ASSESSING AVIAN CONTRIBUTION OF *Escherichia coli* AND NUTRIENT LOADS TO WATERSHEDS

A Dissertation

by

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ABSTRACT

The impairment of waterways by pathogens as indicated by the detection of high *Escherichia coli* (*E. coli*) levels continues to be a problem in Texas. Almost half of the assessed waterbodies designated for contact recreation in Texas are impaired by bacteria. In addition, Texas is in the process of developing nutrient criteria for waterbodies. Avian species such as herons and egrets frequently establish large heronries in close proximity to water. These heronries are potentially major contributors of nutrients and *E. coli* to watersheds. I enumerated *E. coli* in water and fecal samples from four heronries dominated by cattle egrets (*Bubulcus ibis*) during 2011, 2012, and 2013. I compared the fecal sterol profiles of feces to those of water associated with each heronry using sterol ratios, correlation analyses, and principal component analysis. I also analyzed total nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), sulfur (S), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), and boron (B) in water and fecal samples and compared concentrations among sample types. I found that *E. coli* and nutrients deposited through feces from birds at heronries are influenced by the size and location of the heronry. The highest *E. coli* counts in water samples were collected at the two larger heronries, which were both located directly over water. In addition, the highest estimated *E. coli* loads generated by adults ranged between 2×10^{14} and 4×10^{14} Colony Forming Units (CFU) breeding season^{-1}. I also found positive correlations between *E. coli* counts and the sum of bird sterols from water direct under a heronry. N and P concentrations in water samples were as high as 62.4

mg/L and 4.69 mg/L, respectively. K, Ca, Mg, and Fe were most abundant in feces and/or water samples and when birds nested directly over water, concentrations of K, Ca, and Mg were significantly higher than concentrations in water adjacent to birds nesting on islands. The results obtained in this study contribute to furthering the understanding of the potential contributions of bacteria and nutrients from large heronries located on the edge of or near waterbodies.

DEDICATION

This dissertation is dedicated to my parents Anthan and Joyce Telesford who have taught me how to work hard, how to be patient, how to appreciate even the smallest things, how to persevere and most importantly, how to be faithful to God. One of their favorite quotes from the Bible is: "*Trust in the LORD with all your heart and lean not on your own understanding; in all your ways acknowledge Him, and He will direct your path.*" ~ Proverbs 3: 5-6

I also dedicate this to my husband and best friend Julien Checkley who has been there for me and with me every step of this very long journey.

Most importantly, I thank God for granting me the strength and health to complete this journey; this venture would have been impossible without Him.

"*It is God who arms me with strength and makes my way perfect. He makes my feet like the feet of a deer; he enables me to stand on the heights. He trains my hands for battle; my arms can bend a bow of bronze. You give me the shield of Your salvation, and Your right hand has held me up; Your gentleness has made me great. You broaden the path beneath me, so that my feet do not slip."* ~ Psalm 18: 32-36

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I also want to extend my gratitude to the following agencies for granting us access to heronries and for their willingness to provide guidance and assistance: San Jacinto River Authority (Lake Conroe), the City of Taylor – Public Works (Murphy Park), Texas Parks and Wildlife Department (TPWD) at Richland Chambers Wildlife Management Area (Richland Creek), and TPWD at Birch and Nails Creek State Park (Flag Pond). I am also grateful for microbiology assistance and advice from Heidi Mjelde and Emily Martin at the Soil and Aquatic Microbiology Lab and field and/or laboratory assistance from Bradley Kuecker, Laura Siffring, Callie Farrell, and Julien Checkley.

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NOMENCLATURE

B	Boron
BST	Bacteria Source Tracking
Ca	Calcium
CFU	Colony Forming Units
Cu	Copper
GC/MS	Gas Chromatography/Mass Spectrometry
IUPAC	International Union of Pure and Applied Chemistry
K	Potassium
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
P	Phosphorus
S	Sulfur
SWQM	Surface Water Quality Management
TCEQ	Texas Commission on Environmental Quality
TMDL	Total Maximum Daily Load
TSSWCB	Texas State Soil and Water Conservation Board
USEPA	United States Environmental Protection Agency
WPP	Watershed Protection Plan

Zn	Zinc

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CHAPTER I

BACKGROUND AND INTRODUCTION

Water quality refers to the suitability of water based on a particular set of physical, chemical, and biological characteristics, for a designated use such as recreation (Cordy 2001, Telfair II 1993). Factors that affect water quality are related to pollution originating from point or nonpoint sources. Pollution originating from a single source such as an oil spill or discharges from areas such as wastewater treatment facilities and industrial sites are considered point sources because they are easier to locate and identify. However, a greater challenge exists with identifying the origin of pollution from nonpoint sources such as runoff from agricultural areas, city streets, and wildlife areas. Some major types of water pollution include chemicals (such as pesticides and industrial waste), bacteria or pathogens, and excess nutrients.

Nitrogen (N) and phosphorus (P) are two of the most important nutrients in aquatic systems because they are essential for primary production and survival of aquatic life (Downing and McCauley 1992, Tizard 2004). However, excessive amounts of these two nutrients can diminish water quality due to increased growth of aquatic vegetation that eventually decreases oxygen availability and transparency (Downing and McCauley 1992, Elser et al. 2007). In addition, low N: P ratios (< 29:1) in water, commonly caused by excess P, are believed to cause changes in species composition of phytoplankton (Havens et al. 2003, Smith 1983, Tilman et al. 1982). For example, N: P

ratios less than 10:1 create the perfect environment for phytoplankton such as cyanobacteria (*Cyanophycota. spp*.), which are capable of producing harmful toxins. Cyanobacteria, unlike the majority of less harmful types of phytoplankton, are able to fix N from inorganic sources and are therefore capable of surviving in waters where N is in limited supply (Havens et al. 2003, Smith 1983). According to the Environmental Protection Agency (EPA), the amount of N and P pollution entering waterbodies throughout the United States has drastically increased over the last fifty years (Stoner 2011). In an effort to accelerate N and P reduction, the EPA has been urging states to make greater progress at reducing N and P loads to watersheds.

According to a 2009 report generated by an EPA-created nutrient task force (An Urgent Call to Action: Report of the State-EPA Nutrients Innovations Task Force Group), "N and P pollution has the potential to become one of the costliest and most challenging environmental problem" (Gilinsky et al. 2009). The report stated that greater than half of U.S. streams have medium to high levels of N and P and 78% of assessed coastal waters are eutrophic (Gilinsky et al. 2009). The EPA has therefore recommended prioritization of N and P loading reductions in water bodies based on the best available loading estimates (Gilinsky et al. 2009). In response to those recommendations, the Texas Commission on Environmental Quality (TCEQ) is in the process of developing nutrient criteria for streams, reservoirs and other waterways and is considering using concentrations of total N and P as direct indicators of eutrophication (TCEQ 2012a).

However, TCEQ has emphasized the need for more nutrient data from water bodies because N and P have not been routinely measured in the past.

In addition to N and P, other macroelements such as potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), and sulfur (S) and microelements such as zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), and boron (B) are essential for the growth of flora and/or fauna (Epstein 1965, Kapustka et al. 2004, Kopp and Kroner 1968, Otsuki and Wetzel 1974, Wetzel 2001). However, elements present in excess of the required concentrations can be harmful. For example, high Cu concentrations can be toxic to aquatic life (Kapustka et al. 2004), while high Na concentrations are conducive to the growth of some species of cyanobacteria (Allen and Arnon 1955, Wetzel 2001). Kratz and Myers (1954) reported that the threshold level for the optimum growth of several species of cyanobacteria is 4 mg/L of Na and the maximum growth was found at 40 mg/L. In addition, studies show that P enrichment in addition to Na enrichment could be a potential contributor to cyanobacteria bloom (Provasoli 1958, Ward and Wetzel 1975, Wetzel 1965). Excessive amounts of nutrients from colonial birds can also cause changes in plant biomass (Anderson and Polis 1999), as well as changes within plant communities and biodiversity (Ellis 2005, Ellis et al. 2011, Mulder et al. 2011, Żółkoś and Meissner 2008).

Unlike nutrients, pathogens have been routinely monitored for many years in Texas. According to TCEQ, pathogens, as predicted by the presence of high *Escherichia coli*

(*E. coli*) levels, are the most frequent cause of stream impairment in the state (TCEQ 2008, TCEQ 2010a, TCEQ 2012b). Approximately half of assessed streams and rivers designated for contact recreation are listed as impaired because of high *E. coli* levels (TCEQ 2012b).

E. coli is a gram-negative rod that is a common inhabitant in the gastro-intestinal tracts of all warm-blooded organisms (Maier et al. 2009), and is present in the feces of mammals in concentrations of 10^9 colony forming units (CFU) per gram of feces (Edberg et al. 2000). Although *E. coli* is usually considered a harmless organism, some strains can cause a variety of diseases such as septicemia, neonatal meningitis, diarrhea, and dysentery (Maier et al. 2009, Ørskov and Ørskov 1992). The four most common types of pathogenic *E. coli* are enterotoxogenic, enteropathogenic, enteroinvasive and enterohemorrhagic. *E. coli* O157:H7, a well-known virulent strain, belongs to the enterohemorrhagic group (Maier et al. 2009). The strains of *E. coli* referred in this study are assumed non-pathogenic. *E. coli* is used as a bacterial indicator because it can be easily isolated and enumerated, it is excreted in large quantities, and it is more resilient than most pathogenic bacteria and therefore has a longer survival time (Maier et al. 2009). *E. coli* is a type of fecal coliform, therefore, its presence in water indicates fecal contamination and the possibility that other enteric pathogens (such as *Salmonella*, *Campylobacter jejuni*, and *Shigella*) may also be present (Maier et al. 2009). Exposure to these pathogens can cause symptoms ranging from gastroenteritis to severe illnesses or sometimes death (Maier et al. 2009). It is therefore important to have effective water

quality management, including the management of nutrients. In several studies, increased nutrient loads, especially with high N content, have been found to increase the survival time (Lim and Flint 1989) and recovery rate (Bolster et al. 2005) of *E. coli* in water. Sources of *E. coli* contamination include humans, birds, and other wildlife (Ahmad et al. 2009, Alderisio and DeLuca 1999, Benham et al. 2006).

Large heronries of cattle egrets (*Bubulcus ibis*), little blue herons (*Egretta caerulea*), snowy egrets (*Egretta thula*), and great egrets (*Ardea alba*) can potentially be major contributors of *E. coli* and nutrients to watersheds. These birds are known to establish large heronries, frequently numbering in thousands of birds, in coastal areas and inland in close proximity to water (Parkes 2007). Because of large quantities of deposited feces and the potential for runoff, large amounts of nutrients and *E. coli* can be deposited into nearby waterways. In addition to direct deposition, fecal coliform from birds can get into waterways through runoff from bird feces (Alderisio and DeLuca 1999, Fogarty et al. 2003). Several of these birds are migrants and spend the breeding season in Texas. However, many are all-year residents in several areas (Lockwood and Freeman 2004, Parkes 2007, Shackelford and Lockwood 2000, Telfair II and Bister 2004). A public health survey conducted in a recreational lake in Madison, Wisconsin found that high bacteria counts in the water were attributed in part to waterfowl feces transported through runoff from the shore sand to the lake (Standridge et al. 1979). Another study found that wildlife (including birds) was the major contributor of *E. coli* in a watershed dominated by cattle and other agricultural activity (Somarelli et al. 2007).

In addition to *E. coli*, enteric pathogens such as *Salmonella* bacteria (*Salmonella enterica*) are part of the intestinal flora of birds (Makino et al. 2000, Phalen et al. 2010). Phalen et al. (2010) isolated seventeen *Salmonella enterica* subsp. *enterica* serotypes from cultures of the digestive tract, spleen, and liver of cattle egret chicks (Phalen et al. 2010). Another example of wild birds being carriers and potential transmitters of pathogens to the environment is reported in Locke et al. (1974) regarding the establishment of a captive heron colony at the Patuxent Wildlife Research Center in Maryland. Young black-crowned night herons (*Nycticorax nycticorax*), common egrets (*Ardea alba*), snowy egrets (*Egretta thula*) and tricolor herons (*Egretta tricolor*) were obtained from the wild or from captive flocks at the Bronx zoo. Within two weeks of capture, salmonellosis was found to be one of the causative agents of the death of several birds (Locke et al. 1974). *Cryptosporidium parvum* and *Giardia lamblia* are pathogens that are also transmitted through water (Graczyk et al. 1997, Wolfe 1992), but their transmissive stages (oocysts and cysts) are very resilient and difficult to detect in aquatic habitats (Kucerova-Pospisilova et al. 1999, Smith and Rose 1998, Wolfe 1992). Studies show that birds can be hosts and vectors of these pathogens (Graczyk et al. 1998, Graczyk et al. 2008, Kuhn et al. 2002, Słodkowicz-Kowalska et al. 2006, Smith et al. 1993, Zhou et al. 2004). In addition, the prevalence *Cryptosporidium parvum* oocysts in the feces of birds (primarily Canada geese (*Branta canadensis*)), has been reported to be as high as 90% (Graczyk et al. 2008, Kassa et al. 2004).

Studies focused on nutrient contributions, primarily N and P from large avian heronries have also been reported. One example is a study conducted in coastal New South Wales where elevated levels of N and P were found in wetlands supporting heronries composed of four species of egrets, cattle egret, great egret, intermediate egret (*Egretta intermedia*) and little egret (*Egretta garzetta*) (Baxter and Fairweather 1994). Several studies have found a strong correlation between the occurrence of large numbers of colonial birds and high concentration of aquatic N and P (Chaichana et al. 2010, Chaichana et al. 2011), as well as diminished water quality (Baxter and Fairweather 1994, Manny et al. 1994, Portnoy 1990). However, I did not find any studies that report other macro and microelements in the feces of wild birds or their contributions to surface water.

Bacteria source tracking (BST) is a method developed to identify sources of enteric microorganisms in waterbodies (Maier et al. 2009). BST methods are generally divided into two categories, phenotypic and genotypic, and can either be library-dependent or -independent (Maier et al. 2009). Phenotypic methods use physiological characteristics such as antibiotic resistance analysis or carbon utilization patterns to identify sources (Hagedorn et al. 2003, Hagedorn and Weisberg 2009, Maier et al. 2009, Moore et al. 2005, Wiggins et al. 1999). Genotypic methods differentiate sources by looking at genetic patterns of bacteria in the sample (Hagedorn and Weisberg 2009, Maier et al. 2009). Library-dependent methods compare unknown sources to a database of bacterial isolates of known origins. However, library-independent methods do not require

comparison to known isolates (Maier et al. 2009), Library-dependent methods are quantitative and sensitive, and can be used to characterize isolates from a wide variety of sources. However, these methods require considerable time and a large database of isolates. In addition, isolates can be geographically specific and a higher amount of false-positives is obtained compared to library-independent methods (Maier et al. 2009). Library-independent methods produce highly accurate results and results are obtained quickly but depending on the type of analysis, expensive equipment may be required (Maier et al. 2009). In addition to the primary BST methods currently being used in Texas, several additional techniques can be used to identify potential sources of *E. coli* in water. One of these approaches is the use of fecal sterols to distinguish potential sources of fecal contamination.

Sterols are one of three types of steroids that are found in the intestinal track of organisms. Cholesterol is the main type of sterol and originates from diet or is synthesized within the organism (Groh et al. 1993). As cholesterol passes through the intestines, it is chemically reduced by intestinal microorganisms, to different types of sterols. Variability in sterols is caused by a combination of three factors: the animal's diet, synthesis of endogenous sterols, and intestinal flora (Leeming et al. 1996, Leeming and Nichols 1998). Types of fecal sterols include coprostanone, coprostanol, epicoprostanol, cholesterol, cholestanol, campesterol, stigmasterol, fucosterol, β-sitosterol, and stigmastanol (Groh et al. 1993, Isobe et al. 2002, Noblet et al. 2004). Fecal sterol analysis can be a valuable tool in BST studies because there is significant

variation in the composition and type of sterols among warm-blooded organisms (Groh et al. 1993, Leeming et al. 1996).

Coprostanol, for example, is one of the most common types of fecal sterols excreted by humans (Groh et al. 1993, Martin et al. 1973, Murtaugh and Bunch 1967). As a result, this type of sterol has been used as a molecular marker for human fecal contamination. Leeming et al. (1996) found that the major human fecal sterols (60% of total sterols found in feces) were coprostanol and 5β-stanol. These authors also found that the main fecal sterol of herbivores was 24-ethylcoprostanol. The sterol content of birds was reported as being variable with very low amounts of 5β and 5-α stanols but the major sterols were β-sitosterol, cholesterol, campesterol and stigmasterol (Leeming et al. 1996, Subbiah et al. 1972). Noblet (2004) conducted studies in the lower Santa Ana River watershed and the nearby surf zone that was believed to be contaminated by sewage. However, a stronger correlation was found between fecal indicator bacteria and bird fecal sterols than with sewage.

Cattle egrets, and various other heron and egret species have been nesting in large heronries in east and central Texas for over 50 years (Telfair II 1993). These heronries often contain thousands of nests usually constructed in areas in close proximity to water. Heronry sizes range from less than 100 to over 15,000 pairs (Dusi 1978, Parkes 2007, Telfair II 1983, Telfair II et al. 2000). Water is considered an important factor in the formation of heronries (Dusi and Dusi 1968) because water serves as protection from

predators as it reduces predator access (Telfair II 1983). Parkes (2007) reported that approximately 80% of all non-coastal inland colonies nest within 5000 m of a major stream or river. Heronries are usually established in four types of habitats: 1) upland woodlands that may or may not be in close proximity to water, 2) swampy areas with submerged trees, 3) islands containing trees and shrubs that are located inland, and 4) islands with trees and shrubs located in coastal areas (Telfair II 1994).

To my knowledge, no information is available on the N and P or *E. coli* loads deposited by cattle egrets (or other egrets/herons) in their heronries in Texas. Because *E. coli* is found in the feces of all warm-blooded animals, source allocation can be challenging. Nevertheless, several source-tracking methods are available. The objectives of this study were to (1) quantify *E. coli* and nutrient loads originating from large heronries in close proximity to water and (2) test the utility of fecal sterols as a BST method to assess avian contribution of *E. coli* to associated watersheds. The dissertation addresses these objectives in five chapters. Chapter II reports the contribution of *E. coli* from four avian heronries composed primarily of cattle egrets, using fecal sterol analysis. Chapter III reports the N and P contribution from feces originating from these heronries. Chapter IV also focuses on nutrients but reports other macroelements as well as some microelements. Finally, Chapter V summarizes all the results, provides some conclusions, and adds some suggestions for future research.

STUDY AREAS

Heronries were located in four Texas counties, Williamson, Montgomery, Freestone and Lee County. Each heronry contained several species of birds including cormorants, primarily Neotropical (*Phalacrocorax brasilianus*), great egrets, snowy egrets, little blue herons, and anhingas (*Anhinga anhinga*). However, the most common species was the cattle egret, comprising at least 90% of the birds in each heronry. I estimated the population of cattle egrets by counting the number of breeding pairs visible from a fixed point at the water's edge and extrapolating this number to the estimated area of the heronry following the methodology of Gregory et al. (2004). During the 2012 breeding season, a reference site was selected for three of the four heronries. Sites were selected by investigating other water bodies in the area that did not contain cattle egret heronries.

Murphy Park

County: Williamson

Lat. Long.: N 30.5809, W 97.4131

Heronry type: Island

Number of nesting pairs: 1,400 (2011), 1,800 (2012 and 2013)

Nest substrate species: Texas Native Bamboo or Canebrakes (*Arundinaria gigantean*)

Reference site: Bull Branch

Murphy Park (MP) is a city park located in the city of Taylor, Texas. The heronry was situated on a small island in an 809 m^2 pond (Muddy Lake) within the park (Figure 1).

Muddy lake is used for recreational purposes, primarily fishing. Other species of birds that were observed in the colony included a few species of waterfowl.

The reference site Bull Branch Pond, is located upstream (N 30.5871, W 97.4222) (Figure 1) from Murphy Park. Bull Branch Pond is a smaller city pond that is also used primarily for fishing. Although there were no cattle egrets or herons at that site, there were a few species of waterfowl.

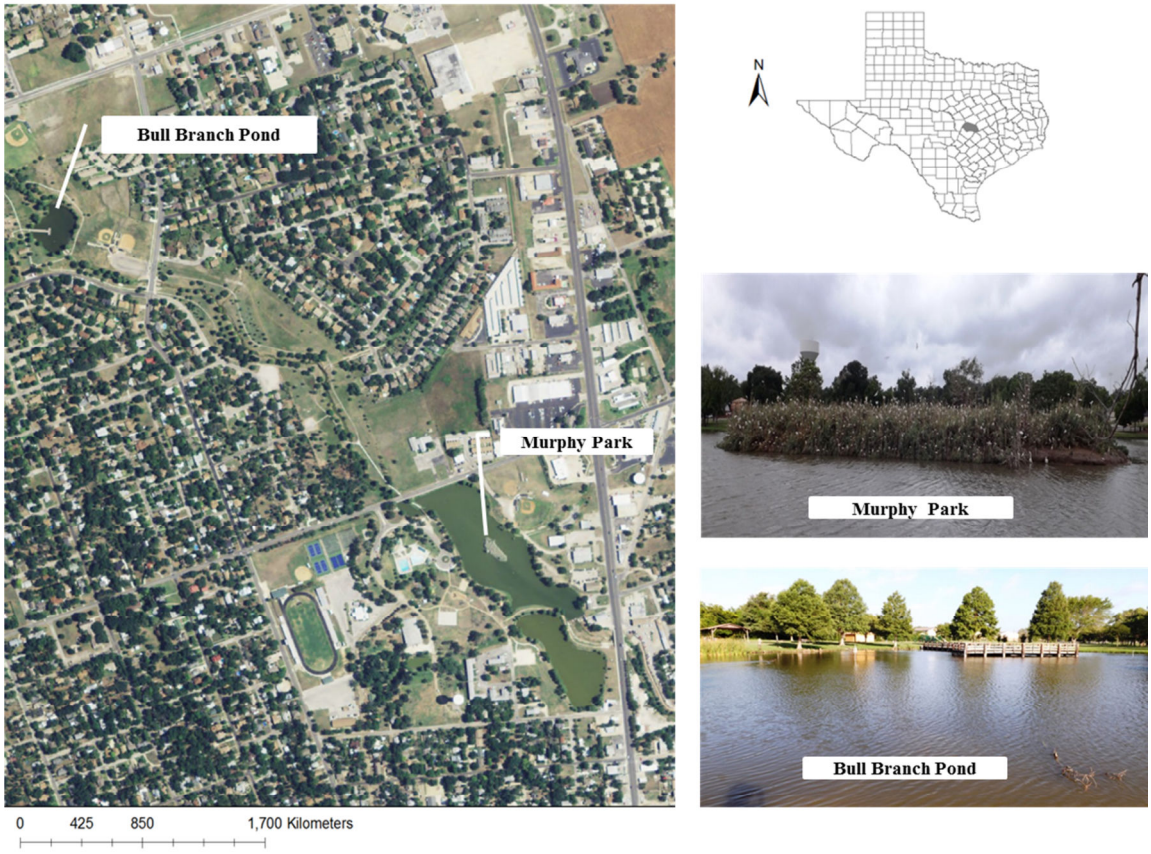


Figure 1: Map and photographs showing the locations of Murphy Park and Bull Branch Pond. Aerial photographs were obtained from ArcMap 10.2.2.

Lake Conroe

County: Montgomery

Lat. Long.: N 30.4035, W 95.5750

Heronry: island

Number of nesting pairs: 1,200 (2011)

Nest substrate species: juniper (*Juniperus sp*.) and willow (*Salix sp*.)

Lake Conroe (LC) is an 80.9 km^2 Lake in Conroe, Texas that is managed by the San Jacinto River Authority. The heronry was located on a small island in the lake (Figure 2). The birds did not return to the heronry during the 2012 and 2013 breeding seasons.

Richland Creek

County: Freestone

Lat. Long.: N 31.9906, W 96.1005

Heronry type: Artificially flooded vegetation

Number of nesting pairs: 20,000 (2011 and 2012), 1,600 (2013)

Nest substrate species: green ash (*Fraxinus pennsyvanica*), burr oak (*Quercus macrocarpa*), buttonbush (*Cephalanthus occidentalis*), and swamp-privet (*Forestiera acuminate*)

Reference site: Richland Creek Ref.

Richland Creek (RC), a tributary to the Trinity River, is a wildlife management area managed by the Texas Parks and Wildlife Department located in Streetman, Texas (Figure 3). Nests were located in trees or shrubs with roots and trunks in water (approximately 1.5 m in most areas).

Figure 2: Map and photographs showing the location of Lake Conroe. Aerial photographs were obtained from ArcMap 10.2.2.

The reference site was also part of the wildlife management area but was located upstream (N 31.9921, W 96.0981) (Figure 3) from the Richland Creek heronry during

the 2012 sampling season. I did not observe cattle egrets, waterfowl, or other species of birds at the reference site during the sampling period. However, about a quarter of the water surface was covered with duckweed *(Lemna sp.*).

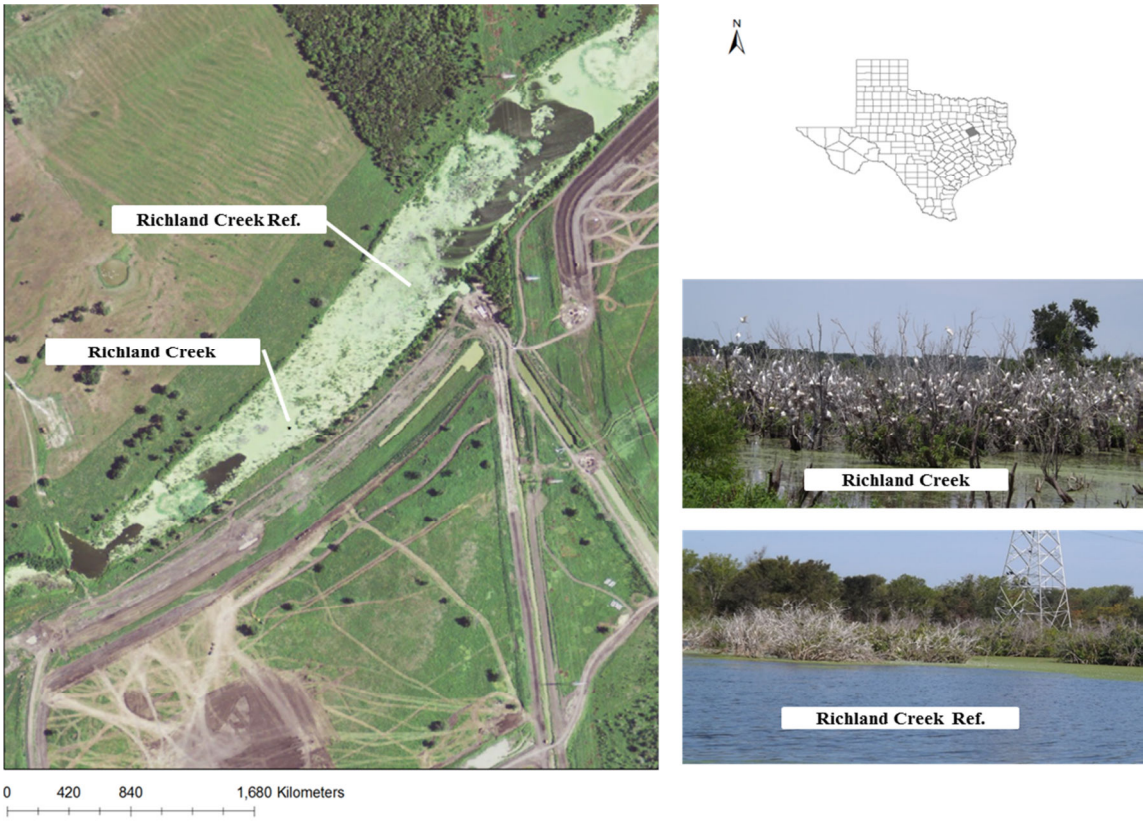


Figure 3: Map and photographs showing the locations of Richland Creek and Richland Creek Ref. Aerial photographs were obtained from ArcMap 10.2.2.

Flag Pond

County: Lee

Lat. Long.: N 30.3063, W 96.6976

Heronry type: Artificially flooded vegetation

Number of nesting pairs: 6,000 (2012)

Nest substrate species: Water Elm (*Planera aquatica*), Siene Bean (*Sesbania drummondiI*)

Reference site: Horse Pond

Flag Pond is a 1.4 km^2 seasonal wetland area managed by the Texas Parks and Wildlife Department (TPWD) that is part of the Birch and Nails Creek State Parks. The heronry was located in a flooded area (Figure 4). The TPWD uses this location as a seasonal wetland for birds wintering in that region. However, due to due to large amounts of rainfall during April and May 2012, the area became flooded and subsequently colonized by cattle egrets and other herons. The pond was drained by park management towards the end of June 2012.

The reference site Horse Pond, is about ¼ the size of Flag Pond. This pond is in an isolated area of Nails Creek State park (N 30.2886, W 96.6688) (Figure 4). No cattle egrets or other birds were observed at Horse Pond during the sampling period. This pond is used as a source of water for horses.

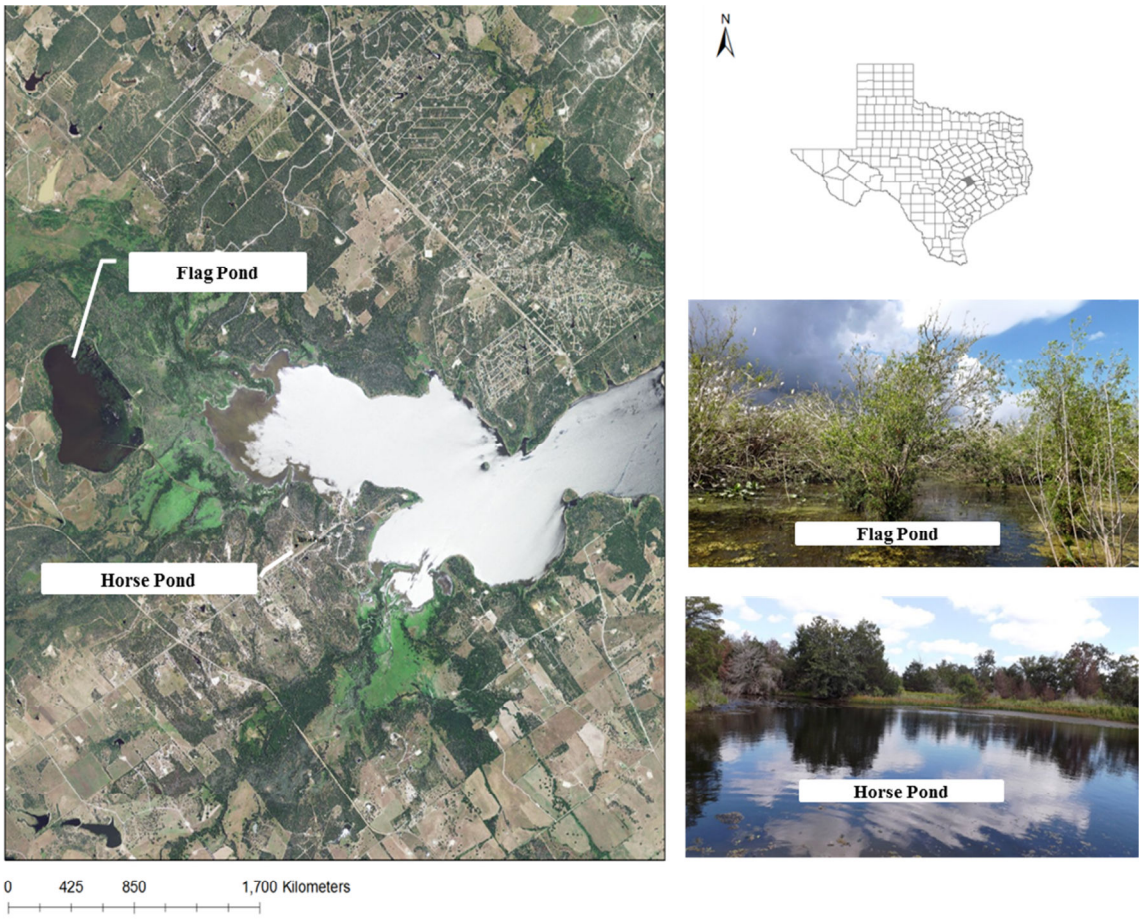


Figure 4: Map and photographs showing the locations of Flag Pond and Horse Pond. Aerial photographs were obtained from ArcMap 10.2.2.

CHAPTER II

AN EVALUATION OF THE CONTRIBUTION OF *Escherichia coli* (*E. coli*) TO WATERSHEDS FROM AVIAN HERONRIES USING FECAL STEROL ANALYSIS

SYNOPSIS

E. coli contained in feces deposited by herons and other species nesting in large heronries can be the source of bacterial contamination of nearby waterways. The deposition of large amounts of fecal material into waterbodies can also degrade water quality. *E. coli* was enumerated in water and fecal samples collected from four heronries during the breeding seasons of 2011, 2012, and 2013. The fecal sterol profiles of fecal samples (2011 and 2012 breeding seasons) was compared to the fecal sterol profile of water associated with heronries to determine the source(s) of fecal material. Sterol ratios and correlation analyses were also used to determine fecal sources. The results obtained in this study indicate that *E. coli* deposited through fecal material from birds at heronries is influenced by the size and location of the heronry. The highest *E. coli* counts were found in water samples collected at the two larger heronries, both of which were located directly over water. In addition, the highest estimated *E. coli* loads generated by adults ranged between 2×10^{14} and 4×10^{14} Colony Forming Units (CFU) breeding season^{-1}. The sterol distribution in the fecal samples was dominated by cholesterol and stigmasterol while the sterol distribution in the water samples was dominated by the cholesterol, coprostanol, and cholestanol. Total sterols ranged from 979 to 5,838 ng/L in

the fecal samples and 13 to 600 ng/L in the water samples. Correlation analyses and the use of sterol ratios yielded positive correlations between *E. coli* counts and the sum of bird sterols (cholesterol, cholesterol, β-sitosterol, and stigmasterol) from water subject to direct fecal deposition by a heronry. In addition, the results of the principal component analysis suggested a strong correlation between *E. coli* and stigmasterol. The results obtained in this study contribute to furthering the understanding of the potential contributions of bacteria from large heronries located on the edge of or near water bodies. These results provide the framework for further studies of bacteria-impaired watersheds, especially those influenced by large heronries because identifying sources of *E. coli* and quantifying loads resulting from various sources are critical tasks in development of restoration measures for impaired watersheds.

INTRODUCTION

In Texas, almost 50% of assessed streams designated for contact recreation are impaired by pathogens as indicated by the presence of high levels of *Escherichia coli* (*E. coli*) (Parkes 2007, TCEQ 2012b). *E. coli* is a type of fecal coliform used as a bacterial indicator; its detection in water indicates that other pathogens may be present. *E. coli* is used by many states including Texas, as a target in bacteria source tracking (BST). Bacterial impairments are derived from fecal contamination from humans, livestock, pets, and wildlife, including birds (Ahmad et al. 2009, Alderisio and DeLuca 1999, Benham et al. 2006). Colonial waterbirds including herons and egrets establish large nesting colonies usually in close proximity to water (Dusi et al. 1971, Parkes 2007).

Because of the deposition of large quantities of feces and the potential for runoff, these heronries are expected to contribute large *E. coli* loads to nearby watersheds.

For decades, cattle egrets (*Bubulcus ibis*), and various other heron and egret species have established large heronries in East and Central Texas (Telfair II and Thompson 1986, Telfair II 1993). These heronries often contain thousands of nests, primarily those of cattle egrets (Dusi 1978, Parkes et al. 2012, Telfair II 1993). According to Parkes et al. 2007, about 80% of all non-coastal inland colonies nest within 5000 m of a major stream or river. These wild birds can be carriers and potential transmitters of pathogens to the environment since other enteric pathogens such as *Salmonella enterica* and *Campylobacter* spp. are part of the intestinal flora of some birds (Phalen et al. 2010, Tizard 2004, Yogasundram et al. 1989). For example, Phalen et al. (2010) isolated seventeen *Salmonella enterica* subsp. *enterica* serotypes from cultures of the digestive tract, spleen, and liver of cattle egret chicks. In addition, in a captive heron colony containing black-crowned night herons (*Nycticorax nycticorax*) and several species of egrets, salmonellosis was found to be the causative agent of the death of several birds (Locke et al. 1974). Currently, *E. coli* loads contributed to Texas watersheds by egrets, herons, and other colonial waterbirds are unknown.

In addition to BST methods, other techniques are used to identify potential sources of *E. coli* in water in Texas. While many of the techniques are similar to current methods (e.g., host specific PCR assays (Lu et al. 2008) and PCR-based DNA fingerprinting

(Dombek et al. 2000), some are more novel and have not been adequately evaluated, specifically with herons such as cattle egrets. One of these approaches is the use of biomarkers, in particular fecal sterols to distinguish potential sources of fecal contamination (Leeming et al. 1996).

Organic compounds such as steroids that are able to remain in a stable chemical or biological form after being released into a different environment are considered biomarkers if their sources can be identified (Leeming et al. 1996). After sterols from fecal materials are deposited into aquatic systems, they either form strong bonds with particulate matter or incorporate into sediments where degradation occurs at a minimum (Bartlett 1987) or degrade within 1-2 weeks under aerobic conditions in the water column (Switzer-Howse and Dutka 1978). Sterols have been used as chemical indicators of fecal pollution because of the high amounts present in feces (Murtaugh and Bunch 1967). The fecal sterol profile is dependent on a combination of three factors, the animal's diet, synthesis of endogenous sterols, and intestinal flora (Leeming et al. 1996, Leeming and Nichols 1998). Cholesterol is the main type of sterol and originates from absorption of dietary steroids and synthesis by the liver (Groh et al. 1993, Murtaugh and Bunch 1967). As cholesterol passes through the intestines, it is transformed by microbes to different types of sterols (Martin et al. 1973, Rosenfeld et al. 1954).

Fecal sterol analysis can be a valuable tool in BST studies because there is significant variation in the composition and type of sterols among warm-blooded organisms (Groh

et al. 1993, Leeming et al. 1996). These factors are responsible for creating a "sterol fingerprint" of specific animal feces (Leeming et al. 1996). Types of fecal sterols include coprostanol (COP), epicoprostanol (eCOP), cholesterol (CHOE), cholestanol (CHOA), campesterol (CAMP), stigmasterol (STIG), and β-sitosterol (bSIT) (Groh et al. 1993, Isobe et al. 2002, Noblet et al. 2004). Some sterols such as CAMP, bSIT, and STIG are found naturally in plants (phytosterols) while sterols such as CHOE and COP are found naturally in animals (zoosterols) (Huang and Meinschein 1979). Studies have linked relative abundances of sterol compounds to individual species of animals and have used such abundances as indication of their origin (Leeming et al. 1996, Subbiah et al. 1972). Coprostanol, for example, is one of the most common types of fecal sterols excreted by humans (it is also produced by other mammals but in smaller proportions) and it has been used as a biomarker for human fecal contamination (Leeming et al. 1996, Martin et al. 1973, Murtaugh and Bunch 1967).

In this study, I investigated the potential impact that large heronries may have on water quality in selected watersheds by quantifying *E. coli* loads originating from four heronries and related the sterol profile of water collected near or below heronries to the sterol profile of feces collected from egrets (mostly cattle egrets). I hypothesized that *E. coli* counts in waters associated with heronries would exceed the Texas state primary contact recreation standard for surface water quality (geometric mean of 126 *E. coli* colony forming units (CFU) per 100 mL), and that the fecal sterol profile (i.e. the dominant sterols) observed from fecal samples would correlate with the profile

observed in the respective water samples. I also hypothesized that fecal sterols in water samples were primarily of avian origin.

Study Site and Heronry Description

Four heronries (Murphy Park, Lake Conroe, Richland Creek, and Flag Pond) were investigated during the breeding seasons of 2011, 2012, and 2013 (Figure 5). Each of the four heronries contained several species of birds including cormorants, primarily Neotropical cormorants (*Phalacrocorax spp*), great egrets (*Ardea alba*), snowy egrets (*Egretta thula*), little blue herons (*Egretta caerulea*), and anhingas (*Anhinga anhinga*). However, the most common species was the cattle egret, comprising at least 90% of the birds in each colony. Two of the four heronries were located on islands and two were located on shrubs and trees with roots and trunks in water. The population of birds at each heronry was estimated by counting the number of breeding pairs visible from a fixed point at the water's edge and extrapolating this number to the estimated area of the heronry following the methodology of Gregory et al. (2004).

Heronry description

The island heronries were Murphy Park (MP) and Lake Conroe (LC) (Figure 5). The heronry at Murphy Park was located on an island in an 809 m^2 pond (Muddy Lake) within a city park in Taylor, Texas. Approximately 700, 900, and 900 nesting pairs of cattle egrets were observed in 2011, 2012, and 2013, respectively. The Lake Conroe heronry was located on an island in a lake (81 km^2) within a residential area

(Montgomery County, Texas). Approximately 600 nesting pairs of cattle egrets were observed in 2011.

The two heronries located in trees or shrubs with roots and trunks submerged in water were Richland Creek (RC) and Flag Pond (FP) (Figure 5). Richland Creek, a tributary to the Trinity River, is a wildlife management area located in Freestone County, Texas. Approximately 10,000 nesting pairs of cattle egrets were observed in 2011 and 2012, but approximately 800 nesting pairs were observed in 2013. Flag Pond, a 1.4 km^2 seasonal wetland, is located within the Birch and Nails Creek State Parks in Lee County, Texas. Approximately 2,500 nesting pairs of cattle egrets were observed in 2012. The pond was drained by park management at the end of June 2012.

During the 2012 sampling season, a reference site was selected for each location. Bull Branch Pond (BB), the site that was selected for Murphy Park, is a smaller pond upstream from Murphy Park. The reference site selected for Richland Creek, RC-c, is located upstream from the Richland Creek heronry. Horse Pond (HP), the site selected for Flag Pond, is located within the Nails Creek State Park and is about ¼ the size of Flag Pond. A few species of waterfowl were observed at Bull Branch Pond, but no cattle egrets or other herons were observed at that site or any of the other reference sites during the sampling period

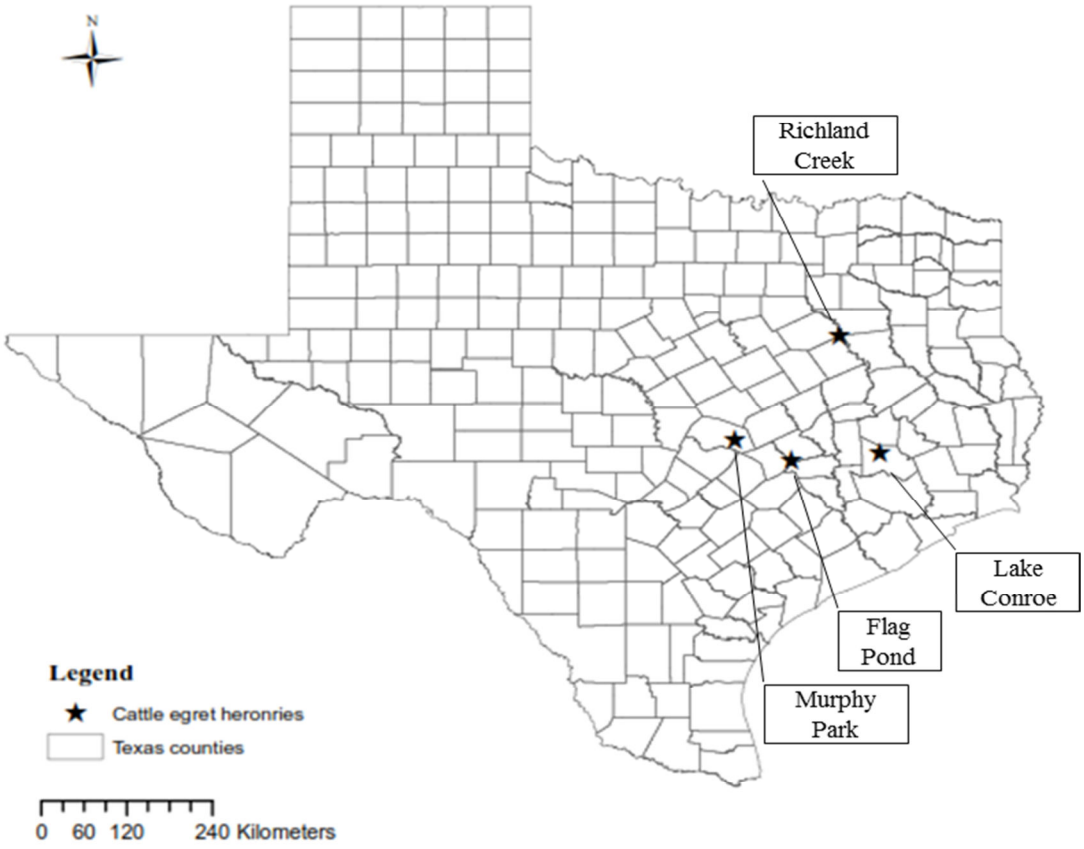


Figure 5: Map showing locations of cattle egret heronries sampled during the 2011 and 2012 breeding seasons.

MATERIALS AND METHODS

Sample Collection Period

Water and fecal samples were collected during the breeding seasons of 2011, 2012, and 2013 from May through August. At Murphy Park, both types of samples were collected during the entire study period. At Lake Conroe, both types of samples were collected in 2011, but because a heronry was not established at that location the preceding years, additional samples were not collected. The heronry at Flag Pond was discovered later

into the breeding season of 2012 (June 2012); water and fecal samples were collected in June 2012 and fecal samples were collected in July, but no water because the pond was drained by park management. A heronry was not established at Flag Pond in 2013. The fourth heronry, which was located at Richland Creek, was discovered late in the 2011 breeding season so samples were only collected once that year (July 2011). The Richland Creek heronry was located over water and feces were difficult to collect; thus, only water samples were collected at this site. Water samples were consistently collected at Richland Creek during the breeding seasons of 2012 and 2013.

Sample Collection and Preparation

Water was collected as grab samples from two ends of the island heronries (Murphy Park and Lake Conroe), approximately 32 km from each island, and from areas directly under the heronries that were located over water (Flag Pond and Richland Creek). At the reference sites, water samples were collected from a central location within the respective waterbody. To collect and store water samples, sterile 250 mL polypropylene screw-cap bottles (for *E. coli* enumeration) and 1L sterile amber glass bottles (for fecal sterol analysis) were used. These samples were stored at approximately 4 ℃ after collection while transporting them to the lab. Water quality parameters were measured (pH, conductivity, temperature, DO) at the same time that the water samples were collected (data presented in Appendix A). An ExStik® DO600 dissolved oxygen meter (EXTECH instruments) was used to measure dissolved oxygen and a PCtestr 35

multiparameter meter (Eutech instruments, Oakton 35425-00) was used to measure pH, temperature, and conductivity.

Fecal samples from several trees and/or nests were collected using sterile forceps and/or syringes, at random locations within the heronry. Fecal samples were also collected using plates (lined with wax paper) mounted on tripods, which were randomly placed under trees with a high density of nests. Fecal samples were collected as composite samples, which represented five fecal events or five birds. These samples were stored in 50 mL sterile polypropylene tubes (for *E. coli* enumeration) and 4 oz sterile amber glass jars (for fecal sterol analysis). Samples were stored at approximately 4 ℃ after collection and during transport to the laboratory.

Samples used for *E. coli* enumeration were analyzed within 3 - 4 hours of collection. However, approximately 5 mL methylene chloride was added to the water samples used for fecal sterol analysis, and the samples were then stored in the refrigerator until analysis. The fecal samples were stored at -80 ℃.

E. coli Enumeration and Quantification

The water samples for *E. coli* were analyzed using modified membrane-thermotolerant *E. coli* agar (mTEC) as described in USEPA Method 1603 (2006). Briefly, a series of diluted samples were filtered through 0.45 µm filters. Each filter was placed in a petri dish containing mTEC, and then incubated for 2 hours at 35 ± 0.5 ℃ and for 22-24 hours

at 44.5 ± 0.2 ℃. The red or magenta colonies were counted then the concentration of *E. coli* in each water sample was determined. The same procedure was followed to analyze the fecal samples by first preparing a diluted solution by adding 1 g of fecal sample to 99 mL of buffer solution.

To estimate the daily *E. coli* load contributed by each bird in the heronry, the enumerated amount was first divided by five since one composite sample represented five birds. To determine the average daily weight of feces produced by each adult bird, the average weight of adults [estimated by Telfair 1994 (270 - 512 g)] was multiplied by 0.02 [Andersen et al. 2003, estimated that avian species such as cattle egrets defecate 2% of their body weight per day]. The average weight of feces was then multiplied by the *E. coli* concentration per bird (CFU/g) of feces to determine the daily *E. coli* load per bird (CFU). Because I was not able to collect feces from the Richland Creek heronry, I used the *E. coli* concentrations from samples collected at Murphy Park (2011 - 2013) to calculate the estimated loads at Richland Creek.

A conservative estimate of the potential *E. coli* load generated by the adults from each heronry during the entire breeding season (~133 days, Blaker 1969, Telfair 1994) was calculated using the following steps. First, the time spent at the heronry was divided into two categories. The first category, pre and post incubation, birds spend 54% of their time at the heronry for a total of 99 days (Blaker 1969, Telfair 1994). To estimate the total *E. coli* load generated by each heronry during that period, I found the product of the

daily *E. coli* load per bird, the time spent at the heronry (0.54), the number of days during the period (99 days), and the number of birds in the respective heronry. The second category, during incubation and rearing, birds spend 77% of their time at the heronry for a total of 34 days (Blaker 1969, Telfair 1994). To estimate the total *E. coli* load generated by each heronry during that period, I found the product of the daily *E. coli* load per bird, the time spent at the heronry (0.77), the number of days during the period (34 days), and the number of birds in the respective heronry. The sum of both categories was then used as the estimated load to each heronry during the breeding season. I did not incorporate juveniles into these estimates because the variability in their population dynamics as the breeding season progresses. These dynamics will be best captured with a simulation model, which will be presented in the manuscript relating to this chapter.

Fecal Sterol Analysis

Samples for fecal sterol analysis were collected during the 2011 and 2012 breeding seasons.

Sterol extraction

The methods described in the USEPA Method 3550C (USEPA 2007a) were used for the fecal sterol analysis of fecal samples. Briefly, approximately 6 g of fecal sample were freeze-dried (Labconco freezone freeze dry system) and then were ground to a fine powder using a mortar and pestle and transferred to 50 ml glass vials. The sterols were ultrasonically extracted using a Branson digital sonifier (model 250) and the following

amounts of solvents were consecutively added to the samples: 25 mL of methanol (MeOH), a 50:50 mixture (25 mL) of MeOH /dichloromethane (DCM), and 25 mL of DCM. After each extraction, the extracts were filtered on glass fiber filters (Whatman GF/F) into clean glass vials. The combined extracts from each sample were then concentrated almost to dryness under a gentle flow of N_2 using a TurboVap LV evaporator (Zymark®) at approximately 40 ℃.

Sterols were extracted from water samples using a modified version of EPA SW-846 Method 3510 (USEPA 1996) (separatory funnel method) by serially extracting the sterols with DCM in separatory funnels and concentrating the extracts to a final volume of approximately 1 mL. Briefly, approximately 80 mL DCM was added to each bottle of water, which was then transferred to 1 L separatory funnels that were vigorously agitated for about 1 minute. The extracts were allowed to separate before they were drained into clean, dry Erlenmeyer flasks. These steps were performed three times per sample while combining the extracts from each sample. N_2 was then used to concentrate each extract almost to dryness (approximately 1 mL).

Sterol quantification

For the quantitative determination of sterols in waters and fecal samples, Gas Chromatography/Mass Spectrometry (GC/MS) was used while following the modified methods described in USEPA SW-846 Method 8270C (USEPA 2007b). Quantitation was performed by GC/MS (Agilent Technologies GC 7890A coupled to an Agilent Technologies 5975C XL MSD in full-scan mode (HP 5975 MSD)). The GC was

temperature-programmed and operated in splitless mode. The capillary column was an Agilent Technologies HP-5MS (30 m long by 0.25 mm ID and 0.25 μm film thickness). The MS was capable of scanning from 35 to 500 AMU every second or less, utilizing 70 volts electron energy in electron impact ionization mode.

Calibration solutions were prepared at three concentrations ranging from 1 to 1000 μg/mL by diluting commercially available neat standards. For each analyte of interest, a response factor (RF) was determined for each calibration level. The response factors were then averaged to produce a mean relative response factor for each analyte. An analytical set consisted of standards, samples, and quality control samples. Each extraction batch was analyzed as an analytical set including samples and some or all of the following quality control samples: method-blank, duplicate, matrix-spike, matrix-spike duplicate, and/or blank spike, blank spike duplicate. Method reporting limits for the sterols analyzed ranged from 46.8 to 55.8 ng/L for water and 2.7 to 3.7 ng/g for fecal samples. Sterol standards (coprostanol, cholesterol, cholestanol, and β-sitosterol) were purchased from Sigma-Aldrich ®. Systematic and common names of sterols are presented in Table 1.

Statistical Analysis

For the *E. coli* data, the values were transformed using the $\log_{10}$ function. ANOVA was then used to test for significant differences in *E. coli* counts (in fecal and water samples) between months from each heronry and associated waters for each year of study. The

same procedure was used to compare differences in *E. coli* levels between years and locations. For the fecal sterol data, the non-parametric Wilcoxon rank-sum test or Kruskal-Wallis test (where the number of samples were greater than two) was used to look for significant differences in detected sterols between months from each heronry and associated waters for each year of study. The same procedure was used to compare differences in fecal sterol concentrations between years and locations. These methods were used because the data were not normally distributed as verified by the Shapiro-Wilks test.

To help with the interpretation of the relationship between fecal sterols and *E. coli* in water and feces, several calculations were made. First, sterol ratios (% COP, COP/(COP+CHOA), and COP/CHOE) were calculated. Second, sterol ratios (COP+eCOP/Σsteroids and CHOE+CHOA+bSIT+STIG/Σsteroids) were graphically compared to the *E. coli* data. Third, Pearson Correlation Analysis of selected sterol ratios and *E. coli* data were used to test for any significant relationships and forth, principal component analysis (PCA) was applied.

To expand on the PCA, for each heronry, the mean value (*E. coli* and fecal sterols) per month was used. The following 26 variables were therefore utilized: Murphy Park = 6 water and 6 fecal, Lake Conroe = 3 water and 3 fecal, Richland Creek = 2 water, Flag Pond = 1 water and 2 fecal, and 3 water samples from the reference sites. The log-transformed *E. coli* data were used for this analysis. In addition, because samples collected in 2013 were not analyzed for fecal sterols, they were not used in any of these

comparisons. For all statistical analyses, the level of significance was set at $p = 0.05$. JMP Pro 11.0.0 ®, Microsoft Excel and XLSTAT version 2014.2.03 were used for all statistical analyses. In instances where there were no significant differences in *E. coli* counts or sterols in water on either side of island heronries, the data were combined for further statistical analysis. Data were combined in all cases except for data from Murphy Park from June 11, July 9, and August 6, 2013.

Table 1: Nomenclature and Mass Spectral data for target compounds used in this study.

Peak number	Common name (Acronym)	IUPAC name	Quantitation ion	Detection limit (solids, ng/g)	Detection limit (solids, ng/L)
1	Coprostanone (cONE)	5β-cholestan-3-one	386	/	/
2	Coprostanol (COP)	5β-cholestan-3β-ol	215	3.72	55.8
3	Epicoprostanol (eCOP)	5β-cholestan-3α-ol	215	/	/
4	Cholesterol (CHOE)	Cholest-5-en-3β-ol	368	2.78	46.75
5	Cholestanol (CHOA)	5α-Cholestan-3β-ol	388	2.65	44.83
6	β-sitosterol (bSIT)	24-ethyl-5-cholesten-3β-ol	368	/	/
7	Stigmasterol (STIG)	24-ethyl-5,22-cholestadiene-3β-ol	414	/	/
8	Campesterol (CAMP)	24-methyl-5-cholesten-3β-ol	386	/	/

RESULTS

E. coli

E. coli counts in fecal samples were not significantly different among heronries except between Murphy Park and Lake Conroe in August of the 2011 breeding season (Figure 6, Appendix B). In water samples, *E. coli* counts at Murphy Park were significantly higher than at Lake Conroe. However, *E. coli* counts were not significantly different among the heronries located directly over water (Richland Creek and Flag Pond). During the 2012 breeding season, *E. coli* counts in water from Flag Pond and Richland

Creek were significantly higher than in water from Murphy Park, except in May 2012 when *E. coli* counts between Richland Creek and Murphy Park were not significantly different (Figure 6, Appendix B). During the 2013 breeding season, *E. coli* counts from water samples at Richland Creek were significantly higher than at Murphy Park. Overall, *E. coli* counts in fecal samples were up to seven orders of magnitude higher than in water samples. In addition, *E. coli* counts in water receiving direct deposition of fecal material from heronries were up to two orders of magnitude higher than those receiving indirect deposition. However, Richland Creek and Flag Pond were also the largest heronries.

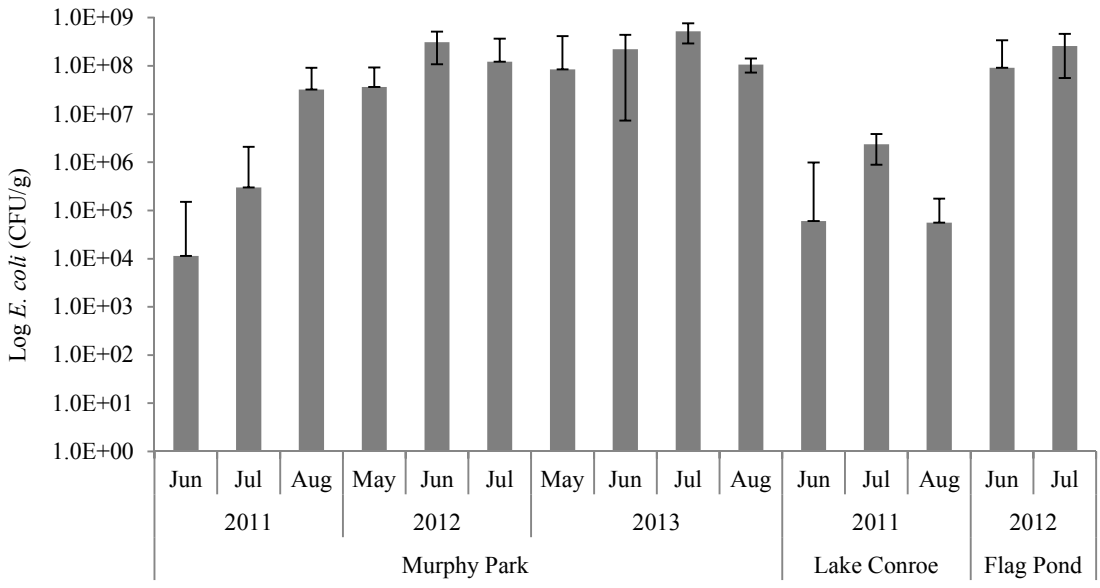


Figure 6: Average *E. coli* counts in fecal samples collected at three of the four study sites. (MP = Murphy Park, LC = Lake Conroe, FP = Flag Pond).

In fecal samples, the highest mean *E. coli* counts occurred at Murphy Park in July 2013 (9×10^8 CFU/g) and the lowest also occurred at Murphy Park (1×10^4 CFU/g) in June 2011. In water samples, the highest mean *E. coli* counts occurred at Richland Creek in July 2012 ($> 8.0 \times 10^4$ CFU/100mL) and the lowest occurred at Lake Conroe in June to August 2011 ($< 1.0 \times 10^2$ CFU/100mL). In 2012, the mean *E. coli* counts at the reference sites for Murphy Park (Bull Branch in July) and Flag Pond (Horse Pond in June) were significantly lower than counts at the corresponding heronries. In May 2013, the mean *E. coli* counts at Bull Branch were significantly lower than counts at Murphy Park but in June, counts were significantly higher at Bull Branch (Figure 7). For the estimated *E. coli* loads, the highest amounts were estimated for heronries with the greatest number of birds. At the Richland Creek heronry (2012), the highest loads ranged between 2×10^{14} and 4×10^{14} CFU breeding season^{-1}, while at the Flag Pond heronry, highest estimated loads ranged between 8×10^{13} and 2×10^{14} CFU breeding season^{-1}. At the smaller heronries located at Murphy Park and Lake Conroe, highest estimates were 3×10^{13} and 6×10^{13} CFU breeding season^{-1} and 2×10^{10} and 4×10^{10} CFU breeding season^{-1}, respectively (Table 2).

Fecal Sterols

Six sterols (coprostanol, epicoprostanol, cholesterol, cholestanol, β- sitosterol, and stigmasterol) were detected in water and fecal samples. There were no significant differences in sterol concentrations in fecal samples between and among heronries during the study period (2011 to 2013), except at Flag Pond. Among island heronries,

there were no significant differences between the sterol concentrations of the water samples from either site (except that epicoprostanol was only detected at Murphy Park in July and August of the 2011 breeding season). Similarly, there were no differences in the sterol concentrations of water samples associated with heronries located over water (except that epicoprostanol was only detected at Richland Creek during the 2012-breeding season). However, concentrations of coprostanol and cholestanol were significantly higher in water at Flag Pond than at Murphy Park (June 2012).

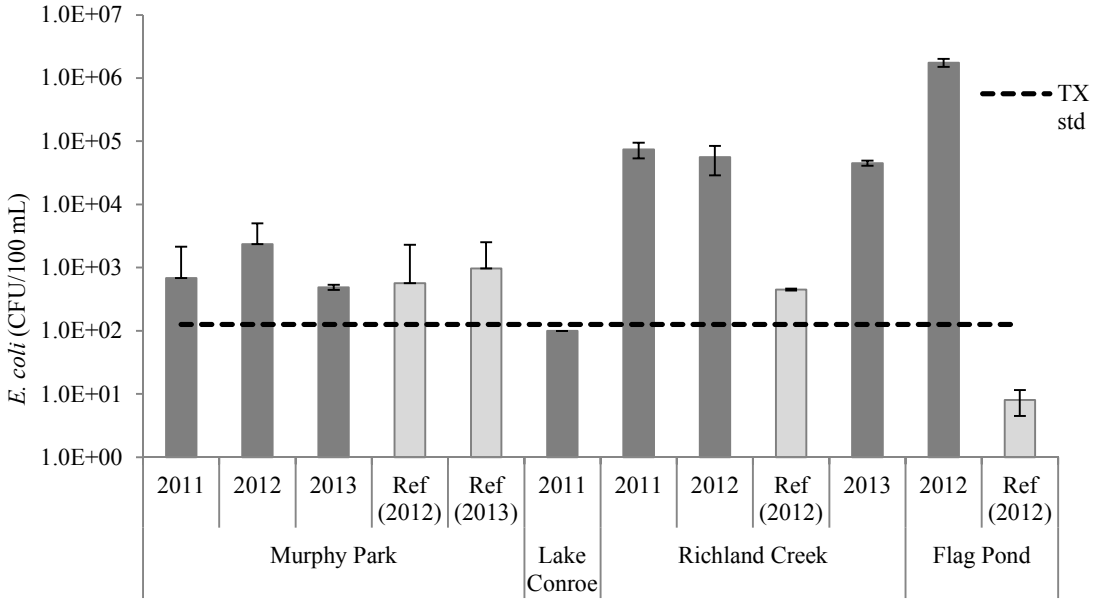


Figure 7: Average *E. coli* counts in water samples collected at the four study sites. The dashed line represents the primary contact recreation standard. Grey bars represent the reference sites.

Table 2: Table showing *E. coli* counts and estimated loads at each heronry during the entire breeding season. Estimated loads are given in ranges based on the average weight of an adult bird being 270 - 512g.

Heronry	Year	Number of birds	E. coli count (CFU/g)[a]	E. coli count (CFU/bird^{-1} g^{-1})[b]	E. coli load (CFU bird^{-1} day^{-1})[c]	E. coli load pre & post incubation & rearing (CFU heronry^{-1})[d]	E. coli load during incubation & rearing (CFU heronry^{-1})[e]	Total E. coli load (CFU heronry^{-1} season^{-1})
	2011	1,400	5×10^5	10×10^4	$5 \times 10^5 – 10 \times 10^5$	4×10^{10} -7×10^{10}	$2 \times 10^{10} – 4 \times 10^{10}$	6×10^{10} - 1×10^{11}
Murphy Park	2012	1,800	1×10^8	2×10^7	$1 \times 10^8 – 2 \times 10^8$	$1 \times 10^{13} – 2 \times 10^{13}$	$6 \times 10^{12} – 1 \times 10^{13}$	2×10^{13} - 3×10^{13}
	2013	1,800	2×10^8	4×10^7	$2 \times 10^8 – 4 \times 10^8$	$2 \times 10^{13} – 4 \times 10^{13}$	$1 \times 10^{13} – 2 \times 10^{13}$	3×10^{13} - 6×10^{13}
Lake Conroe	2011	1,200	2×10^5	4×10^4	$2 \times 10^5 – 4 \times 10^5$	$1 \times 10^{10} – 3 \times 10^{10}$	$7 \times 10^9 – 1 \times 10^{10}$	2×10^{10} - 4×10^{10}
	2011	20,000	5×10^5	10×10^4	$5 \times 10^5 – 10 \times 10^5$	$6 \times 10^{11} – 1 \times 10^{12}$	$3 \times 10^{11} – 5 \times 10^{11}$	8×10^{11} - 2×10^{12}
Richland Creek*	2012	20,000	1×10^8	2×10^7	$1 \times 10^8 – 2 \times 10^8$	$1 \times 10^{14} – 2 \times 10^{14}$	$6 \times 10^{13} – 1 \times 10^{14}$	2×10^{14} - 4×10^{14}
	2013	1,600	2×10^8	4×10^7	$2 \times 10^8 – 4 \times 10^8$	$2 \times 10^{13} – 3 \times 10^{13}$	$9 \times 10^{12} – 2 \times 10^{13}$	3×10^{13} - 5×10^{13}
Flag Pond	2012	6,000	2×10^8	3×10^7	$2 \times 10^8 – 3 \times 10^8$	$5 \times 10^{13} – 1 \times 10^{14}$	$3 \times 10^{13} – 5 \times 10^{13}$	8×10^{13} - 2×10^{14}

[a] Refers to the geometric mean
[b] Refers to the equivalent *E. coli* count in one gram of feces for one bird
[c] Refers to the range of *E. coli* loads from an adult bird with an average weight of 270 - 512 g (Telfair II 1994) and the assumption that the daily rate of excretion per bird is 2% of its body weight (Andersen et al. 2003).
[d] Calculations based on the birds spending 54% of their time at the heronry pre and post incubation and rearing (100 days) (Blaker 1969, Telfair 1994).
[e] Calculations based on the birds spending 77% of their time at the heronry during incubation and rearing (34 days) (Blaker 1969, Telfair 1994).
[*] Since we did not collect fecal samples at Richland Creek, I used the data from Murphy Park

Total sterol concentrations in fecal samples were up to two orders of magnitude higher than total sterols in water samples from the corresponding heronries, except at Flag Pond where the total sterol concentrations in fecal samples were only one order of magnitude higher than in the water samples. Figures 8 and 9 show the mean monthly (part a) and the mean annual (part b) concentrations in fecal and water samples, respectively.

Fecal samples

The highest total sterol concentrations in fecal samples were in Flag Pond, July 2012 (5,838 ng/g) and the lowest in Murphy Park, May 2012 (979 ng/g). Cholesterol and stigmasterol together, represented between 88% (Murphy Park, May 2011) and 95% (Lake Conroe, August 2011 and Murphy Park, July 2011) of the total sterol concentrations. Cholesterol proportions ranged from 31% (Flag Pond, June 2012) to 76% (Lake Conroe, July 2011) and stigmasterol proportions ranged from 18% (Lake Conroe, July 2011) to 60% (Flag Pond, June 2012) (Figure 8). However, cholesterol was present at the highest concentrations in all fecal samples except for those collected at Flag Pond where stigmasterol represented the highest proportion.

Water samples

The highest total sterol concentrations in water were also in Flag Pond, July 2012 (601 ng/g) and the lowest in Lake Conroe, August 2011 (14 ng/g). Cholesterol, coprostanol, cholestanol and epicoprostanol, represented between 84% and 100% of the total sterol concentrations. Overall, concentrations of sterols in water samples were more variable among heronries than what was observed in the fecal samples. For example, at Murphy Park, in June 2011 and May to July 2012, cholesterol, coprostanol and cholestanol

represented between 96% and 99% of the total sterol concentrations in water samples. However, in July and August 2011, epicoprostanol, cholesterol, and coprostanol represented 88% of the total sterol concentrations. Likewise, at Richland Creek, in May 2011, cholesterol, epicoprostanol, and cholestanol represented 88% of the total sterol concentrations, but in June 2011, cholesterol, cholestanol, coprostanol, and epicoprostanol represented 84% of the total sterol concentrations (Figure 9). The sterol distribution in the water samples collected at the three reference sites was dominated by cholesterol, which represents 76 to 84% of the total sterol concentrations. Stigmasterol was only detected at Bull Branch (17.3%) while β-sitosterol was only detected at Horse Pond (< 1%) (Appendix C).

The sterol data were further evaluated by calculating three steroid ratios (Table 3). The mean relative abundance of coprostanol (COP/Σ steroids), used as chemical marker for human fecal pollution (Leeming et al. 1996), ranged from 15% to 37% at Murphy Park, 39% to 40% at Lake Conroe, 8% to 16% at Richland Creek and 16% at Flag Pond. The second ratio COP/ (COP+CHOA), used as an indicator for urban sewage (Grimalt et al. 1990), was calculated to distinguish between sewage and non-sewage pollution. Ratios from water samples ranged from 0.3 (Flag Pond, 2012) to 0.6 (Lake Conroe, August 2011). The third ratio, COP/CHOE, is used to determine if CHOE or COP is produced from biogenic sources such as phytoplankton, zooplankton, and macrophytes instead of humans (Fattore et al. 1996, Nichols et al. 1996).

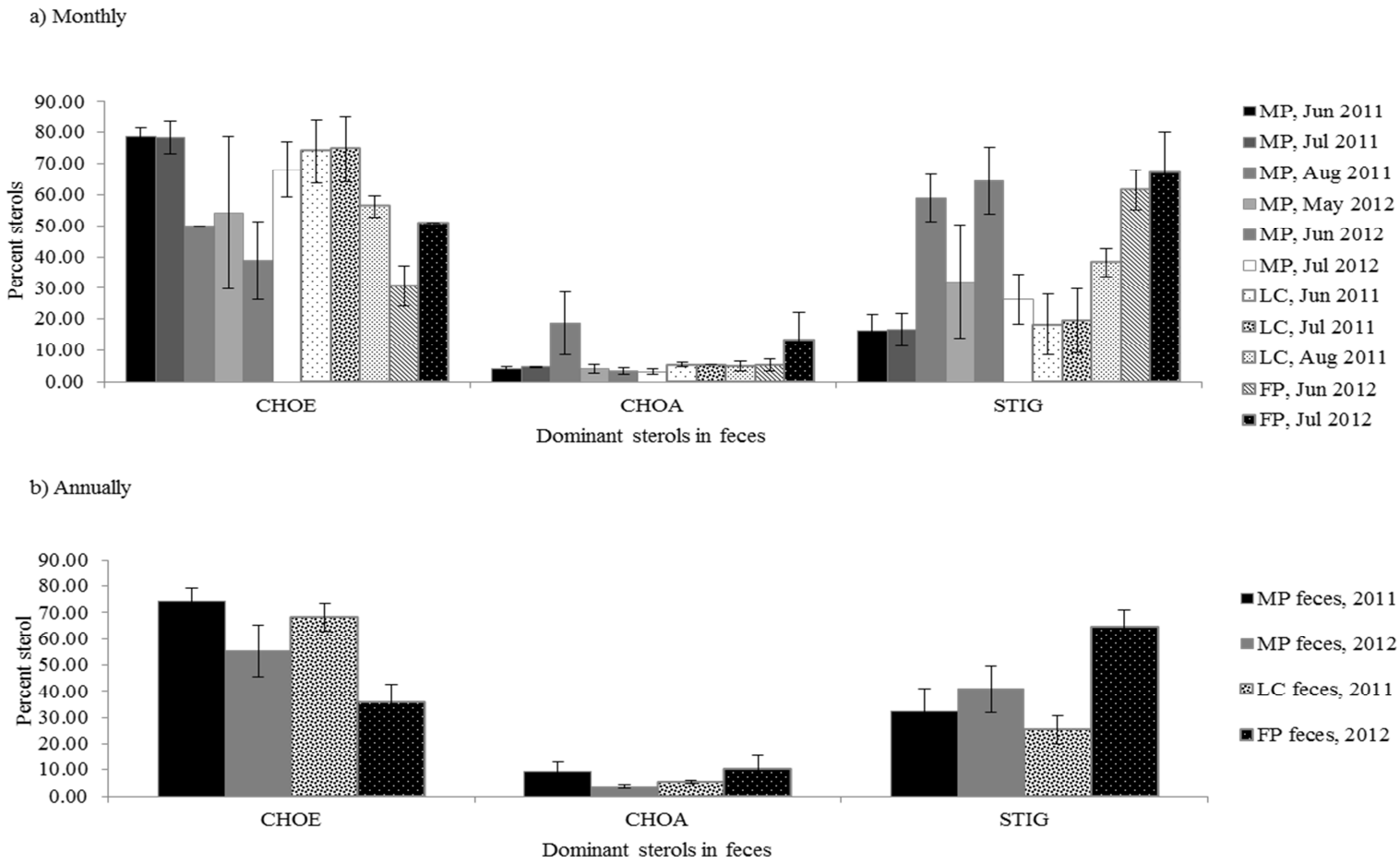


Figure 8: Relative abundance (%) of sterols measured in fecal samples presented in (a) monthly means and (b) annual means, collected at the study sites on the indicated dates.

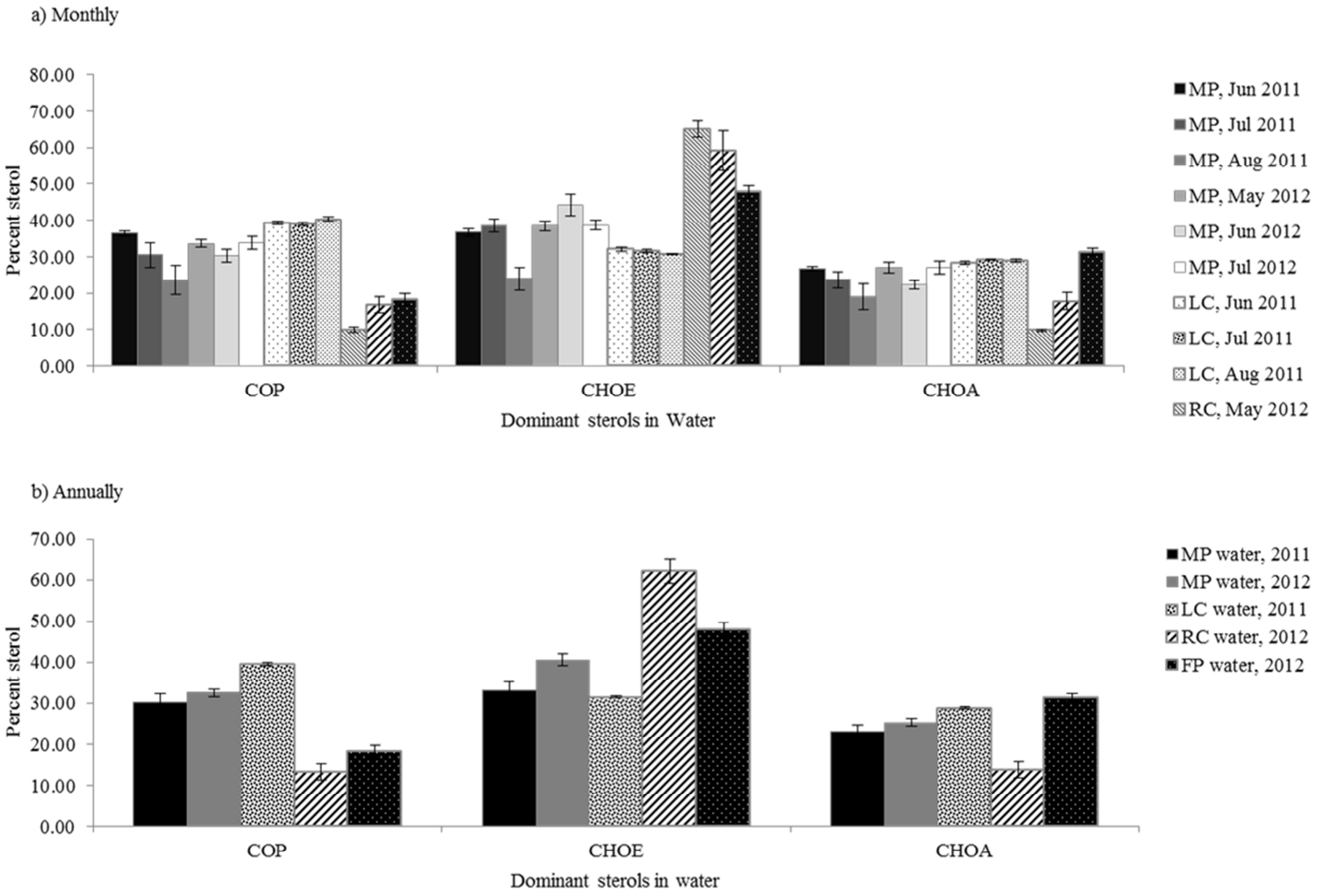


Figure 9: Relative abundance (%) of sterols measured in water samples presented in (a) monthly means and (b) annual means, collected at the study sites on the indicated dates.

Ratios in water samples from this study ranged from 0.15 (Richland Creek, May 2012) to 1.32 (Lake Conroe, August 2011).

Table 3: Calculated steroid ratios for water samples. COP = coprostanol, CHOA = cholestanol, CHOE = cholesterol.

Heronry	Year	Date	%COP	COP/(COP+CHOA)	COP/CHOE
Murphy Park	2011	June 13	36.19	0.58	0.99
		July 7	14.78	0.56	0.73
		Aug 2	15.09	0.56	0.92
	2012	May 23	31.80	0.54	0.84
		June 12	29.66	0.57	0.67
		July 2	31.12	0.51	0.81
Lake Conroe	2011	June 28	39.27	0.58	1.24
		July 21	39.20	0.57	1.25
		Aug 11	40.22	0.59	1.32
Richland Creek	2012	May 30	7.57	0.49	0.15
		June 26	15.55	0.48	0.30
Flag Pond	2012	June 28	16.14	0.33	0.35

Relationships between Fecal Sterols and *E. coli*

Comparing steroid ratios with E. coli counts

The log-transformed *E. coli* data were compared to two sterol ratios, COP+eCOP/Σsteroids, used as an indicator for human fecal pollution (Grimalt et al. 1990) and CHOE+CHOA+bSIT+STIG/Σsteroids, sterols commonly found in bird feces reported in the literature (Noblet et al. 2004) as well as what was found in this study. Comparisons were made by plotting these ratios and the *E. coli* values against time for each heronry except Flag Pond because of insufficient data (Figure 10). These plots were utilized to look for sampling intervals with corresponding peaks or valleys in *E. coli* counts as well as both or one of the sterol ratios. In the data for Murphy Park,

several corresponding areas of increases and decreases were observed between the bird sterols and the *E. coli* data (Figure 10 part a). For example, there was an increase in both *E. coli* levels and those of the bird sterols around July 7, 2011 and a valley around August 2, 2011. No such patterns were observed with the Lake Conroe (graph not presented) and the Richland Creek data (Figure 10 part b). Comparing the *E. coli* data and the sum of the sterol ratios observed in sewage or human feces, there were a few subtle peaks with the Murphy Park data (Figure 10 part a). For example, the slight increase in the sterol ratio and a corresponding increase in the *E. coli* data around June 12, 2012. Corresponding peaks and valleys were not observed in the Lake Conroe or Richland Creek data. An interesting pattern was observed with the bird sterols and human sterols in that there was an inverse relationship in data from all heronries.

At Murphy Park, *E. coli* counts and concentrations of coprostanol were negatively correlated (Pearson's coefficient = - 0.33, $R^2 = 0.110$) (Figure 11 part a). *E. coli* counts were also negatively correlated with the sum of the bird sterols (Pearson's coefficient = - 0.28, $R^2 = 0.08$) (Figure 11 part b). At Richland Creek, although *E. coli* counts were significantly ($p < 0.05$) correlated with both ratios, coprostanol concentrations had a stronger correlation (Pearson's correlation coefficient = 0.67, $R^2 = 0.451$) than the sum of the bird sterols (Pearson's correlation coefficients of 0.44, $R^2 = 0.194$) (Figure 12).

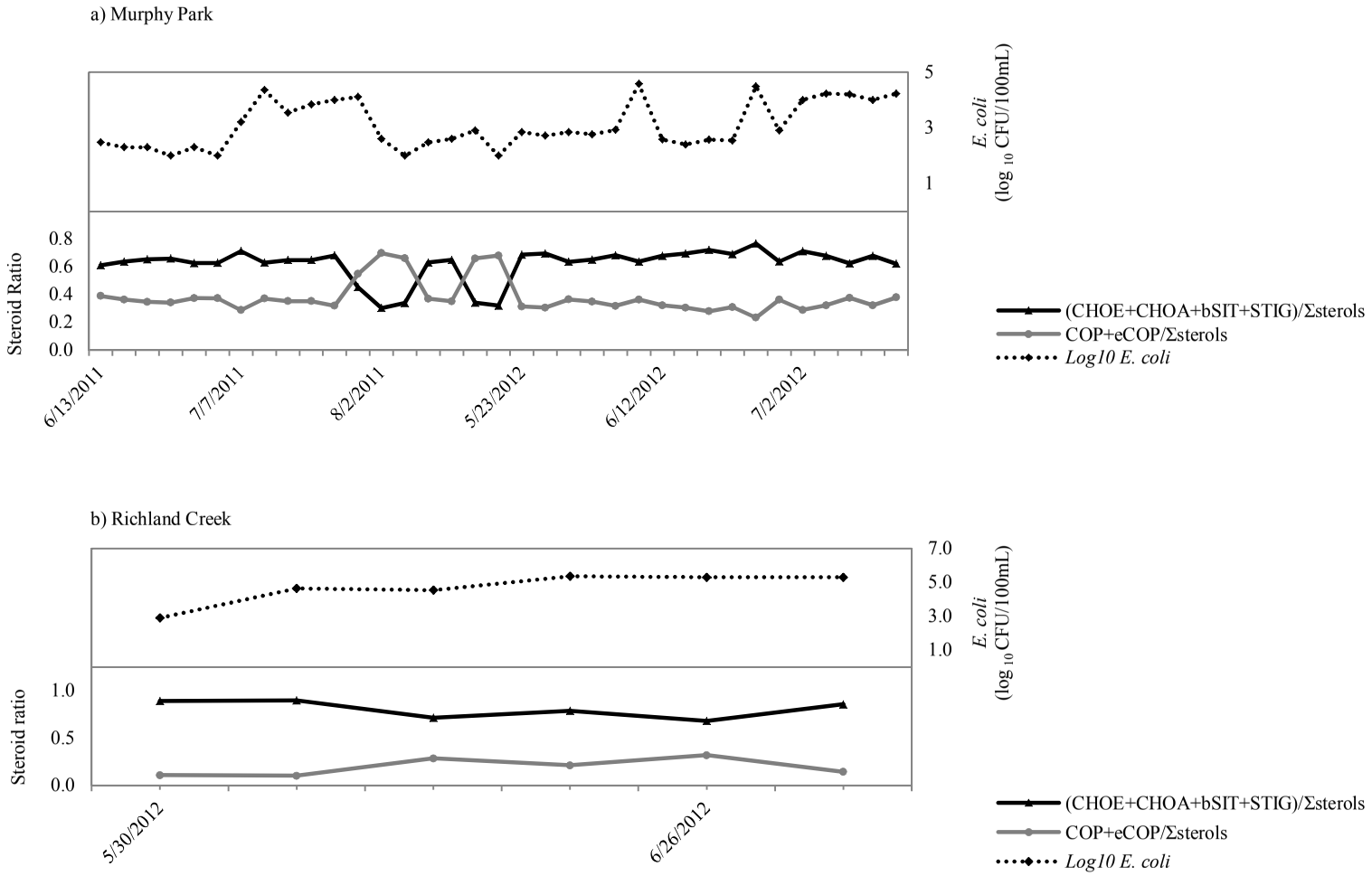


Figure 10: Plot of concentrations of *E. coli* and selected steroid ratios for water samples collected at (a) Murphy Park (MP) and (b) Richland Creek (RC). CHOE = cholesterol, CHOA = cholestanol, bSIT = β- sitosterol, STIG = stigmasterol, COP = coprostanol, eCOP = epicoprostanol.

Because of the low (< 100 CFU/100mL) *E. coli* counts obtained from water samples at Lake Conroe, no correlations were found (graph not presented). Correlations were not calculated for water samples from Flag Pond due to insufficient data.

Principal component analysis

Principal component analysis was used to understand the relationships among fecal sterols and *E. coli* in water and fecal samples as well as locations (Figure 13). The first component explained 51.8% of the variance and the first two components explained 69.8% of the variance. Principal component 1 represented a strong correlation (78.5%) between STIG and *E. coli* contrasted by lower concentrations of COP and CHOA (92.3% correlation). This effect was noticeable by contributions from fecal samples from all three heronries that contained high STIG and *E. coli* amounts in feces contrasted by contributions from Lake Conroe and Murphy Park which contained high COP and CHOA concentrations but lower concentrations of STIG and *E. coli* counts in water (Table 4). Principal component 2 represented a strong relationship between eCOP contrasted by CHOE. This effect was noticeable in two water samples from Murphy Park, which were collected in 2011 with high eCOP concentrations and lower CHOE with contrasting effects seen in water from the reference sites with low eCOP and high CHOE (Table 4).

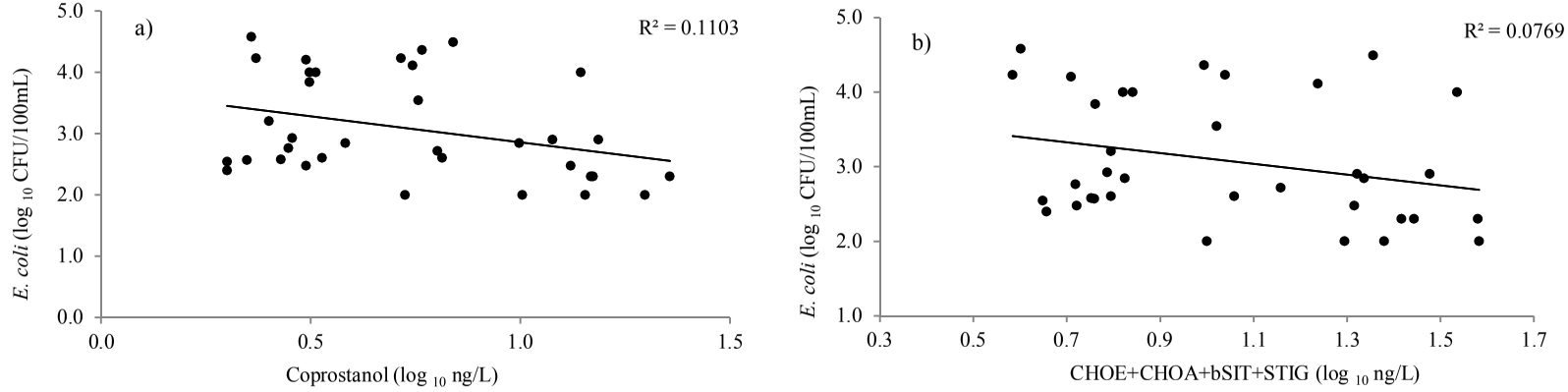


Figure 11: Correlation between the concentrations (log-based) of (a) coprostanol and *E. coli* and (b) The sum of the bird steroids (cholesterol (CHOE), cholestanol (CHOA), β- sitosterol (bSIT), and stigmasterol (STIG) in water samples collected at Murphy Park.

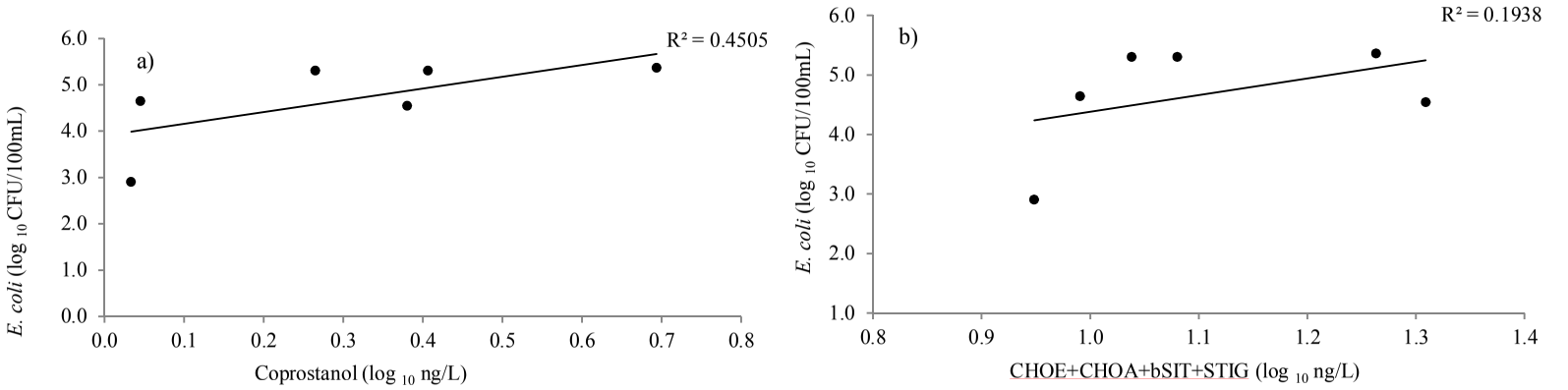


Figure 12: Correlation between the concentrations (log-based) of (a) coprostanol and *E. coli* and (b) The sum of the bird steroids (cholesterol (CHOE), cholestanol (CHOA), β- sitosterol (bSIT), and stigmasterol (STIG) in water samples collected at Richland Creek.

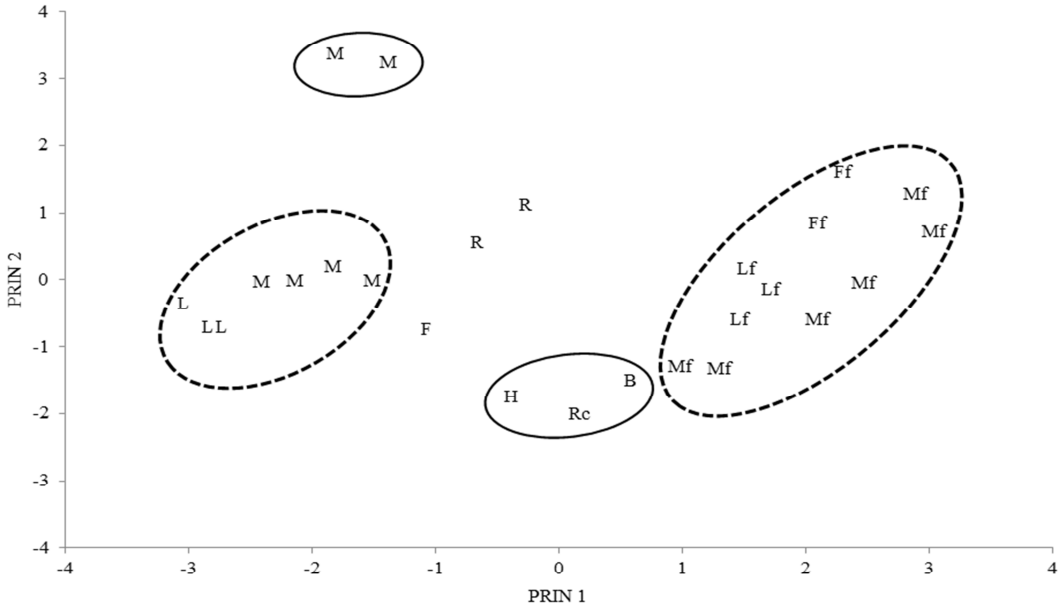


Figure 13: Plot of principal components 1 and 2 based on the principal component analysis of fecal sterols and *E. coli* in water and fecal samples. M = Murphy Park water, L = Lake Conroe water, R = Richland Creek water, F = Flag Pond water, Mf = Murphy Park feces, Lf = Lake Conroe feces, Ff = Flag Pond feces, H = Horse Pond, Rc = Richland Creek reference site, B = Bull Branch.

Table 4: Eigenvectors of the PCA analysis of fecal sterols and log *E. coli* in water and fecal samples from 4 heronries and 3 reference sites.

	PRIN 1	PRIN 2	PRIN 3	PRIN 4
COP	-0.4806	-0.0209	0.3371	0.0626
eCOP	-0.1170	0.6953	-0.5302	0.0743
CHOE	0.2954	-0.6284	-0.3504	0.2021
CHOA	-0.4582	-0.0932	0.3855	-0.0323
bSIT	0.3009	0.2241	0.3706	0.8477
STIG	0.4433	0.1344	0.3105	-0.3477
Log *E.coli*	0.4137	0.2107	0.3166	-0.3305

DISCUSSION

E. coli

The results obtained in this study indicate that the amount of *E. coli* deposited through fecal material from cattle egrets is influenced by both the size and location of the heronry. The highest *E. coli* counts were found in water samples collected at the two larger heronries (Richland Creek and Flag Pond), both of which were located directly over water. These two heronries also had the highest estimated *E. coli* loads for the breeding season. At the two heronries located on islands (Murphy Park and Lake Conroe), higher *E. coli* counts were found in the water samples collected adjacent to the Murphy Park heronry, the larger of the two heronries. Overall, *E. coli* counts at all sites, except Lake Conroe and the reference site for Flag Pond (Horse Pond), exceeded the criterion of 126 CFU/100 mL set by the TCEQ for primary contact recreation in surface water (TCEQ 2010b). The lower counts observed in water from the Lake Conroe samples may have been influenced to some degree by differences in sizes of the adjacent water bodies and differences in precipitation received during the study period. The heronry at Lake Conroe was located in a much larger body of water and the area received more precipitation, particularly during the breeding season of 2011.

The effect of large numbers of migratory birds such as geese and swans on water quality has been studied by several researchers (Leévesque et al. 1993, Standridge et al. 1979, Valiela et al. 1991). For example, Benton et al. (1983) studied the relationship between the number of gulls and *E. coli* counts in water from two lakes in Glasgow, Scotland.

The relationship was found to be highly significant ($r^2 = 0.96$). Leévesque et al. 1993 found an average of 1.0×10^7 CFU/g fecal coliform (FC) in droppings of ring-billed gulls (*Larus delawarensis*); of that amount, greater than 99% were tested positive for *E. coli.* The highest mean *E. coli* counts in my study were an order of magnitude higher (9×10^8 CFU/g) and this amount represented only *E. coli*. In addition, *E. coli* counts in fecal samples were up to four orders of magnitude higher than in water samples and estimated seasonal loads from heronries were as high as 2×10^{14} and 4×10^{14} CFU breeding season^{-1}. Since I did not include loading from juveniles, the *E. coli* load has the potential of being several orders of magnitude higher than this estimated amount because each nesting pair usually have an average clutch size of three (Telfair 1994). Large numbers of other pathogens could have also been present in those samples. Hussong et al. (1979) estimated that in a day a single swan and a goose could excrete up to 10^9 and 10^7 fecal coliforms, respectively. Wright et al. (2009) estimated the amount of enterococci (the fecal indicator for salt water) contributed by birds [Ibis (species not specified), heron (*Ardea herodias, Butorides striatus*, *Egretta caerulae, Egretta tricolor, Nycticorax nycticorax* and *Nycticorax violaceus*), ducks (unidentified species), coots (*Fulica americana*), pelicans (*Pelicanus occidentalis and carolinensis*), gulls (*Larus atricilla and delawarensis*) and pigeons (*Columba leucocephala*)] to a recreational beach. They reported an average enterococci concentration of 4.7×10^5 CFU per bird per event.

Fecal Sterols

Fecal samples

The results from the sterol analysis indicated that the fecal sterol concentrations of fecal samples were not significantly different among and between heronries, except at Flag Pond where stigmasterol was more abundant relative to cholesterol. This overall consistency in sterol proportions indicates similarities in diet among heronries. The sterol profile of birds is reported in the literature as being highly variable both among and within species due to variations in diet (Leeming et al. 1996, Martin et al. 1973). According to the literature, the primary sterols in feces from birds are β-Sitosterol, cholesterol, stigmasterol, isofucosterol, 24-ethylcholesterol, and campesterol (Leeming et al. 1996, Leeming et al. 1997, Subbiah et al. 1972), with proportions of cholesterol and β - sitosterol being most dominant. The most dominant sterols in fecal samples were cholesterol and stigmasterol. Coprostanol was present at low concentrations (≤ 3%) in all fecal samples from this study as mentioned in (Leeming et al. 1996).

Water samples

The highest proportions of coprostanol were measured in water samples collected at Murphy Park and Lake Conroe, the island heronries. At Murphy Park, cholesterol was present at the highest concentrations (except in July and August 2011). However, at Lake Conroe, coprostanol had the highest concentrations in all samples. Coprostanol is primarily produced in the intestines of humans (and some other mammals such as ruminants) by the microbial reduction of cholesterol and is used as a biomarker for human fecal pollution. Human feces are composed of 24-89% COP relative to total

sterols (Leeming et al. 1996). Although coprostanol has not been reported to occur naturally in water, studies show that anaerobic bacteria are capable of transforming cholesterol to coprostanol (Grimalt et al. 1990, Nishimura 1982). Nishimura (1982) reported small amounts (1-2%) of coprostanol in anaerobic sediments. Waterbodies adjacent to the heronries at Lake Conroe and Murphy Park heronry are used for recreation purposes and fecal contamination through leaking septic systems is possible because of nearby homes and businesses. Because of this possibility, the relative amount of coprostanol found in water samples were incorporated in several ratios discussed in another section.

Cholesterol was found at the highest concentrations in water samples from Richland Creek and Murphy Park, and in over 90% of the fecal samples. Cholesterol is an ambiguous sterol because it can also occur in other substrates such as algae, detritus, and phytoplankton (Jardé et al. 2007a, Jardé et al. 2007b, Leeming and Nichols 1998, Volkman 1986). Water samples in this study were not filtered prior to sterol extraction so although a significant amount of cholesterol may have originated from avian feces, a significant amount may have also originated from algae, phytoplankton, and other microorganisms that would have been removed through filtering (Hassett Jr and Lee 1977). Low levels ($\leq 6\%$) of β - sitosterol and stigmasterol were detected in water samples from this study. Although there was direct deposition of feces from the two larger heronries (Richland Creek and Flag Pond), the water samples associated with Richland Creek contained only 0-0.5% stigmasterol, and the total sterols was 20 ng/g

compared to total sterols of 46 ng/g and 53 ng/g at Murphy Park and Lake Conroe, respectively. Water samples collected at Flag Pond contained 6% stigmasterol and the highest total sterols compared to all water samples.

Sterol ratios: Sterol ratios have been used in other studies along with other indices (fecal indicator bacteria etc.) to elucidate sources of fecal pollution (Grimalt et al. 1990, McCalley et al. 1981, Nichols et al. 1996). Because significant levels of coprostanol were found in water samples from Lake Conroe and Murphy Park, the ratio COP/ (COP+CHOA) was calculated to distinguish between urban sewage and non-sewage pollution. According to the literature (Grimalt et al. 1990), values greater than 0.7 are indicative of sewage pollution. The ratios from this study ranged from 0.3 (Flag Pond, 2012) to 0.6 (Lake Conroe, August 2011), meaning that the source of coprostanol is probably not from human fecal pollution. However, the ratios from this study fall within the range of 0.3 and 0.7; this indicates that the sources are unknown (Fattore et al. 1996, Grimalt et al. 1990). The ratio COP/CHOE was then used to test the possibility of secondary sources of coprostanol (from macrophytes, phytoplankton etc.). Ratios >1 are reported in the literature as being indicative of a sewage source (Fattore et al. 1996, Leeming et al. 1996). Several studies reported the ratios of 1.55 to 6.00 in human-induced fecal pollution (Fattore et al. 1996, Jeng and Han 1994, Venkatesan and Kaplan 1990). Based on this ratio, Lake Conroe was the only site with values between 1.24 and 1.32, indicating possible human sources of fecal pollution. Ratios for other sites were <

1 indicating that coprostanol might have originated from a source other than human feces.

The relationship between *E. coli* and fecal sterols: Isobe et al. (2002) examined the relationship between three bacterial indicators in water and sediment from two countries challenged by fecal pollution. They found the strongest correlation with *E. coli* and coprostanol (log-transformed) concentrations ($r^2 = 0.86$) in both locations and concluded that the presence of *E. coli* is more likely to coincide with the source of coprostanol than the other bacterial indicators examined. In that same study, *E. coli* also correlated well with lower coprostanol values measured in groundwater. Noblet et al. (2004) reported significantly moderate ($p < 0.001$) correlations between log-transformed values of *E. coli* and the sum of bird sterols (CHOE+CHOA+bSIT). At Richland Creek, significant ($p < 0.05$) correlations were found between log-transformed values of *E. coli* counts and coprostanol concentrations and the sum or the bird sterols (STIG was added to the formula because it was one of the primary sterols in fecal samples from this study). However, at Murphy Park, values were negatively correlated. Positive correlations between *E. coli* and the sum of the bird sterols were expected at Richland Creek because of the direct deposition of feces from the heronry, but the positive correlation between *E. coli* and coprostanol was unexpected especially since the sterol ratios (COP/CHOE) indicated a biogenic source of coprostanol. However, no apparent relationship was observed at Richland Creek between *E. coli* and the sum of the bird sterols or the sum of the sterols related to humans or sewage compared to those observed at Murphy Park. The relatively small sample sizes from Richland Creek compared to the larger sample

size from Murphy Park may be the reason. Overall, a better seasonal trend may have been observed if samples were collected more frequently during the study period.

In addition, the principal component analysis revealed a strong positive correlation between *E. coli* and STIG in fecal samples but the correlation between *E. coli* and bSIT was not as strong (47.6%). These results indicate that more studies on the relationship between STIG and *E. coli* are warranted. Further, the plot of the first two components resulted in data points clumped by heronry, for the most part. The exception to this pattern seen in Figure 13 is with the two data points for water and fecal samples (M and Mf, respectively) collected at Murphy Park. Interestingly, both types of samples were collected in 2011. Another interesting observation was that there were relatively distinct patterns between island heronries, reference sites and heronries directly over water, suggesting the possibility of significance of location in determining the impact of feces on water.

Inconsistencies and/or misrepresentation of fecal sources using sterol ratios have been reported in past studies (Dutka et al. 1974, Furtula et al. 2012b, Shah et al. 2007). Dutka (1974) investigated the relationship between bacterial indicators and fecal sterols. Although some positive correlations between fecal sterol concentrations and bacterial indicators were found, these correlations were not found to be very consistent. The author concluded instead that the relationships depended on environmental conditions. Standley (2000) experienced a 95% success rate in using a sterol ratio to identify

humans as being the main source of fecal material in effluent. However, the same level of success was not reported for complex environmental samples with non-human and human fecal contributions since a ratio, which was considered a reliable tracer for human sewage, was not able to distinguish agricultural from human sources of fecal pollution. Other studies also reported misinterpretation of fecal sources even when pure effluent was used (Furtula et al. 2012a, Furtula et al. 2012b, Shah et al. 2007).

Overall, fecal sterols have been successfully used to identify sources of contamination primarily because of the presence of fecal stanols (α - cholestanone, β - cholestanone, coprostanol, epicoprostanol, stigmastanol, etc.) produced by the microbial reduction in the digestive tract (Bull et al. 2002, Fattore et al. 1996, Grimalt et al. 1990, Leeming et al. 1996). Furtula et al. (2012) used ten sterol ratios for identifying human and various other sources of fecal contamination. They found multiple instances of human and animal contamination for each study site. Sterol ratios (primarily of 5α and 5β-stanols) have been successfully used by many other researchers (Chan et al. 1998, Reeves and Patton 2005, Standley et al. 2000). However, as seen in this study and reported in the literature (Leeming et al. 1996, Leeming and Nichols 1998), the concentrations of these stanols are very low in the feces of birds probably due to the presence of low numbers of microbial reducers. For this reason, these ratios were not helpful in allocating sources in this study. However, the absence of 5β-stanols such as coprostanol and epicoprostanol in samples with high numbers of fecal bacteria could indicate a non-human source such as birds and dogs (Leeming et al. 1996).

Biache and Philip (2013) used compound specific carbon isotope analysis (CSIA) on β – sitosterol found in river sediments to link fecal contamination to chicken feces. The same principle can be applied to cholesterol by comparing the $\delta^{13}C$ values from cholesterol in known fecal samples to values measured in water samples. Although cholesterol has not been identified as a marker for fecal pollution, Standley et al. (2000) recommended it as a more useful tracer for wildlife fecal matter because it is usually present at higher concentrations and has a greater frequency of detection than coprostanol. In addition to the use of compound-specific stable isotope analysis, I also recommend that (1) sediment samples be analyzed in addition to water samples, (2) samples should be collected at varying distances away from each heronry, and (3) if possible, water samples should be collected around islands at varying intervals before and after the arrival of birds.

My hypotheses that the fecal sterol profile observed from fecal samples would correlate with the profile observed in the respective water samples, and that fecal sterols in water samples are primarily of avian origin, were not supported by the methods of analyses used and the results obtained. However, the strong correlations observed between *E. coli* and STIG in the principal component analysis in addition to the significant positive correlation between *E. coli* and the bird sterols from Richland Creek water samples, suggest that STIG could be an important chemical indicator in determining the source of *E. coli* in water, in relation to cattle egrets and other herons. The objectives of this study

and the data presented contribute to furthering the understanding of the potential contributions of bacteria from large heronries located on the edge of or near water bodies. Results of this study will be particularly useful in bacteria-impaired watersheds in developing Water Protection Plans (WPP) and selecting best management practices. Identifying sources *E. coli* and quantifying loads resulting from various sources are critical tasks in development of restoration measures for impaired watersheds.

CHAPTER III

QUANTIFYING THE NITROGEN AND PHOSPHORUS LOADS DEPOSITED BY CATTLE EGRETS (*Bubulcus ibis*) IN HERONRIES IN TEXAS

SYNOPSIS

Nitrogen (N) and phosphorus (P) contained in feces deposited by cattle egrets (*Bubulcus ibis*) in heronries in Texas are sources of contamination of nearby waterways. N and P concentrations were analyzed in water and fecal samples collected from four heronries during the breeding seasons of 2011, 2012, and 2013. A model was developed to simulate daily and annual rates of fecal deposition at these and 13 other heronries. The results indicated that N and P loads deposited in cattle egret heronries depend primarily on size of the heronry, and that the amount of nutrient loading of nearby water bodies depends primarily on location (over water or over land) of the heronry. Highest (mean ±SD) concentrations of N ($9.94 \times 10^4 \pm 2.72 \times 10^3$ mg/kg) and P ($1.11 \times 10^4 \pm 2.74 \times 10^3$ mg/kg) in fecal samples were up to four orders of magnitude higher than highest concentrations of N (62.4 ± 1.47 mg/L) and P (4.69 ± 0.12 mg/L) in water samples. Concentrations of N and P in water samples collected from heronries located directly above water were significantly higher ($P < 0.05$) than concentrations in water samples collected from heronries located on islands. Simulated N and P loads suggested loads increase linearly with heronry size. Simulated annual N and P loads were as high as 1,965 and 218 kg, respectively, simulated daily loads were as high as 20.66 and 2.30 kg,

respectively, and the most concentrated annual depositions were approximately 195 and 22 g m^{-2} $year^{-1}$, respectively.

INTRODUCTION

Avian species such as herons and egrets establish large heronries in coastal areas and inland in close proximity to water (Parkes 2007). Because of the large quantities of feces deposited and the potential for runoff, the contribution of large nitrogen (N) and phosphorus (P) loads to nearby waterways is inevitable (Chaichana et al. 2010, Hussong et al. 1979, Moss and Leah 1982, Scherer 1995). Numerous studies have reported strong correlations between the occurrence of large numbers of colonial birds near water bodies and high concentrations of N and P (Baxter and Fairweather 1994, Chaichana et al. 2010, Chaichana et al. 2011, McColl and Burger 1976) in the water. In Texas, cattle egrets (*Bubulcus ibis*) establish large heronries during the breeding season, frequently numbering thousands of birds (Parkes 2007, Telfair II 1993).

High concentrations of nutrients increase the production of phytoplankton and aquatic plants and can ultimately lead to oxygen depletion, death of fish and other organisms, decreased lake biodiversity, increased dissolved solids, an increase in undesirable fish and a decrease in desirable fish (Elser et al. 2007, Gould and Fletcher 1978, Prepas and Charette 2003, TCEQ 2010b, Welch and Lindell 2002). Excess concentrations of N and P pose two-times the risk of impairment of biological conditions compared to other nutrients (USEPA 2006), and more than 50% of assessed U.S. streams have medium to

high levels of N and P and 78% of assessed coastal waters are eutrophic (Gilinsky et al. 2009). The Environmental Protection Agency (EPA) has identified nutrient pollution as a major cause of water quality impairment, and has recommended prioritization of N and P loading reductions in water bodies based on the best available loading estimates (Gilinsky et al. 2009).

In Texas, surface water quality standards are developed by the Texas Commission on Environmental Quality (TCEQ), which has established site-specific numerical nutrient criteria based on chlorophyll a for 75 reservoirs (pending approval by the EPA) (SWQM 2012, TCEQ 2012b). In water bodies for which specific nutrient criteria have not been established, nutrient screening levels are based on a combination of narrative nutrient criteria such as relative amount of algae and turbidity, and measurements of NH_3-N, NO_3-N, orthophosphate (OP), (total) P, chlorophyll a (SWQM 2012), and (total) N (TCEQ 2012a). The TCEQ currently is exploring the development of procedures using concentrations of total N and P as direct indicators of eutrophication, and has emphasized the need for more nutrient data (TCEQ 2012a). To my knowledge, no information is available on the N and P loads deposited by cattle egrets in their heronries in Texas.

In this study, I provide initial estimates of the N and P loads deposited by cattle egrets in heronries in Texas and investigate the potential impact these heronries could have on associated watersheds. Estimates are based both on field data collected over a three-year

period at four study sites and on a simulation model, parameterized based on field data, that was used to estimate the N and P loads deposited by cattle egrets at each of 13 other heronries in Texas for which estimates of heronry size were available.

Breeding Biology of Cattle Egrets

The breeding range of cattle egrets is widespread throughout the United States (Telfair II 1993). They migrate to Texas to breed (Telfair II 1983), establishing heronries in four types of habitats: 1) upland woodlands that may or may not be in close proximity to water, 2) swampy areas with submerged trees, 3) islands containing trees and shrubs that are located inland, and 4) islands with trees and shrubs located in coastal areas (Telfair II 1994). Heronry sizes range from less than 100 to over 15,000 pairs (Dusi 1978, Parkes 2007, Telfair II 1983, Telfair II et al. 2000).

In Texas, the breeding season starts in early spring and ends in late August to early September (Dusi et al. 1971, Telfair II 1983). Clutch sizes range from one to nine with an average of about four (Telfair II 1983, Telfair II and Bister 2004, Weber 1975). Incubation lasts 22-28 days (Telfair II 1983, 1994). Males and females both brood and feed nestlings (Blaker 1969, Telfair II 1983), with at least one adult constantly attending the chicks until they are ten days old (Blaker 1969, Telfair II 1994). During this time, adults make an average of two feeding flights per day (Blaker 1969, Dusi et al. 1971, Telfair II 1983), and may carry food back to the nest from a distance of 20-32 km (Telfair II 1983, 1994). Chicks are branchers (they are able to leave the nest, but remain

near) until they are 14-21 days old, can fly by day 25, fledge by day 30, and become independent by day 45, at which time they begin to forage near the colony. After day 60, they are able to fly to foraging areas with adults (Telfair II 1994).

Study Sites

The four heronries that were studied contained several species of birds including cormorants, primarily Neotropical (*Phalacrocorax brasilianus)*, great egrets (*Ardea alba*), snowy egrets (*Egretta thula*), little blue herons (*Egretta caerulea*), and anhingas *(Anhinga anhinga).* However, the most common species was the cattle egret, comprising at least 90% of the birds in each colony. The population of cattle egrets at each heronry was estimated by counting the number of breeding pairs visible from a fixed point at the water's edge and extrapolating this number to the estimated area of the heronry following the methodology of Gregory et al. (2004).

Murphy Park

Murphy Park (MP) is a city park located in Taylor, Texas. The heronry was located on a small island in an 809 m^2 pond (Muddy Lake) within the park (N 30.5809, W 97.4131) (Figure 14). Approximately 700 nesting pairs of cattle egrets were observed in 2011. The size of the heronry increased to approximately 900 pairs in 2012 and 2013. Other species of birds observed in the colony included cormorants, great egrets, snowy egrets, and little blue herons, as well as a few species of waterfowl. Bull Branch Pond, which is located upstream (N 30.5871, W 97.4222) from MP, was identified as a reference site

during the 2012 sampling season. Bull Branch Pond is a smaller pond without cattle egrets; however, a few species of waterfowl were observed.

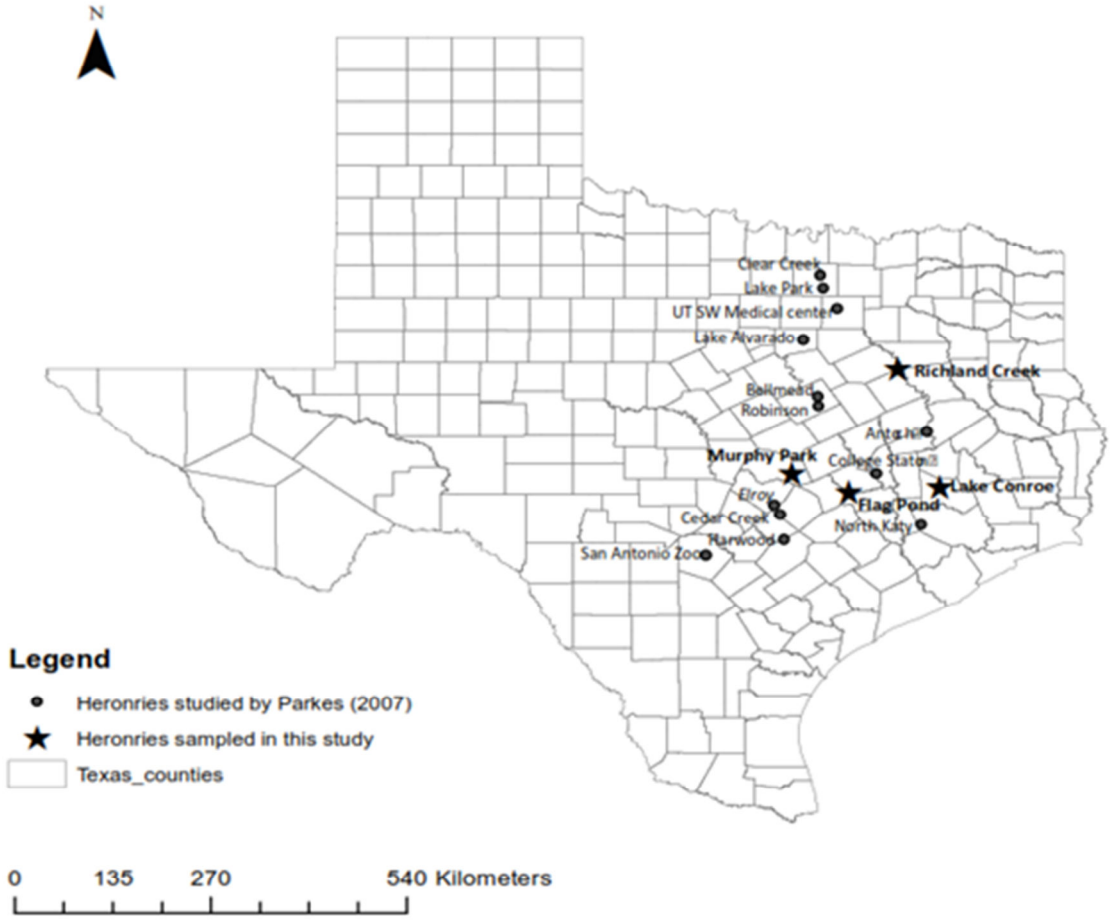


Figure 14: Map showing locations of cattle egret heronries sampled during the 2011, 2012, and 2013 breeding seasons, as well as the 13 other heronries for which estimates of heronry size were available (Parkes, 2007).

Lake Conroe

Lake Conroe (LC) is an 80.9 km^2 Lake in Montgomery County, Texas managed by the San Jacinto River Authority. The heronry was located on a small island in the lake (N 30.4035, W 95.5750) (Figure 14). Approximately 600 nesting pairs of cattle egrets were observed in 2011. Other species of birds observed in the colony included cormorants, great egrets, snowy egrets, little blue herons, and great blue herons. Samples were not collected from this site during the 2012 and 2013 breeding seasons.

Richland Creek

Richland Creek (RC), a tributary to the Trinity River, is a wildlife management area managed by the Texas Parks and Wildlife Department located in Freestone County, Texas (N 31.9906, W 96.1005) (Figure 14). Nests were in trees and shrubs with roots and trunks in water. Approximately 10,000 nesting pairs of cattle egrets were observed in 2011 and 2012. In 2013, the size of the heronry decreased to approximately 800 nesting pairs. Other species of birds observed in the colony included cormorants, great egrets, snowy egrets, little blue herons, great blue herons and anhingas. A reference site located upstream (N 31.9921, W 96.0981) from the Richland Creek heronry was identified during the 2012 sampling season. Cattle egrets, waterfowl, or other species of birds were not observed at the reference site during the sampling period.

Flag Pond

Flag Pond is a 1.4 km^2 seasonal wetland area managed by the Texas Parks and Wildlife Department that is part of the Birch and Nails Creek State Parks in Lee County, Texas. The heronry was located in a flooded area (N 30.3063, W 96.6976) (Figure 14).

Approximately 2,000 to 3,000 nesting pairs of cattle egrets were observed in 2012. Other species of birds observed included cormorants, great egrets, snowy egrets, little blue herons, great blue herons and anhingas. Horse Pond, which is located (N 30.2886, W 96.6688) within the Nails Creek State Park, was identified as a reference site during the 2012 sampling season. Horse Pond is about ¼ the size of Flag Pond. Cattle egrets or other birds were not observed at Horse Pond during the sampling period.

MATERIALS AND METHODS

Field Sampling and Data Analysis

During the 2011 breeding season, water and fecal samples were collected monthly (June to August) from Murphy Park and Lake Conroe. Water samples were collected in July of that year at Richland Creek. During the 2012 breeding season, water and fecal samples were collected monthly (May to July) from Murphy Park and water samples were collected monthly from Richland Creek. At Flag Pond, both fecal and water samples were collected in June but in July, only fecal samples were collected because the pond was drained by park management. During the 2013 breeding season, water and fecal samples were collected twice per month (May to August) from Murphy Park and water samples were collected once per month from Richland Creek.

During each visit, water were collected as grab samples from two ends of the island heronries (Murphy Park and Lake Conroe), approximately 32 km from the shoreline, and from areas directly under the heronries that were located over water (Flag Pond and

Richland Creek). Water samples were collected at a central location of each reference site. Sterile 250 mL polypropylene screw-cap bottles were used to collect and store all water samples. Fecal samples were collected from several trees and/or nests, using sterile forceps and/or syringes, at various locations within the heronry. Samples were also collected with plates (lined with wax paper) mounted on tripods, that were placed under trees with a high density of nests. Fecal samples were placed in 50 mL sterile polypropylene tubes. All sample types were stored at approximately 4℃ while transporting them to the laboratory and prior to analysis.

The Texas A&M AgriLife Extension Service Soil, Forage and Water Testing Laboratory at Texas A&M University analyzed water samples for total N and P using the Kjeldahl digestion method (Parkinson and Allen 1975) and the fecal samples for total N and P using methods described elsewhere (Havlin and Soltanpour 1980, Sheldrick 1986). The non-parametric Wilcoxon tests were utilized to test for significant differences ($p < 0.05$) in N and P in samples collected within and among heronries. Kruskal -Wallis analyses were performed to test for significant differences ($p < 0.05$) in N and P in samples collected in different months and/or years within and among heronries. JMP Pro 11.0.0® software was used for all statistical analyses.

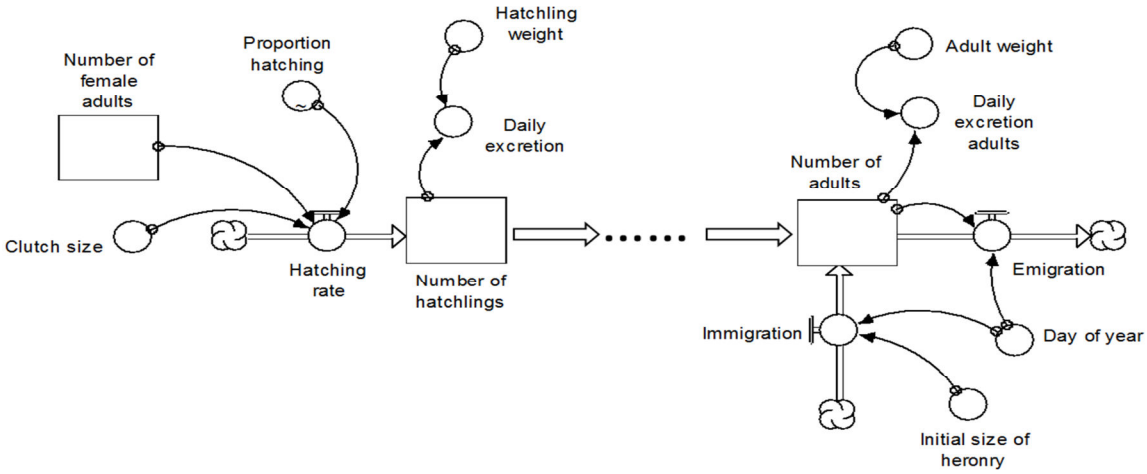
Simulation Model Description

To estimate the daily N and P loads deposited during the breeding season at each of the four heronries that were studied, as well as the 13 other heronries in Texas for which

estimates of heronry size were available (Parkes 2007) (Figure 14), a compartment model was developed based on difference equations. The model represents (1) arrival of cattle egrets to the heronries during early May (day of year 121 to day of year 130 (Blaker 1969, Dusi 1978, Telfair II 1983), (2) nesting and appearance of hatchlings during late May and early June (day of year 147 to day of year 153 (Blaker 1969, Dusi 1978, Telfair II 1983), with an average clutch size of 3 (Telfair II 1983), (3) development of hatchlings into adults (over a 45-day period (Blaker 1969, Dusi 1978, Telfair II 1983)), and (4) emigration from the heronries during early September (day of year 244 to day of year 253 (Blaker 1969, Dusi 1978, Telfair II 1983)) (Figure 15 part a). Daily fecal production depends on the number of birds in each daily age class and their respective body weights (daily fecal production in grams wet weight = 0.02 * live body weight in grams (Andersen et al. 2003). Body weights of simulated birds increase daily until they reach adult size based on information obtained from the literature (Telfair II 1983). The proportions of N and P in the feces change seasonally based on the field data, day-to-day changes in these proportions was estimated by linearly interpolating between field data points. Because the Richland Creek colony was located directly above water and fecal material could be obtained, data from Murphy Park was used to estimate N and P loads at Richland Creek. The proportion of feces deposited in the heronry, rather than in the foraging area, depends on the daily activity budgets of the birds as the breeding season progresses (Figure 15 part b). During the early May immigration period (day of year 121 to day of year 130), adult birds spend roughly half (54%) of each 24-hour period in the heronry and the rest of the day foraging away from

the heronry (Blaker 1969, Telfair II 1993). During the next month (day of year 131 to day of year 164), adults spend about three quarters (77%) of the day in the heronry caring for the nestlings (juveniles) (Blaker 1969, Telfair II 1993). During the last three months prior to emigration (day of year 165 to day of year 253), adults again spend roughly half (54%) of the day in the heronry (Telfair II 1983). Juvenile birds spend all of their time in the heronry (Telfair II 1983). To parameterize the model for each of the different heronries, the appropriate heronry size (the number of adult cattle egrets in the heronry) was specified as well as the appropriate levels of fecal N and P (mg/L dry weight). For each of the four heronries, heronry sizes and concentrations of fecal N and P was estimated based on the collected field data. For the 13 previously studied heronries, the estimates were based of heronry size on information in Parkes (2007) (Table 5), and the estimates of fecal N and P was based on the collected data from Murphy Park, which was averaged over the three consecutive years of sampling.

a)



b)

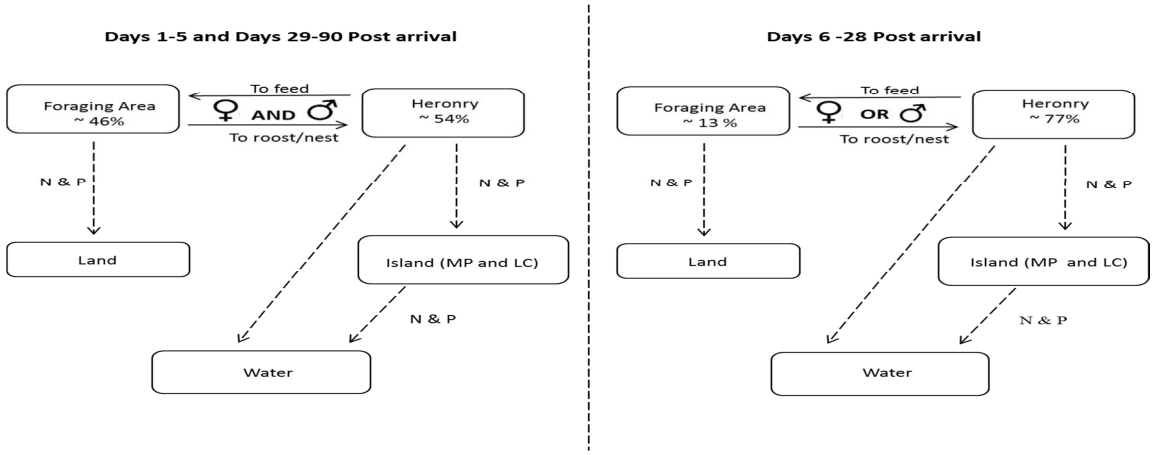


Figure 15: (a) Conceptualization of the compartment model used to estimate daily fecal and nutrient loads generated from each heronry, and (b) diagrammatic representation of the differences between the two types of heronries. In part a, boxes represent state variables, auxiliary variables or constants and arrows represent transfers of materials (thick arrows) or information (thin arrows). In part b, arrows with solid lines represent the movement of birds between the heronry and foraging areas, and arrows with broken lines represent the deposition of fecal material or nutrients. Time spent in the foraging areas varies depending on the stage of the breeding process (incubation, nesting etc.).

Table 5: Descriptions of the other 13 heronries in Texas (Figure 14) for which estimates of population size were available, and simulated daily and annual loads of N and P deposited at each heronry.

Heronry *	Type of Heronry *	Population size * (No. of adult birds)	Area of Heronry * (m^2)	Population Density (birds/m^2)	Maximum daily N load (kg)	Maximum daily P load (kg)	Annual N load (g/m^2)	Annual P load (g/m^2)
Harwood	Residential	21,992	13,742	1.6	20.66	2.30	143.0	15.9
Elroy	Residential	21,396	16,900	1.3	20.10	2.23	113.1	12.6
North Katy	Artificially flooded veg.	15,192	12,000	1.3	14.27	1.59	113.1	12.6
Robinson	Artificially flooded veg.	12,728	9,600	1.3	11.96	1.33	118.5	13.2
Cedar Creek	Residential	11,512	7,195	1.6	10.81	1.20	142.9	15.9
Antioch	Artificially flooded veg.	9,660	165,000	0.1	9.07	1.01	5.2	0.6
Bellmead	Residential	3,520	3,000	1.2	3.31	0.37	104.8	11.7
UT SW Medical Center	Urban	3,240	20,625	0.2	3.04	0.34	14.0	1.6
College Station	Artificially flooded veg.	2,112	3,750	0.6	1.98	0.22	50.3	5.6
Clear Creek	Artificially flooded veg.	1,526	700	2.2	1.43	0.16	194.8	21.6
Lake Alvarado	Residential	800	525	1.5	0.75	0.08	136.1	15.1
Lake Park	Residential	800	1,750	0.5	0.75	0.08	40.8	4.5
San Antonio Zoo	Urban	514	Unknown	-----	0.48	0.05	----	----

* Obtained from Parkes 2007

RESULTS

Field Sampling

Concentrations of N and P in fecal samples were up to four orders of magnitude higher than concentrations in water samples. In addition, concentrations of N and P in water samples collected from heronries located directly above water (Flag Pond and Richland Creek) were significantly higher ($P < 0.05$) than concentrations in water samples collected from heronries located on an island (Murphy Park and Lake Conroe) (Figures 16 to 18, Appendix D). No significant differences ($P > 0.05$) in concentrations of N or P were found in samples collected on either sides of the island heronries, hence these samples were combined ($n = 6$). For the same reason, samples collected in June 2013 at Murphy Park ($n = 12$) as well as at Bull Branch ($n = 6$) were combined. There were no significant differences ($P > 0.05$) in N or P concentrations in fecal samples within or among heronries.

In fecal samples, the highest mean (±SD) concentrations of N ($9.94 \times 10^4 \pm 2.72 \times 10^3$ mg/kg) and P ($1.11 \times 10^4 \pm 2.74 \times 10^3$ mg/kg) occurred at the Lake Conroe heronry in June and July, respectively, of 2011. The lowest mean concentrations of N ($7.48 \times 10^4 \pm 6.04 \times 10^3$ mg/kg) and P ($5.39 \times 10^3 \pm 1.19 \times 10^3$ mg/kg) occurred at the Murphy Park heronry in June of 2012 and August of 2013, respectively (Figure 16, Appendix D).

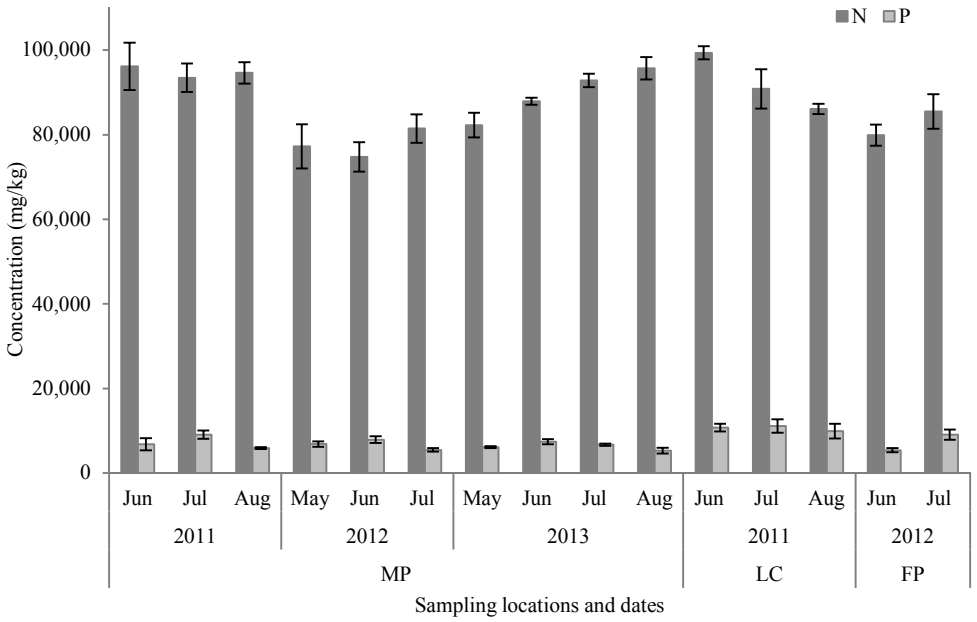


Figure 16: Mean concentrations (mg/kg dry weight) of N and P in fecal samples collected at three of the four study sites on the indicated dates. The error bars represent the standard error of the mean. (MP = Murphy Park, LC = Lake Conroe, FP = Flag Pond).

In water samples, the highest mean (±SD) concentrations of N (62.4 ± 1.47 mg/L) and P (4.69 ± 0.12 mg/L) occurred at the Flag Pond heronry in June 2012 and the lowest mean concentrations of N (0.63 ± 0.27 mg/L) and P (0.05 ± 0.02 mg/L) occurred at the Lake Conroe heronry in July 2011. The mean concentrations of N at the reference sites for Flag Pond (Horse Pond) and Murphy Park (Bull Branch) in 2013 were significantly ($p < 0.05$) lower than concentrations at the corresponding heronries. The mean concentrations of P at the reference site for Flag Pond that year were also significantly ($p < 0.05$) lower than the concentrations at Flag Pond. However, mean concentrations of P for the reference site for Murphy Park in 2013 were significantly ($p < 0.05$) higher in May but significantly lower in July and August (Figures 17 and 18, Appendix D). The

N:P ratio in water samples was as high as 62:1 (Murphy Park, August 2013) and as low as 2:1 (Richland Creek, June 2013) (Appendix D).

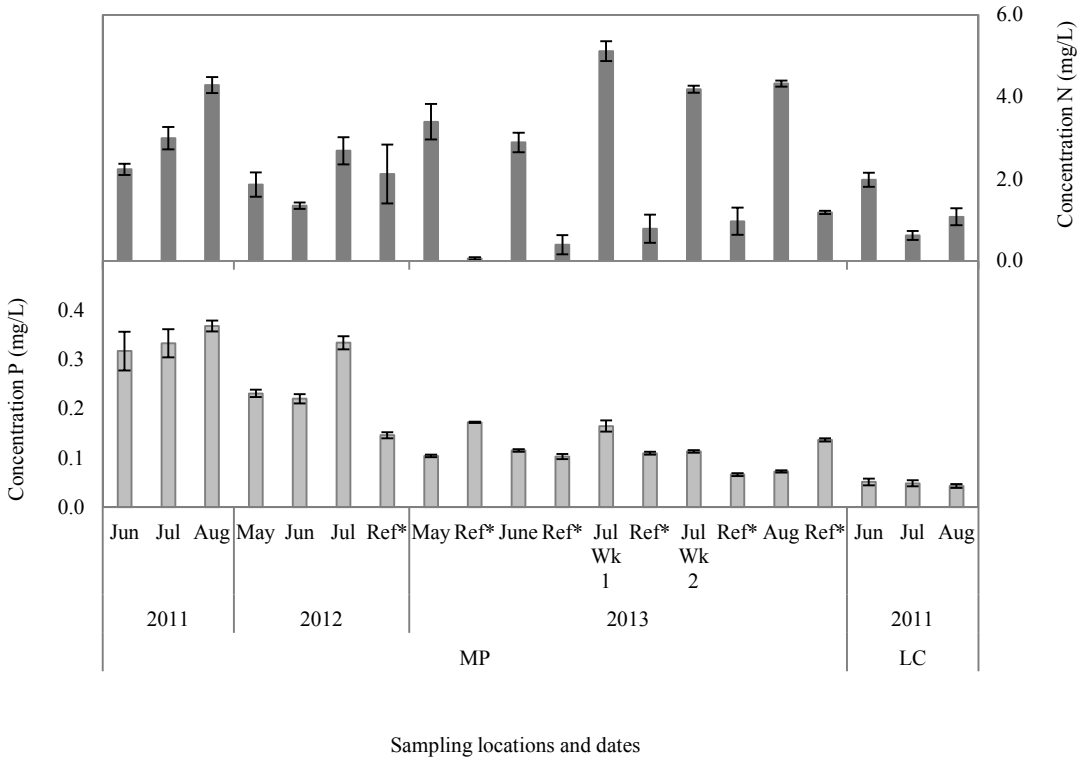


Figure 17: Mean concentrations (mg/L) of N and P in water samples collected at the island heronries and the corresponding reference sites (Ref*) on the indicated dates. The error bars represent the standard error of the mean.

Simulation of N and P Loads

Simulated annual loads of N and P at the four heronries that were studied were variable across sites and years due primarily to differences in heronry size (Figure 19). Highest simulated annual loads occurred at Richland Creek in 2011 (1,884 kg N and 140 kg P)

and 2012 (1,580 kg N and 121 kg P), followed by Flag Pond in 2012 (493 kg N and 43 kg P). Simulated annual loads at Richland Creek decreased drastically in 2013 due to a drastic decrease in heronry size. Simulated annual loads at Richland Creek were different in 2011 and 2012, although the heronry size did not change. Changes in annual loads were not observed at that site because of the lower concentrations (mg/kg dry weight) of N and P measured in the fecal samples at Murphy Park in 2012, which we used for the Richland Creek simulations since no field estimates of fecal N and P were available. Simulated annual loads at Murphy Park remained relatively constant (132-155 kg N and 10-14 kg P).

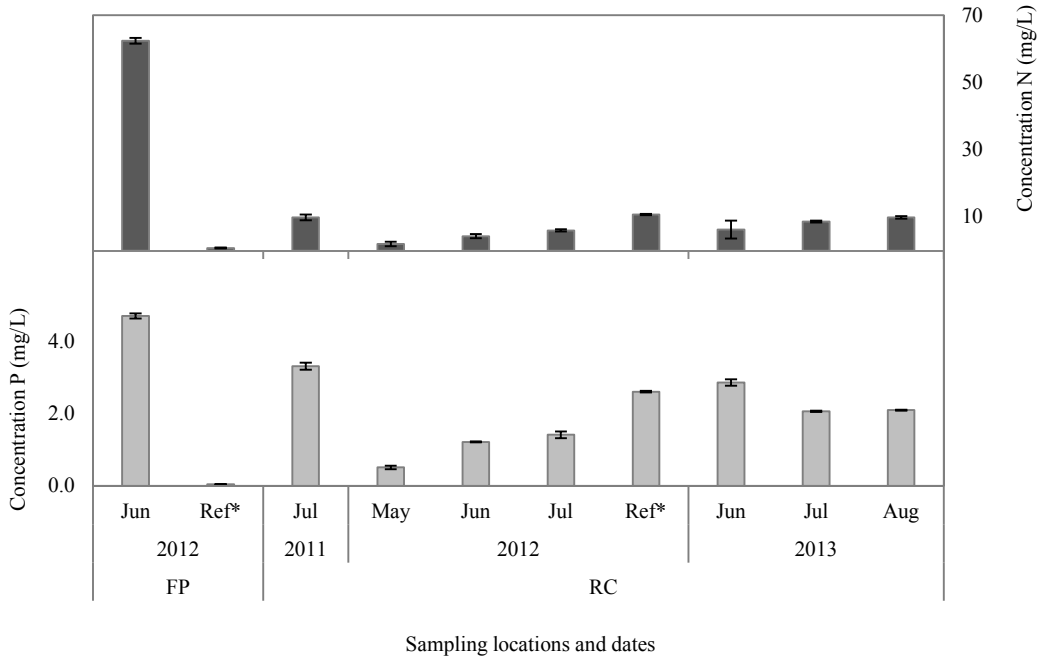


Figure 18: Mean concentrations (mg/L) of N and P in water samples collected at the heronries over water and the corresponding reference sites (Ref*) on the indicated dates. The error bars represent the standard error of the mean.

Simulated daily loads of N and P changed seasonally in a similar manner at all four of the sites (Figure 20), and were directly associated with the shifting daily activity budgets of the birds as the breeding season progressed. Simulated daily N loads at Richland Creek increased to as high as 19.9 kg during June of the 2011 breeding season, and daily P loads increased to as high as 1.9 kg during July of the 2011 breeding season. Simulated daily P loads were lower during the 2013 breeding season compared to previous years. The relative magnitudes of simulated daily loads at the other sites during in the various years followed the same trends exhibited by the annual loads, with the second highest daily loads occurring at Flag Pond (daily N loads peaked at 5.22 kg during July of 2012 and daily P loads peaked at 0.48 kg during July of 2012).

Simulated annual N and P loads at the other 13 heronries for which estimates of heronry size were available increased approximately linearly with heronry size, and were as high as 1,965 kg and 218 kg, respectively (Figure 21). Simulated daily N and P loads at the largest heronry (Harwood, 21,992 adult birds) were as high as 20.66 kg and 2.30 kg, respectively (Table 5). The most concentrated simulated annual depositions of N and P per unit area were approximately 195 and 22 g m^{-2} $year^{-1}$, respectively (Table 5).

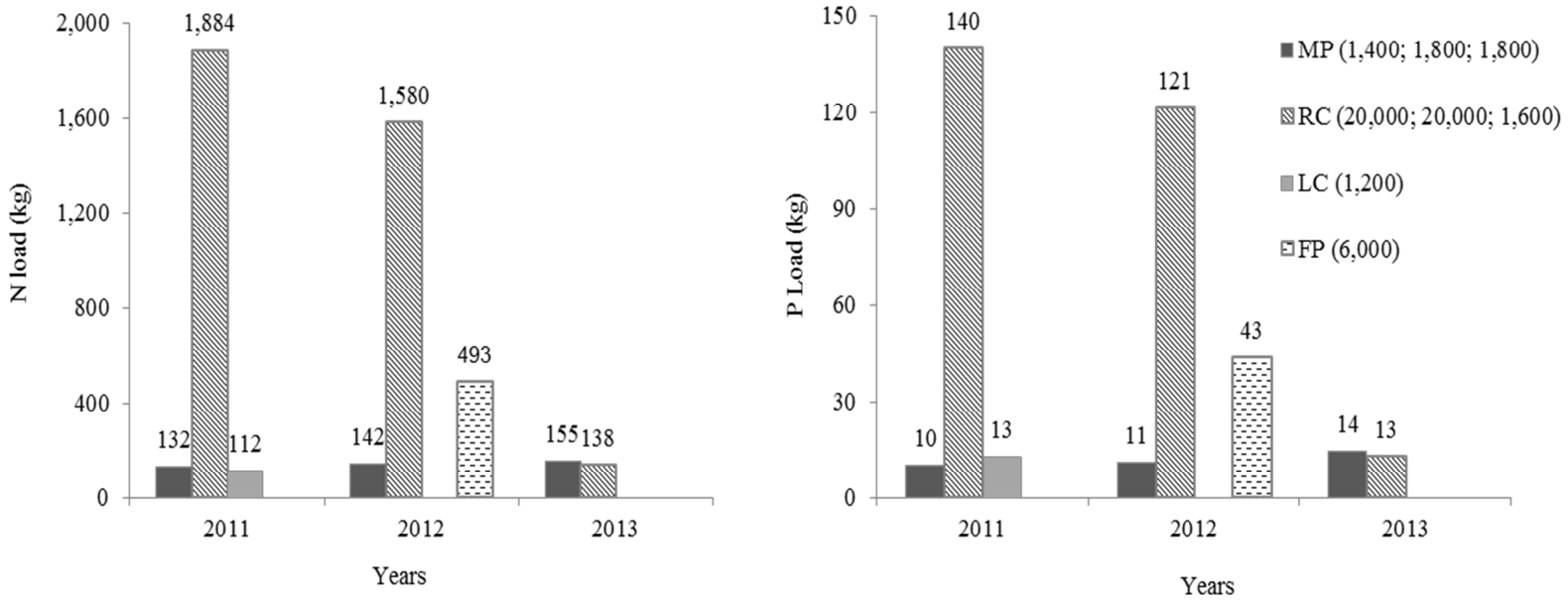


Figure 19: Simulated annual loads of N and P deposited by cattle egrets in each of the four study sites during the indicated years. Numbers in parentheses represent estimated numbers of adult cattle egrets present in 2011, 2012, and 2013, respectively. (Note that LC was sampled only in 2011, and FP was sampled only in 2012.).

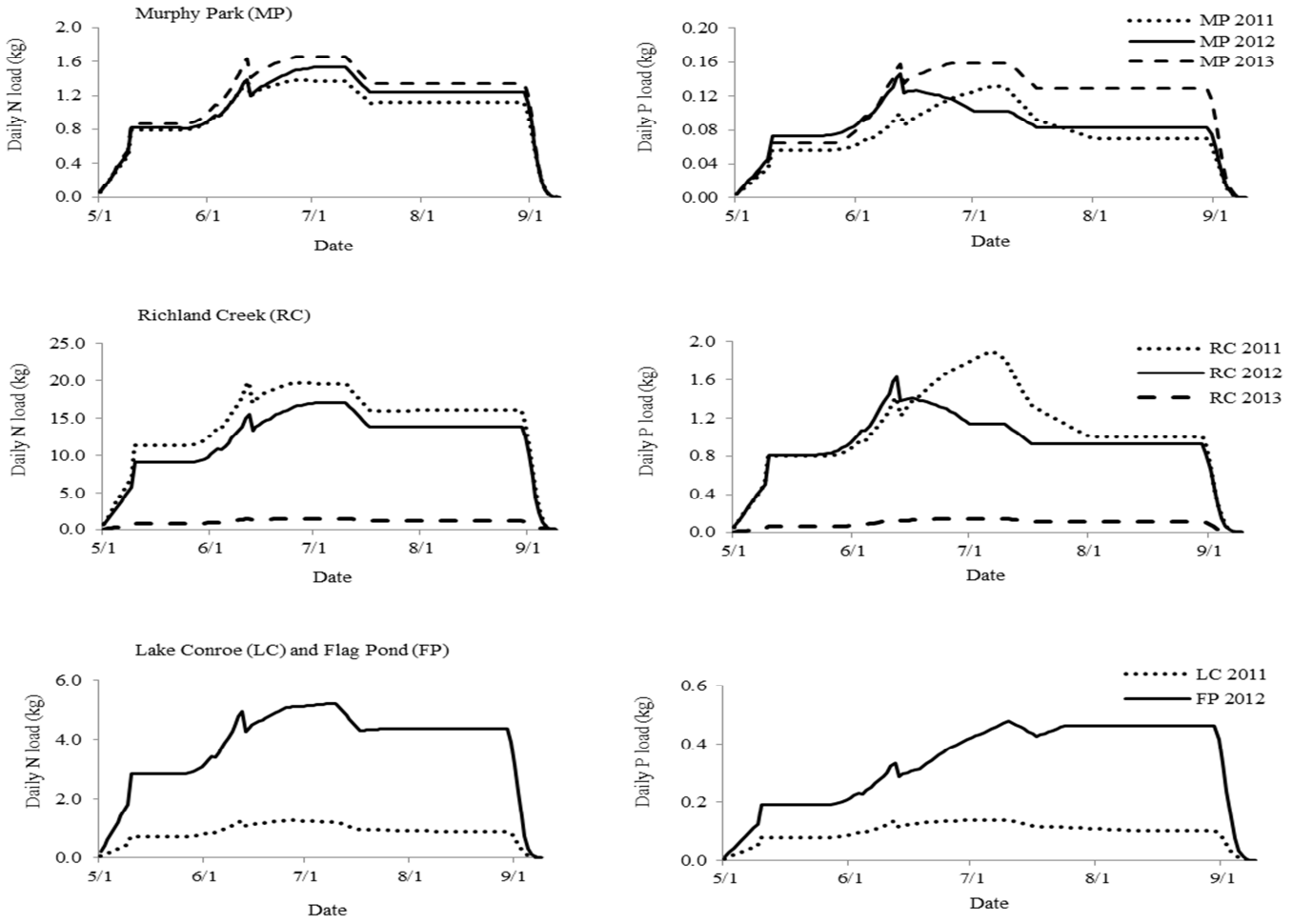


Figure 20: Simulated daily loads of N and P deposited by cattle egrets in each of the four study sites during the indicated years.

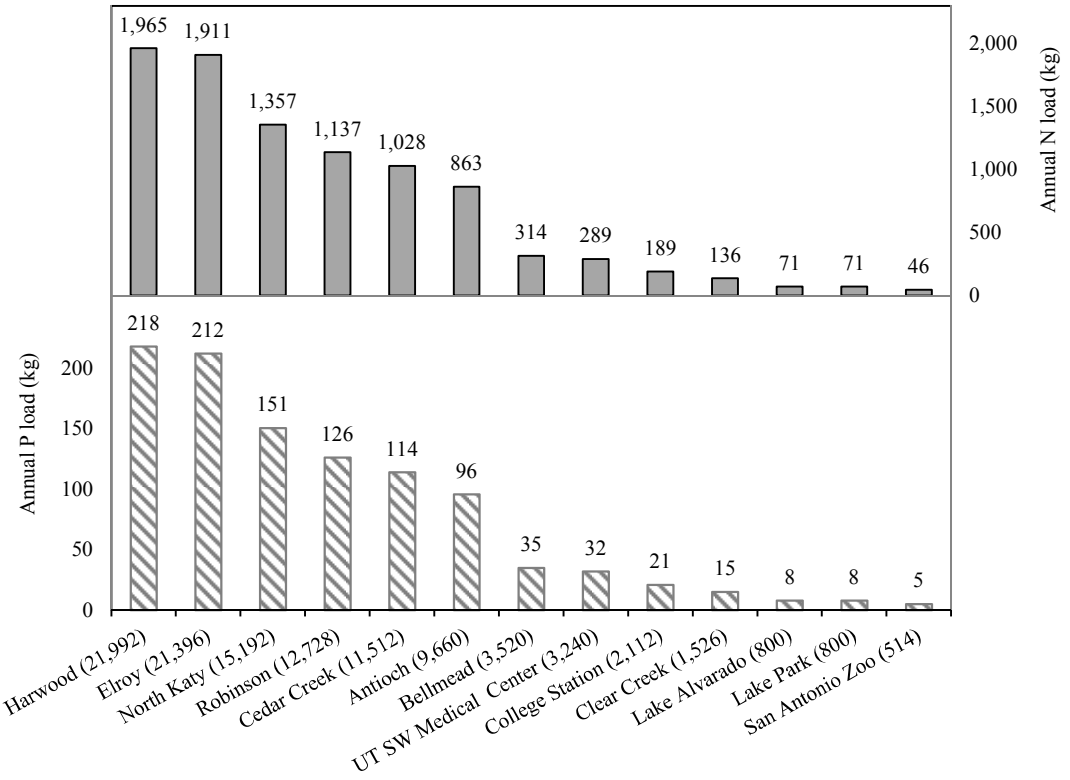


Figure 21: Simulated annual loads (kg) of N and P deposited in each of the other 13 heronries in Texas (Figure 14) for which estimates of heronry size (parenthetical values) were available (Parkes, 2007).

DISCUSSION

The results obtained in this study indicated that amounts N and P deposited by cattle egrets in their heronries depend primarily on size of the heronry, and suggested that amount of nutrient loading of nearby water bodies depends primarily on location of the heronry. Highest N and P concentrations were found in water samples collected at the two larger heronries (Flag Pond and Richland Creek), both of which also were located directly over water. At the two heronries located on islands (Murphy Park and Lake Conroe), higher N and P concentrations were found in water samples collected adjacent

to the larger heronry (Murphy Park). Differences in N and P concentrations in water samples collected at the two island heronries also may have been influenced to some degree by differences in sizes of the adjacent water bodies and differences in precipitation received during the study period. The smaller heronry was in a much larger lake and also received relatively more precipitation, particularly during the 2011 breeding season (approximately 5.1 cm at Lake Conroe vs. 1.3 cm at Murphy Park, 2011 was the hottest and driest 12- month period on record for the State of Texas (Hoerling et al. 2013, NOAA 2014).

Similar studies focused on relating size of cattle egret heronries to amounts of nutrient deposition, were not identified during my review of the literature. However, there have been several studies focused on geese and other types of waterfowl (Bildstein et al. 1992, Post et al. 1998). One such study estimated that annual nutrient loading from migrating waterfowl, mainly geese, increased from 5,540 kg N and 700 kg P (1991-1992) to 8,780 kg N and 1,090 kg P (1995-1996) as waterfowl populations increased to over 40,000 (Post et al. 1998). A second study estimated annual N and P loads contributed by ~ 10,700 geese and ducks to a lake in Michigan at 280 and 88 kg, respectively (Chastain et al. 2001, Manny et al. 1994). For comparison, the simulated N and P loads contributed by ~ 9,660 cattle egrets at Antioch, one of the 13 heronries for which N and P loads were generated, were 863 and 96 kg, respectively. Although the heronry at Antioch was smaller, the simulated N and P loads were greater than the amounts estimated for geese by Manny et al. (1994). Yet another study estimated N and

P loads deposited by 25,946 white ibis into an inlet estuary in South Carolina during the 1994 breeding season at 636.3 and 223.9 kg, respectively (Bildstein et al. 1992). At Richland Creek in 2011, the heronry size was estimated to be ~ 20,000 birds with estimated annual N and P loads at 1,884 and 140 kg, respectively. In this case, the simulated N load at the somewhat smaller Richland Creek heronry was almost three times larger; however, the simulated P load was noticeably smaller.

Variations in N and P loads among different species result in part from differences in diet, which cause differences in the nutrient content of feces (Alderisio and DeLuca 1999). Cattle egrets are omnivorous, feeding mainly on grasshoppers and other insects but also consuming fish, frogs, and small animals (Telfair II 1983), whereas geese are mainly herbivorous. Marion et al. (1994), after studying the N and P content of several species of birds including cormorants, herons, gulls, and starlings (*Sturnus vulgaris*), concluded that the feces of cormorants and herons contained the highest concentrations of N and that the P content was over ten times higher than the other species in the study.

Studies focused on the influence of location of cattle egret heronries on amount of nutrient loading in aquatic systems due to fecal deposition (per se) were also not identified. However, one study reported that organic materials falling into the water directly under a cattle egret heronry, not including feces but including the ~ 85% of cattle egret eggs that did not survive, were responsible for increasing organic N and P by 2 to 9 and 2 to 6 times, respectively, compared to a non-colonized marsh area (Dusi et al.

1971). Another study reported that direct fecal deposition, in addition to eggs and dead birds, accounted for much higher N and P concentrations in water surrounding a colony of Franklin's gulls nesting in cattails (McColl and Burger 1976). Dead birds and unhatched eggs were also observed under each heronry that was investigated in this study. In addition, Phalen et al. (2010) reported the occurrence of dead birds at cattle egret heronries. These conditions probably explain the extremely high N concentrations observed at Flag Pond. The part of the pond directly associated with the heronry appeared to be filled with decaying organic matter. Flag Pond is a seasonal wetland that had only started collecting water about a month prior to colonization by the birds; the high levels of N and P could therefore be attributed to decaying organic matter. The water was dark brown and a large number of dead birds were observed as well as several small fish and frogs that appeared to be left over and/or regurgitated food from the birds. Although a larger number of birds were observed at Richland Creek (2012) compared to Flag Pond, concentrations of N and P were higher at Flag Pond than at Richland Creek probably because there was a greater density of birds at Flag Pond.

Cattle egrets often act as "nutrient loaders," importing nutrients from terrestrial to aquatic habitats (Kitchell et al. 1979, Leentvaar 1967, McColl and Burger 1976, Vanni 2002), thus increasing nutrient loads, and stimulating primary production (Boros et al. 2008, Carvalho et al. 2012, Vanni 2002). At the two heronries that were located over water (Flag Pond and Richland Creek); large areas of duckweed (*Lemna minor L.*) were observed. The association of large areas of duckweed with cattle egret heronries located

over water also has been documented by others (Dusi et al. 1971, Stinner 1983). Among the other thirteen heronries for which loads of N and P were estimated, five were located over water in artificially flooded vegetation (Table 5; North Katy, Robinson, Antioch, College Station, and Clear Creek). Three of these had an estimated density of adult birds sufficiently high to be areas of concern with regard to potential impacts on water quality (Table 5; North Katy > 1/ m^2, Robinson > 1/ m^2, Clear Lake > 2/ m^2) since it is anticipated that higher N and P loads will be deposited directly into the water resulting in higher nutrient concentrations.

In Texas, water quality screening levels for P are 0.69 mg/L for freshwater streams and 0.20 mg/L for reservoirs (SWQM 2012). P limits primary production more than N in most freshwater systems (McColl and Burger 1976, Schindler 1977). Concentrations of P in all the water samples in this study exceeded 0.20 mg/L, with the exception of one year at each of the two island heronries (Murphy Park in 2013 and at Lake Conroe in 2011). Concentrations of P in water samples that were collected at the two heronries located over water exceeded 0.69 mg/L by as much as seven (Richland Creek) and five (Flag Pond) times. Phosphorus is correlated positively with chlorophyll-a levels in phytoplankton (Carvalho et al. 2012, Dillon and Rigler 1974, Schindler 1977), the nutrient-related criteria currently used in Texas for over 70 reservoirs. Water quality screening levels for N (NH_3-N + NO_3-N) are 2.28 mg/L for freshwater streams and 0.48 mg/L for reservoirs (SWQM 2012). Concentrations of N in all the water samples that were collected exceeded 0.48 mg/L (by as much as seven times at Murphy Park) at each

of the two island heronries (Murphy Park and Lake Conroe). Concentrations of N in water samples that were collected at the two heronries located over water exceeded 2.28 mg/L by as much as eight (Richland Creek) and 274 (Flag Pond) times.

Several authors report positive correlations between low N:P ratio (< 29:1) and changes in species composition of phytoplankton (Havens et al. 2003, Smith 1983, Tilman et al. 1982). Ratios of N:P less than 10:1 indicate that N is limiting (Borchardt 1996) and phytoplankton such as cyanobacteria (*Cyanophycota. spp*.), which are able to fix nitrogen, are better adapted to live under such conditions (Havens et al. 2003, Smith 1983). Low N:P ratios are common among streams that receive effluent with high levels of phosphorus from sources such as agricultural runoff and wastewater (Hem 1991, Welch and Lindell 2002). A study conducted on fifteen small streams in the Edwards Plateau, Texas reported that N:P ratios from streams receiving wastewater effluent ranged from 0.6:1 to 7:1, whereas streams that did not receive wastewater effluent had N:P ratios ranging from 35:1 to 558:1 (Mabe 2007). According to that study, N and P concentrations for the least disturbed streams in the Edwards Plateau were estimated at 0.265 and 0.003 mg/L, respectively (Mabe 2007). Low N:P ratios for the heronries over water (Richland Creek and Flag Pond) were observed. At Richland Creek, ratios ranged from 3:1 to 5:1, and at Flag Pond, the ratio was 13:1. Ratios at the island heronries (Murphy Park and Lake Conroe) were more variable. At Murphy Park, N:P ratios ranged from 6:1 to 12:1 during the 2011 and 2012 breeding seasons, and from 20:1 to 62:1 during the 2013 breeding season. At Lake Conroe, ratios ranged from 13:1 to 40:1.

This study provides empirical estimates of the N and P loads deposited at each of four cattle egret heronries, as well as estimates based on the simulation model of loads deposited at thirteen other heronries, in Texas. The model will be useful for providing preliminary estimates of fecal N and P loads from heronries located in watersheds where nutrient concentrations are listed of concern or as impairments in states' Integrated Reports of Surface Water Quality.

CHAPTER IV

AN EVALUATION OF THE CONTRIBUTION OF MACRO AND MICROELEMENTS FROM FECES OF COLONIAL NESTING WATERBIRDS TO SURFACE WATER

SYNOPSIS

Macro and microelements contained in feces of cattle egrets (*Bubulcus ibis*) and other birds nesting in heronries in Texas can be sources of contamination of nearby waterways as well as sources of nutrients to associated soils. Concentrations of macroelements potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), sulfur (S) and the microelements zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), and boron (B) were measured in water and fecal samples collected from four locations containing heronries during the breeding seasons of 2011, 2012, and 2013. Concentrations of K, Ca, Mg, Na, and S in feces were several orders of magnitude higher than in water samples. Concentrations of K in feces ranged from $8.19 \times 10^3 \pm 4.38 \times 10^2$ mg/kg (Murphy Park (MP), Aug 2013) to $4.88 \times 10^4 \pm 7.57 \times 10^2$ mg/kg (Flag Pond (FP), June 2012) while concentrations in water ranged from 3.92 ± 0.05 mg/L (MP, May 2013) to 17.93 ± 0.37 mg/L (Richland Creek (RC), July 2011). Similarly, concentrations of Ca in feces ranged from $4.17 \times 10^3 \pm 1.84 \times 10^3$ mg/L (MP, Aug 2013) to $1.16 \times 10^4 \pm 4.14 \times 10^3$ mg/L (Lake Conroe (LC), Aug 2011) while concentrations in water ranged from 25.28 ± 0.89 mg/L (FP, June 2012) to 67.88 ± 2.02 mg/L (RC, June 2013). When birds nested directly over water, concentrations of K, Ca, and Mg in water were significantly higher

($p < 0.05$) than concentrations in water adjacent to birds nesting on islands. The results from this study show that macroelements from avian feces have the potential of enriching both soil and surface water, which can negatively affect surface water quality similar to nitrogen (N), and phosphorus (P). These results provide information regarding the contribution of nutrients from avian heronries dominated by cattle egrets to watersheds. Such data can be beneficial to water quality management and modeling of surface water quality.

INTRODUCTION

For many years, cattle egrets (*Bubulcus ibis*) and similar species of birds have established large nesting heronries often containing thousands of birds, in east and central Texas (Parkes 2007, Telfair II 1983, 1993). Heronries are often established in upland woodlands, swampy areas with submerged trees, islands that are located inland, and islands located in coastal areas, the majority in close proximity to water (Telfair II 1994). Because of the large number of birds and proximity to water, large amounts of nutrient-rich feces are often deposited in nesting areas or directly into nearby waterways (Chaichana et al. 2010, Hussong et al. 1979, Moss and Leah 1982, Scherer 1995). Other sources of nutrients from bird colonies to the soil and water include feathers, eggshells, unhatched eggs, food remnants, and carcasses (Ellis 2005, Smith and Froneman 2008). Numerous studies have reported substantial fecal deposition in nesting areas and increased nutrient content in the soil (Anderson and Polis 1999, García et al. 2002b, Ligeza and Smal 2003, Litaor et al. 2014) and water (Baxter and Fairweather 1994,

Bedard et al. 1980, Gremillion and Malone 1986). Nutrients are required by plants and animals in large and small quantities for growth and development.

Macroelements are required in large amounts by animals and include nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), and sulfur (S) (Epstein 1965). Nitrogen is a major cellular constituent and like P, it is necessary for the growth of organisms and the productivity of fresh water systems (Wetzel 2001). Calcium is important for the growth and population dynamics of fresh water flora and fauna (Wetzel 2001). Magnesium is required by chlorophyll-containing plants. It is more soluble and found in higher concentrations in water relative to Ca (Otsuki and Wetzel 1974, Wetzel 2001). Both Na and K are required for ion transport and exchange (Maier et al. 2009, Wetzel 2001).

Microelements are required in smaller amounts and include zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), and boron (B) (Epstein 1965). Iron is common in many rocks and is an important component of many soils. Iron is required by both plants and animals and may be a limiting factor for the growth of algae and plants in some waters (USEPA 1986). Copper is naturally occurring and is generally present in surface waters (Nriagu 1979, USEPA 1986). At low concentrations, Cu is necessary for the growth of both plants and animals; however, in higher concentrations, it may become toxic to aquatic life (Kapustka et al. 2004). Small amounts of manganese and boron are also

vital for the growth of both plants and animals (Kopp and Kroner 1968, USEPA 1986), but there is no evidence that boron is required by animals.

Excessive amounts of nutrients from colonial birds cause changes in plant biomass (Anderson and Polis 1999) as well as changes within plant communities and biodiversity (Ellis 2005, Ellis et al. 2011, Mulder et al. 2011, Żółkoś and Meissner 2008). In addition, other soil parameters such as soil humidity, conductivity, and respiration rate have been affected by nutrient-rich avian feces (Anderson and Polis 1999, Ellis et al. 2006, Wait et al. 2005).

There are several studies regarding N and P enrichment associated with feces from colonial waterbirds (Chaichana et al. 2010, Hussong et al. 1979, Scherer 1995); however, other than poultry (Chastain et al. 2001, Zublena et al. 1990), studies that report other macro and microelements in feces of wild birds or their contributions to surface water quality were not identified. The objective of this study was to evaluate the contribution of macro and microelements in feces from cattle egret-dominated heronries and their contributions to watersheds in Central Texas over a three-year period.

Study Sites

Four heronries (Murphy Park, Lake Conroe, Flag Pond, and Richland Creek) were investigated during the breeding seasons of 2011, 2012, and 2013 (Figure 22). Each of the four heronries contained several species of birds including cormorants, primarily

Neotropical, (*Phalacrocorax brasilianus)*, great egrets (*Ardea alba*), snowy egrets (*Egretta thula*), little blue herons (*Egretta caerulea*), and anhingas (*Anhinga anhinga*). However, the most common species was the cattle egret, comprising at least 90% of the birds in each colony. Two of the four heronries were located on shrubs and trees on islands and two were located on shrubs and trees with roots and trunks in water. The population of birds at each heronry was estimated by counting the number of breeding pairs visible from a fixed point at the water's edge and extrapolating this number to the estimated area of the heronry following the methodology of Gregory et al. (2004).

Murphy Park (MP) is a city park located in Taylor, Texas. The heronry was located on a small island in an 809-m^2 pond (Muddy Lake) within the park (N 30.5809, W 97.4131) (Figure 22). The size of the heronry ranged from approximately 700 pairs (in 2011) to 900 pairs (in 2013). The reference site for this location was Bull Branch Pond and was located upstream (N 30.5871, W 97.4222) from MP. The other island heronry, Lake Conroe (LC) was on an 80.9 km^2 Lake in Montgomery County, Texas managed by the San Jacinto River Authority. The heronry was located on a small island in the lake (N 30.4035, W 95.5750) (Figure 22). The size of the heronry was approximately 600 nesting pairs in 2011.

The heronry at Richland Creek (RC) was located in a tributary to the Trinity River, part of a wildlife management area in Freestone County, Texas (N 31.9906, W 96.1005) (Figure 22). Nests were on trees and shrubs with trunks and roots in water. The size of

the heronry ranged from approximately 10,000 in 2011 and 2012 to 800 nesting pairs in 2013. The reference site for this heronry was located upstream (N 31.9921, W 96.0981) from the Richland Creek heronry and was sampled only once in 2012. The fourth heronry, Flag Pond, was located on a flooded area that was part of a 1.4 km^2 seasonal wetland in Lee County, Texas (N 30.3063, W 96.6976) (Figure 22). The size of the heronry was approximately 3,000 nesting pairs in 2012. The reference site for Flag Pond was Horse Pond and was located within the Nails Creek State Park (N 30.2886, W 96.6688).

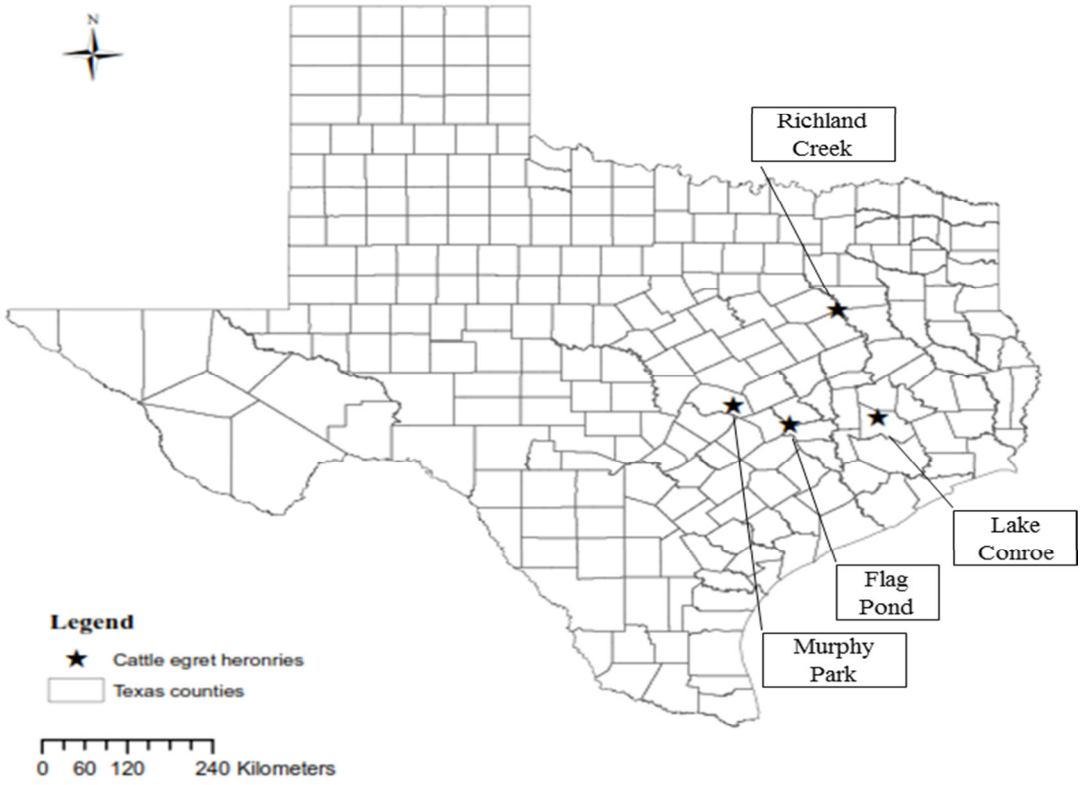


Figure 22: Map showing locations of cattle egret heronries sampled during the 2011 and 2012 breeding seasons.

MATERIALS AND METHODS

Field Sampling and Lab Analysis

During the 2011 breeding season, water and fecal samples were collected monthly (June to August) from Murphy Park and Lake Conroe and in July at Richland Creek. During the 2012 breeding season, water and fecal samples were collected monthly (May to July) from Murphy Park and water samples from Richland Creek. At Flag Pond, both fecal and water samples were collected in June but only fecal samples were collected in July, since the site was drained by park management. During the 2013 breeding season, water and fecal samples were collected twice per month (May to August) from Murphy Park and water samples were collected once per month from Richland Creek.

Water samples were collected from two ends of the island heronries (Murphy Park and Lake Conroe), and from areas directly under the heronries that were located over water (Flag Pond and Richland Creek). Water samples were collected at a central location of each reference site. Sterile 250 mL polypropylene screw-cap bottles were used to collect and store all water samples. Fecal samples were collected from several trees and/or nests, using sterile forceps and/or syringes, at various locations within the heronry. Samples were also collected with plates (lined with wax paper) mounted on tripods, that were placed under trees with a high density of nests. Samples were placed in 50 mL sterile polypropylene tubes and stored along with the water samples, at approximately 4℃ until laboratory analysis.

The Texas A&M AgriLife Extension Service Soil, Forage and Water Testing Laboratory at Texas A&M University analyzed water samples for inorganic elements (Ca, Mg, K, Na, S, Zn, Fe, Cu, Mn, and B) using the Kjeldahl digestion method (Parkinson and Allen 1975). Fecal samples were analyzed for minerals using the methods described in Havlin and Soltanpour 1980 and Sheldrick 1986.

Data Analysis

Because of the relatively small number of sample, inequality of variance and non-normal distributions of data, non-parametric statistical analyses were utilized. The Wilcoxon rank sum test was used when comparing two groups (ex. testing for differences in elemental concentrations in water samples from LC and MP during the 2011 breeding season). The Kruskal-Wallis test was used when comparing more than two groups (ex. testing for differences in elemental concentrations in water samples collected at MP May, June, and July of the 2012 breeding season). In addition, the Steel-Dwass test (Conover 1999), the non-parametric version of the Tukey's HSD was performed to test for significant differences between pairs of results. In instances where the Kruskal - Wallis test indicated significant differences within the group but the Steel-Dwass test did not indicate which pairs were significantly different, the Bonferroni correction was utilized to retest the significance of the p-value (Bland and Altman 1995). If the original p-value was less than the corrected or modified p-value, it was concluded that the difference was significant. In all statistical analyses, P-values of < 0.05 were considered statistically significant.

To determine the effects of micro and macroelements on water quality the results in this study were compared to the aquatic life criteria for elements set by the Environmental Protection Agency (EPA). JMP Pro 11.0.0 ® and R version 3.1.0 ® software were used for statistical analyses.

RESULTS

There were no differences in concentrations of any of the macro and microelements in fecal and water samples collected during each breeding season, at each heronry, (ex. all fecal samples analyzed in 2011 at MP). However, concentrations K, Ca, Na, Fe, Cu, Mn, and B in water and fecal samples varied significantly ($p < 0.05$) when comparing them across heronries (ex. MP and LC in 2011). Overall, K was present at the highest concentrations in feces while Ca was the highest in water. In addition, concentrations of K, Ca, and Mg in water samples collected directly under the heronry at RC were significantly higher than concentrations in water samples not receiving fecal material directly. Of the microelements, Fe was present at the highest concentrations in both water and fecal samples (Figures 23 to 26, Appendices E and F).

Potassium

Concentrations of K in fecal samples ranged from $8.19 \times 10^3 \pm 4.38 \times 10^2$ mg/kg (MP, Aug 2013) to $4.88 \times 10^4 \pm 7.57 \times 10^2$ mg/kg (FP, June 2012). In fecal samples collected at Murphy Park, concentrations of K significantly increased ($p = 0.0038$) between 2011 and 2012 but significantly decreased ($p = 0.0003$) between 2012 and 2013.

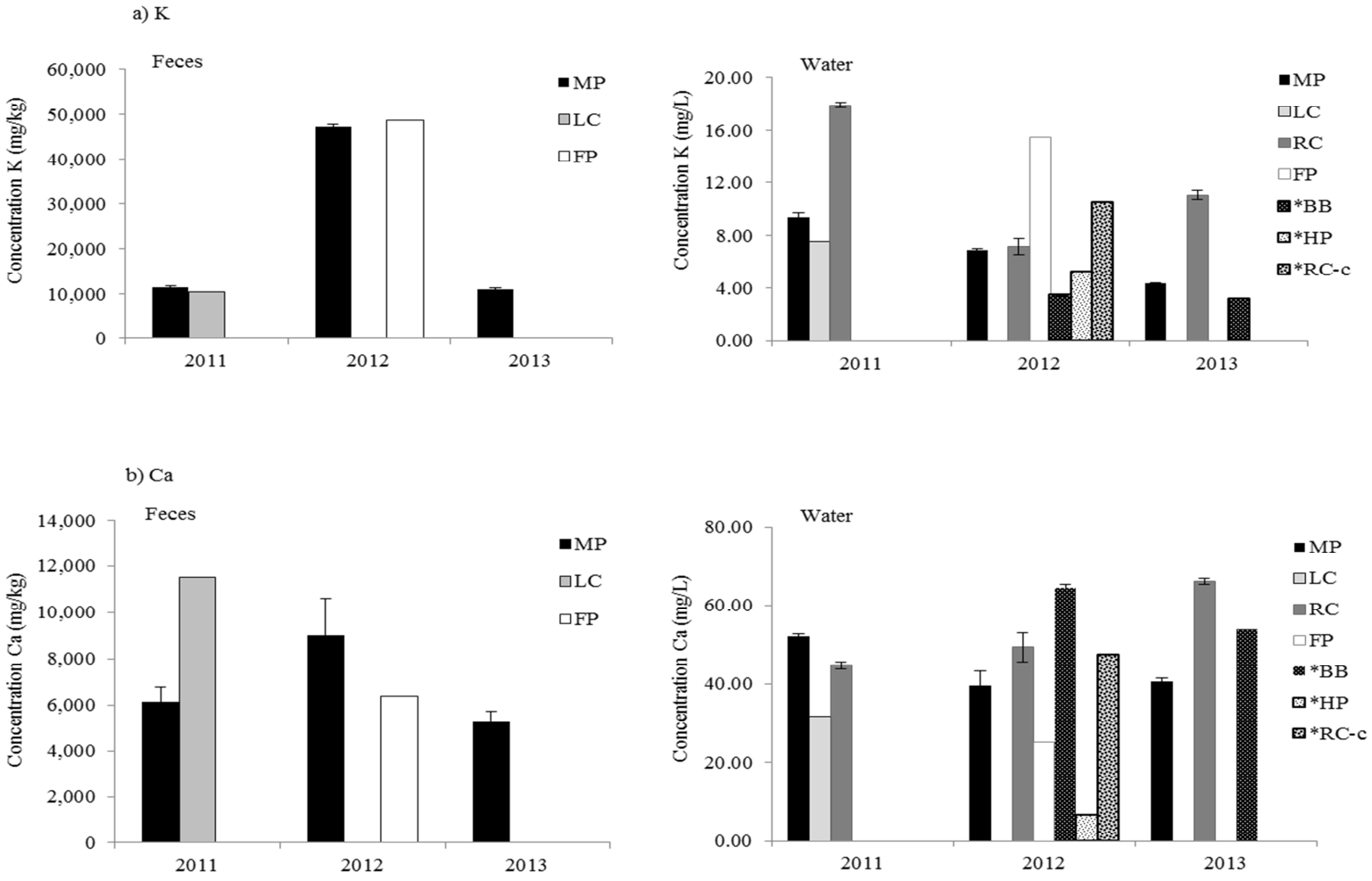


Figure 23: Concentrations of K and Ca from fecal and water samples collected at the study sites. (MP = Murphy Park, LC = Lake Conroe, RC = Richland Creek, FP = Flag Pond). Error bars represent the standard error of the mean. * = reference sites.

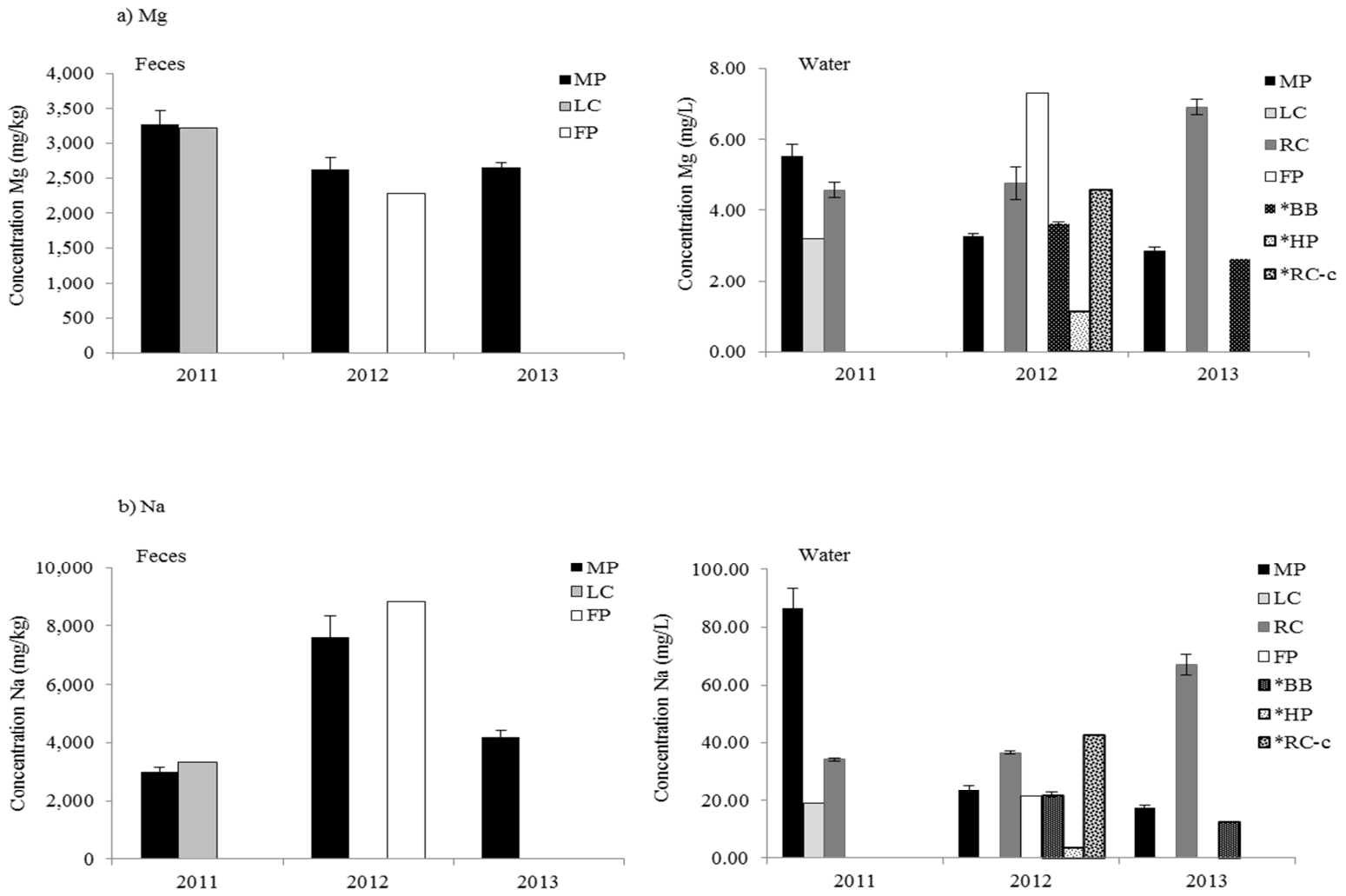


Figure 24: Concentrations of Mg and Na from fecal and water samples collected at the study sites.

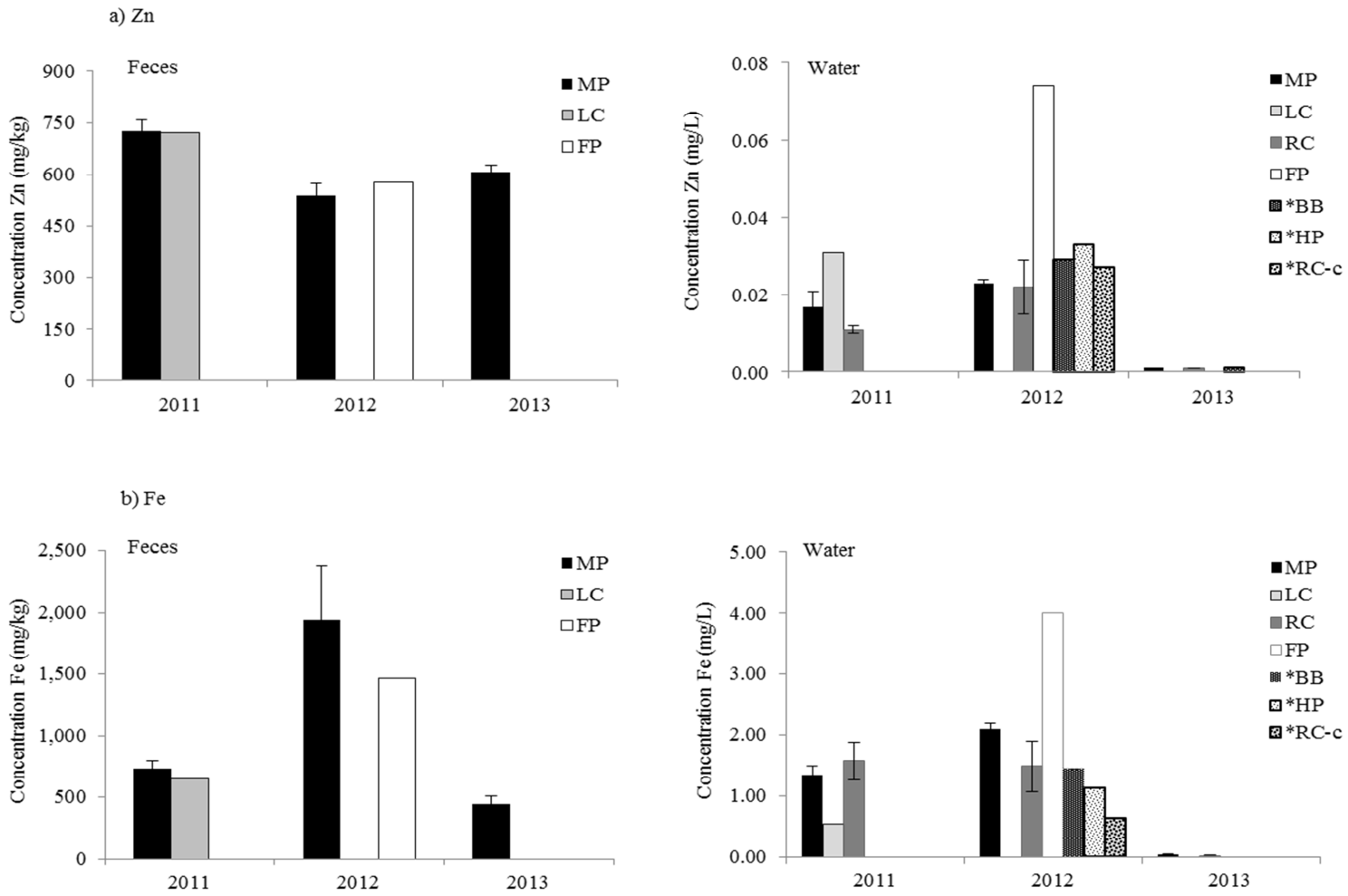


Figure 25: Concentrations of Zn and Fe from fecal and water samples collected at the study sites.

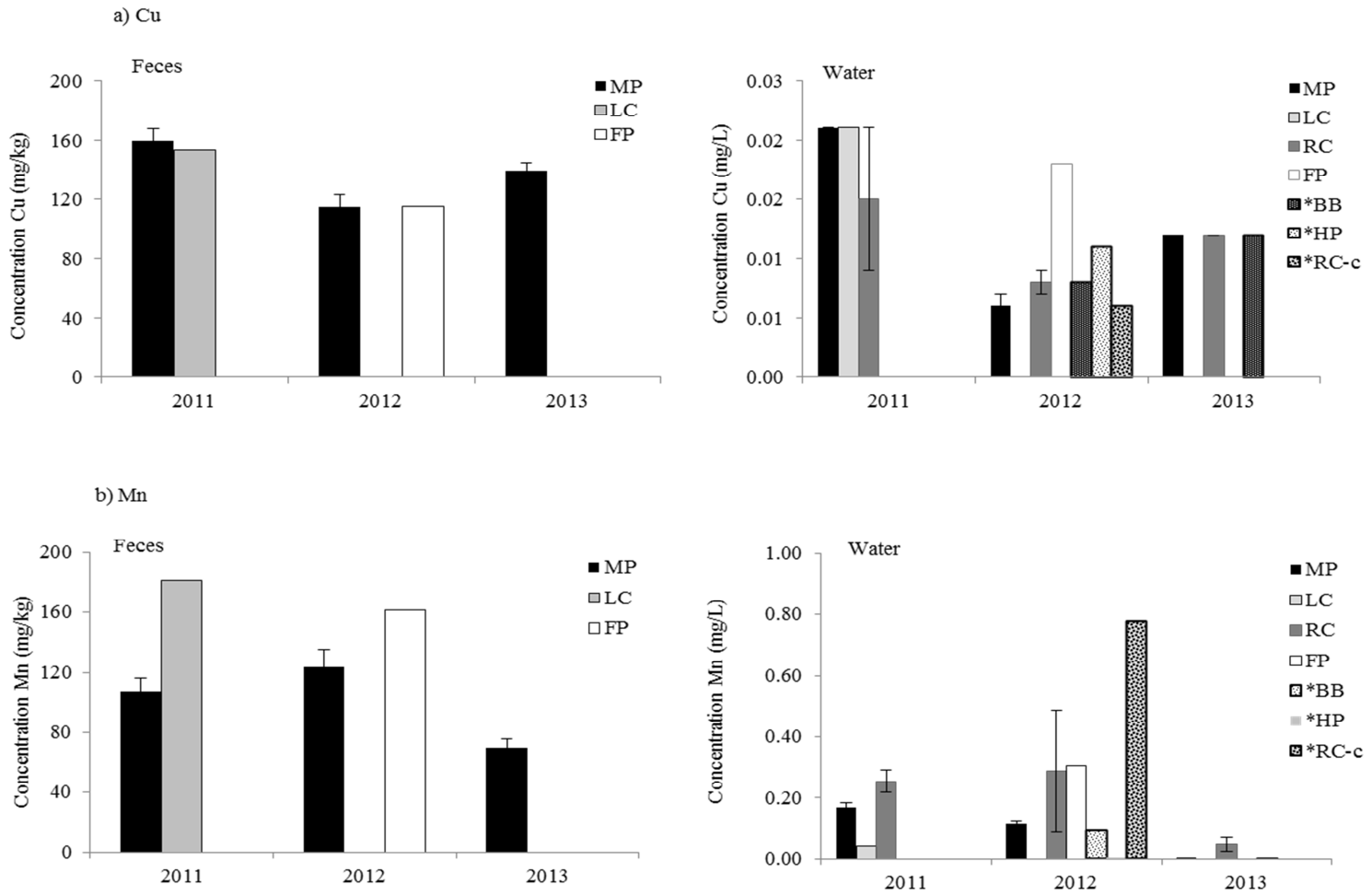


Figure 26: Concentrations of Cu and Mn from fecal and water samples collected at the study sites.

Concentrations of K in water samples ranged from 3.92 ± 0.05 mg/L (MP, May 2013) to 17.93 ± 0.37 mg/L (RC, July 2011). Concentrations of K in water samples from Richland Creek were significantly higher ($p = 0.0003$) than concentrations at Murphy Park during 2013. In addition, concentrations of K were significantly lower ($p < 0.0001$) in water from the reference site, Bull Branch than at Murphy Park (Figure 23 part a).

Calcium

Concentrations of Ca in fecal samples ranged from $4.17 \times 10^3 \pm 1.84 \times 10^3$ mg/kg (MP, Aug 2013) to $1.16 \times 10^4 \pm 4.14 \times 10^3$ mg/kg (LC, Aug 2011). Concentrations of Ca in fecal samples from Lake Conroe were significantly higher ($p = 0.0129$) than concentrations in samples from Murphy Park during 2011. Higher Ca concentrations were measured in feces from the Lake Conroe heronry in 2011 compared to Murphy Park; however, concentrations in water samples from Lake Conroe were significantly lower ($p < 0.0001$) than concentrations in water from Murphy Park that same year. Calcium concentrations in water ranged from 25.28 ± 0.89 mg/L (FP, June 2012) to 67.88 ± 2.02 mg/L (RC, June 2013). Concentrations of Ca in water from Richland Creek were significantly higher than concentrations in water from Murphy Park in 2012 ($p = 0.0259$) and 2013 ($p = 0.0003$). In addition, concentrations of Ca were significantly higher ($p < 0.0001$) in water from Bull Branch than at Murphy Park (Figure 23 part b).

Magnesium

Concentrations of Mg in fecal samples ranged from $2.02 \times 10^3 \pm 2.70 \times 10^2$ mg/kg (FP, June 2012) to $3.76 \times 10^3 \pm 3.84 \times 10^2$ mg/kg (MP, July 2011). In fecal samples collected at Murphy Park, concentrations of Mg significantly decreased ($p = 0.0405$) between

2011 and 2013. Concentrations of Mg in water ranged from 2.06 ± 0.02 mg/L (MP, Aug 2013) to 7.69 ± 0.63 mg/L (RC, Aug 2013) (Figure 24 part a). Concentrations from Lake Conroe water were significantly lower ($p = 0.0014$) than concentrations from Murphy Park in 2011. In addition, concentrations in water from Richland Creek were significantly higher than at Murphy Park in 2012 ($p = 0.0058$) and 2013 ($p = 0.0003$).

Sodium

Concentrations of Na in fecal samples ranged from $2.47 \times 10^3 \pm 3.22 \times 10^2$ mg/kg (LC, July 2011) to $8.89 \times 10^3 \pm 6.88 \times 10^2$ mg/kg (FP, June 2012). In fecal samples collected at Murphy Park, concentrations of Na significantly increased ($p = 0.0487$) between 2011 and 2012, but significantly decreased ($p = 0.0114$) between 2012 and 2013. In water samples, concentrations of Na ranged from 9.23 ± 0.57 mg/L (MP, Aug 2013) to 115.80 ± 5.01 mg/L (MP, Aug 2011) (Figure 24 part b). Concentrations of Na in water from Lake Conroe were significantly lower ($p < 0.0001$) than concentrations in water from Murphy Park in 2011. Significantly lower Na concentrations were also found in water from Murphy Park compared to water from Richland Creek in 2012 ($p = 0.0025$) and 2013 ($p = 0.0003$).

Sulfur

Fecal samples were analyzed for S in samples collected during 2012 and 2013 and concentrations ranged from $5.25 \times 10^3 \pm 1.05 \times 10^3$ mg/kg (MP, July 2012) to $7.11 \times 10^3 \pm 3.40 \times 10^2$ mg/kg (MP, May 2013). Sulfur was analyzed in water samples collected during 2013 and concentrations ranged from 2.22 ± 0.07 mg/L (RC, Aug 2013) to 7.85 ±

0.81 mg/L (MP, June 2013). These data are not presented graphically, but are available in Appendices E and F.

Zinc

Concentrations of Zn in fecal samples ranged from $4.91 \times 10^2 \pm 3.40 \times 10^1$ mg/kg (MP, May 2013) to $8.05 \times 10^2 \pm 1.61 \times 10^2$ mg/kg (LC, June 2011). In fecal samples collected at Murphy Park, concentrations of Zn significantly decreased ($p = 0.0380$) between 2011 and 2012. Concentrations of Zn in water remained relatively low and ranged from < 0.001 mg/L (MP, 2013) to 0.09 ± 0.14 mg/L (LC, June 2011) (Figure 25 part a).

Iron

Concentrations of Fe in fecal samples ranged from $3.36 \times 10^2 \pm 2.35 \times 10^1$ mg/kg (MP, May 2013) to $2.28 \times 10^3 \pm 2.00 \times 10^3$ mg/kg (MP, July 2012). In fecal samples collected at Murphy Park, concentrations of Na significantly increased ($p = 0.0096$) between 2011 and 2012 but significantly decreased ($p = 0.0015$) between 2012 and 2013. In water samples, concentrations of Fe ranged from < 0.002 mg/L (RC, June 2013) to 4.00 ± 1.90 mg/L (FP, June 2012) (Figure 25 part b) and concentrations from Lake Conroe water were significantly lower ($p = 0.0053$) than concentrations from Murphy Park, during 2011. In addition, concentrations of Fe were significantly lower ($p = 0.0089$) in water from Bull Branch than at Murphy Park in 2013.

Copper

Concentrations of Cu in fecal samples ranged from $9.90 \times 10^1 \pm 1.31 \times 10^1$ mg/kg (FP, June 2013) to $1.84 \times 10^2 \pm 1.75 \times 10^1$ mg/kg (MP, Aug 2011) and did not vary significantly throughout the study period. However, concentrations of Cu in water

remained relatively low and ranged from < 0.003 mg/L (LC, Aug 2011) to 0.02 ± 0.01 mg/L (FP, June 2012) (Figure 26 part a). Concentrations of Cu in water from Lake Conroe were significantly higher ($p = 0.0039$) than concentrations from Murphy Park during 2011.

Manganese

Concentrations of Mn in fecal samples ranged from $4.16 \times 10^{1} \pm 1.33 \times 10^{1}$ mg/kg (MP, Aug 2013) to $2.53 \times 10^{2} \pm 1.57 \times 10^{2}$ mg/kg (LC, June 2011). In water samples, concentrations of Mn ranged from < 0.001 mg/L (MP, May to July 2013) to 0.72 ± 1.01 mg/L (RC, July 2012) (Figure 26 part b). Concentrations of Mn from Lake Conroe were significantly lower ($p < 0.0001$) than concentrations from Murphy Park in 2011. In addition, significantly higher ($p = 0.0221$) concentrations of Mn were found in water samples from Richland Creek compared to water from Murphy Park, in 2013.

Boron

Fecal samples were analyzed for B in samples collected during 2012 and 2013 and concentrations ranged from $2.18 \times 10^{0} \pm 8.00 \times 10^{-1}$ mg/kg (MP, Aug 2013) to $3.85 \times 10^{1} \pm 3.16 \times 10^{0}$ mg/kg (FP, July 2012). Concentrations of B in fecal samples collected during 2012 were significantly higher at Murphy Park ($p = 0.0003$) and Flag Pond ($p = 0.0033$) compared to 2013. Boron was analyzed in water samples collected during 2013 and concentrations ranged from 0.03 ± 0.01 mg/L (RC, June 2013) to 0.08 ± 0.03 mg/L (MP, July 2013). These data are not presented graphically but are available in the Appendices E and F.

DISCUSSION

The results of this study show that feces of colonial waterbirds contribute significant concentrations of elements or nutrients to watersheds in addition to N and P. These elements can be beneficial to both aquatic and terrestrial ecosystems. As indicated in the analysis of N and P loads in the previous chapter, the amount of nutrient loading by herons on nearby water bodies depends on location of the heronry. Highest concentrations of K, Ca, and Mg were found in water samples collected at the two larger heronries (Flag Pond and Richland Creek), both of which also were located directly over water. At Murphy Park, higher Na and S concentrations were found in water samples compared to Lake Conroe. Higher concentrations of Zn, Fe, Cu, and Mn were found in water collected at Flag Pond compared to Richland Creek. At reference sites, concentrations of all elements in water samples from Horse Pond were lower than concentrations measured at Flag Pond. However, lower concentrations of K, Fe, Mn but higher concentrations of Ca were found in water samples from Bull Branch compared to Murphy Park. At the reference site for Richland Creek, lower concentrations of Fe and Cu but higher concentrations of K and Mn were found in water samples compared to samples collected at Richland Creek.

Avian feces can be major contributors of elements to both soil and water. Zwolicki et al. (2013) reported that planktivore and piscivore birds affected adjacent tundra soil in different ways with significantly higher P and pH values of soil influenced by piscivores compared to planktivores. Piscivorous birds (cormorants and herons) contributed

significant K enrichment to soils associated with heronries (Ligeza and Smal 2003). Higher K concentrations have also been observed in soils associated with roosting omnivorous blackbirds (*Turdus merula*) (Gilmore et al. 1984). Various studies have reported increased amounts of some macronutrients in soils influenced by avian feces. García et al. (2002a), found twice the amount of K and Ca in soil enriched by feces and other materials from a nesting colony of Audouin's gulls (*Larus audouinii Payr.*) and yellow-legged gulls (*L. cachinnans Pallas*) compared to sites with no seagull activities. Breuning-Madsen et al. (2010) investigated the influence of piscivorous birds such as cormorants on soil nutrient contents and found that soils affected by cormorant feces contained higher Ca and P concentrations relative to the control sites, K and Ca were 30 and 15 times higher, respectively, than soils at control sites.

Many studies have reported the contribution of elements to soil by feces from avian species; however, there were not many studies describing the contribution of elements in avian feces to surface water. Stinner (1983) reported significant amounts of K and Ca in the feces of white ibises (*Eudocimus albus*) in the Okefenokee Swamp ecosystem in Southeast Georgia. One conclusion was that mean K and Ca concentrations in the surface water associated with the rookery were significantly higher than concentrations measured at the reference site (Stinner 1983). Bildstein et al. (1992) studied nutrient transport from terrestrial areas by white ibises into an estuary in South Carolina and concluded that compared to atmospheric sources, nutrient inputs from white ibises into the estuary could be substantial, but varied considerably among years. For poultry

manure (broiler, turkey and duck), Zublena et al. (1990) reported higher concentrations of Ca, Mn and Zn compared to other macro and microelements. Similarly, Chastain et al. (2001) reported higher concentrations of Ca, Zn, and Mn in chicken and turkey litter compared to other macro and microelements.

Contrary to these studies that reported significant increases of K and Ca from avian feces, other researchers found differing results. Brandvold et al. (1976) found that feces from waterfowl were responsible for increased amounts of K to a lake on a wildlife refuge, but Ca concentrations did not change. Leentvaar (1966) reported no increases in the concentration of K from feces deposited by black-headed gulls into an oligotrophic lake in the Netherlands. McColl and Burger (1976) reported nutrient inputs from feces of Franklin's Gulls (*Leucophaeus pipixcan*) nesting in Cattails in the Agassiz National Wildlife Refuge in Minnesota and found increased concentrations of N and P in the pool with gulls. However, there were no changes in the concentrations of Na, K, Ca and Mg in water (McColl and Burger 1976).

An aquatic life criterion lists the chemical concentration necessary to protect surface water quality (Website: http://water.epa.gov/scitech/swguidance/standards/criteria/index.cfm). There are no criteria for K, Ca, Mg, and Na and S. However, studies show that high Na concentrations are conducive to the growth of some species of cyanobacteria (Allen and Arnon 1955, Wetzel 2001). According to Kratz and Myers (1955), the threshold level for the optimum growth of several species of cyanobacteria is 4 mg/L and the maximum

growth was found at 40 mg/L. Concentrations of Na in all water samples from this study, except those collected at one reference site, were higher than 4 mg/L. Concentrations ranged from 9.23 to 115.80 mg/L. Such high concentrations of Na can influence the development of large populations of cyanobacteria. Studies also show that P enrichment in addition to Na enrichment could be a potential contributor to cyanobacteria bloom (Provasoli 1958, Ward and Wetzel 1975, Wetzel 1965). In Chapter IV, I reported that waters associated with the heronry sites were enriched with P.

For the microelements, the criterion for Zn is 47 µg/L (0.047 mg/L) as a 24 hour average and, according to the EPA, the concentration (in µg/L) should not exceed the numerical value given by 0.83 [ln(hardness)]+1.95) at any time (USEPA 1986). This value was only exceeded in samples collected from Lake Conroe (0.09 mg/L). According to Bowen (1985), naturally occurring concentrations of Cu in freshwater systems ranges from 0.20 to 30 µg/L. The highest concentration of Cu was measured at Flag Pond (0.02 mg/L). The criterion for iron is 1.0 mg/L for freshwater aquatic life (USEPA 1986). Iron concentrations in water samples from this study were quite variable and ranged from < 0.002 mg/L to 4.00 mg/L. Depending on the geology of the area and other chemical factors, iron in water is usually present in varying quantities (USEPA 1986). According to the EPA, the tolerance values of aquatic life for Mn range from 1.5 mg/L to over 1000 mg/L, but because manganese ions are found rarely at concentrations above 1 mg/L in surface water, it is not considered a problem in fresh waters (USEPA 1986). Mn concentrations in water samples from this study were less

than 1 mg/L. As stated by the EPA, naturally occurring boron should have no effects on aquatic life (USEPA 1986). However, the maximum concentration found in river and lake waters from various parts of the United States was 5.0 mg/L with mean concentrations of 0.1 mg/L (Kopp and Kroner 1968). The highest B concentrations measured in the water samples from this study was 0.11 mg/L.

This study provides new data that is related to the contribution of macro and microelements in the feces of cattle egrets and similar avian species to watersheds. These results are useful to determine to what extent aquatic birds, particularly colonial waterbirds, could affect water quality, in situations where breeding colonies are specifically established over standing water.

CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

In this dissertation, I assessed the avian contribution of *E. coli* and nutrient loads to watersheds. The study was made up of three components, the details of which were presented in three separate chapters. My first objective was to investigate the potential impact that large heronries may have on water quality in selected watersheds by quantifying *E. coli* loads originating from four heronries and comparing the sterol profile from water collected near or below heronries to the sterol profile from avian fecal material (primarily cattle egrets). My second objective was to provide estimates of the N and P loads deposited by cattle egrets and other herons and investigate the potential impact these heronries could have on associated watersheds. My final objective was to evaluate the contribution of macro and microelements in feces from cattle egret-dominated heronries and their contributions to watersheds over a three-year period. This final chapter serves to summarize the important findings from each chapter and synthesize the results of the entire study.

In Chapter II, I found that there is a strong correlation between *E. coli* counts and stigmasterol concentrations. Although I did not observe a direct correlation between the fecal sterols found in feces and those found in associated waters, the highest *E. coli* counts were found in water samples collected at the two larger heronries, both of which were located directly over water. The fact that the sterol distribution in the fecal samples

was dominated by cholesterol and stigmasterol suggests that further studies on those sterols especially stigmasterol, is warranted. Similar to Chapter II, in the next chapter, Chapter III, I found that the highest concentrations of N and P were in waters receiving direct fecal deposition. In addition, deposition of N and P also increased with the size of the heronry. In the Chapter IV, the results showed that in addition to N and P, feces of colonial waterbirds can contribute significant concentrations of elements to watersheds and, as seen in the previous chapters, the amount of nutrient loading into surface water depended on location of the heronry. The highest concentrations of K were found in feces while the highest Ca concentrations were found in water. Among the microelements, both fecal and water samples contained the highest amounts of Fe. In addition, K, Ca, and Mg appeared to be the most important since they were found in highest concentrations in water samples collected at the two larger heronries.

Overall, the contribution of *E. coli* counts, fecal sterols, and nutrients from feces of avian species depended on both location and size of the heronry. Larger sample sizes and more sampling periods may provide more details in terms of seasonal trends or relationships between fecal sterol profiles and *E. coli* counts. The combined results of my study provides useful insight into the potential effects that large heronries of colonial waterbirds can have on surface water quality. It also creates the framework for further studies of bacteria-impaired watersheds, especially those influenced by large heronries because identifying sources of *E. coli* and quantifying loads resulting from various sources are critical tasks in development of restoration measures for impaired

watersheds. This study provides important data that will be beneficial to water quality managers and stakeholders. It also provides data that will be useful for future studies.

RECOMMENDATIONS

Due to funding and time limitations, the scope of this study was limited to what was presented in this dissertation. I therefore recommend the following for future studies: When possible water samples should be collected at sample sites at least three months prior to and after the birds have left. This data will provide insight into the seasonal variation in nutrients concentration and *E. coli* counts in water.

In order to get a more accurate measure of the percent contribution of nutrients from heronries, all possible sources of nutrients, especially N and P should be analyzed. This involves measuring sediment, soil, and rainwater.

Stable isotope analysis of carbon (C) from fecal sterol extracts can be used to link fecal contamination to avian feces as used by Biache and Philip (2013). Compound specific isotope analysis (CSIA) could be used on stigmasterol found in water samples and those found in fecal samples. The $\delta\ ^{13}C$ values from stigmasterol in fecal samples can then be compared to the $\delta\ ^{13}C$ from stigmasterol in water samples. Although cholesterol has not been identified as a marker for fecal pollution, due to its ubiquitous nature, I believe that CSIA can also be a valuable tool in distinguishing between its different sources.

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APPENDIX A

Mean water quality parameters measured at the four study sites and the corresponding reference sites on the indicated dates. Numbers in parenthesis are standard deviations. The following represent reference sites: BB = Bull Branch, RC-c = Richland Creek ref., HP = Horse Pond. NM = not measured

Heronry	Year	Date	pH	Temp (°C)	Cond (µS)	DO (mg/L)
Murphy Park	2011	June 13	NM	NM	NM	NM
		July 7	8.6	NM	NM	NM
		August 2	8.2	NM	NM	NM
	2012	May 23	8.2	27.8	287.5	7.1
		June 12	8.0	29.2	363.5	4.4
		July 2	8.7	27.2	403.0	5.7
		Ref (BB)	7.5	29.8	485.0	5.0
	2013	May 29	8.4 (0.0)	25.6 (0.0)	301.5 (0.5)	6.0 (0.2)
		Ref (BB)	7.8 (0.0)	25.5 (0.0)	329.0 (0.0)	4.4 (0.1)
		June 11	8.0 (0.1)	29.4 (0.1)	308.0 (0.0)	7.5 (0.2)
		Ref (BB)	7.9 (0.0)	29.2 (0.1)	343.0 (0.0)	8.7 (0.2)
		June 25	8.1 (0.1)	27.9 (0.1)	358.0 (0.0)	6.2 (0.2)
		Ref (BB)	8.1 (0.1)	29.0 (0.1)	361.7 (0.6)	5.4 (0.0)
		July 9	8.2 (0.0)	28.4 (0.1)	360.8 (3.1)	4.6 (1.2)
		Ref (BB)	8.3 (0.0)	29.3 (0.1)	348.7 (0.60	7.4 (0.1)
		July 23	8.2 (0.0)	28.7 (0.1)	260.0 (0.6)	5.0 (0.1)
		Ref (BB)	8.0 (0.0)	28.8 (0.30	306.0 (1.7)	7.1 (0.0)
		August 6	8.2 (0.0)	29.2 (0.2)	240.2 (0.8)	4.2 (0.2)
		Ref (BB)	8.0 (0.0)	30.2 (0.4)	364.7 (0.6)	7.1 (0.2)
Lake Conroe	2011	June 28	8.5	NM	NM	NM
		July 21	8.8	NM	NM	NM
		August 11	8.8	NM	NM	NM
	2012	May 30	7.3	31.9	528.0	1.0
		June 26	7.2	27.7	539.0	4.5
		July 18	NM	NM	NM	NM
		Ref (RC-c)	7.4	29.0	643.0	2.5
Richland Creek	2013	June 4	7.1 (0.3)	27.3 (0.2)	511.0 (79.7)	5.4 (0.4)
		July 2	7.1 (0.1)	25.6 (0.4)	816.3 (72.0)	1.5 (0.7)
		August 13	7.1 (0.0)	28.5 (0.1)	817.7 (2.1)	3.4 (0.2)
Flag Pond	2012	June 28	7.1	29.8	666.0	5.8
		Ref (HP)	9.4	31.7	76.0	8.5

APPENDIX B

Geometric mean of *E. coli* counts in water (CFU / 100 mL) and fecal samples (CFU/g) collected at the four study sites and the corresponding reference sites on the indicated dates. Numbers in parenthesis are ranges. Reference sites are represented by the following: BB = Bull Branch, RC-c = Richland Creek Ref., HP = Horse Pond. NA = not applicable, blank spaces indicate that no fecal samples were available.

Heronry	Year	Date	n (water)	Water	Log_{10} (water)	n (fecal)	Fecal	Log_{10} (Fecal)
Murphy Park	2011	June 13	10	169 (100 - 400)	2.22	3	1×10^4 (1×10^3 - 4×10^5)	3.92
		July 7	10	6,905 (1,600 – 23,000)	3.83	5	2×10^5 (1×10^3 - 6×10^6)	4.99
		August 2	10	232 (100 - 800)	2.34	5	1×10^7 (1×10^6 - 2×10^8)	7.03
	2012	May 23	6	1,295 (520 – 38,000)	3.06	3	4×10^7 (5×10^6 - 2×10^8)	7.53
		June 12	6	820 (250 – 31,000)	2.84	3	3×10^8 (1×10^8 - 8×10^8)	8.48
		July 2	6	13,583 (10,000 – 17,000)	4.13	3	1×10^8 (4×10^7 - 8×10^8)	8.07
		Ref (BB)	3	570 (520 - 660)	2.76	3	NA	NA
		May 29	6	6,987 (800 - 27,000)	3.78	3	8×10^7(6×10^6 - 1×10^9)	7.87
		Ref (BB)	3	485 (440 - 550)	2.68	3	NA	NA
		June 11	3[a]	367 (360 - 370)	2.56	3	2×10^8 (1×10^7 - 1×10^9)	8.27
		Ref (BB)	3	380 (370 - 390)	2.58	3	NA	NA
	2013	June 25	6	525 (450 - 640)	2.72	3	2×10^8 (1×10^8 - 4×10^8)	8.36
		Ref (BB)	3	17,660 (17,000 - 18,000)	4.25	3	NA	NA
		July 9	3[a]	195 (164 - 240)	2.28	3	1×10^8 (8×10^7 - 4×10^8)	8.16
		Ref (BB)	3	637 (560 - 700)	2.80	3	NA	NA
		July 23	6	351 (310 - 420)	2.55	3	9×10^8 (3×10^8 - 2×10^9)	8.95
		Ref (BB)	3	997 (900 – 1,000)	3.00	3	NA	NA
		August 6	3[a]	166 (140 - 210)	2.21	3	1×10^8 (5×10^7 - 2×10^8)	8.03
		Ref (BB)	3	408 (350 - 450)	2.61	3	NA	NA
Lake Conroe	2011	June 28	10	< 100	< 2.00	5	6.1×10^4 (4.0×10^3 - 2.8×10^6)	4.36
		July 21	10	< 100	< 2.00	5	2.4×10^6 (6.3×10^5 - 5.7×10^6)	6.50
		August 11	10	< 100	< 2.00	5	5.6×10^4 (3.0×10^3 - 4.1×10^5)	5.00
Richland Creek	2011	July 26	3	75,235 (53,000 - 120,000)	4.87	3	----	----
	2012	May 30	3	10,720 (800 - 44,000)	3.94	3	----	----
		June 26	3	209,538 (200,000 - 230,000)	5.32	3	----	----
		July 18	3	> 80,000	4.90	3	----	----
		Ref (RC-c)	3	449 (430 - 480)	2.65	3	NA	NA
	2013	June 4	3	41,298 (39,000 - 43,000)	4.62	3	----	----
		July 2	3	63,246 (59,000 - 67,000)	4.80	3	----	----
		Aug. 13	3	35,330 (30,000 - 42,000)	4.55	3	----	----
Flag Pond	2012	June 28	3	1,760,885 (1,300,000 - 2,100,000)	6.25	3	9.1×10^7 (8.0×10^6 - 8.0×10^8)	7.92
		July 25	3	----	----	3	2.6×10^8 (1.2×10^8 - 7.6×10^8)	8.41
		Ref (HP)	3	<100	< 2.00	3	NA	NA

[a] Due to significant differences on either side of the island, only downstream samples were utilized

APPENDIX C

Mean levels and range of fecal sterols measured in (a) water samples (ng/L) and (b) fecal samples (ng/g), collected at the indicated sites and dates. Reference sites are represented by the following: BB = Bull Branch, RC-c = Richland Creek Ref., HP = Horse Pond. Numbers in parenthesis indicate the number of samples in which the indicated sterol was detected. D.L = detection limit, COP = coprostanol, eCOP = epicoprostanol, CHOE = cholesterol, CHOA = cholestanol, bSIT = β-sitosterol, STIG = stigmasterol

a) Water samples

Heronry	Year	Date	n	COP	eCOP	CHOE	CHOA	bSIT	STIG	Total sterols
Murphy Park	2011	June 13	6	16.61 (6)	< D.L	16.81 (6)	12.22 (6)	0.05 (3)	0.17 (3)	45.87
		July 7	6	4.33 (6)	15.36 (1)	5.97 (6)	3.39 (6)	0.09 (1)	0.11 (3)	29.26
		August 2	6	7.29 (6)	27.16 (4)	7.97 (6)	5.77 (6)	0.08 (3)	< D.L	48.27
	2012	May 23	6	4.67 (6)	< D.L	5.54 (6)	3.99 (6)	< D.L	0.48 (2)	14.68
		June 12	6	4.63 (6)	< D.L	6.89 (6)	3.43 (6)	0.05 (2)	0.56 (4)	15.56
		July 2	5	5.54 (5)	< D.L	6.87 (5)	5.23 (5)	0.05 (1)	0.09 (4)	17.77
		Ref (BB)	3	1.64 (3)	1.24 (3)	34.93 (3)	2.33 (3)	< D.L	8.36 (1)	48.49
Lake Conroe	2011	June 28	6	20.73 (6)	< D.L	16.76 (6)	14.88 (6)	0.05 (6)	0.36 (1)	52.79
		July 21	6	6.35 (6)	< D.L	5.10 (6)	4.73 (6)	< D.L	0.08 (4)	16.26
		August 11	6	5.51 (6)	< D.L	4.17 (6)	3.91 (6)	< D.L	0.05 (2)	13.64
Richland Creek	2012	May 30	3	1.53 (3)	5.74 (1)	10.47 (3)	1.62 (3)	0.02 (3)	0.90 (3)	20.28
		June 26	3	3.11 (3)	3.08 (1)	10.35 (3)	3.31 (3)	0.01 (3)	0.09 (3)	19.95
		Ref (RC-c)	3	1.26 (3)	1.09 (2)	22.14 (3)	1.75 (2)	< D.L	< D.L	26.23
Flag Pond	2012	June 28	3	96.85 (3)	< D.L	273.06 (3)	196.25 (6)	0.86 (3)	33.39 (3)	600.41
		Ref (HP)	3	1.92 (3)	0.89 (3)	15.46 (3)	2.15 (6)	0.05 (1)	< D.L	20.48

b) Fecal samples

Heronry	Year	Date	n	COP	eCOP	CHOE	CHOA	bSIT	STIG	Total sterols
Murphy Park	2011	June 13	3	8.05 (2)	8.91 (2)	2814.05 (3)	178.61 (3)	3.54 (2)	985.55 (2)	3998.71
		July 7	3	3.48 (2)	3.43 (1)	1985.55 (3)	129.23 (3)	1.94 (3)	636.12 (3)	2759.75
		August 2	3	12.43 (3)	2.46 (1)	1971.57 (1)	198.33 (3)	70.04 (3)	1068.39 (3)	3323.22
	2012	May 23	3	18.54 (2)	36.65 (2)	586.24 (3)	31.69 (3)	26.45 (3)	279.68 (3)	979.25
		June 12	3	33.57 (2)	72.07 (1)	588.90 (2)	38.65 (2)	21.41 (3)	696.66 (3)	1451.26
		July 2	3	21.92 (3)	14.63 (1)	981.59 (3)	43.26 (3)	1.39 (3)	306.41 (3)	1369.20
Lake Conroe	2011	June 28	3	22.58 (3)	11.48 (1)	3181.42 (3)	245.40 (3)	75.54 (3)	866.94 (3)	4403.36
		July 21	3	3.90 (3)	5.37 (1)	1792.31 (3)	127.93 (3)	1.85 (3)	421.85 (3)	2353.22
		August 11	3	9.85 (3)	2.72 (3)	1967.52 (3)	162.75 (3)	10.31 (3)	1369.07 (3)	3522.22
Flag Pond	2012	June 28	3	41.31 (3)	< D.L	409.34 (3)	74.73 (2)	11.52 (3)	804.34 (3)	1341.23
		July 25	3	13.44 (3)	74.02 (3)	2803.87 (1)	365.77 (3)	22.31 (3)	2558.28 (3)	5837.68

APPENDIX D

Mean concentrations of N and P and N:P ratio (molar) in (a) water samples (mg/L) and (b) fecal samples (mg/kg dry weight) collected at the four study sites and the corresponding reference sites on the indicated dates. Numbers in parenthesis are standard deviations. Reference sites are represented by the following: BB = Bull Branch, RC-c = Richland Creek Ref., HP = Horse Pond.

a) Water Samples

Heronry	Year	Date	n	N	P	N:P
Murphy Park	2011	June 13	6	2.24 (0.33)	0.32 (0.10)	7:1
		July 7	6	3.00 (0.66)	0.33 (0.07)	9:1
		August 2	6	4.29 (0.47)	0.37 (0.03)	12:1
	2012	May 23	6	1.86 (0.72)	0.23 (0.02)	8:1
		June 12	6	1.35 (0.19)	0.22 (0.02)	6:1
		July 2	6	2.69 (0.82)	0.33 (0.03)	8:1
		Ref (BB)	3	2.12 (1.24)	0.15 (0.01)	14:1
		May 29	6	3.39 (1.06)	0.10 (0.01)	34:1
		Ref (BB)	3	0.06 (0.06)	0.17 (0.00)	1:3
		June 11	6	2.44 (0.42)	0.12 (0.01)	20:1
		Ref (BB)	3	0.13 (0.12)	0.11 (0.01)	1:1
	2013	June 25	6	3.34 (0.90)	0.11 (0.01)	30:1
		Ref (BB)	3	0.66 (0.78)	0.09 (0.00)	7:1
		July 9	6	5.12 (0.60)	0.17(0.03)	30:1
		Ref (BB)	3	0.79 (0.59)	0.11 (0.01)	7:1
		July 23	6	4.19 (0.20)	0.11(0.01)	38:1
		Ref (BB)	3	0.97 (0.57)	0.07 (0.01)	14:1
		August 6	6	4.33 (0.18)	0.07(0.00)	62:1
		Ref (BB)	3	1.18 (0.07)	0.14 (0.01)	8:1
Lake Conroe	2011	June 28	6	1.98 (0.43)	0.05 (0.02)	40:1
		July 21	6	0.63 (0.27)	0.05 (0.02)	13:1
		August 11	6	1.08 (0.50)	0.04 (0.01)	27:1
Richland Creek	2011	July 26	3	9.85 (1.49)	3.31 (0.17)	3:1
		May 30	3	1.92 (1.15)	0.51 (0.09)	4:1
	2012	June 26	3	4.27 (1.04)	1.21 (0.03)	4:1
		July 18	3	6.00 (0.57)	1.41 (0.16)	4:1
		Ref (RC-c)	3	10.71 (0.27)	2.60 (0.04)	4:1
	2013	June 4	3	6.25 (4.58)	2.86 (0.15)	2:1
		July 2	3	8.65 (0.48)	2.06 (0.03)	4:1
		Aug. 13	3	9.87 (0.72)	2.09 (0.02)	5:1
Flag Pond	2012	June 28	3	6.24×10^{1} (1.47)	4.69 (0.12)	13:1
		Ref (HP)	3	0.75 (0.23)	< 0.05 (0.01)	15:1

b) Fecal Samples

Heronry	Year	Date	n	N	P
Murphy Park	2011	June 13	3	9.61×10^{4} (9.67×10^{3})	6.83×10^{3} (2.49×10^{3})
		July 7	3	9.35×10^{4} (5.83×10^{3})	9.08×10^{3} (1.65×10^{3})
		August 2	3	9.46×10^{4} (4.40×10^{3})	5.90×10^{3} (3.40×10^{2})
	2012	May 23	3	7.72×10^{4} (9.04×10^{3})	6.85×10^{3} (1.07×10^{3})
		June 12	3	7.48×10^{4} (6.04×10^{3})	7.89×10^{3} (1.40×10^{3})
		July 2	3	8.14×10^{4} (5.79×10^{3})	5.46×10^{3} (7.34×10^{2})
		May 29	3	8.23×10^{4} (5.02×10^{3})	6.14×10^{3} (4.17×10^{2})
		June 11	3	8.80×10^{4} (3.02×10^{3})	8.45×10^{3} (1.52×10^{3})
	2013	June 25	3	8.78×10^{4} (1.25×10^{2})	6.38×10^{3} (5.17×10^{2})
		July 9	3	9.60×10^{4} (2.24×10^{3})	6.97×10^{3} (8.96×10^{2})
		July 23	3	8.96×10^{4} (3.95×10^{2})	6.41×10^{3} (5.06×10^{2})
		August 6	3	9.57×10^{4} (4.60×10^{3})	5.31×10^{3} (1.19×10^{3})
		June 28	3	9.94×10^{4} (2.72×10^{3})	1.08×10^{4} (1.57×10^{3})
Lake Conroe	2011	July 21	3	9.08×10^{4} (8.03×10^{3})	1.11×10^{4} (2.74×10^{3})
		August 11	3	8.61×10^{4} (2.06×10^{3})	9.92×10^{3} (3.04×10^{3})
Flag Pond	2012	June 19	3	7.99×10^{4} (4.32×10^{4})	5.39×10^{3} (8.21×10^{2})
		July 25	3	8.55×10^{4} (7.13×10^{3})	9.10×10^{3} (2.12×10^{3})

APPENDIX E

Mean (± SD) concentrations (mg/kg dry weight) of (a) macroelements and (b) microelements found in fecal samples collected at three study sites on the indicated dates. n = 3. NM= not measured. < = less than

a) Macroelements

Heronry	Year	Date	K	Ca	Mg	Na	S
Murphy Park	2011	June 13	$1.12 \times 10^4 \pm 9.09 \times 10^2$	$5.80 \times 10^3 \pm 1.60 \times 10^3$	$2.95 \times 10^3 \pm 6.48 \times 10^2$	$3.01 \times 10^3 \pm 2.88 \times 10^2$	NM
		July 7	$1.20 \times 10^4 \pm 1.49 \times 10^3$	$7.63 \times 10^3 \pm 1.91 \times 10^3$	$3.76 \times 10^3 \pm 3.84 \times 10^2$	$3.34 \times 10^3 \pm 3.38 \times 10^2$	NM
		August 2	$1.10 \times 10^4 \pm 2.62 \times 10^2$	$5.04 \times 10^3 \pm 1.32 \times 10^3$	$3.12 \times 10^3 \pm 2.52 \times 10^2$	$2.68 \times 10^3 \pm 7.05 \times 10^2$	NM
	2012	May 23	$4.63 \times 10^4 \pm 1.42 \times 10^3$	$7.95 \times 10^3 \pm 2.84 \times 10^3$	$2.86 \times 10^3 \pm 4.55 \times 10^2$	$7.92 \times 10^3 \pm 6.70 \times 10^1$	$5.80 \times 10^3 \pm 8.40 \times 10^2$
		June 12	$4.74 \times 10^4 \pm 7.37 \times 10^2$	$9.09 \times 10^3 \pm 1.70 \times 10^3$	$2.91 \times 10^3 \pm 2.89 \times 10^2$	$8.23 \times 10^3 \pm 2.37 \times 10^2$	$6.47 \times 10^3 \pm 5.38 \times 10^2$
		July 2	$4.82 \times 10^4 \pm 1.27 \times 10^3$	$1.01 \times 10^4 \pm 8.52 \times 10^3$	$2.08 \times 10^3 \pm 4.14 \times 10^2$	$6.69 \times 10^3 \pm 4.19 \times 10^3$	$5.25 \times 10^3 \pm 1.05 \times 10^3$
	2013	May 29	$1.28 \times 10^4 \pm 1.17 \times 10^3$	$4.43 \times 10^3 \pm 2.35 \times 10^3$	$2.62 \times 10^3 \pm 3.35 \times 10^2$	$4.55 \times 10^3 \pm 1.75 \times 10^3$	$7.11 \times 10^3 \pm 3.40 \times 10^2$
		June 11	$1.19 \times 10^4 \pm 1.45 \times 10^3$	$7.71 \times 10^3 \pm 2.36 \times 10^3$	$3.15 \times 10^3 \pm 1.82 \times 10^2$	$3.28 \times 10^3 \pm 8.92 \times 10^1$	$6.55 \times 10^3 \pm 6.76 \times 10^2$
		June 25	$1.01 \times 10^4 \pm 2.22 \times 10^2$	$5.28 \times 10^3 \pm 1.02 \times 10^3$	$2.53 \times 10^3 \pm 1.04 \times 10^2$	$5.16 \times 10^3 \pm 1.35 \times 10^3$	$6.00 \times 10^3 \pm 1.86 \times 10^2$
		July 9	$1.16 \times 10^4 \pm 4.08 \times 10^2$	$5.70 \times 10^3 \pm 1.51 \times 10^3$	$2.65 \times 10^3 \pm 4.81 \times 10^2$	$3.89 \times 10^3 \pm 8.45 \times 10^2$	$6.80 \times 10^3 \pm 3.29 \times 10^2$
		July 23	$1.06 \times 10^4 \pm 9.35 \times 10^1$	$4.35 \times 10^3 \pm 1.12 \times 10^3$	$2.72 \times 10^3 \pm 2.83 \times 10^2$	$4.61 \times 10^3 \pm 7.15 \times 10^2$	$6.74 \times 10^3 \pm 3.09 \times 10^2$
		August 6	$8.19 \times 10^3 \pm 4.38 \times 10^2$	$4.17 \times 10^3 \pm 1.84 \times 10^3$	$2.19 \times 10^3 \pm 3.12 \times 10^2$	$3.59 \times 10^3 \pm 7.91 \times 10^2$	$5.86 \times 10^3 \pm 3.29 \times 10^2$
Lake Conroe	2011	June 28	$9.95 \times 10^3 \pm 1.08 \times 10^3$	$1.16 \times 10^4 \pm 2.16 \times 10^3$	$3.27 \times 10^3 \pm 8.96 \times 10^2$	$3.87 \times 10^3 \pm 8.86 \times 10^2$	NM
		July 21	$1.02 \times 10^4 \pm 1.49 \times 10^3$	$1.15 \times 10^4 \pm 3.80 \times 10^3$	$2.74 \times 10^3 \pm 3.90 \times 10^2$	$2.47 \times 10^3 \pm 3.22 \times 10^2$	NM
		August 11	$1.08 \times 10^4 \pm 1.06 \times 10^3$	$1.16 \times 10^4 \pm 4.14 \times 10^3$	$3.64 \times 10^3 \pm 7.13 \times 10^2$	$3.69 \times 10^3 \pm 5.41 \times 10^2$	NM
Flag Pond	2012	June 19	$4.88 \times 10^4 \pm 7.59 \times 10^2$	$4.53 \times 10^3 \pm 3.27 \times 10^3$	$2.02 \times 10^3 \pm 2.70 \times 10^2$	$8.98 \times 10^3 \pm 6.88 \times 10^2$	$5.48 \times 10^3 \pm 1.23 \times 10^3$
		July 25	$4.87 \times 10^4 \pm 5.42 \times 10^2$	$8.20 \times 10^3 \pm 1.16 \times 10^3$	$2.53 \times 10^3 \pm 8.67 \times 10^2$	$8.71 \times 10^3 \pm 4.41 \times 10^2$	$6.45 \times 10^3 \pm 1.00 \times 10^3$

b) Microelements

Heronry	Year	Date	Zn	Fe	Cu	Mn	B
Murphy Park	2011	June 13	$6.57 \times 10^2 \pm 1.17 \times 10^2$	$7.13 \times 10^2 \pm 1.19 \times 10^2$	$1.40 \times 10^2 \pm 1.52 \times 10^1$	$9.15 \times 10^1 \pm 1.89 \times 10^1$	NM
		July 7	$7.78 \times 10^2 \pm 7.20 \times 10^1$	$8.30 \times 10^2 \pm 3.21 \times 10^2$	$1.53 \times 10^2 \pm 2.03 \times 10^1$	$1.33 \times 10^2 \pm 3.02 \times 10^1$	NM
		August 2	$7.45 \times 10^2 \pm 5.86 \times 10^1$	$6.59 \times 10^2 \pm 9.55 \times 10^1$	$1.84 \times 10^2 \pm 1.75 \times 10^1$	$9.73 \times 10^1 \pm 1.25 \times 10^1$	NM
	2012	May 23	$4.95 \times 10^2 \pm 1.12 \times 10^2$	$1.75 \times 10^3 \pm 1.27 \times 10^3$	$1.07 \times 10^2 \pm 1.96 \times 10^1$	$1.02 \times 10^2 \pm 2.06 \times 10^1$	$3.77 \times 10^1 \pm 2.82 \times 10^0$
		June 12	$6.26 \times 10^2 \pm 6.60 \times 10^1$	$1.80 \times 10^3 \pm 9.12 \times 10^2$	$1.33 \times 10^2 \pm 2.78 \times 10^1$	$1.36 \times 10^2 \pm 1.31 \times 10^1$	$3.65 \times 10^1 \pm 2.78 \times 10^0$
		July 2	$4.97 \times 10^2 \pm 1.08 \times 10^2$	$2.28 \times 10^3 \pm 2.00 \times 10^3$	$1.05 \times 10^2 \pm 2.81 \times 10^1$	$1.32 \times 10^2 \pm 5.99 \times 10^1$	$3.34 \times 10^1 \pm 4.64 \times 10^0$
	2013	May 29	$4.91 \times 10^2 \pm 3.40 \times 10^1$	$3.36 \times 10^2 \pm 2.35 \times 10^1$	$1.18 \times 10^2 \pm 1.69 \times 10^1$	$7.73 \times 10^1 \pm 3.31 \times 10^1$	$7.84 \times 10^0 \pm 5.41 \times 10^0$
		June 11	$6.34 \times 10^2 \pm 6.15 \times 10^1$	$3.09 \times 10^2 \pm 3.85 \times 10^1$	$1.35 \times 10^2 \pm 2.00 \times 10^1$	$8.28 \times 10^1 \pm 1.52 \times 10^1$	$6.96 \times 10^0 \pm 2.75 \times 10^0$
		June 25	$6.05 \times 10^2 \pm 3.25 \times 10^1$	$3.99 \times 10^2 \pm 1.25 \times 10^2$	$1.31 \times 10^2 \pm 9.44 \times 10^0$	$6.41 \times 10^1 \pm 1.02 \times 10^1$	$3.82 \times 10^0 \pm 1.59 \times 10^0$
		July 9	$6.57 \times 10^2 \pm 6.48 \times 10^1$	$3.96 \times 10^2 \pm 8.58 \times 10^1$	$1.41 \times 10^2 \pm 2.28 \times 10^1$	$6.20 \times 10^1 \pm 1.99 \times 10^1$	$3.68 \times 10^0 \pm 9.63 \times 10^{-1}$
		July 23	$6.95 \times 10^2 \pm 1.03 \times 10^2$	$8.99 \times 10^2 \pm 3.95 \times 10^2$	$1.54 \times 10^2 \pm 2.60 \times 10^1$	$9.23 \times 10^1 \pm 2.23 \times 10^1$	$2.22 \times 10^0 \pm 1.94 \times 10^0$
		August 6	$5.55 \times 10^2 \pm 1.58 \times 10^1$	$3.48 \times 10^2 \pm 2.67 \times 10^1$	$1.57 \times 10^2 \pm 2.63 \times 10^1$	$4.16 \times 10^1 \pm 1.33 \times 10^1$	$2.18 \times 10^0 \pm 8.00 \times 10^{-1}$
Lake Conroe	2011	June 28	$8.05 \times 10^2 \pm 1.61 \times 10^2$	$6.74 \times 10^2 \pm 1.06 \times 10^2$	$1.54 \times 10^2 \pm 2.89 \times 10^1$	$2.53 \times 10^2 \pm 1.57 \times 10^2$	NM
		July 21	$6.53 \times 10^2 \pm 1.46 \times 10^1$	$5.95 \times 10^2 \pm 1.25 \times 10^2$	$1.50 \times 10^2 \pm 2.36 \times 10^0$	$1.27 \times 10^2 \pm 3.12 \times 10^0$	NM
		August 11	$7.05 \times 10^2 \pm 1.59 \times 10^2$	$6.82 \times 10^2 \pm 3.76 \times 10^1$	$1.57 \times 10^2 \pm 1.19 \times 10^1$	$1.64 \times 10^2 \pm 2.62 \times 10^1$	NM
Flag Pond	2012	June 19	$5.35 \times 10^2 \pm 5.45 \times 10^1$	$1.87 \times 10^3 \pm 1.60 \times 10^3$	$9.90 \times 10^1 \pm 1.31 \times 10^1$	$1.70 \times 10^2 \pm 5.88 \times 10^1$	$3.58 \times 10^1 \pm 1.70 \times 10^0$
		July 25	$6.22 \times 10^2 \pm 1.59 \times 10^2$	$1.06 \times 10^3 \pm 4.12 \times 10^2$	$1.31 \times 10^2 \pm 1.98 \times 10^0$	$1.53 \times 10^2 \pm 7.61 \times 10^1$	$3.85 \times 10^1 \pm 3.16 \times 10^0$

APPENDIX F

Mean (± SD) concentrations (mg/L) of (a) macroelements and (b) microelements found in water samples collected at the four study sites and the corresponding reference sites on the indicated dates. Reference sites are represented by the following: BB = Bull Branch, RC-c = Richland Creek Ref., HP = Horse Pond., n = 3. NM= not measured. < = less than.

a) Macronutrients

Heronry	Year	Date	K	Ca	Mg	Na	S
Murphy Park	2011	June 13	8.52 ± 1.21	53.19 ± 2.37	6.45 ± 1.52	49.24 ± 1.47	NM
		July 7	8.93 ± 0.99	49.13 ± 4.13	4.89 ± 1.43	95.40 ± 5.88	NM
		August 2	10.78 ± 1.02	54.01 ± 1.50	5.28 ± 0.46	115.80 ± 5.01	NM
	2012	May 23	6.58 ± 0.21	35.36 ± 0.75	2.82 ± 0.09	14.8 ± 1.11	NM
		June 12	6.51 ± 0.18	36.44 ± 1.13	3.17 ± 0.09	24.49 ± 1.46	NM
		July 2	7.60 ± 0.32	47.22 ± 26.31	3.73 ± 0.17	31.94 ± 1.72	NM
		(BB)	3.51 ± 0.12	64.52 ± 0.51	3.58 ± 0.05	21.86 ± 1.01	NM
	2013	May 29	3.92 ± 0.05	43.29 ± 4.73	2.80 ± 0.06	13.63 ± 0.32	6.39 ± 0.15
		Ref (BB)	3.43 ± 0.01	57.91 ± 0.25	2.49 ± 0.02	10.28 ± 0.08	7.04 ± 0.06
		June 11	4.10 ± 0.03	41.13 ± 0.45	3.08 ± 0.01	17.01 ± 0.43	7.07 ± 0.04
		Ref (BB)	2.86 ± 0.03	54.24 ± 0.16	2.76 ± 0.01	13.55 ± 0.30	7.82 ± 0.04
		June 25	4.75 ± 0.05	48.70 ± 1.74	3.60 ± 0.02	22.51 ± 0.48	8.62 ± 0.04
		Ref (BB)	3.00 ± 0.05	53.78 ± 0.13	2.96 ± 0.02	15.42 ± 0.02	7.40 ± 0.04
		July 9	4.95 ± 0.02	41.53 ± 1.51	3.15 ± 0.03	28.93 ± 0.78	9.49 ± 0.05
		Ref (BB)	3.09 ± 0.05	46.93 ± 0.18	3.13 ± 0.02	18.47 ± 0.42	6.75 ± 0.11
		July 23	4.16 ± 0.02	37.03 ± 0.42	2.51 ± 0.01	12.70 ± 0.21	6.13 ± 0.04
		Ref (BB)	3.27 ± 0.13	52.31 ± 0.22	2.19 ± 0.01	8.92 ± 0.85	5.81 ± 0.06
		August 6	4.10 ± 0.04	31.77 ± 0.86	2.06 ± 0.02	9.23 ± 0.57	4.80 ± 0.03
		Ref (BB)	3.95 ± 0.03	58.59 ± 0.51	2.23 ± 0.03	8.75 ± 0.75	5.56 ± 0.02
Lake Conroe	2011	June 28	9.91 ± 1.07	33.20 ± 1.25	4.02 ± 2.23	15.39 ± 3.10	NM
		July 21	6.27 ± 0.13	32.94 ± 2.36	2.80 ± 0.38	20.86 ± 0.71	NM
		August 11	6.37 ± 0.29	29.69 ± 1.05	2.72 ± 0.17	21.07 ± 0.53	NM
	2011	July 26	17.93 ± 0.37	44.82 ± 1.67	4.56 ± 0.38	34.23 ± 0.79	NM
	2012	May 30	9.34 ± 0.14	48.15 ± 9.49	4.70 ± 0.17	35.03 ± 0.81	NM
		June 26	5.29 ± 0.25	45.58 ± 1.43	4.28 ± 0.21	38.57 ± 1.76	NM
Richland Creek		July 18	6.79 ± 0.67	54.52 ± 18.49	5.27 ± 2.62	36.22 ± 0.95	NM
		Ref (RC-c)	10.54 ± 0.75	47.61 ± 0.36	4.55 ± 0.63	42.56 ± 0.93	NM
	2013	June 4	10.40 ± 1.23	67.88 ± 2.02	6.42 ± 0.41	57.94 ± 1.50	7.50 ± 0.80
		July 2	11.05 ± 0.25	63.57 ± 0.27	6.77 ± 0.28	67.15 ± 0.09	4.24 ± 0.23
		August 13	12.16 ± 0.08	67.07 ± 0.16	7.69 ± 0.63	81.66 ± 0.17	2.22 ± 0.07
Flag Pond	2012	June 28	15.48 ± 0.71	25.28 ± 0.89	7.32 ± 0.04	21.38 ± 1.93	NM
		Ref (HP)	5.31 ± 0.17	6.60 ± 0.15	1.15 ± 0.03	3.38 ± 0.71	NM

Appendix F Continued

b) Microelements

Heronry	Year	Date	Zn	Fe	Cu	Mn	B
Murphy Park	2011	June 13	< 0.01	1.26 ± 0.59	< 0.02	0.14 ± 0.02	NM
		July 7	< 0.02	1.56 ± 0.73	< 0.02	0.13 ± 0.04	NM
		August 2	0.02 ± 0.01	1.23 ± 0.48	< 0.02	0.24 ± 0.01	NM
	2012	May 23	< 0.02	1.98 ± 0.19	< 0.01	< 0.07	NM
		June 12	< 0.03	1.85 ± 0.49	< 0.01	< 0.11	NM
		July 2	< 0.02	2.47 ± 0.22	< 0.01	0.16 ± 0.01	NM
		Ref (BB)	< 0.029	1.44 ± 0.43	< 0.008	< 0.095	NM
	2013	May 29	< 0.001	< 0.01	< 0.01	< 0.001	0.05 ± 0.00
		Ref (BB)	< 0.001	0.05 ± 0.03	< 0.012	< 0.001	0.05 ± 0.00
		June 11	< 0.001	< 0.01	< 0.01	< 0.001	0.07 ± 0.00
		Ref (BB)	< 0.001	< 0.002	< 0.012	< 0.001	0.06 ± 0.00
		June 25	< 0.001	0.03 ± 0.02	< 0.001	< 0.001	0.09 ± 0.00
		Ref (BB)	< 0.001	< 0.002	< 0.012	< 0.001	0.07 ± 0.00
		July 9	< 0.001	0.08 ± 0.02	< 0.01	< 0.001	0.11 ± 0.00
		Ref (BB)	< 0.001	< 0.002	< 0.012	< 0.001	0.08 ± 0.00
		July 23	< 0.001	0.03 ± 0.02	< 0.01	< 0.001	0.06 ± 0.00
		Ref (BB)	< 0.001	< 0.002	< 0.012	< 0.001	0.05 ± 0.00
		August 6	< 0.001	0.08 ± 0.05	< 0.01	< 0.01	0.04 ± 0.00
		Ref (BB)	< 0.002	< 0.002	< 0.012	0.02 ± 0.00	0.04 ± 0.00
Lake Conroe	2011	June 28	0.09 ± 0.14	1.02 ± 0.40	< 0.06	0.03 ± 0.02	NM
		July 21	< 0.004	0.27 ± 0.23	< 0.004	0.05 ± 0.00	NM
		August 11	< 0.003	0.35 ± 0.15	< 0.002	0.05 ± 0.02	NM
Richland Creek	2011	July 26	< 0.011	1.58 ± 0.53	< 0.015	0.25 ± 0.06	NM
	2012	May 30	< 0.018	2.23 ± 1.95	< 0.008	< 0.039	NM
		June 26	≪ 0.007	1.42 ± 0.90	< 0.008	0.11 ± 0.02	NM
		July 18	≪ 0.041	0.81 ± 0.07	< 0.009	0.72 ± 1.01	NM
		Ref (RC-c)	≪ 0.027	0.64 ± 0.29	< 0.006	0.78 ± 0.49	NM
	2013	June 4	< 0.001	< 0.002	< 0.012	< 0.061	0.03 ± 0.01
		July 2	< 0.001	0.04 ± 0.03	< 0.012	< 0.063	0.03 ± 0.00
		August 13	< 0.001	0.03 ± 0.01	< 0.012	0.01 ± 0.00	0.04 ± 0.00
Flag Pond	2012	June 28	0.07 ± 0.03	4.00 ± 1.90	0.02 ± 0.01	0.31 ± 0.05	NM
		Ref (HP)	< 0.033	1.13 ± 0.61	< 0.011	≪ 0.005	NM