1-1-2005

The Effects of Dams in the Big Sandy Watershed using a Novel Bacteria-Based Bioindicator of Water Quality

Kathleen Riha Loughman

THE EFFECTS OF DAMS IN THE BIG SANDY WATERSHED USING A NOVEL BACTERIA-BASED BIOINDICATOR OF WATER QUALITY

Thesis submitted to
The Graduate College of
Marshall University

In partial fulfillment of the
Requirements for the degree of
Master of Science
Biological Sciences

by

Kathleen Riha Loughman

Charles C. Somerville, Committee Chair
Frank L. Binder, Committee Member
Ronald E. Gain, Committee Member

Marshall University

April 4, 2005

Abstract

THE EFFECTS OF DAMS IN THE BIG SANDY WATERSHED USING A NOVEL BACTERIA-BASED BIOINDICATOR OF WATER QUALITY

KATHLEEN R. LOUGHMAN. Dept of Biological Science, Marshall University, 1 John Marshall Dr., Huntington, WV 25755

During 2003, water samples from the Big Sandy watershed were collected in conjunction with the United States Army Corps of Engineers (USACE). Objectives were to determine the effects of dams on bacterial populations and to test a bioindicator of water quality based on antibiotic-resistant and fecal indicator bacteria. Thirty-five samples were taken each season within the Big Sandy Watershed, which includes six USACE dams. Total cultivable, ciprofloxacin-resistant, erythromycin-resistant, tetracycline-resistant, total coliform, and fecal coliform bacteria were enumerated. Data on water chemistry and physical parameters were collected by the USACE in the spring and summer seasons. Antibiotic-resistant bacteria and fecal coliform data were used to assign a site impact score (-4 to +4). The scores show significant differences between upstream ($n = 17$) and downstream ($n = 18$) sites in two of three sampling periods analyzed (spring, $P < 0.01$; summer, $P < 0.05$; fall, $P = 1.0$). Sites downstream of dams typically had lower bacterial counts and negative impact scores; whereas, sites upstream had higher bacterial counts and higher impact scores. A significant correlation was repeated in the spring and summer seasons between ciprofloxacin-resistant bacteria and dissolved Kjeldahl Nitrogen (spring, $P < 0.01$; summer, $P < 0.05$) and between turbidity and erythromycin-resistant bacteria ($P < 0.01$). Data on turbidity and weather conditions indicate that bacteria are highly correlated to turbidity, especially under high water and rainfall conditions. This positive correlation suggests an association between bacteria and particulates. The microbiological analyses suggest dams allow particulates and associated bacteria to settle out, leading to an apparent decrease in water impact indicators and bacterial counts.

Acknowledgements

There are many people I would like to thank for bringing me to this point in both my academic career and personal life. First, I would like to extend my gratitude to my advisor Dr. Somerville. It took him no time at all to make me feel welcome in the lab, although it took me a bit longer to call him "Chuck"! In addition to making me feel welcome, he provided me with advice along each step of the process.

I would like to extend a big thanks to all those in the Environmental Microbiology research lab without whom I might never have reached this achievement: Lisa Smith, Heath Damron, Andy Johnson, April Keenan, and Christina Johnson. Not only a thanks for the countless hours of media preparation and lab work, but also for the countless hours outside the lab. Thank you for being great friends!

Another big thanks goes out to Steve Foster with the Army Corp of Engineers. Steve and I spent days collecting samples in the middle of nowhere and he always made it a blast, even when it was raining! He taught me how to drive a boat, identify fish (well, I remember some anyway), and how to karaoke in the truck!

I would like to acknowledge all those who provided additional assistance in the development of my thesis. Thank you to my committee members, Dr. Gain and Dr. Binder, who provided much needed insight after I was tired of looking at my data. Thank you to Dr. May for spending time with me explaining which statistical tests to use and showing me how to perform them! I would also like to extend a thank you to Teresa Fogus in the Herpetology Lab for making my beautiful maps used in this thesis and in my defense.

As always, I would like to thank my family. Even when I would change my mind about my future career path, they provided me encouragement and support to go after whatever would make me happy. Thank all of you for everything and for your continued support in my life.

Now it is time to acknowledge the most important person on my list, my husband Zac. Where do I begin? Without you in my life I might never have found the right path for me. When I would have doubt, you would help me believe in myself. When I would get discouraged, you would provide the encouragement. Thank you for everything: for the many pep talks, for the hugs, and for the celebrations even when there was nothing to celebrate but us! I love you, I admire you, and I thank you.

Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter 1

Literature Review

Introduction

According to the National Park Service, U.S. Department of the Interior, there are currently 3,500,000 miles of rivers in the United States (29). This figure does not represent the many more miles of water found in the country's expansive network of creeks and streams. We interact with this water on a daily basis for a variety of reasons. Water is used world wide as a source of drinking water, recreation, transportation, food supply, and for the removal of sanitary and industrial wastes. Therefore, consistent efforts must be made to ensure the quality of surface waters for the safety of public health as well as the health of the environment. Factors that effect our water supply affect our population. Water quality has been a principal concern on both the environmental and the public health levels. Due to the many potential risks involved with human interaction and dependency on riparian systems, continuous monitoring to ensure that a human health risk is not posed is essential. One such effort that can be made is monitoring the quality of water by examining the presence of bacteriological communities (11).

Water Quality Standards

The standard criteria currently used for microbiological examination of water quality tests for the detection and enumeration of coliform group bacteria (*Escherchia coli,* fecal coliforms and total coliforms), fecal streptococci, enterococci, and

heterotrophic bacteria (11). Fecal coliforms reside in the intestines of warm-blooded animals and are excreted in waste material. High numbers of these bacteria in surface waters indicate a threat to human health from gut-associated pathogens. Additionally, their presence in drinking water is a strong indication of recent sewage or animal waste contamination. *Escherichia coli*, a specific type of fecal coliform bacteria, has long been used as an indicator of fecal contamination in surface waters. Most strains of *E. coli* are not pathogenic, however, some strains exist that produce potent enterotoxins which pose a human health risk when ingested. Research suggests that additional criteria need to be added to the standard methods in order to better represent the quality of water (8, 16, 21).

Calls have been made for the current bacteriological water quality standards to be reviewed to consider new criteria that would incorporate antibiotic resistant bacteria (16). The presence of antibiotic resistant bacteria in fresh waters has been documented in many countries around the world (2, 8, 17, 26, 30, 31, 40). Antibiotic resistance can be found in drinking waters, but in the developed world it is more commonly found in recreational water (40). A South African study over three decades ago called for the re-evaluation of water quality standards due to the possibility of coliforms acting as reservoirs for R-factors (extrachromosomal nucleic acid elements) which may mediate the transfer of antibiotic resistance to pathogens (21). This research pushed for the examination of new water quality standards with the knowledge that the previous assumption of coliforms as harmless indicators of fecal pollution should no longer be made (21).

Antibiotic Resistance

Bacterial antibiotic resistance is a naturally occurring phenomenon; however, concern arises when resistance becomes common or occurs in pathogenic bacteria. Selection for resistance can occur in the presence of antibiotics. Four main sources of antibiotics in water are identified as sewage treatment plants, run-off from such things as manure and fertilizers, aquaculture sources where tetracyclines are a main group used, and pharmaceutical manufacturers (23). Antibiotics have been found in both river water and sewage treatment plant effluents at concentrations up to several micrograms per liter (23). Research out of Africa showed the highest amounts of antibiotic resistant bacteria were isolated from areas near places where antibiotics had been used in animal production (31). Downgradient of a landfill once used for the disposal of pharmaceutical production wastes, pharmaceutical organic compounds have been found in ground water at concentrations up to 5 mg/l (24).

The presence of antibiotics in both ground and surface waters may play a role in the association of antibiotic resistant bacteria and urbanized areas. Thought to reflect localized antibiotic usage, a study of *Aeromonas* spp. in two European rivers showed an association of high incidences of antibiotic resistance to areas with heavy anthropogenic impacts (19). Research performed in Australia found that fecal bacteria have been found in clear spatial patterns of resistance when comparing rural to urban sites along a river, thus further indicating a need to incorporate antibiotic resistance as a bacteriological water quality parameter (8). Antibiotics and antibiotic-resistant bacteria belonging to mammalian commensal flora, mainly *Enterobacteriaceae*, have been documented as being discharged into the water *via* urban effluent (22). A study looking at the effect

urban effluent had on both the antibiotic resistance of *Enterobacteriaceae* (allochthonous river bacteria) and *Aeromonas* spp. (autochthonous river bacteria) in the Arga River showed that the effluent increases antibiotic resistance when comparing percent of resistant strains upstream to the percent downstream of the effluent (18).

In addition to evidence showing a correlation of antibiotic resistance to the presence of urban areas, research has shown that antibiotic resistance is also correlated to heavy metals and industrial pollution. In fact, there may be high potential for antibiotic resistance due to metal contamination because of the overwhelming amounts of metal-contamination in many areas (28). In a comparison of an industrially perturbed stream to an undisturbed reference stream, the amount of antibiotic resistant bacteria was significantly higher ($P < 0.05$) in the industrially impacted stream (28). An established link of genetic association between antibiotic and metal resistance genes in bacteria has been noted on the subject (14). Antibiotic resistance has been shown to be positively correlated ($r^2 = 0.54$, $P = 0.023$) with mercury concentrations in sediments, indicating that mercury concentrations indirectly select for antibiotic resistance in certain bacteria (28). A possible association between mercury resistance and tetracycline resistance is indicated by the outcome of a study showing that 87.5% of mercury-resistant isolates of the Gram-negative coccobacillus *Acinetobacter* were also found to be resistant to tetracycline (14). In the aforementioned study, the genes for the mercury and tetracycline resistance were located on plasmids (14).

Plasmids and Multiple Antibiotic Resistance

Studies have shown that genes coding for antibiotic resistance traits and genes coding for metal resistance are often carried on the same plasmid (38, 39). In addition, multiple antibiotic resistance has commonly been found to be mediated by single transferable plasmids, rather than multiple plasmid bands (17). Transfer of resistance genes among microorganisms, mutation in genes, and increases in selective pressures have been identified as three contributing factors to the development and spread of antibiotic resistant bacteria (22). Even when an absence of specific antibiotic pressures occurs in the environment, multiple-antibiotic resistance has been found among environmental organisms, indicating that the cause of resistance may be from plasmid transfers (17). Transduction, transformation, and conjugation are three processes of gene transfer believed to occur in the aquatic environment, with conjugation being the most studied (13).

Antibiotic resistance is commonly and usually associated with fecal coliforms, such as *Escherichia coli*, suggesting an animal and human reservoir (17). In a study of a community water supply in Tlaxcala, Mexico, coliforms were found exceeding limits set forth by the World Health Organization (W.H.O.) for potable water. The most probable number (MPN) coliform counts were shown to have a direct correlation to rainfall amounts indicating the probability of human and/or animal wastes being washed into the water supply (20). With the knowledge that animal and human feces have been determined to be reservoirs of resistance (*R*-) plasmids (1, 17, 21), the possible source of antibiotic resistant bacteria in surface waters should be examined when evaluating water quality, especially water for human consumption.

In many cases, isolates have been resistant not only to one antibiotic but to more than one antibiotic, indicating an added health risk. In the aforementioned Mexican community water supply study, 52.8% of *E. coli* isolates and 96.2% of *Shigella* isolates were found to be resistant to one or more antibiotics in common use (20). A study in Cairo, Egypt also isolated strains of bacteria from drinking water and effluents, and found that multiple antibiotic resistance (MAR) was demonstrated in 62.4 to 98% of the isolates (16). Even the wastewater treatment process has been shown to have an effect on levels of MAR and individual antibiotic resistance. The percent of fecal coliforms resistant to more than one antibiotic has been observed to be reduced in short-term retention lagoons and mechanical treatment plants; whereas, in long-term retention lagoons there is an observed increase in percent resistance (7). The percent of tetracycline-resistant *E. coli* in a wastewater treatment plant (WTP) was shown to decrease during the treatment process; however, the percent resistance in the wastewater effluent was higher than in the river water (25).

Antibiotics in Study

Antibiotics are natural, that is produced by microorganisms, or synthetic substances that are used to inhibit or kill microorganisms (3). Antibiotics can be classified in several ways, but the most common means of classification involves separating antibiotics into groups based on their chemical structure. The three antibiotics examined in this study for the purpose of determining antibiotic-resistance were taken from three separate classes: tetracyclines, macrolides, and fluoroquinolones.

Tetracyclines are a closely-related four-ringed group of broad-spectrum bacteriostatic agents with similar toxicology (33; Figure 1). The mechanism of action they use is the inhibition of protein synthesis by binding reversibly to the 30 S ribosomal subunits of susceptible microorganisms (6). This binding blocks bacterial translation by distorting the 30 S subunit so that the anticodons of tRNAs can not properly align with the codons of the mRNA. The inability of the aminoacyl-tRNA to bind to the acceptor site on the mRNA-ribosomal complex inhibits protein synthesis by preventing elongation of the peptide chain. Susceptible cells uptake and concentrate the antibiotic by passive diffusion and active transport (3); resistance appears in cells carrying an R-factor that inhibits the uptake of the drug. The specific drug examined in this class for the purposes of this research was tetracycline (33; Figure 1). Tetracycline is a broad-spectrum antibiotic prepared from the cultures of certain *Streptomyces* species with a molecular formula of $C_{22}H_{24}N_2O_8$ and a molecular weight of 444.44g (9). Tetracycline was first patented on January 11, 1955 according to the United States Patent and Trademark Office (12). Studies indicate that patients taking tetracycline excrete 80-90% of the drug unchanged (23).

Macrolide antibioitcs have a basic structure consisting of a large 14-member macrocylic lactone ring containing methyl and hydroxyl groups among others (35; Figure 2). This class of antibiotics is primarily bacteriostatic in action and principally active against gram-positive cocci, with the exception of enterococci, as well as against some gram-negative anaerobes (6). Their mechanism of action involves binding reversibly to the 50 S ribosomal subunits of susceptible organisms, subsequently interfering with protein synthesis. Macrolides inhibit elongation of the protein by either blocking

peptidyltransferase, the enzyme that forms peptide bonds between the amino acids, by preventing the ribosome from translocating down the mRNA to the next codon, or both. The specific drug examined in this class for the purposes of this research was erythromycin (35; Figure 2). Erythromycin was originally found by Waksman and Henrici in a Philippines soil sample and is naturally produced by a strain of *Saccharopolyspora erythraea* (formerly *Streptomyces ervthraeus*). Erythromycin has a molecular formula of $C_{37}H_{67}NO_{13}$ and a molecular weight of 733.94g (9). Erythromycin was the first drug discovered in the class of macrolides. The original patent for Erythromycin was received in 1953 according to the United States Patent and Trademark Office (15). The excretion rate for percent unchanged erythromycin is documented at >60% (23).

Fluoroquinolones are a class of synthetic bacteriocidal antibiotics first developed in the 1980s. Their mode of action involves inhibition of bacterial nuclear DNA synthesis by inhibiting topoisomerase II (DNA gyrase). This enzyme is responsible for the supercoiling and uncoiling of DNA. In order for the long DNA molecule to fit into the cell, it must be compacted by supercoiling. However, in the supercoiled arrangement, the DNA is unable to undergo replication. In order for replication, transcription, and even repair of the DNA to take place, uncoiling of the supercoiled structure must occur. Thus, inhibition of DNA gyrase will eventually lead to cell death by preventing DNA synthesis and repairs within the bacterial cell. The specific drug examined in this class for the purposes of this research was ciprofloxacin (10; Figure 3). Ciprofloxacin, molecular formula $C_{17}H_{18}FN_3O_3$ and molecular weight 331.4g (9), is a broad-spectrum antibiotic that works well against aerobic bacteria. It was the first fluoroquinolone on the

market. Developed by the Bayer pharmaceutical company as Cipro® (4, 5), it became the first oral broad-spectrum antibiotic of this class approved by the US Food and Drug Administration (FDA) on October 22, 1987.

Specific antibiotic production rates are not reported in the literature or by the FDA (personal communication). Each of the three antibiotics included in the study for antibiotic-resistant bacteria are currently listed by the United States Geological Survey as emerging contaminants in US streams (37). In the aquatic environment, erythromycin degradation products have been found at concentrations up to 6 μg/l and tetracycline was not detectable above 50 ng/l due to either high susceptibility to hydrolysis in the aquatic environment or disguise through binding to free ions or sediment (23). In one review, antibiotic substances in surface waters had been estimated and determined for both erythromycin (~1 μg/ml) and tetracycline (~1 μg/ml) (22). Due in part to ciprofloxacin having only been on the U.S. market since late 1987, data is unavailable for ciprofloxacin concentrations in the aquatic environment.

In addition, bacterial resistance has been identified for each of the antibiotics being examined. Among varying *Pseudomonas* strains recovered from drinking water, erythromycin resistance was found to occur in 46.1% to 100% isolates (34). Downstream of a WTP along the Tama River in Tokyo, Japan, tetracycline-resistance was observed increasing due to discharges from the WTP (25). When certain species of fecal coliforms were examined for tetracycline resistance, *E. coli* was the species with the highest percentage of resistance (30). In a study of antibiotic-resistance of *Aeromonas* spp. (normal inhabitant of soil and fresh water) in two European rivers, the highest resistance was found to be to nalidixic acid (59%). This antibiotic is in the quinilone class; the class

of antibiotics used as the first-line drugs against *Aeromonas* infections. Most of the strains in this study were also found to be susceptible to fluoroquinolones (54-98%) such as ciprofloxacin (19).

Study Objectives

Thirty-five water samples were collected in each season of 2003 from the Big Sandy drainage basin in conjunction with the United States Army Corps of Engineers (USACE). The objectives were to gather baseline data for the Army Corp of Engineers and to test a novel bioindicator of water quality based on antibiotic resistant and fecal indicator bacteria (27). Based on these parameters, water quality was examined at sites above and below six USACE dams within the drainage basin.

An additional objective was to determine any effect that dams may have on water quality. "Currently, 600,000 miles of U.S. rivers or 17% of total U.S. river mileage lie behind an estimated 60,000 to 80,000 dams" (29). Anecdotal evidence from Army Corp personnel suggests water quality improves below USACE impoundments. The hypothesis for this study was that impoundments would allow particulates and bacteria associated with particulates to settle out, leading to an apparent decrease of water impact indicators downstream of each dam.

Chapter 2

Methods

Study Area Description

Sampling for this study occurred in the Cumberland Plateau at thirty-five sites within the Big Sandy River Watershed that drains an area along the intersection of Kentucky, Virginia, and West Virginia (Figure 4). The watershed area consists of the Big Sandy River and its two major branches, the Tug Fork and the Levisa Fork. The Big Sandy River and the Tug Fork make up the boundary between the states of West Virginia and Kentucky. The Big Sandy River flows north and enters the Ohio River at river mile 317.1 in Ashland, Kentucky. According to the Ohio River Valley Water Sanitation Commission (ORSANCO), the Big Sandy River is 27 miles in length and its drainage area is 4,280 square mile (32). These figures do not include the portion of the drainage basin comprised by the Tug and Levisa Forks. The Big Sandy River is navigable for commercial shipping, primarily coal. The Tug and Levisa Forks are not commercially navigable. The Tug and Levisa Forks are primarily used for recreational purposes. Located within the watershed are six United States Corp of Engineers (USACE) flood-control dams (Figure 5). These impoundments include Yatesville (KY), Paintsville (KY), Dewey (KY), Fishtrap (KY), J.W. Flanagan (VA), and North Fork of Pound (VA).

The drainage area of the basin extends into seventeen counties across three states (Figure 6). The majority of the basin's land area (54%) lies in ten Kentucky counties which include Boyd, Floyd, Johnson, Knott, Lawrence, Letcher, Magoffin, Martin,

Morgan and Pike counties. Another 23% of the basin is in four Virginia counties, Buchanan, Dickenson, Tazewell and Wise. The remaining 23% of the basin's land area is in McDowell, Mingo and Wayne counties in West Virginia. According to the Kentucky Division of Water's 1999 report on the Big Sandy River Basin, the majority (95.6%) of the basin's land use consists of deciduous forest cover. Accounting for another 3.54% of the land use is croplands and pastures. Strip mines and transitional areas account for 0.77% of the land. Land for urban, industrial, and utilities use (0.06%) and water (0.03%) comprise less than 0.10% of the land usage within the Big Sandy basin.

The basin is primarily rural with relatively low population densities. Total population (318,274) for the Big Sandy River drainage basin was calculated using data taken from the United States Census Bureau's Census 2000 Demographic Profiles on each of the counties within the basin (Table 1). Populations were estimated based on the percent of each county positioned within the basin's boundaries.

Microbiological Sampling

Water samples were taken from thirty-five sites (Appendix A) within the Big Sandy Watershed, eighteen sites in Kentucky, nine in West Virginia, and eight in Virginia. Sampling occurred seasonally during 2003. Winter sampling took place between January 16, 2003 and March 6, 2003. Spring sampling occurred from June 11-18, 2003, summer sampling was performed August 21-28, 2003, and the fall sampling period was November 10-18, 2003. According to standard water collecting procedure, samples were placed on ice and tested in the laboratory within six hours of collection for

the presence of total cultivable, ciprofloxacin-resistant, erythromycin-resistant, tetracycline-resistant, total coliform, and fecal coliform bacteria (Appendix C).

Aliquots (100 µl) of diluted (10^{-2}) water were plated onto R2A agar plus fungizone (375 ng/ml) for the enumeration of total cultivable bacteria. Aliquots (100 or 200 µl) of undiluted water were plated onto R2A plus fungizone and ciprofloxacin (4 mg/L), erythromycin (8 mg/L), or tetracycline (12.5 mg/L) for the enumeration of antibiotic resistant bacteria. All samples were plated in triplicate and incubated at 30°C for one week.

Fecal coliform and total coliform bacteria were enumerated by membrane filtration and cultivation on m-FC (44.5 ± 0.2°C for 24 hours) or m-ENDO broth (35 ± 0.5°C for 24 hours), respectively, according to standard methods. Aliquots (100 µl, 1 ml, 5 ml, 10 ml, 25 ml, or 50ml) used for fecal coliform were different than the aliquots (100 µl, 500 µl, 1ml, 10 ml, 50 ml) filtered for total coliform bacteria in order to obtain countable plates.

All counts were recorded in an Excel spreadsheet for each season. Using the enumeration data for the antibiotic resistant bacteria and fecal coliform bacteria, an impact score (IS) was established for each site. For each population (i.e. fecal coliforms or ciprofloxacin-resistant cells), the average count for a site was ranked within the population data set of all sites during the same season using the percentile rank function in Excel. A percentile score for each data point within the entire population data set was obtained by multiplying the percentile rank by 100.

Boundaries were then chosen to apply to the data. For example, an IS_{90} score weights sites with population counts above the 90^{th} percentile (weight of +1) and below

the 10^{th} percentile (weight of -1). An IS_{80} score weights sites with population counts above the 80^{th} percentile and below the 20^{th} percentile. For the purpose of the proposed bacteria-based biological index, IS_{85} to IS_{90} scores provide a useful signal to noise ratio in the index.

A population score is then assigned to all data points (+1 for data points above the upper percentile boundary; 0 for data points between the chosen boundaries; -1 for data points below the lower percentile boundary). Population scores were determined for antibiotic resistant populations and fecal coliform populations separately by season.

The total impact score (IS) for each site in each season was determined by adding the population scores at each site. For this study, three antibiotics and one fecal indicator was used. Therefore, impact scores can range from -4 to +4. Higher impact scores are indicative of a more impacted water source.

Additional Sampling

In addition to the seasonal microbiological analysis for each site, data was also taken by the USACE on classical measures of water quality; including algae, benthic macroinvertebrates, fish, water chemistry, and physical parameters. Iodine was added to water samples collected in 100 ml jars and sent to Dr. Miriam Steinitz-Kannan at Northern Kentucky University (Highland Heights, KY 41099) for algal analysis.

Benthic macroinvertebrate collections varied depending on stream size at each site. For small streams, four twenty-second kick samples were collected in half-meter 595-micron kick-nets positioned in a riffle section of the stream. For large sites, twelve ten-meter transects were established and the subsequent sampling was performed at each

site in triplicate. Within each of the twelve transects, three targeted-habitat sites were sampled, "grabbed", (2 minutes collection each) with D-frame dip nets. In addition, three twenty-second kicks were performed with the half-meter kick-net. Each collection within the twelve transects was combined to establish the composite sample for the large site location.

Fish were collected during the summer season. Fish sampling is primarily performed during mid to late summer to avoid the spring and fall migratory periods and because stream and river flows are low to moderate with generally little variation as compared to other seasons. Although there are a few exceptions, most fish assemblages tend to remain in the same area in the summer rather than migrate long distances. Like benthic macroinvertebrate sampling, fish collections also varied depending on stream size at each site. Sites were categorized as either "wadeable" sites for shallow water or as "non-wadeable" sites for deeper water. Wadeable sites were collected using either a back-pack shocker system for the small, wadeable streams or an electric seine for the larger, wadeable streams. All wadeable electrofishing was conducted in an upstream direction. At each site, a 150 m section was sampled. Each section was selected to include various habitat types (pools, riffles, and runs) for the fish. Current was passed into the water, using either the backpack or towable unit, and fish were collected using $3/8^{th}$ inch mesh nets. Non-wadeable sites were collected at night using a boom-shocker system on a boat where the fish would be collected using D-frame dip nets. Upon collection at both the wadeable and non-wadeable sites, fish were placed into a livewell until completion of the sampling section. The number of each species collected was

recorded and used to calculate the overall quality of the site based on the Kentucky fish Index of Biological Integrity (IBI).

For each of the thirty-five sites during spring and summer seasons, water samples were taken for water chemistry analyses. The water samples were collected according to protocols established by the water chemistry contractor (BIOCHEM Testing, Inc. 5 WeatherRidge Drive, State Route 34, Hurricane, WV 25526). At each site the following were analyzed: dissolved HCO_3 (mg/l), total solids (mg/l), dissolved solids (mg/l), suspended solids (mg/l), total ammonia (mg/l N), total Kjeldahl (mg/l N), dissolved Kjeldhal (mg/l N), total $NO_2 + NO_3$ (mg/l N), dissolved $NO_2 + NO_3$ (mg/l N), total phosphorous (mg/l), dissolved phosphorous (mg/l), total organic compounds (mg/l), dissolved organic compounds (mg/l), total inorganic carbon (mg/l), dissolved inorganic carbon (mg/l), dissolved calcium (mg/l), dissolved magnesium (mg/l), dissolved sodium (mg/l), dissolved potassium (mg/l), dissolved chloride (mg/l), dissolved sulfate (mg/l), dissolved barium (µg/l), total iron (µg/l), dissolved iron (µg/l), total manganese (µg/l), dissolved manganese (µg/l), total zinc (µg/l), dissolved zinc (µg/l), total aluminum (µg/l), dissolved aluminum (µg/l), total silicon (mg/l), dissolved silicon (mg/l), total titanium (µg/l).

Physical parameters were recorded when water was collected for microbiological, algal, and water chemistry analysis in the spring and summer seasons. Using a portable datasonde, water temperature (°C), turbidity (NTU), specific conductivity (µmho/cm), oxygen (mg/l), and pH were determined. Alkalinity (mg/l) was ascertained using an alkalinity titration kit.

Chapter 3

Results

Impact Scores

Average counts and standard deviations for total cultivable bacteria, ciprofloxacin-resistant bacteria, erythromycin-resistant bacteria, tetracycline-resistant bacteria, fecal coliforms, total coliforms, and fecal streptococci were calculated using Microsoft Excel per site each season (Appendices D-G).

Using the average counts for the antibiotic resistant bacteria and fecal coliforms, an overall site impact score (IS) was determined for each site during each season. An impact score was determined for the spring, summer, and fall seasons at three boundary levels: IS_{85} (Table 2, Figure 7), IS_{90} (Table 3, Figure 8), IS_{95} (Table 4, Figure 9). The IS_{90} provides an appropriate signal-to-noise ratio for the proposed index.

A comparison of all sites upstream of a dam (n = 17) to all sites downstream of a dam (n = 18) sampled within the Big Sandy watershed was made using the IS_{90}. Spring impact scores (range -4 to 4) using the 90^{th} percentile boundary (IS_{90}) showed fifty-seven percent of those sites with positive impact scores were locations upstream and twenty-nine percent of those sites with negative impact scores were locations downstream. A significant difference ($P = 0.001007$) between all upstream and downstream sites' IS_{90} was found using a Student's *t*-test with a two-tail distribution and unequal variance (Figure 10).

Summer impact scores (range -4 to 4) using the 90th percentile boundary (IS_{90}) showed seventy-three percent of sites with positive impact scores were locations upstream and seventy-five percent of those sites with negative impact scores were downstream of an impoundment. A significant difference ($P = 0.034917$) between all upstream and downstream sites' IS_{90} was determined using a student's t-test with a two-tail distribution and unequal variance (Figure 11).

Fall impact scores (range -4 to 4) using the 90th percentile boundary (IS_{90}) showed forty percent of those sites with positive impact scores were locations upstream and seventy-five percent of those sites with negative impact scores were locations downstream. No significant difference ($P = 1.0$) between upstream and downstream sites' IS_{90} was determined using a Student's *t*-test with a two-tail distribution and unequal variance (Figure 12).

Upstream v. Downstream per Dam (Average Counts)

Attention was given to sites directly upstream and downstream of each United States Army Corp of Engineers (USACE) Dam (Table 5). Upstream sites' counts were averaged together to give an upstream value at each dam. The resulting upstream value and downstream count for each dam was used for the determination of any change in antibiotic resistant bacteria and fecal coliform counts from upstream-to-downstream during each season. Significance in changes of counts from upstream-to-downstream were determined using the 95th confidence level (DF = 1; $\chi^2 > 3.84$) for the goodness-of-fit statistical analysis.

During the spring season, average counts for antibiotic-resistant and fecal coliform bacteria decreased significantly ($\chi^2 > 3.84$) from upstream to downstream at the following dams: Yatesville, Paintsville, Fishtrap, and J.W. Flannagan. One exception was that there was no significant change ($\chi^2 = 2.40$) in ciprofloxacin-resistant bacterial counts at the Fishtrap Dam. Average counts for antibiotic-resistant and fecal coliform bacteria increased significantly ($\chi^2 > 3.84$) from upstream to downstream at both the Dewey Dam and the North Fork of the Pound Dam (Table 6, Figures 13 & 14).

During the summer season, average counts for antibiotic-resistant and fecal coliform bacteria decreased significantly ($\chi^2 > 3.84$) from upstream to downstream at the following dams: Yatesville, Dewey, and J.W. Flannagan. One exception was that there was no significant change ($\chi^2 = 2.44$) in tetracycline-resistant bacterial counts at the Dewey Dam. A significant decrease ($\chi^2 > 3.84$) was also observed for the following: ciprofloxacin-resistant bacteria at the Paintsville Dam; fecal coliform, erythromycin- and tetracycline-resistant bacteria at the Fishtrap Dam; fecal coliform, ciprofloxacin- and tetracycline-resistant bacteria at the North Fork of the Pound Dam. A significant increase ($\chi^2 > 3.84$) from upstream to downstream was observed for the following: fecal coliform, erythromycin- and tetracycline-resistant bacteria at the Paintsville Dam; ciprofloxacin-resistant bacteria at the Fishtrap Dam; erythromycin-resistant bacteria at the North Fork of the Pound Dam (Table 7, Figures 15 & 16).

During the fall season, average counts for antibiotic-resistant bacteria and fecal coliforms decreased significantly ($\chi^2 > 3.84$) from upstream to downstream at the following dams: Yatesville, Paintsville, Dewey, and J.W. Flannagan. A significant decrease ($\chi^2 > 3.84$) was also observed for the following: ciprofloxacin- and

erythromycin-resistant bacteria at the Fishtrap Dam. Average counts for antibiotic-resistant bacteria and fecal coliforms increased significantly ($\chi^2 > 3.84$) from upstream to downstream at the North Fork of the Pound Dam. One exception was that there was no significant change ($\chi^2 = 2.34$) in fecal coliform bacteria at this dam. A significant increase ($\chi^2 > 3.84$) from upstream to downstream was also observed for the following: fecal coliform bacteria at the Fishtrap Dam. No change ($\chi^2 = 0$) occurred for the upstream and downstream average counts for tetracycline-resistant bacteria at the Fishtrap Dam (Table 8, Figures 17 & 18).

Upstream v. Downstream per Dam (Percent Antibiotic Resistance)

The average upstream count and downstream count for each dam was compared to the corresponding total cultivable bacteria count to determine the percent of total cultivable bacteria that were ciprofloxacin-resistant, erythromycin-resistant, and tetracycline-resistant upstream and downstream at each dam. Significance in changes of counts from upstream-to-downstream were determined using the 95th confidence level (DF = 1; $\chi^2 > 3.84$) for the chi-square statistical analysis.

During the spring season, percent antibiotic resistance decreased significantly ($\chi^2 > 3.84$) from upstream to downstream at both the Paintsville Dam and the North Fork of the Pound Dam. A significant decrease ($\chi^2 > 3.84$) was also observed for the following: percent erythromycin- and tetracycline-resistant bacteria at both the Yatesville Dam and the Fishtrap Dam. Percent antibiotic resistance increased significantly ($\chi^2 > 3.84$) from upstream to downstream at both the Dewey Dam and the J. W. Flannagan Dam. A significant increase ($\chi^2 > 3.84$) from upstream to downstream was also observed for the

following: percent ciprofloxacin-resistant bacteria at both the Yatesville Dam and the Fishtrap Dam (Table 9, Figures 19 & 20).

During the summer season, percent antibiotic resistance decreased significantly ($\chi^2 > 3.84$) from upstream to downstream at both the Yatesville Dam and the North Fork of the Pound Dam. A significant decrease ($\chi^2 > 3.84$) was also observed for the following: percent ciprofloxacin-resistant bacteria at Paintsville Dam. Percent antibiotic resistance increased significantly ($\chi^2 > 3.84$) from upstream to downstream at the following dams: Dewey, Fishtrap, J.W. Flannagan. A significant increase ($\chi^2 > 3.84$) from upstream to downstream was also observed for the following: percent erythromycin- and tetracycline-resistant bacteria at the Paintsville Dam (Table 10, Figures 21 & 22).

During the fall season, percent antibiotic resistance decreased significantly ($\chi^2 > 3.84$) from upstream to downstream at both the J.W. Flannagan Dam and the North Fork of the Pound Dam. A significant decrease ($\chi^2 > 3.84$) was also observed for the following: percent ciprofloxacin-resistant bacteria at Dewey Dam; percent ciprofloxacin- and erythromycin-resistant bacteria at the Fishtrap Dam. Percent antibiotic resistance increased significantly ($\chi^2 > 3.84$) from upstream to downstream at the Yatesville Dam. A significant increase ($\chi^2 > 3.84$) from upstream to downstream was also observed for the following: percent ciprofloxacin- and erythromycin-resistant bacteria at the Paintsville Dam; percent erythromycin-resistant bacteria at the Dewey Dam. No significant change was observed for percent tetracycline-resistant bacteria at the following dams: Paintsville ($\chi^2 = 2.21$), Dewey ($\chi^2 = 1.54$), Fishtrap ($\chi^2 = 0.02$) (Table 11, Figures 23 & 24).

Physical Parameters

Physical parameter data [water temperature (°C), turbidity (NTU), specific conductivity (µmho/cm), oxygen (mg/l), pH, and alkalinity (mg/l)] gathered for the spring (Appendix H) and summer (Appendix I) seasons was compared to average counts for microbiological data to determine if any correlation occurs. Significant correlations were determined using both the 95th ($P < 0.05$) and the 99th ($P < 0.01$) confidence levels for the correlation coefficient statistical test measuring the strength of association between two variables.

During the spring season, there were significant correlations ($P < 0.05$) between the following variables: turbidity and ciprofloxacin-resistant bacteria; pH and total cultivable bacteria; pH and temperature. There were significant correlations ($P < 0.01$) between all microbiological data and also between the following variables: turbidity and total cultivable bacteria, fecal coliforms, erythromycin-resistant bacteria, tetracycline-resistant bacteria; alkalinity and pH, temperature, specific conductivity; temperature and specific conductivity, oxygen; pH and specific conductivity (Table 12).

During the summer season, there were significant correlations ($P < 0.05$) between the following variables: tetracycline-resistant bacteria and temperature, specific conductivity, alkalinity; total coliforms and specific conductivity, pH; temperature and alkalinity; oxygen and pH. There were significant correlations ($P < 0.01$) between the following variables: erythromycin-resistant bacteria and tetracycline-resistant bacteria, fecal coliforms, turbidity; tetracycline-resistant bacteria

and fecal coliforms; total coliforms and oxygen; pH and alkalinity, temperature, specific conductivity; specific conductivity and alkalinity (Table 13).

Although no physical parameter data was obtained during the fall sampling, a correlation analysis was performed between the microbiological data. A significant correlation ($P < 0.01$) occurred between all microbiological data, except between the total cultivable bacteria and total coliforms (Table 14).

Water Chemistry

Water chemistry data gathered for the spring (Appendix J:1-4) and summer (Appendix K:1-4) seasons was compared to average counts for the antibiotic-resistant bacteria data to determine if any correlation occurs. Significant correlations were determined using both the 95th ($P < 0.05$) and the 99th ($P < 0.01$) confidence levels for the correlation coefficient statistical test measuring the strength of association between two variables. The only significant correlation that was repeated in both the spring (Table 15: A-C) and summer (Table 16: A-C) season was between ciprofloxacin-resistant bacteria and dissolved Kjeldahl Nitrogen ($P < 0.01$ for spring, $P < 0.05$ for summer).

Kentucky Index of Biological Integrity

The Kentucky Index of Biological Integrity (KIBI) scores (range: 0-100) determined for 24 of 35 sites during the summer season (Appendix L) were compared to the summer microbiological average counts and site impact scores to determine if any correlation occurs. No significant correlations were determined using the 95th ($P < 0.05$)

confidence level for the correlation coefficient statistical test measuring the strength of association between two variables (Table 17).

Data Not Reported

The data for some of the classical measures of water quality in this study were not completed at the time of this analysis. Those data not completed were the algae and benthic macroinvertebrate analysis. Samples were sent by the United States Army Corp of Engineers (USACE) to Dr. Miriam Steinitz-Kannan at Northern Kentucky University (Highland Heights, KY 41099) for algal analysis. This data is to be reported to the USACE-Robert C. Byrd Dam Water Quality Unit when completed. The benthic macroinvertebrate samples collected are to be completed and analyzed by the USACE at a later time.

Chapter 4

Discussion

Seasonal Data

An analysis of winter data for 2003 was not conducted due to the long sampling time period and the variability in weather conditions between sampling trips. The winter sampling of the 35 sites within the Big Sandy Watershed was conducted from January 16, 2003 to March 6, 2003. The total time from first day of sampling to the last day of sampling was 50 days. Weather conditions varied within these 50 days especially with regards to rainfall amounts. Mid-sampling, heavy rainfalls occurred causing a skew in the data to be observed between those sites sampled prior to the rainfall and those sites sampled during/after the rainfall. Therefore, due to the noticeable change in rainfall and the large range in sampling days, the winter data was omitted from the seasonal analysis.

Spring, summer, and fall data were used for analyses. Collections of samples from the 35 sites during these seasons were more timely (spring: 8 days; summer: 8 days; fall: 9 days) and weather conditions more constant over the sampling period range. Weather conditions varied from season-to-season and are believed to play a major role in the results observed. During the spring, water levels within the watershed were elevated and turbid due to heavy rainfalls preceding the sampling period. During the summer, water levels were closer to normal and little rainfall occurred around the time of sampling. During the fall, water levels were at the highest. Flood-like conditions and heavy rainfalls occurred around the sampling period.

Microbiological Data

For the purpose of developing the site Impact Score (IS) and comparison of microbiological data upstream versus downstream of each dam, only the antibiotic resistant bacteria and fecal coliform data were used. Total coliform data was not included for the purposes of consistency among the seasonal data. Sampling for total coliforms was not conducted in the spring season due to a lack of funding for necessary media and supplies.

Impact Scores

The development of the impact score system for microbiological data was pioneered in the Environmental Microbiology Research Laboratory at Marshall University by Dr. Charles Somerville. The impact score system was first developed to be used on the Ohio River. Part of this study was to determine if the impact score system used on a large river could be applied to an analysis of an entire watershed with sites ranging from small stream headwaters to large river mainstem.

The impact score system takes into account the traditional microbiological water quality indicator, fecal coliforms, and proposed new indicators, antibiotic-resistant bacteria. Three different antibiotics (ciprofloxacin, erythromycin, and tetracycline) from three separate classes of antibiotics (fluoroquinolones, macrolides, and tetracyclines) were chosen for this study because they appear on the USGS list of emerging contaminants (37).

Since four bacterial populations (fecal coliforms, ciprofloxacin-resistant, erythromycin-resistant, and tetracycline-resistant bacteria) were used, impact scores may

range from -4 to 4. The 90th percentile boundary (IS_{90}) provided an appropriate signal-to-noise ratio for the proposed index. A significant difference between all sites upstream (n =17) of a dam to all sites downstream (n = 18) of a dam was observed for both the spring ($P < 0.01$) and summer ($P < 0.05$) seasons, but not for the fall ($P = 1.0$) season using a Student's *t*-test with a two-tail distribution and unequal variance.

This may be due to the overall weather conditions during the sampling periods. During the spring and summer, water levels were high-to-normal for that time of year. During the fall sampling season, rainfall amounts were high enough to cause flood-like conditions (very high water, high turbidity, heavy rains) at many sites. Significant runoff into waters below dams changes the assumption that water quality in the stream is primarily influenced by water passing through the dam. The weather conditions in the fall may have caused higher bacterial counts than normal both above and below the dams which would have an influence on the overall impact score generated. Thus, no significant difference could be observed between the upstream and downstream sites during the fall season.

Upstream v. Downstream per Dam (Average Counts)

Upstream and downstream averages of microbiological data for each dam (n = 6) were used for the determination of any change in antibiotic resistant bacteria and fecal coliforms (4 variables) during each sampling season. With four variables analyzed at six dams per season, a total of 24 components were analyzed per season. A drop in bacterial counts was expected to occur from upstream to downstream due to sedimentation in the retention area before each dam. Bacteria associated with the sediment would settle out in

the retention area above each dam, thus giving less sediment and associated-bacteria at the output (downstream site) for each dam. The hypothesis of counts decreasing was observed in the majority of components analyzed for each season; any increase is discussed below. Significance in changes of counts from upstream-to-downstream were determined using the 95th confidence level (DF = 1; $\chi^2 > 3.84$) for the goodness-of-fit statistical analysis.

During the spring season, a significant increase ($\chi^2 > 3.84$) was observed for a portion of components analyzed (8 of 24; or 33.3%). Fecal coliforms and antibiotic-resistant bacteria increased at both the Dewey Dam and the North Fork of the Pound Dam. The increase at Dewey Dam could be due to the high turbidity from heavy rainfall prior to sampling the sites associated with this dam. Significant runoff below the Dewey Dam may have influenced the overall bacterial counts. The increase at the North Fork of the Pound Dam is most probably due to the location both of the dam within the watershed and the location of the downstream site. Of the six dams, this dam is closest to the headwaters region in the watershed. Therefore, the upstream sites of this dam would be expected to have relatively low bacterial counts in comparison to the rest of the sampling sites. In addition, between the dam and the downstream site there is a secondary tributary (not sampled) that flows into the waterway. This tributary may be a cause of the increased bacterial counts occurring at the downstream site.

During the summer season, a significant increase ($\chi^2 > 3.84$) was observed for a portion of components analyzed (5 of 24; or 20.8%). Fecal coliforms and antibiotic-resistant bacteria increased at the Paintsville Dam, and ciprofloxacin-resistant bacteria increased at the Fishtrap Dam. One explanation for the increases at the Paintsville Dam

and Fishtrap Dam could be due to the location of the outfalls for these dams. A selective withdrawal system is in place at both of these dams, that is, the location of where the water is being drawn from for downstream flow is variable (low, middle, or surface). Knowledge for where the water was being drawn from prior to and at the time of sampling is not known. Water may have been drawn from near the sediment-rich bottom. If this was the case, it would be expected that an increase in certain bacteria would be expected if associated with the sediment.

During the fall season, a significant increase ($\chi^2 > 3.84$) was observed for a portion of components analyzed (4 of 24; or 16.7%). Fecal coliforms and antibiotic-resistant bacteria increased at the North Fork of the Pound Dam, and fecal coliforms increased at the Fishtrap Dam. An explanation for the possible increase at the North Fork of the Pound Dam was given in the explanation for the increase observed at this dam during the spring season and remains applicable in this case as well (see above). A possible explanation for the increase in fecal coliforms at the Fishtrap Dam could be due to a potential of localized fecal contamination prior to or at the time and site of sampling.

Upstream v. Downstream per Dam (Percent Antibiotic Resistance)

The average upstream count and downstream count for each dam (n = 6) was compared to the corresponding total cultivable bacteria count to determine the percent of total cultivable bacteria that were ciprofloxacin-resistant, erythromycin-resistant, and tetracycline-resistant upstream and downstream at each dam. It was expected that the percent antibiotic resistance would be the same from upstream to downstream at each dam. The reasoning behind this hypothesis is that as the total cultivable bacteria would

decrease/increase, the antibiotic-resistant bacteria would decrease/increase in the same proportion as they are part of the total cultivable bacteria population. Significance in changes of counts from upstream-to-downstream were determined using the 95th confidence level (DF = 1; $\chi^2 > 3.84$) for the chi-square statistical analysis.

During the spring and summer seasons, a significant ($\chi^2 > 3.84$) increase or decrease was observed for percent antibiotic resistance at each dam. During the fall season, there was a significant ($\chi^2 > 3.84$) differences in percent antibiotic resistance at each dam with the exception that there was no significant change in percent tetracycline-resistance at the following dams: Paintsville ($\chi^2 = 2.21$), Dewey ($\chi^2 = 1.54$), Fishtrap ($\chi^2 = 0.02$). No explanation for the observed significant increases/decreases could be ascertained. The apparent selective settling of some populations is not fully understood.

Of additional note, a study looking at species specific (*Escherichia coli*) resistance found resistance to tetracycline to be greater than resistance to ciprofloxacin (36). However, among total cultivable bacteria, percent ciprofloxacin resistance was greater at all six dams than was percent tetracycline resistance in this study. One explanation could be that their findings differ because they looked at species specific resistance.

Physical Parameters

Physical parameter data [water temperature (°C), turbidity (NTU), specific conductivity (µmho/cm), oxygen (mg/l), pH, and alkalinity (mg/l)] was gathered for the spring and summer seasons. A correlation analysis was performed between all microbiological data and physical parameter data. Significant correlations were

determined using both the 95th ($P < 0.05$) and the 99th ($P < 0.01$) confidence levels for the correlation coefficient statistical test measuring the strength of association between two variables. Although no physical parameter data was obtained during the fall sampling, a correlation analysis was performed between the microbiological data for that season. The discussion will focus on any correlations associated with the microbiological data.

During the spring season, all microbiological populations (total cultivable bacteria, antibiotic-resistant bacteria, fecal coliforms) correlated to each other significantly ($P < 0.01$). In addition, turbidity was significantly correlated to all microbiological populations. Because of the weather conditions around the time of sampling, turbidity levels in the spring were high (range: 4-1300 NTU). High counts of bacteria across the spectrum would be expected with high turbidity levels due to the association of bacteria to sediment particles. Because all counts are high due to high turbidity, it would be expected that a significant correlation would exist. In addition, total cultivable bacteria was significantly ($P < 0.05$) correlated with pH. The pH for all sites (range: pH 7-8.1) was within the acceptable range (pH 6-9) set by the Environmental Protection Agency for water quality standards. This correlation was not observed in the summer season although a similar pH range was observed (pH 6.5-8.1), thus the correlation observed in the spring could be due the consistently high total cultivable bacteria counts caused by the weather conditions.

During the summer season, a significant ($P < 0.01$) correlation occurred between the following microbiological populations: fecal coliform, erythromycin-resistant, and tetracycline-resistant bacteria. One explanation could be that these populations overlap. Turbidity was significantly ($P < 0.01$) correlated to erythromycin-resistant bacteria. This

correlation may suggest that the erythromycin-resistant bacteria are more closely associated with particulates in the water. Turbidity was associated with more populations in the spring; however, the turbidity levels in the summer sampling were much lower (range: 0-250 NTU). Tetracycline-resistant bacteria were significantly ($P < 0.05$) correlated to temperature, specific conductivity, and alkalinity. Although these correlations were not observed in the spring season, the weather conditions in the summer involved less rainfall and lower water levels than in the spring. The more normal weather conditions may be one explanation why these correlations appear in the summer and were not visible in the spring.

Although no physical parameter data were obtained during the fall sampling, a correlation analysis was performed between the microbiological data. A significant correlation ($P < 0.01$) occurred between all microbiological data, except between the total cultivable bacteria and total coliforms. The weather conditions around the time of sampling involved heavy rainfall and high water levels, similar to the spring. Although no turbidity data was obtained, the similar trend for the spring and fall seasons between the weather conditions and significant correlations among the microbiological populations suggests that heavy rainfalls causes the populations to be correlated. One possible explanation for the lack of correlation between the total cultivable bacteria and total coliforms could be that these populations do not overlap. Since no total coliform analysis was conducted for the spring, the absence of a correlation between the total cultivable bacteria and total coliforms ($P = 0.2027$) in the fall can not be compared to the spring.

Water Chemistry

Water chemistry data was obtained for the spring and summer seasons only due to a lack of funds for the fall season. This data was compared to average counts for the antibiotic-resistant bacteria data to determine if any correlation occurs. Significant correlations were determined using both the 95^{th} ($P < 0.05$) and the 99^{th} ($P < 0.01$) confidence levels for the correlation coefficient statistical test measuring the strength of association between two variables.

The only significant correlation that was repeated in both the spring and summer seasons was between ciprofloxacin-resistant bacteria and dissolved Kjeldahl ($P < 0.01$ for spring, $P < 0.05$ for summer). All other significant correlations were not consistent from season-to-season. This emphasizes the need to take multiple samples over many seasons before any conclusions should be made. Taking samples in one sampling period only would most likely result in false conclusions drawn from correlation data. For this reason, no conclusions will be drawn from the correlation data obtained from the water chemistry analysis. Although ciprofloxacin-resistant bacteria and dissolved Kjeldahl were significantly correlated in both the spring and summer seasons, more sampling would need to be performed to verify if this phenomenon remains consistent from season-to-season and from year-to-year.

Kentucky Index of Biological Integrity

The Kentucky Index of Biological Integrity (KIBI) scores (range: 0-100) determined for 24 of 35 sites during the summer season were compared to the summer microbiological average counts and site impact scores to determine if any correlation

occurs. Significant correlations were determined using the 95th ($P < 0.05$) confidence level for the correlation coefficient statistical test measuring the strength of association between two variables.

No significant correlation occurred with the traditional measure of water quality, fecal coliforms, or the proposed bacteria-based index for water quality, impact scores (IS-85, IS-90, IS-95). An explanation for the lack of correlations can be that the KIBI is based on fish populations which are good indicators of any long-term (multiple years) effects; whereas, bacteria are more indicative of short-term effects due to their rapid life cycles and susceptibility to a number of environmental factors. Therefore, measuring water quality with only one traditional measure is not enough to determine overall water quality. It is necessary to use multiple measures of water quality, including microbiological populations, to determine water quality for a given location with any sense of confidence.

Chapter 5

Conclusions

The objectives of this study were to gather baseline data for the United States Army Corp of Engineers (USACE), to test a novel bioindicator of water quality based on antibiotic resistant and fecal indicator bacteria, and to determine any effect that dams may have on water quality.

The first objective was completed by providing microbiological data at 35 sites within the Big Sandy Watershed. The idea was to provide the Corp with baseline data based on seasons; however, a more important aspect of the data was observed. That aspect is microbiological data based on water level and rainfall amounts. Seasonally, this data was not as sound due to the variation in the weather variables. All samples must be taken under similar weather and flow conditions for normalization of the data. However, due to the fluctuation in water levels and rainfall amounts between the sampling periods, this data should be viewed more along the weather variables.

The second objective was completed by analyzing the results of site impact scores within the watershed. The impact scores were generated following the proposed protocol for the bacteria-based bioindicator index. The scores showed a significant difference between upstream and downstream sites in two of the three sampling periods. The trend of the scores followed the trend shown in actual bacterial counts. That is, sites downstream typically had lower bacterial counts and a negative impact score; whereas, sites upstream had higher bacterial counts and higher impact scores.

Some concerns with using this proposed index for watershed analysis is that of waterbody size and site location. Sites ranged from small streams to navigable rivers within the watershed. The comparison of bacterial counts used to generate an impact score between such sites should be considered in the development of the index. In other water quality indices (i.e. Kentucky Index of Biological Integrity), waterbody size is calculated into the development of the site score. In addition, it was observed that small streams located at the headwaters of the watershed upstream of any dam scored negative impact scores. This would be expected; however, when a comparison of upstream to downstream was performed to determine significance, these sites may have skewed the results.

The third objective was to test the hypothesis that water quality improves downstream of a dam. This was shown at the majority of dams within the watershed in each sampling period. On average, fecal coliform and antibiotic-resistant bacteria counts decreased downstream of a dam. Using data on turbidity and weather conditions, it was determined that bacteria are highly correlated to turbidity, especially under high water and rainfall conditions. This positive correlation suggests an association between bacteria and particulates. With this knowledge, it can be postulated that the decline in bacterial counts downstream of dams is due in part to the settling of particulates and bacteria associated with particulates in the retention area upstream of dams.

Literature Cited

1. **Anderson, E. S., G. O. Humphreys, and G. A. Willshaw.** 1975. The molecular relatedness of R factors in Enterobacteria of human and animal origin. J Gen Microbiol **91:** 376-382.

2. **Ash, R. J., B. Mauck, and M. Morgan.** 2002. Antibiotic resistance of gram-negative bacteria in rivers, United States. Emerg Infect Dis **8(7):**713-716.

3. **Atlas, R. M.** 1997. Principles of Microbiology. Second Edition. United States: Wm. C. Brown Publishers/McGraw-Hill Company. Pp. 21, 481-482, 1189.

4. **Bayer Co.** 1983. Production of the antibiotic ciprofloxacin. German patent 3,142,854.

5. **Bayer Co.** June 1987. Production of the antibiotic ciprofloxacin. U.S. patent 4,670,444.

6. **Beers, M. H. and R. Berkow.** 1999. The Merck Manual of Diagnosis and Therapy. Seventeenth (Centennial) Edition. Section 13 Infectious Diseases, Chapter 153 Antibacterial Drugs. Whitehouse Station, New Jersey: Merck & Company, Inc.

7. **Bell, J. B., G. E. Elliot, and D. W. Smith.** 1983. Influence of sewage treatment and urbanization on selection of multiple resistance in fecal coliform populations. Appl Environ Microbiol **46(1):** 227-232.

8. **Boon, P. I. and M. Cattanach.** 1999. Antibiotic resistance of native and faecal bacteria isolated from rivers, reservoirs and sewage treatment facilities in Victoria, south-eastern Australia. Let Appl Microbiol **28:**164-168.

9. **Budavari, S.** 1996. The Merck Index. Twelfth Edition. Whitehouse Station, New Jersey: Merck & Company, Inc.

10. **Chemical and Biological Warfare Information (CBWInfo)Website.** 2005, copyright. Pharmaceuticals: Ciprofloxacin. [Online.] http://www.cbwinfo.com/Pharmaceuticals/Cipro.html [23 April 2005, last date accessed.]

11. **Clesceri, L. S., A. E. Greenberg, and A. D. Eaton.** 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edition. United States: American Public Health Association, American Water Works Association, Water Environmental Federation.

12. **Conover, Lloyd H.** January 1955. Production of the antibiotic tetracycline. U.S. patent 2,699,054.

13. **Coughter, J. P. and G. J. Stewart.** 1989. Genetic exchange in the environment. Antonie van Leeuwenhoek **55:** 15-22.

14. **Dhakephalkar, P. K. and B. A. Chopade.** 1994. High levels of multiple metal resistance and its correlation to antibiotic resistance in environmental isolates of *Acinetobacter*. BioMetals **7:**67-74.

15. **Eli Lilly & Co.** 1953. Production of the antibiotic erythromycin. U.S. patent 2,653,899.

16. **El-Zanfaly, H. T.** 1991. The need for new microbiological water quality criteria. Wat Sci Tech **24(2):**43-48.

17. **French, G. L., J. Ling, K. L. Chow, K. K. Mark.** 1987. Occurrence of multiple antibiotic resistance and R-plasmids in gram-negative bacteria isolated from faecally contaminated fresh-water streams in Hong Kong. Epidem Inf **98:**285-299.

18. **Goñi-Urriza, M., M. Capdepuy, C. Arpin, N. Raymond, P. Caumette, and C. Quentin.** 2000. Impact of an urban effluent on antibiotic resistance of riverine Enterobacteriaceae and *Aeromonas* spp. Appl Environ Microbiol **66(1):**125-132.

19. **Goñi-Urriza, M., L. Pineau, M. Capdepuy, C. Roques, P. Caumette, and C. Quentin.** 2000. Antimicrobial resistance of mesophilic Aeromonas spp. isolated from two European rivers. J Antimicrob Chemother **46(2):**297-301.

20. **González, R. G., M. L. Taylor, and G. Alfaro.** 1982. Bacteriologic study of a community water supply; detection of enterotoxigenic activity and resistance to antimicrobial drugs. Bulletin Pan Amer Health Org **16(4):**341-355.

21. **Grabow W. O. K., W. Prozesky, L. S. Smith.** 1974. Drug resistant coliforms call for review of water quality standards. Water Res **8:**1-9.

22. **Halling-Sørensen, B., S. Nors Nielsen, P. F. Lanzky, F. Ingersley, H. C. Holten Lützhøft, and S. E. Jørgensen.** 1998. Occurrence, fate and effects of pharmaceutical substances in the environment—a review. Chemosphere **36:**357-393.

23. **Hirsch, R., T. Ternes, K. Haberer, and K.-L. Kratz.** 1999. Occurrence of antibiotics in the aquatic environment. Sci Tot Environ **225:**109-118.

24. **Holm, J. V., K. Ruegge, P. L. Bjerg, T. H. Christensen.** 1995. Occurrence and distribution of pharmaceutical organic compounds in the groundwater downgradient of a landfill (Grindsted, Denmark). Environ Sci Technol **5:**1415-1420.

25. **Iwane, T., T. Urase, and K. Yamamoto.** 2001. Possible impact of treated wastewater discharge on incidence of antibiotic resistant bacteria in river water. Wat Sci Tech **43(2):**91-99.

26. **Kelch, W. J. and J. S. Lee.** 1978. Antibiotic resistance patterns of gram-negative bacteria isolated from environmental sources. Appl Environ Microbiol **36(3):**450-456.

27. **Loughman, K. R., A. N. Johnson, and C. C. Somerville.** 2004. Testing a bacteria-based bioindicator of water quality above and below USACE impoundments in the Big Sandy Watershed, abstr. N-190. *In* Abstracts of the 104th General Meeting of the American Society for Microbiology 2004. American Society for Microbiology, Washington, D.C.

28. **McArthur, J. V. and R. C. Tuckfield.** 2000. Spatial patterns in antibiotic resistance among stream bacteria: effects of industrial pollution. Appl Environ Microbiol **66(9):**3722-3726.

29. **National Park Service Website.** 7 January 2005, last modification date. River and water facts. [Online.] http://www.nps.gov/rivers/waterfacts.html [2 March 2005, last date accessed.]

30. **Niemi, M., M. Sibakov, S. Niemela.** 1983. Antibiotic resistance among different species of fecal coliforms isolated from water samples. Appl Environ Microbiol **45(1):**79-83.

31. **Ogan, M. T. and D. E. Nwiika.** 1993. Studies on the ecology of aquatic bacteria of the lower Niger delta: multiple antibiotic resistance among the standard plate count organisms. J Appl Microbiol **74:**595-602.

32. **Ohio River Valley Water Sanitation Commission Website.** http://www.orsanco.org/rivinfo/basin/tributaries.htm [19 April 2005, last date accessed.]

33. **Oklahoma State University Website.** 16 April 2001, last modification date. Tetracycline. [Online.] http://opbs.okstate.edu/~petracek/Chapter%2027%20protein [23 April 2005, last date accessed.]

34. **Papapetropoulou, M., J. Iliopoulou, G. Rodopoulou, J. Detorakis, and O. Paniara.** 1994. Occurrence and antibiotic-resistance of *Pseudomonas* species isolated from drinking water in southern Greece. J Chemother **6(2):**111-116.

35. **Purdue University School of Veterinary Medicine Website.** 28 October 2002, last modification date. Macrolide Derivatives. [Online.] http://www.vet.purdue.edu/bms/courses/lcme510/chmrx/macrohd.htm [23 April 2005, last date accessed.]

36. **Roe, M. T., E. Vega, S. D. Pillai.** 2003. Antimicrobial resistance markers of class 1 and 2 integron-bearing *Escherichia coli* from irrigation water and sediments. Emerg Infect Dis **9(7):**822-826.

37. **United States Geological Survey Website.** 5 January 2005, last modification date. Toxic Substances Hydrology Program. [Online.] http://toxics.usgs.gov/regional/contaminants.html [2 March 2005, last date accessed.]

38. **Wireman, J., C. A. Liebert, T. Smith, and A. O. Summers.** 1997. Association of mercury resistance with antibiotic resistance in gram-negative fecal bacteria of primates. Appl Environ Microbiol **63:**4494-4503.

39. **Yurieva. O., G. Kholodii, L. Minakhin, Z. Gorlenko, E. Kalyaeva, S. Mindlin, and V. Nikiforov.** 1997. Intercontinental spread of promiscuous mercury resistance transposons in environmental bacteria. Mol Microbiol **24:**321-329.

40. **Young, H.-K.** 1993. Antimicrobial resistance spread in aquatic environments. J Antimicrob Chemother **31:**627-635.

Table 1. Demographic profiles within the Big Sandy Watershed.

State, Counties, Country	Percent Individuals Below Poverty	Median Household Income	Population	% of County in Basin	Adjusted Population
WV	**17.9**	**29,696**	**1,808,344**		
McDowell	37.7	16,931	27,329	100	27,329
Mingo (3)	29.7	21,347	28,253	90	25427.7
Wayne (6)	19.6	27,352	42,903	33	14157.99
VA	**9.6**	**46,677**	**7,078,515**		
Buchanan (1)	23.2	22,213	26,978	100	26,978
Dickenson (5)	21.3	23,431	16,395	100	16,395
Tazewell	15.3	27,304	44,598	10	4459.8
Wise (2)	20	26,149	40,123	40	16049.2
KY	**15.8**	**33,672**	**4,041,769**		
Boyd	15.5	32,749	49,752	33	16418.16
Floyd (2)	30.3	21,168	42,441	100	42,441
Johnson (3)	26.6	24,911	23,445	100	23,445
Knott	31.1	20,373	17,649	33	5824.17
Lawrence (7)	30.7	21,610	15,569	90	14012.1
Letcher	27.1	21,110	25,277	5	1263.85
Magoffin	36.6	19,421	13,332	5	666.6
Martin	37	18,279	12,578	100	12,578
Morgan (2)	27.2	21,869	13,948	15	2092.2
Pike (4)	23.4	23,930	68,736	100	68,736
US	**12.4**	**41,994**	**281,421,906**		
Total Big Sandy Watershed Population					**318,274**

[a] Numbers taken from the US Census Bureau: Census 2000 Demographic Profiles.
[b] Numbers in parentheses indicate the number of sample sites in these counties.

Table 2. Seasonal Impact Scores (range -4 to 4) using the 85th Percentile (IS_{85}).

SITE ID	DESIGNATION	SPRING IS_{85}	SUMMER IS_{85}	FALL IS_{85}
BSR 0265	D	0	-2	0
BSR 1005	D	0	-2	0
BSR 1685	D	0	3	0
BSR 2595	D	0	2	4
DEW 0003	Up	-1	2	3
DEW 0004	Up	-4	0	0
DEW 0005	Up	-3	0	3
DEW 0049	D	0	0	-2
FRL 0002	Up	3	3	-4
FRL 0032	D	1	1	-3
JWF 0001	D	-1	-4	-4
JWF 0002	D	0	-3	0
JWF 0003	Up	-2	0	0
JWF 0021	D	1	1	-2
LFR 0017	D	1	0	-2
LFR 0024	D	1	0	-1
LFR 0025	D	0	0	4
LFR 0026	D	4	-3	4
LFR 0027	D	0	-1	4
NFP 0008	Up	-4	-3	-4
NFP 0009	Up	-4	1	0
PIV 0003	Up	0	0	1
PIV 0005	Up	0	1	0
PIV 0012	D	-1	1	0
TFV 0003	Up	0	1	0
TFV 0004	Up	0	-1	0
TFV 0042	Up	3	0	0
TFV 0043	Up	4	0	0
TFV 0044	Up	3	-2	0
YBC 0010	D	-3	0	-2
YBC 0024	Up	1	0	1
YBC 0053	Up	1	2	0
YBC 0054	Up	0	3	0

[a] Designation of Up (Upstream-water not passed through a dam) or D (Downstream-water passed through a dam) assigned to each site based on whether water at that location had passed through a USACE dam within the watershed. Sites receiving a score of zero during all three seasons not listed.

Table 3. Seasonal Impact Scores (range -4 to 4) using the 90th Percentile (IS_{90}).

SITE ID	DESIGNATION	SPRING IS_{90}	SUMMER IS_{90}	FALL IS_{90}
BSR 0265	D	0	-2	0
BSR 1005	D	0	-1	0
BSR 1685	D	0	1	0
BSR 2595	D	0	1	4
DEW 0003	Up	-1	1	1
DEW 0004	Up	-3	0	0
DEW 0005	Up	-3	0	3
DEW 0049	D	0	0	-2
FRL 0002	Up	3	3	0
FRL 0032	D	1	0	-2
JWF 0001	D	-1	-3	-3
JWF 0002	D	0	-2	0
JWF 0021	D	0	0	-1
LFR 0017	D	0	0	-2
LFR 0024	D	1	0	-1
LFR 0025	D	0	0	4
LFR 0026	D	4	-3	4
LFR 0027	D	0	-1	0
NFP 0008	Up	-4	-3	-4
NFP 0009	Up	-1	1	0
PIV 0003	Up	0	1	0
PIV 0005	Up	0	1	0
PIV 0012	D	0	1	0
TFV 0003	Up	0	1	0
TFV 0004	Up	0	-1	0
TFV 0042	Up	1	0	0
TFV 0043	Up	3	0	0
TFV 0044	Up	3	0	0
YBC 0010	D	-3	0	-1
YBC 0053	Up	0	1	0
YBC 0054	Up	0	3	0

[a] Designation of Up (Upstream-water not passed through a dam) or D (Downstream-water passed through a dam) assigned to each site based on whether water at that location had passed through a USACE dam within the watershed. Sites receiving a score of zero during all three seasons not listed.

Table 4. Seasonal Impact Scores (range -4 to 4) using the 95th Percentile (IS_{95}).

SITE	DESIGNATION	SPRING IS_{95}	SUMMER IS_{95}	FALL IS_{95}
BSR 1005	D	0	-1	0
BSR 1685	D	0	1	0
BSR 2595	D	0	1	3
DEW 0004	Up	-1	0	0
FRL 0002	Up	2	2	0
FRL 0032	D	1	0	-2
JWF 0001	D	0	-2	-1
JWF 0021	D	0	0	-1
LFR 0017	D	0	0	-1
LFR 0025	D	0	0	2
LFR 0026	D	1	0	3
NFP 0008	Up	-3	-3	-2
PIV 0012	D	0	1	0
TFV 0004	Up	0	-1	0
TFV 0042	Up	1	0	0
TFV 0043	Up	1	0	0
TFV 0044	Up	2	0	0
YBC 0010	D	-3	0	0
YBC 0053	Up	0	1	0
YBC 0054	Up	0	1	0

[a] Designation of Up (Upstream-water not passed through a dam) or D (Downstream-water passed through a dam) assigned to each site based on whether water at that location had passed through a USACE dam within the watershed. Sites receiving a score of zero during all three seasons not listed.

Table 5. Upstream and downstream sites sampled per dam.

USACE DAM	Upstream Site(s)	Downstream Site
Yatesville	YBC 0024	YBC 0010
	YBC 0053	
	YBC 0054	
Paintsville	PIV 0003	PIV 0012
	PIV 0004	
	PIV 0005	
Dewey	DEW 0003	DEW 0049
	DEW 0004	
	DEW 0005	
Fishtrap	FRL 0002	FRL 0032
J.W.Flannagan	JWF 0002	JWF 0001
	JWF 0003	
North Fork of Pound	NFP 0008	JWF 0002
	NFP 0009	

[a] Counts for these sites were used for individual dam data for the determination of any change in bacterial counts from upstream-to-downstream. Upstream sites averaged together to give an upstream value.

Table 6. Spring average counts with standard deviations per dam.

Yatesville	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	1666.7	-	2172.2	378.7	3692.2	208.3	2437.8	221
Downstream	0.0	-	1336.7	83.9	323.3	158.9	210.0	17.3
Paintsville	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	3233.3	-	1905.6	792.8	2361.1	420.2	1783.3	193.1
Downstream	1200.0	-	696.7	159.5	1480.0	365.9	1010.0	137.5
Dewey	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	566.7	-	365.6	258.9	786.7	115.7	288.9	73.0
Downstream	2200.0	-	1900.0	581.0	2213.3	751.6	886.7	49.3
Fishtrap	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	4750.0	353.6	6353.3	1020.3	10640.0	105.8	2350.0	581.0
Downstream	1850.0	1202.1	6180.0	834.5	5240.0	421.4	1666.7	115.9
JWFlannagan	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	1000.0	-	1816.7	116.5	2211.7	381.4	686.7	139.0
Downstream	0.0	-	1143.3	125.0	1450.0	70.7	466.7	41.6
NFofPound	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	125.0	-	508.3	69.5	688.3	138.2	228.3	81.6
Downstream	1200.0	-	2466.7	177.9	3270.0	660.2	1056.7	141.5

[a] Data used for figures 13 & 14.
[b] Upstream and downstream average counts for fecal coliform (FC), Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet) bacteria with standard deviations (S.D.). All counts reported are per 1 ml, except when indicated by *. (* = per 100 ml; "-" = no standard deviation, only one plate countable).

Table 7. Summer average counts with standard deviations per dam.

Yatesville	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	386.7	45.8	1350.0	586.9	1274.7	185.3	247.7	61.0
Downstream	220.0	28.3	213.0	63.5	625.0	35.4	130.0	20.0
Paintsville	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	140.0	-	3325.0	1508.2	959.0	240.4	171.3	44.9
Downstream	663.0	126.6	920.0	329.1	1263.0	414.0	400.0	26.5
Dewey	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	465.6	134.0	830.3	54.0	1518.3	233.3	86.3	18.7
Downstream	133.3	61.1	520.0	230.7	1290.0	144.2	67.0	5.8
Fishtrap	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	740.0	56.6	500.0	34.6	1950.0	278.7	360.0	14.1
Downstream	210.0	14.1	833.0	95.0	1227.0	185.0	305.0	63.6
JWFlannagan	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	155.0	47.8	200.0	70.1	632.5	25.3	103.5	11.5
Downstream	40.0	-	100.0	56.6	307.0	51.3	33.0	25.2
NFofPound	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	283.4	117.2	235.0	101.6	580.0	64.8	195.0	40.0
Downstream	60.0	40.0	133.0	30.6	595.0	7.1	70.0	17.3

[a] Data used for figures 15 & 16.
[b] Upstream and downstream average counts for fecal coliform (FC), Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet) bacteria with standard deviations (S.D.). All counts reported are per 1 ml, except for FC counts indicated by *. (* = per 100 ml; "-" = no standard deviation, only one plate countable).

Table 8. Fall average counts with standard deviations per dam.

Yatesville	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	643.3	72.8	1517.8	225.7	2317.8	292.9	666.1	14.0
Downstream	37.0	32.1	505.0	7.1	823.3	80.8	296.7	70.2
Paintsville	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	2173.3	113.1	1926.7	160.6	2502.2	231.2	563.9	103.7
Downstream	130.0	36.1	720.0	108.2	970.0	212.1	150.0	40.0
Dewey	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	2930.0	268.7	3405.0	363.0	5715.6	330.0	852.2	97.0
Downstream	40.0	34.6	220.0	43.6	2693.3	687.1	80.0	40.0
Fishtrap	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	117.0	28.9	406.7	51.3	806.7	20.8	46.7	11.5
Downstream	217.0	160.7	86.7	41.6	586.7	238.6	46.7	30.6
JWFlannagan	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	178.5	44.9	1266.7	102.4	1865.0	223.2	130.0	48.6
Downstream	30.0	26.5	340.0	43.6	586.7	75.7	40.0	0.0
NFofPound	FC*	FC*-S.D.	Cip	Cip-S.D.	Erythro	Erythro-S.D.	Tet	Tet-S.D.
Upstream	106.5	11.5	668.3	82.6	880.0	247.0	150.0	75.6
Downstream	130.0	43.6	1016.7	95.0	1873.3	142.9	186.7	76.4

[a] Data used for figures 17 & 18.
[b] Upstream and downstream average counts for fecal coliform (FC), Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet) bacteria with standard deviations (S.D.). All counts reported are per 1 ml, except for FC counts indicated by *. (* = per 100 ml).

Table 9. Spring percentages of antibiotic resistance per dam.

Yatesville	Cip	Erythro	Tet
Upstream	1.34%	2.28%	1.50%
Downstream	8.18%	1.98%	1.29%
Paintsville	Cip	Erythro	Tet
Upstream	1.57%	1.95%	1.47%
Downstream	0.68%	1.45%	0.97%
Dewey	Cip	Erythro	Tet
Upstream	0.30%	0.64%	0.24%
Downstream	1.09%	1.27%	0.51%
Fishtrap	Cip	Erythro	Tet
Upstream	2.43%	4.08%	0.90%
Downstream	3.03%	2.57%	0.82%
J.W.Flannagan	Cip	Erythro	Tet
Upstream	1.42%	1.73%	0.54%
Downstream	5.44%	6.90%	2.22%
NF of Pound	Cip	Erythro	Tet
Upstream	2.65%	3.60%	1.19%
Downstream	2.13%	2.84%	0.92%

[a] Data used for figures 19 & 20.
[b] Percentages of total cultivable bacteria that are Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet) upstream and downstream at each dam.

Table 10. Summer percentages of antibiotic resistance per dam.

Yatesville	Cip	Erythro	Tet
Upstream	1.81%	1.71%	0.33%
Downstream	0.18%	0.53%	0.11%
Paintsville	Cip	Erythro	Tet
Upstream	2.29%	0.66%	0.12%
Downstream	0.87%	1.19%	0.38%
Dewey	Cip	Erythro	Tet
Upstream	1.45%	2.65%	0.15%
Downstream	6.24%	15.48%	0.80%
Fishtrap	Cip	Erythro	Tet
Upstream	0.32%	1.25%	0.23%
Downstream	1.02%	1.50%	0.37%
J.W.Flannagan	Cip	Erythro	Tet
Upstream	0.08%	0.25%	0.04%
Downstream	1.18%	3.61%	0.39%
NF of Pound	Cip	Erythro	Tet
Upstream	1.21%	2.97%	1.00%
Downstream	0.04%	0.19%	0.02%

[a] Data used for figures 21 & 22.
[b] Percentages of total cultivable bacteria that are Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet) upstream and downstream at each dam.

Table 11. Fall percentages of antibiotic resistance per dam.

Yatesville	Cip	Erythro	Tet
Upstream	3.19%	4.87%	1.40%
Downstream	3.52%	5.74%	2.07%
Paintsville	Cip	Erythro	Tet
Upstream	5.34%	6.93%	1.56%
Downstream	6.55%	8.82%	1.36%
Dewey	Cip	Erythro	Tet
Upstream	2.68%	4.49%	0.67%
Downstream	2.13%	26.06%	0.77%
Fishtrap	Cip	Erythro	Tet
Upstream	3.49%	6.91%	0.40%
Downstream	0.72%	4.89%	0.39%
J.W.Flannagan	Cip	Erythro	Tet
Upstream	3.10%	4.57%	0.32%
Downstream	1.76%	3.03%	0.21%
NF of Pound	Cip	Erythro	Tet
Upstream	4.83%	6.36%	1.08%
Downstream	3.02%	5.56%	0.55%

[a] Data used for figures 23 & 24.
[b] Percentages of total cultivable bacteria that are Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet) upstream and downstream at each dam.

Table 12. Spring correlations: microbiological and physical parameter data.

Totals	**Totals**										
Cipro.	$2.68E^{-05}$	**Cipro.**									
Erythro.	$3.34E^{-07}$	$2.64E^{-09}$	**Erythro.**								
Tet.	$4.47E^{-07}$	$6.06E^{-05}$	$4.49E^{-09}$	**Tet.**							
FC	$2.16E^{-07}$	$2.80E^{-05}$	$4.38E^{-09}$	$5.65E^{-08}$	**FC**						
Temp. (°C)	0.09336	0.4215	0.9482	0.9446	0.6182	**Temp. (°C)**					
Turbid. (NTU)	$6.00E^{-06}$	0.01721	$3.18E^{-05}$	$1.85E^{-05}$	$2.957E^{-04}$	0.3803	**Turb. (NTU)**				
Specific Conduct.	0.8027	0.5408	0.211	0.01669	0.1099	0.002738	0.2288	**Spec. Cond.**			
Oxygen (mg/l)	0.1416	0.6486	0.8167	0.7497	0.517	$3.00E^{-05}$	0.8139	0.576	**Oxygen (mg/l)**		
pH	0.03615	0.1586	0.2578	0.7914	0.4779	0.02322	0.686	$7.08E^{-05}$	0.9433	**pH**	
Alkalin. (mg/l)	0.3637	0.922	0.6072	0.1838	0.5726	1.72E-05	0.4912	$2.69E^{-12}$	0.05584	$1.00E^{-05}$	**Alk. (mg/l)**

[a] *P*-values based on the correlation coefficient statistical test measuring the strength of association between two variables. Variables include microbiological data (No Total Coliform data available) and physical parameters. Values in red are significantly correlated at $P<0.01$; Values in blue are significant at $P<0.05$

Table 13. Summer correlations: microbiological and physical parameter data.

	Totals											
Cipro.	0.9899	**Cipro.**										
Erythro.	0.06554	0.2985	**Erythro.**									
Tet.	0.5807	0.1119	0.009804	**Tet.**								
FC	0.9714	0.8468	0.004992	0.00702	**FC**							
TC	0.1075	0.793	0.02297	0.8489	0.001574	**TC**						
Temp. (°C)	0.08091	0.806	0.5589	0.04446	0.7808	0.1664	**Temp. (°C)**					
Turbid. (NTU)	0.8957	0.8208	0.00414	0.3633	0.5937	0.7858	0.6231	**Turb. (NTU)**				
Specific Conduct.	0.7946	0.2471	0.5779	0.03616	0.8446	0.0314	0.3461	0.3887	**Spec. Cond.**			
Oxygen (mg/l)	0.6799	0.2363	0.5818	0.7373	0.06408	0.008375	0.13	0.2225	0.3294	**Oxyg. (mg/l)**		
pH	0.07551	0.7394	0.2192	0.1108	0.3399	0.0399	$6.735E^{-04}$	0.6414	$3.833E^{-04}$	0.03553	**pH**	
Alkalin. (mg/l)	0.0671	0.8745	0.1417	0.01894	0.6918	0.06553	0.0142	0.7366	$1.86E^{-06}$	0.5188	$1.94E^{-07}$	**Alk. (mg/l)**

[a] *P*-values based on the correlation coefficient statistical test measuring the strength of association between two variables. Variables include microbiological data and physical parameters. Values in red are significantly correlated at $P<0.01$; Values in blue are significant at $P<0.05$

Table 14. Fall correlations: microbiological data.

	Totals					
Cipro.	$2.75E^{-06}$	**Cipro.**				
Erythro.	$6.47E^{-06}$	$1.81E^{-13}$	**Eythro.**			
Tet.	$2.26E^{-04}$	$1.59E^{-11}$	$2.15E^{-10}$	**Tet.**		
FC	$4.85E^{-05}$	$5.68E^{-12}$	$3.00E^{-11}$	$2.86E^{-09}$	**FC**	
TC	0.2027	$1.13E^{-04}$	$2.84E^{-09}$	$7.61E^{-05}$	$8.88E^{-06}$	**TC**

[a] *P*-values based on the correlation coefficient statistical test measuring the strength of association between two variables. Variables include microbiological data. No physical parameter data available. Values in red are significantly correlated at $P<0.01$

Tables 15 A-C. Spring correlations: antibiotic resistance and water chemistry data.

(A)

	HCO_3, Diss. (mg/L)	Solids, Tot. (mg/L)	Solids, Diss. (mg/L)	Solids, Susp. (mg/L)	NH_3, Tot. (mg/L N)	Kjeldahl, Diss. (mg/L N)	Kjeldahl, Tot. (mg/L N)	NO_2+NO_3, Tot. (mg/L N)	NO_2+NO_3, Diss. (mg/L N)	Phos, Tot. (mg/L)	Phos, Diss. (mg/L)
Cipro.	0.6893	0.08007	0.3759	0.008973	0.6303	0.001702	$5.95E^{-04}$	0.9129	0.5643	0.00396	0.3896
Erythro.	0.418	0.006069	0.1076	$5.85E^{-06}$	0.8621	$3.06E^{-05}$	$3.55E^{-07}$	0.6538	0.9189	$4.65E^{-05}$	0.009419
Tet.	0.09479	0.05548	0.01031	$1.78E^{-05}$	0.3927	$7.17E^{-06}$	$2.29E^{-04}$	0.9928	0.4396	$3.99E^{-06}$	0.01912

(B)

	TOC (mg/L)	DOC (mg/L)	Carbon Inorg. Tot. (mg/L)	Carbon Inorg. Diss. (mg/L)	Calcium, Diss. (mg/L)	Magnesium, Diss. (mg/L)	Sodium, Diss. (mg/L)	Potassium, Diss. (mg/L)	Chloride, Diss. (mg/L)	Sulfate, Diss. (mg/L)	Barium, Diss. (µg/L)
Cipro.	0.03218	0.04447	0.6906	0.7029	0.5138	0.09344	0.06833	0.3476	0.5927	0.248	0.2281
Erythro.	0.001299	$7.26E^{-04}$	0.972	0.9315	0.1973	0.05378	0.5494	0.2703	0.568	0.09679	0.973
Tet.	$1.68E^{-04}$	$6.42E^{-05}$	0.2403	0.2174	0.02274	0.006882	0.2908	0.1038	0.07105	0.007519	0.0736

(C)

	Iron, Tot. (µg/L)	Iron, Diss. (µg/L)	Manganese, Tot. (µg/L)	Manganese, Diss. (µg/L)	Zinc, Diss. (µg/L)	Zinc, Tot. (µg/L)	Aluminum, Tot. (µg/L)	Aluminum, Diss. (µg/L)	Silicon, Diss. (mg/L)	Silicon, Tot. (mg/L)	Titanium, Tot. (µg/L)
Cipro.	0.02003	0.2507	0.0228	0.0716	0.7036	0.009142	0.01894	$4.36E^{-04}$	0.3506	0.008783	0.00246
Erythro.	$2.32E^{-05}$	0.0134	$1.74E^{-04}$	0.01993	0.4744	$7.68E^{-06}$	$2.07E^{-05}$	$3.40E^{-06}$	0.07554	$4.44E^{-06}$	$2.62E^{-06}$
Tet.	$1.90E^{-05}$	$7.56E^{-05}$	$4.89E^{-04}$	0.01464	0.2465	$2.36E^{-05}$	$2.88E^{-05}$	$1.6E^{-04}$	0.001682	$3.35E^{-06}$	$5.37E^{-06}$

[a] *P*-values based on the correlation coefficient statistical test measuring the strength of association between two variables. Variables include antibiotic-resistance and water chemistry. Values in red are significantly correlated at $P<0.01$; Values in blue are significant at $P<0.05$ Tot. = Total; Diss. = Dissolved; Susp. = Suspended; Inorg. = Inorganic

Tables 16 A-C. Summer correlations: antibiotic resistance and water chemistry data.

(A)

	HCO_3, Diss. (mg/L)	Solids, Tot. (mg/L)	Solids, Diss. (mg/L)	Solids, Susp. (mg/L)	NH_3, Tot. (mg/L N)	Kjeldahl, Diss. (mg/L N)	Kjeldahl, Tot. (mg/L N)	NO_2+NO_3, Tot. (mg/L N)	NO_2+NO_3, Diss. (mg/L N)	Phos, Tot. (mg/L)	Phos, Diss. (mg/L)
Cipro.	0.7251	0.1364	0.1506	0.4241	0.4825	0.01483	0.05691	0.1864	0.142	0.4158	0.1221
Erythro.	0.1803	0.8843	0.931	0.3746	0.08341	0.7541	0.6844	0.323	0.3183	0.1872	0.3552
Tet.	0.007908	0.07883	0.08685	0.4803	0.8559	0.5075	0.4013	0.05524	0.07838	0.3532	0.05157

(B)

	TOC (mg/L)	DOC (mg/L)	Carbon Inorg. Tot. (mg/L)	Carbon Inorg. Diss. (mg/L)	Calcium, Diss. (mg/L)	Magnesium, Diss. (mg/L)	Sodium, Diss. (mg/L)	Potassium, Diss. (mg/L)	Chloride, Diss. (mg/L)	Sulfate, Diss. (mg/L)	Barium, Diss. (µg/L)
Cipro.	0.3108	0.7803	0.9426	0.8643	0.4226	0.325	0.4206	0.6566	0.09926	0.116	0.192
Erythro.	0.9403	0.7856	0.1441	0.1309	0.7671	0.898	0.2285	0.3251	0.3154	0.9807	0.05641
Tet.	0.5358	0.8303	0.004808	0.005934	0.06964	0.1518	0.05263	0.071	0.4762	0.07545	0.0934

(C)

	Iron, Tot. (µg/L)	Iron, Diss. (µg/L)	Manganese, Tot. (µg/L)	Manganese, Diss. (µg/L)	Zinc, Diss. (µg/L)	Zinc, Tot. (µg/L)	Aluminum, Tot. (µg/L)	Aluminum, Diss. (µg/L)	Silicon, Diss. (mg/L)	Silicon, Tot. (mg/L)
Cipro.	0.9521	0.7239	0.7371	0.944	0.4207	0.4703	0.543	0.5313	0.3433	0.4655
Erythro.	0.1295	0.9793	0.5554	0.3489	0.8553	0.8018	0.2707	0.6832	0.4363	0.1894
Tet.	0.7995	0.2359	0.2721	0.1308	0.1204	0.08325	0.3179	0.7723	0.4863	0.9914

[a] *P*-values based on the correlation coefficient statistical test measuring the strength of association between two variables. Variables include antibiotic-resistance and water chemistry (No Titanium data). Values in red are significantly correlated at $P<0.01$; Value in blue are significant at $P<0.05$ Tot. = Total; Diss. = Dissolved; Susp. = Suspended; Inorg. = Inorganic

Table 17. Summer correlations: microbiological, impact score, and KIBI data.

	Kentucky Index of Biological Integrity
Totals	0.4913
Ciprofloxacin	0.1317
Erythromycin	0.9677
Tetracycline	0.07076
FC	0.2398
TC	0.2818
IS_{85}	0.8288
IS_{90}	0.3972
IS_{95}	0.5587

[a] *P*-values based on the correlation coefficient statistical test measuring the strength of association between two variables. Variables include microbiological data, Impact Score data (IS_{85}, IS_{90}, IS_{95}), and Kentucky Index of Biological Integrity.

Figure 1. Chemical Structure of Tetracycline

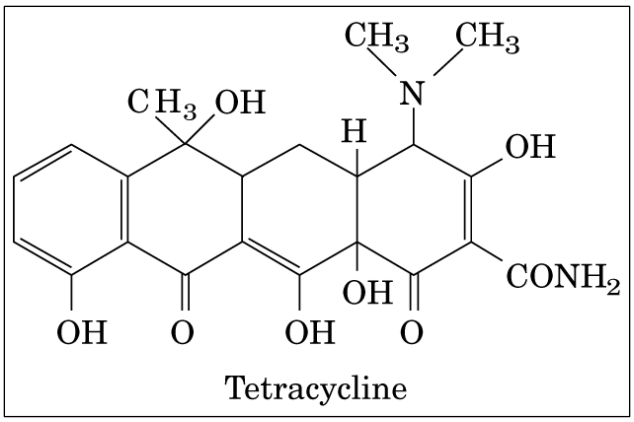


Figure 2. Chemical Structure of Erythromycin

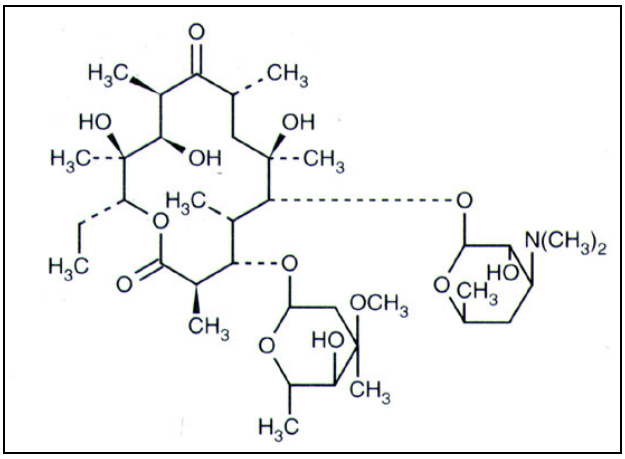


Figure 3. Chemical Structure of Ciprofloxacin

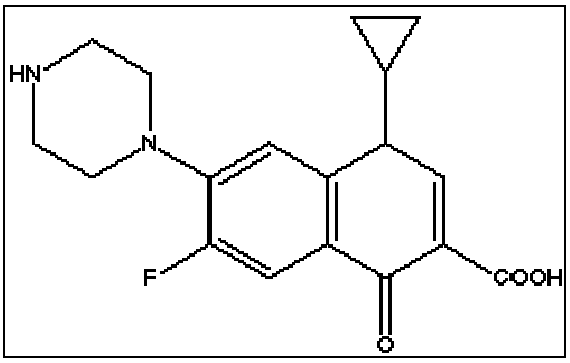
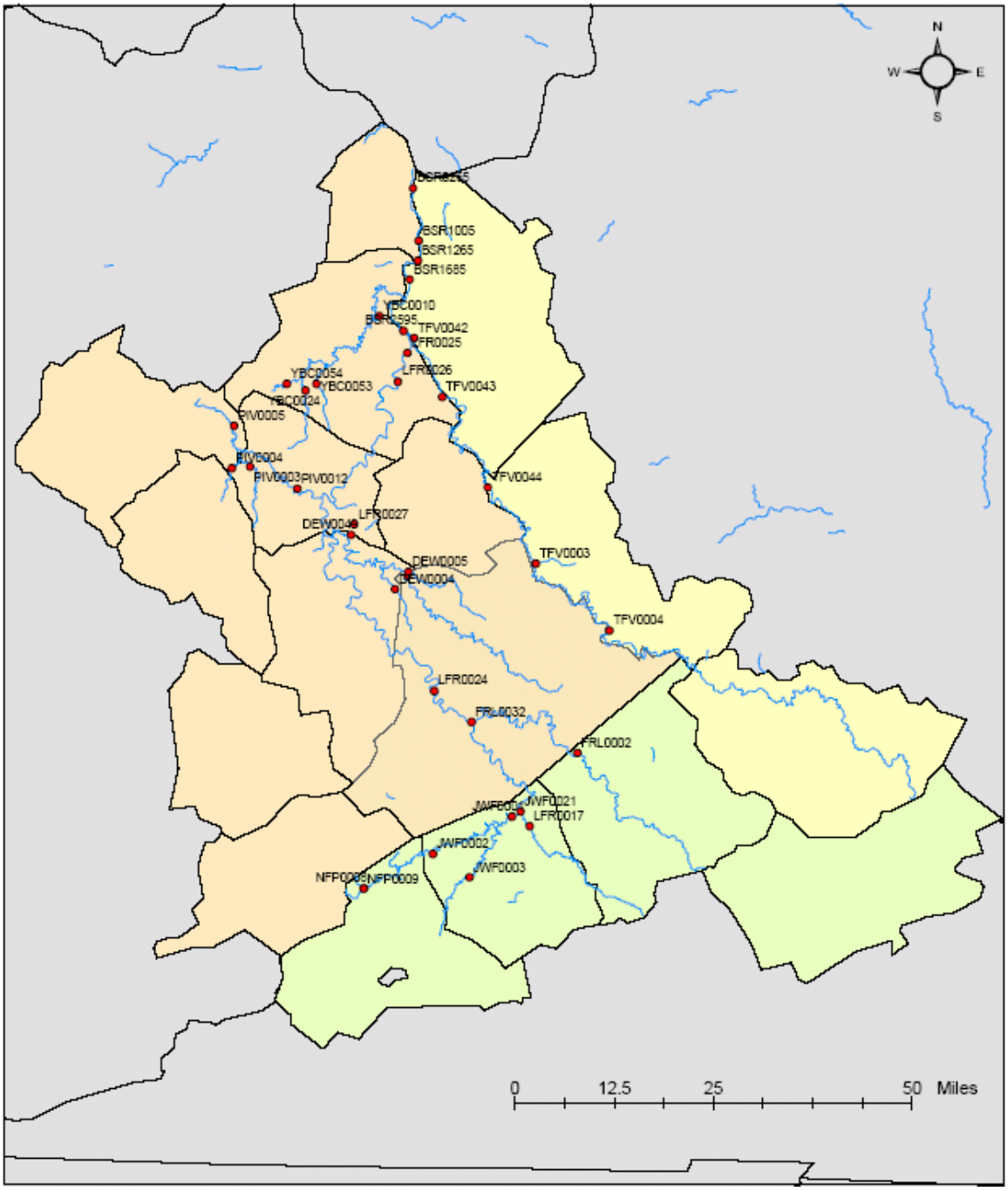
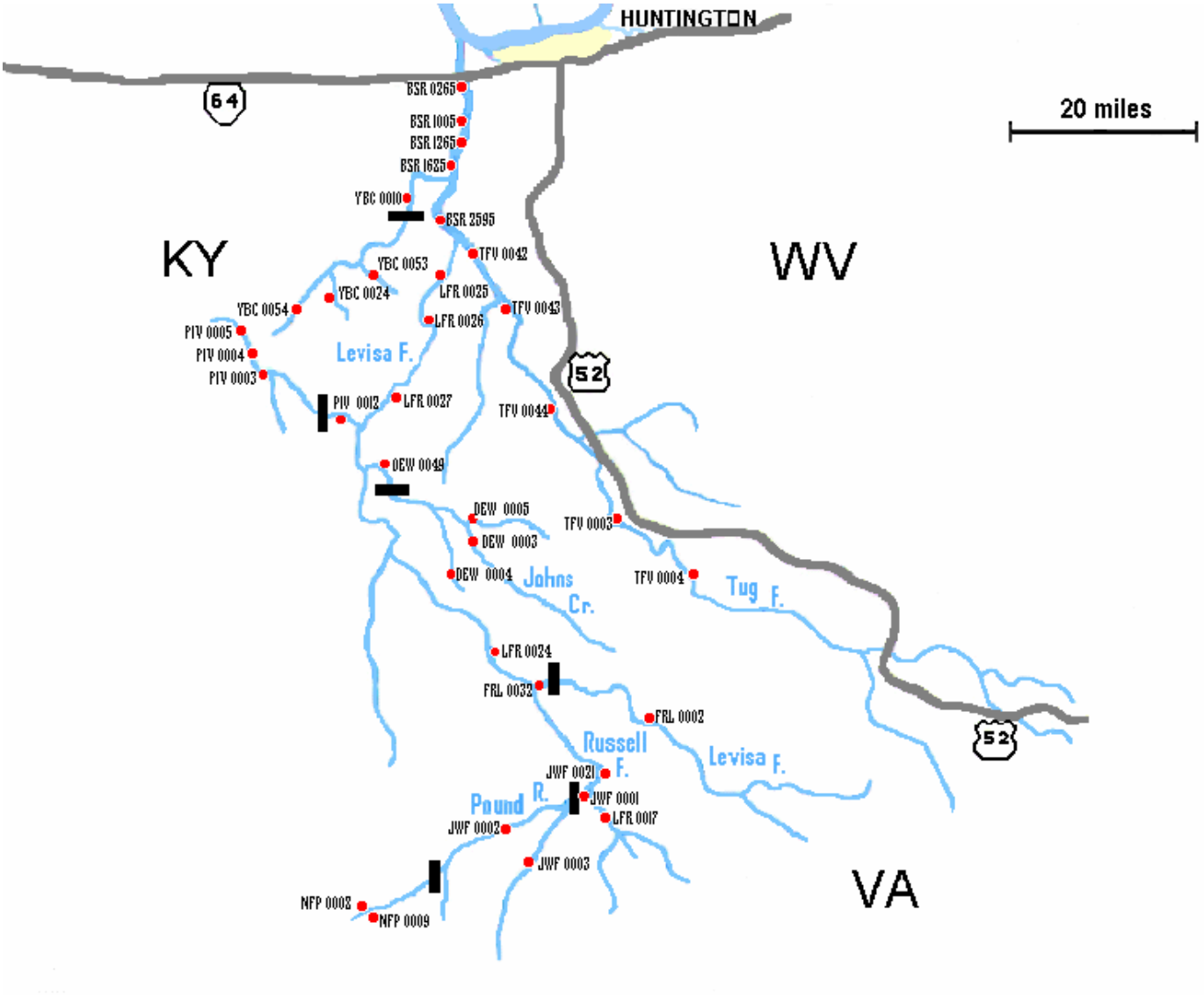


Figure 4. Site location map of the Big Sandy Watershed.



[a] Kentucky: peach; Virginia: green; West Virginia: yellow
[b] The thirty-five sampling sites are marked with red dots (UTM coordinates in GIS) and labeled with their site ID. Not all streams within the watershed are shown on the map.

Figure 5. USACE dam location map of the Big Sandy Watershed

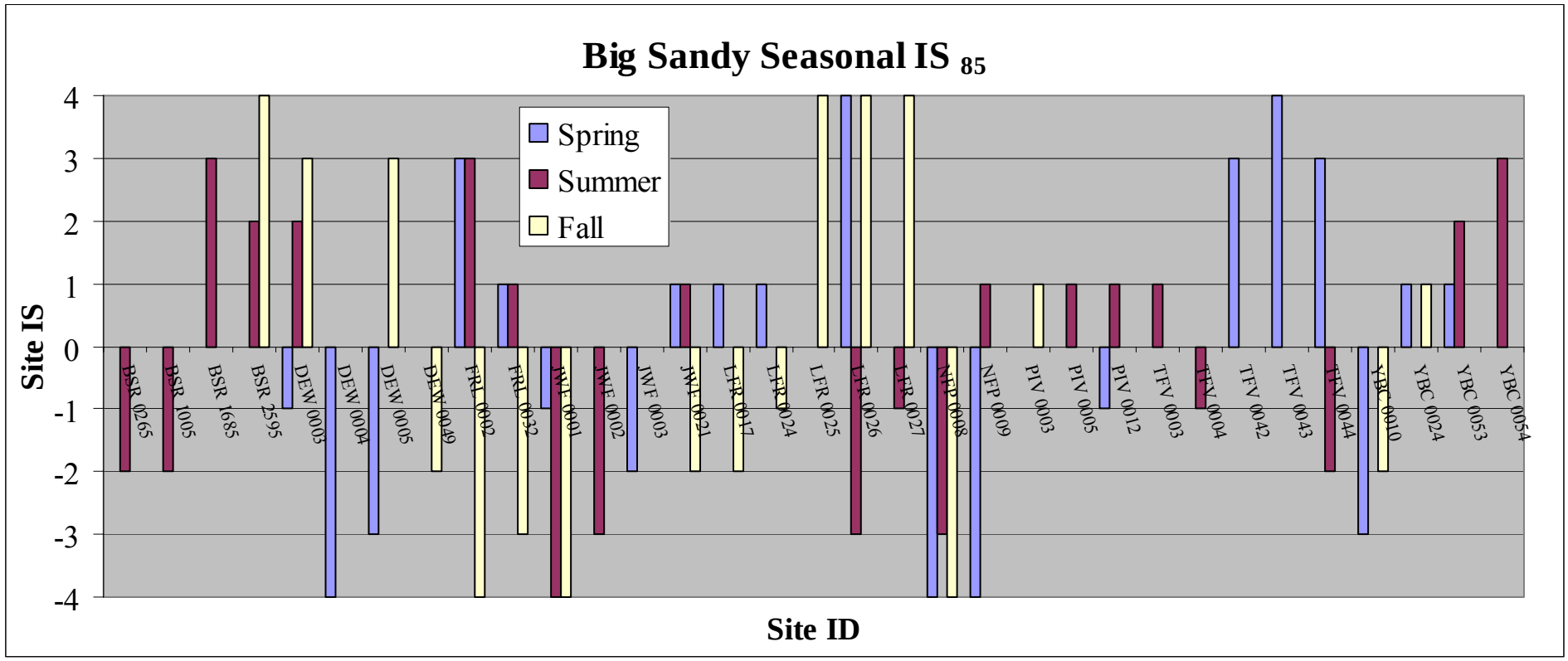


[a] The thirty-five sites where water samples were taken are marked with red dots (approximated coordinates) and labeled with their site ID. The six USACE dams are marked with horizontal and vertical black bars, and are from north-to-south as follows: Yatesville, Paintsville, Dewey, Fishtrap, J.W. Flannagan, and North Fork of the Pound. Not all streams within the watershed are present on map.

Figure 6. County location map of the Big Sandy Watershed.

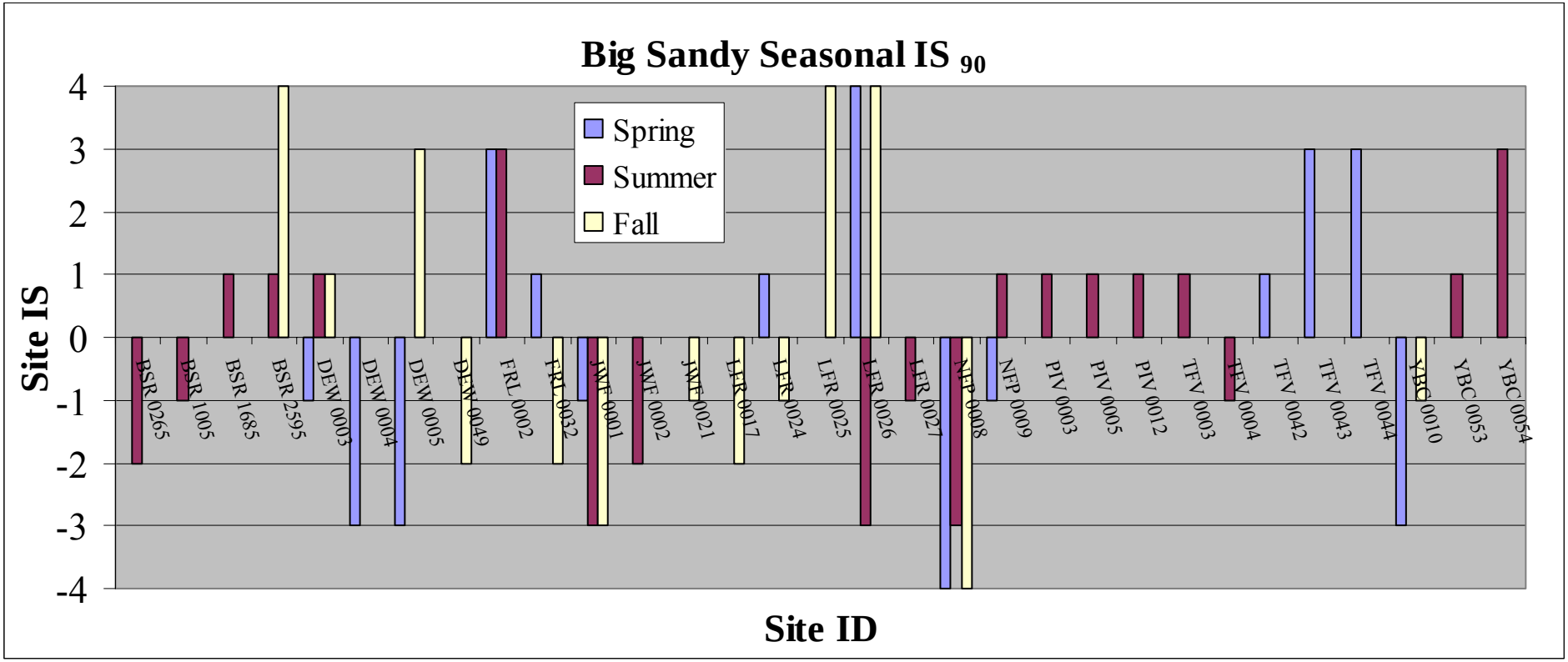
[a] Kentucky: peach; Virginia: green; West Virginia: yellow
[b] The thirty-five sampling sites are marked with red dots (UTM coordinates in GIS). Not all streams within the watershed are present on map.

Figure 7. Seasonal Impact Scores using the 85th Percentile (IS_{85}).



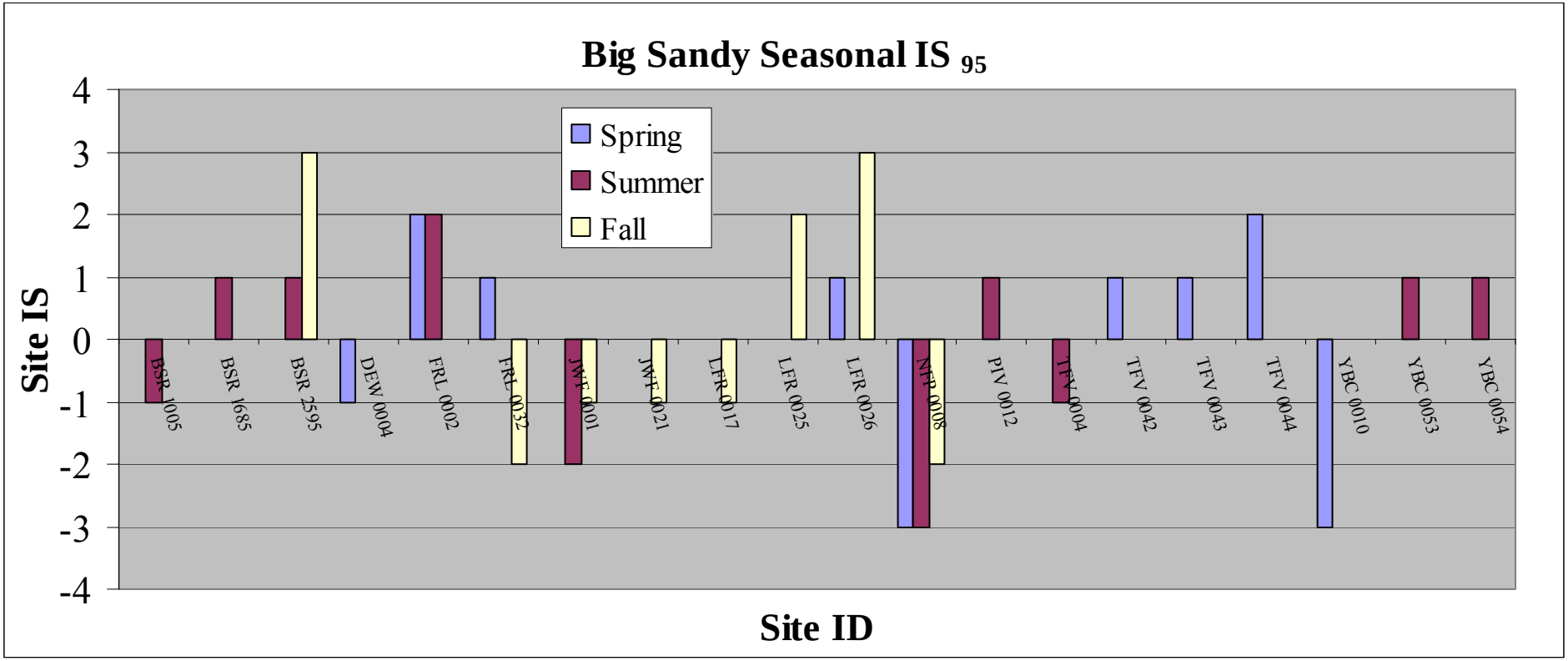
[a] Range -4 to +4
[b] Sites receiving a score of zero during all three seasons not included.

Figure 8. Seasonal Impact Scores using the 90th Percentile (IS_{90}).



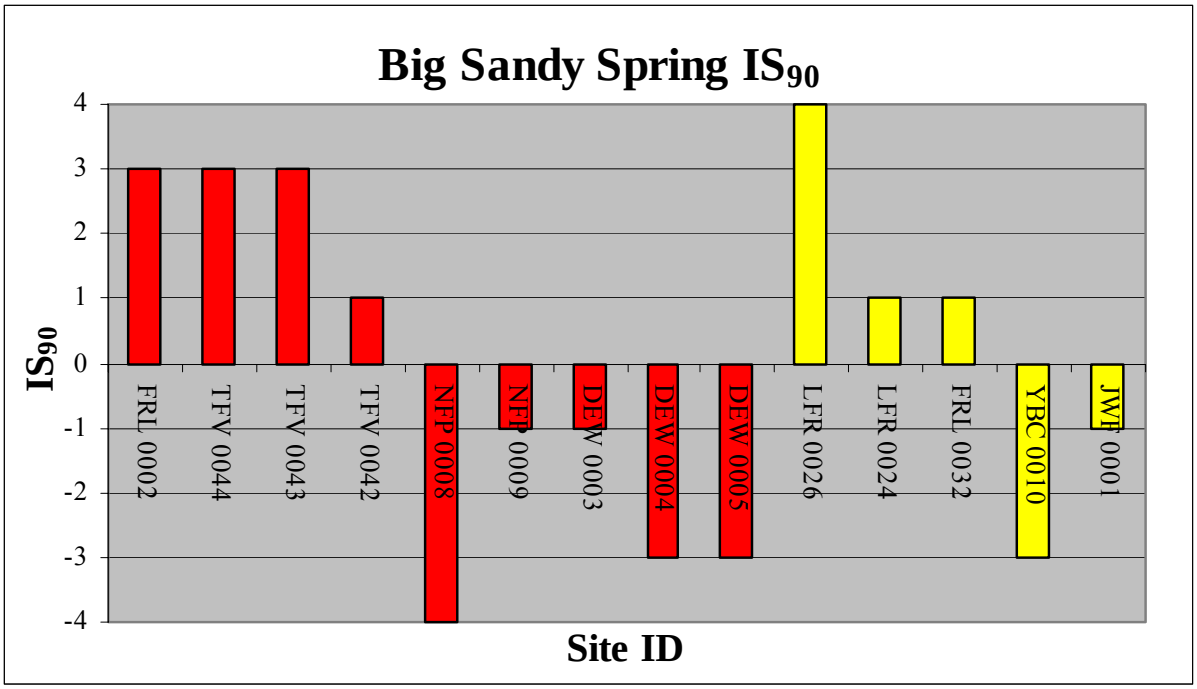
[a] Range -4 to +4
[b] Sites receiving a score of zero during all three seasons not included.

Figure 9. Seasonal Impact Scores using the 95th Percentile (IS_{95}).



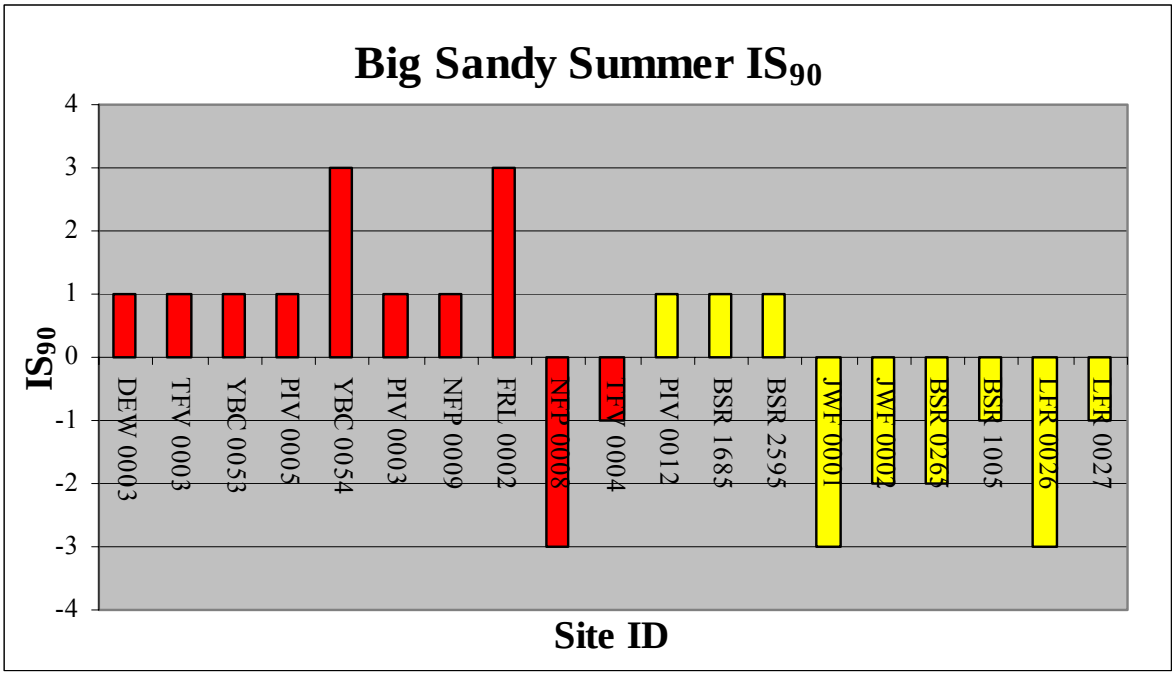
[a] Range -4 to +4
[b] Sites receiving a score of zero during all three seasons not included.

Figure 10. Spring Impact Scores using the 90th Percentile (IS_{90}).



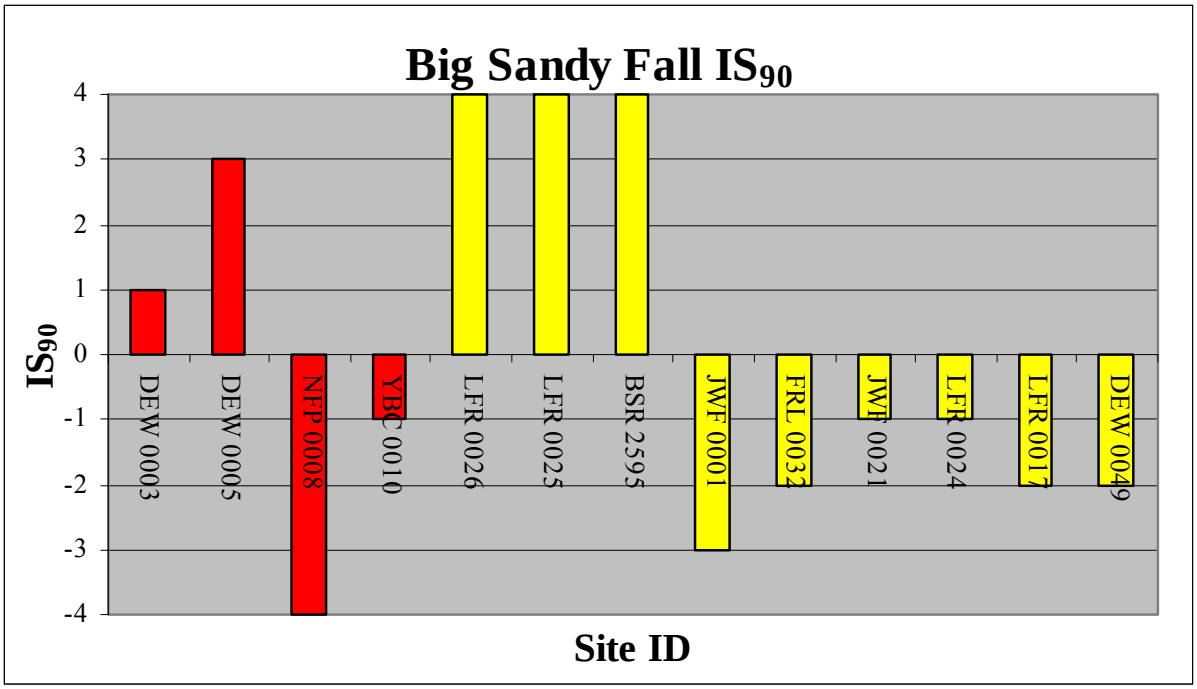
[a] Range -4 to +4
[b] Sites receiving a score of zero were not included in this figure.
[c] Red = upstream sites; Yellow = downstream sites. Fifty-seven percent of those sites with positive impact scores were locations upstream of USACE impoundments. Twenty-nine percent of those sites with negative impact scores were locations downstream of USACE impoundments. There is a significant difference ($P = 0.001007$) between the influenced and the uninfluenced sites.

Figure 11. Summer Impact Scores using the 90th Percentile (IS_{90}).



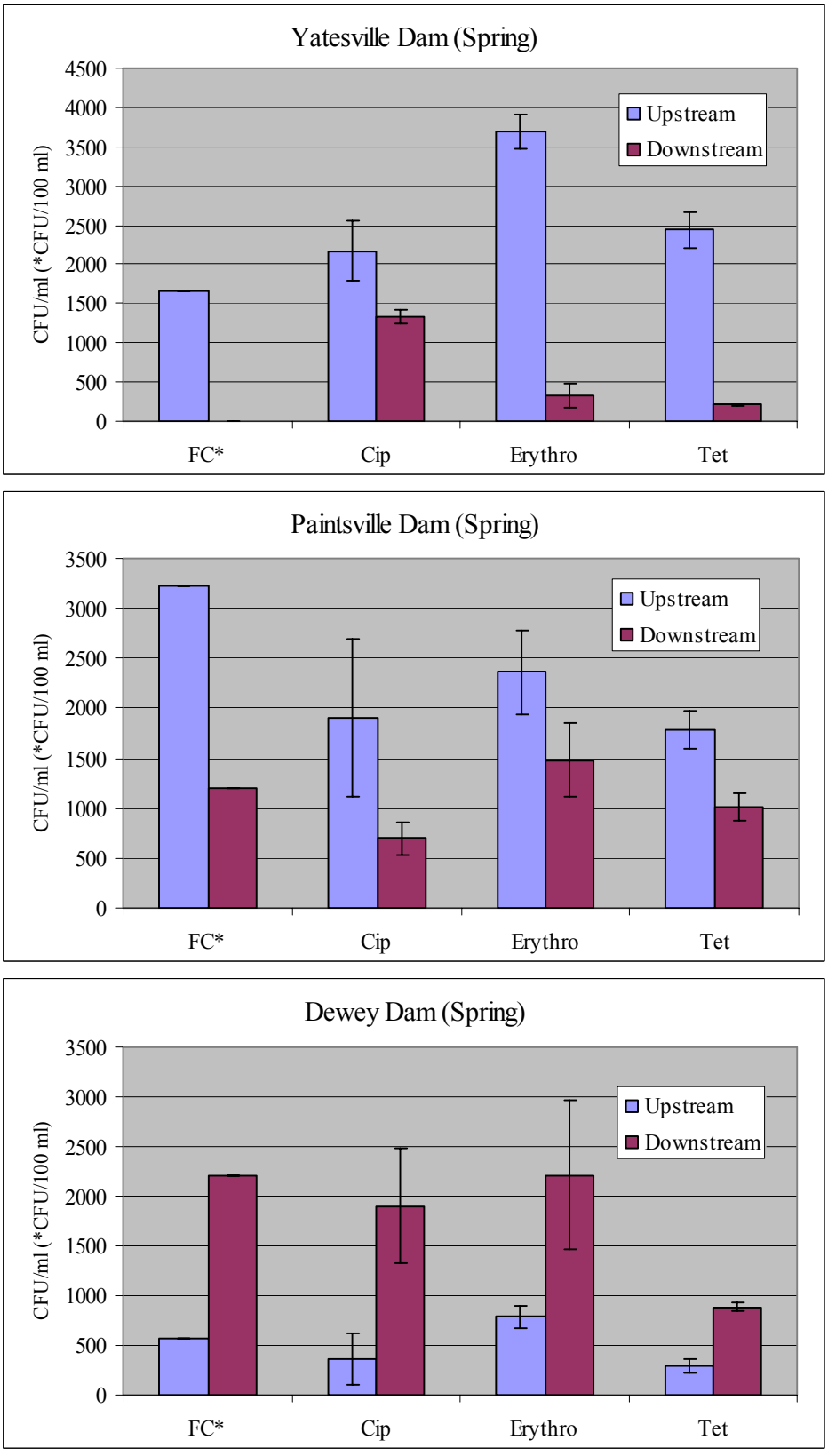
[a] Range -4 to +4
[b] Sites receiving a score of zero were not included in this figure.
[c] Red = upstream sites; Yellow = downstream sites. Seventy-three percent of those sites with positive impact scores were locations upstream of USACE impoundments. Seventy-five percent of those sites with negative impact scores were locations downstream of USACE impoundments. There is a significant difference ($P = 0.034917$) between the influenced and the uninfluenced sites.

Figure 12. Fall Impact Scores using the 90th Percentile (IS_{90}).



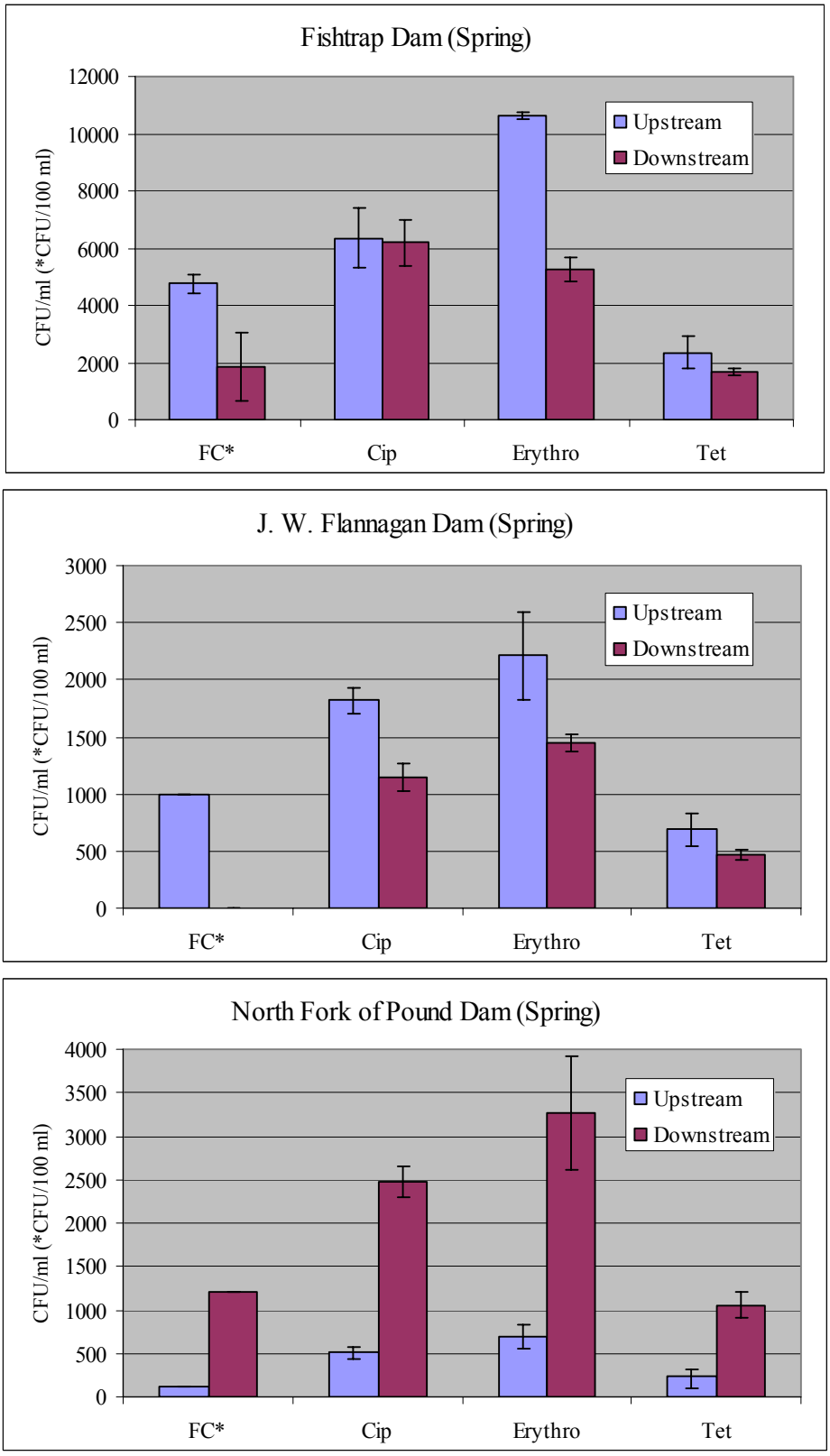
[a] Range -4 to +4
[b] Sites receiving a score of zero were not included in this figure.
[c] Red = upstream sites; Yellow = downstream sites. Forty percent of those sites with positive impact scores were locations upstream of USACE impoundments. Seventy-five percent of those sites with negative impact scores were locations downstream of USACE impoundments. There is no significant difference ($P = 1.0$) between the influenced and the uninfluenced sites.

Figure 13. Spring average counts (3 northern dams)



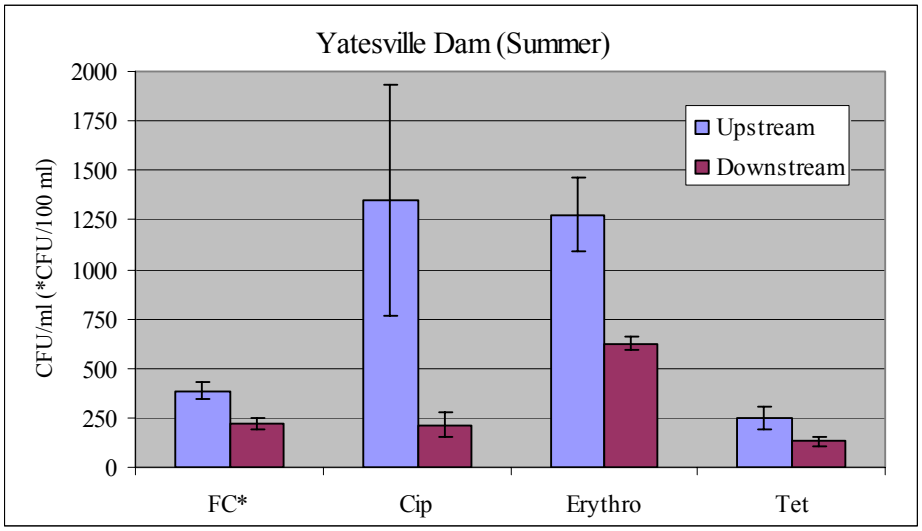
[a] Fecal coliforms (FC), Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet) bacteria. Standard deviation not available for FC. All changes in counts from upstream-to-downstream are significant at the 95th confidence level for the goodness-of-fit statistical analysis (DF = 1).

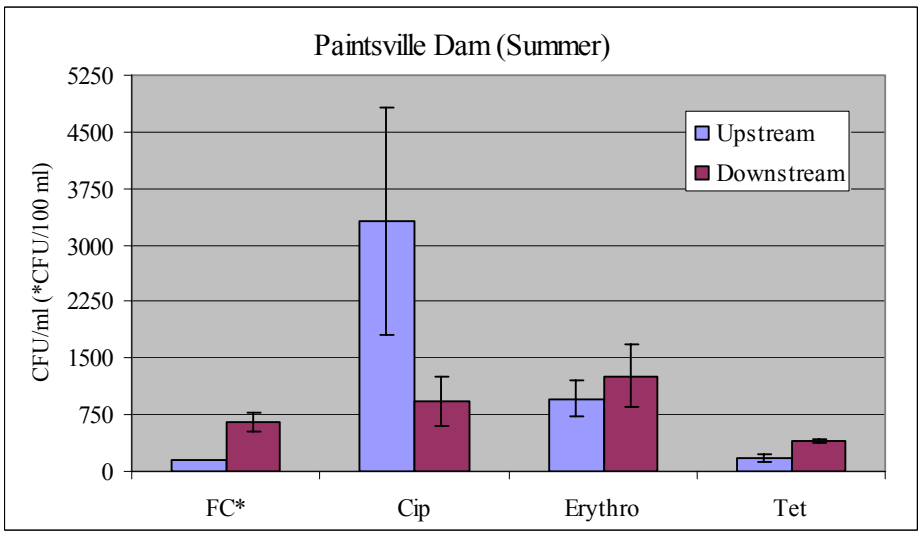
Figure 14. Spring average counts (3 southern dams).

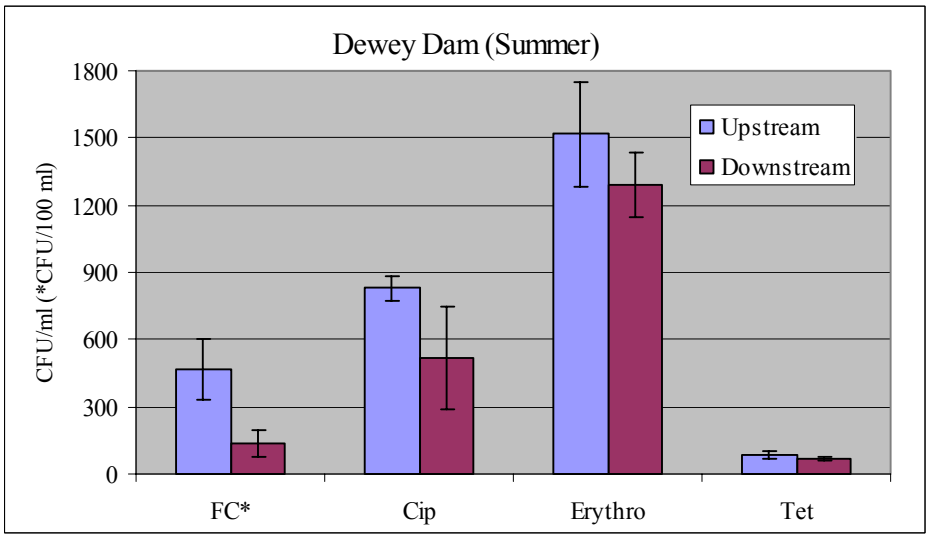


[a] Fecal coliforms (FC), Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet) bacteria. Standard deviation not available for FC, except at Fishtrap Dam. Changes in counts from upstream-to-downstream are significant at the 95th confidence level for the goodness-of-fit statistical analysis (DF = 1), except change of Cip at Fishtrap Dam not significant.

Figure 15. Summer average counts (3 northern dams).

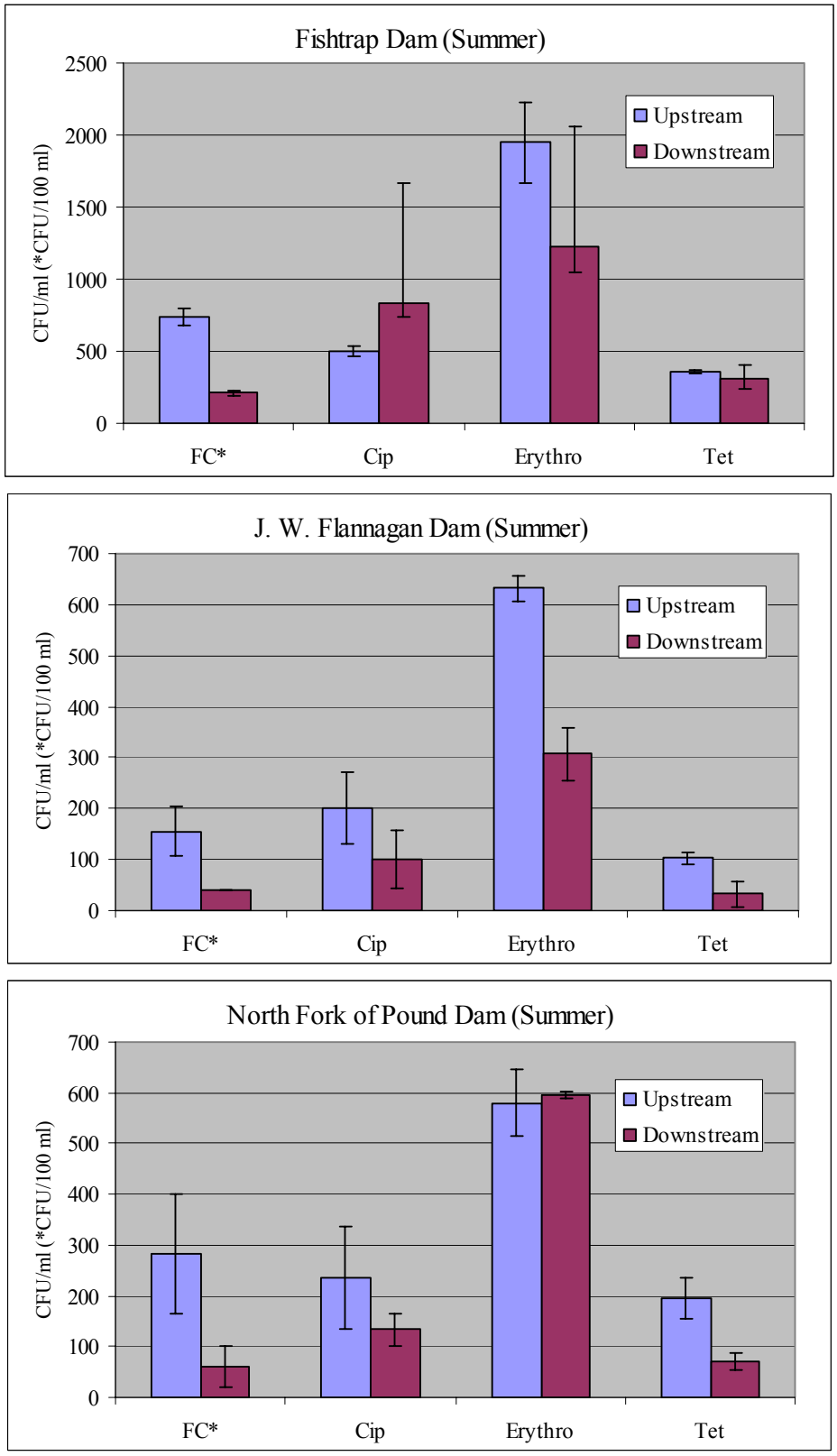






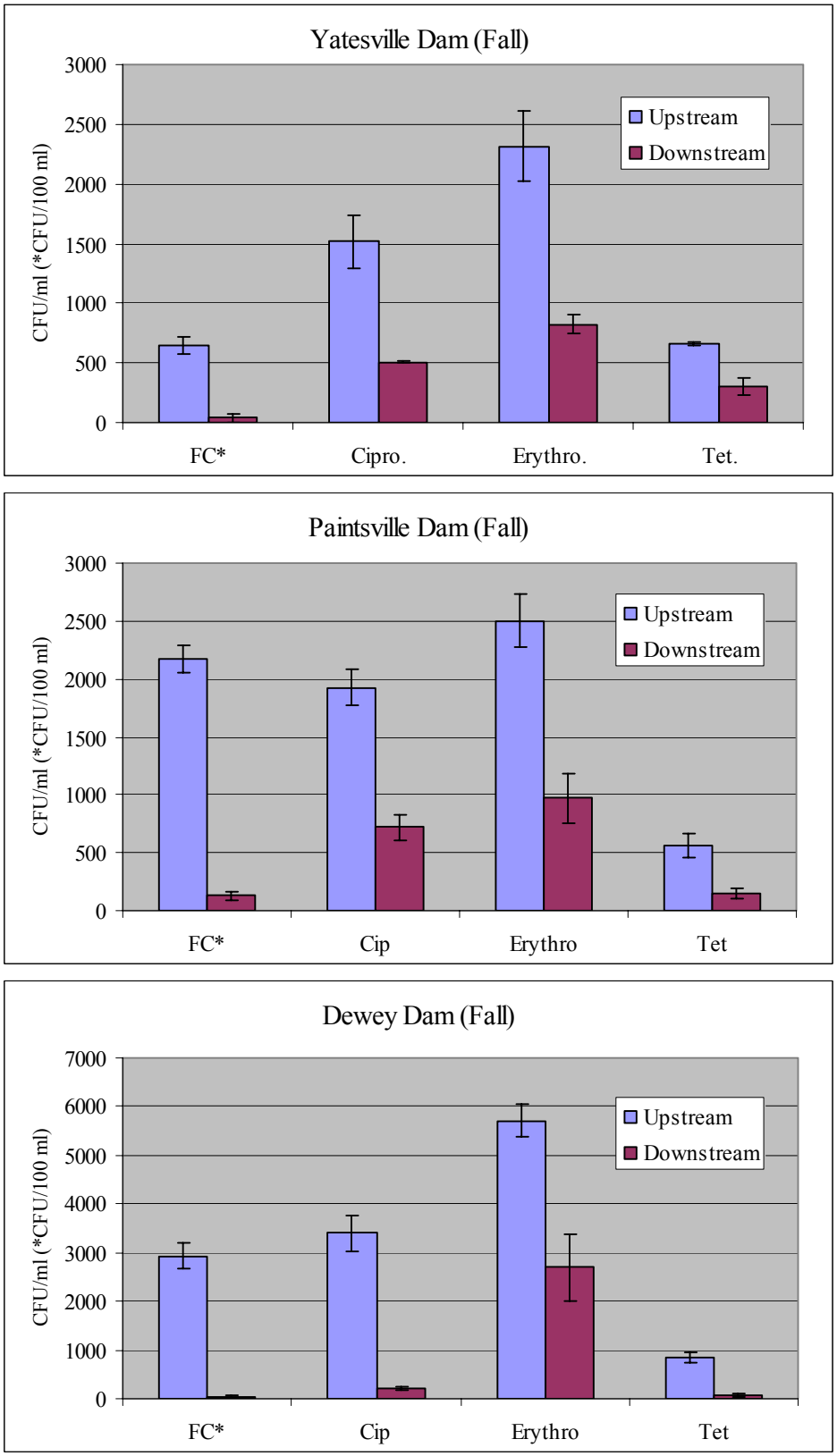
[a] Fecal coliforms (FC), Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet) bacteria. Standard deviation not available for Paintsville Dam Upstream FC. Changes in counts from upstream-to-downstream are significant at the 95th confidence level for the goodness-of-fit statistical analysis (DF = 1), except change of Tet at Dewey Dam not significant.

Figure 16. Summer average counts (3 southern dams).



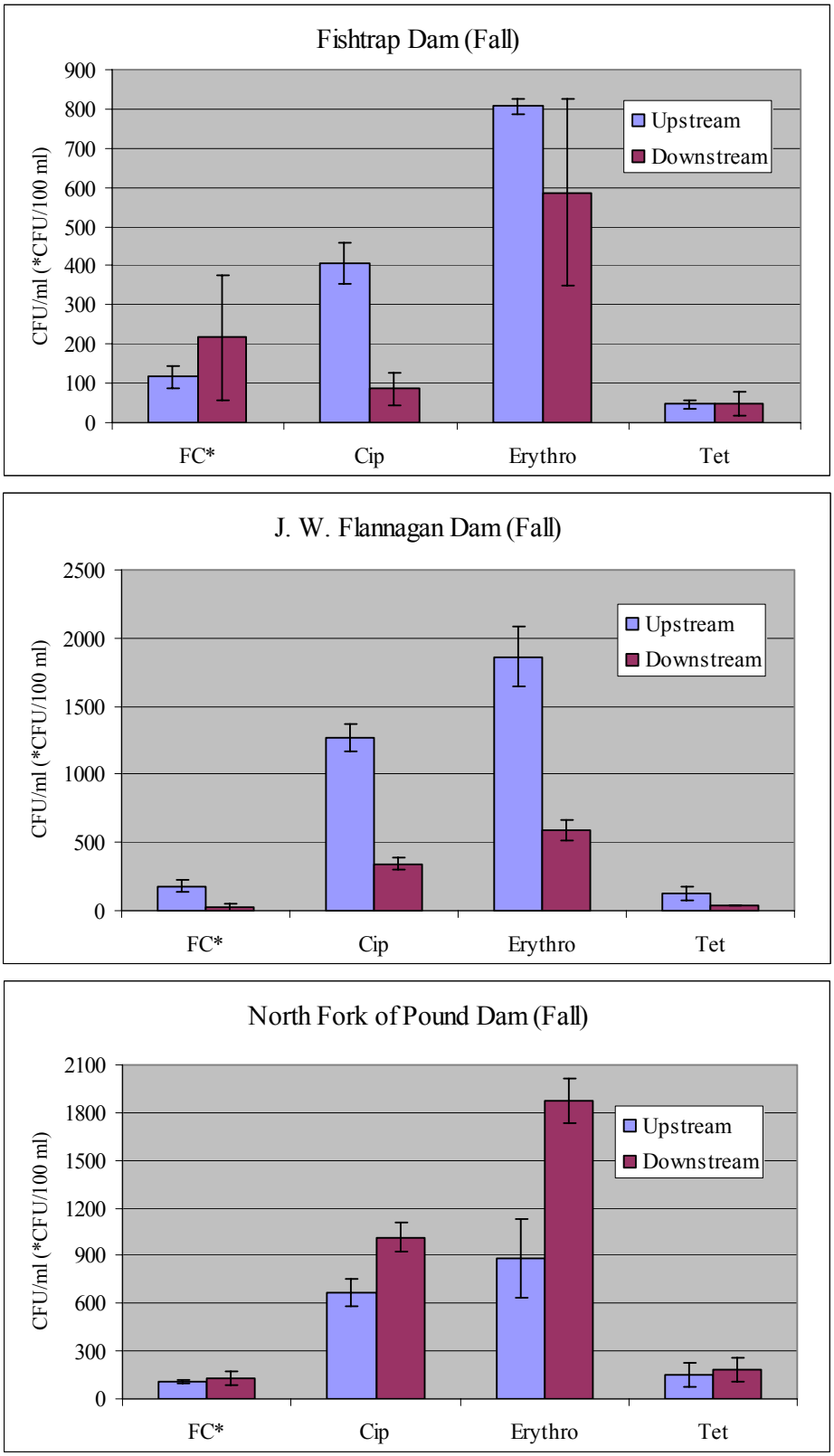
[a] Fecal coliforms (FC), Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet) bacteria. Standard deviation not available for J.W. Flannagan Dam Downstream FC. All changes in counts from upstream-to-downstream are significant at the 95th confidence level for the goodness-of-fit statistical analysis (DF = 1).

Figure 17. Fall average counts (3 northern dams).



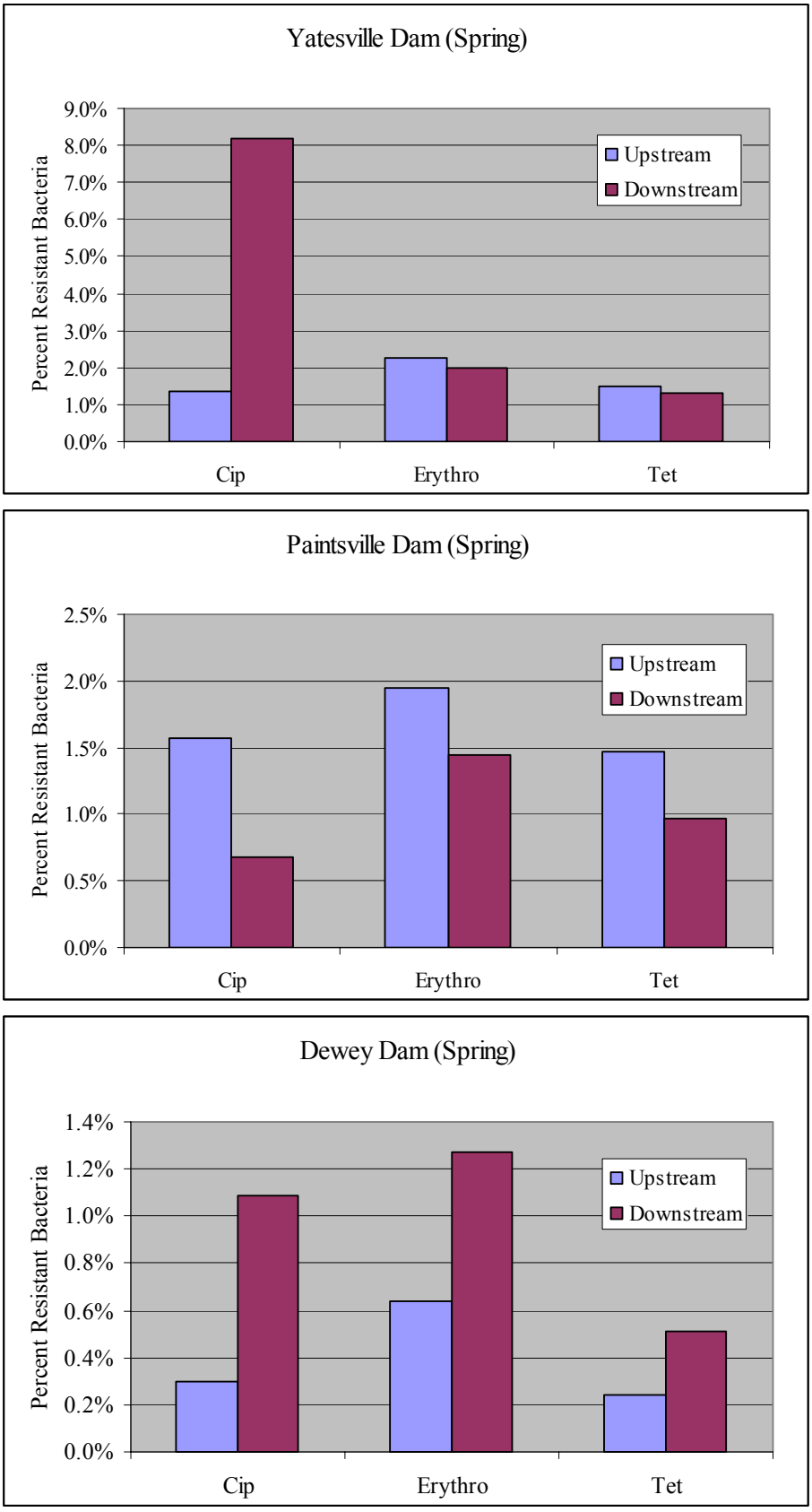
[a] Fecal coliforms (FC), Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet) bacteria. All changes in counts from upstream-to-downstream are significant at the 95th confidence level for the goodness-of-fit statistical analysis (DF = 1)

Figure 18. Fall average counts (3 southern dams).



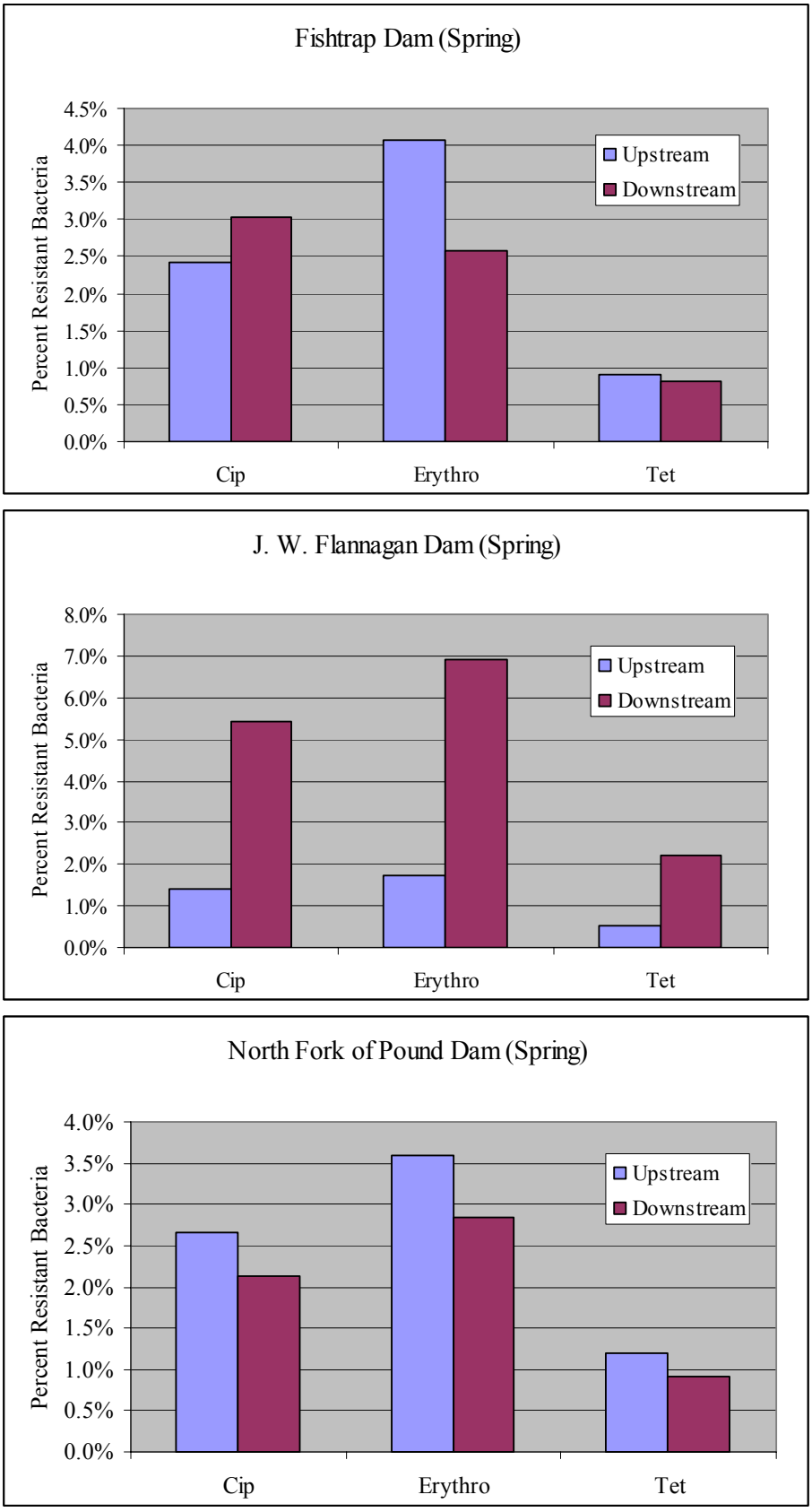
[a] Fecal coliforms (FC), Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet) bacteria. Changes in counts from upstream-to-downstream are significant at the 95th confidence level for the goodness-of-fit statistical analysis (DF = 1), except change of Tet at Fishtrap Dam and change of FC at North Fork of Pound Dam not significant.

Figure 19. Spring percent antibiotic resistance (3 northern dams).



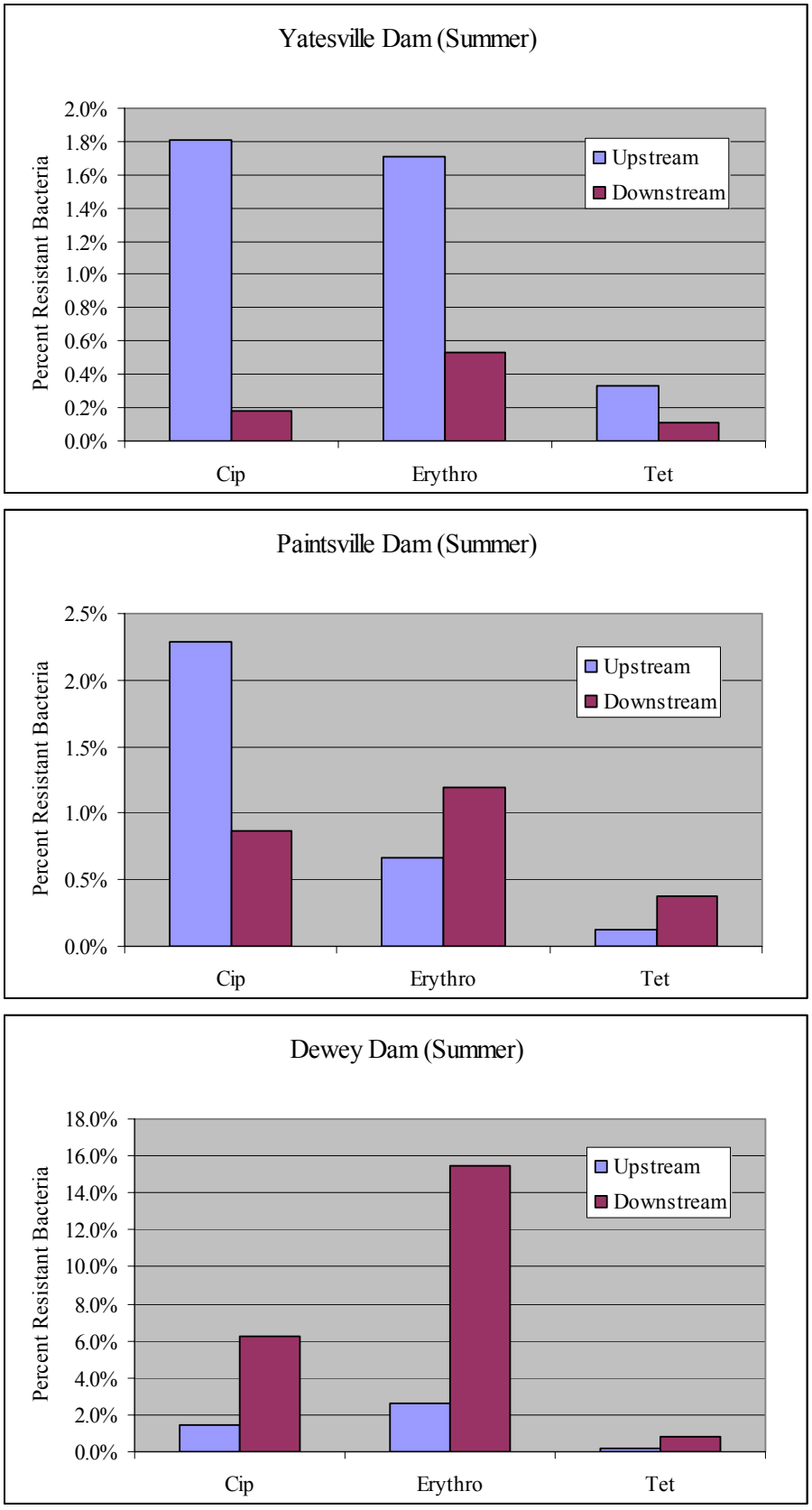
[a] Percent of total cultivable bacteria that were Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet). All changes in percentages from upstream-to-downstream are significant at the 95th confidence level for the chi-square statistical analysis (DF = 1).

Figure 20. Spring percent antibiotic resistance (3 southern dams).



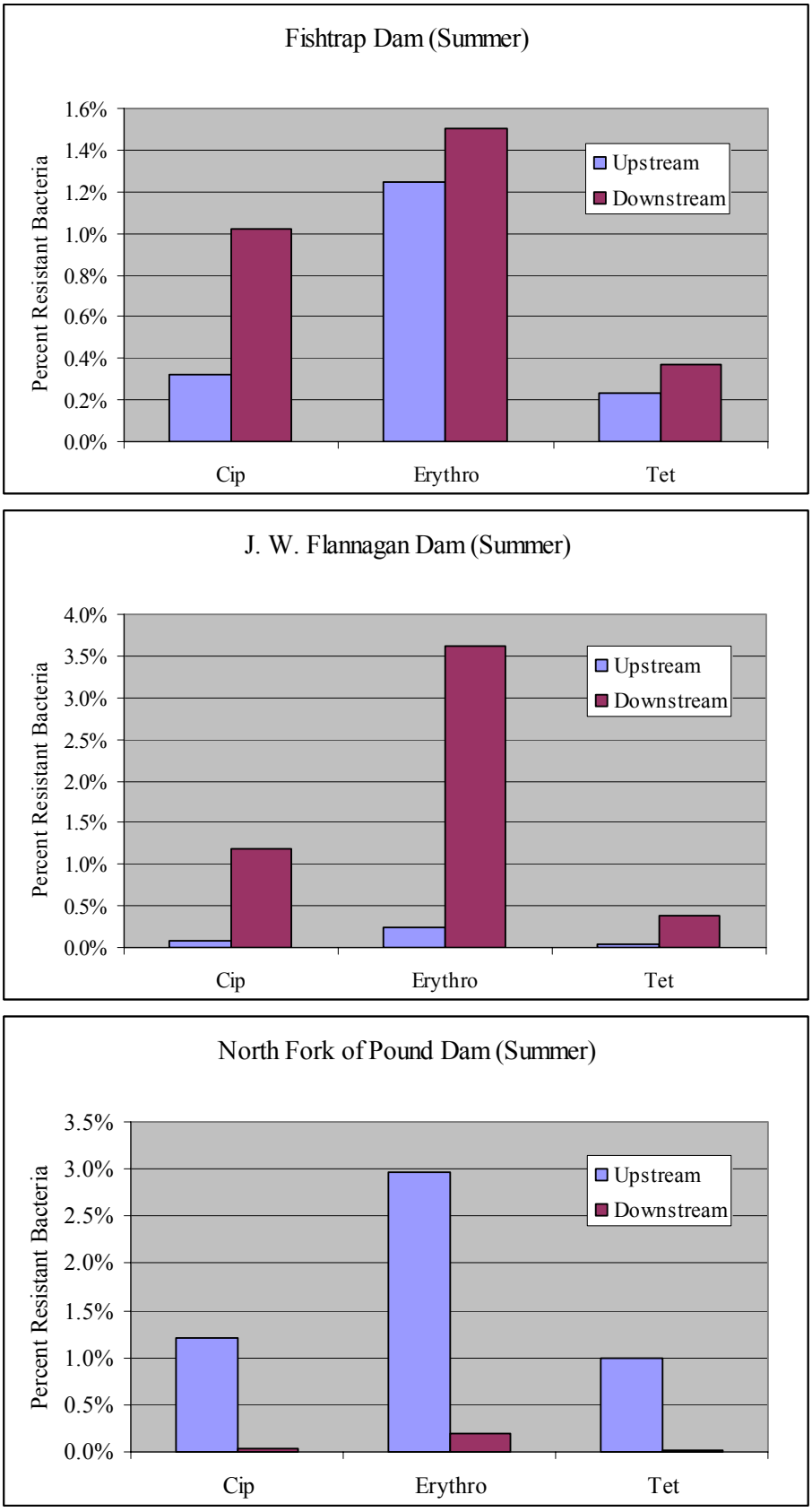
[a] Percent of total cultivable bacteria that were Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet). All changes in percentages from upstream-to-downstream are significant at the 95th confidence level for the chi-square statistical analysis (DF = 1).

Figure 21. Summer percent antibiotic resistance (3 northern dams).



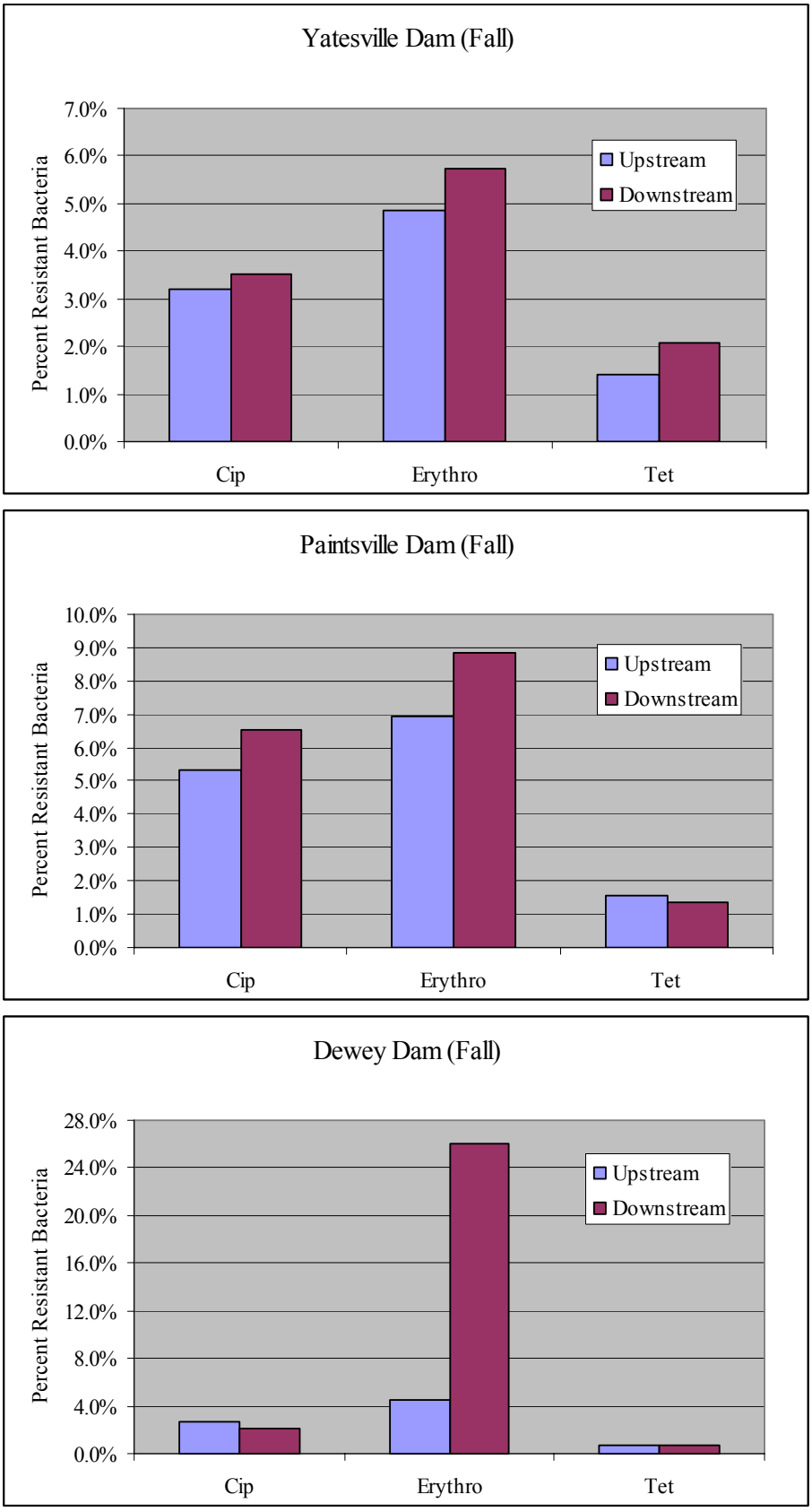
[a] Percent of total cultivable bacteria that were Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet). All changes in percentages from upstream-to-downstream are significant at the 95th confidence level for the chi-square statistical analysis (DF = 1).

Figure 22. Summer percent antibiotic resistance (3 southern dams).



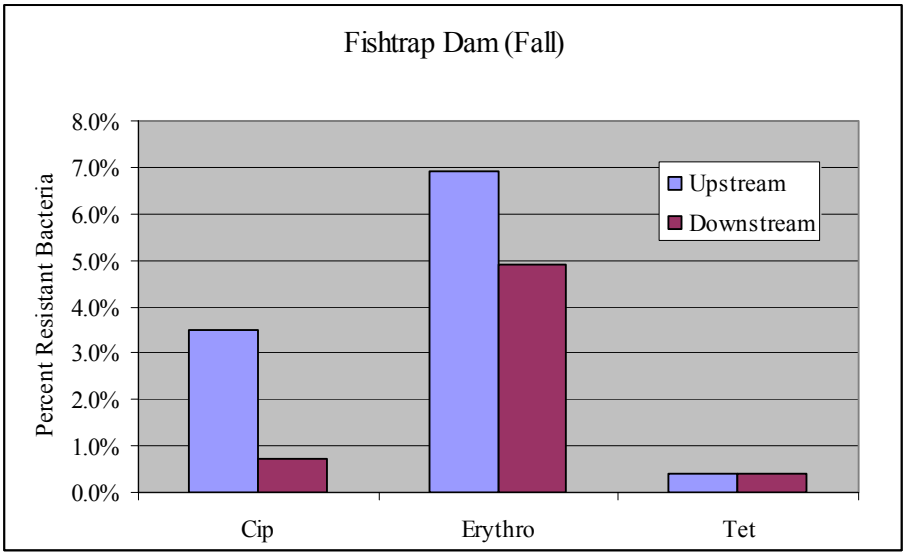
[a] Percent of total cultivable bacteria that were Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet). All changes in percentages from upstream-to-downstream are significant at the 95th confidence level for the chi-square statistical analysis (DF = 1).

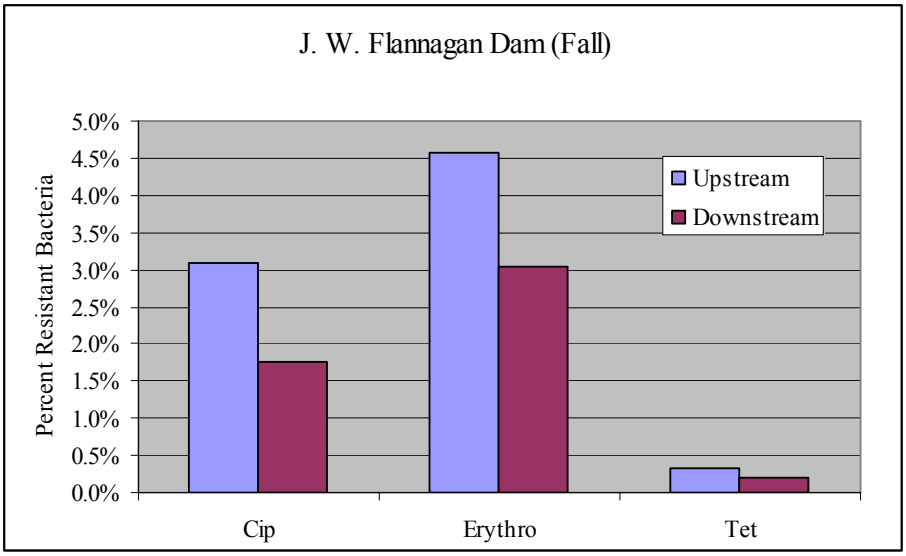
Figure 23. Fall percent antibiotic resistance (3 northern dams).

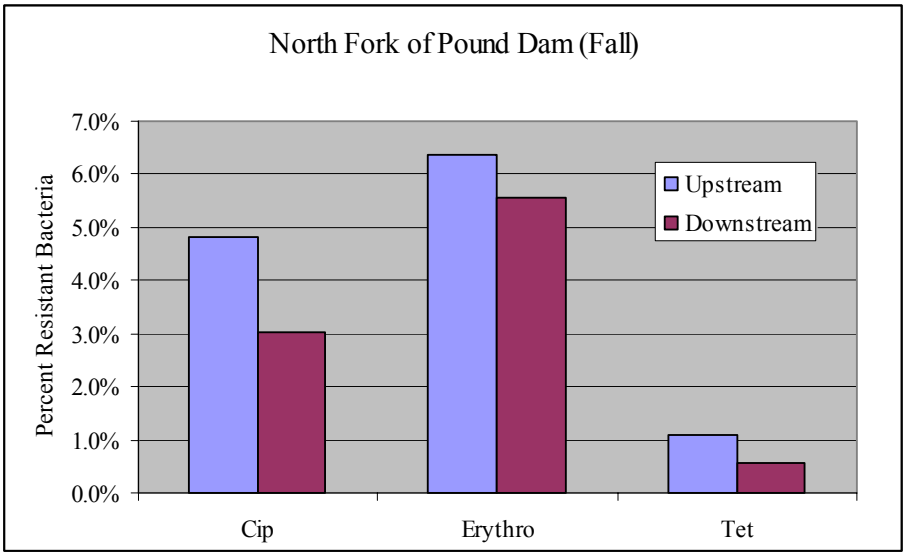


[a] Percent of total cultivable bacteria that were Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet). All changes in percentages from upstream-to-downstream are significant at the 95th confidence level for the chi-square statistical analysis (DF = 1), except change of Tet at Paintsville and Dewey Dams.

.**Figure 24.** Fall percent antibiotic resistance (3 southern dams).







[a] Percent of total cultivable bacteria that were Ciprofloxacin-resistant (Cip), Erythromycin-resistant (Erythro), and Tetracycline-resistant (Tet). All changes in percentages from upstream-to-downstream are significant at the 95th confidence level for the chi-square statistical analysis (DF = 1), except change of Tet at Fishtrap Dam.

Appendix A: Site ID List with latitudinal/longitudinal coordinates for the 35 sites sampled within the Big Sandy Watershed.

Site ID	Site Name	State	County	Latitude			Longitude			Directions to Stream
BSR0265	Big Sandy River	WV	Wayne	38	22	53	82	35	37	Site is at located at mile 2.6. Site is located just upstream of the I-64 Bridge.
BSR1005	Big Sandy River	WV	Wayne	38	17	9.7	82	34	45	Site is at mile 10.
BSR1265	Big Sandy River	WV	Wayne	38	14	58.68	82	34	49.51	Site is located at mile 12.6. Site is located at old Lock and Dam 2.
BSR1685	Big Sandy River	WV	Wayne	38	12	54.06	82	35	53.01	Site is located at mile 16.8.
BSR2595	Big Sandy River	WV	Wayne	38	7	15.3	82	36	38.6	Site is located just below low head dam at Louisa at mile 25.9.
DEW0003	Johns Creek (Inflow)	KY	Pike	37	40	22	82	35	38	Located on Johns Creek of Big Sandy R. of Ohio R. It is 18.75 mi. above Dewey Dam, off Rt. 304 at first bridge on right traveling south after crossing Brushy Fork.
DEW0004	Buffalo Creek	KY	Floyd	37	38	54	82	37	10	Located on Buffalo Creek of Johns Creek of Big Sandy River of Ohio River. It is 4.86 mi. upstream from confluence of Buffalo and Johns Creek, near Endicott.
DEW0005	Brushy Fork	KY	Pike	37	40	50	82	35	23	Located on Brushy Fork of Johns Creek. It is 0.01 miles above the confluence of Brushy Fork and Johns Creek, at a small bridge crossing Brushy Fork.
DEW0049	Johns Creek (Outflow)	KY	Floyd	37	44	47.5	82	43	22.5	Go south on Rt. 302 off of Rt. 3. 200 meters south on Rt 302 there is a small unused road that cuts back toward Johns Creek. This road ends at a small unused bridge. Site is downstream of bridge.
FRL0002	Levisa Fork	VA	Buchanan	37	21	14	82	11	44	Located on Levisa Fork of Big Sandy R. of Ohio R. It is about 0.6 mi. below bridge at Big Rock. Access by US rt. 460, about 0.4 mi. west of Big Rock Bridge.
FRL0032	Levisa Fork	KY	Pike	37	24	29.9	82	26	22.3	Site is located downstream of FRL dam 2.7 miles. Site is under old Rt 460 Bridge, not new Rt 460 Bridge.
JWF0001	Pound River (Outflow)	VA	Dickenson	37	14	13	82	20	36	Located on Pound River of Russell Fork of Levisa Fork of Big Sandy River of Ohio River. It is about 1.5 stream miles above confluence with Russell Fork.
JWF0002	Pound River	VA	Dickenson	37	9	57	82	31	23	Located on Pound R. of Russell Fork of Levisa Fork of Big Sandy R. of Ohio R. It is at the USGS gauging station at the confluence with Camp Ck. at SR. 624 bridge.

JWF0003	Canes Nest River	VA	Dickenson	37	7	26	82	26	18	Located on Cranes Nest River of Pound River of Russell Fork of Levisa Fork of Big Sandy River of Ohio River. It is about 1.5 stream miles above SR. 83 bridge. About 150 ft. above Big Branch Creek.
JWF0021	Russell Fork	VA	Dickenson	37	14	45	82	19	26	Located on Russell Fork of Levisa Fork of Big Sandy River of Ohio River. It is 0.2 mi. above the mouth of the Pound River, at Bartlick.
LFR0017	Russell Fork	VA	Dickenson	37	13	11	82	18	7	Located on Russell Fork of Levisa Fork of Big Sandy River of Ohio River. It is 1.2 mi. below McClure River.
LFR0024	Levisa Fork	KY	Pike	37	27	51	82	31	33	Site is located at Pikeville Gage at the Rt 1426 Bridge.
LFR0025	Levisa Fork	KY	Lawrence	38	4	50.19	82	36	1.11	Take Rt 3 south at Louisa. Turn right on Rt 644. Go 1.3 miles on Rt 644 until you cross 1 lane bridge. After crossing bridge park on left hand side next to railroad tracks. D/S of Bridge.
LFR0026	Levisa Fork	KY	Lawrence	38	1	42.21	82	37	14.84	From LFR0025 go south on Rt 1690 for 5.5 miles. Turn right on 2307 go 2.4 miles. Cross R&R and turn right toward park. Site is 50 m D/S of old bridge pillar.
LFR0027	Levisa Fork	KY	Johnson	37	45	58.56	82	42	56.14	Take Rt 23 to Rt 321. Go 7 miles on Rt 321. Turn right on bridge. Park on right after crossing bridge. Site is upstream of bridge.
NFP0008	Bad Creek	VA	Wise	37	6	4	82	40	45	Located on Bad Creek of North Fork of Pound River of Russell Fork of Levisa Fork of Big Sandy River of Ohio River. It is near Gilley, VA.
NFP0009	North Fork of Pound R.	VA	Wise	37	6	1	82	40	45	Located on North Fork of Pound River of Russell Fork of Levisa Fork of Big Sandy River of Ohio River. It is at Cane Patch Church, just above Bad Creek.
PIV0003	Little Paint Creek	KY	Johnson	37	52	5	82	57	29	Located on Little Paint Creek of Paint Creek of Levisa Fork of Big Sandy River of Ohio River. It is 0.45 mi. above Oil Branch and 0.8 mi. mi. below Hargis Creek.
PIV0004	Big Mine Fork	KY	Morgan	37	51	51	83	0	2	Located on Big Mine Fork of Little Paint Creek of Paint Creek of Levisa Fork of Big Sandy River of Ohio River. It is 0.95 mi. above Little Mine Fork.
PIV0005	Open Fork Paint Ck.	KY	Morgan	37	56	31	82	59	51	Located on Open Fork Paint Creek at Relief, just upstream of Patoker Branch.
PIV0012	Paint Ck. (Outflow)	KY	Johnson	37	49	43	82	50	57	Located on Paint Creek of Levisa Fork of Big Sandy River of Ohio River. It is 1.9 mi. below the Paintsville Dam.
TFV0003	Tug Fork	WV	Mingo	37	41	59.3	82	17	47.6	Turn onto Rt 14 off of Rt 119. Go 0.4 miles. Park at pull off on left. Site is at bottom of trail.

TFV0004	Tug Fork	WV	Mingo	37	34	43	82	7	30	From 119 turn left onto 52. Turn right onto 49, go 6 miles, turn right onto Country Club Road. After crossing RR and immediately turn left. Go 0.4 miles until you come to Golf cart trail. Site is at bottom of hill.
TFV0042	Tug Fork	WV	Wayne	38	6	30.1	82	35	10.7	Rt 23 to Louisa. Rt 3 over bridge to Fort Gay. 0.3 miles on Rt 37. When Rt 37 turns left go straight onto Tug River Road 0.8 miles. Pull off on right, cross tracks to site.
TFV0043	Tug Fork	KY	Lawrence	38	0	2.6	82	31	7.5	10.1 miles on Rt 3 from Louisa. Turn left onto Yellow Creek/Tug Fork road (no road sign). 0.1 miles to turn off on left.
TFV0044	Tug Fork	WV	Mingo	37	50	15.3	82	24	33.5	At Kermit WV, before crossing Rt 292 bridge into Kentucky turn left onto Lincoln Street. Turn right in large parking lot. Site is over bank just upstream from bridge.
YBC0010	Blaine Creek	KY	Lawrence	38	8	50	82	40	1	Located on Blaine Creek of Big Sandy River of Ohio River. It is 0.1 mi. below Backbone Branch.
YBC0024	Hood Creek	KY	Lawrence	38	0	32	82	50	3	Located on Hood Creek of Blaine Creek of Big Sandy River of Ohio River. Travel 201 south from Blaine about 2.1 mi. to unimproved dirt road. The station is at the Highway bridge.
YBC0053	Brushy Creek	KY	Lawrence	38	1	16	82	48	34	No directions recorded.
YBC0054	Blaine Ck. (Inflow)	KY	Lawrence	38	1	12.9	82	52	41.6	Site is located 1.8 miles upstream of YBC08 at small bridge that crosses stream at campground.

Appendix B: Supply List for Microbiological Analysis performed.

Supplies/Equipment	Product No.	Misc. product information	Company/ Manufacturer	Company Location
47 mm Petri dish w/absorbent pad	Cat. No. PD1004705	Petri-Pad	Millipore	Bedford, MA 01730
100 × 15 mm Petri Dish	Falcon ® 35-1029		Becton Dickinson	Franklin Lakes, NJ 07417
17 × 120 mm,15 ml polystrene conical tube	Falcon ® 35-2095	Blue Max™ Jr.	Becton Dickinson	Franklin Lakes, NJ 07417
30 × 115 mm 50 ml polypropylene conical tube	Falcon ® 35-2070	Blue Max™	Becton Dickinson	Franklin Lakes, NJ 07417
0.2 µm syringe filter	Cat. No. 09-719C	-	Fisher Scientific	Fair Lawn, NJ 07410
Filter Funnels,100ml w/47mm cellulose nitrate membrane, 0.45µm pores	Cat. No. 09-740-30D	Nalgene No. 145 0045	Fisher Scientific	Florence, KY 41042
5 mm Glass Beads, Solid	Cat. No. 11-312C		Fisher Scientific	
Fungizone (250 µg/ml)	Cat. No. 17-836R	BioWhittaker™	Cambrex Bio Science Walkersville, Inc.	Walkersville, MD
Ciprofloxacin HCl Powder (809.00 µg/mg)	Cat No. 61-277-RF	Cellgro	Medlatech, Inc.	Herndon, VA 20171
Erythromycin (Biotech Research Grade)	BP920-25	FisherBiotech	Fisher Scientific	Fair Lawn, NJ 07410
Tetracyclie Hydrochloride (Biotech Research Grade)	BP912-100	FisherBiotech	Fisher Scientific	Fair Lawn, NJ 07410
Difco™ R2A agar	Cat. No. 218263		Becton Dickinson	Sparks, MD 21152
m-FC Medium with Rosolic Acid, 2 ml	Cat. No. M00000P2F	Culture Medium in Plastic Ampules	Millipore	Bedford, MA 01730
m-ENDO Broth, 2 ml	Cat. No. M00000P2E	Culture Medium in Plastic Ampules	Millipore	Bedford, MA 01730
Fisher Water Bath, 20L (48°C)	Ser. No. 311269	-	Fisher Scientific	Pittsburgh, PA
Precision mechanical convection incubator 30 MR (30°C)	Cat. No. 51221103	Ser. No. 600041445	Jouan, Inc.	Winchester, VA 22602
Boekel Incubator (35 ± 0.5°C)	Cat. No. 131600	Ser. No. 01288-46	Boekel Industries, Inc.	-
Autoblot® Micro Hybridization Oven (44.5 ± 0.2°C)	Cat. No. 7930-00110	Ser. No. BMH0J-1651	Bellco Glass, Inc.	Vineland, NJ
Hirayama Autoclave	-	HIClave™ HV-110	Amerex Instruments, Inc.	Lafayette, CA 94549
Analytical Scale M-120	Ser. No. P0114032	-	Denver Instrument Co.	Arvada, CO 80004
Top Loading Scale APX-1502	Ser. No. A12032001	-	Denver Instrument Co.	Arvada, CO 80004

Appendix C: Preparation and Analysis Standard Operating Procedures (SOP).

Antibiotic Stock Solutions

1. The antibiotics, solvents, and concentrations used are shown in Table 1.

Table 1. Antibiotics used and recommended concentrations.

Antibiotic	Catalog No.	Solvent[a]	Stock Conc.	Working Conc.
Fungizone	BioWhitaker 17-836R	N/A	250 µg/ml	375 ng/ml
Ciprofloxacin	Cellgro 61-277-RF	DMSO	4 mg/ml	4 µg/ml
Erythromycin	Fisher BP920-25	EtOH:H_2O	8 mg/ml	8 µg/ml
Tetracycline Hydrochloride	Fisher BP912-100	EtOH:H_2O	12.5 mg/ml	12.5 µg/ml

[a] Fungizone is purchased as a stock solution, it is stored frozen and thawed before use. DMSO = dimethylsulfoxide (Certified ACS). EtOH:H_2O = a mixture of equal parts ethanol (100% USP) and reagent grade water (18 MΩ).

2. Using an analytical balance, weigh out sufficient antibiotic to make a 10 ml stock (see Table 1 and note below) and transfer the antibiotic powder to a sterile 15 ml plastic centrifuge tube (Falcon 2095; Becton Dickinson, Sparks, MD or equivalent).

Note – for determining amount of antibiotic powder to use

a. Be sure to account for the purity of the antibiotic powder by dividing the weight of pure antibiotic required by the purity. For example, ciprofloxacin may be provided as a powder that contains 803 mg ciprofloxacin per gram. To achieve a stock concentration of 4 mg ciprofloxacin per ml, it is necessary to add 4.98 [or 4.0 mg cipro x (1000 mg powder / 803 mg cipro)] mg powder per ml of stock solution.

3. Add 10 ml of the appropriate solvent (see Table 1) to the tube, and vortex to mix.

4. In some cases (e.g. when making stock solutions of ciprofloxacin) the tube can be placed in a bath sonicator to facilitate dissolution of the solute. Take care to be certain that all of the antibiotic has gone into solution.

5. Draw the antibiotic solution into a sterile 10 ml syringe, and sterilize by forcing the solution through a sterile, 0.2 µm syringe filter (Fisher Scientific cat. no. 09-719C or equivalent) into a second sterile plastic centrifuge tube. ***Do not*** *filter sterilize antibiotics dissolved in DMSO.*

6. Store the antibiotic stocks at -20°C until used. Replace antibiotic stocks each month.

Media Preparation (for Total Cultivable Bacteria and Antibiotic-resistant Bacteria)

1. Suspend 9.1 grams Difco R2A agar (Becton Dickinson, Sparks, MD; cat no. 218263) in 500 ml of purified water in a 1,000 ml capacity glass Erlenmeyer flask.

2. Add a magnetic stir bar, cover the flask with aluminum foil, place a piece of autoclave tape on the foil, and mark the name of the antibiotic to be added (if appropriate) on the foil.

3. Swirl the flask to evenly hydrate the suspended powder, and autoclave at 121°C and 15 psi for 20 minutes on a slow exhaust cycle.

4. Move the medium from the autoclave to a 48°C water bath, and hold for at least 30 minutes but not more than 4 hours.

5. While the medium is cooling, remove the appropriate antibiotic stock solutions from the freezer and thaw on ice (all antibiotics except ciprofloxacin) or at room temperature (ciprofloxacin).

6. Place the flask on a magnetic stir plate and stir gently until the medium is well mixed. Be careful not to introduce bubbles. Test the temperature of the medium by touching the side of the flask briefly with your bare hand. It should be warm, but not hot. If the flask is hot to the touch, return it to the water bath until it has cooled enough to be handled comfortably. Do not allow the medium to cool below 48°C.

7. Wear disposable latex gloves for the remaining steps of media preparation. When properly tempered, again move the medium to the magnetic stirrer. While stirring gently, ***aseptically*** add 750 µl of fungizone stock.

8. Continue stirring for 15 to 30 seconds after the addition of the fungizone to the medium. Tilt the flask to insure that all the fungizone stock solution is transferred to the medium.

9. If you are preparing R2A plus fungizone for the enumeration of total cultivable bacteria, aseptically pour 25 ml per plate into pre-sterilized 100 x 15 mm Petri dishes (Falcon 1029, Becton Dickinson, Sparks, MD or equivalent).

10. If you are preparing R2A plus fungizone and an additional antibiotic for the enumeration of a particular resistant population, ***aseptically*** add 500 µl of the appropriate antibiotic stock to the flask. Stir gently for an additional 15 seconds and tilt the flask to insure that all the antibiotic stock is transferred to the medium.

11. Pour the plates as described in step 9.

12. Clearly mark the plates to indicate media content. E.g. “R2Af” can be used to indicate R2A agar plus fungizone, and “R2Afc” to indicate R2A agar plus fungizone and ciprofloxacin, etc.

13. Allow plates to cure at room temperature for at least 48 hours before use. Plates should be inoculated no later than seven days after pouring.

Sample Collection

1. Whole water samples must be collected in sterile containers with secure, leak-proof lids. Containers must be clearly labeled with a sample number, and the sample number must be recorded in a notebook in which the location, date and time of sampling are clearly and fully described. If available, include additional information such as: latitude and longitude, air temperature, water temperature, weather conditions, turbidity, level of boating activity, land use patterns, etc.

2. The container should be opened so that the opening is pointing downward, and the inside of the lid does not come into contact with any non-sterile surfaces.

3. Continue holding the opening downward while passing the container through the surface tension layer.

4. When the container is fully submerged, invert it so that it fills with water.

5. Pour off enough water to leave approximately a 10% air headspace.

6. Seal the container and place on ice. Samples should be cultivated within 6 hours of collection.

Enumeration of Total Cultivable Bacteria

1. Remove a sample bottle from the ice chest and mix by inversion to re-suspend any sediment that may have settled out during transit.

2. Aseptically transfer 0.1 ml of sample to a sterile 9.9 ml dilution blank in a screw-cap test tube.

3. Tightly cap the tube and mix at full speed on a vortex mixer for at least 5 seconds.

4. Aseptically transfer 0.1 ml of diluted sample to each of three plates of Difco R2A agar plus 375 ng/ml fungizone.

5. Spread the diluted water sample on the surface of the agar plates using a sterile glass spreading rod, a pre-sterilized inoculating loop, or five sterile glass beads (5 mm; see note) until all of the liquid has been absorbed.

Note – for use of sterile glass beads

a. Place six glass beads (Fisher Scientific cat no. 11-312C) into a 1000 ml pipette tip (Biolog cat no. 3001; other tips should be tested for suitability). One set of beads is required for each plate inoculated.

b. Place the tip with beads into the original pipette box, cover all the tips with a sheet of aluminum foil, place the cap on the box, place a piece of autoclave tape on the box, and autoclave at 121°C and 15 psi for 15 minutes.

c. When plating – open the pipette tip box, roll back the aluminum foil to expose a single row of pipette tips, remove one tip at a time, lift the lid of an inoculated plate, and pour the sterile beads onto the agar surface. Normally, one bead remains stuck in the bottom of the tip.

d. Repeat step c for all replicate plates.

e. Cover the plates and stack them. Then shake the plates by moving them in a quick back and forth motion while keeping the bottom plate in contact with the bench top - *it is important to avoid allowing the beads to run in a circular motion around the outer edge of the plate*. Shake five times, then rotate the plates by one-quarter turn and shake again five times. Repeat shaking and turning the plates a total of five times.

f. Invert the plates and collect the used beads in a beaker containing 70% ethanol.

6. Plates must be clearly marked with sample number and date of inoculation.

7. Wrap each set of three plates with parafilm and incubate inverted at 25°C for one week (see note)

Note – for incubation of R2A plates

a. R2A agar plates inoculated with river or lake water will continue to develop new microcolonies for 5 to 6 days after inoculation. Therefore, incubation for at least seven days is recommended. Incubation at temperatures above 25°C is not recommended as it may reduce the number of colony forming units.

8. After incubation, count the number of colony forming units (CFU) on each plate and record in a laboratory notebook.

9. Determine the mean and standard deviation of CFU counts on replicate plates and record in a laboratory notebook.

10. Determine the CFU per ml of total cultivable bacteria in the original sample by multiplying the average CFU value by a dilution factor of 1,000 (accounts for the initial 10^{-2} dilution and the plating volume of 0.1 ml). Record this value in the laboratory notebook.

Enumeration of Antibiotic Resistant Bacteria

1. Remove a sample bottle from the ice chest and mix by inversion to re-suspend any sediment that may have settled out during transit.
2. Aseptically transfer 0.1 to 0.2 ml (see note) of undiluted sample to each of three plates of Difco R2A agar plus 375 ng/ml fungizone, plus the appropriate concentration of a single antibiotic (see Table 1).

   Note – for selection of plating volume

   a. Preliminary tests to determine the volume of sample to be plated are recommended. A plating volume of 0.1 ml is the default volume, but if the number of antibiotic resistant colony forming units is consistently less than 30 per plate, the volume should be increased to 0.2 ml

3. Spread the undiluted water sample on the surface of the agar plates using a sterile glass spreading rod, a pre-sterilized inoculating loop, or five sterile glass beads (5 mm; see note above) until all of the liquid has been absorbed.

4. Plates must be clearly marked with sample number and date of inoculation.

5. Wrap each set of three plates with parafilm and incubate inverted at 25°C for one week (see note above).

6. After incubation, count the number of colony forming units (CFU) on each plate and record in a laboratory notebook.

7. Determine the mean and standard deviation of CFU counts on replicate plates and record in a laboratory notebook.

8. Determine the CFU per ml of total cultivable bacteria in the original sample by multiplying the average CFU value by a dilution factor of 10 (for a plating volume of 0.1 ml) or 5 (for a plating volume of 0.2 ml). Record this value in the laboratory notebook.

Enumeration of Total Coliform & Fecal Coliform Bacteria

1. Label the 47 mm Petri dishes with absorbent pads (Millipore, cat. no. PD1004705) with media type (i.e. mFC or mENDO), date, sample ID, and aliquot amount to be sampled.

2. Place the m-FC Medium with Rosolic Acid, 2 ml plastic ampules (Cat. No. M00000P2F, Millipore) and the m-ENDO Broth, 2 ml plastic ampules (Cat. No. M00000P2E, Millipore) on ice and set aside until step 6.

3. Pour sterile tap water into a 100 ml capacity analytical test filter funnel with 47mm cellulose nitrate membrane, 0.45µm pore size (Fisher Scientific, cat. no. 09-740-30D or equivalent) until the membrane is covered to an approximate depth of 5-10 mm.

4. Remove a sample bottle from the ice chest and mix by inversion to re-suspend any sediment that may have settled out during transit.

5. Aseptically transfer 0.1 to 50 ml (see note) of undiluted sample to the sterile tap water in the analytical filter funnel, swirl gently to evenly distribute the sample, and filter the water through the funnel. Rinse the sides of the funnel with sterile tap water at least two times and filter through membrane.

   Note – for selection of plating volume

   a. Preliminary tests to determine the volume of sample to be plated are recommended. Plating volumes of 0.1 ml, 0.5 ml, and 1.0 ml are the default volumes for triplicate sampling. However, if the number of colony forming units does not consistently fall within the 20-60 colonies per membrane standard, the volume should be adjusted accordingly.

6. Open m-FC Medium with Rosolic Acid, 2 ml ampule or m-ENDO Broth, 2 ml ampule and squeeze contents onto the absorbent pad in the pre-labeled corresponding 47 mm Petri dish with absorbent pad.

7. Remove the disposable funnel wall and aseptically transfer the membrane (using a 95% ethyl alcohol flame-sterilized flat forcep) to the pre-labeled corresponding 47 mm Petri dish with absorbent pad soaked with the appropriate medium.

8. Incubate the plates as follows: m-FC (44.5 ± 0.2°C for 24 hours) or m-ENDO broth (35 ± 0.5°C for 24 hours).

9. After incubation, count the number of colony forming units (CFU) on each plate and record in a laboratory notebook. For the m-FC plates, count only the blue colonies. For the m-ENDO plates, count all red colonies with a metallic (golden) sheen over all or part of the colony.

10. Determine the mean and standard deviation of CFU counts on replicate plates and record in a laboratory notebook.

11. Determine the CFU per 100 ml of fecal coliform and total coliform bacteria in the original sample by multiplying the average CFU value by a dilution factor (i.e. DF of 10 for a plating volume of 0.1 ml). Record this value in the laboratory notebook.

Determination of Impact Scores

1. Enter enumeration data for fecal indicators and antibiotic resistant bacteria into an Excel spreadsheet.

2. For each population (i.e. fecal coliforms or ciprofloxacin resistant cells), rank the average count for a site within the population data set of all sites using the PERCENTRANK function. Multiply the PERCENTRANK output by 100 to achieve a percentile score for each data point within the entire population data set (see note).

Note – on determining percentile scores

a. The PERCENTRANK function in Excel can not simply be copied and pasted from cell to cell. If the function is transferred it will carry the original array size, but the array will be offset and the function will calculate an inappropriate rank. *Therefore, you must set the array to contain the entire population data set for each individual data point.*

3. Choose the boundaries that you wish to apply to the data. For example, an IS_{90} score weights sites with population counts above the 90^{th} percentile and below the 10^{th} percentile. An IS_{80} score weights sites with population counts above the 80^{th} percentile and below the 20^{th} percentile. In our hands, IS_{85} to IS_{90} scores provide a useful signal to noise ratio in the index.

4. Assign a population score of 1 to all data points that fall above the upper percentile boundary.

5. Assign a population score of -1 to all data points that fall below the lower percentile boundary.

6. Assign a population score of 0 to all data points that fall between the chosen boundaries.

7. Repeat the determination of population scores for all microbial populations enumerated, i.e. for each antibiotic resistant population measured and for the fecal indicator population.

8. Determine the total impact score (IS) by adding the population scores. For studies that include three antibiotics and one fecal indicator, impact scores can range from -4 to +4. Higher impact scores are indicative of a more impacted water source.

Appendix D: Winter microbiology data with average counts (ave.) and standard deviations (S.D.).

* = No standard deviation, only one plate countable; *N.D.* = No Data for site; † = per 100 ml (all other counts per 1ml)

Site	Totals ave.	Totals S.D.	Cipro. ave.	Cipro. S.D.	Erythro. ave.	Erythro. S.D.	Tet. ave.	Tet. S.D.	FC † ave.	FC † S.D.	TC † ave.	TC † S.D.
BSR 0265	229000.0	31796.2	1103.3	267.3	1310.0	290.5	571.7	495.1	330.0	*	933.3	495.0
BSR 1005	254000.0	24980.0	1721.7	142.7	1496.7	226.6	765.0	270.7	302.7	91.8	1833.3	707.1
BSR 1265	206666.7	9504.4	1768.3	297.2	1375.0	277.5	703.3	223.7	440.0	*	800.0	989.9
BSR 1685	178500.0	10606.6	1940.0	109.0	1335.0	127.6	291.7	505.2	470.0	*	1266.7	495.0
BSR 2595	164333.3	40451.6	1963.3	528.6	1151.7	112.5	383.3	336.5	330.0	*	1733.3	321.5
DEW 0003	134666.7	168387.5	413.3	145.0	743.3	81.3	160.0	58.9	8.7	6.4	130.7	15.0
DEW 0004	21666.7	2309.4	385.0	42.7	280.0	43.6	65.0	31.2	61.3	23.4	406.7	102.8
DEW 0005	35333.3	17214.3	1000.0	249.3	481.7	163.6	241.7	69.3	10.7	8.1	256.0	12.2
DEW 0049	33000.0	9165.2	161.7	86.1	303.3	100.2	23.3	22.5	6.0	4.0	14.0	3.5
FRL 0002	13666.7	3214.6	81.7	37.5	1241.7	212.2	55.0	35.0	13.3	2.3	2.7	4.6
FRL 0032	131000.0	14106.7	160.0	43.6	775.0	108.2	81.7	18.9	27.3	8.3	121.3	5.8
JWF 0001	92333.3	7023.8	41.7	14.4	230.0	50.7	16.7	7.6	0.0	0.0	12.0	2.0
JWF 0002	16666.7	9073.8	131.7	59.7	805.0	31.2	75.0	13.2	1.3	1.2	9.3	3.1
JWF 0003	17666.7	2516.6	143.3	36.9	816.7	103.7	73.3	12.6	13.3	3.1	22.0	14.0
JWF 0021	28333.3	13316.7	76.7	20.2	458.3	84.6	40.0	37.7	0.0	0.0	41.3	7.0
LFR 0017	38000.0	6245.0	81.7	42.5	490.0	60.6	33.3	11.5	9.3	5.0	157.3	22.7
LFR 0024	333.3	577.4	1018.3	193.9	1560.0	181.9	546.7	184.8	34.7	13.0	8.0	9.2
LFR 0025	238000.0	74953.3	650.0	106.1	1153.3	222.6	368.3	319.8	288.0	*	2266.7	680.7
LFR 0026	180666.7	25423.1	641.7	68.1	1046.7	103.7	473.3	410.0	440.0	*	1533.3	57.7
LFR 0027	144333.3	30353.5	957.5	123.7	1026.7	88.1	461.7	441.6	236.0	*	2366.7	152.8
NFP 0008	3666.7	2886.8	10.0	13.2	48.3	25.2	25.0	10.0	0.0	0.0	0.7	1.2
NFP 0009	12000.0	4358.9	133.3	41.9	368.3	50.3	83.3	12.6	50.0	16.0	84.0	31.7
PIV 0003	18000.0	7000.0	336.7	56.2	501.7	132.9	121.7	10.4	18.7	18.9	*N.D.*	*N.D.*
PIV 0004	14666.7	7234.2	190.0	65.4	618.3	143.6	116.7	16.1	203.3	27.2	*N.D.*	*N.D.*
PIV 0005	9666.7	2516.6	150.0	85.3	350.0	30.4	66.7	10.4	12.7	6.4	*N.D.*	*N.D.*
PIV 0012	17666.7	2886.8	226.7	53.5	768.3	44.8	58.3	25.7	94.7	31.4	*N.D.*	*N.D.*
TFV 0003	132000.0	3605.6	1908.3	270.2	1356.7	207.9	283.3	50.1	649.3	56.8	4443.3	1329.4
TFV 0004	121666.7	37527.8	1023.3	718.4	995.0	52.7	218.3	51.1	372.0	40.6	930.0	101.5
TFV 0042	177000.0	48538.6	1908.3	65.1	1226.7	175.0	270.0	79.4	358.7	118.1	5316.7	1461.3
TFV 0043	190666.7	20256.7	2121.7	339.3	1040.0	285.8	271.7	48.0	468.0	91.5	6756.7	427.7
TFV 0044	106500.0	707.1	1490.0	796.2	1086.7	399.1	228.3	20.2	558.7	60.2	5506.7	332.3
YBC 0010	19666.7	2081.7	132.5	24.7	255.0	47.7	63.3	22.5	0.0	0.0	*N.D.*	*N.D.*
YBC 0024	12333.3	1154.7	178.3	99.3	428.3	77.5	86.7	5.8	2.0	2.0	*N.D.*	*N.D.*
YBC 0053	10666.7	2886.8	190.0	18.0	363.3	77.7	78.3	16.1	56.7	20.2	*N.D.*	*N.D.*
YBC 0054	17333.3	3055.1	216.7	28.4	553.3	138.4	56.7	12.6	36.7	20.4	*N.D.*	*N.D.*

Appendix E: Spring microbiology data with average counts (ave.) and standard deviations (S.D.). Total Coliform (TC) not tested.
* = No standard deviation, only one plate countable; *N.D.* = No Data for site; † = per 100 ml (all other counts per 1ml)

Site	Totals ave.	Totals S.D.	Cipro. ave.	Cipro. S.D.	Erythro. ave.	Erythro. S.D.	Tet. ave.	Tet. S.D.	FC † ave.	FC † S.D.
BSR 0265	224666.7	4509.2	1453.3	909.4	2096.7	90.2	1066.7	321.5	*N.D.*	*N.D.*
BSR 1005	301000.0	16522.7	2973.3	1138.5	3343.3	193.5	2186.7	89.6	*N.D.*	*N.D.*
BSR 1265	213000.0	106503.5	2853.3	440.0	4370.0	485.7	2250.0	249.8	2600.0	*
BSR 1685	238000.0	8185.4	3623.3	1353.2	3940.0	70.0	2386.7	491.4	1100.0	*
BSR 2595	251000.0	12530.0	3473.3	937.4	3176.7	50.3	1563.3	387.3	2700.0	*
DEW 0003	151333.3	14742.2	630.0	487.7	1210.0	216.6	423.3	110.6	800.0	*
DEW 0004	48000.0	23000.0	136.7	72.3	446.7	109.7	213.3	73.7	300.0	*
DEW 0005	167000.0	12165.5	330.0	216.6	703.3	20.8	230.0	34.6	600.0	*
DEW 0049	174666.7	59534.3	1900.0	581.0	2213.3	751.6	886.7	49.3	2200.0	*
FRL 0002	261000.0	30805.8	6353.3	1020.3	10640.0	105.8	2350.0	581.0	4750.0	353.6
FRL 0032	203666.7	62915.3	6180.0	834.5	5240.0	421.4	1666.7	115.9	1850.0	1202.1
JWF 0001	21000.0	3605.6	1143.3	125.0	1450.0	70.7	466.7	41.6	0.0	*
JWF 0002	115333.3	28676.4	2466.7	177.9	3270.0	660.2	1056.7	141.5	1200.0	*
JWF 0003	140000.0	19924.9	1166.7	55.1	1153.3	102.6	316.7	136.5	800.0	*
JWF 0021	255666.7	19008.8	4463.3	710.0	5293.3	966.7	1676.7	145.0	3700.0	*
LFR 0017	278000.0	63174.4	3363.3	494.0	6393.3	742.2	2073.3	280.2	3700.0	*
LFR 0024	257000.0	60224.6	5913.3	55.1	5660.0	991.4	1843.3	41.6	3650.0	495.0
LFR 0025	230000.0	27184.6	2840.0	820.7	2383.3	500.6	1120.0	147.3	1800.0	*
LFR 0026	312333.3	11015.1	5010.0	844.5	8473.3	441.1	3460.0	629.5	5000.0	1414.2
LFR 0027	211666.7	12342.3	1510.0	350.0	2163.3	637.2	1120.0	308.1	1300.0	*
NFP 0008	4333.3	1527.5	133.3	83.9	306.7	92.9	60.0	30.0	50.0	*
NFP 0009	34000.0	3605.6	883.3	55.1	1070.0	183.6	396.7	133.2	200.0	*
PIV 0003	238666.7	17156.1	2230.0	499.3	3443.3	868.0	2873.3	220.3	4500.0	*
PIV 0004	77666.7	19756.9	2170.0	646.5	2043.3	371.7	1423.3	196.6	2500.0	*
PIV 0005	47666.7	16921.4	1316.7	1232.7	1596.7	20.8	1053.3	162.6	2700.0	*
PIV 0012	102333.3	67485.8	696.7	159.5	1480.0	365.9	1010.0	137.5	1200.0	*
TFV 0003	313000.0	106066.0	3270.0	481.4	4656.7	20.8	2650.0	52.0	1250.0	1767.8
TFV 0004	212000.0	40853.4	3043.3	629.6	6220.0	398.5	2473.3	355.7	3100.0	141.4
TFV 0042	287000.0	35355.3	4023.3	515.2	6570.0	521.6	3723.3	1578.4	4700.0	424.3
TFV 0043	447000.0	38974.4	4096.7	1457.2	8133.3	964.4	3463.3	448.8	6000.0	*
TFV 0044	348000.0	89437.1	2446.7	1095.1	7100.0	330.0	3610.0	240.2	5250.0	1060.7
YBC 0010	16333.3	1154.7	1336.7	83.9	323.3	158.9	210.0	17.3	0.0	*
YBC 0024	171000.0	19467.9	3653.3	300.2	5413.3	61.1	3413.3	201.3	2500.0	*
YBC 0053	141333.3	11547.0	1340.0	268.5	4146.7	300.2	2966.7	388.9	1800.0	*
YBC 0054	174000.0	22516.7	1523.3	567.5	1516.7	263.5	933.3	73.7	700.0	*

Appendix F: Summer microbiology data with average counts (ave.) and standard deviations (S.D.).

* = No standard deviation, only one plate countable; *N.D.* = No Data for site; † = per 100 ml (all other counts per 1ml)

Site	Totals ave.	Totals S.D.	Cipro. ave.	Cipro. S.D.	Erythro. ave.	Erythro. S.D.	Tet. ave.	Tet. S.D.	FC † ave.	FC † S.D.	TC † ave.	TC † S.D.
BSR 0265	26333.3	9291.6	536.7	86.2	513.3	231.6	23.3	15.3	263.3	47.3	6900.0	141.4
BSR 1005	16333.3	4041.5	240.0	99.0	596.7	125.0	0.0	0.0	300.0	100.0	600.0	565.7
BSR 1265	29000.0	10440.3	450.0	28.3	1080.0	424.3	63.3	15.3	273.3	180.4	*N.D.*	*N.D.*
BSR 1685	43666.7	7767.5	1120.0	70.7	1673.3	273.2	145.0	35.4	900.0	*	17000.0	*
BSR 2595	21000.0	9899.5	800.0	84.9	1613.3	118.5	173.3	23.1	1110.0	410.1	19000.0	*
DEW 0003	93333.3	38436.1	1295.0	21.2	1903.3	246.8	113.3	15.3	560.0	226.3	28000.0	*
DEW 0004	26500.0	707.1	373.3	64.3	1246.7	92.4	53.3	5.8	640.0	84.9	34000.0	*
DEW 0005	52000.0	12490.0	823.3	76.4	1405.0	360.6	93.3	35.1	196.7	90.7	10000.0	*
DEW 0049	8333.3	4163.3	520.0	230.7	1290.0	144.2	66.7	5.8	133.3	61.1	12000.0	*
FRL 0002	156333.3	22723.0	500.0	34.6	1950.0	278.7	360.0	14.1	740.0	56.6	*N.D.*	*N.D.*
FRL 0032	82000.0	19000.0	833.3	95.0	1226.7	185.0	305.0	63.6	210.0	14.1	*N.D.*	*N.D.*
JWF 0001	8500.0	3214.6	100.0	56.6	306.7	51.3	33.3	25.2	40.0	*	*N.D.*	*N.D.*
JWF 0002	319000.0	26870.1	133.3	30.6	595.0	7.1	70.0	17.3	60.0	40.0	7500.0	*
JWF 0003	190500.0	2121.3	266.7	109.7	670.0	43.6	136.7	5.8	250.0	55.7	*N.D.*	*N.D.*
JWF 0021	*N.D.*	*N.D.*	365.0	7.1	703.3	89.6	103.3	11.5	723.3	242.1	*N.D.*	*N.D.*
LFR 0017	187333.3	19857.8	195.0	7.1	866.7	202.3	170.0	28.3	720.0	169.7	*N.D.*	*N.D.*
LFR 0024	346666.7	30859.9	486.7	50.3	1370.0	168.2	190.0	55.7	366.7	57.7	*N.D.*	*N.D.*
LFR 0025	269000.0	48590.1	1060.0	84.9	1090.0	95.4	180.0	*	590.0	438.4	19000.0	*
LFR 0026	19333.3	4163.3	360.0	113.1	493.3	98.7	23.3	5.8	60.0	*	11000.0	*
LFR 0027	243666.7	47543.0	120.0	40.0	826.7	92.9	36.7	25.2	150.0	*	14000.0	*
NFP 0008	6000.0	2645.8	53.3	35.1	183.3	70.9	60.0	20.0	0.0	*	1600.0	*
NFP 0009	33000.0	0.0	416.7	168.0	976.7	58.6	330.0	60.0	566.7	117.2	*N.D.*	*N.D.*
PIV 0003	164000.0	0.0	1786.7	692.4	1016.7	263.1	180.0	34.6	130.0	*	7000.0	*
PIV 0004	178500.0	33234.0	6505.0	3811.3	1096.7	193.0	176.7	65.1	10.0	*	4800.0	*
PIV 0005	93500.0	17677.7	1683.3	20.8	763.3	265.0	156.7	35.1	280.0	*	8400.0	*
PIV 0012	106000.0	1414.2	920.0	329.1	1263.3	414.0	400.0	26.5	663.3	126.6	*N.D.*	*N.D.*
TFV 0003	2120666.7	395779.4	1005.0	148.5	1945.0	403.1	83.3	11.5	400.0	*	20000.0	*
TFV 0004	67000.0	7211.1	426.7	90.7	820.0	34.6	20.0	0.0	160.0	*	11400.0	*
TFV 0042	1132000.0	67882.3	666.7	185.8	1503.3	210.3	76.7	20.8	460.0	*	30000.0	*
TFV 0043	1128000.0	214960.5	293.3	85.0	920.0	70.7	110.0	20.0	230.0	*	19000.0	*
TFV 0044	99500.0	7778.2	170.0	84.9	626.7	66.6	25.0	21.2	200.0	*	11000.0	*
YBC 0010	117000.0	10440.3	213.3	63.5	625.0	35.4	130.0	20.0	220.0	28.3	200.0	*
YBC 0024	57000.0	28284.3	793.3	58.6	806.7	102.6	113.3	40.4	166.7	61.1	17600.0	6222.5
YBC 0053	111000.0	0.0	740.0	216.6	2106.7	255.4	283.3	70.2	193.3	30.6	1600.0	1979.9
YBC 0054	55333.3	8144.5	2516.7	1485.6	910.0	198.0	346.7	72.3	800.0	*	21000.0	*

Appendix G: Fall microbiology data with average counts (ave.) and standard deviations (S.D.).

* = No standard deviation, only one plate countable; *N.D.* = No Data for site; † = per 100 ml (all other counts per 1ml)

Site	Totals ave.	Totals S.D.	Cipro. ave.	Cipro. S.D.	Erythro. ave.	Erythro. S.D.	Tet. ave.	Tet. S.D.	FC † ave.	FC † S.D.	TC † ave.	TC † S.D.
BSR 0265	157333.3	16165.8	1826.7	590.0	2760.0	138.6	466.7	145.7	1180.0	452.5	24200.0	2545.6
BSR 1005	173333.3	35571.5	1863.3	300.1	2636.7	442.4	383.3	80.2	1440.0	226.3	19500.0	707.1
BSR 1265	192333.3	35232.6	2393.3	45.1	2943.3	229.0	416.7	94.5	640.0	226.3	19500.0	2121.3
BSR 1685	138500.0	16263.5	1606.7	254.2	2080.0	113.6	363.3	119.3	670.0	183.8	13700.0	2404.2
BSR 2595	262500.0	10606.6	6750.0	495.0	9960.0	385.7	4800.0	226.3	6100.0	*	*N.D.*	*N.D.*
DEW 0003	151000.0	44530.9	3740.0	374.7	6346.7	482.2	710.0	104.4	4700.0	*	73000.0	*
DEW 0004	111500.0	3535.5	2155.0	544.5	3946.7	174.7	550.0	60.8	1190.0	268.7	34000.0	*
DEW 0005	119000.0	12727.9	4320.0	169.7	6853.3	333.1	1296.7	125.8	2900.0	*	70000.0	*
DEW 0049	10333.3	577.4	220.0	43.6	2693.3	687.1	80.0	40.0	40.0	34.6	74000.0	*
FRL 0002	11666.7	4041.5	406.7	51.3	806.7	20.8	46.7	11.5	116.7	28.9	1066.7	115.5
FRL 0032	12000.0	4582.6	86.7	41.6	586.7	238.6	46.7	30.6	216.7	160.7	2433.3	1914.0
JWF 0001	19333.3	4725.8	340.0	43.6	586.7	75.7	40.0	0.0	30.0	26.5	0.0	0.0
JWF 0002	33666.7	13650.4	1016.7	95.0	1873.3	142.9	186.7	76.4	130.0	43.6	833.3	288.7
JWF 0003	48000.0	14730.9	1516.7	109.7	1856.7	303.5	73.3	20.8	226.7	46.2	533.3	503.3
JWF 0021	11333.3	2081.7	486.7	15.3	870.0	212.1	33.3	23.1	76.7	25.2	0.0	0.0
LFR 0017	23666.7	577.4	650.0	155.2	593.3	170.4	23.3	15.3	166.7	115.5	566.7	493.3
LFR 0024	64500.0	13435.0	323.3	15.3	1263.3	253.2	63.3	20.8	826.7	70.2	6066.7	3534.6
LFR 0025	273666.7	15821.9	6400.0	1697.1	7773.3	46.2	2760.0	198.0	9000.0	*	*N.D.*	*N.D.*
LFR 0026	318333.3	35118.8	5700.0	*	8866.7	1372.5	2876.7	242.1	8000.0	*	*N.D.*	*N.D.*
LFR 0027	159000.0	42426.4	2745.0	318.2	6613.3	83.3	890.0	117.9	3600.0	*	90000.0	*
NFP 0008	2666.7	2081.7	183.3	5.8	306.7	153.7	40.0	69.3	3.3	5.8	66.7	115.5
NFP 0009	25000.0	4358.9	1153.3	159.5	1453.3	340.3	260.0	81.9	210.0	17.3	1300.0	264.6
PIV 0003	40000.0	12727.9	2233.3	321.5	2933.3	219.4	646.7	162.0	3300.0	*	14000.0	*
PIV 0004	34000.0	8185.4	1766.7	45.1	2766.7	292.6	490.0	85.4	2600.0	*	30000.0	*
PIV 0005	34333.3	10066.4	1780.0	115.3	1806.7	181.5	555.0	63.6	620.0	113.1	21000.0	*
PIV 0012	11000.0	4242.6	720.0	108.2	970.0	212.1	150.0	40.0	130.0	36.1	7100.0	1555.6
TFV 0003	254000.0	19924.9	1470.0	100.0	2286.7	245.8	263.3	61.1	1220.0	254.6	8800.0	1131.4
TFV 0004	100333.3	29263.2	760.0	353.6	1533.3	115.9	125.0	21.2	270.0	175.2	2300.0	1179.0
TFV 0042	247333.3	39803.7	1310.0	113.1	2060.0	272.2	120.0	*	363.3	183.4	8566.7	6046.8
TFV 0043	216666.7	61614.4	1670.0	204.2	2340.0	438.4	260.0	34.6	336.7	40.4	9433.3	5651.8
TFV 0044	204666.7	56712.7	1463.3	66.6	2626.7	299.4	250.0	90.0	716.7	119.3	11600.0	5091.2
YBC 0010	14333.3	2309.4	505.0	7.1	823.3	80.8	296.7	70.2	36.7	32.1	14800.0	282.8
YBC 0024	57000.0	5000.0	1593.3	185.0	2873.3	374.3	810.0	14.1	1290.0	155.6	18200.0	282.8
YBC 0053	38666.7	5773.5	950.0	153.9	1576.7	226.8	685.0	7.1	120.0	34.6	8900.0	141.4
YBC 0054	47000.0	8888.2	2010.0	338.1	2503.3	277.5	503.3	20.8	520.0	28.3	7800.0	3959.8

Appendix H: Spring physical parameter data obtained at time of sampling.

Site	Temperature (deg C)	Turbidity (NTU)	Spec. Conduct. (umho/cm)	Oxygen (mg/l)	pH	Alkalinity (mg/l)
BSR 0265	20	100	390	9.1	7.8	88
BSR 1005	19.8	250	379	8.7	7.8	96
BSR 1265	19.8	195	381	8.8	7.8	88
BSR 1685	19.7	195	394	8.8	7.8	100
BSR 2595	19.4	185	397	8.9	7.8	92
DEW 0003	19.1	30	460	7.9	7.8	116
DEW 0004	20.8	4.4	1004	9.6	8.1	180
DEW 0005	19.6	15	755	8.2	8	160
DEW 0049	21.6	250	465	7.9	7.9	104
FRL 0002	17.3	160	351	9.5	8	84
FRL 0032	20.4	170	476	9.6	7.9	100
JWF 0001	14.8	6	605	10.4	7.6	84
JWF 0002	17.6	65	418	9.5	7.7	60
JWF 0003	18.7	23	705	9.4	8	120
JWF 0021	18.4	360	371	9.7	7.9	84
LFR 0017	19.3	450	306	9	8	84
LFR 0024	18.2	220	427	9.6	8	102
LFR 0025	19.1	130	411	9	7.8	90
LFR 0026	19.3	900	297	8.2	7.5	80
LFR 0027	19.5	45	460	8.3	7.8	104
NFP 0008	15.1	4	28	9.9	7.7	12
NFP 0009	16.8	21	228	9.5	7.5	40
PIV 0003	18.3	60	136	9.2	7.6	56
PIV 0004	17.6	26	136	9.5	7.8	60
PIV 0005	17.6	45	126	9	7	40
PIV 0012	16.4	33	139	9.4	7.6	48
TFV 0003	16.9	1300	231	9.6	7.7	64
TFV 0004	16.6	1300	226	9.8	7.7	60
TFV 0042	18.1	750	264	9	7.6	80
TFV 0043	17.9	1300	273	9	7.8	80
TFV 0044	17.2	1200	218	9.3	7.8	54
YBC 0010	17.8	13	140	8.8	7.1	48
YBC 0024	18.2	60	137	9	7.5	52
YBC 0053	18.5	65	118	9.2	7.4	44
YBC 0054	18.2	11	260	9.3	7.4	56

Appendix I: Summer physical parameter data obtained at time of sampling.
N.D. = No Data for site.

Site	Temperature (deg C)	Turbidity (NTU)	Spec. Conduct. (umho/cm)	Oxygen (mg/l)	pH	Alkalinity (mg/l)
BSR 0265	25.9	38	602	*N.D*	7.8	132
BSR 1005	25.4	20	612	*N.D*	7.9	140
BSR 1265	25.1	39	617	*N.D*	7.8	136
BSR 1685	24.8	h55	617	8.3	7.8	148
BSR 2595	24.6	45	623	8.8	7.9	152
DEW 0003	22.7	47	786	7.7	7.9	164
DEW 0004	22.4	5	1717	8.4	8	168
DEW 0005	21.5	3	1592	8.1	8.2	288
DEW 0049	26.5	25	619	5.6	7.5	96
FRL 0002	24.5	17	433	9.7	8.5	104
FRL 0032	25.9	22	564	8.2	7.8	120
JWF 0001	19	0	592	11.1	7.8	92
JWF 0002	22.9	5	1400	*N.D*	8.2	146
JWF 0003	21.9	3	1010	*N.D*	8.1	160
JWF 0021	23.4	35	501	10.2	8.3	132
LFR 0017	24.4	11	441	7.8	8.2	164
LFR 0024	25.9	29	631	8.2	7.9	124
LFR 0025	26.1	120	576	7.8	7.9	100
LFR 0026	25.4	21	565	8.2	7.8	124
LFR 0027	25.3	28	601	7.4	7.9	104
NFP 0008	19.7	0	24	*N.D*	6.1	28
NFP 0009	20.8	3	501	*N.D*	7.3	68
PIV 0003	22.8	7	269	6.3	7.5	88
PIV 0004	22.8	6	331	7.2	7.9	136
PIV 0005	23.2	2	214	6.1	7.2	72
PIV 0012	15	4	98	9.6	6.9	48
TFV 0003	26.1	18	634	7.6	8.3	192
TFV 0004	25.9	16	627	8.2	8.4	188
TFV 0042	26	52	645	7.6	8.1	176
TFV 0043	26.3	29	648	7.6	8.1	184
TFV 0044	26.3	21	658	7.7	8.2	188
YBC 0010	22.2	8	150	6.2	7.2	48
YBC 0024	21.7	7	202	7.7	7.5	80
YBC 0053	20.8	250	203	6.1	7.1	84
YBC 0054	24.7	5	406	7.5	7.3	88

Appendix J-1: Spring water chemistry data. Tot. = Total; Diss. = Dissolved; Susp. = Suspended; *N.D.* = No Data

Site	HCO_3, Diss. (mg/L)	Solids, Tot. (mg/L)	Solids, Diss. (mg/L)	Solids, Susp. (mg/L)	NH_3, Tot. (mg/L N)	Kjeldahl, Diss. (mg/L N)	Kjeldahl, Tot. (mg/L N)	NO_2+NO_3, Tot. (mg/L N)
BSR0265	84.8	315	273	42	0.11	*N.D.*	0.07	0.44
BSR1005	81.2	460	305	155	0.24	*N.D.*	0.06	0.45
BSR1265	83	375	260	115	0.2	0.1	0.17	0.46
BSR1685	88	403	266	137	0.19	*N.D.*	0.09	0.46
BSR2595	80.2	449	326	123	0.16	*N.D.*	0.14	0.45
DEW0003	94	328	308	20	0.02	0.08	0.11	0.79
DEW0004	172	913	913	0	0	0.13	0.15	0.79
DEW0005	158	566	556	10	0	*N.D.*	0.12	1.11
DEW0049	92.3	506	331	175	0	*N.D.*	0.21	0.51
FRL0002	71.4	373	228	145	0.02	0.31	0.44	0.41
FRL0032	87.6	365	305	60	0.04	0.33	0.31	0.33
JWF0001	141	481	468	13	0	0.14	0.16	0.54
JWF0002	53	327	279	48	0	*N.D.*	0.28	0.34
JWF0003	111	591	575	16	0.02	*N.D.*	0.14	0.48
JWF0021	78.2	574	365	209	0.02	*N.D.*	0.37	0.31
LFR0017	75	612	221	391	0.09	0.4	0.62	0.41
LFR0024	77	471	298	173	0	*N.D.*	0.48	0.44
LFR0025	78.6	325	283	42	0.03	0.1	0.15	0.44
LFR0026	59	774	250	524	0	0.73	0.8	0.42
LFR0027	89.1	413	355	58	0.06	0.17	0.14	0.43
NFP0008	3.6	16	16	0	0	*N.D.*	0.14	0.02
NFP0009	30.8	151	141	10	0	0.15	0.16	0.16
PIV0003	42.2	119	108	11	0.06	*N.D.*	0.13	0.2
PIV0004	42.9	110	103	7	0	*N.D.*	0.14	0.13
PIV0005	25.9	121	101	20	0	0.14	0.15	0.19
PIV0012	27.4	145	130	15	0.02	0.18	0.16	0.17
TFV0003	50.3	787	181	606	0.02	0.4	0.62	0.58
TFV0004	49.3	823	167	656	0	0.37	0.71	0.55
TFV0042	61.2	648	202	446	0	0.43	0.62	0.49
TFV0043	67	1050	102	948	0	0.61	0.71	0.55
TFV0044	51.6	679	39	640	0	0.44	0.71	0.54
YBC0010	26.2	100	94	6	0.13	0.12	0.25	0.07
YBC0024	35.4	126	111	15	0.08	*N.D.*	0.09	0.39
YBC0053	35.4	100	90	10	0.1	*N.D.*	0.05	0.18
YBC0054	45.1	181	175	6	0.02	0.11	0.1	0.26

Appendix J-2: Spring water chemistry data. Tot. = Total; Diss. = Dissolved; Inorg. = Inorganic; *N.D.* = No Data

Site	NO_2+NO_3, Diss. (mg/L N)	Phos, Tot. (mg/L)	Phos, Diss. (mg/L)	TOC (mg/L)	DOC (mg/L)	Carbon Inorg. Tot. (mg/L)	Carbon Inorg. Diss. (mg/L)	Calcium, Diss. (mg/L)
BSR0265	0.45	0.12	0.006	2	2	14.6	13.9	35.7
BSR1005	*N.D.*	0.196	0.007	2.4	2.7	14.3	13.7	35
BSR1265	0.44	0.211	0.006	2.4	2.2	14.8	14.2	35.2
BSR1685	*N.D.*	0.123	0.006	2.4	2.1	15.1	15	36.3
BSR2595	*N.D.*	0.109	0.004	2.1	2.3	13.6	13	36.4
DEW0003	0.8	0.029	0.006	1.4	1.5	16.5	16.4	40.8
DEW0004	0.8	0.006	0	2.2	2.2	29	28.8	96.9
DEW0005	1.11	0.023	0.009	1.9	2	27.3	27	61.2
DEW0049	*N.D.*	0.02	0.004	2.6	2.8	16.2	15.6	39
FRL0002	0.48	0.05	0.008	1.9	1.7	15.2	14.6	28.3
FRL0032	0.35	0.066	0.004	1.6	1.8	18.3	17.7	41.2
JWF0001	0.47	0.016	0.006	1.8	1.8	15.5	13.5	56.5
JWF0002	*N.D.*	0.075	0.006	2.2	2.2	11	10.6	38.6
JWF0003	0.52	0.037	0.01	2.3	2.3	21.3	22.2	66.4
JWF0021	*N.D.*	0.078	0.008	2.6	2.4	12.6	15.1	33.7
LFR0017	0.43	0.116	0.022	3.2	3.3	15.3	14.8	25.3
LFR0024	*N.D.*	0.067	0.01	2.1	2.2	16.6	15.7	38.8
LFR0025	0.43	0.072	0	1.8	2.1	13.2	13.2	38.7
LFR0026	0.4	0.192	0.017	3.6	5.3	12.3	11.8	25
LFR0027	0.45	0.061	0.006	1.7	1.7	15.3	15.3	42.7
NFP0008	*N.D.*	0.009	*N.D.*	1	1.2	1.5	1.3	1.6
NFP0009	0.17	0.019	0.006	1.1	1.5	6.5	6.3	18.7
PIV0003	0.22	0.034	0.006	2.6	2.6	7.4	7.4	13.4
PIV0004	*N.D.*	0.022	0.005	1.9	2.4	7.4	7.4	13.9
PIV0005	0.21	0.024	0.006	2	1.9	4.6	4.5	10.3
PIV0012	0.2	0.037	0.004	2.3	2.4	5.1	4.8	11.9
TFV0003	0.55	0.177	0.014	2.7	3	10	9.7	21.8
TFV0004	0.51	0.203	0.051	2.2	2.9	10.3	9.6	20.3
TFV0042	0.45	0.137	0.012	2.9	3.3	12	11.6	22.7
TFV0043	0.54	0.22	0.013	2.9	3.3	12.9	12.9	24.5
TFV0044	0.49	0.183	0.022	3	4.2	10.4	9.9	19.8
YBC0010	0.08	0.015	0.003	2.7	2.8	4.8	4.9	10.3
YBC0024	0.37	0.06	0.006	2.1	2.4	6.5	5.9	11.9
YBC0053	0.18	0.061	0.006	1.9	1.9	6.3	6	10.4
YBC0054	0.25	0.014	0.004	2.1	2.1	8.1	7.9	17.8

Appendix J-3: Spring water chemistry data. Tot. = Total; Diss. = Dissolved

Site	Magnesium, Diss (mg/L)	Sodium, Diss (mg/L)	Potassium, Diss (mg/L)	Chloride, Diss (mg/L)	Sulfate, Diss (mg/L)	Barium, Diss (ug/L)	Iron, Tot (µg/L)	Iron, Diss
BSR0265	14.8	18.6	2.6	9.2	102	40	2210	26
BSR1005	14.5	17.7	2.6	8.3	98.7	39	5070	37
BSR1265	14.7	18.2	2.6	8.1	97	39	4800	36
BSR1685	15.2	18.6	2.7	8.3	103	40	4040	32
BSR2595	15.6	17.7	2.6	9.2	112	38	4540	26
DEW0003	16.5	31	3.2	10.2	134	46	1130	22
DEW0004	82.6	10.2	8	10.1	426	45	322	11
DEW0005	54	24.8	5	19	243	47	644	11
DEW0049	21.8	21.9	3.2	10.7	134	42	5810	32
FRL0002	11.5	23.3	2.1	15.2	82.8	42	4950	40
FRL0032	17.4	31.3	2.6	14.7	133	50	2190	20
JWF0001	37.3	20.5	3	5.9	201	33	209	0
JWF0002	23.1	11.8	2.3	5.6	147	28	2260	52
JWF0003	46.4	18.6	3.6	8.1	259	36	663	18
JWF0021	16	20.1	2.3	6.9	103	40	8480	57
LFR0017	11.1	17.1	2.1	6.7	71.2	37	13200	78
LFR0024	18.8	20.3	2.6	8.6	128	40	6920	31
LFR0025	17.1	17.6	2.6	9	118	38	2520	20
LFR0026	12.7	11.8	2.5	6	79.4	32	15200	127
LFR0027	19.8	22.8	2.8	10.6	136	42	1470	17
NFP0008	0.9	0.4	0.5	0.8	5.3	30	69	14
NFP0009	11.8	4.3	1.2	5.5	67.8	30	525	42
PIV0003	4.6	4.5	1.6	6	19	21	1470	105
PIV0004	4.7	4	1.4	6	18.7	28	591	101
PIV0005	6.1	2.1	1.4	1.9	31.4	21	1100	113
PIV0012	6.3	3.1	1.4	4.2	33.5	19	889	57
TFV0003	9.5	7.5	2.2	3.1	59.2	30	27300	99
TFV0004	9.4	7.6	2.1	3.2	56.3	30	27700	65
TFV0042	10.4	11.2	2.3	4.3	66.5	34	20010	82
TFV0043	10	10.6	2.3	3.6	64.9	34	36000	80
TFV0044	8.4	7.7	2	3.5	51	29	29400	101
YBC0010	5.6	3.2	1.5	5.5	32	35	613	44
YBC0024	5.4	3.5	1.7	5.5	25	20	1440	77
YBC0053	4.6	1.7	1.3	1.7	19.7	20	1410	88
YBC0054	10.2	10.8	1.9	21.9	48.6	32	877	109

Appendix J-4: Spring water chemistry data. Tot. = Total; Diss. = Dissolved

Site	Manganese, Tot. (µg/L)	Manganese, Diss. (µg/L)	Zinc, Diss. (µg/L)	Zinc, Tot. (µg/L)	Aluminum, Tot. (µg/L)	Aluminum, Diss. (µg/L)	Silicon, Diss. (mg/L)	Silicon, Tot. (mg/L)	Titanium, Tot. (µg/L)
BSR0265	101	14	0	9	1210	37	3.51	4.59	14
BSR1005	207	9	0	17	2690	45	3.45	6.17	28
BSR1265	197	6	3	18	2530	43	3.49	5.93	27
BSR1685	211	7	0	17	2020	42	3.48	5.62	22
BSR2595	201	6	3	23	2370	36	3.34	5.83	26
DEW0003	66	37	0	4	465	37	3.98	4.31	0
DEW0004	69	63	0	0	73	22	2.65	2.71	0
DEW0005	60	44	0	0	215	23	2.98	3.08	0
DEW0049	302	53	0	18	3560	52	2.82	6.57	42
FRL0002	221	14	0	26	3340	64	3.96	7.34	26
FRL0032	132	37	0	12	1350	58	3.2	4.53	14
JWF0001	84	5	0	7	146	22	2.88	2.95	0
JWF0002	369	148	0	15	1170	48	3.45	4.5	0
JWF0003	109	66	0	6	395	75	3.76	3.95	0
JWF0021	262	16	0	30	4700	78	3.25	8.07	36
LFR0017	407	19	0	47	7230	81	3.1	10.9	55
LFR0024	311	4	0	24	3660	48	3.44	7.96	33
LFR0025	144	9	3	10	1190	32	3.44	4.72	14
LFR0026	581	7	5	51	7330	98	3.81	12	65
LFR0027	97	20	0	7	754	35	3.39	4.05	0
NFP0008	8	4	4	5	70	20	2.72	2.64	0
NFP0009	253	218	5	10	392	49	3.59	3.81	0
PIV0003	54	31	0	5	698	49	4.38	5.25	10
PIV0004	34	23	0	4	300	54	4.41	4.56	0
PIV0005	110	80	0	5	492	43	4.42	4.74	0
PIV0012	69	31	0	5	407	36	3.5	3.79	0
TFV0003	839	14	7	87	14900	90	4.19	18.1	85
TFV0004	878	14	0	85	14600	63	4.17	17.8	92
TFV0042	622	9	3	65	9390	72	4	14.9	88
TFV0043	1050	6	0	122	18900	79	3.73	22	103
TFV0044	780	7	3	86	16100	76	3.91	19.2	90
YBC0010	452	417	0	0	200	0	3.64	3.6	0
YBC0024	50	26	0	4	711	35	4.08	4.61	0
YBC0053	73	57	0	4	629	32	4.25	4.77	0
YBC0054	93	78	0	0	167	0	3.87	3.94	0

Appendix K-1: Summer water chemistry data. Tot. = Total; Diss. = Dissolved; Susp. = Suspended; *N.D.* = No Data

Site	HCO_3, Diss. (mg/L)	Solids, Tot. (mg/L)	Solids, Diss. (mg/L)	Solids, Susp. (mg/L)	NH_3, Tot. (mg/L N)	Kjeldahl, Diss. (mg/L N)	Kjeldahl, Tot. (mg/L N)	NO_2+NO_3, Tot. (mg/L N)
BSR0265	133	424	402	22	0.04	0.15	0.18	0.54
BSR1005	123	421	412	9	0.03	0.09	0.14	0.53
BSR1265	142	429	407	22	0.04	0.12	0.13	0.52
BSR1685	144	456	418	38	0.05	0.11	0.19	0.52
BSR2595	142	427	375	52	0.02	0.1	0.12	0.49
DEW0003	167	570	550	20	0.03	0.14	0.11	1.21
DEW0004	214	1596	1589	7	0	0.25	0.22	0.49
DEW0005	289	1250	1250	0	0	*N.D*	0.15	1.39
DEW0049	122	436	431	5	0.05	*N.D*	0.22	0.14
FRL0002	97.4	284	276	8	0	0.03	0.06	0.3
FRL0032	112	362	356	6	0.06	0.06	0.08	0.29
JWF0001	112	789	783	6	0.02	*N.D*	0	0.34
JWF0002	183	1140	1140	0	0	0.11	0.07	0.82
JWF0003	180	1060	1051	9	0	0.02	0.1	0.75
JWF0021	*N.D.*	325	321	4	0	0.08	0.07	0.35
LFR0017	137	419	413	6	0	0.04	0.07	0.32
LFR0024	137	776	720	56	0.02	*N.D*	0.09	0.17
LFR0025	119	477	394	83	0	0.28	0.34	0.34
LFR0026	120	405	395	10	0	*N.D*	0.18	0.34
LFR0027	121	507	411	96	0.02	0.18	0.24	0.34
NFP0008	6.8	17	11	6	0	0.02	0.06	0.05
NFP0009	63.6	508	502	6	0	*N.D*	0.03	0.07
PIV0003	95.3	153	153	0	0	0.18	0.22	0.05
PIV0004	131	182	182	0	0	0.23	0.19	0.01
PIV0005	72.6	117	117	0	0	0.17	0.16	0.06
PIV0012	35.2	130	126	4	0	0.05	0.05	0.2
TFV0003	196	428	415	13	0.05	0.08	0.1	0.34
TFV0004	202	415	403	12	0	0.08	0.1	0.26
TFV0042	175	455	426	29	0	0.12	0.11	0.56
TFV0043	184	435	417	18	0	0.11	0.14	0.55
TFV0044	192	441	427	14	0	0.09	0.08	0.46
YBC0010	37.9	119	119	0	0.08	0.13	0.1	0.17
YBC0024	74.1	139	139	0	0	0.09	0.12	0.08
YBC0053	86.8	151	147	4	0.06	0.13	0.11	0.09
YBC0054	80	256	254	2	0	0.15	0.22	0.17

Appendix K-2: Summer water chemistry data. Tot. = Total; Diss. = Dissolved; Inorg. = Inorganic; *N.D.* = No Data

Site	NO_2+NO_3, Diss. (mg/L N)	Phos, Tot. (mg/L)	Phos, Diss. (mg/L)	TOC (mg/L)	DOC (mg/L)	Carbon Inorg. Tot. (mg/L)	Carbon Inorg. Diss. (mg/L)	Calcium, Diss. (mg/L)
BSR0265	0.62	0.057	0.021	1.6	1.6	25.4	25.1	51.3
BSR1005	0.51	0.027	0.025	1.5	1.8	27	24.5	52.5
BSR1265	0.54	0.029	0.01	1.5	1.8	27.4	27.3	53.6
BSR1685	0.52	0.042	0.014	1.6	1.8	27	27.2	54.6
BSR2595	0.48	0.047	0.008	1.6	1.8	24	27.8	54.3
DEW0003	1.25	0.03	0.013	2	2.3	32.1	31.7	66.1
DEW0004	0.51	0.013	0.008	2.6	2.6	40.8	40.5	189
DEW0005	1.42	0.009	0.006	2.3	2.4	55.8	54.7	97.3
DEW0049	*N.D*	0.02	0.007	2.4	2.4	24.3	23.4	51.1
FRL0002	0.29	0.017	0.008	1.3	1.3	17.4	18.2	38.5
FRL0032	0.29	0.022	0.009	1.6	2	22	21.4	49.2
JWF0001	*N.D*	0.007	0.01	2	1.9	11.3	*N.D*	58.5
JWF0002	0.82	0.01	0.01	1.6	1.8	32.3	32.9	132
JWF0003	0.8	0.01	0.012	1.6	1.6	25.4	*N.D*	113
JWF0021	0.36	0.014	0.008	1.9	1.9	22.7	22.4	44
LFR0017	0.34	0.018	0.017	1.8	1.9	24.7	24.2	32.7
LFR0024	*N.D*	0.022	0.007	2	2	12	15.7	54.5
LFR0025	0.34	0.078	0.006	1.8	2	23.2	23	51.6
LFR0026	0.34	0.022	0.007	1.8	1.9	23.1	22.5	49.9
LFR0027	0.36	0.067	0.005	1.8	2.1	24.6	24.4	52.6
NFP0008	0.05	0.009	0.007	0.9	1.1	2	0	2.4
NFP0009	*N.D*	0.022	0.011	1.6	1.5	6.9	11.4	51.2
PIV0003	0.05	0.016	0.008	2.3	2.2	18.6	18.1	*N.D*
PIV0004	0.01	0.01	0.005	1.9	1.7	25.2	25.1	43.1
PIV0005	0.06	0.01	0.004	1.8	1.8	14.6	14.3	20.5
PIV0012	0.2	0.011	0.011	1.9	1.9	7.4	7.4	13.6
TFV0003	0.34	0.037	0.014	1.2	1.2	37.3	39.3	53.7
TFV0004	0.26	0.022	0.009	1.2	1.2	38.3	37.7	51.5
TFV0042	0.55	0.042	0.014	1.4	1.3	34.2	33.3	55.2
TFV0043	0.53	0.036	0.015	1.3	1.3	35.1	35.1	53.3
TFV0044	0.45	0.027	0.014	1.2	1.3	37.1	37	53
YBC0010	0.19	0.02	0.011	2.9	2.8	8	8.3	12.8
YBC0024	0.1	0.015	0.008	1.9	1.9	15	14.6	20.2
YBC0053	0.09	0.039	0.011	1.7	1.8	17.5	18	21.5
YBC0054	0.17	0.008	0	2.3	2.1	15.2	16.4	27

Appendix K-3: Summer water chemistry data. Tot. = Total; Diss. = Dissolved; *N.D.* = No Data

Site	Magnesium, Diss. (mg/L)	Sodium, Diss. (mg/L)	Potassium, Diss. (mg/L)	Chloride, Diss. (mg/L)	Sulfate, Diss. (mg/L)	Barium, Diss. (µg/L)	Iron, Tot. (µg/L)	Iron, Diss. (µg/L)
BSR0265	22.4	48.3	4.4	24.6	148	67	1150	0
BSR1005	22.8	42.3	4.2	21.4	159	66	526	0
BSR1265	22.8	41	4.2	21.2	158	67	916	5
BSR1685	23.5	42.6	4.5	21.4	159	68	1190	8
BSR2595	24.1	42.4	4.4	20.4	164	68	1500	7
DEW0003	25.8	70	6.6	23.1	234	66	1180	11
DEW0004	203	22.1	16.8	15	846	97	442	7
DEW0005	111	117	12.7	86.1	540	77	172	14
DEW0049	32.8	31.2	4.6	15.6	194	68	197	26
FRL0002	12.9	33.3	2.6	24	96.8	56	299	15
FRL0032	18.6	42.4	3.4	27.3	146	64	378	0
JWF0001	37.3	18.2	3.3	8.8	221	34	61	0
JWF0002	89.6	72.2	7	19.2	660	38	168	8
JWF0003	82.3	30.3	5.9	20	481	48	313	0
JWF0021	21.6	33.8	3.1	9.5	143	51	174	14
LFR0017	15.8	37.8	3	10.2	111	59	241	25
LFR0024	22.9	43.8	3.8	25.7	186	65	424	0
LFR0025	25.5	36.5	4.1	20.6	158	61	2950	29
LFR0026	23.2	34.2	3.9	19.2	155	58	566	29
LFR0027	25.5	36.5	4.2	18	170	60	1300	10
NFP0008	1.1	0.4	0.5	1.6	7.3	31	77	12
NFP0009	31.2	8.9	2.4	10.1	190	42	402	32
PIV0003	*N.D*	*N.D*	*N.D*	22.8	17.8	*N.D*	379	156
PIV0004	8	14.1	3.2	28.8	12.8	80	192	71
PIV0005	8.3	6	2.5	7.3	32.8	36	540	310
PIV0012	6	5.3	1.6	9	29.1	32	304	91
TFV0003	21.5	54.1	4.1	10.5	145	67	458	8
TFV0004	20.9	54.4	3.9	10.9	143	67	788	6
TFV0042	24.6	49	4.6	17.9	168	67	1210	7
TFV0043	23.4	50.4	4.4	14.5	158	66	749	0
TFV0044	22.3	56.6	4.4	12.9	155	66	534	5
YBC0010	5.8	4.8	1.9	9.3	24.2	41	1320	941
YBC0024	7.3	6	2.7	11.6	21.4	32	504	224
YBC0053	8.6	3.6	2.4	5.3	24.2	36	1580	518
YBC0054	11.8	27.9	3.2	59.8	35.4	53	1040	181

Appendix K-4: Summer water chemistry data. Tot. = Total; Diss. = Dissolved

Site	Manganese, Tot. (µg/L)	Manganese, Diss. (µg/L)	Zinc, Diss. (µg/L)	Zinc, Tot. (µg/L)	Aluminum, Tot. (µg/L)	Aluminum, Diss. (µg/L)	Silicon, Diss.(mg/L)	Silicon, Tot. (mg/L)
BSR0265	106	50	12	14	642	37	3.83	4.2
BSR1005	46	18	10	12	303	36	3.51	3.66
BSR1265	56	20	13	16	499	35	3.36	3.66
BSR1685	66	16	13	15	665	37	3.45	3.89
BSR2595	83	22	12	14	828	38	3.45	4.05
DEW0003	125	90	13	16	543	34	3.44	3.91
DEW0004	140	132	69	68	62	0	1.91	1.88
DEW0005	71	70	50	59	45	28	1.62	1.57
DEW0049	325	323	17	15	48	0	3.18	3.06
FRL0002	22	10	4	4	194	48	3.37	3.05
FRL0032	135	86	8	8	238	35	2.91	2.82
JWF0001	300	93	16	15	42	0	3.33	2.82
JWF0002	112	104	36	37	85	40	2.18	1.97
JWF0003	118	94	35	37	198	89	2.85	2.72
JWF0021	47	19	9	9	108	26	3.23	2.76
LFR0017	22	15	6	5	141	29	3.15	2.83
LFR0024	68	34	10	10	273	34	2.73	2.66
LFR0025	183	40	12	19	1400	43	2.7	3.99
LFR0026	69	34	13	13	291	38	2.44	2.65
LFR0027	107	33	10	13	669	34	2.25	2.84
NFP0008	10	4	0	0	63	0	2.72	2.36
NFP0009	107	92	15	15	117	50	3.03	2.91
PIV0003	133	81	6	7	55	0	1.3	2.02
PIV0004	43	33	7	8	57	21	2.23	2.11
PIV0005	116	116	9	8	25	0	3.92	3.73
PIV0012	162	159	4	0	58	0	3.79	3.55
TFV0003	46	30	10	11	261	57	3.7	3.61
TFV0004	64	24	10	13	421	52	3.3	3.65
TFV0042	54	7	16	12	662	50	4.02	4.88
TFV0043	37	9	10	11	417	51	3.92	4.07
TFV0044	37	18	9	11	304	65	3.74	3.84
YBC0010	428	441	4	0	54	0	3.51	3.32
YBC0024	238	251	0	0	38	0	3.3	3.06
YBC0053	530	556	3	3	98	0	3.79	3.59
YBC0054	195	199	9	8	33	45	3.67	3.52

Appendix L: Kentucky Index of Biological Integrity scores for fish collections at 24 of 35 sites during the summer sampling season. Score may range from 0 (worst) to 100 (best). *N.D.* = No Data for site.

Site	Kentucky Index of Biological Integrity
BSR 0265	14
BSR 1005	14
BSR 1265	16
BSR 1685	17.5
BSR 2595	18.5
DEW 0003	*N.D.*
DEW 0004	45
DEW 0005	39
DEW 0049	*N.D.*
FRL 0002	*N.D.*
FRL 0032	19
JWF 0001	15
JWF 0002	38
JWF 0003	29
JWF 0021	29
LFR 0017	28
LFR 0024	*N.D.*
LFR 0025	16
LFR 0026	16
LFR 0027	*N.D.*
NFP 0008	68
NFP 0009	62
PIV 0003	50
PIV 0004	57
PIV 0005	*N.D.*
PIV 0012	*N.D.*
TFV 0003	*N.D.*
TFV 0004	16
TFV 0042	14.5
TFV 0043	*N.D.*
TFV 0044	*N.D.*
YBC 0010	31
YBC 0024	39
YBC 0053	46
YBC 0054	*N.D.*