Investigation of water quality and sources associated with the Buck Creek Watershed

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SUMMARY

The Buck Creek watershed drains a portion of northeast Allegan County and southwest Kent County. This watershed encompasses nearly 51 miles and eventually feeds into the Grand River (Grand Valley Metropolitan Council 2003). Two sites were selected for microbiological water quality assessment, BCK-11 and BCK-12, based on prior *E. coli* water quality analysis. Sampling results from 2005 and 2006 indicate that fifteen of sixteen samples taken at BCK-11 and BCK-12 exceeded Michigan's total body contact standards (300 *E. coli* CFU/100 ml). The Water Quality and Environmental Microbiology Laboratory of Michigan State University (MSU) investigated this watershed to help identify potential sources of contamination. Our lab used a tool box approach which includes a variety of bacteria and molecular indicators to asses the water quality during this project. Both sites are being impacted by bovine feces. At BCK-12, four of six (67%) samples taken at BCK-12 were positive for the *Bacteroides* cow marker and four of six (67%) samples tested positive for bovine polyomavirus. At BCK-11 one sample (17%) was positive for the *Bacteroides* cow marker and three of six (50%) samples tested positive for bovine polyomavirus. BCK-12 was influenced also by human sources detected by the Enterococci esp gene marker and the *Bacteroides* human marker. One sample (17%) taken at BCK-12 on January 16th, 2008 returned a positive result for the human *Bacteroides* marker. Samples taken on February 6th and 14th (29% of samples tested for esp) at site BCK-12 tested positive for the presence of the Enterococci esp human marker. Physical data were compared to fecal indicator data to identify correlations. The strong correlations identified for each site gives some insight to the mechanics of the watershed. The *C. perfringens* levels detected were found to be correlated with 24 hour precipitation and turbidity.

INTRODUCTION

Bacterial indicators, such as *Escherichia coli (E. coli)*, enterococci, and fecal coliform, are currently used throughout the US and world as assessors of potential illnesses associated with water use and pollution. Fecal water quality indicators are based on the premise that each will be found consistently in fecal waste, e.g. sewage or manure, and have similar survival and transport properties as the pathogen known to cause disease (Colford 2007). Thus indicators should be correlated to the presence of the pathogen. However, survival rates and regrowth potential of some bacteria varies depending on water temperature, sunlight, nutrient status, and turbidity (McLellan 2007) and bacterial indicators have been poor predictors for the presence of viruses and parasites. In addition, the indicators have not been able to identify the source of the contamination.

Table 1 describes the indicators and their application. All of the indicators used by the MSU laboratory are found in the feces of humans or animals and are referred to as fecal indicators.

As a pathogen indicator, *E. coli* has served as the workhorse and is now used in conjunction with other indicators and pathogens such as viruses and protozoa (Schwab 2007). Other bacteria serve as indicators such as enterococci (which may survive longer than *E. coli*), *Clostridium perfringens* (a spore former that does not regrow in the environment and can survive for decades), and Coliphage (a virus indicator which has a finite life and can not regrow in the environment). Microbial source tracking (MST) can use a host specific approach based on

unique characteristics found within a particular host's fecal bacteria (Scott et al. 2002). At the Water Quality and Environmental Microbiology Laboratory at Michigan State University, we have been evaluating several targets for MST such as a bovine and human *Bacteroides* marker, the human sewage-specific *esp* enterococci gene, and the human and bovine specific adenoviruses. MST targets help determine the source of fecal pollution and whether human fecal material is present in the water.

The Buck Creek watershed drains a portion of northeast Allegan County and southwest Kent County, nearly 51 square miles in total. Appendix 1 indicates the Buck Creek watershed location in Michigan and appendix 2 represents a more detailed picture of the watershed. Two sites within this watershed were selected for microbiological water quality assessment, BCK-11 and BCK-12. The Water Quality and Environmental Microbiology Laboratory of Michigan State University (MSU) investigated this watershed in order to identify potential sources of contamination.

TABLE 1. Indicators and their Applications

INDICATOR	DEFINITION	RECREATIONAL USE STANDARD OF MICHIGAN	ADVANTAGE OF USE AS AN INDICATOR	DISADVANTAGE OF USE AS AN INDICATOR
E. coli	A type of coliform bacteria that naturally occurs in the human intestinal tract Many strains exist but only a few are pathogenic	A geometric mean of at least 5 samples may not exceed 130 cfu/100 ml A single sample may not exceed 300 cfu/100 ml	Used as an indicator of bacteriological quality in both drinking and recreational waters Found to have a high correlation with gastroenteritis associated with bathing in freshwater Source tracking methods have bee developed	May grow in the soil of tropical locations Found to be poorly correlation to gastroenteritis in marine waters *E. coli* presence does not always correlate with the presence of enteric viruses and parasites
Enterococci	A gram positive, non-spore forming member of the Streptococci bacteria Commonly found in the feces of warm blooded animals Multiple strains, many of which are not harmful	A geometric mean of at least 5 samples may not exceed 33 cfu/100 ml A single sample may not exceed 61 cfu/ 100 ml	Enterococci may die at a slower rate than fecal coliforms in water and sediments, providing more reliable indications of recent pollution Multi-site epidemiological studies have shown that enterococci have a higher correlation with gastroenteric disease related to swimming in fresh and marine waters than fecal coliforms	Can regrow in the environment Not as well researched as *E. coli*
Clostridium perfringens	Obligate anaerobic gram-positive bacteria that forms endospores and does not carry out dissimilatory sulfate reduction Found in sewage and highly impacted waters An opportunistic pathogen that produces enterotoxin	FRESHWATER STANDARDS USED IN HAWAII: A single sample may not exceed 50 cfu/100 ml	*C. perfringens* spores could be an index parameter for the occurrence of persistent intestinal pathogens like viruses and oocysts of protozoa Useful in such specific situations as the examination of chlorinated waters or industrial waters that may contains compounds lethal to non-spore forming bacterial indicators, samples that cannot be processed within 12 hours and the detection of recent or long term inputs of fecal pollution.	May be too conservative an indicator which may not adequately protect human health Often found in low concentrations
Coliphage	Viruses whose hosts are strains of the bacteria *E. coli* Found wherever fecal contamination occurs	A single sample may not exceed 100 pfu/100 ml	A good indicator of enteroviruses due to similar seasonal variation, propensity for removal and resistance to environmental stress	Coliphage is not specific to human sewage

METHODS

The water quality and environmental microbiology laboratory of Michigan State University, applied a tool box approach while investigating the Buck Creek watershed. This tool box approach involves looking at fecal pollution indicators *(E. coli*, enterococci, *Clostridium perfringens*, coliphage), molecular source tracking markers (*Bacteroides*, adenovirus, polyomavirus, and Enterococci esp gene), and physical data (precipitation, pH, turbidity, etc.). All of these resources were then analyzed to draw conclusions on potential impacts associated with the watershed.

Sample Collection:

Two sampling sites were chosen on the Buck Creek watershed because of past water quality standard exceedances. Half of the sampling events were conducted to coincide with a 48 hour precipitation event of greater than .25 inches. These data were collected from a local weather station on-line (Weather Underground 2008). Other data collected at the time of sampling include water turbidity, air and water temperature, pH, and general weather conditions.

Grab samples were obtained at the river/creek using sterile sample bottles. Samples were placed on ice and brought to the MSU Water Quality and Health laboratory for analysis. The samples were kept at 4^{o} C until processing on the same day as collection.

Table 2. Sampling sites and ID

Water Sample ID	Waterbody	Location Description	Dates Collected
BCK-11	Buck Creek	Northeast of Division Road and 60th Street 42.8559, -85.6636	1/16/2008 2/6/2008 2/14/2008 3/19/2008 3/26/2008 4/9/2008 4/29/2008
BCK-12	Pine Hill Creek	Northeast of Division Road and 52nd Street 42.8712, -85.6641	1/16/2008 2/6/2008 2/14/2008 3/19/2008 3/26/2008 4/9/2008 4/29/2008

Bacterial analysis

Samples were analyzed for *E. coli* using mTEC membrane filtration method (American Public Health Association 1998, US EPA. 2005). *Enterococci* were analyzed using membrane filtration according to USEPA Method 1600 (*Enterococci*) (US EPA. 2002). *Clostridium perfringens* were analyzed using membrane filtration and mCP agar (EPA 1995, Bisson 1979). Coliphage were analyzed via a modification of USEPA Methods 1601 and 1602 (US EPA 2001). Table 3

includes the media and incubation conditions used for these indicators. Volumes for bacteriological analysis via membrane filtration ranged from 1 ml to 100ml.

Table 3. Media and Methods used for Microbial Indicator Testing

Test	**Media**	**Incubation**	**Reference**
E. coli	mTEC	24-28 hours at 37°C	APHA Standard Method 9223B (American Public Health Association 1998, US EPA. 2005)
Enterococci	mEI agar	24 hours at 41°C	USEPA Method 1600 (US EPA. (2002)
Clostridium perfringens	mCP	24 hours at 45°C	EPA 1995, Bisson 1979
Coliphage	Tryptic Soy Agar	16 – 24 hours at 37°C	USEPA Method 1601/1602 (US EPA 2001)

Coliphage Analysis

Agar overlays were utilized to detect coliphage present in the samples. Non-filtered water samples were used to enumerate coliphage. Two types of overlays were conducted one using *E. coli* F^+amp as a host, the other using *E. coli* CN-13. The F^+amp is known as male specific coliphage and it infects the host at the F-pili. Somatic coliphage (CN-13) infect the host bacteria at the outer cell wall.

One-half ml of host (CN-13 or F^+amp) and 2 ml of sample were added to melted top agar before mixing and pouring onto a tryptic soy agar plate (TSA). Coliphage samples were analyzed using at total of 11 ml of sample per host (10 ml of undiluted sample and 10 ml of a 1:10 diluted sample). Thus, 22 ml of sample per site were assayed for coliphage during each sampling event. Two negative control plates were made, one with each host, by adding 1.5 ml host to the top agar, mixing and pouring onto a TSA plate. A positive control was run for each host type by adding 1.5 ml host to the top agar, mixing and pouring onto a TSA plate. Stock phage was spotted onto the hardening agar layer. Overlays were incubated at 37°C for 24 hours, and then assessed for plaque formation.

Molecular Analysis

Bacteroides

Two liters of water were centrifuged for 30 minutes at 4000 rpm. Mobio mega soil DNA kit was used to extract the DNA from the pellet. PCR amplification was performed on the extracted DNA. Primers for both human and cow *Bacteroides* sequences were used (Bernhard 2000). Gel electrophoresis was performed on the PCR product and run on a 1.2% w/v agarose gel at 95 V for approximately one hour.

Enterococci esp Marker

The enterococci bacteria which grew up on the membrane filter as assayed above were washed off the membrane and extracted (Kumar, L. 2007, Scott 2005). The primers specific for the *esp*

gene in *E. faecium* previously developed and examined for specificity to human fecal pollution were used in a polymerase chain reaction [PCR] (Scott 2005). The forward primer: (5'-TAT GAA AGC AAC AGC ACA AGT-3') and the conserved reverse primer (5' –ACG TCG AAA GTT CGA TTT CC-3') were used for all reactions. Gel electrophoresis was performed on the PCR product and run on a 1.2% w/v agarose gel at 95 V for approximately one hour.

Adenovirus and polyomavirus Analysis

Two human specific enteric viruses have been used to address human sewage and health risk in water (Theng and Lipp 2005). Between 1.5 to two L of water samples were filtered through a 90mm, negatively charged HA membrane with pore size of 0.45-µm (Millipore, Billerica, Mass). After filtration, 100 mL of 0.5 mM H_2SO_4 was filtered to rinse out excess ions, and viral particles were eluted from the filter and stored at - 20°C before further concentration. For further purification and concentration, the elutant was thawed and dispensed into an Amicon Ultra 100K concentrator column (Millipore). Eluate was centrifuged at 4000 x g for 1 minute and the resulting filtrated was discarded and column filled with 10 ml PBST (pH 8.0). Column was centrifuged again at 4000 x g for 1 min. After centrifuging, the sample concentrated was removed and stored at –80 °C until further processing. Adenovirus RNA/DNA was extracted using Qiagen RNA viral kit. The sample was concentrated using an Amicon Ultra-15 concentrator column and centrifuged for 20 minutes at 4000x g. Process was repeated until all the eluate is used up. DNA was subsequently extracted using the UltraClean Isolation Kit (Carlsbad, CA). A nested PCR reaction was performed to amplify the hexon gene of all 51 human adenovirus prototype strains. Nested PCR reactions were performed to amplify the hexon gene of bovine adenovirus prototype strains (Maluquer de Motes 2004). Gel electrophoresis was performed on the PCR product and run on a 1.2% w/v agarose gel at 90 V for approximately one hour.

RESULTS

A total of seven sampling events occurred during this project, fourteen total samples at two sites. The samples collected on February 6th, February 14th, and April 9th were classified as wet weather samples because greater than 0.25 inches of precipitation fell in the 48 hours prior to sampling. The other four sampling events were classified as dry flow samples because less than 0.25 inches of precipitation were recorded during the last 48 hours. The physical measurements, including precipitation data, are summarized below in table 4. Precipitation data are the total for each time frame (i.e. total rain fall in past 48 hours, total rain fall in past 72 hours).

FECAL POLLUTION INDICATORS

The sites were analyzed using *E. coli,* enterococci, *C. perfringens*, and coliphage as the fecal pollution indicators. According to the bacterial analysis, each body of water has high levels of fecal contamination. The average *E. coli*, Enterococci*, and C. perfringens* values at site BCK-12 were 610, 3100, and 230 (colony forming units/100 ml), respectively. The average *E. coli*, Enterococci*, and C. perfringens* values at site BCK-11 were 280, 1300, and 190 (colony forming units/100 ml), respectively. The average results are also shown below in table 5. Based on the geometric mean average values of each site, BCK-12 has greater fecal contamination than site BCK-11 but this was not statistically tested due to the low sample size. The coliphage and enterococci data for this site indicate very strong contamination. Data collected from both sites

suggests a constent source of pollution as levels were routinely elevated. The coliphage levels at each site were routinely high which further suggests a consistent source of pollution to each site because coliphage has a limited life and is unable to regrow in the environment.

The bacterial results were compared to the physical measurements to identify factors impacting the water quality of Buck Creek. Due to the small sample size, these results are statistically insignificant. Strong positive correlations were found to exist at site BCK-11 between the somatic coliphage (CN-13) and the temperature of the water and air (r=.737 and r=.800, respectively). *C. perfringens* were found to be positively correlated with 48 hour precipitation at BCK-11 (r=.802). At BCK-12, 24 hour precipitation was found to be positively correlated with turbidity (r=.897) and *C. perfringens* (r=.845). *C. perfringens* were also found to be linked with turbidity levels (r=.748). The correlation for BCK-11 and BCK-12 can be found in Appendix 3 and 4, respectively. Again, as there was a small sample size these are not considered to be statistically significant.

Table 4. Physical measurements summary for Buck Creek

	Water temperature (°C)	**Air Temperature(°C)**	**Turbidity (NTU)**	**pH**	**24 hour precipitation (inches)**	**48 hour precipitation (inches)**	**72 hour precipitation (inches)**
BCK-11							
1/16/2008	0.0	-4.0	9.8	10.2	0.08	0.18	0.39
2/6/2008	1.4	-1.2	28	8.7	0.14	0.41	0.59
2/14/2008	1.0	-2.8	11	8.3	0.00	0.28	0.28
3/19/2008	5.8	5.7	7.2	8.9	0.17	0.19	0.19
3/26/2008	5.5	4.6	4.8	8.7	0.01	0.01	0.01
4/9/2008	8.1	3.5	6.0	7.5	0.38	0.38	0.38
4/29/2008	8.9	6.4	50.1	7.65	0.00	0.00	0.07
BCK-12							
1/16/2008	1.2	-4.2	13	8.9	0.08	0.18	0.39
2/6/2008	1.2	-1.2	29	8.7	0.14	0.41	0.59
2/14/2008	-1.2	-2.8	6.0	8.7	0.00	0.28	0.28
3/19/2008	5.9	3.1	4.6	8.9	0.17	0.19	0.19
3/26/2008	4.4	4.6	8.6	8.4	0.01	0.01	0.01
4/9/2008	7.7	3.7	110	7.7	0.38	0.38	0.38
4/29/2008	9.6	6.4	1.79	8.22	0.00	0.00	0.07

Table 5. Summary of Bacterial concentrations for Buck Creek (CFU or PFU/100 ml)

	E. coli	Enterococci	*C. perfringens*	Coliphage (F^+amp)	Coliphage (CN-13)
BCK-11					
1/16/2008	3.5E+02	3.3E+02	9.0E+01	1.9E+05	<2.0E-01
2/6/2008	2.8E+02	6.9E+03	5.1E+02	<7.7E-02	5.5E+02
2/14/2008	3.9E+02	4.6E+02	2.7E+02	<7.7E-02	<7.7E-02
3/19/2008	2.5E+02	4.3E+02	1.9E+02	7.0E+01	2.0E+02
3/26/2008	2.4E+02	5.4E+01	1.3E+02	1.3E+02	3.0E+02
4/9/2008	3.0E+02	8.5E+02	1.2E+02	2.6E+02	5.5E+01
4/29/2008	1.2E+02	1.1E+02	5.2E+01	1.0E+02	5.5E+02
ARITHMETIC AVERAGE	**2.8E+02**	**1.3E+03**	**1.9E+02**	**2.7E+04**	**2.4E+02**
BCK-12					
1/16/2008	1.4E+03	4.1E+02	3.6E+02	3.6E+05	5.3E+05
2/6/2008	7.6E+02	1.5E+04	2.1E+02	5.9E+02	1.3E+02
2/14/2008	2.1E+02	6.8E+02	<2.1E-02	<7.7E-02	<7.7E-02
3/19/2008	4.6E+02	1.0E+03	2.8E+02	<8.3E-02	5.4E+02
3/26/2008	4.2E+02	7.7E+02	1.9E+02	2.5E+02	1.4E+02
4/9/2008	8.5E+02	3.9E+03	5.1E+02	<9.1E-02	2.3E+02
4/29/2008	1.4E+02	2.2E+02	7.5E+01	2.0E+02	1.6E+02
ARITHMETIC AVERAGE	**6.1E+02**	**3.1E+03**	**2.3E+02**	**5.2E+04**	**7.6E+04**

MOLECULAR RESULTS

In addition to bacterial indicators, each site was analyzed for *Bacteroides*, esp enterococci gene, and the adenovirus. Adenovirus and *Bacteroides* were tested on 6 sampling dates. The *Bacteroides* results indicate the major source of fecal contamination at both sites is cow manure. Cow *Bacteroides* were identified in four of the six samples taken from BCK-12 and one of the six samples taken from BCK-11. The sample collected on January 16th at BCK-12, tested positive for human and bovine *Bacteroides*. BCK-12 tested positive for the human source only once.

The polyomavirus results also indicate the Buck Creek watershed is experiencing bovine impacts. Three BCK-11 samples tested positive for the bovine polyomavirus marker. Four BCK-12 samples tested positive for the bovine polyomavirus marker. No samples tested positive for human or bovine adenovirus. A summary of the polyomavirus and adenovirus results are presented in appendix 5.

The human sewage marker for Enterococci is the esp gene. The esp results tested positive for two samples, both at BCK-12. The Enterococci esp gene was detected on February 6th and February 14th. All other sampling events were negative for the Enterococci esp gene. A summary of the Enterococci esp, Bacteroides (human and bovine), and the polyomavirus results for BCK-11 and BCK-12 are shown in table 6.

Table 6: Human and Bovine source tracking results

		HUMAN		BOVINE	
Site location	**Date**	**Bacteroides**	**Enterococci esp human**	**Bacteroides**	**Polyomavirus bovine**
BCK-11					
	1/16/2008	-	-	-	+
	2/6/2008	-	-	-	-
	2/14/2008	-	-	-	-
	3/19/2008	-	-	-	-
	3/26/2008	-	-	+	+
	4/9/2008	-	-	-	+
	4/29/2008	ND	-	ND	ND
BCK-12					
	1/16/2008	+	-	+	-
	2/6/2008	-	+	-	-
	2/14/2008	+	+	+	+
	3/19/2008	-	-	+	+
	3/26/2008	-	-	+	+
	4/9/2008	-	-	-	+
	4/29/2008	ND	-	ND	ND

ND samples not assayed

DISCUSSION/SUMMARY

Using a tool box approach to evaluate two sites on the Buck Creek watershed, our lab has determined the primary impact is coming from bovine sources. While there does appear to be some human fecal material present, the level is less pronounced than bovine material. Both sites are being impacted by bovine feces.

Site BCK-12 is also influenced by human sources as indicated by the presence of the Enterococci *esp* gene marker and the *Bacteroides* human marker. One sample (17%) taken at BCK-12 on January 16th, 2008 returned a positive result for the human *Bacteroides* marker. At BCK-12, samples taken on February 6th and 14th (29% of samples tested for esp) tested positive for the presence of the Enterococci esp human marker. This site may have human sources while site BCK-11 does not for a number of reasons. Each site is situated on different tributaries of the Buck Creek and may drain different land use areas. The presence of the Enterococci *esp* gene marker and the *Bacteroides* human marker may indicate illicit sewage discharges or cross connections with storm drains.

Physical data were compared to fecal indicator data to identify correlations. The strong correlations identified for each site gives some insight to the mechanics of the watershed. The *C. perfringens* levels detected were found to be correlated with 24 hour precipitation and turbidity. Higher levels of *Clostridium perfringens* were associated with increased turbidity (r=.748) as seen in BCK-12. *C. perfringens* is found in human and animal feces which may be washed into the water bodies during rainfall events and lead to elevated turbidity and organism levels. Bacteria have been shown to persist and grow in sediments (Ishii et al. 2007). The increase in turbidity may also be caused from sediment being mixed into the water and elevating bacteria.

Rain may also be playing another role with the water quality of the Buck Creek. The temperature of each site decreased with rainfall which may allow the bacteria to survive for longer periods of time.

As a result of the multifaceted approach taken on the Buck Creek watershed, possible impacts to the water quality include human sewage, bovine manure, and stormwater runoff. The human sewage may be entering the water body from failing septic tanks, combined sewer overflows, illicit discharges, or cross connections to a storm drain. Bovine impacts enter the watershed from field and lot runoff. Stormwater runoff can also wash fecal material from roads, parking lots, residential yards, etc into the water system. All of these are possible sources of pollution which lead to elevated bacterial levels in surface waters.

Future efforts on the Buck Creek Watershed should include monitoring up stream of BCK-11 and BCK-12 to identify specific outfalls or sources, land use surveys with the aid of GIS, and stormwater remediation as turbidity/ precipitation appears to be a large factor in this system. Future studies should include sediment testing for bacteria levels in order to identify whether the sediment is acting as a bacteria sink. Other studies to improve the Buck Creek watershed should include sampling during a rainfall event and each day after the event to identify the period of greatest water quality impairment.

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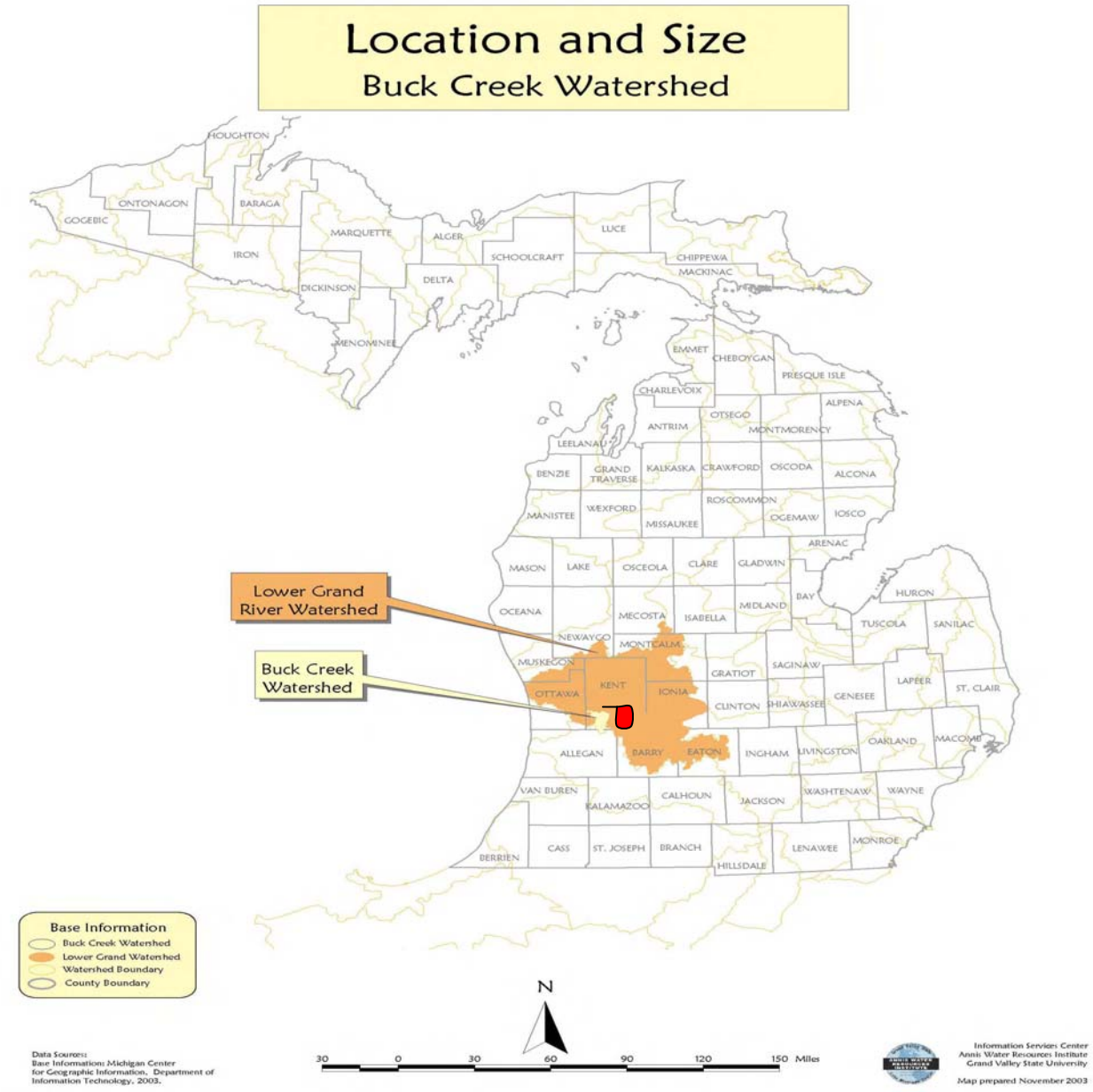
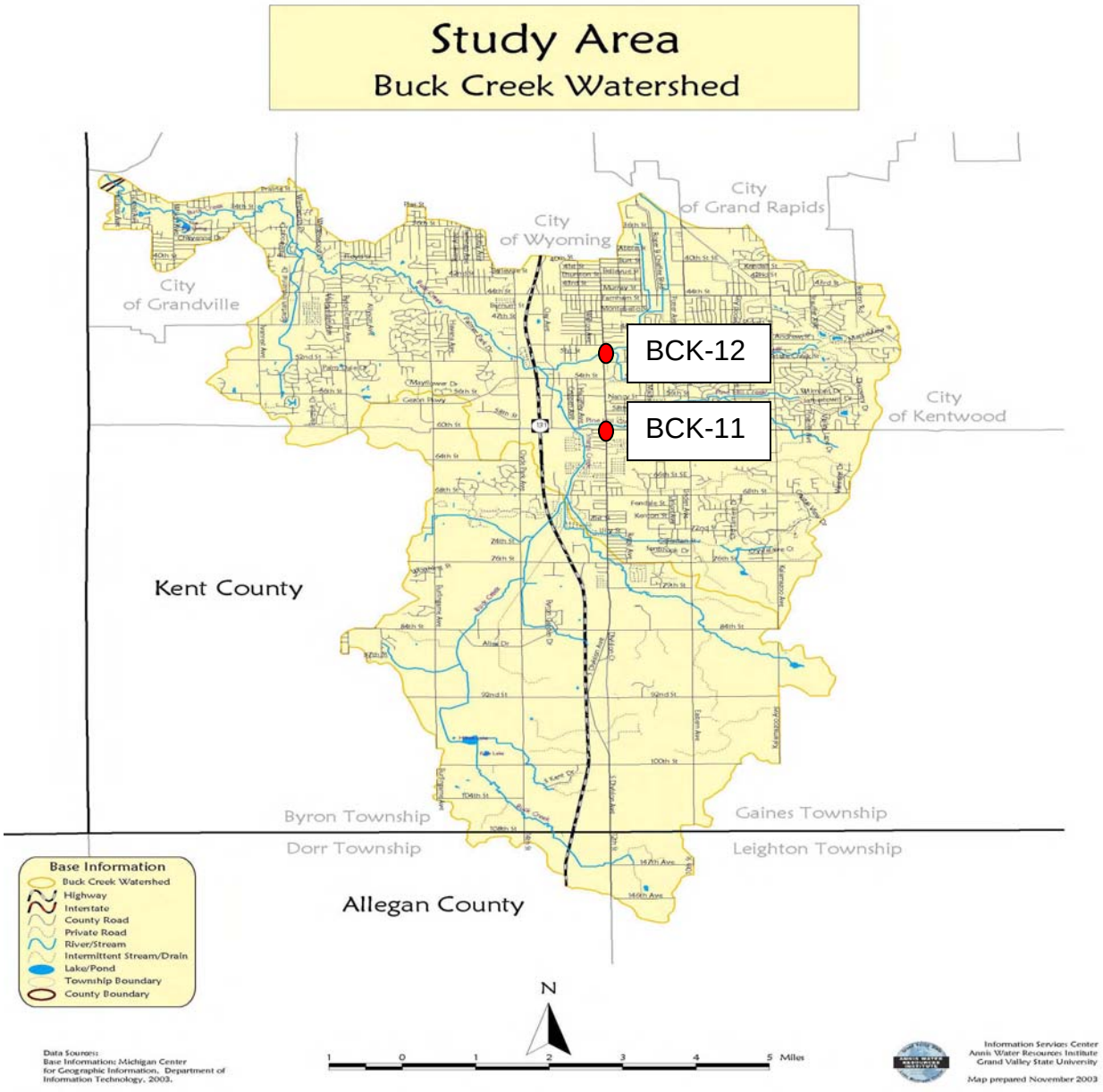
Appendix 1: Lower Grand River Watershed and Buck Creek Watershed



Appendix 2: Buck Creek Watershed



Appendix 3: BCK-11 Correlation data

	E. coli	*Enterococci*	*C. perfringens*	*Coliphage (F amp)*	*Coliphage (CN-13)*	*Water temperature*	*Air Temperature*	*Turbidity*	*pH*	*24 hour precipitation*	*48 hour precipitation*	*72 hour precipitation*
E. coli	1.000											
Enterococci	0.075	1.000										
C. perfringens	0.307	**0.900**	1.000									
Coliphage (F amp)	0.377	-0.171	-0.294	1.000								
Coliphage (CN-13)	**-0.920**	-0.397	-0.526	-0.335	1.000							
Water temperature	**-0.757**	-0.362	-0.518	-0.540	0.737	1.000						
Air Temperature	**-0.822**	-0.319	-0.414	-0.590	**0.800**	0.927	1.000					
Turbidity	-0.679	0.253	0.045	-0.182	0.590	0.279	0.226	1.000				
pH	0.415	0.037	0.061	0.801	-0.402	-0.730	-0.560	-0.357	1.000			
24 hour precipitation	0.155	0.182	0.047	-0.100	-0.381	0.304	0.141	-0.348	-0.265	1.000		
48 hour precipitation	0.602	0.611	**0.802**	0.016	-0.638	-0.790	-0.779	0.045	0.193	-0.243	1.000	
72 hour precipitation	0.114	0.523	0.287	0.690	-0.295	-0.575	-0.592	0.316	0.609	-0.122	0.362	1.000

Appendix 4: BCK-12 Correlation data

	E. coli	*Enterococci*	*C. perfringens*	*Coliphage (F amp)*	*Coliphage (CN-13)*	*Water temperature*	*Air Temperature*	*Turbidity*	*pH*	*24 hour precipitation*	*48 hour precipitation*	*72 hour precipitation*
E. coli	1.000											
Enterococci	0.208	1.000										
C. perfringens	0.738	0.113	1.000									
Coliphage (F amp)	0.803	-0.223	0.328	1.000								
Coliphage (CN-13)	0.802	-0.224	0.329	1.000	1.000							
Water temperature	-0.259	-0.249	0.282	-0.329	-0.329	1.000						
Air Temperature	-0.544	-0.223	0.028	-0.604	-0.604	**0.901**	1.000					
Turbidity	0.357	0.274	**0.748**	-0.134	-0.134	0.288	0.137	1.000				
pH	0.145	0.027	-0.346	0.404	0.404	-0.643	-0.613	-0.754	1.000			
24 hour precipitation	0.422	0.304	**0.854**	-0.102	-0.101	0.320	0.121	**0.897**	-0.503	1.000		
48 hour precipitation	0.022	0.534	-0.436	0.015	0.014	**-0.847**	**-0.767**	-0.191	0.470	-0.243	1.000	
72 hour precipitation	0.670	0.456	0.125	0.690	0.689	-0.348	-0.569	-0.154	0.412	-0.122	0.362	1.000

Appendix 5: Polyomavirus and adenovirus data summary

Sampling date	Sample ID	Sample Type	Volume filtered (ml)	Concentrate volume (ml)	Total concentrate volume (ml)	Dilution	Equivalent vol. extracted (ml)	Equil. volume assayed (ml)	Detection limit (copies/ml)	Real time PCR (copies/ml)	Conventional PCR		
										HAdV	Bovine PyV	Bovine AdV	Human AdV
01/16/08	Bck 11_1	Water	2270	0.50	0.50	1.00	635.60	52.97	0.02	<0.02	+	-	-
01/16/08	Bck 12_1	Water	1650	0.80	0.80	1.00	288.75	24.06	0.04	<0.04	-	-	-
02/06/08	Bck 11_2	Water	1720	0.46	0.51	0.90	425.87	35.49	0.03	<0.03	-	-	-
02/06/08	Bck 12_2	Water	2460	0.33	0.50	0.66	454.61	37.88	0.03	**0.03**	-	-	-
02/14/08	Bck 11_3	Water	3680	0.68	0.68	1.00	757.65	63.14	0.02	<0.02	-	-	-
02/14/08	Bck 12_3	Water	3050	0.68	0.68	1.00	627.94	52.33	0.02	**0.07**	+	-	-
03/19/08	Bck 11_4	Water	4160	1.49	1.49	1.00	390.87	32.57	0.03	<0.03	-	-	-
03/19/08	Bck 12_4	Water	2800	1.16	1.16	1.00	337.93	28.16	0.04	<0.04	+	-	-
03/26/08	Bck 11_5	Water	3300	0.49	0.49	1.00	942.86	78.57	0.01	<0.01	+	-	-
03/26/08	Bck 12_5	Water	2100	0.33	0.50	0.66	388.08	32.34	0.03	<0.03	+	-	-
04/09/08	Bck 11_6	Water	2750	0.20	0.53	0.38	274.12	22.84	0.04	<0.04	+	-	-
04/09/08	Bck 12_6	Water	1160	1.28	1.28	1.00	126.88	10.57	0.09	<0.09	+	-	-