Microbial partitioning in urban stormwaters

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Abstract

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Contamination by high concentrations of fecal indicator bacteria has been identified as one of the most common causes of surface water quality impairment in the United States; however, there is currently very little quantitative data available for use in designing watershed restoration plans that detail microbial transport in receiving waters. In this study, association with settleable particles ("partitioning"), a behavior frequently neglected in water quality models that can affect in-stream fate and transport, is more thoroughly characterized through the analysis of samples from several watersheds.

Results suggest that while intermittent, stormwater flows contribute the majority of indicator organism inputs to receiving waters, as cumulative storm loadings can be equal to several years' worth of equivalent background loadings. Loadings of microorganisms associated with settleable particles appear to be largely transported in the initial "first flush" of storm events. Observations of particle association by fecal indicator bacteria appear to be a reasonable approximation of the partitioning behavior of *Salmonella*; however, *Salmonella* bacteria, as well as the protozoan pathogens *Cryptosporidium* and *Giardia*, were readily recoverable from samples meeting current water quality standards.

Monitoring data from two suburban detention basins suggest that settleable indicator organisms and *Salmonella* are removed at a higher rate than their free-phase counterparts, indicating that sedimentation may be an important microbial removal mechanism in stormwater treatment structures. However, despite mean removals by one pond near the USEPA's typical rate of 65%, effluent concentrations remained several orders of magnitude greater than recommended levels.

Comparisons of free phase and settleable *E. coli* concentrations as measured by a culture-based technique and the quantitative polymerase chain reaction (qPCR) may support previous studies suggesting that particle association reduces cell die-off in addition to accelerating sedimentation in the water column, although further investigation of potential inhibition of the PCR reaction is required. Despite significant differences between enumeration techniques in free phase *E. coli* concentrations, measures of total concentration were equivalent and produced similar conclusions regarding water body impairment. Regardless of detection method or indicator organism used in assessment, compiled data indicate that all four study watersheds will be in violation of recommended standards following storm events.

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I. Introduction

1. Indicator organism partitioning in stormwaters

Over 8,500 water bodies in the United States are currently listed as contaminated due to high concentrations of fecal indicator organisms (USEPA 2009). Nonpoint source loadings of microbes, particularly from stormwater generated flows, have long been suspected as responsible for a significant fraction of these downstream water quality violations (Davis et al. 1977; Geldreich et al. 1968; Weibel et al. 1964). Elevated concentrations of both indicator organisms (Hunter et al. 1992; Kim et al. 2005; Noble et al. 2003) and pathogens (Atherholt et al. 1998; Ferguson et al. 1996; Gales and Baleux 1992; Kistemann et al. 2002; Rouquet et al. 2000) have been observed in receiving waters following storm events, and increased precipitation has been linked to outbreaks of waterborne disease (Gaffield et al. 2003; MacKenzie et al. 1994; Rose et al. 2000).

Under section 303(d) of the 1972 Clean Water Act, all US surface waters must be assessed to verify that appropriate water quality standards are achieved. Water bodies with chronic or significant violations of standards are designated by states as impaired and require development of watershed-scale remediation plans known as Total Maximum Daily Loads ("TMDLs"). In watersheds with high levels of pathogen indicators (e.g. fecal coliforms), TMDL plans aim to reduce indicator concentration, and presumably human health risk, to an acceptable level through the identification of contaminant origins and their transport pathways to receiving waters. Water quality models are generally used to model microbial transport to determine potential interventions that

would reduce downstream concentrations. Despite the relatively large impact of nonpoint source pollution on microbial contamination, quantitative data describing transport are relatively sparse and limit the accuracy of water quality models (Dorner et al. 2006; Jamieson et al. 2004a; Pachepsky et al. 2006). Specifically, microbes have generally been modeled as individual free cells of near neutral buoyancy, though increasing evidence suggests that many microorganisms actually partition between a particle-associated and free phase.

Quantifying the relative fractions of particle-associated and free phase organisms is potentially of critical importance in modeling efforts as observational evidence suggests that association with larger, denser particles can result in the accelerated removal of microbes from the water column via sedimentation (Gannon et al. 1983; Rouquet et al. 2000). A better understanding of this phenomenon could be used to improve water quality through the strategic placement of appropriate best management practices ("BMPs", e.g. detention basins) designed to intercept and treat overland runoff. Although frequently installed as stormwater treatment structures, previous investigations of detention basins and their ability to reduce influent microbial loadings are somewhat limited in their discussion of sedimentation (Davies and Bavor 2000; Tufford and Marshall 2002). More quantitative information describing stormwater loadings of particle-associated microbes is required to determine the effectiveness of sedimentation as a microbial removal mechanism in order to maximize BMP efficiency.

Previous studies investigating microbial partitioning have generally separated microbes from the free and particle-associated phases using filtration (Auer and Niehaus 1993; Jeng et al. 2005; Schillinger and Gannon 1985). Filtration separates microbes and

particles solely on the basis of size, neglecting the effects of density which also influence settling velocity. Characklis et al. (2005) calibrated a centrifugation technique to quantify the partitioning of several indicator organisms to denser, “settleable”, particles in the water column of receiving waters under dry- and wet-weather conditions. Separation based both on size and density via centrifugation provides data that can be used to estimate sedimentation characteristics and are therefore more useful in fate and transport modeling.

While microbial partitioning has been evaluated previously in single grab samples, there have been no attempts to examine multiple samples over storm duration. As water quality varies throughout a storm (Characklis and Wiesner 1997; Weibel et al. 1964), changes in partitioning behavior are possible, though these relationships have yet to be identified. Studies have suggested that the concentration of organisms entering receiving waters is highest during the early phases of a storm event (Davis et al. 1977; Soupir et al. 2006), but no work has examined whether a similar “first flush” effect applies to the fraction of organisms associated with particles. Transport of the majority of settleable, and likely removable, microbial loadings within the early stages of the storm hydrograph may indicate that capture of the “first flush” by BMPs would produce disproportionately large reductions in downstream concentration.

The first phase of the present work collected multiple samples over the course of three storm events at two stream sites in separate watersheds. Samples were partitioned via centrifugation and analyzed for a suite of indicator organisms (fecal coliforms, *E. coli*, enterococci, *C. perfringens* spores, and total coliphage). Analysis of multiple storm samples allowed for estimations of total and settleable loadings of microorganisms, as

well as identification of potential changes or trends in partitioning behavior. Observations of microbial loadings, and in particular the confirmation of a moderate “first flush” effect for settleable organisms, will be useful to engineers in designing more cost-effective solutions for stormwater treatment.

2. Suitability of indicators as pathogen surrogates

Because the detection of actual waterborne human pathogens is often difficult, time-consuming, and expensive, water quality standards and accompanying monitoring programs use indicator organism (e.g. fecal coliforms, *E. coli*) concentrations as surrogates for potential human health risk (Pruss 1998; Wade et al. 2003; Wiedenmann et al. 2006). Computer models simulating microbial loadings to receiving waters, which often play a prominent role in regulatory programs, therefore use available data on the location and concentration of indicator organisms to estimate input parameters. The suitability of indicator organisms as surrogates for actual waterborne pathogens has previously been assessed primarily through the comparison of indicator and pathogen incidence and concentration (Ferguson et al. 2003; Griffin et al. 2001). However, the ability of hydrologic models to accurately predict expected downstream health risks also rests on the assumption that indicator transport behavior in the natural environment is similar to that of pathogens, an assumption for which there is little observational evidence. Without confirmation of similar transport behavior, it is difficult to determine whether reductions of indicator organisms attributable to upland interventions such as BMPs actually translate into reduced pathogen loadings downstream and commensurate improvements in water quality.

While there have been several lab and field-scale examinations of indicator organism partitioning (Characklis et al. 2005; Jeng et al. 2005; Krometis et al. 2007; Schillinger and Gannon 1985), the majority of previous investigations of pathogen particle-association have focused only on protozoan parasites without concurrent examination of indicator behavior, and have been largely conducted under laboratory conditions (Dai and Boll 2003; Medema et al. 1998; Searcy et al. 2005). Laboratory techniques that have been used to separate and enumerate particle-associated and free phase indicator organisms have generally differed from techniques used to partition pathogens. Because each technique used assumes a different operational definition of particle association (e.g. removal by filter of given pore size, particle retention on antibody-coated filter, sedimentation in gravitational fields, etc.) results describing indicator organism partitioning are not directly comparable with those for pathogens. One recent field study did directly compare the partitioning behavior of *Cryptosporidium* and *Giardia* with the behavior of several indicator organisms (Cizek et al. 2008), but there was no examination of the relationship between microbial particle-association and microbial removal by stormwater BMPs.

The relationship between microbial-particle association and removal by sedimentation-based BMPs is important as these structures remain one of the primary strategies for stormwater treatment. Previous investigations of microbial partitioning and BMP effectiveness have been inconclusive and largely focused on indicator organisms or protozoans (Borden et al. 1998; Brookes et al. 2006; Davies et al. 2003). While protozoans are important from a public health perspective, waterborne outbreaks of bacterial etiology remain a substantial concern in the United States as well (Craun et al.

2005). *Salmonella* bacteria infections alone are responsible for over 40,000 cases of illness yearly (CDC 2008), and though the disease is most commonly foodborne, a recent salmonellosis outbreak linked to drinking water in Colorado indicates that waterborne transmission is still of concern (Berg 2008).

In this work, the incidence and partitioning behavior of waterborne *Salmonella* spp. is compared with that of six indicator organisms (fecal coliforms, *E. coli*, enterococci, *C. perfringens* spores, somatic coliphage, and male-specific coliphage) in field samples. Water samples were collected from Northeast Creek, a local freshwater stream in an urban watershed currently requiring TMDL development to address high fecal coliform concentrations. Use of a single centrifugation-based separation technique allowed for direct comparison of the partitioning behavior of indicators and *Salmonella* spp. bacteria. Inflow and outflow samples from two suburban detention ponds in the impaired watershed were also analyzed to determine whether these structures provide similar reductions in indicator and *Salmonella* loadings and whether higher rates of particle-association result in higher rates of removal. Although there have been numerous investigations of BMP removal of indicator bacteria (Davies and Bavor 2000; Hunt et al. 2008; Stenstrom and Carlander 2001; Tufford and Marshall 2002), no previous examinations of pathogenic bacteria removal by detention ponds have been identified in the literature. Results from this study should be useful in the evaluation of the suitability of the targeted indicator organisms as surrogates for *Salmonella* in urban watershed modeling, and provide information for use in the design and maintenance of stormwater treatment BMPs.

A small subset of in-stream samples from the Northeast Creek watershed were additionally analyzed for the presence and partitioning behavior of the protozoan pathogens *Cryptosporidium parvum* and *Giardia lamblia*. Analysis of these samples was used to confirm field observations of protozoan partitioning by Cizek et al. (2008) and to determine whether these pathogens are a potential environmental health problem in local urban watersheds.

3. Comparison of culture-based and molecular techniques

The epidemiological studies used to establish current microbial water quality standards have used culture-based methods to quantify indicator organism exposure (e.g. membrane filtration, Colilert) (Pruss 1998; USEPA 1986; Wade et al. 2003). These methods identify targeted microorganisms via cellular metabolism of a given substrate and subsequent reproduction resulting in macroscopic endpoints (e.g. colony forming units, fluorescence) (Rompre et al. 2002). Because cell division and growth require time, results from culture-based analyses of water quality are not available for 18-24 hours or more. This time lag limits the ability of water quality managers to protect the public health in recreational areas, as results identifying contamination are not available until the day after swimmers have been exposed (Leecaster and Weisberg 2001). The analytical limitations of traditional microbial detection methods may also compromise the efforts of less time sensitive water quality monitoring programs (e.g. TMDLs) targeting non-recreational areas, as culture-based methods do not account for viable but non-culturable (VBNC) cells which may still pose a threat to the public health (Oliver 2000).

The relatively recent development of molecular techniques (e.g. the polymerase chain reaction or PCR, microarrays) for microbial detection and quantification has

offered rapid, highly sensitive alternatives to culture-based testing; however, relatively little is known regarding how results from molecular methods might compare to those from the culture-based methods originally used to develop regulatory standards. To avoid regulatory chaos, implementation of molecular methods will require a comparison of molecular and culture-based methods ideally correlating observations via the new technique and historical approaches (Noble and Weisberg 2005; Wade et al. 2003).

There have been several previous studies comparing the quantification of enterococci in marine and fresh water via quantitative-PCR (qPCR) and culture-based methods. Concentrations as measured by qPCR were generally several orders of magnitude greater than those detected by culture-based methods (Haugland et al. 2005; He and Jiang 2005; Khan et al. 2007; Morrison et al. 2008; Noble et al. 2006), as qPCR detects the nucleic acids of metabolically active, VBNC, and lysed cells indiscriminately. Despite these differences in analytical endpoints, correlations in total indicator bacteria concentrations as identified by qPCR and culture-based methods have generally been significant and strong, with R^2 values ranging from 0.68 to 0.925 (Haugland et al. 2005; Morrison et al. 2008). Although *E. coli* remains the recommended indicator for freshwater monitoring (USEPA 1986; Wade et al. 2003), comparisons of *E. coli* detection via culture-based and PCR analysis have generally focused only on positive detection and have not included attempts to correlate concentration measurements obtained by the two techniques (Bej et al. 1991; Frahm and Obst 2003; Lleo et al. 2005). In addition, all previous studies comparing culture-based and molecular techniques have only compared measures of total concentration. While useful in monitoring, these results do not compare

concentrations of settleable and free phase microbes, which would be valuable in predicting and interpreting fate and transport.

Past investigations of indicator organism or pathogen partitioning behavior have quantified concentration and particle-association using either culture-based methods (Characklis et al. 2005; Fries et al. 2006; Jeng et al. 2005; Schillinger and Gannon 1985) or direct microscopic enumeration (Cizek et al. 2008; Maki and Hicks 2002; Medema et al. 1998) rather than molecular approaches. The nature of the partitioning techniques used to separate settleable (particle-associated) and free phase cells prior to enumeration may yield different results when molecular techniques are used to quantify these fractions of total concentration.

The objective of this section of the study was to identify the bias between molecular and culture-based techniques when examining particle-attached versus free-phase *E. coli* in samples from urban freshwater streams. Samples were collected from four local watersheds of varying levels of contamination during dry weather and storm events, partitioned, and analyzed concurrently for *E. coli* concentration and particle-association via the Colilert-2000® defined substrate technique and qPCR. Analysis via qPCR targeted the *uidA* gene coding for the enzyme *β*-glucuronidase that metabolizes 4-methyl-umbelliferyl (MUG), which is responsible for the fluorescent signal indicating *E. coli*-positive wells in the Colilert assay. Because both methods use the presence of the same enzymatic ability to identify *E. coli*, potential differences in concentration are likely due to differences in physiology rather than the presence of non-MUG strains. Quantification of both free phase and settleable concentrations allowed for comparisons

of the potential impacts of particle-association on these measurement techniques in addition to simple detection.

4. Monitoring for microbiological impairments

Throughout the nation, insufficient monitoring data is available to accurately identify water quality impairments. Inadequate numbers of water quality observations inevitably lead to the mis-classification of water bodies as impaired or unimpaired, resulting in inappropriate allocations of resources (Keller and Cavallaro 2008) and compromising the ultimate success of TMDL development and implementation (Benham et al. 2008). Because individual states are responsible for setting water quality targets for all potential contaminants, including microorganisms, standards may differ by region and provide varying levels of protection for public and ecosystem health (Keller and Cavallaro 2008).

This project required the collection and analysis of numerous fresh water samples from local watersheds of varying impairment status, providing a unique data set for the assessment of water quality. Comparison of the observed data to North Carolina fecal coliform-based standards and recommended USEPA *E. coli*-based standards yielded insights into the effects of selected indicator and sampling conditions on waterbody impairment designation. Additionally, qPCR and Colilert measures of total *E. coli* concentration were compared to determine the potential effect of using qPCR in water quality impairment designation. Although PCR has been used as a microbial source tracking tool in watershed remediation efforts directed by the TMDL program (Domingo et al. 2007; Field and Samadpour 2007), there have been no investigations of the potential implementation of molecular techniques in efforts to identify water quality impairments

for TMDL development. Re-assessment of the relative severity of microbial impairments in the four study watersheds provided perspective on the current TMDL program's potential for success and the importance of this project's focus on stormwater transport of microbial loadings.

II. Research Goals

Contamination by high concentrations of fecal indicator organisms has been identified by the national TMDL program as one of the most common causes of surface water quality impairment in the United States. Despite the ubiquity of these impairments throughout the country and the potential threat they pose to the public health, there is currently very little quantitative data available for use in designing watershed restoration plans that detail microbial transport in receiving waters. In this study, microbial association with settleable particles ("partitioning"), a behavior frequently neglected in water quality models that can affect both in-stream fate and transport and potential removal by upland stormwater BMPs, is more thoroughly characterized through the analysis of water samples from several North Carolina watersheds. Specific goals of these analyses included:

- Confirmation that stormwater is the primary source of fecal indicator organisms responsible for water quality impairment designations in targeted watersheds;
- Identification of intra-storm variability in the partitioning rates of indicator organisms (fecal coliforms, *E. coli*, enetrococci, *C. perfringens* spores, coliphage) and estimation of total and settleable stormwater loadings of these microorganisms;
- Comparison of the incidence and partitioning behavior of indicator organisms and three human pathogens (*Salmonella* spp., *Cryptosporidium parvum*, *Giardia*

lamblia) in order to determine the suitability of the proposed indicator organisms as surrogates for each in water quality modeling;

- Evaluation of stormwater detention pond effectiveness in reducing both influent indicator organism and *Salmonella* spp. concentrations;
- Simultaneous determination of *E. coli* incidence and partitioning in environmental waters via culture-based (Colilert) and molecular (qPCR) methods to determine the effect of using new molecular detection techniques to monitor indicator organism incidence and describe microbial particle-association;
- Re-assessment of the microbial water quality in targeted watersheds as compared to North Carolina and USEPA recommended standards for the identification of freshwater impairments requiring TMDL development.

III. Methodology

1. Site selection

Single grab samples had been collected previously from two streams in Orange and Durham County, NC for partitioning analysis (Figure 1): Booker Creek, Meeting of the Waters Creek, and the Eno River (Characklis et al. 2005). Streamflow for two of these streams, Meeting of the Waters Creek and the Eno River, is currently monitored by USGS streamflow gages (USGS gages #02097517 and #02085070, respectively) and data is available on-line in real-time. Because streamflow data were essential for hydrograph monitoring and estimation of microbial loadings, intrastorm samples were collected only from these two sites, although samples were taken from Booker Creek for later stages of the project.

Accurate evaluation of microbial partitioning requires relatively high concentrations of targeted organisms. Higher concentrations of actual waterborne human pathogens would be expected in waters with higher indicator organism concentrations. While both Booker Creek and Meeting of the Waters Creek are currently included on the North Carolina 303(d) list for biological impairments, neither is listed for elevated indicator organism concentrations. “Biological impairments” are used by North Carolina to address general degradation of the stream ecosystem without targeting specific pollutants. In other states, these types of impairment may be referred to as “general” or “benthic” as the impairment is often indicated by a loss in stream macroinvertebrate

abundance or diversity, and focuses on ecological health issues rather than human health risk (Wagner et al. 2007). A fourth local stream specifically included on the North Carolina 303(d) list for elevated concentrations of indicator bacteria, Northeast Creek, was therefore selected for collection of samples for pathogen partitioning analysis (Figure 1). Northeast Creek was also desirable as it is USGS-gaged (gage #0209741955) with real-time streamflow data available.

Samples for qPCR and culture-based analysis of *E. coli* partitioning behavior were collected from in-stream sites in all four watersheds during a variety of weather conditions to maximize the range of bacteria concentrations in analyzed samples. Comparisons of specific watershed characteristics and designated impairments are included below.

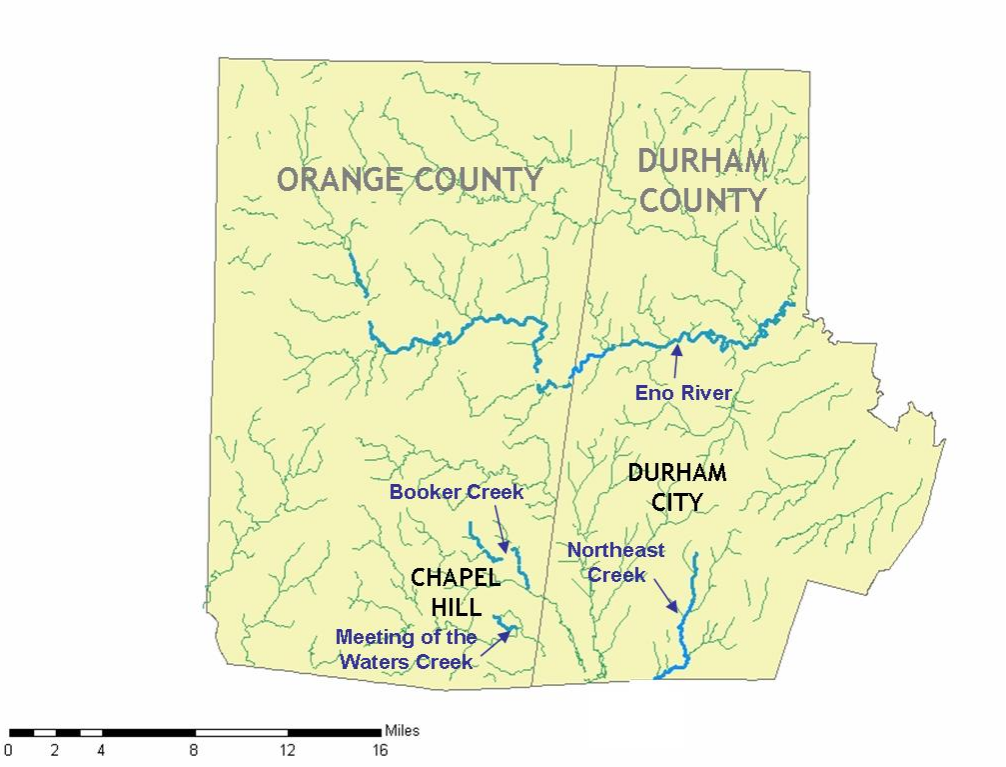


Figure 1. Project stream sites

Booker Creek

Booker Creek is an ungaged stream in the town of Chapel Hill, NC within the Cape Fear River basin. Samples were collected just downstream from a large shopping center with upstream landuse primarily classified as commercial and residential. The stream is included on the current North Carolina 303(d) list as requiring TMDL development to address a "biological impairment" (NCDWQ 2006).

Eno River

The Eno River is located in the Neuse River basin and has an average baseflow of approximately 1.0 m^3/s. Upstream landuse is roughly 10% impervious, and classified as mostly low density residential. The stream is not currently included on the state's impairment list (NCDWQ 2006).

Meeting of the Waters Creek

Meeting of the Waters Creek is a small stream in the Cape Fear River basin with an average baseflow of approximately 0.5 m^3/s. Upstream landuse is dominated by the University of North Carolina at Chapel Hill, primarily classified as institutional, and 5% impervious. The stream is included on the North Carolina 303(d) list as requiring TMDL development to address a biological impairment (NCDWQ 2006).

Northeast Creek

The most extensive sampling for this project occurred at multiple sites within the Northeast Creek watershed. The watershed is approximately 116 km^2 and located within the larger Cape Fear River basin. Landuse data from 2001 characterizes the watershed as 30% urban, 50% forest, 9% wetland, and 11% other (agricultural, barren, water, etc). The current (2007) fraction of urban landuse is likely higher, given rapid development in the

watershed. The stream currently suffers from multiple designated impairments, including high pathogen indicator organism (fecal coliform) concentrations, high turbidity, impaired biological integrity, and low dissolved oxygen (NCDWQ 2006). In North Carolina, a water body is defined as impaired by indicator organisms if the fecal coliform concentrations of more than 20% of grab samples exceeds 400 CFU/100 mL or if the geometric mean of the fecal coliform concentration exceeds 200 CFU/100 mL for any 30-day period (NCDWQ 2007). Water quality in Northeast Creek is of particular concern because it is a tributary flowing into Jordan Lake, a drinking water source and popular recreational area, and so the state has designated these impairments as "high priority".

During investigations of pathogen partitioning, samples were collected at two points along the stream (sites 1 and 2) and from two wet ponds (i.e. detention ponds) permitted as stormwater treatment structures by Durham County (Figure 2). The wet ponds are located less than a kilometer apart in a suburban community. Both ponds were permitted as stormwater treatment structures by the City of Durham. Pond 1 has an estimated surface area of 140 m^2 and an estimated pond volume of 153 m^3. Pond 2 has a permitted dry weather surface area of 1,195 m^2 and a permitted maximum pond volume of 1,303 m^3. Two potentially significant sources of microbial loadings exist between stream site 1 and stream site 2: a wastewater treatment plant (permitted to discharge 23,000 m^3/day) and a waterfowl impoundment.

In order to analyze a sufficient number of samples for *E. coli* concentration via Colilert and qPCR, samples were collected from a third stream site, designated site 1a, in addition to the two stream sites described previously. Site 1a is located between sites 1 and 2, downstream from the wastewater treatment plant outfall, but upstream from the

waterfowl impoundment (Figure 2). Sites from all three Northeast Creek stream sites as well as from sites in the other three watersheds were collected for this section of the project.

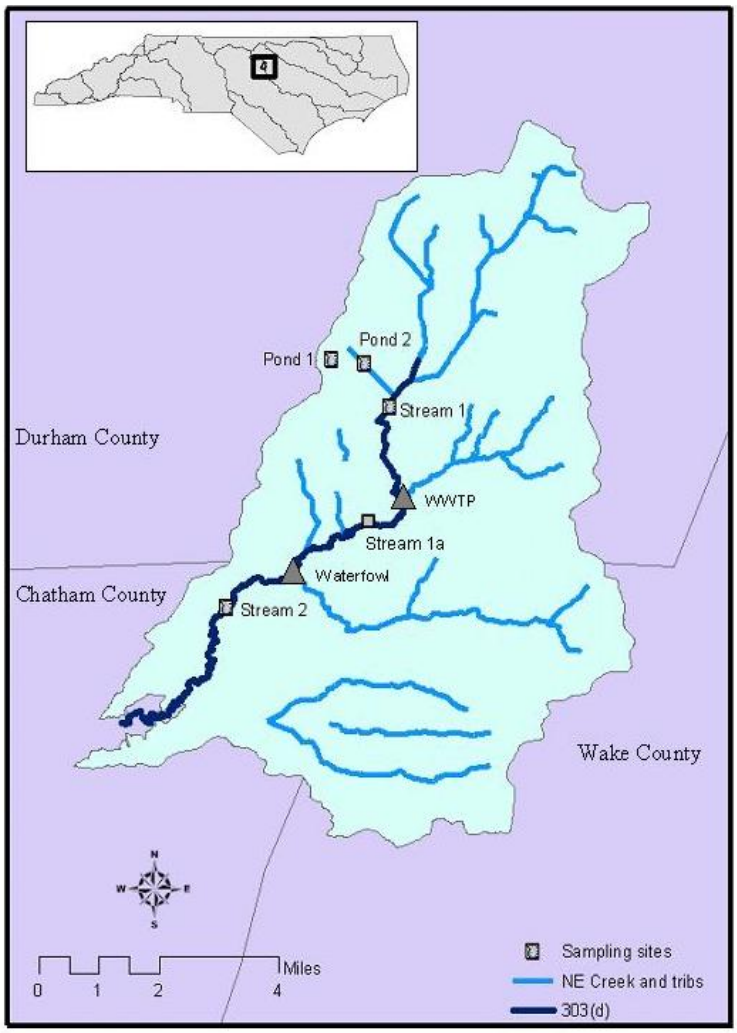


Figure 2. Northeast Creek watershed and sampling sites

2. Sampling regimen

Sample collection and handling

Water samples were collected from all sites using either a pre-sterilized bottle or a bucket rinsed with distilled water and gently poured into cubitainers or 4.0 L storage

bottles for transport to the laboratory. Upon arrival at the laboratory, all samples were stored at 4 C before processing to minimize cell die-off and carbon degradation. Prior to microbial and physical analyses, each sample was gently inverted three times to create a reasonably homogenous suspension without disrupting potential particle or microbial-particle aggregates. Dilutions for particle analysis were completed using graduated cylinders rather than pipettes to minimize potential aggregate disruption. All relevant analyses were completed within one week, with bacterial and particle analyses generally occurring the day of or the day after sampling.

Intrastorm analysis

Both Meeting of the Waters Creek and the Eno River were sampled four to six times over the course of three individual storm events in the summer and fall of 2004, with an attempt to capture samples during the rising limb, peak, and recession of each storm hydrograph. A storm event was operationally defined as a fourfold or higher increase in streamflow following at least three days of no appreciable precipitation. Data from previously collected dry weather samples at both sites (Characklis et al. 2005) was used to estimate background concentrations when calculating of total contaminant storm loadings.

Salmonella analysis

Samples for *Salmonella* analysis were collected from Northeast Creek in-stream sites 1 and 2 and three locations around both wet ponds: the street gutters directly feeding the storm sewer; the point at which the storm sewer empties into the pond (pond inflow); and the point at which water leaves the pond (pond outflow). Samples from all sites were

collected on six occasions during dry weather and during seven to eight storm events during the summers of 2006 and 2007.

Protozoan analysis

Because of the time and expense associated with protozoan analysis, samples for *Cryptosporidium* and *Giardia* analysis were only collected from Northeast Creek at site 1. An initial background screening of samples indicated that (oo)cysts were present in similar concentrations at all stream sites. There is some evidence that the antibodies used for immunomagnetic separation in the USEPA Method 1623 may capture species of *Cryptosporidium* other than *C. parvum* (Carey et al. 2004). Consequently, stream site 1 was selected for further sampling to avoid potential misidentification of avian *Cryptosporidium* species downstream from the waterfowl impoundment as this targeted human pathogen.

Five dry weather samples and four storm event samples were collected and analyzed for (oo)cyst concentration and partitioning behavior. Due to record-breaking drought conditions, no further samples were collected.

qPCR analysis

Samples were collected from all four watersheds, including all three Northeast Creek in-stream sites, during the summer and fall of 2008 for *E. coli* analysis via Colilert and qPCR. A total of ten rounds of samples were collected (dry weather = 7; storm events = 3), although only the first nine could be analyzed via qPCR due to a filter supply shortage. Combined with the occasional exclusion of a site during a sampling round due to insufficient flow or access difficulties, 47 samples were partitioned and their respective raw and supernatant subsamples analyzed by both Colilert and qPCR.

3. Partitioning technique

Overview

Intrastorm samples and samples analyzed for *Salmonella* partitioning behavior were partitioned using a technique originally calibrated by Characklis et al. (2005) to separate free-phase and settleable particle-associated indicator organisms. Protozoan pathogens, including *Cryptosporidium* and *Giardia*, though of similar density, are four to ten times the size of these indicator microorganisms (Table 1). Consequently, there was concern that the original method might remove free phase (oo)cysts from suspension, resulting in an overestimation of protozoan association with particles. Therefore, the method was re-calibrated at a lower centrifugation speed detailed below. Because this lower speed provided a more conservative estimate of settleable microorganisms and sufficient discrimination of bacterial sized particles of typical organic and inorganic densities, the modified technique was also used to assess *E. coli* partitioning in the final section of the project.

Table 1. Typical particle and microbial sizes and densities

	Equiv. spherical diamater (um)	**Density, g/cm^3**	**References**
Inorganic particles	*variable*	2.6	Chapra, 1997
Organic particles	*variable*	1.01 - 1.2	Chapra, 1997
Fecal coliforms	1 - 4		Linsley et al, 1992
E. coli	1 - 2.5	1.09 - 1.13	Bratbak & Dundas, 1984; Holt, 1994
Cryptosporidium	4 - 6	1.06	AWWA, 1999; Metge et al 2003
Giardia	8 - 15		AWWA, 1999
C. perfringens spores	1 - 3	1.23 - 1.38	Lovins et al, 2002; Tisa et al, 1982
Norwalk virus	0.02 - 0.03	1.39 - 1.40	AWWA, 1999
MS-2 coliphage	0.025	1.33 - 1.46	Lovins et al, 2002; Rohrmann & Krueger, 1970

Original technique

Water samples were collected in four-liter cubitainers, transported on ice to the laboratory, and stored at 4^{o} C until analysis. Prior to microbial and physical analysis, samples were partitioned using a calibrated centrifugation technique designed to separate microbes attached to denser particles from microorganisms in the free phase or those attached to less dense particles (Figure 3). Two 1.0-L aliquots were removed from the cubitainer, with one aliquot set aside for analysis as the "raw", or unmodified, sample. The second aliquot was centrifuged at 1164x*g* (*g*=gravity, 9.81 m/s^2; 2000 rpm) for 10 min at 4 C (Sorvall RC-3B centrifuge with a H-6000A rotor) with a brake of 4 (approx 5 min deceleration time). Following centrifugation, the top 700 mL of supernatant was removed via a vacuum pipette. Raw and supernatant samples were analyzed concurrently for microbial, particle, and TOC concentrations.

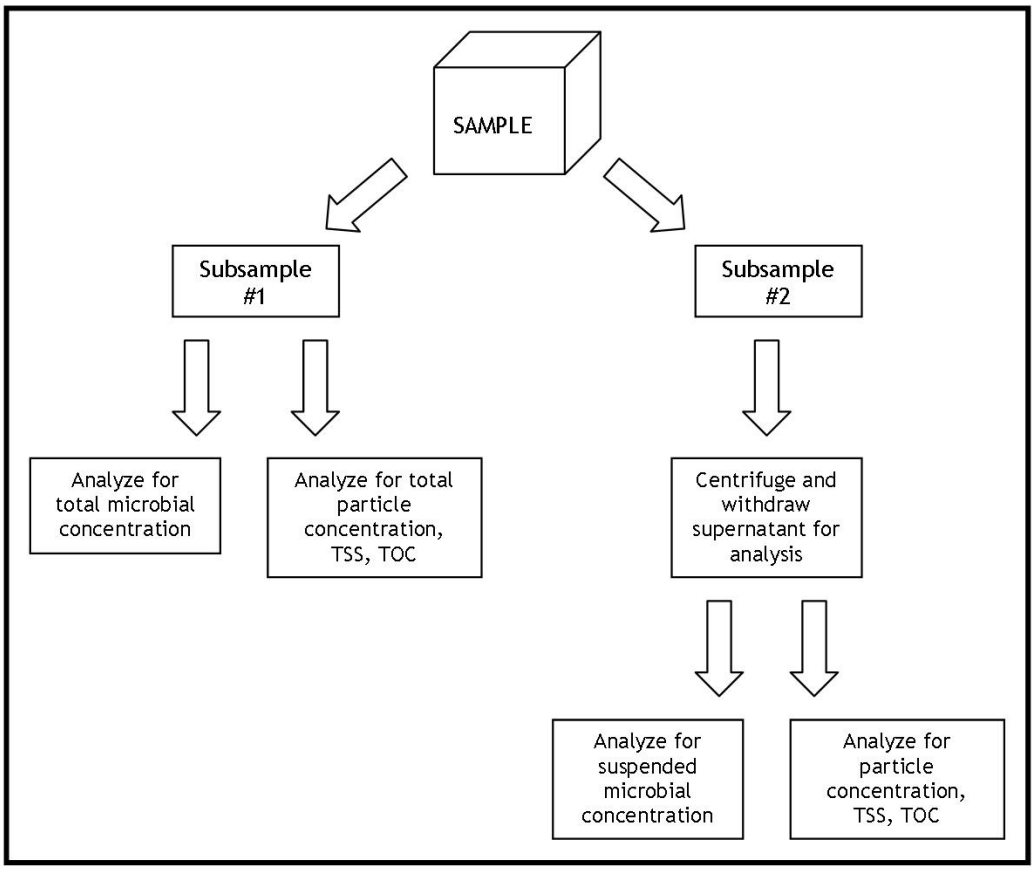


Figure 3. Partitioning technique

The selection of centrifugation settings is based on experiments using standardized particle suspensions detailed in Characklis et al (2005) and plotted in Figure 4. Glass particles (density = 2.65 g/cm^3, diameters = 5–60 µm) were used as a surrogate for inorganic particles such as clays or silicates, while latex particles (density = 1.05 g/cm^3, diameters = 5–40µm) were used as a surrogate for organic particles (density = 1.01-1.2 g/cm^3) and/or free phase microorganisms (density = 1.05-1.3 g/cm^3) (AWWA 1999; Bratbak and Dundas 1984; Chapra 1997; Holt 1994; Linsley et al. 1992; Lovins et al. 2002; Metge et al. 2003; Rohrmann and Krueger 1970; Tisa et al. 1982). Centrifugation via this regimen removed over 97% of the glass particles from suspension,

but left over 80% of the latex particles in suspension (including essentially all latex particles less than 10 µm in diameter).

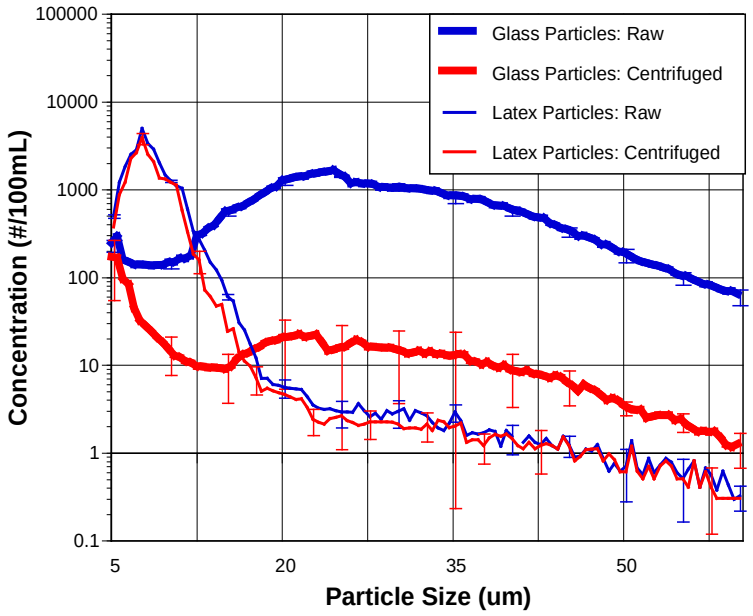


Figure 4. Calibration of 2000 rpm (1164g) partitioning technique (Characklis et al. 2005)

Subsequent application of this procedure to stormwater grab samples from Meeting of the Waters Creek, Booker Creek, and the Eno River led to the removal of approximately 90% of particles, but less than 10% of the organic carbon, indicating that the vast majority of the particles removed were inorganic (Characklis et al 2005). Comparable results were obtained from later analysis of Northeast Creek samples, with an average of 95% of particles removed and only 5% of TOC removed. Analysis of raw environmental samples, centrifuge supernatant, and centrifuge pellets using a mass-balance approach confirmed that microbial population sizes and particle size distribution were essentially unaltered by the procedure, indicating no significant loss of microorganism viability via centrifugation (Fries et al. 2006).

A direct relationship between the fraction of microorganisms defined as settleable by the partitioning regimen and particle concentration might indicate that differential settling, rather than cell-particle associations, was responsible for microbial removal by centrifugation. The settleable fraction of fecal coliforms, *E. coli*, and enterococci was correlated with total particle concentration in samples from Northeast Creek (Spearman's test, α=0.05). Because this relationship did not extend to samples collected from other stream sites or other microorganisms, it is unlikely that increases in cell-particle collisions resulted in a significant amount of differential settling. Instead, the relationship observed in the Northeast Creek samples may have been due to increased availability of attachment sites, or other environmental factors (i.e. microbial strain/origin, soil chemistry) that were not specifically targeted by this project.

Partitioning for protozoan analysis

Because *Cryptosporidium* oocysts and *Giardia* cysts are up to three times the size of the bacteria previously separated in partitioning studies, there was concern that the original centrifugation calibration settings might remove free (oo)cysts from samples. USEPA Method 1623 (USEPA 2005) for *Cryptosporidium* and *Giardia* detection requires centrifugation of water samples at 1500g for concentration of (oo)cysts, a setting just slightly higher than the original 1164g centrifugation setting. Consequently, samples for parasite enumeration and partitioning were centrifuged at a lower speed (73g force).

Standardization experiments were conducted to confirm that this reduced centrifugation speed provided sufficient discrimination between organic and inorganic particles and are detailed in Cizek et al. (2008). Centrifugation at 73g (500 rpm) for 10 min removed 99% of particles in a polydispersed suspension of glass beads (size range 2-

20 µm; density of 2.65 g/cm^3) (Figure 5). Results from centrifugation of a monodispersed suspension of latex beads (sizes: 5, 10, 20, and 43 µm; density of 1.05 g/cm^3) are given in Figure 6. Roughly 90% of the particles remained in suspension after centrifugation, with 98% of 5 µm particles recovered and 77% of 10 um particles recovered. Since indicator organisms and (oo)cysts are less than 10 µm in diameter and of similar density to latex particles, it was assumed that the majority of unassociated, free organisms would remain in suspension.

Samples collected for comparison of *E. coli* detection via Colilert and qPCR were also partitioned using this reduced speed centrifugation method. Calibration experiments appeared to show sufficient discrimination between likely organic and inorganic densities, and a lower centrifugation speed provides a more conservative estimation of the number of settleable particles and microorganisms. On average in these samples, 55% of particles greater than 5 µm in diameter were removed, while 93% of TOC remained in suspension.

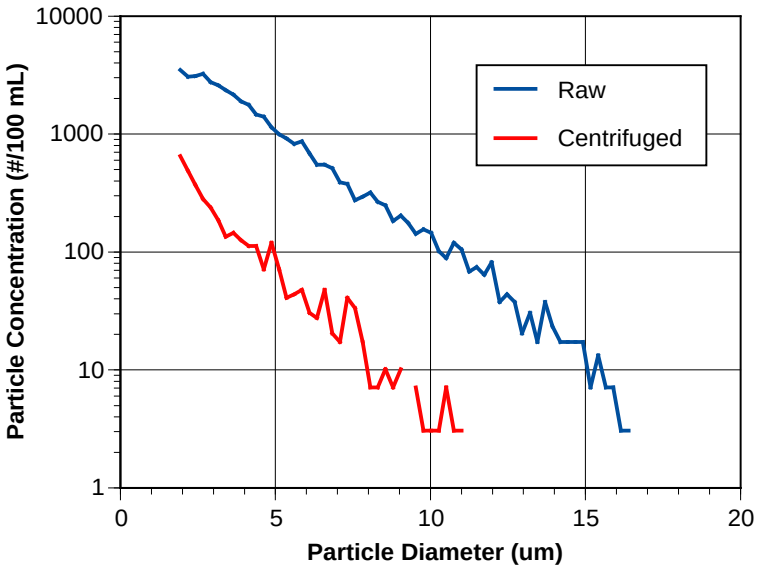
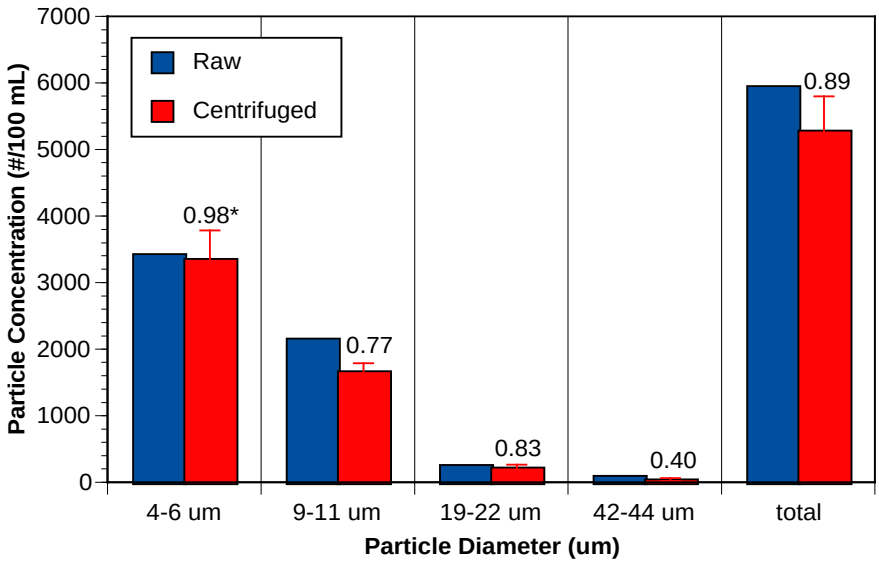


Figure 5. Calibration of 500 rpm (73g) partitioning technique for glass beads



*average supernatant fraction recovery

Figure 6. Calibration of 500 rpm (73g) partitioning technique for latex beads

4. Microbial analyses

Following partitioning, intra-storm raw samples and the supernatants from Meeting of the Waters Creek and Eno River were analyzed separately for five different indicator organisms (fecal coliforms, *E. coli*, enterococci, *C. perfringens* spores, and total coliphage) Northeast Creek samples were simultaneously analyzed for six indicators (fecal coliforms, *E. coli*, enterococci, *C. perfringens* spores, somatic and F+, or male-specific, coliphage) and *Salmonella* spp. bacteria. A subset of Northeast Creek samples were analyzed for two protozoan parasites in addition (*Cryptosporidium parvum*, and *Giardia lamblia*). Indicator organism and *Salmonella* analyses began within 24 hours of sampling. Samples for protozoan analysis were processed through concentration, purification, and staining steps within 72 hours. Microscopic enumeration of (oo)cysts was completed within one week.

Indicator organisms

Fecal coliform, *E. coli*, and enterococci concentrations were determined using the Colilert-2000® and Enterolert® procedures, respectively (IDEXX, Westbrook, Maine, USA). Two Quanti-trays were used for Colilert® and Enterolert® analyses to double the sample size and thereby reduce MPN confidence intervals. For detection of only thermotolerant fecal coliform and *E. coli*, the Colilert® method was modified through incubation of Quanti-trays at 37^{o}C for 4 hours followed by 20 hours at 44.5^{o} C (Chihara et al. 2004; Yakub et al. 2002). The Enterolert® procedure for enterococci detection was not modified, with all trays incubated at 41^{o} C (Simmons III et al. 2003; Yakub et al. 2002).

Subsample aliquots for *C. perfringens* spore detection were heated at 65^{o} C for 20 minutes to inactivate vegetative bacteria. After heating, an MPN procedure using iron-milk medium was used to estimate spore concentration. MPN tubes were incubated at 41^{o} C for 18 to 24 hours and then examined for stormy fermentation with visible gas production (positive result) (AOAC 1995; St John et al. 1982).

Coliphage concentrations were enumerated using EPA Method 1602 (USEPA 2000). Briefly, samples are added to liquid tryptic soy agar supplemented with magnesium chloride and a bacterial host, allowed to solidify, and then incubated at 37^{o} C for 18 to 24 hours. After incubation, clear zones of lysis (plaques) were enumerated. Intrastorm samples from Meeting of the Waters Creek and Eno River were analyzed for total coliphage concentrations using *E. coli* C3000 as a host. Frequent overgrowth by waterborne bacteria at times made the plates difficult to read. Because much heavier microbial contamination was expected in Northeast Creek, somatic and F+ (male-specific) coliphage concentrations were determined separately in these samples using antibiotic resistant hosts to prevent growth by non-host aquatic flora. Liquid agar was supplemented with nalidixic acid and *E. coli* CN13 for somatic coliphage enumeration and streptomycin-ampicillin and *E. coli* F_{amp} for F+ coliphage enumeration.

Pathogens

Salmonella concentrations were determined for each water sample using a multiple-tube method similar to that described in work by Hill and Sobsey (2001). Triplicate sets of buffered peptone water bottles were inoculated with four sample volumes (100 mL, 10 mL, 1 mL, 0.1 mL) and incubated at 37^{o} C for 21 +/- 3 hours as a pre-enrichment to recover and propagate injured cells. After incubation, 100 µL of each

bottle of enriched sample was transferred to a tube of selective Rappaport-Vassilades broth and incubated at 41 ^{o} C for 24 hours. One loopful of liquid from each tube was streaked onto Salmonella-Shigella (SS) agar and incubated for 24 hours at 37 ^{o} C. Suspect colonies (circular, black, surrounded by clear ring of lysis) were confirmed as *Salmonella* spp via the Enterotube biochemical test (Beckton, Dickinson & Co., NJ). The presence of one or more *Salmonella* colonies on the SS agar indicated a positive tube. Four-tube MPN tables were used to determine concentration (lower detection limit = 0.4 MPN/100 mL).

Cryptosporidium and *Giardia* concentrations were determined simultaneously via EPA Method 1623 (USEPA 2005). Samples for protozoan analysis were collected in quantities of at least 12 liters, providing ten liters for protozoan and two liters for indicator partitioning and analysis. After partitioning,a 5.0 L raw water sample and the 5.0 L supernatant sample were each filtered through a 1 micron pore size filter for (oo)cyst collection (Envirochek HV, Pall Corp., East Hills, NY, USA). (Oo)cysts were then eluted using an elution buffer and wrist-action shaking, concentrated by centrifugation at 1500g, separated from particulate matter by immuno-magnetic separation (Dynabeads GC Combo, Invitrogen Corp., Carlsbad, CA, USA), stained using an antibody-based, FITC fluorescent stain (AquaGlo GC, Waterborne, Inc., New Orleans, LA, USA), and visualized using epifluorescence microscopy. Internal positive controls (ColorSeed, BTF Pty Ltd, Sydney, Australia) were added to all appropriate samples, raw and centrifuged, prior to (oo)cyst analysis. ColorSeed vials provide a flow-cytometer confirmed quantity of fluorescently-labeled (Texas Red) cysts and oocysts that can be distinguished from wild-type parasites during microscopic enumeration via the use of

different epifluorescent filters. Enumeration of these labeled (oo)cysts provides a percent recovery value for each sample analyzed. Recovery values for ColorSeed (oo)cysts are similar to recoveries of wild-type (oo)cysts (Warnecke et al. 2003), and allowing calculation of actual total sample concentrations.

5. Quantitative PCR

Sample collection

Two-liter samples were collected from six in-stream sites and partitioned into raw and supernatant fractions via the calibrated centrifugation technique detailed previously. An aliquot from each fraction was appropriately diluted and analyzed for fecal coliform and *E. coli* concentrations via the previously described Colilert-2000® defined substrate method (Figure 7). An additional volume of sample from each fraction was filtered through a 47 mm, 0.45 µm polycarbonate filter (Millipore Isopore, Fisher Scientific), washed with 25-50 mL laboratory grade distilled water, aseptically transferred to a Whirl-Pak bag, and stored at -20^{o} C until qPCR analysis (Figure 7). Filtered volumes ranged from 1 mL to 100 mL of sample, as heavy contamination by suspended sediments periodically resulted in filter clogging and an inability to filter a full 100 mL. An effort was made using previously collected water quality data to capture a minimum of 50 cells per filter. Filters for qPCR analysis were prepared in duplicate with resultant concentrations averaged for analysis (Appendix G). Analysis of filter-captured cells via qPCR occurred within three months of sample collection.

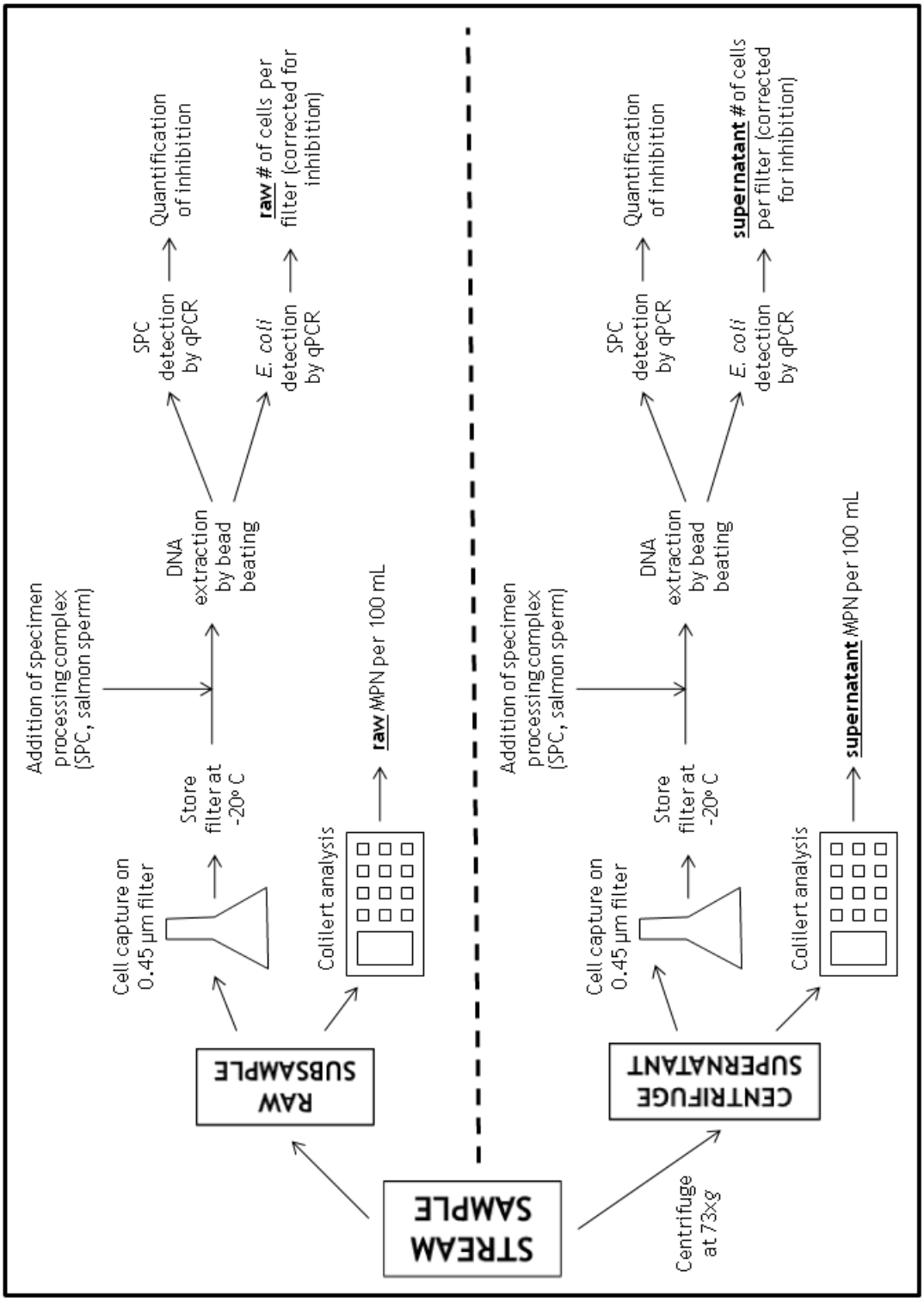


Figure 7. Comparison of Colilert and qPCR measures of *E. coli* concentration

DNA extraction

Prior to qPCR analysis, DNA from the filter-captured cells was extracted via a bead-beating technique similar to that described in Haugland et al. (2005). Sample filters were transferred from the Whirl-Pak bags to 2 mL screw-top microcentrifuge tubes containing 0.3 g of pre-sterilized 1 mm silica zirconium beads (BioSpec Corp., Bartlesville, OK). Using a pipette, 500 µL of AE buffer (QIAGEN, Valencia, CA) was added to each tube, which was then homogenized at maximum speed for 2 minutes using a BioSpec 8-place bead beater. Tubes were removed, centrifuged at 12,000x*g* for 1 minute to remove cellular debris, and the top 125 µL of supernatant was transferred to a sterile 1 mL microcentrifuge tube. These tubes were centrifuged for an additional five minutes at 12,000x*g* to further purify the sample. The top 100 µL of supernatant was transferred to a new sterile 1 mL microcentrifuge tube and stored at 4^{o} C until qPCR analysis. Samples were analyzed within 72 hours of extraction.

Exogenous control for quantification of potential inhibition

Inhibition of the qPCR reaction by humics or other sample matrix molecules is a frequent problem in the analysis of environmental samples that can result in false negatives or reduced detectable concentrations (Haugland et al. 2005; Noble and Weisberg 2005; Reynolds et al. 1997). Quantification of and subsequent correction for inhibition in an unknown sample matrix can be achieved through the addition of a known quantity of nonindigenous cells to the samples prior to processing and analysis (exogenous control). In the present study, chum salmon (*Oncorhynchus keta*) sperm cells, which have been used as an exogenous control in previous studies targeting indicator bacteria in natural waters (Haugland et al. 2005; Morrison et al. 2008), were used

quantify and correct for potential PCR-inhibition by components of the sample matrix. Lyophilized *O. keta* sperm cells were obtained from Sigma-Aldrich Co., reconstituted in water, and stored at -20 C until analysis. *O. keta* cells were spiked into the AE buffer prior to DNA extraction at a concentration of 10 ng (10^5 cells), with *O. keta* DNA thereby extracted with *E. coli* DNA simultaneously via bead beating (Figure 7). Following qPCR analysis, samples were considered inhibited if the *O. keta* threshold cycle (C_t) value was more than 1.5 cycles greater than the average C_t value from a non-diluted (10^5 cells) *O. keta* calibration value (example calculation, Appendix F). Inhibited samples were subjected to a ten-fold dilution with reagent-grade water and re-analyzed via qPCR to confirm sufficient removal of inhibitor compounds.

Generation of standard curves

To control for possible loss of DNA during bead beating or incomplete extraction, 500 µL of AE buffer spiked with 10 ng *O. keta* cells was added to a 2 mL screw-top centrifuge tube containing a sterile 0.45 µm 47-mm polycarbonate filter and 0.3 g of sterile 1-mm silica zirconium beads. Following extraction, serial log dilutions of this *O. keta* positive sample were analyzed via qPCR and used to generate a standard calibration curve. Calibration curves were used to determine exponential amplification and efficiency of the qPCR reaction, and analysis of the undiluted *O. keta* positive controls provided uninhibited C_t values for comparison with *O. keta* values from potentially inhibited spiked environmental samples (example calculations, Appendix F). Efficiencies ranged from 86-98%.

E. coli for generation of standard curves was grown at 37^o C and formalin fixed. Following enumeration via SYBR Green (Noble and Fuhrman 1998), 10^5 cells were

filtered through a 0.45 µm 47 mm filter and stored at -80 C. The filter was subjected to the bead-beating extraction technique and the resultant extract serially diluted and analyzed to generate a standard calibration curve. Efficiencies ranged from 88-90%.

qPCR analysis

Extracted samples were analyzed for *O. keta* sperm DNA (exogenous control) concentration to assess potential inhibition, diluted if necessary, and then analyzed for *E. coli* concentration (Figure 7). Scorpion® primer-probe complexes were designed for *O. keta* sperm whole DNA matrix control after the Taqman chemistry design presented in Haugland et al. (2005). Forward and reverse primers and probes for *O. keta* detection and lyophilized Omnimix beads containing deoxynucleiotides, magnesium chloride, buffer, and *Taq* polymerase (Cepehid, Sunnydale, CA) were appropriately diluted in reagent grade water to create a master mix for *O. keta* detection. Using a pipette, 20 µL of this master mix and 5 µL of sample were transferred to 25 µL optical reaction tubes and inserted into a Cepheid Smart Cycler II system. Thermal cycling conditions were as follows: 120 s at 95^{o} C followed by 45 cycles of 15 s at 95^{o} C and 30 s at 60^{o} C.

qPCR analysis for *E. coli* was conducted using Cepheid Scorpion® primer and probe lyophilized beads (Cepheid, Sunnydale, CA). This set of forward and reverse primers and probe targets the *uidA* gene, which codes for the enzyme *β*-glucuronidase which is responsible for 4-methyl-umbelliferyl (MUG) metabolism. Metabolism of MUG results in the fluorescent signal indicating positive *E. coli* growth in the Colilert method (IDEXX, Westbrook, Maine). Primer and probe sequences are proprietary. Cepheid *E. coli* lyophilized beads containing the *E. coli*-specific primer and probe set and Omnimix lyophilized beads were appropriately diluted in reagent-grade water to make a qPCR

master mix. Using a pipette, 20 µL of this master mix and 5 µL of sample were transferred to 25 µL optical tubes and inserted into a Cepheid Smart Cycler II system. Thermal cycling conditions were as follows: 120 s at 95^{o} C followed by 45 cycles of 5 s at 95^{o} C followed by 43 s at 62^{o} C. Threshhold cycle (C_t) values for each sample were determined after manually adjusting the threshold fluorescence value to 8 units, which corresponded to the point of maximum slope of the cycle-fluorescence curve. Samples were considered nondetectable if the fluorescence curve did not cross the threshold following 45 cycles.

Calculation of E. coli concentration

E. coli concentrations were determined using the cycle threshold values obtained during qPCR analysis. Measurements were corrected for potential inhibition of the qPCR reaction by components of the sample matrix through calculations of the relative difference of *E. coli* and *O. keta* (exogenous control) C_t values as described in Haugland et al. (2005). Briefly, the *E. coli* concentration is calculated via the following equation:

$$C_{observed} = C_o * EA^{-\Delta\Delta Ct} \qquad (Eq'n.\ 1)$$

Where $C_{observed}$ = sample concentration; C_o = concentration of *E. coli* in the nondiluted positive control; EA = exponential amplification; and $\Delta\Delta C_t$ = to the difference in ΔC_t values between the observed sample values and the calibration values, or:

$$\Delta\Delta C_t = (\Delta C_t)_{observed} - (\Delta C_t)_{calibration} \qquad (Eq'n.\ 2)$$

Where ΔC_t is equal to the difference in C_t values of the target (*E. coli*) and the exogenous control (*O. keta* sperm cells), or:

$$\Delta C_t = C_{t,\ target} - C_{t,\ exogenous\ control} \qquad (Eq'n.\ 3)$$

Because only one *uidA* gene should be present per *E. coli* cell, concentration units are reported as number of cells per volume. An example calculation is provided in Appendix F.

6. Physical analyses

In addition to microbial analysis, both the raw sample and centrifuge supernatant were analyzed for particle number concentration and total organic carbon (TOC) concentration during all sampling efforts. Particle analysis for intrastorm samples was performed via a Met-1 light-blockage instrument, with a measurement range of 5 to 100 µm. Analysis of all subsequent project samples was conducted using a Coulter Multisizer I (Coulter Electronics Ltd., Luton, England), with a measurement range of 2 µm to 60 µm. The Coulter Multisizer provides total particle number, surface area, and volume for each diameter size class. TOC concentrations were measured according to Standard Method 5310B using a Shimadzu TOC-5000 Combustion-Infrared analyzer. TSS concentrations for intra-storm samples and samples for comparison of *E. coli* analysis by Colilert and qPCR were evaluated using Standard Method 2540D (StandardMethods 1998). In the last data set comparing *E. coli* detection by IDEXX and qPCR, TSS data and particle volume data from the Coulter Multisizer were used to estimate average particle densities. Water temperature was determined for all samples on site using a field thermometer.

7. Statistical tests

Nonparametric statistical tests are generally considered most appropriate for analysis of microbial data from environmental samples, which are not generally normally

distributed (Tillett et al. 2001). The Shapiro-Wilk test was used to confirm non-normality in the microbial data sets collected for this project. Spearman rankings are among the most common statistic used to test for relationships between pathogens and indicator organisms (Brookes et al. 2005; Horman et al. 2004; Lemarchand and Lebaron 2003; Payment et al. 2000; Rouquet et al. 2000) and so were used to assess relationships between *Salmonella*, *Cryptosporidium*, and *Giardia* concentrations and indicator organisms. This test compares the directionality of data via rankings, and coefficients can range from -1 (perfect inverse relationship) to +1 (perfect direct relationship). The Wilcoxon matched-pairs signed-rank test was used to assess potential relationships between matched pairs of data across separate events (e.g. whether increases/decreases in concentration between two sites were consistent for all storms). In general, unless otherwise indicated, results were considered statistically significant at $\alpha = 0.05$.

IV. Results

1. Intrastorm variability in microbial partitioning and microbial loading rates

Geometric means for raw concentration data for the three sampled storms are provided in Tables 2 and 3, as well as average dry weather (background) conditions available from a previous study (Characklis et al. 2005). The full data set for each storm at each site, including sampling times and associated streamflows, is available in Appendix A.

Table 2. Geometric means and 95% confidence intervals for intrastorm microbial parameters

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perfringens* spores (MPN/100 mL)	Total Coliphage (PFU/100 mL)
Meeting of the Waters Creek		61,833	9,064	13,015	1,496	128
		34,621	**4,349**	**7,721**	**914**	**62**
	storm (n=17)	19,384	2,087	4,581	558	31
		9,741	469	383	70	25
		3,333	**163**	**183**	**26**	**14**
	dry (n=3)	1,140	56	87	9	8
Eno Rover		21,033	4,070	16,618	884	186
		17,542	**3,240**	**13,077**	**518**	**110**
	storm (n=14)	14,631	2,579	10,291	304	66
		1,424	178	111	176	15
		833	**42**	**32**	**20**	**5**
	dry (n=3)	488	10	9	2	2

Table 3. Geometric means and 95% confidence intervals for intrastorm physical parameters

		Particle Concentration (#/100 mL)	TSS (mg/L)	TOC (mg/L)
Meeting of the Waters Creek	storm (n=17)	93,790	270	5.1
		73,732	**160**	**4.6**
		57,963	95	4.2
	dry (n=3)	10,757	9.1	3.0
		10,338	**4.6**	**2.9**
		9,936	2.3	2.9
Eno Rover	storm (n=14)	85,356	83	6.2
		64,329	**53**	**5.4**
		48,482	34	4.6
	dry (n=3)	25,536	8.2	5.1
		18,065	**5.3**	**3.3**
		12,780	3.4	2.2

Initial partitioning analysis involved averaging the data from all samples at all sites to gain some idea of the general variability in partitioning behavior. Results are presented in a box and whisker plot in Figure 8 illustrating 10^{th}, 25^{th}, 75^{th}, and 90^{th} percentiles. In this case, the three bacterial indicators exhibited relatively consistent behavior on average, with the mean fraction of organisms associated with settleable particles ranging between 40% and 50%. Nonetheless, the fraction of settleable organisms observed extended over a relatively broad range. The average fraction of *C. perfringens* associated with settleable particles was also around 50%, but the median was close to 75% and the observed range was quite wide. Total coliphage showed the least evidence of particle association, with a median settlable fraction value of 5% and was somewhat less variable than the bacterial or protozoan indicators. The high fractions of particles and TSS removed, in combination with the very low rates of TOC removal, suggest that the vast majority of settleable material in the water column is inorganic. Low TOC removal by centrifugation suggests that a large quantity of the organic matter in the streamwater may be dissolved or colloidal.

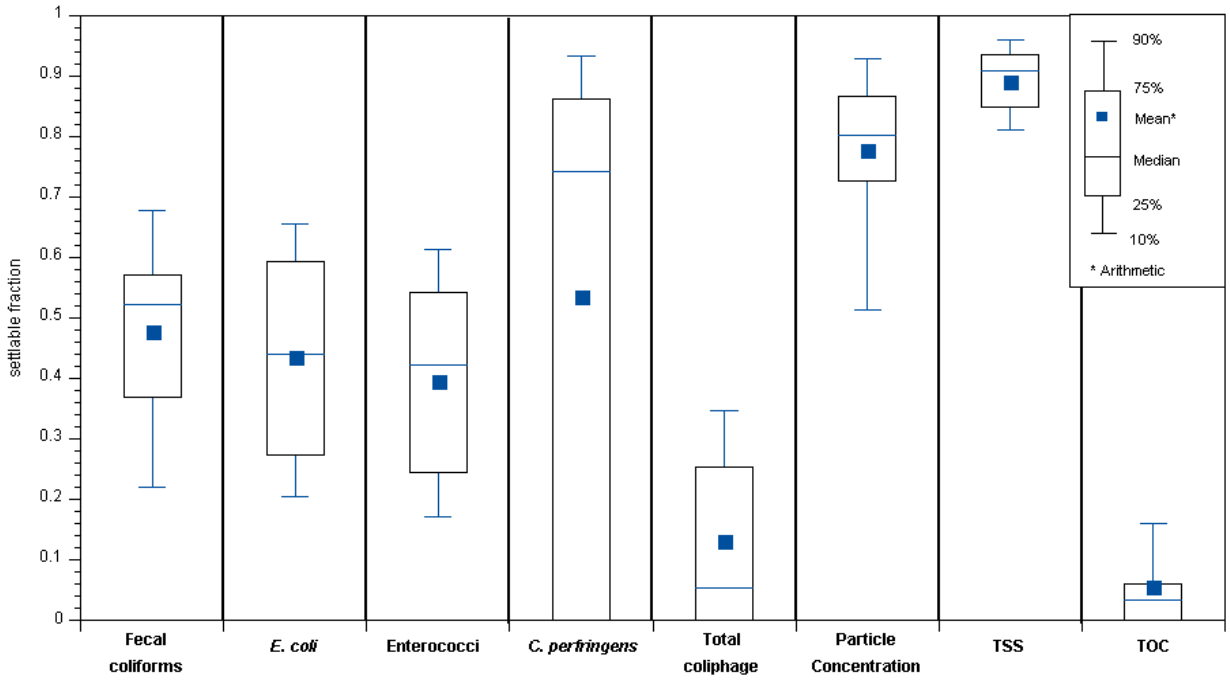


Figure 8. Average settleable fraction of stormwater concentrations in intrastorm samples

Data were also assembled to allow for an inspection of how the concentration (both total and settleable) of each parameter varied throughout the duration of individual storms (Figure 9). Measures of all eight parameters of interest (microbes, TOC, TSS, and particle concentration) over the course of three storms at both sites, led to the development of a total of 48 profiles similar to that in Figure 9. A visual inspection of these profiles suggested some trends in the data, most notably that settleable microbial concentrations (with the exception of total coliphage), and the settlable concentrations of particles, TSS and TOC, were generally highest in the period soon after the storm began. In order to quantitatively evaluate these trends over all storms at both sites, each storm was described in terms of three separate hydrograph stages: 1) the rising limb – the period of time from the beginning of hydrograph flow increase to one hour before peak flow; 2) peak – one hour before the maximum flow was recorded until one hour after; and

3) recession – one hour after the peak flow value until flow returns to baseline levels (Figure 9).

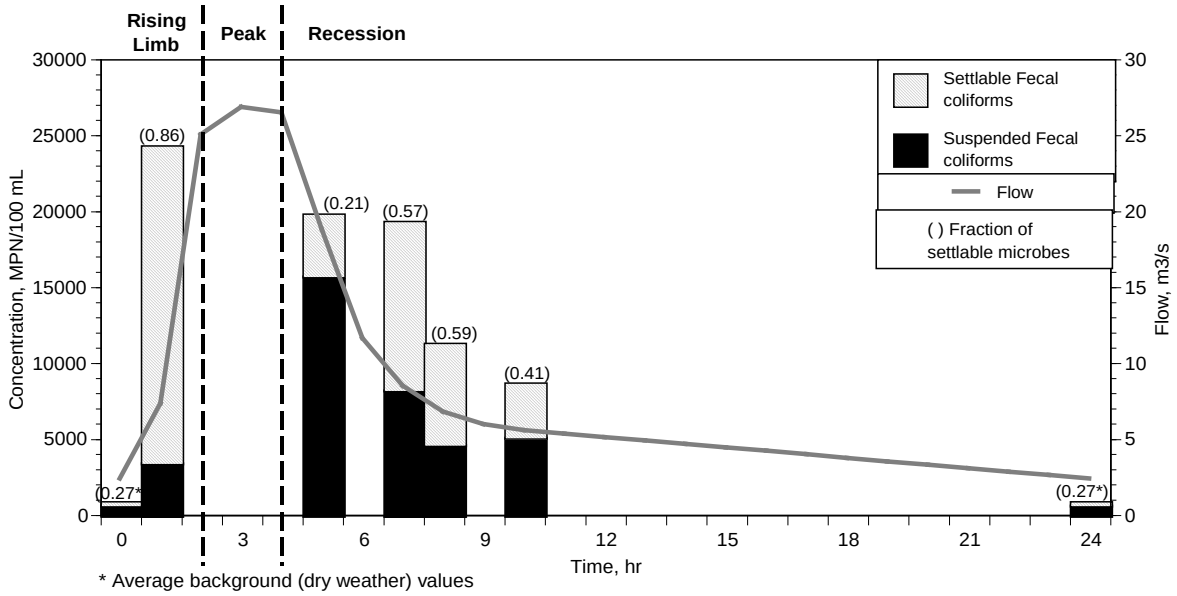


Figure 9. Sampling times and fecal coliform concentrations for Storm 1 at the Eno River

Concentrations of each parameter were averaged by respective storm stage. To preserve statistical independence, only one sample from each site was taken from each stage for any particular storm: these were the earliest sample within the rising limb stage, the sample closest to zero for the peak stage, and the last sample for the recession stage. This yielded a maximum of six values for each stage (one sample per storm per site). Data from previous work (Characklis et al. 2005) which involved single grab samples from the MWC and Eno sites were used in addition to the intra-storm data to increase sample size. These samples were collected during the same season, albeit one year earlier, and were analyzed using the same analytical procedures. The inclusion of these data increased the number of observations in the rising limb, peak, and recession stages to

six, eight, and twelve, respectively. Box and whisker plots illustrating 10th, 25th, 75th, and 90th percentiles are provided in Figure 10.

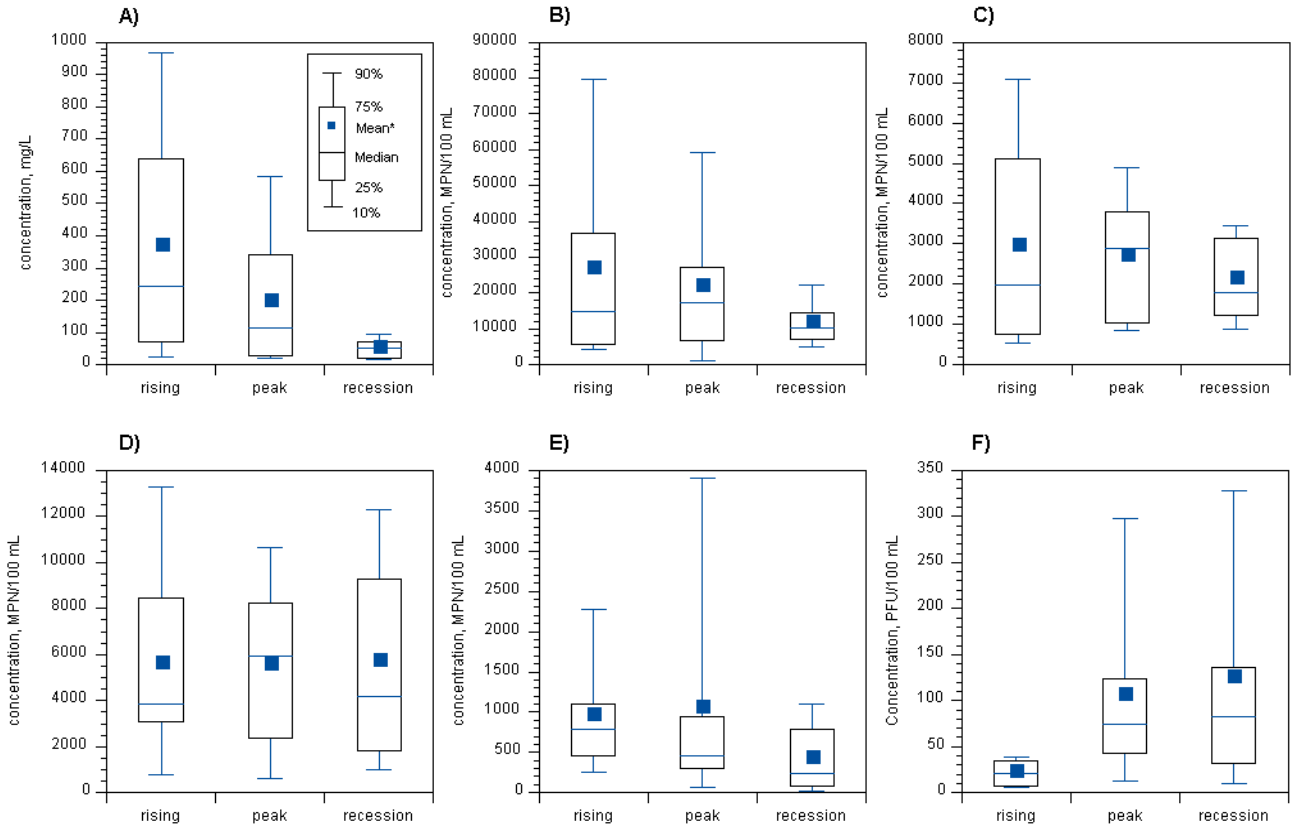


Figure 10. Intrastorm trends in the stormwater concentrations for A) total suspended solids (TSS) B) fecal coliforms C) *E. coli* D) enterococci E) *C. perfringens* and F) coliphage

Patterns in average total concentration appeared to differ across parameters. Average concentrations of TSS, fecal coliforms, and *E. coli* appeared to decrease as the storm progressed (Plates A, B, & C), which would be consistent with a “first flush” phenomenon. However, average enterococci and *C. perfringens* concentrations seemed to remain fairly constant (Plates D & E). Total coliphage behavior differed considerably from the bacterial and protozoan indicators, with averaged concentration increasing as the storm progressed (Plate F).

To further explore intra-storm trends in partitioning, average settleable fractions were determined for each storm stage (Figure 11). The settleable fraction of fecal coliform and *E. coli* remained relatively constant throughout storm duration (Plates B & C), while the average settleable fraction of TSS, enterococci and *C. perfringens* decreased (Plates A, D & E) as the storm progressed. It is interesting to note that the average concentrations of fecal coliforms and *E. coli* were highest in the early stages of a storm, so that even though the fraction of settleable organisms remained constant over the storm's duration, the loading rate of settleable organisms is highest in the rising limb. In the case of enterococci and *C. perfringens*, average concentrations were relatively constant throughout the storms, but the average fraction of settleable microorganisms was highest in the earlier stages. These results again suggest that the highest loading rates for settleable microorganisms occur during the rising limb. These results led to increased interest in both the rate of microbial loading throughout storms and the cumulative microbial loading occurring as result of a storm.

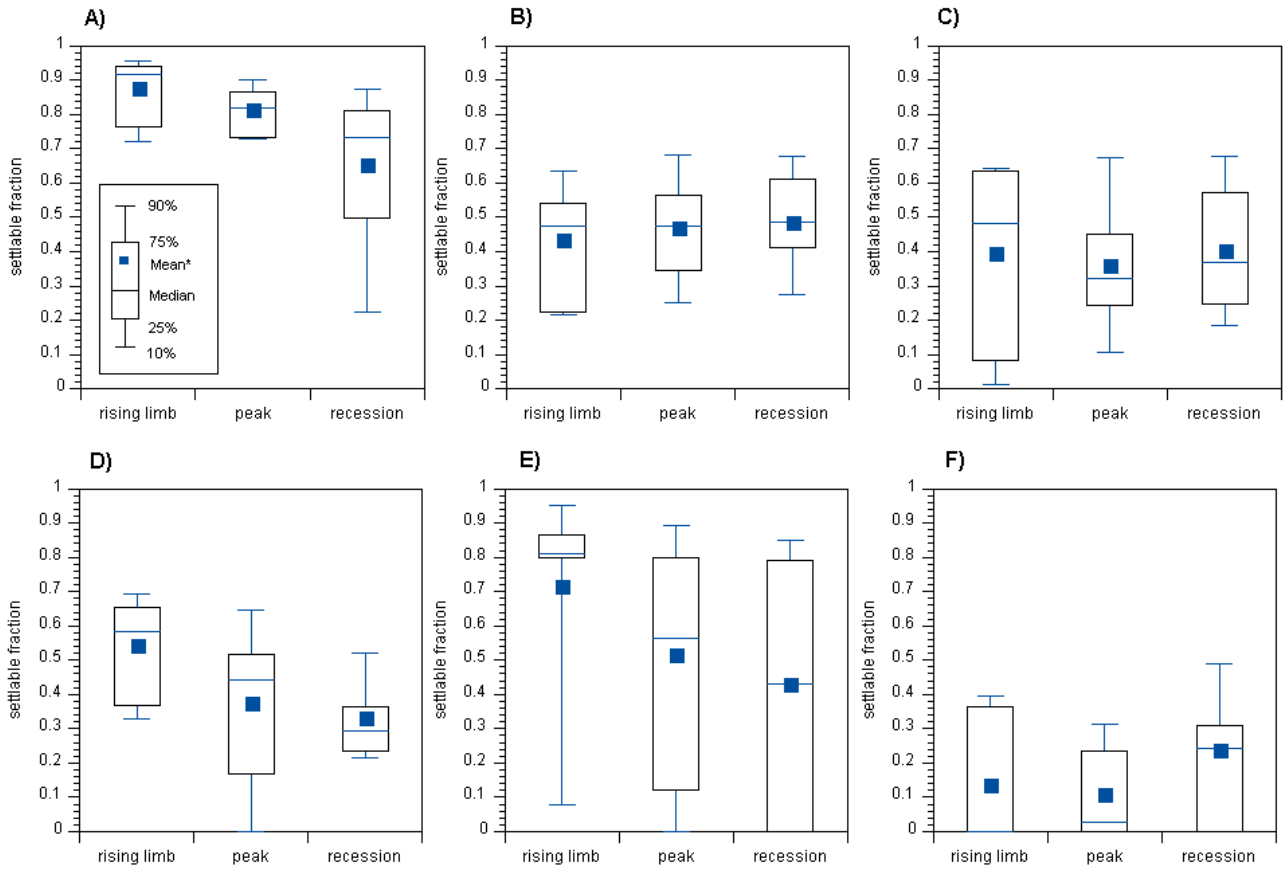


Figure 11. Intrastorm trends in the settleable fraction of stormwater concentrations for A) total suspended solids (TSS) B) fecal coliforms C) *E. coli* D) enterococci E) *C. perfringens* and F) coliphage

The cumulative storm loading of microbes entering these streams is one measure of stormwater impact on water quality. In order to calculate cumulative loads, the concentration of each parameter was estimated at points throughout the storm by linearly interpolating between measured concentrations. For example, if the microbial concentrations measured one and three hours after the onset of the storm were 10,000 and 20,000 organisms per 100 ml (with 40% and 50% of these organisms settleable, respectively), then the concentration two hours after the onset of the storm was estimated at 15,000 organisms per 100 mL (with 45% of these estimated as settleable). Average dry weather concentrations were taken as representative of the concentrations existing at

the beginning and end of the storm (i.e. when stream flow first increases and when it returns to baseline levels). Concentrations were estimated at 15 minute intervals (matching USGS flow data) and all concentration values (both estimated and measured) were then combined with flow data to estimate loadings for each interval over the course of the storm. These quantities were then summed to generate cumulative loading estimates for each parameter over each storm.

Dry weather microbial loadings for an equivalent period were also estimated by multiplying average background concentrations by average baseflow and summing these over the length of the storm. Subsequently, the cumulative wet-weather loading was divided by dry-weather loading to yield information on the relative magnitude of microbial contributions to the stream under both sets of conditions. For example, if one 20-hour period of storm loading was estimated at 10^{15} organisms, and one 20-hour period of background (dry-weather) loading was estimated at 10^{13} organisms, then the storm loading would be equivalent to the loading in 100 background periods. This type of comparison may be useful in determining how to most efficiently direct limited watershed restoration funds, by providing a measure of the relative contributions of more continuous, dry weather, microbial sources and the intermittent loadings attributable to storm events.

Sharp increases in the relative loadings of microbes, particles, and organic carbon strongly implicate storm induced mobilization from nonpoint sources as the primary contributor of contaminant loading in both watersheds (Figure 12). In some cases, wet-weather microbial loadings were over a thousand times that of dry weather loadings over the period of a storm. As most storm hydrographs represented a period of around 24

hours this indicates that a single day's worth of wet-weather loading can be the equivalent of several years' input during dry weather periods. These findings suggest that water quality improvement efforts in these watershed would be more productively focused on reducing storm-related, nonpoint source inputs to receiving waters, rather than more continuous, dry-weather discharges.

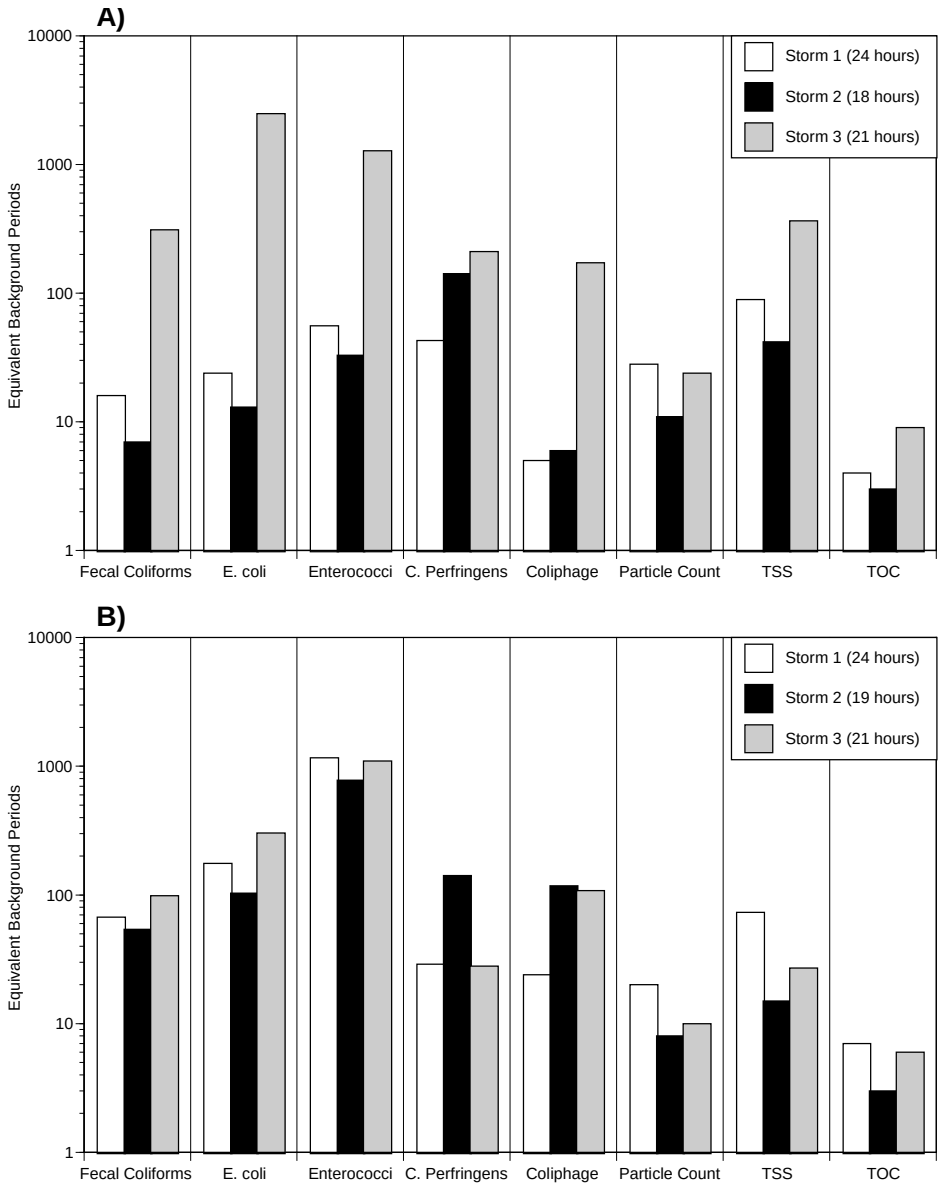


Figure 12. Equivalent periods of background loading for total stormwater loadings observed at A) Meeting of the Waters Creek and B) Eno River

The fraction of cumulative microbial loading associated with settleable particles was estimated in a similar manner, with measured values for the concentration of settleable microbes used to interpolate the settleable concentrations at unmeasured points throughout the storm. Settleable concentration values were also combined with flow data to produce loading estimates at 15 minute intervals, with these loads summed to provide a cumulative storm loading estimate of settleable microbes. These were then divided by the cumulative loading estimates for all microbes (settleable and suspended) to generate estimates of the settleable fraction of cumulative microbial loading for each storm at each site (Figure 13).

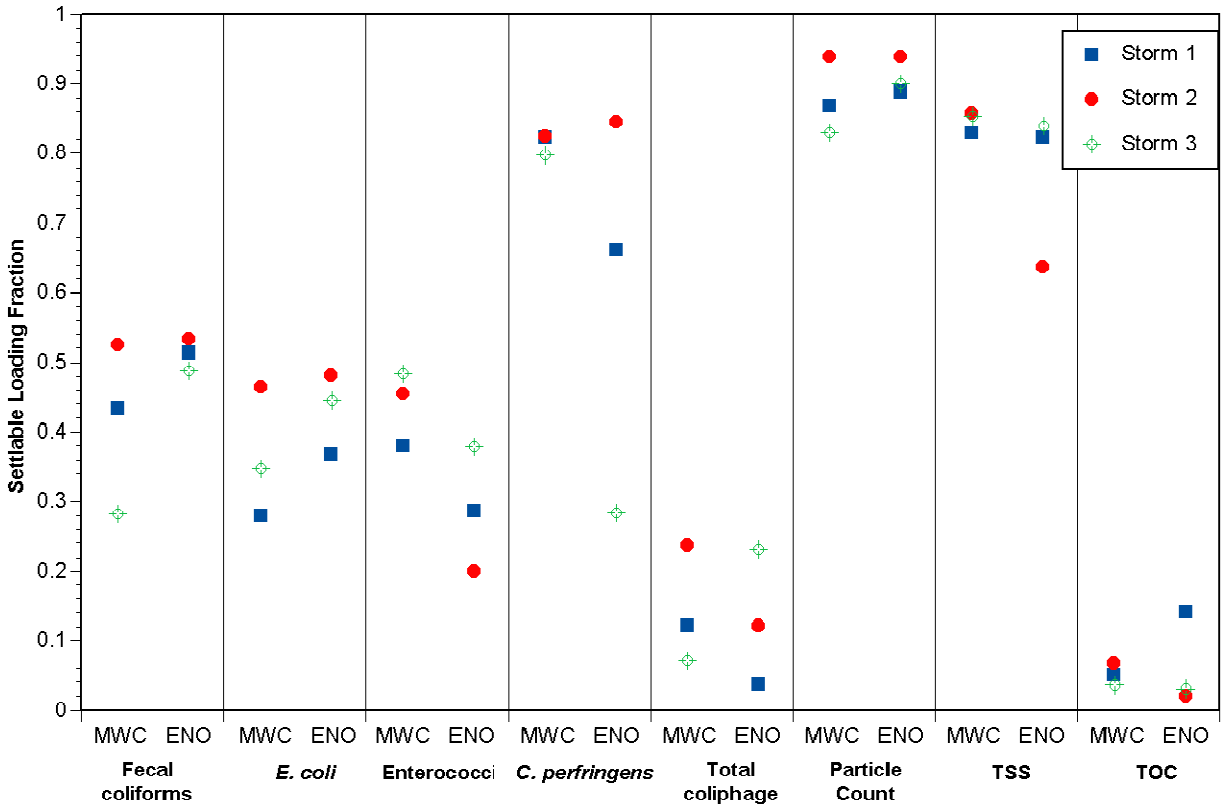


Figure 13. Average fraction of cumulative microbial loadings associated with settleable particles

The fraction of settlable loading across storms and watersheds was remarkably consistent for vegetative bacterial indicators. The settleable fraction of cumulative microbial storm loading for the bacterial indicators averaged 46%, 40%, and 37% for fecal coliforms, *E. coli*, and enterococci, respectively. *C. perfringens* spores, on the other hand, were much more likely to be associated with settleable particles, with settleable spores accounting for over 65% of the cumulative loading in all but one storm. Total coliphage exhibited very different behavior, with less than 15% of the cumulative storm loading classified as settleable. The fraction of settleable particles and TSS was high at both sites (averages of approximately 90% and 80%), while little organic carbon (TOC) was settleable (average = 6%), consistent with observations indicating that the vast majority of settleable particles are inorganic.

While the cumulative loading of both settleable and suspended microbes may provide useful information, microbial loading rate throughout the course of a storm also has important implications. Since it would generally be impractical to treat all of the stormwater generated by a storm, information on loading rate could provide some indication of the effectiveness of measures capable of capturing and treating some portion of total runoff (e.g. detention basins). To compare loading rates across storms of different length and at different sites, mass-volume plots were drawn describing cumulative stormwater loadings for each storm at each site using the methodology presented in (Betrand-Krajewski et al. 1998). Average mass-volume plots were constructed by plotting the average cumulative mass for each cumulative volume value (Figure 14).

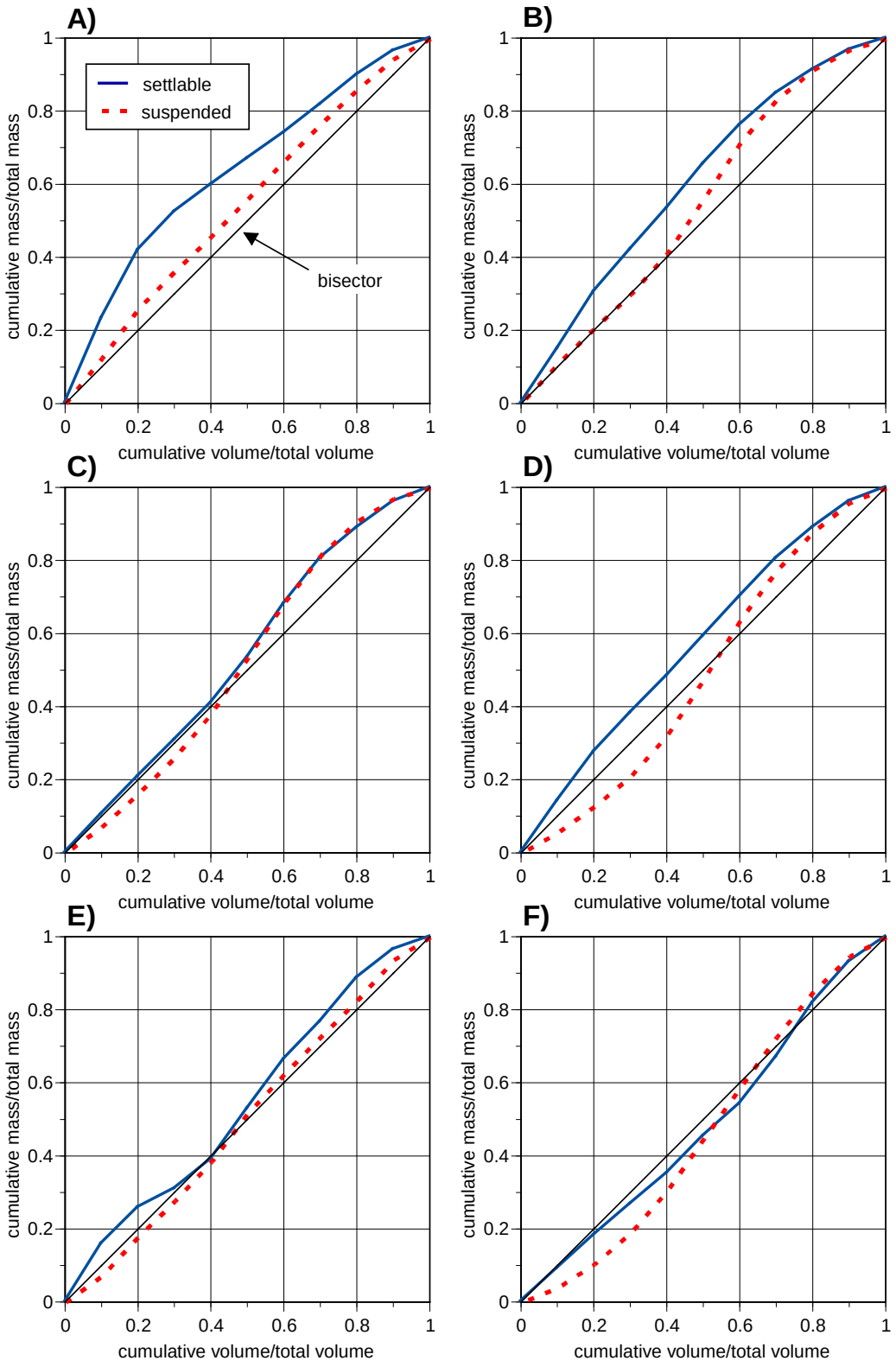


Figure 14. Cumulative loading distributions for A) total suspended solids B) fecal coliforms C) *E. coli* D) enterococci E) *C. perfringens* F) total coliphage

Plate A shows the loading of settleable TSS preceding the loading of nonsettleable TSS, with 50% of settleable solids and 30% of nonsettlable solids contained by the first 30% of stormwater volume. A similar pattern was observed for fecal coliform and enterococci (Plates B, & D), and though less pronounced, for *E. coli*, *C. perfringens* spores, and total coliphage as well (Plates C, E & F). While these results do not fulfill the 80/30 rule (80% of contaminant loading contained by first 30% of stormwater volume) confirming a first flush event (Betrand-Krajewski et al. 1998), they do suggest that the largest proportion of settlable organisms are contained by the first 50% of stormwater volume in these watersheds. If these trends are consistent in other basins of similar size, landuse, and other hydrogeographic conditions, sedimentation may provide an especially cost effective means of reducing the total microbial load entering receiving waters, as a smaller detention basin capturing these earlier flows may remove a disproportionately large fraction of total microbial load. It is important to note however that desired microbial removal rates could not likely be achieved for these specific watersheds based solely on sedimentation as only 50% or less of the total loading of any microorganism (with the exception of *C. perfringens spores*) was particle-associated. Removal of the entire loading of particle-associated organisms would therefore not achieve even a one-log scale reduction in concentration. Nonetheless, this information could be useful in the design of detention basins or other BMPs that depend either entirely, or in part, on sedimentation to affect microbial removal.

The extent to which the microbial partitioning behaviors observed in this study for different microbial indicators (viruses, bacteria and spores as protozoan surrogates) also occur for actual microbial pathogens is uncertain. Therefore, further studies are needed to

determine if the partitioning behaviors observed for these indicators is also true for waterborne enteric microbial pathogens.

As a final attempt to analyze the data, efforts were made to link microbial loading rates (settleable and total) with storm intensity, duration, or antecedent weather conditions, but the results yielded little evidence of strong predictive relationships.

2. Usefulness of indicator organisms as surrogates for *Salmonella* in an urban watershed

Indicator organism and Salmonella incidence

Salmonella spp. were recovered from 23 of 35 (66%) dry weather samples and 45 of 48 (94%) stormwater samples. This storm-related increase in the frequency of *Salmonella*-positive samples was significantly significant (Fischer's exact test, p= 0.001). All sampling points, including street runoff, detention basin inflows and outflows, and in-stream sites were positive for *Salmonella* on multiple occasions. Of the twenty-five samples that met the EPA recommended criterion of 235 *E. coli* per 100 mL for recreational contact (USEPA 1986), fourteen (56%) were positive for *Salmonella* (concentration range: 0.5 - 93 MPN/100 mL) (Figure 15), suggesting that waters in compliance with current indicator organism-based water quality standards may still pose some health risk. All samples with concentrations less than 235 *E. coli* per 100 mL were collected during dry weather conditions, i.e. all storm samples exceeded this threshhold.

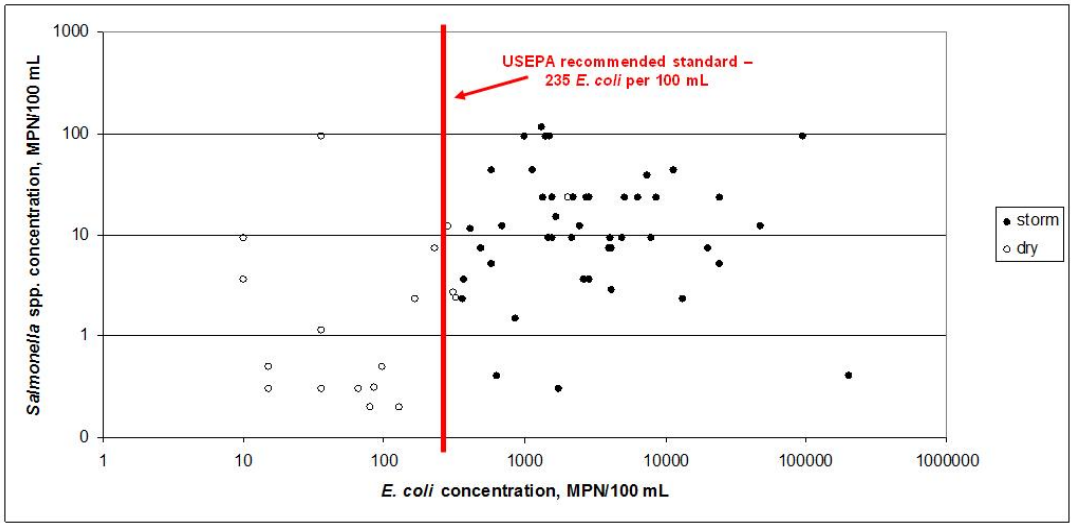


Figure 15. *Salmonella* spp. vs. *E. coli* incidence in the Northeast Creek watershed

Geometric mean concentrations of indicator organisms and *Salmonella* spp. during dry weather sampling were at least one order of magnitude less than concentrations observed during storms (Table 4), suggesting that stormwater loadings are responsible for the majority of microbial loadings to this receiving water. Spatial variability in the concentration of all microorganisms was lower across samples sites during dry weather, but higher during storms, with storm concentrations particularly high in the upland detention ponds. During storm events, mean concentrations of all indicator bacteria and *Salmonella* were higher in the effluent of both suburban pond sites than in samples from stream site 1 just downstream. This difference was statistically significant (Wilcoxon, α=0.05) for fecal coliform, *E. coli*, enterococci, *C. perfringens* spores, and *Salmonella* in pond 1 and for fecal coliforms and *C. perfringens* spores when comparing pond 2 effluent and stream site 1. In contrast to the bacteria, both somatic and male-specific coliphage concentrations were generally higher in the stream site samples than in any of the upland sites.

Salmonella spp. concentrations at stream site 2 were expected to be particularly high relative to the other sites, as this site is just downstream from a waterfowl impoundment and avian species are often suspected carriers of *Salmonella* bacteria (CDC 2008). Although mean *Salmonella* concentrations increased as the creek flowed downstream, the increase was not statistically significant (Wilcoxon, α=0.05). Geometric mean concentrations of all indicator organisms, except fecal coliforms, were actually lower at stream site 2 than the more urbanized stream site 1 (upstream of the impoundment). The highest single sample concentrations of *Salmonella* (115 MPN/100

mL) were detected in samples from the wet ponds. This is notable, as the pond catchment areas are highly urbanized with no wildlife or agricultural areas in evidence.

Table 4. Geometric means of microbial observations and 95% confidence intervals (number of observations in parenthesis)

			Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp., (MPN/100 mL)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)
DETENTION POND 1			141,926	8,846	35,321	497	4	1	868
	runoff		**47,254 (5)**	**1,902 (5)**	**10,801 (5)**	**183 (4)**	**1 (3)**	**0.1 (4)**	**2 (4)**
			15,733	409	3,303	68	0.1	0	0
	influent	storm	119,889	20,844	71,280	671	28	87	218
			43,082 (8)	**5,904 (8)**	**16,262 (8)**	**262 (7)**	**6 (6)**	**2 (6)**	**71 (7)**
			15,482	1,673	3,710	102	1	0	23
		dry	1,995	7	128	1		0.2	5
			721 (6)	**5 (5)**	**37 (6)**	**0.1 (6)**	**0 (5)**	**<0.1 (5)**	**0.2 (5)**
			261	3	10	0		0	0
	effluent	storm	128,090	11,963	69,938	2,546	75	19	1,184
			57,677 (8)	**5,904 (8)**	**29,974 (8)**	**712 (7)**	**3 (6)**	**1 (7)**	**633 (7)**
			25,971	2,914	12,846	199	0.1	0	339
		dry	5,063	34	766	234	1	1	39
			2,138 (6)	**22 (5)**	**375 (6)**	**95 (6)**	**0.1 (5)**	**<0.1 (5)**	**1 (5)**
			902	14	184	38	0	0	0
DETENTION POND 2			463,128	927	45,955	1,008	27	235	1,047
	runoff		**95,956 (4)**	**383 (4)**	**26,139 (4)**	**468 (3)**	**3 (3)**	**2 (3)**	**153 (3)**
			19,881	158	14,868	218	0.4	0	22
	influent	storm	210,761	30,521	78,525	2,522	63	42	653
			73,055 (8)	**7,112 (8)**	**30,356 (8)**	**1,585 (7)**	**3 (7)**	**4 (7)**	**263 (6)**
			25,323	1,657	11,735	997	0.2	0	106
		dry	16,784	134	354	1,467	18	6	20
			7,509 (5)	**26 (4)**	**219 (5)**	**669 (5)**	**1 (5)**	**0.2 (5)**	**6 (4)**
			3,360	5	136	305	0.1	0	2
	effluent	storm	127,848	10,855	43,942	1,017	18	9	443
			51,519 (8)	**4,193 (8)**	**18,189 (8)**	**346 (7)**	**1 (7)**	**1 (7)**	**102 (7)**
			20,760	1,620	7,529	118	0.1	0	24
		dry	11,073	188	1,928	1,464	2		
			2,092 (4)	**79 (3)**	**450 (4)**	**446 (4)**	**1 (4)**	**0 (3)**	**0 (3)**
			395	33	105	136	0.2		
STREAM SITE 1		storm	17,973	2,918	16,049	383	5	41	1,159
			13,305 (11)	**2,138 (11)**	**9,326 (11)**	**233 (10)**	**3 (10)**	**20 (9)**	**850 (10)**
			9,850	1,566	5,419	142	1	9	624
		dry	2,740	248	307	87	1	4	109
			1,322 (10)	**86 (9)**	**149 (10)**	**59 (10)**	**0.1 (10)**	**1 (9)**	**34 (9)**
			638	30	72	40	0	0	11
STREAM SITE 2		storm	38,827	2,963	19,226	177	17	41	1,031
			20,786 (6)	**1,700 (6)**	**8,990 (6)**	**83 (6)**	**9 (6)**	**14 (5)**	**622 (6)**
			11,128	976	4,204	39	5	5	375
		dry	5,026	246	1,779	103	5	32	102
			2,522 (6)	**116 (5)**	**453 (6)**	**51 (6)**	**4 (6)**	**2 (5)**	**36 (5)**
			1,266	55	115	25	0	0	13

Salmonella spp. were significantly correlated (Spearman, α=0.05) with the presence of all the indicator organisms examined with the exception of male-specific

coliphage (Table 5), suggesting that these indicators are reasonable sentinels of *Salmonella* presence in Northeast Creek. Correlations between the incidence of fecal coliform, *E. coli*, enterococci, and somatic coliphage and *Salmonella* were of similar strength (ρ_s=0.46-0.52), while the correlation between *C. perfringens* spores and *Salmonella* incidence was somewhat weaker (ρ_s=0.24), though still significant. Although male-specific coliphage have been identified as strong indicators of fecal pollution and human health risks at recreational beaches (Colford et al. 2007), they are not always present at adequate concentrations to serve as pathogen indicators in waters primarily contaminated by stormwater (Ferguson et al. 1996; Horman et al. 2004). Theoretically, an ideal indicator should always be present at concentrations exceeding pathogen concentrations (Savichtcheva and Okabe 2006), but in Northeast Creek, male-specific coliphage concentrations only exceeded *Salmonella* concentrations in 50% of samples. Additionally, *Salmonella* was actually recovered from 20 of 26 samples (77%) in which no male-specific coliphage were detected.

Table 5. Spearman coefficients (ρ_s) correlating indicator organism and *Salmonella* spp. incidence

Indicator organism	type	ρ_s	p-value	n
Fecal coliforms	bacteria	0.52	<0.0001	83
E. coli	bacteria	0.51	<0.0001	77
Enterococci	bacteria	0.48	<0.0001	83
C. perfringens spores	sporulated bacteria	0.24	0.03	83
Male-specific coliphage	virus	0.17	0.15	74
Somatic coliphage	virus	0.46	<0.0001	76

No minimum or threshold indicator organism concentration could be identified as an entirely reliable measure of pathogen presence or absence (e.g. consistent absence of *Salmonella* when *E. coli* was below a given concentration): *Salmonella* was detected in

samples with *E. coli* concentrations as low as 10 MPN/100 mL. Consequently, despite statistical correlations relating incidence, relatively low concentrations of indicator organisms do not preclude the presence of *Salmonella* bacteria in the Northeast Creek watershed.

Particle association

The centrifugation-based partitioning technique removed a high fraction of total particle number, while the vast majority of total organic carbon (TOC) remained in suspension, confirming that the procedure largely removed inorganic and associated microorganisms (Figure 16). Association with settleable particles appeared to differ by microbial type, which is in keeping with previous studies investigating indicator organism partitioning behavior (Characklis et al. 2005; Cziek et al. 2008; Krometis et al. 2007). The average settleable fraction of *Salmonella* bacteria was most similar to that of the traditional fecal indicator bacteria, fecal coliforms, *E. coli*, and enterococci (25-35% associated). Although *Salmonella* bacteria showed a much wider range in terms of settleable fraction, this may be a result of the uncertainty inherent in current methods of microbial detection, particularly at low concentrations. Higher variability in *Salmonella* behavior would therefore be expected, as observed concentrations were generally two to four orders of magnitude less than that of the fecal indicator bacteria. Similarly, the mean values presented in Figure 16 suggest that male-specific coliphage may associate with particles at a slightly higher rate than somatic coliphage; however, it is worth noting that a smaller number of samples evaluated for male-specific coliphage met the minimum analytical threshhold of 3 microbes per 100 mL for inclusion in partitioning analysis less often, resulting in a smaller sample size. Adding male-specific and somatic coliphage

concentrations to obtain total coliphage values yielded results similar to those observed in earlier studies, with less than 10% of total coliphage identified as particle-associated (Characklis et al. 2005; Krometis et al. 2007).

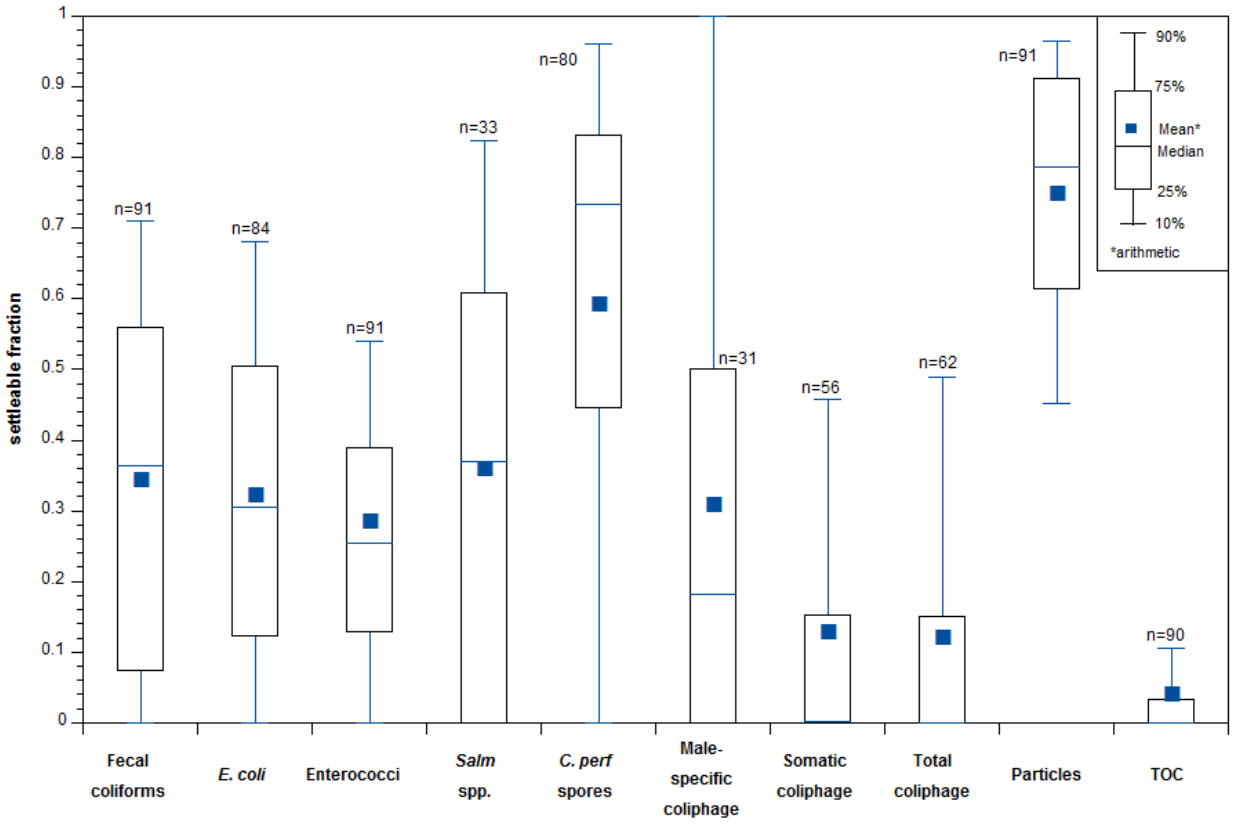


Figure 16. Average settleable fractions of indicator organisms and *Salmonella* spp.

There remains some debate on whether fecal indicator bacteria are the best surrogates for *Salmonella* in receiving waters, mostly centering on the occasional recovery of viable *Salmonella* from waters in which coliforms or enterococci are absent (Morinigo et al. 1990; Polo et al. 1998). However, results from the present study indicate that these bacteria are the most reasonable surrogates from amongst the suite of potential indicator organisms examined here. Although *C. perfringens* spores have been promoted as more conservative markers of fecal pollution due to their prolonged survival in the

environment (Medema et al. 1997), they were only weakly correlated with the presence of *Salmonella* in Northeast Creek. In addition, a much larger fraction of *C. perfringens* spores were particle-associated (>50%) than *Salmonella,* suggesting differences in settling behavior as *C. perfringens* would be likely to settle out of the water column more quickly. While the association between *Salmonella* and somatic coliphage was strong, a much lower fraction of viral particles were particle-associated, indicating that a larger portion of these microbes may remain in suspension for longer periods of time. Only the fecal indicator bacteria (fecal coliforms, *E. coli*, enterococci) exhibited both a strong statistical correlation with *Salmonella* presence and similar partitioning behavior (i.e. presumably similar transport properties), suggesting that these bacterial indicators, while imperfect, remain the most potentially useful surrogates for *Salmonella,* whether from a monitoring or modeling perspective.

Attempts to correlate water quality parameters with observed changes in microbial partitioning behavior provided limited additional insights. Increases in the settleable fractions of fecal coliform, *E. coli,* and enterococci were correlated with increases in total particle concentration (Spearman's test, α=0.05); however, no statistically significant relationship existed between particle concentration and attached fractions of *Salmonella,* coliphage, or *C. perfringens* spores. The lack of consistency in this trend across microorganisms argues against differential settling as responsible for observed microbial reductions after centrifugation. Increases in fecal indicator bacteria settleable fraction may instead be due to increased available sites for attachment. Changes in TOC concentration were not correlated with changes in the settlable fraction of any microorganism. Further investigation would be required to determine whether

other unexplored environmental factors, such as microbial origin/strain (human, wildlife, etc.), contribute to fluctuations in microbial partitioning behavior.

Wet pond efficiency

Samples were collected at the inflow and outflow points of two suburban wet ponds during eight storm events and six times during dry weather. Although North Carolina design regulations indicate that discharge from wet ponds should cease two to five days after significant rainfall, there was sufficient effluent to allow sampling of pond 1 outflow on all dry weather sampling trips. Additionally, effluent microbial concentrations in pond 1 frequently exceeded influent concentrations during dry weather, with the increase statistically significant for *E. coli*, enterococci, and *C. perfringens* spores, suggesting that the pond might actually be a source of microorganisms. While in the case of *Salmonella* there was no statistically significant increase between influent and effluent concentrations in pond 1, *Salmonella* were recovered from the pond effluent on three occasions when influent concentrations were zero. Sufficient outflow for sampling from pond 2 was only present during three of the six dry weather sampling trips. Effluent concentrations from pond 2 exceeded influent concentrations for each bacteria type at least once over the three sampling events; however, the number of samples was insufficient for statistical analysis.

Wet pond removals of all six indicator organisms were highly variable over storm events, with no consistent trends exhibited to conclusively indicate that the ponds were either removing or exporting microbes. Figure 17 illustrates raw effluent vs. influent concentrations for *E. coli*, the primary freshwater regulatory organism, and *Salmonella*. Although the USEPA describes a “typical” microbial removal rate for detention ponds as

65% (2007), microbial concentrations were occasionally higher in the effluent than in the influent for both ponds.

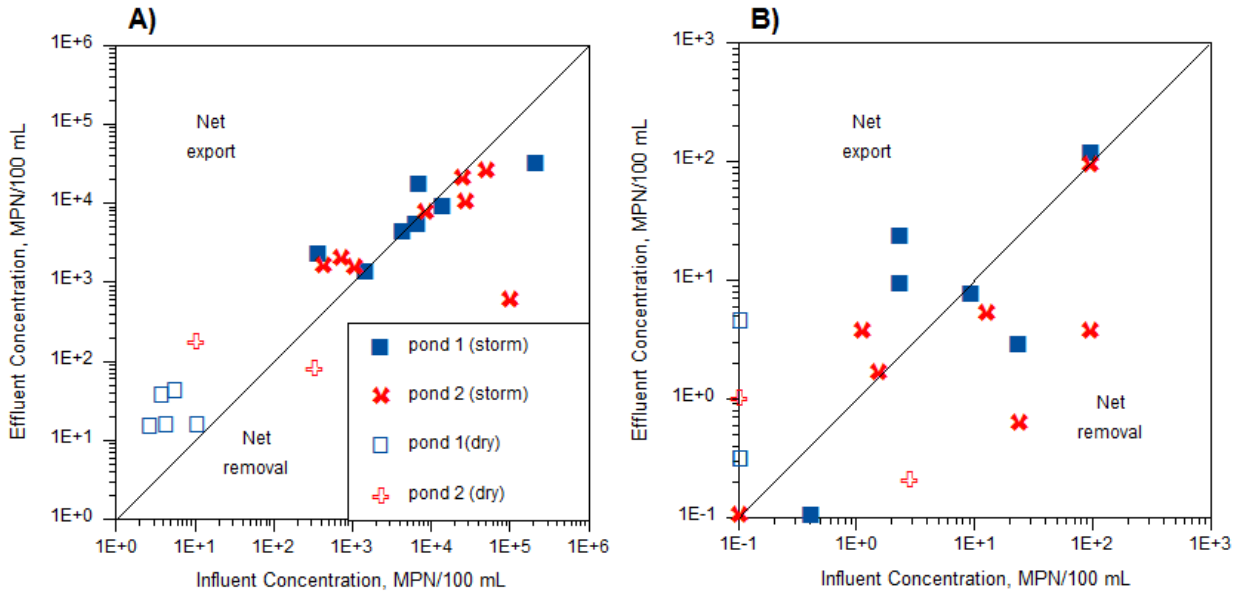


Figure 17. Wet pond influent vs total effluent concentrations during storm events for A) total *E. coli* and B) total *Salmonella* spp.

Single sample microbial removals for each microorganism in each wet pond were calculated for each storm event using an approach commonly employed by the EPA to assess BMP performance (USEPA 2007):

$$SSR = 1 - [C_e/C_i] \qquad (Eq'n.\ 4)$$

where SSR=single sample removal; C_e=effluent concentration; and C_i=influent concentration. Single sample removals were not entirely consistent across microorganism types for a given storm event; i.e. no storm produced simultaneous increases or decreases in pond effluent concentration for all microbes. Particle-associated microbes would be expected to exhibit faster sedimentation velocities, therefore higher fractions of settleable microbes in the influent would be expected to result in higher removal rates. The Spearman rank correlation coefficient was used to investigate potential correlations

between the "settleable" (particle-associated) fraction of microbes in the influent and the observed single sample removal. There was a statistically significant positive correlation between the particle-associated fraction and removal of *C. perfringens* spores in pond 2 ($\alpha<0.05$), and a positive correlation between the particle-associated fraction of *E. coli* and *E. coli* removal was almost significant for both pond 1 and pond 2 ($\alpha=0.09$ and $\alpha=0.06$, respectively). No statistically significant relationships were identified for any of the other microorganisms.

Mean concentration removals are frequently used to assess the performance of stormwater BMPs (USEPA/ASCE 1999) and other water treatment structures (Karpiscak et al. 2001); and the geometric mean is commonly used to average microbial concentrations (Davies and Bavor 2000; Hill and Sobsey 2001; Mallin et al. 2002). Geometric mean concentration removals were used to calculate overall, or "average", pond performance using the following equation:

$$MCR_{geo} = 1 - [C_{e,geo}/C_{i,\ geo}] \qquad \textit{(Eq'n. 5)}$$

where MCR_{geo}=geometric mean concentration removal; $C_{e,geo}$=the geometric mean of all effluent concentrations; and $C_{i,geo}$=the geometric mean of all influent concentrations. Mean concentration removals of settleable concentrations were determined in addition to removals of total (overall) concentration in order to determine whether particle-association leads to greater rates of removal. Both total and settleable mean concentration removals are given in Table 6.

Table 6. Wet pond geometric mean removal of total and settleable microorganisms (n=number of storms). Positive values indicate a net removal of microorganisms, while negative values indicate a net export.

		Pond 1	Pond 2
Fecal coliforms	total	-0.34	0.29
n = 8	settleable	1.0	0.24
E. coli	total	0.00	0.41
n = 8	settleable	0.99	0.67
Enterococci	total	-0.84	0.4
n = 8	settleable	-13	-6.6
***Salmonella* spp.**	total	0.85	0.61
n = 6/7 (pond 1/2)	settleable	-1.3	0.90
***C. perfringens* spores**	total	-1.7	0.64
n = 7	settleable	0.57	1.0
Male-specific coliphage	total	0.84	0.98
n = 7	settleable	0.94	0.98
Somatic coliphage	total	-8.0	0.61
n = 7	settleable	n/a*	0.86

*average concentration of viral particles in influent = 0

Removal of both the total and the settleable concentration of microorganisms appeared to differ between the two detention ponds. While pond 2 exhibited some overall removal of all targeted microorganisms, mean overall removals for pond 1 were either negative (indicating net export) or zero for all microbes except *Salmonella* and male-specific coliphage. Removal of *Salmonella, C. perfringens* spores, and both types of coliphage was greater than 60% for pond 2, and removal of *Salmonella* and male-specific coliphage was greater than 80% in pond 1. Despite these promising average removal rates, it is worth noting that effluent fecal indicator bacteria concentrations from both ponds remained several orders of magnitude greater than typical water quality standards, and *Salmonella* spp. generally remained detectable (Table 4). A two to three log removal (99-99.9%) of microbial influent loadings, much higher than the 65% removal rate described as typical by the USEPA, would likely be required for the pond effluent to meet current water quality standards.

With the exception of enterococci in both ponds and S*almonella* in pond 1, mean concentration removals of settleable microorganisms were equal to or exceeded the total mean concentration removals computed based on single storm samples. This suggests that particle-associated cells are more likely to be removed by detention ponds than their free-phase counterparts, and that sedimentation may be an important microbial removal mechanism. In the case of enterococci however, fairly dramatic increases in settleable concentrations were observed in pond effluents relative to influent concentrations. While there may be many reasons for this increase, previous studies have noted that gram-positive enterococci survive longer in the environment than gram-negative bacteria (fecal coliform, *E. coli*), and that much higher concentrations can be present in bottom sediments (Davies et al. 1995; Jeng et al. 2005). While somewhat speculative, it is possible that this increased persistence could lead to higher levels of particle-associated enterococci becoming resuspended from underlying pond sediment during high flows.

Differences in performance between the two ponds may be attributable to differences in design. Although located less than a kilometer apart, the pond structures differ considerably. The length:width ratio of pond 2 is greater than 2:1, resulting in a more consistent hydraulic residence time, while pond 1 is nearly circular and likely more susceptible to short-circuiting. Also, the effluent pipe of pond 1 is roughly 1 m in diameter, while pond 2 has a small diameter effluent pipe that likely results in a longer hydraulic residence time. It should be noted however that while on average pond 2, which more closely adhered to the county regulations for stormwater BMPs, did reduce influent microbial concentrations, these reductions were relatively modest. Only reductions in male-specific coliphage exceeded 1 log, and the geometric mean

concentration removals of fecal indicator bacteria (fecal coliforms, *E. coli*, enterococci) were roughly equal to or slightly less than the 65% typical removal value given by USEPA for stormwater BMPs (USEPA 2007). Nonetheless, effluent concentrations often exceeded water quality standards by several orders of magnitude, suggesting that detention ponds alone may be insufficient for remediating microbially impaired stormwaters.

Net exports of microorganisms by both ponds were observed several times in both ponds during dry weather and storm events. Several studies have noted that under favorable conditions, indicator bacteria and *Salmonella* may actually reproduce in the environment (Burton et al. 1987; Fish and Pettibone 1995; LaLiberte and Grimes 1982; Lee et al. 2006). As warm temperatures, ponded or low-flow areas, and association with nutrient-rich protective sediments have been identified as conditions particularly conducive to microbial regrowth (He et al. 2007), accumulation and reproduction of microorganisms in these detention ponds between storm inputs seems quite possible. Later resuspension of these microorganisms by high stormflows or wildlife could therefore be responsible for observations of microbial export. Under these conditions, the ponds might actually serve as a source for additional microbial loadings eventually discharged to Northeast Creek.

3. Comparison of indicator organism and protozoan partitioning behavior

Nine samples (five dry weather, four storm events) were collected from Northeast Creek at stream site 1 and analyzed for the protozoans *Cryptosporidium parvum* and *Giardia lamblia* in addition to *Salmonella* spp. and the six indictor organisms targeted previously. Geometric means of microbial, particle, and total organic carbon concentrations are provided in Table 7, while the complete set of data is provided in Appendix D. Only four samples met the USEPA recommended instantaneous standard for *E. coli* (235 *E. coli*/100 mL). At least one of the three targeted pathogens was detected in three of these samples (Figure 18); no pathogens were detected in the sample with the lowest *E. coli* concentration (14 MPN/100 mL). While the average concentration of all indicators, as well as *Salmonella* bacteria and *Giardia* cysts, increased during storm events relative to background levels, the average concentration of *Crypto* oocysts decreased. This result is potentially interesting; however, the sample size is small and a wide range of *Crypto* concentrations were observed during storm events (0-12 oocysts/L).

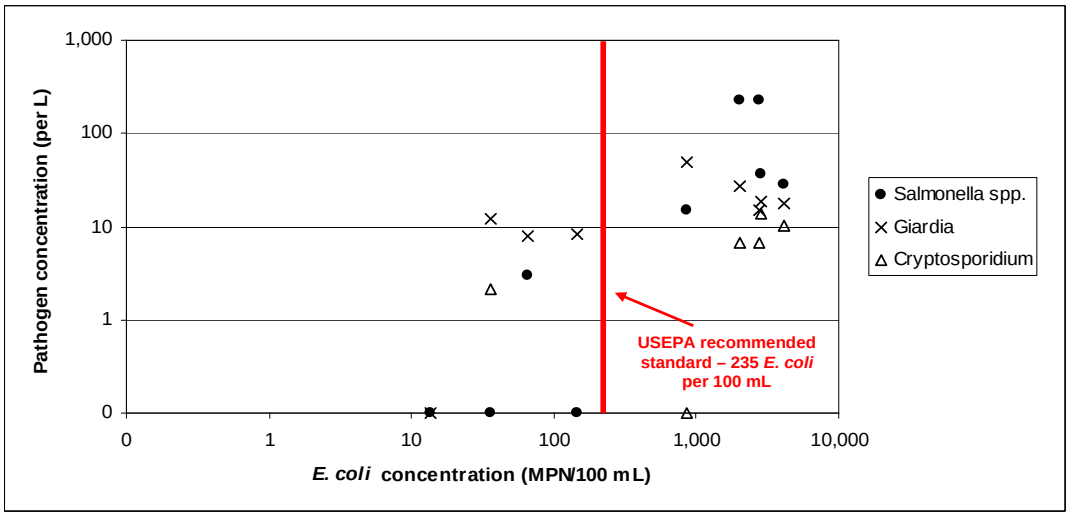


Figure 18. Pathogen vs. *E. coli* incidence at stream site 1, Northeast Creek

Table 7. Geometric means and 95% confidence intervals for protozoan sampling

	Fecal coliforms (MPN/100 mL)	***E. coli* (MPN/100 mL)**	**Enterococci (MPN/100 mL)**	***C. perfringens* (MPN/100 mL)**	**F+ coliphage (PFU/100 mL)**	**Somatic coliphage (PFU/100 mL)**
	5,123	522	584		6	94
dry	**1,260**	**99**	**135**	**43**	**0.4**	**23**
(N = 5)	310	19	31		0	6
	15,133	4,546	12,207	491	45	1,656
storm	**11,312**	**2,301**	**5,175**	**366**	**12**	**757**
(N = 4)	8,506	1,181	2,232	275	4	351
	***Salm.* (MPN/100 mL)**	***Giardia* (cysts/L)**	***Crypto* (oocysts/L)**	**Turbidity (NTU)**	**Particles (#/100 mL)**	**TOC (mg/L)**
	1	66	13	33	1.43E+07	9.4
dry	**0.02**	**2**	**6**	**24**	**7.75E+06**	**7.6**
(N = 5)	0	0	3	18	4.19E+06	6.1
	14	38	82	240	5.86E+07	10.1
storm	**4**	**22**	**1**	**184**	**4.37E+07**	**6.6**
(N = 4)	1	13	0	142	3.27E+07	4.4

When considering this data, it is important to note that protozoan recoveries from stream samples were low, with an average recovery of 24% for *Giardia* and just 8% for *Crypto*. The average recovery for *Crypto* was just below the acceptance criteria (11-100%) set forth in USEPA Method 1623 (USEPA 2005). Similarly low recoveries were observed in another protozoan partitioning study conducted in the same lab over the same time period (Cizek et al. 2008). Repeatedly low recoveries were addressed through analysis of ColorSeed-spiked distilled laboratory grade water in ongoing precision recovery (OPR) assays run in parallel with later samples (n=3). Recovery in these OPR samples was much higher for *Crypto* (28%) and comparable for *Giardia* (25%), suggesting that components of the stream sample matrix may have inhibited oocyst recovery. Because *Crypto* recoveries were particularly low in some natural samples, occasionally only 1 or 2%, three stream samples with an average recovery of less than 3% were excluded from correlation and partitioning analysis. Neither *Crypto* nor *Giardia* recovery was correlated with any water quality parameters, and did not differ significantly between raw and centrifuged or dry weather and storm event samples. Recoveries associated with individual stream samples and OPR samples are provided in detail in Appendix E.

Spearman correlation coefficients comparing protozoan incidence with indicator organism concentrations and water quality parameters are given in Table 8. Because of the small number of samples available, an α of 0.10 was selected as a marker of association. Both protozoans were significantly correlated with *E. coli* and enterococci concentrations, which are the most common freshwater and marine indicator bacteria used in regulatory programs. *Giardia* cysts were also associated with the presence of

several other microorganisms: fecal coliforms, somatic and male-specific coliphage, and *Salmonella*. *Crypto* concentrations were very strongly associated with total organic carbon concentrations ($\alpha < 0.01$), while *Giardia* concentrations were associated with higher particulate and turbidity concentrations. *Crypto* and *Giardia* concentrations were not significantly correlated with one another. Some previous studies of (oo)cyst presence in freshwaters have reported correlations between the presence of the two protozoans (Chauret et al. 1995; Rose et al. 1991), while studies of other watersheds have reported no correlation in presence or absence (Kistemann et al. 2002; Rouquet et al. 2000). This may indicate that the sources of these pathogens in receiving waters are different and perhaps watershed-specific.

Table 8. Spearman coefficients comparing protozoan incidence and water quality parameters (*statistically significant, α=0.10)

	Giardia (n=9)	*Crypto* (n=6)
Fecal coliforms	0.63*	0.49
E. coli	0.67*	0.87*
Enterococci	0.72*	0.78*
C. perfringens	0.47	0.51
Somatic coliphage	0.60*	0.55
F+ coliphage	0.82*	0.20
Salmonella	0.67*	0.47
Giardia	-	-0.12
Crypto	-0.38	-
Particles	0.67*	0.64
Turbidity	0.67*	0.64
TOC	-0.24	0.93*
Temp	-0.43	0.40

As observed throughout this study, average microbial settleable fractions differed by organism type. As expected, attachment rates were slightly lower than those observed previously using a higher centrifugation speed to partition between settleable and free-phase organisms. Roughly 15-30% of bacteria, 40% of *C. perfringens* spores, and less than 20% of coliphage were identified as particle-associated. A consistently higher

fraction of both *Giardia* and *Crypto* (oo)cysts (60%) was removed via the partitioning procedure, suggesting that these protozoans may associate with particles at a higher rate than typical indicator organisms. Partitioning results from this study are very similar to those observed for both fecal indicator bacteria and (oo)cysts by Cizek et al. (2008) in the Kensico Reservoir in New York.

4. Use of culture-based methods and qPCR to determine *E. coli* partitioning behavior

E. coli detection by IDEXX and qPCR

Quantitative PCR (qPCR) offers a rapid, highly sensitive analytical alternative to the culture-based techniques of microbial enumeration typically used in water quality monitoring programs associated with TMDL development. However, correlations between microbial concentration and behavior as assessed by both techniques must be confirmed to ensure that current water quality standards and modeling tools remain appropriate. This section of the study compares *E. coli* concentrations as determined by Colilert and qPCR targeting the *uidA* gene in both raw (total concentration) and supernatant (free-phase) subsamples of partitioned grab samples.

Collection of samples from several watersheds provided a wide and fairly continuous range of *E. coli* concentrations, allowing for a strong comparison of method quantification (Figure 19). While *E. coli* was detectable in all subsamples by Colilert, concentrations were not always detectable by qPCR. These 'non-detects' were assigned a qPCR concentration value of 0.1 for the purposes of plotting and will lie along the x-axis. Equivalent measures of concentration should fall along the 1:1 line. Although linear regression revealed a strong relationship between observations of raw *E. coli* concentration by Colilert and qPCR (R^2=0.81), the relationship between observations of supernatant concentrations was considerably weaker (R^2=0.10). Removal of qPCR non-detects from the linear regression analysis decreased correlation coefficient values slightly (R^2=0.79 and 0.07, respectively).

Differences between measures of total *E. coli* concentration were not statistically significant (Wilcoxon matched pairs signed rank, p=0.59, α=0.05), but qPCR measures of

free-phase *E. coli* concentration were significantly higher than corresponding concentrations determined via Colilert (Wilcoxon, p=0.02, α=0.05). These apparent differences in relative measures of concentration could be due to differences in survival between the particle-associated and free phases of *E. coli* and/or decreased quantities of inhibitors in the supernatant samples.

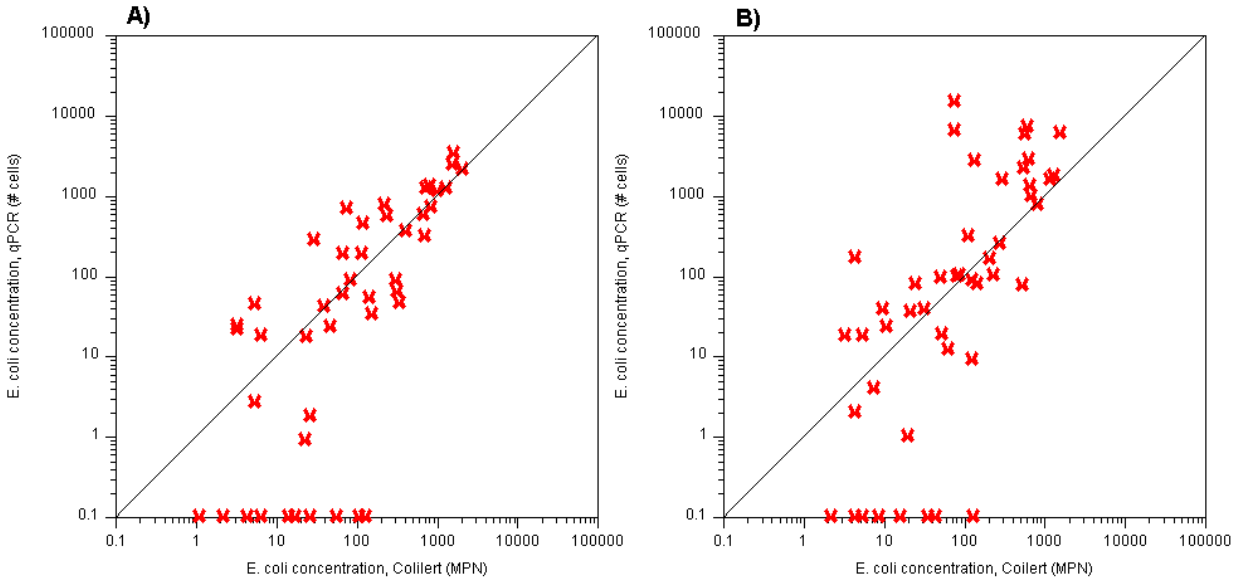


Figure 19. Comparison of A) total (raw) and B) free-phase (supernatant) *E. coli* concentrations (n=47) as measured by Colilert and qPCR

Culture-based detection methods, including Colilert, only measure the number of metabolically active cells in a given sample. There is evidence that cells in the natural environment frequently enter a viable but non-culturable (VBNC) state to maximize survival (Colwell 2000). Previous studies also suggest that microbial particle-association may enhance survival, as measured by extended periods of detection via culture-based methods (Fish and Pettibone 1995; He et al. 2007; Howell et al. 1996; Jamieson et al. 2004b; Roper and Marshall 1978; Sherer et al. 1992). The free-phase counterparts of these longer surviving particle-associated microorganisms may be in a VBNC state rather

than actually inactivated. The significantly higher *E. coli* concentrations as detected by qPCR in the supernatant samples in this study may indicate that a higher proportion of these free-phase cells relative to the total (raw) number are dying off or in a VBNC state not detectable by Colilert. Measures of total concentration may be closer between the two methods as this includes particle-associated cells which are more likely to remain metabolically active as they are protected or nourished by the particles themselves.

Humic materials and other stormwater particulates can interfere with the qPCR reaction, either reducing or preventing the amplification of the target sequence and thereby dampening or eliminating the reporter signal (Haugland et al. 2005; Reynolds et al. 1997; Toze 1999). Inhibition was evaluated and corrected for in this analysis via simultaneous amplification and detection of an exogenous control (*O. keta* sperm cells); however, this control is present in undiluted samples at a concentration of $5x10^3$ cells, generally several orders of magnitude greater than the expected *E. coli* concentrations targeted for detection. Higher concentrations of the exogenous control may have resulted in more success in overcoming inhibitors as compared to the *E. coli* target. The centrifugation procedure used to partition samples prior to *E. coli* analysis resulted in average removals of 35% of particle number, 55% of particle volume, 45% of TSS, and 7% of TOC from the supernatant (free phase) subsample relative to the raw subsample (Figure 20). Removal of a sizable portion of particles during centrifugation would be expected to reduce inhibitor concentration in supernatant samples, allowing for a more accurate measure of the number of *E. coli* cells present.

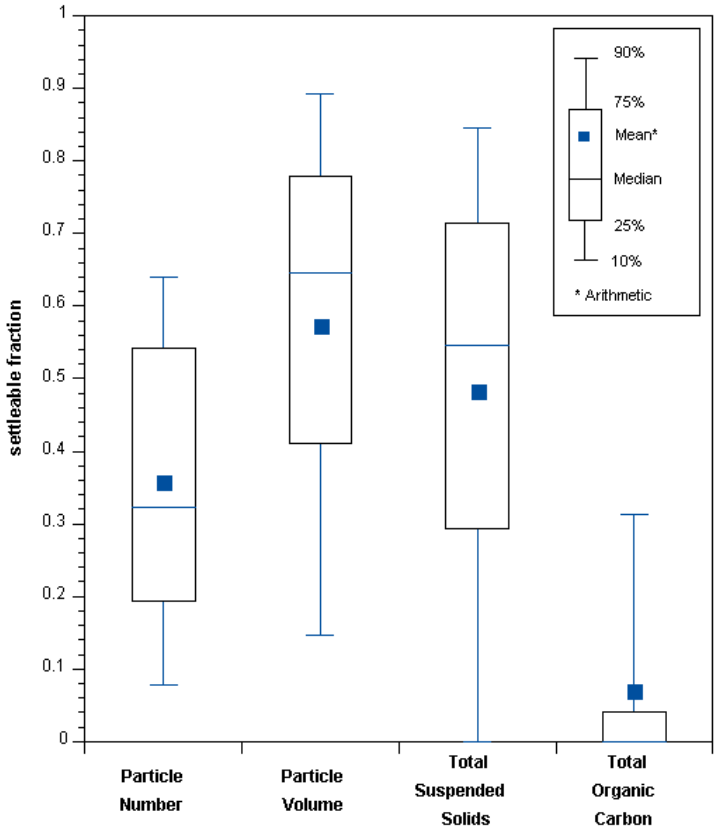


Figure 20. Settleable fractions of particles, TSS, and TOC removed via centrifugation

Total and free-phase *E. coli* concentrations were targeted in order to compare measures of partitioning as determined by qPCR and Colilert. Only samples with qPCR-detectable total and free-phase concentrations were included in the analysis, resulting in a sample size of 32 observations of settleable (i.e. likely particle-associated) fraction. Box and whisker plots, in keeping with previous illustrations of partitioning observations, are provided in Figure 21. Mean values of settleable fraction as calculated using Colilert and qPCR were statistically equal (approx. 10-20% cells associated), though a far greater range of values was calculated using the qPCR data. Perhaps most striking in Figure 21, the median settleable fraction as determined by qPCR was zero. This is due to the large number of samples (22 of 32, or 69%) in which measures of free-phase concentration exceeded measures of total concentration. This is consistent with the finding that free-

phase measures of concentration by qPCR were statistically greater than those determined by Colilert. Because the number of lysed cells responsible for this increase in free concentration is unknown, it is unclear whether there are truly no particle-associated *E. coli* cells in these samples. Similarly, inhibition in the analysis of raw subsamples for total concentration may also have skewed the analysis.

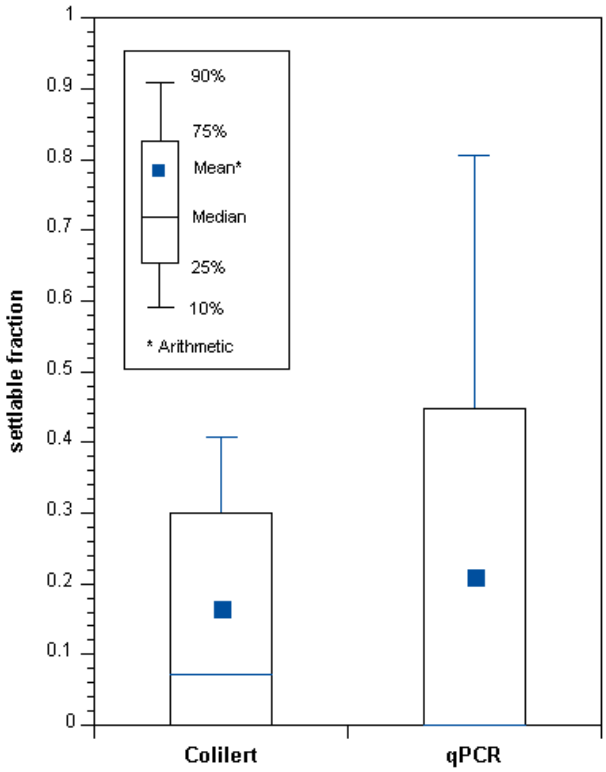


Figure 21. Average fractions of settleable *E. coli* as measured by Colilert and qPCR (n=32)

Comparison of general water quality across sites

The four streams selected for sampling include unimpaired and impaired watersheds representative of differing primary landuses; however, during storm events water quality was uniformly poor across all sites (Table 9). Mean fecal coliform and *E. coli* concentrations as measured by Colilert were at least an order of magnitude greater than corresponding mean dry weather levels at all sites, with nearly every sample

exceeding both the North Carolina instantaneous standard for freshwater of 400 fecal coliform per 100 mL and the USEPA instantaneous standard of 235 *E. coli* per 100 mL. Particle concentrations increased to five to twenty times dry weather concentrations during storms, and increases in TOC, TSS, and turbidity were similarly high. Average particle density, estimated using measures of particle volume and TSS, also increased during storms at all sites, possibly as a result of the mobilization of denser inorganic material by overland flow and/or increased streamflow.

Differences in water quality between streams were only apparent through comparisons of dry weather samples (Table 9). Concentrations of every physical water quality constituent measured were lowest for the Eno River, which is currently classified as unimpaired by North Carolina, although mean fecal coliform concentrations remained high. Samples from the three Northeast Creek sites had the highest mean particle, turbidity, TSS, and TOC concentrations, which was expected as the stream requires TMDL development to address high turbidity and low dissolved oxygen impairments. Interestingly, although only Northeast Creek has been officially identified by the state as impaired due to high indicator organism concentrations, mean concentrations of *E. coli* were higher in Booker Creek and Meeting of the Waters Creek than in any of the three Northeast Creek sites.

Table 9. Geometric mean and 95% confidence intervals for water quality parameters at all sites

		Fecal coliforms (MPN/100 mL)	*E. coli,* Colilert (MPN/100 mL)	Total Particles (#/mL)	TSS (mg/L)	density (g/cm^3)	TOC (mg/L)	Turbidity (NTU)
Booker Creek		50,599	18,203	317,258	130	3.3		124
	storm	**8,810**	**1,916**	**197,061**	**56**	**2.9**	**12.8**	**71**
	n=3	1,534	202	122,403	24	2.6		41
		8,897	1,185	63,199	16	3.8	9.6	25
	dry	**2,933**	**388**	**40,768**	**9**	**2.3**	**7.4**	**13**
	n=7	967	127	26,298	5	1.4	5.7	7
Eno River		110,339	38,731	1,097,521	538	4.6		362
	storm	**6,008**	**1,565**	**152,569**	**57**	**3.5**	**13.2**	**89**
	n=3	327	63	21,209	6	2.7		22
		1,860	144	12,993	3	3.9	5.7	5
	dry	**792**	**76**	**8,927**	**1.3**	**1.9**	**5.1**	**4**
	n=7	337	40	6,134	1	0.9	4.5	3
Meeting of the Waters Creek		90,726	18,915	536,120	324	6.2		302
	storm	**9,472**	**1,158**	**134,591**	**63**	**4.9**	**8.4**	**92**
	n=3	989	71	33,789	12	3.9		28
		3,282	558	50,780	20	5.3	5.5	18
	dry	**1,390**	**230**	**27,145**	**7**	**2.5**	**4.3**	**9**
	n=7	588	95	14,511	3	1.2	3.4	4
Northeast Creek - Site 1		54,074	9,715	552,513	216	6.2		274
	storm	**10,922**	**1,967**	**249,328**	**82**	**4.0**	**12.2**	**144**
	n=3	2,206	398	112,512	32	2.6		75
		2,645	179	77,712	17	4.0	11.9	26
	dry	**1,542**	**92**	**58,582**	**12**	**2.8**	**10.3**	**18**
	n=7	899	47	44,160	8	1.9	9.0	13
Northeast Creek - Site 1a		101,482	11,584	810,107	317	7.9		313
	storm	**9,096**	**1,542**	**234,846**	**89**	**5.5**	**12.3**	**119**
	n=3	815	205	68,081	25	3.8		46
		7,922	519	43,469	20	9.5	10.3	16
	dry	**1,845**	**162**	**31,597**	**12**	**5.1**	**9.5**	**12**
	n=5	430	51	22,968	7	2.7	8.8	10
Northeast Creek - Site 2		89,701	19,087	771,270	311	7.0		327
	storm	**10,027**	**2,293**	**436,805**	**117**	**3.5**	**14.3**	**158**
	n=3	1,121	275	247,382	44	1.8		76
		6,213	301	138,032	44	4.8	10.7	38
	dry	**3,251**	**219**	**89,797**	**24**	**3.8**	**9.7**	**27**
	n=6	1,701	160	58,418	13	3.0	8.8	19

Geometric means of total *E. coli* concentration for dry weather and storm events were calculated in order to compare measures of microbial water quality by qPCR and Colilert between sites (Table 10). It is worth noting that samples in which *E. coli* total concentration was not detectable by qPCR were not included in this analysis. Because

sample particle concentrations were often very high and caused substantial filter-clogging, less than 100 mL was often filter-captured for qPCR analysis. This resulted in lower concentrations of inhibitors, but also much lower numbers of target cells. While the average number of filter-captured cells (as identified by Colilert analysis) in qPCR detectable samples was 321, the average number of cells in samples not detectable by qPCR was only 30. Non-detects in which less than 100 mL (i.e. volume processed by Colilert) was filtered captured for qPCR were therefore excluded from the calculations presented in Table 10.

Table 10. Geometric mean *E. coli* concentrations as measured by Colilert and qPCR

Site		Colilert (MPN/100 mL)	qPCR (cells/100 mL)
Booker Creek			
dry	raw (n=5)	693	852
	supernatant (n=5)	67	144
storm	raw (n=2)	5,876	8,746
	supernatant (n=3)	1,514	14,255
Eno River			
dry	raw (n=3)	124	39
	supernatant (n=5)	666	1,032
storm	raw (n=3)	1,565	4,005
	supernatant (n=3)	2,013	2,062
Meeting of the Waters			
dry	raw (n=5)	355	381
	supernatant (n=5)	337	260
storm	raw (n=2)	3,923	8,143
	supernatant (n=2)	2,703	9,407
Northeast Creek Site 1			
dry	raw (n=1)	121	9
	supernatant (n=3)	135	660
storm	raw (n=3)	1,967	6,225
	supernatant (n=3)	1,611	8,445
Northeast Creek Site 1a			
dry	raw (n=2)	353	2,217
	supernatant (n=2)	337	5,663
storm	raw (n=2)	4,259	1,654
	supernatant (n=2)	3,429	3,999
Northeast Creek Site 2			
dry	raw (n=4)	206	204
	supernatant (n=3)	207	161
storm	raw (n=2)	2,293	2,251
	supernatant (n=2)	3,348	1,152

In general, and in keeping with previously reported findings, mean qPCR values of total *E. coli* concentration exceeded those obtained from Colilert. For most sites, differences between the two measures of concentration appeared greatest during storm events when *E. coli* levels were highest. However, although measures of concentration for samples from Northeast Creek site 2 were very similar, mean Colilert values were

actually higher than qPCR concentrations. This may be due to the particularly poor water quality at this site which may have resulted in greater inhibition of the PCR reaction. Turbidity, TSS, and particle concentration were consistently highest at Northeast Creek site 2. Although the inhibition of polymerases during the qPCR reaction was identified and corrected through use of an exogenous control in this study, as stated earlier, this exogenous control was spiked into samples at higher levels than were observed for *E. coli*. In samples with relatively fewer cells, inhibition of *E. coli* detection may have remained a problem.

5. Designation of impairments for TMDL development

Monitoring data from this project and a previous partitioning project (Dilts 2004) were compiled to provide a detailed data set describing water quality in four local watersheds, three of which currently require TMDL development to address state-identified water quality impairments. The data provide relatively regular measures of both fecal coliform and *E. coli* concentration (determined via Colilert) for comparison to indicator organism regulatory standards. North Carolina currently classifies freshwater bodies as microbially impaired based on standards developed in the 1960s by the Department of the Interior through extrapolation of existing total coliform standards (USEPA 1986). The standards are designed to prevent illness by bathers, regardless of whether full-body contact recreation is likely for a specific stream. Under these standards, a water body is impaired if more than 20% of water samples exceed 400 CFU per 100 mL ("instantaneous standard"), or if the geometric mean of at least five samples during a 30-day period exceeds 200 CFU per 100 mL (NCDWQ 2009). Because an exact monitoring regimen is not specified, these standards are applied irrespective of climatic conditions at the time of sample acquisition.

The USEPA currently recommends implementation of an *E. coli* standard based on 1980s epidemiological studies rather than the fecal coliform standard (USEPA 1986). Assuming North Carolina continued to target full-body contact recreation as a designated use for all state freshwaters, under this standard water bodies would be designated as impaired and require TMDL development if they failed to meet the criteria in Table 11.

Table 11. Indicator organism standards for water body impairment identification

	USEPA, 1986	NCDWQ, 2009
Geometric Mean Standard	126 *E. coli* per 100 mL	200 Fecal coliform per 100 mL
Instantaneous (Single Sample) Standard	235 *E. coli* per 100 mL	400 Fecal coliform per 100 mL

With the exception of one sample from the currently designated as unimpaired Eno River, all samples collected during storm events exceeded the North Carolina instantaneous standard of 400 per 100 mL, often by several orders of magnitude (Table 12). The number of samples exceeding this standard remained high for samples collected at all sites during dry weather, including those not currently identified as impaired due to indicator organisms. Although the geometric means presented in Table 12 represent the entire six year data set rather than a 30-day period as specified by the NCDWQ standard, these observations are still useful in comparing water quality across sites. As stormwater contributes the vast majority of microbial loadings to these streams, dry weather conditions can be considered a “best case scenario” for ambient water quality. However, geometric means at all sites exceeded the 200 per 100 mL standard. Although only Northeast Creek currently requires TMDL development to address a fecal coliform impairment, the data collected here suggest that all four streams would fail to meet the North Carolina standard.

Table 12. Fecal coliform sampling data

SITE	Impairment?	Geometric Mean (no. of samples)		Number of Violations of Single Sample Standard	
		dry	storm	dry	storm
Eno River	none	804 (10)	8,448 (13)	8 (80%)	12 (92%)
Booker Creek	biological only	2,216 (10)	29,200 (7)	10 (100%)	7 (100%)
Meeting of the Waters Creek	biological only	1,038 (13)	13,871 (16)	9 (69%)	16 (100%)
Northeast Creek Site 1	biological, high turbidity, low dissolved oxygen, fecal coliform	1,408 (17)	12,754 (14)	15 (88%)	14 (100%)
Northeast Creek Site 1a		1,081 (6)	3,470 (9)	4 (67%)	9 (100%)
Northeast Creek Site 2		2,864 (12)	16,302 (9)	12 (100%)	9 (100%)

Examination of the corresponding *E. coli* data from the same stream samples results in slightly different conclusions regarding the relative health of the four streams. Although storm geometric means are high (Table 13), with the vast majority of samples exceeding the USEPA instantaneous standard of 235 per 100 mL, dry weather samples frequently meet recommended levels. Geometric mean *E. coli* concentrations from the Eno River and two Northeast Creek sites were equal to or below the 126 per 100 mL standard, indicating lower levels of microbial pollution and, presumably, a lower human health risk than the fecal coliform data would suggest.

Table 13. *E. coli* sampling data via IDEXX defined substrate method

SITE	Impairment?	Geometric Mean (no. of samples)		Number of Violations of Single Sample Standard	
		dry	storm	dry	storm
Eno River	none	64 (10)	1,894 (13)	1 (10%)	12 (92%)
Booker Creek	biological only	1,075 (10)	6,406 (7)	6 (60%)	6 (86%)
Meeting of the Waters Creek	biological only	169 (13)	2,611 (16)	5 (38%)	15 (94%)
Northeast Creek Site 1	biological, high turbidity, low dissolved oxygen, fecal coliform	89 (16)	2,100 (14)	3 (19%)	14 (100%)
Northeast Creek Site 1a		126 (6)	841 (9)	3 (50%)	8 (89%)
Northeast Creek Site 2		164 (11)	1,878 (9)	3 (27%)	9 (100%)

The lower number of *E. coli* standard violations was unexpected as the majority of past studies have observed an *E. coli* to fecal coliform ratio of roughly 0.9, which is greater than the 0.63 ratio of the *E. coli* and fecal coliform standards (i.e. 126 *E. coli*/200 fecal coliform) (Hamilton et al. 2005). In the present study, an average of only 20% of fecal coliform were identified as *E. coli*. This ratio was consistent across analysts, climatic conditions, sampling year, and sample sites (Figure 22).

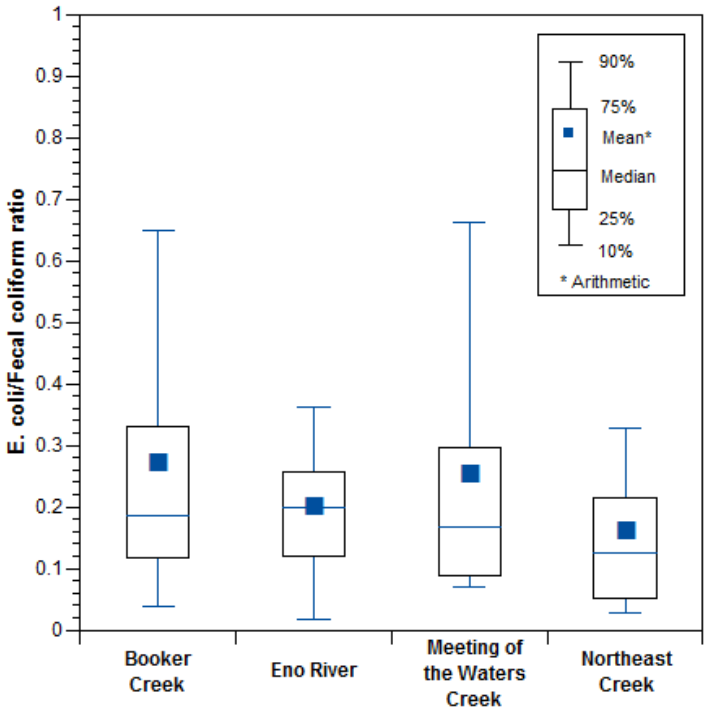


Figure 22. Observed *E. coli*:Fecal coliform ratios for Booker Creek (n=17); Eno River (n=23); Meeting of the Waters Creek (n=29); and Northeast Creek (n=65)

This unexpectedly low *E. coli* to fecal coliform ratio could be the result of slight modifications in the Colilert analysis protocol. In this study, following an initial two hour incubation at 37^{o} C, quantitrays were incubated at 44.5^{o} C for 19-22 hours to select for thermotolerant (fecal) coliforms (Yakub et al. 2002). Although a previous study examining *E. coli* in swine wastewater found that *E. coli* concentrations as enumerated by Colilert did not change as a result of incubation at 44.5^{o} C (Chihara et al. 2004), it is

possible that cells already stressed by the relatively nutrient-poor stream environment would be unable to grow at this elevated temperature. However, this seems somewhat less likely as analysis of a subset of the stream samples during 2008 via qPCR resulted in concentrations statistically equivalent to those provided by Colilert.

High concentrations of coliforms originating from non-fecal origins could also be responsible for the low observed *E. coli* to fecal coliform ratio. Though thermotolerant coliforms are commonly referred to as "fecal" coliforms, recent evidence suggests that some of these bacteria (e.g. *Enterobacter*) are not solely fecal in origin (Leclerc et al. 2001). *E. coli*, which originate solely from the intestines of warm-blooded animals, may simply be present in these streams at much lower levels than naturally occurring "fecal" coliforms. Given the numerous culture-based techniques for recovery and enumeration of *E. coli*, including several alternative types of media and incubation times which may alter results, specification of a single method would render results more comparable between different regions.

Observations of total *E. coli* concentrations obtained through simultaneous Colilert and qPCR analysis were statistically equivalent, though there were variations in observations of individual samples. Regardless, the number of violations was remarkably consistent across detection methods for all sites (Table 14). This suggests that a similar number of impairments would be identified using either detection method. Although the relatively rapid results available via qPCR are attractive, because water quality standards have been developed from studies quantifying indicator organism exposure through culture-based methods, the relationship between qPCR cell concentrations and human health risk is unclear (Noble and Weisberg 2005; Wade et al. 2003). Establishment of a

clear correlation would be necessary before application of qPCR monitoring using existing regulatory standards.

Table 14. Comparison of *E. coli* data as determined by Colilert and qPCR

SITE	Impairment?	Geometric Mean (no. of samples)		Number of Violations of Single Sample Standard	
		Colilert	qPCR	Colilert	qPCR
Eno River	none	441 (6)	396 (6)	3 (50%)	3 (50%)
Booker Creek	biological only	1,276 (7)	1,658 (7)	6 (86%)	5 (71%)
Meeting of the Waters Creek	biological only	705 (7)	913 (7)	6 (86%)	6 (86%)
Northeast Creek Site 1	biological, high turbidity, low dissolved oxygen, fecal coliform	979 (4)	1,222 (4)	3 (75%)	3 (75%)
Northeast Creek Site 1a		1,227 (4)	1,915 (4)	4 (100%)	4 (100%)
Northeast Creek Site 2		579 (7)	572 (7)	4 (57%)	5 (71%)

V. Conclusions

Numerous analyses of stormwater grab samples, including those in the present study, have shown indicator and pathogen concentrations several orders of magnitude greater than dry weather (background) levels. Further characterization of this trend was completed through estimates of cumulative microbial storm loadings in Meeting of the Waters Creek and Eno River using streamflow and intra-storm sampling data. Results suggest that wet weather periods, though intermittent, are likely to contribute a much greater fraction of the total annual load of indicator organisms to a waterway than lengthier dry weather periods. In these streams roughly one day of storm loading can be equivalent to months or even years of dry-weather loading. A disproportionately large fraction of the total settleable loading of microorganisms was contained in the runoff eluting during the rising limb of each storm, suggesting that stormwater BMPs may be relatively effective even if just treating this "first flush", particularly in situations when treating the entire stormwater volume is infeasible.

Statistically significant correlations were observed between fecal indicator bacteria (fecal coliforms, *E. coli*, enterococci) presence and the presence of *Salmonella*, *Cryptosporidium*, and *Giardia* in grab samples collected for this study; however, it is worth noting that all three pathogens were readily detectable in samples meeting current indicator organism guidelines. While similar fractions of the fecal indicator bacteria and *Salmonella* were identified as particle-associated (25-35%), a greater fraction of the protozoans (*Cryptosporidium, Giardia*) appeared to associate with settleable particles

(60%). This suggests that while these indicator bacteria may be reasonable surrogates for *Salmonella* spp. in fate and transport modeling, observations of their behavior may be inadequate in predicting the fate and transport of protozoan pathogens.

Higher removal of settleable indicator organisms and *Salmonella* bacteria by suburban detention ponds suggests that partitioning behavior may be an important consideration in estimating expected BMP removal of microorganisms from stormwater. Despite mean microbial removals near the USEPA's typical rate of 65%, pond effluent concentrations remained several orders of magnitude above recommended water quality standards, and occasional net exports of microorganisms from the ponds were observed for both indicators and *Salmonella* during dry weather and storm events.

Comparisons of free-phase and total *E. coli* concentration as measured by the culture-based Colilert assay and qPCR detection of the *uidA* gene seem to support previous observations of differences in viability between free-phase and particle-associated cells. Free-phase concentrations detected by qPCR were significantly higher than those detected by Colilert though total concentrations were statistically equivalent, suggesting that there may be a larger number of metabolically inactive or lysed cells in the free fraction. Although an exogenous control was used to identify and correct for inhibition of the PCR reaction by humics and other components of the sample matrix, particle concentrations in the samples were often quite high, and some inhibition of *E. coli* detection, particularly at low *E. coli* concentrations, may have occurred.

Improved estimates of microbial partitioning will aid in efforts to model the fate and transport of microorganisms in receiving waters in conjunction with the United States Total Maximum Daily Load (TMDL) program. Compilation of the indicator organism

monitoring data collected for this study revealed that impairment designations may be influenced by the selection of specific indicator organisms, climatic conditions during monitoring, and method of organism detection. Although these observations are not surprising, they are rarely explicitly stated and are often not considered in the design of water quality monitoring programs to identify impairments. As unidentified impairments may be a public health threat and unnecessary impairments can result in an inefficient allocation of available resources for watershed remediation, effective methods of assessment must be more consistent and highly specified.

Appendix A: Intrastorm Sampling Data

Data for intrastorm sampling at Meeting of the Waters Creek and the Eno River. Flow is given in m^3/s. Background values are averages of grab sample data taken by MacKenzie Dilts (Dilts 2004).

Meeting of the Waters Creek (2-3 May 2004)

		Fecal coliforms (MPN/100mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100mL)	*C. perf.* spores (MPN/100mL)	Total coliphage (PFU/100mL)	Particles (#/100mL)	TSS (mg/L)	TOC (mg/L)
Background		0	37	88	10	6	9,931	2	2.9
t = 16:00	Raw	**4,504**	**208**	**206**	**32**	**15**	**10,342**	**5**	**2.9**
Q = 0.45		9,271	379	325	54	24	10,753	8	3
		199	33	138	11	4	2,196	0	2.9
	Supernatant	**3,037**	**324**	**182**	**18**	**9**	**2,893**	**2**	**3**
		5,875	615	226	25	15	3,590	3	3.1
Sample 1		65,817	1,584	3,285	180	2	130,166	607	7.1
t = 16:45	Raw	**84,392**	**2,194**	**4,249**	**1,100**	**6**	**132,683**	**638**	**7.6**
Q = 0.88		108,209	3,038	5,495	4,100	13	135,199	668	8.1
		51,483	485	851	37	3	17,085	25	8.6
	Supernatant	**66,295**	**804**	**1,281**	**150**	**8**	**23,182**	**26**	**8.7**
		85,369	1,334	1,929	420	16	29,279	27	8.8
Sample 2		51,483	1,369	3,008	420	1	128,262	454	3.5
t = 18:30	Raw	**66,295**	**1,929**	**3,915**	**>1,100**	**4**	**132,643**	**462**	**3.6**
Q = 1.90		85,369	2,719	5,097	-	10	137,023	470	3.7
		19,587	531	1,114	37	5	13,437	57	3.4
	Supernatant	**25,515**	**866**	**1,613**	**150**	**10**	**18,630**	**62**	**3.4**
		33,236	1,412	2,336	420	18	23,822	66	3.4
Sample 3		17,747	1,493	1,249	90	5	116,429	220	2.5
t = 19:45	Raw	**22,944**	**2,083**	**1,781**	**460**	**10**	**122,409**	**229**	**3.5**
Q = 3.34		29,662	2,905	2,540	2,000	18	128,389	237	4.5
		14	711	573	18	2	16,571	13	2.9
	Supernatant	**5,865**	**1,101**	**921**	**93**	**6**	**18,385**	**19**	**2.9**
		7,427	1,706	1,481	420	13	20,198	25	2.9
Sample 4		21,503	1,853	4,517	90	27	115,271	109	3.8
t = 21:00	Raw	**28,174**	**2,522**	**5,727**	**460**	**38**	**125,774**	**115**	**4**
Q = 3.43		36,914	3,433	7,262	2,000	52	136,277	121	4.2
		9,926	1,377	2,315	18	26	12,139	26	3.5
	Supernatant	**12,403**	**1,939**	**3,081**	**93**	**37**	**19,133**	**31**	**3.8**
		15,499	2,732	4,102	420	51	26,127	36	4.1
Sample 5		13,557	1,417	5,332	90	35	117,885	63	4.4
t = 1:00	Raw	**17,171**	**1,989**	**6,710**	**460**	**48**	**130,622**	**136**	**4.5**
Q = 1.56		21,748	2,792	8,444	2,000	64	143,358	209	4.6
		18,106	2,244	4,259	9	28	8,601	30	
	Supernatant	**23,446**	**2,996**	**5,417**	**43**	**39**	**11,780**	**32**	**4.2**
		30,360	4,000	6,890	180	53	14,958	34	
Sample 6		10,853	1,121	3,567	90	20	82,980	41	4.1
t = 9:00	Raw	**13,596**	**1,622**	**4,586**	**460**	**24**	**88,500**	**43**	**4.3**
Q = 0.62		17,032	2,346	5,897	2,000	43	94,021	45	4.5
		6,189	573	2,229	37	15	9,184	23	4.2
	Supernatant	**7,750**	**921**	**2,978**	**150**	**24**	**9,697**	**24**	**4.3**
		9,705	1,481	3,978	420	34	10,210	24	4.4

Eno River (May 2-3 2004)

		Fecal coliforms (MPN/100mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100mL)	*C. perf.* spores (MPN/100mL)	Total coliphage (PFU/100mL)	Particles (#/100mL)	TSS (mg/L)	TOC (mg/L)
Background		465	4	0	0	0	11,794	3	1.9
t = 17:00	Raw	**893**	**63**	**45**	**53**	**7**	**18,664**	**6**	**3.5**
Q = 2.32		1,322	122	92	134	14	25,533	8	5.1
		358	0	3	0	1	2,974	0	2.2
	Supernatant	**651**	**66**	**36**	**45**	**10**	**4,514**	**3**	**3.4**
		944	135	69	120	19	6,054	6	4.6
Sample 1		18,736	3,129	16,239	90	19	129,307	169	4.8
t = 18:30	Raw	**24,325**	**4,061**	**20,844**	**460**	**29**	**130,895**	**176**	**7.2**
Q = 20.78		31,583	5,271	26,754	2,000	42	132,483	182	9.6
		2,611	1,195	7,615	4	15	4,465	15	5.3
	Supernatant	**3,439**	**1,714**	**9,500**	**15**	**23**	**6,884**	**17**	**5.5**
		4,528	2,458	11,851	42	35	9,302	20	5.7
Sample 2		15,510	3,476	14,000	42	18	123,361	101	4.9
t = 21:30	Raw	**19,835**	**4,477**	**17,770**	**240**	**27**	**126,836**	**130**	**7.5**
Q = 23:50		25,366	5,767	22,554	1,000	39	130,311	159	4.9
		12,491	2,615	22,032	90	36	5,892	24	7.4
	Supernatant	**15,744**	**3,443**	**28,902**	**460**	**49**	**9,148**	**32**	**7.5**
		19,844	4,534	37,915	2,000	65	12,405	40	7.6
Sample 3		15,164	3,247	20,507	42	50	112,535	48	7.7
t = 0:00	Raw	**19,359**	**4,202**	**26,795**	**240**	**65**	**118,928**	**154**	**8**
Q = 8:44		24,715	5,439	35,011	1,000	83	125,321	260	8.3
		6,586	1,865	11,353	42	73	8,489	19	7.8
	Supernatant	**8,234**	**2,537**	**14,246**	**240**	**91**	**10,136**	**23**	**7.8**
		10,295	3,450	17,876	1,000	112	11,783	27	7.8
Sample 4		9,073	1,971	8,873	90	66	112,708	65	7.9
t = 1:15	Raw	**11,320**	**2,666**	**11,069**	**460**	**83**	**113,854**	**83**	**7.9**
Q = 6.65		14,125	3,605	13,808	2,000	103	114,999	101	7.9
		3,613	811	6,849	90	64	11,051	18	7.4
	Supernatant	**4,642**	**1,231**	**8,556**	**460**	**81**	**14,717**	**19**	**7.5**
		5,964	1,867	10,689	2,000	101	18,384	21	7.6
Sample 5		6,976	1,055	6,903	180	47	51,136	73	6.7
t = 3:00	Raw	**8,712**	**1,539**	**8,622**	**1,100**	**61**	**87,942**	**79**	**7.2**
Q = 5.23		10,880	2,245	10,770	4,100	78	124,748	85	7.7
		4,009	763	5,273	18	49	19,995	14	7.1
	Supernatant	**5,117**	**1,168**	**6,639**	**93**	**64**	**24,836**	**15**	**7.2**
		6,531	1,790	8,358	420	80	29,677	17	7.3

Meeting of the Waters Creek (17-18 September 2004)

		Fecal coliforms (MPN/100mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100mL)	*C. perf.* spores (MPN/100mL)	Total coliphage (PFU/100mL)	Particles (#/100mL)	TSS (mg/L)	TOC (mg/L)
Background		0	37	88	10	6	9,931	2	2.9
t = 15:00	Raw	**4,504**	**208**	**206**	**32**	**15**	**10,342**	**5**	**2.9**
Q = 1.08		9,271	379	325	54	24	10,753	8	3
		199	33	138	11	4	2,196	0	2.9
	Supernatant	**3,037**	**324**	**182**	**18**	**9**	**2,893**	**2**	**3**
		5,875	615	226	25	15	3,590	3	3.1
Sample 1		56348	2451	8906	900	42	118133	558	4.4
t = 16:40	Raw	**72389**	**3245**	**11110**	**4600**	**56**	**125379**	**627**	**5.6**
Q = 3.09		92996	4298	13860	20000	73	132625	695	6.7
		24382	1767	3913	180	27	11048	44	3.8
	Supernatant	**32095**	**2417**	**5002**	**930**	**38**	**14113**	**58**	**4.0**
		42248	3307	6394	4200	52	17179	73	4.2
Sample 2		12840	1572	3882	90	50	79983	57	5.0
t = 19:05	Raw	**16209**	**2179**	**4964**	**460**	**65**	**88078**	**67**	**5.3**
Q = 2.01		20461	3021	6349	2000	83	96174	76	5.6
		6737	485	1664	37	35	1906	3	5.2
	Supernatant	**8419**	**804**	**2292**	**150**	**48**	**3491**	**10**	**5.3**
		10521	1334	3157	420	64	5076	17	5.5
Sample 3		8781	1095	2593	1800	74	56465	25	5.5
t = 21:50	Raw	**10953**	**1589**	**3417**	**11000**	**92**	**63224**	**38**	**5.7**
Q = 1.59		13663	2306	4502	41000	113	69983	52	6.0
		3882	362	2065	180	47	1827	6	5.3
	Supernatant	**4964**	**636**	**2779**	**1100**	**61**	**2404**	**12**	**5.4**
		6349	1119	3741	4100	78	2981	18	5.6
Sample 4		7725	1108	2038	90	42	55403	21	5.1
t = 1:15	Raw	**9636**	**1605**	**2746**	**460**	**56**	**60340**	**32**	**5.3**
Q = 1.53		12020	2326	3702	2000	73	65276	42	5.4
		4188	611	1231	90	40	2481	2	5.2
	Supernatant	**5332**	**971**	**1759**	**460**	**53**	**2953**	**13**	**5.5**
		6789	1544	2513	2000	69	3425	24	5.8
Sample 5		10876	758	2768	180	40	78358	88	5.0
t = 8:45	Raw	**13626**	**1163**	**3627**	**1100**	**53**	**86017**	**97**	**5.4**
Q = 1.16		17071	1783	4753	4100	69	93677	105	5.8
		5150	173	1377	42	29	1206	13	4.8
	Supernatant	**6491**	**362**	**1939**	**240**	**40**	**1945**	**20**	**5.0**
		8180	759	2732	1000	54	2684	27	5.2

Eno River (17-18 September 2004)

		Fecal coliforms (MPN/100mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100mL)	*C. perf.* spores (MPN/100mL)	Total coliphage (PFU/100mL)	Particles (#/100mL)	TSS (mg/L)	TOC (mg/L)
Background		465	4	0	0	0	11,794	3	1.9
t = 14:00	Raw	**893**	**63**	**45**	**53**	**7**	**18,664**	**6**	**3.5**
Q = 1.50		1,322	122	92	134	14	25,533	8	5.1
		358	0	3	0	1	2,974	0	2.2
	Supernatant	**651**	**66**	**36**	**45**	**10**	**4,514**	**3**	**3.4**
		944	135	69	120	19	6,054	6	4.6
Sample 1		9312	802	5549	90	113	30938	7	4.1
t = 17:20	Raw	**11623**	**1219**	**6973**	**460**	**135**	**36481**	**18**	**4.2**
Q = 8.24		14507	1852	8762	2000	160	42023	30	4.2
		5924	754	2929	90	133	1921		3.9
	Supernatant	**7428**	**1157**	**2188**	**460**	**157**	**2447**	**5**	**4.1**
		9313	1776	3920	2000	184	2972		4.3
Sample 2		17908	1748	15322	90	215	69658	7	4.1
t = 18:25	Raw	**23169**	**2394**	**12172**	**460**	**245**	**75121**	**25**	**4.3**
Q = 8.98		29975	3280	19286	2000	278	80585	43	4.5
		8645	1095	8275	42	243	2155		4.3
	Supernatant	**10783**	**1589**	**10320**	**240**	**275**	**2500**	**15**	**4.4**
		13449	2306	12870	1000	309	2845		4.4
Sample 3		21869	3688	20136	1800	416	73618	30	4.8
t = 21:15	Raw	**28678**	**4732**	**26280**	**11000**	**457**	**78528**	**47**	**5.0**
Q = 4.90		37607	6071	34297	41000	501	83439	63	5.1
		10659	1644	19153	42	388	2724		4.6
	Supernatant	**13345**	**2267**	**24909**	**240**	**428**	**3302**	**25**	**4.8**
		16708	3127	32394	1000	471	3880		4.9
Sample 4		17747	2403	13311	180	567	65877	31	5.2
t = 0:45	Raw	**22944**	**3188**	**16839**	**1100**	**615**	**70590**	**63**	**5.4**
Q = 3.37		29662	4230	21303	4100	666	75303	96	5.6
		7029	1523	9517	180	428	4714		5.3
	Supernatant	**8777**	**1042**	**11883**	**1100**	**469**	**5159**	**10**	**5.3**
		10961	2226	14836	4100	513	5604		5.4

Meeting of the Waters Creek (13 October 2004)

		Fecal coliforms (MPN/100mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100mL)	*C. perf.* spores (MPN/100mL)	Total coliphage (PFU/100mL)	Particles (#/100mL)	TSS (mg/L)	TOC (mg/L)
Background		0	37	88	10	6	9,931	2	2.9
t = 6:00	Raw	**4,504**	**208**	**206**	**32**	**15**	**10,342**	**5**	**2.9**
Q = 0.43		9,271	379	325	54	24	10,753	8	3
		199	33	138	11	4	2,196	0	2.9
	Supernatant	**3,037**	**324**	**182**	**18**	**9**	**2,893**	**2**	**3**
		5,875	615	226	25	15	3,590	3	3.1
Sample 1		27,911	5,826	6784	420	29	46,153	891	3.2
t = 9:18	Raw	**36,760**	**7,309**	**8476**	**2,400**	**40**	**46,503**	**1,003**	**3.6**
Q = 1.19		48,414	9,169	10591	10,000	54	46,852	1,116	4.1
		13,370	1,918	2,866	14	15	15,408	70	3.0
	Supernatant	**16,919**	**2,601**	**3,746**	**92**	**24**	**15,931**	**77**	**3.1**
		21,410	3,528	4,894	380	16	16,454	83	3.2
Sample 2		20,038	4,117	7,625	180	304	45,715	348	3.5
t = 10:35	Raw	**26,143**	**5,247**	**9,512**	**1,100**	**339**	**46,179**	**410**	**3.7**
Q = 9.71		34,108	6,686	11,866	4,100	377	46,643	472	4.0
		12,568	906	3,808	18	279	7,893	57	3.3
	Supernatant	**15,846**	**1,351**	**4,876**	**93**	**313**	**9,673**	**65**	**3.4**
		19,979	2,015	6,243	420	350	11,453	73	3.5
Sample 3		372,189	142,112	72,599	420	987	41,818	329	4.5
t = 11:55	Raw	**485,591**	**180,562**	**93,135**	**2,400**	**1,050**	**42,134**	**398**	**4.8**
Q = 8.98		633,544	229,416	119,480	10,000	1,115	42,450	468	5.0
		235,226	119,846	29,525	90	931	5,763	40	4.5
	Supernatant	**309,358**	**150,743**	**38,848**	**460**	**992**	**6,951**	**53**	**4.6**
		406,853	189,606	51,114	2,000	1,056	8,138	66	4.6
Sample 4		470,933	180,551	75,100	180	472	43,240	182	4.4
t = 12:42	Raw	**608,276**	**233,743**	**96,397**	**930**	**516**	**43,680**	**195**	**4.6**
Q = 6.68		785,673	302,604	123,732	4,200	563	44,119	208	4.7
		361,454	105,092	40,643	42	570	4,163	32	4.7
	Supernatant	**472,192**	**131,517**	**52,819**	**240**	**618**	**5,620**	**38**	**4.7**
		616,855	164,587	68,641	1,000	669	7,076	44	4.8
Sample 5		18,333	5,692	13,311	42	183	37,726	60	4.1
t = 17:30	Raw	**23,762**	**7,146**	**16,839**	**240**	**211**	**38,234**	**73**	**4.2**
Q = 2.10		30,800	8,971	21,303	1,000	241	38,742	86	4.3
		13,413	3,074	9,338	42	155	2,041	10	4.1
	Supernatant	**16,977**	**3,995**	**11,656**	**240**	**180**	**3,579**	**20**	**4.1**
		21,487	5,192	14,550	1,000	208	5,118	30	4.1
Sample 6		12,191	3,160	8,275	42	178	31,522	36	4.1
t = 20:25	Raw	**15,346**	**4,098**	**10,320**	**240**	**205**	**32,795**	**55**	**4.1**
Q = 1.61		19,319	5,314	12,870	1,000	235	34,069	74	4.2
		7,496	2,299	6,623	90	117	1,718	21	4.1
	Supernatant	**9,352**	**3,063**	**8,279**	**460**	**139**	**2,181**	**25**	**4.1**
		11,668	4,080	10,350	2,000	164	2,645	29	4.2

Eno River (13 October 2004)

		Fecal coliforms (MPN/100mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100mL)	*C. perf.* spores (MPN/100mL)	Total coliphage (PFU/100mL)	Particles (#/100mL)	TSS (mg/L)	TOC (mg/L)
Background		465	4	0	0	0	11,794	3	1.9
t = 6:00	Raw	**893**	**63**	**45**	**53**	**7**	**18,664**	**6**	**3.5**
Q = 0.77		1,322	122	92	134	14	25,533	8	5.1
		358	0	3	0	1	2,974	0	2.2
	Supernatant	**651**	**66**	**36**	**45**	**10**	**4,514**	**3**	**3.4**
		944	135	69	120	19	6,054	6	4.6
Sample 1		15,690	4,012	11,034	90	24	42,404	98	3.5
t = 10:05	Raw	**20,083**	**5,120**	**13,830**	**460**	**34**	**42,580**	**112**	**3.8**
Q = 3.94		25,707	6,535	17,336	2,000	48	42,756	125	4.2
		5,692	1,385	3,754	18	33	7,054	0	3.5
	Supernatant	**7,146**	**1,950**	**4,811**	**93**	**45**	**7,386**	**7**	**3.8**
		8,971	2,744	6,166	420	60	7,718	13	4.1
Sample 2		14166	2,727	4,856	42	73	38,710	26	3.9
t = 11:25	Raw	**17995**	**3,578**	**6,136**	**240**	**91**	**38,950**	**37**	**4.1**
Q = 13.34		22859	4,695	7,753	1,000	112	39,191	48	4.2
		3,808	1,216	2,691	90	83	2,072	0	3.8
	Supernatant	**4,876**	**1,740**	**3,534**	**460**	**102**	**2,490**	**7**	**3.9**
		6,243	2,490	4,643	2,000	124	2,907	15	3.9
Sample 3		16,322	2,768	7,936	42	139	26,626	3	4.0
t = 13:20	Raw	**20,959**	**3,627**	**9,898**	**240**	**163**	**28,508**	**10**	**4.0**
Q = 12.15		26,913	4,753	12,344	1,000	190	30,391	17	4.1
		11,803	1,417	4,993	42	100	1,336		3.9
	Supernatant	**14,835**	**1,989**	**6,301**	**240**	**121**	**2,216**	**0**	**4.0**
		18,646	2,792	7,951	1,000	145	3,096		4.1
Sample 4		13,743	4,053	11,518	90	282	39,662	34	4.5
t = 16:45	Raw	**17,421**	**5,169**	**14,461**	**460**	**316**	**40,015**	**42**	**4.6**
Q = 3.37		22,084	6,594	18,157	2,000	353	40,368	50	4.8
		7,003	2,540	8,047	42	175	2,916	0	4.3
	Supernatant	**8,746**	**3,353**	**10,038**	**240**	**202**	**3,243**	**10**	**4.3**
		10,921	4,427	12,518	1,000	232	3,570	20	4.4
Sample 5		9,715	2,218	7,937	42	86	36,143	14	4.6
t = 20:55	Raw	**12,134**	**2,964**	**9,899**	**240**	**105**	**36,578**	**25**	**4.7**
Q = 2.41		15,156	3,962	12,346	1,000	127	37,013	36	4.8
		4,370	1,417	5,580	37	93	5,401	3	4.4
	Supernatant	**5,551**	**1,989**	**7,010**	**150**	**113**	**5,837**	**7**	**4.5**
		7,050	2,792	8,807	420	136	6,274	11	4.5

Appendix B: Northeast Creek Dry Weather Grab Sample Data

All dry weather samples were collected following at least three days of no appreciable precipitation (no change in baseflow). Greyed entries indicate that no data was available.

8 June 2006

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
POND 1 INFLOW	Raw	236		3	1				3,119	3.8
		314		**10**	**7**	**0**			**3,427**	**3.9**
		417		40	18				3,734	4.0
	Supernatant	157		3	5	1			1,567	4.6
		218		**10**	**23**	**3**			**1,733**	**4.7**
		302		40	94	10			1,900	4.7
POND 1 OUTFLOW	Raw	1,642		416	40	1			10,800	6.6
		2,108		**529**	**210**	**3**			**13,680**	**6.6**
		2,708		674	430	10			16,560	6.7
	Supernatant	1,346		327	5	1			4,912	7.3
		1,703		**422**	**23**	**2**			**5,467**	**7.4**
		2,155		546	94	7			6,021	7.5
POND 2 INFLOW	Raw	2,870		94	42	0			81,659	10.9
		3,778		**139**	**240**	**1**			**95,720**	**11.0**
		4,972		207	1,000	3			109,781	11.1
	Supernatant	689		40	9				19,507	10.5
		860		**68**	**38**	**0**			**19,987**	**10.7**
		1,074		118	110				20,466	10.8
POND 2 OUTFLOW	Raw	5,809		1,277	90	2			14,621	10.7
		7,457		**1,611**	**460**	**10**			**14,933**	**10.8**
		9,571		2,033	2,000	43			15,246	10.9
	Supernatant	3,834		869	18	1			5,865	12.7
		4,994		**1,084**	**93**	**2**			**6,127**	**13.0**
		6,505		1,352	420	7			6,388	13.2
NE CREEK SITE 1	Raw	1,136		165	37	1			65,766	7.9
		1,425		**228**	**150**	**4**			**68,080**	**7.9**
		1,788		314	420	17			70,394	8.0
	Supernatant	514		149	17	1			7,279	
		648		**208**	**75**	**3**			**7,540**	**7.1**
		816		290	200	10			7,801	
NE CREEK SITE 2	Raw	1,959		105	17	2			121,406	8.3
		2,551		**154**	**75**	**8**			**123,800**	**8.4**
		3,323		224	200	33			126,194	8.4
	Supernatant	762		48	4	2			4,977	6.5
		950		**80**	**15**	**10**			**5,400**	**6.7**
		1,185		133	42	43			5,823	6.8

19 June 2006

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
		536	2	4			1		3,573	3.8
	Raw	**674**	**5**	**8**	**0**	**0**	**2**	**0**	**3,733**	**3.8**
POND 1		848	13	17			7		3,894	3.9
INFLOW		299	2	2					2,994	6.7
	Supernatant	**390**	**5**	**5**	**0**	**0**	**0**	**0**	**3,133**	**6.9**
		508	13	12					3,273	7.2
		1,289	20	56	42		5	184	15,554	7.9
	Raw	**1,628**	**41**	**91**	**240**	**0**	**10**	**212**	**15,827**	**8.0**
POND 1		2,055	82	146	1,000		18	243	16,099	8.1
OUTFLOW		1,372	5	66	1			179	5,028	8.6
	Supernatant	**1,738**	**15**	**103**	**3**	**0**	**0**	**206**	**5,220**	**8.9**
		2,203	47	162	11			236	5,412	9.2
		6,794	235	70	90	6	1	1	37,615	5.8
	Raw	**8,709**	**312**	**109**	**460**	**27**	**2**	**2**	**38,780**	**5.8**
POND 2		11,164	415	169	2,000	110	7	7	39,945	5.9
INFLOW		3,126	242	40	4	5		6	14,868	5.8
	Supernatant	**4,105**	**321**	**69**	**15**	**17**	**0**	**12**	**14,893**	**5.8**
		5,392	425	119	42	55		21	14,918	5.9
		5,305	48	137	37	1			34,408	10.8
	Raw	**6,824**	**80**	**193**	**150**	**2**	**0**	**0**	**34,873**	**10.9**
POND 2		8,778	133	272	420	7			35,339	11.1
OUTFLOW		1,556	36	153	18	0			5,723	11.3
	Supernatant	**1,990**	**63**	**212**	**93**	**1**	**0**	**0**	**6,027**	**11.4**
		2,545	111	295	420	4			6,330	11.6
		2,715	248	140	17	8	6	194	79,177	9.8
	Raw	**3,576**	**329**	**197**	**75**	**24**	**12**	**222**	**87,453**	**9.8**
NE CREEK		4,709	434	277	200	69	21	253	95,730	9.9
SITE 1		772	122	85	4	0	14	244	11,392	8.7
	Supernatant	**963**	**174**	**127**	**15**	**1**	**22**	**276**	**11,593**	**8.9**
		1,201	249	192	42	3	33	311	11,795	9.1
		4,852	213	146	5	29		235	248,377	8.5
	Raw	**6,258**	**286**	**204**	**23**	**122**	**0**	**266**	**252,180**	**8.7**
NE CREEK		8,073	384	285	94	523		300	255,983	8.8
SITE 2		856	115	135	1	11	1	256	5,595	8.0
	Supernatant	**1,067**	**166**	**191**	**7**	**51**	**2**	**288**	**5,853**	**8.2**
		1,331	240	269	20	239	7	323	6,112	8.4

12 July 2006

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
		1,462	3	269				0	6,816	3.5
	Raw	**2,082**	**10**	**353**	**0**		**0**	**2**	**6,893**	**3.5**
POND 1		2,672	40	464				7	6,970	3.6
INFLOW		939	1	206	0			0	2,683	3.4
	Supernatant	**1,217**	**5**	**278**	**4**		**0**	**2**	**2,800**	**3.6**
		1,519	36	374	18			7	2,917	3.7
		11,282	5	370	42				17,297	4.8
	Raw	**14,915**	**15**	**474**	**240**		**0**	**0**	**17,833**	**4.9**
POND 1		19,717	47	608	1,000				18,370	5.1
OUTFLOW		6,794	3	281			1		5,529	5.0
	Supernatant	**8,709**	**10**	**367**	**0**		**4**	**0**	**12,682**	**5.1**
		11,164	40	481			10		19,836	5.3
		15,972	5	200	420	1		6	28,852	4.8
	Raw	**24,370**	**15**	**270**	**2,400**	**5**	**0**	**12**	**29,973**	**4.8**
POND 2		37,185	47	364	10,000	21		21	31,095	4.9
INFLOW		525			9	2		1	14,711	
	Supernatant	**661**	**0**	**0**	**29**	**10**	**0**	**4**	**14,807**	
		832			94	43		10	14,902	
	Raw									
POND 2										
OUTFLOW										
	Supernatant									
		1,706	61	95	5	1		149	48,300	7.1
	Raw	**2,198**	**98**	**140**	**23**	**5**	**0**	**174**	**48,507**	**7.1**
NE CREEK		2,832	155	208	94	21		202	48,713	7.1
SITE 1		916	44	61	1	6	1	144	9,151	7.4
	Supernatant	**1,143**	**75**	**97**	**9**	**27**	**4**	**168**	**9,347**	**7.4**
		1,426	126	154	38	110	10	195	9,543	7.5
		2,952	86	181	42	0	34	30	160,094	7.4
	Raw	**3,884**	**129**	**247**	**240**	**2**	**46**	**42**	**161,333**	**7.5**
NE CREEK		5,109	194	337	1,000	5	61	57	162,573	7.6
SITE 2		817	24	130	9	0	62	24	7,087	6.9
	Supernatant	**1,018**	**47**	**184**	**43**	**2**	**78**	**34**	**7,333**	**7.0**
		1,270	90	261	180	5	97	48	7,580	7.1

15 August 2006

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
POND 1 INFLOW	Raw	3,720	2	94					7,896	4.0
		4,852	**4**	**117**	**0**	**0**	**0**	**0**	**8,033**	**4.0**
		6,329	36	179					8,171	4.1
	Supernatant	2,952	1	73					4,444	4.3
		3,884	**3**	**100**	**0**	**0**	**0**	**0**	**4,513**	**4.4**
		5,109	105	191					4,583	4.5
POND 1 OUTFLOW	Raw	2,151	17	1,003	9	19			11,532	5.1
		2,817	**36**	**1,253**	**43**	**44**	**0**	**0**	**11,607**	**5.2**
		3,690	76	1,566	180	102			11,682	5.2
	Supernatant	2,504	5	682	1	15	0	0	4,327	10.5
		3,296	**15**	**852**	**7**	**36**	**2**	**2**	**4,527**	**10.5**
		4,340	47	1,064	20	89	7	7	4,726	10.6
POND 2 INFLOW	Raw	9,387	3	275	90	0		15	95,937	6.8
		12,163	**10**	**360**	**460**	**3**	**0**	**24**	**97,553**	**6.9**
		15,760	40	472	2,000	22		36	99,170	6.9
	Supernatant	5,809	8	128	1	44	6	17	8,901	6.7
		7,457	**20**	**182**	**9**	**147**	**12**	**26**	**9,180**	**6.8**
		9,571	54	259	38	487	21	38	9,459	6.8
POND 2 OUTFLOW	Raw									
	Supernatant									
NE CREEK SITE 1	Raw	627	3	44	37	2	0	2	60,308	7.6
		785	**10**	**74**	**150**	**6**	**2**	**6**	**61,107**	**7.6**
		982	40	126	420	25	7	13	61,905	7.6
	Supernatant	794	1	17	5		2		11,035	9.4
		990	**5**	**36**	**23**	**0**	**6**	**0**	**11,153**	**9.4**
		1,234	36	75	94		13		11,272	9.5
NE CREEK SITE 2	Raw	2,791	168	472	9	7	3	9	70,136	7.7
		3,675	**230**	**597**	**43**	**20**	**8**	**16**	**70,613**	**8.1**
		4,839	317	755	180	59	16	26	71,091	8.4
	Supernatant	948	90	267	5	43	1	14	7,541	9.8
		1,183	**134**	**351**	**23**	**143**	**4**	**22**	**7,900**	**9.9**
		1,476	200	461	94	473	10	33	8,259	9.9

25 October 2006

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
POND 1 INFLOW	Raw	288	2	48	0				5,236	3.6
		422	**4**	**67**	**4**	**0**	**0**	**0**	**5,353**	**3.6**
		608	87	145	18				5,470	3.6
	Supernatant	256	1	54	--				2,808	4.2
		378	**3**	**74**	**<3.0**	**0**	**0**	**0**	**2,980**	**4.3**
		551	106	156	10				3,152	4.3
POND 1 OUTFLOW	Raw	711	5	351	18	0		5	4,476	4.3
		887	**15**	**452**	**93**	**3**	**0**	**10**	**4,533**	**4.3**
		1,107	47	582	420	21		18	4,591	4.3
	Supernatant	1,022	11	291	18			15	2,408	4.7
		1,277	**26**	**379**	**93**	**0**	**0**	**24**	**2,473**	**4.8**
		1,597	61	495	420			36	2,539	4.8
POND 2 INFLOW	Raw	1,886	3	262	180		3	0	6,654	3.9
		2,449	**10**	**344**	**1,100**	**0**	**8**	**2**	**6,813**	**4.0**
		3,181	40	453	4,100		16	7	6,972	4.1
	Supernatant	2,316	8	204	42	2	2		3,755	5.2
		3,044	**20**	**275**	**240**	**6**	**6**	**0**	**3,833**	**5.3**
		4,000	54	370	1,000	25	13		3,911	5.3
POND 2 OUTFLOW	Raw	1,520	117	1,224	420	6			137,200	5.3
		1,940	**168**	**1,541**	**2,400**	**23**	**0**	**0**	**142,080**	**5.4**
		2,476	242	1,940	10,000	93			146,960	5.5
	Supernatant	1,346	48	574	18	6	0	0	8,451	5.1
		1,703	**80**	**721**	**93**	**23**	**2**	**2**	**8,627**	**5.2**
		2,155	133	904	420	93	7	7	8,802	5.3
NE CREEK SITE 1	Raw	459	53	188	18	0	1	65	25,552	7.7
		582	**86**	**255**	**93**	**3**	**4**	**82**	**25,813**	**7.8**
		737	140	347	420	22	10	102	26,075	7.9
	Supernatant	493	24	181	4	0	0	78	5,691	7.9
		623	**47**	**247**	**15**	**3**	**2**	**96**	**7,647**	**8.0**
		786	90	336	42	22	7	117	9,602	8.0
NE CREEK SITE 2	Raw	402	17	100	5	4	0	14	21,016	8.0
		512	**36**	**147**	**23**	**11**	**2**	**22**	**21,313**	**8.2**
		654	76	216	94	36	7	33	21,611	8.3
	Supernatant	467	14	104	17		5	6	4,363	9.4
		592	**31**	**152**	**75**	**0**	**10**	**12**	**4,573**	**9.5**
		749	69	222	200		18	21	4,784	9.5

3 April 2006

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
POND 1 INFLOW	Raw	124	1	7				29	3,213	3.5
		156	**3**	**11**	**0**	**0**	**0**	**40**	**3,460**	**3.6**
		196	6	16				54	3,707	3.7
	Supernatant	64		1				0	3,878	3.9
		80	**0**	**2**	**0**	**0**	**0**	**2**	**3,980**	**4.0**
		101		5				7	4,082	4.1
POND 1 OUTFLOW	Raw	581	10	156	4			0	55,777	7.5
		746	**15**	**217**	**15**	**0**	**0**	**2**	**56,687**	**8.9**
		957	22	279	42			7	57,596	10.2
	Supernatant	979	10	109	9	4		1	31,038	8.1
		1,273	**14**	**158**	**43**	**30**	**0**	**4**	**32,893**	**8.4**
		1,656	21	199	180	216		10	34,748	8.7
POND 2 INFLOW	Raw				180				691,500	
					1,100	**0**			**727,533**	
					4,100				763,566	
	Supernatant				180	2			660,412	
					1,100	**9**			**711,067**	
					4,100	39			761,721	
POND 2 OUTFLOW	Raw	138	17	52	42				54,903	9.5
		194	**36**	**85**	**240**	**0**	**0**	**0**	**56,500**	**9.6**
		248	47	107	1,000				58,097	9.8
	Supernatant	58	8	28	9	4			4,392	9.5
		93	**20**	**52**	**43**	**30**	**0**	**0**	**5,040**	**9.6**
		115	28	67	180	216			5,688	9.7
NE CREEK SITE 1	Raw	261	17	39	9		1	20	23,518	5.5
		344	**36**	**67**	**43**	**0**	**4**	**30**	**24,380**	**5.6**
		453	47	84	180		10	43	25,242	5.7
	Supernatant	150	14	3	1	2	3	19	8,881	7.4
		209	**31**	**10**	**9**	**9**	**8**	**28**	**9,187**	**7.6**
		269	41	16	38	39	16	40	9,493	7.7
NE CREEK SITE 2	Raw	1,712	40	9,768	9		1	9	59,527	7.4
		2,206	**69**	**12,697**	**43**	**0**	**4**	**16**	**61,307**	**7.6**
		3,211	87	16,504	180		10	26	63,086	7.9
	Supernatant	630	32	11,910	1			5	5,107	7.6
		788	**58**	**15,892**	**9**	**0**	**0**	**10**	**5,187**	**7.7**
		1,010	73	21,206	38			18	5,267	7.8

Appendix C: Northeast Creek Storm Event Grab Sample Data

3 June 2006

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
		14,939	138	2,710					44,690	13.3
	Raw	**19,045**	**307**	**3,557**					**45,347**	**13.4**
POND 1		24,280	684	4,668					46,003	13.5
RUNOFF		9,713	49	1,562					10,447	14.7
	Supernatant	**12,129**	**151**	**2,167**					**10,687**	**14.9**
		15,146	470	3,005					10,927	15.0
		54,529	5,361	18,563					47,398	13.5
	Raw	**70,088**	**6,743**	**24,078**					**48,300**	**13.5**
POND 1		90,087	8,482	31,230					49,202	13.6
INFLOW		14,400	3,740	11,270					8,034	13.3
	Supernatant	**18,309**	**4,794**	**14,135**					**8,227**	**13.7**
		23,278	6,143	17,727					8,419	14.0
		93,482	13,312	41,830					68,363	12.9
	Raw	**121,092**	**16,837**	**54,279**					**68,740**	**13.1**
POND 1		156,856	21,295	70,432					69,117	13.3
OUTFLOW		20,502	7,011	27,075					9,556	13.5
	Supernatant	**26,780**	**8,754**	**35,659**					**10,040**	**13.7**
		34,981	10,929	46,963					10,524	14.0
		6,912	25	13,148					32,918	4.4
	Raw	**8,632**	**101**	**16,616**					**34,473**	**4.6**
POND 2		10,780	403	20,999					36,028	4.8
RUNOFF		4,012	53	6,897					5,698	3.4
	Supernatant	**5,120**	**86**	**8,614**					**6,220**	**3.5**
		6,533	141	10,757					6,742	3.5
		8,177	398	8,151					35,375	7.2
	Raw	**10,195**	**686**	**10,162**					**36,187**	**7.3**
POND 2		12,712	1,183	12,671					36,998	7.4
INFLOW		3,840	403	6,016					6,252	5.8
	Supernatant	**4,913**	**693**	**7,538**					**7,827**	**6.0**
		6,286	1,191	9,444					9,401	6.2
		22,019	1,344	10,599					127,060	7.2
	Raw	**28,875**	**1,898**	**13,264**					**128,300**	**7.3**
POND 2		37,867	2,681	16,600					129,540	7.4
OUTFLOW		8,422	482	7,062					27,653	5.7
	Supernatant	**10,501**	**800**	**8,816**					**29,273**	**5.8**
		13,093	1,328	11,006					30,894	5.9
		6,977	890	3,425					368,836	7.9
	Raw	**8,712**	**1,331**	**4,415**					**375,187**	**7.9**
NE CREEK		10,877	1,990	5,691					381,537	8.0
SITE 1		1,312	398	664					10,046	6.8
	Supernatant	**1,859**	**686**	**1,040**					**10,727**	**7.1**
		2,633	1,183	1,630					11,407	7.3
	Raw									
NE CREEK										
SITE 2										
	Supernatant									

25 June 2006

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
		101,536	8,671	36,894	90		3	240	44,604	11.3
	Raw	**132,460**	**10,812**	**48,141**	**460**		**0**	**210**	**45,787**	**11.7**
POND 1		172,801	13,483	62,817	2,000		0	183	46,970	12.0
RUNOFF		97,352	12,079	31,696	42		3	228	12,103	11.7
	Supernatant	**126,505**	**15,195**	**41,610**	**240**		**0**	**198**	**12,207**	**11.9**
		164,389	19,115	54,624	1,000		0	171	12,310	12.1
		106,079	4,937	52,932	42		18	376	39,171	9.4
	Raw	**139,058**	**6,232**	**68,088**	**240**		**10**	**338**	**40,147**	**9.5**
POND 1		182,290	7,866	87,585	1,000		5	303	41,123	9.6
INFLOW		47,038	2,979	41,830	42		10	382	10,539	9.5
	Supernatant	**60,743**	**3,880**	**54,279**	**240**		**4**	**344**	**10,620**	**9.6**
		78,442	5,054	70,432	1,000		1	309	10,701	9.7
		65,410	4,155	67,480	42		3	447	41,568	10.0
	Raw	**83,850**	**5,291**	**86,501**	**240**		**0**	**406**	**42,020**	**10.2**
POND 1		107,488	6,738	110,884	1,000		0	367	42,472	10.4
OUTFLOW		71,889	3,577	52,932	90		3	479	9,989	9.7
	Supernatant	**92,190**	**4,598**	**68,088**	**460**		**0**	**436**	**10,273**	**9.9**
		118,223	5,909	87,585	2,000		0	396	10,558	10.1
		142,952	403	47,038	42		3		38,508	9.8
	Raw	**201,416**	**693**	**60,743**	**240**	**>122**	**0**	**768***	**39,380**	**9.8**
POND 2		283,789	1,191	78,442	1,000		0		40,252	9.9
RUNOFF		153,281	280	29,409	9	8	3		10,464	9.4
	Supernatant	**224,982**	**520**	**38,687**	**43**	**24**	**0**	**806***	**10,793**	**9.9**
		330,221	968	50,893	180	69	0		11,123	10.3
		153,281	36,894	101,536	180	29	274		56,544	11.0
	Raw	**224,982**	**48,141**	**132,460**	**1,100**	**122**	**242**	**1104***	**56,660**	**11.3**
POND 2		330,221	62,817	172,801	4,100	523	212		56,776	11.6
INFLOW			51,386	79,286	90	29	228		16,052	12.2
	Supernatant	**>294,969**	**66,158**	**101,877**	**460**	**122**	**198**	**960***	**16,660**	**12.4**
			85,176	130,904	2,000	523	171		17,268	12.5
			18,921	71,889	180	11	73		57,020	7.0
	Raw	**>294,969**	**24,578**	**92,190**	**1,100**	**51**	**56**	**792***	**57,887**	**7.1**
POND 2			31,925	118,223	4,100	239	42		58,753	7.1
OUTFLOW			34,848	51,386	42	29	100		12,997	7.7
	Supernatant	**>294,969**	**45,583**	**66,158**	**240**	**122**	**80**	**632***	**13,153**	**7.8**
			59,626	85,176	1,000	523	63		13,310	7.9
		24,347	1,818	17,519	42	29	21		357,296	7.5
	Raw	**32,039**	**2,479**	**22,619**	**240**	**122**	**12**	**976***	**365,667**	**7.6**
NE CREEK		42,161	3,380	29,204	1,000	523	6		374,037	7.8
SITE 1		20,988	1,905	7,832	18	11	28		15,386	7.8
	Supernatant	**27,454**	**2,585**	**9,767**	**93**	**51**	**18**	**888***	**15,573**	**7.8**
		35,912	3,507	12,179	420	239	11		15,760	7.9
		16,018	320	1,767	17	11	68	340	1,002,990	9.8
	Raw	**20,532**	**578**	**2,416**	**75**	**51**	**52**	**304**	**1,005,787**	**10.0**
NE CREEK		26,317	1,042	3,305	200	239	39	271	1,008,583	10.1
SITE 2		6,578	49	969	90	11	36	327	9,026	9.5
	Supernatant	**8,223**	**152**	**1,430**	**460**	**51**	**24**	**292**	**10,240**	**9.5**
		10,279	471	2,111	2,000	239	15	259	11,454	9.6

*TNTC; best estimate counting one quadrant on each plate

25 July 2006

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
		142,952	9,240	33,869	42	15	0	784	44,644	9.1
	Raw	**201,416**	**11,530**	**44,354**	**240**	**36**	**0**	**840**	**45,893**	**9.2**
POND 1		283,789	14,386	58,085	1,000	89	3	899	47,143	9.3
RUNOFF		133,736	5,238	27,825	18	4	0	800	13,612	11.2
	Supernatant	**183,678**	**6,595**	**36,638**	**93**	**11**	**0**	**856**	**13,713**	**11.3**
		252,271	8,303	48,242	420	35	3	915	13,815	11.5
		167,805	142,952	125,725	170	0	24	659	58,665	9.1
	Raw	**294,969**	**201,416**	**169,732**	**750**	**4**	**34**	**710**	**61,507**	**9.1**
POND 1		518,497	283,789	229,144	2,000	26	48	764	64,348	9.1
INFLOW		142,952	17,364	97,352	90	0	32	657	8,228	10.3
	Supernatant	**201,416**	**22,403**	**126,505**	**430**	**3**	**44**	**708**	**8,967**	**10.6**
		283,789	28,905	164,389	1,800	21	59	762	9,705	10.8
		167,805	23,136	125,725	420		22	666	70,231	8.6
	Raw	**294,969**	**30,402**	**169,732**	**2,400**	**0**	**32**	**718**	**71,813**	**8.7**
POND 1		518,497	39,950	229,144	10,000		45	772	73,395	8.8
OUTFLOW		142,952	10,039	86,537	180		25	672	9,470	9.3
	Supernatant	**201,416**	**12,545**	**111,575**	**930**	**0**	**36**	**724**	**9,573**	**9.4**
		283,789	15,676	143,859	4,200		50	779	9,677	9.5
		153,281	358	16,230	90	0	17	155	61,867	9.0
	Raw	**224,982**	**630**	**20,826**	**460**	**4**	**26**	**180**	**64,700**	**9.1**
POND 2		330,221	1,111	26,722	2,000	26	38	208	67,533	9.3
RUNOFF		142,952	280	15,187	17		17	155	15,003	9.1
	Supernatant	**201,416**	**520**	**19,385**	**75**	**0**	**26**	**180**	**15,220**	**9.2**
		283,789	968	24,744	200		38	208	15,437	9.2
		513,870	19,178	153,854	420		0	534	119,966	8.9
	Raw	**661,588**	**26,003**	**196,582**	**2,400**	**0**	**2**	**580**	**124,740**	**9.0**
POND 2		851,770	35,256	251,175	10,000		7	629	129,514	9.0
INFLOW		234,135	11,827	129,193	46		6	381	18,672	9.8
	Supernatant	**307,790**	**16,982**	**163,106**	**230**	**0**	**12**	**420**	**19,667**	**9.9**
		404,615	24,384	205,921	940		21	462	20,662	9.9
		133,736	8,031	116,451	180		2	173	60,533	5.0
	Raw	**183,678**	**10,014**	**154,750**	**930**	**0**	**6**	**200**	**61,120**	**5.1**
POND 2		252,271	12,486	205,645	4,200		13	230	61,707	5.3
OUTFLOW		93,482	5,396	79,286	420	5	0	118	10,822	5.2
	Supernatant	**121,092**	**6,785**	**101,877**	**2,400**	**15**	**0**	**140**	**11,147**	**5.3**
		156,856	8,533	130,904	10,000	40	3	165	11,471	5.5
		15,637	1,161	30,243	14	6	55	900	510,401	7.0
	Raw	**20,005**	**1,672**	**39,758**	**92**	**15**	**70**	**960**	**513,373**	**7.2**
NE CREEK		25,593	2,406	52,266	380	42	88	1,023	516,346	7.4
SITE 1		6,294	837	24,120	180	6	42	920	13,595	7.6
	Supernatant	**7,876**	**1,262**	**31,733**	**930**	**15**	**56**	**980**	**14,933**	**7.6**
		9,855	1,905	41,750	4,200	42	73	1,043	16,272	7.6
		67,480	733	27,825	14	15	8	1,250	1,144,571	9.2
	Raw	**86,501**	**1,130**	**36,638**	**92**	**36**	**14**	**1,320**	**1,147,653**	**10.0**
NE CREEK		110,884	1,741	48,242	380	89	23	1,393	1,150,736	10.9
SITE 2		21,452	525	20,058	2	71	22	1,355	15,968	8.7
	Supernatant	**28,095**	**858**	**26,163**	**36**	**205**	**32**	**1,428**	**16,707**	**9.1**
		36,795	1,401	34,127	180	590	45	1,504	17,445	9.4

5 September 2006

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
POND 1 RUNOFF	Raw									
	Supernatant									
POND 1 INFLOW	Raw	29,409	946	4,933	87	224	196	25	23,997	12.6
		38,687	**1,401**	**6,226**	**290**	**933**	**224**	**36**	**24,333**	**12.6**
		50,893	2,076	7,860	940	3,889	255	50	24,669	12.7
	Supernatant	23,729	851	4,674	37	19	125	35	7,197	13.3
		31,206	**1,281**	**5,915**	**150**	**44**	**148**	**48**	**7,720**	**13.4**
		41,040	1,928	7,485	420	102	174	64	8,243	13.4
POND 1 OUTFLOW	Raw	19,586	896	4,257	420	364	65	421	30,609	13.4
		25,505	**1,338**	**5,414**	**2,400**	**1,152**	**82**	**462**	**31,287**	**13.6**
		33,214	1,998	6,885	10,000	3,644	102	506	31,964	13.7
	Supernatant	24,070	653	3,238	90	98	110	406	6,464	14.0
		31,667	**1,026**	**4,190**	**430**	**427**	**132**	**446**	**6,573**	**14.1**
		41,661	1,612	5,424	1,800	1,864	157	489	6,682	14.3
POND 2 RUNOFF	Raw									
	Supernatant									
POND 2 INFLOW	Raw	7,222	207	2,468	420	6	29	29	14,257	4.8
		9,012	**414**	**3,265**	**2,400**	**15**	**40**	**40**	**14,793**	**4.9**
		11,247	827	4,320	10,000	41	54	54	15,329	4.9
	Supernatant	7,086	208	2,104	90	0	24	46	5,876	5.2
		8,845	**416**	**2,827**	**430**	**3**	**34**	**60**	**5,960**	**5.3**
		11,041	830	3,796	1,800	21	48	77	6,044	5.4
POND 2 OUTFLOW	Raw	8,859	1,088	4,947	180	6	0	81	22,215	6.3
		11,048	**1,581**	**6,244**	**930**	**16**	**0**	**100**	**22,387**	**6.3**
		13,779	2,295	7,881	4,200	43	3	122	22,558	6.4
	Supernatant	9,473	569	3,689	180	11	0	155	6,231	6.1
		11,825	**916**	**4,732**	**930**	**24**	**0**	**180**	**6,400**	**6.1**
		14,759	1,475	6,069	4,200	55	3	208	6,569	6.2
NE CREEK SITE 1	Raw	11,598	1,076	22,566	180	2	69	1,308	256,135	9.6
		14,562	**1,565**	**29,625**	**930**	**6**	**86**	**1,380**	**259,427**	**9.7**
		18,285	2,276	38,892	4,200	25	106	1,455	262,719	9.9
	Supernatant	11,114	793	10,563	180	19	71	1,367	11,189	9.7
		13,932	**1,207**	**13,218**	**930**	**74**	**88**	**1,440**	**11,487**	**9.7**
		17,463	1,837	16,541	4,200	291	108	1,516	11,784	9.8
NE CREEK SITE 2	Raw	9,264	1,595	11,939	46	57	14	958	464,887	8.9
		11,559	**2,207**	**15,011**	**230**	**231**	**22**	**1,020**	**469,093**	**9.1**
		14,423	3,053	18,873	940	938	33	1,085	473,300	9.3
	Supernatant	9,033	997	11,767	46	96	19	900	22,577	8.4
		11,267	**1,466**	**14,785**	**230**	**385**	**28**	**960**	**23,007**	**8.5**
		14,055	2,155	18,576	940	1,546	40	1,023	23,436	8.7

25 September 2006

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
POND 1 RUNOFF	Raw									
	Supernatant									
POND 1 INFLOW	Raw	1,829	171	49	5	6		9	16,055	4.9
		2,493	**359**	**152**	**23**	**23**		**16**	**16,547**	**4.9**
		3,241	470	222	94	93		26	17,038	5.0
	Supernatant	901	76	49		19		15	6,260	5.0
		1,344	**203**	**152**	**0**	**74**	**0**	**24**	**6,380**	**5.1**
		1,683	284	222		291		36	6,500	5.1
POND 1 OUTFLOW	Raw	10,068	1,584	4,371	5	22	0	186	14,567	7.6
		12,582	**2,193**	**5,550**	**23**	**92**	**2**	**214**	**14,680**	**7.6**
		16,343	2,825	7,195	94	382	7	245	14,793	7.6
	Supernatant	9,033	1,767	3,476	5	22	0	51	3,617	7.5
		11,267	**2,416**	**4,477**	**23**	**92**	**2**	**66**	**3,713**	**7.6**
		14,534	3,135	5,861	94	382	7	84	3,809	7.7
POND 2 RUNOFF	Raw									
	Supernatant									
POND 2 INFLOW	Raw	110,234	75,983	56,385	420	224	0	256	69,764	13.6
		138,135	**94,770**	**70,794**	**2,400**	**933**	**2**	**288**	**70,680**	**13.7**
		173,099	118,202	88,886	10,000	3,889	7	323	71,596	13.9
	Supernatant	39,493	14,989	53,940	46	44		369	14,234	12.0
		50,442	**20,889**	**67,835**	**230**	**147**	**0**	**408**	**14,333**	**12.1**
		510,786	29,790	85,309	940	487		450	14,433	12.2
POND 2 OUTFLOW	Raw	7,186	320	2,983	5	15		41	47,589	6.7
		8,968	**578**	**3,885**	**23**	**36**	**0**	**54**	**48,853**	**6.8**
		11,498	732	5,110	94	89		70	50,117	6.8
	Supernatant	4,640	322	1,986	1	98		55	6,653	7.2
		5,874	**580**	**2,683**	**9**	**427**	**0**	**70**	**6,793**	**7.2**
		7,596	735	3,505	38	1,864		88	6,934	7.3
NE CREEK SITE 1	Raw	11,178	1,930	6,393	14	10		668	105,187	8.5
		14,014	**2,615**	**7,997**	**92**	**42**	**0**	**720**	**109,700**	**8.7**
		18,386	3,410	10,254	380	184		775	114,213	8.8
	Supernatant	3,756	354	3,238	2	10		803	9,600	7.5
		5,287	**743**	**4,190**	**36**	**38**	**0**	**860**	**9,640**	**7.6**
		7,656	1,022	5,500	180	153		919	9,680	7.7
NE CREEK SITE 2	Raw	14,893	2,131	9,097	9	57		248	608,528	10.6
		18,982	**2,859**	**11,349**	**38**	**231**	**0**	**280**	**611,267**	**11.3**
		26,289	3,747	14,645	110	938		315	614,005	12.0
	Supernatant	4,298	482	4,439	5	98		400	8,058	9.2
		5,463	**800**	**5,633**	**23**	**427**	**0**	**440**	**8,107**	**9.3**
		7,086	1,001	7,297	94	1,864		483	8,156	9.5

28 October 2006

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
POND 1 RUNOFF	Raw									
	Supernatant									
POND 1 INFLOW	Raw	18,647	3,607	30,243	37	57	25	90	361,819	13.6
		25,358	**6,345**	**39,758**	**150**	**231**	**36**	**110**	**362,940**	**13.7**
		34,483	11,163	51,677	420	938	50	133	364,061	13.7
	Supernatant	7,554	490	25,256	17	224	30	105	15,911	12.1
		11,586	**1,518**	**33,255**	**75**	**933**	**42**	**126**	**17,347**	**12.3**
		17,769	4,705	43,923	200	3,889	57	150	18,782	12.5
POND 1 OUTFLOW	Raw	36,670	2,803	32,919	180	11	29	1,121	1,311,976	14.0
		47,055	**5,212**	**43,157**	**1,100**	**28**	**40**	**1,188**	**1,317,720**	**14.3**
		60,381	9,691	55,730	4,100	76	54	1,258	1,323,464	14.5
	Supernatant	20,096	2,803	28,603	9	57	27	1,147	14,187	13.4
		27,116	**5,212**	**37,647**	**43**	**231**	**38**	**1,214**	**14,500**	**13.8**
		36,589	9,691	49,161	180	938	52	1,284	14,813	14.1
POND 2 RUNOFF	Raw									
	Supernatant									
POND 2 INFLOW	Raw	20,375	4,806	9,419	370	4	3	243	33,478	10.0
		27,454	**7,981**	**13,964**	**1,500**	**11**	**8**	**274**	**34,033**	**10.1**
		36,994	13,255	20,700	4,200	36	16	308	34,588	10.3
	Supernatant	9,527	1,058	2,772	17	7	0	203	12,781	10.2
		14,100	**2,544**	**5,167**	**75**	**20**	**0**	**232**	**13,013**	**10.2**
		20,867	6,118	9,632	200	59	3	264	13,246	10.2
POND 2 OUTFLOW	Raw	19,585	4,360	10,780	40	15	1	511	24,213	9.2
		26,497	**7,378**	**13,498**	**210**	**36**	**4**	**556**	**24,613**	**9.3**
		35,847	12,488	20,129	430	88	10	604	25,014	9.3
	Supernatant	12,653	3,587	9,473	9	4	0	499	10,455	9.3
		18,008	**6,317**	**11,825**	**43**	**15**	**2**	**544**	**10,513**	**9.5**
		25,629	11,126	18,065	180	49	7	592	10,572	9.6
NE CREEK SITE 1	Raw	4,261	911	6,248	18	3	5	891	374,615	8.1
		5,418	**1,358**	**7,820**	**93**	**9**	**10**	**950**	**383,440**	**8.3**
		9,960	4,486	13,049	420	29	18	1,012	392,265	8.5
	Supernatant	2,708	525	2,615	5	4	2	827	13,903	8.1
		3,554	**858**	**3,443**	**23**	**11**	**6**	**884**	**14,247**	**8.2**
		7,492	3,846	7,342	94	36	13	944	14,590	8.2
NE CREEK SITE 2	Raw	7,405	1,007	3,514	5	22	0	639	666,602	8.9
		9,239	**1,479**	**4,521**	**23**	**92**	**2**	**690**	**670,320**	**9.2**
		14,842	4,651	8,782	94	382	7	743	674,038	9.4
	Supernatant	2,667	245	2,091	9	6	6	672	7,864	
		3,506	**470**	**2,810**	**43**	**23**	**12**	**724**	**8,133**	**8.9**
		7,426	3,556	6,483	180	93	21	779	8,402	

8 November 2006

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
		34,766	281	4,901	9	0	1		78,394	8.9
	Raw	**44,771**	**367**	**6,189**	**43**	**3**	**6**	**0**	**80,460**	**9.2**
POND 1		57,655	481	7,815	180	22	18		82,526	9.4
RUNOFF		20,244	277	3,223	37				30,383	8.9
	Supernatant	**27,296**	**363**	**4,173**	**150**	**0**		**0**	**31,500**	**9.0**
		36,804	475	5,403	420				32,617	9.1
		79,262	8,920	10,915	90	6		6	153,092	8.8
	Raw	**98,834**	**13,331**	**13,673**	**430**	**23**	**0**	**12**	**154,767**	**8.9**
POND 1		123,237	19,924	17,127	1,800	93		21	156,442	9.0
INFLOW		70,343	3,607	9,677	37	6		14	16,667	9.0
	Supernatant	**87,818**	**6,345**	**12,083**	**150**	**23**		**22**	**17,313**	**9.1**
		109,635	11,163	15,088	420	93		33	17,959	9.3
		136,393	5,296	17,863	420	57		2,717	359,765	10.1
	Raw	**172,771**	**8,636**	**23,099**	**2,400**	**231**	**0**	**2,820**	**365,207**	**10.3**
POND 1		218,852	14,083	29,870	10,000	938		2,926	370,649	10.5
OUTFLOW		181,367	3,587	13,177	90	10		2,685	23,725	10.4
	Supernatant	**234,816**	**6,317**	**16,655**	**430**	**42**		**2,788**	**24,447**	**10.4**
		304,015	11,126	21,052	1,800	184		2,893	25,168	10.5
		168,411	383	17,225	180	19	9	17	81,145	10.4
	Raw	**216,741**	**490**	**22,209**	**930**	**73**	**16**	**26**	**83,713**	**10.5**
POND 2		278,941	627	28,634	4,200	285	26	38	86,282	10.6
RUNOFF		141,077	335	22,566	90	19		9	37,539	10.3
	Supernatant	**179,109**	**433**	**29,625**	**430**	**73**		**16**	**37,973**	**10.4**
		227,395	558	38,892	1,800	285		26	38,407	10.4
		86,307	17,667	23,864	420	57	2	138	55,734	8.1
	Raw	**107,622**	**24,164**	**31,671**	**2,400**	**231**	**6**	**162**	**56,660**	**8.2**
POND 2		134,202	33,051	42,033	10,000	938	13	189	57,586	8.3
INFLOW		95,547	13,211	10,901	46	22		131	21,602	7.9
	Supernatant	**119,279**	**18,699**	**15,827**	**230**	**92**		**154**	**21,667**	**8.0**
		148,906	26,467	22,978	940	382		180	21,732	8.1
		78,179	14,269	15,637	180	2	5	103	39,844	5.3
	Raw	**97,489**	**20,004**	**20,005**	**930**	**6**	**10**	**124**	**40,227**	**5.4**
POND 2		121,569	28,044	25,593	4,200	24	18	148	40,610	5.4
OUTFLOW		72,638	16,230	14,650	46	15		83	12,907	5.8
	Supernatant	**90,641**	**22,411**	**18,649**	**230**	**61**		**102**	**12,973**	**5.9**
		113,106	30,944	23,740	940	244		124	13,040	6.0
		17,707	3,883	7,050	37	2	17	630	194,142	11.9
	Raw	**22,881**	**4,964**	**8,801**	**150**	**6**	**26**	**680**	**195,027**	**12.0**
NE CREEK		29,567	6,347	10,987	420	25	38	733	195,911	12.1
SITE 1		19,586	1,304	8,157	9	4		638	12,049	11.4
	Supernatant	**25,505**	**1,849**	**10,170**	**43**	**15**		**688**	**12,193**	**11.5**
		33,214	2,622	12,680	180	49		741	12,338	11.7
		17,364	3,052	6,185	42	19	11	680	260,513	13.4
	Raw	**22,403**	**3,968**	**7,744**	**240**	**73**	**18**	**732**	**261,493**	**13.4**
NE CREEK		28,904	5,158	9,694	1,000	285	28	787	262,474	13.5
SITE 2		19,153	2,410	5,200	5	10		703	15,678	14.1
	Supernatant	**24,901**	**3,195**	**6,549**	**21**	**38**		**756**	**15,933**	**14.3**
		32,375	4,237	8,248	42	151		812	16,189	14.4

17 April 2007

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
		8,057	1,395	2,354	36	0			45,043	
	Raw	**10,357**	**1,769**	**3,128**	**240**	**3**	**0**	**0**	**45,353**	
POND 1		13,315	2,245	4,114	1,300	21			45,664	
RUNOFF		4,187	854	2,037	71				18,284	
	Supernatant	**5,432**	**1,064**	**2,745**	**460**	**0**	**0**	**0**	**18,327**	
		7,049	1,327	3,590	2,400				18,370	
		12,638	3,126	25,922	150	22		35	112,838	
	Raw	**17,083**	**4,105**	**34,150**	**1,100**	**92**	**0**	**48**	**113,893**	
POND 1		23,091	5,392	72,420	4,800	382		64	114,949	
INFLOW		9,789	2,504	6,089	71	10		15	16,923	
	Supernatant	**12,726**	**3,296**	**9,683**	**460**	**38**	**0**	**24**	**17,187**	
		16,545	4,340	12,427	2,400	153		36	17,450	
		10,778	2,064	20,244	150	19		385	117,052	
	Raw	**15,672**	**4,129**	**27,296**	**1,100**	**74**	**0**	**424**	**118,400**	
POND 1		20,867	5,422	44,836	4,800	291		466	119,748	
OUTFLOW		6,089	759	15,255	71	10		259	12,834	
	Supernatant	**9,683**	**2,025**	**21,216**	**460**	**42**	**0**	**292**	**13,007**	
		12,427	2,594	30,424	2,400	184		327	13,179	
	Raw									
POND 2										
RUNOFF										
	Supernatant									
	Raw									
POND 2										
INFLOW										
	Supernatant									
	Raw									
POND 2										
OUTFLOW										
	Supernatant									
		6,301	528	1,724	36	4	15	244	383,197	
	Raw	**7,884**	**862**	**2,364**	**240**	**15**	**24**	**276**	**438,267**	
NE CREEK		10,112	1,076	3,062	1,300	49	36	311	493,337	
SITE 1		6,451	243	1,753	30	4	17	324	124,080	
	Supernatant	**8,068**	**468**	**2,399**	**150**	**15**	**26**	**360**	**127,420**	
		10,344	601	3,111	440	49	38	399	130,760	
	Raw									
NE CREEK										
SITE 2										
	Supernatant									

3 June 2007

		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/100 mL)	Enterococci (MPN/100 mL)	*C. perf.* spores (MPN/100 mL)	*Salm.* spp (MPN/L)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	Particles (#/mL)	TOC (mg/L)
POND 1 RUNOFF	Raw									
	Supernatant									
POND 1 INFLOW	Raw									
	Supernatant									
POND 1 OUTFLOW	Raw									
	Supernatant									
POND 2 RUNOFF	Raw									
	Supernatant									
POND 2 INFLOW	Raw	115,768	252	17,401	71	224			40,561	8.3
		145,351	**1,008**	**26,655**	**460**	**933**	**0**	**TNTC**	**41,427**	**8.6**
		182,493	4,026	40,832	2,400	3,889			42,292	8.8
	Supernatant	57,176	1,385	23,814	15	994			22,864	7.6
		78,520	**1,949**	**35,048**	**93**	**4,622**	**0**	**TNTC**	**23,440**	**7.9**
		107,831	2,742	51,581	380	21,510			24,016	8.2
POND 2 OUTFLOW	Raw	99,209	490	5,012	30	224		0	39,227	7.1
		123,942	**1,518**	**9,665**	**140**	**933**	**0**	**2**	**39,240**	**7.2**
		154,841	4,705	18,638	370	3,889		7	39,253	7.3
	Supernatant	89,027	759	3,541	71	98	1	6	14,610	7.3
		111,038	**2,025**	**7,433**	**460**	**427**	**4**	**12**	**15,460**	**7.4**
		138,491	5,404	15,604	2,400	1,864	10	21	16,310	7.5
NE CREEK SITE 1	Raw	12,427	2,024	2,394	71	57	0	1,534	294,936	6.2
		15,656	**2,729**	**3,176**	**460**	**231**	**2**	**1,612**	**298,307**	**6.6**
		19,723	3,680	4,214	2,400	938	7	1,693	301,678	7.0
	Supernatant	4,978	802	2,144	15	44	3	1,773	20,591	6.6
		6,281	**1,218**	**2,874**	**93**	**147**	**8**	**1,856**	**21,227**	**6.7**
		7,926	1,851	3,853	380	487	16	1,942	21,862	6.8
NE CREEK SITE 2	Raw									
	Supernatant									

Appendix D: Northeast Creek Protozoan Sampling Data

Storm Event Samples

Date		Fecal coliforms (MPN/ 100 mL)	*E. coli* (MPN/ 100 mL)	Enterococci (MPN/ 100mL)	*C. perf.* spores (MPN/ 100 mL)	F+ coliphage (PFU/ 100 mL)	Somatic coliphage (PFU/ 100 mL)	*Salm.* spp. (MPN/L)	*Giardia* (cysts/L)	*Crypto* (oocysts/L)	Turbidity (NTU)	Particles (#/mL)	TOC (mg/L)
12 April 2007	raw	8,382	2,076	3,208	70	25	570	11				447,301	8.8
		12,646	**4,147**	**5,787**	**390**	**36**	**618**	**28**	**18**	**10**	**229**	**454,480**	**9.6**
		19,080	8,285	10,441	1,300	50	669	76				461,659	10.3
	cent	3,607	1,064	4,833	150	37	732	57				123,146	9.1
		6,345	**2,555**	**8,018**	**930**	**50**	**786**	**231**	**10**	**4**	**149**	**138,607**	**9.4**
		11,163	6,133	13,301	3,800	66	843	938				154,067	9.8
15 April 2007	raw	6,301	528	1,724	36	15	244	4				383,197	
		7,884	**862**	**2,364**	**240**	**24**	**276**	**15**	**49**	**bt**	**160**	**438,267**	**3.6**
		10,112	1,076	3,062	1,300	36	311	49				493,337	
	cent	6,451	243	1,753	30	17	324	4				124,080	
		8,068	**468**	**2,399**	**150**	**26**	**360**	**15**	**6**	**1**	**41.1**	**127,420**	**3.3**
		10,344	601	3,111	440	38	399	49				130,760	
12 May 2007	raw	8,414	2,145	13,063	70	8	1,127	15				598,095	7.0
		10,491	**2,876**	**16,502**	**430**	**14**	**1,194**	**36**	**19**	**13**	**231**	**630,267**	**8.3**
		13,080	3,855	20,847	2,100	23	1,264	89				662,438	9.6
	cent	4,049	940	13,285	10	5	1,102	98				131,071	7.3
		5,164	**1,394**	**16,800**	**90**	**11**	**1,168**	**427**	**6**	**bt**	**150**	**140,953**	**7.7**
		6,585	2,067	21,245	360	20	1,237	1,864				150,836	8.0
3 June 2007	raw	12,427	2,024	2,394	71	0.2	1,534	57				294,936	6.2
		15,656	**2,729**	**3,176**	**460**	**2**	**1,612**	**231**	**15**	**7**	**136**	**298,307**	**6.6**
		19,723	3,680	4,214	2,400	7	1,693	938				301,678	7.0
	cent	7,397	664	3,252	71	6	1,737	98				20,591	5.9
		9,228	**1,040**	**4,207**	**460**	**12**	**1,820**	**427**	**12**	**5**	**73.2**	**21,227**	**6.3**
		11,513	1,630	5,444	2,400	21	1,906	1,864				21,862	6.6

Dry Weather Samples

Date		Fecal coliforms (MPN/100 mL)	*E. coli* (MPN/ 100 mL)	Enterococci (MPN/ 100mL)	*C. perf.* spores (MPN/100 mL)	F+ coliphage (PFU/100 mL)	Somatic coliphage (PFU/100 mL)	*Salm.* spp. (MPN/L)	*Giardia* (cysts/L)	*Crypto* (oocysts/L)	Turbidity (NTU)	Particles (#/mL)	TOC (mg/L)
3 April 2007		261	17	39	7	1	20					23,518	5.5
	raw	**344**	**36**	**67**	**43**	**4**	**30**	**0**	**12**	**2**	**22**	**24,380**	**5.6**
		453	76	116	210	10	43					25,242	5.7
		236	32	21	4	8	5	0				26,719	7.3
	cent	**313**	**58**	**41**	**23**	**14**	**10**	**3**	**6**	**6**	**19.3**	**27,067**	**7.3**
		416	105	82	120	23	18	21				27,414	7.3
22 May 2007		2,010	99	496	7		81					71,228	7.7
	raw	**2,622**	**145**	**626**	**43**	**0**	**100**	**0**	**8**	**bt**	**21.6**	**74,940**	**7.8**
		3,420	214	790	210		122					78,652	7.9
		2,439	95	200	1		67	0				50,597	7.9
	cent	**3,209**	**141**	**270**	**9**	**0**	**84**	**4**	**2**	**bt**	**19**	**54,153**	**8.1**
		4,223	208	365	36		104	26				57,710	8.3
23 July 2007		9,254	1,452	837	7	3	69	57				144,278	7.7
	raw	**11,547**	**2,031**	**1,044**	**43**	**7**	**86**	**231**	**27**	**7**	**29.2**	**149,220**	**8.0**
		14,409	2,842	1,301	210	14	106	938				154,162	8.4
		6,522	1,443	680	30		25	22				96,720	7.9
	cent	**8,154**	**2,021**	**849**	**150**	**0**	**36**	**92**	**7**	**3**	**19.7**	**102,500**	**9.0**
		10,195	2,829	1,061	440		50	382				108,280	10.1
28 August 2007		1,073	52	14	7	0.2	6	0				107,529	
	raw	**1,409**	**65**	**31**	**43**	**2**	**12**	**3**	**8**	**13**	**38.3**	**118,587**	
		1,849	82	68	210	7	21	22				129,645	
		634	28	14	15	0.2	15	0				60,782	
	cent	**901**	**41**	**31**	**93**	**2**	**24**	**3**	**3**	**bt**	**32.9**	**63,047**	
		1,280	59	68	380	7	36	22				65,311	
17 October 2007		169	9	25	7		0.2					82,274	
	raw	**217**	**14**	**33**	**43**	**0**	**2**	**0**	**bt**	**bt**	**16**	**86,220**	**9.41**
		279	20	44	210		7					90,166	
		117	9	20	14							74,519	
	cent	**163**	**16**	**30**	**75**	**0**	**0**	**0**	**bt**	**bt**	**15.1**	**76,633**	**6.67**
		226	27	45	230							78,747	

Appendix E: Protozoan Recovery Data

Storm Event Samples

			Filtration			ColorSeed			Wild type		
Date	Sample type		initial vol. filtered (L)	pellet vol. (mL)	# slides	Claimed num.	Count num.	Recovery (%)	Raw Count	Corrected Count	Corrected Count per L
12 April 2007	raw	Crypto	5	0.75	3	101	12	12%	6	51	10
		Giardia	5	0.75	3	100	21	21%	19	90	18
	cent	Crypto	3.5	0.75	3	101	10	10%	2	20	4
		Giardia	3.5	0.75	3	100	27	27%	13	48	10
	IMS+	Crypto	N/A		1	101	42	42%	N/A		
		Giardia			1	100	0	0%			
	IMS-	Crypto	N/A		1	0	0	N/A	N/A		
		Giardia			1	0	0				
	OPR	Crypto	N/A			N/A			N/A		
		Giardia									
15 April 2007	raw	Crypto	5	1.1	4	101	4	4%	0	0	0
		Giardia	5	1.1	4	100	9	9%	22	244	49
	cent	Crypto	3.5	0.5	2	101	19	19%	1	5	1
		Giardia	3.5	0.5	2	100	34	34%	10	29	6
	IMS+	Crypto	N/A		1	101	57	56%	N/A		
		Giardia			1	100	50	50%			
	IMS-	Crypto	N/A		1	0	0	N/A	N/A		
		Giardia			1	0	0				
	OPR	Crypto	N/A			N/A			N/A		
		Giardia									
12 May 2007	raw	Crypto	5	1.5	5	101	6	6%	4	67	13
		Giardia	5	1.5	5	100	14	14%	13	93	19
	cent	Crypto	3.5	1.1	3	101	7	7%	0	0	0
		Giardia	3.5	1.1	3	100	34	34%	10	29	6
	IMS+	Crypto	N/A		1	101	53	52%	N/A		
		Giardia			1	100	56	56%			
	IMS-	Crypto	N/A		1	0	0	N/A	N/A		
		Giardia			1	0	0				
	OPR	Crypto	N/A			N/A			N/A		
		Giardia									
3 June2007	raw	Crypto	5	0.75	3	101	3	3%	1	34	7
		Giardia	5	0.75	3	100	8	8%	6	75	15
	cent	Crypto	3.5	0.5	2	101	12	12%	3	25	5
		Giardia	3.5	0.5	2	100	24	24%	14	58	12
	IMS+	Crypto	N/A		1	101	56	55%	N/A		
		Giardia			1	100	51	51%			
	IMS-	Crypto	N/A		1	0	0	N/A	N/A		
		Giardia			1	0	0				
	OPR	Crypto	10	0	1	101	49	49%	N/A		
		Giardia	10	0	1	100	29	29%			

Dry Weather Samples

			Filtration			ColorSeed			Wild type		
Date	Sample type		initial vol. filtered (L)	pellet vol. (mL)	# slides	Claimed num.	Count num.	Recovery (%)	Raw Count	Corrected Count	Corrected Count per L
3 April 2007	raw	Crypto	5	0.5	2	100	19	19%	2	11	2
		Giardia	5	0.5	2	100	47	47%	28	60	12
	cent	Crypto	3.5	0.5	2	100	25	25%	7	28	6
		Giardia	3.5	0.5	2	100	59	59%	19	32	6
	IMS+	Crypto				100	59	59%			
		Giardia		N/A		100	46	46%		N/A	
	IMS-	Crypto				0	0				
		Giardia		N/A		0	0	N/A		N/A	
	OPR	Crypto									
		Giardia		N/A			N/A			N/A	
22 May 2007	raw	Crypto	5	0.5	2	101	1	1%	0	0	0
		Giardia	5	0.5	2	100	44	44%	18	41	8
	cent	Crypto	3.5	0.2	1	101	1	1%	0	0	0
		Giardia	3.5	0.2	1	100	35	35%	3	9	2
	IMS+	Crypto				101	49	49%			
		Giardia		N/A		100	61	61%		N/A	
	IMS-	Crypto				0	0				
		Giardia		N/A		0	0	N/A		N/A	
	OPR	Crypto	10	0	1	101	30	30%			
		Giardia	10	0	1	100	26	26%		N/A	
23July 2007	raw	Crypto	5	0.5	2	101	6	6%	2	34	7
		Giardia	5	0.5	2	100	6	6%	8	133	27
	cent	Crypto	3.5	0.2	1	101	14	14%	2	14	3
		Giardia	3.5	0.2	1	100	3	3%	1	33	7
	IMS+	Crypto									
		Giardia		N/A			N/A			N/A	
	IMS-	Crypto				0	0				
		Giardia		N/A		0	0	N/A		N/A	
	OPR	Crypto									
		Giardia		N/A			N/A			N/A	
28 August 2007	raw	Crypto	5	0.5	2	101	3	3%	2	67	13
		Giardia	5	0.5	2	100	20	20%	8	40	8
	cent	Crypto	3.5	0.5	2	101	3	3%	0	0	0
		Giardia	3.5	0.5	2	100	19	19%	3	16	3
	IMS+	Crypto				101	20	20%			
		Giardia		N/A		100	50	50%		N/A	
	IMS-	Crypto				0	0				
		Giardia		N/A		0	0	N/A		N/A	
	OPR	Crypto									
		Giardia		N/A			N/A			N/A	
17 October 2007	raw	Crypto	5	0.5	2	100	2	2%	12	600	120
		Giardia	5	0.5	2	100	10	10%	0	0	0
	cent	Crypto	3.5	0.2	1	100	1	1%	3	300	60
		Giardia	3.5	0.2	1	100	14	14%	3	21	4
	IMS+	Crypto				100	59	59%			
		Giardia		N/A		100	41	41%		N/A	
	IMS-	Crypto				0	0				
		Giardia		N/A		0	0	N/A		N/A	
	OPR	Crypto	10	0	1	100	5	5%			
		Giardia	10	0	1	100	19	19%		N/A	

Appendix F: qPCR Example Calculations

Calibration

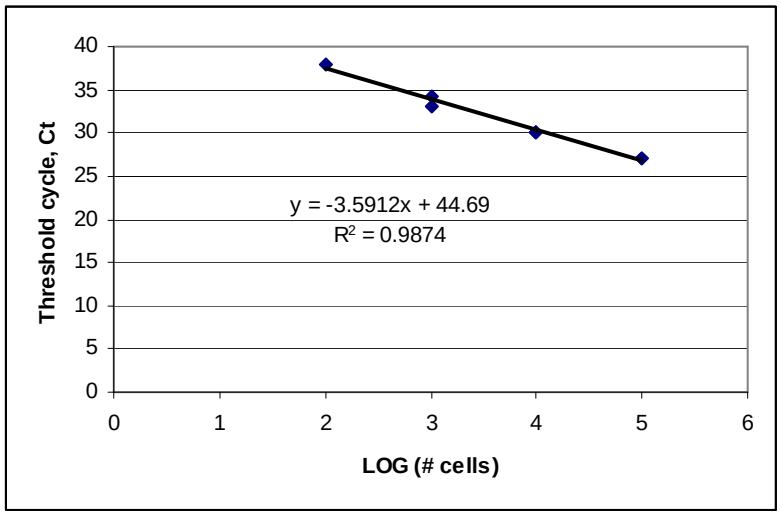
Sample values were calculated based on a calibration curve generated via extraction and subsequent dilution of *E. coli* from a filter holding 10^5 cells. This calibration curve is used to confirm the qPCR reaction and calculate exponential amplification and efficiency.

Example: *E. coli* curve from October 2008 (C_t = threshold cycle)

Raw Data:

Dilution	# cells	C_t
1:1	10^5	27.02
1:1	10^5	26.96
1:10	10^4	30.14
1:10	10^4	29.98
1:100	10^3	34.22
1:100	10^3	33.14
1:1,000	10^2	38
1:1,000	10^2	ND

Linear regression of log(# cells) vs. threshold cycle:



Exponential amplification = $10^{-1/m}$ where m = slope
Exponential amplification = $10^{-1/-3.5912}$ = 1.90

Efficiency = exponential amplification – 1
Efficiency = 1.90 – 1 = 0.90 → 90% efficiency

Inhibition threshold

Samples were considered inhibited if the specimen processing complex (*O. keta*) threshold cycle (C_t) value was more than 1.5 cycles greater than the average C_t value from the non-diluted (10^5 cells) *O. keta* calibration value.

Example: October 2008 data

O. keta 1:1 C_t values = 26.23, 25.80

Inhibition threshold = [0.5*(26.23+25.80)] + 1.5 = 27.52 cycles

Number of cells

The inclusion of an exogenous control (*O. keta* sperm cells) allowed correction for potential loss in bead beating and/or amplification. *E. coli* values are calculated through comparison of the sample *E. coli* and *O. keta* threshold cycle (C_t) values with the known C_t from the *O. keta* and *E. coli* calibrations.

Example: October 2008 data; Booker Creek, 8/26/08, centrifuge supernatant sample 1, 50 mL filtered

Calibration data: C_t for *E. coli* calibration, 10^5 cells = 26.99
C_t for *O. keta* calibration, 10^5 cells = 26.02
Exponential amplification (EA) = 1.978 (98% efficiency)

Sample data: C_t (*E. coli*) = 38.49
C_t (*O. keta*) = 27.42

E. coli cells in sample = $10^5 * (EA)^{-\Delta\Delta Ct}$

Where $\Delta\Delta C_t = [(C_{t,\ E.\ coli} - C_{t,\ O.\ keta})_{sample} - [(C_{t,\ E.\ coli} - C_{t,\ O.\ keta})_{calibration}]$

For the above example, $\Delta\Delta C_t = (38.49 - 27.42) - (26.99 - 26.02) = 10.10$

E. coli cells in sample = $10^5 * (1.978)^{-10.10}$ = 102 *E. coli* cells/sample

E. coli cells per 100 mL = [# cells in filtered sample/100]*filtrate volume

For the above example, # *E. coli* per 100 mL = (102/100)*50 = 204

Appendix G: qPCR Data

qPCR samples analyzed on 11-14 August 2008 were spiked with different O. keta concentrations due to an experimental error. Appropriate calibration data are provided before each data set.

August 2008: Data Set 1

E. coli calibration (88% efficiency)

Dilution	# cells	C_t
1:1	100000	26.64
1:1	100000	-
1:10	10000	29.92
1:10	10000	29.91
1:100	1000	34.11
1:100	1000	33.87
1:1,000	10	37.26
1:1,000	10	-

O. keta calibration (95% efficiency)

Dilution	# cells	C_t
1:1	100000	25.56
1:1	100000	26.06
1:10	10000	29.7
1:10	10000	30.43
1:100	1000	33.1
1:100	1000	33.25
1:1,000	10	36.44
1:1,000	10	36.14

Samples were considered inhibited if the O. keta C_t > 27.31. No samples exceeded this value.

The average number of cells per filter was calculated as the arithmetic mean of the concentrations for both filter duplicated if both were detectable via qPCR. If one filter was nondetectable while its duplicate was detectable, the nondetect value was ignored if the expected *E. coli* concentration captured by the filter was ≥ 10 MPN. If the expected concentration was < 10 MPN, the nondetectable duplicate was assigned a value of 0.5 cells for the purposes of averaging.

Sampling: 22 May 2008

Site	Sample	Volume filtered (mL)	expected MPN per filter	Dilution	C_t (*O. keta*)	C_t (*E. coli*)	# cells per filter	average # cells per filter
Booker Creek	raw - 1	10	43	1:1	26.29	39.68	23	
	raw - 2	10	43	1:1	26.15	0	ND	23
	cent - 1	10	46	1:1	25.98	38.11	54	
	cent - 2	10	46	1:1	25.63	36.33	139	96
Eno River	raw - 1	10	5	1:1	26.29	0	ND	
	raw - 2	10	5	1:1	25.81	41.51	5	3
	cent - 1	10	5	1:1	26.15	39.95	18	
	cent - 2	10	5	1:1	26.46	0	ND	18
Meeting of the Waters Creek	raw - 1	10	6	1:1	26.21	39.95	18	
	raw - 2	10	6	1:1	26.35	0	ND	18
	cent - 1	10	7	1:1	26.05	0	ND	
	cent - 2	10	7	1:1	26.3	41.37	8	4
Northeast Creek - Site 1	raw - 1	10	6	1:1	26.42	0	ND	
	raw - 2	10	6	1:1	26.61	0	ND	ND
	cent - 1	10	8	1:1	26.48	0	ND	
	cent - 2	10	8	1:1	27.08	0	ND	ND

Sampling: 11 June 2008

Site	Sample	Volume filtered (mL)	expected MPN per filter	Dilution	C_t (*O. keta*)	C_t (*E. coli*)	# cells per filter	average # cells
Booker Creek	raw - 1	20	61	1:1	26.59	36.44	245	
	raw - 2	20	61	1:1	25.93	36.76	128	186
	cent - 1	20	59	1:1	25.82	0	ND	
	cent - 2	20	59	1:1	25.52	39.94	12	12
Eno River	raw - 1	20	4	1:1	25.59	0	ND	
	raw - 2	20	4	1:1	25.76	0	ND	ND

August 2008: Data Set 2

E. coli calibration (88% efficiency)

Dilution	# cells	C_t
1:1	100000	26.64
1:1	100000	-
1:10	10000	29.92
1:10	10000	29.91
1:100	1000	34.11
1:100	1000	33.87
1:1,000	10	37.26
1:1,000	10	-

O. keta calibration (93% efficiency)

Dilution	# cells	C_t
1:1	100000	20.51
1:1	100000	
1:10	10000	24.09
1:10	10000	
1:100	1000	27.38
1:100	1000	
1:1,000	10	31.12
1:1,000	10	

Samples were considered inhibited if the O. keta C_t > 22.01. No samples exceeded this value.

Sampling: 11 June 2008

Site	Sample	Volume filtered (mL)	expected MPN per filter	Dilution	C_t (*O. keta*)	C_t (*E. coli*)	# cells per filter	average # cells
Eno River	cent - 1	20	5	1:1	19.71	0	ND	
	cent - 2	20	5	1:1	19.94	0	ND	ND
Meeting of the Waters Creek	raw - 1	20	132	1:1	19.96	40.33	9	
	raw - 2	20	132	1:1	20.03	36.7	100	54
	cent - 1	20	132	1:1	19.76	36.94	71	
	cent - 2	20	132	1:1	20.14	37.06	85	78
Northeast Creek - Site 1	raw - 1	20	4	1:1	19.9	0	ND	
	raw - 2	20	4	1:1	20	0	ND	ND
	cent - 1	20	4	1:1	19.67	0	ND	
	cent - 2	20	4	1:1	19.98	0	ND	ND
Northeast Creek - Site 2	raw - 1	20	36	1:1	21.36	38.48	74	
	raw - 2	20	36	1:1	20.58	40.89	9	42
	cent - 1	20	23	1:1	20.62	37.64	79	
	cent - 2	20	23	1:1	20.78	0	ND	79

Sampling: 17 June 2008

Site	Sample	Volume filtered (mL)	expected MPN per filter	Dilution	C_t (*O. keta*)	C_t (*E. coli*)	# cells per filter	average # cells per filter
Booker Creek	raw - 1	20	1,458	1:1	20.7	32.07	3,221	
	raw - 2	20	1,458	1:1	20.57	31.81	3,507	3,364
	cent - 1	20	1,183	1:1	19.93	32.58	1,392	
	cent - 2	20	1,183	1:1	20.34	32.35	2,117	1,754
Eno River	raw - 1	20	16	1:1	20.22	0	ND	
	raw - 2	20	16	1:1	19.89	0	ND	ND
	cent - 1	20	10	1:1	19.86	38.79	23	
	cent - 2	20	10	1:1	0	39.85	ND	23
Meeting of the Waters Creek	raw - 1	20	78	1:1	19.88	35.74	170	
	raw - 2	20	78	1:1	19.8	40.31	8	89
	cent - 1	20	74	1:1	19.67	36.11	116	
	cent - 2	20	74	1:1	19.63	36.57	84	100
Northeast Creek - Site 1	raw - 1	20	13	1:1	20.17	0	ND	
	raw - 2	20	13	1:1	20.1	0	ND	ND
	cent - 1	20	9	1:1	19.45	40.54	5	
	cent - 2	20	9	1:1	19.79	37	70	38
Northeast Creek - Site 2	raw - 1	20	27	1:1	21.57	0	ND	
	raw - 2	20	27	1:1	21.41	36.48	285	285
	cent - 1	20	41	1:1	20.49	0	ND	
	cent - 2	20	41	1:1	20.49	0	ND	ND

Sampling: 7 July 2008

Site	Sample	Volume filtered (mL)	expected MPN per filter	Dilution	C_t (*O. keta*)	C_t (*E. coli*)	# cells per filter	average # cells
Booker Creek	raw - 1	1	2	1:1	19.42	0	ND	
	raw - 2	1	2	1:1	19.28	0	ND	ND
	cent - 1	1	4	1:1	19.22	41.28	3	
	cent - 2	1	4	1:1	19.59	0	ND	2
Eno River	raw - 1	5	3	1:1	19.49	0	ND	
	raw - 2	5	3	1:1	19.22	38.05	24	24
	cent - 1	5	4	1:1	19.43	36.15	97	
	cent - 2	5	4	1:1	19.9	35.22	242	169
Meeting of the Waters Creek	raw - 1	1	1	1:1	19.28	0	ND	
	raw - 2	1	1	1:1	19.41	0	ND	ND

August 2008: Data Set 3

E. coli calibration (88% efficiency)

Dilution	# cells	C_t
1:1	100000	26.64
1:1	100000	-
1:10	10000	29.92
1:10	10000	29.91
1:100	1000	34.11
1:100	1000	33.87
1:1,000	10	37.26
1:1,000	10	-

O. keta calibration (86% efficiency)

Dilution	# cells	C_t
1:1	100000	24.04
1:1	100000	24.14
1:10	10000	27.78
1:10	10000	27.65
1:100	1000	31.92
1:100	1000	32.05
1:1,000	10	35.3
1:1,000	10	34.7

Samples were considered inhibited if the O. keta C_t >25.59. No samples exceeded this value.

Sampling: 7 July 2008

Site	Sample	Volume filtered (mL)	expected MPN per filter	Dilution	C_t (*O. keta*)	C_t (*E. coli*)	# cells per filter	average # cells per filter
Meeting of the Waters Creek	cent - 1	1	2	1:1	23.31	0	ND	
	cent - 2	1	2	1:1	23.39	0	ND	ND
Northeast Creek - Site 1	raw - 1	1	5	1:1	23.29	38.26	44	
	raw - 2	1	5	1:1	23.31	0	ND	44
	cent - 1	1	3	1:1	23.21	39.66	18	
	cent - 2	1	3	1:1	23.26	0	ND	18
Northeast Creek - Site 2	raw - 1	1	3	1:1	23.22	39.33	22	
	raw - 2	1	3	1:1	23.08	0	ND	22
	cent - 1	1	2	1:1	23.75	0	ND	
	cent - 2	1	2	1:1	23.25	0	ND	ND

Sampling: 14 July 2008

Site	Sample	Volume filtered (mL)	expected MPN per filter	Dilution	C_t (*O. keta*)	C_t (*E. coli*)	# cells per filter	average # cells
Booker Creek	raw - 1	10	22	1:1	24.45	40.91	17	
	raw - 2	10	22	1:1	24.19	0	ND	17
	cent - 1	10	15	1:1	24.03	0	ND	
	cent - 2	10	15	1:1	24.96	0	ND	ND
Eno River	raw - 1	50	24	1:1	23.63	0	ND	
	raw - 2	50	24	1:1	23.4	0	ND	ND
	cent - 1	50	18	1:1	23.92	44.72	1	
	cent - 2	50	18	1:1	23.37	0	ND	1
Meeting of the Waters Creek	raw - 1	20	62	1:1	24.65	38.54	86	
	raw - 2	20	62	1:1	24.13	39.65	31	59
	cent - 1	20	48	1:1	23.4	39.69	19	
	cent - 2	20	48	1:1	23.98	0	ND	19
Northeast Creek - Site 1	raw - 1	20	24	1:1	24.28	44.96	1	
	raw - 2	20	24	1:1	24.25	43.87	2	2
	cent - 1	20	29	1:1	23.81	0	ND	
	cent - 2	20	29	1:1	23.7	38.85	39	39
Northeast Creek - Site 2	raw - 1	10	21	1:1	23.42	0	ND	
	raw - 2	10	21	1:1	24.06	44.63	1	1
	cent - 1	10	20	1:1	23.74	0	ND	
	cent - 2	10	20	1:1	23.66	38.93	36	36

October 2008: Data Set

All samples analyzed 13-15 October 2008 were spiked with the same O. keta level.

E. coli calibration (90% efficiency)

Dilution	# cells	C_t
1:1	100000	27.02
1:1	100000	26.96
1:10	10000	30.14
1:10	10000	29.98
1:100	1000	34.22
1:100	1000	33.14
1:1,000	10	38
1:1,000	10	ND

O. keta calibration (98% efficiency)

Dilution	# cells	C_t
1:1	100000	25.8
1:1	100000	26.23
1:10	10000	29.38
1:10	10000	29.1
1:100	1000	32.81
1:100	1000	32.81
1:1,000	10	36.08
1:1,000	10	36.07

Raw samples were considered inhibited if the O. keta C_t >27.52. Inhibited samples were diluted at 1:10. These diluted samples were then considered inhibited if the O. keta C_t > 30.74. No diluted samples exceeded this value.

26 August 2008

Site	Sample	Volume filtered (mL)	expected MPN per filter	Dilution	C_t (*O. keta*)	C_t (*E. coli*)	# cells per filter	average # cells per filter
Booker Creek	raw - 1	50		1:1	27.16	0.00	ND	
				1:10				
	raw - 2	50	52	1:1	28.11			
				1:10	29.19	0.00	ND	ND
	cent - 1	50		1:1	27.42	38.49	102	
				1:10				
	cent - 2	50	80	1:1	27.30	0.00	ND	
				1:10				102
Eno River	raw - 1	100		1:1	26.50	0.00	ND	
				1:10				
	raw - 2	100	289	1:1	26.52	38.25	65	
				1:10				65
	cent - 1	100		1:1	26.25	37.48	92	
				1:10				
	cent - 2	100	210	1:1	27.07	38.00	112	102
				1:10				
Meeting of the Waters Creek	raw - 1	100		1:1	27.08	34.91	931	
				1:10				
	raw - 2	100	1,213	1:1	27.43	34.53	1,532	
				1:10				1,231
	cent - 1	100		1:1	27.87	35.79	875	
				1:10				
	cent - 2	100	1,081	1:1	26.86	33.39	2,260	
				1:10				1,568
Northeast Creek - Site 1	raw - 1	50		1:1	29.30			
				1:10	29.29	0.00	ND	
	raw - 2	50	104	1:1	28.78			
				1:10	29.34	0.00	ND	ND
	cent - 1	50		1:1	27.90			
				1:10	29.36	0.00	ND	
	cent - 2	50	63	1:1	28.40			
				1:10	29.59	0.00	ND	ND
Northeast Creek - Site 1a	raw - 1	25		1:1	27.57	36.47	449	
				1:10				
	raw - 2	25	450	1:1	26.87	0.00	ND	
				1:10				449
	cent - 1	25		1:1	27.36	37.80	157	
				1:10				
	cent - 2	25	450	1:1	26.24	35.07	471	
				1:10				314
Northeast Creek - Site 2	raw - 1	30		1:1	28.36			
				1:10	29.48	0.00	ND	
	raw - 2	30	356	1:1	27.56	37.71	191	
				1:10				191
	cent - 1	30		1:1	27.20	40.96	16	
				1:10				
	cent - 2	30	382	1:1	27.11	44.82	1	
				1:10				9

27 August 2008

Site	Sample	Volume filtered (mL)	expected MPN per filter	Dilution	C_t (*O. keta*)	C_t (*E. coli*)	# cells per filter	average # cells per filter
Booker Creek	raw - 1	10		1:1	28.17			
				1:10	29.61	36.28	2,054	
	raw - 2	10	910	1:1	28.16			
				1:10	29.34	39.21	231	1,143
	cent - 1	10		1:1	27.11	35.29	733	
				1:10				
	cent - 2	10	613	1:1	27.25	34.03	1,905	
				1:10				1,319
Eno River	raw - 1	20		1:1	30.11			
				1:10	30.06	36.17	3,010	
	raw - 2	20	1,899	1:1	28.89			
				1:10	29.24	36.60	1,283	2,146
	cent - 1	20		1:1	27.88	32.69	7,306	
				1:10				
	cent - 2	20	1,439	1:1	27.48	32.89	4,852	
				1:10				6,079
Meeting of the Waters Creek	raw - 1	10		1:1	28.57			
				1:10	29.48	35.89	2,453	
	raw - 2	10	1,405	1:1	30.29			
				1:10	30.05	36.51	2,370	2,411
	cent - 1	10		1:1	27.41	33.11	3,981	
				1:10				
	cent - 2	10	573	1:1	27.71	32.03	10,207	
				1:10				7,094
Northeast Creek - Site 1	raw - 1	10		1:1	28.40			
				1:10	29.32	37.54	713	
	raw - 2	10	786	1:1	28.50			
				1:10	29.83	0.00	ND	713
	cent - 1	10		1:1	27.60	34.17	2,199	
				1:10				
	cent - 2	10	500	1:1	28.02			
				1:10	29.54	0.00	ND	2,199
Northeast Creek - Site 1a	raw - 1	5		1:1	27.70	38.93	92	
				1:10				
	raw - 2	5	282	1:1	27.55	38.92	83	
				1:10				87
	cent - 1	5		1:1	28.02			
				1:10	29.53	39.99	155	
	cent - 2	5	189	1:1	26.03	36.37	168	
				1:10				161
Northeast Creek - site 2	raw - 1	5		1:1	27.03	35.58	570	
				1:10				
	raw - 2	5	630	1:1	27.03	0.00	ND	
				1:10				570
	cent - 1	5		1:1	28.52			
				1:10	29.77	43.54	16	
	cent - 2	5	482	1:1	25.55	36.19	137	
				1:10				77

6 September 2008

Site	Sample	Volume filtered (mL)	expected MPN per filter	Dilution	C_t (*O. keta*)	C_t (*E. coli*)	# cells per filter	average # cells per filter
Booker Creek	raw - 1	20		1:1	28.05			
				1:10	29.62	36.64	1,618	
	raw - 2	20	759	1:1	28.37			
				1:10	29.75	37.39	1,060	1,339
	cent - 1	20		1:1	29.52			
				1:10	29.90	37.99	780	
	cent - 2	20	740	1:1	28.35			
				1:10	29.32	0.00	ND	780
Eno River	raw - 1	10		1:1	31.03			
				1:10	30.05	37.04	1,651	
	raw - 2	10	678	1:1	30.03			
				1:10	29.80	37.81	823	1,237
	cent - 1	10		1:1	27.44	33.04	4,262	
				1:10				
	cent - 2	10	586	1:1	28.01			
				1:10	29.35	36.61	1,373	2,818
Meeting of the Waters Creek	raw - 1	20		1:1	28.05			
				1:10	29.63	0.00	ND	
	raw - 2	20	219	1:1	28.00	36.60	550	
				1:10				550
	cent - 1	20		1:1	28.28			
				1:10	29.50	0.00	ND	
	cent - 2	20	255	1:1	27.05	36.81	249	
				1:10				249
Northeast Creek - Site 1	raw - 1	10		1:1	-	-	-	
				1:10				
	raw - 2	10	207	1:1	27.70	35.81	769	
				1:10				769
	cent - 1	10		1:1	27.52	34.54	1,618	
				1:10				
	cent - 2	10	271	1:1	28.02			
				1:10	29.48	36.60	1,511	1,564
Northeast Creek - Site 1a	raw - 1	20		1:1	29.55			
				1:10	29.41	39.00	280	
	raw - 2	20	643	1:1	29.02			
				1:10	30.00	39.28	346	313
	cent - 1	20		1:1	29.22			
				1:10	29.39	37.21	937	
	cent - 2	20	624	1:1	30.28			
				1:10	29.79	37.45	1,045	991
Northeast Creek - site 2	raw - 1	10		1:1	27.70	39.93	46	
				1:10				
	raw - 2	10	310	1:1	29.02			
				1:10	29.42	0.00	ND	46
	cent - 1	10		1:1	28.05			
				1:10	29.37	40.68	87	
	cent - 2	10	116	1:1	28.37			
				1:10	29.58	0.00	ND	87

2 October 2008

Site	Sample	Volume filtered (mL)	expected MPN per filter	Dilution	C_t (*O. keta*)	C_t (*E. coli*)	# cells per filter	average # cells per filter
Booker Creek	raw - 1	50		1:1	27.28	36.52	ND	
				1:10				
	raw - 2	50	375	1:1	27.28	0	ND	
				1:10				356
	cent - 1	50		1:1	27.78	33.3	4,501	
				1:10				
	cent - 2	50	516	1:1	27.28	32.12	7,158	
				1:10				5,830
Eno River	raw - 1	100		1:1	28.02			
				1:10	29.44	43.9	10	
	raw - 2	100	141	1:1	28.02			
				1:10	31.03	42.94	58	34
	cent - 1	100		1:1	28.97			
				1:10	29.27	32.61	19,919	
	cent - 2	100	69	1:1	29.76			
				1:10	29.51	33.85	10,068	14,994
Meeting of the Waters Creek	raw - 1	100		1:1	27.61	0.00	ND	
				1:10				
	raw - 2	100	98	1:1	28.17			
				1:10	28.90	0.00	ND	ND
	cent - 1	100		1:1	28.19			
				1:10	29.16	0.00	ND	
	cent - 2	100	121	1:1	27.33	0.00	ND	
				1:10				ND
Northeast Creek - Site 1	raw - 1	35		1:1	30.88			
				1:10	29.33	0	ND	
	raw - 2	35	120	1:1	31.19			
				1:10	29.32	0	ND	ND
	cent - 1	35		1:1	29.08			
				1:10	29.40	36.22	1,854	
	cent - 2	35	125	1:1	29.91			
				1:10	29.28	35.14	3,569	2,712
Northeast Creek - Site 1a	raw - 1	25		1:1	27.52	35.8	685	
				1:10				
	raw - 2	25	69	1:1	28.59			
				1:10	29.15	0	ND	685
	cent - 1	25		1:1	27.78	32.1	10,207	
				1:10				
	cent - 2	25	70	1:1	27.41	33.75	2,573	
				1:10				6,390

Appendix H: qPCR Water Quality Data Summary

22 May 2008

		Colilert		qPCR	Particles				
Date		Fecal coliforms (MPN/ 100 mL)	*E. coli* (MPN/ 100 mL)	*E. coli* (# cells/ 100 mL)	Number per mL	Volume per mL	TSS (mg/L)	TOC (mg/L)	Turbidity (ntu)
Booker Creek		1,960	551		33,405	3,122,199			
	Raw	**1,556**	**426**	**232**	**32,660**	**2,939,307**	**11.5**	**6.1**	**11.5**
		1,236	330		31,915	2,756,415			
		1,394	588		31,245	3,363,181			
	Cent	**1,118**	**457**	**964**	**28,553**	**3,136,087**	**9.2**	**6.4**	**10.6**
		896	356		25,862	2,908,992			
Eno River		522	90		14,191	2,904,775			
	Raw	**402**	**47**	**27**	**12,793**	**1,693,627**	**3.0**	**4.6**	**7.66**
		310	24		11,396	482,478			
		472	97		11,339	1,388,597			
	Cent	**360**	**52**	**176**	**10,293**	**1,168,087**	**10.9**	**4.5**	**6.31**
		275	28		9,248	947,577			
Meeting of the Waters Creek		984	104		44,598	10,523,127			
	Raw	**786**	**58**	**183**	**40,760**	**8,305,580**	**22.3**	**4.7**	**19.3**
		628	32		36,922	6,088,033			
		911	119		20,271	2,974,346			
	Cent	**726**	**69**	**40**	**17,393**	**2,726,100**	**7.3**	**4.6**	**12.3**
		579	40		14,515	2,477,854			
Northeast Creek Site 1		1,271	111		110,918	8,115,228			
	Raw	**1,019**	**63**	**ND**	**85,980**	**7,443,920**	**21.4**	**8.7**	**35**
		817	36		61,042	6,772,612			
		744	133		89,622	7,362,099			
	Cent	**588**	**80**	**ND**	**79,120**	**6,973,333**	**12.4**	**8.8**	**30.9**
		464	48		68,618	6,584,568			
Northeast Creek Site 1a	Raw								
	Cent								
Northeast Creek Site 2	Raw								
	Cent								

11 June 2008

		Colilert		qPCR	Particles				
Date		Fecal coliforms (MPN/ 100 mL)	*E. coli* (MPN/ 100 mL)	*E. coli* (# cells/ 100 mL)	Number per mL	Volume per mL	TSS (mg/L)	TOC (mg/L)	Turbidity (ntu)
Booker Creek		3,156	406		19,924	2,324,927			
	Raw	**2,432**	**305**	**932**	**19,460**	**2,093,907**	**4.4**	**6.2**	**5.94**
		1,873	229		18,996	1,862,886			
		2,860	392		20,270	1,202,613			
	Cent	**2,219**	**293**	**58**	**19,840**	**1,037,747**	**2.0**	**6.1**	**4.89**
		1,721	219		19,410	872,880			
Eno River		1,757	54		14,776	1,642,614			
	Raw	**1,402**	**20**	**ND**	**14,267**	**1,162,620**	**3.1**	**5.0**	**2.75**
		1,118	8		13,757	682,626			
		1,788	61		14,953	474,730			
	Cent	**1,425**	**25**	**ND**	**14,367**	**401,407**	**3.1**	**5.1**	**2.14**
		1,136	11		13,781	328,084			
Meeting of the Water's Creek		3,588	829		22,969	5,548,831			
	Raw	**2,743**	**659**	**271**	**21,767**	**4,155,107**	**16.9**	**3.5**	**8.9**
		2,097	523		20,565	2,761,382			
		3,106	832		9,725	439,628			
	Cent	**2,396**	**661**	**390**	**9,307**	**344,060**	**4.3**	**3.4**	**4.78**
		1,848	525		8,889	248,492			
Northeast Creek Site 1		2,121	27		32,281	4,939,434			
	Raw	**1,590**	**19**	**ND**	**29,933**	**4,571,073**	**8.7**	**11.7**	**9.76**
		1,191	14		27,585	4,202,713			
		1,746	26		24,913	1,872,196			
	Cent	**1,337**	**18**	**ND**	**23,653**	**1,640,540**	**5.0**	**11.1**	**6.72**
		1,024	13		22,394	1,408,884			
Northeast Creek Site 1a		528	28		41,423	9,018,693			
	Raw	**358**	**17**		**40,867**	**6,149,233**	**22.0**	**9.4**	**13.7**
		242	10		40,310	3,279,773			
	Cent								
Northeast Creek Site 2		5,392	258		162,156	12,857,445			
	Raw	**4,105**	**182**	**209**	**155,027**	**11,999,947**	**32.3**	**9.7**	**41.1**
		3,126	128		147,898	11,142,448			
		3,106	178		74,827	4,893,713			
	Cent	**2,396**	**116**	**396**	**73,367**	**3,464,613**	**21.4**	**10.6**	**24**
		1,848	76		71,907	2,035,513			

17 June 2008

		Colilert		qPCR	Particles				
Date		Fecal coliforms (MPN/ 100 mL)	*E. coli* (MPN/ 100 mL)	*E. coli* (# cells/ 100 mL)	Number per mL	Volume per mL	TSS (mg/L)	TOC (mg/L)	Turbidity (ntu)
Booker Creek		76,344	9,143		80,443	7,210,098			
	Raw	**59,052**	**7,289**	**16,821**	**78,233**	**6,257,720**	**12.1**	**12.2**	**13.1**
		45,676	5,811		76,024	5,305,342			
		74,313	7,485		80,261	6,272,975			
	Cent	**57,412**	**5,915**	**8,772**	**73,107**	**5,269,620**	**11.4**	**12.6**	**9.82**
		44,356	4,674		65,953	4,266,265			
Eno River		5,689	133		6,973	362,083			
	Raw	**4,340**	**80**	**ND**	**6,527**	**262,267**	**1.7**	**4.6**	**2.03**
		3,311	48		6,080	162,450			
		5,109	97		7,027	756,976			
	Cent	**3,884**	**52**	**113**	**6,440**	**400,393**	**1.0**	**5.1**	**1.75**
		2,952	28		5,853	43,811			
Meeting of the Waters Creek		3,323	510		18,515	2,896,781			
	Raw	**2,551**	**392**	**444**	**17,787**	**1,532,500**	**8.8**	**6.7**	**9.05**
		1,959	301		17,058	168,219			
		3,690	487		13,873	552,148			
	Cent	**2,817**	**372**	**499**	**13,047**	**395,600**	**2.6**	**7.8**	**7.43**
		2,151	285		12,221	239,052			
Northeast Creek Site 1		2,965	111		60,437	6,120,714			
	Raw	**2,294**	**63**	**ND**	**58,760**	**5,473,527**	**16.0**	**10.4**	**12.6**
		1,775	36		57,083	4,826,339			
		1,268	90		47,597	2,898,362			
	Cent	**1,017**	**47**	**189**	**43,827**	**2,325,280**	**7.1**	**17.9**	**7.87**
		816	24		40,056	1,752,198			
Northeast Creek Site 1a		4206	528		31,680	915,663			
	Raw	**2868**	**360**		**31,313**	**837,827**	**13.9**	**10.2**	**11.4**
		1955	246		30,947	759,990			
	Cent								
Northeast Creek Site 2		8,964	199		104,562	9,643,257			
	Raw	**6,973**	**133**	**1,424**	**102,080**	**7,597,453**	**45.0**	**10.0**	**29.4**
		5,424	89		99,598	5,551,650			
		5,689	286		41,969	2,842,939			
	Cent	**4,340**	**204**	**ND**	**39,367**	**1,652,593**	**7.1**	**10.8**	**17.9**
		3,311	146		36,764	462,248			

7 July 2008

Date		Colilert		qPCR	Particles				
		Fecal coliforms (MPN/ 100 mL)	***E. coli* (MPN/ 100 mL)**	***E. coli* (# cells/ 100 mL)**	**Number per mL**	**Volume per mL**	**TSS (mg/L)**	**TOC (mg/L)**	**Turbidity (ntu)**
Booker Creek		2,173	542		130,219	11,156,775			
	Raw	**1,480**	**204**	**ND**	**129,467**	**9,965,940**	**26.7**	**10.6**	**42.3**
		1,008	77		128,714	8,775,105			
		2,584	756		105,559	8,362,410			
	Cent	**1,818**	**360**	**171**	**104,527**	**7,710,473**	**16.0**	**10.0**	**35.9**
		1,279	172		103,494	7,058,537			
Eno River		405	75		23,056	1,965,196			
	Raw	**308**	**60**	**484**	**21,987**	**1,522,813**	**7.0**	**8.9**	**25.1**
		235	47		20,917	1,080,431			
		617	103		16,835	1,728,059			
	Cent	**472**	**82**	**3,382**	**16,707**	**1,294,007**	**3.0**	**7.4**	**21.6**
		362	66		16,578	859,954			
Meeting of the Waters Creek		1,858	403		42,157	3,755,769			
	Raw	**1,224**	**101**	**ND**	**41,247**	**3,260,100**	**20.4**	**7.5**	**35.5**
		807	25		40,337	2,764,431			
		2,755	542		33,633	1,424,947			
	Cent	**1,959**	**204**	**ND**	**33,233**	**1,251,047**	**9.0**	**8.4**	**27**
		1,394	77		32,833	1,077,146			
Northeast Creek Site 1		3,005	899		125,030	9,199,886			
	Raw	**2,167**	**468**	**4,398**	**120,547**	**8,630,080**	**51.0**	**14.8**	**84**
		1,562	243		116,063	8,060,274			
		4,064	686		104,557	4,199,166			
	Cent	**3,051**	**309**	**1,751**	**103,537**	**4,087,103**	**28.8**	**14.1**	**67.4**
		2,290	139		102,518	3,975,040			
Northeast Creek Site 1a		1,705	811		90,278	6,533,751			
	Raw	**850**	**202**		**89,080**	**5,135,547**	**39.4**	**13.2**	**67.1**
		423	50		87,882	3,737,343			
	Cent								
Northeast Creek Site 2		1,944	686		335,680	32,224,318			
	Raw	**1,294**	**309**	**2,163**	**324,667**	**28,395,360**	**52.0**	**14.3**	**84.3**
		861	139		313,653	24,566,402			
		1,705	471		94,292	5,919,308			
	Cent	**1,101**	**152**	**ND**	**92,809**	**4,197,797**	**32.0**	**15.6**	**66.3**
		711	49		91,325	2,476,285			

14 July 2008

		Colilert		qPCR	Particles				
Date		Fecal coliforms (MPN/ 100 mL)	*E. coli* (MPN/ 100 mL)	*E. coli* (# cells/ 100 mL)	Number per mL	Volume per mL	TSS (mg/L)	TOC (mg/L)	Turbidity (ntu)
Booker Creek		2,860	310		106,718	4,785,768			
	Raw	**2,219**	**224**	**174**	**102,213**	**4,322,773**	**34.0**	**7.7**	**76.1**
		1,721	162		97,709	3,859,778			
		2,106	223		74,934	3,171,436			
	Cent	**1,666**	**153**	**ND**	**71,187**	**2,611,733**	**31.4**	**7.9**	**69.4**
		1,318	105		67,440	2,052,030			
Eno River		275	62		5,867	1,281,397			
	Raw	**214**	**48**	**ND**	**4,437**	**955,177**	**1.7**	**5.1**	**3.38**
		166	38		3,008	628,957			
		205	47		3,146	294,903			
	Cent	**162**	**36**	**2**	**2,780**	**211,997**	**2.4**	**5.8**	**2.92**
		128	27		2,414	129,092			
Meeting of the Waters Creek		2,665	412		141,291	8,498,341			
	Raw	**2,077**	**310**	**293**	**137,753**	**7,751,387**	**35.1**	**3.6**	**40.7**
		1,619	233		134,216	7,004,432			
		4,460	326		67,938	2,081,372			
	Cent	**3,386**	**238**	**97**	**64,607**	**1,955,833**	**20.0**	**4.3**	**22.8**
		2,571	174		61,275	1,830,295			
Northeast Creek Site 1		2,691	151		71,771	6,408,133			
	Raw	**1,908**	**121**	**9**	**67,480**	**4,799,487**	**15.0**	**12.5**	**17.8**
		1,353	97		63,189	3,190,840			
		2,085	183		47,988	2,211,489			
	Cent	**1,508**	**146**	**197**	**46,927**	**2,024,980**	**4.0**	**12.7**	**13.6**
		1,090	116		45,866	1,838,471			
Northeast Creek Site 1a	**Raw**								
	Cent								
Northeast Creek Site 2		3,790	292		135,280	10,144,087			
	Raw	**2,890**	**209**	**9**	**130,620**	**10,007,280**	**40.9**	**10.7**	**36.1**
		2,204	150		125,960	9,870,473			
		3,003	282		61,257	2,051,480			
	Cent	**2,321**	**201**	**365**	**59,353**	**1,791,580**	**14.0**	**10.9**	**24.4**
		1,794	144		57,450	1,531,680			

26 August 2008

Date		Colilert		qPCR	Particles				
		Fecal coliforms (MPN/ 100 mL)	*E. coli* (MPN/ 100 mL)	*E. coli* (# cells/ 100 mL)	Number per mL	Volume per mL	TSS (mg/L)	TOC (mg/L)	Turbidity (ntu)
Booker Creek		4,839	162		31,497	3,845,212			
	Raw	**3,675**	**104**	**ND**	**29,973**	**3,024,953**	**9.3**	**6.3**	**12.4**
		2,791	66		28,450	2,204,695			
		4,583	232		22,045	864,694			
	Cent	**3,480**	**160**	**204**	**20,680**	**770,647**	**5.0**	**7.9**	**10.1**
		2,642	110		19,315	676,599			
Eno River			379		6,300	1,189,355			
	Raw	**>2,500**	**289**	**65**	**5,848**	**941,720**	**2.0**	**6.4**	**2.21**
			220		5,396	694,085			
			269		6,003	538,432			
	Cent	**>2,500**	**210**	**102**	**5,373**	**518,665**	**<1.0**	**6.5**	**1.84**
			163		4,743	498,899			
Meeting of the Waters Creek		8,778	1,515		10,964	2,148,346			
	Raw	**6,824**	**1,213**	**1,231**	**10,467**	**1,048,800**	**4.6**	**3.9**	**3.54**
		5,305	971		9,970	-50,746			
		7,049	1,348		9,668	691,312			
	Cent	**5,432**	**1,081**	**1,568**	**8,200**	**459,773**	**<1.0**	**4.5**	**2.66**
		4,187	867		6,732	228,235			
Northeast Creek Site 1		5,538	163		87,528	6,842,752			
	Raw	**4,221**	**104**	**ND**	**85,027**	**6,651,013**	**8.0**	**8.9**	**18.3**
		3,217	66		82,525	6,459,275			
		2,635	111		64,610	4,500,708			
	Cent	**2,055**	**63**	**ND**	**62,133**	**4,338,233**	**2.0**	**9.0**	**10.9**
		1,603	36		59,657	4,175,759			
Northeast Creek Site 1a		37,185	579		50,546	5,282,947			
	Raw	**24,370**	**450**	**1,794**	**47,087**	**4,629,660**	**14.0**	**8.9**	**14.3**
		15,972	349		43,628	3,976,373			
		14,424	528		15,613	1,680,009			
	Cent	**11,186**	**407**	**1,255**	**15,387**	**941,727**	**2.0**	**8.9**	**7.52**
		8,674	314		15,160	203,445			
Northeast Creek Site 2		8,534	467		106,233	7,718,640			
	Raw	**6,629**	**356**	**637**	**98,820**	**7,263,940**	**22.0**	**8.7**	**26.6**
		5,149	271		91,407	6,809,240			
		4,340	498		38,020	1,586,357			
	Cent	**3,296**	**382**	**29**	**36,040**	**1,471,740**	**7.0**	**9.5**	**17.2**
		2,504	293		34,060	1,357,123			

27 August 2008

		Colilert		qPCR	Particles				
Date		Fecal coliforms (MPN/ 100 mL)	*E. coli* (MPN/ 100 mL)	*E. coli* (# cells/ 100 mL)	Number per mL	Volume per mL	TSS (mg/L)	TOC (mg/L)	Turbidity (ntu)
Booker Creek		28,262	11,352		353,041	39,383,498			
	Raw	**21,940**	**9,098**	**11,427**	**300,387**	**36,705,827**	**120**	**15.3**	**112**
		17,032	7,291		247,732	34,028,156			
		24,312	7,741		166,666	12,643,336			
	Cent	**19,069**	**6,127**	**13,193**	**160,013**	**12,483,067**	**38**	**10.5**	**58.6**
		14,956	4,850		153,361	12,322,798			
Eno River		33,373	11,840		269,033	25,321,057			
	Raw	**25,620**	**9,493**	**10,731**	**249,800**	**23,734,860**	**76**	**19.6**	**91.6**
		19,668	7,611		230,567	22,148,663			
		22,023	9,026		129,936	3,954,285			
	Cent	**17,380**	**7,193**	**30,396**	**114,207**	**3,782,067**	**17**	**7.6**	**46.4**
		13,715	5,732		98,477	3,609,848			
Meeting of the Waters Creek		85,176	17,614		519,711	80,704,401			
	Raw	**66,158**	**14,048**	**24,115**	**472,953**	**75,181,040**	**322**	**9.3**	**283**
		51,386	11,204		426,195	69,657,679			
		28,518	7,268		193,266	6,453,270			
	Cent	**22,125**	**5,734**	**70,939**	**183,153**	**6,139,347**	**65**	**7.3**	**133**
		17,165	4,523		173,041	5,825,423			
Northeast Creek Site 1		38,892	9,834		516,300	56,110,310			
	Raw	**29,625**	**7,858**	**7,134**	**490,660**	**54,738,493**	**220**	**10.1**	**262**
		22,566	6,280		465,020	53,366,677			
		23,749	6,385		176,967	5,747,010			
	Cent	**18,655**	**4,996**	**21,990**	**170,167**	**5,499,267**	**75**	**6.6**	**171**
		14,654	3,909		163,367	5,251,523			
Northeast Creek Site 1a		68,576	7,153		802,018	82,404,334			
	Raw	**52,780**	**5,638**	**1,747**	**769,293**	**80,283,493**	**320**	**11.5**	**318**
		40,623	4,444		736,569	78,162,653			
		46,963	4,923		226,197	6,817,543			
	Cent	**35,659**	**3,770**	**3,227**	**214,013**	**6,562,987**	**85**	**6.4**	**154**
		27,075	2,888		201,830	6,308,431			
Northeast Creek Site 2		78,442	15,743		792,980	78,653,985			
	Raw	**60,743**	**12,597**	**11,392**	**780,240**	**75,210,747**	**290**		**306**
		47,038	10,079		767,500	71,767,509			
		43,788	12,025		298,497	9,041,588			
	Cent	**33,255**	**9,643**	**1,530**	**272,440**	**8,143,933**	**85**	**9.9**	**171**
		25,256	7,732		246,383	7,246,279			

6 September 2008

		Colilert		qPCR	Particles				
Date		Fecal coliforms (MPN/ 100 mL)	*E. coli* (MPN/ 100 mL)	*E. coli* (# cells/ 100 mL)	Number per mL	Volume per mL	TSS (mg/L)	TOC (mg/L)	Turbidity (ntu)
Booker Creek		27,035	4,953		231,400	20,695,445			
	Raw	**21,053**	**3,795**	**6,693**	**196,773**	**18,854,873**	**53.5**		**76.4**
		16,394	2,908		162,147	17,014,302			
		24,744	4,836		116,039	5,360,880			
	Cent	**19,386**	**3,698**	**3,898**	**113,847**	**4,622,433**	**24.0**		**40.5**
		15,187	2,827		111,655	3,883,987			
Eno River		35,912	8,523		680,600	130,157,907			
	Raw	**27,454**	**6,777**	**12,372**	**646,613**	**122,340,787**	**355.0**		**302**
		20,988	5,389		612,627	114,523,667			
		22,023	7,425		244,020	11,488,359			
	Cent	**17,380**	**5,864**	**28,177**	**234,080**	**10,460,127**	**36.0**		**95.1**
		13,715	4,632		224,140	9,431,894			
Meeting of the Waters Creek		13,080	1,699		142,277	9,073,474			
	Raw	**10,491**	**1,096**	**1,054**	**124,980**	**8,564,260**	**38.0**		**78.2**
		8,414	707		107,683	8,055,046			
		11,487	1,920		115,631	5,329,482			
	Cent	**9,207**	**1,275**	**1,247**	**114,200**	**4,533,780**	**20.0**		**67.5**
		7,380	846		112,769	3,738,078			
Northeast Creek Site 1		25,995	2,889		272,466	18,892,321			
	Raw	**20,298**	**2,071**	**7,690**	**262,047**	**18,483,967**	**50.0**		**135**
		15,849	1,484		251,627	18,075,612			
		25,995	3,658		211,357	6,890,503			
	Cent	**20,298**	**2,711**	**15,643**	**187,833**	**6,277,747**	**44.0**		**89.5**
		15,849	2,009		164,309	5,664,991			
Northeast Creek Site 1a		21,219	4,263		191,814	11,643,159			
	Raw	**16,780**	**3,217**	**1,566**	**189,007**	**10,613,333**	**56.7**		**79.8**
		13,270	2,428		186,200	9,583,507			
		20,918	4,146		129,148	4,536,396			
	Cent	**16,555**	**3,119**	**4,956**	**124,353**	**4,121,120**	**24.0**		**62**
		13,102	2,347		119,559	3,705,844			
Northeast Creek Site 2		16,040	4,123		334,294	18,046,405			
	Raw	**12,829**	**3,100**	**463**	**329,000**	**17,272,973**	**106.7**		**152**
		10,261	2,331		323,706	16,499,542			
		9,329	1,782		201,328	5,621,500			
	Cent	**7,443**	**1,162**	**867**	**182,253**	**4,967,233**	**48.0**		**107**
		5,938	758		163,178	4,312,966			

2 October 2008

		Colilert		qPCR	Particles				
Date		Fecal coliforms (MPN/ 100 mL)	*E. coli* (MPN/ 100 mL)	*E. coli* (# cells/ 100 mL)	Number per mL	Volume per mL	TSS (mg/L)	TOC (mg/L)	Turbidity (ntu)
Booker Creek		3,239	940		44,757	5,167,374			
	Raw	**2,491**	**750**	**444**	**43,600**	**4,432,907**	**6.4**		**12.6**
		1,916	599		42,443	3,698,439			
		3,257	1,287		37,786	4,363,975			
	Cent	**2,504**	**1,032**	**11,660**	**37,450**	**3,663,730**	**bt**		**13.3**
		1,926	828		37,114	2,963,485			
Eno River		689	208		18,257	1,991,907			
	Raw	**542**	**141**	**34**	**17,107**	**1,567,733**	**bt**		**5.89**
		426	95		15,957	1,143,560			
		406	119		9,393	1,429,704			
	Cent	**305**	**69**	**14,994**	**9,047**	**915,033**	**bt**		**5.24**
		229	40		8,700	400,363			
Meeting of the Waters Creek		764	155		35,610	3,468,629			
	Raw	**605**	**98**	**ND**	**31,227**	**2,578,693**	**0.8**		**6.55**
		478	61		26,843	1,688,758			
		402	184		33,951	5,069,982			
	Cent	**301**	**121**	**ND**	**30,827**	**3,132,653**	**bt**		**5.47**
		226	80		27,702	1,195,325			
Northeast Creek Site 1		2,162	450		68,936	2,673,177			
	Raw	**1,708**	**342**	**ND**	**64,580**	**2,510,440**	**14.8**		**33.1**
		1,349	259		60,224	2,347,703			
		2,035	469		46,191	1,553,060			
	Cent	**1,613**	**358**	**7,748**	**40,830**	**1,382,180**	**2.4**		**30.1**
		1,278	273		35,469	1,211,300			
Northeast Creek Site 1a		1,972	374		30,958	2,227,261			
	Raw	**1,566**	**278**	**2,739**	**28,427**	**2,137,947**	**12.9**		**16.5**
		1,243	206		25,895	2,048,632			
		1,744	376		22,007	885,858			
	Cent	**1,391**	**280**	**25,559**	**20,000**	**750,687**	**1.3**		**14.5**
		1,110	208		17,993	615,515			
Northeast Creek Site 2		3,690	461		79,582	5,794,126			
	Raw	**2,817**	**351**		**75,773**	**5,560,180**	**25.7**		**26.7**
		2,151	267		71,964	5,326,234			
		2,168	444		36,951	1,148,965			
	Cent	**1,712**	**337**		**35,693**	**1,096,453**	**1.5**		**20.4**
		1,352	255		34,435	1,043,941			

6 October 2008

Date		Colilert		qPCR	Particles				
		Fecal coliforms (MPN/ 100 mL)	*E. coli* (MPN/ 100 mL)	*E. coli* (# cells/ 100 mL)	Number per mL	Volume per mL	TSS (mg/L)	TOC (mg/L)	Turbidity (ntu)
Booker Creek		533	133		29,328	5,232,692			
	Raw	**412**	**80**		**28,180**	**3,135,000**	**3.7**		**6.51**
		318	48		27,032	1,037,308			
		529	133		27,483	2,183,920			
	Cent	**408**	**80**		**25,353**	**2,007,147**	**2.5**		**5.93**
		314	48		23,224	1,830,373			
Eno River		359	122		9,082	9,671,987			
	Raw	**275**	**98**		**8,547**	**3,939,567**	**1.1**		**4.92**
		210	78		8,011	0			
		334	105		6,960	2,271,587			
	Cent	**256**	**84**		**5,680**	**951,480**	**<0.1**		**4.08**
		197	67		4,400	0			
Meeting of the Waters Creek		273	79		15,762	1,745,206			
	Raw	**212**	**63**		**15,287**	**1,612,993**	**2.5**		**2.78**
		165	50		14,812	1,480,780			
		253	70		10,520	2,709,678			
	Cent	**198**	**55**		**9,587**	**1,415,013**	**<0.1**		**2.35**
		155	43		8,653	120,349			
Northeast Creek Site 1		527	239		44,191	1,814,720			
	Raw	**406**	**166**		**42,253**	**1,489,353**	**5.4**		**15.6**
		313	115		40,316	1,163,987			
		461	301		34,848	1,089,189			
	Cent	**351**	**217**		**29,113**	**876,507**	**2.8**		**14.1**
		267	156		23,379	663,824			
Northeast Creek Site 1a		694	215		19,627	1,482,036			
	Raw	**546**	**146**		**18,387**	**1,263,647**	**4.0**		**8.17**
		430	99		17,147	1,045,257			
		609	186		9,948	882,137			
	Cent	**475**	**122**		**9,107**	**598,700**	**0.4**		**5.63**
		370	81		8,266	315,263			
Northeast Creek Site 2		957	251		34,862	1,761,402			
	Raw	**764**	**176**		**33,873**	**1,687,060**	**5.9**		**11.6**
		610	123		32,885	1,612,718			
		802	224		15,296	720,119			
	Cent	**636**	**154**		**13,493**	**512,073**	**2.8**		**8.44**
		504	105		11,691	304,028			

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