ESCHERICHIA COLI REGROWTH AND MACROINVERTEBRATE HEALTH IN URBAN AND RURAL STREAMS

A Thesis

by

KATHRYN JORDAN MCCRARY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2011

Major Subject: Water Management and Hydrological Science

ESCHERICHIA COLI REGROWTH AND MACROINVERTEBRATE HEALTH IN URBAN AND RURAL STREAMS

A Thesis

by

KATHRYN JORDAN MCCRARY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Chair of Committee,	Jacqueline Aitkenhead-Peterson
Committee Members,	Terry J. Gentry
	Sam Feagley
	Francis Gelwick
Chair of Intercollegiate Faculty,	Ronald Kaiser

May 2011

Major Subject: Water Management and Hydrological Science

ABSTRACT

Escherichia coli Regrowth and Macroinvertebrate Health in Urban and Rural Streams.

(May 2011)

Kathryn Jordan McCrary, B.S., University of Miami

Chair of Advisory Committee: Dr. J A Aitkenhead-Peterson

Over the last few decades, increased urbanization has led to a new recognition in stream health – the urban stream or the urban stream syndrome. Understanding urban water quality is important for identifying those factors or sources that contribute to impairment. Many streams are listed as impaired because of the increased concentrations of pathogens. While wastewater treatment plants (WWTPs) discharge effluent that has been disinfected, often downstream from WWTPs point sources are high numbers of indicator bacteria, *Escherichia coli.* This study collected data on the recovery and regrowth of *E. coli* by collecting ultraviolet light treated effluent from the Carters Creek WWTP and spiked it with three different concentrations of DOC derived from a leaf and grass extract. *Escherichia coli* were enumerated at 6, 12, 18, 24, 36, 48, and 72 hours. After 6 h growth for each of the grass treatments, except for the control and high grass treatment exceeded the primary contact recreation standard for surface water quality. At 18h the low and high leaf treatments exceeded the primary contact recreation standard for surface water quality. The chemistry of each flask was analyzed for DOC, total N, NO_3-N, NH_4-N, Na^+, K^+, Mg^{+2}, Ca^{+2}, F^-, Cl^-, SO_4^{-2} and PO_4^{-3} at t=0

and t=72 h. CNP values for both leaf and grass treatments ranged from 2.22 -36.5. Regrowth was not observed in those treatments with a CNP value below 5. Biodegradability of the treatments was examined to identify the limiting nutrient. By focusing on reducing the CNP value below 5 of the receiving water, recovery and regrowth of *E. coli* downstream from WWTPs can be reduced. The biodegradability test suggested that in the presence of excess DOC, N is the limiting nutrient.

Certain macroinvertebrate species, *Ephermeroptera, Trichoptera, and Plecoptera* (EPT), are indicators of good stream health. Macroinvertebrates were collected at nine watersheds within the Bryan/College Station area, a rapidly urbanizing community, upstream and downstream from WWTPs and analyzed for relative abundance of pollution intolerant (%EPT) and pollution tolerant species. All sites downstream from a WWTP had %EPT present in the collection.

DEDICATION

In memory of my sister Jade.

I dedicate this thesis to all those who believed in me.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Aitkenhead-Peterson, and my committee members, Dr. Gentry, Dr. Feagley, and Dr. Gelwick for their patience, guidance and support throughout the course of this research. I would also like to acknowledge Greg Wall and staff of Carters Creek Waste Water Treatment plant, College Station TX, for helping me navigate the plant. I would like to thank Nina Stanley for her help with the chemical analyses in the nutrient and water analysis laboratory and Heidi Mjelde for help with *E. coli* methodology and quantification. I especially thank Jim Totten and Meredith Bilek for all their advice and editing skills. I wish them luck in their pursuits of their PhDs. Thank you also to Dr. Valeen Silvy and Dr. Ronald Kaiser for believing in me and my desire to be a part of the water program.

Thanks go also to my friends and colleagues and the Water Management and Hydrologic Science department faculty for making my time at Texas A&M University a great experience. Special thanks to Dr. Brian Williams and group who never let me give up and supported me through the development, defense, and editing of this document. Also, thanks to Max who stuck with me and was patient and supportive.

Thanks to my entire extended family, my Disney family, and friends who have watched me grow and told me I could do great things. Thanks to my Nana, Pap and Mama Marge for instilling me with a love of nature, and to my brother who never failed to ask me how I was doing. Finally, thanks to my mother and father for their endless support and encouragement

NOMENCLATURE

BOD	Biological Oxygen Demand
CSO	Combined Sewer Overflow
CFU	Colony Forming Units
DDW	Double Distilled Water
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
EC	Electrical Conductivity
EPT	*Ephemeroptera*, *Plecoptera*, and *Trichoptera*
NPDES	National Pollutant Discharge Elimination System
TCEQ	Texas Commission on Environmental Quality
TDN	Total Dissolved Nitrogen
TSS	Total Suspended Solids
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
WWTP	Wastewater Treatment Plant

TABLE OF CONTENTS

Page

ABSTRACT iii

DEDICATION v

ACKNOWLEDGEMENTS vi

NOMENCLATURE vii

TABLE OF CONTENTS viii

LIST OF FIGURES x

LIST OF TABLES xi

1. INTRODUCTION 1

    1.1 Effect of Urbanization on Rivers and Streams 2
        1.1.1 Point source and nonpoint source pollution inputs 5
        1.1.2 Wastewater treatment plant effluent 7
        1.1.3 Wastewater disinfection 9
    1.2 *E. coli* as an Indicator of Pathogens 11
        1.2.1 Pathogens, a growing problem and increasing risk to human health 13
    1.3 Urban Water Quality Effects on Benthic Invertebrates 16
    1.4 Objectives of this Study 19
        1.4.1 *E. coli* regrowth and biodegradability study 19
        1.4.2 Benthic invertebrate in urban watershed study 19

2. *ESCHERICHIA COLI* REGROWTH AND BIODEGRADABILITY OF FRESHWATER NUTRIENTS 20

    2.1 Introduction 20
    2.2 Materials and Methods 26
        2.2.1 *E. coli* regrowth rates 26
        2.2.2 Effect of chemical constituents and nutrient ratios on *E. coli* regrowth in laboratory incubations and *E. coli* numbers in field conditions 28
        2.2.3 Biodegradability of carbon, nitrogen, and orthophosphate-P 29

Page

2.2.4 Chemical analyses 30
2.2.5 Statistical analysis 31
2.3 Results 32
2.3.1 *E. coli* regrowth 32
2.3.2 C/N/P values on *E. coli* 37
2.3.3 Biodegradability of carbon, nitrogen, and orthophosphate-P 45
2.4 Discussion 47
2.4.1 *E. coli* regrowth and DOC 47
2.4.2 *E. coli* and C/N/P values 50
2.4.3 Biodegradabilty 52

3. BENTHIC INVERTEBRATES IN URBAN WATERSHEDS 56

3.1 Introduction 56
3.2 Materials and Methods 59
3.2.1 Site description 59
3.2.2 Field sampling 60
3.2.3 Laboratory analyses 61
3.2.4 Chemical analyses 61
3.2.5 Statistical analyses 63
3.3 Results 63
3.3.1 Invertebrate sampling 63
3.3.2 Relationship between invertebrates and stream chemistry 67
3.4 Discussion 72
3.4.1 Relative abundance of %EPT and stream chemistry 73
3.4.2 Landuse and invertebrate health 75
3.4.3 Limitations of the study 77

4. CONCLUSIONS 78

4.1 Conclusions 78

REFERENCES 79

APPENDIX I: *E. COLI* COUNTS AND C AND N IN SUBCATCHMENTS 89

APPENDIX II: *MICROCOSOMS* AND CHEMISTRY 93

VITA 101

LIST OF FIGURES

FIGURE Page

2.1 *Escherichia coli* growth over the 72 hour period 34

2.2 Effect of incubation water C/N/P value on *E. coli* regrowth in A)grass extract and B) leaf extract 40

2.3 Relationship between *E. coli* 72 h and initial incubation solution C/N/P value 41

2.4 Watersheds used for examining stream C/N/P value and *E. coli* CFUs 42

2.5 Relationship between stream log *E. coli* and C/N/P value..... 44

2.6 Biodegradability of DOC, TDN and PO_4-P under control, low, medium, and high DOC concentrations 46

3.1 Relationship between the percentage relative abundance of pollution intolerant benthic macroinvertebrates and the C:N, C:P, N:P ratios and C/N/P values of stream chemistry 71

3.2 Relationship between the percentage relative abundance of pollution tolerant benthic macroinvertebrates and the C:N, C:P, N:P ratios and C/N/P values of stream chemistry 72

LIST OF TABLES

TABLE		Page
2.1	Mean volumes of grass and leaf extract and ultra-pure water used in incubation flasks for the *E. coli* regrowth experiment	27
2.2	Average *E. coli* colony forming units (Log_{10} CFU/100 mL) at each time period with control, low, medium, and high DOC concentrations of A. grass extract and B. leaf extract	33
2.3	Probability values using one-tail t-tests to examine the effect of extract on *E. coli* growth	35
2.4	Correlation of initial individual chemical constituents (t=0) and nutrient ratios on *E. coli* regrowth over time	37
2.5	Mean concentrations of DOC, TDN, and PO_4-P, mass of C, N and P, and nutrient ratios in incubation flasks at t=0, n=3 for each treatment	38
3.1	Selection of invertebrate orders based on their pollution tolerance levels.	58
3.2	Geographic locations of the stream sites divided up by streams without WWTP and those with WWTP	60
3.3	Numbers of individual benthic invertebrates collected and number of species at each stream site	65
3.4	Number of individuals and relative abundance of benthic invertebrates in streams sites	66
3.5	Stream chemistry at the time of benthic invertebrate collection	68
3.6	Effect of stream chemistry on %EPT abundance	70

1. INTRODUCTION

More than seventy-nine percent of the United States population lives in an urban area (Population Reference Bureau 2010). In the year 2007, the United Nations suggested that 50% of the global population of humans would reside in cities by the year 2008 (United Nations 2007). This shift in population location from rural to urban centers is putting a stress on one of our fundamental natural resources, water. Urbanization has been identified as one of the biggest causes of impairment to our water bodies (Paul and Meyer 2001). The water quality in streams and rivers in many urban watersheds is so impaired that it has major consequences for downstream reaches affecting aquatic health, wildlife health, and human health (e.g. Trueman and van den Hurk 2010; Davis et al. 2007). Anthropogenic activities associated with urbanization include clearing land for sub-divisions and adding non-native vegetation species that sometimes add to diversity (Hope et al. 2003), and increasing coverage of impervious surfaces. These changes alter the hydrology and radically increase the proportion of point source nutrient and pollution inputs across the landscape relative to diffuse source inputs leading to the degradation of rivers and streams within urban areas (Warkentin 2001). The complex interactions among biological, chemical, and ecological processes within a watershed further compound degradation (Gómez et al. 2008). This is apparent from studies of the water chemistry, pathogens loads, and aquatic communities of urban ecosystems (e.g. Brett et al. 2005; Petersen et al. 2006; Meyer et al. 2005a).

This thesis follows the style of *Aquatic Sciences.*

1.1 Effect of Urbanization on Rivers and Streams

The impacts of urbanization on rivers and streams are issues warranting further research at the scale of ecosystems (e.g. Aitkenhead-Peterson and Volder 2010). Urbanization can affect riparian function (Stander and Ehrenfeld 2010), stream hydrology, chemistry, geomorphology, temperature, and aquatic life (Paul and Meyer 2001; Burian et al. 2010; Steele et al. 2010; Roy et al. 2010). The physical response of surface water to increased impervious cover in urban watersheds includes decreased lag time and increased peak levels in discharge after precipitation events (Leopold 1968; Arnold and Gibbons 1996), which typically result in geomorphologic changes to stream channels through accelerated erosion and increases in channel width and depth (Hammer 1972; Dunne and Leopold 1978; Booth and Jackson 1997). Increases in water temperature related to increased impervious surface area (Galli 1991) combine within in a watershed to decrease fish diversity (Klein 1979; Schueler and Galli 1992) and benthic macro-invertebrate diversity (Klein 1979; Jones and Clark 1987; Schueler and Gall 1992) thus compromising biotic integrity. Even watersheds with as little as 2% effective impervious surface can impact algal biomass and diatom assemblages relative to similarly sized, but non-urbanized, watersheds (Newall and Walsh 2005; Walsh et al. 2005). The term "urban stream syndrome" was coined by Walsh et al. (2005) in recognition of the collective degradation effects of urbanization on streams and creeks. They suggested that although the impact of urbanization on surface waters can be quite complex, the major driver for surface water degradation was storm water.

Urban ecosystems are not going to disappear (Aitkenhead-Peterson and Volder 2010), yet few researchers have risen to the challenge to examine the ecology of urban ecosystems, preferring to seek out pristine landscapes (Corbyn 2010). Effects of urban stream syndrome can be mitigated with the tools we currently possess (Walsh et al. 2005). These tools include public education about stream ecology, implementing low-impact designs for housing sub-divisions, and reducing inputs of nutrients and pollutants through best management practices (Walsh et al. 2005). Although some modifications can be made that lower impacts of development in urban and suburban areas (Bedan and Clausen 2009; Sun et al. 2010), such as use of pervious concrete (Viswanathan et al. 2011) and inclusion of stream restoration objectives (Klocker et al. 2009; McBride et al. 2010; Suren 2009) it is unlikely that urban streams will be returned to their pristine state. Often studies of urban stream quality have been conceptualized as a gradient of interrelated components and compared to gradients in rural areas (e.g. Aitkenhead-Peterson et al. 2009, 2011; Kiulia et al. 2010; Kuhl et al. 2010; O'Brien and Wehr 2010). Particular among those commonly recognized as responsible for differences in water quality, is dissolved organic carbon (e.g. Aitkenhead-Peterson et al. 2005; Perakis and Hedin 2007); others are soil type, which determines the hydraulic flow route through soil; parent material, which determines the base flow water chemistry; channel dynamics, which may determine sediment concentrations and loads; climate, vegetation types, and associated soil microbiology; watershed aspect and more recently, the effect of irrigation water chemistry (Aitkenhead-Peterson et al. 2009, 2011). Degraded or impaired surface waters may also have elevated *E. coli*, nutrients, metals, and numerous

organic pollutants, and lower oxygen concentration relative to EPA or individual state criteria (Brett et al. 2005).

Some of the major changes to streams observed after urbanization are in the stream chemistry. For example, higher concentrations of sodium and chloride have been observed in temperate and sub-tropical urban streams and rivers (e.g. Kaushal et al. 2005; Steele and Aitkenhead-Peterson in press). Increased nutrient concentrations and loading rates have also been reported (e.g. Ulseth and Hershey 2005; Klocker et al. 2009; Aitkenhead-Peterson et al. 2011). Within an urbanized watershed, nutrients have several sources. Delivery of nitrogen is postulated to be via impervious surface runoff, from fertilized lawns, stormwater runoff, and sewage effluents (Ulseth and Hershey 2005). Increased human population density is correlated with wastewater inputs contributing to increased nitrogen in streams (Klocker et al. 2009). These higher nitrogen loads override what would otherwise be a nutrient limitation to production in benthic macro-invertebrate and microbial communities in fresh water aquatic ecosystems (Klocker et al. 2009). As inputs of phosphorous and nitrogen into freshwater bodies increase, the increased risk of eutrophication is transferred downstream as channels widen, the canopy opens to allow more sunlight, and water velocity decreases allowing more time for substrate water column interaction and shorter nutrient spiraling distances. Increased nutrients lead to decreased dissolved oxygen in urban streams. Carbonaceous biological oxygen demand (BOD), suspended sediments and dissolved ammonia are all factors that contribute to low dissolved oxygen (DO) concentrations in freshwaters (Viessman and Hammer 2005). The depletion of DO in the water affects the production

of fish and other aquatic life, the odor of decomposing organic matter can eliminate a streams intended beneficial use (Viessman and Hammer 2005) and the creation of low DO lead to dead zones for fish and benthic communities.

Metal concentrations can sometimes be high in urban storm water runoff resulting from contact with roofs, buildings, cars and other anthropogenic materials (e.g. Calabro 2010; Sakata et al. 2010). Heavy metals in concentrations above normal can cause a disruption in physiological functions that lead to detrimental and/or permanent damage to organs. These metals, particularly if methylated, and other organic pollutants carried by storm water can lead to increased health risks and mortality rates in both humans and wildlife, depending on accumulation rates within body tissues and organs (Novotny 2003).

One of the major challenges to rehabilitating impaired surface waters is to discover the dominant sources of impairment across two categories – point source and nonpoint source.

1.1.1 Point source and nonpoint source pollution inputs

Point and nonpoint sources of pollution are managed differently, but each contributes to water quality degradation in urban streams under different conditions and on different time schedules. Each source must be considered to identify the management strategies needed to improve water conditions.

Diffuse or nonpoint source pollution originates from the atmosphere, land surface, and subsurface systems. Warkentin (2001) stated, *"Diffuse pollution arises on*

the land; the amounts of pollutants entering water bodies depend upon the conditions of the land surface". Nonpoint sources include atmospheric deposition, runoff from land use and management practices that include agriculture, silviculture, mining, construction, urban impervious areas, lawns and gardens, golf courses, failing septic systems, and wildlife feces (Scheriber et al. 2001). Nonpoint or diffuse pollution cannot be traced back to a specific source. The transport of bacteria entrained in surface runoff is documented. For example, *Escherichia coli* attached to larger soil particles (> 45 µm) was not as easily moved across the surface during overland flow as those unattached *E. coli* (Muirhead 2006). Nonpoint source pollution is more difficult to counteract because it is often the responsibility of everyone within a watershed to understand how their practices might affect local water resources. Best management practices were recommended throughout the watershed to abate the effects of nonpoint pollution (Novotny 2003).

More easily identifiable and controllable are point sources of water degradation. Two characteristics of point source pollution are that it enters a receiving water body at identifiable locations and that it carries pollutants (Novotny 2003). Primary point sources of surface water pollution are permitted municipal wastewater systems, industrial and agricultural wastes, and point-source leachates from landfill sites (Viessman and Hammer 2005). Other point sources of pollution include wastewater effluent and combined sewer overflows (CSOs) that are characterized by having a National Pollutant Discharge Elimination System (NPDES) permit (Viessman and Hammer 2005).

Pollutant sources that are controlled by government regulation and require monitoring include municipal and industrial wastewater effluents, runoff and leachate from solid waste disposal sites, runoff and infiltrated water from concentrated animal feeding operations, runoff from industrial sites, storm sewers, CSOs, mine drainage, and runoff from construction sites (Novotny 2003). Point sources can be associated with the polluter pays principle, which requires a measure of clean up before discharge.

The divergent regulatory systems for managing each type of pollutant reflect the challenges associated with each pollutant stream. Widespread general management practices are applied for diffuse sources that have multiple inputs, whereas direct monitoring and onsite treatments are applied to manage localized or point sources. Although both diffuse and point source pollutants follow different routes into urban streams their impacts are similar. Regardless of the source, increased loading from nutrients, sediments, thermal effluent or pathogens all negatively impact the water

1.1.2 Wastewater treatment plant effluent

One of the most commonly recognized point source of discharges is the municipal waste water treatment plant (WWTP). Raw wastewater contains pathogens, nutrients, solids, and heavy metals, as well as personal care products that are recognized as dangers to surface water health and consequently regulated to remove them via wastewater treatment facilities. Waste water treatment plants provide different levels of treatment that have advanced with new technology, and regulations imposed on

treatment facilities have greatly improved the conditions and overall pollutant loadings of effluents entering river and streams (Novotny 2003).

One type of discharge that is most detrimental to stream health is raw untreated wastewater (Novotny 2003). The primary treatment of municipal wastewater removes larger solids by screening and settling, which removes about 25% of the biodegradable organic matter (Jjemba 2004). During secondary treatment, soluble organic material is degraded and mineralized through both aerobic and anaerobic processes. Aeration stimulates aerobic bacteria to form an activated sludge that consumes biodegradable organic material and bind less soluble material. Flocculated solids are then settled out and depending upon the treatment plant design, are either aerobically or anaerobically digested. The solids from primary and secondary treatment are flocculated, settled and then subjected to anaerobic digestion driven by bacteria that hydrolyze and ferments the sludge (Jjemba 2004). Effluent is then typically ready for disinfection, but some treatment plants utilize an optional tertiary treatment. Tertiary treatment removes non – biodegradable organic pollutants. Several different methods are implemented to remove the different types of nitrogen, carbon, and phosphorus pollutants. Activated carbon can remove synthetic pollutants such as chlorophenols and polychlorinated biphenyls. Additional areas such as constructed wetlands within the treatment plant, where denitrification and removal of excess nitrates can occur. Addition of lime and iron or aluminum sulfates to the effluent result in the precipitation of phosphorus from the effluent. Depending on the environment into which effluent is being discharged, tertiary treatment may be needed to remove specific nutrient and organic pollutants perceived as

a problem in local waters. Wastewater treatment plant discharges also deliver organic matter and nutrients into receiving waters (Wakelin et al. 2008). As a final step to treatment, WWTPs depend on natural systems to dilute and further purify the water. For example, permits allow greater discharge from WWTPs during rain events than during the dry summer months. Wastewater treatment plants use disinfection to reduce incidences of waterborne diseases caused by pathogens that otherwise might be discharged into streams. However, disinfection by-products and sometimes created and released from these treatment plants, but the benefits to society have been decided to outweigh the risk of these byproducts (Hrudey 2009). There are three major types of disinfectant treatments used in WWTPs in the United States; chlorine, ultraviolet (UV) radiation, and ozone. In accordance with state and federal law, each type of plant is required to monitor for formation of disinfectant byproducts, and meet the mandated minimum concentration levels.

1.1.3 Wastewater disinfection

Chlorination is most popular for disinfection treatment to kill pathogens in wastewater discharge (Alonso et al. 2004). During chlorination, bacterial cells are severely injured to the point of lysis (Chambers 1971). However some bacteria are not killed because their cell walls remain intact, although their metabolic processes cease under exposure to chlorine (USEPA 2001). Damaged bacteria can be considered viable, but not culturable, and are a puzzle to microbiologists (Bolster et al. 2005). Generally, chlorine is considered an effective treatment, but in the presence of organic matter and

ammonia-nitrogen it can cause disinfection byproducts (DBPs) to form (Mezzanotte et al. 2007; Alonso et al. 2004). The interaction of hypochlorous acid and hypochlorite with humic and fulvic acids leads to formation of trihalomethanes which can compromise the quality of the water discharged from a WWTP particularly if receiving surface water is abstracted downstream for a water supply (Hasan et al. 2010). Trihalomethanes are a high risk to human health, as they are linked to cancer and reproductive disorders (Hasan et al. 2010).

In response to the concern for human health, and the effect of DBP's, the properties of ultraviolet light were recognized and developed as a treatment. Because UV radiation is not chemical, there are no toxic substances to handle or worry about when discharging treated wastewater. Furthermore, the exposure time needed for the effectiveness of the treatment is shorter than for chlorine and other treatment types such as ozone (USEPA 2003). Fundamentally, the treatment is exposure of pathogenic organisms to light within the UV spectrum; ideal wavelength range is 200 to 300 nm (USEPA 2003). Disinfection by UV light and chlorine treatment differ in how they target the cells of pathogens. Chlorine targets cellular structures in order to interfere with metabolism and normal cell functions, whereas UV light damages DNA or RNA preventing cell replication (USEPA 2003), but in some cases, microorganisms can repair their DNA (Antonelli et al. 2008). Currently, researchers are seeking to understand the mechanisms behind bacteria's ability to reverse the UV damage in the presence of light, photoreactivation, and dark repair (Antonelli et al. 2008). Effectiveness of a UV system depends upon the intensity of the UV radiation, exposure time to UV light, and the

configurations of the reactor (USEPA 2003). Positive results of UV radiation that achieve damage to viruses, spores, and cysts, depend on radiation being absorbed by microorganisms. Therefore, effluent that contains high amounts of total suspended solids (TSS) can reduce in the effectiveness of UV by protecting microorganisms via shading effects (Antonelli et al. 2008). Other inorganic and organic compounds affect efficiency of disinfection by absorbing UV radiation, thus reducing its intended absorbance by microorganisms (Antonelli et al. 2008).

Ozone is another type of disinfection treatment that involves bubbling ozone gas through the effluent. Its benefit is cell lysis in microorganisms and viruses by destruction of the nucleic acids purine and pyrimidine (Alonso et al. 2004). Its cost is the great amount of energy needed to produce ozone that has an average stability of only about 20 minutes in water (Alonso et al. 2004). While ozone is very powerful at eliminating the pathogenic organisms during the treatment process, once oxidized, it loses effectiveness as a disinfectant and cannot prevent regrowth of pathogens (Alonso et al. 2004). Continued advances in wastewater treatment technology should improve the quality of effluents being discharged to streams and rivers, and reduce their effects on the receiving waters (Abel 1989).

1.2 *E. coli* as an Indicator of Pathogens

Wastewater management involves constant tests of effluents for bacterial counts and water chemistry in order to deliver high quality water that does not degrade or impair receiving waters (USEPA 1986). This increases in importance over time

because water resources are finite, whereas our population is growing exponentially. One way to monitor bacterial loads is through the use of an indicator organism. As the EPA has directed, current water quality tests rely on monitoring of *Escherichia coli* (*E. coli*) as in indicator of fecal contamination (USEPA 1986). Many types of bacteria are present in effluent, but only a few types are actually harmful to humans; theses are collectively termed pathogens. Testing for a specific bacterial pathogen can be lengthy (24-48hrs) and expensive (USEPA 2001), which is further complicated by the viable but not culturable (VBNC) nature of bacteria in post-treatment wastewater. Use of an indicator organism has reduced the need for these tests. Scientists and public health officials have chosen to monitor non-pathogenic bacteria that are commonly associated with pathogens transmitted through fecal contamination, but that also are more easily sampled and measured. Such organisms are assumed to indicate the probability that human pathogenic organisms are present (USEPA 2001). Coliforms are the main group of indicator organisms that live in the gut of humans and other animals (USEPA 2001).

Two indicator organisms, *E. coli* and *Enterococci*, showed a strong correlation with pathogen presence; the former is in fresh waters only; the latter is in both fresh and marine waters (USEPA 2001). However, it is important to realize that water meeting the quality standards for counts can still contain *Cryptosporidium parvum, Giardia, Shigella,* sp. and *E. coli* O157:H7 (USEPA 2001). Therefore, the efficacy of these as indicator organisms is flawed; moreover *E. coli* commonly survives in the natural environment (Blatchley et al. 2007). The standards set for *E. coli* colony forming units (CFU) provide a chance to reduce probability of an infection, but do not guarantee

pathogen free water. Other nontraditional tests have been suggested by researchers and water quality managers to ensure public health and safety such as detection by nucleic acid material with polymerase chain reaction amplification (Theron and Cloete 2002). This type of test has been used to detect and distinguish between pathogenic and non pathogenic bacteria but is not yet approved by the EPA as an approved detection test (Theron and Cloete 2002).

As part of the 1972 National Clean Water Act, a section was created for the listing of impaired water bodies (Keller and Cavallaro 2008). The 303(d) list is a national registry of streams that are listed as impaired due to a variety of different factors which include high numbers of pathogens, excessive nutrients, or high sediment concentrations (Keller and Cavallaro 2008). Each state is required to maintain this list and for those streams listed, either develop a watershed protection plan or a total maximum daily load (TMDL) plan to address these impairments (Keller and Cavallaro 2008). Many streams, rivers, and creeks are listed as 303(d) impaired because they exceed the national standards for indicator organisms (Hathaway et al. 2009). Behavior of *E. coli* after wastewater disinfection, and its relationship as an indicator of pathogens, is important in protecting human health.

1.2.1 Pathogens, a growing problem and increasing risk to human health

Excess pathogens account for the majority of surface waters being placed on the 303(d) list for impairments (USEPA 2004). Pathogens are defined as disease causing organisms that affect animals and plants (Singh and McFeters 1992). Pathogens

typically occur among the taxa of bacteria, protozoans, viruses, or worms (Rosen 2000). They are present in human and animal feces and enter surface waters through direct defecation, and indirectly via transport in surface or subsurface flow (Rosen 2000). The survival rate of pathogens is based on quality of the receiving water due to turbidity, dissolved oxygen and nutrient concentrations, temperature, and the presence of inorganic and organic pollutants (Rosen 2000).

As early as the Romans, humans practiced waste management by installing sewer systems to transport storm runoff (Novotny 2003), but only as late as 1972, was the Clean Water Act passed, which required WWTPs to provide secondary treatment to reduce pathogens in effluent (Blatchley et al. 2007). The concerns for reducing human infection rates have increased and are a health priority. Untreated domestic wastewater may contain enteric bacteria, viruses, and protozoan cysts, which can cause illness if they enter the human body (USEPA 1999).

The most common waterborne diseases worldwide are the result of infections by *Shigella, Salmonella,* enterotoxigenic *Eshcerichia coli, Campylobacter jejuni, and Vibrio cholera* (Singh and McFeters 1992). Protozoa of concern are *Giardia lamblia* and *Cryptospyridium*, because infections are often fatal to humans (Singh and McFeters 1992). All of these pathogens can cause diarrhea, fever, vomiting and severe abdominal pain (Singh and McFeters 1992). Comparatively, the amount of harmful pathogens does not outnumber nonpathogenic bacteria in the environment (Blatchley et al. 2007). Among individuals at higher risk, such as the elderly and children five years old and younger, these pathogens can cause death or long term health problems. The

unintentional ingestion of these pathogens is commonly by fecal-oral routes (Singh and McFeters 1992). In Alpine Wyoming, an outbreak of *E. coli* O157:H7 had detrimental long term effects on four residents (Olsen et al. 2002). It is believed that local wildlife contaminated the city's drinking water through surface water that infiltrated the unconfined aquifer that supplied drinking water (Olsen et al. 2002). Effects of the *E. coli* O157:H7 led to hemolytic urea syndrome (HUS) that eventually causes renal failure (Olsen et al. 2002).

Bacteria can be either challenged by the natural environment after being damaged by treatment levels of chlorine or ultraviolet light, or recover and even thrive. The survival and detection of fecal indicators has been documented in soil and streambeds (e.g. Davies et al. 1995; Walters et al. 2007), but the recovery and regrowth of *E. coli* specifically after WWTP disinfection still requires more research. In an urban stream bacteria are challenged by their new aqueous environment where effluent is diluted into the receiving water.

It is difficult to identify the post-treatment fate of these severely damaged cells (USEPA 2001). The goal of measuring water quality is that water bodies meet their intended uses, and to reduce risks to human health. Humans can be infected by ingesting contaminated fish and shellfish, and through skin contact or ingestion of contaminated water (USEPA 2001). The factors that affect *E. coli* survival in the natural environment must be understood before it can be a reliable indicator organism (Barcina et al. 1989). Although the spread of waterborne disease has decreased dramatically since the beginning of the 20th century, concerns for outbreaks remain (Du Preez et al. 1995). The

most important waters to monitor for pathogenic contamination were identified by EPA (2001) as those designated for recreation (primary and secondary contact), public water supply, aquifer protection, and protection of fish, shellfish, and wildlife.

1.3 Urban Water Quality Effects on Benthic Invertebrates

Benthic macro-invertebrate health has been studied more along the urban corridor than any other benthic community (Paul and Meyer 2001). An advantage of sampling invertebrate communities to monitor aquatic ecological health is the relatively low cost of collection and analyses (Carone et al. 2009). Benthic invertebrates are useful indicators for stream pollution because some species are sensitive to pollution and others are tolerant (e.g., Hoiland and Rabe 1992; Cuffney et al. 2010).

Although dissolved oxygen concentration in streams is the most important parameter in the survival of invertebrates in impaired streams (Novotny 2003), the invertebrate community also responds to changes in physical habitat, turbidity, hydrology, and the concentrations of organic and inorganic contaminants (see Paul and Meyer 2001 review paper). A general response of invertebrates to urbanization is decreased diversity due to toxins, temperature change, siltation, and organic nutrients (Paul and Meyer 2001 review paper). Typically, in highly polluted streams the abundant taxa are those tolerant of pollution (Walsh et al. 2005). Due to their relative immobility, although some drift is often observed, macroinvertebrate communities can help to identify different stressors in addition to known point sources of pollution (Carone 2009).

Changes in the urban landscape that lead to increased sediment transport to the stream channel and increased velocity accelerate channel erosion, which in turn influences nutrient spiraling, particularly for phosphorus (Walsh et al. 2005). Change in the hydrologic regime affects assemblage composition of invertebrates, depending on their mobility and adaptations for living in unstable versus stable habitats (Paul and Meyer 2001).

Point versus nonpoint sources of pollution can differentially affect assemblages of macroinvertebrates, which is useful information indicating to watershed managers the quality of surface water. Historically, one issue often associated with the decline in the diversity of benthic invertebrates was discharge of organic carbon from WWTPs that provided only primary treatment (Gücker et al. 2006). Since the passage of the Clean Water Act, the regulatory mandate for secondary treatment, and the increase in incentives to provide tertiary treatment, water quality issues now typically focus on inorganic pollutants (Gücker et al. 2006).

Land management practices and types of land cover and land use within a watershed can have profound effects on benthic macroinvertebrates. For example, downstream of a textile industry located on Don Carlos Stream in Argentina, permitted discharge affected the temperature, turbidity, BOD and ammonium concentrations of the stream (Gómez et al. 2008). The change in stream chemistry shifted the diversity and type of feeding groups of the benthic macroinvertebrate community in the channel downstream from the textile effluent (Gómez et al. 2008). For example, *Diptera* became

more abundant, which is a common response in streams with high levels of organic pollution (Gómez et al. 2008).

Raw and treated wastewater can alter surface water chemistry, thereby affecting benthic macroinvertebrate diversity and populations, but not always. Roberts et al. (2009) examined the effect of trout farm effluent on benthic macroinvertebrates and concluded that it was not the chemistry of the effluent that impacted macroinvertebrate diversity, but the volume of discharged sediments that filled the interstices of the stream bed. In a study conducted by Gücker et al. (2006), the aquatic insect community downstream from a WWTP that had modern technology and used tertiary treatment was found to be stable, and the strength of the change in community dynamics was influenced by the change in flow rates, chemistry of the WWTP effluent, as well as pre-discharge pollution and human population density. They concluded that nonpoint sources had a greater impact on the benthic macroinvertebrate assemblages than did the current modern effluent. Although benthic invertebrate numbers were significantly higher at effluent discharge sites, the taxa identified were typically dominated by Chironomidae and Oligochates (Gücker et al. 2006).

Bacteria are consumed by benthic invertebrates (Rosen 2000). When discharge from a WWTP still contains residual chlorine, it can kill members of the lower trophic levels in the food chain, and eventually affect higher trophic levels. Although benthic macro- invertebrates are one of the most highly studied groups in urban streams, little is known about the actual mechanism of decreased diversity. The invertebrate community is important because it influences the rest of the food chain. Also, the ability of organic

pollutants to bioaccumulate up the food chain should be considered when monitoring invertebrates.

1.4 Objectives of this Study

1.4.1 E. coli regrowth and biodegradability study

1. To evaluate the effect of dissolved organic carbon (DOC) quantity (or concentration) and quality (or type of vegetation extract) on *Escherichia coli* regrowth by using two types of extracts prepared from different organic material – a) highly managed Saint Augusting grass (*Stenotaphrum secundatum)* and b) Live Oak (*Quercus fusiformis*) leaves.

2. To evaluate the effects of nutrient ratios on *Escherichia coli* regrowth in UV treated wastewater effluent and on *E. coli* numbers in urban and rural streams.

3. To examine biodegradability of C, N and orthophosphate-P in treated effluent and it's receiving water.

1.4.2 Benthic invertebrate in urban watershed study

1. To investigate the relative abundance of benthic macroinvertebrates upstream and downstream of wastewater treatment plants.

2. To determine if stream chemical constituents; DOC, dissolved organic nitrogen (DON), NH_4-N, NO_3-N^-, $CaCO_3$, PO_4-P, Ca^{2+}, Mg^{2+}, $K^{+,}$ Na^+, $SO_4^{2-}$and Cl^- change the relative abundances of benthic macroinvertebrate taxa.

2. *ESCHERICHIA COLI* REGROWTH AND BIODEGRADABILITY OF FRESHWATER NUTRIENTS

2.1 Introduction

The number one cause of stream impairment in Texas is elevated numbers of pathogens (Texas Commission on Environmental Quality [TCEQ] 2010). Elevated pathogens prevent a stream or river from being used as a potable water source, or for contact recreation, because of their potential to increase risk of illness (TCEQ 2010). As directed by the United States Environmental Protection Agency (USEPA) (1986), water quality managers rely on indicator organisms, particularly *Escherichia coli* to indicate probability of fecal contamination and the presence of pathogens in the water body. In the State of Texas, contact recreation standards are calculated by the geometric mean number of *E. coli* in water samples (Texas Administrative Code 2000), which should not exceed 126 cells per 100 mL.

Many potential sources of pathogenic impairment relate to both point and non-point sources. While sources of these pathogens are still being evaluated and tracked, wildlife is presently considered a large contributor to elevated *E. coli* counts (Meyer et al. 2005b). In a watershed studied by Somarelli et al. (2007), geese and deer were found to be major sources of bacterial pollution during the winter, spring and summer. Source tracking appears to be able to identify animals responsible for bacterial pollution (Somarelli et al. 2007). However, wildlife sources of pathogens are so readily mitigated as are point sources of pollution (Meyer et al. 2005b).

Point source pollution is more accessible for regular monitoring and locations for action are readily identifiable. Despite the fact that wastewater treatment plants (WWTPs) disinfect water to kill pathogens before it is discharged, elevated numbers of colony forming units (CFU) of *E. coli* are typically detected in the watershed (Petersen et al. 2006). Thus, wastewater treatment plants tend to be identified as contributors to pathogen pollution. Petersen et al. (2006) examined the bacteria of two bayous within the Houston, TX area to determine the most likely causes of impairment. After evaluating data for 59 monitoring stations between 1973 and 2001, they concluded that WWTPs were a major contributor of fecal coliform to the bayous under dry weather conditions of discharge.

A growing concern of water quality managers is the potential for regrowth and recovery of pathogens injured after chlorination, and the photoreactivation and DNA repair after UV treatment, commonly used to disinfect waste water before it is discharged (DuPreez et al. 1995). Because UV treatment produces no chemical by products, it is a popular alternative to chlorination as a water disinfectant (Antonelli et al. 2008). During a pilot scale study (Antonelli et al. 2008) at a WWTP in Italy, the efficiency of UV treatment was evaluated. Results indicated that UV treatment efficiency was not linear at high UV doses and did not depend on total suspended solid (TSS) concentration (Antonelli et al. 2008). The effectiveness of UV treatment is still being examined, including studies of photoreactivation and DNA repair by pathogens in effluents (Antonelli et al. 2008). Bacterial regrowth, specifically regrowth of *E. coli*

discharged in effluents from WWTPs needs to be evaluated as a possible source of contamination by pathogens.

Harclerode (2009) reported greater numbers of *E. coli* colony forming units in urban streams in south-central Texas after rain events relative to numbers at low flow, suggesting the cause of high *E. coli* might be attributed to watershed sources rather than point source inputs such as WWTPs. Carters Creek (Segment 1209C) was first listed as impaired by bacteria in 1999 and continues to remain on the 303d list for this reason (TCEQ 2008). The Carters Creek WWTP effluent contributes significant flow during dry periods (TCEQ 2010); therefore, recovery and regrowth of *E. coli* could be considered as a possible reason for these regularly high counts of *E. coli*.

While DOC has been shown to stimulate growth in heterotrophic bacteria (Kirchman et al. 1997; Boualam et al. 2003), there is still a question of its exact role in the regrowth of colonies. Lim and Flint (1989) studied the survival behavior of *E. coli* in filtered and unfiltered lakewater from Tocil Lake at the University of Warwick, U.K. by adding synthetic sewage. From their survival experiments, it was surmised that C sources alone did not influence the survival of the ML30 *E. coli* strain (Lim and Flint 1989). In their earlier research it was discovered that competition for N by microorganisms was a major factor in the survival of *E. coli* in fresh water (Lim and Flint 1989). A further study conducted in the Pacific Ocean collected water samples along two transects and placed into bottles with different amounts of amino acids, ammonium, and glucose (Kirchman et al. 1997). After incubation, the results reported that glucose alone stimulated more abundance of heterotrophic bacteria than did

ammonium additions alone which lead to no growth observed (Kirchman et al. 1997). Researchers have found that *E. coli* increases in the water column after storm events are due to resuspension of *E. coli* when the bottom sediment or substrate of a stream or river is disturbed by high velocity flows and resuspended in the water column (Hendricks and Morrison 1967; Stephenson and Rychert 1982).

It is also important to consider that the receiving water chemistry also may affect the growth rate and recovery of bacterial pathogens in effluent that is discharged, and some studies support these effects. Bolster et al. (2005) examined microcosms filled with estuarine water from nine different locations along the sea coast of New Hampshire. After exposure to the many different nutrient combinations that the sites naturally afforded, bacterial recovery rates tended to increase as concentrations of nutrients and DOC increased (Bolster et al. 2005). Lower salinity concentrations (0 to 10.5 ppt) also led to higher recovery rates of *E. coli* in chlorinated effluent (Bolster et al. 2005). The study concluded that concentrations of culturable *E. coli* exposed to chlorine could increase significantly over a three-day period under the right receiving water conditions (Bolster et al. 2005).

Aquatic and terrestrial bacteria are able to use the carbon in natural organic matter as an energy source (McDowell et al. 2006). The origin of dissolved organic matter (DOM) also has an effect on the behavior of coliform bacteria (Boualam et al. 2003). The growth rates of ten different coliform bacteria in three different types of water: drinking water, diluted river water, and a supernatant of algal bloom water with

various concentrations of organic matter were significantly different because of the different labile characteristics of the DOM (Boualam et al. 2003).

In a study in Cucamonga Creek, a concrete-lined urban stream in southern California (Surbeck et al. 2010) dry and wet weather runoff contributed 100% of the indicator bacteria, and effluent from a WWTP contributed nutrients, DOC, and ammonium . As DOC concentrations increased, bacterial counts also increased and were positively correlated with DOC (Surbeck et al. 2010). Furthermore, the concentration of DOC and P in runoff was found to be a pertinent factor in bacterial survival in urban streams (Surbeck et al. 2010). The presence of nutrients, salinity, temperature, sunlight, suspended solids, substrate composition, and many other factors might have the ability to stimulate or inhibit recovery and regrowth of bacterial cells that were previously damaged during wastewater treatment. Further analyses of the relationship between *E. coli* regrowth rates and receiving water chemistry are therefore needed to further understand the mechanisms behind these links.

Hypotheses to be tested:

Objective 1: To evaluate the effect of dissolved organic carbon (DOC) quantity (or concentration) and quality (or type of vegetation extract) on *Escherichia coli* regrowth by using two types of extracts prepared from different organic material – a) highly managed Saint Augusting grass (*Stenotaphrum secundatum)* and b) Live Oak (*Quercus fusiformis*) leaves.

Ho1 There is no significant difference in regrowth rates of UV treated E. coli in sewage effluent when exposed to different concentrations and qualities of DOC.

H1 The regrowth of E. coli is affected by the concentration and quality of DOC made from different extracted vegetations. Turf grass extract will have a significantly higher regrowth rate than the leaf extract.

Objective 2: To evaluate the effects of nutrient ratios on *Escherichia coli* regrowth in UV treated wastewater effluent and on *E. coli* numbers in urban and rural streams.

Ho1 Nutrient ratios will have no effect on increasing E. coli colony forming units in laboratory incubations and urban and rural freshwater streams.

H1 The value of C/N/P will significantly affect E. coli regrowth in laboratory incubations and further will be related to increased colony forming units in urban and rural streams.

Objective 3: To examine biodegradability of C, N and orthophosphate-P in treated effluent and it's receiving water.

Ho1 Biodegradability of carbon, nitrogen and orthophosphate-P in grass and leaf extracts are equal.

H1 Grass extract carbon has greater biodegradability than leaf extract carbon.

2.2 Materials and Methods

2.2.1 E. coli regrowth rates

Dissolved organic carbon (DOC) stock solutions were prepared before the start of the experiment and kept in the refrigerator until completion of the experiment. Turf grass DOC stock was prepared by soaking air-dried thatch (100 g) collected from a highly managed Saint Augustine turfgrass neighborhood lawn in 2 L of double distilled water (DDW) representing a 20:1 water:vegetation mass ratio, for 48 h and stirring occasionally. The solution was then vacuum filtered through a 1.2 µm filter. The leaf litter DOC stock was made by soaking 100 g of air-dried ornamental Live Oak (*Quercus fusiformis*) leaves in 2 L of DDW for 48 h stirring occasionally. The solution was then vacuum filtered through a 1.2 µm filter. After filtration both extract solutions were autoclaved at 120 °C for 20 minutes to destroy any microbes present in the solution, and then capped and refrigerated to avoid outside contamination. Four 50 mL aliquots of each of the stock solutions were analyzed using high temperature platinum-catalyzed combustion with a Shimadzu TOC-VCSH and Shimadzu total measuring unit TNM-1 (Shimadzu Corp. Houston, TX, USA). Dissolved organic carbon was measured as non-purgeable C which entails acidifying the sample (250 µL 2M HCl) and sparging for 4 min with C-free air. The turfgrass DOC stock concentration was 1320 mg L^{-1} and total N was 186 mg L^{-1} giving a C:N ratio of 7.1. The leaf litter DOC stock concentration was 919 mg L^{-1} and total N concentration was 13.88 mg L^{-1} giving a C:N ratio of 66.2. The stock concentrations determined the DOC spike amount to be added to be added to each of the treatments. Three liters of UV treated wastewater effluent was collected from

Carters Creek Wastewater Treatment Plant, just prior to the start of the experiment on May 24, 2010. Three replicate flasks of each high, medium, and low DOC concentration treatments were prepared for each of the DOC extracts by combining 150 ml of waste water effluent (obtained just after UV treatment and prior to discharge to receiving waters) with DDW and a corresponding low, medium, or high concentration spike of DOC extract (Table 2.1). The incubator was set at 30 °C for 24 h before starting the experiment to stabilize the incubator

Table 2.1 Mean volumes of grass and leaf extract and ultra-pure water used in incubation flasks for the *E. coli* regrowth experiment. Effluent spikes of 150 mL were added to obtain a final volume of 500 mL for each flask. DOC concentrations at time = 0 are included. Control is effluent and DDW only with no DOC spike and represents the concentration of DOC in the effluent.

	Grass Extract			**Leaf Extract**		
Treatment	Grass Extract mL	DDW mL	DOC mg/L t = 0	Leaf Extract mL	DDW mL	DOC mg/L t = 0
Control	0	350	2.8	0	350	2.8
Low	8	342	16	5	345	13
Medium	41	309	73	24	326	62
High	82	268	138	48	302	103

Five hundred mL conical Erlenmeyer flasks were used for the incubation vessels. The flasks were acid washed and covered with Parafilm® to avoid outside contamination. Dissolved organic carbon spikes, effluent and DDW were added to each flask (Table 2.1) and the flasks swirled to mix prior to removal of 50 mL of sample for chemical analysis and analysis of E. coli growth at time zero. These samples were frozen

until analyses. Flasks were then placed in a static incubator at a temperature of 30° C for a total period of 72 h. Flasks were then placed in a static incubator at a temperature of 30° C for a total period of 72 h. The collected raw effluent was enumerated for colony forming units (CFUs) per 100 mL, 150 mL and 200 mL. After each of 6, 12, 18, 24, 36, 48 and 72 h, the flasks were removed from the incubator to test for *E. coli* growth. To determine *E. coli* growth, 50 mL samples were removed after thoroughly swirling the flask to mix and filtered through a sterile 0.45 μm Millipore filter and incubated on membrane Thermotolerant *E. coli*. (m TEC) agar for 2 hr at 35 ^{o}C and 22-24 h at 44.5^{o}C according to EPA Method 1603 (USEPA 2002).

For every flask sample, at least three dilutions were performed to obtain an accurate colony count. Dilutions varied depending on counts from prior method development experiments and from observing previous time samples. Flasks were replaced in the incubator after every time = x sample enumeration. The m TEC Agar Plates were counted for CFUs 24-26 h after sampling points and results were recorded. After the t=72 h enumeration, 100mL of sample was collected and frozen prior to chemical analyses.

2.2.2. Effect of chemical constituents and nutrient ratios on E. coli regrowth in laboratory incubations and E. coli numbers in field conditions

The individual chemical constituents in the incubation solution was examined at time = 0 against *E. coli* regrowth at each collection time (t = 0 h to t = 72 h) using Pearson bivariate correlation analysis to determine those chemical constituents that

might be responsible for *E. coli* regrowth. Furthermore, because of the potential for nutrient limitation which may affect *E. coli* regrowth, the *E. coli* at 72 h were examined using ratios of C:N, C:P, N:P and the C/N/P value to determine which set of nutrient ratios might best describe *E. coli* regrowth. The C/N/P value was determined by dividing the mass of C by the mass of N by the mass of P (Equation 1).

$$C/N/P = \frac{mass\ of\ C/mass\ of\ N}{mass\ of\ P} \qquad \text{(Eq. 1)}$$

Unpublished counts of *E. coli* colony forming units were taken in September 2007 from several independent streams around Bryan/College Station described in Aitkenhead-Peterson et al. (2009, 2011). This *E. coli* data was used with the water chemistry in water samples obtained on the same day to determine if field conditions for *E. coli* regrowth at that time were similar to those achieved in the laboratory.

2.2.3 Biodegradability of carbon, nitrogen and orthophosphate-P

Several studies have examined the biodegradability of DOC, some with added supplemental nutrients and others relying on the nutrients supplied in the incubation solution (e.g., McDowell et al. 2006). To examine the potential for nutrient limitation, the biodegradability of DOC, total nitrogen and orthophosphate-P was examined by deducting the mass of C, N or P in the output (O) solution or t = 72 h from the mass of C, N or P in the input solution (I) or t = 0 h (Equation 2)

$$\%Biodegradability = \frac{I_{CNP} - O_{CNP}}{I_{CNP}} \times 100 \qquad \text{(Eq. 2)}$$

Where: CNP is either carbon (C), total nitrogen (N) or orthophosphate-P (P) and I is mass of C, N or P in the flask at t = 0 h assuming a volume of 500 mL and O is mass of C, N or P in the flask at t = 72 h also assuming a volume of 500 mL.

2.2.4 Chemical analyses

Chemical analyses were performed on the t=0 h and t=72 h samples collected from each flask. Dissolved organic carbon and total dissolved nitrogen (TDN) were measured using high temperature Platinum-catalyzed combustion with a Shimadzu TOC-VCSH and Shimadzu total measuring unit TNM-1 (Shimadzu Corp. Houston, TX, USA). Dissolved organic carbon was measured as non-purgeable C, which entails acidifying the sample (250 µL 2M HCl) and sparging for 4 min with C-free air. Ammonium was analyzed using the phenate hypochlorite method with sodium nitroprusside enhancement (USEPA method 350.1) and nitrate was analyzed using Cd-Cu reduction (USEPA method 353.4). Alkalinity was quantified using methyl orange (USEPA method 310.2). Alkalinity was converted to the major carbonate species (AqQA, Rockware Inc., Denver, CO) which, in this study was bicarbonate. All colorimetric methods were performed with a Westco Scientific Smartchem Discrete Analyzer. Calcium, magnesium, potassium and sodium were quantified by ion chromatography using an Ionpac CS12A analytical and Ionpac CG12A guard column for separation and 20 mM methanosulfonic acid as eluent at a flow rate of 1 mL min^{-1} and injection volume of 25 µL (DIONEX ICS 1000). Fluoride, chloride, bromide, sulfate and phosphate were quantified using Ionpak AS20 and Ionpak AG20 analytical and

guard columns for separation with 35 mM KOH as eluent at a flow rate of 1 mL min^{-1} and an injection volume of 25 μL (DIONEX ICS 1000). Dissolved organic nitrogen was estimated by deducting inorganic-N (NH_3-N + NO_3-N) from TDN. NIST traceable check standards and water blanks were analyzed every 12^{th} sample for QA/QC on instrument precision and coefficient of variance between replicates.

2.2.5 Statistical analysis

One-way-analysis-of–variance (ANOVA) with DOC concentration treatments as a factor was run to determine if the concentration of DOC had a significant effect on *E. coli* regrowth rate. Because there were only two levels (grass and leaf) for the categorical quality factor it was not possible to run a univariate analysis of variance and examine interactions between DOC quantity and quality. A one-way-analysis-of-variance using the value obtained using Equation 2 was run to determine the effect of the C/N/P value on *E. coli* regrowth rate. Post-hoc Tukey tests at $\alpha = 0.05$ were applied to the ANOVA results. Student one-tail t-tests were used to compare the grass and leaf treatments for differences in *E. coli* CFU numbers, to test the alternative hypothesis that grass extract would result in significantly greater *E. coli* regrowth rates. Pearson bivariate correlation analysis was used to examine correlations between *E. coli* regrowth rates over each time period and either individual chemical constituents or nutrient ratios analyzed at $t = 0$.

2.3 Results

2.3.1 Escherichia coli regrowth

One-way-analysis-of-variance found that the extract concentration of DOC did have a significant effect on *E. coli* regrowth at 12 h through 72 h ($p = 0.002 – 0.0004$) but not at t = 6 h ($p = 0.10$). One-way-analysis-of-variance found that for grass extract, significantly higher *E. coli* regrowth occurred relative to the control after 12 h of incubation (Table 2.2A) but *E. coli* regrowth was not significantly increased with the leaf extract until after 36 h of incubation and this was in the high DOC treatment only (Table 2.2B).

The recovery and regrowth of *E.* coli is plotted against the time elapsed representing the average growth kinetics of each of the treatment type (Figure 2.1). At t=0, the treated effluent alone had a minimum detection level of 2.5 CFU 100mL^{-1} or Log_{10} value of 0.40 *E. coli* CFU 100 mL^{-1}. For the control, no *E. coli* CFUs were detected over the course of the experiment at a minimum detection level of 5 CFU 100 mL^{-1} or Log_{10} value of 0.70 *E. coli* CFU 100 mL^{-1}. For the first 6 h none of the grass treatments exceeded the126 (2.1 Log_{10}) CFU 100 mL^{-1} state allowed limit. After 12 h the low and medium grass treatments exceeded the126 (2.1 Log_{10}) CFU 100 mL^{-1} state allowed limit for primary contact recreation. For our leaf extract, none of the treatments exceeded the mandated 126 CFU 100 mL^{-1} allowed limit until 18 h Figure 2.1). For most of the incubation period the medium leaf treatment remained below the allowed limit but at 18 h both the high and low leaf treatments exceeded the allowed limit for primary recreation (Figure 2.1).

Table 2.2 Average *E. coli* colony forming units (Log_{10} CFU/100 mL) at each time period with control, low, medium, and high DOC concentrations of A. grass extract and B. leaf extract. Different superscript lower-case letters indicate a significant difference ($\alpha < 0.05$) within each time period.

A		**Grass Extract**			
		Control	Low	Medium	High
	DOC (mg/L)	2.8	16.2	73	138.2
		E. coli (Log_{10} CFU/100 mL)			
Hour	6	0^{a}	1.37^{a}	1.70^{a}	0^{a}
	12	0^{a}	3.13^{b}	4.63^{b}	0^{a}
	18	0^{a}	4.73^{b}	5.57^{b}	0.67^{a}
	24	0^{a}	4.80^{b}	6.07^{b}	0.70^{a}
	36	0^{a}	4.96^{bc}	6.23^{c}	1.37^{ab}
	48	0^{a}	4.93^{b}	6.10^{b}	1.77^{a}
	72	0^{a}	4.90^{b}	5.97^{b}	1.83^{ab}
B		**Leaf Extract**			
		Control	Low	Medium	High
	DOC (mg/L)	2.8	12.6	52.4	102.7
		E. coli (Log_{10} CFU/100 mL)			
Hour	6	0^{a}	0^{a}	0.23^{a}	0.70^{a}
	12	0^{a}	1.23^{a}	0.77^{a}	2.00^{a}
	18	0^{a}	2.20^{a}	1.33^{a}	3.57^{a}
	24	0^{a}	3.27^{a}	1.80^{a}	5.07^{a}
	36	0^{a}	3.57^{ab}	2.50^{ab}	6.00^{b}
	48	0^{a}	2.83^{ab}	1.76^{ab}	6.26^{b}
	72	0^{a}	2.80^{ab}	1.77^{ab}	6.03^{b}

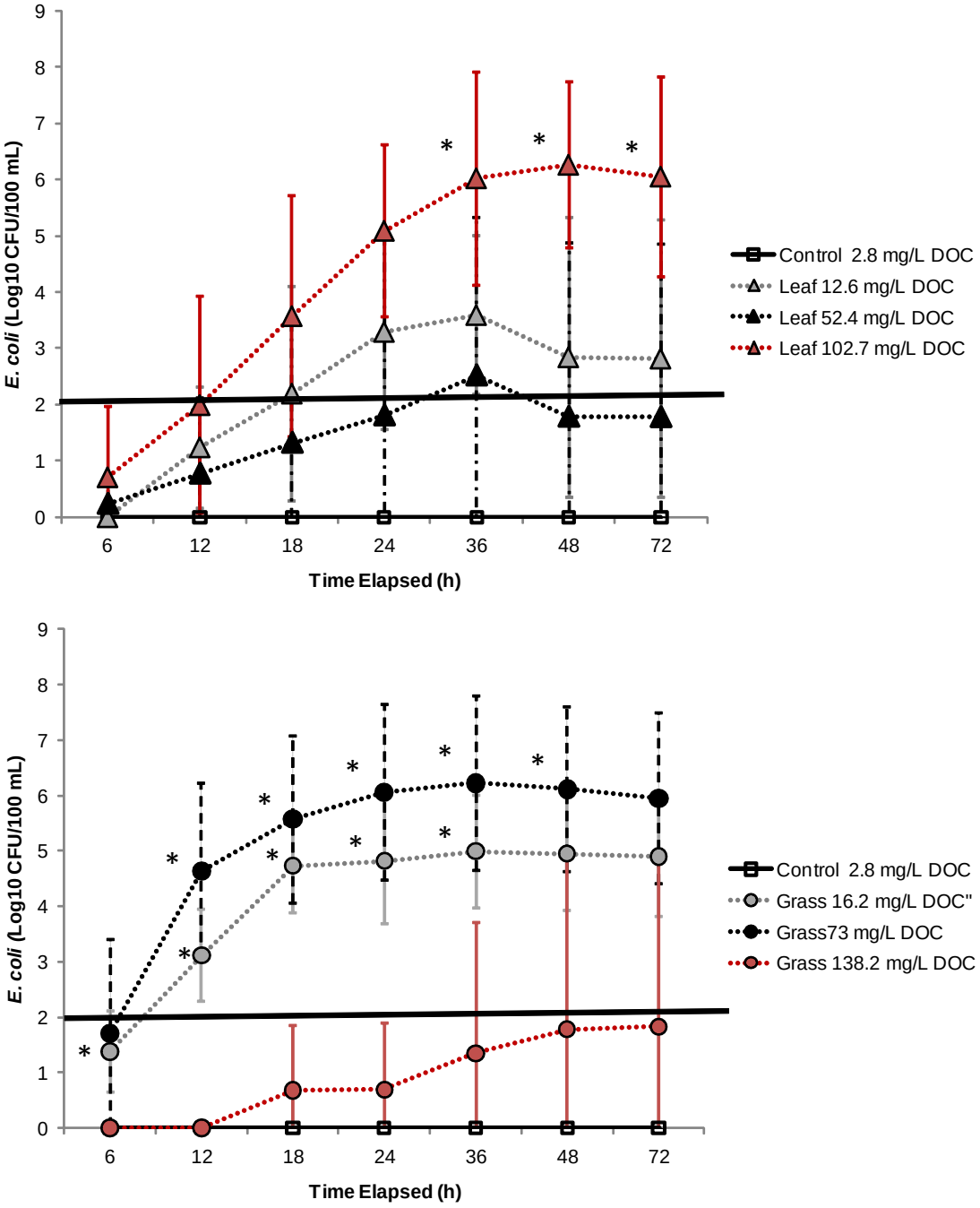

Figure 2.1 *Escherichia coli* growth over the 72 hour period. Error bars are standard deviation. *E. coli* growth with A) leaf extract and B) grass extract where * represents a significant difference at $a < .05$ from the control within each time period. The solid black horizontal line indicates the Log_{10} value for the EPA standard of 126 *E.coli* CFU per 100 mL.

The null hypothesis that quantity of DOC would not affect *E. coli* regrowth was rejected. Based on the alternative hypothesis that grass extract would promote significantly higher regrowth than leaf extract, one-tailed t-tests on *E. coli* were performed between each extract type for each time period (Table 2.3).

Table 2.3 Probability values using one-tail t-tests to examine the effect of extract on *E. coli* growth. L1 is Leaf Low DOC, L2 is Leaf Medium DOC, L3 is Leaf High DOC and G1 is Grass Low DOC, G2 is Grass Medium DOC and G3 is Grass High DOC. Bold values indicate a significant difference at $p < 0.05$ within a time period.

Time =	6	12	18	24	36	48	72
			Grass vs. Leaf 1 tail t-test				
G1vL1	**0.02**	**0.04**	0.05	0.14	0.12	0.12	0.13
G2vL2	0.11	**0.02**	**0.03**	0.05	0.06	**0.05**	0.05
G3vL3	0.19	0.07	0.05	**0.01**	**0.03**	**0.04**	0.06

There were some significant differences in extract quality (leaf vs. grass) on *E. coli* growth over the course of the incubation (Table 2.3). For example, there was significantly higher *E. coli* growth in the low DOC grass treatment relative to the low DOC leaf treatment after 6 and 12 h of incubation, and significantly greater *E. coli* growth in the medium DOC grass treatment relative to the medium DOC leaf treatment during the 12-18 h incubation. However the high DOC leaf treatment had significantly greater *E. coli* regrowth than the high DOC grass treatment between the 24 and 48 h incubation period (Table 2.3).

Because the high DOC treatment for grass extract did not elicit a strong regrowth of *E. coli* compared to the other treatments, the effect of other nutrients and nutrient ratios was examined on *E. coli* regrowth (Table 2.4). Using Pearson bivariate correlation analysis for the full dataset of controls and leaf and grass extracts, *E. coli* values at each collection time were significantly correlated with solution turbidity (Table 2.4). The incubation solution N:P ratio was negatively correlated to *E. coli* regrowth; as the N:P ratio increased in our incubation solutions, *E. coli* regrowth decreased (Table 2.4). The incubation solution C:N and C:P ratios only became important for *E. coli* regrowth in the latter stages of the experiment (Table 2.4). On examination of the leaf extract incubation solutions using Pearson bivariate correlation it was found that several chemical constituents in the incubation solution had a positive and significant correlation with *E. coli* regrowth over the different time periods (Table 2.4).

Table 2.4 Correlation of initial individual chemical constituents (t=0) and nutrient ratios with *E. coli* regrowth over time. Values are strength of correlation (R) and * $p < 0.05$ ** $p < 0.01$. ǂ = no significant correlation with *E. coli* regrowth.

	6	12	18	24	36	48	72
	Time (h)						
Leaf and Grass							
Turbidity	0.56**	0.51*	0.44*	0.41*	0.41*	0.41*	0.41*
C:N	ǂ	ǂ	ǂ	ǂ	ǂ	0.46*	0.46*
C:P	ǂ	ǂ	ǂ	ǂ	ǂ	0.43*	0.42*
N:P	ǂ	-0.48*	-0.47*	ǂ	ǂ	-0.41*	-0.45*
Leaf Extract	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
Ammonium-N	ǂ	ǂ	ǂ	0.60*	0.68*	0.70*	0.67*
Potassium	ǂ	ǂ	ǂ	0.61*	0.69*	0.71**	0.70*
Magnesium	ǂ	ǂ	ǂ	0.60*	0.69*	0.70*	0.69*
Calcium	ǂ	ǂ	ǂ	ǂ	ǂ	0.67*	0.65*
Conductivity	ǂ	ǂ	ǂ	0.58*	0.66*	0.70*	0.68*
Orthophosphate-P	ǂ	ǂ	ǂ	0.59*	0.68*	0.70*	0.68*
DOC	ǂ	ǂ	ǂ	0.58*	0.67*	0.68*	0.67*
C:N	ǂ	ǂ	ǂ	ǂ	0.66*	0.67*	0.65*
C:P	ǂ	ǂ	ǂ	ǂ	0.67*	0.67*	0.65*
N:P	ǂ	ǂ	ǂ	-0.58*	-0.66*	-0.65*	-0.63*
C/N/P	ǂ	ǂ	ǂ	ǂ	0.64*	0.63*	0.61*
Grass Extract	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
Turbidity	0.59*	ǂ	ǂ	ǂ	ǂ	ǂ	ǂ
Fluoride	ǂ	ǂ	ǂ	-0.58*	-0.58*	ǂ	ǂ
C/N/P Value	0.63*	0.78**	0.87**	0.86**	0.85**	0.83**	0.82**

2.3.2 C/N/P values on *E. coli*

This experiment did not find that chemical constituents in the grass extract had the same significant correlation with *E. coli* (Table 2.4); instead mass ratio's of nutrients, particularly the C/N/P value showed a high and significant correlation with *E. coli* regrowth over the course of the incubation where it only showed a significant relationship with *E. coli* in the leaf extract incubation solution after t = 36 (Table 2.4).

Fluoride had a significant negative correlation with *E. coli* in the grass extract incubation solution at 24 and 36 h (Table 2.4).

Table 2.5 Mean concentrations of DOC, TDN, and PO_4-P, mass of C, N and P, and nutrient ratios in incubation flasks at t = 0, n = 3 for each treatment.

	DOC	TDN	PO_4-P	C mass	N mass	P mass	C/N/P Value	C:N	C:P	N:P
	mg L^{-1}			mg $Flask^{-1}$				Ratio		
				t= 0 h						
GRASS										
Control	2.77	7.09	1.17	0.41	1.06	0.18	2.22	0.4	2.3	5.9
Grass 1	16.16	8.65	2.07	2.56	1.37	0.32	5.85	1.9	8.0	4.3
Grass 2	73.00	14.82	6.10	13.95	2.83	0.94	5.21	4.9	14.8	3.0
Grass 3	138.19	22.34	10.66	32.09	5.19	1.65	3.75	6.2	19.4	3.1
LEAF										
Control	2.77	7.09	1.17	0.41	1.06	0.18	2.22	0.4	2.3	5.9
Leaf 1	12.65	7.26	1.27	1.96	1.12	0.20	8.84	1.8	9.8	5.6
Leaf 2	52.36	7.76	1.65	9.11	1.35	0.26	26.41	6.7	35.0	5.2
Leaf 3	102.70	8.49	2.14	20.34	1.68	0.33	36.48	12.1	61.6	5.1
				t = 72 h						
GRASS										
Control	2.97	6.98	1.09	0.30	0.69	0.11	4.00	0.4	2.7	6.3
Grass 1	7.04	6.71	1.77	0.70	0.67	0.18	5.93	1.0	3.9	3.7
Grass 2	29.71	4.63	4.96	2.97	0.46	0.50	12.93	6.5	5.9	0.9
Grass 3	68.54	9.68	9.34	6.85	0.97	0.93	7.58	7.1	7.4	1.0
LEAF										
Control	2.97	6.92	1.07	0.30	0.69	0.11	4.00	0.4	2.7	6.3
Leaf 1	6.00	6.57	1.08	0.60	0.66	0.11	8.43	0.9	5.5	6.0
Leaf 2	27.35	3.60	1.14	2.74	0.36	0.11	66.36	7.6	24.9	3.3
Leaf 3	71.75	2.73	1.46	7.18	0.27	0.15	179.59	26.6	47.9	1.8

With zero *E. coli* regrowth at a mass C/N/P value of 2.22 and low regrowth at a mass C/N/P value of 3.7, nutrient limitation for regrowth may be a factor. The relationship between *E. coli* regrowth and incubation water C/N/P value was able to explain *E. coli* regrowth behavior better than DOC concentration alone.

The control had the lowest C/N/P value (Table 2.5). The grass treatments had a slightly higher range 3.7 – 5.8, but the highest DOC grass treatment had the lowest C/N/P value (Table 2.5). The leaf treatment had the highest range of C/N/P value for the three treatments and ranged from 8.8 to 36.5, with a steadily increasing value from the lowest DOC treatment to the highest DOC treatment (Table 2.5). Regrowth of *E. coli* was not significantly different in the leaf treatment (Figure 2.2). The C/N/P value of grass extract at 5.2 ±0.05 and 5.8 ±0.21 displayed significantly higher regrowth compared to the control and extract with a C/N/P value of 3.7±0.17 (Figure 2.2). The relationship between *E. coli* regrowth and C/N/P value was examined at the microcosm scale using regression analysis (Figure 2.3)

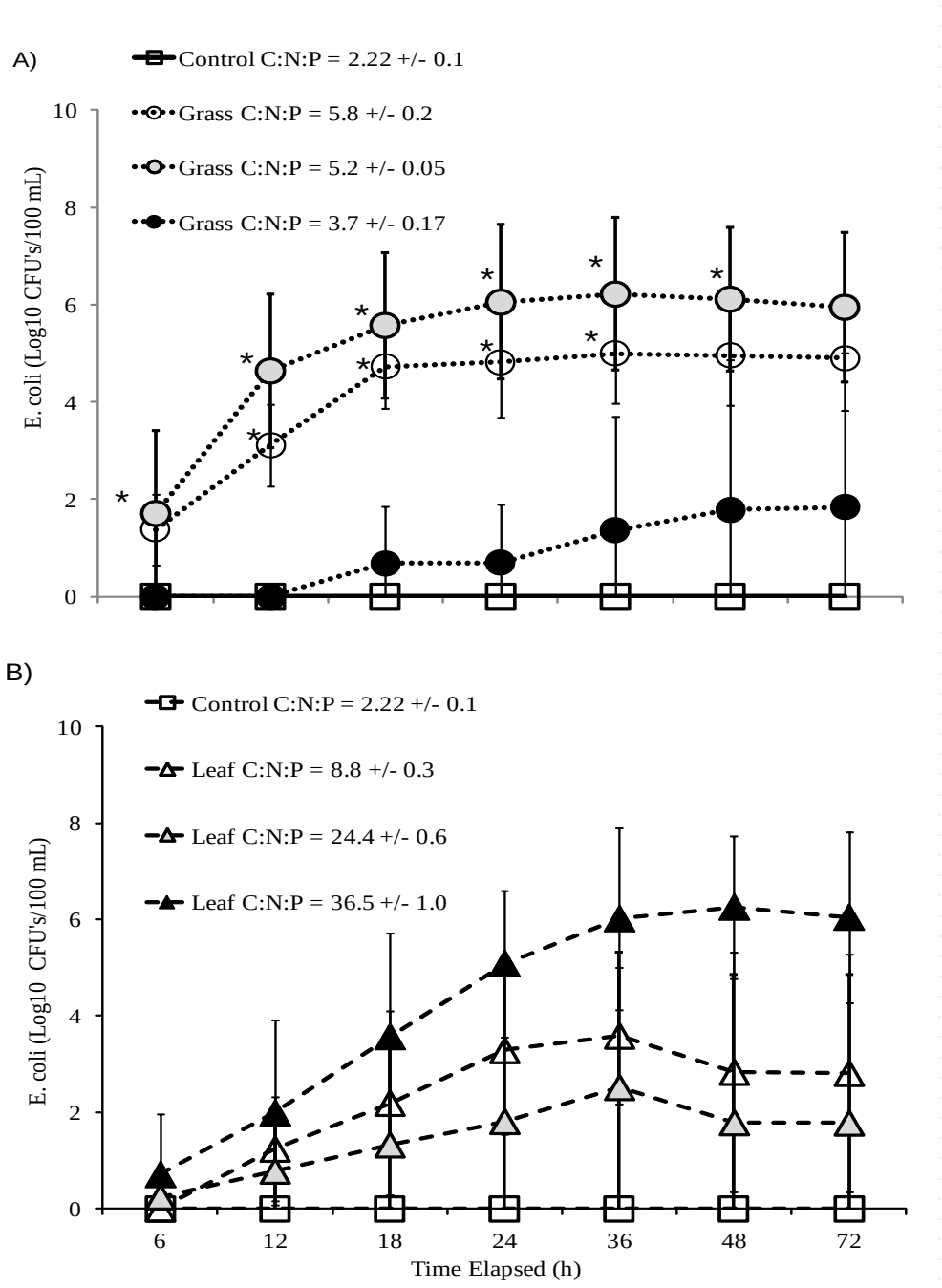

Figure 2.2 Effect of incubation water C/N/P value on *E. coli* regrowth in A) grass extract and B) leaf extract. Error bars are standard deviation. * indicates a significant difference at $\alpha < 0.05$ between control samples and treatment samples.

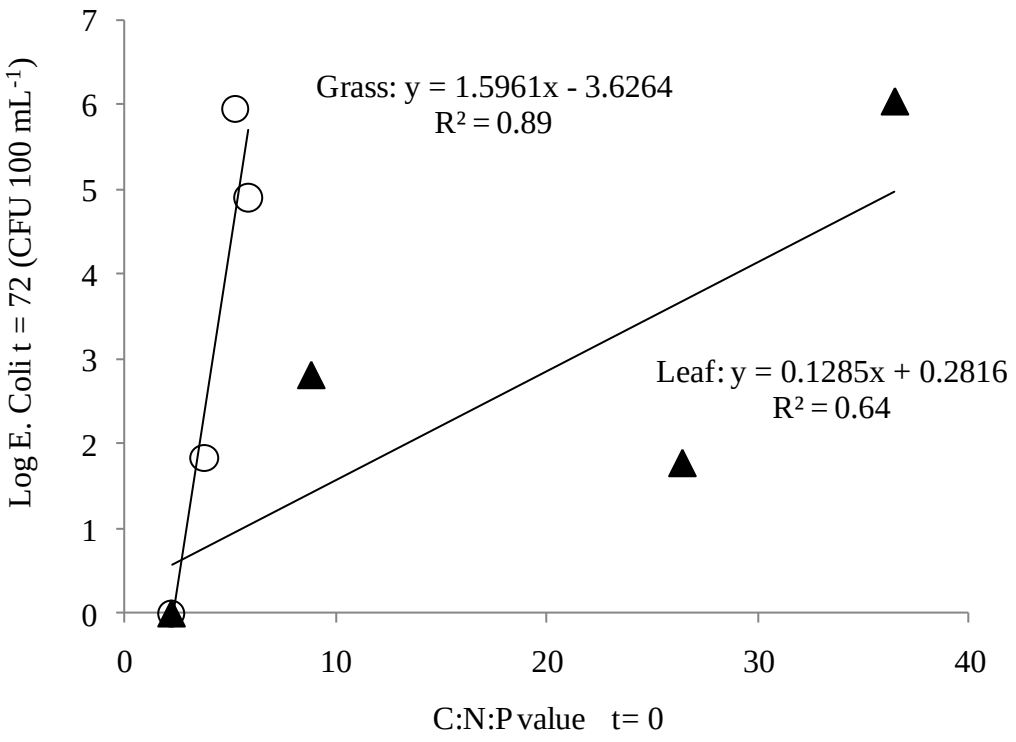

Figure 2.3 Relationship between *E. coli* 72 h and initial incubation solution C/N/P value

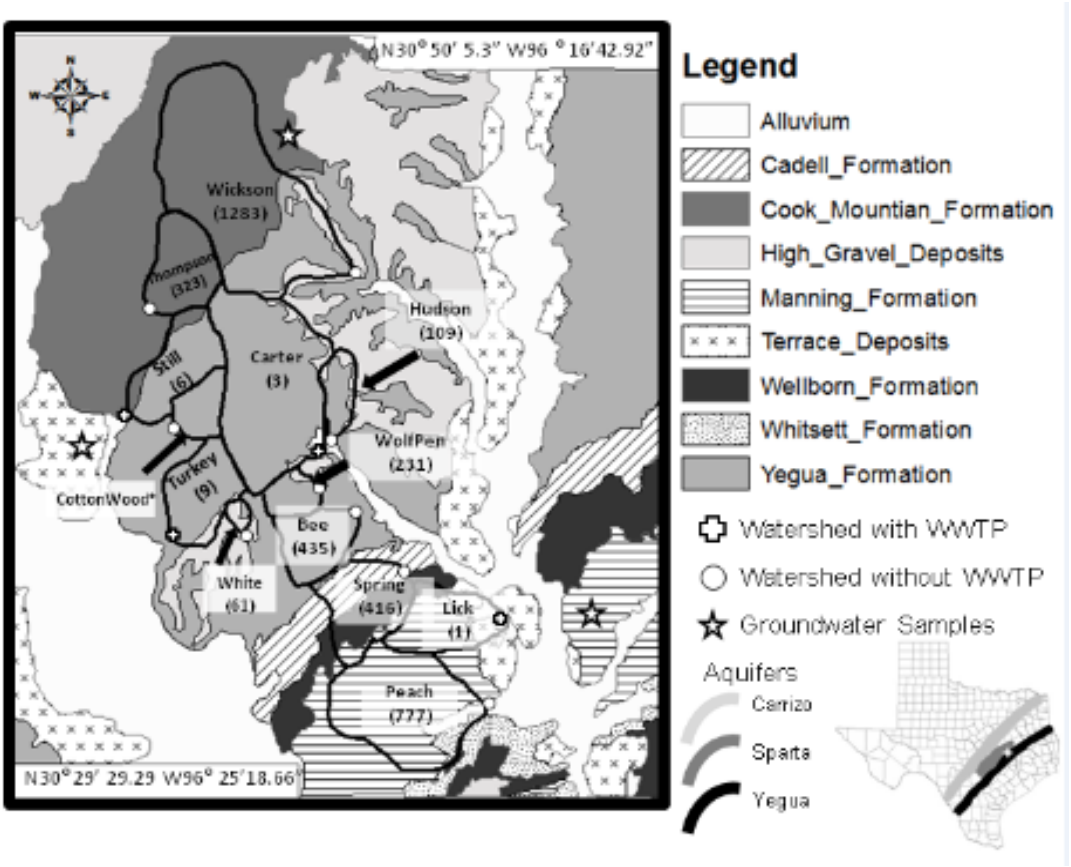

Figure 2.4 Watersheds used for examining stream C/N/P value and *E. coli* CFU's. Adapted from Aitkenhead-Peterson et al. (2011). *There was no flow on the date of sampling at Cottonwood.

Data available from unpublished *E. coli* collections in September 2007 from independent streams (Figure 2.4) in the Bryan/College Station region described by Aitkenhead-Peterson et al. (2009, 2011) were used with stream sample chemistry collected on the same day. The relationship between *E. coli* regrowth and C/N/P value was also examined at the watershed scale (Figure 2. 5). When the stream data downstream of five wastewater treatment plants described in Aitkenhead-Peterson et al. (2009, 2011) that had a single 'snapshot' sampling of *E. coli* were examined it was found that 97% of the variance in *E. coli* was explained by stream C/N/P value on the

day of sampling (Figure 2.5A). Here the stream C/N/P value ranged from 0.82 to 9.0. In those streams without wastewater treatment plants described in Aitkenhead-Peterson et al. (2009, 2011) it was found that the stream C/N/P value on the day of sampling ranged from 61 to 1283. To understand why there was a positive pattern of *E. coli* numbers and low stream C/N/P values and a negative pattern with *E. coli* numbers and high stream C/N/P values the land uses within the watersheds were examined. The most rural streams, Peach Creek, Wickson Creek and Thompson Creek drained rangelands and improved pasture and urban open areas were 9% or less of the total watershed land use. Then the data was plotted by *E. coli* and stream C/N/P value using a subjective <10% and >10% urban turfgrass to differentiate between urban and rural watersheds. There was no indication of a relationship between *E. coli* and C/N/P value in those streams with > 10% of the watershed land use as turfgrass (R^2 = 0.02; Figure 2.5B). In those watersheds with <10% of the watershed under turfgrass there was a strong inverse relationship between *E. coli* and stream C/N/P value (Figure 2.5B). Overall, for those streams without a WWTP it was found that as C/N/P value increased that *E. coli* numbers decreased (R^2 = -0.56; Figure 2.5B).

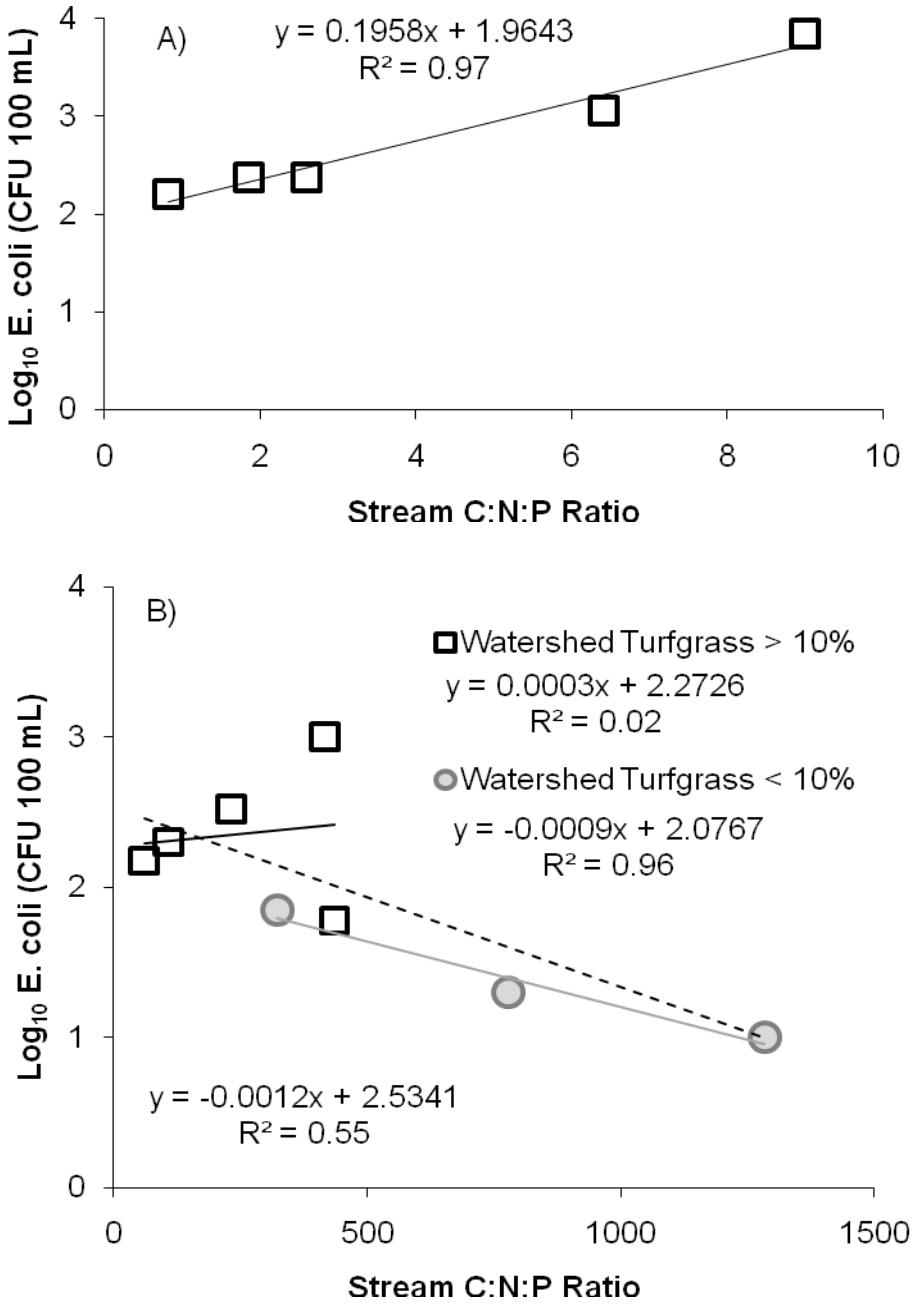

Figure 2.5 Relationships between stream log *E. coli* and stream C/N/P value. A) streams below WWTP and B) streams without a WWTP in the watershed.

2.3.3 Biodegradability of carbon, nitrogen and orthophosphate-P

The quantity or concentration of substrate DOC significantly affected DOC biodegradability ($p < 0.01$). There was a significant interaction between substrate quality and DOC quantity on DOC biodegradability ($p < 0.01$). Quality of substrate did not significantly affect biodegradable TDN ($p = 0.18$) but quantity of TDN did ($p < 0.001$). There was a significant interaction between TDN quantity and quality of substrate on TDN biodegradability ($p = 0.03$). Biodegradability of orthophosphate-P was significantly affected by substrate quality ($p < 0.001$), substrate PO_4-P quantity ($p = 0.014$) and an interaction between substrate quality and quantity of PO_4-P.

There was significantly greater biodegradability of DOC in the medium and high concentration DOC grass treatments relative to the leaf treatments (Figure 2.6A). Biodegradable total N was not significantly different among the grass and leaf treatments (Figure 2.6B). There was significantly greater biodegradability of orthophosphate-P in the leaf treatments relative to the grass treatments (Figure 2.6C). There was some loss or biodegradability of N and P in the control (effluent and water only) (Figure 2.6). For the Low treatment DOC substrate appears to be limiting *E. coli* growth. For the Medium and High treatments the limiting nutrient appears to be N.

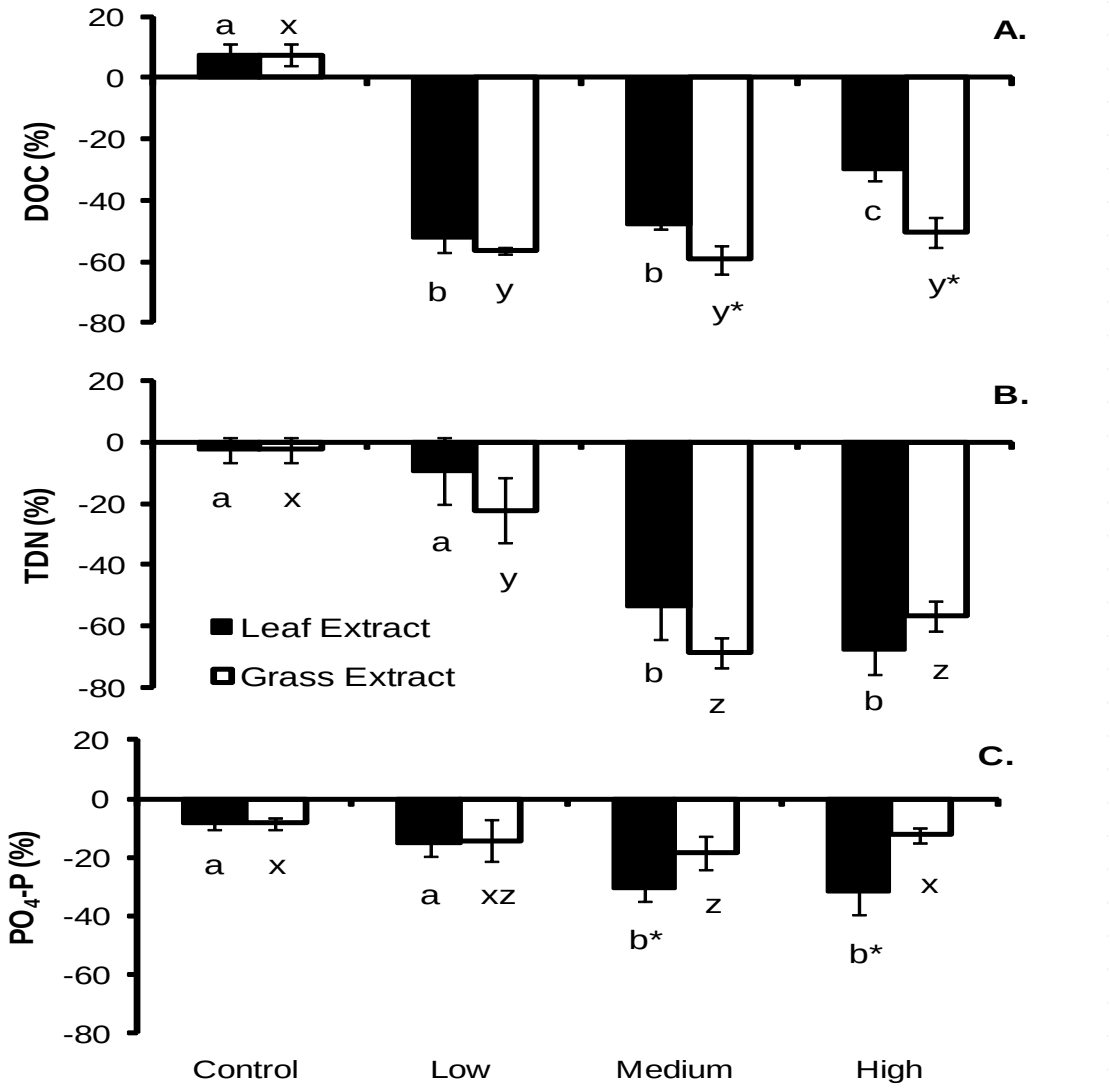

Figure 2.6 Biodegradability of DOC, TDN and PO_4-P under control, low, medium, and high DOC concentrations. Error bars are standard deviation. Difference in lowercase letters (abc) indicate a significant difference between leaf extracts; between (xyz) grass extracts and * a significant difference between leaf and grass extracts at each quantity of initial DOC. Significant difference is $\alpha < 0.05$.

2.4 Discussion

This study investigated the effects of different concentrations and quality (grass vs. leaf extract) of DOC spikes on the regrowth of *E. coli* within UV treated wastewater. This work follows the work of Bolster et al. (2005) where *E. coli* regrowth was found in estuarine water spiked with sewage effluent treated by chlorine. Although DOC quality and quantity was a significant factor for *E. coli* regrowth in each of the microcosms, other possible contributors to regrowth were investigated by evaluating other water chemical constituents and nutrient ratios, biodegradability of nutrients, in part to examine the implications and expected occurrence within a real watershed scenario. The results of these experiments have implications for understanding the regrowth of *E. coli* in urban streams where WWTP effluent is discharged.

2.4.1. E. coli regrowth and DOC

Dissolved organic carbon was hypothesized to be the nutrient attributing to the regrowth of bacteria. Autoclaving is not a natural process in the environment; however, autoclaving was necessary in this experiment to destroy any bacteria present in the treatment solutions that were made from environmental leaf litter and grass cuttings. Autoclaving DOC stocks may have resulted in changes to the DOC molecules and thereby impacted their utilization by bacteria. In a study conducted with wastewater from the Muskiz sewage treatment plant in Biskay, Spain, bioassays were performed to study the effects of disinfection upon the quality of DOC (Arana et al. 2000). The *E. coli* strains were found to be very sensitive to changes in the molecular structure of DOC

(Arana et al. 2000). One of the elements they considered was the treatment method of disinfection by filtering and then autoclaving or autoclaving then filtering before the addition of *E. coli* strains (Arana et al, 2000). These two methods did have varying amounts of DOC due to the breakdown of particulate organic C during autoclaving (Arana et al. 2000). Each of the methods did provoke growth for indigenous bacterial populations from wastewater (Arana et al. 2000). Although the DOC molecular structures may have been changed during autoclaving in this study, it was clearly not significant enough to repress growth or enhance growth as compared to ultra-filtered water and the WWTP effluent.

While the results of this experiment indeed attest that the addition of C does stimulate regrowth, it was observed that the magnitude of regrowth is dependent upon something more than DOC alone. In all treatments, with the exception of the control, *E. coli* regrowth was found to surpass the contact recreation standard of 126 CFU per100mL. A study of the South Central Texas watersheds reported natural DOC mean annual concentrations ranged from 20 to 52.5 mg C L^{-1} and had individual sample highs of > 100 mg C L^{-1} (Aitkenhead-Peterson et al. 2009). These DOC concentrations could attribute to regrowth of bacteria downstream of wastewater treatment plant in this region; however DOC alone is not a reliable predictor of bacterial regrowth. The control treatment only contained the nutrients and DOC (2.8 mg L^{-1}) already present in the effluent and alone resulted in no regrowth of bacteria. These results contrast to Boulam et al. (2003), where growth of bacteria at initial DOC concentrations of 1.6 mg L^{-1} was witnessed during the first 100 h of their experiment. They used three types of water, 1)

drinking, 2) river, and 3) algal bloom water, for conducting their experiment of growth (Boulam et al. 2003). They inoculated water with coliform and autochthonous bacteria and observed their growth and how long the communities remained culturable (Boulam et al. 2003). The magnitudes of growth were different for each solution: the algal bloom water had the highest amount of growth even though the initial DOC concentration was the same as the river water (Boulam et al. 2003). This suggests that even though the DOC is a factor in regrowth the other nutrients within DOM play a role in bacterial growth. While Boulam et al. (2003) did not publish N and P concentrations for the different types of water they used, it should be expected that algal bloom water might have higher N and P because algal blooms typically occur in eutrophic waters. Both this study and that of Boulam et al. (2003) found a pattern of increased regrowth as concentrations of DOC increased. The high DOC grass treatment did not have the greatest magnitude of growth as compared to the medium and low grass treatment which suggests that nutrient composition may play a strong role in regrowth. The Lim and Flint (1989) survival experiment also alluded to the fact that the other nutrients in the water also had a profound effect on the survival of the bacteria. Other potential explanations for the decreased growth rate under the highest concentration of DOC include a bacterial growth inhibitor in the grass samples that was only effective in high doses, osmotic shock from the combined compounds found in the high grass treatments or perhaps competition from other heterotrophic microorganisms from the substrate. The further chemical analysis and research of the DOC substrates could provide insight into the other potential causes.

2.4.2 E. coli regrowth and C/N/P values

The importance of C, N, and P balances on microbial growth has long been recognized even though it is still not fully understood. The lack of significant regrowth of *E. coli* colonies under UV treated effluent and ultra filtered water treatments indicate that something within the treatment solutions caused regrowth; however, the results of our indicated C alone could not explain the variability in re-growth patterns. Examination of solution chemistry, nutrient concentrations, and ratios indicated that the C/N/P value was the best single predictor of *E. coli* regrowth in this study. This suggests that regrowth of bacteria from wastewater treatment plants will be dependent on the chemistry of the receiving water which is controlled by non-point and point source contributors.

Liebig's Law of the Minimum Principle states that growth is controlled not by the total amount of resources, but by the resource that is limiting. Lim and Flint's (1989) *E. coli* survival experiment results exhibited how important the presence of N was to survival in combination with C sources. Bolster et al. (2005) combined the results of their regrowth data from their chlorine treated microcosm to produce possible models by multiple regression analysis. Their multiple regression model with the highest R^2 (0.92) and P-value (0.07) was a combination of TDN, NH_4^+, DOC, PO_4, and salinity (Bolster et al. 2005). The data from this experiment supports the findings of Bolster et al. 2005) in that C, N and P are all important for regrowth.

The highest grass treatment had a lower C/N/P value than did the low and medium DOC concentration treatments, which may explain the lower bacterial counts.

The C/N/P value was only significant for leaf leachates at later time periods and may have resulted from the higher C/N/P values found in the solution compared to grass extract. Leaf extract is darker in color suggesting greater concentrations of humic acids which comprise larger and more aromatic compounds that are more difficult to break down.

The effect of C/N/P value on bacterial regrowth downstream of wastewater treatment plants was supported by bacterial counts in urban and rural streams and areas from a 'one-off' stream sample analysis of *E. coli* in the streams reported in Aitkenhead-Peterson et al. (2009, 2011). The results of this experiment suggest that the effluent itself will not regrow *E. coli* and this is likely due to a limitation of C. Furthermore, the results suggest that regrowth of *E. coli* in effluent is likely dependent upon the C derived from watershed runoff; thus, the WWTP is providing the nutrients and the watershed the C substrate. The relationship between *E. coli* numbers and stream C/N/P downstream of wastewater treatments is extremely strong and significant and highlights the role watersheds, specifically the runoff from them, may play in biological and chemical dynamics of surface waters.

Although the WWTP is a source for the bacteria, albeit injured and not viable or culturable, it appears that it is the stream conditions in terms of the nutrient ratios that are enabling the bacteria to repair themselves. Of equal interest was the relationship between *E. coli* and stream C/N/P values in streams without WWTP. Opposite patterns were observed for streams without wastewater treatment plants, where as the C/N/P value increased there was an overall decline in *E. coli* numbers which is classic evidence

of Liebigs Law of the Minimum (de Baar 1994). These watersheds had C/N/P values 100 times larger than the values observed downstream of WWTPs. Urban watersheds generally have a significant proportion of urban open areas in the form of turfgrass golf courses, sports parks, neighborhood lawns and aesthetic strips in commercial areas. In the central Texas watersheds, as proportion of turfgrass in a watershed increased then the C/N/P value of its surface water decreased to <500, which may better support growth of bacteria in the environment which are flushed to streams. Since no direct source of *E. coli,* such as WWTP discharges, is identifiable in watersheds it is unlikely the source is from regrowth, rather than the bacteria present may be a result homeowner pets or wildlife fecal bacteria derived from runoff (Donnison et al. 2004).

2.4.3 Biodegradability

Biodegradability of nutrients is important in identifying the limiting nutrient for regrowth of *E. coli* as a move towards restoring streams. Problems in urban streams need to be mitigated for the health of our urban ecosystems. Understanding the biodegradability of the available C substrate and available nutrients will help in decision making to ameliorate *E. coli* regrowth.

Less research has been conducted on the biodegradability of C and nutrients in surface waters (e.g. Boulam et al. 2003; Wiegner et al. 2006). Weigner et al. (2006) examining the bioavailability of DOC in nine northern rivers found that only 4% of the DOC was biodegradable. More research has been conducted on biodegradable DOC in watershed soils (e.g. Qualls 2005; McDowell et al. 2006) where the percentage of

biodegradable DOC ranges from 12 to 56%. Vegetation extract DOC was between 50 and 59% biodegradable in the grass extract and between 30 and 53% biodegradable in the leaf extract. This study suggests that most available terrestrial DOC is readily degraded by aquatic bacteria within the first 24 h when it enters surface waters based upon the high regrowth rates in this period. Thus most DOC in surface waters is likely relatively refractory or unable to be degraded by aquatic bacteria because of its molecular size or presence of aromatic rings. Wiegner et al. (2006) also examined biodegradability of N in the same nine, northern streams and found that of the total N, DON was the form most taken up by aquatic microbes. Wiegner et al. (2006) did not examine the effect that low P might have had on freshwater biodegradable C and N. In stream water and soil solution, the largest components of DOC tend to be humic and fulvic acids (Qualls 2005). The complex mixture of these fractions within DOC substrates likely affects the labile and refractory nature of these solutions for microbes (Qualls 2005). Boulam et al. (2003) found different biodegradability of DOC in each of their three types of water used in their *E. coli* survival experiment. The algal bloom water had the highest biodegradability of DOC at 32%, followed by river water at 20%, and lastly drinking water at 6%. They concluded that biodegradability was heavily dependent on the environmental events and origin of the organic matter (Boulam et al. 2003). The biodegradability data from this experiment somewhat supports the conclusions of Boulam et al. (2003) in that we found significantly greater biodegradability of grass extract DOC relative to leaf extract DOC when initial concentrations were high.

An essential part of each riverine system is DOM. Riverine DOC and DON provide energy and nutrients to bacteria and algae (Weigner et al. 2006). The utilization or consumption of DON was eight times more than that of DOC in northern streams (Weigner et al. 2006). Weigner et al. (2006), hypothesized that this could take place for two reasons that either bacteria selectively cleave N from the functional groups or that bacteria prefer the N rich molecules. It was recognized that a sufficient amount of C must be available to support the uptake of DON by bacteria (Weigner et al. 2006). This experiment also found that the proportion of total N consumed for the medium and high grass and leaf treatment was greater than the proportion of C. Nitrogen is an essential element needed for the growth and regrowth of *E. coli*. The assimilation of ammonium by *E. coli* supports the fastest growth rate of the microorganism (Reitzer 2003). Without N the bacteria can become starved and nutrient limited in their growth. The biodegradable N in this study ranged from 9 to 68% in leaf extract and from 22 to 69% in grass extract. Most of the total N utilized was nitrate-N. It appears in this study, data that the low DOC treatment was C limited and the medium and high treatments were N limited as seen in Figure 2.6 A and B. This relates back to Liebigs Law of the Minimum in that the limiting nutrient controls growth. In an urban environment there are large fluxes of N being pumped into the system from various sources (Kaushal et al. 2008). By regulating these sources of N, water managers can control the amounts of N being stored within stream ecosystem (Kaushal et al. 2008).

Other researchers have shown that the chemical composition of DOM affects the potential for the biodegradability of nutrients. By extracting DOM from three different

sources (leaf litter, algae, and macrophytes) with rainwater, the fresh leachates were compared to riverine samples of DOM from 10 sites on the Ogeechee River, GA (Sun et al. 1997). The results of the comparison indicated that the DOM contained in the freshly prepared leachates was more bioavailable than riverine DOM (Sun et al. 1997). Sun et al. (1997) also recognized that the bioavailability of DOM was highly dependent upon its chemical composition which is related to the source of the DOM. Our biodegradability data supports the chemical composition effect as both the grass and the leaf had significantly different rates of biodegradability of DOC, TDN, and PO_4-P rates between the two treatments.

Lim and Flint (1989) examined *E. coli* survival in a freshwater study and concluded that while phosphate is important in the organisms' cellular structure and metabolism, *E. coli*'s survival was dependent on other nutrients besides P. While our orthophosphate-P biodegradability ranged from 15 to 32% in the leaf extract and 12 to 19% in the grass extract it is evident that to utilize available C and N, that P is also needed.

3. BENTHIC INVERTEBRATES IN URBAN WATERSHEDS

3.1 Introduction

Water quality and chemistry greatly affect the survival of freshwater invertebrates. When plans for improving the health of local streams and rivers are formulated and implemented, there is a critical need to assess the biological integrity of these streams (Hawkins 2006). Often, macroinvertebrates are used in biomonitoring studies because of their ease of collection and identification (Thorp and Covich 2009). Rupprecht (2009) conducted a study to re-populate some third order streams in Hessen Germany that were highly polluted with industrial and domestic wastewater and trash. After several purification plants were installed to help stream chemical recovery, native *Plecoptera* had not returned to the streams after several years of expected recovery. While the concentration of DO in the water had increased over time, in spite of purification of industrial discharge to the streams there were still elevated concentrations of pesticides, heavy metals, ammonium and orthophosphate (Rupprecht 2009). A further issue is that the survival of fisheries is based on the availability of aquatic macroinvertebrates (Kolbe and Luedke 2009).

Since macroinvertebrates are keys to the survival of an ecosystem, they are ideal candidates for studying the biotic health of a stream (Kolbe and Luedke 2009). Macroinvertebrates can be classified by their tolerance to pollution in water bodies to assess organic pollution and water quality by collecting and evaluating macroinvertebrate assemblages through assigning pollution values to the species identified (Hilsenhoff 1988). For example, *Ephemeroptera*, *Trichoptera* and *Plecoptera*

are considered important invertebrate species because they are considered to be indicators of good water quality (Rosenberg and Resh 2003) and are intolerant to pollution (Table 3.1). Polluted surface waters have shown a decreased number of invertebrate species (Roy et al. 2003). This can be especially true in highly urbanized areas (Roy et al. 2003). In the Piedmont region of Georgia, urban development has significantly increased and affected macroinvertebrate assemblages (Roy et al. 2003). After sampling in three different catchments within the Piedmont area, Roy et al. (2003) concluded that increased urbanized impervious land cover of ≥15% resulted in the biotic index to go from good or very good, to fair or fairly poor. Low DO and high organic matter concentrations have been identified as a cause of reduced invertebrate communities (Smith 2001). Benthic communities typically respond to urbanized streams by a reduction in species richness and diversity as well as increased populations of pollution tolerant species (Walters et al. 2009). Walters et al. (2009) studied the same Piedmont area described in the Roy et al. (2003) study but used multiple regression analysis to investigate the predictive power of water quality parameters, geomorphic conditions, and land cover. They concluded, of the water quality measures tested, that electrical conductivity and temperature were the most useful predictors of macroinvertebrate assemblages (Walters et al. 2009). No direct link has been made between DOC concentrations and macroinvertebrate assemblage health or the relative abundance of any particular macroinvertebrate orders which would be logical when one considers that DOC is a substrate for aquatic microorganisms and that macroinvertebrates are sustained by these same microorganisms.

Table 3 1. Selection of invertebrate orders based on their pollution tolerance levels. (Adapted from Hilsenhoff's (1988) biotic index)

Pollution Sensitive/Intolerant	**Mid Pollution Tolerant**	**Highly Pollution Tolerant**
Ephemeroptera	*Decapoda*	*Hirudinea*
Trichoptera	*Amphipoda*	*Diptera Chironimadae*
C oleoptera	*Coleoptera Elmidae*	*Oligochaeta*
Tubellaria	*Diptera Tipulidae*	*Gastropoda*
Neoptera	*Odonata -Anisoptera*	*Diptera Simuliidae*
Plectopera	*Odonata-Zygoptera*	

The objectives of this study were to:

1. To investigate the relative abundance of benthic macroinvertebrates upstream and downstream of wastewater treatment plants.

2. Evaluate whether stream chemical constituents; DOC, DON, NH_4-N, NO_3-N-, $CaCO_3$, PO_4-P, Ca^{2+}, Mg^{2+}, K^+ Na^+, $SO_4^{2-}$and Cl^- may have an impact on the relative abundance of benthic macroinvertebrates.

Hypothesis to be tested:

Objective 1. To investigate the relative abundance of benthic macroinvertebrates upstream and downstream of wastewater treatment plants.

Ho1: There will be no difference in the relative abundance of macroinvertebrates upstream and downstream of wastewater treatment plants

H2: Relative abundance of pollution intolerant benthic macroinvertebrates will be significantly reduced and relative abundance of pollution tolerant benthic macroinvertebrates will be significantly increased downstream of a wastewater plant

Objective 2. Evaluate what stream chemical constituents; DOC, DON, NH_4-N, NO_3-N, $CaCO_3$, PO_4-P, Ca^{2+}, Mg^{2+}, K^+ Na^+, $SO_4^{2-}$and Cl^- may have an impact on the relative abundance of benthic macroinvertebrates.

Ho2: Stream water chemistry has no effect on the relative abundance of benthic macroinvertebrate orders.

H2: The relative abundance of macroinvertebrate will increase with a higher amount of C:N, C:P, and N:P present in the stream.

3.2 Materials and Methods

3.2.1 Site description

The sample sites chosen were surrounded by Bryan and College Station in south-central Texas (northern point N 30° 50036.63 W 96° 23053.87 and southern point 30° 29010.46 W 96° 16016.15, Table 3.2). Soils in the region include several soil series, but are dominated by Alfisols underlain with marine clays. Dominant Soil series include

Booneville (fine sandy loam on 1-3% slopes), Booneville-Urban land complex (0-3% slopes), Zack-Urban complex (1-5% slopes), and Rosanky-urban land complex (1-5% slopes). The climate is humid subtropical having a mean annual temperature of 20°C (Aitkenhead-Peterson et al. 2009). Nine sample sites were selected in the Bryan/College Station Region. Sites varied between rural and urbanized areas. Peach Creek was a rural site with no direct WWTP discharge, Lick Creek and Bird Pond were evaluated after WWTP effluent entered the streams; Carters Creek, Still Creek and Turkey Creek streams were evaluated before and after WWTP effluent entered the stream.

Table 3.2 Geographic locations of the stream sites divided up by streams without WWTP and those with WWTP.

Stream	Peach	Carters 1	Still 1	Turkey 1
	30°30'48.58" N	30°40'4.09" N	30°40'16.32" N	30°37'11.66" N
	96°11'50.96" W	96°19'12.84" W	96°24'34.68" W	96°23'11.04" W

Stream	Lick WWTP	Bird Pond WWTP	Carters 2 WWTP	Still 2 WWTP	Turkey 2 WWTP
	30°33'11.60" N	30°36'11.13" N	30°35'19.17" N	30°39'40.56" N	30°36'55.96" N
	96°11'11.26" W	96°15'0.87" W	96°13'29.18" W	96°26'5.62" W	96°23'33.23" W

3.2.2 Field sampling

Riffles were located because they typically indicate a higher possibility for invertebrate life relative to pools. Five minute kick samples, using a D-frame kicknet were performed in these riffles along the banks, in the deep area and along vegetation if present. Stones were also rubbed with the toes of the waders to dislodge any organisms. Organisms were then placed in white water trays to sort and collect individuals; these

were then placed into 100 mL plastic bottles for transport. A 100 mL water sample was also collected from just upstream of the invertebrate sampling site for chemical analysis.

3.2.3 Laboratory analyses

Organisms were transported back to the laboratory within two h of collection. The organisms were sorted by species using a dissecting kit and a dissecting microscope. Then the total number of individuals and of each species was recorded for each site. The water samples were analyzed for pH and electrical conductivity (EC) on unfiltered aliquots. Stream water samples were filtered through an ashed 0.7 μm Whatman GF/F filters and frozen until chemical analysis.

3.2.4 Chemical analyses

Dissolved organic carbon and TDN were measured using high temperature platinum-catalyzed combustion with a Shimadzu TOC-VCSH and Shimadzu total measuring unit TNM-1 (Shimadzu Corp. Houston, TX, USA). Dissolved organic carbon was measured as non-purgeable C which entails acidifying the sample (250 μL 2M HCl) and sparging for 4 min with C-free air. Ammonium-N was analyzed using the phenate hypochlorite method with sodium nitroprusside enhancement (USEPA method

350.1) and nitrate-N was analyzed using Cd-Cu reduction (USEPA method 353.4). Alkalinity was quantified using methyl orange (USEPA method 310.2). Alkalinity was converted to the major carbonate species (AqQA, Rockware Inc., Denver, CO) which, in this study was bicarbonate. All colorimetric methods were performed with a Westco Scientific Smartchem Discrete Analyzer. Calcium, Mg^{2+}, K^{+}, and Na^{+} were quantified by ion chromatography using an Ionpac CS12A analytical and Ionpac CG12A guard column for separation and 20 mM methanosulfonic acid as eluent at a flow rate of 1 mL min^{-1} and injection volume of 25 µL (DIONEX ICS 1000). Fluoride, Cl^{-}, Br^{-}, SO_4^{-2}, and orthophosphate were quantified using Ionpak AS20 and Ionpak AG20 analytical and guard columns for separation with 35 mM KOH as eluent at a flow rate of 1 mL min^{-1} and an injection volume of 25 µL (DIONEX ICS 1000). Dissolved organic nitrogen was estimated by deducting inorganic-N (NH_4-N + NO_3-N) from TDN.

3.2.5. Statistical analyses

The number of individuals for each benthic macroinvertebrate order was counted and then the relative abundance of each order was calculated. The % EPT was calculated as an indicator group of pollution intolerant species. Two-tail student's t-tests were run using number of individuals for each of the upstream/downstream sites with WWTP, a significant difference between the upstream and downstream site was set at $\alpha < 0.05$.

To determine if stream water chemistry had any effect on the number of individuals and the relative abundance of each order, a Pearson Bivariate correlation analysis was run after testing the data set for normality.

3.3. Results

3.3.1 Invertebrate sampling

The most species were found at Still Creek location below the wastewater treatment plant. This was also the site where the most individuals were collected (Table 3.3). There were no species located at Still Creek before wastewater treatment. Throughout the whole sample (n=306) for the 7 sites, 77 individuals belonged to the orders of *Ephemeroptera, Trichoptera*, and *Plecoptera* (EPT). None of the individuals sampled belonged to the *Plecoptera* order. Seventy of the individuals collected belonged to *Ephemeroptera* (Mayflies) order and only 7 individuals were *Trichoptera* (caddisflies). The other individuals belonged to the orders of *Odonata, Diptera, Lumbricina, Plolecithorphora, Coleopteran, Amphipoda, Basommatophora* and *Acarina.*

The relative abundance of the orders, including the combined % *Ephemeroptera*, *Plecoptera* and *Trichoptera* (%EPT) are listed in Table 3.4. The streams with the more than 50% EPT were Carters Creek downstream of a wastewater treatment plant and Lick Creek, also downstream of a wastewater treatment plant. The stream with the lowest %EPT was Still Creek upstream of the wastewater treatment plant because no individuals were collected. In streams where individuals were collected, Turkey Creek before wastewater treatment discharge had the lowest %EPT where none of the individuals collected belonged to those orders. In all of the streams after wastewater treatment there was at least 10% of the population that belonged to the orders EPT. The null hypothesis of this experiment was accepted that a WWTP did not affect the numbers individuals or relative abundances of benthic macroinvertebrates (Table 3.4) and rejected the alternative hypothesis that a WWTP would increase pollution tolerant species and decrease pollution intolerant species.

Table 3.3 Numbers of individual benthic invertebrates collected and number of species at each stream site. The WWTP samples are sampled downstream of a wastewater treatment plant. Superscript lowercase letters indicate significant differences in number of total individuals upstream and downstream of WWTP.

Stream	Peach	Lick WWTP	Bird Pond WWTP	Carters 1	Carters 2 WWTP	Still 1	Still 2 WWTP	Turkey 1	Turkey 2 WWTP
Date of collection	1/5/2010	1/5/2010	2/10/2010	2/10/2010	1/5/2010	3/3/2010	3/3/2010	3/5/2010	3/5/2010
Blackfly Larva	60	0	0	0	0	0	0	0	0
Caddisfly	0	0	1	3	0	0	0	0	3
Damselfly	0	0	0	0	0	0	2	0	0
Dragonfly nymph	0	0	0	0	0	0	2	0	0
Flatworms	0	0	0	3	0	0	0	0	0
Mayfly	3	15	8	14	20	0	10	0	0
Midge Larva	8	0	5	6	10	0	29	7	0
Midge pupa	0	0	0	0	0	0	6	0	0
Mosquito	0	0	0	0	0	0	0	0	0
Redworm	0	6	17	8	0	0	37	6	5
Riffle Beetle	0	0	0	0	0	0	2	0	1
Scud	0	0	1	2	0	0	1	0	0
Snail	0	0	0	1	0	0	0	0	0
Water Mite	0	0	0	0	0	0	4	0	0
Total Individuals	**71**	**21**	**32**	**37**[a]	**30**[a]	**0**[a]	**93**[b]	**13**[a]	**9**[a]
# Species	**3**	**2**	**5**	**7**	**2**	**0**	**9**	**2**	**3**

Table 3.4 Number of individuals and relative abundance of benthic invertebrates in stream sites

	WWTP	Individuals	EPT	*Ephemeroptera*	*Diptera*	*Odonata*	*Trichoptera*	*Lumbricina*
						%		
Peach	No	71	4	4	96	0	0	0
Lick	Yes	21	71	71	0	0	0	29
Bird Pond	Yes	32	28	25	16	0	3	53
Carters 1	No	37	46	38	16	0	8	0
Carters 2	Yes	30	67	67	33	0	0	0
Still 1	No	0	0	0	0	0	0	0
Still 2	Yes	93	11	11	38	4	0	40
Turkey 1	No	13	0	0	54	0	0	46
Turkey 2[a]	Yes	9	33	0	0	0	33	56

[a]11% were of the order *Coleoptera*

3.3.2 Relationship between invertebrates and stream chemistry

No correlation was found between numbers of individuals found at each sampling site and stream chemistry (Table 3.5) when using all sites, streams downstream of a WWTP or streams with no WWTP. There was a significant negative relationship between %EPT and DOC concentration when all sites were examined (R = -0.67; $p = 0.048$; n = 9), but this relationship was lost when examining only those streams with WWTP (R = -0.53; $p = 0.36$; n = 5) or those without a WWTP (R = -0.77; $p = 0.22$; n = 4). Relative abundance of *Odonata* was strongly and significantly related to ammonium-N concentration across all sites (R = 0.95; $p < 0.001$; n = 9) and the relationship remained significant in only those streams with a WWTP (R = 0.97; $p = 0.008$; n = 5). *Lumbricina* were not correlated to any stream chemistry over all sites or the sites with a WWTP. However, a strong significant negative relationship was found between *Lumbricina* and pH in those sites without a WWTP; as the pH of stream water decreased the relative abundance of *Lumbricina* increased (R = -0.99; $p = 0.005$; n = 4). When all sites were examined a relatively strong positive relationship was found between *Trichoptera* and orthophosphate-P (R = 0.70; $p = 0.037$; n = 9), but this relationship was lost when examining only those sites with a WWTP (R = 0.68; $p = 0.21$; n = 5) yet was strengthened when using only those sites without a WWTP (R = 1.0; $p = 0.001$; n = 4). *Trichoptera* was also significantly and positively correlated to stream nitrate concentration (R = 0.97; $p = 0.03$; n = 4) in streams without WWTP. *Diptera* was not significantly correlated to stream water chemistry either across all sites

Table 3.5 Stream chemistry at the time of benthic invertebrate collection

Stream		Peach	Lick	Bird Pond	Carters 1	Carters 2	Still 1	Still 2	Turkey 1	Turkey 2
pH		7.80	7.95	7.83	7.89	8.20	7.79	7.73	7.48	7.96
Conductivity	µS/cm²	610	1060	590	320	1084	330	990	650	1180
NO_3-N	mg/L	0.63	6.88	4.15	0.13	12.73	1.17	11.19	0.07	13.40
NH_4-N	mg/L	0.09	0.11	0.30	0.11	0.18	0.14	1.07	0.08	0.37
DOC	mg/L	11.69	6.05	8.54	11.61	13.80	17.25	13.78	18.42	17.05
DON	mg/L	0.40	0.82	0.89	0.34	1.87	0.73	1.15	0.57	2.11
TDN	mg/L	1.12	7.81	5.33	0.58	14.78	2.04	13.42	0.73	15.88
$CaCO_3$	mg/L	62.80	186.58	163.80	122.48	180.99	90.51	193.54	92.62	188.57
Na^+	mg/L	72.09	146.98	89.31	46.48	169.73	23.36	134.70	79.92	180.16
K^+	mg/L	6.52	9.28	4.24	2.53	8.05	4.75	8.86	6.21	8.36
Mg^{2+}	mg/L	5.95	5.35	1.87	1.29	2.66	3.94	5.65	7.41	4.88
Ca^+	mg/L	21.94	20.59	10.40	8.76	11.89	14.20	16.66	24.41	17.20
F^-	mg/L	0.15	0.51	0.42	0.17	0.66	0.08	0.79	0.37	0.45
Cl^-	mg/L	80.48	100.23	36.93	31.93	106.32	30.75	94.10	61.50	101.69
SO_4	mg/L	77.87	81.98	17.61	12.67	48.28	33.30	55.66	103.88	68.46
PO_4-P	mg/L	0.12	0.56	0.86	0.16	1.65	0.12	1.40	0.09	2.39

or in those sites containing a WWTP. *Diptera* was however significantly and positively correlated to stream water chloride concentration (R = 1; $p < 0.003$; n = 4) in those streams without a WWTP. *Ephemeroptera* was positively correlated to pH across all sites (R = 0.67; $p = 0.05$; n = 9) and negatively correlated to DOC (R = -0.70; $p = 0.03$; n = 9). These significant correlations were lost when examining just those streams with WWTP or just those streams without WWTP were analyzed.. In streams without WWTP a strong positive correlations was found between *Ephemeroptera* and nitrate-N (R = 0.96; $p = 0.036$; n = 4) and orthophosphate-P (R = 0.99; $p = 0.005$; n=4).

When evaluating the % *Ephemeroptera*, higher pH before wastewater treatment pH tended to fall in the range of 7.7-7.9. After wastewater treatment on the different streams the pH range was greater from 7.45-8.2. But evaluating only pH was not a good predictor of the % *Ephemeroptera* found in a stream. In urban streams benthic macroinvertebrates are not good indicators of upstream WWTP. After wastewater treatment the EC ranged from 560-1180, yet the reach sampled prior to the wastewater treatment the EC ranged from 320 – 650 µS cm^{-2}. The best correlation between % abundance of EPT was pH (Table 3.6).. The data collected from the invertebrate study did not reveal any immediate patterns for prediction of invertebrate population or health.

There were differences between the chemistries of sites with and without WWTP. The sites with WWTP had higher conductivity, nitrate-N, total-N, sodium, fluoride and orthophosphate-P. But no significant differences were found between the invertebrate populations based on these chemical differences.

Table 3.6 Effect of stream chemistry on %EPT abundance. Values are strength of correlation (R) and * $p < 0.05$ ** $p < 0.01$. ł represents no significant correlation.

	pH	EC	NO_3-N	PO_4-P	$CaCO_3^-$	K^+	Na^+
Relative % EPT	0.80 *	ł	ł	ł	ł	ł	ł

Regression analysis using the percentages of relative abundance of pollution intolerant and pollution tolerant benthic invertebrates with nutrient ratios led to some interesting results (Figures 3.1 and 3.2). There was a greater percentage of pollution intolerant species with low stream C:N and C:P ratios (Figure 3.1) but stream N:P ratio did not show any relationship with pollution intolerant relative abundance. Here, between 51 and 55% of the variance in the relative abundance of pollution intolerant species is explained by stream C:N and stream C:P ratios. The relative abundance of the pollution tolerant species which was assigned as *Diptera*, and *Lumbricina*, were not as affected by stream nutrient ratios (Figure 3.2). The exception was stream N:P ratio where, when the stream N:P ratio increased, the relative abundance of pollution tolerant decreased.

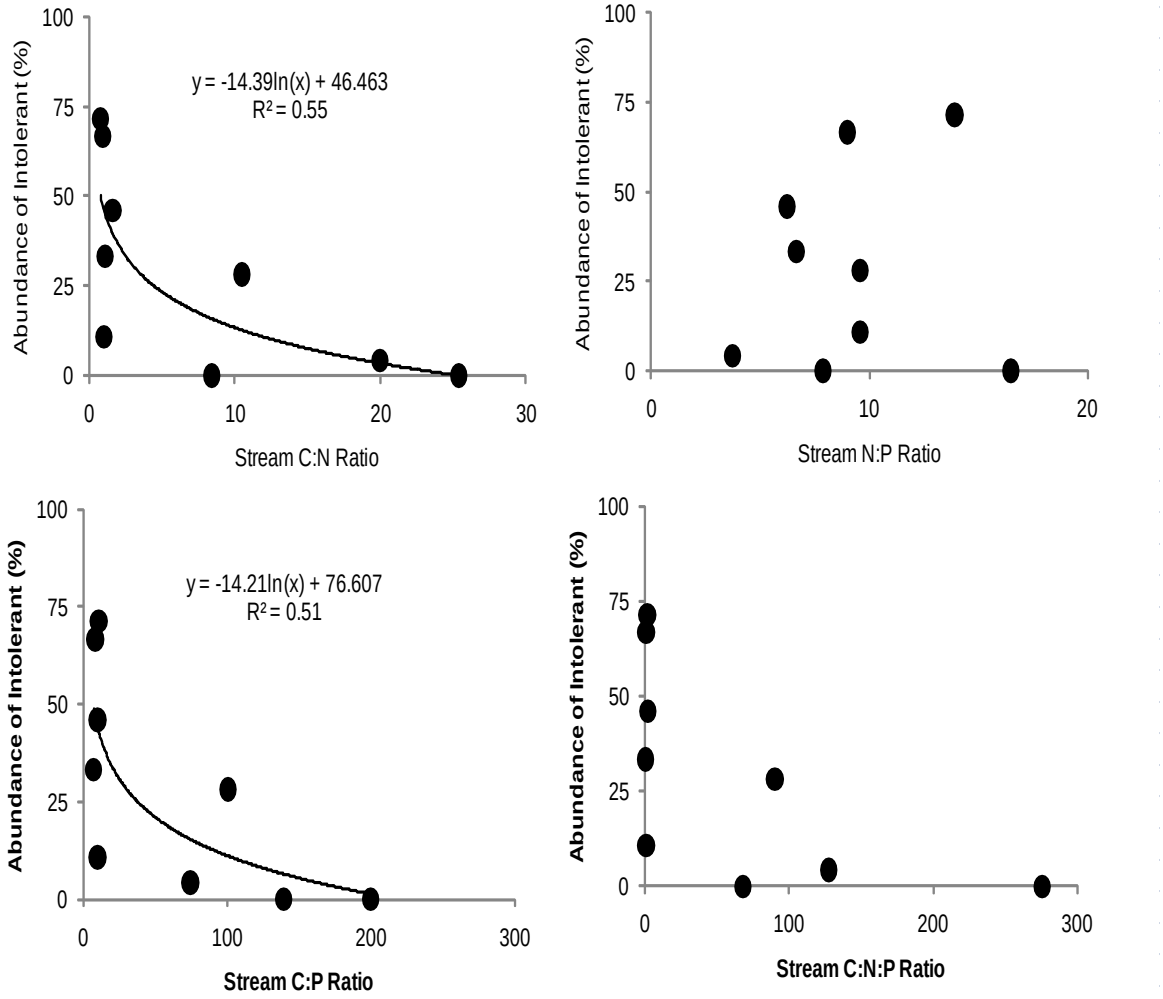

Figure 3.1 Relationships between the percentage relative abundance of pollution intolerant benthic macroinvertebrates and the C:N, C:P, N:P ratios and C/N/P values of stream chemistry.

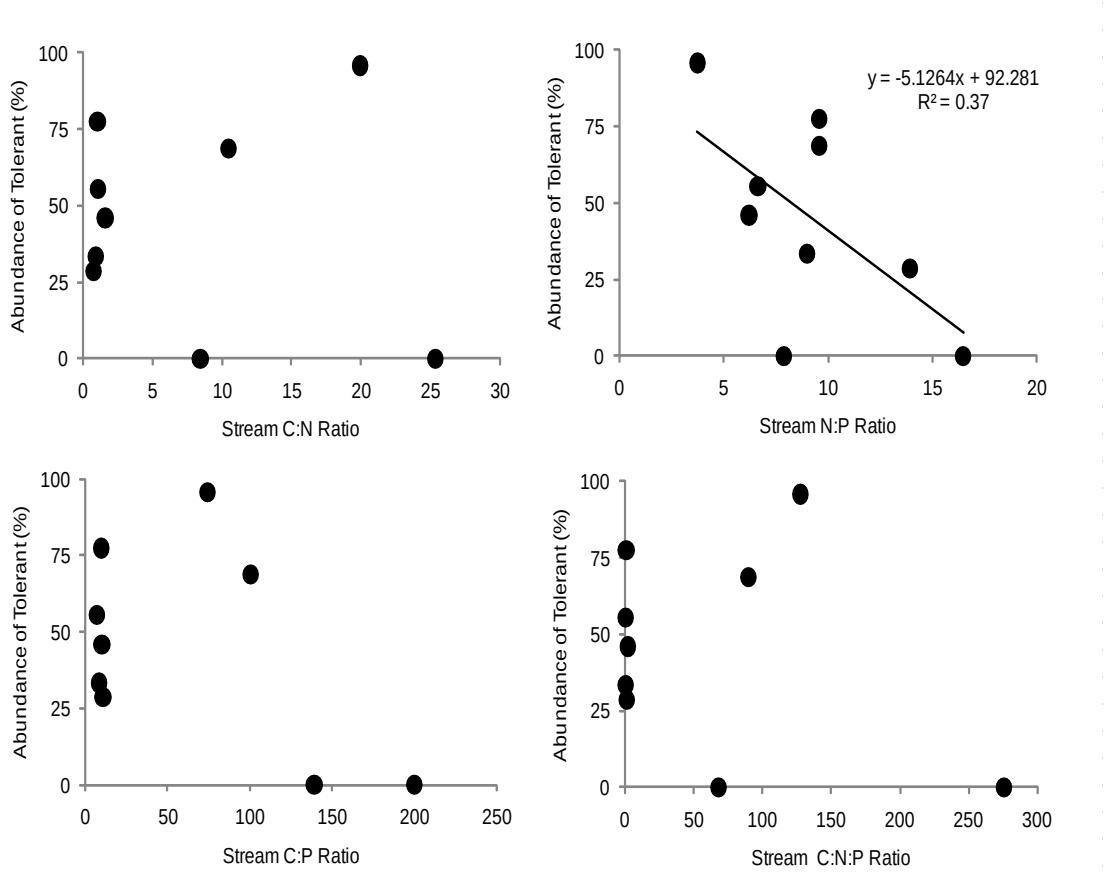

Figure 3.2 Relationships between the percentage relative abundance of pollution tolerant benthic macroinvertebrates and the C:N, C:P, N:P ratios, and C/N/P values of stream chemistry.

3.4 Discussion

The health of urban stream macroinvertebrates assemblages does not seem to be dependent on the chemical constituents within the stream alone. The study sought to measure the health of macroinvertebrates populations in relation to presence or absence of WWTP effluent, water chemistry and nutrient ratios. Increasing species richness and high percentages of pollution tolerant species such as *Ephemeroptera*, *Plecoptera* and

Trichoptera (EPT) taxa generally indicate a healthy stream where macroinvertebrate populations are thriving (Bode et al. 1991).

3.4.1 Relative abundance of %EPT and stream chemistry

Stream chemistry and the abundance of EPT appeared to have little to no noticeable effect across the different geographical sites. Munn et al. (2009) developed nonmetric multidimensional models to identify stream characteristics that were related to geographical location. One of the geographic influences in the Texas region is high stream DOC that occurs in combination with the presence of *Diptera,* which is attributed to the fine substrates in the Texas region (Munn et al. 2009). We did not find any correlation between *Diptera* and DOC but they were the most numerous taxa observed across all sites. Instead a better relationship was found between *Diptera* and stream chloride.

Another finding from the Munn et al. (2009) geographic study was the importance of EC. Over many of the models they developed for the different regions, EC was an important variable identified. This study supported the work of Munn et al. (2009) in that this study found that as stream EC increased so did the percentage of EPT. Electrical conductivity is influenced by nutrients and ions that carry over to indication of degraded and high quality water (Munn et al. 2009).

Macroinvertebrate survival in streams is highly dependent upon the conditions of their environment. During a chronic 30-day pH test on nine species of aquatic insects, each of the species TL_{50} was determined across pH values ranging from 1 – 7.8 (Bell

1971). The most tolerant species with a TL_{50} pH of 2.45 was a member of the *Trichoptera* class, *Brachycentrus americanus*; the most intolerant species was *Ephemerella subvaria* at a TL_{50} at pH of 5.38 (Bell 1971). Bell (1971) concluded that generally as pH of the water decreased the percent of aquatic invertebrates survival rate decreased as well. The pH data somewhat agreed with this principle, although the lowest pH value was 7.73 at Still Creek 2 which is below a WWTP had the greatest number of individuals collected. This was most likely not due to pH but due to other factors such as nutrient concentrations often observed in effluent. The high pH in the study streams is most likely due to the highly sodic soils within our watersheds which is likely induced by irrigation water chemistry (Aitkenhead-Peterson et al. 2009) plus the underlying geology of the watersheds (Aitkenhead-Peterson et al. 2011). Increases in the percentage of EPT however have mainly been found in studies investigating streams impacted with acid mine drainage (Chadwick et al. 1986, DeNicola and Stapleton 2000) suggesting an inverse relationship between EPT and pH. This study's pH levels are not highly acidic nor are they highly basic, and fall within the normal range for streams of 6.5 – 9.0 (TCEQ 2009). While there was a drop in the number of individuals collected at the site with the highest pH (8.2), it had a relatively high %EPT at 67% and the highest number of species.

Habitat quality is a primary factor in determining the health of an aquatic ecosystem for macroinvertebrate assemblages', however a combination of nutrient concentrations and habitat quality is necessary (Heatherly et al. 2007). Heatherly et al (2007) examined habitat quality in 53 streams in Illinois, USA yet they were unable to

identify habitat quality as a primary factor in determining the health of the streams based up macroinvertebrate assemblages. They did not observe a decrease in %EPT with increasing anthropogenic disturbance which they based on surrounding land use in their watersheds (Heatherly et al. 2007). While Heatherly et al. (2007) reported statistically significant different chemistries between water samples from above and below wastewater treatments; these differences were found not to be factors in the relationship with %EPT.

The presence of the species *Ephemeroptera, Plecoptera, and Trichoptera* have been associated with good habitat for pollution intolerant species (Rosenberg and Resh 2003). This study shows that effect of WWTP effluent alone is not indicative of the health of a macroinvertebrate community. All locations within this study below a WWTP had at least one member belonging to the orders included in the EPT taxa. This precludes that perhaps the addition of WWTP effluent is not the sole cause of habitat impairment for invertebrates. It might imply that WWTPs within the sampling area are not determining the invertebrate populations. The invertebrate sampling revealed that locations throughout the watershed contained different characteristics and assemblages.

3.4.2 Land use and invertebrate health

Land use surrounding sampling sites have a greater effect on invertebrate populations than stream chemistry alone (Steur et al. 2010). Steur et al. (2010) acquired macroinvertebrate collections from 261 streams that varied in gradients of increasing urbanization across nine different metropolitan areas in Oregon, Massachusetts and

Texas. The nine metropolitan areas included a wide range of physiographic settings with different elevations and climates and mean annual precipitation. Stream health was evaluated by using %EPT and the authors concluded that EPT richness declined with increasing watershed imperviousness. Steur et al. (2010) also reported that the addition of percentage agriculture and grassland in a watershed as a type of imperviousness to their statistical model increases the reliability of the model. Agricultural and grassland soils are not impervious unless there has been high compaction of the soils through large machinery or high grazing thus it would appear that Steur et al. (2010) merely added these land uses in to achieve a stronger and more significant relationship between %EPT and impervious surfaces. Sites with the highest EPT richness were located in areas draining from forest and wetlands (Steur et al. 2010).

The land use surrounding the invertebrate sampling sites determines the volume of runoff and sediment transport that a sampling site may receive. Urbanization often leads to increased flows and straightening of the channels to deal with increased runoff from impervious surfaces (Paul and Meyer 2001). These extreme changes in hydrology in terms of velocity and stage height are also associated with population loss and survival (Munn et al. 2009). While watershed land use was not a focus on in this study beyond the location of WWTP effluent discharges, the addition of land use data with a focus on urbanization, rural, and agriculture data is perhaps another area that should be considered a factor in percentage EPT abundance and stream health. Peach Creek, the most rural site studied is surrounded by mostly pasture and grassland. But its dominant land cover is forest (37%) followed by agricultural pasture (28%) (Aitkenhead-Peterson

et al. 2009), and was dominated by the order of *Diptera.* The combination of chemistry, hydrologic conditions, and land use appear to be the factors most commonly correlated to healthy macroinvertebrate communities.

The study of aquatic invertebrate health should be a concern for future researchers. As the scientific community begins to evaluate strategies implemented to reduce nutrient and sediment loads, invertebrate health should be monitored for recovery. Stream restoration is going to play an important role in the future as the infrastructure of our urban areas continues to age and be improved. Invertebrate health needs to be studied as these mitigation plans are implemented to ensure that invertebrate populations are surviving.

3.4.3 Limitations of the study

There were several limitations in this study that prevented the finding of more significant results. Replicate samples should have been taken at each of the sites such as 3 replicates from riffles and 3 replicate samples from pools which would have strengthened the ability to perform statistical analysis.

4. CONCLUSIONS

From the hypothesis of the two studies included in this thesis several conclusions can be made. These findings can be applied to other urban streams in regards to *E. coli* regrowth and macroinvertebrate relative abundance of EPT.

4.1 Conclusions

1. A considerable reduction in stream *E. coli* numbers downstream of wastewater treatment plants could be achieved by reducing the high concentrations of N and P in the receiving waters and effluent thereby increasing the stream C/N/P value and rendering N and P limiting for *E. coli* regrowth.

2. Greater proportions of turf grass in a watershed will lower the runoff C/N/P value and may result in better conditions for recovery and regrowth of *E. coli*. Thus maximizing aeration of turfgrass for water infiltration and public education on reducing runoff from turfgrass to adjacent impervious surfaces should help to mitigate this problem.

3. Further research is needed to understand the factors that affect the health of macroinvertebrate populations in urban and rural streams, but it appears that WWTP effluent is not an independent variable in the prediction of macroinvertebrate health.

REFERENCES

Abel PD (1989) Water Pollution Biology. New York: Ellis Horwood; Halsted Press.

Aitkenhead-Peterson JA, Alexander JE, Clair TA (2005) Dissolved organic carbon and dissolved organic nitrogen export from forested watersheds in Nova Scotia: Identifying controlling factors. Glob Biogeochem Cycles, 19(4):1-8

Aitkenhead-Peterson JA, Steele MK, Nahar N, Santhy K (2009) Dissolved organic carbon and nitrogen in urban and rural watersheds of south-central texas: Land use and land management influences. Biogeochem, 96(1-3):119-129

Aitkenhead-Peterson JA, Nahar N, Harclerode C, Stanley N (2011) Chemistry of urban and rural watersheds in central Texas. Urban Ecosystems. doi 10.1007/s11252-010-0147-2.

Aitkenhead-Peterson JA, Volder A (Eds) (2010) Urban ecosystem ecology. Agronomy Monograph 55. ASA, CSSA, SSSA Madison WI, USA

Alonso E, Santos A, Riesco P (2004) Micro-organism re-growth in wastewater disinfected by UV radiation and ozone: A micro-biological study. Environmental Technology, 25(4), 433-441.

Antonelli M, Mezzanotte, V, & Nurizzo, C (2008) Wastewater Disinfection by ultraviolet Irradiation: Short and Long Term Efficiency. Environmental Science Engineering , 25 (3):363-373.

Arana I, Santorum P, Muela A, Barcina I (2000) Effect of disinfection upon dissolved organic carbon (DOC) in wastewater: Bacterial bioassays. Letters in Applied Microbiology, 31(2):157-162.

Arnold CL, Gibbons CJ (1996) Impervious surface coverage: The emergence of a key environmental indicator. Journal of American Planning Association, 62(2):243-156.

Barcina I, Gonzalez JM, Iriberri J, Egea L (1989) Effect of visible light on progressive dormancy of Escherichia coli cells during the survival process in natural fresh water. Applied and Environmental Microbiology, 55(1):246-251.

Bedan ES, Clausen JC (2009) Stormwater runoff quality and quantity from traditional and low impact development watersheds(1) . Journal of American Water Resources Association 45(4):998-1008.

Bell HL (1971) Effect of low pH on the survival and emergence of aquatic insects. Water Research 5(6):313-319.

Blatchley ER III, Gong W, Alleman, JE, Rose JB (2007) Effects of wastewater disinfection on waterborne bacteria and viruses. Water Environment Research, 79(1):79-81.

Bode RW, Novak MA, Abele LE (1991) Methods for rapid biological assessment of streams. Albany, NY: NYS DEC Stream Biomonitoring Unit.

Bolster CH, Bromley JM, Jones SH (2005) Recovery of chlorine-exposed Escherichia coli in estuarine microcosms. Environ Sci Techno 39(9):3083-3089.

Booth DB, Jackson CR (1997) Urbanization of Aquatic Systems:: degradation thresholds, stormwater detection, and the limits of mitigation. Journal of the American Water Resources Association, 33(5):1077-1090.

Boualam M, Fass S, Saby S, Lahoussine V (2003) Organic matter quality and survival of coliforms in low-nutritive waters. American Water Works Association Journal, 95(8):119.-127.

Brett M, Arhonditsis G, Mueller S, Hartley D, Frodge J, Funke D (2005) Non-point-source impacts on stream nutrient concentrations along a forest to urban gradient. Environmental Management, 35(3):330-342.

Burian SJ, Pomeroy CA (2010) Urban impacts on water cycle and potential green infrastructure implications. In Aitkenhead-Peterson JA, Volder A (eds.), Urban Ecosystem Ecology (1st ed., pp. 277-296) Madison WI: ASA, CSSA, SSSA.

Calabro PS (2010) Impact of mechanical street cleaning and rainfall events on the quantity and heavy metals load of street sediments. Environmental Technology, 31(11):1255-1262

Carone MT, Simoniello T, Manfreda S, Caricato G (2009) Watershed influence on fluvial ecosystems: An integrated methodology for river water quality management. Environmental Monitoring and Assessment, 152(1-4):327-342.

Chadwick JW, Canton SP, Dent RL (1986) Recovery of benthic invertebrate communities in silver bow creek, montana, following improved metal mine wastewater treatment. Water, Air, & Soil Pollution, 28(3):427-438.

Chambers C (1971) Chlorination for control of bacteria and viruses in treatment plant effluents. Journal - Water Pollution Control Federation, 43(2):228-241.

Corbyn, Z (2010) Ecologist shun the urban jungle. Nature. doi:10.1038/news.2010.359

Cuffney T, Brightbill R, May J, Waite I (2010) Responses of benthic macroinvertebrates to environmental changes associated with urbanization in nine metropolitan areas Ecological Applications, 20(5):1384-1401.

Davis JA, Hetzel F, Oram JJ, McKee LJ (2007) Polychlorinated biphenyls (PCBs) in San Francisco Bay Environ Res 105(1):67-86

Davies CM, Long JAH, Donald M, and Ashbolt NJ (1995) Survival of fecal microorganisms in marine and freshwater sediments. Applied Environmental Microbiology 61(5):1888-1896.

de Baar, HJW (1994) Von liebig's law of the minimum and plankton ecology (1899–1991) Progress in Oceanography, 33(4):347-386.

DeNicola DM, Stapleton MG (2002) Impact of acid mine drainage on benthic communities in streams: The relative roles of substratum vs. aqueous effects. Environmental Pollution, 119(3):303-315.

Donnison A, Ross C, Thorrold B (2004) Impact of land use on faecal microbial quality of hill-country streams. New Zealand Journal of Marine and Freshwater Research, 38:845-855.

Du Preez M, Kfir R, Coubrough P (1995) Investigation of Injury of Coliforms after Chlorination. Water Sci Techno 3 (5-6):115-118.

Dunne T, Leopold LB (1978) Water in Environmental Planning. San Francisco, CA: W.H. Freeman.

Galli FJ (1991) Thermal Impacts Associated with Urbanization and Stormwater Management Best Management Practices. Washington, DC: Washington Council of Government.

Gómez N, Sierra MV, Cortelezzi A, Rodrigues Capítulo A (2008) Effects of discharges from the textile industry on the biotic integrity of benthic assemblages. Ecotoxicology and Environmental Safety, 69(3):472-479.

Gücker B, Brauns M, Pusch MT (2006) Effects of wastewater treatment plant discharge on ecosystem structure and function of lowland streams. Journal of the North American Benthological Society, 25(2):313-329.

Hammer TR (1972) Stream channel enlargement due to urbanization. Water Resources Research, 8(6):1530-1540.

Harclerode C (2009) A geographical approach to tracking Escherichia coli and nutrients in a Texas coastal plains watershed. Thesis, Texas A&M University.

Hasan A, Thacker N, Bassin J (2010) Trihalomethane formation potential in treated water supplies in urban metro city. 168(1):489-497.

Hathaway JM, Hunt W, Jadlocki S (2009) Indicator bacteria removal in storm-water best management practices in charlotte, north carolina. Journal of Environmental Engineering, 135(12):1275-1285.

Hawkins C (2006) Quantifying Biological Integrity By Taxonomic Completeness: Its Utility In Regional and Global Assessments. Ecological Applications, 16 (4):1277–1294.

Heatherly T Jr, Whiles M, Royer T, David M (2007) Relationships between water quality, habitat quality, and macroinvertebrate assemblages in illinois streams. Journal of Environmental Quality, 36(6):1653-1660.

Hendricks CW, Morrison SM (1967) Multiplication and growth of selected enteric bacteria in clear mountain stream water. Water Research, 1(8-9):567-576.

Hilsenhoff WL (1988) Rapid field assessment of organic pollution with a family-level biotic index. Journal of the North American Benthological Society, 7:65–68.

Hoiland W, Rabe F (1992) Effects of increasing zinc levels and habitat degradation on macroinvertebrate communities in 3 north idaho streams. Journal of Freshwater Ecology, 7(4):373-3810.

Hope D, Gries C, Zhu W, Fagan WF, Redman CL, et al. (2003) Socioeconomics drive urban plant diversity. Proceedings of the National Academy of Science, 100(15):8788-8792.

Hrudey SE (2009) Chlorination disinfection by-products, public health risk tradeoffs and me. Water Research, 43(8):2057-2092.

Jjemba P (2004) Environmental Microbiology : Principles and Applications. Enfield, NH: Science Publishers.

Jones RC, Clark CC (1987) Impact of watershed urbanization on stream insect communities. Water Resour. Bull. 23:1047–1055.

Kaushal, SS, Groffman PM, Likens GE, Belt KT, Stack WP, et. al. (2005) Increased salinization of fresh water in the northeastern United States. Proceedings of the National Academy of Sciences 102:13517-13520.

Kaushal SS, Groffman PM, Band LE, Shields RP, Morgan RP, et al. (2008) Interaction between Urbanization and Climate Variability Amplifies Watershed Nitrate Export in Maryland. Environmental Science & Technology 42(16):5872-5878

Keller AA, Cavallaro L (2008) Assessing the US Clean Water Act 303(d) listing process for determining impairment of a waterbody. Journal of Environmental Management. 86:699-711.

Kirchman DL, Rich JH (1997) Regulation of Bacterial Growth Rates by Dissolved Organic Carbon and Temperature in the Equatorial Pacific Ocean. Springer New York.

Kiulia N, Netshikweta R, Page N, Van Zyl W, Kiraithe M, Nyachieo A, et al. (2010) The detection of enteric viruses in selected urban and rural river water and sewage in kenya, with special reference to rotaviruses. Journal of Applied Microbiology, 109(3):818-828.

Klein RD (1979) Urbanization and stream quality impairment. Water Resour. Bull. 15:948–63

Klocker C, Kaushal S, Groffman P, Mayer P, Morgan R (2009) Nitrogen uptake and denitrification in restored and unrestored streams in urban maryland, USA. Aquatic Sciences, 71(4):411-424.

Kolbe CM, Luedke M (2009) A Guide to Freshwater Ecology. TCEQ Publication Austin, TX.

Kühl AM, Mello Sartori Cardoso da Rocha CL, Gaeta Espíndola EL, Lansac-Tôha FA (2010) Rural and urban streams: Anthropogenic influences and impacts on water and sediment quality International Review of Hydrobiology, 95(3):260-272.

Leopold LB (1968) Hydrology for Urban Land Planning - A Guidebook on the Hydrologic Effects of Urban Land Use. Washington D.C: Geological Survey Circular 554 Department of Interior.

Lim CH, Flint KP (1989) The effects of nutrients on the survival of Escherichia coli in lake water. Journal of Applied Microbiology, 67(6):650-653.

McBride M, Hession WC, Rizzo DM (2010) Riparian reforestation and channel change: How long does it take? Geomorphology, 116(3-4):330-340.

McDowell WH, Zsolnay A, Aitkenhead-Peterson JA, Gregorich EG, Jones DL, et al. (2006) A comparison of methods to determine the biodegradable dissolved organic carbon from different terrestrial sources. Soil Biology and Biochemistry, 38(7):1933-1942.

Meyer JL, Paul MJ, Taulbee WK (2005) Stream ecosystem function in urbanizing landscapes. Journal of the North American Benthological Society, 24(3):602-612.

Meyer KJ, Appletoft CM, Schwemm AK, Uzoigwe JC, Brown EJ (2005) Determining the source of fecal contamination in recreational waters. Journal of Environmental Health, 68(1):25-30.

Mezzanotte V, Antonelli M, Citterio S, Nurizzo C (2007) Wastewater disinfection alternatives: Chlorine, ozone, peracetic acid, and UV light. Water Environment Research, 79(12):2373-2379.

Muirhead RW, Collins RP, Bremer PJ (2006) Interaction of Escherichia coli and soil particles in runoff. Applied and Environmental Microbiology, 72(5):3406-3411.

Munn M, Waite I, Larsen D, Herlihy A (2009) The relative influence of geographic location and reach-scale habitat on benthic invertebrate assemblages in six ecoregions. Environmental Monitoring and Assessment, 154(1-4):1-14.

Newall R, Walsh CJ (2005) Response of epilithic diatom assemblages to urbanization influences. Hydrobiologia 532:53-67.

Novotny V (2003) Water Quality: Diffuse Pollution and Watershed Management (2nd ed.) Hoboken, NJ: J. Wiley.

O'Brien P, Wehr J (2010) Periphyton biomass and ecological stoichiometry in streams within an urban to rural land-use gradient. Hydrobiologia, 657(1):89-105.

Olsen SJ, Miller G, Breuer T, Kennedy M, Higgins C, Walford J, et al. (2002) A waterborne outbreak of Escherichia coli O157:H7 infections and hemolytic uremic syndrome: Implications for rural water systems. Emerging Infectious Diseases, 8(4): 370-375.

Paul MJ, Meyer JL (2001) Streams in the urban landscape. Annual Review of Ecology & Systematics, 32:333-365.

Perakis SS, Hedin LO (2007) State factor relationships of dissolved organic carbon and nitrogen losses from unpolluted temperate forest watersheds. J.Geophys.Res., 112(G02010):1-7

Petersen TM, Suarez MP, Rifai HS, Jensen P (2006) Status and trends of fecal indicator bacteria in two urban watersheds. Water Environment Research, 78(12):2340-2355.

Qualls RG (2005) Biodegradability of fractions of dissolved organic carbon leached from decomposing leaf litter. Environ. Sci. Technol. 39:1616-1622.

Reitzer L. (2003) Nitrogen assimilation and global regulation in *Escherichia coli*. Annu Rev Microbiol 27:155-176.

Roberts L, Boardman G, Voshell R (2009) Benthic macroinvertebrate susceptibility to trout farm effluents. Water Environment Research, 81(2):150-159.

Rosen BH (2000) Waterborne Pathogens in Agricultural Watersheds. Burlington, Vt.: USDA−NRCS Watershed Institute, University of Vermont

Rosenberg DM, Resh VH (2003) Freshwater Biomonitoring and Benthic Macroinvertebrates. New York: Chapman and Hall.

Roy AH, Rosemond AD, Paul MJ, Leigh DS, Wallace JB (2003) Stream macroinvertebrate response to catchment urbanisation (Georgia, U.S.A.) Freshwater Biology, 48(2):329-346.

Roy AH, Paul MJ, Wenge SJ (2010) Urban stream ecology. In Aitkenhead-Peterson JA, Volder A (eds.), Urban Ecosystem Ecology (1st ed., 341-352) Madison, WI: ASA, CSSA, SSSA.

Rupprecht, Rainer (2009) Attempts to re-colonise water insects in German brooks, Aquatic Insects, 31(1):429-441.

Sakata M, Takagi T, Mitsunobu S (2010) Evaluation of loads and sources of heavy metals in Tama River, Tokyo. Water, Air, & Soil Pollution 213(1):363-373.

Schreiber JD, Rebich RA, Cooper CM (2001) Dynamics of diffuse pollution from US southern watersheds. Water Research, 35(10):2534-2542.

Schueler TR Galli J (1992) Environmental impacts of stormwater ponds. In Watershed Restoration Source Book. ed. P Kumble, T Schueler, Washington, DC: Metropol. Wash. Counc. Gov

Singh A, McFeters GA (1992) Detection of waterbourne pathogens. In R. Mitchell (Ed.), Environmental Microbiology. New York: Wiley-Liss.

Smith DG (2001) Pennak's Freshwater Invertebrates of the United States: Porifera to Crustacea, 4th Edition. 4 Ed. New York: Wiley.

Somarelli JA, Makarewicz JC, Sia R, Simon R (2007) Wildlife identified as major source of Escherichia coli in agriculturally dominated watersheds by BOX A1R-derived genetic fingerprints. Journal of Environmental Management, 82(1):60-65.

Stander EK, Ehrenfeld JG (2010) Urban riparian function. In J. A. Aitkenhead-Peterson, & A. Volder (Eds.), Urban Ecosystem Ecology (1st ed., pp. 253-276) Madison WI: ASA, CSSA, SSSA.

Steele MK, Aitkenhead-Peterson JA (submitted) Long-term sodium and chloride surface water exports from the Dallas/Fort Worth region. Science of the Total Environment. (in press)

Steele MK, McDowell WH, Aitkenhead-Peterson, JA (2010) Chemistry of urban, suburban, and rural surface waters. In Aitkenhead-Peterson JA, Volder A (eds.), Urban Ecosystem Ecology (1st ed., pp.297-340) Madison, WI: ASA, CSSA, SSSA.

Stephenson GR, Rychert RC (1982) Bottom sediment: A reservoir of Escherichia coli in rangeland streams. Journal of Range Management, 35(1):119-123.

Steuer J (2010) A generalized watershed disturbance-invertebrate relation applicable in a range of environmental settings across the continental United States. 13(4):415-424.

Sun L, Perdue EM, Meyer JL, Weis J (1997) Use of elemental composition to predict bioavailability of dissolved organic matter in a georgia river. Limnology and Oceanography, 42(4):714-721.

Sun SW, Deng ZQ, Gang DD (2010) Nonpoint source pollution. Water Environment Research, 82(10):1875-1894.

Surbeck CQ, Jiang SC, Grant SB (2010) Ecological control of fecal indicator bacteria in an urban stream. Environmental Science & Technology, 44(2):631-637

Suren AM (2009) Using macrophytes in urban stream rehabilitation: A cautionary tale. Restoration Ecology, 17(6):873-883.

Texas Commission on Environmental Quality (2008) Texas Integrated Report for Clean Water Act Sections 305(b) and 303(d) Retrieved from http://www.tceq.state.tx.us/compliance/monitoring/water/quality/data/08twqi/twqi08.html

Texas Commission on Environmental Quality (2010) Improving Water Quality in Carters Creek and Burton Creek – A TMDL Project for Bacteria. http://www.tceq.info/assets/public/implementation/water/tmdl/85carters/85-carterscreek_po.pdf

Thorp J, Covich A (ed) (2009) Ecology and Classification of North American Freshwater Invertebrates, Third Edition. 3 ed. London: Academic Press.

Theron J, Cloete TE (2002) Emerging Waterborne Infections: Contributing Factors, Agents, and Detection Tools. Critical Reviews in Microbiology. 28(1):1-26.

Trueman PS, van den Hurk P (2010) Xenoestrogen Exposure and Effects in Bluegill from the Reedy River, South Carolina, USA: Archives of Environmental Contamination and Toxicology. 58(1):165-175

Ulseth AJ, Hershey AE (2005) Natural abundances of stable isotopes trace anthropogenic N and C in an urban stream. Journal of the North American Benthological Society, 24(2):270-289.

USEPA (1986) Criteria and Standards Division.. Ambient Water Quality Criteria for Bacteria 1986 (EPA Publication No. 440/5-84-002) Washington, D.C.: USEPA Office of Water Retrieved from http://www.epa.gov/waterscience/beaches/files/1986crit.pdf

USEPA (1999) Wastewater Technology Fact Sheet – Ultraviolet Disinfection. (EPA Publication No 832-F-99-064) Washington D.C.: USEPA Office of Water.

USEPA (2001) Protocol for developing pathogen TMDLs. 1st ed. EPA 841−R−00−002. Washington, D.C.: USEPA Office of Water

USEPA (2002) Method 1603: Escherichia coli (E. coli) in Water by Membrane Filtration Using Modified membrane-Thermotolerant Escherichia coli Agar (Modified m TEC) (EPA Publication No. 821-R-02-023) Washington, D.C.: USEPA Office of Water Retrieved from http://www.epa.gov/microbes/1603sp02.pdf

USEPA (2003) Ultaviolet Disinfection Guidance Manual (EPA Publication No 815-D-03-007)Washington, D.C.: USEPA Office of Water.

USEPA (2004) National water quality inventory:2004 report. Available at http://water.epa.gov/lawsregs/guidance/cwa/305b/2004report_index.cfm.. Washington D.C.: USEPA Office of Water.

Viessman W Jr, Hammer MJ (2005) Water Supply and Pollution Control (7th ed.) Upper Saddle River, NJ: Pearson/Prentice Hall.

Viswanathan B, Volder A, Watson T, Aitkenhead-Peterson JA (2011) Impervious and pervious pavements increase soil CO_2 concentrations and reduced root production of american sweetgum (liquidambar styraciflua) urban forestry and urban greening. Urban Forestry and Urban Greening. In press.

Wakelin SA, Colloff MJ, Kookana RS (2008) Effect of wastewater treatment plant effluent on microbial function and community structure in the sediment of a freshwater stream with variable seasonal flow. Applied and Environmental Microbiology, 74(9):2659-2668.

Walsh CJ, Roy AH, Feminella JW, Cottingham PD, Groffman PM, Morgan RP (2005) The urban stream syndrome: Current knowledge and the search for a cure. Journal of the North American Benthological Society, 24(3):706-723.

Walters SP, Gannon VPJ, Field KG (2007) Detection of *Bacteroidales* fecal indicators and the zoonotic pathogens *E. coli* 0157:H7, *Salmonella,* and *Campylobacter* in river water. Environmental Science and Technology 41:1958-1962.

Walters DM, Roy AH, Leigh DS (2009) Environmental indicators of macroinvertebrate and fish assemblage integrity in urbanizing watersheds. Ecological Indicators, 9(6):1222-1233.

Warkentin BP (2001) Diffuse pollution: Lessons from soil conservation policies. Water Science and Technology, 44(7):197-202.

Wiegner TN, Seitzinger SP, Glibert PM, Bronk DA (2006) Bioavailability of dissolved organic nitrogen and carbon from nine rivers in the eastern United States. Aquatic Microbial Ecology, 43(3):277-287.

APPENDIX I

E. COLI COUNTS AND C AND N IN SUBCATCHMENTS

		Time (h)						
		t=6	t=12	t=18	t=24	t=36	t=48	t=72
Treatment	Dilution mL	CFU's 100 mL^{-1}						
Control 1	20	0	0	0	0	0	0	0
Control 1	10	0	0	0	0	0	0	0
Control 1	1	0	0	0	0	0	0	0
Control 2	20	0	0	0	0	0	0	0
Control 2	10	0	0	0	0	0	0	0
Control 2	1	0	0	0	0	0	0	0
Leaf Low 1	20	0	15	x	x	x	x	x
Leaf Low 1	10	0	4	128	TMTC	x	x	x
Leaf Low 1	1	0	3	21	103	x	70	67
Leaf Low 1	0.1	x	x	2	12	11	9	8
Leaf Low 1	0.01	x	x	x	x	1	1	2
Leaf Low 1	0.001	x	x	x	x	0	x	x
Leaf Low 1	0.0001	x	x	x	x	x	x	x
Leaf Low 2	20	1	7	x	x	x	x	x
Leaf Low 2	10	0	7	175	TMTC	x	x	x
Leaf Low 2	1	0	0	18	241	277	281	269
Leaf Low 2	0.1	x	x	1	35	52	46	41
Leaf Low 2	0.01	x	x	x	x	4	8	4
Leaf Low 2	0.001	x	x	x	x	x	x	x
Leaf Low 2	0.0001	x	x	x	x	x	x	x
Leaf Low 3	20	0	0	x	x	x	x	x
Leaf Low 3	10	0	0	0	2	x	x	x
Leaf Low 3	1	0	0	0	0	1	0	0
Leaf Low 3	0.1	x	x	0	0	0	0	0
Leaf Low 3	0.01	x	x	x	x	0	0	0
Leaf Low 3	0.001	x	x	x	x	x	x	x
Leaf Low 3	0.0001	x	x	x	x	x	x	x
Leaf Mid 1	20	0	0	x	x	x	x	x
Leaf Mid 1	10	0	0	0	x	x	x	x
Leaf Mid 1	1	0	0	0	0	1	0	0
Leaf Mid 1	0.1	x	x	0	0	0	0	0
Leaf Mid 1	0.01	x	x	x	0	0	0	0
Leaf Mid 1	0.001	x	x	x	x	x	x	x

Leaf Mid 1	0.0001	x	x	x	x	x	x	x
Leaf Mid 2	20	1	41	x	x	x	x	x
Leaf Mid 2	10	0	19	TMTC	x	x	x	x
Leaf Mid 2	1	0	2	90	TMTC	x	TMTC	x
Leaf Mid 2	0.1	x	x	7	192	TMTC	203	111
Leaf Mid 2	0.01	x	x	x	26	35	22	21
Leaf Mid 2	0.001	x	x	x	x	4	x	3
Leaf Mid 2	0.0001	x	x	x	x	x	x	x
Leaf Mid 3	20	0	0	x	x	x	x	x
Leaf Mid 3	10	0	0	2	x	x	1	x
Leaf Mid 3	1	0	0	0	15	0	0	0
Leaf Mid 3	0.1	x	x	0	0	0	1	0
Leaf Mid 3	0.01	x	x	x	0	0	x	0
Leaf Mid 3	0.001	x	x	x	x	x	x	x
Leaf Mid 3	0.0001	x	x	x	x	x	x	x
Leaf High 1	20	0	0	x	x	x	x	x
Leaf High 1	10	0	0	2	x	x	x	x
Leaf High 1	1	0	0	0	22	x	x	x
Leaf High 1	0.1	x	x	0	4	7	36	x
Leaf High 1	0.01	x	x	x	0	4	1	x
Leaf High 1	0.001	x	x	x	x	0	0	0
Leaf High 1	0.0001	x	x	x	x	x	x	0
Leaf High 1	0.00001	x	x	x	x	x	x	0
Leaf High 2	20	0	27	x	x	x	x	x
Leaf High 2	10	0	9	TMTC	x	x	x	x
Leaf High 2	1	0	0	74	TMTC	x	x	x
Leaf High 2	0.1	x	x	4	298	TMTC	x	x
Leaf High 2	0.01	x	x	x	54	TMTC	x	x
Leaf High 2	0.001	x	x	x	x	83	94	88
Leaf High 2	0.0001	x	x	x	x	x	12	11
Leaf High 2	0.00001	x	x	x	x	x	0	0
Leaf High 3	20	28	TMTC	x	x	x	x	x
Leaf High 3	10	14	TMTC	TMTC	x	x	x	x
Leaf High 3	1	0	71	TMTC	TMTC	x	x	x
Leaf High 3	0.1	x	x	TMTC	TMTC	x	x	x
Leaf High 3	0.01	x	x	35	TMTC	TMTC	x	x
Leaf High 3	0.001	x	x	150	150	190	170	154
Leaf High 3	0.0001	x	x	x	x	19	17	20
Leaf High 3	0.00001	x	x	x	x	x	2	2
Grass Low 1	20	1	40	x	x	x	x	x

Grass Low 1	10	0	8	313	x	x	x	x
Grass Low 1	1	0	3	58	TMTC	x	x	x
Grass Low 1	0.1	x	x	4	4	7	7	6
Grass Low 1	0.01	x	x	x	3	0	2	4
Grass Low 1	0.001	x	x	x	x	1	1	0
Grass Low 1	0.0001	x	x	x	x	x	x	x
Grass Low 2	20	4	251	x	x	x	x	x
Grass Low 2	10	1	116	TMTC	x	x	x	x
Grass Low 2	1	0	13	TMTC	x	x	x	x
Grass Low 2	0.1	x	x	106	100	138	134	101
Grass Low 2	0.01	x	x	x	13	21	16	7
Grass Low 2	0.001	x	x	x	3	2	0	3
Grass Low 2	0.0001	x	x	x	x	x	x	x
Grass Low 3	20	28	TMTC	x	x	x	x	x
Grass Low 3	10	8	TMTC	TMTC	x	x	x	x
Grass Low 3	1	3	93	TMTC	x	x	x	x
Grass Low 3	0.1	x	x	TMTC	TMTC	x	TMTC	TMTC
Grass Low 3	0.01	x	x	25	70	64	75	84
Grass Low 3	0.001	x	x	x	18	5	9	8
Grass Low 3	0.0001	x	x	x	x	1	x	x
Grass Mid 1	20	11	x	x	x	x	x	x
Grass Mid 1	10	1	TMTC	TMTC	x	x	x	x
Grass Mid 1	1	2	81	TMTC	x	x	x	x
Grass Mid 1	0.1	x	5	216	TMTC	x	x	x
Grass Mid 1	0.01	x	x	x	136	160		153
Grass Mid 1	0.001	x	x	x	24	24	16	11
Grass Mid 1	0.0001	x	x	x	x	1	1	6
Grass Mid 1	0.00001	x	x	x	x	x	0	x
Grass Mid 2	20	TMTC	x	x	x	x	x	x
Grass Mid 2	10	183	TMTC	TMTC	x	x	x	x
Grass Mid 2	1	25	TMTC	TMTC	x	x	x	x
Grass Mid 2	0.1	x	TMTC	TMTC	TMTC	x	x	x
Grass Mid 2	0.01	x	285	x	TMTC	TMTC	x	TMTC
Grass Mid 2	0.001	x	x	150	287	264	TMTC	237
Grass Mid 2	0.0001	x	x	x	x	51	35	23
Grass Mid 2	0.00001	x	x	x	x	x	11	x
Grass Mid 3	20	0	x	x	x	x	x	x
Grass Mid 3	10	0	TMTC	TMTC	x	x	x	x
Grass Mid 3	1	0	35	158	x	x	x	x
Grass Mid 3	0.1	x	0	10	22	x	x	x

Grass Mid 3	0.01	x	x	x	1	0	x	2
Grass Mid 3	0.001	x	x	x	0	0	0	0
Grass Mid 3	0.0001	x	x	x	x	0	0	0
Grass High 1	20	0	x	x	x	x	x	x
Grass High 1	10	0	0	11	12	x	x	x
Grass High 1	1	0	0	1	1	116	x	x
Grass High 1	0.1	x	0	0	0	6	170	282
Grass High 1	0.01	x	x	x	x	1	22	32
Grass High 1	0.001	x	x	x	x	x	4	2
Grass High 1	0.0001	x	x	x	x	x	x	x
Grass High 2	20	0	x	x	x	x	x	x
Grass High 2	10	0	0	0	0	x	x	x
Grass High 2	1	0	0	0	0	0	x	x
Grass High 2	0.1	x	0	0	0	0	0	0
Grass High 2	0.01	x	x	x	x	0	0	0
Grass High 2	0.001	x	x	x	x	x	0	0
Grass High 2	0.0001	x	x	x	x	x	x	x
Grass High 3	20	0	x	x	x	x	x	x
Grass High 3	10	0	0	0	0	x	x	x
Grass High 3	1	0	0	0	0	0	x	x
Grass High 3	0.1	x	0	0	0	0	0	0
Grass High 3	0.01	x	x	x	x	0	0	0
Grass High 3	0.001	x	x	x	x	x	0	0
Grass High 3	0.0001	x	x	x	x	x	x	x

x - not plated TMTC - To many to count 0 - no colonies detected

APPENDIX II

MICROCOSOMS AND CHEMISTRY

Treatment	NPOC	TDN	NO_3-N	NH_4-N	DON	PO_4-P
	t=0					
	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
Effluent	8.091	22.982	19.734	0.150	3.098	3.665
Control 1	2.739	7.069	6.824	0.083	0.161	1.170
Control 2	2.833	7.147	6.269	0.088	0.790	1.184
Leaf Low 1	12.512	7.215	6.409	0.106	0.699	1.280
Leaf Low 2	12.638	7.303	6.435	0.126	0.742	1.297
Leaf Low 3	12.787	7.251	6.021	0.119	1.110	1.246
Leaf Mid 1	51.955	7.785	6.609	0.161	1.015	1.653
Leaf Mid 2	52.296	7.802	6.926	0.158	0.717	1.664
Leaf Mid 3	52.829	7.697	5.854	0.157	1.685	1.634
Leaf High 1	103.568	8.364	6.609	0.263	1.492	2.181
Leaf High 2	103.184	8.397	6.590	0.227	1.580	2.125
Leaf High 3	101.351	8.710	6.661	0.221	1.828	2.123
Grass Low 1	16.232	8.702	7.080	0.396	1.227	2.141
Grass Low 2	15.887	8.601	6.969	0.375	1.257	1.969
Grass Low 3	16.367	8.635	6.240	0.368	2.027	2.086
Grass Mid 1	72.506	14.886	6.485	1.521	6.880	5.973
Grass Mid 2	74.553	15.041	6.497	1.597	6.947	6.165
Grass Mid 3	71.930	14.543	6.930	1.495	6.119	6.165
Grass High 1	138.915	22.142	5.955	2.938	13.248	10.273
Grass High 2	141.345	22.651	6.272	2.993	13.386	10.924
Grass High 3	134.310	22.230	5.875	3.007	13.348	10.794

Treatment	t=72 NPOC mg L^{-1}	TDN mg L^{-1}	NO_3-N mg L^{-1}	NH_4-N mg L^{-1}	DON mg L^{-1}	PO_4-P mg L^{-1}
Control 1	2.995	6.724	5.572	0.087	1.064	1.0819
Control 2	2.927	7.319	6.393	0.096	0.829	1.0544
Leaf Low 1	6.483	7.143	5.859	0.105	1.179	1.1319
Leaf Low 2	6.057	6.880	5.774	0.141	0.964	1.1196
Leaf Low 3	5.449	5.686	4.662	0.136	0.889	0.9971
Leaf Mid 1	26.116	3.366	1.835	0.139	1.392	1.0870
Leaf Mid 2	27.651	2.959	1.571	0.170	1.218	1.1329
Leaf Mid 3	28.290	4.484	2.920	0.188	1.375	1.2124
Leaf High 1	72.890	2.961	0.066	0.084	2.811	1.4949
Leaf High 2	75.448	3.241	0.075	0.091	3.074	1.2879
Leaf High 3	66.921	1.997	0.066	0.078	1.854	1.6030
Grass Low 1	7.001	5.802	4.232	0.213	1.357	1.6631
Grass Low 2	6.803	6.757	5.258	0.399	1.100	1.7753
Grass Low 3	7.325	7.574	6.121	0.382	1.071	1.8671
Grass Mid 1	30.657	5.098	0.070	0.938	4.090	5.2270
Grass Mid 2	33.130	5.070	0.073	0.319	4.678	4.7192
Grass Mid 3	25.327	3.722	0.066	0.349	3.307	4.9364
Grass High 1	73.252	10.208	0.106	0.093	10.009	9.2181
Grass High 2	73.231	10.466	0.068	1.128	9.271	9.6668
Grass High 3	59.139	8.364	0.085	0.083	8.196	9.1426

	t=0			
Treatment	C:N	C:P	N:P	CNP Value
Effluent	0.352	2.208	6.271	0.640
Control 1	0.388	2.342	6.044	2.209
Control 2	0.396	2.393	6.037	2.232
Leaf Low 1	1.734	9.777	5.638	8.754
Leaf Low 2	1.730	9.744	5.631	8.618
Leaf Low 3	1.763	10.262	5.819	9.142
Leaf Mid 1	6.673	35.339	5.296	26.080
Leaf Mid 2	6.703	35.331	5.271	26.019
Leaf Mid 3	6.864	36.360	5.297	27.142
Leaf High 1	12.383	60.748	4.906	36.674
Leaf High 2	12.288	62.120	5.055	37.354
Leaf High 3	11.636	61.075	5.249	35.405
Grass Low 1	1.865	7.747	4.153	5.627
Grass Low 2	1.847	8.245	4.464	6.060
Grass Low 3	1.895	8.017	4.230	5.869
Grass Mid 1	4.871	14.984	3.076	5.267
Grass Mid 2	4.957	14.928	3.012	5.194
Grass Mid 3	4.946	14.403	2.912	5.182
Grass High 1	6.274	20.283	3.233	3.945
Grass High 2	6.240	19.408	3.110	3.690
Grass High 3	6.042	18.665	3.089	3.616

	t=72			
Treatment	C:N	C:P	N:P	CNP Value
Control 1	0.445	2.769	6.215	0.026
Control 2	0.400	2.776	6.941	0.021
Leaf Low 1	0.908	5.728	6.311	0.025
Leaf Low 2	0.880	5.410	6.144	0.026
Leaf Low 3	0.958	5.465	5.703	0.031
Leaf Mid 1	7.760	24.026	3.096	0.104
Leaf Mid 2	9.346	24.407	2.612	0.147
Leaf Mid 3	6.309	23.334	3.698	0.073
Leaf High 1	24.618	48.760	1.981	0.255
Leaf High 2	23.282	58.583	2.516	0.158
Leaf High 3	33.503	41.748	1.246	0.644
Grass Low 1	1.207	4.210	3.489	0.082
Grass Low 2	1.007	3.832	3.806	0.069
Grass Low 3	0.967	3.923	4.057	0.061
Grass Mid 1	6.014	5.865	0.975	1.051
Grass Mid 2	6.534	7.020	1.074	0.866
Grass Mid 3	6.805	5.131	0.754	1.759
Grass High 1	7.176	7.947	1.107	0.816
Grass High 2	6.997	7.576	1.083	0.853
Grass High 3	7.071	6.468	0.915	1.195

	t=0			
Treatment	Na^+ mg L^{-1}	K^+ mg L^{-1}	Mg^{2+} mg L^{-1}	Ca^+ mg L^{-1}
Effluent	204.081	9.475	2.040	8.987
Control 1	92.484	4.508	0.836	3.598
Control 2	70.261	3.182	0.630	2.678
Leaf Low 1	67.318	3.015	0.627	2.752
Leaf Low 2	68.553	4.050	1.090	3.363
Leaf Low 3	70.205	4.330	1.102	3.068
Leaf Mid 1	65.951	4.071	1.158	3.195
Leaf Mid 2	71.020	8.337	3.065	4.410
Leaf Mid 3	70.493	9.066	3.413	4.458
Leaf High 1	64.453	8.255	3.456	4.242
Leaf High 2	69.722	14.188	5.909	5.228
Leaf High 3	71.719	14.890	6.460	5.571
Grass Low 1	71.360	15.227	6.538	5.493
Grass Low 2	72.142	8.019	1.798	3.702
Grass Low 3	60.032	5.952	1.134	3.254
Grass Mid 1	66.850	6.705	0.987	3.097
Grass Mid 2	72.338	19.999	2.441	5.797
Grass Mid 3	75.535	23.511	2.688	5.977
Grass High 1	75.155	23.029	2.623	5.710
Grass High 2	75.292	37.103	4.415	8.387
Grass High 3	76.538	39.920	4.756	8.529
Grass High	75.035	41.786	5.141	9.107

	t=72			
	Na^+	K^+	Mg^{2+}	Ca^+
Treatment	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
Control 1	67.529	3.722	1.124	2.713
Control 2	64.120	2.835	0.591	2.333
Leaf Low 1	78.983	3.965	1.082	3.199
Leaf Low 2	79.396	4.421	1.054	2.745
Leaf Low 3	64.488	3.176	1.080	2.785
Leaf Mid 1	73.005	11.168	3.024	4.433
Leaf Mid 2	81.238	9.079	3.299	4.602
Leaf Mid 3	76.529	9.063	3.321	4.095
Leaf High 1	65.796	13.279	5.798	5.154
Leaf High 2	50.927	9.813	4.248	4.348
Leaf High 3	61.331	13.101	5.106	6.943
Grass Low 1	60.788	10.182	1.386	3.921
Grass Low 2	62.012	5.394	0.872	3.177
Grass Low 3	73.721	6.321	0.968	3.449
Grass Mid 1	74.696	20.014	2.278	5.785
Grass Mid 2	51.191	13.249	1.600	11.437
Grass Mid 3	64.083	20.562	2.641	6.340
Grass High 1	70.608	31.411	4.105	8.775
Grass High 2	77.660	40.426	4.389	9.099
Grass High 3	65.123	30.003	4.218	8.105

			t=0			
	EC	pH	Turbidity	F^-	Cl^-	SO_4
Treatment	µS/cm²		NTU	mg L^{-1}	mg L^{-1}	mg L^{-1}
Control 1	321	8.320	0.390	0.297	32.613	13.499
Control 2	304	8.600	0.530	0.282	29.230	10.327
Leaf Low 1	328	8.190	1.200	0.879	ND	13.283
Leaf Low 2	322	8.690	1.250	0.189	29.460	17.486
Leaf Low 3	305	8.530	0.820	0.839	31.712	10.793
Leaf Mid 1	369	8.310	0.690	4.044	ND	14.157
Leaf Mid 2	373	8.370	0.810	0.302	34.577	13.275
Leaf Mid 3	356	8.180	1.320	4.568	31.788	14.744
Leaf High 1	428	8.140	1.040	0.367	35.328	12.844
Leaf High 2	415	8.020	0.940	0.357	36.119	12.075
Leaf High 3	412	8.330	1.220	0.316	35.355	16.550
Grass Low 1	335	8.250	0.600	0.081	36.632	12.390
Grass Low 2	285	8.470	0.950	0.141	28.808	9.927
Grass Low 3	351	8.920	0.630	0.155	32.622	11.202
Grass Mid 1	457	8.250	1.430	0.143	41.347	19.681
Grass Mid 2	447	8.290	3.510	0.142	44.647	20.257
Grass Mid 3	430	8.300	0.770	0.123	44.296	20.017
Grass High 1	502	8.090	1.330	0.131	48.814	25.175
Grass High 2	546	7.840	1.610	0.134	53.180	26.690
Grass High 3	526	7.750	1.950	0.137	50.113	25.052

ND – not detected

Treatment	EC µS/cm²	pH	t=72 Turbidity NTU	F^- mg L^{-1}	Cl^- mg L^{-1}	SO_4 mg L^{-1}
Control 1	330	8.57	0.76	0.301	29.695	11.651
Control 2	309	8.52	0.7	0.261	27.307	10.565
Leaf Low 1	384	8.47	1.02	0.277	34.599	13.065
Leaf Low 2	374	9.02	0.85	0.275	34.697	13.056
Leaf Low 3	308	8.38	1.01	0.259	28.018	10.494
Leaf Mid 1	378	9.11	4.38	0.593	35.626	10.554
Leaf Mid 2	399	9.32	4.13	0.545	37.556	19.634
Leaf Mid 3	391	8.26	4.26	0.657	36.993	9.773
Leaf High 1	389	8.24	6.71	1.198	32.735	13.032
Leaf High 2	394	8.27	8.06	0.987	25.352	9.966
Leaf High 3	367	8.05	6.67	0.889	31.820	13.987
Grass Low 1	290	8.35	2.31	0.402	30.392	13.593
Grass Low 2	312	8.53	2.28	0.291	28.185	8.665
Grass Low 3	375	8.48	1.5	0.283	33.380	13.510
Grass Mid 1	441	8.15	5.19	0.419	41.267	15.738
Grass Mid 2	339	7.93	3.86	0.350	27.845	14.893
Grass Mid 3	386	7.91	5.16	0.477	37.616	19.692
Grass High 1	498	7.58	8.26	0.659	43.099	24.251
Grass High 2	562	7.59	8.31	0.761	53.002	30.088
Grass High 3	479	7.55	8.23	0.854	41.664	22.303

VITA

Name:	Kathryn Jordan McCrary
Address:	Department of Soil Crop and Sciences c/o Dr. Jacqueline Aitkenhead-Peterson Texas A&M Department of Soil and Crop Sciences 370 Olsen Blvd. Mailstop #2474 College Station, TX 77843-2474
E-mail Address:	Jordan.Mccrary@gmail.com
Education:	B.S. Conservation Biology University of Miami, 2005 M.S. Water Management and Hydrological Science Texas A&M University, 2011