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CONTROL AND PASSIVE TREATMENT OF RUNOFF FROM HORSE MUCK
STORAGE STRUCTURES USING RAIN GARDENS

THESIS

A thesis submitted in partial fulfillment of the requirements
for the degree of Masters of Science in Forestry
in the College of Agriculture
at the University of Kentucky

By

Hillary Otte

Lexington, Kentucky

Director: Dr. Chris Barton, Associate Professor of Forestry

Lexington, Kentucky

2012

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ABSTRACT OF THESIS

CONTROL AND PASSIVE TREATMENT OF RUNOFF FROM HORSE MUCK STORAGE STRUCTURES USING RAIN GARDENS

Runoff from livestock operations may contain a variety of pathogens and high levels of nutrients and other harmful contaminants, and is of particular concern in central Kentucky as watersheds are threatened by waste generated from a high concentration of equine activity. Rain gardens are a type of stormwater management tool used to capture and passively treat runoff. This project aimed to incorporate rain gardens into the horse muck storage structures at a thoroughbred facility in the Cane Run watershed in Lexington, Kentucky. Water quality data from soil water within two rain garden muck pads and two control pads, and grab samples from the stream were compared. No significant differences were observed, but trends revealed higher levels of nitrate and phosphate in rain gardens compared to controls, while total organic carbon and *E. coli* levels were lower in the rain gardens, suggesting that the rain gardens are trapping nutrients while reducing organic matter and killing bacteria. *E. coli* populations were lower in stream sample locations near rain garden muck pads compared to further downstream near controls. Management recommendations include further improvement of muck storage structures, replacing old muck pads, and changing management and housekeeping habits and attitudes towards environmental responsibility.

KEYWORDS: stormwater runoff, rain garden, horse muck, muck pad, *E. coli*

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December 4, 2012

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TABLE OF CONTENTS

Acknowledgements.....	iii
List of Tables.....	vi
List of Figures.....	vii
Chapter 1: Introduction.....	1
Evolution of the Livestock Industry.....	2
Manure Pollution.....	4
Nutrients.....	5
Pathogens.....	6
Other Pollution Indicators.....	7
Regulations.....	8
Compliance and Management Options.....	9
Wetlands Mechanisms for Wastewater Treatment.....	12
Rain Gardens.....	15
Equine Waste in Kentucky.....	17
Cane Run Watershed.....	18
Site Description.....	19
Purpose.....	25
Chapter 2: Methods.....	26
New Muck Storage Pad Design.....	26
Lysimeters and Tensiometers.....	37
Sampling and Analysis.....	43
Statistical Analysis.....	49
Statistical Package and Methods.....	49
Stream Water Quality.....	49
Muck Storage Pads.....	50
Plants.....	51
Chapter 3: Results and Discussion.....	52
Weather.....	52
Stream Water Quality.....	53
Muck Storage Pads.....	65
Plants.....	75
Chapter 4: Conclusions.....	77
The Case for Proper Livestock Waste Management.....	77
Future Work.....	82
Recommendations and Improvements for Best Management Practices.....	85
Appendix.....	95
Appendix 1: Stream <i>E. coli</i> Populations for Each Sample Date.....	95
Appendix 2: Stream Dissolved Oxygen for Each Sample Date.....	110
Appendix 3: Stream Temperature for Each Sample Date.....	120

Appendix 4: Box and Whisker Plots for <i>E. coli</i> Before and After June 2011.....	130
Appendix 5: <i>E. coli</i> Time Series for Each Stream Sample Location...	138
Appendix 6: Time Series of Water Chemistry Parameters: X Pads vs. C Pads.....	143
Appendix 7: Box and Whisker Plots. X pads vs. C pads for <i>E. coli</i> and Water Chemistry.....	150
Bibliography/References.....	164
Vita.....	170

LIST OF TABLES

Table 3.1	Average levels of water quality parameters in stream before and after June 2011.....	61
Table 3.2	Tensiometer readings from experimental and control pads.....	67

LIST OF FIGURES

Figure 1.1	Typical muck storage pad at Victory Haven, with concrete pad, walls, and approach.....	22
Figure 1.2	Spillage from approach end of muck storage pad at Victory Haven.....	22
Figure 1.3	Muck storage pad at Victory Haven showing flimsy wooden walls with gaps, allowing for excess spillage and seepage of liquid waste.....	23
Figure 1.4	Overflowing wooden muck storage pad t Victory Haven, showing drainage and close proximity to on-site stream.....	23
Figure 1.5	Extreme algal growth in stream at Victory Haven.....	24
Figure 2.1	Side-view cross-section diagram of experimental muck pad design.....	28
Figure 2.2	Front-view cross-section diagram of experimental muck pad design.....	29
Figure 2.3	Beginning construction of concrete walls surrounding existing muck pad X1.....	30
Figure 2.4	Completed walls at X2, before installation of new concrete pad.....	30
Figure 2.5	Hole in wall of X1, showing drainage from inside of pad.....	31
Figure 2.6	Excavation of rain garden basin at X1.....	31
Figure 2.7	Excavation of rain garden basin at X1.....	32
Figure 2.8	Planting of vegetation in rain garden at pad X1.....	34
Figure 2.9	Control pad C1.....	36
Figure 2.10	Control pad C2 with drainage leading to stream.....	36
Figure 2.11	Top-view diagram of experimental muck pad design, showing locations of lysimeters and tensiometers in the rain garden basin.....	39
Figure 2.12	Diagram of lysimeter and tensiometer placement within the rain garden basin at X pads.....	40
Figure 2.13	Picture of lysimeter and tensiometer in the rain garden at X1....	40

Figure 2.14	Top-view diagram of control muck pad design, showing locations of lysimeters and tensiometers in native soil behind the pad.....	41
Figure 2.15	Diagram of lysimeter and tensiometer placement within the native soil at control pads.....	42
Figure 2.16	Picture of lysimeter and tensiometer in valve box behind pad C1.....	42
Figure 2.17	Aerial Photograph of Victory Haven showing stream, sampling locations, and muck pads on the property.....	45
Figure 3.1	Measured <i>E. coli</i> populations over the sequence of stream sample locations on July 29, 2010.....	55
Figure 3.2	Dissolved oxygen levels over the sequence of stream sample locations on August 8, 2011.....	57
Figure 3.3	Groundwater seep in stream bank at Victory Haven.....	57
Figure 3.4	Water temperature over the sequence of stream sample locations on January 19, 2012.....	59
Figure 3.5	Water temperature over the sequence of stream sample locations on July 14, 2012.....	59
Figure 3.6	Time series of <i>E. coli</i> populations at stream sample location 6, showing lowest populations during winter months.....	64
Figure 3.7	PO ₄ ⁻³ concentrations in C pads and X pads.....	70
Figure 3.8	NO ₃ ⁻ concentrations in C pads and X pads.....	70
Figure 3.9	Box and Whisker plot of nitrate levels in C pads vs. nitrate levels in X pads.....	73
Figure 3.10	Box and Whisker plot of phosphate levels in C pads vs. phosphate levels in X pads.....	73
Figure 3.11	Box and Whisker plot of total organic carbon in C pads vs. total organic carbon in X pads.....	74
Figure 3.12	Box and Whisker plot of <i>E. coli</i> populations in C pads vs. <i>E. coli</i> populations in X pads.....	74
Figure 3.13	Total nitrogen concentrations for rain garden plants from greenhouse control and second year growth.....	76
Figure 3.14	Total phosphorus concentrations for rain garden plants from greenhouse control and second year growth.....	76

Figure 4.1	Old wooden-walls muck pad at Victory Haven.....	80
Figure 4.2	Spillage and overflow at pad C2 due to infrequent emptying...	80
Figure 4.3	Drainage ditch leading runoff from C2 to nearby Cane Run tributary.....	81
Figure 4.4	Runoff escaping from the approach end of pad X2.....	87
Figure 4.5	Spillage, mud, and tire dredging surrounding pad C2.....	89
Figure 4.6	Spillage and overflow at pad X2.....	92
Figure 4.7	Overflow and trash in the rain garden at pad X2.....	92
Figure 4.8	Overflow and wooden pallets stacked in the rain garden at pad X1.....	93

Chapter 1: INTRODUCTION

In 2011, the world's human population reached and surpassed 7 billion individuals, and as a result, is continuing to outgrow methods traditionally used to sustain it. One of the most important resources under stress is the world's water supply (The Economist, 2010). Overpopulation, increasing area of impervious surfaces, agricultural needs, and inappropriate waste disposal all contribute to water resource issues. Increasing concern is being directed towards the agricultural industry, particularly the livestock sector. As farms continue to become larger and house more animals in a single location, many operations have struggled to keep up with the amount of waste they produce. Improperly managed animal waste can create a wide array of health and environmental problems, especially when it contaminates surface and groundwater. Increasing support for environment health has inspired an initiative to develop practices that lessen these negative environmental impacts.

Wetlands are natural filtration and flood control systems and are increasingly being built specifically to be incorporated into treatment of wastewater from a variety of sources. Wetland function has inspired the development of a specific type of constructed wetland called a rain garden. Rain gardens utilize wetland principles through a passive, slow release system to offer a solution to mitigate stormwater pollution in urban and residential areas where space is a limiting factor, or to accommodate smaller amounts of runoff. Both of these methods may be an attractive option for livestock waste management. This project aims to use fundamental elements of both constructed wetlands and rain gardens, including plant uptake and passive infiltration, to create a manure and muck storage system which aims to improve the quality of stormwater runoff

and reduce stream contamination at a central Kentucky horse boarding and training facility.

EVOLUTION OF THE LIVESTOCK INDUSTRY

For most of human history, agricultural operations have been small, closed-loop systems that may have only supported one or a few families. Crops provided fodder to feed the livestock; in return, animals provided products and services to run and support the farm. Animals were machinery, precursors to cars and tractors. They provided clothing and food with their skins, wool, milk, eggs, and meat, and they fertilized crops with their manure. This time-tested system generated little to no waste, as everything produced within the farm had a specific use.

In the mid-1900s, the world population experienced a marked increase in growth. This population explosion, coupled with a global migration toward Western diet standards of daily meat consumption, sometimes at every meal, sparked an increase in meat and dairy demand. Developing countries have as much as tripled their meat consumption in the past two decades, and per capita milk consumption has increased by over fifty percent (The World Bank, 2005). Based on population growth data, it has been predicted that annual production of livestock products will have to grow by another 200 billion liters of milk and 100 million tons of meat in order to keep up (Martinez, et al., 2009). Because of this demand, traditional agriculture systems have become inefficient, and ultimately, obsolete. Crops and livestock are separated from one another and reared in specialized, intensive operations, focused on one crop or one type of animal. This shift continues, as small farms cannot compete with large commercial productions (USEPA,

2000). According to the most recent US Census of Agriculture, there are 2.2 million farms in the United States; only 125,000 of these produce 75% of the value of US agriculture production (USDA, 2009).

These large operations are classified into two categories based on the number of animals present, confinement strategies, vegetative cover, and waste disposal methods. An animal feeding operation, or AFO, is defined by the EPA as: a lot or facility where animals have been, are, or will be stabled or confined and fed or maintained for a total of 45 days or more in any 12-month period; and where crops, vegetation, forage growth, or post-harvest residues are not sustained over any portion of the lot or facility in the normal growing season. AFOs may house beef or dairy cattle, swine, sheep, horses, or poultry (USEPA, 2000). Depending on the characteristics of an AFO, it may be designated as a confined animal feeding operation, or a CAFO. CAFOs are determined on a case by case basis by assessing animal type and number, and waste disposal practices. It is estimated that approximately 20% of the farms in the United States are AFOs (USEPA, 2008). This figure is most likely more than that for worldwide animal operations, as developing countries in East Asia and Latin America are experiencing the fastest growth rates in livestock production and consumption (The World Bank, 2005).

This period of rapid development that has been taking place since the mid-1900's, widely known as the Livestock Revolution, eventually gave way to questions about its effects on the environment. The Environmental Movement, beginning in the 1960s, shed light on the detrimental consequences of unchecked agricultural growth and natural resource use and concern continues to grow as farming and livestock operations become larger and more concentrated. Due to the demise of the closed-loop farm and separation

of crop and livestock, one of the many issues raised was the topic of livestock waste management. AFOs produce over 500 million tons of manure annually (USEPA, 2008); however, due to the fact that not all farm operations qualify as AFOs, this number underestimates the amount of manure produced on an annual basis. Manure is still a popular choice in fertilization, although this method must compete with chemical fertilizers, concerns with sanitation and pathogen outbreaks, and logistic issues concerning the distance between crop and livestock operations.

MANURE POLLUTION

Manure does not go straight from the animal to the crop; it must be stored, at least temporarily, until it can be applied. Storage of manure poses some concerns, because untreated manure can harbor several contaminants that have the potential to pollute soil, groundwater, and surface water. Livestock manure is rich in organic matter and has a high biochemical oxygen demand (BOD) (Burkholder, et al., 2007). On a BOD scale, one dairy or beef cow is equal to 18-25 people; one hog, 2-3 people; and 10-15 chickens are equivalent to one person (Loehr, 1967). Since the rising concern for pollution associated with the Livestock Revolution, there have been extensive studies undertaken regarding the makeup of wastes from livestock operations, including manure. Manure can contribute to high levels of nitrogen and phosphorus compounds, turbidity, and pathogenic organisms (Loehr, 1967; Cole, et al., 2000; Hooda, et al., 2000; Raloff, 2000; Hutchison, et al., 2004; The World Bank, 2005; Burkholder, et al., 2007; Kato, et al., 2009). Each of these contaminants causes its own lists of problems arising from their presence in the environment.

NUTRIENTS

The nutrient content of manure will vary depending on the species, breed, sex, and size of the animal, as well as what it is fed. Manure from a 1,000 lb. (approximately 454 kg) beef cow is generally composed of 0.5-0.6% nitrogen and 0.2% phosphorus. A 200 lb. (approximately 91 kg) adult pig will produce manure with approximately 0.6-0.7% nitrogen and 0.2-0.3% phosphorus. Manure from a 1,000 lb. (approximately 454 kg) horse can contain 0.5-0.6% nitrogen and 0.1% phosphorus (Gillespie, 2002). High levels of nitrate and nitrite in drinking water is accepted to be a direct cause of methemoglobinemia, or blue baby syndrome (Burkholder, et al., 2007; WHO, 2011). Nitrite may react in the body and form compounds that are carcinogenic to humans, and high levels of nitrate may completely inhibit iodine uptake (WHO, 2011). The current accepted standards for drinking water are no more than 45 mg L⁻¹ for nitrate and no more than 3 mg L⁻¹ for nitrite and are based on thresholds for methemoglobinemia (WHO, 2011). At low pH, ammonia compounds occur in very low levels in drinking water and there is currently no guideline value (WHO, 2011); however, at a higher pH, it only takes a concentration of 0.02 mg L⁻¹ to be toxic to fish and other aquatic wildlife (Hooda, et al., 2000). Phosphorus has high soil retention in comparison to nitrogen, and most large-scale phosphorus losses occur because of application of manure applied to saturated, poorly drained, or frozen soils (Hooda, et al., 2000). Both excess nitrogen and excess phosphorus, combined with the high BOD of livestock manure, can stimulate extreme algal and cyanobacterial growth, contributing to intensive eutrophication and depleted oxygen availability (Hooda,

et al., 2000; The World Bank, 2005). Algal and cyanobacteria blooms are associated with red tide phenomena around the world, especially in Asia, and the release of cyanotoxins that may lead to widespread fish and other aquatic life die-offs (The World Bank, 2005; Burkholder, et al., 2007; WHO, 2011).

PATHOGENS

Animal excrement is a known source of pathogenic organisms, many of which are asymptomatic in livestock species, but cause fatal infections in humans (The World Bank, 2005). If these agents are able to contaminate water supplies, effects can be widespread and devastating to those exposed. It is estimated that water-borne diarrhea causes approximately 6,000 deaths every day, mostly in children (The World Bank, 2005). Pathogens found to originate from manure include *Escherichia coli* and *Salmonella* (Hooda, et al., 2000; The World Bank, 2005; WHO, 2011). *Escherichia coli* are a diverse group of bacteria that inhabit the intestines of warm-blooded animals. When in their native environment, *E. coli* are harmless and essential to digestive health; however, elsewhere in the body, an *E. coli* infection can cause urinary tract infections, bacteremia, and meningitis. If consumed and introduced to a foreign digestive system, several strains are enteropathogenic and can result in severe vomiting and diarrhea (WHO, 2011). *E. coli* is used as an indicator organism to determine water quality, possible fecal contamination, and suggest the presence of other pathogens (WHO, 2011). *Salmonella* spp. is another diverse group of bacteria that may originate from livestock manure. *Salmonella* has an array of serotypes, infection by which

can result in any of four conditions: gastroenteritis, bacteremia or septicemia, typhoid/enteric fever, or an asymptomatic carrier state (WHO, 2011). Other pathogens that have been found in livestock manure include *Cryptosporidium* and *Giardia*, which also introduce health risks with their contamination (Hooda, et al., 2000; Burkholder, et al., 2007). These and other organisms can persist in the environment long after a leak or spill by finding refuge and thriving within settled sediments (Burkholder, et al., 2007).

OTHER POLLUTION INDICATORS

There are other measureable inorganic and organic parameters that are indicators of water quality and may be harmful at high or low levels. Electrical conductivity is a measure of dissolved salts and ions in water and may include chloride, nitrate, sulfate, phosphate, sodium, potassium, and magnesium, among other elements and compounds. High electrical conductivity may be an indicator of septic or other forms of organic pollution (USEPA, 2012). Elevated levels of chloride may corrode metals in distribution systems, increasing concentrations of heavy metals in water (WHO, 2011). A pH of a stream outside the range of 6.5-8 greatly reduces biological diversity. A low pH may allow certain toxic elements and compounds, especially heavy metals, to become more mobile and available for uptake by various organisms (USEPA, 2012.) The alkalinity of a stream determines its ability to neutralize acidic pollution and may be influenced by geology, salts, and certain industrial wastewater discharges (USEPA, 2012). Water hardness is due to high concentrations of cations, most commonly Ca^{+2} and

Mg⁺². There are no apparent biological or human health concerns with water hardness. Most issues are aesthetic regarding taste, lather, and deposits left in plumbing and other surfaces (WHO, 2011).

Dissolved oxygen is the amount of oxygen in the water and is influenced by both physical and biological factors. Dissolved oxygen varies with temperature, with cold water holding higher levels of oxygen than warm water. Dissolved oxygen is also influenced by water movement. The churning action of running water introduces more oxygen, while still water usually has much lower dissolved oxygen. It is also affected by biological activity. Respiration by aquatic animals and decomposition of organic matter can lower dissolved oxygen levels. The amount of oxygen consumed by these processes is the biochemical oxygen demand. Large amounts of organic pollution increases BOD and lowers dissolved oxygen levels and may indicate the presence of animal waste, decaying vegetative matter, and pesticides (USEPA, 2012).

REGULATIONS

Due to the many threats that pollution sources pose to the environment, and because water is often the main vector of contaminant transport, many countries have regulations that aim to protect the quality of their surface waters and to police potential polluters. Most of these regulations in the United States are overseen by the United States Environmental Protection Agency (USEPA) via the Federal Water Pollution Control Act, or the Clean Water Act (CWA), herein referred to as the CWA, with the goal of “restoring and maintaining the chemical, physical, and biological integrity of the

nation's waters" (Federal Water Pollution Control Act, 2011). The CWA includes monitoring, compliance, and enforcement protocol and periodically releases a National Water Quality Inventory, The 305(b) Report, to report which waters are healthy, threatened, or impaired, and how or why. These reports are used to form the Threatened and Impaired Waters List, The 303(d) List, and thus manipulate future regulations and determine operations of environmental concern. The USEPA has used the CWA to develop various permitting and implementation strategies that cover point- and nonpoint-sources of pollution. Section 319 of the CWA approves grants for efforts made to mitigate non-point sources of pollution including livestock associated runoff, unless it originates from an operation large enough to qualify as a CAFO (Federal Water Pollution Control Act, 2011). The National Pollutant Discharge Elimination System (NPDES) applies to point-sources, or direct discharges to surface waters. Under the NPDES, CAFOs qualify as point-sources (i.e. direct discharge from a single, identifiable, fixed source) and are required to comply with stringent permitting elements including effluent limits, best management practices (BMPs), compliance schedules, and monitoring and reporting requirements (Federal Water Pollution Control Act, 2011).

COMPLIANCE AND MANAGEMENT OPTIONS

These stringent rules have inspired many livestock owners to seek out practical uses for excess manure and develop effective, but affordable ways to manage waste onsite. Because of the high amounts of organic matter and nutrients in livestock manure, it is popular as a natural fertilizer option and is often spread or sprayed over fields, or injected into the soil. However, due to the separation of livestock and crop operations,

transportation costs, and pretreatment of the manure to help remove pathogens and other potentially harmful contaminants, this option is viewed as expensive and relatively inconvenient when compared to using synthetic chemical fertilizers. Even without the constraints of competition, sanitation, and cost, the application rates required to accommodate the amount of manure produced far exceed crop demands. This excess overwhelms the nutrient recycling ability of the environment and leads to nutrient losses as most of the applied fertilizer either washes away via stormwater runoff or leaches through the soil into groundwater; thus resulting in a surplus of nutrients in the water supply and the associated complications (Hooda, et al., 2000; Burkholder, et al., 2007). Even if rates or volumes are not excessive, application to saturated soils or terrain with a high slope may generate large amounts of contaminated runoff.

Composting is another popular option for manure management because it produces a useful product that can be used by small or large scale operations. Unfortunately, as with field spreading, composting has obstacles: transportation costs can outweigh the environmental benefits of compost, and commercially sold soil conditioners are often cheaper options (Martinez, et al., 2009).

Regardless whether or not field spreading or composting is an option, temporary to long-term onsite storage is still necessary until the manure can reach its final destination. Because of requirements by the Clean Water Act, storage methods must effectively contain manure and other waste products and prevent spillage, leaking, or any other exposure to the water supply. Certain storage methods are preferred based on type, amount, and storage time of the waste. The Natural Resources Conservation Service

(NRCS) uses these criteria to provide guidelines for constructing animal waste storage facilities to comply with federal, state, and local regulations.

Anaerobic lagoons are one of the oldest methods of waste confinement and are most often used for slurries and wetter material (Loehr, 1967). Lagoons are common but they offer no treatment besides what is achieved through the settling of sediments and minor destruction and stabilization of organic matter (Loehr, 1967; Robbins, et al., 1972); additional treatment is required in order to remove pathogenic organisms and nitrogen (Loehr, 1967). Anaerobic lagoons are the norm, but systems may incorporate aerobic treatment as well. Aerobic systems use either a rotor or aeration system to provide oxygen to the sludge, but are less popular because they require a constant power source and a greater land area to operate effectively (Loehr, 1971). Either type of lagoon is also prone to spills and blowouts if not designed properly. As lagoon effluent may still be rich in pathogens, nitrogen, oxygen-demanding materials, and other harmful contaminants, these spills can be devastating to receiving ecosystems (Horrigan, et al., 2002). Other methods for manure management may utilize both aerobic and anaerobic lagoons or holding tanks, or in-house treatment units for confined animal housing (Loehr, 1971).

While lagoons are the preferred method for liquid wastes (most often washout from confined swine and poultry production), they are not optimal for materials with a more solid composition. Animals like cattle and horses produce waste with high dry matter content and may include soiled bedding and other dry materials. These types of materials are often best for compost, but still require temporary storage, often in an aboveground holding structure. Ideally, these structures sit on an impermeable surface to

prevent leaching, and are surrounded by walls of a sturdy and solid enough material to prevent leakage and to support the weight of the manure. Usually only three walls are incorporated to allow for easy loading and unloading of contents. These structures are traditionally designed purely for storage. They offer no treatment and their one open side makes them susceptible to contaminated runoff in heavy precipitation. Overloading and spillage can occur if the pads are not emptied on a regular basis and can introduce contaminants to the environment and cause degradation of the structure itself. Regardless of the management option chosen, many do not offer any benefits other than compliance, if maintained properly, and rarely provide returns equal to the cost and effort of operation (Martinez, et al., 2009).

WETLAND MECHANISMS FOR WASTEWATER TREATMENT

Because of the potential problems associated with undertreated stormwater runoff and the expense of current treatment options, there has been an effort to develop an affordable, yet effective system for removing contaminants from effluent polluted by livestock waste. Research has begun to look to nature for inspiration. Wetlands occur in a variety of ecosystems, bordering water features such as springs, streams, rivers, lakes, and estuarine areas and perform valuable and irreplaceable functions. Wetlands trap and slow down fast-flowing stormwater runoff and floodwaters, allowing for filtration through plants, roots, and soil, and removing sediments and other substances that may be potentially harmful. Due to their pollutant-removal capabilities, wetlands are becoming popular mechanisms for wastewater treatment. Some natural wetlands have been successfully managed to remove excess nitrogen from municipal and agricultural

wastewater (Gale, et al., 1993; Elder and Goddard, 1996), but most other treatment applications use manmade wetlands. These systems, often referred to as constructed or artificial wetlands, receive and treat heavily polluted wastewater from a variety of sources through complex interactions between chemical, physical, and biological means. Constructed wetlands may be specifically designed to receive high concentrations of particular pollutants, and are successfully used to treat municipal wastewater (Gale, et al., 1993; Green, et al., 1998), runoff from agricultural fields (Elder and Goddard, 1996; Moore, et al., 2000), oil sands industry effluent (Bendell-Young, et al., 2000), mine drainage (Sobolewski, 1996), aquaculture wastewater (Lin, et al., 2002), and landfill leachate (Yalcuk and Ugurlu, 2009). Most treatment mechanisms are performed by the host of microorganisms that thrive in wetland water, substrate, and rhizosphere, and facilitate nutrient-breakdown pathways, predate harmful pathogens, and contribute to overall wetland functions. Contaminants not intercepted by microbiological activity can chemically decompose or become bound in soil and sediments.

Nitrogen compounds can be broken down in several ways, mostly by microbial methods. In anaerobic zones, organic nitrogen is converted to ammonium nitrogen, which can then take multiple pathways: ammonium can be taken up by plants or bacteria and converted back into organic nitrogen, or it can be oxidized into NO_2^- by nitrification. Once NO_2^- is formed, it can be denitrified into N_2 and fixed by bacteria or released into the atmosphere, or nitrification can continue and convert it into NO_3^- . NO_3^- is either utilized by plants or denitrified to N_2 . Because of the complexity of wetlands, coexisting aerobic and anaerobic zones support simultaneous nitrification and denitrification.

Studies have shown that wetland treatment can achieve upwards of 94% nitrogen removal (Conley, et al., 1991; Carleton, et al., 2000; Harrington and McInnes, 2009).

Wetlands are also effective at trapping other nutrients, particularly sulfur and phosphorus. Most sulfur is held stable in organic forms, but if it is mineralized and released into the environment it may precipitate as metal sulfides and stored in sediment, or volatilize as hydrogen sulfide. Trapping sulfur is important in the treatment of acid mine drainage (Sobolewski, 1996). Phosphorus is a relatively stable element and does not readily go through reactions. If phosphorus is able to be released into a soluble state, it is lost in effluent, used by plants, or accreted within the substrate. Wetlands have been shown to remove as much as 90% of influent phosphorus from aquaculture and livestock wastewater (Adler, et al., 1996; Harrington and McInnes, 2009). Microorganisms are not the only contributor in breaking down nutrient compounds; plants are integral, with up to 90% of nutrient uptake accomplished by plants (Rogers, Bet al., 1991). Pitcairn, et al. (1998) found that plants closest to livestock barns contained the highest concentrations of nitrogen when compared to plants found further from the barns, and suggests planting nitrophilous species to encourage uptake from runoff. Pitcairn's study took place in Scotland, but appropriate species could be identified for North America. A dense cover of plants is what slows down runoff enough for it to infiltrate and be treated, but plants are important mostly because of the environment created by their roots. The rhizosphere is home to a large diversity of microorganisms and their interactions with each other and with the plant roots produces a variety of conditions conducive to nutrient trapping and the removal of other contaminants (Conley, et al., 1991; Cheng, et al, 2009). The

addition of plants may significantly improve the function of wastewater treatment systems (Harvey and Fox, 1973; Wolverton and McDonald, 1979).

Most aquatic ecosystems have multi-dimensional structures that consist of complex relationships between many trophic levels. The characteristics of a wetland makes it an ideal habitat for microorganisms: the wet environment allows biofilms to form on the surfaces of plants and substrate media; soil pores provide shelter; and the occurrence of oxidized and anoxic zones within the rhizosphere provides conditions for both aerobic and anaerobic bacteria. While wetland food webs are still complex, they are limited to shallow water or saturated soils, leaving little or no room for larger animals. The majority of trophic activity within the water and substrate is confined to microorganisms and small macroinvertebrates. Because of this, wetlands are perfect environments for capturing and reducing pathogens in wastewater. According to wastewater treatment research, wetlands are capable of removing up to 99% of fecal coliforms, as well as *salmonella*, *giardia*, and *cryptosporidium* cysts and oocysts (Pundsack, et al., 2001; Quiñónez-Díaz, et al., 2001; Nokes, et al., 2003; Tunçsiper, 2007). Constructed wetlands are also effective at breaking down and removing pesticides, and reducing suspended solids and biochemical oxygen demand (Conley, et al., 1991; Green, et al., 1998; Moore, et al., 2000; Lin, et al., 2002).

RAIN GARDENS

A treatment system that exhibits some traits of a constructed wetland is receiving notable attention and becoming a trend in small-scale stormwater runoff treatment. Commonly called rain gardens, they are used mostly in urban and residential landscapes

with large areas of pavement and other impervious surfaces where more complex, large-scale constructed wetlands are not an option. They may be installed on rooftops, along sidewalks and streets, and in yards. Rain gardens use passive treatment and collect and hold water for only up to a few days after a rain event, as opposed to constructed wetlands that require a constant source of water. Rain gardens are also designed to remain dry for extended periods of time in between precipitation events. Excess stormwater runoff that is not absorbed by soil or falls on impermeable surfaces is diverted into rain gardens, and while technically rain gardens do not qualify as wetlands, they do employ some of the basic principles to treat water (Hunt, 2001). As water is trapped and slowly infiltrated into and through the substrate, sediments settle and are held, and nutrients and other particles adhere to mulch and soil. Organic matter may be broken down and pathogens may be killed by microbial processes, especially in the rhizosphere. There is a growing movement to develop wastewater treatment systems that are effective and environmentally friendly but also easily managed for small areas and operations, and rain gardens seem to offer a promising alternative.

Research involving passive treatment of livestock-associated runoff is emerging as traditional lagoon systems are deemed inadequate and new and more stringent pollution regulations are placed on the livestock industry (Loehr, 1967; Robbins, et al., 1972; Hooda, et al., 2000; The World Bank, 2005; Burkholder, et al., 2007; Kato, et al., 2009). In 1991, the Natural Resources Conservation Service issued technical guidelines for designing constructed wetlands and other similar systems specifically used to manage livestock waste (USDA, 2002). In general, these guidelines are intended to provide blueprints for structures that will prevent, collect, or dispose of seepage that may pollute

surface or groundwater (USDA, 2002). There is little knowledge about the feasibility of installing and managing a rain garden to receive large amounts of livestock waste and successfully remove a significant amount of contaminants. The ease of changing an establishment's infrastructure depends heavily on the size of the operation; smaller farms generally have more options for efficient waste management, and can alter their methods more easily. Rain gardens are small, require relatively little maintenance, and would be an easy transition for those looking to renovate a waste management system.

EQUINE WASTE IN KENTUCKY

Most of the publicity given to livestock waste management revolves around large-scale production of animals for food. Feedlots and CAFOs receive the most attention, but the scope of impact extends to other forms of animal agriculture. Kentucky has a thriving, world-renowned horse industry. Racing alone makes up 46% of the Total Economic Impact for Kentucky, and when combined with other equine business is responsible for over half of the state's economy (American Horse Council, 2005). The Kentucky horse community is comprised of approximately 190,000 residents and provides over 140,000 jobs. Kentucky is home to over 300,000 horses, mostly in the central region of the state (American Horse Council, 2005). As these estimates are outdated, the University of Kentucky's Equine Initiative is partnering with the University of Louisville's Equine Business Program and the National Agricultural Statistics Service to conduct a comprehensive survey of Kentucky's equine industry. Results are expected by early 2013 and will hopefully offer a more accurate depiction of the equine industry's impact on Kentucky (UK Equine Initiative, 2012). One 1,000 lbs. (454 kg) horse can

produce 50 lbs. (23 kg) of manure per day and, if housed, 20 lbs. (9 kg) of soiled bedding. Considering the estimated number of horses in the state, this can equal as much as 21 million lbs. (over 9,500 metric tons) of waste, statewide, on a daily basis (Higgins, et al., 2008).

Composting is a popular option for horse manure and muck primarily due to its low moisture content, but requires large amounts of land for implementation. Considering the high demand for and value of land in Central Kentucky, composting is not a priority for most landowners.

CANE RUN WATERSHED

Central Kentucky's watersheds are bearing the burden of the heavy equine activity in the area. Of particular concern is the Cane Run Watershed. Cane Run is a third order stream that flows into North Elkhorn Creek and eventually to the Kentucky River. The main stem of Cane Run is 16.95 miles (27 km) with a 28,562 acre (115.6 square km) watershed (Cane Run and Royal Spring Watershed Restoration Plan, 2006). Cane Run lies within Fayette and Scott counties; and while it contains urban areas in Lexington and Georgetown, it is approximately 75% rural (CRRSWRP, 2006). The area is underlain with the phosphatic, shaly, Ordovician-aged Lexington Limestone formation (CRRSWRP, 2006). The limestone-shale geology encourages karst features; large sinkholes and swallets are common, but difficult to identify, and allow pollution to be spread rapidly between surface and groundwater. This karst system is responsible for the direct link between Cane Run and the Royal Spring Aquifer, which underlies much of the surface water basin and supplies drinking water for Georgetown, Kentucky. Cane Run is

listed on the Kentucky Department of Water's 2010 Integrated Report to Congress on the Condition of Water Resources in Kentucky 303(d) List of Impaired Waters for impaired uses: warm water aquatic habitat (nonsupport), primary contact recreation water (nonsupport), and secondary contact recreation water (partial support) (KDOW, 2011). Listed pollutants are fecal coliform, nutrient/eutrophication biological indicators, organic enrichment (sewage) biological indicators, specific conductance, and sedimentation/siltation (KDOW, 2011). Suspected sources are livestock, urban stormwater, roads and highways, construction, and agriculture (KDOW, 2011).

Cane Run has been set aside in central Kentucky as an example and educational opportunity for watershed health and restoration. The Cane Run and Royal Spring Watershed Restoration Plan was adopted in 2006 and contains project proposals aimed at identifying and reducing potential sources of contamination, and ultimately removing Cane Run from the 303(d) List. One of the initiatives in the plan addresses proper management of livestock waste within the watershed. There are an estimated 2,000 cattle and 1,300 horses within Cane Run and the manure and muck from these animals are likely contributors to watershed impairments (CRRSWRP, 2006; KDOW, 2011).

SITE DESCRIPTION

Victory Haven Training Center is a thoroughbred training facility located on Russell Cave Road in northern Lexington, KY. The property has the capacity to house over 300 horses and is mapped by The Cane Run and Royal Spring Watershed Restoration Plan as a confined feeding operation. A Cane Run tributary flows through the property and is labeled as an intermittent stream on the United States Geological

Survey topographic map (KGS, 2012). The stream flows roughly southeast to northwest through the property, dropping approximately twenty feet in elevation. Like most of central Kentucky, Victory Haven is underlain with the Lexington Limestone formation, while the onsite stream is flanked with Quaternary Alluvium deposits (KGS, 2012). This type of geology is known for karst features which may directly connect surface and groundwater. The Cane Run tributary that flows through Victory Haven is partly fed by several seeps in the stream banks and many streams in the area contribute to underground water features such as the Royal Spring Aquifer. The Victory Haven facility is watered by onsite wells; five unlabeled wells appear on the area map by Kentucky Geological Survey, as well as a domestic well immediately downstream of Victory Haven on the adjacent property (KGS, 2012). Victory haven can be found on the FEMA Flood Insurance Rate Map for Lexington-Fayette Urban County Government, Kentucky, No. 2100670126D, effective date 9/17/2008 (FEMA, 2008).

Victory Haven sells their composted horse muck after it spends up to twelve months in on-site windrows; however, before the muck is applied to the windrows, it is stockpiled in a storage structure, designed per the NRCS Technical Guidelines for Kentucky (NRCS, 2003). These structures are placed directly outside of most of the barns at the facility, and muck is stored for several weeks to over a month until it is transferred to the windrows. These structures consist of an impermeable concrete pad approximately six inches (15 cm) thick surrounded by three concrete or wooden walls, approximately 6 feet (2m) tall, and are accessed at the open side by trucks, tractors, and other manure handling equipment (Figure 1.1). Spillage from the approach side of the storage pads is common and has been documented (Figure 1.2), as is overflow over and

through gaps in the walls (Figure 1.3). As several of the muck pads are close to the onsite stream (some within 50 ft), pollution from spillage and runoff is a legitimate concern (Figure 1.4). The stream on the Victory Haven property displays visible evidence of sedimentation and eutrophication, most likely a result of manure contamination (Figure 1.5).



Figure 1.1: Typical muck storage pad at Victory Haven, with concrete pad, walls, and approach.



Figure 1.2: Spillage from approach end of muck storage pad at Victory Haven.



Figure 1.3: Muck storage pad at Victory Haven showing flimsy wooden walls with gaps, allowing for excess spillage and seepage of liquid waste.



Figure 1.4: Overflowing wooden muck storage pad at Victory Haven, showing drainage and close proximity to onsite stream (photograph taken from stream bank).



Figure 1.5: Extreme algal growth in stream at Victory Haven.

PURPOSE

This project was designed to expand past and current research in livestock waste treatment to include Kentucky's prosperous horse industry and potentially alleviate some of the environmental issues that it faces. The purpose of this project was to design and construct a temporary muck storage structure that will utilize rain gardens and wetland/phytotechnologies to control and treat contaminated runoff from stockpiled horse manure and bedding at a horse boarding and training facility within the Cane Run watershed. The objectives are: 1) Design and build two temporary muck storage pads that will divert stormwater runoff into a rain garden basin for passive treatment, 2) Determine the effect of new muck storage methods on the onsite stream at Victory Haven using analysis of bacterial indicator populations and water chemistry parameters, and 3) Determine the rain garden's ability to trap and/or break down excess nutrients and pathogens introduced by contaminated runoff. This project was initiated July 2010 and concluded July 2012.

Chapter 2: METHODS

NEW MUCK STORAGE PAD DESIGN

Two new muck storage structures were designed and built to replace two existing pads at Victory Haven that used wooden walls, as they were in poor shape and exhibited the most spillage and seepage. The new structures were built using the same basic guidelines suggested by NRCS to build the original structures (NRCS 2003). However, the new design would force runoff coming from the pads into a rain garden basin and provide passive treatment of infiltrated water through an engineered substrate and potentially through uptake from vegetation planted in the basin (Figure 2.1 and Figure 2.2). One of these new structures kept the original pad and was retrofitted with the new rain garden design (herein called experimental pad 1, or pad X1), and the other was torn down and moved to a new location (experimental pad 2, or pad X2). The new design replaced the wooden walls with three concrete walls 6 ft (1.8 m) high and 5.5 inches (14 cm) thick (Figure 2.1, Figure 2.2, Figure 2.3, and Figure 2.4). The new concrete pad on X2 was 21 ft (6.4 m) by 24 ft (7.3 m) and 6 inches (15.2 cm) thick. Three-inch (7.6 cm) diameter holes were drilled throughout the base to allow runoff to escape into the surrounding rain garden (Figure 2.5). Following installation of the containment walls, a ditch 2 ft (0.6 m) deep and 6 ft (1.8 m) wide was excavated surrounding the walled sides of the pads to serve as the rain garden basin (Figure 2.6 and Figure 2.7). The bottom and sides of the basin were lined with geotextile fabric and filled with 6 inches (15 cm) of sand, 6 inches (15 cm) of #57 (3/4") stone, and approximately 12 inches (30.5 cm) of #3 (1 1/2") stone (Figure 2.1 and Figure 2.2). This substrate configuration was chosen with the intent of facilitating rapid filtration through the larger rock, and slowing water

movement as it moved through the sand and approached native soil. The design was calculated to hold all water entering the basin from a 50-year 24-hour rain event (Hershfield, 1961), allowing little opportunity for surface runoff and overland flow originating from the muck pad.

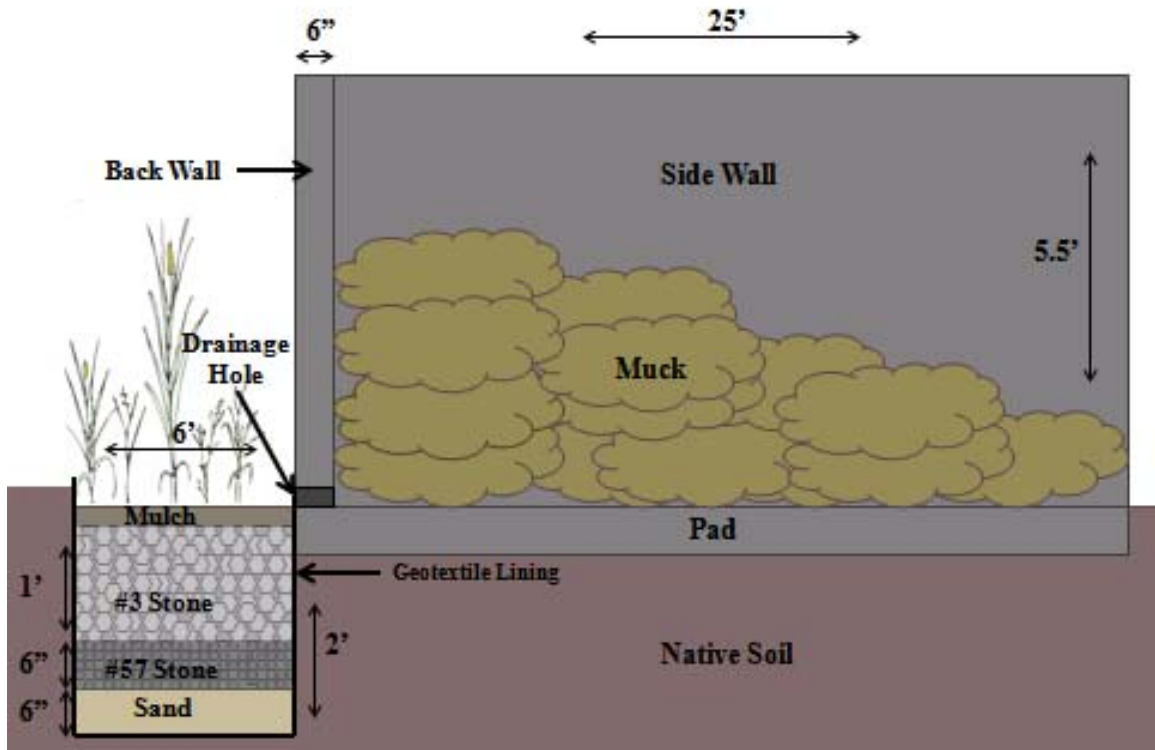


Figure 2.1: Side-view cross-section diagram of experimental muck pad design.

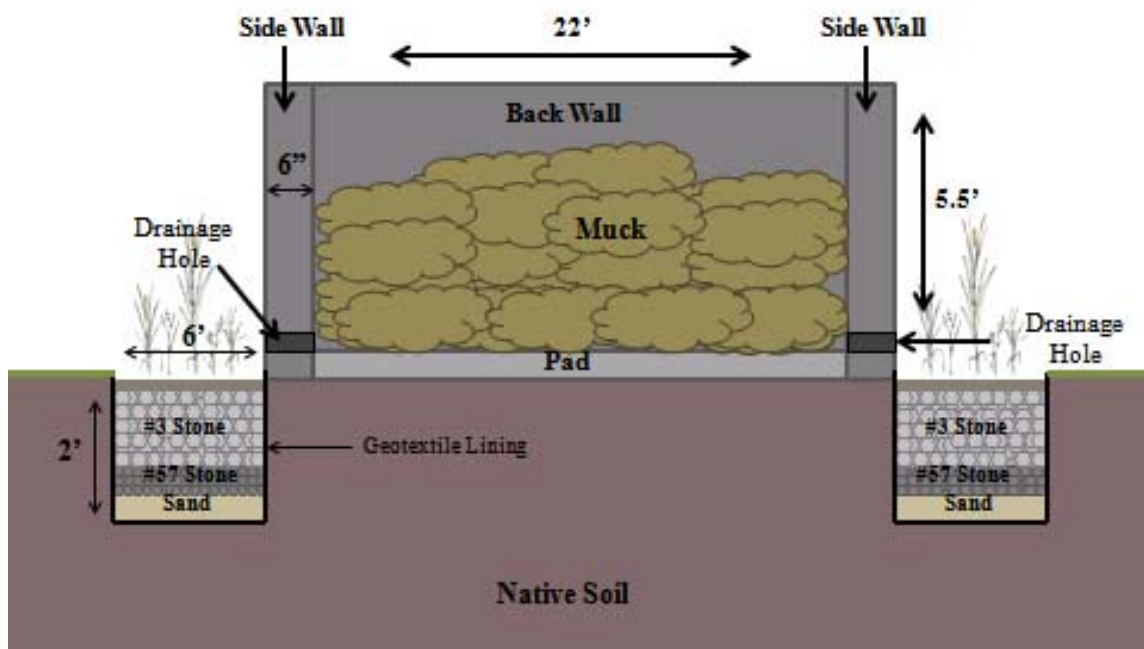


Figure 2.2: Front-view cross-section diagram of experimental muck pad design.



Figure 2.3: Beginning construction of concrete walls surrounding existing muck pad X1.



Figure 2.4: Completed walls at X2, before installation of new concrete pad.



Figure 2.5: Hole in wall of X1, showing drainage from inside of pad.



Figure 2.6: Excavation of rain garden basin at X1.



Figure 2.7: Excavation of rain garden basin at X1.

The surface of the rain gardens were covered with a thin layer of cypress mulch to support a variety of facultative wetland species that were planted in the rain garden basins of the new pads (X1 and X2) (Figure 2.8). Seeds were planted in pots and grown in the University of Kentucky Department of Forestry on-campus greenhouse until plants were mature enough and onsite conditions were appropriate for planting. Plants were grown and planted in both spring/summer of 2011 and spring/summer of 2012. A hole, approximately twice the size of the potted plant, was excavated in the rock and filled with the containerized plant and potting soil backfill. The plant species used are all native to Central Kentucky and include fox sedge (*Carex vulpinoidea*), frank's sedge (*Carex Frankii*), mist flower (*Eupatorium coelestinum*), Illinois bundleflower (*Desmanthus illinoensis*), bur marigold (*Bidens cernua*), river oat (*Chasmanthium latifolium*), and slender mountain mint (*Pycnanthemum tenuifolium*). All seeds were purchased from Dropseed Nursery in Louisville, Kentucky.



Figure 2.8: Planting of vegetation in rain garden basin at pad X1. Cypress mulch (approximately 4 inches (10 cm) thick) was subsequently placed on top of rock and between plants.

Some of the existing pads at Victory Haven already had concrete walls in place instead of wooden walls. Two of these, located downstream of the new pads, were chosen as control structures (pad C1 and pad C2) in order to obtain an idea of Victory Haven's original muck storage pads' effect on groundwater and surface water quality (Figure 2.9 and Figure 2.10). The controls were built on and surrounded by native soil and had no passive treatment system. Any runoff from control pads flowed over highly compacted gravel driveways and mowed grassed areas to the onsite stream.



Figure 2.9: Control pad C1.



Figure 2.10: Control pad C2 with drainage leading to stream.

LYSIMETERS AND TENSIOMETERS

Suction lysimeters (Model 1900 Soil Water Samplers, SoilMoisture Equipment Corp.) were used to collect water from the surrounding substrate of experimental and control pads for analysis. Each lysimeter consisted of a porous ceramic cup attached to the bottom of a 24-inch (61 cm) PVC tube and sealed with a Santoprene stopper. A vacuum was applied, causing water from the substrate to enter through the porous ceramic cup and collect at the bottom of the lysimeter until extracted for analysis. Each lysimeter was paired with a tensiometer (Model 2630AL24K, SoilMoisture Equipment Corp.) to measure soil matric potential. Each tensiometer consisted of a porous ceramic cup attached to a water column in a sealed tube. The pressure head in the water column changes as water enters and exits the ceramic cup with varying soil saturation and is measured with a SW-010 tensiometer (Soil Measurement Systems, Tucson, AZ). Four sets of paired lysimeters and tensiometers were installed within the rain garden basin at each experimental pad (Figure 2.11, Figure 2.12, and Figure 2.13). Pad X1's basin contained lysimeter and tensiometer sample locations X1A, X1B, X1C, and X1D; pad X2's basin contained lysimeter and tensiometer sample locations X2A, X2B, X2C, and X2D (Figure 2.11). After the sand layer was poured into the excavated basin, the porous cups for both instruments were installed in the sand layer within the basins and the remaining rock layers were filled in around the equipment. Paired lysimeters and tensiometers were also installed at the control pads. Each control pad was outfitted with two sets of paired suction lysimeters and tensiometers, installed immediately down gradient of the structure (Figure 2.14). Each tensiometer and lysimeter pair was installed to a depth of 2 ft (61 cm) into the native soil. The annulus of each hole was filled with

silica powder to the tops of the ceramic cups, and in-situ native soil to the ground level. Each pair was then sealed with a bentonite plug and an Orbit® WaterMaster® Extension Valve Box (Model 53213, Orbit Irrigation Products, Inc. North Salt Lake, UT) was anchored above with cement to protect the equipment from mowers (Figure 2.15 and Figure 2.16). Pad C1 contained lysimeter and tensiometer sample locations C1A and C1B, and pad C2 contained lysimeter and tensiometer sample locations C2A and C2B (Figure 2.14). Construction of the new muck storage structures, installation of sampling equipment, and planting of first year wetland species was completed in July 2011.

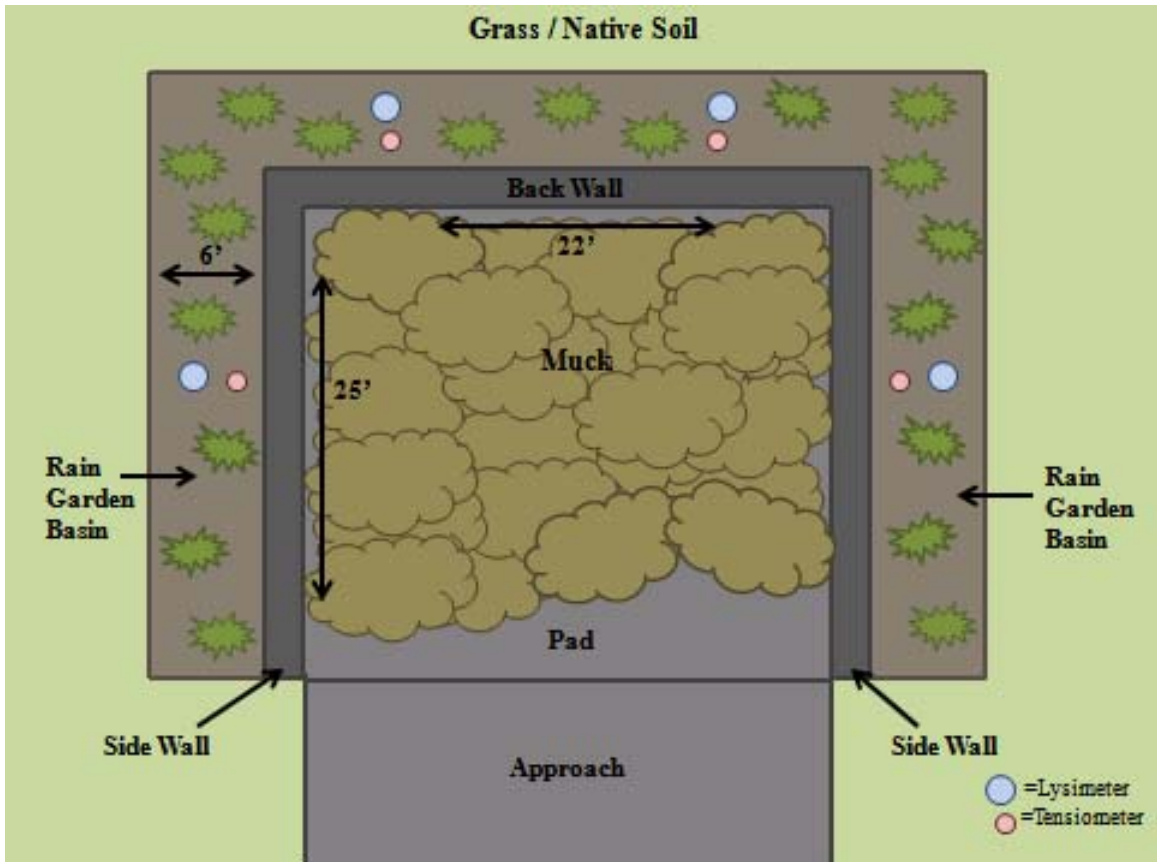


Figure 2.11: Top-view diagram of experimental muck pad design, showing locations of lysimeters and tensiometers in the rain garden basin.

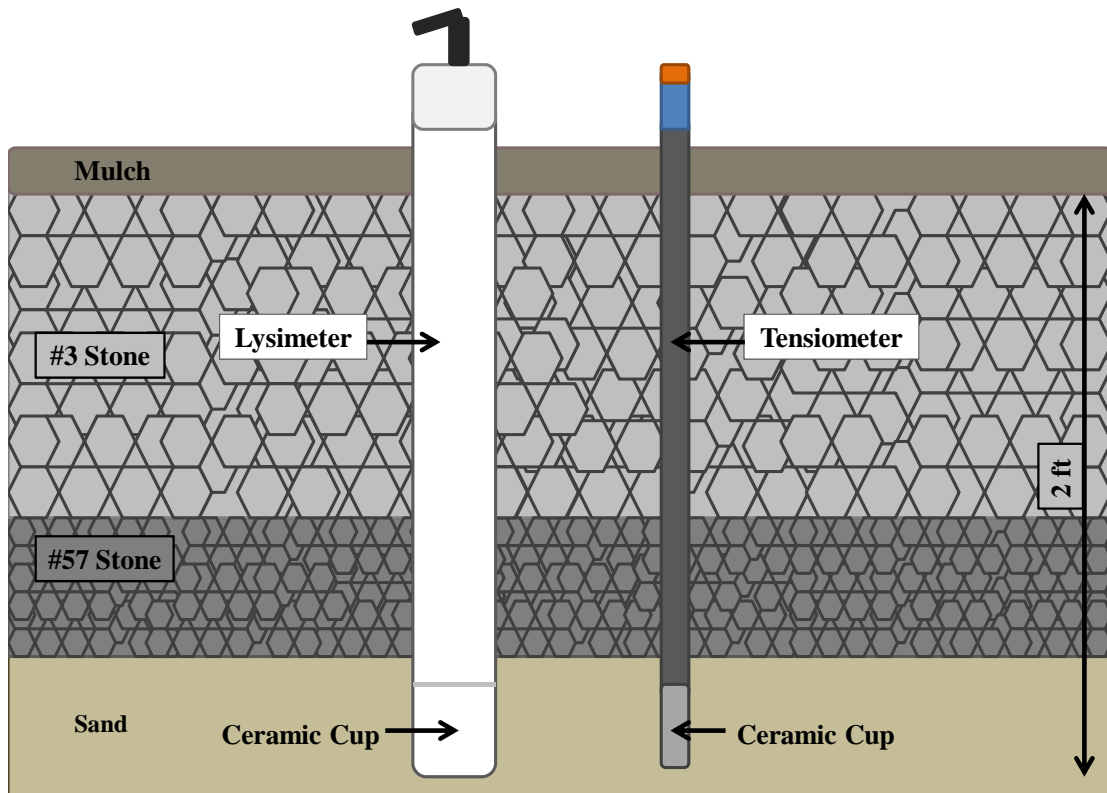


Figure 2.12: Diagram of lysimeter and tensiometer placement within the rain garden basin at X pads.



Figure 2.13: Picture of lysimeter and tensiometer in the rain garden at X1.

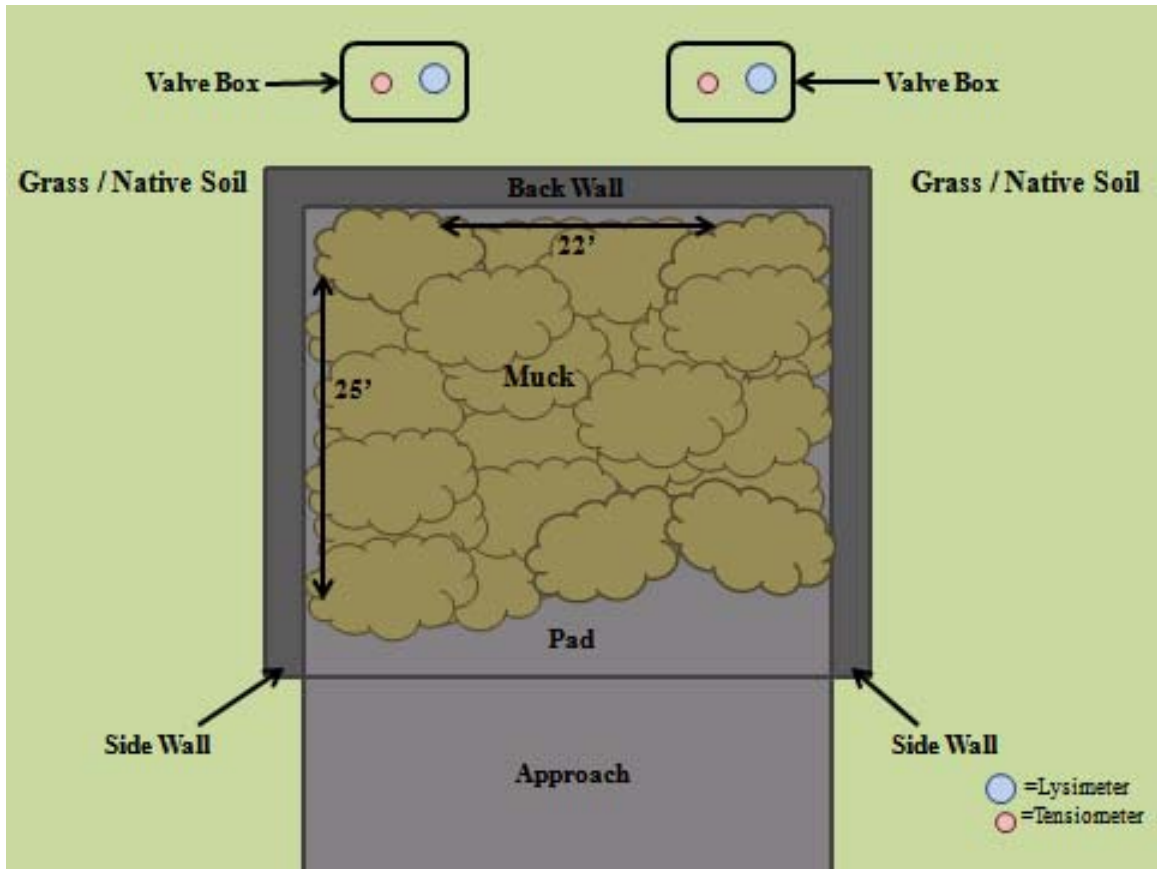


Figure 2.14: Top-view diagram of control muck pad design, showing locations of lysimeters and tensiometers in native soil behind the pad.

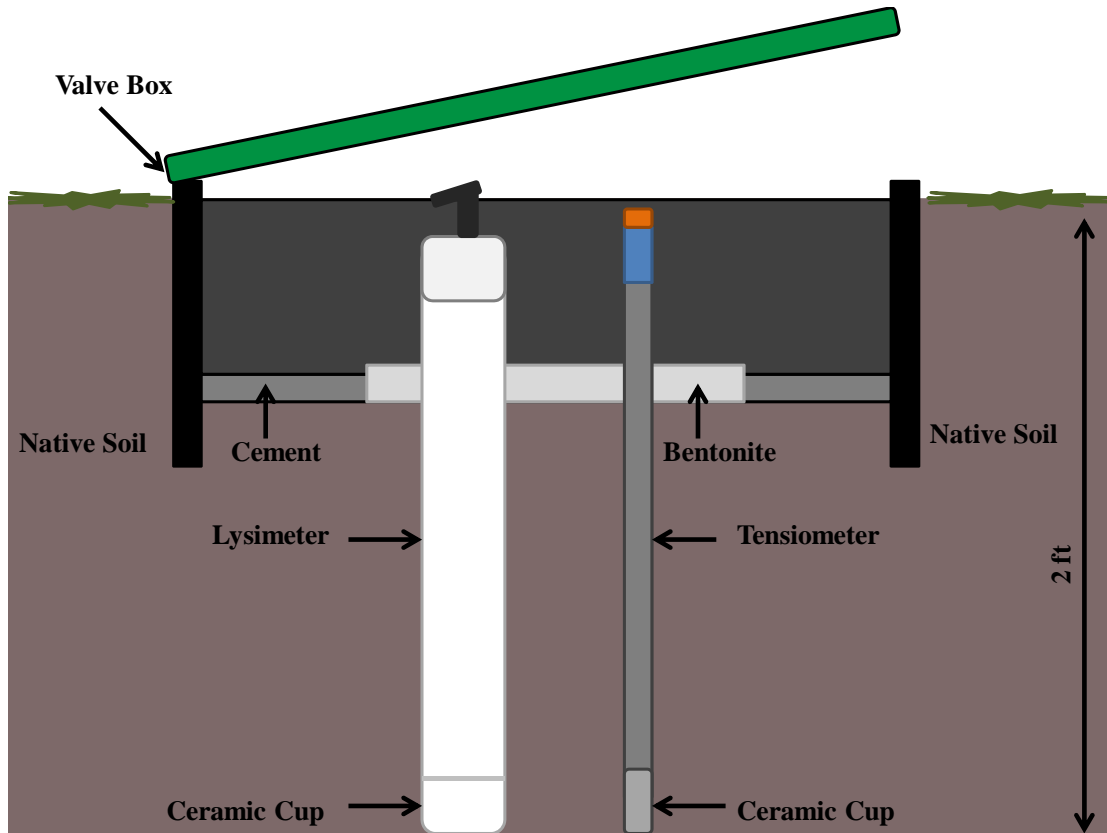


Figure 2.15: Diagram of lysimeter and tensiometer placement within the native soil at control pads.



Figure 2.16: Picture of lysimeter and tensiometer in valve box behind pad C1.

SAMPLING AND ANALYSIS

Immediately following rain events, or when soil tension was suitable as dictated by tensiometer readings (< -50 centibars), a vacuum pressure of approximately -50 kPa was applied to each lysimeter with a Nalgene® mityvac® pump. It was the original goal of the project that lysimeters were sampled at least twice a month. Weather patterns did not always permit bi-monthly sampling; however, the lysimeters were sampled at least once a month. Water samples for bacterial analysis were extracted from all twelve lysimeters with sterile BD 10mL Syringes (Becton Dickinson and Company, Franklin Lakes, NJ) and sterile Nalgene® tubing, and dispensed into sterile Corning® 15mL Centrifuge Tubes (Corning® Inc., Corning, NY) for transport. All sampling equipment used for bacterial analysis was autoclaved a day prior to sampling and stored and transported in sterile plastic sample bags until used. Water samples for water chemistry analysis were also extracted with a Nalgene® mityvac® pump and Nalgene tubing and dispensed into clean 250mL Nalgene® bottles for transport. Lysimeter samples were collected from the time of installation in July 2011 until May 2012. Tensiometer readings were also taken on dates of sampling.

Ten grab-sample locations were chosen along the onsite stream starting at the point where surface flow enters the property and ending where it exits (Figure 2.17). Sample sites were selected based on proximity to muck storage pads and inflows from other areas of the property. Samples for bacterial analysis were placed in sterile Corning® 15mL Centrifuge Tubes (Corning® Inc. Corning, NY) for transport. Samples for water chemistry analysis were placed in clean 250mL Nalgene® bottles. Grab samples were taken from the stream at each site at least twice a month for the first year,

from July 2010 to July 2011. These samples served as preliminary data to evaluate the state of the onsite stream prior to installation of the new structures and to attempt to identify specific points of contamination. Sampling continued following installation of the new muck storage structures from July 2011 through May 2012. In situ water chemistry (dissolved oxygen, pH, temperature, electrical conductivity) was also measured at each site with a YSI® environmental monitor (556 Model) (YSI, Inc. Yellow Springs, OH).



Figure 2.17: Aerial Photograph of Victory Haven, showing stream, sample locations, and muck pads on the property.

All water samples from Victory Haven were placed on ice and transported to the University of Kentucky for analysis. All samples were tested for water chemistry and for total coliforms and *E. coli* following methods outlined in Standard Methods for Examination of Water and Wastewater (Greenberg, et al., 1992). Water chemistry parameters included chloride, magnesium, potassium, calcium, sodium, sulfate, nitrates, ammonium nitrogen, phosphate, total organic carbon, alkalinity, and pH. Total organic carbon (TOC) was analyzed with a Shimadzu TOC 5000A Analyzer (Shimadzu Corporation, Maryland). Calcium (Ca^{+2}), magnesium (Mg^{+2}), sodium (Na^{+}) and potassium (K^{+}) were measured using a GBC SDS 270 Atomic Adsorption Spectrophotometer (AAS) (GBC Scientific Equipment, Illinois). A Dionex Ion Chromatograph (IC) 2000 (Dionex Corporation, California) was used to determine the concentrations of sulfate (SO_4^{-2}) and chloride (Cl^{-}). Alkalinity was found using an auto titrator with a tritnant endpoint pH of 4.6, and an Orion pH meter. Nitrate (NO_3^{-}) and ammonium (NH_4^{+}) were analyzed using colorimetric analysis and a Bran+Luebbe Autoanalyzer (Bran+Luebbe, Analyser Division, Germany).

The bacterial indicators quantified and used to evaluate water quality were total coliforms and *E. coli*. Microbial populations in water are traditionally tested using the Standard Methods Most Probable Number (MPN) method. The products Colilert® and Quanti-Tray®/2000, developed by IDEXX Laboratories, Inc., offer the same accuracy of traditional MPN tests but are less labor-intensive. Colilert® uses the patented Defined Substrate Technology® which simultaneously detects coliforms and *E. coli*. Two nutrient-indicators, ONPG (o-nitrophenyl and β -D-galactopyranoside) and MUG (4-methyl-umbelliferyl and β -D-glucuronide), are metabolized by coliform enzyme β -

galactosidase and *E. coli* enzyme β -glucuronidase. As coliforms grow in Colilert® solution, they will use β -galactosidase to metabolize ONPG and change the solution from colorless to yellow. *E. coli* will use β -glucuronidase to metabolize MUG and create a fluorescent solution. Most non-coliforms do not have the appropriate enzymes to process ONPG and MUG, and those that do are suppressed by Colilert®'s matrix. When performed correctly, this procedure eliminates false positives and false negatives often associated with traditional MPN media. When used with Colilert® media, the Quanti-Tray®/2000 operates on the same statistical model as a traditional 15-tube serial dilution. The Quanti-Tray®/2000 has a counting range from 1 to 2,419 per 100mL sample and a 95% confidence limit. These methods are approved by the USEPA, Standard Methods for Examination of Water and Wastewater, and the Association of Analytical Communities (IDEXX, 2012). Colilert® and the Quanti-Tray®/2000 will be used to determine coliform and *E. coli* populations in water samples taken from Victory Haven. 10mL samples were collected and tested from each stream location and each lysimeter.

In June 2012, plant tissue samples were collected and analyzed for total nitrogen and total phosphorus content. Samples were taken from each species planted in year 1 (July 2011), year 2 (June 2012), and control plants grown in the University of Kentucky Forestry greenhouse in April-June 2012. For nitrogen analysis, the samples were ground into a coarse powder in a ball grinder (SPEX 8000M Mixer/Mill), approximately 0.250 g of which was weighed and placed into a volumetric test tube. Each sample was mixed on a vortexer with three or four selenized boiling chips and 7 mL of concentrated sulfuric acid. After mixing, 3 mL of 30% hydrogen peroxide was added to each tube, and sample was again spun again on the vortexer. The tubes were placed into the block digester at

approximately 630° C for about an hour and then cooled. Once cool, deionized water was added to each tube to a total volume of 75 mL, mixed well and placed into a labeled storage container. The total nitrogen was analyzed colorimetrically using the Bran + Luebbe autoanalyzer II. The sample data was collected comparing peaks to a standard curve. Samples for total phosphorus analysis were ground using the same methods as for total nitrogen. Approximately 0.250 g was weighed and placed into a Coors crucible. The crucibles were placed in a 500° C muffle oven at overnight. After cooling the crucibles to room temperature, a small amount of deionized water was added to each crucible to stabilize ash while moving the crucibles. 10 mL of 6 M nitric acid was added to each crucible and contents are brought to a 'boil' on a hot plate for about 15 minutes, agitating the contents of the crucibles occasionally. The contents of the crucibles were then filtered through Whatman 42 filter paper into a 50 mL volumetric flask, and filters were rinsed with deionized water at least three times still collecting the contents in the volumetric flasks. Samples were brought to a total volume of 50 mL using deionized water and the flasks were mixed and the contents were poured into a labeled storage container. The total phosphorus was analyzed colorimetrically using the Bran + Luebbe autoanalyzer II and the sample peaks were compared to a standard curve to determine concentration.

STATISTICAL ANALYSIS

STATISTICAL PACKAGE AND METHODS

Raw data was recorded and manipulated in Microsoft Excel 2010. All statistical analysis was performed with JMP 9 statistical software from SAS. Statistical analysis methods included box and whisker plots and Wilcoxon two-group tests to measure significance at $\alpha=0.05$. A box and whisker plots is a statistical method used to visually display the distribution of a data set by depicting the five-number summary (minimum, first quartile, median, third quartile and maximum). The box and whisker plots used in this analysis also identify the mean and potential outliers. The Wilcoxon two-group test was chosen due to the highly variable nature of the data and of the sampling sets.

STREAM WATER QUALITY

Raw data includes detected levels of each water quality parameter (In situ dissolved oxygen, in situ temperature, in situ electrical conductivity, in situ pH, total coliforms, *E. coli*, EC, Cl^- , SO_4^{-2} , Mg^{+2} , Ca^{+2} , K^+ , Na^+ , ALK, pH, NO_3^- , NH_4^+ , TOC, and PO_4^{-3}) for each stream sample location on each sample date beginning July 15, 2010 and ending January 24, 2012. Dissolved oxygen, temperature, total and *E. coli* were each plotted over the series of stream sample locations (1-10, excluding 5) for each sample date to evaluate contamination behavior in the stream.

Total coliforms, *E. coli*, and all water chemistry parameters (EC, Cl^- , SO_4^{-2} , Mg^{+2} , Ca^{+2} , K^+ , Na^+ , ALK, pH, NO_3^- , NH_4^+ , TOC, and PO_4^{-3}) for each stream sample location were graphed on time series of each sample date and examined for patterns. In order to determine the effects of the new muck storage pad design, all data in these time series

were split into before June 2011 and after June 2011, when use of first two traditional muck pads at Victory Haven ceased and construction began to replace them with new experimental pads X1 and X2. Because stream sample locations 3 and 4 are in closest proximity (3 immediately upstream, 4 immediately downstream) to pad X1, differences between stream water quality data from the sampling period before June 2011 and data from the sampling period after June 2011 for sample locations 3 and 4 were tested for statistical significance.

Total coliform and *E. coli* population data for stream water samples before and after June 2011, for sample locations 2-10, were converted into Box and Whisker plots to compare possible patterns or differences in distribution.

MUCK STORAGE PADS

Raw data includes tensiometer readings and detected levels of each water quality parameter (total coliforms, *E. coli*, EC, Cl⁻, SO₄⁻², Mg⁺², Ca⁺², K⁺, Na⁺, ALK, pH, NO₃⁻, NH₄⁺, TOC, and PO₄⁻³) in water samples taken from each of the twelve total lysimeters at both X pads and C pads for each sample date beginning July 26, 2011 and ending May 15, 2012. Tensiometer readings for each pad were averaged for each sample date to determine comparisons of soil moisture between pads C1, C2, X1, and X2.

Box and Whisker plots of each parameter were constructed for each of the four monitored muck storage pads to determine any visible differences between the data. Differences in each parameter were also tested for statistical significance. Values for each water quality parameter from each lysimeter were combined into two groups, one for the C pads and one for the X pads. For each sampling event, values corresponding to

either C or X pads were averaged to obtain a single value representing the C pads and a single value representing the X pads. These values were plotted in a time series for the entire sampling period to reveal trends within and between C and X pads. Differences in each water quality parameter between the X pads and the C pads were tested for statistical significance.

PLANTS

Because samples of plant tissue were composites of several specimens within each rain garden basin and there was only one sample per species collected for each sample period, statistical tests could not be performed on that data. Raw laboratory data was observed for patterns and differences in phosphorous and nitrogen content for each sample period.

Chapter 3: RESULTS AND DISCUSSION

WEATHER

Because this project focused on stormwater runoff, its efficacy and the interpretation of results were heavily dependent on weather and precipitation patterns, and any prolonged unusual or extreme weather may have skewed the results. Weather data for Lexington, KY was monitored and downloaded from the Kentucky Mesonet Fayette County Spindletop location (Kentucky Mesonet, 2012). Central Kentucky averages approximately 40-50 inches (101-127 cm) of precipitation annually. Monthly averages are spread fairly evenly, usually falling between 3-5 inches (7-13 cm); spring months (March-June) are the wettest and late summer through early autumn months (August-early October) are most often the driest. This project saw several weather anomalies over the course of its two year timeframe. Preliminary sampling began in a very wet July in 2010, over 3 inches (7.6 cm) above average, followed by a drought, with just over 3 inches (7.6 cm) of rainfall for August, September, and October combined. This dry period was followed by an extremely wet spring, with over 13 inches (33 cm) of rainfall in April alone. Most of 2011 was unusually wet, totaling 65.2 inches (165 cm) of precipitation for the year. Winter 2011-2012 was the fourth warmest winter on record for the United States, with Kentucky's lowest monthly average at only 47°F (8.3°C). The warm winter and spring were followed by extreme summer heat, with fourteen highs above 95°F (35°C), six of which reached over 100°F (37.8°C), making for the hottest July and the third hottest summer on record.

STREAM WATER QUALITY

Preliminary sampling of the stream water at Victory Haven, beginning in July of 2010, confirmed the presence of high populations of *E. coli* within the stream, with counts regularly exceeding 20,000 bacteria 100 mL⁻¹. The Surface Water Standards Administrative Regulations for Kentucky (KDOW, 2012) limit *E. coli* populations in primary contact recreation waters to no more than 130 colonies 100 mL⁻¹ as a geometric mean based on not less than five samples taken during a thirty day period, and no more than twenty percent of those samples may exceed 240 colonies 100 mL⁻¹. There are no *E. coli* limits for secondary contact recreation waters, but fecal coliforms may not exceed an average 1,000 colonies 100 mL⁻¹ over a thirty day period, and 2,000 colonies 100 mL⁻¹ in twenty percent or more of samples. Stream water samples were not collected five times per month, but even if they were, and all other *E. coli* counts were 0 colonies, the 30-day average would still be above the set limits for both primary and secondary recreation waters. The Kentucky Administrative Regulations also list criteria for concentrations of common chemical water pollutants. The substances of concern for this project that are listed are nitrate and sulfate. Limits for domestic water supplies are 10 mg L⁻¹ for nitrate and 250 mg L⁻¹ for sulfate (KDOW, 2012). Measured concentrations of nitrate and sulfate never exceeded these limits in the stream water samples; however, nitrate levels periodically measured above 8 mg L⁻¹. The Kentucky Administrative Regulations also state that surface waters supporting warm water aquatic habitats shall maintain a dissolved oxygen instantaneous minimum of no less than 4.0 mg L⁻¹. Dissolved oxygen in the Victory Haven stream regularly fell below this limit, occasionally measuring less than 2.0 mg L⁻¹. As confirmed by the data, this stream carries heavy loads of bacterial

pollution and experiences low oxygen levels, and although nutrient concentrations are not in excess of established limits, it is clear that the current amounts are having an observable effect on algal growth within the stream.

E. coli counts were graphed over the sequence of stream sample locations (1-10, excluding 5) for each sampling event (Appendix 1). One would expect *E. coli* levels to steadily increase over the course of the onsite stream; this did not occur, but the stream does regularly experience spikes in *E. coli* populations at several points along the stream's path, most notably at sample location 6. Figure 3.1 shows *E. coli* populations representative of the trends in the stream at Victory Haven. Sample location 6 is located at the confluence of the stream with a swale originating from a pipe that drains the horse washing stations (sample location 5) (Figure 2.17). The spikes in sample location 6 reflect high *E. coli* levels in water samples taken directly from the drain pipe. Sample location 5 also consistently displays alarmingly higher concentrations of the measured water chemistry parameters than all other stream sample locations. This trend suggests that wash stations may be heavily contributing to stream pollution at horse facilities, a possibility which was not predicted, or even considered, at the beginning of the project.

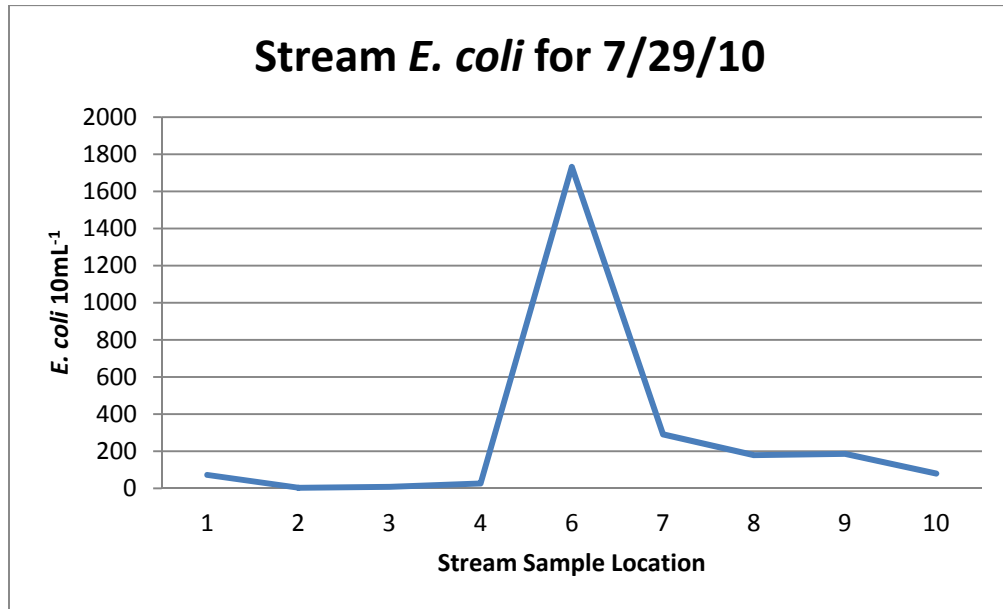


Figure 3.1: Measured *E. coli* populations over the sequence of stream sample locations on July 29, 2010, showing a large increase at sample location 6. Note that concentration of *E. coli* is expressed in colonies per 10mL.

In-situ dissolved oxygen and temperature were also graphed over the sequence of stream sample locations for each sampling date (Appendix 2 and Appendix 3). On most sampling events, there is a dip in dissolved oxygen around sample locations 3 and 4, followed by a gradual increase downstream (Figure 3.2). This may have more to do with streamflow dynamics than with contaminant levels within the stream. This vicinity of the stream, between sample locations 2 and 4, regularly experiences low flow in comparison to downstream. This increase in flow downstream is most likely due to several seeps in the streambank that introduce additional water. Also, inputs of water from horse washing and other activities at the facility could be partially responsible. This increase in flow would improve aeration and introduce more oxygen to the water. Groundwater is also usually cooler than surface water, and the ability of colder water to hold more oxygen could also increase dissolved oxygen levels (Figure 3.3).

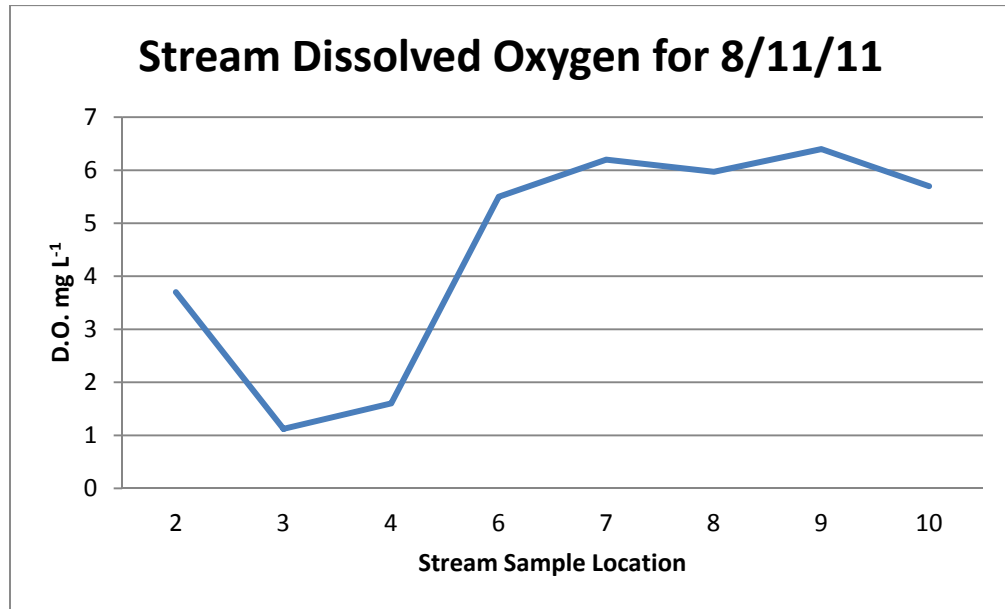


Figure 3.2: Dissolved oxygen levels over the sequence of stream sample locations on August 8, 2011, showing decrease at sample locations 3 and 4, followed by a gradual increase downstream.



Figure 3.3: Groundwater seep in stream bank at Victory Haven.

The stream temperature data also displayed trends when graphed over the course of the stream. Generally speaking, stream temperature increased over the stream's course during the winter, and decreased during the warmer months (Figures 3.4 and 3.5). Decreasing temperatures during warm weather may be related to the same phenomenon controlling dissolved oxygen, where groundwater may be increasing streamflow and cooling the stream via seeps in the bank. Cooler temperatures downstream may also be influenced by increased vegetation and shading of the streambed. Warming of the stream during cold weather may be influenced by several factors. Microbial activity keeps the muck piles much warmer compared to ambient temperature, and it is likely that runoff from the storage pads is at a higher temperature than the stream and is contributing to its warming. Groundwater would have a similar controlling effect in winter as in summer, seeping warmer water from underground into the stream. Warm water may also be introduced via wash stations and other on-site maintenance activities and would alter the stream temperature most dramatically.

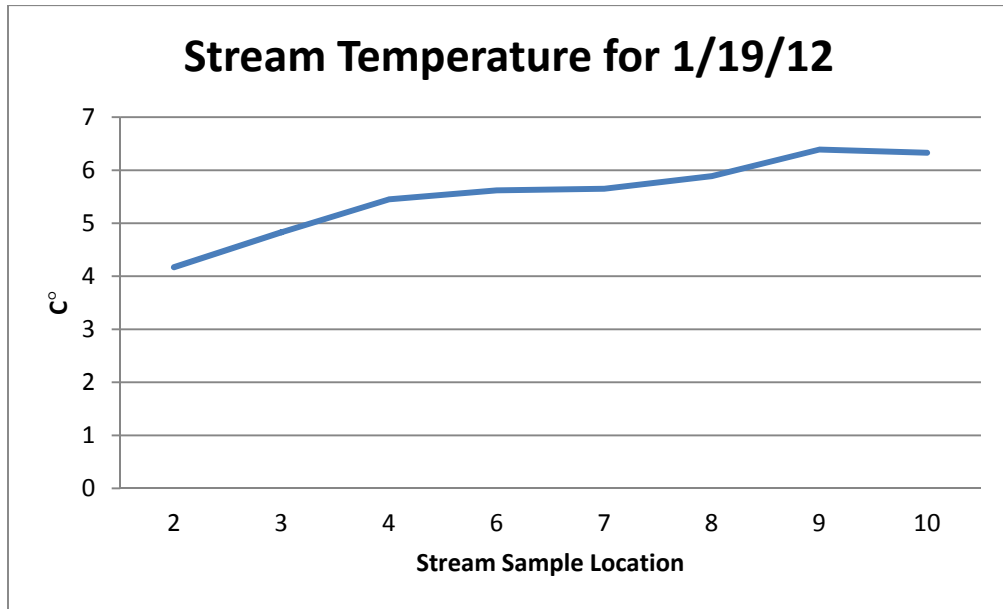
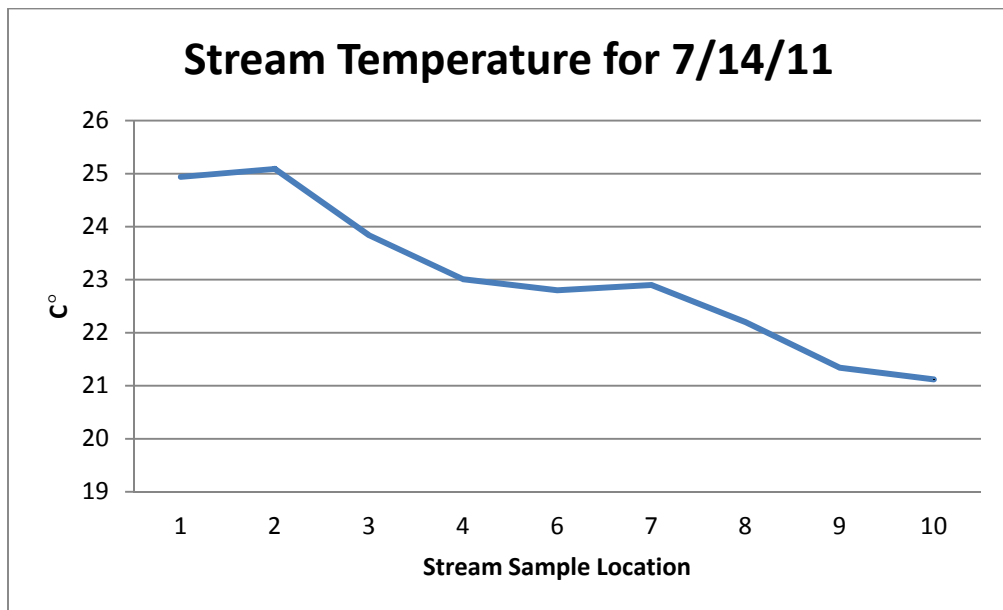


Figure 3.4: Water temperature over the sequence of stream sample locations on January 19, 2012, showing a gradual increase in temperature during winter.



Figures 3.5: Water temperature over the sequence of stream sample locations on July 14, 2012, showing a gradual decrease in temperature during warm weather.

For each stream sample location, *E. coli* and water chemistry parameters were plotted over a time series of the entire length of sampling, but few patterns of interest were observed in the raw data. This raw data was further manipulated to compare water quality before and after June 2011, when use of the first two traditional muck pads on the property ceased and they were replaced with the new design. Table 3.1 displays average levels for water quality parameters before and after June 2011. Box and Whisker plots were made for each sample location showing *E. coli* data before and after June 2011 for stream sample locations 2-10 (Appendix 4). At sample locations 2, 3, and 4, the means after June 2011 are lower than the means before. Maximum *E. coli* counts and interquartile ranges were also less in sample locations 2-4. Sample locations 6-10, where traditional muck storage methods remained in use and where the majority of the horse washing and boarding occur, did not show the same consistency and distributions were sometimes higher after installation of the new pads. This difference in the data could be due to the new muck pads capturing runoff, but it is also likely that declining *E. coli* populations in this part of the stream are affected by a weep berm system that now surrounds the composting field. This weep-berm was installed as a part of another project with University of Kentucky to mitigate the effect of runoff from the compost operation at Victory Haven. The berms were successfully able to contain all storm events from the time they were installed in June of 2011. Because of these differences in the stream data between sample locations 2-4 and sample locations 6-10, differences in data from before and after June 2011 were tested for statistical significance using a Wilcoxon two group test. Each water chemistry parameter was tested, as well as *E. coli*; none were significantly different.

Table 3.1: Average levels of water quality parameters in stream before and after June 2011

(Electrical Conductivity, chloride, sulfate, magnesium, calcium, potassium, sodium, alkalinity, pH, nitrate, ammonium, total organic carbon, phosphate, and *E. coli*) at each sample location for before and after June 2011. All water chemistry measurements are mg L⁻¹, and *E. coli* is colonies 10 mL⁻¹.

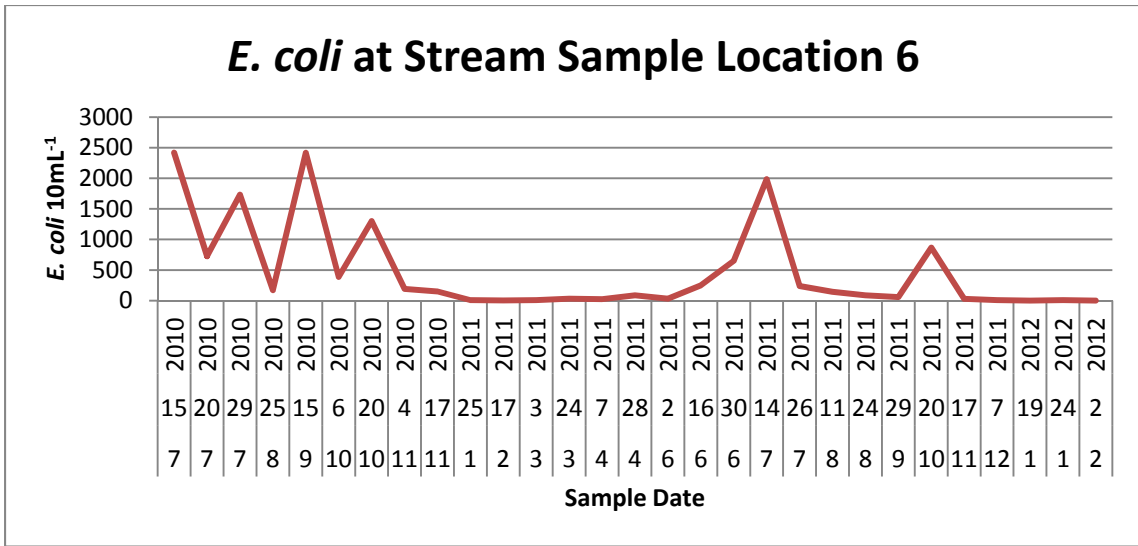
Sample Location	EC	Cl	SO ⁴	Mg	Ca	K	Na	ALK	pH	NO ³	NH ⁴	TOC	PO ⁴	<i>E. coli</i>
Before June 2011														
2	355	18	19	4.6	48	2.2	5.7	593	7.3	2.7	0.4	50	5.7	178
3	363	19	22	4.1	56	2.3	4.7	547	7.1	4.2	0.15	61	3.8	84
4	362	20	21	4.2	56	1.9	5.9	619	7.2	2.7	0.15	61	2.8	229
6	383	18	22	4.3	51	2.4	6.5	694	7.2	2.2	0.39	60	3.2	644
7	384	16	21	4.3	54	2.8	6.4	689	7.3	2.2	0.13	54	2.6	213
8	390	17	21	4.3	56	2.1	9.1	723	7.2	2.1	0.09	53	2.7	242
9	395	19	22	4.3	56	2.3	6.7	647	7.5	2.4	0.09	67	2.3	424
10	394	18	22	4.3	56	2.3	6.4	708	7.5	2.3	0.09	47	2.6	137
mean	378	18	21	4.3	54	2.2	6.4	652	7.3	2.6	0.19	57	3.2	269
After June 2011														
2	326	18	18	5.1	39	2.1	5.2	450	7.1	2.5	0.24	32	12	24
3	349	20	19	6.3	40	2.3	5.2	463	7	2.9	0.21	31	13	34
4	352	19	19	4.8	44	1.9	5.5	461	7.1	2.8	0.18	32	12	37
6	356	18	19	4.8	42	1.9	5.7	532	7.1	2.4	0.12	33	9.8	311
7	356	18	19	4.7	42	1.9	5.4	501	7.2	2.4	0.12	34	9.9	254
8	367	16	20	6.1	43	2.4	5.2	535	7.2	2.4	0.09	33	9.6	259
9	384	16	22	5.9	44	2.4	6.2	600	7.3	2.6	0.07	32	11	259
10	380	16	22	5.7	44	2.5	5.7	565	6.3	2.5	0.06	30	11	159
mean	359	18	20	5.4	42	2.2	5.5	514	7	2.6	0.14	32	11	167

It is speculated that dry weather in late summer influenced transport of contaminants to and within the stream by the notion that if there is no rain, then there is no runoff to carry pollution to the stream or to leach into groundwater. For each sampling date, *E. coli* levels were plotted against the sequence of stream sample locations (Appendix 1). On numerous occasions, there are apparent spikes in *E. coli* levels at certain sample locations, but many of these spikes are followed by large drops, suggesting that the bacteria are not making it further downstream in high numbers during dry weather. Because this trend is most dramatic in the dry months of July-October, especially in 2010, and is less apparent during wetter weather, it may be attributed to low or no-flow stream levels due to lack of precipitation. Several stream sample locations, most notably locations 3 and 4, were dry on multiple occasions in late summer. These trends are still observed during other time periods but are less pronounced, suggesting that downstream decreases in *E. coli* populations may also be influenced by a dilution effect.

E. coli levels for each sample location were plotted over a time series of each sampling event from the beginning of stream sampling to conclusion (Appendix 5). These graphs were highly variable, but a few trends were observed and may be due to precipitation and/or temperature. One trend that was consistent throughout most sample locations were discernible declines in viable coliforms during the colder months of the year, while most peaks were in the warm months of late spring, summer, and early autumn (Figure 3.6). These trends are consistent with the findings of studies concerning the thermal niche of *E. coli* where populations experience increased fitness and survival in warmer temperatures and are decreased in cold environments. Because *E. coli* bacteria

inhabit the intestinal tracts of warm-blooded animals, their populations thrive most in warmer temperatures (Gallagher, et al., 2012; Bennet, et al., 1992). It is typical for *E. coli* populations in water and soil to be high in warm months and low in winter, increasing from January-July, and decreasing from July onward (Koirala, et al., 2008; Byappanahalli, et al., 2006).

Even with little runoff and population declines during winter, there is still contamination risk. Studies have shown that natural water and soil environments can process low concentrations of *E. coli*, mainly through predation by other microorganisms, but high loads may overwhelm the system and allow *E. coli* populations to survive and even grow (Henis, et al., 1989; Byappanahalli, et al., 2003). *E. coli* has a thermal niche of about 19-42°C, and can persist in temperate soils after introduction (Bennet and Lenski, 1993; Byappanahalli, et al., 2006). Although unable to grow and reproduce in extreme cold temperatures, *E. coli* may survive even across seasons and regrow following spring thaw (Byappanahalli, et al., 2006; Adhikari, et al. 2007). Because of this persistence, *E. coli* residing in soil may be released into a stream or groundwater system long after the contamination source is gone. Any contaminants that do reach the stream may survive in pools and sediment along the stream bed and banks that remain within the stream bed during no-flow periods as long as the sediments remain moist. Byappanahalli, et al. (2003) discovered that the highest concentrations of *E. coli* in streams exist in sediments. Sediments and pools provide a safe haven for bacteria in low-flow periods and then allow them to be carried downstream as sediments are disturbed or when flow increases during subsequent rain events.



Figures 3.6: Time series of *E. coli* populations at stream sample locations 6, showing lowest populations during winter months.

Unusual weather does not just threaten the results of an experiment, but also complicates the logistics of the entire project. Originally, construction of the new muck storage pads was set to begin in the spring of 2011, when the ground had thawed and was dry enough to support excavation. Due to the extremely wet weather during early 2011, coupled with issues regarding the reliability of the hired contractors, construction was not completed until July 2011, and the project lost a full four months of sampling opportunity for the new pads. Because of this delay, the intended full year of sampling following was not accomplished and was reduced to nine months. This setback also caused problems regarding the plants planted in the new rain garden basins. Seeds were sown in early spring 2011, with intention of installation soon after the completion of construction. The four month construction delay forced the plants to remain in the Department of Forestry's greenhouse for some of the hottest months of the year, resulting in the mortality of many specimens and the reduced health of the plants that did make it to the rain garden basins.

MUCK STORAGE PADS

Construction of the new muck storage pads with rain garden basins was completed in July 2011 and monitoring began immediately. Tensiometers were read at every sampling event and results showed that the basins do hold a considerable amount of soil moisture after a rain event, although it was observed that long periods between rain resulted in dry substrate conditions (Table 3.2). The data also showed that the average moisture for tensiometers at the control pads was higher than the averages for the tensiometers in the rain garden basins. The increased moisture at the control pads could be due to several factors: increased compaction due to mowing, close proximity to

shallow water table, higher soil clay content, and lower infiltration rate than the rain garden substrate. As the compacted soil adjacent to the control pads was often observed to be saturated, the rain garden basins would provide a greater, less compacted area for runoff to disperse.

Table 3.2: Tensiometer readings from experimental and control pads.

Readings are in centibars. Blank cells represent occasions where faulty equipment prevented readings.

Date	X1A	X1B	X1C	X1D	X2A	X2B	X2C	X2D	C1A	C1B	C2A	C2B
7/26/2011	-51	-50	-49	-52	-80	-74	-79	-80	-5	-20	-25	-24
8/11/2011	-77	-77	-71	-75	-94	-90	-94	-96	-22	-32	-59	-53
8/24/2011	-77	-79	-76	-83	-88	-85	-87	-89	-32	-42	-98	-84
9/29/2011	-62	-58	-50	-54	-85	-83	-85	-85	-11	-22	-33	-23
10/20/2011	-43	-42	-10	-38	-72	-69	-71	-91	0	15	2	2
12/7/2011	--	-16	-11	-16	-40	0	-52	0	0	-35	0	0
1/24/2012	-44	-50	-49	-54	-84	-74	-77	-74	-7	-22	-25	-26
2/2/2012	-51	-50	-45	-51	-84	-79	-81	-47	-12	-14	-26	-25
3/1/2012	-14	-45	11	-15	-65	-35	-28	9	-14	-33	-27	-32
3/27/2012	-65	-76	-65	-87	-91	-79	--	-96	-30	-37	-54	-61
4/3/2012	-83	-87	-84	-84	-95	-89	-88	-90	-15	-43	-73	-63
4/17/2012	-51	-73	-86	-106	-92	-87	-51	-80	-13	-38	-83	-67
4/26/2012	-42	-66	-84	-90	-92	-81	--	-71	-1	-49	-99	-134
5/8/2012	4	6	2	-2	-1	-4	1	4	-3	-3	-21	0
mean	-50	-54	-48	-58	-76	-66	-66	-63	-12	-27	-44	-42

Samples from each lysimeter were analyzed to determine differences between soil water at the control pads and soil water in the rain garden basins at the experimental pads. Concentrations of each water chemistry parameter for each lysimeter were averaged to obtain a single value representing the control pads and the experimental pads for each sampling event. These values were plotted on a time series for the entire sampling period (Appendix 6). The most notable trend in the data showed increases in PO_4^{-3} and NO_3^- concentrations for the experimental pads, while the control pads stayed fairly constant (Figure 3.7 and Figure 3.8). This was not surprising, as nutrient-heavy runoff is being forced directly from the storage pads into the basins, and nutrients may accumulate as the basin environment develops and stabilizes. As long as these nutrients will eventually be utilized by the biota within the basin and not leach into the groundwater or be carried to the stream via surface runoff, the basins are functioning as desired. Total organic carbon increased over time in both the experimental pads and the control pads. It makes sense that TOC would increase in the rain garden basins, as more manure-laden runoff is introduced and the biological communities in the substrate continue to develop, but there are no definite conclusions as to why TOC is also increasing at the control pads. It could be due to increasingly poor maintenance, which seems to have gotten worse over the course of the project. Greater accumulations of muck with a longer time period to break down could be adding organic carbon to the soil. The sampling period may also be having an effect on soil TOC. Lysimeter sampling began in July, when the most decomposition occurs. Sampling continued through fall, winter, and spring until the beginning of the following summer. During the colder months, there were still additions of manure and runoff, but less decomposition, allowing organic carbon to accumulate.

Most other water chemistry patterns (Na^+ , Mg^{+2} , SO_4^{-2} , Cl^- , EC) at the experimental pads varied greatly, while their concentrations at the control pads remained stable. This high variability at the experimental pads may be due to sediments washing off of the unweathered rocks that were installed in the basins or simply because these environments within the basins are relatively young and have not been able to mature and stabilize yet.

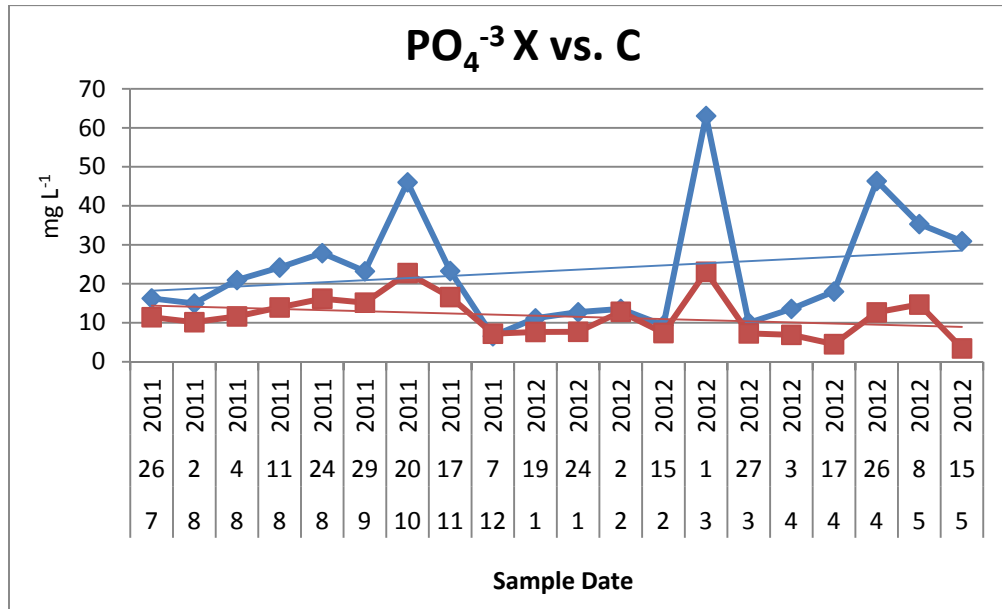


Figure 3.7: PO₄⁻³ concentrations in C pads (red) and X pads (blue), showing increases in X pads while C pads remain constant.

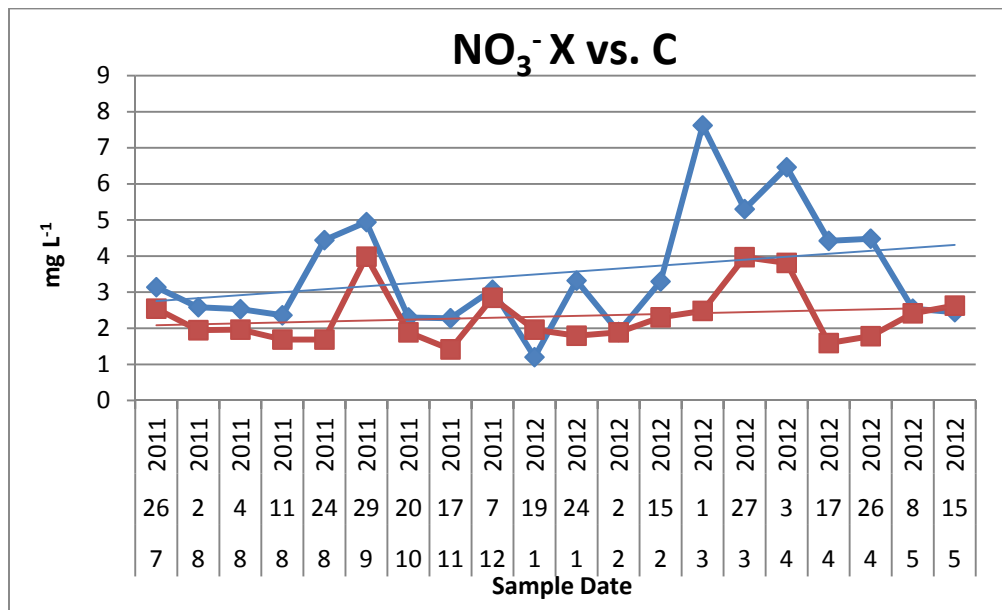


Figure 3.8: NO₃⁻ concentrations in C pads (red) and X pads (blue), showing increases in X pads while C pads remain constant.

Box and whisker plots were made for each water quality parameter to examine treatment differences between control and experimental pads (Appendix 7). The box and whisker plots give a visual representation of the data distribution. The horizontal line within the box represents the median. The diamond within the box is the 95% confidence diamond, and a horizontal line drawn through the middle of the diamond identifies the mean. The bracket outside of the box represents the shortest half, or the most dense 50% of the data. The entire length of the box contains the interquartile range (IQR), with the bottom of the box at the lower quartile, or Q1 (25th percentile) and the top of the box at the upper quartile, or Q3 (75th percentile). The whiskers extend to include the values that fall within the range or $Q1 - 1.5IQR$ or $Q3 + 1.5IQR$. Any values outside of this range are represented by point values on the graph.

Box and whisker plots for nitrate, phosphate, total organic carbon, and *E. coli* are displayed in Figures 3.9-3.12. Both nitrate and phosphate showed wider ranges, higher means, and higher maximums in the experimental pad rain gardens than the controls (Figure 3.9 and Figure 3.10). This trend was likely observed because the experimental muck pads were designed to force nutrient-laden runoff directly into the rain gardens. Total organic carbon in the experimental pads had a lower distribution, with a lower mean and maximum than in the control pads (Figure 3.11). This may be explained by organic carbon being utilized and broken down in the highly oxidized substrate of the rain garden, while organic carbon is being retained in the anaerobic soil environment surrounding the control pads. Observed distributions, mean, and maximum of *E. coli* population were lower in the experimental pads than the control pads (Figure 3.12). The large pore size and quick infiltration rate of the rain gardens allow the substrate to get

fairly dry during long periods between rain events and may be encouraging die-off of *E. coli* populations. In a study by Gallagher, et al. (2012), *E. coli* populations in a low moisture environment (4%) experienced the quickest rate of decay, showing only 5% of their initial concentration in as little as 3-4 hours. Tensiometers were typically read following precipitation events, but still displayed pressures anywhere between 0-100 centibars (Table 3.2). This indicates that the rain garden basins hold moisture after rain events but also experience dry conditions. The low observed concentrations of *E. coli* in the rain garden basins may mean that the new muck storage pads are effective and are functioning by desiccating *E. coli* populations during dry periods. The differences in each water chemistry parameter between the experimental pads and control pads were tested for significance using Wilcoxon two group tests; while some did display large differences, none were statistically significant. Differences in total coliform and *E. coli* data between experimental and control pads were also tested for statistical significance using Wilcoxon two group tests, and also showed no significant differences.

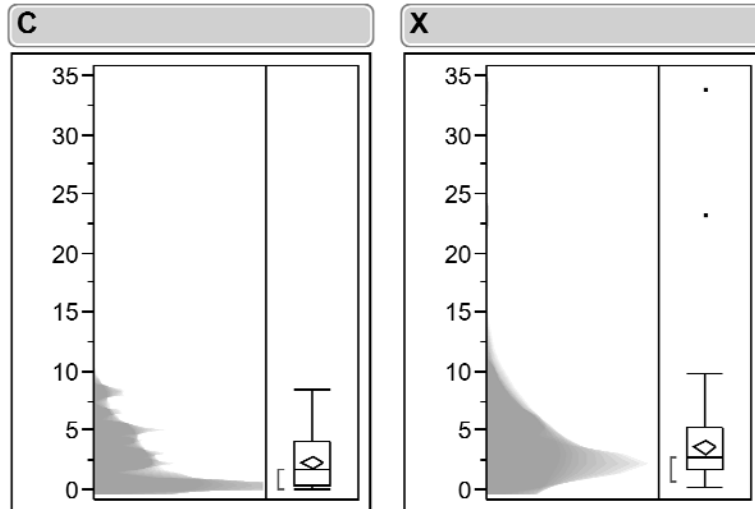


Figure 3.9: Box and Whisker plot of nitrate levels (mg L^{-1}) in C pads vs. nitrate levels (mg L^{-1}) in X pads, showing a higher distribution in X pads.

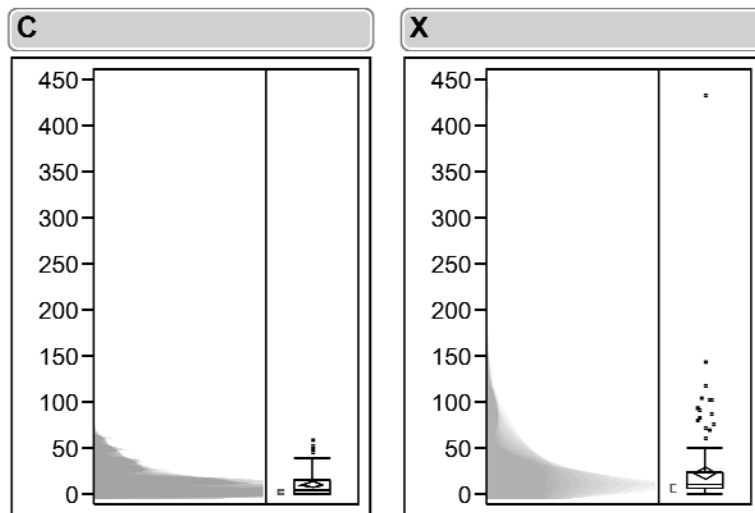


Figure 3.10: Box and Whisker plot of phosphate levels (mg L^{-1}) in C pads vs. phosphate levels (mg L^{-1}) in X pads, showing a higher distribution in X pads.

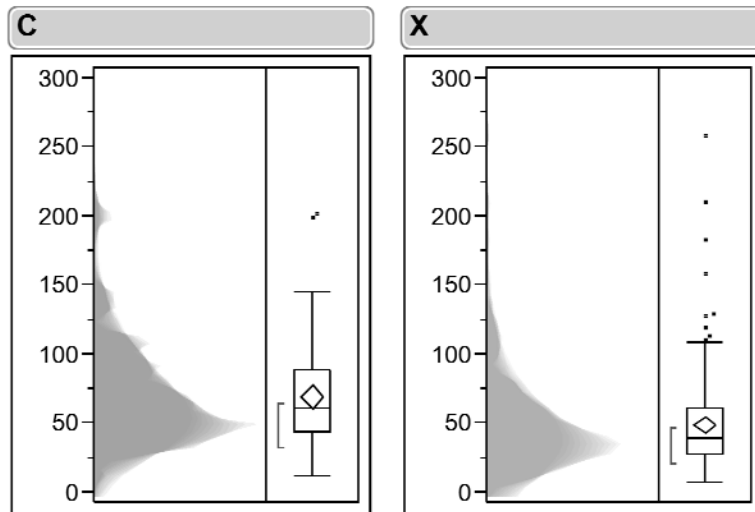


Figure 3.11: Box and Whisker plot of total organic carbon (mg L^{-1}) in C pads vs. total organic carbon (mg L^{-1}) in X pads, showing a lower distribution in X pads.

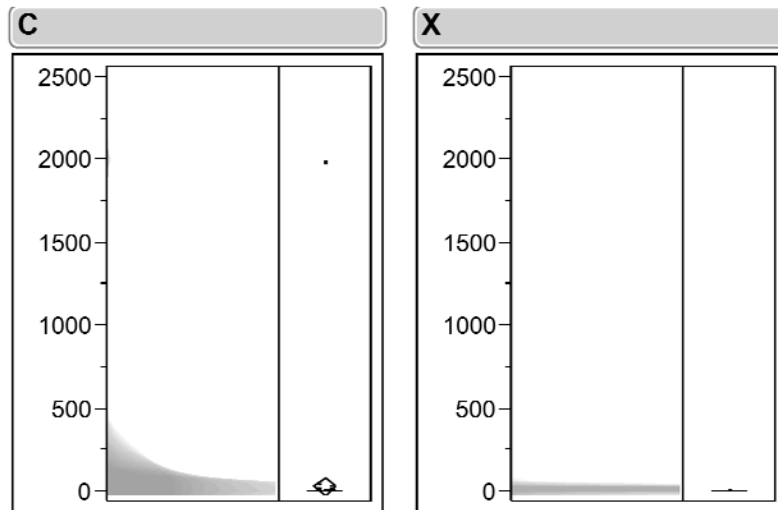


Figure 12: Box and Whisker plot of *E. coli* populations ($\text{colonies } 10\text{mL}^{-1}$) in C pads vs. *E. coli* populations ($\text{colonies } 10\text{mL}^{-1}$) in X pads, showing a greater concentration of *E. coli* in C pads.

PLANTS

Raw data for total nitrogen and total phosphorus from plant tissue samples was analyzed for trends. Content of samples taken from plants grown in the greenhouse was compared to content of plants in their second growing season in the rain garden basins at Victory Haven. Tissue samples from plants in their first growing season at Victory Haven were not considered because these specimens were not planted at the same time, and thus did not have the same amount of exposure to contamination. Total nitrogen content increased in every species in their second year of growth compared to greenhouse control samples (Figure 3.14). Total phosphorus content was highly variable and did not show any trends; content increased in some species and decreased in others (Figure 3.15). There are several possible explanations for the variability of the total phosphorus data. Spillage and overflow of the muck pads caused an unequal distribution of manure and soiled bedding within the rain gardens, resulting in some plants having more exposure than others. Other plants are much closer to the nutrient laden runoff coming from the wall drains. This discrepancy in location may have an effect on nutrient uptake, especially phosphorus. Phosphorus is not as readily available as nitrogen in soil systems, with only a small fraction able to be absorbed by plants; uptake may also be inhibited by the high alkalinity of the rain garden environment (Busman, et al., 2002). However, considering the total nitrogen data, it is believed that plant uptake could have a profound effect on decreasing nutrient levels. In the future, it will be important to increase and develop vegetation within the rain gardens, allow the rain garden alkalinity to decrease and stabilize, and continue to monitor nutrient uptake.

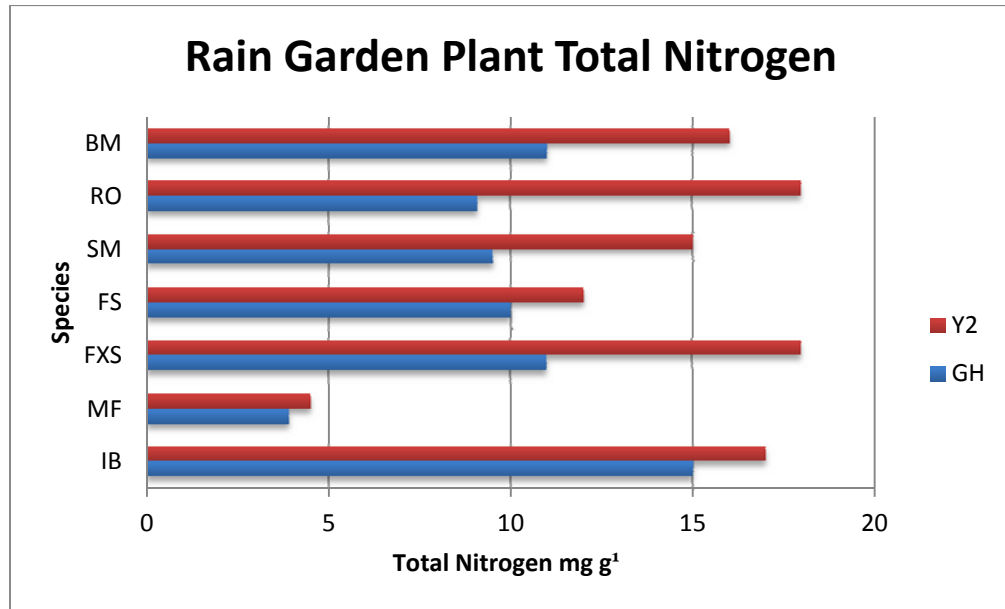


Figure 3.13: Total nitrogen concentrations for rain garden plants from greenhouse control (GH) and second year growth (Y2). For species Bur Marigold (BM), River Oat (RO) Slender Mountain Mint (SM), Frank’s Sedge (FS), Fox Sedge (FXS), Mist Flower (MF), and Illinois Bundleflower (IB).

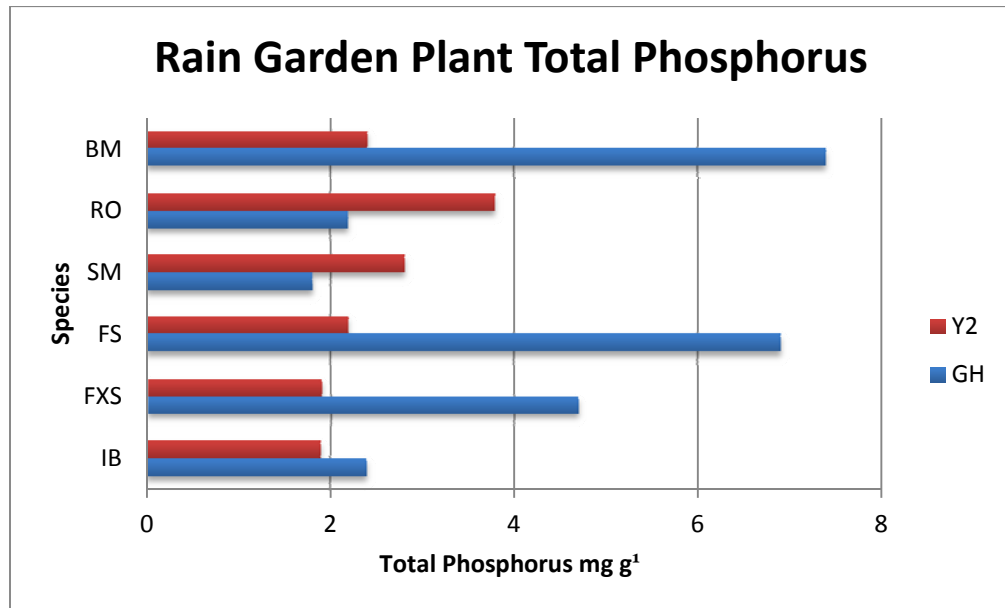


Figure 3.14: Total phosphorus concentrations for rain garden plants from greenhouse control (GH) and second year growth (Y2). For species Bur Marigold (BM), River Oat (RO) Slender Mountain Mint (SM), Frank’s Sedge (FS), Fox Sedge (FXS), Mist Flower (MF), and Illinois Bundleflower (IB).

Chapter 4: CONCLUSIONS

THE CASE FOR PROPER LIVESTOCK WASTE MANGEMENT

Overall, the new muck storage pads did not make any significant difference on contamination levels in the stream at Victory Haven. No significant differences between soil water taken from the control pads and water taken from the rain garden basins at the experimental pads were observed either. However, this lack of significance does not suggest that the methods were ineffective, or that the results were not conclusive. Contamination risks at Victory Haven are obvious and exist as the result of a combination of poor choices regarding location and design of manure storage structures and inadequate waste management operations at the facility. These issues are likely influencing the effectiveness of the new muck storage pads built for this project. Careful management of these types of facilities is important and necessary for them to work properly. Many wastewater treatment systems fail because they are not adequately maintained. A surface flow wetland constructed in Mississippi to treat water for the Mississippi Gulf Coast Regional Wastewater Authority failed to adequately reduce NH_4^+ -N, and fecal coliforms were higher in the outflow compared to the inflow (Kadlec and Knight, 1996). A natural treatment wetland in Florida receiving wastewater from Walt Disney World had to cease operation on one of its wetland systems in 1989 because of its inability to accomplish reductions. In fact, that site exhibited increases in BOD, TOC, NH_4^+ , total nitrogen, and total phosphorus concentration as water moved through the treatment system (Kadlec and Knight, 1996).

From an environmental standpoint, waste storage facilities should be situated to minimize impacts to surface and groundwater features, especially at a facility such as

Victory Haven, which is located on a flood plain and lies within the boundaries of recognized FEMA Flood Zones (FEMA, 2008). From an economic standpoint, waste storage facilities should also be located as close to the source as possible to reduce transport risk, cost, and effort. Unfortunately, the barns at Victory Haven were built in very close proximity (less than 50 ft (15 m) in some instances) to the onsite tributary of Cane Run, which led to construction of temporary muck storage pads in environmentally undesirable locations. The close proximity of these pads to the stream channel not only enhances the possibility of surface water contaminations via runoff, but the physical setting (within a flood plain in an active, karst geologic setting) provides many opportunities for contamination through below surface transport processes (interflow and/or groundwater recharge). These risks regarding location are amplified by a poor design of the muck pads themselves and lack of maintenance.

When this project began, two of the muck pads at Victory Haven had wooden walls, which were flimsy, full of gaps, and allowed for a large amount of spillage and seepage (Figure 4.1). Although the wooden walls were removed as part of this project, retrofitted concrete-walled pads provided little improvement due to mismanagement. It is the assumption that each muck pad handles the waste from one barn, as there are seven barns and seven muck pads at Victory Haven. Stalls per barn vary from 13-74. If one muck pad holds waste from barn 4, which has 48 stalls, that muck pad could be receiving well over 3,000 lbs. (1,300 kg) of manure and soiled bedding per day. Manure is fairly dense, and one horse may only produce 0.04-0.05m³ per day, totaling approximately 2m³ for a full 48-stall barn, but the bulk of the muck comes from the soiled straw bedding. If each horse required only 1m³ of loose bedding per day, a muck pad servicing 48 horses

would receive as much as 50m³ of manure and bedding each day. The amount of muck being placed in each 25ft x 25ft x 6ft (7.6m x 7.6m x 1.8m) (115m³) muck pad should dictate that they be emptied every few days to avoid overflow, but muck pads at Victory Haven are consistently left for weeks at a time without emptying (Figure 4.2).

Inadequate maintenance, combined with no runoff abatement or treatment in the control pads resulted in visible runoff to surface water (Figure 4.3) and explains at least a portion of the water contamination issues at Victory Haven.



Figure 4.1: Old wooden-walled muck pad at Victory Haven.



Figure 4.2: Spillage and overflow at pad C2 due to infrequent emptying.



Figure 4.3: Drainage ditch leading runoff from C2 to nearby Cane Run tributary.

This project also helped identify contamination sources other than the muck storage pads. Victory Haven eventually uses the muck generated by their horses to run a composting operation in several of the onsite fields (Figure 2.17). The weep berm system surrounding the compost windrows was installed in 2011 and is functioning as desired, but until then, runoff from the compost fields went unchecked and was most likely a considerable contributor to water contamination and may be in the future if not maintained. This project also revealed that horse wash stations may be a significant source of contamination at horse facilities. Sample location 5 was a pipe that drained runoff from horse washing stations at Victory Haven, and there are several other wash stations that are not piped. Water samples from this location consistently displayed the highest levels in all measured water chemistry parameters and *E. coli* populations compared to other stream sample locations. Decreased water quality, specifically spikes in *E. coli* populations, was also observed immediately downstream at sample location 6.

FUTURE WORK

While this project was a valuable learning experience, it addressed only some options for mitigating contamination at Victory Haven and offers many opportunities for future work. Extended and more thorough experimentation will give a more complete idea of the types of contamination that equine facilities may encounter, their possible travel pathways, and their effects on the environment.

The water quality parameters investigated in the project were relatively limited when compared to the wide variety of contaminants that may originate from livestock waste. There are several other methods that would give a more accurate characterization

of the contamination at Victory Haven. The only pathogens tested for this project were coliform bacteria, but livestock manure may spawn a variety of pathogens including *giardia*, *cryptosporidium*, and *salmonella* (Hooda, et al., 2000; The World Bank, 2005; Burkholder, et al., 2007; WHO Guidelines, 2011). *E.coli* is used as an indicator species of pathogenic pollution, but does not confirm or refute the existence of other harmful pathogens. A study of the bacterial population of the water and soils at Victory Haven could add valuable information to the literature on the risks of pathogens originating from livestock waste. Benthic macroinvertebrates are often used as indicators of water quality and ecological function. An initial survey and continued monitoring of the benthic macroinvertebrate population over time would help classify the current state of the stream and the effects of any future work done on or in the vicinity of Victory Haven.

Specific pollution pathways were not investigated in this project, but doing so would further aid in developing a solution to runoff contamination. It was assumed that contamination originated at the muck pads and flowed in a straight path to the stream, but in reality it may be much more complicated. Dye and nitrogen isotope tracers added to the muck piles, accompanied by widespread groundwater and stream sampling, could provide a considerably more intricate understanding of hydrologic movement throughout the site. Well nests and piezometer fields could provide a better understanding of shallow groundwater flow and its influence on contaminant transport. Based on years, if not decades, of waste mismanagement, it is suspected that contamination is an extensive problem at Victory Haven and may not be confined only to current muck storage areas. Combined with tracer studies, analyzing soil samples would help establish the scale and magnitude of groundwater and soil contamination.

This project at Victory Haven is part of a larger effort to mitigate pollution and degradation of Cane Run watershed. Cane Run encompasses a wide variety of land uses, but as it is 75% rural, Victory Haven is an ideal setting for a study of agricultural streams and their stressors. A large area of concern regarding agricultural streams is the effect of the presence or absence of an adequate riparian zone. As most landowners want to maximize the land use potential of their property, riparian areas are often made as small as possible, and are sometimes eliminated completely. In the case of Victory Haven and many other livestock operations, streams are excluded from pasture enclosures, allowing plenty of room for riparian areas. Unfortunately, this opportunity was not capitalized at Victory Haven. Vegetated riparian zones are a popular bioengineering solution to prevent water pollution, and it is widely accepted that a combination of varying plant size and structure both above and below ground contributes to stream bank stabilization, slowing of runoff, temperature moderation, and filtration of contaminants (Higgins, et al., 2011). Victory Haven currently does not maintain riparian areas and periodic mowing extends as close to the edge of the stream bed as possible. This site is located within a heavily agricultural area, and would be ideal for project inquiring into and possibly adding to the literature regarding the ideal structure and species diversity for a riparian area handling agricultural waste, particularly originating from livestock.

Because this project is the first of its kind in central Kentucky, it can serve as an educational opportunity for other owners of small livestock operations in the area. With cooperation from the owners, upkeep, and future development of the muck storage system, Victory Haven could set an example for proper muck and manure management.

However, significant changes to current waste management practices would first need to be employed.

RECOMMENDATIONS AND IMPROVEMENTS FOR BEST MANAGEMENT PRACTICES

Regardless of any future studies or experimentation, there are many improvements that can be made to the waste storage system and management practices at Victory Haven to alleviate stream and groundwater contamination. There is a wide array of options for best management practices that could be employed at Victory Haven to reduce the risk of contamination.

There may be opportunities to improve on the design and functionality of the new muck storage structures themselves that could further reduce runoff contamination. Plants within the basin remained relatively sparse, and few volunteers germinated in following growing seasons. The addition of more plants may encourage greater plant uptake of water and nutrients and thus, greater detention of runoff. Aside from the holes drilled into the base of the walls, the muck pads have no other drainage system. As the pads are still exhibiting some runoff escaping from the approach end (Figure 4.4), they would benefit from a more structured drainage system. Re-grading the slope of the pad at a greater angle towards the back wall of the structure would force runoff to the back of the pad and through the drainage holes rather than flowing from the front end. If re-grading is not an option, the approach portion of the pad may be outfitted with a grated drain that would catch runoff and divert it towards the rain garden basin where it would be contained and treated. Green roofing may also be an advantageous addition to the

muck storage system at Victory Haven. By allowing precipitation to fall directly onto a vegetated roof instead of onto the stored muck, a well-designed and executed green roof could potentially eliminate all chance of contaminated runoff.



Figure 4.4: Runoff escaping from the approach end of pad X2.

Victory Haven's stormwater runoff control system is virtually nonexistent, aside from swales and ditches naturally established where runoff flow concentrates. Because there is no controlled drainage system at the site, tire dredging, mud accumulation, and ponding of water is common in high traffic areas of driveways and parking areas. This excess mud and water is of special concern around the muck storage pads because there is a considerable increase in contamination risk (Figure 4.5). In order to eliminate these issues, the soil and gravel in traffic areas should be reinforced or replaced with a more resilient material. Soil cement is an inexpensive option made of soil, cement, and water that forms a durable surface that won't sustain mud and dredging but will still allow for some permeability. As there are many opportunities for runoff to become contaminated throughout Victory Haven, water quality at the facility would greatly benefit from a site-wide runoff diversion system. A series of diversion ditches surrounding barns, muck pads, parking and driveways would collect runoff and transport it efficiently while preventing contact with hazardous areas. Riprap lining, check dams, and vegetation would slow runoff, allowing for settling of sediments and contaminants before they reach the stream. And even more effective treatment option would be to direct all of the runoff diversion ditches to a large on-site constructed wetland or other type of small wastewater treatment facility.



Figure 4.5: Spillage, mud, and tire dredging surrounding pad C2.

It is known and accepted that wash stations may be significant contributors to water pollution at horse facilities (Higgins, et al., 2007). As confirmed by data gathered for this project, the wash stations at Victory Haven are introducing large amounts of *E. coli*, nutrients, and other chemical contaminants directly into the stream. Wash stations are usually floored by soil or concrete. Since compacted soil saturates quickly and traditional concrete is impermeable, both encourage large volumes of runoff during washing. Pervious concrete has been developed as a solution to problems surrounding wash station runoff (Higgins, et al., 2007). A pervious concrete wash station consists of a dry, sandless concrete mixture placed over a rock-lined drainage. This structure would operate on the same principles as the rain garden basins surrounding the muck pads. Water infiltrates directly into and through highly porous concrete which provides habitat for communities of microorganisms that may be beneficial in removing pathogens and contaminants. Any water that makes it through enters a drain and may be diverted to a vegetated filter strip or lined drainage ditch before reaching surface water. However, given the housekeeping habits at Victory Haven and that pervious concrete does require regular maintenance to function properly, this option may also be a challenge. There are currently no regulations addressing this type of runoff, but because they have been proven to be contributors to water contamination, it is in the interest of environmental stewardship to alleviate the risk of contamination associated with horse washing stations.

Many of these suggested best management options involve installation or modification of structures, but the easiest and most affordable solution to contamination may simply be changing onsite housekeeping habits. Muck storage pads may go for weeks or months without being emptied, but ideally they should be cleaned daily in order

to prevent overflow. Cleaning of muck pads in anticipation of heavy rain events would also reduce the contamination risk. Regardless of which options are chosen, alleviation of contamination at Victory Haven will require effort and commitment from both management and staff. Unfortunately, support in that respect has been one of the biggest obstacles to the success of this project, and it seems that even the simplest solutions are not of interest to the property managers. Many boarders at Victory Haven commented on the appeal of the new muck storage pads, but requests for cooperation from management and staff were met with disregard, and even disrespect. Following installation, the new muck pads were still not emptied frequently enough to prevent overflow, and the rain garden basins were somehow perceived as a dump area for unwanted items and garbage (Figures 4.6, Figure 4.7, and Figure 4.8). Unfortunately, because there is such a lack of motivation to make changes at Victory Haven, the most effective solution may be regulatory action by the appropriate authorities. Victory Haven does not maintain best management practices, does not have a visible runoff control or water quality protection plan, and has several point sources on the property. They are in violation of the Clean Water Act and the Kentucky Agriculture Water Quality Act, and it is surprising that they have avoided fines and enforcement thus far.



Figure 4.6: Spillage and overflow at pad X2.



Figure 4.7: Overflow and trash in the rain garden basin at pad X2.

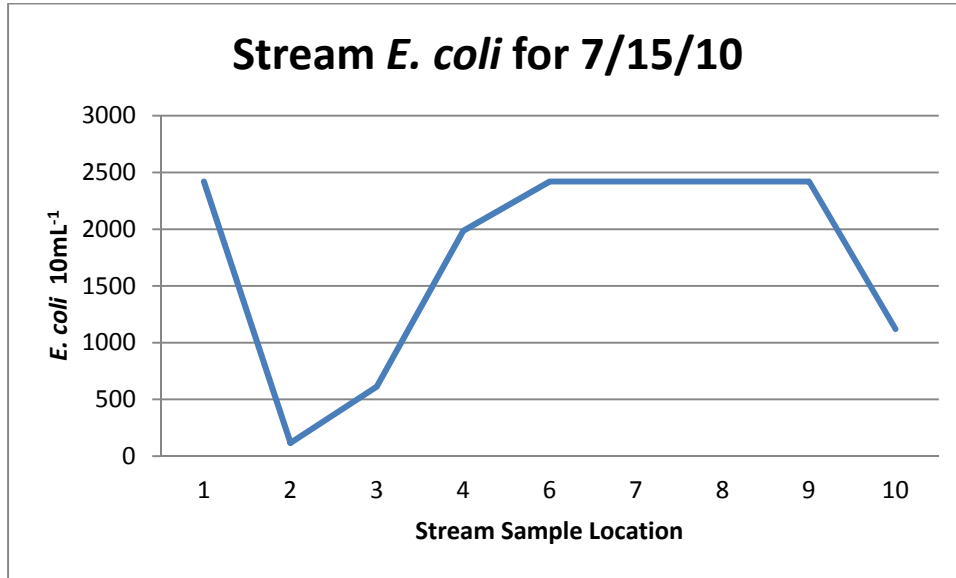


Figure 4.8: Overflow and wooden pallets stacked in the rain garden basin at pad X1.

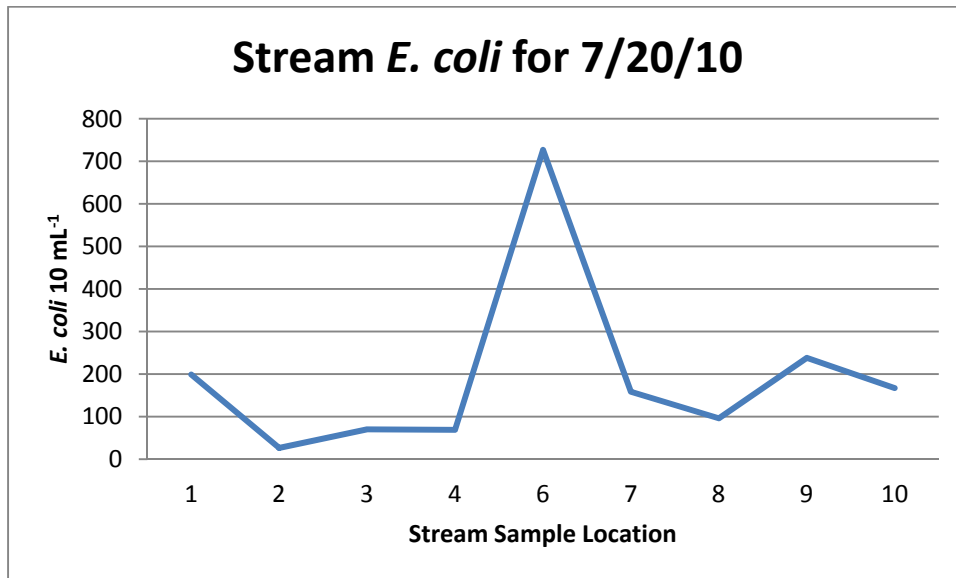
With proper dedication and further development, this project has potential to be a valuable educational tool and example of proper waste management to owners of small to medium sized livestock operations. This project began as a proposal for the first steps toward a technical solution to runoff contamination, and while the significance of the results were important to the conclusions drawn about preventing water contamination, this project also evolved into a lesson in environmental ethics. Even if not used for public education purposes or further scientific study, a properly managed muck storage system at Victory Haven could at least evoke a sense respect for and pride in the individual property, the Kentucky horse industry, and maintaining a clean, safe, and environmentally responsible operation.

APPENDIX 1. Stream *E. coli* Populations for Each Sample Date.

Appendix 1.A. Stream *E. coli* populations over sequence of stream sample locations for 7/15/2010.

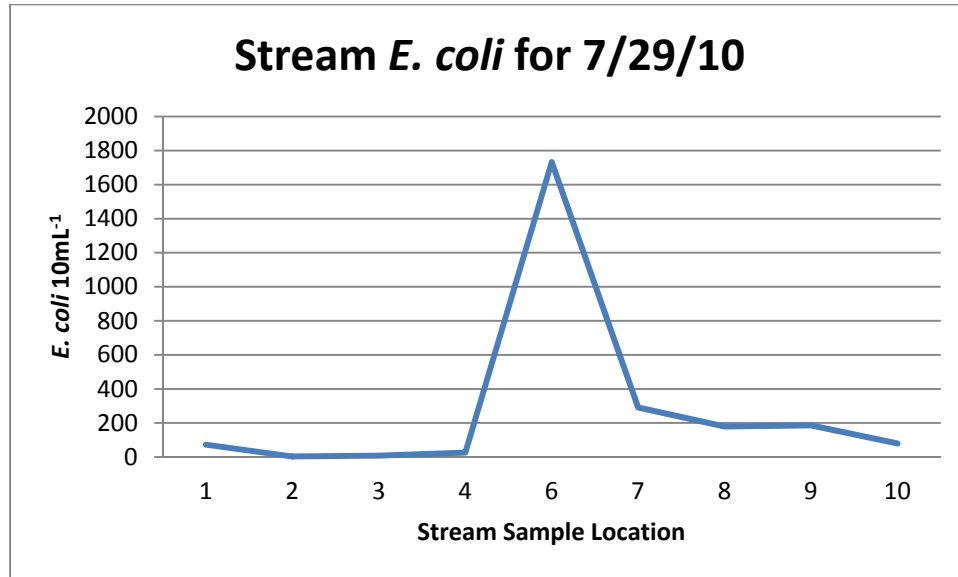


Appendix 1.B. Stream *E. coli* populations over sequence of stream sample locations for 7/20/2010.



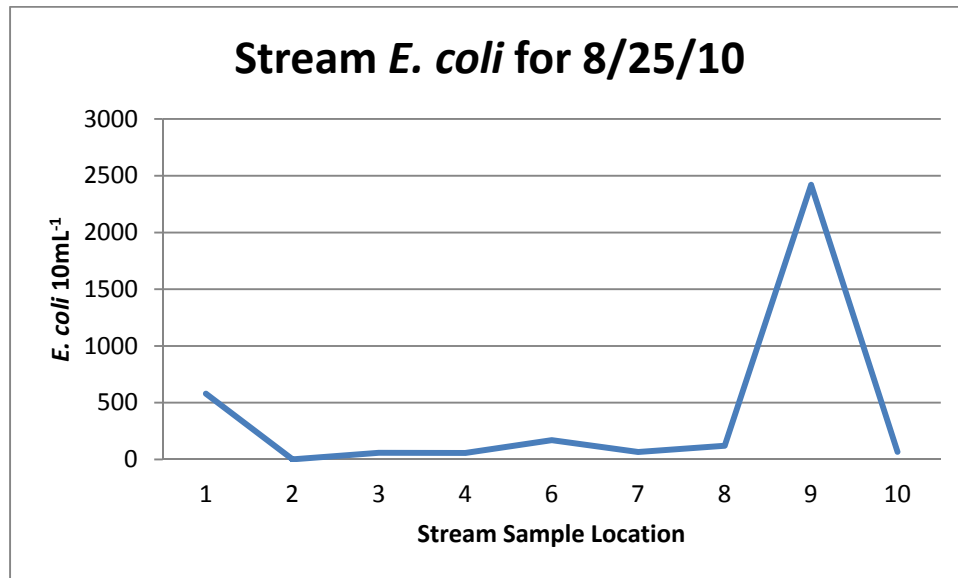
Appendix 1.C.

Stream *E. coli* populations over sequence of stream sample locations for 7/29/2010



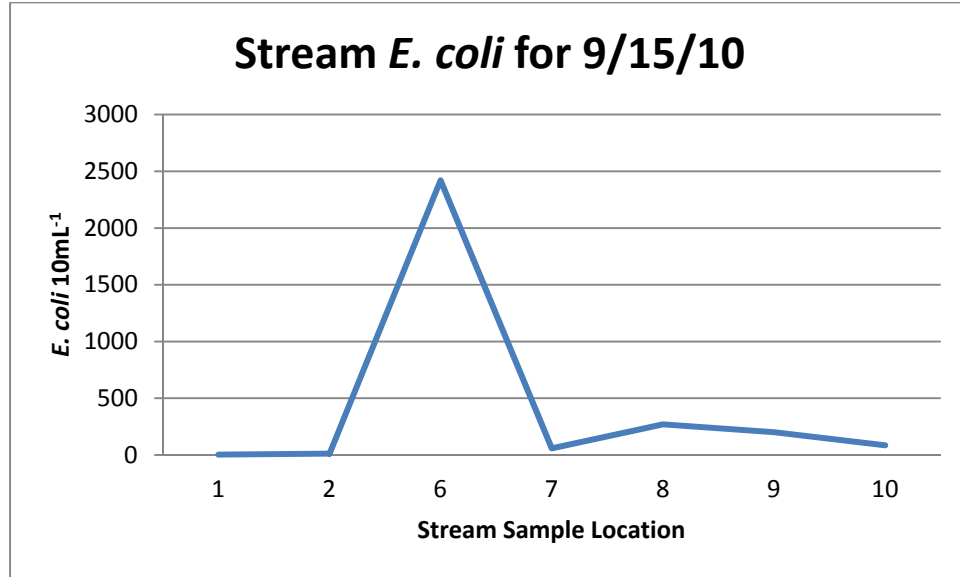
Appendix 1.D.

Stream *E. coli* populations over sequence of stream sample locations for 8/25/2010



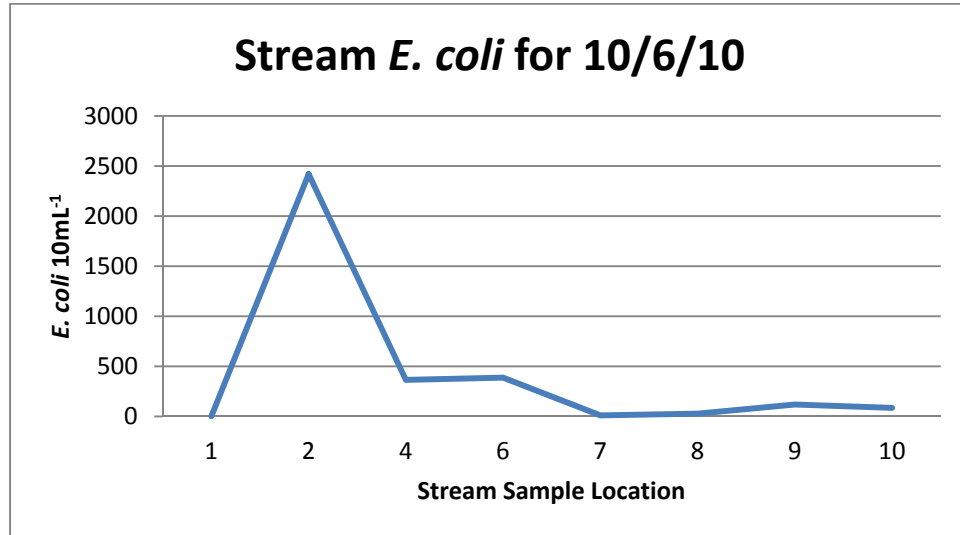
Appendix 1.E.

Stream *E. coli* populations over sequence of stream sample locations for 9/15/2010



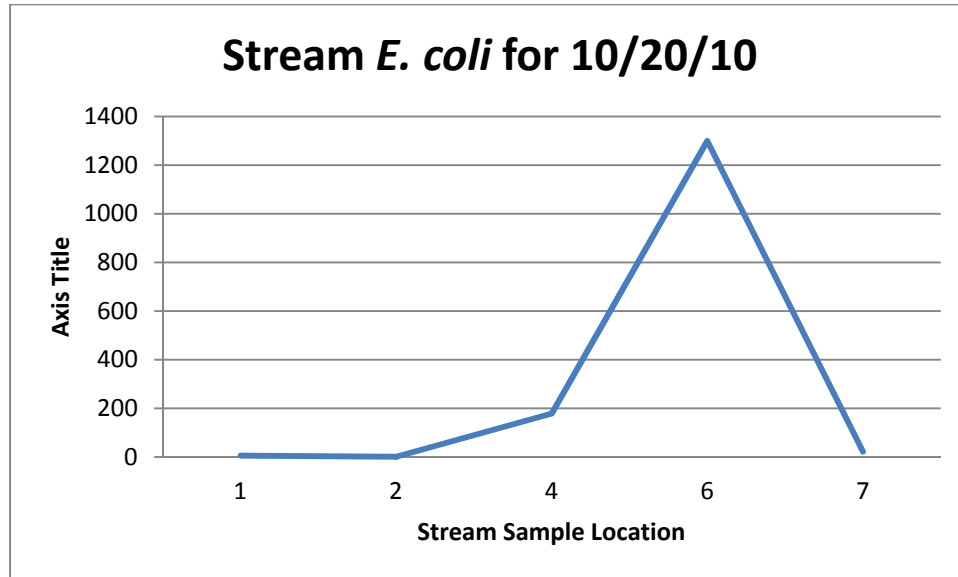
Appendix 1.F.

Stream *E. coli* populations over sequence of stream sample locations for 10/6/2010



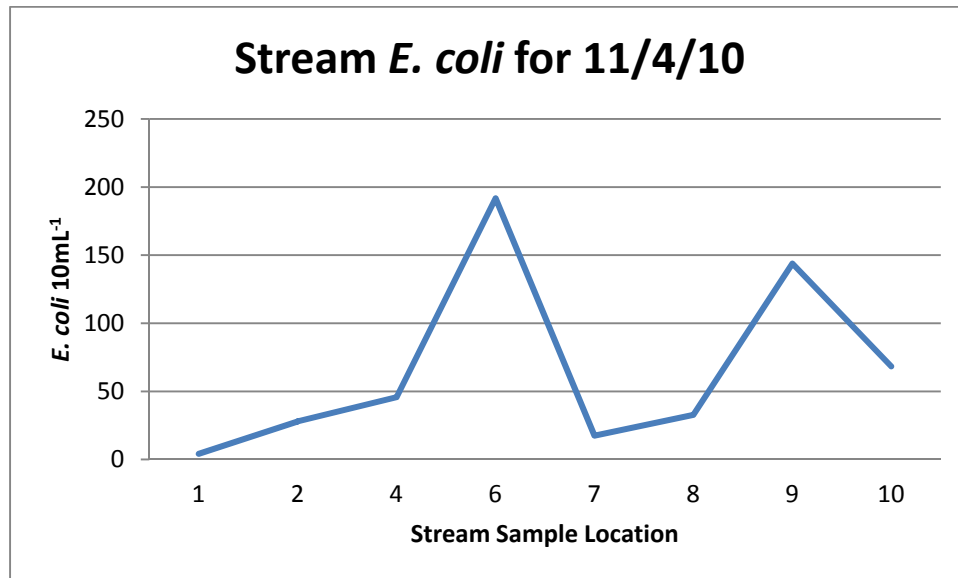
Appendix 1.G.

Stream *E. coli* populations over sequence of stream sample locations for 10/20/2010



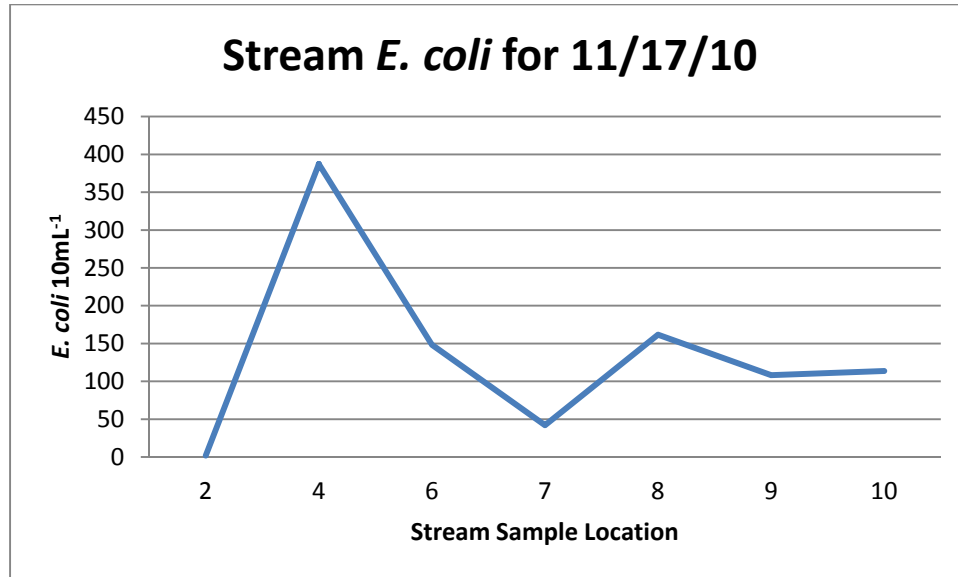
Appendix 1.H.

Stream *E. coli* populations over sequence of stream sample locations for 11/4/2010



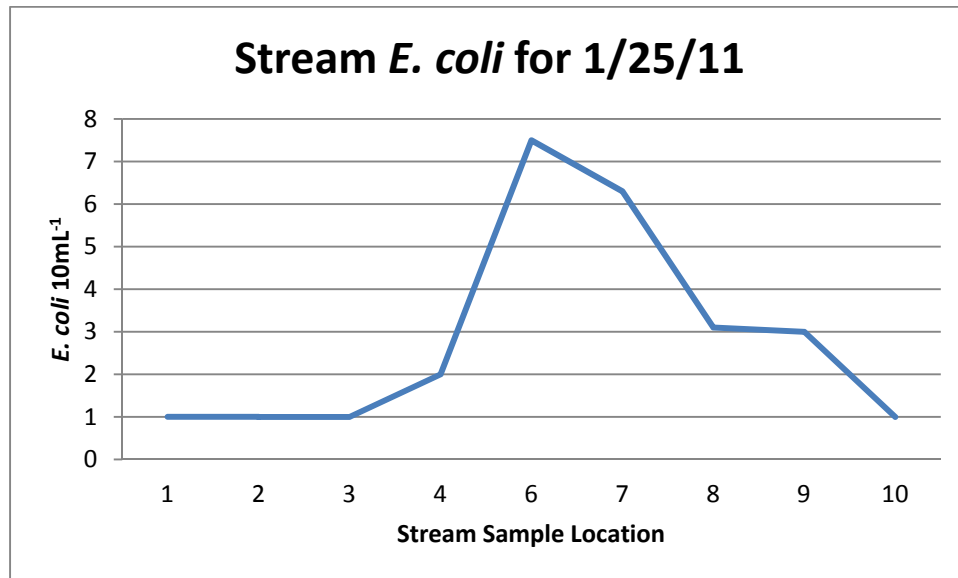
Appendix 1.I.

Stream *E. coli* populations over sequence of stream sample locations for 11/17/2010

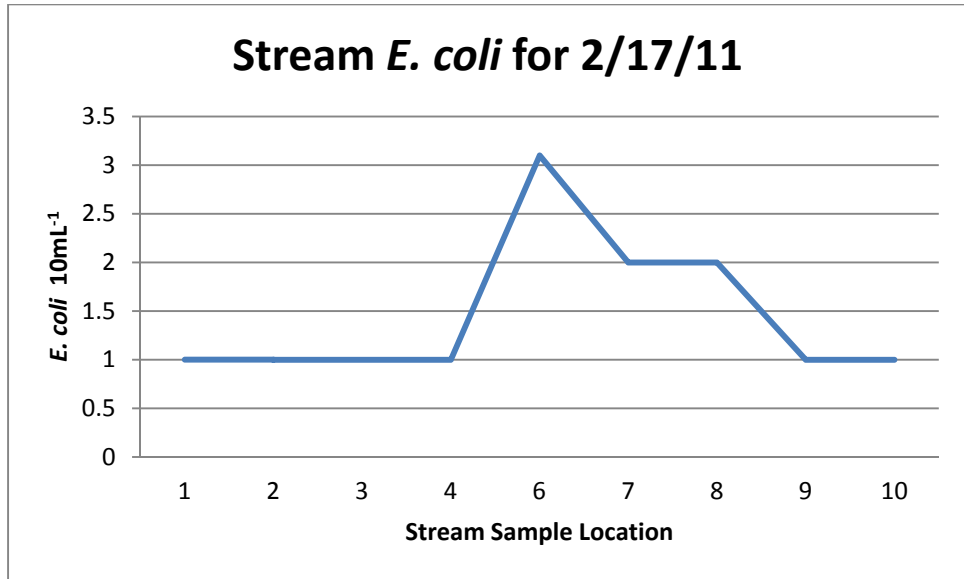


Appendix 1.J.

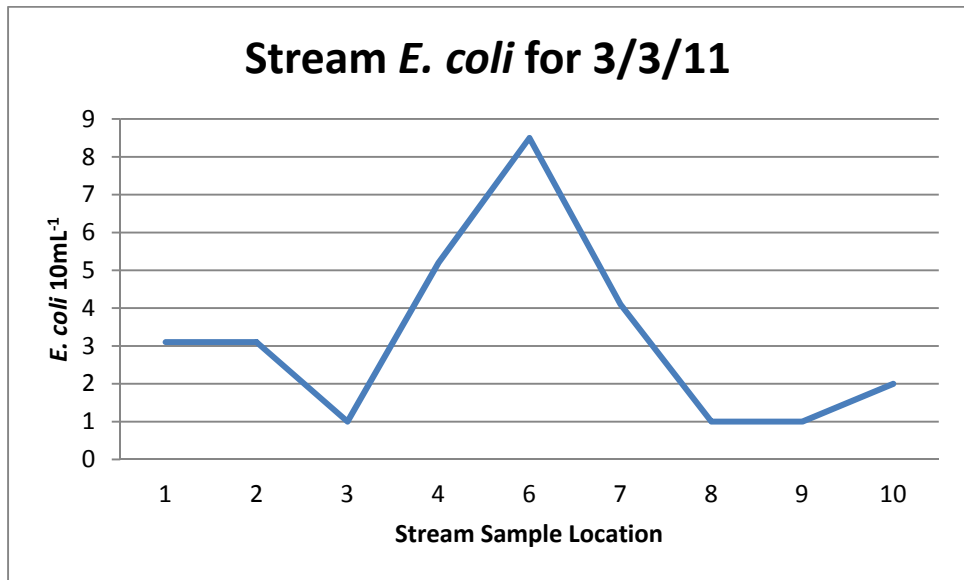
Stream *E. coli* populations over sequence of stream sample locations for 1/25/2011



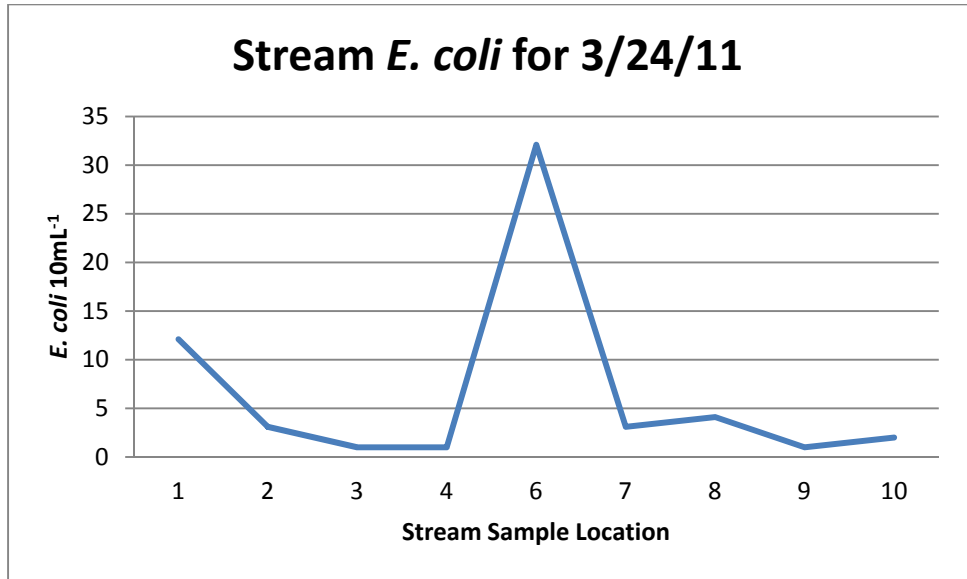
Appendix 1.K. Stream *E. coli* populations over sequence of stream sample locations for 2/17/2011



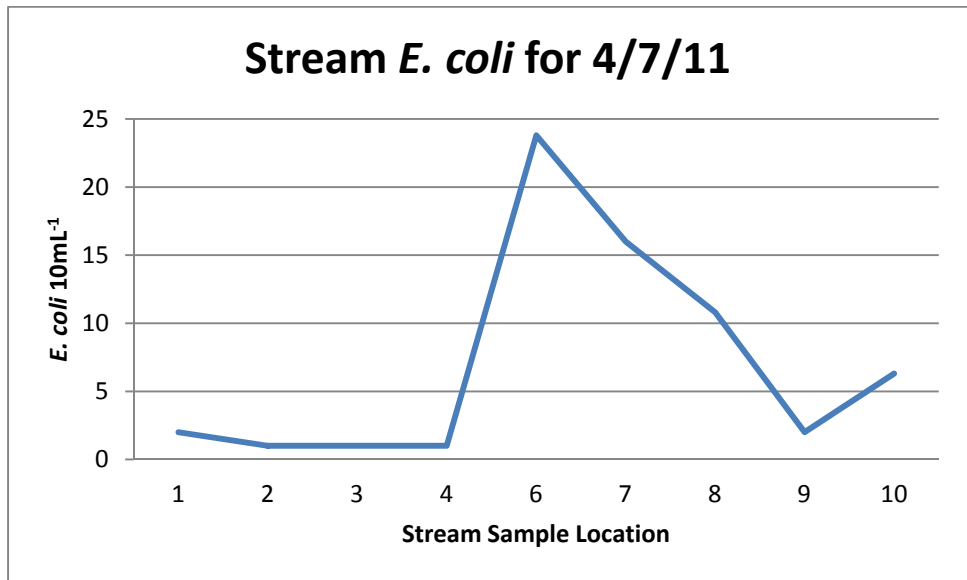
Appendix 1.L. Stream *E. coli* populations over sequence of stream sample locations for 3/3/2011



Appendix 1.M. Stream *E. coli* populations over sequence of stream sample locations for 3/24/2011

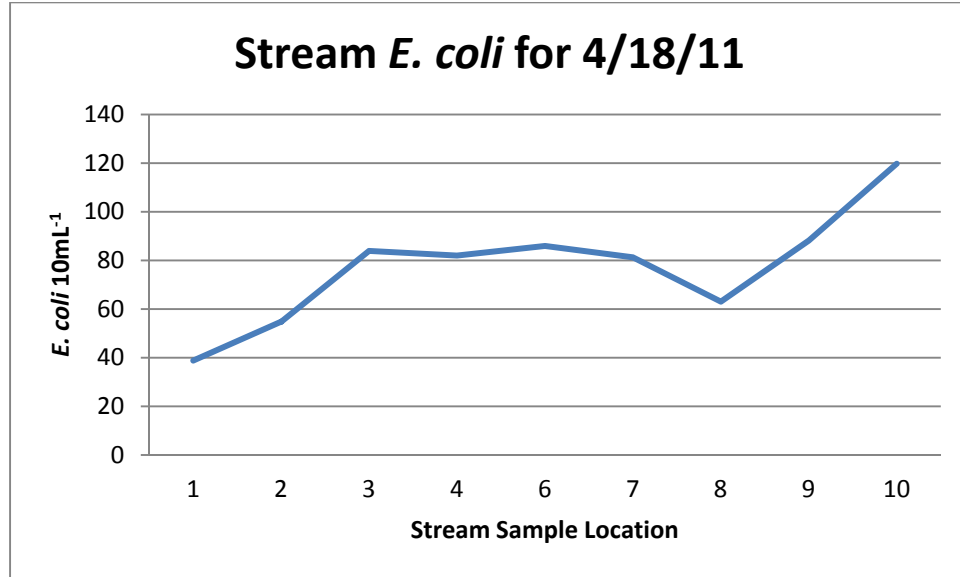


Appendix 1.N. Stream *E. coli* populations over sequence of stream sample locations for 4/7/2011



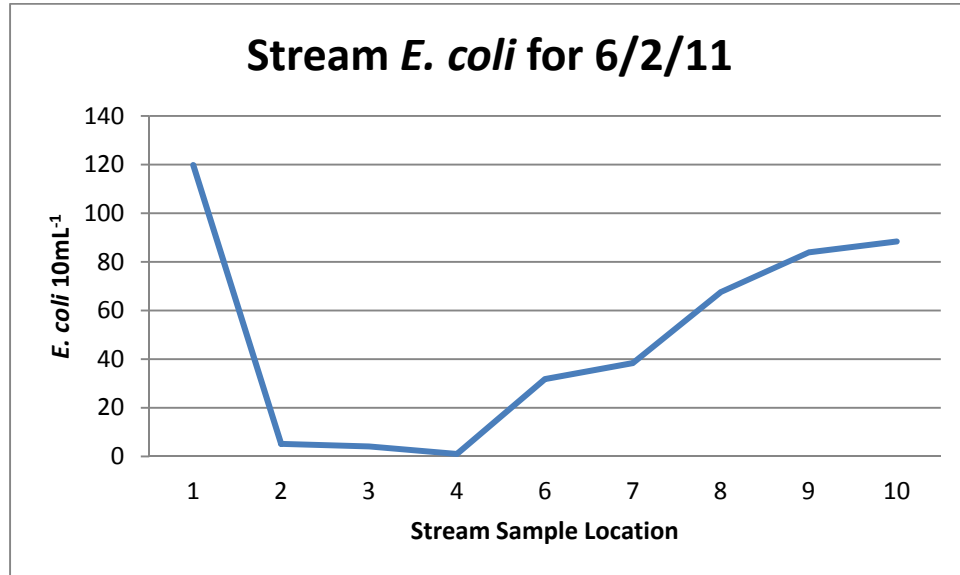
Appendix 1.O.

Stream *E. coli* populations over sequence of stream sample locations for 4/18/2011



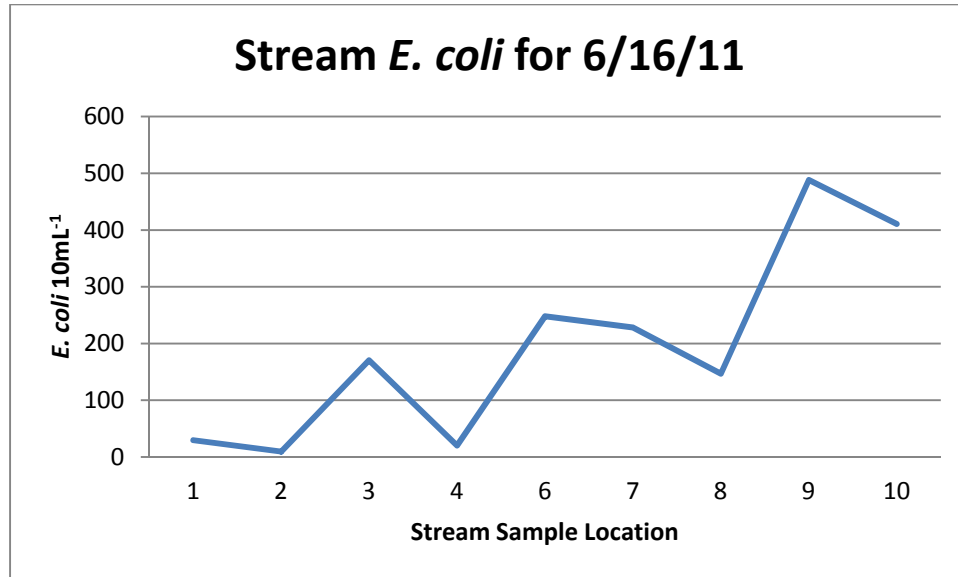
Appendix 1.P.

Stream *E. coli* populations over sequence of stream sample locations for 6/2/2011



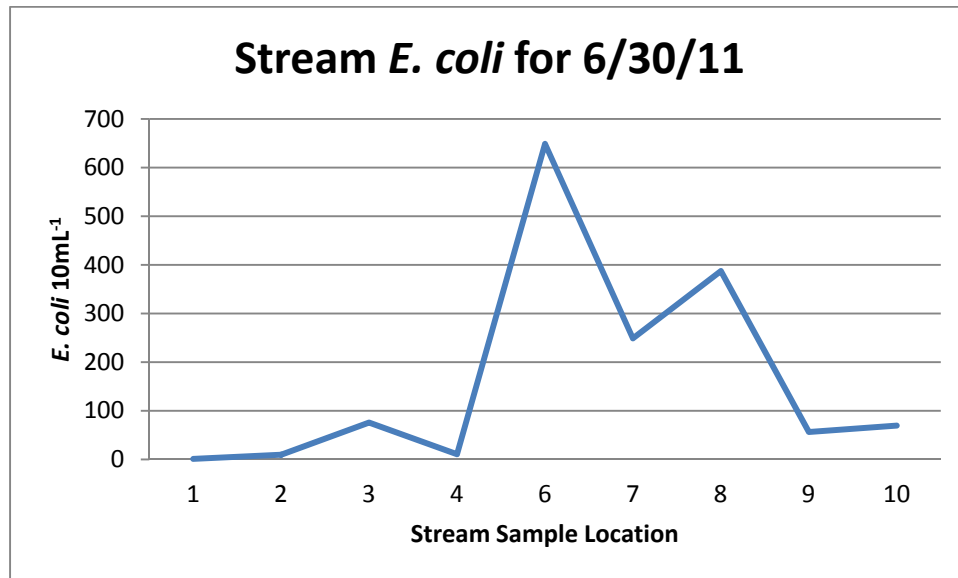
Appendix 1.Q.

Stream *E. coli* populations over sequence of stream sample locations for 6/16/2011



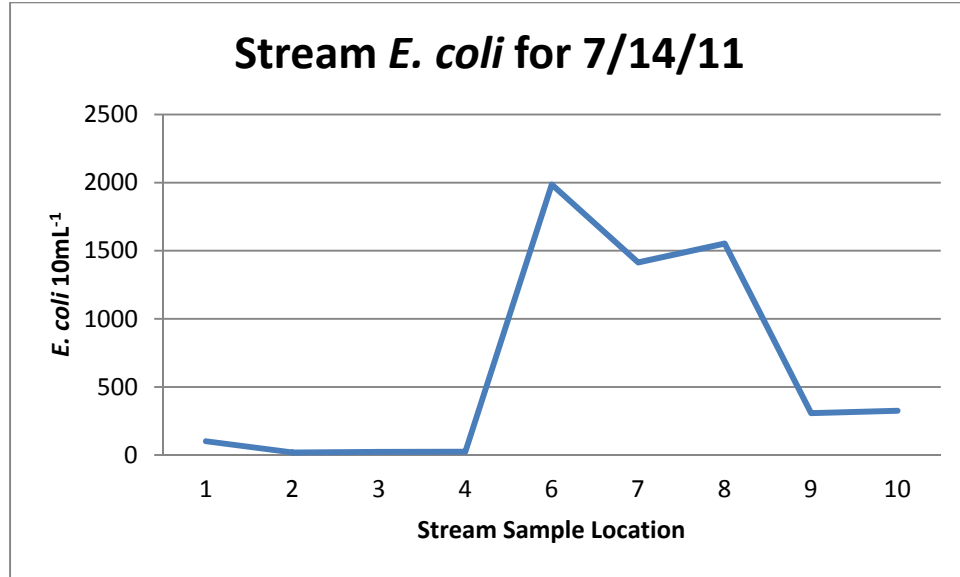
Appendix 1.R.

Stream *E. coli* populations over sequence of stream sample locations for 6/30/2011



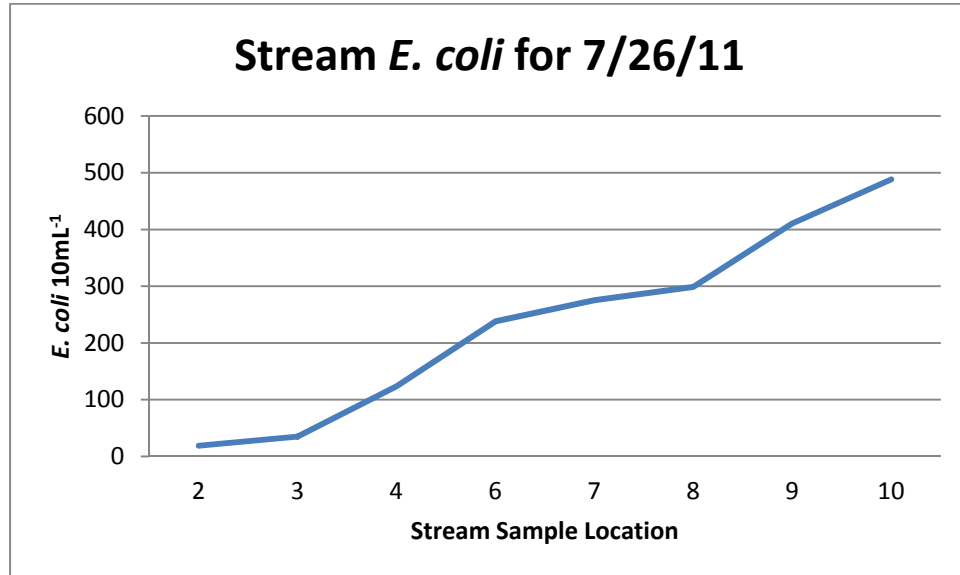
Appendix 1.S.

Stream *E. coli* populations over sequence of stream sample locations for 7/14/2011



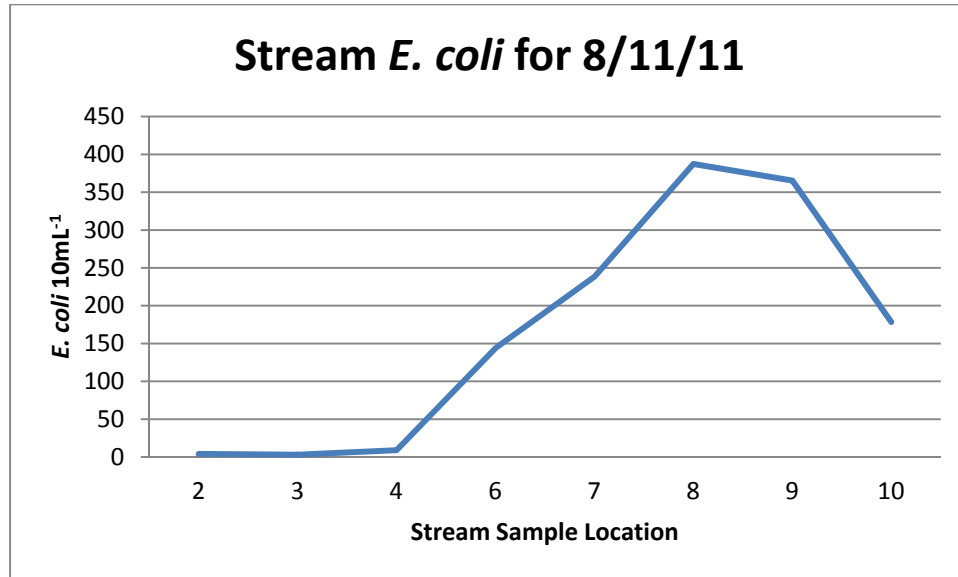
Appendix 1.T.

Stream *E. coli* populations over sequence of stream sample locations for 7/26/2011



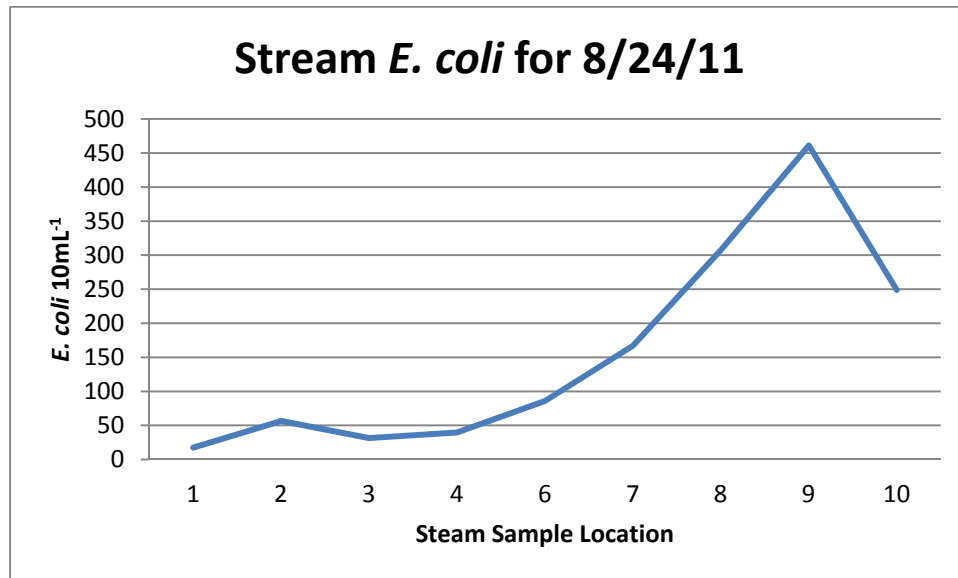
Appendix 1.U.

Stream *E. coli* populations over sequence of stream sample locations for 8/11/2011

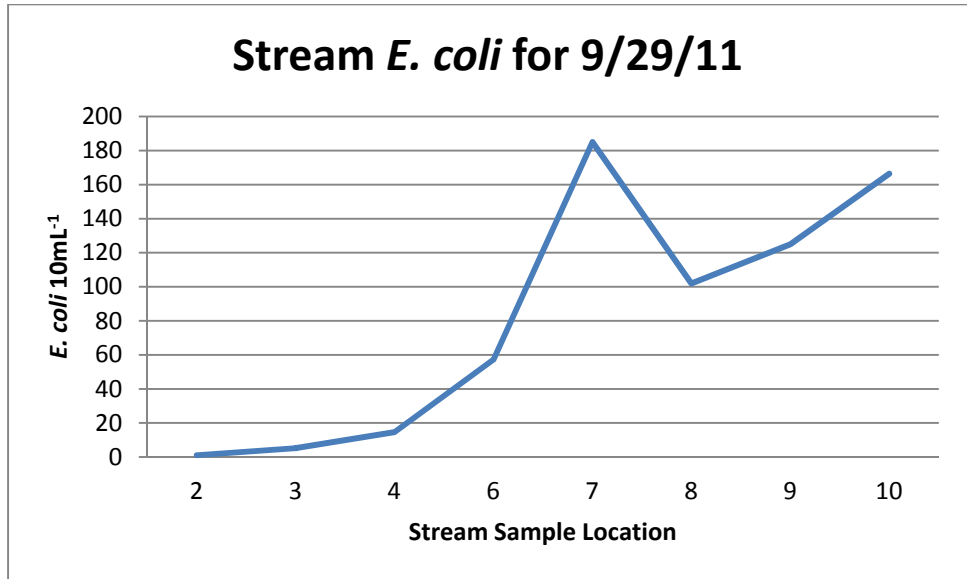


Appendix 1.V.

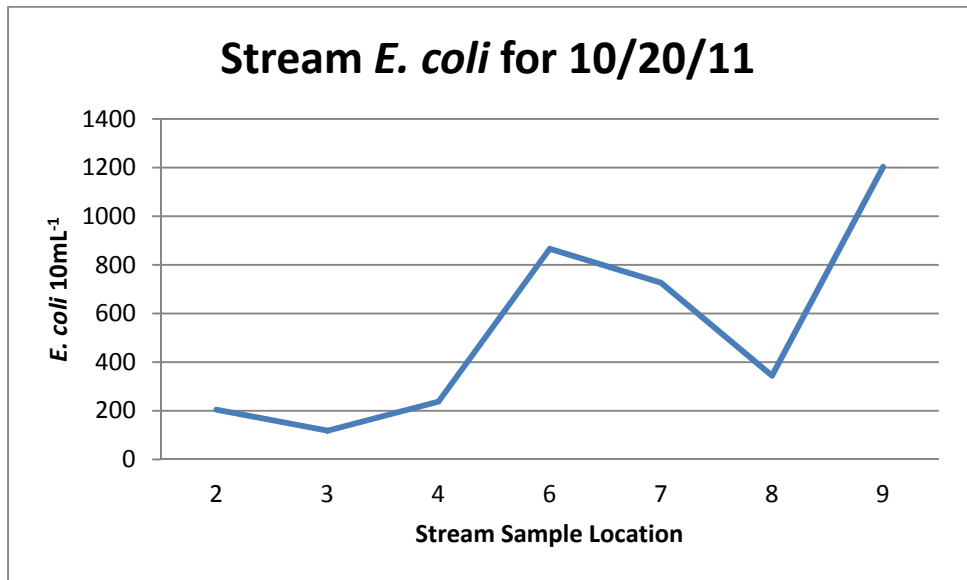
Stream *E. coli* populations over sequence of stream sample locations for 8/24/2011



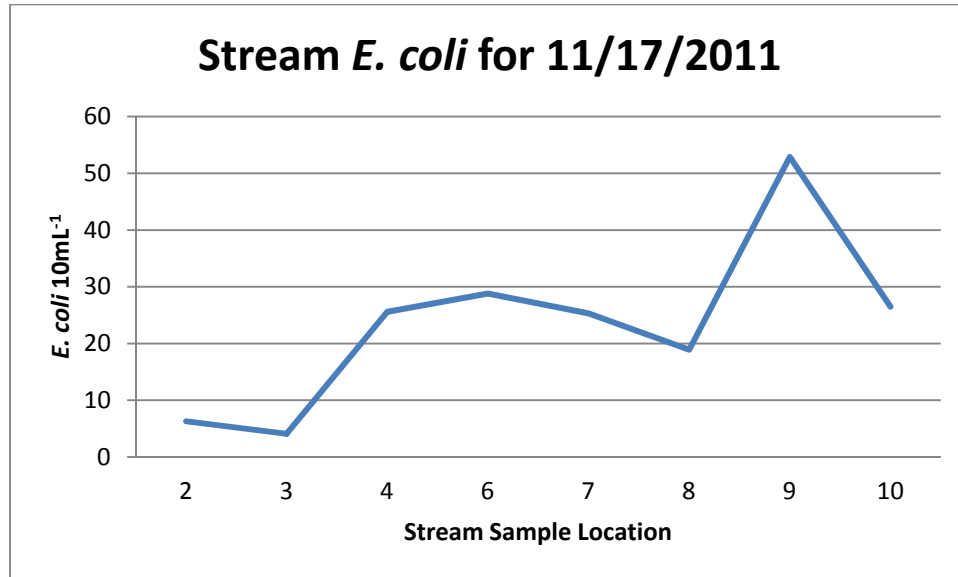
Appendix 1.W. Stream *E. coli* populations over sequence of stream sample locations for 9/29/2011



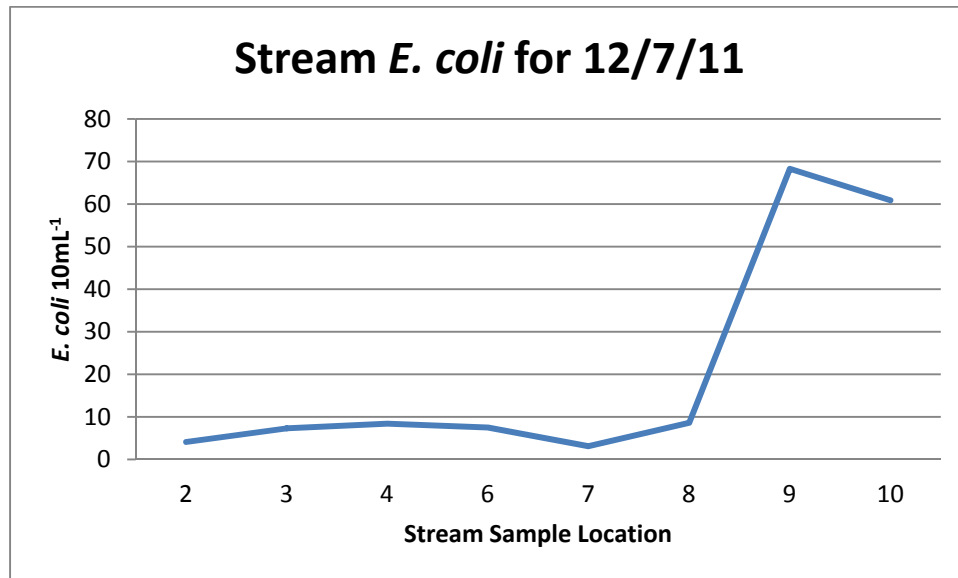
Appendix 1.X. Stream *E. coli* populations over sequence of stream sample locations for 10/20/2011



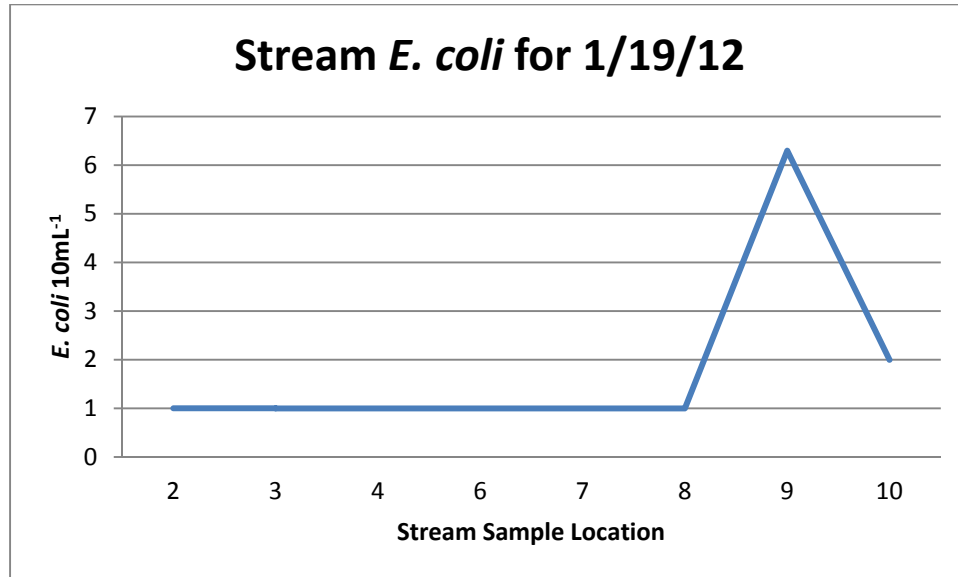
Appendix 1.Y. Stream *E. coli* populations over sequence of stream sample locations for 11/17/2011



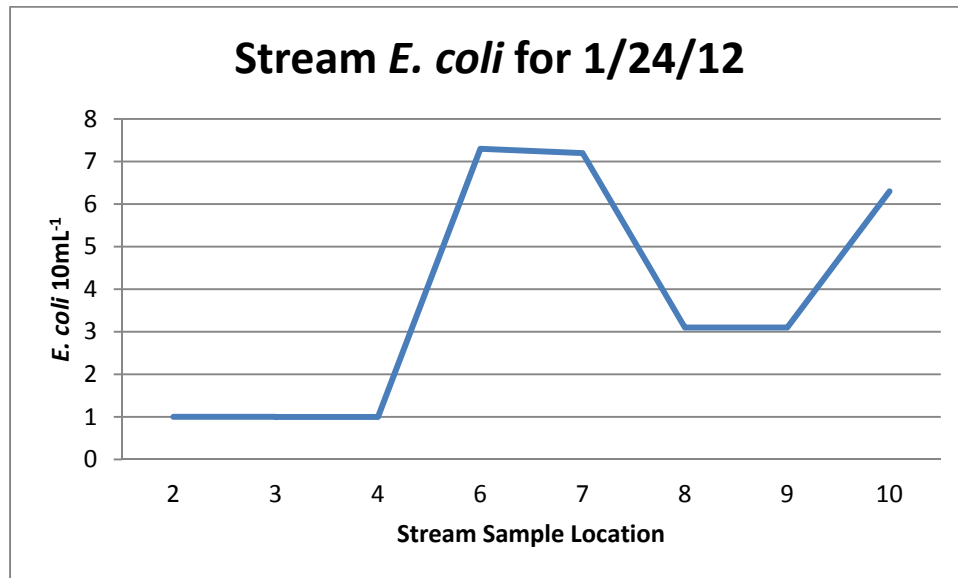
Appendix 1.Z. Stream *E. coli* populations over sequence of stream sample locations for 12/7/2011



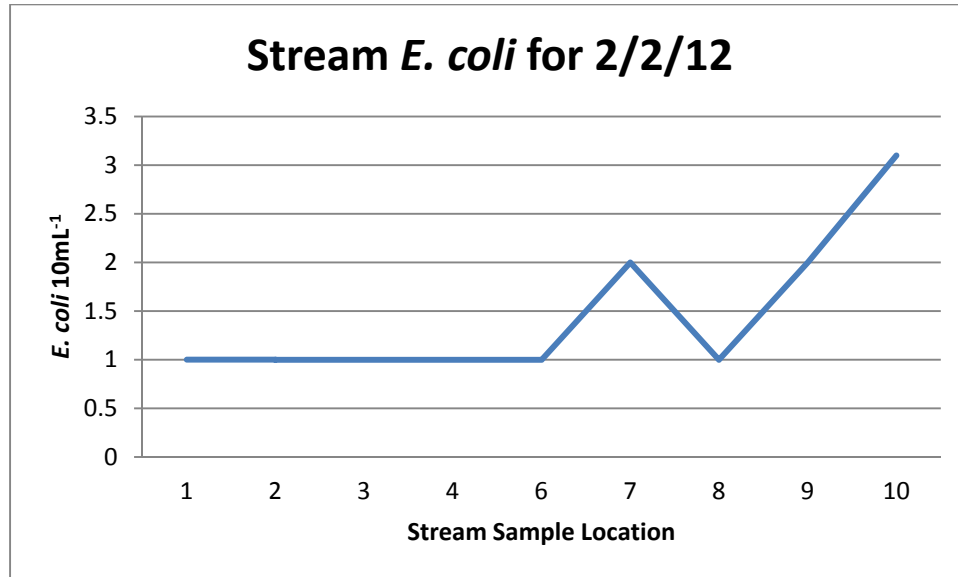
Appendix 1.AA. Stream *E. coli* populations over sequence of stream sample locations for 1/19/2012



Appendix 1.BB. Stream *E. coli* populations over sequence of stream sample locations for 1/24/2012

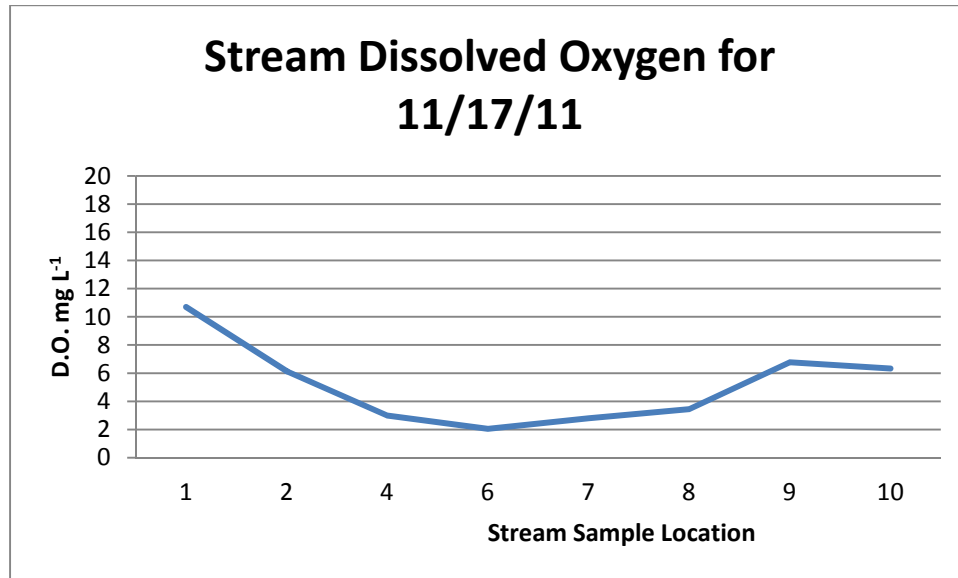


Appendix 1.CC. Stream *E. coli* populations over sequence of stream sample locations for 2/2/2012

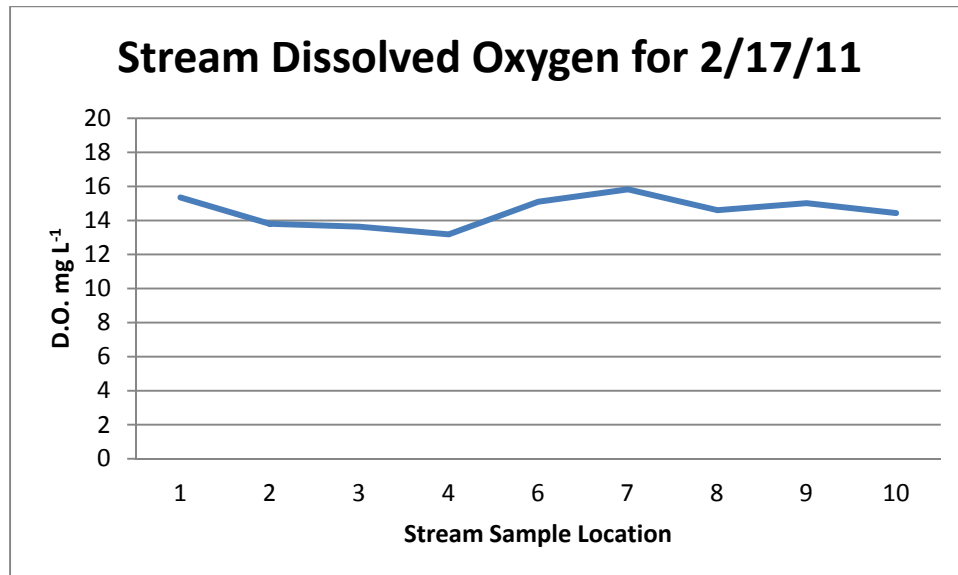


APPENDIX 2. Stream Dissolved Oxygen for Each Sample Date.

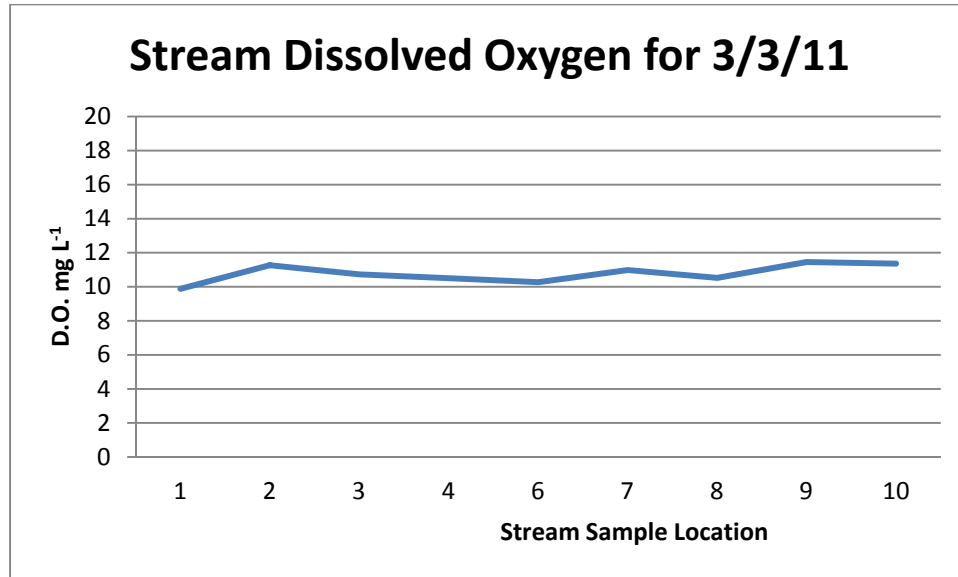
Appendix 2.A. Stream dissolved oxygen levels over sequence of stream sample locations for 11/17/2011



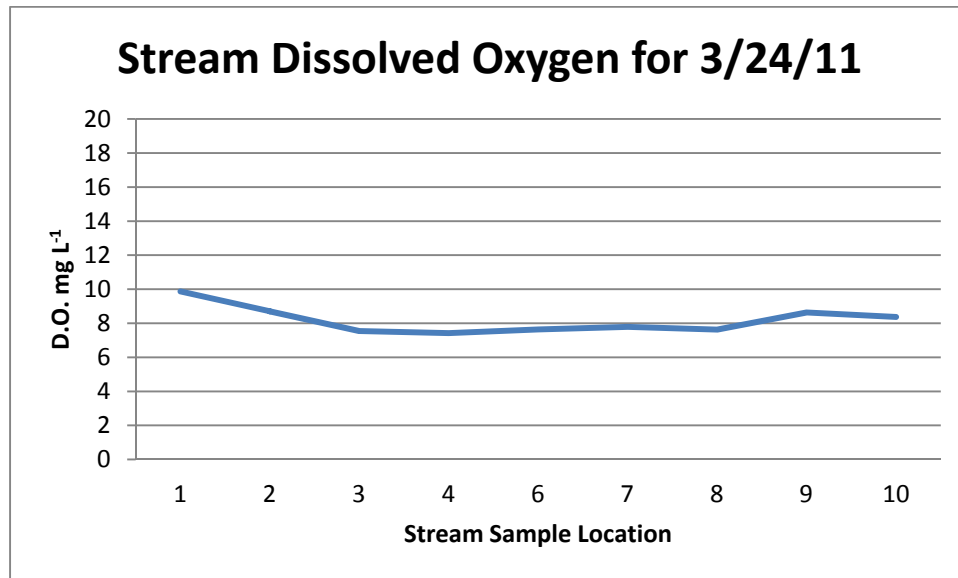
Appendix 2.B. Stream dissolved oxygen levels over sequence of stream sample locations for 2/17/2011



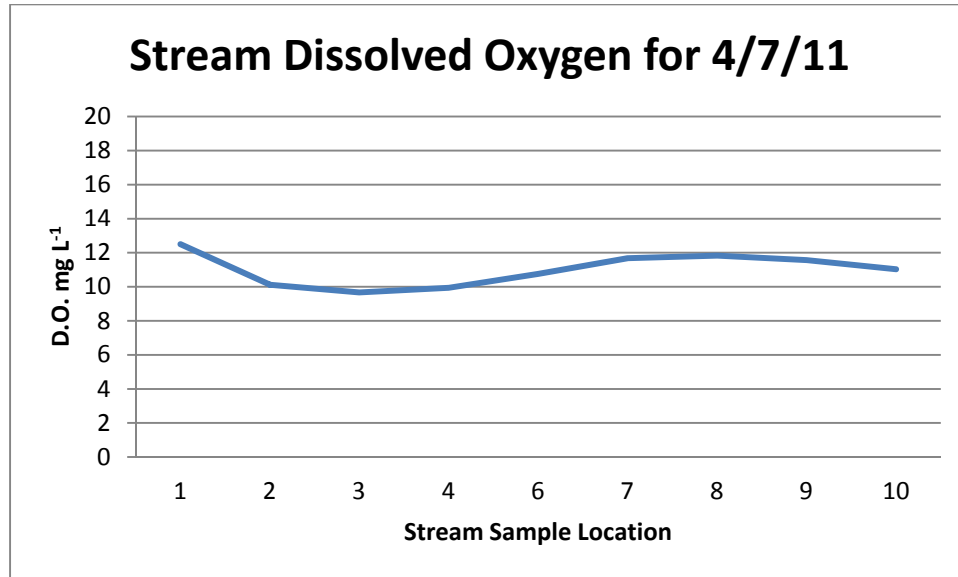
Appendix 2.C. Stream dissolved oxygen levels over sequence of stream sample locations for 3/3/2011



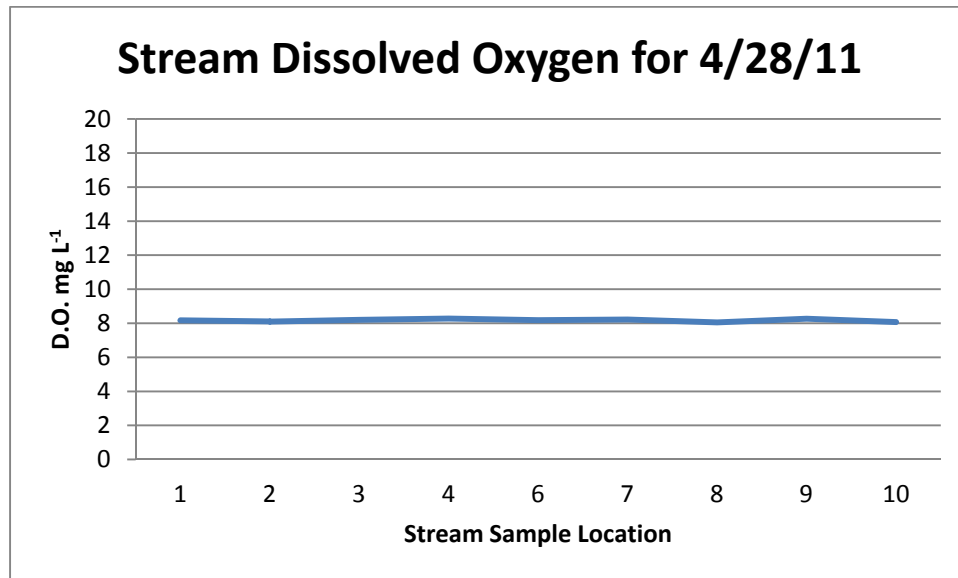
Appendix 2.D. Stream dissolved oxygen levels over sequence of stream sample locations for 3/24/2011



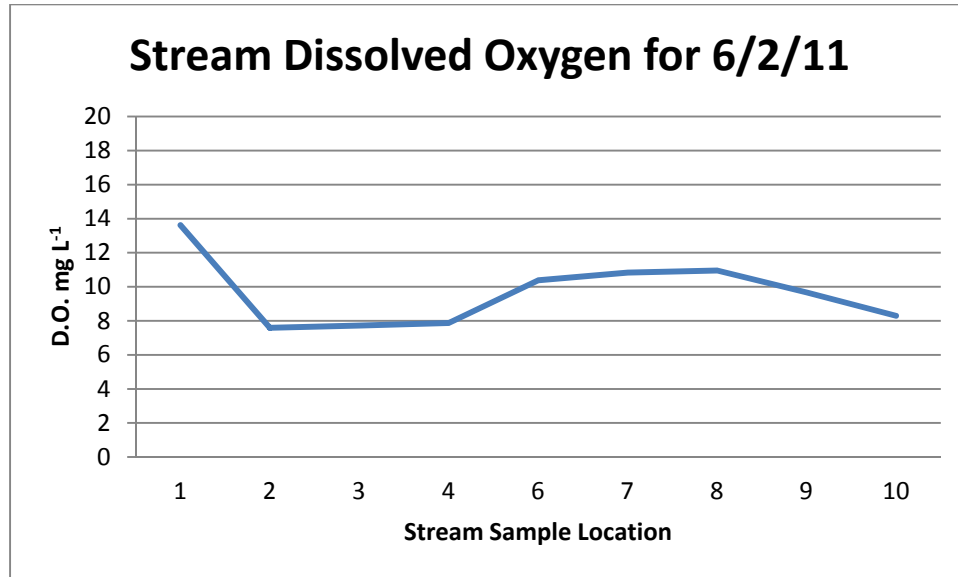
Appendix 2.E. Stream dissolved oxygen levels over sequence of stream sample locations for 4/7/2011



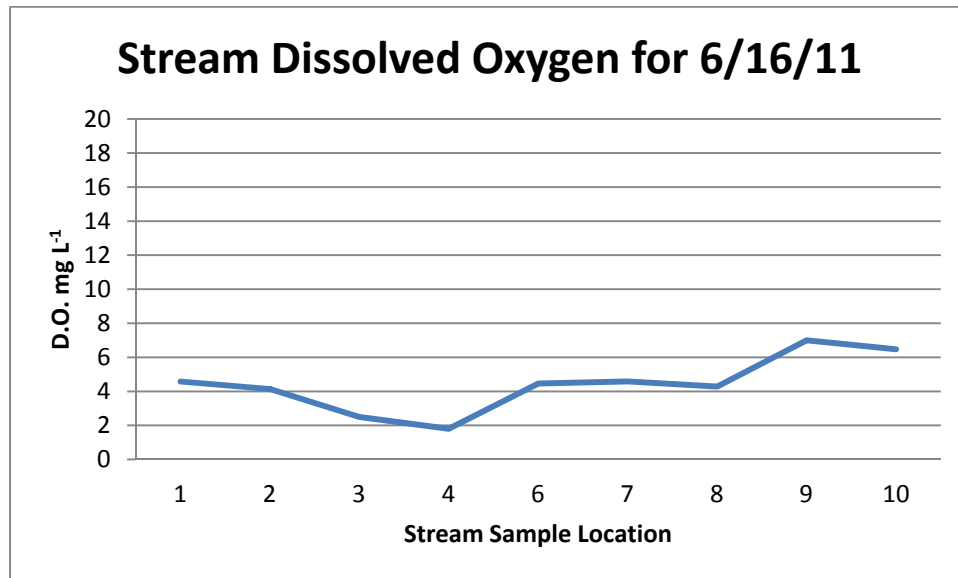
Appendix 2.F. Stream dissolved oxygen levels over sequence of stream sample locations for 4/28/2011



Appendix 2.G. Stream dissolved oxygen levels over sequence of stream sample locations for 6/2/2011

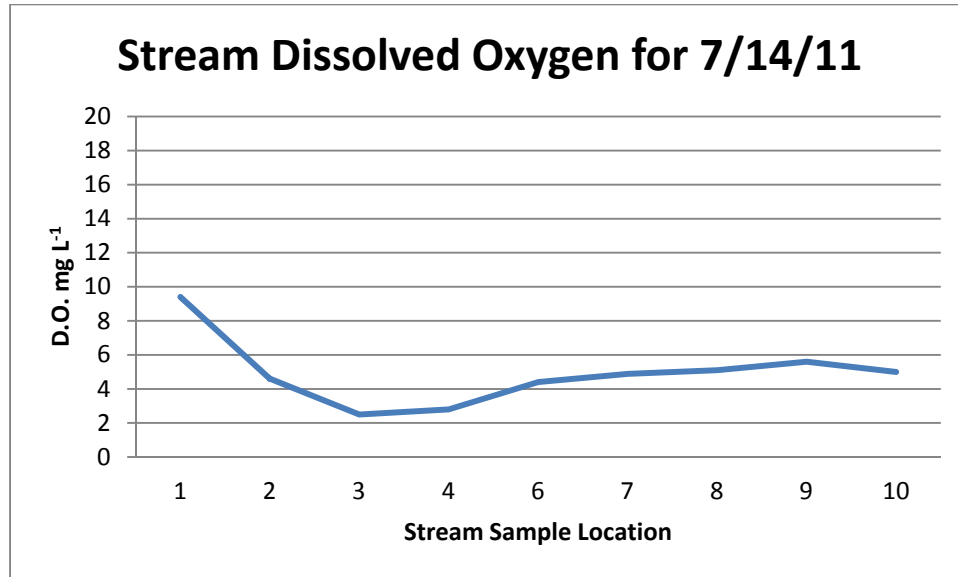


Appendix 2.H. Stream dissolved oxygen levels over sequence of stream sample locations for 6/16/2011



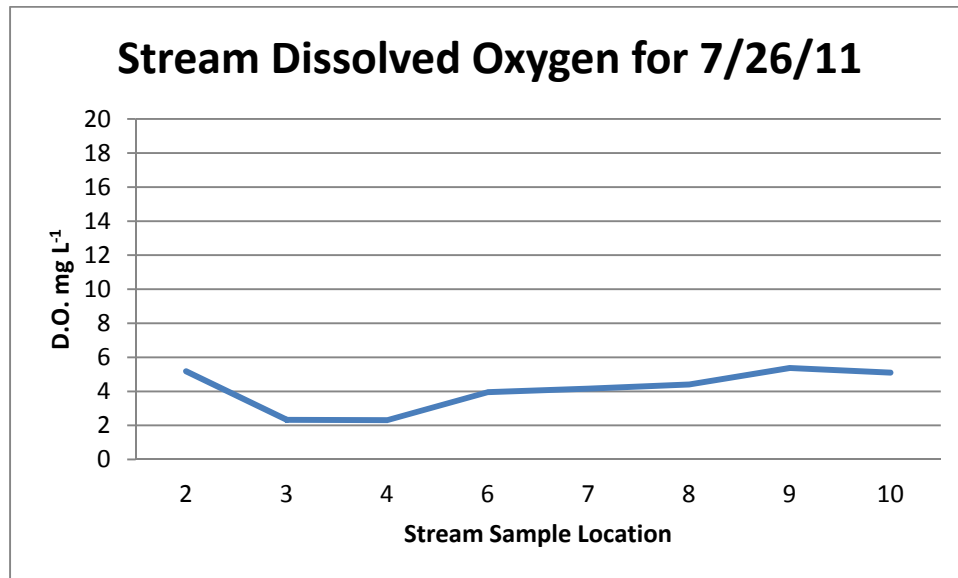
Appendix 2.I.

Stream dissolved oxygen levels over sequence of stream sample locations for 7/14/2011

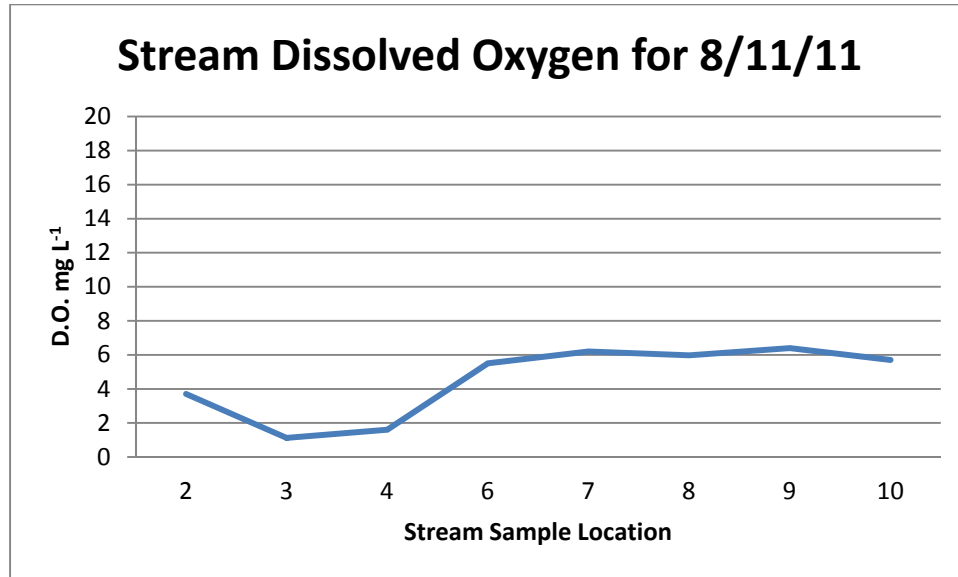


Appendix 2.J.

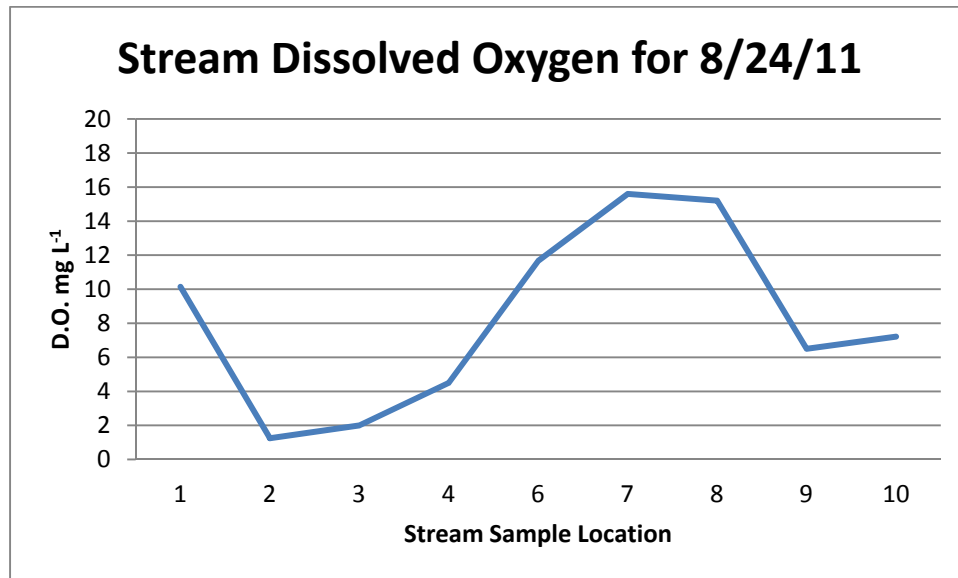
Stream dissolved oxygen levels over sequence of stream sample locations for 7/26/2011



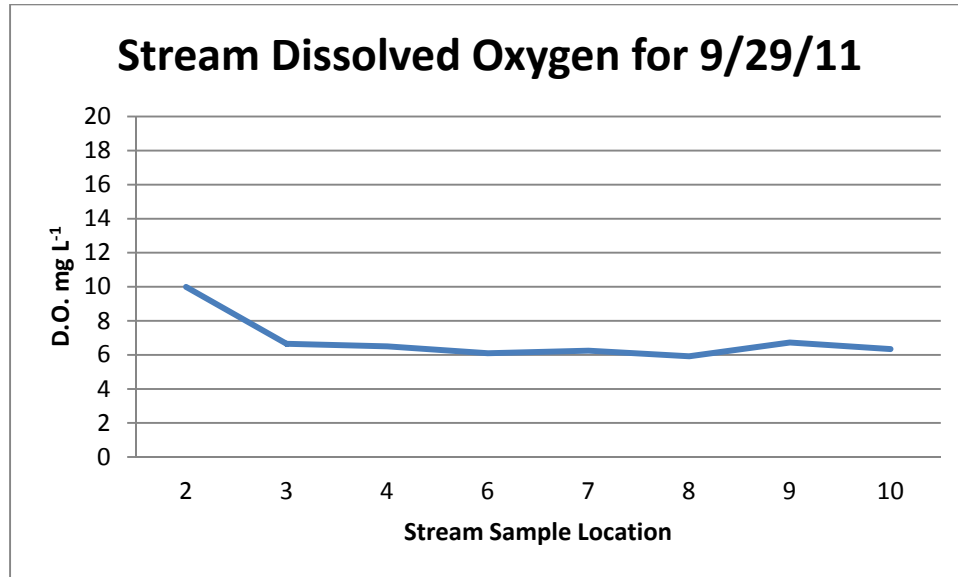
Appendix 2.K. Stream dissolved oxygen levels over sequence of stream sample locations for 8/11/2011



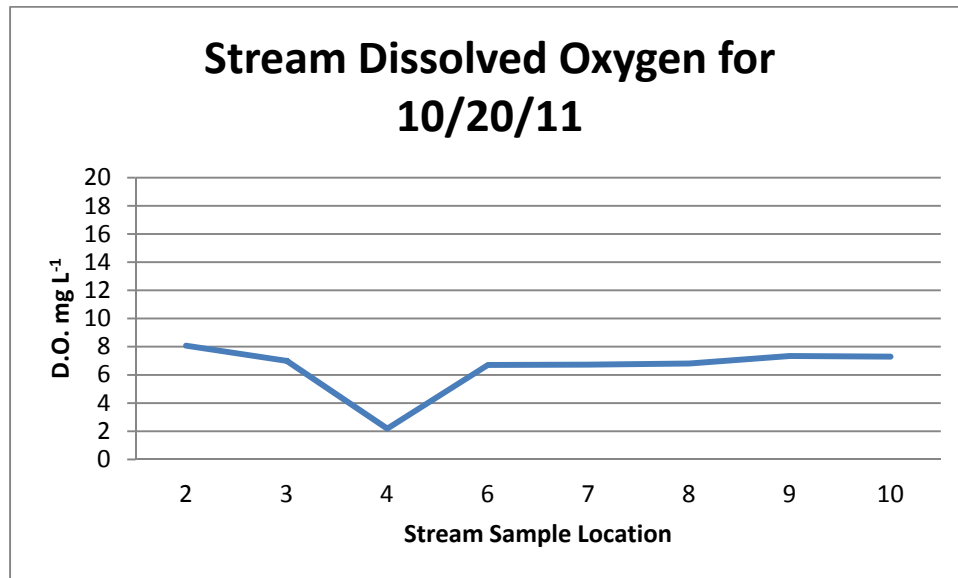
Appendix 2.L. Stream dissolved oxygen levels over sequence of stream sample locations for 8/24/2011



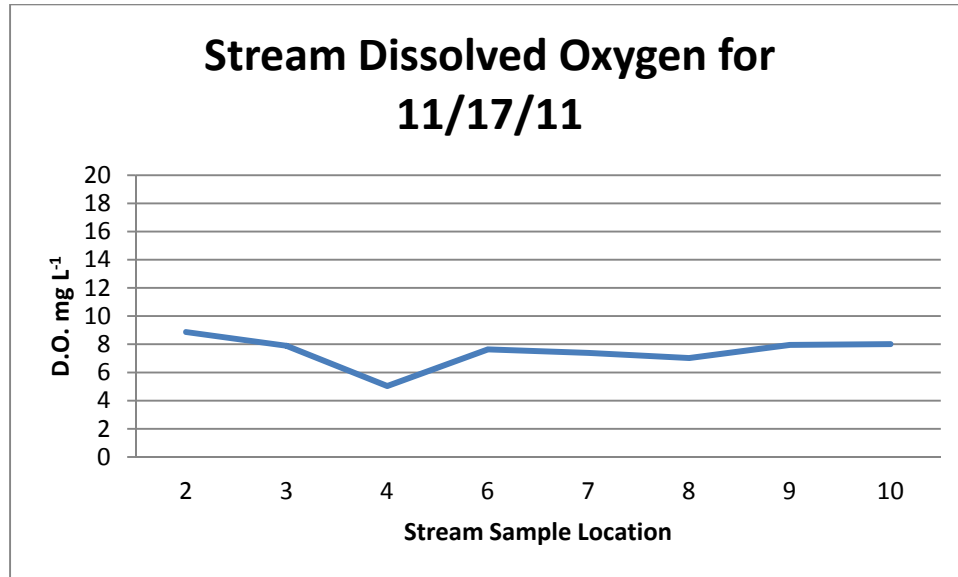
Appendix 2.M. Stream dissolved oxygen levels over sequence of stream sample locations for 9/29/2011



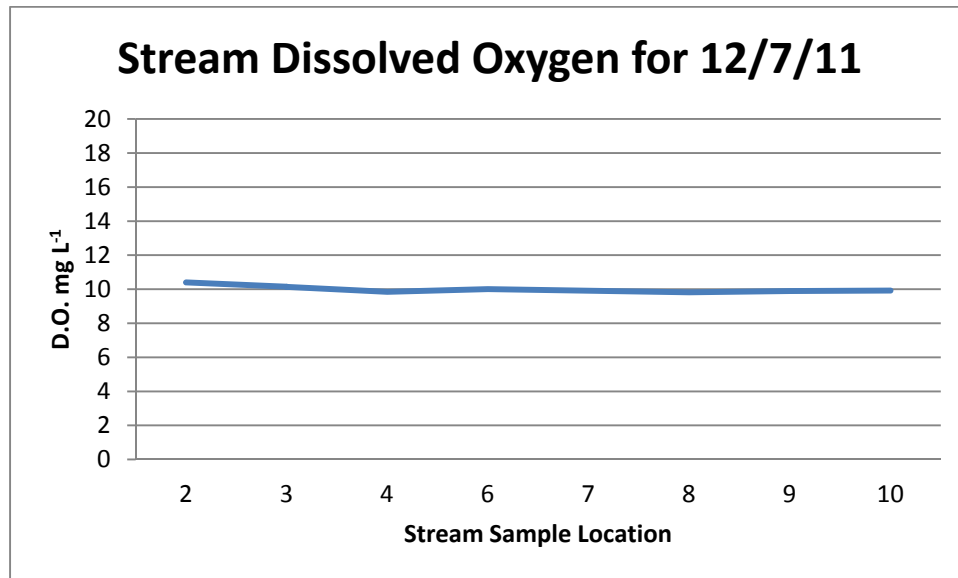
Appendix 2.N. Stream dissolved oxygen levels over sequence of stream sample locations for 10/20/2011



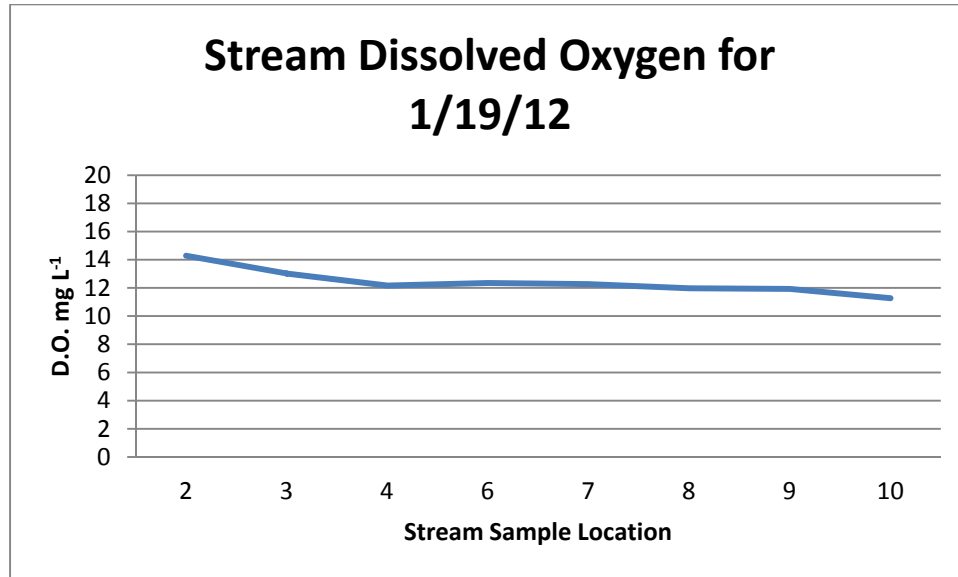
Appendix 2.O. Stream dissolved oxygen levels over sequence of stream sample locations for 11/17/2011



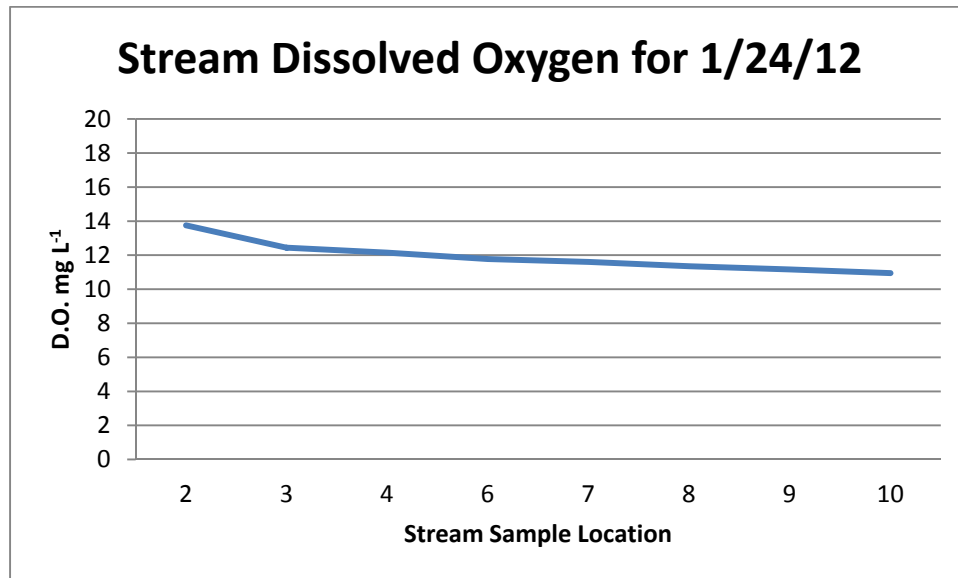
Appendix 2.P. Stream dissolved oxygen levels over sequence of stream sample locations for 12/7/2011



Appendix 2.Q. Stream dissolved oxygen levels over sequence of stream sample locations for 1/19/2012

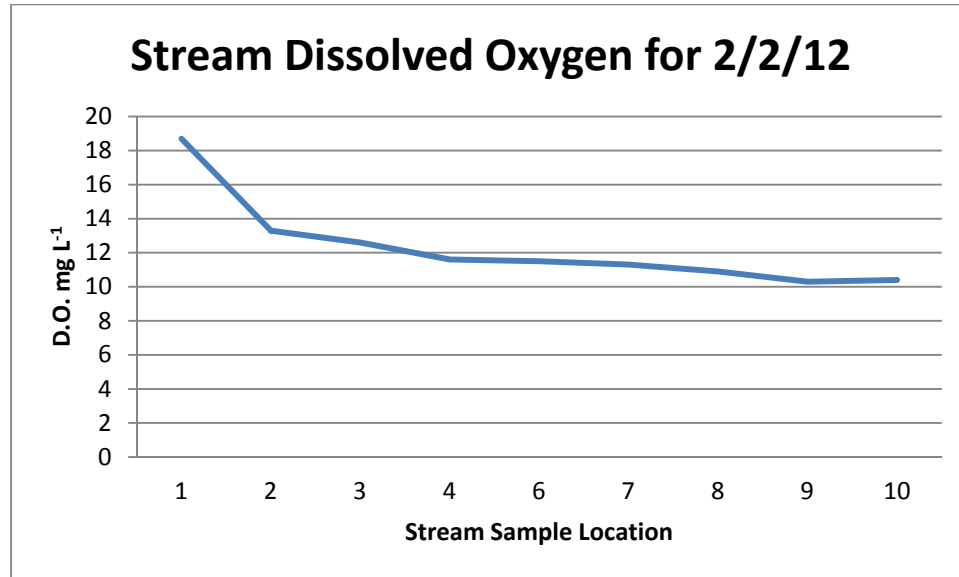


Appendix 2.R. Stream dissolved oxygen levels over sequence of stream sample locations for 1/24/2012



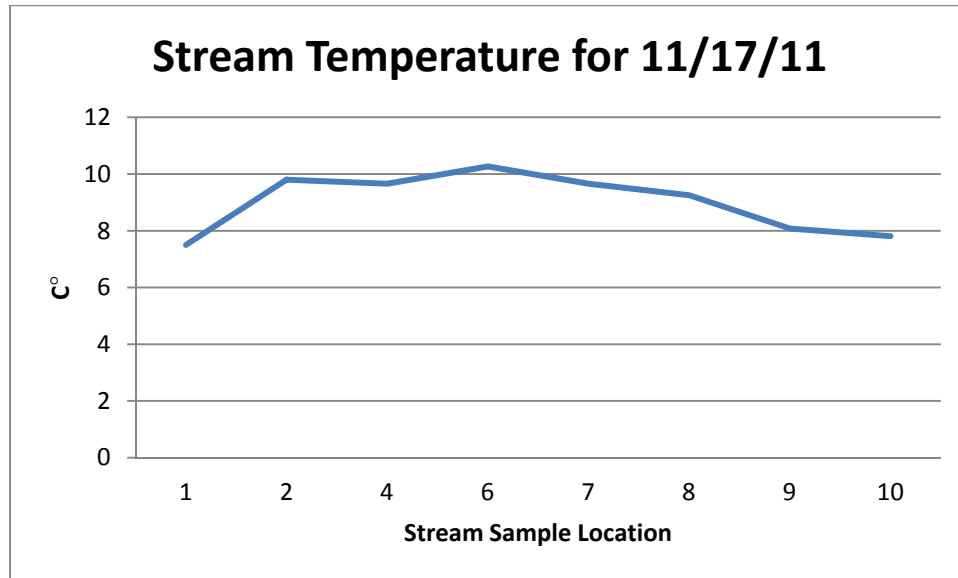
Appendix 2.S.

Stream dissolved oxygen levels over sequence of stream sample locations for 2/2/2012

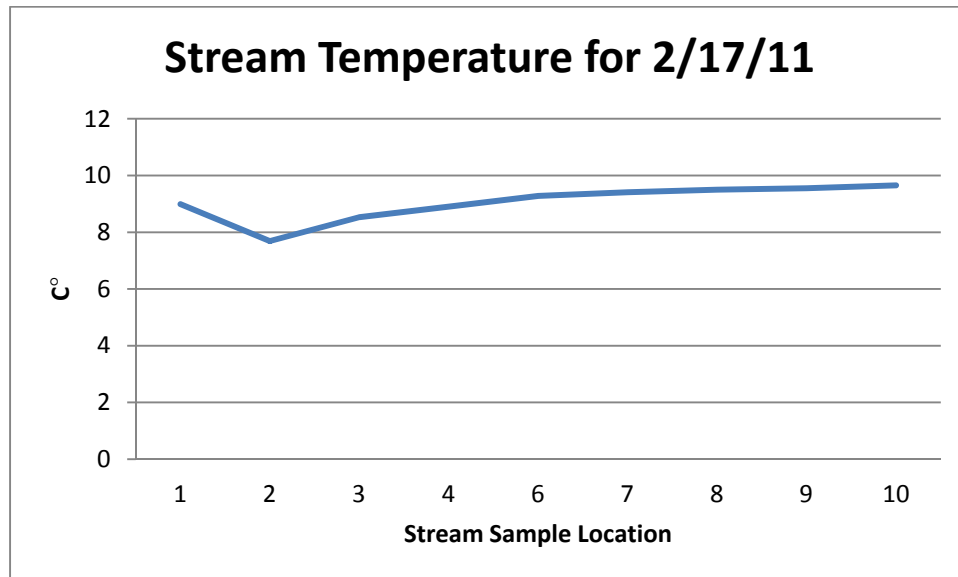


APPENDIX 3. Stream Temperature for Each Sample Date

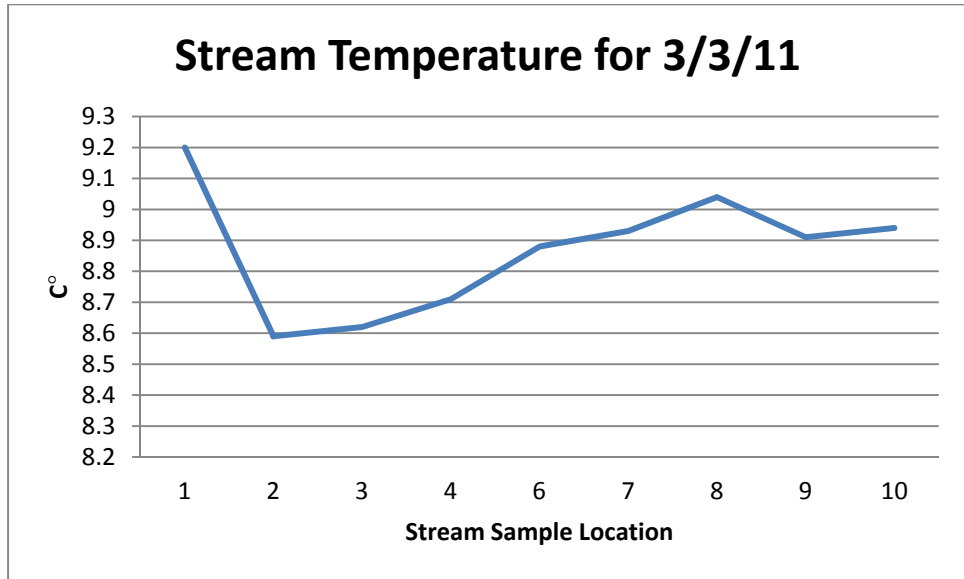
Appendix 3.A. Stream temperature over sequence of stream sample locations for 11/17/2010



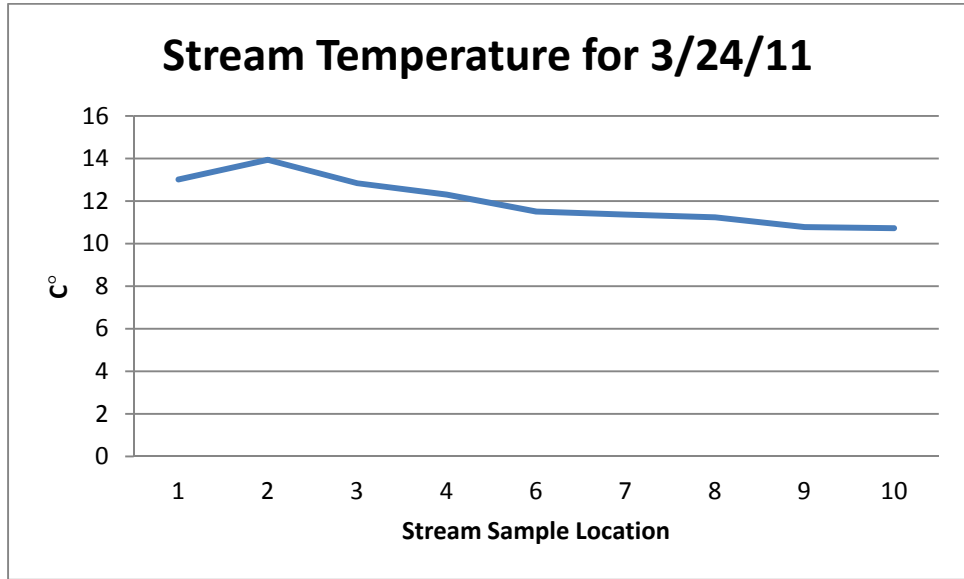
Appendix 3.B. Stream temperature over sequence of stream sample locations for 2/17/2011



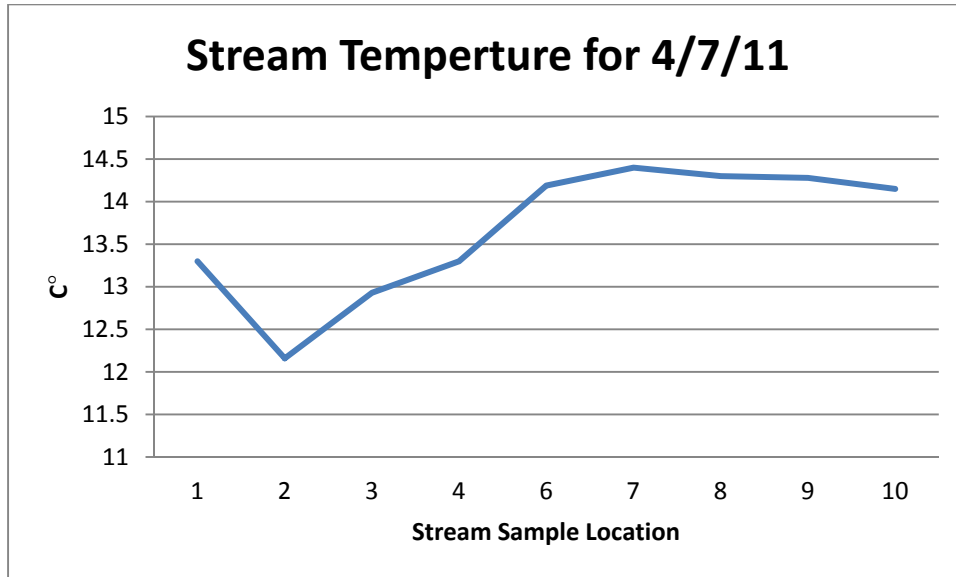
Appendix 3.C. Stream temperature over sequence of stream sample locations for 3/3/2011



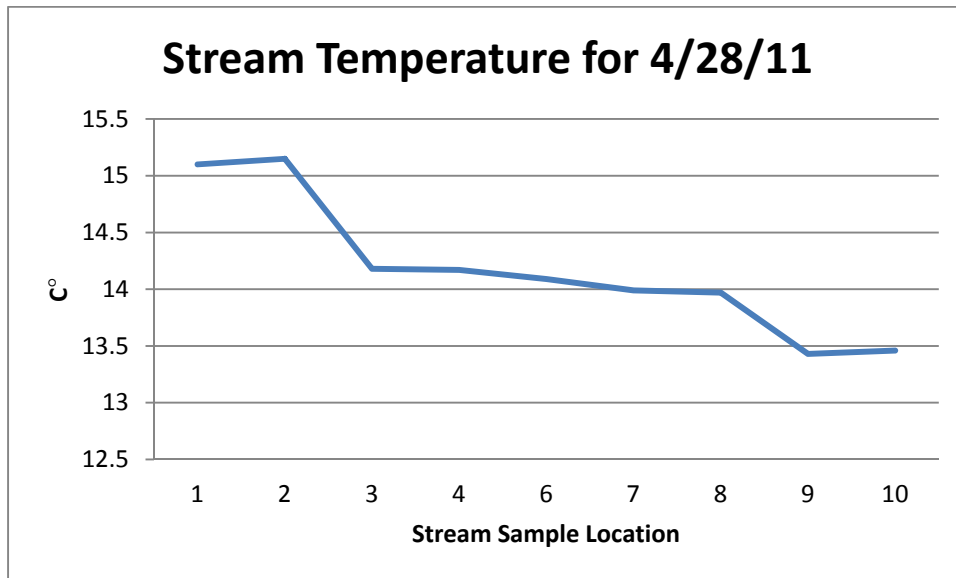
Appendix 3.D. Stream temperature over sequence of stream sample locations for 3/24/2011



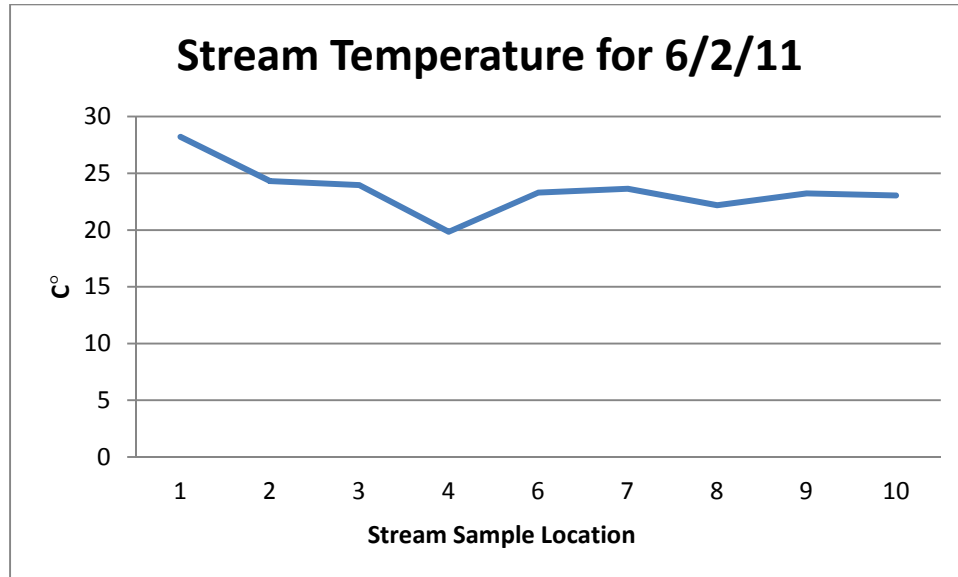
Appendix 3.E. Stream temperature over sequence of stream sample locations for 4/7/2011



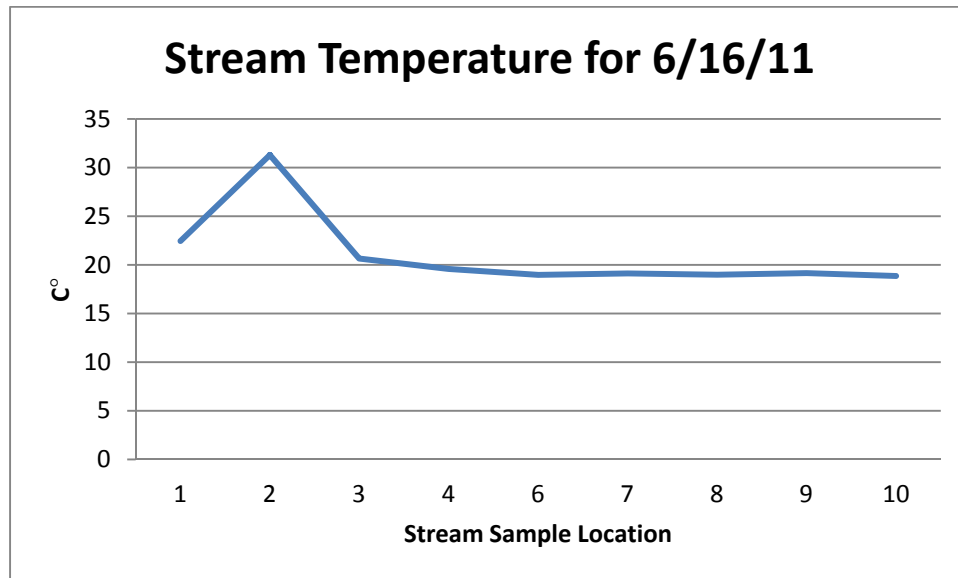
Appendix 3.F. Stream temperature over sequence of stream sample locations for 4/28/2011



Appendix 3.G. Stream temperature over sequence of stream sample locations for 6/2/2011

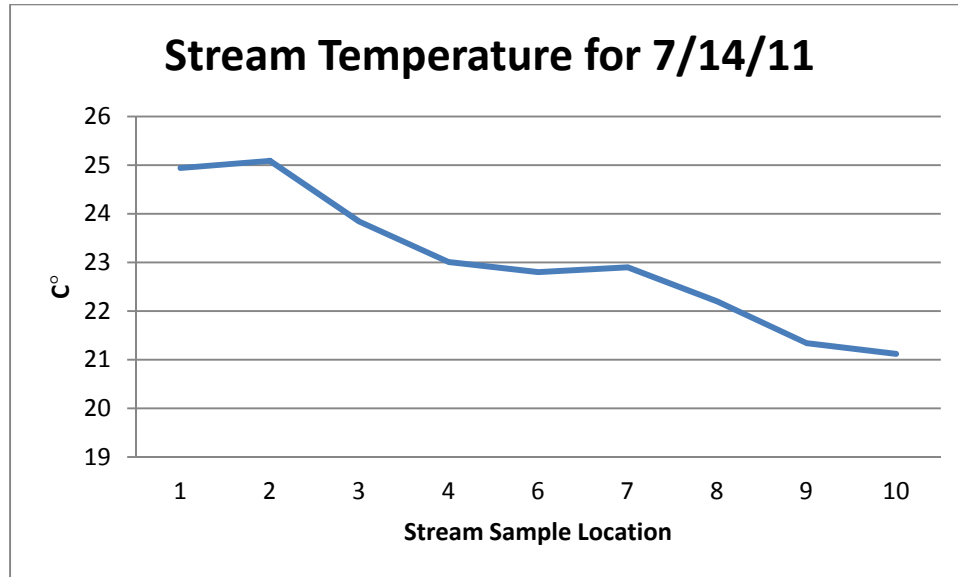


Appendix 3.H. Stream temperature over sequence of stream sample locations for 6/16/2011



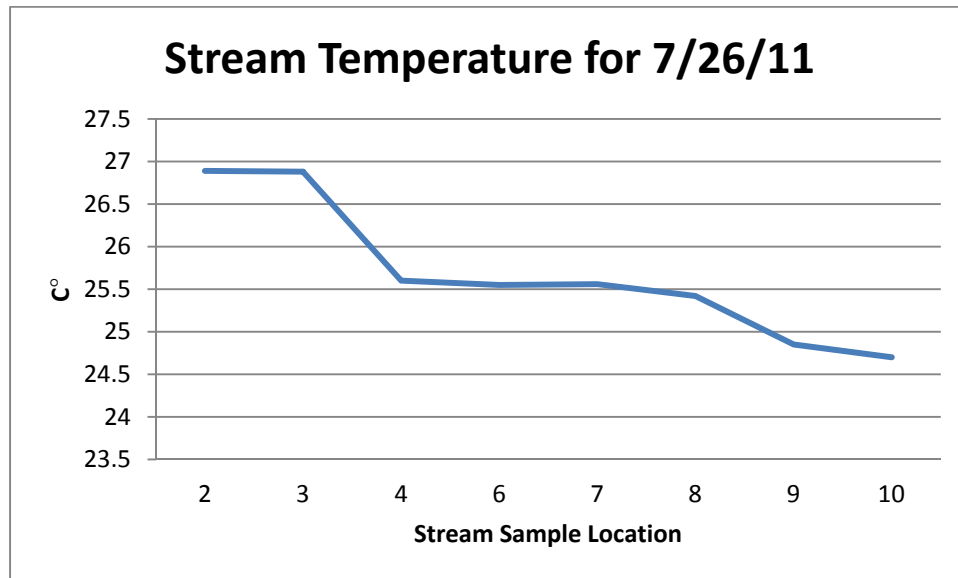
Appendix 3.I.

Stream temperature over sequence of stream sample locations for 7/14/2011

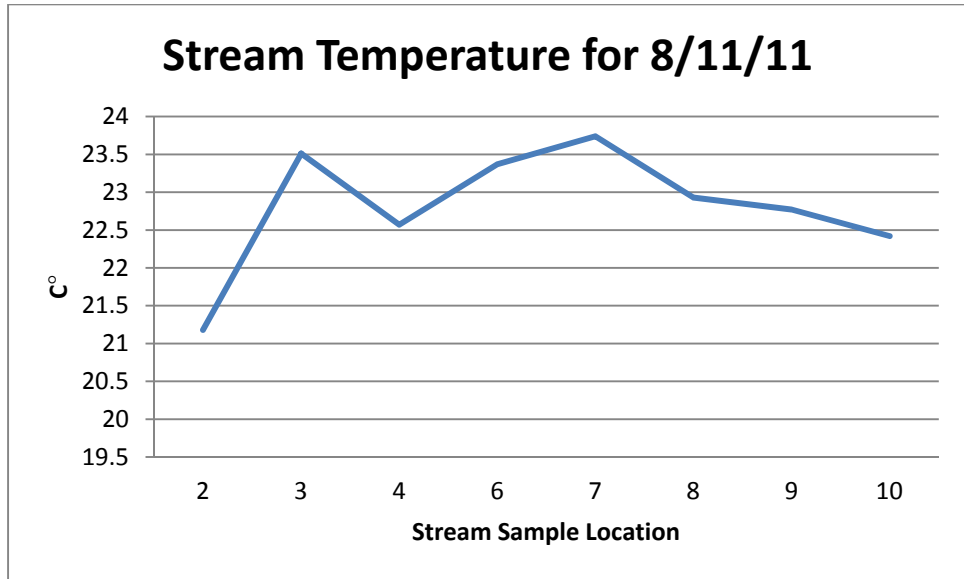


Appendix 3.J.

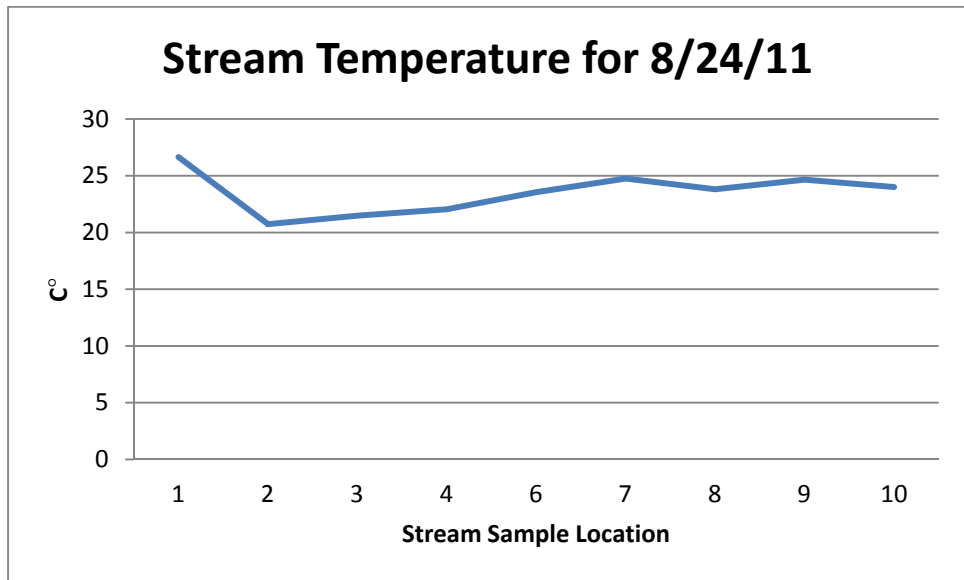
Stream temperature over sequence of stream sample locations for 7/26/2011



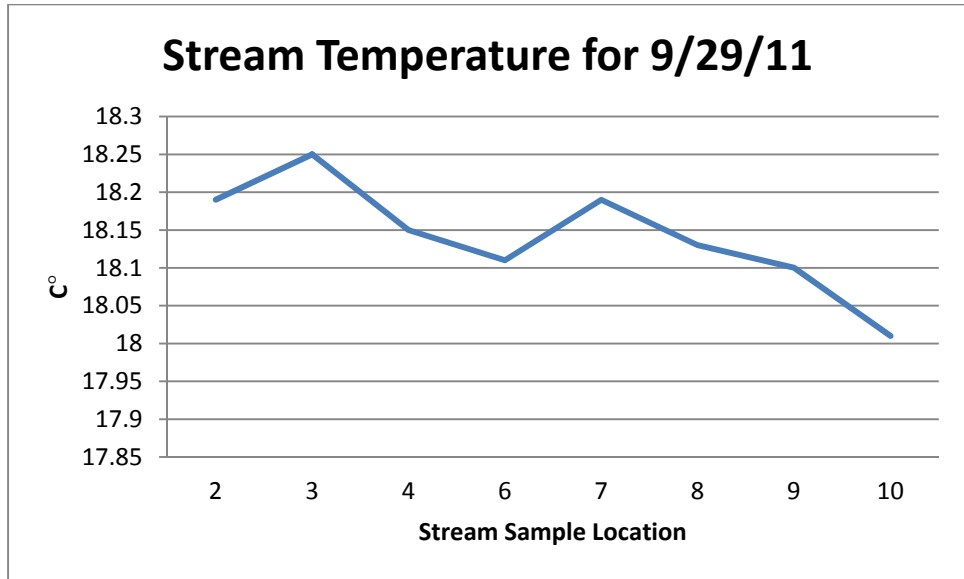
Appendix 3.K. Stream temperature over sequence of stream sample locations for 8/11/2011



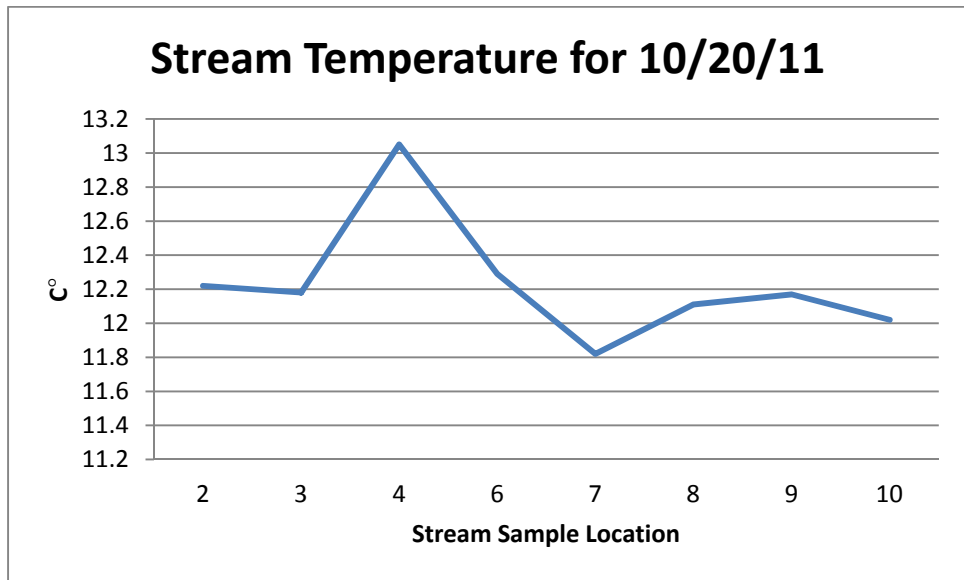
Appendix 3.L. Stream temperature over sequence of stream sample locations for 8/24/2011



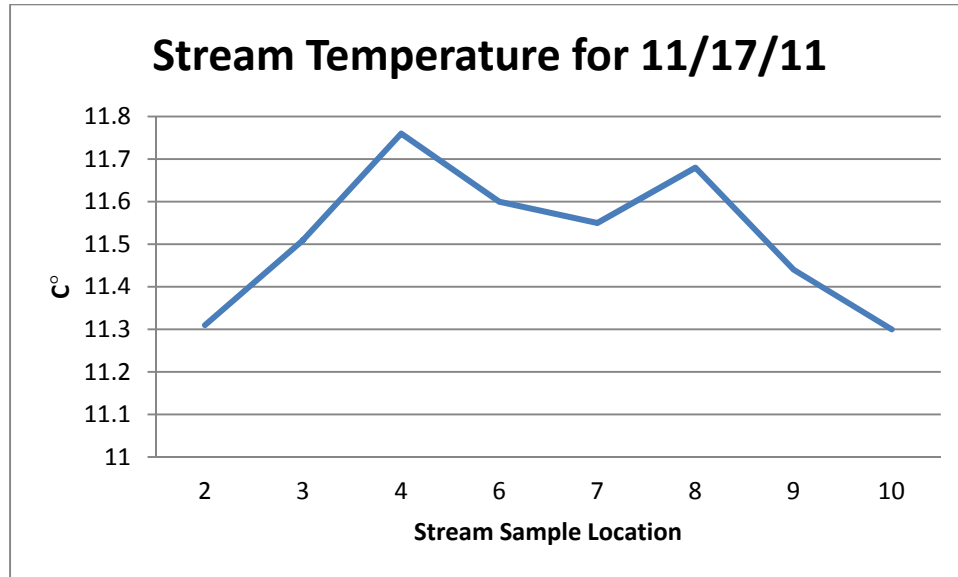
Appendix 3.M. Stream temperature over sequence of stream sample locations for 9/29/2011



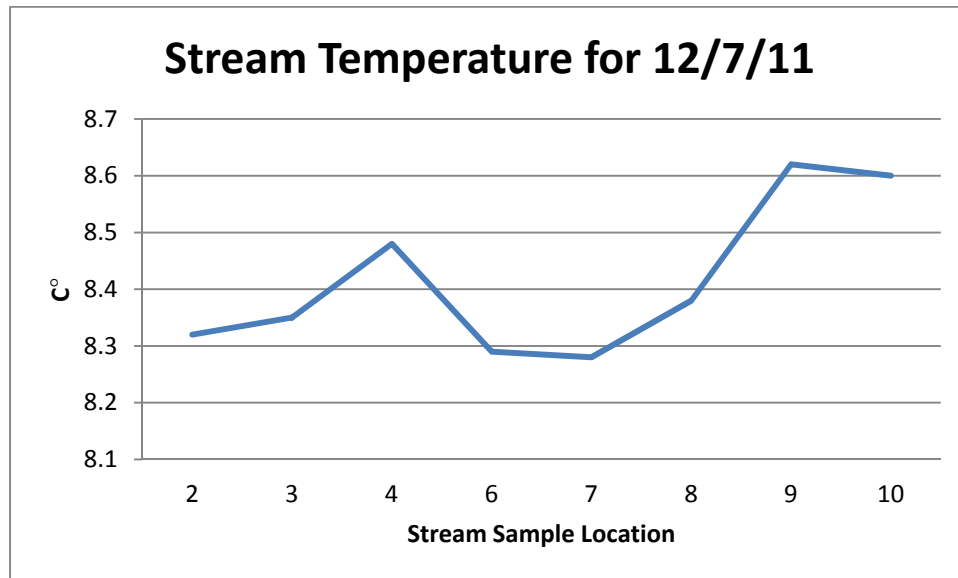
Appendix 3.N. Stream temperature over sequence of stream sample locations for 10/20/2011



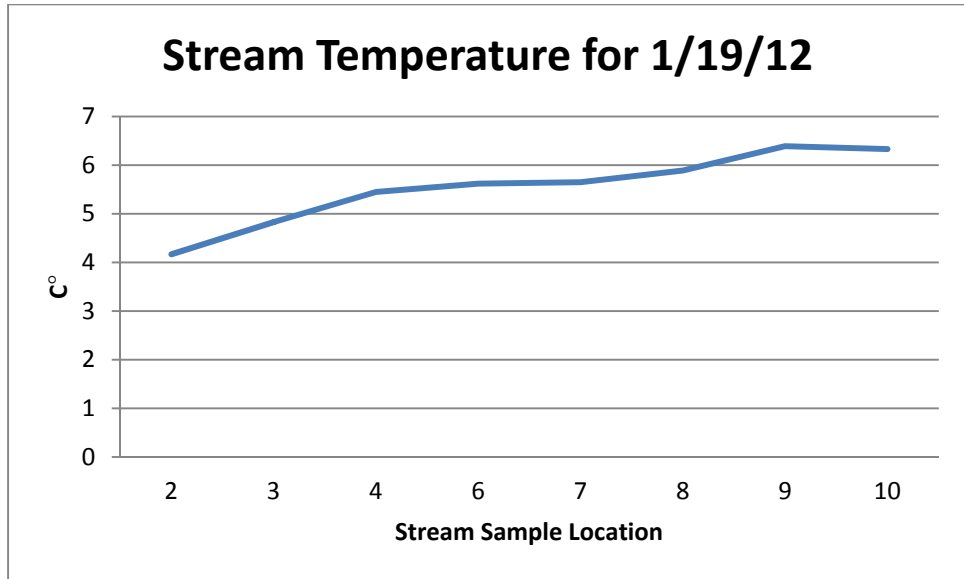
Appendix 3.O. Stream temperature over sequence of stream sample locations for 11/17/2011



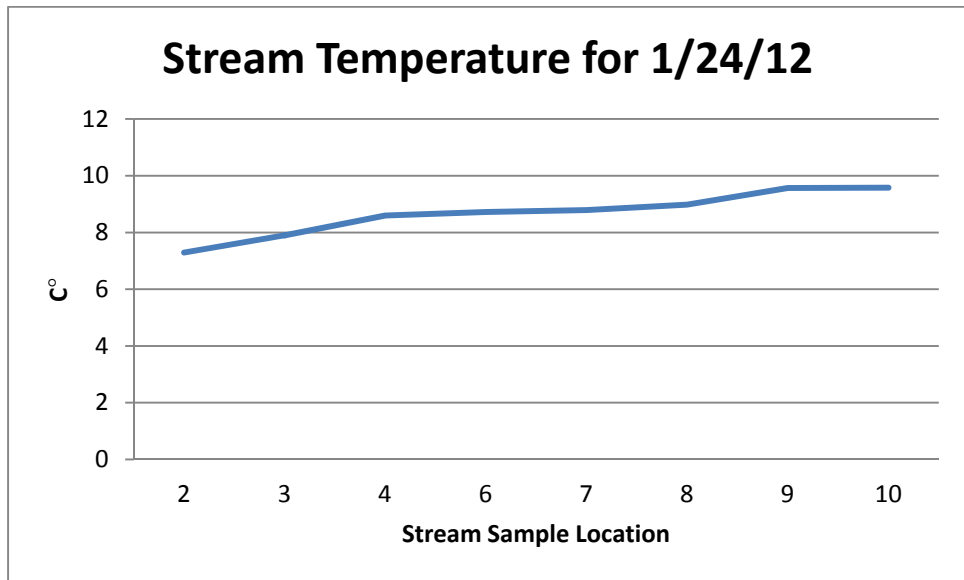
Appendix 3.P. Stream temperature over sequence of stream sample locations for 12/7/2011



Appendix 3.Q. Stream temperature over sequence of stream sample locations for 1/19/2012

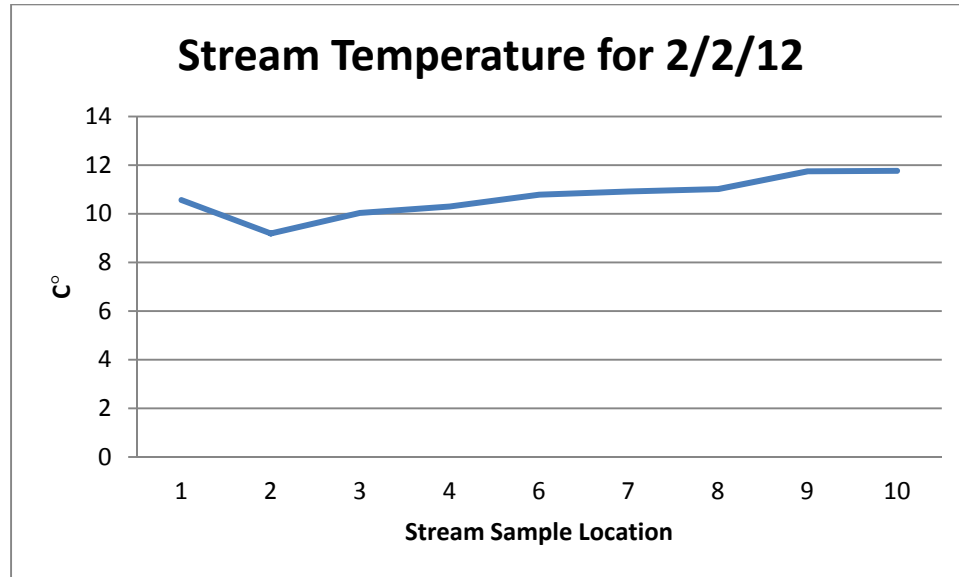


Appendix 3.R. Stream temperature over sequence of stream sample locations for 1/24/2012



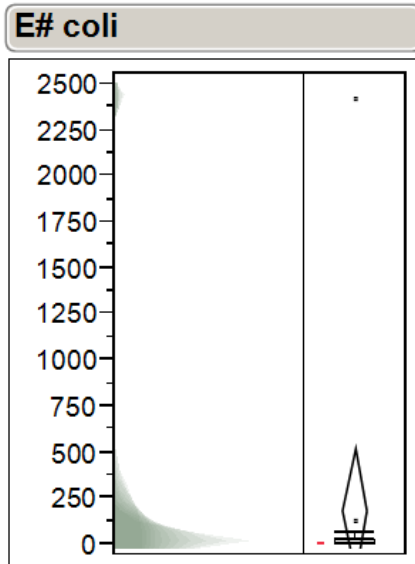
Appendix 3.S.

Stream temperature over sequence of stream sample locations for 2/2/2012



APPENDIX 4. Box and Whisker Plots for *E. coli* Before and After June 2011 for Each Stream Sample Location.

Appendix 4.A. Sample Location 2 *E. coli* Before (left) and After (right) June 2011

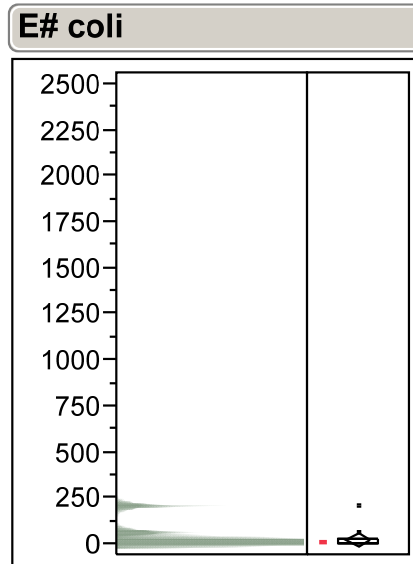


Quantiles

100.0% maximum	2419.6
99.5%	2419.6
97.5%	2419.6
90.0%	1037.98
75.0% quartile	27.9
50.0% median	3.1
25.0% quartile	1
10.0%	1
2.5%	1
0.5%	1
0.0% minimum	1

Moments

Mean	178.19333
Std Dev	620.85657
Std Err Mean	160.30448
Upper 95% Mean	522.01224
Lower 95% Mean	-165.6256
N	15

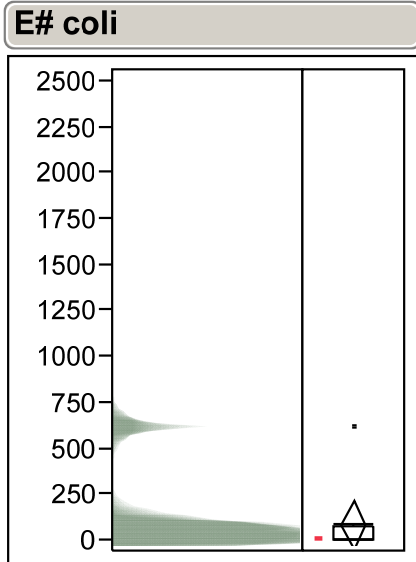


Quantiles

100.0% maximum	204.6
99.5%	204.6
97.5%	204.6
90.0%	130.55
75.0% quartile	18.45
50.0% median	5.75
25.0% quartile	1
10.0%	1
2.5%	1
0.5%	1
0.0% minimum	1

Moments

Mean	24.371429
Std Dev	53.864965
Std Err Mean	14.396018
Upper 95% Mean	55.472134
Lower 95% Mean	-6.729277
N	14

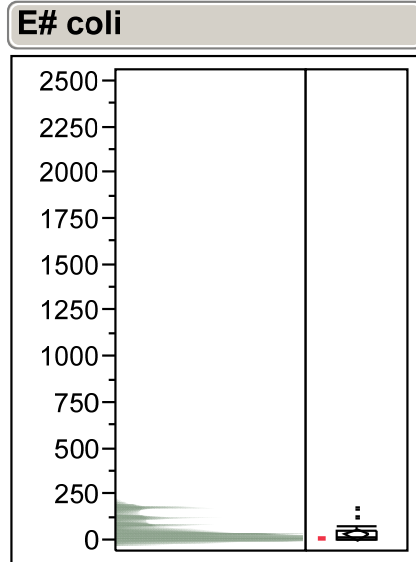


Quantiles

100.0%	maximum	613.1
99.5%		613.1
97.5%		613.1
90.0%		560.18
75.0%	quartile	73.475
50.0%	median	4.65
25.0%	quartile	1
10.0%		1
2.5%		1
0.5%		1
0.0%	minimum	1

Moments

Mean	83.89
Std Dev	188.84542
Std Err Mean	59.718167
Upper 95% Mean	218.98188
Lower 95% Mean	-51.20188
N	10

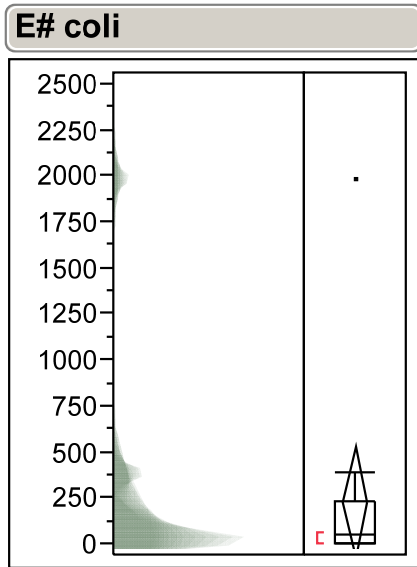


Quantiles

100.0%	maximum	170.5
99.5%		170.5
97.5%		170.5
90.0%		144.35
75.0%	quartile	45.225
50.0%	median	6.25
25.0%	quartile	2.5
10.0%		1
2.5%		1
0.5%		1
0.0%	minimum	1

Moments

Mean	34.314286
Std Dev	52.011979
Std Err Mean	13.900786
Upper 95% Mean	64.345108
Lower 95% Mean	4.2834633
N	14

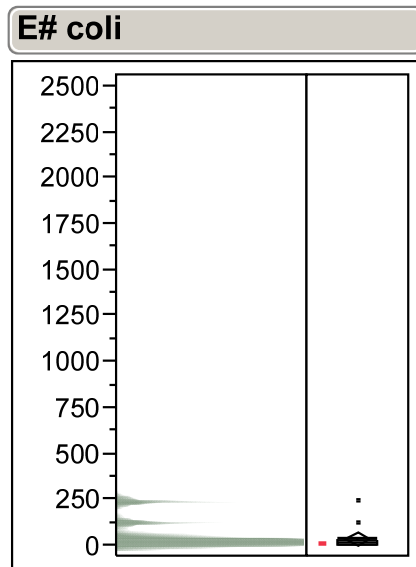


Quantiles

100.0%	maximum	1986.3
99.5%		1986.3
97.5%		1986.3
90.0%		1186.8
75.0%	quartile	225.525
50.0%	median	51.7
25.0%	quartile	1.75
10.0%		1
2.5%		1
0.5%		1
0.0%	minimum	1

Moments

Mean	229.18571
Std Dev	522.06362
Std Err Mean	139.52737
Upper 95% Mean	530.61628
Lower 95% Mean	-72.24485
N	14



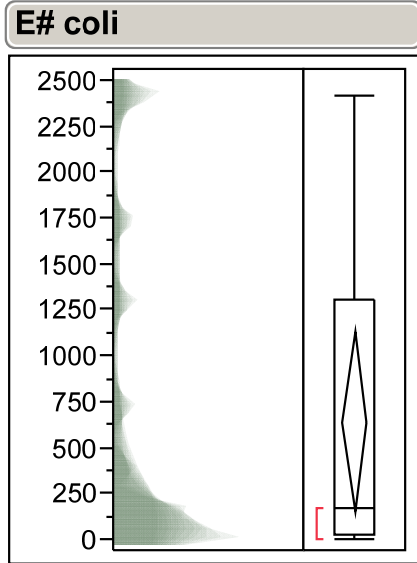
Quantiles

100.0%	maximum	238.2
99.5%		238.2
97.5%		238.2
90.0%		180.9
75.0%	quartile	29.075
50.0%	median	12.7
25.0%	quartile	1
10.0%		1
2.5%		1
0.5%		1
0.0%	minimum	1

Moments

Mean	36.992857
Std Dev	65.950675
Std Err Mean	17.626059
Upper 95% Mean	75.071643
Lower 95% Mean	-1.085929
N	14

2011

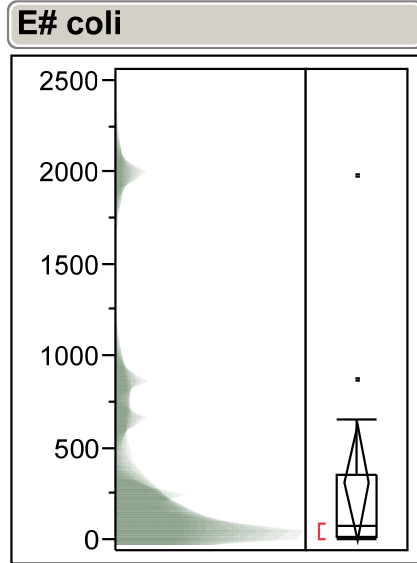


Quantiles

100.0%	maximum	2419.6
99.5%		2419.6
97.5%		2419.6
90.0%		2419.6
75.0%	quartile	1299.7
50.0%	median	170.2
25.0%	quartile	23.8
10.0%		5.74
2.5%		3.1
0.5%		3.1
0.0%	minimum	3.1

Moments

Mean	643.82667
Std Dev	884.74405
Std Err Mean	228.43993
Upper 95% Mean	1133.7816
Lower 95% Mean	153.87174
N	15

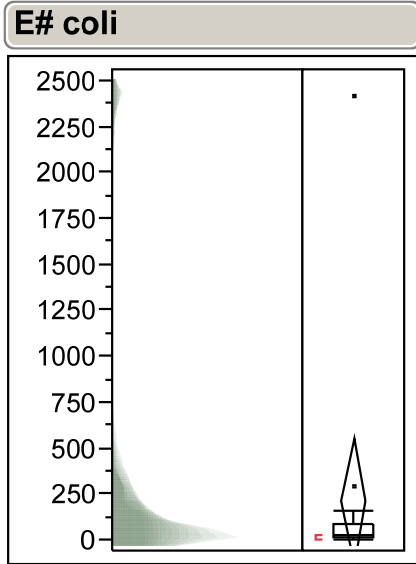


Quantiles

100.0%	maximum	1986.3
99.5%		1986.3
97.5%		1986.3
90.0%		1426.35
75.0%	quartile	348.275
50.0%	median	71.65
25.0%	quartile	7.45
10.0%		1
2.5%		1
0.5%		1
0.0%	minimum	1

Moments

Mean	310.88571
Std Dev	548.81065
Std Err Mean	146.67582
Upper 95% Mean	627.75955
Lower 95% Mean	-5.988121
N	14

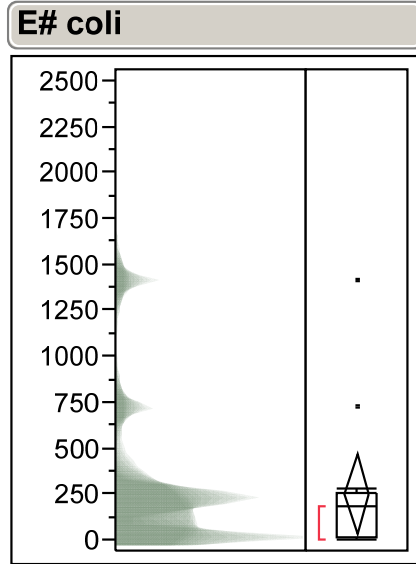


Quantiles

100.0%	maximum	2419.6
99.5%		2419.6
97.5%		2419.6
90.0%		1142.38
75.0%	quartile	81.3
50.0%	median	21.8
25.0%	quartile	6.3
10.0%		2.66
2.5%		2
0.5%		2
0.0%	minimum	2

Moments

Mean	213.21333
Std Dev	615.26076
Std Err Mean	158.85964
Upper 95% Mean	553.93338
Lower 95% Mean	-127.5067
N	15

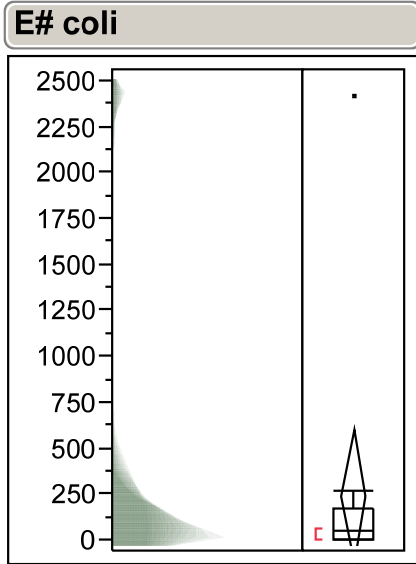


Quantiles

100.0%	maximum	1413.6
99.5%		1413.6
97.5%		1413.6
90.0%		1070.3
75.0%	quartile	255.55
50.0%	median	175.95
25.0%	quartile	6.175
10.0%		1.5
2.5%		1
0.5%		1
0.0%	minimum	1

Moments

Mean	254.30714
Std Dev	385.53778
Std Err Mean	103.0393
Upper 95% Mean	476.91003
Lower 95% Mean	31.704259
N	14

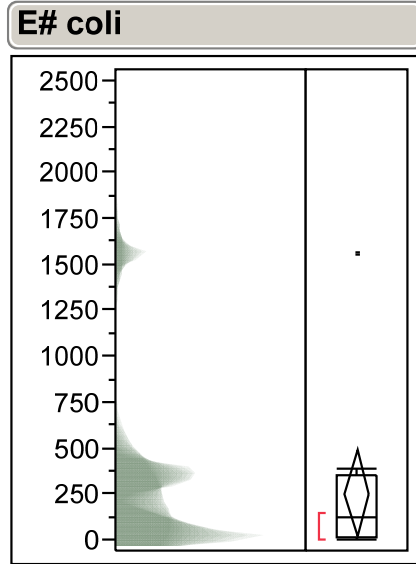


Quantiles

100.0%	maximum	2419.6
99.5%		2419.6
97.5%		2419.6
90.0%		1344.8
75.0%	quartile	165.925
50.0%	median	47.9
25.0%	quartile	3.85
10.0%		1.5
2.5%		1
0.5%		1
0.0%	minimum	1

Moments

Mean	242.25714
Std Dev	632.06612
Std Err Mean	168.92678
Upper 95% Mean	607.20125
Lower 95% Mean	-122.687
N	14

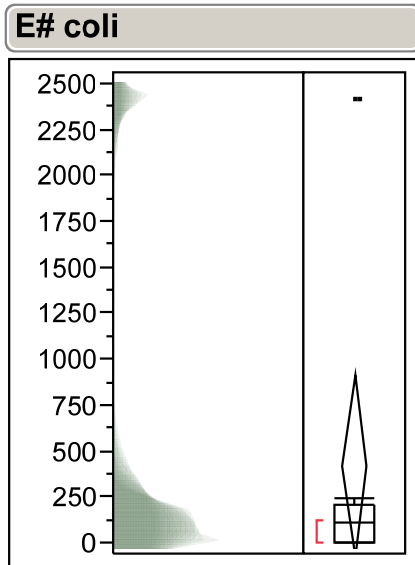


Quantiles

100.0%	maximum	1553.1
99.5%		1553.1
97.5%		1553.1
90.0%		970.2
75.0%	quartile	355.425
50.0%	median	124.3
25.0%	quartile	7.225
10.0%		1
2.5%		1
0.5%		1
0.0%	minimum	1

Moments

Mean	259.11429
Std Dev	403.04636
Std Err Mean	107.71867
Upper 95% Mean	491.82632
Lower 95% Mean	26.402249
N	14

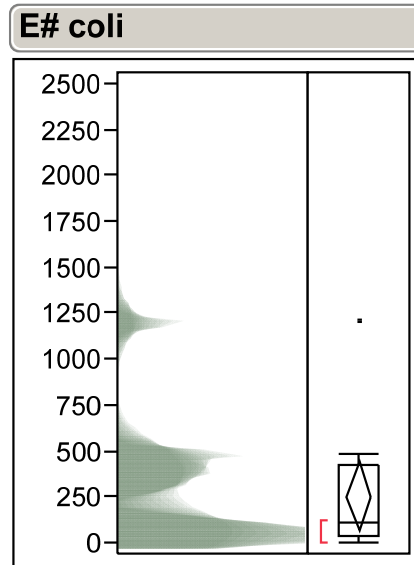


Quantiles

100.0%	maximum	2419.6
99.5%		2419.6
97.5%		2419.6
90.0%		2419.6
75.0%	quartile	210.6
50.0%	median	113.95
25.0%	quartile	1.75
10.0%		1
2.5%		1
0.5%		1
0.0%	minimum	1

Moments

Mean	423.77143
Std Dev	849.48919
Std Err Mean	227.03554
Upper 95% Mean	914.25188
Lower 95% Mean	-66.70903
N	14



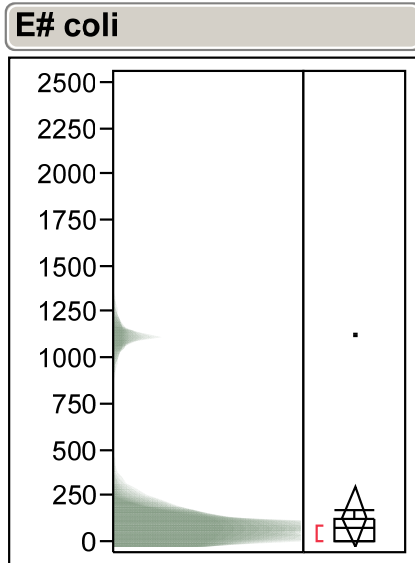
Quantiles

100.0%	maximum	1203.3
99.5%		1203.3
97.5%		1203.3
90.0%		845.85
75.0%	quartile	423.225
50.0%	median	104.45
25.0%	quartile	41.25
10.0%		2.55
2.5%		2
0.5%		2
0.0%	minimum	2

Moments

Mean	259.6
Std Dev	326.48345
Std Err Mean	87.256372
Upper 95% Mean	448.10593
Lower 95% Mean	71.094068
N	14

2011

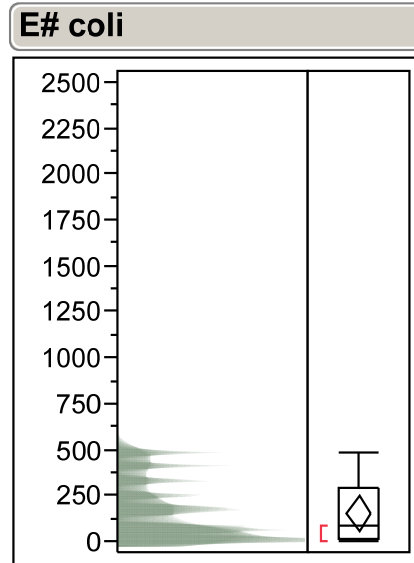


Quantiles

100.0%	maximum	1119.9
99.5%		1119.9
97.5%		1119.9
90.0%		643.45
75.0%	quartile	115.225
50.0%	median	74.05
25.0%	quartile	2
10.0%		1
2.5%		1
0.5%		1
0.0%	minimum	1

Moments

Mean	137.10714
Std Dev	287.76207
Std Err Mean	76.907647
Upper 95% Mean	303.25601
Lower 95% Mean	-29.04173
N	14



Quantiles

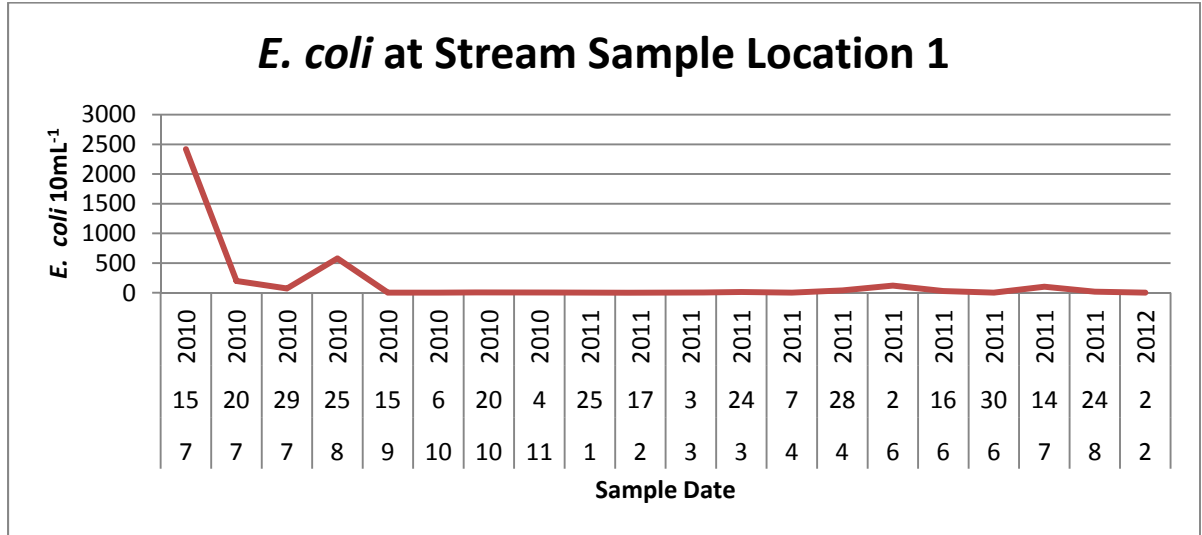
100.0%	maximum	488.4
99.5%		488.4
97.5%		488.4
90.0%		457.28
75.0%	quartile	287.2
50.0%	median	88.4
25.0%	quartile	16.4
10.0%		2.44
2.5%		2
0.5%		2
0.0%	minimum	2

Moments

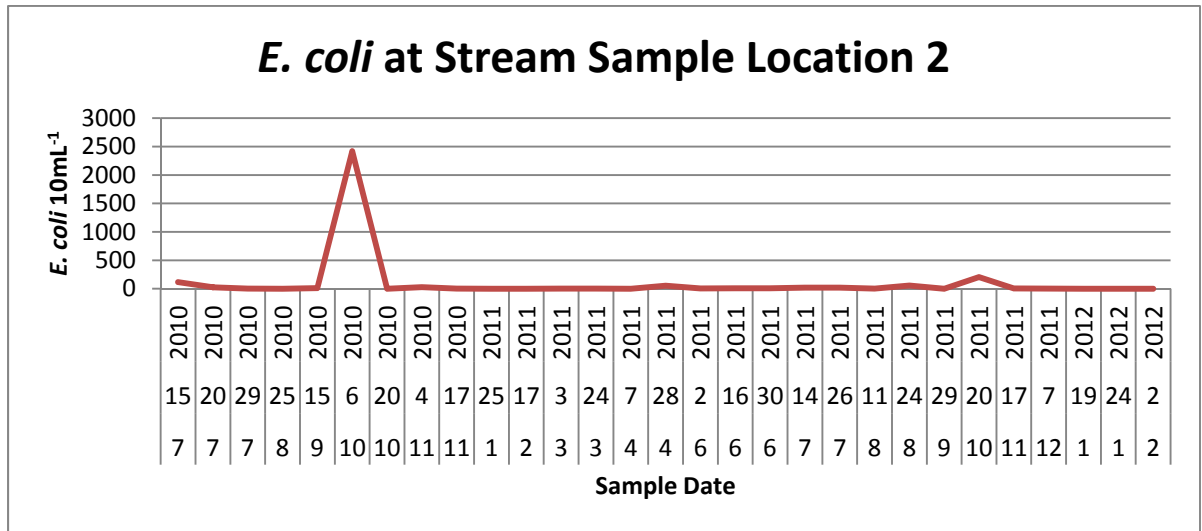
Mean	159.63077
Std Dev	163.41431
Std Err Mean	45.322974
Upper 95% Mean	258.38105
Lower 95% Mean	60.880492
N	13

APPENDIX 5. *E. coli* Time Series for Each Stream Sample Location

Appendix 5.A. *E. coli* populations for each sample date at sample location 1

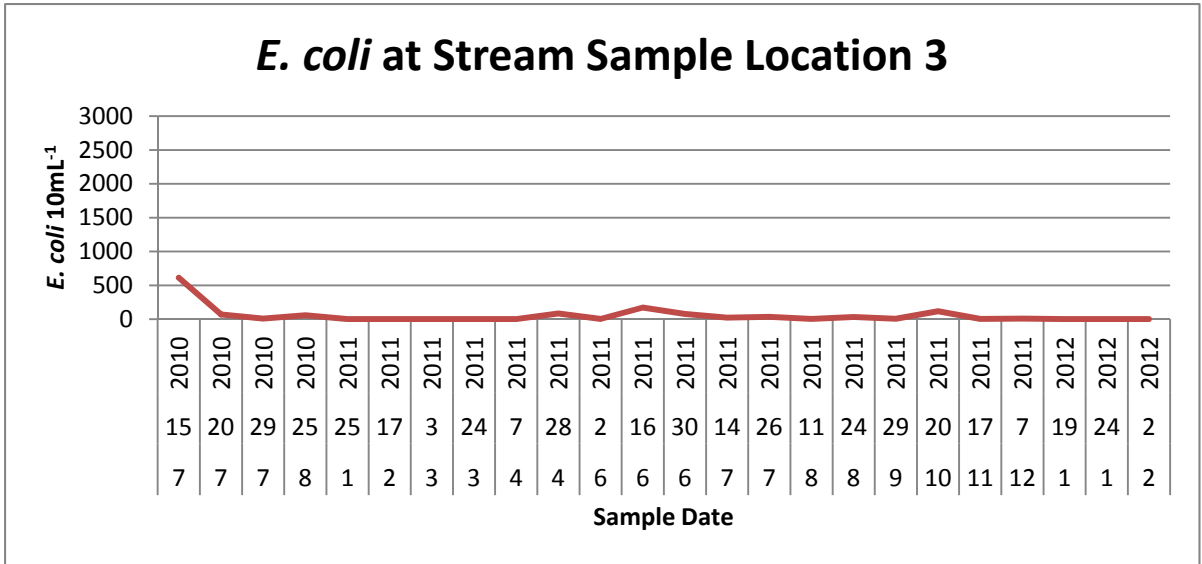


Appendix 5.B. *E. coli* populations for each sample date at sample location 2



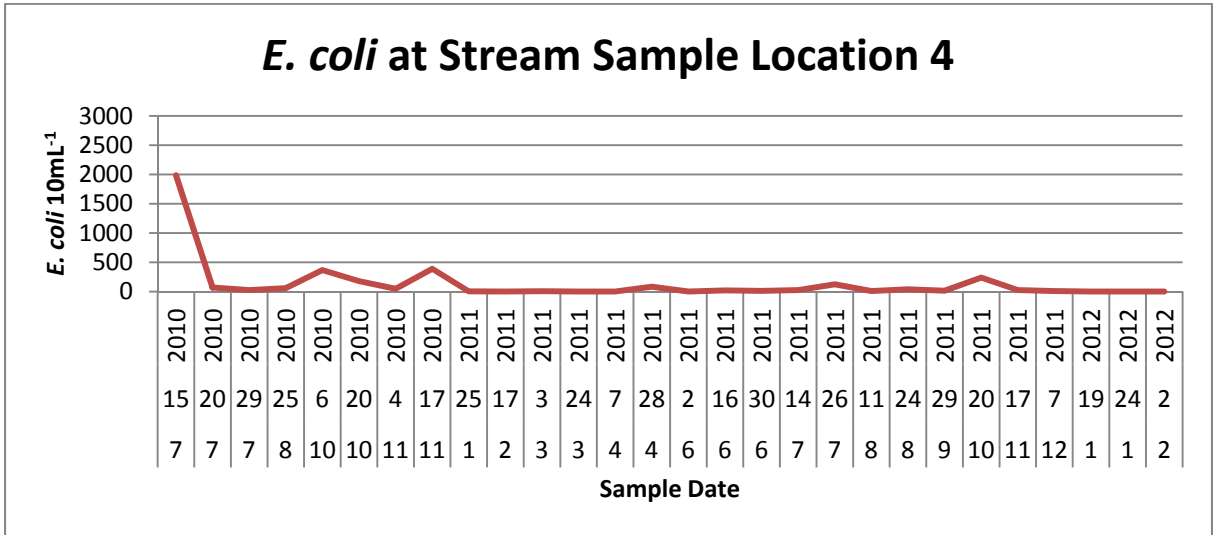
Appendix 5.C.

E. coli populations for each sample date at sample location 3



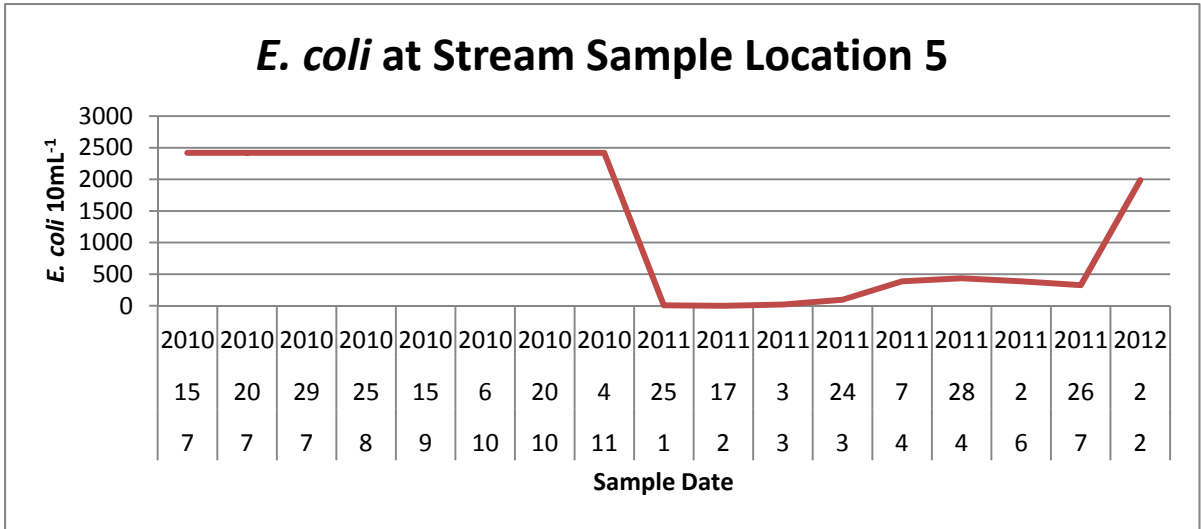
Appendix 5.D.

E. coli populations for each sample date at sample location 4



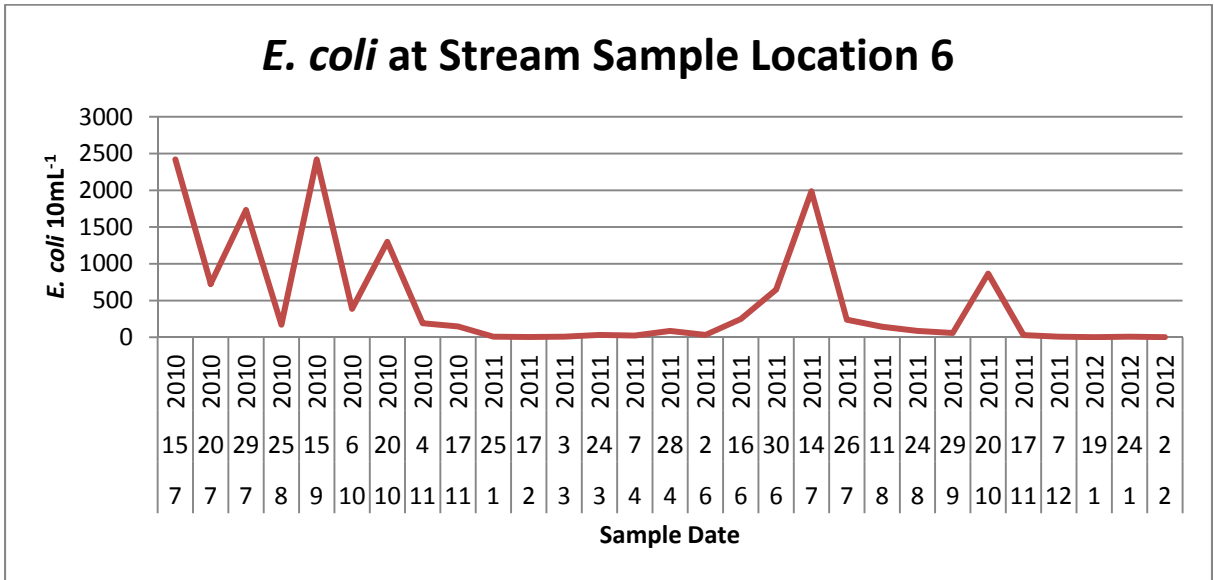
Appendix 5.E.

E. coli populations for each sample date at sample location 5



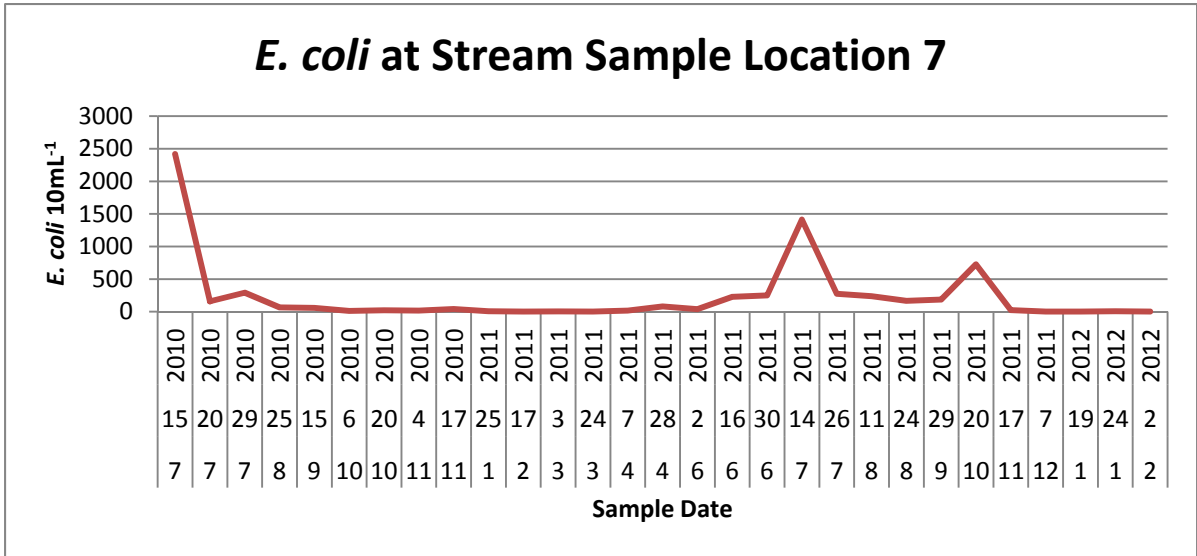
Appendix 5.F.

E. coli populations for each sample date at sample location 6



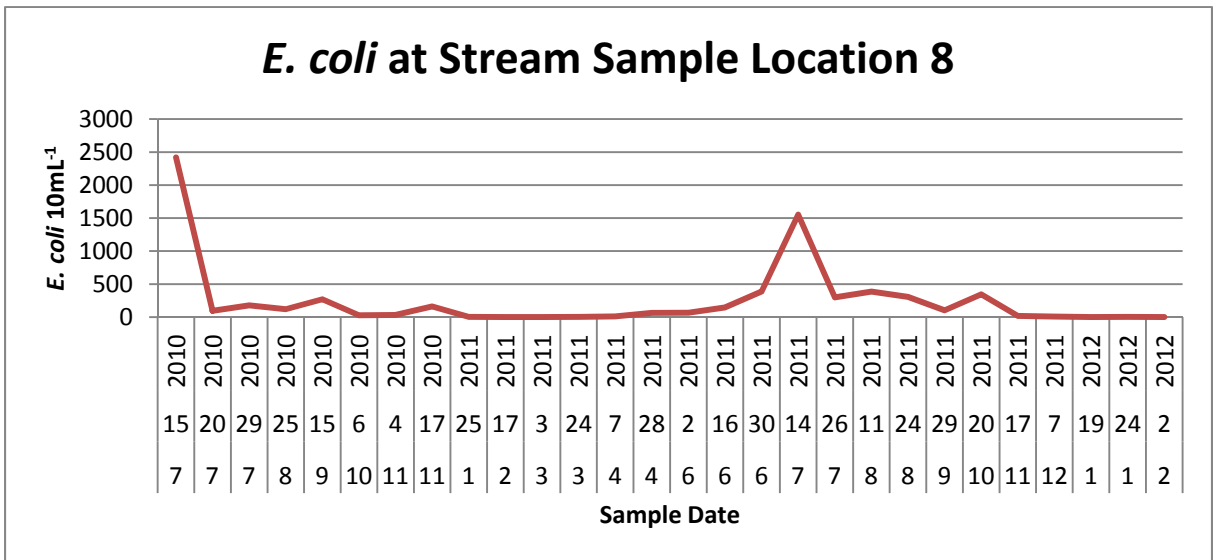
Appendix 5.G.

E. coli populations for each sample date at sample location 7



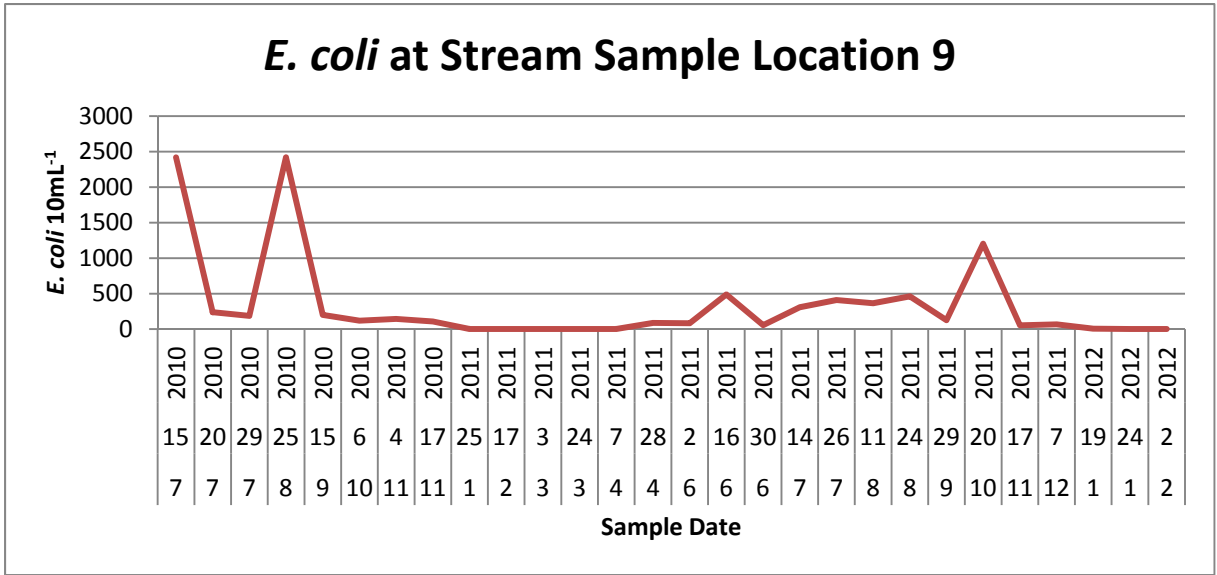
Appendix 5.H.

E. coli populations for each sample date at sample location 8



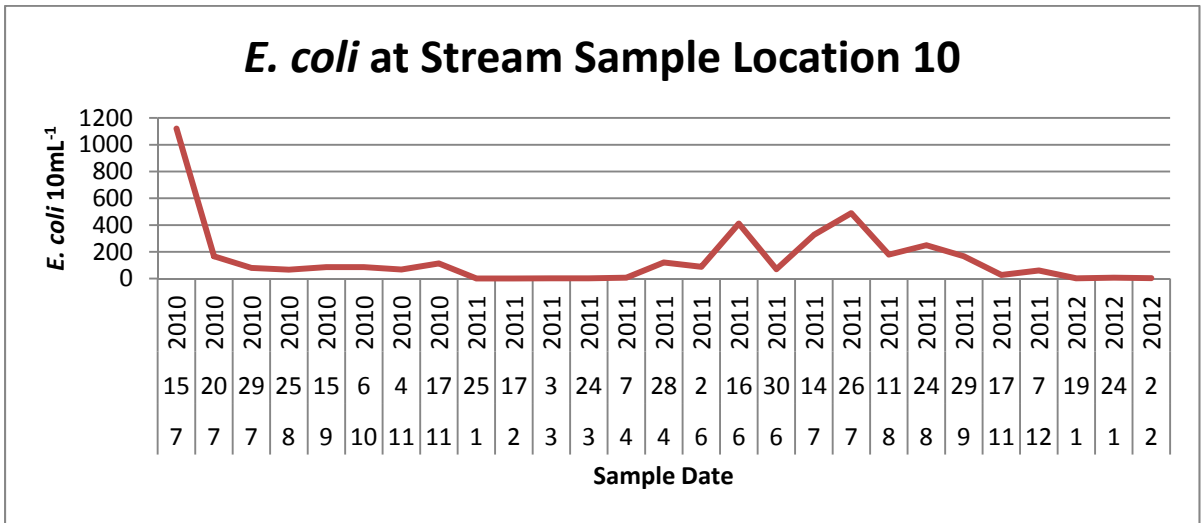
Appendix 5.I.

E. coli populations for each sample date at sample location 9



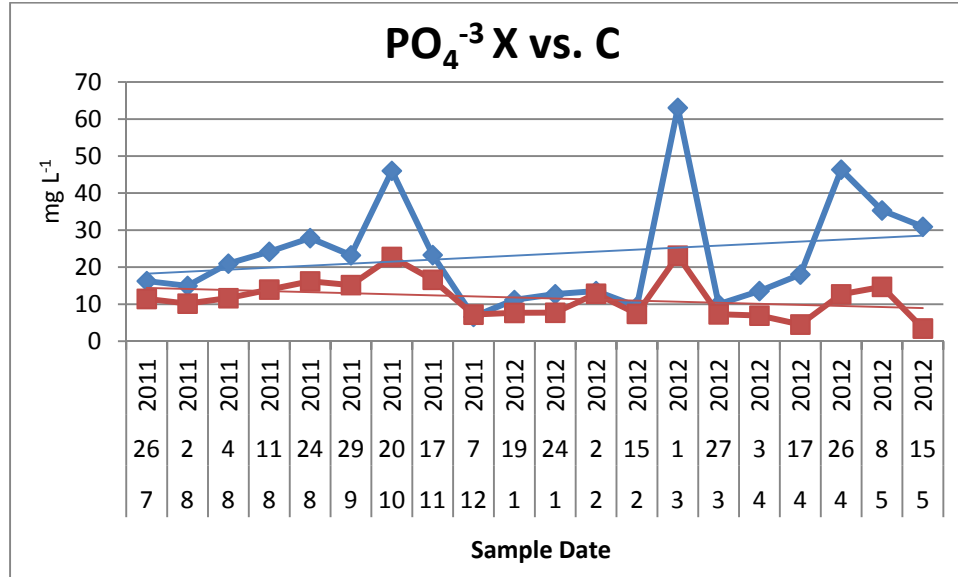
Appendix 5.J.

E. coli populations for each sample date at sample location 10

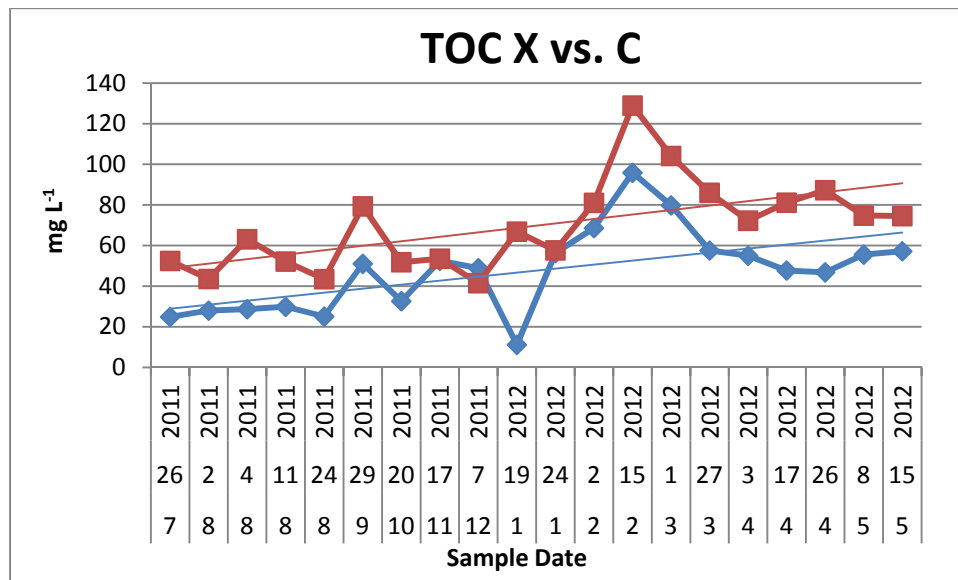


APPENDIX 6. Time Series of Water Chemistry Parameters: X Pads vs. C Pads.

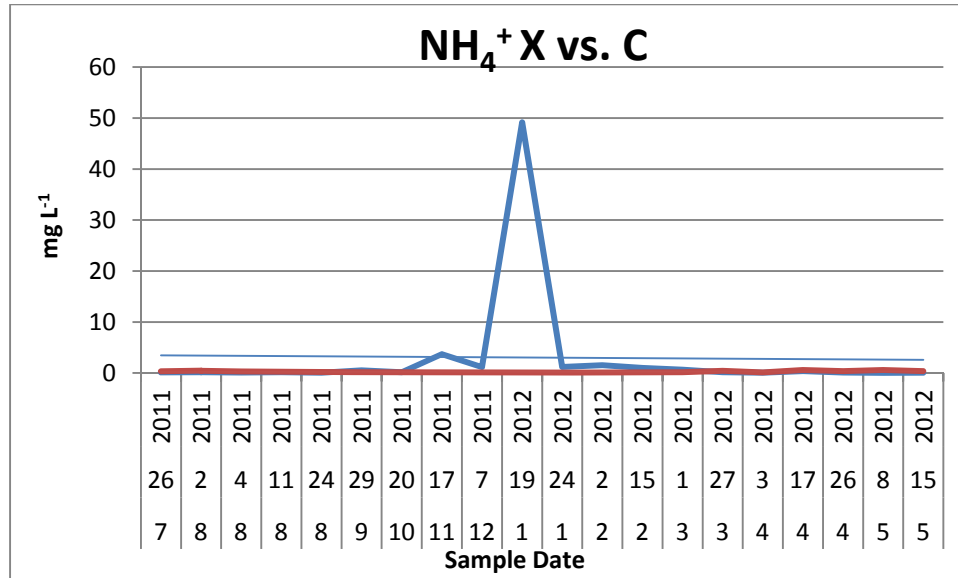
Appendix 6.A. PO_4^{-3} levels for X pads (blue) vs. C pads (red) on each sample date



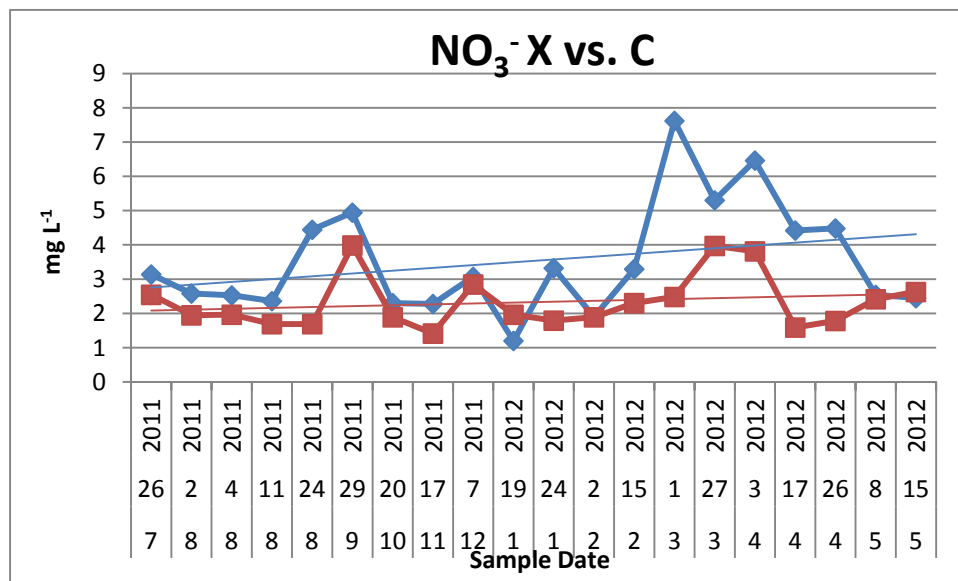
Appendix 6.B. Total Organic Carbon levels for X pads (blue) vs. C pads (red) on each sample date



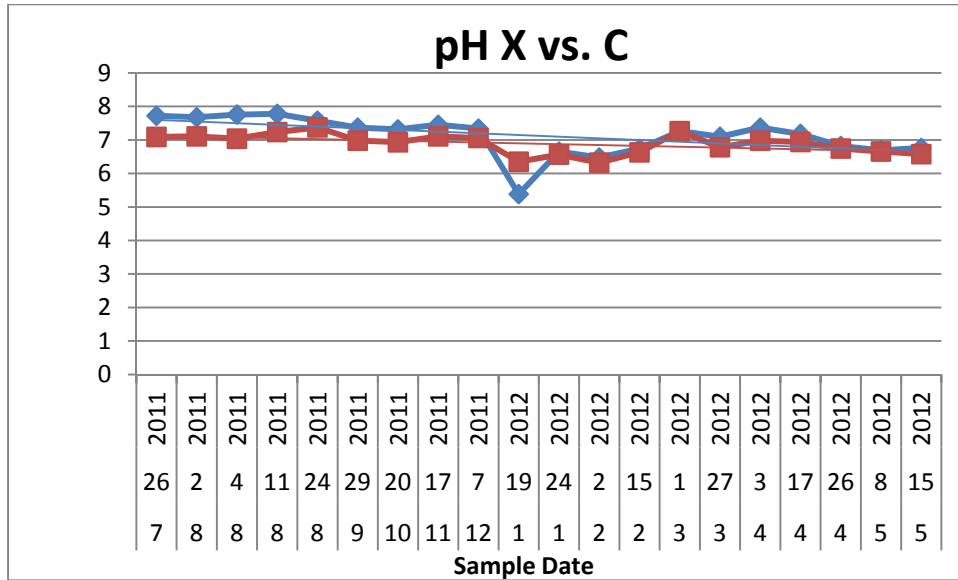
Appendix 6.C. NH_4^+ levels for X pads (blue) vs. C pads (red) on each sample date



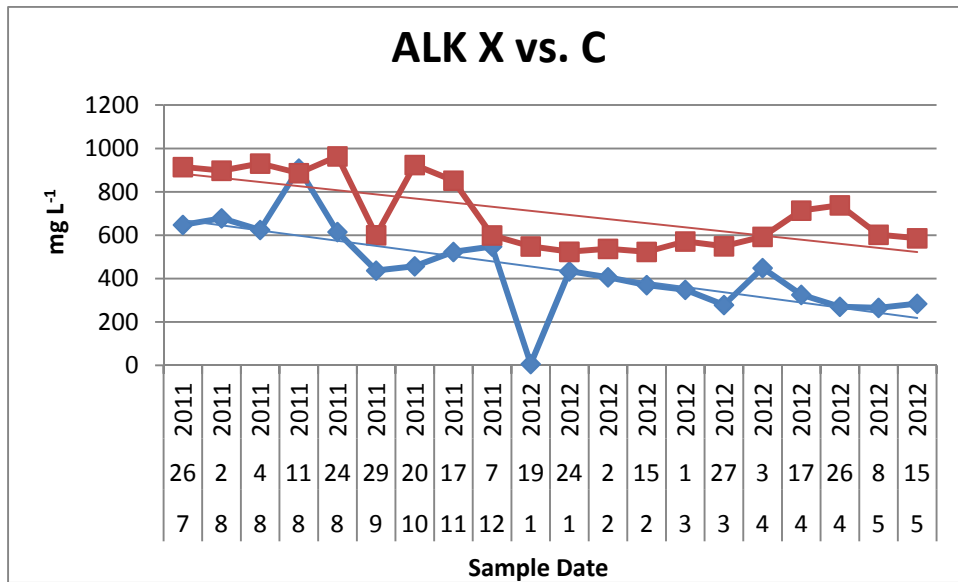
Appendix 6.D. NO_3^- levels for X pads (blue) vs. C pads (red) on each sample date



Appendix 6.E. pH levels for X pads (blue) vs. C pads (red) on each sample date

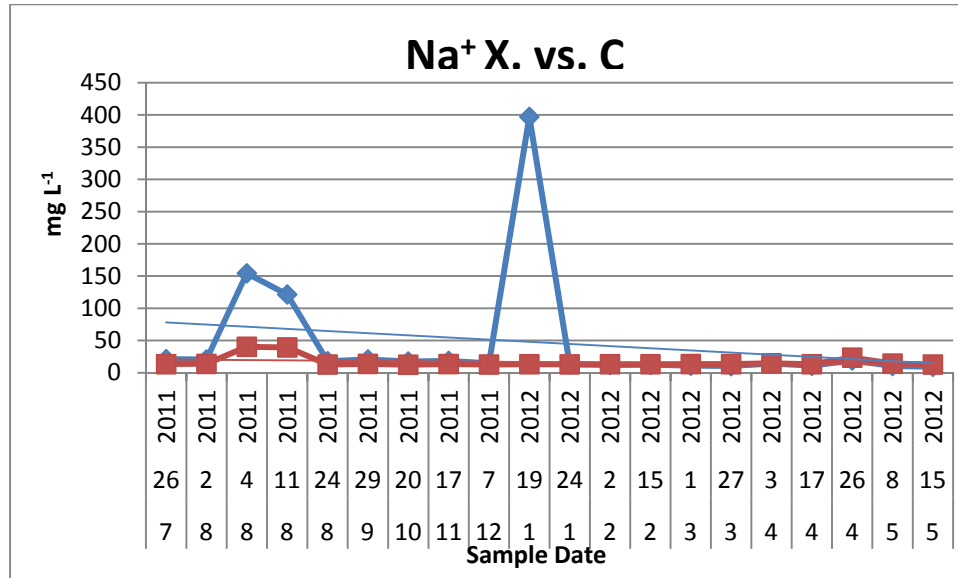


Appendix 6.F. Alkalinity levels for X pads (blue) vs. C pads (red) on each sample date



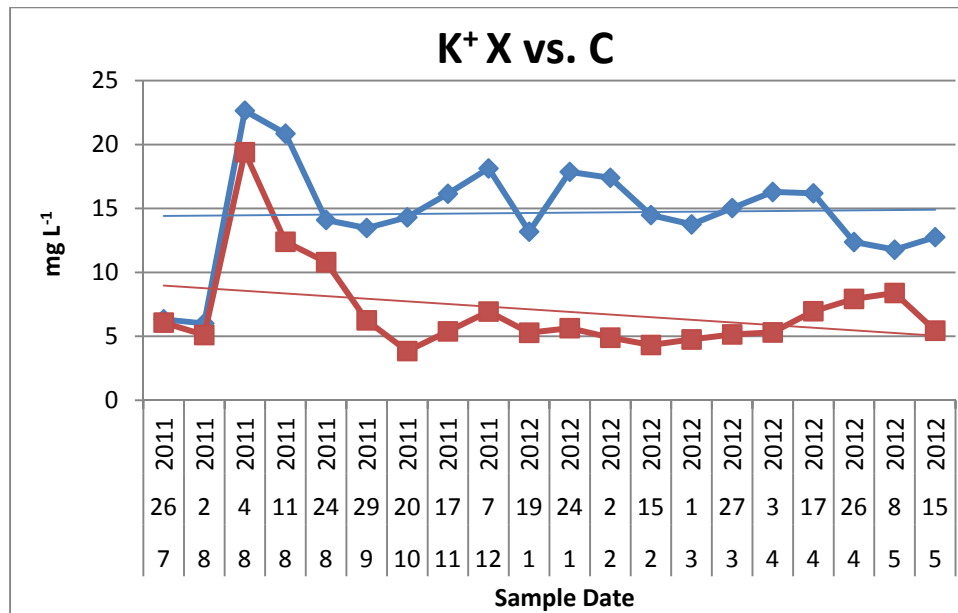
Appendix 6.G.

Na⁺ levels for X pads (blue) vs. C pads (red) on each sample date



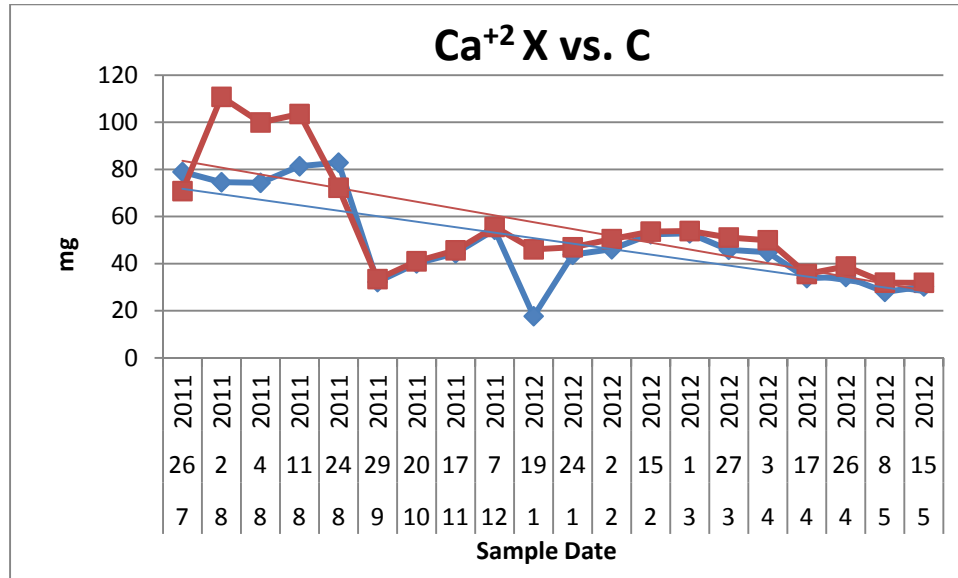
Appendix 6.H.

K⁺ levels for X pads (blue) vs. C pads (red) on each sample date



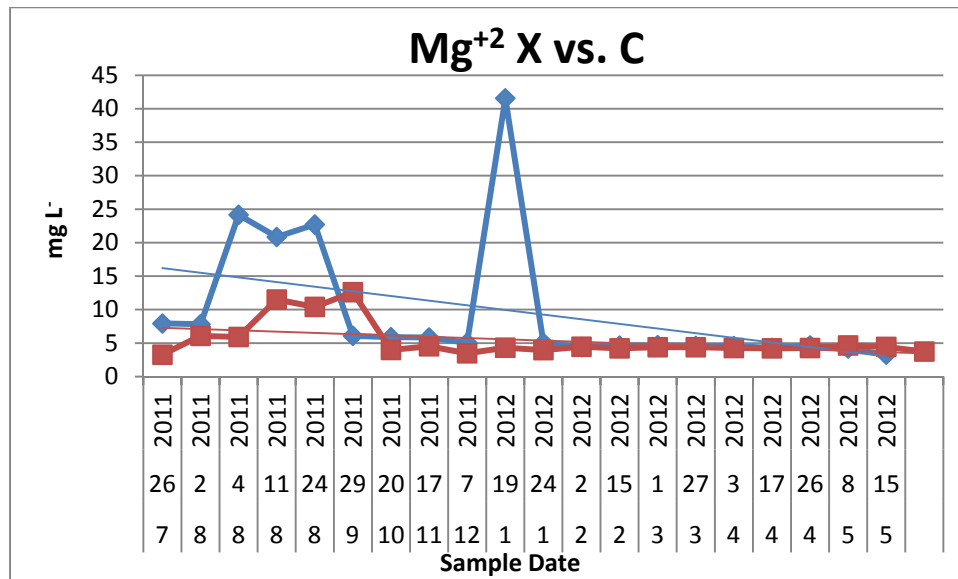
Appendix 6.I.

Ca⁺² levels for X pads (blue) vs. C pads (red) on each sample date



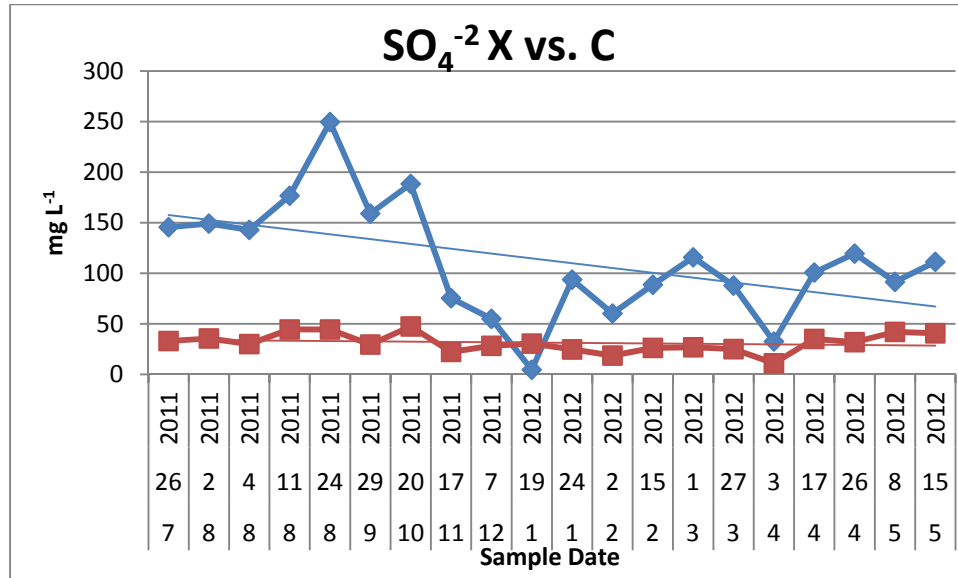
Appendix 6.J.

Mg⁺² levels for X pads (blue) vs. C pads (red) on each sample date



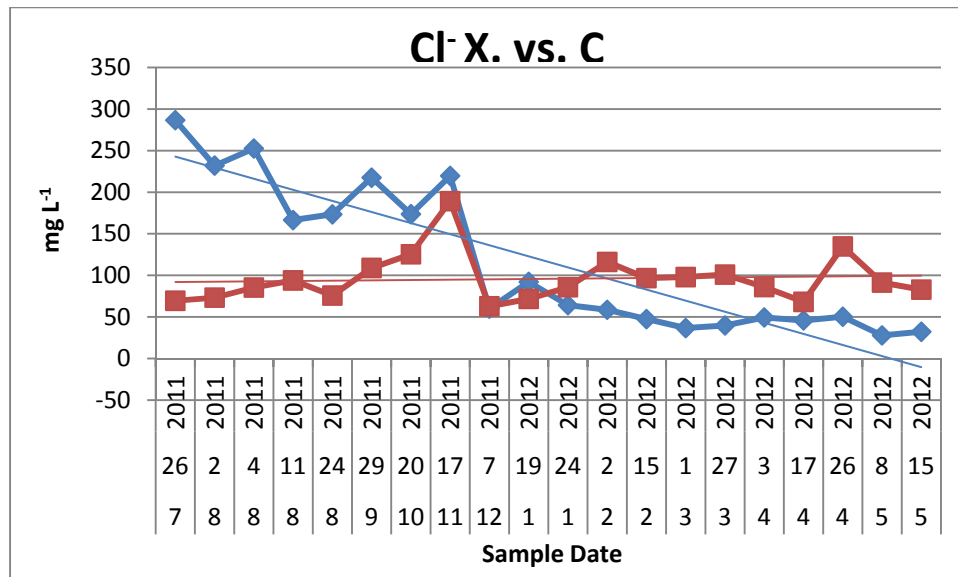
Appendix 6.K.

SO₄⁻² levels for X pads (blue) vs. C pads (red) on each sample date

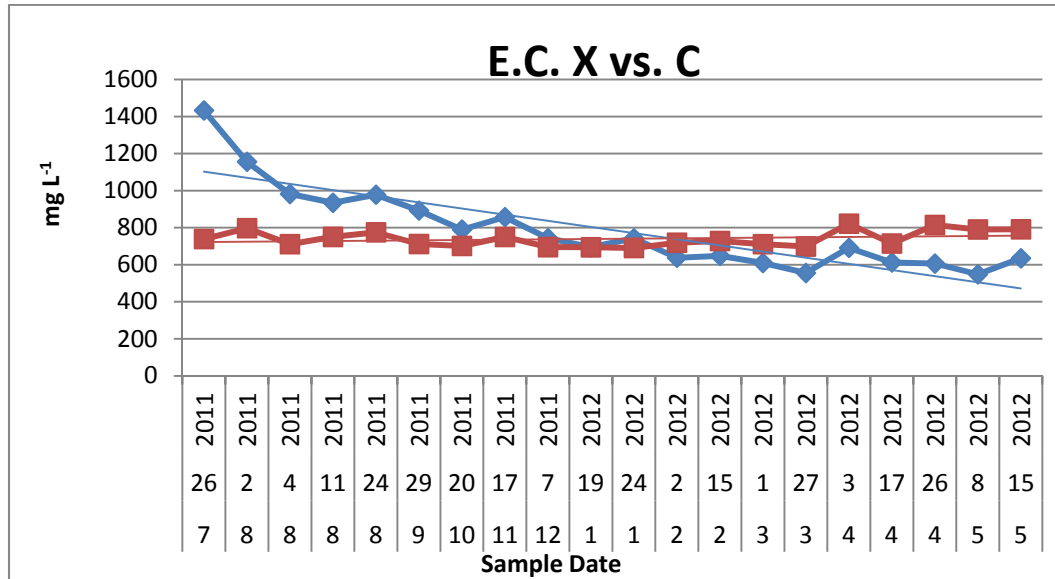


Appendix 6.L.

Cl levels for X pads (blue) vs. C pads (red) on each sample date



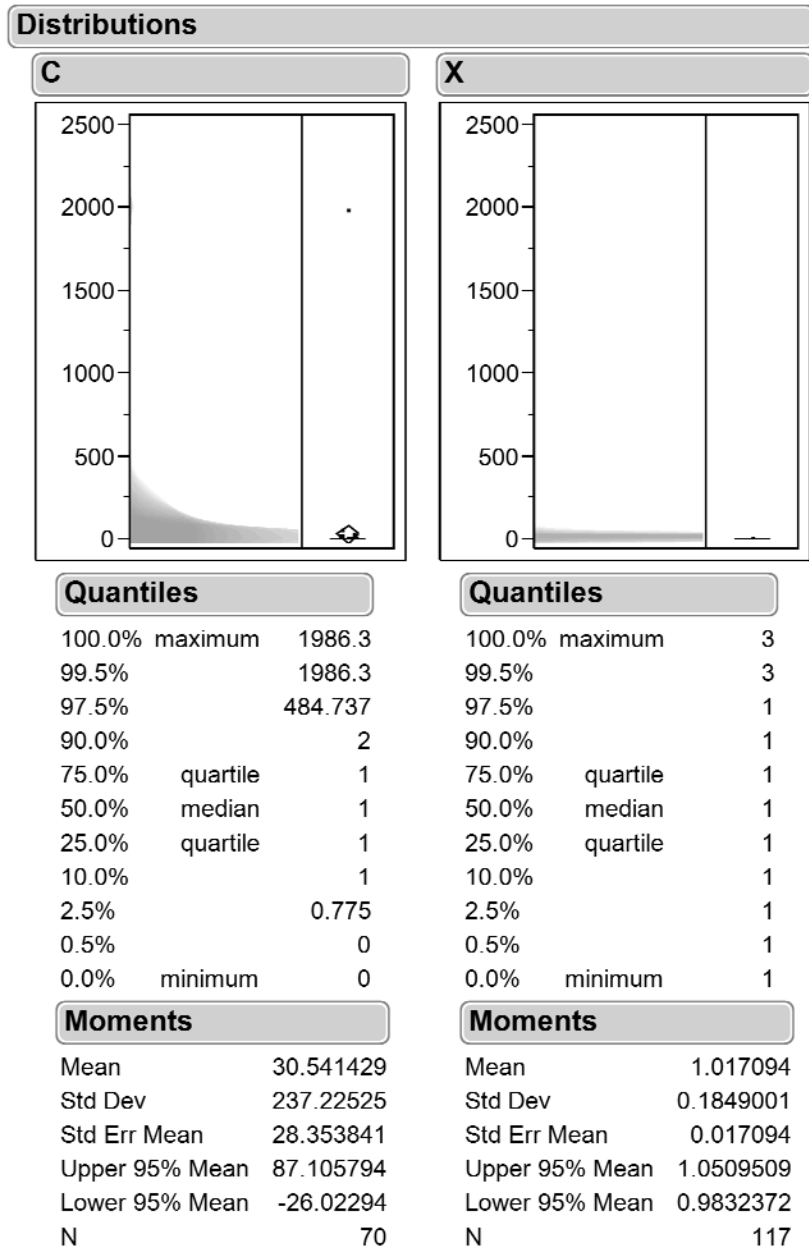
Appendix 6.M. Electrical Conductivity for X pads (blue) vs. C pads (red) on each sample date

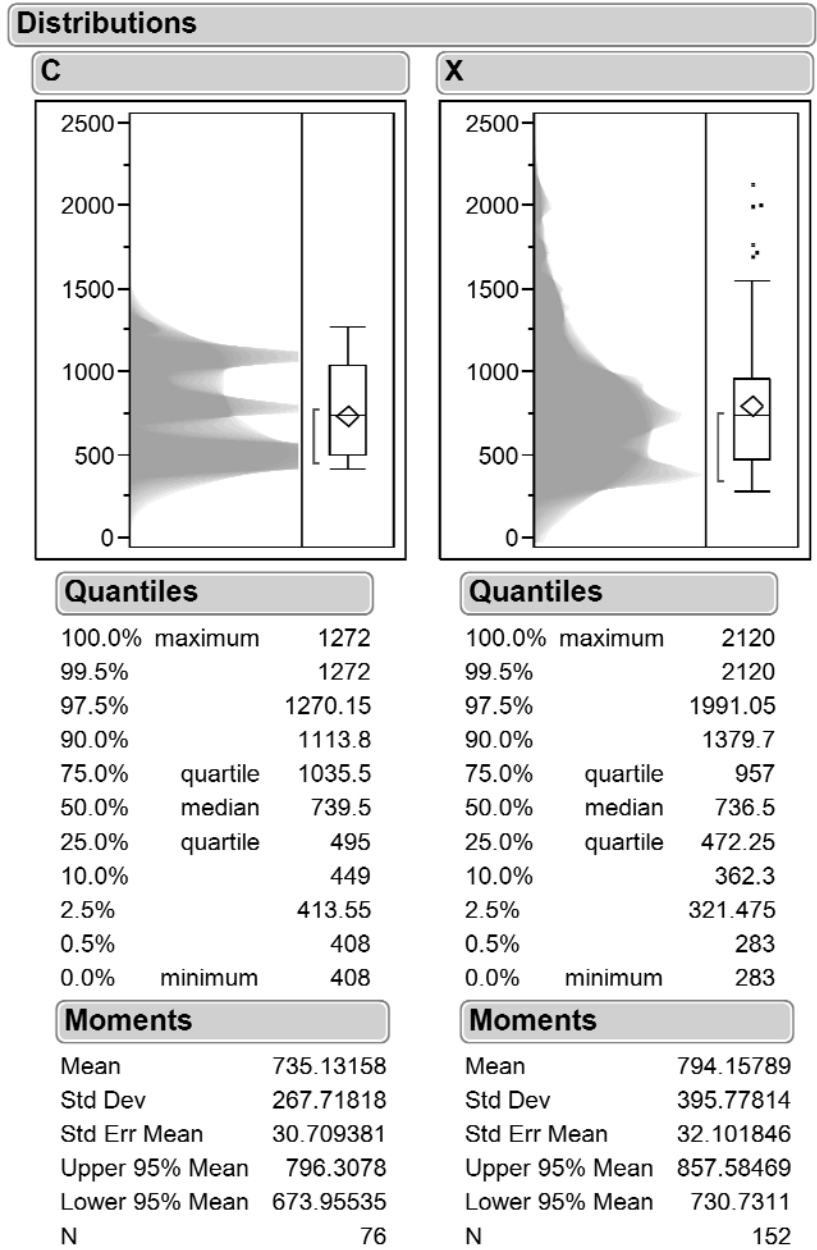


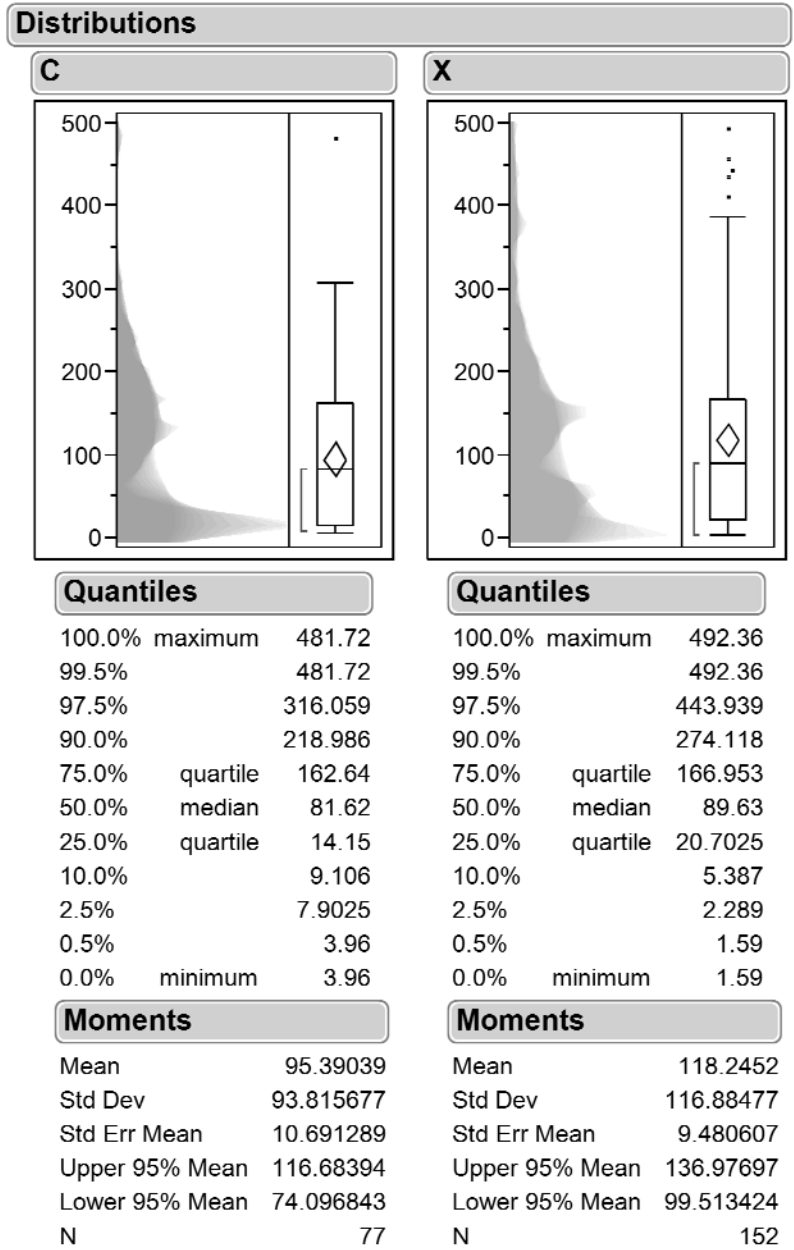
APPENDIX 7. Box and Whisker Plots. X pads vs. C pads for *E. coli* and Water Chemistry

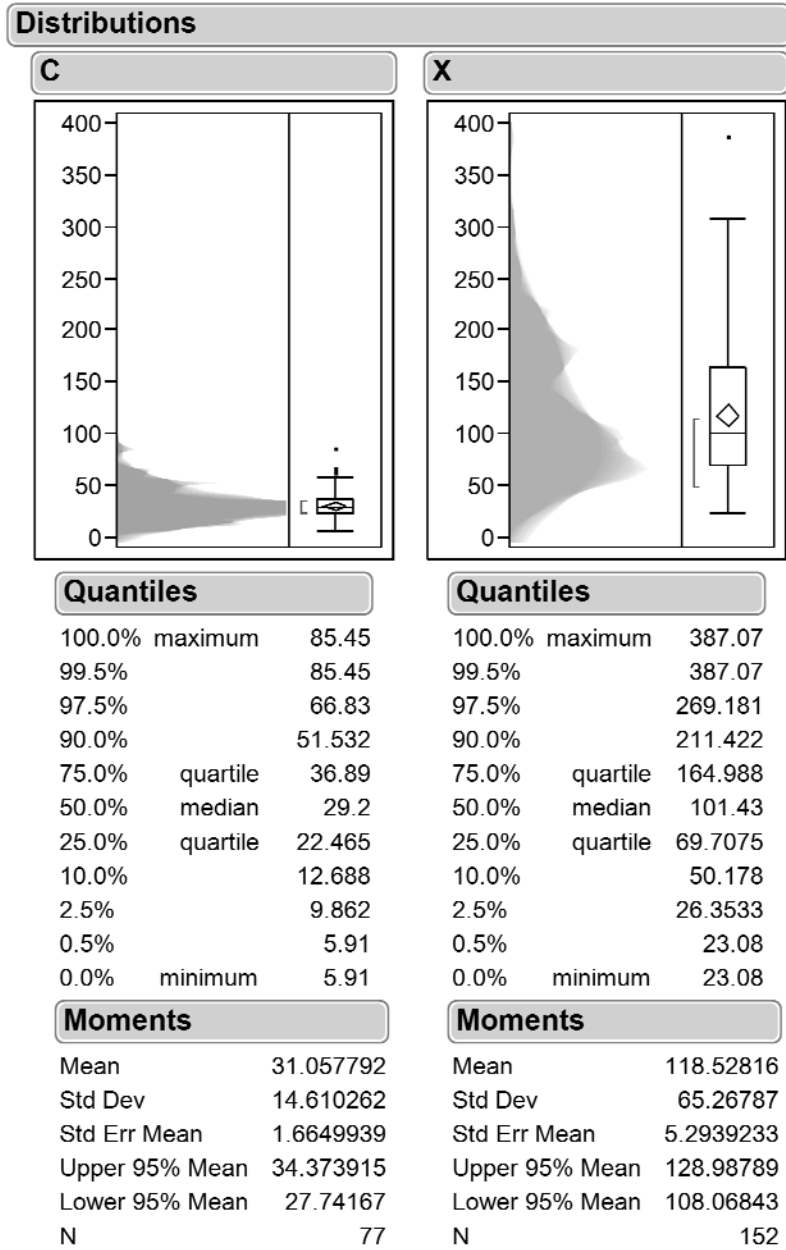
Note: *E. coli* measured per 10m. All water chemistry parameters measured in mg L⁻¹, except for pH.

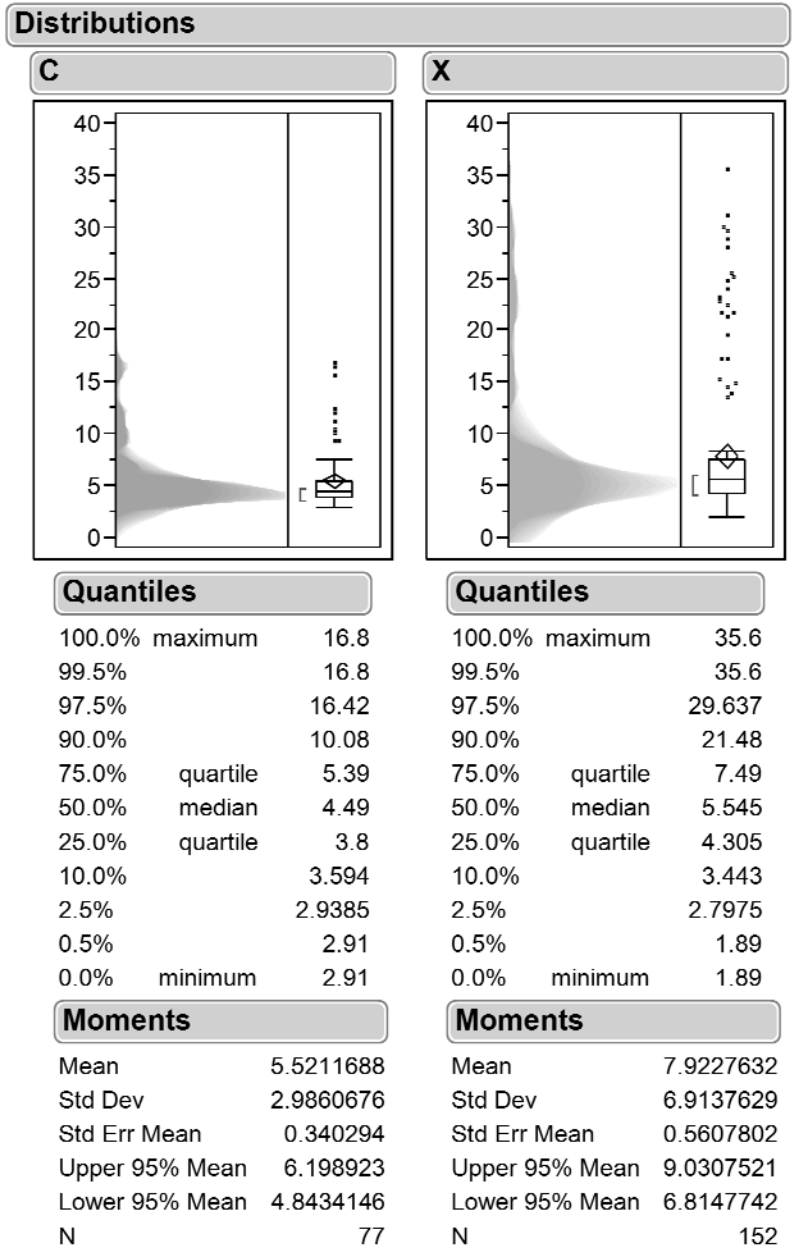
Appendix 7.A. *E. coli* X pads vs. C pads

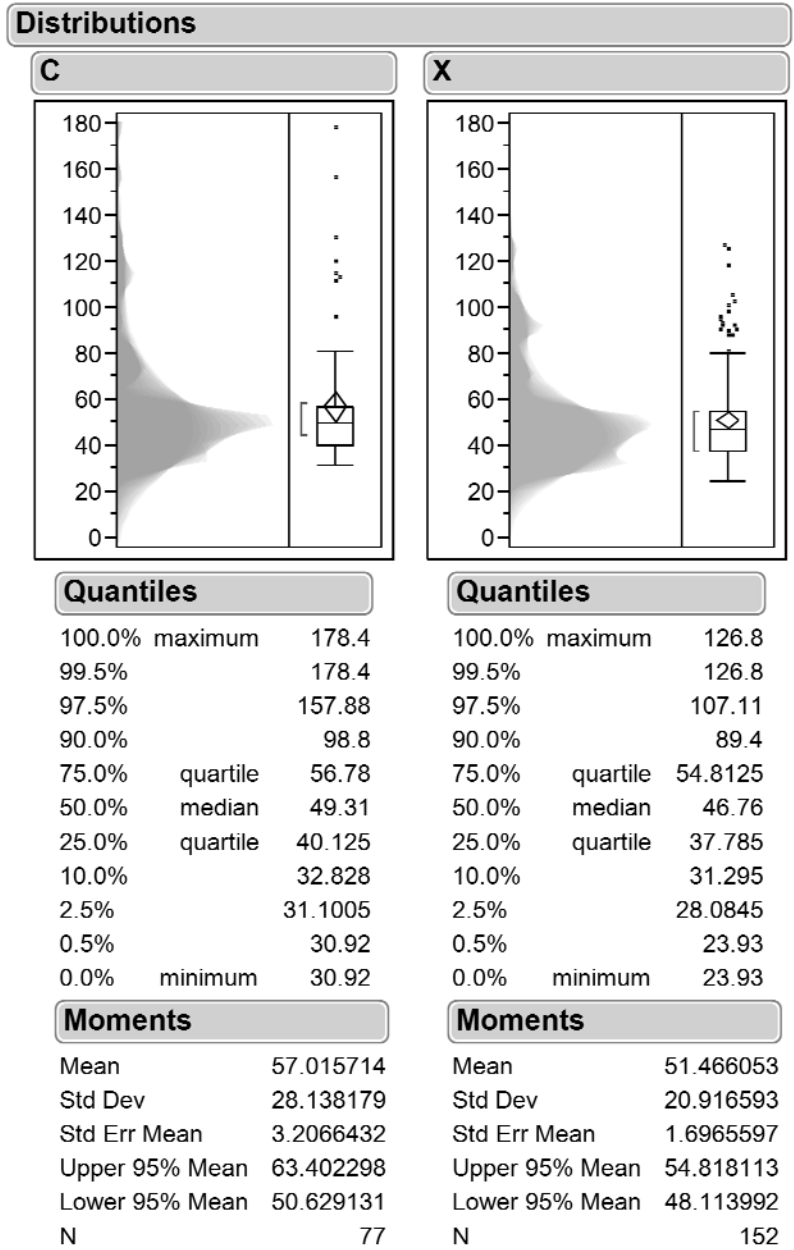


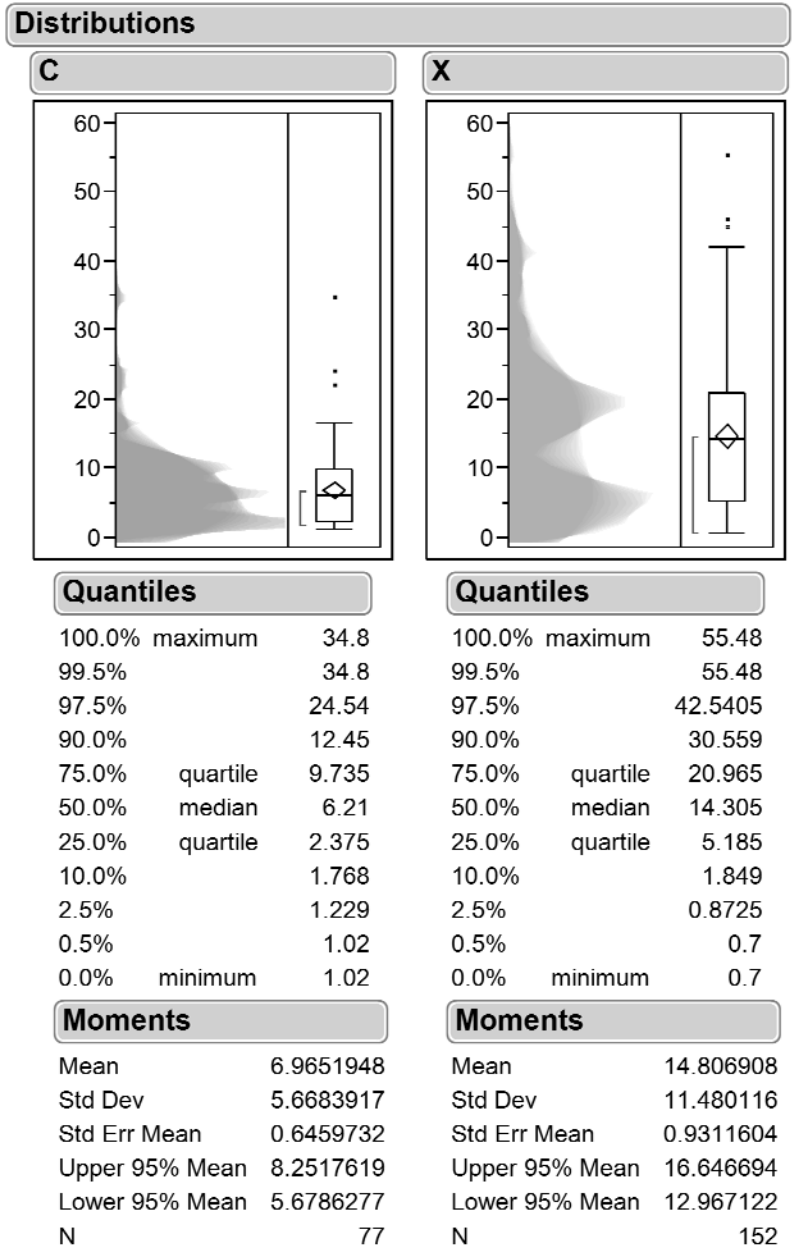


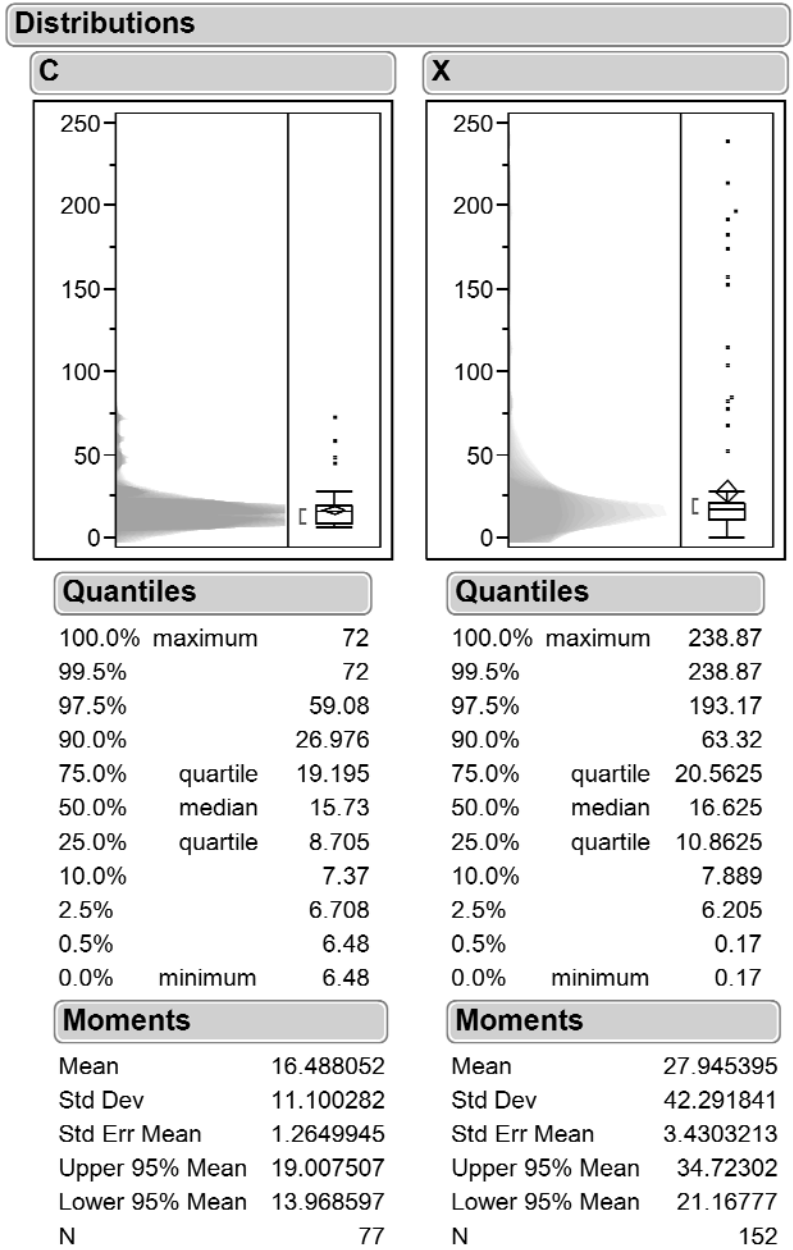


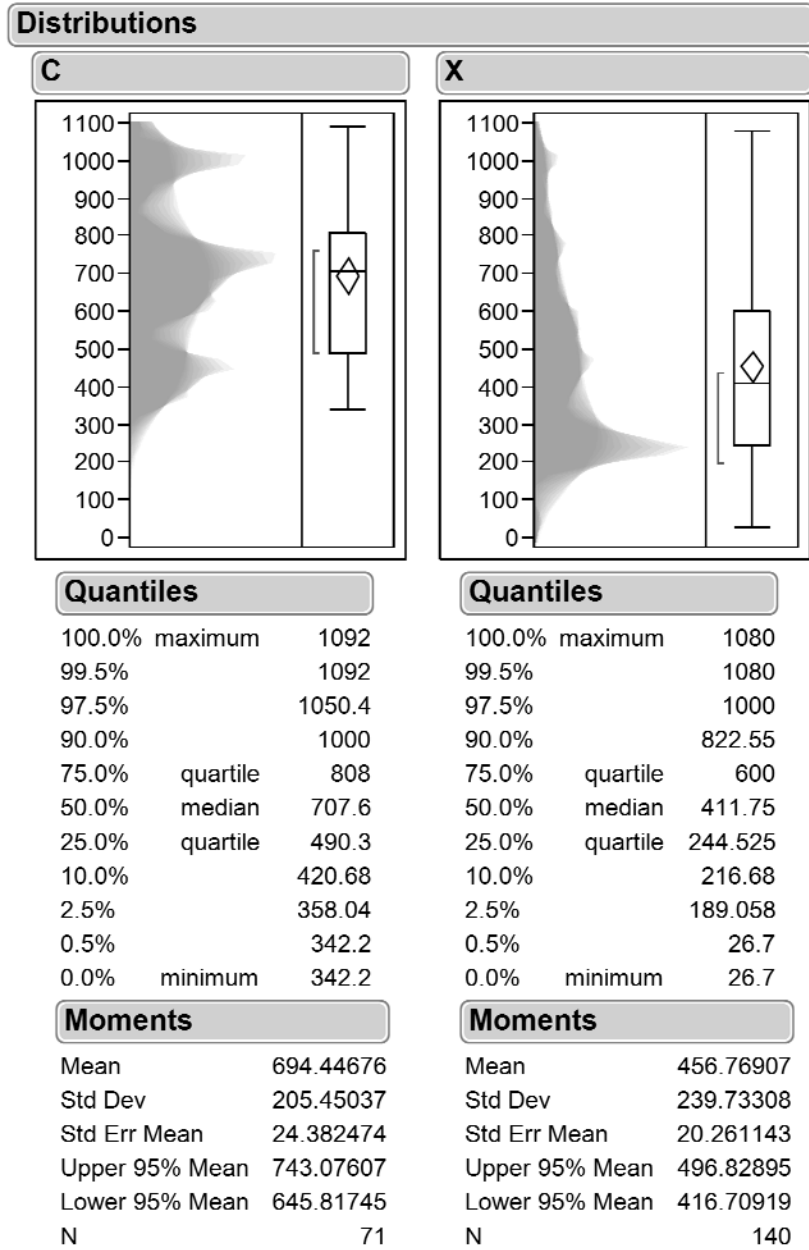


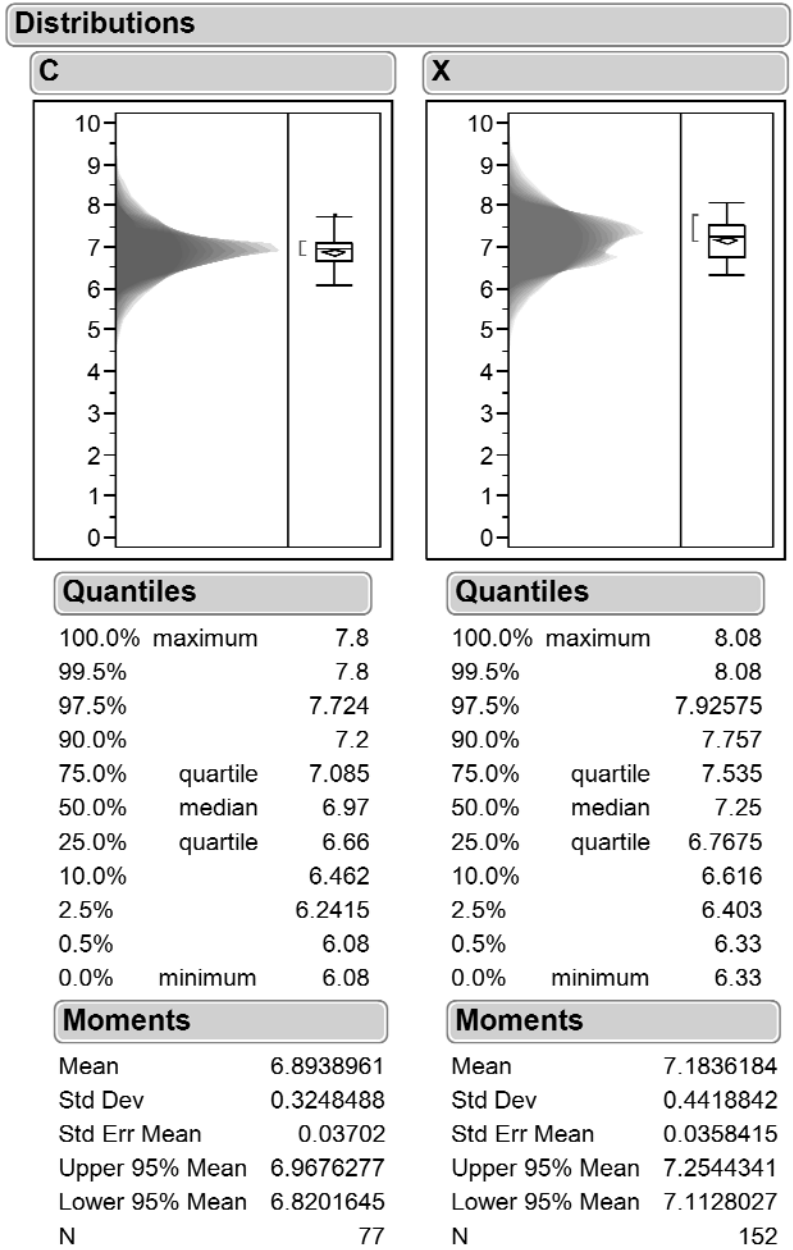


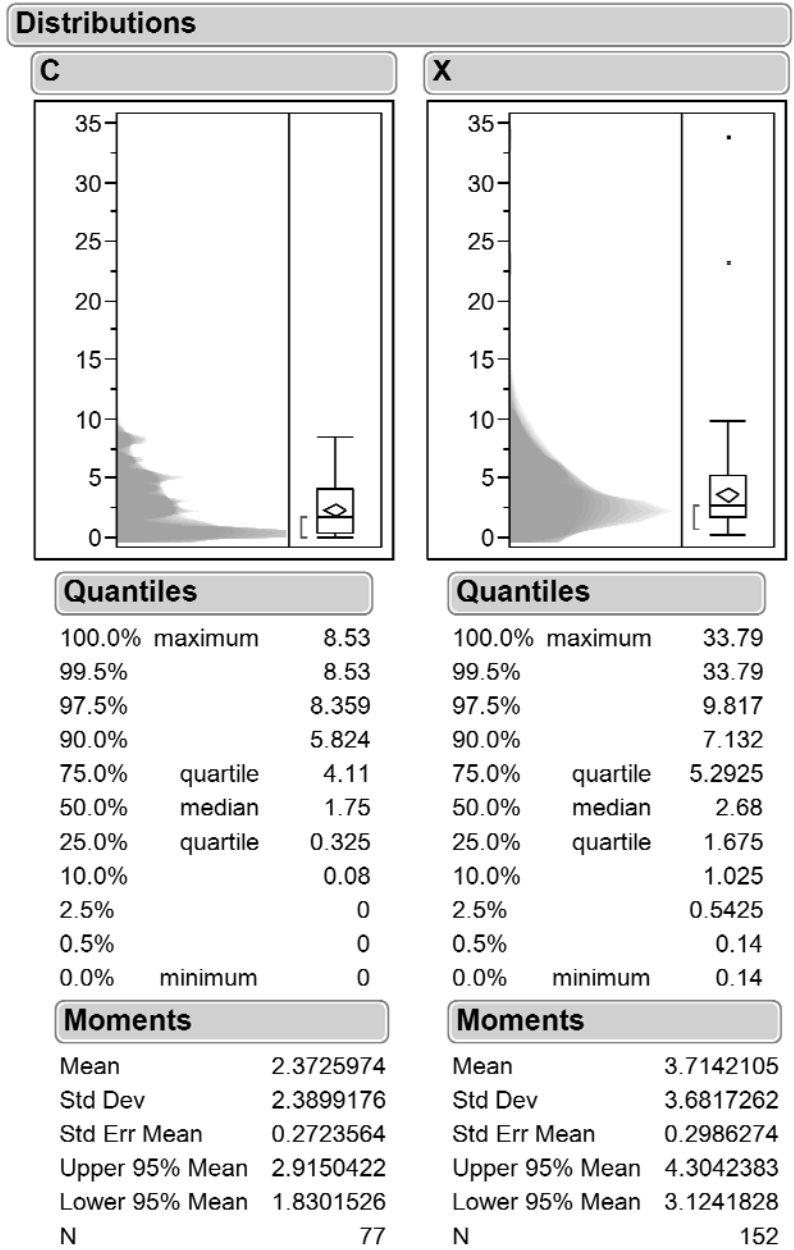


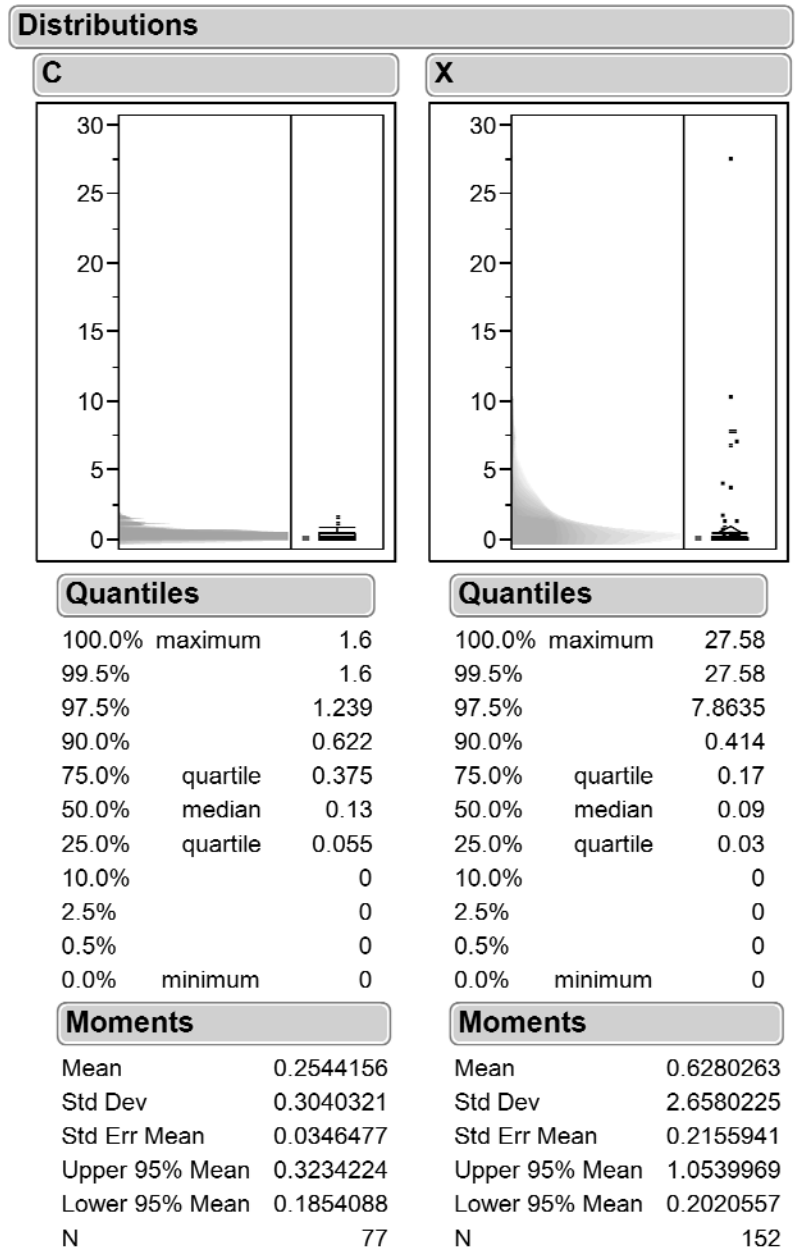




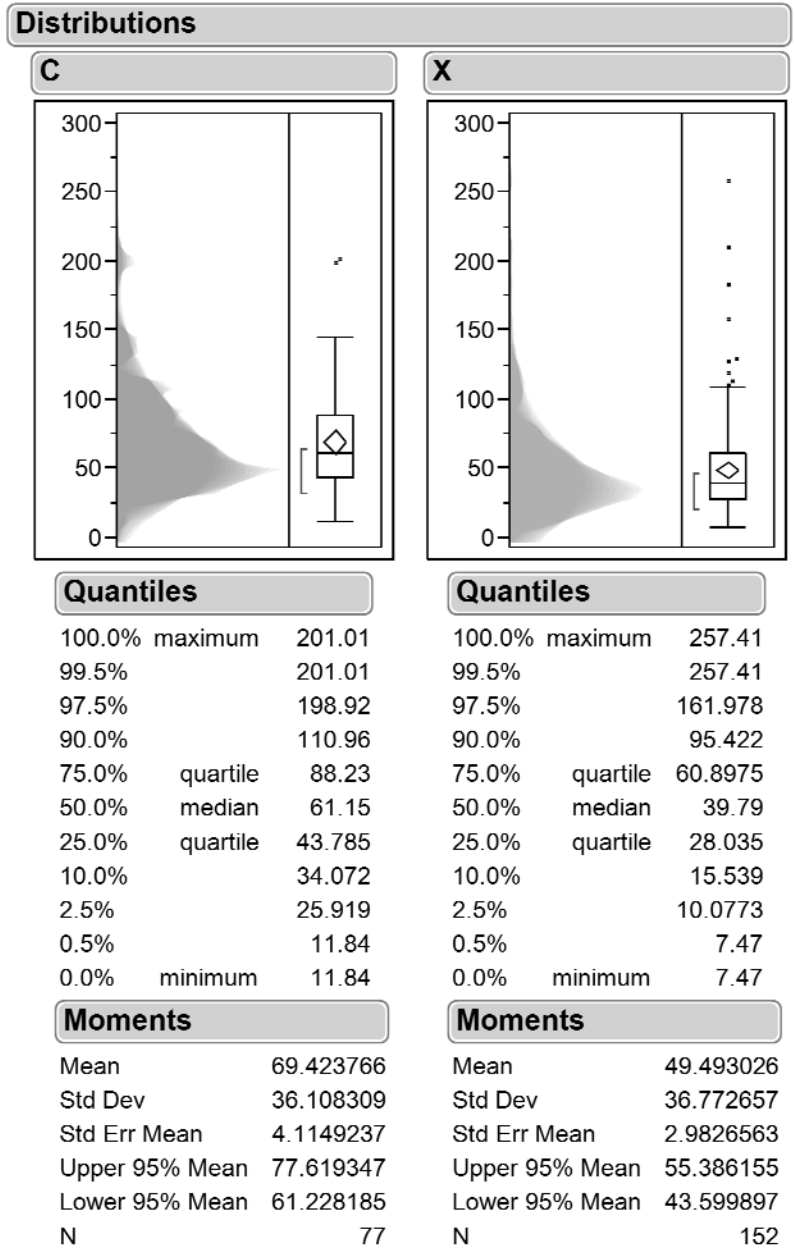


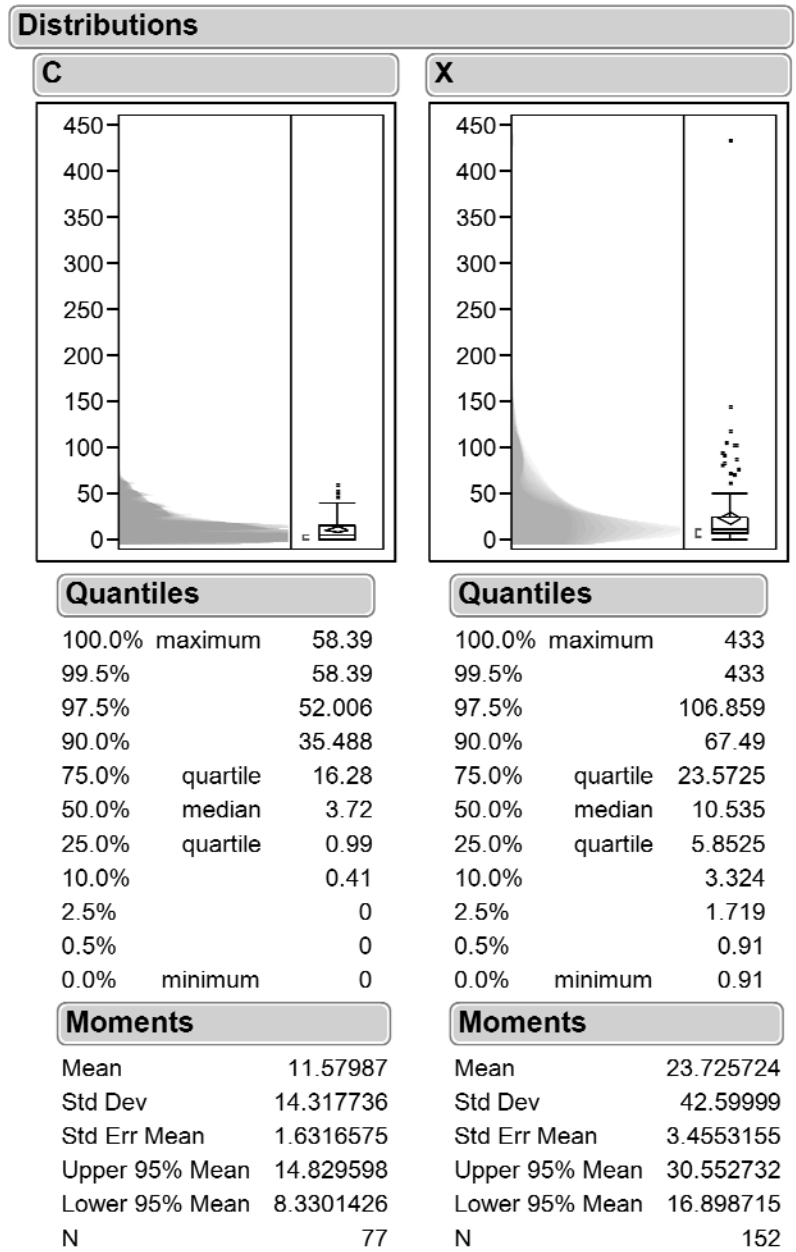






Appendix 7.M. Total Organic Carbon X pads vs. C pads





BIBLIOGRAPHY/REFERENCES

- Adhikari, H., D. Barnes, S. Schiewer, and D. White. (2007) Total Coliform Survival Characteristics in Frozen Soils. *Journal of Environmental Engineering* 133: 1098-1105.
- Adler, Paul R., Steven T. Summerfelt, D. Michael Glenn, and Fumiomo Takeda. (1996) Evaluation of a Wetland System Designed to Meet Stringent Phosphorous Discharge Requirements. *Water Environment Research* 68: 836-840.
- American Horse Council. (2005) The Economic Impact of the Horse Industry on the United States.
- Bendell-Young, L. I., K. E. Bennett, A. Crowe, C. J. Kennedy, A. R. Kermode, M. M. Moore, A. L. Plant, and A. Wood. (2000) Ecological Characteristics of Wetlands Receiving an Industrial Effluent. *Ecological Applications* 10: 310-322.
- Bennet, Albert F., Richard Lenski, and John E. Mittler. (1992) Evolutionary Adaptation to Temperature I. Fitness Responses of *Escherichia coli* to Changes in its Thermal Environment. *Evolution* 46: 16-30.
- Bennet, Albert F. and Richard E. Lenski. (1993) Evolutionary Adaptation to Temperature II. Thermal Niches of Experimental Lines of *Escherichia coli*. *Evolution* 47: 1-12.
- Burkholder, JoAnn, Bob Libra, Peter Weyer, Susan Heathcote, Dana Kolpin, Peter Thorne, and Michael Wichman. (2007) Impacts of Waste from Concentrated Animal Feeding Operations on Water Quality. *Environmental Health Perspectives* 115: 308-312.
- Byappanahalli, Muruleedhara, Melanie Fowler, Dawn Shively, and Richard Whitman. (2003) Ubiquity and Persistence of *Escherichia coli* in a Midwestern Coastal Stream. *Applied Environmental Microbiology* 69: 4549-4555.
- Byappanahalli, Muruleedhara, Richard L. Whitman, Dawn A. Shively, Michael J. Sadowsky, and Satoshi Ishii. (2006) Population structure, persistence, and seasonality of autochthonous *Escherichia coli* in temperate, coastal forest soil from a Great Lakes watershed. *Environmental Microbiology* 8: 504-513.
- Cane Run and Royal Spring Watershed Restoration Plan. Cane Run Watershed. (2011) www.canerunwatershed.org. Accessed 1 January 2012.
- Carleton, James N., Thomas Grizzard, Adil N. Godrej, Harold E. Post, Lex Lampe, and Pamela P. Kenel. (2000) Performance of a Constructed Wetlands in Treating Urban Stormwater Runoff. *Water Environment Research* 72: 295-304.

- Cheng, Xiu-Yun, Wen-Yin Chen, Bin-He Gu, Xu-Cheng Liu, Fang Chen, Zhang-He Chen, Xian-Ye Zhou, Yu-Xiang Li, Hua Huang, and Yun-Jin Chen. (2009) Morphology, ecology, and contaminant removal efficiency of eight wetland plants with differing root systems. *Hydrobiologia* 623: 77-85.
- Cole, Dana, Lori Todd, and Steve Wing. (2000) Concentrated Swine Feeding Operations and Public Health: A Review of Occupational and Community Health Effects. *Environmental Health Perspectives* 108: 685-699.
- Conley, Lucy M., Richard I. Dick, and Leonard W. Lion. (1991) An Assessment of the Root Zone Method of Wastewater Treatment *Research Journal of the Water Pollution Control Federation* 63: 239-247.
- Elder, John F., and Gerald L. Goddard. (1996) Sediment and Nutrient Trapping Efficiency of a Constructed Wetland Near Delavan Lake, Wisconsin, 1993-1995. U.S. Department of the Interior – U.S. Geological Survey Fact Sheet FS-232-96.
- Federal Emergency Management Agency. United States Department of Homeland Security. (2008) FEMA Map Service Center. Current FEMA Issued Flood Maps. Lexington-Fayette Urban County Government. No. 2100670126D, effective date 9/17/2008. Accessed 13 June 2012.
- Federal Water Pollution Control Act. (2011) Chapter 26: Water Pollution Prevention and Control. Title 33: Navigation and Navigable Waters.
- Gale, P. M., K. R. Reddy, and D. A. Graetz. (1993) Nitrogen Removal from Reclaimed Water Applied to Constructed Wetland Microcosms. *Water Environment Research* 65: 162-168.
- Gallagher, Meghan, A., R. Karthikeyan, and S. Mukhtar. (2012) Growth Kinetics of Wildlife *E. coli* Isolates in Soil and Water. *Journal of Environmental Protection* 3:838-846.
- Gillespie, James R. (2002) *Modern Livestock and Poultry Production* Sixth Edition. Delmar, Albany.
- Green, M. B., P. T. O'Connell, and P. Griffin. (1998) Upgrading and Rescuing Small Wastewater Treatment Facilities by Adding Tertiary Treatment Reed Beds. *Water Environment Research* 70: 1307-1313.
- Greenberg, A.E., et al. (1992) *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association.
- Harrington, Rory, and Robert McInnes. (2009) Integrated Constructed Wetlands (ICW) for livestock wastewater management. *Bioresource Technology* 100: 5498-5505.
- Harvey, Richard M., and Jackson L. Fox. (1973) Nutrient Removal Using Lemna Minor. *Journal (Water Pollution Control Federation) Annual Conference Issue* 45: 1928-1938.

- Henis, Yigal, Koteswara R. Gurijala, and Martin Alexander. (1989) Factors Involved in Multiplication and Survival of *Escherichia coli* in Lake Water. *Microbial Ecology* 17: 171-180.
- Hershfield, David M. (1961) Rainfall Frequency Atlas of the United States: Technical Paper No. 40. Weather Bureau, U.S. Department of Commerce.
- Higgins, Steve, Steve Workman, and Robert J. Coleman. (2007) Pervious Concrete as a Flooring Material for Horse Handling Areas. Cooperative Extension Service, University of Kentucky College of Agriculture.
- Higgins, Stephen, Stephen Workman, V. Nicole Gallagher, Donald L. Stamper, and Robert Coleman. (2008) Composting Horse Muck. Cooperative Extension Service, University of Kentucky College of Agriculture.
- Higgins, Steve, Sarah Wightman, and Ray Smith. (2011) Enhanced Vegetative Strips for Livestock Facilities. Cooperative Extension Service, University of Kentucky College of Agriculture.
- Hooda, P. S., A.C. Edwards, H.A. Anderson, and A. Miller. (2000) A review of water quality concerns in livestock farming areas. *The Science of the Total Environment* 250: 143-167.
- Horrigan, Leo, Robert S. Lawrence, and Polly Walker. (2002) How Sustainable Agriculture Can Address the Environmental and Human Health Harms of Industrial Agriculture. *Environmental Health Perspectives* 110: 445-456.
- Hunt, William F. (2001) Designing Rain Gardens (Bio-Retention Areas). Cooperative Extension Service, North Carolina A&T State University.
- Hutchison, M. L., L. D. Walters, S. M. Avery, B. A. Synge, and A. Moore. (2004) Levels of zoonotic agents in British livestock manures. *Letters in Applied Microbiology* 39: 207-214.
- IDEXX Laboratories, Inc. Water Microbiology. (2012) Scientific Basis: How Colilert Works. http://www.idexx.com/view/xhtml/en_us/water/colilert.jsf. Accessed 10 September 2012.
- IDEXX Laboratories, Inc. Water Microbiology. (2012) Scientific Basis: How Quanti-Tray Works. http://www.idexx.com/view/xhtml/en_us/water/quanti-tray.jsf. Accessed 10 September 2012.
- Kadlec, Robert H., and Robert L. Knight. (1996) *Treatment Wetlands*. Lewis Publishers, Boca Raton.
- Kato, Tasuku, Hisao Kuroda, and Hideo Nakasone. (2009) Runoff characteristics of nutrients from an agricultural watershed with intensive livestock production. *Journal of Hydrology* 368: 79-87.

- Kentucky Division of Water. (2011) 2010 Integrated Report to Congress on the Condition of Water Resources in Kentucky. Volume II 303(d) List of Surface Waters.
- Kentucky Division of Water. (2012) Chapter 10:031 Surface Water Standards. 401 Kentucky Administrative Regulations Water Quality Standards.
- Kentucky Mesonet. (2012) Monthly Climatological Survey. www.kymesonet.org. Accessed 23 July 2012.
- Koirala, Shesh R., Randall W. Gentry, Edmund Perfect, John. S. Schwartz, and Gary S. Saylor. (2008) Temporal Variation and Persistence of Bacteria in Streams. *Journal of Environmental Quality* 37: 1559-1566.
- Lin, Ying-Feng, Shuh-Ren Jing, Der-Yuan Lee, and Tze-Wen Wang. (2002) Removal of Solids and Oxygen Demand from Aquaculture Wastewater with a Constructed Wetland System in the Start-Up Phase. *Water Environment Research* 74: 136-141.
- Loehr, Raymond C. (1967) Effluent Quality from Anaerobic Lagoons Treating Feedlot Wastes. *Journal (Water Pollution Control Federation)* 39: 384-391.
- Loehr, Raymond C. (1971) Alternatives for the Treatment and Disposal of Animal Wastes. *Journal (Water Pollution Control Federation)* 43: 668-678.
- Martinez, Jose, Patrick Dabert, Suzelle Barrington, and Colin Burton. (2009) Livestock waste treatment systems for environmental quality, food safety, and sustainability. *Bioresource Technology* 100: 5527-5536.
- Moore, M.F., J.H. Rogers, C.M. Cooper, S. Smith Jr. (2000) Constructed wetlands for mitigation of atrazine-associated agricultural runoff. *Environmental Pollution* 110: 393-399.
- Natural Resources Conservation Service. KY. (2003) Conservation Practice Standards, Waste Storage Facility, Code 313. Field Office Technical Guide Section IV.
- Nokes, Rita L., Charles P. Gerba, and Martin M. Karpiscak. (2003) Microbial Water Quality Improvement by Small Scale On-Site Subsurface Wetland Treatment. *Journal of Environmental Science and Health A38*: 1849-1855.
- Pitcairn, C. E. R., I.D. Leith, L. J. Sheppard, M. A. Sutton, D. Fowler, R. C. Munro, S. Tang, and D. Wilson. (1998) The relationship between nitrogen deposition, species composition and foliar nitrogen concentrations in woodland flora in the vicinity of livestock farms. *Environmental Pollution* 102: 41-48.
- Pundsack, Jonathan, Richard Axler, Randall Hicks, Jerald Henneck, Delwin Nordman, and Barbara McCarthy. (2001) Seasonal Pathogen Removal by Alternative On-Site Wastewater Treatment Systems. *Water Environment Research* 73: 204-212.

- Quiñónez-Díaz, María de J., Martin M. Karpiscak, Eric. D. Ellman, and Charles P. Gerba. (2001) Removal of Pathogenic Indicator Microorganisms by a Constructed Wetland Receiving Untreated Domestic Wastewater. *Journal of Environmental Science and Health A36*: 1311-1320.
- Raloff, Janet. (2000) More Waters Tests Positive for Drugs. *Science News* 157: 212.
- Robbins, Jackie W. D., David Howell, and George J, Kriz. (1972) Stream Pollution from Animal Production Units. *Journal (Water Pollution Control Federation)* 44: 1536-1544.
- Rogers, K. H., P. F. Breen, and A. J. Chick. (1991) Nitrogen Removal in Experimental Wetland Treatment Systems; Evidence for the Role of Aquatic Plants. *Research Journal of the Water Pollution Control Federation* 63: 934-941.
- Sobolewski, André. (1996) Metal species indicate the potential of constructed wetlands for long-term treatment of metal mine drainage. *Ecological Engineering* 6: 259-271.
- The Economist. (2010) A special report on water.
- The World Bank: Agriculture and Rural Development Department. (2005) *Managing the Livestock Revolution: Policy and Technology to Address the Negative Impacts of a Fast-Growing Sector*.
- Tunçsiper, Bilal. (2007) Removal of nutrient and bacteria in pilot-scale constructed wetlands. *Journal of Environmental Science and Health Part A*. 42: 1117-1124.
- Natural Resources Conservation Service, United States Department of Agriculture. (2002) *Constructed Wetlands 210-VI-NEH*. *Environmental Engineering National Engineering Handbook Part 637*.
- United States Department of Agriculture. (2009) *Census of Agriculture: United States Summary and State Data, Vol. 1, Geographic Area Series, Part 51*.
- United States Environmental Protection Agency. (2000) *Profile of the Agricultural Livestock Production Industry*.
- United States Environmental Protection Agency. (2008) *National Pollutant Discharge Elimination System Permit Regulation and Effluent Limitation Guidelines and Standards for Concentrated Animal Feeding Operations in Response to the Waterkeeper Decision; Final Rule*. *Federal Register Vol. 68, No. 29*. 40 CFR Parts 9, 122, 123, and 412.
- United States Environmental Protection Agency. (2012) 5.2 Dissolved Oxygen and Biochemical Oxygen Demand. *Water Monitoring and Assessment*. Accessed 10 October 2012.
- United States Environmental Protection Agency. (2012) 5.4 pH. *Water Monitoring and Assessment*. Accessed 10 October 2012.

- United States Environmental Protection Agency. (2012) 5.9 Conductivity. Water Monitoring and Assessment. Accessed 10 October 2012.
- United States Environmental Protection Agency. (2012) 5.10 Total Alkalinity. Water Monitoring and Assessment. Accessed 10 October 2012.
- University of Kentucky. College of Agriculture. (2012) Kentucky Equine Initiative Kentucky Equine Survey. kgs.uky.edu/kgsmap/kgsgeoserver/viewer.asp. Accessed 7/10/2012.
- University of Kentucky. Kentucky Geological Survey. Kentucky Geologic Map Information Service. Accessed 4/18/2012.
- World Health Organization. (2011) Guidelines for Drinking-water Quality, 4th Edition.
- Wolverton, B. C., Rebecca C. McDonald. (1979) Upgrading Facultative Wastewater Lagoons with Vascular Aquatic Plants. *Journal (Water Pollution Control Federation)* 51: 305-313.
- Yalcuk, Adra, and Aysenur Ugurlu. (2009) Comparison of horizontal and vertical constructed wetland systems for landfill leachate systems. *Bioresource Technology* 100: 2521-2526.

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