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AN ANALYSIS OF FECAL COLIFORM BACTERIA
AS A WATER QUALITY INDICATOR

by

Janet Heyl Vail

A Dissertation
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirements for the
Degree of Doctor of Philosophy
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Western Michigan University
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AN ANALYSIS OF FECAL COLIFORM BACTERIA AS A WATER QUALITY INDICATOR

Janet Heyl Vail, Ph.D.

Western Michigan University, 1998

The focus of this study is to assess the efficacy of fecal coliform bacteria as a microbiological water quality indicator. The scientific and educational context of fecal coliform bacteria is explored through analysis of large sets of water quality data, focused field monitoring projects, and review of commonly used resources for school-based and volunteer water-related studies. Analyzed data sets include long term sampling (10 years), multiple sites, daily samples, storm event samples, and other parameters monitored in parallel with bacteria. Special attention is given to data sets from Kent County, Michigan. A background reference site in Michigan unimpacted by humans was monitored for two years. Field comparisons of membrane filtration with other bacteriological methods for *Escherichia coli* were performed.

Results of exploratory data analysis of long-term monitoring data established station profiles (box plots) for expected fluctuations in levels of bacteria. Typically, stations on the main reaches of Michigan rivers exceed fecal coliform bacterial levels of 200 colonies per 100 mL between 30 and 60% of the time.

Tributary stations vary from 30 to 80% exceedance, and drains are in a range of 70 to 97% exceedance. The "background" site had significant levels of fecal coliform bacteria, *E. coli*, and total coliforms throughout the year that were not associated with humans. The direct inoculation of a water sample onto a plastic film with gel (Petrifilm™) had satisfactory performance and could be effectively used as an alternative method to membrane filtration for student monitoring.

Neither fecal coliform bacteria nor *E. coli* appears to meet all of the basic criteria for a credible water quality indicator, especially for Michigan. Additionally, misconceptions about indicator bacteria are commonly found in educational materials. Further analysis of the efficacy of federal and Michigan water quality standards for the protection of human health in recreational areas is needed. Collection of additional epidemiological evidence and consideration of site-specific standards that correlate with precipitation events should be part of this analysis. Extensive knowledge about the watershed is a key to interpretation of microbiological monitoring results as they relate to human health considerations.

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Janet Heyl Vail

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CHAPTER I

INTRODUCTION

Background

Stream and lake monitoring for educational, as well as regulatory, purposes sometimes involves assessing the microbial quality of water and the risk of possible transmission of waterborne infectious diseases. Pathogenic organisms are generally present in water in very low numbers, and analytical tests to detect them are expensive and difficult. Levels of fecal coliform bacteria in streams and lakes are often used as indicators of microbial water quality instead of specific monitoring for disease-causing organisms. The primary reference for stream monitoring programs in schools, the *Field Manual for Water Quality Monitoring* (Mitchell and Stapp, 1995), lists fecal coliform analysis as one of nine water quality tests whose results make up a water quality index.

The presence of fecal coliform bacteria in water has both public health and policy implications. Fecal coliform bacteria in water samples are regarded as an indicator of potential contamination by human waste. The presence of this waste increases the risk of finding human pathogens. However, fecal coliform bacteria are also naturally present in the intestines and feces of many warm-blooded animals

(Pipes, 1982). Also, Prescott et al. (1946) reported fifty years ago that it was difficult to find a river in inhabited regions that does not contain hundreds to thousands of bacteria per milliliter.

High levels of bacteria are a reason for closing areas to full body contact recreational activity. Prior to 1994, the Michigan Surface Water Quality Standards specified that all waters of the state shall contain not more than 200 fecal coliform colonies per 100 milliliters of water. The Michigan Department of Environmental Quality (MDEQ) can suspend this limit from November 1 through April 30 upon determining that the designated uses for the water body will be protected. In 1994, MDEQ changed the microbiological contaminant indicator group to *Escherichia coli*. *E. coli* is a common coliform organism associated with the human intestinal tract. The new levels are not more than 130 *E. coli* per 100 milliliters of water, as a 30 day geometric mean. Monitoring programs for water quality indicator bacteria are left largely to the discretion of local health departments and wastewater treatment plants, and they are also part of volunteer efforts by citizens or students.

A common study unit for environmental education, as well as science classes, is a stream or lake ecosystem. Typically, students form "stream teams" to monitor water quality and often take action to clean up their adopted section of a stream. This type of experience may be the only direct experience students have with environmental education and hands-on science in an outdoor setting. Environmental awareness, knowledge, values, attitudes, and skills are hallmarks of contemporary

environmental education that can be developed through monitoring studies (Environmental Education Citizens' Advisory Committee, 1992; Hungerford and Volk, 1990).

The goal of environmental education is *for* the environment, while science education is *about* the environment (Lucas, 1980). Environmental education has traditionally been based upon a behaviorist perspective with little attention to the constructivist interpretation of learning that is prevalent in science education (Robertson, 1994). This is not to say that environmental education lessons inherently lack a constructivist focus. On the contrary, they lend themselves well to active construction of knowledge through real life problems, student-centered instruction, productive group interaction, and authentic assessment of student progress (Klein and Merritt, 1994). In stream and lake monitoring, students assess the water quality of a stream (real problem), collect and analyze data themselves (student centered), share information with others (group interaction), and often compile an action plan to improve stream conditions based on their monitoring results (authentic assessment).

Students often sample streams or lakes only once or twice a year. A one time student monitoring event is unlikely to give a true picture of the bacterial dynamics of a stream or lake unless viewed in the context of a larger data set. In more sophisticated regional monitoring programs, students share their results by way of the Internet and at student congresses. Local health departments, wastewater treatment

plants, and water treatment facilities may have water quality long-term data available for student use that would be a valuable adjunct to single episode monitoring.

Stream monitoring can lend itself to ecological experimentation but it is rarely used in this manner. The monitoring results (observations) generally drive students to seek explanations for what is observed. There are likely to be alternative explanations for observed phenomena, and "weak inference" is often used to reach conclusions. Teachers need to be sensitive to the perils of merely gathering evidence to support a hypothesis in lieu of rigorous testing through controlled experiments. Statistics should not be used to "bolster the conclusion" if a valid experiment has not been performed (Hairston, 1989). Stream and lake monitoring data analysis should thus be viewed in the context of these concerns and analyses selected accordingly. Graphs and charts may be more effective than displays of numbers and elaborate statistics for development of concepts related to water quality.

Statement of the Problem

Large quantities of monitoring data on fecal coliform bacteria have been collected by stream teams, public health departments, lake watch groups, wastewater treatment plants, and other entities. Monitoring for *Escherichia coli* also adds to the accumulation of data. However, these data have seldom been analyzed and put into a meaningful context. There are four main issues relating to these data:

1. What framework needs to be in place for students to understand the context of fecal coliform and *Escherichia coli* data?
2. What is the real significance of finding fecal coliform bacteria or *Escherichia coli* in a given water sample?
3. How can monitoring data be translated into a usable format for understanding and decision-making?
4. Do current microbiological water quality standards have a rational basis and universal applicability?

In order for fecal coliform bacteria and *Escherichia coli* to be valid water quality indicators for human pathogens, there should be a strong correlation between densities of the bacteria in water and presence of human wastes. However, fecal coliform bacteria are found in both the feces of humans and other warm-blooded animals. There is evidence of additional sources of fecal coliform bacteria and *Escherichia coli* in the environment as well as possible reproduction outside the intestinal tract. Enteric microorganisms are known to grow and reproduce in freshwater sediments which could be significant bacterial habitats (Matson et al., 1978).

Questions have been raised about the use of fecal coliform bacteria as a water quality indicator in the tropics (Rivera et al., 1988). Fecal coliform bacteria, specifically *Escherichia coli*, were found to be part of the phyllosphere microflora and not merely transient in this environment. The degree to which the natural

environment in the middle latitudes may also harbor fecal coliform bacteria has not been clarified. There appears to be reason to question the utility of fecal coliform bacteria or *E. coli* as a universal indicator of water quality. The foundation of the standard needs to be critically examined. Additionally, the significance of an absolute count of 200 colonies per 100 milliliters needs to be more closely related to specific conditions in a watershed.

Without a proper context, single samples taken by students for the analysis of bacteria have questionable value. Considerations such as sterile technique, deviations from usual trends at a site, and uncertainty about sources of bacteria are important. Monthly and bi-weekly monitoring programs by county health or environmental health departments and by municipal wastewater treatment plants can provide a useful context for student data. These monitoring programs may be undertaken to alert the public to unsafe areas or as part of discharge permit requirements. On the whole, these data have not been analyzed to any great extent, but they could provide a database for developing models that relate watershed characteristics to fecal coliform levels and for comparison with student monitoring data that are collected on a less frequent basis.

Use of exploratory data analysis including graphical interpretations and concept maps could provide the missing element for what students can do with monitoring data. When the dynamics of bacterial contamination have been characterized, modeling of watersheds offers some assistance in identification of

sources of bacteria. Models are not only useful in helping to explain phenomenon, but their development by students has "profound" educational implications such as how distortions of models can lead to misconceptions and how models can be refined to increase accuracy (Stevens and Collins, 1980). Model revising problem solving, as found in Hafner's (1991) work, has become a fertile field of research in science education.

Based on a more comprehensive view of fecal coliform bacteria than is normally found in traditional stream team or lake monitoring teaching units, it is envisioned that this research would result in a structural model for bacterial contamination in water and a frame of reference for student monitoring results. Structural models are used to study qualitative relationships of a problem in a given context and they are not solely dependent on numerical data for input as is the case for dynamic models (Wallick, 1982). Another result of this research will be to critically assess current water quality standards as to their nation-wide applicability.

Research Strategy

An Analysis of Fecal Coliform Bacteria as a Water Quality Indicator is a blend of basic and applied research in science and in education. The "basic" aspect will fill in knowledge gaps about the dynamics of fecal coliform bacteria in aquatic systems. The "practical" aspect will draw conclusions about the efficacy of bacterial indicators of human contamination and help to enrich stream and lake monitoring

programs. The latter will be done through the development of a mechanism for teachers and students to synthesize information from their water monitoring data in a meaningful way through exploratory data analysis. A more holistic model of indicator bacteria will be developed through graphical analysis and concept maps.

The two primary issues for this science education dissertation are:

1. *Science* - Based on scientific evidence, is the 200 colonies per 100 mL fecal coliform standard reasonable as a nation-wide standard for surface water quality?
2. *Education* - What curriculum framework is necessary for students to understand the decision-making context of monitoring results for fecal coliform bacteria?

The specific research strategy included the following steps:

1. Prepare a literature review of fecal coliform bacteria in surface water to ascertain the reason for using fecal coliform bacteria as a water quality indicator, to identify parameters that influence fecal coliform levels in surface water, and to determine the educational context of student monitoring efforts.
2. Critically evaluate resources used by educators for water quality monitoring, and identify misconceptions (naive conceptions) about indicator bacteria.
3. Identify and obtain data sources for long term monitoring of fecal coliform bacteria as well as data from special studies for a defined geographic region.

4. Characterize the sampling sites using exploratory data analysis which involves statistical and graphical analyses to determine significant relationships between various parameters and fecal coliform levels.
5. Identify and monitor a site unimpacted by human activity to establish baseline bacterial counts.
6. Pilot test alternative monitoring methods for student use.
7. Provide a conceptual model and framework for the assessment of fecal coliform bacteria as a water quality indicator for student water quality studies, as well as for a regulatory basis.

Significance of the Study

Effective water quality monitoring is not simply data collection; it should also have a problem-solving component. To solve a problem, students need to know the limitations of the data they are collecting. The proposed research can help to clarify the dynamics of fecal coliform bacteria in a stream or lake ecosystem. Additionally, this work will help to go beyond mere data collection, to dispel student and teacher misconceptions about fecal coliform bacteria, and to foster the use of higher order thinking skills through conceptualizing a holistic model for a water quality parameter.

Additionally, in-depth information for assessing microbiological water quality will be available for regulatory agency personnel in their decision-making regarding enforcement and development of standards. It will be helpful in

evaluating water quality standards in the context of the basic criteria for a credible water quality indicator that adequately reflects risks to public health. According to

Feachem et al., 1983, p. 53, the indicator should be:

- (a) A normal member of the intestinal flora of healthy people,
- (b) Exclusively intestinal in habitat, and hence exclusively fecal in origin when found in the environment,
- (c) Absent from nonhuman animals,
- (d) Present whenever fecal pathogens are present, and present only when fecal pathogens might reasonably be expected to be present,
- (e) Present in higher numbers than fecal pathogens,
- (f) Unable to grow outside the intestine,
- (g) A die-off rate slightly less than that of fecal pathogens,
- (h) Resistant to natural antagonistic factors and to water and wastewater treatment process to the degree equal to or greater than that of fecal pathogens,
- (i) Easy to detect,
- (j) Nonpathogenic.

Most importantly, the indicator meeting these criteria should provide a realistic assessment of the health risk associated with a body of water. Background concentrations of this indicator in the natural environment should not be at levels that would obscure its utility as a water quality indicator.

CHAPTER II

REVIEW OF RELATED LITERATURE

Watershed Education

Education about water for K-12 students has evolved from a human-centered to an ecosystem-based approach. In the 1950s and 60s, water education programs focused on water as it relates to human uses such as drinking water, water conservation, and water resources. In the 1970s and 80s, acute and chronic water quality problems of concern included industrial discharges, heavy-sediment loads, discharges of raw sewage from combined sanitary and stormwater sewers, and dilapidated sewer systems. A holistic, basin-wide approach to education using the watershed concept has become the national paradigm for the 1990s (U.S. EPA, 1996).

Water in the context of the hydrologic cycle, with emphasis on the interconnection between surface water and ground water, is a theme for the hydrosphere strand of current science standards (American Association for the Advancement of Science, 1993; National Research Council, 1996). There has been increasing emphasis on nonpoint source pollution, wetlands, stormwater management, and the role of agriculture in stream pollution. From an

environmental education perspective, education about water has been recast as watershed education.

Watershed education has become a way that students can not only learn science by “doing science” but also help to improve conditions in a watershed. This improvement could be through direct action (stream restoration, pollution prevention) or indirect methods (bringing problems to the attention of the public resulting in changes in public perception/policy). This also ties into the concept of service learning that is gaining in popularity.

However, watershed education in the United States is fragmented. It varies from none at all in a given school to a year-long science curriculum based around field monitoring of streams, lakes, or a watershed. There are numerous water education efforts throughout the country that are beginning to produce substantial awareness of water issues among school-age children. Beiswenger et al. (1991) identified eight major water education programs in the United States: *Investigating Your Environment*, *Project Wild Aquatic*, *The WET Program* (currently Project WET), *Project Water*, *Water and Man*, *Minnesota Water Topic Areas*, *Water Education*, and *Water Precious Water*. There is empirical evidence that water education involves a body of knowledge that is not embedded in traditional education programs (Brody, 1995). Educators may frequently harbor naive conceptions (misconceptions) about water due to lack of formal training in water issues.

Water education is seen as interdisciplinary, integrated, relevant to actual problems, and reliant on a blend of concepts and skills with attention to the affective domain (Brody, 1995). These interdisciplinary school-based programs have contributed to community activities and public education, and these successes are being shared on the Internet and at National Conferences such as *Watershed '96: A National Conference on Watershed Management*, the *1996 National Volunteer Monitoring Conference*, the *1996 National Conference on Nonpoint Source Pollution Information/Education Programs*, and the *1997 Water Resources Education, Training, and Practice Conference*. Water Festivals for children and the public are becoming common throughout the country as well as interactive programs such as kiosks and exhibits (Murphy and Kirschner, 1996). Millions of dollars are being spent on watershed education by the U.S. Environmental protection Agency (EPA) as illustrated by the Rouge River Project in Michigan (Murphy and Kirschner, 1996).

At a 1997 symposium of the American Water Resources Association (Warwick, 1997), many speakers addressed the challenge of effectively communicating with the public about water quality issues. Incorporating water knowledge in elementary and secondary education can serve to also educate parents. The family unit seems to be the most influential force regarding sustained behavioral change (Groundwater Foundation, 1994).

Key elements for outdoor watershed education appear to be proximity of a place to go, adequate equipment, and age-appropriate and curriculum aligned learning activities. Providing an emotional connection to the environment prior to teaching scientific concepts as well as interactions with professionals in the water quality field are important elements of many of the watershed education programs. However, students will be more receptive to what they, themselves, can do about water quality issues rather than what others should do (Warwick, 1997). Watershed education is especially important because needed solutions to problems transcend the usual political jurisdictional boundaries. This education can support the call for intergovernmental cooperation to undertake needed actions regarding watershed management.

Perspective on Volunteer Monitoring

Watershed education programs often involve students in the monitoring of streams and lakes. Volunteer monitoring contributes to data gathering for a number of general categories: physical/chemical measurements in a water column, microbiological measurements, visual ecological surveys, fish and shellfish surveys, benthic macroinvertebrate surveys, and primary productivity studies (Ellett and Mayo, 1990).

Environmental monitoring by volunteers had early origins with training of National Weather Service volunteers to report daily measurements at weather

stations (Lee, 1994). Some of the earliest volunteer water monitoring programs were started by the Izaak Walton League in 1929. This evolved into the national Save Our Streams (SOS) program (Firehock, 1990; Izaak Walton League, 1994). Stream and lake monitoring became especially popular beginning in the late sixties and early seventies as the environmental movement began to take shape. This was the era of the formation of the Environmental Protection Agency and the passage of the Clean Water Act in 1972.

States such as Maine, Minnesota, Vermont, New Hampshire, New York, and Illinois have ongoing lake monitoring programs mostly based on Secchi disk readings (Lee, 1994). Michigan began its Self-Help for lakes program in 1974. The state does not use water quality monitoring data collected by volunteers except for this program (Goudy, 1994).

In the eighties, three important estuary monitoring programs - Rhode Island Salt Pond Watchers, the Chesapeake Bay Citizen Monitoring Program, and Maine's Clean Water Program - were founded. The importance of these programs is that bacterial monitoring was included in response to specific concerns (Lee, 1994). River Watch Network (RWN) was organized in 1987 as a multi-state effort to provide a network and technical advice for river projects. Ohio developed its biological Stream Quality Monitoring Program in 1983 (Kopec and Lewis, 1989).

School-based monitoring programs that began in the eighties include: Washington State's Adopt-a-Stream (1985), Colorado River Watch Network (1988),

and the Interactive Rouge River Water Quality Project (1986). Kentucky Water Watch expanded activities to include school groups. The Rogue River project is one of the earliest examples of an urban river project linking schools (Lee, 1994).

The theme of the nineties has been integration of monitoring of streams, lakes, estuaries, wetlands, and groundwater on a watershed basis (Lee, 1994). In 1996, the Environmental Protection Agency sent a clear message to the nation that watersheds are the unit of consideration for water quality issues (U.S. EPA, 1996). Programs like that of the Tennessee Valley Authority and the Chesapeake Bay Citizens Monitoring Program are holistic models that integrate monitoring of lakes, bays, rivers, and streams (Lee, 1994). The Connecticut River Watch Program coordinates *E. coli* monitoring of waterways (U.S. EPA, 1994). Even surfers such as those in the Surf Riders Foundation are doing bacterial monitoring, and they have home pages on the Internet.

One of the premier bacterial monitoring programs is through the University of Maine Cooperative Extension which set up coliform testing labs in high schools (Stancioff, 1991). Adults collect samples and students do the analyses. Split samples are run with the Department of Resources in Maine.

Some of the many volunteer monitoring programs in Michigan include the Burt Lake Watershed Water Quality Monitoring Program of the University of Michigan Biological Station, Friends of the Rouge, the Michigan Self-help Water Quality Monitoring Program, Mullet Lake Watershed Project, Saginaw Bay

National Watershed Initiative, and the Tip of the Mitt Watershed Council Volunteer Lakes Monitoring Program (Lee and Ely, 1990; Goudy, 1994). In Kent County, Michigan, student Stream Teams have been active since the late 1970s, and the West Michigan Environmental Action Council (WMEAC) has implemented its Adopt-a-Stream Project. This project involves citizen initiated clean up, restoration, and biological monitoring of about 350 miles of Kent County, Michigan, streams.

The Global Rivers Environmental Education Network (GREEN) offers an international model for water quality monitoring that specifically involves schools. GREEN evolved from the Rogue River Project and is associated with Dr. William Stapp of the University of Michigan School of Natural Resources. The 1996 GREEN Conference in Ann Arbor, Michigan, brought together hundreds of educators to learn and strategize about water quality monitoring.

Much of the organized school-based water quality monitoring draws on the GREEN model. For this model, students use Mitchell and Stapp's *Field Manual for Water Quality Monitoring* (1995) and a specially designed water quality index to gauge the health of their monitoring site. Their procedures are semi-standardized and they use test kits by the Hach and Millepore Companies.

A spin off of GREEN is computer access via the Internet for groups monitoring water quality. The GREEN home page has a communications web that allows students to easily connect with a their peers throughout the world. Another

Internet site of note is that the HiC computer group of the University of Michigan. This site has the *River Bank* program which can be used to easily calculate the Mitchell and Stapp (1995) water quality index.

The volunteer monitoring movement in the United States has received support from the United States Environmental Protection Agency (U.S. EPA) through its Office of Water. A 1994 national directory of volunteer monitoring by that office listed over 500 programs (U.S. EPA, 1994). *The Volunteer Monitor* newsletter is supported by U.S. EPA and there is Internet access to the Office of Water. Five National Citizens' Volunteer Monitoring Conferences have been organized since 1988.

States and U.S. EPA are becoming concerned with generation of quality data using accepted techniques and quality assurance protocols. A recent U.S. EPA publication, *The Volunteer Monitor's Guide to Quality Assurance Project Plans* (U.S. EPA, 1996d) guides groups in setting up their quality assurance programs.

Joyce Tugel, a high school chemistry teacher summed up the value of using volunteer monitoring data in the classroom as follows:

Many students enter the classroom unable to identify dependent variables, independent variables, or constants, and needing practice in constructing tables and graphs. Monitoring data sets provide a perfect tool for teaching these concepts in a meaningful way. What's more, working with monitoring data encourages students to learn the skills of true investigation.
(Tugel, 1995, p. 3)

Water Quality Indices

Students in monitoring programs such as the GREEN program not only collect and graph data but they also calculate a water quality index. This index can be used both spatially and temporally to assess the health of a system. The basic premise of an index is that it should allow the user to compare gradations in water quality at different locations, as well as at different points in time, at the same location. "Perfect" water quality is generally rated at 100 for most indices. Fecal coliform bacteria are sometimes a part of a given water quality index and their relative importance varies with the index.

Qualitative water quality indices are found in the literature as far back as 1848, but numerical indices appear to be a relatively new phenomenon beginning with Horton's 1965 index (Ott, 1978a). Landwehr's (1974) doctoral thesis was one of the first comprehensive review of indices. Indices in the literature fall into four categories: general water quality indices, specific-use indices, planning indices, and statistical approaches. Indices have been used for resource allocation, ranking of locations, enforcement of standards, trend analysis, public information, and scientific research (Ott, 1978a).

Development of a water quality index involves several steps which include selection of variables, establishment of a rating scale, and assignment of relative weights to the variables. Water quality indices differ greatly in the selection of

variables used. Commonly used chemical variables are dissolved oxygen, BOD, hardness, iron, nitrates, phosphates, chlorides, pH, and various organic chemicals. Physical parameters include temperature, turbidity, specific conductance, dissolved solids, and color. Biological variables are generally fecal and total coliforms. However, benthic macroinvertebrates, diatoms, and oligochaetes are also used for monitoring water quality as well as, especially when diversity indices are calculated (Weber, 1973; Goodnight, 1973). The mathematical structure of indices is generally based on nonlinear (implicit and explicit) subindex functions with a weighted linear sum aggregation function of differing scales and ranges (Ott, 1978a).

Examples of indices include those of Horton (1965), Dinius (1972), O'Connor (1972), Walski and Parker (1974), Nemerow and Sumitomo (1970), Harkins (1974), and Stoner (1978). Other indices are the public water supply index (Deininger and Landwehr, 1971), River Pollution Index (McDuffie and Haney, 1973), Pollution Potential Index (Zoeteman, 1973), Composite Pollution Index (Shoji et al, 1966), and the National Sanitation Foundation Water Quality Index (Brown et al., 1970; Ott, 1978a; Cogger et al., 1975). The NSF-WQI is the most used index.

The NSF WQI is the basis for the Mitchell and Stapp (1995) index used by many school monitoring programs. Results from nine water quality tests provide input for this WQI. These parameters include dissolved oxygen (DO), fecal

coliforms, total solids, turbidity, pH, biochemical oxygen demand (5-day), temperature change, total phosphate, and nitrate. A "Q-value" for each parameter is determined from extrapolation from curves on charts. Q-values are multiplied by a weighting factor for the relative importance of the test to overall water quality. The WQI ranges from 0 (worst) to 100 (best) with 90-100, excellent; 70-90, good; 50-70, medium; 25-50, bad; and 0-25, very bad. A minimum WQI of 50-58 could meet most state water quality standards (Mitchell and Stapp, 1995). Fecal coliform bacteria have a weight of 16% of the index.

The NSF WQI was modified for use in Michigan with the maximum attainable upper limit being slightly lower for the state than for other areas. The report states that it is "unlikely that Michigan waters in even the most remote and natural settings will reach 100 units on the water quality index scale" (MDNR, 1976a). The index was used in the 1976 report in a map showing WQI values throughout Michigan as well as in graphs of ranges of WQI values for various rivers and overall changes in water quality over time.

In summary, there are many iterations and/or variations of water quality indices that have been proposed since the early 1970s (Ott, 1978a). However, there is currently no national index such as that for air quality. The water quality index used in Mitchell and Stapp (1995) is likely to be the most familiar to K-12 students who are doing water monitoring since other indices are not found in readily

available K-12 curricular materials. It is based on an index by the National Sanitation Foundation (NSF) that has been used in state water quality reports.

Microbiological Water Quality Indicators

In accessing water quality, various combinations of chemical, physical, and biological parameters are used. Student monitoring programs sometimes involve microbiological monitoring. From a public health standpoint, water borne pathogens represent a significant risk and degrade the water. Of most concern are human pathogens such as those found in raw sewage. According to Heukelekian and Faust (1961, p. 938), important waterborne pathogens include: (a) cholera vibrio, (b) typhoid and paratyphoid bacteria (*Salmonella*), (c) dysentery bacteria (*Shigella*), (d) amoebic dysentery (*Entamoeba histolytica*), (e) tuberculosis bacteria (*Mycobacterium tuberculosis*), (f) viruses causing infectious hepatitis and poliomyelitis, and (g) parasitic worms. In more recent years, outbreaks of *Cryptosporidium* and *Giardia* have been of concern.

Low levels of pathogens and the large number of types of pathogenic bacteria and viruses in surface waters make it difficult to monitor for these organisms. Enumeration methods for many pathogens are either unavailable, expensive, imprecise, and/or time-consuming. Consequently, bacteriological water quality has generally been assessed by use of indicator organisms such as coliform

bacteria, fecal coliform bacteria, fecal streptococci, *Escherichia coli*, *Enterococcus*, and *Clostridium perfringens*.

Common Water Quality Indicators

The ideal water quality indicator needs to correlate with the health hazard of a pollution source as supported by epidemiological studies (see page 10 for the extensive set of criteria noted by Feachem et al., 1983). A briefer list of the most pertinent requirements for a water quality indicator was suggested by Cabelli (1977, p. 222):

1. The indicator should be consistently and exclusively associated with the source of the pathogens.
2. It must be present in sufficient numbers to provide an "accurate" density estimate whenever the level of each of the pathogens is such that the risk of illness is unacceptable.
3. It should approach the resistance to disinfectants and environmental stress, including toxic materials deposited therein, of the most resistant pathogen potentially present at significant levels in the source.
4. It should be quantifiable in recreational waters by reasonably facile and inexpensive methods and with considerable accuracy, precision, and specificity.

Some of the earliest work on water quality indicators was that of Escherich who identified *Bacillus coli* (renamed *Escherichia coli*) in stool samples in 1885 (Mack, 1977). The coliform group as an indicator of water quality was discussed as early as 1895 (Smith, 1895). Smith hypothesized that all coliform bacteria have their origin in the guts of warm-blooded animals and their presence elsewhere indicates fecal pollution.

Three principal genera make up the coliform group of organisms that are present in stool samples and water polluted by feces: *Escherichia*, *Aerobacter*, and *Klebsiella*. However, over a hundred different species of bacteria may be found in human stools (Farmer and Brenner, 1977). Primary genera and species in adult human feces are: *Bacteroides*, *Lactobacillus*, *Escherichia coli*, and *Enterococcus*. Secondary organisms include *Citrobacter-Levinea*, *Klebsiella*, *Enterobacter*, *Clostridium*, *Staphylococcus*, *Bacillus*, yeasts, and molds (Leclerc et al., 1977).

The fecal coliform test mainly selects for *E. coli* and *Klebsiella*. Fecal coliform bacteria in human feces are reported to be 90-95 percent *E. coli*, yet raw sewage may contain only 25 to 33 percent *E. coli* (Dufour, 1977). There are situations where positive tests for fecal coliform were not associated with human or warm-blooded animal fecal pollution. *Klebsiella* species are frequently reported in these samples (Dufour, 1977).

A controversy has brewed since the early 1900s as to the significance of fecal versus nonfecal coliforms and their relative danger to human health. Contamination of groundwater by enteric microorganisms is a common reason for restricting the supply of this resource for human consumption. Although the movement of enteric bacteria may be limited in fine-structured soils, significant migration can occur in coarse sandy soils, gravel and in fractured or karst terrain (Rusin et al., 1992). Hagedorn et al. (1978) found that fecal coliform bacteria can move long distances in a relatively short period of time, maximum populations of indicator bacteria are

associated with the rise of the water table following rain events, and *Escherichia coli* can survive in appreciable numbers in saturated soil.

The choice of a standard (*Escherichia coli* or fecal coliforms) varies throughout the world. Countries such as Denmark, Belgium, England, and France have used *E. coli* as a water quality indicator, whereas the United States and Canada have preferred fecal coliforms until recently when *E. coli* has been widely adopted. A defense of fecal coliform as an indicator for recreational water quality was given in a study of Buffalo Lake in Canada by Geldreich (1972b). However, there is no world-wide consensus on the best water quality indicator for recreational water use (Pike, 1993).

Tropical countries have a problem with *E. coli* as an indicator. *E. coli* and *K. pneumoniae* may exist in high densities in the absence of pathogens or fecal sources. These organisms seem to be naturally occurring in some tropical areas. The presence of both bacteria in pristine natural waters is indicative of their being autochthonous to tropical environments. Diffusion chamber studies with *E. coli* in Puerto Rico revealed that this bacterium can survive indefinitely in tropical freshwater (Hazen et al., 1987). In addition, many pathogens have been shown to have shorter survival rates than *E. coli*, their presumed indicator. Thus, the use of coliform and even fecal coliform bacteria as indicators of fecal pollution may be misleading when applied to countries with tropical climates (Hazen and Toranzos, 1990).

Other Water Quality Indicators

The search for adequate water quality indicator species is a continuing quest. Possible indicators fall into four groups: (1) fecal indicators, (2) enteric pathogens whose source is exclusively fecal, (3) human pathogens whose source is the aquatic environment where they can multiply, and (4) environmental parameters which might index the ability of these pathogens to multiply in water (Geldreich, 1979a, p. 14-5).

According to researchers, candidates for microbiological water quality indicators include (a) *Escherichia coli*, (b) total coliforms, (c) fecal coliforms, (d) fecal streptococci, (e) *Clostridium perfringens*, (f) *Aeromonas hydrophila*, (g) *Pseudomonas aeruginosa*, (h) acid-fast organisms, (i) yeasts, (j) lactobacilli, (k) *Bifobacterium bifudus*, (l) *Bacteroides*, (m) bacteriophages, (n) glutamic acid decarboxylase (GAD), (o) fecal sterols (coprostanol), and (p) optical brighteners (Geldreich, 1980; Geldreich 1979; Miescier and Cabelli, 1982; Borrego et al., 1983; Churchland et al., 1982; Thrailkill et al., 1985; Geldreich and Kenner, 1969; Wheeler et al., 1979; Kenner and Clark, 1974; Kreader, 1994; Wiggins, 1996; Borrego et al., 1983; Murtaugh and Bunch, 1967).

Specific recreational water quality indicators most frequently suggested are: enterococci, *Escherichia coli*, total coliforms, fecal coliforms, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, and *Clostridium perfringens* (Miescier and Cabelli, 1982). Marine studies have linked enterococci and *E. coli* densities with incidence of

swimming-associated gastroenteritis. Miescier and Cabelli (1982) suggest that *Enterococcus* bacteria are consistently more related to fecal wastes in waters downstream of sewer populations than any other of the bacterial indicators.

Fecal streptococci are sometimes monitored along with fecal coliform bacteria. Geldreich and Kenner (1969) found that fecal streptococcus counts were significantly higher in animal feces as compared with human feces. A ratio of fecal coliform to fecal streptococcus (FC/FS) was calculated, with 4.0 or higher being indicative of human waste, whereas a ratio of 0.7 or lower is thought to be associated with animals and stormwater runoff. Ratios between 0.7 and 4.0 are difficult to interpret. The FC/FS ratio is most reliable in locations near the point of discharge.

Coliphage detection, polymerase chain reaction, and gene probe techniques are other methods being considered for defining fecal contamination (Bej et al., 1991; Kreader, 1995; Wiggins, 1996). Some species of the bacterial genus *Bacteroides* may live only in the human intestine. *Bacteroides* DNA probes have been used to distinguish human from nonhuman feces (Kreader, 1994). *Bacteroides* probes appear to be more useful than fecal coliform tests to distinguish sewage from farm runoff but the current state of the technique makes it unrealistic for all but special studies. Discriminant analyses of antibiotic resistance patterns in fecal streptococci have also been used to differentiate human and animal sources of fecal pollution (Wiggins, 1996). Several days are needed for results of these profiles limiting usefulness to identification of chronic, not acute, fecal contamination.

Detection of Fecal Coliform Bacteria

The basic definition of coliform bacteria is that they include all aerobic and facultative anaerobic, gram-negative, non-spore forming rods that ferment lactose with gas production within 48 hr \pm 3 hr at 35°C (American Public Health Association, 1992). Fecal coliform bacteria are a sub-set of the coliform group.

In 1904, Eijkman suggested that fecal coliform bacteria can be characterized by gas production from glucose at 46°C (Sloat and Ziel, 1992). Coliform bacteria from the gut of warm-blooded animals produced gas from glucose at 46°C, while coliform strains from nonfecal origin failed to grow (Geldreich, 1965).

According to current definition, fecal coliforms are those bacteria that ferment lactose with gas production at 44.5 \pm 0.5°C which is a more inclusive category than use of strain specific *Escherichia coli* (Geldreich, Bordner, et al., 1962). The elevated temperature test (44.5°C) differentiates between fecal and non-fecal coliforms. It detects not only *E. coli* but also other coliform types from warm-blooded animals. The fecal coliform test mainly selects for *E. coli* and *Klebsiella*.

Estimation of numbers of fecal coliform bacteria was originally performed using multiple tube (Most Probable Number) procedures. Multiple tube fermentation involves inoculation of medium with known dilutions of the sample of water, and incubation for a prescribed period of time (44.5°C for 24 hr). The number of tubes where gas production is observed is reported, and a chart is used to transform number

of tubes to the Most Probable Number of bacteria in the sample. The whole procedure for coliforms takes three days.

In 1965, Geldreich et al. described a membrane filtration procedure for fecal coliform bacteria. The fecal coliform membrane filtration procedure considerably shortens the time for bacteriological analysis. The membrane filtration procedure (MF) is based on an actual count of colonies for a sample volume. In membrane filtration, a water sample is filtered through a 45 μm pore-size membrane filter. The filter is then transferred to a petri dish with an absorbent pad saturated with growth medium. The inoculated plate is incubated at $44.5 \pm 0.2^\circ\text{C}$ for 24 hr after which the number of blue colonies (colony forming units or CFU) are counted. There may be nonfecal coliform colonies on the plate that are cream or gray-colored. The procedure takes advantage of the production of acids by fecal coliform via lactose fermentation which will cause a color change in an indicator chemical in the mFC medium. The m-Coli Blue24™ membrane filtration broth give simultaneous results for total coliforms and *E. coli* in one petri dish.

Absolute numerical values from multiple tube fermentation and membrane filtration techniques are not likely to agree, yet there are often no statistically significant differences between values from the two methods (Middlebrooks et al., 1978). Advantages of the membrane filtration procedure over the multiple tube fermentation method are that a larger sample size can be used, precision can be improved by filtering larger samples, the technique is more reproducible, it takes less

time and materials, and costs are less. Disadvantages of the technique are that turbidity obscures results, noncoliform bacteria can interfere with colony growth, toxic metals can bind to the membranes to inhibit growth, and the initial capital outlay for equipment can be expensive (Geldreich, 1978; Middlebrooks et al, 1978). Membrane filtration was the method of choice for the laboratories that provided data for this dissertation.

Detection of *Escherichia coli*

Escherichia coli is a difficult species to define with its many variations with respect to biochemical reactions, antigens, antimicrobial susceptibilities, and other properties (Farmer and Brenner, 1977). The former Chief of the National Laboratory for Enteric Bacteriophage Typing of the U.S. Public Health Service cautions those who consider future microbiological standards for water to avoid definitions based on bacterial species and continue to use operational terms such as coliform group and fecal streptococci (Farmer and Brenner, 1977). This avoids taxonomic arguments while retaining precise meaning.

E. coli identification involves many tests such as the Gram stain, oxidase reduction, and the indole production, methyl red, Voges-Proskauer, and citrate utilization tests (APHA, 1989). This test series is known as IMViC. There are five categories of *E. coli* that are, themselves, associated with gastrointestinal illness. They are the enterohemorrhagic (EHEC), enteradherent (EAEC), enteropathogenic

(EPEC), enteroinvasive (EIEC), and enterotoxigenic (ETEC) groups (U.S. EPA, 1990). The EIEC are a cause of dysentery and EHEC include the 0157:H7 serotype that is frequently in the news as a contaminant in meat. *E. coli* serogroups have been used as a means of tracing fecal coliform and coliform pollution to determine the degree, location and variation of the load entering a lake from two small streams (Glantz and Jacks, 1968).

In a comparative study of feces from humans, cows, pigs, sheep, chicken, turkeys, and ducks, Geldreich, Bordner, et al. (1962) employed the indole, methyl red reaction, acetyl-methyl-carbonol (Voges-Proskauer), and growth in citrate (IMViC) tests in the characterization. The IMViC series takes as long as five days to run as opposed to elevated temperature fecal coliform detection methods that take 24 hr to complete. An organism of the coliform group with IMViC ++- would be *E. coli* which has positive indol and methyl red reactions but negative Voges-Proskauer and citrate reactions. Although human samples showed the greatest variety of coliform types, 87.2% of the almost 4,000 human strains, 95.6% of about 2,000 livestock strains, and 97.9% of almost 2,000 poultry strains tested were coliform type ++- (Geldreich, Bordner, et al., 1962). However, the IMViC method does not differentiate fecal coliforms of human origin from those of other warm-blooded animals (Geldreich, 1965; Silvey et al, 1974; Geldreich et al., 1968).

Newer tests for *E. coli* draw on the ability of most strains to produce *B*-glucuronidase which is an enzyme that hydrolyzes conjugates such as 4-methyl-

umbelliferyl-*B*-D-glucuronide (MUG). *E. coli*, *Shigella*, *Salmonella*, and some *Yersinia* produce this enzyme. A positive test for *E. coli* is indicated by fluorescence of the medium under long-wave ultraviolet light (Sloat and Ziel, 1992). Leclerc et al. (1977) explored an automated method for *E. coli* detection based on glutamic acid decarboxylase (GAD) biosynthesized by *E. coli*.

Coliscan™, mTEC agar, m-Coli Blue, and Petrifilm™ plates are also used for *E. coli* enumeration. However, at present, there is not a single standard method used for *E. coli* detection in surface and effluent waters, although a U.S. EPA document on test methods uses mTEC (U.S. EPA, 1985). Gaudet et al. (1996) evaluated the following test media for *E. coli* monitoring: mTEC, mTEC with 4-methyl-umbelliferyl-*B*-D-glucuronide (MUG), lauryl tryptose agar (LTA) with MUG, LTA with 5-bromo-4-chloro-3-indolyl-*B*-D-glucuronide (X-GLUC), EC medium with MUG, and lauryl tryptose broth with MUG. Best *E. coli* recoveries were from LTA-MUG and LTA-X-GLUC. These methods have been applied successfully to determination of the impact of sewage on coastal waters (Fiksdal et al., 1994). Another medium commonly used is the m-Coli Blue™ broth which is a dual test for *E. coli* and total coliforms (Grant, 1997).

Epidemiology

As previously mentioned, a valid water quality indicator will be one that correlates with incidence of disease. Specifically, water quality criteria have been

developed based on the strength of the relationship between an estimate of bacterial indicator counts and gastrointestinal illness rates, as measured with the Pearson-Product Correlation Coefficient (U.S. Environmental Protection Agency, 1986).

Recreational water quality criteria are defined as a quantifiable relationship between the density of an indicator in the water and the potential human health risks involved in the water's recreational use (Cabelli, 1977).

The following relationships are implied when relating fecal material in water to incidence of waterborne disease in a user population:

1. The more fecal material in the water the higher the densities of fecal indicator bacteria in the water.
2. There is a more or less stable ratio of the density of a particular fecal indicator to the density of a particular pathogen in sewage from a large population which is a function of the incidence of the disease to the contributing population.
3. The greater the density of the pathogens in the water being used the greater the incidence in the user population.

(Drexel University, 1978. p. 2.)

It is important to know the base level of illness in a community to determine if there is an increase with a particular water use. Illnesses can be grouped into eye, ear, nose and throat; gastrointestinal; and skin irritation. The degree of infection is dependent upon the number of infectious organisms, their virulence, and immune status of the potential host (Edberg, 1996). Beachwater pollution can threaten the public's health. Pathogens in sewage-contaminated waters can cause a wide range of diseases, including gastroenteritis, dysentery, hepatitis, ear, nose, and throat problems, and respiratory illness. The consequences of these swimming-associated

illnesses can be greater for children, elderly people, and those with weakened immune systems. However, mere contact with a pathogen does not automatically result in infection, nor is disease likely to be induced by a single bacterium or virus.

Surveillance of infectious diseases in the United States is heavily dependent upon voluntary collaboration between the Federal Centers for Disease Control (CDC) and state and local health departments, which in turn depend on physician-initiated reporting of a limited number of specific, recognized infectious diseases. Reporting is generally incomplete and most outbreaks are associated with drinking water. Coliform monitoring is routine for drinking water supplies, but there is evidence that this monitoring is not effective in actually preventing waterborne disease outbreaks (Batik et al., 1983).

Examples of two recent outbreaks of disease are the *E. coli* O157:H7 incident in the Pacific Northwest and the Milwaukee *Cryptosporidium* problem. Early in 1993, hamburgers contaminated with the bacterial pathogen *Escherichia coli* O157:H7 and served at a fast-food restaurant chain caused a multi-state outbreak of hemorrhagic colitis (bloody diarrhea) and serious kidney disease. This resulted in the deaths of at least four children. Dairy cattle and their feces appear to be a principal reservoir of *E. coli* O157:H7 (Wang et al., 1996). In the spring of 1993, contamination of a municipal water supply with the intestinal parasite *Cryptosporidium* caused the largest recognized outbreak of waterborne illness in the

history of the United States. An estimated 403,000 persons in Milwaukee, Wisconsin, had prolonged diarrhea, and approximately 4,400 persons required hospitalization (Centers for Disease Control (CDC), 1994).

Lack of surveillance and limited availability of appropriate diagnostic tests have interfered with public health efforts to prevent and control outbreaks. Both *E. coli* O157:H7 and *Cryptosporidium* were first recognized as significant human pathogens in the early 1980s, but neither had received adequate public health attention (CDC, 1994).

Specific studies on linkage of water quality indicators to disease in fresh water are limited. In the 1940s and 1950s, the United States Health Service conducted studies at bathing beaches in Chicago, Illinois (Lake Michigan); Dayton, Kentucky (the Ohio River); and Long Island Sound. Swimming activity and illness rates (gastrointestinal, respiratory, and other symptoms such as skin irritation) were correlated for a control and impacted beaches. There were no excess illnesses for swimmers at beaches with median coliform densities of 91 colonies per 100 mL and 180 colonies per 100 mL over the swimming season, yet there were significantly greater illness rates when mean coliform densities were 2,300 colonies per 100 mL. (U.S. Environmental Protection Agency, 1986).

A seminal study by the United States Environmental Protection Agency (EPA) in 1973-1974 evaluated potential water quality indicators for marine bathing beaches (Cabelli, 1977). Approximately 9,300 individuals were involved in the

studies. The highest correlation with gastrointestinal symptoms were for *E. coli* ($r = .95$) and enterococci ($r = .95$). The enterococci include *Streptococcus faecalis* and *S. faecium*. Correlation coefficients for fecal coliforms ($r = .08$) and total coliforms ($r = .33$) was symptoms were low (Cabelli, 1977).

In the U.S. EPA studies of marine and freshwater bathing beaches conducted in 1973 through 1982, regression analysis was used to define quantitative relationships between swimming-associated health effects and bacterial indicator densities. The EPA subsequently revised the bacteriological ambient water-quality criteria for marine and fresh waters with a recommendation to replace fecal coliform and total coliform standards for state water quality criteria with *E. coli* and enterococci (U.S. Environmental Protection Agency, 1986).

A prospective cohort epidemiological-microbiological study was carried out at 10 beaches in Ontario, Canada (Palmer and Dewey, 1987). Water and sediment samples collected at the beaches were analyzed for fecal coliforms, fecal streptococci, heterotrophic bacteria, *Pseudomonas aeruginosa*, and total staphylococci. Mean fecal coliform levels in the surface water of the lakes were within accepted guidelines. Morbidity among swimmers was shown to be related to staphylococcal counts, to fecal coliform levels, and, somewhat less strongly, to fecal streptococcal counts.

A U.S. EPA document (Dufour, 1984) on health effects criteria for freshwater recreational areas stated that *E. coli* densities show a direct relationship

to swimming associated gastrointestinal illness. Enterococci also showed good correlation with swimming-associated illness. This document provides a basis for the U.S. EPA perspective on health effects.

Numerical Water Quality Standards

Development of water quality standards is based on the command and control philosophy. The early thoughts on water quality standards in the 1970s were somewhat reactionary where "contaminant" and "pollutant" were synonymous and any contamination was too much. This idea has recently gained favor with zero discharge concepts related to Lake Superior. Most standards are set with reference to best available control technology (BAT). Epidemiological evidence is rarely used to relate levels of risk from exposure to a pollutant to incidence of health effects. Risk-based standards, however, are rapidly gaining favor, particularly as they relate to groundwater remediation.

A historical survey of how health departments select standards indicated that although most agencies had no analytical data for their limits, epidemiological experience under their standards has been good (Garber, 1956). Standards varied from zero coliforms to limits greater than 2,400 colonies per 100 mL. By 1979, the use of fecal coliform bacteria as a water quality indicator had been adopted by most states (Cabelli et al., 1983). The current trend is towards substituting the more specific *E. coli* for fecal coliform bacteria.

Unlike the Pollution Standards Index (PSI) for air pollution and National Ambient Air Quality Standards (NAAQS), there is currently no uniform water quality index or standards for all waters of the United States. Each state sets water quality standards based on use classifications of the water and water quality criteria. Standards are established by a governmental authority which has responsibility for prevention of water pollution and abatement. Water quality criteria are the scientific evidence on which a decision is made as to suitability of water to support a designated use (National Technical Advisory Committee, 1968). Ideally, these criteria should be developed in the context of risk analysis and epidemiological evidence. In other words, once an acceptable rate of swimming associated illness/indicator amount is accepted, then the criterion (relationship) is used to derive a guideline (upper limit for an indicator) that can be enacted into a standard (Cabelli et al., 1983).

Federal water quality criteria are not enforceable standards. There are, however, nationwide effluent standards set on an industry-by-industry basis and standards for publicly owned wastewater treatment plants. Geographic variations, different uses of water, and the large number of variables affecting water quality make it difficult to settle on a single nationwide water quality index.

Other standards for recreational water quality have been developed by organizations such as the World Health Organization (Suess, 1977). European total body contact (bathing water) standards for *E. coli* parallel WHO guidelines (Pike,

1993). Canadian Water Quality Guidelines (CCREM, 1987; Health and Welfare Canada, 1992) also involve *E. coli*. The California Ocean Plan (California State Water Resources Control Board, 1990) sets water contact standards in ocean waters for total coliforms and fecal coliforms.

Guidelines and standards for recreational water quality have commonly been expressed as (a) a medium or \log_{10} mean and (b) a second value that cannot be exceeded more than 10 percent of the time (Cabelli, 1977). These are to be regarded as separate and distinct guidelines, each with its own level of acceptable risk. An example is found in the 1968 National Technical Advisory Committee (NTAC) guidelines for direct contact with recreational waters (NTAC, 1968). The NTAC coliform water quality index was developed by translating the early U.S. Public Health Service studies into a fecal coliform index based on the ratio of fecal coliforms to coliforms at the 1949 Ohio River site. About 18% of the coliforms were fecal coliforms and this was related to the 2,300 coliform colonies per 100 mL density associated with gastrointestinal illness. Thus the fecal coliform count would be 400 colonies per 100 mL.

Since detectable risk is not desirable, the NTAC recommended half the density where a health risk occurred which would be 200 fecal coliforms per 100 mL (U.S. Environmental Protection Agency, 1986). According to the NTAC (1968, p. 12):

The studies at the Great Lakes (Michigan) and the Inland River (Ohio) showed an epidemiologically detectable health effect of 2,300 to 2,400 coliforms per

100 mL. Later work on the stretch of the Ohio River...indicated that the fecal coliforms represented 18 percent of total coliforms. This would indicate detectable health effects may occur at a fecal coliform level of about 400 per 100 mL; a factor of safety would indicate that the water quality should be better than that which would cause a health effect.

Based on not less than five samples taken over not more than a 30-day period, the NTAC guidelines recommend that fecal coliform levels not exceed 200 per 100 mL, nor should more than 10 percent of the total samples during any 30-day period exceed 400 per 100 mL (National Technical Advisory Committee, 1968). The second guideline is especially problematic in areas with frequent rainfall resulting in combined sewer overflows.

U.S. EPA Standards

The United States Environmental Protection Agency (U.S. EPA) used the NTAC criteria in establishing bacteriological standards under the Clean Water Act (U.S. EPA, 1976). Surface water quality standards in the United States are regulated by the Clean Water Act (Title 33 *United States Code* Part 1251 et seq.) which has a goal of making all surface waters in the United States fishable and swimmable. Criteria for water quality are dependent upon the use of the water (e.g., primary water contact, support for fish and wildlife, public water supply, agricultural and industrial use). These criteria are found in Title 40 of the *Code of Federal Regulations* Part 125.

States are required to set water quality standards which need to be approved by the United States Environmental Protection Agency. States are to enforce these standards with respect to the intended use of the water and to review standards every three years (40 *CFR* 131). There is great inconsistency among state standards and there is no federal requirement for states to monitor recreational areas or notify the public when water quality standards are being violated (NRDC, 1997).

As mentioned, the fecal coliform standard was originally adopted by most states but this is changing (Cabelli et al., 1983). In *Ambient Water Quality for Bacteria - 1986* (U.S. EPA, 1986), the U.S. EPA changed its opinion on bacteriological water quality standards. Rather than fecal coliform bacteria, U.S. EPA recommended that states begin a transition process to *E. coli* or enterococci for fresh waters and enterococci, only, for marine waters. The specific recommended criteria for recreational waters for bathing full body contact are:

Based on a statistically sufficient number of samples (generally not less than 5 samples equally spaced over a 30-day period), the geometric mean of the indicated bacterial densities should not exceed one or the other of the following:

<i>E. coli</i>	126 per 100 mL; or
enterococci	33 per 100 mL;

no sample should exceed a one sided confidence limit (C.L.) calculated using the following as guidance:

designated bathing beach	75% C.L.
moderate use for bathing	82% C.L.
light use for bathing	90% C.L.
infrequent use for bathing	95% C.L.

based on a site-specific log standard deviation, or if site data are insufficient to establish a log standard deviation, then using 0.4 as the log standard deviation for both indicators.

(U.S. EPA, 1986, p. 16)

Confidence levels relate to the chance that a beach will be left open when the protection is adequate. The smaller the confidence level, the more stringent (i.e. lower) the single sample maximum. The maximum acceptable bacterial density for a single sample is higher than the geometric mean to avoid unnecessary beach closures based on single samples (U.S. EPA, 1986).

Only one indicator (*E. coli* or enterococci) should be used. The state regulatory agencies can determine which is the appropriate indicator for its conditions. Sampling frequency should be related to the intensity of water body use with weekly samples for heavy-use areas and bi-weekly or monthly samples in less used areas. There can be great variation and day-to-day fluctuations in bacterial counts. Samples need to be collected in steady state conditions (dry weather) versus wet weather conditions where counts will be naturally elevated. In terms of noncompliance issues, a site may be in compliance for a long-term geometric mean but single samples may exceed a maximum, or all readings may be below a maximum but they are greater than the geometric mean (U.S. EPA, 1986). For the Great Lakes, EPA recommends a geometric mean of 33 *Enterococcus* bacteria per 100 mL or 126 *E. coli* bacteria per 100 mL of water. Water just meeting these

standards could cause an estimated eight illnesses per 1,000 swimmers (NRDC, 1997).

The Natural Resources Defense Council (1997) is calling for a national program to require states to: (a) adopt the EPA's recommended criteria, (b) to regularly monitor beachwater quality, and (c) to notify the public when water quality standards are violated. This could be part of the upcoming reauthorization of the Clean Water Act.

Michigan Standards

Michigan's water pollution control legislation is found in Act 451 (formerly Part 4. Water Quality Standards, Public Act 245 of 1929, as amended in 1990). Some early standards for coliform were: 100-500 coliforms per 100 mL, interpreted as indicating a bathing water free from detrimental pollution (counts might be attributed to land wash); 1,000 per 100 mL, suspicious (but dangerous in proximity to fresh sewage pollution); and 10,000 per 100 mL, a menace to health (Michigan Stream Control Commission, 1933).

Currently, all waters of Michigan are designated for total body contact recreation from May 1 to October 31 (Goudy, 1994). Prior to 1994, fecal coliform bacteria were the basis of water quality standards. Fecal coliform levels were not to exceed 200 fecal coliform per 100 mL, but this limit could be suspended from November 1 through April 30 if designated uses were protected. Also, this

concentration could be exceeded if uncontrollable nonpoint pollution sources were the cause. Compliance with the fecal coliform standards was determined on the basis of the geometric average of any series of five or more consecutive samples taken over not more than a 30-day period (MDNR, 1992).

New Michigan rules adopted in April 1994 have changed the bacteriological standards. Currently, waters of the state protected for total body contact recreation should not contain more than 130 *Escherichia coli* per 100 milliliters, as a 30-day geometric mean. Total body contact recreation is defined as activities that normally involve direct contact with water to the point of complete submergence, particularly immersion of the head, with considerable risk of ingesting water. At no time should these waters contain more than a maximum of 300 *E. coli* per 100 milliliters. Compliance with the standard is based on the geometric mean of all individual samples taken during five or more sampling events representatively spread over a 30-day period. Each sampling event consists of three or more samples taken at representative locations within a defined sampling area. This means that the total number of samples per month under the new standards has increased from five samples to fifteen samples.

The new rules also have provision for "partial body contact" activities that do not normally involve immersion of the head or ingesting water. These activities include wading, fishing, dry boating, hunting or similar recreational pursuits. The standards for partial body contact are a maximum of 1,000 *E. coli* per 100

milliliters. Compliance is based on the geometric mean of three or more samples, taken during the same sampling event, at representative locations within a defined sampling area. This means potentially a dual classification of streams and adjustment of health warning signs to reflect partial or total body contact.

Water quality standards for human sewage discharges continue to employ fecal coliform bacteria. These discharges should not contain more than 200 fecal coliform bacteria per 100 milliliters, based on a geometric mean of all of five or more samples taken over a 30-day period, nor more than 400 fecal coliform bacteria per 100 milliliters, based on the geometric mean of all of three or more samples taken during any period of discharge not to exceed seven days. This subrule may be suspended from November 1 to April 30, but the previous subrule must still be met.

There is no state monitoring system for bacteria and only a few Michigan counties such as Kent County have year around monitoring programs. Of the 41 Michigan counties that border the Great Lakes, only ten actively monitor beaches and three have limited programs (NRDC, 1997). In 1997, the U.S. Environmental Protection Agency indicated a willingness to address the lack of beach monitoring throughout the nation through a series of new initiatives that could lead to enhanced monitoring. The U.S. EPA will be requesting additional information from the states through an annual beach survey. As indicated in the 5 January 1998 *Federal Register* p. 244, a pilot survey of the Great Lakes will happen in 1998.

Limitations of Water Quality Criteria

There has been some criticism of the early epidemiological studies that have been used to establish water quality criteria (Cabelli, 1977; Committee on Water Quality Criteria, 1972). Specifically, there was concern about the quality of the database, lack of control groups, and paucity of information. Associations of illness and fecal coliform counts were not as strong as for other indicators. Pike (1993) concluded that there is a problem in setting standards for water quality for recreational use throughout the world due to the difficulty of defining acceptable risk of exposure to pathogens.

Environmental factors play a role in the interpretation of water quality measurements. For instance, bathers can be pollution sources and wild or domestic animals contribute to the bacterial load (Cabelli, 1977). Small point sources of pathogens from infected individuals can contribute to illness with no real elevation of fecal coliform levels. The pathogen-indicator ratio that is present in large discharges of raw sewage will show much more fluctuation in small populations or epidemic conditions (U.S. EPA, 1986). The U.S. EPA recommends both sanitary and epidemiological studies to determine sources of the indicator bacteria as they relate to human health risks (U.S. EPA, 1990). Not all fecal coliform bacteria pose the same health risk, nor are they necessarily indicative of fecal pollution (Dufour and Cabelli, 1976; Huntley et al., 1976; Caplenas and Kanarek, 1984).

Some authors challenge the methodology for enumeration of bacteria.

Santiago-Mercado and Jazem (1987) tested four membrane filter methods. The enumeration of fecal coliforms were compared for accuracy, specificity, and recovery. Differences of one to three orders of magnitude in the levels of fecal coliforms were observed in some samples by different recovery techniques. All of the methods tested were unacceptable for the enumeration of fecal coliforms in tropical fresh and marine waters. Since *Escherichia coli* appears to be a normal inhabitant of tropical waters, fecal contamination may be indicated when none is present.

Habitat of Fecal Coliform Bacteria

Microbiological water quality standards are set by looking at the risk of human disease. Yet, the normal habitat of fecal coliform bacteria is the intestinal tracts of both humans and warm-blooded animals. Additionally, there is evidence that coliform bacteria can survive and multiply in the natural environment. This makes interpretation of their origin in a water sample difficult.

There are many approaches in the literature applicable to conceptualizing bacterial populations in the environment. Mackay (1991) proposes a simple four compartment system for modeling and a more complex eight compartment system to represent the real environment. This first system is: air, soil, water, and sediment. The second, more realistic system includes: air, particulate matter or aerosols, water,

aquatic biota, sediment, suspended sediment, soil, and terrestrial biota. These phases may be well mixed (homogeneous) or poorly mixed (heterogeneous). Even within an area, there may be internal variations such as the main channel of a river being vertically mixed but not horizontally mixed. Conditions at a site at a given time may represent a steady state or a varying environment.

Air, soil, water, and sediment all factor into bacterial dynamics. Bacterial aerosols can transport coliform bacteria (Sorber et al., 1976; Adams et al., 1978; and Hickey and Reist (1975a, b). Airborne microorganisms can survive and be carried long distances by wind currents. In studies of wastewater spray irrigation, there is little correlation between the generation or transport of microbiological aerosols and traditional wastewater monitoring methods (Johnson et. al, 1980). Evidence is lacking, however, as to health risks associated with these aerosols but wastewater disinfection is recommended before spraying.

Soil can harbor fecal coliform bacteria (Presnell and Miescier, 1971; VanDonsel et al., 1967). Data from 26 states and 3 foreign countries indicated that fecal coliforms are usually absent, or present in comparatively small numbers (less than two fecal coliforms/gram), in unpolluted soils but there is a marked increase in polluted soils (Geldreich, Huff, et al., 1962). Soil appears to play a major role in bacterial contamination (Geldreich et al., 1968). Survival of indicator bacteria in soil is tempered by: (a) presence of competing or antagonistic soil organisms, (b) temperature, (c) frequency of rainfall, (d) soil moisture, (e) soil pH, (f) organic

matter, (g) frequency of recontamination of a soil site, and (h) sunlight exposure (Geldreich et al.; 1968 Bell and Bole, 1976; Geldreich, 1972a). Soil is the most likely source of the high concentrations of indicator bacteria that are naturally present in freshwater streams in Hawaii (Hardina and Fujioka, 1991).

McDonald et al. (1982) confirm that river banks and channels may act as major sinks for bacteria. Results of English stream studies suggest, but do not prove, that there is a direct causal link between stream water fecal coliform levels and the magnitude of bacterial input at the river bank (Hunter et al., 1992).

Goyal et al. (1977) reported that bottom sediments in a shallow canal system act as reservoirs for enteric bacteria. These bacteria can become resuspended in response to various environmental factors as well as recreational activity. Heavy rainfall brought about large increases in bacteria in both the sediments and water.

According to Goyal et al. (1979), four factors might contribute to high concentrations of enteric bacteria in sediments: (1) decreased die-off in areas when sunlight does not penetrate, (2) more organic nutrients in sediments than in water columns, (3) settling of bacteria from the water column, and (4) adsorption of bacteria to silt and other solids. Bacteria in sediments include those firmly bound to particles, bound in extensive flocs, and aggregates of the original fecal matter (Schillinger and Gannon, 1982).

In a study of mountain streams in North Carolina, McSwain (1977) postulated that seasonal cycles of enteric bacteria were caused by multiplication in sediments in

response to increased stream temperatures. Japanese studies also indicated correlation of temperature and fecal coliform counts (Hirotsu et al., 1992). Elevation of fecal coliform counts during storm events appeared to be more related to bottom sediment disturbances than to streambank flushing.

The role of sediments in harboring enteric bacteria has been confirmed by numerous other research studies such as those of Struck, 1988; VanDonsel and Geldreich, 1971; Hendricks, 1971; Horak, 1974; LaLiberte and Grimes, 1982; and Matson et al., 1978. Sediment provides a favorable, nonstarvation environment for fecal coliform bacteria, and in the absence of predators, fecal coliform bacteria may be capable of growth in sediments (Davies et al., 1995).

Enumeration of bacteria in bottom sediment appears to be important in recreation water quality analyses. For instance, at Ontario beaches, bacterial densities were found to be approximately ten times higher in the sediment than in the corresponding surface water samples (Seyfried et al., 1985). Surface water samples alone do not appear to give a true indication of the potential bacterial quality of an aquatic ecosystem used for recreational purposes.

Sources of Fecal Coliform Bacteria

The simplest model of contamination of water by fecal coliform bacteria is that human waste represents the source of the bacteria and the main transportation routes include combined sewer overflows, unchlorinated wastewater effluent, and

faulty septic systems. This narrow view does not take into account animal sources of fecal coliform bacteria and the whole issue of nonpoint sources.

Land use influences bacterial contamination and agricultural contributions are very significant. In relation to land use, Faust and Goff (1977) determined the following percentages of fecal coliform inputs for the Rhode River rural watershed: pasture 68%, forest 17%, and cultivated land 15%. High levels of fecal coliform bacteria are also measured in stormwater originating in agricultural areas and forested watersheds. Levels of fecal coliform bacteria in excess of accepted standards were commonly found which substantiates the need for a reappraisal of this test as an indicator of pathogenic organisms. These data also point to the importance of recognizing the streamflow regime in the analysis of water quality sampling data since observations taken during storm periods usually indicate high bacteriological loads.

Over one third of the land area of the continental United States is used for grazing livestock and this land receives 50% of all livestock wastes (Doran and Linn, 1979). The occurrence of fecal coliforms was found to be related directly to the presence of cattle on summer range and winter pastures (Stephenson and Street, 1978). Fecal coliform bacterial counts in streams adjacent to pastures were found to increase shortly after cattle were turned in and remained high for several months after cattle were removed (Stephenson and Street, 1978). Hunter and McDonald (1991) found that enteric bacteria can survive for a sufficient period outside parent

fecal material to provide a semi-permanent land store that might then be capable of contaminating upland waters following transport by hydrological processes.

Three critical factors that are involved in the rate of discharge and concentration of fecal bacteria in land drainage have been identified: (1) rate of drainage discharge, (2) number of bacteria in or on the soil and vegetation, and (3) application to the land of large volumes of semiliquid animal excrement over short periods of time (Evans et al., 1968). Most total coliform bacteria are native soil organisms, whereas the fecal coliform and fecal streptococci bacteria originate from the feces of wild and domestic animals.

Stormwater bacteriological water quality sampling indicates that high fecal coliform loads are common in stormwater runoff (Buckinham and Betson, 1970). According to Mallard (1980), all storm runoff contains a variety of bacteria, including total coliform, fecal coliform, and fecal streptococci, which are derived from the land over which the water flows. In "clean" residential areas, sustained high fecal coliform loads were observed during stormwater runoff (Buckinham and Betson, 1970). The source of these loads was associated with overland flow, and it appeared that staggered contributing times accounted for the sustained high loads. However, runoff from snowmelt does not necessarily have high fecal coliform counts even when contaminated with human waste (Elles, 1997).

Birds can also contribute to the bacterial load of a water body. Duck feces contain large amounts of coliforms, pathogenic enteric bacteria and unstable organic

matter (Gates, 1963). Palmer (1983) found that statistical testing of river cross-section geometric means showed wild birds had a significant effect on river fecal coliform levels. Interestingly, a study of a migratory wildfowl refuge revealed that the presence of large numbers of birds did not affect the concentrations of fecal coliform bacteria (averages of 3,200 - 64,000 per 100 mL) and the bird habitat pond had a decreased number of bacteria during and following its period of maximum use (Briweley et al., 1975). However, the problem with the study area was that it received a high sediment and bacterial load from upstream that could have obscured the impact of the birds.

Bacterial contamination of the swimming beach at Marina del Rey, California, caused closing of the area to swimmers. Weekly water sampling revealed unacceptable levels of total and fecal coliform and enterococci bacteria. An environmental audit of potential sources of the high bacterial levels identified shorebird defecation as the most probable cause. Following the installation of overhead monofilament fishing lines, shorebird populations generally abandoned the site, bacterial counts declined significantly, and the beach was reopened (Smith and Charness, 1990).

Fecal coliform bacteria can occur in great numbers in fish, especially those living in either a polluted stream environment or bottom feeders and scavengers in relatively clean streams (Geldreich, 1965). Water quality and feeding habits were shown to be the source of coliforms in the intestinal tracts of fish. There does not,

however, seem to be a permanent coliform flora in the intestinal tract of fish (Geldreich and Clarke, 1966).

During periods of rainfall, coliform bacteria associated with vegetation can impact streams (Geldreich, 1965; Mundt, 1963). Bacteria on plants could be as a result of insect contact. Geldreich et al. (1964) found that typical coliforms of the type found in the human intestine contributed a relatively small percentage of the organisms associated with vegetation (14.1%) and insects (14.9%). This would indicate other sources of coliform bacteria, most likely wildlife, or perhaps domestic animals. Samples collected from water accumulated in leaf axillae and on leaf surfaces of bromeliads (epiphytic flora) in a tropical rain forest harbored fecal coliforms (Rivera et al., 1988). Fecal coliform bacterial counts from 200 to 400 coliforms per 100 mL in an unpolluted pristine western stream coincided with a bloom of an algal mat which could have provided nutrients (McFeters et al., 1978).

Fecal Coliform Population Dynamics

It has been demonstrated that *E. coli* can multiply naturally outside of the digestive tract. A suggested mechanism for bacterial regrowth is that aggregates of suspended matter disintegrate releasing viable bacterial cells. The major fecal strain, *E. coli*, requires a more complex set of growth factors than a nonfecal strain such as *Enterobacter aerogenes* which can grow on a minimal supply of nutrients (Evans et

al., 1968). Preliminary experiments indicated that water temperature must be above 15°C before multiplication occurs (Evans et al., 1968).

Regrowth of coliform bacteria has been observed in wastewater, stormwater, secondary effluent, and advanced wastewater treatment plant effluent that has been chlorinated (Kinney et al. 1978). The mechanism of regrowth was attributed to recovery in damaged cells rather than growth of surviving cells. The authors suggest that chlorination may not be needed for effluent discharge where bacterial death occurs naturally in a stream.

Although coliform bacteria can multiply in stream water, there is a sharp decline that occurs after the peak density is reached (Kittrell and Furfari, 1963). After one day in the summer, as little as 15 to 25 percent of the bacteria remain with 0.3 to 1 percent after five days. The major influences on the shape of the survival curve are changes in nutrient levels and bacterial predators. The increase in bacterial density can be attributed to the upset in balance of predator-prey relationships in the stream. Although the bacteria continue to multiply, reduction in nutrients and an increase in predators inhibit further increases in bacteria. Bacteria decrease more rapidly in polluted than in clean water. Rates of bacterial decrease are greater for populations of bacteria that have higher initial densities (Kittrell and Furfari, 1963).

Fecal coliform net surveys and *in situ* total coliform disappearance tests in Lake Michigan indicated a rapid decline of enteric organisms when introduced to the lake system from a single discharge point where three rivers enter the lake in

Milwaukee (Zanoni et al., 1978). The estimated time for a 90% reduction in total coliforms in the in situ sample was 380 minutes.

Kittrell and Furfari (1963) reviewed water studies on the Ohio River and the midwest from 1914–1925 to clarify the dynamics of coliform bacteria in streams below sources of wastewater discharges. They dispel the common adage that water purifies itself every seven miles (11.3 km). Some of their conclusions were that there is an increase in coliform bacteria that occurs in raw sewage which is followed by a further increase in the receiving stream. The presence of riffles and substrate with attached biota of predators in small streams could act as a trickling filter system. Muddy or sandy bottom streams may behave more like large streams (Kittrell and Furfari, 1963).

Stream self-purification is a complex and ill-defined process involving predation, bacterial adsorption with sedimentation, dilution, hydrologic tributary effects, water temperature, and solar radiation (Geldreich, 1978). Natural self-purification that is effective during warm temperatures and limited precipitation can be poor during seasonal wet periods and cooler temperatures. Geldreich (1978) presents an example, the Red River, where stream self-purification takes 25 to 30 miles in the summer and 100 to 125 miles in the winter for a 99% reduction in microbial hazard. However, it is possible the difference is partly an artifact of higher (faster) stream flow in the winter as compared to low (slower) summer flow.

Interaction of Variables

Modeling of fecal coliform populations must consider several water quality and physical variables that affect bacterial die-off rates as well as biological considerations (Mackay et al., 1983). A model has yet to be developed that makes such a comprehensive analysis and any model would need to be calibrated on a watershed-specific basis. Walkington (1986) points out that modeling for fecal coliform does not automatically correlate with pathogens, which is really the crux of the water quality problem.

Along a stream reach or in a lake, chemical parameters are not as likely to differ as much as biological water quality indicators. In general, living organisms are seldom distributed randomly, or uniformly, in nature with patchy or clumped distribution as the usual situation (Slack et al., 1973).

Competition, predation, mortality, and natality play a significant role in biological dynamics. Coler and Gunner (1969) found that the common soil ciliate protozoan, *Colpoda* sp., readily consumes soil-water bacteria, e.g. *E. coli* and *Aerobacter aerogenes*. Gram-negative bacteria are preferred prey for many common predators (Tate, 1978). In a study of storm drain sediment, competition and antagonism appeared to account for 66-77% of the biotic effect on fecal coliform survival with the remaining percentages due to predation (Marino, 1989). The stable counts between storm events were thought to be due to fecal coliform multiplication

at a rate equal to predation. Another explanation might be that these stable counts could be a result of deterioration of colonies with separation of cells causing more colony forming units (CFUs).

Many factors that play a significant role in the survival and die-off or disappearance of wastewater enteric bacteria in lakes and streams have been identified: (a) algal toxins, (b) bacteriophage, (c) predators, (d) sedimentation, (e) adsorption, (f) flocculation, (g) bacterial nutrients, (h) sunlight, (i) temperature, (j) dissolved solids, (k) chemical constituents 1X, (l) pH, and (m) antibiotics (Canale et al., 1973; Faust, 1976; Zannoni et al., 1978; Bowles et al., 1979; Canale et al., 1993). A modeling assumption is that survival follows a first-order decay where “K” is a first-order rate coefficient that varies seasonally and is a function of water temperature. In die-off and disappearance studies, dilution is a key factor. Dilution of bacteria in a stream can be illustrated by the successive random dilution process where bacterial concentrations decrease exponentially when released into the water (Ott, 1995).

According to Polprasert et al. (1983), a comprehensive die-off model for a pond should include: (a) algal biomass concentration, (b) temperature, (c) organic loading, (d) sunlight intensity, (e) sunlight duration, (f) hydraulic detention time, (g) substrate degradation rate, and (h) pond dispersion number. The basic bacterial density mass balance is: $\text{input} = \text{output} + \text{disappearance by pond action} + \text{accumulation}$. The episodic nature of their origin and the transience of the receiving water response make modeling of fecal coliform contamination events particularly

challenging. Discharge and velocity are important parameters that determine the dilution and downstream movement of fecal coliform bacteria (White and Dracup, 1977).

Kelch and Lee (1978) developed statistical models for predicting fecal coliform levels based on environmental factors, bacterial data, recreational use data, and antibiotic resistance data for the bacteria isolated. Statistical models based on probabilistic relationships appear to be better suited to fecal coliform monitoring than deterministic models which are based on exact mathematical relationships (Mahloch, 1974).

Models for bacterial contamination of water use a number of variables. Water quality in streams in Santa Clara County, California was assessed to ascertain factors causing variations (Sylvester, 1986). Factors causing variations were rainstorms, urban and rural runoff, basin geology and geomorphology, riparian and in-stream vegetation, dry season effects, and reservoir storage and release of water. In a suburban watersheds in Utah, fecal coliform levels were characterized by unit pollution factors relating to number of people, domestic animals, vehicles, disposal systems, other constructed facilities, and natural background levels (Glenne, 1984). The Nonpoint Source Model (NPS) integrates parameters such as runoff value from pervious and impervious surfaces, precipitation data, soil moisture status, and land use categories (Litwin and Donigian, 1978).

Coliform densities depend not only on discharge, but also on factors such as whether the river stage is rising or falling, whether a flood is in an early or late phase, and the volume of the current peak relative to earlier peaks (Elder, 1987). The "first flush" phenomenon as an important indicator for stream loading of bacteria (Westerdahl and Perrier, 1987). Factors such as temperature, hydrologic proximity of pollution sources, livestock management practices, wildlife activity, fecal deposit age, and channel and bank storage, all affect bacterial densities in runoff (Westerdahl and Perrier, 1987).

A model for Nebraska farmland uses inputs from a geographic information system (GIS) to attempt to identify predictive factors for high fecal coliform levels (Gilliland and Baxter-Potter, 1987; Baxter-Potter and Gilliland, 1989). The resulting pollution potential maps are useful for communication with a non-technical audience and illustrating concepts.

Bannerman et al. (1993) presented detailed information that characterizes specific levels of *E. coli* in stormwater runoff from streets, roofs, lawns, driveways, and parking lots that could be useful for model development. One could conceptualize a simple computer model for student use that uses these *E. coli* values to simulate change in land use in a watershed as it affects bacterial counts. Commercial software such as *Stella*™ that is easy to use is available for student model development. However, modeling even a simple system for bacterial contamination is a major challenge.

Water Quality Monitoring

Detection of trends over time depends on the acquisition of water quality data from a properly designed monitoring program as well as application of appropriate statistical methods and a good understanding of water quality relationships (Reckhow et al., 1993). Fixed monitoring networks are common throughout the United States. Six objectives of fixed networks are to:

1. Characterize and define trends in the physical, chemical, and biological condition of the State's surface waters, including significant publicly owned lakes and impoundments.
2. Establish baselines of water quality.
3. Provide for a continuing assessment of water pollution control programs.
4. Identify and quantify new or existing water quality problems or problem areas.
5. Aid in the identification of stream segments as either effluent limited or water quality limited.
6. Act as a triggering mechanism for intensive surveys, enforcement proceedings or other actions.

(National Water Monitoring Panel, 1975, p. II-7)

Fixed station water quality monitoring typically consists of a defined network of sampling points (stations) at which samples are taken at monthly intervals. Several different water quality variables are usually measured (Ward and Loftis, 1983).

There has been much discussion about the value of whether data collected at routine monitoring stations should be used to detect extremes in water quality for compliance purposes, or whether statistical means are more relevant to ascertain trends (Ward et al., 1979). Short term, intensive studies are appropriate for enforcement actions, determination of causal relationships, and definition of

mechanisms for stream recovery. However, these intensive studies may not reflect normal water quality variations (Van Belle and Hughes, 1983).

It was concluded from a study of beaches in Lake Erie that, in order to investigate health effects of recreational water as related to some microbiological variables, one needs to know the specific time of day and day of the weekend that an individual was exposed (Brenniman et al., 1981; Northrop et al., 1981).

Differences in bacteriological indicator levels between sampling time of day and day of weekend can probably be attributed to such variables as wind direction, wind speed, wave height, precipitation, water temperature and waste sources. Geldreich (1971) suggests that bacteriological sampling needs to be done daily (preferably every six hours in a shifting time schedule) in order to get optimum correlations with stormwater runoff and intermittent discharge from pollution sources.

Burton (1982) indicated that sampling programs must be geared toward critical time periods and areas; i.e., summer months, storm flows, feeder streams, agricultural and urban runoff, and swimming areas, including water and sediments. Also, frequency of sampling should be dictated by variability of water conditions, confidence level of data, and extent of human contact. While the cause remains unknown, coliform populations exhibit daily and annual cycling. Regardless of the specific peak periods, the implication of these fluctuations to management and research programs is clear: individual samples represent the sanitary status of a stream only at the time of sampling (Bohn and Buckhouse, 1985).

A unique alternative to sampling the water column and/or natural sediments is the use of sediment bags suspended in the water column that can act to integrate water quality data with respect to fecal coliform concentrations (Nix et al., 1993). Furthermore, sediment bags retain coliform bacteria after their initial attachment to the sand substrate. As a result, they can identify contaminant sources even though the sampling survey is carried out after the pollution event. More research is needed on this concept, however.

The validity of a single fecal coliform count or single site is uncertain. Limited monitoring along a stretch of river or one sample at the mouth of a tributary cannot be predictive of the whole watercourse. Single sampling stations cannot represent both upstream and downstream conditions. Describing entire streams as "safe" or "unsafe" should be avoided if data are limited (Kent County Health Department, 1990).

Hines et al. (1977) point out the importance of characterization of hydrology - every river has a unique hydrology that controls water quality both in time and space. Low flow bacterial contamination events are most likely attributed to direct discharge of municipal, industrial or septic tank wastes and urban runoff after rainstorms. High flow contamination results from urban and rural overland runoff and hydraulically overloaded septic tank fields and wastewater treatment plants (Hines et al., 1977; Duda et al., 1982).

Large sets of monitoring data can provide fertile ground for studying water quality trends and stream dynamics. The research for this dissertation relies heavily on sets of monitoring data from multiple sources during all times of the year. There is, however, a field study component of the dissertation research that provides baseline monitoring data to establish a control site at which manipulative experimentation could take place.

CHAPTER III

DESIGN AND METHODOLOGY

The educational and the scientific contexts of fecal coliform bacteria as a water quality indicator were explored by three methods. These methods included an examination of water quality educational materials and misconceptions about microbiological water quality indicators, analysis of large sets of water quality data and related special studies, and focused field monitoring.

Educational Aspects of Microbiological Monitoring

The purpose of this qualitative research component was to ascertain misconceptions about indicator bacteria both by educators, students, and in curriculum materials, and to evaluate resources being used by teachers and informal educators for microbiological water quality monitoring. This is accomplished through a review of resources and supplementary curriculum materials, surveys and personal interviews with teachers and watershed educators, and search of the Internet for case studies relevant to monitoring of bacteria in water. Part of this research included attendance at the Global Rivers Environmental Education Network '96 (GREEN) Conference, the Fifth National Citizen's Volunteer

Monitoring Conference in 1996 (see U.S. EPA, 1997), the 1996 National Conference on Nonpoint Source Pollution Information/Education Programs, and the 1997 Water Resources Education, Training, and Practice Conference (see Warwick, 1997). This provided a nationwide perspective on the educational use and issues surrounding microbiological monitoring.

Evaluation of resources included: (a) a critique of what is presented in the educational material, and (b) its applicability to the conceptual framework needed to properly integrate microbiological water monitoring into the classroom.

Supplementary curriculum resources of national significance were reviewed.

A subset of attendees at the GREEN '96 conference were given a survey instrument to provide a sense of how microbiological monitoring is being integrated into the classroom and what barriers are encountered. An informal survey for teachers at the Grand Valley State University Water Resources Institute workshops on water was also conducted. Neither survey was meant to be quantitative, but rather they were designed to provide a sense of the knowledge and activity levels of educators in respect to microbiological water quality monitoring.

The Internet also served as a research tool. Searches were performed using a variety of search engines to identify groups that had fecal coliform monitoring data posted on home pages and other Internet resources such as research papers by students and teachers. The purpose of this research element was to provide insight

as to the value of this tool for student and teacher use as well as to identify misconceptions that may be present at various Internet sites.

Monitoring Data

To assess fecal coliform as a water quality indicator, it was necessary to identify and obtain data sources for long term monitoring of fecal coliform bacteria, as well as to perform field work. Monitoring stations on a main river system, as well as tributaries and headwaters, were desired for this analysis. These sites should be within a defined geographic area where there is also a long-term meteorological record available. Ideally, there should be sufficient regional interest in fecal coliform bacteria as an issue of concern, and specialized fecal coliform studies should be available. As a regional control for background levels for bacteria, monitoring of a site unimpacted by human activity to establish baseline bacterial counts was also a priority.

To answer the research question as to the utility of the microbiological standards specifically for the State of Michigan, data were sought that met the following criteria: (a) location in the lower peninsula of Michigan, (b) data set of at least eight samples per site, (c) data collected within the last 25 years, and (d) data in "raw" form for use in further analysis. Data sets that provided long term sampling (10 years), multiple sites, paired sites, and other parameters monitored along with bacteria were desirable. Other data sets of interest were daily samples,

year around records, and event sampling on a 24 hour basis. Special attention was given to data sets from Kent County, Michigan. This county has been well characterized by the author's home institution, the Grand Valley State University Water Resources Institute (GVSU-WRI) in Allendale, Michigan. The Kent County information ties into the author's special field studies on a groundwater seep in Kent County, Michigan.

Study Area

Using the above criteria, county-wide water quality monitoring sites and a groundwater seep in Kent County, Michigan provided the basis for this study. Surface water is monitored throughout the county by both the City of Grand Rapids and the Kent County Health Department. Climatological data are available from the Kent County International Airport which is just southeast of Grand Rapids, Michigan.

Located in west Michigan, Kent County occupies approximately 862 square miles in a rectangular area of 24 by 36 miles. Kent County is about 30 miles east of Lake Michigan. Grand Rapids, the second largest city in Michigan, is located on the major river of the county - the Grand River.

The Grand River has its headwaters in Jackson, Michigan which is in southeast Michigan (Appendix A, Figure 1). As the second largest river system in the state, the Grand River traverses the lower peninsula of Michigan as it flows

through Lansing, Grand Rapids, and Grand Haven to Lake Michigan in a northwesterly direction. The Grand River is over 300 miles long with a total fall of over 500 feet. The chemistry of Grand River is predominantly calcium and bicarbonate based. Average hardness is above 250 ppm and average temperature is 53°F.

Major tributaries in Kent County that drain into the Grand River are the Thornapple, Flat, Rogue, and Coldwater Rivers (Appendix A, Figure 2). Smaller streams in the area are Plaster Creek, Indian Mill Creek, Bear Creek, Mill Creek, and Buck Creek.

The City of Grand Rapids Wastewater Treatment Plant and the Kent County Health Department water quality monitoring sites are found on the Grand River and its tributaries. Sites in the Bear Creek Watershed which enters the watershed above the Rogue River and Plaster Creek were also subjects of this study (Appendix A, Figures 3-4). For perspective, USGS Station 250050 at M-45 is 25 river miles from the mouth of the Grand River and USGS Station 250120 at the Northland Drive Bridge is 50 miles from the mouth. About 4,900 of the 5,570 square miles of Grand River drainage lies upstream of Grand Rapids (Stramel et al., 1954).

Climatological data for Grand Rapids (Latitude 42°53'N; Longitude 85°31'W) in Kent County was obtained for this study from the National Climatic Data Center. These data are collected by the National Oceanic and Atmospheric Administration (NOAA) as part of their nationwide weather network. The current

monitoring location is at the Kent County International Airport at an elevation of 784 feet above sea level. Weather information is available from as far back as 1849 (NOAA, 1986).

Background Reference Site

A location in Kent County that represented an area relatively unimpacted by human inputs was selected for intensive study. The intent of this type of location was to provide reference for the background levels of coliform bacteria.

Theoretically, if the midwest system is like the dynamics of the river system that the author studied in California, this site should have no fecal coliform or *E. coli* during most sampling events. This site would ideally be in an undisturbed area where there is direct groundwater discharge.

The groundwater seep with its "wetland" origin which was selected for this study is located in the northwest 1/4 of Section 6, T6N, R9W, Lowell Township, Kent County (Appendix A, Figure 5). The seep is at an elevation of about 760 feet and drains southwest directly into the Grand River at an elevation of 636 feet. The Grand River is within one mile of the site. The seep is surrounded by over 30 acres of woods with oak, maple, dogwood, and sassafras as dominant vegetation. There is no agricultural drainage impacting the seep area and the nearest human habitation is a single family house over 1,000 feet away at an elevation of 832 feet. The house has a septic system and well logs from that location indicate that the

system sits on 60 feet of yellow sand followed by 25 feet of clay gravel, 25 feet of blue clay, 20 feet of grey sand, and 10 feet of clean coarse sand. The soil in the area is Oakville fine sand with a surface layer of very friable fine sand about 6 inches thick with a subsoil of loose fine sand about 34 inches thick underlain by 60 inches of light yellow fine sand (USSCS, 1986). The static water table at the house is 85 feet below the surface.

There is no reason to believe the septic system at the house has detectable influence on the seep. The system serves two people who work outside of the home. The system was pumped prior to sampling and is well maintained. Septic systems are most likely to contribute to contamination of groundwater when they are in thin soil above permeable bedrock, in extremely permeable soil such as gravel, and/or when the water table is within a couple of feet of the surface (Fetter, 1994). Coliform organisms only penetrate a few yards in medium grained sand and finer materials (Krone et al., 1958). In a study of glacial outwash deposits, coliform bacteria and enterococci were normally removed by passage through 20 ft (6.7 m) of soil. Complete removal was certain when pollutant loads were filtered through 50 ft (6.7 m) of outwash.

The general soil profile in the seep area is Plainfield sand with a surface layer of very dark grayish brown, very friable sand about 1 inch thick. The subsoil is about 20 inches of loose sand. Permeability is rapid in this soil with medium surface runoff. The "wetland" area feeding the seep has about 2 cm of muck

covering the sand. The seep, itself, scours to a sand base in heavy rain events and collects mucky sediment in less intense events. Organic matter such as leaves, insect parts, and twigs collect in the seep channel.

Field Study Sampling and Analysis Methods

Sampling and analysis for the author's field studies in this research follows *Standard Methods for the Examination of Water and Wastewater* (American Public Health Association (APHA), 1992) with reference to U.S. EPA methods as found in the Code of Federal Regulations Title 40, Part 136 (6). The analysis method used was the standard membrane filtration procedure described in the Section 9000 series of *Standard Methods* (APHA, 1992) and the Hach water analysis manual (Hach, 1992) for procedures not in *Standard Methods*.

Sampling Location

The specific sampling location for the seep water was approximately 30 feet downstream from the seep's groundwater origin in the "wetland" area (Appendix A, Figure 5). This site selected was along the "active" reach of the seep. At this point, the stream width is about 30 to 50 cm. Water level in the seep varies from about 2 to 5 cm in depth. It was possible to sample both the seep and the muck "wetland" year around. The average temperature of groundwater in this area of Michigan is 1 to 2°C higher than the mean annual air temperature with seasonal

fluctuations to soil depths of 10 to 25 m (Heath, 1982). Fluctuations are greatest near the surface and may be on the order of 5 to 10°C. If the mean temperature in the Kent County area is 8.6°C (47.5°F), then the predicted groundwater temperature would be 9.6 to 10.6°C with a maximum range of about 0 to 20°C. Indeed, the seep water temperature varied from 0 to 22°C during the duration of the study.

Samples were taken at least once a month for two years from July 1995 through July 1997. On each sampling day, approximately 100 mL of water from the seep was collected in a sterile polyethylene Whirlpak™ bag. To collect surface samples from the seep, the sampling bag was held by the metal strap and plunged downward below the water surface. It was filled by turning the top of the bag slightly upward into the current.

In times of low or no flow, the bag was rested on the seep bed and gently swept through the water to collect the sample. All water samples were filtered within thirty minutes of collection. Water temperature of the seep was recorded at each sampling event with a calibrated mercury thermometer.

A sample quantity for bacterial analysis was determined based upon knowledge of the sampling site. This varied from 1 mL to 100 mL. Appropriate sample size for seep water was generally 10 mL. As necessary, samples were diluted with sterile well water from the aquifer that feeds the seep with the goal of achieving fecal coliform densities of between 20 and 60 colonies/sample. As per

Standard Methods (APHA, 1985), just prior to analysis, water samples were thoroughly mixed by rapidly making about 25 back-and forth movements which ensure equal distribution of the bacteria.

Soil and sediment samples were collected in the wetland area. It has been suggested that the sampling depth for fecal coliform bacteria in sediment be standardized at 0-1 cm (Albinger, 1991) which was the case for this research. In most sampling events, a volume of approximately ten milliliters of wetland soil/sediment was collected from the top one centimeter of substrate with a sterile sampling spatula, and was transferred to a sterile Whirlpak™ polyethylene bag. For presence/absence determination for litter and vegetation, 207 mL Whirlpak bags were filled half full of the sample. If transport time was more than five minutes, the samples were placed in a cooler with ice.

Upon arrival at the laboratory, 100 mL of sterile well water from the aquifer that feeds the seep was added to the samples. The samples in the sampling bags were gently shaken 25 times back and forth, and then allowed to settle for fifteen minutes before analysis commenced. These samples represented suspended and dissolved sediment. Either one or ten milliliters of the supernatant was filtered through a 0.45 µm Gelman cellulose acetate filter. Samples were filtered within forty-five minutes of collection. Results of analysis are based on the levels of bacteria associated with the soil volume of approximately ten milliliters as reflected in the 100 mL of added dilution water. Interpretation of these results is best approached on a

relative presence/absence basis. Dry weight of the soil or sediment would be necessary if these data were intended to be truly quantitative instead of semi-quantitative.

Laboratory Analysis

The membrane filtration technique is an approved method for enumeration of bacteria in water and is described in *Standard Methods* in the Section 9000 series (APHA, 1992). Sterile filtration units were used at the beginning of each filtration series. These plastic units were those suggested by the Global Rivers Environmental Education Network (GREEN) river monitoring network in the *Field Manual for Water Quality Monitoring* (Mitchell and Stapp, 1995). Decontamination of the filtration apparatus was through steam sterilization for 15 minutes.

Using sterile forceps, a 45 μm Gelman membrane filter was placed on the filtration apparatus. A sample from the seep or wetland water was taken with a sterile pipette and filtered through the membrane filter. At least twice, volumes of 20 to 30 milliliters of sterile well water from the nearest well to the seep were drawn through the filter to rinse the sides of the filter holder. The filter was removed from the filter holder with sterile forceps and immediately placed in a sterile petri dish on top of a sterile absorbent pad saturated with 2 mL of m-FC broth or 2 mL of m-Coli Blue™ broth. These media were supplied in individual sterile ampoules from the Hach Company. The m-FC broth is specific for enumeration of fecal coliform

bacteria as indicated by formation of blue colonies. The m-Coli Blue™ broth is a dual test for *E. coli* which produces blue colonies and coliforms which show up as red colonies. Non-coliform, bacteria may appear on filters as gray to cream colored colonies.

The inoculated m-FC petri dishes were inverted and placed in a dual chamber Millipore Environmental Incubator for incubation at $44.5 \pm 0.2^{\circ}\text{C}$ for 24 ± 2 hours. The m-Coli Blue™ dishes were incubated at $35.0^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 24 ± 2 hours. This incubator has a rated accuracy of ± 0.2 for 44.5°C and ± 0.5 for 35.0°C . Inversion of dishes during incubation restricts the formation of "spreaders" due to condensed water vapor.

After 24 hours, blue colonies on the FC membrane filter were counted. The number of colonies were converted to colony forming units (CFU) per 100 mL. Any gray to cream-colored colonies were not counted as fecal coliform bacteria (APHA, 1985). Colony counts were made under 10x magnification and were aided by a hand-held colony counter.

Sample size is critical in the MF procedure. High concentrations of algae and suspended solids can interfere with the technique. The optimum colony count for a membrane filter is between 20 and 60 colony forming units (CFUs). Excessive numbers of colonies result in a "too numerous to count" (TNTC) designation for reported data. More than 60 colonies on a membrane filter risk crowding,

confluency. and competition for nutrients. Random sampling variations are risked with counts less than 20 colonies (Geldreich et al., 1965).

The blue colonies on the m-Coli Blue™ pads were counted as *E. coli* (EC). The red colonies were counted as coliforms (C) with total coliforms being the sum of all of the red and blue colonies (TC).

Regardless of the original sample volume, the following formula was used to convert to colonies counts:

$$\text{Colonies per 100 mL} = \frac{\text{colonies counted} \times 100}{\text{mL of sample filtered}}$$

The term TNTC was used when the number of colonies was "too numerous to count." Counting becomes difficult when there are more than 200 colonies on a plate. A range of between 20 and 60 colonies was desired for fecal coliform plates and 20 to 80 colonies for total coliforms.

Quality Control/Assurance

Field procedures for this research involved replicate samples (samples from similar sites), duplicate samples from the same site, and documentation of location, seep temperature, and field observations. The laboratory procedures were standardized as to how samples were handled when they reached the laboratory and how they were processed. Aseptic techniques were followed including steam sterilization of equipment, disinfection of working surfaces, and flame sterilization of

transfer forceps. Filtration apparatus and dilution water were autoclaved at 121°C for a minimum of 15 minutes.

As verified by Lin (1977), it is important to use the same brand of membranes since there is variation in the recovery of fecal coliforms with different manufacturer's membranes. Gelman membranes were used throughout this study. The percent verification of fecal coliform colonies captured on the Gelman product was 92.5% (Lin, 1977).

Time and temperature charts were kept for the incubator to verify that the 35°C and 44.5°C chambers were within the appropriate range.

The medium used for the fecal coliform analysis was m-FC broth containing 1% rosolic acid. Individual PourRite™ ampoules of m-FC broth from the Hach Company were used in the analysis. Each lot of media was shipped with an expiration date and a Certificate of Analysis. The medium was stored in a refrigerator at about 5°C. Stock cultures of *E. coli* and *Enterococcus* were diluted and run for each new batch of medium.

Field blanks, well water blanks, and split samples were run in conjunction with the samples. Periodic negative and positive control plates were part of the analysis. Sterile well water needed to have a zero count for coliforms. Verification of selected colonies was through gas production in lauryl tryptose broth incubated at 35°C for 24 hours followed by inoculation into EC broth with incubation at 44.5°C for 24 hours (APHA, 1992).

Plates were read twice under magnification. Only definitive fecal coliform, *E. coli*, or total coliform colonies were counted. The colony counting followed procedures from *Standard Methods* (1992), and the Hach and 3M literature. For large numbers of colonies, readings needed to be within 10% of each other. The highest count of duplicate plates was recorded which differs from *Standard Methods* (1992) where averages are calculated but better reflects the Mitchell and Stapp (1995) methods.

Analysis Errors

There are inherent limitations to the accuracy of counts and the overall membrane filtration methodology is fashioned to minimize these errors. The plating error is the combined effect of all errors encountered during the course of the analysis with the exception of dilutions (Prescott et al., 1946). These errors include human errors of faulty technique, lack of precision of pipettes, and counting the colonies. The nature of the bacteria also poses difficulties. The organisms may be clumped leading to underestimation of counts, there may be loss of viability, and colonies can overcrowd a plate. When colonies occur in chains, each chain should be considered a single colony and each spreader would be one colony (Prescott et al., 1946). Even though samples are shaken, there is a distribution error due to different positions of bacteria in the sample.

Replicate plates with higher dilutions of samples can obviate some of these problems. However, this introduces a dilution error which is related to tolerances of pipettes and the nature of dilution blanks. Plating errors usually vary from 0 to 20% with approximately a 2.8% dilution error for each dilution (Prescott et al., 1946).

Alternative Methods of Analysis

Parallel plates for the m-Coli Blue™, Petrifilm™, and Coliscan™ analyses were run to determine the suitability of using these methods for student use. Water samples from the seep and the wetland were used for these tests as well as cultures of *E. coli* that were in the range of 200 - 600 colonies per 100 mL. For the environmental samples, 10 mL of water was used for the m-Coli Blue™ analysis and 1 mL was used in the other two methods.

The one milliliter samples for the Petrifilm™, and Coliscan™ analyses were taken in two ways:

1. For routine seep and wetland monitoring, an extra milliliter was drawn into a pipette that delivered the water for the membrane filtration analysis and was used to inoculate Petrifilm™ or Coliscan™.
2. For special samples, one milliliter was obtained with a mechanical pipette directly in the ambient water and it was immediately transferred to the Petrifilm™ pad or the Coliscan™ medium bottle.

Petrifilm™ plates from the 3M Corporation consist of plastic films with grids which are coated with nutrients and gelling agents. Two tests in one (coliform and *E. coli*) are performed with the Petrifilm™ plates. The gel contains β -glucuronidase indicator for confirmed detection of *E. coli*. Originally designed for food analysis, these plates are also appropriate for water sampling. Petrifilm™ methods have been collaboratively tested and are included in the Association of Official Analytical Chemists Official Methods of Analysis (Curiale et al., 1989).

The methodology for using the Petrifilm™ plates is to: (a) inoculate and spread one milliliter of water on the gel, (b) incubate the plate at a temperature of 32 - 35°C (90 - 95°F) for 24-48 hours, and (c) read the plates using a Quebec-type colony counter or other magnification.

Coliform colonies appear red due to an indicator dye and the top film traps the gas produced by the coliforms. *E. coli* colonies are characterized by a blue precipitate and usually there is gas formation. The total coliform count is the red and blue colonies with gas. Blue colonies without gas formation at an incubation temperature of 35°C would not be classified as *E. coli* whereas at a 44.5°C incubation temperature, confirmation for non-gas formers is needed. The desired counting range is 15 to 150 colonies per plate. When colonies counts are greater than 150, the count should be estimated. The growth area on the Petrifilm™ plate is approximately 20 cm². The average number of colonies in a square (1 cm²) can be multiplied by 20 to obtain a total count per plate.

The Coliscan™ Easygel™ system from Microbiology Laboratories takes advantage of the fact that general coliforms produce the enzyme galactosidase in lactose fermentation and *E. coli* produces glucuronidase in addition to galactosidase. The procedure for this analysis is to: (a) add a 1 mL water sample to the liquid Coliscan™ medium, (b) pour the water-medium mix into a petri dish, and (c) incubate at room temperature or higher (85 - 99°F) for 24 to 48 hours. Usually 1 mL of test sample is added to the medium but up to 5 mL can be used. Coliform colonies will be a pink color and fecal coliforms (*E. coli*) will appear as purple colonies. The "general" coliforms are *Citrobacter*, *Enterobacter*, and *Klebsiella*. Total coliforms are all of the pink and purple colonies.

Short-term Monitoring Data

Many short term studies have been performed in Plaster Creek, a well-known heavily contaminated tributary to the Grand River. The creek drains a watershed of approximately 60 square miles in southern Kent County (Appendix A, Figure 9). It includes portions of the cities of Kentwood, Wyoming, and Grand Rapids, as well as Cascade, Gaines, and Caledonia Townships. Fecal coliform bacteria in urban drains in Plaster Creek were surveyed in two projects in 1989-90; one by the City of Wyoming and another by the Grand Rapids Waste Water Treatment Plant (Madden, 1990; Barton, 1990). The Wyoming project involved daily grab samples of Lee Drain and Burton Street Drain that both enter Plaster

Creek and, eventually, the Grand River. Data from these studies are available in "raw" form. The Grand Rapids Wastewater Treatment Plant provided fecal coliform monitoring information that was collected using continuous sampling devices for the Silver Creek drain which also flows into Plaster Creek.

Long Term Monitoring Data

Kent County Health Department

The Kent County Health Department (KCHD) and the Grand Rapids Wastewater Treatment Plant both have substantial data sets for this analysis. The Kent County Health Department's surface water monitoring program began in 1989 as a result of a series of surface water contamination events and concern about the health effects of combined sewer overflows from the Grand Rapids Wastewater Treatment Plant (KCHD, 1990).

Sampling by the Kent County Environmental Health Division is performed from April to October with two samples collected two times a month from each location. From 1989 through 1994, monitoring was for fecal coliform bacteria and the water quality standard for total body contact in recreational surface waters was 200 fecal coliform colonies per 100 mL (KCHD, 1995). In 1995, the County changed to monitoring for *E. coli*. This was in response to changes in the state water quality standards from fecal coliform to *E. coli* with new limits of 130 *E. coli*

per 100 mL for total body contact and 1,000 *E. coli* per 100 mL for partial body contact uses. What makes this data set interesting is the comparative aspect of the fecal coliform versus *E. coli* sampling and how this relates to whether the body of water is considered "safe".

The original KCHD program had 14 different sampling locations; it now has 32 stations. The original criteria for sampling sites were locations (a) involving significant human contact with surface water, (b) for which water quality information is not available from different sources, (c) proximate to known or potential pollution sources, (d) which allow for measurement of water quality of streams in Kent County, and (e) with convenient access to sampling points (Kent County Health Department, 1988). Whereas the City of Grand Rapids sites are close to the Grand River, the Health Department sites are scattered throughout the tributaries of the county. Streams in numerous parks in the county are sampled in this program. Stations have been added and dropped based on monitoring results (KCHD, 1993). Eight years of data were available for analysis.

Through a visit to the Kent County Health Department Laboratory and presence during a sampling collection event, the author of this dissertation observed the following about KCHD procedures. Sampling by the Kent County Health Department is performed pursuant to the methods described in the U.S. EPA microbiology methods manual, Section II, a (5) (U.S. EPA, 1978). Samples are collected by a sanitarian. Great care is taken to avoid contamination and a special

sterile sampling apparatus has been designed for sampling off of bridges. Some sites are sampled from bridges and at other sites, the technician either samples with a pole or enters the stream with waders. Samples are stored at 4°C as they are transported to the County Laboratory for analysis by membrane filtration.

The Kent County Health Department samples are run at the Kent County Health Department Laboratory. Fecal coliform bacteria are enumerated using standard membrane filtration procedures with m-FC medium. *E. coli* detection used the membrane filtration technique and mTEC medium (U.S. EPA, 1985). Details of these methods and standard operating procedures can be found on file at the Kent County Health Department Laboratory. A count of zero colonies is reported as less than 10 colonies per 100 mL.

Grand Rapids Wastewater Treatment Plant

The Grand Rapids Wastewater Treatment Plant monitors 15 locations on the Grand River and its tributaries on a monthly basis. Data on fecal coliform bacteria are available from 1985 to the present (1998). A pair of GR WWTP technicians conducts the water sampling on a monthly basis (2nd Wednesday of the month) throughout the year. There are normally no adjustments in the schedule for weather conditions other than the inability to take samples at stations where water has frozen in the winter (Personal communication with Grand Rapids Wastewater Treatment Plant, 1996).

The author accompanied the Grand Rapids Wastewater Treatment Plant technicians on a sampling run and made the following observations. Samples for bacteriological analysis are collected in a Van Dorn sampler. This sampler is made of PVC with rubber closures. It is not a standard sampling apparatus for bacteria. Other than flushing with the water of the next station prior to sampling, there is no sterilization of the sampler between sites. The samples are, however, transferred to sterile bottles. Technicians generally wear the same pair of rubber gloves at each sampling station.

Most of the water samples are taken from bridges. All sampling sites with the exception of the M-11 (Wilson Avenue) site (Station 5) are downstream of a bridge. The bridges are marked with the sampling location for consistency as to where the samples are to be taken. The order of sampling does not reflect the numerical order of the stations, but is related to the geographic locations of the sites.

The City of Grand Rapids also uses membrane filtration for its fecal coliform analysis. Their standard operating procedure on file at the Wastewater Treatment Plant has information on sample handling and preservation, procedures, and preparing the plates.

All plates are counted but only those having counts between 20 and 60 colonies are reported. Plates with ≥ 150 colonies are recorded as too numerous to count. If a plate from a smaller sample has fewer than the acceptable colony range

and a plate of a larger sample has more than the acceptable range, the results from the smaller volume are reported. Dilutions of samples for membrane filtration that were "too numerous to count" (TNTC) are recorded as 15,000. For a 1 mL dilution, the highest calculated count would thus be 15,000. Given this, any reading of 15,000 could only be interpreted as a place holder and not an actual count. The actual count could very well be more than 15,000. This limits estimates of means to a 15,000 maximum, and lowers estimates of means based on any samples with actual values greater than 15,000. This point should be kept in mind when comparing data from the wastewater treatment plant with data from other monitoring programs.

Exploratory Data Analysis

The approach to data analysis in this project is one of systematic examination of the data to ascertain relationships and trends. The most revealing trend will be the percent exceedance of the fecal coliform standard at a given station. For the purpose of this research, the term "exceedance" is used in a limited way; it reflects whether a single sample has greater than 200 colonies per 100 mL for fecal coliform bacteria and greater than 130 colonies per 100 mL for *E. coli*. It is not used in the strict sense of the state standards that have a time frame and number of samples associated with them. Exceedance rates have a direct relationship to the regulations and tie into the public health aspects of bacterial

contamination. For a standard to be reasonable, there should be a high probability that it can be attained. If, for some reason, the overall watershed conditions are not naturally conducive to meeting the standard, even if best management practices are in place and point sources are controlled, then some reexamination of the standard is in order.

The basic statistical parameters for analysis of water quality data are outlined by Sylvester et al. (1962). Calculated values for each station and parameter included maximum, minimum, median, mean, weighted mean according to flow, variance, standard deviation, skewness, and confidence limits. Both arithmetic and geometric distributions were used. Comparisons between categories was accomplished mostly through linear vs. linear power function correlations.

In exploratory data analysis (EDA), attention is given to determining what scale (e.g., square root, logarithmic) would simplify data analysis. Emphasis on graphical display is also a feature of this analysis. Unlike classical statistics, EDA involves successive iterations that involve refinement of an initial value to bring it closer to a final answer (Hoaglin et al., 1983).

The analysis procedure had the following steps:

1. Prepare data for analysis. This involves: (a) any zero values for bacteria are changed to 1; (b) the data set is adjusted to eliminate dates with missing values; (c) if the number of data points per time period at a station is greater than one, each time period is summarized with the mean or median when necessary; (d)

stations are aligned based on sampling dates; and (e) appropriate logarithmic transformations are made.

2. Calculate basic descriptive statistics (central tendency, dispersion, skewness, kurtosis). Note: graphical analysis will suggest appropriate measures
3. Examine the data graphically. This includes preparation of histograms (exploration of data structure), bivariate scatter plots (time series), and box plots (station comparison) using normal and logarithmic transformation.
4. Ascertain relationships and trends. Methods used include (a) correlation, (b) regression, (c) t-tests.
5. Compare results with other geographic areas (Meta-analysis)

Graphical Analysis

Pictorial diagrams, photographs, line drawings, line graphs, bar graphs, scatterplots, combination graphs, 3-D graphs, and sector diagrams are all graphical ways to convey results of water quality monitoring (Ingram and Bartsch, 1960; U.S. EPA, 1994; Kerr, 1995). The basic scatterplot is an effective way to convey monitoring data. Tufte (1983) estimates that 75% of the graphs used in science are scatterplots. These plots show single data points relating to trends as well as outliers, and patterns that are obscured by summary statistics. According to Berthouex and Brown (1994, p. 32), "suitable graphs of data and the human mind are an effective combination; endless tables of data and the mind are not."

Histograms are a way for the analyst to get a sense of the distribution of the data even before other statistics are calculated. Skewness of the data will be very evident in this visual representation of data. The location of the center of the sample, symmetry, dispersion, and outliers will all be visible (Reckhow et al., 1993). Important analysis questions to be answered by construction of a histogram relate to whether the data have a normal and uniform distribution and what influence outliers have in the data set. Since water quality data are frequently right skewed, a logarithmic transformation plotted on a histogram can give an indication of whether the distribution (on a log scale) approaches normal.

Of special use to school volunteer monitoring groups is the box plot which summarizes these statistics in graphical form. The box plot is useful in display of water quality data since it can summarize a large amount of data in a compact form. Box plots visually display ordered data highlighting the median, variability, and skew as well as the size of the data set and the statistical significance of differences between medians (Mc Gill et al., 1978; Ellett and Mayo, 1990). Reckhow and Chapia (1983) outline the basic steps in the production of box plots.

The data are ordered from lowest to highest for box plot construction. Elements of a box plot include the median, maximum/minimum values, 75 percent level and 25 percent level, and the interquartile range (Simpson, 1991). The shape of the box plot gives an indication of skew if there is a lack of symmetry. Outliers can also be represented with the basic box plot. Plots can contain notches to

indicate the approximate 95% confidence interval for comparison of medians. A statistical test of significance for the difference between two medians can be ascertained by the lack of vertical overlay between notches (Reckhow et al., 1993). Vertical overlay of notches indicates that the medians are not significantly different. Comparison of the shape and relative location of a series of box plots can give an insightful comparison of sampling stations.

Statistical Analysis

Volunteer monitoring programs generally use statistics for (a) summarizing and reporting monitoring results, (b) evaluating quality assurance/control programs, and (c) interpreting data and drawing conclusions (Rector, 1995). Commonly used statistics for monitoring include measures of central tendency such as the arithmetic mean, geometric mean, and median; and measures of variation including range and standard deviation (Berthouex and Hunter, 1981). Accuracy and precision of measurements are also important elements for volunteer monitoring information.

Calculation of a mean is confounded by missing values and values that are "below detection limit" or "above a certain limit." Other characteristics of water quality data that complicate analysis include non-normal distributions, flow relatedness, seasonality, and serial correlation in time and between constituents (Hirsh et al., 1982). Serial correlations are seen when high values tend to follow high values and low values follow low values. Other data problems related to

environmental monitoring include aberrant values, large amounts of data from numerous sources, large measurement errors, nonconstant variance, complex cause and effect relationships, changes in measurement and sampling methods, moved sampling locations, and lurking, unmeasured variables (Berthouex and Hunter, 1983; Berthouex and Brown, 1994).

For analysis purposes, data sets for this dissertation were edited to remove sampling dates with incomplete data when series of stations were compared to another. Situations where it was obvious that an analyst reported "greater than" were noted.

Coliform counts have been expressed as arithmetic means, medians, and geometric means. Most commonly, U.S. Environmental Protection Agency documents use the term "log mean" for fecal coliform standards (U.S. EPA, 1976). The log mean is also referred to as the log average, logarithmic mean, and geometric mean in state water quality standards (Landwehr, 1978). The term "average" is sometimes used alone without explanation which adds confusion. Often there are no explicit definitions of the terms used in the standards.

Large data sets of coliform counts from monitoring stations, more often than not, are best described through log transformation. Typically, there are high counts that are extreme from the rest of the distribution. Standard deviations tend to be proportional to the mean. Geometric means are more appropriate than arithmetic means for describing this type of data.

The geometric mean of the numbers is not the same as the arithmetic mean of the logarithms of the numbers. The strict definition of the geometric mean is the n th root of the product of n values (Landwehr, 1978). For water quality analysis, an alteration of this definition is necessary since values of zero will make a geometric mean impossible to compute. Following the advice given to wastewater treatment plant operators (Karn, 1977), all zero fecal coliform, *E. coli*, and coliform values are assigned a value of 1.

The geometric mean has also been calculated as the antilog of the mean of logarithmically transformed observations which is the procedure used in this research (Reckhow et al., 1993). In the antilog method, the geometric mean is calculated by taking the logarithm (log) of each sample. The logs are then added together and the sum divided by the number of samples. The antilogarithm of the quotient is calculated which results in a value for the mean (geometric average).

The skewness of the data relates to the symmetry of the data set. A normal distribution has a skew of zero. If the skewness value is greater than zero, the data are skewed to the right (positive skew). Skewness values less than zero indicate data are skewed to the left (negative skew). Lake and stream data frequently are skewed due to the many factors that affect water conditions including seasonal variations, weather conditions, and activity in the water (U.S. EPA, 1991).

Kurtosis is a measure of the peakedness of the data. It is a dimensionless value used to compare the height of the peak of two distributions. A normal

distribution has a kurtosis value of 3.0. Skewness and kurtosis provide information about the shape of the distribution.

The variability of data around the central tendency is generally represented by the standard deviation. Standard statistical analysis computer programs will calculate the standard deviation based on the arithmetic mean. Due to the positive skew common to bacterial monitoring data, this is not as appropriate a measure as the interquartile range. The interquartile range is less influenced by outliers and asymmetry. Based on order statistics, the interquartile range is the difference between the observations at the 75th percentile (upper quartile) and the 25th percentile (lower quartile). The interquartile range is more conservative than the simple range (Hinkle et al., 1988).

Correlation coefficients are useful for ascertaining possible relationships between parameters. Interpretation of the size of a correlation coefficient follows guidelines proposed by Hinkle et al. (1988). Interpretations are categorized as very high correlation (.90 - 1.00), high (.70 - .90), moderate (.50 - .70), low (.30 - .50), and little or no correlation (.00 - .30). Regression analysis describes the dependence of a variable Y on an independent variable X. It lends support to possible causation of changes in Y by changes in X, and serves as a predictive measure.

For hypothesis testing, nonparametric tests are more appropriate than parametric tests for bacterial data. These tests do not depend upon the form of the

underlying distribution of data but are not as powerful as parametric tests (Hipel, 1988). Nonparametric t-tests evaluate the null hypothesis that two samples come from populations having the same distribution and are appropriate for raw microbiological monitoring data. Although not used in the present analysis, the Seasonal Kendall test and its modifications for serial dependence is appropriate for nonparametric bacterial monitoring data (Hirsch and Slack, 1984). Analysis of variance (ANOVA), a parametric test, is appropriate for log transformed data that exhibit homogeneity of variance and normal distribution.

Software packages used for the analysis include Excel 5 (PC), StatView (MAC), MINITAB, and PC-ORD. PC-ORD Version 3.0 is a Windows-based program for multivariate analysis of ecological data produced by MjM Software Design. The package has many ordination and classification techniques as well as standardization, relativizations and smoothing utilities that are not available in other statistical analysis packages. Many of the PC-ORD macros are based on programs from the Cornell Ecology Program series.

CHAPTER IV

RESULTS OF EDUCATIONAL ASPECTS REVIEW

The research strategy for the educational aspect of microbiological monitoring was to network with watershed educators through national conferences and local workshops as well as to survey existing monitoring manuals for bacterial monitoring methods and, more importantly, how fecal coliform monitoring is presented to students. It was anticipated that this review would provide insight into common misconceptions (naïve conceptions) about microbiological monitoring. The guides reviewed represent simplified approaches to stream and lake monitoring that are used by school groups as well as adult volunteers. Other resources such as computer software and the Internet have also been examined.

National Water Education Conferences

Elevated counts of fecal coliform and *E. coli* bacteria are clearly a national issue that is of interest to students and the general public. The keynote speaker at the 1997 American Water Resources Association Conference, Dr. Penney Firth from the National Science Foundation, noted that bacterial pollution ranks number one for miles of river contaminated in the United States. Teachers and informal

educators need to have a clear understanding of the meaning of bacterial contamination as they teach about water quality issues. However, individual teachers often lack knowledge about indicator bacteria. This is caused by available information sources that are incomplete, greatly simplified, and, in some cases, misleading and inaccurate. Brody (1995, p. 20) noted that "some teachers feel they do not have adequate knowledge to teach about water and water resources and there is an existing gap in the water education field." According to Rakow (1984), teachers will give the highest priority in their curricula to topics about which they are most knowledgeable. Given this, it is not surprising that teachers would be hesitant about involving students in monitoring for bacteria.

Using the AWRA symposium as an example, there were many instances where speakers or authors displayed a naive view of fecal coliform bacteria and interpretation of water quality data. For instance, fecal coliform bacteria were lumped with "water chemistry" tests in one paper. The author presented a weighted Water Quality Index involving nine weighted parameters (Excellent, Good, Medium, Bad, Very Bad) with no reference as to how these judgments were made. Another speaker described what appears to be a typical student stream monitoring project where once a year students take a single sample for fecal coliform analysis. That single sample becomes part of an overall water quality index that is then put on the Internet as representative of the water quality of the stream for the entire year.

An overall impression from this conference, as well as the Global Rivers Environmental Education Network '96 (GREEN) Conference, the Fifth National Citizen's Volunteer Monitoring Conference in 1996 (see U.S. EPA, 1997), and the 1996 National Conference on Nonpoint Source Pollution Information/Education Programs, is that, although there is active interest in bacterial monitoring, the level of sophistication about monitoring techniques is not great.

Analysis of Water Quality Curriculum Materials

The following are some of the current resources used for information on monitoring. Presentation of information in each source regarding indicator bacteria is reviewed. The materials are mostly developed by governmental and nonprofit organizations, and they are written for teachers and/or for the general public.

U.S. Environmental Protection Agency Manuals

The United States Environmental Protection Agency has put forth a major effort in the 1990s to produce a number of water quality manuals on lakes (U.S. EPA, 1991; 1996b), streams (U.S. EPA, 1995 draft), wetlands (1996c), and quality assurance for monitoring (1996d). The U.S. EPA *Volunteer Lake Monitoring Manual* (1991) devotes only two pages to bacterial monitoring with a discussion centering on bathing beaches. Volunteers are urged to work with local health departments on monitoring protocol and no specific methods are outlined. The

manual makes some serious errors - it states that another microbiological indicator, enterococcus, "are a subset of the fecal coliform group". This is not true. The enterococcus group is not even a coliform subset (Gram-negative rods); it includes *Streptococcus faecalis* and *S. faecium* (Gram-positive spherical or ovoid cells) (APHA, 1985).

The basic lake test mentioned in most manuals is transparency as measured with a Secchi disk (U.S. EPA, 1991). Frequently, monitoring is done two times a month from May to October. A lake water quality index, the Carlson Trophic State Index (Carlson, 1977), is an index of the trophic status of lakes that is based upon Secchi disk transparency, total phosphorus concentration, and chlorophyll *a*. This index uses a log transformation of Secchi disk values as a surrogate for algal biomass. Suspended solids and nutrients (nitrogen and phosphorus) are frequently mentioned as other monitoring parameters for lakes. The U.S. EPA Trophic Index for lakes does not use coliform but draws upon the following six variables: total phosphorus, dissolved phosphorous, inorganic nitrogen, Secchi disk transparency, minimum dissolved oxygen, and chlorophyll *a* (U.S. EPA, 1974).

Little mention is made regarding bacteria in the U.S. EPA streams (U.S. EPA, 1995 draft), wetlands (1996c), and monitoring quality assurance (1996d) documents. The absence of attention to bacteria is unfortunate given the usefulness of these manuals for aquatic education. However, the quality assurance manual should be mandatory reading for all teachers involved in water monitoring. It

presents the stepwise development of a quality assurance project plan (QAPP) with procedures for data collection and analysis so information collected through water monitoring will have validity.

Field Manual for Water Quality Monitoring

The manual used by many student stream teams to generate a water quality index is the *Field Manual for Water Quality Monitoring* (Mitchell and Stapp, 1995). The specific skills and understanding intended to be developed through use of the manual are:

1. to understand the meaning of nine important tests for measuring water quality;
2. to become familiar with important sources of water pollution and ways to help solve those problems;
3. to learn how to run accurate water quality tests; to determine how the tests relate to each other;
4. to run the test on the river safely; and to understand what the test results mean in terms of human uses of the river.

(Mitchell and Stapp, 1995, p. 10)

There is some mention of integration of other items related to river water quality including benthic macroinvertebrates, land use, human impact, and stream surveys. A case study on the Interactive Rouge River Water Quality Project gives insight on integrating concepts into a student water quality network.

Fecal coliform bacteria are one of the nine water quality tests that make up the manual's water quality index. The section on fecal coliform bacteria in this manual recognizes non-human origin of the bacteria as well as nonpoint pollution

sources. The element of "chance" of contracting disease due to possible association with pathogens in high fecal coliform count waters is also made. A brief section on sampling design gives guidance in relating the purpose of sampling with the sampling location(s). Although there is a discussion of sewer systems, there is not an elaboration on nonpoint bacterial contamination which is now thought to be even more significant than point sources.

The sampling procedures outlined in the *Field Manual for Water Quality Monitoring* could be improved by continually noting that equipment such as sampling bottles, forceps, filtration system, and pipettes need to be sterile. An alternative frequently used by volunteer monitors is sterile plastic bags. The manual needs to stress that the person sampling needs to be downstream of the sample location.

There are many improvements that could be made in the fecal coliform testing procedures listed in the *Field Manual for Water Quality Monitoring*. For instance, there is less chance of contamination if petri dishes with absorbent pads already in them are used instead of having students put in the pads. The procedure states that the filtration system should be rinsed with distilled water - perhaps another chance for contamination. It is not clear that small sample volumes need to be diluted with sterile buffered water and that the samples need to be thoroughly mixed. The traditional three rinses of 20 to 30 mL with sterile buffered dilution water from *Standard Methods* (APHA, 1992) are not mentioned. The suggested

river sample sizes of 0.1 to 5.0 mL are conservative and may result in too few colonies; 10 mL often suffices to give 20 to 60 colonies which translates to 200 to 600 colonies per 100 mL.

The directions for transferring the filter to the petri dish need improvement. The method described for transfer of the filter is to "slide" the filter onto to absorbent pad which is not a standard procedure. The filter should be centered with a slight rolling motion making sure air is not trapped beneath the surface. The temperature range for incubation was incorrect in that it should be $\pm 0.2^{\circ}\text{C}$ not $\pm 0.25^{\circ}\text{C}$.

The directions for counting colonies do not recognize the common problem students experience in conceptualizing what a "blue spot" really means. They are told to count the "spots" without an explanation of what a colony (spot) really represents (many bacteria - not a single organism). However, the *Manual* does have drawings of plates to diagnose problems with the membrane filtration procedure which are helpful.

A chart to interpret fecal coliform results is given in the procedure for calculating the overall water quality index. The x-axis is a logarithmic scale that may not be obvious to students. If the Q value corresponds at all to the overall water quality index, then anything above 60 colonies per 100 mL would be considered "bad" water even though standards are 200 colonies per 100 mL.

Finally, a point is made that "it is important to report the highest fecal coliform value rather than an average value" (Mitchell and Stapp, 1995, p. 42). There is a risk of accepting counts from contaminated samples as true values if this is followed. Also, this statement is made with no explanation of the applicability of the geometric mean for analysis of bacterial data. Some of the confusion surrounding the quantification of bacteria is not unlike the problem of interpretation of pH as a water quality indicator. The logarithmic aspects of pH as well as the actual definition of pH are frequently lost in the pursuit of "numbers." Lists of pH values are added and statistics are calculated that ignore the actual numerical meaning of the numbers where a change in one pH unit represents a tenfold change in acidity. Students have difficulty making the connection between hydronium ions (or H^+ for convenience) and pH values, and they often have no concept of ions and ionization. The analogous difficulty with bacteria is the use of the log mean in water quality standards.

Water Quality Testing Manual for the Project SEARCH Student Monitoring Program

A water quality testing manual was developed for the Project SEARCH Student Monitoring Program in Connecticut (Beauchene et al., 1996). In this National Science Foundation funded project, high school teachers were given 144 hours of training to conduct student water quality monitoring projects by the

Science Center of Connecticut. The SEARCH data collected by students have been used in Connecticut Bureau of Water Management reports.

The manual for the project has a chapter on fecal coliform bacteria (Beauchene et al., 1996). The authors note that coliform bacteria can live and reproduce in soil and water so they are less precise as indicators of fecal contamination than fecal coliforms. High levels of fecal coliform bacteria in water are attributed to recent fecal contamination, but not necessarily of human origin. Methods used in the manual are from a number of sources (Millipore, U.S. Geological Survey, U.S. EPA) but not specifically *Standard Methods* (APHA, 1992). Some liberty is taken with methods such as substituting sterile stream water for buffered water and using the same funnel rinsed thoroughly for subsamples. The manual suggests running a positive blank from a toilet sample - a novel approach. It also cautions about sampling between October and May when sewage treatment plants are not chlorinating effluent, and to avoid stirring up sediments.

The analysis procedures are the most detailed of any of the manuals reviewed but phrases such as "squirt about 10 - 20 mL of sterile stream water into the funnel to provide a cushion" detract from the narrative and do not reflect *Standard Methods* (APHA, 1992). Also, the use of three (1 mL, 10 mL and 100 mL) subsamples is usually not necessary for normal stream studies. Overall, the manual effectively integrates analysis methods, safety guidelines, quality

assurance/control, and report writing in a much richer sense than the *Field Manual for Water Quality Monitoring* (Mitchell and Stapp, 1995).

Volunteer Monitoring: Water Quality Protocol Manual

A Rhode Island stream monitoring manual, *Volunteer Monitoring: Water Quality Protocol Manual*, provides one of the more in depth presentations on fecal coliform bacteria (Kerr and Lee, 1992). Sources of the bacteria were listed as decaying vegetation in soils, feces of many kinds of organisms, leachate from failed septic systems, direct discharges of untreated sewage, leaking sewers, and sanitary landfills. The method of analysis used by the Rhode Island Department of Health is the most probable number (MPN) per 100 mL. When sampling, users of the manual are cautioned to avoid stirring up the bottom, algae mats or debris on the water surface, oil slicks or scum, water fowl or bird droppings, and prop wash (Kerr and Lee, 1992). Users are told that there is no way to differentiate fecal coliform bacteria from non-human wastes such as that from waterfowl, livestock, and family pets (Kerr and Lee, 1992).

A common thread in most stream monitoring manuals is macroinvertebrates. Neither the Maryland Streamwalk Manual (Hosmer, 1988) nor the Ohio Stream Quality Monitoring Program (Kopec and Lewis, 1989) listed bacteria as a measured parameter. The Maryland program emphasized sediment stabilization.

Testing the Waters

Behar (1996) of the River Watch Network takes a different approach to water quality monitoring for bacteria in her monitoring manual, *Testing the Waters*. Instead of focusing on water quality tests and indices per se, her goals are to provide:

- (a) solid background information that educators can use to design their own curriculum around a river monitoring program and community volunteers can use to gain a better understanding of what, how and what to monitor;
 - (b) clear information and technical procedures for implementing a river monitoring and protection program;
 - (c) activity ideas dealing with concepts underlying the water quality study to help teachers better integrate a river monitoring program into their curriculum.
- (Behar, 1996 p. 2)

Instead of mechanically using standard water quality tests, Behar's and the River Watch Network's approach is to begin with the premise that how and why you monitor depends on the specific questions one has about a river or stream. Study design, including determining the purpose of monitoring, what will be monitored, data goals, sample analysis methods, monitoring location and time, and quality assurance/control are all necessary elements to be in place before students go to the field for any monitoring. This planning element is not as evident in the manual by Mitchell and Stapp (1995). Behar (1996) approaches data collection from the point of view of creating "information" from the raw numbers through use of graphs and statistics. This was a featured topic at the 1996 National Volunteer Monitoring

Conference and it is receiving a lot of attention in stream studies throughout the nation.

Fecal indicator bacteria are discussed in *Testing the Waters* in a separate section, although detailed methods are not given. This discussion is more sophisticated than other resources for teachers, and is based on why a site is being monitored (i.e., for health reasons or to see if the site meets state standards). Behar (1996) indicates that fecal indicator bacteria are found in cold as well as warm-blooded animals. The ubiquitous nature of bacteria and the interference of *Klebsiella* on fecal coliform counts are pointed out. She also mentions other impacts of fecal material on water bodies such as cloudy water, unpleasant odors, and increased oxygen demand. There is a credible discussion of point and nonpoint bacterial contamination. The link between rainfall, flow, and bacterial counts is made. The importance of collecting flow and weather information while sampling is noted. In a discussion of how coliform colonies are measured, Behar (1996, p. 166) makes an important point that Mitchell and Stapp (1995) miss: "each bacterium on the filter grows into a colony, which becomes visible to the eye." This is a simple, but important, statement that should be in all discussions about using membrane filtration in monitoring for bacteria.

There are, however, some problems with the fecal coliform narrative. Behar's (1996, p. 164) statement that *E. coli* "does not grow in the natural environment under ordinary circumstances" is not substantiated in the literature.

Her use of a the Vermont *E. coli* standard of 77 colonies per 100 mL as well as the risk numbers she presents are misleading based on the U.S. EPA standard. Also, her use of mTEC medium for both fecal coliform and *E. coli* is not standard use since this medium is more appropriate for just *E. coli* (U.S. EPA 1985). However, overall, *Testing the Waters* (1996) represents a credible approach to monitoring.

Project WET

Project WET has rapidly become a nationwide influence on how water issues are integrated into the classroom. Its impact is likely to be on the order of two other major environmental education programs, Project WILD and Project Learning Tree. The Project WET curriculum framework has three major areas: conceptual, affective, and skills with field-tested activities that are aligned with standards, such as *Benchmarks for Scientific Literacy* from the American Association for the Advancement of Science (AAAS, 1993).

Although there are no specific activities on monitoring for bacteria, Project WET takes a health-based approach to bacteria. In the *Project WET Curriculum & Activity Guide* (Project WET, 1995), there are activities for tracing the source of contagious disease using the 1854 London cholera epidemic as an example ("Poison Pump") and follow-up activities on waterborne disease ("Super Sleuths") and transmission of disease ("No Bellyachers"). Transport of bacterial contamination is mentioned in a groundwater study ("Get the Groundwater Picture"). Another

activity links water quality and water treatment while developing water quality concepts related to dilution ("Reaching Your Limits"). Strategies to remove contaminants from groundwater are covered ("Sparkling Water"). A strong environmental education component is found in an exercise where students do in-depth research on problems of increased demands on a community wastewater treatment plant ("Super Bowl Surge"). These types of activities are valuable contextual extensions for student monitoring of bacteria. However, to be most effective, many WET activities require a solid science background which elementary teachers, in particular, often lack.

Water Sourcebook

The Georgia Water Wise Council facilitated the development of a 1997 publication of water lesson plans for high school teachers, *Water Sourcebook* (Auburn University et al., 1997). The publication has the endorsement of the U.S. Environmental Protection Agency as well as the Water Environment Federation. This book had directions for testing for fecal coliform bacteria that simplify the process to the point where any results would be suspect. Many of the normal precautions on sterility were not there. Forceps were called "tweezers", and the need to maintain a constant incubation temperature was glossed over.

A diagram of the membrane filtration procedure in the *Water Sourcebook* has the following six-step sequence which is critiqued:

1. *Collect sample. Dilute, if necessary with water.* The picture shows water from a tap being poured into a Whirlpak™ bag. Is the student to assume that tap water is used for dilutions?

2. *Mix medium. Add medium to petri dish.* These directions are unclear. The way this should read is "prepare the medium and pour it on a sterile absorbent pad in the petri dish."

3. *Vacuum-filter sample through membrane filter. Rinse filter.* There is no indication that a filter needs to be placed in the filter holder and no diagram of what a membrane filter looks like. How is the water sample transferred to the filter holder? What is used for rinsing? What volume is used?

4. *Incubate filter on medium in petri dish.* The picture shows an incubator that looks more like a microwave oven than an incubator. Water baths and heat sink incubators are the preferred heat sources. How does the filter (which again isn't adequately illustrated) get into the petri dish? Is it picked it up by fingers?

5. *Count typical colonies at 10-15 magnification.* A picture of a dissecting microscope and a petri dish is shown. However, the contents of the petri dish are represented by a vague criss-cross pattern. Students will actually be viewing and counting discrete colonies on a grid, yet it isn't clear what a colony is.

6. *Or confirm colonies and report results.* The picture shows a petri dish with some circles, dots, and short lines with no clear indication of what is

represented. What does "confirm colonies" mean? Why would this be done instead of a count? How are results reported?

The result for a student looking at this diagram would be confusion and a high probability that misconceptions about the membrane filtration procedure would develop. What is disconcerting about this diagram is that it is from a new publication that had many reviewers who should have easily noted the deficiencies. This casts some doubt about the credibility of the rest of the document. It is a puzzle as to how these methods were able to pass review by both the U.S. Environmental Protection Agency and the Water Environment Federation.

Nonpoint Source Pollution Prevention Environmental Resources Guides

The Air & Waste Management Association *Nonpoint Source Pollution Prevention Environmental Resources Guides* for K-12 students (A&WMA, 1992) present activities for teachers to use in nonpoint pollution lessons. These "teacher friendly" materials include lesson plans and fact sheets. One of the activities centers around testing water samples for coliforms and describing ways to control livestock pollution ("Manure Matters"). The guide states that *E. coli* are not harmful by themselves which is confusing based on human deaths from *E. coli* contaminated food. One would hope teachers take note of the health warnings alluded to elsewhere in the narrative.

Instructions for testing for bacteria are minimal. *Coli-test*[™] or similar unspecified fecal coliform kits are recommended for sampling at multiple sites. Students are told to "collect the samples", period, - in what? what volume? The directions contain a note that the teacher should take samples or that students need to carefully disinfect their hands after sampling. Sampling with disposable plastic gloves would be a better strategy. There is no mention of sterile technique and disposal of samples. Essentially, the activity is a presence/absence test that might be only appropriate for a cursory view of coliform problems for lower grades. It is doubtful that an inexperienced teacher would have sufficient information to do much with this lesson. This could lead to frustration and a reluctance to be involved in monitoring for fecal coliform bacteria.

Model-It Computer Program

The A&WMA (1992) fact sheet on bacterial water pollution initially approaches the problem from a decomposition angle. The principal detrimental effect to a water body by bacteria is said to be depletion of oxygen. This is mirrored in a computer program, *Model-It*, for middle and high school students. The program will soon be available on the Internet. This interactive program uses factors which are measured or calculated to discern both immediate and long-term water quality relationships. Fecal coliform colonies are one of the factors for the modeling program. Unfortunately, the simulations of the relationship of fecal

coliform colonies to the other water quality parameters are not appropriate. Specifically, the program developer has linked oxygen depletion by decomposition bacteria directly to the presence of fecal coliform bacteria. Although bacteria and decomposing organic matter are associated, fecal coliform bacteria do not function as major decomposers of stream detritus. Generally, fecal coliforms are intestinal bacteria, and organic matter in water columns in streams is not the usual food supply for these organisms. Rather than fecal coliform bacteria, the organisms for modeling purposes that relate to dissolved oxygen decreases are the quickly growing, rich-food-loving mixed populations of sewage associated organisms including bacteria, fungi, algae, protozoa, and rotifers. In streams severely polluted with organic matter, the "slime" layer is likely to be a mix of bacteria such as *Sphaerotilus*, *Zoogloea*, and *Beggiatoa* as well as aquatic fungi and ciliated protozoans, but not fecal coliform bacteria (Tarzwell and Gauvin, 1967).

Internet and Computer Resources

The Internet is used by students throughout the world to communicate water quality results and is quickly becoming a research tool. The Internet can be a connection to both scholarly research as well as information of dubious value that perpetuates misconceptions. A search for "fecal coliform" on the Internet revealed numerous sites that can be categorized as fact sheets about fecal coliform bacteria, scientific research results, university course materials, and stream/lake monitoring

results. For instance, the Hillsdale-Lenawee-Monroe Math Science Center in Michigan has an interactive web site for students to enter their monitoring data. WETNET is a project to display and manage water quality data from 60 schools in Michigan's Saginaw Basin. This project uses the Mitchell and Stapp (1995) water quality index. The Vermont Rivers Project also maintains a student monitoring database. Stream Watch in Australia is another example of a community water quality monitoring program.

Both the Hillsdale and the Australian sites imply that *E. coli* is harmless which is misleading given the pathogenic forms of *E. coli*. The Australian site, however, points out non-human sources of *E. coli*, mentions the possibility of airborne *E. coli*, and recognizes the contribution of sediments to bacterial contamination. Individual fecal coliform monitoring results can be found in data sets of these and other Internet sites, but there does not appear to be any long term monitoring of a single location.

Another reference is the Internet site for a Woodrow Wilson National Fellowship Foundation teacher institute held in the summer of 1997 and a subsequent student paper on fecal coliform. Teachers at the 1997 Woodrow Wilson National Fellowship Foundation Environmental Science Institute selected fecal coliform contamination as their study topic. They developed an Internet site to highlight their study of fecal coliform bacteria in runoff water on Lake Carnegie in Princeton, New Jersey (Woodrow Wilson, 1997). The teacher team established

links to stream flow data, USGS maps, and USGS station information at their site. They described their monitoring efforts using Coliscan™ Easygel™. Their zero counts were recorded as "none detected (ND <33)", which relates to a 3 mL sample size.

The site has an online environmental simulation model for fecal coliform levels using *Stella*™ software. The model relates coliform runoff and rainfall to water in the lake, coliforms entering the lake, and coliform concentration. Terms in the concept map for the model such as "coliform from rain", "coliform running in", and "coliform running out" do little to explain the dynamics of the system and the simulation numbers are not substantiated by a strong basis in reality.

Misconceptions or incomplete information noted for this site are: the idea that *E. coli* bacteria are the most abundant of a number of coliform bacteria in both animals and humans, inadequate discussion of environmental sources of fecal coliforms, equating "most probable number" to "colonies per 100 mL", and imprecise methods for use of Coliscan™. The writing lacks references and the overall style illustrates a degree of naiveté, it is not scientific, and it has poor structure (e.g., poor structure example "In 1994-1995, fecal coliform was the leading contaminant most commonly found in excess in water of federal health standards").

A very serious error is that the teachers counted total coliform colonies on the Coliscan™ pour plates and then came to the conclusion that "levels of fecal

coliform were different after rainfall" (Woodrow Wilson, 1997, p. 10). There is no basis for this conclusion since Coliscan™ only screens for total coliforms and *E. coli*, not fecal coliforms. Nevertheless, this Internet site illustrates one educational use of microbiological monitoring and provides an example for dissemination of student study results.

A student paper on the Internet entitled "A study of fecal coliform in Bean Creek over the course of twenty-five weeks" is an interesting case study that illustrates use of the scientific method but also contains misconceptions (Lay, 1997). The purpose of this study, by a high school student from Michigan, was to determine whether fecal coliform counts in a local creek remained consistent over a period of time. He monitored for fecal coliform bacteria using membrane filtration at five different sites along Bean Creek in Michigan. His paper on the Internet was well organized with an abstract, introduction, materials and methods, results, discussion, and references.

As with the Woodrow Wilson group, there were some inaccuracies, misconceptions, and unsubstantiated statements in his report (Lay, 1997, p. 1-5). Examples are: *E. coli* was called "echinonococcus" and "a member of the *E. coli* family is fecal coliform." Also, "the fecal coliform colonies are harmless by themselves, but carriers of disease" and "they can only spread by contact and are not airborne." Additional misconceptions are: "Fecal coliform is a naturally occurring bacteria caused by the digestion of food in warm blooded animals" and

"fecal coliform is the primary test for water quality because of it's possible harmfulness to biological organisms." There were problems with data analysis: "data gathered from one sample has a variability of up to 30%." Results for colonies per 100 mL were given to two decimal places, i.e., 130.83 colonies per 100 mL. Some statements did not make sense: "the origin of water in a storm sewer pipe is suspected to originate from several manhole covers in the vicinity."

Although the student seems to have a good grasp of sterile technique as well the concepts of replicates and controls for his fecal coliform laboratory work, some of his descriptions of his methods are a bit unorthodox . For example, "I squirted an ampoule of fecal food onto the pad" (Lay, 1997, p.3). To determine colonies per 100 mL, the student "multiplied them by their division into 100" (Lay, 1997, p. 4). He calculated arithmetic means rather than geometric means and was inconsistent in the use of whole numbers for colony counts.

Standard deviations were calculated with the comment made "the high standard deviation could also be attributed to luck by getting large numbers in the initial weeks followed by a little luck in the closing weeks getting smaller numbers" (Lay, 1997, p. 5). Standard deviation is not a particularly useful statistical parameter to use with fecal coliform data. Standard deviations are very sensitive to outliers, in particular to high counts that are typical of these data. Much of the data discussion could have been better presented in a graphical format. On a positive note, Lay (1997) recognizes the limitations of his study and suggests some further

research such as sampling at different depths, 24 hr sampling, and correlating fecal coliform levels with precipitation and flow rates from drains.

The Internet examples raise some serious issues about the Internet as an accurate source of information for students. Myths and misconceptions can become perpetuated through Internet sites, and there is often a lack of well-informed scientific peer review of studies published on the Internet. Internet misinformation has the potential to undermine scientific knowledge in that the student may be led astray by accepting anything as valid science.

The lessons learned from the analysis of student and teacher efforts is that there are many points of confusion regarding fecal coliform bacteria. These can be categorized as taxonomic relationships, relationships between fecal coliform bacteria and pathogens, habitat of fecal coliform bacteria, laboratory methods, and analysis of monitoring data.

All of the above are illustrated in an article by a student intern with the West Michigan Environmental Action Council (DeGoot, 1997, p. 1) in *Adopt-a-Stream* entitled *E. coli and Our Water Systems*. This publication has local distribution to teachers and adults involved in the council's stream monitoring and restoration program. Quotes from the article followed by the dissertation author's comments are as follows: "*E. coli* belongs to a family of bacteria called the fecal coliforms." [she is unclear about taxonomy]. "When *E. coli* enters our water systems it begins to decay. In the decomposition process dissolved oxygen is used up and ammonia

is released to the stream." [*E. coli* is confused with decomposers]. "*E. coli* does not cause diseases." [not true]. "*E. coli* is essential to food digestion." [not true]. "Regional health departments take monthly samples from Michigan's lakes and streams to test for *E. coli*." [most health departments do not monitor and they are more likely to be bi-monthly from April to October]. "In 1996 *E. coli* levels were significantly lower than 1995." [where?]. "In order to draw effective conclusions from *E. coli* test results, the system of testing would have to be elaborated and reorganized. This would require exhaustive studies that would be very costly." [nebulous and vague statements made without a reason, what studies?] It is evident that the article's author has a number of misconceptions about indicator bacteria.

Instructor Survey

Teachers are justifiably concerned about high fecal coliform levels if they are doing stream sampling. Ed Arnold, a Michigan teacher, stated that he does not take his classes to a nearby creek anymore since Health Department sampling revealed high counts of fecal coliform bacteria. He states in an article in the *Grand Rapids Press* (Sinkevics, 1995, February 5) that "with this high bacteria count, it has scared me off. I'm concerned for the health of my students."

However, in a survey of attendees at the 1996 GREEN Conference, teachers were found to avoid sampling for bacteria, not because of health reasons, but, for lack of a suitable incubator, difficulties with sterile technique, and expense of the

tests. This hesitancy to do bacterial monitoring was also reflected by some of the participants at the U.S. EPA National Volunteer Monitoring Conference in 1996. The dual chamber incubator and equipment used in the research for this dissertation had a cost of over \$2,000. In an established fecal coliform volunteer monitoring program in Maine, the greatest cost was for the incubator (\$800), and a germicidal UV bulb in a box that was used for sterilization (Stancioff, 1991). Some groups, such as the fourth grade students involved in the Great Lakes Education Program of Sea Grant Extension in Michigan, use a presence/absence test for bacterial monitoring instead of the membrane filtration or multiple tube fermentation. This test does not require elaborate equipment or precise temperatures for incubation.

Participants in a bacterial monitoring workshop at the GREEN '96 conference ($n = 36$) completed a survey about their monitoring programs. The participants included predominantly high school teachers, river project managers, and university faculty. All but two of the participants were monitoring for bacteria and almost all used membrane filtration. One teacher used the *Colilert*TM system and another used Millipore paddles. Most of the monitoring was for fecal coliforms but three respondents monitored total coliforms, one monitored both FC and TC, and one monitored *E. coli*. Zero to 100 colonies per 100 mL for FC and zero to 1,000 colonies per 100 mL for TC were the general ranges given for bacterial counts of the streams monitored by the participants who were from throughout the United States. A teacher in New Hampshire noted that her total

coliform counts were formerly 0 to 16,000 colonies per 100 mL in 1984 but are now 0 to 1,000 colonies per 100 mL. Frequency of monitoring varied from "when we can borrow equipment" or once a year to 20 times per year.

Monitoring problems encountered included consistency, expense of equipment, complex procedures, data interpretation, spoilage of media, membrane contamination, lack of training, sterility, expense of duplicate samples, and cost of the incubator. Student conceptual difficulties mentioned included fuzzy understanding of what a "blue dot" on a membrane filter represents, inability to grasp the connection between fecal coliform and pathogens, unwarranted extrapolation from a single sample result to the overall quality of a stream, difficulties in converting colony counts to colonies per 100 mL, and confusion involving relationships between coliforms, fecal coliforms, and *E. coli*.

Participants in teacher training workshops by the Grand Valley State University Water Resources Institute in 1995 ($n = 23$) were surveyed prior to the workshop about their knowledge of monitoring for bacteria. Workshop participants were mainly K-12 teachers and two student teachers representing an equal distribution of grade levels. None of the teachers had ongoing monitoring programs for bacteria. The participants had zero to 30 years of teaching experience. Only 56% of the participants were able to identify coliforms, *E. coli* or fecal coliforms as indicators of microbiological water quality. Approximately 30% of the participants associated agar plates or petri dishes with testing for water

quality bacteria. Only one participant seemed well informed on the overall method of sampling and incubation. Of particular concern is a student teacher with 15-20 science courses who thought *Oscillatoria* (blue green algae) was the indicator for bacterial contamination and that pH testing somehow was used to test for bacteria (his/her other answers were reasonable, however). Another respondent indicated that the oxygen content in water is the test for the presence of bacteria which relates back to equating decomposers to pathogens. In general, middle school and high school teachers seemed more knowledgeable about monitoring for bacteria than lower elementary teachers, but they also reported a greater number of science courses. The number of science courses taken by participants was significantly correlated with correct/reasonable responses ($r = 0.63$). In general, attendees at these training workshops that the author facilitated appeared to have both a lack of knowledge and a number of misconceptions regarding microbiological monitoring.

Summary

In summary, numerous sources of misconceptions, inaccuracies, and superficial coverage regarding fecal coliform and *E. coli* bacteria, were found in water education materials. A survey of Internet resources, computer software, student projects, and teacher knowledge about indicator bacteria also revealed difficulties with concepts relating to microbiological water quality indicators. This is unfortunate since *E. coli*, in particular, has been frequently mentioned in the

popular press. The usual context is food contamination, but it is also associated with "no swimming" warning signs posted at streams and lakes. Students frequently hear about beach closings due to bacterial contamination, yet available teacher materials and student activities do not always reflect the microbiological conditions of water. Teachers would be unable to present an accurate portrayal of the bacterial contamination problem to their students if they were to rely solely on some of the commonly used publications. In some of the newest publications, there is the wide range of presentation of concepts regarding fecal coliform bacteria from very good to unacceptable and misleading.

CHAPTER V

RESULTS OF FIELD STUDIES

Background Reference Site Sampling

It is important for teachers have a context in which to frame their bacteriological monitoring data, especially if they have only a few monitoring events per year which seems to be typical of many student monitoring projects. Ideally, the headwaters of a stream should have zero or very low fecal coliform counts. Reaches of streams with elevated counts should be associated with specific pollution sources. However, typical fecal coliform counts in Michigan and many parts of the midwest agricultural regions more often than not will exceed water quality standards throughout a watershed. There may not be any particular source that can explain the high counts. This raises the question as to whether there is an "endemic" background level of fecal coliform bacteria.

Groundwater Seep

To answer this question, two years of monitoring for fecal coliform, *E. coli*, and total coliform bacteria were performed on a groundwater seep located in Lowell Township, Michigan. The specific location has been described in the

Design and Methodology section. The monitoring involved year-round sampling of the seep and the seep drainage origin, henceforth referred to as the "wetland" area. Monitoring of bacteria during storm events, sediment sampling, litter sampling, transects from the seep to its banks, slides placed *in situ* in the seep, and focused spot sampling in the seep to determine the origin of the bacteria were all performed. Monitoring results in the seep as they relate to ambient daily air temperature means, daily precipitation, and seep temperature were explored. In all, there were 162 sampling events from July 1995 through July 1997.

The seep was selected as a possible reference site for the midwest climatic conditions. Within the San Lorenzo River system in a Mediterranean climatic region of California which the author monitored, there was a reference site, Waterman Gap (Appendix A, Figure 8), which consistently had zero or very low counts of fecal coliform bacteria. The hypothesis tested was that fecal coliform and *E. coli* counts in the Michigan seep would be essentially zero throughout the year if this site follows the pattern of the California site. If counts were consistently above zero, then the site would be more similar to tropical sites.

In 1995-97, monitoring was performed at a designated site in the "seep" where water flows throughout the year and at one of the origins of the seep which is a year-around mucky wetland area upstream of the previous sampling site. Even in mid-winter, the temperature of the wetland area was warm enough to melt the snow and there were areas of standing water. The temperature of the seep water

varied throughout the year from about 0°C to 22°C. Much of the seep retained snow cover in winter but water flowed in a channel underneath the snow. Thus, sampling was possible throughout the year in the seep and the wetland even when snow cover was present. Sampling of the seep did not generally present difficulties, except in January and February, when air temperatures were below 0°F and snowcover was present, or in very low flow periods related to drought, as found in September 1995. At times, the seep had an accumulation of leaves and muck, at other times the seep bed was scoured revealing a sandy bottom. There did not appear to be any particular correlation between the substrate and the bacterial counts.

As shown in Table 1, the fecal coliform (FC) bacterial count for the seep location (n = 57) varied from 0 to 14,000 colonies per 100 mL. The geometric (log) mean for fecal coliform colonies was 524 colonies per 100 mL with an arithmetic mean of 1,841 for the 1995-97 sampling period. The *E. coli* (EC) counts in the seep (n = 54) varied from 5 to over 11,600 colonies per 100 mL (too numerous to count) with no samples with zero colonies. The geometric mean for *E. coli* colonies was less than that for fecal coliforms at 219 colonies per 100 mL with an arithmetic mean of 824 colonies per 100 mL for 1995-97. Total coliform counts (n = 53) for the seep ranged from 55 to over 33,900 colonies per 100 mL (too numerous to count). The geometric mean for the coliform (TC) colonies was 1,122 colonies per 100 mL with a mean of 3,042 for 1995-97. The ratio of geometric means was 2.14 TC to 1 FC to 0.42 EC. This is consistent with the fact

that total coliforms is the most inclusive group followed by the fecal coliforms and *E. coli*, which, as a single species, is the least inclusive.

Table 1
Summary Statistics for Groundwater Seep Bacterial Monitoring, 1995-97

Statistic	Fecal Coliform	<i>E. coli</i>	Total Coliform
Geometric Mean, colonies per 100 mL	524	219	1,122
Exceedance of standard*	74%	65%	41.5%
Arithmetic Mean, colonies per 100 mL	1,841	824	3,042
Standard Error	336	233	824
Median	1,000	265	1,280
Mode	0	20	70
Standard Deviation	2,533	1,714	6,001
Sample Variance	6,418,582	2,938,417	36,017,526
Kurtosis	9.28	30.21	18.42
Skewness	2.68	5.03	4.15
Range	14,000	11,595	33,845
Minimum	0	5	55
Maximum	14,000	11,600	33,900
Sum	104,963	44,493	161,202
Count	57	54	53

* 200 colonies per 100 mL for FC, 130 colonies per 100 mL for EC, and 1,000 colonies per 100 mL for TC.

Some other statistical parameters found in Table 1 are difficult to interpret since they were calculated based on the arithmetic mean which is higher than the geometric mean for this type of environmental data. Note also that the median values are also greater than the geometric means. In order to translate the standard deviation, variance, and confidence limits to a geometric mean, a log transformation of the bacterial counts would have to happen followed by calculation of the summary statistics for the log values. These statistics would then have to be transformed back to the original scale by taking antilogarithms of the values with additional conversion equations. The utility of this conversion for student monitoring data is questionable at best. Graphical analysis is better suited for ascertaining data trends as demonstrated in the following discussion.

Since a normal distribution of data is important for statistical purposes, the distribution of the log of the bacterial counts from low to high has been plotted for visual examination (Figure 1). Logarithmic transformations are useful when data are markedly skewed which they appear to be (FC skewness = 2.68; EC = 5.03; TC = 4.15 from Table 1). Neither the FC, EC or TC log distribution is a straight line which would be expected for a normal distribution. The shape and the tails of the curve are similar for each of the three groups of bacteria that were monitored.

For the two years of sampling, the seep fecal coliform counts exceeded 200 colonies per 100 mL 74% of the time (Figure 2). Counts below 200 colonies per 100 mL were not recorded in July through November. Winter and spring counts

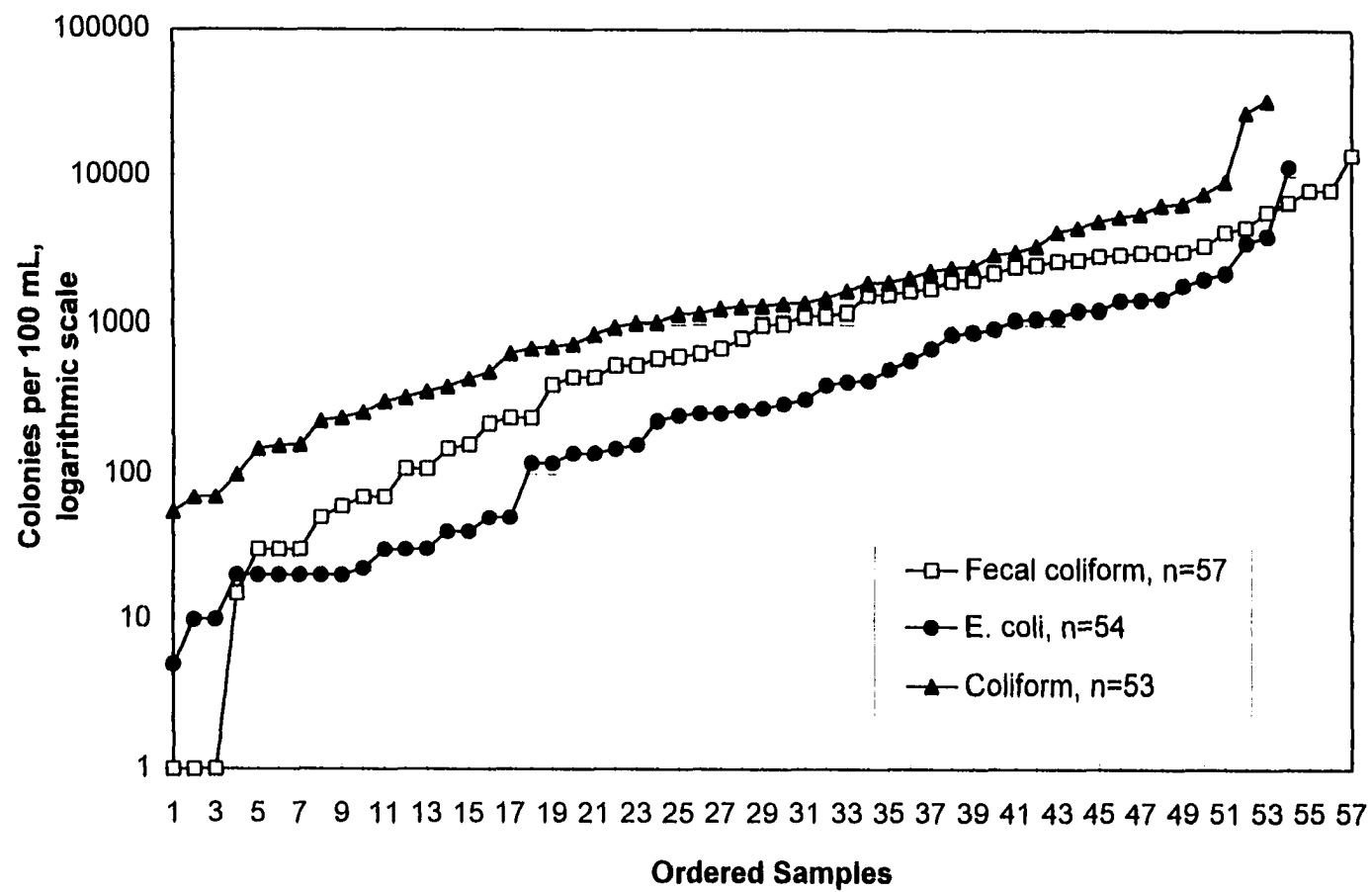


Figure 1. Distribution of Bacterial Counts in the Groundwater Seep, 1995-97.

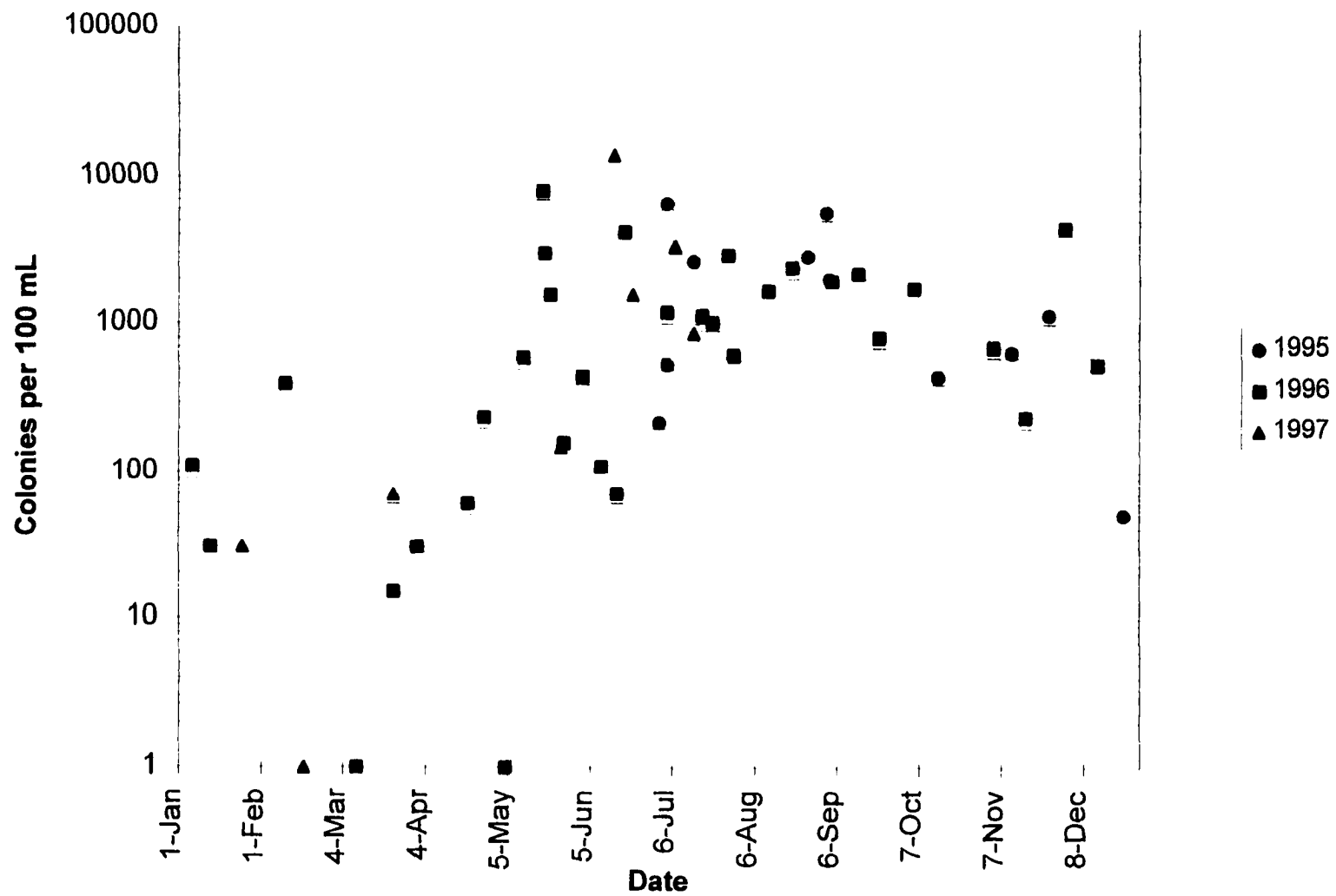


Figure 2. Fecal Coliform Counts in the Groundwater Seep, 1995-97.

were more likely to be below 200 colonies and highest counts were between May and October. However, a fecal coliform count of 4,460 colonies per 100 mL was recorded in December 1996. There were only three occasions (3/9/96, 5/4/96, and 2/17/96) when the seep fecal coliform counts were zero. There appears to be a cycle of high counts during the warmer months and lower counts in the cold months.

During the course of the study, there were some unusual weather conditions from 17 August 1995 through 16 September 1995. There was no significant rainfall during a time when normal summer rainfall is about three inches. This made sampling of the seep difficult but it also provided an opportunity to look at the system in a precipitation regime similar to Santa Cruz, California. Water samples in the seep sampling site taken when the water was stagnant had fecal coliform counts ranging from 2,000 to 5,700 colonies per 100 mL.

During 1995-97, *E. coli* counts ($n = 54$) in the seep exceeded 130 colonies per 100 mL 65% of the time with no zero counts (Figure 3). Like the fecal coliform counts, there appears to be a summer peak followed by a slight decrease and then an increase in late fall. Low counts were scattered throughout the year, but were more likely in the winter months. Highest counts occurred in June, July, September, and November. The high fecal coliform count in December 1996 was reflected in an equally high *E. coli* count of 3,900 colonies per 100 mL.

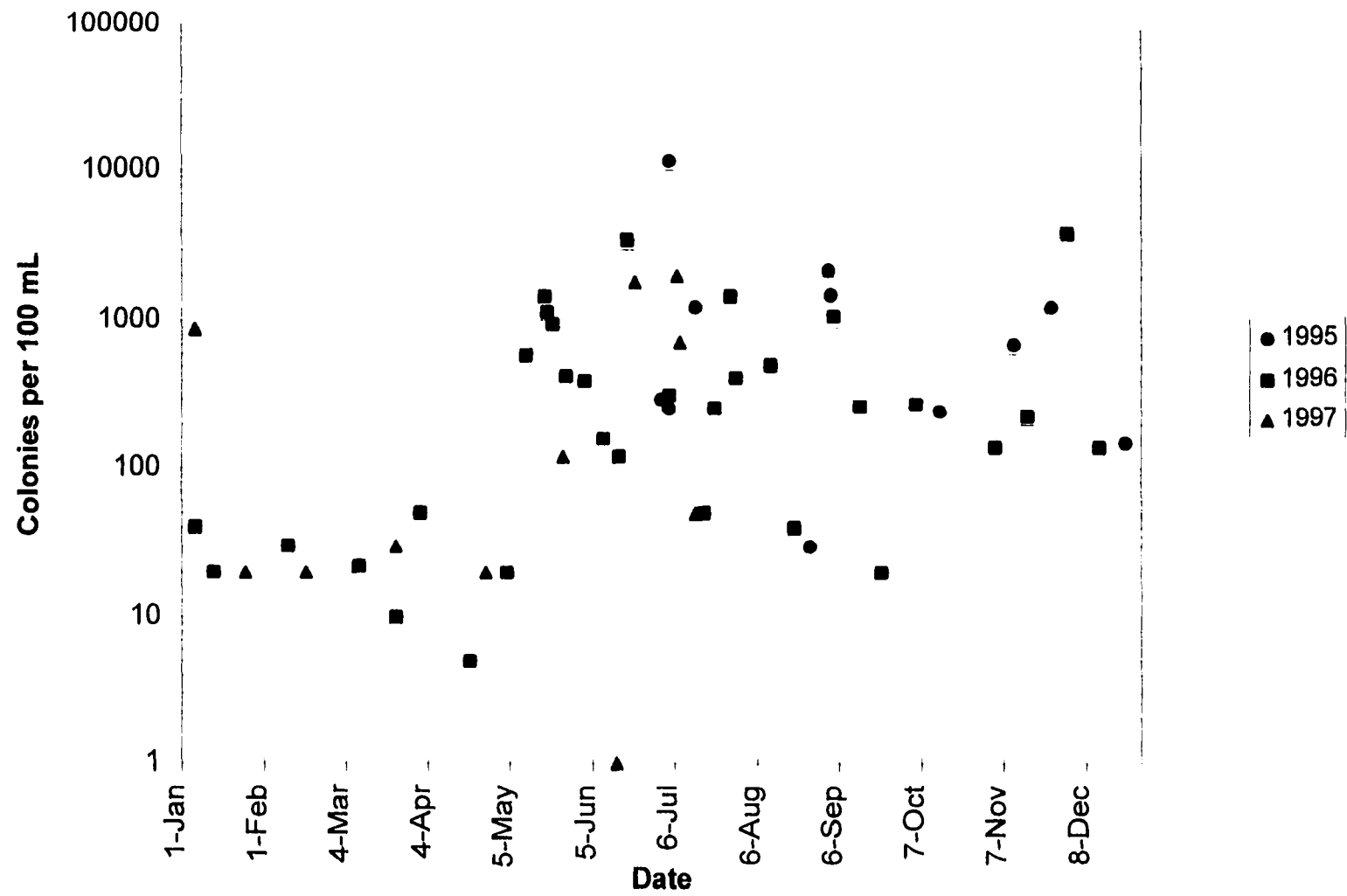


Figure 3. *E. coli* Counts in the Groundwater Seep, 1995-97.

Total coliform counts were consistently higher than either fecal coliform or *E. coli* in the seep and had the greatest range of colony counts (Figure 4). All samples had coliform colonies and frequently it was difficult to get an accurate colony count due to the preponderance of coliform colonies which is inherent in the m-Coli Blue™ methodology. Both *E. coli* and the other coliforms are on a single plate so in order to have *E. coli* counts within a range of 20 to 80 colonies, there were often three times as many coliforms to count. There were six seep samples where the total coliforms were too numerous to count. These data are, therefore, of use only for comparing relative amounts of total coliforms and should not be regarded with the same reliability as the fecal coliform and *E. coli* counts. Low counts were in the winter months and higher counts were in June through October reflecting the trends of fecal coliforms and *E. coli*.

During each sampling event, the temperature of the groundwater seep was measured. The predicted groundwater temperature for the study area was between 9.6 to 10.6°C with a range of about 0 to 20°C. The seep water temperature varied from just above 0°C to 22°C. Pearson correlation coefficients for sample temperatures and the bacterial counts were 0.41 for fecal coliform, 0.16 for *E. coli* and 0.21 for total coliform. The correlation coefficient was 0.19 for all of the bacterial counts. This suggests that higher bacterial counts occurred at warmer ambient sample temperatures (Figure 5). Linear regression analysis revealed a relationship of $y = 105.52e^{0.127x}$ ($R^2 = 0.1011$).

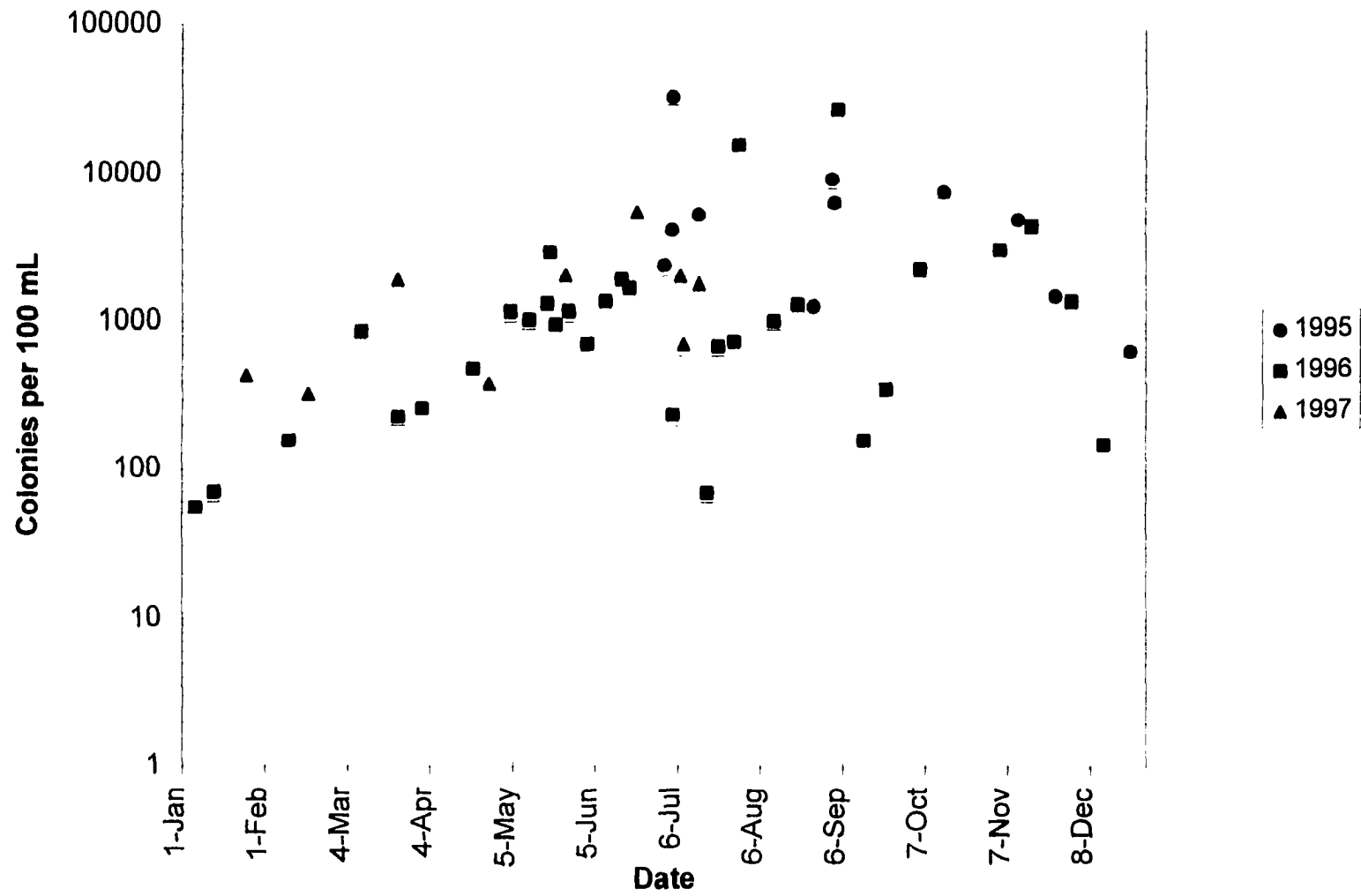


Figure 4. Total Coliform Counts in the Groundwater Seep, 1995-97.

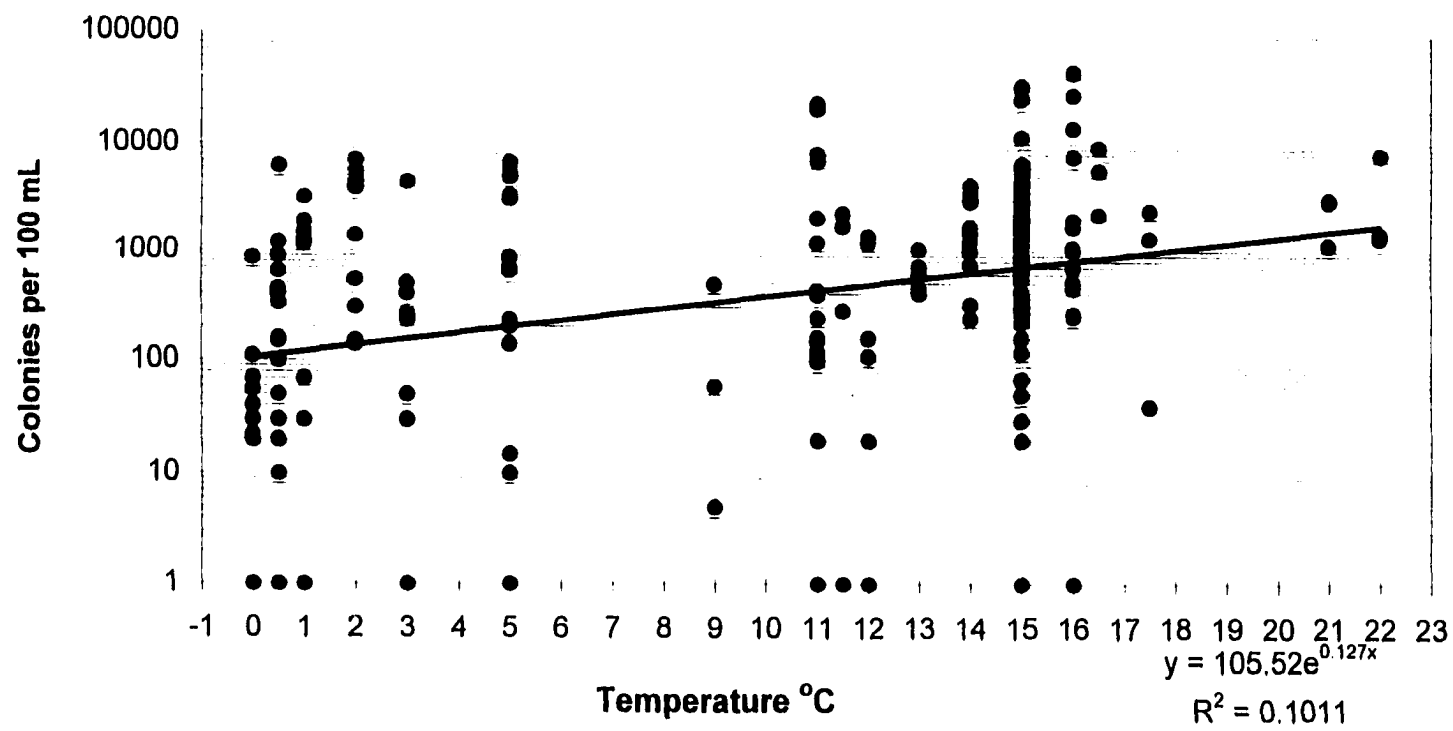


Figure 5. Relationship Between Seep Temperature and Bacterial Counts in the Groundwater Seep.

Wetland

Results of the wetland studies should be interpreted in the sense that they are essentially a presence/absence test. This is due to the methodology of collecting the soil and the difficulty in using one plate for both *E. coli* and coliforms. On an individual plate, there might be a single *E. coli* colony with hundreds of non-*E. coli* coliforms. Exceedance percentages are not appropriate for wetland analysis since these were not water samples, per se. Other than water that was associated with the soil, laboratory water made up the main volume of a sample for the analysis.

The fecal coliform counts in the wetland area ($n = 29$) were lower than the seep with a mean of 201 colonies per 100 mL and a geometric mean of 35 colonies per 100 mL for the 1995-97 sampling period (Table 2). Fecal coliform counts ranged from 0 to 2,010 FC colonies per 100 mL. Of the samples, 72% were below 200 colonies per 100 mL with 17% showing no fecal coliform. The FC analysis was not performed on all of the sample dates when the seep was also sampled. However, parallel EC and TC counts were done on a routine basis for the seep samples.

The *E. coli* counts in the wetland ($n = 42$) varied from 0 to 1,270 colonies per 100 mL with 31% of the samples having no *E. coli* colonies and 9% with greater than 200 colonies per 100 mL. The geometric mean for *E. coli* was 9 colonies per 100 mL for 1995-97 and the mean was 85 colonies per 100 mL.

Table 2
Summary Statistics for Wetland Bacterial Monitoring, 1995-97

Statistic	Fecal coliform	<i>E. coli</i>	Total coliform
Geometric Mean, colonies per 100 mL	35	9	1,445
Arithmetic Mean, colonies per 100 mL	201	85	2,273
Standard Error	77	35	304
Median	30	10	2,110
Mode	0	0	N/A
Standard Deviation	416	225	1,797
Sample Variance	173,309	50,490	3,229,107
Kurtosis	13.13	19.59	1.91
Skewness	3.42	4.16	1.22
Range	2,010	1,270	7,950
Minimum	0	0	50
Maximum	2,010	1,270	8,000
Sum	5,824	3,557	79,545
Count	29	42	35

All of the wetland samples had non - *E. coli* coliform colonies and there were many occasions where the colonies were too numerous to count. Total coliform counts for the wetland (n = 35) ranged from 50 to over 50,000 colonies

per 100 mL (estimated from "too numerous to count"). Coliform colonies in 17% of the samples were too numerous to count. The geometric mean for the coliform colonies for all but the TNTC plates was 1,445 colonies per 100 mL and a mean of 2,273 for 1995-97. The sampling of the wetland area indicates that there are viable fecal coliform, *E. coli*, and coliform colonies in wet soil throughout the year. No seasonal trends were evident for the *E. coli* and fecal coliform counts (Figures 6 and 7).

There is compelling evidence that fecal coliform bacteria flourish in the seep environment. For example, August and September 1995 were unusually dry periods for the seep area. On 2 September 1995, the usual sample site had virtually no flow yet the water column FC count was 5,700 colonies per 100 mL and EC was 2,200 colonies per 100 mL. The wetland sediment counts were 1,000 fecal coliform and 600 *E. coli* per 100 mL. A transect of the dry banks at 10 cm intervals at sampling site revealed counts ranging from 300 to 400 FC and EC colonies per 100 mL on the bank's slope to zero at the top of the bank which is less than 30 cm above the seep.

A key finding is that in some of the seep water column samples taken in 1995-97, EC and FC colonies were found to be associated with sediment particles. The sediment was due to disturbance when taking samples at times of low flow. Leaf litter samples taken from the banks of the seep had counts as high as 2,500 FC colonies per 100 mL. Litter samples near the seep taken before and after a rainstorm

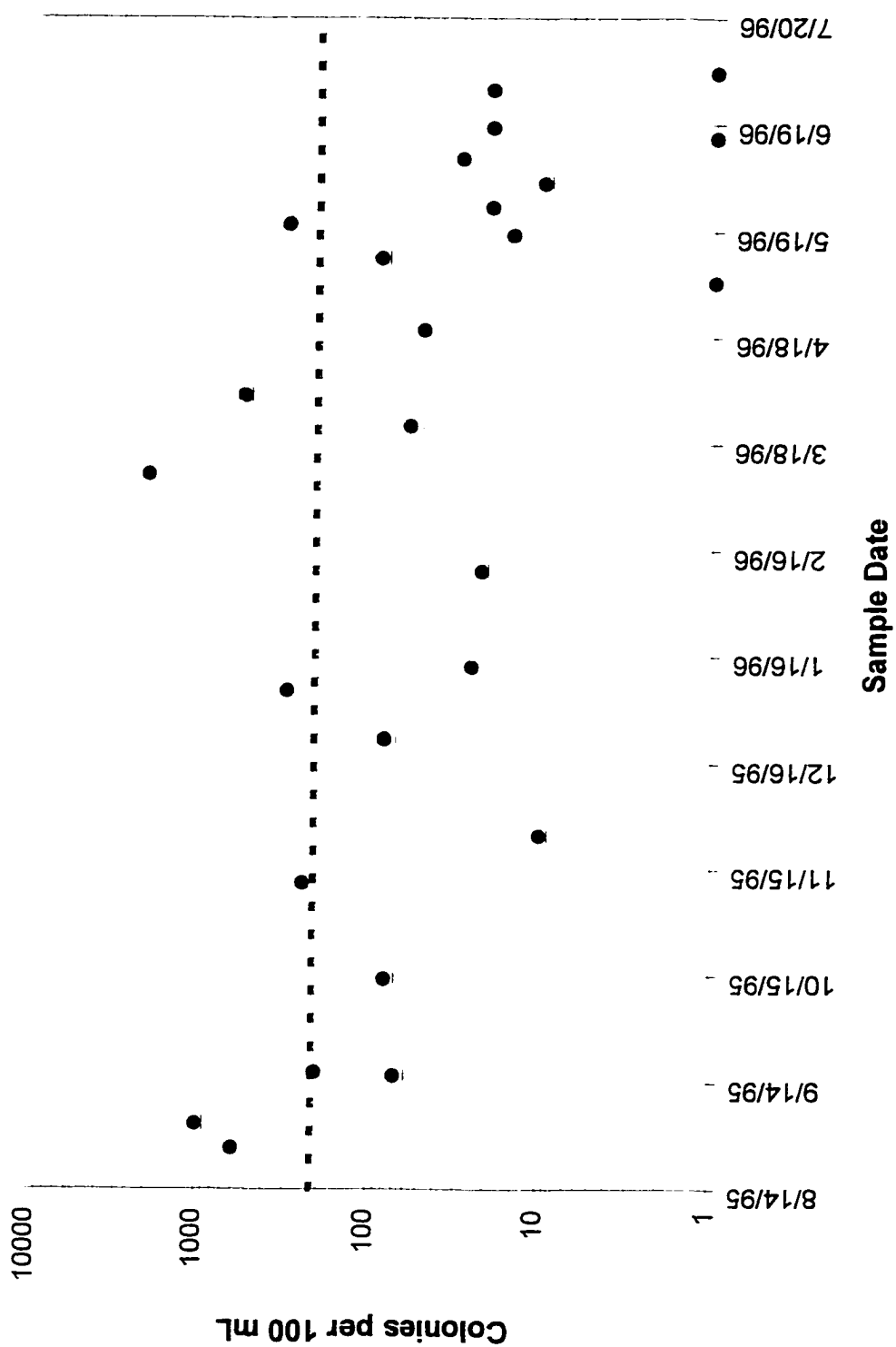


Figure 6. Fecal Coliform Counts in the Groundwater Seep Wetland, 1995-97.

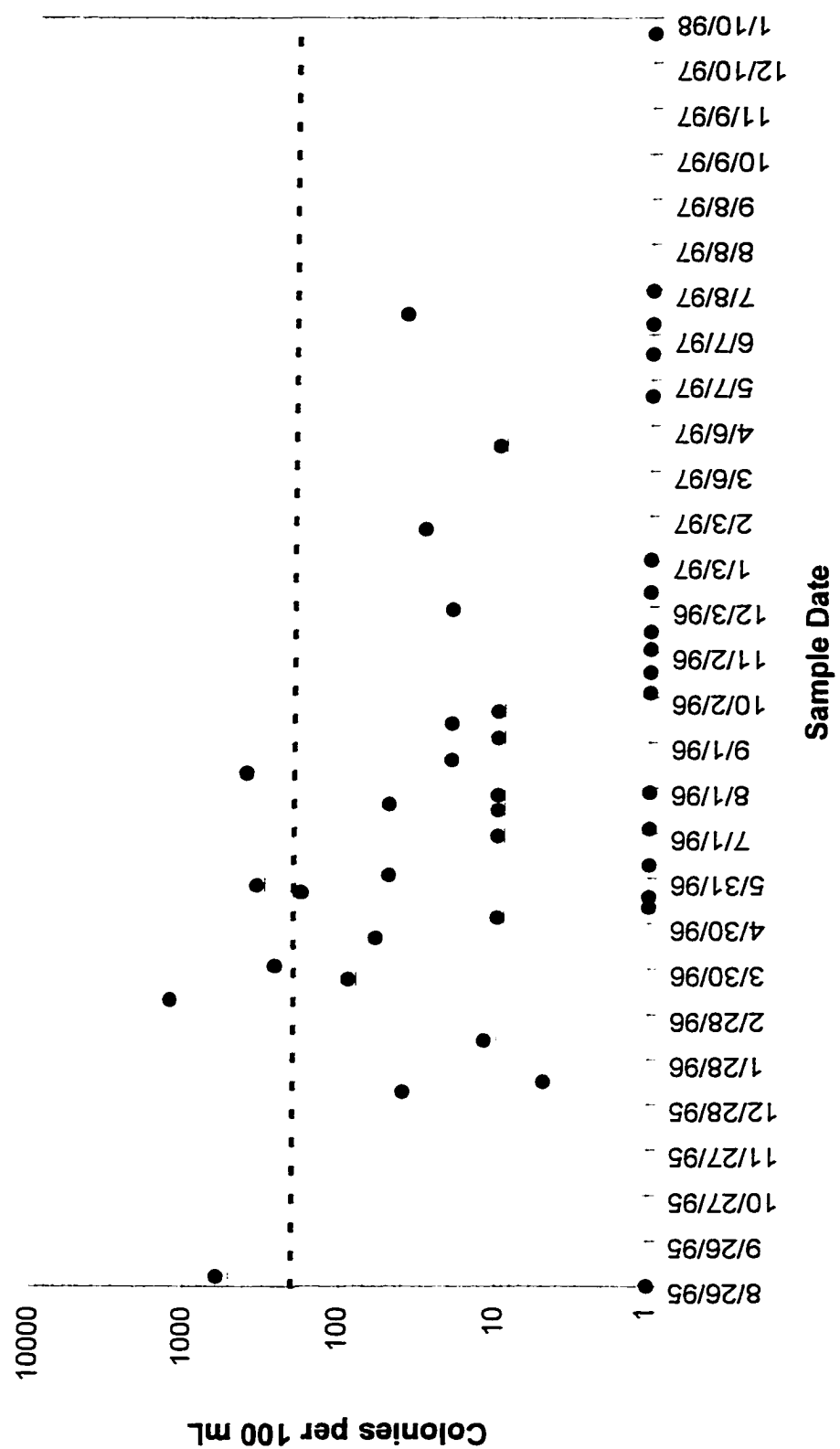


Figure 7. *E. coli* Counts in the Groundwater Seep Wetland, 1995-97.

revealed low levels of FC (52 CFU/100 mL) and EC (28 CFU/100 mL) prior to the storm and no FC and EC after a storm deposited 0.64 inches of precipitation.

In contrast, samples of litter, vegetation, and sediment from the California reference site at Waterman Gap (Appendix A, Figure 8) revealed no EC or FC with the exemption of one bank sample where there were 70 EC colonies per 100 mL. In contrast, another California site, Pacific Street, showed some similarities to the seep. This site is along the San Lorenzo River in California where ducks are frequently sighted and there is illicit human camping activity. Pacific Street had “too numerous to count” levels of fecal coliform in 10 mL samples for bank samples, vegetation, and litter. This is similar to the elevated FC counts typical of the midwest environment.

Stream Banks

As these data were gathered, the GVSU Water Resources Institute was conducting fecal coliform analyses in the Bear Creek watershed in Cannon Township which is just north of Lowell Township. Split samples were run with the GVSU Water Resources Institute Laboratory and the Kent County Health Department. In conjunction with that study, bank soil at the Bear Creek sampling sites was sampled on 11 September 1995 during a drought period. A volume of 10 mL of soil was collected at each site and mixed with 100 mL of sterile well water. It should not be construed that bacterial counts of bank samples have a direct relationship to water column counts since the sampling procedures are in no way comparable.

Comparisons of relative counts and treating bank samples on a presence/absence basis are all that it is really appropriate.

Fecal coliform bacteria were found in the banks of all but two of the fifteen sites. Fecal coliform counts in the water at the Bear Creek sites ranged from 100 to 3,125 FC colonies per 100 mL and EC ranged from 25 to 325 colonies per 100 mL. The highest count in the bank samples was 1,000 FC colonies per 10 mL of soil mixed in 100 mL of water (Waddell Creek at Cannonsburg) while water column counts of 250 FC colonies per 100 mL were noted. The second highest count was a bank sample reading of 530 FC colonies per 100 mL at Bear Creek (Cannonsburg) that corresponded to a water column count of 125 colonies per 100 mL. The two sites with no detectable FC (Bear Creek tributary at old Kreuter and Bear Creek at Cannonsburg) had water column counts of 50 and 100 colonies per 100 mL. The main significance of this study is that fecal coliform bacteria were present in the soil of the banks.

Another study on banks was performed in the sub-watershed to the east of the groundwater seep. A small stream that drains into the Grand River was studied. Sampling was done in a drought situation on 16 September 1995. Samples from a sandy bank had counts of 200 FC colonies per 100 mL, a muck area - 270, dry bank - 10, and the water column - 120. This is consistent with a study on the levels of total coliform and *Escherichia coli* bacteria in the surface soils of two upland catchments in North Yorkshire (Hunter and McDonald, 1991). These authors found

significant differences in bacterial counts between wet and dry soil moisture/vegetation zones in both catchments. This was explained by the more favorable survival conditions when soil moisture content was high enough to prevent the rapid desiccation and death of enteric bacteria. Hunter and McDonald (1991) also found significant differences between the counts of total coliform and *Escherichia coli* in soil, and they concluded that the use of the total coliform group alone may overestimate the true extent of contamination.

A transect of the bank of the seep sampling site in one foot intervals under drought conditions (3 September 1995) revealed counts of 300, 400, and 0 fecal coliform colonies in 10 mL volume samples mixed with 100 mL of sterile well water. Relative moisture conditions along the transect were wet, moist, and dry. That day, the seep itself had 2,000 FC colonies per 100 mL. This pattern on the bank transect was again found on 16 September 1995 with much lower counts (20, 10, 0 respectively). Sampling after a rain event on 17 September 1995 revealed counts of 1,000 FC CFU/100 mL in the seep water which was less than the previous day, and bank counts of 160 to 200 CFU/100 mL that were higher than the previous day. Additional bank counts in September and October 1995 were similar to 16 September (40 - 50 in wet areas and 0 in dry areas). It was not until December, when about 2.5 inches of snow was on the ground, that bank counts were zero. Again, fecal coliform is associated with soil on the moist banks of seeps and streams. This mirrored the results of studies by Hunter et al. (1992) in England that suggest a direct causal link

between stream water bacterial levels and the magnitude of bacterial input from the banks of the stream.

Climate Considerations

Other parameters that might be associated with bacterial counts were explored. An analysis was made of ambient mean air temperature and precipitation for the sampling date and the previous two days as compared to the bacterial counts (Table 3). The values for temperature and precipitation were obtained from the National Weather Service Station at Grand Rapids, Michigan. For precipitation, the highest correlation was for total coliform and the precipitation on the sample day ($r = 0.26$). There was a pattern of fecal coliform counts in the seep that appeared to be related to ambient mean temperature for the sampling day, day before, and two days before ($r = 0.32, 0.32, 0.34$). Interestingly, a negative correlation coefficient was found for fecal coliform and ambient temperature in the wetland ($r = -0.29, -0.25, -0.17$) as well as for *E. coli* and ambient temperature ($r = -0.26, -0.23, -0.15$). The overall seep bacterial counts exhibited weak positive correlations with temperature and with precipitation on the sample day. The wetland correlation coefficients were frequently negative. The correlation coefficients do not suggest a strong influence of ambient temperature or precipitation on the bacterial counts in the seep or the wetland.

Table 3

Correlation Matrix for Groundwater Seep and Wetland Bacteria With
Precipitation and Ambient Air Mean Temperature for
Sampling Day and Previous Two Days

Bacteria Type	n	Precipitation			Temperature		
		Two Days Before	One Day Before	Sample Day	Two Days Before	One Day Before	Sample Day
All Bacteria*	336	-0.027	-0.046	0.080	0.079	0.099	0.091
Seep FC	56	0.168	0.002	0.094	0.336	0.316	0.315
Seep EC	73	0.043	-0.004	0.095	0.156	0.156	0.111
Seep TC	68	-0.098	-0.061	0.258	0.205	0.261	0.245
Wet FC	28	-0.197	-0.010	-0.113	-0.173	-0.248	-0.291
Wet EC	57	-0.120	0.058	0.033	-0.152	-0.233	-0.261
Wet TC	53	-0.133	-0.086	0.033	-0.093	-0.066	-0.059
Seep Bacteria	198	0.010	-0.029	0.153	0.182	0.203	0.178
Wet Bacteria	138	-0.068	-0.065	-0.038	-0.040	-0.027	-0.016

* Fecal coliform (FC), *E. coli* (EC), and totals coliforms (TC)

Alternative Monitoring Methods

Many participants at the author's workshop on *Perspective on Fecal Coliform and E. coli Monitoring* at GREEN '96 indicated that they avoided

bacterial monitoring due to lack of equipment (especially an incubator), cost, and sterilization concerns. Four methods of fecal coliform and *E. coli* analysis have been identified as being used for student and volunteer monitoring efforts. These include multiple-tube fermentation, membrane filtration, direct inoculation into plastic plates with gel (Petrifilm™), and direct inoculation in a media transferred to a petri dish (i.e., Coliscan™). All of these methods except the first method were explored in this study.

The m-Coli Blue membrane filtration method was compared to the direct inoculation Petrifilm™ method for samples taken at the groundwater seep and for standard *E. coli* cultures. Moderate correlation ($r = 0.58$) was found between m-Coli Blue and Petrifilm™ for *E. coli* counts ($n = 20$) in the groundwater seep. The geometric mean for m-Coli Blue was 158 colonies per 100 mL and 177 colonies per 100 mL for Petrifilm™. Results of a paired t-statistic test assuming unequal variance showed no statistically significant difference ($p > 0.05$) in fecal coliform recovery between the two methods.

The conceptual advantage of Petrifilm™ is that the plates are similar in shape and size (5 cm diameter) to membrane filters (4.5 diameter) and are complete with grids. The colony colors in both methods are similar for *E. coli* and total coliform. With three simple steps, the Petrifilm™ method is simple to perform in both the laboratory and the field. With a flat substrate and a pipette, the Petrifilm™ can be directly inoculated in the field. The gel on the plate is initially solid and sets

quickly with the addition of water. To deliver the 1 mL of water, a hand-held pipette with a sterile plastic tip works well with this system and provides a less expensive alternative to individual pipettes. The wide range of incubation time (24 - 48 hr) and temperature (32 - 35°C) gives needed versatility for school use. The overall film packet is compact (7.7 cm x 10.1 cm), thin (about 1 mm), and stackable so many packets will fit easily into an incubator. The 1997 price per plate was about \$1.40.

E. coli colonies are easily differentiated, quite evident, and well formed in the Petrifilm™ system. Extraneous colonies other than the characteristic blue colonies were not found. The 3M™ Company provides a color interpretation guide for bacterial enumeration. There is versatility in the Petrifilm™ method in that air sampling, swab contact, direct contact, and surface sampling are all possible.

The main disadvantage of the Petrifilm™ system is that only one milliliter of water is used. Since colony counts are given in colonies per 100 mL, counts are recorded to the nearest 100. This means that 1 colony would translate to 100 colonies per 100 mL and 2 colonies would be 200 colonies per 100 mL. This is problematic for serious water quality monitoring given the 130 colonies per 100 mL standard for *E. coli*. Any contamination is greatly magnified by this procedure.

Another difficulty with Petrifilm™ is that total coliform colonies in environmental samples are frequently so numerous that they are difficult to count. Underestimation of the number of coliform colonies is likely in this method. False

positives can also be problematic when there is not gas formation or if gas formation disrupts the colonies.

The m-Coli Blue membrane filtration method was also compared to the Coliscan™ method for samples taken at the groundwater seep as well as standard *E. coli* cultures. The Coliscan™ method did not perform as well as Petrifilm™. Correlation between m-ColiBlue and Coliscan™ seep samples was 0.18 with the geometric mean of 348 colonies per 100 mL for m-ColiBlue and 188 for Coliscan™ ($n = 20$). However, neither a single factor analysis of variance or a t-test assuming unequal variances indicated a significant difference between the methods ($p > 0.05$).

Although Coliscan™ contains inhibitors, many white or green non-coliform colonies appeared in the plates and spreaders were frequently found. Light pink colonies were easy to overlook and new colonies appeared well after the 48 hour incubation period. Correspondence of colony counts with other methods was weak. There does not appear to be documentation of collaborative testing of Coliscan™.

Although the Coliscan™ medium bottles are easily inoculated in the field, it is not recommended that the medium be transferred to petri dishes in the field. The medium in the dishes is easily spilled prior to solidification. The dishes have no grids to help with counting and they are much larger (9 cm) than the other systems. There is not the visual emulation of this system with the membrane filtration method. The petri dishes and the medium bottles make up more bulk than the other systems which impacts storage, transport, and disposal.

Given the ease of use by students, the Petrifilm™ method looks the most promising. The cost is favorable, the method is simple, and supplementary equipment is minimal. However, use of Petrifilm™ should be limited to *E. coli* counts since coliforms are difficult to enumerate accurately with this method.

CHAPTER VI

RESULTS OF MONITORING DATA ANALYSIS

Kent County Monitoring Data

Students need a frame of reference to understand the variation in number of bacteria at their monitoring sites. Until sufficient sampling is performed at a given site, use of other long-term data sets might help with the interpretation of bacterial counts. The primary data sets for long-term analysis are from the eleven years of monitoring data for the Grand River and its tributaries in Kent County from the Grand Rapids (Michigan) Wastewater Treatment Plant (GR WWTP) and four years of data from throughout the county on fecal coliforms and *E. coli* from the Kent County Health Department (KCHD). River Flow data were obtained from the City of Grand Rapids Wastewater Treatment Plant for the United States Geological Survey Station 04119000 on the Grand River. This station is located on the right bank upstream from the bridge on Fulton Street in downtown Grand Rapids at an elevation of 585.70 feet above sea level where the river has a drainage area of 4,900 square miles.

The data analysis methods for the fecal coliform data sets center around exploratory and confirmatory data analysis with attention to robust statistical

methods. Exploratory data analysis gained an important role in recent years as methods became more standardized. The intent of this type of analysis is to isolate patterns and features of data in a way allowing flexibility for matching the analysis to the data structure. Confirmatory analysis draws on other data or new data to assess the reproducibility of observed patterns or effects (Hoaglin, 1983).

The purpose of analyzing long-term monitoring data is to establish a station profile of expected fluctuations in bacterial levels. It is in this context that students can select suitable streams for monitoring that do not represent unreasonable health risks and for regulatory agencies to detect where there are problems in stream reaches. Extremely high, persistent, and unusual counts for a particular sampling station or vast differences from adjacent stations act as triggers for more sampling and a search for sources of contamination (Kebabjian, 1994).

Special studies are also included in the analysis to provide insight into trends that cannot be seen in monthly or biweekly sampling. These include raw data from the: (a) Grand River study upstream and downstream of the Grand Rapids Wastewater Treatment Plant, (b) daily sampling of two storm drains in Kent County, Michigan, and (c) storm event sampling in Plaster Creek, Michigan.

Grand Rapids Wastewater Treatment Plant

The City of Grand Rapids Wastewater Treatment Plant monitors the Grand River and its tributaries from 12 miles upstream of the wastewater treatment plant

to 19 miles downstream as shown in Figure 8. For analysis purposes, the log of the raw fecal coliform data was used along with geometric means. A logarithmic transformation normalizes the data so that analysis of variance (ANOVA) techniques can be used.

It is not assumed that there is independence of sites along the river since upstream bacterial contamination is carried downstream. However, the sites in the tributaries are independent of the sites on the main river. Individual tributaries are affected by the "spottiness" of thunderstorms in Kent County. It is not infrequent that one area of the county may get over an inch of rain whereas the rest of county may remain dry. The overall weather conditions (storm events) will also affect the system as a whole. Since there are different dynamics operating in the tributaries, as compared to the main river, the GR WWTP plant data were partitioned for analysis into six river run stations and seven tributary stations.

Overall, there were 1,831 fecal coliform data points that made up the complete data base from 1985 through 1996. Samples were routinely taken on the third Wednesday of the month regardless of the weather. The fecal coliform counts exceeded 200 colonies per 100 mL 57.0% of the time. The geometric mean was 295 colonies per 100 mL and the arithmetic mean was 1,102 colonies per 100 mL. The data were skewed to the right and ranged from 0 to 35,400 colonies per 100 mL. Right-skewed concentration distributions (a long tail of high values) are common in environmental sampling and "a lognormal process is one in which the random

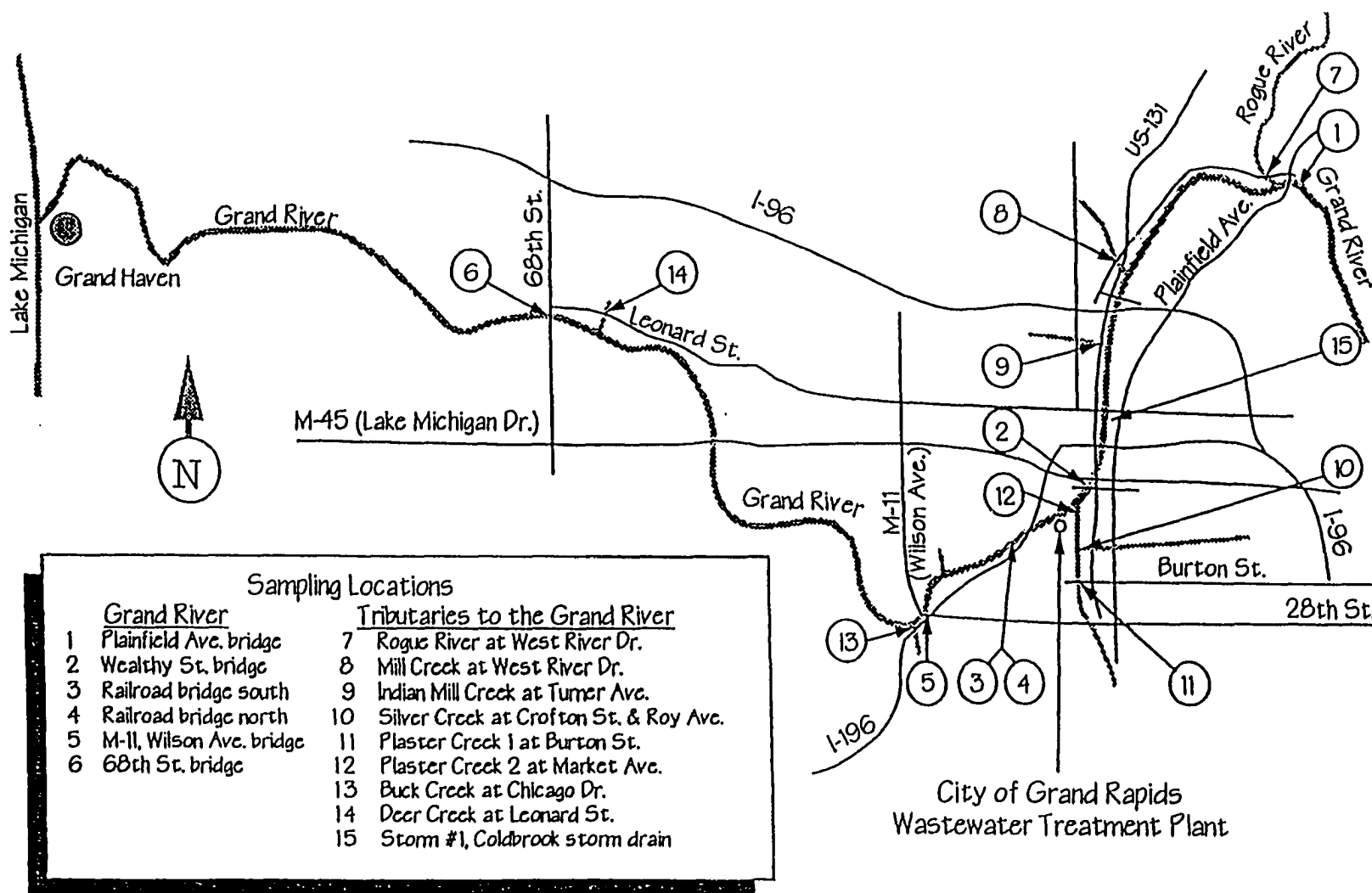


Figure 8. City of Grand Rapids Wastewater Treatment Plant Monitoring Stations.

variable of interest results from the product of many independent random variables multiplied together" (Ott, 1995, p. 253).

For comparison, the San Lorenzo River watershed fecal coliform counts (n=2,439) had a 42.2% exceedance rate with a geometric mean of 163 colonies per 100 mL. The San Lorenzo River *E. coli* (n=363) exceedance rate was 60.1% with a geometric mean of 185 colonies per 100 mL.

River Run Stations

The GR WWTP monitors six sites along 31 miles of the Grand River (Figure 8). A subset of the data collected by the Grand Rapids Wastewater Treatment Plant on these six river run sites was constructed. Days when not all of the six stations were sampled were eliminated. The subset had information for all stations in 119 monthly sampling runs between 17 July 1985 and 16 October 1996.

Station 1 in Plainfield Township is the furthest upstream where the main channel of the Grand River is sampled from the Northland Drive bridge (T.8N.-R.11W., Section 23, Plainfield Township). This station is approximately 50 river miles from Lake Michigan. There is a small amount of commercial development upstream but most of the area is sandy lowlands. The width of the river at this point is about 0.1 km with Bear Creek as the nearest upstream tributary. The major soil type on the south bank is Abscota loamy sand with a gravel pit further upstream. Sloan loam which is prone to flooding and Algansee loamy fine sand

dominate the north side. There are trees along the river upstream of the sampling site. Common trees found in these soil types are quaking aspen, silver maple, swamp white oak, white ash, green ash, and eastern cottonwood.

The Grand River upstream of the wastewater treatment plant is sampled one mile upstream at Station 2, the Wealthy Street Bridge (T.7N.-R.12W., Border of Sections 25 and 36). This station is about 11 miles downstream from Station 1 where river width is about 0.12 km. The surrounding is highly urbanized with industrial complexes along the shoreline and residential areas inland. Upstream and downstream of the bridge sampling site are sea walls that channelize the river. A small island is upstream of the sampling site. The area is served by a sewer system.

Two samples from a railroad bridge are taken about one mile downstream from the Grand Rapids Wastewater Treatment Plant. Station 3 samples are from the south side of the railroad bridge and Station 4 is on the north side of the bridge. Velocity of the river is slower on the south side. The samples are generally taken within ten minutes of each other from marked sites on the bridge. One mile of the Grand River near the sampling site at Johnson Park has been identified as being impacted by combined sewer overflows and pathogens (MDNR, 1992). Earlier studies (Michigan Grand River Watershed Council, 1972) reported fecal coliform counts near this site almost continuously at above 200 colonies/100 mL from 1968 through 1970.

Station 5 is located at the M-11 Wilson Avenue bridge (T.6N.-R.12W., Section 18, City of Grandville) about 4 1/2 miles downstream from the GR WWTP. The Wyoming Waste Water Treatment Plant is located upstream of this sampling site. Station 6 is in Ottawa County at 68th Street at Eastmanville (T.7N.-R.14W., Section 3, Allendale). North of the river is Mancelona-Nester-Belding-Iosca soil association which is gently sloping to hilly, well drained to somewhat poorly drained soils. There are sandy and loamy soils in the uplands. To the south is the Rubicon-Granby-Crosswell-Au Gres soil association which includes well-drained to poorly drained sandy soils of lake and outwash plains (U.S. SCS, 1972). This stretch of the river is impacted by upstream dairy farming. River residents have reported heavy sediment loads at this site during rain events.

Although not presently an active station, the M-45 sampling station is about 25 river miles from Lake Michigan. Historical data suggest that slightly elevated fecal coliform levels at M-45 are due to impact of the City of Grand Rapids. However, improper collection and treatment of sanitary sewage and enriched runoff from dairy-farming and beef-cattle production have also been implicated as sources of bacteria (Michigan Grand River Watershed Council, 1972).

Basic descriptive statistics were calculated for each of the six river run stations and are summarized in Table 4 with a graphical display in a scatterplot (Figure 9). What is most striking are the exceedance levels. The State of Michigan water quality standards imply that fecal coliform counts over 200 colonies per 100

Table 4
Summary Statistics for GR WWTP River Run Fecal Coliform Counts, 1985-87

Statistics	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
% Exceedance	60.3%	31.3%	40.0%	57.1%	55.5%	46.6%
Geometric Mean, colonies per 100 mL	269	117	165	269	257	237
Arithmetic Mean, colonies per 100 mL	919	205	316	626	646	687
Standard Error	165	23	41	108	111	120
Median	275	106	158	250	250	182
Mode	20	70	200	300	100	100
Standard Deviation	1,921	267	470	1,246	1,304	1,379
Sample Variance	3,689,393	71,289	220,735	1,553,466	1,699,714	1,901,585
Kurtosis	23.25	12.59	18.37	22.87	29.74	18.87
Skewness	4.24	3.22	3.75	4.60	4.99	4.12
Range	14,999	1,692	3,499	8,390	10,399	8,799
Minimum	1	8	1	10	1	1
Maximum	15,000	1,700	3,500	8,400	10,400	8,800
Count	136	134	130	133	137	133

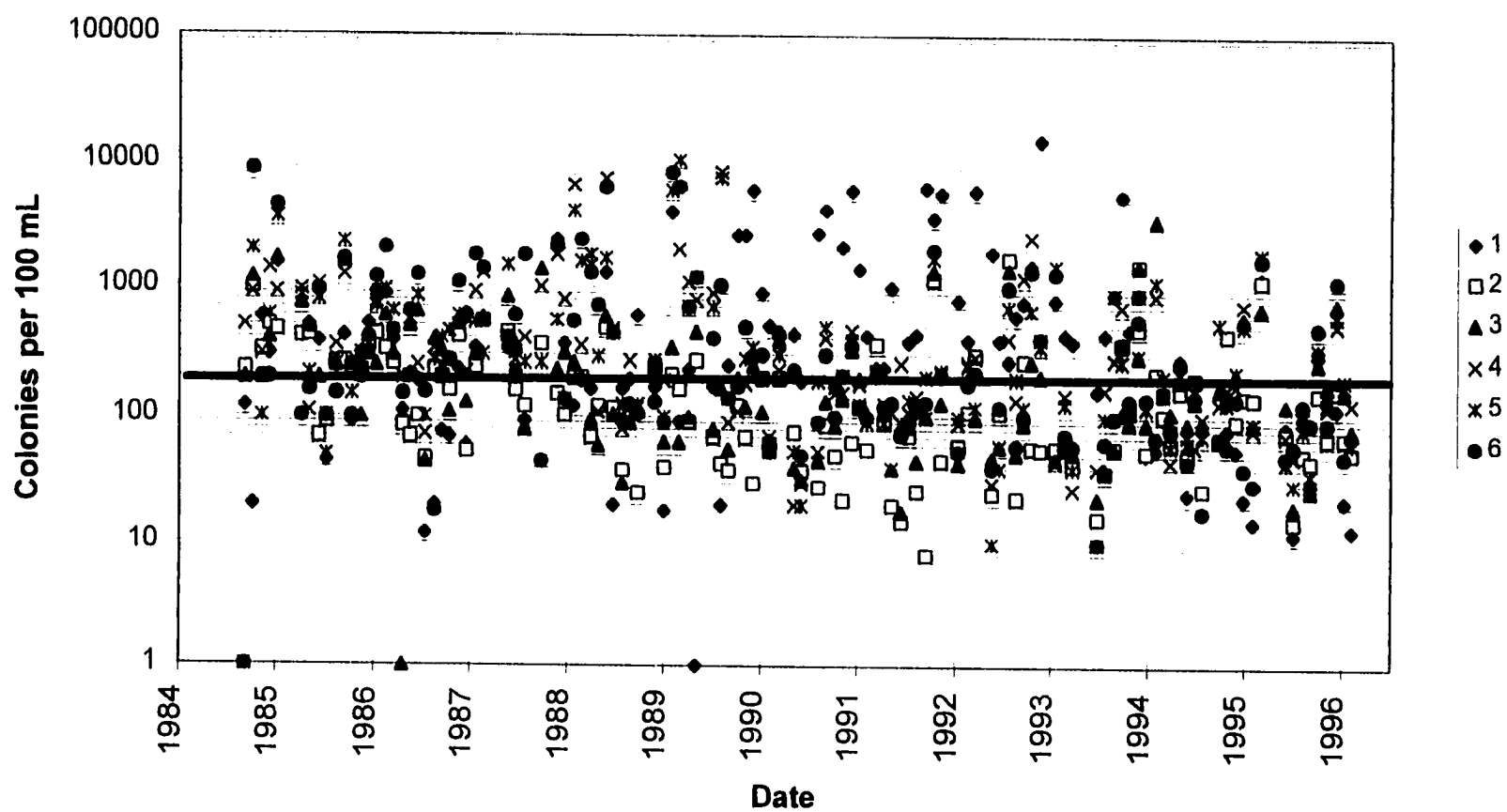


Figure 9. Distribution of Fecal Coliform in GR WWTP River Run Stations, 1985-96.

mL should be the exception, not the rule. The GR WWTP data suggest otherwise in that three of the six stations exceeded this number over 50% of the time and the other three stations ranged from 31.3% to 46.6%. There does not appear to be any dramatic downward or upward trend in the data as illustrated by the scatterplot. For the entire river run data set, exceedance rates were 47.3%. The geometric mean of the data set ($n = 714$) was 207 colonies per 100 mL and the arithmetic mean was 573 colonies per 100 mL.

Station 1 had the greatest range (1 to >15,000 colonies per 100 mL) and highest fecal coliform counts (Station 1, geometric mean = 269 colonies per 100 mL). Inspection of the scatterplot shows the high counts for Station 1 are partly a result of unusually high counts during the years 1990 - 1994; the cause of the elevation is undetermined. Station 1 is furthest upstream and it drains a mix of agricultural and sparsely populated residential areas. Upstream of the site is Bear Creek which has been identified for special studies on bacterial contamination by the Michigan Department of Environmental Quality and the GVSU Water Resources Institute. Somewhat surprising is the low geometric average count of Station 2 at Wealthy Street (117 colonies/100 mL; range = 8 to 1,692 colonies/100 mL) in downtown Grand Rapids. This would indicate that there is some purification from upstream stations that might be expedited as a result of water passing over a series of falls. Additionally, the city is sewered and served by a central Wastewater Treatment Plant whose major outfall is below this station.

The skewness statistic for the six stations ranged from 3.22 to 4.99 with the mean greater than the median. This suggests a positively (right) skewed distribution with numerous low values and few high values. The measure for skewness at the river run six stations is within the range of other bacterial data that have been analyzed. Specifically, Landwehr (1978) reported a range of skewness from 1.8 to 5.5 for seven bacterial monitoring stations as compared with 1.21 to 4.12 for the Grand River data. The skew is significant and should be considered in the description of data. Histograms for the river run (Figure 10) and tributary data (Figure 11) confirm the data structure as being positively skewed. Figures 12 and 13 provide insight into the distribution of fecal coliform bacterial counts for the GR WWTP data as compared with the San Lorenzo River data from California. Both have similar shapes with similar ranges of values. This is typical of environmental data and reinforces the necessity of calculating geometric means for comparative purposes.

Much of the data found in Table 4 is more easily visualized in a notched box plot (Figure 14). Instead of endless numbers, the visual display allows for easy comparison of stations that is more in tune with the way the human mind can best assimilate information (Berthouex and Hunter, 1981). Interpretation of box plots, as created in StatView® for the Macintosh, has five elements:

1. The top of the box is the 75 percent value (upper quartile) and the bottom of the box is the 25 percent (lower quartile limit).

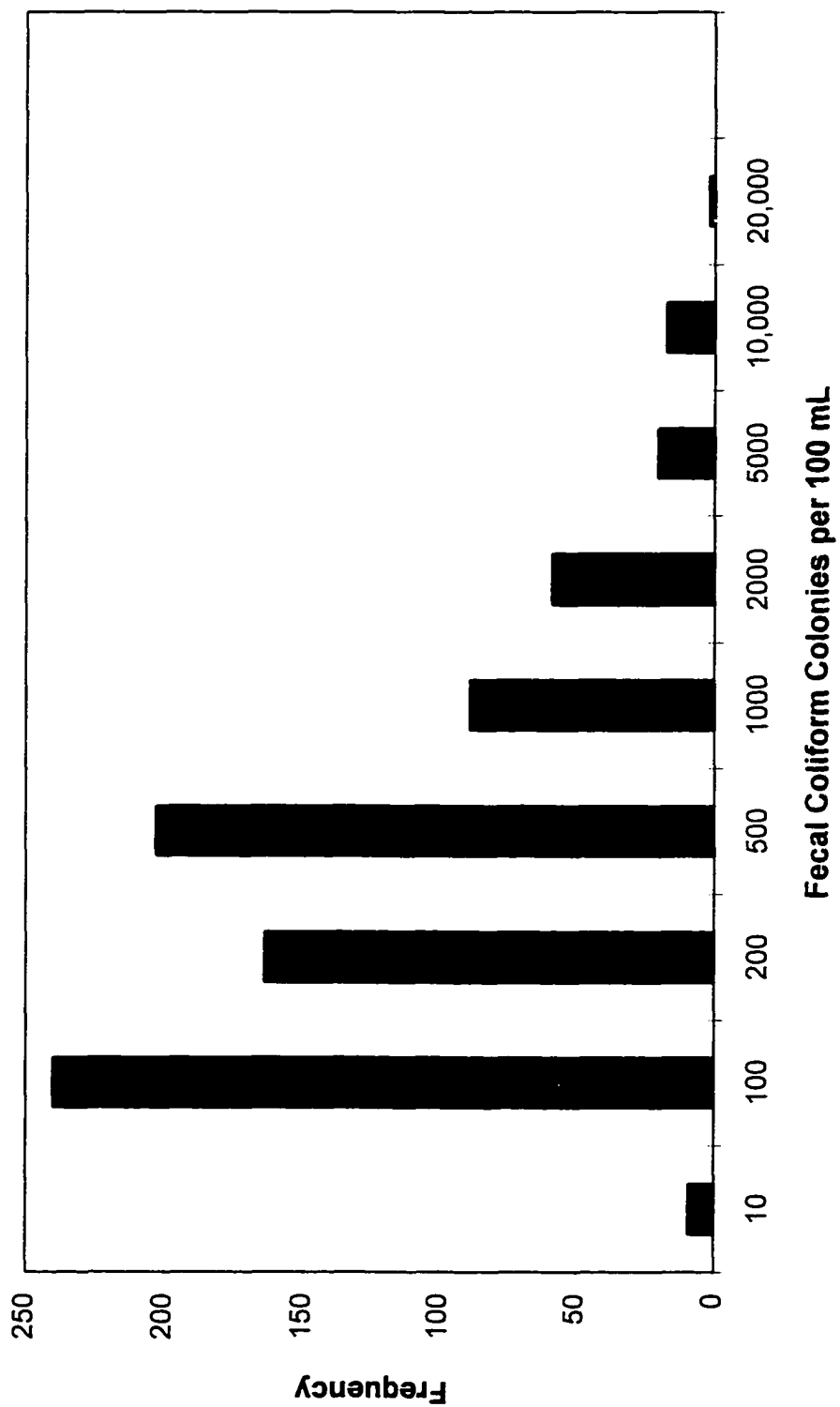


Figure 10. Frequency Distribution of GR WWTP River Run Data, 1985-96.

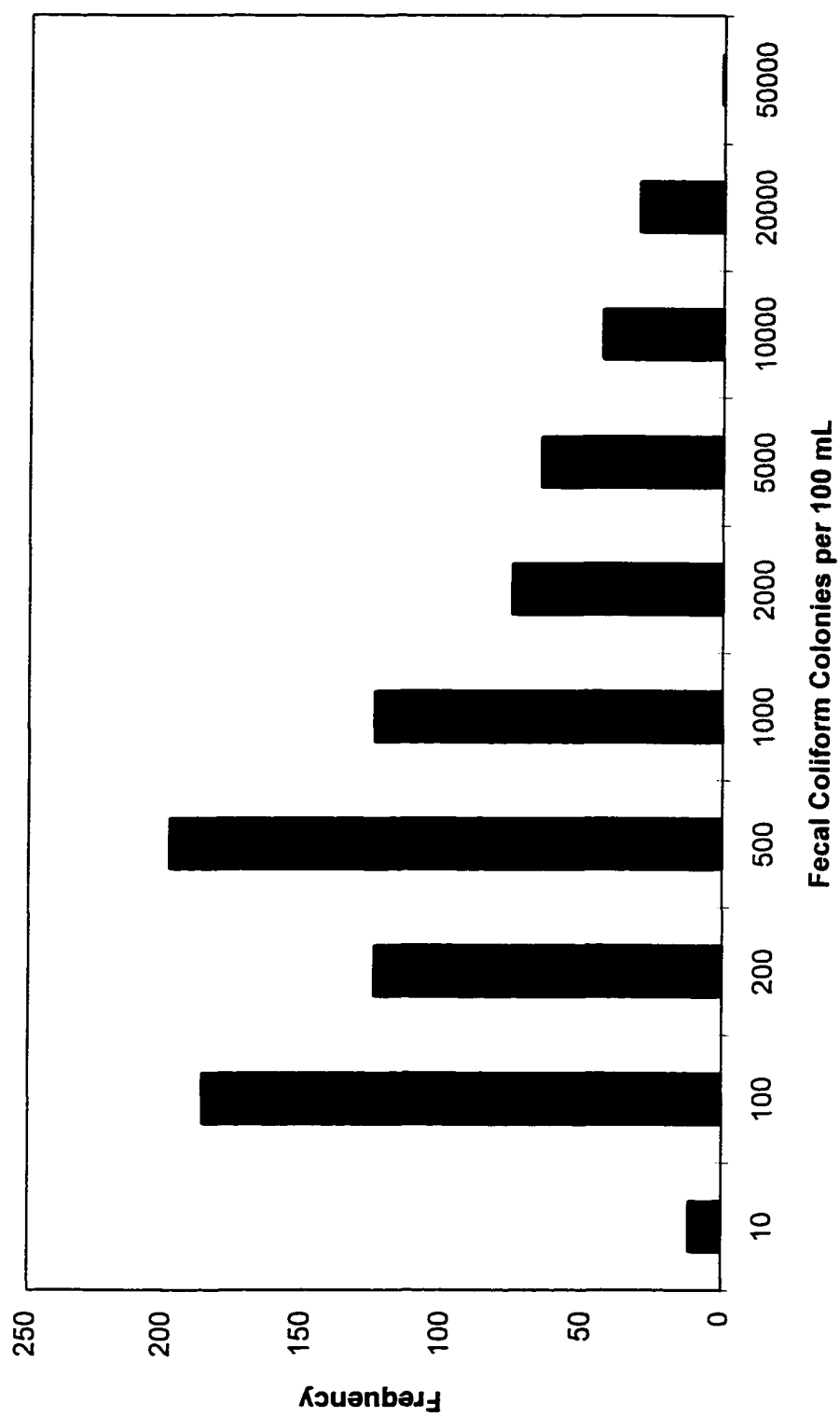


Figure 11. Frequency Distribution of GR WWTP Tributary Data, 1987-96.

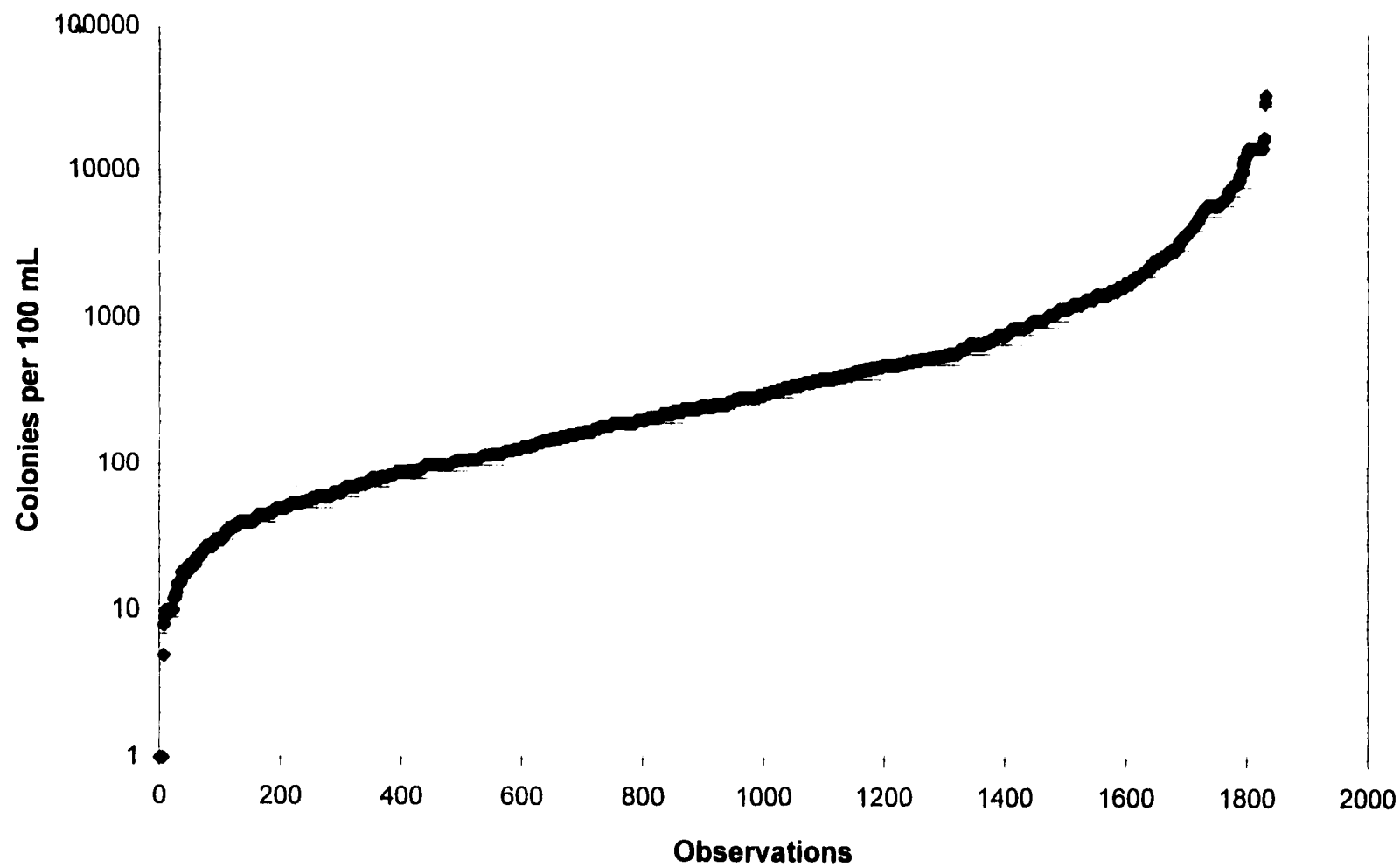


Figure 12. Ordered Distribution of Fecal Coliform Bacteria in the Grand River and Tributaries, 1985-96.

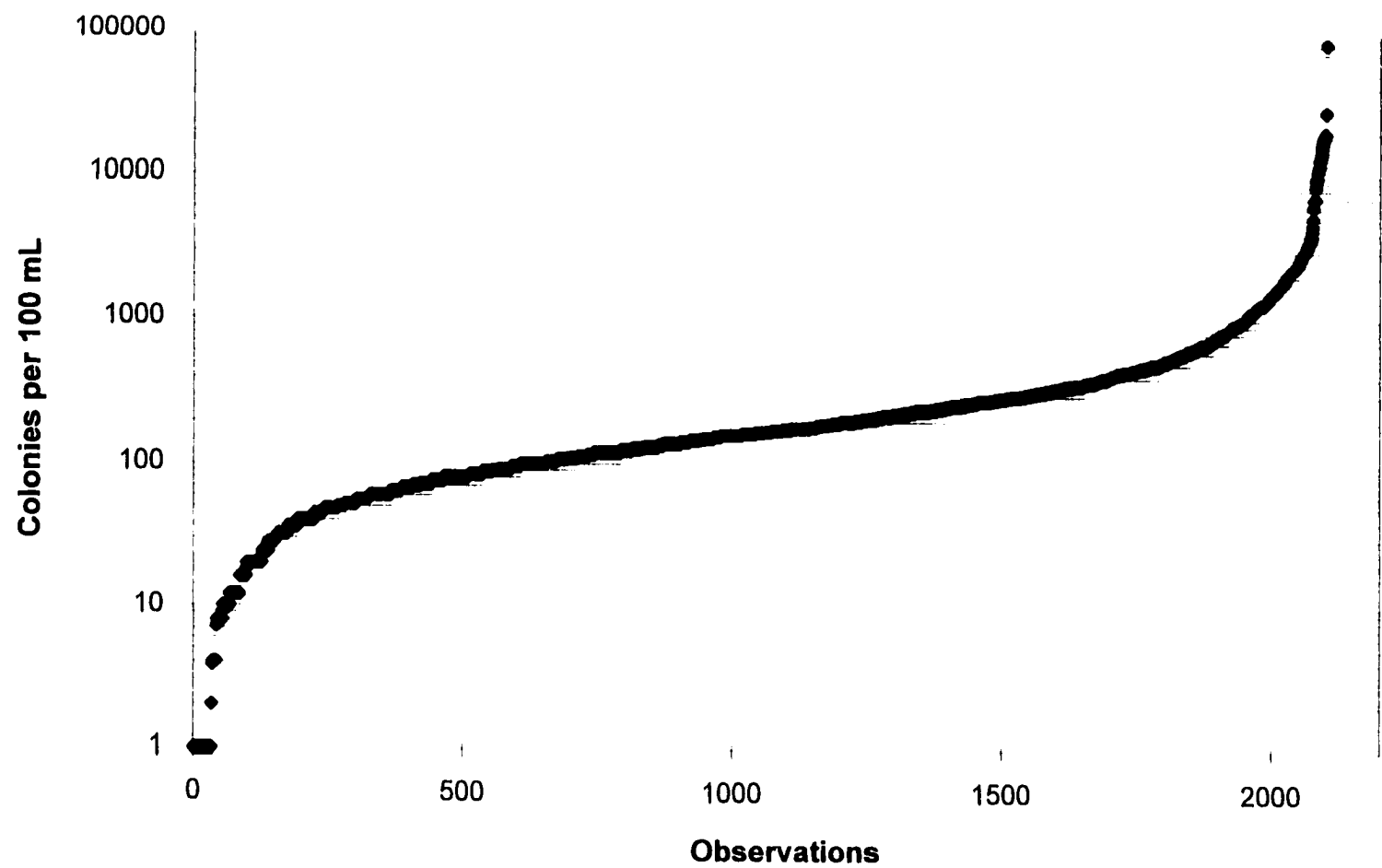


Figure 13. Ordered Distribution of Fecal Coliform Bacteria in the San Lorenzo River and Tributaries, 1994-96.

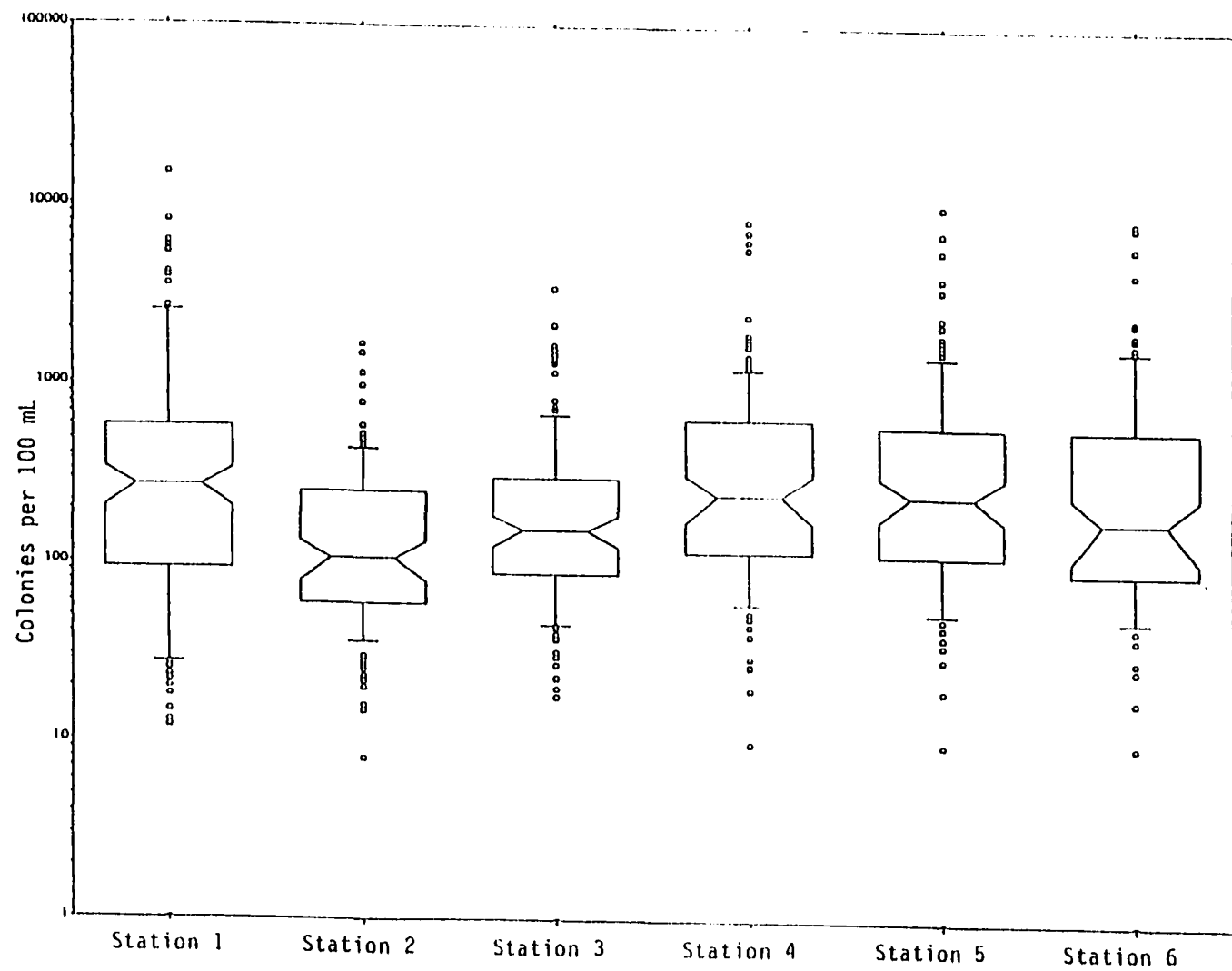


Figure 14. Notched Box Plots of GR WWTP River Run Fecal Coliform Monitoring Stations, 1985-96.

2. The line at the notch in the box is the median.
3. The "t" line is the outlier control which eliminates the extreme 20% of the values, with ten percent below the 10th percentile and ten percent above the 90th percentile.
4. The single data points represent outliers with the maximum and minimum points defining the range.
5. The notch height reflects a 95% confidence interval for comparison of box medians. When notches for any boxes overlap in a vertical sense, the medians are not significantly different at about a 95% level (Reckhow and Chapia, 1983).

The notched box plots reveal similar patterns of overall shape with upper interquartile ranges greater than lower interquartile ranges for all of the stations downstream from Station 1. Station 1 is noticeably different with the lower interquartile range being greater than the upper range, as noted in Figure 9. This suggests that the high "spikes" of bacteria are influencing the statistics at Station 1 to a greater degree than at the other stations.

According to the alignment of the notches on the boxplots, Station 2 with the lowest median value is significantly different from the other stations. The range of Station 2 is less than the other stations. Stations 3 and 6 also had median values that were less than other stations. The correlation coefficient between these Stations 2 and 3 was 0.56. Other significant correlations were between Stations 4, 5, and 6, downstream of the wastewater treatment plant ($r = 0.52$ to 0.68).

Interestingly, there was a low correlation between Stations 3 and 4 ($r=0.16$) which were sampled from the same railroad bridge in the river. A two-sample t-test assuming unequal variances indicated significant differences between the railroad bridge stations ($p<0.01$). On seven occasions, counts on the north side were between 1,000 and 7,000 colonies per 100 mL higher than the south side. The geometric mean of Station 3 (south) was 165 colonies per 100 mL and that of Station 4 (north) was 269 colonies per 100 mL. Exceedance of the fecal coliform limit of 200 colonies per 200 mL happened in 40% of the samples for Station 4 and 57.1% for Station 3. This raises some serious issues about what sampling results are really saying about the condition of the river. It is disconcerting to think that samples from the same reach of the river taken within minutes of each other could have so much variation.

Along with fecal coliform bacteria, the Grand Rapids Wastewater Treatment Plant also runs analyses of monthly samples for other parameters including: (a) silver, (b) biochemical oxygen demand (BOD), (c) chloride, (d) conductivity, (e) silver, (f) chromium, (g) copper, (h) nickel, (i) iron, (j) hardness, (k) ammonia, (l) nitrate, (m) nitrite, (n) pH, (o) phosphate, (p) dissolved oxygen, and (q) water temperature. A question that would be answered by these data is whether one of the other monitored parameters is associated with bacterial counts and whether that parameter might give an early warning signal for elevated counts. Of course, other than perhaps nutrients, there is not a proximate connection between the above

parameters and fecal coliform bacteria. However, there may be specific tributaries such as Plaster Creek whose contribution to river contamination can be tracked by a surrogate for fecal coliform bacteria such as heavy metals. An assumption is that the bacterial load of Plaster Creek is higher than that of the portion of the Grand River into which it drains. This is particularly true of storm events with the exception of times of combined sewer overflows.

Correlation coefficients were calculated for the ten year GR WWTP data set (Table 5). Few significant correlations could be found for fecal coliform. Copper ($r = 0.32$) and nickel (0.35) were the highest correlations which might be explained by the Plaster Creek and Silver Creek Drain inputs which have high levels of bacteria as well as metals. The nutrients, nitrites ($r = 0.28$) and phosphates ($r = 0.30$), were the next highest correlations. Other studies found positive correlations of coliforms with phosphate and sulfate, and a negative correlation with chloride (Brasfeild, 1972). Of note are the high correlations of conductivity with chloride ($r = 0.95$), and total suspended solids with iron ($r = 0.9238$). There was a strong negative correlation of dissolved oxygen with temperature as would be expected as gas solubility is inversely related to temperature ($r = -0.74$).

There have been indications that water temperature, flow, and precipitation influence levels of fecal coliform bacteria. To determine if these may be factors in the Grand River, correlation coefficients were calculated for fecal coliform levels and river flow, ambient air temperature, and precipitation. Flow data for the Grand

Table 5

Correlation Matrix for GR WWTP River Run Parameters, 1985-96

	FC	Ag	BOD	Cl	Cond	Cr	Cu	DO	Fe	Hard	NH ₃	Ni	NO ₂	NO ₃	pH	PO ₄	Temp
FC	1																
Ag	.1945	1															
BOD	.1977	.2525	1														
Cl	.1992	.3341	.2207	1													
Con	.1686	.3400	.1043	.9528	1												
Cr	.2423	.0565	.0191	.3250	.3261	1											
Cu	.3235	.1518	.3695	.2223	.1569	.1844	1										
DO	-.1756	.0256	-.0111	.1818	.1500	-.1078	-.1607	1									
Fe	.1676	-.027	.3189	-.0375	-.1167	-.0414	.2211	-.0587	1								
Hard	-.0787	.0879	-.3229	.1131	.3633	.1021	-.2141	-.1751	-.2801	1							
NH ₃	.1123	.1099	.3253	.2975	.2490	.0000	.1240	.3136	.0180	-.1588	1						
Ni	.3507	.1694	.0199	.3126	.3050	.5205	.1675	-.0798	.0448	.1232	.0543	1					
NO ₂	.2756	.1062	.4764	.3061	.2663	.1172	.2634	.0121	.1428	-.1109	.5770	.1242	1				
NO ₃	-.0279	-.007	-.3761	.0758	.1327	.3108	-.1738	.3152	-.1679	.2205	.0784	.2797	-.0394	1			
pH	-.1098	.0546	.2064	-.2903	-.2766	.0220	.0018	-.3119	-.0288	.0673	-.0977	.0547	-.0139	-.1399	1		
PO ₄	.2974	.0080	.2182	.1709	.0980	.0906	.2991	.0335	.4772	-.2165	.1975	.0948	.2953	-.0439	-.2424	1	
Temp	.1403	-.140	.1287	-.1323	-.0989	.0575	.1353	-.7372	.0330	.1801	-.2652	.0400	.0384	-.3598	.3033	.0807	1
TSS	.1544	-.025	.3605	-.0890	-.1796	-.0405	.1718	-.1374	.9238	-.3091	-.0091	.0499	.1661	-.2458	.0501	.4557	.2333

River at the Grand Rapids Station and meteorological data from the weather station at the Kent County International Airport were integrated with the GR WWTP data base for this analysis.

Precipitation is generally in the form of snow from mid-November through mid-March and rain during the rest of the year. Average precipitation in the Grand Rapids area is 34.4 inches with 2.5 inches or more monthly except in January and February. Precipitation of more than 0.01 inch is expected during 153 days with 16 days of snow of over one inch. There are two seasons when flooding is more prevalent: the January-February thaw and March-April snow melt. Droughts occur occasionally during late spring or summer. In an average year, 22% of the days are clear and 78% are cloudy or partly-cloudy.

Thunderstorms and localized rain events are frequent during the summer. It is not uncommon to have a rain event where over an inch of rain falls in one area of Kent County and there is little or no rain in another area. Snowfall patterns can also be spotty. The immediate lakeshore of Lake Michigan is influenced by "lake effect" snow. In a 1996 snow event, lakeshore communities had numerous school closings due to 12-18" of snow but eastern Kent County received no snow. These localized effects pose some difficulty when correlating precipitation at a specific site with precipitation data from the airport.

Additional confounding variables include the complex hydrology of a river system as large as the Grand River which drains 5,570 square miles and has its

headwaters over 225 miles from the mouth. The physiography of Kent County is predominantly glacial drift (a mixture of sand, gravel, clay and till) deposited during the Wisconsin glacial period (United States Soil Conservation Service (USSCS), 1986). A complex interlobate moraine system reflects the meeting of the Saginaw and Michigan lobes of the Wisconsin ice sheet. Elevations in the county range from 600 to 1,060 feet above sea level (USSCS, 1986). Approximate thickness in Kent County ranges from a few feet to 300 feet. Small bodies of well-sorted sand and gravel can be found in the drift. Three physiographic regions are found in the County: outwash plains and lake plains with bedrock near the surface, hilly moraine rising from level valleys, and upland till plains (USSCS, 1986).

In the Grand Rapids area, the water table slopes into streams allowing groundwater to slowly drain into the Grand River system. Recharge of aquifers is through precipitation. Calcium and magnesium predominate as cations in groundwater with variable amounts of sulfate anions (Stramel et al., 1954). In most area wells, the water temperature is between 50 and 52°F.

River run stations correlation results are found in Table 6. For river flow (cfs), there was little correlation for the sampling date and the days before and after (range of $r = -0.24$ to -0.29) For temperatures from the sampling date and each of two days before, there were low correlations for Station 1 ($r = 0.31, .31, .34$) but little correlation for other stations. Precipitation totals for the sampling date and each of two days before revealed a moderate correlation of 0.52 for two days after

Table 6

Correlation Matrix for GR WWTP River Run Bacteria With
Discharge, Temperature, and Precipitation, 1985-96

Parameter	Station					
	1	2	3	4	5	6
Discharge Day Before	-0.2361	0.1734	-0.0548	-0.0737	-0.0222	0.0233
Discharge Sample Day	-0.1016	0.2369	0.0610	0.0219	-0.0223	0.0724
Discharge Previous Day	-0.2055	0.2858	0.0048	-0.0543	-0.0091	0.0345
Water Temperature	0.1569	-0.0253	0.0680	-0.0394	-0.0689	-0.0846
Mean Air Temperature Sample Day	0.3114	-0.0488	0.1024	-0.1061	-0.1266	-0.1229
Mean Air Temperature Previous Day	0.3051	-0.0569	0.1051	-0.1021	-0.0798	-0.0725
Mean Air Temperature Previous 2 Days	0.3414	-0.0069	0.1593	0.0075	-0.0019	-0.0163
Precipitation Sample Day	0.0117	-0.0487	-0.0441	0.0192	0.3326	0.2510
Precipitation Previous Day	0.0285	0.1877	0.1830	0.0259	0.1108	0.1286
Precipitation Previous 2 Days	0.0284	0.5231	0.2049	0.0279	-0.0200	0.0241

sampling events at Station 2 (Wealthy Street) and 0.33 for the sampling day at Station 5. None of the above factors appear to be universally useful for development of a model for predicting fecal coliform levels in the Grand River.

Long-term data sets can give clues as to seasonality associated with fecal coliform counts. An overall geometric mean was calculated for each month for the data from river run Stations 1 through 6 for 1985-96 ($n = 803$, Figure 15).

Correlation coefficients of fecal coliform bacteria and weather parameters are:

mean monthly air temperature ($r = 0.17$) and mean monthly precipitation (0.32).

The lowest geometric means of bacterial counts were in February (108 colonies per 100 mL), May (126 colonies per 100 mL), and October (158 colonies per 10 mL).

Overall geometric means exceeded 200 colonies per 100 mL in January, April,

July, August, September, November, and December. There appears to be an August-September peak preceding the highest peak in November. August average monthly precipitation is generally one of the highest of the year (3.45 inches) along with April and June. February has the lowest monthly precipitation of 1.53 inches.

Chlorination of wastewater effluent ceases in October, and this may be a factor in the November peak. Another influence on the November peak is the fall salmon/trout run. The fish may contribute directly to bacterial load by feces and indirectly by disturbing the sediments. However, lack of support for this factor is that the peak is less evident in the stations immediately below the dam and fish-ladder where fishing activities are expected to be most concentrated.

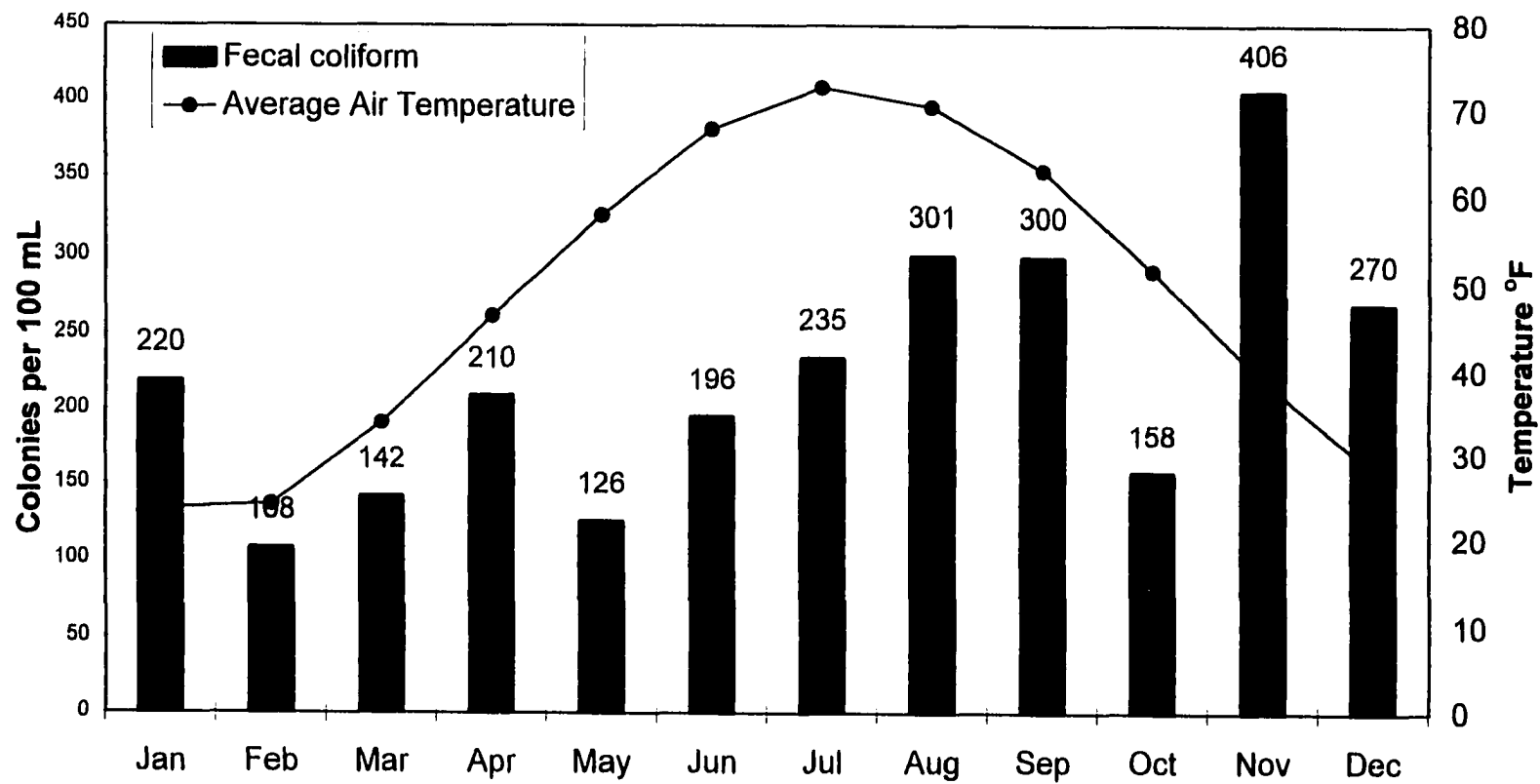


Figure 15. Monthly Fecal Coliform Geometric Means for GR WWTP River Run Stations, 1985-96.

The river run stations do not contribute equally to the bacterial load as shown in Figure 16 where the band widths indicate the mean load in a given month for 1985-96. Stations 2 and 3 experience a relatively constant load throughout the year. With the exception December (geometric mean = 209 colonies per 100 mL), average counts at Station 2 were below 200 colonies per 100 mL. Station 5 had geometric mean monthly levels above 200 colonies for ten months of the year; Station 4, nine months; Station 1, seven months; Station 6, six months; and Station 3, three months. A very evident peak on November is the result of contributions from the upstream station (Station 1) as well as the three downstream stations (Stations 4 - 6). This suggests other things are happening in the watershed to elevate counts other than cessation of chlorination of wastewater.

The stations do not always have the same trends. An examination of band widths of Station 1 as compared to Stations 4, 5, and 6 shows opposite trends most clearly in the March - April period. A decrease occurs at Station 1 while there are increases at Stations 4, 5, and 6. The May - June increase at Station 1 does not occur at Stations 5 and 6.

Tributaries

The Grand Rapids Wastewater Treatment Plant (GR WWTP) presently monitors eight tributaries of the Grand River. The longest records are for the Rogue River, Mill Creek, Indian Mill Creek, Silver Creek, Plaster Creek, and

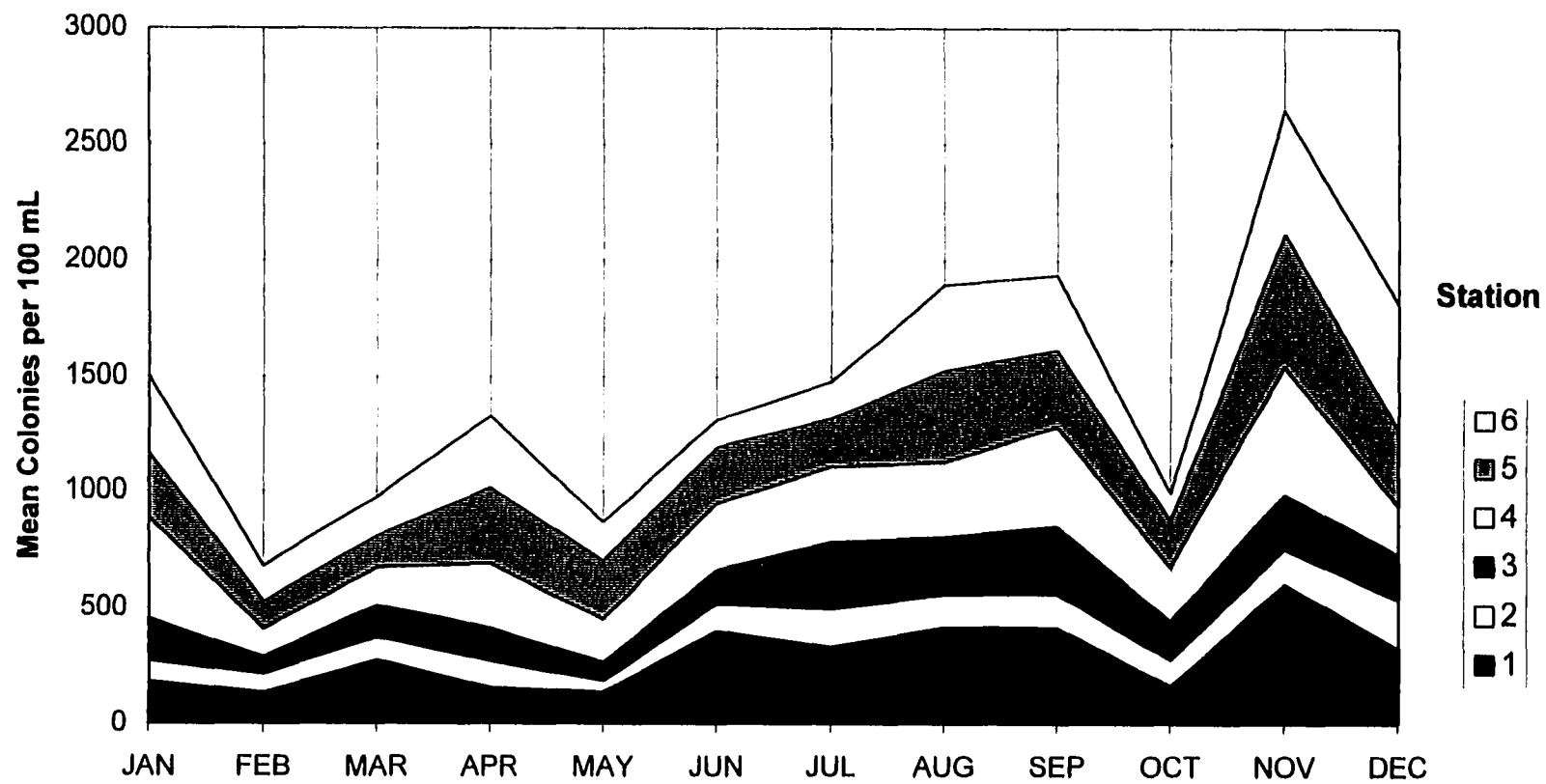


Figure 16. Seasonal Distribution of Fecal Coliform for GR WWTP River Run Stations, 1985-1996.

Buck Creek. Shorter records are available for Deer Creek in Ottawa County and the Coldbrook storm drain near the Monroe Street water treatment plant. As with the river data, a subset of the tributary data was constructed. Parallel sampling runs ($n = 86$) were available from 18 November 1987 through 16 October 1996. The most common reason for eliminating a sampling date was incomplete information due to frozen conditions in mid-winter.

Rogue River. The furthest upstream tributary sampled by the GR WWTP is the Rogue River just west of Grand River Station 1 at Plainfield Avenue. The Rogue River joins the Grand River on the north bank near Verta Drive and West River Drive (T.8N.-R.11W., Section 22, Plainfield Township). The sample is taken from the West River Drive bridge. A golf course is found on the east side of the Rogue River and a narrow wooded section with light residential development is to the west. Major soil types near the sampling site are Abscota loamy sand and Boyer loamy sand. Trees typically associated with these soils are northern red oak, white ash, silver maple, eastern cottonwood, sycamore, sugar maple, and white oak. The Rogue is heavily wooded upstream.

Mill Creek. The next major tributary sampled is Mill Creek which is about six miles downstream from Station 1. The river flows from north to south at this juncture and Mill Creek joins the river on the west side. The Mill Creek watershed covers about twenty square miles with the stream's origin in Cranberry Lake.

Seven major tributaries and two lakes are part of the watershed (Grand Valley State College, 1978). Approximately 80% of the watershed is in Alpine Township which has a rapidly developing commercial area.

The Mill Creek stream mouth station is in Comstock Park (T.8N.-R.11W., Section 31, Plainfield Township) and is taken at an abandoned railroad bridge between West River Drive and the Grand River. Upstream is Dwight Lydell Park. The park contains a mix of lawn and wooded vegetation on either side of the stream with a Udipsamment (loamy sand to gravelly sand) soil type. Chelsea loamy fine sand is also found upstream. Trees associated with this soil type are white oak, red pine, jack pine, quaking aspen, and northern red oak. The lower part of Mill Creek is mixed residential and the upper reaches are agricultural with fruit and grain crops predominating. In general, there is a buffer of trees along the banks.

Indian Mill Creek. Indian Mill Creek flows through residential, commercial, and industrial areas of the city of Grand Rapids (T.7N.-R.12W., Section 13, City of Walker/City of Grand Rapids). The sampling point is the bridge at Turner Avenue on the west side of the Grand River. The area in the city that impacts on the creek is sewered. The soil is classified as urban land mixed with the Chohoctah complex. Upstream land use includes a golf course and agriculture. Seven miles of the creek have been reported to have sedimentation, biological degradation, and nonpoint source pollution problems (MDNR, 1992).

Plaster Creek. Plaster Creek is sampled at the bridge on Market Avenue just north of the Grand Rapids Wastewater Treatment Plant (T.7N-R.12.W, Section 35). The stream is highly impacted by trash, debris, and fallen vegetation. For instance, sixteen shopping carts were found in an area of the creek near a shopping center (Madden, 1990). Plaster Creek shows biological degradation, sedimentation, nonpoint source pollution, and high levels of pathogens for twelve miles (MDNR, 1992). A second sampling location on Plaster Creek is two miles upstream at Burton Street.

Silver Creek. The GR WWTP sample is taken from a manhole at Crofton and Roy Streets (T.7N.-R.12W., Section 36, City of Walker/City of Grand Rapids). Low flow is a problem in sampling this site. On the occasion where the author was present, the sampling device had to be used five times to extract the required sample volume. The Silver Creek Drain joins Plaster Creek about 1/4 mile from the sampling site. This juncture is about 1 mile from the Grand River.

Although it was once a natural creek, Silver Creek now is largely enclosed and underground in a concrete drain system. In 1990, a report on Silver Creek was issued by the GR WWTP that assessed the drain system (Barton, 1990). Walk-through observations, a business survey, dye testing, an initial study, and long term monitoring were elements of the project. Sampling was done in eight manholes with long term monitoring via ISCO samplers at two locations. Heavy metals,

biochemical oxygen demand, cyanide, pH, suspended solids, and fecal coliform bacteria were measured. Fecal coliform counts varied from 171 to 6,800 colonies/100 mL. After rainstorms, oil sheens were evident in the drain along with automotive fluids, litter, animal wastes and debris (Barton, 1990).

Buck Creek. Buck Creek joins the flow of the Grand River on the south side approximately 4 1/2 miles downstream of the Grand Rapids Wastewater Treatment Plant (T.6N.-R.12W., Section 18, City of Grandville). It flows through a commercial and residential area downstream and agricultural land upstream. Fourteen miles of Buck Creek have been identified by the Michigan Department of Natural Resources as being impacted by biological degradation, sedimentation, nonpoint source pollution, and pathogens (MDNR, 1992).

Summary. Figure 17 provides a composite visual summary of the fecal coliform counts from 1987-96 for the tributaries. Again, the pattern is similar to that of the river run stations. As with the river run stations, basic descriptive statistics were calculated. The statistics are summarized for the tributaries in Table 7. Of note is that all but two of the tributaries exceed 200 fecal coliform colonies per 100 mL more than 50% of the time. Only the Rogue River (36.2%) and Mill Creek (30.9%) were less than 50% and five of the tributaries had greater than 70% exceedance rates. The Rogue River and Mill Creek have the least industrial impacts, and neither watershed has many animal-husbandry operations. The lower

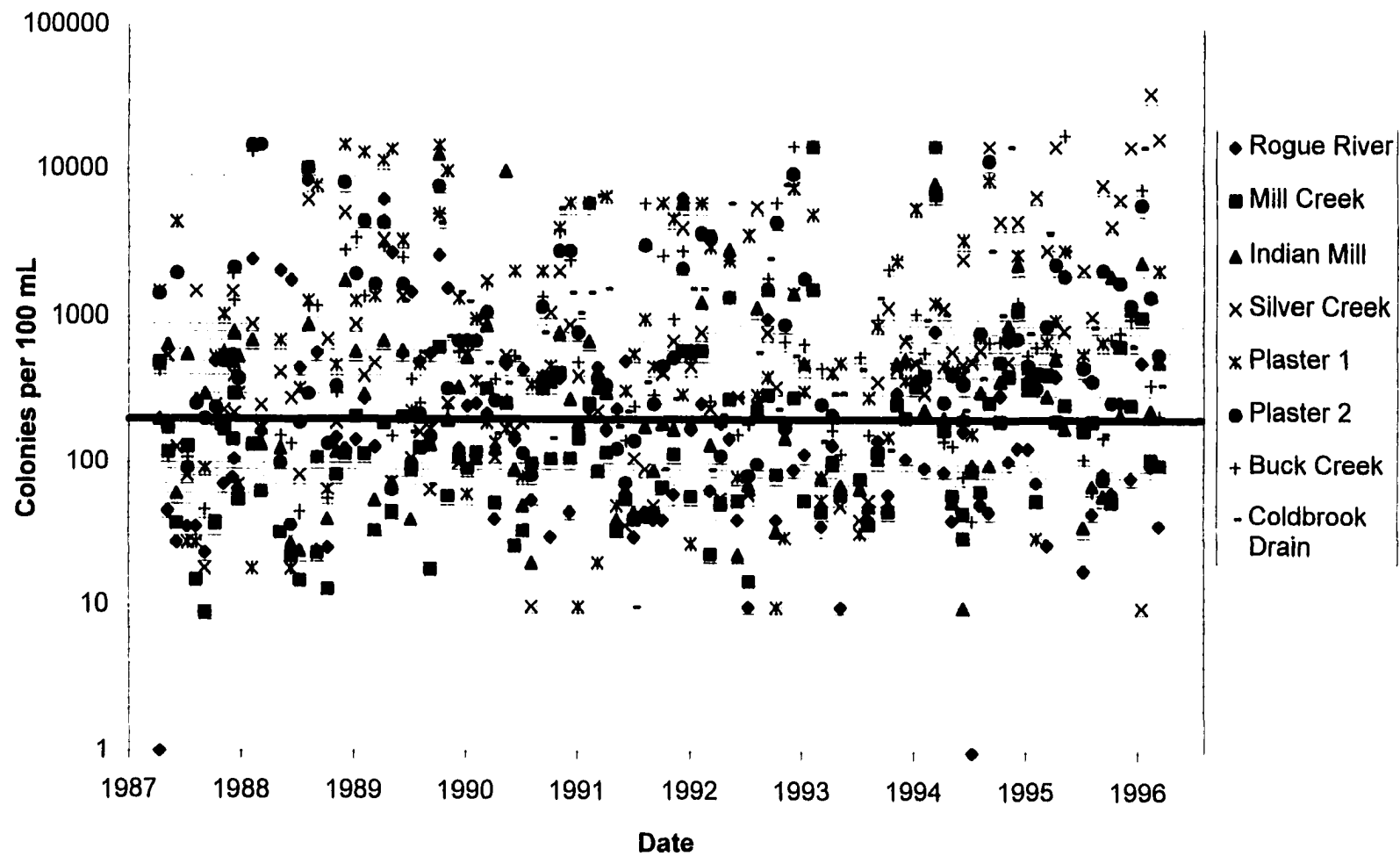


Figure 17. Distribution of Fecal Coliform in GR WWTP Tributary Stations, 1987-96.

Table 7

Summary Statistics for GR WWTP Tributary Monitoring for Fecal Coliform Bacteria, 1987-96

Parameter	Rogue River	Mill Creek	Indian Mill	Silver Creek	Plaster Creek 1	Plaster Creek 2	Buck Creek	Deer Creek	Coldbrook
Exceedance	36.2%	30.9%	54.1%	73.1%	74.1%	82.3%	81.0%	76.4%	81.5%
Geometric Mean, colonies/100 mL	143	117	246	600	576	763	611	519	656
Arithmetic Mean, colonies/100 mL	598	342	872	2,483	2,244	2,097	1,955	1,454	1,869
Standard Error	151	113	214	485	354	317	344	377	380
Median	126	110	220	505	500	650	560	510	580
Mode	40	260	500	15,000	173	15,000	15,000	3,000	15,000
Standard Dev.	1,721	1,181	2,258	5,045	3,632	3,527	3,612	2,793	3,419
Kurtosis	42.36	55.65	23.23	17.83	4.56	6.30	8.47	14.60	8.92
Skewness	5.99	7.28	4.69	3.73	2.25	2.62	2.94	3.66	3.03
Range	14,999	10,291	14,990	35,390	14,990	14,964	17,973	14,990	14,990
Minimum	1	9	10	10	10	36	27	10	10
Maximum	15,000	10,300	15,000	35,400	15,000	15,000	18,000	15,000	15,000
Count	130	110	111	108	105	124	110	55	81

Plaster Creek sample had a 71.4% exceedance rate while the upper sample was 82.3%. It is clear that creeks in urban areas such as Plaster Creek and Silver Creek are very impacted by fecal coliform bacteria. These creeks drain directly into the Grand River above the Grand Rapids Wastewater Treatment Plant and they cannot help but contribute to the bacterial load in the river. Buck Creek (81.5 % exceedance) and Deer Creek (76.4%) are similar in that they drain agricultural regions. Drains in Indian Mill Creek (54.1% exceedance) may be contributing to fecal coliform counts in that watershed.

Silver Creek had the greatest range of fecal coliform counts (35,390 colonies per 100 mL) but the other stations also had large ranges between 10,291 and 17,973 colonies. Geometric means of the tributaries ranged from 117 colonies per 100 mL at Mill Creek to 763 colonies per 100 mL at the upper Plaster Creek station. All stations showed a positive skew (range: 2.25 to 7.28). The histogram for the tributaries is similar to that of the river run stations except that there are more high counts (compare Figures 10 and 11). The geometric mean for the tributary data set ($n = 934$) was 352 colonies per 100 mL with a 59.8% exceedance rate. The arithmetic mean was 1,455 colonies per 100 mL. This suggests that, overall, tributaries are more likely to have higher fecal coliform counts than main river stations.

Notched box plots for six of the tributaries graphically display the differences between the stations (Figure 18). Silver Creek, Plaster Creek, and

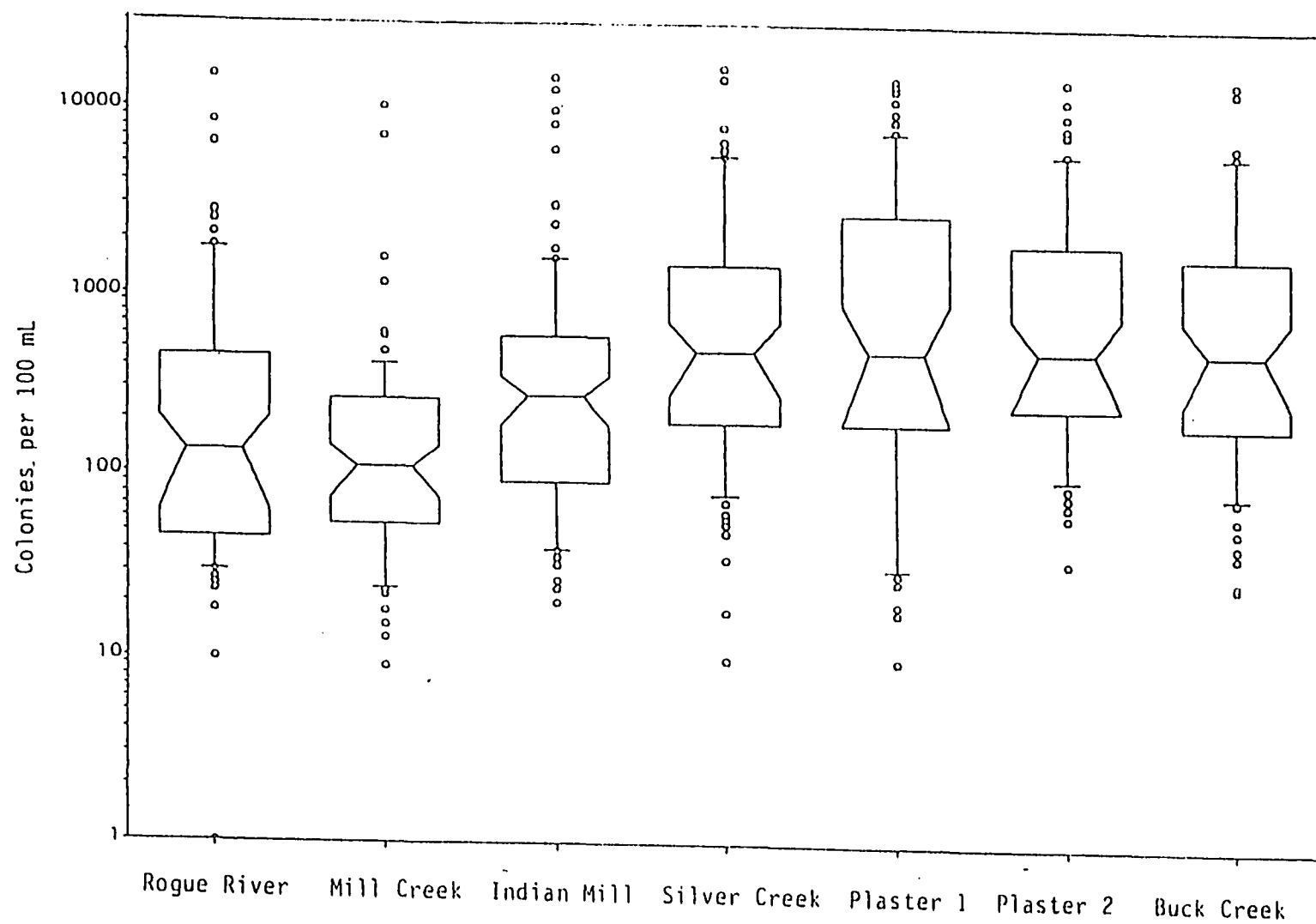


Figure 18. Notched Box Plots of GR WWTP Tributary Fecal Coliform Monitoring Stations, 1987-96.

Buck Creek are clustered with similar means that are not significantly different from each other ($p > 0.05$). The downstream Plaster Creek station had a proportionally large upper interquartile range which is characteristic of a data set with a preponderance of high counts. In contrast, Indian Mill Creek was similar to the upstream station (Station 1) in that the lower interquartile range was less than the upper range. The Rogue River and Mill Creek means were not significantly different ($p > 0.05$). The box plots reveal that the overall range of outliers varies between the stations and each station has a unique box plot with Buck Creek and Silver Creek being the most similar. Silver Creek is mostly an enclosed system where fecal coliform bacteria could multiply. The following studies on drains illustrate their importance to understanding fecal coliform dynamics.

Urban Drain Studies

Urban drains in Kent County were surveyed in two projects in 1989-90: one by the City of Wyoming and another by the Grand Rapids Waste Water Treatment Plant (Madden, 1990; Barton, 1990). Drains offer a perspective on fecal coliform bacteria that cannot be found in natural streams. Given the influence of light on the survival of the bacteria, the darkness of drains removes a variable. Natural purification of streams by ultra-violet radiation is inhibited in a drain situation. Low flows are the norm in drains which are, of course, constructed to carry excess

surface water during storm events. Portions of drains may or may not be impacted by groundwater depending upon the integrity of the system. Access to drains is limited, and they are not perceived as a public health problem except when they transport water into open water. The presence of animals in drain systems is problematic since their wastes can contribute pathogens to the water and their activities can block drains.

The City of Wyoming project involved analysis of two storm drain systems, Burton Street and Lee, that enter Plaster Creek and eventually, the Grand River (Madden, 1990). The drains were initially constructed in 1919 with expansions and upgrades in 1931 and 1952. Illegal connections were thought to be responsible for water quality complaints associated with these drains. The Grand Rapids project focused on Silver Creek Drain (Barton, 1990). Both projects provided fecal coliform data for analysis, but they both suffer from lack of sterile technique.

Burton Street and Lee Drains

The Burton Street Drain system is an extensive maze of drains under more than 30 streets (Appendix A, Figure 7). The Lee Street system is less complex with the main drain running along Lee Street connecting portions of about ten streets. The land use of the Lee system is primarily residential with some shopping centers, an abandoned aluminum extrusion plant, gas stations, a food processor, and old industrial sites. Groundwater infiltration into the Lee system is evident and

leads to a constant clear flow (Madden, 1990). The Burton Street land use system is also primarily residential, mixed with major business districts, which include numerous auto service facilities, and a metal working facility with a lagoon.

Daily grab samples of Lee and Burton Street drains, that drain into Plaster Creek, were taken from 13 June 1990 through 30 August 1990 (Madden, 1990). The drains were accessed via manholes for sampling.

Geometric means for fecal coliform counts ($n = 53$) in Burton Drain were 754 colonies per 100 mL with a range of 50 to 15,800 colonies (Table 8). The Lee Drain geometric mean for fecal coliforms ($n = 53$) was 818 colonies per 100 mL with a range of 10 to 31,200. As indicated by a t-test, there were no significant differences between the two drains ($p > 0.05$, $r = 0.52$). The box plots for the two drains are similar and have an overlapping range at the notches which represent the 95% confidence level (Figure 19). Percent exceedances above the fecal coliform standard of 200 colonies per 100 mL were 73.6% for Burton Drain and 79.2% for Lee Drain. Skew varied from 1.96 for Burton Drain to 3.58 for Lee Drain which is typical of a log normal distribution. Lee Drain had the highest kurtosis value (13.36) of all of the data sets which is a result of the high count of 31,200 colonies per 100 mL contributing to the peak in the data.

Unique to this data set are the daily values as shown in Figure 20. No particular pattern is evident when the data are plotted on a log scale. Correlations were nearly zero for fecal coliform counts with precipitation ($r = 0.0086$ for Lee

Table 8

Summary Statistics for Lee Drain and Burton Drain Fecal Coliform Counts, 1990

Statistic	Burton Drain	Lee Drain
% Exceedance	73.6	79.2
Geometric mean, colonies per 100 mL	754	818
Mean, colonies per 100 mL	2,545	3,034
Standard Error	565	865
Median	600	840
Mode	200	850
Standard Deviation	4,114	6,300
Sample Variance	16,925,387	39,687,261
Kurtosis	2.85	13.36
Skewness	1.96	3.58
Range	15,750	31,190
Minimum	50	10
Maximum	15,800	31,200
Sum	134902	160809
Count	53	53

and 0.14 for Burton) and with ambient mean air temperature ($r = 0.0169$ for Lee and -0.057 for Burton). However, a plot of the data on a linear scale with precipitation revealed elevated fecal coliform counts prior to rain events generally

decreased dramatically after the events (Figure 21). The three highest peaks in coliforms precede peaks in rainfall and occur after a week or more of less than 0.1 inches of precipitation per day. This suggests the possibility that there was some rain washing of large amounts of fecal coliforms before the flushing of the drain system and/or dilution took place. The elevated fecal coliform counts were attributed to animal fecal matter deposited in storm drains. Specifically, the researchers noted numerous raccoons in the storm drains (Madden, 1990).

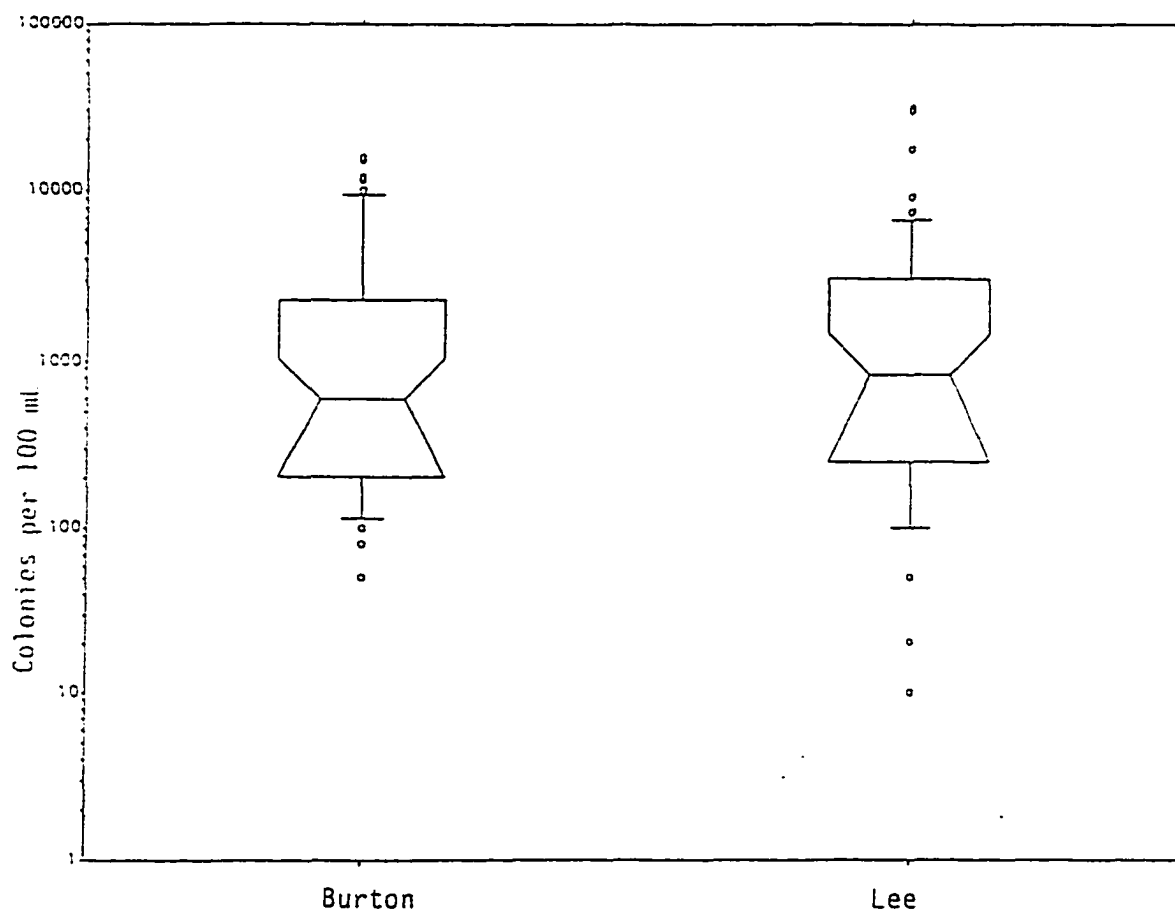


Figure 19. Notched Boxplots of Burton Drain and Lee Drain Fecal Coliform Bacteria, 1990.

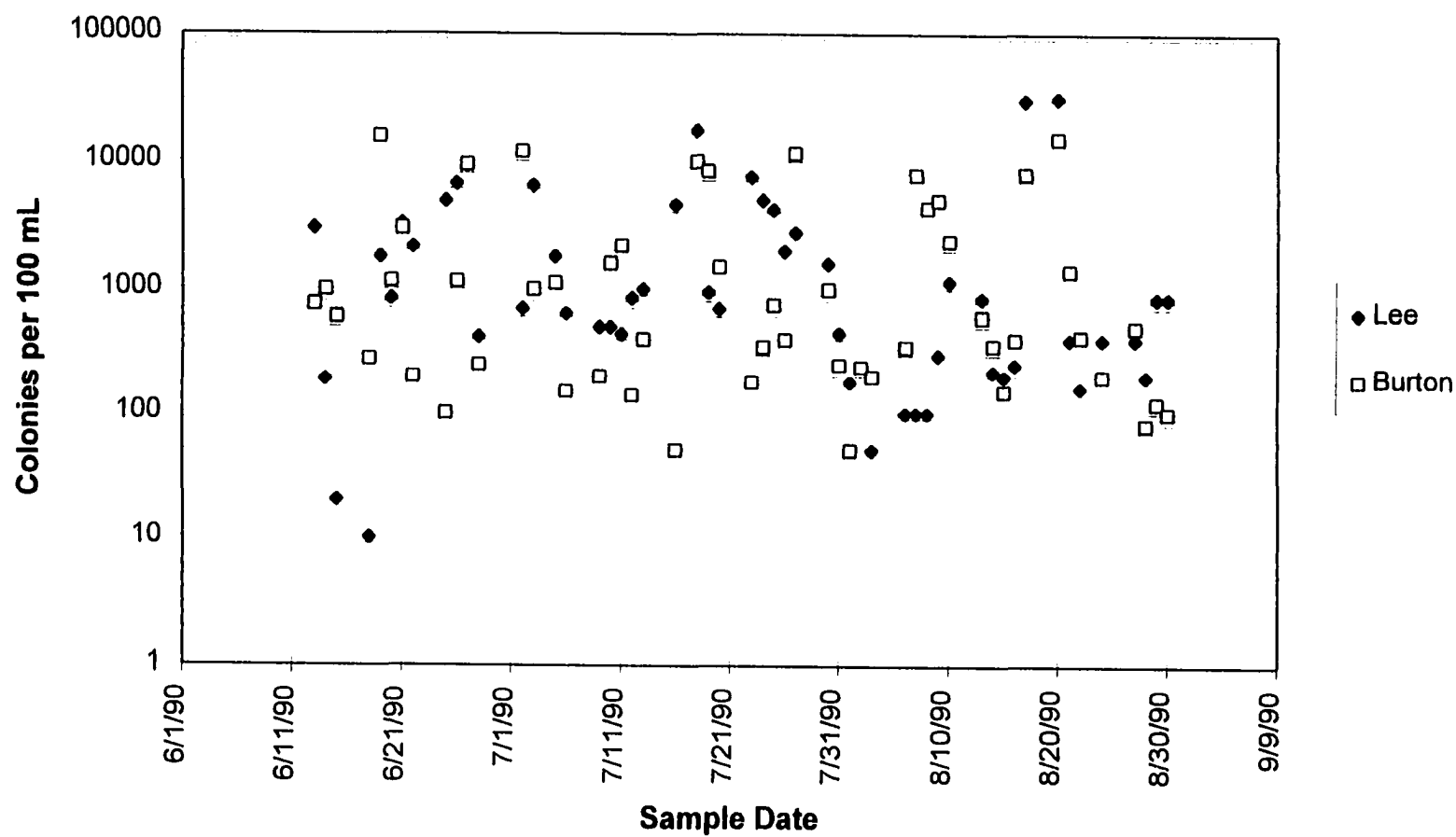


Figure 20. Daily Sampling of Fecal Coliform Bacteria at Lee and Burton Drains, 1990.

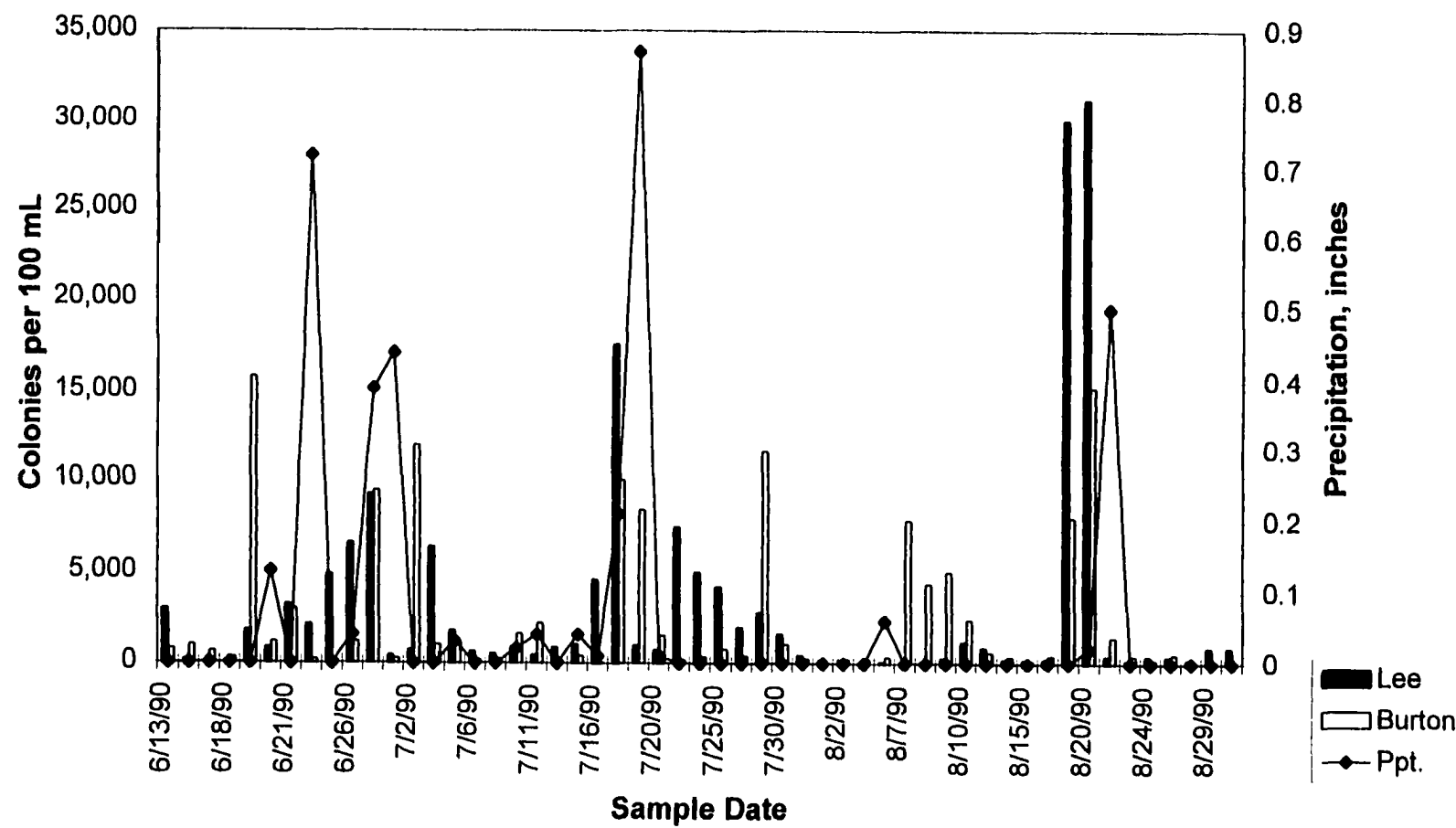


Figure 21. Daily Sampling of Fecal Coliform Bacteria at Lee and Burton Drains as Related to Precipitation, 1990.

Appendix B documents a rainfall event study showing that a rise in bacteria occurs with the beginning of a hydrographic rise in Plaster Creek. The rise preceding rainfall noted in the present study may be an artifact of the method of recording rainfall in climatological records. Even with precision in rainfall recording of the National Weather Service, the rainfall data are, nevertheless, measured at an airport southeast of Grand Rapids. Some lag in time for rainfall in many storm events due to the general movement of local rain clouds and attendant rainfall from west to east.

What is significant about this study is (1) the high concentration of fecal coliform bacteria harbored in drains, and (2) the relative stability of the drain fecal coliform population at levels above 200 colonies per 100 mL. Additionally, use of several types of graphical analysis of the data revealed more about the drain bacteria than a single graph.

Silver Creek Drain

Also draining into the Plaster Creek and the Grand River is Silver Creek. The creek merges with Plaster Creek north of the Lee and Burton Drain systems. Silver Creek was a natural creek until the 1920s when it was buried and enclosed. It is a major drainage system for southeast Grand Rapids and the City of East Grand Rapids. The upstream portion of the drain watershed has heavily populated residential areas, and the lower reaches are impacted by old industrial sites, some

of which were electroplating operations. Most of the drain is a concrete structure approximately 11 feet wide that varies between 5.5 and 8 feet in height. Silver Creek emerges from underground in a small area in East Grand Rapids.

Groundwater contributes to a small dry weather flow. The drain system cannot handle all of the drainage during rain storms and there are back-ups of runoff resulting in lowland flooding (Barton, 1990).

Intensive monitoring to assess both the delivery of fecal coliform to the Grand River and the impact of that delivery on the river was performed by the staff from the City of Grand Rapids Wastewater Treatment Plant (Barton, 1990). The Silver Creek Drain study was unique in that it involved a walkthrough of 15,000 feet of the drain. This study identified 226 connections to the drain (Barton, 1990). The study team noted that heavy rains can clean the streets, yards, parking lots and "junk piles" of anything that can be washed away (Barton, 1990). Wash-off of animal debris, litter, automobile fluids, and general debris was evident. Equipment washing contributed to dry weather flows and a constant oil sheen was observed at the Silver Creek outfall to Plaster Creek. Runoff from a major expressway and groundwater contaminated with fuel also contributed to sheen (Barton, 1990).

An upstream site (Fuller and Adams) and downstream site (Chicago and Clyde Park) in Silver Creek were sampled between June 1989 and September 1990 with special attention given to heavy metals. The upstream site drains residential and commercial areas, but no industrial areas. The downstream site receives all of

the Silver Creek drainage. Two ISCO continuous samplers taking discrete samples were used in the study. They were emptied at programmed intervals of time.

Results of the study indicated that the upstream station exceeded fecal coliform standards 97.6% of the time and the exceedance rate for the downstream site was 95.2% (Table 9). At no time were colony counts at zero. The upstream site only had two samples below 200 colonies per 100 mL and the downstream site had four samples below the standard. The geometric mean of fecal coliform colonies was 1,694 colonies per 100 mL for the upstream site ($n = 84$) and 1,190 for the downstream site ($n = 84$). A two sample t-test assuming unequal variance revealed no significant differences between the two sampling locations ($p > 0.05$). Correlation between the two sites as illustrated in Figure 22 was moderate ($r = 0.60$). Figure 22 also illustrates the differences and scatter in the fecal coliform counts in these two sites. There was not a pattern of the upstream site counts being higher than the downstream counts or vice versa. These studies reveal consistently high contributions of fecal coliform from this storm drain. For health reasons, students would be wise to avoid enclosed drain systems such as Silver Creek.

Special Studies

A common question by the public that can be answered by the GR WWTP data set is: Is the water quality improving or becoming worse? This type of analysis is difficult even with this large data set ($n = 1,831$). Timing of samples

Table 9
Summary Statistics for the Silver Creek Drain Study
Fecal Coliform Counts, 1989-90

Statistic	Upstream	Downstream
% Exceedance	97.6	95.2
Geometric Mean, colonies per 100 mL	1,694	1,190
Mean, colonies per 100 mL	3,233	3,145
Standard Error	444	492
Median	1,567	895
Mode	15,000	15,000
Standard Deviation	4,069	4,505
Sample Variance	16,554,638	20,297,204
Kurtosis	3.07	1.73
Skewness	2.00	1.74
Range	14,894	14,913
Minimum	106	87
Maximum	15,000	15,000
Sum	271,553	264,216
Count	84	84

and sample location may or may not reflect the average condition of the river or its tributaries. There is a finite probability that in a given year, sampling days may

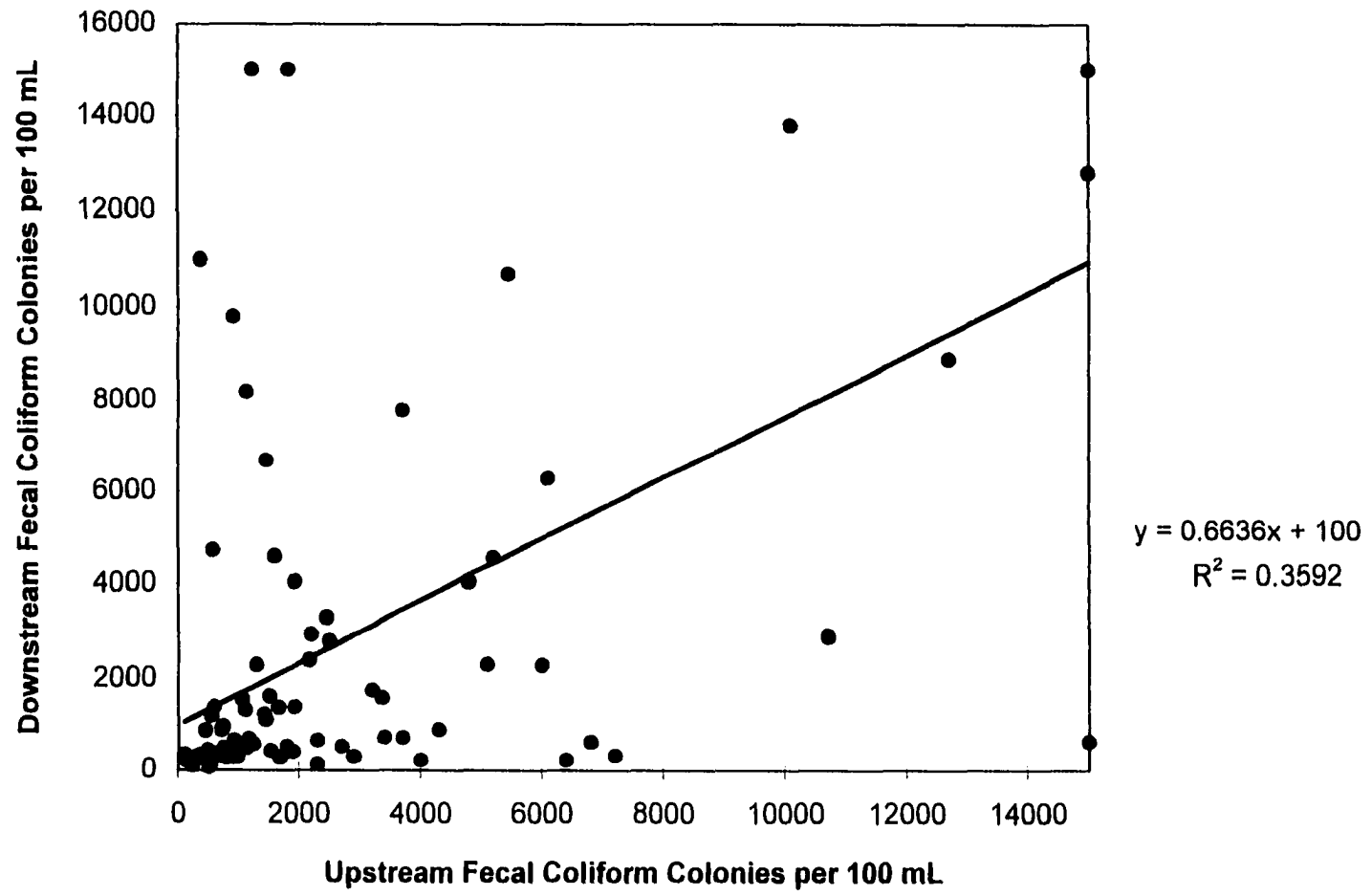


Figure 22. Comparison of Upstream and Downstream Stations at Silver Creek, 1989-90.

coincide with unusual weather or river flow conditions. This is the constraint of a fixed-day sampling regime. Also, the database does not reflect equal sampling of all stations throughout a year (e.g., samples could not be taken at some stations in January and February). However, a preliminary conclusion might be that the microbiological quality is improving based on a comparison of geometric means and exceedance rates from 1985 through 1996 (Figure 23). The regression equation for the trendline is: $y = -10.973x + 377.57$ ($R^2 = 0.5561$). However, this conclusion may be due to stochastic (random) or cyclic climate parameters. The specific sample location within a river or stream may also be a major variable. Data from special studies by the Grand Valley State Water Resources Institute and by the Grand Rapids Wastewater Treatment Plant shed some light on this issue. A description of the GVSU stormwater sampling study at Plaster Creek is provided in Appendix B. This study monitored storm events as they related to fecal coliform levels.

In 1985, the City of Grand Rapids Wastewater Treatment Plant (WWTP) performed an intensive study on the Grand River from 1.25 miles upstream of the plant to four miles downstream (Barton et al., 1986). Unlike other studies where samples were taken from bridges, a boat and a weighted Wildco stream sampler were used to sample eight sampling locations. There were four upstream stations of the WWTP and four downstream (Appendix A, Figure 6). Both the Grand Rapids Wastewater Treatment Plant and the Wyoming Wastewater Treatment Plant

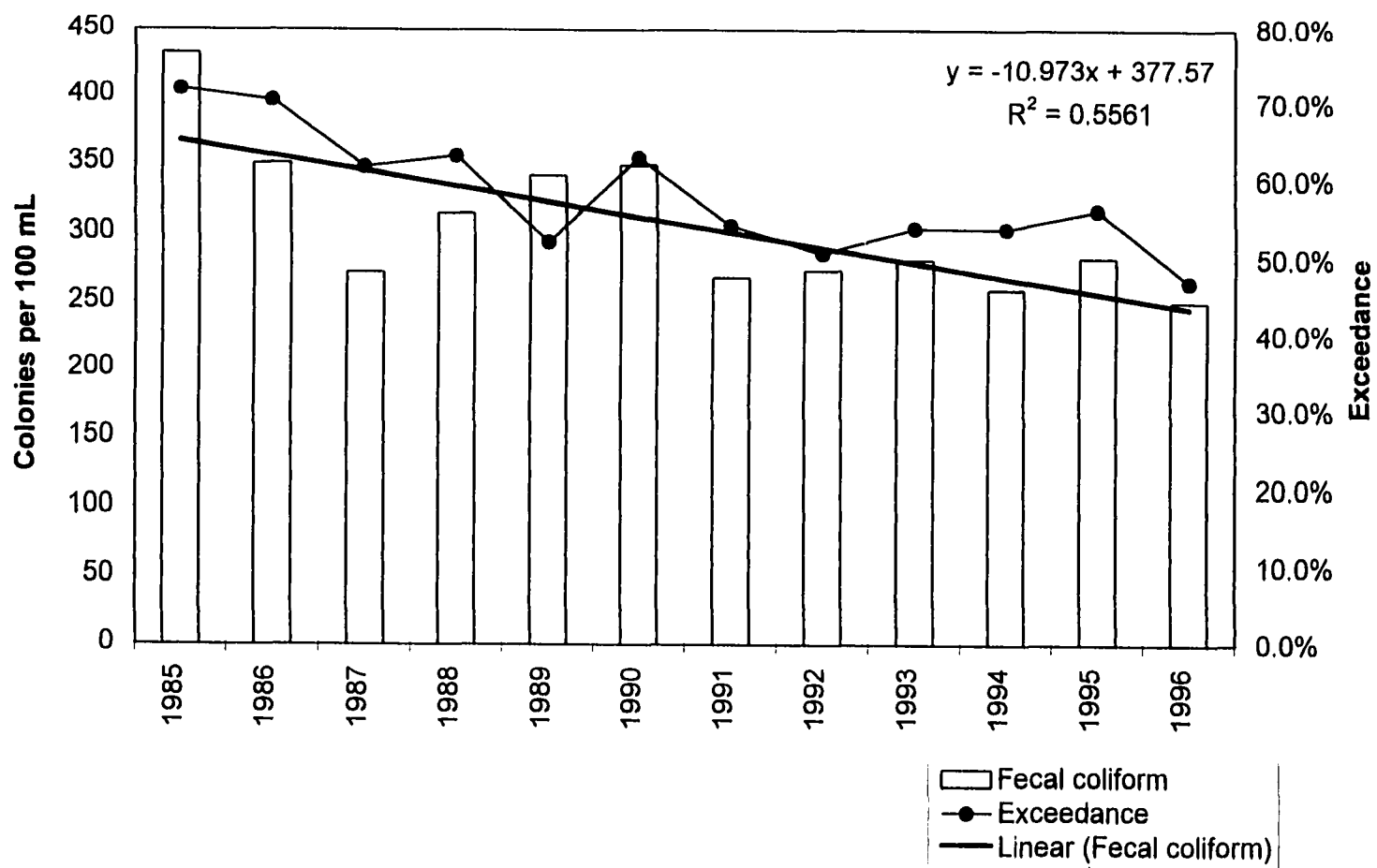


Figure 23. Yearly Geometric Means and Exceedance Rates for GR WWTP River and Tributary Database, 1985-96.

discharge effluent along this stretch of the river. Seven water samples were taken at each location with a surface transect of five samples, a midstream/mid-depth sample, and a midstream/bottom sample. The water samples were collected while the WWTP effluent was still being chlorinated (15 October 1985) and after chlorination had stopped for the season (29 October 1985). Membrane filtration of one mL and ten mL samples was performed at the GR WWTP laboratory.

On 15 October 1985, when the plant was still chlorinating effluent, the fecal coliform bacterial counts in the downstream Stations 5 through 8 (Geometric mean = 339 colonies per 100 mL) were significantly lower ($p < 0.01$ for t-test) than upstream Stations 1 through 4 (Geometric mean = 454 colonies per 100 mL). On 29 October 1985, the reverse was true with the upstream stations having significantly lower means ($p < 0.01$) of 396 colonies per 100 mL than downstream stations with 795 colonies per 100 mL.

There were significant differences in the fecal coliform counts depending on where the sample was taken in the river. On 15 October 1985, the FC counts from the south bank were consistently higher ($p < 0.01$) than those of the north bank (Figure 24). This is not borne out by the long-term monitoring data from the GR WWTP of the nearby railroad bridge sampling sites where the geometric mean of the 1985-96 samples on the south side was 165 FC colonies per 100 mL and the north side was 269 FC colonies per 100 mL.

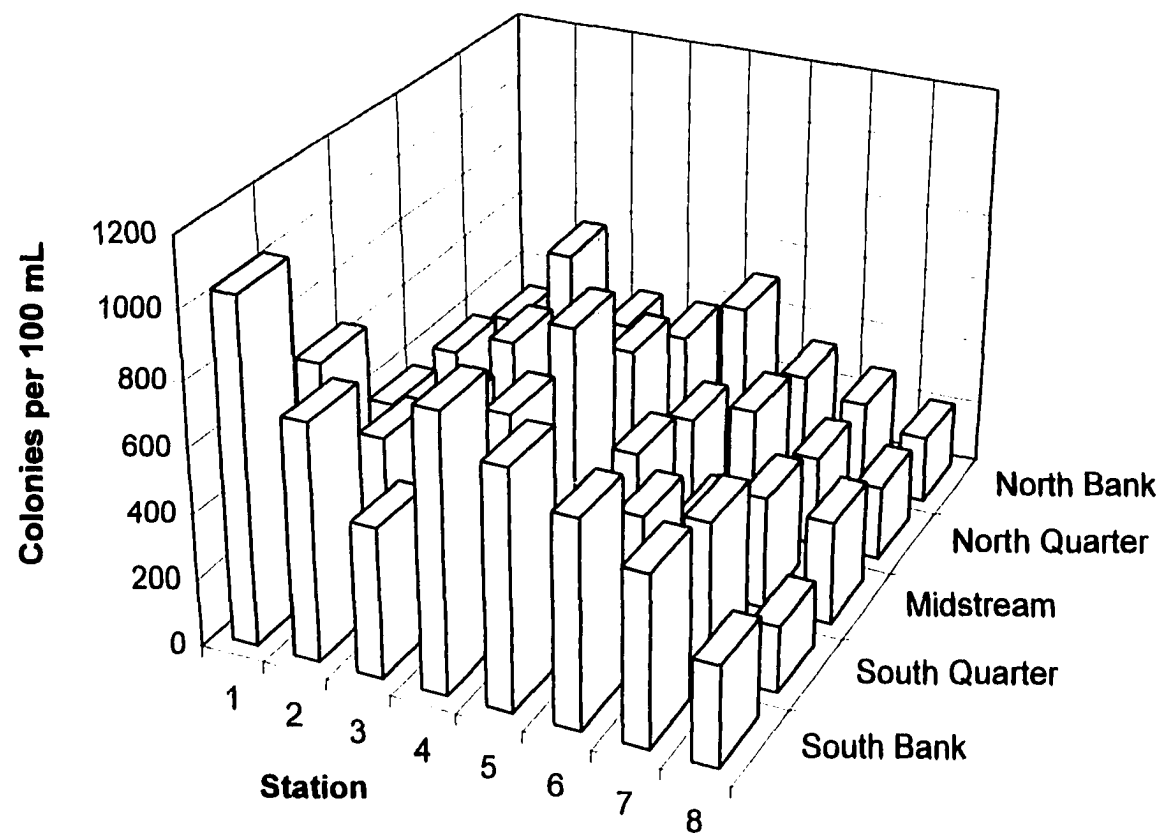


Figure 24. Cross-Section Fecal Coliform Counts in the Grand River, 15 October 1986.

An anomaly in the 29 October 1985 sampling event resulted in the composite mean for the north bank being elevated (Figure 25, note different colony scale). At location 3 above the WWTP, a high count in the north bank sample of 15,000 FC colonies per 100 mL was measured with 6,000 FC colonies per 100 mL at the next station. Above that station, the counts were 230 colonies per 100 mL and midstream sample at Station 3 was only 170 colonies per 100 mL. Upstream from Station 3 on the north side of the river is a channel with the Butterworth overflow regulator which was probably allowing intermittent flows. This is consistent with the long term monitoring data at the Railroad Bridge which suggest higher counts on the north side of the river.

Vertical FC counts at each station in the Grand River were made midstream at three different depths: surface, 1/2 depth, and bottom. The overall midstream surface counts on 15 October were 377 FC colonies per 100 mL, 1/2 depth was 343 and the bottom depth was 320 which is a slight but not significant ($p > 0.05$) decrease in counts with depth (Figure 26). A similar sampling regime was also performed on 29 October 1997. One value was missing from the bottom and mid-depth samples. Overall midstream surface counts on 15 October were 507 colonies per 100 mL, 1/2 depth was 591 and the bottom depth was 446 (Figure 27, note change of colony scale). Midstream surface samples that are typical of the GR WWTP's monthly sampling regime appear to generally reflect what may be a greater depth and appear to be adequate to locate major sources of fecal

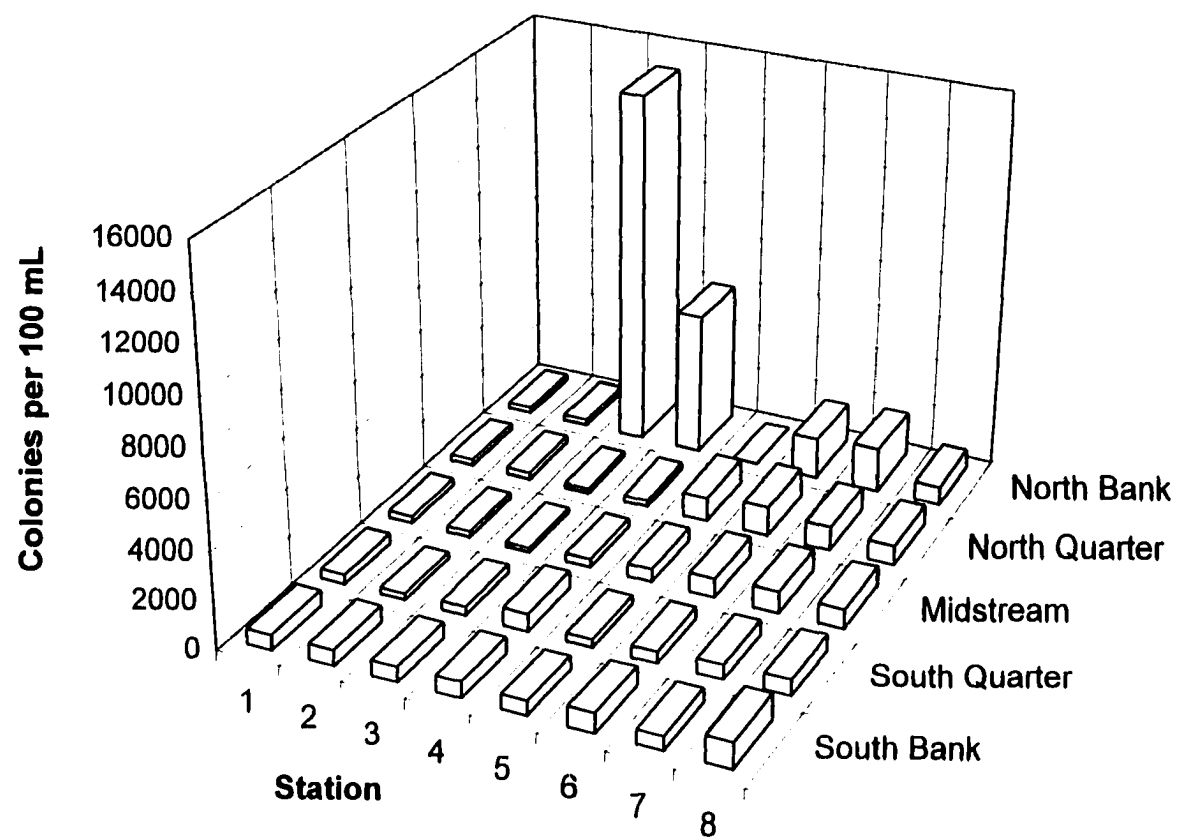


Figure 25. Cross-Section Fecal Coliform Counts in the Grand River, 29 October 1986.

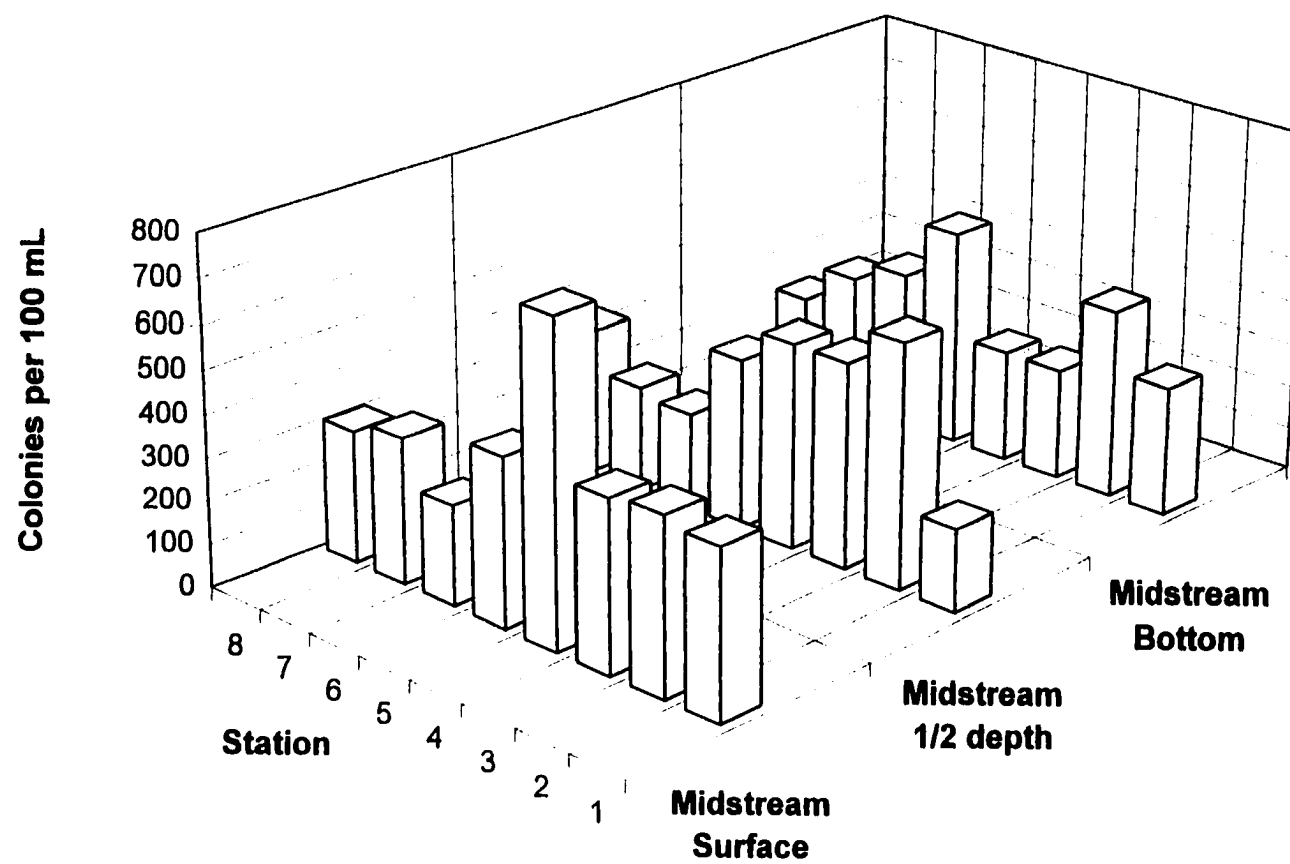


Figure 26. Vertical Fecal Coliform Counts in the Grand River, 15 October 1986.

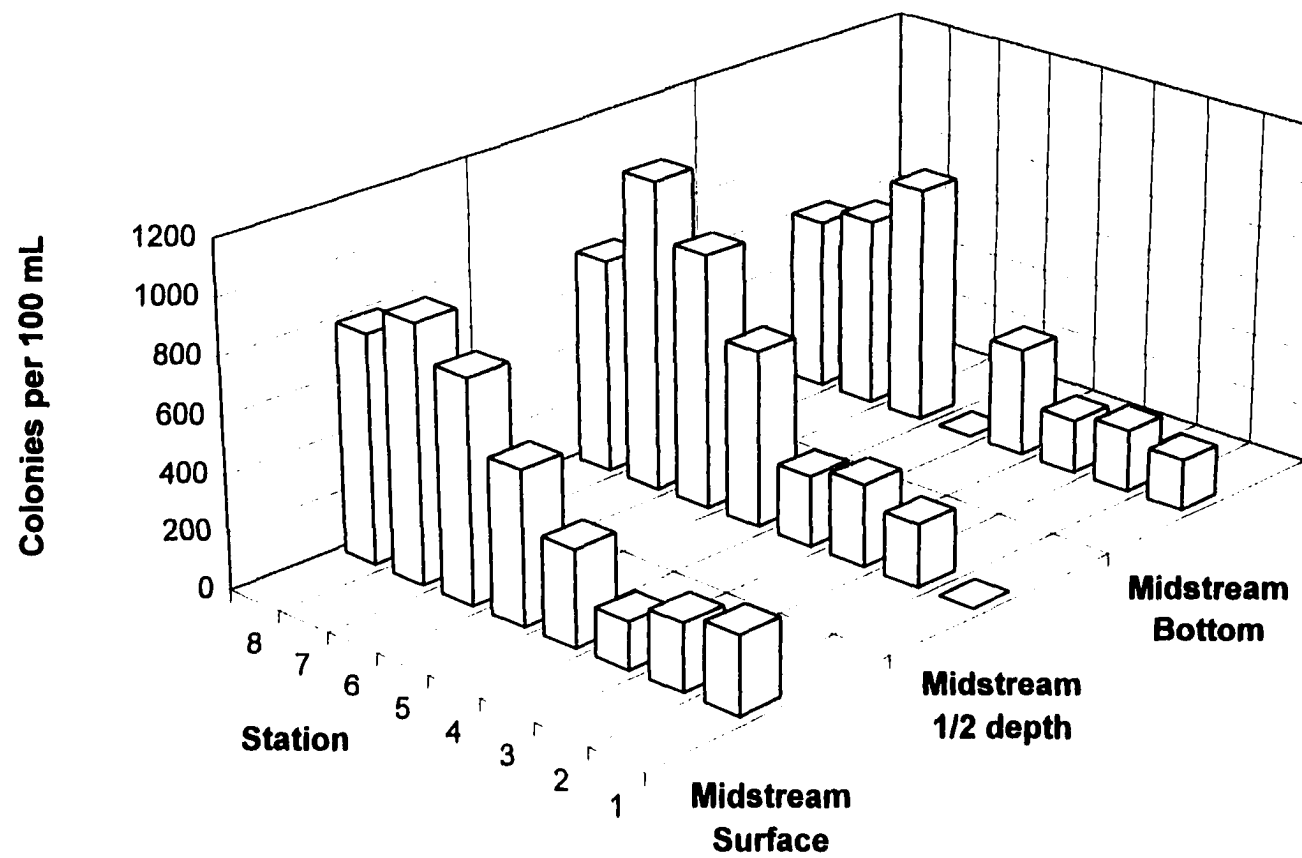


Figure 27. Vertical Fecal Coliform Counts in the Grand River, 29 October 1986.

contamination. However, they do not detect intermittent or low volume pollution along banks or differences such as those found between the higher counts on the south than the north banks. Data from studies such as this provide much insight into river dynamics and are an important context for the interpretation of monthly monitoring data.

E. coli - Fecal Coliform Relationship

In conjunction with the GVSU Water Resources Institute studies on Bear Creek in Kent County, parallel samples of fecal coliform bacteria and *E. coli* were taken at ten locations in 1995-96. These were the regular Kent County Health Department sites at Townsend Park (Station 8), Giles Avenue (Station 29), Egypt Valley (Station 30), and Chauncy Street (Station 31), and six other sites. Locations of these sites as well as other Bear Creek sampling sites are found in Appendix A. Analysis of *E. coli* was performed by the Kent County Health Department and fecal coliform analysis was done at the GVSU Water Resources Institute.

As previously mentioned, fecal coliform monitoring has largely been replaced by *E. coli* monitoring. In order for historical fecal coliform data to be compared to *E. coli* data, there needs to be a conversion factor. A 0.65 conversion factor (EC to FC, or 1.54 FC to EC) is implicit in the 130 EC colonies versus 200 FC colonies per 100 mL standards.

For the four Kent County sites, *E. coli* geometric means for the April through October sampling periods exceeded 130 colonies per 100 mL for all stations. The a range was 265 to 494 colonies per 100 mL in 1995, and 354 to 501 colonies per 100 mL in 1996. Overall geometric means for all ten sites in 1995 were 239 colonies per 100 mL for fecal coliform and 293 colonies per 100 mL for *E. coli*. In 1995, the correlation between FC and EC was high ($r = 0.85$) with an average EC/FC ratio of 1.19 ($n = 21$). Station-specific patterns were evident at the ten stations with several stations having consistently high counts for both EC and FC and other stations being consistently low (Figure 28).

Parallel FC-EC samples for the seep and the wetland in 1995-97 revealed no consistent relationship between individual pairs of *E. coli* and fecal coliform bacterial counts. However, fecal coliform counts were generally greater than *E. coli* and less than total coliform counts (see Figure 1). The average EC/FC ratio ($n = 49$, $r = 0.57$) was 0.71 with 77.5% of the FC counts being greater than the EC counts. Methods used for this comparison were membrane filtration using m-FC medium and Hach's m-ColiBlue 24 medium for coliforms and *E. coli*. The m-ColiBlue 24 medium compares favorably to media such as mTEC and m-Endo-NA-MUG used in other studies (Grant, 1997).

Given the variation of the EC/FC ratios, little can be concluded about the relationship of a single sample of fecal coliform bacteria to the level of *E. coli*.

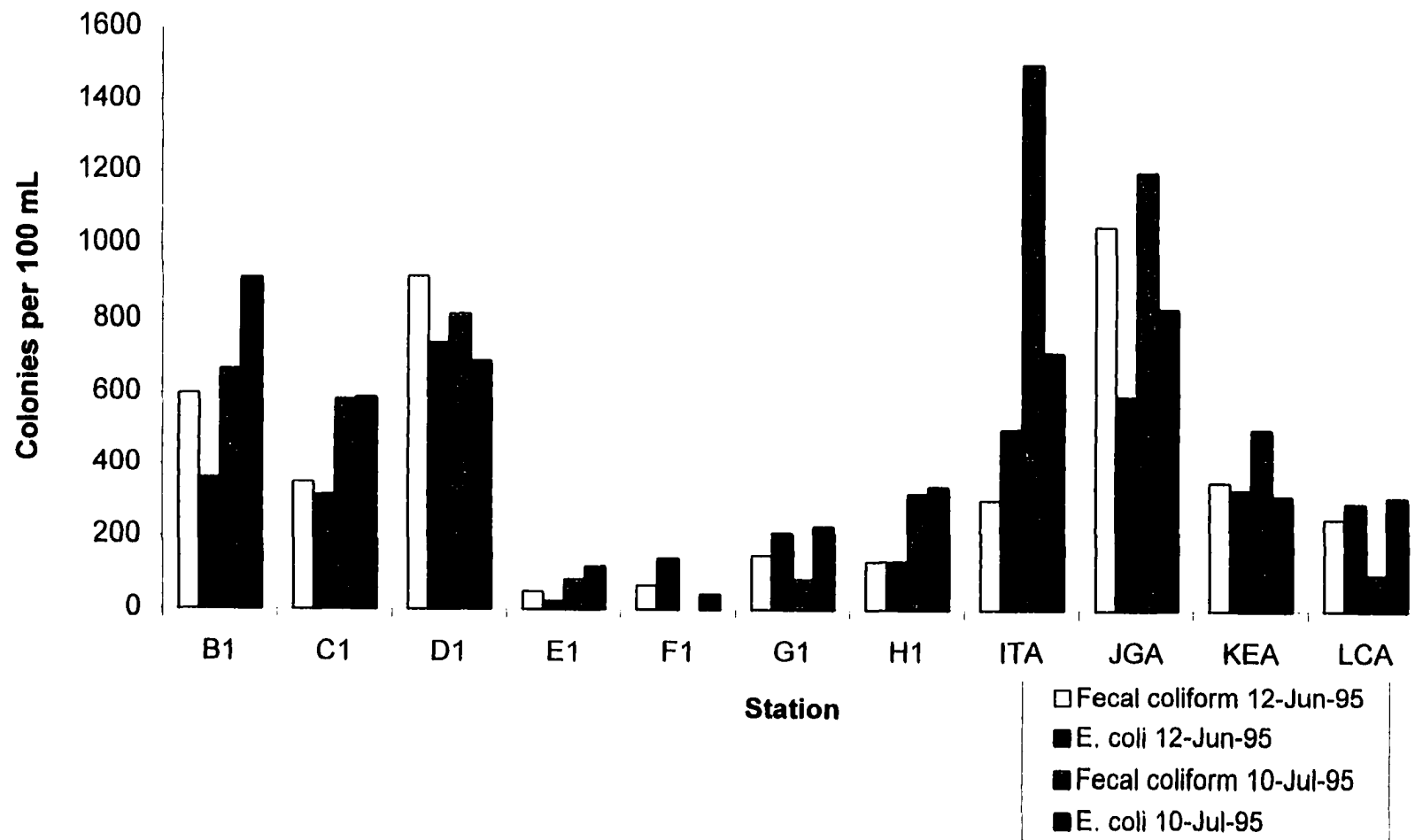


Figure 28. Comparison of *E. coli* and Fecal Coliform Counts at Monitoring Stations in the Bear Creek Watershed, 1995.

The difficult aspect of calculating this ratio are that zero counts or even one colony per 100 mL can skew the ratios to an unacceptable extent. Although not commonly done, another approach would be to take a sum of the total number of bacterial colonies for FC and EC and calculate the EC/FC ratio. This adjustment is shown in Table 10 which is essentially a regression towards the mean. Even with this adjustment, EC/FC ratio is more than 1.0 for the California data set (San Lorenzo River) and the Michigan data (Seep/wetland and Bear Creek) is less than 1.00. The seep and wetland ratios appear to be closest to the theoretical ratio of 0.65 that has been suggested by the U.S. EPA studies. The relative sensitivity of the methodologies for detecting *E. coli* and fecal coliforms might be responsible for the apparent anomaly where the subset of fecal coliforms (*E. coli*) has higher counts than the fecal coliform bacteria.

Table 10

Comparison of Different Methods for Calculating EC/FC Ratios

Location	Average of Individual EC/FC Ratios	Composite EC/FC Ratios
Seep/Wetland (1995-97)	0.71	0.54
Bear Creek (1995)	1.19	0.86
San Lorenzo River (1994-96)	1.18	1.04

Kent County Health Department

The Kent County Health Department data set provides a basis for comparing fecal coliform and *E. coli* monitoring data. A Kent County Health Department report (KCHD, 1992, page 1) summed up the situation in 1988 when the County was planning the sampling program:

Very little background information was currently available from any source which described the "normal" water quality of Kent County streams and rivers. This lack of background information made it virtually impossible to determine what degree of risk, if any, was experienced by persons using local surface waters for various recreational activities. Without such background information, it was also extremely difficult to judge when the effects of a specific contamination episode has subsided to "normal" levels.

Although data are available back to 1989, this analysis is limited to two years of fecal coliform monitoring before the standard changed (1993-94) and two years of *E. coli* monitoring after the standard changed (1995-96). Data are available from early April through mid-October for these four years and there are historical data available back to 1989. There were 14 sampling events in 1993 and in 1994, and 7 events in 1995 and in 1996. In all, 2,113 samples were included in this analysis (1993: $n = 770$, 1994: $n = 440$, 1995: $n = 441$, 1996: $n = 462$). Not all of the 32 stations were monitored each year, and there were some special sampling

The locations of the stations are summarized in Appendix A, Figure 3. Current sampling locations are at the river below Grand Rapids and along the major tributaries of the Grand River which include (from upstream to downstream) Flat

River, Thornapple River with branches (Tyler Creek, Coldwater River), Bear Creek, Rogue River and its far upstream tributary (Duke Creek), Mill Creek, Plaster Creek, and Buck Creek. A reference station at Johnson Park (Station 32) is close to where Grand Rapids monitors the Grand River (river run Station 5). Fecal coliform yearly exceedance rates for the reference station for 1993-4 (53.8%) are comparable with the long term rates found by the GR WWTP (55.5%).

Yearly geometric means for the composite of all stations were higher in 1993 (310 colonies per 100 mL and 1994 (252 colonies per 100 mL) than in 1995 (205 colonies per 100 mL) and 1996 (236 colonies per 100 mL when the county changed from fecal coliform to *E. coli* monitoring (Figure 28). These means are above the fecal coliform (200 colonies per 100 mL) and *E. coli* (130 colonies per 100 mL) standards for total body contact. Percent exceedance of full body contact standards for all of the samples in a given year varied from 58.2% in 1994 to 70.6% in 1996 (Table 11). What is of note is that exceedance rates for *E. coli* are significantly higher than those of fecal coliform ($p < 0.05$). All things being equal, this implies that the *E. coli* standard may be more difficult to meet.

The pattern of fecal coliform and *E. coli* for all stations for 1993-96 is shown in Figure 29. Shapes of the distribution of counts are similar for all years. Lowest counts were found in 1995-96 when *E. coli* was measured. However, 1995 was a drought year for the area which acts as a confounding variable to this sort of analysis. The 1996 pattern is more similar to previous years. A seasonal pattern is

Table 11

Percent Exceedance of Fecal Coliform (200 colonies/100 mL) and
E. coli (130 colonies/100 mL) Standards for Kent County
 Health Department Sites

Station	Fecal Coliform		<i>E. coli</i>	
	1993	1994	1995	1996
1 Plaster	82.9	—	—	85.7
2 Buck	—	90.5	85.7	—
3 Mill	77.1	52.4	57.1	76.2
4 Rogue	60.0	47.6	71.4	76.2
5 Duke	57.1	61.9	71.4	61.9
7 Rogue	45.7	38.1	14.3	57.1
8 Bear	57.1	76.2	85.7	81.0
9 Flat	42.9	4.8	0	33.3
11 Coldwater	51.4	66.7	95.2	85.7
13 Thornapple	37.1	0	0	28.6
16 Buck	80.0	—	—	85.7
20 Plaster	—	85.7	85.7	—
21 Plaster	—	76.2	80.9	—
22 Plaster	80.0	—	—	90.5
23 Rogue	62.9	33.3	38.1	71.4
24 Rogue	42.9	33.3	57.1	61.9
25 Rogue	60.0	57.1	85.7	—
29 Bear	65.7	76.2	85.7	71.4
30 Bear	74.3	61.9	81.0	85.7
31 Bear	65.7	76.2	76.2	85.7
32 Grand	60.0	47.6	52.4	42.9
33 Tyler	80.0	85.7	85.7	81.0
34 Coldwater	—	57.1	71.4	76.2
All Samples	59.8	58.2	65.1	70.6

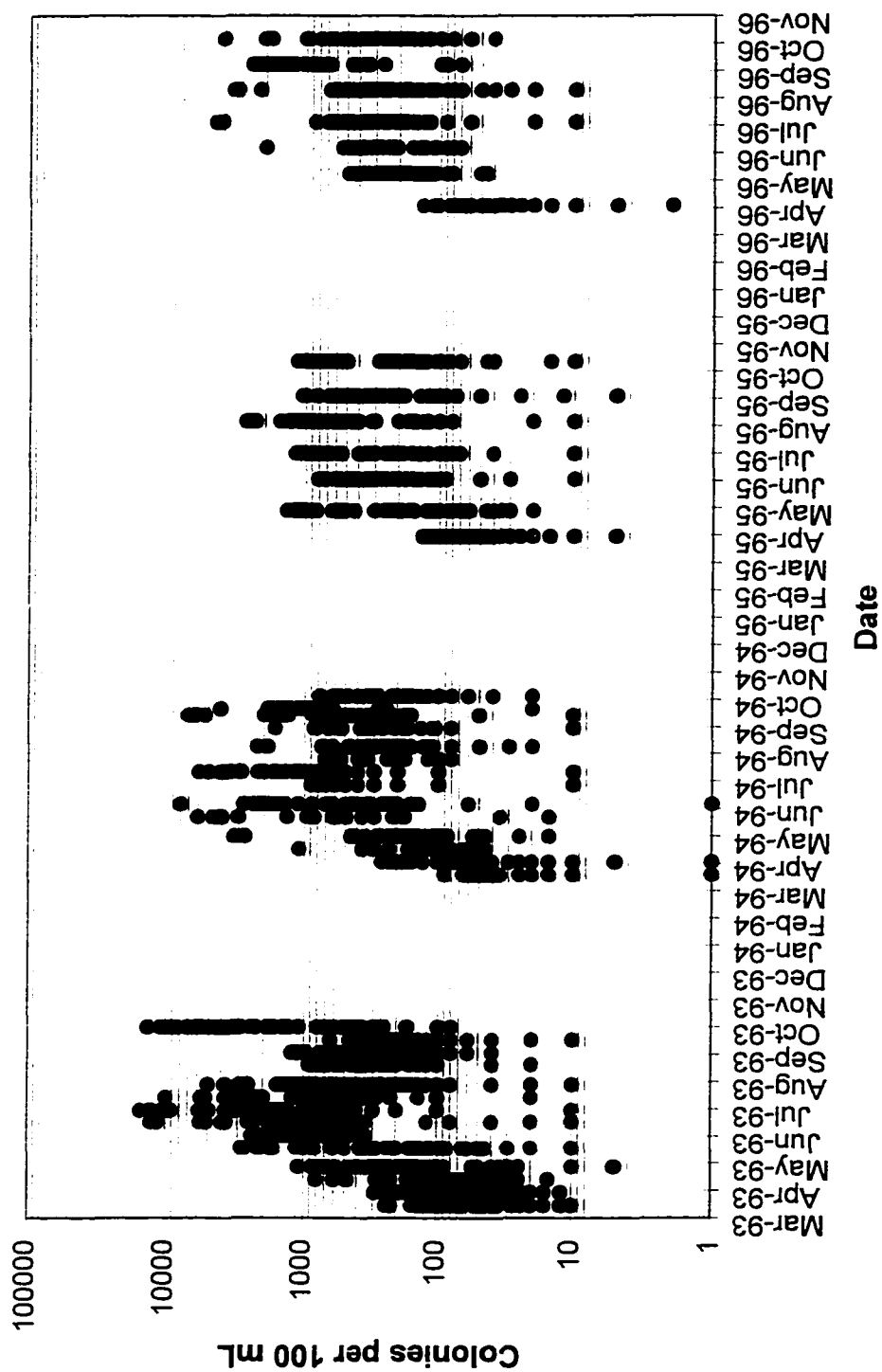


Figure 29. Distribution of Fecal Coliform (1993-94) and *E. coli* (1995-96) for Kent County Health Department Stations.

evident for all of the years with bacterial levels gradually increasing from April through October as quantified in regression equations (Figures 30-33). For fecal coliform bacteria, there appears to be a peak in mid-summer followed by a decline in late August and a subsequent increase in October (Figures 30 and 31). For *E. coli*, the peaks are not as evident which could be related to the decreased frequency of sampling (Figures 32 and 33).

The partial body contact standard is 1,000 *E. coli* per 100 mL. Exceedance rates for partial body contact of 1,000 colonies per 100 mL were ranged from 22.9% in 1993 to 7.9% in 1995 (See Figure 34). Although the partial body contact exceedances were low in 1995, they increased to 1993 levels in 1996 (20.6%). In 1995, "Partial Body Contact" warnings were posted in only one area (Plaster Creek) where standards were exceeded three of the seven sampling months (Kent County Health Department, 1996). In 1996, two areas in Plaster Creek and three areas in Bear Creek at Townsend Park were posted for exceeding "Partial Body Contact" standards (Kent County Health Department, 1997).

There was high degree of variability among individual stations (Figure 35). The highest counts were found in Buck Creek (Station 2) and Plaster Creek (Stations 1, 21, and 22). This is consistent with the monitoring of the Grand Rapids WWTP although their values were generally higher.

The Thornapple River (Station 13) and the Flat River (Station 9) consistently had the lowest counts. The Thornapple River site is below a dam in Ada.

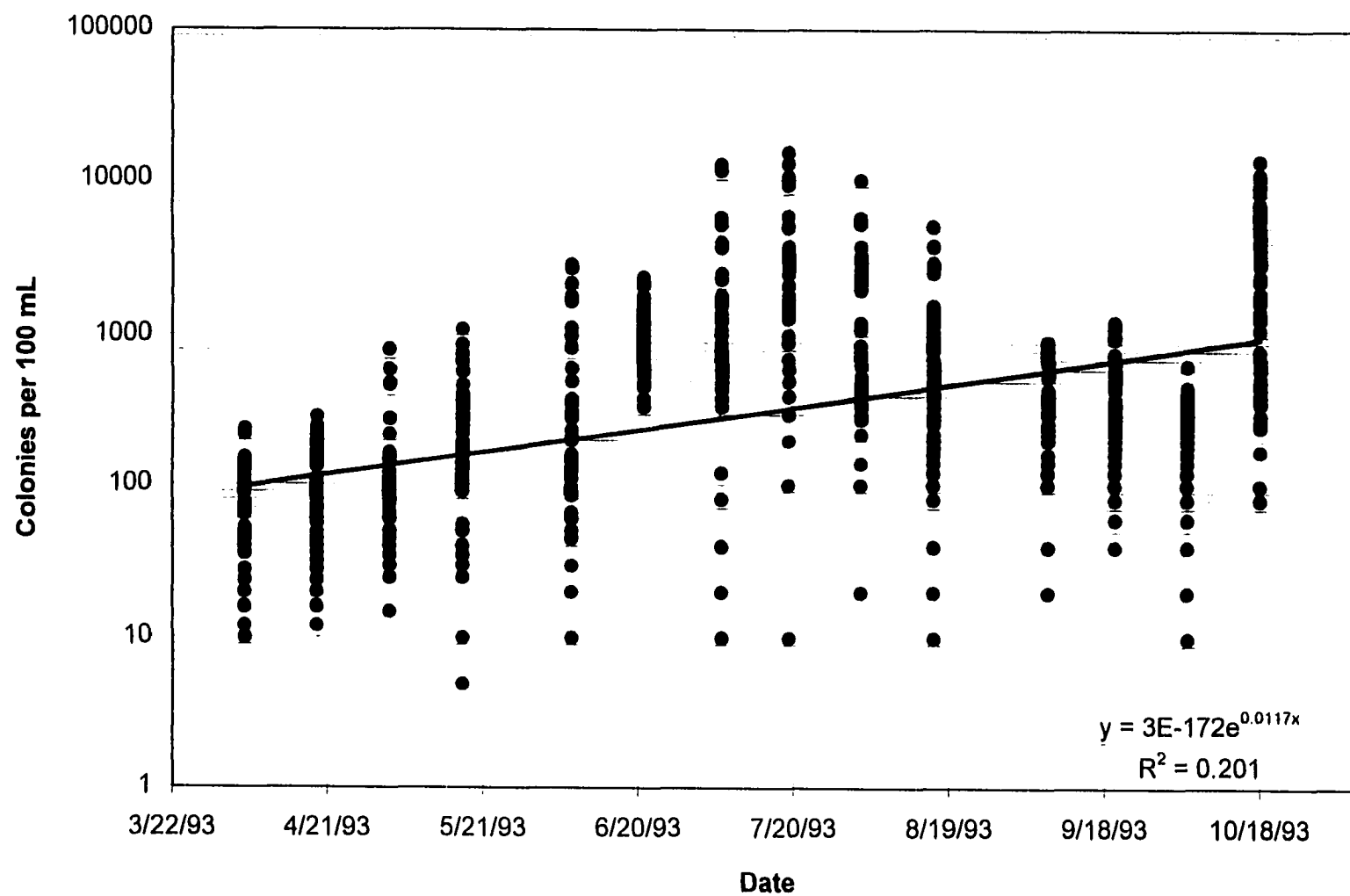


Figure 30. Distribution of Fecal Coliform for Kent County Health Department Stations, 1993.

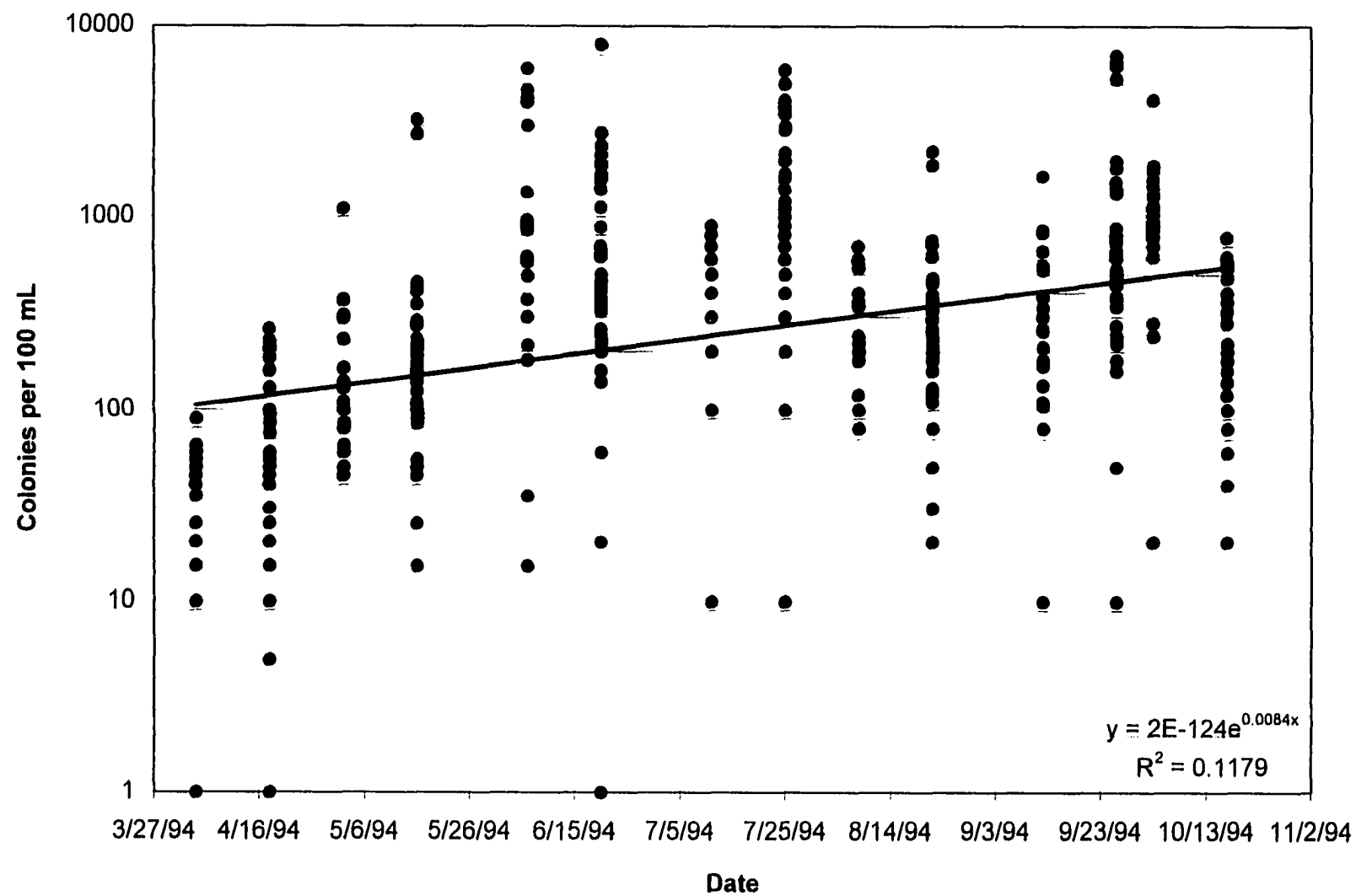


Figure 31. Distribution of Fecal Coliform for Kent County Health Department Stations, 1994.

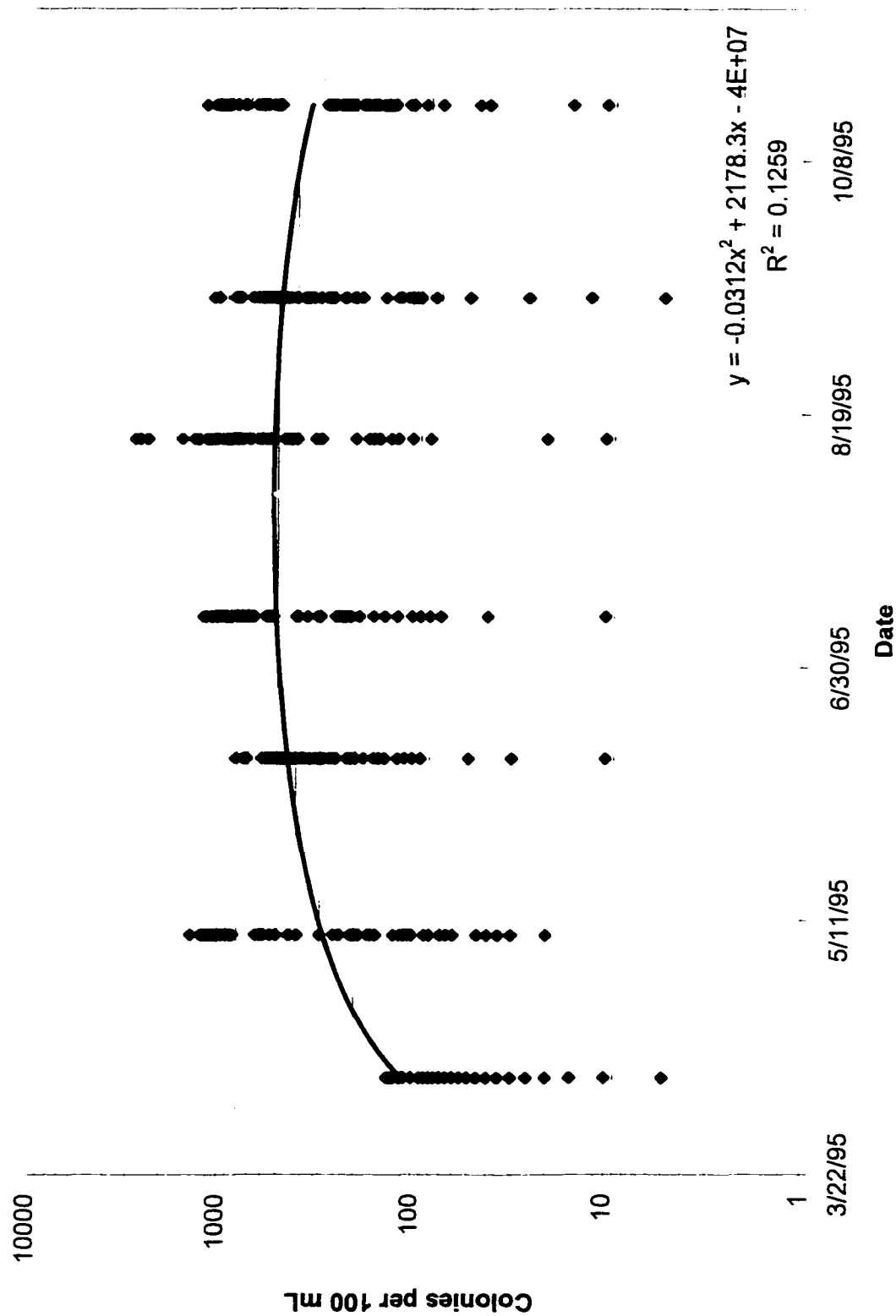


Figure 32. Distribution of *E. coli* for Kent County Health Department Stations, 1995.

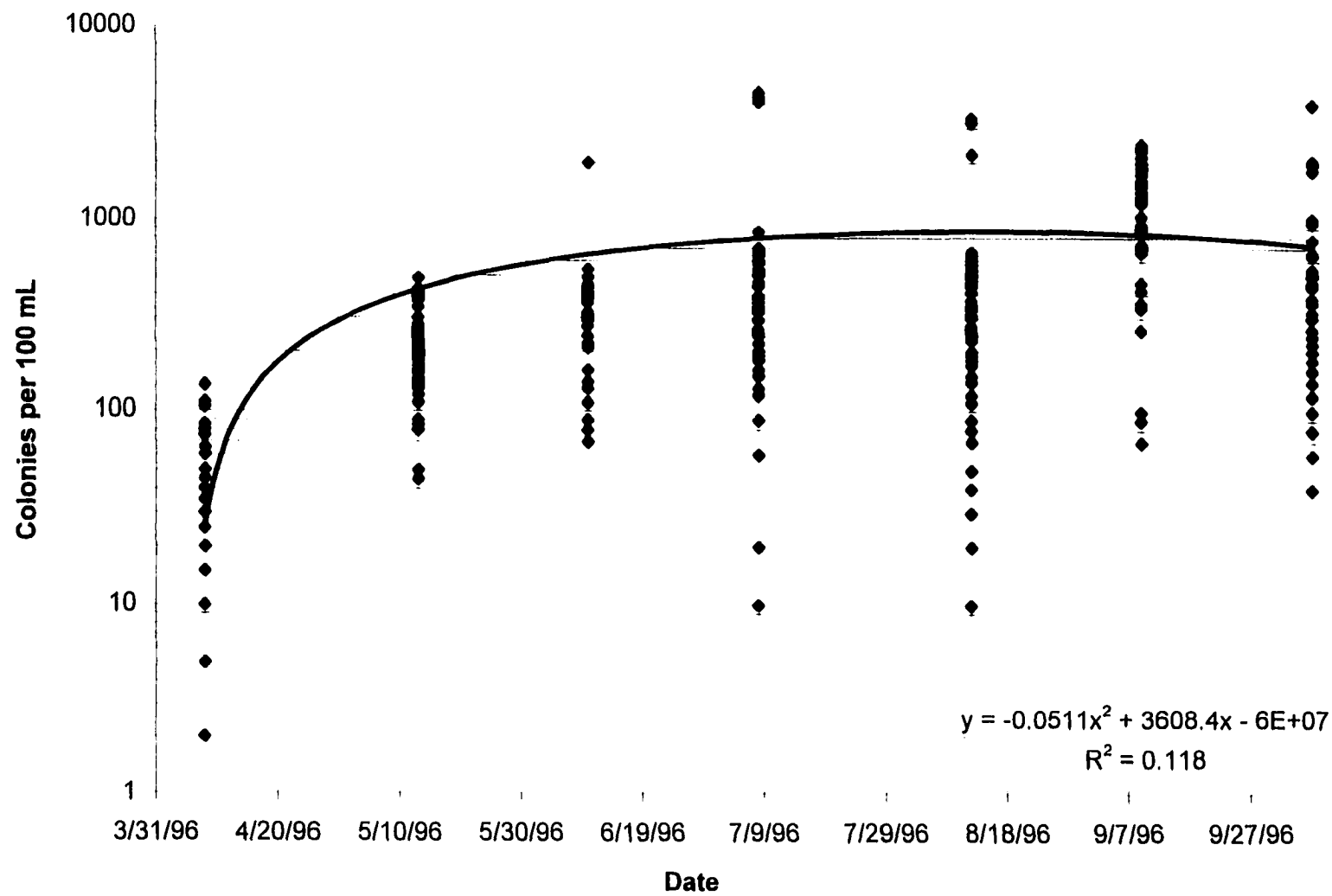


Figure 33. Distribution of *E. coli* for Kent County Health Department Stations, 1996.

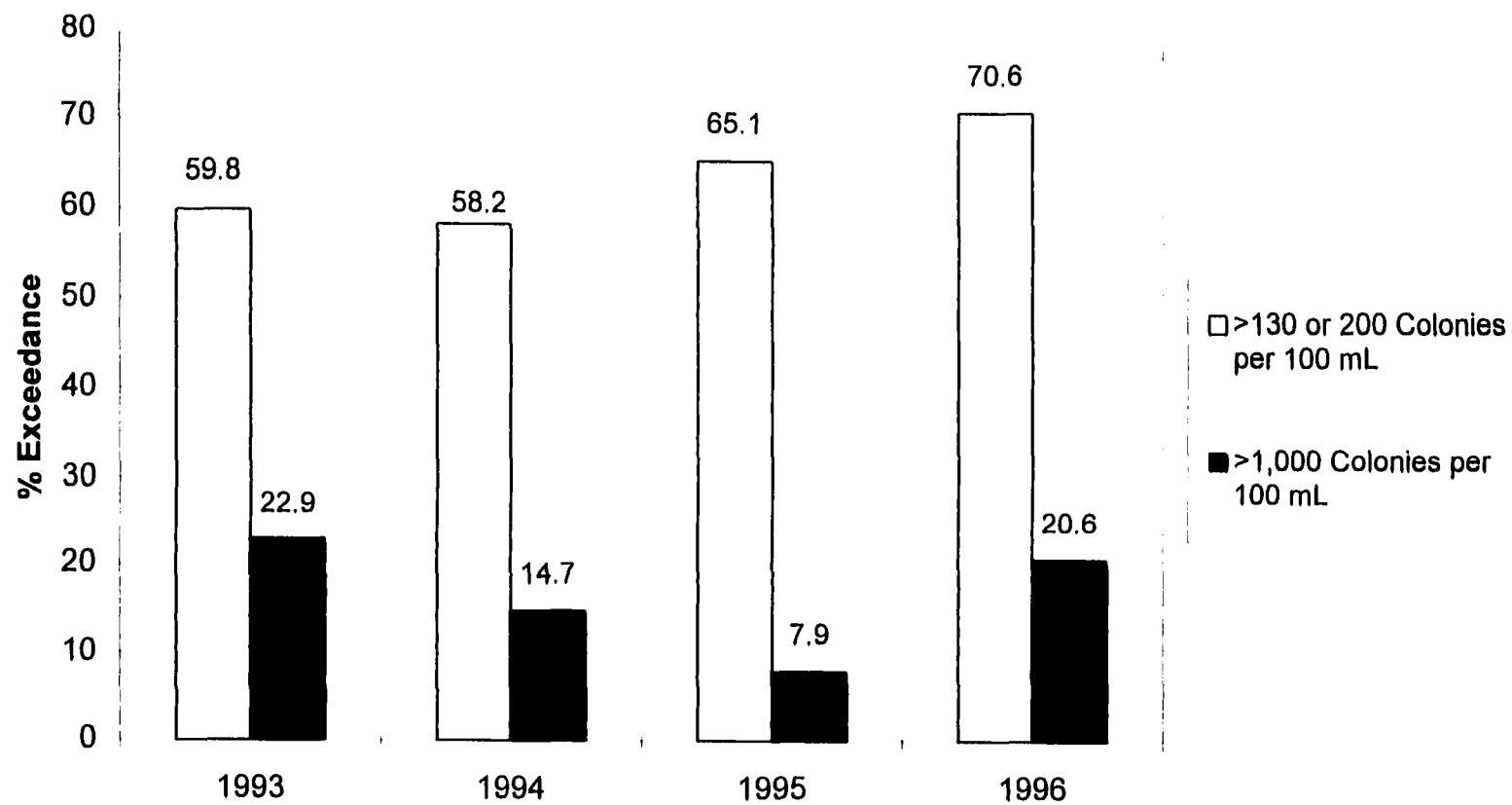


Figure 34. Exceedance Rates for Fecal Coliform (1993-94) and *E. coli* (1995-96) at Kent County Health Department Stations.

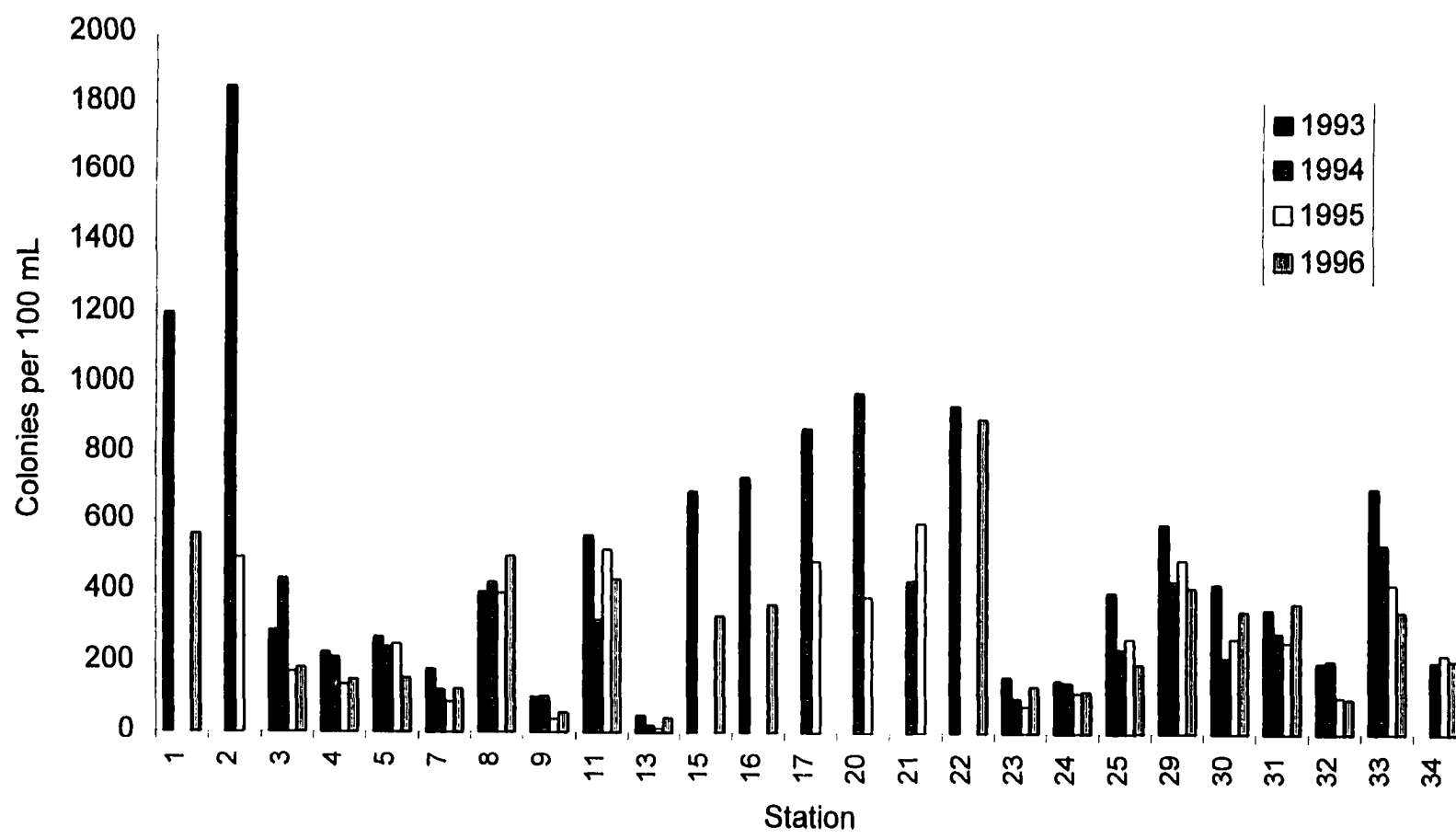


Figure 35. Geometric Means for Fecal Coliform (1993-94) and *E. coli* (1995-96) for Kent County Health Department Individual Stations.

Stabilization pond studies offer a clue as to the mechanisms responsible for the decline in coliform numbers compared with upstream sites in the Thornapple River ((Prescott et al., 1946; Fitzgerald and Rohlich, 1958). The hypothesis that the dam on the Thornapple River above the Ada Bridge is acting as a sink for fecal coliform is borne out by upstream sampling of the river where exceedance rates were higher than the Ada site (KCHD, 1992; Wagendorp, 1992).

Since the Kent County Health Department changed their standard from fecal coliform (FC) to *E. coli* (EC), it is important to establish a ratio of EC to FC so that the new monitoring data can be compared to the old data. As previously discussed, it would be predicted that the EC/FC ratio would be 0.65 using the 130 EC colonies per 100 mL to 200 FC colonies per 100 mL relationship. Comparison of all of the 1995-96 EC data with the 1993-94 FC data gives a ratio of 0.64 which is in line with the prediction. Although the overall ratio is consistent, individual stations had EC/FC ratios from 0.27 to 1.38 (Table 12).

Of note is the Plaster Creek (Station 21) *E. coli*/fecal coliform ratio of 1.38. This sampling point is in a wetland area in a park leased to a city (Kentwood) from Kent County. It is unlike any of the other sites in that it is wooded swamp prone to flooding. The other stations that have ratios greater than one were a site at Bear Creek (Station 8) and two sites on the Coldwater River. The Bear Creek site is located in Townsend Park in a relatively wide, shallow stream. Other Bear Creek ratios ranged from 0.88 to 0.99. One of the Coldwater River sites is an old dam

Table 12

Comparison of Geometric Means of *E. coli* (1995-96) and Fecal Coliform
(1993-94) for Kent County Health Department Stations

Station	<i>E. coli</i> (colonies per 100 mL)	Fecal coliform (colonies per 100 mL)	Ratio
1 Plaster	562	1200	0.47
2 Buck	495	1847	0.27
3 Mill	179	362	0.49
4 Rogue	145	222	0.65
5 Duke	204	259	0.79
7 Rogue	108	153	0.71
8 Bear	447	411	1.09
9 Flat	49	104	0.47
11 Coldwater	477	441	1.08
13 Thornapple	28	37	0.76
15 Buck	334	687	0.49
16 Buck	366	728	0.50
17 Buck	486	875	0.56
20 Plaster	386	978	0.39
21 Plaster	596	432	1.38
22 Plaster	904	941	0.96
23 Rogue	109	133	0.82
24 Rogue	122	152	0.80
25 Rogue	237	323	0.73
29 Bear	454	515	0.88
30 Bear	313	323	0.97
31 Bear	320	324	0.99
32 Grand	108	210	0.51
33 Tyler	389	621	0.63
34 Coldwater	222	210	1.06
Average	321	499	0.64

site; the other is a wide, sandy bottom area at Coldwater River Park. The lowest ratio (0.27) was found in Buck Creek at Linus Palmer Park (Station 2). As a high use area for people, it is of special concern that the ratio is different from the predicted ratio, especially since that geometric mean for *E. coli* for 1995-6 was 495 colonies per 100 mL.

Future Predictions

If there are long-term patterns at a site, historical values for each station should predict 1997 exceedance rates. The 1997 sampling regime for the Health Department was 22 sampling stations once a month from April through October (KCHD, 1998). Special studies of sites for "total body contact" happen only when the data indicate a strong probability of the standard being exceeded and when the site is known or suspected to be used for total body contact activities. Based on historical data for exceedance rates, 44% of 1997 station exceedance rates fell within the range that would be predicted from the 1993-96 monitoring data. The 44% prediction rate does not seem especially useful since random assignment of the approximately 58 to 70 exceedance rates of 1993-1996 to new exceedance rates would give a prediction rate of over 40%. With the exception of Plaster Creek which had a 95.2% exceedance rate, 1997 exceedance values at stations that were outside the expected range were lower than in the previous years. Of note is that 1996 exceedance rates for the Grand River were 42.9% in 1996 and only 7.1% in

1997. This was, by far, the greatest difference in any of the stations. The 1997 precipitation regime most closely approximated 1995, a drought year, in which two stations showed no exceedances of water quality standards. Additionally, the summer of 1997 was relatively mild.

CHAPTER VII

DISCUSSION

Perspective on Modeling

The art of successful environmental modeling lies in the selection of the best, or "least worst" set of assumptions which yields a model which is not too complex, yet is sufficiently detailed to be useful.

(Mackay, 1991, p. 5)

For data on bacterial contamination to be interpreted in the context of the ambient environment, teachers and students need a broader view of aquatic systems than just that of the water column, itself. Environmental modeling attempts to explain complex, imperfectly understood ecosystems. A model is an abstraction or a simplification of a system, or it can be a formalization about the knowledge of a system (Hall and Day, 1977). Creation of a conceptual model involves physical knowledge of the structure of a system and this model is validated through real observations (Ott, 1995).

Initially, it was hoped that this research would lead to creation of dynamic model (e.g., mathematical model and simulation) for fecal coliform bacteria in lakes and streams. This implies looking at changes in a system through time and predicting trends. Creation of a dynamic model involves advanced mathematics and computer programs. This research has indicated that the many unknowns and variables

associated with fecal coliform bacterial contamination would result in models that are far too complex for student use.

Still, a structural model can be fashioned. As previously mentioned, structural models are used to study qualitative relationships in a given context, and they are not solely dependent on numerical data for input (Wallick, 1982). Elements of building a structural model include: identification and study of the components, analysis of relationships among the parts, and creation of some graphical or tangible product to represent the complex system (Watson, 1978).

Too often structural environmental models are not comprehensive - they focus solely on just the lithosphere, hydrosphere or atmosphere. This is especially true as students develop models in relation to fecal coliform bacteria. The implicit message garnered through their monitoring activities is that these bacteria are found in the water - period. It is vital to make the connection between bacterial counts in water and the transport of bacteria which may be via land runoff, sediments, tributaries, and even air deposition. The transient water-soil interface during precipitation events is especially important in predicting fecal coliform levels in surface waters. For instance, Tate (1978) notes that *E. coli* cells found in muck were threefold greater than those in sand with greatest survival of *E. coli* in flooded soil. He found a positive association of organic matter and coliform survival with multiplication of *E. coli* in sterile soil.

The perspective of Hines et al. (1977) presents the essence of general modeling of streams and rivers in a simple fashion. He uses stream flow, water temperature, and channel morphology to define the "immediate environment." This environment is in constant flux due to seasonal changes and reach-to-reach changes in the hydrologic regime. The "generative environment" incorporates all hydrological and terrain properties of the river basin which are related to precipitation, vegetation cover, erosion potential, soil type, and land use. In general, low flow and high temperatures accentuate problems associated with wastewater discharges. Rainy high flow periods are associated with erosion, sediment transport, and turbidity, with heavy inputs from the generative environment. In other words, point sources (with the exception of combined sewer overflows) are likely to be important contributors to the bacterial load in low flow, while nonpoint sources and combined sewer overflows assume a large role in bacterial contamination problems during wet periods resulting in high flow.

Other literature and the Michigan seep studies also suggest a multi-route model for fecal coliform/*E. coli* dynamics. McDonald et al. (1982) confirm that river banks and channels may act as major sinks for bacteria. The year around presence of fecal coliform, *E. coli*, and coliform bacteria in the seep water column, the groundwater-surface water interface, drains, sediment, and even the surrounding vegetation suggest a more complex approach to bacterial modeling. As contrasted with the wetland samples, where predatory protozoans such as *Colpoda* were found,

microscopic analysis of the seep water did not reveal obvious predators on the bacteria although pennate diatoms were frequently found. This might be an important factor in the maintenance of high levels of bacteria in the seep environment. As for as the original source of the bacteria, the abundant wildlife (deer, raccoons, rabbits, mice, moles), sighted near the study site, could easily provide environmental fecal coliform bacteria. The implication of this for student studies is that they can sharpen their observational powers by looking for animal signs that might give a clue to sources of bacteria.

The pedagogical implications of these findings are several. Students should characterize both the point and nonpoint sources of bacteria in their watersheds, sample beyond the water column, and sample during both wet and dry periods. The Petrifilm™ method is especially suited for aerosol and soil/sediment sampling. The discovery that bacteria are ubiquitous to the ecosystem is vital to student understanding and making connections between actions in other parts of the ecosystem and their study site. Students should also perform microscopic analysis on water samples to reveal whether there are predators present and to get a sense of the possible interactions of other biota and bacteria.

However, conclusions of speakers at the August 1997 national conference on *E. coli* in surface water were that there is presently no easy and reliable way to characterize fecal coliform and *E. coli* bacteria as to their specific source and behavior within an aquatic system, thus model development remains problematic

(Inter-Agency Technical Task Force on *E. coli*, 1997). What was even more disconcerting was the general skepticism as to the utility of fecal coliform and *E. coli* bacteria for public health purposes at this 1997 conference.

A general approach to this dilemma would be to design a descriptive study based on the concept of risk assessment instead of a model. The study could mirror elements suggested by Hines et al. (1975) for studies of algal problems and it could incorporate epidemiological work. The algal problem was approached by (a) compilation and analysis of historical data to relate past to present conditions as well as to anticipate problems; (b) maintenance of a network of stations for frequent monitoring of organisms, physical conditions, and hydrology including groundwater inputs for low flow events; and (c) intensive studies of algal production and enrichment bioassays. Monitoring high flow events, estimation of background values, definition of competition and predator-prey relationships, die-off rates, categorization of land use, and scrutiny of health records as they relate to stream/river use are relevant for bacterial monitoring. The ultimate importance of a model or descriptive study, beyond its educational purposes, is its use by planners, decision-makers, and the public to effectively manage the watershed and make wise decisions when planning for the future.

Some teaching aids have been developed based on the results of this research. A diagram that should obviate the confusion regarding the taxonomic relationships of fecal indicator bacteria is presented in Figure 36. Although the diagram implies that

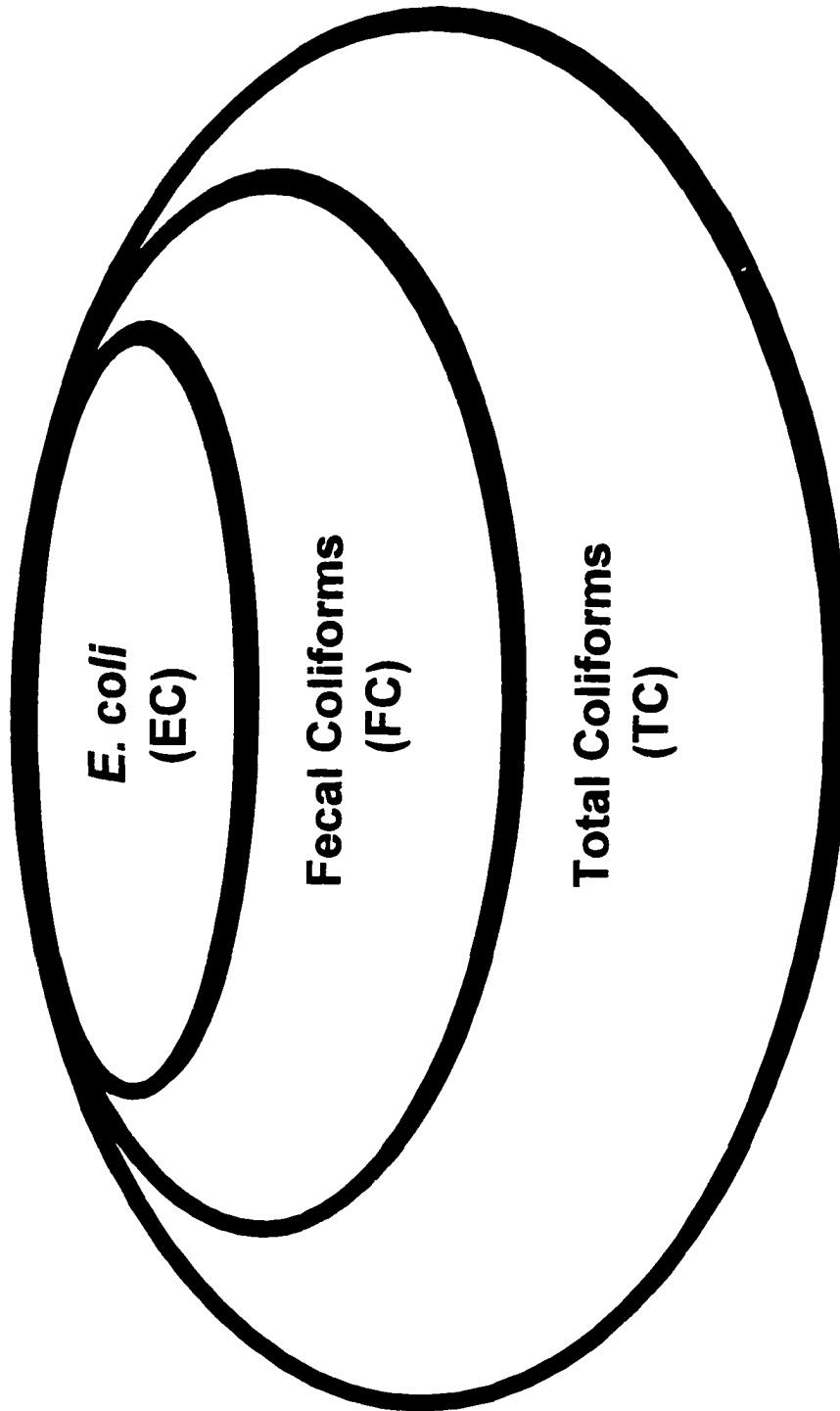


Figure 36. Taxonomic Relationships of Indicator Bacteria.

total coliforms > fecal coliforms > *E. coli*, random sampling error and differences in laboratory analysis procedures may obscure these relationships when real monitoring data are analyzed.

Three diagrams (Figures 37, 38, and 39) capture the many variables involved with interpretation of bacteriological data. Figure 37 summarizes the sources of fecal coliform bacteria, population dynamics, and transport mechanisms as they relate to levels of bacteria in water, soil, and sediment. Figure 38 is a more complex presentation. The focal point is water where fecal coliform levels are actually monitored. Pathways for the contributions from humans (right side of diagram) as well as animals and insects (left side of diagram) are both illustrated. Although not specifically linked in Figure 38, non-human sources of fecal coliform bacteria are also associated with storm drains. Deposition is classified as dry (direct contact of solids or aerosols with water and soil) or wet (precipitation-related). Bold lines indicate transfer via water; gray lines are air-related, and thin lines are direct deposition of solids. Figure 39 illustrates specific human sources. The main impacts of fecal coliform bacteria on humans are through contamination of food and water. Water considerations include not only drinking water but contact through recreational use.

These diagrams suggest ways to approach interpretation of bacteriological data in a larger context than just water. Combined with the box plots, bar graphs, frequency distributions, and scatterplots from the previous section of this dissertation, these aids assist students in developing a deeper understanding of bacterial dynamics.

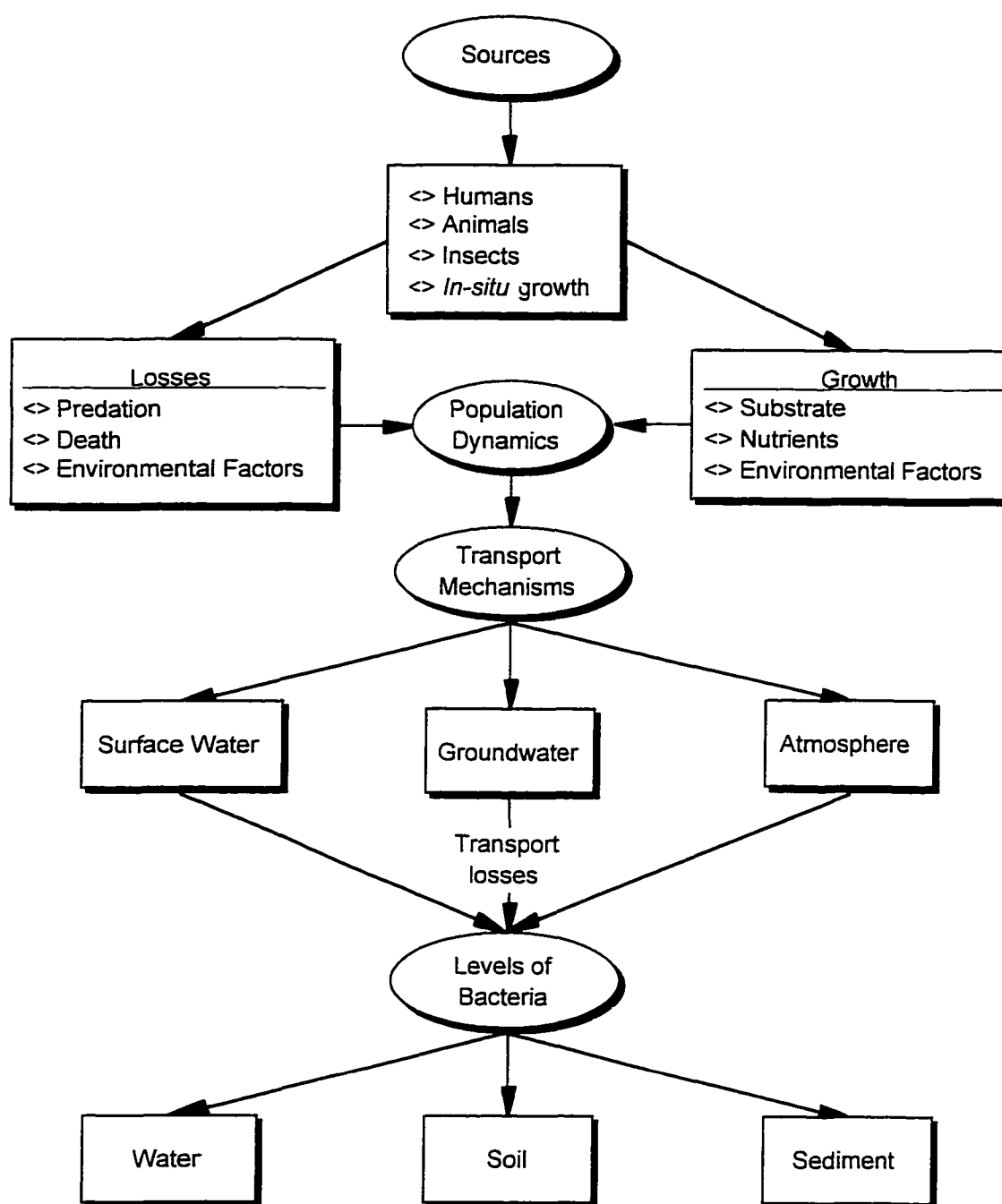


Figure 37. Relationships of Indicator Bacteria and the Environment.

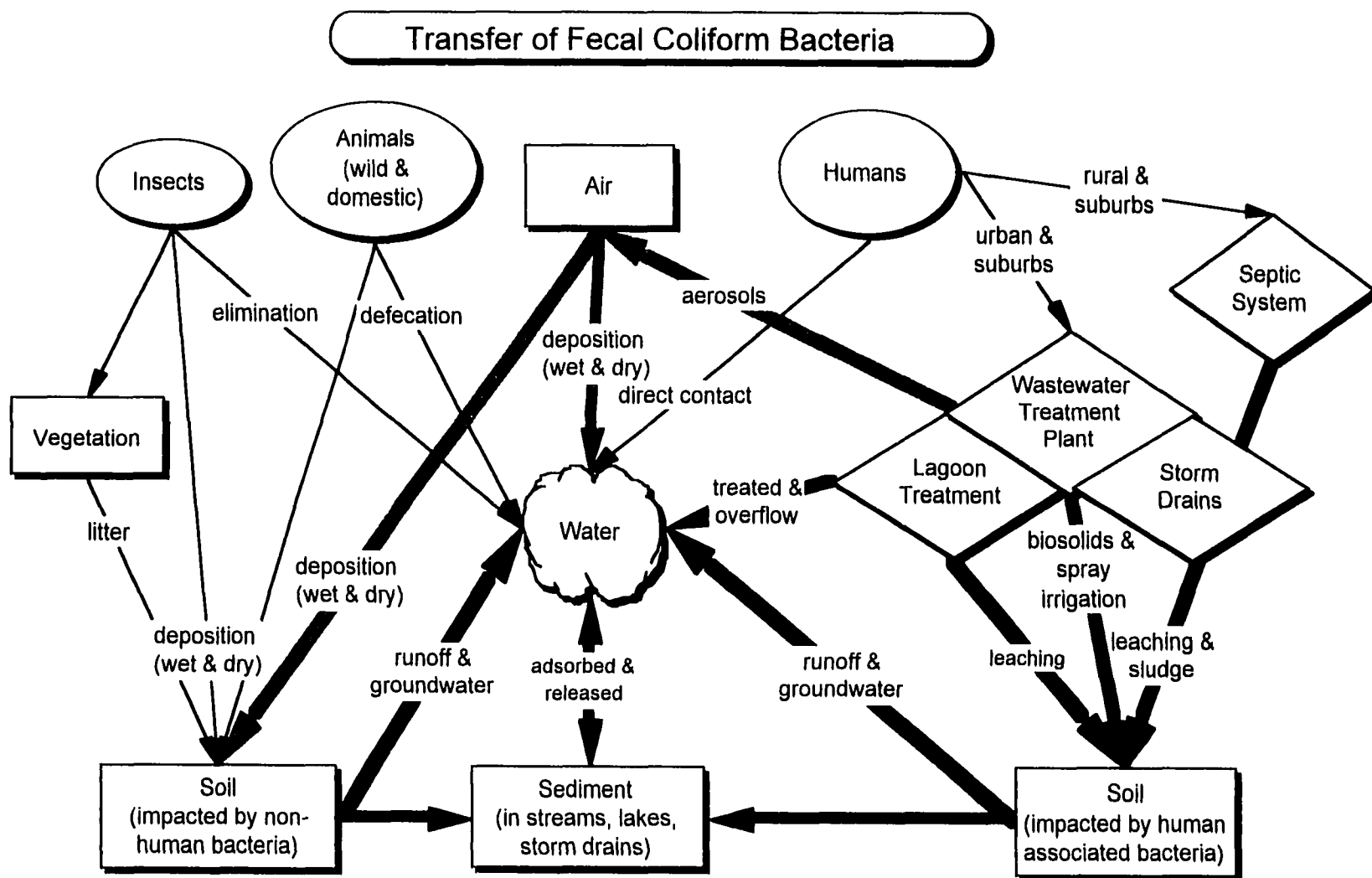


Figure 38. Concept Map for Transfer of Fecal Coliform Bacteria in the Environment.

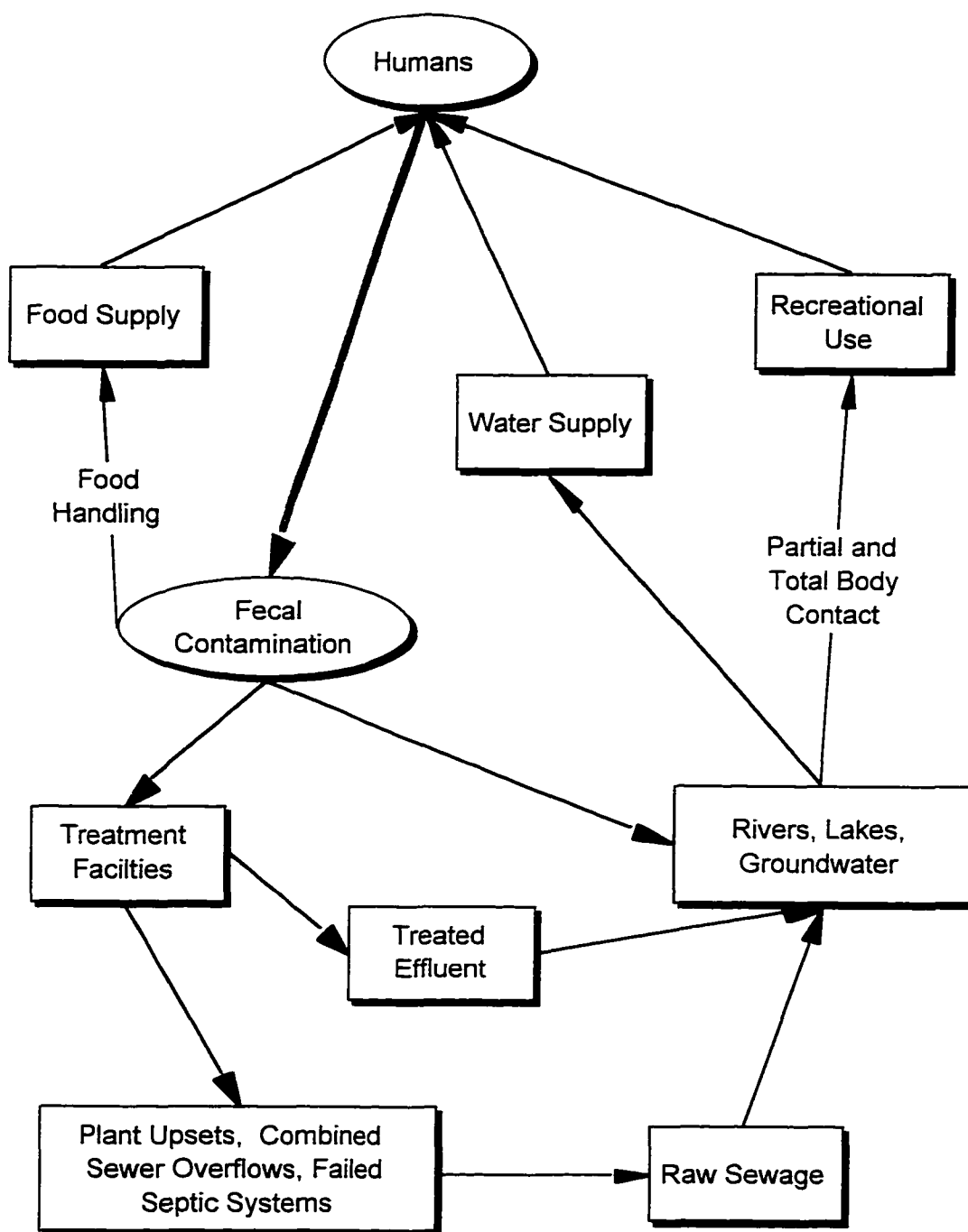


Figure 39. Interaction of Human Sources of Fecal Coliform Bacteria and the Environment.

Significance of Water Quality Monitoring Data

There needs to be some context to water quality data - both temporal and spatial. For instance, how does a sample in January from Mill Creek in Kent County compare with other times of the year and other years as well as with other sites in the Kent County, Michigan, and other areas of the United States? The methods and the results of this research represent a way to analyze data for comparative purposes.

There are numerous factors which cause measured water quality parameters to vary over time: (a) actual trends (decreasing, increasing); (b) seasonal cycles; (c) daily cycles; (d) hydrology (variations in streamflow, lake levels); (e) natural (unexplained) variability; (f) measurement error; and (g) sample location (Rechow et al., 1993). Difficulties in measuring changes are lack of continuous records, differences in techniques of observation and analysis throughout the years, changes in location and frequency of observations, lack of correlation with hydrologic behavior, and incomplete knowledge of "natural background" or temporal variation.

Trends are distinguished from the daily and seasonal patterns in that they are persistent changes in the level of a water quality variable. In order for water quality trends to be ascertained through statistical means, as many as 10 consecutive years of data are needed to distinguish short-term variability from long-term changes (Lettenmaier et al., 1991; Smith et al., 1993). In their study of 1980-89

monitoring for fecal coliform bacteria in the United States by the U.S. Geological survey and state monitoring programs, Smith et al. (1993) found that fecal coliform counts in more than a third of all streams sampled in 1989 exceeded limits.

A recent U.S. EPA report on environmental indicators of water quality (U.S. EPA, 1996a) listed 18 indicators of water quality that relate to attainment of national water quality objectives. These are analogous to economic indicators and should serve as a basic framework for data collection and analysis. The indicators are expected to be reported yearly to show water quality trends over time.

A lingering question is how much data are necessary to prove whether an individual value is representative of a long-term trend or merely a reflection of the variability in water quality data. A single sample such as that done by students in a classroom study cannot answer this question and is contextually void. A frame of reference with other data sets is necessary.

Kent County Sampling Site Trends

Kent County is one of the few counties in Michigan that regularly tests for bacterial contamination in its streams and rivers. The river and stream stations provide contrasting land uses for comparison of urban, suburban, and rural impacts. Stream and river data are perhaps best viewed in the context of risk. In other words, is the water safe for recreational purposes as defined by the fecal coliform standard? Mack (1973) noted that most streams entering Lake Michigan originated in remote

areas with little human habitation. He concluded that coliforms in these streams are coming from animal populations, thus fecal coliforms would not indicate human contamination.

As previously noted, analyses used to generate risk information must take into account temporal and spatial variability. There is seasonal as well as daily variability. If analyses are properly structured to account for the seasonal and storm-related variability, then daily variability would be used as the background variability in any analyses of pattern and trend. There are at least two levels of spatial variability to consider. Individual stations often have distinct characteristics that reflect the areas they drain. At a somewhat larger scale, various reaches of the river may differ from each other, again reflecting the subwatersheds they drain. The large amount of variability makes it difficult to draw simple conclusions about patterns and trends in indicators of risk. The daily station-by-station data are suited for ongoing compliance and health management, but the composite raw data can have considerable variation and appear confusing.

Analyses, performed on the entire set of sampling stations, could be used to group stations with similar contamination patterns. These would not necessarily be spatially contiguous sets of stations. Instead such station groups are likely to contain similar stations scattered throughout the County. This would directly address the question, "Where are the riskiest areas?" It would then be up to

managers and scientists to interpret and describe these relative degrees of risk in terms meaningful to the public.

Another analysis approach is to compare the relative risk associated with groups of stations from pre-defined regions of the County. This could be accomplished by simply presenting the area averages and confidence limits of the transformed indicator values. These averages could also be compared more analytically using analysis of variance (ANOVA) procedures.

An important aspect of assessing relative risk is determining whether apparent risk is increasing, decreasing, or staying the same over time. This question is equally applicable to individual stations, to groups of similar stations, and to pre-defined areas. The most straightforward approach to this question would be to examine confidence limits around the smoothed curves of indicator values over time at the monitoring stations. Average curves for station groups and pre-defined areas could also be compared from one time period to the next. If specific time periods must be compared (e.g., one year to the next), then a simple t-test on the two sets of data would be appropriate. More sophisticated questions could be addressed with time series analysis.

Given the above context, three questions are directed at the analysis of the Kent County data:

1. How do current counts for bacteria compare with the past?

2. How do Kent County counts for bacteria compare with other areas of Michigan?
3. How do Kent County levels of fecal coliform bacteria differ from other areas of the country?

Temporal Trends

In 1968-1972, the Michigan Grand River Watershed Council began monitoring once a month at over 100 stations in the Grand River system with 44 stations in Kent County alone (MGRWC, 1972). In Kent County, the river downstream of the Grand Rapids Wastewater Treatment Plant had fecal coliform levels exceeding standards almost continuously and was deemed unsuitable for recreational use. Upstream, nutrients were elevated and the river was "marginal" for swimming (MGRWC, 1972). Between Grand Rapids and Grand Ledge, water quality of the Grand River did not meet standards for partial body contact recreation such as boating and fishing. In the Lansing area, fecal coliform standards were also exceeded (MGRWC, 1972).

A 1985 Michigan Department of Natural Resources study on small watersheds in the Grand Rapids area revealed significant nonpoint source impacts in the Grand River from Silver Creek Drain, Coldbrook Drain, Indian Mill Creek, Buck Creek, and Plaster Creek. Oxygen depletion, coliform bacterial contamination, sedimentation, heavy metal toxicity, and turbidity were problems.

Impairment of these streams was thought to be 30% from point sources, 5% from agriculture, 35% from urban sources, and 30% from combined sewer overflows (MDNR, 1985).

The report of the first monitoring year for the Kent County Health Department (KCHD, 1990) indicated that some stations from locations throughout the county were free from persistent high levels of fecal coliform bacteria. However, there were short term increases in counts at all stations attributed to run-off of stormwater. Also, Plaster Creek Buck Creek, Bear Creek, and Mill Creek frequently had fecal coliform counts above 200 colonies per 100 mL.

After five years of monitoring, the Kent County Health Department concluded that there had not been major changes in the Kent County water quality (KCHD, 1994). Acceptable water quality streams remained acceptable and those with unacceptable water quality did not improve. Acute public health advisories decreased as the City of Grand Rapids made improvements in its sewer system and wastewater treatment plant. Problems were still evident at Plaster Creek and Buck Creek as well as portions of Bear Creek, Coldwater River, Rogue River, Mill Creek, and the Grand River. These problems persist through 1997.

The state of streams and rivers in Kent County is adequately captured in a quote from *The Grand Rapids Press* "it pays to be cautious because any stream can contain disease-causing organisms depending on weather conditions, sewage spills, and other factors" (Sinkevics, 1996). The County, however, chose to gauge water

quality by partial (not total) body contact standards when it changed from monitoring fecal coliform to *E. coli*. Only 8% of all sampling runs had *E. coli* counts above 1,000 colonies per 100 mL in 1995, which was, incidentally, a drought year with low stream flows and lack of frequent wash-off. This resulted in the County removing some no swimming signs. The media coverage, however, did not make it clear to readers that *E. coli* bacteria are a sub-set of fecal coliform bacteria so it is logical that, all things being equal, the counts would appear to have decreased since 1994.

Influence of Point Sources

In an effort to explain the elevated levels of fecal coliform bacteria in areas of Kent County, point sources of contamination need to be defined. The Grand Rapids Wastewater Treatment Plant has the greatest potential for permitted and unpermitted discharges (combined sewer overflows) of bacteria in Kent County. The plant is an activated sludge secondary treatment facility that discharges an average of 54 million gallons of water per day into the Grand River (Tabor, 1992). The community is well informed of the operations of the Grand Rapids Wastewater Treatment Plant. Its monthly operating reports are comprehensive and contain over 65 pages of information (City of Grand Rapids, 1997). River survey monitoring data, NPDES permit compliance, discharge monitoring reports, and combined sewer overflow reports can all be found in these documents.

The study of Barton et al.(1986) indicated minimal impact on the river with normal plant operations. Since 1980, the GR WWTP has exceeded its final effluent fecal coliform geometric monthly average of 200 colonies per 100 mL four times and its seven day moving averages of 400 colonies per 100 mL nine times. The most serious problems happen during combined sewer overflow events. Combined sewer overflows happen when storm events overload wastewater treatment plant capacity. Fecal coliform counts in the tens of thousands per 100 mL have been recorded in the Grand River during overflow events (Black et al., 1990).

The City of Grand Rapids, however, has made great strides in correcting the CSO problem since it gained prominence in 1988 when the city first began reporting CSO events to the public. Billed as "sewer gate", a fisherman discovered raw sewage entering the Grand River which raised community awareness of the problem (Lloyd, 1988, September 16). In the event of a combined sewer overflow, the City contacts officials in the Department of Environmental Quality who then, in turn, make a determination as to whether to contact local health departments. Former director of the Kent County Environmental Health Division, George Pio admitted that "we do not have a scientifically precise process for determining whether a health advisory is or is not warranted. There is an element of judgment involved" as to assessing risk (Sinkevics, 1988, August 20).

In recent years, decreased frequency and magnitude of combined sewer overflows have been realized through methods such as constructing a retention basin,

separating combined sewers, limiting discharges, evaluating sewer systems, and passing local ordinances. The 30 million gallon retention basin was completed in March, 1992. The effect of the basin and other improvements, including separation of almost 95% of the combined sewers on the city's west side, was a 90% reduction in CSO flows in four years by Grand Rapids (City of Grand Rapids, 1995). This represents a decrease from 340 million gallons (MG) of overflow in 1991-2 to 39 MG in 1994-5 (Grand Valley State University, 1995).

There is little question that there are times when the assimilative capacity of the river is exceeded but these occasions are generally well documented by the City of Grand Rapids. Also, downstream communities are alerted to the magnitude of CSO events and can act accordingly, which may mean issuing warnings for contact with the river.

As previously discussed, the City samples the Grand River above and below the main outfall from the Wastewater Treatment Plant. Since sampling is on a fixed schedule, it is difficult to associate monitoring data with CSO events. However, there are data that can indicate the general impact of the GR WWTP. The closest upstream river station is the Wealthy Street Bridge, one mile upstream from the GR WWTP. The Wealthy Street Bridge lies in the center of downtown Grand Rapids. Unfortunately, Plaster Creek with its high fecal coliform counts drains into the Grand River just above the GR WWTP which confounds the analysis. The closest downstream river stations are at the Railroad Bridge about one mile downstream from

the GR WWTP. The river front is largely forested and undeveloped between the GR WWTP and the Railroad Bridge and there is only a single, small unmonitored tributary on the north side of the river.

The effect of high levels of fecal coliform bacteria in Plaster Creek obscures interpretation of the results for the downstream stations. The Wealthy Street station, however, provides a reasonable background station for ascertaining effects of urban nonpoint pollution from up the river. There are no major contributing tributaries for three miles above the station. The special study by the GR WWTP (Barton et al., 1986) indicated that fecal coliform counts downstream did not increase when the effluent was chlorinated. When the effluent was not chlorinated, fecal coliform counts downstream exceeded upstream values. Compared to the fecal coliform loads from the tributaries both upstream and downstream of the GR WWTP, it does not appear that the general operation of the Wastewater Treatment Plant during periods of chlorination has a major impact on impairment of the river by high levels of fecal coliform bacteria. During the winter months when there is no chlorination of effluent, there is also minimal use of the river involving body contact so public health concern is minimized.

Major point source dischargers into the water are required to obtain a National Pollution Elimination Discharge Permit (NPDES) from the State of Michigan. Research into point sources of pollution in the Grand River Watershed revealed that there were 52 facilities in the Grand River watershed that filed

discharge monitoring reports for fecal coliform bacteria and 7 facilities have self-monitoring (Vail, 1993). Virtually all of these facilities were wastewater treatment plants or sewage lagoons with the exception of the Grand Haven Bureau of Power and Light Island Station. The lack of monitoring information makes it difficult to assess the contribution of these sources.

Influence of Nonpoint Sources

Nonpoint source coliform contamination appears to overshadow point source contamination of Kent County streams. Schaftlein (1985) created a preliminary profile of nonpoint sources in the Grand Rapids area and easily found multiple point and nonpoint sources from industrial outfalls and wastewater treatment plants, combined sewer overflows, uncontrolled urban stormwater, and rural runoff. There are likely to be major sources of coliforms from the nearly random, spotty distribution of contaminated soil that impact the tributaries feeding the main river. This contamination is largely from domestic and wild animal activities, and contributes to heavy bacterial loads in runoff water into streams.

The complex matrix of agricultural drains in Kent County are other possible reservoirs of bacteria. For example, in terms of overall delivery of fecal coliform and impact on the Plaster Creek water quality, the results from the limited sampling of storm events indicate that fecal coliform bacteria from the rural areas in the watershed may be producing a pronounced impact on fecal coliform levels in the

Creek (see Appendix B). Fecal coliform levels were 6-8 times higher during wet weather conditions than prior to the monitored storm event, highlighting the nonpoint source origin of bacteria. The most upstream Plaster Creek monitoring location gave results that were consistently higher than the downstream monitoring location.

Sampling during a summer storm event revealed the highest fecal coliform contamination of the creek. The combination of warm water temperatures and a long antecedent period of no rainfall enable the fecal coliform bacteria to accumulate and become available for washoff. Concentrations of bacteria may be greatest in the headwaters as the water levels recede (although total coliforms may not be as great as in the first flush from rain or meltwater).

Storage of water occurs in stream channels, surface soil, and groundwater (Linsley et al., 1982). Reversal of flow in some areas of the stream bottom may occur as the water table rises following significant precipitation or snowmelt. The position of the groundwater table is a critical factor in the transmission of pollutants (Leopold, 1968). Areas of sedimentation may become areas of resuspension. Slow flow of the most contaminated water nearest the water-sediment interface means a delay of peak contamination reaching downstream locations as compared to the hydrologic peak.

Downstream, the depths of the water column are greater, and velocity remains constant or increases (Allan, 1995). There is less friction due to a smoother bottom resulting from erosion and sedimentation. The sediment shifts from coarser material

upstream to finer material downstream, and there is less resuspension of materials, especially as the water level recedes. This, along with the high volume of water relative to the bottom and distance from soil inputs from the headwaters, allows coliform mortality to keep main stream nonpoint source levels relatively low.

Pettibone and Irvine (1996) studied levels of indicator bacteria in the Buffalo River Area of Concern in the context of upstream sources. There appeared to be continuous input of fecal coliform bacteria from various sources year around, possibly associated with the 500 farms along the tributaries. The level of FC in tributaries in the winter exceeded state water quality guidelines (200 FC/100 mL) in 66% of the samples. The authors, as well as investigators working on the St. Mary's River Area of Concern (Dutka and Marsalek, 1993), concluded that, as compared to rural sources, urban sources of bacteria have only a minor impact on water quality especially during the summer months. Sharp increases in fecal coliform bacteria were correlated with storm and snowmelt events.

Pettibone and Irvine (1996) note that runoff processes, rather than resuspension of sediments, are probably responsible for elevated counts in the bedrock or sand streambeds of the upper watershed. However, solids present in the water column may be a vehicle by which bacteria are kept in suspension and transported downstream (Auer and Niehaus, 1993). Brettar and Hofle (1992) go as far as stating that suspended particles offer an environment that promotes bacterial growth and protects bacteria from predation. It is evident from the results of this

present research that there is an association of indicator bacteria with particles as seen in the distribution of colonies on the membrane filters. Frequently, it was observed that characteristic blue fecal coliform bacterial colonies were found around the edges of particles that were caught on the filters. Conceptually, this is an important idea. If sedimentation of streams in Kent County can be controlled, then perhaps levels of fecal coliform bacteria in the water will be reduced.

None of the studies undertaken so far have been specifically designed to pinpoint sources and origin of fecal coliform contamination in Kent County. The Kent County Health Department sampling sites have been selected on the basis of recreational areas, the Grand Rapids WWTP monitoring locations are part of their major river waters sampling program, and the locations for the storm event study were selected on the basis of sites with available hydrological information. The Plaster Creek Nonpoint Study sites were the most extensive in terms of possible sources of fecal coliform bacteria (Schaftelein and Bittrick, 1989). Noted problem areas included hog and cattle operations, drains, and septic drain fields.

Comparison With Other Areas of Michigan

Is Kent County unique in its problem with fecal coliform bacteria? Section 305(b) of the Clean Water Act requires each state to make a biennial, statewide assessment of designated-use support of water and to submit findings to U.S. EPA which compiles the information into a report to Congress. In the 1992 report to

Congress (Browner, 1994), 60,804 miles of the 642,881 assessed miles of rivers and streams in the United States were reported by state water quality agencies to be impaired by pathogen indicators. The 1994 Michigan 305(b) report indicated 229 miles with major impact and 156.5 miles with moderate/minor impact from pathogens.

Comparison with studies from counties adjacent to Kent County, as well as with adjacent and other drainage areas of interest were made to answer the question of how Kent County compares with other areas. Monitoring data were analyzed for five counties in the Grand River Watershed (Ottawa, Barry, Eaton, Montcalm, and Ingham counties), Southwest Michigan, Grand Traverse Bay, and Southeast Michigan as summarized in Appendix C. Levels of fecal coliform bacteria above 200 colonies per 100 mL are frequently reported in the waters of Michigan. Although the frequency of monitoring is not as comprehensive as that of Kent County, all of the other areas of Michigan that were reviewed had levels of fecal coliform bacteria comparable to those found in Kent County.

In summary, it is realistic to expect that the fecal coliform standard of 200 colonies per 100 mL will be often exceeded in the surface waters in the State of Michigan. Clearly there are areas of the state where it would be extremely difficult to meet health standards for bacterial contamination of recreational waters and major improvements are not likely by the U.S. EPA target date for clean water in the year 2005 (U.S. EPA, 1996a).

Comparison of Michigan and California Watersheds

There are other areas of the United States where microbiological water quality standards are more consistently being met, background stations consistently show zero or very low levels of fecal coliform bacteria, and exceedances of water quality standards can generally be attributed to specific causes. One such area is the San Lorenzo River watershed in California.

In order to understand why fecal coliform levels in Plaster Creek in Kent County are above the public health limit of 200 colonies per 100 mL, a comparison will be made with the San Lorenzo River watershed where counts are consistently below 200 colonies per 100 mL. A frame of reference for this comparison is the author's work as a water quality analyst for the County of Santa Cruz Environmental Health Department in the San Lorenzo River watershed in Santa Cruz County, California (Appendix A, Figure 8).

Monitoring data From 1985 to the present for a series of stations in this watershed are available from the County of Santa Cruz Environmental Health Department. Currently, the county routinely tests 15 sites per week throughout the year. Sites are in rivers, creeks, and the Pacific Ocean. Another 30 sites are tracked to detect trends and contamination. The county runs a variety of microbiological tests including fecal coliform, coliforms, fecal streptococcus, *E. coli*, and *Enterococcus*.

Located in northern California, the San Lorenzo River drains a mountainous and forest-covered area with the major human point sources being septic systems. However, it has been estimated that 75 % of the high bacterial levels in the San Lorenzo Watershed result from background bacterial contamination associated with dense development and disturbance in close proximity to stream channels (County of Santa Cruz, 1989). The Plaster Creek watershed encompasses 60 square miles, whereas the San Lorenzo River watershed is 136 square miles. The Plaster Creek drainage system has been extensively altered through drains and baseflow sustains the Creek year around. (Camp, Dresser, and McKee, 1991). There are few agricultural drains in the San Lorenzo River watershed and baseflow maintains river levels in the summer.

The typical climatic regime of the San Lorenzo River Watershed is that of a cool summer Mediterranean climate. There is minimal rain from May through November and yearly totals vary considerably in different locations throughout the basin. For instance, average rainfall in Santa Cruz at the mouth of the San Lorenzo River is 28.25 inches and 56.34 inches in Ben Lomand in the middle of the watershed (Thomas, 1961). Annual average temperatures are between 55 and 59°F with warmest months in July, August and September. Summer stream and river conditions are normally low flow conditions with elevated water temperatures. Fall is characterized by falling temperatures, accumulation of leaves and organic matter in streams, and low streamflow. Winter brings high rainfall, flushing of streams,

and lower water temperatures. Spring is a period of time when the ground is saturated, intermittent streams are flowing, baseflows are high, and temperatures are rising (County of Santa Cruz, 1989).

Fecal coliform counts in this watershed are very predictable and there are reference sites where surface water counts for fecal coliform bacteria are zero throughout much of the year. Upstream, high fecal coliform counts are easily associated with causes such as rainfall and septic system failures. At the river mouth, high levels are related to the presence of urban drains. Fecal coliform values in the watershed generally increase during periods of storm runoff and the increase can be as much as 1,000 to 100,000% above mean levels. Statistical analysis reveals seasonal variations of rainfall, temperature, and streamflow could explain most of the variation in bacterial levels (County of Santa Cruz, 1989).

There are many differences between the San Lorenzo and Plaster Creek watersheds (Table 13). A key difference in the watersheds that accounts for the low California counts is that there is virtually no summer rainfall in California. In contrast, summer storm events in Michigan happen almost weekly. Bacteria are thus continually flushed into Plaster Creek at high levels. It is hypothesized that sampling after a long dry period might reveal low fecal coliform counts.

The San Lorenzo River watershed has steep slopes and excess water from rainfall events quickly runs off in contrast to the level to gentle aspect of Plaster Creek where there can be slower drainage which further contributes to the fecal

Table 13

Comparison of Two Watersheds in California and Michigan

Parameter	San Lorenzo River (Santa Cruz County, CA)	Plaster Creek (Kent County, MI)
Fecal Coliform	Typically 0-200 colonies/100 mL; upstream station 0; highest averages at river mouth (90-5000 colonies/100 mL)	Consistently above 200 colonies/100 mL; no low background station; counts high throughout watershed
Elevation/Slope	Sea level to 3000 feet: steep slopes	590 to 705 feet; level areas and gentle slopes
Land Use	Forested (85%), little agriculture (5%), urban only at mouth (10%)	Mostly agriculture upstream (20%); suburban, urban, industrial, commercial (75%); forest (5%)
Vegetation	Predominantly evergreen	Deciduous, fields
Industrial Use	Only near the mouth, few industries	Numerous industries near mouth
Drains	Relatively few	Many throughout: 24 major storm drains
Sewers/Septic Systems	City sewers at mouth; many septic systems upstream with failures	Good portion of the area sewerred; combined sewers in some areas
Rainfall	No summer rainfall; 30 inches per year at mouth, 60 inches in mountains	Frequent summer rain events; 36 inches per year
Average Temperature	55 to 59°F	24°F Winter; 69°F Summer
Flow	Minimal summer flow	Variable, winter ice

coliform load. In the upper reaches of small tributaries, flow is slow during periods of low runoff, especially near the bottom substrate, resulting in minimal dilution and slow flushing. Highest coliform concentrations would often be associated with sediments which are trapped in the irregular surfaces of the substrate. If the land slopes are steep, as in the California example, temporary storage of sediment in depressions may be nearly absent (Leopold, 1968). If the tributary (or drain) contains little water except during storms, sediment will accumulate and movement of sediment during storms will be great. Rainfall suspends coliforms from the soil and sediment, moving them downstream with increasing velocity.

Land use patterns of the Plaster Creek watershed also differs considerably from the San Lorenzo River watershed. The San Lorenzo River watershed is heavily forested so rainfall velocity is decreased by leaves and the porous soil, there is little agricultural nonpoint source pollution, and most of the highly urbanized areas are downstream near the Pacific Ocean. The mouth of the San Lorenzo River consistently has the highest fecal coliform counts due to runoff from streets and faulty drains. Sporadic elevated counts upstream are often attributed to failing septic systems and waterfowl. The fecal coliform sources are more easily identified in the San Lorenzo Valley watershed due to lack of summer rainfall and low flows enabling investigators to walk the streams when necessary.

The Plaster Creek watershed has multiple sources of fecal coliform contamination from highly urbanized areas via combined sewer systems and street

runoff as well as from agricultural land via feed lots, agricultural drains, and questionable management practices. The number of urban and agricultural drains in the Plaster Creek watershed far exceeds those in the San Lorenzo River. It is as if the main function of Plaster Creek has been to be an urban and agricultural drainage system. One might say that Plaster Creek represents the "worst case scenario" as far as fecal coliform counts are concerned.

Sources of fecal coliform bacteria in Plaster Creek are more cosmopolitan than those in the San Lorenzo system. Decrease in coliform counts will require long term solutions such as conducting sanitary surveys of septic systems, finding a way to periodically "flush" the drains, and implementing best management practices in the agricultural areas.

Summary

It is clear from studies in other areas of Michigan that bacterial contamination in waterways is not unique to Kent County; it is just that other counties in Michigan generally do not have aggressive monitoring programs. According to an editorial in the *Grand Rapids Press*, "no one will understand the extent of pollution until all counties start monitoring" ("Testing the Waters," 1994). A combination of combined sewer overflows, stormwater inputs, and what appears to be an endemic bacterial population all contribute to this region-wide problem.

Tributaries to rivers appear to be contributing heavily to the bacterial load. The rivers, however, exhibit a dilution and purifying effect, so that, in the absence of excessive tributary contributions, counts along main reaches tend to fall within the standards except in wet weather events. Areas where fecal coliform levels rarely exceed standards include undeveloped stretches, areas with limited agricultural or urban land use, dam areas, and river reaches without tributaries. The *Grand Rapids Press* concludes that:

The question isn't whether streams are polluted around the state but how much. If legislators won't address pollution from farms, construction sites, roads and homes, they should at least demand that streams be tested so that the public is aware of the danger. ("Testing the Waters," 1994. p. A10).

The background reference site monitored for two years by the author in Kent County had significant levels of fecal coliform bacteria, *E. coli*, and total coliforms throughout the year that are not associated with humans. Field notes indicate the presence of deer, raccoons, mice, moles, turtles, frogs, toads, rabbits, chipmunks, and squirrels in the surrounding forest. Deer paths are common near the seep area. Wildlife scats were frequently found in the vicinity of the sampling area. In the two years of study, there did not appear to be any direct evidence of animal defecation in the seep or on the banks within one foot of the seep.

As noted by Simmons (1997), rigorous sampling of land/water interfaces, creek beds, marshes, and every tiny rivulet is necessary to define source areas of fecal contamination. There are no simple answers as to where these sources are

located and what may have initially caused them. Fecal coliform bacteria from animal excrement can pass through soil, into sub-surface drains, and into watercourses. Kress and Gifford (1984) found that cowpies that were 100 days old were potential sources of fecal coliform bacteria and releases from them exceeded water quality standards. Fecal deposits appear to be a protective medium for the bacteria inside (Thelin and Gifford, 1983). Niemi and Niemi (1991) found significant levels of fecal indicators in half the samples from pristine areas in Finland. They attributed this to contamination by wild animals, especially elk and deer. This leads to speculation that the dynamics of the seep involves a complex relationship of soil types that harbor fecal coliform bacteria, source bacteria from animal droppings, and a precipitation regime conducive to movement of bacteria into the watercourse.

What is a Reasonable Standard?

Points of view on water quality standards differ as to whether it is the opinion of a wastewater treatment plant, industry, general public, or government agency. Economics plays a key role in the concern of the first two groups. The public is concerned with health and aesthetics but is asked to pay for water quality improvements. The last group is charged with setting enforceable standards. A historical survey of how health departments select standards indicated that most agencies had no analytical data for their limits, but epidemiological experience under

the standards had been good (Garber, 1956). Standards varied from: no coliforms should be found, to limits greater than 2,400 per 100 mL.

Lee et al. (1982) feel that technically valid, attainable, and site specific water quality standards make more sense than cookbook reliance on U.S. Environmental Protection Agency *Quality Criteria for Water* which are also known as the "Red Book" criteria (U.S. EPA, 1976). The Red Book criteria are often single values and worst-case assumptions (Lee et al., 1982). Water quality standards need to be related to beneficial use of a particular body of water such as swimming, fishing, or recreation.

In a discussion about improving water quality, Lee et al. (1982) state that the first step is to define the problem; that is, how are the beneficial uses of a particular water being impaired and what is the cause of this abuse? Points to bear in mind are that each water body has its own assimilative capacity and that contaminant load allocations need to be related to this capacity. Also, decisions need to be made as to the amounts of money and other resources that should be spent to address the problem and to what degree the problem will be solved. These decisions are best made at a local level with case-by-case analysis (Lee et al., 1982).

The expenditure of public and private funds that result in no appreciable water quality improvement is an underlying issue in meeting standards. A balance between expenditures on point and nonpoint pollution control appear to be needed. For instance, Geldreich (1979a) suggests a dual health criteria for sewerage and non-sewerage stormwater runoff be investigated.

Questions need to be posed as pollutant impacts with reference to the entire ecosystem and the hydrologic conditions. While fecal coliform bacteria are considered indicative of a human health hazard, how do they relate to the ecosystem dynamics and actual use of a body of water? Will the waters really be used for swimming and/or fishing? Is a river reach impacted because of basic hydrological conditions? Do high levels of fecal coliform bacteria change biological interactions such as the predator-prey dynamics? What is the biological integrity of the system?

Basic risk assessment takes into account concentration and duration of exposure. The problems of regrowth, background concentrations of bacteria, the nature and use of the receiving water, and greater variability in indicator organism levels than in pathogen levels have all made it difficult to assess health risks (Garber, 1956). A range of values for a water quality parameter in a given system could be related to cost-benefit for various levels of protection.

The universal application of water quality standards was strongly questioned by Nuzzi and Burhans (1997) - "the desire for a national standard, while laudable, must be approached with caution." High numbers of fecal coliform and other indicator bacteria may indicate the presence of human sewage with a resulting direct public health relationship, or they could be attributed to other contamination such as animal wastes and indigenous populations that have less public health significance.

Nuzzi and Burhans (1997) reported on a New York study that compared the total and fecal coliform standards for freshwater and seawater bathing beaches with a

proposed *Enterococcus* standard. Use of the *Enterococcus* standard would have resulted in twice the potential beach closures than would the FC standard, and yet, the *Enterococcus* standard was derived for beaches impacted by municipal sewage, which was not the case for the study beaches. The authors concluded that additional research is needed to evaluate the relationship between indicator organisms and public health effects in waters that are subject mainly to nonpoint source inputs. Because of a demonstrated relationship between rainfall, tides, and bacterial numbers, they suggest that it may be more appropriate to control beach use on the basis of rainfall data supplemented with knowledge gained from random sampling data and the historical monitoring of a database for a particular area that represents all weather conditions.

In this present research, the location where the standard is most important to the greatest number of people is at Grand Haven State Park at the mouth of the Grand River on Lake Michigan. North Beach Park also attracts summer visitors. Thousands of bathers converge on these beaches throughout the summer. The plume of the Grand River flows through a channel adjacent to Grand Haven State Park to the south, and North Beach Park is about a mile north of the pier. Although these beaches have not been closed, data from the Natural Resources Defense Council (1997) indicate that during 1996, at U.S. ocean, bay, and Great Lakes beaches, there were at least 2,596 individual closings and advisories, sixteen extended (6-12 weeks) closings and advisories, and twenty permanent (over 12 weeks) closings and

advisories. Including the days of extended closings, the yearly total comes to over 3,685 closings and advisories.

Approximately 83 percent of beach closings and advisories in 1996 were based on monitoring that detected bacterial levels exceeding beachwater quality standards. An estimated 13 percent were in response to a known pollution event (without solely relying on monitoring results) and 4 percent were precautionary beach closures due to rain that carried pollution to swimming waters (NRDC, 1997). Major pollution sources responsible for 1996 beach closings and advisories include: polluted runoff from non-urban areas, sewer spills and overflows, urban stormwater runoff, and combined sewer overflows.

The Ottawa County Environmental Health Department's 1996 bi-weekly sampling program from May through August recorded *E. coli* counts between 4 and 2,800 EC colonies per 100 mL at Grand Haven State Park and 2 to 432 colonies per 100 mL at North Beach Park. The high value of 2,800 colonies per 100 mL was on a rainy day in June when the air temperature was 65°F which is an unlikely time for swimming in the cold waters of Lake Michigan. Colony counts exceeded the *E. coli* standard of 130 colonies per 100 mL in 30% of the samples taken at Grand Haven State Park and in 20% of the samples at North Beach. Spring Lake and Pottawattomie Bayou which feed into the Grand River above Grand Haven had no exceedances with EC counts ranging from 0 to 77 colonies per 100 mL. There is a strong relationship between precipitation on the sampling day (Figure 40) as recorded

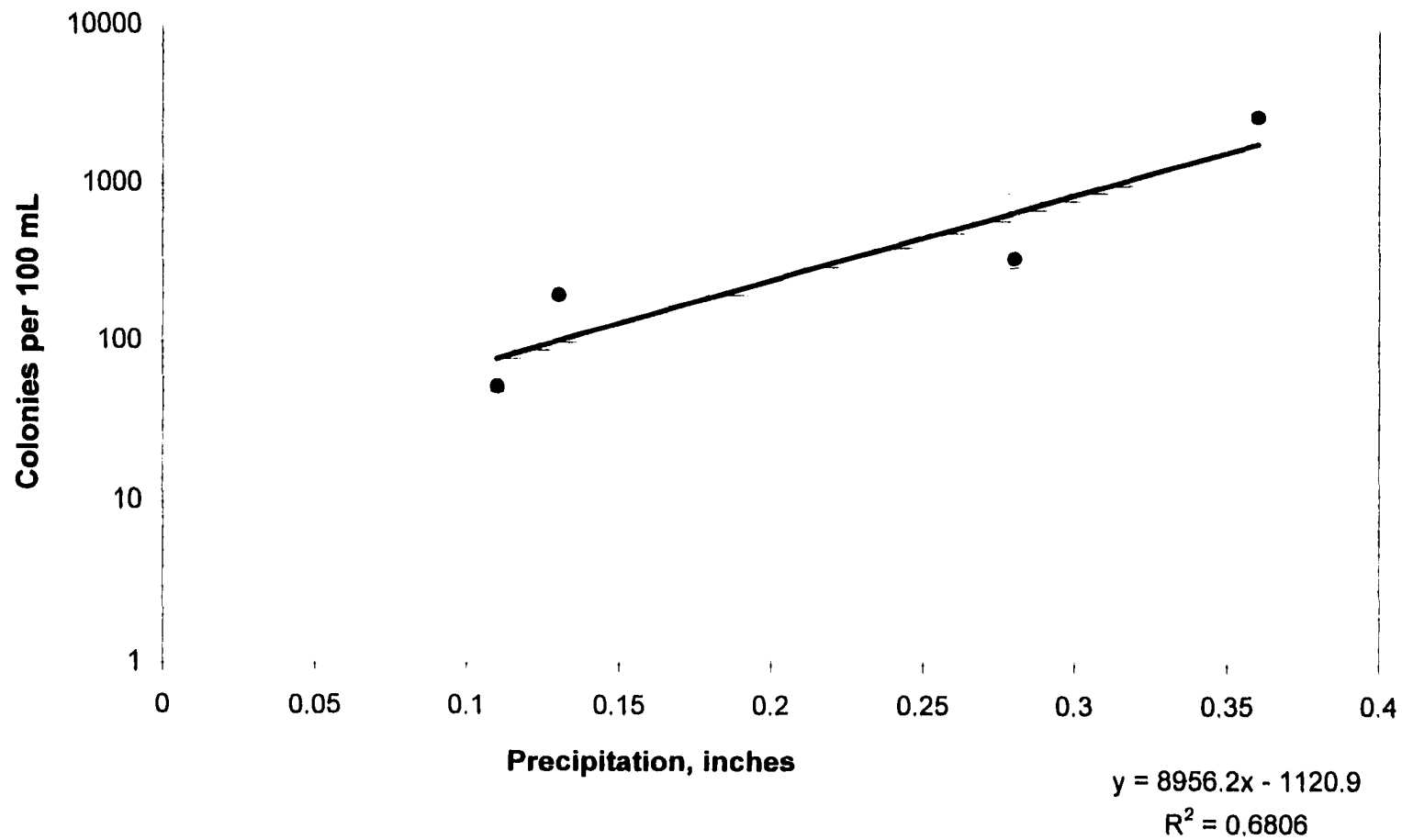


Figure 40. Correlation of Rain Events in 1996 With *E. coli* Counts in Lake Michigan at Grand Haven State Park, 1996.

at the airport in Grand Rapids and *E. coli* counts ($r = .83$). Linear regression revealed a relationship of $y = 8956.2x - 1120.9$. Average EC count on days where no rain was recorded was 24.8 colonies per 100 mL.

There is reason to be concerned about the influence of the Grand River on use of Lake Michigan beaches since there is the potential for microbiological inputs from runoff and combined sewer overflows. A conservative approach is to immediately post the beach when there is an accidental release of sewage and continue posting until the time of travel of the spill indicates that the episode has passed and there has been sufficient dilution. A recommendation would be to work on a model for this area similar to the dynamic two-dimensional finite difference water quality model developed to predict the fecal coliform densities resulting from storm-water discharges at the Toronto, Ontario, Eastern Beaches (Palmer and Dewey, 1987). Site-specific data collected for use in the model included local bathymetry, currents, dispersion, fecal coliform mortality rates, winds, receiving water fecal coliform densities, and discharge coliform densities. Proximity to large urban areas and patterns of lake currents in Lake Erie have been related to total coliform concentrations (Esterby and El-Shaarawi, 1984). It is of note that Scarce (1965) found that the central portion of Lake Michigan had very few coliform bacteria yet total bacterial densities approached 5,000/100 mL.

Elevated fecal coliform levels associated with rainfall events and runoff from two large catchments upstream were also successfully modeled for a beach in

Ottawa, Canada (Palmer and Dewey, 1984). The National Resource Defense Council (1997, p 2) notes that "issuance of preemptive rainfall advisories where a correlation between rainfall and water quality exists in anticipation of elevated bacterial levels is an important measure to protect public health and should be part of every beach monitoring program."

Yet, there remain unknowns such as where do the coliforms in open water originate? Are there organisms in the open lake that are concentrated along the shore by wave action? What is the proportion of organisms that enter the lake from runoff, rivers, and streams? The new monitoring strategy proposed by the Michigan Department of Environmental Quality puts emphasis on collection of *E. coli* data from bathing beaches, including sampling, compiling a statewide database, assistance to public health department monitoring programs, and special studies that might be able to answer these questions (MDEQ, 1997). Also included is support for training and coordination of volunteer monitoring programs. However, what is missing in the strategy is specific mention of epidemiological studies.

A recent study on Santa Monica Bay in California (Haile et al., 1996) is perhaps one of the most comprehensive cohort studies on adverse health effects of bathing in a contaminated area and whether health risks were associated with urban runoff from storm drains. The study attempted to answer the question "How safe is it to swim in Santa Monica Bay?" There were 11,686 people interviewed for analysis from three beaches. Water samples were analyzed for total and fecal coliforms,

enterococci, and *E. coli*. Water sampling established a gradient of improving water quality (as measured by indicator bacteria) with distance from drains. A survey of a coastal lagoon in San Diego, California, also implicated a storm drain as the likely source of bacterial contamination during dry periods (Gersberg et al., 1995). The study team concluded that there were, indeed, positive associations of adverse health effects with elevated indicator bacterial levels directly related to distance from the drains. The study has caught the attention of California legislators and the 1997 California Assembly Bill 411 calls for the establishment of uniform requirements throughout coastal California for monitoring bacterial contamination (total coliform, fecal coliform, enterococci) and for public notification of adverse conditions.

The study team's conceptual model relates the issue of contamination to policy (Haile et al., 1996). An understanding of where contaminants come from, how they work their way through the ecosystem, and how they affect humans is fundamental to monitoring design. Such "conceptual models" help focus monitoring on key processes or parameters and on specific kinds of information most useful for decision making. They also identify critical assumptions and uncertainties that set limits on how monitoring data can be interpreted.

However, if there is no public health problem, it would appear unnecessary and counterproductive to use a standard that would result in increased beach or stream reach closures without a clear public health benefit. The lag time in receiving laboratory results makes the readings merely a historical record and do not provide

for immediate action. Contaminated beaches are likely to remain open during the peak bacterial counts. In other words, merely collecting a sample out of the context of climatic and environmental conditions is almost meaningless. The suggestion of coordinating beach closings with real time rainfall readings would appear to be of greater public health significance than the present methodology (Nuzzia and Burhans, 1997). An example of this is the San Diego lagoon which is opened for tidal flushing from April through August. It is posted for one or two weeks following opening and for one week after any significant rain event (Gersberg et al., 1995).

Local health departments in Michigan were contacted by MDEQ regarding closing of public swimming areas and incidence of waterborne disease (Goudy, 1994). During 1992-1993, Michigan health departments reported public swimming area closures in 47 locations in 16 counties. In Kent County, there were five locations on Buck Creek, four on Plaster Creek, four on the Rogue River, and one each on the Grand River, Coldwater River, Duke Creek, and Mill Creek where closures were reported. Ottawa County reported that the entire length of the Grand River from the Kent/Ottawa County line to Lake Michigan was reported as unsafe on three different occasions in 1991 due to combined sewer overflows from the City of Grand Rapids Wastewater Treatment Plant. Cause of closures in Michigan included high counts for bacteria, sewage contamination, combined sewer overflows, treatment plant/sewer malfunctions, water stagnation, and unknown causes. Most health departments do not have actual water quality monitoring

programs for beaches, but responded to complaints. Of the reported waterborne disease incidents, swimmers itch and *Giardia* were reported in five counties (Goudy, 1994).

The Kent County Health Department (1990, p. 20) took the position that "it is almost impossible to designate a particular stream monitoring location as being consistently 'safe' or 'unsafe' for total body contact uses on an unlimited basis." Elevated levels of fecal coliform bacteria existed for several days after rainfall events in virtually all of the streams tested. During these times, the Health Department warns against total body contact but other recreational activities were not deemed as hazardous.

Based on studies in the Huron River, Gannon (1986) recommended that the State of Michigan retain the 200 colonies per 100 mL fecal coliform standard, rather than adopt an *E. coli* standard, or at least have a dual standard until firmer relationships between these indicator organisms are established for Michigan conditions and waters. In a study of a pond in the Ann Arbor, Michigan area, Gannon (1986) attributed elevated levels of fecal coliform to urban stormwater runoff from drains. No definitive answer was found as to where the organisms come from that discharge into the drains. It may be that these organisms grow in the sewers and can accumulate in the dry periods prior to flushing by rainfall events. Domesticated and non-domesticated warm-blooded animal excrement, illegal sewer connections, and growth of fecal coliform bacteria in the sediment are

possibilities for the “original” origin. Fecal coliform levels were positively correlated with the amount and intensity of rainfall. Fecal coliform/fecal streptococcus ratios suggest animal origin of the bacteria.

Gannon (1986) concluded that the most practical means of predicting high fecal coliform levels is precipitation which should be the basis of establishing restrictions in the use of water. He went on to propose a type of standard which would be to restrict use of the water body for a two-day period following a daily precipitation in the range of 0.1 to 0.49 inches, for a four-day period following precipitation in the range of 0.5 to 0.99 inches, and five days when daily precipitation is greater than 1.0 inch.

In a study on the Huron River in Michigan, the geometric mean EC/FC (*E. coli*/fecal coliforms) ratios were in the range of 0.82-1.34, well above the ratio of 0.63 calculated using the U.S. EPA recommended level for *E. coli* of 126/100 mL (Gannon and Busse, 1989). One would not expect these ratios to be greater than one since *E. coli* is a subset of the fecal coliform group. Variability in samples and differences in specificity of the culture media for *E. coli* and fecal coliforms are possible explanations. If the *E. coli* standard had been applied to this system, there would have been many more periods of water use restrictions. From their studies, Gannon and Busse (1989) concluded that if the intent is to maintain the currently accepted illness rate, additional results from other areas are necessary to refine the *E. coli* and enterococci levels for water quality standard development purposes.

For the St. Mary's River which borders Ontario and Michigan, Dutka and Marsalek (1993) found significant correlations between fecal coliform and *E. coli* during both dry weather ($r^2 = 0.78$ to 0.94) and wet weather ($r^2 = 0.98$ to 0.996). EC/FC ratios varied from 0.48 to 1.0 in dry weather with an average of 0.74, and between 0.42 to 0.84 in wet weather with an average of 0.63.

The conclusion that use of *E. coli* would result in more water use restrictions was also reached by the Environmental Health Service in Santa Cruz, California. In the author's monitoring in the San Lorenzo River in 1987, *E. coli* counts using mTEC agar averaged 125% of the fecal coliform counts. This may be attributed to holding the *E. coli* plates at 35°C for two hours prior to the subjecting them to elevated temperature of 44.5°C. The initial lower temperature is said to assist the resuscitation of injured or stressed bacterial cells (Dufour, Strickland, and Cabelli, 1981).

It would be expected that *E. coli* counts should be consistently less than fecal coliform which was not the case with the California results. However, linear regression analysis showed *E. coli* levels were directly related to fecal coliform levels ($r = 0.97$). Monitoring data from 1994-96 in the San Lorenzo River System ($n = 321$) showed an average of 2.7 for the EC/FC ratio, but 57% of the EC/FC ratios were 1.0 or less. By removing ten highest outliers, this ratio is 1.18 which is close to the author's 1987 results. In the San Lorenzo River parallel studies for different water quality indicators ($n=312$) in 1994-96, there was a correlation of

0.91 between FC and TC, 0.58 for *Enterococcus* and FC, and *Enterococcus* and EC (0.32).

According to the Environmental Health Department at Santa Cruz County, "there is no evidence to suggest that such a tighter [*E. coli*] standard is needed, or even appropriate for the San Lorenzo Watershed" (County of Santa Cruz, 1989). Additionally, the County concluded that "based on studies here and in other areas, the presence of high fecal coliform levels does not reliably indicate the actual public health threat or source of contamination. There have been limited actual reports of illness that could be linked to swimming in local waters" (County of Santa Cruz, 1996).

The statistical probability of a given concentration occurring is a better approach than water quality criteria based on concentration alone. Risk would thus be expressed as the probability that a given stream standard will be violated (Ward and Loftis, 1983). According to Ott (1995, p. 5), "it is impossible to design a regulatory program that can guarantee that any reasonable standard *never* will be violated, and there is a growing awareness that probabilistic concepts should be an integral part of the standard setting process." Confidence limits on means or extremes for water quality data clarify uncertainties in data. Variation in water quality data reflects sampling, laboratory analysis, and actual conditions. To account for the stochastic nature of water quality hydrology and the statistical nature of

sampling, working towards water quality guidelines that are water body specific seems to be a reasonable goal for management strategies (Ward and Loftis, 1983).

Researchers in Wisconsin found that even in relatively clean streams, water quality standards for fecal coliform bacteria are not met all of the time (Wible, 1980). This resulted in a recommendation of a 90% compliance level as the criterion for those indicators which do not directly affect aquatic life such as phosphorus and fecal coliform organisms. This probabilistic approach to water quality standards was recommended for use as a supplement to the current exemption in Wisconsin standards for low flow conditions.

Strobel's (1968) work on shellfish growing waters indicated that correlations for relating fecal coliform limits to present coliform standards must take into account the particular characteristics of each shellfish growing area. This research indicates site specific differences that should be factored into water quality criteria. Clearly, lessons from the past and the information derived from long-term data sets should be applied in the current thinking on water quality standard setting.

CHAPTER VIII

CONCLUSION

Implications for Science Educators

The pedagogical question that needs to be asked is whether students are monitoring for bacteria to learn, in general, about their environment and to gain experience in the way a scientist works, or are they collecting data to be used in community and government agency decision-making. Given the difficulty and expense of generating “good data” from a regulatory standpoint, the former seems to be a more realistic expectation.

If educators can accept *E. coli* counts from a presence/absence or relative abundance perspective, then a system like the Petrifilm™ method would be a cost effective, relatively error-free, simple means of monitoring for bacteria. Conceptually and appearance-wise, the system is similar to membrane filtration. Petrifilm™ has the added advantage of a lower (and more forgiving) incubation temperature. The trade off is that "colonies per 100 mL" can only be recorded in intervals of 100. However, groups like Florida Lake Watch have not found this to be a major problem, and the Petrifilm™ method appears to be very effective for their bacteriological monitoring.

Fecal coliform or *E. coli* counts should be interpreted in the context of long-term regional monitoring data. Long-term databases are valuable for their temporal and spatial aspects as well as for identification of new pollution sources and generation of predictive models. As illustrated by the contrast between the Michigan and California studies, it is evident that standards for bacterial counts in one area are not necessarily realistic for other areas. There is compelling evidence from the Michigan seep study that fecal coliform and *E. coli* bacteria are normal inhabitants of midwest ecosystems throughout the year, and that high counts sometimes have no association with human contamination. The climatic regime, soils, and non-human sources in the Michigan study contribute to a flourishing fecal coliform and *E. coli* population that results in a significant background count for these organisms. Unlike the California system where headwaters of streams had counts of zero for fecal coliform, the headwaters in southern Michigan rarely have zero counts. It is highly probable that random samples from Michigan streams, seeps or rivers will exceed water quality standards. In the Midwest, a general assumption might be that just about any stream is likely to have significant levels of fecal coliform and *E. coli* unless proven otherwise by long-term monitoring.

The science educator who is involved in stream or lake monitoring should consider doing the following:

1. Establish baseline conditions for bacteria in the stream or lake through year-around monitoring. Although classroom sampling is not normally done in the summer, the data collected are valuable for comparative purposes.
2. Find and analyze long-term data sets on bacterial counts for streams and lakes in the region, and use an appropriate regional conversion factor for *E. coli* and fecal coliform comparisons.
3. Run fecal coliform or *E. coli* tests at the sampling site prior to any student monitoring to get a sense of the bacterial levels.
4. Avoid bringing groups to streams or lakes during or after rainfall events, particularly in areas where storm drains are present.
5. Incorporate *E. coli* monitoring using a simple system such as Petrifilm™ in each monitoring episode for other parameters such as dissolved oxygen and pH..
6. Have students analyze bacterial monitoring results in the context of the entire watershed and multi-source inputs to the stream or lake.

Clearly, it is essential for the educator to know whether the stream or lake being monitored is impacted predominantly by potential human contamination (combined sewer overflows, storm drains with heavy urban runoff, septic systems) or nonpoint sources (agricultural runoff, tile drain systems). Monitoring of drains and urban runoff should be part of a sampling strategy. It is important to keep in mind that sediment, soil, plants, air (e.g., near wastewater treatment plants), and water, as well as feces, can all harbor fecal coliform bacteria. The search for

sources of fecal coliform bacteria should include observations of relative abundance and activity of birds, wildlife, and domestic animals. Scat (fecal pellets) can be located and enumerated. Indeed, generating and testing hypotheses about sources of bacteria begins with these sorts of observations. An excellent framework for interpreting fecal coliform data can be found in an article by George Heufelder of the Barnstable (Massachusetts) County Department of Health and the Environment (Heufelder, 1997).

The concept of the watershed as a multiple storage and release system (Jenkins et al., 1984) is a good starting point for student understanding of complex relationships, in particular nonpoint source pollution. The land surface receives direct fecal input by excretion from animals and from agricultural practices such as slurry spreading. The concentration of bacteria in water is increased by fecal input and washout from sediment and is reduced by settling, predation, and die-off. If nutrient conditions are favorable, numbers of bacteria in sediment are increased by settling and reproduction. They are reduced by die-off, predation, and washout. Lakes can act as stabilization ponds to reduce numbers of bacteria.

As soon as a rain drop touches the earth's surface, bacterial contamination begins. Runoff from plowed land and roadways may contain several hundred thousand bacteria per milliliter. Although groundwater contributions and natural stream purification diminish and dilute the bacterial load, "it is difficult, however,

to find a river in inhabited regions which does not contain several hundreds or thousands of bacteria to the milliliter" (Prescott et al. , 1946 p. 4).

Within an aquatic system, there are four distinct populations of bacteria: (1) planktonic, (2) particle-associated, (3) surface associated (slime on rocks and vegetation), and (4) sediment/soil-associated (Costerton and Geesey, 1979). High fecal coliform bacterial counts in the water column are related to numbers of planktonic and particle-associated bacteria. However, water column samples may fail to reflect surface and sediment-associated bacteria.

The role of sediment in indicator bacterial dynamics needs to be emphasized. Indeed, the bacteria bound in the sediment should be treated with equal importance to that in the water column (LaLiberti, 1982). The importance of not disturbing bottom sediment when sampling the water column has been illustrated in many studies such as that of Grimes (1975) who cautioned that dredging of bottom sediment could cause a temporary enteric pathogen health hazard downstream.

Students need to view the presence of bacteria in an ecological context as well as human health context. This should be done within a framework of relative risk. Activities such as those from the WET curriculum help relate bacterial contamination to human society. Point source inputs from wastewater treatment plants and septic systems are much more of a direct public health risk than background concentrations from nonpoint sources. A comprehensive sanitary

survey with the following four elements can help clarify the overall health of a stream: (1) Bacteriological - fecal coliform or *E. coli* counts; (2) Biological - nuisance aquatic plants, algal blooms, types of macroinvertebrates; (3) Chemical - toxic substances, low dissolved oxygen, abnormal pH, odors; and (4) Physical - scum, floating solids, debris, oil, sludge, turbidity. A good summary of all of the elements of a sanitary survey is found in VanOrden (1994). Particularly in urban streams, the assessment strategy should include a streamwalk to identify illicit connections and storm drains as well as a biological survey (Duda et al., 1982). Detailed storm-event monitoring is also revealing but not recommended for safety reasons.

Dates (1995, p. 2-3) presents a valuable perspective about what to do with data from bacteria and other water quality monitoring parameters. "Findings" are observations about the data and "conclusions" explain why the data look the way they do. Using this framework, the approach is to use the data to answer questions related to findings and conclusions such as:

1. Which sites consistently did not meet the water quality criteria? By how much? What is different about stations that have high and low values?
2. Were there sampling dates on which most or all sites did not meet water quality criteria? Does weather appear to influence results? For example, do problem levels coincide with intense rainstorms? Do levels coincide with rising flow?

3. Does the time of day when samples are taken affect results?
4. Do levels increase or decrease in a consistent manner from upstream to downstream?
5. Do bacterial results consistently show safe levels for swimming and/or partial body contact?
6. For multiple years of data, are there overall trends?
7. Can sources of contamination be identified?

Other questions along this line are found in *Testing the Waters* (Behar, 1996).

Effective teaching of science should lead students to ask questions and not to expect simple answers. Water quality is not a simple matter of "bad" or "good" implied by water quality indices, but rather the degree to which a water body is affected by pollution and how this compares to designated uses of the water. The blend of science and society is a very compelling reason for the use of bacterial monitoring in an educational setting. The ambiguity and the "mystery" of the specific causes of fecal coliform bacterial contamination in water can be a useful science education teaching tool. Additionally, lively discussions can ensue about the risk of contracting a waterborne disease versus the economic ramifications of closing beaches and rivers to public use when bacterial levels are high.

Initial student conceptions about water quality indicator bacteria can be broadened through actual project work which leads to meaningful learning and conceptual change. Linkage of ideas helps to eliminate contradictions and to create

more robust and powerful ideas (Eylon and Linn, 1988). An example of how to integrate concepts of indicator bacteria into the classroom is illustrated in a project in the Bear Creek watershed that involved students in dye testing of septic systems.

From February through April 1997, the "Explore 4-5" gifted and talented class from an elementary school in Rockford, Michigan joined the Bear Creek Watershed project staff from GVSU-WRI in conducting a septic system dye test survey at homes and businesses along Bear Creek and its tributaries. The purpose of the study was to determine whether failing septic systems add to the chronic bacterial contamination of Bear Creek. The students sent letters to riparian owners to solicit participation, conducted a survey and visual inspections, and worked with the staff on follow-up. Seven of twenty-one sites showed positive results indicating septic system failure. These systems generally fit into three categories: (1) location within 50 feet of the creek, (2) installation on a steep slope, and (3) age of more than 25 years. This project captured the interest of the media and a 30-minute documentary featuring the project aired on PBS stations in June 1997. According to a GVSU research assistant, "the children are learning from the homeowners and the homeowners are learning from the children."

Development of new understanding requires an authentic experience that challenges prior knowledge and beliefs (Stofflett and Stoddart, 1994). The study of bacterial contamination through monitoring and action projects can do much towards achievement of scientific and environmental literacy. However, educators

must be certain that they, themselves, have a thorough understanding of the nuances of bacterial monitoring and ecosystem dynamics before involving students in this type of monitoring.

Implications for Water Quality Standards

A question frequently asked in regards to monitoring for bacterial contamination is: what does it all mean? It is clear that there are regional trends and association of high counts with nonpoint sources (e.g., agriculture). Ambiguity about the significance of finding fecal coliform in water is not new. Compelling evidence for reexamination of current water quality standards comes as far back as 1917 when a researcher reported that *Escherichia* and *Aerobacter* organisms were surviving in water nine months after inoculation with feces (Prescott et al., 1946).

Active surveillance, investigation, and control of waterborne disease in the United States does not appear to be a high priority. Fewer than half of the states have a designated coordinator for waterborne disease outbreaks. This aspect of the standards has been left to the state and local health departments to manage. In a 1993 survey, no states were found to use computerized illness data from health maintenance organizations as a waterborne disease surveillance tool (Frost et al., 1996). As noted in the 1993 cryptosporidiosis outbreak in Milwaukee, Wisconsin, waterborne disease outbreaks, though infrequent, can affect hundreds of thousands of people. Agencies need to have the knowledge to respond to such outbreaks.

Stronger linkages of illness to bacterial contamination need to be made if fecal coliform bacteria and *E. coli* are to continue as water quality indicators .

The "one standard fits all" concept does not appear to reflect reality. Recognizing that stream purification is a complex and ill-defined process, Geldreich (1978, p. 16) states that "it would be desirable to make the decision regarding sewage effluent disinfection on a case by case basis rather than enforcing an arbitrary, uniform zone for all water courses." This is echoed in current literature where researchers state that "the use of coliforms as indicators of the presence of feces-derived pathogenic bacteria and viruses has shortcomings which can make them untrustworthy indicators in a variety of situations" (McCorquodale and Bumry, 1996, p.1). In Michigan, there seems to be a ubiquitous background count of fecal coliform and *E. coli* bacteria in the surface water that complicates decision-making on levels for standards.

Epidemiological evidence linking fecal coliform or *E. coli* counts with disease does not always seem to be a credible and cost effective way to determine waterbody closings to protect health. Questions such as those posed for marine recreational water quality criterion (Cabelli et al., 1983, p. 1311) are relevant to freshwater conditions:

1. Does swimming in the water carry with it an increased risk of illness, and if so, to what type?
2. Is there an association of the illness rate to pollution from domestic wastewater, and if so, to what type of illness?
3. Which, if any, of the potential indicators of water quality best defines the illness symptomology to water quality?

4. Can the relationship of swimming-associated health effects to the quality of the water, as determined by a microbial or chemical indicator, be quantified sufficiently to produce a health effects, quality criterion for recreational waters?

We must not lose the real sense of a standard which is to protect the population from infectious diseases. Such diseases are more likely to be associated with raw sewage than with ambient bacteria. A reasonable approach is to generate bacteriological profiles of individual streams identifying point and nonpoint sources of pollution, work towards elimination of specific human inputs (faulty septic systems, combined sewer overflows), and relate warnings of contamination more directly to rainfall events, flooding or snow melt events. Brief heavy storms increases bacterial contamination as the rain washes bacteria from the land, and combined sewer overflows can happen during these storms. A prolonged moderate rain initially washes away bacteria followed by dilution of the stream water which can decrease bacterial levels.

The work of Barbe and Francis (1995), who developed predictive equations relating river discharge to coliform concentrations, leads the way in providing timely estimates of fecal coliform counts for regulatory posting purposes. Figure 41 provides a framework for decision-making regarding bacterial contamination. Inputs for decisions about allowable human use of water bodies involve not only bacteriological monitoring but also precipitation data, epidemiological considerations, and unpredictable events such as spills, combined sewer overflows,

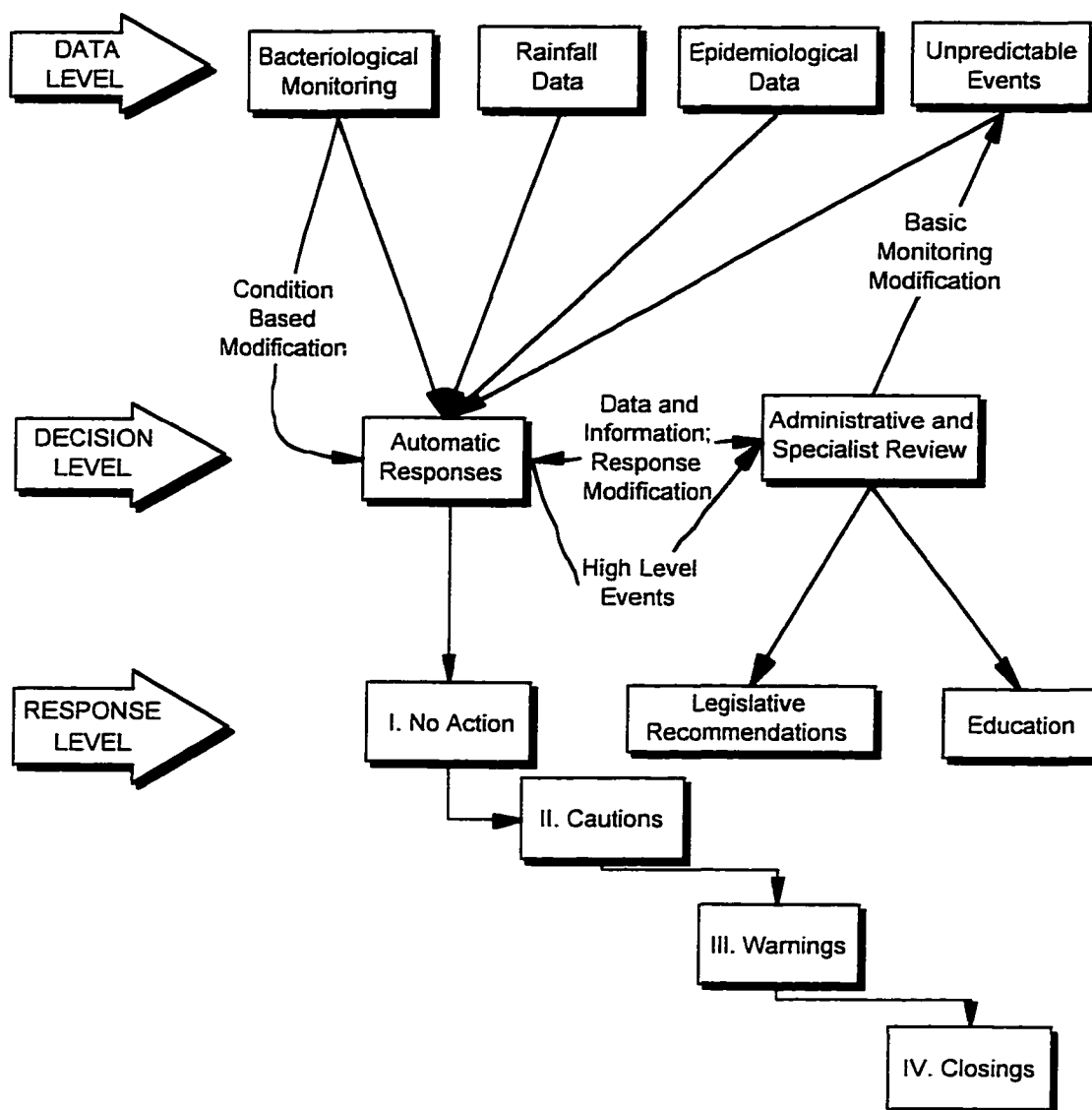


Figure 41. Decision Matrix for Responses to Microbiological Contamination of Recreational Waters.

and wastewater treatment plant failures. A possible way to integrate these inputs would be to assign numerical values based on level of concern. For instance, ratings for rainfall amounts and duration, bacteriological monitoring results,

presence of waterborne diseases and epidemics, and types of unpredictable incidences could be developed. The composite rating along with professional judgment would determine the appropriate action level to take.

The framework for a holistic view of bacterial monitoring was set over fifty years ago, but somehow the message has been lost in the narrower view of current water quality regulations. In 1946, the textbook *Water Bacteriology*, which was first published in 1904, stated that the significance of coliforms in water should be analyzed with regard to the following considerations:

1. Bacteria conforming to the definition of coliform organisms are by no means confined to the animal body but are widely distributed in nature.
2. The finding of a few coliforms in large samples of water, or their occasional discovery in small samples, does not necessarily have any special significance.
3. The detection of coliforms in a considerable proportion of 1 mL samples is imperatively required as an indication of recent sewage pollution.
4. The number of coliform organisms in water, rather than their mere presence or their type, should be used as the principal criterion of recent pollution by sewage.

(Prescott et al., 1946, p. 188)

Initially, the presence of indicator bacteria led health departments to do a sanitary survey to seek the cause of the contamination. However, the current regulatory structure favors beach closings and health warning postings upon detection of these bacteria, but not surveys to determine sources of contamination. Due to frequent fluctuations and variability in routine monitoring, high levels of

bacteria in the absence of any known sewage spill should be carefully investigated before action is taken (Kebabjian, 1994).

Prescott et al, 1946, p. 248, continues with: "conclusions based on arbitrary bacteriological standards will never completely fit all circumstances." Streams, in particular, cannot be generalized and "stream standards cannot be applied wholesale to all rivers without regard to their widely varying natural self-purification capacities" (Nemerow, 1974). Available evidence in 1946 also warranted the conclusion that "although the presence of *E. coli* in water may be considered, in general, more indicative of recent and dangerous pollution than is the presence of other coliforms, this generally is so subject to exception that its usefulness in the practical interpretation of coliform results is, save in a few particular instances, problematical" (Prescott et al., 1946, p. 187). There does not seem to be sufficient present day evidence to alter this statement.

In conclusion, neither fecal coliform bacteria nor *E. coli* appears to meet all of the basic criteria for a credible water quality indicator (Feachem et al., 1983, p. 53) as summarized in Table 14. In light of the research results, a re-analysis of the efficacy of federal and Michigan water quality standards for the protection of human health in recreational areas should be performed. Consideration of site-specific standards and correlation with precipitation events needs to be part of this analysis.

Table 14

Evaluation of Fecal Coliform Bacteria and *E. coli* as
Microbiological Water Quality Indicators

Criterion (from Feachem et al., 1983, p. 53)	Analysis
A normal member of the intestinal flora of healthy people	True
Exclusively intestinal in habitat, and hence exclusively fecal in origin when found in the environment	Not true
Absent from nonhuman animals	Not true
Present whenever fecal pathogens are present, and present only when fecal pathogens might reasonably be expected to be present	Uncertain
Present in higher numbers than fecal pathogens	Probably true
Unable to grow outside the intestine	Not true
A die-off rate slightly less than that of fecal pathogens	Uncertain but probably not true
Resistant to natural antagonistic factors and to water and wastewater treatment process to the degree equal to or greater than that of fecal pathogens	Uncertain
Easy to read and detect	True
Nonpathogenic	Not true for all strains

Additionally, a regional epidemiological study on the order of the Santa Monica Bay study (Haile et al., 1996) is recommended. In tune with the latest views of bacterial monitoring, the latest County of Santa Cruz plan for the San Lorenzo River recommends continuation of basic monitoring, rainy weather sampling, source investigations, sediment sampling, beach sampling, a health risk survey, and purchase of equipment for performing the polymerase chain reaction methodology (County of Santa Cruz, 1996).

Perhaps Simmons' (1997) recent work on DNA fingerprinting of *E. coli* could salvage or at least clarify the fecal coliform and *E. coli* standards. Comparing *E. coli* from stream samples with a DNA "library" of strains, Dr. George Simmons of Virginia Tech was able to trace fecal coliform sources to deer and raccoon. Both species are present at the Michigan groundwater area seep studied in this project. Most profound is that after removal of several hundred animals, including deer, raccoon and muskrat in the winter, the spring fecal coliform counts in streams decreased by one or two orders of magnitude (Simmons, 1997). Simmons admits to bias going into the study "it took me about a year to come to grips with the fact that the fecal coliform were not coming from a leaky septic tank or other effluent...It got to the point where I was climbing trees to see if I could see any potential sources (i.e., houses), but there weren't any" (Simmons, 1997, p. 20).

Simmons believes his technology can solve the mystery of fecal coliform sources. However, Howard Kator, an environmental microbiologist from Virginia Institute of Marine Science, questions the ultimate value of DNA fingerprinting, but not because of the methodology. Kator is one of the numerous scientists who believe that "*E. coli* is not a good indicator of water quality...[DNA fingerprinting] doesn't address the fundamental issue of determining health risks" (Kratch, 1997, p. 26). Kator continues with "indicators we have now have never been evaluated" and standards were essentially "plucked out of hats" (Kratch, 1997, p. 26).

The message of this dissertation is illustrated in a study comparing total coliform, fecal coliform, and fecal streptococci indicator bacterial counts in lakes and streams in the wilderness of Yosemite National Park (Holmes, 1976). Typical bacterial counts in waters in the wilderness ranged from virtual sterility in early July snowmelt to light and moderate levels in August and September samples to heavy levels in storm runoff. Little correlation of human use with increased indicator levels was shown. This was thought to be due either to masking by background levels of indicators from the native warm-blooded species or to lack of substantial human bacterial inputs to surface and ground waters. Compelling evidence from mountain streams in Montana draws the same conclusions (Stuart et al., 1971). Fecal coliform counts were actually lower in areas open to public use (less wild animals) than in areas closed to humans. Elk and bear droppings were significant environmental sources of indicator bacteria. Heufelder (1997, p. 18)

notes that "fecal pellets act like little time capsules, slowly releasing fecal coliforms."

Epidemiological evidence is sorely needed to support recreational water quality standards that currently apply to all bodies of water in a state. Indeed, the Kent County Health Department in 1993 stated that

In consideration of scientific evidence which suggests that fecal coliform levels in streams do not correlate well with the incidence of human disease, the department should advocate the adoption of a new water quality indicator by the State of Michigan which more accurately describes the level of risk associated with water contact. (KCHD, 1993. p. 81)

A conservative approach would be to close many beaches and streams permanently since endemic fecal coliform levels are always high. Another approach is to realistically assess the risk based on suspected sources of contamination coupled with in-depth epidemiological studies. If there is a lesson educators can teach regarding bacterial contamination, it is that "accurate perceptions of risk are necessary for the attainment of a risk-literate society" (Reichard, 1993, p. 12).

In the meantime, the Alabama Water Watch (1996, p. 5) sums up the current state of affairs in the following way: "a single high *E. coli* [or fecal coliform] number does not necessarily mean anything as the *E. coli* could be from excrement left by a deer or another animal a short time before the sample was collected. However, a series of elevated samples is a reason for concern." Extensive knowledge about the watershed is the key to interpretation of microbiological monitoring results.

Appendix A
Location of Sampling Sites

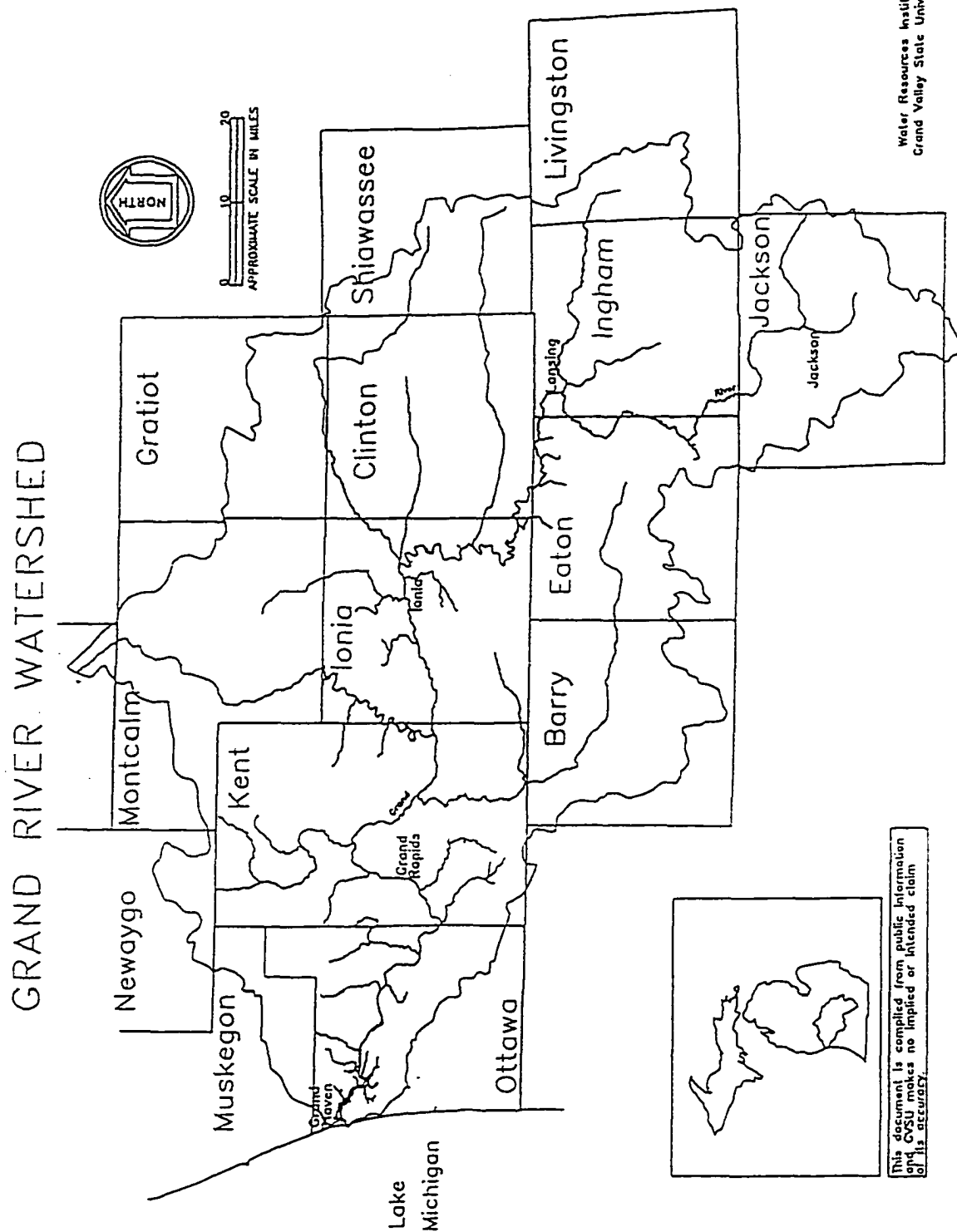


Figure 1. Grand River Watershed, Michigan.

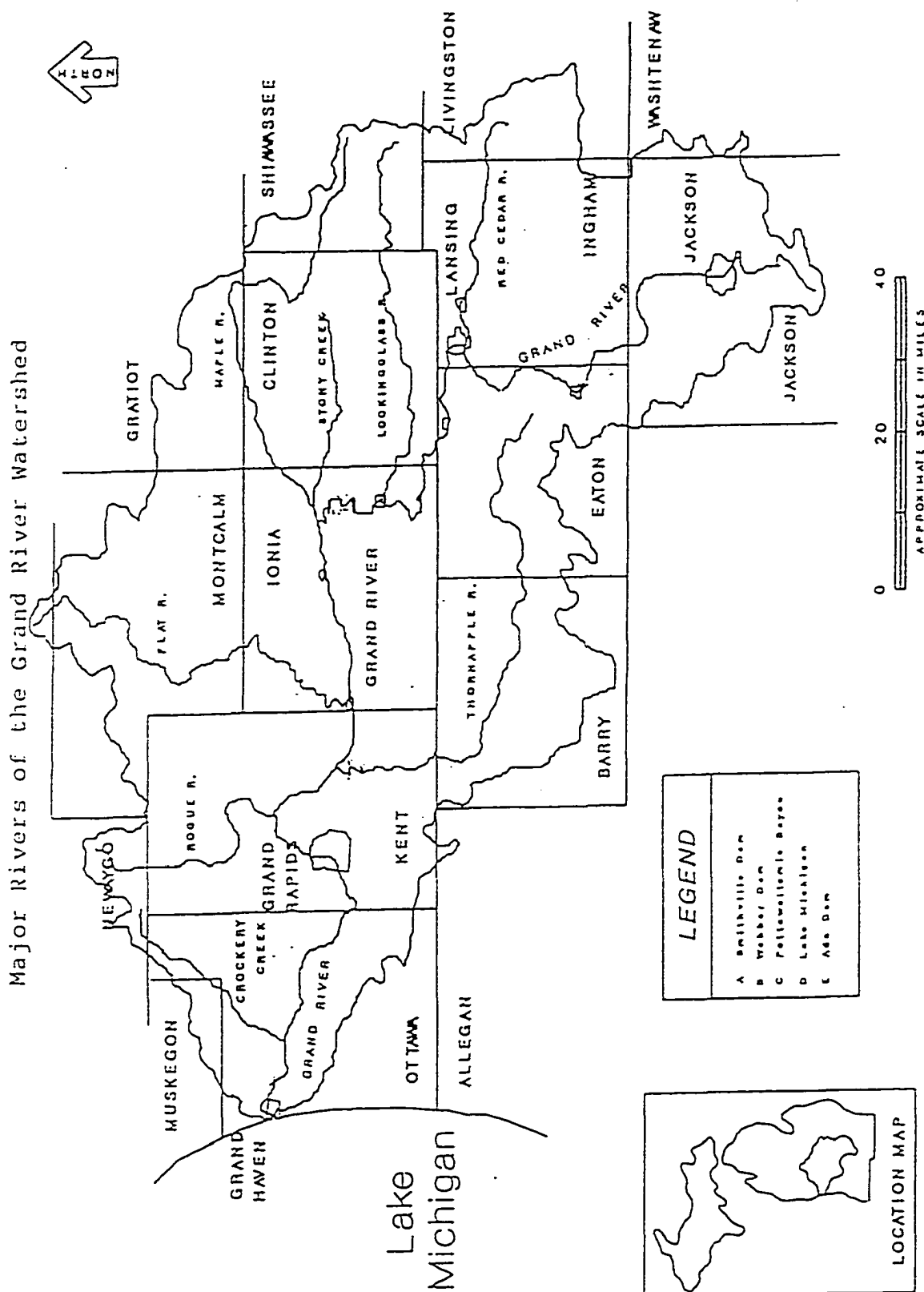


Figure 2. Major Rivers in the Grand River Watershed, Michigan.

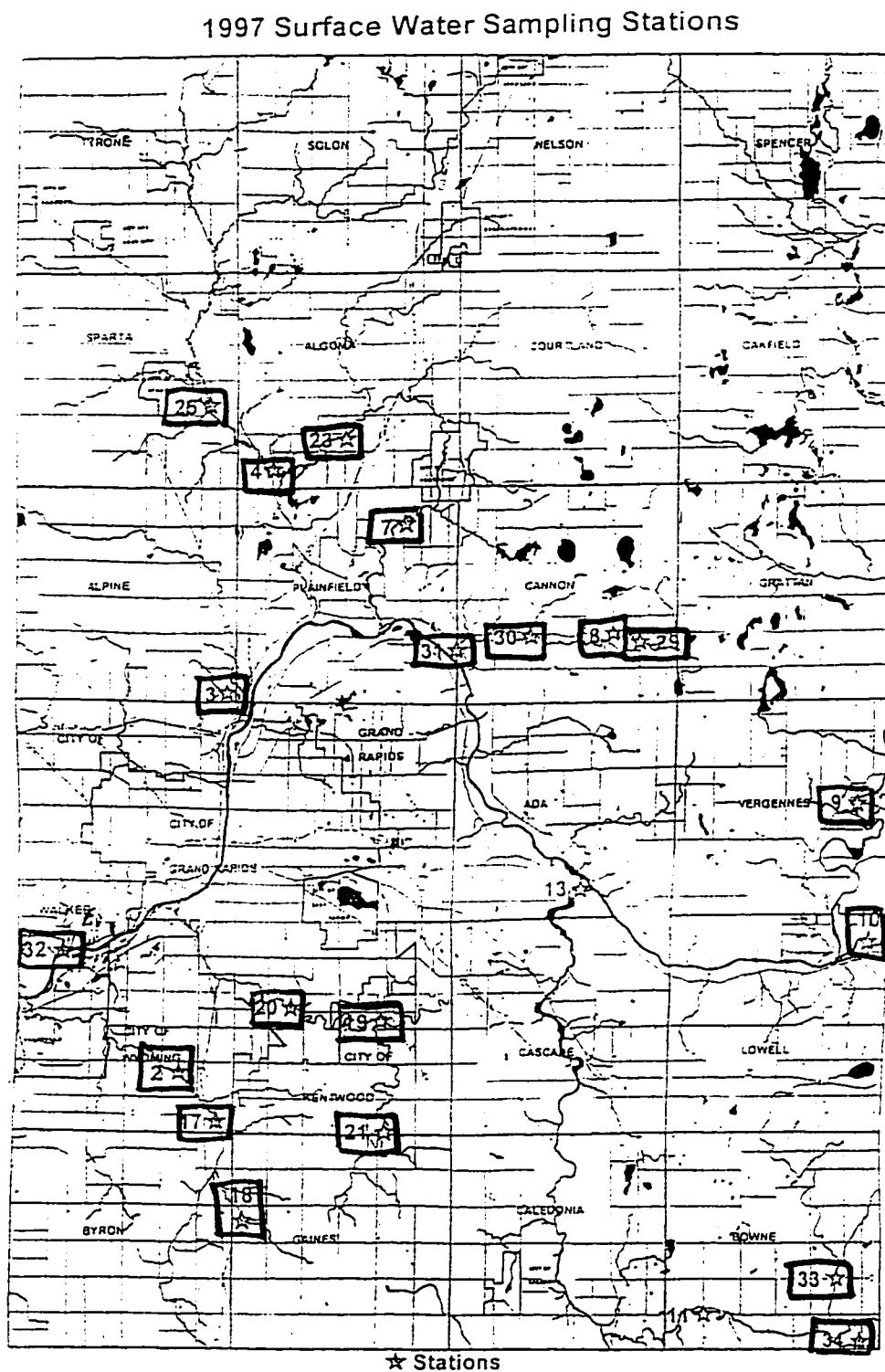
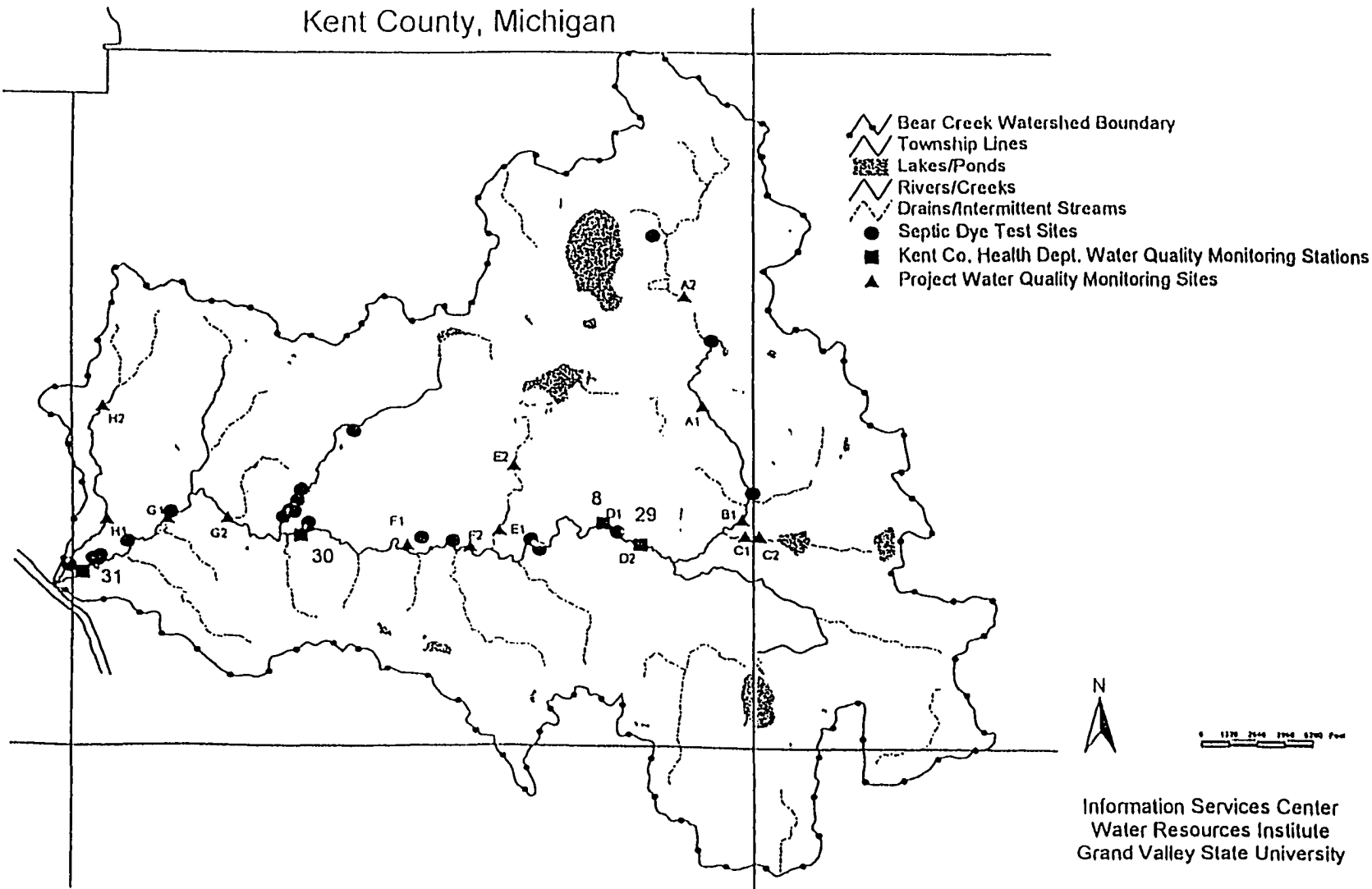


Figure 3. Kent County Health Department Sampling Sites.

Figure 4. Bear Creek Watershed, Kent County, Michigan.



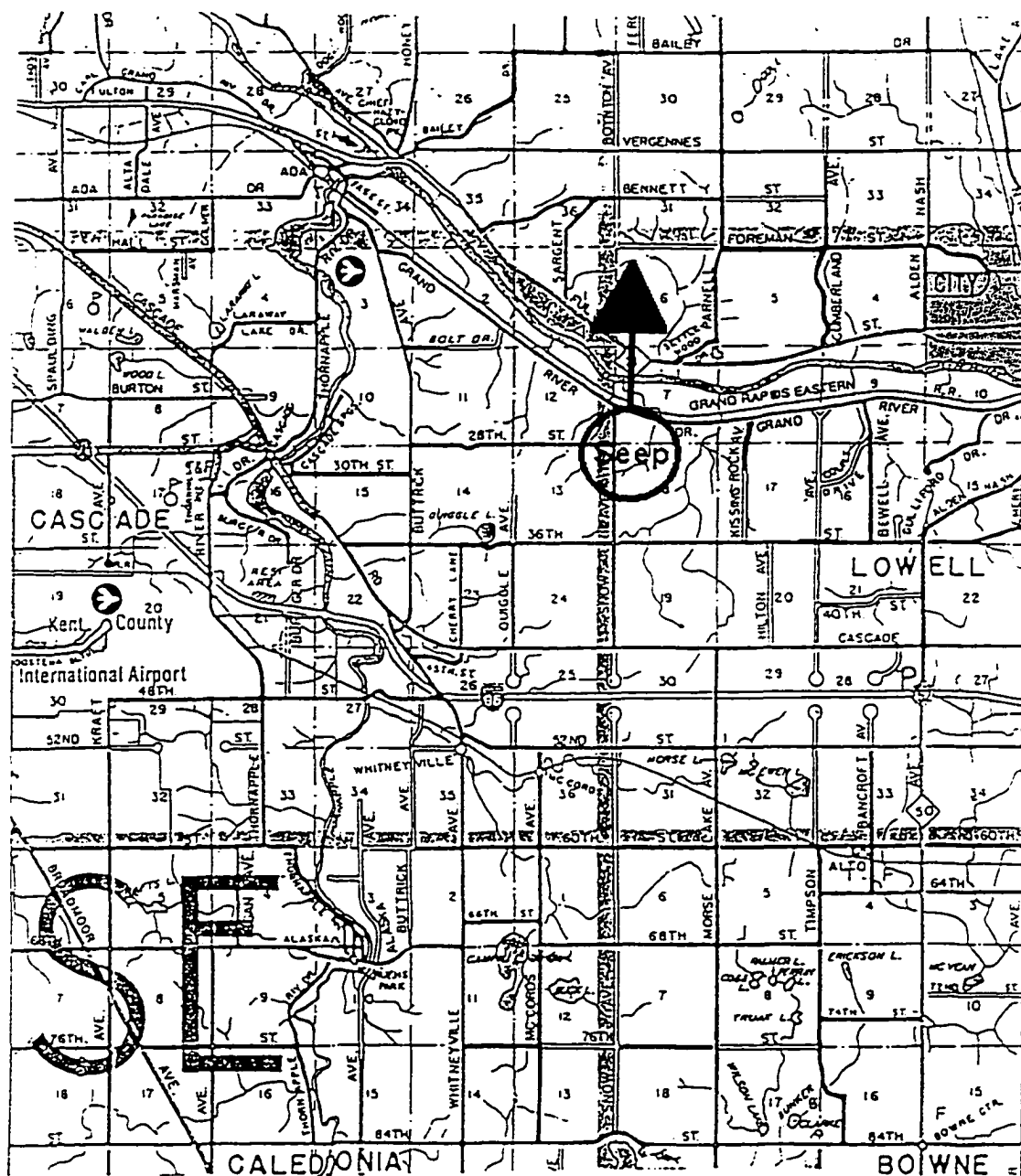


Figure 5. Location of Groundwater Seep in Lowell Township, Kent County.

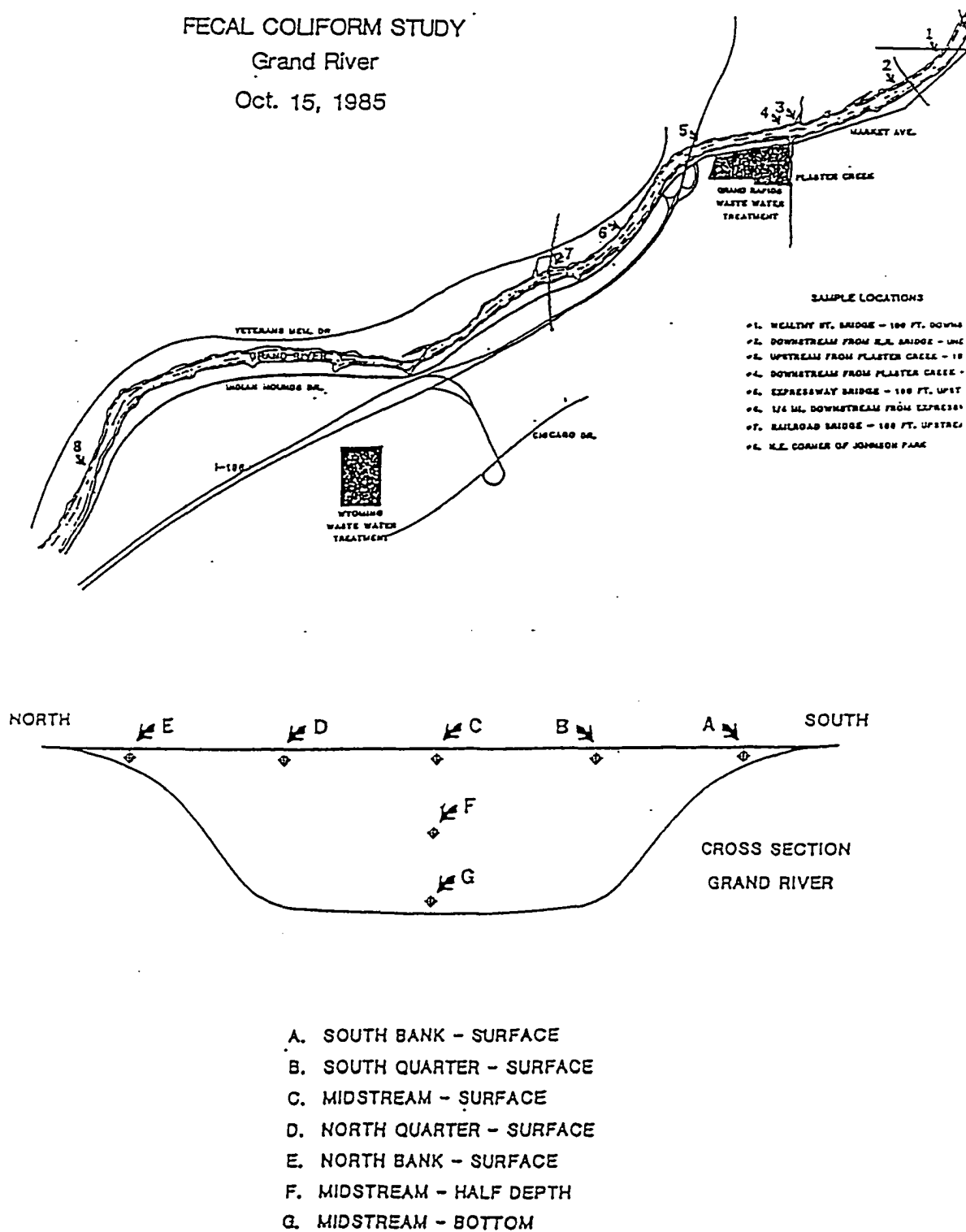


Figure 6. Grand River Fecal Coliform Study Site.

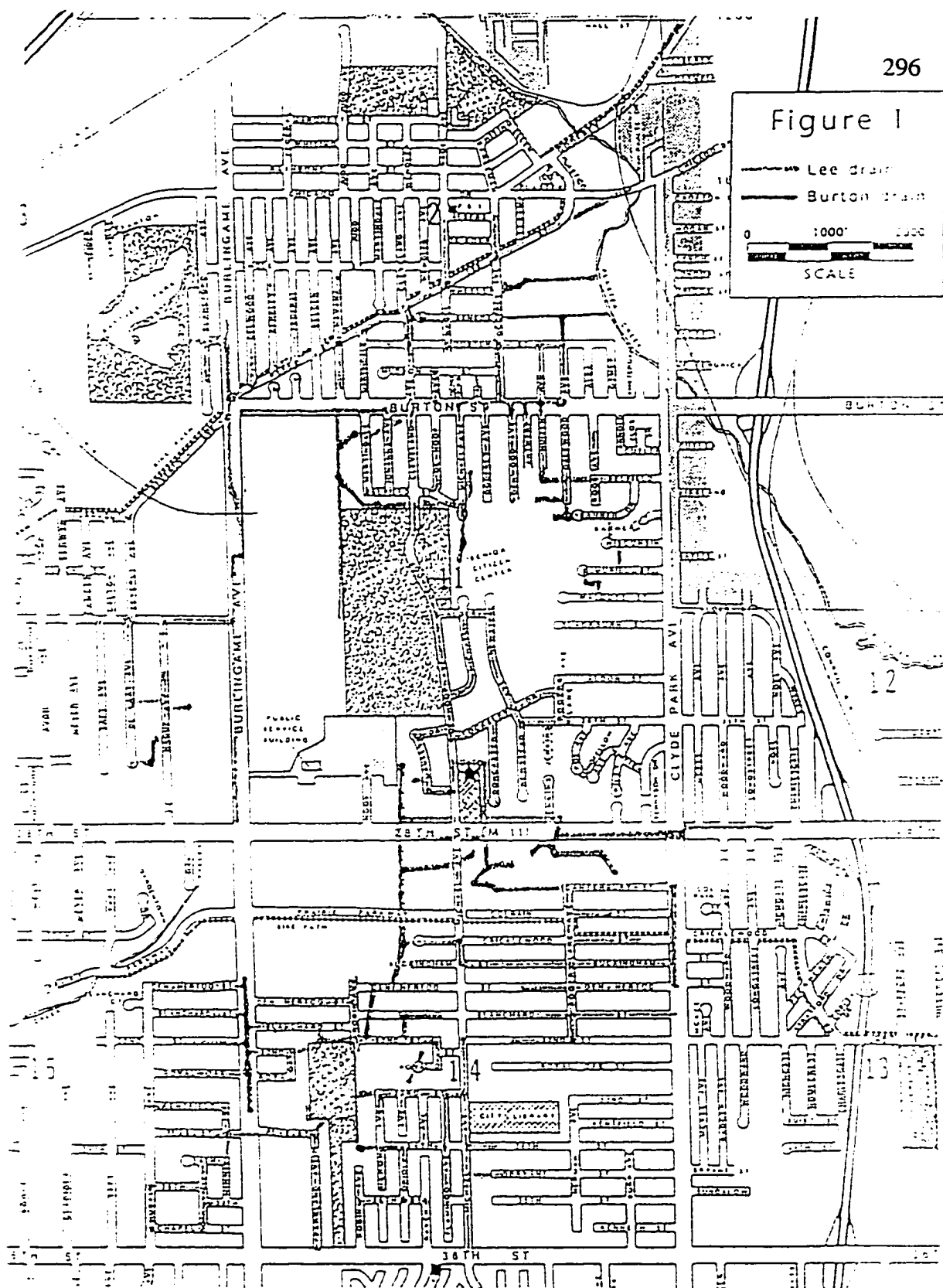


Figure 7. Lee Drain and Burton Drain Systems in Kent County, Michigan.

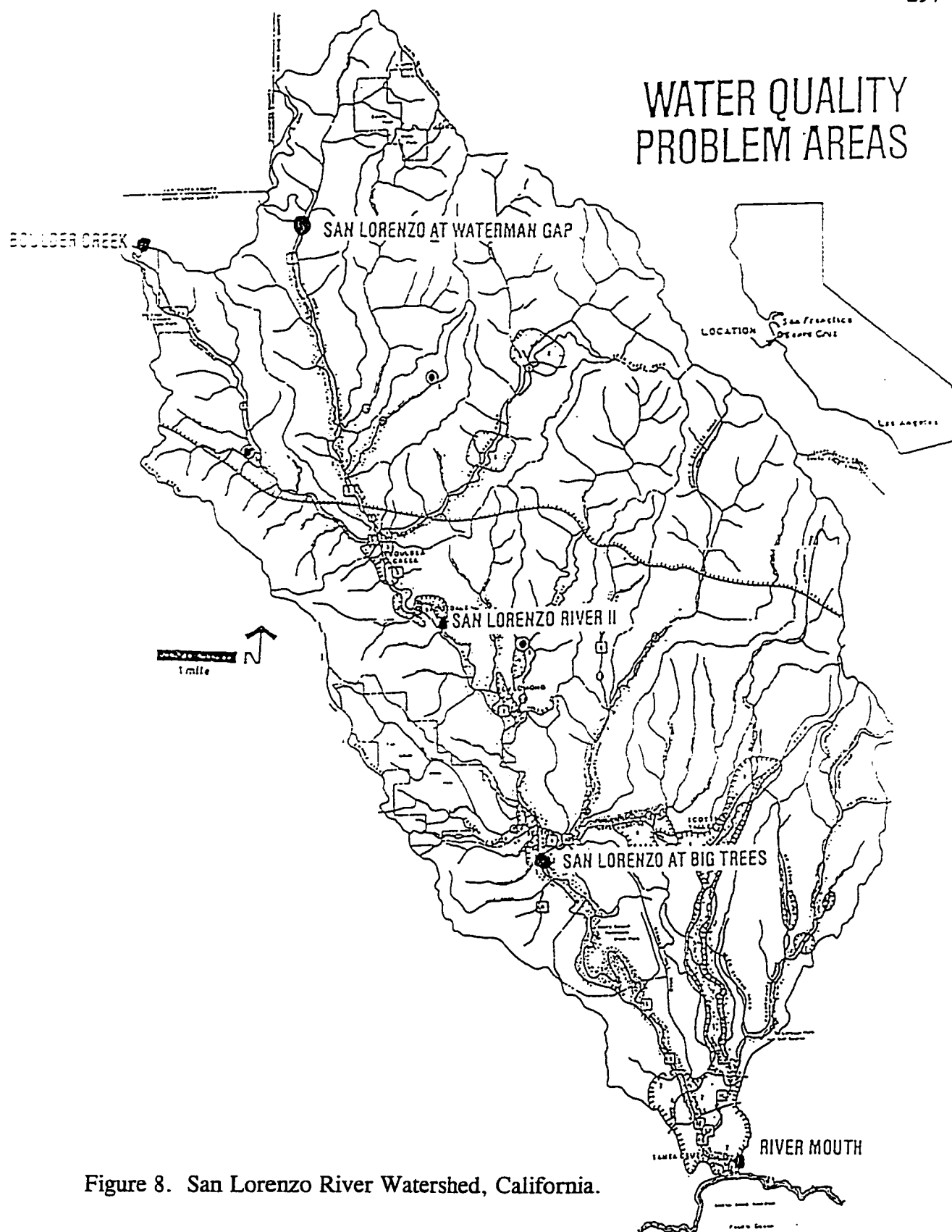


Figure 8. San Lorenzo River Watershed, California.

Watershed Management Units Kent County, Michigan

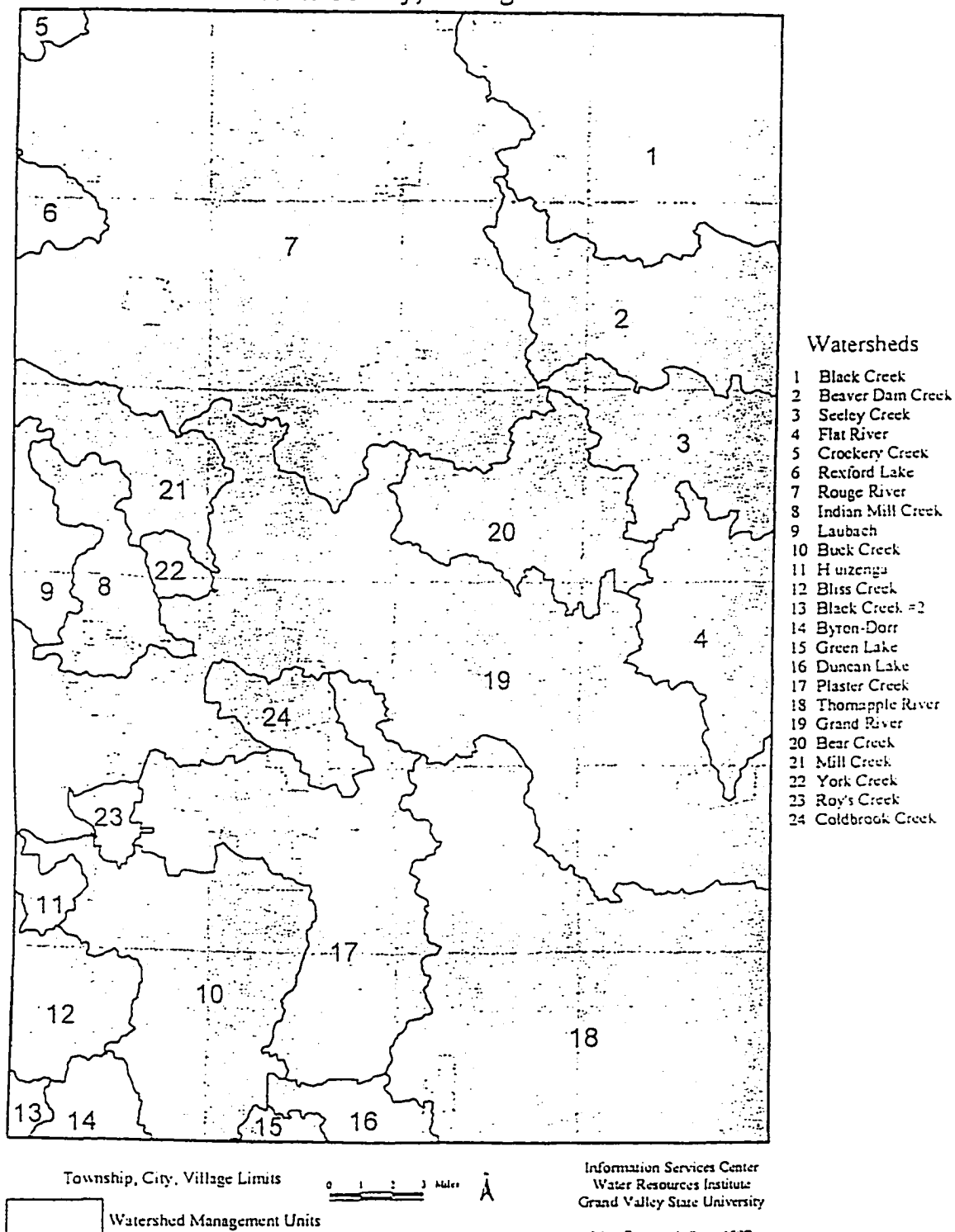


Figure 9. Watershed Management Units in Kent County, Michigan.

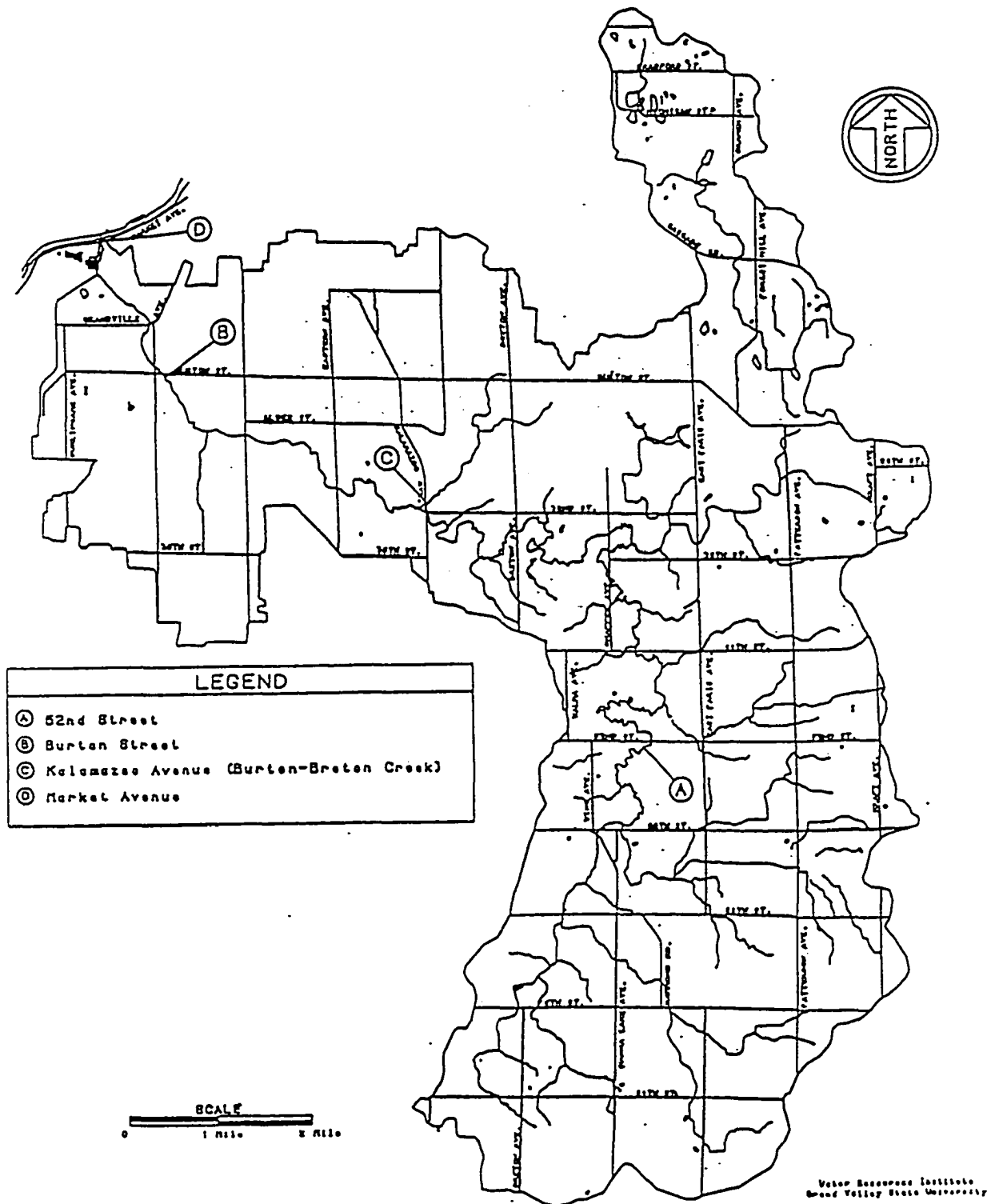


Figure 10. Plaster Creek Sampling Sites.

Appendix B
Plaster Creek Storm Event Study

Results of Storm Event Sampling in Plaster Creek
Grand Valley State University
Water Resources Institute
1991

The effect of storms on fecal coliform counts was studied by the Grand Valley State University Water Resources Institute in 1991. Plaster Creek is a major tributary to the Grand River with its mouth just south of the City of Grand Rapids. It is a 60 square mile watershed that is bounded by Buck Creek to the west and the Thornapple River watershed to the east (Appendix A, Figure 9). The City of Grand Rapids WWTP data from 1985 through 1994 reveal Plaster Creek counts of fecal coliform bacteria exceeded 200 colonies per 100 mL 67% of the time at one station and 79% of the time at another station. The Kent County Health Department reported similar exceedance rates. These monitoring programs reveal seasonal variation of fecal coliform levels in the Creek, but provide little information on short term fluctuations of fecal coliform levels associated with storm events.

The purposes of this study were to:

1. Characterize fecal coliform bacterial contamination in Plaster Creek at selected locations during storm events.
2. Assess the time taken for fecal coliform levels in the Creek to return to pre storm levels.
3. Use existing information from the Kent County Health Department, the City of Grand Rapids WWTP, and available river discharge information to assess the

relationship between river discharge changes and fecal coliform bacterial loading.

Three sampling locations were monitored on the main Plaster Creek channel and one location on Burton-Breton Creek (Appendix A, Figure 10). These sites were selected because they are the monitoring sites used in other Plaster Creek stormwater studies performed by the GVSU Water Resources Institute.

Site A at the 52nd Street Bridge represents an upstream sampling location in the watershed that drains primarily agricultural and undeveloped area. Site B at Burton - McKee Streets is in an urban area of the watershed upstream from Silver Creek Drain. Site C is located on a small Tributary creek to Plaster Creek draining a predominantly residential area. Site D at Market Avenue is upstream of confluence of Plaster Creek with the Grand River and downstream of Silver Creek Drain.

Spring storm events(April/ May) and summer storm events (July/August) were monitored. Exact timing of the events depended on precipitation conditions. Monitoring of the event occurred if the precipitation was expected to exceed 0.1 inches and following an antecedent dry period of at least 72 hours.

Decisions to mobilize resources for a storm sampling event was influenced by the above considerations in combination with information on local weather conditions. Local weather information was obtained from Weather Radio Station KIG63 of the National Weather Service, broadcasting from the Grand Rapids office. The extended forecasts provided a basis for sample trip preparation. The up-to-date weather forecasts influenced the final decision to initiate sampling.

Samples for fecal coliform analyses were collected from the Plaster Creek and Burton-Breton Creek by taking a manual grab sample of stream water using sterilized glass bottles. The methods of collection, sample storage conditions and sample analyses followed *Standard Methods* (American Public Health Association, 1989). The samples were collected by Water Resources Institute staff, stored in containers with ice immediately after collection and delivered to the Kent County Health Department Laboratory for analyses by KCHD staff within 24 hours of collection.

Samples were intended to be collected at hourly intervals at sample sites B (Burton Street) and D (Market Avenue), 30 minute intervals at site C (Kalamazoo Avenue on Burton-Breton Creek) and at approximately two hourly intervals at site A (52nd Street), to reflect the same sampling strategy as the other water quality parameters in GVSU-WRI Plaster Creek studies. The different sampling times were designed to reflect the different hydrograph responses at each location. However it was not logistically possible to keep to this sampling strategy and the sampling times at each location reflected the travel times between the sampling sites and daylight sampling hours. Samples were taken at longer time intervals on the recession limb of the hydrograph and ceased when the next consecutive rain event occurred.

The hydrological data loggers at the sites were queried during the sampling periods to determine the change in discharge regime. Hourly precipitation information for the sampling events was obtained from the National Weather Service Station at Grand Rapids, located at Kent County International Airport. The rain gage

data for this location is representative of the rainfall intensity and duration in the Plaster Creek Watershed (Camp, Dresser, McKee, 1991).

Results

The results from fecal coliform sampling at the four stations during these storm events are presented in relation to the date of the storm event. The events where sampling was performed were: 9-11 April 1991, 5-8 May 1991, and 29 July through 1 August 1991.

Storm Event of 9-11 April 1991

There was a rapid and dramatic change in the discharge regime of Plaster Creek during this storm event which was 1.2 inches, with a maximum intensity of 0.25 inches per hour. A maximum river discharge of 250 cfs was recorded at the upstream 52nd Street location for most of 9 April 1991. The Creek at this location was flowing at maximum channel capacity and may even have been in excess of 250 cfs since this represents the limit of calibration of the data logger. Further downstream at the monitoring location at Burton Street, the discharge of the Creek had increased by over 1,000 cfs from pre storm level to reach a maximum event discharge of 1,500 cfs on 10 April 10 1991. Stream water temperatures at the time of sampling were generally between 9-10°C.

Fecal coliform levels in Plaster Creek during this event were over 10,000 colonies per 100 mL at 52nd Street during peak discharge, and gradually receded to 2,000 colonies per 100 mL forty hours later (Appendix B - Figure 1). At the Burton

Street sampling site fecal coliform concentrations remained lower than the upstream 52nd Street site (Appendix B - Figure 2).

At the peak of the discharge event at 1,000 cfs the fecal coliform levels were between 2,000-4,000 colonies per 100 mL. These lower levels probably represent an interaction between fecal coliform source exhaustion and volume dilution by increased runoff delivery to the Creek. The highest fecal coliform level (7,900 colonies per 100 mL) recorded at Burton Street during this storm event was sampled at 19:40 hours on the 10 April 1991. The decline of fecal coliform bacteria numbers to pre-storm event levels occurred within 24 hours of the storm event.

The first sample from the tributary (Burton-Breton Creek) at the start of the storm event recorded 9,400 colonies of fecal coliform bacteria per 100 mL (Appendix B - Figure 3). This value is surprisingly high for this small tributary drainage area and is comparable to the level recorded at 52nd Street location during the same time period. The fecal coliform levels in the Burton-Breton Creek rapidly declined to 450 colonies per 100 mL forty five hours later. Early storm event samples for the 9 April 1991 event are not available for the Market Avenue site because of contamination problems during sampling (Appendix B - Figure 4). The later samples for Market Avenue closely reflect the quantities and trend of fecal coliform bacteria found at Burton Street during the same time period.

Storm Event of 5-8 May 1991

This storm event was characterized by 0.45 inches of rainfall which occurred

after an antecedent dry period of six days. Although the precipitation total for this event total was less than the previous event sampled, the maximum rainfall intensity was greater with 0.33 inches being recorded in an hour. Water temperatures at the time of sampling were 9-10°C.

At the upstream station (52nd Street), the fecal coliform counts were eight times higher than the pre storm levels reaching a peak value of 3,200 colonies per 100 mL at 17:00 hrs on 5 May 1991 (Appendix B - Figure 5). Counts of over 1000 colonies per 100 mL were still being recorded 24 hours after the peak discharge. The results for the Burton Street monitoring location reveal there is a close correlation between discharge and fecal coliform bacteria concentrations. Nearly 4,000 FC colonies per 100 mL were recorded at maximum discharge, representing a four times increase compared to pre storm levels (Appendix B - Figure 6).

At the Market Avenue sampling site, which is located near the confluence of Plaster Creek with the Grand River, the fecal coliform counts were similar to the Burton Street station with respect to timing and magnitude of fecal coliform levels during the storm event. However, the Market Avenue counts were generally higher (Appendix B - Figure 7). The two sampling sites are within 1.9 river miles of each other. Downstream of the Burton Street monitoring location, a major urban storm drain, the Silver Creek drain, discharges into the Creek. The Silver Creek drain is a known source of fecal coliform bacteria delivery during wet weather conditions as previously discussed.

Sampling of the Burton-Breton Creek, the small tributary of Plaster Creek, again revealed some of the highest fecal coliform levels recorded throughout the duration of the sampling period. The highest value was over 10,000 colonies per 100 mL (Appendix B - Figure 8).

Storm Event 29 July 1991 - 1 August 1991

In this summer rain event, water temperatures of the Creek were between 16 - 17°C. Fecal coliform levels of samples from this storm event were an order of magnitude higher than the previous two monitored events (Appendix B - Figures 9, 10, 11, 12). Two counts of 240,000 colonies per 100 mL were recorded at 52nd Street during this sampling period indicating considerable contamination of the Creek.

Summary

In terms of overall delivery of fecal coliform and impact on water quality of the Creek, the results from the limited sampling of storm events indicate that fecal coliform from the rural areas in the watershed may be producing a pronounced impact on fecal coliform levels in the Creek. The 9 - 11 April 1991 and the 29-July - 1 August 1991 storm events both showed that the highest fecal coliform loadings monitored during the storm events are not correlated with peak discharge at the Burton Street location. They occurred on the recession limb of the storm hydrographs, some time after peak discharge. Stream tracer study information was not available to provide accurate information on possible travel times of contaminants.

The decline in fecal coliform bacteria following rain events is gradual, but given the rainfall pattern of the watershed, the numbers of bacteria are unlikely to return to levels below the state standard before a new delivery reaches the stream channel and perpetuates the chronic level of contamination. The rainfall record for the 1990-1991 monitoring period illustrates the frequent storm events. The average number of storm events in the Plaster Creek Watershed is 127 per year (Camp, Dresser, McKee, 1991).

It is clear from the storm event sampling that fecal coliform levels in Plaster Creek far exceed water quality standards. Counts continue to be high for several days after significant rain events. This study points out the public health concern associated with rain events. Especially in streams that normally exceed water quality standards, students should avoid sampling after rain events unless there is strict adherence to protective measures against direct stream water contact.

Figure 1. **FECAL COLIFORM IN PLASTER CREEK
FLOOD EVENT APRIL 9 - 11, 1991**

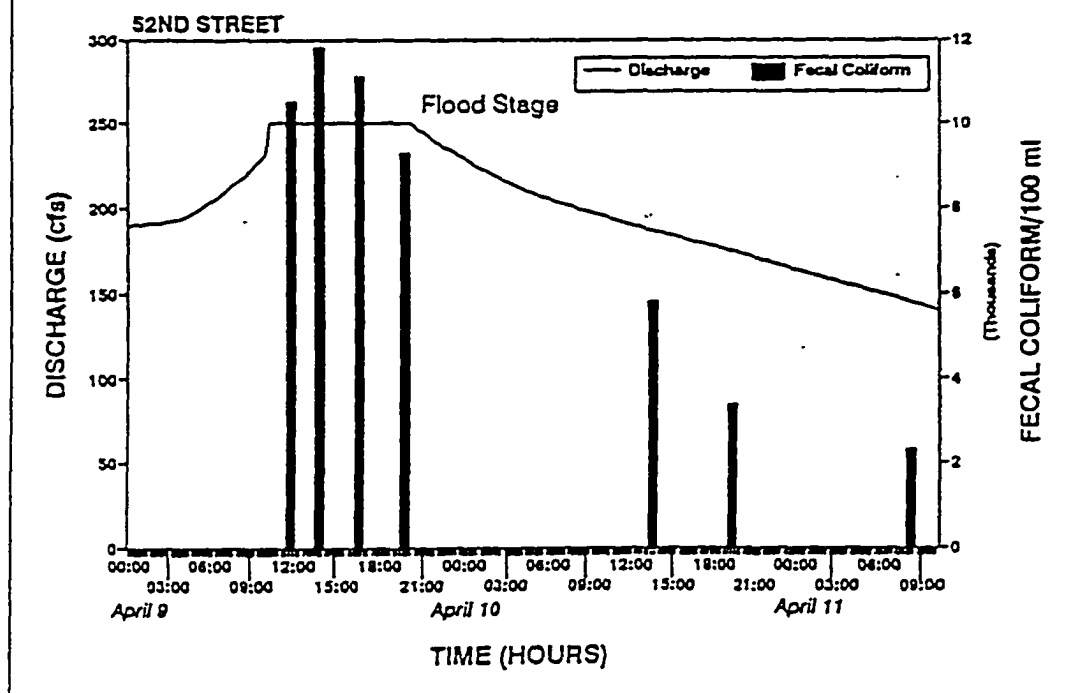


Figure 2. **FECAL COLIFORM IN PLASTER CREEK
WATER SAMPLES, APRIL 9-11, 1991**

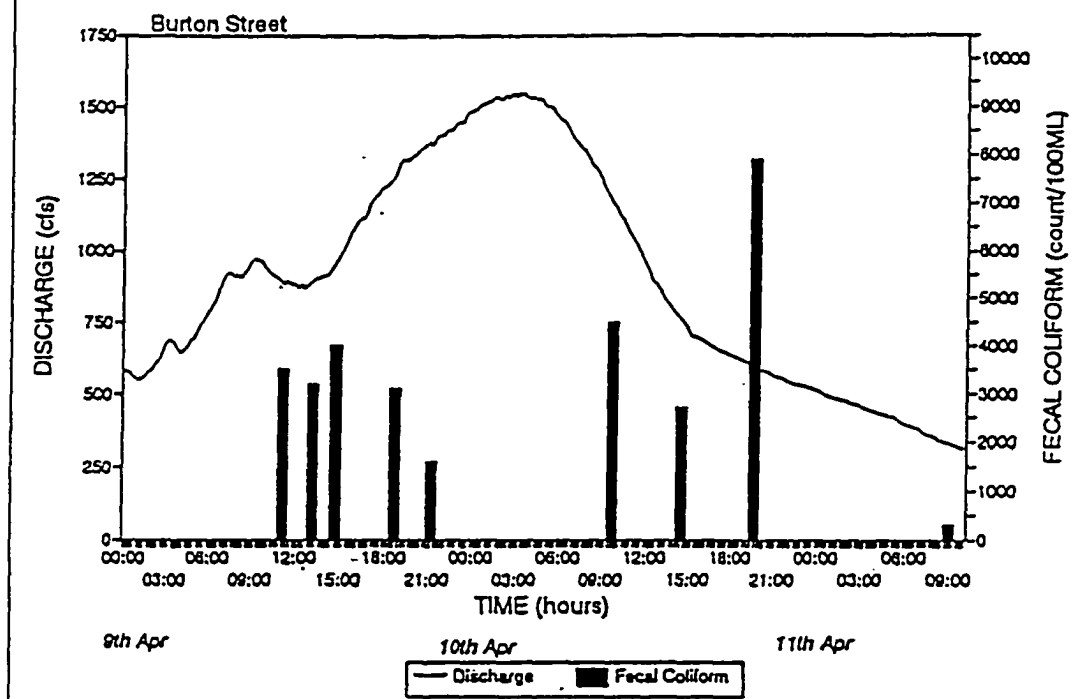


Figure 3. **FECAL COLIFORM IN BURTON-BURTON CREEK
WATER SAMPLES, APRIL 9-11, 1991**

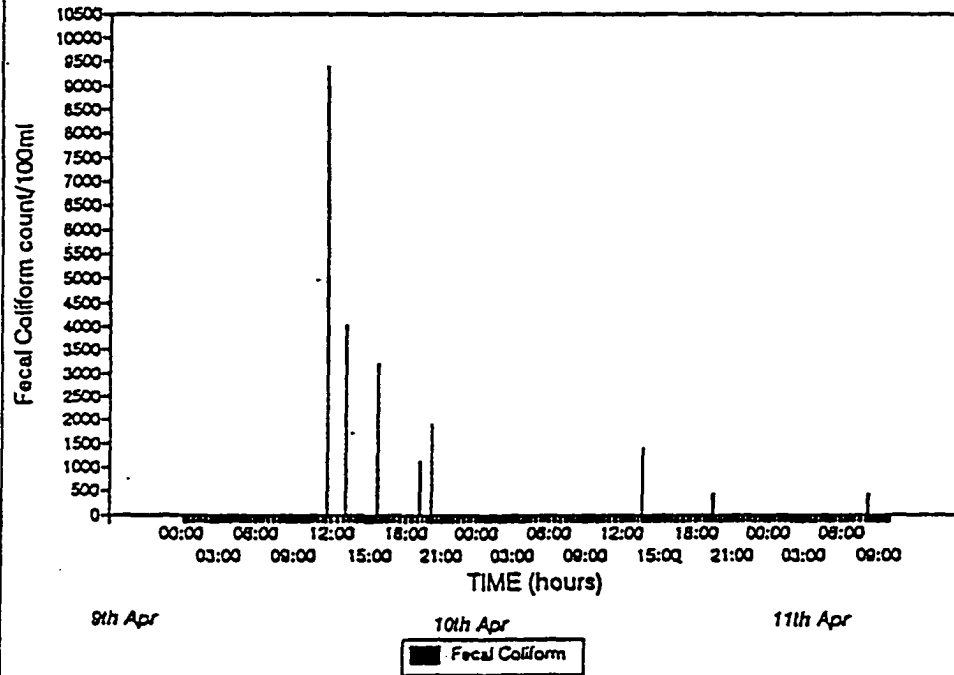


Figure 4. **FECAL COLIFORM IN PLASTER CREEK
WATER SAMPLES, APRIL 9-11, 1991**

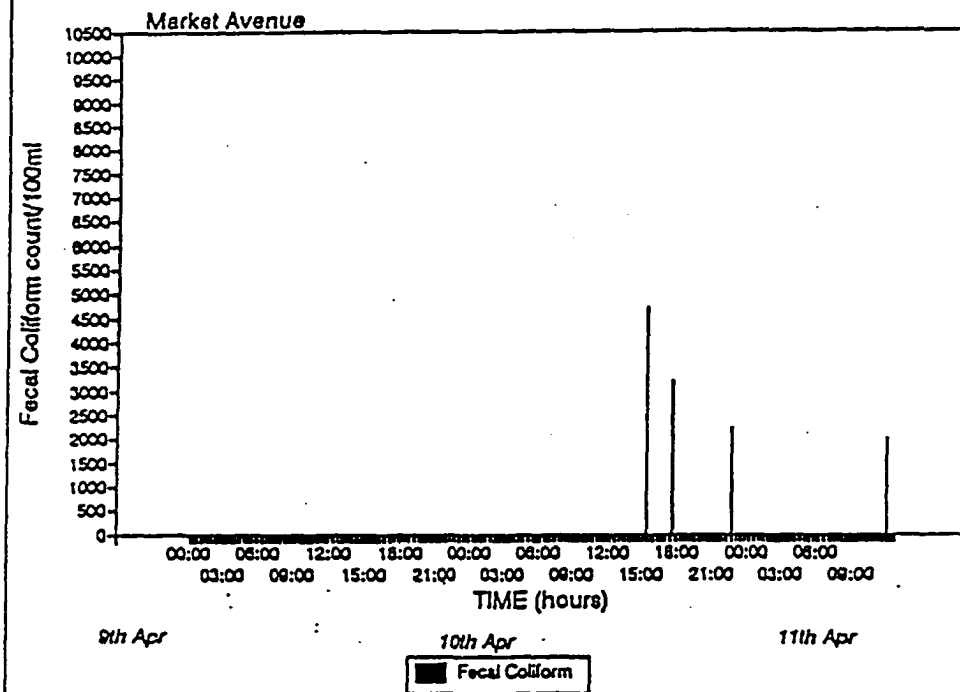


Figure 5. FECAL COLIFORM IN PLASTER CREEK
WATER SAMPLES, MAY 5-8, 1991

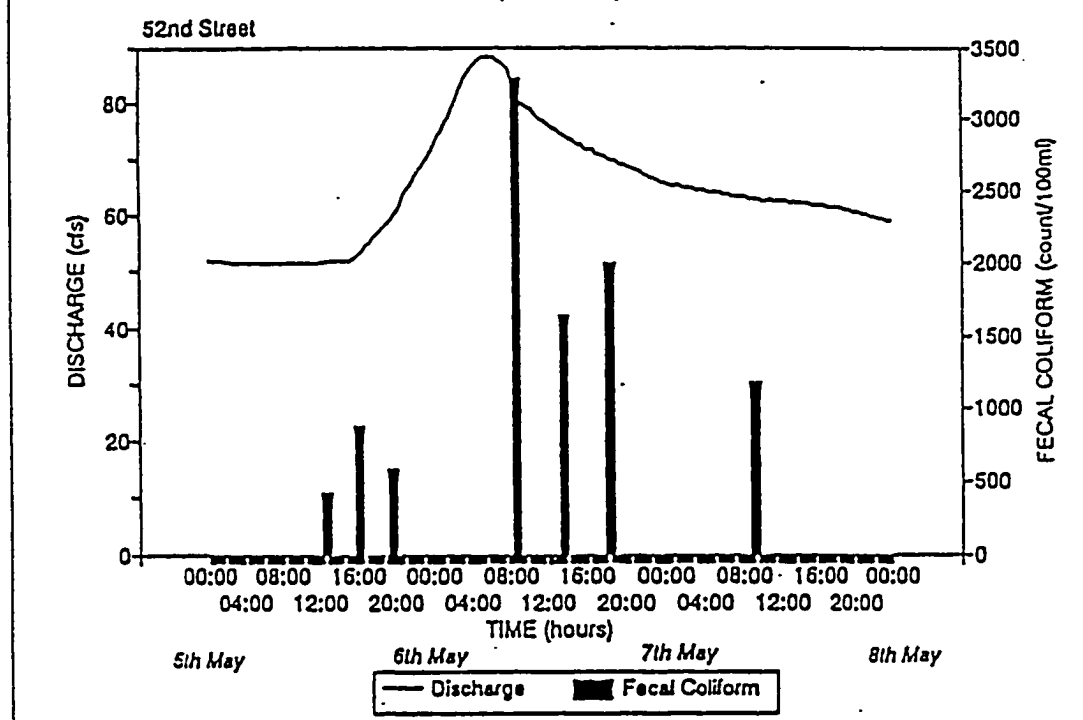
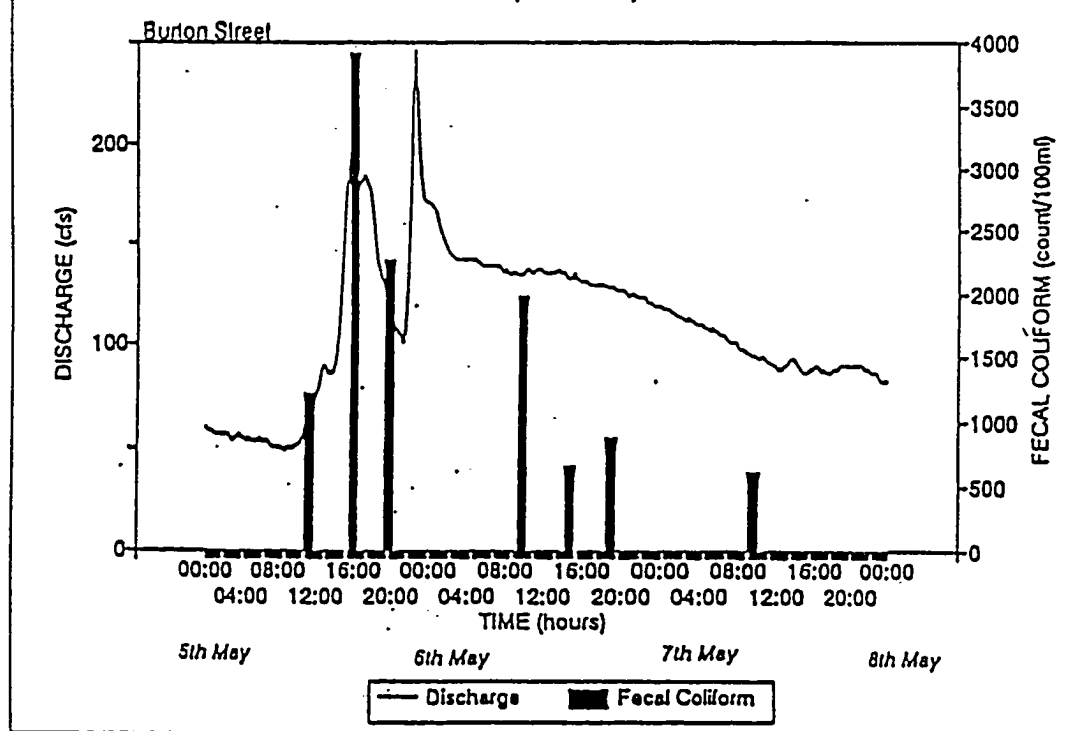


Figure 6. FECAL COLIFORM IN PLASTER CREEK
WATER SAMPLES, MAY 5-8, 1991



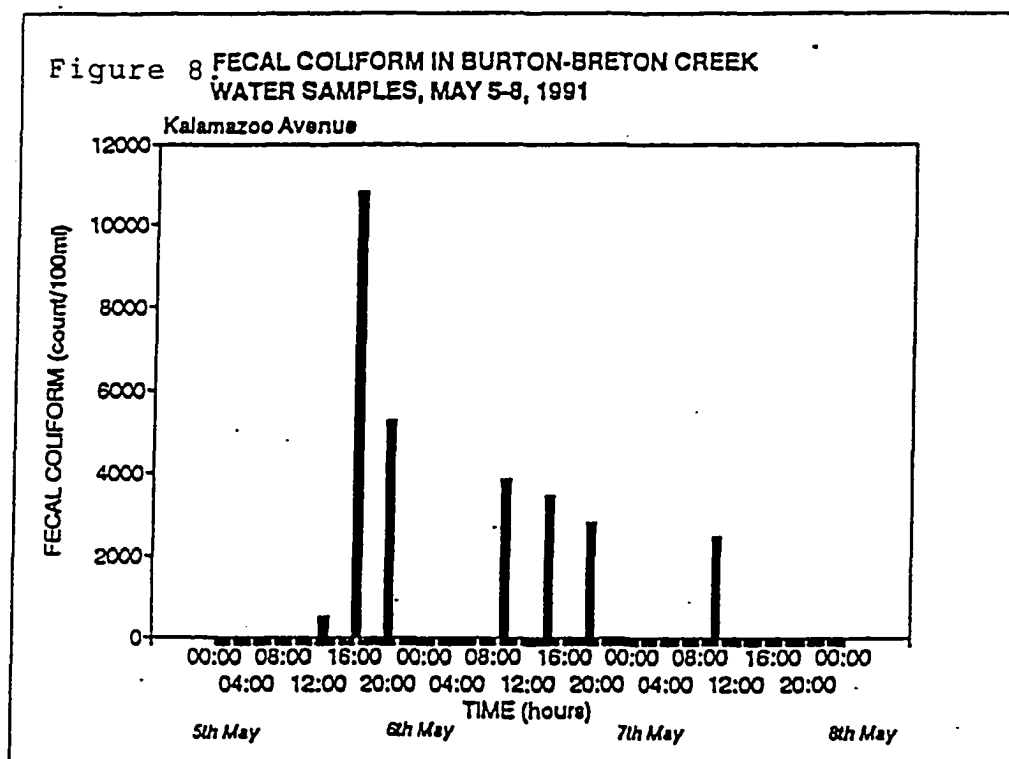
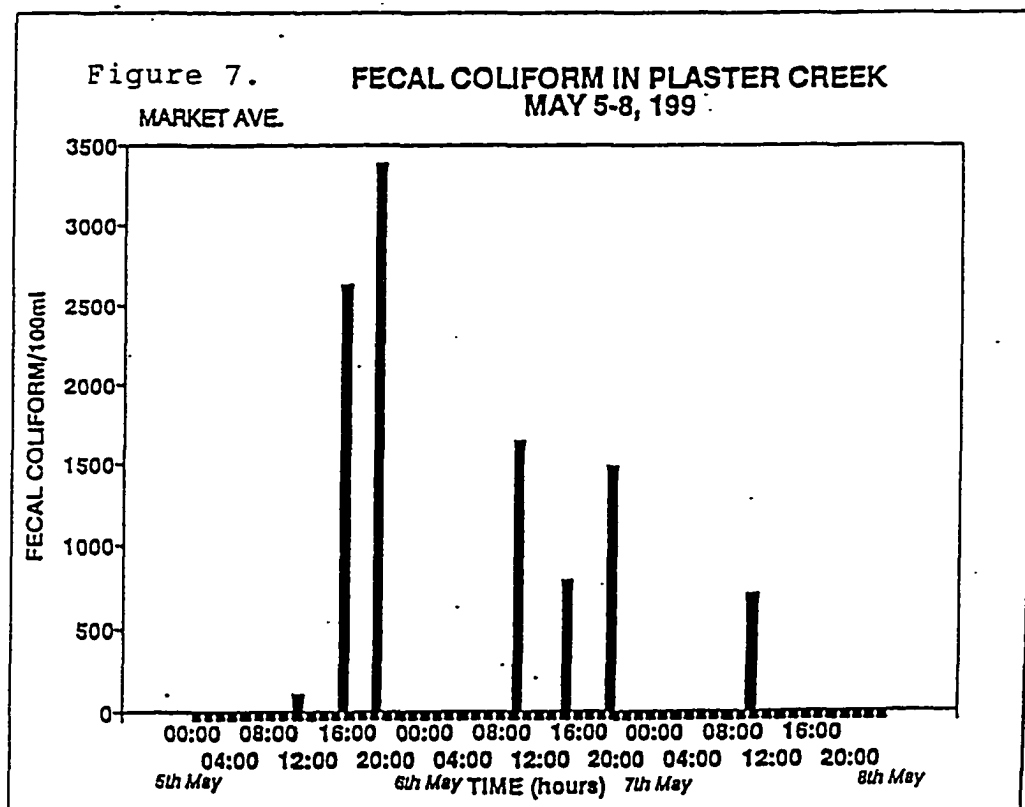


Figure 9. FECAL COLIFORM IN PLASTER CREEK
WATER SAMPLES, JULY 29-AUGUST 1, 1991

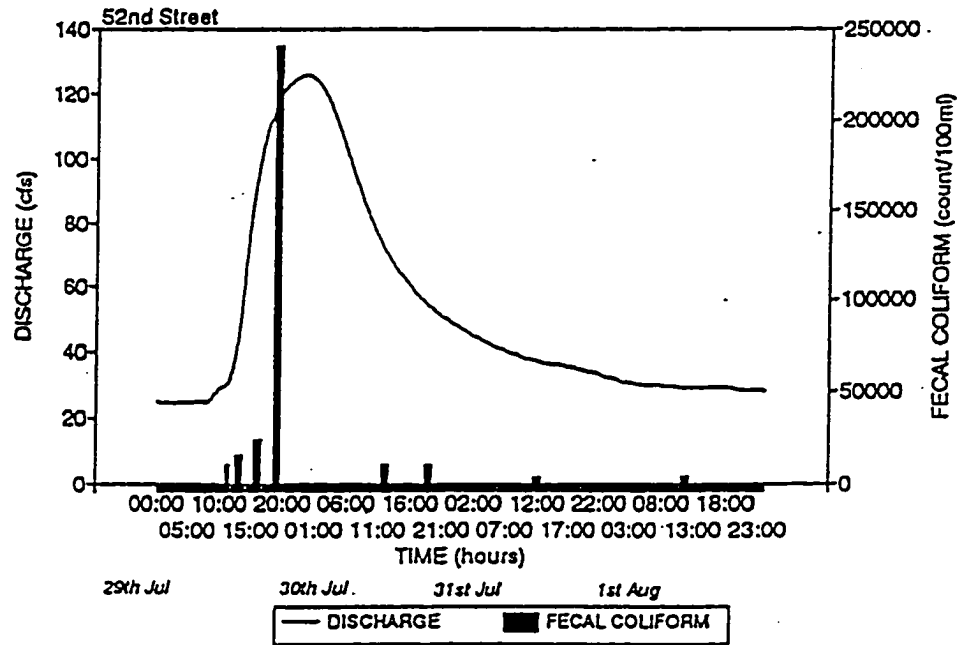


Figure 10. FECAL COLIFORM IN PLASTER CREEK
WATER SAMPLES, JULY 29-AUGUST 1, 1991

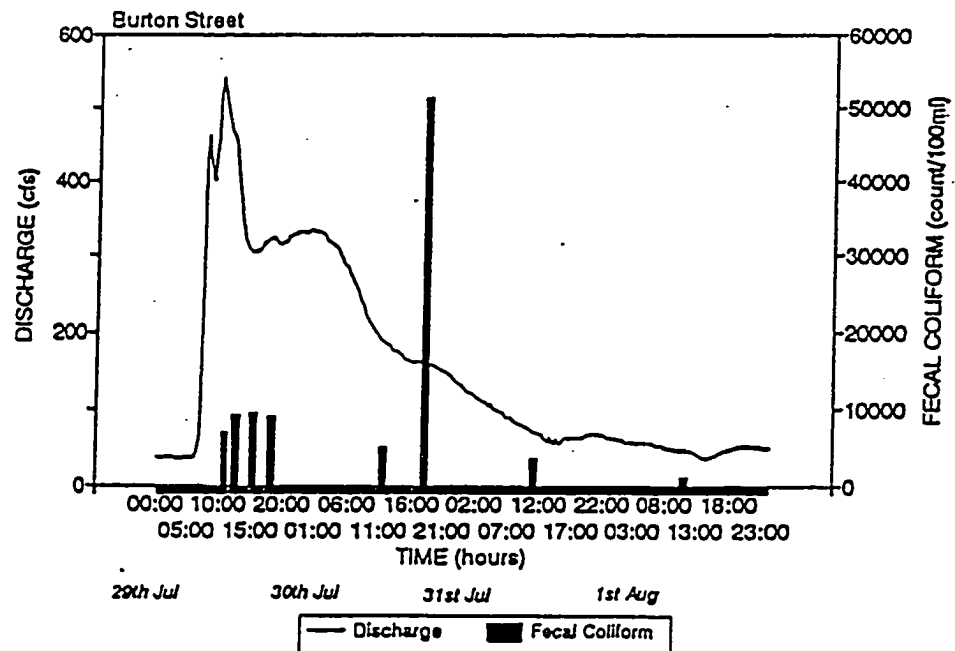


Figure 11. **FECAL COLIFORM IN BURTON-BRETON CREEK**
WATER SAMPLES, JULY 29-AUGUST 1, 1991

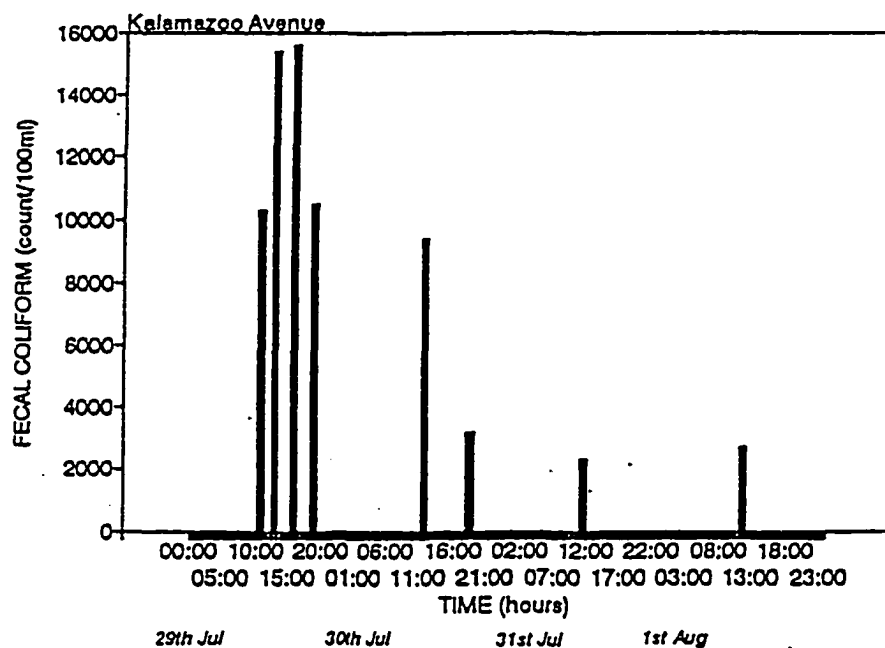
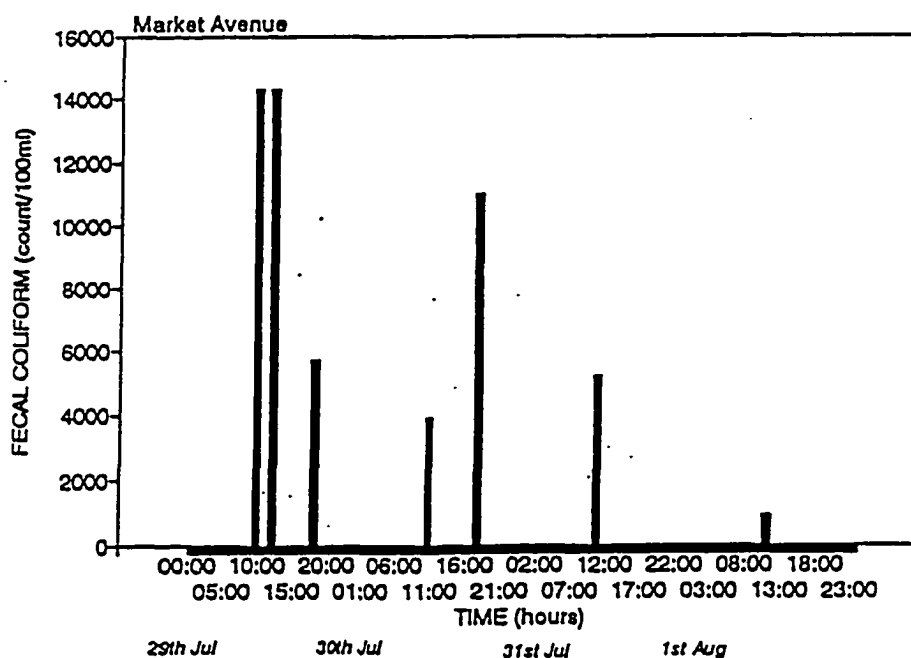


Figure 12. **FECAL COLIFORM IN PLASTER CREEK**
WATER SAMPLES, JULY 29-AUGUST 1, 1991



Appendix C
Meta-Analysis of Bacterial Contamination
in Michigan

Meta-Analysis of Bacterial Contamination In Michigan

Monitoring data were analyzed for five counties in the Grand River Watershed (Ottawa, Barry, Eaton, Montcalm, and Ingham counties), Southwest Michigan, Grand Traverse Bay, and Southeast Michigan. The following are representative studies for these areas for comparison with data from Kent County, Michigan.

Ottawa County

Unlike Kent County, Ottawa County (the adjacent county to the west) has not undertaken extensive microbiological monitoring. In the past, citizens in downstream Ottawa County have tended to blame fecal coliform contamination on the Grand Rapids Wastewater Treatment Plant and its combined sewer overflows. Activist groups in Ottawa County such as People Outraged by the Overflow of Poop (P.O.O.P) and North Ottawa Families Living Under Sewage Hazards (N.O.F.L.U.S.H.) sprang up in the late 1980s through the early 90s.

As a result of testing by a local newspaper in 1995, it was discovered that Ottawa County *E. coli* levels exceed state standards in many areas which "shocked" the activist groups (Sinkevics, 1995, September 3). Testing at 17 sites in late July and mid-August 1995 revealed *E. coli* levels above 130 colonies per 130 mL at eight sites and above 600 in four creeks. Beaver Creek had an average concentration of 8,333 *E. coli* bacteria in three samples taken over 30 days. The

MDEQ has found numerous failed septic systems along this stream. Every *E. coli* count was above 130 colonies per 100 mL in a spring fed stream in the middle of an agricultural region. According to Fred Eyer, district Michigan Department of Environmental Quality supervisor, "The small streams in Ottawa County and Lake Macatawa are the biggest water quality problems facing that county. It's just that they're not well known" (Sinkevics, 1995, September 3, p. A 21).

In an editorial in 1995, The *Grand Rapids Press* urged Ottawa County to test their waters ("Test the waters", 1995, December 15). The paper cited additional studies by students at a local high school that also found bacterial contamination in a local creek. As a result of the sampling done by the newspaper, Ottawa County Health Department began a sampling program for *E. coli* in 1996 (King, 1996, March 8). Tests began in May and continued through mid-August and were limited to ten biweekly samples at sixteen sites. The overall *E. coli* counts ranged from 0 to 2,800 colonies per 100 mL. The 2,800 count was in Lake Michigan at Grand Haven State Park which is a serious issue given the thousands of people who swim at that park in the summer. The *E. coli* count at three Lake Michigan sites were greater than the *E. coli* standard of 130 colonies per 100 mL 10 to 30% of time. Lake Macatawa, a drowned river mouth in Holland, exceeded the standard *E. coli* count 20% of the time. Both Spring Lake and Pottawattomie Bayou, embayments draining into the Grand River near Grand Haven, had zero *E. coli* counts above 130 colonies per 100 mL. This might be explained by the fact

that the Spring Lake shoreline is residential (not agricultural), and the Bayou is a park surrounded by woods. The lower Grand River EC exceedance rates varied from 0 to 20%. The problem areas, as in other studies, were the tributaries: Pine Creek (100% exceedance), Bass River (100%), and Sand Creek (80%).

Studies by the Grand Valley State University Water Resources Institute provide fecal coliform monitoring data on Grand River rural tributaries in Ottawa County: Sand Creek, Deer Creek, Crockery Creek, and Bass Creek (Cooper and Rediske, 1996). The GR WWTP river station at Eastmanville was also sampled along with a Grand River site approximately one half mile east of the river's confluence with Lake Michigan. In the duration of the study from March 1994 through February 1995, bimonthly fecal coliform bacterial counts for Sand Creek ranged from 150 to 3,400 colonies per 100 mL ($n = 21$, exceedance = 81%); Deer Creek, 98 to 6,000 colonies per 100 mL ($n = 18$, exceedance = 83%); Crockery Creek, 0 to 6,000 colonies per 100 mL ($n = 19$, exceedance = 63%); and Bass Creek, 21 to 4,270 colonies per 100 mL ($n = 20$, exceedance = 85%). Grand River samples revealed fecal coliform counts ranging from 8 to 6,000 colonies per 100 mL at Eastmanville ($n = 21$; exceedance = 62%) and from 0 to 4,095 near the river's mouth ($n = 21$, exceedance = 33%).

The magnitude of the contribution of Ottawa County tributaries to the fecal coliform load of the Grand River overshadows all but the larger volume combined sewer overflows from the City of Grand Rapids which are likely to quickly move

downstream in a slug to Lake Michigan. There is evidence that the river rapidly dilutes and dissipates the raw sewage from combined sewer overflows which has puzzled the Ottawa County health officials (Sinkevics, 1988, July 21). Elevated counts after a CSO event at Eastmanville fell to well within water quality standards near the river mouth.

Even with the high tributary loads, the river seems to have the ability to purify itself since the river mouth exhibits relatively low counts. What is somewhat surprising is that four of the river mouth samples were zero colonies per 100 mL and 28% of the samples were below 10 colonies per 100 mL. The presence of an extensive bayou system in the lower Grand River might act as a buffer for nonpoint source pollution and contribute to settling of bacteria resulting in lower counts.

Barry and Eaton Counties

Barry County is directly southeast of Kent County, and Eaton County is just east of Barry County. Nineteen sites along three rivers in Barry and Eaton Counties were sampled weekly in the summer of 1992 (Wagendorp, 1992). Both fecal coliform (FC) and fecal streptococcus (FS) bacteria were monitored. Geometric means of all Barry County Thornapple River sites were below 200 colonies per 100 mL and most counts were below the 200 limit. Barry County does not have much agriculture compared to Eaton County. Wagendorp attributes some of the low counts to prevailing dry conditions where most of the contribution to the

river is from groundwater from up-slope shallow aquifers with little or no surface water runoff. Mill ponds, undeveloped river fronts, and absence of tributaries are associated with these low counts.

The Eaton County sites showed mixed results, with the Grand River having lower counts than the Thornapple and the Battle Creek Rivers. Monitoring of the Battle Creek River was at the headwaters in an agricultural area. Soils in Eaton County tend to be heavy and have drains feeding into the tributaries. Two upper reach Thornapple River sites had geometric means above 500 colonies per 100 mL. The river takes on the characteristic of a county agricultural drain upstream.

Counts for almost all of the sites were elevated during the 11 September 1992 sampling event and to some extent on 31 August 31 1992. There was no rainfall reported in nearby Grand Rapids on 11 September 1992 but there were 0.15 inches on 8 September, 1.61 inches on 9 September, and 0.13 inches on 10 September. Rain events prior to 31 August were 1.76 inches on 27 August, 0.41 inches on 28 August, and 0.19 inches on 29 August.

Exceedance of 200 colonies per 100 mL occurred in 56.2% of the 64 Thornapple River samples, 41.3% of the 46 Grand River samples, and 81.2% of the 22 Battle Creek River samples. The highest counts were 8,900 colonies per 100 mL for fecal coliform and 1,460 colonies per 100 mL for fecal streptococcus in the Grand River on 9 July which correlate with an accidental release from the upstream Lansing Waste Water Treatment Plant. Another high fecal coliform count of over

3,000 on Carrier Creek reflected a release from the Delta Waste Water Treatment Plant. At all sites, geometric means of fecal coliform were greater than those of fecal streptococcus. FC/FS ratios greater than 4.0 are said by some researchers to indicate human fecal contamination when close to the source of contamination.

Montcalm County

Bacterial contamination of water courses was reported in a 1975 Michigan Department of Natural Resources study on Tamarack Lake and Tamarack Creek in Montcalm County (MDNR, 1977). Located northeast of Kent County, Montcalm County is predominantly agricultural. The county produces one fifth of Michigan's potato crop and has one of the largest gas storage fields in the country. The county has over 200 lakes and 21,000 acres of public land.

Tamarack Lake is a eutrophic 310 acre lake that discharges to the west to Tamarack Creek which is a tributary of the Muskegon River. Its water source is primarily groundwater but there is a diversion from upper Tamarack Creek from the east that contributes water to the lake during high flow. During the summer of 1975, 68 stations along the lake and creek were sampled. There were four main sampling events: a preliminary investigation (24 July 1975), dry weather survey (12 August 1975), survey following a storm (3 September 1975), and a final survey (23 September 1975).

Geometric means of lake samples following the September storm ranged from 13,000 to 23,000 fecal coliform colonies per 100 mL. Pond overflow to the lake on that date had readings of 108,000 colonies per 100 mL. Fecal coliform levels were consistently above 11,000 colonies per 100 mL in all areas of the lake with 100% exceedance of water quality standards. However, fecal streptococcus levels were generally less than 200 colonies per 100 mL with the exception of a sample near the pond.

Dry weather samples of the lake revealed fecal coliform levels generally less than 10 colonies per 100 mL. Percent exceedance of the standard was 11% with one sample near the food processing plant pond at 21,000 colonies per 100 mL. Fecal streptococcus levels were also less than 10 colonies per 100 mL with the exception of the pond sample (1,330 colonies per 100 mL). The study revealed three sources of fecal coliform bacteria that impact Tamarack Lake: a pond receiving swamp drainage and runoff from a food processing (pickle) plant, lakeside residences, and a high flow diversion of Tamarack Creek (MDNR. 1977).

Nutrient concentrations and fecal coliform levels were high in the upstream areas of the creek. Cattle have access to the headwaters of the creek. In the dry weather sampling event, the nine stations on the creek exceeded the fecal coliform standard of 200 colonies per 100 mL. Geometric means of the five samples from each station ranged from 900 to 3100 colonies per 100 mL. Counts in the 3 September 1975 storm event were even higher than the dry weather samples with a

range of 12,000 to 34,000 colonies per 100 mL yet fecal streptococcus levels were less than 10 colonies per 100 mL. In the late September sample, the creek station fecal coliform geometric means ranged from 40 to 200 colonies per 100 mL. The lake outlet contributed levels of fecal coliform exceeding standards during the dry and storm sampling episodes. The lake storm samples at the outlet revealed 17,000 colonies per 100 mL for fecal coliform and 3,300 for fecal streptococcus.

Ingham County

Ingham County is in the middle of southern Michigan west of Kent County. A major University and the State Capitol are in this county. The purpose of the Ingham County Surface Water Sampling Project, which began in 1989, was to establish baseline data on total coliforms, fecal coliform, and fecal streptococci (File in Ingham County Environmental Health Department). Sixteen sites were sampled monthly in the Grand River, Red Cedar River, and Sycamore Creek. Average fecal coliform counts for the Grand River sites in 1991 were 389 colonies per 100 mL; Red Cedar, CFU/100 mL; and Sycamore Creek, 373 CFU/100 mL. In the Grand River, monthly counts in 1989-91 ranged from less than 10 to 13,600 colonies per 100 mL; the Red Cedar range was 10 to 16,800 CFU/100 mL; and the Sycamore Creek range was 20 to 8,720 CFU/100 mL. One Grand River site had an average of 961 fecal coliform colonies per 100 mL and exceeded standards 97% of time with the "best" station exceeding 44% of the time. Average fecal

coliform counts at 15 out of the 16 stations were above 200 colonies per 100 mL. Sampling by the City of East Lansing at three sites on the Red Cedar River in 1990-92 also reflected high counts with ranges from 40 to 40,800 FC colonies per 100 mL, means of 282 to 1,306 FC colonies per 100 mL, and exceedances of 33% to 100%.

Southwest Michigan

Between 1975 and 1976, background water quality was assessed at nineteen stations in the Kalamazoo, Macatawa, Paw Paw, and Black River Basins which are located south and southwest of Kent County (MDNR, 1976b). Portions of Ottawa, Allegan and Kalamazoo counties were in the study area.

In summer samples, fecal coliform counts exceeded 200 colonies per 100 mL 78.9% of the time; fall samples, 51.3%; early winter, 31.6%; and mid-winter, 31.5%. Of the nineteen stations, 78.9% had mean fecal coliform counts greater than 200 colonies per 100 mL. All but one station had counts below 200 colonies per 100 mL at some time during the sampling period with seven stations reporting counts of 10 or less.

Maximum counts ranged from 190 to 5000 colonies per 100 mL. Augusta Creek in Kalamazoo County had the lowest counts and was consistently below the standard (Mean = 80 colonies per 100 mL; Range = 0 to 190 colonies per 100 mL), and the Little Rabbit River in Allegan County had the highest mean (1200

colonies per 100 mL) with all samples above the standards (Range = 210 to 5,000 colonies per 100 mL).

Grand Traverse Bay

Often referred to as an "area of quality" in Michigan, the Grand Traverse Bay is located in the northwest part of Michigan's lower peninsula adjacent to Traverse City. The Bay is a cold water portion of Lake Michigan into which the Elk and Boardman Rivers, flow along with 14 other tributaries. There are no significant industrial discharges to the Bay, but there have been seasonal discharges from the fruit processing industry which are now sent to sewers or spray irrigation fields. In 1976, the municipal treatment systems of Traverse City, Elk Rapids, and Sutton's Bay discharged directly into the Bay. However, it has been estimated that 95% of the flow into the bay is groundwater. Total surface area of the Bay is 681.6 km² and its mean depth is 55 meters (Auer et al., 1976). Known primarily as a tourist, recreation, and agricultural area, the Grand Traverse Bay is currently experiencing rapid growth and development.

Michigan Sea Grant selected the Grand Traverse Bay in Lake Michigan for development of a model for a portion of a Great Lakes ecosystem. A separate chapter in the Sea Grant report (Auer et al., 1976) is devoted to coliform bacteria. Historical high summer levels which exceeded 45,000 colonies per 100 mL in 1964 were associated with discharges of organic wastes from the fruit processing

industry. The Boardman River and city storm sewers had relatively small inputs of bacteria in comparison to the load from the fruit industry when they were discharging to the bay. Direct discharge was eliminated by 1972.

Immediately after rainfall events, high fecal coliform counts have been reported near storm sewer outfalls and beaches (Gannon and Meier, 1974). Highest counts happen during the first flush and water quality improves with dilution and depletion of sources of contaminants. Gannon and Meier (1974) concluded that storm sewer outflow is now the most important source of fecal coliform during wet periods. Fecal coliform counts in the Boardman River increased downstream with total coliform counts increasing significantly below the sewage treatment plant. Although the fecal coliform counts in the Bay generally meet state standards, there are numerous short-term exceedances during wet periods that are associated with sewer overflows. In 1972, four of eight monitoring stations on the Boardman River had geometric means ranging from 1,346 to 3,091 colonies per 100 mL while the other stations were below 200 colonies per 100 mL. Shoreline fecal coliform counts had a geometric mean of 403 colonies per 100 mL at one site with six other stations below 100 colonies per 100 mL.

A very important statement from the Sea Grant report that challenges the efficacy of coliforms as a sanitary indicator is as follows:

The coliform bacteria were once thought to be entirely of fecal origin, and for many years were used to define safe limits for drinking and recreational use of water. However, it has recently been shown that certain soil bacteria such as *Aerobacter* are included in this group, and therefore the presence of

fecal contamination is not always directly linked to coliform group.
(Auer et al., 1976, p 113)

Bacterial contamination in the Grand Traverse Bay does not appear to persist beyond the immediate source. In situ bottle experiments by Gannon and Meir (1974) suggest that survival of fecal coliform bacteria varies with storage temperature, dilution factors, and illumination.

Canale et al. (1973) performed a modeling study on the Grand Traverse Bay in upper Michigan to predict total coliform densities. Long-term monitoring indicated an August total coliform peak which mirrored the large seasonal human population as well as the fruit canning season. Keys to modeling are the availability of long-term data as well as a rational understanding of the mechanisms for the transport and die-off of coliform bacteria. Material balance models were explored with attention to advective and dispersive transport processes, growth/death kinetics, and location and concentration of all coliform sources. Sources in the Bay include discharge from a river, direct industrial waste inputs, stormwater discharges, waterfowl, private and commercial watercraft, groundwater seepage, and swimmers and bathers.

Southeast Michigan

Smith and Twedt (1971) conducted studies on the Saline and Huron Rivers in southeast Michigan which looked at the natural relationships of indicator organisms and *Salmonella*. Sampling was performed at ten representative sites in the Saline

River in Washtenaw and Monroe Counties and twenty sites along the Huron River.

These rivers pass through mixed rural, suburban, urban, and industrial areas.

In the Saline River sampling, fecal coliform bacteria averaged less than 16% of the coliform bacteria, and the fecal coliform to fecal streptococcus ratios (generally less than 4.0) suggested animal origin for the bacteria. Average FC counts for samples taken from June through October 1968 ranged from 920 to 3,500 colonies per 100 mL. In the upper Huron River, FC comprised less than 10% of the coliforms. Most Huron River samples had FC:FS ratios between 4.0 and 0.7 with average counts ranging from 46 to 500 colonies per 100 mL. Although salmonellae were isolated from some samples, no salmonellae were found when the FC count was less than 200 colonies per 100 mL in the Saline River or 100 colonies per 100 mL in the Huron River. This is consistent with Geldreich's suggestion that 200 FC colonies per 100 mL is a limiting concentration that may be a useful water quality value (Geldreich, 1970).

A correlation matrix of factors studied in the lower Huron River revealed the most positive correlations as being: (a) TC to FC to flow, (b) FC to FS to rainfall to flow, (c) FS to flow rate, (d) rainfall to flow rate. The Saline River data provided positive correlations for TC to FC, FC to flow rate, and rainfall to flow rate. Negative correlations for the Huron River were found with: (a) FC to pH, (b) FS to pH, (c) DO to temperature, and (d) rainfall to temperature.

Other studies have looked at correlations between fecal coliform densities and temperature, flow, algal densities, DO, BOD, TOC, ammonia, and nitrate with varying degrees of success (Geldreich, 1976). Flow, suspended solids, and turbidity are often mentioned to be highly correlated with fecal coliform counts (Geldreich, 1980).

A continuation of the Smith and Twedt research with the addition of survival studies was conducted by Smith, Twedt, and Flanigan (1973) in 1969-70. *Salmonella* species were isolated when FC counts were as low as 52 colonies per 100 mL in the Saline River and 4 colonies per 100 mL in the Huron River. The differences in the two rivers relates to different pollution patterns in the two basins which was also reflected in opposite seasonal patterns of FC/FS ratios. Another observation was that the probability for *Salmonella* isolation decreased as the fecal coliform concentration increased which may be a result of competitive microflora or chemical wastes. Smith et al. (1973, p. 1743) caution that "the level of 200 FC colonies per 100 mL may not be applied without qualification to all waters."

A study in the Detroit-Ann Arbor area (Burm and Vaughan, 1966; Benzie and Courchaine, 1966) revealed total coliform densities of combined sewer overflows sometimes approach those of raw wastewater whereas coliforms in runoff from the storm sewers of separate sewer systems is on the order of one tenth that of combined sewers. Bacterial quality of discharges did not seem to be related to storm intensity, duration, or total rainfall. Fecal coliforms approached 100% of the coliforms in some

of the combined sewer overflow samples, but more often, the densities were 20% of the total coliforms. Maximum counts were 61 million organisms per 100 mL for combined sewers and 4.3 million per 100 mL for separate storm sewers. Combined sewer discharge contained approximately 40 times more fecal coliforms than separate storm sewer discharges (Burm and Vaughan, 1966). The ratio of fecal streptococcus to fecal coliform in the separate system was 0.6 to 1.0 and in the combined system, 4.7 to 1.0 (Benzie and Courchaine, 1966). This suggests a lower impact of human sources in the separate sewer system.

Additional conclusions for the Detroit studies are that the effects of combined sewer overflows in terms of coliform bacterial density are still evident several days after overflows have ceased (Burm, 1967). This may be due to re-suspension from the river bottom and banks. The duration of increased coliform counts was proportional to the intensity of the storm; from three days for a moderate storm to six days for a severe storm. Effects of the storms were negligible in receiving waters above the outfalls, but fecal coliform densities increased between ten and one hundred fold below outfalls. Fecal coliform counts approached 100,000 organisms per 100 mL and they made up about 10% of the total coliform densities (Burm, 1967).

In 1994, the Rouge River Project team sampled 46 areas in the Rouge River system in the Detroit area (Krinn, 1994). The current sewer system in Detroit and all but the newest communities in the watershed collect residential, industrial, municipal, and stormwater which is sent to the Detroit Wastewater Treatment

Plant. This combined sewer system serves three million people in four counties. When stormwater from significant rainfall events adds to the flow, the excess bypasses the WWTP and dumps directly into the river (combined sewer overflows).

The CSO problem on the Rouge is well documented but there are other sources of bacteria. Sites for this study were selected based on Oakland County Health Division's records of previous residential septic failures, and areas where residents were not connected to sewer lines. The results of sampling showed geometric average fecal coliform counts in the range of 1,800 to 12,200 colonies per 100 mL throughout the study area. More than 90% of the water samples in eight branches of the Rouge River had fecal coliform counts exceeding surface water quality standards. The headwaters were clearly not pristine and often failed water quality standards for bacteria. This is consistent with monitoring in 1986-87 by the Michigan Department of Natural Resources (MDNR, 1988). Dye testing on septic systems revealed many system failures. The elevated fecal coliform counts were attributed largely to septic leachate from residential septic systems.

There are areas in lower Michigan where water quality standards are met more often. One of them is in the Saginaw Bay area on Lake Huron. With the exception of sporadic increases in fecal coliform levels, the Saginaw Bay has mostly acceptable levels with a 25% exceedance rate of FC standards (Majeske, 1986). There are, however, elevated levels upriver of Bay County that were attributed to an improperly operated wastewater treatment plant.

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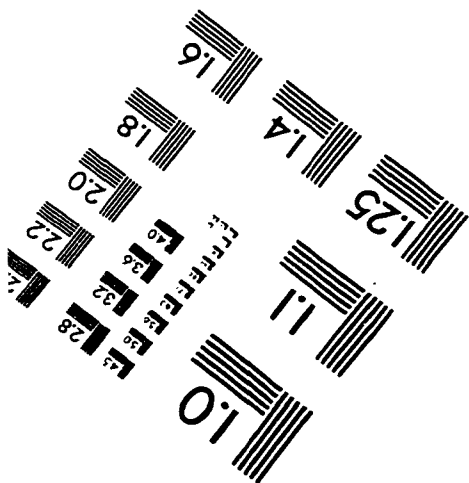
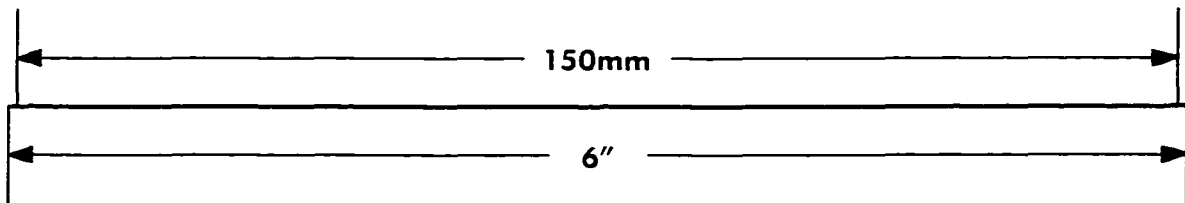
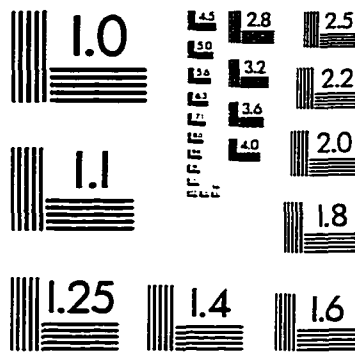
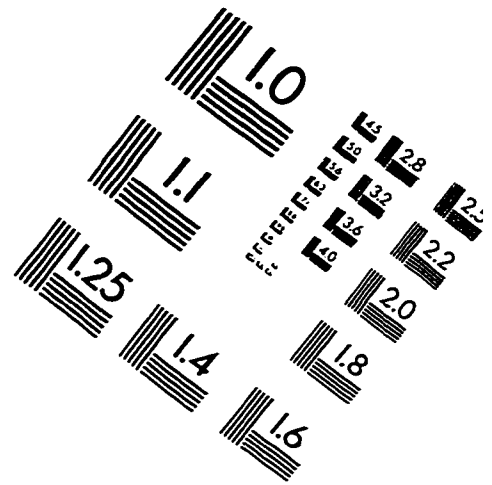
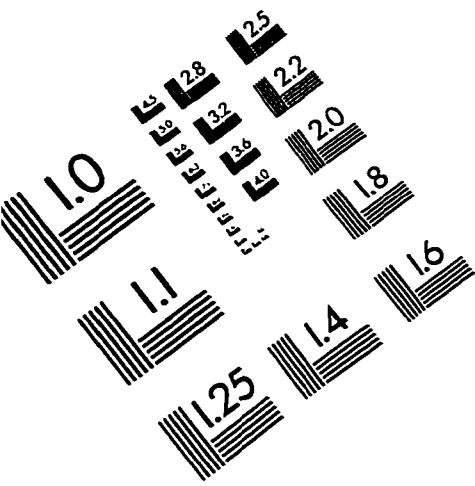
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IMAGE EVALUATION TEST TARGET (QA-3)



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