MEASURING MICROBIAL WATER QUALITY RESPONSES TO LAND AND CLIMATE USING FECAL INDICATOR BACTERIA AND MOLECULAR SOURCE TRACKING IN RIVERS AND NEAR-SHORE SURFACE WATERS OF MICHIGAN

By

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ABSTRACT

MEASURING MICROBIAL WATER QUALITY RESPONSES TO LAND AND CLIMATE USING FECAL INDICATOR BACTERIA AND MOLECULAR SOURCE TRACKING IN RIVERS AND NEAR-SHORE SURFACE WATERS OF MICHIGAN

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Current recreational water quality science in the Great Lakes relies on measuring *E. coli* concentrations via cultivation techniques to estimate the risk associated with swimming in a particular waterbody. However, this dissertation showed such approaches inaccurately describe water quality across the entire beach and fail to represent underlining water quality issues. Water, sediment, and algae samples were collected in creeks, rivers, and beaches from multiple watersheds around Michigan. Samples were analyzed for cultivation based *E. coli*, enterococci, *Clostridium perfringens*, and coliphage as well as molecular markers for *E. coli*, *Enterococcus* spp., enterococci surface protein gene (human), Bacteroides (human and bovine) and *Bacteroides thetaiotaomicron* mannanase gene (human).

In the Saginaw Bay, microbial indicators at four beaches were generally highest to lowest in stranded algae mats, sediment, shallow water, and deep water, respectively. Contamination in algae mats and sediment was identified in part as human specific using the enterococci surface protein gene. Higher concentrations of *E. coli* and enterococci in algae mats and sediment, compared to shallow and deep waters, were attributed largely to sediment bound bacteria and bacterial regrowth or persistence. Results demonstrated the potential for sediment and algae mats to act as non-point sources of pollution in the nearshore zone.

Water and sediment samples collected from Mitchell Creek and Traverse City State Park beach quantified fecal indicator bacteria across space and time. Fecal indicator bacteria concentrations represented widespread, long-term, and recent fecal contamination in the Mitchell Creek. Despite the close proximity of the Mitchell Creek discharge to the Traverse City State Park beach, microbial concentrations were significantly lower ($p < 0.01$) in beach water which was partially impacted by creek discharge. Assessment of land use type at the watershed scale failed to identify consistent correlations with fecal indicator bacteria. However, *Bacteroides thetaiotaomicron* detections in both waterbodies indicated fecal contamination was partially human. Additional analysis on a subset of data identified significant disconnect between molecular and cultivation based results in creek and beach water. However, across all waterbodies, cultivated enterococci would have resulted in the greatest number of regulatory actions compared to cultivated *E. coli* and molecular based *Enterococcus* spp.

A snapshot survey of 64 rivers discharging to the Great Lakes quantified *E. coli* and *Bacteroides thetaiotaomicron* under baseflow conditions. *Bacteroides thetaiotaomicron* was detected in all samples ($\overline{X} = 5.1 \log_{10}$ Cell Equivalents 100 ml^{-1}). The *E. coli* geometric mean across all rivers (1.4 $\log_{10}$ MPN 100 ml^{-1}) suggests a potential regional reference condition. Classification And Regression Tree analysis indicated the total number of septic system in a watershed significantly impacted *Bacteroides thetaiotaomicron* concentrations under baseflow conditions. Land use characteristics better predicted microbial water quality than land use type.

This work coupled molecular tools and novel monitoring strategies of unique environments (algae mats, sediments, beaches, small creek systems, and large river watersheds) to better understand the impact of human activities on Great Lakes water quality. Land use characteristics, not land use type, were related to qPCR markers in rivers which are transported to nearby beaches. Finally, septic systems, algae mats, and sediments were identified as non-point sources of pollution in Michigan surface water systems.

This dissertation is dedicated to my family who unknowingly taught me the ability of water to unite communities, my friends who supported me during all of my endeavors, and the Great Lakes which generated a thirst for water science eclipsed only by a really thirsty person.

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Although this work is already dedicated to my family and friends, they deserve special recognition here as they provided personal support during my graduate school carrier. They stood beside me while I chased microorganisms across Michigan and allowed me to accomplish far more than I could have on my own.

A number of graduate students provided vital sample collection and processing support essential to the development of my research. Asli Aslan, Sibel Zeki, Rebecca Ives, Chris Wendt, and Stephanie Longstaff helped immensely during the final leg of my Mitchell Creek research. Anthony Kendall and Sherry Martin were instrumental in the development of chapters three and five.

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CHAPTER 1.

THE GREAT LAKES BASIN: POLICY, POLLUTION, AND WATER QUALITY

1.1. The Laurentian Great Lakes

The Great Lakes, prior to European settlers, were largely separated from outside surface waters but connected together through rivers, straits, and waterfalls. As population increased during the American industrial revolution (Voth 2003), the Great Lakes became an important waterbody for transportation and waste disposal (Page and Walker 1991). Sewage from the City of Chicago polluted drinking water sources and led to the development of the Chicago Sanitary and Ship Canal (Ashworth 1986). This canal opened the Mississippi river to the Great Lakes and redirected the flow of surface water away from Lake Michigan, Chicago's only source of drinking water (completed in 1900) (Ashworth 1986). As the population of the Midwestern region grew so did the need for faster and more efficient transportation. In response, the St. Lawrence Seaway (1959) was opened to direct nautical shipping from the Atlantic Ocean to the Great Lakes (Whitfield and Kolenosky 1978). As a result of the increased connectivity and human population, the Great Lakes ecosystems became increasingly stressed.

The Laurentian Great Lakes have recently been described in terms of great flux (Karrow and Calkin 1985; Larson and Schaetzl 2001). The land-water interface, lake level, percent ice coverage, and surface water temperature are constantly changing due to natural and anthropogenic influences. The most recent measurements of these parameters for each of the lakes are detailed in Table 1.1. Land use in the Great Lakes basin has also undergone change in recent decades with one study reporting a 9.8% decrease in agriculture land use since 1982 and 49.3% increase in urban developed land use (Wolter et al. 2006).

Table 1.1. Measurements of key physical parameters for each of the Laurentian Great Lakes during modern times.

Great Lake	Surface area (km^2)[A]	Average depth (m)[B]	Lake levels (m)[C]	Surface water temperature (range)[D]	Ice coverage (%)[E]	Water retention time (years)[A]
Lake Erie	25,700	19	174.0 - 174.3	0.0 - 23.0 °C	87.5	2.6
Lake Huron	59,600	59	176.3 - 176.6	0.0 - 18.0 °C	68.1	22
Lake Michigan	57,800	85	176.3 - 176.6	0.0 - 25.5 °C	42.7	99
Lake Ontario	18,960	86	74.5 - 75.1	0.0 - 20.1 °C	32.5	6
Lake Superior	82,100	147	183.3 - 183.6	0.0 - 15.5 °C	70.1	191

[A]USEPA 2006; [B]Grady 2007; [C]United States Army Corp of Engineers 2011; [D]Schwab et al. 1999. [E]Ferris and Andrachuk 2009

Recommended beneficial uses for the Great Lakes include: aquatic life; fish and shellfish consumption; drinking water supply; primary contact recreation; secondary contact recreation; and agriculture (USEPA 1992). Designated beneficial uses are specific to a waterbody and are the desired uses that water quality should support (USEPA 1992). At minimum, all waters of the Great Lakes should meet the swimmable and fishable uses as set forth in the Clean Water Act.

One of the most valued beneficial uses and utilized areas in the Great Lakes is for recreation in the nearshore zone, respectively, which includes beaches and shallow waters. The Great Lakes include 8851 km (Dorfman and Rosselot 2011) of some of the world's greatest sandy beaches (Chrzastowski et al. 1994; Folger et al. 1994), but a growing trend of increasing beach closures (Dorfman and Rosselot 2011) has plagued many coastal communities. Nationally, tourism has become a primary factor driving economic activity, job creation, wealth, and investment

(Houston 2008) and the economic value gained from Great Lakes beach tourism can be seen in the fiscal impacts of beach closures. Song et al. (2010) estimated closing all Lake Michigan beaches located in the state of Michigan would result in an economic loss of $2.7 billion. Another Great Lakes Basin study estimated beach closures cost the surrounding community nearly $228,000 per beach closure (Murray et al. 2001). Austin et al. (2007) suggested a 20% reduction in Great Lakes beach closures would result in an economic benefit of at least $130 million per year. Therefore, Great Lakes beaches are not only a treasured natural resource but also a vital economic driver to the surrounding states which require protection against further degradation.

Significant federal and state policies were required to address decades of misuse and degradation of America’s water systems. Over the latter half of the 20th century, policy slowly emerged that partially addressed the most significant stressors including invasive species (Mills et al. 1993; Ricciardi 2006), toxins (Baumann and Whittle 1988), nutrient loads (Wiley et al. 2010), and pathogens associated with human fecal pollution, mainly from wastewater infrastructure or combined sewer overflows (Dreelin et al. 2007; Patz et al. 2008). Water quality policy specifically, slowly developed, with each new act building upon previous legislation (Table 1.2.). However, it was not until the 1970’s when a more unified water quality protection act addressing ambient waters was enacted via the development of the Clean Water Act.

Initial laws governing United States water quality protection date back to the 1880s. In 1886, Congress enacted the first federal environmental law known as the River and Harbor Appropriations Act, later renamed the Rivers and Harbors Act (1899) which gave water quality protection power to the Army Corp of Engineers. The Act aimed to reduce pollution of navigable waters by classifying refuse discharges misdemeanors and requiring a permit for any alterations to harbors or channels. The Act did not address most liquid wastes, an area that would later affect many American waterways. Nonetheless, the Rivers and Harbors Act was an important first step in protecting American water systems that would stand for over 50 years.

After five decades, the Rivers and Harbors Act had failed to reach environmentalists expectations. During the late 19th and early 20th century, following lax enforcement and widespread unmonitored discharges, America's surface waters were increasingly stressed from human derived pollution (Dempsey 2004). Between 1900 and 1970, rivers throughout the Great Lakes watershed, including the Cuyahoga, Rouge, and Buffalo Rivers, had reportedly caught fire on multiple occasions (Adler 2002). In the 1960s, American surface waters were perceived unsafe for swimming or fishing and the current standards provided inadequate protection (Copeland 2010). In addition to point sources, non-point pollution such as agricultural runoff also resulted in the annual loss of soil and the deposition of nutrients into surface waters which was estimated in the billions of tons (USDA-NRCS 1997). A lack of environmental protection and enforcement required government assistance beyond the River and Harbors Act if the highly polluted surface waters were to recover.

Table 1.2. History of U.S. water quality public laws.

Public Law	Official Title	Primary Goals
33-403	Rivers and Harbors Appropriation Act of 1899	Addressed dumping of trash to waterways
80-845	Water Pollution Control Act of 1948	Cooperative programs between state and federal agencies; limited federal enforcement and financial assistance
84-660	Federal Water Pollution Control Act of 1956	Cooperative programs between state and federal agencies; limited federal enforcement and financial assistance
87-88	Federal Water Pollution Control Act of 1961	Strengthens federal enforcement and support to states; Established Department of Health, Education, and Welfare
89-234	Water Quality Act of 1965	Directs States to develop standards for interstate waters
89-753	Clean Water Restoration Act of 1966	Study the effects of sedimentation and pollution on designated uses
91-190	National Environmental Policy Act of 1969	Establish a national policy for environment and the Council of Environmental Quality
91-224	Water Quality Improvement Act of 1970	Established stricter limits on pollutant discharge to waters
92-500	Federal Water Pollution Control Act Amendments of 1972	Established NPDES
95-217	Clean Water Act (CWA) of 1977	Provides pollution control authority to EPA; Funded wastewater treatment plant construction; Discharge without permit to navigable waters became illegal
96-510	Comprehensive Environmental Response, Compensation, and Liability Act (1980)	Addresses environmental issues from accidental spills or releases
100-4	Water Quality Act of 1987	Required states to develop numeric criteria for water body segments where toxic pollutants were negatively affect designated uses; antidegradation policy
106-284	Beaches Environmental Assessment and Coastal Health Act (2000)	Criteria development by States; Standard modification; Monitoring notification; Appropriations

The Rivers and Harbors Act (1899) stood as the only federal legislation protecting American waterways until the passage of the Federal Water Pollution Control Act (1948) which strengthened the Federal government's authority of pollution control and aimed to enhance water quality through the creation of national pollution control and prevention policy. Federal authority improved with the creation of the 1956 Federal Water Pollution Control Act, Federal Water Pollution Control Act of 1961, the Water Quality Act (1965), the Clean Water Restoration Act (1966), and the Water Quality Improvement Act (1970). These Acts allowed Federal government to file lawsuits against polluting entities, established national enforceable standards, imposed monetary fines for polluters, and mandated States to develop and adopt antidegradation standards. All of these acts laid the foundation for water quality improvement, protection, and enforcement but the multiple amendments hindered the Federal government's ability to effectively implement and enforce regulations.

Following decades of cobbled policies and a plethora of highly publicized environmental problems, policymakers undertook the long process of cleaning, protecting, and rehabilitating public surface waters and the surrounding environment. This process culminated with the National Environmental Policy Act (NEPA) which established the Council of Environmental Quality and paved the way for future measures aimed at protecting the environment. The Council of Environmental Quality acts as a moderator between environmental federal agencies and reports to the president of the United States on the progress of environmental conditions. NEPA was instrumental in raising environmental awareness which eventually resulted in the creation of the Federal Water Pollution Control Act Amendments (known as the Clean Water Act of 1972 and amended in 1977). After decades of gross misuse and impacts on human health, America’s surface waters finally received substantial federal governmental attention.

Under the Clean Water Act (CWA), programs and funding sources were developed to establish, implement, and enforce chemical, biological, and physical integrity for America’s surface waters. Waterbodies that failed to meet designated use criteria were to be listed on an impaired waters list (303(d)) and receive further control measures including Total Maximum Daily Loads (TMDL) which established maximum pollutant levels that could be discharged daily to a waterbody and still meet water quality standards. The National Pollutant Discharge Elimination System (NPDES) permitting program enforced water quality rules limiting point source pollution discharges. The Clean Water Act and associated programs sought to improve water quality to swimmable and fishable standards by 1983 and eliminate the discharge of pollutants to navigable waters by 1985. The key goals of the CWA were to provide financial assistance for wastewater treatment plant construction; regulate pollution discharge (NPDES); and achieve water quality safe for swimming and fishing.

Following the passage of the CWA, it became evident that additional environmental protection was required. As, part of the superfund (Comprehensive Environmental Response, Compensation, and Liability Act of 1980) program, the U.S. Environmental Protection Agency (EPA) was authorized to address toxic spills which threatened water quality or human health. In 1987, Congress addressed the need to improve stormwater and required municipal treatment plants to obtain NPDES permits.

The CWA established recreational water quality regulations, health based criteria, and state standards, however many beaches remained heavily impacted by polluted water and required additional protection. In response, Congress passed a CWA amendment, the Beach Environmental Assessment and Coastal Health (BEACH) Act, in 2000. It required coastal States, including those along the Great Lakes, to adopt recreational water quality standards equally protective as established Federal criteria. The BEACH Act also established a monitoring appropriation for beaches to identify acute and chronic pollution issues. The BEACH Act was authorized at $30 million per year but annual appropriations are often less than $10 million. Since its initiation, the BEACH Act has supported Great Lakes beach monitoring in Michigan with awards totaling $2.82 million (USEPA 2011). Furthermore, the Act included a timeframe for criteria revisions that must occur at least every five years and required beach monitoring result to be reported in a timely fashion.

NEPA and subsequent water quality public laws have been instrumental in the protection and enhancement of surface water quality throughout the United States. Since the enactment of the CWA, publicly owned water treatment (POWT) plants have increased in number, resulting in decreased biological oxygen demand (BOD) in surface waters across the country (Najjum 2009). Increasing POWT plants reduced the number of point source pollution discharges to surface waters and subsequently reduced nutrient and pathogen loads.

Separate from the CWA but equally important for Great Lakes protection were the International Joint Commission, the Great Lakes Water Quality Agreement, and the Great Lakes Basin

Compact. These entities offered additional protection for the Great Lakes water and ecosystem at a regional/basin wide scale, as described below.

The International Joint Commission (IJC) was established under the Canada-U.S. Boundary Waters Treaty of 1909 to resolve water quality disputes between the two nations (IJC 2005). The IJC was tasked with informing each national government of emerging Great Lakes issues that have potential to escalate into disputes and providing recommendations on projects that may alter the flow and levels of boundary waters (IJC 2005). In 1914, the IJC undertook an extensive bacteriological survey of the Great Lakes to identify causation and sources of pollution in boundary waters and to identify remediation actions for water quality improvement (Durfee and Bagley 1997; Dreelin 2008). This survey identified sewage entering surface waters, crossing geographic boundaries, and causing human illnesses (IJC 2008). Unfortunately, the results from this study were never incorporated to policy and Great Lakes pollution was not addressed until later in the century. Following World War II, the IJC again addressed stark pollution concerns in the Great Lakes and eventually formed a binational agreement focused on improving the Great Lakes water quality (IJC 2008). Since its inception, the IJC has worked on over 120 international requests for boundary water applications (Clamens 2005). Over the last 100 years, the IJC has been instrumental in protecting the transboundary waters of the Great Lakes through research, intervention, and neutral political recommendations.

The Great Lakes Basin Compact is a regional organization representative of the eight US States (Public Law 90-419) and 2 Canadian Provinces bordering the Great Lakes. The Compact is led by a board of directors and commissioners composed of US representatives and associated members from Canada. The Compact, originally established in 1955 between the US and Canada, was intended to:

- Direct the development and conservation of the Basin
- Provide a Basin wide development plan
- Provide Basin residents with lakes access and all of their associated benefits to the maximum possible extent
- Advise on the appropriate level of each beneficial use to maintain proper balance between all uses
- Establish a governmental organization to meet the Compact purposes and goals.

Later, the Great Lakes Compact (Great Lakes-St. Lawrence River Basin Water Resources Compact) was created between the eight Great Lakes states and signed into law in 2008. This US interstate Compact addresses water use, withdrawal, and management of the Great Lakes; its impact will be measured over time as demand for clean water increases with national population growth.

The Great Lakes Water Quality Agreement was first signed by the United States and Canada in 1972 and affirmed each countries commitment to protecting and enhancing the Great Lakes. The Great Lakes Water Quality Agreement initially focused on phosphorus and toxic substance reduction and aimed to improve the Great Lakes by taking an ecosystem based approach (Krantzberg 2007). The Great Lakes Water Quality Agreement created a Great Lakes Water Quality Board and a Research Advisory Board which advices the International Joint Commission on progress of the Agreement’s objectives and programs. Under the Great Lakes Water Quality

Agreement, the IJC was tasked with examining land use impacts on water quality and analyzing, assessing, and reporting water quality information to the United States and Canadian governments every two years (IJC 2005). Since the inception of the original Agreement, the Great Lakes have seen a reduction in phosphorus and toxic substance loads (Botts and Muldoon 2005). This critical binational agreement bridged political boundaries between Canada and the United States addressed transboundary pollution and put in place measures that will protect the Great Lakes for generations.

Water quality policy in the 1800's was largely nonexistent and water quality degradation resulted in waterborne diseases, beach closures, and new stressors for the Great Lakes. Slowly water quality was protected and improved through a series of federal policy, epidemiological studies, water criteria and standards, and local authoritative associations. Most notably, the CWA improved water quality and protection by designating standards, funding surveillance, and establishing pollution reduction measures. Great Lake's water has been further protected by the IJC, the Great Lakes Water Quality Agreement, and the Great Lakes Basin Compact. Recreational health was addressed by the BEACH Act which significantly increased water quality monitoring through annual appropriations. Surface waters will continue to improve as long as political protection, science, and technology continue to evolve.

1.2. Indicators of Water Quality

1.2.1. Water quality and health studies

It is known that exposure to poor water quality at recreational beaches can result in acute human illnesses. Between 1997 and 2006, 100 outbreaks and 3,021 cases of illness (e.g. gastrointestinal, skin irritation, respiratory/ear/eye infection) were associated with ambient recreational waters of the United States (Barwick et al. 2000; Lee et al. 2002; Yoder et al. 2004; Dziuban et al. 2006; Yoder et al., 2008). Fleisher et al. (1998) reported that only up to 22.2% of bathers seek medical attention for illness associated with contaminated recreational water and symptoms remain for up to eight days.

Epidemiological studies performed since the 1950's provided vital information for recreational water quality criteria development. Stevenson (1953) first described that illness incidences occurred more frequently in swimmers than in non-swimmers and suggested a fecal coliform criterion. In 1982 and 1984, three epidemiological studies were undertaken in the United States that investigated gastrointestinal illness rates in individuals at recreational beaches. Cabelli et al. (1982) performed studies at marine bathing beaches in New York, Massachusetts, and Louisiana. Cabelli et al. (1982) reported enterococci densities were related to gastroenteritis. Dufour et al. (1984) performed epidemiological studies in Lake Erie (Pennsylvania) and Keystone Lake (Oklahoma). They reported enterococci and *E. coli* densities were statistically related to gastroenteritis and noted *E. coli* had a slightly greater correlation coefficient. All of these studies illustrated the most commonly reported illnesses were associated with infections of the eyes, ears, and the upper respiratory system (Favero 1985). These early epidemiological studies established a foundation for future studies and protective health criteria.

Reviews of the multiple recreational epidemiological studies by Prüss (1998), Wade et al. (2003), Zmirou et al. (2003), and Wade et al. (2006) found strongest correlations between the GI illness and predictors (enterococci and *E. coli*) which supported the EPA's total body contact criteria (USEPA 2009a) below which no illness could be observed. Additional studies identified young swimmers (children) were at an increased risk of illness compared to adult swimmers due to less developed immune systems (Wade et al. 2008; Parkins et al. 2003; White and Fenner 1994). Associated risks with waters impacted by different fecal sources (e.g. animals, humans, etc.) were not explicitly assessed during early studies, but are a concern for scientists and public health officials. Calderon et al. (1991) was unable to identify a statistical difference between risks of swimming in waters impacted by human versus animal fecal material. Pruss (1998) identified water quality measured by bacterial indicators and exposure caused gastrointestinal illness symptoms regardless of the apparent source. Using new molecular technology, a more recent study conducted at Great Lakes beaches suggests a positive association between enterococcus using rapid DNA detection and GI illness (Wade et al. 2006). This study also reported time of day and time spent swimming (exposure time) increased correlation strength between enterococci and illness (Wade et al. 2006). Contamination sources however remained elusive and were seen as an important concern during risk assessment, for restoration and water quality protection, but the epidemiological studies have improved the connection between water quality conditions and human health risk.

Guided by epidemiological results, the EPA published recreational water quality criteria for marine and freshwater. Marine water criteria were set at a single sample maximum of 104 enterococci 100 ml^{-1}. Freshwater criteria were set at a single sample maximum of 61 enterococci 100 ml^{-1} and 235 *E. coli* 100 ml^{-1} (USEPA 1986; Wade et al. 2008). Recreational water quality criteria were proposed to states as a suggestion for protection of human health.

Each state is responsible for developing and adopting standards for “swimmable” waters under the Clean Water Act. These standards must be as protective, based on the risk of illness, as the EPA criteria. Michigan’s *E. coli* total body contact standard has been set at a daily maximum (geometric mean of three individual samples spatial representative of a defined swim area) of 300 colony forming unit (CFU) 100 ml^{-1}. Michigan’s monthly total body contact standard was set at 130 CFU 100 ml^{-1} as a geometric mean of at least five sample dates. Unlike federal criteria, State recreational water standards are enforceable and help protect human health from exposure to contaminated water. In Michigan, beach managers conduct routine beach monitoring for bacteria to assess water quality conditions during the swim season. Samples are collected in waist-deep water at least once per week (May - September). Closures or advisories are issued as a response to potential health risks from fecal indicator bacteria densities that exceed state water quality standards. A yearly summary starting in 2001of Michigan Great Lakes beach closures/advisories is presented in Table 1.3. (MDEQ 2007; MDEQ 2011).

Table 1.3. Michigan’s Great Lakes beach closure or advisory days per year.

Year	Closures or advisory days
2001	122
2002	211
2003	140
2004	578
2005	474
2006	886
2007	1568
2008	697
2009	1596
2010	1003
2011	789

1.2.2. Fecal indicator bacteria

The most widely used tools for assessing water quality and risk of illness in recreational waters, as mentioned above, are the fecal indicator bacteria. Fecal indicator bacteria are measured as surrogates of true waterborne pathogen presence in water (Griffin et al. 2001). Fecal indicators are generally easier to detect than their pathogen counterpart and their respective detection methods tend to be cheaper. Optimal indicator species are based on the following premises: 1. They are present only when fecal pathogens are present; 2. They are present in greater concentrations than pathogens; 3. They are more resistant to treatment and environmental surroundings than pathogens; 4. They are easily detected; 5. They represent a specific group of pathogens and; 6. They are evenly distributed and grow independently in samples (Bonde 1966; Colford et al. 2007). Indicators are supposed to be present in greater concentrations, thus their detection often requires a smaller sample volume. Currently no indicator system meets all criteria.

Natural shortfalls of fecal indicators limit their usefulness in recreational water quality monitoring. Increased understanding of pathogens, particularly viruses and protozoa, identification of regrowth potential, and improved laboratory techniques have increased scrutiny of the use of a single fecal indicator for environmental assessments (Schwab 2007). Survival rates and regrowth potential of fecal indicator bacteria varies depending on water temperature, sunlight, nutrient availability, turbidity and specific biological properties of each bacterium (McLellan et al. 2007). Detection of cultivation based indicator bacteria involves incubation periods of 18-26 hours and do not identify the source of pollution. Not one organism is capable of showing the whole picture, requiring a combination of approaches (McLellan 2004). For such reasons, scientists are beginning to use multiple fecal indicator bacteria and viruses.

Contact with polluted waters has been associated with illness for many years, as previously described, but determining which microbial indicator best represents human health risk is still under debate. From the 1950s to mid-1980s, the fecal indicator of choice in the US was coliforms and fecal coliforms in both marine and freshwater (Stevenson 1953). In 1986, *E. coli* and enterococci were adopted as the leading fecal indicators for freshwater and marine water, respectively (USEPA 1986). Additionally, microbial indicators of fecal contamination have included *Clostridium perfringens* (Cabelli 1978) and bacteriophages (Lipp et al. 2001). Each suggested fecal indicator bacteria have strengths and weaknesses as a tool for water quality assessment. A brief description of each indicator is provided in the following paragraphs.

Escherichia coli

E. coli are a subgroup of the fecal coliforms, gram negative and facultative anaerobic bacteria. They are commensal in the lower intestine of warm blooded mammals and birds (Winfield and Groisman 2003). *E. coli* are distinguishable from other fecal coliforms by their ability to grow at 45°C, an absence of urease, and the presence of B-D glucuronidase which catalyzes B-D-glucopyranosiduronic acid (Toranzos and McFeters 1997). Most strains of *E. coli* are harmless but some can cause diseases. These strains include the enterhemorrhagic, enterotoxigenic, enteropathogenic, enteroinvasive, enteroadherent, and enteroaggregative *E. coli* (Rice 2003). Most infectious *E. coli* are spread via fecal-oral route (Bischoff et al. 2005) such as exposure to fecal matter while swimming in contaminated water. *E. coli* has been and remains the workhorse for water quality investigations despite several issues that fail the true fecal indicators premise.

Recent research has questioned the continued use of *E. coli* as a fecal indicator. The disadvantages of *E. coli* include: their ability to grow in the water environment; their presence only when pathogens are present; and their uneven distribution. *E. coli* has been shown to replicate in water outside of its natural host (McLellan et al. 2001; Vital et al. 2008). In regards to uneven distribution, beach monitoring samples are routinely taken in the waste deep water (i.e. swimmable water), but waste deep water have significantly different *E. coli* densities than other nearshore depths (Whitman and Nevers 2004). *E. coli* are generally found in lower densities in deeper waters due to settling and sunlight inactivation (Thupaki et al. 2010). Furthermore, current cultivation methods require an 18-24 hour incubation time, reducing the effectiveness of protecting bathers in real-time.

Advantages of using *E. coli* as a fecal indicator are primarily due to its long term use in monitoring including during epidemiological studies which correlated *E. coli* with gastroenteritis in freshwater, as previously stated. Although *E. coli* can regrow, Winfield and Groisman (2003) showed it does not survive as well in these secondary environments compared to inside mammalian guts. Current methods for *E. coli* detection are fairly inexpensive and produce MPN or CFU results (Rompre et al. 2002).

Enterococci

Enterococci are gram positive, non-spore forming members of the Streptococci bacteria commonly found in the feces of warm blooded animals. Enterococci can be distinguished from Streptococci by their ability to grow in sodium chloride (6.5%), at high pH (9.6), and at 45°C (Toranzos et al., 2007). There are multiple strains of enterococci, many of which are not harmful to humans. Compared to *E. coli*, enterococci are more resistant to chlorination and environmental stress and persist longer in the environment (Gleeson and Gray 1997). Enterococci have distinguishing characteristics which allow them to be isolated and used as a fecal indicator in recreation water. The selective media mEI, uses Indoxyl-B-D-glucoside as a chromogen which reacts with by B-D-glucosidase to produce a blue halo around positive enterococci colonies (USEPA 2002).

Enterococcus plays an important role in recreational water quality monitoring, but like *E. coli*, have significant shortfalls as a true fecal indicator. Enterococci can regrow in the environment under favorable conditions (Desmarais et al. 2002). Enterococci concentrations rapidly fluctuated by as much as 140 MPN 100 ml^{-1} in a single minute or by as much as 345 MPN 100 ml^{-1} in ten minutes (Boehm 2007). Furthermore, streptococci are often less numerous than *E. coli* in human fecal material (Pipes 1982) which may make them more difficult to detect in surface waters. Regrowth and lower organism concentrations limited enterococci's ability to be used as a fecal indicator of recent fecal contamination.

Despite their shortfalls, using enterococci as a fecal indicator offer key advantages over *E. coli*. Kinzelman et al. (2003) reported that when US EPA enterococci and *E. coli* total body contact threshold levels were applied to beach monitoring more closures were identified using enterococci than *E. coli*. Thus, indicating a more protective estimation of water quality compared to *E. coli* and a better statistical relationship to human health. Another advantage is that many groups have identified relationships between enterococci results using culture based methods and rapid (< 3 hours) methods (Whitman et al. 2010; Byappanahalli et al. 2010; Haugland et al. 2005). These comparisons are crucial for integrating technology with monitoring to lower illness risks from contaminated recreational water exposure.

Clostridium perfringens

Clostridium perfringens (*C. perfringens*) are obligate anaerobic, rod shaped, gram positive, spore forming, and sulfate reducing organisms. They are opportunistic pathogens that produce enterotoxins (Gleeson and Gray 1997). The spores do not regrow in the environment and are resistant to high temperatures and disinfection treatments (Payment and Franco 1993). *C. perfringens* are found in sewage and highly impacted waters (Lisle et al. 2004). Detection is accomplished using phenolphthalein diphosphate which reacts with an acid phosphate enzyme elaborated by *C. perfringens*. After the bacterium is exposed to ammonium hydroxide fumes, the diphosphate bond is cleaved and the reaction becomes visible by the absorption of Indoxyl-B-D-Glucoside (Sartory 1986; Bisson and Cabelli 1979).

Similar to *E. coli* and enterococci, *C. perfringens* has advantages and disadvantages as a fecal indicator. *C. perfringens* spores can be an index parameter for persistent intestinal pathogens (e.g. viruses and oocysts of protozoa) (Fujioka and Shizumura 1985). Since the spores are highly resistant to disinfection (Cabelli 1978), suitable applications include the assessment of:

chlorinated waters; industrial waters which contain compounds lethal to non-spore forming bacterial indicators; samples that cannot be processed within 12 hours; and the detection long term inputs of fecal pollution. Conversely, *C. perfringens* are often found in low concentrations (Garrido-Perez et al. 2008) making them more difficult to detect and the spores may provide a far too conservative estimate for protecting human health. Furthermore, there has not been much application of *C. perfringens* as a fecal indicator in recreational waters, except in Hawaii where traditional fecal indicators are ubiquitous (Mahin and Pancorbo 1999), requiring significant investment prior to their application in monitoring plans.

Viruses

Bacteriophages are viruses that infect only bacteria while the bacteriophage coliphage specifically infects *E. coli*. Coliphage have a finite life, do not regrow in the environment, and physically resembles many enteric viruses (Havelaar 1987). Two coliphage types (T and F), defined by infection mechanisms, have been widely used in the water quality field (Guzman et al. 2008; Donnison and Ross 1995). Coliphage serve a unique role in water quality monitoring for potential enteric pathogens and show promise as a fecal indicator. Phages can be measured as a lytic unit in a lawn of their respective bacterial host grown in tryptic soy agar (TSA) using a double agar overlay according to EPA method 1601 (USEPA 2001) and reported as plaque forming units (PFU) 100 ml^{-1}. Similar to other fecal indicators, current method for detecting coliphage require 24 hour incubation to produce results.

Coliphage have been employed as fecal indicators during recreational water quality monitoring with some success. Brion et al. (2002) identified one serotype of F+RNA coliphage was related to untreated human fecal material. Another study identified coliphage as an indicator of noroviruses in freshwater exhibiting similar seasonal variation, propensity for removal and resistance to environmental stress (Allwood et al. 2003). Whitman et al. (2008) used FRNA coliphage in a Great Lakes study and found their concentrations increased during wet weather summer periods', suggesting fecal contamination was impacting water quality following heavy rain events. Viruses are increasingly being implicated in water related outbreaks (Hlavsa et al. 2011) and using coliphage as a virus indicator is becoming increasingly important to reduce the risk of exposure to contaminated water.

Despite the promising results supporting coliphage as a fecal indicator, there are disadvantages to its application including non-human sewage specific occurrence (Allwood et al. 2003). Additionally, coliphage results are not consistent across studies (Ashbolt et al. 2001), leaving their meaning and future use questionable. Ashbolt et al. (2001) further remark that such differences are likely the results of inconsistent techniques, temperature, pH, and original densities of coliphage and bacteria host. As methods become more standardized, coliphage shortfalls may be improved upon and their use in water quality monitoring may grow.

1.2.3. Molecular Source Tracking

The inability of fecal indicator bacteria to identify pollution sources has emerged as a significant gap in water quality microbiology, leading to the development of molecular source tracking. Molecular source tracking (MST) is a field of study that seeks to identify the origin of fecal waste. Current methods quantify species specific gene targets generally using Polymerase Chain Reaction (PCR) approaches (Scott et al. 2002), known as library-independent host specific source tracking. Host specific source markers are specific DNA sequences that can be extracted from water and assayed with PCR to indicate the presence of feces from a single species. Advantages of using PCR based MST over cultivation methods include a high sensitivity and specificity, quicker result outputs, and in most methods, a more automated processes (Girones et al. 2010). Current molecular approaches do no distinguish between viable and non-viable organisms, producing mixed interpretations of molecular results for human health risk (Girones et al. 2010). Source tracking has gained popularity throughout the Great Lakes region as fecal source identification becomes more critical for maintaining or improving water quality.

Library-independent source tracking methods involve the detection of one species using one or more of the following: chemicals, sterols, viruses, and bacterial genes (Santo Domingo et al. 2007). In the case of bacterial genes, the DNA sequences first are identified and then undergo validation testing to evaluate the specificity of the sequence as unique to a particular species (Walters and Field 2006), but no marker has been or is likely to be completely validated. Water samples are assayed for specific source markers, generally through a non-culture based method (Santo Domingo et al. 2007) and DNA amplification through polymerase chain reactions (PCR). The library-independent method can use conventional or real-time PCR (quantitative method) to detect the specific gene sequence. Library-independent methods return less false positive and false negative results than the library dependent method (Griffith et al. 2003). Originally, few specific markers were available (Field and Samadpour 2007) and these markers were not always present in large quantities throughout the environment (Scott et al. 2005). However the field is expanding dramatically. Discussed below are two popular MST markers for recreational water quality monitoring.

One bacterial group that has become widely used and reported in water quality analysis is Bacteroides. Bacteroides (Order Bacteroidales) are anaerobic bacteria found in high concentration throughout the intestinal tract and feces (Field et al. 2003) of most animals. Bacteroides are anaerobic and thus do not survive long in surface waters, but using PCR method their presence can be detected in water for long periods of time (Okabe and Shimazu 2007). Bell et al. (2009) suggests microorganism predation and temperature result in the greatest reduction of *Bacteroides* (AllBac) marker densities in water. Balleste and Blanch (2010) confirmed that temperature was a significant factor in *Bacteroides* DNA degradation. Dick et al. (2010) used microcosm experiments to expose seeded water to varying simulated conditions and concluded the BacHum source marker decayed faster, relative to cultivated *E. coli*, under sunlight, sediment, and decreased predation treatments. All of these studies have predicted that the signal can last in the environment at least 200 days. Due in part to molecular methods, the identification of Bacteroides persistence in surface waters has led to the popular use of Bacteroides as a marker of fecal contamination in water.

Water quality investigations are increasingly turning to molecular detection of Bacteroides for improved understanding of water environments. Bernhard and Field (2000a, 2000b) identified unique gene sequences (Bac32F, Bac708R, Bac303R) highly specific to human and ruminant feces. Ahmed et al. (2008) confirmed two other markers (HF 183 and HF 134) were highly specific to humans and nearly absent in a multitude of animals. Ahmed et al. (2009) used the HF183 to differentiate human sewage and animal waste and found the marker had a host specificity of 98%. Another marker, *B. thetaiotaomicron*, α-1-6 mannanase, described by

Yampara-Iquise et al. (2008) was found to be a good indicator of sewage impacted waters with a low method detection limit (9.3 copies per reaction). Additionally, Srinivasan et al. (2011) found significant correlations between this marker and traditional cultivation based *E. coli* and enterococci concentrations throughout the wastewater treatment processes. Reischer et al. (2008) applied a Bacteroides marker (BacR) to determine that ruminants were the leading cause of fecal contamination in a large catchment. Additional Bacteroides source tracking research has targeted specific gene markers including gulls (Jeter et al. 2009), pigs (Gourmelon et al. 2007), horses (Dick et al. 2005), muskrat (Marti et al. 2011), and dogs (Kildare et al. 2007). Table 1.4. describes popular MST sequences and their respective targeted organism. Cumulatively, these studies represent the common development of the Bacteroides marker, each one developing, refining, and applying molecular source tracking methods to improve our understanding of water quality pollution in complex watersheds.

Table 1.4. Sequences and targeted organisms for select molecular source tracking markers.

Marker ID	Gene sequence (5'-3')	Target microorganism (Specificity)	Reference
Bac32F	AACGCTAGCTACAGGCTT	Bacteroides-Prevotella (General)	Bernhard and Field 2000b
Bac303R	CCAATGTGGGGGACCTTC	Bacteroides-Prevotella (Cow)	Bernhard and Field 2000a
Bac708R	CAATCGGAGTTCTTCGTG	Bacteroides-Prevotella (Human)	Bernhard and Field 2000b
HF183	ATCATGAGTTCACATGTCCG	*Bacteroidales* species (Human)	Bernhard and Field 2000a
BacR	GCGTATCCAACCTTCCCG	*Bacteroidales* (Ruminant)	Reischer et al. 2006
AllBac	GAGAGGAAGGTCCCCCAC	*Bacteroidales* (General)	Layton et al. 2006
BacHum	TGAGTTCACATGTCCGCATGA	*Bacteroidales* (Human)	Kildare et al. 2007
BtH	CATCGTTCGTCAGCAGTAACA GTAATTGCTACACCTGCTGAA ACCACTGTCCCT TTTTCTTGG	*Bacteroides thetaiotaomicron* α-1-6, mannanase (Human)	Yampara-Iquise et al. 2008

The Bacteroides have offered a substantial benefit to water quality investigators by targeting specific source(s) of fecal contamination; however, there are disadvantages to their use in beach monitoring. The Bac32F, Bac708R, Bac303R were shown to have little or no correlation with fecal coliforms, *E. coli*, or enterococci in river water samples (McQuaig et al. 2006). Drawing from this study, it is uncertain whether human health risk and Bacteroides levels are related. In order to achieve an understanding of human health risk in recreational water, Bacteroides must be monitored in conjunction with a health based fecal indicator in specialized studies as suggested by Rose et al. (1997). This would add significant cost to beach monitoring and regress from the concept of water quality monitoring using an inexpensive indicator. Improving analytical methods will allow for increased use of Bacteroides during routine monitoring, but until then their use remains limited.

Another MST marker is the enterococci surface protein (esp) gene. Esp is a potential virulence gene involved in biofilm formation and possibly involved in intestinal colonization (Heikens et al. 2007). The protein gene marker has been suggested as a useful tool for human fecal source tracking (Scott et al. 2005). This reportedly human-specific marker has been consistently detected in sewage and septage and inconsistently detected in animal feces (Ahmed et al. 2008, Whitman et al. 2007). Given the ability of esp to colonize intestinal tracts and cause gastrointestinal illness, the esp gene may be used to improve water quality and human health protection.

Supporters of the esp gene as a human fecal marker claim a high degree of human specificity which accurately describes human fecal contamination. Kim et al. 2010 claimed the *E. faecium* esp gene had a specificity of 100%. The same group also assayed 237 enterococcus species isolates from thirty-four human, chicken, pig, cow, and goose samples and found significant genetic differences between human and animals. The esp gene was absent in all tested animals by Scott et al. (2005) and Masago et al. (2011). Additionally, the marker was found in all or nearly all sewage samples (Scott et al. 2005, Ahmed et al. 2008) and in 67-97% of septic samples (Ahmed et al. 2008, Scott et al. 2005). With the esp, false negatives may be potentially problematic (Goodwin et al. 2008).

Acceptance of esp as an indicator of human fecal contamination has been received with some skepticism. Byappanahalli et al. (2008) concluded the esp gene was inconsistent and did not accurately distinguish between animal and human sources. Furthermore, they argue the esp marker has inconsistent findings between studies and a low specificity between animal and human fecal sources. These conclusions were based on a study which detected the esp gene in 29% of seagulls, 14% mice, and 9% songbirds by swabbing fecal material then diluting and processing (Byappanahalli et al. 2008). As new markers are developed, it is important that they are critically reviewed and tested to ensure they accurately describe the source of contamination. The esp gene may be a reliable indicator of human fecal contamination but first a standard method is required to produce consistent results across all studies.

Fecal indicator bacteria have provided information on the wide spread and continued pollution of the Great Lakes for over half a century. Improved scientific and molecular technology has allowed for increased accuracy, precision, and source identification in the water quality field. Although much work is still required to improve and fully understand the sources of fecal contamination in the Great Lake and health risks, the foundation has been laid for long term improvements. Sewage has often been the focus of pollution studies, but nonpoint sources (e.g. contaminated urban, agriculture, or industrial runoff, atmospheric deposition, or seepage from contaminated subsurface sites), have been a challenge to identify. While the use of geographic mapping of nonpoint source pollution and land use has improved the understanding of water quality degradation in the Great Lakes, the need for further research is still of great interest.

1.3. Nonpoint Source, Land, and Weather Impacts on Water Quality

Implementation and enforcement of the CWA for the past 40 years has provided considerable improvements in the management of point source discharges, especially NPDES, CSOs, and SSOs. As improvements continue to reduce contamination from identified sources, regulators and stakeholders in the Great Lakes have begun the arduous task of addressing nonpoint sources of pollution. Nonpoint source pollutants are now the leading cause of impaired waters (USEPA 2009b). One type of nonpoint source pollution is contaminated runoff or the overland flow of water transporting pollution from the landscape to surface waters. Land use and land cover (LULC) management can alter the natural percolation and runoff patterns along with the movement of pollutants in water. Dreelin et al. (2007) stated land use decisions affect the sources and transport of pathogens into environments. Understanding LULC and influences on hydrology and nonpoint source pollutants is vital to protecting water quality in the Great Lakes, but there are also key pollutant reservoirs such as sediment and algae. Sediment resuspension, algae accumulation in the littoral zone, and contaminated runoff are among the most problematic in the Great Lakes, generating significant concern for water quality managers (Kinzelman et al. 2004), but they do not have well-defined impacts on public health (Verhougstraete et al. 2010).

1.3.1. Algae

Algae mats act as a significant reservoir of pollutants for nearshore zones. Many studies have discovered high levels of fecal indicator bacteria in nearshore algae mats (Garrido-Perez et al. 2008; Whitman and Nevers 2003; Englebert et al. 2008a; Englebert et al. 2008b; Ishii et al. 2006; Olapade et al. 2006). Bacteria may regrow in algae and can influence surrounding water quality when disturbed. This water quality change occurs in the absence of recent fecal contamination, potentially generating false positive results regarding risk during beach monitoring. Science is progressing to identify the potential risk posed by algae presence in the nearshore area on human health and recreational water quality monitoring. Algae samples collected around the Great Lakes have confirmed the presence of elevated fecal indicator bacteria in the mats (Englebert et al. 2008b; Byappanahalli et al. 2007; Olapade et al. 2006; Ksoll et al. 2007; Whitman et al. 2003). In addition to fecal indicator bacteria, human pathogens, specifically Shiga Toxin producing *E. coli* (STEC) and *Shigella* (Ishii et al. 2006a), *Salmonella* (Byappanahalli et al. 2009; Ishii et al. 2006), *Clostridium botulinum* type E (Byappanahalli and Whitman 2009), and *Campylobacter* (Ishii et al. 2006) have been detected in algae. The microorganisms identified in algae, the respective Great Lakes studies, and the detected concentrations are presented in Table 1.5. (Verhougstraete et al. 2010).

Table 1.5. Studies and the microorganisms identified in *Cladophora* collected within the Great Lakes*.

Study	*E. coli*	Enterococci	*Salmonella*	*Campylobacter*	*Shigella*	STEC
Byappanahalli et al. 2007	2.55-3.09 log CFU/g ^{A}					
	Laboratory					
Byappanahalli et al. 2009			0.16-89.46 MPN/g ^{A}			
			Env.			
Whitman et al. 2003	5.3 ± 4.8 log CFU/g ^{A}	4.8 ± 4.5 log CFU/g ^{A}				
	Env.	Env.				
Byappanahalli et al. 2003a	1.3-4.3 log CFU/ml	1.3-3.3 log CFU/ml				
	Laboratory	Laboratory				
Englebert et al. 2008a	8.0-9.0 log CFU/ml		5.0-6.0 log CFU/ml		7.0-8.0 log CFU/ml	
	Microcosm		Microcosm		Microcosm	
Englebert et al. 2008b	2.9-3.4 log MPN/100 ml					
	Env.					
Olapade et al. 2006	38,000 CFU/100 g ^{B}					
	Env.					
Ishii et al. 2006	5.8 ± 5.5 log MPN/g ^{A}		1.5 log MPN/g ^{A}	39 cells/g ^{A}	^{C}	^{C}
	Env.		Env.	Env.	Env.	Env.
Ksoll et al. 2007	294,000 CFU/cm^{2}					
	Microcosm					

A: dry weight; B: wet weight; C: organisms detected but not quantified. **Laboratory**: Bacterial analysis performed under laboratory settings; **Microcosm**: Bacterial analysis performed under simulated conditions on a minute scale; **Env. (environmental)**: Bacterial analysis performed on natural samples; *Reproduced from Verhougstraete, M.P., Byappanahalli, M.N., Rose, J.B., and Whitman, R L. (2010). '*Cladophora* in the Great Lakes: Impacts on beach water quality and human health'. *Water Science and Technology*, 62 (1), 68-76, with permission from the copyright holders, IWA Publishing.

Bacteria not only accumulate in algae mats they likely persist longer in algae mats than in surrounding water and can regrow. *E. coli* was shown to survive less than four days in lake water (Olapade et al. 2006) but as long as 45 days in algae mats (Englebert et al. 2008a). Many theories have been proposed for this occurrence including increased attachment points for bacteria in algae mats (Brettar and Höfle 1992; Weinbauer and Höfle 1998; Signoretto et al. 2004), increased substrates vital for bacterial growth in algae leachate (Byappanahalli et al. 2003a), and increased protection from sunlight in algae mats thicker than 6mm (Whitman et al. 2003). Additionally, Whitman and Byappanahalli (unpublished) demonstrated that *E. coli* can survive in dried algal mats stored at 4 °C for over two years. *Salmonella* was shown to persist for 10 days in algae manipulated in a laboratory microcosm environment (Englebert et al. 2008a). Englebert et al. (2008a) showed, via viability tests, that *Shigella* survived up to 2 days in an algae microcosm. The fate of fecal indicator bacteria in algae mats is becoming increasingly understood, the next step is to identify how presence and regrowth in algae mats will impact nearshore water quality.

Algae impact on nearshore water quality is not as clear as bacteria accumulation, persistence, and regrowth in algae. Two studies have reported contrasting conclusions regarding the impact of *E. coli* found in algae and water quality. Whitman et al. (2003) demonstrated floating algae mats have the ability to release bacteria to surrounding waters and influence water quality when present in the nearshore. In contrast, Englebert et al. (2008b) and Olapade at al. (2006) report that water quality was not significantly influenced by the presence of algae, regardless of *E. coli* concentrations in the algal mats or amount of algae material present. *E. coli* adherence to algae material was suggested as the main reason that the impacts to water quality were minimal

(Englebert et al. 2008b) and it was demonstrated by Whitman et al. (2003) that algae required multiple washes to remove nearly all *E. coli* cells. Despite the contrast in conclusions from these cases, each identified bacteria in algae may be released to surrounding waters as a result of environmental conditions. Whitman et al. (2003) identified that bacteria were released from floating mats as a result of wave action while Englebert et al. (2008b) noted strong wind and wave influences were required to dislodge the bacteria from algae mats. The contrast in conclusions from each of these cases shows the highly variable interactions between algae amounts and water quality that cannot be captured through a single study.

1.3.2. Sediment

For decades, scientists have known that sediment harbors chemicals, toxins, and nutrients which have the potential to impact overlying water (Smart and Barko 1978; Mortimer 1971; Marvin-DiPasquale and Agee 2003). The relationship between bacteria and sediment or the impact of such bacteria on surrounding water quality is not as clear. But bacteria can accumulate, regrow and potentially be released to the overlying water. Standards for bacteria analysis in sediments do not exist which have resulted in multiple reporting formats including bacteria numbers per gram of wet weight, gram of dry weight, or milliliters. Such reporting inconsistencies cannot easily be compared across studies as illustrated in the summary below.

Fecal indicator bacteria concentrations observed in beach sand and sediment are often higher than in surrounding water. Zehms et al. (2008) found *E. coli* concentrations as high as 21,670 CFU 100 g^{-1} dry weight in the sand at one GL beach. Whitman and Nevers (2003) reported nearshore sand had higher *E. coli* concentrations (4000 CFU 100 ml^{-1}) than the surrounding water (43 CFU 100 ml^{-1}). Alm et al. (2003) found *E. coli* was up to 38 times higher in beach sand than the water column and *E. coli* concentrations decreased with sediment depth. Garrido-Perez et al. (2008) found proximity to contamination source has a significant influence on sediment bacterial densities. They also reported beaches with low energy and circulation had a high accumulation of sediment and high bacterial concentrations (Garrido-Perez et al. 2008). The *E. coli* density range in Great Lakes sediment studies are provided in Table 1.6. Cumulatively these results indicate sediment exhibit a higher density of fecal indicator bacteria compared to surrounding waters.

Table 1.6. *E. coli* density ranges identified in Great Lakes sediment.

Study	*E. coli* density ranges
Alm et al. 2003	12-80 CFU GDW^{-1}
Byappanahalli et al. 2003b	1-119 MPN GDW^{-1}
Kinzelman et al. 2004	0-20000 CFU GDW^{-1}
Ge et al. 2010	10-10^5 CFU 100 ml^{-1}
Whitman and Nevers 2003	10^2-10^6 CFU 100 ml^{-1}
Byappanahalli et al. 2006	1-1657 MPN 100 GDW^{-1}
Zehms et al. 2008	1800-21670 CFU GDW^{-1}

GDW: Gram dry weight

In addition to bacterial storage, sediments can provide a suitable habitat for some bacteria, and potentially MST markers, to persist and/or regrow. Kinzelman et al. (2004) detected a relatively low number of *E. coli* clonal patterns isolates from Lake Michigan beach sediment, indicating *E. coli* was accumulating in the sediment and not regrowing. LaLiberte and Grimes (1982) used reduction rates in sediment to conclude bacteria detected in surface waters may not always be the result of fresh fecal contamination. Craig et al. (2002) found enterococci, *E. coli*, and coliphage all persisted longer in sediment than in water. One group removed and replaced contaminated sand at a Chicago beach with *E. coli* free sand and found that *E. coli* concentrations in the sand returned to historical levels within two weeks (Whitman and Nevers 2003). Alm et al. (2006) found *E. coli* densities increased fivefold in two days and remained above 2 X 10^5 CFU g^{-1} wet weight for 35 days in an in situ experiment. Garzio-Hadzick et al. (2010) found faster *E. coli* decay rates in water than in sediment, regardless of temperature. In the same study, undertaken in a field experiment, *E. coli* reached a density of 7.5 X 10^5 CFU g^{-1} for at least 48 days. Yamahara et al. (2007; 2009) showed enterococci regrew in wetted beach sand and doubled in as little as 1.1 days in sediment exposed to diurnal tidal wetting. This same study identified the origin of enterococci contamination in sediment was human based on the presence of the esp gene (Yamahara et al. 2007). It was also demonstrated that moisture and sand temperature had a significant impact on enterococci and *E. coli* decay rates (Mika et al. 2009).

The fate of molecular source tracking markers in sediment is not well understood. In fact, only one study has attempted to investigate the persistence of source markers in water while taking into account their presence in sediments (Dick et al. 2010). This study used Bacteroides markers in microcosms controlled for light, sediment exposure, temperature, and predation. The authors found, amongst other findings, that the decay rate of source markers in river water exposed to sediments was slower than the decay rate of the same markers without sediment. One important finding of this investigation was that the AllBac markers had a longer 2-log reduction time in the sediment exposed water than any other condition or marker. Cumulatively, these studies implicate sediment as a bacterial sink which encourage bacterial persistence and growth. The accumulation and growth of bacteria can have significant impacts on surface waters, as described below.

Fecal indicator bacteria present in nearshore sediment can result in significant water quality impairments during resuspension. Whitman and Nevers (2003) found lake water quality is significantly influenced by foreshore sand and may raise health concerns even in the absence of recent human or animal fecal material. LeFevre and Lewis (2003) and Kinzelman et al. (2004) identified fecal indicator bacteria accumulated in sediment significantly contributed to surrounding water quality as a result of wave action. More specifically, wave action in the nearshore swash zone was used to explain *E. coli* concentrations in knee deep water at a Lake Michigan beach (Ge et al. 2010). Increased turbidity was shown to increase the survival of *E. coli* (Pote et al. 2009; Garcia-Armisen and Servais 2009), again implicating sediment resuspension as a source of impairment for water quality. Many studies have identified latent sediments impact overlaying waters and sediments are now considered one of the primary sources of bacteria in nearshore waters.

1.3.3. Land-water interface

The surface water hydrologic cycle involves multiple land systems and parameters that influence water quality. A watershed is the total land area that drains to one point in a water body and is often defined by ridges/drainage divides that define the direction of overland flows (Kalff 2002). Within a watershed, overland flow (from precipitation) drains to a river, lake, or wetland. Rivers can be gaining (groundwater percolates into river), losing (river water percolates into groundwater), or intermediate (no continuous source of water) and are composed of headwaters (groundwater and snow/glacier melt), tributaries, and confluences of tributaries before the mouth of the river. Lakes are non-ocean bodies of water that exhibit little to no horizontal movement. The shallow, nearshore zones of lakes, including beaches, are referred to as the littoral zones, the open waters of lakes are referred to as the limnetic zone, and the lake bottom is referred to as the profundal zone. The transport of fecal indicators between the land and water in these zones is of scientific interest regarding links between pollution sources and impact.

Land use changes impact pollutant transport by modifying the surface water hydrologic cycle. Water movement continuously resuspends and deposit sediments and pollutants throughout hydrologic systems. Overland flow rates are determined by the type of the landscape (Lavee and Poesen 1991), precipitation rates and land roughness (Katz et al. 1995), and vegetative cover (Loch 2000). Modification to any of these parameters presents additional pollutant sources and sinks and movement in surface waters. Walters et al. (2011) found *E. coli*, enterococci, and pathogens (*Salmonella* and *E. coli* O157:H7) presence in waters change with land use type and are magnified by rainfall. Landscape changes influence natural percolation/infiltration of precipitation/melt off, causing changes to stream velocity and transport rates of pollutants to surface waters (Desai et al. 2010; Allan 2004). Patz et al. (2000) reviewed the application of land use changes on pathogen and diseases, noting forest removal increases habitat fragmentation and allows for exchange and transmission of pathogens to new areas, including into surface waters. Changes to land use create new sources of pollution, including bacterial sources, which modify the transport and quality of surface water, but investigative scales remains uncertain.

Watershed and LULC investigations have traditionally been costly and required large spatial and temporal scales. The current trend in ecosystem research is to investigate processes at multiple spatial scales (Soranno et al. 2010; Chang 2008) which often require 3-5 years (Spooner and Line 1993). Desai et al. (2010) described the need for longer time scale requirements in order to identify bacterial (*E. coli*) changes in water quality resulting from LULC changes in the watershed. Mehaffey et al. (2005) linked fecal coliform bacteria to urban and agriculture land use, noting land use location relative to the surface water was more significant than watershed percentages. Furthermore, the authors concluded large area watersheds can be evaluated for fecal coliform bacteria using simple geographic information system (GIS) approaches (Mehaffey et al. 2005).

Landscape ecology is emerging in the water quality field as a useful concept that links spatial terrestrial patterns (LULC) patterns and ecological processes. Using global information systems (GIS), connections between LULC and water quality have been identified, often focusing on physical parameters, nutrients, chemicals, and biological indices (e.g. fish and macrophytes) as the metric of water quality (Wang and Yin 1997; Broussard and Turner 2009; Akasaka et al. 2010). For instance, Mattikalli and Richards (1996) found elevated nitrogen concentrations in surface waters were related to changes in land use and fertilizer applications over five decades. Wang et al. (2001) found significant connections with urbanized development proximity to streams and fish communities. LeBlanc (1997) created a decision support tool that predicts the impact of land use changes on surface water temperatures in lotic systems. Linking water quality to landscape patterns has improved scientific understanding of environmental processes and implications on aquatic health, but the connection between land use and microbial densities in water remains unclear.

Few studies have successfully linked bacterial water quality to a specific land use type. A brief summary of studies attempting to connect LULC with microbial water quality is provided in Table 1.7. A few studies identified high concentrations of *E. coli* were characteristic of source sheds dominated by urban landscapes (Mallin et al. 2000; Desai and Rifai 2010; Desai et al. 2010; Wu et al. 2011). Kang et al. (2010) linked bacteria (*E. coli* and enterococci) to urban and industrial land uses. Desai et al. (2010) further noted that *E. coli* concentrations were highest in stream segments with high percent land development (40%), population density (1996 km^{-2}), dog density (513 km^{-2}), and low grassland percentage (20%). Similarly, Goto and Yan (2011) found a distinct urban land use pattern influence on *E. coli*, enterococci, and *C. perfringens* concentrations in Hawaii. Another study in Hawaii identified direct associations between *Salmonella* and agriculture/forested land cover while *Staphylococcus aureus* was directly associated with urban/agriculture land cover (Viau et al. 2011). Mehaffey et al. (2005) directly linked fecal indicator bacteria to both agriculture and urban percent development in a watershed, but noted correlations were stronger for land use located near the reservoir in the catchment than total percentage of the catchment. Hunter et al. (1999) found fecal coliform concentrations increased 100 fold as waters moved through agriculture lands. There is only one known study that addresses and claims a relation exists between microbial source tracking markers (Bac708 and CF128) to LULC (agriculture) (Kirs et al. 2011). In the previously mentioned studies bacteria appear to be ubiquitous in the environment influenced by any LULC change in a watershed. Few studies have successfully linked one microbe to a specific type of land use. This may be a result of the multiple influences not measured including soil characteristics, spatial implications from source, or weighted calculations for sources closer to surface water.

Table 1.7. Key findings of studies attempting to link land use and microbial water quality.

Study location	Key findings	Reference
Derbyshire, England	Fecal coliforms and fecal streptococci increased in agriculture catchments as a result of sediment storage and hydrological transport from landscape	Hunter et al. 1999
New York, USA	Fecal coliforms directly related to percent urban and agriculture in catchment	Mehaffey et al. (2005)
North Carolina, USA	Fecal coliforms linked to population, percent urban, and percent impervious surface in watershed	Mallin et al. 2000
Oregon, USA	Enterococci linked to urban and agriculture land use; Urban land use change resulted in microbial water quality exceedances	Nash et al. 2009
Yeongsan River, Korea	*E. coli* and enterococci are predominately from urban and industrial land use	Kang et al. 2010
Texas, USA	*E. coli* concentrations were higher, less temporally variable, and more spatially variable in urban dominated watersheds compared to grasslands	Desai and Rifai 2010; Desai et al. 2010
Oahu, Hawaii	*E. coli*, enterococci, and *C. perfringens* higher in urban streams compared to forested streams	Goto and Yan 2011
Nelson, New Zealand	Human (HF183) marker found in stormwater drains; Ruminant (Bac709/CF128) marker in agriculture catchments	Kirs et al. 2011
O’ahu, Hawaii	*Salmonella* directly related to forest and agriculture land cover; *Staphylococcus aureus* directly related to urban and agriculture land cover	Viau et al. 2011
California, USA	*E. coli*, enterococci, and *Salmonella* directly related to percent urban in catchment; *E. coli* O157:H7 directly related to percent agriculture in catchment	Walters et al. 2011
Ontario, Canada	*E. coli* O157:H7 related to agriculture density upstream	Wilkes et al. 2011
Massachusetts, USA	Highest human derived *E. coli* (ribotyping) found when urban >30% of catchment; Wildlife sources dominated when natural lands > 54% of catchment	Wu et al. 2011

1.3.4. Weather

Weather patterns and processes influence surface waters on shorter temporal scales and groundwater on longer temporal scales (Johnson et al. 2004) and likely drive the movement of pollutants from land to surface waters. Precipitation events above the 90th percentile were linked to more than half of all waterborne disease outbreaks in the United States, thus implicating the role of rainfall in the transport of waterborne pathogens (Curriero et al. 2001). Poor water quality has resulted from contaminated runoff or combined sewer overflow discharges as precipitation inundates soil or treatment systems (McLellan et al. 2007). In a Florida estuary, Lipp et al. (2001) linked elevated fecal contamination with variability in precipitation, temperature, and stream flow. Understanding weather influences on water quality is becoming more important in the Great Lakes as nonpoint source pollution and the potential association with health risks emerge.

Precipitation and snow melt have the potential to influence microbial water quality. The connection between microbes and precipitation has been demonstrated by many studies throughout the world and in the Great Lakes (Shehane et al. 2005; Cho et al. 2010; Jayawickreme and Hyndman 2007; Dorner et al. 2007; Scopel et al. 2006). Whitman et al. (2008) showed *E. coli* increased 10 and 100 fold following rainfall and snowmelt events, respectively, in an artificial stream system. Haack et al. (2003) identified a 48-72 hour lag between precipitation and elevated *E. coli* concentrations along the Grand Traverse Bay coast. Another study found positive correlations between 72 hour cumulative rainfall and *E. coli*, enterococci, and *C. perfringens* concentrations in urban dominated catchments (Goto and Yan 2011). Wu et al. (2011) showed water quality standards were exceeded more often following wet weather events. Jamieson et al. (2005) found storm events increased sediment resuspension and in turn elevated bacterial concentration in a Canadian stream. Furthermore, Nevers et al. (2007) identified a significant correlation between *E. coli* and barometric pressure in Lake Michigan. Collectively, these results demonstrate the impact precipitation has on natural (e.g. stream flow) and human (e.g. wastewater treatment) systems which led to degraded surface water.

In the Great Lakes, wind direction and speed has also been implicated as a significant influential factor of fecal indicator bacteria concentrations (Crowther et al. 2001; Haack et al. 2003; Nevers et al. 2007). The role wind plays on nearshore water is evident causing both wave action and sediment resuspension. This result has been repeated by the European Union which found increased winds contributed to increased bacterial concentrations in the surrounding water at recreational beaches (Roslev et al. 2008). Furthermore, wind (direction and speed) was shown to dislodge bacteria from Saginaw Bay algae mats and resuspends them into the water column (Verhougstraete and Rose, in prep.). Beach orientation, as opposed to wind direction, has also been suggested as a significant environmental parameter effecting nearshore water quality (Nevers and Whitman 2005; Nevers and Whitman 2008; Wong et al. 2009).

Temperature can affect a microorganism’s ability to survive and grow in water. Pip and Allegro (2010) showed higher concentrations of total coliform bacteria were associated with increased temperature in Lake Winnipeg. Wilkes et al. (2011) detected *Campylobacter* more often when mean air temperatures were cooler. One study addressed temperature (air and water) on *Bacteroides* using molecular and cultivation approaches and found *Bacteroides* organisms were detected more often in lower temperatures and high temperatures were associated with greater organism die-off rates (Balleste and Blanch 2010). Schulz and Childers (2011) also identified Bacteroidales decay rates were faster at higher temperatures using molecular approaches targeting Bacteroidales 16S rRNA gene. A growing concern in the Great Lakes is a shift in long term air and water temperature which may present water quality managers with significant challenges for beach monitoring and human health protection.

In summary, changes to land use land cover alter natural hydrologic process which affect surface runoff and water quality. Precipitation, wind, and temperature are often implicated as factors influencing water quality. Furthermore, sediment and algae mats have been shown to harbor bacteria and even foster regrowth of some fecal indicator bacteria. Increased energy from precipitation or wind can cause such bacteria to be released to surrounding water. Although scientists are investigating the implications of nonpoint source pollution on water quality, their associated human health risks remain unclear. Such associations will require further investigations at multiple spatial and temporal scales throughout the Great Lakes.

1.4. Scientific Needs

The current state of science for recreational water quality in the Great Lakes primarily rely on one fecal indicator microbe (*E. coli*) to inform the public of health risks from swimming in deep water. A trend in recent years to use multiple parameters, microbes, and source specific molecular analysis has been suggestive of a greater understanding of ecosystem and human health risks. Such methods allow scientists to explore important links between fecal indictors and true pathogens and enteric bacteria. Advancements in the tools available for source identification and survey (microbial, spatial, and sanitarian) efforts are needed to remediate fecal contamination impacting beaches.

Algae mat accumulation in the nearshore zone has posed significant challenges to beach managers protecting water quality. A number of pathogens have been identified within *Cladophora* mats, however the relationships between algal mat formation and a variety of fecal indicators, including MST, and water quality along beaches requires further investigation.

Currently, scientists understand that sediment can influence microbial and molecular persistence in the environment. Bacteria and molecular source markers have been shown to exist in higher concentrations and survive longer in sediments than in surrounding water. However, the linking of microbial accumulation and persistence characteristics in environmental sediments to water quality change at various watershed scales are not well studied particularly given both natural and anthropogenic influences.

Protection of surface waters requires an understanding of microbial water quality, nonpoint source pollution, and terrestrial/aquatic processes. Currently there is a significant knowledge gap between microbial contamination and terrestrial landscape patterns. Landscape ecology uses a variety of tools and scales to improve upon the knowledge of relationships between landscape patterns and ecosystem processes, but this approach has not been widely applied to the microbial water quality/landscape interactions. Scientists need to identify the appropriate landscape scale at which significant relationships between one or multiple fecal indicators can be inferred.

Water quality can be inferred by studying microbial (occurrence, fate, survival, and life cycles) and environmental (precipitation, wind, solar radiation, etc.) processes. However, further research focused on the quantification of microbial responses to environmental processes is required in nearshore waters. The scientific queries presented below expand upon current knowledge of parameters that influence non-point source pollution in the Great Lakes. These advancements will feed hydrologic models, address land use/cover impacts on water quality, and describe ambient water quality conditions useful for measuring changes over time. Developing process based water quality models will provide a framework for development of more effective water quality policies and improve the connection between recreational waters and human health.

1.5. Research Objectives

Goal 1

Multiple sources of pollution threaten the Great Lakes and one waterbody that has become dangerously threatened is the Saginaw Bay. This ecosystem is threatened by industrial, urban, and agricultural runoff, wastewater discharge, and algae blooms. Few studies have focused on microbial water quality in Saginaw Bay. A survey of four Saginaw Bay beaches was undertaken to quantitatively describe the fecal contamination in beach sediment, stranded algae mats, and surrounding water using fecal indicator bacteria and source specific DNA markers.

The objectives of this goal were to: 1) determine the occurrence and relationship of fecal indicator bacteria in water, sediment, and algae at beaches in a mixed use watershed; 2)

identify human and bovine sources of fecal contamination using microbial source tracking and; 3) identify the environmental parameters influencing water quality degradation.

Goal 2

Smaller watershed landscape scales may provide better associations between water and sediment quality. However, small scale systems may be subject to rapid temporal changes that may not be identifiable using current methods. Mitchell Creek is a small, flashy system draining mixed use watershed and discharging to the Grand Traverse Bay (Michigan). Mitchell Creek is a known contributor of *E. coli* to the Bay, but the sources of contamination are not well described. Water and sediment samples were collected over multiple seasons from Mitchell Creek sites representative of multiple land use types and assayed for fecal indicator bacteria and molecular source markers.

The objectives were to 1) examine the spatial and temporal distribution of traditional and alternative fecal indicators in a watershed influenced by non-point source pollution, 2) use a quantitative PCR marker to measure human sources associated with fecal bacteria, and 3) assess land use patterns effect on bacterial water quality.

Goal 3

“The valley rules the river” is a phrase ecologists have used for decades. Land use has been reported as a significant factor for non-microbial based water quality measures at multiple scales. However, land use/cover impacts on microbial water quality parameters, at any scale, is not understood. A broad spatial and temporal survey of bacterial water quality across Michigan was undertaken to quantitatively describe fecal pollution concentrations entering the Great Lakes during distinct hydrologic conditions and across multiple landscape scales.

The objectives of this goal were to: 1) Examine the occurrence of *Escherichia coli* (*E. coli*) and a human specific source marker (*B. thetaiotaomicron*) in river systems under baseflow conditions; 2) identify specific land uses that modify reference levels of fecal contamination in rivers; and 3) determine key chemical, physical, environmental, and hydrological variables driving water quality of rivers draining to the Great Lakes.

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CHAPTER 2.

MICROBIAL INVESTIGATIONS OF WATER, SEDIMENT, AND ALGAE MATS IN A MIXED USE WATERSHED

2.1. Introduction

Saginaw Bay, situated on the western shore of Lake Huron is an example of a mixed land use watershed (Figure 2.1.). Like many bays across the United States, water is economically vital to the region. The Bay averages 9.8 m deep, drains over 22,000 km^2 (USEPA 2011), and hosts 43 public beaches. This Bay has numerous key pollution sources including four combined sewer overflow systems (Saginaw Bay Science Committee Pathogen Work Group 2007) and an unknown number of septic systems within the Saginaw Bay coastal zone. The Bay has been heavily stressed by toxins (Yun and Kannan 2011), fish contamination (Jude et al. 2010), nutrient loading (Cha et al. 2010), invasive species (Fahnenstiel et al. 1995), and changes in natural phytoplankton populations (Fishman et al. 2009). In 1986, the Bay was added to the United States Environmental Protection Agency's (USEPA) Areas of Concern and the Remediation Action Plan process began to address the impairment of beneficial uses (including recreation) eutrophication/nuisance algae, degradation of aesthetics, and beach closures (USEPA 2011). Since 2002, 36 Saginaw Bay beaches have been monitored for *E. coli* weekly between June and September, resulting in 894 closure/advisory days (MDEQ 2011). The causative agents for these closures were categorized as unknown (93%), runoff (3%), and agriculture (4%). The nearshore zone receives significant inputs of pollution including sediment contamination and algae mat accumulations which produce highly visible impacts in the swimming area.

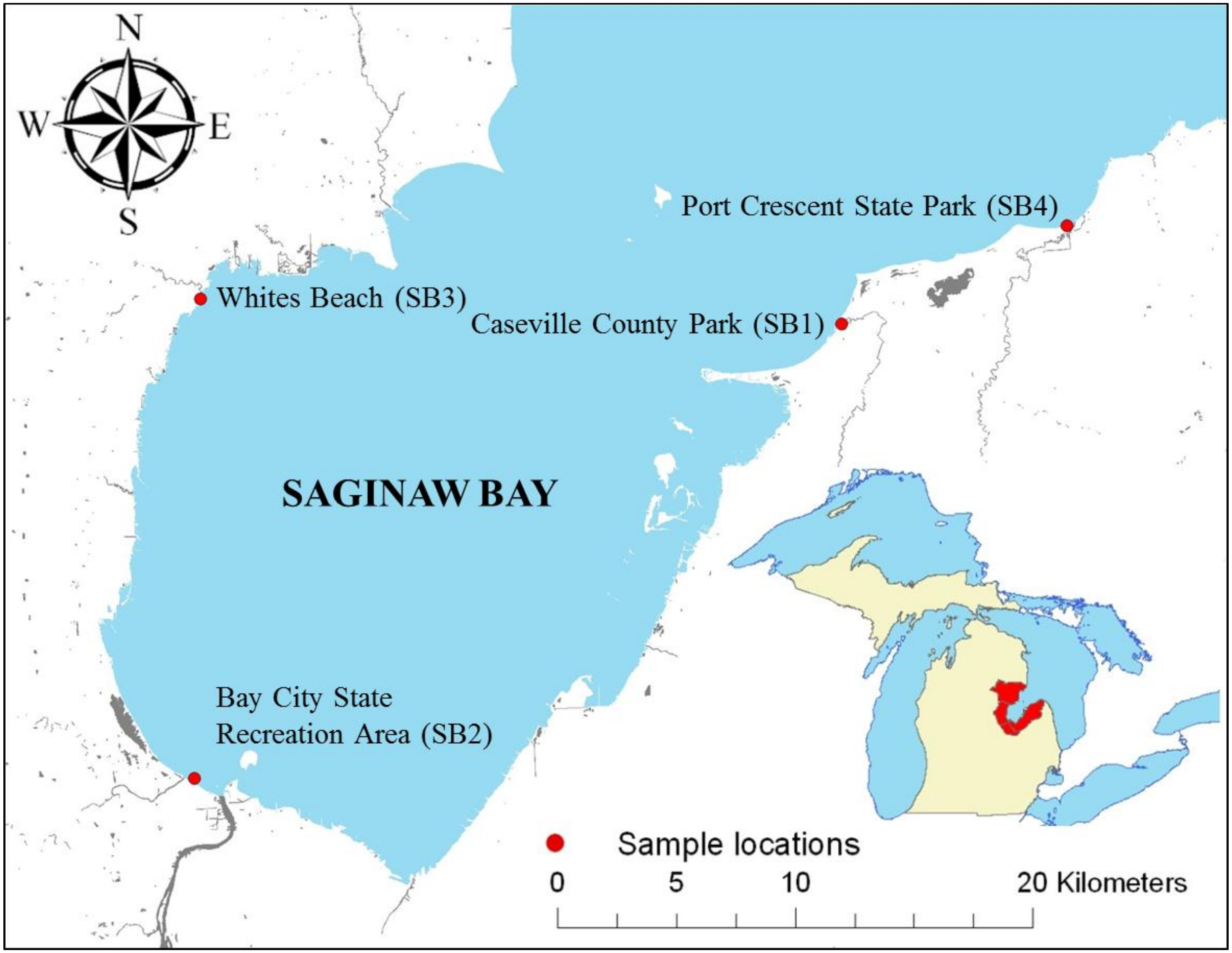


Figure 2.1. Location of beach sites in the Saginaw Bay selected for deep water, shallow water, sediment, and stranded algae mat investigation using fecal indicator bacteria and molecular source tracking. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

Algae mat accumulation on beaches is a growing concern for Great Lakes shorelines and the Saginaw Bay. In the Great Lakes, algae grow rapidly during the warm summer months (May - September). Following environmental forces, algae detaches from substrate to form free floating mats. Many studies have shown algae mats provide a suitable habitat for enteric bacteria such as *Escherichia coli* (*E. coli*), enterococci, *Shigella*, *Campylobacter, and Salmonella*, allowing waterborne pathogens to persist and potentially regrow (Verhougstraete et al. 2010; Byappanahalli et al. 2009; Ishii et al. 2006). As a result of wind and wave action, these microorganisms can detach and enter surrounding waters which influence water quality (Englebert et al. 2008b). In the last decade, algae mat occurrence in the Saginaw Bay has raised concern about possible pathogen occurrence at beaches. Knowledge of pathogen occurrence in algae mats or the impact on surrounding water quality and public health is not well understood (Verhougstraete et al. 2010). To date, there have been no published studies investigating algae mats for molecular source tracking markers to identify the origin of fecal pollution and potential pathogens.

In addition to algae, sediments are being increasingly implicated as a cause of water quality impairments at beaches. Whitman and Nevers (2003) reported nearshore sand had higher *E. coli* concentrations (4000 Colony Forming Units (CFU) 100 ml^{-1}) than the surrounding water (43 CFU 100 ml^{-1}). Alm et al. (2003) found *E. coli* was up to 38 times higher in beach sand than the water column and *E. coli* concentrations decreased with sediment depth. Garrido-Perez et al. (2008) found proximity to contamination source had a significant influence on sediment bacterial densities. Despite evidence linking beach water impacts and bacteria in sediment and algae mats, recreational water quality monitoring continues to focus on deep water.

It is often not possible to directly test for multiple pathogenic microorganisms during routine beach monitoring. A single fecal indicator is generally used as pathogen surrogates to characterize water quality and protect human health (Griffen et al. 2001), limiting the ability to fully characterize pollution and sources of fecal contamination. The Michigan Department of Environmental Quality has set recreational water quality criteria for fresh water at 300 *E. coli* 100 ml^{-1} as a single sample maximum or 130 *E. coli* 100 ml^{-1} as a geometric mean of five or more samples. The USEPA has also suggested enterococci as an indicator for evaluation of public health risks for recreational waters (Wade et al. 2006; Wade et al. 2010). *Clostridium perfringens* (*C. perfringens*) and coliphage viruses have also been used as fecal indicator organisms. *C. perfringens* form spores that do not regrow in the environment and are resistant to high temperatures and disinfection treatments (Payment and Franco 1993). Hawaii adopted a *C. perfringens* regulatory standard of 50 CFU 100 ml^{-1} (Mahin and Pancorbo 1999). Although no regulatory standards exist for coliphage, a virus that infects *E. coli*, they are used to indicate the presence of enteric pathogens in water (Allwood et al. 2003). Monitoring multiple fecal indicators has been shown to provide better recreational water protection (Kinzelman et al. 2003), but routine beach monitoring continues to largely rely on a single indicator.

Molecular source tracking is used to define the cause of fecal pollution. Scott et al. (2005) demonstrated *Enterococci faecium* surface protein (esp) gene was specific to human sewage and septic systems while absent in swine, poultry, and cattle feces using cultivatable enterococci. Bernhard and Field (2000) developed two *Bacteroides - Prevotella* ribosomal DNA markers specific to cow and human feces. The *Bacteroides* species has proven useful for molecular source tracking and in recent years improvements in specificity and analytical techniques allow for more definitive results (Yampara-Iquise et al. 2008). In addition to routine monitoring and source tracking, environmental surveys capture surrounding parameters not identified during microbial and molecular analysis (Field and Samadpour 2007).

Current standards focus only on water and fail to address the multiple other types of pollution threatening the nearshore including sediments and algae mats. Thus, there is a need to better characterize microbial occurrence in the nearshore zones as no microbial standards exist for such matrices. Using multiple emerging analyses across beach transects, this investigation aimed to: 1) determine the occurrence and relationship of fecal indicator bacteria in water, sediment, and algae at beaches in a mixed use watershed; 2) identify human and bovine sources of fecal contamination using microbial source tracking and; 3) identify the environmental parameters influencing water quality degradation.

2.2. Materials and Methods

2.2.1. Description of study area

Four beaches were chosen based on their proximity to rivers with large drainage basins, historically poor beach water quality, and summer popularity for swimming (Table 2.1. and Figure 2.1.). Furthermore, nearshore algae mass accumulation has been recorded at SB1 and SB2. Three equally spaced sites parallel to the shoreline were monitored at each beach. Beach transects samples (sediment, shallow water, deep water, and algae mats) were collected perpendicular to each of the three equally spaced sites.

Table 2.1. Site description, water quality exceedances, and potential pollution influences.

Site ID	Location	Site description (Latitude/longitude)	Water quality standard exceedances[A]	Suspect pollution source(s)
SB1	Caseville County Park beach	(Huron County) Adjacent to the Pigeon River which has been subject to fish kills from sediment and agricultural runoff (43.98964, -83.27540)	5	Sewage Agriculture Algae
SB2	Bay City State Recreation Area beach	(Bay County) North of the Saginaw River which receives significant inputs from CSOs and urban runoff (43.67407, -83.90903)	9	Sewage Algae
SB3	Whites Beach	(Arenac County) Surrounded by dense residential homes relying on septic systems for wastewater management (43.92861, -83.89051)	21	Septage
SB4	Port Crescent State Park-day use	(Huron County) Southwest of the Pinnebog River which receives inputs from multiple agricultural drains (44.00246, -83.06981)	3	Agriculture

A. Closures reported since the creation of Michigan Beach Guard database (circa 2001)

2.2.2. Sample collection and processing

Each beach was sampled eight times between June-September, 2008. Each event included collection of shallow water, waist deep water, sediment, and, when present, stranded algae mats. Using sterile one liter bottles, water column grab samples were collected at depths of 15-20 cm (shallow) and 100 cm (deep) above lake bottom. Sediment and algae samples were collected in the swash zone by inverting a Whirl-Pak®, grabbing a handful of material from three horizontal points along the beach, and then compositing all subsamples in one bag. All Samples were placed on ice (4 °C), stored in a cooler, transported to the Michigan State University Water

Quality, Environmental, and Molecular Microbiology Laboratory (East Lansing, Michigan, USA) and processed within 24 hours.

2.2.3. Water analysis

Microbial analysis of water and sediment included *E. coli*, enterococci, *Clostridium perfringens* (*C. perfringens*), and coliphage (CN-13 and F+amp). Undiluted water samples were filtered through 0.45 µm hydrophilic mixed cellulose esters filters (Pall Corporation 66278). *E. coli*, enterococci, and *C. perfringens* were analyzed using cultivation and selective media mTEC (USEPA 2005), mEI (USEPA 2002), and mCP (USEPA 1995; Bisson and Cabelli 1979), respectively. *E. coli*, enterococci, and *C. perfringens* were reported as colony forming units 100 ml^{-1}. Double agar layers were utilized to detect two coliphage strains following EPA methods 1601 (USEPA 2001). The two selected bacterial hosts were *E. coli* F^{+}amp and *E. coli* CN-13. Clearings in the host lawn were counted and reported as plaque forming units (PFU) 100 ml^{-1}. *Escherichia coli* C-3000 (ATCC 15597), *Enterococci faecium* (ATCC 35667), *Clostridium perfringens* (ATCC 3624), ΦX-174 coliphage were used as a positive controls for verification of media integrity. Sterile water was used as negative controls for verification of method integrity.

2.2.4. Sediment and algae analysis

Sediment and algae samples were diluted with sterile Phosphate Buffered Water (PBW) to a final weight/volume ratio of 10% and 1%, respectively, to obtain countable results. Algae mat samples underwent an initial pulse blending in a sterile blender until homogenized. Each sample and PBW solution was vigorously hand shaken (10 cm radius) for two minutes, allowed to settle for 2 minutes, and the eluent was decanted into a sterile bottle (Shibata et al. 2004; Boehm et al., 2009). An additional volume of PBW was added to the sediment or algae sample, swirled for 10 seconds, allowed to settle for 30 seconds, and the eluent was added to the sterile bottle from the first rinse. *E. coli*, Enterococci, *C. perfringens*, and coliphage were assayed from the diluted sediment or algae solution using the same methods described in the water analysis section and reported as colony or plaque forming units per gram wet weight of material.

2.2.5. Molecular analysis

Water, sediment, and algae samples were analyzed for enterococci surface protein (esp) gene and *Bacteroides* human and bovine specific markers using PCR. The marker, primer sequence, and product size for each assay are described in Table 2.2. The esp analysis was performed from the CFU membranes grown on mEI during enterococci cultivation (described above). For the Bacteroides markers, water and eluted sediment and algae were filtered and DNA was extracted directly from filters containing non-cultivated cells. For all molecular assays, each filter was placed into a 50 ml centrifuge tube containing 20 ml of sterile PBW, vortexed to recover the CFU and cells from the membrane, and then centrifuged (30 minutes; 4000 x *g*) to pellet the cells. Eighteen ml were decanted from the tube and the remaining eluent and pellet were stored at -80°C until DNA extraction.

Table 2.2. PCR assays examined during source tracking in water, sediment, and algae.

Marker name	Primer sequence	Product size (bp)	Reference
Enterococci surface protein	F 5′-TATGAAAGCAACAGCACAAGTT-3′ R 5′-ACGTCGAAAGTTCGATTTCC-3′	680	Scott et al. 2005
Human *Bacteroides*	F 5’-ATCATGAGTTCACATGTCCG-3’ R 5’-CAA TCG GAG TTC TTC GTG-3’	116	Bernhard and Field 2000
Bovine *Bacteroides*	F 5’-CCAACY TTCCCG WTACTC-3’ R 5’-CAATCGGAGTTCTTCGTG-3’	100	Bernhard and Field 2000

After thawing, approximately 50 µl of DNA was extracted from 200 µl of the pellet using QIAamp® DNA mini kit. *Bacteroides* were assayed using polymerase chain reaction (PCR) amplification according to Bernhard and Field (2000). Briefly, analysis were carried out using forward and references primers (0.4 µM), MgCl2 (3 mM), HotStarTaq Master Mix, and molecular grade water to make up a final volume of 22 µl. The bovine and human *Bacteroides* assays were processed using a Bio-Rad PCR thermocycler (iCycler) with a 15 min initial warming step (95°C), followed by 30 cycles of the amplification step (94°C for 30 s, 58°C for 30 s, and 72°C for 60 s), and a final extension step of 8 min (72°C).

Esp was assayed using PCR amplification according to Scott et al. (2005). Briefly, master mix is prepared with forward and reverse primers (3 µM), HotStarTaq Master Mix (Qiagen 203443), and molecular grade water to make up a final volume of 19 µl. Using a Bio-Rad PCR thermocycler (iCycler), esp analysis were carried out under the following conditions: 15min at 95°C, 35 cycles each consisting of 1min at 94°C, 1min at 58°C, and 1min at 72°C, followed by a final extension step for 5min at 72°C.

DNA from sewage, cow manure, and *E. faecium* EL1 templates were used as positive controls for human and bovine *Bacteroides* and esp, respectively. Molecular grade water was used as a negative control for each analysis. Gel electrophoresis for each assay was performed on the PCR product in duplicates, run on a 1.2% w/v agarose gel at 95 V for one hour. Samples with bands at 116 base pairs (bp), 100 bp, and 680 bp were recorded as positives for human *Bacteroides*, bovine *Bacteroides*, and esp, respectively.

2.2.6. Environmental and physical data

During sample collection, wave height was measured as the distance from the trough to the crest. Visual counts of birds on the beach or in the swimming area were also made during sample collection. Precipitation (24, 48, and 72 hour cumulative totals prior to the sampling date) and wind direction and speed at the date and time of sampling were collected from Enviro-weather (www.agweather.geo.msu.edu/mawn/) and NOAA National Weather Service (weather.gov) observation and forecast reports. Observational data for SB2 and SB3 were collected from Enviro-weather station LIN (43.7199, -84.0275) and data for SB1 and SB4 were collected from PIG (43.8992, -83.2667). Daily and hourly observations of total precipitation (mm), wind direction (degress, north = 0°), and wind speed (m s^{-1}) were available at these stations throughout the duration of the project.

2.2.7. Data analysis

Non-detects and concentrations below method detection limits for water and sediment/algae were calculated using equation 1 and 2, respectively, and recorded as the lowest detection limit.

1. $$Detection\ limit\ lower\ \left(\frac{organisms}{100\ ml}\right) = \frac{1}{Vt} * 100$$

2. $$Detection\ limit\ lower\ \left(\frac{organisms}{g\ wet\ weight}\right) = \frac{1}{Vt} * \frac{Vd}{M}$$

Where Vt is the total volume used for analysis, Vd is the dilution volume, and M is the mass of sediment or algae used for dilution and analysis. Results that exceeded the upper detection limit of applied methods were recorded as the upper method detection limit. Microorganism concentrations underwent log-transformation to fit the data to a normal distribution, but not one transformation satisfied all parameters. All water, sediment, and algae microbial results were evaluated for intra-site relatedness and associations with environmental parameters using independent samples-Kruskal-Wallis 1 way ANOVA and bivariate Spearman Rank (r) correlation coefficients. All tests were performed using SPSS Statistic 17.0 software with significance set at $\alpha = 0.05$.

2.3. Results

2.3.1. Fecal indicator organisms in water and sediment

Ninety-six samples were collected from three zones (deep and shallow water, sediment) at four Saginaw Bay beaches (9 stranded algae mat samples were collected from two Saginaw Bay beaches when present, see section 2.3.2). Each sample was assayed for the fecal indicators *E. coli*, enterococci, *C. perfringens*, and coliphage CN-13 and F+amp. At least one fecal indicator was detected during every sample event at each beach and in each zone. Figure 2.2. compares the geometric mean concentrations of each indicator for the three zones at each beach.

Each beach was ranked based on the geometric means, single sample maximum, and percent positive detections for each of the five fecal indicators in sediment, shallow water, and deep water. Based on this ranking scheme, SB1 was determined the most contaminated site. All sediment, deep, and shallow water samples collected at SB1 were positive for *E. coli*, enterococci, and *C. perfringens*. At SB1, the *E. coli*, enterococci, and *C. perfringens* geometric means were 0.41, 0.36, 0.75 $\log_{10}$ CFU 100 g^{-1} (sediment); 2.0, 1.7, and 0.75 $\log_{10}$ CFU 100 ml^{-1} (shallow water); and 0.72, 0.51, and 0.27 $\log_{10}$ CFU 100 ml^{-1} (deep water), respectively. The highest coliphage F+amp (2.56 $\log_{10}$ PFU 100 g^{-1}) and CN-13 (3.00 $\log_{10}$ PFU 100 g^{-1}) single sample measurements were recorded in the sediment at SB1. The SB1 geometric means for coliphage F+amp in the sediment was 0.34 $\log_{10}$ PFU 100 g^{-1} and 1.0 $\log_{10}$ PFU 100 ml^{-1} in shallow and deep water samples. The SB1 geometric means for coliphage CN-13 in the sediment was 0.37 $\log_{10}$ PFU 100 g^{-1} and 1.3 $\log_{10}$ PFU 100 ml^{-1} shallow and deep water samples.

The site ranking second most impacted was SB3. At SB3, the *E. coli*, enterococci, and *C. perfringens* geometric means were 0.86, 0.73, and 0.50 $\log_{10}$ CFU 100 g^{-1} (sediment); 1.64, 1.60, and 0.51 $\log_{10}$ CFU 100 ml^{-1} (shallow water); and 1.01, 0.57, and 0.13 $\log_{10}$ CFU 100 ml^{-1} (deep water), respectively. This beach had the highest percentage of samples positive for CN-13 (70.6%) as well as the highest single sample concentration of *C. perfringens* (2.82 $\log_{10}$ CFU 100 g^{-1}) measured in the sediment. The SB3 geometric means for coliphage F+amp in the sediment was 0.28 $\log_{10}$ PFU 100 g^{-1} and 1.0 $\log_{10}$ PFU 100 ml^{-1} in shallow and deep water, respectively. The SB3 geometric means for coliphage CN-13 in the sediment was 0.41 $\log_{10}$ PFU 100 g^{-1} and in the shallow and deep water samples 1.6 and 1.1 $\log_{10}$ PFU 100 ml^{-1}, respectively.

The site ranking third most impacted was SB2. At this site *E. coli*, enterococci, and *C. perfringens* geometric means were 0.87, 1.1, and 0.31 $\log_{10}$ CFU 100 g^{-1} (sediment); 2.2, 1.5, and 0.99 $\log_{10}$ CFU 100 ml^{-1} (shallow water); and 0.65, 0.35, and 0.38 $\log_{10}$ CFU 100 ml^{-1} (deep water), respectively. The SB2 coliphage F+amp geometric mean in the sediment was 0.31 $\log_{10}$ PFU 100 g^{-1} and in the shallow and deep water samples the means were 1.1 and 1.0 $\log_{10}$ PFU 100 ml^{-1}, respectively. The SB2 geometric means for coliphage CN-13 in the sediment, was 0.31 $\log_{10}$ PFU 100 g^{-1} and in the shallow and deep water samples 1.3 and 1.0 $\log_{10}$ PFU 100 ml^{-1}, respectively. In comparison to the other three beaches and their respective zones, SB2 had the highest geometric means for *E. coli* (sediment), enterococci (shallow water), and *C. perfringens* (shallow and deep water). Furthermore, the highest single sample measurements of *E. coli* and enterococci of the entire project (> 4.34 $\log_{10}$ CFU 100 g^{-1}) were recorded in the sediment at SB2.

The site ranking fourth and least impacted was SB4. At this site *E. coli*, enterococci, and *C. perfringens* geometric means were 0.09, 0.07, and 0.01 $\log_{10}$ CFU 100 g^{-1} (sediment); 1.06, 0.47, 0.32 $\log_{10}$ CFU 100 ml^{-1} (shallow water); and 0.99, 0.35, and 0.23 $\log_{10}$ CFU 100 ml^{-1} (deep water), respectively. The lowest *E. coli*, enterococci, *and C. perfringens* geometric means in shallow water and sediment were measured at SB4. However, this site had the highest percentage of samples positive for F+amp (35.3%) across all zones. The SB4 geometric means for coliphage F+amp in the sediment was 0.32 $\log_{10}$ PFU 100 g^{-1} and in the shallow and deep water samples 1.1 and 1.3 $\log_{10}$ PFU 100 ml^{-1}, respectively. The geometric means for coliphage CN-13 in the sediment was 0.42 $\log_{10}$ PFU 100 g^{-1} and in the shallow and deep water samples 1.4 and 1.3 $\log_{10}$ PFU 100 ml^{-1}, respectively.

In summary, SB1 was the most polluted site followed by SB3, SB2, and SB4 based on geometric means, total number of positive samples, and single sample maximum of all indicators. In comparing beaches, *E. coli*, enterococci, and *C. perfringens* concentrations were statistically different amongst all beaches in sediment and deep water ($p \leq 0.017$ and $p \leq 0.024$, respectively). However, enterococcus was the only indicator different across all beaches in the shallow water ($p \leq 0.026$). Although as a whole, coliphage means were different between zones, they were not statistically different between beaches ($p > 0.05$); likely due to the detection limits, small assay volumes (~10 ml), and the number of non-detects (72.7%).

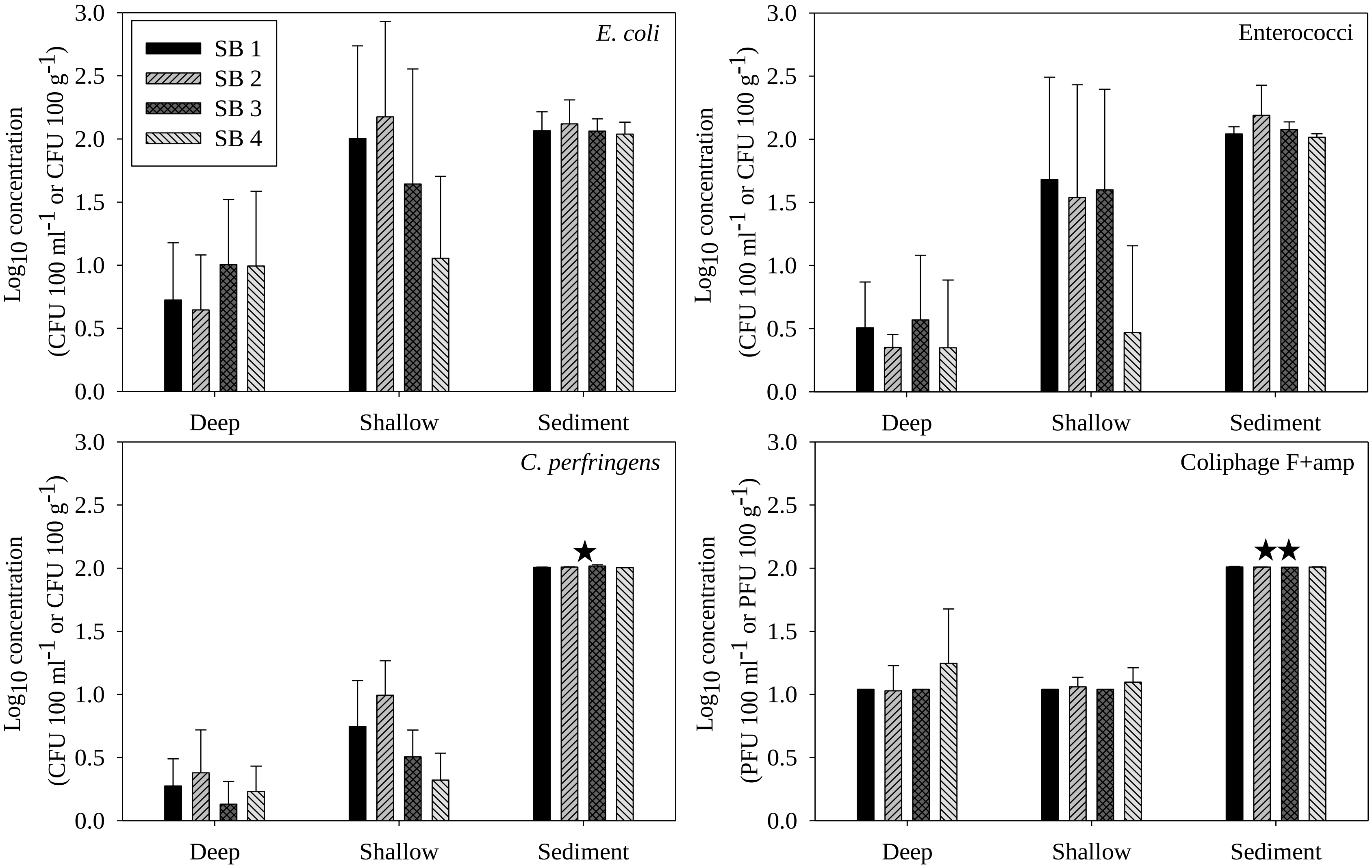
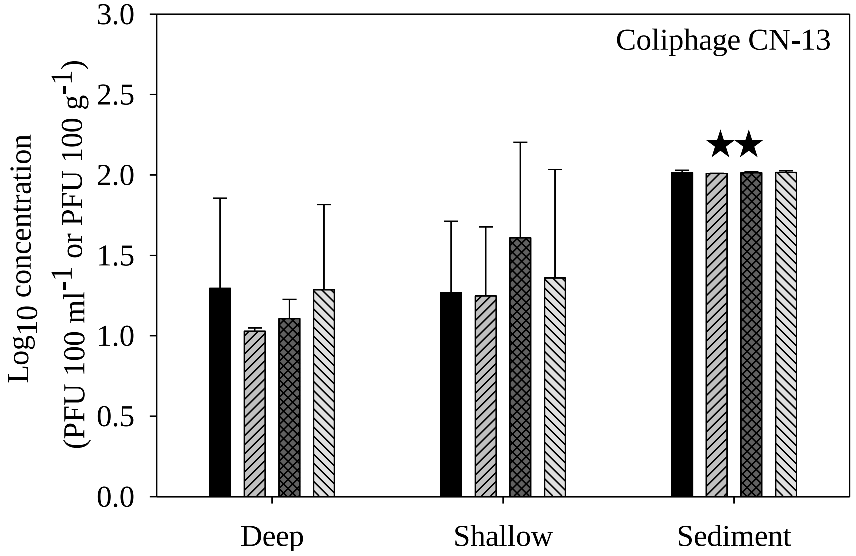


Figure 2.2. Geometric mean concentrations of fecal indicator organisms in shallow water (20 cm; n = 32), deep water (1 meter; n = 32), and sediment (n = 32) at SB1, SB2, SB3, and SB4. ★: Short error bars result of small *C. perfringens* standard deviations; ★★: No standard deviation due to high percentage of non-detects; Non-detects were recorded at the detection limit; Water reported as $\log_{10}$ CFU or PFU 100 ml^{-1}; Sediment reported as $\log_{10}$ CFU or PFU 100 g^{-1} wet weight.

Figure 2.2. (cont'd)



The first objective was to determine the occurrence and relationship of fecal indicator bacteria in water, sediment, and algae. *E. coli*, enterococci, and *C. perfringens* were detected in 99, 88, and 87 percent of all (i.e. water, sediment, and algae) tested samples, respectively. With respect to all water, sediment, and algae samples, coliphage F+amp and CN-13 were detected in 15 and 45 percent of samples, respectively. Not surprisingly, deep water had the lowest number of enterococci, *C. perfringens*, and coliphage CN-13. Kruskal-Wallis tests confirmed all microorganisms were statistically different across zones ($p \leq 0.005$). Based on *E. coli*, enterococci, and *C. perfringens* concentrations, the most contaminated zone was sediment followed by shallow water and finally deep water. However, *E. coli* concentration ranges in each zone showed shallow water quality was the most variable (ranged over 2.78 log$_{10}$ CFU 100 ml^{-1}), followed by deep water (ranged over 1.75 log$_{10}$ CFU 100 ml^{-1}), and sediment (ranged over 0.50 log$_{10}$ CFU 100 g^{-1}). This trend was also found with enterococci, *C. perfringens*, and coliphage CN-13. However, coliphage F+amp deviated from this trend and were most variable in deep water followed by sediment and then shallow water, but the large percentage of F+amp non-detects likely influenced such results.

Comparisons of microorganism concentrations across zones were undertaken to better characterize each zone and identify potential interactions. Sediment-shallow water pairwise comparisons indicated that 70% of sediment samples had higher *E. coli* levels compared to the shallow water. This percentage increased with the examination of enterococci (81%) and *C. perfringens* (90%). The high number of F+amp (100%), and CN-13 non-detects produced less meaningful results and were not considered statistically significant. Shallow-deep water pairwise comparisons found higher levels of *E. coli*, enterococci, and *C. perfringens* in shallow water during 90%, 67%, and 83% of samples, respectively. Using CN-13 and F+amp, shallow water was more contaminated than deep water in 50% and 8% of paired samples (n = 12), respectively. The low F+amp percentage occurred because of the significant number of non-detects in both zones (83% deep and 94% shallow). In comparison, coliphage CN-13 was not detected in the 38% and 75% of shallow and deep water samples, respectively. Cumulatively, these results indicate sediment acts as a sink for fecal indicator bacteria in the nearshore of Saginaw Bay which can occasionally be released to the shallow water with some eventually entering deep water.

Correlation analysis attempted to define associations of bacteria to each other across the beach transect. All the bacteria were positively correlated with each other in the sediments and shallow water but not in the deep water. The correlation coefficient between *E. coli* and enterococci in the sediment was r = 0.760 (p = 0.001) and in shallow water r = 0.585 (p = 0.001). Correlations between *E. coli* and *C. perfringens* in the sediment was r = 0.640 (p = 0.002) and r = 0.379 (p = 0.032) in the shallow water. Between enterococci and *C. perfringens* in the sediment r = 0.617 (p = 0.004) and r = 0.453 (p = 0.009) in the shallow water. The greatest correlations were found between the two gram negative bacteria (*E. coli* and enterococci) in sediments followed by shallow water.

F+amp and CN13 were positively correlated with each other in deep water (r = 0.704, p = 0.011), but showed no relationships to each other in shallow water, sediments, nor were they correlated to any other bacterial indicators.

2.3.2. Occurrence of fecal indicator organisms in algae and source tracking markers

Algae mats were present and collected at SB1 (n = 2) and SB2 (n = 7), but absent at sites SB3 and SB4. Higher levels of all indicator bacteria were found in algae from SB2 compared to SB1. All algae mat samples were reported in grams wet weight. At SB1, algae mats exhibited geometric means of *E. coli* , enterococci, *C. perfringens*, coliphage F+amp, and coliphage CN-13 of 1.23 CFU 100 g^{-1} wet weight, 2.11 CFU 100 g^{-1} wet weight, -0.91 CFU 100 g^{-1} wet weight, 0.07 PFU 100 g^{-1} wet weight, and 0.07 PFU 100 g^{-1} wet weight, respectively. At SB1, algae mat geometric mean concentrations of *E. coli*, enterococci, *C. perfringens*, colipohage F+amp, and coliphage CN-13 were 2.20 CFU 100 g^{-1} wet weight, 2.57 CFU 100 g^{-1} wet weight, 1.90 CFU 100 g^{-1} wet weight, 2.02 PFU 100 g^{-1} wet weight, and 1.97 PFU 100 g^{-1} wet weight, respectively. When algae mats were present, 85% of all microbial measurements (including coliphage) at that site were higher in the algae mat than the underlying sediment. Likewise, when algae mats were present, 55% of all microbial measurements at that site were higher in the algae mats than shallow water; demonstrating a shift in microbe concentration trends compared to sites and dates when algae were not present during sample collection. *E. coli*, enterococci, and *C. perfringens* were detected in every algae mat sample (Table 2.3.). No statistical correlations were identified between fecal indicators in algae mats as statistical analysis was likely limited by sample size.

Table 2.3. Range and geometric mean concentrations of fecal indicator organisms in algae samples at SB1 (n =2) and SB2 (n = 7).

Site	Zone		*E. coli* (log$_{10}$) CFU 100 g^{-1} or 100 ml^{-1}	Enterococci (log$_{10}$) CFU 100 g^{-1} or 100 ml^{-1}	*C. perfringens* (log$_{10}$) CFU 100 g^{-1} or 100 ml^{-1}	Coliphage F+amp (log$_{10}$) PFU 100 g^{-1} or 100 ml^{-1}	Coliphage CN-13 (log$_{10}$) PFU 100 g^{-1} or 100 ml^{-1}	Esp (+/-)
SB1	Algae	Range	0.71-1.85	1.98-2.25	-	-	-	1/1*
		Mean**	1.15	2.11	0.05	0.33	0.33	
		% positive	100	100	100	0	0	
	Sediment	Mean**	0.22	0.40	0.16	0.28	0.28	
	Shallow water	Mean**	1.46	0.83	0.75	1.04	1.04	
	Deep water***	Mean**	0.59					
SB2	Algae	Range	1.08-2.92	0.97-3.38	0.73-4.04	1.04-3.52	1.04-3.52	1/6
		Mean**	2.17	2.52	1.62	1.89	1.84	
		% positive	100	100	100	60	60	
	Sediment	Mean**	0.96	1.23	0.31	0.31	0.31	
	Shallow water	Mean**	2.16	1.80	1.09	1.07	1.25	
	Deep water	Mean**	0.78	0.33	0.68	1.04	1.04	

Sediment and algae reported in wet weight; coliphage detection limit: 90 PFU 100 g^{-1} wet weight; *ESP also detected in one sediment sample at SB1 (9-30-2008). **Geometric mean. ***Deep water samples were collected by the local health department on the two days when algae mats were present and processed for *E. coli*. Sediment, shallow water, and deep water geometric mean concentrations when algae mats were present are shown for comparison purposes.

Another goal was to identify the source(s) of pollution. Thus, human and bovine *Bacteroides* and enterococci surface protein gene markers were employed. Twenty-seven samples were tested for the *Bacteroides* marker and 48 samples were assayed for the esp gene. The esp gene was identified in three samples, all non-water samples (i.e. algae and sediment) at SB1 and SB2. Human and bovine *Bacteroides* were not identified in any of the samples collected in 2008. However, bovine and human feces were identified in water and algae mats sampled from Saginaw Bay beaches in 2007 using the same markers and methods (Singh and Rose 2007).

2.3.3. Fecal indicator associations with environmental parameters

As previously described, non-parametric correlation analysis were used to determine significant relationships between microbial concentrations and environmental conditions (objective 3). The most recurrent variables related to indicator concentrations were wave height and wind speed/direction. Precipitation (24 and 72 hour totals) was the next most recurring variable. Occasionally, bird populations (at the beach or in the water) and temperature (water, mean daily air temperature) were significant parameters associated with fecal indicator densities. The top two most influential parameters at each beach and zone are presented in Table 2.4.

Table 2.4. Significant correlations identified between fecal indicator organisms and environmental parameters.

Site	Zone	Fecal indicator	Parameters	r	p	Fecal indicator	Parameters	r	p
SB 1	Shallow	Entero.	Precip.[B]	0.762	0.028	*C. per.*	Birds	0.732	0.039
	Sediment	*E. coli*	Precip.[A]	0.862	0.006	*E. coli*	Wave height	0.764	0.027
SB 2	Deep	Entero.	Wind speed	0.798	0.018	*C. per.*	Wind speed	0.833	0.010
	Shallow	*E. coli*	Wave height	0.845	0.033	Entero.	Wind direction	0.831	0.011
	Sediment	*E. coli*	Precip.[A]	0.849	0.008	F+amp/CN13	Precip.[B]	0.898	0.002
SB 3	Shallow	CN-13	Temp.[C]	0.810	0.015				
	Sediment	*C. per.*	Precip.[A]	0.768	0.026	*E. coli*	Wind speed	-0.708	0.050
SB 4	Deep	*E. coli*	Wind speed	-0.812	0.050				
	Shallow	Entero.	Wave height	-0.864	0.006	*E. coli*	Wave height	-0.832	0.010
	Sediment	CN-13	Precip.[A]	0.788	0.020	CN13	Wind speed	-0.791	0.019

A. 24 hour precipitation; **B**. 72 hour precipitation; **C**. Mean daily air temperature; **D**. Water temperature at time of sampling. **SB1**: Caseville County Park; **SB2:** Bay City State Recreation Area; **SB**3: Whites Beach; **SB4**: Port Crescent State Park. r: bivariate Spearman Rank correlation coefficient and p values ≤ 0.05. No significant associations were found in deep water samples at SB1 and SB3.

Microbial concentrations were influenced by wind or waves in at least one zone of every beach. However, the association strength between these parameters and microbes varied throughout the Saginaw Bay depending on the beach, which will be described in more detail below. Wave height ranged from 0.0 to 0.46 m with an average of 0.14 m. The highest wave heights were all recorded at SB4 on July 22 and September 30, 2008. When wave height was above average (> 0.14m), deep water enterococci concentrations were statistically lower (mean = 0.38 $\log_{10}$ CFU 100 ml^{-1}) than when wave height was below average ($\overline{X}$ = 0.88 $\log_{10}$ CFU 100 ml^{-1}). Wind speed ranged from 0.0 to 14.5 kmh with an average of 3.89 kmh out of the south-southwest (212.6°). The highest wind speed measurements were recorded at SB2 on July 15 and September 30, 2008. The lowest wind speeds (0.0 kmh) were recorded at SB1 and SB3 on 7 different dates.

At the start of the project a wet weather threshold was set at 6.4 mm of cumulative rainfall in the 48 hours prior to sample collection, per local health department recommendations. Wet weather events above this thresholds accounted for 40% of all sampling dates. The greatest variation between wet and dry weather concentrations were seen in the shallow water of SB1 (range 2.82 $\log_{10}$ Enterococci 100 ml^{-1}). During wet weather monitoring, deep water enterococci concentrations averaged 0.31 $\log_{10}$ CFU 100 ml^{-1} and were statistically higher ($p = 0.015$) than enterococci measured during dry weather in the same zone (mean of 0.05 $\log_{10}$ CFU 100 ml^{-1}). Interestingly, twenty-four hour precipitation totals were related to an increase in one or more fecal indicators in the sediment at all sites. Specifically, coliphage F+amp and CN-13 concentrations were statistically different between wet and dry conditions in the sediment (p <

0.001). Outside of enterococci in deep water and both coliphage in the sediment, no other indicators were statistically different between wet and dry conditions ($p \geq 0.102$) in any zone.

Sites SB1 and SB2 showed similar responses to environmental parameters. At SB1, precipitation (72 hour) influenced microbe concentrations in the shallow water, while precipitation (24 hour) and wave height were the most influential parameters on sediment microbes. At SB2, wind speed was the primary influence on enterococci and *C. perfringens* in deep water. In the shallow water, wave height, precipitation (24 hour), and wind direction were directly correlated with *E. coli*. In algae mats, *E. coli* concentrations were indirectly correlated with wind direction ($r = -0.937$, $p = 0.002$). At site SB3, shallow water coliphage concentrations were positively influenced by daily mean air temperature. *C. perfringens* and coliphage CN-13 concentrations in sediment at SB3 and SB4 were directly influenced by precipitation (24 hour).

SB4 showed very difference responses to the environmental parameters associated with wind and the *E. coli* and enterococci (as well as coliphage in sediments) concentrations were inversely influenced by wind speed and wave height. At SB4, deep water *E. coli* concentrations were inversely associated with wind speed and shallow water enterococci and *E. coli* were inversely associated with wave height.

2.4. Discussion

This study aimed to determine the occurrence of fecal indicators and define their relationships between various areas across the beach. As expected, and previously shown (Whitman et al.

2003; Ishii et al. 2007), *E. coli*, enterococci, *C. perfringens*, and coliphage (CN-13 and F+amp) were highest in algae mats and sediment. In this study, *E. coli*, enterococci, *C. perfringens*, and coliphage levels were routinely 1 log greater in sediments than shallow water, also shown by Whitman and Nevers (2003), Alm et al. (2006), and Ishii et al. (2007) regardless of the presence of algal mats. The results from the current study supported previous studies attributing elevated bacteria in shallow waters to sediment and algae mats occurrence (Whitman and Nevers 2003; Boehm 2007; Engelbert et al. 2008b; Whitman et al. 2011) and found bacteria diminished with increasing water depth (Whitman and Nevers 2004). Measured levels of *C. perfringens* indicate chronic pollution in algae and sediment zones since *C. perfringens* persists and do not readily regrow in water environments (Fujioka and Shizumura 1985; Davies et al. 1995; Desmarais et al. 2002). On the other hand, coliphage was detected in only about 23% of all sediment, shallow water, and deep water samples, indicating sporadic fecal contamination at selected beaches.

Although *E. coli*, enterococci, and *C. perfringens* were consistently present in algae mats and sediments, recent fecal contamination was not always suspected. Previous findings demonstrate bacterial regrowth, accumulation, and persistence in algae mats absent of fresh fecal material (Byappanahalli et al. 2003; Ishii et al. 2006a; Englebert et al. 2008a; Byappanahalli et al. 2009). Detection of the esp gene in algae mats (SB2) when good water quality was expected (i.e. no recorded rainfall in previous 5 days, above average air temperatures, and generally calm wind conditions) supports the concept that bacteria are growing in the Saginaw Bay.

Fecal indicator bacteria in these media create a conundrum for beach managers and researchers attempting to identify and remediate pollution sources. For instance, Whitman and Nevers (2003) removed and replaced sand at one beach only to find that *E. coli* had recolonized within two weeks. Eliminating bacteria in sediments or algae mats is not feasible or practical at any natural beach. However, using best management practices including moderate beach grooming, and removing stranded algae mat can improve water quality. Based on identified drivers it is recommended that beach grooming measures be undertaken in the Saginaw Bay during minimal wind and wave action, in absence of recent or near future precipitation, and in the morning to allow for sunlight inactivation of bacteria prior to peak bather loads.

Overall, microorganism correlation coefficients indicated microbial levels in sediments were influencing shallow water microorganism concentrations. However, most of the previous studies focused on *E. coli* and enterococci which continuously fail to represent the true presence of fecal contamination or pathogens as shown by Yamahara et al. (2012) who identified weak associations between fecal indicator organisms (*E. coli*, enterococci, and F+amp coliphage) and pathogen (*Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus*, and methicillin-resistant *S. aureus*) presence in sand from 53 California beaches. In the current study two types of coliphage (somatic and F+) were used because they have been suggested as surrogates for human enteric viruses (Wiedenmann et al. 2006; Krometis et al. 2010) and have exhibited strong associations with pathogens (Borrego et al. 1987), noroviruses (Allwood et al. 2003), and illness outcomes following exposure in water (Colford et al. 2007). Krometis et al. (2010) illustrated that somatic coliphage were less associated with particles and were more likely to remain in suspension for longer periods of time compared to other measured microorganisms including F+ coliphage. This may be the reason coliphage were not found readily in the sediments of Saginaw Bay.

Collectively, these results indicate sediment and algae mats acted as non-point sources of fecal indicator bacteria and influence shallow water in the Great Lakes. However, coupling detected bacteria results (*E. coli,* enterococci, and *C. perfringens*) with the overwhelming number of coliphage non-detects, and the weak correlations between indicators and pathogens in sand (Yamahara et al. 2012), does not explicitly imply that pathogens were consistently present throughout the Saginaw Bay. Therefore, we recommend future pathogen testing be included in parallel with beach monitoring across the beachscape.

No beach exceeded 300 *E. coli* 100 ml^{-1}, Michigan's single sample maximum standard for recreational water, in deep water. Additionally, there were no samples collected from deep water that exceeded 61 enterococci 100 ml^{-1}, EPA's single sample maximum criterion for recreational water. However, in shallow water, seven samples exceeded *E. coli* standard and 12 samples exceeded enterococci criteria. In total, 27% and 40% of shallow water samples exceeded *E. coli* standard and enterococci criteria, respectively. Seventy-one percent of samples exceeding the *E. coli* standard were associated with at least one measurement of wave height, wind speed, and precipitation (24 hour) above average. Eighty-three percent of samples exceeding the enterococci criteria were associated with at least one measurement of wave height, wind speed, and precipitation (24 hour) above average. Interestingly, six (86%) *E. coli* exceedances were associated with offshore wind direction relative to each beach with the one exception occurring at SB1 during above average wind speed (5.6 kmh) directed onshore. *E. coli* and enterococci concentrations agreed with regulatory outcomes during 76% of samples with respect to their individual criterion (i.e. both indicators either meet or exceeded thresholds). An additional five exceedances would have occurred if monitoring included only enterococci in respect to monitoring only for *E. coli*. Coliphage F+amp were not detected in the same shallow water samples collected during the *E. coli* and enterococci exceedances. However, coliphage CN-13 measurements were above the coliphage CN-13 geometric mean during four *E. coli* and six enterococci exceedances. There were no shallow or deep water quality exceedances based on the Hawaii surface water standard for *C. perfringens* (50 CFU 100 ml^{-1}). In summary, all exceedances for *E. coli* and enterococci were reported in the shallow water, currently an unmanaged source of contamination which evades current recreational water regulations. In order to adequately protect bathers, samples should be collected in the shallow water (15-20 cm).

Molecular source tracking methods employed at each beach failed to routinely identify the source of fecal contamination. The enterococci surface protein (esp) marker was detected in sediments and algae, suggestive of human fecal material present in a small percentage of samples (6%). However, it is not clear if this was due to *Enterococci* spp. regrowth, as described above, or the addition of recent fecal contamination. This method detects the esp gene present in cultivated enterococci which are absent in chlorinated wastewater and in water with generally less than 100 enterococci CFU per 100 ml (Masago et al. 2011). Therefore, the esp marker may be better suited for point source dominated watersheds where examinations focus on disinfected versus non-disinfected wastewaters.

The fact that bovine and human specific markers were regularly absent throughout this project indicates either poor method approaches or other significant contributing sources in the Saginaw Bay. Identified limitations of molecular methods include: reduced assay volumes during filtration from excess suspended solids which were exacerbated by blending algae samples and likely increased inhibiting substances (Girones et al. 2010; Toze 1999); method results used in this study were presence/absence and gave no quantitative measurements (Villari et al. 1998); and methods likely produced a high number of false negatives (Toze 1999; Yang and Rothman 2004). Previous sample collections at the same beaches were successful at detecting human and bovine sources of contamination in water and algae mats. However, results were not duplicated in the current study using similar processing methods. This may suggest an intermediate presence of bovine feces in the Saginaw Bay. Wildlife, domesticated pets, cattle, and endemic waterfowl populations have been recorded in the Saginaw Bay watershed (Johnson et al. 1997; Singh and Rose 2007; Kraus et al. 2009), suggesting other animals are significant contributors to fecal pollution in the Bay and signaling the need for additional source markers. Fecal source tracking is further complicated by constantly changing source inputs, hydrology, environmental influences, and algae mat source/occurrence (Fishman et al. 2009). Given the large amount of variability in the Saginaw Bay dynamics and source tracking techniques (Girones et al. 2010), detecting a single source of contamination illustrates the need for more frequent monitoring using improved source tracking methods (i.e. qPCR) and markers including *B. thetaiotaomicron* (Yampara-Iquise et al. 2008) and Norovirus (Wolf et al. 2010).

The final objective of this project focused on identifying environmental factors associated with water quality in the Saginaw Bay. The predominant mechanisms driving microorganism concentrations and water quality degradation in the Saginaw Bay were associated with wind and waves. These dependent variables represent a source of energy often associated with impaired beaches (Frick et al. 2008). In the shallow water of Saginaw Bay, surface water currents respond rapidly to wind (within minutes) and persist for approximately eight hours before returning to normal current patterns dominated by water entering and leaving the bay (Danek and Saylor 1977). During the current study, average wind direction at each beach followed similar patterns described by Danek and Saylor (1977) with average wind directed offshore at SB3, parallel to shore at SB1 and SB2, and onshore at SB4. Although wind and waves were influential at all beaches, they were most visible at SB2 between algae mats and shallow water since SB2 routinely had the highest wind speeds ($\bar{X}$ = 7.4 KPH) and stranded algae mats were consistently present in the shallow water. Additionally, *E. coli* and coliphage CN-13 levels in the sediment at SB3 and SB4 were inversely related to wind speed and wave height, respectively, indicating bacteria settle out of the water column during low energy conditions. This cyclical process of deposition, accumulation, and resuspension explains the abundance of fecal indicator bacteria in shallow waters even in absence of fresh fecal inputs. Due to the inconsistent presence of algae mats at SB1 and the continuous presence at SB2, it was difficult to determine what role wind and wave action have on algae presence or associated bacteria concentrations. Wind induced surface water currents drive the movement of pollution throughout the waterbody until the polluted water becomes detected in the nearshore zone, but the specific current dynamics (i.e. parallel perpendicular to the shore) could not be identified under the current project design. Additional analysis focused on measuring microbial concentrations and nearshore currents at hourly intervals (or potentially shorter) is required to define such movement in the nearshore.

Precipitation was another recurrent influential environmental factor throughout the Saginaw Bay. Overall monthly precipitation totals in the Saginaw Bay during the project (July – 81 mm; August – 69 mm; September – 105 mm) were similar to long-term (1899 and 2011) monthly averages (July – 76 mm; August – 91 mm; September – 97 mm) (retrieved on July 2, 2012 from weather.com). However, 24 and 72 hour precipitation totals prior to sample collection averaged just 2.5 mm and 12.4 mm, respectively, indicating a significant portion of rainfall events were not captured during this project. Precipitation transports pollution from the land to surfaces waters which eventually enters the Bay primarily via rivers. Increased fecal indicator levels in water were seen 24-72 hour after rainstorms, indicating transport mechanisms take 1-3 days to transfer pollution from land to beach. Investigations aimed at rainfall intensity may provide further insight into precipitation effect on microbial water quality, especially important under climate change predictions for the Great Lakes which include increases in precipitation intensity and dry periods between rainstorm events (Mortsch et al. 2003). If Saginaw Bay beach managers continue using *E. coli* cultivation methods, these results suggest a sample collection shift of at least one day following rain storms and illustrate the need for predictive beach water quality models that incorporate wind and precipitation (Ge et al. 2012) to improve bather protection.

Addressing beach orientation and specific land use impacts on water quality is beyond the scope of this manuscript, but it is important to mention their potential for influencing water quality. Sites SB1 and SB2 are located near the Pigeon and Saginaw Rivers mouths (respectively), each has a large urban composition in their lower reaches. Interestingly, these beaches were the only sites closed during the project as a result of elevated *E. coli* levels (per local government monitoring) and the only locations where human markers were detected. At SB4, relationships between fecal indicators and wind/wave associates were inversely related, contrary to the other sites. This beach, situated near the Saginaw Bay/Lake Huron boundary, has virtually no protective coastal barriers (e.g. piers or peninsulas). It is suspected that the exposed characteristics of this beach increases wind fetch and allow lake currents to continuously dilute and circulate water, resulting in lower fecal indicator bacteria concentrations in water. Together these results demonstrate beaches situated near rivers and in areas with low circulation/renewal exhibit elevated fecal indicator organisms. It is evident that surrounding land use, including upstream areas drained by nearby rivers, and beach orientation influence water quality. Future Saginaw Bay investigations should focus on these characteristics during water quality monitoring by coupling microbial surveys at more spatial transects on the beach and upstream river sites with GIS based land use composition and empirical based current models.

Microbial indicators, previously linked to human health, were described across multiple beach zones and partially identified as human specific using the esp gene. This project demonstrated the potential for sediment and algae mats to act as non-point sources of pollution in the nearshore zone. Higher concentrations of traditionally monitored indicators were found in shallow waters than in deep water in large part due to sediment bound bacteria levels and potentially regrowth of the bacteria. Despite such evidence, governing bodies and beach managers continue to focus beach monitoring efforts in deep water. The following suggestions are based on findings presented in this manuscript:

1. The USEPA should: reevaluate recreational criteria with a nearshore health focus aimed at defining the potential for this zone to influence the traditionally monitored deep water zone;
2. State governments should: investigate shallow water and sediment at a regional scale, taking into consideration wind, waves, precipitation, and temperature as primary drivers;
3. Local health officials should: utilize newer molecular source tracking methods during routine beach monitoring to pinpoint pollution sources and focus remediation efforts for long-term

water quality improvements; and adjust routine beach monitoring to coincide with conditions known to produce the greatest bather risk such as precipitation, wind and wave action;

4. Scientists should: continue to evaluate coliphage as a potential indicator of pathogen presence at Great Lakes beaches; develop and improve source tracking marker techniques to a greater suite of sources; and measure microbe concentrations at incremental distances from known pollution sources (e.g. wastewater treatment outfall) down river and to nearby beaches.

2.5. Conclusions

After assessing multiple fecal indicators, molecular source tracking markers, and environmental surveys across four beachscapes, this study was able to conclude that: 1) stranded algae mats and sediment harbor the highest levels of fecal indicator organisms and can act as localized non-point sources of bacteria; 2) human sewage is partially contributing to the fecal contamination in the Saginaw Bay; and 3) Saginaw Bay water quality is significantly impacted by wind, waves, and precipitation.

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CHAPTER 3.

LINKING LAND-USE AND MICROBES IN A SMALL DIVERSE WATERSHED USING INDICATOR BACTERIA AND MOLECULAR SOURCE TRACKING

3.1. Introduction

Small stream systems and their associated discharges and catchment areas, are often overlooked as significant sources of pollution to larger receiving bodies. However, previous studies have shown small systems to be significant sources of microbes, nutrients, and sediments (Kistemann et al. 2012**;** Edwards et al. 2012**;** Wilkes et al. 2011; Nadal-Romero et al. 2008). Bacteria that enter small systems attached to particles settle into underlying sediment when stream velocity is low (Bai and Lung 2005). As stream velocities increase, bacteria are resuspended and transported downstream and eventually enter larger receiving bodies (Rehmann and Soupir 2009; Muirhead et al. 2004), in essence acting as nonpoint sources of bacterial pollution.

Understanding microbial water quality of small systems is complicated by anthropogenic pressure in watersheds. It was first reported in the 1970's that watershed impervious surface coverage exceeding 10% resulted in a rapid decline of water quality and biodiversity (Klein 1979). Nearly forty years later, connections between land use and water quality primarily focus on chemicals and nutrients as the metric of water quality (Wang and Yin 1997; Mehaffey et al. 2005; Broussard and Turner 2009; Akasaka et al. 2010), but linking land use with microorganisms has proven particularly difficult. Hunter et al. (1999) and Boyer and Pasquarell (1999) linked fecal and total coliforms in water to agriculture land use. Desai and Rifai (2010) found noticeably higher concentrations of *E. coli* in urban dominated sites compared to grassland sites. For instance, Kang et al. (2010) used constrained least squares models and a comparative statistics to link *E. coli* and enterococci concentrations with urban and industrial land uses and added that *E. coli* and enterococci concentrations attributed to land use decreased as the size of the watershed increased. Additionally, Mehaffey et al. (2005) used regression analysis to directly correlate fecal coliform bacteria to urban and agriculture land use, but noted that land use position relative to the waterbody was more important than percent land use in the entire watershed. These studies implicate the potential for all land use types to contribute bacteria to water. Variable findings of microbe-land use relationships can be partially explained by the spatial scale at which investigations occur but also show the difficulty of separating multiple sources acting simultaneously in a watershed with general bacteria. Accounting for appropriate land use scale during microbial water quality assessment is vital, especially in watersheds with mixed land use patterns.

Land use and sediment pollution require transport mechanisms to influence water quality. Pollution is primarily transported from the landscape and sediment to waterbodies via weather and environmental forces. Specific driving forces are highly variable between watersheds and depend on landscape characteristics (Lavee and Poesen 1991), vegetative cover (Loch 2000), and precipitation rates and land roughness (Katz et al. 1995). The primary transport mechanism for landscape pollution is precipitation and runoff, defined by surface characteristics, landscape slope, and soil hydraulic conductivity. Previous studies have reported fecal indicator bacteria typically exhibited proportionally greater concentrations during large storms (based on rainfall intensity, total volume, and/or discharge rates) compared to baseflow conditions (Cho et al. 2010; Traister and Anisfeld 2006; Schilling et al. 2009). Likewise, the majority of *E. coli* movement to downstream waters occurred during storm events and was attributed to resuspension of sediment-bound bacteria (McKergow and Davies-Colley 2010). More specifically, Stumpf et al. (2010) found *E. coli* loads were 30 times greater during storm events than during baseflow conditions with statistically different concentrations between each condition. Wilkes et al. (2011) detected multiple pathogens on dates when total rainfall of the previous week exceeded the 62nd percentile (~27 mm in the previous 7 days) and also showed a positive correlation between pathogen (*Cryptosporidium* and *Giardia*) densities and surface water discharge.

Eutrophication, beach closures, algae blooms, and sediment loading were common water quality responses to landscape-associated pollution (Stoermer et al. 1978; MacGregor et al. 2001; U'Ren 2005; Rediske 2010). Traditional statistical approaches for modeling ecosystem responses have failed to define explicit links between pollution and source, scale, and driving force of microbial water quality. An emerging tool called Classification and Regression Tress (CART) has proven useful for environmental exploration in complex systems (De'ath and Fabricius 2000). CART models use multiple explanatory variables to correlate the variation of one response variable in relationship to multiple parameters by repeatedly splitting the data into two groups based on defined splitting criteria (Breiman et al. 1984). CART has been used to describe the source of fecal pollution in water via chemical indicators (Gregor et al. 2002), to link *E. coli* O157:H7 with relatively high pasture density (Wilkes et al. 2011), and to define significant physical-chemical variables (including dissolved oxygen and turbidity) influencing fecal and total coliforms in beach water (Bae et al. 2010). To date, no studies have reported on CART's ability to link fecal indicator bacteria, molecular source tracking markers, environmental conditions, climate variables, and land use in small complex systems.

This project aims to determine the dynamics of water quality change and identify factors influencing microorganism transportation throughout the watershed. Water quality was assessed using fecal indicator bacteria and molecular source tracking markers in a diverse watershed (Mitchell Creek) draining to Lake Michigan. The objectives were to 1) examine the spatial and temporal distribution of traditional and alternative fecal indicators in a watershed influenced by non-point source pollution, 2) use a quantitative PCR marker to measure human sources associated with fecal bacteria, and 3) assess land use pattern effects on bacterial water quality.

3.2. Materials and Methods

3.2.1. Sampling strategy

The Grand Traverse Bay, located in northwest Lower Michigan, is currently facing water quality concerns following beach closures and pollutant loading from recreational, urban, industrial, and agricultural stormwater runoff (U'Ren 2005). Water quality degradation concerns were raised following a 2008 local health department survey of the Mitchell Creek that detected multiple sites with elevated *E. coli* levels. The Mitchell Creek accounts for 1.6% of the Grand Traverse Bay watershed area, but is considered a major source of polluted stormwater input to the Bay (U'Ren 2005). The Creek discharges into the southern end of East-Grand Traverse Bay, 300 m west of Traverse City State Park's designated swimming area. The Traverse City State Park (TCSP) beach is heavily utilized for recreation during summer months. This beach has exceeded Michigan water quality standards fifteen times since 2001, the majority of which were attributed to stormwater runoff (MDEQ 2012; U'Ren 2005).

Water samples were collected from eight Mitchell Creek sites and one Grand Traverse Bay beach between: June and November (2009); June and August (2010); November (2010); and

March (2011) (Table 3.1. and Figure 3.1.). Additionally, sediment samples were collected between June and August (2010) and March (2011) at Mitchell Creek sites 2, 3, 4, 5, 6, 8, and TCSP. No sediment samples were collected from MC1 (rocks composed the entire creek bottom) or MC7 (sampling locations was at a metal culvert). Beach samples were collected in ankle deep water (15 cm) in triplicates (left, center, and right) from the designated swim area. Creek samples were collected 2 m from shore using an extendable arm. Sediment samples were collected from the top 3 cm of benthos immediately after water sample collection. All samples were collected using aseptic techniques and sterile Nalogene bottles, placed on ice (4°C), and brought to Michigan State University's Water Quality, Environmental, and Molecular Microbiology Laboratory. Samples were kept at 4°C and processed for fecal indicator organisms within 12 hours of collection.

An intensive ten day study began on August 7, 2010 at sites MC2, MC3, MC4, MC5, MC6, MC8, and TCSP. Water and sediment samples were collected from each site hourly for 12 hours on day one followed by one sample from each site at hours 24, 72, 120, 168, and 240 (n = 17 per site). Each water sample was assayed for *E. coli*, enterococci, and *Bacteroides thetaiotaomicron* α-1-6 mannanase (*B. theta*), while sediment analysis included *E. coli* and enterococci.

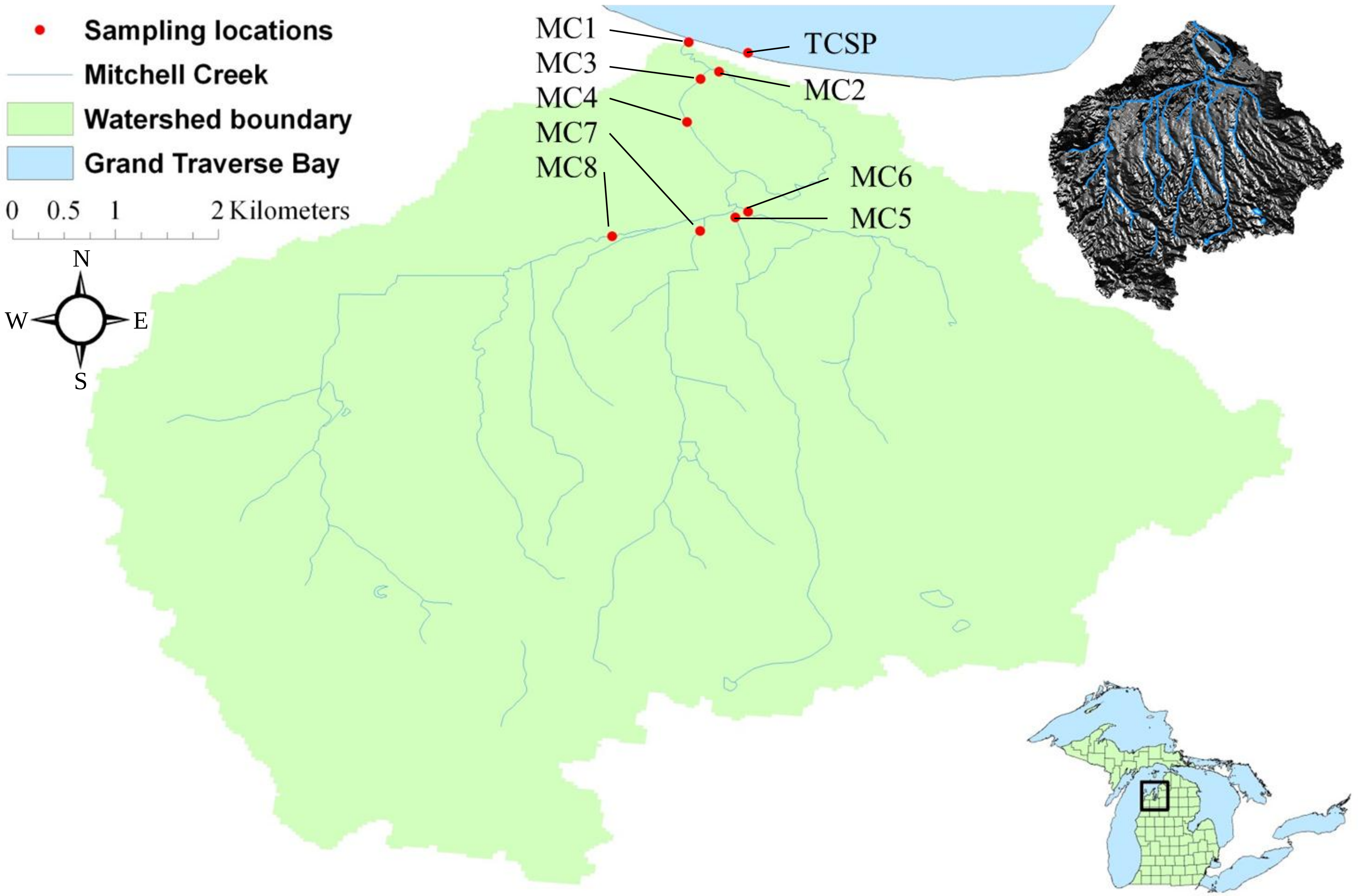


Figure 3.1. The Mitchell Creek watershed with sampling site locations. Upper inset image: The Digital Elevation Model of the Mitchell Creek watershed. Bottom inset image: Location of the Mitchell Creek watershed in Michigan and the Great Lakes.

Table 3.1. Description of Mitchell Creek watershed including land use and number of samples collected for each site. Mitchell Creek flows from sites 8, 7, 6, and 5 (headwater catchments), through sites 4 and 3 or 2, before discharging near site 1 (upstream of outlet).

Site name (Site ID)	Physical description	Basin Size (km^2)	Site location		Sample number		Land use (%)				
			Lat.	Long.	Water	Sediment	Urban	Ag.	Open/ Forest	Wetland	Water
Traverse City State Park (TCSP[A])	Swimming beach; sandy shoreline and lake bottom; 500 m long	0.6	44.749	-85.555	45	29	19.4	0.8	26.7	12.0	41.2
Mitchell Creek 1 (MC1)	Modified rocky embankments and bottom; 120 m upstream of creek mouth	39.7	44.745	-85.560	28	0	23.4	37.7	24.7	14.0	0.1
Mitchell Creek 2 (MC2)	Sandy embankments and bottom; 570 m upstream of creek mouth	37.9	44.748	-85.558	44	29	20.6	39.3	25.4	14.7	0.1
Mitchell Creek 3 (MC3)	Modified rocky embankments and bottom; 567 m upstream of creek mouth	39.6	44.748	-85.559	44	29	23.3	37.8	24.8	14.0	0.1
Mitchell Creek 4 (MC4)	Steep eroded embankments and sandy bottom; 1.1 km upstream from Creek mouth	36.7	44.743	-85.560	44	19	22.0	40.4	24.0	13.5	0.1
Mitchell Creek 5 (MC5)	Concrete embankments and sandy bottom; 2.5 km upstream of creak mouth	35.3	44.735	-85.556	44	29	20.9	41.6	23.7	13.6	0.1

A. Reference condition due to it close proximity to the mouth of the Mitchell Creek (MC1) and its popularity as a public swimming beach during summer months

Table 3.1. (cont'd)

Site name (Site ID)	Physical description	Basin Size (km^2)	Site location		Sample number		Land use (%)				
			Lat.	Long.	Water	Sediment	Urban	Ag.	Open/ Forest	Wetland	Water
Mitchell Creek 6 (MC6)	Deciduous woodland surrounding and organic rich bottom; 2.6 km upstream of creek mouth	9.2	44.735	-85.554	44	29	8.1	35.7	40.9	15.1	0.3
Mitchell Creek 7 (MC7)	Residential wetland outlet at culvert; rocky bottom; 2.6 km upstream of creek mouth	3.3	44.733	-85.559	24	0	33.3	36.7	14.2	15.3	0.5
Mitchell Creek 8 (MC8)	Grassy embankments and modified rocky embankments; 3.2 km upstream of creek mouth	14.6	44.733	-85.566	44	29	25.1	48.2	16.4	10.3	0.0

3.2.2. Environmental monitoring

Bather load, bird presence, wave height, and water and air temperature were recorded at time of sample collection. Wind speed/direction (0° = north, 180° = south), barometric pressure, and relative humidity were collected from NOAA's National Weather Service (Traverse City, Cherry Capital Airport) (NOAA 2012). Hourly Precipitation data were extracted from the Gaylord, Michigan Next Generation Radar (NEXRAD) through the National Climate Data Center (http://www.ncdc.noaa.gov/nexradinv/). This station has a base reflectivity 0.50 degree with an elevation range of 124 nautical miles. Samples were collected during dry and wet periods throughout the summer. Sampling events were considered wet weather when 48 hour precipitation totals were equal to or greater than 5.1 mm, following local beach manager recommendations and Haack et al. (2003).

Streamflow was measured at each site during four sampling events using an Acoustic Doppler Current Profiler (ADCP) or current-meter via wading following USGS protocol (Rantz 1982). River discharge was calculated from flow velocities and reported as $m^3 s^{-1}$. For all other events, stream discharge at the nearby Boardman River from U.S. Geological Survey (USGS) daily records (gage 04126970) was used. A statistically related dependent factor was calculated using gage recorded discharges on the Boardman River and measured discharges in the Mitchell Creek on the same day. Scaling of the Boardman River mean daily discharge to estimate daily mean flow at each Mitchell Creek site was performed using this dependent factor (Fulcher 1991).

Daily flows (million gallons) were collected from the Traverse City wastewater treatment plant. Wastewater Treatment Plant (wwtp) discharge was used as a proxy for human population density, which is considered a stressor of water quality (Smith et al. 2003; Nobre 2009). Although not perfect, this method was used in lieu of outdated and delayed census results that fail to grasp seasonal and tourist populations. Other sewage based indicators of human population have been suggested including caffeine and coprostanol (Daughton 2012), but such methods were beyond the scope of this project.

3.2.3. Cultivation analyses

Microbial analysis included *E. coli,* enterococci, *Clostridium perfringens* (*C. perfringens*), and coliphage CN-13. Undiluted water samples were filtered through 0.45 µm hydrophilic mixed cellulose esters filters (Pall Corporation). Sediment samples were assayed by weighing 100 g wet weight and mixing with 600 mL sterile Phosphate Buffered Water (PBW) using shaker arm for 2 minutes. The samples were allowed to settle for 30 seconds and the eluent was poured into a sterile bottle using caution not to mix sediment into eluent. An additional volume of PBW (400 mL) was added to the sediment, swirled for 10 seconds, and allowed to settle for 30 seconds. The eluent was added to the first rinse to achieve a 10% weight/volume dilution. Microbial assessment was made directly from final eluent.

E. coli were assayed using IDEXX Colilert defined substrate method and reported as Most Probable Number (MPN) 100 ml^{-1} (water) or MPN 100 g^{-1} dry weight (sediment). Enterococci were analyzed using membrane filtration and cultivation with selective media mEI (USEPA 2002) and reported as CFU (colony forming unites) 100 ml^{-1} (water) or CFU 100 g^{-1} dry weight (sediment). *C. perfringens* were assayed via membrane filtration (no pretreatment), cultivated using selective media mCP (USEPA 1995; Bisson and Cabelli 1979) and reported as CFU 100

ml^{-1} (water) or CFU 100 g^{-1} dry weight (sediment). Double agar layers were utilized to detect coliphage strains following USEPA method 1601 (USEPA 2001) using *E. coli* F+amp (male-specific coliphage) and *E. coli* CN-13 (somatic coliphage) as host bacteria. Clearings in the host lawn were counted and reported as plaque forming units (PFU) 100 ml^{-1} (water) or PFU 100 g^{-1} dry weight (sediment). *Escherichia coli* C-3000 (ATCC 15597), *Enterococci faecium* (ATCC 35667), *C. perfringens* (ATCC 3624), ΦX-174 coliphage were used as a positive controls for verification of media integrity. Sterile water was used as negative controls for verification of method integrity.

3.2.4. Molecular analyses

Samples were analyzed for the human specific marker *B. thetaiotaomicron* α-1-6 mannanase (5' CATCGTTCGTCAGCAGTAACA 3') following a modified procdure from Yampara-Iquise et al. (2008) as described by Srinivasan et al. (2011). Analysis was performed by filtering 1 L of sample through a 0.45 µm hydrophilic mixed cellulose esters filter. The filter was placed into a 50 mL centrifuge tube containing 20 mL of sterile PBW, vortexed, and centrifuged (30 minutes; 4000 x *g*; 21°C). Eighteen mL were decanted from the tube, and the remaining eluent and pellet were stored at -80°C. DNA was extracted from 200 µL (10%) of the thawed suspended pellet via QIAamp® DNA mini kit protocol. Quantitative Polymerase Chain Reaction (qPCR) for *B. theta* was performed on a Roche Light-Cycler® 2.0 Instrument (Roche Applied Sciences) according to Yampara-Iquise et al. (2008) and a primer modification (below) from Srinivasan et al. (2011). Each *B. theta* assay was carried out with 10 µL of LightCycler 480 Probe Mastermix (Roche Applied Sciences), 0.4 µL forward and reverse primers, 0.2 µL probe #62 (Roche Applied Sciences Universal Probe Library), 1.0 µL Bovine Serum Albumin, 3.0 µL nuclease free water, and 5.0 µL of extracted DNA and processed in triplicates. The qPCR analyses included a 15 minute, 95°C pre-incubation cycle, followed by 50 amplification cycles, and a 0.5 minute, 40°C cooling cycle. A diluted plasmid standard was included during each qPCR run as a positive control and molecular grade water was used in place of DNA template for negative controls. *B. theta* results were reported as copies 100 ml^{-1}.

3.2.5. Spatial analysis

ArcMap 9.2 was used to delineate catchments for sampling location and to quantify the landscape patterns in each watershed. In this project, land cover was defined as the cover of a landscape, physical or biological, and land use was defined as the anthropogenic activities and changes implemented on a specific land cover type (Di Gregorio and Jansen 1997). The shape files and layers employed for this proposal were obtained from NOAA's Coastal Change Analysis Program (C-CAP) Regional Land Cover dataset (NOAA Coastal Services Center 2001; http://www.csc.noaa.gov/digitalcoast/data/ccapregional/) and Michigan's Center for Geographic Information Library (MDTMB 2002). Digital elevation models were obtained from NASA's Land Processes Distributed Active Archive Center (USGS 2012) and USGS's Seamless data warehouse National Elevation Dataset (USGS 2010).

Catchment delineation

Catchments were delineated for each sampling point using ArcMap's spatial analyst watershed tool based on 1/3 Arc-Second (NED 1/3) resolution contour lines on a GCS North American coordinate system. Each catchment includes the entire upstream landscape that contributes water (and subsequently pollution) to each sampling point. Each catchment was further refined to include distal buffers upstream of each sampling point at distances of 500 m, 1000 m, and 5000 m.

Land use delineation

Land use in each catchment was determined using C-CAP land use imagery at a 5 meter resolution (see section 3.2.5). The land use contained in each catchment was reclassified into the following categories: *Anthropogenic* (residential, commercial, industrial landscape classifications), *Agriculture* (orchard, fields, and agriculture landscape classifications), *Natural* (Forest, grassland, and prairie landscape classification, beaches), *Wetlands*, or *Water* as described in Michigan Land cover/use classification (MSU 2010).

3.2.6. Statistical analysis

The percent moisture content by mass was determined for each sediment sample by measuring approximately 10 g wet weight sediment in a pre-weighed aluminum dish. Samples were placed in a 45°C incubator for 24 hours and reweighed. Percent dry weight was calculated by subtracting the dish weight from the dry and wet weights then dividing the dry weight by the wet weight. Mean daily air temperatures were calculated as an average of hourly observations recorded at Traverse City, Cherry Capital Airport (NOAA's National Weather Service; http://w1.weather.gov/data/obhistory/KTVC.html) over a 24 hour period.

When microbial and molecular analysis results were below method detection, a value equal to the method detection limit was reported. Microorganism concentrations underwent $\log_{10}$ transformation to fit the data to a normal distribution, but not all data met normality tests. Sample sets meeting normality assumptions were assayed for relatedness using Levenne's test for equality of variance, Pearson correlation, and one-way ANOVA with Bonferroni post hoc tests. When normality tests were not met, measurements were evaluated for relatedness using independent samples-Kruskal-Wallis one-way ANOVA and bivariate Spearman Rank correlation tests. These tests were performed using SPSS Statistic 17.0 software with significance set at (α) 0.05.

An agglomeration hierarchical cluster analysis was applied to surface water geometric means of *E. coli*, enterococci, *C. perfringens*, and coliphage at each site. Sites were grouped into clusters based on the linkage between-groups measured by Euclidean distance. Clusters were then used to classify sites and illustrated with a dendrogram.

Examination of microbial water quality associations with independent parameters was achieved using Classification And Regression Tree (CART) analysis following Martin et al. (2011). CART is a trial and error method that attempts to split dependent variables into homogeneous categories based on independent variables that influence the dependent variable (target organism). All CART analyses were performed using R software system (R foundation for Statistical Computing). CART has been previously used to investigate pathogenic bacteria and parasite relationships with environmental and land use factors (Wilkes et al. 2011), to classify lakes based on chemistry and clarity (Martin et al. 2011), and to predict the occurrence of total/fecal coliforms and enterococci with respect to physiochemical variables (Bae et al. 2010).

Models start out with a parent or root node which contains all available date. CART then looks at all independent variables (splitting variables) and selects the single variable that produces the two most different groups of dependent variables based on predefined splitting criterion and regression analysis. In this study, splitting criteria were developed using recursive partitioning algorithm and a 10-fold cross validation. A 10-fold cross validation breaks all data into 10 subsets and calculates the split based on nine of the ten subsets. Each time a group is split per above criteria the binary splits are called child nodes. This method is used for each group until a stopping rule is reached. For this project, the stopping criterion was set at a minimum of five observations per subgroup (Martin et al. 2011). A terminal node is defined as a child node which has met the defined stopping rule.

Fully grown trees often require pruning to ensure significant variable associations are not missed as a result of following the splitting and stopping criteria (Lemon et al. 2003). Pruning is the process of growing trees until they reach stopping criteria and then cutting less statistically significant results back. Trees were pruned according to the 1-standard error rule (Breiman et al. 1984; Venables and Ripley 1999; De'ath and Fabricous 2000). This rule minimizes the cross-validated error of the model which has been shown to produce optimal sized trees and produce more stable tree sizes across replications compared to the 0-SE pruning method and (Breiman et al. 1984; Questier et al. 2005).

Investigating detailed CART outputs, competitor and surrogate variables can be identified for each node. Competitor splits are those variables that have similar complexity parameter values compared to the primary split. A complexity parameter compares the complexity (number of terminal nodes) to the cross-validated error for each group. For this project the complexity parameter was set at 0.05. Surrogate splits are alternative variables that split the subgroup into very similar groups.

An example of a CART output is presented below (Figure 3.2.). At the top of the tree is the parent or root node with the primary splitting variables and values described for each child node. At the bottom of the tree, terminal nodes include the mean concentration and number of target organism cases in each node.

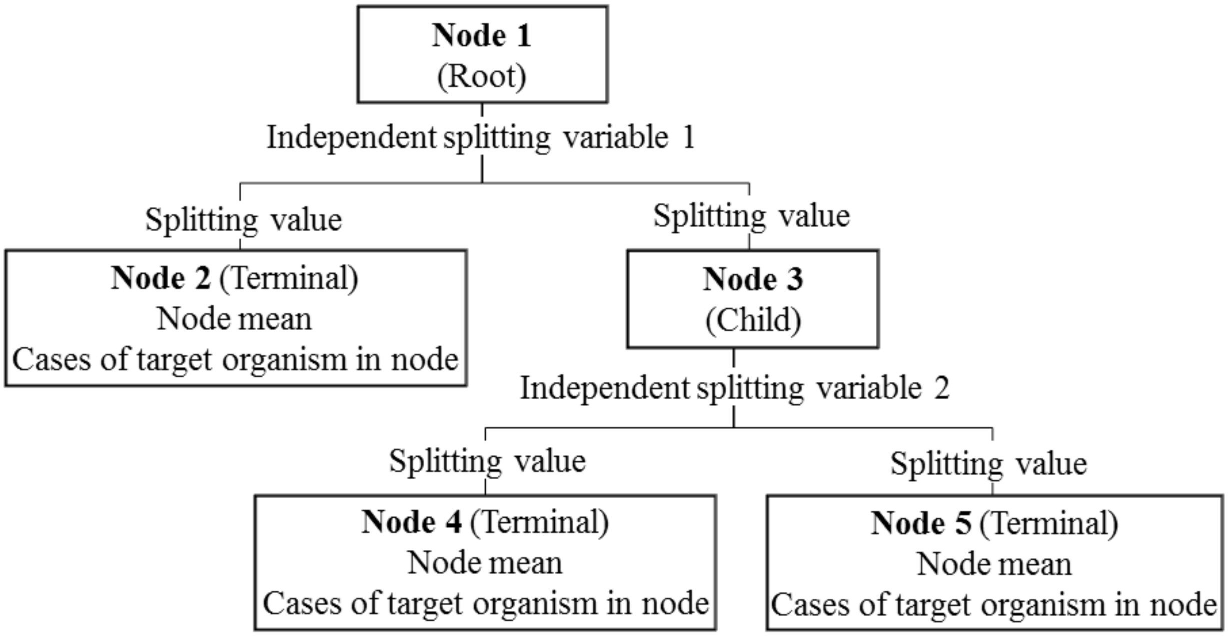


Figure 3.2. Classification And Regression Trees are composed of root nodes that contain all available data and are split into binary groups using recursive partitioning algorithm and 10-fold cross validation with a complexity parameter value of 0.05. Primary splitting variables and values are described for each child node. Terminal nodes (bottom of the tree) include the mean concentration and number of target organism cases in each node. Each node was derived based on mean value of each response variable, group size, and defining variables.

3.3. Results

3.3.1. Land use and cover

Land use composition of each catchment included multiple land cover types categorized as urban, agricultural, natural (forest and shrub), wetland, and water (Table 3.1. and Figure S.3.1.). Land use trends illustrate urban development primarily near the lower reaches (MC1, MC2, MC3, and MC4), wetlands in the middle reaches (MC5, MC6, MC7, and MC8) and agriculture near the headwaters of the watershed. Water quality from the agriculturally dominated headwaters was captured by sites in the middle reaches as they represent the drain point for the upstream land use. Spatial variation within sub-catchments was identified by applying distal buffers to upstream catchments of each sampling point and comparing land use composition across scales (Figure S.3.1.).

All Mitchell Creek sites, except MC6, exceeded 20% urban development at the catchment, with an average of 22.1% urban development amongst all Mitchell Creek sites. Overall, the dominate catchment land use was agriculture (39.7%), with a high of 48.2% at MC8. MC6 was composed of 40.9% natural land use, the highest of all sites at the catchment scale. MC7 had the highest urban (33.3%), wetland (15.3%), and aquatic (0.5%) compositions at the catchment scale.

In the Mitchell Creek, urban development had the highest land use composition average across all sites at the 5000 m (30.0%), 1000 m (38.9%), and 500 m (35.3%) scales. MC8 had the highest urban (34.7%) and agriculture (19.3%) compositions at the 5000 m scale. MC6, MC7, and MC1 had the highest natural (23.3%), wetland (12.0%), and water (30.3%) coverage, respectively, at the 5000 m scale. Urban coverage was highest at the 1000 and 500 m scales around MC4 (63.0%

and 65.0%, respectively). At the 1000 and 500 m scales, MC8 had the highest agriculture (16.9% and 15.5%, respectively) and natural (26.3% and 32.5%, respectively) land use coverage. MC6 and MC1 had the highest wetland (45.5%) and water (42.3%) coverage at the 1000 m scale, respectively. Spatial contrasts between the upper and lower catchments were exhibited at the 500 m buffer as urban composition exceeded 43% near MC1 but decreased to 18% at MC8.

Comparing urban development in the Mitchell Creek at the catchment scale (22.1%) to the 5000 m (28.9%), 1000 m (36.7%), and 500 m (33.5%) scales, confirms greater development near the river, more so in lower reaching sites. Agriculture (cropland and orchard) was the dominant land use at MC8 across all scales. Natural land use was highest in the MC6 catchment at the larger scales (i.e. catchment and 5000 m) but highest in the 1000 m and 500 m surrounding MC8. Wetland composition at all sites ranged between 10.3% and 15.3% with an average of 13.8%. TCSP had the greatest water coverage (41.2%) which was attributed to the beach orientation in the landscape and the surrounding topography. A Chi-square comparison of water coverage between Mitchell Creek sites generated a Chi value below 0.001 (7 degrees of freedom, p = 0.05), resulting in acceptance of the null hypothesis that water coverage is similar at all Mitchell Creek sites.

3.3.2. Spatial analyses of water and sediment quality

In total, 361 water samples and 193 sediment samples were collected from eight Mitchell Creek sites and one beach site, the Traverse City State Park beach (TCSP). *E. coli,* enterococci, *C. perfringens,* and coliphage CN-13 results for each site are shown in Figure 3.3. MC6 was the only Mitchell Creek site distinguishable from other sites based on *E. coli* and enterococci concentrations in water ($p < 0.05$). Coliphage CN-13 was not statistically different between any Mitchell Creek sites ($p > 0.05$).

Fecal indicator concentrations in water

E. coli densities ranged between 0.30 and 3.5 $\log_{10}$ MPN 100 ml^{-1} and had an overall geometric mean of 2.4 $\log_{10}$ MPN 100 ml^{-1}. Enterococci ranged between 0.29 and 4.5 $\log_{10}$ CFU 100 ml^{-1} with an overall geometric mean of 2.5 $\log_{10}$ CFU 100 ml^{-1}. *C. perfringens* ranged from 0.05 to 2.5 $\log_{10}$ CFU 100 ml^{-1} and exhibited an overall geometric mean of 1.1 $\log_{10}$ CFU 100 ml^{-1}. Coliphage CN-13 ranged from 1.0 to 3.9 $\log_{10}$ PFU 100 ml^{-1} and had an overall geometric mean of 1.8 $\log_{10}$ PFU 100 ml^{-1}. The highest *E. coli*, enterococci, *C. perfringens*, and coliphage individual measurements were found in samples collected at MC2, MC3, MC3, and MC5, respectively. The highest site specific *E. coli*, enterococci, *C. perfringens*, and coliphage geometric means were all found at MC5.

The *B. theta* marker was detected at every assayed site except MC7 (Figure 3.3 and Table 3.2.). In total, 118 Mitchell Creek water samples were tested for *B. theta* with an overall geometric mean of 4.1 $\log_{10}$ copies 100 ml^{-1}. *B. theta* concentrations in the Mitchell Creek ranged from 2.9 to 6.5 $\log_{10}$ copies 100 ml^{-1}. The highest *B. theta* geometric mean concentration was recorded at MC2 and the highest single sample concentration was measured at MC3 (6.5 $\log_{10}$ copies 100 ml^{-1}). No MC4 samples were assayed for *B. theta* as it was thought that any human contamination present at this site would be captured at MC3 (~500 m downstream). More samples were tested at TCSP, MC2, MC3, MC5, and MC6 compared to MC1, MC4, MC7, and MC8 because the former were included in additional qPCR analysis stemming from the intensive study.

TCSP was selected as a reference location because it is a popular designated swimming area next to the mouth of the Mitchell Creek. The lowest single water measurements of *E. coli* (< 0.30 $\log_{10}$ MPN 100 ml^{-1}), enterococci (< 0.30 $\log_{10}$ MPN 100 ml^{-1}), *C. perfringens* (0.05 $\log_{10}$ CFU 100 ml^{-1}), and coliphage CN-13 (< 1.0 $\log_{10}$ PFU 100 ml^{-1}) were recorded at TCSP. Furthermore, *E. coli* and enterococci geometric means (1.12 $\log_{10}$ MPN 100 ml^{-1} and 1.11 $\log_{10}$ MPN 100 ml^{-1}, respectively) amongst all sites were significantly lower than those in all Mitchell Creek sites ($p > 0.05$). The TCSP *C. perfringens* geometric mean (0.246 $\log_{10}$ CFU 100 ml^{-1}) was statistically lower than individual geometric means computed at each Mitchell Creek site except MC6 (0.797 $\log_{10}$ CFU 100 ml^{-1}) and MC7 (0.679 $\log_{10}$ CFU 100 ml^{-1}). The TCSP coliphage mean (1.13 $\log_{10}$ PFU 100 ml^{-1}) was lower than each Mitchell Creek site except MC6 (1.41 $\log_{10}$ PFU 100 ml^{-1}). Interestingly, in contrast to the other indicators, the TCSP *B. theta* geometric mean (4.2 $\log_{10}$ copies 100 ml^{-1}; $n = 35$) was slightly higher than the overall Mitchell Creek watershed geometric mean (4.1 $\log_{10}$ copies 100 ml^{-1}), however this was not statistically significant ($p = 0.262$).

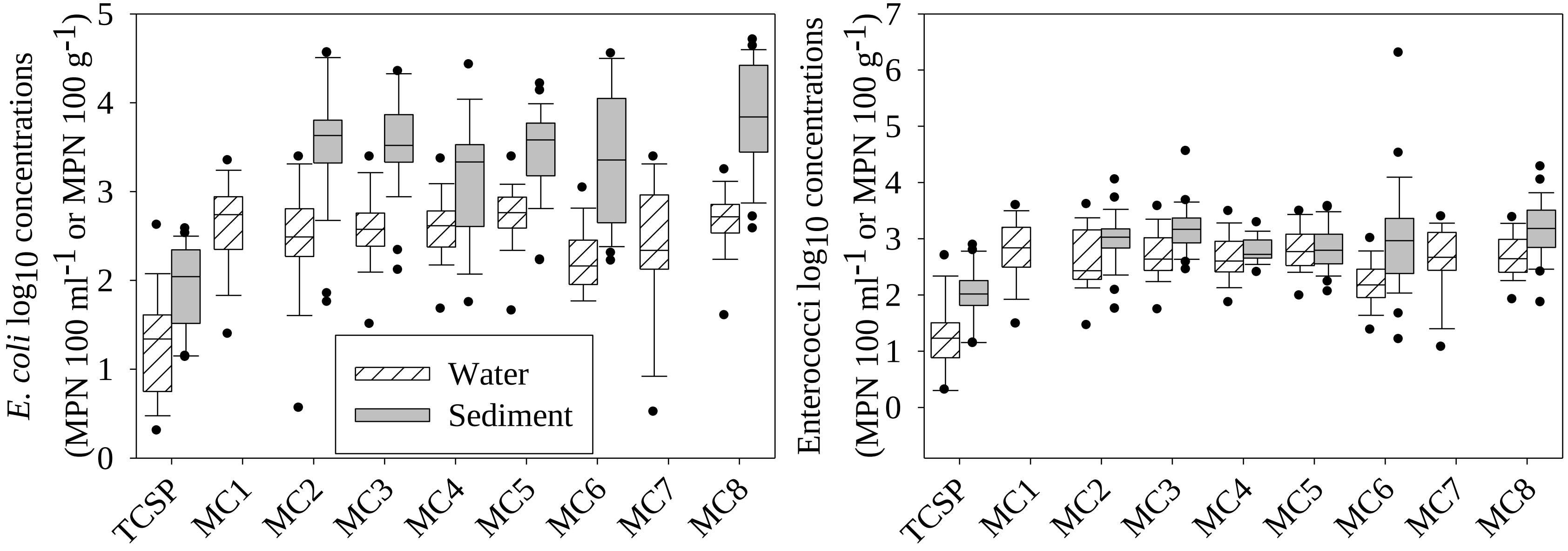


Figure 3.3. Box plots illustrating the ranges of fecal indicator organisms and *B. thetaiotaomicron* concentrations measured in the water and sediment (*E. coli* and enterococci only) at each site. The lower, middle, and top box edges correspond to the 25th, median, and75th percentiles of each measurement. The whiskers indicate the 10th and 90th percentile. The points indicate measurements outside the 5th and 95th percentiles. *E. coli*, enterococci, *C. perfringens*, and coliphage were measured in sediment and reported per 100 g^{-1} dry weight. The number of samples assayed for each cultivated microorganism at each site is presented in Table S.3.1. The number of *B. theta* assays for each site is presented in Table 3.2.

Figure 3.3. (Cont'd)

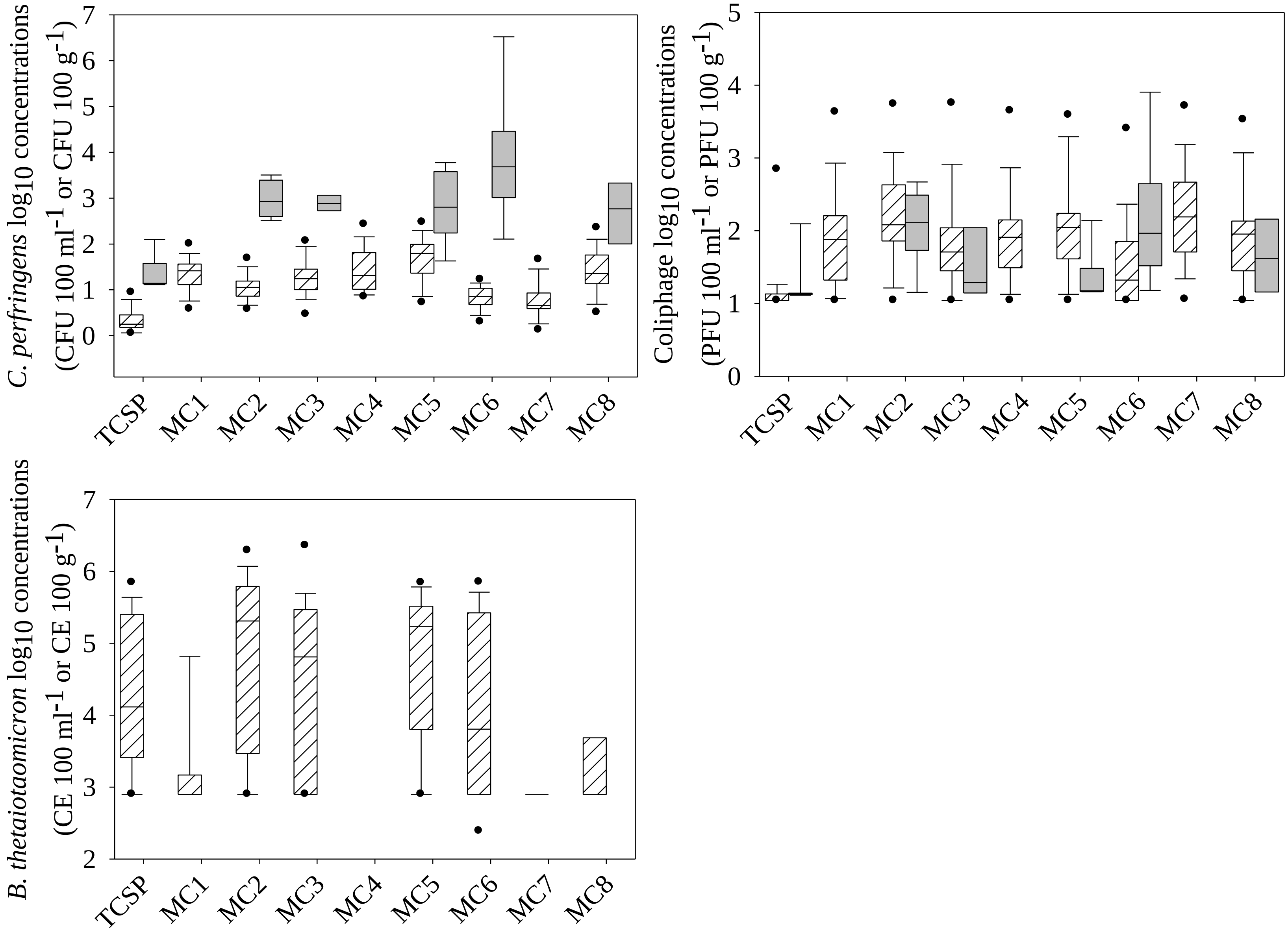


Table 3.2. *B. thetaiotaomicron* results in Grand Traverse Bay and Mitchell Creek water samples.

Site ID	Number of Samples	Log mean concentrations ($\log_{10}$ copies 100 ml^{-1})	Concentration range ($\log_{10}$ copies 100 ml^{-1})	Number samples positive (> 2.9 $\log_{10}$ copies 100 ml^{-1})
TCSP	35	4.2	2.9 – 6.0	29
MC1	9	3.1	2.9 – 4.8	2
MC2	26	4.6	2.9 – 6.4	22
MC3	23	4.3	2.9 – 6.5	15
MC4	0	NA	NA	NA
MC5	21	4.6	2.9 – 5.8	17
MC6	25	4.0	2.9 – 5.9	16
MC7	3	2.9	-	0
MC8	7	3.1	2.9 – 3.8	2

NA: No samples assessed for *B. theta* as it was assumed water quality would be captured at MC3 situated 500 m downstream from MC4.

Fecal indicator concentrations in sediment

Fecal indicators were routinely recovered in the sediment of each sampling site. At TCSP, the geometric means for *E. coli,* enterococci, *C. perfringens*, and coliphage in the sediment were 1.9 $\log_{10}$ MPN 100 g^{-1}, 2.0 $\log_{10}$ CFU 100 g^{-1}, 1.3 CFU 100 g^{-1}, and 1.2 PFU 100 g^{-1}, respectively. *E. coli* and enterococci in the sediment of all tested Mitchell Creek sites were statistically higher than TCSP sediment ($p \leq 0.003$). In general, *E. coli* and enterococci were not statistically different between Mitchell Creek sites ($p > 0.05$). In the Mitchell Creek sediment, *E. coli* ranged from 1.7 (MC2 and MC4) to 5.9 (MC6) $\log_{10}$ MPN 100 g^{-1} and exhibited an overall geometric mean of 3.4 $\log_{10}$ MPN 100 g^{-1}. On average amongst TCSP and Mitchell Creek sites, *E. coli* concentrations in the sediment were 1.5 times higher than *E. coli* concentrations in the water column. Specifically, *E. coli* averaged 1.4 times higher in sediment than in water in the Mitchell Creek and 1.8 times higher at TCSP. Enterococci ranged from 1.2 (MC6) to 6.3 (MC6) $\log_{10}$

CFU 100 g^{-1} in the Mitchell Creek with an overall geometric mean average of 3.0 $\log_{10}$ CFU 100 g^{-1}. When combining the TCSP and Mitchell Creek sites, enterococci concentrations in the sediment were 1.4 times higher than enterococci concentrations in the water column. Specifically, sediment enterococci averaged 1.3 times higher than water in the Mitchell Creek and 1.9 times higher at TCSP. *C. perfringens* had an overall geometric mean of 2.5 $\log_{10}$ CFU 100 g^{-1} in the Mitchell Creek and ranged from 1.1 to 6.5 $\log_{10}$ CFU 100 g^{-1}. Overall TCSP and Mitchell Creek sites, *C. perfringens* concentrations in the sediment were 3.6 times higher than *C. perfringens* concentrations in the water column. Specifically, sediment *C. perfringens* averaged 3.4 times higher than water in the Mitchell Creek and 4.5 times higher at TCSP. CN-13 ranged between 0.4 and 3.9 $\log_{10}$ PFU 100 g^{-1} with a geometric mean of 1.6 $\log_{10}$ PFU 100 g^{-1}. On average amongst TCSP and Mitchell Creek sites, coliphage CN-13 concentrations in the sediment were 1.0 log higher than CN-13 concentrations in the water column. Specifically, CN-13 averaged 1.0 log higher in sediment than water in the Mitchell Creek and 1.1 times higher at TCSP. Preliminary *B. theta* analysis returned a significant number of non-detects (n = 116; non-detect = 91%) in sediment samples suggesting the need for further method developments and potentially a high inhibition, thus further analysis were discontinued due to timing constraints.

3.3.3. Temporal analysis

Water and sediment quality changes

Daily microbial averages in water and sediment of the creek were examined over time. Daily *E. coli* geometric means over time in sediment and water amongst all Mitchell Creek sites are presented in Figure 3.4, along with precipitation (24 hour totals) which will be discussed in the next section. All *E. coli*, Enterococci, *C. perfringens,* and coliphage CN-13 measurements in water and sediment from all sites are presented in Table S.3.2. and Table S.3.3., respectively.

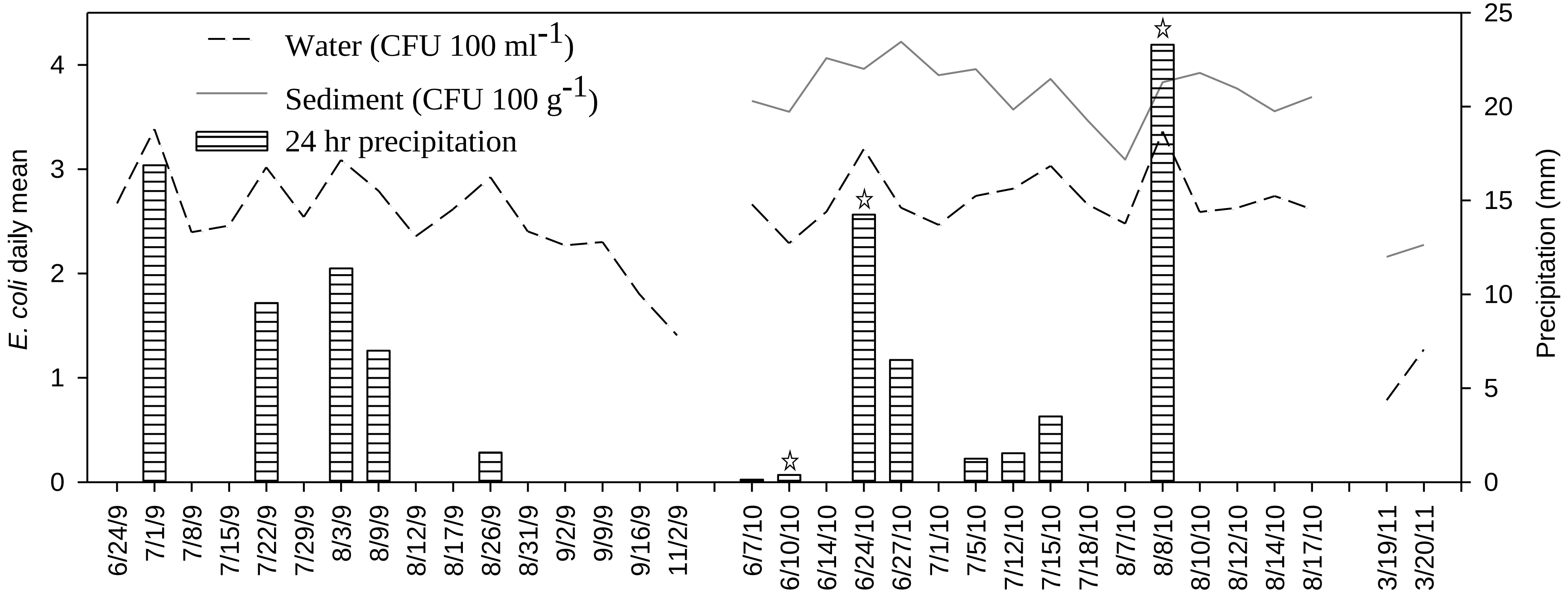


Figure 3.4. Temporal variation of *E. coli* in the Mitchell Creek. Water daily geometric means reported as $\log_{10}$ MPN 100 ml^{-1} and sediment reported as $\log_{10}$ MPN 100 g^{-1} dry weight. Enterococci trended similarly to *E. coli*. Minor temporal variations were identified using *C. perfringens* and coliphage CN-13. Precipitation is shown as cumulative rainfall in 24 hours prior to sample collection. ☆ denotes dates when TCSP water samples exceeded Michigan *E. coli* water quality standards. No sediment samples were collected in 2009.

Sediment showed less variability in the bacterial concentrations over time and in general did not change dramatically between morning and afternoon, collection hour, or day of month. In regards to water quality, *E. coli*, enterococci, and *C. perfringens*, averaged across all sites, were significantly higher in pre-noon samples (n = 262) compared to post-noon samples (n = 99; p = 0.002) likely due to inactivation in the water column, but coliphage was not significantly different (p = 0.241). Daily and monthly variations of microorganism concentrations were also identified and suggestive of *E. coli* and enterococci regrowth during warm summer months. Monthly differences of *C. perfringens* and coliphage CN-13 densities were generally insignificant.

3.3.4. Drivers of bacterial water quality

Environment and weather conditions

During sample collection, Mitchell Creek water temperature ranged from -0.5 °C to 23.5 °C and air temperatures ranged from -0.8 °C to 34.5 °C. Air and water temperature were highly related to each other (r = 0.87, p = 0.01). Descriptive statistics of environmental parameters recorded throughout the study period are presented in Table S.3.4. Correlation between independent parameters (air and water temperature at time of sampling, mean daily air temperature, cumulative precipitation totals [1, 2, 3, 4, 6, 8, 12, 16, 24, 48, and 72 hour] prior to sample collection, barometric pressure, relative humidity, daily mean solar radiation, and wastewater treatment plant daily discharge) and fecal indicators were generally positive but small or insignificant. Correlation results for independent variables and microbes in water and sediment are presented in TableS.3.5. and Table S.3.6, respectively.

Hydrology

Discharge in each catchment was directly associated with 24-72 hour precipitation totals. Total 72 hour precipitation described most of the discharge-precipitation relationship ($R^2 = 0.539$, $p < 0.001$). On June 14, 2010, the largest discharges were recorded at each site which was preceded by the largest 72 hour cumulative precipitation total. Eleven sampling events occurred after 48 hour cumulative precipitation exceeded 5.1 mm, the predetermined wet weather threshold. The wettest month of the project was June 2010 (169 mm total monthly precipitation), far greater than the long term monthly average of 84.3 mm (Midwest Regional Climate Center 2012). Discharge and precipitation results recorded throughout the project period are detailed in Table S.3.7 and Table S.3.8., respectively.

An intensive study began on August 7, 2010 and included hourly sample collection for 12 consecutive hours. On hour 10, it began to rain and at hour 12, the 95th percentile of the project *E. coli* distribution was exceeded at TCSP. The following day (8-8-2010; hour 24), precipitation totals for the previous 4-24 hours exceeded the 99th percentile and microbial responses included: 1) Mitchell Creek water *E. coli* exceeded the 95th percentile, 2) Mitchell Creek water enterococci exceeded the 90th percentile, 3) Mitchell Creek sediment *E. coli* and enterococci exceeded the 95th percentile, 4) TCSP water quality failed Michigan's single sample *E. coli* standard (300 *E. coli* 100 ml^{-1}), and 5) site MC2 exceeded water *E. coli* and enterococci 95th percentiles. On August 10 (hour 72), cumulative *B. theta* averages for all sites reached a project high and MC4 exceeded water *E. coli* and enterococci 90th percentiles. On August 12 (hour 120) MC5 exceeded water *E. coli* 95th percentile.

Human population and WWTP

The wastewater treatment facility discharges to the Boardman River and drains to the west branch of the Grand Traverse Bay (Latitude: 44.75754, Longitude: -85.61270). The TCSP and mouth of the Mitchell Creek are located in the east branch of the Grand Traverse Bay (separated from the west branch by the ~27 km long Old Mission Peninsula) and thus the WWTP discharge does not affect the Mitchell Creek watershed. Therefore, WWTP discharge was used as a surrogate for short term human population presence in the study area which partially relies on septic tanks. Daily discharge volumes from the WWTP were statistically higher from June through October compared to December through May ($p < 0.001$). Daily effluent flows averaged over each month and per day of week are illustrated in Figure S.3.2 (A and B, respectively). Weekend flows were statistically lower than weekday flows ($p < 0.001$), illustrating the significant presence of commerce in the watershed. Increases of daily flow existed during the first week of July each year, attributed to population increase for an annual weeklong festival. In the Mitchell Creek, statistically significant ($p \leq 0.002$) and direct correlations were shown to exist between daily WWTP discharges and concentrations of *E. coli* ($r = 0.242$), enterococci ($r = 0.527$), and *C. perfringens* ($r = 0.237$) in water. WWTP discharges were not statistically related to coliphage CN-13 ($p = 0.871$) or *B. theta* ($p = 0.993$) levels in the Mitchell Creek. At TCSP, WWTP discharges were significantly related to coliphage CN-13 in water ($r = 0.361$; $p = 0.015$), but not to *E. coli*, enterococci, *C. perfringens*, or *B. theta* ($p \geq 0.194$).

3.3.5. Microbial responses to sources and drivers

Environmental impacts on water quality

Mitchell Creek water quality is influenced rapidly by precipitation (Table S.3.9.) and *E. coli* and enterococci measurements from sediment and water displayed weak positive correlations to precipitation with the most significant responses generally associated with 16 hour cumulative precipitation (Table S.3.10.). At TCSP, significant associations between rainfall and fecal indicators in water and sediment were not identified. However, during three of the 11 wet weather sampling events (June 10, 2010, June 24, 2010, and August 8, 2010), TCSP water quality exceeded Michigan's *E. coli* standard (300 *E. coli* 100 ml^{-1}) as shown in Figure 3.4. There were no exceedances of the same standards during dry weather sampling events (n = 34).

Associations between the four fecal indicator bacteria and independent variables were tested using CART. The independent variables temperature (water and air), precipitation, collection timing, humidity, and wastewater flows were considered indicators of temporal variation while land use and scale represented of spatial variability. Temporal variables dominated the root node splits in three of the four Mitchell Creek water models targeting fecal indicator bacteria (Figure 3.5). CART results explained 65% of *E. coli*, 74% of enterococci, 62% of *C. perfringens*, and 59% of coliphage CN-13 variations in Mitchell Creek water samples.

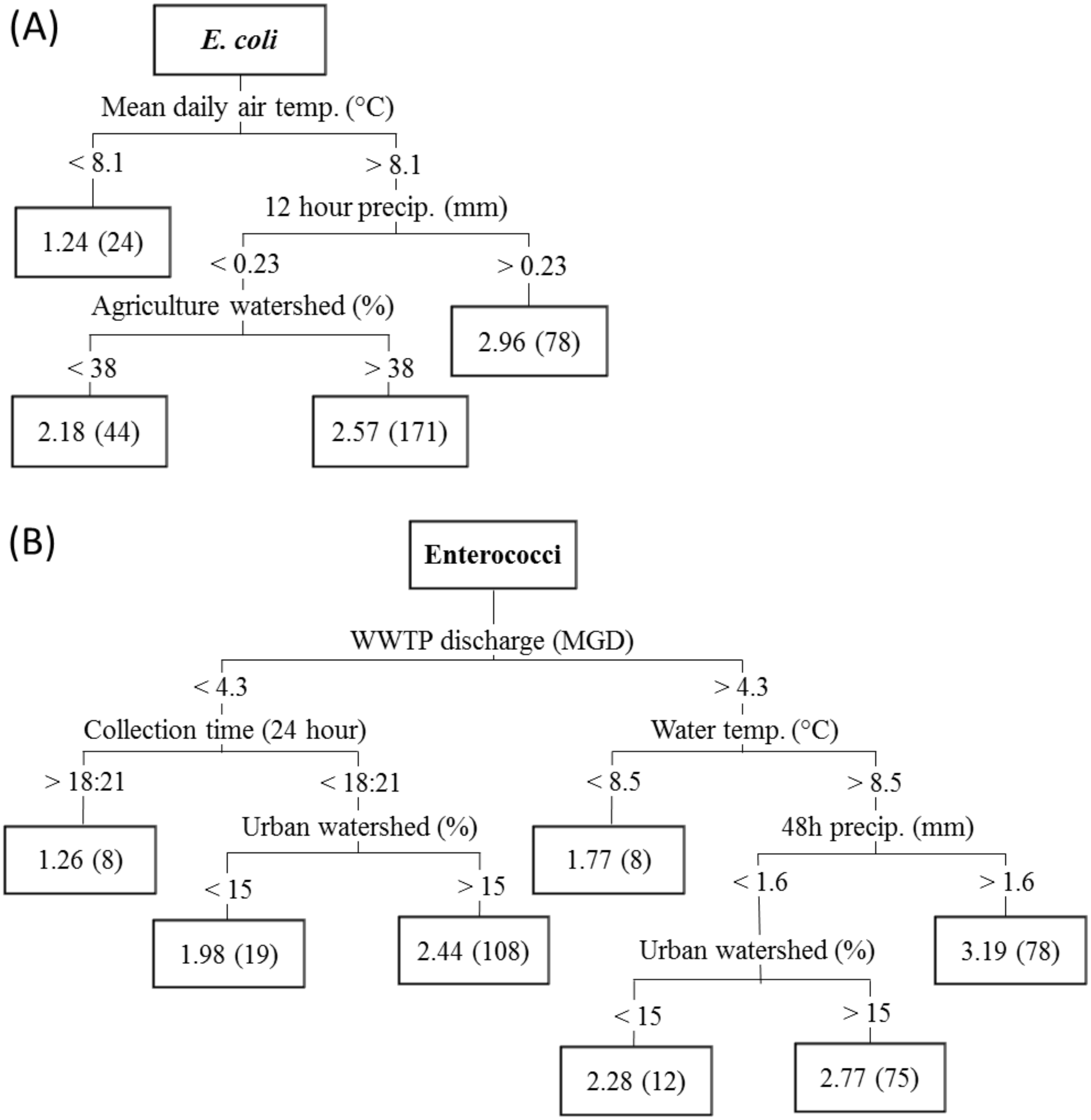


Figure 3.5. Mitchell Creek water CART outputs for (A) *E. coli*, (B) enterococci, (C) *C. perfringens*, (D) coliphage (CN-13), and (E) *B. theta*. Each split is labeled with splitting variable and value. Terminal nodes (bottom rectangle) are labeled with means and cases of target organism in each group.

Figure 3.5. (cont'd)

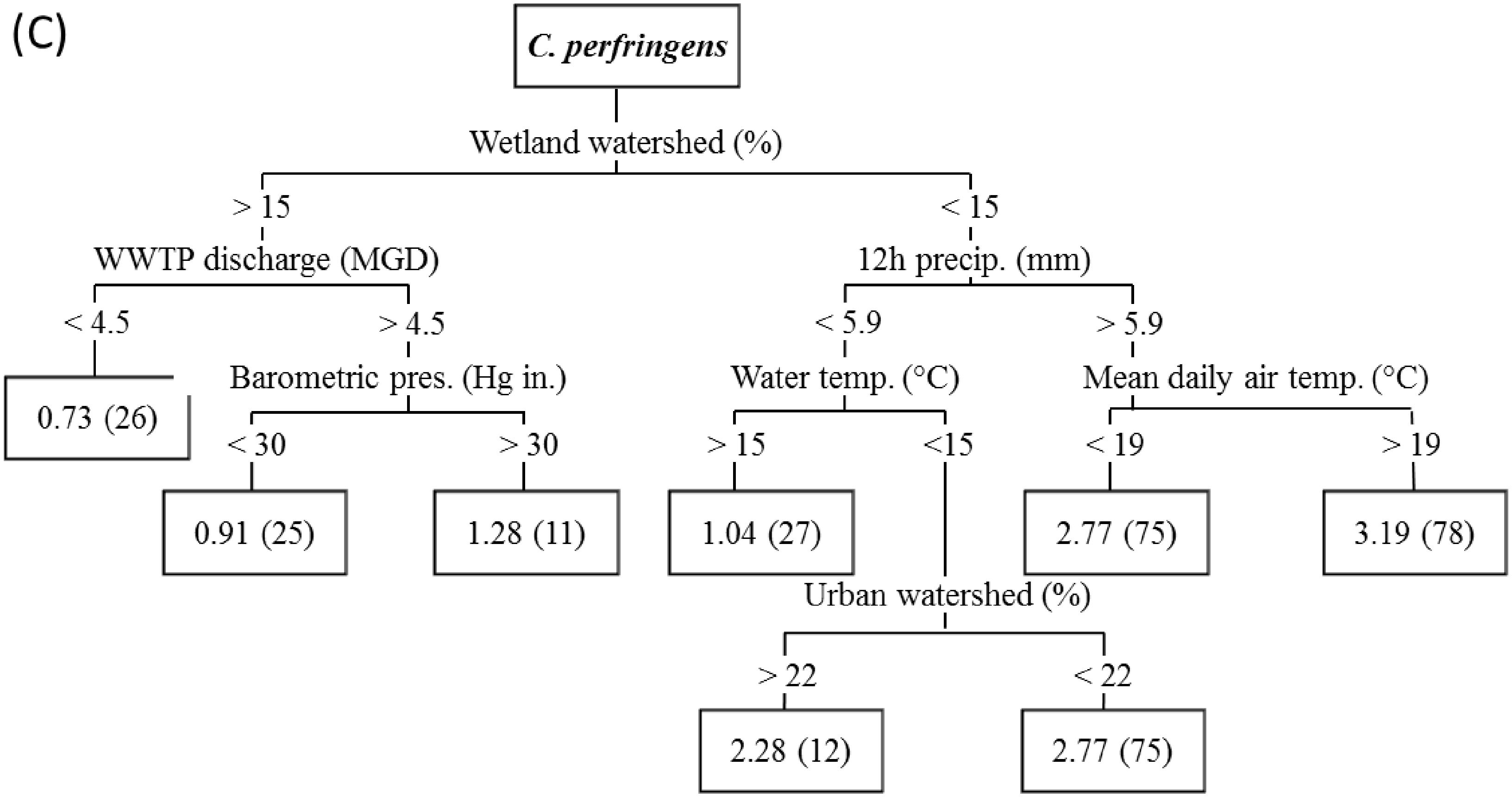
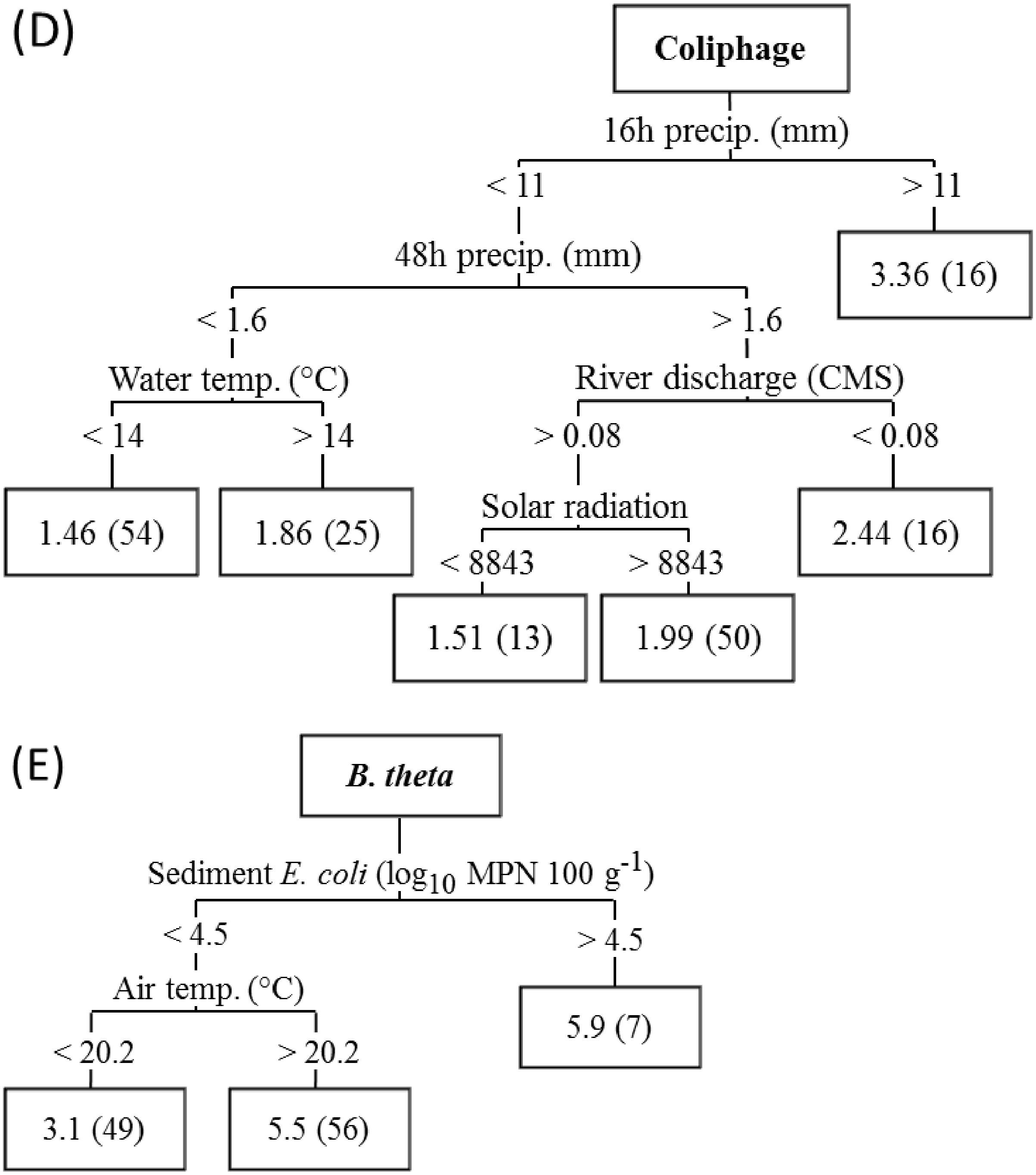


Figure 3.5. (cont'd)



E. coli was best explained by mean daily air temperature (R^2 = 0.457) and 12 hour cumulative precipitation (R^2 = 0.136). Further investigation of CART outputs indicated *E. coli* levels were equally explained by mean daily air temperature, 12, 16, and 24 hour precipitation totals, percent agriculture in the watershed and 1000 m buffer, and percent urban coverage in the watershed. Enterococci was best explained by human population using Traverse City's WWTP daily discharge (R^2 = 0.280) and water temperature (R^2 = 0.122). Watershed wetland coverage best explained *C. perfringens* levels (R^2 = 0.273) while coliphage CN-13 was almost wholly explained by precipitation (16 and 48 hour totals) and discharge. When Mitchell Creek sediment *E. coli* was above 4.5 $\log_{10}$ MPN 100 ml^{-1}, the highest *B. theta* levels (5.9 log10 CE 100 ml^{-1}) were also expected, although this variable explained just 14% of *B. theta* variation, suggesting at least a portion of *B. theta* occurrence in water is coming from the sediments. For *E. coli*, enterococci, and *C. perfringens*, a vast majority of land use nodes also included competitor variables of other land use types and scales (agriculture, urban, wetlands, and natural).

The highest means of fecal indicator bacteria in the Mitchell Creek (i.e. highest contamination and human health risk) were associated with a mixture of spatial and temporal parameters. The highest fecal indicator means, as defined by CART, were explained by average daily air temperature, water temperature, and wastewater discharge. Precipitation partially explained the highest concentrations of each fecal indicator in water. The only land use variable identified as a root node split for any bacteria was wetland coverage in the entire catchment. Wetland coverage below 15% was the primary driver of *C. perfringens* above 1.24 $\log_{10}$ CFU 100 ml^{-1}.

Microbes in TCSP water were compared to independent variables using CART (Figure 3.6). Land use was excluded from TCSP analysis because of the small catchment size and homogeneous land use patterns across all scales (Figure S.3.1.). CART models explained between 11.9% and 36.9% of fecal indicator bacteria detections at TCSP water, far less than Mitchell Creek water. However, significant explanatory parameters were identified including discharge from Mitchell Creek and wind direction. The root nodes of *E. coli* and coliphage CN-13 were split by wind direction and wind speed, respectively. Enterococci and *C. perfringens* in TCSP water were primarily explained by enterococci at MC3 water and discharge from MC1, respectively. *B. theta* was the only microorganism influenced by both the environment (wind direction) and the Mitchell Creek (MC1 discharge). Varying the number of Mitchell Creek parameters input to TCSP CART analysis resulted in similar explanatory variables at TCSP with overall explanatory powers slightly shifted. After analyzing multiple tree variations, it was determined that *C. perfringens* and *B. theta* at TCSP were highly influenced by the Mitchell Creek while *E. coli,* enterococci, and coliphage CN-13 were only moderately affected.

To better understand the timing of Mitchell Creek's influence on swimming at TCSP, time lags were imposed during statistical analysis for the 12 consecutive hourly samples collected as part of the intensive study. Water quality comparisons between TCSP and Mitchell Creek measurements from the previous hour produced the strongest relationships, specifically with MC2 ($p < 0.05$), MC3, MC4, and MC5 ($p < 0.01$), but not with the upstream sites MC6 and MC8 $p > 0.05$).

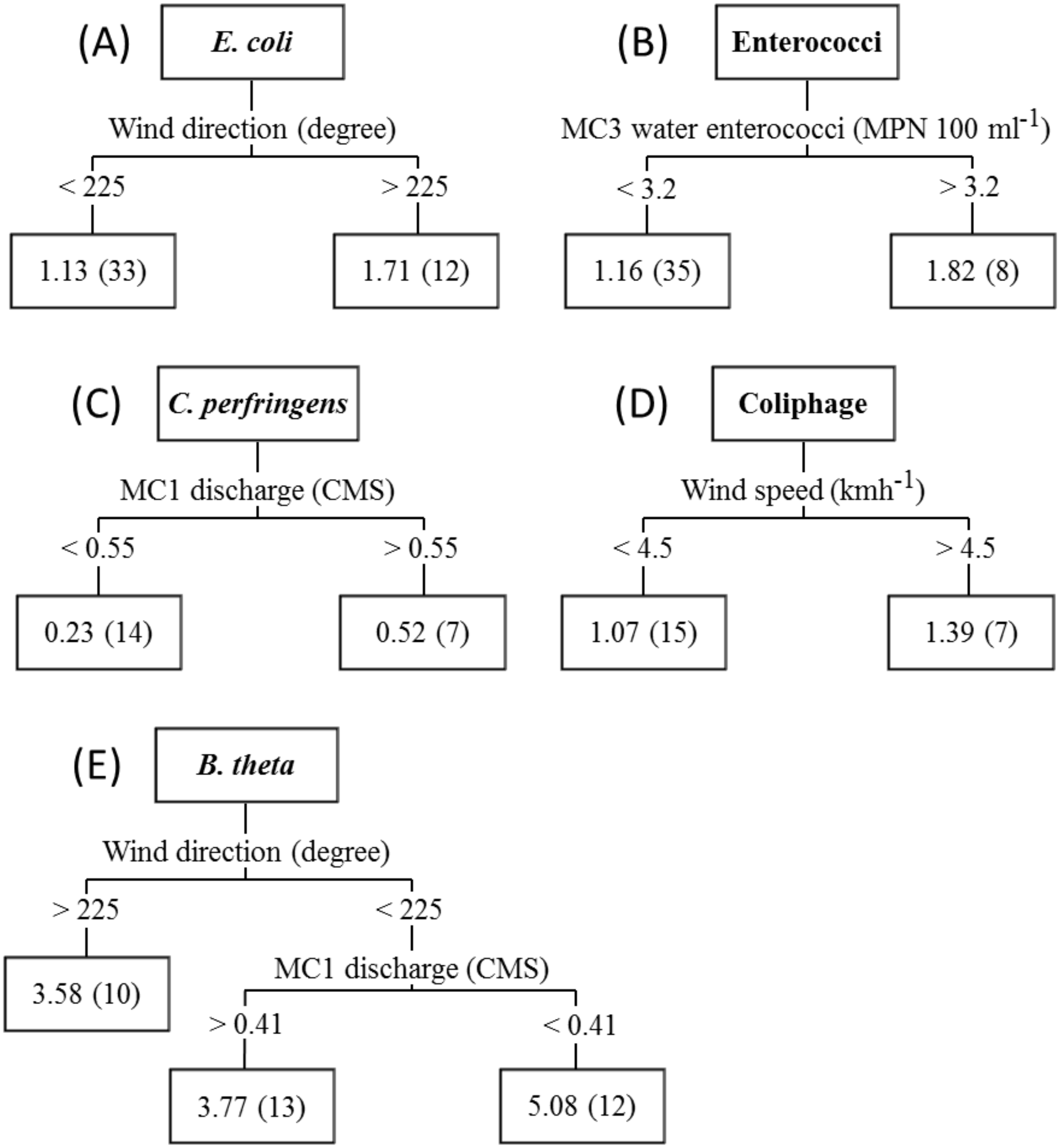


Figure 3.6. TCSP water CART outputs for (A) *E. coli*, (B) enterococci, (C) *C. perfringens*, (D) coliphage (CN-13), and (E) *B. theta*.

Sites were grouped into clusters using an agglomeration hierarchical analysis. Four distinct groupings were identified between the clusters (Figure 3.7.). TCSP (Cluster 1) was the least contaminated, followed by MC1 and MC7 (Cluster 2), and MC6 (Cluster 3). Cluster 4 was the most contaminated group and consisted of MC5, MC8, MC2, MC3, and MC4. Each cluster was defined by surface water geometric means of fecal indicators, but also shared common and unique root node splitting variables in CART. Temperature was a common root node split in the Mitchell Creek (clusters 2, 3, and 4) but was not important at TCSP. *E. coli* and enterococci splitting variables in Cluster 2 were all variants of temperature (air, water, or mean daily air temperature). Splitting variables at Cluster 3 included temperature (air and water), precipitation (72 hour), and wastewater discharge. Cluster 4 split significantly on wastewater discharge as well as air temperature and precipitation (48 and 72 hour).

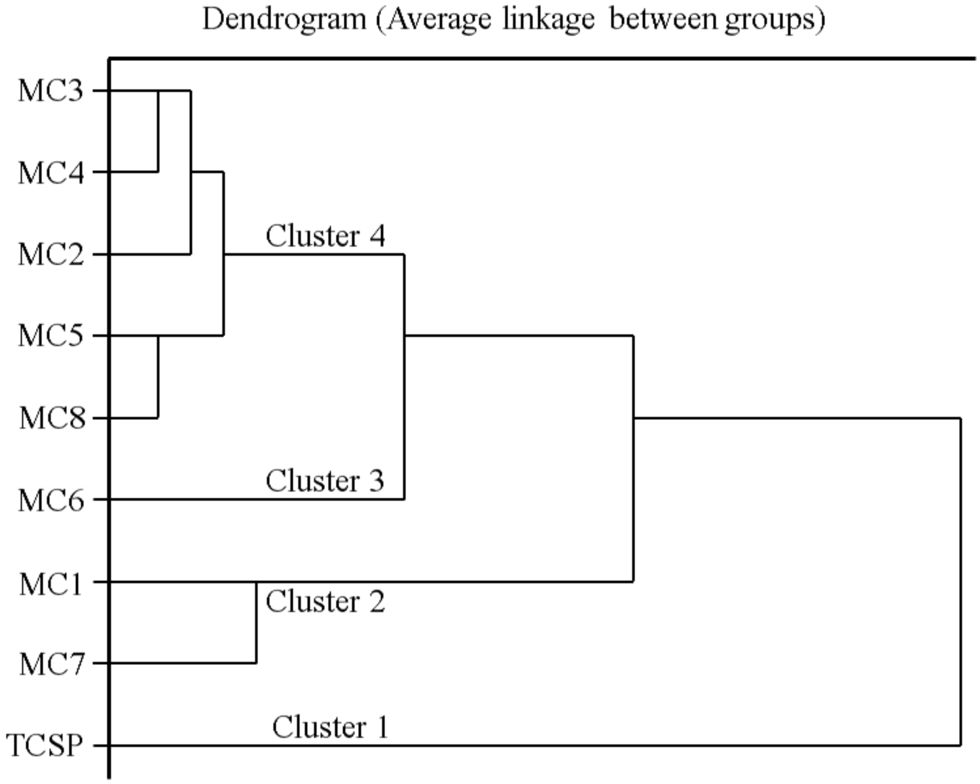


Figure 3.7. Dendrogram showing clusters based on geometric mean indicator concentrations at each site. TCSP is a designated swimming area with relatively low indicator organism concentrations and root node splits on wind (direction and speed) and MC parameters (discharge and water/sediment microbes). Clusters 2, 3, and 4 are sites within the Mitchell Creek watershed and are not designated as primary contact recreation sites.

Sediment implications for water quality

CART analysis was again used to investigate associations between independent variables and microbes occurrences, this time in the sediment. In regards to the Mitchell Creek, CART results explained a lower percentage of fecal indicator presence in sediments compared to water models, likely due to the steady state of microbial concentrations found in the sediments which were more resistant to change from outside forces. Sample collection month explained 28.0% of sediment *E. coli* detections in Creek sites. Precipitation (24 hour), wastewater discharge, and wetland coverage explained 15.1%, 25.8%, and 19.1% of enterococci, *C. perfringens*, and coliphage CN-13 detection in Mitchell Creek sediments, respectively. In the sediment of Mitchell Creek, the highest fecal indicator bacteria means were associated with mean daily air temperature above 5.3 °C, 24 hour precipitation totals above 0.07 mm, small wastewater discharge volumes, and wetland coverage greater than 14.5% in the watershed. CART results for Mitchell Creek sediment are presented in Figure 3.8. (A-D).

In the sediment at TCSP, CART analysis were slightly better than Mitchell Creek models at explaining *E. coli* (37.9%) and enterococci (29.5%) levels. Insufficient data were available to perform CART analysis on *C. perfringens* and coliphage assays at TCSP. Wave height explained approximately 30% of *E. coli* occurrences. Enterococci concentrations in water explained 30% of enterococci in sediment, but wind direction was a strong competitor variable. Results for each fecal indicator at TCSP sediment are presented in Figure 3.8 (E and F). Multiple models were again developed by varying the number of Mitchell Creek parameters input to CART. All model variations produced similar results for *E. coli* and enterococci in TCSP sediment, indicating sediment fecal indicator organisms act independent of the Mitchell Creek sediment.

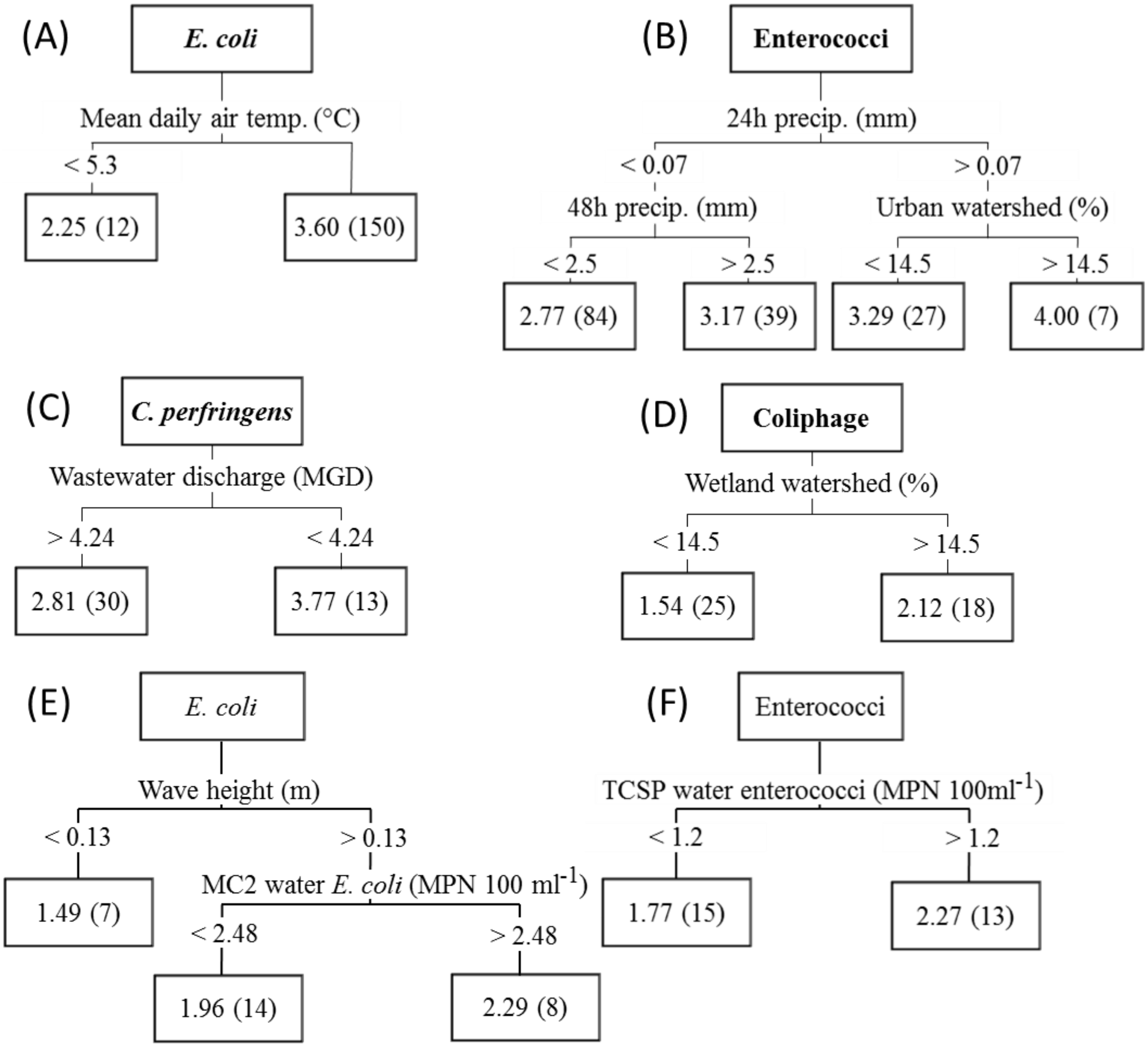


Figure 3.8. Sediment CART outputs for (A) Mitchell Creek *E. coli*, (B) Mitchell Creek enterococci, (C) Mitchell Creek *C. perfringens*, (D) Mitchell Creek coliphage (CN-13), (E) TCSP *E. coli*, and (F) TCSP enterococci. Insufficient data was available to perform CART analysis on *C. perfringens* and coliphage assays at TCSP.

Comparisons of water and sediment pairwise samples identified 95% of *E. coli* and 87% of enterococci results were greater in sediment than in the overlying water column, but the majority of differences were less than 1 order of magnitude. The largest difference between sediment and water *E. coli* and enterococci concentrations occurred at MC6. To identify sediment contributions to water, water microbe concentrations from each site were compared to microbial concentrations in sediment, discharge, and precipitation. Significant positive correlations between sediment and water were identified at MC2, MC5, and MC8 (Table 3.3.). Sediment impacts on water were better explained using *E. coli* than enterococci.

Table 3.3. Sites identified were water quality could be partially explained by sediment levels (CART) and the associated correlations and significance.

Site	Water Fecal indicator	Sediment fecal indicator	p	R^2
MC2*	*E. coli*	Enterococci	0.022	0.174
MC3	*E. coli*	*E. coli*	0.267	0.012
MC4	*E. coli*	Enterococci	0.177	0.030
MC5*	*E. coli*	*E. coli*	0.011	0.196
MC8**	*E. coli*	*E. coli*	0.001	0.120
MC8**	Enterococci	*E. coli*	0.005	0.017

* Significance level ≤ 0.05; ** Significance level ≤ 0.01

3.4. Discussion

This project aimed to quantify fecal contamination in a small mixed use watershed through spatial and temporal monitoring of *E. coli*, enterococci, *C. perfringens*, and coliphage CN-13. Based on suggested geometric mean thresholds of *E. coli* (2.10 $\log_{10}$ MPN 100 ml^{-1}, USEPA 1986), enterococci (1.52 $\log_{10}$ MPN 100 ml^{-1}, USEPA 1986), *C. perfringens* (1.70 $\log_{10}$ CFU 100 ml^{-1}), and coliphage CN-13 (1.56 $\log_{10}$ PFU 100 ml^{-1}), the Mitchell Creek was deemed unsafe for swimming. The *C. perfringens* threshold comes from the Hawaii standard (Mahin and

Pancorbo 1999) and the coliphage threshold was developed by Love et al. (2010) following a statistical equivalence comparison of coliphage to USEPA freshwater enterococci criteria.

E. coli and enterococci occurrences represented widespread fecal contamination in the Mitchell Creek. *C. perfringens* levels indicated a long-term chronic input of fecal contamination and the lower concentrations of coliphage CN-13 (geometric mean = 1.83 $\log_{10}$ PFU 100 ml^{-1}) were indicative of fresh fecal contamination. It was speculated, and further described below, that wastewater infrastructure was the leading source of the overall fecal pollution in the Mitchell Creek. Water quality at TCSP beach was generally regarded safe for recreational activities. *E. coli*, enterococci, *C. perfringens*, and coliphage met the suggested geometric mean criteria described above. Greater than 82% of samples met suggested *E. coli*, enterococci, and CN-13 single sample criterion, indicating TCSP was generally clean but for sporadic incidences of elevated bacteria.

There are more than 1600 on-site septic systems in the Mitchell Creek watershed but a portion of residents rely on a wastewater treatment plant which discharges outside of the watershed. Therefore, this study used daily wastewater treatment plant (WWTP) discharge as a surrogate for shorter term human population presence in the study area. This method is not completely accurate for estimating small area populations since wastewater infrastructure is inherently leaky and transient changes are not captured, but these gaps play into this project's goals of identifying human fecal contamination, regardless of input paths. While poor correlations existed between WWTP discharge volume and *B. theta* ($p = 0.993$) and coliphage CN-13 ($p = 0.871$) in Mitchell Creek, direct associations ($p \leq 0.002$) were identified between WWTP discharge volumes and *E.*

coli, enterococci, and *C. perfringens* concentrations in Mitchell Creek water. Interestingly, there are only four reported discharge points in Mitchell Creek watershed, none of which involve sewage, suggestive of non-point sources of pollution (i.e. leaking wastewater infrastructure or illicit discharges) as the primary sources of human fecal material.

In the current study, land use and microbe concentration analysis indicated the most appropriate spatial scale for investigating *E. coli*, enterococci, *C. perfringens*, and coliphage CN-13 in the Mitchell Creek was generally at the catchment scale. Specifically, sites with agriculture cover above 38% in the catchment exhibited higher *E. coli* concentrations ($> 2.49 \log_{10}$ MPN 100 ml^{-1}) in water compared to sites with less than 38% agriculture cover. However, thorough analysis indicated wetlands, urban, agriculture, and natural land types appear to be acting as sources of *E. coli*, enterococci, *C. perfringens*, and coliphage in the Mitchell Creek. Differentiating between specific land uses at any scale does not seem plausible using *E. coli* and enterococci alone, as supported by Kang et al. (2010). Additional monitoring with a larger suite of molecular source markers coupled with land use analysis at the catchment scale is recommended.

While land use was not helpful for identifying water quality microbial impairments, cluster analysis showed that the most polluted cluster (MC2, MC3, MC4, MC5, and MC8) had the highest average of agricultural cover (41%) at the catchment scale. Each site in this cluster was composed of less than 40% wetland and forested cover combined and more than 20% urban development in the catchment. Of special note in this cluster, MC5 had the highest overall *E. coli*, *C. perfringens*, and *B. theta* geometric means recorded amongst all sites and coliphage was detected in 100% of water samples, suggesting a chronic input of human feces entering Mitchell

Creek near MC5. In fact, a spatial survey of the surrounding area identified an old wastewater lift station with structural integrity concerns. The lower river flow rates observed at MC6 may be allowing time for *C. perfringens* spores and coliphage viruses to settle out of the water column and accumulate in the sediment, resulting in the stark differences between sediment and water concentrations not observed at any other site. These results demonstrate, and Goto and Yan (2011) support, sediment in forested creek stretches contain more bacteria than creek sediments in urban areas.

Characterization of environmental and physical parameters in parallel with microbial analysis revealed precipitation significant relationships between *E. coli*, enterococci, *C. perfringens*, coliphage CN-13, and *B. theta* levels in Mitchell Creek and TCSP water ($p < 0.01$). Furthermore, temporal analysis indicated precipitation had a more rapid effect on water quality (*E. coli* and enterococci responded to rainfall in 1 hour) than sediment quality (*E. coli* and enterococci responded rainfall in 12-24 hours). These results were not surprising given a number of studies have previously demonstrated precipitation driven microbe concentrations in water (Fong et al. 2007; Converse et al. 2011; Walters et al. 2011). In a small watershed (16 km^2) with river flow rates similar to those seen in the Mitchell Creek, Goto et al. (2011) reported that 72 hour cumulative rainfall was a strong positive driver of *E. coli*, enterococci, and *C. perfringens* in urban and agriculture dominated catchments, but not in catchments dominated by forests.

It is likely that the slower microbial response to precipitation seen in sediments was the result of either creek flow rates, particularly the slowing of flows, and the settling of *E. coli* and enterococci out of the water column following initial loading of runoff and bacteria or the introduction from shallow groundwater. As indicated by enterococci and coliphage concentrations, precipitation impacts on water quality were more pronounced in the creeks lower reaches which were directly and significantly related to creek discharge rates ($p < 0.002$). A discharge threshold for Mitchell Creek was identified (86th percentile; range = 0.025 to 0.509 $m^3 s^{-1}$) which was observed when enterococci (33%) and coliphage CN-13 (89%) samples exceeded 95th percentiles of this project data set distribution. This threshold is recommended to guide future sampling as the rate represents an important statistical threshold, above which water samples should be assessed for enterococci, *C. perfringens*, coliphage CN-13, and *B. theta*. Analysis should also measure *Salmonella* spp. which was detected more often when river discharge exceeded the 83rd percentile in an agriculturally dominated stream (Wilkes et al. 2011). Similar discharge thresholds may be applied to other small, flashy, and highly mixed watersheds throughout the Great Lakes.

Transport mechanisms at the TCSP beach were different than those observed in the Mitchell Creek. Specifically, *E. coli* and coliphage CN-13 levels at TCSP were transported by wind (direction and speed) while enterococci and *C. perfringens* were influenced by the Mitchell Creek (water quality and discharge). Interestingly, *B. theta* was driven by both wind and Mitchell Creek. It was speculated that *C. perfringens* and *B. theta* were more influenced by the Mitchell Creek because their associated methods detected persistent targets that do not readily grow in water (Tallon et al. 2005; Desmarais et al. 2002; Ballesté, and Blanch 2010; Converse et al. 2009), whereas *E. coli* and enterococci associations may have been masked by growth and coliphage by inactivation. Dwight et al. (2002) reported correlations between discharge and enterococci were highest at beaches next to river outlets. Results presented here strengthen such findings as *C. perfringens*, *B. theta*, and enterococci from the Mitchell Creek negatively impacted the nearby TCSP water.

It is recommended that in addition to discharge threshold sampling, *E. coli* and coliphage CN-13 monitoring at TCSP should be conducted when 12 hour cumulative precipitation exceeds 0.23 mm, wind is out of the southwest, and wave height exceeds 0.13 m to coincide with the greatest potential risk to bathers and *E. coli* levels. It is strongly suggested that TCSP beach monitoring expand to include analysis near the mouth of the Mitchell Creek and include molecular source tracking and multiple fecal indicators (*E. coli*, enterococci, and coliphage CN-13). Eventually, this type of data should feed into mechanistic and predictive models at TCSP beach to better understand the timing and quantity of pollution entering the TCSP designated swimming area.

Results indicated both die-off and regrowth of bacteria need to be considered when developing sampling strategy. Daily fecal indicator concentrations (*E. coli* and enterococci) were lower when sampled in the afternoon, potentially from increased temperatures or solar radiation. Thus, it is recommended that morning is the most appropriate time to collect the samples. On the other hand, during peak recreational activity, bacteria were likely regrowing as observed temperature ranges were consistent with previously reported growth thresholds for *E. coli* (18 - 44.5 °C) and enterococci (9 - 47.8 °C) (Johnson and Lewin 1946; Fisher and Phillips 2009; Borrego et al. 2002). This is another reason why coliphage and *C. perfringens* should be included as part of the sampling in watersheds that are supporting high levels of traditional enteric bacteria.

Local concerns surrounding the Mitchell Creek's influence on TCSP bathing water was the initially reason for this study. Regression analysis showed a portion of TCSP water quality could be explained by the previous hours Mitchell Creek water quality while birds and contaminated runoff from outside the Mitchell Creek may also be contributing fecal contamination, as previously suggested by Haack et al. (2003), this should be further investigated particularly with the coliphage markers. However, the Creek water may be more influential then test results indicate as great uncertainty was associated with the number and timing of sampling events which were not frequent enough to define associates between the creek and beach due to their close proximity. Long-term water quality improvement in the Mitchell Creek, and subsequently at TCSP, should focus on 1) the remediation of wetlands throughout the watershed while adopting minimum catchment wetland coverage (14.5%) policy to minimize the effects of runoff and reduce water flow rates and 2) investigate wastewater and stormwater infrastructure, including on-site septic systems, for structural integrity and illicit connections as human fecal contamination was identified throughout the watershed.

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APPENDIX

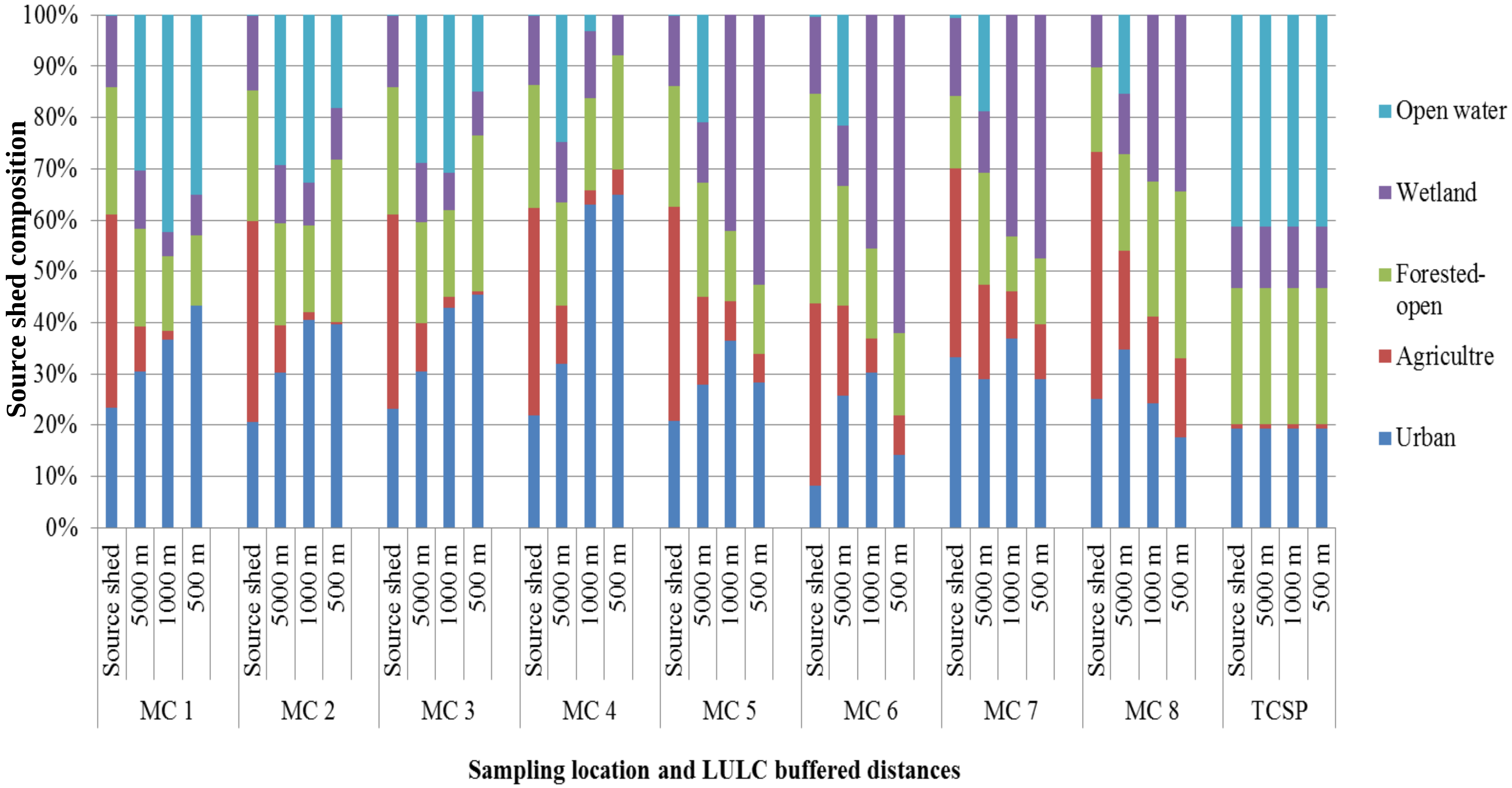

Figure S.3.1. Land use patterns at multiple scales for each sampling location.

Table S.3.1. Number of water and sediment samples assayed for *E. coli*, enterococci, *C. perfringens*, and coliphage CN-13.

Microorganism	Medium	Site								
		TCSP	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8
E. coli	Water	43	26	42	42	42	42	42	22	42
	Sediment	29	0	29	28	19	29	29	0	28
Enterococci	Water	41	25	41	41	41	41	41	21	41
	Sediment	28	0	28	27	19	28	28	0	27
C. perfringens	Water	21	23	22	22	22	22	21	19	22
	Sediment	9	0	9	8	0	9	9	0	8
CN-13	Water	22	22	22	22	22	22	22	20	22
	Sediment	9	0	9	8	0	9	9	0	8

Fewer sediment samples were collected in comparison to water samples as sediment analysis was added to the project in 2010 only. No sediment samples collected at MC1and MC7 because the bottom substrate was cobbled rock or a culvert, respectively. MC1 and MC7 were also not included in the intensive study, explaining the lower number of water and sediment samples at these two sites. MC7 was also an intermediate stream site and water was not always available for sample collection at this site. MC4 was included in the intensive study, however, prior to that event sediment samples were not collected.

Table S.3.2. Water *E. coli*, enterococci, *C. perfringens*, and coliphage CN-13 recordings for each event at TCSP and in the Mitchell Creek.

Date	Indicator	TCSP	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8
6/24/2009	*E. coli*	0.48	2.86	2.24	2.94	2.89	3.38	1.99	2.19	3.24
	Enterococci		2.69	2.37	2.71	2.51	2.70	1.96	2.51	2.65
	C. perfringens		1.11	0.75	1.27	1.32	0.90	0.64	0.93	1.33
	CN-13	1.04	2.34	1.91	2.15	1.96	2.12	1.32	1.04	1.04
7/1/2009	*E. coli*	1.44	3.38							
	Enterococci	1.50	3.62							
	C. perfringens	0.25	1.68							
	CN-13									
7/8/2009	*E. coli*	1.41	2.71	1.79	2.76	2.86	2.99	1.78	2.10	2.51
	Enterococci	2.39	2.84	2.43	3.12	3.06	3.19	1.95	2.33	2.29
	C. perfringens	0.53	1.16	0.91	1.24	1.31	1.45	1.15	0.59	1.24
	CN-13	1.04	1.71	1.71	1.71	2.21	1.61	1.96	1.71	1.04
7/15/2009	*E. coli*	0.71	2.74	1.90	2.66	2.79	3.02	2.02	2.04	2.79
	Enterococci	2.65	3.01	2.71	3.24	3.12	3.33	2.36	2.68	3.12
	C. perfringens	0.05	1.13	1.03	1.23	1.24	1.67	1.00	1.37	1.16
	CN-13	1.13	1.61	2.05	1.32	1.61	1.04	1.04	1.32	1.49
7/22/2009	*E. coli*	0.48	3.24	3.24	3.24	3.30	2.94	2.20		3.15
	Enterococci	1.29	3.52	3.74	3.52	3.49	3.05	2.77		3.19
	C. perfringens	0.39	1.67	1.35	1.67	2.15	2.04	0.29		1.38
	CN-13	1.04	1.32	2.00	1.32	1.32	2.05	1.04		1.85
7/29/2009	*E. coli*	0.97	2.76	2.27	2.81	2.84	2.84	2.04	2.32	2.59
	Enterococci	0.29	3.08	2.78	3.16	2.95	3.14	2.39	2.53	2.91
	C. perfringens	0.29	2.03	1.03	2.06	1.99	2.26	1.04	0.74	1.85
	CN-13	1.32	1.85	1.04	1.80	1.49	1.96	1.32	1.85	2.08
8/3/2009	*E. coli*	0.30	3.24	2.79	3.19	3.19	3.11	3.05	3.08	3.08
	Enterococci	0.51	3.49	3.21	3.58	3.54	3.71	2.94	3.28	3.49
	C. perfringens	0.05	1.53	1.16	1.63	2.16	2.50	1.03	0.87	2.38
	CN-13	1.04	3.18	2.05	3.18	3.15	3.54	2.52	3.20	3.27

♦ mCP auger not available; *Average of 12 samples collected hourly starting at 07:00 EST; *E. coli* reported as MPN 100 ml-1 dry sediment weight; Enterococci reported as CFU 100 ml^{-1} dry sediment weight; Due to time constraints and processing logistics, *C. perfringens* and coliphage CN-13 were not assayed during intensive study beginning August 7, 2010.

Table S.3.2. (cont'd)

Date	Indicator	TCSP	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8
8/9/2009	*E. coli*	1.23	2.81	3.08	2.64	2.76	2.84	2.61	2.74	2.86
	Enterococci	0.79	3.20	3.20	3.21	3.23	3.40	3.01	3.09	3.27
	C. perfringens		1.87	0.87	2.06	2.47	2.30		0.64	2.20
	CN-13									
8/12/2009	*E. coli*	1.11	2.56	2.29	2.44	2.61	2.59	1.76	2.29	2.41
	Enterococci	2.07	3.32	3.21	3.36	3.28	3.49	2.73	3.12	3.39
	C. perfringens	0.31	1.56	1.07	1.34	1.26	1.58	0.80	0.83	1.30
	CN-13	1.04	2.18	2.60	1.85	1.91	2.08	1.32	2.62	1.96
8/17/2009	*E. coli*	1.03	3.11	3.38	2.69	2.69	2.81	1.80	2.20	2.59
	Enterococci	0.30	3.15	3.38	3.02	3.02	3.08	2.38	3.11	2.99
	C. perfringens♦									
	CN-13	1.04	2.21	2.97	2.30	2.05	1.96	1.32	1.85	1.71
8/26/2009	*E. coli*	1.75	3.02	2.74	3.38	2.61	2.81	2.81	3.08	2.99
	Enterococci	1.56	3.20	3.13	4.50	3.28	3.36	2.72	2.94	3.25
	C. perfringens	0.19	1.67	1.70	1.05	1.79	1.90	0.82	1.18	1.73
	CN-13	1.04	1.61	2.34	1.49	2.15	2.26	1.85	2.57	2.61
8/31/2009	*E. coli*	0.48	2.30	3.48	2.42	2.37	2.32	2.07	2.15	2.34
	Enterococci	1.32	2.84	3.64	2.81	2.63	3.11	2.39	2.91	3.02
	C. perfringens♦									
	CN-13	1.04	2.08	2.51	2.15	2.21	2.71	1.49	3.00	2.08
9/2/2009	*E. coli*	0.79	2.24	2.86	2.14	2.16	2.38	1.92	2.29	2.27
	Enterococci	0.85	2.49	3.26	2.54	2.39	2.57	2.28	2.44	2.51
	C. perfringens	0.10	1.41	1.04	1.35	1.71	1.97	1.15	1.66	1.67
	CN-13	1.04	1.04	1.71	1.04	1.04	1.32	1.04	2.05	1.32
9/9/2009	*E. coli*	1.74	2.29	2.99	2.05	2.21	2.51	1.79	2.49	2.27
	Enterococci	1.30	2.50	3.45	2.57	2.49	2.74	2.18	2.61	2.60
	C. perfringens	0.62	1.30	1.57	1.41	1.56	1.69	1.23	1.45	1.63
	CN-13	1.04	1.32	1.04	1.04	1.04	1.04	1.04	2.68	1.04
9/16/2009	*E. coli*	1.76	2.40	0.48	2.34	2.18	2.36	1.82		2.42
	Enterococci	1.56	2.83	2.56	2.84	2.74	2.86	2.63		2.80
	C. perfringens	0.18	1.45	1.18	1.37	1.60	1.92	1.14		1.89
	CN-13									

Table S.3.2. (cont’d)

Date	Indicator	TCSP	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8
11/2/2009	*E. coli*		1.66	1.42	1.39	1.65	1.56	1.08	1.23	1.37
	Enterococci		1.80	2.10	1.69	1.82	1.64	1.58	1.71	1.87
	C. perfringens		1.43	1.17	0.93	1.00	0.70	0.83	0.64	0.71
	CN-13		1.61	2.28	1.49	1.32	1.71	1.04	1.61	1.91
6/7/2010	*E. coli*	1.59	2.59	2.84	2.69	2.61	3.05	2.16	2.79	2.66
	Enterococci	1.23	2.21	3.10	2.54	2.66	2.82	1.95	2.67	2.30
	C. perfringens	0.43	1.52	1.08	1.25	1.68	1.97	0.94	0.70	1.59
	CN-13	1.04	2.23	3.12	1.71	2.15	2.23	2.00	1.71	2.18
6/10/2010	*E. coli*	2.61	2.40	2.00	2.42	2.42	2.69	1.94	2.35	2.21
	Enterococci	2.26	2.49	1.95	2.43	2.39	2.42	1.97	2.71	2.08
	C. perfringens	0.95	1.21	1.00	1.20	1.29	1.26	0.85	0.56	1.30
	CN-13	1.04	1.91	2.72	2.00	1.71	2.18	1.32	1.49	2.00
6/14/2010	*E. coli*	2.17	2.76	2.51	2.69	2.66	2.71	2.16	2.69	2.59
	Enterococci	1.86	2.72	2.32	2.69	2.71	2.77	2.25	2.44	2.71
	C. perfringens	0.83	0.56	0.83	1.02	0.85	0.83	0.50	0.61	1.05
	CN-13									
6/24/2010	*E. coli*	2.89	3.30	2.47	3.38	3.38	3.38	2.99	3.38	3.38
	Enterococci	2.74	3.24	2.63	3.31	3.41	3.47	2.71	3.27	3.28
	C. perfringens	0.48	0.64	0.62	0.41	1.00	1.90	0.43	0.26	0.67
	CN-13	3.11	3.71	3.85	3.85	3.73	3.60	3.56	3.74	3.57
6/27/2010	*E. coli*	2.01	2.76	2.81	2.50	2.61	2.72	2.81	2.12	2.79
	Enterococci	2.70	2.72	2.55	2.58	2.56	2.68	2.11	2.43	2.57
	C. perfringens	0.24	1.45	0.56	1.58	1.88	2.30	0.85	0.66	1.86
	CN-13	1.04	1.91	2.46	1.91	2.08	2.05	1.85	2.43	2.05
7/1/2010	*E. coli*	1.11	2.54	2.24	2.44	2.40	2.76	2.22	2.51	2.64
	Enterococci	1.18	2.53	2.31	2.43	2.41	2.48	2.46	2.51	2.44
	C. perfringens	0.34	1.22	1.13	0.74	1.12	1.90	0.72	0.44	1.42
	CN-13	1.13	1.32	1.91	1.04	1.49	1.61	1.04	2.23	1.32
7/5/2010	*E. coli*	1.31	2.74	2.69	2.51	2.79	2.94	2.47	3.02	2.84
	CN-13	1.13	1.13	2.00	1.95	2.10	2.10	1.13	2.69	1.95

Table S.3.2. (cont'd)

Date	Indicator	TCSP	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8
7/12/2010	*E. coli*	1.49	2.66	3.08	2.64	2.71	2.81	2.49	3.24	2.94
	Enterococci	1.01	3.13	3.26	2.83	2.85	2.84	2.57	3.17	2.71
	C. perfringens	0.18	1.01	1.23	1.00	0.85	1.37	0.74	0.62	1.17
	CN-13	1.13	1.04	1.61	1.49	1.49	1.79	1.04	2.15	1.85
7/15/2010	*E. coli*	1.32	3.15	3.38	2.84	2.91	2.99	2.81	3.38	2.84
	Enterococci	0.85	3.47	3.16	2.94	2.94	2.41	2.79	3.41	3.06
	C. perfringens	0.18	0.94	1.25	0.91	0.99	1.58	0.99	0.12	0.48
	CN-13	1.13	2.21	2.12	1.49	1.91	2.59	1.71	2.42	2.59
7/18/2010	*E. coli*	0.61	2.79	2.51	2.61	2.71	2.61	2.66		2.71
	Enterococci	0.30	2.76	2.40	2.64	2.54	2.69	2.12		2.61
	C. perfringens	0.18	1.11	0.82	1.00	1.01	1.32	0.56		0.87
	CN-13	1.13	1.96	2.81	1.61	1.71	1.61	1.04		2.12
8/7/2010**	*E. coli*	1.12		2.44	2.45	2.47	2.67	2.17		2.71
	Enterococci	1.15		2.27	2.39	2.36	2.55	1.95		2.47
8/8/2010	*E. coli*	2.61		3.38	3.38	3.38	3.38	3.38		3.24
	Enterococci	2.26		3.36	3.33	3.11	3.46	3.27		3.31
8/10/2010	*E. coli*	1.47		2.27	2.74	2.69	2.96	2.17		2.81
	Enterococci	1.16		2.31	2.68	2.68	2.78	2.17		2.60
8/12/2010	*E. coli*	1.48		2.74	2.42	2.51	2.71	2.64		2.76
	Enterococci	1.16		2.59	2.61	2.60	2.80	2.29		2.73
8/14/2010	*E. coli*	1.61		2.49	2.81	2.99	3.05	2.24		2.96
	Enterococci	1.44		2.69	2.70	2.80	2.94	2.28		2.83
8/17/2010	*E. coli*	1.34		2.43	2.79	2.61	2.91	2.18		2.84
	Enterococci	0.88		2.62	2.77	2.81	2.92	2.30		2.91
3/19/2011	*E. coli*	0.30	1.12	0.30	0.86	0.61	1.13	1.03	0.48	1.45
	Enterococci	0.30	1.26	1.08	1.03	0.98	1.89	1.21	1.03	1.56
3/20/2011	*E. coli*	0.30	1.83	0.79	1.82	1.72	1.94	0.61	0.61	2.05
	Enterococci	0.30	1.95	1.32	2.32	2.46	2.71	1.32	1.19	2.81

Table S.3.3. Sediment *E. coli*, enterococci, *C. perfringens*, and coliphage CN-13 recordings for each event at TCSP and in the Mitchell Creek.

Date	Indicator	TCSP	MC2	MC3	MC4	MC5	MC6	MC8
6/7/2010	*E. coli*	2.50	3.20			3.80	4.00	
	Enterococci	2.20	3.10			3.60	4.00	
	C. perfringens	2.10	3.50			3.57	4.97	
	CN-13	< 2.10	< 2.11			2.14	< 2.90	
6/10/2010	*E. coli*	2.20	2.70	3.10		3.70	5.90	3.20
	Enterococci	2.70	2.90	3.10		3.50	6.30	2.80
	C. perfringens	< 1.11	3.00	2.94		3.78	6.52	2.87
	CN-13	< 1.11	< 1.15	< 1.14		1.18	< 3.90	< 1.14
6/14/2010	*E. coli*	2.30	4.50	4.30		4.00	4.10	3.40
	Enterococci	2.30	3.70	3.40		3.10	3.30	3.00
	C. perfringens	< 1.14	3.47	3.10		3.58	3.84	1.81
	CN-13							
6/24/2010	*E. coli*	2.50	3.80	3.80		3.70	4.50	4.00
	Enterococci	2.90	3.30	3.70		3.40	4.00	3.80
	C. perfringens	1.57	3.31	2.76		3.46	3.45	1.97
	CN-13	< 1.11	2.67	2.19		1.49	2.39	1.61
6/27/2010	*E. coli*	1.90	3.60	5.40		4.10	4.30	3.90
	Enterococci	2.20	2.90	4.50		3.20	3.40	2.90
	C. perfringens	< 1.15	2.93	4.71		2.80	3.95	3.48
	CN-13	< 1.15	2.59	3.10		1.17	< 1.94	< 1.15
7/1/2010	*E. coli*	< 1.1	3.80	3.50		3.60	4.10	4.60
	Enterococci	1.40	3.30	3.20		2.70	3.40	4.30
	C. perfringens	< 1.13	2.57	2.88		2.39	3.60	2.98
	CN-13	< 1.13	2.39	1.43		< 1.18	< 1.85	2.75
7/12/2010	*E. coli*	1.90	3.80	3.20		3.50	2.90	4.70
	Enterococci	1.10	3.00	3.20		3.00	2.70	3.70
	C. perfringens	1.13	2.92	2.89		2.18	2.57	3.44
	CN-13	< 1.13	1.97	< 1.15		< 1.16	< 1.18	1.63

*Average of 12 samples collected hourly starting at 07:00 EST; *E. coli* reported as log$_{10}$ MPN 100 g^{-1} dry sediment weight; Enterococci and *C. perfringens* reported as log$_{10}$ CFU 100 g^{-1} dry sediment weight; Coliphage CN-13 reported as log$_{10}$ PFU 100 g^{-1} dry sediment weight; Due to time constraints and processing logistics, *C. perfringens* and coliphage CN-13 were not assayed during intensive study beginning August 7, 2010.

Table S.3.3. (cont'd)

Date	Indicator	TCSP	MC2	MC3	MC4	MC5	MC6	MC8
7/15/2010	*E. coli*	1.40	3.80	3.70		3.10	4.50	4.40
	Enterococci	2.10	3.20	3.30		2.80	4.50	3.50
	C. perfringens	1.59	2.63	2.72		1.63	3.69	2.09
	CN-13	< 1.13	1.84	1.61		1.17	< 1.97	2.31
7/18/2010	*E. coli*	2.40	3.70	3.00		3.90	3.40	3.40
	Enterococci	1.10	3.50	2.80		3.20	3.00	3.20
	C. perfringens	1.14	2.51	2.70		2.30	2.11	2.67
	CN-13	< 1.14	1.62	< 1.14		1.47	< 1.18	< 1.18
8/7/2010*	*E. coli*	2.00	3.50	3.70	3.10	3.20	2.80	3.60
	Enterococci	2.10	2.90	3.10	2.80	2.50	2.30	2.80
8/8/2010	*E. coli*	1.10	4.60	3.70	3.40	3.60	3.50	4.40
	Enterococci	1.80	4.00	3.20	2.60	3.10	3.00	3.70
8/10/2010	*E. coli*	2.00	3.70	3.60	4.40	3.90	3.50	4.50
	Enterococci	1.80	2.80	3.30	3.30	3.00	2.60	3.50
8/12/2010	*E. coli*	2.20	4.00	3.40	3.50	4.20	3.50	4.10
	Enterococci	1.60	3.00	3.00	3.00	2.90	2.70	3.30
8/14/2010	*E. coli*	1.70	3.80	3.50	2.60	4.00	3.40	4.40
	Enterococci	2.80	2.90	3.60	3.10	3.50	3.40	3.70
8/17/2010	*E. coli*	2.00	3.80	3.30	4.00	3.60	3.10	4.50
	Enterococci	2.00	3.30	2.70	2.70	2.80	3.00	3.50
3/19/2011	*E. coli*	< 1.2	1.70	2.10	2.10	2.20	2.20	2.70
	Enterococci	1.60	2.40	3.40	2.70	2.80	3.60	4.00
3/20/2011	*E. coli*	< 1.2	1.80	2.30	1.70	2.20	3.20	2.60
	Enterococci	1.20	1.70	2.80	2.40	2.80	3.20	3.40

Table S.3.4. Summary statistics of physical and environmental properties for all sites.

Variable name	Unit	n	Minimum	Mean	Maximum	Standard Deviation
Air temp.	°C	361	-0.80	18.00	34.50	8.02
Water temp.	°C	362	-0.50	14.43	26.00	4.70
Air temp. (day mean)	°C	361	0.00	18.47	27.70	5.87
Barometric pressure	Hg in.	360	0.00	28.88	32.67	3.72
Relative humidity	%	359	30.45	79.99	97.50	17.76
Solar radiation (day mean)	$\mathrm{kJ\ m^{-2}}$	360	1981.30	18284.95	28038.00	5758.52
WWTP	MGD	378	3.75	4.46	5.03	0.32
Wind speed	$\mathrm{Km\ h^{-1}}$	378	0.00	5.55	24.14	6.22
Wind direction	Degree	378	-	154.65	-	87.75
Wave height*	m	45	0.00	6.55	1.83	0.33
Precipitation 1 hrs	mm	336	0.00	0.13	4.13	0.65
Precipitation 2 hrs	mm	336	0.00	0.24	5.63	1.00
Precipitation 3 hrs	mm	336	0.00	0.42	8.42	1.46
Precipitation 4 hrs	mm	336	0.00	0.87	18.08	3.11
Precipitation 6 hrs	mm	336	0.00	0.96	18.08	3.23
Precipitation 8 hrs	mm	336	0.00	1.01	18.08	3.24
Precipitation 12 hrs	mm	336	0.00	1.62	23.29	4.19
Precipitation 16 hrs	mm	336	0.00	2.06	23.29	4.77
Precipitation 24 hrs	mm	336	0.00	2.08	23.29	4.77
Precipitation 48 hrs	mm	336	0.00	5.31	29.21	6.48
Precipitation 72 hrs	mm	336	0.00	9.42	73.88	13.87

*Measured at TCSP only

Table S.3.5. Spearman's correlation matrix of environmental, weather, and microorganisms in water (Mitchell Creek and TCSP).

	E. coli	Enterococci	*C. perfringens*	Coliphage	Air temp.	Water temp.	Daily air temp.	Barometric P.	Humidity	Solar radiation	Discharge	1 hour precip.	2 hour precip.	4 hour precip.	8 hour precip.	12 hour precip.	16 hour precip.	24 hour precip.	48 hour precip.
Enterococci	**0.80**																		
C. perfringens	**0.37**	**0.46**																	
Coliphage	**0.54**	**0.47**	*0.18*																
Air temp.	**0.25**	0.05	0.11	**0.22**															
Water temp.	0.05	0.06	**0.34**	0.10	**0.74**														
Daily air temp.	**0.34**	**0.22**	0.02	0.07	**0.64**	**0.56**													
Barometric P.	**0.21**	0.05	0.13	0.06	**0.64**	**0.47**	**0.49**												
Humidity	**0.20**	**0.34**	0.03	0.04	0.40	*0.13*	0.04	**0.47**											
Solar radiation	0.01	0.05	0.06	0.03	**0.32**	**0.21**	**0.16**	*0.12*	**0.29**										
Discharge	**0.20**	**0.15**	0.02	**0.28**	0.05	**0.20**	*0.12*	*0.11*	0.03	0.01									
1 hour precip.	**0.17**	*0.14*	0.01	0.02	0.03	0.07	**0.17**	**0.14**	**0.17**	**0.37**	0.04								
2 hour precip.	**0.25**	**0.20**	0.01	0.02	0.03	0.09	**0.23**	0.04	**0.20**	**0.29**	0.02	**0.89**							
4 hour precip.	**0.27**	**0.26**	0.09	0.02	0.08	**0.19**	**0.30**	0.06	**0.15**	**0.34**	0.03	**0.77**	**0.89**						
8 hour precip.	**0.30**	**0.27**	0.03	0.03	*0.13*	**0.19**	**0.42**	**0.17**	**0.14**	**0.36**	0.01	**0.68**	**0.78**	**0.87**					
12 hour precip.	**0.45**	**0.43**	0.06	**0.29**	**0.22**	**0.27**	**0.27**	**0.17**	**0.18**	0.30	0.04	**0.54**	**0.64**	**0.72**	**0.80**				
16 hour precip.	**0.46**	**0.44**	0.01	**0.33**	**0.23**	**0.28**	**0.26**	**0.17**	**0.18**	**0.29**	0.04	**0.50**	**0.60**	**0.68**	**0.78**	**0.99**			
24 hour precip.	**0.44**	**0.39**	0.02	**0.37**	**0.16**	**0.22**	*0.13*	0.10	**0.19**	**0.24**	0.07	**0.47**	**0.56**	**0.64**	**0.72**	**0.93**	**0.94**		
48 hour precip.	**0.20**	**0.15**	0.08	**0.47**	**0.26**	**0.25**	0.08	0.08	**0.16**	**0.23**	0.06	**0.26**	**0.35**	**0.45**	**0.35**	**0.48**	**0.49**	**0.58**	
72 hour precip.	**0.15**	*0.13*	0.06	**0.46**	**0.16**	**0.22**	0.01	0.03	0.07	**0.24**	0.08	*0.13*	**0.22**	**0.32**	**0.20**	**0.31**	**0.32**	**0.40**	**0.77**

Values *italics* indicate correlations significant at the 0.05 level; Values in **bold** indicate correlations significant at the 0.01 level; Shaded boxes represent inverse (negative) correlation coefficients; Refer to Table S.3.4. for variable units.

Table S.3.6. Spearman's correlation matrix of environmental, weather, and microorganisms in sediment (Mitchell Creek and TCSP).

	E. coli	Enterococci	*C. perfringens*	Coliphage	Air temp.	Water temp.	Daily air temp.	Barometric P.	Humidity	Solar radiation	Discharge	1 hour precip.	2 hour precip.	4 hour precip.	8 hour precip.	12 hour precip.	16 hour precip.	24 hour precip.	48 hour precip.
Enterococci	**0.72**																		
C. perfringens	**0.70**	**0.68**																	
Coliphage	**0.76**	**0.75**	**0.59**																
Air temp.	0.03	0.12	0.18	0.04															
Water temp.	**0.23**	**0.26**	**0.47**	**0.36**	**0.74**														
Daily air temp.	**0.22**	0.04	0.24	0.15	**0.64**	**0.56**													
Barometric P.	0.01	0.02	0.27	0.25	**0.64**	**0.47**	**0.49**												
Humidity	**0.32**	**0.27**	0.01	0.13	**0.40**	**0.13**	0.04	**0.47**											
Solar radiation	0.05	0.07	0.09	0.21	**0.31**	**0.21**	**0.16**	*0.12*	**0.29**										
Discharge	**0.42**	**0.29**	*0.35*	**0.56**	0.05	**0.20**	*0.12*	*0.11*	0.03	0.01									
1 hour precip.	0.14	0.13	0.04	0.08	0.03	0.07	**0.17**	**0.14**	**0.17**	**0.37**	0.04								
2 hour precip.	*0.15*	*0.15*	0.04	0.08	0.03	0.09	**0.23**	0.04	**0.20**	**0.29**	0.02	**0.89**							
4 hour precip.	*0.15*	*0.15*	0.04	0.08	0.08	**0.19**	**0.30**	0.06	**0.15**	**0.34**	0.03	**0.77**	**0.89**						
8 hour precip.	*0.16*	0.15	0.01	0.01	*0.13*	**0.19**	**0.42**	**0.17**	**0.14**	**0.36**	0.01	**0.68**	**0.78**	**0.87**					
12 hour precip.	**0.20**	**0.24**	0.01	0.08	**0.22**	**0.27**	**0.27**	**0.17**	**0.18**	**0.30**	0.04	**0.54**	**0.64**	**0.72**	**0.80**				
16 hour precip.	**0.20**	**0.24**	0.02	0.11	**0.23**	**0.28**	**0.26**	**0.17**	**0.18**	**0.29**	0.04	**0.50**	**0.60**	**0.68**	**0.78**	**0.99**			
24 hour precip.	*0.18*	**0.29**	0.06	0.09	**0.16**	**0.21**	*0.13*	0.09	**0.18**	**0.24**	0.07	**0.47**	**0.56**	**0.64**	**0.72**	**0.93**	**0.94**		
48 hour precip.	0.08	0.04	0.24	0.23	**0.26**	**0.25**	0.08	0.08	**0.16**	**0.23**	0.06	**0.26**	**0.35**	**0.45**	**0.35**	**0.48**	**0.49**	**0.58**	
72 hour precip.	0.05	0.06	*0.30*	0.21	**0.16**	**0.22**	0.01	0.03	0.07	**0.23**	0.08	*0.13*	**0.22**	**0.32**	**0.20**	**0.31**	**0.32**	**0.40**	**0.77**

Values *italics* indicate correlations significant at the 0.05 level; Values in **bold** indicate correlations significant at the 0.01 level; Shaded boxes represent inverse (negative) correlation coefficients; Refer to Table S.3.4. for variable units.

Table S.3.7. Average daily discharge for each Mitchell Creek site (m^3 s^{-1}).

Date	MC1	MC2	MC3	MC4	MC5	MC6	MC7	MC8
6/24/2009	0.623	0.041	0.583	0.587	0.345	0.244	0.082	0.256
7/1/2009	0.743	0.049	0.695	0.699	0.411	0.291	0.098	0.305
7/8/2009	0.605	0.04	0.566	0.570	0.335	0.237	0.080	0.248
7/15/2009	0.593	0.039	0.555	0.558	0.328	0.233	0.078	0.243
7/22/2009	0.623	0.041	0.583	0.587	0.345	0.244	0.082	0.256
7/29/2009	0.581	0.038	0.543	0.547	0.321	0.228	0.077	0.238
8/3/2009	0.641	0.042	0.599	0.604	0.355	0.251	0.084	0.263
8/9/2009	0.605	0.04	0.566	0.570	0.335	0.237	0.080	0.248
8/12/2009	0.665	0.044	0.622	0.626	0.368	0.261	0.088	0.273
8/17/2009	0.629	0.041	0.588	0.592	0.348	0.247	0.083	0.258
8/26/2009	0.611	0.04	0.571	0.575	0.338	0.240	0.080	0.251
8/31/2009	0.641	0.042	0.599	0.604	0.355	0.251	0.084	0.263
9/2/2009	0.695	0.046	0.650	0.654	0.384	0.273	0.091	0.285
9/9/2009	0.623	0.041	0.583	0.587	0.345	0.244	0.082	0.256
9/16/2009	0.563	0.037	0.527	0.530	0.311	0.221	0.074	0.231
11/2/2009	**0.534**	**0.035**	**0.499**	0.502	**0.295**	0.209	**0.070**	**0.219**
6/7/2010	0.893	0.058	0.835	0.841	0.494	0.350	0.118	0.366
6/10/2010	**0.599**	**0.039**	**0.560**	**0.564**	**0.331**	**0.235**	0.079	**0.246**
6/14/2010	0.635	0.042	0.594	0.598	0.351	0.249	0.084	0.260
6/24/2010	**1.043**	**0.068**	**0.975**	**0.982**	**0.577**	**0.409**	0.137	**0.427**
6/27/2010	0.683	0.045	0.639	0.643	0.378	0.268	0.090	0.280
7/1/2010	0.647	0.042	0.605	0.609	0.358	0.254	0.085	0.265
7/5/2010	0.563	0.037	0.527	0.530	0.311	0.221	0.074	0.231
7/12/2010	0.522	0.034	0.487	0.491	0.288	0.204	0.069	0.214
7/15/2010	0.486	0.032	0.454	0.457	0.268	0.190	0.064	0.199
7/18/2010	0.480	0.031	0.448	0.451	0.265	0.188	0.063	0.197
8/7/2010	0.468	0.031	**0.437**	**0.440**	**0.258**	**0.183**	0.062	**0.192**
8/8/2010	0.456	0.03	0.426	0.429	0.252	0.179	0.060	0.187
8/10/2010	0.456	0.03	0.426	0.429	0.252	0.179	0.060	0.187
8/12/2010	0.456	0.03	0.426	0.429	0.252	0.179	0.060	0.187
8/14/2010	0.456	0.03	0.426	0.429	0.252	0.179	0.060	0.187
8/17/2010	0.456	0.03	0.426	0.429	0.252	0.179	0.060	0.187
3/19/2011	0.456	0.03	0.426	0.429	0.252	0.179	0.060	0.187
3/20/2011	0.456	0.03	0.426	0.429	0.252	0.179	0.060	0.187

Values in bold represent actual discharge measurements; All other values were estimated using a statistically related dependent factor based on the Boardman River gage (04126970) and measured Mitchell Creek discharges.

Table S.3.8. Precipitation details for the Mitchell Creek watershed.

Month	Year	Project daily average (mm)	Project monthly total (mm)	Long term monthly average (mm)
March	2009	1.35*	6.8*	50.3
April	2009	0.10	72.2	69.1
May	2009	0.10	72.1	58.4
June	2009	0.10	73.6	84.3
July	2009	0.08	59.9	79.8
August	2009	0.14	103.4	86.1
September	2009	0.07	50.2	90.9
October	2009	0.16	117.8	74.7
March	2010	0.00*	0.0*	50.3
April	2010	0.14	100.2	69.1
May	2010	0.09	69.1	58.4
June	2010	0.23	168.6	84.3
July	2010	0.12	92.3	79.8
August	2010	0.11	81.7	86.1
September	2010	0.17	125.6	90.9
October	2010	0.11*	16.8*	74.7

*Average calculated for partial month

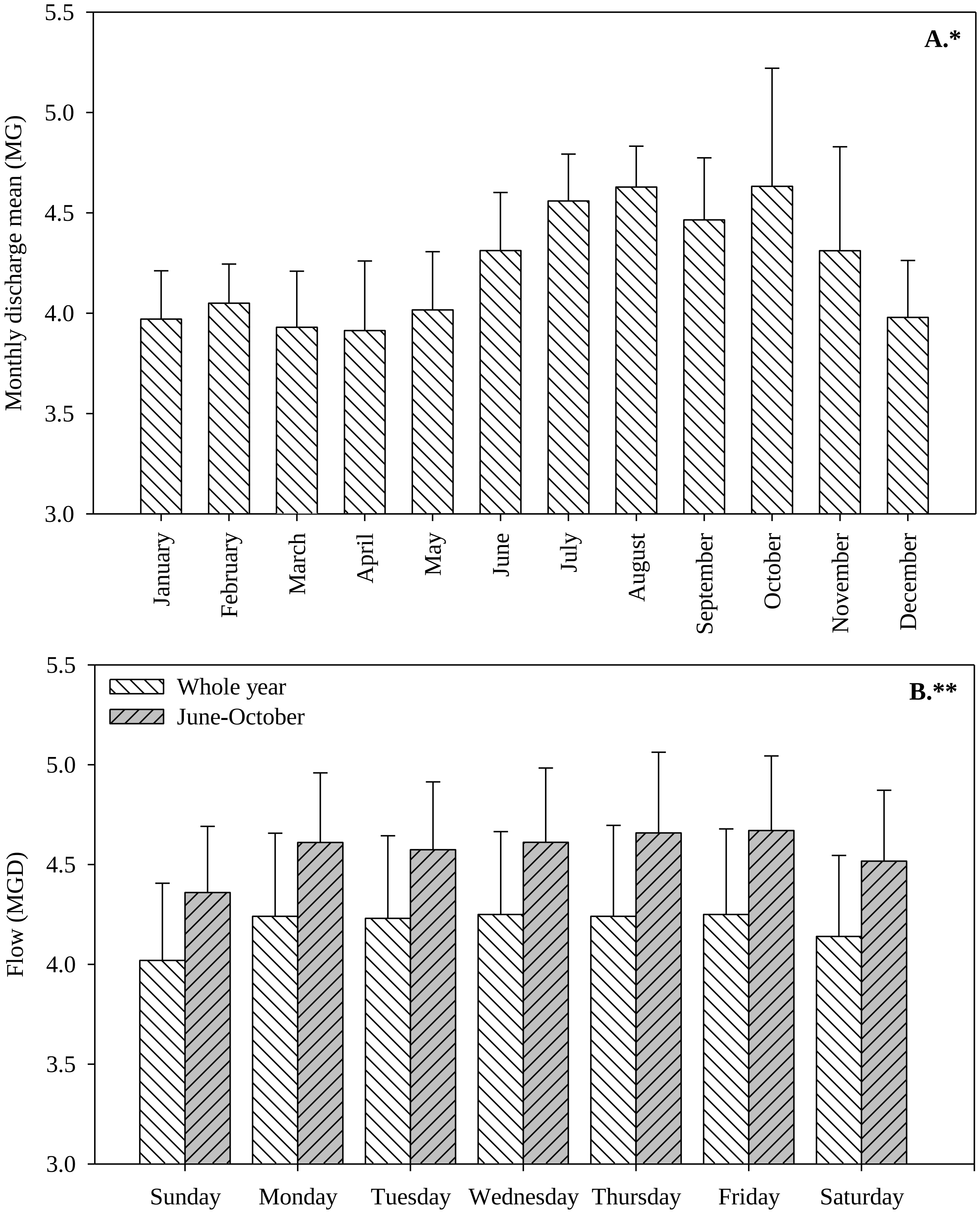

Figure S.3.2. WWTP daily discharge flows (A.) averaged per month and (B.) averaged per day of week. *between January 1, 2009 and April 29, 2011; **whole year and tourist season (June to October)

Table S.3.9. Fecal indicator bacteria exceedances of the 95th percentile and associated discharge percentile.

	Date	Site	Concentration ($\log_{10}$)	Discharge percentile
E. coli (MPN 100 ml^{-1})	8/31/2009	MC2	3.5	69.6
Enterococci (CFU 100 ml^{-1})	7/1/2009	MC1	3.6	87.1
	7/22/2009	MC2	3.7	71.7
	8/3/2009	MC5	3.7	65.2
	8/3/2009	MC3	3.6	65.2
	8/26/2009	MC3	4.5	93.6
	8/31/2009	MC2	3.6	69.6
C. perfringens	NA			
Coliphage (PFU 100 ml^{-1})	8/3/2009	MC5	3.5	65.2
	6/24/2010	MC1	3.7	87.1
	6/24/2010	MC2	3.8	91.3
	6/24/2010	MC3	3.9	91.3
	6/24/2010	MC4	3.7	91.3
	6/24/2010	MC5	3.6	91.3
	6/24/2010	MC6	3.6	91.3
	6/24/2010	MC7	3.7	86.2
	6/24/2010	MC8	3.6	91.3

E. coli 95th percentile: 3.4 $\log_{10}$ MPN 100 ml^{-1}; Enterococci 95th percentile: 3.5 $\log_{10}$ CFU 100 ml^{-1}; *C. perfringens* 95th percentile: 2.5 $\log_{10}$ CFU 100 ml^{-1}; Coliphage CN-13 95th percentile: 3.3 $\log_{10}$ PFU 100 ml^{-1}; NA: No samples exceeded 95th percentile

Table S.3.10. Spearman's correlation coefficients and significance levels between precipitation and *E. coli* and enterococci in water and sediment from the Mitchell Creek.

Total precipitation time (hour)	Water (r, p)			Sediment (r, p)	
	E. coli	Enterococci		*E. coli*	Enterococci
1	0.242, 0.000	0.175, 0.003		0.137, 0.070	0.132, 0.084
2	0.325, 0.000	0.249, 0.000		0.150, 0.050	0.149, 0.052
3	0.321, 0.000	0.242, 0.000		0.150, 0.046	0.149, 0.052
4	0.350, 0.000	0.324, 0.000		0.147, 0.051	0.148, 0.053
6	0.364, 0.000	0.322, 0.000		0.175, 0.020	0.148, 0.053
8	0.384, 0.000	0.349, 0.000		0.158, 0.036	0.148, 0.054
12	**0.553, 0.000**	**0.539, 0.000**		**0.200, 0.008**	0.235, 0.002
16	**0.559, 0.000**	**0.543, 0.000**		**0.202, 0.007**	**0.243, 0.001**
24	0.523, 0.000	0.474, 0.000		0.182, 0.015	**0.284, 0.000**
48	0.224, 0.000	0.183, 0.000		-0.081, 0.284	-0.039, 0.617
72	0.179, 0.000	0.175, 0.000		0.053, 0.483	0.064, 0.409

Data in bold indicates the highest correlation coefficients. Individual Mitchell Creek sites, responded to precipitation on similar time frames. Other significantly influential parameters included: wind direction, air temperature, water temperature, barometric pressure, relative humidity, river discharge, and turbidity.

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CHAPTER 4.

FINE TUNING MICROBIAL WATER QUALITY CRITERIA FOR LOCAL WATERSHEDS

4.1. Introduction

Molecular microbiological methods provide a promise of improved water quality assessment. However, cultivation based detection of fecal indicator bacteria continue to be used for recreational water quality assessment throughout the world (WH0 2003; USEPA 2011; EC 2006). Current scientific literature focusing on indicator organisms results in mixed conclusions, calling into question the continued use of indicator systems for human health protection. Multiple reports identify inconsistencies between culture and molecular based concentrations, indicator and pathogen associations, and human health implications (Lavender and Kinzelman 2009; Byappanahalli et al. 2010; Wilkes et al. 2011).

Approved methods for beach monitoring include both cultivation and molecular technologies (USEPA 2011; USEPA 2002a). The most commonly used standard method for routine beach monitoring relies on the detection of cultivatable fecal indicator bacteria requiring incubation for 18-48 hours. Incubation periods delay water quality advisories and do not provide real-time water quality information, and may result in unnecessary beach closures (Rabinovici et al. 2004; Colford et al. 2012). To compensate, fecal indicator bacteria are increasingly being detected using quantitative polymerase chain reaction (qPCR) which eliminates the cultivation process (Siefring et al. 2008; Field and Samadpour 2007). This method can be used to detect most microorganisms and produce quantitative results in a few hours (Girones et al. 2010). However, qPCR detects both viable and non-viable organisms, potentially limiting its usefulness as a method for assessing risk.

Discrepancies between methods have prompted a search for a common numerical factor connecting cultivation and molecular methods. Whitman et al. (2010) suggests an empirical relationship can be developed between cultivation and molecular *Enterococci* spp. following a water survey from 37 US states. The authors do not provide a specific numerical factor; rather, they suggest site specific function based on colony forming units (CFU) and CFU-CCE (calibrated cell equivalents) coefficients reflective of background CCE persistence and CFU variance (Whitman et al. 2010). Byappanahalli et al. (2010) and Haugland et al. (2005) report strong positive correlations between cultivation and molecular methods for *Enterococci* spp. at Lake Michigan beaches (r = 0.65 and r = 0.68, respectively) with CE measurements consistently higher than CFU measurements (5-10 times higher as reported by Byappanahalli et al. 2010 and 16 times higher as reported by Haugland et al. 2005). Although relationships between qPCR and culture based methods are suggested, each study reports that associations varied with respect to environment, method, or pollution type. For example, Converse et al. (2012) found positive correlations between culture and molecular based approaches for *Enterococci* spp., but note association strength depends on whether the waterbody is dominated by point (r = 0.38 to 0.83) or non-point sources (r = 0.19 to 0.34). Several studies and reviews report inconsistent relationships between bacterial levels measured using non-cultivation techniques (i.e. qPCR) and fecal indicator bacteria measured via cultivation methods (Santo Domingo et al. 2007; Haack et al. 2009; Stapleton et al. 2009). To date, a single, universally accepted relationship between cultivation and molecular based methods has not been adopted.

In addition to the relationship between indicators and methods, several studies focused on relationships between pathogens and fecal indicator organisms with conflicting results.

Schriewer et al. (2010) found qPCR detection of *Bacteroidales* had direct and significant correlations with *Cryptosporidium* spp. occurrences in estuary and river environments (r = 0.21, p = 0.013). However, multiple studies report that indicator organisms rarely correlate with pathogen detection. For instance, Harwood et al. (2005) and Wilkes et al. (2009) report routine detection of fecal indicators (*Escherichia coli* (*E. coli*), *Clostridium perfringens,* enterococci, total and fecal coliforms, and F-specific coliphage) in absence of pathogens (*Campylobacter* spp., *Salmonella* spp., *Giardia*, *Cryptosporidium*, *Listeria monocytogenes*, and *E. coli* O157:H7) using cultivation methods. Hellein et al. (2011) measured enterococci using two United State Environmental Protection Agency (USEPA) approved methods (cultivation and molecular) to conclude both techniques produced poor correlations with molecular based *Campylobacter* spp. presence.

Finally, uncertainty surrounds microbial monitoring and human health implications. *E. coli* and enterococci, the most common microorganisms for determining recreational water quality safety, were directly linked to adverse health outcomes via epidemiological studies at multiple beaches throughout the US (Cabelli et al. 1982; Cabelli 1983). A meta-analysis of 27 studies concluded that *E. coli* and enterococci consistently represented gastrointestinal illness in fresh and marine waters, respectively (Wade et al. 2003). Using qPCR and cultivation techniques to measure *Enterococcus*, Wade et al. (2008) noted significant associations between gastrointestinal illness in swimmers and bacterial concentrations in both marine and freshwater recreational beaches; specifically a single $\log_{10}$ increase of daily averages for *Enterococcus* or *Bacteroidales* (16S rRNA sequence from Dick and Fields 2004) (calibrated cell equivalents, CCE) doubled the risk of gastrointestinal illness in swimmers (Wade et al. 2010). One study investigating method specific health implications claimed molecular and cultivation methods produced similar correlations between water exposure and gastrointestinal illnesses (Colford et al. 2012). Others support these findings and suggest that molecular and culture methods represent similar implications for beach actions (i.e. beach closure or advisory numbers) (Shibata et al. 2010; Lavender and Kinzelman 2009). However, Colford et al. (2007) have also reported no existing associations between illnesses and enterococci via cultivation or molecular methods during a cohort study at a single marine beach. Cumulatively, implications for water quality management following modification of microbial monitoring programs remain unclear, regardless of detection organism or method. The contrast in bacteria and health relationships for studies focused on single or multiple sites illustrates the need for fine tuning criteria at more localized scales.

The USEPA suggests new water quality criteria that include the use of qPCR for marine and freshwater beach monitoring (USEPA 2011). Following epidemiological studies at nine beaches from the Great Lakes and marine waters influenced by sewage treatment discharge, the USEPA made public a new molecular method and criteria for beach closures/advisories targeting *Enterococci* spp. with a geometric mean of 475 CCE 100 ml^{-1} and a statistical threshold level of 1000 CCE 100 ml^{-1} (USEPA 2011) which represented the 75th percentile of the microbial data distributions found during the epidemiological studies. However, this value for the molecular methods (versus cultivation criterion that is already used in the states) may not be appropriate at sites with diffuse sources of pollution and watersheds with heterogeneous landscape patterns. This manuscript aims to 1) evaluate molecular and cultivation based regulatory criteria in a flashy watershed receiving primarily non-point source pollution, 2) investigate potential subcriteria for molecular markers not included in current regulations, and 3) analyze a long term monitoring data set for implications of water quality interpretation following a method shift.

4.2. Materials and methods

4.2.1. Sampling location and collection

The Mitchell Creek (Michigan, USA) drains a watershed composed of urban (23.4%), agriculture (37.7%), forest/open (24.7%), wetlands (14.0%), and water (0.1%) (Figure 4.1.). Surface water grab samples (n = 111) were collected from four Mitchell Creek sites and one Grand Traverse Bay beach site between June and September 2010 using sterile one liter Nalgene bottles. All samples were placed in coolers on ice, transported to the laboratory, and analyzed within four hours. Samples were collected under wet and dry conditions based on a predetermined threshold of 5.1 mm cumulative rainfall in the 48 hours prior to sample collection. These threshold levels were associated with rapid changes in surface water velocity and discharge.

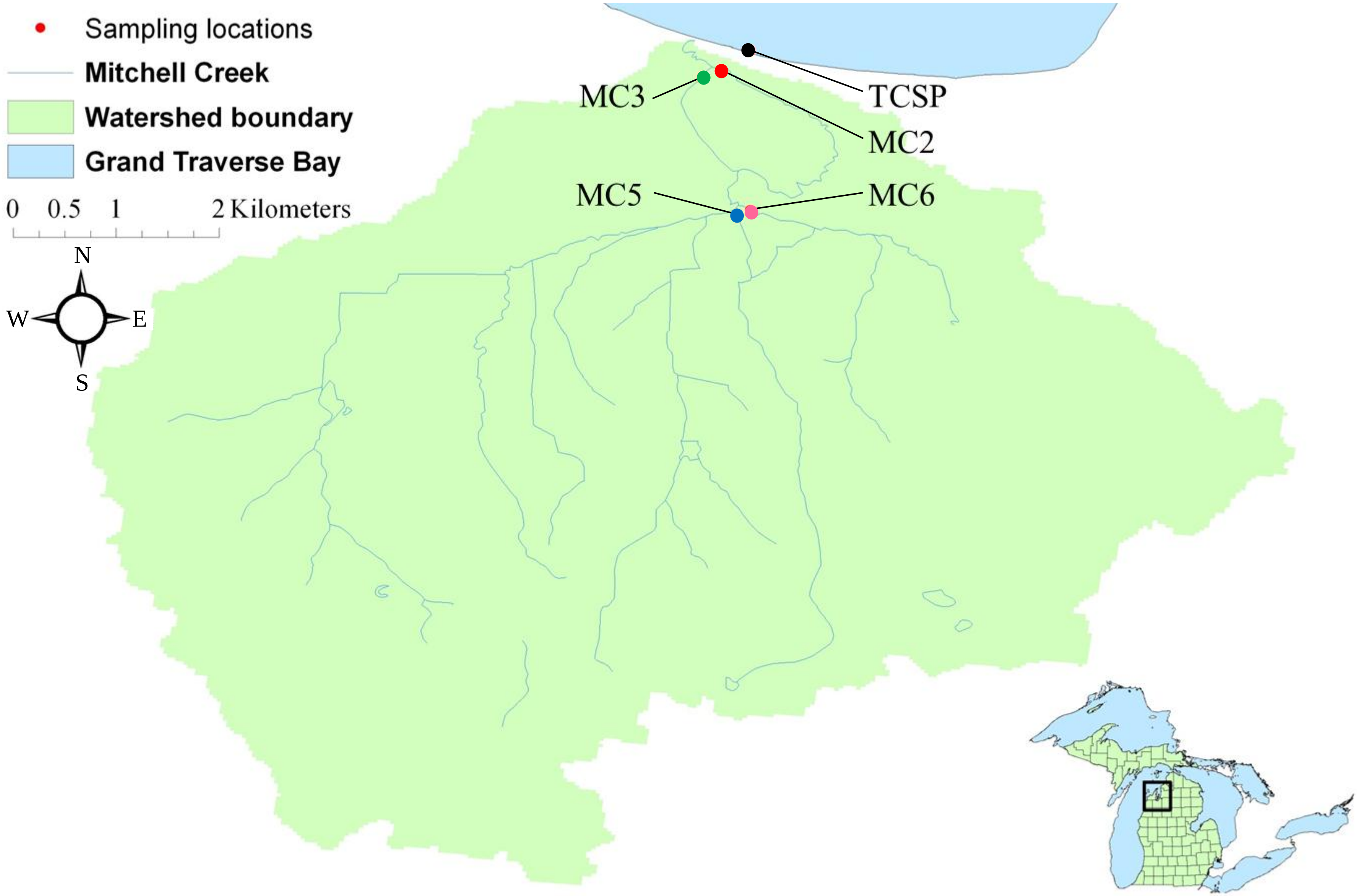


Figure 4.1. Mitchell Creek watershed and sampling locations within Michigan and the Great Lakes.

4.2.2. Enumeration of bacteria using cultivation techniques

Water subsamples (100 ml) were analyzed for *E. coli* and enterococci using chromogenic substrate methods Quanti-Tray 2000 Colilert® and Enterolert®, (IDEXX Laboratories, Inc., Westbrook, ME) respectively. *E. coli* and enterococci trays were incubated for 24 hours at 35 °C and 41 °C, respectively. Wells that fluoresced yellow were counted as *E. coli* positive and wells that fluoresced blue were count as enterococci positive in their respective trays. *E. coli* and enterococci concentrations were calculated from IDEXX Quanti-Tray®/2000 MPN table and reported as Most Probable Number (MPN) 100 ml^{-1}. Stock cultures of *E. coli* (ATCC 15597) and *Enterococcus faecium* (ATCC 35667) were used as positive controls.

4.2.3. Enumeration of bacteria using molecular techniques

A total of 900 ml per sample was filtered through a 47 mm 0.45 µm pores size nitrocellulose membrane filter and then immersed into 25 ml of sterile phosphate buffer solution in a 50 ml centrifuge tube. The solution and filter was vortexed at high speed (3200 RPM) for 2 minutes, followed by filter removal, and the suspension was centrifuged at 4500 x *g* for 20 minutes. Using sterile pipettes, 23 ml of supernatant was decanted and the remaining volume re-suspended to form a 2 ml concentrate. A volume of 200 µl of the concentrated suspension was then used for DNA extraction by QIAamp Mini DNA kit according to the manufacturer's instructions (Qiagen, Valencia, CA). The total volume of 200 µl of the concentrated samples were extracted for DNA and stored in -20 °C until analyzed with qPCR. A negative control was included during filtration and extraction, consisting of molecular grade water in lieu of sample product.

Standards for qPCR were prepared by extracting DNA from bacterial strains *Escherichia coli* ATCC 15597 and *Enterococcus faecalis* ATCC 19433. *Bacteroides thetaiotaomicron* genomic DNA was purchased from ATCC (29148D-5). The *E. coli uidA*, *Enterococci* spp. 23S rRNA, and *B. thetaiotaomicron a-mannanase* genes were amplified separately using published primer sets (Srinivasan et al. 2011; Frahm and Obst 2003; Yampara-Iquise et al. 2008). Polymerase chain reaction (PCR) was performed in a 25 µl total reaction mix which contained 15 µl Hotstart DNA Polymerase Mastermix, 0.4 mM of each primer, 2 µl of the template DNA, and molecular grade water (QIAGEN, Valencia, CA, USA) to a final volume of 25 µl. The amplified PCR products for all three target genes were cloned into TOPO PCR 2.1 and transformed with the TOPO10 competent cells (Invitrogen Inc., Carlsbad, CA, USA) according to the protocol provided by the manufacturer. Plasmids were extracted with QIAprep Spin MiniPrep kit (QIAGEN, Valencia, CA, USA) and were sequenced at the Research Technology Support Facility (RTSF) at Michigan State University to confirm the insertion of the target inside the vector. The plasmids were quantified using Nano-Drop spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) and then serially diluted ten-fold to construct qPCR standard curves. Triplicates of plasmid concentrations ranging from 10^0 to 10^6 copies per 5 µl were used for the standard curve.

E. coli qPCR assay targeting the *uidA* gene (Srinivasan et al. 2011) had a detection limit of 100 copies per 5 µl. The *E. coli* qPCR reaction mix consisted of 10 µl of Taqman Light Cycler Mastermix (Roche, Indianapolis, IN), 0.2 µM each of forward and reverse primers (Eurofins MWG Operon, USA), 0.1 µM of probe (Eurofins MWG Operon, USA), 5 µl of template, and nuclease free water to a final volume of 20 µl. The assay was carried out in LightCycler 2.0 ®

(Roche, Indianapolis, IN) through the following temperature profiles: initial denaturation for 10min at 95 °C followed by 45 cycles of denaturation for 6s at 95 °C; annealing for 8s at 58 °C and extension at 72 °C for 8s.

Enterococcus qPCR assay targeting the *23S rRNA* gene (Frahm and Obst 2003) had a detection limit of 10 copies per 5 µl. The enterococcus qPCR reaction mix consisted of 10 µl of Taqman Light Cycler Mastermix (Roche, Indianapolis, IN), 0.2 µM each of forward and reverse primers (Eurofins MWG Operon, USA), 0.1 µM of the probe (Eurofins MWG Operon, USA), and 5 µl of template and nuclease free water to a final volume of 20 µl. The assay was carried out in LightCycler 2.0 ® (Roche, Indianapolis, IN) through the following temperature profiles: initial denaturation at 10min at 95 °C followed by 45 cycles of denaturation for 15s at 95 °C; annealing for 30s at 60 °C and extension at 72 °C for 15s.

The α-1-6, mannanase (BT 3501) targeting *B. thetaiotaomicron* qPCR assay (Yampara-Iquise et al. 2008) had a detection limit of 10 copies per 5 µl. The reaction mix for qPCR *B. thetaiotaomicron* consisted of 10 µl of Taqman Light Cycler Mastermix (Roche, Indianapolis, IN), 0.2 µM each of forward and reverse primers (Eurofins MWG Operon, USA), 0.1 µM of the probe (Eurofins MWG Operon, USA), and 5 µl of template and nuclease free water to a final volume of 20 µl. The assay was carried out in LightCycler 2.0 ® (Roche, Indianapolis, IN) through the following temperature profiles: initial denaturation at 10min at 95°C followed by 50 cycles of denaturation for 15s at 94 °C; annealing for 60s at 60 °C and extension at 72 °C for 5s. Triplicate analysis was done for all dilutions, positive controls, and negative controls for all three markers.

The copies of the corresponding genes were converted to cell equivalents (CE). In the cases of *E. coli* and *B. thetaiotaomicron*, only one copy of the target gene is present in a cell, thus one copy number corresponds to one cell. Viau and Peccia (2009) suggest four copies of 23S rRNA present per enterococci cell, therefore DNA copies-to-cell conversions of enterococci qPCR targets were based on a 4:1 ratio. All final concentrations for qPCR analyses were reported as qPCR cell equivalents (CE) 100 ml^{-1}.

To examine DNA extract for inhibitory substances, five replicates from each sample DNA extract initially negative for *E. coli*, *Enterococci* spp. or *B. thetaiotaomicron* DNA were pooled. All samples were then diluted 10 and 100 times. Molecular grade water (control), undiluted, 10- and 100- dilutions of DNA were spiked with known amounts of *E. coli*, enterococcus, or *B. thetaiotaomicron* DNA and analyzed by real time PCR. The threshold cycle values of these spiked DNA samples were compared to those of the DNA samples from distilled water spiked with the same concentration of the target DNA of the respective assay.

4.2.4. Statistical analysis

Cultivation (*E. coli* and enterococci) and molecular (*E. coli*, *Enterococci* spp., and *B. thetaiotaomicron*) results were compared by space, time, and detection technique to identify differences and associations using independent t-tests, Kruskal-Wallis one-way ANOVA, and Spearman rank correlation coefficient tests. Classification And Regression Tree (CART) analysis was also used to compare *E. coli* (molecular), *Enterococci* spp. (molecular), *B. thetaiotaomicron* (molecular), *E. coli* (cultivation) and enterococci (cultivation) results following Martin et al.

(2011) and Wilkes et al. (2011). CART is a trial and error method that attempts to split dependent variables into homogeneous categories based on independent variables that influence the dependent variable (target organism). All CART analyses were performed using R software system (R foundation for Statistical Computing). CART has been previously used to investigate pathogenic bacteria and parasite relationships with environmental and land use factors (Wilkes et al. 2011) and to predict the occurrence of fecal indicator bacteria with respect to physiochemical variables (Bae et al. 2010).

CART models start out with a parent or root node which contains all available data. CART then looks at all independent variables (splitting variables) and selects the single variable that produces the two most different groups of dependent variables based on predefined splitting criterion and regression analysis. In this study, splitting criteria were developed using recursive partitioning algorithm and a 10-fold cross validation. A 10-fold cross validation breaks all data into 10 subsets and calculates the split based on nine of the ten subsets. Each time a group is split per above criteria the binary splits are called child nodes. This method is used for each group until a stopping rule is reached. For this project, the stopping criterion was set at a minimum of 5 observations per subgroup (Martin et al. 2011). A terminal node is defined as a child node which has met the defined stopping rule.

Fully grown trees often require pruning to ensure significant variable associations are not missed as a result of following the splitting and stopping criteria (Lemon et al. 2003). Pruning is the process of growing trees until they reach stopping criteria and then removing less statistically significant results from the analysis. Trees were pruned according to the one-standard error rule

(Breiman et al. 1984; Venables and Ripley 1999; De'ath and Fabricous 2000). This rule minimizes the cross-validated error of the model which has been shown to produce optimal sized trees and produce more stable tree sizes across replications compared to the 0-SE pruning method and (Breiman et al. 1984; Questier et al. 2005).

Competitor and surrogate variables were identified for each node by investigating detailed CART outputs. Competitor splits are those variables that have similar complexity parameter values compared to the primary split. A complexity parameter compares the complexity (number of terminal nodes) to the cross-validated error for each group. For this project the complexity parameter was set at 0.05. Surrogate splits are alternative variables that split the subgroup into very similar groups. An example of a CART output is presented below in Figure 4.2.

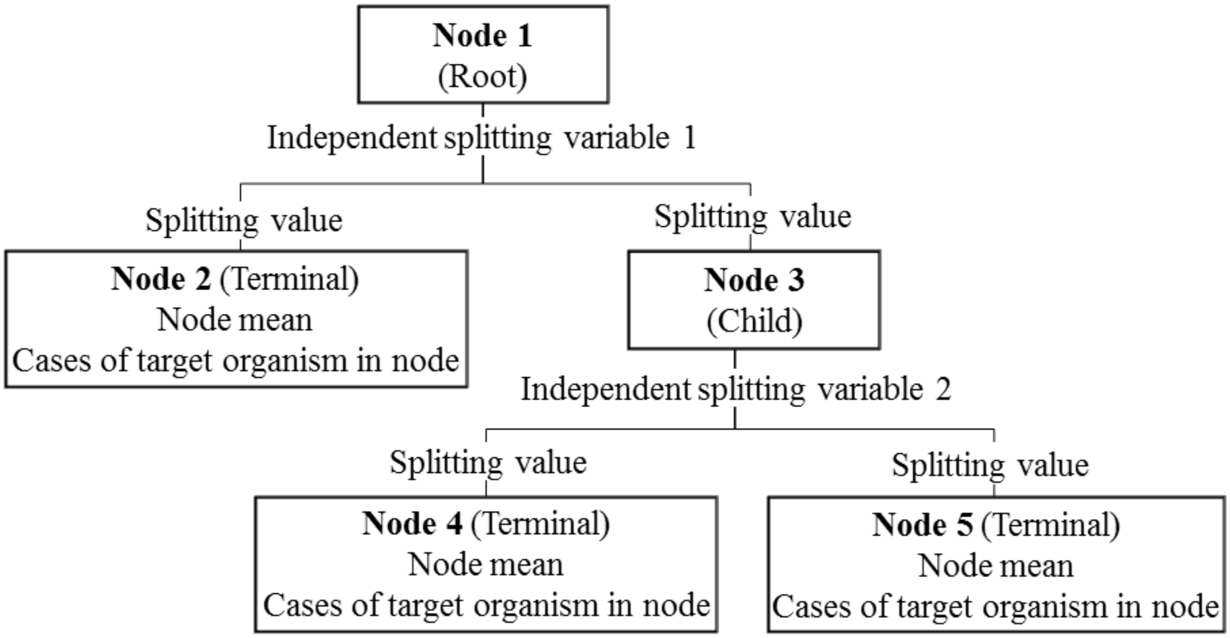


Figure 4.2. Example of a Classification And Regression Tree (CART) output. Root nodes contain all available data and are split into binary groups using recursive partitioning algorithm and 10-fold cross validation with a complexity parameter value of 0.05. Primary splitting variables and values are described for each child node. Terminal nodes (bottom of the tree) include the mean concentration and number of target organism cases in each node. Each node was derived based on mean value of each response variable, group size, and defining variables.

Regulatory criteria outcomes were compared between samples based on USEPA suggested water quality criteria for *E. coli* (235 MPN 100 ml^{-1}), enterococci (61 MPN 100 ml^{-1}) and *Enterococci* spp. (1000 cell equivalents 100 ml^{-1}). Regulatory actions were defined as any act (i.e. closure, advisory, or warning) that would result from water quality exceeding the suggested criteria. Ratios were developed by diving cultivation results by qPCR results and then compared. Significant differences between regulatory outcomes were identified using independent t-tests and Chi-square power tests. Binomial regression tests were used to calculate the probability of increased bacteria incidences when one regulatory criterion was exceeded.

E. coli daily geometric mean concentrations (MPN 100 ml^{-1}) measured between 2001 and 2011 (n = 189) at Traverse City State Park beach (TCSP) were collected from Michigan Beach Guard database (http://www.deq.state.mi.us/beach/). Independent t-tests were used to compare *E. coli* cultivation results from the long term dataset and this project dataset at TCSP to identify if water quality measured during this project accurately represented historical water quality.

Sample concentrations below method detection were assigned a value equal to half of the detection limit with respect to each method. The geometric means from all molecular assay replicates were calculated and used for statistical analysis. Kruskal-Wallis one-way ANOVA, independent t-tests, Chi-square power tests, and correlation coefficients were calculated using IMB SPSS Statistics (v.19.0) or SigmaPlot (v.11.0). Significance threshold was set at an (α) of 0.05.

4.3. Results

4.3.1. Comparing culture versus molecular results

In total, 111 samples were analyzed for *E. coli* (cultivation and qPCR), enterococci (cultivation and qPCR), and *B. thetaiotaomicron* (qPCR). Results for each assay are summarized in Table 4.1. The highest single sample and geometric mean concentrations for both cultivation assays (*E. coli* and enterococci) were reported at MC5, the site with the highest percent agricultural land use (41%) in the upstream catchment. For all molecular assays, the highest geometric mean and single sample concentrations were detected at MC2 (570 m upstream from Creek outlet) and TCSP, respectively. At the TCSP beach, cultivation based *E. coli* and enterococci means were statistically lower than cultivation *E. coli* and enterococci averaged across all creek sites ($p < 0.05$) and molecular based *Enterococci* spp. means were statistically lower than molecular based *E. coli* and *B. thetaiotaomicron* ($p < 0.05$). Amongst all data, all three molecular assay means were statistically different from both cultivation means ($p < 0.001$). However wet weather sample means of all assays were not statistically different from dry weather samples ($p \geq 0.134$).

Table 4.1. Log_{10} transformed bacterial concentrations from a Great Lakes water system and the respective number of regulatory exceedances.

Assay	Method	Sample number	Range	Geometric Mean	Regulatory exceedance[1] (n)
E. coli	Cultivation (MPN 100 ml^{-1})	111 (23)	0.30-3.38 (0.30-2.89)	2.20 (1.22)	65 (2)
Enterococci spp.	Cultivation (MPN 100 ml^{-1})	111 (23)	0.78-3.47 (0.78-2.74)	2.14 (1.22)	89 (3)
E. coli	qPCR (CE 100 ml^{-1})	111 (23)	3.02-5.72[2] (3.26-5.72)	4.23 (4.27)	NA[4]
Enterococci spp.	qPCR (CE 100 ml^{-1})	111 (23)	1.53-4.47[3] (1.82-4.47)	2.86 (2.90)	66 (12)
B. thetaiotaomicron	qPCR (CE 100 ml^{-1})	111 (23)	1.43-6.84[3] (1.92-6.84)	3.77 (3.85)	NA

[1] Values in parenthesis represent measurements from TCSP beach only. [1] Regulator exceedances based on USEPA suggested criteria for freshwater recreational waters resulted in advisories at TCSP but not in Creek sites. [2] Method detection limit of 3.9 $\log_{10}$ copies 100 ml^{-1}. [3] Method detection limit of 2.9 $\log_{10}$ copies 100 ml^{-1}. Non-detections were reported as half the method detection limit. [4] NA represents the absence of established health based criteria.

Cultivatable *E. coli* and enterococci concentrations followed close spatial and temporal patterns (Figure 4.3.) which were not statistically different over time (except on August 8, 2010) or space throughout Mitchell Creek (except at TCSP beach) ($p \geq 0.066$). Similarly, the molecular assays *B. thetaiotaomicron*, *E. coli*, and *Enterococci* spp. followed spatial and temporal trends with each other (Figure 4.3.) and were not statistically different over space ($p \geq 0.276$) or time ($p < 0.001$). Spearman rank correlations of assay concentrations (Table S.4.1.) indicate strong, positive relationships between (1) cultivation *E. coli* and cultivation enterococci ($r = 0.877$, $p < 0.001$) and (2) all molecular assays ($r = 1.000$, $p < 0.001$).

During dry weather, moderate correlations were identified between (1) molecular *Enterococci* spp. and cultivation *E. coli* ($r < 0.69$, $p < 0.05$) and (2) molecular *Enterococci* spp. and molecular *E. coli* ($r < -0.87$, $p < 0.05$). During wet weather, moderate correlations were found between molecular *B. thetaiotaomicron* and molecular *E. coli* ($r > 0.87$, $p < 0.05$), but cultivation enterococci were inversely related to *B. thetaiotaomicron*, molecular *E. coli*, and molecular *Enterococci* spp. ($p < 0.05$). Overall, *E. coli* (molecular), *Enterococci* spp. (molecular), and *B. thetaiotaomicron* (molecular) means were not significantly different between beach and creek sites ($p \geq 0.746$), but *E. coli* (cultivation) and enterococci (cultivation) were significantly different between creek sites and the beach site ($p < 0.001$).

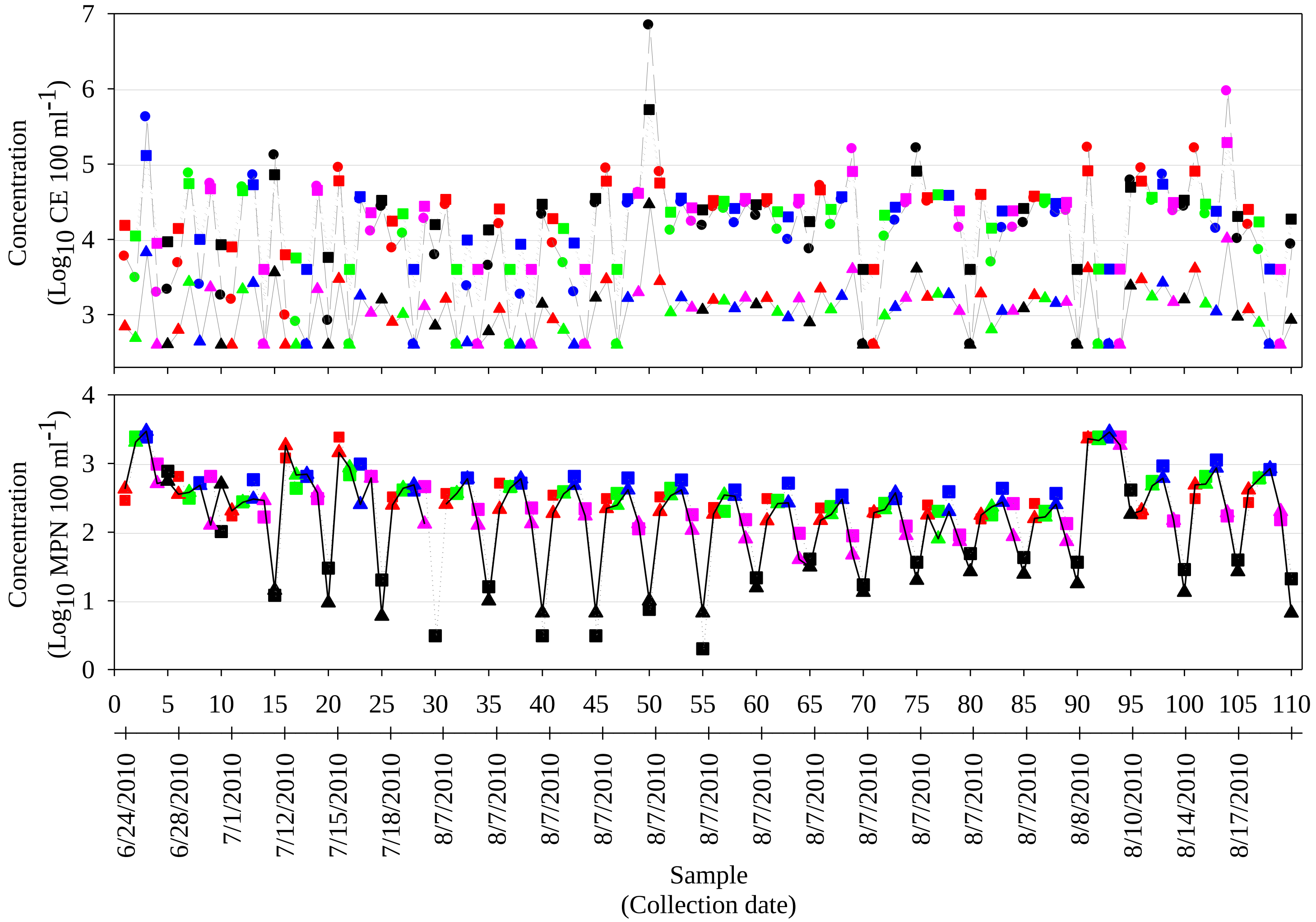


Figure 4.3. A spatial and temporal depiction of molecular assays (TOP FIGURE) *B. thetaiotaomicron* (CIRCLE), *E. coli* (SQUARE), and *Enterococci* spp. (TRIANGLE) and cultivation assays (BOTTOM FIGURE) *E. coli* (SQUARE) and enterococci (TRIANGLE) concentrations. Lines are presented to discern between organisms (*B. thetaiotaomicron*-DASHED, enterococci-SOLID, *E. coli*-DOTTED). Each collection date (n = 11) depicts results from four creek sites (MC2-RED, MC3-GREEN, MC5-BLUE, MC6-PINK) and one beach (TCSP-BLACK). On August 7, 2010 consecutive hourly samples (n = 12) were collected from each site for a total of 60 samples. In total 111 samples were collected during this study.

Molecular and cultivation indicator ratios were highly variable amongst all data. The two cultivation tests used for regulatory purposes were compared to the qPCR signal for *Enterococci* spp. (which as previously mentioned could also be used for regulatory purposes). For the complete data set, cultivation *E. coli* per molecular *Enterococci* spp. ratios averaged 0.84 and cultivation enterococci per molecular *Enterococci* spp. ratios were slightly lower and averaged 0.81 ($p < 0.001$). Ratios were not different between wet (n = 75; *E. coli* MPN: *Enterococci* spp. qPCR average ratio = 0.84; enterococci MPN: *Enterococci* spp. qPCR average ratio = 0.83) and dry (n = 36; *E. coli* MPN: *Enterococci* spp. qPCR average ratio = 0.84; enterococci MPN: *Enterococci* spp. qPCR average ratio = 0.80) weather samples ($p \geq 0.424$).

The ratios for TCSP beach and creek sites specifically were significantly higher in the creek (*E. coli* MPN: *Enterococci* spp. qPCR average ratio = 0.93; enterococci MPN: *Enterococci* spp. qPCR average ratio = 0.90) than at the beach (*E. coli* MPN: *Enterococci* spp. qPCR average ratio = 0.51; enterococci MPN: *Enterococci* spp. qPCR average ratio = 0.47; $p < 0.001$).

4.3.2. Indicators and criteria

Regulatory outcomes based on USEPA single sample maximum criteria were applied to all samples (n = 111) and identified 65 samples above the *E. coli* criterion (235 MPN 100 ml^{-1}), 89 samples exceeded the enterococci criterion (61 MPN 100 ml^{-1}), and 66 samples exceeded the *Enterococci* spp. criterion (1000 CCE 100 ml^{-1}). Regulatory outcomes based on *Enterococci* spp. qPCR results agreed with *E. coli* and enterococci cultivation regulatory outcomes in 50.5% and 55.9% of samples, respectively. In comparison, 78.4% of samples were in regulatory agreement using *E. coli* and enterococci cultivation based methods. Thirty-four percent of samples exceeded all three criteria while 8.1% of samples were below all three criteria. Comparisons of regulatory outcomes between each criterion are described in Figure S.4.1.

Based on binomial regression analysis, it was determined that cultivation based criteria (i.e. *E. coli* and enterococci) exceedances were not predictive of increased molecular incidence of *B. thetaiotaomicron*, *E. coli*, and *Enterococci* spp. ($p \geq 0.357$). Similarly, molecular *Enterococci* spp. criteria exceedances were not predictive of increased cultivation *E. coli* or enterococci levels ($p \geq 0.162$). Conversely, comparisons of criteria to organisms detected using the same method (i.e. cultivation verse cultivation OR molecular verse molecular) demonstrated highly significant relationships ($p < 0.001$; $Chi^2 \geq 82.1$; df = 1).

4.3.3. Implications for use historical data sets

Analysis aimed at identifying implications for comparing water quality datasets following a method shift was performed on TCSP beach sample datasets. Cultivation *E. coli* results from long term beach monitoring data (2001-2011, n = 189) were compared to cultivation based *E. coli* results measured during this project (2010, n = 23) at the same location, as shown in Figure 4.4. At TCSP beach, the long term *E. coli* mean (1.29 $\log_{10}$ MPN 100 ml^{-1}) was not statistically different ($p = 0.116$) from this project *E. coli* mean (1.12 $\log_{10}$ MPN 100 ml^{-1}). In comparison, molecular *E. coli* results measured during this project (4.26 $\log_{10}$ CE 100 ml^{-1}) were statistically different than cultivation *E. coli* results from the long term data set ($p < 0.001$).

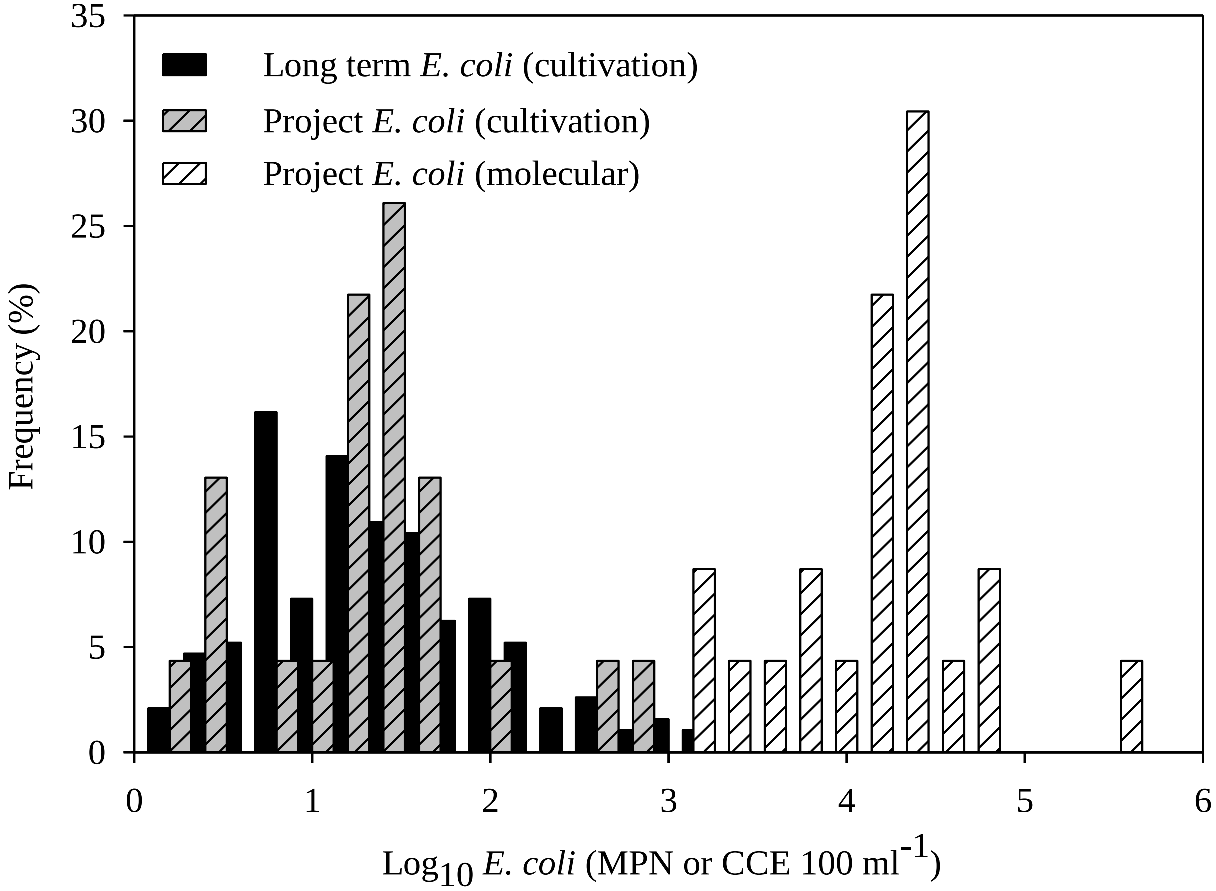


Figure 4.4. A frequency distribution of cultivation *E. coli* measurements from a long term beach monitoring database (2001-2011, n = 189), cultivation *E. coli* measurements during project (2010, n = 23), and molecular *E. coli* measurements during project (2010, n = 23) at TCSP beach during 2 time periods. Cultivation *E. coli* concentrations between time periods were within the normal expected distribution ($p = 0.116$) while molecular based *E. coli* concentrations from the project were outside expected normal distributions of long term cultivation *E. coli* ($p < 0.001$).

Classification and Regression Tree (CART) analysis was used to further investigate relationships between detection methods. CART results showed cultivation results mostly explained other cultivation concentrations and molecular results mainly explained other molecular results (Figure 4.5.). Molecular results did not include any significant surrogate or primary splits associated with cultivation variables. Similarly, cultivation results did not include any significant primary or surrogate splits associated with molecular variables. To test the predictive ability of cultivation based organisms for molecular organism concentrations, CART models targeting molecular bacteria in the root node were developed using only cultivation based organisms as independent variables. Likewise, to test the predictive ability of molecular based organisms for cultivated bacteria concentrations, CART models targeting cultivated organisms in the root node were developed using only molecular based organisms as independent variables. These reduced models identified that cultivation results predicted no more than 6% of molecular concentrations and molecular variables predicted less than 12% of cultivation variables (Table S.4.2.). Cumulatively, these models suggest qPCR assays poorly predict cultivation results and vice versa, likely being driven by the beach analyses.

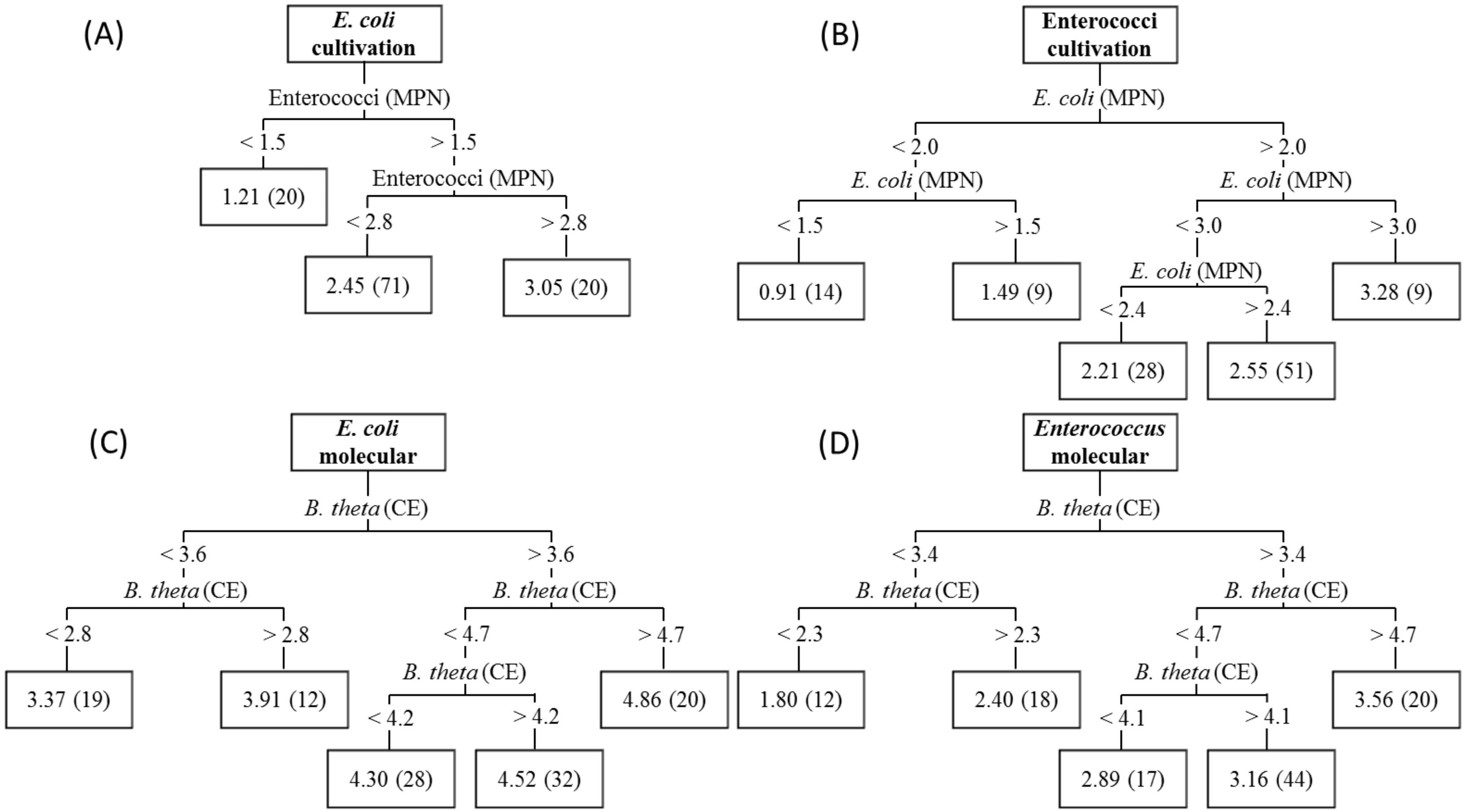
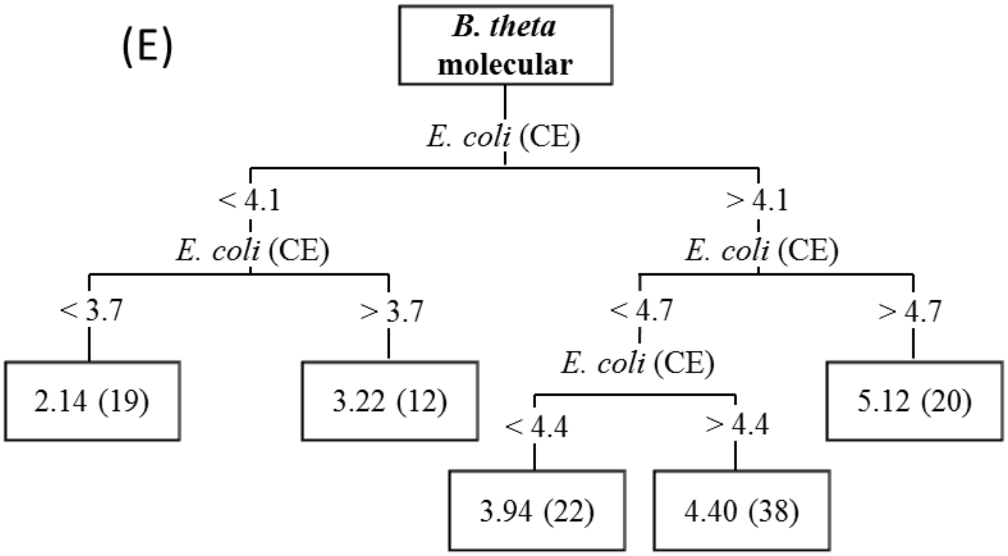


Figure 4.5. Classification And Regression Tree (CART) analysis of (A) *E. coli* MPN, (B) enterococci MPN, (C) *E. coli* CE, (D) *Enterococci* spp. CE, and (E) *B. theta* CE. Binary splitting of variables identified best categories according to splitting criteria. The target organism is bolded in the top rectangle. Independent splitting variables and splitting value are presented for each branch of the tree. Target organism means and target organism cases (in parenthesis) are described for each terminal node (bottom rectangle).

Figure 4.5. (cont'd)



4.4. Discussion

Using the USEPA suggested criteria for cultivation *E. coli*, cultivation enterococci, and molecular based *Enterococci* spp., water quality in the study area was unsafe for total body contact during the project timeframe. Molecular *E. coli* measurements at the beach ($\overline{X}$ = 4.27 $\log_{10}$ copies 100 ml^{-1}) and across all creek sites ($\overline{X}$ = 4.23 $\log_{10}$ copies 100 ml^{-1}) were greater than levels reported by Lee et al. (2012) at four other Great Lakes beaches (~3.0 $\log_{10}$ copies 100 ml^{-1}). Concentrations of the *B. thetaiotaomicron* marker averaged 3.77 $\log_{10}$ copies 100 ml^{-1} across all sites, within reported ranges of a human impacted urban creek in the US (Yampara-Iquise et al. 2008) and similar to levels reported in tertiary treated sewage effluent (Srinivasan et al. 2011). Together, these results represent elevated health risks in the study area stemming from the presence of human fecal contamination.

Although direct correlations between *E. coli* MPN and *Enterococci* spp. CE concentrations were identified, CART analysis demonstrated weak predictive power between assays. Specifically, no molecular variables predicted cultivated variables and no cultivation variables predicted molecular variables. Interestingly, *Enterococci* spp. (CE) concentrations were more closely related to cultivation *E. coli* than cultivation enterococci amongst all sites. Biochemical based cultivation methods targeting enterococci used during the current study favor the growth of *E. faecium* and *E. faecali*s (USEPA 2002B), with higher concentrations of these species found in human feces (Scott et al. 2002). In comparison, the molecular *Enterococci* spp. target is not specific to humans as others have reported finding it in chickens at levels of > 4.46 $\log_{10}$ copies g^{-1} wet weight (Wise and Siragusa 2007) in cattle at 10^6 copies g^{-1} dry weight(Rogers et al. 2011), and in gulls at levels up to 10^6 CE g^{-1} wet weight. *E. coli* is found in virtually all warm blooded animals and gulls (Winfield and Groisman 2003). Perhaps the strong relationships between molecular *Enterococci* spp. and cultivation *E. coli* (i.e. two general targets) are due to the non-point sources which dominate this watershed.

B. thetaiotaomicron α-1-6 mannanase is a human sewage specific marker (Yampara-Iquise et al. 2008) with a reported human pollution specificity of 97% (Aslan and Rose, 2012). *B. thetaiotaomicron* results indicate a significant presence of human fecal contamination. Interestingly, no point source sewage discharges are located directly in the creek watershed but there are approximately 1600 on-site septic systems in the watershed (Luscz and Hyndman, in prep.). These results suggest human fecal contamination is entering the Creek from faulty infrastructure such as septic systems, sewer pipes, or illicit stormwater connections. However, *B.*

thetaiotaomicron increases were not associated with cultivation *E. coli* and cultivation enterococci when they exceeded their respective criterion; suggesting the *B. thetaiotaomicron* marker was not a suitable sub-criterion for cultivation based *E. coli* or enterococci and their associated health risk implications. However, the *B. thetaiotaomicron* measured during this project are indicative of increased human health risk in both the Mitchell Creek and at TCSP beach as the geometric means were higher than those reported at three marine beaches ($\overline{X}$ = 2.95, 2.99, and 3.11 log10 CE 100 ml^{-1}) where a direct association between *Bacteriodales* daily averages and gastrointestinal illnesses was demonstrated (Wade et al. 2010).

The findings of this project do not support the use of a single universal factor which describes the mathematical relationship between cultivation and molecular results and illustrates the difficulty of defining a generalized relationship factor on a national or global scale, as supported by Converse et al. (2012). Whitman et al. (2010) demonstrated high uncertainty and poor correlations between molecular and cultivation methods when microbial concentrations were close to 1.0 $\log_{10}$ CFU 100 ml^{-1} and associations between methods were influenced by local factors. However, in the current study, such trends were not identified as overall cultivation *E. coli* and cultivation enterococci concentrations were generally greater than 2.1 $\log_{10}$ MPN 100 ml^{-1}. At TCSP beach, *E. coli* and enterococci means were generally low (1.2 $\log_{10}$ MPN 100 ml^{-1}) and the ratio variability was high. Overall, qPCR to cultivation ratios at creek sites were closer to one and nearly two-times higher than those found at TCSP beach. This indicates qPCR and cultivation concentrations were more similar in creek water than beach water. These results support those of Byappanahalli et al. (2010) who reported more agreement in qPCR to cultivation ratios at a river site than to a nearby Great Lakes beach using similar microbial methods.

It was initially theorized that the different rates of DNA persistence and viable organism survival significantly influenced molecular and cultivation method relationships, especially when comparing beach to creek results. Walters et al. (2009) reported enterococci CE took eight times longer than cultivated enterococci to decline by the same order of magnitude (T_{90} = 8.28 days and 1.04 days, respectively) in a microcosm experiment. Cultivated *E. coli* was shown to decay slower/faster than molecular *E. coli* in water (T_{90} = 21.7 hours and T_{99} = 5.65 days, respectively) (Jin et al. 2004; Liang et al. 2012). Parallel research in the study area (Chapter 3) measured river discharge rates which were used to estimate the average transport time from the most upstream sampling point to the creek mouth (3.2 linear km) at 6 hours. Additional analysis from the same project indicated microbial contamination entering the Grand Traverse Bay from the Mitchell Creek can impair the TCSP designated swim area (500 linear m) in less than 1 hour. Comparing such temporal transport estimates to previously reported decay rates of the assayed markers, indicates there was not sufficient time for bacterial or DNA degradation to significantly influence results. However, the conditions required for the rapid transport of pollution from the creek to the TCSP beach (wind and high creek discharge) were not always present during sample collection, leaving open the theory that transport DNA persistence and viable organism survival rates may influence molecular and cultivation relationships.

A comparison of the current project dataset with respect to regulatory criteria showed cultivation of enterococci would have resulted in the greatest number of regulatory based actions (i.e.

closure, advisory, or warning), suggesting the enterococci cultivation method may offer the most protection of public health but also potentially the result of false-positive as suggested by Kinzelman et al. (2003). These results support findings by Kinzelman et al. (2003) who compared cultivation *E. coli* and enterococci threshold levels per USEPA suggestions and found monitoring for enterococci would have resulted in an additional 56 water quality advisories compared to *E. coli*. In the current study, three-quarters of samples tested for cultivation *E. coli* and enterococci were in agreement with criteria based regulatory outcomes. However, 55.1% and 45.6% of *Enterococci* spp. CE criteria exceedances agreed with cultivation based criteria exceedances in the creek and at the beach, respectively. Although the total numbers of regulatory outcomes during the project were similar between *Enterococci* spp. (qPCR) and *E. coli* (MPN), monitoring for *Enterococci* spp. (qPCR) alone would have resulted in 65% fewer regulatory actions (i.e. water quality above criteria) in the creek and 91% more regulatory actions at the beach compared to monitoring only for cultivated *E. coli*.

This project focused on a small watershed dominated by non-point source pollutants, much different than those used for criteria development (USEPA 2011). Nearly all the beaches selected for epidemiological studies during USEPA criteria development were impacted by sewage and the non-point source impacted beach was found to have poor illness to indicator correlations (USEPA 2010). The current study identified direct associations between molecular *Enterococci* spp. and cultivation *E. coli* assays, supporting previous research by Lavender and Kinzelman (2009), Whitman et al. (2010), and Converse et al. (2012). Furthermore, the total number of individual regulatory exceedances was similar for cultivation *E. coli* and molecular *Enterococci* spp. but the pairwise agreement between was much lower when beaches and creek sites were separated, implying these two criterions and methods do not provide similar levels of human health protection across all water types. It is recommended that further studies focus on molecular methods which develop a regulatory target as well as persistence studies that address the transport and fate of molecular markers from rivers to beaches.

APPENDIX

Table S.4.1. Spearman's rank correlation matrix among microorganism detection methods.

		E. coli (MPN 100 ml^{-1})	Enterococcus (MPN 100 ml^{-1})	*E. coli* (CE 100 ml^{-1})	Enterococcus (CE 100 ml^{-1})	*B. thetaiotaomicron* (CE 100 ml^{-1})
E. coli (MPN 100 ml^{-1})	r coefficient					
	p Value					
	Sample n					
Enterococcus (MPN 100 ml^{-1})	r coefficient	0.877				
	p Value	< 0.001				
	Sample n	111				
E. coli (CE 100 ml^{-1})	r coefficient	-0.100	-0.187			
	p Value	0.298	0.049			
	Sample n	111	111			
Enterococcus spp. (CE 100 ml^{-1})	r coefficient	-0.099	-0.188	1.000		
	p Value	0.299	0.048	< 0.001		
	Sample n	111	111	111		
B. thetaiotaomicron (CE 100 ml^{-1})	r coefficient	-0.101	-0.189	1.000	1.000	
	p Value	0.290	0.047	< 0.001	< 0.001	
	Sample n	111	111	111	111	

		Enterococci spp. (1000 CE 100 ml^{-1})	
		Yes	No
E. coli (235 MPN 100 ml^{-1})	Yes	38	27
	No	28	18

		Enterococci spp. (1000 CE 100 ml^{-1})	
		Yes	No
Enterococci (61 MPN 100 ml^{-1})	Yes	53	36
	No	13	9

		Enterococci (61 MPN 100 ml^{-1})	
		Yes	No
E. coli (235 MPN 100 ml^{-1})	Yes	65	0
	No	24	22

Figure S.4.1. Analysis of regulatory based outcomes occurring during entire project according to USEPA suggested criteria. Values presented represent number of cases (total n = 111).

Table S.4.2. CART results describing the ability of different methods to predict microorganism concentrations. Model results include model target assay and interactions between assays measured using the different method (i.e. molecular (target) associations with cultivation only and vice versa). Refer to methods and Figure 4.2. for interpretation of CART analysis.

Model target assay	Root node	Complexity parameter (R^2)	Child node	Complexity parameter (R^2)	Total predictive Value (R^2)
B. thetaiotaomicron (CE 100 ml^{-1})	Enterococcus (MPN 100 ml^{-1})	0.06	-	-	0.06
E. coli (CE 100 ml^{-1})	Enterococcus (MPN 100 ml^{-1})	0.06	-	-	0.06
Enterococcus spp. (CE 100 ml^{-1})	Enterococcus (MPN 100 ml^{-1})	0.06	-	-	0.06
E. coli (MPN 100 ml^{-1})	*B. thetaiotaomicron* (CE 100 ml^{-1})	0.05	*B. thetaiotaomicron* (CE 100 ml^{-1})	0.05	0.10
Enterococcus (MPN 100 ml^{-1})	*B. thetaiotaomicron* (CE 100 ml^{-1})	0.07	*B. thetaiotaomicron* (CE 100 ml^{-1})	0.05	0.12

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CHAPTER 5.

MICROBIAL RESPONSES TO LAND, PHYSICAL, CHEMICAL, ENVIRONMENTAL, AND HYDROLOGICAL FACTORS

5.1. Introduction

Natural landscapes are defined using geological properties including topography and soil composition (Saatchi et al. 2009). However, human impacts modify natural land use/cover resulting in permanent changes of hydrogeological cycles (Breuer et al. 2009; Vörösmarty and Sahagian 2000). Specifically, decreased precipitation infiltration (i.e. increased surface runoff) was associated with deforestation (Germer et al. 2009) and increased impervious surface area (Arnold and Gibbons 1996). Overland flows concentrate pollutants and rapidly transport them down gradient where they eventually enter larger systems and become magnified in aquatic environments, impacting water quality (Falkenmark 2011; Evers et al. 2011). The greatest potential for pollution transport across the landscape is the physical movement of water itself (Falkenmark 2011). A number of models have been developed to calculate overland and surface water flows (Katz et al. 1995; Ray et al. 2010) and nutrient/chemical transport (Cha et al. 2010), but few studies have focused on microbial movement from land to water, particularly non-traditional coliform bacteria.

Larger waterbodies receive multiple inputs (rivers, point sources, and non-point sources) are often at highest risk of long term and chronic water degradation. Microbial loading and deposition posed the greatest risks near recreational areas, drinking water intakes, and fishing/shellfish harvesting zones where human exposure was highest (Kistemann et al. 2002; Wong et al. 2009; Almeida and Soares 2012). These highly visible areas receive more attention than the actual source(s) since identifying the origin of all pollution in complex watersheds would require extensive time and intellectual investments. For instance, Soranno et al. (2011) suggested addressing water quality concerns in diverse watersheds would require comprehensive investigations at multiple temporal and spatial scales during various environmental and hydrological conditions, coupled with predictive models. Grayson et al. (1997) suggested another technique referred to as a ‘snapshot’ approach. This method captures water quality characteristics at a single point in time or condition across broad spatial areas, while providing information regularly missed during routine monitoring. Compared to long term comprehensive investigations, the snapshot approach reduces the number of samples, cost, and personnel resources required to address pollution sources.

Escherichia coli (*E. coli*) are commonly used to describe relative risk during routine monitoring in lieu of pathogen detection. However, *E. coli* is not source specific and tracing pollution in water bodies to a specific land use has proven difficult. These types of studies have rarely produced definitive conclusions because bacteria respond rapidity to flows, are not specific to one source, and have finite lives but can regrow under certain environmental conditions (Vega et al. 1998; Alm et al. 2006). Using molecular approaches (DNA detection via qPCR), specific source targets can be isolated in complex systems and have recently been used to investigate land use and water quality impairments (Peed et al. 2011). Furtula et al. (2012) demonstrated ruminant, pig, and dog fecal contamination in an agriculturally dominated river (Canada) using multiple *Bacteroides* markers. More specifically, the molecular based marker *Bacteroides thetaiotaomicron* α-1-6 mannanase (*B. thetaiotaomicron*) gene was shown to be highly specific to human fecal material (Yampara-Iquise et al. 2008; Srinivasan et al. 2011), but no studies have used this particular marker to link water quality to specific land use patterns.

Reference conditions are defined as the condition that exists under minimal disturbance and representative of a group with similar physical, chemical, and biological characteristics (Reynoldson et al. 1997). Reference conditions have been used to assess aquatic resources and habitat expectations by measuring the presence of macroinvertebrates, fish, and diatoms (Reynoldson et al. 1997; Davies and Jackson 2006; Carlisle et al. 2008). However, microbial reference conditions have not been adequately explored or defined. Tiefenthaler et al. (2009) suggested microbial reference conditions based on 15 low-impaired California streams with geometric mean concentrations of cultivatable *E. coli* (1.0 $\log_{10}$ MPN 100 ml^{-1}) and enterococci (1.2 $\log_{10}$ MPN 100 ml^{-1}) below State water quality thresholds and were considered a low potential health risk. In the Great Lakes, no *E. coli* reference conditions have been described, however a health threshold has been suggested by the USEPA at a geometric mean of 2.10 $\log_{10}$ *E. coli* MPN 100 ml^{-1} for recreating in freshwater. Ideally, in truly pristine water there would be no *B. thetaiotaomicron* as it is a highly human specific molecular marker (Yampara-Iquise et al. 2008), but the marker can be detected in sewage effluent following complete treatment and disinfection (Srinivasan et al. 2011). Microbial reference conditions could help fine-tune general water quality criteria for specific watersheds, water quality changes over time associated with degradation, and assessing management actions and goals.

In response to increased water degradation from human stressors and the lack of microbial reference conditions in the Great Lakes basin, this paper aims to 1) Examine the occurrence of *Escherichia coli* (*E. coli*) and a human specific source marker (*B. thetaiotaomicron*) in river systems under baseflow conditions; 2) identify specific land uses that modify reference levels of fecal contamination in rivers; and 3) determine key chemical, physical, environmental, and hydrological variables driving water quality of rivers draining to the Great Lakes. To address these objectives, land use, hydrological, geological, physical, chemical, biological, and microbial variables measured from spatially independent rivers under baseflow conditions were coupled with Classification And Regression Tree analysis. This statistical approach was investigated to better understand relationships in a variety of watersheds with the hope to eventually support water and landscape decision making and reduce human health risk from pathogen exposure.

5.2. Materials and methods

5.2.1. Study area

This study involved Lower Peninsula (Michigan, USA) river watersheds draining to the Great Lakes. Watersheds were selected using the following criteria: 1) 30 large watersheds were deemed essential as they represented 80% of land cover of the Lower Peninsula; and 2) of the remaining smaller watersheds, 70 were randomly selected around the state. Smaller watersheds were further filtered in the field according to timing and personnel logistics. In total, 64 river systems were selected and represented 84% of Michigan’s Lower Peninsula drainage area (Figure 5.1.). All sampling locations were located at bridge crossings and selected on the criteria that each was reasonably accessible, had adequate flow which could be measured using an Acoustic Doppler Current Profiler, river water dominated discharge, and the maximum amount of upstream land use was captured while meeting the above criteria.

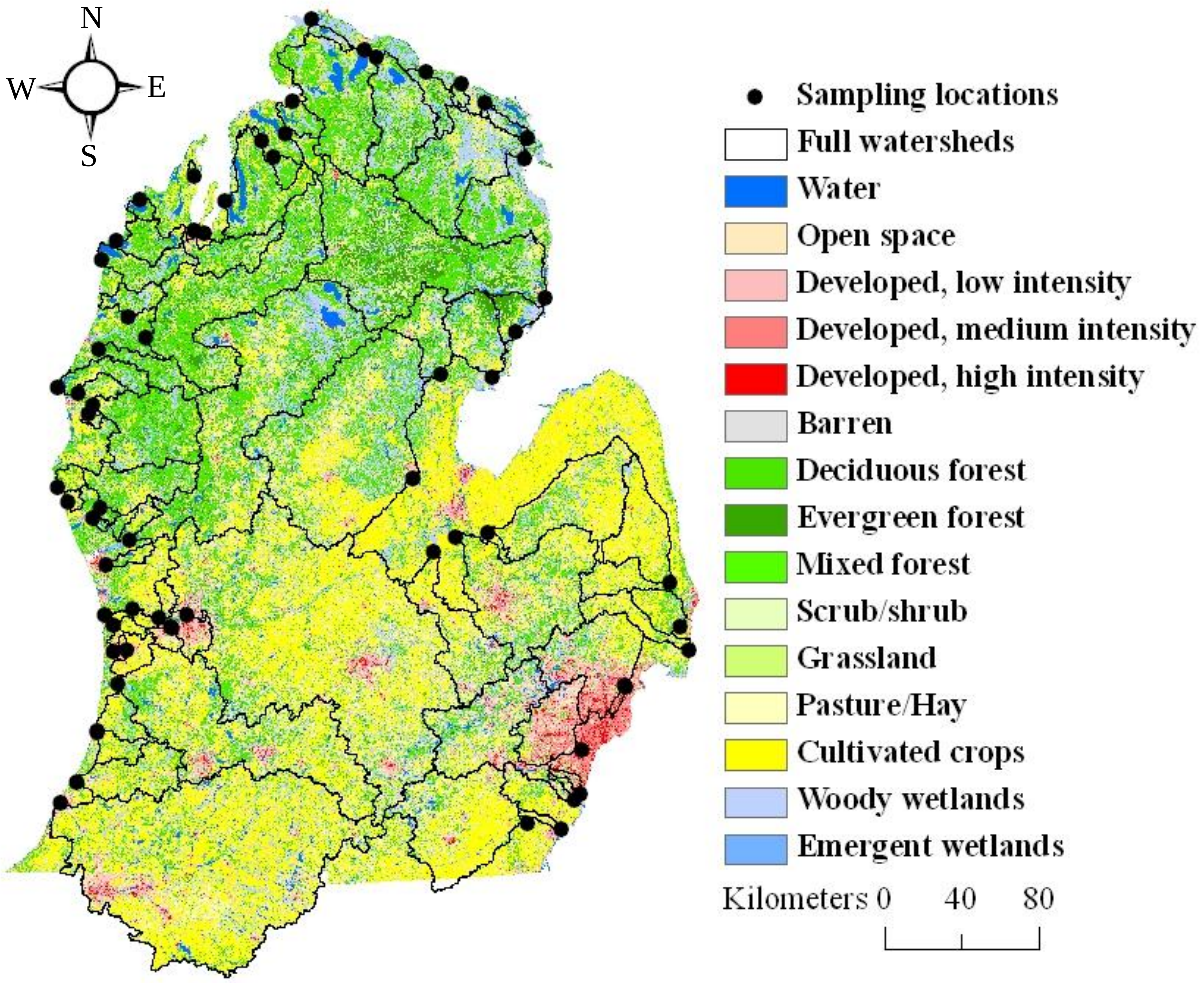

Figure 5.1. Sampled river systems and catchment areas in Michigan (USA) and NLCD 2006 land use in Michigan.

5.2.2. Water sample collection

Grab samples were collected from each river between October 1 and October 13, 2010 during baseflow hydrologic stage conditions. Baseflow was determined using gage data and reasonable assumptions gained from team member's prior field experiences in Michigan and the Great Lakes. Water temperature (°C), specific conductance (microsiemens per centimeter), and dissolved oxygen (mg l^{-1}) were measured on site using YSI 600R Sonde (YSI Incorporated, United States). Field samples were placed on ice in coolers and transported to Michigan State University for analysis at respective laboratories. The same methods were used for samples collected during spring thaw (March 4-23, 2011) and after summer rainfall (June 1-28, 2011).

5.2.3. Water analysis

Each sample was assayed for water chemistry as summarized in Table 5.1. Microbial analyses were performed within 24 hours of collection, whereas DNA analysis was performed at a later date on frozen sample concentrate (described below). All samples were tested for *E. coli* using IDEXX Colilert® Quanti-Tray 2000®. Following incubation at 35°C (± 0.5 °C) for 24 hour (± 2 hour), yellow and fluorescent wells were reported positive for *E. coli*, compared to a most probable number (MPN) table, and reported as MPN 100 ml^{-1}. *Escherichia coli* C-3000 (ATCC 15597) were used as positive controls for verification of media integrity. Sterile water was used for negative controls to verify method integrity. *E. coli* measurements below method detection capabilities (1.0 MPN 100 ml^{-1}) were assigned a value equal to the detection limit.

Samples were analyzed for the human specific marker *B. thetaiotaomicron* α-1-6 mannanase (5'CATCGTTCGTCAGCAGTAACA3'; 5'CCAAGAAAAAGGGACAGTGG3') according to Yampara-Iquise et al. (2008). Analysis was performed by filtering 900 ml of water through a 0.45 µm hydrophilic mixed cellulose esters filter. Each filter was placed into a 50 ml centrifuge tube containing 20 ml of sterile Phosphate Buffered Water, vortexed, and centrifuged (30 minutes; 4000 x *g*; 21 °C). Eighteen ml were decanted from the tube and the remaining eluent and pellet were stored at -80 °C. DNA was extracted from 200 µl of the thawed pellet via QIAamp® DNA mini kit protocol. Quantitative polymerase chain reaction (qPCR) for *B.*

thetaiotaomicron was performed following Yampara-Iquise et al. (2008) with a probe modification (Srinivasan et al. 2011) using a Roche Light-Cycler® 2.0 Instrument (Roche Applied Sciences). Each *B. thetaiotaomicron* assay was carried out with 10 µl of LightCycler 480 Probe Mastermix (Roche Applied Sciences), 0.4 µl forward and reverse primers, 0.2 µl probe #62 (6FAM-ACCTGCTG-NFQ; Roche Applied Sciences Universal Probe Library), 1.0 µl Bovine Serum Albumin, 3.0 µl nuclease free water, and 5.0 µl of extracted DNA and processed in triplicates. The qPCR analyses included a 15 minute, 95 °C pre-incubation cycle, followed by 50 amplification cycles, and a 0.5 minute 40 °C cooling cycle. A diluted plasmid standard was included during each qPCR run as a positive control and molecular grade water was used in place of DNA template for negative controls. *B. thetaiotaomicron* gene copies were converted to cell equivalents (CE) and reported as qPCR CE 100 ml^{-1}. For *B. thetaiotaomicron*, one copy of the target gene is present in each cell, thus one copy number corresponds to one cell.

E. coli and *B. thetaiotaomicron* results were reported as concentrations instead of loads to be consistent with USEPA's total maximum daily load (TMDL) recommendations. The USEPA suggests a recreational water quality threshold for *E. coli* of 2.37 $\log_{10}$ MPN 100 ml^{-1}, above which full body submersion is not recommended.

Table 5.1. Summary of chemical and nutrient methods with respective references.

Assay	Units	Method description	Reference
Ammonia	µg l^{-1}	Phenate method	Standard Methods 4500-NH_3-G*
Calcium	mg l^{-1}	Flame atomic absorption spectrophotometry	Wetzel and Likens 2000
Chlorine (Cl-)	mg l^{-1}	Dionex membrane-suppression ion chromatography	Wetzel and Likens 2000; Hamilton et al. 2009
Magnesium	mg l^{-1}	Flame atomic absorption spectrophotometry	Wetzel and Likens 2000
Nitrate/nitrite	µg l^{-1}	Cadmium reduction	Standard Methods 4500-NO_3-E*
Pheophytin corrected chlorophyll a	µg l^{-1}	Fluorometry with pheophytincorrection following ethanol extraction	Standard Methods 10200.H*
pH		Hydrolab multisonde	Hamilton et al. 2009
Potassium	mg l^{-1}	Flame atomic absorption spectrophotometry (0.5% HNO_3 preservative)	Hamilton et al. 2009
Sodium	mg l^{-1}	Flame atomic absorption spectrophotometry (0.5% HNO_3 preservative)	Hamilton et al. 2009
Soluble reactive phosphorus	µg l^{-1}	Ascorbic acid method	Standard Methods 4500-P.E.*
Sulfate (SO4)	µg l^{-1}	Dionex membrane- suppression ion chromatography	Hamilton et al. 2009
Total dissolved nitrogen	µg l^{-1}	Second derivative spectroscopy following persulfate digestion	Crumpton et al. 1992
Total dissolved phosphorus	µg l^{-1}	Ascorbic acid method following persulfate digestion	Standard Methods 4500-P.E and 4500-N.C*
Total nitrogen	µg l^{-1}	Second derivative spectroscopy following persulfate digestion	Crumpton et al. 1992
Total phosphorus	µg l^{-1}	Ascorbic acid method following persulfate digestion	Standard Methods 4500-P.E and 4500-N.C*
Total chlorophyll a	µg l^{-1}	Fluorometry following ethanol extraction	Standard Methods 10200.H*

*APHA (1999)

5.2.4. Hydrometry

Hourly precipitation data were extracted from Next Generation Radar (NEXRAD) through the National Climate Data Center (http://www.ncdc.noaa.gov/nexradinv/). The radar stations were located in Grand Rapids, Gaylord, and Detroit (Michigan) and had a base reflectivity of 0.50 degree with an elevation range of 124 nautical miles and 16 km^2 cells. Hourly precipitation averages across each watershed were used to calculate total rainfall with weighted averages applied to watersheds partially contained in NEXRAD cells. Precipitation was categorized into cumulative hourly totals (mm) prior to sample collection and reported as mm per time prior to sample collection.

Real-time river velocity was measured at each site during sample collection using an Acoustic Doppler Current Profiler (ADCP), USGS stream gauges, or current-meter via wading following USGS protocol (Jarrett 1991). River discharge was calculated from flow velocities and reported as $m^3 s^{-1}$.

5.2.5. Land use

Land use, watershed delineation, and septic system estimates were defined using ESRA ArcMap GIS software. The spatial analyst watershed tool was used to develop surface watersheds for each sampling point at 1/3 Arc-Second resolution contour lines on a GCS North American coordinate system. Two watersheds were defined for each river and referred to as 1) full watersheds which included the entire upstream drainage area and 2) reduced watersheds which included drainage boundaries upstream of the sampling site to the nearest lakes, reservoirs, and ponds, referred to as ‘lakes’ from here on. A 60 m riparian buffer (referred to as ‘buffer’ from here on) was applied to each watershed. A digital map of land cover from Landsat imagery at 30 meter resolution and the National Land Cover Database (2006) was used to define land use of each watershed and buffer. Land use was categorized using NLCD classification system with 16 categories and further refined to seven categories using Anderson Land Cover Classification System Level 1 (Anderson et al. 1976). Table 5.2. describes the Anderson classifications and equivalent NLCD categories.

Table 5.2. Anderson level 1 land use classifications and descriptions.

Classification	Description	Examples	Associated NLCD classifications (Code)
Urban	Intensive use with structures covering the majority of land	Cities, shopping, industrial, and commercial centers	Developed open space (21) Developed low intensity (22) Developed Medium intensity (23) Developed high intensity (24)
Agricultural	Land used for food production	Pasture, row crop, orchards, confined feeding operations	Pasture and hay (81) Cultivated crops (82)
Open	Predominant natural vegetation is grass or shrubs	Herbaceous, shrub, brush	Shrub and scrub (52) Grassland and herbaceous (71)
Forest	Closed canopy at least 10% from timber quality trees	Deciduous, coniferous, and mixed forested	Deciduous forest (41) Evergreen forest (42) Mixed forest (43)
Water	Area predominantly cover by water throughout year	Streams, lakes, bays, and reservoirs	Water (11)
Wetland	Land with water table near land surface for significant portion of year	Marshes, swamps, perched bogs	Woody wetland (90) Emergent herbaceous wetland (95)
Barren	Land that has less than one-third vegetative cover.	Beaches, exposed rock, gravel pits	Barren (31)

Using GIS programs, household locations from the 2010 US Census data were compared to current municipal sewage treatment infrastructure locations to produce a list and location of households that likely utilize on-site septic systems to treat wastewater (Luscz and Hyndman, in prep.). Estimated septic system numbers (per watershed) and densities (per km^2) in catchment and 60m buffered areas were calculated for each of the 64 river systems.

The National Pollutant Discharge Elimination System (NPDES) from the Environmental Protection Agency's Enforcement and Compliance History was used to estimate ammonia and total phosphorus loads (kg $year^{-1}$) from point source effluents discharging upstream of the sampling point for each river (Luscz and Hyndman, in prep.).

5.2.6. Statistical analysis

A constant value of one was added to *E. coli* and *B. thetaiotaomicron* concentrations prior to log transformation and statistical analysis. Soil hydraulic conductivity underwent natural log transformations prior to statistical analyses. Spearman Correlation tests were used to examine relationships amongst physical, chemical, weather, river discharge, land use, estimated pollution discharges and microbial measurements. Significance threshold was set at (α) 0.05. Descriptive statistics were performed using IBM SPSS Statistics software (Version 19.0).

Classification And Regression Tree (CART) analysis was used to compare *E. coli* and *B. thetaiotaomicron* results to chemical, hydrological, physical, environmental, and land use variables. Three *E. coli* and *B. thetaiotaomicron* model scenarios were created: 1) only land use variables; 2) only nutrient, chemical, hydrological, precipitation, and physical variables; and 3) all variables combined.

CART attempts to split dependent variables into homogeneous categories based upon the influence of independent variables on the dependent variable (target organism). CART was previously used to investigate pathogenic bacteria and parasite relationships with environmental and land use factors (Wilkes et al. 2011), to classify lakes based on chemistry and clarity (Martin et al. 2011), and to predict the occurrence of fecal indicator bacteria with respect to physiochemical variables (Bae et al. 2010).

CART models started out with a parent or root node containing all available data. Then all independent variables were examined and the variable that produced the two most different groups of dependent variables, using regression analysis based on R^2 and pre-defined splitting criteria were selected (splitting variables). In this study, splitting criteria were developed using recursive partitioning algorithm and a 10-fold cross validation. A 10-fold cross validation broke all data into 10 subsets and calculated the split based on nine of the ten subsets. Each time a group split (per above criteria) the binary splits were called child nodes. This method was used for each group until reaching a stopping rule set at a minimum of five observations per subgroup (Martin et al. 2011). A terminal node was defined as a child node which met the defined stopping rule.

Fully grown trees often required pruning to ensure significant variable associations were not missed as a result of following the splitting and stopping criteria (Lemon et al. 2003). Pruning is the process of producing the trees until they reach the stopping criterion and then removing the less statistically significant results from the analysis. Pruning followed the 1-standard error rule (Breiman et al. 1984; Venables and Ripley 1999; De'ath and Fabricous 2000) which minimized the cross-validated error of the model. This approach was shown to produce optimal sized and more stable tree sizes across replications compared to the 0-standard error pruning method (Breiman et al. 1984; Questier et al. 2005).

Competitor and surrogate variables were identified for each node by investigating detailed CART outputs. Competitor splits are those variables with similar complexity parameter values compared to the primary split. A complexity parameter compares the number of terminal nodes (complexity) to the cross-validated error for each group. For this project the complexity parameter was set at 0.05. Surrogate splits were alternative variables that split the subgroup into very similar groups. All CART analyses were performed using R software system (R foundation for Statistical Computing).

An example of a CART output is presented below (Figure 5.2.). At the top of the tree, a parent or root node is presented with the primary splitting variables and values described for each child node. At the bottom of the tree, terminal nodes include the mean concentration and number of target organism cases in each node.

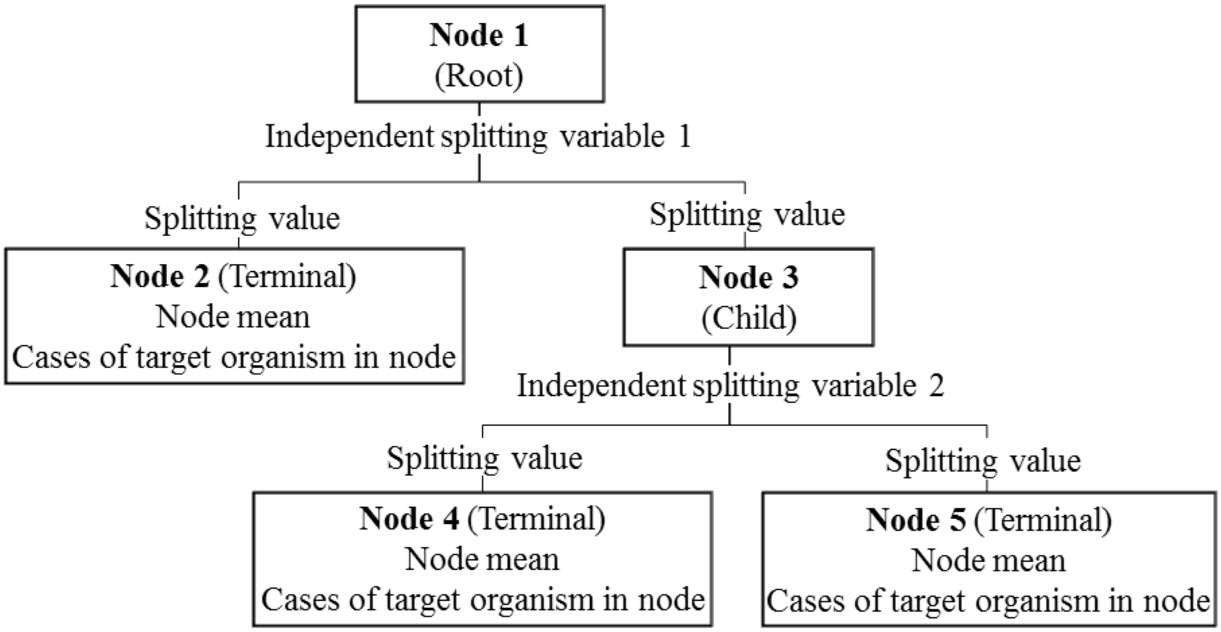

Figure 5.2. Classification And Regression Tree analysis output example. Root nodes contain all available data and are split into binary groups using recursive partitioning algorithm and 10-fold cross validation with a complexity parameter value of 0.05. Primary splitting variables and values are described for each child node. Terminal nodes (bottom of the tree) include the mean concentration and number of target organism cases in each node. Each node was derived based on mean value of each response variable, group size, and defining variables.

5.3. Results

Sixty-four river catchments were sampled during baseflow, spring thaw, and summer rain conditions. *E. coli* and *B. thetaiotaomicron* results for all sites under each of the three conditions are presented in Table S.5.1. However, this manuscript and all presented calculations and results, address the baseflow conditions only. Baseflow conditions offer an opportunity to define reference conditions which provide a measuring point for temporal changes and management goals. Future work will compare microbial analysis across baseflow, spring thaw, and summer rainfall events.

5.3.1. Microbial water quality

The first goal of this project was to examine *E. coli* CFU and *B. thetaiotaomicron* cell equivalent concentrations in rivers under baseflow conditions from 64 rivers systems in the Lower Peninsula of Michigan (USA). *E. coli* ranged between 0.20 and 3.0 $\log_{10}$ MPN $100ml^{-1}$ with a geometric mean of 1.4 $\log_{10}$ MPN $100ml^{-1}$. *E. coli* levels were below the detection limit (< 1 MPN 100 ml^{-1}) at four rivers. *B. thetaiotaomicron* concentrations ranged between 4.2 and 5.9 $\log_{10}$ CE 100 ml^{-1} with a geometric mean of 5.1 $\log_{10}$ CE 100 ml^{-1}. Interestingly, *B. thetaiotaomicron* was present in all samples even in the absence of *E. coli*. Figure 5.3. illustrates the ranges of *E. coli* and *B. thetaiotaomicron* measured in each river system.

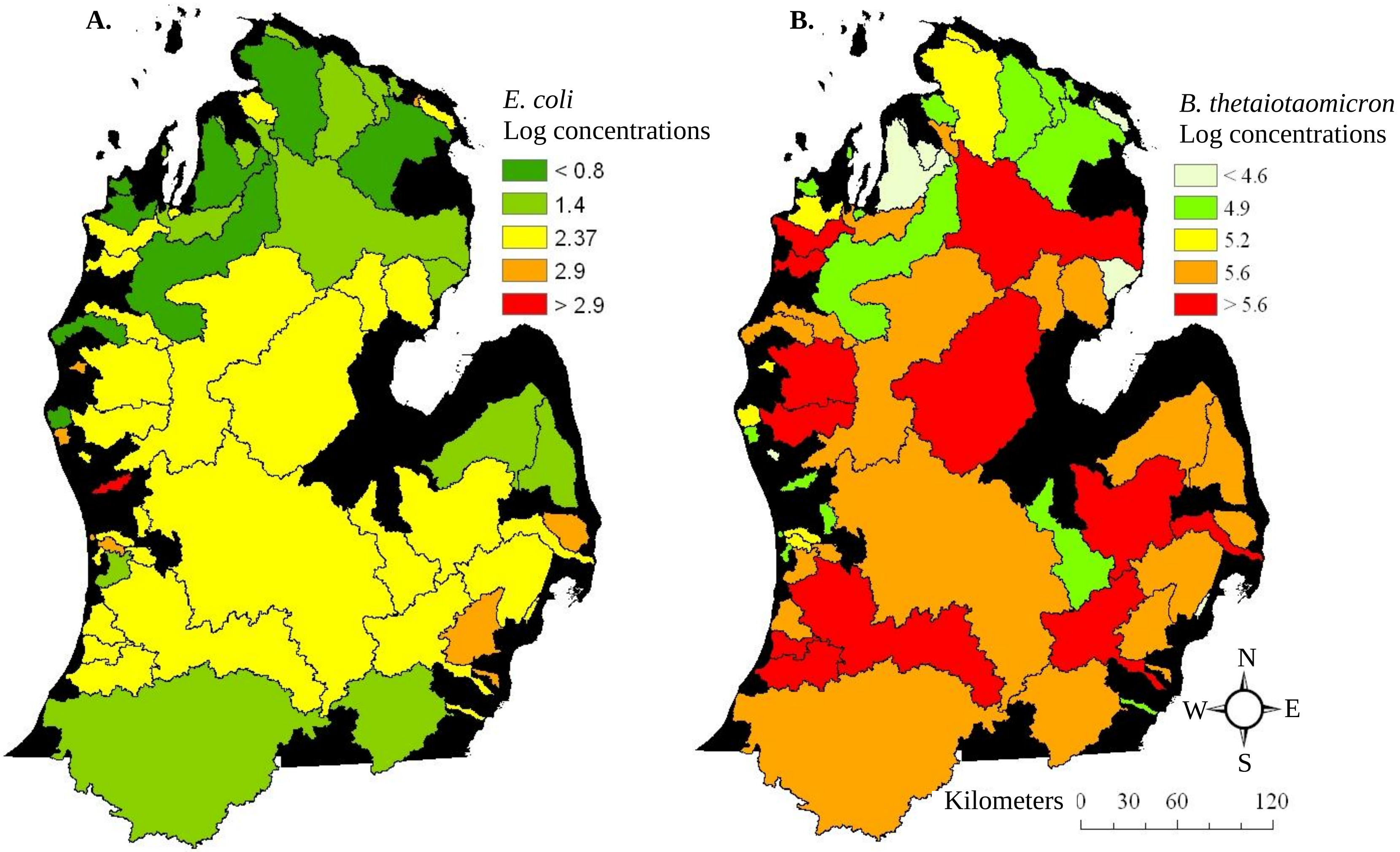

Figure 5.3. (A.) *E. coli* ($\log_{10}$ MPN 100 ml^{-1}) and (B.) *B. thetaiotaomicron* ($\log_{10}$ CE 100 ml^{-1}) concentrations measured at 64 river catchments under baseflow conditions. *E. coli* and *B. thetaiotaomicron* categories were evenly split across the concentration range. Areas in black were not sampled. The USEPA health exposure criterion for *E. coli* is 2.37 $\log_{10}$ MPN 100 ml^{-1}, shown as the two highest categories in the *E. coli* figure and was detected at nine rivers. No single river sample had measurable concentrations of both microorganisms in the highest concentration categories.

Nine rivers exceeded USEPA's suggested criterion for safe contact (2.37 $\log_{10}$ *E. coli* MPN 100ml^{-1}). At these nine rivers, *E. coli* ranged from 2.4 to 3.0 $\log_{10}$ MPN 100ml^{-1} with a geometric mean of 2.3 $\log_{10}$ MPN 100 ml^{-1} and *B. thetaiotaomicron* ranged from 4.6 to 5.6 $\log_{10}$ CE 100 ml^{-1} with a geometric mean of 5.0 $\log_{10}$ CE 100 ml^{-1}. In the rivers meeting USEPA criterion ($n = 55$), *E. coli* and *B. thetaiotaomicron* geometric means were 1.3 $\log_{10}$ MPN 100 ml^{-1} and 5.2 $\log_{10}$ CE 100 ml^{-1}, respectively. *E. coli* ranged from 0.30 to 2.3 $\log_{10}$ MPN 100 ml^{-1} and *B. thetaiotaomicron* ranged from 4.2 to 5.9 $\log_{10}$ CE 100 ml^{-1}. A comparison of microorganism geometric means at sites above and below criterion indicated *E. coli* were statistically different ($p < 0.001$) while *B. thetaiotaomicron* were not different ($p = 0.433$) between the two groups. Correlation analysis between *E. coli* and *B. thetaiotaomicron* at sites below criteria were statistically related ($r = 0.308$, $p = 0.022$) while *E. coli* and *B. thetaiotaomicron* at sites above *E. coli* criteria were not statistically related ($r = 0.159$, $p = 0.683$).

5.3.2. Land use

Land use classifications for each river system at the full watershed, reduced watershed, and reduced watershed riparian buffer are summarized in Table 5.3 and detailed in Table S.5.2 according to Anderson Land Use Classification systems level one. The land use composition over the entire project area is also illustrated in Figure 5.1. Overall, full watershed sizes ranged from 2.9 km^2 to 12853 km^2 ($\overline{X} = 1377$ km^2). Urban development averaged 16.7% and 21.3% in the full and reduced watersheds, respectively. In the reduced watersheds, urban coverage exceeded 90% at four sites while 34 sites had less than 10% urban coverage. Agriculture in the full and reduced watersheds averaged 27.9% and 27.2%, respectively. Forest, water, and wetland cover in full watersheds averaged 31.3%, 2.67%, and 14.0%, respectively. Forest, water, and wetland cover in reduced watersheds averaged 29.0%, 1.61%, and 13.9%, respectively. In the reduced watershed and buffered areas, significant associations were identified between *E. coli* and percent agriculture ($r > 0.345$, $p < 0.005$) and water ($r > -0.311$, $p < 0.01$) coverage. *B. thetaiotaomicron* was also associated with agriculture cover at the reduced watershed and buffered area ($r > 0.250$, $p < 0.05$).

Impervious surface coverage averaged 5.5% in the buffers and 7.5% in the watersheds with a low of 0.0% and a high of 55.9%. Impervious surface coverage in the buffer and watershed were correlated to septic density at the same spatial scale ($r \geq 0.370$, $p < 0.001$). In the reduced watersheds, septic system numbers in the reduced watersheds ranged between 0 and 63624 systems per watershed ($\overline{X}$ = 6063 systems per watershed). Similar septic system densities were observed in the buffer ($\overline{X}$ = 17 systems km^{-2}), reduced watershed ($\overline{X}$ = 19 systems km^{-2}), and full watersheds ($\overline{X}$ = 16 systems km^{-2}). *B. thetaiotaomicron* was statistically related to the number of septic systems in the watershed ($r = 0.634$, $p < 0.001$). The average soil hydraulic conductivity in the buffer and watershed was 1.78 m day^{-1} and 2.21 m day^{-1}, respectively. Soil conductivity ranged from 0.40 to 4.7 m day^{-1} in the buffer and 0.52 to 4.7 m day^{-1} in the watershed. No *E. coli* or *B. thetaiotaomicron* correlations were identified with impervious surface coverage, septic system density per km^{2}, soil hydraulic conductivity, or estimated total point source loadings of total nitrogen and phosphorus.

Of the 64 sampled rivers, six were located in Areas of Concern (AOC) as identified by the Michigan Department of Environmental Quality (http://www.michigan.gov/deq/0,4561,7-135-3313_3677_15430-240913--,00.html). The *E. coli* geometric mean for these six sites (1.9 $\log_{10}$ MPN 100 ml^{-1}) was not statistically different than the overall *E. coli* geometric mean for the entire project ($p = 0.345$). However, the *B. thetaiotaomicron* geometric mean (5.6 $\log_{10}$ CE 100 ml^{-1}) was statistically higher than non-AOC sites ($p = 0.002$). No significant land use correlations existed with either microorganism at these six sites ($p < 0.05$), indicating another land use characteristic was associated with increases of this human fecal marker.

Table 5.3. Land use summary for full watersheds, reduced watersheds, and reduced watersheds riparian buffers (60 m).

Scale-Parameter	Minimum	Mean	Maximum	Standard deviation
Watershed [A]				
Area (km^2)	2.88	1377	12854	2431
Septic density (Number km^{-2})	0.00	16	114	19.5
Impervious surface (km^2)	0.41	5.13	56.9	9.80
Urban (%)	3.16	16.7	99.7	0.21
Agriculture (%)	0.00	28.0	74.2	0.22
Open (%)	0.00	6.97	20.1	0.05
Forest (%)	0.19	31.4	70.7	0.18
Water (%)	0.00	2.68	23.7	0.04
Wetland (%)	0.07	14.0	48.3	0.10
Barren (%)	0.00	0.31	2.45	0.00
Watershed [B]				
Area (km^2)	0.15	366	4065	630
Septic density (Number km^{-2})	0.00	19.0	102	18.1
Impervious surface (km^2)	0.40	7.50	55.9	13.6
Urban (%)	3.10	21.3	99.7	26.2
Agriculture (%)	0.00	27.2	77.4	24.0
Open (%)	0.00	6.16	18.8	5.27
Forest (%)	0.00	29.0	71.2	19.5
Water (%)	0.00	1.61	15.4	3.32
Wetland (%)	0.00	13.9	47.9	12.1
Barren (%)	0.00	0.77	31.1	3.87

[A] Entire upstream drainage area including lakes; [B] Watersheds were defined as the total upstream area to the nearest lake draining to each rivers respective sampling point

Table 5.3. (cont'd)

Scale-Parameter	Minimum	Mean	Maximum	Standard deviation
60 m riparian buffer $^{\mathbf{B}}$				
Area (km^2)	0.06	46.0	497	78.3
Septic density (Number km^{-2})	0.00	17.0	161	29.0
Impervious surface (km^2)	0.00	5.50	42.7	9.64
Urban (%)	0.00	18.9	98.3	23.0
Agriculture (%)	0.00	21.4	72.1	21.7
Open (%)	0.00	3.64	19.4	3.80
Forest (%)	0.00	22.1	62.6	14.9
Water (%)	0.00	6.09	63.2	12.0
Wetland (%)	0.00	27.3	76.3	17.9
Barren (%)	0.00	0.59	24.9	3.12

5.3.3. Physical, chemical, environmental, and hydrology

Variables thought to be influencing water quality in Great Lakes tributaries were measured according to Table 5.1. Dissolved oxygen ranged from 5.9 to 13.3 mg l^{-1} ($\overline{X}$ = 9.8 mg l^{-1}), potassium ranged from 0.43 to 9.8 mg l^{-1} ($\overline{X}$ = 2.2 mg l^{-1}), and total phosphorus ranged from 7.7 to 396 mg l^{-1} ($\overline{X}$ = 37.8 mg l^{-1}). Water temperatures ranged between 7.0°C and 17.5°C ($\overline{X}$ = 13.1 °C) and were directly correlated to urban ($r = 0.466$) and water ($r = 0.328$) coverage while inversely correlated to open ($r = -0.580$), forest ($r = -0.429$), and wetland ($r = -0.440$) coverage at the watershed scale ($p < 0.008$), demonstrating the urban heat effect.

Analysis of the data was undertaken during base flow when precipitation was not significantly influencing water quality or quantity. Six hour cumulative precipitation amounts ranged from 0.0 to 9.2 mm and averaged 0.14 mm. River discharge ($\overline{X}$ = 6.7 m^3s^{-1}) ranged from 0.01 to 57 m^3s^{-1} and discharge per area ranged from 0.0 to 84 $m^3s^{-1}km^{-2}$. Discharge for each river system is provided in Table S.5.1. Descriptive statistics for all physical, chemical, and hydrological variables are provided in Table S.5.3. A complete chemical, nutrient, environmental, and hydrological analysis is provided in Martin et al. (in preparation for publication).

5.3.5. CART analysis of microbial water quality

CART analysis was used to determine associations between water quality variables and microorganisms for full and reduced watersheds. Reduced watershed analysis was performed only on river systems where sampling points were not located at the lake outlet (n = 53). Eleven sites were located at lake outlets resulting in defined watershed which averaged 108 km^2, significantly smaller than the overall watershed size ($\overline{X}$ = 366 km^2). In comparison to the other 53 systems, these eleven watersheds had 9X greater water coverage (6% watershed and 23% buffer) and 5X less agriculture coverage (6% watershed and 4% buffer). These eleven sites were removed from the reduced watershed and buffer CART analysis as it was assumed retention time in the lentic water systems reduced microbe inputs derived from land use activity. CART analyses for each model scenario are summarized in Table 5.4. and presented in Figure S.5.1.

Table 5.4. CART analyses for *E. coli* and *B. thetaiotaomicron* as dependent variables and land use, nutrient, chemical, hydrological, and environmental as independent variables.

Watershed scale	Model Scenario	Target organism (Total CP)	Primary split	Split value	Number of target organisms	Geometric mean ($\log_{10}$ CE or MPN)
Full (n=64)	All data	*E. coli* (47.4%)	Total phosphorus	$< 19.0^{A}$	24	0.98
			Total phosphorus	$> 19.0^{A}$	40	2.02
		B. thetaiotaomicron (36.5%)	Septic system #	$< 1622^{D}$	19	1.73
			Septic system #	$> 1622^{D}$	45	5.32
	Land use data	*E. coli* (28.8%)	Forest	$< 42.5^{C}$	43	1.91
			Forest	$> 42.5^{C}$	21	1.06
		B. thetaiotaomicron (51.8%)	Septic system #	$< 1622^{D}$	19	1.73
			Septic system #	$> 1622^{D}$	45	5.32
	Nutrient, chemical, precipitation, and physical	*E. coli* (63.8%)	Total phosphorus	$< 19.0^{A}$	24	0.98
			Total phosphorus	$> 19.0^{A}$	40	2.02
		B. thetaiotaomicron (25.8%)	Dissolved oxygen	$< 10.0^{B}$	36	5.35
			Dissolved oxygen	$> 10.0^{B}$	28	4.89

^{A} µg l^{-1}; ^{B} mg l^{-1}; ^{C} percent land cover; ^{D} total number of estimated septic systems in the watershed. *Reduced watersheds excluded upstream lakes and reservoirs and included 53 river systems as 11 sampling points were located at the lake outlet, resulting in substantially smaller watersheds and minimizing the influence of land use characteristics on water.

Table 5.4. (cont'd)

Watershed scale	Model Scenario	Target organism (Total CP)	Primary split	Split value	Number of target organisms	Geometric mean (log10 CE or MPN)
Reduced* (n=53)	All data	*E. coli* (36.4%)	Potassium	$< 0.91^{B}$	15	1.29
			Potassium	$> 0.91^{B}$	38	2.04
		B. thetaiotaomicron (50.2%)	Septic system #	$< 1912^{D}$	25	4.87
			Septic system #	$> 1912^{D}$	28	5.50
	Land use data	*E. coli* (22%)	Mixed forest	$< 1.28^{C}$	32	2.04
			Mixed forest	$> 1.28^{C}$	21	1.50
		B. thetaiotaomicron (50.2%)	Septic system #	$< 1912^{D}$	25	4.87
			Septic system #	$> 1912^{D}$	28	5.50
	Nutrient, chemical, precipitation, and physical	*E. coli* (58.7%)	Potassium	$< 0.91^{B}$	15	1.29
			Potassium	$> 0.91^{B}$	38	2.04
		B. thetaiotaomicron (33.5%)	Dissolved oxygen	$< 10.0^{B}$	28	5.45
			Dissolved oxygen	$> 10.0^{B}$	25	4.93

CART models developed using only land variables indicated low forest coverage (< 42.5% in the watershed) had the strongest association with the highest *E. coli* levels ($\overline{X} > 1.91$ $\log_{10}$ MPN 100 ml^{-1}). Septic system numbers had the strongest association with elevated *B. thetaiotaomicron* concentrations. The number of septic tanks required to impair water quality varied depending on whether upstream lakes were included (i.e. full watershed; > 1621 septic systems per watershed) or excluded (i.e. reduced watershed; > 1912 septic systems per watershed). The number of septic systems located within the 60 m riparian buffer was a competitor variable for *B. thetaiotaomicron* in the full watershed model (improvement difference between primary and competitor variable = 0.79%), but no competitor variables were identified for *E. coli*.

CART models developed with only nutrient, chemical, and hydrological independent variables identified the highest *E. coli* concentrations in the full watershed ($\overline{X} = 2.02$ $\log_{10}$ MPN 100 ml^{-1}; $R^2_{total} = 0.64$) were associated with total phosphorus levels above 19.0 μg l^{-1}, while in the reduced watersheds the highest *E. coli* concentrations were associated with potassium levels above 0.91 mg l^{-1} ($\overline{X} = 2.31$ $\log_{10}$ MPN 100 ml^{-1}; $R^2_{total} = 0.545$). Dissolved oxygen below 10 mg l^{-1} explained the highest levels of *B. thetaiotaomicron* ($\overline{X} > 5.35$ $\log_{10}$ CE 100 ml^{-1}) in the full and reduced watersheds. No strong competitor variables were identified for *E. coli* or *B. thetaiotaomicron*.

Finally, models were developed that incorporated all independent variables. Interestingly, *E. coli* outputs for these models were nearly identical to models developed with only nutrient, chemical, and hydrological variables. However, *B. thetaiotaomicron* outputs developed with all variables were nearly identical to the models developed with only land use variables and were heavily influenced by the number of septic systems located in the watershed. Septic system numbers in the full watershed were directly correlated to *B. thetaiotaomicron* concentrations (R^2 = 0.338). No competitor variables were identified for *E. coli* in this model, but as seen in the *B. thetaiotaomicron* land use model, the number of septic systems located within the 60 m riparian buffer was a competitor variable.

5.4. Discussion

E. coli is commonly used as an indicator of fecal contamination and pathogens in freshwater rivers and lakes. As shown in this study, *E. coli* can be found in a variety of stream systems under baseflow conditions. In general, observed *E. coli* densities were below USEPA water quality criteria. The *E. coli* levels in this study were within previously observed and reported ranges in Great Lakes tributary rivers (Byappanahalli et al. 2003; Byappanahalli et al. 2006; Nevers et al. 2007). The overall *B. thetaiotaomicron* geometric mean was a single log higher than secondary treated sewage effluent, while the highest concentrations in the current study were 1.5 logs higher than biologically treated septage effluent (Srinivasan et al. 2011). Collectively, the *E. coli* and *B. thetaiotaomicron* results suggest human fecal contamination was impairing river water quality under baseflow conditions.

A comprehensive review by Wade et al. (2003) found *E. coli* levels in freshwater below 2.23 $\log_{10}$ MPN 100 ml^{-1} were associated with low relative risks for swimmers compared to non-swimmers. Since the *E. coli* geometric mean concentration observed in this study (1.4 $\log_{10}$ MPN 100ml^{-1}) was below the safety level reported by Wade et al. (2003), it is suggested that a reference condition for *E. coli* be established at 1.4 $\log_{10}$ MPN 100ml^{-1} for Michigan rivers. Wade et al. (2006) reported positive associations between occurrence of illness and molecular detection of *Bacteroides* at one Great Lakes beach with a geometric mean concentration of 3.08 $\log_{10}$ CE 100 ml^{-1}, but the authors note the associations were statistically weak ($p < 0.1$). Additionally, Yampara-Iquise et al. (2008) reported *B. thetaiotaomicron* levels ranged from 5.8 to 9.8 $\log_{10}$ copies 100 ml^{-1} in multiple urban, agricultural, and small town creek systems representing various levels of human impact. In the current study, the mean *B. thetaiotaomicron* concentration (5.1 $\log_{10}$ CE 100 ml^{-1}) was 1.6 times higher than levels measured by Wade et al. (2006) but below the range reported by Yampara-Iquise et al. (2008). Therefore, establishing a *B. thetaiotaomicron* reference condition for Michigan Rivers will require additional sample collection and analysis. While the concept of a reference condition lies in the notion of minimal impact (Reynoldson et al. 1997), this study examined a variety of river types including highly urbanized systems, as it is widely recognized that few streams are truly unimpaired in the Great Lakes. It is also understood that *E. coli* levels in watersheds will likely differ from the reference condition depending on temporal changes and geographic, natural, and anthropogenic characteristics. However, establishing such levels in the Great Lakes is important to define acceptable disturbance levels, support management decisions, and define long term water quality changes.

On average, *E. coli* concentrations were 0.5 logs higher in rivers with less than 1.27% forest coverage in the 60 m riparian buffer suggests that the presence of a small forest cover will improve microbial water quality, perhaps by slowing the transport of bacteria through soil or overland. However, the overall watershed forest coverage also had a significant (indirect) influence on *E. coli* concentrations; suggesting that forests are bacteria sinks or more likely, the land use replacing forest are a source of bacteria. Overall, land use had relatively little influence on baseflow *E. coli* concentration as shown between the three CART scenario outputs. This was not surprising since precipitation, the primary driver of microbes from land to water, was purposefully excluded from sampling events.

It was thought that specific land use characteristics would better explain microorganism occurrence in water compared to overall land use. However, the number of septic systems per watershed was the only land use characteristic associated with *B. thetaiotaomicron* using CART. The direct and significant correlation identified between total number of septic systems in the full watershed and *B. thetaiotaomicron* concentrations illustrates a significant problem for Michigan with an estimated 1.4 million on-site septic systems (MDEQ 2009). Similar amounts of *B. thetaiotaomicron* variation were explained regardless of lake presence in the upstream watershed (i.e. full verse reduced watersheds). However, the number of septic systems associated with elevated *B. thetaiotaomicron* concentrations increased when upstream lakes were removed from model development; indicating lakes are a *B. thetaiotaomicron* sink under baseflow conditions. Although *B. thetaiotaomicron* was primarily explained by the number of septic systems per watershed, dissolved oxygen was also identified as an explanatory variable, indicating the presence of other potential sources of organic material leading to lower dissolved oxygen (i.e. sewage discharge or combined sewer overflows) as reported by Hvitved-Jacobson (1982) and Gammons et al. (2011). It is suggested that future analysis include incremental spatial assessment of *B. thetaiotaomicron* upstream and downstream from waste water treatment plant discharges.

Five of the 64 sampled river systems were placed on the AOC list due in part to beach closures, but only one river was explicitly characterized as having chronic bacterial contamination (Rouge River). At this site, *E. coli* was above the 95th percentile for microbial distributions of the project dataset (2.68 $\log_{10}$ MPN 100 ml^{-1}). In comparison to the overall geometric means, this watershed had 3X more septic systems (n = 20175), 4X more urban coverage (85%), 5X more impervious surface coverage (35%), and 4X more total nitrogen and total phosphorus loads associated with point source discharges (715149 and 128982 kg $year^{-1}$, respectively); indicating extreme watershed specific influences were masked during CART modeling. Further investigations should focus on smaller spatial scales, specifically on the effects of combined sewer overflows (although likely not an issue during baseflow conditions), waste water treatment infrastructure, local low impact development and fertilizer use policies in relation to water quality.

CART analysis identified higher levels of potassium and lower discharge rates resulted in the highest measurements of *E. coli*. Inverse relations between discharge and *E. coli* ($r = -0.517$, $p < 0.001$) suggest that *E. coli* could accumulate in streams when discharge rates are below 0.66 $m^3 s^{-1}$, above which *E. coli* would be transported downstream. Potassium has been linked to water softener regeneration waste, domestic sewage, forest clear cutting, and leaching of bio-solid and manure application in agriculture fields (Lynch and Corbett 1990; Wang et al. 1999; Thomas 2000; Chambers et al. 2002). Specifically, potassium levels reported in the literature averaged 25 mg l^{-1} in secondary sewage effluent (Emongor and Ramolemana 2004), 38.5 mg l^{-1} in septic tank effluent (Brandes 1977), 6.78 mg l^{-1} in a river with a watershed comprised predominantly by agriculturale (Neal et al. 2000), and 16 mg l^{-1} in a sewage impacted river (Gunkel et al. 2007). In the current study, elevated *E. coli* levels were associated with potassium levels above 0.91 mg l^{-1}, but the overall potassium average was 2.2 mg l^{-1}, much lower than previous reports. Observed potassium levels were more similar to those presented by Katz et al. (2011) and Wolf et al. (2004), indicative of contaminated groundwater. Thus, potassium levels above 0.91 mg l^{-1} may be a suitable indicator of elevated *E. coli* levels resulting from contaminated groundwater entering rivers during baseflow conditions.

Using a snapshot approach, this study found multiple Great Lake tributary rivers contained human fecal contamination under baseflow conditions. Results suggest a regional *E. coli* reference condition could be established below the current USEPA freshwater recreational criterion. Furthermore, the impact septic systems have on surface water quality was shown, highlighting the need for increased monitoring of on-site wastewater treatment systems. Michigan does not have a statewide sanitary code which has allowed septic systems to go unchecked for decades. If these systems are not addressed at a state level, continued chronic water quality impairments are expected.

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APPENDIX

Table S.5.1. *E. coli* and *B. theta* levels measured in 64 Michigan rivers under baseflow, spring thaw, and summer rain conditions.

Site ID	River system	*E. coli* (MPN 100 ml^{-1})			*B. thetaiotaomicron* (CE 100 ml^{-1})			Discharge (m^3 s^{-1})
		Baseflow	Spring thaw	Summer rain	Baseflow	Spring thaw	Summer rain	Baseflow
1	St. Joseph	1.0	1.4	1.8	5.3	4.4	3.1	57.28
2	Paw Paw River	1.8	1.7	1.1	5.7	4.2	3.6	8.01
3	Kalamazoo	1.6	1.6	1.5	5.9	4.0	3.3	0.00
4	Grand	1.5	1.3	1.1	5.5	4.1	3.5	50.69
5	Muskegon	1.8	1.8	1.6	5.4	3.9	3.7	37.77
6	White River	2.1	1.6	2.0	5.8	4.2	3.6	7.70
7	Pere Marquette	2.2	2.0	2.0	5.8	4.1	3.3	12.20
8	Big Sable River	0.7	0.3	0.3	5.3	3.7	3.5	2.87
9	Little Manistee	1.4	1.4	1.7	5.4	3.8	3.2	4.49
10	Manistee	0.2	0.2	1.5	4.7	3.6	3.4	42.76
11	Bear Creek	2.1	1.3	2.2	5.9	3.6	3.0	3.01
12	Betsie	2.0	0.9	1.9	5.8	4.0	3.1	22.83
13	Platte	0.2	0.2	1.0	5.2	3.7	3.3	4.82
14	Boardman	1.1	0.2	1.6	5.3	3.7	3.4	6.68
15	Elk-Torch	0.2	0.2	0.7	4.5	3.3	2.6	12.82
16	Cheboygan	0.2	0.2	1.0	4.9	3.2	3.3	26.05
17	Black	1.2	0.3	1.4	4.8	3.6	3.3	11.58
18	Thunder Bay	0.5	1.0	1.0	4.9	4.0	3.3	12.59
19	Au Sable	1.2	0.5	1.2	5.6	3.9	3.5	32.46
20	Au Gres	2.1	1.1	1.9	5.3	2.6	2.6	1.07
21	Rifle	1.6	1.6	2.3	5.5	4.1	3.8	5.04
23	Black River	1.1	2.8	-	5.4	4.1	-	0.66
24	Pine River	2.6	2.3	1.7	5.4	3.5	2.6	0.02

Table S.5.1. (cont'd)

Site ID	River system	*E. coli* (MPN 100 ml^{-1})			*B. thetaiotaomicron* (CE 100 ml^{-1})			Discharge (m^{3} s^{-1})
		Baseflow	Spring thaw	Summer rain	Baseflow	Spring thaw	Summer rain	Baseflow
25	Belle River	2.0	2.5	1.7	5.6	3.4	3.3	1.36
26	Clinton River	2.0	1.8	1.9	5.5	4.4	3.1	0.00
27	River Rouge	2.7	2.9	2.8	5.5	4.5	3.5	0.32
28	Huron	1.9	1.3	1.9	5.8	4.7	3.5	7.70
29	Raisin	1.4	1.9	2.3	5.4	3.9	2.6	4.73
31	South Branch Black River	2.3	2.5	1.6	5.9	3.5	3.2	1.10
32	North Branch Black River	2.2	2.2	1.7	5.6	4.1	3.2	1.49
33	Macatawa River	1.3	2.8	2.1	5.6	4.1	2.6	0.16
34	Pine Creek	2.1	2.6	2.3	4.9	3.6	3.3	0.27
35	Pigeon River	2.7	2.9	2.3	5.0	4.0	3.1	0.08
36	Rush Creek	2.3	2.1	2.5	5.4	4.0	2.6	0.12
37	Buck Creek	2.2	2.2	2.3	4.6	3.7	3.6	0.64
39	Sand Creek	2.3	2.9	1.9	4.9	4.1	2.6	0.31
40	Bass River	2.1	2.4	2.3	5.1	4.2	2.9	0.20
41	Little Pigeon Creek	2.5	2.2	2.3	4.9	4.0	3.5	0.03
43	Black Creek	3.0	2.0	2.1	4.8	3.9	3.9	0.66
48	Silver Creek	1.7	1.8	1.8	4.4	3.5	2.6	0.37
51	Flower Creek	2.5	2.2	2.7	4.6	3.7	2.6	0.34
52	Stony Lake Outlet	0.5	1.3	1.1	5.2	4.3	3.7	1.26
55	Swan Creek	2.5	2.7	2.7	4.9	3.8	3.4	0.34
56	Lincoln River	2.2	1.5	2.5	5.0	3.8	2.6	0.66
57	Crystal River	0.7	0.6	1.2	4.6	3.8	2.6	1.57
59	Belangers Creek	1.3	1.7	1.8	4.7	3.3	2.6	0.08

Table S.5.1. (cont'd)

Site ID	River system	*E. coli* (MPN 100 ml^{-1})			*B. thetaiotaomicron* (CE 100 ml^{-1})			Discharge (m^3 s^{-1})
		Baseflow	Spring thaw	Summer rain	Baseflow	Spring thaw	Summer rain	Baseflow
60	Mitchell Creek	2.2	0.9	2.6	4.8	4.0	3.1	0.29
62	Jordan River	0.9	0.8	1.6	4.4	2.6	2.6	4.11
63	Monroe Creek	1.2	1.2	2.3	4.5	2.6	2.6	0.08
64	Boyne River	1.2	0.5	1.5	5.4	2.6	2.6	1.75
65	Bear River	1.5	0.9	1.9	4.8	3.2	3.5	1.58
66	Carp River	1.0	0.8	1.8	5.0	3.9	2.9	1.76
67	Ocqueoc River	0.9	0.7	1.7	4.7	4.1	2.6	2.39
69	Trout River	1.3	0.9	1.6	4.8	3.7	2.9	0.41
70	Little Trout River	2.4	1.0	2.1	4.9	4.0	3.3	0.06
71	Long Lake Creek	1.8	0.8	1.1	4.2	3.5	3.1	0.03
73	Tawas River	1.2	0.8	2.0	4.5	4.1	2.6	1.59
91	Harrington Drain	2.0	1.9	3.0	4.5	4.3	3.4	0.01
94	Marsh Creek	2.4	1.5	2.5	5.3	4.1	2.6	0.13
97	Sandy Creek	2.2	1.7	2.4	4.9	3.8	2.6	0.02
101	Cass River	1.2	2.7	2.5	5.4	3.9	2.6	1.95
102	Flint River	1.9	2.7	2.1	5.7	5.2	2.6	6.31
103	Shiawassee River	2.0	2.5	2.1	4.7	4.4	2.6	4.36
104	Tittabawassee River	1.9	1.0	2.5	5.6	4.4	2.6	17.47

Table S.5.2. Land use composition of defined river catchments using Anderson Land Use Classification systems.

Site ID	Site Description	Area (km^2)	Urban (%)	Agriculture (%)	Rangeland (%)	Forest (%)	Water (%)	Wetland (%)	Barren (%)
1	St. Joseph	11061	14.3	59.5	1.1	10.4	2.4	12.2	0.2
2	Paw Paw River	1027	11.5	47.5	2.6	21.1	1.4	15.6	0.3
3	Kalamazoo	5002	14.1	47.8	1.8	21.5	2.1	12.4	0.4
4	Grand	12854	12.7	55.3	1.0	16.6	1.5	12.7	0.3
5	Muskegon	6418	7.6	19.6	9.6	40.5	3.9	18.6	0.1
6	White River	1049	5.2	20.3	9.7	49.7	0.7	14.3	0.1
7	Pere Marquette	1790	5.0	9.3	8.3	61.6	1.2	14.5	0.1
8	Big Sable River	476	5.3	11.5	8.0	52.4	5.0	17.0	0.8
9	Little Manistee	526	4.7	3.9	12.5	68.6	0.7	9.6	0.1
10	Manistee	3559	5.7	9.6	15.9	56.4	1.4	10.9	0.1
11	Bear Creek	350	6.3	13.8	20.1	37.6	2.3	19.7	0.1
12	Betsie	618	8.1	7.6	13.1	46.2	9.9	15.0	0.1
13	Platte	471	6.6	9.9	13.4	56.2	7.5	6.1	0.2
14	Boardman	716	10.8	10.4	18.8	46.7	2.1	10.9	0.2
15	Elk-Torch	1308	7.6	14.4	13.6	45.4	11.3	7.4	0.2
16	Cheboygan	2317	6.4	8.2	11.6	51.0	8.1	14.5	0.2
17	Black	1509	5.5	4.4	12.1	47.1	3.9	27.0	0.1
18	Thunder Bay	2241	6.3	11.0	8.8	40.2	2.7	31.0	0.1
19	Au Sable	5287	8.4	3.2	14.5	58.9	2.0	12.8	0.1
20	Au Gres	987	6.4	23.3	7.6	37.7	2.2	22.0	0.7
21	Rifle	858	9.3	16.5	8.9	44.2	1.6	19.4	0.1
23	Black River	1250	6.2	74.2	1.2	10.6	0.1	7.5	0.1

Table S.5.2. (cont'd)

Site ID	Site Description	Area (km^2)	Urban (%)	Agriculture (%)	Rangeland (%)	Forest (%)	Water (%)	Wetland (%)	Barren (%)
24	Pine River	440	9.0	46.5	3.2	33.3	0.3	7.5	0.1
25	Belle River	512	9.5	59.7	1.7	19.0	0.3	9.7	0.2
26	Clinton River	1880	51.5	20.2	1.3	14.9	2.8	8.6	0.7
27	River Rouge	1033	82.9	5.4	0.5	7.2	0.7	2.9	0.3
28	Huron	2298	32.5	24.5	1.2	21.8	4.2	15.1	0.6
29	Raisin	2683	10.8	67.4	0.8	11.1	1.4	8.3	0.2
31	South Branch Black River	313	9.1	45.8	4.4	22.8	1.2	16.5	0.2
32	North Branch Black River	398	7.0	43.6	5.6	24.8	1.7	17.1	0.2
33	Macatawa River	292	23.5	67.8	0.7	4.0	0.2	3.1	0.9
34	Pine Creek	48	48.4	30.9	1.1	12.1	0.3	6.1	1.1
35	Pigeon River	102	11.0	66.0	2.0	15.3	0.1	5.1	0.5
36	Rush Creek	152	56.4	31.5	0.4	7.6	1.1	2.3	0.6
37	Buck Creek	3	91.3	0.0	0.6	1.4	0.9	5.8	0.0
39	Sand Creek	142	19.1	60.8	0.8	11.3	0.2	7.6	0.3
40	Bass River	127	11.1	63.6	2.1	16.0	0.2	6.6	0.5
41	Little Pigeon Creek	14	18.9	16.4	6.2	41.9	0.0	16.3	0.3
43	Black Creek	136	14.9	34.8	5.3	29.9	4.8	10.1	0.2
48	Silver Creek	41	11.7	0.6	15.2	63.7	4.2	4.4	0.2
51	Flower Creek	79	10.2	45.6	10.7	27.7	0.6	3.4	1.8
52	Stony Lake Outlet	160	10.1	37.7	11.7	35.1	1.0	4.1	0.3
55	Swan Creek	54	5.5	57.9	8.2	15.5	1.3	11.5	0.0

Table S.5.2. (cont'd)

Site ID	Site Description	Area (km^2)	Urban (%)	Agriculture (%)	Rangeland (%)	Forest (%)	Water (%)	Wetland (%)	Barren (%)
56	Lincoln River	215	5.6	33.2	11.8	30.6	2.1	16.5	0.2
57	Crystal River	110	4.7	3.4	8.7	53.7	23.7	3.3	2.4
59	Belangers Creek	25	6.7	38.4	12.7	30.8	1.5	9.9	0.0
60	Mitchell Creek	38	28.3	22.8	16.3	19.4	0.2	13.0	0.1
62	Jordan River	174	3.2	7.8	6.5	70.7	0.0	11.8	0.1
63	Monroe Creek	27	4.2	22.3	8.8	44.5	2.2	18.1	0.0
64	Boyne River	199	8.3	16.1	10.8	54.5	0.6	9.4	0.2
65	Bear River	293	6.4	13.3	7.0	48.5	6.6	18.1	0.2
66	Carp River	119	6.2	8.6	7.7	22.0	7.0	48.3	0.1
67	Ocqueoc River	369	4.7	6.5	11.5	43.4	2.2	31.4	0.3
69	Trout River	82	4.6	13.5	9.5	28.8	0.1	43.1	0.3
70	Little Trout River	28	5.4	27.8	7.5	14.3	0.1	44.6	0.3
71	Long Lake Creek	162	5.7	11.7	7.1	20.7	15.7	39.1	0.1
73	Tawas River	403	8.4	7.1	6.9	51.6	2.0	24.0	0.0
91	Harrington Drain	53	99.7	0.0	0.0	0.2	0.0	0.1	0.0
94	Marsh Creek	78	72.0	4.7	1.7	15.4	0.0	5.9	0.2
97	Sandy Creek	82	26.2	58.7	1.3	10.6	0.0	2.7	0.4
101	Cass River	2174	6.9	57.4	2.2	19.7	0.2	13.5	0.1
102	Flint River	3206	21.0	40.6	2.0	24.1	1.6	10.4	0.3
103	Shiawassee River	1517	15.7	52.5	0.7	17.0	2.2	11.4	0.4
104	Tittabawassee River	6211	8.6	32.8	7.3	30.6	1.5	19.1	0.2

Table S.5.3. Descriptive statistics of physical-chemical, and hydrological variables measured during baseflow conditions at 64 rivers.

Parameter	UNIT	Count	Minimum	Mean	Maximum	Standard deviation	5th Percentile	95th Percentile
Ammonia	µg l^{-1}	63	0.00	23.6	280.0	45.57	0.00	98.50
Calcium	mg l^{-1}	63	30.04	62.4	160.6	21.56	33.82	98.22
Chlorine (Cl-)	mg l^{-1}	63	3.44	42.3	291.8	54.43	5.85	174.79
Dissolved oxygen	mg l^{-1}	64	5.90	9.82	13.3	1.66	7.15	12.21
Dissolved organic carbon	mg l^{-1}	63	1.60	6.14	26.8	4.23	2.12	15.55
Magnesium	mg l^{-1}	63	7.03	18.4	34.2	6.27	10.28	29.05
Nitrate/nitrite	µg l^{-1}	64	0.00	858.3	5638.9	1310.26	0.00	4095.58
Pheophytin corrected chlorophyll a	µg l^{-1}	59	0.03	0.82	4.42	1.04	0.07	3.42
pH		63	7.90	8.20	8.38	0.11	7.99	8.35
Potassium	mg l^{-1}	63	0.43	2.20	9.79	1.90	0.45	6.02
Sodium	mg l^{-1}	63	3.03	27.0	199.3	36.86	3.40	113.00
Soil hydraulic conductivity	m day^{-1}	64	0.523	2.21	4.66	1.14	0.620	4.20
Specific conductance	µS cm^{-1}	63	257.00	527.0	1589.0	264.16	265.20	1039.80
Soluble reactive P	µg l^{-1}	64	0.86	23.3	266.0	45.03	2.10	87.01
Sulfate	µg l^{-1}	63	2.39	32.1	169.8	30.47	5.55	89.58

*Precipitation measured at hourly averages from 16 km^2 NEXRAD cells and reported in cumulative mm per time

Table S.5.3. (cont'd)

Parameter	UNIT	Count	Minimum	Mean	Maximum	Standard deviation	5th Percentile	95th Percentile
Total dissolved N	µg l^{-1}	64	0.00	1423.3	6033.7	1346.46	337.55	5414.14
Total dissolved P	µg l^{-1}	64	3.11	25.2	292.3	38.57	3.94	58.04
Total N	µg l^{-1}	64	81.82	1082.1	5583.1	1129.31	110.80	3610.59
Total P	µg l^{-1}	64	7.70	37.8	395.5	52.44	8.91	102.54
Total chlorophyll a	µg l^{-1}	59	0.07	1.57	7.76	1.92	0.20	7.41
Precipitation*								
6 hour	mm	64	0.00	0.14	9.20	1.15	-	-
12 hour	mm	64	0.00	1.89	77.89	10.20	0.00	7.91
18 hour	mm	64	0.00	3.42	78.61	11.64	0.00	30.8
24 hour	mm	64	0.00	4.42	78.61	11.86	0.00	31.0
2 day	mm	64	0.00	6.04	78.61	11.85	0.00	31.0
3 day	mm	64	0.00	7.66	80.07	13.66	0.00	34.2
4 day	mm	64	0.00	8.26	80.46	13.45	0.00	34.2
6 day	mm	64	0.00	9.00	87.30	14.06	0.029	34.2
8 day	mm	64	0.00	11.62	92.55	16.46	0.029	57.2
Discharge	$m^3 s^{-1}$	63	0.00	6.74	57.28	12.48	0.017	43.4
Water temperature	°C	64	6.98	13.09	17.50	2.56	8.2	16.6

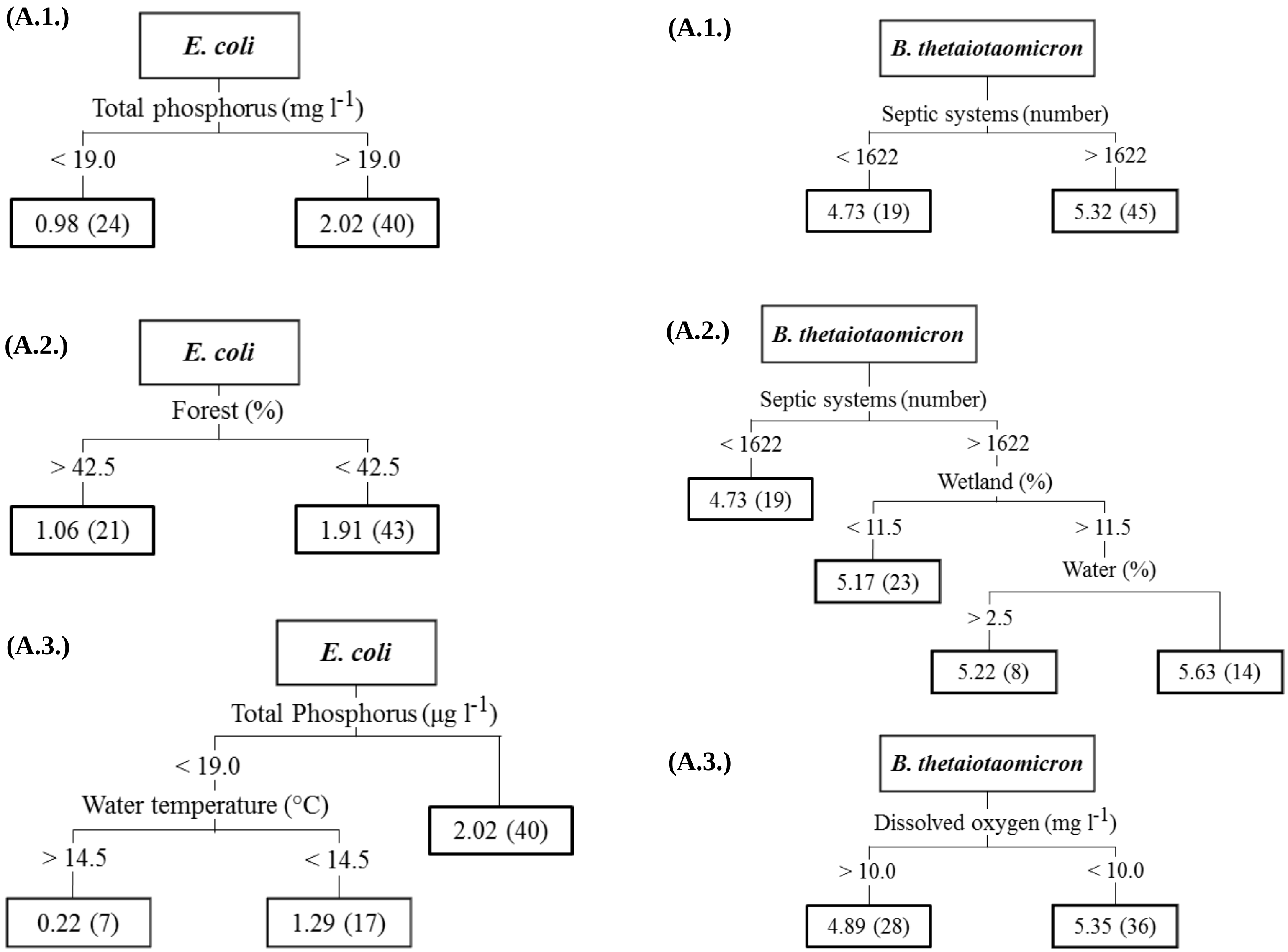
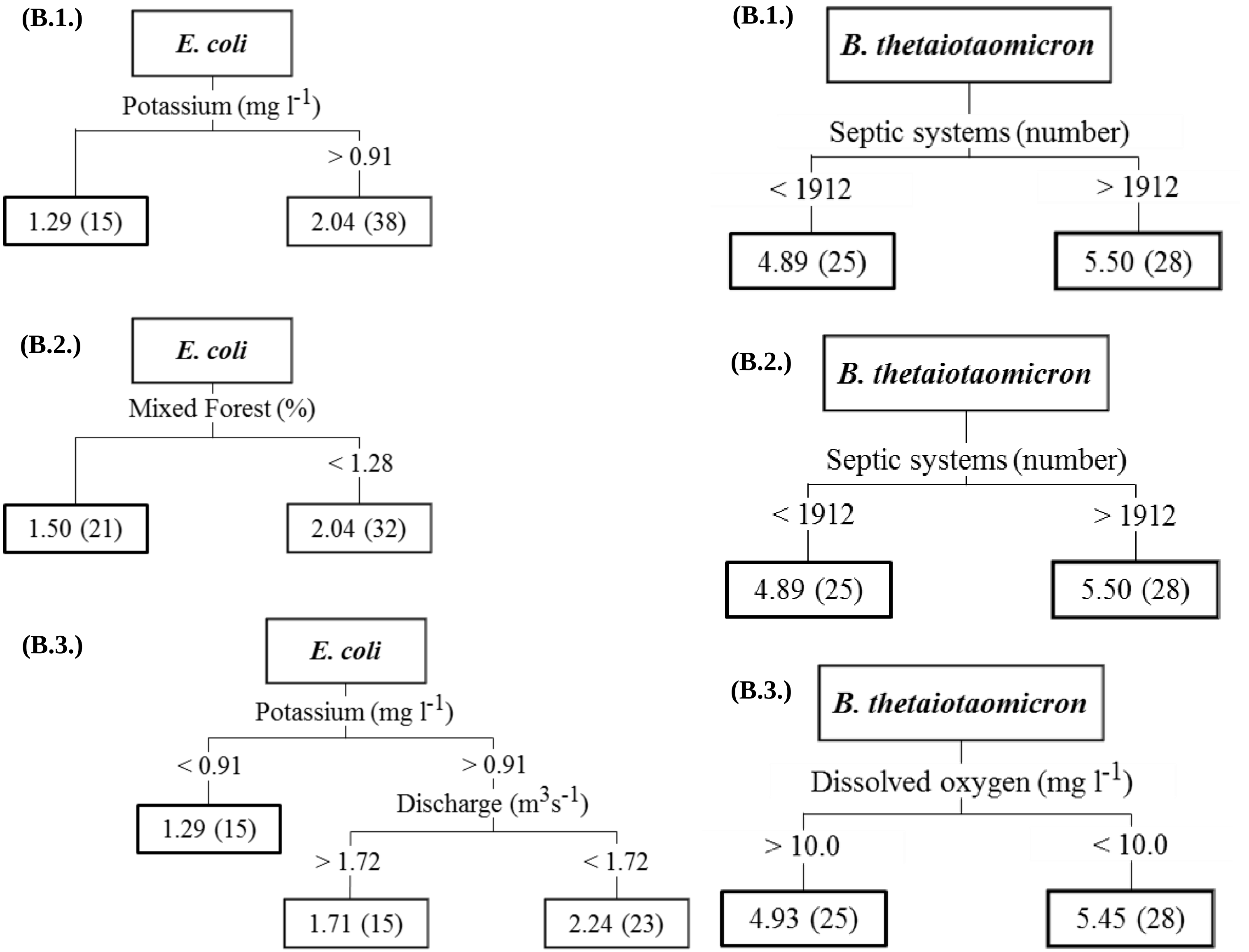


Figure S.5.1. CART tree outputs for *E. coli* and *B. thetaiotaomicron* at the (A) full and (B) reduced watersheds developed with (1) all data, (2) land use variables only, and (3) nutrient, chemical, and environmental variables only.

Figure S.5.1. (cont'd)



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CHAPTER 6.

CONCLUSION

6.1. Goals of the Research and Results of the Multi-scale Studies.

Overall, this project aimed to measure and assess fecal indicator bacteria in water and describe their response to land and climate variability. Focused investigations occurred at various spatial and temporal scales across multiple watersheds in order to describe causes, sources, and processes associated with the fate of fecal pollution in water.

Investigations in the Saginaw Bay, a shallow wind driven system that receives agriculture, urban, and forested runoff, focused on a narrow spatial scale directly at the beach. The tools used in this study included *E. coli*, enterococci, *C. perfringens*, coliphage CN-13 and F+amp, enterococcus surface protein (esp), and Bacteroides human and bovine markers. Results identified sediment and stranded algae mats were acting as reservoirs of *E. coli*, enterococci, *C. perfringens*, and coliphage in the nearshore area. These reservoirs had the greatest influence on microbial water quality when precipitation, wind, and wave action resuspended sediments and detached bacteria into the water column. Elevated concentrations of *E. coli*, enterococci, and *C. perfringens* were observed in these reservoirs (compared to the surrounding water column) in absence of recent contamiantion, potentially as a result of regrowth and persistence. Positive detections of the enterococci surface protein gene marker confirmed that some human fecal contamination was impacting Saginaw Bay water quality. It was clear from this work that in addition to the nearshore reservoirs, upstream activities required further investigations to accuaretly describe contamination sources. This required going from the beach environment to the watershed level and adding more sensitive genetic markers and land use investigations.

The sequential investigation increased the spatial and temporal assessment and focused on a single watershed (Mitchell Creek) draining to the Grand Traverse Bay. This highly modified and flashy watershed discharges to the East Grand Traverse Bay near the Traverse City State Park beach. Using the fecal indicator *E.coli*, microbial water quality at Traverse City State Park beach routinely met water quality safety thresholds but the nearby Mitchell Creek was deemed unsafe for swimming at all sampling locations. Enterococci, *C. perfringens*, coliphage CN-13, and *Bacteroides theataiotaomicron* were added to the toolbox to further assess water quality.

Major findings identified that the Mitchell Creek was heavily impacted by human population and human fecal contamination. This contamination was primarily precipitation driven and had the ability to influence Traverse City State Park beach water quality. Results confirmed environmental conditions (precipitation, air and water temperature, river discharge, and solar radiation) had significant effects on microbial water quality. Furthermore, land use coverage (urban, agriculture, and wetlands) and wastewater treatment discharge (as a proxy for human population) were identified as potential sources of fecal contamination in this area. As there are no sewage discharges located directly in this watershed, non-point sources (e.g. septic tanks, leaky sewers) were considered the likely source of the human sewage marker. Although it was proposed that the microbes detected in water originated from land, a single land use type was not identified as a primary source of bacteria using *E. coli*, enterococci, *C. perfringens*, or coliphage. At the conclusion of this project, it was not clear whether the results observed in this system could be directly translated to larger-scale, basin wide assessment using the same tools; leading to the design and implementation of a broader spatial survey.

A survey of Michigan Lower Peninsula rivers draining to the Great Lakes was conducted under baseflow hydrologic conditions. The study design included analysis of nutrients, ions, isotopes, physical parameters, land use characteristics, and microbes (*E. coli* and *Bacteroides thetaiotaomicron*) from 64 rivers. This approach was used as a way to improve scientific understanding of water pollution processes at the state level. At this scale, land use characteristics (primarily the number of septic systems in the watershed) more accurately predicted *Bacteroides thetaiotaomicron* concentrations than land use type. Total phosphorus and potassium best predicted *E. coli* at the entire watershed and entire watershed excluding upstream lakes, respectively. Specifically, phosphorus was retained in lakes while potassium, presumably from agriculture and septic system leachate, was transported through groundwater into rivers. Similarly, *B. thetaiotaomicron* was retained in lake systems as a result of increased water retention time, increased residential density, and DNA degradation. *B. thetaiotaomicron* detected in rivers likely entered via groundwater from nearby septic systems and sewage discharge. Strong correlations between *B. thetaiotaomicron* and the total number of septic systems illustrated the significant influence of septic systems on microbial water quality. Baseflow analysis represented a microbial reference condition for Michigan rivers which can be used to assess future changes to water quality stemming from climate change or anthropogenic influences.

Overall, the Sagianw Bay project idenfied the processes of microbial contamination at the beach but failed to identify the processes by which water initially became impacted. In the Mitchell Creek, sources and transport mechanisms led to impacted beaches but specific land use types were not implicated using general bacteria. The statewide survey identified correlations between land use characterizations (i.e. septic system numbers per watershed) and microbial water quality, indicating land use types were too general to draw significant links with fecal indicator bacteria.

6.2. Implications for managing and improving water quality in the Great Lakes

The Clean Water Act set the lofty goal of making all waters safe for fishing and swimming. While progress has been made in many systems, the overall goal has not been reached. In order to eliminate water pollution, significant and continuous efforts, enforcement, and funding must occur using a top down approach. While it is important to protect and improve the water quality of large waterbodies and systems, this dissertation illustrated how the health of small streams can have drastic implications for surrounding communities and waterbodies. By allocating greater attention to small systems, improvements in Great Lake water quality can be achieved and costly, large scale projects can be avoided. Only through continuous monitoring, application, enforcement, and perseverance of the Clean Water Act can the health of the Great Lakes be guaranteed for future generations.

A series of regulatory based outcome analysis in the Grand Traverse Bay area using multiple indicator organisms determined that monitoring for enterococci using cultivation methods would result in the greatest number of regulatory actions according to the USEPA suggested criteria. It is not assumed that the greater number of closures represented a greater protection for bathers compared to *E. coli* outcomes. Analysis identified a significant disconnect between molecular and cultivation based results in different water system types (i.e. creek or beach). Specifically in beach water, analysis of criteria for *E. coli* cultivation, enterococci cultivation, and *Enterococcus* spp. molecular methods suggest that molecular based *Enterococcus* spp. would have resulted in the most regulatory actions. Unlike cultivation based enterococci, this molecular approach may provide a greater level of protection as results can be produced more rapidly, allowing for water quality managers to make regulatory decisions on the same day.

Climate investigations found that watershed and site specific precipitation thresholds exist for microbial water quality. Projected temperature increaes in the Great Lakes basin will lead to more frequent algae blooms as surface water flow descreases and nutrient loading increases. As shown in this dissertation, algae mat occurrence can impair nearshore water and represents a potential threat to human health. Additionally, more frequent and intense precipitation events are projected, which will lead to shallow groundwater contamination and require a shift in the design and implementation of on-site wastewater treatment systems. Climate change, population growth, and land use development in the Great Lakes will drive the availability and quality of water, compounding water infrastructure stress. Such forecasts indicate the need to anticipate long term water use shifts and prepare for their associated implications on infrastructure. Congress should mandate integrated assessments for watersheds most at risk and fund more effective stormwater management based on current drinking and wastewater infrastructure capacities.

6.3. Recommentations

Based on the cummulatve results from these studies, the following management actions are recommended:

1. Stranded algae mats should be removed from the nearshore area early in the morning under low wind speed and wave height and when 48 hour anticendent precipitation is below 6.4 mm. Such actions would allow for solar inactivation of bacteria, reduce risk to bathers entering water later in the day, and improve beach water quality. Significant financial and managerial attention must focus on combined sewer overflow and on-site wastewater treatment systems in order to reduce the discharge, direct or diffuse, of human fecal material to the Saginaw Bay.

2. Michigan must adopt a statewide sanitary code to level the playing field between all counties, eventually leading to improved design, maintanence, and governance of on-site wastewater treatment systems. Creation of a statwide sanitary code would bridge the knowledge gap between those who know about potential environmental hazards and those who need to know in order to provide effective changes in policy and technology that would improve water quality throughout Michigan.

3. Molecular and cultivation based monitoring methods in the nearshore areas must continue to be evaluated both at large spatial scales and at site-specific beaches. Weak relationships between molecular and cultivation results confirm criteria are not interchangeable between all water systems and provide different levels of protection. Further exploration of the suggested recreational water quality monitoring marker Enterococcus spp. is reguired at beaches impacted by non-point sources of pollution.

4. Michigan must implement a surveylence system for on-site wastewater treatment systems. This system will allow prioritization of remediation efforts and promote more effective treatment systems on a large scale. A new framework should include detailed history of each system across the state inclusive of molecular source tracking tools but should not be contstrained by political boundaries. Ideally this system would facilitate local level assessments, actions, and adaptations through land and water management plans at a watershed scale.