DEFINING LINKAGES BETWEEN LAND-USE, *SALMONELLA*, AND *CAMPYLOBACTER* IN THE SATILLA RIVER BASIN (GEORGIA, USA)

by

ETHELL VEREEN, JR.

(Under the direction of Erin K. Lipp)

ABSTRACT

From August 2007 to August 2009 a study of the Satilla River Basin (SRB), a mixed-use rural watershed located in the coastal plain of southern Georgia (USA), was conducted to provide an understanding of landuse and existing water quality in these important surface waters. Watersheds were sampled that: 1) represent agricultural areas receiving poultry litter application, 2) agricultural areas with poultry houses and receiving poultry litter application, 3) reference areas with little or no agricultural activity, 4) a small watershed receiving direct discharge from a wastewater treatment plant (WWTP); and 5) larger watersheds on the main channel of the Satilla and Seventeen Mile River's. Landuse differences were responsible water quality and nutrient variability. Eighty-three of the dissolved oxygen (DO) samples (35.78%, N=232) were below the Georgia Environmental Protection Division DO minimum, indicating that the SRB did not support its designated recreational use. *Salmonella* and *Campylobacter* were detected at all 13 sites monitored, and their prevalence varied by location (i.e., upstream vs. downstream of the WWTP). Pathogens were more frequently present among the sites with agricultural landuse and poultry production. *Salmonella* were recovered from 43% (129 of 299) of all samples.

Salmonella serogroups identified were serogroup C (59%), B (14%), D (14%), and E (13%). *Salmonella enterica* serotype Montevideo (23%), Braenderup (14%) and Saint Paul (13%) were the most frequently detected of 16 different serotypes identified. *Campylobacter* were recovered from 62% (96 of 156) of all samples. *C. jejuni* (30%) was the most prevalent species detected followed by *C. lari* (22%), *C. upsaliensis* (11%), and *C. coli* (3%). Enterococci were reasonably predictive of *Salmonella* and *Campylobacter* presence; however 62% (80/129) of *Salmonella* were detected when *E. coli* were below EPA standards and 73% (70/96) of *Campylobacter* were detected when fecal coliforms were below EPA standards. These results highlight the drawback of the current fecal indicator system as proxies for enteric bacteria such as *Salmonella* and *Campylobacter*, which were highly prevalent in this area. This study indicates that these pathogens likely arise from numerous sources, including humans, and that there may be risk of human exposure through recreational contact.

INDEX WORDS: *Salmonella*, *Campylobacter*, *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, S. Montevideo, S. Braenderup, S. Typhimurium, S. Javiana, S. Newport, S. Muenchen, *Salmonella* serotype, *Salmonella* serovar, antimicrobial susceptibility, Satilla River Basin, Satilla River, Seventeen Mile River, Poultry, Poultry Litter, Poultry Production, Georgia Coastal Plain, Waterborne Pathogen, Waterborne Transmission, Animal Waste, Wastewater treatment, WWTP, Agriculture, Microbial Ecology

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December 2010

DEDICATION

To my grandparents,

Henry & Eva Vereen
Edward & Bertha-Mae Bennett

And whatsoever ye do in word or deed,

do all in the name of the Lord Jesus,

giving thanks to God and the Father by him.

Col 3:17

Ruth Ella Moore, PhD

Born in 1903 in Columbus Ohio, Ruth Ella Moore was the first African American woman in the United States to earn a Ph.D. in the natural sciences. Her doctorate was in the field of bacteriology from Ohio State University.

Her dissertation on tuberculosis earned her a doctorate in bacteriology in 1933 [also from Ohio State University]. Dr. Moore was hired as an assistant professor at Howard University Medical College in 1940. Her research at Howard focused on blood groups and Enterobacteriaceae, a family of bacteria which includes Salmonella.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

INTRODUCTION

The United States leads the world in poultry production (17). Broiler chickens (often referred to as 'Broilers') have been selectively bred and reared for their meat rather than eggs. Broiler production is a rapidly expanding industry in the southern Coastal Plain of Georgia; as well as in the Coastal Plain regions of other states in the southeast, such as Arkansas, Alabama, North Carolina and Mississippi (10). Georgia produces roughly 15 percent of the country's broilers annually; and according to a U.S. Department of Agriculture (USDA) report on broiler production and value, Georgia produced 1.4 billion broilers; more than any other state valued at $2.7 billion in 2006 (14). Additionally, an immense quantity of waste is generated in association with broiler, or poultry production. Considering that one chicken produces 1.5 kg of poultry litter (a mixture of raw poultry feces/manure and bedding material) annually (12), more than 2.1 million metric tons of poultry litter is produced as a by-product of this industry.

While treatment measures such as anaerobic digestion or composting may be practiced, application of poultry litter as a fertilizer, on pasture and cropland is most often used as streams are the "final" receivers. Poultry litter use as a fertilizer is accepted as good agronomic practice as it contains important plant nutrients, i.e. nitrogen (N), phosphorus (P), and potassium (K) (2-4). However, studies have shown that animal manure application on agricultural lands may lead to contamination of field level resources (2, 4, 6, 13), and may even increase the risk of transport

of pathogens to downstream water resources if these wastes contain pathogenic microorganisms such as *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Clostridium perfringens*, *E. coli* O157:H7, and the protozoan pathogens *Cryptosporidium parvum* and *Giardia lamblia* (1, 5-9, 11, 16). As poultry production is increasing rapidly in the Coastal Plain region of Georgia, the potential for agricultural wastes to contaminate surface waters must be explored.

The Satilla River Basin (SRB) is located in southeast Georgia and is flanked by the Altamaha River Basin to the north and the Suwannee and St. Mary's River Basins to the south. The upper region of the SRB is heavily agricultural with row-crops, hayfields, poultry houses, and beef cattle production; but there are differences in sub-watersheds based on the amount of cropland and the number of poultry houses. The city of Douglas is in the middle part of the basin and home to a broiler chicken processing plant that empties to the city wastewater treatment plant (WWTP), and discharge from the WWTP enters a small stream that flows into Seventeen Mile River, a major tributary of the Satilla River. The Satilla River empties into Cumberland Sound and National Seashore on the Georgia Atlantic Coast and provides an ideal setting for determining the effects of mixed-use agriculture, dominated by animal production, on pathogen transport.

During a water quality survey begun in 2003, campylobacter-like organisms (CLO) were found in the headwaters of the SRB (15). Mean CLO counts and overall prevalence were highest downstream from the WWTP that handled both human and poultry slaughterhouse waste (up to 595 CFU ml^{-1}, 100% of samples positive) (15). Isolations of pathogens from the surface waters of an area give rise to questions as to the origins of the organisms, their survival or persistence, and their relevance to public health, which prompted the present study. The purpose

of this research was to identify and investigate the prevalence of *Salmonella* and *Campylobacter*, and their transport in streams associated with agriculture and wastewater treatment in the Satilla River Basin. An examination of the SRB for these pathogens provides essential information for management of water quality in Coastal Plain and other watersheds with large amounts of animal agriculture.

A comprehensive review of the literature including the production of poultry and poultry litter, the microbial ecology of *Salmonella* and *Campylobacter,* and a discussion on the potential transport of these pathogens in poultry wastes, is presented in Chapter 2; with a brief description of the Satilla River Basin in the Georgia Coastal Plain province. In Chapter 3, the current stream water quality of the Satilla River Basin is assessed. In Chapter 4, the prevalence and distribution *Salmonella* and *Campylobacter* in the Satilla River Basin is examined. In Chapter 5, the focus shifts to targeted investigation of *Salmonella* and *Campylobacter* to identify serotypes and species of environmental and clinical significance. In addition, we evaluated antimicrobial susceptibility patterns among *Salmonella* isolates. This chapter also discusses the potential public health risk of the pathogens in the Satilla River Basin. The last chapter, Chapter 6, provides concluding remarks on these studies and their significance as a whole.

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CHAPTER 2

LITERATURE REVIEW

INTRODUCTION

Globally, poultry production is one of the fastest growing segments of the animal industry and the United States is the largest producer in the world (77). Poultry litter is a mixture of manure and bedding material used during the poultry production cycle. As poultry litter contains significant amounts of nutrients, especially phosphorus; it is commonly used as a soil fertilizer for crop production (21, 23, 26). Poultry litter may also contain pathogenic microorganisms, such as *Salmonella* and *Campylobacter* (49, 53, 73), and concerns exist about the potential runoff of microbial pathogens from land-applied animal waste, including poultry litter (21, 26, 31, 45, 61).

The challenges associated with microbial contamination of water resources and the roles that science plays in addressing those challenges in general are becoming more significant. In a recent review of the literature, Smith and Perdek (60) concluded "a significant body of research is needed to understand the uncertainties in pathogenic (microorganism) processes at the watershed scale… ". At a workshop convened by the Environmental Protection Agency (3), work group members noted that, "the principles of microbial ecology must be considered in water quality assessment." Considering the significant growth of the poultry industry and the large output of poultry waste generated that may contain pathogenic microorganisms that are then used as a soil fertilizer, the potential for microbial contamination of water resources at the watershed scale should be explored.

This chapter reviews the microbial ecology of *Salmonella* and *Campylobacter* with a discussion on the potential transport of these pathogens in poultry litter. A brief description of the Satilla River Basin in the Georgia Coastal Plain province is also included as this is a region where the long term trend is expected to be a continuing increase of poultry production.

HISTORY AND TAXONOMY OF *CAMPYLOBACTER*

Campylobacter is derived from the Greek word "kampylos," which means curved (71). The organism was given this name because of the spiral or S-shaped morphology of the cells (71). The first recognized identification of *Campylobacter* was made by McFadyen and Stockman in 1913 (as cited in (46, 75)) in association with abortions in sheep. Confirmatory tests were carried out by Smith in 1919 when similar organisms were isolated from aborted bovine feces (as cited in (46)). Smith originally identified these organisms as *Vibrio fetus* because of morphological similarities with vibrios (46, 75). In the subsequent decades, similar organisms were found and included in the genus *Vibrio* as *Vibrio jejuni*, *Vibrio coli*, *Vibrio sputorum*, *Vibrio bubulus* and *Vibrio fecalis* (75). It was not until 1963 that a new Genus *Campylobacter* was proposed by Sebald and Véron (as cited in (75)) for *V. fetus* and *V. bulbus* to differentiate them from traditional members of the Genus *Vibrio* because of their different microaerophilic growth requirements and their nonfermentative metabolism. In 1991, a number of presumed *Campylobacters* were moved to the new genus *Arcobacter* and *Helicobacter* on the basis of ribosomal sequence analysis (70).

The genus *Campylobacter* is placed in the bacterial family *Campylobacteraceae;* and in 1994, minimal standards were proposed for describing new species of the family (67). Currently, the genus *Campylobacter* comprises 15 species (one of which is still disputed) and 6 subspecies: *Campylobacter coli, C. concisus, C. curvus, C. fetus* subsp. *fetus, C. fetus* subsp. *venerealis, C.*

gracilis, C. helveticus, C. hyoilei (controversial), *C. hyointestinalis* subsp. *hyointestinalis, C. hyointestinalis* subsp. *lawsonii, C. jejuni* subsp. *jejuni, C. jejuni* subsp. *doylei, C. lari, C. mucosalis, C. rectus, C. showae, C. sputorum and C. upsaliensis* (75).

DESCRIPTION OF *CAMPYLOBACTER*

All members of the genus *Campylobacter* are microaerophilic, generally requiring lower oxygen tensions for growth; the optimal growing conditions are a gas mixture of 10% CO_2, 80% N_2, and 10% H_2 (28). *Campylobacters* are gram-negative, oxidase positive, indole negative and reduce nitrate. The bacterial cells of *Campylobacters* are slender, helical or curved rods (0.2-0.8 µm X 0.5-5.0 µm) (75). Certain species have cells that are straight rods (i.e., *C. showae*), and in *C. jejuni* strains, cells that are occasionally straight rods (75). In older cultures or cultures exposed to environmental stresses such as atmospheric oxygen, the cells can change to spherical or coccoid forms. *Campylobacters* are slow growing (24 to 48 hours) with an optimal growth temperature ranging from 30^{o}C to 42^{o}C.

ECOLOGY AND EPIDEMIOLOGY OF *CAMPYLOBACTER*

Under natural conditions, *Campylobacter* growth is only achieved within a suitable host. The natural habitats of most *Campylobacter* species are the intestines of birds and other warm-blooded animals (46, 75). The microaerophilic nature and temperature dependence of *Campylobacter* prevents growth outside of their intestinal niches. Domestic and farm animals such as pigs, cattle, dogs and cats may also harbor *Campylobacter* within their intestinal tracts (10, 62, 75); however, the favored environment appears to be the intestines of avian species, including wild birds, chickens, turkeys, quails, and ducks (1, 11, 48, 74). *Campylobacters* appear to have evolved to optimally colonize the intestinal mucosa, especially the cecum of birds (body temperature 42^{o}C) and are usually commensal within their avian hosts (46, 48, 75), with

the possible exception of ostriches (72). *Campylobacters* may also act as a pathogen when organisms colonize the intestine of mammals with lower body temperatures.

Eleven of the current *Campylobacter* species and subspecies are considered pathogenic to humans (9, 75). Campylobacteriosis, gastroenteritis caused by the thermotolerant *Campylobacter* spp. (*C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*), is the most commonly reported gastrointestinal bacterial disease in developed countries throughout the world (15, 46). Although, numbers of infections have declined slightly in some parts of the world during recent years, the overall disease burden is still significant (5, 46). The major pathogens, *C. jejuni* and *C. coli* account for about 90% and 10% of human campylobacteriosis cases, respectively (46). While *C. lari* and *C. upsaliensis* cause far fewer cases of disease, both species have been recognized as emerging human pathogens (38, 76). Typically, cases are self-limited characterized by diarrhea, abdominal pain, and fever. However, approximately 1 in 2,000 *C. jejuni* infections may be complicated by Guillain-Barré syndrome (GBS, an inflammatory disorder of the peripheral nerves) (8, 46).

Campylobacter is transmitted via the fecal-oral route. The most common mode of transmission is ingestion of food or water that has been contaminated with human or animal feces (46, 75). This transfer includes raw and undercooked poultry or pork, inadequately treated drinking water, and raw milk and raw milk products (46, 75). However, any food contaminated with the bacteria can be a source of infection. A very small number of *Campylobacter* organisms (fewer than 500) can cause illness in humans (12). Campylobacteriosis is the leading cause of bacterial foodborne illness worldwide (15) and the second leading cause of bacterial foodborne illness in the United States (50).

HISTORY AND TAXONOMY OF *SALMONELLA*

The Salmonellae are a diverse group of bacteria within the family *Enterobacteriaceae* that are named after Dr. Daniel E. Salmon, an American veterinary medical scientist who pioneered research in bacterial diseases and in immunology (22, 35). In 1885 Dr. Theobald Smith working under Dr. Salmon isolated what became known as *Salmonella choleraesuis* from the intestine of a pig (22, 35). It is argued that this bacteria should in fairness be called Smithella, since it was Dr. Smith, who was the true discoverer of the first member of the Salmonellae (35). However, French bacteriologist Dr. Joseph Léon Marcel Lignières suggested in 1900, that the group of bacteria represented by the swine cholera organisms in the paper, "The bacterium of swine plague" written by Dr. Salmon be named in his honor (35, 58).

Salmonella nomenclature is complex, and the species concept in the Genus *Salmonella* has developed in several phases as scientists have used different systems to communicate about this genus (14, 22, 36, 41). However, uniformity in *Salmonella* nomenclature is necessary for communication among scientists, health officials, and the public. The original taxonomy of the genus was not based on DNA relatedness, rather names were given according to clinical considerations, e.g., *Salmonella typhi-murium* (mouse typhoid fever), *Salmonella cholerae-suis* (hog cholera), *Salmonella abortus-ovis* (abortion in sheep), and so on (41, 63, 64). During the 1920s microbiologists began to work towards a unified taxonomy by serological comparison of representative cultures. Significant contributions were made in this field by Philip Bruce White in London, and by Fritz Kauffmann in Copenhagen, and general agreement was finally achieved under the guidance of the *Salmonella* Subcommittee of the International Society for Microbiology in 1933 (as cited in (22, 56)). The different *Salmonella* were then classified under the Kauffmann-White scheme, and newly identified members of the genus were named after the

place where they were first recognized e.g., *Salmonella london*, *Salmonella panama*, *Salmonella Stanleyville* (as cited in (22, 56)). When serological analysis was adopted into the Kauffmann-White scheme in 1946, each serotype was considered a separate species on the basis of the serologic identification of O (somatic) and H (flagellar) antigens (as cited in (22, 56). Later, DNA relatedness studies demonstrated that all *Salmonella* serovars formed a single DNA hybridization group with seven subgroups (as cited in (22, 56); and Le Minor et al. (39) proposed *S. choleraesuis* as the species name, having seven subspecies. Noting the potential confusion in using *S. choleraesuis* to indicate organisms that cause very different diseases, e.g., serovar Choleraesuis versus serovar Typhi, *S. enterica*, the name first proposed by Kauffman and Edwards in 1952, was eventually proposed as the sole species name based on numerical taxonomy and DNA relatedness studies (39). Subsequently, Reeves et al. (1989) proposed that *Salmonella bongori* be elevated to species status on the basis of molecular characterization (as cited in (22, 56, 64). Although these proposals have never been officially sanctioned, this nomenclature is used widely and has been adopted by the Centers for Disease Control and Prevention (CDC), the American Society for Microbiology (ASM), and the World Health Organization (WHO) Collaborating Centre for Reference and Research on *Salmonella*, which is responsible for updating the Kauffmann-White scheme (22, 64). Pursuant to this considerable change in nomenclature, only two species of the genus *Salmonella* are currently recognized, *Salmonella bongori* and *Salmonella enterica*, and encompasses over 2, 500 known serotypes. *Salmonella enterica* is further divided into six subspecies: *Salmonella enterica* subsp. e*nterica* (subsp. I), *Salmonella enterica* subsp. *salamae* (subsp. II), *Salmonella enterica* subsp. *arizonae* (subsp. IIIa), *Salmonella enterica* subsp. *diarizonae* (subsp. IIIb), *Salmonella enterica* subsp. *houtenae* (subsp. IV), *Salmonella enterica* subsp. *indica* (subsp. VI). Serovars of *Salmonella*

enterica subsp. *enterica* are written in Roman letters (not italicized) and the first letter is capitalized (22, 64). Given that only *Salmonella enterica* subsp. *enterica* strains are named, various shortened versions have become acceptable, such as *Salmonella enterica* serovar Typhimurium, *Salmonella* Typhimurium, or serovar Typhimurium.

DESCRIPTION OF *SALMONELLA*

Salmonella are gram-negative, oxidase negative, indole negative bacteria that do not form spores and reduce nitrate. The bacterial cells of *Salmonella* are straight rods, 0.7-1.5 µm X 2.0-5.0 µm and generally 2-4 mm in diameter with parallel sides and rounded ends (56). *Salmonella* are facultatively anaerobic and usually motile having peritrichous flagella. Serovar Gallinarum however is always nonmotile (56). Most *Salmonella* are aerogenic, commonly producing hydrogen sulfide; however, a few serovars do not (e.g. serovar Typhi never produces a gas) (56). *Salmonella* serotypes are designated according to the conventions of the Kauffmann-White Scheme (55). They are composed of numbers and letters given to the different O (somatic), Vi (capsular), and H (flagellar) antigens (22, 56).

ECOLOGY AND EPIDEMIOLOGY OF *SALMONELLA*

Although some *Salmonella* serovars are strictly host adapted, a characteristic feature of this organism is its broad host spectrum, that consist of most animal species, including both warm and cold-blooded animals, mammals, birds and humans (18, 22, 56). Some *Salmonella* are localized in a particular region of the world (e.g., serovar Sendai in the Far East, serovar Berta in North America), but others are ubiquitous (e.g., serovar Typhimurium) (18, 22, 56). Similar to *Campylobacter*, *Salmonella* live primarily in the intestinal tracts of animals. Common hosts are domestic and farm animals such as pigs, cattle, dogs and cats (19, 24, 42), but reptiles such as

iguanas, turtles, snakes and lizards are also carriers of these bacteria and harbor *Salmonella* within their intestinal tracts (42).

In humans, serovars Typhi, Paratyphi and Sendai cause enteric fever (typhoid), while most other serovars are commonly associated with acute gastroenteritis (salmonellosis) (18). The most common mode of transmission of *Salmonella* is ingestion of contaminated foods from animal origins including meat, poultry, eggs, and dairy products (18, 22). *Salmonella* is transmitted via the fecal-oral route, and ingestion of a small number of organisms (fewer than 1000) may cause illness in humans (13). Infections are generally self-limiting and the most common symptoms of salmonellosis include sudden onset of abdominal cramps and nausea followed by diarrhea (sometimes bloody), fever, and sometimes vomiting (18).

SATILLA RIVER BASIN CHARACTERISTICS

Physiography and basin boundaries

The Satilla River Basin is located in the southeastern part of Georgia, occupying an area of 3,940 miles^2 (10,204 km^2) and is flanked by the Altamaha River basin to the north and the Suwannee and St. Mary's River basins to the south (Figure 2.1). The SRB covers all or part of 15 Georgia counties (Figure 2.2); however, only two counties Bacon and Pierce are entirely within the basin. The other counties are: Appling, Atkinson, Ben Hill, Brantley, Camden, Charlton, Clinch, Coffee, Glynn, Irwin, Jeff Davis, Ware, and Wayne. Population growth in the SRB has exceeded 20% per decade in recent years, although the watershed is still primarily rural (6). According to the 2009 U.S. Census Bureau: State and County QuickFacts (66) the counties in the basin are experiencing steady population growth, ranging from 1.5% per annum (Irwin County) to 4.5% per annum (Camden County). Significantly, two of the counties in the basin

that are either on the coast of Georgia (Camden, 4.5%) or immediately adjacent to the coastal tier of counties (Brantley, 3.2%) are experiencing the highest growth rates.

The SRB lies within the Coastal Plain physiographic province, which extends throughout the southeastern United States. The structure and function of lotic (flowing water) ecosystems in the southeastern United States Coastal Plain result from geological repeated periods of land submergence and climatic forces working throughout time, with some recent direct influence from human activities. There are five rivers in Georgia's Coastal Plain forming a collective Black Water river basin: the Ochlockonee, Ogeechee, Satilla, St. Mary's, and Suwannee (44). The black water rivers and streams, named for the tea or black color of their deep water, are tinted by organic acids leached from the swamps on the tributary floodplains (43, 52). This water has a low content of suspended sediments but a high concentration of dissolved organic matter (DOM). These rivers often drain regions with sandy soils that do not retain DOM leached from terrestrial vegetation; hence the DOM is washed into the river, where it imparts a color to the water (7). These river systems are also characterized by low topographic gradients and from late spring to late autumn, by high temperatures and low flows (43). They are also characterized by low DO concentrations and low concentrations of suspended sediments (43). Three of the five black water rivers in Georgia's Coastal Plain, the Ogeechee, Satilla, and St. Mary's Rivers, drain to the Atlantic Ocean, while the remaining two, the Suwanee and Ochlockonee, are Gulf Coast drainages (44).

The topography of the SRB is relatively flat and is characterized by nearly level, well drained upland soils that are sandy and porous, to poorly drained sandy and very poorly drained clayey soils (6, 25). The upper part of the Satilla Basin is in the Tifton-Vidalia upland of the Coastal Plain with dense dendritic stream networks, and riparian wetlands along streams. The

SRB is characterized by mild winters and hot summers. The mean annual temperature is 68^{o}F (20^{o}C). Mean annual precipitation ranges from 116.84 to 137.16 cm (46 to 54 inches) per year. Rainfall is fairly evenly distributed throughout the year, but a distinct dry season occurs from mid-summer to late fall, and rainfall is greatest in March and least in October. Water tables are commonly at or near the surface during wet seasons, and the soils are subject to flooding or often ponded (6, 25).

Agricultural land use

Prior to European settlement, the Coastal Plain was covered by extensive longleaf pine forests, that were gradually converted to agriculture in the years prior to the twentieth century (6). Over the past 100 years much of the land of the SRB has been converted from agriculture to forest, although much of the forest is in pine plantations. Modern mechanized silvicultural and agricultural practices have disturbed natural hydrologic regimes and soil drainage through plowing and extensive ditching used to convert seasonally flooded wetlands into pine plantations, and through increased use of irrigation (25).

Agriculture, however, is a key component of the SRB's economy. In 2007, the market value of agricultural products sold contributed over $5.3 million to the local economy (68). Agriculture in the SRB is a varied mix of animal operations and commodity production. In 2007 there were 967,991 acres of farm land in the SRB dedicated to agriculture (68). All major commodities that are grown in Georgia (peanuts, corn, cotton, oats, rye, sorghum, soybeans, and tobacco) are produced in the SRB (25, 68, 69). Irwin County ranks 1st in the state of Georgia in corn production (for grain). Five of the counties in the SRB (Appling, Atkinson, Coffee, Pierce, and Wayne) rank in the top 10 of tobacco production in the state (68). In addition to commodity production, animal agriculture is prominent in the SRB as well. Coffee county, a major

agricultural county, ranks among the state's highest producers in cattle, swine, broiler, and layer production (68, 69). Appling county has significant milk production from dairies ranking 5th in the state of Georgia in value of sales by commodity group (68).

Along with significant agricultural production, however comes an increased potential for agricultural non-point source pollution. Animal operations in the counties that comprise the SRB used 2.27 million gallons of water per day in 1995 (25). Additionally, 2.9 million tons per year of animal waste was generated on these operations (25). Producers handle animal waste through various management activities that utilize nutrients, and other soil amendments to benefit agricultural production. The common agricultural practice of spreading livestock manures to land presents an obvious and well-described mode of contamination of water resources at the field level. If these wastes contain pathogenic microorganisms such as *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Clostridium perfringens*, *E. coli* O157:H7, and the protozoan pathogens *Cryptosporidium parvum* and *Giardia lamblia* then this contamination can lead to transport of pathogens to downstream water resources (10, 29, 30, 37, 40).

Water quantity (availability and use)

Groundwater resources in the SRB are supplied by the Floridan aquifer system, one of the most productive ground water reservoirs in the United States (6, 25). It is used as a major water source throughout most of South Georgia. Wells in this aquifer system are generally high-yielding and are extensively used for irrigation, municipal supplies, industries and private domestic supply (25, 44). A more detailed description of the Floridan aquifer system has been reviewed by Miller and Survey (44).

The main streams of the SRB are the Satilla River itself and its largest tributaries, the Little Satilla River, the Alabaha River, Seventeen Mile River, and Hurricane Creek. The Satilla rises in Ben Hill and Coffee counties at an elevation of about 350 feet (6, 25). The Satilla flows generally east-southeast for 418.43 km (260 miles) and empties into the Atlantic Ocean through St. Andrews Sound, north of Cumberland Island (6, 25). In the upper reaches, the river is bordered by swamps, except where it's touched by bluffs, which sometimes reach to a height of 50 feet above the river (6). From a width of 0.40 km (0.25 miles) at river km 11.27 (mile 7), the Satilla gradually widens, becoming approximately 2.41 km (1.5 miles) across at the mouth. The lower reaches of the river are bordered by a salt marsh and have a maximum width of about 4.83 km (3 miles). The streams of the SRB are typical of the Coastal Plain. They generally lack the riffles and shoals that are common to streams in the Piedmont Province to the north, and exhibit more extensive floodplain development and greater sinuosity (bending or curving) (6, 25).

The areas of least surface water availability are the headwaters of most of the tributaries along the northern boundary of the SRB. As the tributaries approach their lower extremities and merge with other streams, the available flow becomes much more reliable. However, the topographical characteristics of the Satilla and other Coastal Plain basins are such that they do not produce surface runoff to the extent that the basins of the Piedmont, Valley and Ridge and Blue Ridge Mountains provinces do (25). Relatively small stream flow rates during low-flow periods characterize rivers in the Coastal Plain; as many have virtually no flow during extended dry periods (6, 25).

Alber and Smith (7) compiled information on water use in the SRB from the Georgia Water Use Program (http://ga.water.usgs.gov/), which regularly surveys both water sources (groundwater and surface water) and water uses (domestic, commercial, industrial, mining,

irrigation, livestock, thermoelectric, and hydroelectric) as part of the USGS National Water Use Synthesis (http://water.usgs.gov/watuse/). In 1995, total water withdrawals in the SRB amounted to 43 million gallons per day (mgd); of that 74% (32 mgd) was withdrawal of groundwater and 26% (11 mgd) came from surface water (7). The largest withdrawals in the SRB were for public use (26%, 11 mgd) and irrigation (49%, 21 mgd). Of the 28 mgd of water that was actually consumed, 75% (28 mgd) was due to irrigation reflecting the magnitude and extent of agriculture in the SRB (7). Alber et al. (6) more recently noted that there were probably large changes in water use patterns in the SRB prior to the time that adequate records have been kept; and, future changes in land use and population growth will likely change these patterns.

POULTRY PRODUCTION, POULTRY LITTER, AND PATHOGENS

Poultry production and poultry litter

Globally, poultry production is one of the fastest growing segments of the animal industry and in 2005 the production of poultry meat world wide was more than 81 million tons (77). The largest poultry producer in the world is the United States (US) followed by China, Brazil, Mexico, and France (77). Together these countries accounted for a combined output of 57% of the total poultry meat produced in 2005 (77). As the world continues to shift towards a global market economy (more focused on international trade in poultry products), poultry production will continue to increase in those countries that have the natural resources, and that are economically competitive and politically open to business investments (4).

Poultry production in the US is concentrated in the southeastern and south-central states because of the favorable climate, low labor costs, and comparative advantage in feed production (51). Together, these two regions produce more than 85% of the broiler meat supply found in

the United States. The leading broiler-producing states, Georgia, Arkansas, Alabama, Mississippi, and North Carolina account for more than 60% of all of the broiler meat produced in the US (51) and poultry production is increasing rapidly in the Coastal Plain region of Georgia.

Poultry litter refers to the mixture of bird feces/manure and material used by the poultry producers for bedding during the production cycle. The litter material is typically sawdust, wood shavings, wheat straw, peanut hulls, or rice hulls (21, 54). During broiler production, the accumulating manure is mixed with the litter. To clean out the poultry manure, one must necessarily remove the mixture of bedding material and manure. Based on a study of eight broiler houses in Georgia, Perkins et al. (54) determined that an average of 1.5 kg (dry basis) litter per bird was produced annually. A typical broiler house containing 20,000–25,000 broilers per flock with a maximum of five flocks a year produces from 125 to 150 tons of litter material annually (51, 54).

The majority of poultry litter (>90%) is applied to agricultural lands no more than a few miles from where it was produced (47). Application of poultry litter as a fertilizer on pasture and cropland is accepted as good agronomic practice as it contains important plant nutrients, i.e. nitrogen (N), phosphorus (P), and potassium (K) (21, 23, 26, 47). Although not a common practice in the Southeast, it can be fed to beef cattle as a feed supplement (21, 57). Poultry litter is applied by broadcasting (surface application) methods, which leaves the litter exposed to environmental processes; and the litter application rate varies based on the crops planted and the existing soil quality (47, 51).

Most environmental concerns about poultry litter management either have focused on the effects of nutrients, especially N and P, on water quality or have emphasized odor problems and air quality (21, 47, 51, 53). Research has tied the spreading of livestock manures to land with

nutrient runoff, as the disposal of these wastes have been shown to contaminate water resources at the field level; and may lead to contamination of downstream water resources (2, 21, 26, 51).

Pathogens in poultry litter and transport

Microbes (pathogens) from manure are often low on the priority list for control and remediation, despite the fact that several outbreaks have been traced directly or indirectly to livestock operations (16, 26, 53). As an example, the Walkerton, Canada, waterborne outbreak of 2000 resulted from entry of *Escherichia coli* O157:H7 and *Campylobacter* spp. from neighboring farms into the town water supply (17). Additionally, it has been shown that when poultry litter is used as a soil amendment, contamination of produce can occur (31, 32). Survival studies by Islam et al (32) detected *Salmonella* persistence in soil amended with an avirulent strain of *Salmonella enterica* serovar Typhimurium added to poultry compost after seeds were sown for 84 and 203 days on radishes and carrots. Islam et al. (31) also showed that *Salmonella* persisted for 161 and up to 231 days in soils amended with an avirulent strain of *Salmonella enterica* serovar Typhimurium added to poultry composts on which lettuce and parsley, respectively, were grown. Furthermore, *Salmonella enterica* serovar Typhimurium was detected for up to 63 days and 231 days on lettuce and parsley, respectively.

As it is impractical to monitor water for every individual pathogen, contamination is often based on the presence of indicator organisms such as total coliform, Enterococci, *Escherichia coli* (*E. coli*), and fecal coliform bacteria (65). Several researchers have reported on the presence of fecal bacteria from poultry litter in runoff from field soils (20, 27, 34, 45, 47). Rainfall simulations in these studies of field plots amended with poultry litter resulted in elevated concentrations of indicator organisms. It may be inferred then, that runoff from agricultural fields to which poultry litter has been applied could have unacceptable concentrations of these

fecal indicator bacteria; as well as the pathogens that these indicators are used to demonstrate the potential presence or absence of (33).

Presence of *Salmonella* and *Campylobacter* in poultry litter is not ubiquitous. Although Shepherd et al. (59) showed the persistence of *Salmonella* in composted poultry litter heaps (59), *Salmonella* was not detected in second-phase composting; suggesting that a complete compost cycle may limit *Salmonella* detection. Additionally, neither *Salmonella* nor *Campylobacter* were detected from two loads of poultry litter applied under the conditions of drought and conservation tillage by Jenkins et al (33). The researchers did however detect elevated concentrations of fecal bacteria following a storm event and noted that concentrations of pathogens may vary widely between poultry litter based on operational practices (e.g. management and storage of litter).

SUMMARY

Although salmonellosis and campylobacteriosis are more commonly associated with the consumption of contaminated food products such as meat, poultry, eggs, milk, seafood, and fresh produce, transmission of *Salmonella* and *Campylobacter* can occur by many routes including transmission from animals to humans when manure is used as a fertilizer for food crops eaten raw, by storm water runoff from land applied manure to surface wasters, or by pathogens percolation to ground water. Broiler production is a rapidly expanding industry in the southern coastal plain of Georgia; and much of the resulting poultry litter is applied to fields and pastures. More than 90% of poultry litter is applied to agricultural lands no more than a few miles from where the litter was generated. Thus in states with a large or growing poultry production industry, such as the Coastal Plain of Georgia, increasing demands are being imposed on producers to efficiently manage this waste. The potential for movement of *Salmonella* and

Campylobacter from nonpoint sources such as land applied animal manures exist and the presence of these pathogens in the Coastal Plain must be examined to determine their level of risk to public health.

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FIGURE LEGEND

Figure 2.1. Map of Satilla River Basin

Figure 2.2. USGS Map of Satilla River Basin Counties

Figure 2.1.

Map reprinted from (25)

Figure 2.2.

Map reprinted from (25)

CHAPTER 3

SATILLA RIVER BASIN ASSESSMENT: IMPACT OF AGRICULTURAL LANDUSE AND MUNICIPAL WASTEWATER TREATMENT ON STREAM WATER QUALITY [1]

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ABSTRACT

From August 2007 to August 2009 a study of the Satilla River Basin (SRB), a mixed-use rural watershed located in the coastal plain of southern Georgia (USA), was conducted to provide an understanding of the existing conditions, constraints, and water quality found therein. Watersheds were sampled that represented: 1) agricultural areas receiving poultry litter application, 2) agricultural areas with poultry houses and receiving poultry litter application, 3) reference areas with little or no agricultural activity, 4) a small watershed receiving direct discharge from a wastewater treatment plant (WWTP), which receives municipal and poultry slaughterhouse waste, and 5) larger watersheds on the main channel of the Satilla River and a major tributary Seventeen Mile River. Land use differences between the watersheds were responsible for the differences in water quality and nutrient variables measured. Eighty-three of the Dissolved Oxygen (DO) samples (35.78%, N=232) were below the Georgia Environmental Protection Division (GAEPD) DO minimum standard. This percentage would indicate that during the period of study, the SRB did not support its designated use based on low DO levels. Nitrogen and phosphorus concentrations were higher at the WWTP and generally the presence of nutrients in the SRB was concentrated in the areas where agriculture production and poultry production were concentrated. Microbial indicators were consistently detected from all sites studied (13 total sites) and indicators were detected at levels above water quality thresholds in 28% of samples based on fecal coliform bacteria, 34% of samples based on *E. coli* levels, and 74% of samples based on enterococci levels.

INTRODUCTION

The Satilla River Basin (SRB) lies within the Coastal Plain physiographic province, which extends throughout the southeastern United States. The SRB is located in the southeastern part of Georgia, covering an area of 3,940 miles^2 (10,204 km^2) and is flanked by the Altamaha River basin to the north and the Suwannee and St. Mary's River basins to the south. The SRB covers all or part of 15 Georgia counties. Population growth in the SRB has exceeded 20% per decade in recent years, although the watershed is still primarily rural (3). The local linkages between land use and water quality have cumulative effects within a region, its watershed, and the receiving coastal waters. The effects of these linkages vary as the cultural and ecological landscape varies with population growth, changes in land use, and climatic events. Land use changes, often resulting in changes to land cover, are significant because land cover provides many ecosystem goods and services, including the production of food and fiber, clean air and water, energy resources, and natural ecosystems that provide for both biodiversity and recreation (14).

Over the past 100 years much of the land of the SRB has been converted from agriculture to forest, although much of the forest is planted pine. Modern mechanized silvicultural and agricultural practices have disturbed natural hydrologic regimes and soil drainage through plowing and extensive ditching used to convert seasonally flooded wetlands into pine plantations, and through increased use of irrigation (10). Agriculture, however, still remains a key component of the SRB's economy as well as employment to many residents. In 2007, the market value of agricultural products sold contributed over $5.3 million to the SRB local economy (33). Agriculture in the basin is a varied mix of animal operations and commodity production. In 2007 there were 967,991 acres (3,917 km^2) of farm land in the SRB dedicated to

agriculture (33); and all major commodities that are grown in Georgia (peanuts, corn, cotton, oats, rye, sorghum, soybeans, and tobacco) are produced in the SRB (10, 33, 34).

In addition to commodity production, animal agriculture is prominent in the SRB as well. Coffee county, a major agricultural county, ranks among the state's highest producers in cattle, swine, and poultry (broiler and layer) production (33, 34). In 2002, Coffee county produced 37,401,458 broilers (34). Broilers are chickens that have been selectively bred and reared for their meat rather than eggs. Considering that one chicken produces 1.5 kg yr^{-1} poultry litter (a mixture of raw poultry manure and bedding material such as sawdust, wood shavings or rice hulls, and feathers) (26), more than 56,000 metric tons of poultry litter was produced in Coffee county in 2002.

Application of poultry litter as a fertilizer on pasture and cropland is accepted as good agronomic practice as it contains important plant nutrients, i.e. nitrogen (N), phosphorus (P), and potassium (K) (8, 9, 11). Poultry litter is applied by broadcasting (surface application) methods, which leaves the litter exposed to environmental processes. A large body of research has tied the spreading of livestock manures to land with nutrient runoff, as the disposal of these wastes may contaminate water resources at the field level; and may even lead to contamination of downstream water resources (2, 8, 11, 16, 24).

Much of the concern with regard to nonpoint source pollution in the SRB revolves around broiler production (34). Although trends have changed with recent developments in the poultry industry, the long term trend is expected to be increased poultry production in the Coastal Plain of Georgia. The SRB provides a model for examining the potential effects of this future expansion. The purpose of this research was to define the current state of knowledge of the SRB with specific emphasis on the upper portion of the SRB by means of monitoring the water quality

of watersheds with varying levels of agricultural landuse and poultry production. Watersheds were sampled that represented: 1) agricultural areas receiving poultry litter application, 2) agricultural areas with poultry houses and receiving poultry litter application, 3) reference areas with little or no agricultural activity, 4) a small watershed receiving direct discharge from a wastewater treatment plant (WWTP), which receives municipal and poultry slaughterhouse waste, and 5) larger watersheds on the main channel of the Satilla River and a major tributary Seventeen Mile River. An examination of the water quality of the SRB provides a useful reference point against which the effects of agricultural development in other coastal plain areas within the region may be assessed.

METHODS AND MATERIALS

Description of sample sites in the Satilla River Basin

The U.S. Geological Survey (USGS) has divided the SRB into three sub basins or 8-digit Hydrologic Unit Codes (HUCs): Satilla River (03070201), Little Satilla River (03070202), and the Cumberland-St. Simons (03070203). This study was conducted in the upper portions of the Satilla River HUC. Thirteen sampling sites were established (Figure 3.1) and watershed characteristics are shown in Table 3.1. The differences among land cover and land use types were based on 1998 land use classification (Georgia GIS clearinghouse). Additionally, 2009 crop year data for cotton was obtained from the USDA Farm Service Agency (FSA) to map the spatial distribution of cotton in the SRB (Figure 3.2).

The largest city in the study area is Douglas, GA (Figure 3.1) with a population of 10,639 (2000 Census). Douglas is home to a broiler bird processing plant and is supported by over 112 poultry producers and over 440 poultry houses. These poultry houses are operated on a variety of farms and given the large amount of cropland in the upper SRB, the majority of the poultry

litter from the poultry houses is spread within the watershed (B. Bannister, USDA-NRCS personal communication).

Reference site

Site 1, the control or reference site, is on Rocky Creek, which is located adjacent to the northern part of the SRB in Coffee County. Rocky Creek is part of a Nature Conservancy owned property named Broxton Rocks Preserve. The Nature Conservancy is working to restore the original longleaf pine-wiregrass community through prescribed fire and planting.

Agricultural areas receiving poultry litter application

Sites 2 and 3 are agricultural watersheds with no poultry houses. Site 2 is on Little Hurricane Creek. Site 3 is on the Upper Satilla Creek. Site 2 is predominately in Jeff Davis County and experienced a 12% decrease in the total number of farms from 2002 to 2007, however, this decrease was counterbalanced by a 17% increase in the average size of farms (33). The Upper Satilla Creek, site 3, has a drainage area of 45.17 km^2 in Irwin and Ben Hill Counties in the northwestern part of the SRB study area. The principal agricultural enterprise in both of these watersheds is cotton and peanut production.

Agricultural areas with poultry houses and receiving poultry litter application

Sites 4 and 5 are watersheds with the largest numbers of poultry houses in the SRB; the number of poultry houses per km^2 is similar for these two watersheds. Site 4, Pudding Creek watershed, has a drainage area of 204 km^2 and has a total of 50 poultry houses. Site 5, Hurricane Creek has a total of 21 poultry houses in a drainage area of 45.70 km^2.

Additional sample locations

A mixture of domestic, light-industrial and agricultural influent wastewater (site 6) to the City of Douglas WWTP and effluent (site 13) from the WWTP were sampled. Site 14 is from a

pond that receives the effluent from site 13 directly and was considered part of the continuum of WWTP sites. Wastewater from the broiler processing plant is piped to the treatment plant and discharge from the treatment plant enters a small stream that flows into Seventeen Mile River, a major tributary of the Satilla River (Figure 3.1). The WWTP has a permitted discharge of 6 million gallons per day (MGD) and handles both human and poultry slaughterhouse waste, of which about 50% is provided by wastewater from the broiler processing plant facility (Delnee Wilcox, personal communication).

The other 5 watershed sites (sites 8, 9, 10, 11 and 12) were on larger streams above and below the WWTP. Sites 8 and 9 were on Seventeen Mile River, above and below the WWTP, respectively (Table 3.1 and Figure 3.1). Sites 10 and 11 were on the Satilla River, above and below the confluence with Seventeen Mile River, therefore above and below the Douglas WWTP. Site 12 was on the main channel of the Satilla, below the confluence of the Satilla River and Hurricane Creek (Table 3.1 and Figure 3.1) and was the most downstream station sampled.

Description of hydrology and water quality monitoring

From August 2007 to August 2009 monthly samples were obtained on a regular schedule from all sites located within the upper SRB, as well as the influent to the WWTP and the direct effluent from the WWTP. No sampling was obtained during November of each year due to low flow conditions. The following water quality measurements were taken *in situ* with a YSI® 6600 Multiparameter Sonde (Yellow Springs, OH): temperature (^{o}C), conductivity (mS cm^{-1}), pH, dissolved oxygen (DO, mg L^{-1}), turbidity (nephlometric turbidity units, NTU), oxidation reduction potential (ORP, mV), and chlorophyll *a* (mg L^{-1}). The probe was placed at about 30 cm depth in the deepest part of the stream channel and used to record instantaneous values after stabilization.

The Satilla River (03070201) has a calibrated USGS continuous stream flow (discharge) monitoring station at the Satilla River, near Waycross, GA (02226500). This stream gage is approximately 8 miles (13 km) downstream from sampling site 12 and the watershed above site 12 is about 91% of the watershed at the USGS gage. Using discharge measured (ft^3 sec^{-1}, cfs) at this gage, we calculated the predicted area proportional discharge for all of the water quality monitoring sites as [(drainage area of sample site) / (drainage area of USGS gage at Waycross, GA)] * (measured discharge at USGS gage at Waycross, GA). A HOBO® Data Logging Rain Gauge (Bourne, MA) was installed in the SRB to measure rainfall; however, as a result of vandalism an incomplete series of rainfall was recorded. Basin-wide rainfall was estimated using data retrieved from a National Climatic Data Center (NCDC) weather station in Douglas, Georgia (Cooperative Station Identification # 092783) (www.georgiaweather.net). In order to examine the potential impact of rainfall on other study variables, several measures of precipitation were compiled for analyses: daily rainfall, rainfall on the day preceding the sampling, total rainfall in the 7 days before the day sampled, and total daily rainfall 30 days preceding the sampling.

Water quality analysis

All water samples were collected in sterile 3-liter polypropylene bottles as discrete surface grabs and transported on ice to the University of Georgia National Environmentally Sound Production Agriculture Laboratory (NESPAL). Sample processing began immediately upon return to the lab; and the 3 liter composite sample was split into one liter samples for microbial indicator and nutrient analyses.

Nutrient analyses

One liter was filtered through Whatman 934 AH glass microfiber filters (0.45 micron effective pore diameter) in preparation for dissolved nutrient analysis. An aliquot of the filtrate was stored for nutrient analysis. In addition, an aliquot of the unfiltered sample was frozen for analysis of total Kjeldahl N (TKN) and total P in a digestate. The filtered sample was analyzed for nitrate-N (NO_3-N), ammonium-N (NH_4-N), dissolved molybdate reactive P (ortho P), and chloride using Environmental Protection Agency (EPA) approved colorimetric techniques (4) on a Lachat Flow Injection Analyzer within seven days of collection. The unfiltered sample was analyzed for TKN and total P using digestion and colorimetric techniques adapted from EPA approved methods (4). Filtered samples were analyzed for total carbon (TC), inorganic carbon (IC), and dissolved organic carbon (DOC) using a Shimadzu model 5050 TOC analyzer.

Microbial indicator analyses

Concentrations of *E. coli*, and enterococci were determined with the Colilert and Enterolert Quanti-Tray system (from the IDEXX system most probable number estimation) (IDEXX Laboratories, Inc., Westbrook, ME, USA). Trays were inoculated and incubated for 24 hours at 35^{o}C and 41^{o}C, for *E. coli* and enterococci respectively, according to the manufacturer's guidelines. Water samples were screened for fecal coliform bacteria by membrane filtration and growth on mFC agar following Standard Methods (13). Plates were incubated for 24 ± 2 h in a 44.5^{o}C water bath. All blue colonies were counted as fecal coliform bacteria and enumerated as colony forming units (CFU) per 100 ml.

Description of data analysis and statistics

Data were analyzed using Statistical Analysis System (SAS) release 9.1 (Cary, NC). The concentration data (nutrient and fecal indicator bacteria) were tested for normal distribution

using the Univariate Procedure of SAS. The concentration data were not normally distributed so typical analysis of variance was not used. Instead, the NPAR1WAY Procedure of SAS with the Kruskal-Wallis test (the nonparametric equivalent of a one-way ANOVA) was used to test for significant differences among the measured parameters between sampling sites. The Tukey's honestly significant differences test (Tukey's HSD) was used as a post-hoc test for pair-wise comparison and letters were assigned to indicate significant differences for presentation. The Spearman's rank correlation coefficient was used to determine measures of association. Spearman's is a non-parametric measure of statistical dependence between two variables and an analog of the Pearson product-moment correlation coefficient (38). When levels were below the limit of detection a value of zero was used for statistical analysis. Only those environmental parameters that showed significant differences or relationships of at least the 0.05 level were reported (p values ≤ 0.05).

RESULTS

From August 2007 to August 2009, 232 samples were examined from the Satilla River basin to characterize the watershed and determine whether varying landuse and agricultural production impact water quality. The total number of water samples tested per site varied from 14 to 23 (mean = 19) as some sites (especially the smaller streams) experienced zero flow conditions more often than others. Data from the influent to the City of Douglas WWTP (site 6) was not included in water quality analysis as the raw, untreated wastewater influent sample was characterized *a priori* to be polluted. From December 2008 to March 2009 water samples could not be analyzed for pH, or chlorophyll *a* from April 2009 to June 2009 due to instrument errors.

Spatial differences in hydrology and water quality

Table 3.2 shows the means and range for measured water quality parameters for each site. Using Kruskal Wallis a significant effect of sample site on discharge, temperature, conductivity, DO, pH, turbidity, ORP, and chlorophyll *a* ($p < 0.05$) was observed. Lower temperatures were observed from the control and agricultural stations upstream of the WWTP (sites 2, 3, and 5), excluding site 4 at Pudding Creek. The highest temperatures were observed from the WWTP effluent stations (sites 13 and 14). Conductivity measured at site 14 was also significantly higher than all other sites. Site 13, the direct effluent station at the treatment plant was significantly higher than all other sites for measures of DO, pH, turbidity, and chlorophyll *a*. Nutrient concentrations also showed a significant effect of sample site (Table 3.3). Concentrations were significantly higher from the WWTP effluent stations (sites 13 and 14) than all other sites for all measured nutrients, except total carbon and dissolved organic carbon. DOC concentrations from these sites were significantly lower than all other stations. In general, nutrient concentrations as well as other measured water quality parameters were higher at the WWTP effluent stations (sites 13 and 14) than all other sites observed and were considered outliers to the overall statistical analysis. As a result, sites 13 and 14 were omitted from subsequent total basin statistical analysis and analyzed separately.

Using Kruskal Wallis, temperature ($H = 18.58$, 9 d.f., $p = 0.03$), conductivity ($H = 34.74$, 9 d.f., $p = < 0.05$), ORP ($H = 26.01$, 9 d.f., $p = 0.002$), and chlorophyll *a* ($H = 47.61$, 9 d.f., $p = < 0.05$) were the water quality parameters that varied significantly when the WWTP stations (sites 13 and 14) were removed from statistical analyses (See Appendix A, Table A.3.1). TKN and TP were the only nutrients that did not vary significantly when the WWTP stations were removed from statistical analyses. Although there was a significant effect of sample site on temperature,

NH_4 (H = 25.65, 9 d.f., p = <0.05), and NO_3 (H = 13.27, p = < 0.05) within the sites excluding the WWTP stations, temperature, ammonium and nitrate concentrations could not be significantly discerned on a pairwise basis (p > 0.05). Conductivity, ortho P, chloride, and inorganic carbon measured at site 9, located on Seventeen Mile River downstream from the WWTP, were significantly higher than all other sites suggesting effluent from the treatment plant may be affecting downstream waters. Additionally, we observed a trend among water quality and nutrients as there were significant groupings between the SRB study area sites, with smaller watersheds (sites 1 to 5) and the larger downstream stations (sites 8 to 12) separating into two distinct clusters (See Appendix A, Table A.3.1). Concentrations of nutrients were higher in the agricultural watersheds with poultry houses and the sites downstream of the WWTP. Figure 3.2 shows the different mean nutrient concentrations in the agricultural watersheds without poultry houses (sites 2 and 3) and with poultry houses (site 4 and 5) compared with the control station (site 1) and the water sites downstream of the WWTP station (sites 9, 10, 11, 12 and including site 8 on Seventeen Mile River that is downstream of the agricultural sites but upstream of the WWTP).

To investigate the difference between these watershed categories further, statistical analysis was conducted with the smaller watersheds and larger watersheds separately. There was a significant effect of sample site location on conductivity and chlorophyll *a* (p<0.05) for both the smaller (sites 1, 2, 3, 4, 5) watershed sites and the large watershed sites downstream of the WWTP as described previously including the control (site 1) (See Appendix A, Table A.3.2). In the small watershed sites conductivity (H = 26.24, 4 d.f., p < 0.05), ORP (H = 15.39, 4 d.f., p < 0.05), pH (H = 9.93, p = 0.04) and chlorophyll *a* (H = 29.16, 4 d.f., p < 0.05) were significantly different between sites. In the smaller watershed sites conductivity and pH were significantly

highest at site 3 and ORP was significantly the lowest at this site. Chlorophyll *a* was significantly highest at site 4, Pudding Creek. ORP was significantly higher at site 2 than the other small watershed sites and pH at site 2 was significantly lower than all other small watershed sites. Although there was a significant effect of sample site on chlorophyll *a* ($H = 11.77$, 5 d.f., $p = 0.04$) in the larger watershed sites, concentrations could not be significantly discerned on a pairwise basis ($p = 0.34$). Conductivity ($p < 0.05$) measured at site 9 however was significantly higher than all of the large watershed sites.

The nutrients NH_4, NO_3, ortho P, chloride, TC, IC, and DOC were also significantly different among the individual smaller watershed and larger watershed analyses (See Appendix A, Table A.3.3). While there was a significant effect of sample site on NH_4 ($H = 21.96$, 4 d.f., $p < 0.05$), NO_3 ($H = 13.27$, $p < 0.05$), ortho P ($H = 39.8117$, 4 d.f., $p < 0.05$), and chloride ($H = 11.20$, 4 d.f., $p = 0.02$) within the small watershed sites, concentrations could not be significantly discerned on a pairwise basis [NH_4 ($p = 0.12$), NO_3 ($p = 0.35$), ortho P ($p = 0.19$), chloride ($p = 0.24$)].

Total carbon and DOC levels in the small watershed sites were significantly higher at sites 2, 4 and 5 than the control station site 1 and site 3. Inorganic carbon levels were highest at site 3 and lowest at site 2. In analyzing the larger watershed sites separately, Tukey's HSD test did not yield significant difference among NH_4 ($p = 0.31$) or total carbon ($p > 0.05$) concentrations. Nitrate ($p < 0.05$), ortho P ($p < 0.05$), chloride ($p < 0.05$), and inorganic carbon ($p < 0.05$) concentrations however were significantly higher at site 9 than at all other stations (See Appendix A, Table A.3.3). Total phosphorus concentrations at site 9 were also significantly higher than all the large watershed sites, although using kruskal wallis a significant site effect was not discerned ($H = 10.36$, 5 d.f., $p = 0.07$).

As expected there were minimal significant differences between water quality and nutrient parameters measured from the WWTP effluent stations. Turbidity ($H = 10.19$, 1 d.f. $p = < 0.05$) and conductivity ($H = 12.21$, 1 d.f., p value < 0.05). Turbidity was significantly higher at site 13 the direct effluent from the WWTP. Conductivity was higher at site 14.

Temporal differences in hydrology and water quality

Rivers across the state of Georgia experienced moderate to severe hydrologic drought in 2007. Daily discharge data obtained for the Satilla River sampling station near Waycross, GA (Gauge 02226500) from the U.S. Geological Survey (USGS) are presented in Figures 3.4 with mean predicted area proportional flow for the SRB. Stream levels and discharge were low throughout 2007. The Satilla River remained relatively low, but incrementally rose to and above median daily flows over the course of the study period (September 2007 to October 2007; March 2008, November 2008 to December 2008, and April 2009 to June 2009) based on 71 years of data (Figure 3.4). Over the period of study, the mean flow at the USGS gage at Waycross was 989.33 cubic feet per second (cfs), with a peak flow of 41,100 cfs on 5-April 2009. In the seven days prior to this maximum flow, there was approximately 15.95 cm (6.28 inches) total of rain recorded at the USGS gauge. Historic widespread flooding occurred throughout South Georgia from a storm event beginning in the late evening of 27-March and continuing through 3-April 2009 (20). Maximum gauge height of 6.78 m (22.24 ft) also occurred on 5-April 2009. Mean predicted area proportional flow was positively correlated with 7-day antecedent rainfall ($r = 0.23$, $p < 0.05$) and 30-day antecedent rainfall ($r = 0.36$, $p < 0.05$). Figure A.3.1 in Appendix A shows mean monthly discharge from the USGS gage at Waycross, mean monthly predicted area proportional flow and 30-day antecedent rainfall.

Time series of mean water quality conditions at the small watersheds (sites 1, 2, 3, 4, 5), large watersheds (sites 8, 9, 10, 11, 12) and WWTP stations (sites 6, 13, 14) are shown in Appendix A, Figures A.3.2 and A.3.3. Water temperatures in the small watershed sites closely parallel those in the large watershed sites and the WWTP. Seasonal high temperatures coincided with seasonal low dissolved oxygen concentrations in both the small and large watersheds. DO concentrations were lowest in 2007 when temperatures were higher throughout the SRB reflecting drought conditions. Coincidentally, DO concentrations were not associated with discharge from either the small or large watershed stations (Spearman's rank correlation p value > 0.05); and were frequently below the Georgia Environmental Protection Division (GAEPD) standard of 4 mg L^{-1} (See Appendix A, Table A.3.1). DO concentrations were negatively correlated with temperature ($r = -0.28$, $p = <0.05$) and several measures of rainfall (See Appendix A, Table A.3.4). The correlation matrix of association between significant ($p = <0.05$) measured parameters at all sites (excluding the WWTP station sites) is shown in Appendix A, Table A.3.4.

In May of 2008 there was a spike in chlorophyll *a* concentrations at the WWTP stations. Photosynthetic pigments, like chlorophyll a, are measured as a surrogate for algal biomass (36). Field notes of physical conditions at the time of sampling confirm the presence of an algal bloom at the treatment plant on this date. DO concentrations at the WWTP correspondingly decreased following the algal bloom (Figure 3.5). Total Phosphorus concentrations at site 8 upstream from the treatment also peaked prior to the algal bloom and TP concentrations at the WWTP coincided with the bloom.

Fecal indicator bacteria.

Fecal indicator bacteria were detected at every station sampled. The mean and range for the indicators are shown in Table 3.4. As expected, fecal indicator concentrations from the WWTP influent (site 6) were significantly greater than all other sites, including the WWTP effluent stations (*E. coli* ($H = 91$, 12 d.f. $p < 0.05$), enterococci ($H = 77$, 12 d.f., $p < 0.05$), and fecal coliforms ($H = 86$, 12 d.f., $p < 0.05$)). Excluding site 6, fecal indicator concentrations were significantly greatest from site 13 the direct effluent from the WWTP than all other stations (Table 3.4). The next highest concentrations of all indicators were from site 14. Enterococci levels were statistically greater for stations 13, 14, 9 (below the WWTP) and the reference station (1) than for all other stations (Table 3.4). None of the indicator levels varied significantly by site when all of the WWTP stations (site 6, 13 and 14) were removed from statistical analyses. Using the same metric of the small watershed sites and the large watershed sites for group statistical analyses, none of the indicators varied significantly by site in the smaller watershed. Fecal coliforms ($H = 9.33$, 5 d.f., $p = 0.10$) was not significantly different between site in the large watershed stations. While there was a significant effect of sample site location on *E. coli* ($H = 17.26$, 5 d.f., $p < 0.05$), and enterococci ($H = 20.02$, 5 d.f., $p < 0.05$) within the large watershed sites, concentrations could not be significantly discerned on a pairwise basis [*E. coli* ($p = 0.73$) and enterococci $p = 0.48$)]. With all data included in analysis, enterococci concentrations were the only indicator that varied significantly by sample date ($p < 0.05$) (See Appendix A, Figure A.3.4). The highest mean enterococci level was 16, 638 MPN 100 ml^{-1} in August 2007; while the lowest was 223 MPN 100 ml^{-1} in April 2008. Without the WWTP data included in the analysis, enterococci and fecal coliform bacteria both varied significantly by sample month (See Appendix A, Figure A.3.5). The highest mean fecal coliform level was 896

MPN 100 ml^{-1} in December 2008. Mean fecal coliform concentrations measured in August 2007, January 2008, August 2008, October 2008, May 2009, and August 2009 were not significantly different from each other and were all significantly higher than the remaining months (See Appendix A, Figure A.3.5).

Georgia water quality standards establish a fecal coliform bacterial criterion of a geometric mean (four samples collected over a 30-day period) of 100 MPN 100 ml^{-1} for coastal waters (littoral recreational waters on the ocean side of the Georgia coast (1)) and 200 MPN 100 ml^{-1} for recreational waters (waters used for general recreational activities such as water skiing, boating, and swimming, or for any other use requiring water of a lower quality, such as recreational fishing (1)). The waters in the SRB study area are classified as recreational (1). The number of samples collected was not adequate to satisfy 'four samples collected over a 30-day period.' In this case, the US EPA recommends the use of a one-time sample maximum of 400 MPN 100 ml^{-1} for fecal coliform bacteria, 61 CFU 100 ml^{-1} for enterococci and 235 CFU 100 ml^{-1} for *E. coli* (31, 32), which were used to evaluate data for the period of study. Fecal indicator bacteria were detected above the one-time sample maximum standard from all sites sampled. The percentage of samples above the water quality guidelines ranged from 28% (83 out of 299) of fecal coliform samples to 74% (221 out of 299) of the enterococci samples. *E. coli* was detected above the standard from 34% (103 out of 299) of the samples.

DISCUSSION

In 1972, Congress passed the Federal Water Pollution Control Act, more commonly called the Clean Water Act (CWA). The goal of this act was to "restore and maintain the chemical, physical, and biological integrity of the Nation's waters." The State of Georgia Environmental Protection Division (GAEPD) is responsible for setting and enforcing water

quality standards compliance with the CWA in Georgia. Georgia's water quality standards are found in Chapter 391-3-6-.03 of the Georgia Rules and Regulations for Water Quality Control. Streams in the SRB study area are classified as "recreational use" according to GAEPD standards. GAEPD assesses water quality data to determine if water quality standards are met and if the water body supports its classified use. The assessed waters are described in three categories: waters supporting designated uses, waters partially supporting designated uses, and waters not supporting designated uses. If monitoring data shows that standards are not achieved, depending on the frequency with which standards are not met, the water body is said to be not supporting or partially supporting the designated use. Water bodies are placed on the partially supporting list if the data (dissolved oxygen, pH, temperature) indicate a departure from a water quality standard in 11 percent to 25 percent of the samples collected (10). Generally, a stream reach is placed on the not supporting list if the data (dissolved oxygen, pH, temperature) indicate a departure from a water quality standard in greater than 25 percent of the samples collected (10).

GAEPD mandates DO concentration for water quality at a minimum of 4.0 mg L^{-1}, with a daily average of 5.0 mg L^{-1}. DO concentrations measured in the SRB ranged from 0.17 mg L^{-1} to 13.46 mg L^{-1} and were frequently below the 4.0 mg L^{-1} minimum established by GAEPD. Eighty-three of the DO samples (35.78%, N=232) were below the GAEPD DO minimum standard. This frequency would indicate that during the period of study, the SRB did not support its designated use based on low DO levels. Low DO is most often associated with elevated stream temperature or elevated biochemical oxygen demand from municipal, industrial or feedlot wastes or from storm water runoff (6, 18, 30). However, evidence suggests that low DO may be a natural phenomenon in black water streams like those in the SRB (21, 30). Levels of DO

below the 5 mg L^{-1} daily average limit have been shown in several studies, even those in largely forested watersheds or where there is extensive riparian vegetation (18, 21, 30). The GAEPD threshold for pH is a range from 6 to 8.5. pH in the SRB was measured within this acceptable range over the course of the study.

Concentrations of nutrients were higher at the sites downstream of the WWTP, suggesting that constant loading midstream from the treatment plant effluent discharge into the stream network are supplying a constant flux of nutrients (chemicals) to downstream waters. The presence of nutrients in the SRB is suspected to originate from agricultural fields where crops were grown and the use of poultry litter and other fertilizers is rather frequent. The most common use of poultry litter in the SRB is application to pasture and cotton cropland within 5 to 10 miles of poultry houses; however, litter may also be applied to cropland throughout the watershed (B. Bannister, USDA Natural Resources Conservation Service, personal communication). Figure 3.2 shows cotton land cover for 2009 in the agricultural watersheds with the location of poultry houses in and around their respective drainage area.

Fluctuations and peaks in water quality and nutrient concentrations in the SRB tended to coincide also with the processing cycle of broilers. The decrease in nutrient concentrations may be attributed to the temporary closure of the broiler processing plant in May of 2009 (15, 25). The plant closure was announced in February 2009 and there was a steady decline of nitrogen and phosphorus at the WWTP. Ammonia levels at the WWTP flattened after May 2009. The broiler processing plant recently announced the plant will be reopening prior to January 2011 (27).

Fecal indicator bacteria such as enterococci and *E. coli* are measured in surface waters because they are thought to indicate the presence of fecal matter, and potential pathogens (5, 29,

35, 37). Fecal indicator bacteria were detected above the one-time sample maximum standard from all sites sampled, and dominant pollutant sources in the SRB are likely humans, wildlife and agricultural inputs. If a water source is contaminated with high numbers of fecal enterococci the most likely host sources are humans and birds (19). This would explain the high levels of enterococci from both the control site and the WWTP. The control site located in the Broxton Rocks Preserve is home to over 100 species of birds (28) and the WWTP receives municipal waste from the City of Douglas. Fecal indicator concentrations in the agricultural watersheds with poultry houses were also higher than the agricultural watershed with no poultry houses. Several researchers have reported on the presence of fecal indicator bacteria from poultry litter in runoff from field soils (7, 12, 17, 22, 23) in rainfall simulation studies. The general consensus from these studies being that greater loads of poultry litter applied to fields resulted in higher concentrations of fecal indicators being detected. Increased levels of all of these indicators were frequently detected throughout the SRB and although there is continued debate as to efficacy of all of the microbial indicators used (5, 29, 35, 37); the presence of these indicators at this frequency does indicate a substantial risk for human exposure to enteric pathogens in the SRB.

CONCLUSION

Surface water quality monitoring provides an integrated evaluation of the physical, chemical, and biological characteristics of an aquatic system in relation to human health concerns, ecosystem, and designated uses. Agriculture, including commercial livestock and poultry farming, is the source of many pollutants in surface waters and groundwater. Monitoring of the contaminants of surface water in agricultural watersheds is a necessity to maintain a healthy nexus between the farm and the surrounding waters. Land use differences between watersheds are likely responsible for the differences in water quality and nutrient variables

measured in the present study. Although nutrient concentrations generally decreased over the course of the study, higher concentrations were more likely to occur in the smaller watershed stations where agriculture and poultry production were centered, as well as downstream from a wastewater treatment plant that process both human and poultry slaughterhouse waste. Dissolved oxygen concentrations were frequently below conventional minimum levels; although this standard may not be reflective of naturally occurring DO concentrations in black water streams. When agricultural operations are sustainably managed, they can help preserve and restore critical habitats, protect watersheds, and improve soil health and water quality. Conversely, agriculture practices (such as the spreading of livestock manures to lands) may present one of the greatest threats to ecosystems. Proper management and an understanding of not only how a given agricultural practice benefits crop production or waste disposal but the potential degradative impact being placed on the surrounding waters are also of importance. This study again shows the benefits of surface water monitoring in determining the impact of agricultural activity and landuse on water quality and ecosystem health.

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TABLES

Table 3.1. Description of the sample sites within the Satilla River Basin study area

Site No.	Stream Name (Sampling location)	Area (km^2)	Upstream/ Downstream To WWTP	Number of Poultry Producers	Number of Poultry Houses	Agricultural[b] Landuse (%)	Forest[c] Landuse (%)	Urban[d] Landuse (%)	Other[e] Landuse (%)
1	Rocky Creek (remote site)	9.10	Upstream	0	0	5	83	0	12
2	Little Hurricane (County Rd 552)	64.24	Upstream	0	0	22	65	5	8
3	Upper Satilla Cr. (Whitley Rd)	45.17	Upstream	0	0	52	31	8	9
4	Pudding Cr. (US Hwy 441)	204.80	Upstream	12	50	30	52	7	11
5	Upper Hurricane Cr. (Squirrel Bridge Rd)	45.70	Upstream	4	21	20	67	7	7
8	Seventeen Mile River 1 (GA Hwy 32)	433.50	Upstream	22	98	33	50	10	7
9	Seventeen Mile River 2 (GA Hwy 158)	462.10	Downstream	22	98	32	51	10	7
10	Satilla River 1 (GA Hwy 64)	927.10	Upstream	42	173	32	51	7	10
11	Satilla River 2 (GA Hwy 158)	2306.00	Downstream	91	368	25	57	7	10
12	Satilla River 3 (US Hwy 1)	2922.00	Downstream	112	440	23	59	7	11
6	Influent to WWTP (pipeline)	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]
13	Effluent from WWTP (pipeline)	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]	NA[f]
14	Effluent from WWTP (Sears Rd)	14.54	Downstream	0	0	23	45	27	6

a Landuse is based on 1998 landuse classification (Georgia GIS Clearinghouse)

b Includes row-crops and pasture land

c Includes deciduous, evergreen, mixed, forested wetlands

d Includes low and high density

e Includes open water, transportation, utility, clearcut/sparse vegetation and golf courses

f Not applicable

Table 3.2. Mean [a] and range of water quality parameters measured [b] in Satilla River Basin

Site		Discharge [c] (cfs)		Temperature (°C)		Conductivity (mS cm^{-1})		Dissolved Oxygen (mg L^{-1})		pH		ORP [d] (mV)		Turbidity+ NTU		Chlorophyll *a* (mg L^{-1})	
1	Mean	3.94	B	15.50	B	0.06	C	6.51	AB	4.88	BC	176.40	AB	33.01	B	9.50	B
	Min	0.00		7.65		0.00		0.17		0.00		93.00		0.00		4.40	
	Max	43.04		24.55		0.12		12.13		8.19		246.00		235.40		27.60	
2	Mean	27.55	B	15.56	B	0.07	C	4.13	AB	4.64	C	189.33	A	16.81	B	12.61	B
	Min	0.00		7.37		0.05		0.18		0.00		120.00		0.00		8.80	
	Max	303.84		25.35		0.12		9.68		7.39		250.00		178.30		16.70	
3	Mean	19.49	B	14.57	B	0.10	C	4.65	AB	5.14	AB	129.71	D	16.38	B	8.08	B
	Min	0.00		6.67		0.05		0.40		0.00		96.00		0.00		4.40	
	Max	213.64		25.53		0.15		10.58		7.77		210.00		98.70		12.80	
4	Mean	88.61	AB	19.10	AB	0.10	C	3.91	B	5.58	ABC	159.05	ABCD	9.43	B	21.58	B
	Min	0.00		7.48		0.06		0.30		0.00		104.00		0.00		4.90	
	Max	968.65		28.64		0.17		13.26		7.51		229.00		80.10		61.60	
5	Mean	19.77	B	15.40	B	0.10	C	5.24	AB	5.16	ABC	140.40	ABCD	29.81	B	15.83	B
	Min	0.00		7.70		0.06		0.20		0.00		16.00		0.00		4.40	
	Max	216.15		23.88		0.15		10.89		7.76		232.00		345.30		38.40	
8	Mean	187.56	AB	17.26	AB	0.10	C	4.57	AB	5.52	AB	143.33	BCD	36.63	B	10.28	B
	Min	0.00		6.59		0.00		0.94		0.00		89.00		0.00		5.50	
	Max	2050.34		25.40		0.52		11.20		7.74		207.00		345.60		14.90	
9	Mean	199.93	AB	17.58	AB	0.30	BC	4.57	AB	5.57	ABC	148.65	BCD	4.75	B	10.60	B
	Min	0.00		6.45		0.00		0.30		0.00		84.00		0.00		4.90	
	Max	2185.61		25.89		1.00		11.94		7.66		206.00		30.00		17.40	
10	Mean	401.12	AB	19.01	AB	0.09	C	4.10	AB	5.73	ABC	158.48	ABCD	4.90	B	10.56	B
	Min	0.00		7.19		0.00		0.25		0.00		92.00		0.00		4.40	
	Max	4384.93		27.88		0.27		13.07		8.22		243.00		52.90		19.40	
11	Mean	997.72	AB	19.64	AB	0.12	BC	5.01	AB	5.69	ABC	154.70	ABCD	16.96	B	12.36	B
	Min	0.00		7.04		0.00		0.41		0.00		85.00		0.00		4.40	
	Max	10906.76		29.07		0.27		12.08		8.01		228.00		338.70		16.90	
12	Mean	1264.78	A	20.13	AB	0.10	C	5.45	AB	5.46	BC	173.17	ABC	36.95	B	11.70	B
	Min	7.15		7.27		0.05		0.56		0.00		86.00		0.00		3.20	
	Max	13820.27		29.27		0.19		13.46		7.90		233.00		784.40		19.80	
13	Mean			22.82	A	0.36	B	7.40	A	6.05	A	135.95	CD	242.09	A	115.56	A
	Min			16.37		0.01		0.67		0.00		104.00		0.00		3.30	
	Max			28.14		2.30		11.01		8.13		184.00		1358.60		463.10	
14	Mean			22.25	A	0.81	A	5.78	AB	5.94	AB	141.22	BCD	7.83	B	25.06	B
	Min			12.41		0.02		0.56		0.00		99.00		0.00		4.40	
	Max			32.13		1.36		12.95		7.93		190.00		40.40		183.60	
All Sites	Mean	321.05		18.62		0.21		5.12		6.94		154.17		37.92		22.47	
	Min	0.00		6.45		0.00		0.17		5.16		16.00		0.00		3.20	
	Max	13820.27		32.13		2.30		13.46		8.22		250.00		1358.60		463.10	

a Means with the same letter in a column are not significantly different ($p<0.05$) using Tukey's HSD test

b December 2008 to March 2009 water samples collected could not be analyzed for pH due to instrument errors; nor chlorophyll *a* from April 2009 to June 2009

c Discharge not measured at Wastewater Treatment Plant Stations (Site 13 and Site 14)

d Oxidation reduction potential, ORP

Table 3.3. Mean [a] and range of nutrient [b] parameters measured in Satilla River Basin

Site		NH_4 (mg L^{-1})	NO_3 (mg L^{-1})	PO_4 (mg L^{-1})	Cl (mg L^{-1})	TKN (mg L^{-1})	TP (mg L^{-1})	TC (mg L^{-1})	IC (mg L^{-1})	DOC (mg L^{-1})
1	Mean	0.01 C	0.46 B	0.08 C	9.80 B	0.62 B	0.05 B	19.84 E	2.70 C	17.25 E
	Min	0.00	0.00	0.00	4.06	0.07	0.00	11.07	0.22	10.62
	Max	0.03	6.20	1.04	30.70	1.27	0.14	29.09	11.60	23.79
2	Mean	0.14 C	0.01 B	0.03 C	11.94 B	1.00 B	0.06 B	39.52 A	2.39 C	37.13 A
	Min	0.00	0.00	0.01	6.81	0.08	0.00	23.07	0.26	22.29
	Max	1.08	0.07	0.07	18.75	3.22	0.23	59.06	7.40	51.66
3	Mean	0.13 C	0.17 B	0.01 C	13.19 B	0.76 B	0.04 B	23.75 DE	5.64 BC	18.11 E
	Min	0.01	0.00	0.00	3.48	0.03	0.00	11.72	0.93	9.49
	Max	0.50	1.49	0.04	17.25	2.28	0.20	35.41	13.67	23.48
4	Mean	0.08 C	0.19 B	0.19 C	13.53 B	2.04 B	0.20 B	38.88 AB	3.23 BC	35.84 AB
	Min	0.00	0.00	0.01	3.79	0.26	0.00	21.13	0.42	17.24
	Max	0.24	2.72	1.91	26.60	13.90	2.59	55.05	7.24	49.80
5	Mean	0.09 C	0.03 B	0.04 C	13.07 B	0.89 B	0.06 B	36.61 ABC	3.32 BC	33.30 ABC
	Min	0.00	0.00	0.01	7.80	0.04	0.00	12.71	0.38	11.49
	Max	0.19	0.13	0.11	21.20	1.57	0.20	55.22	6.86	50.97
8	Mean	0.11 C	0.04 B	0.01 C	12.36 B	1.12 B	0.03 B	30.11 ABC	3.19 BC	27.36 CD
	Min	0.00	0.00	0.00	7.52	0.12	0.00	20.97	0.55	18.82
	Max	0.66	0.24	0.06	22.80	3.65	0.09	47.26	7.80	45.16
9	Mean	0.78 BC	0.29 B	1.85 BC	32.09 B	1.95 B	0.58 AB	30.22 ABCD	9.71 B	20.89 DE
	Min	0.01	0.00	0.00	7.53	0.54	0.00	16.80	0.58	9.78
	Max	12.40	2.27	8.29	139.00	9.40	2.84	44.16	30.13	34.18
10	Mean	0.08 C	0.04 B	0.03 C	11.22 B	1.54 B	0.13 B	30.59 ABCD	3.37 BC	27.39 CD
	Min	0.00	0.00	0.00	5.37	0.03	0.00	10.01	0.33	7.22
	Max	0.26	0.50	0.14	18.70	7.40	2.03	46.86	11.20	45.67
11	Mean	0.22 C	0.03 B	0.07 C	15.60 B	2.18 B	0.06 B	32.74 ABCD	2.96 C	29.84 ABC
	Min	0.00	0.00	0.00	3.59	0.12	0.00	12.49	0.34	10.86
	Max	2.75	0.17	0.23	34.44	18.50	0.15	46.21	9.55	41.94
12	Mean	0.07 C	0.02 B	0.07 C	14.83 B	1.19 B	0.10 B	31.63 ABCD	2.65 C	28.99 BC
	Min	0.01	0.00	0.00	4.89	0.04	0.00	8.14	0.31	6.63
	Max	0.27	0.07	0.53	32.50	4.11	0.79	49.67	9.99	48.97
13	Mean	5.54 A	0.99 AB	4.05 A	92.23 A	6.08 A	1.47 A	29.24 BCDE	20.37 A	9.40 F
	Min	0.01	0.00	0.01	15.00	0.57	0.04	16.29	1.44	5.58
	Max	27.50	4.67	15.20	271.00	17.40	8.94	62.47	45.98	20.64
14	Mean	3.75 AB	1.80 A	3.40 AB	77.78 A	4.02 AB	0.87 AB	28.54 CDE	20.76 A	8.37 F
	Min	0.03	0.00	0.07	22.75	0.05	0.05	15.50	8.87	3.08
	Max	24.70	9.50	15.52	208.50	12.90	2.95	54.42	39.55	14.99
All Sites	Mean	1.07	0.35	0.95	31.53	2.07	0.33	31.06	7.01	24.05
	Min	0.00	0.00	0.00	3.48	0.03	0.00	8.14	0.22	3.08
	Max	27.50	9.50	15.52	271.00	18.50	8.94	62.47	45.98	51.66

a Means with the same letter in a column are not significantly different ($p<0.05$) using Tukey's HSD test

b Ammonium (NH_4), nitrate (NO_3), phosphate (PO_4), total Kjeldahl N (TKN), total phosphorus (TP), total carbon (TC), inorganic carbon (IC), and dissolved organic carbon (DOC)

Table 3.4. Mean [a] and range of microbial [b] indicators measured in Satilla River Basin

Site		E. coli MPN 100 ml^{-1}		Enterococci MPN 100 ml^{-1}		Fecal coliforms CFU 100 ml^{-1}	
	Mean	300	B	1158	B	210	B
1	Min	0		0		21	
	Max	1396		17329		1273	
	Mean	224	B	336	B	162	B
2	Min	0		0		16	
	Max	826		1373		700	
	Mean	1253	B	540	B	167	B
3	Min	0		0		26	
	Max	17329		4884		410	
	Mean	225	B	711	B	235	B
4	Min	0		0		18	
	Max	809		4838		510	
	Mean	515	B	372	B	420	B
5	Min	0		0		9	
	Max	4838		1169		3500	
	Mean	234	B	515	B	274	B
8	Min	0		0		33	
	Max	1373		2827		2100	
	Mean	200	B	447	B	171	B
9	Min	66		31		27	
	Max	977		1624		800	
	Mean	192	B	472	B	202	B
10	Min	13		41		20	
	Max	651		1850		1200	
	Mean	176	B	330	B	250	B
11	Min	20		24		23	
	Max	1159		2092		2400	
	Mean	191	B	226	B	219	B
12	Min	20		10		16	
	Max	1540		1298		1500	
	Mean	257614	A	22659	A	4267247	A
6	Min	0		0		0	
	Max	686700		90900		17272727	
	Mean	28960	B	3250	B	25386	B
13	Min	10		15		0	
	Max	241920		41060		230000	
	Mean	4413	B	1717	B	1831	B
14	Min	24		132		63	
	Max	72700		4884		14000	
	Mean	3074		840		2461	
All	Min	0		0		0	
Sites	Max	241920		41060		230000	

a Means with the same letter in a column are not significantly different ($p<0.05$) using Tukey's HSD test

FIGURE LEGEND

Figure 3.1. **Map of Satilla River Basin (Georgia, USA) and sampling stations**

Figure 3.2. **2009 Cotton coverage in Site 2, Site 4 and Site 5**

Figure 3.3. **Mean nutrient concentrations of landuse categorized stations**

Figure 3. 4. **USGS 02226500 Satilla River near Waycross, GA measured daily discharge (feet^3 sec^{-1}) from August 2007 to August 2009.**

Figure 3.5. **Chlorophyll *a* and DO concentrations at the effluent of the Wastewater Treatment Plant**

Figure 3.1.

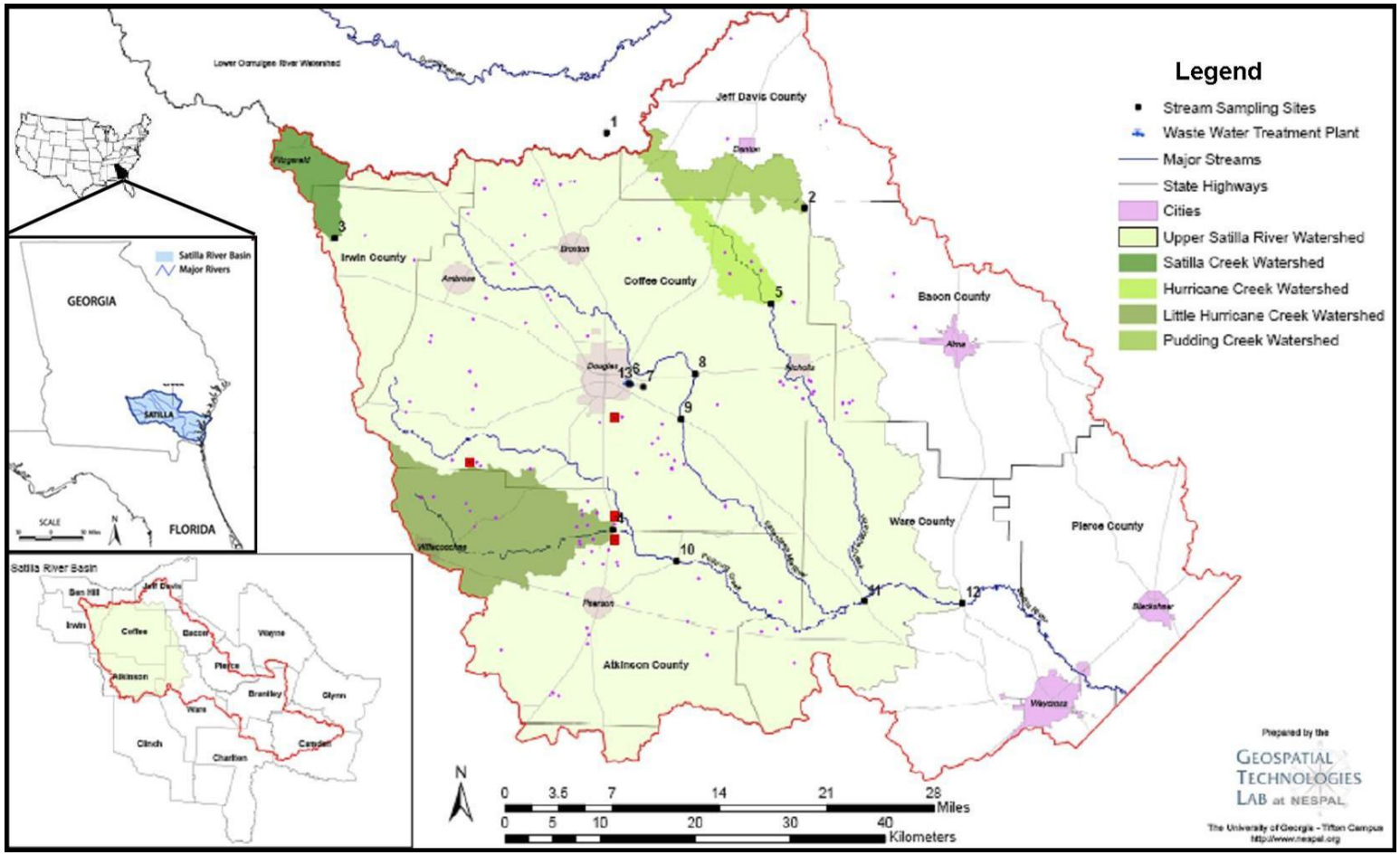
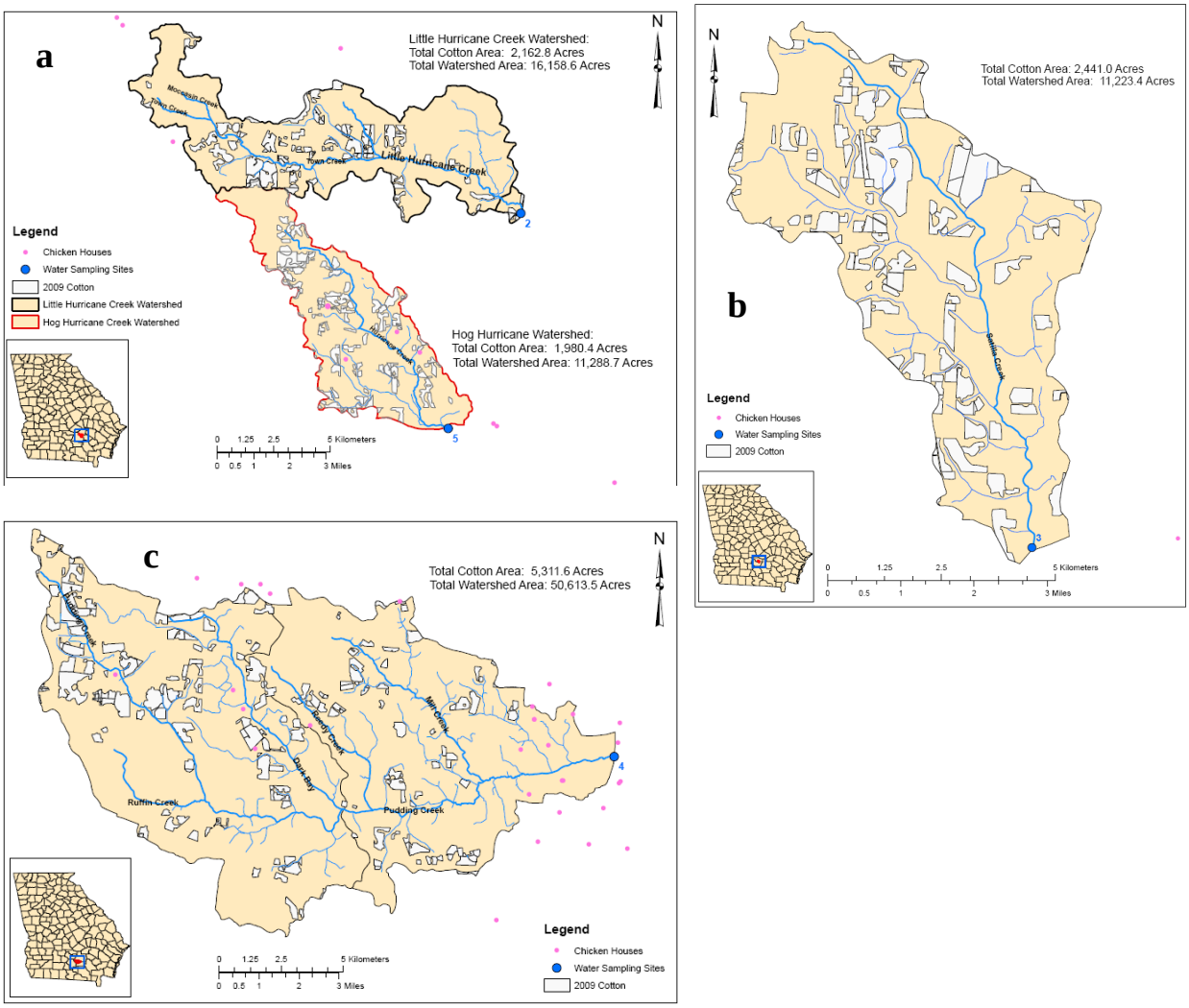

Legend
Stream Sampling Sites
Waste Water Treatment Plant
Major Streams
State Highways
Cities
Upper Satilla River Watershed
Satilla Creek Watershed
Hurricane Creek Watershed
Little Hurricane Creek Watershed
Pudding Creek Watershed
Lower Ocmulgee River Watershed
Jeff Davis County
Irwin County
Coffee County
Bacon County
Ware County
Pierce County
Atkinson County
Wilcox County
Satilla River Basin
Major Rivers
GEORGIA
FLORIDA
SATILLA
Satilla River Basin
Ben Hill
Jeff Davis
Irwin
Coffee
Bacon
Wayne
Atkinson
Pierce
Brantley
Glynn
Ware
Clinch
Charlton
Camden
0 3.5 7 14 21 28 Miles
0 5 10 20 30 40 Kilometers
Prepared by the
GEOSPATIAL TECHNOLOGIES LAB at NESPAL
The University of Georgia - Tifton Campus
http://www.nespal.org

Figure 3.2.

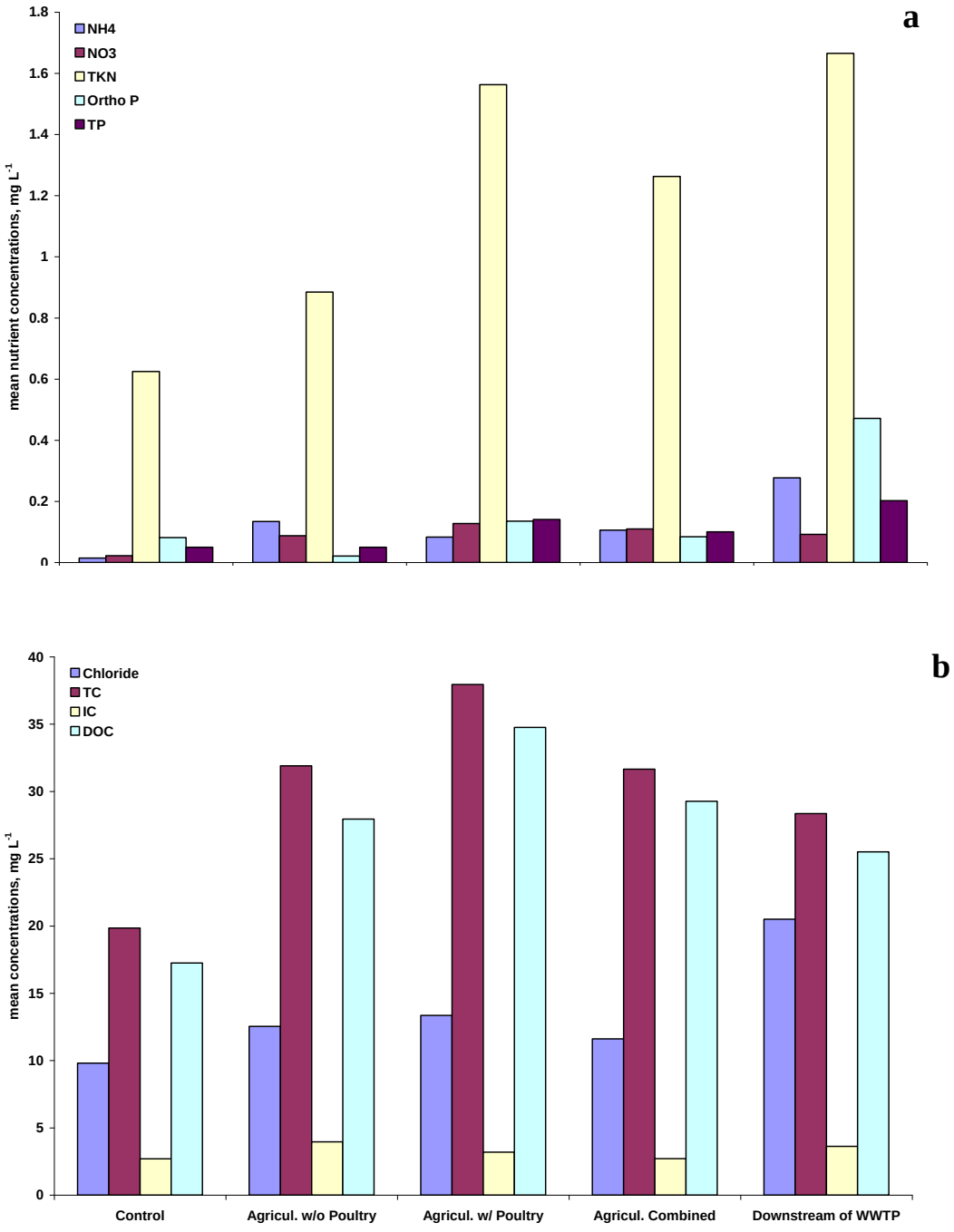


a Site 2 (Little Hurricane Creek, agriculture with no poultry houses) and Site 5 (Hog Hurricane Creek, agricultural with poultry houses)

b Site 3 (Upper Satilla Creek, agricultural with no poultry houses)

c Site 4 (Pudding Creek, agricultural with poultry houses)

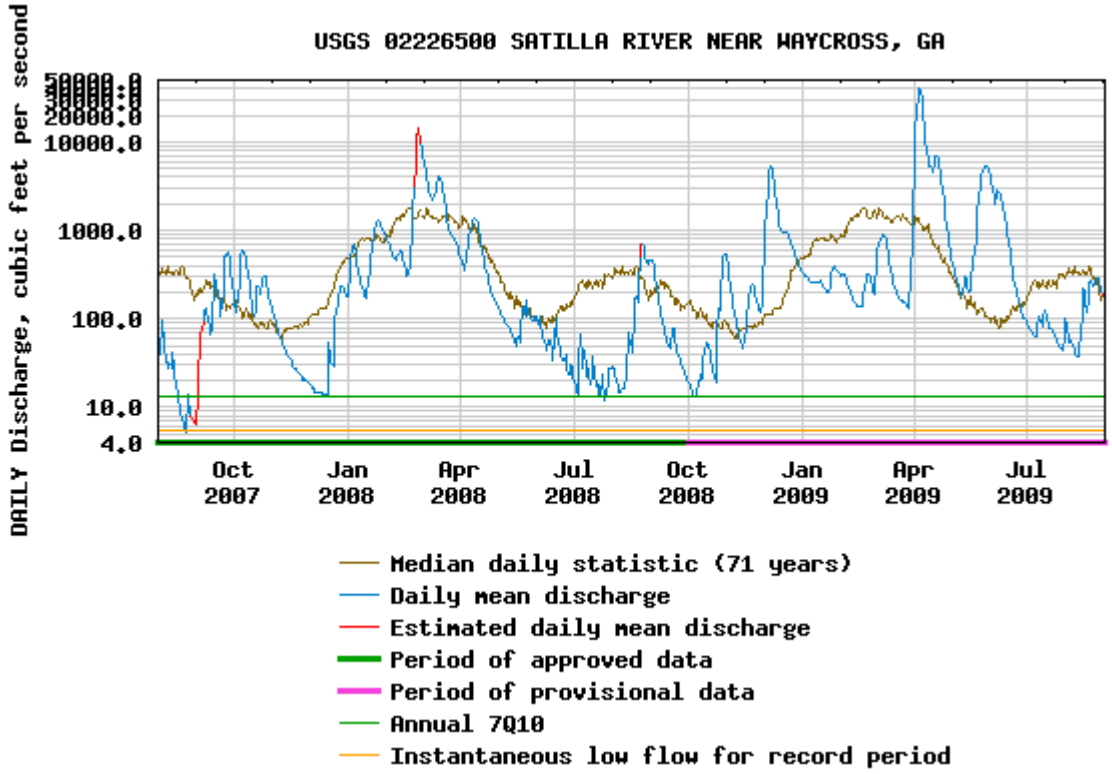
Figure 3.3.



a Ammonia (NH_4), nitrate (NO_3), orthophosphate (ortho P), total Kjeldahl N (TKN), total phosphorus (TP)

b Chloride, total carbon (TC), inorganic carbon (IC), dissolved organic carbon (DOC)

Figure 3.4.


USGS 02226500 SATILLA RIVER NEAR WAYCROSS, GA
DAILY Discharge, cubic feet per second
50000.0
20000.0
10000.0
1000.0
100.0
10.0
4.0
Oct 2007
Jan 2008
Apr 2008
Jul 2008
Oct 2008
Jan 2009
Apr 2009
Jul 2009
Median daily statistic (71 years)
Daily mean discharge
Estimated daily mean discharge
Period of approved data
Period of provisional data
Annual 7Q10
Instantaneous low flow for record period

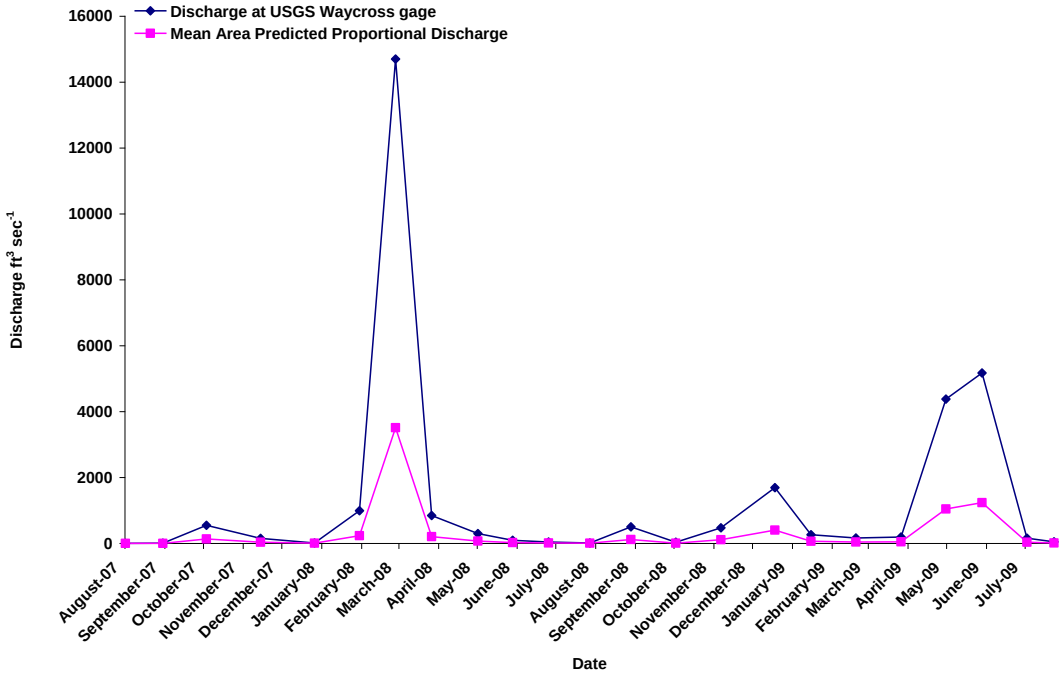
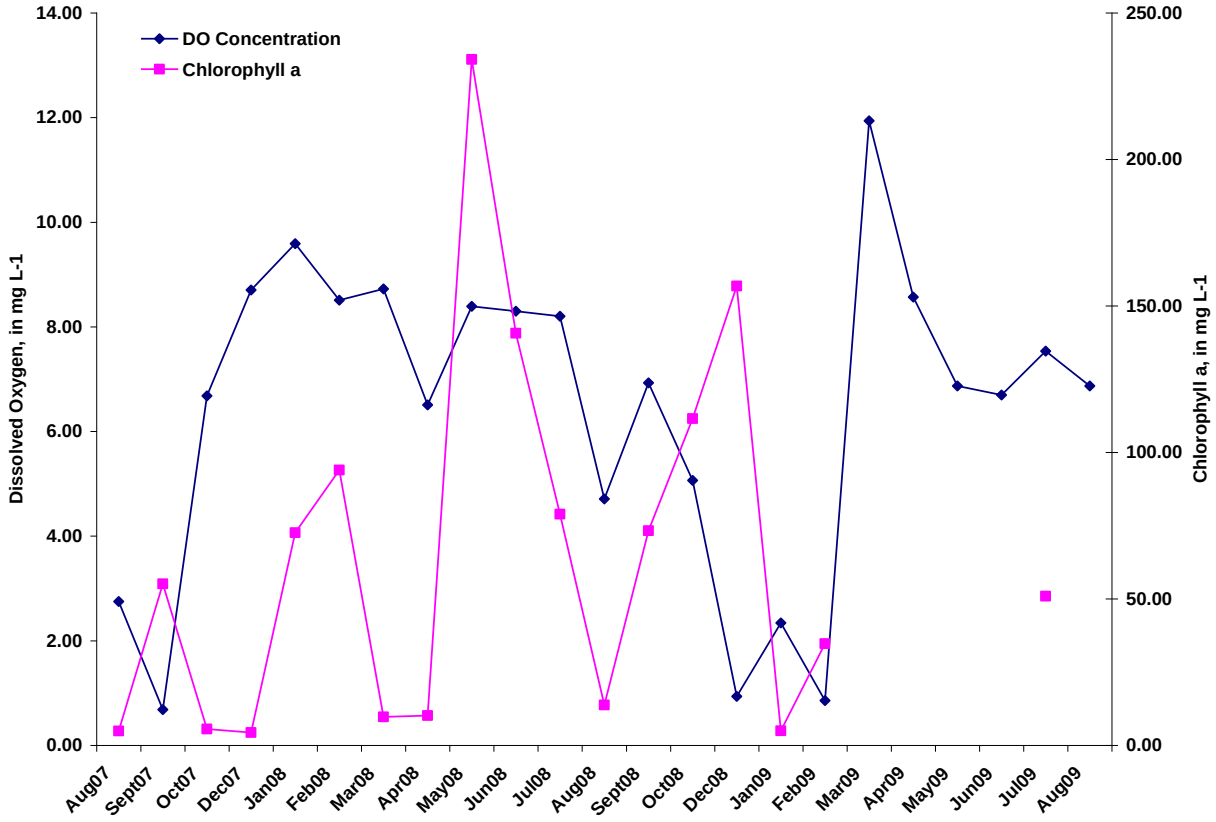

Discharge at USGS Waycross gage
Mean Area Predicted Proportional Discharge
Discharge ft3 sec-1
16000
14000
12000
10000
8000
6000
4000
2000
0
August-07
September-07
October-07
November-07
December-07
January-08
February-08
March-08
April-08
May-08
June-08
July-08
August-08
September-08
October-08
November-08
December-08
January-09
February-09
March-09
April-09
May-09
June-09
July-09
Date

Figure 3.5.


DO Concentration
Chlorophyll a
Dissolved Oxygen, in mg L-1
Chlorophyll a, in mg L-1
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
250.00
200.00
150.00
100.00
50.00
0.00
Aug07
Sept07
Oct07
Dec07
Jan08
Feb08
Mar08
Apr08
May08
Jun08
Jul08
Aug08
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Oct08
Dec08
Jan09
Feb09
Mar09
Apr09
May09
Jun09
Jul09
Aug09

CHAPTER 4

ASSESSING THE IMPACT OF AGRICULUTURE AND COASTAL WATER QUALITY ON SALMONELLA AND CAMPYLOBACTER PREVALENCE IN A MIXED USE WATERSHED [1]

[1] Ethell Vereen, Jr., R. Richard Lowrance, Michael Jenkins, Sreekumari Rajeev, and Erin K. Lipp. To be submitted to *Applied and Environmental Microbiology*.

ABSTRACT

The presence of *Salmonella* and *Campylobacter* in the stream network of the Satilla River Basin (SRB) was monitored monthly from August 2007 to August 2009 to study the potential relationships between these bacterial pathogens and varying levels of agriculture and poultry processing facilities within the SRB. Watersheds were sampled that represent agricultural areas, agricultural areas with poultry houses, a small watershed receiving direct discharge from a municipal wastewater treatment plant (WWTP), larger watersheds on the main channel of the Satilla River and a major tributary (Seventeen Mile River), and reference areas with little or no agricultural activity. Enrichment and conventional cultural methods were used for confirmation and identification of *Salmonella,* while *Campylobacter* detection consisted of enrichment and conventional polymerase chain reaction (PCR). *Salmonella* and *Campylobacter* were frequently detected at all 13 sites monitored with pathogens more frequently present among the downstream sites with agricultural landuse and poultry production. *Salmonella* was present in 43% (129 of 299) of all samples, and *Campylobacter* was present in 62% (96 of 156) of all samples. Enterococci were reasonably predictive of *Salmonella* and *Campylobacter* presence; however 62% (80/129) of *Salmonella* were detected when *E. coli* were below EPA standards and 73% (70/96) of *Campylobacter* were detected when fecal coliforms were below EPA standards. These results highlight the weakness with the current fecal indicator system in flowing inland waters as adequate proxies of pathogen loading; *Salmonella* and *Campylobacter* are highly prevalent in surface waters of a mixed-use watershed even when fecal indicators are low. These results may be indicative of drought effects given that one year (2007 to 2008) was unusually dry; other months (2008 to 2009) were unusually wet and the pattern of occurrence remained spatially distributed during the drought months.

INTRODUCTION

Foodborne illnesses are a substantial health burden in the United States, with *Campylobacter, Escherichia coli* and *Salmonella* reported among the major bacterial foodborne pathogens. An estimated 76 million food-borne illnesses occur each year in the United States (27, 30). Nearly 2.4 million cases are caused by *Campylobacter* species, 1.4 million cases are caused by nontyphoidal *Salmonella* serovars, and 270,000 cases are caused by pathogenic *E. coli*, including *E. coli* O157:H7 (3). While most of these illnesses are undiagnosed and unreported, approximately 325,000 cases result in hospitalization, and 5,000 cases are fatal resulting in death (27).

Food from animal origin is one of the main sources of *Salmonella* and *Campylobacter* caused infection, predominately from ingestion of contaminated food products, such as undercooked beef, pork, chicken, seafood, and eggs (12, 14, 19, 33, 40). Infections are also contracted following consumption of fresh fruits or vegetables that have been treated (or irrigated) with contaminated fertilizer or water (39, 40). Contact with pets and farm animals have also been implicated as potential sources of infection (5, 48).

Although food is a much more significant route of exposure, water may be directly or indirectly associated with *Salmonella* and *Campylobacter* transmission. While *Campylobacter* has a strict host requirement for growth, there is ample opportunity for *Campylobacter* to contaminate environmental water, including streams, rivers, and lakes given its widespread distribution in the intestinal tracts of a wide variety of hosts (23, 28, 49). *Campylobacters* presence in the environment is evidence of fecal contamination and *Campylobacter* has been detected in fecally contaminated environments and natural waters such as streams, rivers, and lakes due to discharges from wastewater treatment plants, runoff from pastures after rain and

direct contamination by wild birds and animals (3, 16, 18, 20, 23). *Salmonella* are well adapted to both host conditions and the environment and are constantly released into the environment from infected humans, farm animals, pets, and wildlife (6, 8). *Salmonella* are frequently isolated from water sources, including rivers, coastal water and lakes (4, 15, 17, 25) which serve as bacterial reservoirs and may possibly aid transmission between hosts.

U.S. broiler production is concentrated in the south, particularly in Arkansas, Georgia, Alabama, North Carolina and Mississippi (32). According to the U.S. Department of Agriculture (USDA), Georgia produced 1.4 billion broilers, more than any other state, valued at $2.7 billion in 2006 (43). Georgia produces roughly 15 percent of the country's broilers annually. This rapidly expanding industry in the coastal plain of Georgia results in much of the poultry litter being applied to pastures and crop land because of its recognized value as a soil amendment (7, 9, 13). The common agricultural practice of spreading livestock manures to land presents an obvious and well-described mode of contamination of water resources at the field level (7); however, little to no research has been done to determine the potential for pathogen contamination of streams at the watershed level from these animal manures. The lack of information puts the public at risk of disease as poultry manure is a known source of *Salmonella* and *Campylobacter* (2, 7, 21, 23) and increased application of poultry litter to agricultural fields may lead to an increased load of pathogens contaminating surface and subsurface waters. The primary objectives of this study were to examine the effect of specific land-use characteristics at the sub-watershed level on the relative loading of fecal indicator bacteria and the enteric bacterial pathogens, *Campylobacter* and *Salmonella*. The secondary objective was to assess the utility of traditional indicator bacteria as proxies for these pathogens among flowing in-land waters of the Coastal Plain.

METHODS AND MATERIALS

Watershed and sample site description

The study area and the Satilla River Basin (SRB) are described here briefly (see Chapter 3 (45) for a more detailed description). Thirteen sampling sites were established in the SRB (45). Watershed characteristics are shown in Table 3.1.(45). Eleven of the sites were in-stream and mapped to specific sub-watersheds. Watersheds were sampled that represented: A) a control or reference area with little or no agricultural activity (Site 1), B) agricultural areas receiving poultry litter application (Sites 2 and 3), C) agricultural areas with poultry houses and receiving poultry litter application (Sites 4 and 5), D) Influent (site 6) to the City of Douglas WWTP and effluent (sites 13 and 14) from the WWTP; and the remaining 5 watershed sites were on larger streams above and below the WWTP. Sites 8 and 9 were on Seventeen Mile River, above and below the WWTP, respectively (Figure 3.1) (45). Sites 10 and 11 were on the Satilla River, above and below the confluence with Seventeen Mile River, therefore above and below the Douglas WWTP (Figure 3.1) (45). Site 12 was on the main channel of the Satilla, below the confluence of the Satilla River and Hurricane Creek (Figure 3.1) and was the most downstream station sampled. Sample collection, water quality, nutrient and fecal indicator methods and results are discussed in Chapter 3 (45).

Microbial analyses

Isolation and identification of Salmonella

A one liter sample was mixed by gentle inversion several times and 250 ml of the sample were then filtered through a 0.45 µm 47 mm nitrocellulose membrane. The filters were aseptically cut in half and placed into 25 ml of buffered peptone water (BPW). The BPW was then incubated at 37^{o}C for 24 ± 2 hours for non-selective pre-enrichment. One hundred

microliters of the BPW pre-enrichment was then transferred to 10 ml of Rappaport-Vassiliadis (RV) broth for selective enrichment at 42°C for 24 ± 2 hours. This was followed by streaking from RV broth onto xylose lysine tergitol (XLT) agar and incubating plates at 37°C for 24 to 48 hours. Following incubation, three to five presumptive *Salmonella* colonies per XLT plate were picked and saved (identified as black colonies). Suspect *Salmonella* colonies were identified by O antigen screening, and using the Sensititre Microplate System (AP 80; Trek Diagnostics, Westlake, OH). Following a positive reaction with polyvalent 'O' antiserum, the isolates were also sub typed using individual *Salmonella* antisera for 'O' groups B, C, D, and E (results and analysis of *Salmonella* serogroups discussed in Chapter 5 (46)). Isolated cultures identified as *Salmonella* were preserved in CryoCareBacteria Preservers (Stamford, Texas) containing a cryopreservative fluid of TSB + glycerol and stored in a -80°C freezer. Each isolate was later subcultured on a tryptic soy agar slant and forwarded to the National Veterinary Services Laboratories of the Department of Agriculture in Ames, Iowa for specific serotyping [results and analysis of *Salmonella* serotyping discussed in Chapter 5 (46)].

Campylobacter enrichment and PCR

An additional 250 ml of sample were also filtered through a second 0.45 µm nitrocellulose membrane filter. The membranes were cut in half and placed into 10 ml of Campylobacter enrichment broth (CEB). The CEB was then incubated at 42°C for 48 ± 2 hour enrichment. A 1.5 ml aliquot of each CEB enrichment was used for DNA extraction (MoBio Power Soil DNA Extraction kit, MOBIO Laboratories, Carlsbad, CA). The primers and PCR conditions used were described by Eyers et al. (10, 11) and from Savill et al. (36). Primers targeted the 23S rRNA gene of *Campylobacter* and allow detection of the four thermotolerant *Campylobacter* species (*C. jejuni subsp. jejuni, C. coli, C. lari,* and *C. upsaliensis*) by using one

primer pair: THERM 1 (5'-TATTCCAATACCAACATTAGT-3') and THERM 2 (5'-CGGTACGGGCAACATTAG-3'). All four thermotolerant *Campylobacter* species yield a 222 bp product when amplified with this primer set. Additional primers used as discussed in Chapter 5 (46) allow discrimination between the four thermotolerant species (22).

PCR was performed with a Bio-rad® thermal cycler (PTC-200, Waltham, Massachusetts). The amplification consisted of an initial DNA denaturing step at 95°C for 1 minute 30 seconds, followed by a 40-cycle reaction (PCR denaturing at 95°C for 1 minute 30 seconds, PCR annealing at 50°C for 30 seconds, and PCR extension at 72°C for 1 minute). The cycling included a final extension step at 72°C for 5 minutes to ensure full extension of the product. PCR products were analyzed by electrophoresis at 110 V for 1 hour through 1% (wt/vol) agarose gel. Products were visualized by staining with ethidium bromide under UV light and a 100-bp DNA ladder was used as a molecular weight marker.

Description of data analysis and statistics

Data were analyzed using Statistical Analysis System (SAS) release 9.1 (Cary, NC). For all statistical analyses the presence/absence of *Salmonella* and *Campylobacter* were treated as the dependent variable (i.e., a binary variable). When a target organism was present, it was assigned the value 1, and when a target organism was absent, it was assigned the value 0. The total number of samples positive for each month was calculated by summing the number of samples positive for all sites for a given month. Fisher's exact test and binary logistic regression models were used to statistically evaluate the presence and absence of *Salmonella* and *Campylobacter* with environmental variables and fecal indicator levels reported in Chapter 3 (45). The likelihood ratio method was used to estimate the p value. This method is more robust than the Wald chi square, and appropriate for small sample sizes in that it uses the difference between the

probability of obtaining the observed results under the logistic model and the probability of obtaining the results in a model with no relationship between the independent and dependent variables (26). For all measures of association, p values $\leq$ 0.05 were considered significant. Only those environmental parameters and fecal indicator organisms that showed significant relationships with either *Salmonella* or *Campylobacter* were reported.

RESULTS

Spatial and temporal distribution of pathogens

Salmonella and *Campylobacter* were frequently detected at all 13 sites monitored in the stream network of the Satilla River Basin. Initial trials attempted to detect *Campylobacter* with a modified direct-plating method as described by Rosef et al. (35). Evaluation of water samples for the presence of *Campylobacter* was challenging as TSA plates would routinely become overgrown with fungus and other contaminants. Subsequent trials determined the Campylobacter enrichment and PCR method to be effective in detecting thermotolerant *Campylobacters*. As a result of these trials, the direct plating method was suspended from subsequent sampling and reported are only August 2008 to August 2009 results for *Campylobacter* as this was the study period when the Campylobacter enrichment and PCR method was used exclusively. *Salmonella* was detected over the entire study period from August 2007 to August 2009.

Overall, 43% (129 of 299) of all samples were positive for *Salmonella*, and 62% (96 of 156) of all samples were found to be positive for *Campylobacter*. Table 4.1 shows the prevalence of *Salmonella* and *Campylobacter* by site. Among the 11 in-stream sites, *Salmonella* ranged from 17% positive (4/23) at site 2 to 61% positive (14/23) at sites 9 and 10. *Campylobacter* detection ranged from 50% positive (6/12) at site 2 to 75% positive (9/12) at site

8. Among the WWTP stations, *Salmonella* was positive in 43% (10/23) of the samples from both sites 6 (influent) and site 14 (pond effluent), and 35% (8/23) of the samples from site 13 (direct effluent). *Campylobacter* was positive in 75% (9/12) of the samples from site 6, and 50% (6/12) of the samples from sites 13 and 14.

Comparing the smaller upstream stations (sites 1 to 5) with the WWTP stations (site 6, 13, and 14) and the larger downstream stations (sites 8 to 12), the differences in *Salmonella* and *Campylobacter* presence in the SRB showed substantial difference in pathogen prevalence (Figures 4.1 and 4.2). In general, prevalence increased downstream of the WWTP effluent.

Over the two year study period, there were not significant changes observed in *Salmonella* prevalence (Fisher's p value 1.0) with sample month. Likewise, no significant changes were observed in *Campylobacter* prevalence (Fishers p value 1.0) with sample month. *Salmonella* by month ranged from a low of 0% positive (0/13) in April of 2009 to 85% (11/13) positive in December 2008 (Figure 4.3). *Campylobacter* were detected in all study months and ranged from a low of 8% (1/13) positive in August 2009 to the highest, 100% (13/13) in March 2009 (Figure 4.3).

Relationships between the occurrence of pathogens and environmental variables including fecal indicator organisms

Although there was no significant difference between monthly prevalence of *Salmonella*, binary logistic regression models using DO as the independent variable fit the frequency for presence and absence of *Salmonella* at z = -0.10 with 59.6% model concordance (p value 0.03) and an odds ratio (OR) of 0.91. Additionally rainfall totals for the day of sampling, (z = -0.72, 33.5% model concordance, OR 0.48, p value 0.03), and 30 days prior to sampling (z = -0.15, 36.3% model concordance, OR 0.86, p value <0.05) showed significant fit with *Salmonella*

presence and absence. *Campylobacter* presence was successfully fit with several measures of association including temperature (z = -0.07, model concordance 62.4%, OR 0.93, p value <0.05), DO (z = -0.09, 57.2% model concordance, OR 0.91, p value = 0.05), pH (z = 0.86, 62.7% model concordance, OR 2.38, p value <0.05), rainfall on the day of sample collection (z = -2.54, 35.6% model concordance, OR 0.08, p value = 0.03), and rainfall 30 days prior to sampling (z = 0.10, 48.7% model concordance, OR 1.11, p value <0.05) (Table 4.2). Figure 4.3 shows the monthly frequency of *Salmonella* and *Campylobacter* prevalence with 30-day antecedent rainfall. When WWTP data (sites 6, 13, and 14) were excluded *Campylobacter* was additionally associated with ORP (z = -0.01, 58.9% model concordance, OR 0.99, p value 0.04); however there was no longer an association with ammonium, orthophosphate, chloride or total phosphorus (Table 4.2).

To determine if there were any spatially unique associations upstream of the WWTP vs. downstream of the WWTP effluent we also ran separate models of the smaller upstream stations (sites 1 to 5) and the larger downstream stations (sites 8 to 12) separately (Table 4.3). Temperature, conductivity, DO, pH, ORP, chloride, TKN, TC, and IC were significantly associated with *Salmonella* and *Campylobacter* presence upstream of the WWTP effluent compared with only pH, TKN, TC, and IC from the downstream stations (Table 4.3). Additionally, the binary logistic regression fit was greater for the small sites upstream of the WWTP effluent than that noted in the downstream sites (Table 4.3).

The binary logistic regression models did not fit *Salmonella* or *Campylobacter* presence/absence when any of the fecal indicator organisms were used as independent variables. Table 4.4 shows the frequency of the pathogens detected when the indicator organisms were detected above or below threshold standards for “safe” water (41, 42). Of the 129 samples

positive for *Salmonella*, 88% (113/129) were detected when enterococci were above EPA thresholds and only 12% (16/129) were detected when enterococci were below. *E. coli* and fecal coliform bacteria were not as predictive. Sixty-two percent (80/129) *Salmonella* positive samples and 67% (87/129) samples positive for *Salmonella* were detected when *E. coli* and fecal coliform bacteria, respectively, were at levels below the EPA standards. Of the 96 samples positive for *Campylobacter*, 90% (86/96) were detected when enterococci levels were above EPA thresholds and only 10% (10/96) of *Campylobacter* were detected when enterococci were below the EPA standard. As with *Salmonella* detection, *E. coli* and fecal coliform bacteria were not as predictive for *Campylobacter*. Fifty-nine percent (57/96) of the time when *Campylobacter* was detected, *E. coli* levels were below the EPA standard and 73% (70/96) *Campylobacter* positive samples were detected when fecal coliform bacteria were below the EPA standard.

DISCUSSION

The presence of the pathogens *Salmonella* and *Campylobacter* in the stream network of the Satilla River Basin (SRB) were monitored monthly from August 2007 to August 2009 to ascertain potential relationships between these pathogens and varying levels of agriculture and poultry processing facilities within the SRB.

Salmonella and *Campylobacter* were frequently detected at all 13 sites monitored in the stream network of the SRB. Pathogens were frequently detected from the larger downstream stations and the sites with agricultural landuse and poultry production. Of the watersheds sampled, site 2 has the smallest agricultural landuse (22% of area) and no poultry houses (Table 4.1). *Salmonella* was detected at its lowest rate from this site (17% positive, 4/23) of all stations monitored, including the control station in the Broxton Rocks Nature Preserve. *Campylobacter* was also detected less frequently from this site with only half of the samples testing positive

(6/12). Conversely, both pathogens were detected more frequently from site 4, which has the most poultry houses (N=50) of the smaller watersheds and supports 30% agricultural landuse.

One of the common assumptions regarding enteric pathogens such as *Salmonella* and *Campylobacter* loading in surface waters is that livestock production is one of the primary sources of contamination. In this study *Salmonella* was detected twice as frequently from the agricultural sites with poultry production (sites 4 and 5) than the agricultural sites without poultry production (sites 2 and 3). These results are consistent with a recent survey conducted by Sigua et al. (37), where *Salmonella* was detected more frequently from an area of the Pinahal River in Brazil influenced by pigs, poultry and crops compared with other areas of the sub-basin without animals and areas largely dominated by animals and humans. Additionally, in an area of the Llobregat River in the Mediterranean where pigs and poultry are the most numerous livestock, with 500,000 pigs and 70,000 poultry, Rodriguez and Araujo (34) isolated *Campylobacter* from 82% (N=55) of river samples.

Other sources may also be contributing to the pathogen load in the SRB, as a relatively high proportion of samples from the stations downstream of the WWTP were also found to be positive for *Salmonella* and *Campylobacter*. Although only 43% (10/23) of the influent to the WWTP (site 6) tested positive for *Salmonella*, 75% (9/12) of the samples from this site were positive for *Campylobacter*. The WWTP has a permitted discharge of 6 million gallons per day (MGD) of which about 50% is provided by wastewater from the broiler processing plant (Delnee Wilcox, personal communication) and likely contributes to the pathogens present at the treatment plant. The discharge from the WWTP enters a small stream that flows into a pond behind the plant and then to Seventeen Mile River. Thirty-five percent (8/23) of the water samples were positive for *Salmonella* from the direct effluent from the WWTP at site 13, compared to 43%

positive samples from the WWTP discharge at site 14, the pond area, and 61% (14/23) of the samples below the WWTP at site 9 on Seventeen Mile River. Similarly, *Campylobacter* was detected in 50% (6/12) of the samples from sites 13 and 14, compared to 67% positive (8/12) from site 9 on Seventeen Mile River below the treatment plant. *Salmonella* and *Campylobacter* have frequently been found in poultry wastewater (23, 34) and sewage discharge (29, 34, 38, 44).

As discussed earlier both pathogens were detected more frequently from site 9 on Seventeen Mile River below the WWTP. Pathogen detection rates increased downstream where these larger downstream stations (11 and 12) have the most poultry houses within their watershed area and therefore receive inputs from upstream sources (including wastewater) as well as agricultural loading. Additionally, more than half of the landuse at these downstream stations includes forested areas home to a variety of animals. Both domestic and wild animals have been found to harbor *Salmonella* and *Campylobacter* (1, 3, 5, 6, 8, 24) and these animals may be contributing the pathogens to these downstream sources, in addition to the accumulation of inputs from the nested upstream stations. There is the potential that transmission of *Salmonella* and *Campylobacter* to humans may occur through contact with animals and this environment. These stations are frequently visited year-round for outdoor leisure activities such as fishing, hunting, canoeing/kayaking, picnicking and other forms of nature-based recreation.

Salmonella and *Campylobacter* were more frequently isolated during the late autumn and winter months than in the spring and summer months. Other studies have shown this pattern as well and suggested that seasonal differences were caused by the effects of rainfall on the input from agricultural run-off in winter months and bacterial die off due to warmer temperatures in late spring and summer (15, 31, 38). In a previous survey, in this watershed Vereen et al. (44) isolated Campylobacter (using only traditional culture techniques) more frequently during the

summer months, which is consistent with the trends for clinical cases of campylobacteriosis (24, 28), but contrary to the present study and other studies (15, 31, 38) that have routinely detected *Campylobacter* environmentally during fall and winter months. Rivers across the state of Georgia experienced moderate to severe hydrologic drought in 2007, and this likely impacted the pathogens relationship with water quality variables at individual stations (especially the smaller sites as they experienced minimal to no flow more often than others during summer and warmer months). Additionally, the length and intensity of this drought may have affected the seasonal distribution of these pathogens and may also contribute to the differences noted for *Campylobacter* prevalence here and in the previous study by Vereen et al. (44). *Salmonella* prevalence was generally higher in 2008 and 2009 as drought conditions subsided and rainfall increased (Figure 4.3)

Microbial water quality standards are based upon fecal indicator bacteria e.g., fecal coliform bacteria, *E. coli* and enterococci. It is assumed that these bacteria can "indicate" the presence of pathogens, and associated human health risks. For a variety of reasons bacterial indicators may not always be an effective surrogate for the presence of bacterial pathogens, including *Salmonella* and *Campylobacter*. In the present study, *E. coli* and fecal coliforms were not as predictive as enterococci. Enterococci have primarily been suggested as an indicator for marine waters (47), and *E. coli* for fresh waters (47). Although the SRB is in the coastal plain of Georgia, the sites sampled were freshwater streams and these suggested indicators may not adequately predict the presence of *Salmonella* and *Campylobacter*.

CONCLUSION

Land application of animal manures, such as poultry litter, has been implicated as a contributing factor of non-point source pollution. This practice has the potential to introduce

pathogenic microorganisms, such as *Salmonella* and *Campylobacter* to the environment; with a subsequent risk of transmission to exposed humans or animals. *Salmonella* and *Campylobacter* were frequently detected in the stream network of the Satilla River Basin and the presence of the pathogens appears to be a function of location in the watershed and associated inputs. Both pathogens were more frequently detected from downstream stations and those stations influenced by agricultural and poultry production. There was also an association with monthly rainfall indicating that rainfall related inputs (mainly from surface run-off) may also be transmitting pathogens to the streams. As other studies have previously shown, indicator organisms are not ideal proxies to predict the presence of *Salmonella* and *Campylobacter* and our findings generally coincide with that; the exception being enterococci that were generally predictive of the pathogens presence. The purpose of this report was to identify specific land-use characteristics at the sub-watershed level on the loading of *Campylobacter* and *Salmonella*. To that end we have found *Salmonella* and *Campylobacter* to be highly prevalent in agrarian areas where livestock manures are commonly used as a fertilizer. The pathogens have been detected throughout the watershed indicating the potential for transmission not only at the farm level but also to downstream waters. More focused investigation is needed to build on our understanding of agricultural practices (such as the spreading of animal manures) that present a substantial risk to contaminating water resources.

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TABLES

Table 4.1. Watershed description[a] and presence of *Salmonella* and *Campylobacter* by site

Site	Number of Poultry Producers	Number of Poultry Houses	Agricultural[b] Landuse (%)	% + for Salmonella, N=23 (# of positives)	% + for Campylobacter, N=12 (# of positives)
1	0	0	5	35% (8)	58% (7)
2	0	0	22	17% (4)	50% (6)
3	0	0	52	26% (6)	58% (7)
4	12	50	30	57% (13)	58% (7)
5	4	21	20	39% (9)	67% (8)
8	22	98	33	39% (9)	75% (9)
9	22	98	32	61% (14)	67% (8)
10	42	173	32	61% (14)	58% (7)
11	91	368	25	57% (13)	67% (8)
12	112	440	23	48% (11)	67% (8)

a Landuse is based on 1998 landuse classification (Georgia GIS Clearinghouse)

b Includes row-crops and pasture land

Table 4.2. Relationships between the occurrence of *Salmonella* and *Campylobacter* and environmental variables by all sites and without the wastewater treatment plant (WWTP, sites 6, 13, and 14) in the Satilla River Basin

	Binary Logistic Regression model for occurrence of *Salmonella*			
	All Sites		Without WWTP sites	
Variable	z (p-value)	Model concordance (Odds Ratio)	z (p-value)	Model concordance (Odds Ratio)
Dissolved Oxygen (mg L^{-1})	-0.10 (P = 0.03)	59.6% (0.91)		
Rainfall (total, in)				
on sample day		33.5% (0.38)	-1.18 (P = 0.03)	33.5% (0.31)
day before sample	-0.72 (P < 0.05)	36.3% (0.48)	-0.75 (P < 0.05)	35.5% (0.47)
30 day's before sample	-0.15 (P < 0.05)	56.6% (0.86)	-0.15 (P < 0.05)	56.6% (0.86)

	Binary Logistic Regression model for occurrence of *Campylobacter*			
	All Sites		Without WWTP sites	
Variable	z (p-value)	Model concordance (Odds Ratio)	z (p-value)	Model concordance (Odds Ratio)
Temperature (^{o} C)	-0.07 (P < 0.05)	62.4% (0.93)	-0.08 (P < 0.05)	62.8% (0.93)
Dissolved Oxygen (mg L^{-1})	-0.09 (P = 0.05)	57.2% (0.91)	-0.10 (P = 0.05)	56.1% (0.91)
pH	0.86 (P < 0.05)	62.7% (2.384)	1.00 (P < 0.05)	66.3% (2.72)
ORP			-0.01 (P =0.04)	58.9% (0.99)
NH4 (mg L^{-1})	-0.27 (P = 0.02)	53% (0.77)		
Ortho P (mg L^{-1})	-0.39 (P = 0.01)	54.6% (0.68)		
Chloride (mg L^{-1})	-0.01 (P = 0.01)	61.77% (0.99)		
TKN (mg L^{-1})	-0.31 (P < 0.05)	59.1% (0.73)	-0.39 (P = 0.02)	55.4% (0.67)
TP (mg L^{-1})	-1.09 (P = 0.02)	64.1% (0.34)		
TC (mg L^{-1})	-0.07 (P < 0.05)	67.7% (0.94)	-0.06 (P < 0.05)	67.6% (0.94)
IC (mg L^{-1})	-0.14 (P < 0.05)	74.6% (0.87)	-0.48 (P < 0.05)	78.4% (0.62)
Rainfall (total, in)				
on sample day	-2.54 (P = 0.03)	35.6% (0.08)	-3.00 (P = 0.03)	36.9% (0.05)
day before sample				
30 day's before sample	0.10 (P < 0.05)	48.7% (1.11)	0.13 (P < 0.05)	51.2% (1.14)

Table 4.3. Relationships between the occurrence of *Salmonella* and *Campylobacter* and environmental variables by the small watershed sites (sites 1, 2, 3, 4 and 5) and the large watershed sites (sites 8, 9, 10, 11, and 12) in the Satilla River Basin

	Binary Logistic Regression model for occurrence of *Salmonella*			
	Small Watershed Sites		Large Watershed Sites	
Variable	z (p-value)	Model concordance (Odds Ratio)	z (p-value)	Model concordance (Odds Ratio)
Conductivity (mS cm^{-1})	16.06 (P = 0.04)	64.7% (>999.99)		
Chloride (mg L^{-1})	0.11 (P = 0.03)	64.4% (1.12)		
Rainfall (total, in)				
day before sample			-0.81 (P = 0.02)	36.6% (0.45)
30 day's before sample			-0.17 (P = 0.01)	58.6% (0.85)

	Binary Logistic Regression model for occurrence of *Campylobacter*			
	Small Watershed Sites		Large Watershed Sites	
Variable	z (p-value)	Model concordance (Odds Ratio)	z (p-value)	Model concordance (Odds Ratio)
Temperature (^{o} C)	-0.08 (P < 0.05)	62.8% (0.93)		
Dissolved Oxygen (mg L^{-1})	-0.10 (P = 0.05)	56.1% (0.91)		
pH	1.00 (P < 0.05)	66.3% (2.72)	0.74 (P = 0.05)	62.0% (2.09)
ORP	-0.01 (P = 0.04)	58.9% (0.99)	-0.01 (P = 0.04)	
TKN (mg L^{-1})	-0.39 (P = 0.02)	55.4% (0.67)	-0.47 (P = 0.05)	57.5% (0.63)
TC (mg L^{-1})	-0.06 (P < 0.05)	67.6% (0.94)	-0.07 (P < 0.05)	67.9% (0.93)
IC (mg L-1)	-0.48 (P < 0.05)	78.4% (0.62)	-0.39 (P < 0.05)	76.0% (0.68)
Rainfall (total, in)				
30 day's before sample	0.15 (P = 0.02)	54.8% (1.17)	0.12 (P = 0.03)	49.5% (1.13)

Table 4.4. Frequency (%) of *Salmonella* and *Campylobacter* prevalence when fecal indicator organisms are above and below EPA standards [a] for safe water

Indicator organism	EPA Threshold (one-time maximum)	*Salmonella* (N=129) present and indicator		*Campylobacter* (N=96) present and indicator	
		above standard	below standard	above standard	below standard
E. coli	235	49 (38%)	80 (62%)	39 (41%)	57 (59%)
Enterococci	61	113 (88%)	16 (12%)	86 (90%)	10 (10%)
Fecal coliform	400	42 (33%)	87 (67%)	26 (27%)	70 (73%)

a based on a single sample maximum for any 30-day period as cited by the Environmental Protection Agency (41, 42)

FIGURE LEGEND

Figure 4.1. Map of *Salmonella* distribution in the Satilla River Basin

Figure 4.2. Map of *Campylobacter* distribution in the Satilla River Basin

Figure 4.3. Monthly distribution of *Salmonella* and *Campylobacter*

Figure 4.1.

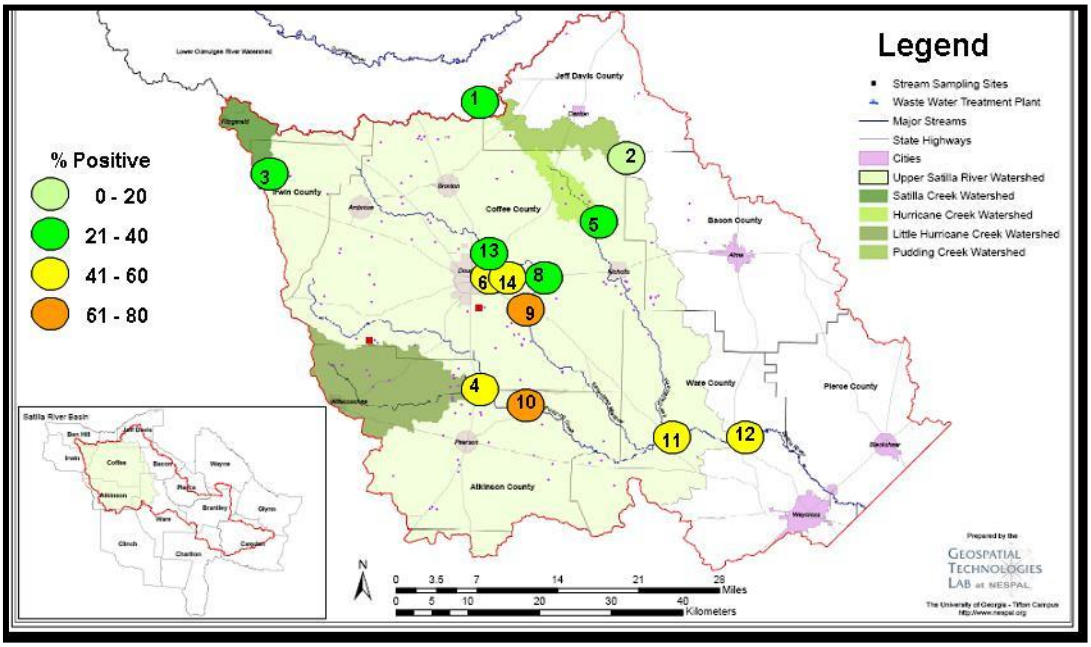


Figure 4.2.

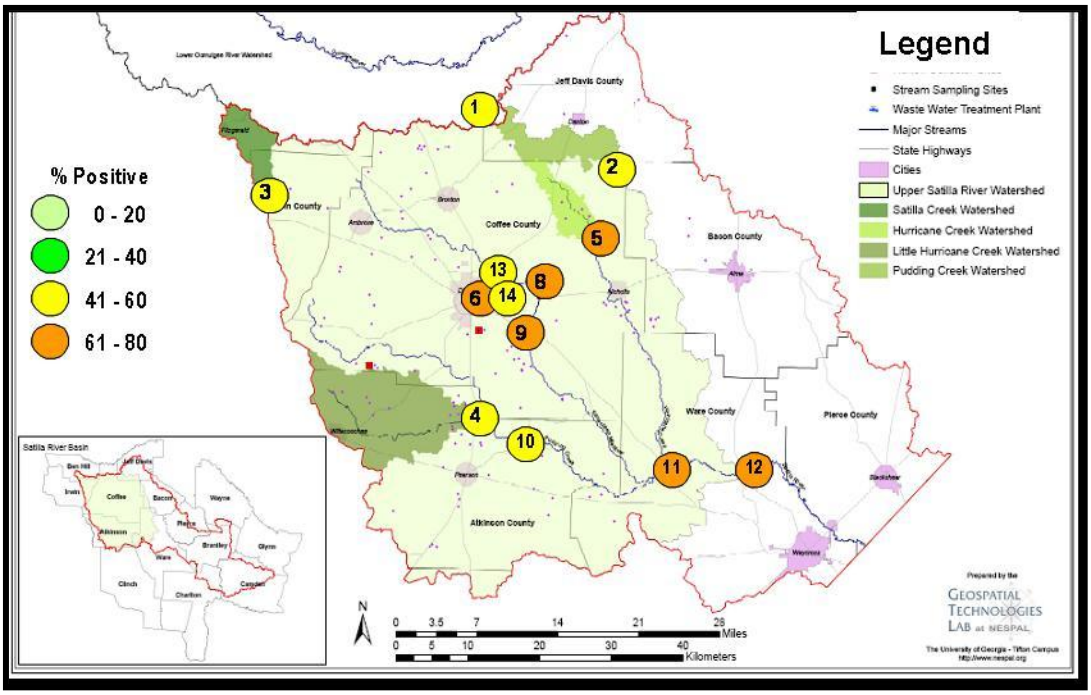
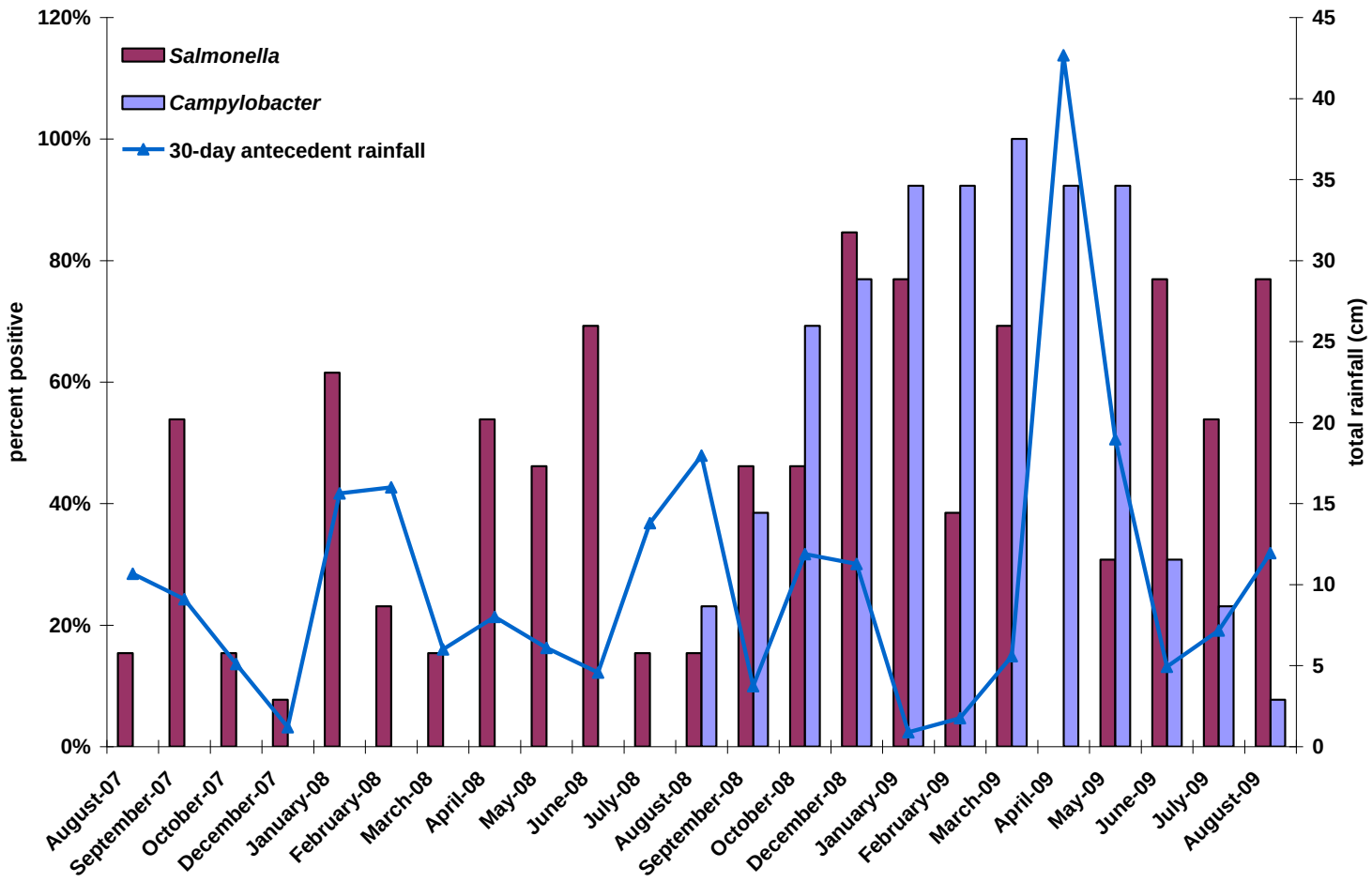


Figure 4.3.


Salmonella
Campylobacter
30-day antecedent rainfall
percent positive
total rainfall (cm)
120%
100%
80%
60%
40%
20%
0%
45
40
35
30
25
20
15
10
5
0
August-07
September-07
October-07
December-07
January-08
February-08
March-08
April-08
May-08
June-08
July-08
August-08
September-08
October-08
December-08
January-09
February-09
March-09
April-09
May-09
June-09
July-09
August-09

CHAPTER 5

DISTRIBUTION AND DIVERSITY OF IN-STREAM LEVELS OF SALMONELLA AND CAMPYLOBACTER IN THE SATILLA RIVER BASIN (GEORGIA, USA) [1]

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ABSTRACT

Although salmonellosis and campylobacteriosis are more commonly associated with contaminated foods and feeds than with water; both *Salmonella* and *Campylobacter* have frequently been found in effluent from sewage treatment plants, in industrial wastes, and in streams that receive a variety of inputs. The presence of the pathogens *Salmonella* and *Campylobacter* in the stream network of the Satilla River Basin (SRB) were monitored monthly from August 2007 to August 2009 to determine the occurrence of these pathogens in the surface waters of the region. A combination of enrichment and conventional cultural methods was used for confirmation and identification of *Salmonella*, while *Campylobacter* detection consisted of enrichment and conventional polymerase chain reaction (PCR). Antimicrobial susceptibility patterns and serotypes were determined for the *Salmonella* isolates. *Salmonella* spp. was recovered from 43% (129 of 299) of all samples. The most common serogroups identified were C (25%), B (6%), D (6%) and E (5%). Sixteen different serotypes identified included *Salmonella enterica* serotype Montevideo (23%), Braenderup (14%) and Saint Paul (13%). Serotype diversity generally peaked in the fall and winter months. No antimicrobial resistance to fifteen antimicrobials in the NARMS Panel was detected in *Salmonella* isolates. *Campylobacter* spp. were recovered from 62% (96 of 156) of all samples. *C. jejuni* was the most prevalent species detected (30%) followed by *C. lari* (22%), *C. upsaliensis* (11%), and *C. coli* (3%) Pathogens were more prevalent in the areas where agriculture and poultry production were concentrated, as well as nature based recreational activities (e.g. fishing, canoeing) indicating a potential public health risk of *Salmonella* and *Campylobacter* from surface waters in a rural mixed land-use watershed.

INTRODUCTION

The global incidence of foodborne disease is difficult to estimate, but it has been reported that in 2005 alone 1.8 million people died from diarrheal diseases, and majority of those cases may be attributed to contamination of food and drinking water (57). Estimates from the US Centers for Disease Control and Prevention (CDC) indicate that there are approximately 1.4 million cases of salmonellosis each year in the US and 2.4 million cases of campylobacteriosis (34). The true incidence is undoubtedly much higher as many more cases go undiagnosed and unreported. The CDC estimates that for every confirmed case of *Salmonella*, there are 38 cases that go unreported (34).

Campylobacteriosis, gastroenteritis caused by the thermotolerant *Campylobacter* spp. (*C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis*), is the most commonly reported gastrointestinal bacterial disease in developed countries throughout the world (11, 37). Although, numbers of infections have declined slightly in some parts of the world during recent years, the overall disease burden is still significant (2, 37). The genus *Campylobacter* currently includes 15 species and 6 subspecies; 11 of these are considered pathogenic to humans (5, 55). The major pathogens, *C. jejuni and C. coli* account for about 90% and 10% of human campylobacteriosis cases, respectively (37). While *C. lari* and *C. upsaliensis* cause far fewer cases of disease, both species have been recognized as emerging human pathogens (31, 56). Typically, cases are self-limited characterized by diarrhea, abdominal pain, and fever. However, approximately 1 in 2,000 *C. jejuni* infections may be complicated by Guillain-Barré syndrome (GBS, an inflammatory disorder of the peripheral nerves) (3, 37).

In humans *Salmonella* infection may cause: enteric fever (typhoid), resulting from bacterial invasion of the bloodstream, and acute gastroenteritis, resulting from a foodborne

infection/intoxication (25, 34). Salmonellosis is the second leading cause of bacterial foodborne illness worldwide (usually slightly less frequent than *Campylobacter* infection) (11) and the leading cause of bacterial foodborne illness in the United States (39). The genus *Salmonella* consists of two separate species, *Salmonella bongori* and *Salmonella enterica*, and encompasses over 2, 500 known serotypes, all of which are considered potential human pathogens (8, 26). Proliferation of *Salmonella enterica* serotype Enteritidis and *Salmonella enterica* serotype Typhimurium has increased since the second half of the twentieth century, derived from two changes in the epidemiology of salmonellosis that occurred worldwide: the emergence of human infections caused by food contaminated with serotype Enteritidis (11, 47), and the emergence of resistance against multiple antibiotics in serotype Typhimurium strains (11, 17, 49).

Antimicrobial resistance is an increasingly global problem, and emerging antimicrobial resistance has become a public health issue worldwide (29). A variety of foods and environmental sources harbor bacteria that are resistant to one or more antimicrobial drugs used in human or veterinary medicine and in food-animal production (4, 7). Although salmonellosis and campylobacteriosis are more commonly associated with the consumption of contaminated food products such as meat, poultry, eggs, milk, seafood, and fresh produce than with water, transmission of *Salmonella* and *Campylobacter* to humans can occur by many routes including consumption of food, animal products, or raw produce contaminated with animal waste; contact with animals and their environment; and contact with contaminated water (9, 28, 33, 39, 45). This includes surface waters that are potential reservoirs and transmission routes for these pathogens (28, 33).

During a water quality survey begun in 2003, campylobacter-like organisms (CLO) were found in the headwaters of the Satilla River basin (SRB) in a mixed-use rural watershed located

in the coastal plain of southern Georgia (52). Mean CLO counts and overall prevalence were highest downstream from a wastewater treatment plant (WWTP) that handled both human and poultry slaughterhouse waste (up to 595 CFU ml^{-1}, 100% of samples positive) (52). Isolation of pathogens from the surface waters of an area give rise to questions as to the origins of the organisms, their survival or persistence, and their relevance to public health, which prompted the present study. Here, the distribution and diversity of *Salmonella* and *Campylobacter* in the SRB was further examined following an analysis of their frequency distribution in relation to land use in this watershed(53). In addition, we evaluated antimicrobial susceptibility patterns among *Salmonella* isolates. Our overall goal was to determine how landuse practices shape the distribution of specific strains of *Salmonella* and *Campylobacter* and to evaluate the potential relationship of environmental prevalence with clinical cases in this rural watershed.

METHODS AND MATERIALS

Previous chapters to this report described a range of water quality, nutrient, and fecal indicator methods and analysis including a detailed description of the study area (Chapter 3, (53)). The reader is referred to these chapter's for discussion of that material (53, 54).

Isolation and identification of Salmonella

The methods for *Salmonella* detection are described here briefly (see Chapter 4 (54) for a more detailed description). From each sample 250 ml was concentrated onto a 0.45 µm 47 mm nitrocellulose membrane filter. The filters were placed in 25 ml of buffered peptone water (BPW) and incubated at 37^{o}C for 24 ± 2 hours for non-selective pre-enrichment. One hundred microliters of the BPW pre-enrichment was then transferred to 10 ml of Rappaport-Vassiliadis (RV) broth for selective enrichment at 42^{o}C for 24 ± 2 hours. This was followed by streaking from RV broth onto xylose lysine tergitol (XLT) agar and incubating plates at 37^{o}C for 24 to 48

hours. Colonies (3–5) exhibiting typical *Salmonella* morphology were transferred to blood agar plates for biochemical and serological confirmation. Confirmed isolates were serotyped at the National Veterinary Services Laboratory (U.S. Department of Agriculture, Animal Plant Health Inspection Service, Ames, Iowa, USA).

Testing Salmonella for Antimicrobial Susceptibility

Antimicrobial susceptibility patterns of *Salmonella* isolates were determined using a panel of 15 antimicrobial agents in the National Antimicrobial Resistance Monitoring System (NARMS) (Trek Diagnostic Systems, Westlake, Ohio). Antimicrobials tested included amikacin (concentration range 0.5–64 µg/ml), gentamicin (0.25–16 µg/ml), kanamycin (8–64 µg/ml), streptomycin (32–64 µg/ml), ampicillin (1–32 µg/ml), amoxicillin/clavulanic acid (1–32 µg/ml), ceftiofur (0.12–8 µg/ml), ceftriaxone (0.25–64 µg/ml), cefoxitin (0.5–32 µg/ml), sulfisoxazole (16–256 µg/ml), trimethoprim/sulfamethoxazole (0.12–4 µg/ml), chloramphenicol (2–32 µg/ml), ciprofloxacin (0.015–4 µg/ml), nalidixic acid (0.5–32 µg/ml), and tetracycline (4–32 µg/ml)[cited 29 Oct 2008].

Available from:Minimal inhibitory concentrations (MIC) were determined by the broth-microdilution method using the Sensititre system (Trek Diagnostic Systems, Westlake, Ohio). *Salmonella* isolates were classified as susceptible, intermediate, or resistant (38, 51).

Campylobacter enrichment and PCR

The methods for *Campylobacter* detection are described here briefly (see Chapter 4 (54) for a more detailed description). 250 ml of each sample was concentrated onto a 0.45 µm 47 mm nitrocellulose membrane filter. The filters were placed into 10 ml of Campylobacter enrichment broth (CEB) and incubated at 42^{o}C for 48 ± 2 hour enrichment. A 1.5 ml aliquot of each CEB enrichment was used for DNA extraction (MoBio Power Soil DNA Extraction kit, MOBIO

Laboratories, Carlsbad, CA) and polymerase chain reaction (PCR). The primers and PCR conditions used have been described by Eyers et al. (21, 22) and from Savill et al. (45). Additional primers (Table 5.1) were used to discriminate between the four thermotolerant species of *Campylobacter* (30). PCR was performed with a Bio-rad® thermal cycler (PTC-200, Waltham, Massachusetts). The amplification and visualization protocols are detailed in Chapter 4 (54).

Georgia public health data for Salmonella and Campylobacter

Georgia is divided into 159 counties and 18 public health districts (PH); the Satilla River Basin is located within Georgia PH (8-1) and PH (9-2). We obtained data on the number of *Salmonella* and *Campylobacter* cases reported from 2007 to 2009 at the county level for PH (8-1) and PH (9-2) in the SRB to determine if the frequency of *Salmonella* and *Campylobacter* detection and distribution from environmental samples in the SRB were similar to the frequency of clinical cases reported. Incidence for each pathogen was calculated as the total annual number of monthly cases of each bacterial pathogen, divided by the group-specific population estimates based on the 2000 census (50). To account for year-to-year variation, the mean annual incidence rates were also calculated.

Statistical analysis

For all analyses of *Salmonella* serovars and *Campylobacter* species when a target organism was present, it was assigned the value 1, and when a target organism was absent, it was assigned the value 0. The total number of samples positive for each month was calculated by summing the number of samples positive for all sites for a given month. Fisher's exact test was used to statistically evaluate the presence and absence of *Salmonella* serogroups and *Campylobacter* species. Using SAS release 9.1 (Cary, NC) statistical software $\underline{p}$ values ≤ 0.05

were considered significant. “Percent resistant” was calculated as the number of isolates classified as “intermediate” or “resistant” divided by the total number of isolates tested and expressed as a percentage.

RESULTS

Salmonella

Salmonella spp. were recovered from 43% (129 of 299) of all samples. *Salmonella* occurrence ranged from 17% (4 out of 23) at site 2 (no poultry houses) to 61% (14 out of 23) positive at two sites, one immediately downstream of the WWTP (site 9) and the other upstream of the WWTP on the Satilla River within a sub-watershed containing 173 poultry houses (site 10) (Table 4.1 as cited in Chapter 4 (54)). *Salmonella* isolates (N=263) were serogrouped by the slide agglutination test with the use of O-antigen antiserum. 112 *Salmonella* isolates belonged to serogroup C (66 positives, 59%), B (16 positives, 14%), D (16 positives, 14%), and E (14 positives, 13%) in decreasing order. More than one serogroup was detected from three sites but only on two separate sample occasions (August and September 2007). Serogroups C and E were detected from site 6 (wastewater influent August 2007) and site 4 (September 2007). Serogroups C and D were both isolated at site 9 in August 2007; and serogroups B and C were detected in September 2007. The remaining 151 presumptive *Salmonella* isolates could not be typed using individual *Salmonella* antisera for ‘O’ groups B through E (See Appendix B, Table B.5.1).

Seventy-eight of the 112 *Salmonella* isolates belonging to serogroups C, B, D, and E were serotyped at the National Veterinary Services Laboratories of the Department of Agriculture in Ames, Iowa. Of the 78 isolates, 16 different serotypes were identified. *Salmonella enterica* serotype Montevideo was the predominant serotype, represented by 18

isolates (23%), followed by serotype Braenderup (11 isolates [14%]) and serotype Saint Paul (10 isolates [13%]) (Table 5.2). Although samples from all sites tested positive for *Salmonella,* all samples were not confirmed to serotype. As few as zero serotypes were detected at sites 2 and 5 with a maximum of four different serotypes detected at sites 6 and 12. Serotype distribution also varied between sites and was most often separated between the larger sites downstream of the WWTP stations and the smaller sites upstream of the treatment plant; with more serotypes being detected from the downstream stations. The most frequently detected serotype was Montevideo, detected from six different sites (sites 1, 8, 9, 10, 11, and 12). Braenderup, the second most common serotype, was detected from four different sites (sites 6, 8, 12, and 13).

The distribution of serogroups B, C, D, and E by month did not vary significantly (Fisher's p value 1.0). The number of isolates recovered ranged from a low of one in May 2008 to a high of 17 in September 2007 and December 2008 (Figure 5.1). From August 2007 to August 2008 most isolates were detected in the fall months of August, September and October; while from August 2008 to August 2009 most isolates were detected in the winter months of December and January (no sampling in November of either year) (Figure 5.1). Among individual serotypes a seasonal trend was not observed (See Appendix B, Figure B.5.1).

All of the isolates tested from the SRB (78/78, 100%) were susceptible to the antimicrobials.

Campylobacter

Presence of *Campylobacter* spp. was detected in 62% (96 of 156) of all samples. *Campylobacter* occurrence ranged from 50% positive (N = 12) at three sites including site 2 (no poultry houses) and both effluent sites at the WWTP (site 13 and 14) to 75% (N = 12) positive at site 6 the direct influent to the WWTP and site 8 upstream of the WWTP on Seventeen Mile

River within a sub-watershed containing 98 poultry houses (Table 4.1 as cited in Chapter 4 (54)). Of the 96 samples positive for thermotolerant *Campylobacter* spp., *C. jejuni* was the most prevalent species detected (29 positives, 30%) followed by *C. lari* (21 positives, 22%), *C. upsaliensis* (11 positives, 11%), and *C. coli* (3 positives, 3%).

Forty-seven of the samples positive for thermotolerant *Campylobacter* spp. did not yield a positive species result. Additionally, four of the 156 total samples tested produced a positive species result, despite testing negative for the broader thermotolerant *Campylobacter* spp. primer pair. Of these four, two were *C. jejuni* (Site 6 August 2008 and Site 3 June 2009); and the remaining two were *C. upsaliensis* (Site 4 September 2008) and *C. lari* (Site 13 December 2008).

During the 12-month sampling period, more than one species was detected from seven sites but this occurrence was limited to the sample period from January to May 2009. Multiple species were detected from site 9 below the WWTP in each month from January 2009 to April 2009. Multiple species were only detected on one occasion from the other six sites; four of the sites being the smaller upstream stations (sites 2, 3, 4, and 5). The most frequent multiple species pair detected was *C. jejuni* and *C. lari* (7 out of 10, 70%). The four species were never detected simultaneously from any site at a single sample; and, three species from a single sample were only detected simultaneously once (at site 4, April 2009) only *C. upsaliensis* was not detected.

All four *Campylobacter* species were detected in the SRB; however species diversity (number of different species) varied by site (See Appendix B, Table B.5.2). All four species were only detected at site 4 (small watershed with agricultural and poultry houses). Only one species was detected at the control station, site 1 (*C. upsaliensis*) and at the direct effluent from the WWTP station, site 13 (*C. lari*). *Campylobacter* species distribution (number of species

detected from each site) varied as well. 45% (29/64) of all *Campylobacter* isolates were detected from the larger downstream stations (sites 8, 9, 10, 11 and 12), 19% (12/64) were detected from the WWTP stations (sites 6, 13, and 14), and 36% (23/64) from the remaining smaller agricultural stations (sites 2, 3, 4, and 5) and control station (site 1).

Campylobacter were detected in all study months. The percentage of samples positive ranged from a low of eight percent (1/13) positive in August 2009 to the highest 100% (13/13) in March 2009 (Figure 5.2). *Campylobacter* presence was not significantly different between months (Fisher's p value 1.0). Campylobacter was detected more frequently in January, February, April, and May 2009 (92% positive, 12/13) peaking in March 2009 with 100% of sites testing positive. *Campylobacter* was not detected from site 2 in February 2009 nor from site 14 in January, April, or May 2009. Among individual *Campylobacter* species, the frequency of detection also varied monthly peaking in the winter and spring months (Figure 5.2).

Spatial Distribution

From August 2007 to August 2009, *Salmonella* and *Campylobacter* (beginning in August 2008) were frequently detected at all sites monitored in the stream network of the Satilla River Basin. *Salmonella* spp. were recovered from 43% (129 of 299) of all samples and *Campylobacter* spp. were recovered from 62% (96 of 156) of all samples. The pathogens were most often detected downstream of the WWTP and from the other larger downstream sites on the Satilla River. This pattern was also noted for *Salmonella* serotypes and *Campylobacter* species identified from the various sample sites.

Downstream from the WWTP at site 9 *Salmonella* serogroups B, C, and D were detected. Upstream from the WWTP at site 8 only serogroups C and D were detected and there were fewer isolates than those obtained from the downstream station (site 9) (Figure 5.3). The frequency of

detection among the *Salmonella* serotypes among the smaller sites with varying agricultural landuse and poultry production generally were noted in terms of location in the watershed (i.e., upstream *vs.* downstream of the WWTP) (Figure 5.3). Sites 4 and 5 were the sites with both agricultural landuse and poultry production in the subset of watersheds from sites 1 to 5, site 1 being the control station. Serogroups B, C, and E were isolated from site 4. *Salmonella* from serogroup B was isolated from both sites 3 and 5, but more isolates were detected from site 3 and no serovars were isolated from site 2.

However, if we include those isolates that were not identified by antigen screening but tested positive for *Salmonella*, this adds an additional 10 isolates detected from site 5 and only six and five additional isolates from sites 2 and 3 respectively. The addition of these isolates by mere count alone indicates that more positive isolates were detected from the sites with both agricultural landuse and poultry production. As stated earlier the inability to type these particular isolates presently does not exclude the possibility that these isolates are *Salmonella*.

This trend was also observed upon examination of *Campylobacter* species and does not appear to be pathogen dependent. As with *Salmonella* serogroups, the frequency of *Campylobacter* species appeared to be more a function of location (i.e. upstream vs. downstream of the WWTP effluent) and localized to areas where agriculture and poultry production are concentrated in the SRB (Figure 5.4). There was more variation again at site 4 where all four of the *Campylobacter* species being investigated were identified. *C. jejuni*, *C. lari*, and *C. upsaliensis* were all isolated from sites 3 and 5. *C. jejuni* was isolated more frequently from site 3, again having 50% agricultural landuse and no poultry production. Downstream from site 2, site 5, being agriculturally influenced with poultry production we observed increased variation in the *Campylobacter* species composition between these two sites. *C. jejuni* and *C. upsaliensis*

were both isolated from these sites (2 and 5), however *C. lari* was additionally isolated from site 5 (Figure 5.4). In the same way, *C. jejuni* and *C. lari* were detected upstream from the WWTP at site 8, and downstream at site 9 *C. coli* was also detected with these two *Campylobacter* species.

Pathogen cases reported from Georgia Public Health (PH) District (8-1) and (9-2)

We obtained data on the number of *Salmonella* and *Campylobacter* cases reported from 2007 to 2009 at the county level for PH (8-1) and PH (9-2) in the SRB. The *Salmonella* serotypes and *Campylobacter* species found most frequently during the study period were similar to those serovars and species commonly isolated from reported human cases, as well as clinical animal isolates (15). Of the 16 different *Salmonella* serotypes detected in this study, eight were listed among the 20 most frequently reported serotypes from human sources, 6 were listed among the 20 most frequently reported serotypes from clinical non-human sources, and 7 were listed among the 20 most frequently reported serotypes from non-clinical non-human sources (Table 5.3) (15). The 16 *Salmonella* serotypes identified in the environmental samples were also reported in 51.94% (617 out of 1188) of the *Salmonella* combined cases reported from PH 8-1 and 9-2 (Table 5.4). The majority of *Campylobacter* cases reported (54%, 113 out of 210) in PH 8-1 and PH 9-2 were unknown or not speciated. Forty-four percent (93 out of 210) of reported *Campylobacter* cases were *C. jejuni*. *C. lari* was not detected; and *C. coli* (2 out of 210) and *C. upsaliensis* (1 out of 210) accounted for only 1% each of the total *Campylobacter* cases reported. During the study period salmonellosis and campylobacteriosis (Table 5.5) case rates in these public health districts consistently exceeded the mean rate for both the US and the state of Georgia also. CDC reports the most current estimates of *Salmonella* infection at 7,039 and 6,033 for *Campylobacter* laboratory-confirmed cases of infection, for an incidence per 100, 000 population of 15.19 and 13.02, respectively in the US (14). The most current incidence rates in

Georgia for *Salmonella* are 24.57 per 100, 000 population and 7.58 for *Campylobacter* (14). The *Salmonella* rate is the highest among all states reporting (14).

DISCUSSION

This study was part of a larger investigation into the distribution and diversity of *Salmonella* and *Campylobacter* in the Satilla River Basin within Georgia's coastal plain. We were particularly interested in the prevalence and distribution of *Salmonella* serovars and *Campylobacter* species in an area comprised of varying levels of agricultural landuse and poultry production. While *Salmonella* and *Campylobacter* continue to be the greatest cause of bacterial gastrointestinal infections in the United States, rates are especially high in the southeast and in south Georgia, in particular. Estimates from the US Centers for Disease Control and Prevention (CDC) indicate that there are approximately 1.4 million cases of salmonellosis each year in the US and 2.4 million cases of campylobacteriosis (34). The true incidence is undoubtedly much higher as many more cases go undiagnosed and unreported. The CDC estimates that for every confirmed case of *Salmonella*, there are 38 cases that go unreported (34).

There are multiple sources of *Salmonella* and *Campylobacter* in the SRB. The basin is home to a major broiler bird processing plant; including, 112 poultry producers and over 440 poultry houses. In addition to producing food for both local and national consumption, the intensively farmed area of the SRB also contains a municipal WWTP with a permitted discharge of 6 million gallons per day (MGD) of which about half is provided by wastewater from the broiler processing plant (Delnee Wilcox, plant operator, personal communication). The majority of the poultry litter from the poultry houses is spread within the watershed (B. Bannister, USDA-NRCS personal communication). A large body of research has tied the spreading of livestock manures to land with nutrient runoff, as the disposal of these wastes may contaminate water

resources at the field level; and may even lead to contamination of downstream water resources (1, 19, 24, 27, 41). When animal manure and processing wastes are spread on the land, the microorganisms are exposed to environmental conditions and their survival depends on manure type (solid or liquid), handling and treatment of manure, time of the year, presence or absence of plants, active microbial movement, microbial surface properties, soil water content, and environmental factors (such as soil pH, temperature and permeability) (10). The larger study area sites (8, 9, 10, 11. 12) downstream of the WWTP stations are frequently visited year-round for recreational use such as fishing, hunting, canoeing/kayaking, four-wheeling, picnicking and other forms of nature-based tourism.

Salmonella serogroups and *Campylobacter* species were most often detected from the stations where agricultural landuse and poultry production were greatest in the SRB. Comparing the smaller upstream stations (sites 1 to 5) with the WWTP stations (site 6, 13, and 14) and the larger downstream stations (sites 8 to 12) the difference in *Salmonella* serogroups and *Campylobacter* species also increased in number from upstream to downstream stations with more species and serotypes generally detected at the downstream sites. Site 2 has a drainage area of 64 km^2 with 22% agricultural landuse and no poultry houses. No *Salmonella* serogroups were detected from this station and only two of the *Campylobacter* species were detected from this station compared with site 5 that is downstream of this station where three of the *Campylobacter* species were detected and also *Salmonella* serogroup B was detected from this station. Sites 3 and 5 both have similar size drainage area at 45 km^2 and 46 km^2 respectively. These sites differ in that site 3 has 52% agricultural landuse with no poultry houses while site 5 has 20% agricultural landuse but 21 poultry houses. *Salmonella* serogroups and *Campylobacter* species that were detected from both of these sites were more frequently isolated from site 3

where there is more agricultural landuse. Site 4 was the most downstream of the smaller sites sampled and the station with the most agricultural landuse and poultry houses (50 poultry houses) among all of the small watershed sites (sites 1 to 5). Serotype and species diversity (number of different species or serotypes detected) was highest at this station as was the frequency of serotype and species detection.

The highest frequency of isolation for all serogroups and species were from the most downstream stations (sites 10, 11, and 12); however the diversity of pathogens isolated from these sites was not as varied as those stations upstream in the study area where agricultural landuse and poultry production are highly concentrated.

The exception to this trend was the WWTP watershed, where the untreated influent to the treatment plant (site 6) had not only more isolates of the serogroups and species, but the diversity or type of species and serogroups detected from these samples were also higher than the pathogens detected from the treated WWTP effluent discharge (site 13 and 14). However, the frequent occurrence of *Salmonella* and *Campylobacter* (and fecal indicators as described in chapter 3 (53)) from disinfected and undisinfected discharge from the WWTP is a concern. Because all serotypes of *Salmonella* are potentially pathogenic, the detection of *Salmonella* should be interpreted as a public health risk.

Salmonella are divided into 60 serogroups and over 2,500 known serotypes (16). Forty-three percent (112/263) of the presumptive *Salmonella*-positive isolates in this study were typed by serogroup. The remaining 151 isolates were not typeable using the available polyvalent and group-specific antisera. The lack of reactivity with these antisera does not indicate that these isolates were not *Salmonella*, but that these isolates may potentially possess 'O' antigen that was

not included in the *Salmonella* antisera testing. The strain might not also be *Salmonella;* and future serotyping of these samples will determine the final type strain of these isolates.

The most prevalent serogroup identified in the SRB were isolates belonging to *Salmonella* serogroup C (See Appendix B, Table B.5.1). Common *Salmonella* of serogroup C include Montevideo, Oranienburg, Muenchen and Newport. *Salmonella* from serogroups B and D followed for a combined detection of 12% of the isolates typed in the SRB. The six most common serogroups in the United States are B, C1, C2, D, E, and O13 (23). *Salmonella* from serogroups B and D account for approximately two-thirds of all reported *Salmonella* human infections and include the 2 most common serotypes, *S. enterica* serotype Enteritidis and *S. enterica* serotype Typhimurium, which together cause approximately one-half of all human infections in the United States (36).

The identification of specific *Salmonella* serotype is useful and helps to define the major serotypes circulating in the population and the environment. Serotyping of isolates corroborated the findings that the majority (55%, 43 out of 78) of the isolates were also members of serogroup C. In total, 16 different *Salmonella* serotypes were detected. This is consistent with other studies of *Salmonella* spp. in surface waters that have typically isolated less than 20 serotypes from a survey (26, 33, 35, 42, 46). The most common serotype recovered was *Salmonella enterica* serotype Montevideo (23%, 18 out of 78), followed by serotype Braenderup (14%, 11 out of 78) and serotype Saint Paul (13%, 10 out of 78). In addition, serotypes Senftenberg (n = 7), Anatum (n = 3), and Typhimurium (n = 3) that belong to serogroups E and B were found.

The *Salmonella* serotypes identified were notably similar to those serotypes most commonly identified in clinical, animal, or food samples in the United States. Of the 16 different *Salmonella* serotypes detected in this study, eight are listed among the 20 most

frequently reported serotypes from human sources (such as Montevideo, Typhimurium, Javiana, and Saint Paul), 6 are listed among the 20 most frequently reported serotypes from clinical non-human sources (such as Montevideo, Senftenberg, Mbandaka, and Anatum), and 7 are listed among the 20 most frequently reported serotypes from non-clinical non-human sources (such as Montevideo, Saint Paul, Anatum, Typhimurium, and Muenchen) according to the 2006 *Salmonella* Annual summary (full list shown in Table 5.3) (15).

Data on the number of *Salmonella* and *Campylobacter* cases reported from 2007 to 2009 at the county level for Georgia Public Health (PH) district (8-1) and PH district (9-2) indicated that salmonellosis and campylobacteriosis case rates in these public health districts consistently exceeded the mean rate for both the US and the state of Georgia (Table 5.5). Some ecological descriptive studies have shown that *Campylobacter* rates of infection are higher in rural areas than urban areas and that among rural areas, farming regions have the highest rates (43, 44, 48). Potter et al. (44) showed that persons engaged in poultry and bovine husbandry had increased odds of campylobacteriosis infection and demonstrated a dose-response relationship for poultry and bovine exposure. Although we did not see significant spatial difference among the sites, we did detect pathogens more frequently from the sites with agricultural and poultry influence.

Of the 16 serotypes recovered, 9 were common clinical serotypes reported in Georgia (Montevideo, Braenderup, Saint Paul, Javiana, Anatum, Typhimurium, Bareilly, Muenchen, and Rubislaw), that have also frequently been isolated in environmental samples (15, 26, 35). Additionally, the common serotypes Javiana and Newport are of epidemiological significance given their increasing frequency in human clinical cases (14). Compared with the incidence of infection a decade ago, Javiana reported cases are 120% higher and Newport reported cases are 64% higher (14). Nine of the serotypes identified were also commonly detected by researchers

in an environmental survey of a north Georgia watershed (Muenchen, Rubislaw, Hartford, Mbandaka, Montevideo, Thompson, Typhimurium, Braenderup, and Infantis) (35); and seven serotypes were commonly identified in a south Georgia watershed environmental survey near the SRB (26). The common serotypes from the south Georgia watershed were Muenchen, Rubislaw, Braenderup, Saint Paul, Bareilly, Montevideo, and Anatum. Only four serotypes were commonly identified in all three studies (Muenchen, Rubislaw, Montevideo, and Braenderup). The common presence of Montevideo is significant as this serotype has been among the top 10 human clinical infections reported in the previous 3 years (2007-2009) (12, 14).

Although *Salmonella* recovered from clinical human and animal cases have been examined, few published studies have reported on antimicrobial resistance in *Salmonella* recovered from surface waters (6, 18, 35, 40). In the present study, all of the isolates tested (78/78, 100%) were susceptible, to the 15 antimicrobials tested. This is less than we expected based on levels seen in CDC's surveillance data for 2005 from human and animal clinical non-Typhi *Salmonella* isolates (13). In human clinical cases, 14.8% (304/2052) of isolates were resistant to two or more antimicrobials, and 7.6% (156/2052) were resistant to five or more antimicrobials (13). However, we did find that antimicrobial resistance was observed in 30 out of 60 *Salmonella* isolates obtained from clinical samples from various animal species submitted to Tifton Veterinary Diagnostic Laboratory from this region (data not presented). In a similar survey conducted by Meinersmann et al. (35) in a northeastern Georgia watershed, 37% (7/19) of *Salmonella* isolates detected, were resistant to the antimicrobials tested. Dolejska et al. (18) found that antibiotic resistance was in only 12% (2/14) of *Salmonella* isolates obtained from surface water samples of a pond in the northeastern part of the Czech Republic. Our data indicate that antibiotic resistance is less pronounced and may not be related to a singular event or

factor but dependent on more complex variables such as environmental exposure and carriage rate of human and/or animal source.

C. jejuni was the most prevalent *Campylobacter* species detected (30%, 29 out of 96) followed by *C. lari* (22%, 21 out of 96), *C. upsaliensis* (11%, 11 out of 96), and *C. coli* (3%, 3 out of 96). *C. jejuni* is most often isolated from poultry and poultry products; appearing to have evolved to optimally colonize the intestinal mucosa of birds (body temperature 42^{o}C) where it appears to act as a commensal (55). Among food producing animals, *C. jejuni* predominates among cattle and broilers, whereas *C. coli* is most commonly found among pigs (37). Swine operations are limited in the SRB and the narrow detection of *C. coli* in the present study reflects this. *C. lari* is commonly associated with wild birds (32, 55) and *C. upsaliensis* are found in the intestines of cats and dogs (55). Given the high carriage rate of these *Campylobacter* species in domestic and wild animals, large numbers are excreted and provide a continuous flow into the environment.

CONCLUSION

Frequent isolation of pathogens from the surface waters of an area give rise to questions as to the origins of the organisms, their survival or persistence, and their relevance to public health. The *Salmonella* serotypes and *Campylobacter* species observed in the present study were notably similar to those most frequently identified in clinical, animal and environmental samples. Both pathogens were frequently isolated from areas influenced by agricultural and poultry production; as well as from downstream stations influenced by human recreational activities. This observation indicates that one of the primary sources of the pathogens presence environmentally may be due to human inputs. Conversely, the public may be at risk of becoming infected from *Salmonella* and *Campylobacter* as the population and poultry production

in this region continues to grow. We have previously shown the prevalence of these pathogens in stream flow of the SRB, now we further demonstrate that they can include clinically relevant serotypes and species, which can be linked to the different landuse and agricultural practices that may threaten the health of environmental waters and downstream recreational users.

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TABLES

Table 5.1. PCR primers, primer sequences, and sizes of PCR amplicons used in this study[a]

Primer name	Primer Sequence 5' to 3'	PCR amplicon size
C jejuni	**ACAACTTGGTGACGATGTTGTA**	**331 bp**
C coli	**AGACAAATAAGAGAGAATCAG**	**391 bp**
C lari	**TRCCAAATGTTAAAATAGGCGA**	**233 bp**
C upsaliensis	**AAGTCGTATATTTTCYTACGCTTGTGTG**	**206 bp**
ARkk2m	**CAATCATGDGCDATATGASAATAHGCCAT**	

[a] ***C. jejuni, C. coli, C. lari,*** **and** ***C. upsaliensis*** **are the forward primers used in conjunction with ARkk2m, the reverse primer**

Table 5.2. Frequency (%) of isolation among *Salmonella* serotypes in the Satilla River Basin

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 8	Site 9	Site 10	Site 11	Site 12	Site 13	Site 14	TOTAL
Montevideo	3 (17%)	0	0	0	0	0	1 (6%)	2 (11%)	5 (28%)	6 (33%)	1 (6%)	0	0	18 (23%)
Braenderup	0	0	0	0	0	4 (36%)	3 (27%)	0	0	0	1 (9%)	3 (27%)	0	11 (14%)
Saintpaul	0	0	3 (30%)	3 (30%)	0	0	0	0	0	0	0	0	4 (40%)	10 (13%)
Senftenberg	0	0		7 (100%)	0	0	0	0	0	0	0	0	0	7 (9%)
Mbandaka	0	0	0	0	0	2 (33%)	0	0	0	2 (33%)	0	2 (33%)	0	6 (8%)
Javiana	0	0	0	0	0	3 (60%)	0	0	0	0	0	0	2 (40%)	5 (6%)
Berta	0	0	0	0	0	0	2 (50%)	2 (50%)	0	0	0	0	0	4 (5%)
Hartford	0	0	0	0	0	0	0	0	0	0	4 (100%)	0	0	4 (5%)
Anatum	0	0	3 (100%)	0	0	0	0	0	0	0	0	0	0	3 (4%)
Typhimurium	0	0	1 (33%)	0	0	0	0	0	2 (67%)	0	0	0	0	3 (4%)
Inverness	0	0	0	0	0	0	0	0	0	2 (100%)	0	0	0	2 (3%)
Bareilly	1 (100%)	0	0	0	0	0	0	0	0	0	0	0	0	1 (1%)
Infantis	0	0	0	0	0	1 (100%)	0	0	0	0	0	0	0	1 (1%)
Muenchen	0	0	0	0	0	0	0	0	1 (100%)	0	0	0	0	1 (1%)
Rubislaw	0	0	0	0	0	0	0	0	0	0	1 (100%)	0	0	1 (1%)
Thompson	0	0	0	0	0	0	0	0	0	0	0	0	1 (100%)	1 (1%)
Total	4 (5%)	0 (0%)	7 (9%)	10 (13%)	0 (0%)	10 (13%)	6 (8%)	4 (5%)	8 (10%)	10 (13%)	7 (9%)	5 (6%)	7 (9%)	78 (100%)

a does not include those isolates that were untypeable or untyped

Table 5.3. *Salmonella enterica* isolates – serotype diversity in order of frequency, and the frequency rank each serotype has been found in humans and/or animal cases

August 2007 to August 2009 Satilla River Basin Environmental Isolates detected from surface water			2006 Top 20 CDC Annual Summary[a]					
			Human Sources		Clinical NonHuman Sources		Non-Clinical Non Human Sources	
Serotype	Rank	Isolated (%)	Rank	Reported (%)	Rank	Reported (%)	Rank	Reported (%)
Montevideo	1	18 (23.1%)	7	1061 (2.6%)	6	356 (4.4%)	10	160 (2.4%)
Braenderup	2	11 (14.1%)	12	561 (1.4%)				
Saintpaul	3	10 (12.8%)	11	588 (1.4%)			20	93 (1.4%)
Senftenberg	4	7 (9.0%)			10	232 (2.9%	4	555 (8.3%)
Mbandaka	5	6 (7.7%)			13	172 (2.1%)	12	137 (2.0%)
Javiana	6	5 (6.4%)	5	1433 (3.5%)				
Berta	7	4 (5.1%)						
Hartford	8	4 (5.1%)						
Anatum	9	3 (3.8%)			7	315 (3.9%)	9	179 (2.7%)
Typhimurium	10	3 (3.8%)	1	6872 (16.9%)	1	1592 (19.9%)	3	695 (10.4%)
Inverness	11	2 (2.6%)						
Bareilly	12	1 (1.3%)						
Infantis	13	1 (1.3%)	14	491 (1.2%)	16	159 (2.0%)		
Muenchen	14	1 (1.3%)	8	753 (1.9%)			19	95 (1.4%)
Rubislaw	15	1 (1.3%)						
Thompson	16	1 (1.3%)	15	447 (1.1%)				
# of Common Serotypes			**8**		**6**		**7**	

a Data from CDC as cited (15)

Table 5.4. Frequency (%) of *Salmonella* serotypes isolated in the Satilla River Basin (SRB) from the environment and cases reported to the Georgia Division of Public Health (PH)

Serotype	SRB study area	Case Report			PH Districts
	Environment [a] N=78	SRB Counties [b] N=327	PH District 8-1 N=606	PH District 9-2 N=582	Combined [c] N=1188
Montevideo	18 (23.08%)	12 (3.67%)	20 (3.30%)	50 (8.59%)	70 (5.89%)
Braenderup	11 (14.10%)	10 (3.06%)	4 (0.66%)	16 (2.75%)	20 (1.68%)
Saintpaul	10 (12.82%)	24 (7.34%)	24 (3.96%)	37 (6.36%)	61 (5.13%)
Senftenberg	7 (8.97%)	0 (0.00%)	1 (0.17%)	0 (0.00%)	1 (0.08%)
Mbandaka	6 (7.69%)	2 (0.61%)	2 (0.33%)	2 (0.34%)	4 (0.34%)
Javiana	5 (6.41%)	68 (20.80%)	104 (17.16%)	139 (23.88%)	243 (20.45%)
Berta	4 (5.13%)	0 (0.00%)	1 (0.17%)	0 (0.00%)	1 (0.08%)
Hartford	4 (5.13%)	0 (0.00%)	6 (0.99%)	1 (0.17%)	7 (0.59%)
Anatum	3 (3.85%)	1 (0.31%)	2 (0.33%)	3 (0.52%)	5 (0.42%)
Typhimurium	3 (3.85%)	18 (5.50%)	57 (9.41%)	51 (8.76%)	108 (9.09%)
Inverness	2 (2.56%)	0 (0.00%)	2 (0.33%)	0 (0.00%)	2 (0.17%)
Bareilly	1(1.28%)	1 (0.31%)	6 (0.99%)	0 (0.00%)	6 (0.51%)
Infantis	1 (1.28%)	1 (0.31%)	1 (0.17%)	1 (0.17%)	2 (0.17%)
Muenchen	1 (1.28%)	18 (5.50%)	27 (4.46%)	46 (7.90%)	73 (6.14%)
Rubislaw	1 (1.28%)	2 (0.61%)	8 (1.32%)	6 (1.03%)	14 (1.18%)
Thompson	1 (1.28%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Total		157 (48.01%)	265 (43.73%)	352 (60.48%)	617 (51.94%)

a does not include those isolates that were untypeable or not typed

b Atkinson, Bacon, Ben Hill, Coffee, Irwin, Jeff Davis and Ware Counties

c Public Health District 8-1 and Public Health District 9-2 combined case reports

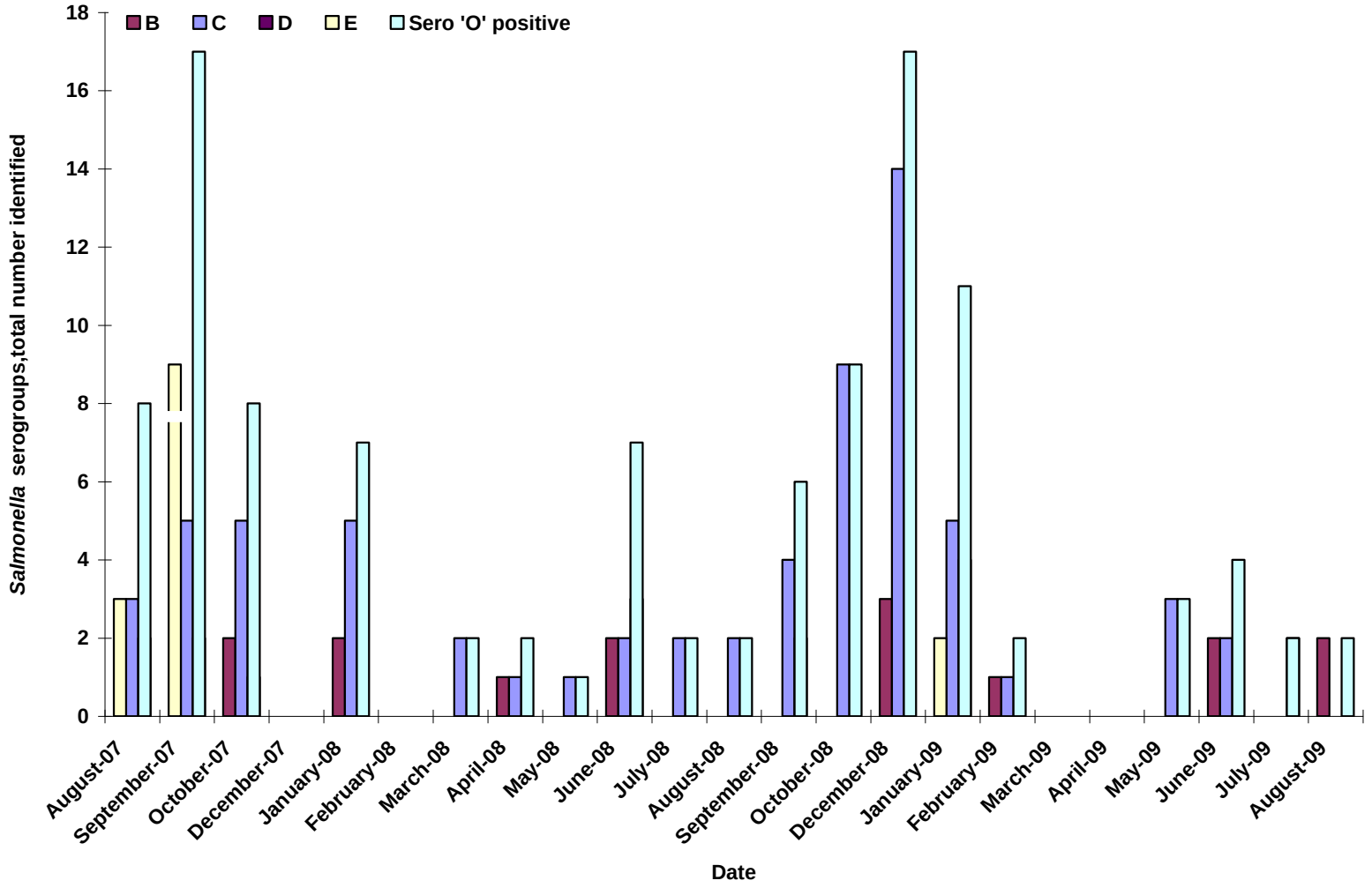
Table 5.5. Incidence of *Salmonella* and *Campylobacter* rates per 100,000 (# cases)

	2007		2008		2009		Average	
	Salmonella	Campylobacter	Salmonella	Campylobacter	Salmonella	Campylobacter	Salmonella	Campylobacter
United States	14.92 (6,790)	12.78 (5,816)	16.20 (7,444)	12.68 (5,825)	15.19 (7,039)	13.02 (6,033)		
Georgia	21.35 (2,033)	7.28 (693)	23.77 (2,302)	7.12 (690)	24.02 (2,361)	7.56 (743)	23.04 (2,232)	7.21 (709)
Public Health District (8-1)	57.46 (139)	12.40 (30)	99.78 (246)	8.92 (22)	88.64 (221)	12.03 (30)	81.96 (202)	10.96 (27)
Public Health District (9-2)	60.65 (209)	11.32 (39)	69.59 (243)	13.75 (48)	62.67 (221)	11.63 (41)	64.30 (224)	12.10 (43)
SRB Counties [a]	63.50 (86)	17.72 (24)	99.72 (136)	21.26 (29)	75.95 (104)	9.49 (13)	79.72 (109)	16.16 (22)

a SRB, Satilla River Basin counties (Atkins, Bacon, Ben Hill, Coffee, Irwin, Jeff Davis, and Ware

FIGURE LEGEND

Figure 5.1.


B
C
D
E
Sero 'O' positive
Salmonella serogroups,total number identified
0
2
4
6
8
10
12
14
16
18
August-07
September-07
October-07
December-07
January-08
February-08
March-08
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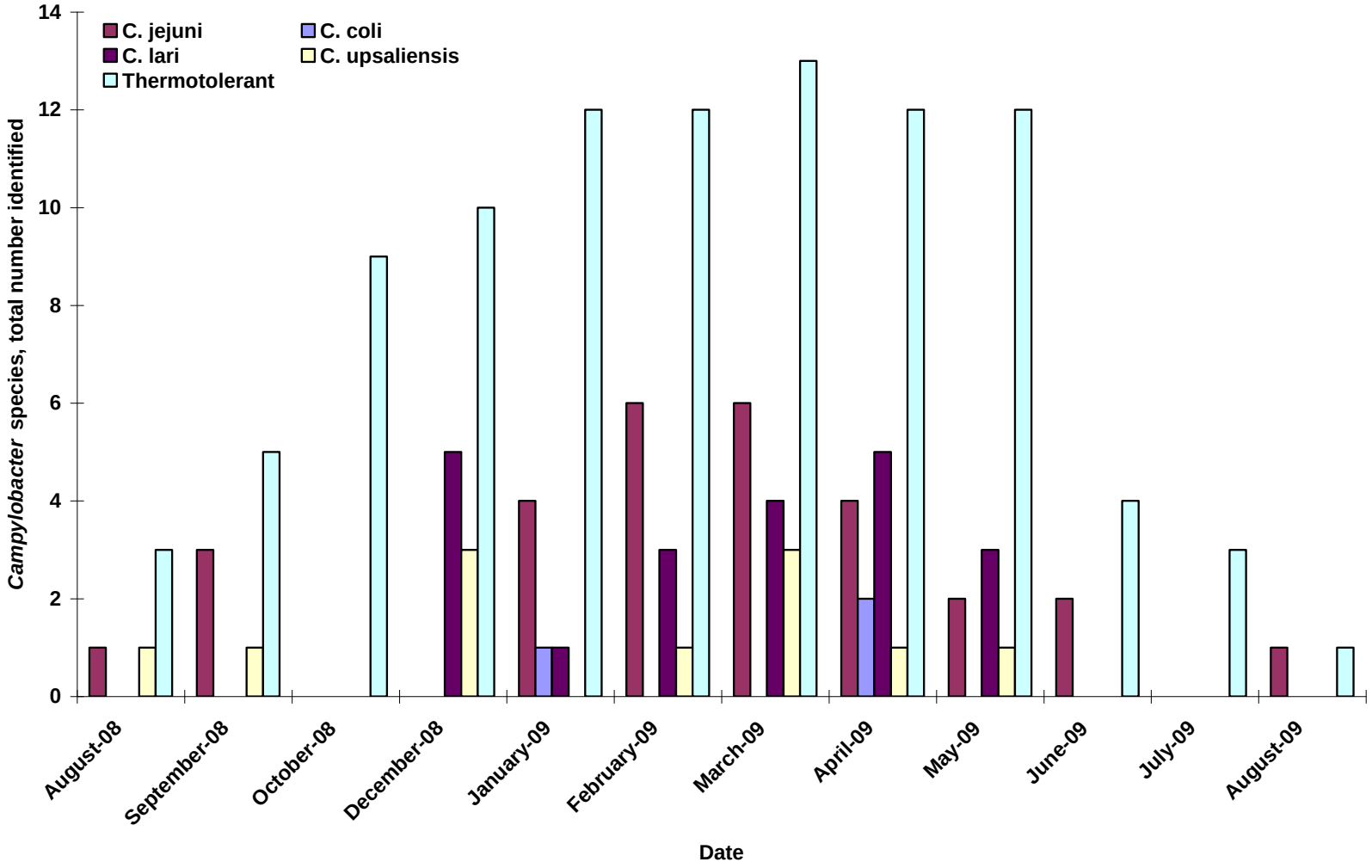
March-09
April-09
May-09
June-09
July-09
August-09
Date

Figure 5.2.


C. jejuni
C. lari
Thermotolerant
C. coli
C. upsaliensis
Campylobacter species, total number identified
0
2
4
6
8
10
12
14
August-08
September-08
October-08
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January-09
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March-09
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July-09
August-09
Date

Figure 5.3.

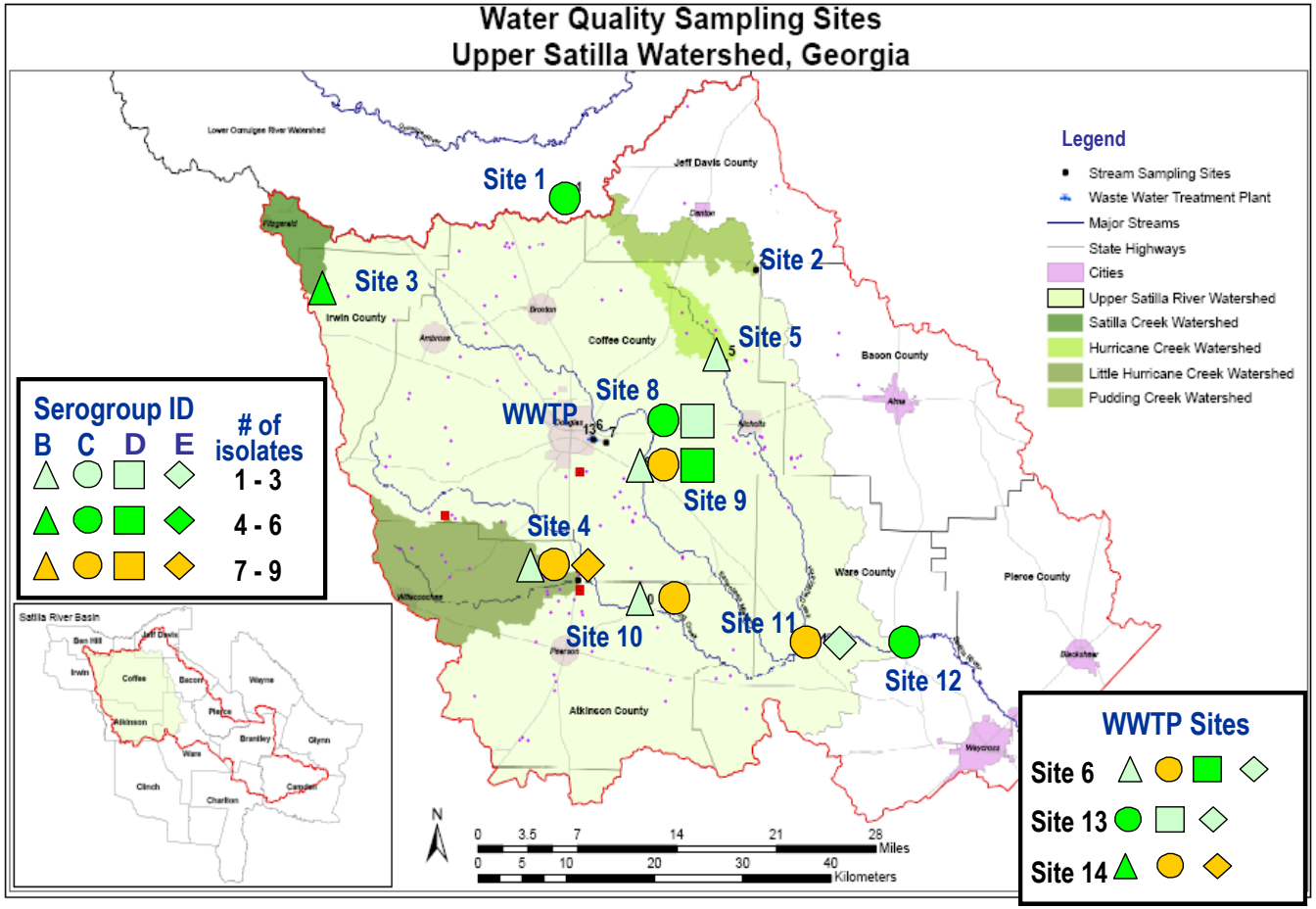
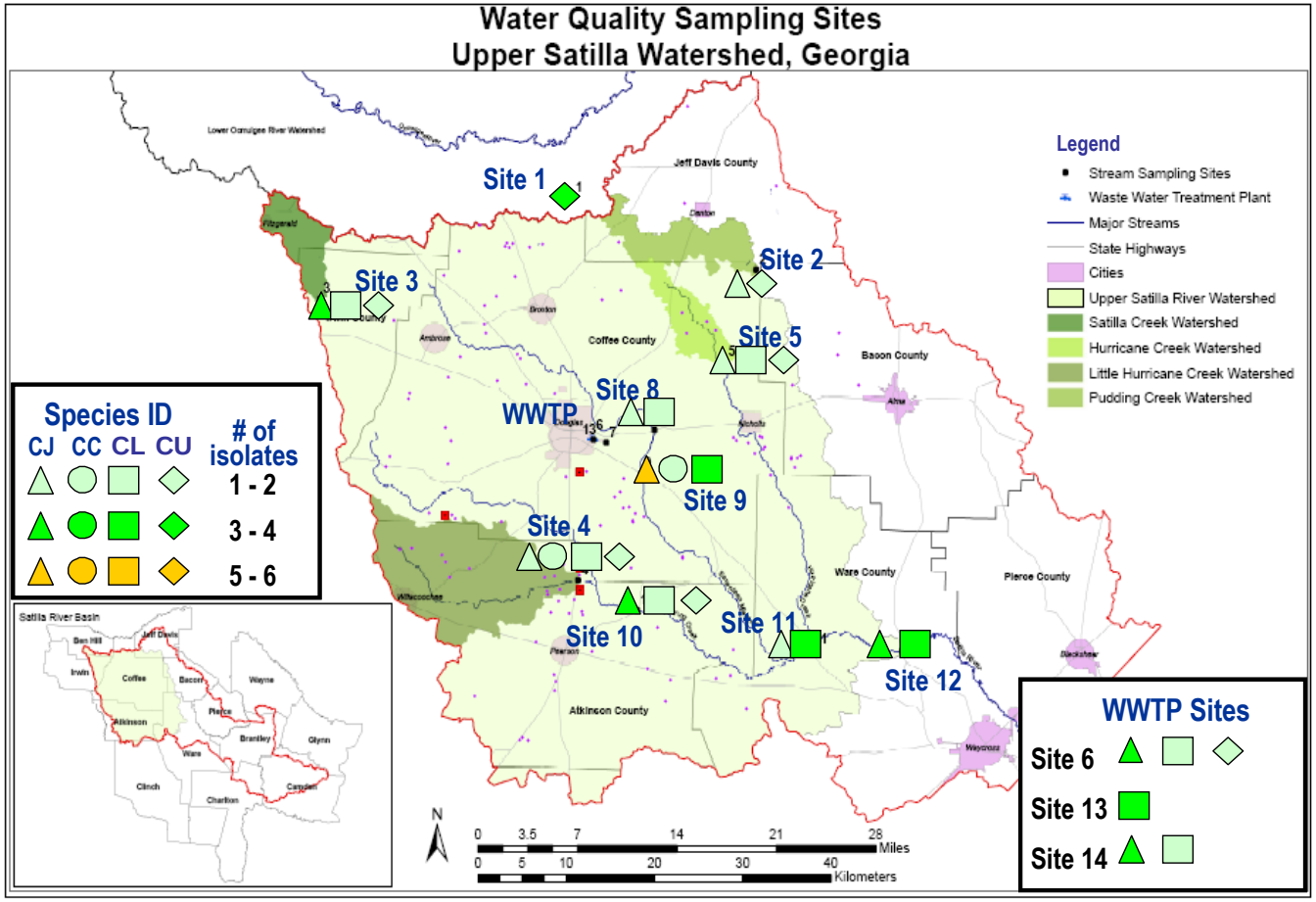

Water Quality Sampling Sites
Upper Satilla Watershed, Georgia
Legend
Stream Sampling Sites
Waste Water Treatment Plant
Major Streams
State Highways
Cities
Upper Satilla River Watershed
Satilla Creek Watershed
Hurricane Creek Watershed
Little Hurricane Creek Watershed
Pudding Creek Watershed
Site 1
Site 2
Site 3
Site 4
Site 5
Site 8
WWTP
Site 9
Site 10
Site 11
Site 12
Jeff Davis County
Irwin County
Coffee County
Bacon County
Ware County
Pierce County
Atkinson County
Serogroup ID
B C D E
# of isolates
1 - 3
4 - 6
7 - 9
Satilla River Basin
WWTP Sites
Site 6
Site 13
Site 14
0 3.5 7 14 21 28 Miles
0 5 10 20 30 40 Kilometers

Figure 5.4.


Water Quality Sampling Sites
Upper Satilla Watershed, Georgia
Legend
Stream Sampling Sites
Waste Water Treatment Plant
Major Streams
State Highways
Cities
Upper Satilla River Watershed
Satilla Creek Watershed
Hurricane Creek Watershed
Little Hurricane Creek Watershed
Pudding Creek Watershed
Site 1
Site 2
Site 3
Site 4
Site 5
Site 8
WWTP
Site 9
Site 10
Site 11
Site 12
Species ID
CJ CC CL CU
# of isolates
1 - 2
3 - 4
5 - 6
WWTP Sites
Site 6
Site 13
Site 14
Jeff Davis County
Coffee County
Bacon County
Ware County
Pierce County
Atkinson County
Satilla River Basin
N
0 3.5 7 14 21 28 Miles
0 5 10 20 30 40 Kilometers

CHAPTER 6

SUMMARY AND CONCLUSION

While poultry production is a large part of the global economy and one of the fastest growing segments of the animal industry (15), poultry litter, a by product of this industry may contain pathogenic microorganisms, such as *Salmonella* and *Campylobacter* (11, 12, 14); and concerns exist about the potential runoff of microbial pathogens from land applied animal waste, including poultry litter (5, 7, 8, 10, 13). Monitoring and assessing agricultural watersheds impacted by poultry production is important because of the wider impact agricultural practices such as spreading of poultry litter may have on water quality. Nonpoint source pollution from agricultural runoff threatens aquatic habitats, and a variety of other beneficial uses of water resources.

An important focus of the Satilla River Basin (SRB) assessment was to identify how the varying levels of agricultural landuse and poultry production in the SRB influenced the presence of *Salmonella* and *Campylobacter*. A limitation of this study was that we were not able to detect the pathogens directly from poultry litter, and there was a severe drought in 2007 that limited sample collection. However, even under drought conditions, we were still able to detect both *Salmonella* and *Campylobacter* throughout the SRB. *Salmonella* and *Campylobacter* were more frequently detected in areas where poultry litter was being applied to agricultural fields near poultry houses, and nutrient concentrations at these stations were also higher suggesting that poultry litter may be a source of these pathogens in the SRB. *Salmonella* and *Campylobacter* prevalence was also associated with several measures of precipitation including, 30-day

antecedent rainfall, indicating that rainfall related inputs (primarily from surface run-off) may also be transporting *Salmonella* and *Campylobacter* to the streams.

Salmonella and *Campylobacter* were also detected more frequently from samples downstream of a small watershed receiving direct discharge from a wastewater treatment plant (WWTP) in the SRB. The WWTP received municipal and poultry slaughterhouse waste and generally, nutrient concentrations as well as measured water quality parameters were higher at the treatment plant effluent stations than all other sites. The constant loading of effluent discharge from the treatment plant into SRB (downstream sites) is likely supplying a constant flux of nutrients and pathogens as an additional source contributing to the presence of *Salmonella* and *Campylobacter*. This hypothesis is generally supported as we observed a decrease in nutrient concentrations and the prevalence of the pathogens downstream of the WWTP when the broiler operation that supplied 50% of the waste (D. Wilcox, plant operator, personal communication) to the treatment plant closed. The closure occurred in the last months of the study and a thorough investigation of water quality and *Salmonella* and *Campylobacter* detection post-closure of the broiler processing plant was not feasible, but is an area where future research is warranted. The plant is scheduled to reopen and the data collected from our study will aid future researchers in continued observations.

Other sources of *Salmonella* and *Campylobacter* in the SRB may include a variety of domestic and wild animals known to harbor *Salmonella* and *Campylobacter* (1-4, 6, 9) as more than half of the landuse at the stations downstream of the WWTP are home to these animals. Additionally, these areas are frequently visited year-round for outdoor nature based recreational activities and there is substantial risk that humans may be contributing the pathogens to the environment; or transmission of *Salmonella* and *Campylobacter* to humans may occur through

contact with animals and this environment. We did observe that clinically relevant *Salmonella* serotypes and *Campylobacter* species detected in the environment were similar to those serotypes and species of clinical cases reported in the SRB.

Water quality is essential to public health, our natural environment, and economic development. Future studies must continue to investigate the microbial ecology of *Salmonella* and *Campylobacter* to better understand their presence environmentally in contaminating surface waters in a mixed use watershed.

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APPENDICES

APPENDIX A: CHAPTER 3 SUPPLEMENTARY INFORMATION

TABLES

Table A.3.1. Significant sample site differences [a] among mean water quality and nutrient variables

	Water Quality Variables[b]								Nutrient Variables[b]													
Site	Temp		SpCond		Chl *a*		ORP		NH_4		NO_3		Ortho P		Chloride		TC		IC		DOC	
1	15.50	A	0.06	B	9.50	BC	176.40	AB	0.015	A	0.022	A	176.40	B	9.801	B	19.840	D	2.697	B	17.251	E
2	15.56	A	0.07	B	12.61	BC	189.33	A	0.141	A	0.008	A	189.33	B	11.936	B	39.518	A	2.393	B	37.126	A
3	14.57	A	0.10	B	8.08	C	129.71	C	0.126	A	0.173	A	129.71	B	13.189	B	23.749	CD	5.641	AB	18.106	E
4	19.10	A	0.10	B	21.58	A	159.05	ABC	0.082	A	0.192	A	159.05	B	13.531	B	38.879	AB	3.229	B	35.839	AB
5	15.40	A	0.10	B	15.83	AB	140.40	BC	0.086	A	0.026	A	140.40	B	13.074	B	36.612	AB	3.315	B	33.296	ABC
8	17.26	A	0.10	B	10.28	BC	143.33	BC	0.113	A	0.040	A	0.013	B	12.365	B	30.110	BC	3.191	B	27.359	CD
9	17.58	A	0.30	A	10.60	BC	148.65	ABC	0.776	A	0.294	A	1.847	A	42.783	A	30.222	ABC	9.712	A	20.888	DE
10	19.01	A	0.09	B	10.56	BC	158.48	ABC	0.079	A	0.043	A	0.025	B	11.646	B	30.594	ABC	3.375	B	27.386	BCD
11	19.64	A	0.12	B	12.36	BC	154.70	ABC	0.224	A	0.030	A	0.069	B	21.972	AB	32.737	ABC	2.965	B	29.844	ABC
12	20.13	A	0.10	B	11.70	BC	173.17	AB	0.073	A	0.019	A	0.065	B	14.425	B	31.630	ABC	2.649	B	28.985	ABCD

a Variables with the same letter in a column are not significantly different ($p<0.05$) using Tukey's HSD test only those variables that showed significant site variation (excluding WWTP sites) using Kruskal-Wallis test reported

b Temperature (Temp), conductivity (SpCond), oxidation reduction potential (ORP), phosphate (ortho P), ammonium (NH_4), nitrate (NO_3), phosphate (Ortho P), total carbon (TC), inorganic carbon (IC), and dissolved organic carbon (DOC)

Table A.3.2. Poultry production, agricultural landuse [a] and significant sample site differences [b] among water quality variables

Watershed	Site	Number of Poultry Producers	Number of Poultry Houses	Agricultural[c] Landuse (%)	Water Quality Variables[d]							
					SpCond		ORP		pH		Chl *a*	
Small	1	0	0	5	0.06	C	176.40	AB	6.66	AB	9.50	B
	2	0	0	22	0.07	BC	189.33	A	6.32	B	12.61	AB
	3	0	0	52	0.10	A	129.71	C	7.20	A	8.08	B
	4	12	50	30	0.10	AB	159.05	ABC	6.82	AB	21.58	A
	5	4	21	20	0.10	AB	140.40	BC	7.03	AB	15.83	AB
Large	1	0	0	5	0.06	B	176.40		6.66		9.50	A
	8	22	98	33	0.10	B	143.33		7.09		10.28	A
	9	22	98	32	0.30	A	148.65		6.96		10.60	A
	10	42	173	32	0.09	B	158.48		6.94		10.56	A
	11	91	368	25	0.12	B	154.70		6.89		12.36	A
	12	112	440	23	0.10	B	173.17		6.61		11.70	A

a Land use is based on 1998 landuse classification (Georgia GIS Clearinghouse)

b Variables with the same letter in a row are not significantly different ($p<0.05$) using Tukey's HSD test

only those variables that showed significant site variation (excluding WWTP sites) using Kruskal-Wallis test reported

c Includes row-crops and pasture land

d Conductivity (SpCond), oxidation reduction potential (ORP), chlorophyll *a* (Chl *a*)

Table A.3.3. Poultry production, agricultural landuse [a] and significant sample site differences [b] among nutrient variables

Watershed	Site	Number of Poultry Producers	Number of Poultry Houses	Agricultural[c] Landuse (%)	Nutrient Variables[d]													
					NH_4		NO_3		Ortho P		Chloride		TC		IC		DOC	
Small	1	0	0	5	0.015	A	0.022	A	176.40	A	9.801	A	19.840	B	2.697	AB	17.251	B
	2	0	0	22	0.141	A	0.008	A	189.33	A	11.936	A	39.518	A	2.393	B	37.126	A
	3	0	0	52	0.126	A	0.173	A	129.71	A	13.189	A	23.749	B	5.641	A	18.106	B
	4	12	50	30	0.082	A	0.192	A	159.05	A	13.531	A	38.879	A	3.229	AB	35.839	A
	5	4	21	20	0.086	A	0.026	A	140.40	A	13.074	A	36.612	A	3.315	AB	33.296	A
Large	1	0	0	5	0.015	A	0.022	B	176.40	B	9.801	B	19.840	B	2.697	B	17.251	C
	8	22	98	33	0.113	A	0.040	B	0.013	B	12.365	B	30.110	A	3.191	B	27.359	AB
	9	22	98	32	0.776	A	0.294	A	1.847	A	42.783	A	30.222	A	9.712	A	20.888	BC
	10	42	173	32	0.079	A	0.043	B	0.025	B	11.646	B	30.594	A	3.375	B	27.386	AB
	11	91	368	25	0.224	A	0.030	B	0.069	B	21.972	AB	32.737	A	2.965	B	29.844	A
	12	112	440	23	0.073	A	0.019	B	0.065	B	14.425	B	31.630	A	2.649	B	28.985	A

a Land use is based on 1998 landuse classification (Georgia GIS Clearinghouse)

b Variables with the same letter in a row are not significantly different ($p<0.05$) using Tukey's HSD test

only those variables that showed significant site variation (excluding WWTP sites) using Kruskal-Wallis test reported

c Includes row-crops and pasture land

d Ammonium (NH_4), nitrate (NO_3), phosphate (Ortho P), total carbon (TC), inorganic carbon (IC), and dissolved organic carbon (DOC)

Table A.3.4. Correlation matrix[a] of water quality and nutrient variables[b]

	Discharge	Temp	SpCond	DO	pH	ORP	Turbidity	Chl *a*	NH4N	NO3N	Ortho P	Chloride	TKN	TP	TC	IC	DOC
Discharge	1																
Temp		1															
SpCond	-0.20248		1														
DO		-0.28365		1													
pH					1												
ORP			-0.1797	0.4774	-0.62895	1											
Turbidity	0.24629	0.22854				0.30243	1										
Chl *a*						0.27371	0.34637	1									
NH4N		0.1959	0.27619	-0.23533		-0.08189	0.3055	0.29547	1								
NO3N	0.3601			0.1893						1							
Ortho P		0.3793	0.19446					0.30165	0.23435	0.24219	1						
Chloride	-0.30028	-0.16362	0.71192			-0.19979	-0.30293				0.20374	1					
TKN			0.16118	-0.23395		-0.17712	-0.21075					0.17361	1				
TP	-0.2931	0.21934	0.17953								0.28228	0.15189	0.29056	1			
TC	0.28861		0.19037			0.20235	0.18675	0.5019	0.42457		0.43771	0.19724		0.21018	1		
IC			0.40488	0.23491								0.3044		0.4132	0.25753	1	
DOC	0.33478					0.26202	0.20637	0.58436	0.35651		0.34053				0.87458		1

a **Only those variables (excluding WWTP sites) that showed significant associations ($p \leq 0.05$) using Spearman's rank correlation coefficient reported**

b **Temperature (temp), conductivity (SpCond), dissolved oxygen (DO), oxidation reduction potential (ORP), chlorophyll *a* (Chl *a*), ammonia-N (NH_4-N), nitrate-N (NO_3-N), dissolved molybdate reactive P (ortho P), total Kjeldahl N (TKN), total P (TP), total carbon (TC), inorganic carbon (IC), and dissolved organic carbon (DOC)**

Table A.3.4. Correlation matrix[a] of water quality and nutrient variables[b] cont.

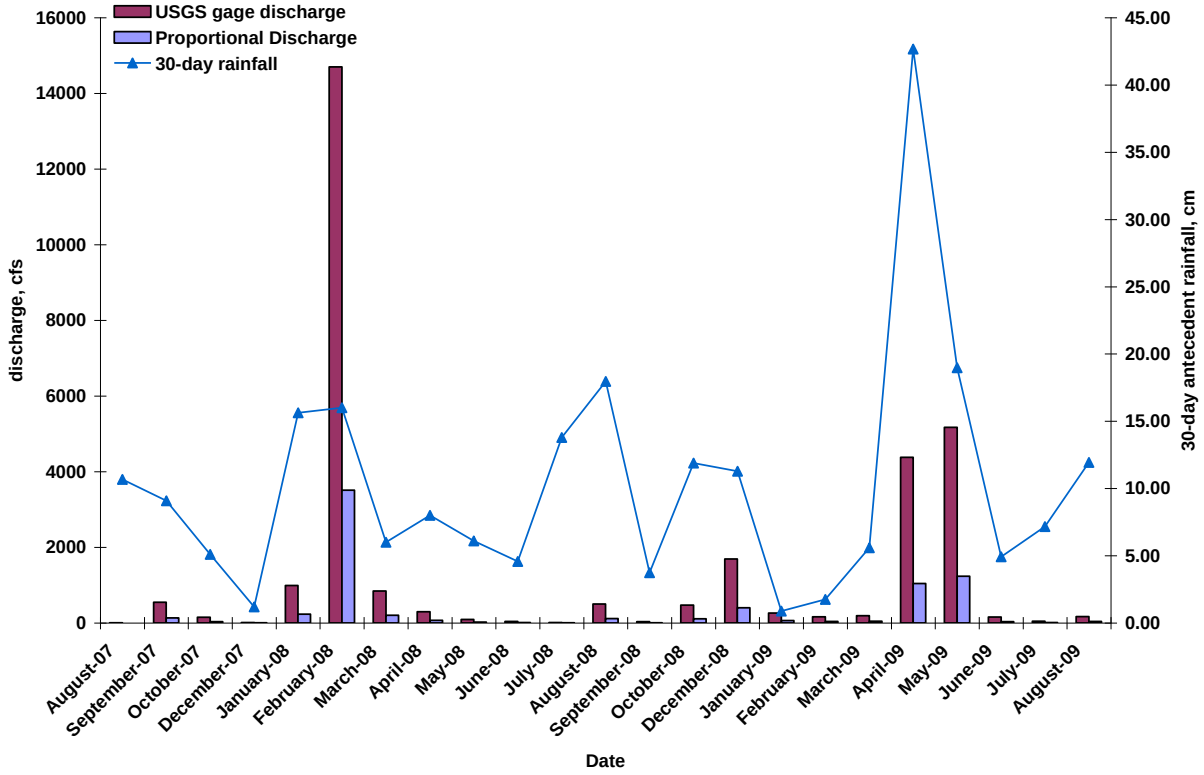
									Rainfall (total) measured to sample date				
	discharge	Temp	SpCond	DO	pH	ORP	Turbidity	Chl a	sample day	day before	7 days prior	10 days prior	30 days prior
discharge	1												
Temp		1											
SpCond	-0.20248		1										
DO		-0.28365		1									
pH					1								
ORP			-0.1797	0.4774	-0.62895	1							
Turbidity	0.24629	0.22854				0.30243	1						
Chl *a*						0.27371	0.34637	1					
sample day		0.28062	-0.20411	0.27579		0.14706			1				
day before		0.32308	-0.20189	0.24493		0.18919			0.93987	1			
7 days prior	0.29153		-0.24596						0.64892	0.60002	1		
10 days prior	0.57216		-0.31112	0.22711		0.26299	0.18673		0.62872	0.55081	0.87562	1	
30 days prior	0.55316		-0.30411	0.33133		0.36612	0.2803		0.61462	0.57874	0.68318	0.90437	1

a Only those variables (excluding WWTP sites) that showed significant associations ($p \leq 0.05$) using Spearman's rank correlation coefficient reported

b Temperature (temp), conductivity (SpCond), dissolved oxygen (DO), oxidation reduction potential (ORP), chlorophyll *a* (Chl *a*)

FIGURE LEGEND

Figure A.3.1.


USGS gage discharge
Proportional Discharge
30-day rainfall
discharge, cfs
30-day antecedent rainfall, cm
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2000
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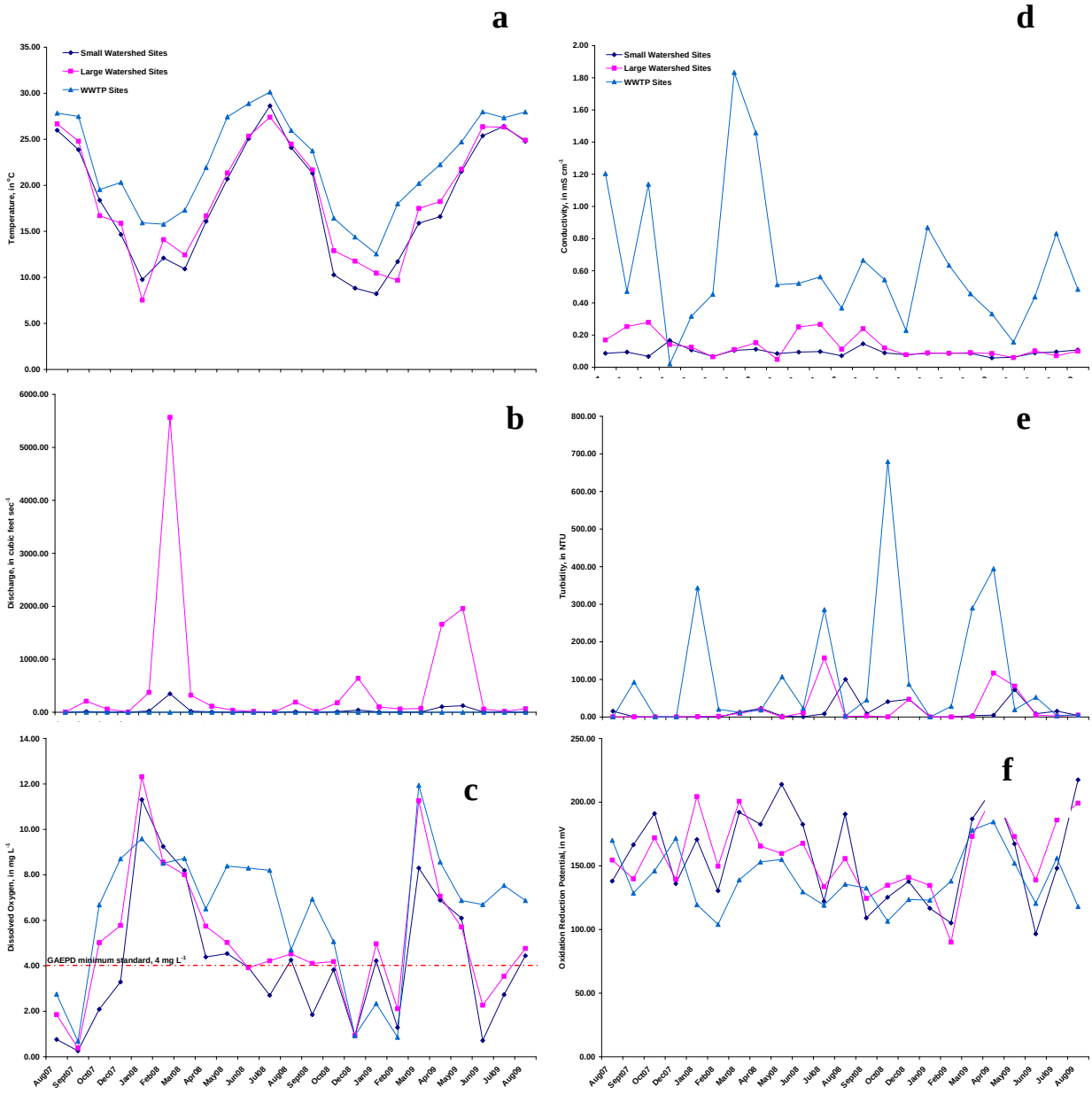
Figure A.3.2.



a Temperature

b Discharge (predicted area proportional)

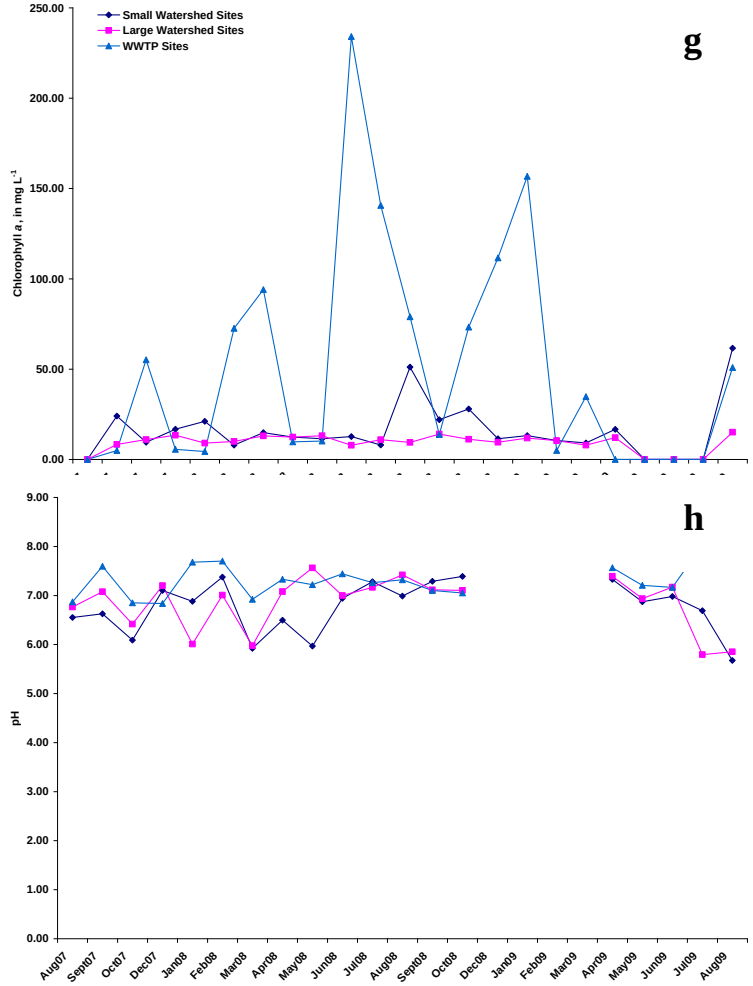
c Dissolved Oxygen

d Conductivity

e Turbidity

f Oxidation Reduction Potential

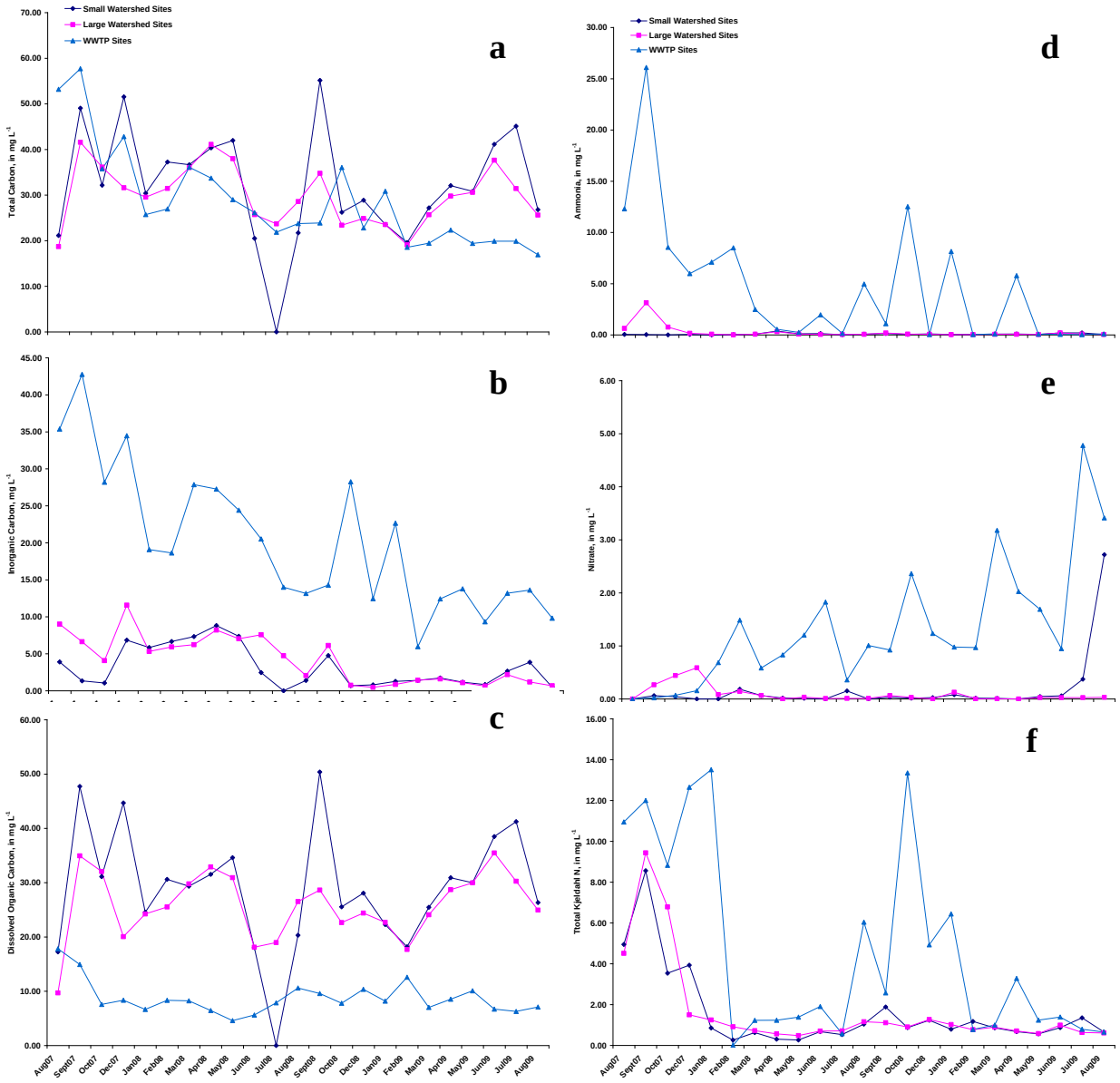
Figure A.3.2. cont.



g Chlorophyll *a*

h pH

Figure A.3.3.



a Total Carbon

b Inorganic Carbon

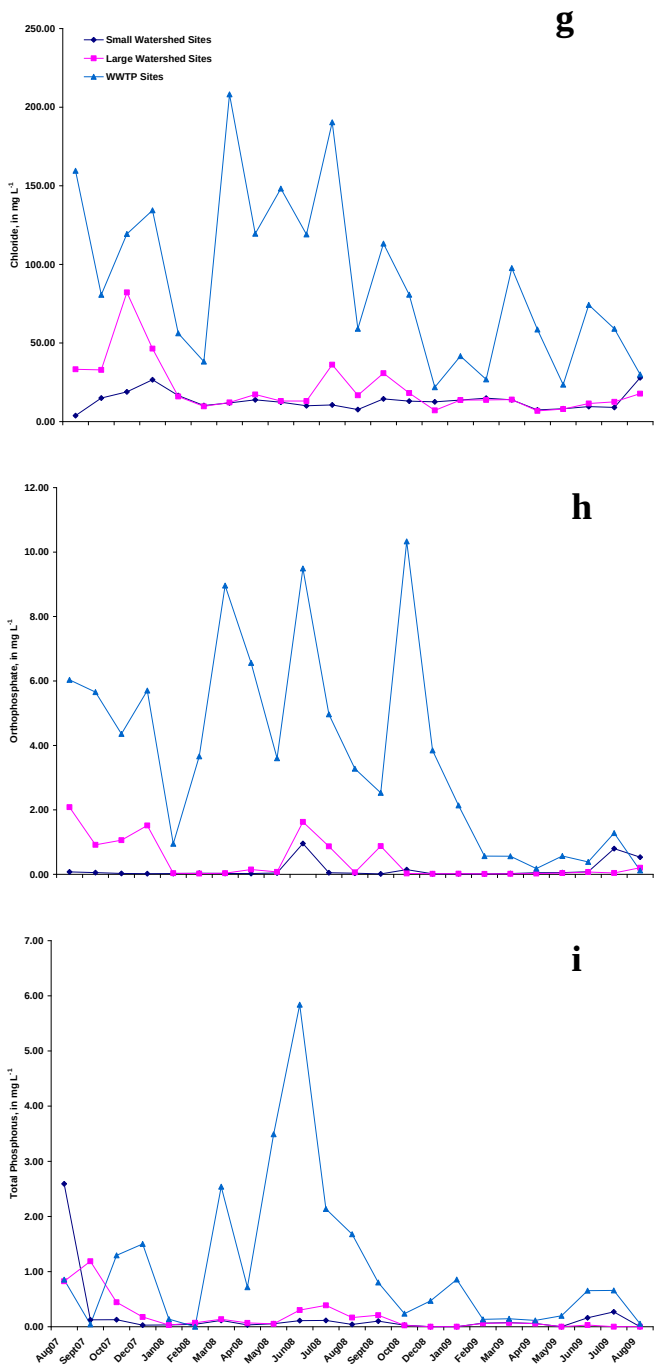
c Dissolved Organic Carbon

d Ammonia-N

e Nitrate-N

f Ttotal Kjeldahl N

Figure A.3.3. cont.

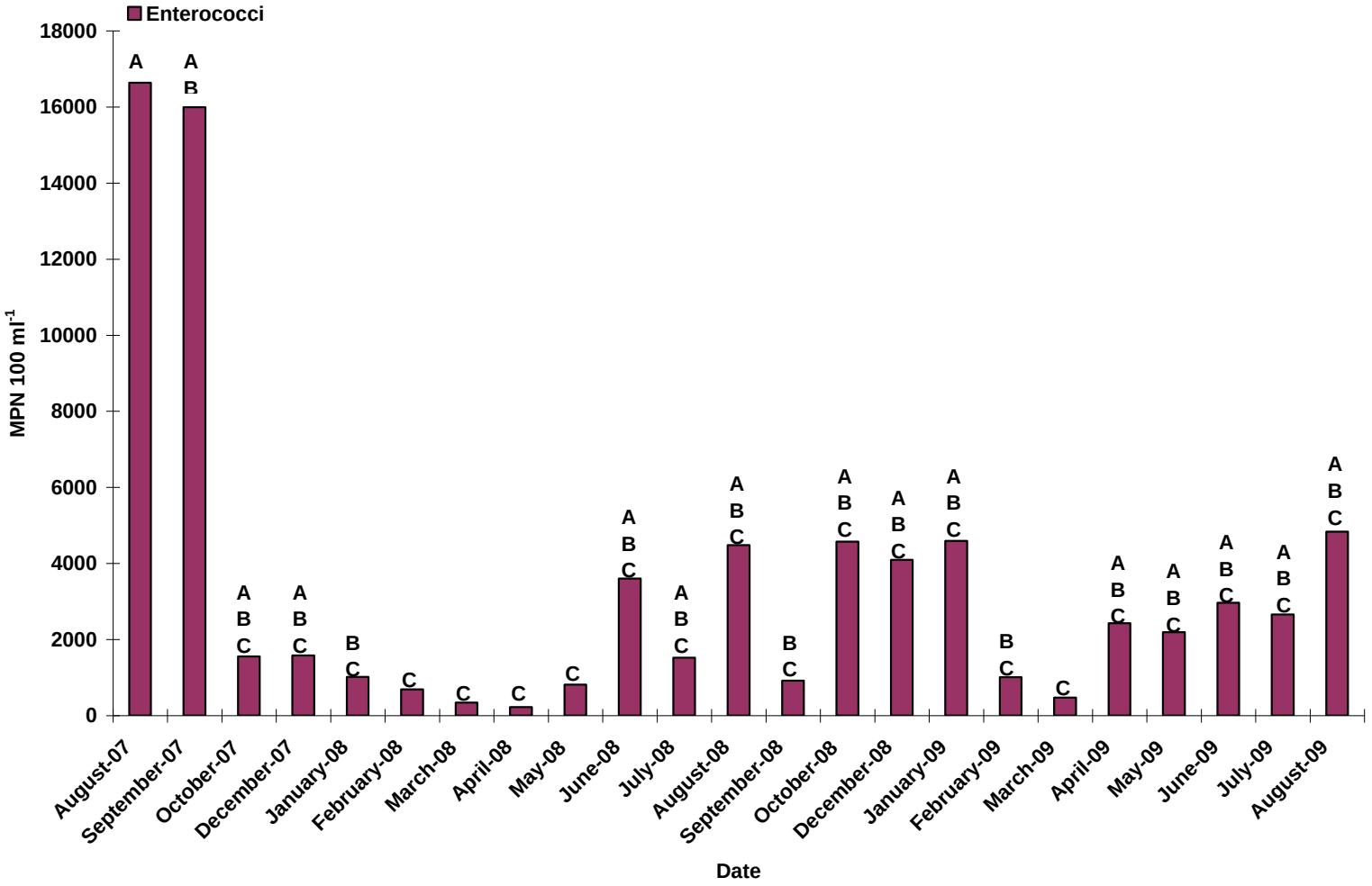


g Chloride

h Orthophosphate

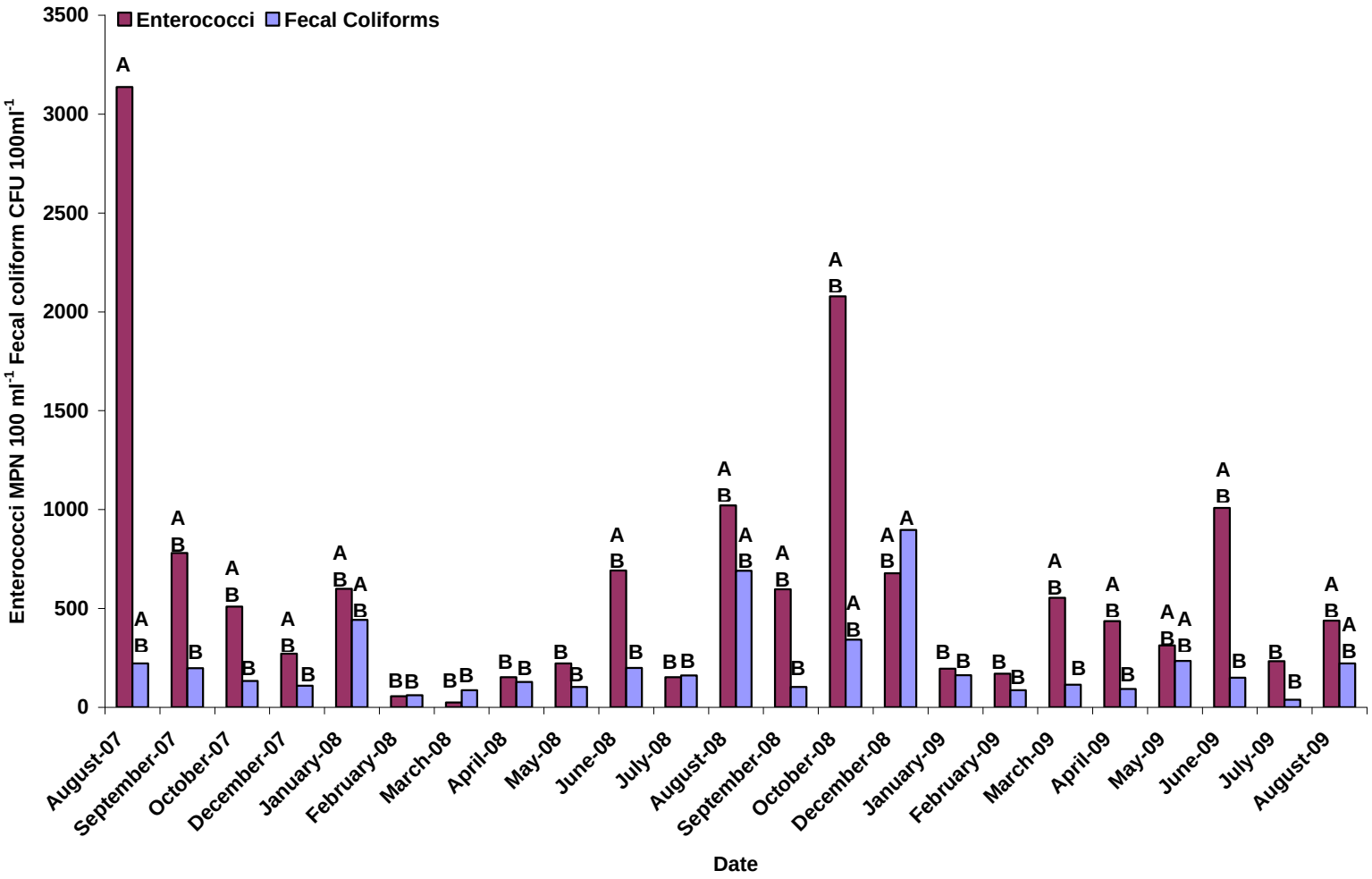
i Total Phosphorus

Figure A.3.4.



a Variables with the same letter in a row are not significantly different ($p<0.05$) using Tukey's HSD test

Figure A.3.5.



a Variables with the same letter in a row are not significantly different ($p<0.05$) using Tukey's HSD test

APPENDIX B: CHAPTER 5 SUPPLEMENTARY INFORMATION

TABLES

Table B.5.1. Frequency (%) of *Salmonella* serogroups at different sample sites in the Satilla River Basin

Serogroup	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 8	Site 9	Site 10	Site 11	Site 12	Site 13	Site 14	Total (%) of Isolates [a]	Total (%) of Serogroup (+) [b]
B	0 (0.0%)	0 (0.0%)	4 (25.0%)	1 (6.3%)	2 (12.5%)	2 (12.5%)	0 (0.0%)	1 (6.3%)	2 (12.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (25.0%)	16 (6.1%)	16 (14.3%)
C	5 (7.6%)	0 (0.0%)	0 (0.0%)	7 (10.6%)	0 (0.0%)	8 (12.1%)	5 (7.6%)	8 (12.1%)	8 (12.1%)	8 (12.1%)	6 (9.1%)	6 (9.1%)	5 (7.6%)	66 (25.1%)	66 (58.9%)
D	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (25.0%)	2 (12.5%)	4 (25.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (12.5%)	4 (25.0%)	16 (6.1%)	16 (14.3%)
E	0 (0.0%)	0 (0.0%)	0 (0.0%)	7 (50.0%)	0 (0.0%)	3 (21.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (21.4%)	0 (0.0%)	1 (7.1%)	0 (0.0%)	14 (5.3%)	14 (5.3%)
BCDE (-)	12 (7.9%)	6 (4.0%)	5 3.3%)	17 (11.3%)	10 (6.6%)	9 (6.0%)	12 (7.9%)	16 (10.6%)	16 (10.6%)	18 (11.9%)	14 (9.3%)	8 (5.3%)	8 (5.3%)	151 (57.4%)	

a N=263, isolates tested Salmonella positive

b N=112, Salmonella positive isolates that were identified by serogroup

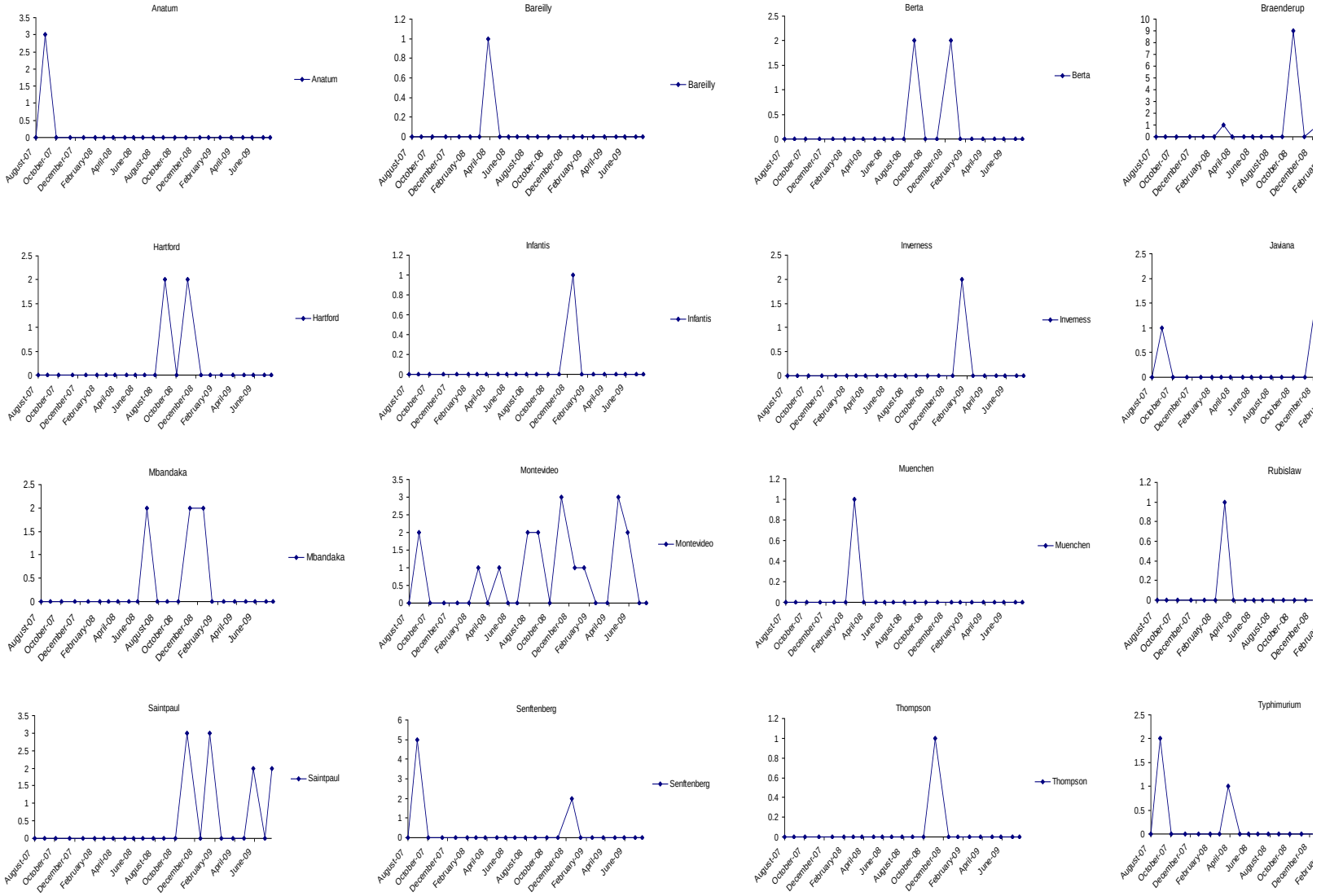
Table B.5.2. Frequency (%) of *Campylobacter* species at different sample sites in the Satilla River Basin

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 8	Site 9	Site 10	Site 11	Site 12	Site 13	Site 14	TOTAL
C. jejuni	0	2 (7%)	4 (14%)	2 (7%)	1 (3%)	3 (10%)	2 (7%)	5 (17%)	3 (10%)	1 (3%)	3 (10%)	0	3 (10%)	29 (45%)
C. coli	0	0	0	2 (67%)	0	0	0	1 (33%)	0	0	0	0	0	3 (5%)
C. lari	0	0	1 (5%)	1 (5%)	1 (5%)	1 (5%)	2 (10%)	3 (14%)	2 (10%)	3 (14%)	3 (14%)	3 (14%)	1 (5%)	21 (33%)
C. upsaliensi	4 (36%)	1 (9%)	2 (18%)	1 (9%)	1 (9%)	1 (9%)	0	0	1 (9%)	0	0	0	0	11 (17%)
Total	4 (6%)	3 (5%)	7 (11%)	6 (9%)	3 (5%)	5 (8%)	4 (6%)	9 (14%)	6 (9%)	4 (6%)	6 (9%)	3 (5%)	4 (6%)	64 (100%)

FIGURE LEGEND

Figure B.5.1. Monthly distribution of *Salmonella* serotypes

Figure B.5.1.


Anatum
Bareilly
Berta
Braenderup
Hartford
Infantis
Inverness
Javiana
Mbandaka
Montevideo
Muenchen
Rubislaw
Saintpaul
Senftenberg
Thompson
Typhimurium
August-07
October-07
December-07
February-08
April-08
June-08
August-08
October-08
December-08
February-09
April-09
June-09