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Escherichia coli as a Water Quality Indicator Organism: A Case for Responsive, Science-Based Policy

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Escherichia coli as Water Quality Indicator Organism: A Case for Responsive, Science-Based Policy

An Environmental Studies All College Thesis

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ABSTRACT

Water quality indicator organisms such as *E. coli* are used in the monitoring of recreational waterbodies to indicate the presence of fecal contamination. In 2009, a 1.5-mile stretch of Plum Creek in Stearns County, MN was classified by the Minnesota Pollution Control Agency as impaired due to high levels of *E. coli*. Citizen science to investigate the source of this contamination began in 2014 and with the addition of student research from CSB/SJU in 2016. This thesis presents results of the 2016 study, supplemented by summary of Minnesota water quality policy and recent research on indicator organisms. The 2016 study confirmed presence of fecal coliform indicator bacteria, but to date no source of contamination has been identified. As a result, a key contention of this thesis is that further research is necessary regarding the influence of sediment *E. coli* on stream *E. coli* in order to establish or maintain policies that protect human health and are fiscally and environmentally effective.

Abbreviations

BMP	Best Management Practices
BWSR	Minnesota Board of Water and Soil Resources
CWA	Clean Water Act
CLMP	Citizens Lake Monitoring Program
CSMP	Citizens Stream Monitoring Program
EPA	U.S. Environmental Protection Agency
FIO	Fecal Indicator Organism
FWPCA	Federal Water Pollution Control Act
MDA	Minnesota Department of Agriculture
MDH	Minnesota Department of Health
MDNR	Minnesota Department of Natural Resources
MNDOT	Minnesota Department of Transportation
MPCA	Minnesota Pollution Control Agency
MPFA	Minnesota Public Facilities Authority
PCNN	Plum Creek Neighborhood Network
SWCD	Soil and Water Conservation District
TMDL	Total Maximum Daily Load
WPLMN	Watershed Pollutant Load Monitoring Network

Terms

Fecal Indicator Organism	bacteria used to detect and estimate the level of fecal contamination of water
Hypohoreic Exchange	is the mixing of surface and shallow subsurface water through porous sediment surrounding a river
Impaired	rivers, lakes, or streams that do not meet one or more water-quality standards and are considered too polluted for their intended uses
Naturalize	to introduce (organisms) into a region and cause them to flourish as if native.
Non-point Source	a source of pollution that issues from widely distributed or pervasive environmental elements
Pathogen	bacterium, virus, or other microorganism that causes disease
Point Source	a localized and stationary pollution source.
Public Waters	waters open to the use of the public managed by the state
Sediment	inorganic particles smaller than 2mm that are deposited on the beds of rivers and streams
Turbulence	the speed of the fluid at a point is continuously undergoing changes in both magnitude and direction
Watershed	is an area of land that captures rainfall and other precipitation and funnels it to a lake or stream or wetland

INTRODUCTION

The biological significance of water cannot be overstated. Liquid water is necessary for life to exist at a universal level. Every organism on the planet requires water to perform biological processes necessary to live. Access to sufficient, reliable, clean water has been a primary objective of human civilizations throughout history. While many modern societies experience a seeming limitless supply of water, billions of people remain water scarce, lacking access to the World Health Organization's recommended 50 liters per day.[1] For those who have access to water, ensuring it is safe to consume is an added challenge. Waterborne disease is a leading cause of death worldwide. There are more than 200 million cases of waterborne illness annually, amounting to 2.1 million deaths.[2]

Americans are not free from the consequences of contaminated water physically or financially: "The Economic Research Service of the United States Department of Agriculture (USDA-ERS) estimated that in 2001, diseases caused by five major bacterial pathogens in the U.S. resulted in a loss of approximately $6.9 billion."[3] Compounding historic challenges to water access are the threats of melting glaciers, salinization, and contamination from industry and agriculture, further diminishing the planet's freshwater reserves. Understanding both our dependence on clean water as well as the crisis of scarcity we imminently face makes clear the imperative to establish strong policies to protect this fundamental resource. The United States has taken a number of measures on both federal

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[1] Peter H Gleick, "Basic Water Requirements for Human Activities: Meeting Basic Needs," *Water international* 21, no. 2 (1996).

[2] Steven L. Percival et al., Microbiology of Waterborne Diseases : Microbiological Aspects and Risks, (Amsterdam ;: Elsevier/Academic Press, 2014), Ebook Library http://public.eblib.com/choice/publicfullrecord.aspx?p=1562325, ebrary

[3] S. Ishii, "Escherichia Coli in the Environment: Implications for Water Quality and Human Health," *Microbes and Environments* 23, no. 2 (2008).

and state levels to monitor and regulate water quality. Minnesota, with its abundance of water, has been a leader in this area.

Yet, while important steps have been taken to improve water quality in the United States, it is critical that policies remain responsive to the progress of relevant scientific findings. Both science and policy are, by definition, dynamic processes. However, the pace of policy often does not equal that of research and as a result, policies may lag behind the most accurate scientific information. The implications of this reality are broad and severe.

The case for responsive, science-based policy is exemplified by the policies in place regulating the management of recreational waterbodies. Biological indicator organisms are used to indicate the presence of contamination in recreational waterbodies. Since the late 1980's *Escherichia coli* (*E. coli*) has been accepted by the United States and many other nations as the most effective indicator organism.[4] However, beginning in the mid-2000s, a body of research began to indicate *E. coli* might have properties that result in false positives in fecal contamination testing.[5] As a result, numerous taxpayer dollars go to investigating and repairing contaminated waterbodies that may pose no human health risk.

The study of the impairment designation of Plum Creek in Stearns County, Minnesota provides an opportunity to thoroughly examine the impacts of this policy in practice. Plum Creek was listed by the Minnesota Pollution Control Agency (MPCA) as impaired for fecal contamination in 2008. Thousands of dollars over three seasons of study indicate that the *E. coli* present in Plum Creek is unlikely to be anthropogenic in source and

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[4] SCL Edberg et al., "Escherichia Coli: The Best Biological Drinking Water Indicator for Public Health Protection," *Journal of Applied Microbiology* 88, no. S1 (2000).

[5] S. Ishii, "Presence and Growth of Naturalized Escherichia Coli in Temperate Soils from Lake Superior Watersheds," *Applied and Environmental Microbiology* 72, no. 1 (2006).

may not be indicative of contamination. Through the case of Plum Creek and an abundance of supporting studies, it is evident that further research is necessary to determine the effectiveness of *E. coli* as a water quality indicator organism.

This paper investigates the effectiveness of *E. coli* as a water quality indicator organism first, by reviewing the history of water quality policy nationally and at the state level. A more thorough examination of the monitoring protocols used in the state of Minnesota puts the use of *E. coli* as an indicator organism in context. Case studies of two Minnesota waterbodies offer a more practical understanding of policy in practice, and set the stage for the ultimate evaluation of *E. coli* as an indicator organism.

BACKGROUND

Humans can contract over two-dozen unique waterborne illnesses from various protozoans, bacteria, and viruses.[6] The vast majority of these organisms are transmitted through the feces of humans or other warm-blooded animals to a water source.[7] The consequences of consuming water contaminated by these organisms can be deadly. Cholera, shigella, dysentery, and typhoid fever are a few of the most common and best understood illnesses contracted from water. These illnesses are diarrheal and can be particularly fatal in children and the elderly.[8]

While originating from feces, the organisms that cause these illnesses can enter water bodies from a number of sources. Sources of pollution are typically identified as

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[6] T. H. Y. Tebbutt, Principles of Water Quality Control, (Boston, Mass. :: ButterWorth-Heinemann, 1998), ebrary http://site.ebrary.com/id/10201891, Google

[7] Percival et al., Microbiology of Waterborne Diseases : Microbiological Aspects and Risks.

[8] Tebbutt, Principles of Water Quality Control.

point source or nonpoint source. Point-source pollution is defined as a single identifiable source of pollution such as industrial or sewage discharge. Nonpoint source pollution cannot be attributed to a single source but is rather the product of run-off or snowmelt. Nonpoint pollution is often agricultural in origin, either via the run-off of feedlots or manure application. These pollutants may enter surface water or seep into groundwater.[9]

The numerous sources of pollution make addressing water quality a particularly challenging task. In the United States, government agencies on multiple scales have implemented various water quality monitoring and regulating policies over the last century years. The evolution of these policies is indicative of the progress of water quality science during the same period, and serves as a reminder of the need for responsive policy.

National Policy

National water policy is a relatively recent addition at the federal level in the United States. The first federal policy in the United States was passed in 1948, and was called the Federal Water Pollution Control Act (FWPCA).[10] Through the FWPCA the Surgeon General developed programs to be implemented on a state level to address pollution. The Federal Works Administrator was authorized to aid states in construction projects that would reduce pollution.[11] In 1956, the FWPCA was amended to increase the government's ability

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[9] Percival et al., Microbiology of Waterborne Diseases : Microbiological Aspects and Risks.
[10] "Cuyahoga River Fire," accessed 1/27/17, https://clevelandhistorical.org/items/show/63.
[11] https://www.fws.gov/laws/lawsdigest/FWATRPO.HTML

to enforce the policy in states. Further revision occurred in 1965 with the passing of the Water Quality Act, which granted the federal government more control of water quality. [12]

Oil and chemicals floating on Ohio's Cuyahoga River burst into flames in 1969, bringing national attention to the issue of water quality.[13] Industrial pollution from the city of Cleveland had produced an oil slick that, when accidentally ignited, resulted in flames five stories high. While not the largest or most damaging river fire to occur in the U.S., the 1969 Cuyahoga fire struck at the end of the first decade of environmentalism, and thus drew significant public attention. [14]

In 1972, the FWPCA underwent several broad amendments resulting in what has since been known as the Clean Water Act (CWA). The 1972 amendments were important in establishing a method of regulation and enforcement for pollution. Point source pollution by an individual became illegal and construction grants were implemented to fund sewage construction. The Environmental Protection Agency (EPA) was given the authority to develop programs for pollution control.[15]

Minnesota Policy

Minnesota has a national reputation for its abundance of freshwater. The 'Land of 10,000 Lakes' is actually home to 11,842 lakes (greater than 10 acres in size), and 105,000

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[12] "Timeline: The Modern Environmental Movement," accessed 1/27/17, http://www.pbs.org/wgbh/americanexperience/features/timeline/earthdays/2/.

[13] Ibid

[14] "Cuyahoga River Fire," *Cleveland Historical*, accessed January 27, 2017, https://clevelandhistorical.org/items/show/63.

[15] "Summary of the Clean Water Act," last modified 2017-09-08, accessed October 10, 2016. https://www.epa.gov/laws-regulations/summary-clean-water-act.

river miles, accounting for nearly 10% of the area of the state.[16] The MPCA notes "Minnesotans have the privilege and, with that, the huge responsibility of living 'upstream' of millions of downstream users of these major waterways."[17] Minnesota's history of strong water quality policies acknowledges this responsibility.

Water quality policy in the state of Minnesota pre-dates legislation at the federal level. In the state's first legal effort to address water policy, the term 'public water' was adopted in Minnesota in 1897 to refer to large bodies of water used for fishing, boating, or consumption. The identification of public waters was intended to protect those water bodies but had the undesirable side effect of promoting conversion of private waters into agricultural land through drainage.[18] The law became problematic, and in 1919 the Office of State Drainage Commissioner was created to shift drainage regulation from counties to the state.

In 1933, the Department of Conservation (now the Department of Natural Resources) took over drainage authority. A drought in the 1930's made it clear the state needed to take control of maintaining the resource of 'waters of the state'. Waters of the state were managed by the state for commercial, industrial, or agricultural uses. Shortly after, this authority was further expanded to include the regulation of work that would alter the "course, current, or cross section" of a water body.

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[16] "Minnesota Facts and Figures," accessed 1/27/17, http://www.dnr.state.mn.us/faq/mnfacts/index.html.

[17] "Minnesota's Water Quality Monitoring Strategy 2011 to 2021: A Report Prepared for the U.S. Environmental Protection Agency," (Minnesota Pollution Control Agency, 2011).

[18] "History of Water Protection," accessed 1/17, 2017.

Significant controversy ensued for the next four decades regarding what was to be considered a 'water of the state'. In order to address the controversy the Department of Natural Resources (DNR) developed specific rules for water work permits and the Minnesota legislature identified a new definition of public waters and implemented the Public Waters Inventory Program. A complicated and drawn out mandatory inventory began in 1976; however, by 1979 Minnesota had yet to have a single county designate any public waters.[19]

During this time, the Minnesota legislature established the Minnesota Pollution Control Agency (MPCA), an agency tasked with identifying and coordinating pollution control efforts within the state. Presently, the MPCA is responsible for overseeing water pollution as well as air and waste within the state. The MPCA's most visible efforts are monitoring and enforcement of environmental regulations. However, the agency is also involved in shaping state policy, educating the public and providing technical and financial support.[20]

While federal and state level policies are important for developing a framework and standard for water quality, implementation of these policies typically takes place on the county level. In Minnesota, the MPCA and DNR set parameters in accordance with EPA guidelines, which are largely implemented by Soil and Water Conservation Districts (SWCD) in each county. Many of the federal and state mandates are termed 'guidelines', meaning they are not binding laws but rather recommendations to voluntarily be responded to on a local level.[21]

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[19] Ibid.

[20] "About the Minnesota Pollution Control Agency," accessed 3/30/17,

[21] Phil Votruba, interview by Sarah McLarnan2017.

WATER QUALITY MONITORING IN THE STATE OF MINNESOTA

The MPCA collaborates with a number of other federal, state and local agencies. The EPA sets the federal standards under which the MPCA operates. At a state level, the MPCA works closely with the Minnesota Department of Natural Resources (MDNR) and Minnesota Board of Water and Soil Resources (BWSR) on monitoring and mitigation efforts. As agriculture is a significant contributor of water pollution, the MPCA also coordinates with the Minnesota Department of Agriculture (MDA). The Minnesota Department of Health (MDH) aids in identifying and communicating human health risks of pollution, while the Minnesota Public Facilities Authority (MPFA) assists in funding and implementing improvement projects.

On a local level, Metropolitan Council (Met Council) is involved with managing pollution and waste in the Twin Cities/metro area. Perhaps the most important partnership exists between the MPCA and the Watershed Districts and county Soil and Water Conservation Districts (SWCDs). As Phil Votruba of the MPCA explains, the relationship between farmers, land owners, citizens and local officials is vital to getting individuals to take the actions necessary to improve water quality.[22]

The MPCA has the responsibility of preserving the quality of water bodies that meet national and state published standards, and improving the quality of waters that do not meet the standards. Water quality standards are developed with regard to legally identified beneficial uses. A beneficial use is determined by the primary use of a water body and

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[22] Ibid.

includes aquatic life, aquatic recreation and aquatic consumption (both humans and wildlife). Waters that do not meet the standards for their designated beneficial use are classified as "impaired". As required by the CWA the MPCA publishes a list every even year of the impaired water bodies in the state, all of which then require a Total Maximum Daily Load (TMDL) study.

A TMDL study is required if a water body has been listed as impaired by the MPCA. TMDL studies evaluate all potential sources of pollution and determine actions necessary to return pollutant levels to below threshold.[23] During this time MPCA officials assess available data, identify areas that require more data, and draft a TMDL report.[24] The draft is then available to the public, and community meetings and a comment period allow individuals to review and respond. The process often takes years before the EPA approves a final report.[25]

Statewide monitoring of water quality still exists for some parameters, such as toxic metals or polychlorinated biphenyls. In general, watershed assessments address threats to aquatic life and recreational uses, while statewide assessments address aquatic consumption and aquatic health threats. Upon completion of the 2014 season TMDL reports, the MPCA published the *Guidance Manual for Assessing the Quality of Minnesota Surface Waters for Determination of Impairment: 305(b) Report and 303(d) List*. This is the most recent document outlining the monitoring protocols implemented by the MPCA. This document is the foundation for the following discussion on the current parameters and programs the MPCA has in place for water quality management.

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[23] Ibid.
[24] Ibid.
[25] Ibid.

Parameters

There are a number of different parameters used to measure water quality, and monitoring strategies often employ different combinations of measures based on the available equipment, funds, probable pollutants, and nature of the waterbody.[26] Table 1 defines some of the most common parameters measured in evaluating surface waters, and describes what these parameters indicate about water quality.[27] Ground water is evaluated for a number of a number of elements as well as a number of anthropogenic products such as DEET, pharmaceuticals, and fragrances.[28] Monitoring of wetlands remains in its early stages but focuses on biotic indicators such as the plant variety and invertebrate species.[29]

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[26] Pam Anderson et al., "Guidance Manual for Assessing the Quality of Minnesota Surface Waters for Determination of Impairment: 305(B) Report and 303(D) List," ed. Minnesota Pollution Control Agency(Saint Paul, MN2014).

[27] S. K. Bhargava, Practical Methods for Water and Air Pollution Monitoring, (New Delhi: New Age International (P) Ltd., 2009), Ebook Library

[28] Anderson et al., "Guidance Manual for Assessing the Quality of Minnesota Surface Waters for Determination of Impairment: 305(B) Report and 303(D) List."

[29] Ibid.

Table 1. Common surface water-quality parameters. Table by author.

PARAMTER	DEFINITION	SIGNIFICANCE
pH	A measure of hydrogen ions (H+) in a solution.	Most organisms require a relatively neutral (pH 6-8.5) environment to survive.
Color	Visible appearance of water, pure water appears light blue.	Color may indicate the presence of organic matter, or metallic ions.
Turbidity	Measurement of the scattering of light cause by suspended particles in water.	Aesthetically displeasing in domestic use and unsuitable for some industrial processes.
Conductivity	The capacity for water to conduct electricity.	Conductivity is directly proportional to the amount of dissolved inorganic solids.
Nitrate	Concentration of NO_3^- in solution.	High levels of nitrate in drinking water causes methemoglobinemia "blue baby syndrome" also contributes to hypoxia in waterbodies.
Dissolved Oxygen	Concentration of oxygen in solution.	Aquatic organisms rely on oxygen for biological processes.
Phosphate	Concentration of phosphorus in solution.	High concentrations indicate pollution and lead to eutrophication.
Ammonia	Concentration of NH_3 in solution.	Indicates the presence of organic contamination, high concentrations are toxic to aquatic life.
Sulfate	Concentration of SO_4^- in solution.	Gives water unpleasant odor and sour taste.

Fecal Indicator Organisms (FIOs)

Fecal indicator organisms are organisms that occur in water in proportion to pathogens and serve as an additional parameter to identify contamination of a waterbody. Bacteria are commonly used as indicator organisms because of their relative ease of enumeration. The criteria of a suitable bacterial indicator include:

- Always present in animal and human feces
- Present in high numbers for higher probability of detection
- Persistence in the environment/drinking water similar to that of pathogens
- Does not multiply rapidly in the environment
- Simple, rapid, accurate, and inexpensive enumeration methods are available.[30]

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[30] Edberg et al., "Escherichia Coli: The Best Biological Drinking Water Indicator for Public Health Protection."

One significant challenge in selecting an indicator is identifying the desirable lifespan of the organism. The lifespan of an indicator must be as long, if not slightly longer than the pathogens, however not so long that it falsely indicates contamination after the lifespan of the pathogen. A number of factors influence the lifespan of both pathogens and indicators including; species, water type, temperature, and UV radiation.[31]

E. coli is widely accepted as the most frequently used indicator organism; however, there are a handful of other organisms present in feces, which may also be used to indicate contamination. *Enterococci* and *Clostridium perfringens* are used worldwide as indicator organisms. Some research also indicates viruses that infect fecal coliform bacteria, termed bacteriophages, may also be suitable indicators. The effectiveness of these organisms as indicators is evaluated primarily upon their survival in natural conditions and the cost and skill required in laboratory testing.

Enterococcus

Enterococcus occur in all human feces and in the colons of mammals at a concentration of approximately 106-107 organisms/gram fecal matter (100-1,000 fold less than *E. coli*), and thus require more sensitive testing. The lifespan of *Enterococcus* is weeks to months, similar to that of most enteric pathogens. Salt resistance characterizes the organism as particularly suitable for testing in marine environments and it is therefore used primarily to monitor marine bathing beaches. *Enterococcus* testing is being considered in addition to *E. coli* as it may provide more accuracy at a low additional cost.

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[31] Ibid.

Clostridium pefringens

Clostridium pefringens spores are a less commonly used indicator. The spores have a lifespan of months to years, reducing the organism's effectiveness as an indicator as they often outlive pathogens. Laboratory procedures for measuring *Clostridium pefringens* spores have been published but not adopted by regulatory agencies. The cost of performing testing for this organism is estimated to be significantly higher than that of *E. coli* or *Enterococci*.[32]

Coliphages

Coliphages are bacteriophages that infect members of the total coliform group (*Escherichia, Enterobacter, Serratia, Citrobacter, Klebsiella*). These organisms have been studied for their use as indicator organisms because of their particularly long life span and abundance relative to other indicator organisms. These qualities are particularly advantageous in ground water testing. However, limitations in establishing a standard method for measurement, and inconclusive correlation with illness makes it unlikely coliphages will become a popular indicator organism.[33]

Escherichia coli

E. coli is infamous due to the few strains that are pathogenic to humans (e.g. O157:H7) . It is relatively common knowledge that raw or undercooked meat is a source of some strains of *E. coli,* which can have serious medical consequences. The use of *E. coli* as an

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[32] Ibid.
[33] Ibid.

indicator organism is not because of pathogenic strains, but for a number of other features that make the organism particularly suitable to indicate the presence of pathogens.

The evolution of *E. coli* as the predominant indicator organism is part of the history of microbiology.[34] Fecal contamination was identified as a source of infection in the mid-1500s.[35] The "Golden Era of Microbiology" occurred in the mid-to-late-1800s. The period was characterized by the identification of a number of pathogens and microbial processes beginning with Louis Pasteur's discoveries of fermentation, pasteurization and biogenesis (all cells arise from preexisting cells).[36]

E. coli was one of the first organisms to be widely used as an indicator organism. *E. coli* has many of the features necessary of an indicator organism; it is found at high concentrations in human feces (10^9organisms/gram) and is significantly correlated with gastrointestinal disease.[37] The initial procedure developed was somewhat misleadingly called the "fecal coliform test". This test indicated the presence of all coliform forming bacteria from the genera *Klebseilla, Enterobacter, Serratia, Citrobacter,* and *Escherichia.* However, only bacteria in the genera *Escherichia* are of fecal origin, the other genera are commonly found in the environment and not indicative of fecal contamination. The fecal coliform test relied on the belief that *E. coli* was the predominant coliform and thus the test was used as an approximation of *E. coli* levels. While substituting total coliform for *E. coli*

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[34] Ibid.

[35] Ibid.

[36] Raymond N. Doetsch, *Microbiology Historical Contributions from 1776 to 1908 by Spallanzani, Schwann, Pasteur, Cohn, Tyndall, Koch, Lister, Schloesing, Burrill, Ehrlich, Winogradsky, Warington, Beijerinck, Smith, Orla-Jensen*(New Brunswick (New Jersey) :: Rutgers University Press, 1960).

[37] Edberg et al., "Escherichia Coli: The Best Biological Drinking Water Indicator for Public Health Protection."

was a sufficient test at the time of its creation, modern monitoring employs more accurate measures.[38]

In 1998, a procedure to detect *E. coli* specifically within water samples was identified. The technique was both inexpensive and required little laboratory experience, qualities necessary in an effective indicator organism. The "availability of sensitive, specific, inexpensive, easy-to-use methods for its detection directly from water samples" has warranted *E. coli* remaining the primary indicator organism for the last 20 years.[39] However, recent discoveries regarding naturalized populations of *E. coli* in streambed sediments have led some to question this method. As Edberg et al. explain,

> In the 1890s, one of the central questions of public health protection was: should one monitor the safety of drinking water for specific pathogens or indicators? Paradoxically, one hundred years later, with recent knowledge regarding parasitic and viral waterborne disease transmission, the same question is being actively re-evaluated. [40]

Naturalized Sediment Populations as a Confounding Factor

The use of *E. coli* as biological indicator organisms is based upon the assumption that these organisms are present in water solely via direct deposition of feces or runoff of feces.[41] However, recent studies have demonstrated *E. coli* can become naturalized to streambed sediments, therefore becoming an unreliable indicator of fecal contamination.[42] The lifecycle of *E. coli* is visualized in Fig. 1. *E. coli* is released in the feces of warm-blooded

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[38] Ibid.
[39] Ibid.
[40] Ibid.
[41] Yakov Pachepsky et al., "Enrichment of Stream Water with Fecal Indicator Organisms During Baseflow Periods," *Environmental Monitoring & Assessment* 189, no. 2 (2017).
[42] Ishii.
Yakov Pachepsky, interview by Sarah McLarnan2017.

animals. Like all organisms, *E. coli* requires the availability of nutrients, specific environmental conditions and is subject to predation. Without the proper conditions *E. coli* bacteria die. However, some *E. coli* may enter soil, sand, sediment, or algae where it can then follow one of three paths: it may be ingested by warm-blooded animals thereby infecting the organism with *E. coli*; it may die; or it may establish a naturalized population within its new environment.[43] It is this final pathway, in which *E. coli* becomes naturalized, that may confound the use of the organism as an indicator of contamination.

In water, the force of gravity causes *E. coli* and other particles to settle to the streambed in a process called gravitational sedimentation.[44] The survival of *E. coli* and other microorganisms is increased when they are attached to sediment.[45] In the sediment, the availability of nutrients, physical protection from predation and thermal insulation allows *E. coli* to survive and multiply significantly past the lifespan of other enteric pathogens.[46] A 2011 study investigated the mechanisms by which

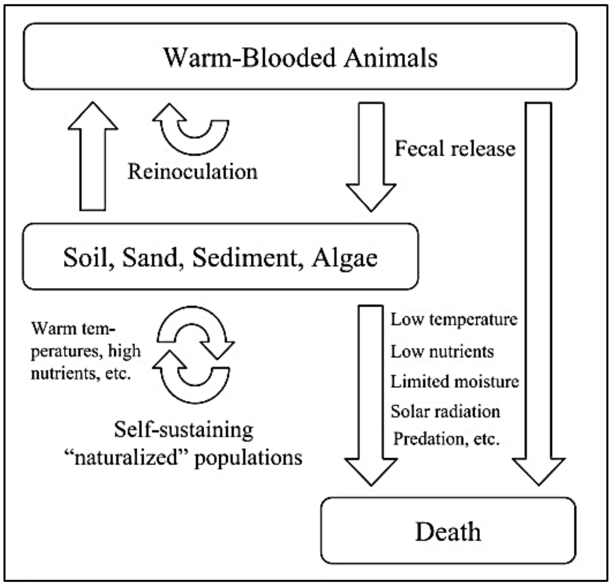

Figure 1. Diagram of the lifecycle of *E. coli.* From Ishii and Sadowsky 2008 Fig 1.

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[43] Ishii, "Escherichia Coli in the Environment: Implications for Water Quality and Human Health."
[44] Ibid.
[45] Stanley B. Grant, Rachel M. Litton-Mueller, and Jong H. Ahn, "Measuring and Modeling the Flux of Fecal Bacteria across the Sediment-Water Interface in a Turbulent Stream," *Water Resources Research* 47, no. 5 (2011).
[46] Ishii, "Presence and Growth of Naturalized Escherichia Coli in Temperate Soils from Lake Superior Watersheds."

naturalized *E. coli* and other FIOs reenter the stream from the sediment layer.[47] Figure 2 demonstrates the four mechanisms identified by Grant *et. al.* for the resuspension of bacteria in a turbulent stream:

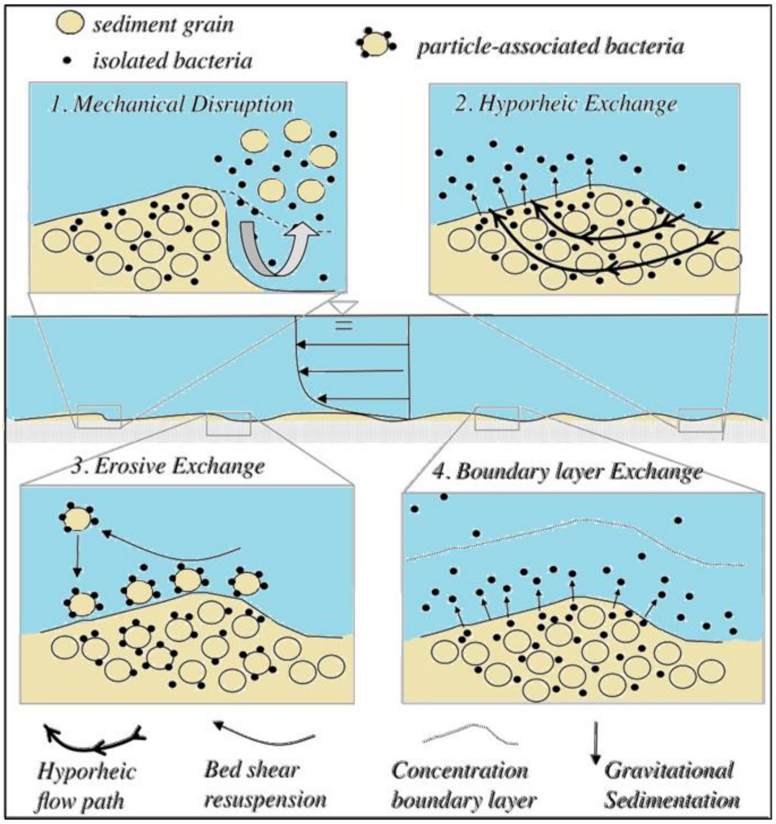

Figure 2. Mechanisms of bacterial exchange across the sediment water interface. Adapted from Grant *et. al* 2011 Fig. 1.

(1) Mechanical Disruption - turbulent water or aquatic life releases bacteria from pores in the streambed

(2) Hyporheic Exchange - the movement of bacteria from pores in the streambed due to the lateral movement of water through the pores

(3) Erosive Exchange - detachment of particle-associated bacteria from the surface of the streambed and settling downstream due to gravitational sedimentation

(4) Boundary layer exchange - slow movement of bacteria from pore spaces into a boundary layer of water

While Grant *et. al.* indicate the movement of bacteria across the sediment water interface occurs in turbulent stream, a 2017 study indicates hyporheic exchange under

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[47] Grant, Litton-Mueller, and Ahn, "Measuring and Modeling the Flux of Fecal Bacteria across the Sediment-Water Interface in a Turbulent Stream."

base flow conditions may also suspended significant amounts of naturalized *E. coli* from the sediment .[48] These findings have led a number of scientists to question whether *E. coli* remains the most reliable method of assessing contamination.[49] "The continuing practice of implementing fecal indicator organisms without understanding their persistence and survivability in the environment has hindered the ability to determine their significance in water and to accurately assess human health risks."[50]

Identifying the source of *E. coli* can ascertain whether it is indicative of fecal contamination or a naturalized population. DNA source tracking is a laboratory technique that uses genetic markers unique to *E. coli* from various sources (e.g. human, cattle, and poultry) as a reference to compare samples and potentially identify their source. Eliminating common *E. coli* sources through DNA source tracking may help identify naturalized populations; however, these techniques require timely and expensive lab techniques unsuitable for routine water quality monitoring.

MPCA Water Quality Monitoring Programs

The MPCA has developed a number of different water quality monitoring strategies over the last five decades, a comprehensive list of which can be seen in Tables 2 and 3. Monitoring strategies are categorized based on the water type: stream, lake, groundwater

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[48] Pachepsky et al., "Enrichment of Stream Water with Fecal Indicator Organisms During Baseflow Periods."

[49] Ibid.

Ishii, "Presence and Growth of Naturalized Escherichia Coli in Temperate Soils from Lake Superior Watersheds."

[50] Donna Ferguson and Caterina Signoretto, "Environmental Persistence and Naturalization of Fecal Indicator Organisms," in *Microbial Source Tracking: Methods, Applications, and Case Studies*(New York, NY : Springer New York : Springer, 2011).

or wetland. While a thorough review of each of these programs is beyond the scope of this paper, it is worth understanding the evolution and implementation of a couple of programs.

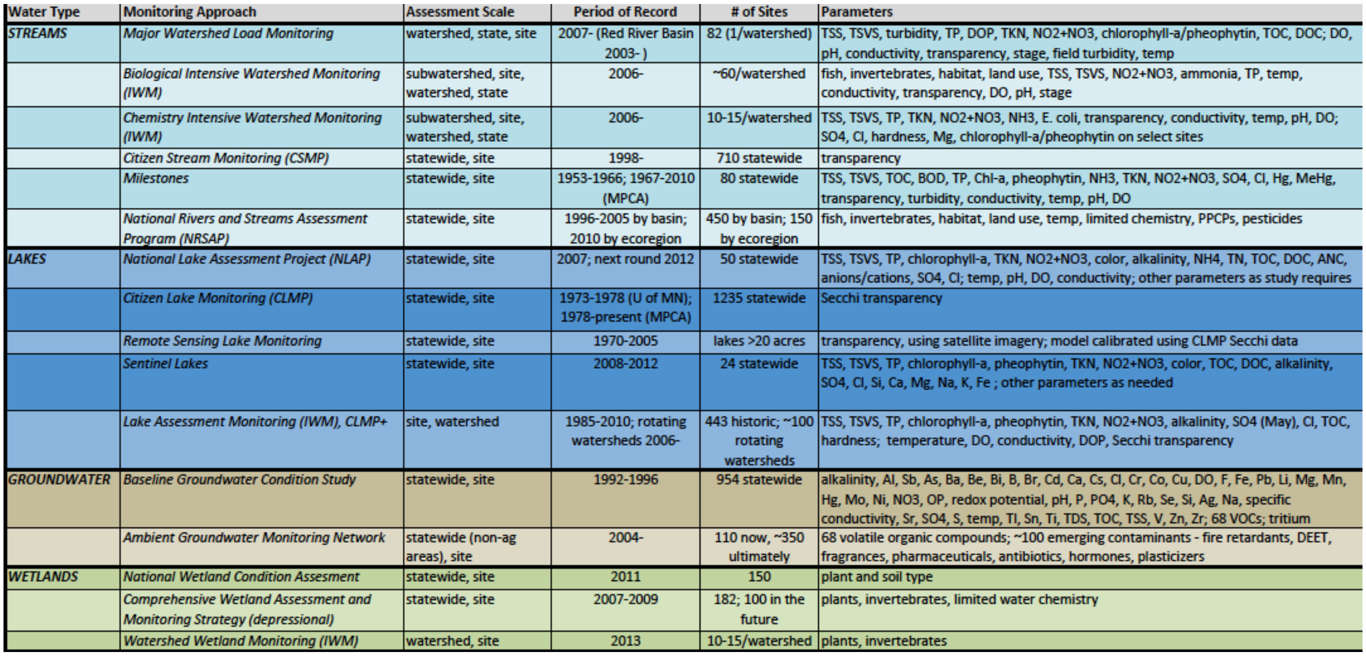
Major Watershed Load Monitoring

Funding made available through the passage of the Clean Water, Land and Legacy Amendment aided in implementing the Watershed Pollutant Load Monitoring Network (WPLMN). The WPLMN has established 201 sampling sites that are measured 25-35 times per year, depending on the scale of the watershed or sub-watershed being measured.

The Citizen Stream Monitoring Program

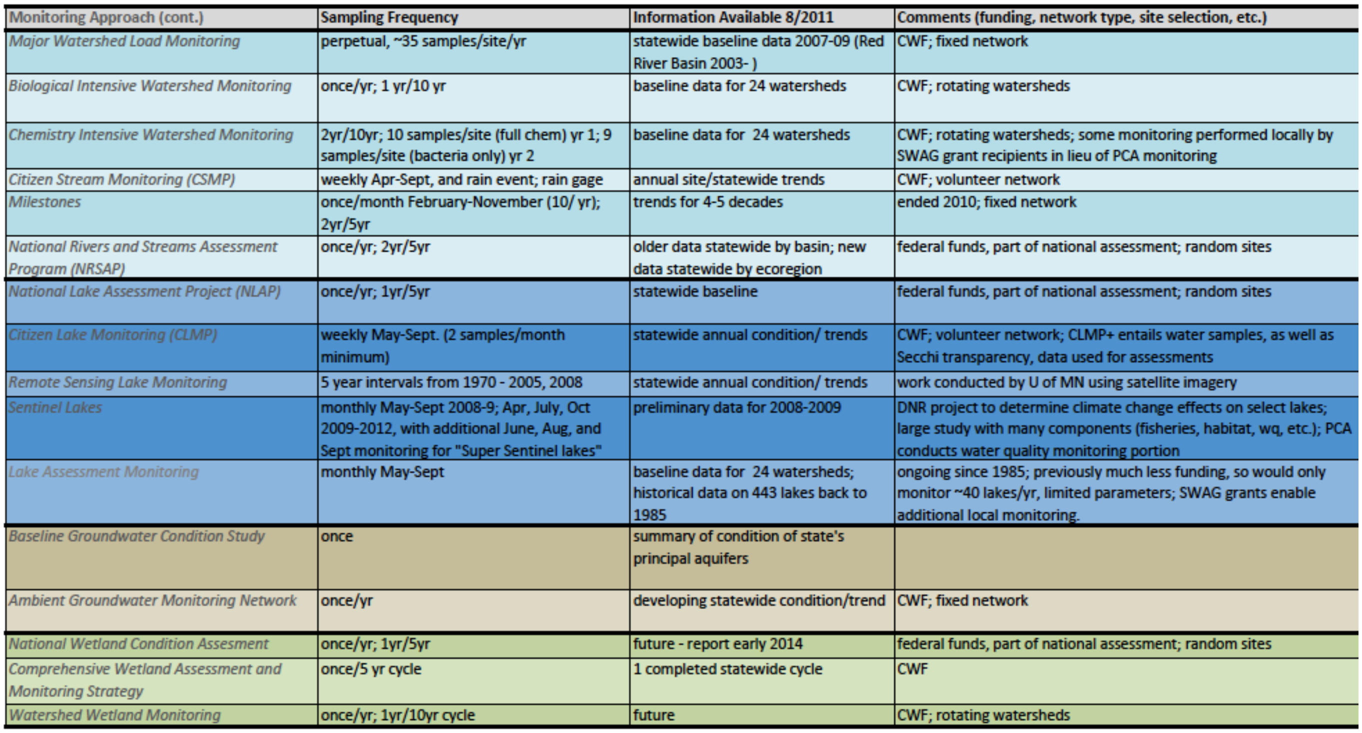
The magnitude of sampling that must occur in order to effectively monitor the numerous water bodies in Minnesota requires the contribution of citizen volunteers. The MPCA oversees the Citizens Lake Monitoring Program (CLMP) and Citizen Stream Monitoring Program (CSMP). Citizen sampling simply monitors the water clarity of water bodies, as this parameter requires limited expertise and equipment. Over 1,400 Minnesotans volunteer through the CLMP and CSMP.

Table 2. Summary of monitoring programs implemented by the MPCA, from MPCA *Minnesota's Water Quality Monitoring Strategy* .



Water Type	Monitoring Approach	Assessment Scale	Period of Record	# of Sites	Parameters
STREAMS	*Major Watershed Load Monitoring*	watershed, state, site	2007- (Red River Basin 2003-)	82 (1/watershed)	TSS, TSVS, turbidity, TP, DOP, TKN, NO2+NO3, chlorophyll-a/pheophytin, TOC, DOC; DO, pH, conductivity, transparency, stage, field turbidity, temp
	Biological Intensive Watershed Monitoring (IWM)	subwatershed, site, watershed, state	2006-	~60/watershed	fish, invertebrates, habitat, land use, TSS, TSVS, NO2+NO3, ammonia, TP, temp, conductivity, transparency, DO, pH, stage
	Chemistry Intensive Watershed Monitoring (IWM)	subwatershed, site, watershed, state	2006-	10-15/watershed	TSS, TSVS, TP, TKN, NO2+NO3, NH3, E. coli, transparency, conductivity, temp, pH, DO; SO4, Cl, hardness, Mg, chlorophyll-a/pheophytin on select sites
	Citizen Stream Monitoring (CSMP)	statewide, site	1998-	710 statewide	transparency
	Milestones	statewide, site	1953-1966; 1967-2010 (MPCA)	80 statewide	TSS, TSVS, TOC, BOD, TP, Chl-a, pheophytin, NH3, TKN, NO2+NO3, SO4, Cl, Hg, MeHg, transparency, turbidity, conductivity, temp, pH, DO
	National Rivers and Streams Assessment Program (NRSAP)	statewide, site	1996-2005 by basin; 2010 by ecoregion	450 by basin; 150 by ecoregion	fish, invertebrates, habitat, land use, temp, limited chemistry, PPCPs, pesticides
LAKES	*National Lake Assessment Project (NLAP)*	statewide, site	2007; next round 2012	50 statewide	TSS, TSVS, TP, chlorophyll-a, TKN, NO2+NO3, color, alkalinity, NH4, TN, TOC, DOC, ANC, anions/cations, SO4, Cl; temp, pH, DO, conductivity; other parameters as study requires
	Citizen Lake Monitoring (CLMP)	statewide, site	1973-1978 (U of MN); 1978-present (MPCA)	1235 statewide	Secchi transparency
	Remote Sensing Lake Monitoring	statewide, site	1970-2005	lakes >20 acres	transparency, using satellite imagery; model calibrated using CLMP Secchi data
	Sentinel Lakes	statewide, site	2008-2012	24 statewide	TSS, TSVS, TP, chlorophyll-a, pheophytin, TKN, NO2+NO3, color, TOC, DOC, alkalinity, SO4, Cl, Si, Ca, Mg, Na, K, Fe ; other parameters as needed
	Lake Assessment Monitoring (IWM), CLMP+	site, watershed	1985-2010; rotating watersheds 2006-	443 historic; ~100 rotating watersheds	TSS, TSVS, TP, chlorophyll-a, pheophytin, TKN, NO2+NO3, alkalinity, SO4 (May), Cl, TOC, hardness; temperature, DO, conductivity, DOP, Secchi transparency
GROUNDWATER	*Baseline Groundwater Condition Study*	statewide, site	1992-1996	954 statewide	alkalinity, Al, Sb, As, Ba, Be, Bi, B, Br, Cd, Ca, Cs, Cl, Cr, Co, Cu, DO, F, Fe, Pb, Li, Mg, Mn, Hg, Mo, Ni, NO3, OP, redox potential, pH, P, PO4, K, Rb, Se, Si, Ag, Na, specific conductivity, Sr, SO4, S, temp, Tl, Sn, Ti, TDS, TOC, TSS, V, Zn, Zr; 68 VOCs; tritium
	Ambient Groundwater Monitoring Network	statewide (non-ag areas), site	2004-	110 now, ~350 ultimately	68 volatile organic compounds; ~100 emerging contaminants - fire retardants, DEET, fragrances, pharmaceuticals, antibiotics, hormones, plasticizers
WETLANDS	*National Wetland Condition Assesment*	statewide, site	2011	150	plant and soil type
	Comprehensive Wetland Assessment and Monitoring Strategy (depressional)	statewide, site	2007-2009	182; 100 in the future	plants, invertebrates, limited water chemistry
	Watershed Wetland Monitoring (IWM)	watershed, site	2013	10-15/watershed	plants, invertebrates

Table 3. Extension of Table 2. Summary of monitoring programs implemented by the MPCA, from MPCA *Minnesota's Water Quality Monitoring Strategy.*



Monitoring Approach (cont.)	Sampling Frequency	Information Available 8/2011	Comments (funding, network type, site selection, etc.)
Major Watershed Load Monitoring	perpetual, ~35 samples/site/yr	statewide baseline data 2007-09 (Red River Basin 2003-)	CWF; fixed network
Biological Intensive Watershed Monitoring	once/yr; 1 yr/10 yr	baseline data for 24 watersheds	CWF; rotating watersheds
Chemistry Intensive Watershed Monitoring	2yr/10yr; 10 samples/site (full chem) yr 1; 9 samples/site (bacteria only) yr 2	baseline data for 24 watersheds	CWF; rotating watersheds; some monitoring performed locally by SWAG grant recipients in lieu of PCA monitoring
Citizen Stream Monitoring (CSMP)	weekly Apr-Sept, and rain event; rain gage	annual site/statewide trends	CWF; volunteer network
Milestones	once/month February-November (10/ yr); 2yr/5yr	trends for 4-5 decades	ended 2010; fixed network
National Rivers and Streams Assessment Program (NRSAP)	once/yr; 2yr/5yr	older data statewide by basin; new data statewide by ecoregion	federal funds, part of national assessment; random sites
National Lake Assessment Project (NLAP)	once/yr; 1yr/5yr	statewide baseline	federal funds, part of national assessment; random sites
Citizen Lake Monitoring (CLMP)	weekly May-Sept. (2 samples/month minimum)	statewide annual condition/ trends	CWF; volunteer network; CLMP+ entails water samples, as well as Secchi transparency, data used for assessments
Remote Sensing Lake Monitoring	5 year intervals from 1970 - 2005, 2008	statewide annual condition/ trends	work conducted by U of MN using satellite imagery
Sentinel Lakes	monthly May-Sept 2008-9; Apr, July, Oct 2009-2012, with additional June, Aug, and Sept monitoring for "Super Sentinel lakes"	preliminary data for 2008-2009	DNR project to determine climate change effects on select lakes; large study with many components (fisheries, habitat, wq, etc.); PCA conducts water quality monitoring portion
Lake Assessment Monitoring	monthly May-Sept	baseline data for 24 watersheds; historical data on 443 lakes back to 1985	ongoing since 1985; previously much less funding, so would only monitor ~40 lakes/yr, limited parameters; SWAG grants enable additional local monitoring.
Baseline Groundwater Condition Study	once	summary of condition of state's principal aquifers	
Ambient Groundwater Monitoring Network	once/yr	developing statewide condition/trend	CWF; fixed network
National Wetland Condition Assesment	once/yr; 1yr/5yr	future - report early 2014	federal funds, part of national assessment; random sites
Comprehensive Wetland Assessment and Monitoring Strategy	once/5 yr cycle	1 completed statewide cycle	CWF
Watershed Wetland Monitoring	once/yr; 1yr/10yr cycle	future	CWF; rotating watersheds

Intensive Watershed Based Monitoring

Beginning in 2006, the MPCA adopted the intensive watershed-based approach for monitoring water quality. Minnesota's 81 major watersheds are divided into subgroups across the state and scheduled for intensive study every 10 years. Figure 3 shows the geographic extent of these watersheds as well as the year of their first intensive study cycle. The process of intensive study and assessment takes approximately 10 years, at which time the watershed will be up for study again. Monitoring programs operate under different sampling frequencies and sites may be selected randomly or rotate on a schedule. Given the abundance of waterbodies in the state of Minnesota, it would be infeasible to sample every stream or lake.[51] Stream waters are sampled in sub-watersheds or minor watersheds.[52] The MPCA aims to sample all lakes greater than 500 acres and at least half of the lakes 100 to 500 acres during each monitoring cycle.[53] Citizen sampling may provide data for waterbodies not measured during a sampling period.

Implementation of the watershed-based monitoring strategy resulted in modification of the assessment strategy in 2010. The assessment approach "is designed to combine computerized data analysis, expert review, and internal and external partner input to use all available data and information to determine the appropriate assessment decisions for a number of beneficial uses."[54] The assessment process is broken down into five steps:

1. Data compilation (pre-assessment) - computerized screening of parameters within a specified area of study and time span to determine if criteria were met.

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[51] Water quality "Minnesota's Water Quality Monitoring Strategy 2011 to 2021: A Report Prepared for the U.S. Environmental Protection Agency."
[52] Ibid.
[53] Ibid.
[54] Ibid.

2. Expert review - quality assurance of the computerized data compilation process, also includes further analysis for a number of parameters.
3. Desktop assessment - specialized staff review the pre-assessments produced by the data compilation and expert review process. The pre-assessment is reviewed with consideration for precipitation, land use, habitat for evaluation.
4. Watershed Assessment Team (WAT) - Meeting of the MPCA staff involved in the desktop assessment, the regional watershed project manager, and staff specialized for stressors specific to the watershed convene to discuss the results of the desktop assessment.
5. Professional Judgment Group (PJG) - A meeting of WAT in addition to local data collectors/government units. The result of the meeting is determination of water bodies to listed or delisted.

Different protocols and parameters are in place depending on whether the beneficial use of a water body is identified as aquatic life, aquatic consumption/drinking water, aquatic recreation or limited resource value waters.

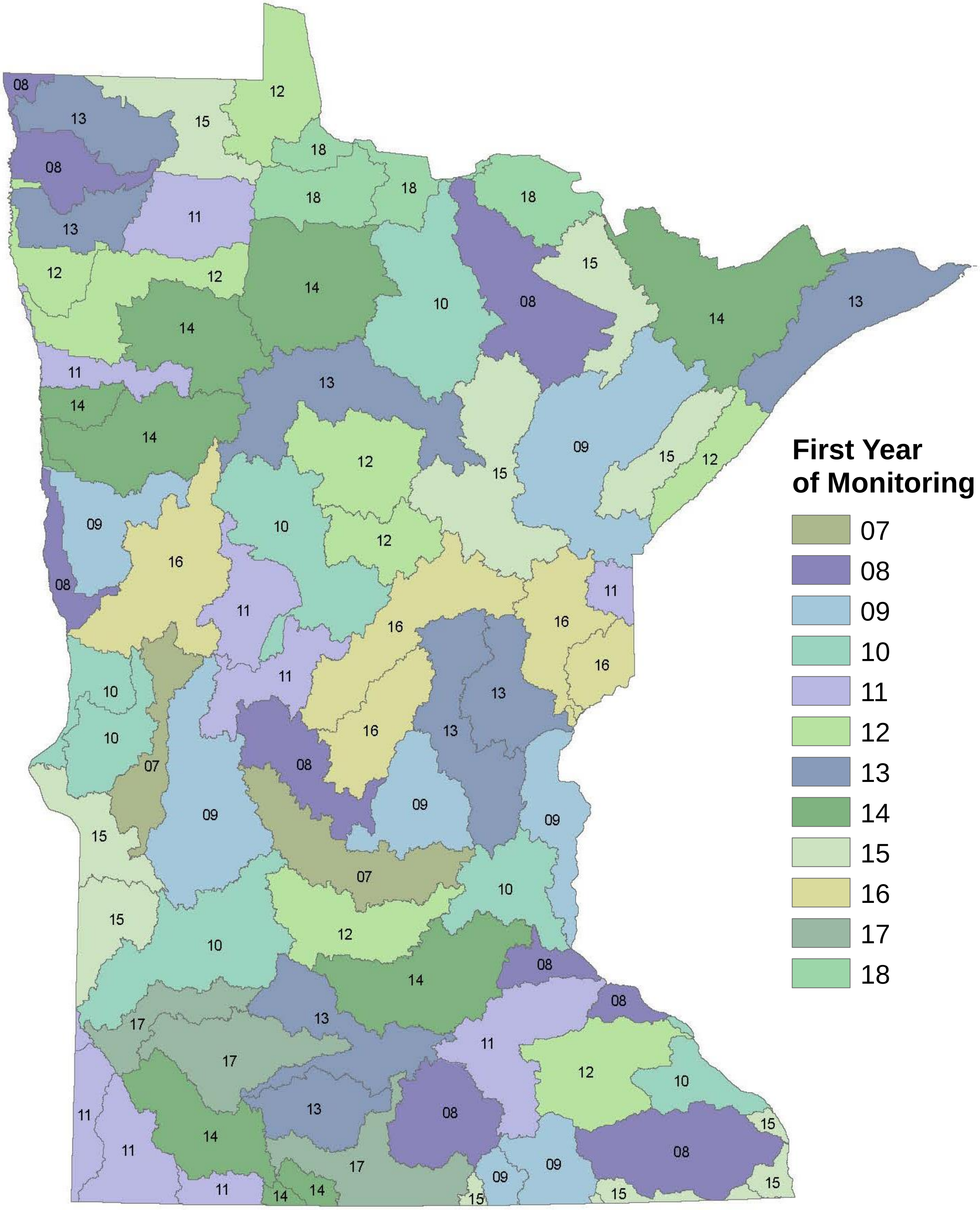

Figure 3. Map of Minnesota watersheds identified by MPCA monitoring start year. Data from MPCA *Guidance Manual for Assessing the Quality of Minnesota Surface Waters for Determination of Impairment.* Map by author.

Aquatic Life

Aquatic life is protected both for the effects of pollution on the aquatic community as well as the wildlife and humans that consume aquatic organisms. Waterbodies are assessed to determine whether they meet the standards to support aquatic life. Water chemistry and biological data are used in the assessment of these water bodies. Pollutants tested for in waterbodies under these parameters include; trace metals, unionized ammonia and chloride. Water bodies are listed if they have "Two or more exceedances of the chronic standard in three years or one or more exceedances above the maximum standard" over the previous 10 years of data.[55]

Other standard parameters used to assess water quality include dissolved oxygen, pH, turbidity, temperature, and biological indicators. The term 'biological indicator' refers to evaluation of the aquatic population in reference to what would occur under natural conditions as a means of assessing water quality.

Aquatic Consumption and Drinking Water

Human consumption is of primary concern in water quality. Human consumption refers to both the consumption of drinking water as well as of fish and other aquatic life, which may accumulate pollutants in their bodies.[56] Low levels of pollutants in water may become concentrated in fish tissue in a process termed 'bioaccumulation'. Low of levels of pollutants are concentrated through the food chain reaching dangerous levels in organisms near the top of the food chain. Common game fish in the state of Minnesota, such as

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[55] Anderson et al., "Guidance Manual for Assessing the Quality of Minnesota Surface Waters for Determination of Impairment: 305(B) Report and 303(D) List."
[56] Ibid.

northern pike and walleye are at the top of their food chain and are sources of concern. The quantity of fish consumed in Minnesota is further cause to monitor this source of pollution. It is estimated the average Minnesota consumes more fish than the average American, therefore the MPCA increased the amount of fish consumed weekly from the E.P.A.'s 17.5-gram standard to 30 grams for calculation purposes.[57]

Standards for a water body are dependent upon whether humans primarily consume the water or aquatic life. Generally, the MPCA tests these water bodies for mercury, polychlorinated biphenyls, dioxins and chlorinated pesticides, and nitrate. As with the standards set for water bodies protected for aquatic life, a water body is classified as impaired if there are two or more exceedances of a standard within a three-year period.

Aquatic Recreation

Water bodies protected for aquatic recreation are primarily evaluated using *E. coli* as a biological indicator organism. Water bodies are defined based on the likelihood of ingesting water: water bodies used for swimming are considered primary, while water bodies used for boating are secondary. Due to the climate in Minnesota, water bodies are evaluated for recreational use from April 1-October 31 annually.[58] The EPA standard is 126cfu[59]/100 mL (2.10 $\log_{10}$(cfu/100mL) when log transformed for normalization). If the geometric mean for a month exceeds 126cfu/100 mL, or if there is one or more instances of greater than 1260cfu/100 mL the water body is classified as impaired. The geometric mean is defined as

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[57] Ibid.

[58] Ibid.

[59] cfu-colony forming units

"the n-th root of the product of n numbers".[60] Geometric means are commonly used on bacterial data in order to normalize data and reduce the effect of very high or low values.

Another measure of waterbodies used for aquatic recreation is the degree of eutrophication. Severe eutrophication renders water bodies unsuitable for recreation. Eutrophication is due to increased nutrients entering fresh waterbodies, often phosphorus. Higher levels of nutrients contribute to large algae blooms, an example of which can be seen in Fig. 4. Algae blooms are not only aesthetically unpleasant, but may also pose health hazards, as in the case of toxic blue green algae.[61] Eutrophication is evaluated by measuring the Total Phosphorus (TP) of a water body, the transparency and chlorophyll-*a* concentration.[62]

Figure 4. Eutrophied River in Minnesota. From the Minnesota Pollution Control Agency Website. Accessed March 19, 2017.

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[60] "Geometric Mean: Definition, Uses, Examples, Formula," 2014, accessed 4/2, 2017.
[61] Donald M. Anderson, Patricia M. Glibert, and Joann M. Burkholder, "Harmful Algal Blooms and Eutrophication: Nutrient Sources, Composition, and Consequences," *Estuaries* 25, no. 4 (2002).
[62] Anderson et al., "Guidance Manual for Assessing the Quality of Minnesota Surface Waters for Determination of Impairment: 305(B) Report and 303(D) List."

Delisting

The objective of Intensive Watershed Based Monitoring, and indeed all MPCA efforts, is to improve the quality of a waterbody such that it meets the standards for its beneficial use classification. When this is successful and standards are met, a water body will be removed from the impaired list. However, Phil Votruba of the MPCA explains that examples of successful cleanup are rare. The cost and challenge of remediating a water body is often prohibitive; ideally the goal is to prevent impairment in the first place.[63]

Depending on the type of impairment a water body is classified for, it must meet a variety of assessment criteria to be considered for delisting. For instance if a water body qualified as impaired for mercury content, it may be delisted if there are a minimum of 5 samples over three years and none exceed the standard.[64] A waterbody impaired for fecal contamination is required to have a minimum of 15 sampling events over a two-year period with a minimum of 5 per month (April-October). Less than 10 percent of samples may exceed the standard during this time. [65]

A water body may also be removed from the list upon completion of the TMDL study, if it is determined the source of pollution is natural, or if it is determined the water body was placed on the list incorrectly.[66]

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[63] Votruba.

[64] Anderson et al., "Guidance Manual for Assessing the Quality of Minnesota Surface Waters for Determination of Impairment: 305(B) Report and 303(D) List."

[65] Ibid.

[66] Ibid.

CASE STUDIES

While there is a great deal of policy in place to monitor and improve water quality, the implementation of these protocols varies on a case-by-case basis. Before examining the details of the Plum Creek case study highlighted in this paper, it is worthwhile to understand how the process works under ideal circumstances.

Reference Study: Clearwater River

In 2002, a 58-mile stretch of the Clearwater River, approximately 15 miles south of St. Cloud, Minnesota, was listed as impaired for high levels of fecal coliform. The contamination was suggested to be coming from a number of non-point sources including livestock, wildlife and drainage from wild rice paddies nearby. Additionally, areas of the watershed had been drained significantly and nearby land had been converted from tall grass prairie to agricultural or urban uses.

The task of cleaning up the river fell upon the Clearwater County and Red Lake County Soil and Water Conservation Districts in coordination with local citizens and farmers. Initial work focused on "implement[ing] a number of best management practices (BMPs) such as residue management, grazing management, nutrient management, grade control structures, side water inlets, stream bank protection, and grassed waterways." The majority of these efforts were targeted at farmers with the goals of reducing erosion and filtering run-off manure and fertilizers. In a TMDL study conducted from 2007 to 2009, all *E. coli* values fell below regulatory limits and in 2010 the MPCA delisted the 58-mile stretch of Clearwater River. Over $1 million dollars were invested in the implementation phase of the Clearwater River project.[67]

Current Study: Plum Creek

History and Geography

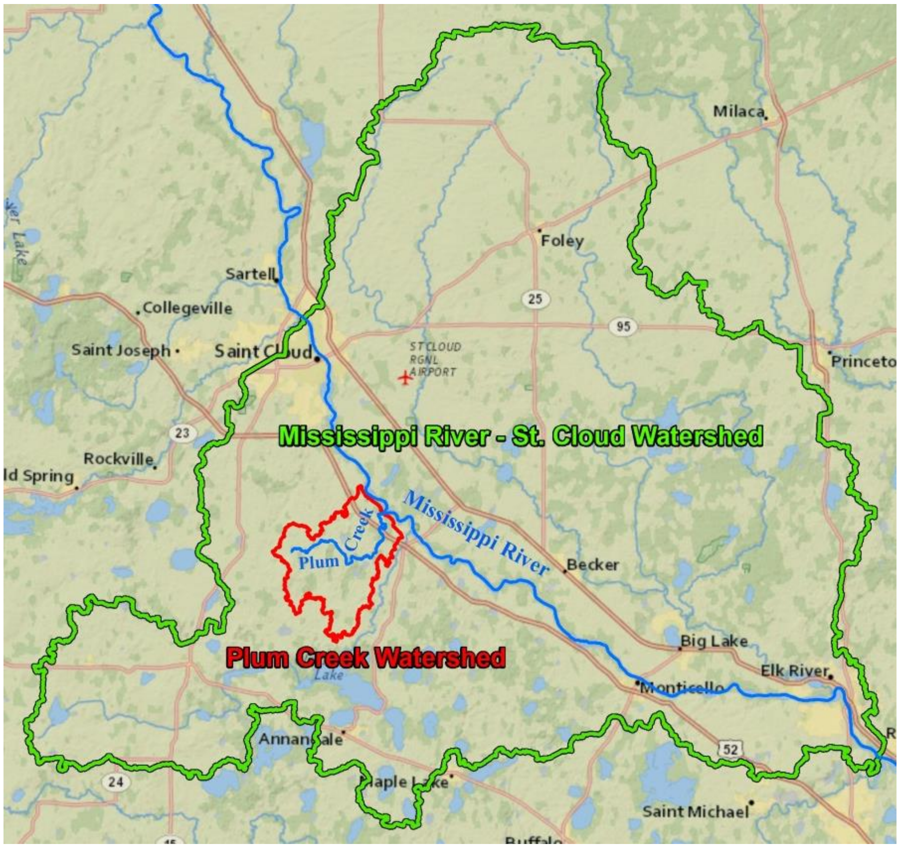

Figure 5. Map of Plum Creek Watershed from Stearns County Soil and Water Conservation District.

Plum Creek is located in southeast Stearns County, Minnesota in the Sauk River Watershed (Fig. 5). The MPCA identifies Plum Creek as a water body protected for aquatic recreation. The stretch of the creek studied begins in Warner Lake, a popular recreation area, and flows northeast, emptying into the Mississippi River. Plum Creek flows from Warner Lake under County Road 143, adjacent to agricultural fields (Fig. 6). In 1970, the Minnesota Department of Transportation (MNDOT) straightened a section of Plum Creek between Warner Lake and the Mississippi River to allow for the construction of Interstate Highway 94 (Yellow line, Fig. 6). North of Interstate 94, Plum Creek flows through additional agricultural fields before crossing under County

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[67] "Water Story: Clearwater River Gets Cleaner," accessed 3/17/17,

Road 75. At Franklin Road County Drainage Ditch 39 joins Plum Creek before the final stretch that passes through a forested rural residential area, which empties into the Mississippi River.

Figure 6. Map of Plum Creek channel construction to accommodate Interstate 94 from Stearns County Soil and Water Conservation District.

In 2008, Plum Creek was scheduled for routine testing as part of the MPCA's intensive watershed based approach (Fig. 3). The MPCA monitors Plum Creek from Warner Lake to the Mississippi River at two sites; County Road 75 and Franklin Road (sites 4 and 7 of current study as seen in Fig. 9). The stretch exceeded the regulatory standard for *E. coli* and the MPCA added the creek to the impaired water bodies list in 2012.[68] The MPCA TMDL study published in 2014, identified hog and poultry livestock, and incorporated manure application on agricultural fields as the primary sources of contamination, and recommended implementing a number of BMPs to improve the water quality.[69]

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[68] Minnesota Pollution Control Agency and Minnesota Department of Health, "Upper Mississippi River Bacteria Tmdl Study & Protection Plan,"(2014).

[69] Ibid.

Upon the creek's listing, Jerry Finch, a Lynden Township Supervisor on the Stearns County Planning Commission began investigating what could be done to remediate the impairment.[70] Finch was primarily concerned the TMDL did not correctly identify the source of *E. coli*, as there are no poultry, hogs or surface manure applications within the Plum Creek watershed. The area of land within the immediate impaired area was insufficient to warrant further study or funding from the MPCA. In the fall of 2013, Finch enrolled in the inaugural University of Minnesota Watershed specialist course online, designed for water quality professionals. Following the course guidelines, Finch enlisted landowners with interest in the project and established the Plum Creek Neighborhood Network (PCNN). The PCNN was initially made up of 24 members from the local community, chaired by two local residents and facilitated by Finch. In 2014, Finch began independent sampling of the impaired area of the creek at five locations along the creek: Warner Lake, CR 143, Interstate 94, CR 75 and Franklin Road. The Lynden Township financed the testing as a matter concerning the health, safety and welfare of the Township's residents.

Finch's findings in 2014 and 2015 found levels of *E. coli* exceeding the threshold on 1 occasion in 2014 (Fig. 7) and 9 occasions in 2015 (Fig. 8). With increased sampling locations relative to MPCA testing, it was found the levels of *E. coli* generally increased from Warner Lake to Franklin Road, suggesting the source of impairment was located within the 1.5-mile stretch.

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[70] Jerry Finch, interview by Sarah McLarnan2017.

Finch sought assistance from the Stearns County Soil and Water Conservation District and experts at University of Minnesota. Dr. Mike Sadowsky, Director of the BioTechnology Institute at the University of Minnesota recommended DNA source tracking in order to identify the potential biological sources of the *E. coli*. In 2014, a water sample from Plum Creek was submitted to Source Molecular in Miami, Florida for analysis. The test analyzed Plum Creek *E. coli* against known markers for poultry, ruminants, and humans. Results were negative for all three.

In March 2016 a team of individuals from the Water Quality Division staff at the MPCA, University of Minnesota Biotechnology Institute, PCNN and Stearns County SWCD and the College of Saint Benedict and Saint John's University developed a research protocol for the 2016 summer study. The goals of this study were to determine whether the stream sediment was a potential source of contamination, and expand upon the spatial resolution of previous *E. coli* data collection with additional sampling locations. Basic stream parameters were also evaluate for any anomalies or correlations with high concentrations of *E. coli.*

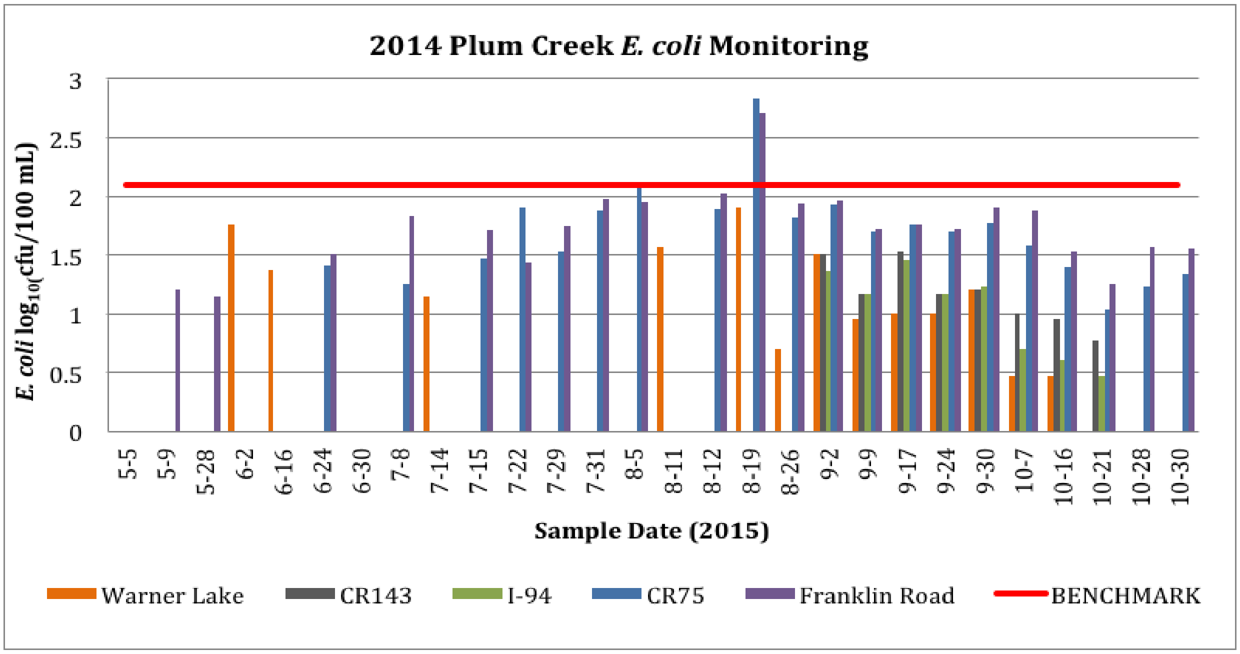

Figure 7. Graph of *E. coli* $\log_{10}$(cfu/100mL) at three sampling locations performed by Jerry Finch in 2014. Modified from Stearns County Soil and Water Conservation District.

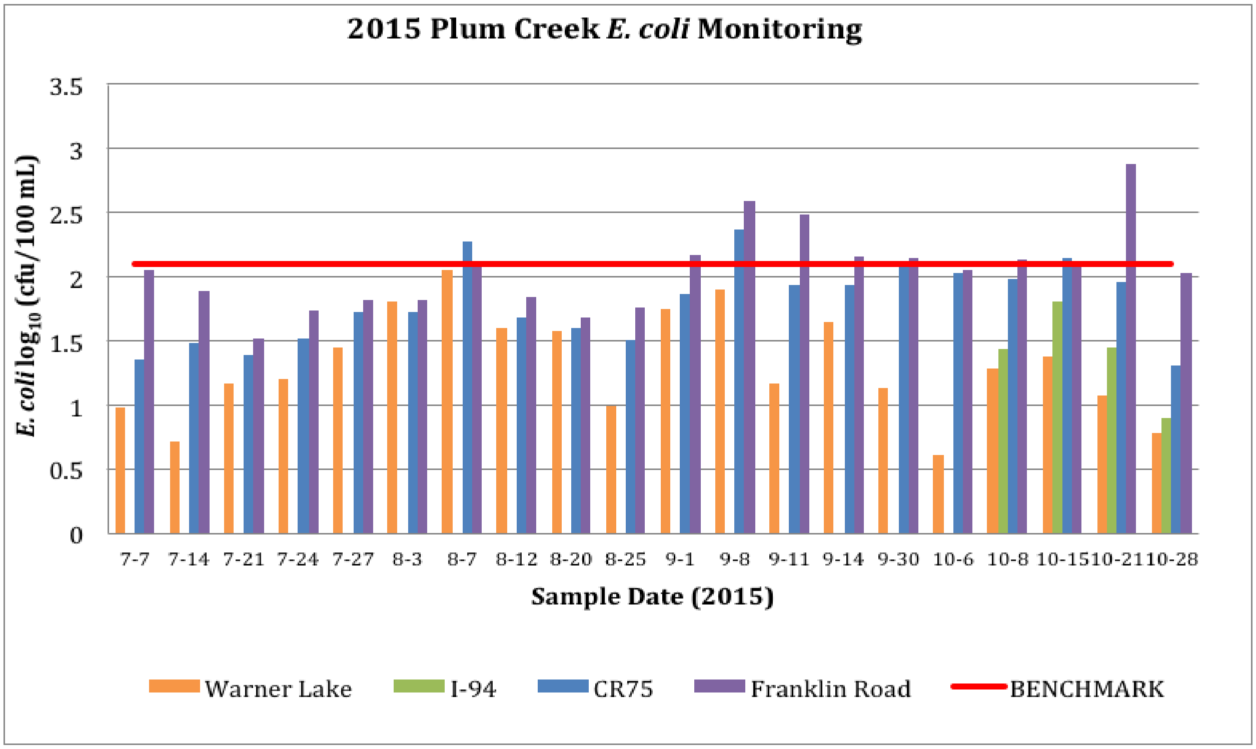

Figure 8. Graph of *E. coli* $\log_{10}$(cfu/100mL) at three sampling locations performed by Jerry Finch in 2015. Modified from Stearns County Soil and Water Conservation District.

Methods and Materials

A site assessment was performed April 20th, 2016 to identify locations for data collection. Seven sites were identified between Warner Lake and the Mississippi River (Fig. 9). Previous sites of study were included to maintain consistency and adhere to MPCA water sampling protocols. Additional sites were selected for nearby features with potential point sources of pollution, such as areas of bank erosion, agricultural fields, or drainage ditches/culverts. Site 2 (Fig. 9) was added after crossing under County Road 143, adjacent to an agricultural field in an area with a culvert that enters the stream at a 90° angle. Site 3 was added after Plum Creek crosses under Interstate 94, adjacent to an agricultural field near an area with visible stream bank erosion. Sites 5-7 were selected to identify the influence of County Ditch 39 on Plum Creek. Site 5 was located at Plum Creek immediately upstream from the confluence with County Ditch 39. Site 6 was located approximately 15 meters upstream of County Ditch 39 near where it enters Plum Creek. Site 7 was located immediately after the confluence of Plum Creek and County Ditch 39 after passing under Franklin Road.

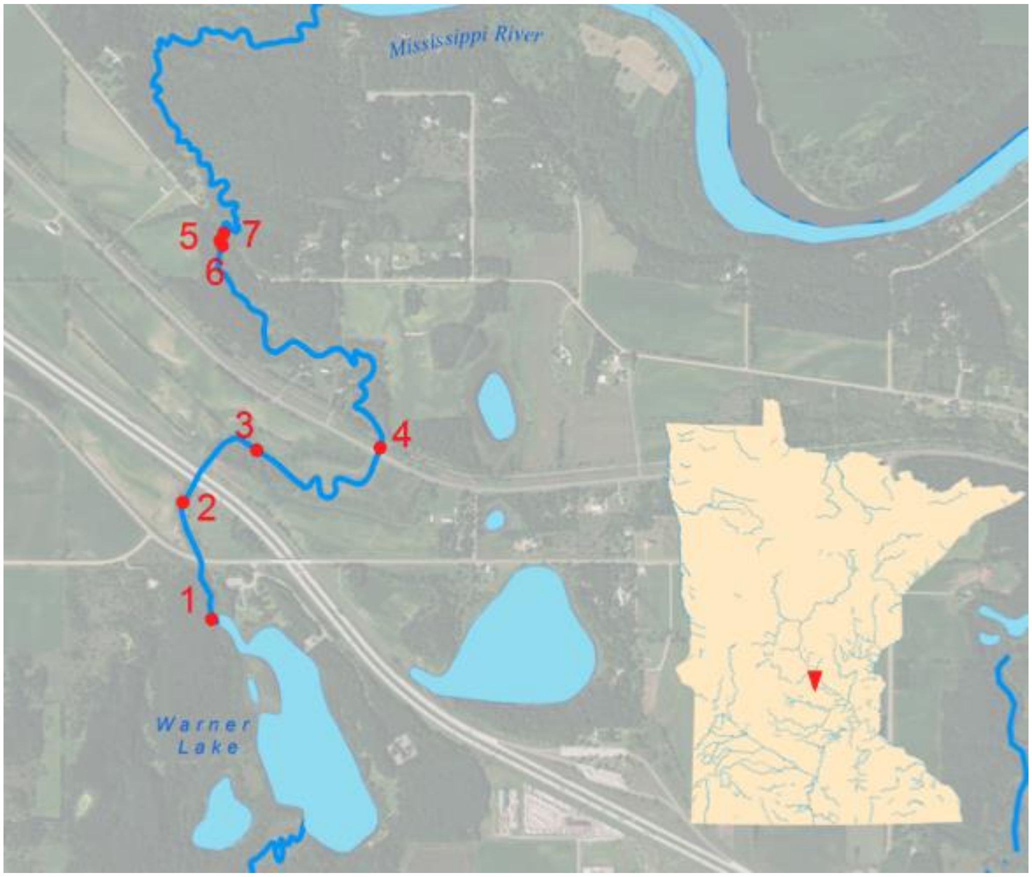

Figure 9. Map of the sampling locations of Plum Creek during the summer of 2016. Map by author

Table 3. Site description of sampling locations of Plum Creek during the summer of 2016. Table by author. *Indicates locations also monitored by PCNN.

Site	GPS Coordinates	Distance from Warner Lake	Sediment (% sand, silt, clay)	Notable Features
1*	45°25’30” N; 94°5’10” W	0 miles	87, 5, 7	Headwaters of Plum Creek
2	45°25’30” N; 94°5’13” W	.25 miles	88, 7, 5	After crossing under CR 143, adjacent to agricultural field, near 90° culver outlet
3	45°25’33” N; 94°5’6” W	.87 miles	83, 15, 2	After crossing under Interstate 94, adjacent to agricultural field, near streambank erosion
4*	45°25’37” N; 94°4’48” W	1.04 miles	79, 13, 8	Immediately below CR75 bridge
5	45°25’55” N; 94°5’10” W	1.67 miles	95, 4, 1.	Near agricultural field and wooden riparian buffer, upstream of County Ditch 39
6	45°25’54” N; 94°5’9” W	1.67 miles	90, 3, 7	County Ditch 39
7*	45°25’55” N; 94°5’9” W	1.68 miles	94, 5, 1	After water from Plum Creek and drainage ditch have combined and gone under Franklin Road

Additional water parameters were collected during the summer 2016 study in order to identify stream characteristics, which could correlate with *E. coli* growth. A Y-Si 556 handheld multi-probe (YSI Incorporated, Yellow Springs, OH) was used to measure pH, dissolved oxygen, and conductivity (Fig. 10A). Turbidity was measured using a Vernier Turbidity Sensor (Fig. 10B) integrated with a LabQuest 2 interface (Vernier Software and Technology, Beaverton, OR). Transparency was measured using a 120cm transparency tube (Fig. 10C). Triplicate samples of both water and sediment were obtained from each site (sediment was collected on two occasions). Sample collection followed EPA *E. coli* enumeration protocol, briefly summarized here. Water samples were membrane filtered using replicate 1, 10 and 100 mL samples, incubated at 37° C for 20-24 hours then counted for coliform forming units (cfu) (Fig. 10D). Sediment samples were kept on ice in sterilized whirlpack bags and transported within 24h to the University of Minnesota Biotechnology Institute. Sediment sample processing and analysis requires the extraction of *E. coli* from the sediment and then followed a similar enumeration procedure to water samples.

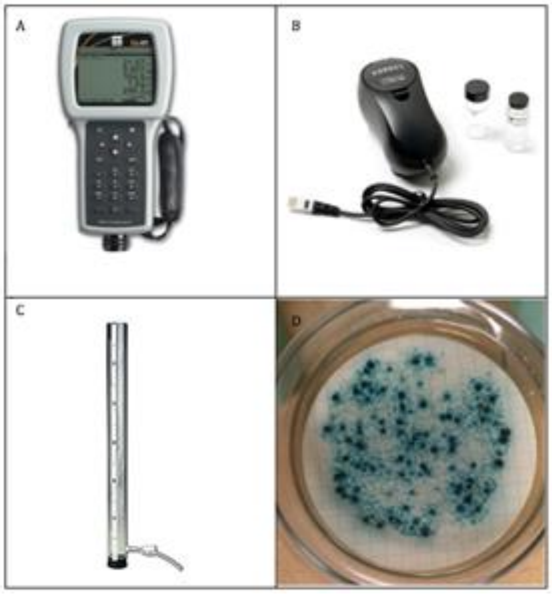

Figure 10. Instrumentation used for this study included (A) Y-Si 556 multi-probe from YSI.com (B) Vernier Turbidity probe from Vernier.com (C) Transparency tube from Grainger.com (D) Incubated membrane filtered sample

Sampling was performed seven times between June and October 2016 (see appendix table 1 for full data). Soil texture was obtained during the July 5th sampling event using the Bouyoucos Hydrometer Method. While water samples were kept chilled on ice and quickly transported to labs at St. John's University for analysis within 24h. Sediment samples were kept chilled on ice and transported to the University of Minnesota Biotechnology Institute for analysis within 24 hours. Logistical challenges arose from need to quickly transport different samples to different labs more than 80 miles apart within 24h. As a result, sediment samples were successfully collected and processed on only two occasions.

Data analysis followed standard procedure for water quality parameters as set by the MPCA. Geometric means were calculated for the 100mL samples from each site after each sampling event. ANOVA tests were performed using StatPlus to evaluate whether site location, date, and sediment *E. coli* concentrations were predictors of *E. coli* levels. Correlation between sediment and water *E. coli* concentrations was evaluated using a Spearman Rank test in SigmaPlot 13.0. A significance level of $P<0.05$was used for all analyses, unless otherwise noted.

Results

Temperature and precipitation data were obtained from the St. Cloud Regional Airport Weather station near the study location (14 km) (Fig. 11).[71] The study location had higher than average temperatures (+2.6°F) during the sampling season (Fig. 11). Rainfall was higher than average in the months of July (+3.43in above average) and August (+4.58in above average) and approximately average for the months of June, September and October. There were six rainfall events greater than 1 inch during the sampling season (6/14; 7/10;

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[71] "St. Cloud Regional, Mn," 2016, accessed March, 18, 2017.

7/11; 7/23; 8/10; 8/29). Stream flow was not a parameter quantitatively measured in this study; however, field observation during sampling events noted an approximately 1-foot increase in depth between sampling on August 2 and September 2; local residents from the PCNN noted this was abnormal for this time of year.[72]

Our preliminary findings were consistent with earlier data collected by the PCNN in the previous two years (Fig. 7&8). Samples exceeded the regulatory threshold of $\log_{10}$(cfu/100 mL) < 2.10 on 3 occasions. Sites 4-7 all exceeded the threshold on at least one occasion, with sites 6 and 7 most frequently exceeding the threshold value (Fig. 12).

Analysis of stream parameters revealed only minor spatial and temporal changes throughout the study. Plum Creek's pH values were consistently elevated at sites 1-4 relative to sites 5-7, with the exception of the final sampling event (Oct. 8). Average pH also decreased over the sampling season. Dissolved oxygen generally decreased from sites 1 to 7 on all sampling events except the final sampling event (Oct. 8). Differences in dissolved oxygen decreased across sites as the sampling season progressed. Average dissolved oxygen declined from 9.05 mg/L in June to 8.06 mg/L in September, rising to 8.51 mg/L on the final sampling event. Conductivity remained consistent across sites 1-4, 6 and 7 (values ranged from .362 mS/cm to .498 mS/cm). However, site 5 consistently varied from the other 6 sites either above or below (values ranged from .313 to .524). Transparency typically increased from sites 1 to 7 and increased throughout the season.

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[72] Finch.

Sediment *E. coli* demonstrated no significant trends spatially or temporally. Values did not increase downstream, nor were they appreciably different between sampling events. University of Minnesota Biotechnology Institute researchers noted sediment values were low relative to similar stream studies they have performed.[73] No correlation was found between stream *E. coli* and sediment *E. coli* concentrations (*P*=0.17).

Statistical analysis indicated strong correlations between a number of measured parameters and *E. coli* concentration. Stream *E. coli* was strongly positively correlated with dissolved oxygen (*P*=0.0006), conductivity (*P*< 0.0001) (Fig. 13) as well as turbidity (*P*= 0.0006) (Fig. 14). Transparency was negatively correlated with *E. coli* concentration (*P*= 0.0174) (Fig. 14). There was no correlation between *E. coli* and pH (*P*= 0.15).

On each sampling event, the *E. coli* concentration increased significantly downstream (*P*< 0.0001), increasing from Warner Lake to Franklin Road. *E. coli* concentration was also correlated with sampling date (*P*< 0.0001), with higher *E. coli* levels occurring during the warm wet months of July and August. Samples were collected for DNA source tracking to be performed by the University of Minnesota Biotechnology Institute during each sampling event in 2016. Results of DNA source tracking analysis were still pending at the time of this report.

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[73] Michael Sadowsky, interview by Sarah McLarnan2017.

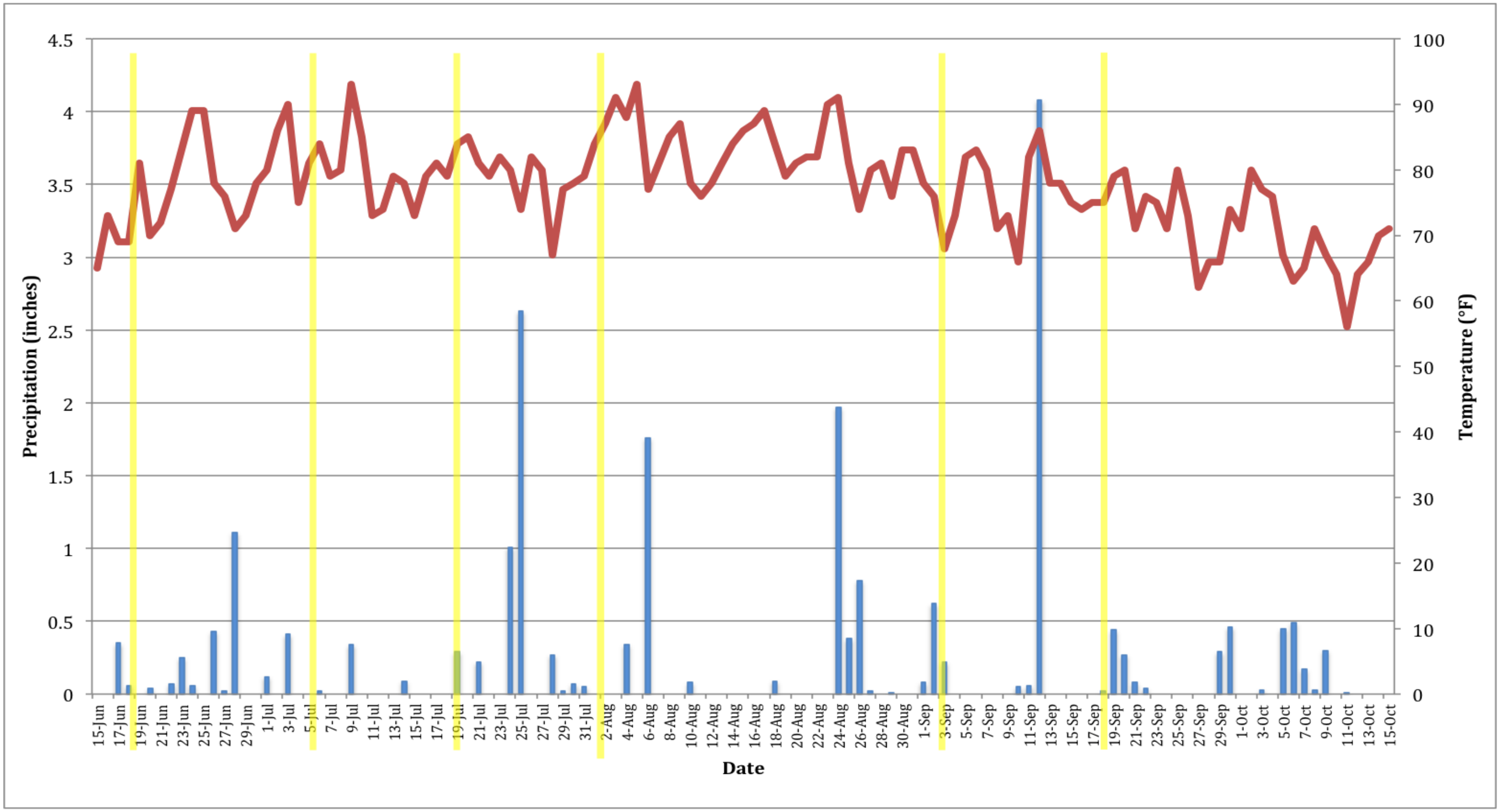

Figure 11. 2016 sample season precipitation (blue bars) and average daily temperature (red line). Sampling events indicated by vertical yellow lines. Data from St. Cloud Regional Airport weather station obtained from weatherunderground.com. Figure by author.

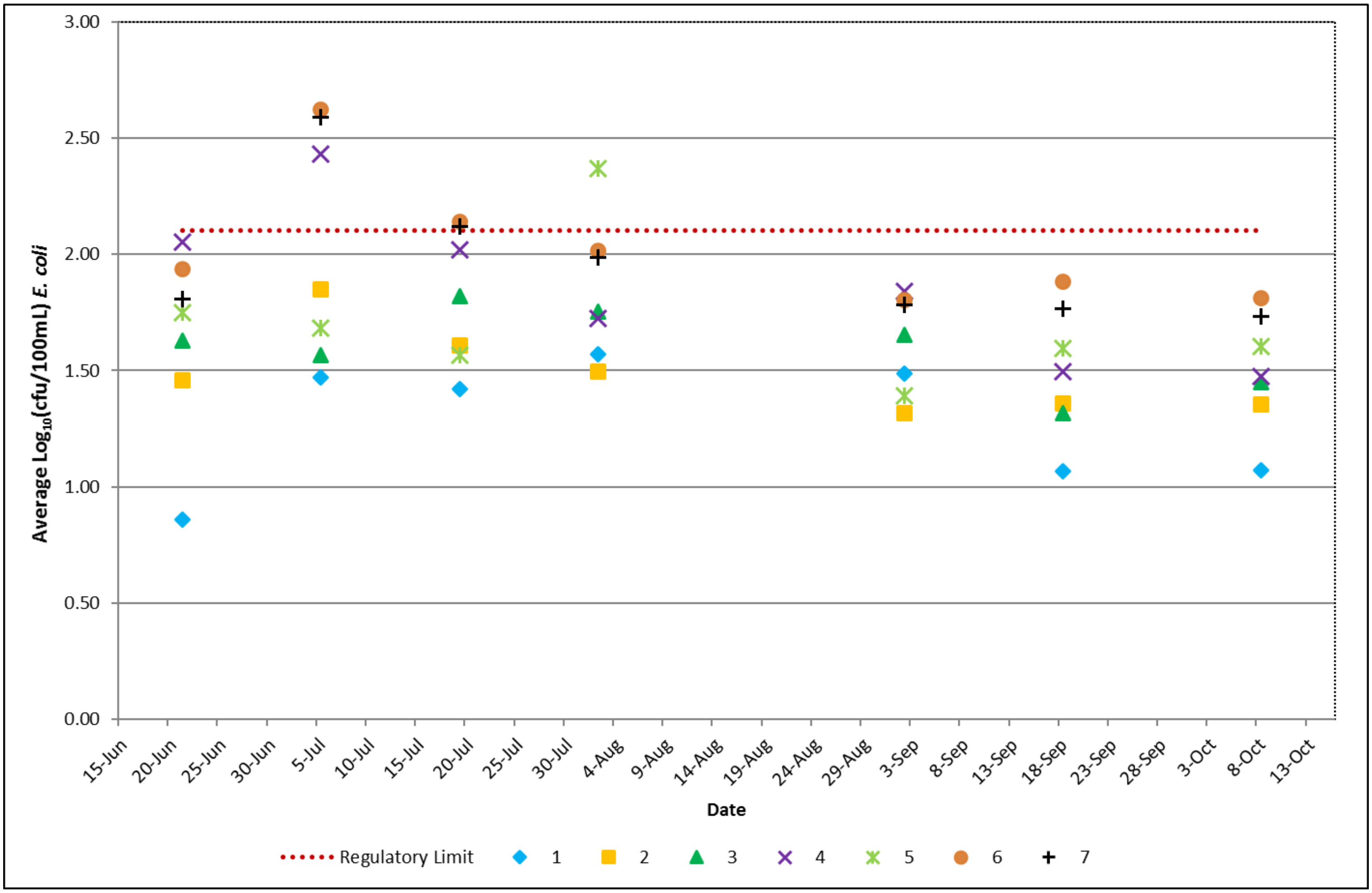

Figure 12. Arithmetic mean of *E. coli* at each sampling location on each sampling date. Figure by author.

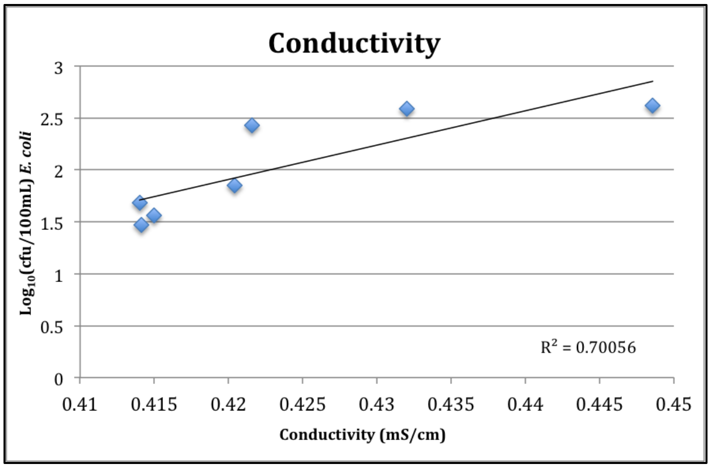

Figure 13. Correlation of *E. coli* concentration and conductivity. Figure by author.

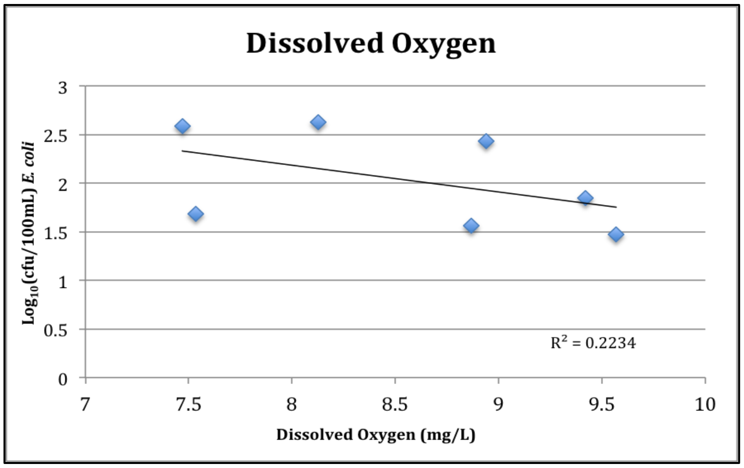

Figure 14. Correlation of *E. coli* concentration and dissolved oxygen. Figure by author.

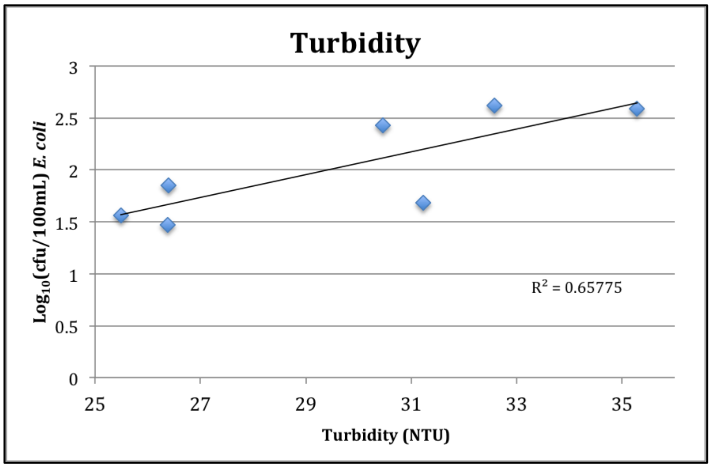

Figure 15. Correlation of *E. coli* concentration and turbidity. Figure by author.

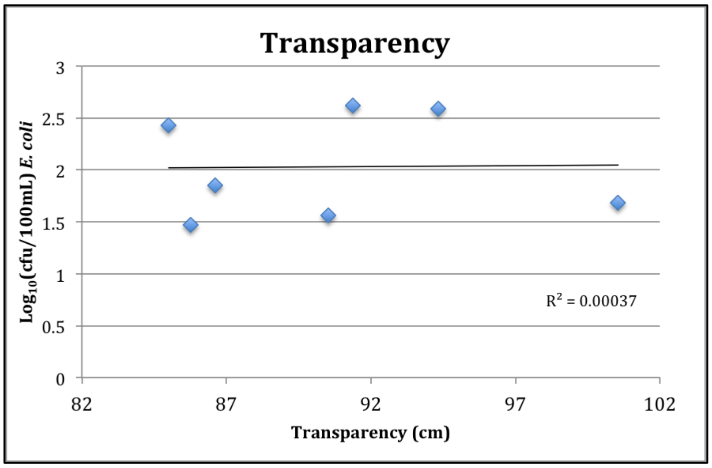

Figure 16. Correlation of *E. coli* concentration and transparency. Figure by author.

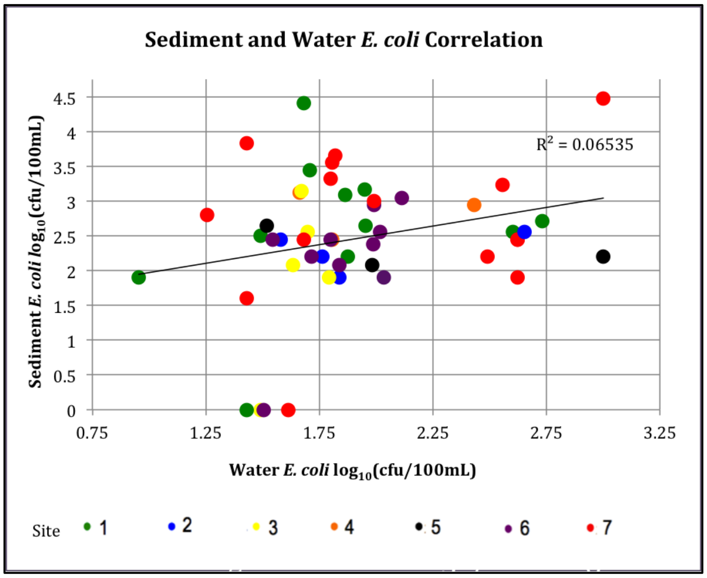

Figure 17. Correlation of sediment and stream *E. coli* concentrations. Figure by author.

Discussion

The 2016 summer study confirmed that Plum Creek *E. coli* levels increases from Warner Lake to Franklin Road, reaching values qualifying the creek for the impaired waterbodies list under MPCA standards. Despite increases in the temporal and spatial data, the study did not identify a specific source of fecal contamination. The gradual increase of *E. coli* downstream indicated no singular source of contamination. Moving forward, results of 2016 DNA source tracking will provide valuable information comparing water samples to *E. coli* from humans, cattle and waterfowl.[74] While

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[74] Ibid.

2014 DNA source tracking was measured on one occasion, samples were taken at multiple sites on multiple sampling events in the 2016 study, thus greater confidence can be drawn from these results.

When comparing *E. coli* values relative to other years it is necessary to take into account the variables that may influence growth from season to season. The characteristics of the 2016 sampling season were conducive to *E. coli* growth. Above average temperatures and precipitation are optimal for the organism. As such, the values obtained in the 2016 sampling season are above the observed average for the creek.

The study of Plum Creek will continue in the summer of 2017. The study will involve less intensive sampling with increased focus on County Ditch 39 (site 6). Further study intends to test whether a naturalized population of *E. coli* is the source of contamination. DNA comparison of stream and sediment *E. coli* will provide a more conclusive indication of the role of sediment *E. coli* in stream *E. coli* in Plum Creek.

While there was no correlation between sediment and stream *E. coli* in 2016 data, these findings do not conclusively eliminate sediment *E. coli* as a potential source of impairment in Plum Creek. The 2017 study *Enrichment of Stream Water with Fecal Indicator Organisms During Baseflow Periods* conducted by Yakov Pachepsky measured levels of FIO at the beginning and end of a 600-meter stretch of creek under baseflow conditions (at least 48 hours after a rain event).[75] Between the three replications stream *E. coli* concentration increased 11.6 to 74.6 times from inlet sampling location and outlet

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[75] Pachepsky et al., "Enrichment of Stream Water with Fecal Indicator Organisms During Baseflow Periods."

sampling location.[76] Increased *E. coli* under baseflow conditions indicates microorganisms are being released from the sediment to the stream.[77] In correspondence with Pachepsky he suggested this would be a relatively easy and effective way to evaluate the influence of sediment *E. coli* in Plum Creek.[78]

Since the listing of Plum Creek as impaired in 2012, considerable resources have gone into attempting to identify and mitigate the sources of fecal coliform. The Stearns County SWCD has invested numerous employee hours to implement the TMDL actions suggested by the MPCA.[79] The Lynden Township has invested up to $3000 annually in the independent research performed by Jerry Finch and the PCNN.[80] More recently, grant funding from the U.S. EPA, employee hours from members at the College of Saint Benedict and Saint John's University and volunteer hours from members of the Institute of Biotechnology at the University of Minnesota were invested in the project.

A conservative estimation of the cost of this investigation amounts to tens of thousands of dollars. At present, there is no evidence confirming a source of fecal contamination threatening to human health. Current analysis indicates the source of *E. coli* in Plum Creek is not anthropogenic. The discrepancy between the financial cost and human health benefits of the Plum Creek impairment listing raises questions regarding the financial effectiveness of *E. coli* as the primary FIO under current monitoring guidelines.

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[76] Ibid.
[77] Ibid.
[78] Pachepsky.
[79] Dennis Fuchs, interview by Sarah McLarnan2017.
[80] Finch.

Taxpayers like Jerry Finch are not the only ones who recognize the implications of improper impairment listings. The MPCA notes, “The possible erroneous placement of a waterbody on the […] impaired list is a concern because of the regulatory and monetary implications of […] listing.”[81] It is in the best interest of all involved to ensure accuracy in the identification of human health threats. With limited funds available and an abundance of water to monitor and manage accurate assessment is imperative to responsibly allocating limited funds.

Solutions

Plum Creek is one of a growing number of cases that demonstrates the limitations of *E. coli* as an indicator organism. Yet while the evidence mounts against the practicality of using *E. coli*, a sensible solution remains elusive. As in the 1800’s, testing water directly for the presence of pathogens remains unfeasible. Yet there is no perfect indicator organism.[82]

In communications with Dr. Yakov Pachepsky and Dr. Mike Sadowsky, both agree there is no organism that provides a reliable alternative.[83] However, each has their own idea for improved methods. Pachepsky believes experimentally proven relationships are superior to theoretical relationships. “Water quality should be evaluated for a specific

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[81] Anderson et al., "Guidance Manual for Assessing the Quality of Minnesota Surface Waters for Determination of Impairment: 305(B) Report and 303(D) List."

[82] Nduka Okafor, Environmental Microbiology of Aquatic and Waste Systems, (Dordrecht ;: Springer, 2011), ebrary http://site.ebrary.com/id/10480451, SpringerLink http://dx.doi.org/10.1007/978-94-007-1460-1.

[83] Pachepsky.
Sadowsky.

application, i.e. recreation/swimming/bathing, irrigation, aquaculture […] etc.. *E. coli* standards have been developed from epidemiological studies, i.e. from the statistical relationship between *E. coli* concentrations and frequencies of [gastrointestinal] sicknesses. No such studies were done for other applications.”[84]

Sadowsky has other ideas. He cites his own 2010 studies *Use of Barcoded Pyrosequencing and Shared OTUs To Determine Sources of Fecal Bacteria in Watersheds*. In this study rather than using DNA source tracking, the relative combination of microorganisms was used to identify the source of fecal contamination. Sadowsky theorizes that this method could be used in addition to *E. coli* testing as a routine measure to assure impairment values are linked to fecal contamination.[85]

Of course, an improved method of detecting contamination must not only account for accuracy but also practicality. Routine monitoring must be inexpensive and require minimal laboratory equipment and technique. Undoubtedly, sacrifices to accuracy will need to be made for any new monitoring efforts to be pragmatic. Whether there is an alternative to *E. coli* that optimizes accuracy and economics is a question that must be answered in order to effectively manage water quality.

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[84] Pachepsky.

[85] Sadowsky.

CONCLUSION

The research done at Plum Creek provides one example of the potential price of policies that do not account for recent scientific research. The 1.5-mile stretch of creek has cost thousands of dollars over the last 5 years, and it is increasingly likely that the contamination identified does not pose a human health risk. As the properties of *E. coli* were understood in the 1980's, it was a model indicator organism. However, for over a decade the evidence has proven some of the assumptions regarding *E. coli*'s ability to persist and multiply in the environment were inaccurate, and yet the relevant policies have not been reevaluated. Nearly 40 years later water quality is still governed by 1980's microbiology.

As the science of *E. coli* as an indicator organism remains in question, policies must allow for flexibility in the evaluation of impairment and the allotment of resources. While, identification of a reliable means of evaluating contamination is critical, it is important recognize the resources lost. Policies based on yesterday's research will result in yesterday's health outcomes and expenses. Today's science offers immense amounts of new information, which is ultimately futile if not implemented

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Appendix

Table 1. 2016 Plum Creek data set. Table by author.

Site	Date	pH	DO (mg/L)	Conductivity (mS/cm)	Turbidity (NTU)	Transparency Tube	Water *E. coli* log$_{10}$(cfu/100ml)			Sediment *E. coli* log$_{10}$(cfu/100g)		
1	21-Jun-16	8.45	10.16	0.42	35.50	63.80	0.85	0.78	0.95			
2		8.46	9.80	0.45	26.30	65.15	1.43	1.46	1.48			
3		8.94	10.33	0.44	24.90	83.35	1.65	1.66	1.57			
4		8.35	8.90	0.45	28.00	80.23	2.16	1.93	2.07			
5		8.02	7.26	0.39	27.20	73.90	1.72	1.73	1.79			
6		8.30	8.37	0.46	25.20	79.60	1.95	1.95	1.90			
7		8.32	8.51	0.46	23.40	98.84	1.72	1.79	1.91			
1	5-Jul-16	8.33	10.35	0.422	22.0	72.00	1.49	1.43	1.49	0.00	0.00	2.51
2		8.44	10.69	0.423	26.9	84.00	1.76	1.84	1.95	2.20	1.90	3.17
3		8.39	9.68	0.426	30.0	67.00	0.95	1.86	1.88	1.90	3.09	2.20
4		8.34	8.66	0.424	37.7	68.00	1.95	2.73	2.60	2.64	2.72	2.56
5		8.02	7.38	0.313	31.0	108.00	1.68	1.71	1.66	4.41	3.45	3.12
6		8.18	7.45	0.432	39.2	72.00	TNTC	2.62	2.62	2.20	2.45	1.90
7		8.10	6.97	0.432	37.3	91.00	2.49	2.62	2.65	2.20	2.45	2.56
1	19-Jul-16	8.38	11.70	0.401	20.3	64.60	1.41	1.52	1.32			
2		8.47	11.12	0.406	23.7	70.40	1.60	1.61	1.60			
3		8.42	10.14	0.362	22.7	70.8	1.78	1.86	1.82			
4		8.31	8.81	0.398	28.1	57.9	2.00	1.96	2.09			
5		8.01	7.27	0.524	34.4	105.4	1.57	1.46	1.66			
6		8.03	6.99	0.431	37.2	74.3	2.15	2.14	2.13			
7		8.01	6.86	0.431	37.7	78.1	2.14	2.10	2.11			

Site	Date	pH	DO (mg/L)	Conductivity (mS/cm)	Turbidity (NTU)	Transparency Tube	Water *E. coli* $\log_{10}$(cfu/100ml)			Sediment *E. coli* $\log_{10}$(cfu/100g)		
1	2-Aug-16	8.37	9.84	0.420	20.8	83	1.58	1.52	1.61	2.45	2.64	0.00
2		8.38	9.31	0.425	33.8	75	1.43	1.51	1.54	1.60	0.00	2.45
3		8.35	8.77	0.427	45.5	93	1.84	1.79	1.63	2.08	1.90	2.08
4		8.33	8.11	0.429	41.0	80	1.67	1.70	1.81	3.15	2.56	2.45
5		8.16	6.97	0.440	31.4	76	2.43	2.56	2.11	2.94	3.24	3.05
6		8.12	7.53	0.498	29.1	100	1.99	2.03	2.02	2.94	1.90	2.56
7		8.14	6.88	0.440	32.3	65.1	1.99	1.98	1.99	2.38	2.08	3.00
1	2-Sep-16	8.05	8	0.413	18.6	111.2	1.43	1.46	1.57			
2		8.05	8.54	0.414	24.3	105.1	1.00	1.41	1.53			
3		8.09	8.44	0.415	20.3	101.8	1.59	1.68	1.69			
4		8.02	10.85	0.417	26	103.9	1.88	1.77	1.87			
5		7.45	7.24	0.393	44.4	120+	1.23	1.52	1.43			
6		8.06	7.77	0.418	31.1	79.5	1.77	1.79	1.85			
7		7.93	7.7	0.418	40.5	87.2	1.65	1.82	1.87			
1	18-Sep-16	7.89	8.43	0.414	41.2	120+	0.90	1.04	1.26			
2		8.01	8.43	0.416	43	120+	1.30	1.15	1.62			
3		7.83	8.31	0.417	27	120+	1.18	1.26	1.52			
4		8.04	8.39	0.418	38.3	120+	1.43	1.41	1.64			
5		7.75	7.68	0.423	22.2	120+	1.23	1.69	1.87			
6		7.35	7.67	0.424	39.2	120+	1.91	TNTC	1.85			
7		7.47	7.56	0.424	39.8	122	1.79	1.69	1.83			
1	8-Oct-16	7.94	8.428	0.413	26.2		1.26	1.00	.95			
2		7.89	8.06	0.414	6.8		1.43	1.43	1.20			
3		7.87	6.4	0.414	8		1.45	1.45	1.45			
4		7.91	8.86	0.416	14.1		1.38	1.49	1.56			
5		7.91	8.93	0.419	28		1.80	1.65	1.36			
6		8.12	11.11	0.482	27	114.3	TNTC	1.82	1.81			
7		8.09	7.8	0.42	36	120+	1.68	1.80	1.71			

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