGROUND

The vast majority of waterborne illnesses in the U.S. go unreported because of difficulties in identifying the etiologic agent and source of gastrointestinal illnesses that may have been acquired via water. Approximately 99 million people in the United States have acute gastrointestinal illnesses each year, and 6% to 40% of these illnesses may be caused by contaminated drinking water (Jones et al., 2006; Levin et al., 2002; Cotte et al., 1999). There may be as many as 26 million infections and 13 million illnesses per year from municipal surface water systems alone (Reynolds et al., 2008).

The occurrence of the intestinal parasites *Cryptosporidium parvum* and *Giardia lamblia* in surface water sources is of particular concern because of the substantial disease risk they pose. Studies have found 17% to 32% of people tested have evidence of *Cryptosporidium* infection by young adulthood (Guerrant 1997), and community seroprevalence in some communities is about 30% (Isaac-Renton et al., 1999). Over 160 waterborne cryptosporidiosis outbreaks have been reported worldwide (Slifko et al., 2000), and some investigators have estimated that as much as 96% of the surface waters in the US are contaminated by protozoan parasites (Hansen and Ongerth, 1991). Although *Giardia lamblia* is the most common intestinal parasite in the United States, infecting approximately 2.5 million persons per year (Furness et al., 2000), *Cryptosporidium* may pose a greater risk of waterborne outbreaks due to more efficient replication in a human host compared to *Giardia.* The infectious dose of *Cryptosporidium* is low, especially in immunocompromised people (Ono et al., 2001; Okhuysen et al., 1999; Olsen et al., 1999). The importance of *Cryptosporidium* is highlighted by two outbreaks that occurred in large populations, and were attributed to the consumption of treated drinking water. In 1987,

13,000 cases of cryptosporidiosis were attributed to a surface water supply in Carrollton, Georgia, where water treatment consisted of coagulation, sedimentation, filtration, and chlorination. In April 1993, *Cryptosporidium parvum* was responsible for the largest waterborne disease outbreak in United States history; an estimated 400,000 people were sickened and another 54 died in Milwaukee, Wisconsin (Hoxie et al., 1997). The suspected sources of oocysts in the source water used by the drinking water utility included cattle waste, slaughterhouse waste, and human sewage. This outbreak occurred despite the fact that the water was disinfected with free chlorine and met the requirements of the Safe Drinking Water Act (Fox and Lytle, 1996).*Cryptosporidium* oocysts are able to survive for long periods in surface water (Chauret et al., 1998), are fecally shed by many animal species, and are resistant to common chemical disinfectants such as chlorine (Finch et al., 1993). As seen in the Milwaukee outbreak, where surface water sources are used for drinking water, oocysts can pass through conventional drinking water treatment processes (MacKenzie et al., 1994) if treatment is insufficient or not optimized.

The Kensico Reservoir in Westchester County, New York is the final stop for approximately 90% of New York City's drinking water supply before it enters the water tunnels that carry it to consumers' taps. Usually, all of the water from the Catskill and Delaware watersheds flows into Kensico, supplying water to nine million people in twenty-seven communities surrounding the nation's largest city (NYCDEP n.d.). Approximately 90% of the drinking water obtained from the New York City watersheds is not filtered as part of the treatment process. For water sources of this type, the Surface Water Treatment Rule mandates the establishment of watershed control programs to minimize pathogen contamination of watersheds. The Delaware and Catskill Watersheds can remain unfiltered as

long as they are in compliance with the USEPA's Filtration Avoidance Determination (FAD) criteria. To qualify for an FAD the system cannot be the source of a waterborne disease outbreak, and it must meet source water quality limits for coliforms, turbidity, and trihalomethanes. It also requires the implementation of a complex watershed control program to minimize microbial contamination of source water (USEPA 2008).Currently, the disinfection requirements (redundant disinfection and adequate disinfection residuals) are satisfied by the addition of chlorine to the water in the drinking water distribution system, making chlorine-resistant pathogens such as *Cryptosporidium* and *Giardia* a high priority for alternative control measures.

Due to their resistance to the levels of hypochlorite routinely used to disinfect drinking water, control of *Cryptosporidium* and *Giardia* is a high priority for the New York City water supply. Testing for these protozoan parasites is costly and time-consuming; it requires specialized equipment and trained laboratory personnel. Finding a potential indicator for *Cryptosporidium* or *Giardia* may be beneficial to organizations with limited funding, staff, or time to analyze water samples for these protozoa. Potential indicators include fecal coliforms and other fecal indicator bacteria (FIB), such as *E. coli* and enterococci, and physiochemical measurements. FIB testing can be completed using much simpler and less expensive assay methods than protozoan parasites. Physicochemical factors such as pH and TOC can often be quantified by machines; while this may incur a larger upfront cost, personnel work time is typically the largest factor in determining the total cost of a testing procedure. However, data on the relationship between protozoan parasites, FIB, and physiochemical measures is limited for the city's watersheds, particularly with regard to the influence of storm events on these variables. Therefore, this study was undertaken to

determine the occurrence of *Cryptosporidium* and *Giardia* in the Kensico Reservoir and the correlation of these protozoan parasites with fecal indicator microbes and various physicochemical water quality measures. The overall objective was to determine if fecal indicator bacteria or physicochemical measurements can be used to predict the presence of protozoan parasites in the watershed that supplies the New York City drinking water system.

OBJECTIVES

- Examine the relationship between the occurrence and concentrations of *Cryptosporidium*, *Giardia* and fecal indicator microbes in waters from tributaries of the Kensico Reservoir,
- Determine if the relationships between *Cryptosporidium, Giardia* and fecal indicator microbes in these waters are different during dry and wet weather periods,
- Determine how physicochemical factors, concentrations of fecal indicator microbes, and concentrations of *Cryptosporidium* and *Giardia* in these waters change over the course of individual storm events
- Determine if fecal indicator bacteria or physicochemical measurements can be used to predict the presence of protozoan parasites in the watershed that supplies the New York City drinking water system.

LITERATURE REVIEW

Contribution of storm events to total loading

Heavy amounts of rainfall disturb sediments and microbes, mobilizing them and carrying them into streams, rivers, and other bodies of water. The stormwater loading poses major waterborne infectious disease risks because one storm's microbial loading can be the equivalent of months or years of dry-weather loading (Krometis et al., 2007; Jamieson et al., 2004). Therefore, it is important to consider the effects of rainfall events in the development of any Best Management Practices for drinking water systems and their watersheds. During storms, rainfall intensity is positively correlated with turbidity and concentrations of fecal indicator bacteria (Reeves et al., 2004), as well as with protozoan parasites (Atherholt et al. 1998). Some studies have linked this phenomenon to the catchment area, contributing impervious area, and rainfall intensity (Lee et al., 2002), while others cite the most important factors as being maximum rainfall intensity and the length of the preceding dry period (Gupta and Saul, 1996). More recent work has shown that microbial contaminants may be carried into and deposited within storm sewer sediments, where they accumulate, die off, and perhaps grow (Reeves et al., 2004) before eventually being eroded by high stormwater flows (Yin and Li, 2008). All of these are potential reasons that rainfall has been continually associated with waterborne disease outbreaks (Thomas et al. 2006, Auld et al. 2004).

"First flush" phenomenon. The concept of a "first flush" phenomenon remains contentious within the scientific community. Previous studies have disagreed on the definition of the term (Bertrand-Krajewski et al. 1998, Lee et al. 2002), but have generally agreed that "first flush" refers to a concentration of a measured variable that is disproportionately higher during the beginning stages of a rainfall event as compared with

later stages. The term has also entered the popular lexicon due to increasing environmental awareness and the popularity of rain barrel catchment systems for home and business use. In this study, "first flush" means the occurrence of a measured microbe or physicochemical factor that is disproportionately higher during the rising limb of a stream's hydrograph or in the beginning stages of a rainfall event, as compared with concentrations that are measured during other storm stages or time periods.

Some of the difficulty in describing and demonstrating first flush is due to a large number of potential contributing factors, as well as many different measured variables. Soller et al. (2005) examined the phenomenon for dissolved metals and other chemical constituents, suggesting that first flush is dependent on sampling site and the length of antecedent dry periods. Results were composites for entire storms, not separated based on the hydrograph, with "first flush" storms being those that were the first of each rainy season. The results of Sansalone and Kim (2008) described an increase in the concentration of suspended particle concentrations during the rising limb of the hydrograph, and these particles have been shown to be associated with dissolved ions (Grant 2003) or suspended microbes (Krometis et al. 2007). Geospatial patterns in rainfall appear to contribute as well, with areas that receive more frequent, lighter precipitation (such as the United States Pacific Northwest) seeing less of an effect than those with heavier and more frequent rainfall events (Livingston 2000). Lee and Bang (2000) found that smaller watersheds (less than 1 km^2) with high percentages of impervious surface area were more likely to exhibit a first flush for pollutants and previous studies have reported similar results (Yousef 1985, Miller 1985). Although the Catskill/Delaware watershed associated with the Kensico Reservoir has an area of more than 4,000 km^2, much of the water that enters the reservoir is through aqueducts and

the rainfall that flows through each sampled tributary does so from across a much smaller catchment. The approximately 25 km^2 subwatershed immediately surrounding the Kensico Reservoir contains housing developments and a regional airport, both of which can be expected to contribute impervious ground cover.

National Urban Runoff Pollution study. In 1983, a five-year meta-analysis called the National Urban Runoff Project was completed to determine the significance of nonpoint source pollution on water quality (USEPA 1983). While NURP did not find evidence of a significant first flush phenomenon, it did note that this effect did occur at some sites and that patterns in ionic concentration were locality-specific. However, NURP only examined sewered districts and urban areas, which somewhat limits its application to the Kensico Reservoir. Additionally, it did not analyze pollutant concentrations within each storm, but rather event mean concentrations (EMCs) that pooled results for each storm. Results from NURP were used to create National Pollutant Discharge Elimination System (NPDES) permitting regulations (USEPA 1990), which required first flush testing for various pollutants during the first 30 minutes of a storm event. The most current NPDES permit application has changed this requirement to the first 20 minutes, in addition to analyzing flow-weighted composite values for each sampling site.

Increasing impact of storm-related contamination. According to the US National Assessment on the Potential Consequences of Climate Variability and Change, determining the role of weather in the incidence of waterborne disease outbreaks is a priority public health research issue (Patz et al., 2000). A waterborne outbreak of giardiasis in Montana was related to rainfall (Weniger et al., 1983), as was the Milwaukee cryptosporidiosis event, where a period of heavy rainfall and runoff created a turbidity level that compromised the

effectiveness of the drinking water treatment plant (MacKenzie et al., 1994). Even relatively small increases in the turbidity of treated drinking water have been associated with an increased occurrence of acute gastrointestinal illness among children and the elderly, even when the water is in compliance with EPA standards (Gaffield et al., 2003). Additionally, waterborne outbreaks due to pathogenic *E. coli* have been linked to rainfall events. The largest reported outbreak of *E. coli* O157:H7 occurred at a New York fairgroundin September 1999 and was linked to contaminated well water. Unusually heavy rainfall, preceded by a drought, coincided with this major outbreak (Patz et al., 2000), as well as an outbreak of giardiasis in Bergen, Norway in 2004 (Roberston et al., 2004). As the evidence for global climate change grows, so does the risk from waterborne pathogens. In the past century, average daily temperatures in the contiguous United States increased by approximately 1°F (Karl et al., 1996). As air becomes warmer, it can hold more moisture, and this has already begun to manifest itself in increases in cloudcover (Karl and Steurer, 1990) and total precipitation (Groisman and Easterling, 1994).

Associations between indicators and protozoan parasites

As the impact of *Cryptosporidium* and *Giardia* becomes more prominent, studies have increasingly begun to focus on ways to improve testing for these organisms () or to find a suitable surrogate that will eliminate the need for their routine testing. Samples have been taken from reservoirs (Keeley and Faulkner 2008) or rivers and from single locations to a wide variety of sampling sites. Horman et al. (2004) found no significance between samples taken from reservoirs and those taken from rivers in the same geographic region, although

Carmena et al. (2006) found occurrences of both *Cryptosporidium* and *Giardia* that were approximately twice as high in rivers as they were in reservoirs.

Several studies have specifically attempted to correlate fecal indicator bacteria with *Cryptosporidium* and *Giardia*, but those that have done so have found disparate results. Atherholt et al. (1998) performed a landmark study in New Jersey, finding significant correlations between 15 different factors and the occurrence of these protozoa. Chauret et al. (1995) found a significant correlation between enterococci and *Cryptosporidium* in a Canadian river, as well as between coliphages and *Giardia.* Payment et al. (2000), which was also conducted on a Canadian river, concluded that these relationships were not significant, instead finding correlations between fecal coliforms, *C. perfringens*, *Cryptosporidium*, and *Giardia.* LeChevallier et al. (1991) characterized the occurrence of these protozoan parasites at water treatment plants across the United States, noting significant relationships between *Giardia* and fecal coliform densities (Spearman rho = 0.70) as well, and between *Cryptosporidium* and turbidity (Spearman rho = 0.75). Many of these correlations have also been found to be significant in estuarine environments as well (Touron et al. 2007).

Attempts at correlating bacteriophage concentrations with either *Cryptosporidium* or *Giardia* have met with mixed results. Coliphages, a type of virus that infects *E. coli* often found in feces, have been proposed as surrogates for enteric viruses in surface waters (WHO 2004). Since they are relatively easy and inexpensive to assay, several studies have investigated both somatic coliphages (which can replicate in the environment) and F-RNA or "male-specific" coliphages (primarily from the intestines of endothermic mammals) as protozoan surrogates. Payment and Franco (1993) found that somatic coliphages were

significantly correlated with *Cryptosporidium* at a water treatment plant, but found no significant relationship between male-specific coliphages and either *Giardia* or *Cryptosporidium.* No significant correlations were found in other studies examined (Harwood et al 2005, Brookes et al. 2005, Ferguson et al. 1996).

NYCDEP conducts routine monitoring of storm events but is not required to perform intrastorm microbial sampling as part of their FAD. Despite the growing recognition of the impact of wet weather events on total surface water loading, relatively few studies have attempted to profile how the correlations between and among indicator microbes and pathogenic protozoa change over the course of a single storm. The most comprehensive study to date was performed by Rees et al. (2006), which attempted to characterize intrastorm *Cryptosporidium* and *Giardia* changes in another non-filtered drinking water supply reservoir in New England. Most of the variables it studied were physicochemical, however, and although it found no reliable surrogate for these pathogens, Rees et al. (1996) did find evidence of a first flush phenomenon.

Zoonotic/agricultural issues

As *Cryptosporidium* and *Giardia* are both zoonotic parasites, many animals may serve as sources of water supply contamination with the potential for human infection (Medema et al., 1998; Ng et al., 2008). Although an accurate estimate of the incidence of interspecies cycling between humans and these parasites is still lacking (Thompson et al., 2008), it has been established that indirect person-to-person or zoonotic transmission involves contamination of water used for recreation, drinking, swimming pools, or food preparation (Li et al., 2005; Goh et al., 2004). The primary sources of surface water

contamination with *Cryptosporidium* and *Giardia* are farm animals through direct fecal input and runoff from agricultural lands, as well as treated and untreated human sewage discharges (Bodley-Tickell et al., 2002). This is of major concern within watersheds such as those of New York City, where agricultural prevails. Cattle, particularly young calves, are considered significant sources of parasites due to the high numbers of (oo)cysts that they shed in their feces (Bradford and Schijven, 2002). Agricultural cropping and grazing land are suspected source areas for several documented waterborne outbreaks of cryptosporidiosis (Smith, 1998; Fricker and Crabb, 1998; Solo-Gabriele and Neumeister, 1996; Richardson et al., 1991; Fayer et al., 2000).

Wildlife is also considered to be a source of contamination in pristine waters (Hansen and Ongerth, 1991). The Kensico Reservoir has well-documented problem with geese and other waterfowl who are attracted the young shoots of the frequently-cut grass in surrounding recreational areas (Kilgannon 2001). Since 1993, NYCDEP has successfully implemented "Operation Goosebusters", a bird harassment program whose ultimate goal is to reduce the waterfowl populations that contribute large numbers of fecal coliforms to the reservoir by leaving their feces (Alderisio and DeLuca 1999).

Jiang et al. (2005) determined that the vast majority of detected *Cryptosporidium* species in three sampled Kensico basins (including two included in the current study, Malcolm Brook and N5) came from animal sources and that only two had ever been documented in humans. It is also important to note, however, that these two genotypes are known to be pathogenic in humans, including a cervine genotype that has recently emerged in humans (Ong et al 2002). *Giardia* has developed the nickname of "beaver fever" due to a

high proportion of waterborne infections originating in pristine water sources (Fayer et al. 2006, Smith et al. 1995).

EXPERIMENTAL DESIGN

Site selection, sample acquisition and handling

Five tributaries located on the Kensico Reservoir watershed were sampled between November 2006 and July 2007. These tributaries were selected based on their historically high relative flow and pathogen levels, as well as spatial location (Figure 1). Sites are designated as *MB-1, N5-1, WHIP, E9,* and *E11* by NYCDEP staff, and these labels were used throughout the study.

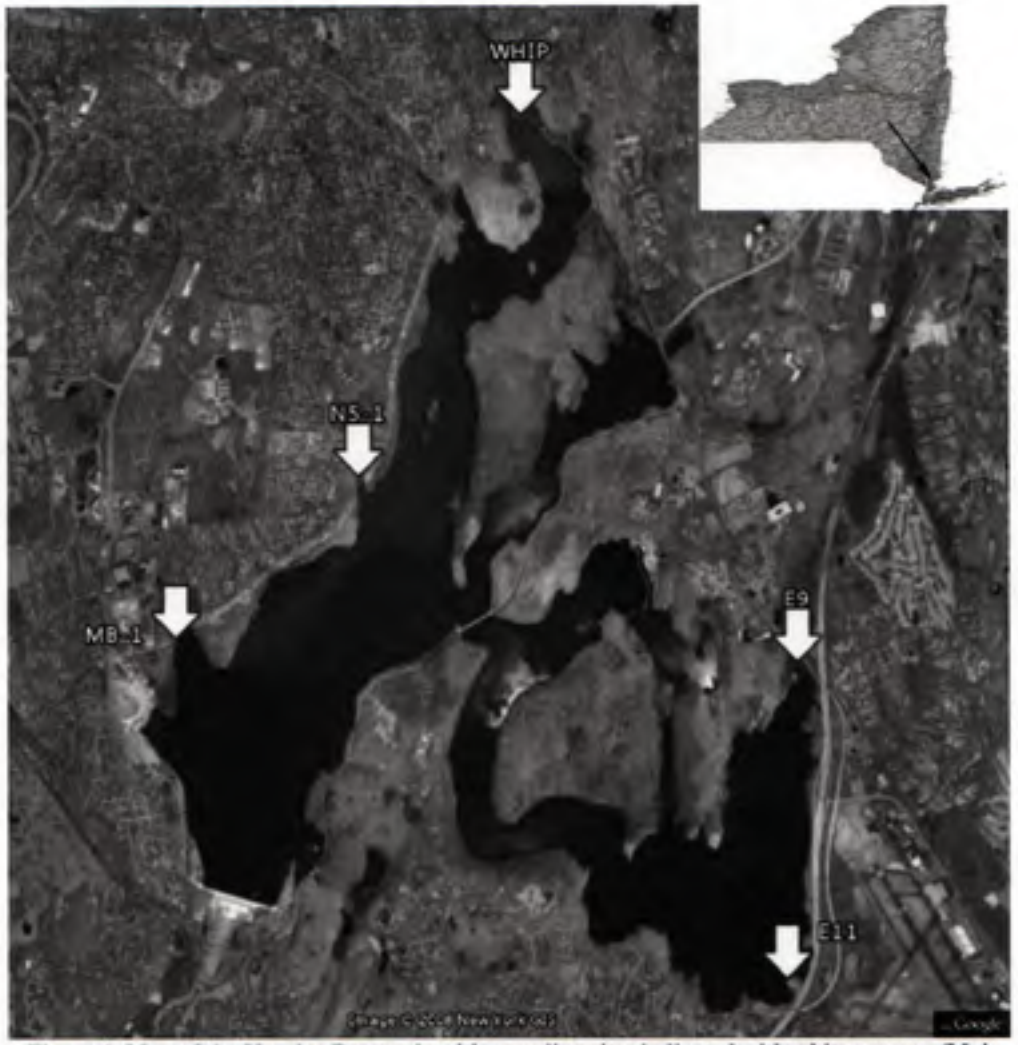


Figure 1. Map of the Kensico Reservoir with sampling sites indicated with white arrows. (Main image courtesy Google Earth; inset courtesy Zeducorp)

Single-sample events

Two simultaneous composite stream water samples were collected at existing DEP sample stations using ISCO auto-samplers. Samples were taken during three separate dry weather periods, defined as a period having <0.2 inches of rain in the preceding 72 hours. The NYCDEP laboratory processed one of these samples immediately for *Cryptosporidium* and *Giardia*, while the second sample was packaged with ice and shipped overnight for analysis at the UNC laboratory. Once the NYCDEP assay was completed, personnel notified UNC staff of the results. If the number of either *Cryptosporidium* oocysts or *Giardia* cysts was above 10 per total sample volume, ("action level") the UNC laboratory performed a subsequent analysis for both protozoa within 72 hours of initial sample collection. Samples received by the UNC laboratory varied in volume between 9L and 24L. All samples shipped to the UNC laboratory were analyzed for thermotolerant (fecal) coliforms, *Escherichia coli*, enterococci, *Clostridium perfringens* spores, male-specific (F^+) coliphages, and somatic coliphages within 72 hours of samples collection. All samples were also analyzed for total particle concentration and total organic carbon (TOC).

Stream water samples were also collected during four wet weather events, defined as s period where there was >0.2 inches of rain in the 24 hours prior to and during sampling. Auto-samplers were controlled by Campbell data loggers, which initiated sampling based on a 20% increase above baseline in stream stage over 3 consecutive 5-minute intervals, indicating a storm event. Two ISCO auto-samplers simultaneously collected a series of separate 1L storm samples at specified intervals throughout the storm. Samples were subsequently composited into 24L Cubitainers to represent the entire storm event before being analyzed by NYCDEP and shipped with ice overnight to the UNC laboratory.

Intrastorm sampling

Three of the original five sites (E9, WHIP, and N5-1) were selected by NYCDEP for intrastorm sampling, which was done using the same method as single-sample storm events. Auto-samplers were triggered by an increase in stream flow that was 20% above baseline (dry weather) levels. Individual 1L samples were then combined by NYCDEP staff so that there were three composite samples, representing approximately one third of each site's hydrograph over the course of a single storm. Samples were analyzed by NYCDEP and UNC as described for single-sample events.

METHODS

Microbial analysis

Enterococci were enumerated using the Enterolert® Quanti-Tray®/2000 system (IDEXX Laboratories Inc., Westbrook, Maine). Enterolert was incubated at 41°C for 25 ±3 hours (Simmons et al., 2003; Yakub et al., 2002). The results were recorded as a series of positive and negative wells, and standard tables provided by IDEXX were used to compute bacterial concentrations as Most Probable Number per 100 mL.

Fecal (thermotolerant) coliforms (FC) and *E. coli* were enumerated using the Colilert® Quanti-Tray®/2000 system. Colilert® was incubated at 37°C for two hours to revive environmentally damaged bacteria and then moved to 44.5°C for the remainder of the 24 ± 3 hour incubation period to allow for growth of thermotolerant coliforms (Chihara et al., 2004; Yakub et al., 2002). Yellow wells were scored as positive for fecal (thermotolerant) coliforms and wells that fluoresced under 365 nm ultraviolet light were scored positive for *E. coli.* The results were recorded as a series of positive and negative wells, and standard tables provided by IDEXX were used to compute bacterial concentrations as Most Probable Number per 100 mL.

Coliphages were quantified using the Single Agar Layer (SAL) method (EPA 2001). One-hundred milliliter samples were separately inoculated with log-phase host-culture strains of *E. coli. E. coli* host strain CN-13 cultivated in media containing nalidixic acid was used to detect somatic coliphages. *E. coli* host strain F-amp cultivated in media containing streptomycin and ampicillin was used to detect male-specific coliphages. For both phage types, 1:1 volumes of molten 2x Tryptic Soy Agar (TSA) and sample with *E. coli* host were combined and distributed equally into four 150mm x 15mm Petri dishes. After the agar

hardened, plates were inverted and incubated for 16-24 hours at 36°C. Coliphages were enumerated by counting plaques in the bacterial lawn. Results were expressed in plaque forming units (PFU) per 100 mL of sample.

Clostridium perfringens spores were quantified using the MPN method with iron milk medium (IMM) (AOAC 1995). A 3-tube MPN with triplicate sample volumes of 10 mL, 1 mL, and 100 µL was used. Samples were heat-treated at 70°C for 20 minutes to inactivate vegetative cells before assay. Tubes were incubated at 41°C for 18-24 hours and observed for stormy fermentation. Results were expressed as MPN/100mL of sample based on an MPN table.

Analysis for *Cryptosporidium* and *Giardia* (oo)cysts was performed according to EPA Method 1623 (EPA 2005). ColorSeed™ (BTF Pty Ltd, Sydney, Australia), which contains a known number of inactivated *Cryptosporidium* and *Giardia* (oo)cysts permanently labeled with fluorescent dye, was used as an internal calibration standard for all samples. ColorSeed was spiked into deionized water to assess precision and intra-assay variability.

For parasite analysis of stormwater, samples of 10 L were collected. Parasites were concentrated from samples by filtering the entire 10L volume through an Envirochek HV Capsule filter (Pall Corporation, East Hills, New York) with a nominal pore size of 1 µm using a bilge-type pump and sterile 3/8" ID Nalgene tubing. Cysts and oocysts were eluted from the filters using wrist-action shaking and the 0.01% Laureth-12 eluent specified in section 7.4.1 of Method 1623. The volume of recovered eluent was approximately 200 mL per filter. (Oo)cysts in eluent samples were concentrated by centrifugation (1817g, 4°C, 15 minutes, brake speed 4). The bottom 5 ml, which included the solid pellet, was transferred to a borosilicate 16x125mm Leighton-type tube (Bellco Glass Inc, Vineland, NJ) for

immunomagnetic separation using the Dynabeads® GC-Combo (Invitrogen Corporation, Carlsbad, California) kit. Any single sample with a pellet volume of more than 0.5 mL was diluted to an appropriate volume and split equally so that the portion of the pellet in each tube was ≤0.5 mL. IMS was performed according to the manufacturer's instructions. Following the IMS procedure, the remaining 50 µL of each sample was transferred to one well on a three-well Merifluor slide (Meridian Biosciences Inc, Cincinnati, Ohio) The slides were stained with Aqua-Glo® G/C epifluorescent stain (Waterborne Inc cat. no. A100FLR, New Orleans, Louisiana) containing fluorescein isothiocyanate (FITC)-labeled monoclonal antibodies, according to the manufacturer's instructions. The slides were then enumerated at 25x magnification with a binocular Leitz Orthoplan 2 light microscope (Leica Microsystems, Wetzlar, Germany) containing Leica I3 and N2.1 fluorescein filter blocks.

Giardia and *Cryptosporidium* counts were corrected for recovery, based on the following equation:

$$R = 100\ x\ \frac{T}{N}$$

where N = the number of cysts or oocysts counted and T = the number of cysts or oocysts spiked. Additionally, ongoing percent recovery (OPR) was determined by the same procedure, with one spiked, distilled water sample analyzed per testing day.

Physicochemical analysis

Single-sample event samples were analyzed by NYCDEP personnel for temperature at the time of sampling, and for pH and turbidity before they were shipped to the UNC laboratory. All samples were analyzed by the UNC laboratory for particle concentration and

total organic carbon (TOC). Particle analysis was performed using a Coulter Multisizer I electric sensing zone device (Beckman Coulter, Inc.), with a measurement range of 2 to 60 μm. Concentrations of TOC were measured according to Standard Method 5310B using a Shimadzu TOC-5000 Combustion-Infrared analyzer (Standard Methods 1998).

Statistical Analysis

Microbial data were analyzed using GraphPad Prism software (GraphPad Software, Inc, La Jolla, California). Values of p less than 0.05 were considered statistically significant in all statistical analyses, unless otherwise stated.

RESULTS

Single-Sample Events

Prevalence of microbial indicators

Fecal (thermotolerant) coliforms, *E. coli*, and enterococci were detected in all single-sample event samples analyzed. *C. perfringens* spores were detected in all except one wet weather sample and three dry weather samples. These four organisms showed arithmetic mean increases in microorganism concentrations from dry periods to wet periods (Table 1). Maximum concentrations of these four organisms also were higher in wet weather than in dry weather, with enterococci concentrations increasing by 66-fold. Although the mean numbers of somatic and male-specific coliphages increased during wet weather versus dry weather, the median of both data sets decreased, indicating that the data are likely skewed due to outliers. Removing the highest male-specific coliphage data point of 517 PFU/mL, for example, changes the mean from 31 PFU/mL to 3.7 PFU/mL, bringing it closer to the mean of the somatic coliphage results. In some wet weather samples, *C. perfringens* spores were above the maximum detection limit of the MPN assay used (1,100 spores/100 mL)

		N	Min	Med	Max	Arithmetic Mean	Geometric Mean	Coeff. of Variation
FC (MPN/100mL)	dry	15	3.09	30.9	945	117	41.3	203%
	wet	19	100	528	8160	1120	513	163%
E coli (MPN/100mL)	dry	15	1.01	30.9	123	50.0	28.5	91.7%
	wet	19	44.2	238	8160	790	301	230.%
Ent (MPN/100mL)	dry	15	2.04	12.0	370.	44.8	17.3	206%
	wet	19	101	630.	3280	1130	620.	99.9%
C perf (MPN/100mL)	dry	15	<3*	43	460	90.9	31.3	140.%
	wet	19	<3*	240	>1100*	359	126	104%
M-S phage (PFU/100 mL)	dry	15	0	2	19	3.2	-	160.%
	wet	19	0	0	517	31.1	-	380.%
Som phage (PFU/100 mL)	dry	15	0	10	20	9.27	-	68.4%
	wet	19	0	4	91	10.2	-	203%

Table 1. Summary statistics for microbial indicators, single-sample wet weather events and dry periods.
*represents the minimum or maximum detection limit of the assay

Physicochemical factors

Mean values for particle concentration and turbidity increased during storm events versus dry weather events. As measured by the coefficient of variation, data from wet weather events showed more variation than data from dry weather events for *E. coli* and both types of coliphages (Table 1). Sample pH was slightly lower during wet weather events (7.3) than during dry weather periods (7.6).

		N	Min	Med	Max	Arith. Mean	Geom. Mean	Coeff. of Variation
Particles (# 1000 / 100mL)	dry	15	356	801	253	1120	932	62.1%
	wet	19	495	7640	35200	979	5170	93.5%
TOC (mg/L)	dry	15	1.47	2.36	6.71	2.99	2.7	50.8%
	wet	19	1.16	3.69	5.81	3.73	3.5	32.4%
Turbidity (NTU)	dry	15	0.7	2.8	4.7	2.8	2.56	40.7%
	wet	18	1.7	18	100	30.4	16.1	101%
pH	dry	15	7.26	7.88	9.58	7.90	7.88	7.36%
	wet	14	7.03	7.35	7.75	7.33	7.32	3.13%
Temperature (°C)	dry	15	3	9	18	10.3	9.2	46.2%
	wet	19	0	7	10	6.3	-	50.2%

Table 2. Summary statistics for physicochemical factors during single-sample wet weather events and dry periods.

Comparisons between indicators and physicochemical factors in samples above and below the action level

When compared with those below the action level of 10 (oo)cysts per sample volume, samples above the action level had higher geometric mean concentrations and identical or higher minimum concentrations of all measured variables (Table 3). With the exception of fecal coliforms, arithmetic mean concentrations were also higher for all variables in samples above the action level as compared with those below the action level. For concentrations of fecal coliforms, *E. coli*, enterococci, particles, and turbidity, many of the summary statistics in Table 3 (minimum, median, maximum, arithmetic mean, and geometric mean) were larger for samples above the action level than for those below. To test for statistical differences between samples above and below the action level, a Mann-Whitney test was performed for each variable. None of the variables analyzed had significantly different median values at the $\alpha = 0.05$ level, but median concentration values for TOC and turbidity were significantly different at the $\alpha = 0.10$ level.

		N	Min	Med	Max	Arith. Mean	Geom. Mean	Coeff. of Variation
FC (MPN / 100mL)	above	15	30.9	287	8160	937	273	222%
	below	19	3.09	101	1760	471	115	132%
E. coli (MPN / 100mL)	above	15	20.4	122	8160	698	158	296%
	below	19	1.01	97.6	1220	278	77.8	145%
Ent (MPN / 100mL)	above	15	3	309	3280	760	233	132%
	below	19	2.04	41.6	2890	562	79.5	180.%
C. perf (MPN / 100mL)	above	15	<3*	92	>1100*	227	66.3	132%
	below	19	<3*	93	>1100*	251	69.8	135%
M-S phage (PFU / 100mL)	above	15	0	1	517	39.3	-	337%
	below	19	0	1	11	2.58	-	136%
Som. Phage (PFU / 100mL)	above	15	0	4	91	12.1	-	190.%
	below	19	0	6	20	7.95	-	79.6%
Particles (#1000 / 100mL)	above	15	481	2530	35200	8050	3230	123%
	below	19	356	1810	21600	4310	1940	139%
TOC (mg /L)	above	15	1.96	3.63	5.81	3.88	3.69	31.4%
	below	19	1.16	2.49	6.71	3.03	2.73	46.9%
Turbidity (NTU)	above	15	1.7	7.6	100	26.9	11.4	119%
	below	19	0.7	3.6	80	11.3	5.11	165%
pH	above	12	7.05	7.3	9.58	7.59	7.56	9.67%
	below	18	7.03	7.58	8.52	7.65	7.64	4.28%
Temperature (°C)	above	15	0	9	18	8.6	-	52.7%
	below	20	1	6.5	18	7.5	6.14	55.8%

Table 3. Summary statistics for fecal indicator microbes and physicochemical factors during single-sample periods, comparing those above and below the action level of 10 (oo)cysts per sample volume.

*Represents the upper or lower detection limit of the assay.

Prevalence of Cryptosporidium *and* Giardia

The occurrence of *Cryptosporidium* and *Giardia* in water samples was lower than that of fecal (thermotolerant) coliforms, *E. coli*, enterococci, or *C. perfringens*. Of 33 total samples collected, 15 samples exceeded the action level of 10 (oo)cysts for either *Giardia* or

Cryptosporidium. This occurred in 11 of 21 wet weather samples (52%) and 4 of 12 dry weather samples (33%). Mean *Giardia* concentration per liter nearly doubled during wet weather events compared to dry weather (Table 4), while *Cryptosporidium* concentration per liter decreased during wet weather events. Median *Giardia* concentration per liter increased by 5-fold.

		N tested (total)	Min	Med	Max	Arith. Mean	Geom. Mean	Coeff. of Variation
Giardia (#/L)	dry	4 (12)	0	2	10.7	4.6	3.00	96.0%
	wet	11 (21)	1	10.3	14.4	8.3	6.13	63.4%
Crypto (#/L)	dry	4 (12)	2.8	7.5	11.5	7.3	6.46	50.4%
	wet	11 (21)	1	5	17.5	6.6	4.98	76.1%

Table 4. Summary statistics for *Cryptosporidium* and *Giardia*, single-sample wet weather events and dry periods. "N" represents the number of samples that were above the action level as tested by NYCDEP.

Comparisons among indicators and physicochemical factors

Since most of the data analyzed in this study was non-normally distributed according to a D'Agostino-Pearson normality test, nonparametric methods were used for all analyses. Spearman rank correlation was used to test possible relationships of physicochemical parameters (particle concentration, total organic carbon, turbidity, pH, and water temperature) and microbial concentration. A higher Spearman Rho (R_s) value indicates a stronger correlation, and its critical value varies with the number of samples. An R_s value of one (1.0) means that the rank order of the two variables match, while a value of zero (0) indicates no relationship.

(a) Combined data for wet and dry samples. When the data for both dry and wet weather events are combined across all sampling sites, several statistically significant correlations were found (Table 5). The vegetative enteric bacteria (fecal (thermotolerant)

coliforms, *E. coli*, and enterococci) are strongly correlated with each other. Fecal coliforms, *E. coli*, and enterococci each also have a significant negative association with pH, which ranged from 7.03 to 9.58 during the sampling period. The other remaining bacterial indicator, *C. perfringens* spores, is also significantly correlated with each of the other three bacteria. Each of these four microbial indicators is significantly correlated with particle concentration, as well as turbidity. Turbidity and particle concentration are also significantly correlated (R_s = 0.92) with one another. Concentrations of somatic and male-specific coliphages are correlated with each other (R_s = 0.69), but not with any other factor, microbial or physicochemical.

	FC	*E coli*	Ent	*C perf*	MS Phage	Som Phage	Part Conc	TOC	Turb-idity	pH
E coli	**0.90**									
Ent	**0.79**	**0.83**								
C perf	**0.45**	**0.56**	**0.57**							
MS Phage	0.06	0.14	-0.07	0.24						
Som Phage	-0.01	0.02	-0.10	0.20	**0.69**					
Part Conc	**0.60**	**0.69**	**0.74**	**0.72**	0.27	0.16				
TOC	0.27	0.24	**0.41**	0.19	0.01	0.07	**0.45**			
Turbidity	**0.63**	**0.70**	**0.73**	**0.70**	0.18	0.11	**0.92**	**0.47**		
pH	**-0.45**	**-0.44**	**-0.70**	-0.34	0.32	0.14	-0.33	-0.30	-0.35	
Temp	-0.19	-0.25	-0.32	**-0.54**	0.06	0.00	**-0.40**	-0.19	**-0.41**	0.16

Table 5. Spearman*rho* correlations (R_s) between and among microbial and physicochemical indicators, with single-sample wet weather event and dry period samples combined into one dataset. Highlighted values are statistically significant ($p < 0.05$).

(b) Wet weather data As in the combined data, each of the vegetative enteric bacteria is significantly correlated with each of the other ones (Table 6) with the exception of fecal coliforms and *C. perfringens*. *E. coli* and enterococci are each significantly correlated with each of the other biological indicators, including coliphages, during wet weather events. *E. coli*, enterococci, and *C. perfringens* each remain significantly correlated with both particle

concentration and turbidity but at a lower R_s. Particle concentration and turbidity show a significant correlation with each other. Male-specific and somatic coliphages are also significantly correlated with each other ($R_s = 0.80$) as they were for the combined data.

	FC	*E coli*	Ent	*C perf*	MS Phage	Som Phage	Part Conc	TOC	Turb-idity	pH
E coli	0.72									
Ent	0.55	0.69								
C perf	0.20	0.50	0.58							
MS Phage	0.33	0.51	0.51	0.38						
Som Phage	0.38	0.53	0.71	0.56	0.80					
Part Conc	0.45	0.57	0.82	0.75	0.42	0.72				
TOC	-0.08	-0.11	0.13	0.04	0.29	0.17	0.03			
Turbidity	0.39	0.54	0.84	0.73	0.40	0.65	0.97	0.19		
pH	-0.14	0.06	-0.03	-0.09	-0.19	-0.41	-0.25	-0.13	-0.28	
Temp	0.02	-0.24	-0.30	-0.39	-0.08	-0.19	-0.37	-0.07	-0.35	-0.08

Table 6. Spearman*rho* correlations (R_s) between and among microbial and physicochemical indicators during single-sample wet weather events. Values highlighted in gray are statistically significant ($p < 0.05$).

(c) Dry weather data. The correlation between *E. coli* and fecal coliforms was significant during dry weather sampling. This is the only relationship between any two fecal indicator microbes that remains significant across all weather conditions (Table 7). Particle concentration and turbidity are significantly correlated in dry weather samples as they were in wet weather samples and for both wet and dry weather samples combined. The parameter of pH was negatively correlated with enterococci and positively correlated with male-specific coliphages.

	FC	*E coli*	Ent	*C perf*	MS Phage	Som Phage	Part Conc	TOC	Turb-idity	pH
E coli	0.75									
Ent	0.34	0.28								
C perf	0.14	0.23	-0.02							
MS Phage	-0.01	0.17	-0.58	-0.01						
Som Phage	0.08	0.12	-0.43	-0.18	0.39					
Part Conc	-0.10	0.18	0.09	0.11	0.36	-0.38				
TOC	0.14	-0.03	0.44	-0.26	-0.32	-0.15	0.38			
Turbidity	0.11	0.26	-0.23	0.24	0.37	-0.23	0.76	0.22		
pH	0.06	0.15	-0.56	-0.32	0.80	0.26	0.25	-0.11	0.34	
Temp	0.27	0.42	0.40	-0.36	0.30	-0.09	0.26	0.03	0.07	0.34

Table 7. Spearman rho correlations (R) between microbial indicators during single-sample dry weather periods. Values highlighted in gray are statistically significant ($p < 0.05$).

Comparisons among indicators and physicochemical factors in samples above and below the action level

Spearman rank correlations were separately calculated for samples above and below the action level of 10 (oo)cysts per sample volume (Table 8,Table 9). A larger number of correlations were significant for samples above the action level as compared with those below it. Notably, *Giardia* was significantly pairwise correlated with concentrations of fecal coliforms, *E. coli*, enterococci, *C. perfringens*, particles, and *Cryptosporidium*, as well as with turbidity and temperature for samples above the action level. Fecal coliforms had significant pairwise correlations with all other indicator microbe concentrations, as well as particles concentration, TOC, and turbidity in these samples.

	FC	*E. coli*	Ent	*C perf.*	MS phage	Som phage	Particles	TOC	Turbidity	pH	Temp	*Crypto* (NYCDEP)
FC												
E. coli	0.81											
Ent	0.68	0.67										
C perf.	0.59	0.55	0.74									
MS phage	0.59	0.66	0.26	0.31								
Som phage	0.62	0.68	0.32	0.49	0.92							
Particles	0.71	0.72	0.73	0.78	0.51	0.64						
TOC	0.53	0.27	0.36	0.19	0.36	0.25	0.34					
Turbidity	0.57	0.63	0.74	0.79	0.37	0.49	0.95	0.31				
pH	-0.34	-0.35	-0.73	-0.47	-0.01	-0.13	-0.23	0.11	-0.24			
Temp	-0.06	-0.05	-0.32	-0.42	0.34	0.19	-0.33	0.11	-0.41	0.44		
Crypto (NYCDEP)	0.12	0.35	-0.08	0.26	0.20	0.32	0.31	-0.03	0.33	0.06	-0.41	
Giardia (NYCDEP)	0.62	0.59	0.55	0.72	0.38	0.50	0.77	0.41	0.79	-0.22	-0.61	0.67

Table 8. Spearman rho correlations between concentrations of fecal indicator microbes, physicochemical factors, and protozoan pathogens for samples that tested above the action level of 10 (oo)cysts per sample volume. Values highlighted in gray are statistically significant at $p < 0.05$.

	FC	*E. coli*	Ent	*C perf.*	MS phage	Som phage	Particles	TOC	Turbidity	pH	Temp	*Crypto* (NYCDEP)
FC												
E. coli	0.87											
Ent	0.72	0.84										
C perf.	0.37	0.62	0.45									
MS phage	-0.28	-0.14	-0.31	0.22								
Som phage	-0.34	-0.29	-0.41	-0.05	0.42							
Particles	0.43	0.64	0.75	0.67	0.06	-0.36						
TOC	0.05	0.12	0.41	0.21	-0.11	-0.01	0.52					
Turbidity	0.50	0.64	0.62	0.73	-0.02	-0.31	0.90	0.43				
pH	-0.52	-0.54	-0.70	-0.30	0.61	0.35	-0.42	-0.54	-0.32			
Temp	-0.27	-0.37	-0.37	-0.57	-0.22	-0.12	-0.51	-0.45	-0.50	0.07		
Crypto (NYCDEP)	0.63	0.55	0.43	0.21	-0.18	-0.23	0.19	-0.17	0.01	-0.25	0.03	
Giardia (NYCDEP)	0.37	0.39	0.54	0.30	-0.24	-0.26	0.45	0.40	0.25	-0.40	-0.57	0.56

Table 9. Spearman rho correlations between concentrations of fecal indicator microbes, physicochemical factors, and protozoan pathogens for samples that tested below the action level of 10 (oo)cysts per sample volume. Values highlighted in gray are statistically significant at $p < 0.05$.

Comparisons between concentrations of indicators and protozoan parasites in samples above the action level

When the data for wet and dry samples are combined, *Giardia* has significant Spearman rank correlations with fecal coliforms, enterococci, and *C. perfringens* (Table 10). *Giardia* also significantly correlates with particle concentration and turbidity in this data set for total (combined wet and dry) samples. For wet weather samples, *Giardia* was also significantly positively correlated with fecal coliforms, enterococci, and *C. perfringens*. Wet weather *Giardia* concentrations were also significantly positively correlated with somatic coliphage, particle concentration, TOC and turbidity. With the exception of *C. perfringens*, the values for Spearman rank correlations of *Giardia* with other parameters are lower in the combined data sets than in the data set containing only wet weather events. The correlation between *Giardia* and enterococci is the only R_s value that is significant in wet, dry, and combined data sets (Table 14).

Cryptosporidium and *Giardia* are significantly correlated in combined samples and in wet weather sampling. Despite having a perfect rank correlation ($R_s = 1$) during dry weather periods, they are significant only at the $p < 0.10$ level in this data set due to a low number of samples that exceeded the action level of 10 (oo)cysts per sample. This is also the case for the correlation between *Cryptosporidium* concentration and enterococci concentration during dry weather. *Cryptosporidium* also had a negative correlation with sample temperature ($R_s = -0.77$), but this correlation is only significant during wet weather events.

	Giardia			*Crypto*		
	Total	Wet	Dry	Total	Wet	Dry
FC	0.58	0.75	0.30	0.45	0.41	0.40
E coli	0.34	0.39	-0.50	0.17	0.09	-0.40
Ent	0.67	0.87	1.00	0.24	0.27	1.00
C perf	0.80	0.69	0.56	0.40	0.29	0.63
MS Phage	0.04	0.56	-0.41	0.01	-0.02	-0.40
Som Phage	0.04	0.79	-0.30	0.16	0.17	-0.40
Part Conc	0.59	0.72	-0.30	0.35	0.37	-0.40
TOC	0.17	0.69	-0.10	0.31	0.40	-0.20
Turbidity	0.55	0.68	-0.80	0.27	0.41	-0.80
pH	-0.58	-0.60	-0.60	0.13	-0.02	-0.80
Temp	-0.52	-0.49	0.30	-0.26	-0.77	0.20
Giardia	-	-	-	0.72	0.74	1.00
Crypto	0.72	0.74	1.00	-	-	-

Table 10. Spearman rho correlations (R) between concentrations of *Cryptosporidium*, *Giardia*, and microbial and physicochemical indicators for different single-sample data sets as analyzed by UNC. These values only represent samples that were above the action level of 10 (oo)cysts per sample volume, as tested by NYCDEP. Values highlighted in gray are statistically significant ($p < 0.05$).

Comparisons between sampling sites and dates

No significant differences (α=0.05) in the median values of indicator microbes were found between sampling sites using a Friedman two-way analysis of variance by ranks. For physicochemical factors, only TOC had median values that were significantly different between sites at α=0.05 level. *Cryptosporidium* and *Giardia* values were not included in this analysis, as data was very limited for some sites.

Comparisons between UNC data and NYCDEP data

Since duplicate water samples were each analyzed by NYCDEP and UNC, results were analyzed to determine potential differences between testing laboratories. Data from NYCDEP was not corrected based on an internal standard, so uncorrected UNC data was used in these analyses. A Mann-Whitney analysis (α = 0.05) was used to determine if

pathogen concentrations were significantly different between the two laboratories. *Giardia* concentrations were not significantly different between the NYCDEP laboratory (median = 0.326 cysts/L) and the UNC laboratory (median = 0.570 cysts/L); however, *Cryptosporidium* concentrations were significantly different at this level (medians 0.044 and 0.430 oocysts/L, respectively).

Pathogen data from the NYCDEP laboratory was combined with UNC laboratory data to determine the relationships, if any, between NYCDEP data and UNC laboratory data for all variables. For *Giardia*, there is no correlation with any indicator that is significant in the same direction (positive or negative) for both UNC data and NYCDEP data during dry weather, wet weather, or combined weather data (Table 11). Notably, particle concentration was significantly positively correlated with *Giardia* during total and wet weather data sets when UNC corrected data was used; however, this correlation is significant and negative when uncorrected data is used. Within both the total data set and the wet weather data set, uncorrected *Giardia* concentrations were significantly associated with the recovery percentage of a spiked internal standard. Additionally, *Giardia* concentration has a significant correlation with *Cryptosporidium* concentration in the total and wet weather data sets, similar to those of the corrected UNC data (Table 10).

As with the corrected UNC *Cryptosporidium* data, there are few significant correlations between uncorrected UNC *Cryptosporidium* data and the concentration of any measured variable other than *Giardia* (Table 12). The recovery percentage for spiked internal *Cryptosporidium* standards is significantly correlated with uncorrected *Cryptosporidium* concentration in the UNC data set. Additionally, *Cryptosporidium* concentration is

significantly pairwise correlated with fecal coliform concentration and *E. coli*, with the dry weather data having a higher R_s value than that of the total data set.

Overall, few correlations exist across corrected UNC data, uncorrected UNC data, and NYCDEP data. The concentrations of the pathogens *Cryptosporidium* and *Giardia* are significantly correlated with each other in many cases, but only within each laboratory.

	UNC			NYCDEP		
	Total	Wet	Dry	Total	Wet	Dry
Fecal coliforms	-0.19	-0.23	0.40	0.52	0.10	0.38
E. coli	-0.37	-0.31	-0.40	0.55	0.25	0.33
Enterococci	-0.42	-0.32	1.00	0.55	0.40	0.13
C perfringens	-0.41	-0.51	0.63	0.29	0.11	0.02
M-S phage	-0.13	-0.14	-0.40	0.04	0.30	-0.08
Som phage	-0.28	-0.36	-0.40	-0.03	0.27	-0.21
Particles	-0.54	-0.50	-0.40	0.55	0.45	0.41
TOC	0.31	0.49	-0.20	0.51	0.34	0.59
Turbidity	-0.47	-0.34	-0.80	0.59	0.60	0.33
pH	0.19	0.16	-0.80	-0.31	-0.48	0.16
Temperature	0.46	0.20	0.20	-0.28	-0.18	-0.19
Giardia recovery %	0.84	0.81	0.74	-0.59	-0.44	-0.50
Crypto recovery %	0.65	0.77	-0.32	-0.33	-0.34	0.50
Giardia (UNC)	-	-	-	-0.36	-0.04	-0.95
Giardia (NYCDEP)	-0.36	-0.04	-0.95	-	-	-
Crypto (UNC)	0.80	0.75	1.00	-0.13	0.14	-0.95
Crypto (NYCDEP)	-0.22	-0.05	n/a	0.62	0.56	0.64

Table 11. Spearman rho correlations (R) between concentrations of *Giardia* and microbial and physicochemical indicators for different single-sample data sets as analyzed by either UNC or NYCDEP. Pathogen correlation values for the UNC laboratory only represent samples that were above the action level of 10 (oo)cysts per sample volume, as tested by NYCDEP. Values highlighted in gray are statistically significant ($p < 0.05$).

	UNC			NYCDEP		
	Total	Wet	Dry	Total	Wet	Dry
Fecal coliforms	0.13	0.19	0.40	0.40	0.14	0.63
E. coli	-0.09	0.04	-0.40	0.47	0.23	0.70
Enterococci	-0.14	-0.02	1.00	0.26	0.07	0.07
C perfringens	-0.13	-0.27	0.63	0.18	0.10	0.13
M-S phage	0.17	0.30	-0.40	-0.02	-0.06	0.23
Som phage	0.02	0.09	-0.40	-0.09	-0.03	0.01
Particles	-0.24	-0.19	-0.40	0.28	0.24	0.23
TOC	0.29	0.42	-0.20	-0.01	-0.19	0.13
Turbidity	-0.25	-0.16	-0.80	0.22	0.23	0.25
pH	-0.08	-0.26	-0.80	-0.11	-0.43	0.40
Temperature	0.30	0.05	0.20	-0.02	-0.09	0.25
Giardia recovery %	0.45	0.37	0.74	-0.27	-0.14	-0.74
Crypto recovery %	0.55	0.82	-0.32	-0.10	-0.37	0.32
Giardia (UNC)	0.80	0.75	1.00	-0.22	-0.05	n/a
Giardia (NYCDEP)	-0.13	0.14	-0.95	0.62	0.56	0.64
Crypto (UNC)	-	-	-	-0.21	-0.06	n/a
Crypto (NYCDEP)	-0.21	-0.06	n/a	-	-	-

Table 12. Spearman rho correlations (R) between concentrations of *Cryptosporidium* and microbial and physicochemical indicators for different single-sample data sets as analyzed by either UNC or NYCDEP. Pathogen correlation values for the UNC laboratory only represent samples that were above the action level of 10 (oo)cysts per sample volume, as tested by NYCDEP. Values highlighted in gray are statistically significant ($p < 0.05$).

Comparisons between microbial indicators, physicochemical factors, and the summation of pathogen concentrations

Another approach to comparing pathogens and indicators is to sum the concentrations of both *Cryptosporidium* and *Giardia* before analyzing for correlations. The results of this analysis are presented in (Table 13) below. Many of the highest correlations from previous analyses appear again in this table, with significant pairwise associations between corrected UNC pathogen data and concentrations of enterococci, particles and *C. perfringens*. While the uncorrected UNC data set has few significant correlations in this analysis, the uncorrected NYCDEP data set has several. For combined data across all weather conditions, combined pathogen concentration is significantly pairwise correlated with concentrations of fecal

coliforms, *E. coli*, enterococci, particles, and TOC, as well as with turbidity. No correlations are significant in all data sets for any laboratory's corrected or uncorrected data.

	UNC corrected			UNC uncorrected			NYCDEP uncorrected		
	Total	Wet	Dry	Total	Wet	Dry	Total	Wet	Dry
Fecal coliforms	0.48	0.63	0.40	-0.07	-0.02	0.40	0.45	0.06	0.38
E. coli	0.27	0.32	-0.40	-0.28	-0.15	-0.40	0.46	0.14	0.33
Enterococci	0.56	0.73	1.00	-0.35	-0.19	1.00	0.49	0.39	0.11
C. perfringens	0.75	0.76	0.63	-0.36	-0.47	0.63	0.28	0.17	0.00
M-S phage	-0.02	0.27	-0.40	-0.02	0.06	-0.40	0.00	0.18	-0.06
Som. Phage	0.21	0.65	-0.40	-0.20	-0.22	-0.40	-0.03	0.22	-0.18
Particles	0.60	0.87	-0.40	-0.50	-0.44	-0.40	0.50	0.47	0.40
TOC	0.34	0.43	-0.20	0.31	0.48	-0.20	0.43	0.27	0.59
Turbidity	0.41	0.62	-0.80	-0.44	-0.25	-0.80	0.52	0.55	0.33
pH	-0.37	-0.61	-0.80	0.01	-0.28	-0.80	-0.31	-0.59	0.18
Temperature	-0.29	-0.60	0.20	0.51	0.29	0.20	-0.20	-0.09	-0.18

Table 13. Spearman rho correlations (R) between combined concentrations of *Cryptosporidium* and *Giardia* and microbial and physicochemical indicators for different single-sample data sets as analyzed by UNC and NYCDEP. Pathogen correlation values for the UNC laboratory only represent samples that were above the action level of 10 (oo)cysts per sample volume, as tested by NYCDEP. Values highlighted in gray are statistically significant ($p < 0.05$).

Intrastorm Samples

Microbial indicators and physicochemical factors

Fecal coliforms, *E. coli*, enterococci, and somatic coliphages all showed increases in intrastorm mean concentrations as compared with single-sample wet weather events (Table 14). A Mann-Whitney analysis was performed to determine if median concentration differences between intrastorm and single-sample wet weather events were significantly different at the $\alpha = 0.05$ level. Median concentrations of fecal coliforms, *E. coli*, enterococci, somatic coliphages, and particles were all significantly higher during sampled intrastorm periods.

		N	Min	Med	Max	Mean	Std Dev
FC (MPN / 100mL)	wet	19	100	528	8160	1120	1830
	intrastorm	28	524	10600	278000	23700	51700
E coli (MPN / 100mL)	wet	19	44.2	238	816	790.	1820
	intrastorm	28	103	2180	1580	3930	4050
Ent (MPN / 100mL)	wet	19	101	630	3280	1126	1125
	intrastorm	28	71	2270	21100	372	500.
C perf (MPN / 100mL)	wet	19	<3*	240	>1100*	359	373
	intrastorm	28	<3*	75	>1100*	167	233
MS phage (PFU / 100 mL)	wet	19	0	0	517	31.1	118
	intrastorm	20	0	0	7	1	2
Som phage (PFU / 100 mL)	wet	19	0	4	91	10.2	20.8
	intrastorm	20	10	145	421	179	114
Part Conc (# 1000 / 100mL)	wet	19	495	7640	35200	9790	9150
	intrastorm	28	369	6850	12300	5930	3420
TOC (mg/L)	wet	19	1.16	3.69	5.81	3.73	1.21
	intrastorm	28	2.2	4.0	8.6	4.4	1.6

Table 14. Comparison of summary statistics for single-sample wet weather events and combined intrastorm sampling.

*indicates minimum or maximum detection limit of assay

Prevalence of Cryptosporidium *and* Giardia

During intrastorm periods, 12 of 28 (43%) of samples analyzed by the NYCDEP exceeded the action level of 10 (oo)cysts per sample (Table 15) for either *Giardia* or *Cryptosporidium* and were subsequently analyzed by the UNC laboratory. This is fewer than during single-sample wet weather events (52%) but higher than single-sample dry weather periods (33%). Mean concentrations of both *Giardia* and *Cryptosporidium* in intrastorm samples were lower than in single-sample wet weather events. To determine if concentrations of *Cryptosporidium* and *Giardia* were significantly different between these two periods, a Mann-Whitney analysis was performed. At the $\alpha = 0.05$ level, the median concentration of

Cryptosporidium was significantly lower for intrastorm sampling periods than for single-sample wet weather sampling periods.

	N tested (total)	Min	Med	Max	Mean	Std Dev
Giardia (#/L)	12 (28)	0	4.5	12.4	4.9	3.0
Crypto (#/L)	12 (28)	0	2.0	7.6	2.5	2.6

Table 15. Summary statistics for *Giardia* and *Cryptosporidium* during intrastorm sampling. Only samples that were above the action level of 10 (oo)cysts (per total sample volume) as tested by NYCDEP were subsequently analyzed by the UNC lab.

Comparisons within indicators

Some of the relationships between microbial indicators that were statistically significant in samples taken during single-sample wet weather events were also significant in samples taken during the intrastorm period (Table 17). Fecal coliforms, which were significantly correlated with *E. coli* within all single-sample data sets, were also significantly correlated during intrastorm sampling. *E. coli* and fecal coliforms were each significantly pairwise correlated with enterococci and *C. perfringens* as well. Of these, only the correlation between fecal coliforms and *C. perfringens* was not significant during single-sample wet weather events. TOC was significantly correlated with *E. coli*, enterococci, and *C. perfringens*; none of these three relationships had significant R_s values during previous single-sample wet weather sampling (Table 6).

	FC	*E coli*	Ent	*C perf*	MS Phage	Som Phage	Part Conc
E coli	0.54						
Ent	0.57	0.83					
C perf	0.41	0.53	0.30				
MS Phage	0.23	0.32	0.37	0.39			
Som Phage	-0.17	0.29	0.18	0.43	-0.09		
Part Conc	0.19	0.25	0.22	0.68	0.34	0.37	
TOC	0.20	0.67	0.39	0.52	0.22	0.37	0.23

Table 16. Spearman*rho* rank correlations (R_s) between and among the mean values of microbial and physicochemical indicators, with all intrastorm samples combined. Values highlighted in gray are statistically significant ($p < 0.05$).

Comparisons between indicators and protozoan pathogens in intrastorm samples

No significant Spearman rank correlations were found between *Giardia* concentrations and those of any other variable during the intrastorm period (Table 17). *Giardia* and *Cryptosporidium* concentrations were not significantly correlated with each other in intrastorm samples, whereas they were significantly correlated in single-sample wet weather and single-sample dry weather samples. During the intrastorm period *Cryptosporidium* concentration was significantly correlated with both *C. perfringens* and male-specific coliphages.

	Crypto	*Giardia*
FC	0.02	0.11
E coli	0.35	-0.06
Ent	0.14	0.36
C perf	0.66	-0.07
MS Phage	0.81	-0.22
Som Phage	-0.48	-0.12
Part Conc	0.22	-0.31
TOC	0.06	-0.21
Crypto	-	0.02
Giardia	0.02	-

Table 17. Spearman rho correlations (R) between *Cryptosporidium*, *Giardia*, and microbial and physicochemical indicators for intrastorm sampling. Values highlighted in gray are statistically significant ($p < 0.05$).

Changes in concentrations and associations over the course of a storm

Due to a small data set and experimental errors in analyzing some samples for coliphages, these organisms were not included in remainder of the intrastorm results.

Categorization of hydrographic limbs

Although it is difficult to strictly classify each storm's three phases into hydrographic flow limbs, each phase was categorized based on a visual inspection of stream flow over the course of sampling. Phases were designated as "rising" limbs if stream flow increased but did not reach a peak during that particular storm. "Peak" limbs included the highest stream flow during that particular storm, and "falling" limbs occurred when stream flow decreased after a peak but had not decreased to a steady level. Storm phases were categorized by limbs without respect to whether they were the first, second, or third chronological phase of each storm.

Qualitative changes in concentration

Taken as a whole, it is difficult to determine a pattern in the concentration of any microbial or physicochemical factor over the course of a storm. One of the sampling sites, designated as WHIP, had concentrations of fecal coliforms,*E. coli*, enterococci, and particles that appear to change with changes in stream flow (Figure 2). Figure 3 illustrates the summary distribution of each variable for rising, peak, and falling hydrographic limbs. Mean concentrations of fecal coliforms, enterococci, male-specific coliphages, *Giardia*, *Cryptosporidium*, and particle concentration are highest for the peak limbs. Both *E. coli* and TOC means decrease from rising to peak limbs, and then again from peak to falling limbs.

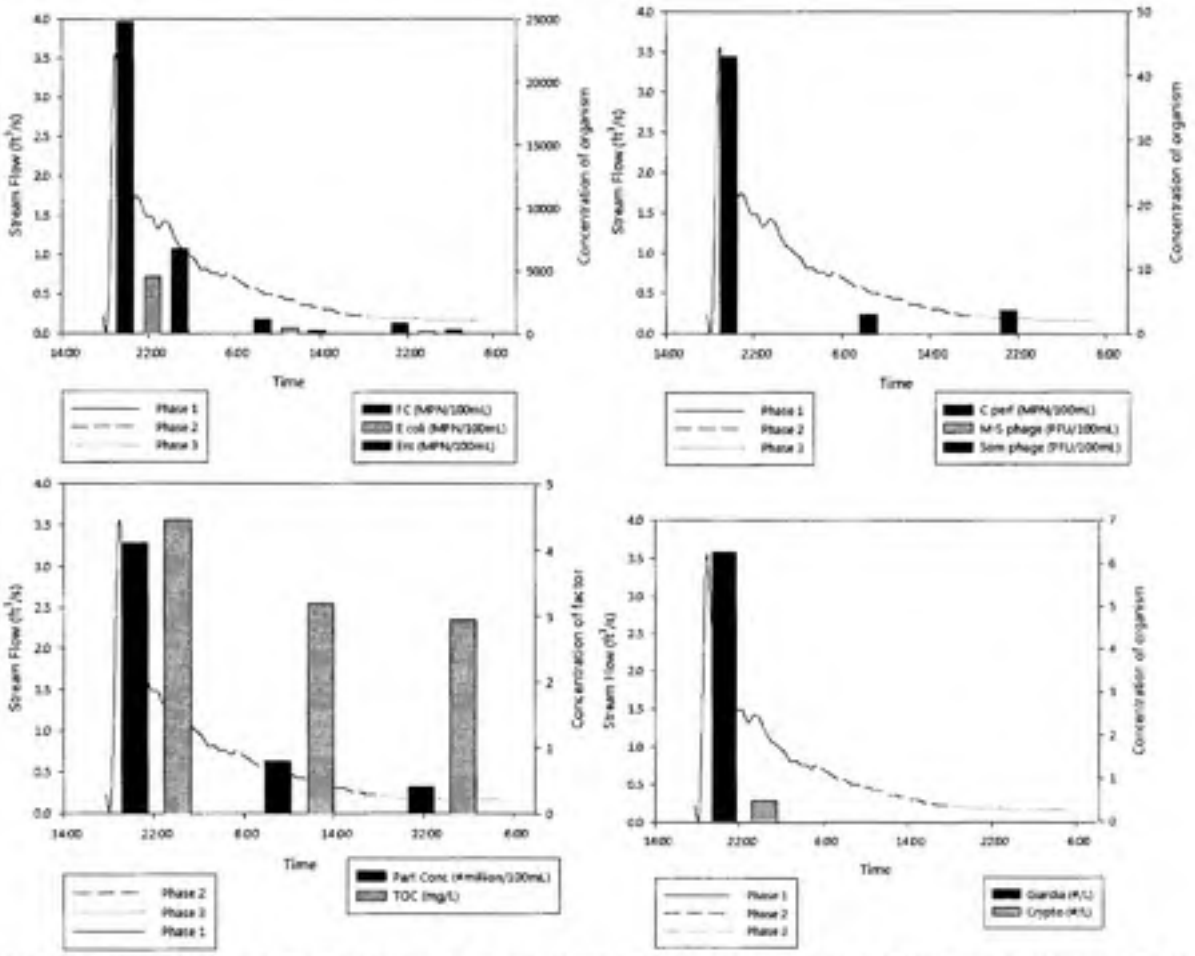
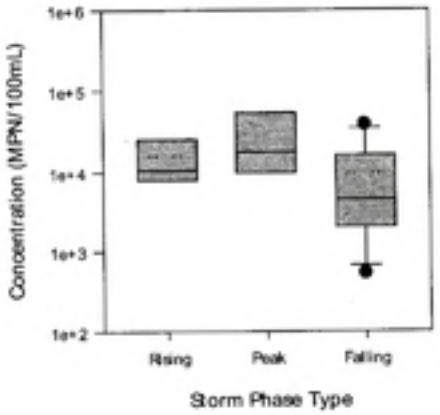
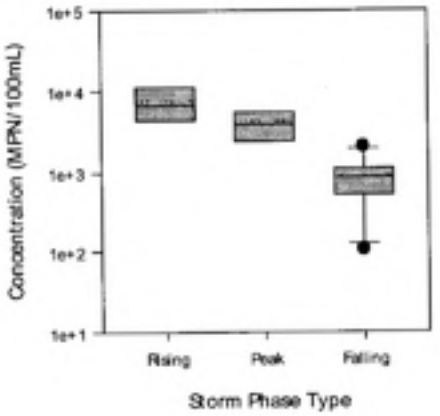


Figure 2. Stream flow and concentrations of microbial indicators, physicochemical factors, and pathogens for the third storm at sampling site WHIP.

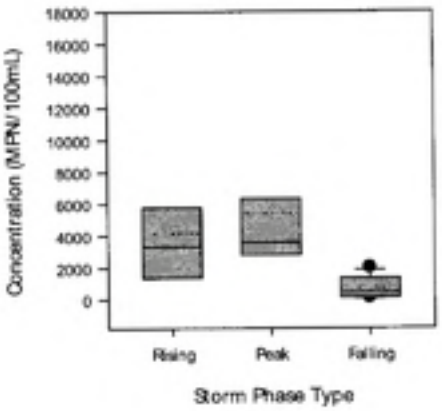
Fecal Coliforms



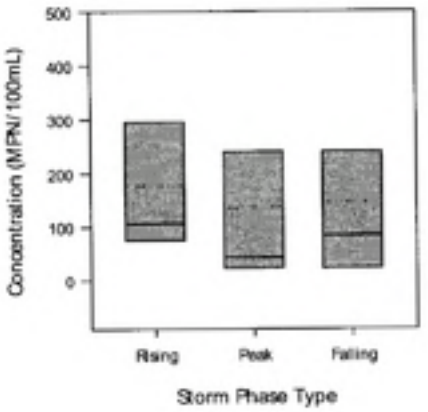
E coli



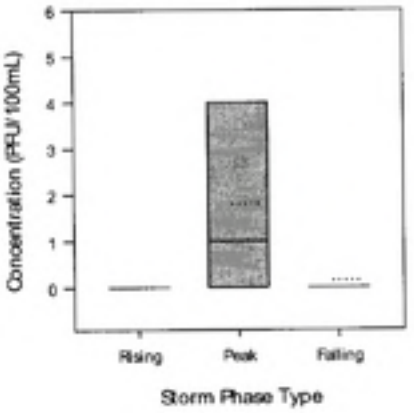
Enterococci



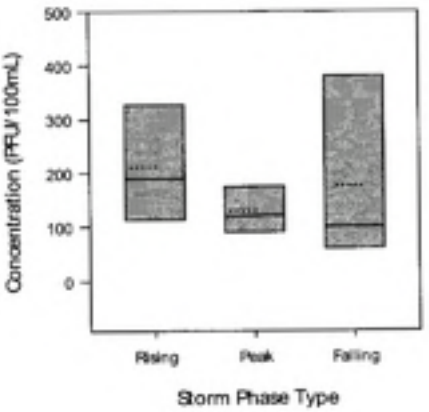
C. perfringens



Male-specific coliphages



Somatic coliphages



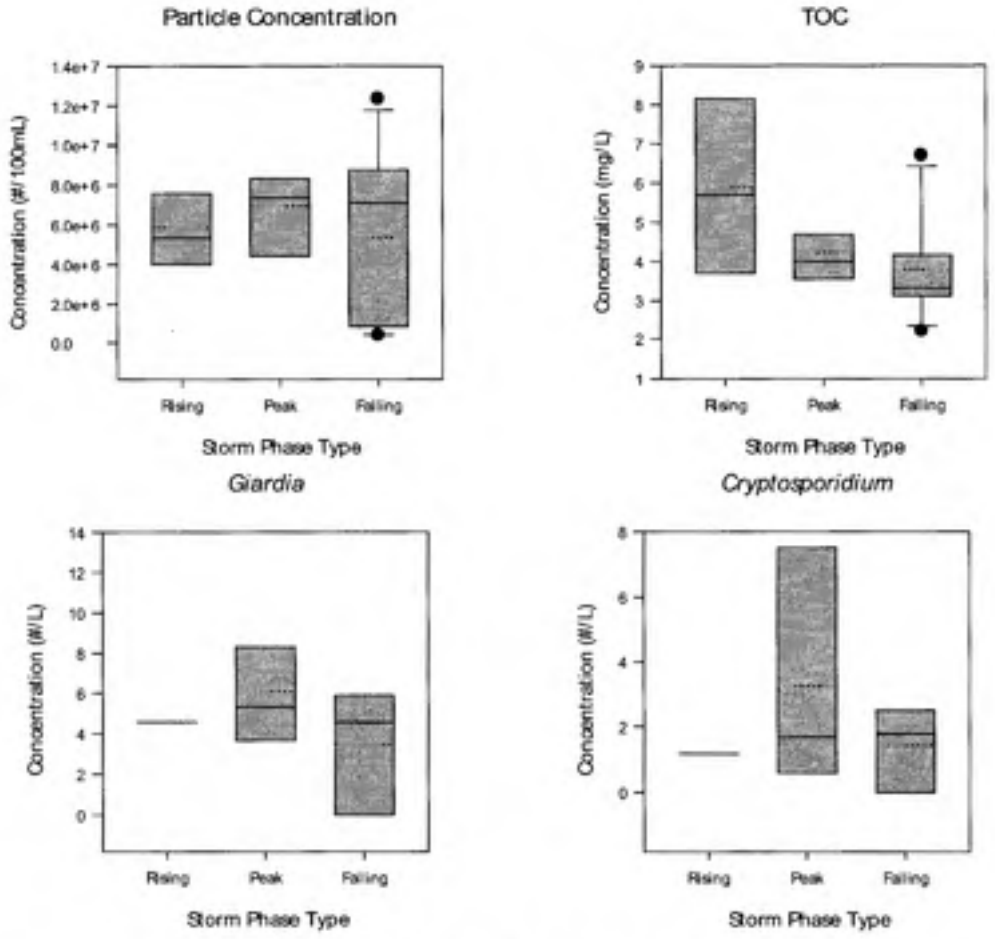


Figure 3. Distributions of microbial indicators, physicochemical factors, and pathogens separated by hydrographic limb type.

Comparisons between total loadings and storm water volume fraction

If all other factors are static, an increase in total water volume (which occurs during storm events) will cause a decrease in the concentration of all measured microbial and physicochemical variables. To determine if changes in concentration were proportional to this increase in stream volume, values for all microbial indicators, physicochemical factors, and pathogens were converted to total loadings based on the total stream volume during their respective phase. If there is a direct relationship between rainfall, stream volume, and total loading, the percentage of each microbe or factor in each phase of a storm should be

approximately equal to the percentage of each storm's volume during that phase. These values are compared in Appendix A.

Comparison of microbial and physicochemical loadings by limb type

Peak hydrographic limbs accounted for the largest average stream volume, as well as the largest total volume sampled of the three limb types (Table 18). This contributes to large total loadings of all microbes and factors, all of which are higher than the associated stream volume fraction, as well as mean loadings that are above the average of all limbs. Hydrographic limbs classified as "rising" contained disproportionate average loadings for both *E. coli* and TOC, as well as mean loadings substantially above the average of all limb types. Falling limbs, which had the lowest total contribution to overall stream volume despite the largest number of samples, accounted for less than 15% of any organism's total loading for the testing period.

		Water Volume (L)[++]	FC (MPN)	*E. coli* (MPN)	Ent (MPN)	*C perf* (MPN)	Particles (#)	TOC (mg)
Total loading (all storms)		4.74E+07	4.49E+12	2.07E+12	1.66E+12	6.38E+10	2.20E+15	1.69E+08
Mean loading (per limb)		1.58E+06	1.61E+11	7.38E+10	5.93E+10	2.28E+09	7.86E+13	6.02E+06
Rising (n=6)	Phase Loading (% total)	1.05E+07 (22%)[+++]	1.19E+12 (26%)	8.78E+11 (42%)	4.53E+11 (27%)	1.48E+10 (23%)	4.97E+14 (23%)	6.31E+07 (37%)
	Mean loading (% difference)	1.74E+06 (+10%)	1.98E+11 (+23%)	1.46E+11 (+98%)	7.55E+10 (+27%)	2.46E+09 (+8%)	8.29E+13 (+5%)	1.05E+07 (+75%)
Peak (n=8)	Phase Loading (% total)	1.71E+07 (36%)	2.71E+12 (60%)	9.99E+11 (48%)	9.76E+11 (59%)	3.56E+10 (56%)	1.29E+15 (58%)	7.63E+07 (45%)
	Mean loading (% difference)	2.14E+06 (+35%)	3.39E+11 (+111%)	1.25E+11 (+69%)	1.22E+11 (+106%)	4.45E+09 (+95%)	1.61E+14 (+105%)	9.54E+06 (+58%)
Falling (n=11)	Phase Loading (% total)	7.03E+06 (15%)	3.16E+11 (7%)	3.92E+10 (2%)	1.96E+10 (1%)	3.96E+09 (6%)	2.99E+14 (14%)	2.14E+07 (13%)
	Mean loading (% difference)	6.39E+05 (-60%)	2.87E+10 (-82%)	3.56E+09 (-95%)	1.78E+09 (-97%)	3.60E+08 (-84%)	2.72E+13 (-65%)	1.95E+06 (-68%)

Table 18. Total and mean contributions of each hydrographic limb type to overall loading of each microbial indicator and physicochemical factor.

[++] *Giardia*, *Cryptosporidium*, and coliphages were not tested for all samples and were not included here.

[+++] Percentages do not add up to 100% in some cases because not all storms were sampled three times, and because limbs that represented baseline flow (n = 3) were not included.

Comparison of microbial and physicochemical loadings by sampling site

Sampling site WHIP accounts for an average total water volume that is substantially higher than the mean for all sample phases, as well as higher average microbial loadings (Table 19). The total contribution of each site to the loading of the Kensico Reservoir was not calculated due to relatively few samples being taken at site E9.

		Water Volume (L)**	FC (MPN)	*E. coli* (MPN)	Ent (MPN)	*C perf* (MPN)	Particles (#)	TOC (mg)
Mean loading (all phases)		1.30E+06	1.61E+11	7.38E+10	5.93E+10	2.28E+09	7.86E+13	6.02E+06
E9 (n=5)	Mean loading	1.17E+06	1.08E+11	8.35E+10	2.15E+10	2.05E+09	5.19E+13	9.27E+06
	% difference	-10%	-33%	+13%	-64%	-10%	-34%	+54%
WHIP (n=11)	Mean loading	2.13E+06	2.44E+11	1.07E+11	7.07E+10	2.97E+09	1.22E+14	8.62E+06
	% difference	+64%	+52%	+45%	+19%	+30%	+55%	+43%
N5-1 (n=12)	Mean loading	5.84E+05	1.06E+11	3.94E+10	6.46E+10	1.74E+09	5.01E+13	2.29E+06
	% difference	-55%	-34%	-47%	+9%	-24%	-36%	-62%

Table 19. Mean contributions of each sampling site to overall loading of each microbial indicator and physicochemical factor, and the percent difference from the mean.

** *Giardia, Cryptosporidium*, and coliphages were not tested for all samples and were not included here.

Comparison of microbial and physicochemical loadings by chronological phase

Regardless of limb type, the first phase of each sampled storm had total and mean loadings of fecal coliforms, *E. coli*, enterococci, and particles that were higher than expected based on stream water volume (Table 20). The mean loadings of *C. perfringens*, particles, and TOC were lowest during the second phase, peaking during the final sample period.

		Water Volume (L)**	FC (MPN)	*E. coli* (MPN)	Ent (MPN)	*C perf* (MPN)	Particles (#)	TOC (mg)
Total loading		4.74E+07	4.49E+12	2.07E+12	1.66E+12	6.38E+10	2.20E+15	1.69E+08
Mean loading		1.58E+06	1.61E+11	7.38E+10	5.93E+10	2.28E+09	7.86E+13	6.02E+06
Phase 1 (n=10)	Phase Loading (% total)	1.09E+07 (23%)***	2.37E+12 (53%)	7.83E+11 (38%)	9.43E+11 (57%)	1.35E+10 (21%)	7.79E+14 (35%)	4.24E+07 (25%)
	Mean loading (% difference)	1.09E+06 (-31%)	2.37E+11 (+47%)	7.83E+10 (+6%)	9.43E+10 (+59%)	1.35E+09 (-41%)	7.79E+13 (-1%)	4.24E+06 (-30%)
Phase 2 (n=10)	Phase Loading (% total)	9.37E+06 (20%)	6.67E+11 (15%)	3.88E+11 (19%)	3.27E+11 (20%)	6.82E+09 (11%)	3.04E+14 (14%)	3.92E+07 (23%)
	Mean loading (% difference)	9.37E+05 (-41%)	6.67E+10 (-58%)	3.88E+10 (-48%)	3.27E+10 (-45%)	6.82E+08 (-70%)	3.04E+13 (-61%)	3.92E+06 (-35%)
Phase 3 (n=8)	Phase Loading (% total)	1.62E+07 (34%)	1.46E+12 (32%)	8.97E+11 (43%)	3.90E+11 (23%)	4.35E+10 (68%)	1.12E+15 (51%)	8.70E+07 (52%)
	Mean loading (% difference)	1.80E+06 (+14%)	1.83E+11 (+14%)	1.12E+11 (+52%)	4.87E+10 (-18%)	5.44E+09 (+139%)	1.40E+14 (+78%)	1.09E+07 (+81%)

Table 20. Total and mean contributions of each chronological storm phase to overall loading of each microbial indicator and physicochemical factor.

****** *Giardia, Cryptosporidium*, and coliphages were not tested for all samples and were not included here.

******* Percentages do not add up to 100% in some cases because not all storms were sampled three times, and because limbs that represented baseline flow (n = 3) were not included.

Precision of EPA Method 1623 for Giardia *and* Cryptosporidium

Recoveries for spiked *Cryptosporidium* and *Giardia* averaged less than 20% for those added to stream samples (Figure 4). Recoveries for positive biological controls (OPR) were higher, although they averaged below 40% for both single-sample and intrastorm periods. The final process before the sample is transferred to a slide is immunomagnetic separation (IMS), which uses a positive combined *Cryptosporidium and Giardia* control provided by the manufacturer. The recoveries from this step alone were higher than samples that had been processed using the entire method, with *Cryptosporidium* recoveries higher than those of *Giardia.*

Arithmetic mean recoveries were higher for samples with turbidities of less than 5 NTU (*Cryptosporidium* = 26%, *Giardia* = 25%) than for samples above 5 NTU

(*Cryptosporidium* = 9%, *Giardia* = 12%). To determine if recoveries were significantly different above and below a turbidity of 5 NTU, a Mann-Whitney analysis was used. The results of this test indicate that there is no significant difference between median recovery efficiencies above or below a turbidity of 5 NTU. Additionally, there were no significant Spearman rank correlations between turbidity and *Cryptosporidium* concentration or turbidity and *Giardia* concentration.

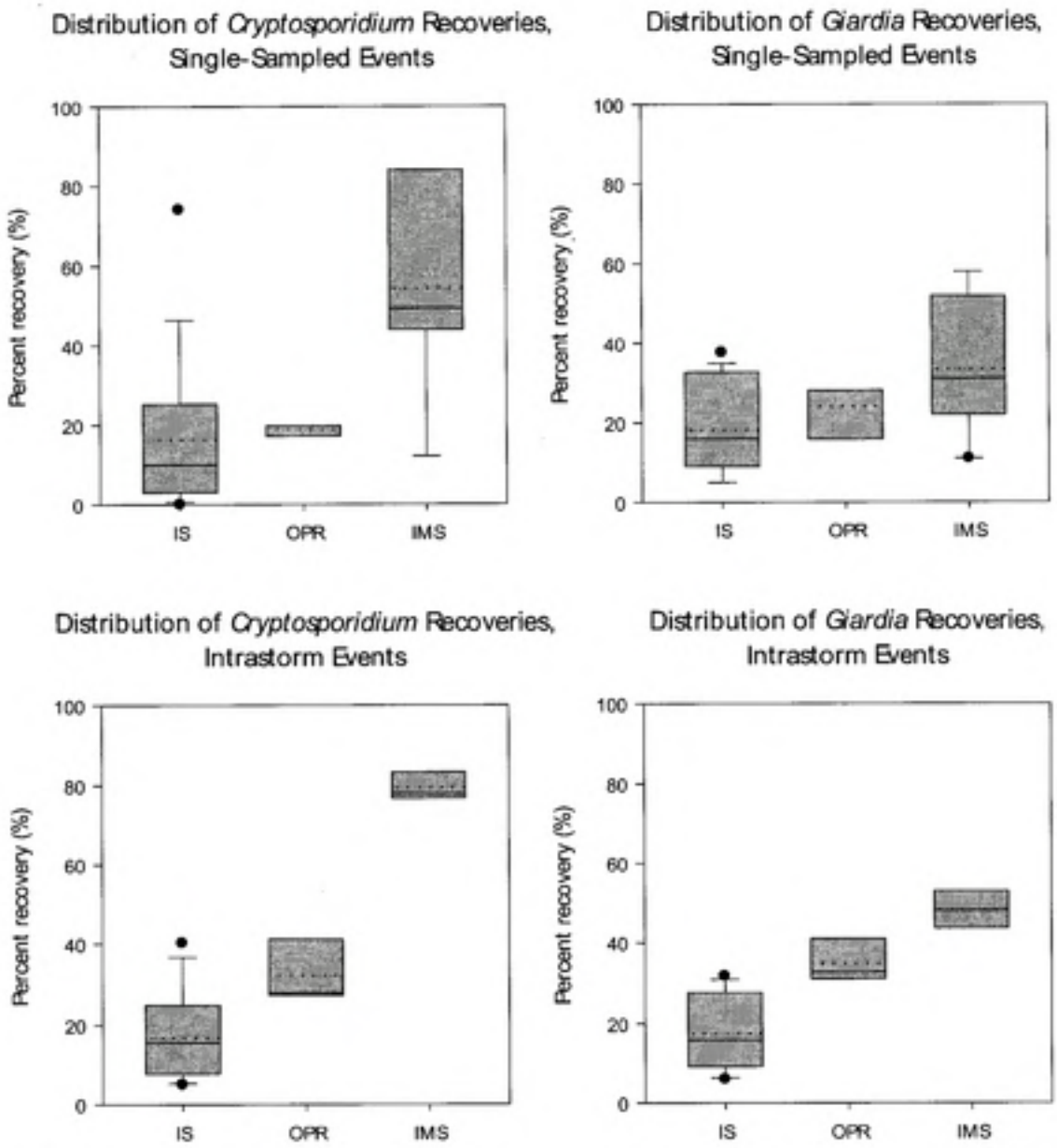


Figure 4. Comparison of the recoveries of spiked internal standard ("IS") and control ("OPR") *Cryptosporidium* and *Giardia* with controls only undergoing IMS analysis.

DISCUSSION

This study presents many potentially important associations between and among fecal indicator microbes, physicochemical factors, and protozoan pathogens in tributaries of the Kensico watershed. High concentrations and stream flows during wet weather events emphasize the particular importance of considering these periods when determining water quality impacts. Some chronological phases and hydrographical limb types within individual wet weather events contribute to total loadings in amounts disproportional to others, as well.

Importance of wet weather events in microbial quality of surface source water for drinking water supply

These results of this study show an increase in the concentrations of fecal indicator bacteria from dry to wet weather periods. These findings are supported by existing literature (Coulliette and Noble, 2008, Kistemann, 2002), who reported varying increases in fecal indicator microbes during wet weather events. With the associated increase in total stream volume, wet weather events contribute a large portion of the total loading of both fecal indicator microbes and physicochemical factors such as particles. The loading of particles has important implications for overall water quality, as high particle concentrations can interfere with microbial testing (DiGiorgio, 2002) and water treatment procedures (Winward et al., 2008). This may be one reason why heavy rainfall has been associated with waterborne disease outbreaks (Thomas et al., 2006; Auld et al., 2004). Because of the disproportionate impact of wet weather events on total microbial loadings, it may be appropriate to direct time and resources to these periods to achieve the maximum reductions in risk and overall disease burden.

Although mean *Giardia* concentration approximately doubled during storm events, *Cryptosporidium* concentrations decreased. An increase in the frequency of samples that exceeded the action level (10 cysts or oocysts per sample volume) as compared with dry weather periods reinforces the important role that wet weather events play in total loading.

Relationships between fecal indicators, physicochemical factors, and pathogens

There were a larger number of significant Spearman rank correlations between studied water quality variables during wet weather events, as compared with dry weather periods. Many of these relationships were remained significant when data for both weather types were combined, and many had higher R_s values in the combined data set than in the wet weather data set. Because there were few statistically significant correlations between study variables when dry weather periods were examined separately, it appears that the storm event data are responsible for many of the significant correlations observed between study variables.

Enterococci. Enterococcus spp. typically inhabit the intestines of mammals, including the dairy cattle that inhabit New York's Catskill and Delaware watersheds (Salminen 2004). One of these species, *E. faecalis*, is found in both humans and dairy cattle and is responsible for as much as 80% of all enterococcus-related infections (Fischetti 2000). Although not spore-forming bacteria, enterococci may persist in the environment, since they are resistant to desiccation, a wide range of pH, and temperatures as high as 60°C (Fischetti 2000). During this study, they were the only indicator organism or physicochemical factor that was significantly associated with *Giardia* during both dry and wet weather periods. Both Molleda et al. (2008) and Briancesco and Bonadonna (2005) also found a significant

correlation between enterococci and *Giardia* in surface waters. Additionally, the latter detected enterococci in chlorinated swimming pools, indicating that this bacterium may survive drinking water chlorination similarly to *Cryptosporidium* and *Giardia.* The relationships observed in this study suggest that enterococci may be a potential surrogate for *Giardia.*

Particle concentration and turbidity. Particle concentrations and turbidity, which were higher during wet weather events versus dry weather periods, were significantly correlated with each other during storms. This is most likely due to the physical movement of falling precipitation as it moves across the ground and through the watershed. Previous studies have suggested that this may be because microbes associate with particles that are subsequently suspended in the water column (Characklis et al., 2005; Jeng et al., 2005). Particle concentration and turbidity each have a significant Spearman rank correlation with *E. coli*, enterococci, *C. perfringens* spores, somatic coliphages, and *Giardia* during wet weather events. Particle concentration and turbidity can each be measured in real-time or rapidly by electronic devices and with very little training, making them candidates for replacing or supplementing costly and time-consuming microbial analyses.

Giardia and Cryptosporidium. Although enterococci is the only microbial indicator that has a significant correlation with either pathogen across all data sets, the impact of storm events on total loadings highlights the importance of wet weather correlations. During these periods, *Giardia* is significantly correlated with fecal coliforms, *C. perfringens*, and somatic coliphages. *Cryptosporidium*, although not significantly correlated with any of these organisms, has a strong rank correlation ($R_s = 0.74$) with *Giardia.* This Spearman rho value

is consistent with other studies that have investigated potential surrogates for *Cryptosporidium* and *Giardia* (Graczyk et al., 1998; Rose et al., 1988).

Intrastorm sampling period. Intrastorm relationships between *Giardia* and *Cryptosporidium* are different from those found during single-sample storm events. *Giardia* is not significantly correlated with any other factor, microbial or physical, during intrastorm sampling. *Cryptosporidium* in intrastorm samples is significantly correlated with both *C. perfringens* ($R_s = 0.66$) and male-specific coliphage ($R_s = 0.81$), despite having no significant correlations with indicator microbes during either wet or dry single-sample periods. In intrastorm samples, the two pathogens are not correlated with each other ($R_s = 0.02$), despite having significant correlations with each other during single-sample events. Fecal indicator microbes had some of the same significant correlations in intrastorm samples as in wet and dry single storm samples. All but one of these pairings (enterococci with *E. coli*) have lower R_s values than during single-sample storm events. TOC is also significantly correlated with *E. coli*, enterococci, and *C. perfringens*, and it is only for these microbial variables that there were significant correlations with TOC.

A possible explanation for the differences in single-sample event results and those of the intrastorm period is seasonality. Intrastorm samples were taken during the summer months, with all four storms occurring between June 4th and July 12th, 2007, while single-sample events occurred between November 28, 2006 and May 9, 2007, which is in the winter and spring seasons. Determining the effects of seasonality on *Cryptosporidium* and *Giardia* correlations is beyond the scope of this work, but concentrations of these protozoa and other indicators that are seasonally dissimilar would affect rank correlations.

Intrastorm trends in microbial water quality

Microbial concentrations in water appeared to match stream water levels more closely at sampling site WHIP than at either site E9 or N5-1. Although quantitative data for land usage is not readily available, these differences are most likely due to differing watershed characteristics at each site. All three sampling locations are near the junction of the Kensico Reservoir and are separate small, flashy urban streams. Site WHIP, however, has a few key differences in catchment morphology. Its watershed lacks a sewer system to buffer changes in water flow, and residential septic tanks present opportunities for direct human fecal contamination. Sampling site E9 is preceded by a large natural wetland, which buffers stream flow and potentially retains a portion of the stream's suspended solid matter. Likewise, Best Management Practices (BMPs) have been implemented prior to sampling site N5-1. A man-made detention basin designed to contain a rainfall event of 1.2" (equivalent to an Average Recurrence Interval of 1 year) provides the potential for flow attenuation and the settling of solids. Since average wet weather microbial loadings for site WHIP are much higher than for the other two sites (Table 19), it should be a primary focus of efforts to reduce the public health impact of fecal contamination in the Kensico Reservoir.

"First flush" phenomenon

The streams sampled in this study had rapid increases in stream flow during precipitation events. Consequently, there were difficulties in sampling water for rising limbs that did not also include peak stream flow. Of the samples taken during the first

chronological phase of each storm, six of ten (60%) were classified as peak limbs. Five of these six (83%) showed total loadings for fecal coliforms, *E. coli*, and particles that were higher than that phase's water volume, and all six (100%) had enterococci loadings that exceeded expected values. Despite accounting for only 13% of the total stream volume across all sites and dates, these six peak limbs contained 40% of all enterococci and 37% of all fecal coliforms detected across all sampling sites and dates. Additionally, five of these six limbs (83%) were above the action level of 10 (oo)cysts per sample volume required for further *Giardia* and *Cryptosporidium* testing. The observed *Giardia* and *Cryptosporidium* levels were considerably higher than the average for all intrastorm samples (48%) and for all other samples (38%). These six limbs account for 72% of total *Giardia* loading and 73% of total *Cryptosporidium* loading of all samples above the action level.

For rising hydrographic limbs that did not include the peak of a storm, concentrations of *E. coli* and TOC were disproportionately higher than total stream volume and the mean concentrations of both *E. coli* and TOC were higher than in any other limb type. This provides evidence of a first flush for these pollutants and stresses the importance of including increasing rainfall rates in stormwater management plans. As the Kensico Reservoir watershed becomes increasingly developed, the increase in impervious surfaces has the potential to influence the water quality impacts of first flush events.

Late-phase storm loading of microbial indicators and physicochemical factors

It is interesting to note that late-phase storm samples contribute higher than average loadings for *E. coli* and *C. perfringens*, each of which was individually correlated with TOC and with particle concentration at significant levels during the intrastorm sampling period

(Table 16). Both of these organisms are considered useful specific indicators of fecal pollution thought to not replicate in the environment in temperate climates (WHO 2004). A late-phase delay in storm loading with these organisms may indicate a reservoir such as a septic tank that is dependent on reaching a critical rainfall level before overflowing, implicating a potential source of reservoir contamination that may be mitigated.

Regardless of limb type, total and mean TOC loading was very similar to stream volume for the first two chronological storm phases (Table 20). During the third and final phase, TOC spiked substantially above the mean for all phases, despite most (75%) being falling limbs. This implies that although TOC increases rapidly with stream flow, its source continues to contribute a disproportionate amount of organic carbon to these tributaries while rainfall is ending. These results may be significant because TOC also plays a significant role in the potential generation of disinfection by-products (DBPs) during the chlorination stage of New York's water treatment process.

Recoveries of *Cryptosporidium* and *Giardia*

EPA Method 1623 for measuring *Cryptosporidium* and *Giardia* uses a spiked internal standard to determine recovery efficiency. This internal standard attempts to account for the losses inherent in the filtration, transfers, and immunomagnetic separation steps of the method, as well as errors and inconsistencies among laboratory analysts and equipment. Mean recoveries in this study (*Cryptosporidium* = 16%, *Giardia* = 18%) were on the lower end of the acceptable range of values (*Cryptosporidium* = 13-111%, *Giardia* = 15-118%), as designated in the Method. Recoveries for positive biological controls (OPR) were higher

(*Cryptosporidium* = 19%, *Giardia* = 24%) and within the Method's acceptance criteria (*Cryptosporidium* = 11-100%, *Giardia* = 14-100%).

Past attempts at studying *Cryptosporidium* and *Giardia* in water have achieved similar recoveries for spiked samples as observed here, particularly when using EPA Method 1623 (McCuin and Clancy 2003). There are potential water constituents that can affect the recoveries of EPA Method 1623. DiGiorgio et al. (2002) examined various turbidity levels, finding that increasing sample turbidity from 20 nephelometric turbidity units (NTU) to 36 NTU decreased the mean recovery of *Giardia* cysts from 46% to 2.6%. *Cryptosporidium* recovery showed less of an effect from increased turbidity, with mean values falling below 50% at only the highest turbidity level of 99 NTU. Hu et al. (2004) found that a moderate amount of turbidity (10 NTU) increased mean *Cryptosporidium* recovery versus a tap water control (0.2 NTU) from 18.1% to 86.2%. Given the size differences of *Giardia* (11-14 μm) and *Cryptosporidium* (4-6 μm), these studies suggest that the filtration step of EPA Method 1623 may not capture *Cryptosporidium* oocysts as well as it does *Giardia* cysts, and that particles in water samples may help retain oocysts on filters to some degree. There appears to be a point of diminishing returns with respect to turbidity effects on *Cryptosporidium* recovery; higher numbers of large particles make filtration more difficult by clogging filters (DiGiorgio et al. 2002) and make it more difficult to identify (oo)cysts among other debris on microscopy slides.

Another potential vulnerability is in the IMS procedure, which relies on the attachment of antibody-coated magnetic beads to *Cryptosporidium* and *Giardia* cell walls. This attachment is based partly on ionic charge, which makes IMS susceptible to charged particles (Dynal Biotech ASA 2003) and ions in the water sample (Yakub and Stadterman-

Knauer 2000). The manufacturer also specifies that the amount of visible solid material must be less than 0.5mL per sample, as physical collisions with particulate matter can dissociate (oo)cysts from magnetic beads.

As in these previous studies, the recovery of both organisms subjected only to the IMS procedure was higher than those that had been analyzed using filtration followed by IMS. During this study, mean recovery values increased for both distilled water spiked samples and IMS positive controls during the analysis of intrastorm samples. The distribution of these recoveries was narrower as well (Figure 4), indicating an increase in laboratory analyst proficiency and precision. Average recovery for *Cryptosporidium* and *Giardia* spiked internal standards remained unchanged across all sampled weather events (*Cryptosporidium* = 16%, *Giardia* = 18%).

Effects of recovery correction on the precision of EPA Method 1623

During this study, *Cryptosporidium* and *Giardia* recoveries were corrected based on the recovery of the internal standard (ColorSeed). This correction attempts to account for losses that occur due to analyst imprecision, water constituents, and any shortcomings of EPA Method 1623 itself. The major assumption in correcting for recoveries is that environmental microbes will have properties that are similar or identical to the internal standard itself, a theory that has a number of potential flaws. *Cryptosporidium* and *Giardia* biological controls are grown, counted, and packaged under controlled conditions, and even variances in manufacturing methods can damage control microbes (BTF Pty Inc. 2008). Nonetheless, Warnecke et al. (2003) demonstrated that ColorSeed controls are resistant to a number of chemical challenges and that the fluorescent labeling system was robust.

Environmental *Cryptosporidium* and *Giardia* are exposed to a range of factors that can affect their viability and detection, including temperature, pH, and physical damage. They also originate in a variety of host species and have a range of genotypes, so it is reasonable to assume that environmental conditions can alter the recovery of *Cryptosporidium* and *Giardia* by EPA Method 1623 in ways that are not yet understood.

After undergoing EPA Method 1623, ColorSeed (oo)cysts glow both green (FITC) and red (Texas Red) under fluorescent microscopy, while wild-type (oo)cysts appear only green. In experimental testing, the FITC dye also attaches to other particles and organisms present in environmental samples, and wild-type (oo)cysts vary more in appearance than ColorSeed (oo)cysts from a manufacturing facility. Because they are labeled twice, it is easier to verify the presence and identities of ColorSeed (oo)cysts than it is wild-type (oo)cysts. This may result in counting a higher percentage of ColorSeed (oo)cysts than the percentage of wild-type (oo)cysts, effectively underestimating the number of wild-type (oo)cysts present in environmental samples. Further complications may have resulted from manufacturer-reported genetic drift in the ColorSeed organisms used in this study, but it is impossible to determine the magnitude of this effect.

Assuming that ColorSeed and wild-type (oo)cysts react similarly to EPA Method 1623, low recoveries decrease the certainties of the corrected counts for two reasons. First, larger corrections magnify potential analyst errors more so than smaller corrections. In other words, each wild-type (oo)cyst missed while visually examining a slide will underestimate the total count by (100/R), where R is the recovery percentage of ColorSeed in that sample. Additional imprecision comes from only being able to count individual (oo)cysts. For example, if there are 22 wild-type *Giardia* cysts in an analyzed sample, and the recovery

percentage of ColorSeed is 10% (10/100 spiked cysts) for the total method, there should theoretically be 2.2 cysts on the examined microscopy slide. The analyst would then count either 2 or 3 cysts, depending on the effect of randomness on the method. The sample would then either have a corrected count of either 20 or 30 cysts, underestimating or overestimating the true value, respectively. The magnitude of this imprecision is dependent on the actual number of wild-type (oo)cysts in the original sample and is demonstrated in Figure 5. As the percentage recovery approaches zero, the likelihood of overestimating the number of wild-type (oo)cysts in a sample increases, especially with lower actual counts. Since many studies have found concentrations of *Cryptosporidium* and *Giardia* that are below 5 (oo)cysts per liter (Table 21), it is necessary to collect large water samples to reduce this effect. For samples with greater than 100 (oo)cysts per total analyzed volume, this effect is virtually eliminated above recovery values of approximately 10% (Figure 6). Gaps in the graph lines indicate points where there is no difference between theoretical overestimation and underestimation. These gaps occur at intervals of (100/N), where N is the number of (oo)cysts present in the sample. As N increases, the frequency of these gaps increases.

Study	Water source	*Cryptosporidium*	*Giardia*

		% positive	Mean conc. (oocysts/L)	% positive	Mean conc. (cysts/L)
Keeley et al. 2008	Reservoir	99%	-	87%	-
Shields et al. 2008	Swimming pools	1.2%	-	6.2%	-
Touron et al. 2007	Estuary	81%	2.7-12[a]	54%	1.3-3.0[a]
Carmena et al. 2006	River	63.5%	1.132	92.3%	65.22
	Reservoir	33%	0.176	55.5%	0.633
Harwood et al. 2005	Pre-treatment	74%		100%	-
Boyer et al. 2003	Karst	53%	3.5-156.8[a]	-	-
Kistemann et al. 2002	Streams	-	0.013-0.1705[ab]	-	0-0.124[ab]
Robertson and Gjude 2001	Raw surface water	16%	1	11.5%	1
Hansen and Ongerth 2001	River	97%	0.2-18.2[a]	-	-
Hsu et al. 2000	Surface waters	46.2%	0.221	46.2%	0.795
Payment at al. 2000	Pre-treatment	-	0.04-2.72[a]	-	0.04-14.38[a]
LeChevallier et al. 1991	Pre-treatment	87%	2.70	81%	2.77
This study,above action level only	Reservoir tributaries	40%	6.20	40%	5.02
This study, uncorrected NYCDEP values	Reservoir tributaries	51.4%	0.167	94.3%	1.19

Table 21. Prevalence and mean concentrations of*Cryptosporidium* and *Giardia* found in previous studies.

[a]Range of mean values from different sampling locations.

[b]Median values.

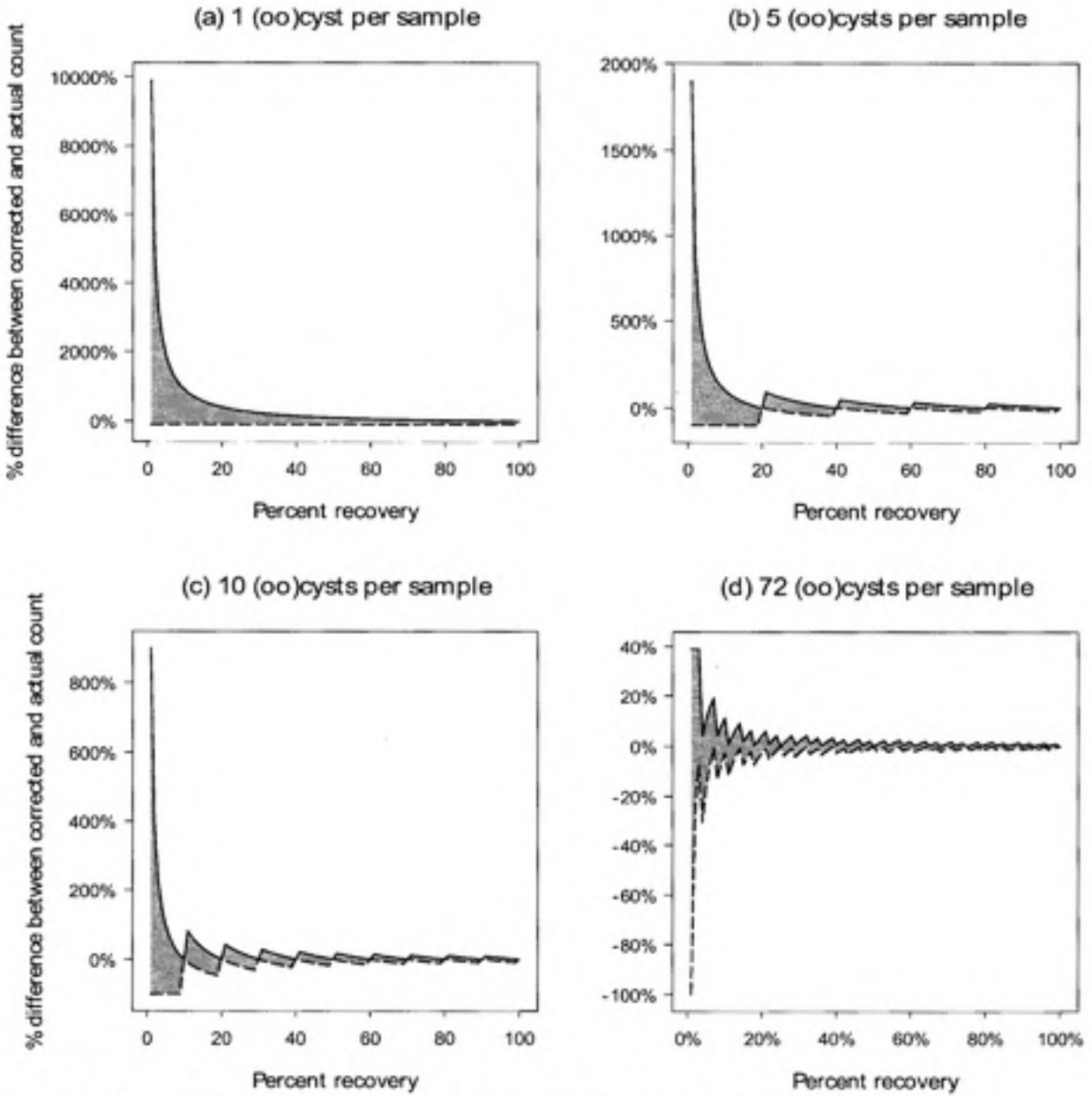


Figure 5. Theoretical errors in EPA Method 1623 due to rounding and recovery correction at various sample (oo)cysts levels. The top (solid) line represents the overestimation of the method in terms of percent difference from the actual (oo)cyst count per tested sample; the lower (dashed) line represents underestimation.

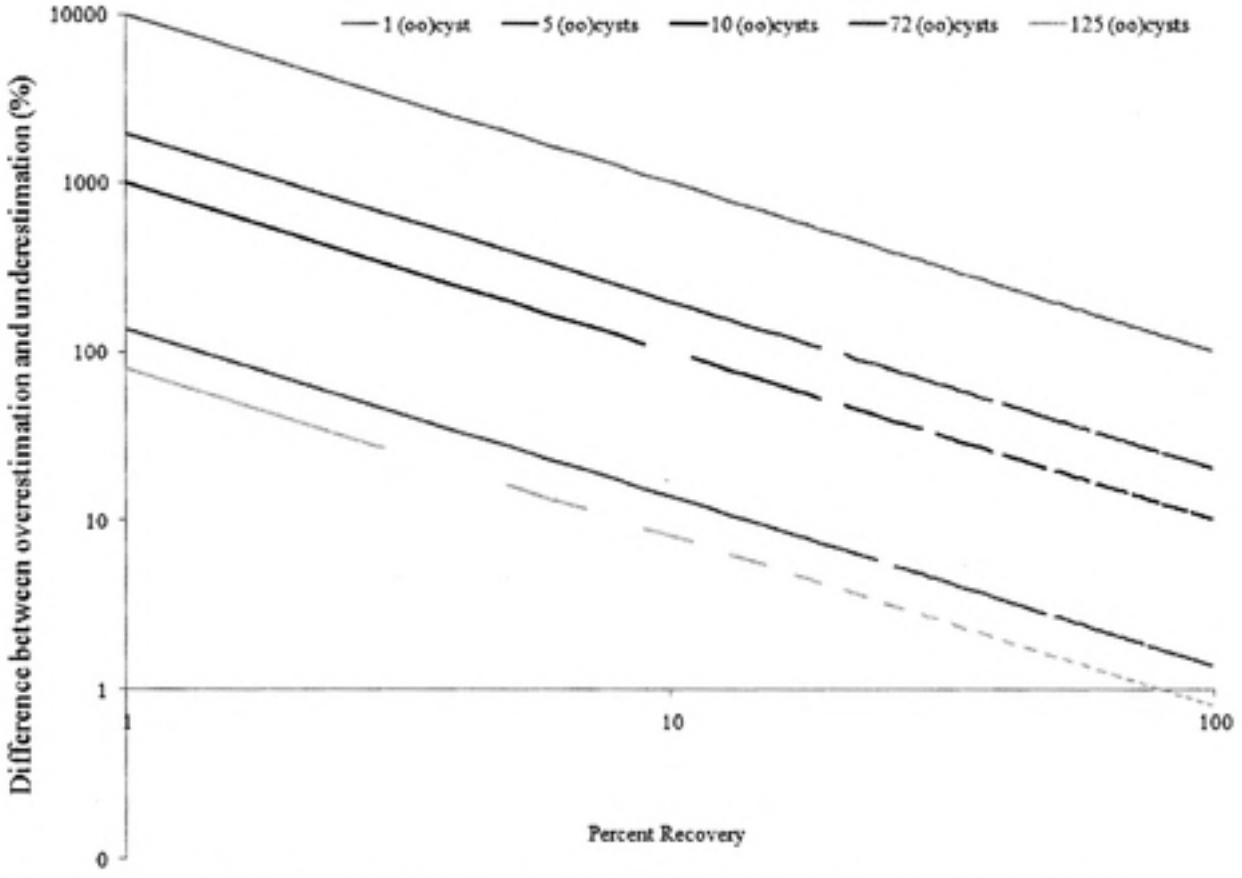


Figure 6. Difference in theoretical overestimation and underestimation at various percent recoveries and actual sample (oo)cyst levels. Gaps in the lines are due to percent differences of 0% and results being displayed on axes that are logarithmic. Similarly, there are no graph points at 100% recovery, as there is no over- or underestimation at any (oo)cyst level and zero values cannot be displayed on a logarithmic graph.

CONCLUSIONS

The conclusions below are presented in terms of study objectives:

Examine the relationship between the occurrence and concentrations of *Cryptosporidium*, *Giardia* and fecal indicator microbes in waters from tributaries of the Kensico Reservoir.

- Fecal indicator microbes were ubiquitous in Kensico tributaries, with prevalences ranging from 57% (male-specific coliphages) to 100% (fecal coliforms, E. coli, and enterococci) of samples. Ninety-four percent (94%) of samples were positive for Giardia cysts and 51% were positive for Cryptosporidium oocysts, with 44% of all samples testing above the action level of 10 (oo)cysts per sample volume.

- The concentration of each of the vegetative enteric bacteria (fecal coliforms, *E. coli*, enterococci, and *C. perfringens*) was significantly pairwise correlated with the others, as well as with particle concentration, and turbidity at the $\alpha = 0.05$ level. The two types of coliphages were only significantly correlated with each other.

- Concentrations of *Giardia* cysts were significantly correlated with turbidity levels, as well as concentrations of *Cryptosporidium* oocysts, fecal coliforms, enterococci, *C. perfringens*, and particles. There was no significant correlation between the concentration of *Cryptosporidium* oocysts and any of the measured variables, with the exception of *Giardia* cysts.

- Samples taken during the intrastorm sampling period were significantly higher than those taken during single-sample wet weather periods for concentrations of fecal coliforms, *E. coli*, enterococci, somatic coliphages, and particles; *Cryptosporidium* concentrations were significantly lower. This may be due to seasonality, as intrastorm samples were taken during summer months, while the single-sample period spanned autumn, winter, and spring seasons. Potential confounders associated with seasonality in the Kensico watershed include temperature, differential rates of rainfall and snow melting, and changes in agricultural practices.

Determine if the relationships between *Cryptosporidium, Giardia* and fecal indicator microbes in these waters are different during dry and wet weather periods.

- Associations between measured variables are different during wet weather periods and dry weather periods, with more associations being significant during wet weather periods. These associations influence the correlation values of the total combined data set, although this study was limited by few dry weather samples that were positive for protozoa. Since wet weather periods contribute substantially higher loadings of potentially harmful organisms, it is worthwhile to focus microbial reduction efforts on periods of heavy rainfall.

- While there are few significant correlations during dry weather periods, the correlation between *Giardia* concentration and enterococci concentration is

significant across all data sets. It is likely that more associations would emerge with a larger dry weather data set. There are no significant correlations for *Cryptosporidium* during dry weather periods, but temperature has a significant negative correlation with *Cryptosporidium* during wet weather events.

Determine how physicochemical factors, concentrations of fecal indicator microbes, and concentrations of *Cryptosporidium* and *Giardia* in these waters change over the course of individual storm events

- Samples taken at the peak of a storm's flow and those that occur at the beginning of a storm event are each responsible for disproportionate microbial loadings. Peak limb samples contribute average loading values for microbial indicators that are significantly higher than rising or falling limb samples, even when corrected for stream flow volume. For peak hydrographical limbs on average, particle concentration was more than double the average of the other limb types. Total organic carbon was 75% greater than the average during rising limbs.

- Despite containing an average stream volume fraction 31% below the mean for all chronological phases, samples taken during the first phase of a storm contributed average loadings of fecal coliforms and enterococci that were higher than the average for all phases (47% and 59% higher, respectively). This occurred regardless of each phase's hydrographical limb type (rising, peak, or falling).

- Disproportionately high average loadings of *E. coli* (52% above the mean), *C. perfringens* (139%), particles (78%), and TOC (81%) occurred during later storm phases, indicating that it may take time for these factors to be mobilized in the Kensico watershed.

- Of the three tributaries sampled during the intrastorm period, site WHIP contributed the largest average stream volume fraction, as well as loadings for all fecal indicators and physicochemical factors that were above the average for all sites. However, these all of these loadings are lower than expected when average stream volume fraction is considered. Despite having an average stream water fraction that is 55% below the average for all sites, sampling site N5-1 had an average enterococci loading that was 9% above average. For site E9, average total organic carbon and *E. coli* loadings were above the average for all sites (+54% and +13%, respectively) despite an average stream volume fraction that was below the mean for all sites (-10%).

Determine if fecal indicator bacteria or physicochemical measurements can be used to predict the presence of protozoan parasites in the watershed that supplies the New York City drinking water system.

- Concentrations of both enterococci and particles are significantly correlated with *Giardia* concentrations during wet weather periods. *Giardia* concentration, as measured by the NYCDEP laboratory, has a significant correlation with these measurements when samples are above the action level of 10 (oo)cysts per sample.

Although the development of a predictive model is beyond the scope of this work, it may be useful to measure enterococci and/or particle concentration when the assay for protozoa is not practical.

- Lower temperatures may be predictive of higher *Cryptosporidium* concentrations, although seasonality may be a confounder. There was not enough pathogen data in this study to reliably determine the relationships between *Cryptosporidium* and other variables, as it was detected in 51% of samples and at low levels.

RECOMMENDATIONS

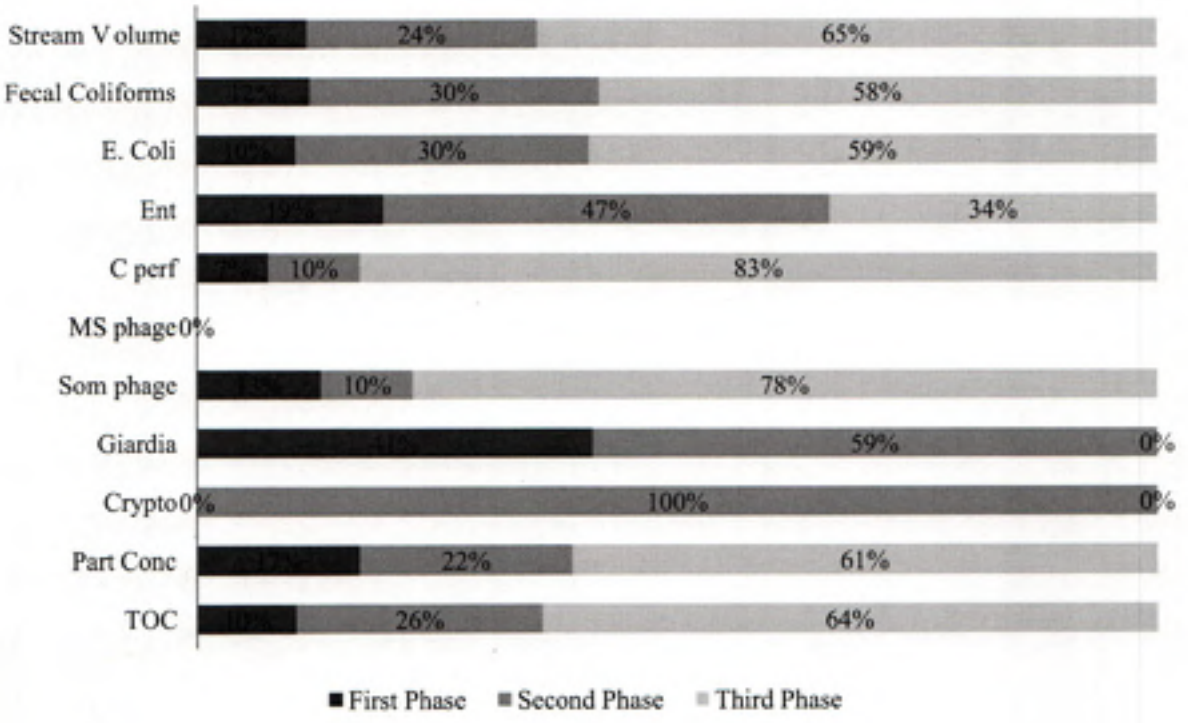
- Limited pathogen data prevented a substantive analysis of intrastorm trends for *Cryptosporidium* and *Giardia*. This was due to a combination of low concentrations, relatively low prevalence, bad luck, and inconsistent sampling technique. The latter factor should be carefully controlled to provide quality data.

- Future studies should better account for land use and antecedent rainfall amounts or dry periods. These are potentially confounding factors that could alter the conclusions of this study.

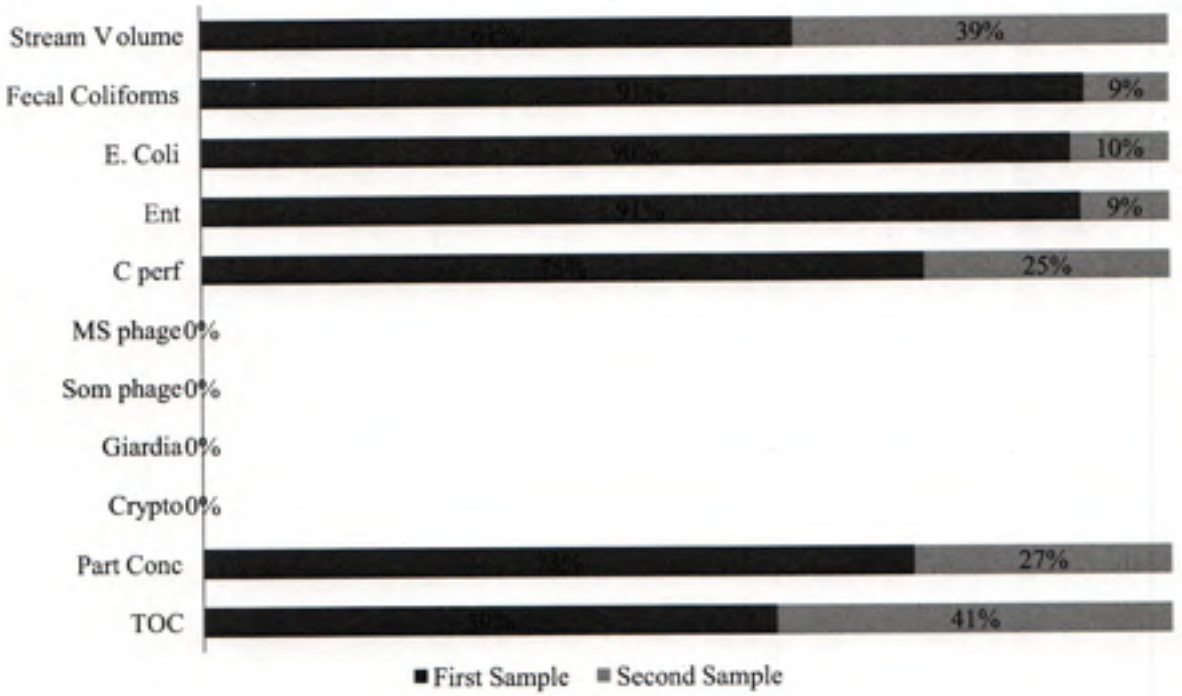
- Although only *Cryptosporidium* and *Giardia* were analyzed at both UNC and NYCDEP, substantial differences were found between laboratories. Any attempt at correlating these pathogens with other potential surrogates should be both watershed- and laboratory-specific for higher reliability.

APPENDIX A: Relative contributions of each chronological intrastorm phase to total per-storm loading of fecal indicator microbes, physicochemical factors, and protozoan parasites

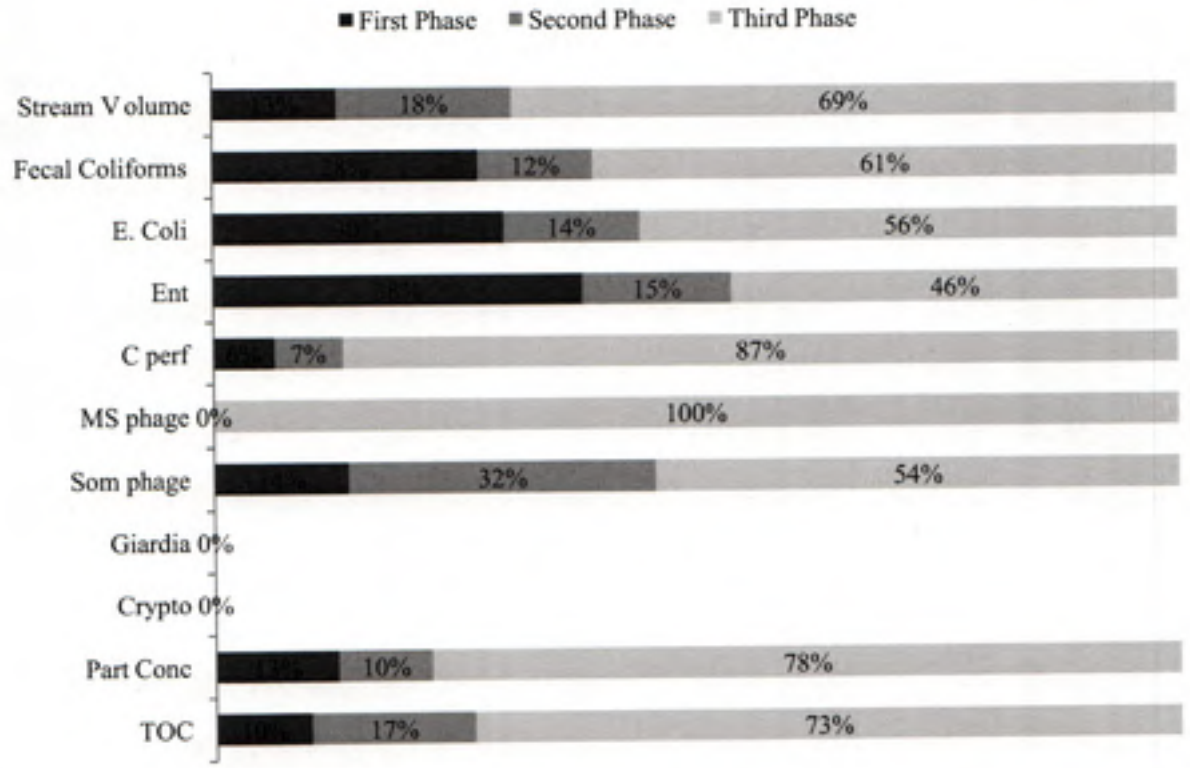
(a) Site E9, Storm 1

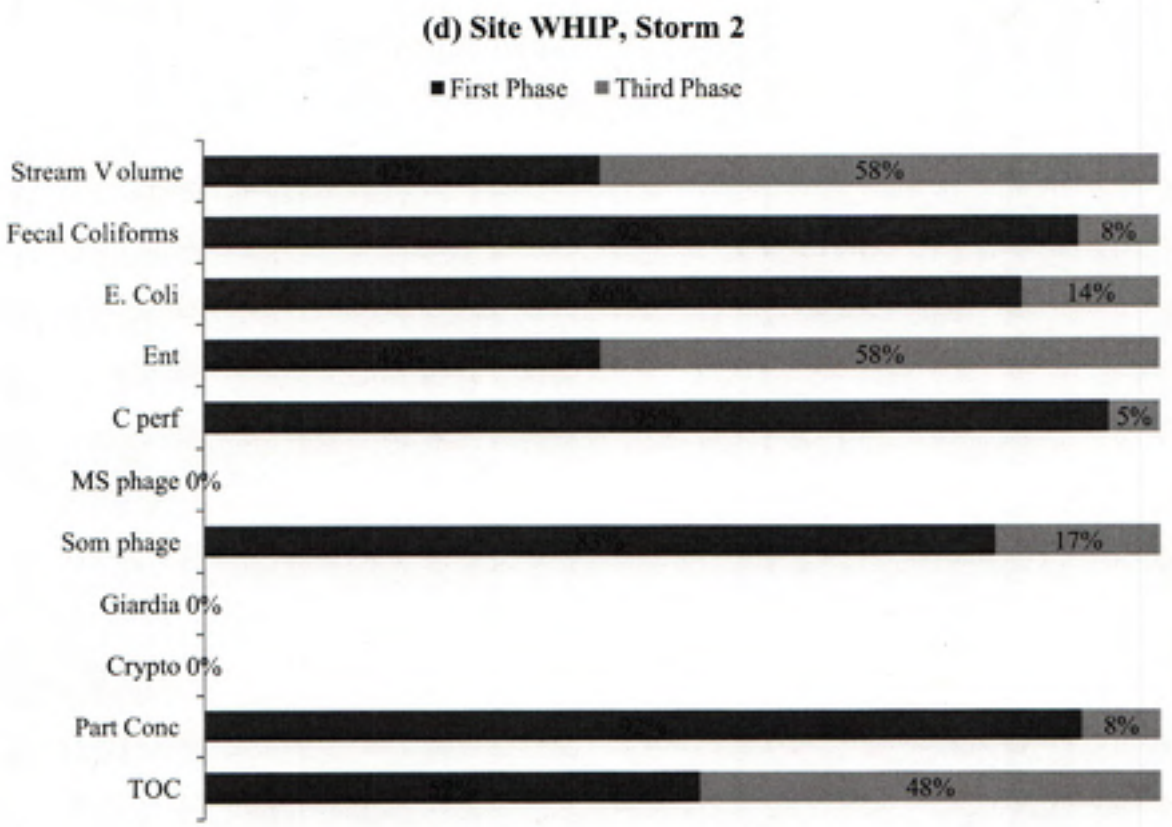


(b) Site E9, Storm 3

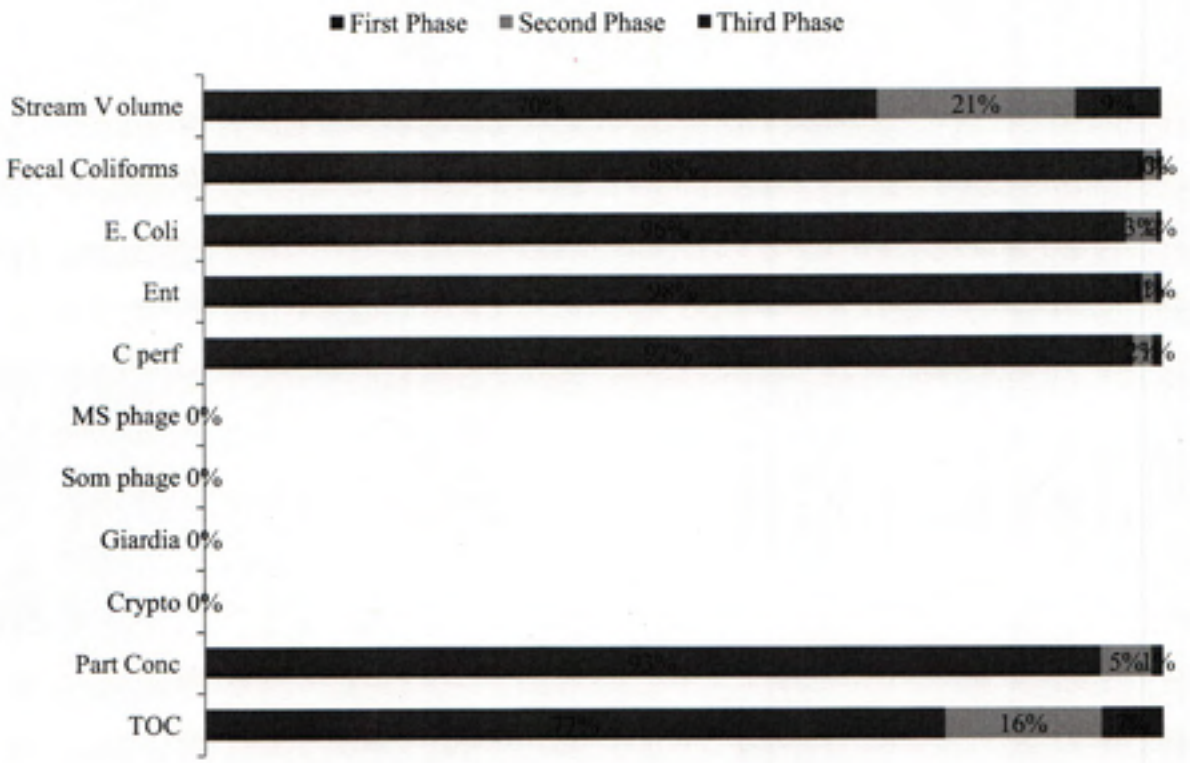


(c) Site WHIP, Storm 1

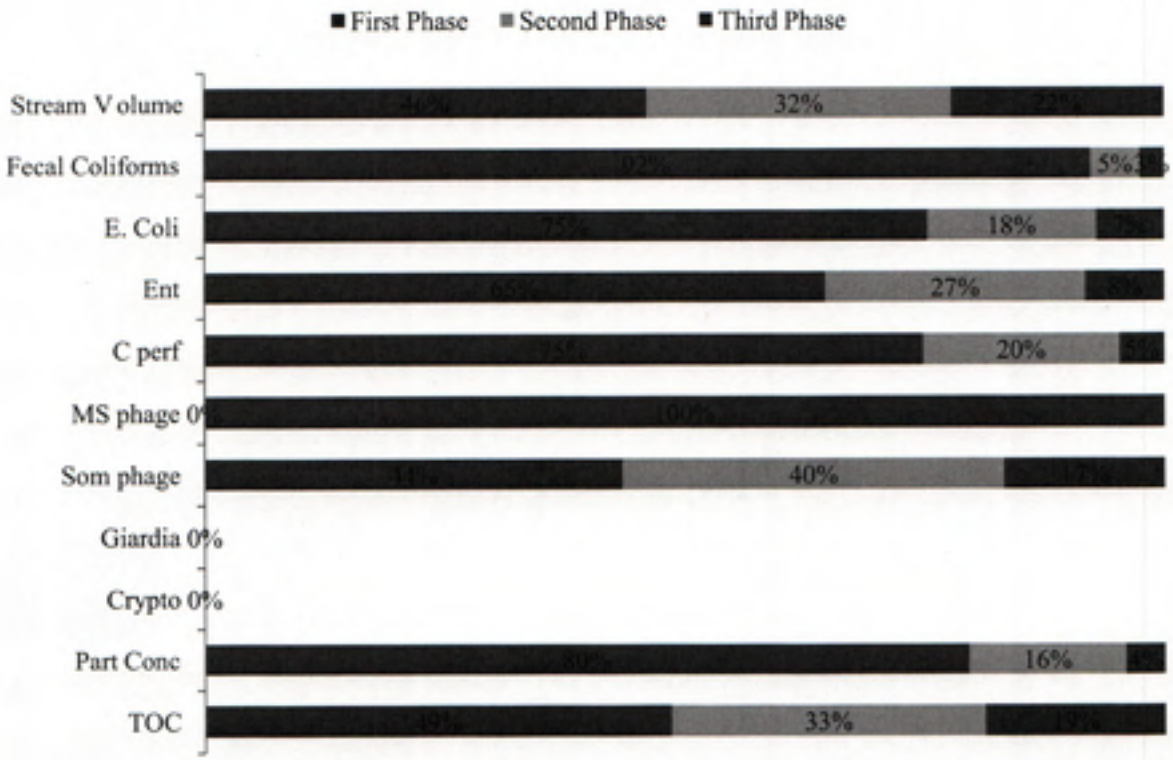



(d) Site WHIP, Storm 2
First Phase
Third Phase
Stream Volume
58%
Fecal Coliforms
8%
E. Coli
14%
Ent
58%
C perf
5%
MS phage 0%
Som phage
17%
Giardia 0%
Crypto 0%
Part Conc
8%
TOC
48%

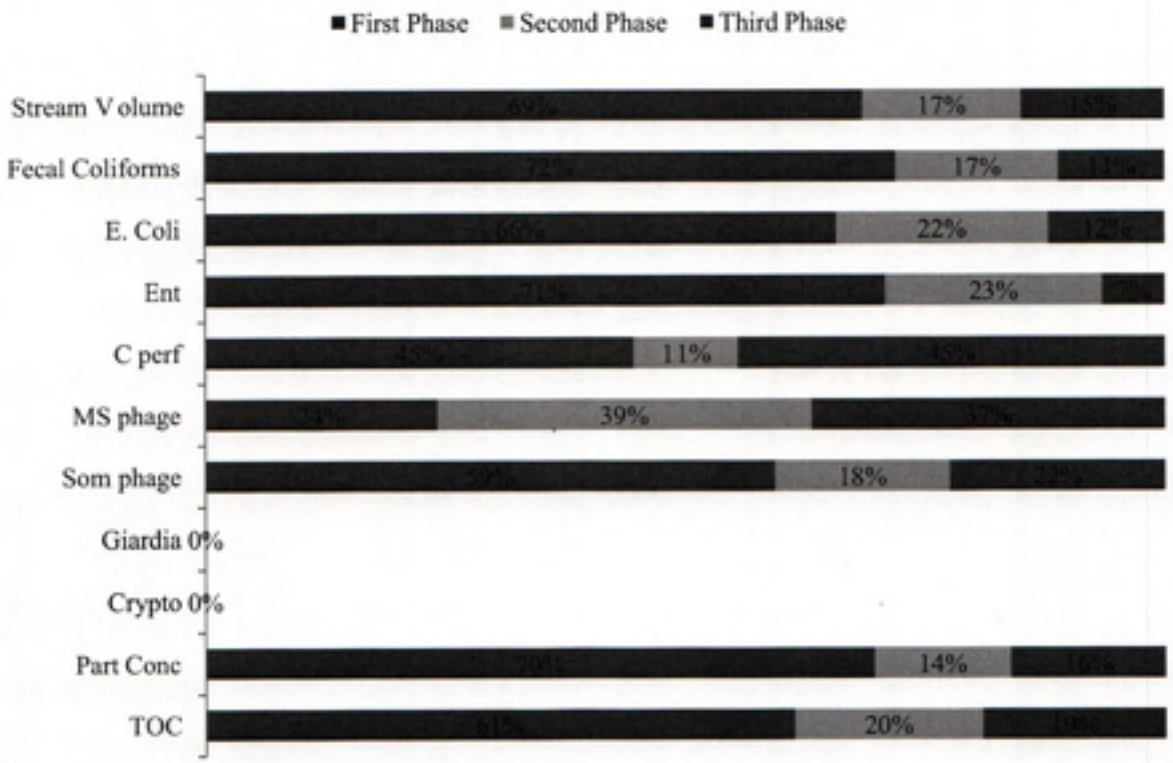
(e) Site WHIP, Storm 3



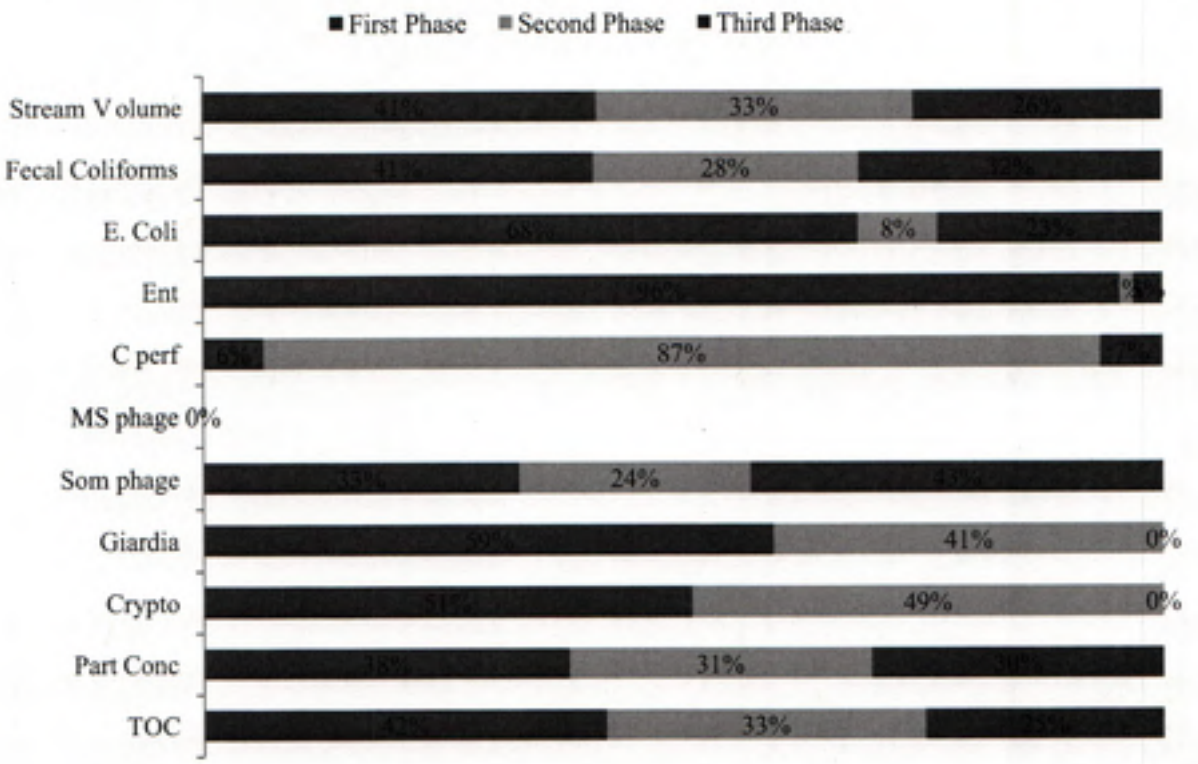
(f) Site WHIP, Storm 4



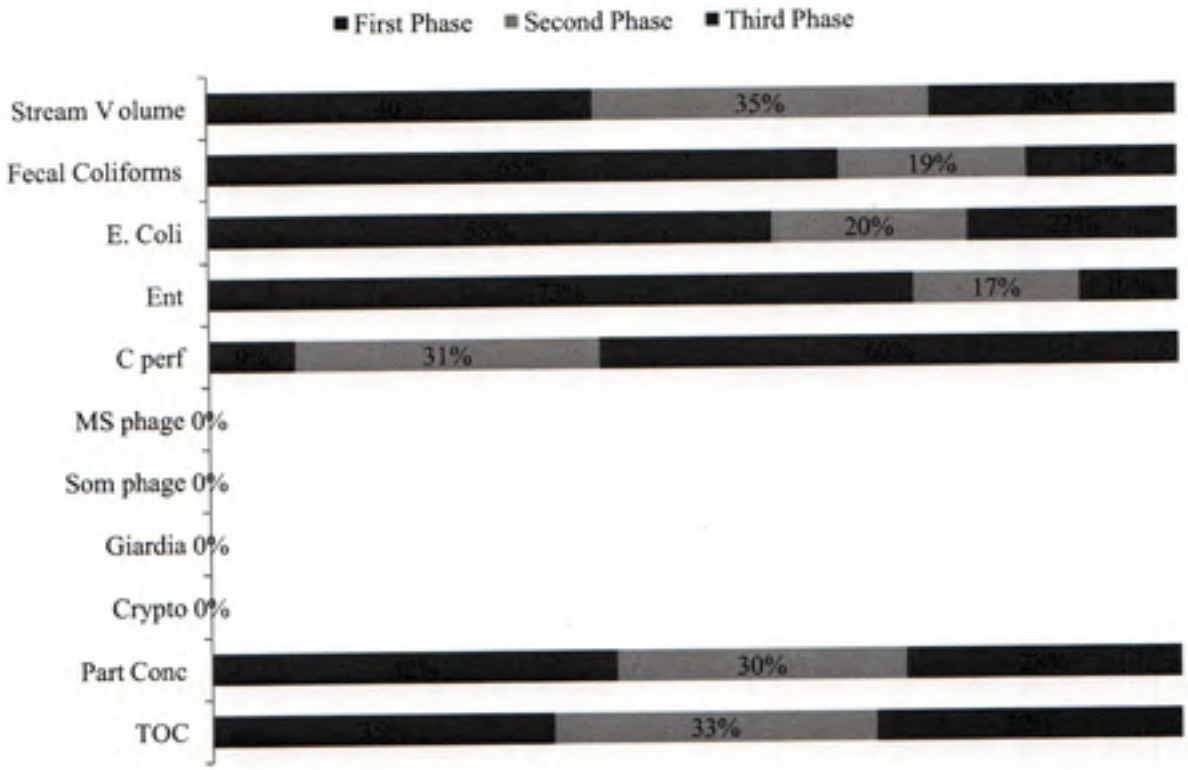
(g) Site N5-1, Storm 1



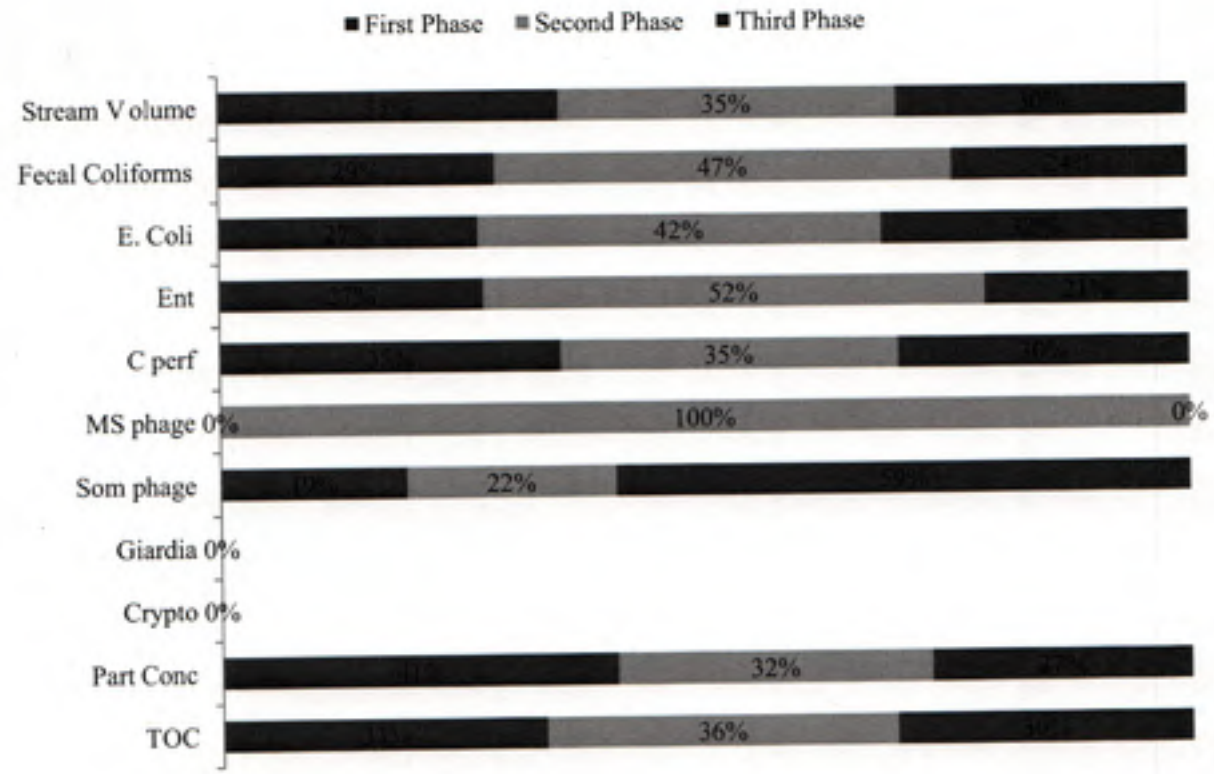
(h) Site N5-1, Storm 2



(i) Site N5-1, Storm 3



(j) Site N5-1, Storm 4



APPENDIX B: Complete data set for all single-sample events

Site	Date	Weather type	FC (MPN / 100mL)	E. coli (MPN / 100 mL)	Ent (MPN / 100mL)	*C. perf* (MPN / 100mL)	M-S Phage (PFU / 100 mL)	Som. Phage (PFU / 100	*Giardia* (# / L)	*Crypto* (# / L)	Particles (# 1000 / 100mL)	TOC (mg/L)
E9	11/28/2006	Dry	10.1	28.3	41.6	9.2	0.0	15.0	bt	bt	860.7	6.7
	12/13/2006	Wet	100.0	100.0	138.1	9.2	0.0	2.0	5.0	5.0	1790.7	4.9
	3/1/2007	Wet	286.8	237.6	524.4	240.0	1.0	3.0	12.5	9.1	5619.3	4.9
	4/10/2007	Dry	3.1	1.0	10.1	93.0	2.0	4.0	bt	bt	2003.3	4.2
	4/12/2007	Wet	100.9	100.9	132.3	460.0	11.0	8.0	bt	bt	2718.7	5.0
	4/16/2007	Wet	1621.5	203.7	308.5	75.0	0.0	4.0	bt	bt	6308.0	4.1
	5/9/2007	Dry	945.1	121.9	63.0	43.0	2.0	10.0	8.0	8.6	1596.0	5.4
E11	11/28/2006	Dry	52.5	18.8	20.4	<3.0	0.0	14.0	bt	bt	533.3	3.8
	12/13/2006	Wet	100.9	100.9	308.5	9.2	0.0	0.0	1.0	2.0	563.3	4.1
	3/1/2007	Wet	357.6	44.2	1074.4	240.0	0.0	2.0	13.2	13.3	12004.0	5.4
	4/10/2007	Dry	30.9	30.9	3.0	43.0	1.0	6.0	1.1	2.8	801.3	3.2
	4/12/2007	Wet	974.8	974.8	630.0	150.0	3.0	4.0	bt	bt	3548.7	3.7
	4/16/2007	Wet	8158.4	8158.4	3277.1	240.0	517.0	28.0	14.4	6.2	8499.3	5.8
	5/9/2007	Dry	123.1	123.1	12.0	<3.0	19.0	12.0	1.3	6.3	2532.7	3.6
WHIP	11/28/2006	Dry	20.2	10.1	20.4	<3.0	0.0	1.0	bt	bt	712.0	2.3
	12/13/2006	Wet	100.9	100.9	109.5	9.2	0.0	2.0	4.0	1.0	495.3	2.0
	3/1/2007	Wet	528.2	377.8	742.5	460.0	0.0	4.0	12.5	17.5	17152.0	3.3
	4/10/2007	Dry	20.2	20.2	2.0	23.0	2.0	15.0	bt	bt	356.0	1.6
	4/16/2007	Wet	591.2	237.6	635.7	460.0	1.0	4.0	10.3	7.6	15894.0	4.0
	5/9/2007	Dry	30.9	30.9	10.1	3.6	5.0	20.0	bt	bt	450.0	1.7
MB-1	11/28/2006	Dry	52.5	20.4	73.6	92.0	0.0	2.0	10.7	11.5	481.3	2.4
	12/13/2006	Wet	100.9	100.9	100.9	3.6	0.0	0.0	2.0	5.0	571.3	2.1
	3/1/2007	Wet	792.6	702.7	2893.9	460.0	8.0	8.0	bt	bt	14847.3	3.3
	4/10/2007	Dry	109.6	97.6	10.1	240.0	1.0	14.0	bt	bt	614.7	2.0
	4/12/2007	Wet	1344.4	1217.4	519.8	>1100	0.0	0.0	bt	bt	7642.7	3.7
	4/16/2007	Wet	2267.0	411.8	2317.5	460.0	46.0	91.0	bt	3.3	17554.0	3.5
	5/9/2007	Dry	19.0	6.4	10.0	43.0	10.0	6.0	bt	bt	1989.3	2.3
N5-1	11/28/2006	Dry	244.4	121.9	369.8	230.0	0.0	0.0	bt	bt	1216.0	2.4
	12/13/2006	Wet	1758.4	203.7	203.7	<3.0	0.0	0.0	bt	bt	664.7	1.2
	3/1/2007	Wet	1336.9	1118.6	2893.9	240.0	1.0	13.0	bt	bt	21630.0	3.9
	4/10/2007	Dry	85.8	85.8	10.1	460.0	4.0	16.0	bt	bt	792.7	1.7
	4/12/2007	Wet	415.4	308.5	2571.4	>1100	0.0	5.0	bt	bt	13262.7	2.5
	4/16/2007	Wet	308.5	308.5	2021.1	1100.0	3.0	16.0	bt	2.9	35172.7	3.6
	5/9/2007	Dry	10.1	32.4	16.1	75.0	2.0	4.0	bt	bt	1810.7	1.5

"bt" denotes samples that were not tested, as they were below the action level of 10 (oo)cysts per sample volume.

APPENDIX C: Complete data set for all intrastorm sampling events

Site and phase	Date	FC (MPN / 100mL)	*E. coli* (MPN / 100 mL)	Enterococci (MPN / 100mL)	*C. perf* (MPN / 100mL)	MS phage (PFU / 100 mL)	Som phage (PFU / 100 mL)	*Giardia* (#/L)	*Crypto* (#/L)	Particles (#1000/100mL)	TOC (mg/L)
E9 A	6/4/2007	9462.5	6656.8	3100.0	120.0	0.0	321.0	5.4	0.0	6496.0	7.2
E9 B	6/4/2007	11643.6	9491.1	3605.1	75.0	0.0	115.0	3.7	2.4	4119.3	8.6
E9 C	6/4/2007	8299.9	6800.3	974.8	240.0	0.0	345.0	bt	bt	4182.7	8.0
N5-1 A	6/4/2007	22121.7	8993.8	16001.1	240.0	1.0	145.0	12.4	7.5	8418.0	3.5
N5-1 B	6/4/2007	21537.2	12541.7	21055.2	240.0	6.7	184.0	bt	bt	7054.7	4.7
N5-1 C	6/4/2007	15644.4	7608.5	6789.4	1100.0	7.0	252.0	2.9	3.5	8988.0	5.1
WHIP A	6/4/2007	21817.2	15805.5	12243.4	93.0	0.0	144.0	bt	bt	6786.0	3.5
WHIP B	6/4/2007	6730.0	5287.3	3511.8	75.0	0.0	234.7	bt	bt	3681.3	4.2
WHIP C	6/4/2007	8966.5	5462.1	2762.2	240.0	7.0	105.0	2.5	7.6	7738.7	4.8
N5-1 A	6/28/2007	4106.4	1357.8	3994.3	14.0	0.0	204.0	6.9	2.1	6922.7	3.3
N5-1 B	6/28/2007	3460.1	203.7	71.2	240.0	0.0	186.0	5.9	2.5	7101.3	3.3
N5-1 C	6/28/2007	5038.8	735.8	203.7	23.0	0.0	421.0	0.0	0.0	8752.7	3.1
WHIP A	6/28/2007	7965.0	865.4	100.9	75.0	0.0	68.0	4.5	1.8	7692.7	3.3
WHIP B	6/28/2007	524.4	103.1	100.9	<3.0	0.0	10.0	bt	bt	490.7	2.2
WHIP C	6/28/2007										
E9 A	7/5/2007	13194.7	5454.1	2571.4	43.0	ee	ee	4.0	1.3	5304.0	6.2
E9 B	7/5/2007	1999.7	974.8	411.8	23.0	ee	ee	bt	bt	3024.7	6.7
E9 C	7/5/2007										
N5-1 A	7/5/2007	12067.9	2762.2	4974.7	23.0	ee	ee	4.4	0.6	11641.3	3.9
N5-1 B	7/5/2007	4143.6	1094.7	1344.4	93.0	ee	ee	bt	bt	9506.0	4.2
N5-1 C	7/5/2007	4481.0	1605.0	1084.5	240.0	ee	ee	bt	bt	12333.3	5.4
WHIP A	7/5/2007	24874.4	4573.0	6800.3	43.0	ee	ee	6.3	0.5	4116.7	4.5
WHIP B	7/5/2007	1217.4	519.8	308.5	<3.0	ee	ee	bt	bt	807.3	3.2
WHIP C	7/5/2007	857.4	203.7	408.2	3.6	ee	ee	bt	bt	410.7	2.9
N5-1 A	7/12/2007	37382.1	1473.1	1473.1	460.0	0.0	105.0	bt	bt	9949.3	3.8
N5-1 B	7/12/2007	62242.2	2317.5	2827.3	460.0	1.0	120.0	bt	bt	7979.3	4.1
N5-1 C	7/12/2007	37382.1	2043.1	1329.9	460.0	0.0	380.0	bt	bt	7664.0	4.0
WHIP A	7/12/2007	278145.4	3236.6	3197.2	23.0	0.0	75.0	bt	bt	3434.0	3.6
WHIP B	7/12/2007	22679.2	1105.2	1958.2	9.2	0.0	100.0	bt	bt	1032.7	3.5
WHIP C	7/12/2007	16001.1	635.7	849.6	<3.0	1.0	60.0	bt	bt	368.7	2.9

bt = below action level of 10 (oo)cysts per sample volume; ee = experimental error; A,B,C = chronological phases 1, 2, and 3

APPENDIX D: Performance and recovery data for single-sample *Cryptosporidium* and *Giardia* testing of samples above the action level

Site E9													
			Filtration			ColorSeed				Wild Type			
Date	Sample Type / Organism		Initial Volume (L)	Pellet Vol (mL)	Vol Subj to IMS (mL)	Lot Num	Claimed Num	Count Num	Recovery %	Raw Count	Corrected Count	Corrected Count per L	
12/13/07	raw	*Crypto*	8	0.5	0.5	88	100	28	28%	12	43	5.4	
		Giardia					100	18	18%	7	39	4.9	
	IMS +	*Crypto*	NA			88	100	84	84%	NA			
		Giardia					100	22	22%				
	IMS-	*Crypto*	NA			NA				0	NA	NA	
		Giardia								0			
	OPR	*Crypto*											
		Giardia											
	blank	*Crypto*											
		Giardia											
03/01/07	raw	*Crypto*	8	1	0.5	88	100	4	4%	4	100	6.3	
		Giardia					100	11	11%	8	73	4.5	
	IMS +	*Crypto*	NA			88	100	44	44%	NA			
		Giardia					100	31	31%				
	IMS-	*Crypto*	NA			NA				0	NA	NA	
		Giardia								0			
	OPR	*Crypto*											
		Giardia											
	blank	*Crypto*											
		Giardia											
05/15/07	raw	*Crypto*	7	0.75	0.75	B94	99	28	28%	17	60	8.6	
		Giardia					101	38	38%	9	24	3.4	
	IMS +	*Crypto*	NA			B94	99	12	12%	NA			
		Giardia					101	11	11%				
	IMS-	*Crypto*	NA			NA				0	NA	NA	
		Giardia								0			
	OPR	*Crypto*	7	-0.5	-0.5		99	17	17%	0	0	0.0	
		Giardia					101	16	16%	0	0	0.0	
	blank	*Crypto*	7	-0.5	-0.5	NA				0	NA	NA	
		Giardia								0	NA	NA	

Site E11

			Filtration			ColorSeed				Wild Type		
Date	Sample Type / Organism		Initial Volume (L)	Pellet Vol (mL)	mL Subj to IMS	Lot Num	Claimed Num	Count Num	Recovery %	Raw Count	Corrected Count	Corrected Count per L
12/13/07	raw	*Crypto*	10	0.5	0.5	88	100	74	74%	14	19	1.9
		Giardia					100	16	16%	2	13	1.3
	IMS+	*Crypto*	NA			88	100	84	84%	NA		
		Giardia					100	22	22%			
	IMS-	*Crypto*	NA			NA				0	NA	NA
		Giardia								0		
	OPR	*Crypto*										
		Giardia										
	blank	*Crypto*										
		Giardia										
03/01/07	raw	*Crypto*	5	1	0.5	88	100	3	3%	2	67	6.7
		Giardia					100	6	6%	4	67	6.7
	IMS+	*Crypto*	NA			88	100	44	44%	NA		
		Giardia					100	31	31%			
	IMS-	*Crypto*	NA			NA				0	NA	NA
		Giardia								0		
	OPR	*Crypto*										
		Giardia										
	blank	*Crypto*										
		Giardia										
04/10/07	raw	*Crypto*	8	0.5	0.5	92	101	9	9%	2	22	2.8
		Giardia					100	33	33%	3	9	1.1
	IMS+	*Crypto*	NA			92	101	66	65%	NA		
		Giardia					100	52	52%			
	IMS-	*Crypto*	NA			NA				0	NA	NA
		Giardia								0		
	OPR	*Crypto*										
		Giardia										
	blank	*Crypto*										
		Giardia										

Site E11, continued

Date	Sample Type / Organism		Filtration: Initial Volume (L)	Filtration: Pellet Vol (mL)	Filtration: mL Subj to IMS	ColorSeed: Lot Num	ColorSeed: Claimed Num	ColorSeed: Count Num	ColorSeed: Recovery %	Wild Type: Raw Count	Wild Type: Corrected Count	Wild Type: Corrected Count per L
04/16/07	raw	*Crypto*	7	1	1	92	101	10	10%	10	101	14.4
		Giardia					100	23	23%	10	43	6.2
	IMS+	*Crypto*	NA			92	101	50	50%	NA		
		Giardia					100	58	58%			
	IMS-	*Crypto*	NA			NA				0	NA	NA
		Giardia								0		
	OPR	*Crypto*	10	-0.5	-0.5	92	101	20	20%	0	0	0.0
		Giardia					100	28	28%	0	0	0.0
	blank	*Crypto*	10	-0.5	-0.5	NA				0	NA	NA
		Giardia								0	NA	NA
05/15/07	raw	*Crypto*	7	0.75	0.75	B94	99	9	9%	4	44	6.3
		Giardia					101	33	33%	3	9	1.3
	IMS+	*Crypto*	NA			B94	99	65	66%	NA		
		Giardia					101	40	40%			
	IMS-	*Crypto*	NA			NA				0	NA	NA
		Giardia								0		
	OPR	*Crypto*	7	-0.5	-0.5	B94	99	17	17%	0	0	0.0
		Giardia					101	16	16%	0	0	0.0
	blank	*Crypto*	7	-0.5	-0.5	NA				0	NA	NA
		Giardia								0	NA	NA

Site WHIP													
			Filtration			ColorSeed				Wild Type			
Date	Sample Type		Initial Volume (L)	Pellet Vol (mL)	Vol Subj to IMS (mL)	Lot Num	Claimed Num	Count Num	Recovery %	Raw Count	Corrected Count	Corrected Count per L
12/13/07	raw	*Crypto*	6	0.5	0.5	88	100	12	12%	1	8	1.4
		Giardia					100	9	9%	2	22	3.7
	IMS+	*Crypto*	NA			88	100	84	84%	NA		
		Giardia					100	22	22%			
	IMS-	*Crypto*	NA			NA				0	NA	NA
		Giardia								0		
	OPR	*Crypto*										
		Giardia										
	blank	*Crypto*										
		Giardia										
03/01/07	raw	*Crypto*	8	3	0.5	88	100	3	3%	3	100	2.1
		Giardia					100	5	5%	7	140	2.9
	IMS+	*Crypto*	NA			88	100	44	44%	NA		
		Giardia					100	31	31%			
	IMS-	*Crypto*	NA			NA				0	NA	NA
		Giardia								0		
	OPR	*Crypto*										
		Giardia										
	blank	*Crypto*										
		Giardia										
04/16/07	raw	*Crypto*	7	3	3	92	101	14	14%	10	72	10.3
		Giardia					100	17	17%	9	53	7.6
	IMS+	*Crypto*	NA			92	101	50	50%	NA		
		Giardia					100	58	58%			
	IMS-	*Crypto*	NA			NA				0	NA	NA
		Giardia								0		
	OPR	*Crypto*	10	-0.5	-0.5	92	101	20	20%	0	0	0.0
		Giardia					100	28	28%	0	0	0.0
	blank	*Crypto*	10	-0.5	-0.5	NA				0	0	0.0
		Giardia								0	0	0.0

Site MB-1

Date	Sample Type		Filtration: Initial Volume (L)	Filtration: Pellet Vol (mL)	Filtration: Vol Subj to IMS (mL)	ColorSeed: Lot Num	ColorSeed: Claimed Num	ColorSeed: Count Num	ColorSeed: Recovery %	Wild Type: Raw Count	Wild Type: Corrected Count	Wild Type: Corrected Count per L
11/28/07	raw	*Crypto*	10	0.5	0.5	86	99	25	25%	29	115	11.5
		Giardia					100	14	14%	15	107	10.7
	IMS+	*Crypto*	NA			86	99	12	12%	NA		
		Giardia					100	11	11%			
	IMS-	*Crypto*	NA			NA				0	NA	NA
		Giardia								1		
	OPR	*Crypto*										
		Giardia										
	blank	*Crypto*										
		Giardia										
12/13/07	raw	*Crypto*	8	0.5	0.5	88	100	25	25%	9	36	4.5
		Giardia					100	33	33%	5	15	1.9
	IMS+	*Crypto*	NA			88	100	84	84%	NA		
		Giardia					100	22	22%			
	IMS-	*Crypto*	NA			NA				0	NA	NA
		Giardia								0		
	OPR	*Crypto*										
		Giardia										
	blank	*Crypto*										
		Giardia										
03/01/07	raw	*Crypto*	6	1	0.5	88	100	ee	ee	ee	ee	ee
		Giardia					100	ee	ee	ee	ee	ee
	IMS+	*Crypto*	NA			88	100	44	44%	NA		
		Giardia					100	31	31%			
	IMS-	*Crypto*	NA			NA				0	NA	NA
		Giardia								0		
	OPR	*Crypto*										
		Giardia										
	blank	*Crypto*										
		Giardia										
04/16/07	raw	*Crypto*	7	1	1	92	101	0	0%	0	NA	NA
		Giardia					100	13	13%	3	23	3.3
	IMS+	*Crypto*	NA			92	101	50	50%	NA		
		Giardia					100	58	58%			
	IMS-	*Crypto*	NA			NA				0	NA	NA
		Giardia								0		
	OPR	*Crypto*	10	-0.5	-0.5	92	101	20	20%	0	0	0.0
		Giardia					100	28	28%	0	0	0.0
	blank	*Crypto*	10	-0.5	-0.5	NA				0	NA	NA
		Giardia								0	NA	NA

Site N5-1													
			Filtration			ColorSeed				Wild Type			
Date	Sample Type		Initial Volume (L)	Pellet Vol (mL)	Vol Subj to IMS (mL)	Lot Num	Claimed Num	Count Num	Recovery %	Raw Count	Corrected Count	Corrected Count per L	
03/01/07	raw	*Crypto*	8	2	0.5	88	100	ee	ee	ee	ee	ee	
		Giardia					100	ee	ee	ee	ee	ee	
	IMS+	*Crypto*	NA			88	100	44	44%	NA			
		Giardia					100	31	31%				
	IMS-	*Crypto*	NA			NA				0	NA	NA	
		Giardia								0			
	OPR	*Crypto*											
		Giardia											
	blank	*Crypto*											
		Giardia											
04/16/07	raw	*Crypto*	7	3	3	92	101	1	1%	0	0	0.0	
		Giardia					100	5	5%	1	20	2.9	
	IMS+	*Crypto*	NA			92	101	50	50%	NA			
		Giardia					100	58	58%				
	IMS-	*Crypto*	NA			NA				0	NA	NA	
		Giardia								0			
	OPR	*Crypto*	10	-0.5	-0.5	92	101	20	20%	0	0	0.0	
		Giardia					100	28	28%	0	0	0.0	
	blank	*Crypto*	10	-0.5	-0.5	NA				0	NA	NA	
		Giardia								0	NA	NA	

APPENDIX E: Performance and recovery data for intrastorm *Cryptosporidium* and *Giardia* testing of samples above the action level

Site E9													
			Filtration			ColorSeed				Wild Type			
Date	Sample Type / Organism		Initial Volume Filtered (L)	Pellet Vol (mL)	Vol Subj to IMS (mL)	Lot Num	Claimed Num	Count Num	Recovery %	Raw Count	Corrected Count	Corrected Count per L	
06/04/07	Phase 1	*Crypto*	7	2	2	B94	99	7	7%	0	0	0.0	
		Giardia					101	32	32%	12	38	5.4	
	Phase 2	*Crypto*	7	3	3	B94	99	18	18%	3	17	2.4	
		Giardia					101	27	27%	7	26	3.7	
	IMS +	*Crypto*	NA			B94	99	76	77%	0	0	0.0	
		Giardia					101	44	44%	0	0	0.0	
	IMS-	*Crypto*	NA			NA				0	0	0.0	
		Giardia								0	0	0.0	
	OPR	*Crypto*	7	-0.5	-0.5	B94	99	27	27%	0	0	0.0	
		Giardia					101	33	33%	0	0	0.0	
	blank	*Crypto*	7	-0.5	-0.5	B94	99	0	0%	0	0	0.0	
		Giardia					101	0	0%	0	0	0.0	
07/05/07	Phase 1	*Crypto*	8	3	3	B94	102	10	10%	1	10	1.3	
		Giardia					100	28	28%	9	32	4.0	
	IMS +	*Crypto*	NA			B94	102	85	83%	0	0	0.0	
		Giardia					100	48	48%	0	0	0.0	
	IMS-	*Crypto*	NA			NA		0	NA	0	0	0.0	
		Giardia						0		0	0	0.0	
	OPR	*Crypto*	7	-0.5	-0.5	B94	102	42	41%	0	0	0.0	
		Giardia					100	31	31%	0	0	0.0	
	blank	*Crypto*	7	-0.5	-0.5	NA		0	NA	0	0	0.0	
		Giardia						0		0	0	0.0	

Site WHIP													
			Filtration			ColorSeed				Wild Type			
Date	Sample Type/ Organism		Initial Volume (L)	Pellet Vol (mL)	Vol Subj to IMS (mL)	Lot Num	Claimed Num	Count Num	Recovery %	Raw Count	Corrected Count	Corrected Count per L	
06/04/07	Phase 3	*Crypto*	7	4	4	B94	99	28	28%	15	53	7.6	
		Giardia					101	29	29%	5	17	2.5	
	IMS+	*Crypto*	NA			B94	99	76	77%	0	0	0.0	
		Giardia					101	44	44%	0	0	0.0	
	IMS-	*Crypto*	NA			NA				0	0	0.0	
		Giardia								0	0	0.0	
	OPR	*Crypto*	7	-0.5	-0.5	B94	99	27	27%	0	0	0.0	
		Giardia					101	33	33%	0	0	0.0	
	blank	*Crypto*	7	-0.5	-0.5	B94	99	0	0%	0	0	0.0	
		Giardia					101	0	0%	0	0	0.0	
06/28/07	Phase 1	*Crypto*	8	0.5	0.5	B95	100	14	14%	2	14	1.8	
		Giardia					100	11	11%	4	36	4.5	
	IMS+	*Crypto*	NA			B95	100	78	78%	0	0	0.0	
		Giardia					100	53	53%	0	0	0.0	
	IMS-	*Crypto*	NA			NA		0	0%	0	0	0.0	
		Giardia						0	0%	0	0	0.0	
	OPR	*Crypto*	7	-0.5	-0.5	B95	100	28	28%	0	0	0.0	
		Giardia					100	41	41%	0	0	0.0	
	blank	*Crypto*	7	-0.5	-0.5	B95	100	0	0%	0	0	0.0	
		Giardia					100	0	0%	0	0	0.0	
07/05/07	Phase 1	*Crypto*	8	1	1	B94	102	27	26%	1	4	0.5	
		Giardia					100	10	10%	5	50	6.3	
	IMS+	*Crypto*	NA			B94	102	85	83%	0	0	0.0	
		Giardia					100	48	48%	0	0	0.0	
	IMS-	*Crypto*	NA			NA		0	NA	0	0	0.0	
		Giardia						0		0	0	0.0	
	OPR	*Crypto*	7	-0.5	-0.5	B94	102	42	41%	0	0	0.0	
		Giardia					100	31	31%	0	0	0.0	
	blank	*Crypto*	7	-0.5	-0.5	NA		0	NA	0	0	0.0	
		Giardia						0		0	0	0.0	

Site N5-1														
			Filtration			ColorSeed				Wild Type				
Date	Sample Type		Initial Volume (L)	Pellet Vol (mL)	Vol Subj to IMS (mL)	Lot Num	Claimed Num	Count Num	Recovery %	Raw Count	Corrected Count	Corrected Count per L		
06/04/07	Phase 1	*Crypto*	7	1	1	B94	99	17	17%	9	52	7.5		
		Giardia					101	7	7%	6	87	12.4		
	Phase 3	*Crypto*	7	1	1	B94	99	20	20%	5	25	3.5		
		Giardia					101	15	15%	3	20	2.9		
	IMS+	*Crypto*	NA			B94	99	76	77%	0	0	0.0		
		Giardia					101	44	44%	0	0	0.0		
	IMS-	*Crypto*	NA			NA				0	0	0.0		
		Giardia								0	0	0.0		
	OPR	*Crypto*	7	-0.5	-0.5	B94	99	27	27%	0	0	0.0		
		Giardia					101	33	33%	0	0	0.0		
	blank	*Crypto*	7	-0.5	-0.5	B94	99	0	0%	0	0	0.0		
		Giardia					101	0	0%	0	0	0.0		
06/28/07	Phase 1	*Crypto*	8	1	1	B95	100	6	6%	1	17	2.1		
		Giardia					100	9	9%	5	56	6.9		
	Phase 2	*Crypto*	8	1	1	B95	100	10	10%	2	20	2.5		
		Giardia					100	17	17%	8	47	5.9		
	Phase 3	*Crypto*	8	1	1	B95	100	5	5%	0	0	0.0		
		Giardia					100	6	6%	0	0	0.0		
	IMS+	*Crypto*	NA			B95	100	78	78%	0	0	0.0		
		Giardia					100	53	53%	0	0	0.0		
	IMS-	*Crypto*	NA			NA		0	0%	0	0	0.0		
		Giardia						0	0%	0	0	0.0		
	OPR	*Crypto*	7	-0.5	-0.5	B95	100	28	28%	0	0	0.0		
		Giardia					100	41	41%	0	0	0.0		
	blank	*Crypto*	7	-0.5	-0.5	B95	100	0	0%	0	0	0.0		
		Giardia					100	0	0%	0	0	0.0		
07/05/07	Phase 1	*Crypto*	8	1	1	B94	102	41	40%	2	5	0.6		
		Giardia					100	20	20%	7	35	4.4		
	IMS+	*Crypto*	NA			B94	102	85	83%	0	0	0.0		
		Giardia					100	48	48%	0	0	0.0		
	IMS-	*Crypto*	NA			NA		0	NA	0	0	0.0		
		Giardia						0		0	0	0.0		
	OPR	*Crypto*	7	-0.5	-0.5	B94	102	42	41%	0	0	0.0		
		Giardia					100	31	31%	0	0	0.0		
	blank	*Crypto*	7	-0.5	-0.5	NA		0	NA	0	0	0.0		
		Giardia						0		0	0	0.0		

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